Oncode Institute

Outsmarting cancer
Impacting lives

Oncode Investigator Annual Reports

2023

Content

Oncode Institute Oncode Investigator Annual Reports - Reporting period: 2023

May 2024

Reuven Agami

Netherlands Cancer Institute

1. General information

Research Focus	Controlling cancer by RNA
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our primary research objective is to identify novel cellular vulnerabilities that can be exploited for cancer therapies. For this purpose, we have developed, and are developing, innovative genomic and genetic tools to functionally interrogate such vulnerabilities. In recent three years, we have made major discoveries in the field of mRNA translation in cancer. We investigated the impact of amino acid shortages on protein production in the context of immune-tumor cell interaction. This has led us to uncover a novel phenomenon that we coined "Sloppiness in mRNA translation in cancer". Cancer cells present a sloppy mRNA translation that, under nutrient stress, is prone to make specific mistakes. The outcome of Sloppiness is the production of two types of aberrant proteins. Chimera polypeptides (in-frame followed by out-of-frame) and specific substitutions. Intriguingly, these aberrant proteins are processed intracellularly to peptides that are presented as neo-epitopes at the surface of cancer cells to immune cells. In 2023, we set up experiments to characterize and broaden our knowledge on Sloppiness in mRNA translation cancer; and in parallel, we use immunological tools to target aberrant proteins to bring our findings to the clinic.

2.2. Major scientific achievements in 2023

a) We have identified additional types of aberrant proteins that indicate cancer processes connected to additional essential amino acids beyond tryptophan to phenylalanine substitutions.

b) We have developed a strategy for utilizing neoepitopes generated by Sloppiness in mRNA translation for cancer immunotherapy. We are discussing options for setting up a clinical trial with clinicians.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A. Aberrant peptides as a tool to enhance anti-tumor immunity.

Dysregulated protein production by oncogenic pathways fuels tumor development. Following induction of tryptophan shortage by Indoleamine 2,3-Dioxygenase 1 (IDO1) enzyme, caused by interferon-gamma (IFNγ) secretion by activated T cells, tryptophan to phenylalanine (W>F) substitutants are induced[1,2]. These defective protein products are processed and presented on Human Leukocyte Antigen (HLA) molecules as neoepitopes. Here, we addressed the potential of substitutant neoepitopes to improve cancer immunotherapy. Adoptive cancer immunotherapy is an attractive treatment mode but suffers the scarcity of shared and highly expressed immunogenic targets, as somatic genetic aberrations in cancer are mostly private and counter-selected for strong immunogenicity[3,4]. Moreover, adoptive T cell therapy against key neoantigens, such as MART1 in melanoma patients, is limited due to high toxicity when used in high concentrations5. Using immunopeptidomics, we identified IFN γ-inducible frequent W>F substitutant neoepitopes presented on HLA-A*24:02, a worldwide prevalent HLA allele that favors binding of peptides containing phenylalanine. In this group, the TMBIM6$^{W>F}$ neoepitope was derived from the gene with the broadest and highest expression in cancer transcriptome datasets. By priming healthy donor T cells, we identified a TCR (TCR$^{TMBIM6W>F.1}$) possessing high affinity and specificity towards TMBIM6$^{W>F}$/HLA-A*24:02, compared to its wild-type counterpart. We demonstrated that TCR$^{TMBIM6W>F.1}$ T cells are activated by IFNγ-mediated tryptophan-depleted cancer cells, provided they express HLA-A*24:02, TMBIM6, and IDO1 and are proficient in antigen presentation. Importantly, this activation was not observed when a collection of non-cancer cells was used, indicating cancer-specific effects. Finally, we provided a proof-of-concept approach for a novel clinical use of substitutants. We show that TCR$^{TMBIM6W>F.1}$ T cells enhance cancer cell killing by TCR^{MART1} T cells in an IDO1-dependent manner. Thus, substitutant neoepitopes are widespread inducible products of aberrant mRNA translation that provide novel means to overcome current limitations in adoptive T-cell therapy.

Project B. Identification of new mRNA translation mechanisms in response to amino acid depletion in lung cancer.

To foster cancer progression and inhibit anti-tumor activity, many types of cancers often suppress the expression of key enzymes involved in generating an essential amino acid. While promoting specific amino-acid-deprivation therapies, the impact of key enzyme suppression on the quality of the tumor proteome remained unknown. We, therefore, interrogated proteomes of cancer patients for related codon reassignments (substitutants) and surprisingly identified a strong enrichment for new essential amino acid substitutions in lung tumors specifically. These events did not coincide with their genetically encoded mutations, but were likely products of tRNA misalignments. In lung cancer, such incidents are highly associated with certain protection and oncogenic pathway mutations. Finally, functional interrogation indicated a key role for these substitutants in cell survival to chemotherapy. Thus, we present here a novel mechanism for enriching lung cancer proteomes with key substitutions that may have vast implications for therapeutic decisions.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) In generating patents and communicating with potential companies.

b) PoC to establish a clinical approach to our findings.

2.4.2. Contribution to the Oncode community in 2023

a) Collaborations within Oncode community.

b) Meetings and conferences that are open to my team .

2.4.3. Key collaborations within Oncode in 2023

a) Daniel Peeper (T cell reagents).

b) Thijn Brumelkamp (ribosome profiling and the identification of SLFN11-mediated apoptosis via tRNA leucine).

c) Rene Bernards (immunopeptidomics)

2.4.4. Major valorization achievements in 2023

a) PCT patent application, Anti-tumor immunity induces the presentation of aberrant peptides.

b) Provisional patent filed for substitutant peptides, Tryptophan depletion induces production and presentation of tryptophan to phenylalanine substitution).

c) Working on putting a priority patent application for the first TCR towards a prominent W>F neoepitope.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
NWO	M1	352,875	352,875	01/2024	48	Choose an item.
AvL foundation		117,250	117,250	02/2021	48	Choose an item.

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Leila Akkari

Netherlands Cancer Institute

1. General information

Research Focus	Immune cells and macrophages in therapeutically challenging tumors
Junior/Senior Oncode Investigator	Junior, senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

The tumor microenvironment (TME) field now appreciates that immune cells evolve in their composition and programming in a tissue, tumor and stage-dependent manner, hampering the efficacy of "one size fits all" strategies and underlying the need to better comprehend the dynamics of these cells to better harness them therapeutically.

We have tackled these challenges by studying the profound complexity of immune cell regulation in the TME as it dynamically adapts within an evolving tumor, based on cancer cell genetics and response to therapeutic intervention, whether these are standard of care treatments or novel targeted/immunotherapies. Our main focus is centered on the role of key immunosuppressive cell types in the TME in brain and liver cancers, two tumor types that scarcely metastasize and that resist current therapeutic approaches employing immunotherapy. To functionally address the complexity and dynamics of immunosuppressive subpopulation phenotype in glioblastoma (GBM) and hepatocellular carcinoma (HCC), and particularly of TAMs, we have developed and make use of genetically-engineered and somatic mouse models, ex vivo co-culture systems and analyses of patient-derived material to functionally assess the longitudinal adaptation their TME undergo. Using these complementary and powerful tools, we revealed that both content and features of myeloid cells are altered as tumors progress and resist treatment, to fuel malignancy.

2.2. Major scientific achievements in 2023

a) **Optimizing the regimen of T-cell centric immunotherapy in radiation-treated GBM:** We identified CD103+ Tregs with increased lipid metabolism as a central cell type restraining the response to IT through CD8+ T cell repression. Treg targeting enhanced CD4+ and CD8+ T cell frequency and functions and unleashed the therapeutic efficacy of radio-immunotherapy. These results support the rational design of therapeutic regimens limiting the induction of immunosuppressive feedback pathways, in GBM (Van Hooren et al, Nat Cancer, 2023).

b) **ERC Synergy grant:** In collaboration with chemical biologist Alex Kros (Leiden) and supramolecular chemist Joost Reek (UvA), we obtained funding for glioblastoma targeted treatment to develop novel toolboxes using catalysis in vivo to target different actors of the protumorigenic glioblastoma environment.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Analyzing and targeting of the tumor microenvironment dynamics in hepatocellular carcinoma (HCC) initiation and progression.

In the overarching goal to decipher how different genetic make-up orchestrates the phenotype of immune cells to the tumor advantage, we set up multiple genetic mouse models of HCC bearing different genetic mutations relevant to the human pathology and employed unbiased approaches (RNA seq, proteomics) to examine the education of infiltrating TAMs and other immune cells in these differently aggressive murine models. This represented a lengthy and uncertain endeavor which Oncode funds supported through covering the salary of a PhD student on this project. We have published the tools to target relevant immune cells in genetically distinct HCC and have prepared a manuscript on this project. In this work, we present the newly built somatic models of HCC we established and used them to show that the MAPK/ERK pathway activation in liver cancer cells regulates the expression and secretion of GMCSF to promote the recruitment and differentiation of myeloid cells that repress T cell activity and protect cancer cells from apoptosis. This work is accepted in Principle at Nature Communications.

Project B) Assessing the heterogenous subpopulations of macrophages in primary and recurrent glioblastomas after radiation therapy

In this project, we employed genetically modified murine models combined with lineage tracing of macrophages to identify the transcriptional changes in infiltrating and resident macrophage subpopulations during glioblastoma radiotherapy/recurrence. We identified unique transcriptional programs in TAM subsets, including a novel sub-population of these cells within the mesenchymal subtype of GBM, which are characterized by altered metabolic networks that co-evolve with tumor cell metabolic demands. These cells present heightened lipid metabolism activities, due to uptake of myelin debris in the TME, that they are then able to transfer in forms of lipid to the tumor cells to fuel proliferation. We have now been able to target these cells showing their therapeutic potential. These costly experiments become the basis of specific targeting of this new subset of macrophages, and several successful grants (NWO-XL, ERC Synergy) Oncode funds were used to support the development of this high-risk, high reward project in brain tumors. This study in now in revision in Cell.

Project C) Implementing immunomodulation strategies in combination with pro-senescence treatment in HCC .

In close collaboration with the group of Rene Bernards, we have previously demonstrated that senescence-inducing therapy (SIT) promotes the infiltration of macrophages and T cells and improves animal survival in a genetic-dependent manner in HCC mouse models. Using the genetically distinct HCC mouse models from project A, we explored the effect of Aurora kinase A (AURKA) inhibition as a broad SIT and found differential a survival benefit across the distinct HCC pre-clinical models. Following AURKAi treatment, both the kinetics and magnitude of senescence induction were different across the genetically distinct HCC models. Interestingly, longitudinal TME analysis revealed a dynamic reprogramming of the immune landscape unique to each HCC. Altogether, our data suggest that cancer cell genetics influences the efficacy of SIT by engaging different senescence programs with distinct non-cell autonomous effects, highlighting a potential for designing new combinatorial strategies that exploit senescence as a tumor-induced vulnerability, while harnessing the immune microenvironment in a more personalized manner.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) We participate to the patient engagement initiative led by Oncode, and have made contact with 2 patients who have joined our discussion of the CRUK/NIH Cancer Grand Challenge grants, weighting in the grant science, patient engagement, strategy and dissemination and will continue to interact with them within our lab.

b) Thanks to the support of our BD Yuva Oz, we have established contact and contract with iTeos, a biotech company, to test novel macrophage immunomodulation in brain and liver cancer (subsidised by them). We also have established contact and set up a contract with Scenic Biotech, in both cases subsidizing a technician working on novel strategies in HCC and glioblastoma.

c) Thanks to the support of Oncode and Yuva Oz, we have submitted a new patent of the important discovery of our group on a novel subset of macrophages in brain cancer and lipid nanoparticle based targeting that we propose can significantly impact therapy response in glioblastoma and beyond, now making several contacts with company to follow up on this novel delivery of drug cargo

d) Together with CRUK, we are taking a leading role on establishing a consortium discussing HCC treatment and future research lines, with the organization in 2024 of a symposium.

2.4.2. Contribution to the Oncode community in 2023

a) I am part of the organization committee of the KIT meeting in 2024 initiated in 2023

b) I gave a seminar at the KIT 2023 as one of the Oncode PI

c) My lab was represented at mutliple Patient Engagement events

d) I am part of the steering committee to organize an alliance between Oncode and CRUK regarding HCC research directions

2.4.3. Key collaborations within Oncode in 2023

	Name Collaborator	Were you already collaborating prior to Oncode?	Subject Collaboration	Reference (if applicable)
1	Rene Bernards	No	Novel therapeutic approaches in liver cancer	Mulero-Sanchez A et al, Mol Oncol. 2023 Jan 17. 10.1002/1878-0261.13377
In this collaboration, we use our genetically-distinct HCC murine models to assess the potential of combination therapies in liver cancers, merging the expertise of the Bernards lab in HCC cancer cell-centric therapies to our tumor microenvironment one. The somatic HCC models in our possession, particularly highly aggressive ones mimicking human tumors, are a highly relevant platform to test cytotoxic therapies and understand the role immune cells undertake to mediate response or resistance. Importantly, our models were instrumental in testing novel pro-senescence therapies identified in the Bernards group, which we now employ in combination with immunomodulation.				
2	Jacco van Rheenen	No	Immunotherapy in brain metastasis	Vennin et al, Cancer Cell, 2023. 2023 Jun 12;41(6):1170-1185.e12. doi:10.1016/j.ccell.2023.05.009.
The van Rheenen lab developed a model of brain metastasis from primary breast cancers, and reached out to us to explore its associated tumor microenvironment in order to identify immune components that may be involved in dampening response to chemotherapy. In this collaboration, we have the opportunity to compare primary brain tumor TME (using our GBM models) and the ones modeling metastatic disease. Our results will provide insights into the dynamic functions of brain specific cells in promoting or halting cancer outgrowth in the context of tumors either arising from glial cells (GBM) or that invade the homeostatic brain (metastasis).				
3	Miao Ping Chen	No	Target-ID in myeloid cells and GBM using re fSCS	
In order to gain mechanistic insights into the elusive education of macrophages instructed by GBM cancer cells and particularly the formation of lipid laden macrophages (LLMs) we have identified in our glioblastoma murine models, we set up a collaboration with the Chen lab to utilize their unique microscopy technologies. In this collaboration, we employ their optochemistry tools to selectively isolate LLMs with different lipid accumulation features following direct co-culture with GBM cells. Single-cell sequencing and proteomics of these annotated cells will identify the range of GBM-mediated education in LLMs, and reveal specific pathways enforcing this TAM subset education.				
4	Sjoerd van der Burg, Karin de Visser, Linde Meyaard (management team) + OI Miao-Ping Chien, Sarah Derks, Carl Figdor, Sjaak Neefjes, Jacco van Rheenen, Jeroen de Ridder, Tineke Lenstra	No	Curing tumors difficult to treat with immunotherapy by mobilizing innate leucocytes	
This Oncode/KWF sponsored Accelerator grant thrives to mobilize different innate immune cell types to unleash the potential of T cell-centric immunotherapy in cancers renowned to resist such treatment approaches. Our model systems and ex vivo set ups will be instrumental in testing the potential of macrophage, and to assess the role of other innate immune cells, including eosinophils, neutrophils and dendritic cells, in brain and liver tumors. Our results will be crossed-checked in other tumor types available in the consortium, and the large sequencing datasets in our possession will be mined by the Meyaard lab to identify putative targets and develop specific agonists/antagonists antibodies.				
5	Elzo de Wit (former OI)	no	Epigenetic rewiring in GBM macrophages	Ms in revision in Cell
Macrophage heterogeneous phenotype is partly regulated at the epigenetic level, with distinct chromatin conformation allowing the integration of cues coming from their surrounding environment. In this collaboration, we explored the chromatin state of LLM vs non LLMs TAMs by performing ATAC seq on sorted cells from GBM tumors. We identified that LLM presented a closed chromatin state which correlated with inhibition of pro-inflammation gene transcription and increased lipid metabolic gene expression. Importantly, these data suggest that rewiring macrophages epigenetically could alter their education, a research area we are currently pursuing.				

2.4.4. Major valorization achievements in 2023

	Activity	Date/ period	Title	Reference (if applicable)
1	Patent	2022-now (renewed)	Discovery of Lipid-Laden Macrophages and Its Correlation with the Mesenchymal Glioblastoma (GBM) Subtype as a Biomarker and Therapeutic Target	applications no: 2029153. P091147NL And in 2023: P099572EP
2	Patient Engagement program in CRUK Grand Challenge grant	2023	Interaction with 3 patient representatives of Oncode's PE program	N/A
3	Iteos collaboration (subsidized)	2023	Testing iTeos-developed TREM2 antibodies to target lipid-laden macrophages in GBM murine models and human ex vivo coculture	N/A
4	Scenic Biotech (subsidized, finalization ongoing)	2023	Understanding QPCTL- mediated regulation of cytokine function in liver cancer and testing QPCTL inhibitors in vivo	N/A

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
ERC SYNERGY	HORIZON EUROPE	10.5M	3,6M	Jan 1ST 2024	72	Main applicant
Best poster Prize Post doc Masami Ando Kuri	ISREC meeting Lausanne	500euros	Masami Andi Kuri *(Salary covered by Oncode funds)*	*N/A*		Main applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT03582514	PreOperative Brain Irradiation in Glioblastoma (POBIG)	2022-04-19	50	Yes	Co-PI

3.3. PhD defenses in 2023

N/A

René Bernards

Netherlands Cancer Institute

1. General information

Research Focus	Functional Cancer Genetics
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

My group uses genome-wide functional genetic approaches to identify powerful drug combinations, new drug targets and mechanisms of drug resistance. In 2023, we have identified druggable vulnerabilities of drug tolerant persister cells as part of the PERSIST-SEQ consortium. Our work on drugging the MAP kinase pathway has resulted in validation of a small molecule MKK4 inhibitor as being highly synergistic with multiple MAP kinase inhibitory drugs. Finally, we have identified powerful new drug combinations based on the concept of hyper-activation of oncogenic signaling in cancer cells. A clinical trial based on this concept will start in Q2 of 2024, made possible in part through an Oncode clinical proof of concept grant. We also found that hyper-activation of oncogenic signaling in cancer cells causes mis-splicing. In collaboration with Reuven Agami's group, we are studying whether this results in neo-antigens. Our findings suggest that hyperactivation of oncogenic signaling would synergize with immune checkpoint inhibition. A clinical trial to study this will start in Q1 of 2024.

2.2. Major scientific achievements in 2023

a) Two new clinical trials were designed based on our research. Both will start in the first half of 2024, one funded through Oncode proof of concept fund.

b) Two PhD defences in 203: Antonio Mulero and Fleur Jochems.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Drugging the MAP kinase pathway

We have progressed our work on inhibition of MAP2K4 in combination with conventional MAP kinase inhibitor drugs for a series of KRAS mutant cancers. We have found considerable synergy with the new selective KRAS G12C inhibitors, drugs that only provide modest survival benefit when used as single agent. We have validated the utility of this combination in animal models. We struggle to bring this combination to the clinic, due to lack of funding. I am in discussion with Emil Pot to find solutions for this problem.

Project B) Exploiting senescence for cancer treatment

We have identified additional senolytic drugs, agents that kill senescent cells. In 2022, we had already identified agonistic DR5 antibodies as senolytic agents. We now have identified salinomycin as senolytic, an agent known to be an ionophore that facilitate the transport of cations. We observed significant synergy between DR5 agonistic antibodies and salinomyin in klining senescent cells.

Project C) Paradoxical activation of oncogenic signaling as a cancer treatment strategy

We have found that LB-100, an inhibitor of protein phosphatase 2A (PP2A) hyper-activates mitogenic signaling in cancer cells. LB-100 treated cells become very sensitive to drugs that inhibit the WEE1 kinase. We will initiate a phase 1 trial to test this combination in 2024. Our recent data indicate that resistance to this drug combination is associated with loss of oncogenic phenotype through downregulation of oncogenic signaling.

Project D) Targeting drug tolerant persister cells

We have completed a CRISPR screen to find vulnerabilities of Drug Tolerant Persister cells (DTPs). We find that DTPS are hyper-sensitive to BRD inhibitor drugs as these drugs further increase Reactive Oxygen Species (ROS) in DTPs, leading to cell death. In vivo, treatment of minimal residual disease (thought to be rich in DTPs) with BRD inhibitors delays onset of drug resistance. Our data identified sequential drug therapy regimen to forestall drug resistance.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Our work on hyperactivation of oncogenic signaling has formed the basis for an ERC advanced grant application (my third). A funding decision is expected in Q2 of 24. We have written clinical trial protocols for two trials based on this work, both of which will start in the first half of 24, one with partial Oncode funding.

b) We have validated the use of HRX-0233, a MAP2K4 inhibitor drug, as highly synergistic with multiple drugs in the MAPK pathway. We are in discussions with Boehringer Ingelheim to fund this research further. A grant was submitted and we expect a decision in Q1 24.

c) We have worked with UMAB and a spin off company co-funded by Oncode (Oncosence) to generate a unique new senolytic antibody. This is currently undergoing pre-clinical testing.

2.4.2. Contribution to the Oncode community in 2023

a) We participate in the IMI call PERSIST-SEQ, collaborating with Alexander van Oudenaarden.

b) With Lodewyk Wessels and Hugo Snippert, we have been awarded new KWF funding to investigate multi low dose drug treatment of MAPK activated cancers. This program is ongoing.

2.4.3. Key collaborations within Oncode in 2023

a) Hugo Snippert and Lodewyk Wessels on multiple low dose therapy of cancer. This was awarded a KWF grant.

b) Alexander van Oudenaarden. Single cell RNAseq of DTPs, manuscript in press.

c) Emile Voest, SHERPA trial. https://www.clinicaltrials.gov/ct2/show/NCT04916236

2.4.4. Major valorization achievements in 2023

a) Research collaboration with Lixte extended for two years with larger budget.

b) Two clinical trials agreed with large pharma.

c) Completing funded research agreement with Verastem.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Mark Foundation	Aspire 2	USD 500,000	USD 250,000	July 2023	24	Main applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT04916236	SHP2 and ERK inhibitors in pancreatic cancer	01/2020	60	Yes	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Antonio Mulero	All roads lead to SHP2
Fleur Jochems	If you stop, you might fall: Senescence as a steppingstone for cancer treatment

Ruben van Boxtel

Princess Máxima Center

1. General information

Research Focus	Cancer Etiology, Mutagenesis and Clonal Evolution
Junior/Senior Oncode Investigator	Junior, senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

The aim of our research is to determine the mechanisms and rate-limiting steps underlying the initiation of cancer. We work on 3 main topics:
Topic 1: Study the etiology of childhood cancers. Why do children get cancer even though their young cells are not yet damaged by aging? We use retrospective lineage tracing using somatic mutations as genetic barcodes to pinpoint and study the initiation of cancer in the life history of childhood leukemia and lymphoma patients.
Topic 2: Characterize the mutagenic effects of cancer treatment in normal cells. How does chemotherapy cause long-term damage in normal tissues and contribute to adverse late effects in cancer survivors? Therapy-related second malignancies are a major cause for long-term mortality in childhood cancer survivors. We have generated a cohort of patients who developed therapy-related leukemia and performed in-depth mutational analyses on their hematopoietic system to find cancer causes.
Topic 3: Dissect the etiology of cancer-associated mutational signatures. Which processes cause specific mutation patterns in cancer genomes, and can we use these for improved diagnostics and treatment? We have developed experimental models to identify clinically relevant signatures, such as a signature caused by antiviral treatment, as well as studying causes of known signatures.

2.2. Major scientific achievements in 2023

a) We published our new method to perform single-cell whole genome sequencing in *Cell Genomics*. To deal with amplification artefacts, we developed a machine-learning based software. This is a major achievement, because this allows us -for the first time- to characterize mutation accumulation in any cell type, including malignant cells.

b) This year three PhD students of our group defended their thesis; Freek Manders (February 21st), Eline Bertrums (November 14th cum laude) and Flavia Peci (December 21st). Furthermore, two of our postdocs received a Veni grant. This is a major achievement because they will be the next generation of great scientists!

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Finding the causes for bone marrow failure in Fanconi anemia.

Patients with Fanconi anaemia (FA) have an increased risk for leukemia, because of an inherited DNA repair defect. Early in life, patients develop bone marrow (BM) failure for which they receive BM transplantation after chemotherapeutic conditioning. However, the clinical presentation of FA can vary greatly among patients. We have initiated this project in collaboration with our clinical colleagues in our centre to determine why the bone marrow fails in these patients. This project is innovative because it required a novel single-cell whole genome sequencing (WGS) method, which first needed to be developed before we could apply for funding. For this, we implemented and improved primary template-direct amplification (PTA; see project C). We have analyzed multiple HSCs of 6 FA patients, including an individual with BRCA2 germline mutations. We combined the result of this project with those obtained in project C and published these this year in *Cell Genomics*.

Project B) The mutational effects of DNA repair throughout the cell cycle

A multitude of mutational signatures have been identified in cancer genomes, which can provide valuable information about causes of cancer and facilitate the selection of effective treatment. However, the mechanisms underlying many signatures remain unknown, which hampers their use in cancer diagnostics. We hypothesized that various mutational signatures share a common mutagenic source but have unique mutation characteristics, because the damage occurs in different phases of the cell cycle. To test this, we rescued knockout models for specific DNA repair components with versions that are only present during certain phases of the cell cycle. Because this is a high risk-project for which we did not have pilot data, it could not be funded with standard grant applications. The postdoc working on this project received a Veni grant and moved to Hugo Snippert's lab. In collaboration, we will finalize the project.

Project C) The PTA Analysis Toolbox for single cell genome sequencing

Our original single-cell genome sequencing method depends on clonal expansion of stem cells, which might cause a selective bias for less damaged cells to grow out. We have therefore implemented and improved a novel single-cell WGS approach based on primary template-directed amplification (PTA). However, this method still generates hundreds of artefacts per genome. We have developed a machine learning tool that can classify individual mutations as genuine or artefact. This tool allows us to retrieve >90% of all mutations (base substitutions, indels and SVs) in a single cell with <5% artefacts left. We could not fund this project using standard grant applications, because we took an existing method and improved it. Nonetheless, this project is innovative, because whereas single cell RNA sequencing has revolutionized biomedical research, single-cell DNA sequencing has lagged. This work was combined with the data of project A and published this year in *Cell Genomic*.

Project D) Mutagenic consequences of probiotic Nissle E. Coli in the gut

In 2020, we reported, in collaboration with Oncode Investigator Hans Clevers, a mutational signature caused by a genotoxic colibactin, which is produced by the pks+ E. coli strain (Pleguezuelos-Manzano et al, Nature 2020). E. coli Nissle 1917 (EcN) remains a commonly used probiotic, despite harboring the same pks+ operon and inducing double strand DNA breaks. We evaluated the mutagenic activity of EcN by an analytical framework based on a novel machine learning approach. EcN showed low, but detectable mutagenic activity compared to previously tested pks+ E. coli strains. We could also show that the colorectal cancer (CRC) gene is preferentially mutated by the E. coli strain and that carriers of this strain get cancer earlier in their lives. After three revision cycles the manuscript is now almost accepted in *Cancer Cell*.

Project E) Tracing cellular plasticity in pediatric leukemia

We set out to test if we could observe cellular plasticity in human cancers *in vivo* using our single-cell WGS method, which is innovative. We combined phylogenetic analyses of somatic mutations with multiparameter flow cytometry. We leveraged the well-defined differentiation states during T-cell development to pinpoint the initiation of T-cell acute lymphoblastic leukemia (T-ALL), an aggressive form of childhood leukemia, and study the emergence of phenotypic plasticity. We demonstrate that during leukemia initiation there is transient differentiation state plasticity, which fuels the phenotypic heterogeneity observed at diagnosis. We could not fund this project using standard grant applications, because I inherited a PhD student working on T-ALL from a group leaving our centre and there is limited time for her to finalize her PhD thesis. A manuscript describing the results has been reviewed at *Cancer Discovery* and we are currently submitting a revised version.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The Oncode Base Fund has allowed us to develop a novel single-cell WGS method, enabling us to profile mutations in the genome of any cell. Whereas we were previously only capable of analyzing healthy stem cells, we can now also sequence individual cancer cells. We are now using this to determine heterogeneity in leukemia and lymphoma as well as to trace back the origin of these cancers. Importantly, this method can be combined with single-cell RNA sequencing, allowing us to assess both mutations as well as their direct phenotypic consequences on gene expression.

b) Together with Oncode Business Developer Amber Liu, we have had several meetings with potential investors for our venture GenomeTOX as well as potential collaborators from industry. This has inspired us to further validate our method for which we received a TechDev fund from Oncode. We finished these tests and have also developed an additional implication for our GenomeTOX. Using artificial intelligence, we have trained an algorithm to predict, pure on structure and chemical properties, whether a compound will be mutagenic or not. We are currently validating this method.

c) We continued our collaboration with Jacco van Rheenen to study whether platinum drugs cause therapy-related colorectal cancer in adult cancer survivors. In the initial patient, we could indeed show that the driver mutation in the *APC* gene was directly caused by cisplatin. We are currently analyzing additional patients. In addition, we initiated a new collaboration with the group of Madelon Maurice where we use our expertise to study the origin of CRC in predisposed patients. For this we have analyzed WGS data of clonal organoid cultures and constructed phylogenetic trees.

2.4.2. Contribution to the Oncode community in 2023

a) Active participation to Oncode's patient engagement program. We currently have 2 patient advocates associated to our group, who we regularly meet.

b) Chair of the brainstorm session on "Tumor plasticitiy/heterogeneity" during the Oncode Investigator retreat.

2.4.3. Key collaborations within Oncode in 2023

a) Madelon Maurice. Subject: Mutation landscapes in RNF43 and LKB1-driven colorectal cancers. Reference: Bugter et al, bioRxiv 2022. We were not collaborating before Oncode.

b) Jacco van Rheenen. Subject: Lineage tracing in human breast tissue and carcinogenic potential of cisplatin exposure. No reference available. We were already collaborating before Oncode.

c) Hans Clevers. Subject: Organoid sequencing. Reference: Geurts et al, Nature Communications 2023. We were already collaborating before Oncode.

d) Wilbert Zwart. Subject: Timing of prostate cancer metastasis using mutational signatures. No reference available. We were not collaborating before Oncode.

2.4.4. Major valorization achievements in 2023

a) Completion of an Oncode TechDev fund to further validate our GenomeTOX assay. This allowed us to further develop the toolbox of GenomeTOX (see above).

b) We obtained a Health~Holland TKI PPP grant together with Xilis BV to find novel treatment options for Hodgkin lymphoma using MicroOrganoSpheres (MOS).

c) We discussed our GenomeTOX idea with ROM Utrecht region as well as the genotoxicity department of Roche.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Freek Manders	Patterns of somatic mutations in normal cells
Eline Bertrums	The clonal dynamics underlying the genesis and regression of myeloid disorders
Flavia Peci	Safety of Transplantation; characterising the Mutational Consequences of Treatment in Hematopoietic Stem Cells

Thijn Brummelkamp

Netherlands Cancer Institute

1. General information

Research Focus	Experimental biomedical genetics
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our group uses genetics in haploid human cells to construct a genetic wiring map for human cells and to study important outstanding questions in cell biology. To achieve this, we measure quantitative cellular traits in haploid cells carrying a gene-disruptive mutation and apply sequencing to link millions of mutations in parallel to cellular phenotypes.
Whereas interesting findings can be derived from individual experiments, we have assigned genetic regulators to >160 quantitative phenotypes enabling comparative analysis. These comparisons point out specific regulators that affect only a limited number of traits and broad genetic regulators affecting many traits and can be used to cluster genes with similar phenotypic output.
Next to a loss-of-function approach we have also studied a compendium of cellular phenotypes using a recently developed gain-of-function approach. Using both methods, we have identified new biological processes that we are characterizing further: (1) a new pathway for the synthesis of triglycerides, (2) a p53-independent pathway for the induction of apoptosis by DNA damage, (3) a new pathway for cellular iron uptake and (4) a new co-activator complex that activates a large series of developmental transcription factors.

2.2. Major scientific achievements in 2023

a) We have identified an alternative triglyceride synthesis pathway consisting of a complex containing TMX1 (regulator) and DIESL (triglyceride synthase). In contrast to the classical pathway, the new pathway is important under conditions of nutrient shortage (McLelland et al, Nature, 2023).

b) We have identified a new factor, C1orf112/FIRRM which is critical for the cellular response to DNA crosslinking agents. C1orf112/FIRRM functions in a late stage of the response to DNA damage and affects the resolution of homologous recombination intermediates (Mazouzi et al, Science Advances, 2023.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Identification of an alternative triglyceride biosynthesis pathway.
Triglycerides constitute the main form of stored energy in cells and their abundance affects the development of diseases including cardiac disease, obesity, and metabolic syndrome. In this project we have identified a new synthesis mechanism for triglycerides in human cells that is under tight control consisting of a complex of TMX1 (regulator) and DIESL (triglyceride synthase) (McLelland et al, Nature, 2023). We are now studying the regulation of this pathway making use of TMX1-deficient mice as well as genetics in human cells.

Project B) Identification and characterization of new DNA repair factors.
In collaboration with the group of Oncode PI Jos Jonkers we have carried out genetic screens to identify genes that affect the cellular response to DNA crosslinking agents. Besides many known players, such as the Fanconi Anemia pathway, we identified C1orf112 as a critical factor. In the last year we have studied the mechanism and relevance of this new factor using experiments in cells and mice (Mazouzi et al, Science Advances, 2023).

Project C) The elusive pathway for p53-independent apoptosis in response to DNA damage.
Excessive DNA damage can result in the induction of apoptosis. Although this response has mainly been linked to p53, it is known that p53-deficient cells also undergo apoptosis in response to DNA damage. To our surprise, there is no clarity on the pathway that triggers cell death in p53-deficient cells in response to DNA damage. Because tumors often have a defective p53 pathway (and are still treated with DNA damaging therapies) we carried out a genetic screen to identify the responsible pathway.

Project D) Linking genes to a compendium of cellular phenotypes using gain-of-function genetics.
Using loss-of-function genetics we have assigned genes to the regulation of more than 160 different cellular phenotypes. This unique dataset is the largest gene-phenotype inventory in human cells. Whereas a loss-of-function genetics highlights those genes that *do* regulate a phenotype, a gain-of-function approach highlights those genes that *can* regulate a phenotype. In addition, it is not affected by genetic redundancy or non-expressed genes. Thus, we have applied gain-of-function genetics to link genes to a compendium of 10 cellular phenotypes. This resulted in the identification of a new pathway for the uptake of iron into cells that we are examining further.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Our broadly applicable genetics approach enables us to address major outstanding questions in diverse areas of biology. While exciting and successful, these initiatives cannot be launched using regular funding schemes because (1) we do not have preliminary data and expertise on such new topics and (2) a genetic approach is often viewed as a fishing expedition.

b) Because we often enter scientific territories that we have no expertise in it is valuable to interact with other Oncode groups for advice as well as collaborations. In 2023 the interactions with Oncode PIs Anastassis Perrakis (structural biology), Lodewyk Wessels (bioinformatics), Jos Jonkers (DNA damage), Hugo Snippert (organoids), Ton Schumacher (T cells) and Michiel Vermeulen (proteomics) were most fruitful.

2.4.2. Contribution to the Oncode community in 2023

a) Participation in Oncode meetings

b) Collaboration with other Oncode groups

2.4.3. Key collaborations within Oncode in 2023

a) For our work on the p53-independent induction of apoptosis in response to DNA damage we have combined expertise within the Oncode network: ribosome profiling (Agami group), organoids (Snippert), T cells (Schumacher). This collaboration started thanks to being part of Oncode which provided the connection as well as the financial freedom to engage in such collaborations.

2.4.4. Major valorization achievements in 2023

a) I am a co-founder, scientific advisor and board observer at Scenic Biotech

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Sjoerd van der Burg

LUMC

1. General information

Research Focus	Immunotherapy; T-cells; immune regulation; clinical trials
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The point of focus of my group is the study on host-tumor interactions which determine the success and failure in immune control of solid cancer in order to improve and develop immunotherapeutic strategies. Several types of fundamental, translational and clinical studies are performed with emphasis on Human Papilloma Virus-induced cancers, Ovarian cancer, Melanoma, Lung cancer and Pancreatic cancer. The main basic research questions which are currently being addressed are: 1) immune therapy driven resistance mechanisms; 2) the role of innate immune cells in immunotherapy driven tumor regression; 3) the function and regulation of T cells expressing NK-like regulatory molecules; 4) how to convert cold to hot tumors. Clinical studies focus on cancer vaccination and adoptive T cell transfer in combination with other treatment modalities such as immunomodulation, chemotherapy, radiotherapy, and checkpoint therapy to enhance the efficacy and specificity of immune responses.

2.2. Major scientific achievements in 2023

a) Bench to bedside translational success in our work on 1) shared neoantigens (TEIPP) for which we have filed two patents, almost completed our clinical trial in patients with lung cancer, and for which we obtained a large grant from KWF to study a two-punch approach of ACT+vaccination; and our work on 2) primary and secondary immune escape mechanisms for which we showed the strong impact of CD163+ macrophages on immunotherapy of cancer in mice and subsequently obtained a substantial PPS grant to develop a compound to target these cells in patients; as well as demonstrated how checkpoint therapy resistance may occur as consequence of regulatory T cell reactivation in treated patients (Science Immunology 2023).

b) Clinical success was achieved by having opened the Apollo trial, in which women with VSCC will have neoadjuvant checkpoint therapy (cPoC); and the publication of our clinical trial on timed adoptive T cell transfer during chemotherapy in patients with recurrent platinum-sensitive epithelial ovarian cancer (JITC 2023) which helped us to initiate and open the TILLC trial on adoptive T cell transfer in lung cancer.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Primary-Secondary resistance to immunotherapy.

An Oncode-financed PhD student (van Elsas, PhD defence Feb 2024) aimed to identify immunologic pathways playing a role in the primary/secondary resistance against immunotherapy. Her work on the immunotherapy-resistance causing CD115-CD163^{hi} macrophages was published and have led to a new LUMC-PPS project in which we, together with a small biotech, will develop compounds for the human setting. In the last two years she has shown that therapy driven induction of tumor-reactive CD8^{+} T cells and pro-inflammatory CD115+ macrophages were of pivotal importance for tumor control, and the underlying mechanisms. The anti-tumor CD115+ macrophage transcriptomic profile was also found single cell data of human cancers, and specifically in patients responsive to immunotherapy. For publication, she teamed up with another non-oncode BF sponsored PhD student who repeated it in another tumor model. The manuscript is currently under revision in Cancer Cell. The project is continued in the Oncode/Synergy project.

Project B) The impact of the TME on the outcome of patients undergoing immunotherapy

A LUMC-fellowship funded PhD student aims to analyse the impact of the TME on the outcome of patients undergoing immunotherapy and *vice versa* using multiplex imaging techniques. She is now working in the clinic again, while continuing to work on her last study. Here, the TME of a series of NR, PR and CR patients with HPV16+ vulvar HSIL treated with a therapeutic HPV16 vaccine, is studied using the new Nanostring CosMX, allowing single cell spatial transcriptomics. After processing and quality control of the data sets, the actual analyses have started. We digitally reconstructed the cellular interaction landscape of the vHSILs and found that the vulvar microenvironment of none of the patients was alike. Yet, the proportion of immunotherapy supporting and resisting cells – spatially organized in clinical response-specific ecosystems – was best balanced in complete responders. Furthermore, the cells making the ecosystems differed between clinical response groups.

Project C) The role CD8 T cell mediated killing during immunotherapy

In project A we found that in some tumor models CD8 T cell based therapy required the attraction/polarization of innate effector cells (macrophages, neutrophils) in order to stop tumor growth, without these cells tumor growth was delayed but tumors were not regressing. As a pilot, we used perforin KO mice and found evidence that in these mice, T cell based therapy was as effective. To understand the exact role of CD8 T cells in T cell based therapy we will embark on studies identifying which effector functions of CD8 T cells are required during therapy, and which of the innate effector cells.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode's base fund helped me to expand our data set on the immune microenvironment of healthy and diseased vulvar tissue, the latter before and after therapeutic vaccination, at a single cell spatial transcriptomic scale. This allows us to understand the biology of disease, what is required to respond to immunotherapy and also how the environment is altered after therapy.

b) Oncode's business team helped us with the consortium contracting of a PPS project that was a spin-off of our BF project A "primary-secondary resistance to immunotherapy" as well as with setting up (still ongoing) discussions with potential collaborators/funding possibilities for the expansion of our KWF/Oncode Accelerator/Synergy consortium and translation to human setting. In addition, with a contract with Genmab and two MTA's, one of which may lead to a new research agreement with a company. Furthermore, they helped with filing two patents for TEIPP and responding to questions of a patent filed in 2022

2.4.2. Contribution to the Oncode community in 2023

a) Workstream leader Consortium Cancer Vaccines in Oncode Accelerator, wrote, presented, organized and pushed to finalize the budgets, description of work and consortium agreement, resulting in the start of our consortium per December 2023.

2.4.3. Key collaborations within Oncode in 2023

a) Visser, Akkari, Meyaard, van Rheenen, de Ridder as a part of the group involved in the KWF/Oncode Synergy (2021-14399) grant : Curing tumors difficult to treat with immunotherapy by mobilizing innate leukocytes. These collaborations were started because of the Oncode Synergy Challenge.

b) Ten Dijke for our studies in the context of oncolytic virus therapy and the impact of TGFb on this, with whom we were be able to procure a shared PhD student (Groeneveldt). A recent shared publication of us was: C. Groeneveldt et al. (2023) Intertumoral Differences Dictate the Outcome of TGF-β Blockade on the Efficacy of Viro-Immunotherapy. Cancer Res Commun. 3:325-337.

c) Neefjes, for state of the art techniques as Spinning-disk confocal microscopy and for CRISPR screens of Qa-1 (mouse NKG2A ligand) receptors. Two shared publications (one on each topic) are: 1) T.J. Harryvan, et al. (2022) Enhanced antigen cross-presentation in human colorectal cancer-associated fibroblasts through upregulation of the lysosomal protease Cathepsin S. J. Immunother. Cancer, 10:e003591; and 2) J. Middelburg et al. (2023) MHC-E is a convergent checkpoint ligand for LILRB1 on antigen-presenting cells and during inflammation for NKG2A on lymphocytes. Cell Reports, 42:113516.

d) Borst, CD4 help signature in TILs and DCs. A shared manuscript is in revision: X. Lei, et al. (2023) Human CD4+ T-cells optimize cDC1 for potent CTL-based cancer immunity via complementary IFN-I and CD40 ligand signaling. Cell Mol Immunol, in revision.

2.4.4. Major valorization achievements in 2023

a) Our work from project A, on immunotherapy resistance-inducing CD163+ macrophages has led to a substantial PPS grant to collaborate with a small biotech in order to develop and test compounds that can be used to modulate CD163+ macrophages in humans.

b) Building on p2019-0016 (CPoC) and p2020-0002 (TDF) we have now obtained a substantial KWF grant to develop and test the concept of a one-two double punch approach using patient-derived autologous in vitro expanded TEIPP-specific CD8+ T cells that following their infusion in the patient will be stimulated using the vaccine developed in p2019-0016. Thus an ACT-vaccine immunotherapy approach based on TEIPP-specific T cells.

c) Led Oncode Accelerator therapeutic vaccine consortium to its start at December 2023, first of all consortia.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
SOAK	Steun Casper	380.000	192.500	12/2023	24	Co-applicant
Genmab	Research contract	250.000	250.000	06/2023	12	Main applicant
Holland Health	LUMC-PPS	600.000	600.000	09/2023	48	Main applicant
KWF	Alpe d'HuZes	716.841	716.841	01/2024	48	Main applicant
Bontius Stichting		500.000	300.000	01/2024	60	Co-applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT03638375	Adoptive TIL therapy with low dose IFN-alpha plus anti-PD1 in metastatic melanoma	07/2018	Present	Yes	Lead
NL75654.000.20	TEIPP-targeting immunotherapy	11/2021	Present	Yes	Lead
NL76592.000.21	MesoPher/mitazalimab-combination therapy in progressive metastatic pancreatic disease (REACtiVe-2 Trial)	05/2021	24	Yes	Co-PI
NL82378.058.22	Neo-adjuvant Pembrolizumab in vulvar squamous cell carcinoma: a clinical proof-of-concept study	06/2023	24	No	Lead
NL83665.000.23	TILLC: Adoptive TIL therapy in combination with chemoimmunotherapy in advanced NSCLC patients	01/2024	60	No	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
M.K. van der Kooij	Immunotherapy in advanced melanoma crossing borders
P.C. Groeneveldt	Harnessing the immunostimulatory properties of oncolytic reovirus for anticancer immunotherapy

Boudewijn Burgering
UMC Utrecht

1. General information

Research Focus	Signal transduction and metabolism
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

PI3K signaling towards FOXO transcription factors is the major focus of my research. With respect to understanding regulation of transcriptional activity through members of the FOXO class, I collaborated with Dr Jurian Schuijers on the role of condensate formation in controlling transcriptional activity in general. This resulted in a publication (see below) and constituted the major part of Dr Gui's thesis defended in 2023. This work is now continued in several directions amongst which the use of small peptides to inhibit b-catenin driven transcription by dissolving b-catenin containing condensates. This work is also supported through additional Oncode funding (see furtheron). In 2023 the first results with single cell proteomics were obtained. A proof of principle was generated on gastruloids in collaboration with Dr Suzan Stelloo (Vermeulen lab) and a revised manuscript has been resubmitted to Cell Stem Cell. In addition we have received a KWF high risk grant to perform single cell proteomics on tumor organoids.
In collaboration with the lab of Dr Tobias Dansen Oncode PhD student Daan van Soest is exploring the relevance of localized H2O2 release in driving cellular response. This work was in part presented at the annual Oncode meeting and has provided some very interesting insights in currently held beliefs surrounding the role of ROS in causing DNA damage and driving many diseases including cancer. A resubmission is now at Nature Communications. In collaboration with Jeroen de Ridder we are working on this subject but now with the perspective of the DNA damage caused by H2O2 (8-oxy-G) a manuscript on this is now in preparation.

2.2. Major scientific achievements in 2023

a) "Targeted perturbation of signaling-driven condensates" Gui T, Fleming C, Manzato C, Bourgeois B, Sirati N, Heuer J, Papadionysiou I, Montfort DIV, Gijzen MV, Smits LMM, Burgering BMT, Madl T, Schuijers J. Mol Cell. 2023 Nov 16;83(22):4141-4157. Biocondensates are still debated in terms of relevance etc. for transcription and but experiments will bring discussion further and I consider this paper an important step in furthering our understanding on this subject.

b) FOXO transcription factors as mediators of stress adaptation. Rodriguez-Colman MJ, Dansen TB, Burgering BMT. Nat Rev Mol Cell Biol. 2024 Jan;25(1):46-64. I am invited by Nature Rev Mol Cell Biol every 8 years or so to write an updated review on FOXO literature and this year I co-wrote with two former post-docs that out of their pos-doc work on FOXO established their own lab

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Biocondensates in transcription.
A paper was published related to the studies we have been doing "Targeted perturbation of signaling-driven condensates" by Gui T, Fleming C, Manzato C, Bourgeois B, Sirati N, Heuer J, Papadionysiou I, Montfort DIV, Gijzen MV, Smits LMM, Burgering BMT, Madl T, Schuijers J. Mol Cell. 2023 Nov 16;83(22):4141-4157. Related to this project Oncode also funded P2021-0061 “Live-cell screening for disruption of oncogenic condensates”. A pilot screen was successfully executed and at present several hits have been validated and will be continued, a large-scale screen is planned in Q1 2024 and will complete the project.

Project B) Cancer metabolism
I am collaborating with Prof. Dennis Klomp on metabolic imaging of cancer patients using 7T MRI and we have published in 2023 one joined paper (see van der Kemp et al. NMR Biomed. 2023 Apr;36(4):e4882).

Project C) Foxo and DNA damage.
Within the FOXO and DNA damage project, the PhD funded by Oncode submitted two studies both dealing with the use of a genetic tool to produce in cells H2O2 in a localized manner. A technical paper was accepted (see van Soest et al. Free Radic Biol Med. 2023 Sep;206:134-142) and a related functional paper was submitted and now revised (Mitochondrial H2O2 release does not directly cause damage to chromosomal DNA. van Soest et al. Nature Comm). Furthermore, a paper on the metabolic control of ATM is in preparation.

Project D) Oncode funding is used to sustain the Metabolic and Proteomics facility within our department. These facilities provide valuable services, and this has resulted in several publications in 2023. To provide context here, although I am the head of these facilities I only co-author if I feel I provided sufficient other input to these studies, otherwise only people employed by the facility that did the work will co-author.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) With the help of Oncode we obtained two patents with respect to our work on biocondensates, covering both a screening platform for potential condensate drugs and the development of condensate-perturbing peptides. Oncode has now mediated in starting a collaboration with Ambagon Therapeutics a SME located in Eindhoven that focusses on the development of molecular glues for 14-3-3:client stabilization. This collaboration has now led to an application for a KWF TKI private-public partnership grant to further fund these efforts.

b) Oncode has been enormously instrumental in enabling single cell proteomics and providing proteomics access to Oncode (but also other) researchers this is done together with Oncode researcher Michiel Vermeulen. This has also helped us to participate in Oncode Accelerator so that we now have financial support to at least by and large continue our proteomics work initiated through Oncode funding.

c) I have been working on ATM regulation by metabolism and unfortunately (or not, depending on. your perspective) we obtained results that do not align with current knowledge/thinking and publications concerning this subject. It is my opinion/experience, that the commonly praised 'self-cleaning capacity of science" is a difficult path to pursue. Working on reproducibility etc. is not well appreciated. It is difficult to publish and obtaining funding for this is hard. Irrespective I think that in the end, when we are able to resolve these matters, this will result in impactful knowledge that provides a new perspective on the interaction between cellular metabolism and DNA damage. This is only possible to do with "free" funding like that provided by the Oncode base fund.

2.4.2. Contribution to the Oncode community in 2023

a) I have been part of the committee that organized the Oncode Annual meeting held in Amersfoort (June) (together with Bas van Steensel Miao-Pie Chien, Wilbert Zwart) and one of my team members (Daan van Soest) contributed with an oral presentation.

b) I participated in the OI retreat held in Veenendaal and updated there on the progress of single cell proteomics.

c) I organize/teach a one-week master course "Concepts in Cancer" (UU/UMCU research school "Cancer, Stem cells and Developmental Biology) and as part of this course participating students (around 25) are invited to join the KIT-meeting organized by Oncode. This also took place this year. These students are always highly impressed by the meeting and also through this course are being, on the role of Oncode in current cancer research etc.

2.4.3. Key collaborations within Oncode in 2023

a) Michiel Vermeulen (RadboudUMC). My group and the group of Vermeulen have a long-standing collaboration within the field of proteomics. We obtained funding from Oncode first phase to start single cell proteomics and we have now obtained funding through the Nationaal Groeifonds funded Oncode accelerator program whereby we (-) consolidate funding for acquiring new mass-spectrometry machines (3) to be used for proteomics, as well as a mass-spectrometry machine for metabolomics (-) and additional funding (3 person (8 years), data storage, LC equipment etc.). This funding at least enables us to continue and capitalize on the initial funding of Oncode. In addition, through this funding we can introduce novel technology (thermal profiling) also to the Oncode community. This technology is suited to study in a semi-unbiased manner off-targets of small molecules.

b) Maria Rodriguez-Colman (UMCU). Maria started years back as a post-doc (appointed in part on Oncode funding) in my group to study metabolism of stem cells using organoids as model system. This collaboration resulted in several publications and successful grant applications. Maria is now an independent group leader and recently has been elected to join Oncode. We will continue to collaborate on diverse subjects related to cancer metabolism and one joined post-doc will start in 2024 (first of february).

c) Holland project but his last (PhD) year is funded by Jeroen's Oncode base fund. Marc has been driving our idea that nanopore sequencing in principle can provide ground truth data on DNA base modifications. Because my interest in redox stress we focused in this project on 8-oxo-G, as this is considered the main DNA modification caused by redox stress. This project is nearly finished (manuscript in preparation). Methylation of Cytosine like oxidation of Guanine is a DNA modification that can be detected through nanopore sequencing. Marc has consequently also been involved in the project using nanopore-mediated detection of DNA methylation to guide surgery decision (see Refs).

2.4.4. Major valorization achievements in 2023

a) Patent application P099240NL “Targeted perturbation of signaling-driven condensates”. This patent covers the design and development of bioactive peptides or other biomolecules that selectively perturb condensate formation of target genes through “monomer saturation”.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF		260K	260K	01-02-2014	18	Main applicant
Oncode accelerator	Groeifonds	350.000K	5000K	01-01-2024	96	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Tianshu Gui	The structure-function paradigm reshaped by the intrinsically disordered domains
MaoJie Wang	Metabolic crosstalk between keraticoncytes and immune cells: new therapeutic options for psoriasis treatment
Marlies Ludikhuize	Metabolic regulation of proliferation and differentiation
Miguel Hernández Quiles	Proteomics approaches for the study of adipose tissue biology

Miao-Ping Chien

Erasmus MC

1. General information

Research Focus	Single cell technology, advanced imaging & quantitative analysis, cancer-driving cells, target discovery
Junior/Senior Oncode Investigator	Junior, senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

Our group develops advanced single-cell technologies for cancer biology and treatment. My research is focused on the creation and application of single-cell and spatial profiling technologies to facilitate the discovery and interpretation of multi-omics ((epi)genomic, transcriptomic and proteomic) profiles of rare and aberrant single cells that are responsible for tumor metastasis and therapy resistance, with the aim of translating the gained knowledge to clinical applications.

Regarding technology development, we are developing technologies to bridge the knowledge gap in current single-cell sequencing and spatial profiling methods, namely microscopy-based functional single-cell sequencing (FUNseq), spatially annotated FUNseq, and multiplexed FUNseq technologies. We are also devising the next-generation FUNseq technology for 3D samples (organoids and tumor tissues), enabling high-content 3D live-cell imaging followed by selective single-cell and spatial profiling.

