EACR
European Association for Cancer Research
Conference Series
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2nd EACR Conference

Defence is the Best Attack

Immuno-Oncology Breakthroughs

11 - 13 March 2019 | Barcelona, Spain

Scientific Programme Committee

Alena Gros, Johanna Joyce,
Joan Seoane, Jedd D. Wolchok

Programme Book

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2nd EACR Conference

Defence is the Best Attack

Immuno-Oncology Breakthroughs

Day 1 - Monday 11 March

12.00 – 13.00	REGISTRATION
13.00 – 14.00	WELCOME LUNCH *A chance to meet fellow participants and enjoy a light buffet lunch before the first session.*
14.00 – 14.15	CONFERENCE WELCOME **Scientific Programme Committee**
	SESSION 1 Chair: Joan Seoane
14.15 – 14.35 Q&A: 14.35 – 14.45	**Pablo Umaña** Roche Innovation Center Zurich, Switzerland "Redirecting T-cells for Cancer Immunotherapy using Next Generation Bispecific Antibodies and Fusion Proteins"
14.45 – 15.05 Q&A: 15.05 – 15.15	**Karin de Visser** NKI, Netherlands "Dissecting cancer cell-intrinsic mechanisms dictating the immune landscape of breast cancer"
15.15 – 15.25 Q&A: 15.25 – 15.30	**Yotam Bar-Ephraim** Hubrecht Institute, Netherlands Proffered Paper 1: "Raising T cells against tumour-derived neo-antigens using epithelial organoid cultures"
15.30 – 15.50 Q&A: 15.50 – 16.00	**Elena Garralda** VHIO, Spain "Challenges in early drug development of IO agents"
16.00 – 16.10	EXHIBITOR INTRODUCTIONS
16.10 – 16.40	COFFEE BREAK
	SESSION 2 Chair: Johanna Joyce
16.40 – 16.50	POSTER SPOTLIGHTS *3 top-scoring abstracts will be presented in short 'flash talks' of 3 minutes each.*

16.50 – 17.00 **Neta Erez** Tel Aviv University, Israel
Q&A: 17.00 – 17.05 Proffered Paper 2: "Shaping the immune landscape: Cancer-Associated Fibroblasts drive Th2 immunity in breast cancer progression and metastasis"

17.05 – 17.25 **Luca Gattinoni** National Cancer Institute, USA
Q&A: 17.25 – 17.35 "Harnessing T memory stem cells for adoptive cell transfer therapy of cancer"

17.35 – 17.55 **Andrea Schietinger** MSKCC, USA
Q&A: 17.55 – 18.05 "Molecular mechanisms defining tumor-specific T cell dysfunction and therapeutic reprogrammability"

18.05 – 18.35 EMBO KEYNOTE LECTURE
Q&A: 18.35 – 18.50 **Richard Flavell** Yale University School of Medicine, USA
"Study of the interface between human cancers and the human immune system in novel humanized mice"

18.50 – 19.30 NETWORKING RECEPTION
with drinks and a hot buffet dinner

19.30 - 21.00 POSTER DISCUSSION SESSION 1
Odd numbered posters will be presented

Day 2 - Tuesday 12 March

09.00 – 09.30 POSTER VIEWING AND TRADE EXHIBITION

09.30 – 09.45 INDUSTRY SPOTLIGHT - NEXCELOM BIOSCIENCE
Sun Yung UK
"Novel plate-based detection method for T cell activation/ proliferation, migration and cytotoxicity assay using image cytometry"

SESSION 3 Chair: Andrea Schietinger

09.45 – 10.05 **Alena Gros** VHIO, Spain
Q&A: 10.05 – 10.15 "Harnessing the personalized antitumor T-cell response to treat cancer"

10.15 – 10.35 **Johanna Joyce** University of Lausanne, Switzerland
Q&A: 10.35 – 10.45 "Exploring and Therapeutically Exploiting the Tumour Microenvironment"

10.45 – 11.15 Q&A: 11.15 – 11.30	KEYNOTE LECTURE **George Coukos** University of Lausanne, Switzerland "Orchestration of antitumor immune response. Lesson for immunotherapy from the tumor microenvironment"
11.30 – 12.05	COFFEE BREAK
	SESSION 4 Chair: Karin de Visser
12.05 – 12.15	POSTER SPOTLIGHTS *3 top-scoring abstracts will be presented in short 'flash talks' of 3 minutes each.*
12.15 – 12.35 Q&A: 12.35 – 12.45	**Joan Seoane** VHIO, Spain "Embryology meets Oncology. The role of LIF in cancer"
12.45 – 12.55 Q&A: 12.55 – 13.00	**Marit Melssen** University of Virginia, USA Proffered Paper 3: "Formation and function of CD49a+ and CD49b+ CD8 T cells in a murine breast cancer model"
13.00 – 13.20 Q&A: 13.20 – 13.30	**Sergio Quezada** UCL Cancer Institute, UK "T cell evolution in non-small cell lung cancer"
13.30 – 14.00	INDUSTRY SYMPOSIUM - THERMO FISHER SCIENTIFIC **Tim Looney** USA "Predicting immunotherapy response and adverse events through next generation sequencing of peripheral blood T cell repertoires"
14.00 – 15.00	LUNCH
15.00 – 16.30	POSTER DISCUSSION SESSION 2 *Even numbered posters will be presented*
	SESSION 5 Chair: Sergio Quezada
16.30– 16.50 Q&A: 16.50 – 17.00	**Graham Pawelec** University of Tübingen, Germany "Immunotherapy of Cancer: Triumphs and Challenges and the Impact of Immunosenescence"
17.00 – 17.10 Q&A: 17.10 – 17.15	**Danique Duits** NKI, Netherlands Proffered Paper 4: "Systemic expansion of pro-metastatic neutrophils is enhanced by MET amplification in breast cancer"
17.15 – 17.35 Q&A: 17.35 – 17.45	**Kris Thielemans** VUB, Belgium "mRNA therapeutics: ex-vivo and in-vivo modification of antigen presenting cells"

17.45 – 18.45

DISCUSSION FORUM

"How to introduce precision medicine principles to immuno-oncology"

The discussion will be led by Joan Seoane, Zlatko Trajanosky and George Coukos, and participants are invited to contribute to the conversation.

19.30

OPTIONAL CONFERENCE DINNER

El Principal del Eixample

For those who have purchased tickets.

Day 3 - Wednesday 13 March

09.00 - 09.30

POSTER VIEWING AND TRADE EXHIBITION

09.30 - 09.45

INDUSTRY SPOTLIGHT - 10X GENOMICS

Hannes Arnold Germany

"Single Cell and Spatial Gene Expression Analysis of the Tumor Microenvironment"

SESSION 6 Chair: Alena Gros

09.45– 10.05
Q&A: 10.05 – 10.15

Núria Lopez-Bigas IRB Barcelona, Spain

"Biological and clinical relevance of tumor mutations"

10.15 – 10.35
Q&A: 10.35 – 10.45

Zlatko Trajanosky Innsbruck Medical University, Austria

"Computing and probing immunity in colorectal cancer"

10.45 – 10.55
Q&A: 10.55 – 11.00

Jitske van den Bulk LUMC, Netherlands

Proffered Paper 5: "Detection of neo-antigen-specific T cell responses in low mutation burden colorectal cancers for personalized therapies"

11.00 – 11.30

COFFEE BREAK

11.30 – 11.50
Q&A: 11.50 – 12.00

Miguel Sanmamed University of Navarra, Spain

"Novel patient-derived tumor models for immunotherapy development"

12.00 – 12.30
Q&A: 12.30 – 12.45

KEYNOTE LECTURE

Laurence Zitvogel Gustave Roussy, France

"The unsuspected role of gut microbiota in cancer therapies"

12.45 – 13.00

SUMMARY & AWARDS

Scientific Programme Committee

13.00

LUNCH & DEPART

Meeting Bursary Awards

EACR-Worldwide Cancer Research Meeting Bursaries provide funds to help early-career EACR members to participate in our conferences.

Congratulations to the recipients of the Meeting Bursaries for this conference. Each winner received a full registration free of charge and funds of up to €500 to assist with the cost of travel and accommodation.

- **Marit Martina Melssen** USA
- **Valeria Quaranta** UK
- **Jonas Van Audenaerde** BELGIUM

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worldwide cancer research

Two **EFIS-EJI Meeting Bursaries**, each with a total value of €1000, were awarded to assist early-career scientists to attend the conference and present their work as an oral or poster presentation. Each bursary includes funds to support registration, travel, accommodation and subsistence costs.

- **Yotam Bar-Ephraïm** NETHERLANDS
- **Jitske van den Bulk** NETHERLANDS

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For more information about EACR Meeting Bursaries visit:
eacr.org/meeting-bursary

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Interactive activities

An important part of the EACR Conference Series is the range of opportunities we aim to provide for participants to interact, discuss, reflect and build relationships and collaborations.

We hope you enjoy the dedicated interactive activities, which are listed below.

Networking Reception

Monday 11 March 18.50 - 19.30

A complimentary buffet dinner will be served for all participants and exhibitors to enjoy on Monday evening. The trade exhibition will be open at this time, and there is the opportunity to continue networking. Poster Session 1 will follow at 19.30.

Discussion Forum

Tuesday 12 March 17.45 - 18.45 "How to introduce precision medicine principles to immuno-oncology"

One of the most important challenges in the field of immune-oncology is the identification of biomarkers to predict response to immunotherapy. This is crucial to selecting the patients to be treated with compounds designed to engage the immune system against tumours. The discussion will focus on this important point and will deal with some of the present controversies in the field. The session will also provide an overview of how to move forward in the future leading to discussions about how to identify novel biomarkers and the limitations (and how to circumvent them) of the present preclinical models.

The discussion will be led by Joan Seoane, Zlatko Trajanosky and George Coukos, and participants are invited to contribute to the conversation.

Poster Spotlights

Monday 11 March 16.40 - 16.50
Tuesday 12 March 12.05 - 12.15

Six high-scoring abstracts have been selected to give Poster Spotlight presentations. These three minute 'flash talks' will be presented in the lecture theatre. All participants are invited to join in with these informal sessions.

Interactive activities

Poster Discussion Sessions

Monday 11 March 19.30 - 21.00
Tuesday 12 March 15.00 - 16.30

The programme features two 90 minute Poster Discussion Sessions which will be an excellent opportunity to present and discuss the latest research. As well as these dedicated sessions, there will be additional opportunities to browse the posters throughout the meeting.

Two EACR Poster Prizes of €100 each will be awarded for the best posters as selected by the judges.

Conference Dinner

Tuesday 12 March 19.30

For participants who have purchased a ticket only.

The conference dinner is an excellent opportunity for participants and speakers to get to know each other in a relaxed and informal environment. The dinner will take place at El Principal del Eixample and will consist of three courses along with wine and coffee.

Venue
El Principal del Eixample
Carrer de Provença 286 - 288
08008 Barcelona

It is only about a 2 minute walk from the conference venue to the dinner venue.

Don't forget to let us have your feedback about these activities in the survey we will send after the conference!

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Meet the Exhibitors

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Represented at the conference by:
Hannes Arnold, Pau Corral

Describe your company in 5 words or less
Biology at True Resolution

Tell us a little bit about your company
10x Genomics offers single cell sequencing and spatial transcriptomic approaches that provide researchers with a detailed, multidimensional view of the tumour and the tumour microenvironment. Unmask the genetic, transcriptomic and epigenetic complexity of cancer at single cell resolution with our comprehensive solutions.

Why are you attending this conference?
Solutions provided by 10x Genomics are directly applicable to the study of cancer and we welcome discussions with attendees working at the various steps; PIs, clinicians, wet-lab researchers and bioinformaticians.

Agilent

Website: www.agilent.com/en/promotions/cell-analysis-technology
Contact: cellanalysis.support@agilent.com
Represented at the conference by:
Alfredo Caro-Maldonado

Tell us a little bit about your company
Agilent is a leader in life science research tools, providing analytical instruments, software, consumables and services for research laboratories worldwide. Agilent products including Seahorse are used in phenotyping, metabolomics, cell metabolism, and mitochondrial toxicity assays; as well as immunotherapy, cancer, and metabolic disease research.

BD

Website: www.bd.com
Contact: cormac.smyth@bd.com
Represented at the conference by: Cormac Smyth, Gemma Coma, Serge Scherrer

Describe your company in 5 words or less
Pioneering single-cell multi-omics

Tell us a little bit about your company
BD is one of the largest global medical technology companies in the world and is advancing the world of health by improving medical discovery, diagnostics and the delivery of care. BD helps customers enhance outcomes, lower costs, increase efficiencies, improve safety and expand access to health care.

Why are you attending this conference?
BD Biosciences is a world leader in flow cytometry, reagents and in single-cell multi-omics. Our unique product range allows both protein and mRNA expression levels in single-cells to be evaluated in one experiment. Here, BD wishes to engage with researchers working with challenging problems at the frontiers of immuno-oncology.

Fluidigm

Website: www.fluidigm.com
Contact: info-europe@fluidigm.com
+33 1 60 92 42 40
Represented at the conference by:
Muge Akpinar, Roberto Spada

Describe your company in 5 words or less
Intuitive, intelligent, inspirational and collaborative

Tell us a little bit about your company
Fluidigm is committed to empowering the cancer community with research tools for the comprehensive interrogation of immune cell function and the tumor microenvironment. Using proven mass cytometry and microfluidic technologies, we provide workflows to identify cell signatures and characterize changes in cellular phenotypes at single cell resolution.

Who would you like to meet at the conference?
Researchers from academic institutions, clinical research laboratories, and pharmaceutical, biotechnology companies worldwide.

Nexcelom BioScience

Website: www.nexcelom.co.uk
Contact: sales@nexcelom.co.uk
+44 161 232 4592
Represented at the conference by:
Scott Cribbes, Sun Yung

Describe your company in 5 words or less
Expertise in Automated Cell Analysis

Tell us a little bit about your company
Nexcelom is focused on providing novel tools to meet the specific needs of today's researchers. Our applications specialists are in the field with customers every day, analyzing new cell types, validating new cell-based assays, and exploring new therapeutic areas to meet emerging customer needs.

Why are you attending this conference?
We would like to meet investigators and scientist involved in immuno-oncology. To learn from them and their experience, to see how we can make their work easier and reach work goals.

Nonacus

Website: www.nonacus.com
Contact: info@nonacus.com
Represented at the conference by: Chris Sale, David Hughes

Describe your company in 5 words or less
Innovating Translational Oncology Research

Tell us a little bit about your company
Nonacus develops liquid biopsy (ctDNA) and primary tumor (FF/FFPE) analysis products. We have developed a complete analysis workflow for liquid biopsies and recently launched our Pan-Cancer (524) gene NGS Panel for analysis of primary tissue or ctDNA for calling variants, Tumor Mutational Burden or MSI.

Why are you attending this conference?
We wish to inform customers about our range of products to assist with genomics analysis of both primary tumor (FFPE) and liquid biopsy (ctDNA). If you are undertaking biomarker discovery or validation or translation research and perform genomics analysis, please visit us.

ProQinase

Website: www.proqinase.com
Contact: info@proqinase.com
+49-761-769996-0
Represented at the conference by:
Anja Baumgart, Kelvin Lam

Describe your company in 5 words or less
Tumor models & In vitro services

Tell us a little bit about your company
A main focus at ProQinase is a versatile immuno-oncology platform based on syngeneic mouse models. A recent innovation lies in new proprietary models with tumors propagated in mice only to conserve tumor-stroma characteristics and original immune cell profiles as close to nature as possible.

Why are you attending this conference?
We want to take the chance to promote our immuno-oncology platform and unique models, answer questions and provide solutions to expedite the pre-clinical development of compounds. We value face-to-face meetings and are excited to talk to scientists and hear firsthand how we can help them.

Thermo Fisher Scientific

Website: www.thermofisher.com
Contact: rosella.petraroli@thermofisher.com
Represented at the conference by: Rosella Petraroli, Chris Allen, Beatriz Bellosillo, Andrea Lucchetti, Nuria Queralt Diaz, Luca Quagliata

Tell us a little bit about your company
Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable customers to make the world healthier, cleaner and safer. We support oncology research with next generation sequencing solutions and innovative technologies for Solid Tumours testing and Liquid-Biopsy and Immuno-oncology applications.

VectorBuilder

Website: en.vectorbuilder.com
Contact: vladimirivosev@vectorbuilder.com
Represented at the conference by:
Vladimir Ivosev

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VectorBuilder is a revolutionary online platform that provides one-stop solutions to all your vector design, custom cloning and virus packaging needs. Besides cloning and virus packaging, VectorBuilder offers other molecular biology services such as BAC modification (recombineering), library construction, DNA/RNA preparation, mutagenesis, and more.