Regarding the biological applications with our technologies, we are i) unravelling previously undiscovered mechanistic insights into metastasis and irradiation-induced abnormal DNA-damage response in head-and-neck squamous-cell carcinoma (HNSCC) as well as ii) the role of immune cell clusters in influencing metastatic capabilities in HNSCC. In collaboration with Dr. Derks, we are applying our technologies to study the driving mechanisms of chromosomal instability in gastric cancers and how chromosomally-unstable cancer cells shape immune responses in these cancers.

2.2. Major scientific achievements in 2023

a) Based on our FUNseq-identified predictive signatures for metastasis in HNSCC, we have received a KWF-TKI grant (€654K) in collaboration with SkylineDx. Leveraging these preliminary results, we also secured a EMC-Synergy Grant (€300K) to identify predictive signatures for metastasis in cutaneous squamous-cell carcinoma(cSCC) and investigate the role of metastatic-cSCC cells in shaping tumor microenvironment.

b) Based on my scientific achievements and the VIDI award I received in 2022, I was promoted to an associate professor in 2023, accompanied by the Aspasia Award, an instrument to accelerate the advancement of women scientists.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Validation of FUNseq-identified predictive gene signatures for metastasis in HNSCC.

We have applied FUNseq to profile invasively-migrating HNSCC cells, from which a number of key driving pathways were found. We further applied advanced models to the FUNseq-curated datasets and identified a small set of gene signatures(~10 genes) that can predict distant metastasis in HNSCC, validated with a small patient cohort(clinical-pathological samples). We are currently increasing the number of patient samples to curate larger gene signatures as well as the to-be-validated patient cohort, in collaboration with SkylineDx, towards clinical translation(funded KWF-TKI grant). The project required extensive validation of the found signatures with clinical data; securing funding for this is difficult. With Oncode's base&TechDev funds, I was able to provide sufficient preliminary results to secure the KWF-TKI grant. These preliminary results also help secure another synergy grant for the application of cutaneous-squamous-cell-carcinoma. Furthermore, FUNseq-curated databases have drawn attention from another private party, Scailyte, with whom we will soon initiate a CDA for further discussion.

Project B) Multiplexed functional & spatial single-cell sequencing to link phenotypes to genotypes.

This project has two parts: one part is the development of a multiplexed functional&spatial single-cell sequencing technology, multi-FUNseq (previously called MUCE-seq; the related patent was filed by Oncode in 2020(EP3943945A1); another part is the development of an in-situ spatial single-cell profiling technology, enabling profiling samples from FFPE tissue slices. With Oncode's unrestricted funding, I was able to hire a PhD with a multidisciplinary background to develop the foundation of Part 1 of the project. Part 2 of the project was initiated in late 2023 by another multidisciplinary PhD, who was mainly funded by Oncode's base fund. These methods, as well as our spatially annotated FUNseq, enable biological applications related to the investigation of the unique spatial clustering of specific cell types or cell-cell interactions influencing the tumor microenvironment. These technologies and applications have drawn great attention from the spatial-omics community (invited talks in many international spatial-omics conferences).

Project C) Multi-omics (DNA/RNA) FUNseq profiling of tripolar-dividing breast cancer cells.

The project will be completed early this year, and the manuscript of this work will be submitted afterward. We have applied multi-omics (DNA/RNA) FUNseq to profile tripolar-dividing breast cancer cells. From this work, a number of new insights have been discovered from the used cell model, including the underlying mechanisms of tripolar division, driving pathways of cytokinesis failure, as well as a novel innate immune pathway associated with tripolar division. This is a high-risk/high-gain project and difficult to secure funding for. With Oncode's base fund, I was able to hire a PhD student to perform the project and generate sufficient preliminary results to secure my NWO VIDI grant, which requires some of the technologies and data from this project. In addition, the preliminary results further laid part of the foundation for my ERC Consolidator project, submitted last year (invited interview, but it wasn't awarded) and this year.

Project D) Investigation of the role of immune cell clusters in influencing metastatic capabilities in HNSCC.

This is an ongoing project that was initiated last year. Leveraging our spatially annotated FUNseq technology, we have applied it to profile tumor and immune cells in the immune cell clusters of tumor tissue slices between metastatic and non-metastatic HNSCC patients. We have identified a couple of immune-related signaling pathways that were differentially downregulated in metastatic patients. This is also a high-risk/high-gain project and difficult to secure funding for. With Oncode's base fund, I was able to use it for some consumables and sequencing

costs in the project. The preliminary results laid the foundation of a KWF project, aiming to be submitted this year, in collaboration with a tumor immunologist, Reno Debets, at our institute.

Project E) Multi-omics (DNA/methylation/RNA) FUNseq profiling of chromosomally-unstable gastric cancer cells and investigation of how these cells shape the tumor microenvironment.

This is an ongoing project that was initiated last year. Built upon the knowledge discovered in Project C, we further applied multi-omics (DNA/methylation/RNA) FUNseq to profile patient-derived chromosomally-unstable gastric cancer cells, namely cells displaying micronuclei or chromosomal bridges. Additionally, we will investigate how these chromosomally-unstable gastric cancer cells influence the immune response and shape the tumor microenvironment. This project highly relies on the sustainable resources of patient-derived gastric cancer cells, which were accessible through Dr. Sarah Derks, one of the Oncode PIs. Furthermore, I was able to use Oncode's base fund for some consumables and sequencing costs to generate promising preliminary results. These laid part of the foundation of my ERC Consolidator project.

Project F) Cell fate prediction by deep learning.

The project is still ongoing and is expected to be completed this year. The aim of the project is to use AI to predict the cell fate of cells that will undergo abnormal division caused by chromosomal instability or cells that are chemoresistant (two different applications). With that, we can isolate and profile these cells before abnormal division occurs or they become chemoresistant so that the molecular mechanisms that drive such abnormality can be unravelled. This is a high-risk, high-reward project. If successful, it will yield unprecedented insights into the causative mechanisms of abnormal mitosis and chemoresistance. However, the risky nature of the project makes it difficult to secure funding elsewhere for execution, which was made possible with Oncode's base fund. The promising preliminary results generated from this project partially helped me secure my NWO VIDI grant.

Project G) Functional pooled optical CRISPR screening for target discovery.

In addition to applying FUNseq to profile chromosomally-unstable cells and identify their associated molecular mechanisms (Project C and E), we are also collaborating with Roderick Beijersbergen (at NKI) in combining our technologies/inventions (functional pooled optical CRISPR screening) to identify actionable targets related to chromosomal instability. Oncode's network and base fund enabled me to establish this new collaboration and to start this high-risk/high-gain project. We have identified a few potential targets and have so far validated one of them. Furthermore, this functional pooled optical CRISPR screening platform and the generated data have laid the foundation for the communication with Novo Nordisk, with whom we will soon enter an exploratory conversation.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) New collaborations and pilot projects have been initiated. Through Oncode's network, I was able to establish (new) collaborations with Oncode PIs with complimentary expertise. In 2023, we initiated three pilot projects: i) one was with Dr. Akkari, working on the investigation of how lipid transfer from lipid-laden macrophages affect glioblastoma malignancy; ii) another one was with Dr. Derks, working on the investigation of how chromosomally-unstable gastric cancer cells influence the immune response in the tumor microenvironment; and iii) the last one was with Dr. Noordermeer, working on how cells choose which double-strand break (DSB) repair pathway to take after UV irradiation. With Oncode's base fund, we were able to generate promising preliminary results, which will be further used for upcoming grant applications and discovering new targets. For example, with Dr. Noordermeer, we submitted a ENW-M2 grant in 2023.

b) Interactions with industry: our group has developed proprietary technologies that can be further valorized via a number of routes: i) licensing out signatures or targets found through our technologies; ii) start-up for target/biomarker & drug discovery; iii) contract research organization; iv) commercializing equipment. Oncode's valorization team has actively helped me explore viable options and interact with industrial parties. Because of this, we are collaborating with Leica Microsystems, working on a cutting-edge light-sheet microscope, and with SkylineDx in a funded KWF-TKI project (potential to license out our newly found signatures to SkylineDx). We are currently discussing with a startup in San Diego regarding joining forces of our venture with theirs to execute the abovementioned four valorization routs. Oncode's business developer is helping with this regard.

c) Oncode-supported single-cell sequencing facilities and startups: Our research highly relies on single-cell genomic and transcriptomic sequencing. Thanks to Oncode-funded facilities/startups, e.g. Single Cell Core facility and Single Cell Discoveries, we were/are able to routinely use and pay for these services to speed up our research and generate publications (including the ones in 2023).

2.4.2. Contribution to the Oncode community in 2023

a) UFO Biosciences facility (Oncode & Erasmus MC facility): FUNseq pipeline consists of a custom-built microscope, called Ultrawide Field-of-view Optical (UFO) microscope; Oncode's valorization team has helped me in setting up the facility (in 2021), offering our proprietary UFO and FUNseq technology to the Oncode community (or outside of the Oncode community). Through this facility, we have collaborated with 10 Oncode PIs and 2 Oncode-related facilities/startups (Single Cell Core, Single Cell Discoveries), from which we have so far generated 3 collaborative publications and secured 2 Oncode-assisted target projects (*Oncode Accelerator Project, P2021-0023*).

b) Several collaborations within Oncode critically rely on our technology or contributions because state-of-the-art methods cannot satisfy the demands posed by the scientific questions the collaborations address. Because of this, we created new pilot projects in 2023: i) with Dr. Akkari for the investigation of how macrophages educate glioblastoma cell fate by modulating their lipid metabolic profiles; ii) with Dr. Derks for the investigation of how chromosomally-unstable gastric cancer cells influence the tumor microenvironment; iii) with Dr. Noordermeer for the investigation of how cells choose different DSB repair pathways upon UV irradiation.

c) I was one of the scientific committee members for the Oncode Annual Meeting in 2023. My group members also assisted with poster award assessment and managed microphones during Q&A sessions.

2.4.3. Key collaborations within Oncode in 2023

a) With Leila Akkari: the investigation of how macrophages educate glioblastoma cell fate by modulating their lipid metabolic profiles. The collaboration has started thanks to my affiliation with Oncode.

b) With Sarah Derks: the investigation of how chromosomally-unstable gastric cancer cells influence the tumor microenvironment. The collaboration has started thanks to my affiliation with Oncode

c) With Sylvie Noordermeer: the investigation of how cells choose different DSB repair pathways upon UV irradiation. The generated preliminary results have been included in the ENW-M2 grant application, submitted in 2023. The collaboration has started thanks to my affiliation with Oncode.

2.4.4. Major valorization achievements in 2023

a) Industry engagement: collaborations with Leica Microsystems & with SkylineDx (potential to license out our newly found signatures to them); connections/communication with General Inception and JnJ.

b) Venture creation activities: we are in the negotiation phase regarding licensing our UFO and FUNseq technologies to a startup in San Diego, accompanying by Dr. Chien as a co-founder of the startup

c) UFO Biosciences service facility: we have created new pilot projects (described in 2.4.3) through our UFO facility, facilitated by Oncode

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Erasmus MC	EMC Synergy Grant	300K	163K	02/2024	36	Main applicant
Aspasia award	NWO	40K	40K	07/2023	N.A.	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Ruud Delwel

Erasmus MC

1. General information

Research Focus	Molecular genetics of AML
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

We study the mechanisms of defective gene regulation in acute myeloid leukemia (AML). We focus on the function of enhancers that are hijacked by putative oncogenes as the result of chromosomal rearrangements. More than 10 enhancers have been discovered that are frequently hijacked by the same oncogene, EVI1. Other oncogenes frequently use the same enhancer to become overexpressed. The question we address is: Can we interfere with the enhancer activity? We identified a number of compounds that are able to block enhancer activity and with that inhibit oncogene EVI1 expression. This year KWF has awarded us a research grant to study the role of those hijacked enhancers in AML and uncover ways to target those enhancers with specific molecules.
Another approach to affect oncogenes, is to target the oncoprotein itself. Therefore, targeting EVI1 protein in AMLs with aberrant expression may be another way to cure the disease. We found EVI1/CTBP2 interaction to be essential and found a way to break EVI1/CTBP binding and inhibit tumor growth in a mouse model. This study, which is in press in ScienceAdvances, demonstrates a way to specifically inhibit tumor growth. In collaboration with Sebastian Pomplun, we are testing small peptide-compounds to inhibit leukemic growth.

2.2. Major scientific achievements in 2023

a) Thesis entitled “epigenetic regulation in normal and malignant hematopoiesis”, by Roger Mulet-Lazaro. Roger received, the Jaap Steenbergen award, for the best Hematology thesis (6 published chapters) in 2023. Part of the introduction was published as a review in Hemasphere. Another part is under consideration by Blood Cancer Discovery.

b) Part of the work that we were able to carry out thanks to the base funding of Oncode has now been awarded by the KWF as a research grant. This project is a logical follow-up on the drug-repurposing compound screen, which resulted in the identification of molecules able to target of EVI1 expression in AML.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Targeting refractory AML by interfering EVI1 function.
AML with 3q26 rearrangements and consequently overexpression of EVI1 are among the most severe forms of the disease. In fact, 75% of those patients never get into remission upon chemotherapy. We demonstrated that those leukemia cells die when the gene is turned off. Therefore, targeting EVI1 protein in AMLs with aberrant expression may be another way to cure the disease. We found EVI1/CTBP2 interaction essential and found a way to break EVI1/CTBP binding and inhibit tumor growth in a mouse model. This study is in Press in Science Advances. A second manuscript demonstrating how the EVI1/CTBP complex represses transcription of key genes is in progress. Together with Sebastian Pomplun (New young PI from Leiden) we are generating peptides/compounds to interfere the interaction in primary leukemia cells. This work is not funded yet by any organization. We hope to apply for grants this year.

Project B) Understanding p300 driven superenhancer activity driving oncogene expression.
We generated models in which endogenous EVI1, under control of a hijacked enhancer, was tagged by GFP. We demonstrated that two distinct p300/CBP inhibitors in combination were able to block EVI1(GFP) expression and closing the chromatin at the enhancer. Importantly, these inhibitors did not affect the expression of EVI1 in normal bone marrow hematopoietic stem cells. We will now study the effects of those inhibitors of primary AML cells with EVI1 under control of the MYC-superenhancer (translocation t(3;8) in vivo using a xenotransplant model (Collaboration with Dr. Richard Groen from Amsterdam UMC). This work too, is not funded by any organization yet. If the xenotransplant studies will be positive, this work will be ready for the next step of funding as well.

Project C) Identifying and targeting regulators upstream of hijacked superenhancers driving EVI1 expression.
We study the mechanisms of defective gene regulation by hijacked enhancers in acute myeloid leukemia (AML). More than 10 enhancers have been discovered that are frequently hijacked by the oncogene EVI1. The question that we address is: Can we interfere with the enhancer activity? We identified a number of compounds, using the drug repurposing screen, that are able to block enhancer activity and with that inhibit oncogene EVI1 expression. This year KWF has awarded us a research grant to study the role of those hijacked enhancers in AML, study the effects of identified compounds and uncover ways to target those enhancers with those specific molecules in vitro and in vivo.

Project D) EVI1 in Ovarium Cancer.
We recently discovered that EVI1 is frequently overexpressed in Ovarian Cancer. We have evidence that this is caused by amplifications or rearrangements in which the EVI1 gene becomes regulated by hijacked or newly formed enhancers. The exact mechanism behind this aberrant gene control requires further study. This work is not yet at a fundable level for organizations such as KWF, but it shows that abnormal gene control driven by altered regulatory elements is not restricted to leukemia. The outcome of Project C and the availability of drug repurposing libraries will be of benefit of this project.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Although it has been more than a year ago, the compound repurposing screen has been essential for me to obtain a new KWF grant. This project will now be funded by KWF, which allows us o use Oncode funding for a new project, i.e. EVI1 in Ovarian Cancer.

b) A very fruitful collaboration has started with Dr. Sebastian Pomplun, a chemist and new Oncode investigator. Together we will apply for new grants studying the effects of small peptide/compounds in EVI1 AMLs.

2.4.2. Contribution to the Oncode community in 2023

a) Member of RMC.

b) Launched bi-weekly lecture series at Erasmus which has been made available for all Oncode scientists.

c) Member of promotion committees of PhDs within Oncode in and outside Erasmus MC.

2.4.3. Key collaborations within Oncode in 2023

a) Collaboration with Sebastian Pomplun, Chemist at Leiden UMC. This collaboration started with a cooperation with Prof. Mario van der Stelt. The specific expertise of Sebastian Pomplun was essential for this collaboration. Sebastian is now also a young Oncode PI.

2.4.4. Major valorization achievements in 2023

a) The valorization discussions on the distinct projects still need to be done. Hopefully we will finish those this year.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF		780.000	780.000	February 1	48	Main applicant
KWF		800.000	400.000	January 1	48	Co applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Roger Mulet-Lazaro	Epigenetic regulation in normal and malignant hematopoiesis
Tim Grob	Molecular stratification and residual disease detection in acute myeloid leukemia

Sarah Derks

Amsterdam UMC

1. General information

Research Focus	GEA, genome-immunome interactions, intratumor heterogeneity, targeted-immunotherapy
Junior/Senior Oncode Investigator	Junior, senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

To understand the biology of resistance to (immune/radio/chemo)therapy in GEA we work on the following topics:

1) Improve success of chemoradiotherapy in GEAs:
   topic 1.1: Determine whether tumoral oncolytic virus injections induce T cell infiltration and thereby improve response to neoadjuvant chemoradiotherapy.
   topic 1.2: Determine whether a metabolic intervention can activate the immune microenvironment and improve response to neoadjuvant chemoradiotherapy.

2) Re-activate antitumor immunity in GEA:
   topic 2.1: identify cancer-intrinsic pathways of immune suppression, by
   (a) analyzing the secretome and transcriptome of cancer cells in immune-excluded tumor areas
   (b) perform a CRISPR screen to identify novel targetable genes and pathways to improve anti-tumor immunity
   topic 2.2: Improve response to checkpoint inhibitors by
   (a) identifying the effect of chemotherapy on the immune microenvironment and response to PD-1 inhibition in MSI GEAs
   (b) determine the role of tertiary lymphoid structures (TLS) in primary and peritoneal metastatic GEA.
   (c) test whether targeting pro-angiogenic factors improves response to PD-1 inhibition using mouse models.

3) Develop and improve patient derived model systems to test novel immunemodulatory strategies ex vivo.
   (a) build an organoid – T cell biobank
   (b) test new T cell activating strategies in microfluidic systems

2.2. Major scientific achievements in 2023

a) Using spatial transcriptomics we identified that lipid metabolisms is associated with immune exclusion/suppression in EAC (manuscript in preparation, oral presentation ITOC in 2024). We will test whether metformin can alter the metabolic state and activate anti-tumor immunity in EAC in a CCA-funded (2023_11) Clinical Proof of Concepts study (MEMENTO-Trial).

b) I acquired a competitive NWO vidi grant in July 2023 entitled; "The REACT study: Reviving gastroEsophageal AdenoCarcinoma T cell immunity", which allows me to perform additional spatial transcriptomics, secretome analyses and optimize microfluidic systems to test novel T cell activating strategies.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Defining the role of Tertiary Lymphoid Structures in diffuse type gastric cancers to understand their pro- or anti-inflammatory role in the course of the disease.

This project aims to phenotype Tertiary Lymphoid Structures (TLS) in the immune microenvironment of primary gastric cancers (GCs) and peritoneal metastases to understand why TLS are profoundly observed in GCs with a poor prognosis and response to immune checkpoint blockades (ICB); an observation that contrasts results in other cancer types. The project was not accepted for funding elsewhere due to lack of pilot data. To acquire pilot data we used Oncode base funding to start the project and performed spatial transcriptomics (together with oncode PIs Joep Grootjans and Daniela Thommen) and identified that the cellular composition and maturation status of TLS varies between patients and that primary cancer of patients with stage IV disease and peritoneal metastatic most often contain immature TLS that do not support antitumor immunity. Thereby this study confirms the suppressive state of the TIME of GCs and that the role of TLS is tumor type and tissue location specific. This is a novel finding as TLS are mostly studied in diseases (such as melanoma and lung cancer) in which TLS are mature and support antitumor immunity. For GC, the immature character of TLS needs to be taken into account when optimizing immunomodulatory strategies for metastatic GC. The manuscript that describes these findings will be submitted in the following weeks.

Project B) Identify cancer cell intrinsic pathways of immune suppression in gastro-esophageal cancers to identify targetable immune suppressive factor to re-initiate an antitumor immune response.

As the majority of GEAs are characterized by an immune excluded microenvironment we hypothesize that GEAs express or excrete immune suppressive factors and that targeting these factors might revert the immune suppressive state. To identify these factors we took an multipronged approach in which we 1) map the secretome of GEAs by plating freshly dissociated tumor biopsies and use the supernatant 48h later for proteomics and 2) determine transcriptional differences between T cell inflamed and non-inflamed tumor areas. Using Oncode base funding and the technology access program we could pilot spatial whole transcriptome analysis (GEOmx) which led to the observation that tumors with low T cells scores are enriched for CXCL5 expression and IDO1 and have suppressed antigen presentation. Furthermore we identified that immune excluded tumor areas are enriched for lipid metabolism pathways, that might have a direct immune suppressive effect. We are currently performing functional studies to test the metabolic state of cancer cells in association with the immune microenvironment in patients-derived tissues. A manuscript describing these data is in preparation. Furthermore, the data were used as preliminary data for my Vidi grant application (granted July 2023) and for a clinical proof of concept study testing the effect of metformin treatment on the immune microenvironment in patients with EAC (granted Nov 2023). Thereby this project is an example of how Oncode base funding could be used to start a project and acquire data to needed with continue with additional funding.

Project C) Performing a CRISPR screen to identify pathways of immune suppression in GEA.
In this project we aimed to take an unbiased approach to identify novel cancer-intrinsic immune suppressive mechanisms by performing a CRISPR screen on MART-1 expressing GEA cell lines (MART1 reactive T cells) to determine how we can improve their immunogenicity. The project started with support of Daniel Peeper based on the experience with this approach and support of Oncode base funding to get the project started. In 2022 the lab received a philanthropic gift from a patient which allowed me to appoint PhD candidate Jasper Sanders on the project and cover the experimental cost. At this moment all GEA cell lines have be transduced and can be used for the CRISPR screen. Based on project B we will focus on a CRISP screen to identify how to alter the metabolic state of these cancer cells.

Project D) Determine the immune suppressive role of a proangiogenic factor using a new immunogenetic GEA mouse model.
This project aims to test the role of overexpression of a pro-angiogenic factor in response to checkpoint inhibitors using an immunogenic GEA specific mouse model I generated during my postdoc at DFCI in Boston. This project has shown that high expression of a pro-angiogenic factor induces resistance to checkpoint inhibitors which is very relevant for GEA. The project needs some last experiments to confirm these findings and get published but these experiments were stopped during the covid pandemia. This year we restarted the mouse experiments at Amsterdam UMC with help of Oncode-funded postdoc Jens Seidel, in collaboration with oncode-PI Joep Grootjans. We currently confirmed previous findings in the Amsterdam UMC mouse facility and included peritoneal tumor location in the study. Anti-PD-1 treatment experiments are planned to understand how this overexpression lead to anti PD-1 resistance and whether targeting this factor can reverse this resistance. This project could not start before I hired a dedicated postdoc which was possible with Oncode Base funding.

Project E) Defining the role of intratumor heterogeneity on the immune infiltrate and response to immunotherapy
Together with Daniel Miedema (Louis Vermeulen lab) I started a project to understand the association between chromosomal instability (CIN), intratumor heterogeneity (ITH) and antitumor immunity using genome and transcriptome data from a divers set of metastatic tumors sequenced by the Hartwig Medical Foundation. A manuscript that describes the tumor type dependency of this association is in preparation. As a follow-up project I started a collaboration with Oncode PI Miao-Ping Chien to use a single-cell omics approach to determine the level of intratumor heterogeneity in CIN EACs and identify how this heterogeneity impacts the T cell response on a single cell level. This project started as a pilot supported by Oncode base funding. We currently shared 3 patients derived cultures from CIN EACs that will be analyzed on a single cell level on a multiomics level. After mapping the lever of intratumor heterogeneity we will perform a cancer-cell co-culture setting to determine heterogeneity in T cell response.

Project F) Building a patient-derived GEA organoid biobank and in vitro culture systems to study tumor-immune associations in GEA.
This year we further build upon our organoid biobank and 3d patient-derived organotypic model systems and managed to start expanding intratumoral T cell from the same tumors from which we generate organoids with a high dose Il-2 rapid expansion for future co-culture purposes. We managed to generate 15 organoids with TILs which are currently used to test our microfluidic systems. Via a new collaboration with React4Life as part of a MSCA doctoral network programme (PI Maarten Bijlsma) we will work together with PhD Rok Ziberna to test the new MIVO organ on a chip system. Our organoid-T cell cultures also form the basis for collaborations with oncode-PIs Jacco van Rheenen (besides Miao-Ping Chien).

Project G) Use cyclomics to identify cell free DNA from chromosomal instable tumors to predict treatment outcome.
In this project we explore the use of cyclomics to measure and analyze cell free DNA from chromosomal instable cancers such as GEA. The project was delayed due to an update of our MTA/CDA in which we added Prof. van Laarhoven as collaborator. In Q4 of 2022 we were able to continue the sequencing of cfDNA which are used in a manuscript that will be summited soon by the group of Jeroen de Ridder. Furthermore we started a new pilot to test whether we can use cyclomics to measure tumor-derived DNA in fluid from a peritoneal wash during a diagnostic laparoscopy to improve stageing and prevent surgery in patients with peritoneal metastatic disease. This pilot was started in collaboration with Joep Grootjans and Jeroen de Ridder and will be funded with Oncode base fund.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode's networking and scientific events in 2023 were great places to meet other Oncode PIs and start new collaborations such as with Miao-Ping Chien and Jacco van Rheenen. The collaboration with Jacco van Rheenen started after an organized speed date at the retreat. Also the brainstorm session led to an idea to work collaboratively on 3D tumor-immune co-culture models which will be very important for my lab.

b) As a (junior) PI it is important to get input from a broad scientific community. It was very helpful to present early data at the Annual conference and discuss the design of a new clinical proof of concept study. Furthermore, different Oncode PIs provided feedback on research grants which helped me to improve their quality.

c) Oncode base funding has been crucial in generating pilot data needed to apply for other grants such as the VIDI grant, the Clinical Proof of Concept grant (CCA-funded) but also investigator-initiated studies such as the LOAD study.

2.4.2. Contribution to the Oncode community in 2023

a) I chaired 2 brainstorm sessions during the OI retreat 2023.

b) I recorded a "vlog" about how Oncode supports my research for Oncodes Major donor program (together with KWF).

c) I am involved in the organization of two workshops that will be organized in 2024.

2.4.3. Key collaborations within Oncode in 2023

a) My collaboration with Jeroen de Ridder (for description see project G) started after a meet-up at an Oncode event. He was not part of my network before Oncode. The data we generated so far was supported by Oncode base funding and will be used for applying external funding in the future. The results are described in a manuscript that will be submitted soon.

b) My collaboration with Jacco van Rheenen started after a meeting at the OI retreat in 2923. In this collaboration we will share our treatment-naïve GEA organoids and matching (expanded) intratumoral T cells to test the hypothesis that taxanes stimulated T cell-mediated killing of GEA organoids in vitro. This hypothesis is based on a previous publication of the van Rheenen group (https://doi.org/10.1016/j.ccell.2023.05.009) which described that taxanes can trigger T cells to secrete cytotoxic extracellular vesicles. As docetaxel is an important part of the treatment of gastric cancer in stage II/III disease we test whether taxane treatment of tumor-derived TILs is sufficient to eradicate matching patient-derived organoids.

c) My collaboration with Miao-Ping Chien started in 2023 after an Oncode meeting. The project is described under 2.3.1 as Project E. The goal of the project is to measure the level of intratumor heterogeneity in chromosomal instable (CIN) GEAs and determine whether the level of CIN influences T cell function. Therefore, we shared patient-derived esophageal organoids that were selected based on high levels of copy number variation (determined by shallow DNA sequencing). We first cultures organoids cells from 3D into 2D which worked for 3 organoid lines. We will first map the level of genomic/epigenomic heterogeneity and then co-culture organoid and matched TILs to determine how genomic-epigenomic variations influence T cell function.

d) The collaboration with Bas van Steensel started as a discussion at an Oncode dinner and evolved as one of Oncodes Synergy projects that could start due to external funding. The project aims to establish the overall importance of non-coding mutations in cancer, and to develop a powerful genomics pipeline for screening and functional interpretation of such mutations. The project is a cooperative effort from seven Oncode labs. My contribution to the project is that we aim to find non-coding mutation in diffuse gastric cancers (DGC). In the first years we focus on the generation of organoid lines from genome stable gastric cancers. In the last 1.5 year we generated 35 GEA lines, among which 8 genome stable lines. We aim to establish 7 additional lines in the following year.

2.4.4. *Major valorization achievements in 2023*

a) After finalizing the agreement with ORCA therapeutics for the IIT-LOAD trial (Local oncolytic virus ORCA-010 administration to sensitize the immune microenvironment of esophageal adenocarcinoma for chemoradiotherapy (CRT), € 1.298,676) we worked on all documents needed to apply for IRB and GMO approval. We hope to get approval in 2024_Q2.

b) Based on our finding that T cell exclusion in EACs is associated with fatty acid metabolism of tumor and immune cells, we designed the MEMENTO study (MEtformin as a MEtabolic iNTervention in Oesophageal adenocarcinomas to improve response to neoadjuvant chemoradiotherapy) which was granted for funding by the CCA in 2023/11.

c) As a physician scientist I mostly work with patient-derived tissues. As this is not possible for all researcher, I share our GEA organoid-T cell biobank (n=49) to support other research groups such as the group of Tanja de Gruijl (Amsterdam UMC), Jacco van Rheenen, and Miao-Ping Chien. Besides that, we share cell free DNA with the group van Jeroen de Ridder and fresh gastric cancer tissue with Linde Meyaard (as part of her KWF-LAIR project).

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
NWO	VIDI	€ 796.970		01/2024	60	Main applicant
Cancer Center Amsterdam	CPOC program	€ 277.468,20		07/2024	12	Main applicant
European Union	MSCA Doctoral Network		€ 235,490	02/2024	48	Co-applicant
Cancer Center Amsterdam	Philanthropy program	€ 50.000		09/2023	24	Main applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
EU trial number 2021-001181-38	Anti-PD1, capecitabine, and oxaliplatin for the first-line treatment of dMMR esophagogastric cancer (AuspiCiOus-dMMR): A proof-of-principle study (AuspiCiOus).	10/2021	48	Yes	Lead
EU trial number 2024-510754-25	Local oncolytic virus ORCA-010 administration to sensitize the immune microenvironment of esophageal adenocarcinoma for chemoradiotherapy	Expected 09/2024	36	No	Lead
Follows shortly	MEtformin as a MEtabolic iNTervention in Oesophageal adenocarcinomas to improve response to neoadjuvant chemoradiotherapy.	Expected 05/2024	12	No	Lead

3.3. PhD defenses in 2023

N/A

Peter ten Dijke

LUMC

1. General information

Research Focus	Chemical Signaling
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our research efforts are focussed on:

(1) To elucidate the molecular and cellular mechanisms that control tumor (microenvironment) cell behaviour
(2) To manipulate signaling pathways to develop more effective cancer therapies
(3) To use extracellular vesicles as cancer biomarkers

The main questions that are being addressed:

(1) How is epithelial-mesenchymal cancer cell plasticity controlled and contributes to metastasis and immune evasion?
We continue to uncover pivotal determinants by which the cytokine TGF-b switches non-invasive cancer cells into aggressive and immune therapy resistant cancer cells.
(2) How can we antagonize TGF-b-induced pro-tumorigenic responses without perturbing tissue homeostasis?
We are developing bifunctional proximity-inducing modalities for precise signaling manipulation to inhibit pro-tumorigenic responses in a cell type specific manner.
(3) How can we harness E3 ubiquitin ligases and/or proteasome to specifically degrade tumor promoting proteins?
We are developing bi-functional small molecules that recruit target proteins directly to the ubiquitin receptors of the proteasome for degradation.
(4) Can we specifically isolate tumor-derived extracellular vesicles from blood of cancer patients and use their number and characteristics as cancer biomarker?
We identified cancer selective sugar protein modification on tumor-derived extracellular vesicles that we are validating on cancer patients and control plasma samples.

2.2. Major scientific achievements in 2023

a) We elucidated the mechanism by which a parasite evades host immune responses by secreting TGF-β mimics that interact with TGF-β receptors in a cell type selective manner. These insights are now providing unexpected perspectives on how TGF-β signaling can be modulated for therapy (DOI: 10.1073/pnas.2302370120; DOI: 10.1101/2023.11.13.566701; DOI: /10.1101/2023.12.22.573140).

b) We identified LncRNAs that acts as gatekeeper of epithelial cancer cell integrity, or in contrast, enforce an aggressive mesenchymal cancer cell phenotype (DOI: 10.1126/scisignal.adf1947;DOI: 10.15252/embj.2022112806). We uncovered a new layer by which epithelial-mesenchymal plasticity (EMP) is controlled. Some of these lncRNAs controlling EMP can be explored as therapeutic targets.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Long coding and circular RNAs in cancer progression.

Using genetic screens and mining of data bases (generated by ourself or publicly available) we identified multiple novel long non coding RNAs (LncRNAs) and circular RNAs (cirRNAs) that potently inhibit, mediate or promote TGF–β-induced epithelial-mesenchymal plasticity. Identification and initial mechanistic studies were performed using base funds. Subsequent research on using insights lncRNAs/circRNAs towards targeting mesenchymal cancer cells is now funded by ZON-open competition grant.

Project B) Understanding and targeting immune evasive pancreatic ductal adenocarcinoma.

We have performed a genome-wide CRISPR-Cas9 library knock out screen to understand why mesenchymal cancer cells are more efficient than epithelial cancer cells in evading killing by cytotoxic T cells. Multiple targets were identified followed up functionally (DOI: 10.1126/sciadv.adf9915. Additional hits from genetic screens that determine ability of CD8 T cells to kill epithelial cancer cells are now followed up, and is funded in part by a CSC fellowship.

Project C) Targeting ETS transcription factors to block tumor growth.

ETS transcription factors can lead to tumorigenesis either through mutation or through changes in their level of expression. Another important tumorigenesis-promoting mechanism results from a mutation in the regulatory region of the TERT promoter rather than in the ETS protein itself. This mutation creates an ETS transcription factor binding site and is one of the most common mutations reported in all solid tumors. It has been shown that illicit binding of ETS factor to the mutant promoter drives tumorigenesis. We have performed several high throughput screens to identify unique ETS inhibitors, which have yielded a number of promising small molecule leads which are currently being characterized in relevant biological assays. In parallel, in collaboration with Gluetacs/Shanghai Tech University, we will use a new methodology developed in the last two years, termed oligonucleotide -PROTACs, to target ETS factor for degradation. A personal fellowship grant was awarded to investigate and target ETS transcription factor. The preliminary data created using with Oncode base fund will be used for external funding next year.

Project D) Towards cell type specific targeting of TGF-β signaling.

We elucidated the mechanisms by which parasite-derived TGF-β mimics (TGMs) selectively suppress host immune response. TGMs directly interact with signaling TGF-β receptors via distinct domains, but need a co-receptor for high affinity and cell selective binding. This project has allowed us to rational design cell type specific TGF-β (in)activators. Based on obtained results we have submitted a grant application entitled "Redirecting TGF-β signalling, inspired by adaptive evolution of parasite mimics" and we will apply for Tech Dev funding, together with Daniela Thommen and Ton Schumacher.

Project E) Harnessing the proteasome to induce targeted protein degradation.
Proteolysis targeting chimeras (PROTACs) are bifunctional molecules that recruit an E3 Ubiquitin ligase to target protein for proteasomal degradation. We developed UbRTACs that recruit target proteins selectively and directly to the ubiquitin (Ub) receptors (R) of the proteasome for degradation. Proof of concept studies for UbRTAC) have been completed and together with Selvita company (supported by Oncode base funds) an in silico screen was performed to identify new ligands for ubiquitin receptors. At present, compounds are being synthesized and will be tested for proteasome binding, converted to UbRTACs and tested for selectivity and potency. When successful, we will apply for additional funding next year.

Project F) Extracellular vesicles in cancer progression and cancer biomarkers.
Tumor-derived extracellular vesicles (tdEVs) have emerged as key mediators of cancer progression. Moreover, tdEVs have promise as cancer biomarkers. We have increased our mechanistic understanding of tdEV-induced immune evasion and metastasis, and explored their clinical utility as cancer biomarkers (DOI: 10.1038/s41467-022-31250-2; DOI: 10.15252/embj.2021108791). A KWF project was funded that is aimed at quantifying and profiling circulating tdEVs from pancreatic cancer patients using a fluorescence-based probe that is also used in fluorescence-guided surgery. Moreover, we have identified a sugar modification of proteins that is specific for tumors and were able to show that this allows for specific detection of tdEVs in cancer patients. When more preliminary data is obtained, we will apply for additional funding.

Base funding has allowed for significant progress to be made on all of these innovative projects and this has allowed sufficient preliminary data to secure outside funding (Project A, B, C and G) or funding has been applied for (Project E).

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Grant applications support and facilitating academic/industrial collaborations - Ten Dijke (and also post-docs) received valuable support from Oncode to improve grant applications, including aspects on knowledge utilization, valorization, societal impact and FAIR data plan. The business development team continues to help ten Dijke lab with contacts with biotech industry. Via the Oncode Patient Engagement program we were able to obtain support letters from a patient.

b) Access to excellent collaborators, meetings/work-shops and equipment/facilities Group members greatly benefitted from base funds and access to Oncode collaborators with complementary expertise. For example, with obtaining access to HTS facility/drug repurposing library, Jos Jonkers on mouse cancer models, Michiel Vermeulen on proteomics and van der Burg on insights into immune-oncology. Moreover, our group benefitted from presenting/participation (and receive feedback) at annual and technical workshops.

c) Facilitating the translation of basic discoveries into therapeutic targeting –Support from Oncode base fund has facilitated our studies to redirect specific signalling events using small molecules for therapeutic anti-cancer gain. Examples are (1) development of bifunctional proximity-inducing modalities for precise signaling manipulation to inhibit pro-tumorigenic responses in a ell type specific manner. (2) proof of concept studies to harness the proteasome to target cancer promoting proteins for degradation using bispecific molecules that simultaneously bind a protein of interest and ubiquitin receptors of the proteasome.

2.4.2. Contribution to the Oncode community in 2023

a) Patient engagement – Ten Dijke group actively participated in cancer patient-researcher interaction. This year ten Dijke organized a thematic patient meeting on “early detection and monitoring of cancer” at Boerhaave museum in Leiden in which Agustin Enciso Martinez (ten Dijke lab), Oncode PI Jeroen de Ridder and Prof. L Terstappen were speakers. Agustin Enciso Martinez and Ten Dijke participated in an interactive session on cancer patient engagement with biomedical students from Utrecht University. Ten Dijke gave a presentation of experiences of his group to interact with (ex)cancer patients. Together with Colette ten Hove, manager on Oncode Patient engagement programme, ten Dijke participated in a panel discussion at Figon Dutch medicine days 2023 (Oss, Netherlands) to share the challenges, opportunities and experiences within the Oncode patient engagement programme.

b) Providing advice/support for young Oncode researchers –Ten Dijke lab continues to assist PhD students and post-doc researchers for advice on grant applications/projects. He has participated in opposition committees for PhD students from Oncode Investigators (Vermeulen, Neefjes, van den Burg).

2.4.3. Key collaborations within Oncode in 2023

a) Ruud Wijdeven (Neefjes group) and Zhengkui Zhang (Peeper group). Elucidating molecular and cellular mechanisms and targeting immune evasion of mesenchymal pancreatic cancer cells; this new collaboration because of Oncode resulted in two 2023 publications: DOI: 10.1038/s41423-023-00980-8 and DOI: 10.1126/sciadv.adf9915.

b) Julia Houthuijzen and Jos Jonkers. Mechanism of action and targeting of Gremlin in xenograft and spontaneous breast cancer models; this new collaboration because of Oncode resulted in one 2023 publication: DOI: 10.1007/s12079-023-00777-4

c) Michiel Vermeulen. Identification of ITD-1, a potent inducer of the degradation of TGF-β type II receptor protein. New collaboration because of Oncode. We are using the newly developed isothermal proteomic profiling method of Vermeulen lab to identify ITD-1 binders.

d) Sjoerd van der Burg. Improving anticancer immunotherapy and oncolytic viral therapy using TGF-b antagonists. New Oncode collaboration. This research resulted in one 2023 publication: DOI: 10.1158/2767-9764.CRC-23-0019.

e) Sjaak Neefjes. Interplay between TGF-β-induced EMT and E3 ubiquitin ligase RNF26 controlling TGF-β receptor-mediated endocytosis. A new collaboration because of Oncode, a manuscript is in preparation.

f) Wouter de Laat. New Oncode collaboration to map genomic DNA regions that communicate with a enhancer lncRNA using Chromosome conformation capture combined with high-throughput sequencing (4C-seq).

2.4.4. Major valorization achievements in 2023

a) After proof-of-concept studies for UbRTACs, we identified novel putative ligands for UbRs by in silico screening (performed by Selvita and funded by my Oncode base funding). After synthesis, we will test for proteasome binding and PROTAC-like conversion. We will apply for a TKI-LSH match grant together with Gluetacs.

b) We continued our public private partnership with UCB company on targeting of Gremlin (alone or combined with immunotherapy) to inhibit breast cancer.

c) We developed an approach for cell specific manipulation of TGF-β signaling responses. We are preparing a Tech Dev fund application to generate and test bispecific antibodies that selectively inhibit TGF-β receptor function in CD8 T cells to boost anti-cancer immune response. If successful, we will apply for patent.

d) In collaboration with ProSion and Dept. Chemistry, Univ. Cologne, we successfully generated bi-specific targeting agents based on proline-rich motifs involved in protein-protein interactions. We will apply for a TKI-LSH match grant.

e) Consultant activities for Laekna Therapeutics on targeting liver fibrosis and cancer, Agomab on targeting lung fibrosis, Thirona Biosciences on inhibiting skin fibrosis.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Chinese Ministry of Education	Chinese Scholarship Council *https://www.universiteitleiden.nl/en/scholarships/sea/csc-leiden-university-scholarship*	80K	80K	01-10-2023	01-10-2027	Co-applicant
Chinese Ministry of Education	Chinese Scholarship Council *https://www.universiteitleiden.nl/en/scholarships/sea/csc-leiden-university-scholarship*	80K	80K	01-10-2023	01-10-2027	Co-applicant
KWF	Onderzoek & Implementatie (Behandel)technieken	490K	200K	01-12-2023	01-12-2026	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Adilson Fonseca Teixeira	Exosomes mediate TGF-β signaling activity and promote breast cancer progression
Kseniya Glinkina	In search of synergy: novel therapy for metastatic uveal melanoma
Dieuwke L. Marvin	Understanding the complexity of TGF-b signaling in cancer
Jessie S. Kroonen	Targeting SUMO signaling to wrestle cancer
Yang Hao	Photodynamic Therapy Based combinations with Immunotherapy in colon cancer treatment
Fredrik Trulsson	Deciphering the Ubiquitin code by Mass Spectrometry

Jarno Drost

Princess Máxima Center

1. General information

Research Focus	Molecular dissection of childhood solid tumors
Junior/Senior Oncode Investigator	Junior, senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

Although survival rates for children with cancer have increased in recent decades, cancer is still the leading cause of disease-related deaths in children. Survivors suffer from side effects of the, in most cases, intensive treatment regimens. Hence, there is an urgent need to develop new therapies. However, therapeutic innovation is hampered by the lack of cell models representative of native tumor tissue. My lab pioneers the use of organoid technology for pediatric cancer research.

Many childhood tumors originate in the developing fetus. They are likely caused by a block in processes driving lineage-specification and differentiation. In most cases, the cells from which the tumors originate are only present during short, specific time-windows in development, which makes it challenging to identify the processes initiating and driving tumorigenesis. We aim to identify the origin of childhood cancer and to increase our understanding of the processes that underpin their development. To this end, we take a multi-disciplinary approach making use of our unique in vitro models, access to unique patient material, in vivo orthotopic xenograft models, as well as state-of-the-art (single-cell) omics and lineage tracing technologies. Ultimately, we aim to develop new, less toxic therapies to treat children with cancer.

2.2. Major scientific achievements in 2023

a) Applying a multi-omics approach to patient-derived tissues and organoids, we mapped the epigenome of malignant rhabdoid tumors (MRT). We were the first to demonstrate intertumoral heterogeneity on the epigenetic level as a driver of oncogene expression fueling tumor growth (Liu, Paassen & Custers et al., Nat. Commun. 2023).

b) We investigated the clonal architecture of Wilms tumor using high-resolution DNA-sequencing. We found that the clonal architecture of Wilms tumor in infants is different from that in older children, resembling normal tissues. We also identified a novel genetic driver of newborn Wilms tumor (Lee-Six et al., in revision).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) mmunomodulation of pediatric tumors.

Pediatric tumors are considered “cold” tumors making it challenging to obtain funding for immunomodulation research. Oncode base funds have allowed us to:

- map the TME of pediatric tumors using single-cell transcriptomics (DeMartino et al., Nat. Commun. 2023). Due to their rarity, patient numbers remained limited. Oncode base funds allowed me to test a new scRNAseq technology (10x Genomics), allowing for the retrieval of high-quality single cell gene expression data from FFPE specimens. The pilot was successful, and we are currently applying this to a large cohort of archived specimens.
- apply cell surface proteomics to tumor- and normal tissue-derived organoids to find tumor-specific antigens that could serve as therapeutic targets. We identified an MRT-specific protein and engineered antigen-specific CAR-T cells that we are currently testing in co-culture experiments (Buhl et al., in preparation). We are applying a similar approach to other pediatric malignancies.

Project B) Genome architecture of MRT.

Both project B and C require expensive single-cell sequencing readouts. For these projects, preliminary data generated by Oncode base funds served as the basis for my successful NWO-Vidi application (2021) (used as main funding for project C). In collaboration with the De Wit group (Netherlands Cancer Institute and former Oncode PI), we published a paper describing, for the first time, intertumoral heterogeneity on the level of the epigenome driving tumorigenesis. More specifically, chromosome conformation capture experiments revealed patient-specific looping of distal enhancer regions with the promoter of the MYC oncogene. We also showed that this can be pharmacologically targeted (Liu, Paassen & Custers et al., Nat. Commun. 2023). We are expanding our study to other subtypes of MRT such as ATRT. In ATRT, we again find intertumoral epigenetic heterogeneity, but activating expression of oncogenes other than MYC (ongoing work)

Project C) Epigenetic heterogeneity in SWI/SNF-mutated childhood tumors.

This project is now primarily funded by my NWO-Vidi grant. We successfully applied scMultiome (gene expression + ATAC) to several SWI/SNF-mutated childhood tumors. Our unique dataset now includes rhabdomyosarcoma (n=15), synovial sarcoma (n=8), MRT (n=9) and ATRT (n=8) of primary tumors, relapses and metastases, pre- and post-treatment. Sample and data acquisition are still ongoing to further increase our cohort. Preliminary analyses indicate extensive inter- and intra-tumoral epigenetic heterogeneity, as well as signaling trajectories that might be involved in tumor growth. Functional validations of some of the identified trajectories will be performed in 2024..

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The Oncode valorization team impacted my research in several ways. Through them, Cancer Research Horizons approached me in 2022 to talk about our research resulting in an invitation to submit a CYP Therapeutic Catalyst Grant Proposal (P2022-0054) which was recently accepted for funding. In addition, the team facilitated many new academic (including Memorial Sloan Kettering Cancer Center, KiTZ Heidelberg, Yonsei University, Centre de recherche en cancérologie de Lyon, UMCU, and St. Anna Children’s Cancer Research Institute) and industry (Arima Genomics) collaborations by arranging the legal frameworks.

b) Oncode base funding has been critical for our research. As described in 2.3.1, it allowed me to continuate, finalize (DeMartino et al., Nat. Commun. 2023; Liu et al., Nat. Commun. 2023) and initiate new (based on the FFPE scRNAseq pilot) research projects.

c) Participating in the Oncode events (see 2.4.2) resulted in several new collaborations. For instance, during the Oncode Annual meeting I discussed our research results (2.3.1 – project A) with OI Jacco van Rheenen, which was the basis of a new collaboration (see 2.4.3).

2.4.2. Contribution to the Oncode community in 2023

a) I have attended several Oncode events such as the Oncode Retreat (Veenendaal), the Oncode Annual Meeting (Amersfoort), and the Oncode Annual Conference (Amsterdam). During the Annual Meeting, Jop Kind and I presented during the knowledge session organized by Oncode and KWF to secure future funding.

b) As a member of the scientific committee, together with Rebekka Schneider and Anne Rios, I helped organizing Oncode's Annual Conference..

c) My group is participating in Oncode's Patient Perspective Programme (PPP). Together with one of my PhD students, Charlotte op 't Hoog (working on project A), I participated in the Annual meeting of PPP, which was held at the Princess Máxima Center. Charlotte presented her work to the attendants during a poster session.

2.4.3. Key collaborations within Oncode in 2023

a) Jacco van Rheenen. As described above, this collaboration was initiated during the Annual meeting – and therefore started thanks to my affiliation with Oncode. It involves two projects, 1) studying the effect of taxanes on CAR-T cells and TILs in our tumor organoid/immune cell co-culture models (project A), and 2) applying the FFPE scRNAseq technology to mouse models generated by the Van Rheenen lab. Both projects are ongoing.

b) Alexander van Oudenaarden. We collaborate on lentiviral barcode tracing (Kester et al., Cell Genomics 2022 & ongoing work) and (single-cell) epigenomics technologies to map epigenetic reprogramming in pediatric cancer (Liu et al., Nat. Commun. 2023; Ganpat et al., pre-print on bioRxiv (2023)). This collaboration has started thanks to my Oncode affiliation (initially by making use of the Oncode single-cell (epi) genome sequencing facility).

c) Hans Clevers. We collaborated on a project in which we performed CRISPR screens in intestinal organoids to find transcription factors responsible for enteroendocrine differentiation (Lin et al., Science 2023). This collaboration is independent of my affiliation with Oncode.

d) Jop Kind. We collaborate with the Kind group to show proof-of-principle of their MISC-seq technology using our MRT organoid and orthotopic transplantation models (Tech Dev Fund project (P2021-024)). This collaboration has started thanks to my Oncode affiliation. As part of this collaboration, we aim to characterize the niche in which primary tumors and metastatic clones thrive. The project is still ongoing.

e) Monika Wolkers. This collaboration involves developing co-cultures of tumor organoids and patient-matched immune cells for immunotherapy development (project A). Together with the Wolkers group, we isolate and expand tumor-infiltrating lymphocytes of different patient tumors. In parallel, we establish organoids from the same tissue and establish co-cultures of both cell types. The project is still ongoing and started thanks to my affiliation with Oncode.

2.4.4. Major valorization achievements in 2023

a) Patient and society engagement. We participate in Oncode's PPP, which is very inspiring for me, my team, and patient partners. I facilitated visits for parents who have/had a child with cancer, where I tell them about our research. Finally, I presented at Oncode/KWF (2.4.2) and KiKa (vrijwilligersdag) fundraising events.

b) Clinical implementation. We explore(d) possibilities to initiate preclinical research-driven clinical trials. Several in vivo trials in PDX models are still ongoing.

c) Oncode-Accelerator. I am Platform lead Organoids and Program Board member. We will build large collections of well-characterized tumor organoid models. These will be linked to clinical data and made available to academia and industry to provide a platform for testing of therapeutic targets thereby accelerating development of cancer therapies.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KiTZ-Maxima Research funds	KiTZ Hopp Kindertumorzentrum Heidelberg and Princess Maxima Center funding for collaborative projects	250K	125K	2/2024	24	Main applicant
CRUK CYP Therapeutic Catalyst	Cancer Research Horizons	81,5K	75,7 K	3/2024	12	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Michael Meister	En route to better treatment of Rhabdomyosarcoma: signaling pathways, tumor organoid models and single-cell RNA-sequencing

Carl Figdor

Radboudumc

1. General information

Research Focus	Chemical Immunology, antigen presenting cells, Immunotherapy
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our research is centered around the main question how to exploit the immune system to fight against cancer. Although immunotherapy has now successfully entered the clinic still a large number of patients do not benefit. Current research is focused on the design novel nanomedicines to more effectively activate the immune system? We work on three completely novel approaches:

a. Biodegradable nanoparticles that contain tumor antigen and adjuvants to activate dendritic cells and iNKT cells in order to evoke a strong immune response. This trial was initiated in the clinic.

b. We extended our work on synthetic dendritic cells, which consist of polymer filaments on which pMHC and immunostimulants are adjoined by click chemistry techniques. These are designated 'immunofilaments' We demonstrated that these not only can stimulate in vitro but also in vivo in preclinical mouse models

c. We develop injectable immune niches, with the idea that we can create lymph node likes structure at will anywhere in the body to boost immune responses. We exploit cryogel based structures to study this in vitro and in vivo.

2.2. Major scientific achievements in 2023

a) A high impact paper (ACSnano 2023) titled: Direct In Vivo Activation of T Cells with Nanosized Immunofilaments Inhibits Tumor Growth and Metastasis. We show for the first time that immunofilaments work in vivo. The paper is a result of more than five years of work.

b) Competitive PPP/KWF grant obtained for 3 years of work to further develop immunofilaments toward the clinic in collaboration with Simmunext.

2.3 Oncode research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Technology Development Project.

This project was completed this year. It was a study to get further insight in the behavior of immunofilaments (IF). We studied the intracellular distribution of IF and found that a large proportion is internatized. Furthermore, we tested the biocompatibility of IF and showed that they distributed all over the body and also reached immunological organs such as spleen and lymph nodes. Finally, we also made a start on getting information of the degradability of IF as this will be of major importance when designing.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The techdev helped to get a better characterization of the IF. This is of major importance when translating this to the clinic, as especially regulatory bodies such as CMC and CBG that will have to judge on safety of the product that we develop

b) Also the help of Ian and Shobit with advice and guidance of Simmunext Biotherapeutics is invaluable.

2.4.2. Contribution to the Oncode community in 2023

a) several of my PhD and postdocs participated in Oncode activities.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Lude Franke

UMCG

1. General information

Research Focus	Functional genomics
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

In 202 we developed novel algorithms and resources to better understand complex diseases and cancer

- We published a new algorithm to identify hidden contexts that mediate the effects of genetic variants on gene expression. This is valuable, because it enables to get a better understanding in what particular situation germline genetic risk factors for different types of cancer are exerting their detrimental effect on disrupting downstream molecular pathways. For instance, maybe some of these effects only work during a specific immune stimulation, or in only a highly specific cell type. Our method, called "PICALO", is able to identify these, without the need to have any prior evidence on these contexts. Our method works by maximizing the explaining interaction variance (PICALO: Principal Interaction Componant Anlysis through Likelihood Optimization) of a set of identified eQTLs. We show in our paper (Vochteloo et al, Genome Biology 2024), that our method works successfully in both brain and blood. When applying our method to these tissues we observed that it is capable of identifying individual cell-types and immune stimulations, that have a profound effect on the effect size of eQTLs. Our method can be applied to any well-powered QTL dataset. We are currently employing it to gut eQTL datasests, and since we have made the software publicly available, we envision others will use it in other tissues as well. In the near future we will apply it to different cancer eQTL datasets, and aim tot study to what extent the effect of germline risk factors for cancer show different molecular downstream effects in cancer tissue, as compared to matching, but healthy tissue.
- We developed a new algorithm to make inferences on causal relationships between genes, using a mendelian randomization framework (Van der Graaff et al, BioRXiv 2024) that is robust to various forms of pleiotropy. We apply it to various functional genomics datasets and show that it is able to make the appropriate causal inferences between genes, proteins and metabolites. We envision these algorithms are valuable, particularly in the context of ever expanding functional genomics datasets. It opens up avenues to get a much better and detailed overview of the causal relationships that exist within individual cells.
- We progressed well on our very large-scale eQTL meta-analysis (eQTLGen) and completed full trans-eQTL analysis in over 25,000. In 2024 we will wrap up this project, and plan to make this resource available for the scientific community, as a successor to our previous eQTLGen resource (Vosa et al, Nature Genetics 2021, cited > 1000x). Currently, interim results are already being used by various other groups, since we aim to disseminate the results of our project as fast as possible. Our companion project, single-cell eQTLGen, is progressing as well. Within that project we can study in detail in what specific cell-type eQTLs are showing up. We are aiming to grow that project in the next few years substantially.
- We completed our study on how germline genetic risk factors for breast cancer, prostate cancer, colorectal cancer and skin cancer affect the well-established somatic driver genes. We observed that this is the case when using tissue specific gene regulatory networks (Urzua Traslaviña et al,. MedRXiv 2023).

2.2. Major scientific achievements in 2023

a) Discovery: Strong individual variation in how cells operate: We previously developed methodology to ascertain how genetic variation affects the wiring of individual cells. Through our single-cell eQTLGen consortium we now studied this in 1,500 individuals and observed this to be a very widespread phenomenon. This might also explain why drugs typically work well in only a minority of people.

b) Funding: VIDI for Monique van der Wijst: Team member Monique van der Wijst obtained a highly competitive VIDI grant. It enables her to expand her fundamental work on how gene regulatory networks and memory of immune cells, which also has immediate relevance for our work within Oncode.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Functional genomics interpretation of germline risk factors.

In 2023 we have worked on perform very large-scale trans-eQTL mapping in bulk and single-cells. We also developed new statistical algorithms for taking advantage of these large-scale functional genomics datasets. A major realization was that we observed that many genetic risk factors for disease change the wiring within cells, changing how different genes and proteins work together. We aim to follow-up this in more detail to determine to what extent this might also partially explain why drugs typically work in only a subset of individuals who get such drugs prescribed. In 2024 we will release several resources to the scientific community that describe these effects. We expect these resources to be used by a large number of researchers, and that they help to get insight into the molecular downstream consequences of many genetic variants that have been found through genome-wide association studies.

Project B) In vitro high-throughput functional follow-up of individual variants.

We have been working on setting up experimental essays to follow-up individual genetic variants for whom we have observed that they are changing the wiring of individual cells. We have been able to make substantial progress on this and are expecting results on their proper functioning in the next couple of months. Later this year we also hope to get the first results of our base editing experiments, where we use single-cell RNA-seq as a read-out.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode has enabled us to pursuit our fundamental work on understanding how genetic variation has molecular consequences. We have been able to observe that germline genetic risk factors for cancer converge, through tissue-specific networts, on somatic mutations that confer risk for these tumors. This indicates that different classes of genetic variation are linked to each other, and that different inherent properties also explain why these variants typically act in different types of genes.

b) Our collaboration on non-coding somatic mutations with the teams of Bas van Steensel and Jeroen de Ridder has led to a considerable interest in how sequence-based models can be of use. We would not have been able to do so without Oncode, because through Oncode we have gotten in touch with each and got this project going.

c) Together with several Dutch pharmaceutical companies we are now scaling up our fundamental work on eQTLs to apply it to cancer samples as well. Oncode Accelerator has been instrumental in bringing us in touch with these companies.

2.4.2. Contribution to the Oncode community in 2023

a) Lude Franke has been a grant reviewer for KWF.

b) We have participated in several Oncode community events

c) Lude Franke gave a lecture on data visualization to a large number of Oncode investigaters.

2.4.3. Key collaborations within Oncode in 2023

a) We have been very actively participating with Bas van Steensel (NKI) and Jeroen de Ridder (UMCU) in the context of non-coding genetic variation and the application of sequence-based models to make inferences on the molecular consequences of private and (ultra) rare genetic variants.

b) Together with the team of Jeroen de Ridder we have started work on applying variational autoencoders to large-scale bulk RNA-seq datasets, to ascertain to what extent these methods can help to better understand gene expression variation.

2.4.4. Major valorization achievements in 2023

a) We engaged in a new public private collaboration with Roche and Biogen on single-cell eQTL mapping in brain samples..

b) X

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Roche + Biogen	Public private partnership	€350,000	€350,000	12/2023	18	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Laura Heitman
Leiden University

1. General information

Research Focus	GPCR, allosteric modulation, target binding kinetics, small molecules
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

I focus on the theme "Novel receptor concepts to target membrane proteins" with the ultimate aim to make medicines work better. Thus, having the potential for a large societal impact. I have selected membrane-bound proteins, such as G protein-coupled receptors (GPCRs), as many drugs act via these and they play a pivotal role in disease. I have been able to develop an array of novel *in vitro* equilibrium and kinetic binding assays, as well as (label-free) functional assays to investigate "Novel Mechanisms of Action" (i.e. concepts) to target membrane proteins, such as target binding kinetics and allosteric modulation. My research can basically be split in two lines: 1) Intervening with receptor function by small molecules, and 2) Understanding (variant) receptor pharmacology.
To this end, we combine and integrate different expertise and research domains relevant for modern future molecular pharmacology, such as receptor pharmacology, molecular and cellular biology, organic chemistry and computational chemistry. Here the implementation of future key technologies and methodologies is crucial, such as high throughput kinetic binding and signalling assays, both at cell membranes and on living cells. My team collaborates with crystallographers, cell biologists and in vivo pharmacologists, both from academia and pharmaceutical industry. Of note, medicinal chemistry research in academia typically has few, if any, links with the clinic, but the concepts that I work on have a large translational potential. Hence, I keep a keen eye on collaborating within Oncode, but also with research hospitals and pharmaceutical companies to go from an idea to proof-of-concept and continue to make my research translational in nature, next to being fundamental.

The concepts that I work on are in principle 'disease-agnostic', i.e. the proteins that I have selected have a place in many diseases. However, I am applying (part of) my research lines to cancer. This is actually quite logical, as it is becoming clear that GPCRs and their subsequent signalling mechanisms play an important role in regulating cellular functions, which are related to known hallmarks of cancer. Notably, GPCRs are not only (over-)expressed on cancer cells themselves, but also on cell-types in the tumour microenvironment, including cancer-associated fibroblasts and inflammatory cells. Moreover, recent work has shown that GPCRs present in patient isolates are sensitive to mutation, i.e. GPCRs are mutated in an estimated 20% of all cancers. In addition, we and others have seen that GPCRs can have a wide variety of post translational modifications (PTMs) such as N-glycosylation and phosphorylation, where these PTM "patterns" differ in various cell backgrounds. This fuels further interest in the role of GPCRs in tumour biology.

The main scientific questions are:
1. Which kind of chemical modality, e.g. allosteric, covalent, is best suited to efficaciously target a protein?
2. Does in vitro optimization of a drug's mechanism of action benefit in vivo efficacy?
3. Where is the drug target expressed and in what form?
4. How does a mutation or PTM affect a drug target's pharmacology? And does this alter its druggability?

2.2. Major scientific achievements in 2023

a) I published in Nature Communications on the resolving several cannabinoid CB2 receptor structures with different agonists together with Profs Mario van der Stelt and Tian Hua (PMID: 36922494). Combined with mutagenesis, functional and binding kinetics experiments this work enlightened the molecular mechanism for CB2R activation by selective agonists from different chemical classes. Moreover, it showed a role of ligand lipophilicity in CB2R engagement, which may have implications for GPCR drug design.

b) I co-organized the first workshop on small molecule protein degraders (e.g. PROTACs) together with Prof Alessio Ciulli from the University of Dundee (i.e. he is one of the first scientists to discover protein degradation as an avenue for drug discovery). This course was fully booked and a great success, which led us and the invited lecturers to write a paper about it (PMID: 37817354).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Functional characterization of (mutant) GPCRs in breast cancer a focus on adenosine A2A and serotonin 5HT2C receptors.

- A1: Functional characterization of wild-type and mutant adenosine A2A receptor (A2AAR) in breast cancer
  The effects of cancer-associated A2AAR mutations on ligand binding and receptor functionality were characterized and the results was published in 2022. As the follow-up research, in 2023 we continued to investigate the role of A2AAR in breast cancer models, focusing on the effects of wild-type and mutant receptors on cell proliferation and the response to receptor agonists/ antagonists. We found there were multiple factors that could influence the cellular response to A2AAR activation/inhibition, such as the endogenous expression level of A2AAR and receptor subtype selectivity of compounds.
- A2: Characterization of cancer-associated mutations of serotonin 5-HT2c receptor
  We retrieved missense mutations of 5-HT2c receptor among cancer patients from the Genomic Data Commons Data Portal. A list of 12 mutations were chosen for further investigation. Wild-type and mutant 5-HT2c receptors were over-expressed in mammalian cell lines. Radioligand displacement binding assays were used to determine the affinity of ligands at different receptor variants. Whole cell-based calcium flux assays were used to characterize receptor activation and inhibition. We found that cancer-associated 5-HT2c mutations have diverse effects. Mutations in the orthosteric binding pocket tend to affect ligand binding affinity and indirectly affect receptor functionality. Mutations in the conserved motifs are likely to cause change in receptor conformation and lead to subsequent changes in ligand binding. The druggability of a mutant receptor were determined by several factors including the relative change in affinity of the agonist versus the inhibitor, and the constitutive activity of the mutant. Our study provides insights into cancer-associated 5-HT2C mutations on receptor pharmacology level, and invites further research to reveal the effects of mutations on cancer cell proliferation and metastasis, which will facilitate drug discovery and personalized medicine. The manuscript has been written and is under internal revision.

Project B) Artificial intelligence and structure-based approaches to characterize the role of selected membrane proteins in cancer

- B1. Developing 3D dynamic protein descriptors (3DDPDs) for bioactivity prediction in GPCRs
  An open-source python package has been published on GitHub to compute 3DDPDs for GPCRs. The complete development of these protein descriptors and the analysis of their performance in proteochemometric bioactivity prediction models, as well as in mutant GPCR description, has been published in the Journal of Cheminformatics in August 2023.
- B2. Investigating the effect of cancer-related mutations in glutamate transporter SLC1A3/EAAT1 structure and ligand binding
  The results from this project where we analyzed the effect of cancer- and ataxia-related EAAT1 mutations in protein structure, function, and pharmacological intervention were published in Frontiers in Molecular Biosciences in November 2023.
- B3. Annotating mutant bioactivity data from ChEMBL database to perform proteochemometric modelling in RTKs
  We revised and expanded our mutant annotation strategy upon talks with staff at ChEMBL responsible for variant annotation. Moreover, we expanded the analysis strategy to comprehensively explore the coverage and effect of mutant data on the ChEMBL database at different levels, including protein families, organisms, individual proteins, and variants. We focused the analysis on EGFR and three other proteins of different families. We found for these examples that our analysis pipeline is very successful at portraying the effect of variants in bioactivity changes across different areas of the chemical space. On top of this, we developed an open-source Python package with easily reusable notebooks to facilitate the analysis of any protein of interest to the user. This tool can be very useful in hypothesis generation and data preprocessing for modelling. We also modelled all annotated proteins using machine learning to predict bioactivity. We found that taking mutants into account in modelling is crucial to improve model performance. These results have been condensed in a manuscript and are currently under internal revision for submission.
- B4. Constructing a knowledge graph to prioritize RTK mutations to target selectively
  We designed the structure of a complex network that integrates several layers of knowledge that can be exploited to prioritize RTK mutations to increase selectivity. These layers include a phosphorylation network, cancer-related mutations, differential expression in different cancer types, structural information, and mutant-derived bioactivity differences (from project B3). We extracted data for each of these layers from public databases and we are currently defining the best connection types for the knowledge graph.

Project C) Design and characterization of insurmountable antagonists for GPCRs – a case for CCR2, A2aR and CCR5.

- C.1 Design and synthesis of CCR2 PROTACs
  Various PROTACs that target CCR2 for degradation were designed and synthesised. The affinity of the potential PROTACs was evaluated using membranes from cells expressing CCR2 and previously characterized using radioligand binding assays. Furthermore, we used a label-free functional assay (xCELLigence) to determine indirectly the permeability of the developed PROTACs through measurement of CCR2 inhibition in a whole-cell assay. Finally, luminescence-based HiBit assays were carried out to evaluate the degradative capacity of the designed PROTACs. in live-cells over 24 hours. The final assays are being carried out to further elucidate the mechanism of action of selected PROTACs and the manuscript is currently in preparation.
- C.2 Design and synthesis of novel allosteric CCR5 ligand
  Novel selective ligands targeting the intracellular allosteric pocket of CCR5 were designed using an approach combining computational and organic chemical techniques. Initially, a combination of docking, molecular dynamics and virtual screening studies identified a potential ligand for CCR5. Next, nineteen derivatives were designed based on the initial hit. Synthesis of the derivatives was successful. The binding affinity for CCR5 was evaluated in a β-arrestin functional assay. Binding of the ligands to CCR2 was also evaluated using a radioligand binding assay to determine the selectivity of the synthesized ligands. This was done since CCR2 and CCR5 have high percentage of homology. The evaluations identified a potentially selective ligand. The results of this study are currently being prepare in a manuscript.
- C.3 Design and synthesis of PROTACs targeting the A2aR
  Within this project we pursue a novel method to degrade a membrane bound GPCR. By combining an agonist for the GPCR of interest and an E3 ligase (CRBN) ligand, we envision that the agonist induced internalization will be followed by proteasome-mediated degradation. Within this project we focused on the A2AR for a proof of concept. Linkers of varying lengths and chemical compositions were introduced to create an initial library of A2AR PROTACs. The library is currently being synthesized. Upon completion of the initial library, we will carry out radioligand binding, western blotting and ELISA assays to evaluate the binding affinity to A2AR and the degradative capacity of the novel compounds.
- C.4 Design and synthesis of orthosteric CCR2 covalent ligands
  Within this project we aim towards developing a covalent CCR2 ligand that binds the orthosteric pocket. We modified a previously discovered CCR2 orthosteric ligand to include a covalent warhead. We performed docking studies on the known ligand to accurately to design an initial library of covalent CCR2 ligands. An initial library of 12 compounds is currently being synthesized. The binding affinity and potential covalency of the initial library will be evaluated using single and full curve radioligand displacement assays. Discovery of a novel covalent CCR2 orthosteric ligands can be a starting point to create molecular tools such affinity-based probes to further unravel the expression and function of CCR2.

Project D) Novel GPCR targets and compounds for TNBC treatment.

- D1. Phenotypic screening of repurposing library on TNBC cell lines
  Drug repurposing (also known as drug repositioning or drug reprofiling) is the application of an existing therapeutic to a new disease indication, and holds promise of rapid clinical impact at a low cost. Additionally, several drugs in preclinical and clinical phases have been repurposed for TNBC via target-based repurposing methods. Strikingly, phenotypic screening seems still lacking in the drug repurposing activities. The “Drug repurposing library”, collected and distributed by Oncode Institute, contains more than 6000 drugs in various stages of clinical development (FDA-approved, clinical, pre-clinical trials). This library provides us with the possibility to rapidly identify novel indications for known drugs and compounds with potential effects on the target of interest. By screening of this compound library on cancer cell proliferation, we might be able to identify drugs to have a novel indication in TNBC.
- D2. Phenotypic screening of GPCR ligand library on TNBC cell lines
  Altered expression of GPCRs has been detected in several TNBC cell lines. Therefore, GPCRs may contribute to new generation of diagnostic tools and therapeutic options for combatting TNBC. We purchased a GPCR compound library with a unique collection of 1325 small molecules targeting GPCRs, focusing on not only on well-studied GPCRs, but also orphan GPCRs with unknown functionality. This library has been used in research on various cancer types. All of the compounds have been well-characterized with biological and pharmaceutical activity. We performed phenotypic screening of this compound library on cancer cell proliferation and migration. We also investigated cytoskeleton rearrangement upon compound treatment. We aim to identify GPCR-targeting drugs with potential regulatory effects in TNBC.
- D3. Establishment of stable FRET reporter TNBC cell lines
  Both GPCRs and post-GPCR signalling mechanisms have been shown to play a role in regulating cellular functions integral to the hallmarks of cancer. In this project, we established stably expressing fluorescence resonance energy transfer (FRET)-based biosensors in TNBC cell lines for second messengers, cAMP, Rho GTPase or Ca2+. We validated these reporter cell lines on cell proliferation and migration.

Currently, we are investigating their capability on reporting the receptor signalling of endogenous GPCRs. By using the FRET biosensor imaging technique, we will be able to investigate GPCR signalling networks in TNBC cells.As a follow-up of D2, hit compounds obtained will be further characterized on their GPCR-mediated signalling pathways using these FRET reporter cell lines.

Project E) Investigating the effect of post-translational modifications (PTMs) and protein-protein interactions (PPIs) on CCR2 functioning.

- E1. Development of a CCR2-targeting affinity-based probe
  A CCR2-targeting small molecular probe has been developed that allows detection of endogenous CCR2 on various cell lines, possibly tissues, in a variety of biochemical assays, such as SDS-PAGE (Western Blot) and proteomic pull-down experiments.
- E2. Utilization of the above-mentioned affinity-based probe in biochemical assays
  Assay optimization has been performed in which the optimal conditions were investigated for the detection of the CCR2 with the above-mentioned chemical probe. These assay will be utilized in the future for further characterization of PTMs and PPIs of the CCR2, and their effect of the CCR2-induced intracellular signaling pathways

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) New network: Oncode gave me the opportunity to translate my fundamental research to (pre-)clinical disease models. Specifically, I can obtain proof that my 'molecules' or concepts can make a difference in a clinically relevant setting, as well as select new GPCRs that seem important to target based on (clinical) expression data available within the Oncode network.

b) Business development: I have monthly contact with my Oncode Business developer (Alexander Turkin) during which we discuss ongoing activities: 1) novel ligands, 2) novel targets, 3) grant opportunities. Moreover, the Oncode business development team provides support in contacting and negotiating with new industrial partners. Lastly, several new probes (in-house synthesis programs) have been licensed by HelloBio, who now sell our ligands to the community. Lastly, it offers support in grant applications

2.4.2. Contribution to the Oncode community in 2023

a) Novel drug targets: Unlock importance of GPCRs as 'anti-cancer' targets.

b) Mutant drug targets: Explore the impact of somatic mutations in cancer on GPCR function, and thus intervention.

c) Novel concepts to target GPCRs in cancer using small molecules, e.g. molecules that have been kinetically optimized or bind at non-competing (allosteric) binding sites, or are designed to degrade the GPCR (i.e. PROTACs).

d) Novel methods to detect GPCRs in cancer, such as the development of small molecular probes. Such compounds can function as tool compounds to aid investigations of GPCRs that play a role in cancer.

2.4.3. Key collaborations within Oncode in 2023

a) Ovaa-lab (part of my network before Oncode): Discovery and development of CCR2 PROTACs. Initial compounds were synthesized in the Ovaa-lab, after which further synthesis and biological evaluation was done in my lab. Currently, we are writing the paper with Monique Mulder, who took over this project from Huib after his passing.

b) Mario van der Stelt (part of my network before Oncode): continues collaboration on the development of novel CB2R ligands and probes.

c) Karin de Visser (new connection): A postdoc in Karin's lab has examined GPCR expression in the bulk RNAseq dataset of tumors and their different transgenic mouse models. This resulted in a heatmap, showing that the GPCR expression pattern is quite different between the models. Currently, we are deciding which GPCR should be prioritized and what experiments can be done.

d) Michiel Vermeulen (new connection): Michiel Vermeulen has recently development a protein conjugate, ProtA - Turbo-ID (PMID: 34408139, PMID: 36224470), for the biotinylation of proteins interacting with any antibody of interest. We would like to utilize this concept for the investigation of all proteins that are interacting with the CCR2. Therefore we are currently looking into the production of Turbo-ID conjugates, coupled to our in-house developed probes, to bind CCR2 and label all CCR2-interacting proteins for detection.

e) Jos Jonkers (new connection): The DRP screen in TNBC cell lines is yet to start, but once we have hits, we will make use of 3D tumor spheroids and TNBC PDX models to test and validate the efficacy of the selected candidates

2.4.4. Major valorization achievements in 2023

a) We have established a license with HelloBio to sell several of our in-house compounds targeting GPCRs; 5 covalent ligands and 3 clickable probes.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Jan Hoeijmakers

Erasmus MC

1. General information

Research Focus	DNA repair
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our group studies genomic instability and its consequences for cancer and aging: the main healthcare problems in all developed societies. DNA damage (DD) occurs continuously in every cell, at massive scale. It leads to mutations that initiate and fuel carcinogenesis including onset of therapy resistance frustrating effective cure. DD also triggers cell death, senescence and interferes with genome function causing functional decline and aging-related diseases. We discovered that DD is a main cause of aging and predicted that DNA-damaging chemo- and radiotherapy would accelerate aging, later confirmed in long-term cancer survivors. Previously, we found that dietary restriction and fasting induce a surprisingly powerful protective 'survival response', which suppresses growth and prioritizes resilience mechanisms, reduces DD load, delays aging and provides acute protection from surgery-associated ischemia/reperfusion-injury and chemo/radiotherapy. By studying molecular mechanisms and mouse models for human DNA repair syndromes, generated in our own team, we intend to reach full understanding of this response, derive rational-based effective nutritional and pharmacological strategies, that promote overall healthy aging and -within ONCODE- reduce severe short/long-term side-effects in children/adults treated for cancer and hence significantly improve daily quality of life. Particular interest is in cognitive decline as we found neurofunctioning to benefit disproportionally from nutritional interventions. Importantly, these nutritional interventions are easily implementable and can be applied to all cancer patients, globally

2.2. Major scientific achievements in 2023

a) Winnie van den Boogaard, our first Máxima PhD graduate, defended her thesis on the effects of short-term fasting in cells, organoids, mice and men to reduce chemotherapy-induced toxicity in pediatric oncology. The results will contribute to developing protocols for nutritional preconditioning prior to chemotherapy to reduce chemotherapy-induced side effects.

b) Genome-wide accumulation of stochastic endogenous DNA damage during aging physically stalls RNA polymerase, lowering and skewing transcriptional output in a gene-length-dependent manner, which largely determines the age-related transcriptome and affects key aging hallmark pathways, disclosing on how DNA damage functionally underlies major aspects of normal ageing. (Nature Genetics: https://doi.org/10.1038/s41588-022-01279-6)

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Pre-treatment fasting as a means to prevent chemotherapy-induced side effects.

Although improving survival, chemotherapy also causes numerous features of accelerated aging, impacting quality of life (Van den Boogaard, Komninos, and Vermeij 2022 Cancers). Dietary restriction and fasting induce a highly conserved 'survival' response, which potentiates resilience and stress resistance and delays aging. We examine whether -counterintuitively- fasting prior to chemotherapy reduces side-effects. Fasting prior to cisplatin treatment prevented the loss of appetite and body weight of young ad-libitum-fed mice , and strongly enhanced survival: whereas all ad-libitum-fed animals succumbed in 6 days from high-dose cisplatin, 50% of fasted mice survived >6 months. Nephro- and hepatotoxicity, including tubule deformation, -necrosis and karyomegaly, were alleviated by pre-treatment fasting. Interestingly, gene expression analysis of kidneys three days after cisplatin treatment revealed a senescence-like response, which appears exacerbated but also more transient by pre-treatment fasting. Although senescence is generally associated with aging pathology, an acute senescence-like response enhanced by pre-therapy fasting may, paradoxically, contribute to protection from chemotherapy-induced toxicity and accelerated aging, and promote survival. Additional experiments with doxorubicin and irinotecan yielded similar positive effects strongly supporting nutritional preconditioning in cancer care, involving unexpected benefits of a transient senescence-like response.

Project B) Measuring functional metabolism in pediatric oncology

Metabolism is frequently altered in cancer (e.g., the Warburg effect, the tendency to overuse glycolysis at the expense of oxidative phosphorylation) and chemotherapy and nutrition (such as short-term fasting) could differentially influence metabolism in cancer and healthy tissues. Monitoring the response to each of these treatments using Seahorse metabolic read-outs in various cell types helps understanding the interplay between DNA damage induction and metabolism. We monitored functional metabolism parameters in various pediatric cancer cell-lines and patient cells (of hematological, solid tumor and neuronal origin, as well as sensitive versus resistant) to discover energy source dependency of specific pediatric cancers and thereby identify new metabolic targets for precision medicines and/or classes of patients which might benefit from targeted nutritional interventions. Most canonical chemotherapy drugs are included in this study. Samples with/without nutrient restriction-mimicking *in vitro* culture conditions are treated with chemotherapeutics and measured in real-time to determine metabolic differences.

Project C) Dissecting the effect of DNA damage, DNA repair deficiency and nutrition on transcription and translation stress.

Previously, we have developed technology and bioinformatic pipelines to analyze the effect of aging on transcription, and found that transcription declines with aging in mouse liver, and particularly long genes (Vermeij WP et al 2016 Nature). We demonstrated that this age-related gene-length-dependent transcriptional decline is directly caused by accumulating stochastic DNA damage physically hampering elongation. This novel aging-associated phenomenon was also discovered in human aging brain (including Alzheimer disease), aging kidney in mice and even in *C.elegans*, demonstrating its universal occurrence. Transcription stress explains >50% of all aging-related transcription changes in numerous organs and tissues, which are largely post-mitotic, and hence will accumulate DNA damage in time (Gyenis A et al 2023 Nat Genet). The affected pathways include virtually all previously identified 'hallmarks of aging', revealing its enormous impact. Strikingly, this response was largely prevented by dietary restriction (DR), which also delays aging, explaining the anti-aging effect of DR, which has been

mysterious since its discovery almost a century ago. We are now extending this to the level of translation by ribosome profiling technology and analysis pipelines to explore how DNA damage, aging and dietary restriction influence various aspects of translation.

Project D) Fasting intervention for children with Unilateral Renal Tumors to reduce Toxicity.

Renal tumors represent ~7% of all childhood cancers. The majority (90%) are Wilms tumors. Current treatment is pre-operative chemotherapy followed by surgery (SIOP-RTSG-2016 UMBRELLA protocol). For Wilms tumors, this has resulted in excellent prognosis with an overall five-year survival approaching 90%. Priority is now to decrease toxicity, which has severe long-term side effects and affects quality of life (QoL). Dietary restriction (DR; reduced food intake without malnutrition) is associated with extended lifespan, lower risk of age-related diseases, improved fitness, and increased resistance to acute stress. DR and short-term fasting (STF) also reduce short- and long-term side effects of chemotherapy, increases expression of cytoprotective genes and boosts immuno-modulation via anti-inflammatory cytokine production. In recent clinical trials, nutritional preconditioning has been proven feasible and safe in well-nourished adult patients before surgery. We initiated a clinical study on preoperative fasting to improve recovery of postoperative renal function and reduce the incidence of Acute Kidney Injury. As surgery is an important part of renal tumor treatment, we are conducting nutritional preconditioning at PMC to determine feasibility and improve patients' recovery and therapy.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The Oncode support and its expertise were/are crucially important to achieve our missions. It provides manpower and means for fundamental research deciphering the cause of transcription stress, a stochastic process impairing longer genes more than shorter genes, that further supports the DNA damage accumulation theory of aging and provides insight into the short- and long-term side-effects of chemo-, radiotherapy. In addition, it enabled preclinical research on translation of basic insights on the effect of nutritional preconditioning to cancer treatment (surgery and chemotherapy) as a prerequisite for application in the clinic.

b) Oncode inspired, stimulated, enabled, helped, and facilitated. The Oncode Patient Engagement brainstorm session and patient evening meeting on Nutrition and Cancer has inspired us to join the Patient Engagement Program. Currently, we have two childhood/adolescent cancer survivors coupled to our group and have regular contact with them. They gave valuable feed-back, inspired our research team and helped us with disseminating results.

c) Scientifically, our research has yielded important insights into mechanisms of repair and the biological impact of DNA damage: most recently, the connection with aging (i.e., the main risk factor for cancer) and the unexpected potential of nutritional interventions. The latter aspects, which globally get increasing attention with >200 clinical trials ongoing are unique within Oncode and provide realistic opportunities for direct clinical translation for improved cancer treatment and long-term benefit for virtually all (ex-)cancer patients.

2.4.2. Contribution to the Oncode community in 2023

a) Last fall, Wilbert Vermeij, senior investigator in my team, has co-organized together with Colette ten Hove (Oncode), the Annual Patient Engagement Meeting. They brought together patients and researchers for a pleasant and fruitful day of presentations and networking within the Princess Máxima Center. Special focus was placed on young researchers to further improve their informing skills and make research accessible to a non-scientific audience.

b) Last spring, two members of my team, Ziqin Tang and Wilbert Vermeij, organized a Seahorse Technology workshop available to all Máxima and Oncode researchers. Over 20 researchers from more than 15 research groups including Oncode affiliated groups were informed that day about cell metabolism changes, possibilities of Seahorse Technology, and e.g., its use for assessing CAR-T cell fitness.

c) As senior Oncode PI, I have adopted the task to coach several bright junior PI's by training them for critical interviews to obtain prestigious grants -hitherto with significant success- and by advising them on grant applications and other practical or strategic decisions. This includes all PMC and several EMC junior PIs. Also, I take part in internal scientific review boards to improve project applications for KWF and KiKa and chair the internal scientific integrity committee.

2.4.3. Key collaborations within Oncode in 2023

a) Together with Oncode PIs Jurgen Marteijn, Michiel Vermeulen, and Titia Sixma and former Oncode PI Wim Vermeulen, we identified DDA1 as novel factor in transcription-coupled repair and unraveled the dynamic molecular interation with CRL4-CSA and regulation of this complex at DNA damage-stalled RNA polymerases. DOI: 10.21203/rs.3.rs-3385435/v1

b) Given the high toxicity of Doxorubicin, we explored together with Oncode PIs Sjaak Neefjes and Jarno Drost, if two orthologous Aclarubicin and Dimethyl-doxo could equally well be used for killing of pediatric renal tumors while yielding lower toxicity using patient derived Organotypic Tissue Slices and Organoids of both tumor and healthy tissue. Additionally, the different Anthracyclines were tested on a panel of DNA repair-deficient patient fibroblasts to further explore their mechanisms of action and capacity of inducing DNA damage. This collaboration illustrates the potential of working together based on complementary expertise.

c) As dietary restriction and fasting can induce a very powerful protective response in healthy tissues protecting against stress, we also explored together with Máxima PI Stefan Nierkens and Oncode/Máxima PI Anne Rios if such nutritional intervention could benefit the production of CAR T cells. Isolated T-cells from three different donors indeed turned out to become better activated and thus likely more potent in killing tumor cells.

2.4.4. Major valorization achievements in 2023

a) Within our ongoing clinical trial for children with a renal tumor (FIURTT-study) we noted that fewer patients were eligible for participation in our study. The main reason seemed that many tumors were more advanced presumably as a consequence of Covid19-related issues. Therefore, we have shifted our current focus to the first included patients to show that fasting is safe and feasible for children with cancer.

b) Previously, we revealed enormous clinical benefit from reducing calorie intake in the first patient suffering from the DNA repair syndrome trichothiodystrophy (TTD) with spectacular improvements in her overall health condition, most impressively her neurological performance (cognition, motor capability, disappearance of tremors), enormous improved quality of life and unquestionable lifespan extension (now >5,5 years she is in stable health, and doing extremely well). Although in the beginning highly controversial, the nutritional guidelines for children with defects in transcription-coupled repair, TTD and Cockayne syndrome (CS) have been completely revised based

on our results: reduce instead of increase calorie intake in these severely growth-retarded patients. This is done in close consultation with parents, parent family organizations (Amy&Friends, UK, NL, Em'ma Vie, Fr), dieticians, clinicians, and researchers. Currently, we are engaged in clinical application of our findings and in measuring the energy expenditure and demands of these children and improve our understanding of the underlying mechanisms.

c) In addition, in the framework of an international study we explore the translation of nutritional interventions to other genome instability disorders, such as Fanconi anemia, Ataxia telangiectasia and others. Since we found that calorie restriction lowers the DNA damage load, we predict beneficial potential for these devastating conditions as well.

d) Also, as spin-off from our findings of the importance and enormous benefit of appropriate feeding for TTD and CS, we have initiated a multi-disciplinary expertise center for genome instability disorders at the Erasmus MC, which is now officially recognized. Regularly, we participate in evaluating and advice on treatment of patients from within and outside our country who visit with their parents our clinic.

e) Finally, besides (0nline) presentations and seminar for various scientific conferences and research departments, we also frequently give lectures to the general public (local societies, students form high schools), as well as patient family organizations about our research, participate in courses for undergraduate, graduate, PhD students and postdocs, retreats of research groups, etc. Moreover, we appear in articles on our findings or on scientific developments that attract general attention in non-scientific journals or other public media.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
EU Marie Sklodowska-Curie	COFUND; Máxima Butterfly *(jointly with o.a. various Oncode PIs)*	3.7 M *(€)*	150 K *(€)*	09/2023	48	Co-applicant
NWO	NWA-ORC; CureQ	5.5 M *(€)*	345 k *(€)*	05/2023	48	Co-applicant
ZonMW	Multidisciplinaire Consortia van het Onderzoeksprogramma Dementie; MODEM	6.25 M *(€)*	280 k *(€)*	09/2023	48	Co-applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NL9422	Fasting Intervention for children with Unilateral Renal Tumours to reduce Toxicity (FIURTT)	22-04-2021		Yes	Lead
NL9092	KETOgenic diet therapy in patients with HEPatocellular adenoma (KetoHeppy)	01-02-2021		Yes	Co-PI
NL9262	Fasting before live kidney donation, effect on donor wellbeing and postoperative recovery (FAST)	01-03-2021		Yes	Lead
NL9262	Living kidney donation and the immune-modulatory effect of fasting (FAST-IMMOLATE)	15-09-2021		Yes	Co-PI
NL7657	Resting energy expenditure in children with cancer (ENERGICE)	11-04-2019		Yes	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Winnie van den Boogaard	Nutritional interventions as a means to reduce chemotherapy-induced toxicity in paediatric cancer patients

Jos Jonkers

Netherlands Cancer Institute

1. General information

Research Focus	Mouse models of breast cancer
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

My group studies human breast cancer development and progression, as well as therapy response and resistance, in genetically engineered mouse models (GEMMs) and patient-derived tumor xenograft (PDX) models. We have developed mouse models for BRCA1/2-associated breast cancer and invasive lobular carcinoma (ILC), which are used to (1) study tumor cell-intrinsic and -extrinsic mechanisms of breast cancer development and progression; (2) develop novel therapeutic strategies for prevention and treatment of breast tumors; (3) study mechanisms of acquired resistance to targeted therapeutics.

2.2. Major scientific achievements in 2023

a) Hutten et al. A living biobank of patient-derived ductal carcinoma in situ mouse-intraductal xenografts identifies risk factors for invasive progression. Cancer Cell 2023; 41(5):986-1002.e9.

Ductal carcinoma in situ (DCIS) is a non-obligate precursor of invasive breast cancer (IBC). Due to a lack of biomarkers able to distinguish high- from low-risk cases, DCIS is treated similar to early IBC even though the minority of untreated cases eventually become invasive. To study the biology of DCIS, we generated 115 patient-derived xenograft models of DCIS. Using these models, we could identify multiple prognostic factors for high-risk DCIS.

b) Mazouzi et al. FIRRM/C1orf112 mediates resolution of homologous recombination intermediates in response to DNA interstrand crosslinks. Science Advances 2023; 9(22):eadf4409.
Resolution of DNA interstrand crosslinks (ICL) requires the coordination of various DNA repair mechanisms. We identified FIRRM/C1orf112 as a novel and essential factor in in the response to ICLs. FIRRM promotes resolution of homologous recombination (HR) intermediates and thus plays a critical role in the response to ICLs encountered during DNA replication.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Truncated FGFR2 is a clinically actionable oncogene in multiple cancers.
We have previously shown that truncation of fibroblast growth factor receptor 2 exon 18 (FGFR2-E18), caused by focal FGFR2 amplifications or rearrangements, acts as a potent driver mutation in cancer (Zingg et al., Nature 2022). To elucidate the mechanism by which loss of the carboxy-terminal tail (C-tail) renders FGFR2 oncogenic, we analyzed a compendium of Fgfr2-E18 variants. While permutation of previously annotated C-terminal FGFR motifs did not recapitulate the tumorigenicity of FGFR2ΔE18, our functional annotation efforts led to the discovery of a novel C-terminal phenylalanine–serine motif that mediates binding of the C-tail to the kinase domain and thereby suppresses FGFR2 kinase activity. Permutation of this kinase domain-binding and suppression (KDBS) motif in conjunction with other FGFR2-regulatory C-terminal sites fully phenocopied the oncogenic competence of FGFR2ΔE18, thus delineating how the C-tail prevents FGFR2 from aberrant oncogenic activation (*Zingg et al, manuscript submitted*).

Project B) MYC promotes immune-suppression in triple-negative breast cancer via inhibition of interferon signaling.
We previously found that MYC overexpression suppresses inflammatory signaling induced by BRCA1/2 inactivation (*Zimmerli et al., Nature Communications 2022;13:6579*). We are currently investigating the mechanisms underlying MYC-dependent and MYC-independent immune evasion in BRCA1-deficient triple-negative breast cancer (TNBC). In addition, we are investigating if we can therapeutically intervene with immune evasion of TNBCs to enhance ICI efficacy. For this, we are running preclinical tumor intervention studies with combinations of PARPi, ICI and STING agonists.

Project C) Targeting resistance to PARP inhibitors.
To investigate which BRCA1/2-independent mechanisms drive PARP inhibitor (PARPi) resistance in vivo, we combined molecular profiling with functional homologous recombination (HR) analysis of matched PARPi-naive and PARPi-resistant mouse mammary tumors harboring large intragenic deletions that prevent reactivation of BRCA1/2. HR restoration occurred in 62% of PARPi-resistant BRCA1-deficient tumors but none of the BRCA2-deficient tumors. Moreover, we found that 53BP1 loss is the prevalent resistance mechanism in BRCA1-deficient tumors with HR-restoration, whereas resistance in BRCA2-deficient tumors is mainly induced by PARG loss (*Bhin et al., Cell Reports 2023; 42(5):112538*). Using functional screens, we identified EXO1 and FEN1 as major synthetic lethal interactors of PARPi-resistant BRCA2-deficient tumors with PARG loss. EXO1/FEN1 targeting may be a useful strategy for treating PARPi-resistant tumors that have lost PARG activity, or for enhancing the effect of PARG inhibitors in HR-deficient tumors (*Andronikou et al., EMBO Journal 2024; online ahead of print*).

Project D) Exploring novel genetic interactions in the DNA damage response.
Together with Thijn Brummelkamp, we are exploring novel synthetic lethal interactions in the DNA damage response network. Using complementary genetic screens, we identified FIRRM/C1orf112 as an indispensable factor in in the response to DNA interstrand crosslinks (ICLs). FIRRM deficiency leads to hypersensitivity to ICL-inducing compounds, accumulation of DNA damage during S-G2 phase of the cell cycle, and chromosomal aberrations. In addition, FIRRM is recruited to ICLs, controls MUS81 chromatin loading, and thereby promotes resolution of HR intermediates. Thus, FIRRM plays a critical role in the response to ICLs encountered during DNA replication (*Mazouzi et al., Science Advances 2023; 9(22):eadf4409*). To identify novel modulators of PARPi response, we performed drop-out screens in BRCA1/53BP1 double-knockout HAP1 cells exposed to PARPi. This screen yielded several novel candidates, which are currently being validated and functionally characterized.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode has stimulated interactions and collaborations with other Oncode groups. We have active collaborations with 10 Oncode groups (Thijn Brummelkamp, Peter ten Dijke, Roland Kanaar, Puck Knipscheer, Sylvie Noordermeer, Jacco van Rheenen, Titia Sixma, Karin de Visser, Lodewyk Wessels and Wilbert Zwart).

b) Oncode base funding enabled us to investigate PARP inhibitor resistance and explore novel interactions in the DNA damage response network. This work has resulted in 3 publications in 2023 and 2024 (Bhin et al, Cell Rep 42:112538; Mazouzi et al, Sci Adv 9:eadf4409; Andronikou et al, EMBO J, online ahead of print).

c) The Oncode Valorization Team has been helpful in establishing research collaborations with Artios Pharma on the development of polymerase theta inhibitors; with UCB on the development of Gremlin1 inhibitors; and with Foundation Medicine, Incyte and Debiopharm to study FGFR2 rearrangements in human cancers.

d) The Oncode TechDev fund has enabled us to initiate a collaboration with Cancer Research Horizons to develop small-molecule inhibitors of DNA Damage Repair (DDR) proteins to enhance response and overcome resistance to PARP inhibitors. This collaboration has led to the establishment of a new company, which is focused on the development of DDR-targeted therapeutics.

2.4.2. Contribution to the Oncode community in 2023

a) We provide infrastructure and facilities for preclinical in vivo studies (generation of new mouse models, in vivo testing of organoids, preclinical imaging and drug efficacy studies in mice), which are used by several Oncode groups.

b) Together with Oncode, I participated in 4.UNCAN.eu, a Coordination and Support Action funded by Horizon Europe to prepare the UNCAN.eu platform.

c) Stefan Hutten gave a presentation on our breast cancer research for the Oncode/BVN/BOOG Patient Advisory Group meeting on January 10th, 2024

2.4.3. Key collaborations within Oncode in 2023

a) Together with Jacco van Rheenen and Lodewyk Wessels, we characterized 115 patient-derived mouse-intraductal (MIND) models of ductal carcinoma in situ (DCIS). Utilizing the possibility to follow the natural progression of DCIS combined with omics and imaging data, we reveal multiple prognostic factors for high-risk DCIS including high grade, HER2 amplification, expansive 3D growth, and high burden of copy number aberrations. In addition, sequential transplantation of xenografts showed minimal phenotypic and genotypic changes over time, indicating that invasive behavior is an intrinsic phenotype of DCIS (Hutten et al., 2023; 41(5):986-1002).

b) Together with Lodewyk Wessels, we investigated which BRCA1/2-independent mechanisms drive resistance to PARP inhibitors (PARPi) in vivo. We combined molecular profiling with functional HR analysis of matched PARPi-naive and PARPi-resistant mouse mammary tumors harboring large intragenic deletions that prevent reactivation of BRCA1/2. We observed restoration of HR in 62% of PARPi-resistant BRCA1-deficient tumors but none in the PARPi-resistant BRCA2-deficient tumors. Moreover, we found that 53BP1 loss is the prevalent resistance mechanism in HR-proficient BRCA1-deficient tumors, whereas resistance in BRCA2-deficient tumors is mainly induced by PARG loss (Bhin et al., Cell Reports 2023; 42(5):112538).

c) Together with Thijn Brummelkamp, we performed genetic screens to map the machinery involved in the response to ICLs and identified FIRRM/C1orf112 as an indispensable factor in maintaining genome stability. FIRRM deficiency leads to hypersensitivity to ICL-inducing compounds, accumulation of DNA damage during S-G2 phase of the cell cycle, and chromosomal aberrations. In addition, FIRRM is recruited to ICLs, controls MUS81 chromatin loading, and thereby affects resolution of HR intermediates. Thus, FIRRM plays a critical role in the response to ICLs encountered during DNA replication (Mazouzi et al., Science Advances 2023; 9(22):eadf4409).

d) Together with Lodewyk Wessels, we showed that MYC activation drives resistance to mTOR inhibitors (mTORi) in breast cancer. Multiomic profiling revealed recurrent Myc amplifications in mouse mammary tumors that acquired resistance to the mTORi AZD8055. MYC activation counteracted mTORi-induced translation inhibition by promoting translation of ribosomal proteins. In vitro and in vivo induction of MYC conferred mTORi resistance in mouse and human breast cancer models. Notably, MYC status was significantly associated with poor response to everolimus therapy in metastatic breast cancer patients (Bhin et al., Journal of Experimental Medicine 2023; 220(11):e20211743).

The above-mentioned collaborations were already part of my network before I joined Oncode.

2.4.4. Major valorization achievements in 2023

a) Collaboration with Artios Pharma on development of polymerase theta inhibitors, and with UCB on development of Gremlin1 inhibitors.

b) We have been awarded an Oncode TechDev grant for the development of DDR inhibitors to enhance response to PARP inhibitors (PARPi) and overcome PARPi resistance (collaboration with Cancer Research Horizons).

c) The collaboration between Oncode and Cancer Research Horizons has resulted in the creation of, a new next-generation precision oncology company focused on developing first-in-class therapeutics targeting the DNA damage response (DDR) for the treatment of patients with hard-to-treat solid cancers.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Oncode	TechDev grant	150,000.00	150,000.00	01/2023	18	Main applicant
Artios	Research collaboration	169,238.00	169,238.00	10/2023	18	Main applicant
SNF	International Co-Investigator Scheme	1,000,000.00	347,873.00	10/2023	48	Co-applicant
NWO	Open Technology Programme	848,493.00	395,565.00	01/2024	72	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Mariana Paes Dias	Using functional genetic screens to understand and overcome PARP inhibitor resistance
Metamia Ciampricotti	Dissecting the immune microenvironment of breast cancer

Roland Kanaar

Erasmus MC

1. General information

Research Focus	Molecular radiation biology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

We define and dissect the molecular circuits of the DNA damage response (DDR) to identify molecular targets for designing novel mechanism-based anti-cancer interventions.
We address fundamental mechanistic aspects of the DDR with special attention to homologous recombination, DNA mismatch repair and DNA replication stress. We study the dynamic molecular interactions that are responsible for the assembly and disassembly of the molecular factories that guard and repair the genome both at the biochemical and cellular level.
Our translational research involves proton therapy and cancer-on-chip studies. We are characterizing the proton DNA damage response and testing hypotheses on combination therapies. Our cancer-on-chip studies are aimed at making chips compatible with optical imaging such that functional probes, for biological pathways, can be imaged in ex vivo tumor slices from patients.

2.2. Major scientific achievements in 2023

a) We resolved the structure of the cancer-germline-antigen HSF2BP in complex with its BRCA2 interaction domain by cryo-EM and X-ray crystallography. Upon binding to BRCA2, HSF2BP forms octameric rings that are able to interlock into a 3-ring oligomer. Our results explain why ectopic expression of HSF2BP in cancer cells inhibits BRCA2-mediated-recombination. (Science Advances 2023, doi: 10.1126/sciadv.adi7352).

b) Chromatin is dynamically reorganized when DNA replication forks are challenged. We discovered how de novo heterochromatin assembly and disassembly at stalled forks ensures fork stability: A checkpoint-regulated cascade of chromatin signaling activates a histone-methyltransferase to catalyze heterochromatin assembly at stressed replication forks, while a histone-demethylase, facilitates heterochromatin disassembly upon fork restart. (Nature Cell Biology 2023, doi: 10.1038/s41556-023-01167-z).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Ectopically expressed meiotic proteins and cancer.
We discovered that the cancer-germline-antigen HSF2BP when ectopically expressed in cancer cells causes degradation of BRCA2 thereby inhibiting homologous recombination due to lack of RAD51 loading, making cancer cells sensitive to specific chemotherapeutics. Interestingly, in meiocytes HSF2BP promotes homologous recombination. We determined the structure HSFF2BP in complex with its BRCA2 interaction domain by cryo-EM and X-ray crystallography. Upon binding to BRCA2, HSF2BP forms octameric rings that are able to interlock into a 3-ring oligomer. Addition of the meiosis-specific BRME1 proteins leads to dissociation of the ring structure and cancels the disruptive effect of HSF2BP on cancer cell resistance to DNA damage. It also prevents BRCA2 degradation during interstrand DNA crosslink repair in Xenopus egg extracts. We propose that, during meiosis, the control of HSF2BP-BRCA2 oligomerization by BRME1 ensures timely assembly of the ring complex that concentrates BRCA2 and controls its turnover, thus promoting homologous recombination.

Project B) Modeling mechanisms of countering mutations through DNA mismatch repair
DNA mismatch repair (MMR) corrects misincorporated nucleotides during DNA replication. Dysfunctional MMR causes a hereditary predisposition to cancer, called Lynch syndrome, and is often observed in sporadic cancers. Furthermore, the MMR-status of tumors determines their sensitivity and resistance towards immunotherapeutics and chemotherapy. To functionally interpret VUS in MMR genes, it is important to understand which amino acids are critical for complex formation between MMR proteins. As a model system we use the E. coli MMR system to study the interaction between the MutL and MutH MMR proteins. We employed a hybrid approach for predicting critical interface residues between the proteins: in silico interface prediction using HADDOCK (Molecular Dynamics Driven Binding Simulations) and AlphaFold (AlphaFold Multimer: Deep Learning Multimer Structure Prediction). We experimentally verified predicted important amino acids which resulted in a new model for how the proteins interact. This project is funded through base funding to generate preliminary data.

Project C) Re-organization of chromatin architecture in response to cancer treatment induced DNA replication stress.
Chromatin is dynamically reorganized when DNA replication forks are challenged. However, the process of epigenetic reorganization and its implication for fork stability is poorly understood. We discovered a checkpoint-regulated cascade of chromatin signalling that activates the histone methyltransferase EHMT2/G9a to catalyse heterochromatin assembly at stressed replication forks. Using biochemical and single molecule chromatin fibre approaches, we showed that G9a together with SUV39h1 induces chromatin compaction by accumulating the repressive modifications, H3K9me1/me2/me3, in the vicinity of stressed replication forks. This closed conformation is also favoured by the G9a-dependent exclusion of the H3K9-demethylase JMJD1A/KDM3A, which facilitates heterochromatin disassembly upon fork restart. Untimely heterochromatin disassembly from stressed forks by KDM3A enables PRIMPOL access, triggering single-stranded DNA gap formation and sensitizing cells towards chemotherapeutic drugs. These findings may help in explaining chemotherapy resistance and poor prognosis observed in patients with cancer displaying elevated levels of G9a/H3K9me3.

Project D) Can modulation of RAD51 result in chemoresistance in BRCA2 deficient tumors?.
Cancer cells rewire essential cellular pathways against chemotherapeutic challenges. BRCA2 deficient tumor cells lack efficient homologous recombination repair pathway and replication fork protection leading to genome instability due to defective RAD51 loading which can be targeted for chemotherapeutic intervention. However, BRCA2 deficient tumor cells are highly prone to chemoresistance- the mechanisms of which are poorly understood. In this project we are exploring whether modulators of RAD51 can be involved in driving chemoresistance in BRCA2 deficient cells, including resistance of PARP inhibitors. If alternative mechanisms are uncovered that can lead to alternate mechanism of RAD51 loading in BRCA2 deficient cells they could be the cause of chemoresistance in these tumors. This is an exploratory project to generate sufficient preliminary data.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode provides us with the opportunity to bring our fundamental discoveries on the DNA damage response, as well as our more translational results to clinical implementation. Clinical proof of concepts funds allow us to spike the interest of Erasmus MC clinicians. Because of these Oncode funds we are now applying our functional assay on viable ex vivo patient tumor material to select breast cancer patients for Talazoparib treatment and we are translating our discovery that Olaparib synergies for cell killing with ^{177}Lu-Dotatate peptide radionuclide therapy by testing its effect in patients with neuroendocrine tumors.

2.4.2. Contribution to the Oncode community in 2023

a) I am taking an active role together with Jos Jonkers and Alexander Turkin from the BD team to set up the DDR alliance, a collaborative project with industry on the topic of the DNA Damage Response.

2.4.3. Key collaborations within Oncode in 2023

a) Our key collaboration is with Puck Knipscheer. This collaboration is active for a number of years now and was initiated due to our memberships of Oncode. Our different techniques synergize to determine the mechanism though which a cancer testis antigen sensitizes tumors cells to chemotherapeutics, inlcuding PARP inhibitors. In 2023 this resulted in a manuscript in Science Advances (.doi: 10.1126/sciadv.adi7352).