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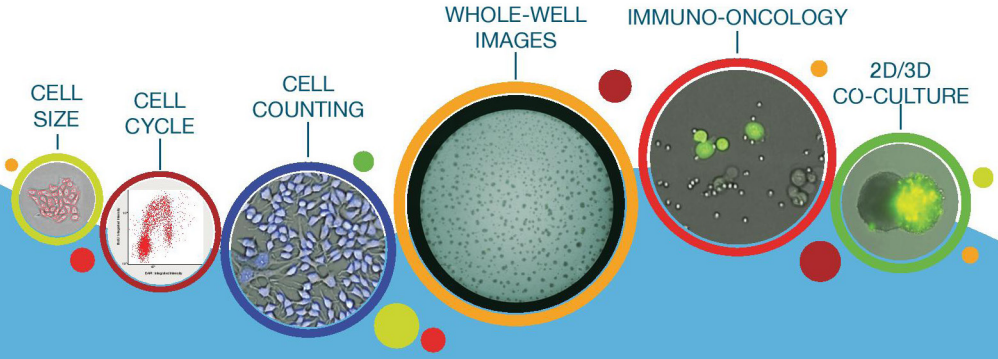

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Novel plate-based detection method for T cell activation/proliferation, migration and cytotoxicity assay using image cytometry.

Tuesday 12 March at 09:30-09:45
Presented by Sun Yung, PhD, *Nexcelom Bioscience*

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Industry Symposium - Elite Sponsor

We are pleased to announce that **Thermo Fisher Scientific** will not only be exhibiting at the conference but also inviting participants to join their Industry Symposium.

Tuesday 12 March, 13.30 - 14.00

Tim Looney Thermo Fisher Scientific, USA

"Predicting immunotherapy response and adverse events through next generation sequencing of peripheral blood T cell repertoires"

Industry Spotlight - Premium sponsors

Tuesday 12 March, 09.30 - 09.45

Sun Yung Nexcelom Bioscience, UK

"Novel plate-based detection method for T cell activation/proliferation, migration and cytotoxicity assay using image cytometry."

Wednesday 13 March, 09.30 - 09.45

Hannes Arnold 10x Genomics, Germany

"Single Cell and Spatial Gene Expression Analysis of the Tumor Microenvironment"

EACR Industry Partners

The European Association for Cancer Research gratefully acknowledges the organisations that support the Association as Industry Partners. Industry Partners offer ongoing support to the EACR and provide the means for the Association to develop important initiatives. The EACR Conference Series is an example of this.

Speaker abstracts

Redirecting T-cells for Cancer Immunotherapy using Next Generation Bispecific Antibodies and Fusion Proteins

Pablo Umaña[1]
[1] *Roche Glycart AG, Schlieren, SWITZERLAND*

This talk will discuss a new generation of therapeutic approaches to redirect and enhance T cells attack on cancer cells. The approaches are based on combinations of “off-the-shelf”, systemically administered, recombinant proteins that have been engineered to enhance their activity for stimulating T cells in tumors and/or lymph nodes vs. normal tissues.

Dissecting cancer cell-intrinsic mechanisms dictating the immune landscape of breast cancer

Karin de Visser[1]
[1] the Netherlands Cancer Institute, Amsterdam, NETHERLANDS

A great challenge in the field of immuno-oncology is the unexplained inter-patient heterogeneity in the intratumoral and systemic composition and functional state of the immune system. Given the surge of interest in utilizing immunomodulatory drugs for the treatment of cancer, it is critical to understand the underlying tumor characteristics that dictate the inter-tumor heterogeneity in immune landscapes, since these insights will help to 1) select the right patients for the right immune-intervention strategy; 2) uncover novel actionable pathways that can be exploited to convert tumor-supportive immune landscapes into those that favor anti-tumor immunity. In our lab, we study how mammary tumor-induced systemic inflammation influences breast cancer metastasis formation. Utilizing pre-clinical mouse models that faithfully recapitulate human breast tumorigenesis in combination with immune profiling studies in breast cancer patients, we have previously discovered that breast tumors elicit a systemic inflammatory cascade to dampen anti-tumor T cells and promote metastasis formation. Cancer cell-intrinsic mechanisms that lie at the basis of heterogeneity in the systemic immune milieu within tumor types are largely unknown. To determine how pro-metastatic systemic inflammation is influenced by genetic aberrations in mammary tumous, we utilized a panel of 16 genetically engineered mouse tumor models (GEMMs) for breast cancer that harbor different tissue-specific mutations. Our findings provide novel mechanistic insights into the impact of the genetic makeup of breast cancer on the systemic immune landscape, and open new avenues for the development of therapeutic strategies to unleash anti-tumor immunity and to inhibit metastatic disease that are tailored to the genetic makeup of the tumor.

PROFFERED PAPER 1

Raising T cells against tumour-derived neo-antigens using epithelial organoid cultures

Yotam Bar-Ephraim[3,4], Kai Kretzschmar[3,4], Wei Wu[7], Laura Demmers[7], Henk Van den Toorn[7], Priyanca Asra[3,4], Kim Boonekamp[3,4], Evelien De Jongh[3,4], Jarno Drost[5], Apollo Pronk[1], Joost Van Gorp[1], Niels Smakman[1], Inez Gan[6], Zsolt Sebestyen[6], Jurgen Kuball[6], Robert Vries[2], Albert Heck[7], Hans Clevers[3,4]
[1] Diakonessenhuis Hospital, Utrecht, NETHERLANDS, [2] Foundation Hubrecht Organoid Technology (HUB), Utrecht, NETHERLANDS, [3] Hubrecht Institute, Utrecht, NETHERLANDS, [4] Oncode Institute, Utrecht, NETHERLANDS, [5] Princess Maxima Center for Pediatric Oncology, Utrecht, NETHERLANDS, [6] University Medical Center, Utrecht, NETHERLANDS, [7] Utrecht University, Utrecht, NETHERLANDS

Colorectal carcinoma (CRC) is one of the most prevalent forms of cancer which develops in a multi-step process from lesions in healthy colon tissue. While mutations in cancerous epithelial cells drive the process of tumorigenesis, interaction of the tumour with the immune system and subsequent evasion from immune-mediated destruction is essential for tumour progression. There are several subtypes of CRC, distinguished amongst others by microsatellite stability. While microsatellite stable (MSS) tumours are generally less infiltrated by the immune system, the opposite is true for hyper-mutated microsatellite instable (MSI) tumours.

Epithelial organoids provide a platform which allows culturing of cancerous and healthy epithelium while retaining tissue-of-origin identity over prolonged culture periods. Organoids are thus a reliable system to model many biological processes, ranging from normal epithelial differentiation to tumour development. Also, tumor-derived organoids have shown potential to be used in patient-specific drug screens, making a critical step towards personalised medicine.

As tumour-derived organoids retain their genetic signature in culture, we set out to investigate whether we could use organoids to find tumour-derived neo-antigens which could be used to raise tumour-reactive $CD8^+$ T cell clones. To this end we analysed peptides presented in the context of MHC-I on MSI tumour organoids and compared them to profiles obtained from the matching healthy colon organoids. As MSI tumour organoids retain their mutation rate *in vitro*, we analysed four different clonal organoid lines from the same tumour line. Only peptides present on all four clonal tumour lines while absent on the healthy organoids have been considered to be true tumour-derived neo-antigens. Neo-antigens have furthermore been confirmed as such by whole-genome sequencing of the different clones.

In conclusions, we show that using organoids, tumour-derived neo-antigens can be defined and tumour-specific T cell clones can be raised against them, opening up new avenues for personalised medicine.

Challenges in early drug development of IO agents

Elena Garralda[1]
[1] *VHIO, Barcelona, SPAIN*

-- The abstract had not been received at the time of printing --

PROFFERED PAPER 2

Shaping the immune landscape: Cancer-Associated Fibroblasts drive Th2 immunity in breast cancer progression and metastasis

Ophir Shani[1], Noam Cohen[1], Yael Raz[1], Neta Erez[1]
[1] *Tel Aviv University, Tel Aviv, ISRAEL*

Immune modulation in the tumor microenvironment plays a central role in determining disease outcome. Cancer-associated fibroblasts (CAFs) are prominent players in the microenvironment of breast cancer, and were demonstrated to facilitate tumor progression and metastasis by multiple mechanisms, including by mediating tumor-promoting inflammation. However, the functional interactions of CAFs with immune cells are still largely unknown. We profiled the transcriptome of CAFs isolated from primary breast tumors and from lung metastases in mouse models of breast cancer, and found novel signaling axes between fibroblasts, cancer cells and immune cells that drive an immunosuppressive microenvironment. We demonstrate that Chi3L1 is highly upregulated in CAFs isolated from mammary tumors and pulmonary metastases of transgenic mice, and in the stroma of human breast carcinomas. Genetic ablation of Chi3L1 in fibroblasts *in vivo* attenuated tumor growth, macrophage recruitment and reprogramming to an M2-like phenotype. Moreover, analysis of T cell infiltration and cytokine secretion indicated that CAF-derived Chi3L1 shifts the balance of the immune milieu towards type 2 immunity. At the metastatic microenvironment, transcriptome profiling of metastases-associated fibroblasts revealed upregulation of interleukin-33 (IL-33), a cytokine with potent immune modulating properties. Moreover, we found that stromal IL-33 is upregulated in human breast tumors and lung metastases. By characterizing its function, we demonstrated that targeting of IL-33 signaling *in vivo* significantly attenuated lung metastatic relapse, and modified the immune milieu at the metastatic site, indicating that CAF-derived IL-33 drives a metastases-permissive microenvironment in lungs via activation of Th2 immunity. Taken together, our findings implicate fibroblast-derived factors as key players in the complex reciprocal interactions between stromal and immune cells that facilitate tumor progression and metastasis, and suggest that CAFs are central in modulating the immune microenvironment in breast cancer.

Harnessing T memory stem cells for adoptive cell transfer therapy of cancer

Luca Gattinoni[1]
[1] *National Cancer Institute, Bethesda, USA*

T memory stem (T_{SCM}) cells are a rare subset of memory lymphocytes endowed with the stem cell-like ability to self-renew and the multipotent capacity to reconstitute the entire spectrum of memory and effector T cell subsets. Cumulative evidence in mice, non-human primates and humans indicates that T_{SCM} cells are minimally differentiated cells at the apex of the hierarchical system of memory T lymphocytes. I will describe emerging findings demonstrating that T_{SCM} cells, owing to their extreme longevity and robust potential for immune reconstitution, are central players in many physiological human processes. I will also discuss how T_{SCM} cell stemness could be leveraged therapeutically to enhance the efficacy of adoptive T cell therapies for cancer.

Molecular mechanisms defining tumor-specific T cell dysfunction and therapeutic reprogrammability

Andrea Schietinger[1]
[1] *Memorial Sloan Kettering Cancer Center, New York, NY, USA*

T cell responses to cancers differ depending on the nature of the target antigen: tumor antigens that are self-proteins are generally weakly immunogenic due to pre-existing self-tolerance, whereas tumor antigens that are tumor-specific (e.g. mutated proteins) are potentially highly immunogenic because the immune system has not been exposed to these antigens. We recently demonstrated that tumor-specific T cells differentiate to a non-responsive state following initial encounter with tumor antigen, even before the emergence of a pathologically-defined tumor. While this state is initially reversible, it progresses to a fixed state that cannot be rescued, and the reversible and fixed non-responsive states were defined by discrete chromatin states. This pathway resembles what we observe in peripheral self-tolerance in which self-reactive T cells differentiate to an epigenetically-encoded tolerant state after encountering self-antigen. Thus, the adaptive immune system is under strong pressure to permanently neutralize self-reactive T cells through a specific, epigenetically-enforced differentiation program, and while this differentiation pathway effectively prevents auto-immunity for the most part, unfortunately, it just as effectively subverts tumor-specific anti-cancer responses. New insights into the epigenetic and molecular programs underlying hyporesponsiveness in self-reactive and tumor-specific T cells will be discussed.

EMBO KEYNOTE LECTURE

Study of the interface between human cancers and the human immune system in novel humanized mice

Richard Flavell[2,1]
[1] Howard Hughes Medical Institute, New Haven, CT, USA, [2] Yale University, New Haven, CT, USA

Immunodeficient mice transplanted with human hematopoietic stem and progenitor cells represent a promising approach for studying human immune function and diseases, particularly cancer in vivo. Knock-in of the genes encoding human signal regulatory protein alpha (SIRPA) and the human cytokines IL-3/GM-CSF, M-CSF and THPO in the MISTRG mouse led to improved development and function of human myeloid and NK cells. Human macrophages infiltrated human tumor xenografts in MISTRG mice in a manner similar to that observed in tumors obtained from human patients. Knock-in of human IL-15 further improved human NK cell and T cell development. These mice can be engrafted with human tumor material as well as the hematopoitic system of the tumor patient enabling These humanized mouse models may be used to model the human immune system in many scenarios of health and pathology, and may enable evaluation of therapeutic candidates in an in vivo setting relevant to human cancer.

Harnessing the personalized antitumor T-cell response to treat cancer

Alena Gros[1]
[1] *Vall d'Hebron Institute of Oncology (VHIO), Barcelona, SPAIN*

Cancer immunotherapy has shown encouraging antitumor responses in patients with metastatic melanoma, renal cancer, non-small cell lung cancer and other tumor types, and it is currently being evaluated in combination with chemotherapy and radiotherapy for the treatment of a diverse range of cancers. Cytotoxic T lymphocytes, which play an important role in the antitumor activity of cancer immunotherapies, can mediate specific tumor recognition and lysis through the recognition of tumor rejection antigens. Accumulating evidence suggests that T cells targeting neoantigens derived from tumor-specific mutated products play an important role in the antitumor responses observed following treatment with immunotherapy. Moreover, the administration of highly enriched populations of neoantigen-specific lymphocytes has demonstrated antitumor responses in selected patients with epithelial cancers such as cholangiocarcinoma, colorectal cancer and breast cancer. Given the importance of tumor-reactive and neoantigen-specific lymphocytes for the development of effective cell-transfer immunotherapies, our group as well as others have focused on identifying phenotypic traits that ca be used to guide the identification of neoantigen specific lymphocytes. This talk will summarize the current status of the use of phenotypic markers to identify and enrich for neoantigen-specific lymphocytes from the tumor and peripheral blood of cancer patients, and will compare some of the most commonly used strategies to select and screen for the presence of neoantigen-specific lymphocytes.

Exploring and Therapeutically Exploiting the Tumour Microenvironment

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Cancers develop in complex tissue environments, which they depend upon for sustained growth, invasion and metastasis. Different tumour microenvironments (TME) are populated by diverse cell types including innate and adaptive immune cells, fibroblasts, blood and lymphatic vascular networks, and specialised organ-specific cell types, which collectively have critical functions in regulating tumorigenesis. We are interested in determining how reciprocal communication between cancer cells and diverse immune and stromal cell types in the TME regulates tumour initiation, progression, and metastasis, and additionally modulates the response to therapeutic intervention. Our latest findings will be presented, with a focus on our identification of critical roles for innate immune cells in controlling these processes.

KEYNOTE LECTURE

Orchestration of antitumor immune response. Lesson for immunotherapy from the tumor microenvironment

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Tumor immunogenicity arises due to successful coordination of the tumor and its stroma components to foster an adaptive immune response. Based on *in situ* T cell presence, tumors have been categorized as immunogenic or inflamed and non-immunogenic or non-inflamed. Approximately half of ovarian cancers are infiltrated by tumor-infiltrating lymphocytes (TILs). Our mechanistic studies show that successful T-cell infiltration in ovarian cancer requires tumor cell-derived CCL5 and is amplified by myeloid cell-secreted IFN□-inducible CXCL9, dependent on activation of tumor-infiltrating lymphocytes (TILs). Constitutive CCL5 expression by tumor cells is associated with intraepithelial TILs and CCL5 status defined the immunophenotype of ovarian cancer. Spontaneous, epigenetically-mediated loss or stable knockdown of *ccl5* leads to CD8+ T-cell loss and immune desertification in the mouse, while forced *ccl5* expression prevented loss of *cxcl9* and TILs, and leads to attenuated tumor growth. Accordingly, CCL5-CXCL9 co-expression revealed immunoreactive tumors with prolonged survival and response to checkpoint blockade. Our studies provide mechanistic insights explaining how the genetic makeup of ovarian cancer can shape its own local T cell immunity and reveal immunogenicity programs which render ovarian carcinomas vulnerable to immune checkpoint blockade. The impact of DNA repair pathways, homologous recombination deficiency and BRCA loss on the establishment of immunoreactive tumors will be also discussed.

Embryology meets Oncology. The role of LIF in cancer

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LIF is a cytokine with a crucial role in embryogenesis and embryo implantation in the uterus. Previous work has identified LIF as an oncogenic factor that regulates cancer-initiating cells, cancer-associated fibroblasts and chemo- and radioresistance. We have observed that in tumors expressing high levels of LIF, the blockade of LIF inhibited tumor growth and caused tumor regression through the engagement of an immune response. The anti-tumor effect of LIF neutralization was mediated by the tumor-associated macrophages through the regulation of a set of cytokines that impacted on the T cell response. The LIF-mediated immune suppression was observed in several tumor types with an important incidence in glioblastoma. Our results identify LIF as a critical cytokine in the immune response to cancer and a promising therapeutic target.

PROFFERED PAPER 3

Formation and function of CD49a+ and CD49b+ CD8 T cells in a murine breast cancer model

Marit Melssen[2,1], Robin Lindsay[2], Anthony Rodriguez[2], Salwador Cyranowski[2], Cornelis Melief[1], Craig Slingluff Jr[2], Victor Engelhard[2]
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Integrins CD49a and CD49b can mediate retention of lymphocytes in peripheral tissues by binding to collagens IV and I, respectively. Their expression is upregulated on CD8+ tumor infiltrating lymphocytes (TIL) compared to circulating lymphocytes and the presence of CD49a+ TIL improves patient outcome. Little is known about how expression of these integrins is regulated and the functional capacity of integrin-expressing cells. We hypothesized that CD49a expression identifies more functional T cells in the tumor microenvironment (TME) and expression is induced on effectors and/or early memory cells. To address these hypotheses, CD8 TIL from an implantable breast carcinoma model were evaluated at day (d) 14 and d23 for expression of CD49a, CD49b and functional markers, and tumors were assessed for collagen expression. In early stage tumors (d14), T cells were predominantly CD49b single positive (SP) or CD49aCD49b double positive (DP). Later (d23), CD49b SP cells largely disappeared and CD49a SP cells appeared, while DP cells remained unchanged. After treatment with FTY720, to block further T cell infiltration, the switch from CD49b SP to CD49a SP cells was even more pronounced, suggesting the change is not due to newly infiltrating CD49a SP cells. Interestingly, regardless of timepoint, CD49b SP cells were antigen responsive, whereas DP and CD49a SP cells had features of exhaustion. These data suggest that, contrary to our hypothesis, during tumor progression, CD8 TIL gain CD49a and lose CD49b as they become dysfunctional. Preliminary data showed that expression of collagen I decreased over time, suggesting changes in ligand availability may play a role in the decrease of antigen-responsive CD49b+ TIL. Taken together, the data suggest that CD49b+ cells may be the most crucial anti-tumor TIL in stroma-dense tumors such as breast cancer. Future experiments will assess whether collagens in the TME are crucial for support of CD49b+ CD8 TIL.

T cell evolution in non-small cell lung cancer

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Persistent antigen exposure has a detrimental effect on immune function, associated with CD4 differentiation skewing ($CD4^{DS}$) resulting from decline of early progenitors and gain in abundance of later differentiated, dysfunctional subsets. This process has been developed in the context of chronic vital infections but not been studied in the context of the tumour evolutionary process and microenvironment. Combining high dimensional flow cytometry, exome, bulk and single T cell RNA sequencing from patients within independent cohorts of early non-small cell lung cancer, we found $CD4^{DS}$ to occur amongst intra-tumour T cells and to associate with tumour mutational burden, regulatory T cell infiltration and worse outcomes.

Immunotherapy of Cancer: Triumphs and Challenges and the Impact of Immunosenescence

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It is “common knowledge” that the human immune system deteriorates over time, resulting in an increased frequency of and susceptibility to infectious disease, autoimmunity and cancer, and in poorer responses to vaccines. However, although there is a considerable weight of evidence supporting the idea that the elderly may deal poorly with novel infectious disease (i.e. with neoantigens), evidence for the other claims is surprisingly poor in humans. However, there is a valid concern that anti-cancer immunity may be compromised in the elderly due to i) low amounts of naïve T-cells (potentially leading to holes in the repertoire), ii) “exhaustion” of tumour-specific memory T-cells, and iii) accumulations of suppressive T-cells and myeloid cells. Disentangling the contributions of age *per se* from the result of a lifetime´s exposure to pathogens and other environmental challenges is no simple matter. This presentation will consider the clinically highly relevant question of the impact of immune ageing on responses of patients with solid cancers to immunomodulatory antibody therapy, which has revolutionized medical oncology. It has been regularly assumed and commonly stated, without much actual supporting evidence, that immunity against cancer is compromised in the elderly. However, a growing body of clinical experience, including our own, suggests that older melanoma patients respond to CTLA-4 and PD-1-directed immunotherapy just as well as or better than younger patients. It is also often considered that infection with Cytomegalovirus (CMV), the frequency of which increases with age in industrialized countries, accelerates “immunosenescence”. However, we find that CMV-seropositivity does not compromise immunomodulatory antibody therapy and may actually have a beneficial influence on the outcome. It may thus represent a mechanism by which older patients experience a better clinical course. Recent findings suggest that many tumour-infiltrating lymphocytes are specific for viruses like CMV rather than tumour antigens, potentially explaining some of these results.

PROFFERED PAPER 4

Systemic expansion of pro-metastatic neutrophils is enhanced by MET amplification in breast cancer

Danique Duits[2], Max Wellenstein[2], Martine van Miltenburg[2], Linda Henneman[2], Seth Coffelt[1], Chris Doornebal[2], Renske de Korte-Grimmerink[2], Anne Paulien Drenth[2], Marieke van de Ven[2], Cheei-Sing Hau[2], Kim Vrijland[2], Jos Jonkers[2], Karin de Visser[2]
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Spontaneous amplification of tyrosine kinase receptors such as the HGF receptor c-MET is frequently observed in human breast tumors. Amplification of the MET gene results in an invasive subtype of breast carcinoma with poor prognosis. Similar as in breast cancer patients, we observed spontaneous amplification of MET in breast tumors of *K14Cre;Trp53$^{F/F}$* (KP) and *K14Cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* (KEP) mice. We have previously shown that mammary tumors induce a systemic inflammatory cascade, characterized by neutrophil accumulation, that promotes metastatic disease. However, it is unknown whether and how the genetic make-up of primary breast tumors can influence systemic pro-metastatic inflammation. Therefore, we set out to dissect the impact of breast cancer cell-intrinsic MET amplification on the intratumoral and systemic immune landscape, and how this influences metastasis formation, using the pre-clinical mouse models for breast cancer KP, *K14Cre;Brca1$^{F/F}$;Trp53$^{F/F}$* (KB1P), and *WapCre;Brca1$^{F/F}$;Trp53$^{F/F}$* (WB1P), and their MET-amplified counterparts KP-MET, KB1P-MET and WB1P-MET. We demonstrate that systemic neutrophil levels are increased in MET-amplified tumor-bearing mice. Treatment of KEP and KB1P mice with a c-MET inhibitor strongly reduced systemic neutrophilia. Transcriptomic analysis revealed enrichment of immune-related signaling pathways and genes in KB1P-MET tumors compared to KB1P tumors. In line with these findings, the intratumoral immune cell compartment of MET-amplified tumors showed to be different from MET-normal tumors. Additionally, substantial changes were found in serum cytokine levels when comparing MET-normal and MET-amplified breast tumor-bearing mice. We are currently validating the role of MET-induced candidate genes and cytokines in the tumor microenvironment and whether the immune-regulatory effects of MET contribute to the metastatic potential of MET-amplified breast tumors. Together, these findings indicate that MET plays a profound role in the intratumoral crosstalk between cancer and immune cells leading to enhanced activation of systemic neutrophil expansion.

mRNA therapeutics: ex-vivo and in-vivo modification of antigen presenting cells

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Modification of dendritic cells (DC) with mRNA allows their loading with tumor antigens and their functional programming. To reprogram immature DC towards potent antigen (Ag) presenting cells, we designed a set of molecules that mimic closely activation of these cells during the initiation of an adaptive immune response. We provide 3 molecular adjuvants: mRNA coding for a constitutive active variant of TLR4, mimicking TLR-4 activation; CD40L mRNA, mimicking the 'licensing' of DCs when Th cells and DCs interact and CD70 to provide an extra stimulus for the priming of CD8+ T cells and inducing their proliferation and survival. The mixture of these three mRNA's is referred to as 'TriMix'.

mRNA encoding the full-length tumor specific or associated antigens is used to direct the immune system to the desired targets. To enhance a broad immune response and provide help to the CTLs, we ensure HLA-class-I and class-II presentation by modifying the antigen sequence by adding a DC-LAMP-derived lysosomal targeting sequence.

Dendritic cells modified *ex vivo* have been used in numerous clinical trials. The results of the investigator initiated studies performed in Brussels for the treatment of melanoma will be presented. But mRNA can also be used directly, i.e. by injection of naked mRNA *in vivo* to reprogram and load the professional antigen presenting cells in lymph nodes or at the tumor site. The latter approach is now being implemented in clinical studies.

Biological and clinical relevance of tumor mutations

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Somatic mutations are the driving force of cancer genome evolution. The rate of somatic mutations appears to be greatly variable across the genome due to variations in chromatin organization, DNA accessibility and replication timing. In addition, other variables that influence the mutation rate in a local scale are starting to emerge. I will discuss recent findings from our lab on how DNA-binding proteins, nucleosomes and differences in exons and introns influence mutation rate. These findings have important implications for our understanding of mutational and DNA repair processes, genome evolution and in the identification of cancer driver mutations.

Given the evolutionary principles of cancer, one effective way to identify genomic elements involved in cancer is by tracing the signals left by the positive selection of driver mutations across tumours. We analyze thousands of tumor genomes to identify driver mutations in coding and non-coding regions of the genome. The analysis of tumor cohorts provide valuable information to improve the interpretation of individual variants detected in newly sequenced tumors in clinical or research settings. We have developed CancerGenomeInterpreter.org, a tool designed to identify driver mutations and biomarkers of drug response in individual tumors.

Computing and probing immunity in colorectal cancer

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Colorectal cancer (CRC), a cancer with 1.4 million new cases diagnosed annually worldwide, is refractory to immunotherapy (with the exception of a minority of tumors with microsatellite instability). This is somehow paradoxical as CRC is a cancer for which we have shown that it is under immunological control and that tumor infiltrating lymphocytes represent a strong independent predictor of survival. Based on our previous work showing that the immunophenotypes are determined by the genotypes, we hypothesized that mutations are rewiring signaling pathways and thereby modulate the recognition of tumor cells by T cells.

In order to investigate rewiring of signaling networks and their interference with immunity for individual patients, we developed an experimental-computational framework using perturbation experiments with patient-derived tumor organoids and comprehensive multidimensional molecular and cellular profiling. A biobank of CRC organoids was generated from histologically verified tumor samples, normal tissue, and liver metastases obtained from CRC patients (n=22). Comprehensive characterization of the organoids (exome sequencing, RNA sequencing and proteomics) and of the tumors (multiplexed immunofluorescence for 6 immune cell types) was carried out and the resulting data used to prioritize perturbation experiments. Organoids were then perturbed with kinase inhibitors (MEKi, PI3Ki, mTORi, TBK1i, IKKi, BRAFi, and TAKi) and large-scale phosphoproteomic profiling using data-independent acquisition (SWATH-MS) was carried out. Integration of independent datasets of mutations, transcriptional changes, and phosphoproteomics activities revealed patient-specific signaling rewiring and interference with actionable pathways, suggesting possible pharmacological modulation by approved targeted agents to induce immunogenic effects.

We show for the first time that systematic and comprehensive analysis of the signaling rewiring can provide a mechanistic rationale for immunotherapy-based combination regimens in CRC. This work is an important step towards the development of a precision immuno-oncology platform that integrates tumor organoids with high-throughput and high-content data, and machine learning for making therapeutic recommendations for individual patients.

PROFFERED PAPER 5

Detection of neo-antigen-specific T cell responses in low mutation burden colorectal cancers for personalized therapies

Jitske van den Bulk[3], Dina Ruano[3], Marieke E. IJsselsteijn[3], Natasja L. de Vries[3], Marten Visser[3], Ruud van der Breggen[3], Koen Peeters[3], Thomas Duhen[1], Rebekka Duhen[2], Andrew D. Weinberg[1,2], Sjoerd H. van der Burg[3], Els M.E. Verdegaal[3], Noel F. de Miranda[3]
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Innovative treatment options are required to improve cure rates in advanced colorectal cancer patients. The efficacy of immune checkpoint blockade therapy (anti-PD-1) is restricted to a minority of colorectal cancers with high mutation burden (mismatch repair-deficient). No immunotherapeutic strategies are currently available for patients diagnosed with low mutation burden colorectal cancer. We hypothesized that if neo-antigen-specific T cells would be present in these patients, the latter could benefit from immunotherapeutic interventions that stimulate neo-antigen recognition and the triggering of a robust anti-tumour immune response.

Whole exome and RNA next-generation sequencing were performed in cancer and healthy tissues from five colorectal cancer patients with low mutation burden. Corresponding neo-epitopes were synthesized and tested for their ability to induce immune cell activation in T cells isolated from the tumour tissues (TIL) and from peripheral blood. Neo-antigen-specific T cell responses were identified in patients that presented with tumours carrying 20 to 55 transcribed, non-synonymous variants. Furthermore, we demonstrate that neo-antigen-specific T cells can be discriminated by specific immunophenotypes that include CD103 and CD39 expression. Single-cell and imaging CyTOF analysis confirms the presence of these phenotypes in colorectal cancers with low mutation burden, albeit in lower numbers when compared to mismatch repair-deficient cancers.

In conclusion, we developed a neo-antigen screening pipeline to unlock the immunogenic potential of colorectal cancers with low mutation burden. We have detected a relatively high number of neo-antigens that are recognized by T cells in mismatch repair proficient, low mutation burden colorectal cancer patients, and show the relevance of focussing on specific cell subsets for neo-antigen-based immunotherapies. These findings warrant further exploration of the potential to employ neo-antigen-targeted therapies to improve clinical outcomes of colorectal cancer patients.

Novel patient-derived tumor models for immunotherapy development

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One of the main limitations in evaluating and developing new immunotherapies is the absence of suitable animal models in which human immune responses against cancer can be interrogated. Patient-derived tumor xenograft (PDX) models are considered the most suitable to reproduce the complexity of patients' tumor microenvironments (TME), but these models lack a human immune system. In this regard, PDX models do not reflect the dynamic process of tumor-immune surveillance and immune-mediated editing, limiting to some extent the translation of anticancer results. To overcome these important limitations, there are different approaches to enrich a PDX model with human immune cells: co-engrafting patient-derived peripheral blood mononuclear cells (immune-avatar PDX), co-engrafting hematopoietic stem progenitor cells (humanized PDX) or using the tumor infiltrating leukocytes contained in the patient derived tumor xenograft in situ or after ex-vivo expansion (immune-PDX).Overall, these models have different strengths and weaknesses that are important to know for obtaining preclinical results that are meaningful for clinical translation.

KEYNOTE LECTURE

The unsuspected role of gut microbiota in cancer therapies

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We recently highlighted the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer. Chemotherapeutic agents facilitate the gut permeability and selective translocation of Gram positive bacteria in secondary lymphoid organs. There, anti-commensal pathogenic TH17 T cell responses are primed, facilitating the accumulation of TH1 helper T cells in tumor beds post-chemotherapy as well as tumor regression (Viaud S, *Science* 2013). These findings have been extended to platinum salts as well as to a combination of anti-IL-10R mAb+CpG (Iida et al. Science Nov 2013). The immune checkpoint blockers (ICB) anti-CTLA4 Ab and anti-PD1/PD-L1 Ab are first-in class compounds approved for reinstating cancer immunosurveillance and prolonging survival in metastatic patients. Zitvogel's group showed that antibiotics compromise the efficacy of these ICB in mice and patients and unveiled the immunomodulatory role of distinct commensals from the intestinal ecosystem in mobilizing anticancer immunosurveillance. Vétizou et al. showed that the antitumor effects of CTLA4 blockade, largely dependent upon Toll like receptor (TLR)2/TLR4 receptors, markedly rely on the regulatory commensal *Bacteroides fragilis* (in coordination with *Burkholderia cenocepacia, Science Nov 2015*). Next, the demonstration of the deleterious role of antibiotics in the clinical efficacy of PD-1 blockade in lung, kidney and bladder cancer patients was brought up, highlighting the role of *Akkermansia muciniphila* as the main player in the immunomodulatory effects of pembrolizumab or nivolumab (Routy et al. Science 2017 Nov2). The mechanisms by which *A. muciniphila* restores gut dysbiosis will be discussed, involving CCR9 and IL-12. From these findings, we infer that oncomicrobiotics and/or fecal microbial transplantation could be considered as adjuvants to the current oncological armamentarium in dysbiotic cancer bearers.