2.4.4. Major valorization achievements in 2023

a) DDR alliance (a collaborative project with industry on the topic of DDR) discussed, strategy devised together with Jos Jonkers and the BD team.

b) DDR project: novel synthetic lethal combination discovered, strategy discussed extensively with the valorisation team, prior art search performed indicating narrow space for filing a patent application (to be done at later stages when the manuscript is ready).

c) Senescence reporter: marketing activities performed (BioEurope), one partner eager to collaborate, conversations ongoing.

d) Radiobiology: a number of conversations with industry initiated about potential research collaboration to perform radiobiological studies of novel radiopharmaceuticals.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
P2018-0008	Selection of advanced breast cancer patients for carboplatin treatment using the functional repair capacity (RECAP) test: the CAREFUL study	6/1/2020	41	Yes	Co-PI
P2019-0035	Improving Peptide Receptor Radionuclide Therapy with PARP inhibitors: the PRRT-PARPi study	1/1/2021	56	Yes	Co-PI

3.3. PhD defenses in 2023

N/A

Jop Kind
Hubrecht Institute

1. General information

Research Focus	Spatiotemporal regulation of genomic function
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Epigenetics plays a crucial role in the priming, consolidation and maintenance of transcriptional programs. In our lab we develop and implement single-cell methods to obtain better insight into the epigenetic mechanisms that govern cellular decision making in early mammalian development and in cancer.
Preimplantation development involves epigenetic reprogramming of the parental genomes upon fertilization followed by the subsequent transcriptional activation of the genome and the first lineage specification event in life. In our group we are very interested to understand the epigenetic basis that govern totipotency, the "awakening" of the genome and the processes that directs lineage choice into cells that constitute the embryo proper and the supporting material. To achieve this goal, an important activity in our group involves the development of single-cell epigenomic technologies to record such processes in detail in individual blastomeres. The development of these methods also offers opportunities beyond studying early embryogenesis. For example, we collaborate with the Drost group (PMC) to implement our methods to determine the epigenetic states associated with rhabdoid tumorigenesis. Furthermore, we are attempting to develop a diagnostic approach to classify tumour of origin in liquid biopsies based on multiplexed epigenetic recordings in blood plasma.

2.2. Major scientific achievements in 2023

a) We developed a method to measure at high resolution two chromatin states in the same cell by combining scDam&T-seq with sortChIC. Particularly exciting about the integration of these methods is the introduction of a temporal axis which allows for studying transitions in chromatin states in the same cell (https://doi.org/10.1101/2023.12.15.571749).

b) We uncovered the mechanisms by which the spatial positioning of the genome is established and reprogrammed upon exit of totipotency at the 2-cell stage. This process involves the coordination between broad domains of H3K27me3, DNA affinity for nuclear lamina associations and available space at the nuclear periphery (https://doi.org/10.1101/2023.02.06.527307).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Multi-modal single-cell profiling.
We have finished and published the project to profile 6 chromatin modalities in single cells. This was the main project of Silke Lochs who has is currently finalizing her thesis to defend this year. We will continue improving this technology and we are in the finalizing stages of negotiations to establish a spin off based on the commercialization of this technology (doi.org/10.1038/s41592-023-02090-9).

Project B) Single-cell DNA repair detection.
We have finished a project to profile DNA double-strand break (DSB) repair in single cells. We have submitted this project and we are currently working towards submitting a revised manuscript within a month. This was the main project of Kim de Luca who is now finalizing her thesis to defend this year https://doi.org/10.1101/2023.05.10.540169).

Project C) Understanding DNA damage responses.
Based on project B, Carlota joined our team in 2023 to build on the work by Kim and implement the technology to map DNA DSBs in single cells to understand how cells make decisions based on their underlying damage profile. The first challenge is to implement the method to profile DSBs. Then the step after this will be to combine live-imaging with single-cell sequencing. For the latter, we have initiated collaborations with the Tanenbaum and Kops groups at the Hubrecht and Miao-Ping Chien (Erasmus MC) to set this up technically and explore possibilities. We also initiated a collaboration with Julie Nonnekens to take advantage of our method to better understand cellular responses upon therapeutic exposure. As soon as we have those assays established, we plan to apply for additional funding via a Oncode Tech-dev and through KWF.

Project D) cfDNA diagnostics.
This is on ongoing effort to optimize conditions in blood plasma to perform multiplexed epigenetic profiling to classify -cancer- cell of origin based in liquid biopsies. We have optimized all enzymatic steps that are required to perform MAbID (project A) in blood plasma (which is not trivial due to the harsh conditions). We have decided to take one step back with this project to first take advantage of the optimized conditions with another approach. Next, the same experiments will be performed with MAbID. This work was funded by Oncode after which we obtained independent funding (KNAW). We are currently seeking additional funding to continue this project. Upon establishment of the protocol, we will work together with JP Medema (AMC) to perform pilot experiment to test for the sensitivity and accuracy to classify colon cancer subtypes based on patient material.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The Oncode base funds provided us with the flexibility to pursue high-risk research for which it was difficult to secure funding. This enabled us to finalize project B and build on the technology to yield insights into cell-fate responses following a DNA-damage insult. This work can have implications for optimizing cancer therapeutic strategy and we've initiated contacts with Julie Nonnekens to take action into this direction. We have recruited Carlota on Oncode base funding and she is now performing pilot experiments to explore different research directions. Based on her initiating work we plan to apply for follow-up funding.

b) One of the most important aspects of being part of Oncode is the research community. Through Oncode we have initiated many new collaborations. We have established a collaboration with Jeroen de Ridder (UMC) who I've met through the Oncode junior program and the Oncode valorization team (Amber) brought us in contact with Julie Nonnekens and Miao-Ping Chien (both EMC). Such cross-disciplinary collaborations would not have been established if we were not part of Oncode.

c) Finally, we are currently in the finalizing phase of founding a spin-off (sCellgen) based on the MAbID technology (project A & D). This entire process is spearheaded by Amber and the Oncode valorization team. Without their support we would not have patented the method (this was on Veerle's initiative) and we would not have had the knowledge/time to establish the NewCo.

2.4.2. Contribution to the Oncode community in 2023

a) We collaborate with many research groups within Oncode to advise on, and provide expertise on how to design single-cell experiments. One very successful example is a collaboration with the Tanenbaum group where we used our expert know-how gained through establishing other protocols to help develop a new method to integrate live-cell imaging and single-cell sequencing of the same cell (coined WALDO).

b) Franka Rang presented her work at the annual Oncode meeting on the mechanisms behind the atypical organization of the spatial genome at the 2-cell stage. See section 2.2.b.

2.4.3. Key collaborations within Oncode in 2023

a) A collaboration with Jarno Drost (PMC) to epigenetically profile Rhabdoid tumor progression and metastasis. This is an ongoing effort which was initiated by the Oncode valorization team and is supported by a TechDev. From my group Marta performs the computational analyses and Giulia in Jarno's group performs the experiments. Marta is also well-integrated in Jeroen de Ridder group (UMC) to acquire computational knowledge and profit from the intellectual environment. Jeroen also helps in providing his knowledge on deep-learning. The project is also supported by the single-cell core at the Hubrecht in processing our samples with T-ChIC (developed by Alexander van Oudenaarden).

b) A collaboration with the Tanenbaum group (Hubrecht) where we used our expertise in single-cell sequencing techniques to help develop a new method to integrate live-cell imaging and single-cell sequencing of the same cell (coined WALDO). Technically this project is established and looks very promising. Silke Lochs (partly paid on Oncode base funds) and Pim Rullens contributed to this and Silke wrote a chapter based on this technology in her thesis.

c) We have just submitted a collaborative effort with the van Oudenaarden group (Hubrecht). This involves measuring two chromatin states at high resolution in the same cell by combining scDam&T-seq with sortChIC. Particularly exciting about the integration of these methods is the introduction of a temporal axis which allows for studying transitions in chromatin states in the same cell.

2.4.4. Major valorization achievements in 2023

a) We are in the finalizing stages of establishing a NewCo (sCellgen) with General Inception (US) and Oncode Bridge Fund as partners. The company is based on our development of MAbID which was a project that was largely funded by the Oncode base fund (first phase).

b) We are working towards establishing an assay with which we can relate cell-fate responses of cells to the underlying DNA damage profiles. We anticipate that knowledge about how cells take decisions upon an DNA damage insult will help uncover vulnerabilities to more efficiently target cancer cells

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Puck Knipscheer

Hubrecht Institute

1. General information

Research Focus	Molecular mechanisms and regulation of DNA repair
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

My group focusses on deciphering the molecular details of cellular processes that maintain genome integrity. This is critical to understand cancer development and could contribute to novel strategies for cancer treatment. We use a powerful biochemical system to recapitulate complex biochemical pathways under physiological conditions in vitro, enabling their molecular dissection.
We study various genome maintenance pathways. A major topic is the repair of DNA interstrand crosslinks (ICLs), toxic DNA lesions that form endogenously but are also induced in cancer chemotherapy. The classical pathway of ICL repair is the FA pathway affected in the cancer predisposition disorder Fanconi anemia. However, we and others have recently identified additional ICL repair pathways. Our current research focusses on: 1) Novel factors and the molecular mechanism of the FA pathway. 2) Characterization of new pathways that repair ICLs induced by reactive aldehydes. 3) Development and use of high throughput sequencing techniques to examine chromatin dynamics and mutation induction. Another main research line focusses on the resolution of mutagenic secondary DNA structures (G4 structures). We developed methods to study the resolution of these structures, identified a mechanism that unwinds these structures during DNA replication, and recently defined a replication-independent mechanism that regulates these structures

2.2. Major scientific achievements in 2023

a) I was appointed professor of Biochemistry of Genome Maintenance at the department of Human Genetics at the LUMC at the start of 2023. This was in large part based on the scientific achievements and collaborative spirit of the entire Knipscheer group.

b) In 2023 my lab took an important step to complement our biochemical approaches with cell-based methods. This is a new direction that allows us to validate our findings and further study these genome maintenance pathways. We have started to implement NGS techniques such as Cut&Tag and scEdUSeq in our studies and foresee this to develop into a powerful combination of approaches.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) The mechanism of ICL repair by the Fanconi anemia pathway.

- In our efforts to decipher the mechanism of translesion synthesis (TLS) during ICL repair we found that polymerase kappa (PolK), acts specifically during repair of ICLs induced by reactive aldehydes (DOI: 10.1021/jacs.2c10070). In addition, we defined the specific domains of PolK that are required for this, as well as domains that are less important. A manuscript on this work is in preparation.
- We also set out to define the role of the proteins that are part of the Fanconi core complex. Our first experiments indicate that this complex may have other functions in addition to its role as an E3 ubiquitin ligase for FANCI and FANCD2.
- We have an extensive collaboration with the laboratory of Roland Kanaar. We further studied the role of the BRCA2 interacting protein HSF2BP in homologous recombination (DOI: 10.1126/sciadv.adi7352), and pinpointed requirements for a newly identified damage-dependent BRCA2 modification.
- In collaborations with Thijn Brummelkamp (genetic screens), Michiel Vermeulen (BioID-Mass spec), and Markus Raschle (plasmid pulldown proteomics, TU, Kaiserslautern) we are searching for novel players in ICL repair pathways. Oncode funding enables us to perform initial research into promising hits from these efforts and respond to collaboration requests quickly.

Project B) The mechanism of ICL repair by novel pathway(s).

- We have been testing several factors from genetic and proteomic screens that are potentially involved in the new pathway of ICL repair we have recently identified (DOI: 10.1038/s41586-020-2059-5). Unfortunately, so far this has not yielded successful candidates. Currently this project is on hold and we hope to continue when new opportunities arise.

Project C) Mutagenicity of ICL repair pathways.

- We developed sequencing methods to analyse mutations induced during ICL repair in our biochemical system. We are currently using these methods to determine the effect of depletion of TLS polymerases on the mutational signature of ICL repair for various different ICL types. Development of this method required the help of Oncode base funding.

Project D) The resolution mechanisms of alternative DNA structures.

- Recently, we discovered a DNA replication-independent mechanism that resolves stable mutagenic G4 structures in our extract system. This involves R-loop formation across from the G4, homologous recombination factors, nucleolytic incisions and the G4 unwinding helicases DHX36 and FANCJ. To validate this mechanisms in cells we generated DHX36/FANCJ knockout cell lines and showed their depletion in cells causes strong enhancement of genomic G4 structures (by Cut&Tag) which leads to replication slow down and DNA damage, but also transcriptional upregulation of many lncRNAs. We are currently finalizing data analysis and are preparing a manuscript for submission. We also published a review on this topic in 2023 (DOI: 10.1016/j.dnarep.2023.103552).
  Furthermore, have recently biochemically characterized three mutations in DHX36 that were found in patients with a previously undescribed monogenic developmental disorder. We have shown that these patient variants are defective in duplex and G4 unwinding and act in a dominant negative fashion. Thanks to Oncode base funding we quickly started analysing these patient mutations and a manuscript describing this work will be submitted in the coming months

Project E) Nucleosome dynamics in DNA replication and repair.

- In the past few years we have developed methods to determine nucleosome position and dynamics during DNA replication and repair in our Xenopus egg extract system. We have used this to characterize nucleosome dynamics during ICL repair and we have started investigating the role of the nucleosome chaperone CAF-1 in DNA replication and repair (doi: 10.1093/nar/gkad171). Initial results indicate

that immunodepletion of CAF-1 from our extract compromises DNA replication-dependent nucleosome assembly providing us with important tools to study the mechanism of this process.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode base funding has enabled us to perform important preliminary work for my Vici application. I successfully passed the first stage of evaluation and awardees will be announced in February 2024.

b) We greatly benefit from the active oncode community in stimulating collaborative projects. We have several successful ongoing collaborations with other Oncode groups (initiated after the start Oncode).

2.4.2. Contribution to the Oncode community in 2023

a) I helped to organize and contributed to 2 major donor events for Oncode/KWF at the Hubrecht Institute. These took place in March and October 2023

b) I took part in a brainstorm session for an Oncode DDR alliance during the Oncode OI retreat in September 2023.

c) I was part of the Junior OI assessment round in March 2023

2.4.3. Key collaborations within Oncode in 2023

a) We have a very fruitful collaboration with the group of Roland Kanaar that started after Oncode affiliation. Subject of this collaboration is the role of HSF2BP and BRCA2 in DNA interstrand crosslink repair and homologous recombination. This has so far resulted in 3 co-publications of which one in 2023 (DOI: 10.1126/sciadv.adi7352).

b) Since 2022 we are collaborating with the group of Ruben van Boxtel on the mechanism of mutation induction by ganciclovir. My PhD student, together with a PhD student from the van Boxtel lab initiated this collaboration which is currently at the stage of method development.

c) After establishing knockout cell lines for DHX36 and FANCJ, helicases involved in the resolution of G4 structures, we started a collaboration with the group of Alexander van Oudenaarden to investigate replication speed with their single cell nascent strand sequencing approach. This lead to the interesting finding that depletion of these helicases reduces replication speed, particularly in early S-phase, indicating that these helicases are likely involved in the resolution of pre-exsiting G4 structures

2.4.4. Major valorization achievements in 2023

a) I contributed to a brainstorm session for a potential Oncode DDR alliance during the Oncode OI retreat in September 2023.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Geert Kops
Hubrecht Institute

1. General information

Research Focus	Cell division & chromosome segregation
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Errors in chromosome segregation cause aneuploidy. ~80% of all human tumors are aneuploid, and they harbour subclones with different karyotypes. This strongly suggests that cancers experience ongoing chromosome missegregations, a.k.a chromosomal instability (CIN), and that CIN is beneficial for cancer cells.
Central questions to research in the Kops lab are: 1) How do cells ensure high fidelity chromosome segregation? We use cell biological, structural and imaging techniques to examine the chromosomal structures and signaling pathways that promote successful cell division. Current focus is on the fibrous coronas of kinetochores and on centromeric chromatin architecture. 2) What are causes and consequences of CIN in cancer? We use three models: a) human organoids; b) a mouse model of CIN; c) various cell lines.
All questions are related to how cell division control mechanisms prevent tumor formation. The answers are sought through state-of-the-art approaches and extensive collaborations, with potential for clinical impact, all objectives of Oncode.

2.2. Major scientific achievements in 2023

a) Acquired KWF grant on work started with Oncode funding: see project E in 2.3. Project was initiated after clinical workshop on colorectal cancer, and initial collaboration was funded through Oncode base funding. Successful prelim work was then submitted to KWF, and was awarded €918k

b) Publication on identification of new mechanism for spindle assembly in mitosis and meiosis, impacting our understanding of the origins of aneuploidy in cancer and development. Doi: 10.1016/j.cub.2023.01.010.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Single cell DNA sequencing to examine evolution in chromosomal copy numbers during cancer progression and therapy.
Paper submitted + paper published: doi: 10.1053/j.gastro.2023.02.047. Oncode funding used to pay technician and consumables to perform single cell karyotype sequencing.

Project B) Genome editing for tagging cell division genes in human organoids
Successfully established nanoblade technology (DOI: 10.1016/j.omtn.2023.06.004) for gene tagging in human organoids. Now using it to tag genes involved in chromosome segregation to investigate origins of chromosomal instability in colorectal cancer. Oncode funding used to pay postdoc salary.

Project C) Centromere architecture and its impact on chromosome segregation
In this project that examines how centromeres are organised in order to ensure error-free chromosome segregation during cell divisions, we found that human centromeres are built from two chromatin domains organised by condensin and cohesin. We used Oncode base funds in 2023 on PhD student salary. A manuscript was published on the preprint server BioRxiv (ID 502248) in 2022 and is currently under revision at Cell.

Project D) Chromosomal instability and karyotype evolution in colorectal adenomas.
Understanding genome evolution at the chromosomal level and how it impacts tumor development is difficult to probe in humans due to challenges in obtaining living material from the early stages of tumor formation. In a collaboration with researchers from NKI (Meijer/Carvalho group) that was started at an Oncode clinical workshop, we continued to examine CIN and karyotype heterogeneity during early stages of human CRC progression, from early to late adenoma. Due to depleted Oncode funds in 2022, this project was continued with Hubrecht core funding, but KWF grant funding was secured in July 2023. Initial data suggest in vitro evolution of several organoids to karyotypes that are seen also in vivo, providing an exciting model to follow chromosome aneuploidy evolution and impact of early stage cancer.

Project E) Chromosome-specific sensitivities to centromere/kinetochore defects.
This project was newly started at start of Oncode phase 2. During cell division, our 46 replicated chromosomes divide equally, ensuring that each progeny cell inherits one complete copy of the genome. Chromosomes vary in fidelity of segregation, and mistakes in this process cause cancer and developmental disorders. To properly segregate, chromosomes use large multi-protein structures known as kinetochores that assemble on centromeric DNA and connect to the segregation apparatus. The size of both the centromere and the kinetochore differs widely between chromosomes, yet little is known about how this affects robustness of their segregation. We aim to uncover the relationship between human centromere size, kinetochore size and a chromosome's ability to robustly connect to the segregation apparatus, by using chromosome-specific quantitative imaging and perturbations. The results will uncover vulnerabilities of kinetochore function for individual chromosomes and its impact on their segregation fidelities.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Ian Bell ensures great relationship with ONO pharmaceuticals, with whom we share an interest in the CIN tumors. This resulted in a collaborative research project, and in 2024 we will discuss possibilities of continued funding.

b) Community: through Oncode and its meetings and low-threshold culture of accessing expertise and data before publication, we engage in several collaborations with other OIs or with clinical research groups.

c) In 2023 we used several Oncode-initiated facilities: the Oncode single cell core for single cell ChiC-seq and scKaryo-Seq, and the proteomics facility.

2.4.2. Contribution to the Oncode community in 2023

a) Many activities as Head of Institute, Scientific Director, chair of RMC.

b) Collaborations (expertise/reagents) with several OIs

2.4.3. Key collaborations within Oncode in 2023

a) Susanne Lens + Rene Medema, on a synergy project application revolving around chromosomal instability in cancer, proposed to ONO pharma. With Lens, we also collaborate on analyses of adenoma organoids. The proposal was due to Oncode activities and discussions

b) Tassos Perrakis, on structural biology of mitotic regulators. Existed before Oncode.

c) Rios, Maurice, Van Rheenen, Snippert: together in a NWO-Gravitation project, preliminary work by our lab made possible by Oncode base funding

d) Wouter de Laat: on 3D organisaion of the mitotic centromere. Work made possible by Oncode base funding.

2.4.4. Major valorization achievements in 2023

a) Ongoing collaboration with ONO pharma

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF	Project grant	918000	918000	1-2-2024	48	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Wouter de Laat

Hubrecht Institute

1. General information

Research Focus	Biomedical genomics
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The de Laat lab aims to understand how gene expression is (mis-)regulated in health and disease. Key terms are gene regulation, epigenetics, chromatin, 3D genome and genetic diagnostics. In 2023 we created endometrial organoid cultures that enable conditional depletion of CTCF, to test how this tumor suppressor transcription factor involved in 3D genome folding drives endometrial cancer. With Oncode support we are completing a screening study for long-range enhancer action factors, currently using conditional depletions to test if enhancer distance determines the transcriptional resistance of genes against reduced levels of enhancer-associated factors. Also with Oncode funding we developed novel systems to locally control chromatin loop extrusion, opening exciting new research directions to study factors controlling loop extrusion and the impact of chromatin looping on transcription and epigenetics (in revision). We completed phase 1 of a large validation study for non-invasive prenatal diagnosis for hereditary monogenic diseases, including breast cancer (BRCA) and are collaborating with stakeholders to implement NIPD in the clinic. Also with Oncode funding, we pursued our studies on patented novel powerful gene editing methods for fetal globin gene reactivation, with potential therapeutic applications for thalassemia and sickle cell disease.

2.2. Major scientific achievements in 2023

a) The completion of phase 1 of the clinical validation study for non-invasive prenatal diagnosis, aimed to offer a blood test instead of surgery to risk couples opting for prenatal diagnosis because they carry a severe monogenic disease. With UMC stakeholders steps are ere now taken towards clinical implementation.

b) The development of a system to locally control 3D genome in living cells, enabling us to show how chromatin looping affects gene expression and epigenetic chromatin landscapes (submission in Q1 2024). The two achievements highlight our capacity to combine original basic research (b) and bring this to the clinic (a).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Life cell imaging of long-range gene activation (Tjalsma).
Project supported with Oncode funding, focused on live-cell imaging of chromatin contacts and gene expression was abandoned because of imaging resolution issues and because we prioritized other projects.

Project B) In vivo methods for targeted cohesin recruitment (Han, Huang, Vaandrager, Krijger, Allahyar, Verstegen, Robers, Sofiadis)
High-risk project initiated with Oncode funding. We have developed tools for controlled, site-specific cohesin recruitment to follow individual loop extrusion trajectories in living cells. Results form the basis for an exciting publication (Han et al. BioRxiv and in revision), a new PhD project (Michelle Robers) and for future grant writing.

Project C) Gene activation and repression in complex regulatory landscapes (Huang, Verstegen, Krijger).
Project supported by Oncode funding. We are completing our studies on the mode of action of enhancers controlling the expression of multiple, functionally unrelated, genes. This study led to surprising observations, for which we are currently doing final validation studies. We expect to submit this story in 2024 (is part of the thesis of Yike Huang).

Project D) Therapeutic gene reactivation through genome editing (Felder, Tjalsma, Luce, Krijger).
Project initiated with Oncode funding, later supported by an EU ITN grant. We are completing a first proof-of-principle study that forced enhancer recruitment by CRISPR-Cas deletion of intervening DNA sequences can drive the reactivation of developmentally silenced genes. We show this can reactivate fetal globin gene expression to therapeutically relevant levels. We will submit this paper in Q1-Q2 2024. IP was generated with help from Oncode

Project E) NIPD validation study.
Oncode funding was used to invest in the bioinformatics needed to analyze the complex NIPD datasets. Based on this progress, we together with UMC stakeholders are now organizing the final phase of validation and implementation of NIPD in the clinic.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode motivates and supports us to translate our basic science into clinical applications. As a first example we aim to bring non-invasive prenatal diagnosis (NIPD) to the clinic, to offer a blood test instead of burdensome surgery to pregnant couples who desire prenatal diagnosis (PD) because they carry the same severe monogenic disease (eg hereditary breast cancer). Thanks to Oncode funding we were able to successfully complete phase 1 of a clinical validation study. We are currently organizing stakeholders (UMCs) to complete the validation study and implement NIPD in the Netherlands.

b) As a second example, Oncode greatly helps us accelerating valorisation. Frequent contact with Emil Pot, Oncode Associated Oncode BD, enabled filing two patent application on a method for fetal globin reactivation, as a potential therapeutic for thalassemia and sickle cell anemia patients. With experienced biotech entrepreneurs we are currently investigating possibilities to start a biotech venture.

2.4.2. Contribution to the Oncode community in 2023

a) Participation PI and group members) and presentation (postdoc Ruiqi Han) at the Oncode Annual Scientific Meeting in Amersfoort

b) Participation OI retreat in Veenendaal

2.4.3. Key collaborations within Oncode in 2023

a) Collaboration with Tineke Lenstra group (NKI), ongoing, to visualize cohesin loop extrusion in living cells (collaboration initiated via Oncode).

b) Collaboration with Jeroen de Ridder (UMCU) group to complete multiway HiC contact analysis study.

2.4.4. Major valorization achievements in 2023

a) Completing phase 1 clinical validation study NIPD

b) With Sanquin, proof-of-concept that our patented CRISPR editing method efficiently drives fetal globin gene reactivation to therapeutically relevant levels.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Susanne Lens

UMC Utrecht

1. General information

Research Focus	Genome instability
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Most solid tumours display an abnormal number of chromosomes, known as aneuploidy. Aneuploidy is a frequent consequence of erroneous chromosome segregation during cell division (chromosomal instability, CIN), and both features correlate with tumour heterogeneity, drug resistance, and poor patient prognosis. Our research aims to decipher the molecular principles that ensure error-free chromosome segregation during cell division, and to understand how different (cancer) tissue types can cope with the gains or losses of specific chromosomes. With our fundamental research, we aim to gain novel mechanistic insights into cancer-driving events and to identify targets for potential novel anti-cancer therapies. Specific research topics of the lab are:

- The inner centromere network. The inner centromere is a specialized chromosome structure on which protein activities accumulate that control sister-chromatid cohesion and chromosome-microtubule connections. We investigate inner centromere protein network regulation, and how network perturbations induce CIN. We are currently exploring if mild perturbations of sister-chromatid cohesion could be used as a strategy to target and kill CIN+ cancer cells.
- Cancer-specific aneuploidy signatures. Different cancer types exhibit characteristic subsets of whole or partial chromosomal gains and losses. By inducing or reverting chromosome-specific aneuploidies, we aim to identify drivers of these cancer tissue-specific aneuploidies and to unravel their contribution to cancer progression and maintenance.

2.2. Major scientific achievements in 2023

a) Truong and Cané Gasull et al. (EMBO J. 2023; https://doi.org/10.15252/embj.2022111559); We developed an original, motor protein-based strategy for mis-segregation of specific chromosomes at will during mitosis that was published in EMBO J. The work also resulted in an invited review on classic and novel strategies for inducing or selecting specific chromosomal gains and losses in mammalian cell systems. Truong et al. (Chromosome Res, 2023; https://doi.org/10.1007/s10577-023-09735-7)

b) I am very excited that we have managed to correct a whole chromosome aneuploidy in a patient-derived CRC organoid, and to find that it has dramatic consequences to cancer physiology. This places us in a strong position to study how aneuploidy – a still poorly understood cancer hallmark- contributes to oncogenesis.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Drivers of specific aneuploidy patterns in CRC

Beside the well-studied colorectal cancer (CRC) driver mutations in oncogenes and tumorsuppressor genes, most CRCs also display recurrent alterations of specific chromosome arms or entire chromosomes. However, how these specific aneuploidies arise and contribute to oncogenesis remains unresolved. We recently obtained exciting preliminary data suggesting that the deprivation of specific stem cell niche factors selects for specific chromosomal gains in healthy human colorectal organoids. Strikingly, the observed chromosomal gains are the same as recurrently found in colorectal cancer. This suggests that tissue microenvironment and/or architecture may be important factors that shape the aneuploidy landscape of CRC. These data will support a new grant application.

Project B) Understanding the contribution of recurrent aneuploidies to CRC maintenance

To understand if and how specific chromosomal gains contribute to tumor maintenance, we successfully corrected a highly recurrent CRC aneuploidy (chr 7 trisomy) in a patient-derived colorectal cancer organoid. Reverting chromosome 7 to its normal copy number state is associated with severe growth defects, suggesting that CRCs have become addicted to the aneuploidy. We are in the process of identifying the genes on chr 7 supporting colorectal cancer (organoid) growth and are extending our aneuploidy correction approach to additional patient-derived CRC organoids and to other chromosomes that are frequently trisomic in CRC. Note that this project is now funded through KWF, but got started (i.e. preliminary data that supported the application) with Oncode basefund phase I.

Project C) Tissue neighbourhood responses to CIN and aneuploidy.

We hypothesize that cells in a human tissue may sense and respond to the presence of neighbouring cells that is CIN. Depending on the genetic background of the CIN cell, the response of the 'healthy' neighbours may either act as a barrier for the CIN cell or contribute to field cancerization, or even field immunization. To study this, we developed a mosaic epithelial cell culture system in which we can acutely induce CIN and aneuploidy in a targeted subpopulation of cells and study the response (i.e. transcriptional using RNA seq, and functional by live and fixed cell imaging) of cells that reside in the neighbourhood of these recent CIN cells. RNA seq analysis of FACS-sorted CIN cells and their neighbours suggests that the latter indeed respond to having CIN cells in their proximity. These data will support a new grant application. Moreover, in collaboration with Oncode PI Marvin Tanenbaum, we are exploring the feasibility of smRNA FISH technology to assess the strength and spatial range of CIN-induced innate immune signaling.

Project D) (Inner) centromere network regulation.

We gathered preliminary data to support a KWF project proposal to target centromere cohesion weakness in cancer through the centromere network protein Shugoshin-1 (submitted September 2023).

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The monthly meetings with business developer Yuva Oz have been extremely valuable as it really helps to look at the projects in a different way and to focus on what is needed to make the next step towards valorisation, to apply for specific funding within Oncode, and to meet with potential (industry) partners to discuss certain projects.

b) Oncode base fund allows me to test the viability of new ideas (e.g. neighbourhood responses to CIN and aneuploidy, stem cell niche factors and CRC aneuploidy patterns), and to subsequently gather preliminary data to support new grant applications.

c) The Oncode single cell (epi) genome sequencing facility and the Oncode research community (e.g Sjors Middelkamp) have been professional partners for sequencing and for help and advice on sequencing data analysis.

2.4.2. Contribution to the Oncode community in 2023

a) Participation in the Oncode-CGC Annual Scientific meeting (8 & 9 June 2023; presentation by Paula Cane Gasull) and the Oncode-CGC Annual conference (2 & 3 Nov 2023).

b) Reviewer/evaluator of new junior Oncode PI applications.

c) Oncode PI retreat, 18/19 Sept 2023 (brainstorm session w/ Sarah Derks)

2.4.3. Key collaborations within Oncode in 2023

a) Hugo Snippert (UMCU): Collaborator on KWF research grant 13515 (Delineating the contribution of recurrent aneuploidies to CRC development and maintenance).

b) Together with Geert Kops, René Medema, Jonne Raaijmakers and Floris Foijer (UMCG, not Oncode), we hold yearly Dutch CIN & aneuploidy meetings to explore common grounds for collaboration and to define unique research angles as Dutch CIN & aneuploidy community (last meeting in July 2023)

c) Marvin Tanenbaum (smFISH to quantify innate immune responses in CIN cells and their neighbors).

d) Ruben van Boxtel (inducing chromosome-specific mis-segregations in human hematopoietic stem cells to model the chr7 monosomy observed in myeloid dysplastic syndrome).

2.4.4. Major valorization achievements in 2023

a) Together with Yuva we explored whether potential (industry) partners (Ono Pharmaceutical, Incircular/Grossmann) would be interested in developing peptide-based inhibitors of the centromere protein Shugoshin-1 and probed if they could be potential partners for a TKI application. These meetings taught us that we needed to gather more fundamental data and as such it helped us shape a more explorative grant application (together with Joep de Lange, VUMC) that we submitted to KWF (see also 'project D).

b) This maybe not the valorization you are looking for, but together with Ruben van Boxtel and Maarten Geurts (Oncode/PMC), we are trying to generate RNPs of our dCas9-kinesin fusion and chromosome-specific sgRNAs to see if this allows the introduction of our chromosome-specific missegregation approach into primary cell systems (such as hematopoietic stem cells). If this works it would broaden the applicability of our approach, which is already frequently requested and send to scientists working in the field of genome instability and cancer.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Tineke Lenstra

Netherlands Cancer Institute

1. General information

Research Focus	Transcription dynamics, single-molecule imaging
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

Transcription dynamics in single cells

Gene expression is tightly regulated to ensure that genes are transcribed in the right cell at the right time. Yet, even genetically-identical cells in the same differentiation state and in the same environment exhibit considerable variability and fluctuations in the transcriptional process. This stochastic gene expression variation can influence important cell fate decisions and can also contribute to heterogeneity in tumors. A major source of stochastic gene expression heterogeneity is transcriptional bursting, where genes are transcribed during short periods of high gene activity interspersed by periods of inactivity. The origin and regulators of bursting remain largely unknown. Our lab aims to understand the regulatory mechanisms of transcriptional bursting in single cells. We use a range of single-molecule imaging techniques to directly observe the stochastic behavior of regulatory factors and the process of transcription, as these dynamically occur inside living yeast and mammalian cells. We reveal how bursting is regulated by transcription factor binding dynamics, transcription factor clustering, enhancer-promoter interactions, chromatin, and DNA supercoiling. In addition, we are starting to apply our live-cell imaging approaches to study the function of stochastic heterogeneity in for example cancer plasticity.

2.2. Major scientific achievements in 2023

a) DNA supercoiling restricts the transcriptional bursting of neighboring eukaryotic genes. Patel HP, Coppola S, Pomp W, Aiello U, Brouwer I, Libri D, Lenstra TL. Mol Cell. 2023 May 18;83(10):1573-1587.e8. doi: 10.1016/j.molcel.2023.04.015. In this manuscript, we used dual-color single-molecule imaging to show that transcriptional bursting of adjacent genes is coupled and that this coupling requires rapid release of DNA supercoils by topoisomerases. When DNA supercoils accumulate, transcription of one gene inhibits transcription at its adjacent genes by destabilizing transcription factor binding.

b) Dynamic epistasis analysis reveals how chromatin remodeling regulates transcriptional bursting. Brouwer I, Kerklingh E, van Leeuwen F, Lenstra TL. Nat Struct Mol Biol. 2023 May;30(5):692-702. doi: 10.1038/s41594-023-00981-1. In this manuscript, we showed how remodeling of different promoter nucleosomes regulate specific bursting properties.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Interplay between TC-NER and transcription in single cells.

Combining the expertise of Jurgen Marteijn in the field of transcription-coupled nucleotide excision repair (TC-NER) with our single-molecule transcription imaging expertise, we have a collaborative project to understand what happens with Pol II and transcription when Pol II stalls at DNA damage sites. Thus far this longstanding question was difficult to answer due to a lack of techniques to analyze single Pol II complexes. Using Oncode funds, we have been able to develop a live-cell single-molecule imaging approach to measure the fate of individual Pol II molecules and have generated preliminary data that allowed us to successfully apply for an NWO-M grant. Our results will provide more insight into the mechanisms of DNA damage-induced aging and cancer cell cytotoxicity during chemotherapy.

Project B) Measuring bursting at many promoters in human cells

The first step in understanding gene expression heterogeneity in cancer is to understand the mechanisms that modulate expression heterogeneity. In this project, we aim to understand how different promoter sequences regulate the kinetics and heterogeneity of transcription in human cells. To get more insight into the regulation of bursting in mammalian cells and to make this technique wider available for the (Oncode) community, we have set up a system to easily integrate many promoter variants at a single genomic location in human cells. We are using this system to measure transcriptional bursting of many wildtype or mutated promoters of interest in living cells. In addition, we are applying our novel 3D tracking algorithms to understand how transcription factor binding at these different promoters contribute to the observed kinetics. Oncode funding has allowed us to invest in the setup of such a high-potential system and to develop the required analysis tools.

Project C) Impact of enhancers on transcription dynamics.

In more complex eukaryotes, distal regulatory elements, called enhancers, are essential to activate gene transcription in the right cell type at the right time. Enhancers are often misregulated in cancer cells. However, the mechanisms of enhancer activation, as well as their effect on transcription dynamics are still unclear. To gain more insight into the spatiotemporal mechanisms of enhancer activation, we have set up live-cell imaging of enhancer-dependent transcription at a pluripotency gene in mouse embryonic stem cells. We found that enhancer perturbations had unexpected and dose-dependent effects on transcription dynamics. In collaboration with former OI Elzo de Wit, we are studying at the same locus how enhancer methylation and 3D genome architecture affects transcriptional bursting, and in collaboration with Bas van Steensel, we investigate the how gene position affects transcription dynamics. This project is facilitated by the core funding of Oncode

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The unrestricted Oncode base-funding allows us to explore high-risk ideas and to set up novel imaging tools. For example, we have generated a human reporter cell line to study bursting for many promoters, which resulted in a successful VIDI application, we have developed an imaging tool in collaboration with Jurgen Marteijn. We have also set up transcription imaging in mammalian systems, which has resulted in us joining a consortium including OI Jop Kind.

b) The accessibility of the Oncode network provides visibility and has created the opportunity to start collaborating with several PIs, even at the beginning of my independent career. This allowed us to initiate several projects outside my own field (see key collaborations).

Recently, the benefit of the Oncode network was showcased by several brainstorm sessions with OIs about an idea to test how stochastic transcription affects cancer progression.

c) Oncode provides access to a valorisation team, which has stimulated us to start a conversation with Zeiss, a microscope company to licence our software for use by other researchers and has helped us throughout the whole process of licensing our software. The product is expected to be released March 2024.

2.4.2. Contribution to the Oncode community in 2023

a) Our lab shares expertise in several cutting-edge imaging techniques with the Oncode community, such as single-molecule RNA FISH, single-molecule live-cell transcription imaging, single-particle tracking, and 3D spot tracking. Our lab provides technical support for other OI to setup their experiments on the 3D orbital tracking microscope. After my lab joined Oncode, we initiated many novel collaborations where we were able to apply our expertise for different scientific questions. The majority of these would not have been possible without the networking opportunities or funding support of Oncode. In addition to small-scale collaborations, our lab is part of one of the Oncode Accelerator projects that has recently started.

b) In 2023, I have helped organize the Oncode retreat, and helped with the Major Donor Programme.

c) We perform and publish excellent science, which has resulted in selection for a shared ENW-M grant in 2023. We thereby contribute to the reputation and visibility of Oncode.

2.4.3. Key collaborations within Oncode in 2023

a) Based on our affiliation with Oncode, we started a collaboration with Jurgen Marteijn. Together, we aim to understand what happens with Pol II and transcription when Pol II stalls at DNA damage sites. Using Oncode base funds, we have developed a live-cell single-molecule imaging approach to measure the fate of individual Pol II molecules and have generated preliminary data that allowed us to jointly apply for an ENW-M grant, which was awarded in 2023. Our results will provide more insight into the mechanisms of DNA damage-induced aging and cancer cell cytotoxicity during chemotherapy. Oncode news item: https://oncodeinstitute.nl/news/oncode-investigators-tineke-lenstra-and-jurgen-marteijn-receive-enw-m-collaborative-grant.

b) In a consortium project, that includes Oncode Investigator Jop Kind, we aim to understand whether and how stochastic variability contributes to cell fate decisions. Using mouse gastruloids as a model system of early mammalian development, we dissect the contributions of heterogeneity in chromatin state and stochastic transcriptional fluctuations to the competence of single cells to respond to instructive signaling cues - and to determine causal relationships between these different elements. Our results will provide new conceptual frameworks for cellular identity and lineage specification, which will be important for understanding how cancer cells may use stochastic heterogeneity for acquiring new traits. Oncode based funds allowed us to setup of novel imaging tools that resulted in us joining the consortium. Moreover, the consortium has strongly benefitted from Oncode during the grant writing process, resulting in a successful application for an NWO-XL grant. News item: https://www.nki.nl/news-events/news/three-prestigious-nwo-grants-for-cancer-research/.

c) In a recent collaboration with Wouter de Laat, we aim to visualize extruding cohesin molecules in living cells to learn more about their biophysical properties. We exploit a novel method from the de Laat group to load cohesin at specific loading docks. Using our developed microscopy systems to track single genes, we will image single cohesin molecules as soon as they load onto the DNA and follow their behavior over time as they exclude along the DNA, providing insight into their mobility, residence time and binding frequency. This project was initiated within the Oncode setting.

2.4.4. Major valorization achievements in 2023

a) Licensing of tracking software at Zeiss. Expected release product March 2024.

b) Initiated collaboration with Lumicks

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
NWO	XL	2.055.405	400.000	01/2023	48	Co-applicant
NWO	M	731.164	365.582	03/2024	48	Co-applicant
NWO	Aspasia	40.000	40.000	09/2023	48	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Heta Patel	Spatiotemporal Analysis of Transcription Dynamics

Jurgen Marteijn

Erasmus MC

1. General information

Research Focus	Transcription Stress & Genome Stability
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The goal of my lab is to mechanistically dissect the DNA damage response (DDR), as defects in this pathway are commonly observed in cancer, which can be exploited as Achilles heels in anti-cancer treatments. The central theme of my lab is how cells cope with the severe consequences of transcription-blocking DNA lesions (TBLs). Correct regulation of gene expression is crucial for proper cell function and tissue homeostasis. However, the DNA template transcribed by RNA polymerase II (Pol II) is compromised on a daily basis by numerous types of DNA damaging agents either from environmental (e.g. UV induced-damage, chemicals in food or chemotherapy) or endogenous (reactive oxygen species, aldehydes) origin. If these different TBLs are not resolved properly, prolonged stalling of Pol II can lead to severely disrupted cell function due to the absence of newly synthesized RNA molecules or to the appearance of mutant RNA molecules. In addition, prolonged damage-stalled Pol II induces R-loops and may result in collisions with advancing replication forks resulting in genome instability. We focus on how TBLs are repaired by Transcription-Coupled Repair (TCR) and what their effects are on transcription, genome instability and the related DNA damage response, and test whether inhibition of this pathway can be used to make cancer therapies more effective. Furthermore, we study the effects of chemotherapy-induced DNA damage on transcription and its link with neurotoxicity, an important side-effect of chemotherapy.

2.2. Major scientific achievements in 2023

a) We have identified a new transcription-coupled repair pathway of DNA protein crosslinks (DPCs). DPCs arise from enzymatic intermediates, metabolism or exogenous chemicals like chemotherapeutics. We show that DPCs severely impede RNA polymerase II (Pol II)-mediated transcription, and are repair by a non-canonical TC-NER pathway.

b) Together with the Lenstra lab we obtained a NWO ENW-M2 grant (€750.000) to study the effects of DNA on elongating Pol II at a single molecule level in living cells. This project will allow us for the first time what happens with Pol II when it stalls at DNA damage.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Role of DNA-protein crosslinks in DNA damage-induced cytotoxicity and transcription stress.

DNA-protein crosslinks (DPCs) are caused by covalent and irreversible linkage of proteins to DNA. DPCs can be caused by the exposure to crosslinking agents such as aldehydes. In addition, the cytotoxicity of DPCs is exploited in cancer therapies, for example DPCs that are induced by Pt-based chemotherapeutics or topoisomerase inhibitors. Unrepaired DPCs are expected to obstruct any DNA-based transacting process, such as transcription and replication. Indeed, our studies show that DPCs strongly impede transcription and DPCs in active genes are preferentially repaired by transcription-coupled repair mechanism. Interestingly, these DPCs are repaired by a non-canonical TC-NER mechanism. The flexibility of the Oncode base funding allowed my lab to finalize this study, which otherwise would not have been possible. Our obtained results will subsequently be used to write grant applications on this topic.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The flexibility of the Oncode base funding allowed me to continue with research projects for which there was no funding. This allowed me to completely finish our TC-DPC project which will be published in 2024 in nature cell biology.

b) The highly collaborative Oncode network allowed me to collaborate with the different labs present in Oncode, including for example a recent collaboration with Titia Sixma, which resulted obtaining cryo-EM structures of the complete TC-NER complex, including the newly discovered TC-NER factor STK19.

2.4.2. Contribution to the Oncode community in 2023

a) Part of selection committee of jr OI recruitment 2023.

b) Started with organization of Oncode Major Donor event in EMC, which has not been follow-upped by Oncode thus far.

c) Presented a pitch for the brainstorm session on the OI retreat on chemotherapy-induced neuropathy

2.4.3. Key collaborations within Oncode in 2023

a) Collaboration with Titia Sixma and Michiel Vermeulen on Transcription Coupled Repair. This work resulted in the characterization of STK19 as new TC-NER factor, which we could map in the TC-NER complex by cryo-EM studies of the Sixma lab. This culminated in a collaborative ENW-XL grant application in 2023.

b) Together with Tineke Lenstra we developed a single molecule and live-cell imaging procedure to detect the effects of DNA damage on RNA Polymerase II. This work will be continued as this year our joint NWO-ENW-M2 grant will start in which two PhD students (one in each lab) will study the effects of DNA damage on the Pol II remodeling.

c) Continuous collaboration with Jan Hoeijmakers on DNA damage-induced transcription stress.

2.4.4. Major valorization achievements in 2023

a) Wrote potential research projects for discussions with ONO research

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
NWO	ENW-M2	731.164	365.582	03/2024	48	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Marvin van Toorn	Incision aftercare: Nucleotide excision repair after the cut

Madelon Maurice

UMC Utrecht

1. General information

Research Focus	Mechanisms of cell-cell communication and tissue self-organization
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The overall aim of our work is to gain a fundamental understanding of the dual nature of signals that guide homeostatic tissue renewal and cancer growth. In healthy tissue renewal, a handful of signaling pathways supports the maintenance of small populations of adult stem cells. Deregulation of these pathways due to mutations is strongly linked to cancer development. We aim to gain a mechanistic understanding of how adult stem cells and their progenitors process signaling input received from their environment and how the underlying molecular events are exploited for cancer growth. Main scientific questions:

1) How do stem cells communicate with their niche? We investigate how cells interpret signals received at their cell surface and how dysregulation of receptor-mediated signal relay by mutations leads to cancer.

2) What is the impact of mutations on protein function? By examining how mutations alter protein activity to drive cancer predisposition, initiation and growth we aim to uncover patient-specific disease mechanisms and develop novel cancer-targeting approaches.

3) How can we employ our molecular knowledge to develop tailored treatment strategies? We integrate our fundamental insights with various strategies to interfere with inappropriate cell signaling to translate our findings into applications.

2.2. Major scientific achievements in 2023

a) We investigated the mode of action of a novel antifolate (C1) that, like methotrexate, potently inhibits dihydrofolate reductase (DHFR) and downstream one-carbon metabolism. We uncovered that polyglutamylation-independent antifolates like C1 can be applied to exert selective pressure on FPGS-deficient cells during chemotherapy, employing a vulnerability created by polyglutamylation deficiency (publication link).

b) We examined how LKB1 deficiency, linked to Peutz-Jeghers syndrome (PJS), alters the phenotypical landscape of the intestinal epithelium to mediate an increased cancer risk, using organoid models. We found that mutations in LKB1 predispose the intestinal epithelium to uncontrolled growth along the serrated pathway, providing an explanation for the increased cancer risk in PJS patients (BioRxiv link).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Driver mechanisms of Axin tumor suppressor mutations in Human cancer.

AXIN1 is the primary organizing protein of the destruction complex, a key regulatory complex of WNT signaling that often acquires inactivating mutations in cancer. AXIN1 self-polymerization induces the formation of cytosolic condensates, but how AXIN1 conformation and dynamics within condensates links to turnover of the key WNT regulator ß-catenin remains unknown. Using nuclear magnetic resonance (NMR) spectroscopy, we uncovered three motifs within the intrinsically disordered region (IDR) of AXIN1 that interact with the self - polymerizing DIX domain. Together, our study sheds light on the multivalent interactions that underlie ß - catenin destruction complex activity and indicates that manipulation of AXIN1 condensate-forming capacity may promote Wnt pathway suppression in cancer cells. In general, it is difficult to obtain funding for a project that aims to understand very fundamental protein interactions and function. With these results, we can now start investigating how AXIN1 point mutations affect destruction complex assembly and function in cancer.

Project B) The role of Wnt niches in cancer.

Although expression of WNT7B/10A in pancreatic ductal adenocarcinoma (PDAC) correlates with a more aggressive PDAC subtype and lower patient survival rates, the mechanisms by which these WNTs drive PDAC progression remains unknown. We examined the role of these WNTs using patient-derived PDAC organoids. Using WNT inhibitors and knock-out strategies, we show that tumor growth and survival relies on tumorintrinsic WNT7B/10A expression. WNT7B knock-in reporter organoids reveal that WNT7B-high expressing cells are heterogeneously distributed within PDAC organoids. Furthermore, single-cell profiling uncovers that WNT7B drives a transcriptional program for proliferation and, importantly, prevents expression of a more differentiated, classical PDAC signature that is re-activated upon WNT inhibition. In summary, our work uncovers a role of WNT7B/10A in driving PDAC tumor subtype heterogeneity and argues that WNT inhibition
may be applied to drive a 'class switch' to a more differentiated, less aggressive cancer subtype that correlates to improved therapeutical response. Without convincing data that validate these model systems, it would be difficult to be competitive in funding schemes.

Project C) Targeting undruggable membrane proteins using SureTACs.

We developed a proprietary platform technology for induced degradation of membrane proteins using heterobispecific antibodies (SureTACs) that bring a membrane-bound E3 ubiquitin ligase in close proximity to a target protein to induce ubiquitin-mediated endocytosis and degradation of the target. For SureTACs generation, single chain antibodies against the extracellular parts of E3 and target are incorporated into a bispecific antibody format. To identify optimal E3-target pairs with degrader potency, we used Oncode funding to set up assays and screen for E3s that demonstrate capacity to degrade a hard -to-drug cancer target that belongs to the SLC protein family. Based on these findings, we are currently examining a set of nanobodies generated against membrane E3s and this SLC family member. This strategy is high-risk as there is no guarantee that SureTACs antibodies will show highly potent degrader capacity. Thus, without preliminary data it would have been difficult to obtain funding via regular funding schemes.

Project D) Uncovering the mechanistic basis of cancer predisposition in PJS.

We previously uncovered that LKB1 loss drives the mouse intestinal epithelium into a premalignant program along the serrated pathway, thus providing novel insights in how patients suffering from Peutz Jeghers Syndrome acquire an increased cancer risk (Ms submitted). To obtain an in-depth understanding of how LKB1 loss induces molecular alterations that promote polyposis and tumour formation within human PJS colon epithelia, we collaborated with clinicians of the Amsterdam UMC and KU Leuven to generate a PJS patient - derived colon organoid biobank (>10 patients). We are currently characterizing PJS-specific genotypic and phenotypic alterations, focussing on polyp and non-polyp derived organoids. Without Oncode support, it would have been difficult to initiate this research line as the work is entirely new to my lab; a proven track record is commonly required to acquire funding. With our preliminary findings we obtained KWF funding to continue our efforts.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) A major benefit from being member of Oncode is the regular interactions with the tech transfer team. Over the course of the past year, Oncode greatly facilitated the next steps in the development of our SureTACs platform technology to induce degradation of oncogenic membrane proteins. I received great help with the identification of IP and the writing of a patent application (patent filed beginning of 2024). Also, when presenting our results at scientific meetings or when pitching towards new investors, it has been very useful to be able to interact with the BDs for the preparation of nonconfidential presentations.

b) With help of the Oncode business developers we were able to obtain legal advice and arrange all paperwork to be able to appoint the first employee of our startup company Laigo Bio, a real milestone for the company. I also received very valuable help with questions related to my (future) role for Laigo Bio and to the way of working with our investor ArgoBio.

c) Another great benefit is the regular interactions with Oncode PIs, to learn about latest insights and technological advances within the community. After hearing about the expertise and research plans of the newly recruited Oncode PIs, I became interested in the model systems of Daniela Thommen (NKI), in which she treats patient -derived tumor fragments with immune checkpoint therapies to faithfully predict patient responses in the clinic. I contacted Daniela and we started a collaboration to evaluate the functional activity of our newly generated immune oncology target degrader SureTACs in her ex vivo patient models. Oncode helped to make the appropriate arrangements, such as confidentiality agreements and material transfer documents.

2.4.2. Contribution to the Oncode community in 2023

a) I am a member of the RMC. We convene every month to discuss Oncode activities, current and future strategies and programs, and advice the Oncode management team on these matters. Participation also involves regular reviewing and evaluation of applications for calls, e.g. for clinical proof-of-concept, drug screening proposals etc.

b) I participated within the major donor program of Oncode that took place in a recording studio in Iisselsteijn. This event involved a round-table discussion in which I explained how the Oncode valorization team has helped me to develop SureTACs technology and launch the startup Laigo Bio. Discussion was led by Dionne Stax.

c) I participated in all PI meetings and members of my lab have joined the majority of Oncode meetings and workshops. I was a member of the evaluation and selection committee of former Oncode junior PIs for entry as senior PI in Oncode Phase II. I was a member of the review committee for the selection of new Oncode PIs, which included reviewing.

applications and interviewing candidates.

2.4.3. Key collaborations within Oncode in 2023

a) To uncover mode of action of a novel small molecule that strongly inhibited the growth of some but not all cancer cell lines, we collaborated with Mario van der Stelt which greatly helped us to identify potential targets of the compound. Together with the lab of Michiel Vermeulen, we uncovered this compound as a novel anti-folate that selectively acts on FPGS-deficient cells, thus employing a vulnerability created by treatment with conventional anti-folates such as methotrexate (publication link). Both collaborations started thanks to my affiliation with Oncode.

b) We collaborate with Ruben van Boxtel to assess how patients with Peutz-Jeghers Syndrome (PJS) suffer from an increased cancer risk. We have generated a biobank of polyp and non-polyp organoids derived from PJS patient colon tissues and are currently assessing the mutational landscape of these tissues, including the contribution of mutational signatures of exogenous and endogenous sources. Our insights will be helpful to understand the genetic basis of tumor development in PJS patients. This collaboration only started recently and did not exist before I joined Oncode.

c) Together with Linde Meyaard we are generating SureTACs-based bispecific antibodies aimed at degrading a novel immune oncology target, for treatment of a subtype of leukemia. Although Linde and I know each other for a long time, we started collaborating only recently. The work was also encouraged by our Oncode BD, who helped making the formal arrangements to gain access to e ach other's reagents.