Poster abstracts

1 - Poster Spotlight

Microfluidics and oncoimmunology: new *in vitro* models to study solid tumor immunotherapy

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Immunotherapies against solid tumors face daunting challenges compared with hematological cancers. In solid tumors, immune cells and antibodies need to extravasate from the vasculature; find the tumor cells; and migrate through a dense mass of normal and tumor cells where nutrients are depleted, and waste products build up. All these factors pose significant obstacles for solid tumor immunotherapy, commonly leading to immune exhaustion and compromising the immune response. Thus, finding effective immunotherapies against solid tumors require *in vitro* models that accurately mimic the solid tumor microenvironment. In this work, we show how microfluidic models mimic the tumor architecture in an unprecedented way compared with other traditional *in vitro* models based on Petri dishes. Breast cancer cells were cultured as a dense mass and embedded in a 3D collagen hydrogel in a microfluidic device. Endothelial cells were cultured in lateral flanking lumens to mimic the blood vessels, allowing the perfusion of therapeutic antibodies or effector immune cells (e.g., natural killer cells). Antibody and immune cell extravasation, diffusion, migration and tumor clearance were evaluated in the model. The results showed that a subset of the cancer cells in the solid tumor model were able to internalize the antibodies in intracellular vesicles in order to remain invisible to the immune surveillance. On the other hand, natural killer cells were able to detect the presence of the tumor several hundreds of microns away, exhibiting a directional migration towards the tumor. Once inside the spheroid, real-time microscopy revealed natural killer cells were able to destroy tumor cells at the tumor periphery and, more importantly, also at the innermost layers. Finally, the combination of antibodies and natural killer cells led to an enhanced cytotoxicity, showing the potential of these models to evaluate new immunotherapy combinations.

2 - Poster Spotlight

Dual immune checkpoint blockade synergizes with cisplatin in mouse models of spontaneous breast cancer development and metastasis

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Despite the success of immune checkpoint blockade (ICB) for some cancer patients, objective responses in breast cancer patients have been limited to 5-20%. The current challenge is to understand why these patients fail to respond to immunotherapy. An urgent question to address is whether response and immune evasion mechanisms to cancer immunotherapy strategies are differently regulated in primary breast cancer and metastases. In this study, we aim to elucidate the *in vivo* sensitivity of breast cancer metastases versus primary tumors to a clinically relevant ICB strategy, namely combined anti-PD-1 and anti-CTLA-4 antibody therapy. We make use of the *K14-cre;Ecad$^{F/F}$;p53$^{F/F}$* (KEP) conditional mouse model for spontaneous breast tumorigenesis, and a KEP-based metastasis model in which spontaneous multi-organ metastases arise after primary tumor resection. Whereas primary KEP tumors do not respond to dual ICB, an anti-tumor CD8$^+$ T-cell response could be unleashed when dual ICB was combined with cisplatin, resulting in improved survival. The synergistic effect of cisplatin and dual ICB was characterized by increased numbers of tumor-infiltrating T cells and enhanced IFN-gamma production by CD8$^+$ T cells. We are currently translating these findings to the metastatic cancer setting. In addition, we are monitoring the changes in the immune landscape induced by this treatment strategy, comparing the immune evasion mechanisms and response to therapy between the primary tumor and metastatic nodules in different anatomical locations.

3 - Poster Spotlight

Formation of a fibrotic pre-metastatic niche is mediated by systemic Activin A during pulmonary metastasis of breast cancer

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Advanced metastatic cancers are mostly incurable and available therapies can only prolong life to a limited extent. It is increasingly appreciated that the metastatic microenvironment is crucial in supporting metastases formation. Previous studies demonstrated that changes in the composition and finction of the ECM play a major role in shaping the microenvironment at the metastatic site. Since fibroblasts are central modulators of ECM composition, we set out to study the role of fibroblasts in facilitating pulmonary metastases of breast cancer. Utilizing multiple mouse models of breast cancer metastasis, we show that enhanced collagen deposition is a relatively early event during the formation of the pre-metastatic niche in lungs, which precedes metastases formation. Moreover, we found that tumor-derived secreted factors induce fibroblast activation and upregulation of pro-fibrogenic signaling in fibroblasts. Proteomic analysis of serum from normal and tumor-bearing mice revealed significant changes between the two groups. One of the proteins that were highly upregulated in the serum of tumor bearing mice was Activin A (ActA), known to enhance the expression of pro-fibrogenic genes. We demonstrated that ActA was sufficient to activate normal lung fibroblasts, and its inhibition attenuated this pro-fibrogenic activation. Moreover, we show that systemic ActA levels are gradually upregulated in the circulation during tumor progression in two different mouse models of breast cancer. Importantly, the expression of ActA is upregulated in human breast cancer and in multiple other human cancers. Our findings suggest a functional role for ActA in driving collagen deposition in the lungs and in facilitating lung metastatic relapse in breast cancer.

4 - Poster Spotlight

Non-autonomous induction of endothelial Icosl may underpin immune-mediated senescence surveillance

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Oncogene-induced senescence (OIS) is a tumour suppressor mechanism, with profound effects on the tumour microenvironment (TME) through the SASP (senescence-associated secretory phenotype). The SASP drives T-lymphocyte dependent clearance of senescent cells; failure of this senescence surveillance leads to tumourigenesis. We hypothesise that OIS cells will modulate endothelial behaviour, controlling immune recruitment and behaviour in the TME.

Using transcriptomics in human liver sinusoidal endothelial cells (LSECs) incubated in control or OIS-conditioned media, we find SASP-induced activation of endothelial NF-kB signalling, with upregulation of cytokines, chemokines and the immune co-stimulatory ligand *ICOSLG*. Inhibition of NF-kB prevents the OIS-dependent increase in both gene expression and lymphocyte adherence in the endothelium. We validated OIS-induced NF-kB-dependent upregulation of *ICOSLG* in multiple endothelial cell types.

We utilise hydrodynamic tail-vein (HDTV) injected $NRAS^{G12V}$-containing transposons generating murine hepatocyte OIS, before analysis of LSEC behaviour. Hepatocyte OIS drives the upregulation of NF-kB-dependent genes in LSECs, including *Cxcl1* and *Icosl*, demonstrating OIS hepatocyte to endothelial cell signalling *in vivo.* OIS hepatocytes themselves demonstrate autonomous upregulation of chemokines, but not *Icosl*. Use of Icosl-blocking antibodies after induction of hepatocyte OIS completely abrogates the time-dependent immune-mediated senescence surveillance. Mass-cytometry based deep intrahepatic immunophenotyping showed that OIS hepatocytes drive an intrahepatic enrichment of activated Icos+CD4+ T-lymphocytes and CD11b+Ly6G+ granulocytes, that is lost with Icosl blockade, suggesting that induced endothelial behaviours control immunocyte recruitment to the TME. We are currently confirming the endothelial dependence of Icosl signaling in senescence surveillance.

In conclusion, we propose that senescence drives non-autonomous induction of NFkB-dependent *Icosl* expression in endothelial cells. Induced endothelial *Icosl* has profound effects upon immune-cell recruitment and may control immune-mediated senescence surveillance.

5 - Poster Spotlight

IL-35^{+} B cells establish immunosuppressive network in pancreatic ductal adenocarcinoma

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Despite advances in our understanding of the mutational landscape in pancreatic ductal adenocarcinoma (PDAC), this devastating disease is now the third-leading cause of U.S. cancer-related deaths. While recent successes of cancer immunotherapy have generated considerable excitement, this form of treatment has been largely ineffective in patients with pancreatic cancer. A major barrier for immunotherapeutic approaches is marked immunosuppression within the PDAC milieu. We have previously identified a novel role for IL-35 producing B cells in the pathogenesis of pancreatic cancer. However, little is known about the mechanisms behind IL-35 activity in cancer. Here, we set out to elucidate molecular and cellular mechanisms by which IL-35 facilitates the emergence of pancreatic cancer. Our results demonstrate that IL-35, but not IL-10, potentiates PDAC growth. This correlates with induction of regulatory T cells and suppression of effector T cell activity, suggesting that IL-35 controls endogenous anti-tumor immune responses in PDAC. Furthermore, while IL-35 is expressed by several immune cell types in PDAC, we show that its expression specifically in B cells is essential for suppression of anti-tumor T cell responses. Importantly, while PDAC is typically resistant to anti-PD-1 immunotherapy, we demonstrate robust synergistic reduction in tumor growth when IL-35 deficiency is combined with anti-PD-1 treatment. Insights gleaned from these and further mechanistic studies of IL-35 in PDAC may be expeditiously translated into IL-35 targeted combination immunotherapy.

6 - Poster Spotlight

Mass spectrometry-based phosphoproteomics to uncover PD-1/PD-L1 signaling

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Antibodies, which block the interaction of the co-inhibitory receptors PD-1 and PD-L1, have achieved notable clinical success in various cancer types. However, detailed molecular knowledge about PD-1/PD-L1 signaling in response to immune checkpoint inhibitors is scarce.

In order to advance our understanding of these molecular events, we established a mass spectrometry-based phosphoproteomic workflow to study time-resolved consequences of PD-1/PD-L1 interaction in both immune checkpoint-presenting cells in parallel. In order to distinguish signaling in the two model cell lines - Jurkat$^{PD\text{-}1+}$ and Raji$^{PD\text{-}L1+}$ cells - following co-culture, we used stable isotope labeling by amino acids in cell culture (SILAC) such that one cell was labeled with heavy Lys and Arg and the other with the corresponding light amino acid. After lysis and tryptic digestion, phosphopeptide enrichment was performed by both Fe^{3+}-IMAC and phosphotyrosine-immunoprecipitation (pY-IP). Multiplexing of the different time points of co-culture (0, 2, 10, 60, 120 min) was achieved by labeling digested peptides by tandem mass tags (TMT). LC-MS^3 analysis was performed on an Orbitrap Fusion Lumos followed by phosphopeptide identification and quantification using MaxQuant.

With the above combined SILAC-TMT labeling approach, we can measure phosphorylation-mediated signaling PD-1- and PD-L1- in both cell types side-by-side and in a time-resolved manner. We quantified the levels of 14,000 phosphorylated peptides including 1,000 pY sites. Preliminary analysis of this comprehensive data set revealed an extensive perturbation of cellular signaling downstream of PD-1 and PD-L1 upon interaction illuminating the large uncharted parts of the PD-1/PD-L1 signaling network. The established phosphoproteomic workflow is generic and can be utilized on further cellular systems.

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Oral intake of natural immunostimulants suppresses the 7,12-DMBA/ Croton oil induced two-step skin carcinogenesis in Swiss albino mice

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The immune system is critical in fighting cancer, so, is it possible that, the natural stimulation of this system can slow down the evolution of a cancer? The aim of this study was to evaluate the *in vivo* preventive combined effect of five types of immunostimulants which are the Beta-glucan and Arabinogalactan as polysaccarides and three fungi extracts (Reishi, Maitake and Shiitake) on 7,12-Dimethylbenz[a]anthracene (DMBA)/Croton oil induced two-step skin carcinogenesis in Swiss albino mice. We used a biochemical techniques to determinate the variations of the oxidative stress enzymatic activity (CAT & SOD) which are involved in the fight against the majority of cancers development.

The cutaneous application of the DMBA/Croton oil caused a precancerous hyperplasia in squamous cells also called papilloma which is translated by the creation of a cutaneous tumor on the back of mice. Tumor developpement was accompanied by a decrease in the antioxidant system activity.

Treatment with the indicated immunostimulants significantly decreased the incidence of tumors and also the level of CAT activity, however, total proteins levels, and SOD activity were increased with a clear enhancement of immune system activity. This suggests that the immunostimulants used in our study can have an effective prophylactic effect against skin carcinogenesis via the enhancing of the global function of the immune system.

Key words: Immunostimulants, (DMBA)/croton oil, Oxidative stress, papilloma.

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New small-molecule immune checkpoint inhibitors: A new strategy in cancer immunotherapy

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Immunotherapy is currently a powerful strategy in cancer therapy. In particular, modulation of immune checkpoint receptors have gain special attention. These immune regulators limit proliferation and activity of T cells and other immune cells enrolled in these signaling pathways. Under normal conditions, they are essential in modulation of immune responses; however, they are also one of the major mechanisms used by tumors to evade immune system recognition and destruction. To date, several immune checkpoint receptors have been identified and used as therapeutics in oncology, as programmed cell death protein 1 (PD-1). When engaged by one of its ligands (PD ligand 1 (PD-L1) and PD ligand 2) PD-1 limits autoimmunity. PD-1 ligands are upregulated in many human cancers and their blockade could lead to activation of T cells and therefore enforce tumor recognition. In fact, PD-1/PD-L1 pathway is one of the most successful pathways in the context of clinical cancer immunotherapy with several approved drugs. These successful therapies rely on the use of antibodies. However, despite their outstanding success, they still have numerous disadvantages as severe immune-related adverse events.

Recently, small-molecule modulators have emerged as safer therapeutic alternative. However, limited efforts have been directed toward immune checkpoint receptors. Our study is focus on the discovery of small-molecule inhibitors targeting PD-L1 in order to block PD-1/PD-L1 interaction and therefore overcome antibody therapy disadvantages. Limited structural information of PD-L1 led us to a detailed structural characterization based on *in silico* studies. After assessing structural features and following a computer assisted drug discovery approach we accomplished a structure based virtual screening campaign. Potential PD-L1 inhibitors were selected and their activity have been tested by fluorescence based assay. We were able to identify new small-molecule PD-L1 inhibitors that are currently being tested *in vitro* in several cell lines. Therefore, immune checkpoint blockade using small molecules represent a step forward in cancer immunotherapy.

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Anti-tumoral targeting of granulysin-containing immunotoxins*

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Granulysin is a protein present in the granules of human CTLs and NK cells, with cytolytic activity against microbes and tumors. Previous work from our group demonstrated the *in vivo* antitumoral activity of intratumoral injection of recombinant granulysin in two animal models of tumor development, breast adenocarcinoma and multiple myeloma. In the present work we have developed two types of immunotoxins containing granulysin, one targeting the carcinoembryonic antigen (CEA), or two others targeting the Tn antigen, present in tumor-associated mucin-1 (MUC-1), respectively. The specific binding of the immunotoxins to their specific antigen, purified or expressed on the surface of cells, was analyzed using ELISA, surface plasmon resonance, flow cytometry or fluorescence microscopy. We then proved that the *in vitro* cytotoxicity of the immunotoxins against several cell lines positive for the expression of the targeted antigens was higher than that observed for granulysin alone. Next, granulysin and the anti-CEA immunotoxin were tested as a treatment in *in vivo* models of tumor development, based on subcutaneous injection of colon carcinoma HT-29 cells or HeLa-CEA transfected cells in immunodeficient athymic mice. In the HT-29 model, injections were performed intratumorally, and in the HeLa-CEA model, injections were performed systemically (intraperitoneal). After intra-tumor injection, both granulysin and the anti-CEA immunotoxin were able to inhibit HT-29 tumoral growth, confirming the *in vivo* bioactivity of the immunotoxin. Finally, the anti-CEA immunotoxin demonstrated *in vivo* targeting of granulysin against tumors derived from HeLa-CEA cells after systemic administration, while granulysin alone had no targeting effect. This is the proof of concept for the use of granulysin-containing immunotoxins for systemic cancer treatment.

*A patent application protecting the intelectual property of immunotoxins containing granulysin has been filed to the Spanish Patent Bureau (OEPM application # P201830768; 07/26/2018)

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Transcriptomics changes in the tumor microenvironment in patients with metastatic solid tumors undergoing check-point inhibition

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Background: Check-point inhibition has become a valuable therapeutic tool, however, a considerable fraction of patients does not experience benefit. Therefore, exploring tumor microenvironment is crucial for understanding the different response patterns.

Aim: Explore transcriptomics changes of tumor microenvironment that correlate with response to check-point inhibition.

Material and methods: 50 patients with different metastatic solid tumors receiving check-point inhibition targeting PD-1 – PDL-1 axis were included in this study. Fresh tumor biopsies were taken prior and 6 weeks after treatment initiation. RNA was analysed by GeneChip® Human Genome U133 Plus 2.0 Array. Response was assessed by RECIST criteria 1.1 and analysis of gene expression carried out with Qlucore Ominics Explore version 3.2.

Results: 18 patients responded to the treatment (CR=3, PR=6 and SD=9). Paired tumor biopsies were obtained approximately 40 days after treatment initiation for 15 patients. Median PFS and OS was 4 months (0.00005) and 7.1 months (0.045), respectively. For baseline samples, 150 genes were differentially expressed (DEG; FC>2, p< 0.01). Upregulated genes (n=102) were involved in chemoattraction/adhesion of T-cells (*VCAM, CXCL9, CXCL10*), antigen presentation (*TNFSF4*), T cell proliferation, cytotoxic activity and maintenance of immune response (*IL2RB, ITK, GZMA, CD27*). Enrichment analysis showed upregulation of pathways involved with adaptive immune response, cell adhesion and activation of lymphocytes.

Transcriptomic changes in paired samples comprised 78 DEG, mainly involved in cell adhesion pathways. Interestingly, tumors of patients responsive to treatment showed down-regulation of VEGF-A (tumor cell migration and invasiveness). The VEGF-VEGF-R axis has equally been studied as a chemoattractant for T regs and suppressor of activated T cells, creating a microenvironment where immune evasion is facilitated.

Conclusion: Our results show that transcriptomics changes both prior to treatment start and under therapy can give valuable information about clinical outcome.

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Identification of novel mechanisms of resistance against immunotherapies

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Immunotherapy changed the paradigm of cancer care in recent years, and became the standard of care for many cancer types. However, the mechanisms of resistance to these therapies are not explored yet. Therefore, there is a need to anticipate the mechanisms of response and resistance to these promising immunotherapies. HER2 (also known as ErbB2 or Neu) is a receptor tyrosine kinase overexpressed in ~20% of breast and gastric cancers. One of the most successful therapies against HER2 positive tumors, trastuzumab, is a monoclonal antibody originally designed to inhibit HER2, which has been shown to be effective because not only impairs cell cycle but also promotes an immune reaction. Chimeric Antigen Receptors (CARs) or T Cell Bispecific antibodies (TCBs) are two different strategies designed to boost the immune response against tumors, as they direct cytotoxic T cells against the tumor cells. TCBs are engineered molecules that include, within a single entity, binding sites to the T cell receptor and to a tumor-specific or a tumor-associated antigen, while CARs consist of the antigen-binding domain of an antibody fused to the intracellular signaling motifs that activate T cells. Clinical evidence shows that either TCBs or CARs are effective immunotherapies to treat cancer. In this project, we performed two approaches to obtain a resistance model to an HER2-TCB. The first one is based on a co-culture *in vitro* model of $HER2^+$ cancer cells with lymphocytes, and the second, an $HER2^+$ Patient Derived Xenograft (PDX) *in vivo* model with a reconstituted human immune system (termed as humanized mice). With this novel resistance model, we aim to identify novel immune modulators of resistance to immune therapies, with potential clinical applications, by next generation sequencing techniques. In addition, we aim to further validate our findings with other immune therapies. In conclusion, we generated an "immuno resistance" model that will give new insights to understand more the immunotherapies, and with potential clinical applications.

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Identification of Novel Receptors Tyrosine Kinases (RTKs) Regulating the Hippo Signaling Pathway in Tumorigenesis

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Several studies have shown that the Hippo pathway plays an important role in tissue growth, organ size, and cell death. Deregulation of the Hippo pathway contributes to loss of cell contact inhibition and continuing cell proliferation, which is observed during tumorigenesis. Although many studies have been done to clarify the role of the Hippo pathway in organ size control, cell proliferation and tumorigenesis, currently the connection between the Hippo pathway and its potential upstream kinase regulators is not very clear. Since the overexpression and deregulation of receptor protein kinases (RTKs) have a pivotal role in many cancers, we hypothesize that some receptor tyrosine kinases may be involved in tumorigenesis by inhibiting the Hippo pathway. To test the hypothesis, we developed a biosensor based on split-luciferase complementation assay[1]. To find new RTKs which regulate the Hippo pathway, gain of function and kinase-inhibitor screenings were performed. The screenings revealed several novel RTKs that regulate the Hippo pathway such as FGFR and VEGFR. Remarkably, we found three new tyrosine-phosphorylation sites on YAP, which regulate its function and stability. Our studies indicate that some RTKs also regulate the Hippo pathway through interaction or phosphorylation of LATS, YAP, and TEAD. In conclusion, these findings highlight the pivotal role of the Hippo pathway in mediating RTK-MAPK/PI3K signalling and provide a compelling rationale for targeting YAP/TAZ in RTK-driven cancer therapies.

1) Azad, T. et al. A LATS biosensor screen identifies VEGFR as a regulator of the Hippo pathway in angiogenesis. *Nature communications 9*, 1061 (2018).

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Generation of a new human/mouse cross-reactive anti-PD-L1 chimeric antigen receptor for preclinical studies

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The adoptive immunotherapy of cancer has emerged in recent years as a promising strategy for anticancer modality. A special attention is paid to the developing and application a technology of chimeric antigen receptors (CAR). Another highly interesting immunotherapeutic approach is targeting negative immune checkpoints, such as PD-L1:PD-1 axis. We combine these two approaches in our project.

In our study, we have been investigating CAR-based strategy to target the effector cells against the PD-L1 molecule. We have designed a new second generation CAR targeted against human PD-L1 molecule via a specific scFv fragment. Our anti-PD-L1-CAR molecule consists of two functional domains derived from natural molecules expressed in a T cell (CD28 and CD3ζ), the antigen-recognition domain derived from anti-PD-L1 monoclonal antibody that is linked with the remaining portion of the CAR molecule via IgG4-derived hinge linker structure.

Subsequently, we have shown that primary NK cells can be effectively modified by mRNA electroporation method with the new anti-PD-L1-CAR. We have noticed that NK cells with expression of anti-PD-L1-CAR on their surface are able to recognize and effectively kill PD-L1 harboring human (MDA-MB-231, RAJI-PD-L1) as well as murine (P815) cell lines. Moreover, we have observed that anti-PD-L1-CAR-bearing primary NK cells, undergone potent degranulation after co-incubation with both human and murine target cells harboring PD-L1 molecule on the surface. Using the new anti-PD-L1-CAR-bearing effector cells, recognizing human and mouse cells expressing PD-L1 molecule, might be a useful tool for testing anti-PD-L1-CAR effectiveness and cytotoxicity in in vivo studies, as it is planned in our future investigations.

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Investigation of NK-cell mediated induction of ADCC in HNSCC, with focus on the potential role of HPV-status and cetuximab resistance

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Introduction: The epidermal growth factor receptor (EGFR)-targeted therapies and immunotherapies are now at the forefront for treatment of head and neck squamous cell carcinoma (HNSCC). Besides their direct cytotoxic effect, anti-EGFR monoclonal antibodies (mAbs) can also induce antibody dependent cellular cytotoxicity (ADCC). The aim of this study was to investigate the role of cetuximab resistance and human papillomavirus (HPV)-status on the induction of ADCC. The effect of cetuximab treatment on the expression of immune checkpoint ligands in HNSCC tumor cells is being investigated as well.

Material and methods: Ten HNSCC cell lines, comprising cetuximab sensitive as well as intrinsically and acquired resistant cell lines, differing in HPV-status, were used. A co-culture of healthy donor NK-cells together with tumor cells was created with/without cetuximab and ADCC was monitored in real-time for 48h (xCELLigence). The experiments were performed under normoxia and hypoxia (1% O_2). To examine the influence of cetuximab on the expression of immune checkpoint ligands, single-stain flow cytometry was used for multiple ligands under both normoxic and hypoxic conditions.

Results: Our data show that, under normoxia, addition of cetuximab to the co-culture of HNSCC and NK cells induced an ADCC response ($p<0.012$), ranging from 42% to 84% cell death compared to baseline NK killing, in 9 out of 10 cell lines. Neither cetuximab resistance ($p=0.398$) nor HPV-status ($p=0.846$) significantly influenced the ADCC response under normal oxygen conditions. Induction of ADCC under hypoxic conditions and the effect of cetuximab on the immune profile are currently being investigated.

Conclusion: This study demonstrates that cetuximab resistance mechanisms do not interfere with the ability of NK cells to induce an ADCC response. Additionally, HPV infection is associated with an improved prognosis in a subset of HNSCC patients, our results show that this benefit is not likely due to a higher sensitivity to NK-cell killing.

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Analytical validation and standardization of tumor mutational burden (TMB) assessment using the Oncomine Tumor Mutation Load Assay across eight clinical research laboratories

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Background*:* TMB assessment through whole exome sequencing is challenging to implement in routine clinical practice. The Oncomine Tumor Mutation Load Assay (Oncomine TML) is a PCR-based NGS approach exploring 409 cancer genes (total 1.2 Mb exonic region) to define the TMB. The Immuno-Oncology Consortia is a European collaborative effort of eight translational clinical laboratories involved in the analytical validation and standardization of Oncomine TML. Phase I of the study assessed the accuracy and precision of the assay on control samples.

Methods*:* Each research laboratory received six FFPE tumor cell-line samples and a synthetic control (AcroMetrix Oncology Hotspot Control (AOHC)). Samples were analysed independently in each laboratory. “Expected TMB” was calculated based on the number of true somatic mutations in public databases that are covered by the panel. Expected and measured TMB values were compared for each sample.

Results*:* As shown in the table, measured TMB was consistently close to expected TMB with less than 7% deviation for samples with expected high TMB > 20 mutations/Mb (HCC2998, AOHC). TMB results obtained from the eight labs showed that in samples with TMB >10 mutations/Mb less than 10% CV was observed; whereas on the rest of the samples, CV ranged from 8-17%.

	AOHC	A549	H2228	HCC2998	MCF7	SKMEL2	T47D
Measured Mut/Mb	255.46	7.01	7.75	198.24	3.89	18.98	2.76
% CV across sites	9%	12%	8%	6%	17%	9%	14%
Expected Mut/Mb	271.67	10	10	196.67	12.5	17.5	4.17

Conclusions*:* Phase I results show robust results across the participant laboratories. TMB measures with Oncomine TML Assay were consistently close to expected TMB. Deviation from expected TMB and variability across sites was higher on low TMB (< 10 mutations/Mb) samples. Clinical FFPE samples will be assessed in the next phases of the study to summarize the overall performance of the assay.

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The role of the tetraspanin protein CD151 in the regulation of the immune microenvironment in inflammatory breast cancer

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Abstract withheld at the author's request

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APC-targeted DNA vaccination induce protective CD8+ T cells against multiple myloma in mice

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Multiple myeloma is a second most common hematologic malignancy with poor prognosis, thus there is a great need for new and better treatments. To this end, we try to establish patient specific vaccines for therapy. As a mouse model for these studies we use MOPC315.BM cell line that homes to the bone marrow and expresses M315 monoclonal immunoglobulin. Variable regions of M315 contain tumor specific antigens, which are weak immunogens. In our cancer vaccine, we have connected variable regions from M315 into a scFv format. To improve its immunogenicity, $scFv^{315}$ has been fused to the MIP1α chemokine that binds to its receptors on antigen presenting cells. This targeted vaccine is called vaccibody, and is employed in a DNA format.

Mice were vaccinated by intramuscular injection of MIP1a-scFv315 followed by electroporation. After 2 weeks mice were challenged with luciferase-labeled MOPC315.BM.Luc cells.

When compared to the control group, vaccinated mice showed higher survival rate, and delayed onset of the disease. The tumor-associated luminescence signal was significantly higher in control compared to vaccinated mice. Surprisingly, the level of M315 in sera, which is considered to be a measure of tumor burden, was undetectable in 60-80% of the vaccinated mice. Still, mice had high levels of lambda light chain, suggesting that lack of M315 was a consequence of Ig heavy chain loss. When mice vaccinated with MIP1a-scFV315 were treated with CD8-depleting antibodies, the protective impact of the vaccine was reverted, and phenomenon of heavy chain loss was no longer observed.

Our results suggest that epitopes for CD8 cells are present on the heavy chain of M315 and that vaccine-induced $CD8^{+}$T cells are protective against multiple myeloma. We are currently trying to identify those neo-epitopes in order to produce vaccines with even better protective properties.

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Myc inhibition by an Omomyc-based therapy induces intratumoral immune cell recruitment in models of NSCLC

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Lung cancer is the leading cause of cancer mortality worldwide. Despite the early promise of immunotherapy, many lung cancer patients with Kras-mutated tumors do not respond to treatment and are still in need of effective therapeutic options. Here, we propose to target a central functional node downstream of Kras: the Myc transcription factor. Clinical Myc inhibition has long been considered unfeasible, but we designed a Myc dominant negative called Omomyc and showed that it exerts extraordinary therapeutic impact in various mouse models of cancer. Until today, Omomyc has been considered a proof of principle, but here we show the therapeutic potential of the purified Omomyc mini-protein to treat KRAS-driven Non-Small-Cell Lung Cancer (NSCLC).

Indeed, intranasal administration of the Omomyc mini-protein reduces tumor burden of mice bearing $Kras^{G12D}$-induced adenocarcinomas, shutting down both MYC-dependent and KRAS-driven oncogenic transcriptional signatures, reducing proliferation and increasing apoptosis of cancer cells. Importantly, Omomyc treatment also causes changes in chemokine and cytokine profiles and induces T cell recruitment to the tumor site as early as one week after treatment onset. In fact, Omomyc increases the proportion of $CD4^+$ T cells, which show expression of PD-1 and Tim-3, suggesting that Omomyc induces the expansion of tumor-reactive $CD4^+$ T cells. Interestingly, mice also display higher proportions of Th17 cells, specifically of Th1-Th17 hybrid population, and of dendritic cells. Moreover, using another NSCLC mouse model bearing both Kras and p53 mutations, we show that Omomyc systemic administration not only halts tumor progression, but also increases tumor infiltration of both CD4 and CD8 $PD\text{-}1^+Tim\text{-}3^+$ T cells.

Our findings indicate that Omomyc treatment represents a new opportunity to pharmacologically inhibit Myc in NSCLC, while inducing an antitumor immune response. Combination of our anti-Myc therapy with immunotherapies could overcome - or potentially prevent - immunotherapeutic resistance, thus changing the paradigm of cancer treatment.

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Oncolysis is the predominant mechanism for the therapeutic effect of LCMV-GP-pseudotyped vesicular stomatitis virus in a mouse lung cancer model

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Oncolytic viruses mediate their anti-cancer therapeutic effects mainly by two mechanisms; direct oncolysis due to tumor-selective viral replication and the simultaneous activation of innate and adaptive immune responses with the potential of long-lasting tumor remissions. The chimeric vesicular stomatitis virus pseudotyped with LCMV glycoprotein (VSV-GP) has been previously reported to have both a rapid lytic cycle and a broad tumor tropism. In this study, we demonstrate its therapeutic potential in the syngeneic lung cancer model LLC1.

In vitro, VSV-GP was found to efficiently infect and lyse LLC1 cancer cell-lines. Exogenously applied interferon type 1, however, conferred resistance to VSV-GP action demonstrating a dependence of the oncolytic effect on defects in the IFN response of cancer cells. Using a matched pair of LLC1 wildtype and interferon receptor knockout tumors (LLC1-IFNAR$^{-/-}$) *in vivo*, interferon insensitivity of cancer cells correlated with prolonged intratumoral viral replication and improved therapeutic outcome. Additionally, bio-luminescent imaging revealed successful tumor-to-tumor spread of viral progeny in bilateral tumor models. VSV-GP therapy was associated with enhanced T cell infiltration and upregulation of various immune-associated genes. Interestingly, the efficacy of VSV-GP therapy in treating LLC1-IFNAR$^{-/-}$ tumors was not diminished by the absence of CD8^{+} T cells and cured mice were not immune to tumor rechallenge indicating a predominant lytic effect. In summary, the treatment effect of VSV-GP in LLC1-IFNAR$^{-/-}$ lung cancer model is primarily lytic with negligible contribution of adaptive anti-tumor immunity despite strong activation of both innate and adaptive immune signatures.

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The effect of clinically relevant chemotherapies and the tumour microenvironment on inhibitory immune checkpoint expression on T-cells and oesophageal adenocarcinoma cells

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This novel study examines the expression of inhibitory immune checkpoints (ICs) in oesophageal adenocarcinoma (OAC) to identify potential ICs that could be targeted, as more than two-thirds of patients don't benefit from chemoradiotherapy (CRT). Importantly the effect of clinically relevant chemotherapies and the effect of the hostile hypoxic nutrient deprived tumour microenvironment (TME) on the expression of ICs is examined to provide insight for rationally incorporating immune checkpoint inhibitors (ICIs) into current standards of neoadjuvant care for OAC patients.

CRT significantly decreases PD-1 expression on peripheral blood and tumour-infiltrating T-cells in OAC patients ($p<0.05$) suggesting that PD-1 inhibitors would not be effective post-CRT in OAC patients as the ICI target is removed, highlighting the importance of careful ICI scheduling with current standards of care.

PD-1, CD160 and TIGIT are basally expressed on OAC cells (OE33 cell line) in vitro ($p<0.05$) which could potentially be targeted in OAC. Single agent 5-fluorouracil, capecitabine, cisplatin and oxaliplatin significantly upregulates PD-L1, PD-L2, TIM-3, LAG-3 and VISTA on live OE33 cells in vitro ($p<0.05$). Single agent cisplatin, oxaliplatin and docetaxel significantly increases PD-L1, PD-L2, CD160, TIGIT, PD-1 and LAG-3 on live activated T-cells in vitro ($p<0.05$). PD-1 expression significantly increases on OE33 cells under severe hypoxic and glucose deprived conditions.

This data demonstrates that chemotherapy directly increases IC expression on OAC cells and T-cells highlighting a link between chemotherapy and immune-resistance, providing a rationale for combining ICIs with chemotherapy regimens in OAC. This data offers a starting point for understanding changes in IC expression which could help guide the selection and timing of ICIs with current standards of care for OAC patients.