2.4.4. Major valorization achievements in 2023

a) Based on my seminar at the Institute of Chemical Immunology (ICI) meeting in Leiden (June 2023), I was invited for an interview for the ICI Bulletin about our start-up Laigo Bio. Topics included the basic idea of our technology, the activities to develop SureTACs bispecific reagents to degrade membrane proteins, and how we aim to proceed towards testing our SureTACs reagents in the clinic.

b) We launched the website for Laigo Bio (laigobio.com). Furthermore, with help of the Oncode business developers we were able to obtain legal advice and arrange all paperwork to be able to appoint the first employee of our startup company Laigo Bio, a real milestone for the company.

c) I led the 'Power hour' at the Gord on mee ting for WNT signaling in Barce lona, which is de signed to addre s ways to improve diversity and inclusion in science by providing a safe environment for informal and meaningful conversations amongst colleagues of all career stages. Participants involved around 100 international researchers.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Health Holland	TKI-UMCU	473.400	336.400	11/2023	24	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Jan Paul Medema

Amsterdam UMC

1. General information

Research Focus	Experimental oncology and radiobiology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The laboratory of Experimental Oncology and Radiobiology houses multiple research teams under my supervision and the supervision of L. Vermeulen. These teams study cancers of the gastrointestinal tract, specifically oesophageal, pancreas and colorectal cancer. The aim is to understand the heterogeneity present within cancers and between distinct cancer patients. For pancreas and colorectal cancer we have been instrumental in the design of molecular subtypes that identify patient groups with clearly distinctive tumors from a biological point of view. Understanding the ontogeny, molecular wiring and vulnerabilities of these molecular subtypes is key to our research program.
Next to this patient-to-patient variation we study the intra-tumor heterogeneity from a cancer stem cell perspective. Interaction with the microenvironment, the ECM, nutrients, microvasculature and immune components is part of our studies to unravel biological wiring as well as novel targets for therapy. Moreover, our work involves the regulation of cell death mechanisms specifically zooming into the mitochondria.
For our studies we depend on primary tumor material from patients and hence we have optimal connection to several clinical departments. Moreover, organoids and xenografts derived from tumor material as well as a wide range of mouse models is part of our research infrastructure.

2.2. Major scientific achievements in 2023

a) After years of study we finally managed to identify the specific role PAK2 plays in the mesenchymal subtype of colorectal cancer (J Exp Clin Cancer Res 2023, impact factor 12,5). Targeting this kinase prevents metastatic spread, in vitro and in vivo.

b) We set up a large bank of murine organoid models with distinct genotypes and classified them according to the CMS models. This resulted in the identification of the underlying wiring of the CMS3 subtype. In addition, we used this program to set up highly sophisticated mouse models of CRC in which intra colonic injections are performed with an endoscope that also allows for close monitoring of disease progression.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) CMS in CRC clinical trials.
We have finalized the development of a Nanostring-based classifier that is currently under submission. This classifier is drilled down to a 55 gene analysis on Nanostring and applied in multiple studies into clinical samples to define the role of CMS in the choice and efficacy of therapy. (J Pathol 2023). Interestingly we now show that the actual expression levels of these 55 genes can be used to predict the gen expression of a much larger pool of genes with very high confidence. The current program therefore zooms in on this predictive power and the bioinformatic pipeline that is needed to gain large insight form a small snapshot.

Project B) MCL1 activation in CRC
MCL1 is a crucial regulator of apoptosis in cancer cells, yet recent findings indicate that it is also related to the regulation of metabolism. We have identified a novel escape mechanism that regulates the expression of MCL1 and allows tumor cells to adapt to attack of their mitochondria. In essence cancer cells appear to respond to induction of apoptosis by upregulating their anti-apoptotic machinery (MCL1). We are setting up models to study the mechanism underlying this regulation and will use these to perform drop-out screens to identify the key players.

Project C) Mitochondria as point of entry.
Mitochondria display key changes in cancer that are not completely understood. In addition, they represent the central node where cell death is regulated. We have identified multiple players that either selectively sensitize colorectal cancer cells to mitochondrial-induced apoptosis (GSK3B inhibition and Sulfatide metabolism) and have identified CRC subtype specific therapies that target mitochondria directly to kill cancer cells (elesclomol). These findings are all under review and show that mitochondria in (mesenchymal) cancer cells display selective vulnerabilities that can be exploited to induce cell death.

Project D) ECM as transforming entity.
This project is initiated as a new project based on exciting results obtained in another KWF-funded project. The ECM is a strong determinant of the morphology of colon epithelial cells. Our data now shows that it is also a strong driver of transformation. We found that plating cells on collagen not only induces a transition towards more fetal epithelium, but also makes the cells more vulnerable to mistakes in the cell cycle process. In the case of heterozygous mutations this pushes them towards homozygosity and transformation. The mechanism by which this occurs requires further evaluation. In the last year we have set up human and mouse models to study this process in vitro and in vivo. In addition, we found that several models of CRC contain a similar balance between epithelial and more fetal-like cells and that this is a crucial determinant for metastasis. Understanding the ratio and specifically the role of Notch signaling and CXRC4 in this process is key in our approach

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The change from OI to Head of institute has been an enormous change, which from a scientific point of view has not improved my attention for the research.

b) The ease by which we go off on new paths is still a crucial part of the funding. In the last year we have invested in the equipment and workflow of the most elegant orthotopic model for CRC in mice with endoscopic monitoring and injections. This has strongly broadened and improved our potential for in vivo work.

2.4.2. Contribution to the Oncode community in 2023

a) I have been part of the RMC for the complete year.

b) As of October 1st but in effect already earlier I have become Head of the Institute for effectively 2 days a week.

c) During the first part of the year I have been very strongly involved in the selection of the junior OIs.

2.4.3. Key collaborations within Oncode in 2023

a) Louis Vermeulen, Rene Bernards, Madelon Maurice and Joep Grootjans on EMT and CRC. Several were excisting before Oncode others are Oncode based.

b) Geert Kops on chromosomal instability on ECM. This one is purely Oncode based.

c) Boudewijn Burgering on metabolism, Oncode based

2.4.4. Major valorization achievements in 2023

a) Both the Sunrise and Basalt studies were running in 2023. The Sunrise is now finished and will be communicated soon. The Basalt continues into 2024.

b) Bijlsma from my team is PI of a Marie Curie Doctoral Network that was awarded in 2023 (PRESSURE). The IP negotiations are handled by Oncode.

c) Contracts with SeekIn for the development of a blood test in cancer.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
	Sunrise	15-4-20		No	Lead	
	Basalt	1-10-18		No	Lead	

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Roxan Helderman	The heat and time is on
Leonie Hartl	The ambiguous role of C/EBPdelta in pancreatic cancer

René Medema

Netherlands Cancer Institute

1. General information

Research Focus	Cell division and cancer
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

We study how (epi)genome evolution affects the fitness of tumor cells. In 2023, we showed that epigenetic evolution, achieved via reorganisation of the 3D genome, can play an important role in the acquisition of drug resistance (Manon et al., Cell Rep., 2023). We showed that activation of the multidrug resistance gene ABCB1 is associated with reduced H3K9 trimethylation, increased H3K27 acetylation, and ABCB1 displacement from the nuclear lamina. Merely altering DNA methylation and/or H3K27 methylation did not promote resistance, but disrupting nuclear lamina association did facilitate ABCB1 derepression. Based on these findings we proposed a model in which nuclear lamina dissociation of a repressed gene allows for its activation. In addition, whilst studying genome evolution via defective repair, we found that the meiotic recombination co-factor MND1 facilitates homologous recombination (HR) of two-ended DSBs in somatic cells, but is dispensable for HR-mediated repair of one-ended DSBs. MND1 is specifically active in G2 phase, and the lack of MND1-driven HR repair directly potentiates the toxicity of IR-induced damage, which could open new possibilities for therapeutic intervention, specifically in HR-proficient tumors.

2.2. Major scientific achievements in 2023

a) We showed that nuclear lamina dissociation of a repressed gene allows for its activation, implying that deregulation of the 3D genome topology plays an important role in tumor evolution and the acquisition of drug resistance. This represents an entirely new perspective on tumor evolution, not genetic but epi-genetic.

b) We showed that MND1 is required for HR in somatic cells, in particular for repair of double-ended breaks in G2. Consequently, MND1 loss potentiates the G2 DNA damage checkpoint, causing hypersensitivity to DNA damage during G2 phase. This opens a new avenue to target repair-deficient tumors.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Liabilities that arise with genomic instability.

This project is still ongoing. We continue to study liabilities that arise through repair defects in tumor cells, and by genetic alterations that commonly occur in cancer. Our motivation to do this is that it allows us to uncover important new insights in tumor biology, but also allows us to identify novel targets for anti-cancer therapy. We execute genetic screens to identify genes that are required to tolerate these common genetic alterations, and validate the hits through extensive follow-up experiments. For example, in our screens for repair-associated genes, we identified Mnd1 as a novel HR-associated gene, whose inhibition results in increased sensitivity to sub-classes of DNA damaging agents. We have subsequently identified synthetic lethal interactors of Mnd1. Only once these liabilities are identified and properly validated, funding can be applied for in the standard funding landscape.

Project B) The influence of chromatin modifications on the DNA damage response

This project is still ongoing. We have continued our efforts to better understand the relation between chromatin context and repair pathway choice. By combining knockout screening with a dual MMEJ:NHEJ reporter inserted in 19 different chromatin environments, we identified dozens of DNA repair proteins that modulate pathway balance dependent on the local chromatin state. We showed that in a diversity of human cancer types, loss of several of these proteins alters the distribution of pathway-specific mutations between heterochromatin and euchromatin. We have recently completed a revised manuscript describing these findings. In addition, we have found that DNA lesions result in displacement of DNA from the nuclear envelope. We believe this is critical for the DNA damage response, but since we currently don't know the functional relevance of this finding we will need more information to be able to apply for funding in the standard funding landscape.

Project C) Locus reactivation during epigenetic evolution.

This project is still ongoing. We study how the epigenetic landscape is affected by DNA damaging insults. We found that upon break formation in the ABCB1 gene, the locus moves to the nuclear interior. Strikingly, a subset of cells derepress the ABCB1 gene upon DSB induction. In these cells, ABCB1 is retained in the nuclear interior and acquires active histone modifications. We hypothesize that in a small subset of cells that engage in resection-dependent repair, the epigenome is not faithfully restored after damage induction, leading to ABCB1 gene re-activation and acquisition of taxol-resistance. Once we can definitively prove this link, we can apply for further funding to investigate if/how epigenetic scars that result from DNA damage can promote tumor evolution.

Project D) The contribution of extracellular DNA to tumor heterogeneity.

This project is still ongoing. We established several cellular models to study double minutes. We derived isogenic pairs of cell lines, with and without double minutes as well as models in which double minutes are tagged, which allows their visualization in living cells. We have completed a number of genome-wide screens aimed at identifying vulnerabilities specific to cells containing double minutes and are currently validating the hits. This work is now funded through the standard funding landscape, but the early work on drug sensitivity is still dependent on Oncode base funding.

Project E) The influence of ecDNA on drug sensitivity.

During the course of project D, we discovered that the ecDNA-containing cell lines display enhanced sensitivity to commonly used anti-cancer interventions. We are currently studying this in more detail, as it potentially represents a good way to target tumors that carry ecDNA. This we do using Oncode base funding.

Project F) Karyotype evolution in cancer.
This project is (almost) completed. We have submitted a paper describing commonly mechanisms of adaptation to aneuploidy. We show that aneuploid clones initially display reduced fitness, enhanced levels of chromosomal instability and an upregulated inflammatory response. Intriguingly, after a prolonged period of culturing, all adapted clones displayed reduced CIN and reduced inflammatory signaling, suggesting that these are common aspects of adaptation to aneuploidy. We provide evidence that amplification of oncogenic KRAS promotes adaptation to aneuploidy.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) My team was able to perform a screen for compounds that affect the outcome of a DNA damaging insult thanks to the Oncode drug repurposing program. This screen would not have been possible for us without the support of Oncode.

b) For the two major achievements I listed in this annual report I collaborated with 4 other groups in the Oncode community (Brummelkamp, Zwart, van Steensel and de Wit (currently no longer in Oncode)). Without the access to their technologies and expertise, these achievements would not have been possible.

c) After discussing results from the van Rheenen lab I was informed about at an Oncode event, I engaged in a collaboration that included a total of 5 groups from the Oncode community, to demonstrate that taxanes kill cancer cells through the induction of non-canonical T cell cytotoxicity (Vennin et al., Cancer Cell, 2023).

2.4.2. Contribution to the Oncode community in 2023

a) I have represented the NKI and Oncode Institute in 4.UNCAN.eu, a European consortium of cancer research institutes that 3MEuro to generate a blueprint for UNCAN.eu for the EU Commission. UNCAN.eu is one of the 13 specific objectives of the Mission on Cancer and one of the ten flagships of the Europe's Cancer Beating plan.

b) I participated in the Drug Repurposing Program of Oncode. We successfully applied to the program and executed a drug screen to identify compounds that affect the outcome of a DNA damaging insult. We have validated several hits and are planning further follow up.

c) Several members of my team, including myself, participated in the Oncode Annual meeting.

2.4.3. Key collaborations within Oncode in 2023

a) Collaboration with the van Steensel lab to study the relation between chromatin context and repair pathway choice. I collaborated with the van Steensel lab prior to Oncode, but the launch of Oncode made it possible for us to explore this aspect in greater detail. Vergara et al., under revision but see also Vergara et al., bioRxiv, 2022; doi: https://doi.org/10.1101/2022.10.07.511243

b) Collaboration with the van Steensel and Zwart labs to study 3D genome reorganization during the acquisition of drug resistance. Manjon et al., Cell Rep. 2023; doi 10.1016/j.celrep.2023.113124

c) Collaboration with the van Rheenen, Rios, Akkari and Voest labs to study how taxanes kills tumor cells. Vennin et al., Cancer Cell, 2023; doi:10.1016/j.ccell.2023.05.009

2.4.4. Major valorization achievements in 2023

a) Had regular meetings with Oncode Business Developer to discuss findings with potential for valorization.

b) We have received approval of the department of Radiotherapy for the execution of a clinical trial to validate split fractionation schedules as more efficacious in radiotherapy. The trial is now being prepared for approval by the medical ethical committee (METC).

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF	Exploration	544000	544000	11-2022	48	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
M.S. van Ruiten	Make or Brake it: On the roles of the cohesion acetylation cycle

Linde Meyaard

UMC Utrect

1. General information

Research Focus	Infection & immunity
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

We aim to develop novel strategies targeting inhibitory receptors to benefit more cancer patients. LAIR-1 is an inhibitory collagen receptor and a promising therapeutic target, particularly for those patients with immune-excluded tumors that lack immune cell infiltration. Using Oncode base funds, we discovered that absence of LAIR-1 enhances migration of T cells towards the tumor nest. We now showed that LAIR-1 blockade therapy in combination with PDL-1 blockade enhances anti-tumor response in mice (Singh et al, Cancer Immunol Immunother, 2024). We are currently studying how tumor-associated collagens regulate LAIR-1 signaling to identify patients that benefit from LAIR-1 targeted therapy. LAIR-1 blockade, either or not in combination with blocking PD1 or LILRB4, is in Phase I/II clinical trials in several companies. We discovered that scavenger receptor MARCO is a novel ligand for LAIR-1 that can regulate LAIR-1 function in cis. LAIR-1/MARCO co-expression is associated with poor survival in certain tumors.
Furthermore, using Oncode base funds, we developed a bioinformatics pipeline for the identification of novel inhibitory receptors, which we are exploiting in the Oncode Synergy/KWF consortium project to mobilize myeloid cells for cancer treatment with current focus on eosinophils. Our discoveries of both the LAIR-1/MARCO interaction and the inhibitory receptor pipeline have led to patent applications this year.

2.2. Major scientific achievements in 2023

a) We discovered that scavenger receptor MARCO is a novel ligand for LAIR-1 and can regulate LAIR-1 function in cis. In addition, we showed that LAIR-1 inhibits T cell migration through collagen-rich matrices and thus interferes with tumor killing, implying application of LAIR-1 for the treatment of immune-excluded tumors.

b) Based on our discovery that LAIR-1 ligands are sensitive to modification and that these modifications impact the ability of LAIR-1 to provide inhibitory signals to immune cells, we obtained NWO ENW-M2 funding with Kristina Ganzinger to study the fundamental requirements for signaling by LAIR-1 and other inhibitory receptors.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) LAIR-1 – collagen interaction in the tumor microenvironment
This project studies the collagen side of LAIR:collagen interaction. High collagens concentrations correlate with poor prognosis in for cancer patients. Here, we will characterize tumor-associated collagen modifications and investigate how different collagen modifications impact LAIR-1 binding and signaling to see if LAIR-1 blockade can improve T cell functionality in the presence of tumor-associated collagens. This will lead to better tools to select cancer patients for LAIR-1 targeted immune therapy. In this project we collaborate with Oncode PIs Monika Wolkers, Sarah Derks and Jacco van Rheenen. Currently funded by a KWF grant.

Project B) Inhibitory receptor signaling
The initial events and precise requirements for inhibitory receptor signaling remain unknown. In collaboration with Kristina Ganzinger, we study the temporal and special requirements of inhibitory receptor signaling. Currently funded by an NWO-ENW M2 grant.

Project C) Pipeline for novel inhibitory receptors
We developed a bioinformatics pipeline for the identification of novel inhibitory receptors on which a patent application was submitted. We are exploiting in the Oncode Synergy/KWF consortium project to mobilize myeloid cells for cancer treatment with current focus on eosinophils and in a project on gamma delta T cells. Currently funded in part by KWF and in part by Oncode base fund. In addition, part of project with Jurgen Kuball funded by Oncode Accelerator.

Project D) Inhibitory receptors in non-immune cells
Non-haemopoietic cells are increasingly recognized for their role in cancer immunity. We found also endothelial cells and fibroblasts express inhibitory receptors (von Richthofen and Meyaard, Eur J Immunol, 2023). In this project, we explore the capability of inhibitory receptors on non-haemopoietic cells to suppress immune responses either initiated by these cells, or indirectly though interaction with immune cells. Together with the organoid lab previously headed by Hans Clevers, we have generated organoids that express inhibitory receptors that lack the signaling domain. This project is difficult to fund through the standard funding landscape because it is highly explorative with little preliminary data (only expression data from RNAseq). However, this project potentially has extremely high impact by refocusing immune therapy towards non-immune cells. If we find that indeed non-immune cells have inhibitory (immune) receptors that shape immune responses, these can be targeted in tumors to improve anti-tumor responses. Funded by Oncode basefund.

Project E) Human 3D organ models
Mouse models have been fundamental to the current therapeutics that redirect/reactivate the immune system but they clearly have their limits in translation ability to human disease. Blocking LAIR-1 is currently in the pipeline of multiple companies, but for LAIR-1 mouse models are specifically difficult to translate. While we found absolutely no effect of LAIR-1 blockade in naïve mice, blockade of LAIR-1 was effective in humanized mouse models (Singh et al, 2023). Hence, we need another, better, model to study LAIR-1 responses. We currently develop a fully human 3D model that resembles human skin. The human structural cells produce fully-human extra cellular matrix with native LAIR-1 ligands. In addition, we add human myeloid cells to study the role of LAIR-1 in this tissue. Funded by Oncode basefund. Currently applying for external funding.

Project F) Myeloid cells in cancer Synergy project

Current checkpoint therapies target T cells, while myeloid cells have a major impact on anti-cancer responses. In this project, we aim to identify novel myeloid checkpoints that can be blocked to improve anti-cancer immune responses. Oncode Synergy project in collaboration with several Oncode PIs. Funded in part by KWF and in part by Oncode base fund.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Valorization support. Emil Pot and team greatly supported our lab to obtain private funding from argenx, apply for patents with NGM, and discuss development of blocking-CD200R antibodies. Without the support from the Oncode Valorisation team, we expect that these applications would have been delayed/stalled.

b) Collaborations. Also this year, we had multiple collaborations through Oncode. With Madelon Maurice we explore the option to reduce LAIR-1 expression with the use of SureTag; with Harmjan Vos (Burgering) we performed phospho-proteomics to understand LAIR-1 function on T cells, and with Hans Clevers we explore the function of inhibitory receptors on non-immune cells. In addition we have multiple ongoing collaborations with new Oncode PI Kristina Ganzinger.

2.4.2. Contribution to the Oncode community in 2023

a) UMC Utrecht UMAB and flow facility The CTI houses the antibody facility UMAB (lead by Jeanette Leusen), which is available for the whole Oncode community as a facility. As part of the Oncode equipment and infrastructure program, the central flow facility of the UMC Utrecht applied for and received 100 k€ to invest in a multi-color high speed cell sorter. The central flow facility, including support staff, is embedded in the Center for Translational Immunology (CTI), which matched the additional necessary 380 k€. This core facility is accessible to and used by all UMC Utrecht Oncode investigators.

b) I reviewed the Oncode Junior PI applications in 2023.

2.4.3. Key collaborations within Oncode in 2023

a) Sjoerd van den Burg, Karin de Visser, Leila Akkari, Jacco van Reenen and Jeroen de Ridder. Oncode/KWF accelerator grant: Mobilizing innate leukocytes to cure difficult-to-treat tumors. I did not collaborate with any of these PIs before joining Oncode.

b) Kristina Ganzinger. We collaborate on multiple projects. Our collaboration was started before Kristina joined Oncode.

c) Hans Clevers. We collaborate on inhibitory receptors on non-immune cells. This is a collaboration on a new topic, not started before joining Oncode.

2.4.4. Major valorization achievements in 2023

a) argenx collaboration. Collaborative research agreement signed with argenx on the design, development and functional testing of novel inhibitory receptor agonists, starting with LAIR-1 and SIRL-1. We hired a PhD student, technician and a post-doc, the latter is working partially in the lab of new Oncode PI Kristina Ganzinger.

b) Patent submission. Immune modulating inhibitory receptors. Application based on our bioinformatics pipeline for novel inhibitory receptors. We are currently exploring the opportunities to bring this IP into a NewCo on modulation of engineered T cells (Kuball) and to take a similar approach in other cell types with a different partner, among which Sanofi.

c) Patent submission. METHODS FOR PREDICTING RESPONSIVENESS TO LAIR1-BINDING AGENTS. Based on the work we did with NGM Biopharmaceuticals on MARCO as a novel ligand for LAIR-1. Application is a joint submission of NGM Biopharmaceuticals and UMC Utrecht

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
NWO	ENW-M2	740,121	370,060	02/2024	48	Main applicant
Van Loghem award	Dutch Society of Immunology career award	-	-	-	-	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Ruben Geerdink	Hit the brakes! Inhibitory receptors as therapeutic targets for neutrophil-driven pathology
Helen von Richthofen	Immune inhibitory receptors in blood and barrier tissues: conveyers of context

Sjaak Neefjes

LUMC

1. General information

Research Focus	Cell biology, infection, cancer and cancer drugs
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The Neefjes lab focusses on 3 topics. 1. The molecular mechanisms of MHC class I and class II antigen presentation. This process is at the heart of cancer immunotherapy. We are focussing of the mechanisms of MHC class II transport in cells and the dynamics within an MHC class II compartment. Other aspects include off target binding by tetramers and peptide recycling. 2. Bacteria and cancer. We have shown how and why Salmonella can contribute to gallbladder and colon carcinoma. We have combined epidemiology and lab experiments to link frequent mild infections to colon cancer risk and are now identifying dangerous salmonella subspecies. How bacteria imprint the transformed state in cells remains a major topic. 3. Detoxifying anthracyclines. We have identified a new mechanism of the common anti-cancer drug doxorubicin and chemically separated the two activities to determine that the 'new' activity is the most cytotoxic one. We then showed that some of these drugs lack the major side effects but remained active drugs. We are in the process of making two such drugs for clinical testing.

2.2. Major scientific achievements in 2023

a) We have shown that multivesicular bodies are in fact dynamic structures where the internal vesicles can fuse back to the limiting membrane (retrofusion). The part that does not participate in retrofusion become exosomes. We thus uncovered a fundamental process in cells with various consequences for cell signaling.

b) We have shown that people with frequent mild infections by salmonella that are usually only recognized by the immune system have a higher risk of colon cancer.

c) We have produced GMP aclarubicin that is currently being prepared for clinical studies of R/R AML patients.

d) We have solved the GMP synthesis steps for the production of N,N-dimethyldoxorubicin in the plant. The test batch will be synthesized early 2013.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Modifying Streptomyces for the production of N,N-dimethyldoxorubicin.

We aimed to modify the high produces Streptomyces peucetius strain by including enzymes to generate N,N-dimethyldoxorubicin. The would allow high yield production of this drug, which in fact is a detoxified form of doxorubicin that can also be used for patients that have completed treatment due to the cardiotoxic effects of doxorubicin. In addition, cancer patients can be treated more safely without the long term risk of heart issues. While we were able to modify the strains to make low amounts of the demethylated drug, we failed to improve it further. Further testing showed that the medication generated a strong antibiotic that was killing the producer strain. Our strategy is now to make it by half-chemistry starting with doxorubicin and to select strains that are resistant. The half-chemistry approach is now completed and will move to the plant for synthesis of the test batch for preclinical tox experiments.

Project B) The oral microbiome and metabolome in health and oral cancer

We have generated the tools for the proper and reproducible detection of the metabolome in the oral cavity. This should that the metabolites differ in the morning from later in the day and also from the location where these are sampled. We have made some preliminary tests as to whether the metabolome and the microbiome differs on an oral tumor from the healthy environment. At this point, we do not have indications that this is the case.

Project C) Intracellular lipid transfer and membrane contact sites

Lipids are hydrophobic and have to be distributed around the cell. Transfer from one to the next compartment occurs at membrane contact sites. We have identified 5 ER proteins in control of these contact sites that act in 2 subclusters with different interacting proteins. We have classified the many potential lipid transfer proteins that are -for example- critical for lipid transfer from ER to Golgi for glycolipid biochemistry. The role of these membrane contact sites in the control of cell biology is further expanded in research related to cholesterol transfer and modifications.

2.4 Impact and contribution

Track code	Title of Project	Oncode call (*)	Status	Start date (#)
P2021-0070	HTA assessment of the use of Detoxified Rubicin compounds Aclarubicin and NNdimethyldoxorubicin in patients eligible to receive a 2L rubicin based chemotherapy	Technology Development Fund	Active	1/1/2022
P2022-0030	Identifying the optimal regulatory strategy and prepare for regulatory interactions (EMA or national) to repurpose and register Aclarubicin as an alternative treatment for relapsed AML	Technology Development Fund	Active	5/15/2022
P2018-0016	Molecular mechanisms in action: high-resolution time lapse imaging in living cells	Equipment Grant	Completed	11/15/2019

2.4.1. How Oncode impacted your research in 2023

a) Oncode made it possible to perform research that otherwise would be difficult to fund (ie. high risk/high gain type of research). All three lines that were directly funded illustrate this point.

b) The production of N,N-dimethyldoxorubicin by modifying the producing Streptomyces strain would not have been possible through Oncode support. While the identification of this drug as one superior to what is currently used was made by the Neefjes lab, the modification of the strain requires new technology and knowledge and was acquired in collaboration with the van Wezel lab through a joint PhD student.

c) Technology. Oncode supported a spinning disk microscope that is heavily used and critical for the more cell biological experiments in the lab.

2.4.2. Contribution to the Oncode community in 2023

a) We have produced scripts for the automatic assignment of moving endosomes relative to the ER and made these broadly available.

b) We have generated aclarubicin and synthesized N,N-dimethyldoxorubicin. These drugs have been distributed and used in various drug testing systems (at LUMC, PMC and NKI) for cancer cytotoxic effects.

2.4.3. Key collaborations within Oncode in 2023

Name Collaborator	Were you already collaborating prior to Oncode?	Subject Collaboration	Reference
Peter ten Dijke	no	Endosomal transport and TGF signaling	
Wilbert Zwart	yes	ERalpha mechanisms	
Jannie Borst	sometimes	Cell biology of immune responses	
Mario van der Stelt	hardly	Medchem Flt3 inhibitor	

2.4.4. Major valorization achievements in 2023

a) Aclarubicin is a anthracycline drug that -as we showed- does not induce cardiotoxicity and has a unique treatment effect on hematological cancers. We have produced a GMP batch aclarubicin for clinical trials of R/R AML patients. This batch is now evaluated by AvL Pharmacy for release.

b) N,N-dimethyldoxorubicin has a different tissue distribution than aclarubicin and may be used for solid tumors. We have developed the GMP synthesis route and transferred that to Olon for GMP production. The further development for the transfer to the production plant is completed.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Oncode	Oncode Fase 2	1.020.000	1.020.000	01-01-2023	31-12-2027	Main applicant
reNEW	reNEW		100.000	01-09-2023	31-08-2025	Co-applicant
KWF	KWF	1.156.349,49(	1.156.349,49	01-08-2022	30-09-2024	Main applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
We have made aclarubicine for R/R AML patiens	We are writing an Phase 2B trial together with HOVON and hope to start this halfway 2023. The study can be extended to skin lymphoma and other leukemias			Choose an item.	Choose an item.

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Gabrielle van Tilburg	Exploring the nature of ubiquitin linkage-specific binding. Leiden 14-03-23
Margherita Botto	Chracterization of DNA-replication proteins and their molecular mechanisms: a team business, Leiden 23-03-2023
Yufeng Li	Functional study of the human genome, Leiden 02-10-2023

Sylvie Noordermeer

LUMC

1. General information

Research Focus	DNA damage repair, homologous recombination, BRCA1
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

My group studies how genomic integrity is maintained and how disbalances in the maintenance pathways affect cancer onset and treatment outcome. Our research focusses on DNA double-strand break (DSB) repair. One of the main focusses is to better understand the cellular consequences of the wide variety of BRCA1 mutations observed in tumours. BRCA1 forms multiple protein complexes, all with unique roles in the DSB repair pathway. We study the how, when and why of BRCA1 being part of these complexes to better understand their individual roles. In addition, we study how BRCA1 mutations affect complex formation and consequently DSB repair. With this information, we aim to improve prediction of disease onset and outcome for patients with BRCA1-mutated tumours. In addition, we use this information to identify novel vulnerabilities of BRCA1-mutated tumours.

Another line of research focusses on the determinants of pathway choice during DSB repair. Using high resolution live-cell imaging of the different repair pathways, we aim to better understand which cellular factors drive the activation of a certain pathway. We combine this imaging approach with novel sequencing approaches to evaluate the genomic outcome of repair (e.g. errorfree repair, mutation or structural aberration). Also, we study the functional consequences of (cancer-derived) mutations in DSB repair factors on pathway choice. This information will yield important data on cancer development caused by such mutations and will also provide opportunities to perturb pathway choice to improve gene editing approaches.

2.2. Major scientific achievements in 2023

a) Our project identifying EXO1 as a synthetic lethal interactor of BRCA1-deficient cells was accepted for publication in Molecular Cell. In this manuscript we describe the clinically relevant synthetic lethal interaction and provide a mechanistic explanation of the interaction. This paper is the first published paper of my group which started in 2019.

b) We have finalized a project where we study the function of BRCA1's exon 11 – a frequently mutated region in tumours – via proteomic approaches. We show a novel interaction with TOPBP1 which we show is essential for driving end-resection during homologous recombination. We are about to submit a manuscript for publication.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Live-cell imaging of DSB pathway choice

In this project, we aim to understand how, when and why a cell decides to repair a DSB by one of its 4 available pathways. We do this by visualizing pathway choice by live-cell imaging approaches. In addition, we aim to correlate this pathway choice to repair outcome to better understand how error-free versus error-prone repair is regulated in normal and cancer cells. We have setup a toolbox in which we can induce a DSB and directly follow activation of homologous recombination and non-homologous recombination, 2 of the 4 pathways. Also, in collaboration with Oncode investigator Miao-Ping Chien, we are setting up single-cell sequencing techniques to determine repair outcome in the same cell we used for imaging. Oncode base fund provided essential budget to start this high-risk high-gain innovative project and gain preliminary results. We have used these results for an ENW-M1 application, which was reviewed exceptionally well (expected outcome May '24).

Project B) Studying the synthetic lethal interaction between BRCA1-deficiency and loss of EXO

Using Oncode's base fund and KWF funding we have been able to mechanistically explain our previously identified synthetic lethal interaction between BRCA1 deficiency and EXO1 loss. This project is recently published in Molecular Cell (Van de Kooij et al., 2024) We show that EXO1 loss disrupts single strand annealing (SSA) in BRCA1-mutated cells. This decrease in SSA is not observed in BRCA2-deficient cells, explaining why these cells do not show synthetic lethality with EXO1 loss. We are now focusing on identifying chemical EXO1 inhibitors for future therapeutic use. With the help of Oncode's drug repurposing program, we have conducted a compound screen resulting in one top lead that inhibits EXO1 in vitro and in vivo and results in specific cell killing of BRCA1-deficient cells. We are now actively looking for industrial partners to help us further optimize this compound. For this project, we work together with fellow Oncode investigator Jos Jonkers.

Project C) Structural studies to identify BRCA1 BRCT mutations affecting individual protein complexes

The BRCT domains of BRCA1 – frequently mutated in tumours - bind in a phospho-dependent manner to ABRAXAS and CtIP. Importantly, these two interactors have opposing effects on homologous recombination. We set out to better understand how the interaction with these two partners is regulated. With the help of Oncode's base fund, we purchased a site-saturated library of BRCA1 BRCT variants. We used this library in yeast-two-hybrid screens to identify which amino acids of the BRCT are important for binding CtIP and/or ABRAXAS. The screens identified many novel sites in the BRCT that stimulate the interaction with both proteins, but more importantly also identified unique sites that are specific for one interactor. We are in the progress of validating the data in human cell models. Simultaneously, we are testing the functional consequence of mutations that disrupt either of the two protein complexes on homologous recombination and genome stability.

Project D) Structural studies on the BRCA1-A complex

In this collaborative project with the Sixma lab (Oncode, NKI), we are interested in studying the role of the BRCA1-A complex in genomic stability. This complex has deubiquitinating activity and an inhibitory function during homologous recombination. We have characterized the effect of disrupting this complex on homologous recombination and genomic stability. Interestingly, we show that depletion of the full BRCA1-A complex has a different effect than disrupting the interaction of the complex with BRCA1. This has important implications for understanding this complex in relation to BRCA1 biology. The Sixma lab is currently performing cryo-EM studies on this complex. In our lab we will perform functional analysis to understand the biological role of important motifs in the structure. This project aligns well with project C, as one of the

interactors we study there, ABRAXAS, is part of the BRCA1-A complex. The preliminary data we have obtained will be used for a grant application later this year.

Project E) Technology development: analyzing break induction and repair using Nanopore sequencing

The repair of a DSB often results in a mutational footprint, a so-called 'scar', reminiscent of the pathway used for repair. Until now, these scars are identified using amplication-based short-read sequencing. However, such approach does not allow to look for large structural variants or chromosomal translocations. Furthermore, due to the amplification step, one can only look at the end-product: the repaired chromosomal locus, but not the broken intermediates. That is why we are developing a novel technology in the lab that we called NanoBreakR (NANOpore-based Break REpair Analysis Kit by crispR). This approach uses locus-specific long-read nanopore sequencing to simultaneously sequence repair outcomes but also repair intermediates. Our preliminary data show a high percentage of repair outcomes usually missed by short-read sequencing. Furthermore, by performing time courses, we can follow break induction and repair over time. This project was originally funded by a mini-grant of my department for innovative projects and is now funded by Oncode.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Due to Oncode's network, I was exposed to Miao-Ping Chien's research on microscopic selection of single cells for sequencing. This novel technology holds great potential for our project A. We were able to perform some preliminary experiments together, which greatly helped in writing the ENW-M1 grant application.

b) Oncode is facilitating our contact with industrial partners for EXO1 inhibitor development. The network that Oncode has for this is very helpful and provides me with the unique possibility to translate our fundamental research question into a potential novel therapy. Without the help of Oncode, I would not have the resources nor time to invest in this translational step.

c) I have actively participated in the Oncode junior PI mentoring program. This is a great program that has been very instrumental for my journey of becoming a confident group leader.

2.4.2. Contribution to the Oncode community in 2023

a) I helped organize the first in-person Oncode PI meeting in Oct '23 and am involved in organizing the Oncode-KIT meeting of 2024.

b) We actively participated in the annual Oncode meeting with an oral presentation of our PhD student Anne Schreuder.

c) I have several active collaborations within the Oncode community (see below).

2.4.3. Key collaborations within Oncode in 2023

a) Titia Sixma (NKI): I work together with Titia on multiple projects, either spearheaded by me or Titia (e.g. project C and D above). Our cellular expertise in DNA damage forms a great complementation to Titia's structural biology expertise on BRCA1 complexes. The ongoing collaborations have started after I joined Oncode, although Titia and I have collaborated also previously.

b) Jos Jonkers (NKI): With Jos, we work together to exploit novel therapeutic targets for cancer patients with BRCA1 or BRCA2 mutations. His BRCA1/2 mouse models are essential for us to make the translation of our fundamental research findings into pre-clinical models. Our collaboration was spiked by Oncode's initiatives to start a DNA damage repair (DDR) alliance,

c) Miao-Ping Chien (Erasmus MC) and Tineke Lenstra (NKI): In project A, we complement our DSB repair expertise with Tineke's and Miao-Ping's expertise on high-resolution microscopy and single cell sequencing. With their expertise, we have been able to incorporate innovative techniques to better study DSB pathway choice at the single cell level. Both these collaborations were started because of the interactions we had during the Oncode mentoring program for young group leaders.

2.4.4. Major valorization achievements in 2023

a) X

b) X

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Instruct-ERIC		In kind	In kind	01/2023	Not determined	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Organoids Group
Hubrecht Institute

1. General information

Research Focus	Adult Stem cells and cancer
Junior/Senior Oncode Investigator	N/A

2. Oncode activities

2.1. Research topics and scientific progress

We have established organotypic ex vivo culture systems (organoids) from multiple human and murine epithelial (diseased) organs. Organoids, mini-organs in a dish, allow all laboratory methods that are applied to cell lines, such as transfection, infection with recombinant viruses, imaging, *in vitro* throughput drug-screening, CRISPR-CAS9 modification, etc.

We aim to combine these technologies and thereby exploit the possibilities of the usage of (genetically modified) organoids in fundamental research, regenerative medicine, gene therapy or treating cancer (e.g. drug screenings and the testing of patient-specific drug treatments (personalized medicine).

Moreover, we are generating more complex organoid (disease) models that combine cancer cells with immune cells, defined stromal cells and/or microbes. This will allow us to model more closely -yet in a fully controlled way- the tumour environment and, for instance, the effects of immune modulators on cancers.

2.2. Major scientific achievements in 2023

a) In 2023, we published 42 scientific papers, including four articles in highly ranked journals: Science, Nat. Biotechnology, Med, and Cancer Cell. Moreover, we received funding from the KWF, started with the Oncode-Accelerator grant, and Cayetano Pleguezuelos obtained his PhD degree cum laude

2.3 Oncode research projects

2.3.1. Oncode research projects and progression thereof in 2023

Project A) Cancer: (new) organoids models and their manipulation.

Treatment of Head and Neck Cancer (HNC) consists of surgical resection, chemotherapy and/or radiotherapy, which has a high incidence of disease relapse. So far, targeted therapies have shown limited efficacy in HNC. To identify potential therapies for HNC patients, we screened a >5600 compound drug repurposing library on a genetically diverse biobank of HNC patient-derived organoids in parallel with tumor-adjacent control organoids. Hit validation with ATP-based and image-coupled readouts identified four anti-HNC drug candidates. Validation of these hits in primary in vitro tumour models (MicroOrganoSpheres) confirmed the anti-tumour potential of 2 in primary (tumour material.
Taken together, using patient-derived organoids as a preclinical differential screening platform, we identified specific drugs as potentially efficacious anti-cancer agents in the context of HNC. Next, we plan to perform in vivo studies in mice to confirm the obtained in vitro data.

Project B) Cancer: organoids and the immune system

Chimeric antigen receptor (CAR)-immune cells are a promising approach to treat cancer. With the specificity of CAR and harnessing immune cells' efficient tumour-killing ability, several CAR-T therapies have been approved clinically. However, tumour microenvironmental barriers, heterogeneity among the patients, and difficulties in antigen identification are major hurdles in applying adoptive immune cell therapy for solid tumours. To overcome these limitations, we successfully utilized cancer organoids of different origins to identify cancer-specific tumour antigens and targeted the identified cancer antigens CAR-NK cells. We observed tumour organoid-specific killing.

Project C) Cancer: organoids and bacteria.

We previously showed that the E. coli strain E. coli CRC carrying the polyketide synthase (pks) genomic island can induce specific mutations in intestinal organoids. This signature is also enriched in WGS datasets of tumours from colorectal cancer patients. The genes encoded in the pks island are responsible for synthesizing the genotoxin colibactin, which mediates this mutagenicity. The pks island is present in many strains of E. coli, including the strain E. coli Nissle, which is widely available as a probiotic. Nissle can induce mutations at a lower rate than other pks+ strains (E. coli CRC), which suggests the existence of E. coli regulatory mechanisms that control the ability of colibactin to induce mutations in human cells. We are investigating how this is regulated. In addition, we are studying how enterotoxigenic B. fragilis (ETBF) can degrade the mucus layer that protects the colonic epithelium. Finally, we aim to identify the mutational signature of Helicobacter pylori. H. pylori, causes DNA damage in human cells, but the mechanisms remain elusive. We, therefore, are establishing a model of infection of gastric organoids with H. pylori. We will then use scWGS to identify H. pylori's mutational signature and compare it to whole genome datasets of gastric tumours.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) We received tremendous help from Oncode regarding patent applications, drawing up contracts with external (commercial) parties, etc.

2.4.2. Contribution to the Oncode community in 2023

a) We continue to help establish organoid technology in multiple other labs and have made reagents, protocols and organoids available to the scientific community

2.4.3. Key collaborations within Oncode in 2023

Name Collaborator	Subject Collaboration	Reference
Hugo Snippert	Drug screening on cancer organoids	PMID 37000626
Jarno Drost	Generation and analysis of (cancer) organoids	PMID 37883554 / PMID 37020038
Ruben van Boxtel	Data analysis of (cancer) organoids of different origin	PMID 37435135

2.4.4. *Major valorization achievements in 2023*

a) *Hans Clevers gives many lectures and interviews in newspapers*

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Dutch Government	Nat. Groeifonds	325.000K	7.500K	12-2023	60	Co-applicant
KWF	KWF Res Project	690K	650K	02-2024	48	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Marie Bannier	Modelling the ocular surface with organoid technology
Cayetano Pleguezuelos	Modelling host-microbiota interactions in disease using organoid co-cultures

Alexande van Oudenaarden

Hubrecht Institute

1. General information

Research Focus	Quantitative biology of development & stem cells
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

In 2023 the van Oudenaarden lab focussed on developing novel technology to perform chromatin profiling in single cells. We developed both the experimental (Zeller et al., Nature Genetics, 2023) and computational pipelines (Yeung et al., Nature Biotechnology, 2023) to determine profiles of chromatin modifications in 10,000's of individual cells. Additionally, we started a novel research line focusing on detecting newly replicated DNA during genome replication. These projects are explained in more detail below.

1. Single-cell sortChIC identifies hierarchical chromatin dynamics during hematopoiesis

Post-translational histone modifications modulate chromatin activity to affect gene expression. How chromatin states underlie lineage choice in single cells is relatively unexplored. We develop sort-assisted single-cell chromatin immunocleavage (sortChIC) and map active (H3K4me1 and H3K4me3) and repressive (H3K27me3 and H3K9me3) histone modifications in the mouse bone marrow. During differentiation, hematopoietic stem and progenitor cells (HSPCs) acquire active chromatin states mediated by cell-type-specifying transcription factors, which are unique for each lineage. By contrast, most alterations in repressive marks during differentiation occur independent of the final cell type. Chromatin trajectory analysis shows that lineage choice at the chromatin level occurs at the progenitor stage. Joint profiling of H3K4me1 and H3K9me3 demonstrates that cell types within the myeloid lineage have distinct active chromatin but share similar myeloid-specific heterochromatin states. This implies a hierarchical regulation of chromatin during hematopoiesis: heterochromatin dynamics distinguish differentiation trajectories and lineages, while euchromatin dynamics reflect cell types within lineages.

2. scChIX-seq infers dynamic relationships between histone modifications in single cells

Regulation of chromatin states involves the dynamic interplay between different histone modifications to control gene expression. Recent advances have enabled mapping of histone marks in single cells, but most methods are constrained to profile only one histone mark per cell. Here, we present an integrated experimental and computational framework, scChIX-seq (single-cell chromatin immunocleavage and unmixing sequencing), to map several histone marks in single cells. scChIX-seq multiplexes two histone marks together in single cells, then computationally deconvolves the signal using training data from respective histone mark profiles. This framework learns the cell-type-specific correlation structure between histone marks, and therefore does not require a priori assumptions of their genomic distributions. Using scChIX-seq, we demonstrate multimodal analysis of histone marks in single cells across a range of mark combinations. Modeling dynamics of in vitro macrophage differentiation enables integrated analysis of chromatin velocity. Overall, scChIX-seq unlocks systematic interrogation of the interplay between histone modifications in single cells.

3. Quantifying DNA replication speeds in single cells by scEdU-seq

In a human cell thousands of replication forks simultaneously coordinate the duplication of the entire genome. The rate at which this process occurs might depend on the epigenetic state of the genome and vary between, or even within, cell types. To accurately measure DNA replication speeds, we developed a technology to detect recently replicated DNA using single-cell sequencing. We observed that replication speed is not constant but increases during S-phase of the cell cycle. Using genetic and pharmacological perturbations we are able to alter this acceleration of replication and conclude that DNA damage inflicted by the process of transcription limits the speed of replication during early S-phase. In late S-phase, during which less transcribed regions replicate, replication accelerates and approaches its maximum speed.

2.2. Major scientific achievements in 2023

a) Single-cell sortChIC identifies hierarchical chromatin dynamics during hematopoiesis. P. Zeller, J. Yeung, H. Viñas Gaza, B.A. de Barbanson, V. Bhardwaj, M. Florescu, R. van der Linden, A. van Oudenaarden. Nature Genetics 55, 333-345 (2023).

b) scChIX-seq infers dynamic relationships between histone modifications in single cells. J. Yeung, M. Florescu, P. Zeller, B.A. de Barbanson, M.D. Wellenstein, A. van Oudenaarden. Nature Biotechnology 41, 813-823 (2023).

c) Acceleration of genome replication uncovered by single-cell nascent DNA sequencing. J. van den Berg, V. van Batenburg, A. van Oudenaarden. https://doi.org/10.1101/2022.12.13.520365

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) scRibo-seq (Single-cell Ribosome profiling)

In recent years novel single-cell sequencing methods have allowed an in-depth analysis of the diversity of cell types and states in a wide range of organisms. Due to the continuous optimization of experimental and computational methods by many research groups, it is now possible to sequence the transcriptomes of thousands to millions of individual cells. Albeit an exciting development, transcription only covers the first step in the central dogma. The second step, the process of translation, is currently much harder to explore in single cells. Building upon existing ribosome profiling protocols, our laboratory majorly increased the sensitivity of these assays allowing ribosome profiling in single cells. However, currently no methods exist to determine the translation efficiencies in single cells and to correlate translation efficiencies to tRNA levels and their modifications, RNA bound proteins, and mRNA modifications, all major regulatory mechanisms of translation. We are developing several novel multi-omics approaches to quantify translation in single cells by integrating information on ribosome position along the transcript, tRNA expression levels, tRNA modifications and ribosome structure.

Project B) scEdU-seq (Single-cell Nascent DNA sequencing).

In 2022 we started a new research line to detect DNA replication in single cells. In 2023 this research line has expanded and several new projects have started. In a human cell thousands of replication forks simultaneously coordinate the duplication of the entire genome. The rate at which this process occurs, might depend on the epigenetic state of the genome and vary between, or even within, cell types. To accurately

measure DNA replication speeds, we developed a technology to detect recently replicated DNA using single-cell sequencing. We are currently collaborating with several groups specialized in DNA replication (including the group of Puck Knipscheer) to apply our new technology to open questions in the field of DNA replication. Additionally, we are developing a single-cell sequencing method to jointly detect nascent DNA and transcripts in the same cell and to detect replication of the mitochondrial genome.

Project C) T-ChiC (Jointly detecting transcripts and chromatin modifications in single cells).
Epigenetic mechanisms, including histone modifications, are key regulators of transcription and influence cellular differentiation. While our knowledge concerning cell type-specific histone modifications has constantly increased, we still know very little about the interplay between epigenetics and transcription. To gain understanding regarding this process, we developed T-ChIC (Transcriptome + Chromatin ImmunoCleavage), a method allowing for acquisition of full-length transcripts and histone mark positions of the same single cell. We applied this technique to an in vitro model of gastrulation termed gastruloids. These are aggregates of (mouse) embryonic stem cells, which over the course of several days differentiate into polarised structures with an anteroposterior axis and cell types derived from all three germ layers. Our analysis of H3K27me3 (repressed regions) and H3K4me3 (active promoters) distributions revealed complex chromatin regulation of cell type-specific chromatin stages.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Like in previous years, Oncode is essential for our group when it comes to knowledge transfer. Without Oncode we probably would not have applied for patents for VASA-seq and scRibo-seq, two novel single-cell sequencing technologies developed in our lab. Also this year we have been exploring possibilities to apply for patents for several of our single-cell epigenetics methods.

b) Oncode facilitates collaborations and contact with companies. During the last years we were contacted by several companies and Oncode really helps to streamline these interactions by making sure all paperwork is in order and agreements are clear. In particular Oncode's help with a recent collaboration with Altos is really valuable.

2.4.2. Contribution to the Oncode community in 2023

a) Since the start of our IMI proposal (PERSIST-SEQ) that was granted in 2021 I have been leading this consortium. Oncode has an important role in helping to coordinate this consortium (through the hard work of Alexander Turkin). Other Oncode members in PERSIST-SEQ are Hans Clevers and Rene Bernards. The consortium is now running at full strength with an important role for Single Cell Discoveries.

b) With Geerts Kops and funding from Oncode we founded the single-cell core in 2020. The single-cell core has been serving many reseachers within Oncode and beyond to help with single-cell sequencing technologies. Unfortunately, the Oncode funding ended at the end of 2022. In 2023 I have been exploring ways to avoid closing of this facility. Fortunately, I was able to secure funding to continue the single-cell core. Currently the single-cell core funded by the Hubrecht Institute and external funding.

2.4.3. Key collaborations within Oncode in 2023

a) A collaboration with the Drost lab resulted in the following paper (collaboration initiated after joining Oncode): SMARCB1 loss activates patient-specific distal oncogenic enhancers in malignant rhabdoid tumors. Liu NQ, Paassen I, Custers L, Zeller P, Teunissen H, Ayyildiz D, He J, Buhl JL, Hoving EW, van Oudenaarden A, de Wit E, Drost J. Nat Commun. 2023 Dec 1;14(1):7762. doi: 10.1038/s41467-023-43498-3.

2.4.4. Major valorization achievements in 2023

a) We continue to explore possibilities to apply for patents for our novel single-cell sequencing methods. Currently scRibo-seq and VASA-seq have been patented.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
TKI grant	Health~Holland Match Call PPP Allowance LSH-sector	€ 212.143	€ 212.143	01 september 2023	24 months	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Helena Viñas Gaza	Tracing histone modification dynamics in single cells during differentiation and early development

Daniel Peeper

Netherlands Cancer Institute

1. General information

Research Focus	Functional genomics for rational tumor and immune cell combination therapy
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Notwithstanding clinical advances, unfortunately many patients are not experiencing durably benefit from targeted and immunotherapies, mostly because of early or late resistance. We employ function-based, genome-wide screens and other strategies to develop rational combinatorial cancer treatment, targeting both cancer and immune cells. On the one hand, we are increasing our understanding of how cancer cells rewire their signaling networks, to expose and exploit new pharmacologically tractable tumor susceptibilities, particularly in the context of immunotherapy. On the other hand, we are manipulating various cell types from the patient's own immune system to revert their dysfunction and boost their specific cytotoxicity towards tumor cells. We complement these studies with analyses of clinical samples in collaboration with our clinical colleagues. In this way, we develop new rational combinatorial therapies, which simultaneously eliminate the patients' tumors and harness their immune system, aiming to achieve more durable clinical responses.

2.2. Major scientific achievements in 2023

a) IDO1 inhibitors, intended to revive anti-tumor T cells by restoring tryptophan, failed clinically. We uncovered that IDO1 inhibition protected melanoma cells against T cell-derived interferon-gamma. This study revealed an unforeseen consequence of IDO1 inhibition, which may contribute to the failure of the clinical trial (Kenski et al., Cell Rep Med 2023).

b) We uncovered by CRISPR-Cas9 knockout screening that TSC2 ablation strongly augmented tumor cell sensitivity to T cell attack in vitro and in vivo, suggesting one of its functions is to critically protect tumor cells. Crosstalk between TSC2-mTOR and TRAIL signaling may aid future therapeutic exploration of this pathway in immuno-oncology (Lin et al, EMBO J 2023).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Genetic screens to combat T cell dysfunction

This project supported by Oncode base funding to include translational opportunities addresses a key problem in IO, namely T cell dysfunction, contributing to IO resistance. We have established a T cell dysfunction genetic screening program comprising several genetic screens, which have uncovered an array of factors. They not only shed light on fundamental T cell biology, but some of these factors may also serve as new therapeutic IO targets. We identified several new and critical regulators of dysfunction, including PD-1 regulator TMED-10, which may be targeted pharmacologically, representing a potential drug repurposing opportunity (see TDF P2020-0057). We have recently submitted a revised manuscript. Furthermore, we have made good progress in the characterization of several additional T cell screen hits; also for this study we have recently submitted a revised manuscript. Oncode is in the process of protecting the accompanying IP.