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High-dimensional cytometric analysis of colorectal cancer reveals novel and diverse mediators of anti-tumor immunity

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Checkpoint blockade has revived the potential of immunotherapy for cancer treatment. For optimal application and development of cancer immunotherapies, a comprehensive understanding of the anti-tumor immune response is required. We unraveled local and systemic immune profiles of colorectal cancer (CRC) by multidimensional mass cytometric analysis of 36 immune cell markers at the single-cell level in tumor tissues, tumor-associated lymph nodes, adjacent normal mucosa, and peripheral blood samples from CRC patients. In addition, functional and transcriptional profiles of tumor-infiltrating lymphocytes were investigated by flow cytometry and single-cell RNA-sequencing. We discovered that a previously unappreciated innate lymphocyte population ($Lin^-CD7^+CD127^-CD45RO^+$) is enriched in CRC tissues and displayed cytotoxic activity. This subset demonstrated a tissue-resident ($CD69^+$, $CD103^+$) phenotype, and was most abundant in immunogenic mismatch repair deficient (MMRd) cancers. The presence of these $CD45RO^+$ innate lymphoid cells strongly correlated with the presence of tissue-resident cytotoxic, helper and γδ T cells with a highly similar activated ($HLA\text{-}DR^+$, $CD38^+$, $PD\text{-}1^+$) phenotype in CRCs. These tumor-resident $CD8^+$ and $CD4^+$ T cells demonstrated neo-antigen recognition capacity. Furthermore, PD-1-intermediate and PD-1-high $CD8^+$ T cell subsets represented distinct states of T cell activation and differentiation that further discriminated immunogenic from non-immunogenic CRCs. Remarkably, activated γδ T cells were specific for MMRd cancers, and their potential role in response to PD-1 checkpoint blockade requires further clarification. The non-activated counterparts of tumor-resident $CD103^+PD\text{-}1^+$ cytotoxic and γδ T cells were present in tumor and healthy colorectal tissues. We did not detect any of the aforementioned tumor-resident immune cell populations in lymph node samples, with the exception of tumor-positive lymph nodes. Altogether, these findings provide a blueprint for the detailed characterization of the involved immune cell subsets in anti-tumor immunity in CRC. The coordinated action of innate and adaptive immune cell populations suggests a multi-targeted exploitation of their anti-tumor properties in a therapeutic setting.

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Preclinical development of CD37CAR T-cell therapy for treatment of B-cell lymphoma

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T cells modified to express chimeric antigen receptor (CAR) targeting CD19 have produced remarkable clinical responses in patients with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL). CD19CAR T-cell therapy has also demonstrated prominent effects in B-cell non-Hodgkin lymphoma (B-NHL) patients. However, a subset of patients who relapse after CD19CAR T-cell therapy have outgrowth of CD19-negative tumor cells. Hence, development of alternative CARs targeting other B-cell markers represents an unmet medical need for B-ALL and B-NHL. Here, we confirmed previous data by showing that B-NHL overall have high expression of CD37. A second generation CD37CAR was designed and its efficacy in T cells was compared to that of CD19CAR. In vitro assessment of cytotoxicity and T-cell function upon co-culture of the CAR T cells with different target B-cell lymphoma cell lines demonstrated comparable efficacy between the two CARs. In an aggressive B-cell lymphoma xenograft model, CD37CAR T cells were as potent as CD19CAR T cells in controlling tumor growth. In a second xenograft model, using U2932 lymphoma cells containing a CD19-negative subpopulation, CD37CAR T cells efficiently controlled tumors and cured the mice while CD19CAR T cells had limited effect. We further show that, unlike CD19CAR, CD37CAR was not sensitive to antigen masking. Finally, CD37CAR reactivity was restricted to B-lineage cells. Collectively, our results demonstrated that CD37CAR T cells effectively can eradicate B-cell lymphoma tumors also when CD19 antigen expression is lost, and support further clinical testing for patients with relapsed/refractory B-NHL.

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Enhancing the anti tumour immune response; The effect of Ionising radiation on Immune checkpoint expression in OE33 cells

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Background: The use of Radiotherapy (RT) as definitive or palliative treatment for some malignancies has been well established. The possibility of using RT in combination with immunotherapies has garnered much attention. In addition to control of tumour growth, RT exerts a range of immunomodulatory effects on the tumour and its microenvironment. These serve to prime the tumour for an immune-mediated response. This has led to a renewed focus on the possibility of synergy with older anti-cancer therapies such as radiation therapy.

Methods: We used an isogenic model of oesophageal adenocarcinoma radio-resistance developed in house and fully characterised, with cells irradiated at 3 separate time-points and different dosing regimens (24hrs, 48hrs, 72 hrs). The expression of PD-1, and its ligands PD-L1, PD-L2 were assessed by flow cytometry. We also examined the impact of fractionated dosing regimens on Damage Associated Molecular Patterns (DAMP's).

Results: In all three treatment arms, immune checkpoint expression were increased with exponentially higher levels of checkpoints expressed at 10Gy and 20Gy. PD-L2 increased significantly ($p<0.02$) in the 10 Gy sequential dosing treatment arm and again was statistically significant in the 10 Gy treatment cohort in the 48hr and 72 hr time point post radiotherapy group after flow cytometry analysis. There was also an increase in DAMP expression post radiotherapy.

Conclusions: This study identifies possible avenues for potentiating the anti-tumour effects of the host immune system. It identifies that Radiotherapy increase checkpoint inhibitor expression on OE33 cells and as such identifies a starting point for potentially combining immnotherapy with radiotherapy in the treatment of Oesophageal Carcinoma.

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Germinal center hypoxia develops during immune responses to tumor antigens

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Lymph nodes (LNs) are the earliest site of metastasis, but also serve as hubs of immune response development against tumor antigens. Immunotherapy controls disease in some patients, but such responses rely on pre-existing immunity against the tumor, and it is unknown what conditions in the tumor-draining LN (TDLN) promote the development of effective anti-tumor immunity. Upon stimulation, lymphocytes proliferate within the T cell paracortex or B cell germinal centers of the responding LN, where molecular cues dictate their differentiation into effector or memory cells**.** We hypothesized that low oxygen (hypoxia) developed during lymphocyte expansion in TDLNs, affecting the phenotype of differentiating immune cells, and efficacy of anti-tumor immunity.

Microscopy and/or FC was used to quantify hypoxia in LNs draining murine mammary tumors and in non-involved, TDLNs from breast cancer patients.

Tumor-bearing mice developed hypoxia in the B cell cortex of TDLNs, while LNs from naïve mice were hypoxia-free. To assess whether LN hypoxia reflects the extent of adaptive immune activation against tumor antigens, we injected lethally-irradiated tumor cells undergoing immunogenic cell death into the mammary fat, which induced greater levels of germinal center hypoxia than poorly immunogenic, heat-killed cells. In LNs draining established tumors, hypoxia was associated with more antibody-secreting cells (ASCs) vs. B memory cells, despite similar levels of germinal centers, suggesting that hypoxia promotes preferential B cell differentiation towards ASCs. In TDLNs from breast cancer patients, we detected the hypoxia-inducible protein CA9 specifically within germinal centers, which is the first observation to suggest that humans can develop germinal center hypoxia in response to tumor antigens.

Our results suggest that hypoxia develops in TDLNs, and reflects the extent of adaptive immune activation against the tumor. We describe a novel role for hypoxia in the development of anti-tumor immunity within the LN, whose detection may help predict positive treatment outcomes.

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Evaluation of potential immunogenic cell death-inducing chemotherapeutic agents in non-small cell lung cancer

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Certain chemotherapeutic agents can elicit immune stimulatory changes in the tumor microenvironment by triggering immunogenic cell death (ICD) and enabling tumor-specific cytotoxic T cell responses in the tumor micro-environment. Strategically combining such a chemotherapeutic agent with immunotherapy has the potential to achieve enhanced anti-tumor effects in non-small cell lung cancer (NSCLC) patients, where there is an urgent need for new therapeutic strategies.

For this, the immune stimulatory potential of different chemotherapeutics (cisplatin, oxaliplatin, cyclophosphamide, docetaxel and carboplatin) that are relevant in NSCLC was evaluated. We assessed the most important hallmarks of immunogenic cell death *in vitro* using three human (A549, NCI-1975, NCI-1650) and one murine (LLC) cell line: secretion of ATP (bioluminescence), ecto-calreticulin (ecto-CRT) exposure on the tumor cells (flow cytometry) and HMGB1 release (ELISA) were evaluated. Furthermore, phagocytosis of tumor cells and maturation of dendritic cells (DCs) were assessed using the human cell lines and cytokine secretion of the co-culture was evaluated.

Cytotoxicity experiments demonstrated for all chemotherapeutics divergent sensitivity to NSCLC cell lines. NSCLC cell lines demonstrate varying sensitivity towards induction of ICD hallmarks after treatment with the IC_{50} value of each chemotherapeutic compound, with docetaxel displaying overall the most immune stimulatory profile. Interestingly, the combination of docetaxel with either carboplatin or cisplatin increased induction of these ICD markers *in vitro*. Phagocytosis by and maturation of DCs (mainly CD86) was observed using all human cell lines. TGFβ secretion in the supernatant of the co-culture significantly decreased in almost all chemotherapy-treated tumor cells. An *in vivo* vaccination assay to confirm ICD is still under investigation.

These *in vitro* results demonstrate the immune stimulatory effects of clinically relevant chemotherapeutics, making it worthwhile to further investigate the potential of novel combination strategies of these chemotherapeutic agents and immunotherapy in NSCLC.

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The induction of different types of (immunogenic) cell death by the antioxidant inhibitors APR-246 and auranofin in non-small cell lung cancer cell lines

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Cancer cells have higher steady-state levels of reactive oxygen species (ROS) relative to normal cells which can promote tumor growth. Blocking antioxidant defenses in these tumors decreases their ability to balance out oxidative stress resulting in lethal ROS levels. Therefore, the vulnerability of cancer cells to disturbance in their red-ox status opens a new window for selective antioxidant anticancer strategies.

The main rationale of this study was to compare the competence of two antioxidant inhibitors, APR-246 and auranofin, to induce different types of cell death in p53 mutant NSCLC cell lines, potentially in an immunogenic manner. Our p53 mutant cell line panel consisted of the parental NSCLC cell line NCI-H1299 (p53 deletion, null) and its isogenic derivates stably transfected to express either R273H or R175H p53 mutants. Apoptosis, necroptosis and ferroptosis in response to APR-246 and auranofin treatment were assessed using the IncuCyte ZOOM system. In addition, we characterized the release of 'damage-associated molecular patterns' (DAMPs) associated with immunogenic cell death, that can alert the immune system. The most imporant DAMPs were investigated: ATP secretion (bioluminescence), membrane calreticulin expression (flow cytometry) and HMGB1 secretion (ELISA).

Knock-in of mutant p53 enhanced the cytotoxicity of antioxidant inhibition towards NSCLC cell lines. At present, the exact mechanisms of cell death are under investigation and will be presented at the meeting. Nevertheless, current data show increased membrane expression of calreticulin and the release of ATP after auranofin treatment, suggesting that the NSCLC cell lines die in an immunogenic manner.

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Tumor PD-L1 expression is an independent unfavorable prognostic factor in oral squamous cell carcinoma

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The immune checkpoint PD-1 and its ligand PD-L1 are involved in the induction of immunological tolerance of solid tumors, including oral squamous cell carcinoma (OSCC). The aim of the study was to establish the clinical and prognostic significance of PD-L1 in OSCC. Tissue microarrays of 125 resected OSCC were stained with two different commercially available PD-L1 antibodies (clones E1L3N and 22C3), alongside with PD-1 immunostaining. PD-L1 expression in more than 10% of tumor cells was associated with poorer survival, and established as a clinically relevant cut-off point. This relevant PD-L1 expression was detected in 10-15% OSCC specimens depending on the anti-PD-L1 antibody, and showed an inverse correlation with tobacco and alcohol consumption. In addition, PD-L1 expression was consistently found to associate with tumor recurrence and lower disease-specific survival. Multivariate analysis further revealed that neck node metastasis (HR 2.304, p = 0.009) and tumor PD-L1 expression (HR 2.571, p = 0.01) were significant independent factors for poor prognosis.Taken together, PD-L1 expression in more than 10% of tumor cells was consistently established as a clinically relevant cut-off point by using two different antibodies. More importantly, tumor PD-L1 expression emerges as an independent poor prognosis marker in OSCC patients.

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Changing matrix composition suppresses cancer growth by modifying macrophage behavior

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An emerging field in cancer research consists of the study of the role of extracellular matrix proteins. We found that fibronectin, an extracellular matrix protein, enhances angiogenesis, and, under special circumstances suppresses the immune response against cancer *in vivo* in mice (Rossnagl et al. PLoS Biology 2016). We therefore hypothesized that decreasing fibronectin accumulation in cancer not only slows down cancer progression by suppressing angiogenesis but may additionally diminish cancer size by stimulating the immune response. To test this we knocked-down (Kd) fibronectin in human breast cancer cells (MDA-MB-231) and found a decrease in cancer growth in mice (CT 7±1 vs. Kd 1±0.3 ($x10^6$ RLU); $p<0.0001$). This was associated with an increase in macrophages in tumors (CT 0.8±0.2 vs. Kd 1.5±0.3%, n=16/11, $p<0.05$). In a model of liver fibrosis we had successfully used a peptide derived from bacteria that prevents fibronectin fibril formation and hence matrix accumulation. We therefore injected the inhibitor to mice harboring bone metastatic lesions of breast cancer daily for 10 days. This slowed tumor growth resulting in lesions half the size of control lesions (CT 9±1 vs. inhibitor 4±1 ($x10^6$ RLU), $p<0.05$). Suppressing fibronectin accumulation also led to an increase in macrophages (CT 1±0.1 vs. inhibitor 1.8±0.4%, n=18/6, $p<0.05$). Macrophages from treated mice expressed lower levels of arginase (CT 2.1±0.8 vs. inhibitor 0.4±0.2 $x10^{-3}$, $p<0.05$) as we had found in macrophages from Kd tumors. The mice carry a foxn1 mutation resulting in failure to mature T-cells, the enhancement of cancer suppression in the absence of fibronectin is T-cell independent. In summary, we show that the absence of fibronectin suppresses cancer growth. This is due to an enhanced macrophage-mediated immune response independent of T-cell actions.

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RANKL inhibition in breast cancer induces an anti-tumor immune response orchestrated by cytotoxic T cells

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Abstract withheld at the author's request

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Modulated electro hyperthermia inhibits tumor progression in a triple negative mouse breast cancer model

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Circulating MicroRNA expression profiling in acute myeloid leukaemia patients and healthy donors according to age

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Introduction: MicroRNAs (miRNAs) are important regulators of biological processes such as cell proliferation/apoptosis, immune responses and tumorigenesis. miRNAs dysregulation have been identified in haematological malignancies including acute myeloid leukaemia (AML), which is a disease of older adults. Ageing has been associated with a progressive deterioration of the immune system that limits the capacity to mount an appropriate immune response to pathogens and may affect tumour immunosurveillance. Recently, age-associated changes in miRNA profiles have been described some of them related to immune system function.

Methods: we analysed miRNA expression profiles in AML patients and the effect of aging on miRNA expression.

Results: we identified six miRNAs significantly downregulated and seven miRNAs that were significantly upregulated in AML patients. Some of them have been implicated in cancer pathogenesis.

Conclusions: we demonstrate that AML induces changes in miRNA profile that have the potential to be diagnostic or prognostic biomarkers of disease. In addition, in healthy donors we have found three miRNAs (miR-15b, miR-181a, miR-494) that were significantly decreased with age. However, no age-associated differences were observed in AML patients suggesting that AML-induced changes in miRNA profile surpass the effect of age itself. In conclusion, circulating miRNAs in AML had a distinctive profile that distinguishes patients from healthy donors. Further characterization of circulating miRNAs in AML and the effect of age in their expression are required to use miRNAs as biomarkers of disease and ageing.

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Investigating the contribution of Epstein-Barr virus infection in the development of nasopharyngeal carcinoma using humanized mice model

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Undifferentiated nasopharyngeal carcinoma (NPC) is a type of head-and-neck cancer which is nearly 100% associated with Epstein-Barr virus (EBV) infection and heavily infiltrated with lymphocytes. Moreover, expression of microRNAs encoded from the EBV-*Bam*HI-A rightwards transcripts (miR-BARTs) can constitute up to 45% of total miRNAs in NPC cells. All these imply that the development of NPC involves the interplay of EBV infection and the infiltrated lymphocytes. Humanized mice model offers a platform to study the interaction between human immune cells and EBV-infected NPC cells.

Reconstitution of human immune system in NOD/SCID-IL2R-KO (NSG) mice was achieved by engraftment of CD34+ hematopoietic stem cells. Flow cytometry analysis of blood showed presence of human immune components, including monocytes, NK cells, B cells, and T cells. To understand the contribution of EBV infection and miR-BARTs in affecting the growth of NPC in humanized mice, our laboratory has established a new pair of EBV-positive and EBV-negative NPC cell lines, NPC43(EBV+ve) and NPC43(EBV-ve); and re-infected a NPC-specific strain of EBV (M81) and its mutant form with all the miR-BARTs deleted (M81-ΔBARTs) to the NPC43(EBV-ve).

The tumor growth rate of NPC43-EBV+ was faster in humanized mice than in conventional immunodeficient mice. The tumors formed were heavily infiltrated with lymphocytes, including macrophages, helper and cytotoxic T cells. On the contrary, no tumor was formed with NPC43(EBV-ve) cell line, which may demonstrate a role of EBV in promoting tumorigenicity in NPC. The other two cell lines, NPC43-M81 and NPC43-M81ΔBART, were also injected into humanized mice. NPC43-M81 grew into bigger tumors than NPC43-M81ΔBART, which may also suggest that miR-BARTs provide growth advantages for NPC *in vivo*. Further multiplex immunohistochemistry and flow analysis will reveal the differences in components present in the tumors and give insights into the roles of EBV infection or miR-BARTs in NPC development.

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PPARgamma upregulation as therapeutic target in Mesenchymal Glioblastoma Subtype

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Glioblastoma (GBM) is one of the most aggressive cancer despite the conventional treatments. This unfavorable prognosis mainly due to tumor recurrence caused by heterogeneous nature of the disease with GBM cancer stem cells as well as GBM subtypes. Among those subtypes, proneural and mesenchymal GBMs are recognized to have mutually exclusive gene signatures. Compared to proneural, mesenchymal GBMs are more aggressive and therapy-resistant. Here we report the high expression of peroxisome proliferator activated receptor-gamma (PPARgamma) in mesenchymal subtype of GBM among other nuclear receptors by analyzing the RNA-seq data of proneural and mesenchymal GBM cells. We next confirmed that mRNA and protein level of PPARgamma were significantly higher in mesenchymal GBM by using patient-derived glioblastoma stem cells (GSCs). Further analysis of available transcriptome microarray and The Cancer Genome Atlas glioblastoma database consistently supported high expression of PPARgamma in mesenchymal GBM. Since mesenchymal GBM is associated with poor prognosis, we also found PPARgamma upregulation links to poor overall survival and disease free survival of GBM patients. As known for potential benefits in many cancers, we evaluated therapeutic effect of PPARgamma in a set of glioblastoma stem cells (GSCs). Ligand activation of PPARgamma showed dramatic therapeutic effect decreasing cell survival in Mes GSCs but not in the corresponding PN ones. More interestingly, PPARgamma activation inhibited self-renewal ability of Mes GSCs but no change in PN GSCs. Taken together, our finding not only provides a better understanding about the role of PPARgamma in Mes GSCs but also identifies the therapeutic potential of PPARgamma in Mes type of GBM.

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Tumor mutational burden assessed by a targeted NGS assay predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer

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Background: A subgroup of patients with non-small cell lung cancer (NSCLC) shows prolonged response to immune checkpoint inhibitors (ICI) with improved overall survival. Predictive biomarkers for treatment response are highly desired. Tumor mutational burden (TMB) assessed by whole-exome sequencing was demonstrated to be associated with outcome in patients treated with ICIs. First studies show that targeted panel sequencing, which is more suited for clinical applications, can reliably assess TMB. The goal of our study was to assess clinical validity of using TMB determined by a targeted NGS panel as a predictive biomarker for ICI therapy.

Methods: NSCLC patients treated with second- or further line ICI and with available FFPE tumor specimens for TMB analysis were retrospectively included in the study. TMB was assessed using a 409-gene targeted NGS assay that detects variants in all coding regions (Oncomine TML Assay, Thermo Fisher Scientific). Clinicopathological data including best response to ICI therapy (using RECIST 1.1) were collected and patients were characterized as either having clinical benefit (CB) (defined as complete/partial response or stable disease) or no CB. The correlation between TMB and CB was calculated.

Results: Of 64 patients (median age = 64 years, range 31- 83), 58% were male, 73% smokers and 95% had adenocarcinoma histology. The most commonly used ICI was nivolumab (86%), mainly in the second-line setting (55%). CB rate was 45%. Median TMB was 6.7 mutations per megabase. In patients with CB TMB was significantly higher than in patients without response to therapy (median 8.4 vs 5.9, p=0.019). AUC of receiver operating characteristic (ROC) analysis was 0.67.

Conclusion: Using a 409-gene panel, TMB can be assessed on routine clinical samples and significantly predicts clinical benefit from ICI. Given the retrospective nature of this study, these results need to be confirmed in a larger prospective cohort study.

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Towards the next generation CAR T cells with TEGs: in vivo efficacy – toxicity profile in xenografts of primary human AML disease and healthy bone marrow

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γδT cells mediate cancer immune surveillance by sensing metabolic changes of malignant leukemic blasts and not their healthy counterpart via their γδT cell receptor (TCR). This concept led to the development of next generation CAR T cells, so-called TEGs: αβT cells Engineered to express a defined γδTCR. A particular γ9δ2TCR, isolated from “clone 5”, has been selected as the candidate for clinical testing (TEG001). TEG001 cells showed a strong and broad recognition of hematological malignancies against both cell lines and primary AML.

In order to evaluate the biodistribution and safety profile of TEGs we developed a patient-derived xenograft (PD-X) *in vivo* model by establishing primary malignant AML blasts, which tested positive in an *in vitro* assay for recognition by TEGs, in NSG mice. In addition, healthy stem cells from human cord-blood were in parallel engrafted in a separate set of NSG mice. After engraftment, TEGs were infused and mice followed for additional 50 days. While engrafted primary AML blasts were no longer detectable in the peripheral blood at the end of the study period, all healthy hematological cellular compartments remained unharmed.

Within the limitations of humanized PD-X models, TEGs target acute myeloid leukemia but do neither interfere with engraftment of hematopoietic progenitors nor harm matured subsets of the hematopoiesis. In addition, no additional signs of off-target toxicity were observed in mice. TEGs are a promising addition to the currently available immune therapeutic strategies as they target cancer as a metabolic disorder.

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TRIB1 overexpression in tumour-associated macrophages enhances breast tumour growth *in vivo*

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Background: During tumourigenesis, tumour-associated macrophages (TAMs) are key cells recruited and re-educated within the tumour microenvironment and can comprise up-to 50% of a solid tumour mass. Re-educated TAMs are polarised to facilitate tumour growth by expressing both pro- and antiinflammatory cytokines and Tribbles 1 (TRIB1) have been shown to regulate macrophage polarisation. However, the role of TRIB1 in TAMs and the consequences of this on tumourigenesis has not yet been explored.

Aim: We aim to understand the role of TRIB1 in the polarisation of macrophages, focusing on TAMs, and gain insight into how TRIB1 expression in TAMs may alter tumourigenesis.

Methods: Mammary tumour growth was induced by injecting mammary E0771 tumour cells into the mammary fat pads of wild-type or myeloid-specific Trib1 overexpressing mice. The tumour microenvironment and the phenotype of TAMs were analysed in post-mortem tissue by immunofluorescence staining.

Results: Mammary tumour growth in the myeloid Trib1 over-expressing mice demonstrated significantly enhanced tumour growth in vivo. Additionally, Trib1 overexpression modified the composition of the tumour microenvironment where a trend of increased number of CD3 positive T cells and number of CD31 and F4/80 positive perivascular macrophages. Reduced proportion of pro-inflammatory TAMs was also found in tumours from Trib1 transgenic mice.

Conclusion: Overexpression of Trib1 in myeloid cells facilitated mammary tumour growth in vivo and this enhancement is potentially caused by Trib1 dependent alterations in TAM phenotype and the consequent changes in the composition of cells in the tumour microenvironment.

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Beyond CTLA-4 and PD-1: Nuclear Receptor NR2F6 as an Alternative Cancer Immune Checkpoint in T Cells

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In contrast to cell surface checkpoints like CTLA-4 and PD-1, additional cancer therapeutic targets are located inside the effector immune cells. Targeting these alternative checkpoints in cancer immunotherapy with the goal to strengthen the patient's immune system are likely to extend the benefits of cancer immunotherapy in the near future. Along this line, we have defined and validated the orphan nuclear receptor NR2F6 (nuclear receptor subfamily 2 group F member 6, also called Ear-2) as an intracellular immune checkpoint in effector T cells by analyzing mouse tumor models in vivo, human T cells *ex vivo* and human lung cancer samples. Genetic ablation of *Nr2f6*, particularly in combination with PD-L1 blockade efficiently delayed tumor progression and improved survival in experimental mouse models. In accordance with published data (Hermann-Kleiter 2008, 2010; Kleiter & Klepsch 2016), acute *Nr2f6* silencing in both mouse and human T cells induced hyper-responsiveness that established a non-redundant T cell-inhibitory function of NR2F6 by directly repressing transcription of key cytokine genes in T effector cells relevant for tumor cell rejection, such as IL-2, IFNγ and TNFα. In T cell-infiltrating cells of human NSCLC patients, NR2F6 protein expression was found to be upregulated in 54% of the cases (163 of 303 samples) and significantly correlated with PD-1 and CTLA-4 expression. Our data define NR2F6 as an intracellular immune checkpoint that suppresses adaptive anti-cancer immune responses and set the stage for clinical validation of targeting NR2F6 with low molecular weight compounds for improvement of next generation immune-oncological regimens.

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Characterization of PD-1/PD-L1 immune checkpoint inhibitors: small molecules, macrocyclic peptides and antibodies

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Inhibition of the interaction between PD-1 (programmed cell death protein) and PD-L1 has been reported to be a promising therapeutic approach for treating cancer. When either PD-1 or PD-L1 protein is blocked, the “brakes” on the immune system are released and the ability of T cells to recognize and kill tumor cells is rising. There are several anti-PD-1 and anti-PD-L1 antibodies approved as anticancer drugs, but not a single small-molecule has been successfully evaluated in the clinical trials, so far.

In our study, we investigated the affinity and the activity of small-molecules (BMS-1001; -1166) and bioactive macrocyclic peptides (named: 51; 71 and 99), retrieved from Bristol-Myers Squibb patents. We confirmed the binding of the molecules to molecular target, PD-L1 protein, using differential scanning fluorimetry (DSF) and nuclear magnetic resonance (NMR) methods. We also used X-ray crystallography to obtain a structural insight into the interaction between every tested molecule and PD-L1.

To determine the *in vitro* activity of BMS compounds, we performed the cell-based assay - with Jurkat T cells, overexpressing PD-1 and carrying a reporter luciferase gene under the control of NFAT promoter, and CHO-K1 cells overexpressing PD-L1 and TCR-activator. We have provided evidence, that each of the PD-L1 blockers restore the activation of Jurkat T cells in dose-dependent manner. We also characterized the immunomodulatory effects by calculating EC_{50} values. The compounds’ ability to restore the activation of Jurkat T cells by soluble PD-1 is also confirmed by a lumiensecence measurement.

Two macrocyclic peptides are the most promising PD-1/PD-L1 blockers among the chosen compounds. Moreover, they are potent in elimination a number of side effects and disadvantages of antibodies. We hope, that our results will be used for the preclinical development of new class of anticancer drugs.

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Two Distinct Tumor Subgroups Defined by NK Cells in Renal Cell Carcinoma Patients

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Background: Renal cell carcinoma (RCC) is considered as one of the most immunogenic cancers with the highest number of indel mutations and frequent infiltration of T cells. Since less is known about natural killer (NK) cells in RCC, we aimed to investigate the intratumoral phenotype of NK cells and further assess the overall immune landscape of RCC.

Methods: We used multi-parameter flow cytometry to immunophenotype the tumor, peripheral blood (PB) and adjacent tissue from 35 RCC patients that underwent partial or radical nephrectomies. T cell receptor beta (TCRβ) deep sequencing was carried out with 8 tumors together with their corresponding PB and adjacent tissues (n=24).

Results: Using hierarchical clustering and correlation analysis, we discovered that our cohort clustered into two distinct subgroups defined by a high (n=17; mean 24.3%) and low (n=18; mean 7.5%) abundance of NK cells among the intratumoral lymphocyte population. Accordingly, the NKhigh had a lower percentage of T cells than the NKlow subgroup (mean 51.7% vs 74.2%; $p<0.0001$).

Our TCRβ sequencing results revealed a positive correlation between T cell clonality and the intratumoral T cell percentage. Furthermore, a diverse T cell repertoire in the tumors was observed compared to the corresponding PB and adjacent tissue respectively. Moreover, compared to the adjacent tissue, the tumor counterparts were less clonal, suggesting a more restricted TCRβ chain usage.

The overall immune landscape of RCC showed that tumors have more NK cells compared to their corresponding PB and healthy tissue, supporting our findings that some tumors accumulate NK cells.

Conclusions: Our study has led to the discovery of two distinct RCC tumor subgroups. Prospective bulk RNA sequencing for the NKhigh/NKlow subgroups will be carried out to discover the transcriptomic differences and mutational profiles, which will be supported with our extensive flow cytometry results, as well as through publicly available data.

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HSV-TK/GCV suicide gene therapy of glioblastoma induces an immunosuppressive microenvironment

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Abstract withheld at the author's request

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3D organoid platform optimized for cancer-immunology research

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Malignant tumors are one of the leading causes of death world-wide and elicit enormous socio-economical expanses. Approximately 30% of patients experience tumor recurrence or metastasis. Tumor-microenvironment (TME) including tumor-infiltrating immune cells would confer treatment resistance against both conventional and targeted chemotherapies. Moreover, recent trials on adapting immune-modulators to treat cancers have demonstrated striking clinical efficacies in various cancer types. For comprehensive study of TME and tumor-immunology, in vitro TME-mimicking system would provide great opportunities. Current organoid technology has been developed based on the murine-originated matrixes including Matrigel and BME. However, these matrixes might induce xenogeneic effect on immune cells, especially helper T cells when tumor-immune cells are co-incubation within the matrixes, which could trigger unwanted immune responses.

In this regard, we have tried to develop patient-derived tumor organoids using non-murine matrixes including hydrogel materials and compare the proliferation rate, morphologies and histologic characteristics with murine-matrix-based organoids. In brief, we have established high grade ovarian cancer patient-derived tumor spheres. Also, optimized the culture condition for adapting in matrigel or hydrogel-based 3D organoid culture system. Simultaneously, matrix-embedded tumor fragments cultures have been tested. We next tried to establish tumor-immune multicellular organoids, and investigated whether the distinct cell populations were viable and proliferative in whole mass. Finally, xenogeneic effect on CD4 T cells on various matrixes were evaluated.

In conclusion, to investigate the tumor-immune networks contributing tumor evolution and treatment resistance, we are developing 3D organoid-based translational research platform using primary human tumor tissues and tumor spheres. The parent tumors and organoids were comprehensively annotated by genome and clinical information including treatment histories. Our platform will provide new opportunities in basic and clinical scientist interested in the immuno-oncological researches.

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Phenotypic and functional characterization of immune cell subsets in liver metastases of colorectal cancer by multiparametric flow cytometry

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Colorectal cancer (CRC) progression commonly involve liver metastasis. As multiple immune cells exist in the liver and in conjunction with the success of PD-1 immune checkpoint blockade therapy in a subgroup of CRC patients, we have applied flow cytometric *ex vivo* analyses of immune cell subsets in freshly-isolated cell suspensions of CRC liver metastases and autologous metastasis-free liver controls from a limited number of non-treated patients. Relative to the control, $CD4^+$ T cells were increased in the metastases, whereas $CD8^+$ T cells were decreased. On further subset level, $CD4^+$ $CD127^{dim/-}$ $CD25^+$ Tregs were also elevated in the metastases, but frequencies of Va7.2 TcR^+ MAIT cells, gdT cells and $CD56^+$ NK cells were declined. Metastases and control had similar scarce levels of $CD19^+$ B cells and Va2.4 TcR^+ NKT cells. Upregulation of inhibitory PD-1 in metastases was more robust on Tregs compared to other immunotherapy targets (LAG-3, TIM-3). Increase of these inhibitory markers was not detected on MAIT cells, gdT cells and NK cells, which highly expressed CD69 representative of activation and/or tissue residency. Cytokine production by T cell subsets (IFN-g, IL-2, IL-17, Perforin) were detected upon polyclonal stimulation. Since antigen-presenting cells provide T cell stimulation or inhibition, their presence in metastases may influence T cell functionality. Among $CD163^+$ macrophages/Kupffer cells, the $CD14^+$ subset was especially increased in metastases. $CD11c^+$ dendritic cell (DC) subsets defined by CD1c or CD141/Clec9a remained similar. These macrophages and DCs readily endocytosed and degraded OVA protein, resulting in upregulation of T cell co-stimulatory CD80 and co-inhibitory PDL-1. Similar frequencies of $HLA\text{-}DR^-$ $CD33^+$ myeloid-derived suppressor cells (MDSCs) expressing $CD15^+$ or $CD14^+$, plus $CD33^-$ $CD15^+$ PMNs were detected in metastases and control. In conclusion, this study provides in-depth phenotypic and functional assessment of immune cell subsets co-existing within CRC metastases vs. liver that is prone to metastases.

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The immunotherapeutic role of indoleamine 2,3-dioxygenase (IDO) in head and neck squamous cell carcinoma: a systematic review

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Background**:** Novel cancer immunotherapy applies immune checkpoint inhibitors to harness the body's own immune system and thereby tip the balance in favour of antitumour activity. The intracellular enzyme IDO is a critical regulator of the tumour microenvironment (TME) via tryptophan metabolism. The immune checkpoint, IDO1, breaks down tryptophan into its metabolites which results in TME immunosuppression.