Project B) IDO1 inhibition causes tumor cell protection through reversion of T cell-induced translation stress

Tryptophan restoration via indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors seeks to revive anti-tumor T cells. Despite a phase III trial setback, our reevaluation of IDO1 in T cell-attacked tumors revealed that IDO1 inhibition leads to melanoma cell protection against T cell-derived interferon-gamma (IFNγ). RNA sequencing and ribosome profiling demonstrated that IDO1 inhibition reverses IFNγ-induced shutdown of protein translation. This impaired translation triggers an amino acid deprivation-dependent stress response, resulting in ATF4high/MITFlow signatures, which is also observed in patient melanomas. Single-cell sequencing highlighted MITF downregulation predicting enhanced patient outcomes with immune checkpoint blockade. Conversely, MITF restoration in melanoma cells induced T cell resistance, emphasizing the pivotal roles of tryptophan and MITF in the melanoma response to T cell-derived IFNγ and revealing an unexpected downside of IDO1 inhibition (Kenski, Huang et al, Cell Rep Med 2023).

Project C) SerpinB9 contributes to tumor-intrinsic T cell resistance

Completed and summarized in last year's report.

Project D) TWEAKR as a potential novel immunotherapy target

I received a KWF grant to continue our work on TWEAKR signaling in cancer. A PhD student has started earlier this month and we are currently recruiting a technician.

Project E) Exploring T cell clusters for immunotherapy

From human melanoma metastases, we were able to isolate heterotypic clusters, comprising CD8+ T cells interacting with one or more tumor cells and/or antigen-presenting cells (APCs). CD8+ T cells from tumor cell clusters and APC clusters exerted on average 7.6-fold increased melanoma-killing activity than single T cells, which was associated with enhanced cytokine production. CD8+ T cells from clusters were enriched for tumor-reactive and exhausted gene signatures. Integration with T cell receptor (TCR)-sequencing showed increased clonality of clustered T cells, indicative of expansion upon antigen recognition. Together, these results demonstrate that tumor-reactive CD8+ T cells are enriched in functional clusters with tumor cells and/or APCs, and that they can be isolated and expanded from clinical samples. Being often excluded in cell sorting procedures, these distinct heterotypic CD8+ T cell clusters serve as a valuable source amenable to deciphering functional tumor-immune cell interactions, while they may also be therapeutically explored. Oncode protected the associated IP and awarded a TDF for preclinical translation.

Project F) TSC2 in tumor sensitivity to T cell killing

By CRISPR-Cas9 knockout screening we uncovered an important role for Tuberous Sclerosis Complex 2 (TSC2) in determining tumor susceptibility to T cell killing in melanoma. TSC2-depleted tumor cells had disrupted mTOR regulation following CTL attack, which was associated with enhanced cell death. Wild-type tumor cells adapted to CTL attack by shifting their mTOR signaling balance toward increased

mTORC2 activity, circumventing apoptosis, and necroptosis. TSC2 ablation strongly augmented tumor cell sensitivity to T cell attack in vitro and in vivo, suggesting one of its functions is to critically protect tumor cells. Moreover, a lower TSC2 immune response signature was observed in melanomas from patients responding to immune checkpoint blockade. Our study uncovers a pivotal role for TSC2 in the cancer immune response by governing crosstalk between TSC2-mTOR and TRAIL signaling, aiding future therapeutic exploration of this pathway in immuno-oncology (Lin et al., EMBO J 2023).

Project G) Immunotherapy resistance in lung cancer

Immune checkpoint blockade treatment is now standard-of-care for advanced non-small cell lung cancer without actionable mutations, but over 80% of patients are unable to mount a long-term response. In collaboration with pulmonary oncologist Dr. Willemijn Theelen we have DNA- and/or RNA-sequenced over 200 tumors from patients who never responded or became resistant to ICB . Through comprehensive analysis of our data we aim to better understand which resistance mechanisms are most prevalent pre- and post ICB treatment. Patients whose therapy resistance cannot be explained by the presence of known resistance mechanisms such as HLA loss will be extensively characterized by their genomic data as well as IHC in order to provide possible explanations for their lack of response. Our findings will be used to focus follow-up research addressing ICB resistance in NSCLC.

Project H) Synthetic lethality in cancer

Synthetic lethal interactions (SLIs) can provide a therapeutic index, as illustrated by PARP inhibition of BRCA-deficient cancers. Whereas additional SLIs based on genomic alterations in cancer have been identified, we set out to explore the SLI space as a function of differential RNA expression profiles in cancer and normal tissue. By unbiased computational analyses of publicly available functional genomic and gene expression resources we uncovered a cancer-specific SLI between the paralogs cytidine diphosphate synthase 1 (CDS1) and CDS2. The essentiality of CDS2 for cell survival is observed for mesenchymal-like cancers, which express low levels of CDS1. We confirm the CDS1-2 SLI in a panel of cultured cancer cell lines and in tumor-bearing mice. Our findings reveal that CDS2 may serve as a pharmacologically tractable target in mesenchymal cancers, meriting therapeutic exploration. The manuscript has been submitted and Oncode has protected the IP icw the Sanger Institute. Conversations with CRUK Horizon are ongoing for a potential drug development program.

Project I) NGFR heterogeneity in melanoma therapy resistance

NGFR expression in melanoma has emerged as a dedifferentiation marker associated with resistance to targeted and immunotherapies1. To elucidate its role in phenotype switching, we conducted a comprehensive analysis using single-cell RNA sequencing (scRNAseq) and spatial transcriptomics on four clinical samples. Our study revealed significant inter- and intra-tumor heterogeneity in NGFR+ sorted cells, extending to the gene regulation level. Notably, these populations expressed markers associated with both differentiation (MITF, Melan-A) and dedifferentiation (AXL, PDGFR). Leveraging an in-house dataset and two publicly available bulk-RNAseq cohorts of stage IV melanoma, we observed an enrichment of the NGFR+/PDGFR+ phenotype in immune-active non-responders (NR_HI_IAS), suggesting an involvement in immunotherapy resistance. This finding was validated in a fourth independent cohort and in in vitro cytokine assays. Additionally, analysis of a malignant compartment scRNAseq dataset by Pozniak et al. confirmed that tumor populations closely resembling our defined NR_HI_IAS samples were marked by the NGFR+/PDGFR+ phenotype. The manuscript will be submitted shortly.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode base funding has been, and continues to be, instrumental in supporting the hi-risk/gain research in my group, as it allows for a bypass from the typical delays and challenges of traditional grant funding. It thus provides critical freedom and flexibility to explore new territories and sometimes go against the flow. Furthermore, the benefit of this funding extends to other projects in my group.

b) As pointed out also last year, Oncode funding leverages to obtain funding from other sources. A good example is our work on TWEAKR signaling, which has now been funded by KWF, ensuring continuation of this important translational project.

c) Oncode funding also allows for launching projects from scratch. A good example is our synthetic lethality project on CDS1/2, which has already been submitted, IP-protected by Oncode and currently discussed with Oncode and CRUK Horizon for a possible drug discovery program.

2.4.2. Contribution to the Oncode community in 2023

a) I attended the Oncode annual meeting in Amersfoort and presented my experience with setting up NKI/Oncode spinoff Immagene, together with co-founder Maarten Ligtenberg.

b) I have attended both the Oncode events in Utrecht and retreat.

c) I have been engaged in multiple conversations with Ian Bell and Shobit Dhawan about the possible creation of a newco on T cell therapies.

2.4.3. Key collaborations within Oncode in 2023

a) We started a collaboration with Anne Rios on their 3D imaging platform to test hits from our genetic screens for critical determinants of T cell:tumor cell interactions and T cell antitumor activity. We are also discussing co-application for funding.

b) We collaborate with Karin de Visser through an NWO XL grant on T cell:tumor cell interactions.

c) We collaborate with Sarah Derks, mainly advising on genetic screens.

2.4.4. Major valorization achievements in 2023

a) Patent filed on cluster technology.

b) Patent filed on CDS1/2 synthetic lethality.

c) Patent being filed on T cell dysfunction genes.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant

		(€)	(€)	(mm/yyyy)	(months	(*)
KWF	Exploration	835,169	835,169	01/2024	48	Main applicant
Personal charity	IT resistance in NSCLC	1,000,000	1,000,000	01/2024	60	Main applicant
Oncode	TDF clusters	140,000	140,000	09/2023	12	Main applicant

*) Lung cancer project is a collaboration with Dr Joop de Langen (NKI-AVL)

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
David Vredevoogd (cum laude)	Genetic screens to improve immunotherapy
Disha Rao	Improving immunotherapy for melanoma

Anastassis Perrakis

Netherlands Cancer Institute

1. General information

Research Focus	Structural Biology, Cell Division, Lysolipid Signaling
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Macromolecular structures are key for generating new knowledge that can be translated to novel approaches for cancer therapy. We work on making macromolecular structures more useful for cancer research, by designing and providing methods to increase the information content of macromolecular structures, either predicted by modern AI technologies, or experimental. By making experimental structures of macromolecules more accurate or adding missing information in the AI-predicted models (e.g. ligands, metals, and co-factors) they can be used more efficiently for understanding molecular mechanisms and in the context of lead discovery campaigns. In parallel, we study macromolecular interactions and structures to generate basic knowledge that could lead to novel targets in cancer research. We focus in two biological research themes: the signalling axis established by the Autotaxin (ATX) extracellular phospholipase that produces the lysolipid LPA, which and has numerous physiological roles and therapeutic applications, with a promising role in cancer immunotherapy; and the biochemical basis of dynamic microtubule interactions and modifications in regulating mitotic progression and the mechanisms underlying microtubule detyrosination.

2.2. Major scientific achievements in 2023

a) Last year, we developed AlphaFill (https://alphafill.eu/), which adds various ligands to predicted protein structures, based on sequence similarity with experimental structures. In 2023, we finalized the development of an image analysis-based deep learning algorithm; this AI predicts binding sites of common metal ions and nucleotides, without detectable sequence similarity.

b) This year I was elected EMBO member, a distinction that means a lot to me, especially as a former EMBO long-term fellowship awardee, but also as a former PhD student, EMBO Young Investigator, and team leader at the sister organization of EMBO, the EMBL.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) ATX in liver cancer.
ATX produces the lipid mediator LPA, which regulates multiple biological functions via distinct GPCRs. Based on our previous work on ATX inhibitors, namely on the "Type IV" inhibitor Cpd17, that ameliorated induced liver steatosis and fibrosis in mouse models, we collaborated with OI Leila Akkari, to see if the inhibitor has an application in liver cancer. The project was abandoned based on the results for the first trials, where ATX inhibition delayed the onset of cancer but not the final outcome on survival.

Project B) AI methods for protein-ligand complexes
We have extended our new AI based on 3D image recognition to recognize not only metal ions (small sphere without rotational ambiguity in their placement) but also nucleotides. We thus demonstrated that, given enough learning data, this new Ai can suggest new binding sites for chemical entities that are common in experimental databanks. Importantly, unlike previous methods, this Ai does not use any sequence information. We demonstrated that by finding metal binding sites and nucleotides in the metagenome database of proteins without similarity to know sequences.

Project C) Improving inhibitors of Bub1 Kinase
Bub1 kinase is a central to the progression of cell division, and considered a promising target for cancer therapy. We determined the structures of Bub1 in complex with a new class of inhibitors designed and synthesized by OI Mario van der Stelt. This inhibitor class binds Bub1 covalently, through a C-terminal cysteine residue as we demonstrated based on this new crystal structure.

Project D) Hec1-Mps1 protein-protein interaction inhibitors
We concluded out efforts using fragments bound to Hec1 as seeds to produce new inhibitors of the Mps1-Hec1 interaction, which is central in outer kinetochore interactions during mitosis. We could not find viable inhibitors and the project was abandoned.

Project E) The QRICH1-SephS1 complex in transcriptional activation
OI Thijn Brummelkamp discovered a new complex of two proteins, QRICH and SPEHS1, which mediates transcription through interactions with several zinc finger transcription factors. We determined the structure of QRICH bound to SEPHS1 by cryo-EM, showing an equilibrium between a 1:2 and 2:2 complex. We are currently trying to understand how this complex binds zinc finger transcription factors, employing biophysical methods and cryo-EM.

Project F) Cryo-EM structure and function of the SRBD1 protein.
In collaboration with OI Rene Medema we are seeking to determine by cryo-EM the structure of SRBD1 in chromosome entanglement. We aim to investigate how single- and double-stranded binding of SRBD1 to DNA and to RNA might affect its function, as well as the role of its multiple domains.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) X

2.4.2. Contribution to the Oncode community in 2023

a) X

2.4.3. Key collaborations within Oncode in 2023

a) We continue to work closely with OI Thijn Brummelkamp, adding mechanistic insight that his team discovers through the use of haploid genetics. Current projects include the wort on microtubule modifications and the work described above, on the QRICH protein.

b) We are excited by engaging with OI Rene Medema on the function and structure of SRBD1, which we started recently.

c) Our efforts with Mario van der Stelt and Geert Kops on Bub1 inhibitors and on BubR1 function, in the context of mitotic control for the onset the anaphase in mitosis, continues strong.

2.4.4. Major valorization achievements in 2023

a) X

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Dutch goverment	Oncode Accelerator	320? 628?	Phase 1? Phase 2?	Different for each consortium?	Phase 1, phase 2?	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Jacco van Rheenen

Netherlands Cancer Institute

1. General information

Research Focus	Intravital Microscopy
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The van Rheenen group studies the identity, behavior, and fate of cells that drive tumor initiation, progression, metastasis and the development of therapy resistance. These populations of cells are difficult to study since they are rare, and their behavior (e.g. migration) and traits (e.g. stemness) change over time. To be able to study these dangerous cells, we have developed microscopy techniques to visualize individual cells in real-time in living animals, referred to as intravital microscopy. For example, we developed small imaging windows that can be surgically implanted in mice giving visual access to tissues with cellular precision for several weeks. We combine the latest genetic tumor models with intravital imaging to obtain fundamental knowledge on cancer.
Our research focuses on three areas are (1) The cellular mechanisms of tissue development and homeostasis, tumor initiation, and tumor progression; (2) The cellular mechanisms of migration and metastasis of cancer; (3) The molecular and cellular mechanisms of therapy resistance and side effects.

2.2. Major scientific achievements in 2023

a) Vennin C, Cattaneo CM, Bosch L, Vegna S, Ma X, Damstra HGJ, Martinovic M, Tsouri E, Ilic M, Azarang L, van Weering JRT, Pulver E, Zeeman AL, Schelfhorst T, Lohuis JO, Rios AC, Dekkers JF, Akkari L, Menezes R, Medema R, Baglio SR, Akhmanova A, Linn SC, Lemeer S, Pegtel DM, Voest EE, van Rheenen J. Taxanes trigger cancer cell killing in vivo by inducing non-canonical T cell cytotoxicity. Cancer Cell . 2023 Jun 12;41(6):1170-1185.e12.

Explanation: We identified the in vivo mode of action of taxanes: it directly triggers T cells to selectively kill cancer cells in a non-canonical, T cell receptor-independent manner. We developed an effective therapeutic approach, based on transfer of T cells pre-treated with taxanes ex vivo, thereby avoiding toxicity of systemic treatment.

b) Morgner J, Bornes L, Hahn K, López-Iglesias C, Kroese L, Pritchard CEJ, Vennin C, Peters PJ, Huijbers I, van Rheenen J. A Lamb1Dendra2 mouse model identifies basement-membrane-producing origins and dynamics in PyMT breast tumors. Dev Cell. 2023 Apr 10;58(7):535-549.e5.
Explanation: The basement membrane (BM) forms a barrier to prevent stromal cancer cells invasion. This manuscript draws a new paradigm for tumor BM turnover in which the disassembly happens at a constant rate, and a local misbalance of compensating production leads to reduction or even complete disappearance of the BM.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Taxanes directly induce T-cell mediated killing via release of cytotoxic extracellular vesicles.
The paper that describes the in vivo mode of action of taxanes has been published in Cancer Cell. Here, we demonstrate that in vivo, taxanes directly trigger T cells to selectively kill cancer cells in a non-canonical, T cell receptor-independent manner. Mechanistically, taxanes induce T cells to release cytotoxic extracellular vesicles, which lead to apoptosis specifically in tumor cells while leaving healthy epithelial cells intact. Currently, we exploit these findings to develop an effective therapeutic approach, based on transfer of T cells pre-treated with taxanes ex vivo, thereby avoiding toxicity of systemic treatment. We focus on transfer of tumor infiltrating lymphocytes (TILs), which is financially supported by a TechDev project (Project ID: P2022-0055).

Project B) Hormonal cycle stage determines chemosensitivity in breast cancer both in mice and patients.
The response of breast cancer (BC) to neoadjuvant chemotherapy (NAC) varies significantly even when tumors belong to the same molecular or pathological subtype. Here, we reveal the menstrual cycle as an important factor for this heterogeneity. We observe that in murine BC distinct phases chemosensitivity during the estrous cycle, the murine equivalent of the menstrual cycle. Importantly, we found similar results in retrospective studies of two cohorts of premenopausal patients. We observed improved tumor response in both cohorts who started NAC during the follicular phase compared to those initiated NAC during the luteal phase. Collectively, our study reveals the menstrual cycle as an important infradian rhythm that determines chemosensitivity, which suggests that optimal timing of treatment initiation can be exploited to further improve the benefits of chemotherapy. This paper is at resubmission at Nature and a clinical trial to validate this results has been started.

Project C) Development of a polylox mouse for complex lineage tracing
We have developed a new lineage tracing mouse in which >250 different color bar codes can be induced and which is inherited by all daughter cells. This color bar code can be read out by imaging and sequencing. We have optimized the imaging part, and are currently optimizing the sequencing part with our collaborator Jarno Drost. If successful, we will lineage trace the fate of specific cells pools, such as stem cells, cancer initiating cells microenvironmental, during development, cancer progression, and treatments. Moreover, we are currently developing the next generation polytope mouse model which, when combined with the original one, leads to over 200.000 different color codes.

Project D) Development of T cell activation sensor mouse
We have developed a new mouse model that reports the activation and exhaustion of T cells. T cells are labelled with a membrane-red fluorophore. Upon activation, T cell express a nuclear CFP by the endogenous IL2 gene, so these cells become red and blue. Upon exhaustion, various exhaustion markers are being expressed. We developed two split-cre models, which are expressed by the exhaustion markers TIM3 and Tigit. When both expressed, the membrane-red fluorophore is excises and GFP is expressed. So upon exhaustion, the red-blue cells become green. If we can reactivate the T cells, the cells become green and blue. We have validate the red and blue conversion (i.e. activation), and are currently validating the exhaustion part together with the labs of Monika Wolkers and Sjoerd van der Burg. Once validated, this mouse will be used to study T cell activation and exhaustion during immunotherapy.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Patient engagement: with help of Oncode, I have now two patients advocates engaged to my lab: Jacqueline Jersen and Henk Winkel. They regularly visit my lab for research updates and input, and Jacqueline has participated in our lab retreat. Both the patient advocates and lab members get inspired by these interactions.

b) Due to Oncode I have initiated various collaborations within Oncode, including with the labs of H. Snippert, J.P. Medema, Monika Wolkers, Sjoerd van der Burg, Linde Meyaard, Sarah Derks, Jarno Drost.

c) Due to Oncode, I could develop technologies where patent applications have been filed, including the improved T cell therapies upon taxane treatment. Moreover, we have developed new technologies such as the polytope and T cell reporter mouse. Lastly, we have started a clinical trial to independently validate the role of the menstrual cycle in chemo-sensitivity, however, this requires acquisition of more funds.

2.4.2. Contribution to the Oncode community in 2023

a) I am active member of the patient engagement program.

b) I collaborate with multiple labs within Oncode. Especially our intravital microscopy technology helps multiple projects within Oncode.

c) I am an active members in the Oncode society. For example, I participate in almost all Oncode meetings. I have given a seminar at the KIT meeting

2.4.3. Key collaborations within Oncode in 2023

a) Emile Voest, Jarno Drost, and Sarah Derks. We have collaborated with Emile's group to show that taxanes induce T cells to release cytotoxic vesicles. The collaboration with Emile's group as key to validate our mouse work in patient samples. This paper has been published (Vennin et al, Cancer Cell 2023). Currently, we also collaborate with the groups of Jarno and Sarah to test whether we can use taxanes to enhance the tumor infiltrating lymphocytes (TIL) therapy. These collaborations were initiated at two different Oncode meetings.

b) Linde Meyaard, Sjoerd van de Burg, Karin de Visser and Leila Akkari. We are developing a T cell reporter mouse, which I presented at an Oncode meeting. This got the attention of Sjoerd and Karin, after which I joined the Oncode accelerator project to study the cross-talk between myeloid cells and T cells. Moreover, I also actively collaborate with Linde's lab to help her imaging T cells migration upon LAIR blockage.

c) Jarno Drost and Anne Rios. We have developed the polytope mouse in which we can lineage trace >250 colored barcodes. We collaborate with Anne's lab to image the barcodes. We collaborate with the Drost lab to read out the bar codes by sequencing, in order to establish spatial genomics.

2.4.4. Major valorization achievements in 2023

a) I joined the activity "meet the professor". In this event, professors from the University of Utrecht visit a primary school to make them enthusiastic about science.

b) I gave a seminar for YoungAVL and YoungBlood, which is a network of young scientist who want to work on their career and are interested in extending the borders in healthcare.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Lauira Bornes	Zooming in on Cellular Plasticity: unveiling cancer cell secrets and gaining therapeutic insights using intravital microscopy

Jeroen de Ridder

UMC Utrecht

1. General information

Research Focus	Bioinformatics, Machine learning and AI, Nanopore Sequencing
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

Pillar 1 - Machine learning and bioinformatics for omics data –In our lab, we are leveraging the latest AI developments for personalized medicine. For cancer patient, for instance, individualized decisions can be based on a thorough characterization of the tumor at a molecular level. However, recent artificial intelligence (AI) models, that work so well on images and text, struggle in dealing with the complexity of these molecular data. When it comes to patient-derived molecular profiles there is simply not enough patient data to use modern AI models. To address this, we are trying a new approach. Instead of immediately training on patient data, we first want to teach an AI about molecular disease biology by creating so-called foundation models based on massive amounts of biomolecular data from single cells, molecular profiles of healthy and sick tissues and biomolecular network information. Based on these data we use self-supervised learning (SSL), an important driver of AI. Once the AI has some 'common sense' about molecular disease biology we can finally train it to make predictions such as "what drug should this patient receive?" or "what tumor subtype does this patient have?". Furthermore, we are studying how mutations and modifications affect the functions encoded in the genome and contribute to disease. For instance, we pioneered deep learning modeling of Massively Parallel Reporter Assay (MPRA) data to predict promoter or enhancer activity. These semi-supervised AI models can be used for predicting the effect of non-coding mutations in cancer based on DNA sequence alone. With this research line we aim to leverage the enormous progress in AI and thereby bring truly personalized medicine one step closer.

Pillar 2 - Bringing data-driven cancer diagnostics solutions to the clinic – Our lab aims to short-cut the route from fundamental research results to patient benefit and actively contributes to clinical application of the research. My lab has embraced nanopore sequencing to lower the threshold for routine diagnostic sequencing and developed a nanopore based liquid biopsy test (Published in NPJ Genomic Medicine in 2021), filed a patent on the underlying technology (US20210180109A1) and founded a start-up company Cyclomics BV, aimed at developing the next generation of liquid biopsies. Most recently, we have submitted our work demonstrating that nanopore sequencing can be used for ultra-rapid methylation-based brain cancer classification during the resection surgery and that, by using our deep learning model "Sturgeon", turnaround times of less than 90 minutes are possible (Published in Nature in 2023).

2.2. Major scientific achievements in 2023

a) We have published our paper on intra-operative pediatric brain cancer diagnosis using our unique "Sturgeon" deep learning approach in Nature. This algorithm can deal with extremely sparse methylation data, allowing ultra-rapid diagnosis within 1.5 hours. We prospectively sequenced 25 patients during resection surgery at the Princess Maxima Center (PMC) and Amsterdam Medical Center, in 2023. A patent application (Nov 2022) is submitted.

b) I have written and received an ERC consolidator grant. With this 2M euro grant, I will investigate how we can use the concept of foundation models, that is, AI model that we train to attain a basic understanding of molecular biology, to solve molecular diagnostic tasks with limited samples to learn from.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Sepsis detection from liquid biopsies

At the critically early phase of invasive pulmonary fungal disease, rapid and sensitive microbiological diagnostic tools are lacking, resulting in diagnostic uncertainty and impeded initiation with effective antimicrobials. To evaluate whether hypothesis free microbial cell-free DNA sequencing has diagnostic potential, we developed a blueprint diagnostic workflow to detect fungal etiological agents by unbiased capture and rapid next-generation sequencing of double- and/or single-strand cell-free DNA molecules from liquid biopsy samples. All data analysis is completed. Draft manuscripts detailing the workflow and results on a set of human and foal patients have been completed and will be submitted in February.

Project B) Brain tumor diagnosis using real time methylation sequencing

We published our initial findings in Nature in 2023 (see above). To continue this project we have secured funding from Kika (pilot grant) and are in the process of applying for KWF and additional funding from Kika. To perform clinical validation we will be applying for an Oncode cPOC grant. To expand this research line to liquid biopsies we have successfully applied for a Hanarth grant. Moreover, supported by the Oncode BD team we are discussing a sponsored research contract with Oxford Nanopore Technologies to expand the approach to other cancer types.

Project C) ctDNA sequencing on Nanopore

We finished our proof-of-concept application of NanoRCS (the Genome-wide version of the Cyclomics technology). We applied the technique to three tumor types, Granulosa Cell Tumor (GCT), esophageal adenocarcinoma (EAC) and ovarian cancer (OVCA). We showcased MRD detection, determined tumor fraction and genomic characteristics of these tumors. Moreover, using a 'fragmentomics' approach we determined additional read-based characteristics that can be used to classify tumor-derived from normal cell-free DNA. We further aim to nanopore sequencing of cfDNA to advance diagnosis of gastrointestinal cancers in collaboration with Oncode PI's Joep Grootjans and Sarah Derks. Specifically, we're investigating the potential of TP53 CyclomicsSeq as a screening tool for identifying early colorectal cancer (CRC) lesions in patients with inflammatory bowel disease (IBD), who are at a notable risk of developing CRC. We have sequenced cell-free DNA from plasma of 5 IBD patients with CRC and 5 healthy controls, of which we plan to analyze the data within the coming month. Moreover, we're exploring whether native nanopore sequencing on peritoneal flush fluid can be used to detect peritoneal metastases in colorectal and gastric cancer patients, which is pivotal for guiding timely chemotherapy interventions. We have set up the native cell-free DNA Nanopore sequencing protocol recently within our lab and are currently sequencing DNA from three flush fluid samples of CRC patients.

Project D) Glycosylation-based methylation detection on nanopores

This project is finished and manuscripts are in preparation.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) In 2023 the Oncode institute has been instrumental in driving the developments of our intraoperative CNS tumor classifier. The initial pilot data were generated based on my base fund. To obtain sufficient data for a first manuscript we secured a TechDev fund. This resulted in a very nice publication in Nature. Moreover, we attained funding from KiKA and the Hanarth fund to further pursue this research line.

b) The BD team has been instrumental in protecting the IP related to the Sturgeon algorithm, capable of rapid diagnosis of CNS tumors. Currently the BD team is carrying out the discussions with Oxford Nanopore Technologies for licensing of the IP.

c) The BD team has been instrumental in protecting the Pericode algorithm, capable of predicting expression from sequence alone. Moreover, the BD team supported the follow-up meetings (including with a VC) on how to transform the IP into a viable enterprise. These discussions are still pending.

2.4.2. Contribution to the Oncode community in 2023

a) Presentation by my team at the Oncode Annual meeting of the Sturgeon algorithm.

b) Joining of the Oncode Patient Engagement program.

2.4.3. Key collaborations within Oncode in 2023

a) Bas van Steensel, Emile Voest, Lude Franke, Michiel Vermeulen. Pericode consortium – Using machine learning for predict promoter activity and making sense of non-coding mutations.

b) Sarah Derks and Joep Grootjans – using nanopore sequencing for liquid biopsy monitoring colorectal cancer (CRC) lesions in patients with inflammatory bowel disease (IBD) and analyzing peritoneal flush fluid to detect peritoneal metastases in colorectal and gastric cancer patients.

c) Lude Franke, Lodewyk Wessels – Oncode Accelerator project - Machine learning for personalized treatment selection.

2.4.4. Major valorization achievements in 2023

a) Implementation of the Sturgeon approach in the PMC clinic. Currently approximately 3 cases per week are sequenced and analyzed using our method making a clear and tangible impact on patient lives.

b) The Cyclomics patent was granted in the USA. We have now finished the manuscript on the Genome-wide version of the technology. Submission is imminent.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Hanarth	Research Projects	500k	500k	01-06-2024	48	Main applicant
ERC	Consolidator	2M	2M	01-06-2024	60	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Anne Rios

Princess Máxima Center

1. General information

Research Focus	Cancer, immunotherapy development, high resolution 3D imaging, organoid technology, bioengineering
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

The Rios group forms an interdisciplinary team with a strong emphasis on technological advancements: combining organoid technology, multi-spectral 3D imaging and vision-based multi-omics to identify new actionable targets to improve treatment outcomes, and designing T cell therapeutic strategies for pediatric tumors and breast cancer. To do so at patient-population scale, we invested in the establishment of patient-derived organoid (PDO) biobanks. In addition, we developed 3D live imaging technologies, with our leading platform; BEHAV3D, designed to profile the dynamic behavioral landscape of engineered T cells within immune-organoid co-cultures, and how it correlates with treatment efficacy.

To deliver in vitro solutions for addressing human cancer in a near-native organ environment, we combine organoid technology with bioengineering strategies. For these upcoming models and already implemented co-culture assays, we further advance our multi-colored 3D imaging protocols and accompanying AI-based single-cell omics pipelines to decipher the critical role of intricate spatial organization and cell-to-cell communication, offering new dimensions for understanding and targeting cancer. This includes addressing the complex spatial organization and relationship of cancer cells with their tumor microenvironment (TME) and how it impacts T cell function, by combining advances in mouse models, spatial transcriptomics and multiplexed imaging, focused on diffuse midline glioma (DMG).

2.2. Major scientific achievements in 2023

a) We published a review on how dynamic imaging technologies contribute to a better understanding of cancer treatment and next generation therapy development. This is highly relevant in the current era of cellular immunotherapy; 'living drugs' that depend on their dynamic behavior for achieving tumor control (Nature Reviews Cancer, 2023).

b) We obtained funding for a 4-year research programme dedicated to mapping the efficacy of different T cell therapy concepts against diffuse midline glioma (DMG) using patient-derived organoids, and find leads to improve their mode-of-action against these highly lethal pediatric brain tumors (KiKa research grant, 2023).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) The behavioral-phenotypic landscape of engineered T cells targeted to human cancer organoids

We apply various organoid tumor models and our 3D imaging and transcriptomics platform; BEHAV3D, to map the functional heterogeneity of engineered T cells. With this strategy we aim to gain insight into their dynamic mode-of-action and uncover relevant behavioral sub-populations related to targeting efficacy. We, thereby, take a unique angle from the behavioral perspective to unlock new insight that subsequently can be used for next-generation treatment design via genetic engineering, cell selection, or combinatorial treatments (Nature Reviews Cancer, 2023). Using this approach, we identified relevant targets that we are currently following up on and that form the basis for upcoming grant applications. Importantly, we validated the importance of a previously identified serial-killing T cell population with high morphological plasticity across tumor types, T cell concepts and within clinically relevant treatment schemes. We are now further studying the biology and genetic requirements of this unique T cell population.

Project B) Developing a human mammary gland in a dish using organoid technology and advanced 3D imaging-based bioprinting

We are developing advanced human breast models by generating healthy breast organoids from an alternative tissue source and combining them with bioprinted chips to introduce more complex ductal geometries. Through a collaboration with the Isala clinic, we, furthermore, obtain both healthy, as well as tumor breast tissue that is used for organoid establishment. Together, we aim to create a large-biobank of both tumor and healthy breast tissue organoids to evaluate healthy mammary gland function, as well as breast cancer progression and it's response to treatment, including T cell immunotherapy. We analyze the impact of biomaterial design and organ-architecture for modelling proper functionality and treatment outcomes. Our healthy organoid model derived from a highly accessible cell source, importantly, offers opportunities for healthy-tissue toxicity screening at large scale. Further advanced and validated models will be used in tailored research projects and grant applications.

Project C) Applying patient-derived organoids to dissect the molecular mechanisms underlying heterogeneity of treatment response

Through our advanced computational tools and clonal organoid technology, we have identified both unique treatment resistance mechanisms, as well as those that are shared between multiple donors. The latter, thus, have the potential to enhance treatment outcomes across patients. Therefore, we are currently developing a data-driven CRISPR screen for validating targets of these resistance pathways to ultimately provide molecular indicators of T cell therapy performance in patients and, importantly, combinatorial interventions that have the potential to improve efficacy of T-cell immunotherapy in large groups of patients.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Navigating legalities. Oncode's Valorisation team and dedicated business developer; Saharla Ahmed, have navigated contracting for our projects funded by external partners. In 2023, this included a sponsored partnership with Roche and a research project funded through the Proefdiervrij foundation.

b) Patent and innovation assistance. Oncode's Valorisation team has provided critical advice on novelty and patentability of our inventions and how to obtain additional funding and pre-clinical validation to support proof-of-concept for further development. This includes

multiple disclosures in regard to our newly developed technologies, identified therapeutic leads, and advanced in vitro human models (e.g. PCT/EP2023/073303 HYBRID SUPRAMOLECULAR BIOMATERIAL FOR BIOPRINTING AND CELL CULTURE).

2.4.2. Contribution to the Oncode community in 2023

a) Event organization and scientific programme development. Acted as 1 out of 3 organizing scientific committee members for the Oncode Annual Conference, 2023.

b) Technology transfer. With our unique profile integrating organoid, imaging and AI technologies, we have been involved in many collaborations with other researchers in the Oncode community. 2023 jointly published applications, include a macrophage-organoid co-culture assay for validating kidney tumor macrophage polarization states identified by the Drost group (DeMartio J, …, Rios AC, …, Drost J, Nature Communications, 2023), and T cell-organoid co-cultures and imaging to together with the van Rheenen group investigate the effects of Taxane treatment on human breast cancer (Vennin C, …, Rios AC, …, van Rheenen J, Cancer Cell, 2023). In addition, in an ongoing collaboration with the group of Daniel Peeper, we are exploiting cell-cell communication among T cells, or with tumor cells, to define the most potent tumor-targeting T cells, as well as enhance their interactive strategy towards more efficient antitumor responses.

2.4.3. Key collaborations within Oncode in 2023

a) Jacco van Rheenen; collaboration established via affiliation with Oncode. T cell-organoid co-cultures and imaging analysis to investigate the effects of Taxane treatment on breast cancer (Vennin C, …, Rios AC, …, van Rheenen J, Cancer Cell, 2023).

b) Jarno Drost; already part of our network prior to joining Oncode. Application of our macrophage-organoid co-culture assay to validate macrophage polarization states identified by the Drost group in pediatric rhabdomyosarcoma, using single-cell mRNA sequencing (DeMartio J, …, Rios AC, …, Drost J, Nature Communications, 2023).

2.4.4. Major valorization achievements in 2023

a) We started our collaborative project with Roche funded through their Roche Access to Distinguished Scientists (ROADS) programme, dedicated to understanding and overcoming resistance to T-cell bispecific antibody (TCB) treatment, using patient-derived tumor organoids and BEHAV3D. The project has commenced and we have already achieved the first milestone as outline in the project plan.

b) Together with our collaborator in the field of biofabrication (Riccardo Levato), we filed a priority patent application regarding a novel biomaterial with advanced in vitro modelling properties (PCT/EP2023/073303 HYBRID SUPRAMOLECULAR BIOMATERIAL FOR BIOPRINTING AND CELL CULTURE).

c) I am listed as an inventor on a patent application filed by EUSA based on an anti-G2 antibody (dinutuximab) conjugated to an imaging probe (IRDye800CW) to detect GD2 positive cancers (PATENT APPLICATION NUMBER 2307103.8 METHOD FOR DETECTING A GD2 POSITIVE CANCER).

d) Funded through an NWA Science Communication grant, we developed a collaboration between us, as scientists, an artist, a philosopher and immersive designers to put together an immersive design exhibition (Betweter Festival, Tivoli Vredenburg, Sep 2023) to showcase organoid technology and its applications for cancer research to the general public.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KiKa	Research Grant	550.000	550.000	07/2023	48	Main Applicant
Proefdiervrij	Research Grant	100.000	100.000	10/2023	12	Main Applicant
NOW National Roadmap	Large-scale research infrastructure	15.000.000	807.937	01/2023	60 (-120)	Main Applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
PS21DIN (internal PMC identifier)	Phase I/II clinical trial for testing anti-GD2-IRDye800CW during surgery in patient with neuroblastoma	2024	24	No	Co-PI

3.3. PhD defenses in 2023

N/A

Rebekka Schneider

Erasmus MC

1. General information

Research Focus	Hematopoietic stem cells; leukemia; bone marrow failure; myelodysplastic syndrome; myeloproliferative neoplasms; genetic fate tracing; CRISPR-Cas9; scRNA sequencing
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

The presence of bone marrow fibrosis in chronic blood cancer significantly diminishes patients' survival rates. Our objective is to enhance our comprehension of the molecular and cellular factors driving this disease, with the ultimate aim of directly applying our findings to the development of targeted therapeutics. This is especially crucial for the extensive and growing population of patients experiencing fibrosis and organ failure due to chronic blood cancer or cancer more broadly (schneiderlab.org). Achieving this objective in Oncode phase 1 has fueled our commitment to conducting research that seamlessly translates from bench to bedside, aiming to benefit patients directly.

In our ongoing projects, we are integrating genetic fate tracing with single-cell RNA sequencing to gain a more nuanced spatiotemporal understanding of the disease. Our focus is on identifying the genuine progenitor cell of fibrosis. By dissecting "physically interacting cells," our goal is to target both malignant cells and fibrosis-driving cells, with the overarching aim of restoring normal blood formation and reducing fibrosis. Our most recent research has pinpointed a role for vitamins in the development of bone marrow fibrosis. Furthermore, we have provided insights into the reasons behind the clinical trial failures of Smoothened inhibitors in myeloproliferative neoplasms/myelofibrosis (MPN/MF).

2.2. Major scientific achievements in 2023

a) Publication in Cell Reports "Non-canonical Hedgehog signaling mediates profibrotic hematopoiesis-stroma crosstalk in myeloproliferative neoplasms". Our data highlight the complex interplay between alterations in the blood cancer clone and activation of stromal cells and indicate that Gli1 represents a promising therapeutic target in MPNs

b) Secured an ERC consolidator grant: In Rewind MF, my aim is to rewind even advanced fibrosis in blood cancer. I hope to apply what we learn in blood cancer to other fibrotic cancers too, working with other Oncode researchers to improve outcomes across the board

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Stromal precursor cells as origin of fibrosis in chronic blood cancer

We recently uncovered a spatial relevance of fibrosis-driving cells by combining scRNA sequencing and genetic fate tracing, and identified the origin of fibrosis-driving cells (Banjanin et al. in revision Science Translational Medicine). These results have central impact on a better understanding of the stromal biology in chronic blood cancers associated with fibrosis. This also reveals that the actual progenitor cells need to be targeted in order to halt the disease. *This project included the combination of numerous methods that are well-established in the lab, including advanced genetically engineered mice combined with scRNA sequencing. It would not have been possible to analyze the mice in the depth we did with "regular funding schemes".*

Project B) Dissecting the cellular cross-talk in MPN for improved targeting of malignant cells and fibrosis

The hematopoiesis-stromal cell cross-talk is poorly understood in MPN and fibrosis. We recently sequenced physically interacting cells via genetic fate tracing of CXCL4+ hematopoietic and Gli1+ stromal cells. In this context, we demonstrate that a particular vitamin can slow down the fibrotic phenotype in murine models and in vitro experiments, which will have huge impact on altered therapeutic strategies for patients. We teamed up with clinicians to gain clinical validation on a positive effect of this vitamin on the disease course. *For this project, we had to set up the sequencing of physically interacting cells which was technically challenging in the bone marrow. This preparatory work could not have been funded with "regular funding schemes" as this is considered high-risk.*

Project C) Acute leukemia model for del(5q) MDS (published in Blood advances).

We asked how compound haploinsufficiency for Csnk1a1 and Egr1 in the common deleted region on chromosome 5 affects hematopoietic stem cells. Trp53 was disrupted as the most frequently co-mutated gene in del(5q) MDS using CRISPR/Cas9 editing in hematopoietic progenitors of WT, Csnk1a1-/+, Egr1-/+, Csnk1a1/Egr1-/+ mice. A transplantable acute leukemia only developed in the Csnk1a1-/+ Trp53 edited recipient. A collaborative effect of Csnk1a1 haploinsufficiency and p53 loss on MAPK and Myc upregulation was confirmed. Downregulation of Myc protein expression correlated with efficient elimination of blasts in A51 treatment. *This project a high risk/high gain project (usually not fundable by regular funding schemes) as we had to test the combination of multiple genetically engineered mice with editing of several oncogenes. Only one combination led to an optimal acute leukemia model, which now is invaluable for drug screening.*

Project D) Mechanisms of Tasquinimod in MPN and clinical application (preparation of clinical trial)

This project is a prime example for basic research leading to clinical translation and would not be feasible to this extend with regular funding schemes. We now elucidate the mechanisms of Tasquinimod in MPN using scRNA sequencing for improved targeting. In the Technology Development grant, we are testing combinatorial strategies with first line treatments in order to gain better insights for therapeutic strategies. We performed an early economic evaluation comparing Tasquinimod to first line treatment in MPN funded by Oncode. We are finalizing the study protocol for the clinical trial based on our findings in the laboratory and also with input from patients. In parallel to the clinical trial, we will have a research program on the collected patient samples to look at molecular remission and fibrosis reversal mechanisms.

Project E) Role of Hh signaling in the initiation of bone marrow fibrosis *(published in Cell Reports).*

The role of hematopoietic hedgehog signaling in myeloproliferative neoplasms (MPNs) remains incompletely understood despite data suggesting that Hedgehog (Hh) pathway inhibitors have therapeutic activity in patients. We established that hematopoietic Gli1 has a significant effect on stromal cells, partially mediated through a MIF/CD74 axis. Hh signaling is incompletely understood in myeloid malignancies

and SMO inhibitors show mixed results in clinical trials. We show that Gli proteins can be activated independently of canonical Hh signaling in malignant cells, for the first time explaining the poor response of SMO inhibitors in MPN patients. Our findings are relevant not only for MPN but also hematological malignancies more broadly. *We systematically worked up Hh-signaling in murine models, patient samples and in vitro treatments. This line of research would not have been fundable by conventional measures.*

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Clinical trial "TasquForceMPN" as Hovon trial: It was always my dream to translate our findings to a clinically relevant setting. This dream has now become a reality with the clinical trial we are about to start with Hovon. We now explore our large patient-derived single cell RNA seq data sets in a different way to move quicker to clinical translation. This includes novelty and IP checks and inclusion of a freedom to operate analysis.

b) Early economic evaluation of pharmacological treatments: Thanks to Oncode, we were able to do an economic evaluation of Tasquinimod compared to the first line treatment (JAK inhibitors). Based on this analysis we also talked to Active Biotech to make sure the treatment could be accessible to all patients and is cost-effective.

c) Discussion with patients for preparation of the clinical trial. Within these preparations, I met with MPN patients at various occasions: in the clinic, at MPN meetings organized by us and at a patient platform on Facebook that I am part of. An important question for me is: what are other factors which impair the quality of life that are not represented by measuring spleen size and blood counts. Based on these interviews, we now implemented also an "skin itching score" into our clinical trial as a side parameter.

2.4.2. Contribution to the Oncode community in 2023

a) I was a member of the Scientific Committee of the Oncode Annual Meeting/KIT 2023.

b) I participated in the "round table discussion" moderated by Dionne Stax about the translational efforts within Oncode Institute and how we approach the advancement of basic research finding to clinical application. This includes overview about valorization activities, initiation of start-ups, drug repurposing, initiation of clinical trials, biomarker validation discussed with Jan Paul Medema, Jeroen de Ridder, Veerle Fleskens, Madelon Maurice, Judith Vivié.

2.4.3. Key collaborations within Oncode in 2023

a) with Linde Meyaard on a specific target in tumor inflammation and fibrosis: Linde and I have a common interest in tumor immunology and tumor associated fibrosis and our work converged on a soluble molecule which plays a central role in these processes.

b) with Miao-Ping Chien : Miao Chien employed microscopy-based single cell profiling technology to investigate tumor heterogeneity in head and neck tumors and also identified a druggable axis, similar to previous findings. We are following up on this interesting finding and if targeting the identified pathways has the potential for an even broader application in cancer.

2.4.4. Major valorization achievements in 2023

a) Finalization of Research collaboration agreement with Active Biotech

b) Finalized contract with Hovon to conduct clinical trial (HOVON172): This ensures a high quality clinical trial for a rather rare disease. This is important as the FDA granted orphan drug designation for Tasquinimod in MPN.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
European Research Council; ERC	Consolidator grant 2023	1.98 Mio €	1.98 Mio €	April 2024	60	Main applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
HOVON172	Tasquinimod as second line treatment in MPN/MF	2024		No	Lead

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Stalmann, Ursula	Intrinsic and Extrinsic mechanisms of clonal expansion in del(5q) MDS
Flosdorf, Niclas	The impact of the JAK2 V617F mutation on megakaryocytes and bone marrow fibrosis in an iPS cell model

Ton Schumacher

Netherlands Cancer Institute

1. General information

Research Focus	Molecular Oncology & Immunology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our aim is to understand how immune cell activity – and in particular T cell activity – is regulated in cancer, and how such immune cell activity can be manipulated. Towards this goal we use an engineering-based approach, relying on the development of novel technologies where we feel these could be of value. Over the past year, we have focused on 3 main areas: I. We have developed technology that allows control over the activity of adoptively transferred T cells using an approved small molecule and are using this platform to design next-gen T cell therapies. II. We have developed and used technology to determine how T cell secreted cytokines modulate cell states in tumor tissue, resulting in the concept of local and global tissue modifiers. III. In collaboration with John Haanen and Wouter Scheper we are developing a series of platforms that should ultimately allow the in silico prediction of the antigen-specificity of (tumor-specific) TCRs.

2.2. Major scientific achievements in 2023

a) Demonstrated that the T cell-secreted cytokines TNFa and IFNg influence cell states in tumor tissue in distinct manners, in which TNFa sensing is restricted to a minor fraction of tumor cells near sites of T cell attack, while IFNg forms a global tumor tissue modifier (Hoekstra et al. Cancer Cell In press).

b) Developed and exploited technology for TCR library screening and for robotics-based TCR assembly (Moravec et al. Ms submitted, Messemaker et al. Unpublished), two core platforms towards our long-term goal to develop algorithms to predict the antigen specificity of (tumor-resident) TCRs.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Prediction of TCR – pMHC interactions

The clinical activity of immune checkpoint blockade and TIL therapy in large part rely on the increased TCR recognition of cancer neoantigens that they induce. With this critical role of patient-specific TCR-pMHC interactions firmly established. our main goal for the coming years is to develop and exploit technology to measure such TCR- pMHC interactions, with the aim to ultimately develop algorithms that can predict the antigen specificity of TCRs in silico. Major results obtained in 2023: 1. Development of TCR library screening technology and demonstration of its value in identification of neoantigen specific TCRs in patient material, and 2). Development of robotics-based technology that allows the synthesis of 10,000s TCRs. In addition, we pitched 'Cracking the TCR – cancer recognition code' as one of the 2024 Cancer Grand Challenges, with the ambition to combine efforts in T cell immunology/ engineering with the ongoing rapid developments in protein design/ AI.

Project B) Next-gen T cell therapies

We have previously developed a small molecule-regulated switch domain that can be controlled with low concentrations of lenalidomide, an approved and orally available drug. In the past year, we have optimized this technology for the controlled production of (cytokine) cargoes of interest by CAR-T cells, TCR-T cells and, in collaboration with the Haanen group) TIL. Notably, dynamic range that is achieved using this technology is substantially higher than that obtained with previously described technologies. Furthermore, we have obtained the first evidence that demonstrates how this technology can successfully be used to control T cell activity in vivo.

Project C) Cytokines as tissue modifiers

Cells in the tumor microenvironment influence each other through the secretion and sensing of soluble mediators, such as cytokines and chemokines. While signaling of interferon γ (IFNγ) and tumor necrosis factor α (TNFα) have both been shown to be critical in anti-tumor immune responses, our understanding of the spatiotemporal behavior of these cytokines is limited. In this project, we have developed a single cell transcriptome-based approach to infer which single or combined cytokine signals an individual tumor cell has received. Using this technology, we demonstrated that, contrary to expectations, CD8+ T cell-derived IFNγ is the dominant modifier of the TME relative to TNFα. Furthermore, we demonstrated that tumor cell pools that show abundant IFNγ sensing are characterized by decreased TGFβ signaling, consistent with IFNγ-mediated remodeling of the TME. Collectively, these data provide evidence that CD8+ T cell-secreted cytokines should be distinguished into local and global tissue modifiers.

Note: These projects are not being funded through other research grants. However, indicated projects are in part supported by unrestricted career awards.
All of the above projects rely fully on the development of novel technologies, a research activity that is generally not supported by other funding schemes until first proof of concept/ a first prototype has been obtained.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Worked closely with the Oncode team to support the development of Cell Control. This included discussions with VCs and potential pharma partners

2.4.2. Contribution to the Oncode community in 2023

a) Discussions with Peter ten Dijke and Yuva Oz around the ten Dijke lab project to create a novel class of cell type specific TGFBR inhibitors. Provided scientific input regarding both specific molecule designs and general design considerations.

2.4.3. Key collaborations within Oncode in 2023

a) Emile Voest (collaboration already ongoing): Role of gd T cells in immune checkpoint inhibitor activity in MSI cancers. Nature 2023, doi: 10.1038/s41586-022-05593-1.

b) Emile Voest (collaboration already ongoing): Identification of neoantigen-reactive T cell responses through HLA agnostic screening. Nature Biotech 2023 doi: 10.1038/s41587-022-01547-0.

2.4.4. Major valorization achievements in 2023

a) Developed novel applications of the Rheobrick technology that allows lenalidomide-based control over T cell activity. Technology is currently further developed in the Oncode supported startup Cell Control, and these novel applications have been tested in the context of two Oncode TDF grants.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT03026140	NICHE-2	07/2017	60	Yes	Co-PI
NCT02278887	TIL or Ipilimumab	09/2014	90	Yes	Co-PI
NCT03448835	PANDA	04/2018	38	Yes	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Lianne Kok (promotor)	Genetic-tracing of CD8+ T cell fate decisions
Mirjam Hoekstra (promotor)	How T cells talk to the neighborhood: the spatiotemporal dynamics of T cell-derived cytokines in cancer
David Vredevoogd (co-promotor)	An engineering approach to decode immune responses
Kaspar Bresser (promotor)	Genetic screens to improve immunotherapy

Titia Sixma

Netherlands Cancer Institute

1. General information

Research Focus	Structural Biology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Development of cancer is generally due to errors that occur in cellular pathways. Understanding the mechanisms will help to determine where the errors occur and how they can be treated. We use a combination of biochemical, biophysical and structural methods to provide insights in molecular mechanisms and targets for drug design studies. We focus on ubiquitin conjugation pathways in DNA repair and on DNA mismatch repair.

We combine quantitative biochemical analysis, Cryo-EM single-particle analysis and AI using AlphaFold to study a series of DNA repair complexes for TC-NER, mismatch repair, TLS and dsDNA break repair and mechanistic analyses in ubiquitin conjugation.

2.2. Major scientific achievements in 2023

a) Quantitative analysis of five USPs showed that the third critical residue is dispensable in some USPs and the fourth critical residue is vital instead, with varying roles in nucleophilic attack. This unexpected variety of catalytic mechanisms in DUBs may generate opportunities for selective targeting. Life Science Alliance, accepted for publication; BioRxiv, https://doi.org/10.1101/2023.07.24.550302

b) Structural analysis of DDA1 in the CUL4CSA E3 ligase helped to interpret its role in transcription-coupled DNA repair (TC-NER). This structural and biochemical work is the basis on ongoing structural analyses of TC-NER and their ubiquitination https://doi.org/10.21203/rs.3.rs-3385435/v1

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Structural analysis of BRCA1-A complexes

The BRCA1-A complex is important in the choice between DNA repair pathways after double strand breaks and in replication stress, but it also contains a deubiquitinating enzyme. We generated a novel di-ubiquitin probe that targets metal-dependent DUBs. This allowed structural analysis of substrate bound BRCA1-A and gave insight in its large conformational changes. Currently we are validating the observed ubiquitin chain path, mutations that selectively activate the BCRA1-A complex and the origin of the conformational changes observed.

Project B) Allosteric regulation of USP1

USP1 is a DUB that acts on PCNA and FA proteins. Inhibitors against USP1 are showing promise in BRCA1 deficient tumors, particularly for its role in PCNA deubiquitination. Here we follow kinetics of USP1 against poly-ubiquitinated PCNA, finding differences in kinetics with mono-ubiquitinated PCNA. Structural evaluation of the di-ubiquitin bound USP1 may give insight in these differences.

Project C) TC-NER analysis

Transcription-coupled nucleotide excision repair (TC-NER) is critical for rescue from large blocking lesions such as those resulting from UV. Here we collaborate with the Michiel Vermeulen and Wim Vermeulen labs to study the role of DDA1 in TC-NER.

Project D) Role of STK19 in TC-NER

In collaboration with the Jurgen Marteijn lab we study the role of STK19 in TC-NER. Native gel analysis showed that STK19 stabilizes the TC-NER complex, allowing us to obtain a cryo-EM structure of the RNA polymerase /TC-NER complex, containing 20 proteins and an RNA-DNA hybrid. This structure is currently being refined and analyzed.

Project E) Activation of UvrD by MutL

In DNA mismatch repair, the E. coli MutL protein activates a helicase UvrD to allow repair. Here we established the minimal domain that allows UvrD unwinding and study its contacts. A manuscript will shortly be submitted for publication. This project is terminated as the bacterial work will be discontinued in the lab in favor of work on the human proteins.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) OI investigator retreat was inspiring and allowed efficient exchange with many collaborators.

b) Oncode funding was instrumental to take on a collaborative effort with the Jurgen Marteijn lab to study the role of STK19 in transcription coupled repair. In this work we were able to resolve the cryo-EM structure of the TC-NER complex and understand how this effects its ubiquitination cycle.

c) Oncode funding has been instrumental in our ongoing efforts to understand how BRCA1-A affects DNA repair after double strand breaks and replication stress. Together with our development of a metal-directed ubiquitin probe this is now giving insight in conformational changes and chain specificity for this important DUB complex.

2.4.2. Contribution to the Oncode community in 2023

a) Organization of Oncode Master Class on structural biology, 8 December 2023

b) Initial review of Oncode Institute junior researchers

c) Programme committee Oncode Annual Meeting 2024

2.4.3. Key collaborations within Oncode in 2023

a) Sylvie Noordermeer: Two separate collaboration, both started after Sylvie joined the Oncode network; One on BRCA1-A, testing of mutants in cell lines. the other a collaboration on analysis of BRCA1 and CTIP BRCT domain interactions. Each of these collaborations is expected to lead to a publication in 2024

b) Michiel Vermeulen: collaboration on role of DDA1 in TC-NER, started as NWO project, but further improved thanks to meetings at Oncode events. publication in revision (https://doi.org/)10.21203/rs.3.rs-3385435/v1). Second manuscript planned for 2024. Work has led to new grant proposal to NWO, also with Jurgen Marteijn (see c).

c) Jurgen Marteijn: collaboration on role of STK19 in TC-NER. Started based on work done in the context of the NWO project, but would not have been possible without Oncode funding. Forms the basis of new proposal to NWO. Manuscript planned for first half of 2024

2.4.4. Major valorization achievements in 2023

a) Collaboration CHDI on trapping Different States in the MutSß Conformational Cycle, funded by CHDI

b) Collaboration UbiQ on development of a toolbox for ubiquitin metalloproteases, funded by Health Holland

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Wietske Pieters (copromotor)	Modifiers of tumor development in Lynch syndrome

n

Hugo Snippert

UMC Utrecht

1. General information

Research Focus	Functional heterogeneity in cancer
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Research interest:

Cells within a cancer are highly heterogeneous with respect to their phenotype and can manifest distinct morphological, molecular and functional features. As a consequence, it is challenging to design treatment therapies that target all cancer cells as effectively. The lab main interest is to use patient-derived (cancer) organoids, molecular genetics and advanced imaging to study the cell biological underpinnings of 3 research topics that have a large impact on cancer treatment:

I) Understand the consequence of phenotypic heterogeneity & plasticity on tumor growth
II) Understand the rate and mode of genomic alteration patterns in cancer
III) Study cellular drug response using patient-derived tumor organoids (PDTOs)

Scientific scope: The overall setting to which above questions are applied has transitioned over the last couple of years from late-stage metastatic & resistant colon cancer (CRC) towards basic understanding of the earliest stages of CRC. Currently, we have limited mechanistic understanding of what happens during the malignant transformation of benign adenoma to early-stage cancer and why a subset of early-stage CRC become already metastatic at an early stage. Improving basic understanding of this elusive transitioning state will help to prevent rather than cure malignant stages.

2.2. Major scientific achievements in 2023

a) Acquisition of a highly competitive ERC Consolidator grant named 'TRANSFORMATION' (€2M). Using TRANSFORMATION I will provide in-depth understanding of the evolutionary parameters of an evolving tumor genome (mutation rate, type, patterns, tempo) that underly malignant transformation of benign colon adenomas to malignant cancers.

b) I was awarded the AMMODO Science Award for basic science (€350k) within the discipline of biomedical sciences. The funding can be spent to my own insights on a fundamental research project. Winning such a prestigious award is a fantastic support for my career and the direction that my lab is heading (prevention rather than curing). Former laureates are, among others, Thijn Brummelkamp and Jacco van Rheenen.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Born to be bad: Early stage colorectal cancers with metastatic capacity

Initiated with Oncode 1.0 Base fund. Subsequently acquired NWO VIDI grant to expand this topic (€800k) that started in Jan 2022.

With clinical partners in the UMC Utrecht, we developed a comprehensive approach using multiregional sampling for organoids and parallel examination of the tumor-microenvironment to perform functional studies on early-stage CRC. Each sampled region is representative of a sequential stage along the adenoma-carcinoma sequence of CRC progression (being normal, adenoma, cancer, invasive front), enabling personalized reconstruction of the critical steps that defined the formation of invasive cancer growth.

We now have a preliminary biobank of about 14 early-stage CRCs, of which the majority contains paired samples between cancers and invasive front. 8 patient samples have been sequenced by WGS (30x) as well as single-cell RNA seq to characterize the tumor microenvironment of normal, cancer and invasive front of 5 patients. Growth factor dependency screens are performed for all. Moreover, spatial transcriptomics has been performed on early-stage colorectal cancers to a) validate our organoid findings on the cellular mechanisms that initiate the formation of the invasive front (first sign malignancy) and b) to explore new biomarkers to stratify metastatic patients. Organoid co-cultures are now being established between tumor and microenvironment to perform functional studies.

Project B) Genomic diversification patterns in cancer

Initiated as spin-off of project A with remaining support of Oncode Base fund. Subsequently acquired ERC CoG to expand this topic (€2M) that will start in May 2024.

We are currently trying to experimentally map the evolutionary parameters that underly cancer progression from benign adenoma to malignant cancer, like mutation rate, fluctuations in rate, order, pattern etc.

Among others, we map the cause and consequence of chromosomal instability in normal human colon tissue. Both via the analysis of normal organoids (time-lapse imaging and cell type knock-in/knock-outs), as well as via Karyotype sequencing of all stem cells in human colon crypts where diversity between karyotypes is a direct read-out of ongoing instability.

In addition, within a collaborative setting with colleagues at UCL, we have documented that mutation rate in MLH1-deficient hypermutator CRCs is adaptable. Using >20 clonal organoid lines of a cancer, we identified clonal mutations in DNA repair proteins MSH2, 3 and 6 that are in addition (and not neutral) to MLH1-deficiency. Moreover, these mutations involve frame-shifts in a homopolymer track in their coding region, continuously sliding in-and out of frame and thus acting as a toggle switch that alters mutation rate and propensity (altered signature).

Manuscript is currently accepted in Nature Genetics.

Project C) Real-time single-cell drug response

We are continuously optimizing new strategies to calibrate our biosensors to perform single-cell measurements of live MAPK signaling activity in patient organoids. New strategies can transform our drug response analyses from short-term (hours) to long-term (days) and truly investigate mechanisms of therapy resistance. We have now optimized genetic knock-ins of the biosensor that is compatible with significant brightness and no silencing. This genetic approach is compatible with future in vivo microscopy measurements (with Jacco van Rheenen).

In parallel, we are establishing inducible protein degradation strategies (degron knock-ins), and general knock-in templates to measure immediate effect of protein depletion on MAPK signaling activity or cell cycle progression (Cdk1 activity).

Project D) Cell fate plasticity in normal colon

We have succeeded in making a successful knock-in strategy to visualize (live-cell microscopy) and/or quantify (FACS) the number of LGR5 stem cells in normal human colon organoids, despite low expression of marker genes. Moreover, we demonstrated a new cell-type depletion system that is mechanistically independent and superior to the current method (inducible caspase), enabling us to deplete stem cells (or any other cell type) and monitor the emergence of de novo human stem cells via de-differentiation. We are planning large-scale sc multiome experiments to study the (epi)genetic rewiring during cell fate plasticity, both in normal and well as in cancer derivatives.

Project A, B and C are predominantly funded via additional grants. However, Oncode base fund was used for additional/ high resolution analysis of samples like whole-genome sequencing, scRNA seq, and expensive organoid cultures, as well as additional personnel support for bioinformatic analyses, or technical support to establish new techniques and methods (organoid co-cultures, knock-in templates, etc.)

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Along the Oncode philosophy, I foster vital collaborations with clinicians (e.g. Jeanine Roodhart, oncologist and Leon Moons, gastroenterologist) to obtain access to highly unique tumor resection material to perform state-of-the art functional studies on early-stage colon cancers. My clinical partners ensure that my basic science is dedicated to actual pressing clinical problems, like solving patient stratification of early-stage CRC to prevent overtreatment on non-metastatic cancers.

b) Financial flexibility of the base fund is a key component of my team's success since high-risk projects can be explored without delay and acts as a multiplier once alternative funding sources are acquired to continuate and/or expand the work. Moreover, it helps to onboard talented people in the wake of no immediate alternative funding. Examples are: Arne van Hoeck, former postdoc Cuppen lab who helped me to establish a bioinformatic core in my lab, while he was allowed to finish the last papers of the Cuppen lab (former Oncode PI) that were published in Nature and Nature Genetics in 2023. Sjors Middelkamp, a newly hired postdoc in 2023 that ensured NWO Veni funding from 2024 onwards.

d) We embarked on three collaborations that required immediate investment in resources (organoids, DNA sequencing) and personnel to carry the projects to a successful end. One collaboration is now accepted in Nature genetics (with shared first authorships and co-correspondence for me). The other is in revision at Science (lead author is Oncode PI Thijn Brummelkamp). I cannot oversee how I would have managed if not thanks to the flexibility of the Oncode base fund.

2.4.2. Contribution to the Oncode community in 2023

a) As a group, we aim to be active participants at meetings and within the Oncode community, including presentations and posters by lab members.

b) In additional to existing/long-time collaborators, we intensified a successful collaboration with Thijn Brummelkamp/Reuven Agami (NKI) to validate and explore their finding in patient-derived organoids. Similar collaborations are ongoing with Rene Bernards/Lodewyk Wessels (NKI).

c) I took the initiative to create/ lead a new consortium, dominated by (young) Oncode faculty, to apply for NWO funding (SUMMIT, Single-cell united, ~€40M) to provide long-term continuation for the infrastructure facilities (single-cell/ sequencing) that have been founded by Oncode at various research institutes. This initiative was highly coordinated with the board of Oncode Institute. Although initially not successful, we are exploring alternative options.

2.4.3. Key collaborations within Oncode in 2023

a)	Brummelkamp/Agami	new collaboration thanks to Oncode	manuscript in revision at Science
b)	Bernards/ Wessels	new collab. thanks to Oncode	new KWF grant in 2022
c)	R. van Boxtel	new collab. thanks to Oncode	Nature Genetics, accepted
d)	J. van Rheenen	long-term collaborator	collab. in stem cell biology (NWO gravity)
e)	M. Vermeulen	long-term collaborator	collab. on CRISPR in organoids
f)	G. Kops	long-term collaborator	collab. on CIN in organoids (NWO gravity)
g)	R. Medema	long-term collaborator	collab. on CIN in stem cells
h)	M. Maurice	long-term collaborator	collab. study on RNF43/ optogenetics (NWO TOP/ gravity)
i)	H. Clevers	long-term collaborator	collab. study on Tuft cells (NWO groot)

2.4.4. Major valorization achievements in 2023

a) Continuation of the RASTRIC trial. Started Sept. 2020. Preclinical data was published in March 2023.

b) Organoid biobank of the RASTRIC trial (unique chemo-treated tumor organoid samples) are licensed to Crown (leaded by Jeanine Roodhart).

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
ERC	Consolidator	2M	2M	05/2024	60	Main applicant
AMMODO	Science award	350k	350k	06/2024	36	Main applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Bas van Steensel

Netherlands Cancer Institute

1. General information

Research Focus	Genome Biology and Gene Regulation
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Our Chromatin Genomics lab develops and applies new genomics techniques to study the regulation of gene expression, chromatin architecture and genome maintenance.
We have used our Oncode base funds to develop several novel approaches to gain insights into the role of promoters and enhancers: How do these elements communicate with each other to regulate gene expression? How are they controlled by the layer of transcription factors that interpret incoming signaling events? To tackle these questions, we have set up novel high-throughput reporter assays and efficient transposon-based genome perturbation methods. Importantly, we have also begun to leverage our unique genomics toolbox to systematically identify mutations in cancer genomes that alter the activity of regulatory elements. We also continue to study the interaction of the genome with nuclear structures such as the lamina and nucleoli, and the impact on genome regulation. Finally, using a powerful method to probe double-strand break repair throughout the genome, we elucidated how chromatin context affects the balance between various repair pathways, and how dozens of repair proteins are involved in this interplay. Our efforts yield new tools to study genome biology more efficiently, and offer new biological insights into gene regulation and DNA repair.

2.2. Major scientific achievements in 2023

a) Firmly established powerful technology to move regulatory elements (promoters, enhancers, LoxP sites, other sequences) around in a genomic locus at highly scalable manner. This enables us to understand the logic of domain organization and the positioning of regulatory elements. Manuscript: https://www.biorxiv.org/content/10.1101/2024.01.10.574825v1; second manuscript in preparation.

b) Completed construction of a highly optimized set of reporter constructs for ~50 transcription factors, by testing 36,000 reporter designs. This creates many opportunities for multiplexed tracking of transcription factor activities in developmental biology, cancer models, etc.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Impact of chromatin on double-strand break repair

Repair of double-strand DNA breaks is done by several multi-protein pathways, including NHEJ and MMEJ. Chromatin composition at the break site controls the balance between these pathways. To understand which repair proteins 'sense' the local chromatin state, and alter pathway balance accordingly, we executed a unique CRISPR-based screening approach to probe the impact of >500 repair proteins on NHEJ and MMEJ activity in 19 different chromatin environments. We thus identified 89 proteins that show chromatin context-dependent effects on pathway balance. For each of these proteins we identified the chromatin context that they respond to. Much of our work in 2023 was focused on addressing referee comments towards publication. As part of this, we now demonstrated that these chromatin dependencies broadly impact the accumulation of mutations in genomes of a wide variety of cancers. Collaboration with R. Medema (NKI) and M. Sanders (Erasmus MC) and supported by Oncode base funding.

Project B) Finding mutations in cancer genomes that affect regulatory elements

With the PERICODE consortium of seven Oncode labs we have built a powerful AI-based computational model, trained on our SuRE massively parallel reporter assay, that can predict the activity of human promoters from DNA sequence alone, and even predict how specific mutations alter promoter activity. In the past year we have substantially improved and validated the AI model, and constructed SuRE libraries that greatly simplify the generation of training data in a diversity of cell types (patent application filed). PERICODE is now applying this model to identify functionally non-coding mutations in cancer genomes. Moreover, we have discovered that the model makes spectacularly detailed predictions of the TFs (and their interactions) that control each human promoter. This has numerous applications, both fundamentally and in cancer. Therefore, in 2024 I am re-directing a major part of my lab towards this project, in part using Oncode base funding to maximally speed up this project.

Project C) Monitoring the activity dozens of transcription factors in parallel

In every cell, dozens of transcription factors (TFs) each control the expression of hundreds of genes. Understanding how these complex TF networks control cell function requires precise measurements of TF activity, ideally for many TFs in parallel and in single cells. By systematic optimization (testing of 36,000 reporter designs), we managed to establish a set of highly senstitive and specific reporters for ~50 different TFs that can be detected in parallel by barcoding. In 2024 we will (i) move towards single-cell detection of these TF activities in a differentation system; (ii) use these reporters to validate and improve our AI-based model (project B); (iii) combine these reporters with our TRIP assay to understand how each TF "senses" its local environment of chromatin and nearby regulatory elements. So far, this project seemed poorly compatible with the funding landscape, and hence was supported by Oncode base funding.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The PERICODE project (which emerged from the Synergy/Acceleration program) has changed my research significantly. First, it is much more collaborative than my previous projects, and I very much enjoy coordinating this interdisciplinary team effort. Second, the AI-based technology that we have developed in PERICODE is a game changer for my research, creating numerous new opportunities to tackle fundamental and cancer-related problems

2.4.2. Contribution to the Oncode community in 2023

a) Co-organized the Oncode Annual Scientific Meeting (June 8-9, 2023)

b) Leading PERICODE Consortium (see above and below).

c) Co-lead of Artificial Intelligence discussion at PI retreat

2.4.3. Key collaborations within Oncode in 2023

a) PERICODE consortium (E. Voest, W. Zwart, S. Derks, L. Franke, M. Vermeulen, J. de Ridder, BvS). I am leading this consortium, and accordingly spend a significant proportion of my time on coordinating activities: discussing results, paper writing, planning, maximizing synergies, organizing meetings, etc. It is one of the most exciting projects that I have been involved in.

b) René Medema: joint PhD student (Xabier Vergara), studying chromatin context dependencies of DNA repair.

c) Thijn Brummelkamp: Haploid genetic screen to identify regulators of gene repression in LADs.

2.4.4. Major valorization achievements in 2023

a) Filed patent (together with Jeroen de Ridder) for AI-based algorithm construction to predict promoter activity from DNA sequence

b) Exploring possibilities for spin-out company based on (a). Among others, we presented our platform to a delegation from Syncona.

c) With help from Yuva Oz, we have contacted commercial parties that may be interested in our collection of TF reporters.

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Heta Patel	Spatiotemporal analysis of transcription dynamics
Ruben Schep	A Cas9 TRIP through chromatin
Christ Leemans	TRIP to learn how processes dance to local beats

Mario van der Stelt

University Leiden

1. General information

Research Focus	Medicinal chemistry, drug discovery, chemical biology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

In multidisciplinary research lines organic and medicinal chemistry are combined with machine learning and innovative chemical biology techniques, such as chemical proteomics, to optimize and profile compounds as chemical tools and drug candidates to validate proteins as potential therapeutic anti-cancer targets. My current research interests are focused on the detection and modulation of lipid metabolism and kinase signalling. We have currently several hit-to-lead optimization programs running in the lab based on inhibitors for BuB1-kinase and monoacylglycerol lipase (MAGL), which were identified using high throughput screening. The BuB1 program is guided by structure-based drug design in collaboration with Prof. Perrakis (NKI). Our advanced lead candidate ROB-433 induced senescence and in combination with Navitoclax-treatment showed synergistic killing of triple negative breast cancer cells. Using a chemical genetics approach we discovered that the senescence was induced by an off-target of the compound. Currently, we are optimizing the selectivity profile of the chemical series. Our MAGL program has delivered LEI-515 as the first-in-class peripherally restricted monoacylglycerol lipase inhibitor that reduces paclitaxel-induced neuropathy without inducing central nervous system mediated side effects or physical dependence. The results have been published in Nature Communication in December 2023. Back-up compounds with improved lipophilicity, selectivity and cellular activity have been identified. Finally, we are developing new probes and chemical proteomic methods for cellular target engagement studies of kinase inhibitors.

2.2. Major scientific achievements in 2023

a) Jiang M, et al. A monoacylglycerol lipase inhibitor showing therapeutic efficacy in mice without central side effects or dependence. Nat Commun. 2023 Dec 5;14(1):8039.

Here we report the discovery of the peripherally restricted MAGL inhibitor LEI-515. Our data support targeting peripheral MAGL as a promising therapeutic strategy for developing safe and effective anti-inflammatory and analgesic agents to counteract the side effects of chemotherapy. The valorisation potential of this breakthrough is currently being explored.

b) My former PhD-student, Dr. A. Bakker won the Award of Best PhD-thesis in Medicinal Chemistry 2021-2022 of the Royal Dutch Chemistry Society. This award is a recognition of the quality of the research performed in our department by the Dutch chemistry community.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Discovery of BuB1 modulators for triple negative breast cancer1

ROB-433 was previously identified as a lead candidate with dual BuB1 and AURB inhibition profile in collaboration with the NTRC. The compound induces senescence in various cancer cell lines and inhibits the proliferation of cancers cells as a single agent. We have discovered in 2023 that the senescence induction in triple negative breast cancer (TNBC) cells was not due to BuB1 inhibition using a chemical genetics approach. Several co-crystal structures of this chemotype with BuB1 have been elucidated in collaboration with Prof. Tassos Perrakis (NKI), which guides the design of new, and hopefully more selective compounds with better physico-chemical properties to improve the in vitro ADME properties. A promising new subseries has been identified.

Project B) Discovery of inhibitors of lipid metabolism (MAGL) 1

Monoacylglycerol lipase (MAGL) regulates endocannabinoid 2-arachidonoylglycerol (2-AG) and eicosanoid signalling. MAGL inhibition provides therapeutic opportunities but clinical potential is limited by central nervous system (CNS)-mediated side effects. Here, we report the discovery of LEI-515, a peripherally restricted, reversible MAGL inhibitor, using high throughput screening and a medicinal chemistry programme. LEI-515 increased 2-AG levels in peripheral organs, but not mouse brain. LEI-515 suppressed chemotherapy-induced neuropathic nociception in mice without inducing CNS-side effects of physical dependence. Our data support targeting peripheral MAGL as a promising therapeutic strategy for developing safe and effective anti-inflammatory and analgesic agents to counteract the side effects of chemotherapy. In 2023, using a structure-based design approach, we identified back-up compounds that have an improved in vitro profile compared to LEI-515. There is a significant funding gap to translate fundamental discoveries in academia towards clinical solutions in the Netherlands. We may apply for a Oncode Accelerator demonstrator project.

Project C) Design, synthesis and application of new kinase probes 1

Protein kinases comprise a family of 518 enzymes which are highly attractive for drug discovery. Cellular target engagement and selectivity profiling is essential to correlate inhibitor concentrations at the site of action to pharmacological and phenotypic readouts. We have developed a chemical proteomic assay to determine kinase activity in cells, which enabled us to determine the cellular IC50s of Btk and FLT3 inhibitors across 270 kinases expressed in HEK293T and MV4-11 cells. To increase the kinome coverage we have expanded our probe library to 25 novel activity-based probes and tested their kinase engagement in the cells. We can now identify over 500 kinases. The new probes are currently being patented and we are exploring the set-up of a start-up company. Roche has shown interest our work and we signed a three-year research agreement.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) By becoming an Oncode PI, my scientific network has greatly expanded, which led to several collaborations that have accelerated my research and provided new opportunities. For example, we continued the collaboration with Prof. Kops and Prof. Perrakis on our BuB1 inhibitor program and with Prof. Heitman on Cannabinoid CB2 receptors. In addition, we have sent compounds to Prof. Bernards.

b) The business development team of Oncode Institute has coordinated my valorization activities. They prepared several CDA, MTA and contract research / interinstitutional agreements with various companies (e.g. Roche, NTRC, PPSC). This facilitated my interactions with industry and led to productive collaborations. For example, a three-year research agreement with Roche was established.

c) Arguably, equally important is their help in the coordination and filing of the patent application for our kinase engagement profiling program. This patent application will be filed in 2024 and forms the basis for further exploration of exciting business opportunities (e.g. start-up company).

2.4.2. Contribution to the Oncode community in 2023

a) As a member of the Research Management Committee, I contribute to the management and strategic discussions on the science and organization of Oncode Institute on a regular basis. A major activity was the junior PI recruitment in 2023.

b) As the architect of the small molecule workstream and AI-platform and coordinator of the small molecule workstream of Oncode Accelerator, I have assembled a public-private preclinical small molecule drug discovery pipeline. The collaboration and consortium agreements were signed in 2023, thereby providing funding for the protein facility, the proteomics facility and the drug repurposing facility, which are managed by other OIs. In this way I can help to translate the fundamental findings of Oncode Institute into clinical solutions for patients.

2.4.3. Key collaborations within Oncode in 2023

a) Prof. dr. L. Heitman – long term collaboration on the cannabinoid CB2 receptor. Publication: Li X, Chang H, Bouma J, de Paus LV, Mukhopadhyay P, Paloczi J, Mustafa M, van der Horst C, Kumar SS, Wu L, Yu Y, van den Berg RJBHN, Janssen APA, Lichtman A, Liu ZJ, Pacher P, van der Stelt M, Heitman LH, Hua T. Structural basis of selective cannabinoid CB2 receptor activation. Nat Commun. 2023 Mar 15;14(1):1447. doi: 10.1038/s41467-023-37112-9. PMID: 36922494; PMCID: PMC10017709. Existing collaboration.

b) Prof. dr. A. Perrakis & Prof. dr. G. Kops –Structure-based drug design of BuB1 inhibitors. New collaboration started at an Oncode meeting.

c) Oncode Accelerator led to collaboration with many OIs, including Prof. M. Vermeulen, Prof. A. Perrakis, Prof. B. Burgering, Prof. L. Franke, Prof. L. Wessels, Prof. S. van der Burg, Dr. J. de Ridder, Dr. S. van Heesch and Dr. J. Drost. New collaborations

d) Prof. dr. M. Maurice – We supported Madelon on target identification. Publication: van der Krift F, Zijlmans DW, Shukla R, Javed A, Koukos PI, Schwarz LL, Timmermans-Sprang EP, Maas PE, Gahtory D, van den Nieuwboer M, Mol JA, Strous GJ, Bonvin AM, van der Stelt M, Veldhuizen EJ, Weingarth M, Vermeulen M, Klumperman J, Maurice MM. A novel antifolate suppresses growth of FPGS-deficient cells and overcomes methotrexate resistance. Life Sci Alliance. 2023 Aug 17;6(11):e202302058. doi: 10.26508/lsa.202302058. New collaboration.

2.4.4. Major valorization achievements in 2023

a) New collaboration agreement with Roche (3-year PDF position, signed in Jan 2024)

b) New patent application drafted for kinase probes (to be filed in Feb 2024)

3. Highlights

3.1. External grants & awards awarded in 2023

Awards:

a) Dr. A. Bakker won the Award of Best PhD-thesis in Medicinal Chemistry 2021-2022 of the Royal Dutch Chemistry Society

b) A. Beers, M.Sc. won the Unilever Research prize for best master thesis 2023.

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Timo Wendel	*New chemical tools to illuminate N-acylphosphatidylethanolamine biosynthesis*

Marvin Tanenbaum

Hubrecht Institute

1. General information

Research Focus	Single molecule analysis of gene expression dynamics
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Precise gene expression control is critical for correct cell and tissue function. Defects in gene expression control deregulate the expression levels of proteins, and as such, can contribute to the development of cancer. The ability to precisely measure gene expression, and obtain an understanding of how precise gene expression control is achieved, will provide a better understanding of both physiological cell homeostasis, as well as of cancer development and treatment. Our group is developing and applying cutting-edge imaging-based technologies to study gene expression regulation in single living cells at single-molecule resolution. Using these novel technologies, we aim to understand the regulation, dynamics and heterogeneity of gene expression and to determine how altered gene expression affects cellular functioning in cancer. In addition, we are re-deploying these technologies to study gene expression in RNA viruses as well, and are interested in optimizing RNA viruses for oncolyitic virus therapeutics.

2.2. Major scientific achievements in 2023

a) Technology and discovery: Collisions between ribosomes translating the same RNA are common and need to be resolved to prevent cellular toxicity. Drugs, UV light and chemotherapeutics can induce collisions exaggerating toxicity. Using a newly developed technology, we have discovered new mechanisms that resolve ribosome collisions to prevent toxicity.

b) Zero-Mode Waveguide Nanowells for Single-Molecule Detection in Living Cells. Yang S, Klughammer N, Barth A, Tanenbaum ME, Dekker C. ACS Nano. 2023 Oct 24;17(20):20179-20193. doi: 10.1021/acsnano.3c05959. In this paper, we develop a new method for visualizing single protein molecules inside living cells, a major breakthrough that will help unravel how proteins work in the cellular environment.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Linking genotypes to phenotypes through a unique barcoding scheme.

Project A) Cellular phenotypic heterogeneity is common, and thought to drive cancer therapy resistance. Therefore, it is critical to understand how differences in genotype (DNA sequence, transcriptome, etc) affect phenotypic heterogeneity. We are developing a method that allows real-time imaging of single cells, to assess cellular phenotypes, combined with single cell sequencing of the same cells. This method will be broadly applicable both in the study of genetic control of biological processes and for understanding therapy resistance. This project was made possible through a collaboration with Oncode PI Jop Kind, who provides the single cell sequencing expertise. In the past year, we have also established a collaboration with Oncode PI Boudewijn Burgering (established through a casual discussion at the Oncode annual meeting) to try and link imaging phenotypes to single cell mass spec as well.

Project B) A synthetic biology approach to design potent oncolytic viruses

Oncolytic viruses have show great potential in cancer therapy in recent years. A major goal in oncolytic virus therapy is to design viruses that specifically target cancer cells. So far, efforst have focused on the cellular entry step. In this high risk project, we are trying to engineer viruses that sense the intracellular milieu to determine if they are in a cancer cell, which would allow many new aspects of cancer cells to be targeted (e.g. mutations in Ras oncogene). We have no publication record in oncolytic virus research and do to the very high risk nature of this project, we would unlikely be able to obtain funding for this project. Yet, our expertise on RNA biology, virology and cellular engineering uniquely allow us to undertake this project using the base fund.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The base fund allowed us to start a very high risk high gain project on oncolytic virus engineering

b) Discussions at the Oncode Annual meeting led to a new, very interesting collaboration with the group of Boudewijn Burgering. This collaboration also involves the single cell mass spec facility that was established with help of Oncode funding.

2.4.2. Contribution to the Oncode community in 2023

a) Member of the RMC; evaluation of junior investigators; recruitment of new OIs

b) Taken part of 2x Grote gevers event at the Hubrecht Institute

2.4.3. Key collaborations within Oncode in 2023

a) Jop kind (2.3.1. project A)

b) Boudewijn Burgering (2.3.1. project A)

2.4.4. Major valorization achievements in 2023

a) We are currently collaborating with the RNA

3. Highlights

3.1. External grants & awards awarded in 2023

N/A

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Louis Vermeulen

Amsterdam AMC

1. General information

Research Focus	Cancer molecular subtypes, stem cell dynamics, colorectal cancer, microenvironment
Junior/Senior Oncode Investigator	Junior, Senior per 2024

2. Oncode activities

2.1. Research topics and scientific progress

Our group seeks to elucidate the origin and evolution colorectal cancer (CRC). We have set up and developed a collection of state-of-the-art tools and techniques to study cancer stem cell dynamics. Stem cells are essential for the homeostasis of most adult human tissues. Previously we identified stem-like cells that fuel the growth and progression of CRC. We defined the impact of oncogenic mutations on stem cell dynamics in the intestine. We use this knowledge and state-of-the-art research tools to develop novel, and more effective therapies for CRC

2.2. Major scientific achievements in 2023

a) We investigate the chemopreventive effects of caffeine on the expansion of Apc-mutant clones in the intestine and found that caffeine Limits expansion of Apc-deficient clones in the intestine by NOTUM inhibition (van Driel et al., Cell Mol Gastroenterol Hepatol. 2023).

b) Meta-analyses of Systemic Treatments of Small Intestinal Adenocarcinomas (SIA) showed that adjuvant and palliative chemotherapy were both associated with improved survival of patients with SIA. Also, immunotherapy appears to offer advantages and should be contemplated as a viable option for SIA patients with tumors characterized by defective mismatch repair (De Back et al., JAMA Netw Open. 2023).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Clonal dynamics in developing and established cancers

We aim to better understand and manipulate cell competition in the earliest stage of cancer development, to be able to prevent the disease. We have been studying familiar adenoma polyposis (FAP), but now expanding this effort to other heritable cancer syndromes. Future patient care of heritable cancer syndromes can be shifted from treatment to prevention.

Project B) Wnt-regulated long non-coding RNAs (lncRNAs) in CRC

High-throughput CRISPR-based techniques systematically assess the function of genes or regulatory elements present in the human genome. We developed and published a protocol for identifying essential lncRNAs in cancer using CRISPRi-based dropout screens (Bril et al., STAR Protoc. 2023).

Project C) Molecular cancer subtypes in CRC

We set up the PREDICATE project to generate the scientific evidence and develop the work flows and infrastructure necessary to implement personalized medicine for CRC in the clinic. We collaborate with pharmaceutical and diagnostics companies (such as Novartis, Servier, Pierre Fabre, Incyte, Illumina, Roche, Genomescan) that together sponsor the PREDICATE project with > 1M Euro funding. Also, we obtained financial support from the Oncode Technology Development Fund. In 2023, molecular data (RNAseq, TSO500) of >650 CRC tumor samples has been analyzed and the first paper will be submitted early 2024.

Project D) Peritoneal metastatic disease

Currently, we are further exploring effective treatments of Peritoneal Metastatic Disease (PMD) in collaboration with clinical experts (e.g. Ignace de Hingh, Catharina Hospital), chemists (e.g. Maikel Wijtmans, VU) and drug development experts (e.g. Hans Platteeuw, Avivia BV). In 2023, we obtained a KWF Proof-of-concept grant (in collaboration with Dr. Maarten Bijlsma) where we further explore clinical application of YM155 against peritoneal metastatic disease.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Several contacts and contracts with companies were initiated with help of Oncode business developers. These initiatives resulted in various promising project.

b) Oncode helped us to reach out to companies for potential use/collaboration/licensing of our patented invention on a method for determining intra-tumour heterogeneity/ copy number heterogeneity of a tumour in a patient. In 2023, an agreement with Cergentis was completed for collaborations on new services.

c) Oncode is actively seeking potential partners to fund follow-up trails for our currently ongoing phase II trail on the chemopreventive effect of lithium in FAP (Linssen et al., BMC Gastroenterology, 2022).

2.4.2. Contribution to the Oncode community in 2023

a) Members of my research group attend and actively engage in Oncode meetings

2.4.3. Key collaborations within Oncode in 2023

a) Oncode helped us set up a collaborative research program with Miao-Ping-Chien on mCRC-focussed target discovery project. Using the newly developed state-of-the-art (FUNsice) technology we were able to identify candidate therapeutic targets crucial for the tumor-stroma crosstalk that defines the tumor growth dynamics (publication in preparation).

b) We currently work with Michiel Vermeulen to use the STPP-UP method for drug target identification using protein thermal stability. With this methods we aim to find the target of elesclomol, a compound that we previously found to be effective against peritoneal metastases.

2.4.4. Major valorization achievements in 2023

a) In 2023, my lab obtained a KWF Proof-of-concept grant (in collaboration with Dr. Maarten Bijlsma) where we further explore clinical application of YM155 against peritoneal metastatic disease.

b) We collaborate with Prof. Tom Grossmann (Chemistry, VU) and several companies (PLS, Incircular, EnzyTag) to design and experimentally test first-in-class Wnt inhibitors as novel treatment for CRC.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
NWO	ENW-M	656,298	328,147	10/2023	48	Co-Applicant
KWF	TKI	598,756	598,756	8/2023	36	Scientific Advisor
KWF	TKI	490,013	245,005	9/2023	36	Scientific Advisor
AUMC	Starter Grant	315,000	315,000	1/2024	60	Scientific Advisor
KWF	PoC	149,961	149961	1/2024	18	Scientific Advisor
CCA	Research Project	87,500	87,500	3/2024	18	Scientific Advisor
KWF	TKI	496,060	248,030	3/2024	36	Scientific Advisor
CRUK		650,000	50,000	6/2024	48	Scientific Advisor

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT05402891 2022-000240-30	the chemopreventive effect of lithium in familial adenomatous polyposis	5/2022	11/2024	Yes	Co-PI

3.3. PhD defenses in 2023

N/A

Michiel Vermeulen

Radboud University

1. General information

Research Focus	Proteomics & Chromatin Biology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

The main focus of our lab is to decipher (epi)genetic regulation of gene expression and cell fate in (differentiated) stem cells using integrative omics approaches. Furthermore, we use the same technology to study deregulation of gene expression in cancer. Our lab also develops new proteomics and genomics technology for cancer research, which we make available to the Oncode community. Below, a selection of current research questions/topics is listed:

1. Integration of multi-omics data from colon tumor progression organoids (Jelmer Dijkstra et al., JPR 2023) with publicly available TCGA data uncovered various SMAD4 mutation-dependent oncogenes and tumor suppressors in colorectal cancer, which we are currently further exploring.

2. We are exploring the functional consequences of somatic non-coding mutations that occur with a high frequency in various cancers. These efforts are embedded in the Pericode consortium, which is one of the shortlisted Oncode 'Grand Challenge' projects

3. Development and implementation of new omics technologies for cancer research. Current efforts are focused towards further development of proximity biotinylation technologies and implementation of the STPP-UP thermal proteome profiling method (Dick Zijlmans et al., JBC 2023) to identify cellular on and off-targets for cancer drugs and lead compounds of interest.

2.2. Major scientific achievements in 2023

a) Our method to profile genome-wide transcription factor binding affinities across the genome was published in Nature Biotechnology (PMID 36973556)

b) Our lab enjoyed a prolific year in 2023 (23 publications, 'Michiel Vermeulen' on Pubmed), many of which in leading research journals and numerous in collaboration with Oncode investigators

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) (Interaction) proteomics using proximity biotinylation

We are currently developing a new proximity labelling technology in which we couple two distinct chemistries, one associated with the APEX-based proximity labelling enzyme and one with TurboID in the same cells. This allows sequential affinity enrichments using click-chemistry and streptavidin beads, respectively. This method allows, amongst other things, to identify the shared interactome for two baits of interest (for example a transcription factor with a chromatin modifying enzyme) or exploring the cell surface interactome of tumor cells and immune cells. We also plan to apply this technology to investigate loss of polarity in cancer cells, which in the future may have profound clinical implications, i.e. development of bi-specific antibodies targeting multiple proteins that are proximal to each other specifically on the cell surface of cancer cells. In 2023 we also completed two TurboID proximity biotinylation studies, one for the Polycomb group proteins PRC1 and PRC2 in early development (Zijlmans et al., in review at Cell Reports) and for p300 in mouse gastruloids (Stelloo et al, in revision at Cell Stem Cell). Finally, we are currently completing two interaction proteomics/proximity biotinylation studies in the context of transcription coupled DNA repair (in collaboration with Wim Vermeulen/Jurgen Marteijn and Tita Sixma)

Project B) A genomics method to profile genome-wide TF binding affinities

Genomics profiling methods such as ChIP-sequencing are not quantitative but rather binary in nature, i.e. a protein of interest binds to a particular genomic region or not. We recently developed a new method, called BANC-seq, to determine genome-wide absolute apparent binding affinities of transcription factors to native, chromatinized DNA (Hannah Neikes et al., Nat. Biotech. 2023). In the future, this method can be explored to investigate transcription factor binding across the genome in a truly quantitative manner in health and disease, including cancer-associated expression of oncogenes, such as Myc. Development of this high-risk high-gain methodology was made possible to our Oncode base funds. BANC-seq is currently also being explored in collaboration with Wilbert Zwart, who is interested to use this method in the context of Androgen receptor binding across the genome in prostate cancer cells.

Project C) Interaction proteomics of ADP ribose (ADPr) chains

Poly-ADP-ribosylation (PARylation) is a post-translational modification (PTM), which plays an important role in genome maintenance and the immune response. The recent discovery that survival of certain cancer types depends on PAR-synthesizing activity has triggered an increased interest in PARylation. The great potential of modulating PAR signalling as an anticancer therapy is illustrated by the development of clinically approved PARP inhibitors, such as Olaparib. Proteins comprising modules that bind to PARylated targets (PAR readers) are particularly interesting, as they enable downstream signalling events. Previously we performed the first proteome-wide interaction screenings for linear ADPr chains (Kliza KK et al., Mol. Cell 2021). In addition to linear ADPr chains, branched ADPr chains can also be generated inside cells, but the biological function of these branched chains thus far remains elusive. In collaboration with Dima Fillippov in Leiden, we are currently finalizing, to the best of our knowledge, the first interaction proteomics screening for branched ADPr probes, which provides unique insights into the biological function of branched ADPr. We are also further exploring ADPr baits and Olaparib molecules containing photo-crosslinking groups, which will allow identifying direct ADPr readers and comprehensive profiling of cellular proteins that interact with Olaparib. We also initiated a collaboration with Jos Jonkers to investigate the role of BRCA1/2 in heterochromatin function

Project D) Multi-omics tools to study cell fate switches in (cancer) organoids
In 2023 we published a (single-cell) multi-omics study in which we discovered a transcription factor-mediated regulatory switch that restricts Microfold cell numbers in the small intestinal epithelium. This study sheds new light on the mechanism regulating cell fate balance in the Peyer's patches and provides a powerful blueprint for investigation of cell fate switches in the intestinal epithelium (Luna Velez MV et al., Nucleic Acids Res 2023). In a separate study, using diverse proteomics methods including secretomics, we identified several additional factors that are specific to Microfold cells, one of which is the secreted protein IL4I1. In collaboration with Annemiek van Spriel, we are currently investigating the effects of IL4I1 activity on T cell lineage specification. We also published a multi-omics study of colorectal cancer tumor progression organoids, in which we uncovered putative mediators of cancer progression resulting from SMAD4 inactivation (Dijkstra JJ et al., J Proteome Res. 2023). We are also finalizing a collaborative study with the Clevers lab in which we investigate the molecular mechanisms of human small intestinal cancer driven by a loss of the Menin tumor suppressor gene.

Project E) Towards single cell proteomics
In collaboration with the lab of Boudewijn Burgering we have established a workflow making use of the CellenONE platform and a multiplexed TMT-based quantification strategy to enable proteome measurements in single cells. This is a highly ambitious high-risk high-gain aim with obvious applications in the Oncode community, considering the cellular heterogeneity of tumors. As a proof of principle, we (Suzan Stelloo and Charlotte van Gelder) applied this workflow to mouse gastruloids, and we were able to identify and quantify ~1500 proteins in single cells, which is highly encouraging. This work is currently under revision at Cell Stem Cell.

Project F) Function of non-coding mutations in cancer
In the Oncode Synergy project 'Pericode' we are developing innovative interaction proteomics technologies to investigate specific interactions between proteins and non-coding mutations and the affinity of these interactions using multiplexed quantitative interaction proteomics (i.e. Grawe CG et al., J Proteome Res. 2023). The van Steensel and the de Ridder lab have recently developed a deep-learning model based on massively parallel reporter assays (SuRe technology) to investigate the regulatory principles of human promoters. In the context of this newly developed technology, we are collaborating with the van Steensel lab using our quantitative interaction proteomics technology to experimentally verify predictions made by the model. In the future, we will further explore the model to identify non-coding mutations in cancer that are functionally relevant. Furthermore, the deep learning model has identified a number of novel 'orphan' DNA binding motifs in the human genome that drive gene expression but for which the interacting TFs are currently unknown. Our interaction proteomics technology is perfectly suited to 'de-orphan' these motifs and identify the TFs that bind to these motifs to regulate gene expression.

Project G) STPP-UP: a new method for drug target identification using protein thermal stability
A major bottleneck in cancer drug discovery is the fact that many lead compounds fail in clinical trials. One of the reasons for this is that many compounds are not specific and show substantial off-target effects, which are not always apparent during preclinical testing. A powerful technology that can be used to identify target and off-targets effects for compounds of interest is called Thermal Proteome Profiling (TPP). TPP is based on the principle that proteins denature and become insoluble when subjected to heat. Thermostability of proteins, however, can change upon interactions with small molecules, such as drugs, DNA or other proteins. In TPP, multiplexed quantitative mass spectrometry is used to investigate the melting profile for thousands of cellular proteins in a global, proteome-wide manner. A significant shift in the melting curve for a particular protein upon addition of a compound to cells indicates that this protein may be a direct target for that compound. We have recently implemented TPP in the lab and have also used it in collaboration with various Oncode PIs (Madelon Maurice, Jan-Paul Medema, Peter ten Dijke) to assess the cellular targets for drugs of interest. One of the main problems of TPP, however, is its lack of throughput: ~ 2 days of non-stop data acquisition on a high-end mass spectrometer is required to measure one TPP experiment. To overcome this bottleneck, we recently developed a new TPP method called STPP-UP, which reduces the amount of input material and mass spec measurement time substantially, while retaining the ability to identify direct drug targets. This method was recently published (Zijlmans D et al., JBC 2023). We filed an initial patent for STPP-UP and we have been in touch with a pharmaceutical company that is potentially interested to license the STPP-UP method. STPP-UP will also be key technology in the Oncode Accelerator Growth Fund initiative, in which we participate.

Project H) Context-dependent oncogenes in colorectal cancer
Colorectal cancer (CRC) tumors can be classified into four molecular subtypes (CMS). CMS4 tumors are characterized by epithelial-to-mesenchymal transition (EMT) induced by TGF-β signaling and are associated with increased metastasis and poor prognosis. To investigate the molecular context of TGF-β signaling and EMT in mesenchymal (CMS4) CRC, we mined molecular and clinical data from CRC patients and integrated this with multi-omics data from a CMS4-resembling colon cancer organoid model (Dijkstra JJ et al., J Proteome Research 2023). This analysis revealed a number of context-dependent oncogenes that require SMAD4 mutations to unleash their full oncogenic potential. We are currently investigating the molecular function of these context-dependent oncogenes in CRC, including Hand1 and PTK7. Preliminary work revealed that expression of Hand1 in CRC cells induces an EMT gene expression signature, but only in SMAD4 deficient cells, further supporting our hypothesis that Smad4 mutations are required for Hand1 to act as an oncogenic transcription factor. Additional experiments suggest that Smad4 and Hand1 compete for binding to the same DNA motifs, which we are currently further exploring. In this project, we will collaborate with various Oncode investigators, including Hugo Snippert, Jacco van Rheenen, Emile Voest and Madelon Maurice.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Numerous collaborative studies with various Oncode researchers were published and/or submitted in 2023, which contributes to our national and international visibility. These collaborative studies also enable our lab members to build a scientific network.

b) Our interactions within the Oncode community helped us to develop various project and grant proposals, some of which were submitted in 2023

c) Oncode is closely aligned with the Oncode Accelerator Growth Fund Project in which we participate. This provides us with ample opportunities to further strengthen existing and further develop new collaborations within the oncology community in the Netherlands. These collaborations will also involve various pharmaceutical companies. Furthermore, the Growth Fund funding enables us to modernize our proteomics research infrastructure (we recently purchased an Orbitrap-Astral instrument from Thermo which will be installed in the spring of 2024), which the entire Oncode community will benefit from.

2.4.2. Contribution to the Oncode community in 2023

a) Various Oncode investigators benefited from our proteomics and genomics expertise in 2023 (see collaborations section). Our lab significantly contributes to the collaborative spirit that forms a cornerstone of Oncode

b) Michiel Vermeulen and various lab members presented and/or participated in various online and 'live' Oncode events.

c) Michiel Vermeulen participated in the selection committee for the Oncode 2023 junior PI call

2.4.3. Key collaborations within Oncode in 2023

a) Jurgen Marteijn, Titia Sixma interaction proteomics of the transcription coupled DNA damage response (PMID PMID: 37886519, in revision at Nature Communications) and manuscript in preparation

b) Monika Wolkers, proteomics of tumor infiltrating lymphocytes and mRNA modification m6A interaction proteomics in T cells, work in progress (collaboration started within Oncode)

c) Boudewijn Burgering, single cell proteomics in gastruloids (in revision at Cell Stem Cell)

d) Wilbert Zwart, interaction proteomics of nuclear hormone receptor elements (PMID 37961147 and Nature Communications (in revision)) (collaboration started within Oncode)

e) Carl Figdor, functional characterization of myeloid derived suppressor cells (PMID 37739035) (collaboration started within Oncode)

f) Maria Rodriguez-Colman, role of lactate in CRC, manuscript submitted

g) Emile Voest, secretomics in CRC (PMID 36450103) and work in progress (collaboration started within Oncode)

h) Madelon Maurice, Thermal Proteome Profiling and PTK7 in CRC (PMID 37591722) and work in progress (collaboration started within Oncode)

i) Peter ten Dijke, secretomics in melanoma and thermal proteome profiling (PMID 37240029) and work in progress (collaboration started within Oncode)

j) Hans Clevers, role of Menin in human small intestinal cancer, work in progress

k) Lodewyk Wessels, Thermal Proteome Profiling for multiple low dosage treatments, work in progress (collaboration started within Oncode)

l) Thijn Brummelkamp, interaction proteomics for an 'orphan' DNA motif and its interactor, work in progress (collaboration started within Oncode)

m) Jan Paul Medema, Thermal proteome profiling for Mandipine in CRC, manuscript submitted (collaboration started within Oncode)

n) Bas van Steensel, Jeroen de Ridder, Pericode Oncode Grand Synergy challenge project, work in progress (collaboration started within Oncode)

o) Susanne Lens, role of Flywch1 in heterochromatin, work in progress (collaboration started within Oncode)

p) Leila Akkari, proteomics of lipid-laden macrophages in glioblastoma, work in progress (collaboration started within Oncode)

q) Laura Heitman, interaction proteomics for GPCRs, work in progress (collaboration started within Oncode)

r) Jos Jonkers, interaction proteomics for BRCA1/2 in heterochromatin function, work in progress (collaboration started within Oncode)

2.4.4. Major valorization achievements in 2023

a) The ProteinA-Turbo enzyme that we licensed to Merck/Millipore is now commercially available

b) We filed an initial patent for the STPP-UP Thermal Proteome Profiling technology

c) Our lab became part of the Oncode Patient Engagement Program in 2023. We welcomed a first patient to our lab in December 2023, which was very inspiring.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
National Growth Fund	Oncode Accelerator	300 million euro	4.8 million euro	2023	96	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Jelmer J. Dijkstra	A multiomics perspective on the role of SMAD4 inactivation in colorectal cancer
Cheng Wang	Glucocorticoid regulation of gene expression in innate immune cells

Karin de Visser

Netherlands Cancer Institute

1. General information

Research Focus	Inflammation and cancer
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Through mechanistic understanding of the crosstalk between the immune system and cancer cells, we aim to contribute to the design of novel immunomodulatory strategies to fight metastatic breast cancer. Our main research questions:

1. Understanding the crosstalk between breast cancer and the immune system

We study how tumors shape the immune landscape and how this impacts disease progression. We focus on how the genetic makeup of breast cancer dictates the interaction with the immune system and immunotherapy response, with the aim to move towards personalized immune intervention strategies (Van Weverwijk & De Visser, Nature Reviews Cancer 2023). Moreover, we aim to dissect how tumors induce systemic immunosuppression and how this influences organ-specific metastatic spread.

2. How can we maximize the therapeutic benefit of immunomodulatory treatment strategies for breast cancer

Utilizing clinically relevant mouse tumor models and by collaborating with medical oncologist and researcher Marleen Kok (NKI/AVL), we focus on enhancing the success of immunotherapy for metastatic breast cancer by relieving tumor-induced immunosuppression and by engaging certain myeloid immune cell subsets. We combined our patient immunomonitoring program (flow- and omics- based) with fundamental research, and discovered that eosinophils enhance immunotherapy response of breast cancer (Blomberg et al. Cancer Cell 2023) while Tregs blunt therapeutic efficacy (Blomberg et al. Oncoimmunology 2023).

2.2. Major scientific achievements in 2023

a) With medical oncologist/scientist Marleen Kok we discovered that immunotherapy-induced eosinophils are critical for immunotherapy response of breast cancer. We identified how eosinophils are activated by ICB and we provided proof-of-principle for eosinophil engagement to enhance ICB efficacy of breast cancer (Blomberg et al. Cancer Cell 2023). This work was a team effort bridging the lab with the clinic, and was highlighted in a Preview in Cancer Cell, Nature Reviews Immunology, the Volkskrant, BNR radio. We obtained KWF funding to continue this work, and Olga Blomberg defended her thesis describing this research on January 11th 2024

b) In 2023, we published two invited reviews in prestigious journals (De Visser & Joyce, Cancer Cell 2023; Van Weverwijk & De Visser, Nature Reviews Cancer 2023). Cancer Cell had invited Prof. Johanna Joyce (Lausanne, Switzerland) and me to write a review on the evolving tumor microenvironment, as an introductory review to the 2023 Cancer Cell special issue on the tumor microenvironment. This review is highly cited and gained broad international attention. Nature Reviews Cancer invited us to write a review on mechanisms driving the immunoregulatory function of cancer cells. I wrote this with my postdoc Antoinette van Weverwijk. These invited reviews highlight our international visibility and expertise

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Identifying mechanisms underlying immunotherapy response of breast cancer patients

This project is performed in close collaboration with Marleen Kok, breast cancer oncologist and scientist. In 2023, we published that eosinophils are causally involved in immunotherapy response of breast cancer (Blomberg et al. Cancer Cell 2023). Part of this project was funded by the Oncode basefund (e.g the bioinformatics analysis, establishing the immunomonitoring of patients). The eosinophil-centered follow-up research is now funded by KWF (KWF13191; €585.482 and the Oncode accelerator/KWF project). We are now using our base-fund to explore other avenues within this topic. We are studying how breast cancer subtype and stage affect the composition and functionality of the systemic immune landscape (fully funded by the Oncode basefund). We have discovered intriguing short-term and long-term effects of chemotherapy treatment on the systemic immune landscape, and we have identified differences in neutrophil functionality between breast cancer patients and healthy individuals (Bakker et al. close to submission). PhD student Noor Bakker is funded by the base-fund. In addition, we have initiated a new explorative project to study the importance of lymph nodes in the efficacy of cancer immunotherapy. Marleen Kok is funding the PhD student on this project, and my lab funds the animal experiments and lab resources from the Oncode base-fund. We just started to profile tumor-draining lymph nodes from patients treated with immunotherapy by scRNAseq and spatial transcriptomics. We aim to identify differences in the tissue microenvironment between responders vs non-responders and pre- vs. post-treatment samples. In parallel, we will perform functional and mechanistic studies in our breast cancer mouse models.

Project B) Understanding and disrupting breast cancer-induced systemic inflammation to reduce metastatic spread

In this project, we aim to dissect the step-wise evolution of pro-metastatic neutrophils in breast cancer and to investigate whether their therapeutic reprogramming can reduce metastatic spread. Postdoc Hannah Garner collaborated with the team of ex-Oncode PI Elzo de Wit to perform RNA- and ATAC-sequencing analysis on haematopoietic stem and progenitor cells. These data revealed tumor-induced dysregulation along the neutrophil development trajectory, from the most primitive stem cells to mature bone marrow neutrophils and showed differential expression of key transcription factors critical for neutrophil development in tumour bearing mice compared to controls. Treatment with anti-IL-1b, a clinically relevant anti-inflammatory agent induced normalisation of dysregulated neutrophil development at both the molecular and cellular level and reduced metastatic spread (Garner et al. expected submission February 2024). With help of the Oncode TTO team, we obtained a collaborative agreement with MiNA therapeutics to test a new compound to re-program neutrophils. Thanks to the Oncode basefund we were able to initiate this project, and to lay a very solid foundation, which helped to obtain KWF funding in 2023 to continue this project outside the scope of the Oncode basefund.

Project C) Oncode Accelerator Project/KWF: Curing tumors difficult to treat with immunotherapy by mobilizing innate leukocytes

Part of my Oncode basefund is allocated to the 14339 KWF/Oncode consortium project. I use the basefund for the salary of 0.5 fte technician, Kim Vrijland MSc. In this multi-disciplinary project, Sjoerd van der Burg (Lead), Leila Akkari, Linde Meyaard and I (main applicants), in collaboration with several other Oncode PIs (e.g. Jacco van Rheenen, Jeroen de Ridder), aim to discover effective strategies to mobilize, engage or target myeloid immune cells to attack tumors, as a novel immunomodulatory strategies to fight cancer. This project was initially designed as an Oncode Accelerator project. We are very grateful to KWF that they selected this project for funding, provided that all involved PIs invest part of their Oncode basefund in this project. I recruited PhD student Daniil Anastasopoulos on this project (start date September 2022), and together with technician Kim Vrijland (on the basefund), he is involved in several research lines within this project. Together with the PhD student of Linde Meyaard, he is mining RNA sequencing data from myeloid cells (eosinophils and neutrophils) from our transgenic mouse models and breast cancer patients for the presence of inhibitory ITIM-containing receptors that could serve as inhibitory immune checkpoints on eosinophils, utilizing the antibody production platform. Moreover, we are collaborating with the other involved labs to analyze and compare the presence and phenotype of eosinophils, neutrophils and macrophages across the different tumor models during immunotherapy

Project D) Mechanistic understanding of acquired resistance of breast cancer to immune checkpoint blockade

The overall goal of this project is to study acquired resistance to immune checkpoint blockade in breast cancer. A proportion of breast cancer patients responds to ICB, however, these responses are often not durable. Knowledge about the mechanisms driving acquired resistance to ICB is lacking. Using our transgenic mouse models we aim to study how cancer cell intrinsic and extrinsic parameters change during the acquisition of immunotherapy resistance, and the causal relevance of these parameters will be assessed in mechanistic studies. Through our collaboration with Marleen Kok, we will have access to human datasets and samples to validate our preclinical findings. The ultimate goal is to improve ICB response by preventing or targeting mechanisms of acquired resistance. We have not progressed a lot on this project, as in 2023 we put our priorities more on projects A-C and the bioinformatician has left the lab. We are currently preparing an application for a NWO XS grant, in which we will propose scRNAseq analysis of ICB resistant versus response mammary tumors of our mouse models. If successful, that may boost further investment into this project.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Since the start of Oncode, I have invested a substantial part of the Oncode base fund in building a connection between my lab and the clinic (via a very fruitful collaboration with Marleen Kok), to facilitate the validation of our research findings from the lab to patients, and to initiate novel research lines based on observations in patients. In 2023 we harvested the fruit of this investment, as we published our findings on the essential role of eosinophils in ICB response of breast cancer (Blomberg et al. Cancer Cell 2023). This work has received broad international attention, and has opened several new research lines. We obtained funding from KWF to expand our work on eosinophils, and we use the Oncode basefund to explore novel directions, including the role of lymph nodes in immunotherapy response of breast cancer.

b) Thanks to the Oncode Accelerator Project initiative of a couple of years ago, and thanks to KWF for funding this project, I now actively collaborate with Sjoerd van der Burg, Linde Meyaard, Leila Akkari and Jacco van Rheenen on the KWF/Oncode consortium grant (See project C). The PhD students that are recruited on this project form a great team, and exciting new collaborative experiments are on the way. This projects opens opportunities to 1) understand and compare myeloid cell complexity and functionality across cancer types; 2) work towards clinically relevant strategies to engage or target myeloid cell (sub)types to improve immunotherapy response

c) Since the start of the Oncode patient engagement program, my lab has been actively involved. One cancer patient is directly linked to my lab. We organize meetings in which lab-members share their research plans/findings with 'our' patient, and the patient provides her perspective on having cancer. My postdoc, Antoinette van Weverwijk, coordinates the connection of our patient with the lab. Antoinette gave a guest lecture together with our patient to Biomedical Sciences students in Utrecht, and she presented at the BOOG/BVN/Oncode meeting. Moreover, as part of a Cancer Grand Challenges project application, Leila Akkari and I connected with three additional patients of the Oncode patient engagement program. These patients were very closely involved in the process of writing and preparation of the interview. It is very inspiring to interact with these highly motivated patients. I am happy to see how the Oncode patient engagement program has developed over the last years into a professional program.

2.4.2. Contribution to the Oncode community in 2023

a) Since 2018, I am a member of the Oncode Research Management Committee (RMC). It is a privilege to be an RMC member, as it provides many opportunities to interact with other Oncode team members, and to contribute –together with the other dedicated committee members- to the success and future direction of Oncode beyond performing excellent cancer research. In 2023, we had the opportunity to recruit ten talented novel junior group leaders to Oncode after an intense recruitment procedure. In 2024, my term in the RMC will end. For sure, I will miss being part of the RMC!

b) My lab actively collaborates with various Oncode teams, sharing our expertise and tools/models within the Oncode community. Simultaneously, we benefit from these collaborations, as reflected in Section 2.4.3. As a concrete example, we are sharing our expertise and model systems to study eosinophils in ICB response with Linde Meyaard, Leila Akkari, Sjoerd van der Burg and Jacco van Rheenen (via the KWF/Oncode accelerator consortium grant), which has initiated novel collaborative research lines in their labs.

c) We not only benefit from the Patient engagement program, but we also actively contribute to the program. As mentioned above, my postdoc has lectured together with 'our' patient in the biomedical sciences bachelor program and she gave a talk at the BOOG/BVN/Oncode annual meeting. Team members have participated in the Annual meeting of rhe Patient Engagement program (https://oncodeinstitute.nl/news/a-common-goal-bringing-patients-and-researchers-together).

2.4.3. Key collaborations within Oncode in 2023

a) Sjoerd van der Burg, Linde Meyaard, Leila Akkari, Jacco van Rheenen. Joint project: Oncode Accelerator Project/KWF: Curing tumors difficult to treat with immunotherapy by mobilizing innate leukocytes. (https://www.oncode.nl/news/oncode-collaboration-mobilizing-innate-leukocytes-to-attack-tumors). Goal is to gain mechanistic insights into the role of myeloid immune cells subsets in ICB response across cancer types, and to use this knowledge to target or engage myeloid cells to improve ICB efficacy. This collaboration was started thanks to Oncode.

b) Daniel Peeper. Joint project: NWO-XL consortium - Assessing the T cell- cancer cell interactome (PIs: Daniel Peeper, Maarten Altelaar, me). Goal is to design and utilize in vivo proximity labeling technology (TURBO-ID) to identify novel proteins functionally involved in cancer cell- T cell interactions, and how these interactions are impacted by tumor-associated Tregs and neutrophils. I have not collaborated with Daniel Peeper prior to Oncode. The Oncode basefund helped to generate some preliminary data for the grant application

c) Laura Heitman. We collaborate with Laura Heitman to assess the potential of CCR2 targeting drugs in our mouse breast tumor models and to search for novel GPCR targets in breast cancer (Ortiz Zacarías et al. Trends Pharmacol. Sci. 2021; Den Hollander et al. Biochem. Pharmacol. 2023). This collaboration started thanks to Oncode

d) Emile Voest. My team has analyzed data from blood samples of patients enrolled in the DRUP trial, which we published in Blomberg et al. Cancer Cell 2023. I have not collaborated with Emile Voest prior to Oncode.

e) Wilbert Zwart. With Wilbert, we have performed ChIP seq for p53 binding sites, and RIME analysis to unravel how p53 hotspot mutations alter the immunoregulatory properties of cancer cells (manuscript in preparation, and Wellenstein et al. Nature 2019, Prekovic et al. Nature Communications 2021). I have not collaborated with Wilbert Zwart prior to Oncode

2.4.4. Major valorization achievements in 2023

a) My team is member of the Roche/Genentech imCORE global network – testing of unique next generation immune-modulatory drugs. We are currently preparing a manuscript for publication describing our preclinical studies with the muPD1-IL2v compound that we have performed as part of this network (Blomberg et al. in preparation).

b) My team is actively involved as co-investigator in various clinical trials. In collaboration with Marleen Kok, we perform translational research on breast cancer patients enrolled in immunotherapy clinical trials (e.g. see Blomberg et al. Cancer Cell 2023; Voorwerk et al. Nature Cancer 2023).

c) After extensive negotiations, with help of the Oncode valorization team, we obtained a collaborative agreement with MiNA therapeutics in 2023. Through this agreement, we have access to a novel compound which we will test in our preclinical breast cancer models to modify tumor-induced education of neutrophils (see project B above).

d) My lab is actively involved in the Oncode Patient Engagement program

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF	Project grant	€664.943	€664.943	07/2023	48	Main applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT04159818	Immune induction strategies to improve response to immune checkpoint blockade in triple negative breast cancer (TNBC) patients: the TONIC-2 trial *My lab is involved in the immune profiling of these patients (e.g. see Blomberg et al. Cancer Cell 2023)*	February 2020	ongoing	Yes	Co-PI
NCT03147040	AssessinG Efficacy of Carboplatin and ATezOlizumab in Metastatic Lobular Breast Cancer (GELATO) *My lab was involved in design clinical trial and co-supervision of translational research (Voorwerk et al. Nature Cancer 2023)*	November 2017	Ended July 2022	Yes	Co-PI
NCT03815890	Pre-operative phase II trial for breast cancer with nivolumab in combination with novel IO (BELLINI trial) *Co-investigator involved in design clinical trial and co-supervision of translational research (manuscript in preparation*	October 2019	ongoing	yes	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Kevin Kos (promotor)	Immunosuppresion in breast cancer; a closer look at regulatory T cells
Metamia Ciampricotti (promotor)	Dissecting the immune microenvironment of breast cancer
Olga Blomberg (promotor – thesis defence in January 2024)	Breaking barriers; Unraveling response mechanisms to immunotherapy in breast cancer
Wietske Pieters (co-promotor)	Modifiers of tumor development in Lynch syndrome

Emile Voest

Netherlands Cancer Institute

1. General information

Research Focus	Genomics, precision medicine, organoids, immunotherapy
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

New models to improve immunotherapy: We developed co-culture models to study immune-tumor interactions in individual patients and to generate a better understanding of which tumors are recognized by immune cells. These models are now used to determine the role of gammadelta T cells in recognizing B2M deficient tumor cells, how NK cells recognize tumor cells and how monocytes and NK cells interact with T cells.

Genomic-guided personalized medicine: We created a platform, coined the Drug Rediscovery Protocol (DRUP), to identify early signals of activity in well-defined cohorts of cancer patients. In this multi-pharma, multi-drug, national multi-center study we facilitate access for patients to approved medication based on a genomic profile coupled to a tumor type. We also started the DRUG Access Protocol (DAP) to facilitate systematic data collections of patients treated with the newest drugs.

Understanding the tumor microbiome: The role of the microbiome is well established but it is unclear how the microbiome affects treatment outcome. By employing unique data sets we are now dissecting the dynamics of the microbiome in metastases and the relative contributions of bacteria and their metabolites to tumor progression.

2.2. Major scientific achievements in 2023

a) KRAS G12 mutations were identified as a predictor of resistance to trifluridine/tipiracil treatment in patients with metastatic colorectal cancer. Our study used an exploratory data set, an independent clinical studie and real life data from two countries and in vitro testing in organoids. These findings changed daily clinical practice (Nat Medicine, 2023).

b) We identified gamma delta T cells as important effector cells in tumors that lose their ability to present neoantigens. These findings and subsequent follow up studies highlight the importance of the innate immune system as a potential target for treatment. (Nature, 2023).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Utilizing the microbiome to improve immunotherapy

We feel that we are uniquely positioned to study the impact of the microbiome on cancer treatment because we have generated several special data sets: i) whole genome sequencing (WGS) of >7000 metastases of cancer patients; ii) RNA seq data of >2000 patients included in the WGS cohort; iii) WGS of a cohort of 250 patients of whom we have at least two biopsies and several other cohorts that will help us to better dissect which bacteria in which tumor type may be influencing treatment outcome and how.

We now completed the analysis of microbial presence in metastases which will be a great resource for the scientific community. This resource includes i) whole genome sequencing (WGS) of >7000 metastases of cancer patients; ii) RNA seq data of >2000 patients included in the WGS cohort; iii) WGS of a cohort of 250 patients of whom we have at least two biopsies and several other cohorts. Our computational analyses are supported by IHC. For example, we showed a correlation between hypoxia (HIF1alpha) and anaerobic bacteria. Also, against the background of discussions on computational methods, we developed a state of the art approach for identifying microbial species that can help to advance the field. Our next step is to focus on finding overrepresentation of pathways and their microbial metabolites as key regulators of immune responses.

Project B) Dissecting tumor-immune cell interactions

Our autologous immune-tumor organoids co-culture platform provides unique opportunities to understand how tumors interact with the immune system: we will broaden our platform to include other immune cells such as gamma delta T cells, monocytes, NK cells and fibroblasts. Importantly, we will use pairs of tumor organoids and respective immune cells to perform genome wide CRISPR-Cas and drug screens. The main goal is to see if and how we can enhance the immune response.

This is a more technology driven project. We have broadened our autologous immune-tumor organoids co-culture platform and incorporated several different immune cells to determine immune activation, tumor cell killing and/or communication between immune cells. Immune cells such as alpha beta and gamma delta T cells, monocytes, and NK cells are now part of our model platform. These models are essential to address multiple questions including the modulatory effects of microbial metabolites and the secretome.

Project C) Improving precision medicine

The Drug Rediscovery Protocol (DRUP) will be used to address:

1. Combinatorial approaches with approved drugs based on individual DNA profiles
2. To use RNA sequencing to identify profiles that may benefit from treatment
3. Integrated diagnostics (i.c., machine learning based on DNA, digital pathology, imaging and outcome) may identify features to refine an “actionable” profile

To improve precision medicine we continue with the trials: the DRUG Rediscovery Protocol (DRUP) and the DRUG Access Protocol (DAP). Almost 2000 patients have benefitted from these studies and the have been instrumental in including nivolumab, cemiplimab, larotrectinib and entrectinib in the Dutch health care reimbursement system. We are continuously mining this data for signals of activity, biomarkers and new treatment opportunities. In the next year we will launch DRUP ATTAC which aims to facilitate combinatorial targeted treatment approaches in first and last line of treatment.

Project D) PERICODE: Finding regulatory mutations in the non-coding cancer genome
This project has the ambition to uncover the impact of non-coding DNA regions on coding regions. Ultimately this will uncover a wealth of new potential treatment strategies.
The Pericode project is funded through philanthropy and aims to uncover the regulatory function of the non coding DNA. Our first step is to generate predictive algorithms based on machine learning technologies to better understand the impact of changes in promotor sequences. Next this algorithm will be used to identify promotors that once mutated modulate progression of cancer or make them more vulnerable to the immune system.

Project E) The tumor's secretome as a regulator of immune activation.
This project focuses on the recognition of secreted proteins that may repel the immune system and offer a potential strategy to convert cold tumors to immune responsive tumorsX.
The tumor's secretome is a newly started project with a focus on secreted proteins which may explain different sensitivities to immune cells or regulate the influx of immune cells in so called cold tumors.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) The Oncode Community has greatly facilitated scientific interactions. The list of collaborators who I have interacted with, is long and of mutual benefit. Also important, before embarking on a new high-risk project it is extremely valuable to get input to improve the project. This culture was already present at the NKI but is now actually being broadened to all excellent scientists in the Netherlands.

b) Through its meetings Oncode has created a platform where young scientists can meet each other, exchange ideas and shape networks for the future. Participation of my group's team members in the various meetings is of great value.

c) The Pericode project is a great example of how the Oncode network has helped to establish a consortium with a clear focus on non coding DNA but with very complementary expertises. Pericode is funded through the NKI philatropy network (AvL Foundation) but we included Oncode PI's from several other institutions in the Netherlands because of their excellent contributions and the fact that Oncode was able to bring everyone together. Oncode also facilitates a potential route to a spin out once the data are mature enough.

2.4.2. Contribution to the Oncode community in 2023

a) I initiated a visit of a renowned venture capitalist (Syncona) to discuss opportunities to work together on a number of projects. They came from London with a team of experts and several Oncode PI's had an opportunity to pitch there plans.

b) I serve as the liaison with the funder of the Pericode project (4.5M for 4 years) that allows the Pericode consortium of 7 Oncode PIs to move fast forward.

2.4.3. Key collaborations within Oncode in 2023

a) The key preclinical collaboration is represented by Pericode (D). Started in 2022 this consortium of Oncode PIs (van Steensel, de Ridder, Franke, Vermeulen, Zwart, Derks and Voest) is for me an example of how Oncode brings together top scientists without institutiobal bounderies. It is expected that the consortium will submit its first big manuscript on non coding DNA early 2024.

b) Our co-culture model systems have helped a project to identify a new, chemotherapy-induced activation of T cells. This collaborative effort with Jacco van Rheenen has resulted in a publication in Cancer Cell 2023.

c) Similarly, together with Ton Schumacher, our models have been instrumental in the development of a platform to identify patient-specific T cell neoantigens through HLA-unbiased genetic screens (Nature Biotechnology 2023).

2.4.4. Major valorization achievements in 2023

a) I am co-founder of Mosaic Therapeutics, Cambridge UK, which has secured a series A funding to develop combinatorial treatment approaches with de-prioritized or abandoned drugs.

b) Based on the DAP study cemiplimab, a check point inhibitor, is now reimbursed in the Dutch Health Care system for patients with advanced cutaneous squamous cell carcinoma. After nivolumab for MSI regardless of tumor type, this is the second drug that is now made available to patients in the Netherlands. This is an unprecedented succes.

c) Larotrectinib and entrectinib, two NTRK fusion protein inhibitors, are now available for patients in the Netherlands. These drugs entered a conditional approval program supported by the Dutch Ministery of Health which mandated participation in the Drug Access Protocol (DAP).

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Dutch Cancer Society	C	1.5M	1.5M	ongoing	48 months	Main applicant
AvL Foundation	A	100k	100k	01-2023	24 months	Main applicant
NWO (VENI Dijkstra)	E	500k	500k	11-203	48 months	Co-applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
DRUP	Drug Rediscovery Protocol	09-2016		Yes	Lead
DAP	Drug Acces Protocol	01-2021		Yes	Lead
SHERPA	Combination of a SHP2 inibitor with an ERK inhibitor in KRAS mutated pancreatic cancer	01-2022		Yes	Co-PI
NICHE	Neoadjuvant immune checkpoint inhibition in locally advanced dMMR early-stage colon cancer: the NICHE study			Yes	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Kris Samsom	Bridging the gap: Implementation of whole genome sequencing in routine clinical care 03-05-2023 G.A. Meijer and E.E. Voest; K. Monkhorst and LJW Bosch, co-promotores
Jorn Mulder	The authorisation of anti-cancer medicinal products:Clinical benefit, precision medicine and regulatory flexibility 25-01-2023 Voest EE, de Boer T; Stoyanova V and Pasmooij AMG co-promotores
Hanneke van der Wijngaart	Clinical application of genomics- and phosphoproteomics-based selection of targeted therapy in patients with advanced solid tumors 15-12-2023 Verheul H, Voest EE; Labots M, co-promotor
Maxime van Bergen-Henegouwen	Genomic driven treatment and drug rediscovery in patients with advanced cancer. 05-12-2023. Gelderblom H and Voest EE

Lodewyk Wessels

Netherlands Cancer Institute

1. General information

Research Focus	Computational Biology
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

We aim to quantify and understand treatment response. To this end we pursue three topics. In Topic 1 we develop data-driven, statistical approaches to model molecular interactions and drug response. In Topic 2 we construct semi-mechanistic models to understand cellular decision making and how this affects drug response. In Topic 3 we develop and apply computational approaches for understanding checkpoint blockade response (CPB)

Topic 1: Statistical models for drug response and interaction. 1) We have resubmitted our manuscript on a pan-cancer drug combination screen and proposed a new aggregated measure of combinatorial drug response. 2) We are finalizing DISCOVER-LVM to correct for confounding in interaction analyses.

Topic 2: Semi-mechanistic models of cellular decision making. 1) We have developed scMeMo (single cell Mechanistic Modeler): a mechanistic modeling framework that can leverage diverse sets of measurements in order to infer unobserved variables in heterogeneous single cells. 2) We have performed single cell CyTOF measurements to unravel multiple low dose (MLD) response.

Topic 3: Computational immunology. We have submitted our manuscript describing our approach for analyzing spatial relationships in the tumor micro-environment. This manuscript describes the application to bladder cancer and the validation in head-and-neck cancer.

2.2. Major scientific achievements in 2023

a) We have shown that MYC activation drives resistance to mTOR inhibitors and is significantly associated with poor response to mTOR inhibition in breast cancer patients.

b) We developed scMeMo (single cell Mechanistic Modeler), a modeling framework that uses diverse measurements to infer unobserved variables in heterogeneous single cells.

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Statistical models of genetic interaction

Previously, we devised a new probabilistic model for detecting mutual exclusivity in cancer mutations. This new model detected far fewer mutually exclusive genes than claimed by existing tests. This suggested true genetic interaction is rarer than previously thought. To support this conclusion, we had to ensure that the absence of observed mutual exclusivity is not due to a reduced sensitivity of our new model. Analyses of simulated data have shown that our model overall has overall good sensitivity. In cases where molecular subtypes of the cancer studied correlate strongly with the pattern of mutual exclusivity, sensitivity is reduced. This was expected given the specific correction for subtype in our model. Overall, these analyses confirmed the validity of our model. We plan to publish the results in 2024.

Funding: This research is fundamental in nature, reducing fundability through regular channels. Nevertheless, it has great impact on our understanding of cancer development and progression.

Project B) Single cell perturbation and profiling

In 2023, we tested the P2seq method, integrating site-specific antibody tagging and improved cell blocking to eliminate background, using a 15 antibody panel on BT474 cells treated ± BEZ235. While the total signal per antibody showed expected and robust changes following treatment, the dynamic range for the weaker-staining antibodies was limited. We undertook the following steps to improve the protocol: re-fixation of cells following antibody staining, optimization of reaction temperatures during reverse transcription and ligation steps, and more efficient recovery of the indexed fragments before NGS library generation. We are currently quantifying the resulting fold increase in dynamic range of signal detection in BT474 cells treated with a dose range of BEZ235. We expect this will confirm the usefulness of the P2seq method, justifying application to other projects in our laboratory.

Funding: This project would have been virtually impossible to fund via regular channels as my group had no track record in experimental approaches. Experimental protocols, closely integrated with computational modelling would greatly enhance the impact of our work.

Project C) Modeling spatial relationships

Immune checkpoint blockade (CPB) achieved remarkable clinical results, but how it remodels the tumor microenvironment (TME) remains unclear. We developed a novel approach to describe spatial relationships (SRs) between cells in the TME based on multiplex immunofluorescence (mIF) data by approximating the 1-NN distance distribution with a Weibull distribution. We evaluated the association between the Weibull parameters, densities, and CPB response. The Weibull SR metrics showed a significant association with response, but immune cell density did not. We applied this to 749 breast samples quantified by CyToF with 324 cell type pairs. This revealed subtype-specific SRs for basal, HER2-enriched and luminal A tumors. CyTOF identified many cell types compared to mIF, but some specific to few samples, strongly reducing power. Our data confirm that SRs, compared to density metrics, are robust biomarkers with significant translational relevance.

Funding: This is a novel area of research for us and we are generating preliminary data in this project to position ourselves for the submission of project proposals with other funding agencies.

Project D) Pan-cancer combinatorial drug screens

In collaboration with the Garnett group at the Wellcome Trust Sanger Institute, we screened the largest number of cell lines to date (n = 757) with 51 clinically-relevant combinations and identified responses at the level of individual cell lines and tissue populations. We established three response classes to model effects beyond monotherapy: synergy, Bliss, and independent drug action (IDA). We confirm synergy is rare (11% of responses) and efficacious, whereas Bliss and IDA are more frequent but less efficacious. We introduce Efficacious Combination Benefit (ECB) to describe high efficacy synergy, Bliss, and IDA responses, demonstrating the potential of different response classes. Furthermore, we

identify biomarkers of ECB, overlay clinical trials, and by mining PDX studies, support ECB's predictive power of in-vivo effectiveness. The data resource and framework established here advance our understanding of combination treatments and facilitate their preclinical evaluation and development.
Funding: The experimental work was funded by an ERC grant but the computational analyses had not been completed. Due to its perfect alignment with the Oncode goals, I decided to fund the analyses on Oncode and will submit follow-up grants elsewhere.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) New experimental technology development (Single cell perturbation and profiling). Oncode has enabled my group to implement these technologies (described under Project 2 in 2.3.1) enabling us to establish the capacity to generate rich, original datasets of our own. These data sets, in combination with our computational expertise will allow us to probe cellular decision making, and its effect on (combinatorial) drug response in great detail.

b) New collaborations through experimental capability. We have started a collaboration with Michiel Vermeulen on using thermal protein profiling to understand cellular decision making processes and especially the mechanisms of action of multiple low dose therapy. This collaboration is in the context of the Oncode Accelerator project where we closely collaborate with Lude Franke (Oncode PI), Jeroen de Ridder (Oncode PI) and Skyline diagnostics. These collaborations have been made possible through the Oncode network.

c) New collaborations through single cell technology development. Due to our activities as described in (a) above, we have started a collaboration with Klaas Mulder on the analyses of single cell ID-seq data sets. This has led to the creation of a joint PhD position with Evert Bosdriesz (former PDF in my lab, now Assistant Professor at the VU) on the development of novel computational approaches for single cell data analysis and to a joint KWF project on identifying cell states and using these to understand drug response.

2.4.2. Contribution to the Oncode community in 2023

a) ONCODE-Accelerator. I have provided a significant contribution to the ONCODE-Accelerator Growth fund application by acting as co-lead for the AI for Drug Development and Vaccine tracks and we are now actively participating in the project (We participated in a joint worship with the Jeroen de Ridder group, the Lude Franke group and Skyline Diagnostics).

b) GPU Infrastructure. With Tassos Perrakis, Jeroen de Ridder and Lude Franke, we are actively maintaining the distributed GPU infrastructure to enable Artificial Intelligence and Deep Learning applications in Cancer Genomics, Proteomics, and Structural Cell Biology.

2.4.3. Key collaborations within Oncode in 2023

Name Collaborator	Were you already collaborating prior to Oncode?	Subject Collaboration	Reference
Jos Jonkers	Yes	Predicting response to mTOR inhibitors in breast cancer; In vivo screening for genetic determinants of breast cancer	Publications: Bhin et al, Cell Reports, 2023 Hutten et al, Cancer Cell, 2023 Bhin et al, JEM, 2023 Shared personnel: Roebi de Bruin
Rene Bernards	Yes	Drug response modelling and dissecting Multiple Low Dose treatment.	Shared KWF project on understanding MLD treatment
Emile Voest	Yes	Computational approaches to detect molecular interactions; Integrative approaches for prediction of immune therapy response. Analyses of microbiome data to understand therapy response	Publications: vd Haar, Nat. Medicine , 2023 de Vries et al, Nature 2023 Shared personnel: Tom Battaglia Lindsay Leek Vanessa Botha
Ton Schumacher	Yes	Computational analysis of molecular data to dissect immune therapy response; Neo-antigen prediction pipelines.	Publications: Hoekstra et al, Cancer Cell, 2023
Daniel Peeper	Yes	Integrative predictors for immune response in lung cancer and melanoma	Shared personnel: Alex van Vliet
Hugo Snippert	No	Dissecting Multiple Low Dose response	Shared KWF project on understanding MLD treatment
Anastassis Perrakis	No	GPU infrastructure project	Joint Oncode infrastructure project: A distributed GPU infrastructure to enable Artificial Intelligence and Deep Learning applications in Cancer Genomics, Proteomics, and Structural Cell Biology
Mario van der Stelt, Lude Franke, Jeroen de Ridder, Sjoerd van der Burg	No	Employing AI for Drug and Vaccine Development.	Growth Fund application: ONCODE Accelerator: Preclinical accelerator for cancer treatments.
Michiel Vermeulen	No	Using thermal protein profiling to understand MLD treatment	Collaboration within ONCODE Accelerator.

2.4.4. Major valorization achievements in 2023

a) Patent application filed with the Wellcome Trust Sanger Institute and Mosaic Therapeutics on Combination Therapies.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF	Exploration	680K	250K	1-7-2023	48	Lodewyk Wessels
KWF	Exploration	543K	220K	11-7-2023	48	Klaas Mulder

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT04949113	Neoadjuvant Ipilimumab Plus Nivolumab Versus Standard Adjuvant Nivolumab in Macroscopic Stage III Melanoma (NADINA)	07/2021	12/2024		Computational analyses for translational research
NCT03026140	Neoadjuvant Immune Checkpoint Inhibition and Novel IO Combinations in Early-stage Colon Cancer (NICHE)	03/2017	till 12/2024		Computational analyses for translational research
NCT03387761	Neo-Adjuvant Bladder Urothelial Carcinoma COmbination immunotherapy (NABUCCO)	01/2018	09/2021		Computational analyses for translational research
NCT04159818	Immune Induction Strategies to Improve Response to Immune Checkpoint Blockade in Triple Negative Breast Cancer (TNBC) Patients (TONIC-2)	11/2019			Computational analyses for translational research
NCT03815890	Pre-operative Trial for Breast Cancer With Nivolumab in Combination With Novel IO (BELLINI)	10/2019			Computational analyses for translational research

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Soufiane Mourragui	Computational Models For Clinical Drug Response Prediction

Monika Wolkers

Sanquin

1. General information

Research Focus	T cell effector function, post-transcriptional gene regulation, RNA binding proteins
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

My lab studies how T cell responses against tumors and infections are generated and maintained. We 1) study T cell responses against Non-Small Cell Lung Cancer (NSCLC) and pediatric neuroblastoma, with the aim to bring TIL therapy to the clinic. T cell products are also generated for cancer patients from peripheral-blood T cells for the production of e.g. CAR or TCR-transgenic T cells. Therefore, we 2) investigate the heterogeneity and functionality of blood-derived human T cells, in particular how this heterogeneity translates into their capacity to kill target cells, and how most potent T cell products can be generated and maintained. Furthermore, it is critical to decipher the molecular mechanisms that drive T cell effector function. We therefore 3) investigate how post-transcriptional events define T cell function and how it can be modified, with a specific focus on the role of RNA-binding proteins herein. We recently also set up analysis pipelines to define the role of sequence determinants in transcripts in the protein output from transcripts. Unraveling these regulatory mechanisms in protein expression are thus key to our understanding of T cell functionality

2.2. Major scientific achievements in 2023

a) Popović B, Nicolet BP, Guislain A, Engels S, Jurgens AP, Paravinja N, Freen-van Heeren JJ, van Alphen FPJ, van den Biggelaar M, Salerno F, Wolkers MC. Time-dependent regulation of cytokine production by RNA binding proteins defines T cell effector function. Cell Reports. 2023 42(5):112419.

This study show cases the intricate regulation of cytokine mRNA in T cells by RNA binding proteins (RBPs). In particular it defines how RBPs act in a time and context-dependent manner.

b) Nicolet BP, Jurgens AP, Bresser K, Guislain A, Wolkers MC. Learning the sequence code for mRNA and protein abundance in human immune cells. bioRxiv 2023.09.01.555843. Submitted.

This study uses machine learning to learn the sequence code that predicts mRNA and protein abundance in human immune cells. In particular, it allowed us to use the prediction pipelines to construct synthetic 3'UTRs which, when fused to different constructs boost the protein expression in eukaryotic cells. this study has let to a patent application through Oncode (pending).

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Achieving proof-of-concept for modulating therapeutic protein expression in eukaryotic cells with artificial 3'UTRs.

This proof-of concept study is based on the study that uses machine learning to decipher the sequence features that predicts mRNA and protein abundance in human immune cells (Nicolet et al., bioRxiv 2023.09.01.555843. In particular, this analysis pipeline allowed us to construct synthetic 3'UTRs which, when fused to different constructs, boost the protein expression in eukaryotic cells. Within this proof-of-concept study we have further consolidated our findings from the initial patent filing, and we have updated the patent filing with additional data in December 2023. We are currently extending our findings and are further fine-tuning our observations (Jurgens et al., manuscript in preparation).

Project B) TLS-related immune infiltrates in NSCLC tumor lesions correlate with low tumor-reactivity of TIL products.

Adoptive transfer of tumor infiltrating lymphocytes (TIL) has shown great potential for the treatment of solid cancers. However, the parameters that define if TIL products are tumor-reactive are unknown. We questioned whether the composition of immune cell infiltrates correlated with tumor-reactivity of expanded TIL products. Unbiased flow cytometry data analysis was used to correlate immune infiltrates with the expansion rate, immune cell activation and T cell differentiation stage, and the anti-tumor response of TIL products. The tumor immune infiltrate composition was highly variable between patients, irrespective of the disease stage. Tumors with high B cell infiltrates contained BCL6+ B cells and CXCR5+BLC6+ CD4 T cell infiltrates and an increased percentage of naïve CD8 T cells, indicative of the presence of tertiary lymphoid structures (TLS). Importantly, the tumor-responsiveness of TIL products negatively correlated with the presence of TLS-associated immune infiltrates in tumors. Our finding may thus help improve patient selection for TIL therapy. (Castenmiller, Kanagasabesan et al., to be submitted in January '24).

Project C) Defining the role of RBPs in T cell effector function.

Potent T cell responses against infections and malignancies depend on the release of effector molecules. Because effector molecules can be toxic, their production is tightly regulated through post-transcriptional events at 3' Untranslated Regions (3'UTRs). RNA binding proteins (RBPs) are key regulators herein. With an RNA aptamer-based capture assay from human T cells, we identified >130 RBPs interacting with IFNG, TNF and IL2 3'UTRs in human T cells. T cell activation altered RBP-RNA interactions, revealing its plasticity. Furthermore, we uncovered the intricate and time-dependent regulation of cytokine production by RBPs: whereas HuR supports early cytokine production, ZFP36L1, ATXN2L and ZC3HAV1 dampen and shorten the production duration, each at different time points. Strikingly, even though ZFP36L1 deletion did not phenotypically rescue T cell dysfunction in tumors, the increased production of cytokines and cytotoxic molecules resulted in superior anti-tumoral T cell responses in vivo. Our findings thus show that identifying RBP-RNA interactions reveals key modulators of T cell responses in health and disease (Popovic, Cell reports 2023).

Project D) Identifying interaction partners of the RBP ZFP36L1 in T cells with proximity labeling.

We previously found that the activity of RNA binding proteins (RBPs) is time- and context- dependent, revealing an intricate regulation of T cell effector function by RBPs. Using proximity labelling of one RBP, ZFP36L1, we have now uncovered interaction partners in T cells upon activation. The proteomics and isolation approach is now fully operational, and we will now further fine tune this approach to achieve the time resolution of early and late interactors during T cell activation.

Project E) Define how sequence motifs define protein expression in T cells.
Protein expression in primary human T cells poorly correlates with mRNA expression, irrespective of the T cell differentiation status. We recently identified several features that correlate with the relative RNA expression (Nicolet, PlosOne 2022). We have now developed and optimized a Machine learning analysis pipeline that identifies sequence determinants that accurately predict up to 67% of the protein expression in immune cells and in cell lines interesting for industrial protein expression. We now validated several identified sequences with reporter constructs in primary T cells (Nicolet, submitted. bioRxiv 2023.09.01.555843). We have filed a patent for our findings (project A), and we are currently validating the claims with follow up experiments. This study has also led to a collaboration with the Schumacher lab (Bresser et al, in revision, bioRxiv 2023.09.03.556079).

Project F) Proteomics of tumor-infiltrating T cells.
Current predictions on gene expression in tumor-infiltrating T cells are based on RNA-seq data, sometimes with some proteomics information with CITE-seq or variants thereof. Based on our findings in project E, we hypothesized that the actual protein make up of TILs may be misinterpreted from (sc)RNAseq data. Therefore, generating proteomics data offer the opportunity to 1) define the rules of gene regulation when cells are under stress conditions, and 2) allow to potentially identify putative target proteins for immunotherapy, that thus far may have been overlooked. In collaboration with Michiel Vermeulen (Radboud University), we have mapped the proteome landscape of TILs, and we have achieved a depth of 1900 proteins. Intriguingly, specific metabolic pathways that are non-differentially expressed in (sc)RNAseq data were found to be differentially expressed on protein levels. These exciting results were the basis of a CCA project grant (AmsterdamUMC) granted to Dr. Kaspar Bresser to perform follow up studies. The data will also be used to mine for RBPs that may potentially dictate the altered protein landscape in TILs.

Project G) Define the role of RBPs in translation regulation in human T cells by a Crispr-screen.
Our recent efforts in defining the role of RBPs in human T cells have revealed their critical role in fine-tuning the production of cytokines. Here we will investigate which RBPs drive the translation regulation during T cell activation, and how conditions such as hypoxia influence this regulation. We have now optimized the experimental setups for a CRISPR CAS9 mediated RBP screen in human T cells. Due to personal changes in the group (4 people have been replaced in 2023) the project has a delay. Results from the screen are expected Q1, 2024

Project H) Analysis of m6A RNA methylation on T cell effector function.
M6A methylation is a well described mechanism to regulate gene expression in eukaryotic cells. Only recently, Dr. Foskolou has identified m6A RNA methylation profiles in human T cells. She found that in addition to the well described DRACH motifs, also AU-rich elements are subject to M6A methylation, which modulated RNA and protein expression of target mRNAs. In collaboration with Michiel Vermeulen, we have identified the RBPs that interact with m6A methylated and unmethylated mRNAs and thus regulate the fate of mRNA in T cells. This part of the project has now been finalized and awaits further data from Dr. Foskolou's collaborators (Jerne lab, Crick Institute).

Project I) Defining the RBP interactome in T cells. several studies have highlighted the RBP interactome in eukaryotic cells.
These studies have however primarily been performed under static conditions, in cell lines. We here are defining the RBP interactome in T cells by the OOPS technology. After optimizing and translating the methodology to primary human T cells, we have now mapped the RBPome in human CD8 T cells, and have been able to define the alterations that occur upon T cell activation. We are currently further optimizing this methodology towards subcellular OOPS, i.e. defining whether the RBP interaction occurs in the nucleus or in the cytoplasm. This information is key to identify the mode of action an RBP is exerting, provided that its activity within the nucleus and the cytoplasm is known to differ. Methodology is set up and collaboration with M. Vermeulen lab currently set up for the latter part. We expect results by Q2/3 2024.

Project J) Unraveling the immune responses in pediatric neuroblastoma.
High-risk pediatric neuroblastoma is a difficult to treat tumor, and most patients succumb to this tumor. There is thus a dire need for novel treatment options. We have studied whether TILs of neuroblastoma lesions can be expanded, and whether they are tumor-reactive. Intriguingly, whereas conventional CD4 and CD8 T cells recognize the tumor, they don't show high functionality. However, because we observed also cytokine production upon exposure to tumors, we are currently investigating the cell types that drive the immune responses towards Neuroblastoma, define the best source for generating TIL products (primary tumors versus metastatic BM lesions). Castenmiller et al., Manuscript in preparation, submission expected in Q2, 2024). Together with our collaborator Dr. J. Wienke (PMC), we have applied for a KIKA grant (awarded and to be started in April 2024).

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Our collaborations with the Vermeulen lab have substantially contributed to the progress in several projects. We are continuing with this collaboration to move forward other projects.

b) This also holds true for the collaborations with the Schumacher lab. Other collaborations are starting (see below) and are expected to yield similarly fruitful results.

2.4.2. Contribution to the Oncode community in 2023

a) Junior OI assessment of Oncode, supporting RMC in decision making

2.4.3. Key collaborations within Oncode in 2023

a) Michiel Vermeulen: Defining the protein expression of Non-small-cell lung cancer -derived tumor infiltrating T cells (TILs) by Mass spectrometry. This study has resulted in the identification of novel tumor-specific protein expression nodes, which we currently further investigate. Of note, these gene regulation nodes would not have been identified by RNA sequencing, as the RNA expression of these proteins is identical in tumor or lung T cells. These data have been used for a Amsterdam UMC-CCA innovative research project grant (2y, 175k), which has been granted to Dr. Kaspar Bresser. See: https://www.amsterdamumc.org/en/research/news/cancer-center-amsterdam-foundation-funds-9-innovative-research-projects.htm

b) Michiel Vermeulen. Using previous methods Michiel had set up, we studied the interactions of RNA binding proteins with m6A methylated RNA. We previously found that specific sequences in cytokine mRNA become methylated in T cells upon activation, and these sequences deviate from the classically described m6A sites. With the data that Michiel's lab has provided, we have been able to identify interactors in T cells with these sites. (Foskolou et al., manuscript in preparation). Collaboration on both studies with Michiel have started

thanks to discussions during breaks on Oncode meetings (on a workshop in 2020 (TIL work) and on annual Oncode KIT meeting (m6A study).

c) Ton Schumacher: We helped improve the the prediction pipelines of HLA ligandome with the of Machine-learning pipelines to predict the protein expression. Paper is in revision with Cell Reports, and available at bioRxiv. See: Bresser K, Nicolet BP, Jeko A, Wu W, Loayza-Puch F, Agami R, Heck AJR, Wolkers MC, Schumacher TN. Gene and protein sequence features augment HLA class I ligand predictions. BioRxiv. doi: https://doi.org/10.1101/2023.09.03.556079. Collaboration with Ton on this topic was started thanks to Oncode meeting (of first/second author on conference)

d) Ton Schumacher: ***TCR isolation from NSCLC***. We are supporting the team of T. Schumacher (lead: Dr. Wouter Scheper) in the isolation of TCRs from NSCLC tumors, to further decipher the T cell landscape in these tumors.

e) **Jarno Drost:** studying TILs in rhabdosarcomas. We have expanded TILs for Jarno's group. However, the starting material was too limited, and data were uninterpretable. We are currently discussing better experimental setups. We have also shared with Jarno our recent unexpected findings in pediatric neuroblastoma's, where we have observed that gd T cells are the prime tumor-reactive T cells. we will incorporate this knowledge in our collaboration with Jarno and have proposed alternative strategies to Jarno to study the immune response (and how to reinvigorate it) in December. We expect continuation of this collaboration in the coming months.

f) ***Sjoerd van der Burg.*** Analysis of expanded TIL products from NSCLC for TEIPP-specific T cells. Sjoerd has approached us to use the expanded TIL products from our NSCLC studies to determine the prevalency (and isolate) TEIPP specific T cells, and to further characterize their phenotype. Last month the MTA has been put in place, and the IRB approval has been obtained. We will soon start this study.

2.4.4. Major valorization achievements in 2023

a) Achieving proof-of-concept for modulating therapeutic protein expression in eukaryotic cells with artificial 3'UTRs. Using the machine learning pipeline SONAR, we have been able to construct synthetic 3'UTRs which, when fused to different constructs, boost the protein expression in eukaryotic cells

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
Horizon 2020	Marie Curie fellowship	200.000	200.000 to Iosifina Foskolou	01-03-2023	24	Co-applicant
AmsterdamUMC	Postdoctoral fellowship	175.000	175.000, to Kaspar Bresser	01-04-2024	24	Co-applicant
Horizon 2020	MSCA-ITN	2.900.000	225.000	01-08-2023	36	Co-applicant

3.2. Clinical activities in 2023

N/A

3.3. PhD defenses in 2023

N/A

Wilbert Zwart

Netherlands Cancer Institute

1. General information

Research Focus	Hormones and Cancer
Junior/Senior Oncode Investigator	Senior

2. Oncode activities

2.1. Research topics and scientific progress

Steroid hormone receptors are critical drivers in the development and progression of multiple cancer types, and hormonal interventions represent the very backbone of treatment for many patients with cancer, yet therapy resistance imposes a major clinical challenge. Our research on hormonal regulation spans the entire scientific spectrum, from basic fundamental studies on epigenetic gene regulation, translational research on therapy resistance and novel therapeutic vulnerabilities, and clinical trials to bring our findings towards the patient. Our goals are to better understand hormonal signalling plasticity in cancer development and progression, elucidate the mechanisms of therapy resistance, contribute to personalized treatment, identify of novel therapeutic options and limit over-treatment.

Over the past year, we have published 18 papers; many of which in close collaboration with other Oncode labs. Collaborations with the clinic have extensively been expanded, with the appointment of a new MD PhD student and newly initiated shared projects with the department of nuclear medicine within the NKI. With this, we are gradually closing the gap between research and clinic, better facilitating transfer of our findings towards improving clinical care for patients with cancer.

2.2. Major scientific achievements in 2023

a) Last year was exceptional, with 5 PhD students graduating (Simon Linder receiving Cum Laude, and receiving a DKFZ postdoctoral fellowship), and postdoc Stefan Prekovic starting his own lab with in CMM-UMCU, and receiving a KWF Young Investigator grant. Our lab positions itself as fruitful successful environment for young scientists.

b) For three of our clinical studies (Joosten et al., 2023; Severson et al., 2023; Linder et al., 2023), we identified distinct non-protein coding features that predicted drug response, disease progression of functionally explained the biological contribution of cancer risk SNPs. With this, our reverse translation capacities have now fully matured, not only bringing our research from the bench to the bedside, but now also back again towards new functional insights and newly developed Phase II trials based on these data; Our research is now officially full-circle

2.3 Oncode base fund research projects

2.3.1. Oncode base fund research projects and progression thereof in 2023

Project A) Epigenetic plasticity driving Endometrial tumorigenesis

We analyzed plasticity of enhancer activity, Estrogen Receptor action and 3D genome organization in endometrial tumor development. This project pioneers in functionally annotating the oncogenic contribution of non-coding mutations in human cancers; a highly innovative field of research. We are currently revising our manuscript for Nature Communications, and these data serve as prior work for postdoc Sebastian Gregoricchio, to write a career development grant (NWO-VENI).

Project B) Antifolates as novel therapeutic options for metastatic breast cancer patient relapsing after aromatase inhibitor treatment

We previously employed the Oncode Drug Repurposing library to screen for compounds that would have increased anti-tumor efficacy in breast cancer cell lines with resistance to aromatase inhibitor treatment. We identified a strong acquired sensitivity to anti-folate drugs in the aromatase inhibitor resistant setting, including Pemetrexed; an antifolate normally used for lung cancer treatment. As this drug is off-patent, industry interest is limited. We are currently wrapping up the manuscript reporting these results, and plan to submit a proposal for clinical follow-up to ZONMW, for an affordable healthcare call.

Project C) 3D genomics in human tumor tissue

We successfully charted the 3D genome (using Hi-C) in two tumor types: breast cancer and endometrial cancer. For endometrial cancer, we compared healthy tissue with endometrial tumors, observing large-scale changes. This work was integrated in the manuscript of Project A. For breast cancer, we compared healthy tissue with primary tumors, and two metastatic sites (liver and pleural metastases). This work is currently being finalized towards a manuscript, in collaboration with Elzo de Wit. Basesd on this preliminary work, we aim to submit a grant to KWF.

Project D) Deciphering tissue-context dependence of enhancer activity

Using the STARR-seq (Self-transcribing active regulatory region sequencing) technology we implemented in the lab last year, are currently addressed a long-standing critical question in human biology: how does the same transcription factor act differently in different organs? We designed a STARR-seq library containing all ER sites shared or unique between breast and endometrial cancers. Since this is a plasmid-based readout, we can uncouple chromatin structure from impact of primary DNA sequence or contribution of tissue-selective coregulator expression. We observe intriguing differences in transcription complex composition that may explain tissue selectivity, and are identifying features that may explain why particular DNA-binding sites of hormone receptors are actively engaged in transcription, while others are not. These studies already resulted in three independent manuscripts, and we are currently exploring opportunities to apply for funding.

Project E) 11-keto testosterone as driver of therapy resistance in prostate cancer

11-keto testosterone (11KT) is an oxidized form of testosterone, produced in the adrenal glands, and the most-predominant form of androgen receptor ligand in castrated men. Strangely, 11KT is largely ignored in the clinic and in science. We are currently charting the biological, genomic and clinical roles of 11KT in prostate cancer. These preliminary data suggest that 11KT is a very potent androgen receptor ligand, that should be incorporated in clinical route testing. We are currently working towards a manuscript, and are implementing 11KT testing within a Phase II clinical trial. Based on these results, we aim to apply for grant funding, within the next year.

2.4 Impact and contribution

2.4.1. How Oncode impacted your research in 2023

a) Oncode base funds enabled my lab to explore STARR-seq as a new technology to separate out the contribution of chromatin from the impact of primary DNA sequence in ultimately driving enhancer action and gene regulation. I find this a very exciting new research direction, already leading to three independent manuscripts and many more to come.

b) Through the PERICODE Oncode Synergy project, we very intensely collaborated with numerous other Oncode labs throughout the country, initiating new spin-off collaborations and exciting new projects within our teams.

c) We were granted access to the Oncode Drug Repurposing library, to screen for compound that act synthetic lethal with tamoxifen treatment in breast cancer. Hormonal therapies in breast cancer, such as tamoxifen, represent the very backbone of systemic treatment of 80% of all breast cancer patients, yet these drugs only act cytostatic, not cytotoxic. Identifying a synthetic lethal drug combination together with tamoxifen, would have major clinical implications.

2.4.2. Contribution to the Oncode community in 2023

a) I serve as Scientific Evaluation Committee member of the drug reporposing programme, served in the organizing committee of the Oncode Annual Meeting 2023 (in which many of my labmembers proactively contributed), and participated in the Open Science and Faire data advisory group.

b) I volunteered to serve on the Oncode RMC, for which I hope to hear the outcome soon.

c) I proactively participate and contribute in the patient engagement program of Oncode, interacting with patients and help in outreach to other lay audience groups.

2.4.3. Key collaborations within Oncode in 2023

a) The Pericode consortium (van Steensel, Voest, Derks, Franke, de Ridder, Vermeulen). Within this consortium, we are charting the non-protein coding genome, to identify biological consequences of non-coding mutations (Funded through Saxum Volutum). Through an AI model, we have developed a unprecedented strong predictor for gene activity based on primary DNA sequence, along with the unbiased discovery of transcription factors that drive gene genes. With none of these labs, I collaborated before I joined Oncode.

b) Michiel Vermeulen. With the Vermeulen lab, we are combining our expertise of enhancer profiling and epigenetic regulation in cancer, with their expertise on protein/DNA interactions and the impact of DNA sequence alterations thereof. Manuscripts: Joosten et al., 2023. BioRxiv (revisions for Genome Research), Gregoricchio et al., 2023. BioRxiv (revisions for Nature Communications). We are currently writing a joint grant proposal for ZonMW. Preexisting interactions with this lab, prior to Oncode: none.

c) Ruben van Boxtel. With the Van Boxtel lab, we are analysing mutational signatures in prostate cancer metastases, associated with prior treatment with radiotherapy. In prostate cancer care (but also for other cancer types), relapse after radiotherapy may possibly imply seeding of non-effectively-irradiated tumor cells that metastasized subsequently, or imply a tumor cell seeding that preceded the radiotherapy exposure. This is a long-standing question in cancer care, and addressing this may have profound impact on how patients are treated with radiotherapy. Current status of research: ongoing. Prior interactions with this lab, before Oncode: none.

2.4.4. Major valorization achievements in 2023

a) Through the Oncode Drug Reporposing program, we are identifying therapeutic options that may act as synthetic lethal drugs, together with endocrine therapeutics. As endocrine therapy only acts as cytostatic agent, such synthetic lethal drug interactions may have substantial clinical benefit to enhance cure for patients with hormone-driven breast cancer. Based these results, pharmaceutical pcompanies will be contacted for potential collaborations.

b) With Xilis and Genentech, we are setting up collaborations to comprehensively and systematically chart drug responsiveness, epigenomics and transcriptomics, on single cell level, for malignant pleural effusions from patients with progressive breast cancer. These data will be used to better predict drug response, and facilitate optimal drug selection for individual patients.

c) We are in the process of finalizing a cPOC full proposal, collaboration with Astellas Pharma, to set up a Phase II clinical trial enrolling high-risk prostate cancer patients from adjuvant treatment with Enzalutamide. Standard of care treatment does not involve any systemic therapy approaches, leaving a substantial patient population under-treated. With a gene signature, designed through a previous cPOC application, we now aim to pre-select those patients with a predicted dismal outcome wen treated according to standard-of-care, and who would benefit from intensified early treatment to improve outcome.

3. Highlights

3.1. External grants & awards awarded in 2023

Funding agency	Funding programme	Total grant value	Amount allocated to your group	Anticipated start date	Project duration	Main or co-applicant
		(€)	(€)	(mm/yyyy)	(months	(*)
KWF	exploration round *www.KWF.nl*	711K	711K	01-07-2023	48	Co-applicant
KWF	Young Investigator	680K	0K	01-07-2023	48	Co-applicant

3.2. Clinical activities in 2023

Study identifier	Study title	Study start date	Study duration	First patient dosed?	Role OI
(ref #)		(mm/yyyy)	(months	(€)	(*)
NCT03295565	Optimal Sequencing of Treatment Options for Poor Risk mCRPC Previously Treated With Docetaxel (OSTRICh)	01/05/2017	?	Yes	Co-PI
NCT03223597	Registry of Treatment Outcomes in a non-study population of Symptomatic Metastasized Castration Resistant Prostate Cancer (mCRPC) Patients Treated with Radium-223 (ROTOR-registry)	01/01/2017	?	Yes	Co-PI

3.3. PhD defenses in 2023

Name and Surname	Thesis title
Isabel Mayayo-Peralta	Deciphering the role of steroid hormone receptors, and their co-regulators, across different tumor types: It takes (more than) two to tango
Jeroen Kneppers	The multifaceted face of prostate cancer
Anniek Zaalberg	Unravelling the dynamic interplay between prostate cancer and its microenvironment: A race towards novel therapeutic strategies
Simon Linder (Cum Laude)	Advancing prostate cancer therapies through integrative multi-omics: It's about time.
Emira Visser	Evolution of 14-3-3 molecular glues: from fragment hits to cellularly active leads