Aim**:** To assess the evidence on IDO in head and neck squamous cell carcinoma (HNSCC).

Methods**:** Medline, EMBASE using Ovid, Scopus, Web of Science, Cochrane Library databases and ClinicalTrials.gov were searched from inception until present day.

Results**:** We included 32 studies. Of those, 5 involved cell lines, 7 assessed tumour immunohistochemistry, 6 measured IDO gene transcription, and 14 reported on clinical trials. IDO expression and activation by the Stimulator of Interferon Genes (STING) pathway played a central role in the human cell lines studied (SCC4, SCC15 and SCC25). Retrospective immunohistochemistry studies of lip, oral cavity, tonsil and larynx found that relatively high IDO expression correlated with worse survival. Gene transcription studies showed increased IDO in tumours that expressed programmed death-ligand 1 (PD-L1) and harboured human papillomavirus (HPV). Phase I/II clinical trials showed 1) overall responses (34%) and disease control rates (62%) for IDO1 inhibitor in combination therapy, 2) consistent safety profile and 3) IDO gene expression as a predictive biomarker for response to therapy.

Conclusions**:** IDO is integral to TME immunity in HNSCC particularly in HPV positive cancers. IDO can be used to modulate existing therapies and has applications in combinatorial immunotherapy. Retrospective studies have shown its presence in the TME and suggest a link to HNSCC treatment outcome. However, the exact mechanism of IDO-driven immune modulation in the HNSCC TME remains unclear. We now require prospective longitudinal studies to track IDO activity and expression throughout HNSCC treatment, thence optimise IDO-based immunotherapy.

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NK cells increase T cell infiltration and antigen presenting cell maturation within melanoma tumors

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While checkpoint blockade therapies have demonstrated amazing success treating a variety of cancer patients, significant numbers of patients do not respond to therapy. Increased numbers of T cells within tumors is correlated with longer patient survival and positive response to checkpoint blockade therapy. T cell presence within the tumor can be affected by entry through the endothelium, as well as proliferation or survival within the tumor. What regulates T cell presence within the tumor microenvironment (TME) remains an open area of study. The role of innate immune cells effect on T cell presence within the tumor has not been fully determined. NK cells can produce inflammatory cytokines to alter homing receptor ligands on the vasculature that increase T cell entry into tumors. Additionally, NK cells have been shown to interact with dendritic cells (DCs), which increases DC maturation. Increased DC maturation within the tumor may cause T cell proliferation, survival, and effector function within the tumor. Using an implantable subcutaneous murine melanoma model, NK cells only produced IFNγ at early time points during tumor development. To determine what effect NK cells have on immune cell presence within the TME, we depleted NK cells. Depletion of NK cells resulted in lower number of T cells within the tumor only when depleted prior to tumor implantation, suggesting that NK cells play a role in establishing an early TME that encourages T cell infiltration, proliferation or survival. Depletion of NK cells also resulted in lower levels of DC maturation and cross presentation of antigen within the TME. Overall these results suggest that NK cell contribute to a TME that increases T cell presence and activation. Developing therapies that increase NK cell function within the TME may result in an increase in T cell presence and result in synergy with checkpoint blockade therapies.

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Peripheral blood TCRB chain convergence in chronic viral infection and cancer: Insights from a novel immune repertoire biomarker.

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Background: T cell receptor (TCR) convergence refers to the phenomenon whereby antigen-driven selection enriches for TCRs having shared antigen specificity but different nucleotide sequences. Previous work has demonstrated the potential utility of TCR convergence as a predictive biomarker for response to checkpoint blockade and dendritic cell based immunotherapy for cancer. The extent to which convergence arises owing to chronic viral infection is not yet established. Here we sought to identify features of chronic cytomegalovirus (CMV) infection using TCRB profiling of peripheral blood (PBL) total RNA.

Methods: Total RNA from PBL was obtained from 35 blood donors of known CMV status, then used for TCRB sequencing via the Oncomine TCRB-LR assay (amplicon spanning CDR1, 2 and 3) and the Ion Torrent S5. In parallel, we prepared libraries via the Oncomine TCRB-SR assay (CDR3 only). Data were used to identify TCRB repertoire features correlated with CMV status and compare repertoire features across the two assays. For context, we compare CMV-related convergence to previous reports detailing convergent T cell responses in individuals with cancer.

Results: T cell clone evenness was reduced in CMV positive individuals irrespective of age, predictive of CMV status (AUC=.86, p=2E-4, Wilcoxon), and strongly correlated between LR and SR assays (Spearman cor=.96). TCR convergence was elevated in CMV positive individuals and uncorrelated with evenness (Spearman cor = -.03) such that the combination of convergence and evenness improved the performance of a logistic regression classifier (AUC= .93).

Conclusions: We identify reduced T cell evenness and elevated TCR convergence as features of chronic CMV infection. These results suggest that TCR convergence may be a hallmark of chronic antigen stimulation, both in the context of viral infection and cancer. TCR convergence may have broad utility as a liquid-biopsy compatible biomarker for cancer immunotherapy.

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Exploiting 3D cell-based models to recapitulate Breast Cancer-Macrophage crosstalk

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The tumor microenvironment (TME) promotes tumor progression and metastasis. Monocytes are recruited to TME and enroll a molecular crosstalk, promoting their polarization towards a pro-tumorigenic phenotype. In Breast Cancer (BC), Tumor Associated Macrophages (TAM) infiltration within TME highly correlates with aggressive BC subtypes. Once BC-induced, TAM resemble M2 anti-inflammatory macrophage phenotype, promoting immunosuppression, matrix remodeling and neo-angiogenesis. M2-related functions are mediated by cytokines like IL-4, IL-10 or TGF-β, therefore, TAM emerge as potential targets for immunomodulatory therapies towards this signaling. Due to the lack of relevant *in vitro* and *in vivo* models to study cytokine-mediated mechanisms, we propose a 3D human model to recapitulate tumor-TAM crosstalk.

Recently, our group developed a 3D cell-based model system, based on alginate microencapsulation and stirred-tank vessels, to recapitulate tumor-specific events. We employed the system to establish co-cultures of BT474 or HCC1954 BC cell lines spheroids and human blood-derived monocytes. After 7 days of culture, monocytes differentiated into M2-macrophages, shown by the low detection of M1 (CD80) and high detection of M2 macrophage markers (CD206, CD163). Distinct M2-polarizing ability between both BC cell lines was also observed, with higher detection of M2-macrophage markers in HCC1954-cultured monocytes. By cytokine analysis, distinct secretory profiles were observed between co-cultures. HCC1954 co-culture showed higher IL-4 and VEGF secretion, among others, whereas in BT474 co-culture, higher amounts of IL-10 and TGF-β were observed. Thus, our results suggest distinct cytokine-mediated crosstalk between different BC cell lines and monocytes, which may be correlated with different BC subtypes influence on the surrounding immune microenvironment.

In conclusion, our model can be refined as a tool to identify key mechanisms underlying immunoncological molecular crosstalk, by assessing key cytokines that promote TAM immunomodulation.

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Combined immune checkpoint blockade in mesothelioma: in vivo investigation of in vitro data

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Introduction: Malignant pleural mesothelioma (MPM) is an aggressive cancer that is causally associated with asbestos exposure. Due to its aggressive nature and despite the effectiveness of conventional anti-cancer treatment, the prognosis of patients diagnosed with MPM remains dismal, highlighting the urgent need for new therapeutic strategies. Our group and others have recently demonstrated PD-1 and PD-L1 expression in MPM patients, providing rationale to evaluate their suitability as immunotherapeutic targets in MPM.

Material & methods: Tree human cell lines representative for the epithelioid and sarcomatoid subtypes of MPM were placed in allogeneic co-cultures with healthy donor peripheral blood mononuclear cells. The co-cultures were treated with the following immune checkpoint blocking antibodies: anti PD-1 (Nivolumab®, BMS) or anti PD-L1 (Durvalumab®, AstraZeneca) in combination with anti TIM-3 or anti LAG-3. Supernatant was collected and enzyme-linked immunosorbent assays and multiplex electrochemo-luminescence were used to look at the secretion of 7 cytokines, being IFNg, IL-2/5/6/10, IL-1ß and TNF-a, as well as the enzyme granzyme B.

Results & discussion: Significant differences were found for the secretion of IFNg, granzyme B, IL-2, IL-5 and IL-10. Though the differences were not always significant for the 3 MPM cell lines, the same trends were observed among them. Interestingly, highest concentrations of the aforementioned cytokines were all noticed for monotherapy treatment with anti PD-1, anti PD-L1 or or their combination with anti TIM-3. In vivo investigation of PD-1, PD-L1 and TIM-3 blockade, alone or in combination is required for validation of our in vitro results and is currently ongoing.

Conclusion: Our data show that treatment with anti PD-1, anti PD-L1 or their respective combination with anti TIM-3 resulted in the highest secretion of cytokines and granzyme B, suggesting that these treatments stimulate the antitumor response the most. Results of our in vivo validation are awaited in order to confirm our in vitro findings.

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Increased plasma levels of galectin-1 in pancreatic cancer: potential use as biomarker

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Pancreatic ductal adenocarcinoma (PDA) is the most frequent type of pancreatic cancer and one of the deadliest diseases overall. New biomarkers are urgently needed to allow early diagnosis, one of the only factors that currently improves prognosis. Galectin-1 is a soluble carbohydrate-binding protein overexpressed in PDA tissue samples promoting tumor proliferation, angiogenesis, stroma activation and immune escape. Here we analyzed whether the detection of circulating galectin-1 (Gal-1), can be used as a biomarker for PDA. Gal-1 levels were determined by ELISA in plasma from healthy controls and patients diagnosed with PDA, using three independent cohorts. Patients with chronic pancreatitis (CP) were also included in the study to analyze the potential of Gal-1 to discriminate between cancer and inflammatory process. Plasma Gal-1 levels were significantly increased in patients with PDA as compared to controls in all three cohorts. Gal-1 sensitivity and specificity values were similar to that of the CA19-9 biomarker (the only FDA-approved blood test biomarker for PDA), and the combination of Gal-1 and CA19-9 significantly improved their individual discriminatory powers. Moreover, high levels of Gal-1 were associated with lower survival in patients with non-resected tumors. Collectively, our data indicate a strong potential of using circulating Gal-1 levels as a biomarker for detection and prognostics of patients with PDA.

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Characterizing the role of cancer-associated osteoblasts (CAOs) in facilitating bone colonization of metastasizing breast cancer cells

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Mortality from breast cancer is almost exclusively due to metastasis. Bone metastasis occur in up to 85% of patients with advanced breast cancer, resulting in severe skeletal complications and are mostly untreatable. Breast cancer-induced bone metastases are characterized by changes in the bone metastatic microenvironment and frequently osteolytic lesions. This is partially mediated by osteoblasts, which over-activate osteoclasts to resorb bone, thus releasing growth factors that can in turn help cancer cells to survive and invade. However, the early interactions of metastasizing cancer cells with stromal cells in the bone-marrow and their reprogramming to cancer-associated osteoblasts (CAOs) are largely unresolved.

In this study we investigated the role of paracrine signaling by bone-metastasizing breast cancer cells in activation of osteoblasts. We found that activation of osteoblasts instigated a switch in their morphology and induced the expression of a pro-inflammatory gene signature, reminiscent of CAFs. CAOs demonstrated an upregulation of multiple pro-inflammatory genes and a downregulation of genes associated with osteoblast development, suggesting tumor-induced inhibition of normal osteoblast differentiation and maturation. To study the interactions of breast cancer cells with the bone microenvironment *in vivo*, we generated bone-seeking variants of 4T1 cells (4T1.2 and 4T1.3). Analysis of cell populations in BM of mice with bone metastases revealed an immune-suppressed microenvironment, including increase in the population of granulocytic BMDCs and a decrease in T cells. Interestingly, we found that tumor-activated osteoblasts upregulated immune-suppressive genes, suggesting that activated osteoblasts play a role in the formation of an immune suppressed niche. Uncovering the mechanisms that underlie the early events of breast cancer bone metastasis may provide the mechanistic basis for therapeutic targeting to reduce bone metastatic relapse.

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Novel human NK cell line carrying CAR Targeting EGFRvIII Induces antitumor effects in glioblastoma cellsNovel human NK cell line carrying CAR Targeting EGFRvIII Induces antitumor effects in glioblastoma cells

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Background/Aim: Glioblastoma (GBM) is one of the most common and aggressive primary malignant brain tumors occurring in adults. Immune-based therapy for GBM is a promising alternative to conventional treatments. Natural killer (NK) cells are considered potential antitumor effector cells. The aim of this study was to establish a novel type of a chimeric antigen receptor (CAR) NK cell line (CAR-KHYG-1) specific for epidermal growth factor receptor variant III (EGFRvIII)-expressing tumors and investigate the anti-tumor activity of EGFRvIII-specific-CAR-KHYG-1 (EvCAR-KHYG-1).

Materials and Methods: NK cell line KHYG-1 was cultured in 10% FBS containing RPMI1640. EvCAR-KHYG-1 was established by self-inactivated lentiviral-based transduction of the EvCAR gene and magnetic bead-based purification of EvCAR-expressing NK cells. The anti-tumor effects of EvCAR-KHYG-1 were evaluated using growth inhibition and flow cytometry-based apoptosis detection assays in EGFRvIII-expressing and non-expressing U87MG glioblastoma cell lines

Results: Lentivirus-transduced KHYG-1 showed 11% positivity for EvCAR. Following the first round of magnetic bead sorting, EvCAR-expressing KHYG-1 cells were increased by up to 81.3%, while that increase was up to 89.2% after the second round of magnetic sorting. The NK cells established in this manner were used in the following experiments.

Growth inhibition assays showed that EvCAR-KHYG-1 significantly inhibited U87MG-EGFRvIII growth as compared to parental U87MG. Parental KHYG-1 showed no inhibition of the growth of U87MG or U87-EGFRvIII cells.

Apoptosis detection assays showed that there was a significant increase in apoptosis of U87MG-EGFRvIII induced by EvCAR-KHYG-1 compared to parental KHYG-1.

These findings demonstrated that EvCAR-KHYG-1 inhibited GBM cell-growth via apoptosis in an EGFRvIII-expressing specific manner.

Conclusion: This is the first study to establish a CAR NK cell line based on the human NK cell line KHYG-1. Therapy with EvCAR-KHYG-1 may be an effective treatment option for GBM patients.

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Capability of dendritic cells loaded with induced-pluripotent stem cells to induce cancer-responsive T cells from a donor with HLA class I-A33 alleles *in vitro*

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Background: Dendritic cells (DC) loaded with cancer cell lysate induce the antitumor effect in several cancers *in vivo* but the approach is limited by tumor burden. It is recently reported that irradiated induced-pluripotent stem cells (iPS) elicit the antitumor response in melanoma *via* immune systems since iPS share many tumor-associated antigens (TAA) in mouse and human. It remains unclear whether destroyed iPS-loaded DC (DiPS/DC) induce cancer-responsive T cells in human.

Aim: To investigate whether lysate of DiPS/DC induces cancer-responsive T cell *in vitro.*

Method: Peripheral blood mononuclear cells (PBMC): a human leukocyte antigen (HLA) class I-A33 homo donor's PB was used. DNA microarray: Experiments to generate signatures use Human U133 2.0 Plus GeneChips. iPS generation: iPS were generated by plasmid-based non-viral induction method, were used. Cultures were fed with xenofree medium Stemfit AK02 at laminin 511-E8-coated plate. DiPS lysate:iPS were destroyed by three freeze-thaw cycles and subsequent sonication. DiPS/DC and T cell culture: PBMC were cultured in GM-CSF, IL-4 and autoserum-containing AIM-V for DC induction. Induced-DC were pulsed with DiPS lysate and then stimulated by TNF-α to allow full maturation. DiPS/DC was co-cultured with purified-T cells and were restimulated by DiPS/DC weekly and supplemented with IL-7 and/or IL-2 for 3 weeks. ELISPOT: Immunospot kit h-IFN-γ was used. SW48 (HLA-A33), T47D (HLA-A33), T98G (HLA-A02) were used as target cells.

Results: DNA microarray analysis showed that the established-iPS shared many TAA in SW48 and T47D. Immunofluorescent analysis showed that annexin-V-FITC-labeled DiPS was captured by immature DC within 2 hours. DiPS/DC induced the sufficient number of T cell for analysis. IFM-γ-based ELISPOT assay revealed DiPS/DC-induced T cells responded SW48 and T47D but did not respond T98G.

Conclusion: DiPS/DC induced cancer-responsive T cells in HLA-matched cancer cells. DiPS/DC could be a promising vaccine for cancer immunotherapy.

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Deciphering the contribution of macrophages to follicular lymphoma pathogenesis: new insights into therapy

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Follicular lymphoma (FL) represents the paradigm of a lymphoid neoplasia depending on microenvironment, where its enriched composition in follicular dendritic cells (FDC) and macrophages associates with inferior outcome *(Dave et al, NEJM 2004)*. We have analyzed FL-FDC crosstalk and found that cell culture supernatants were enriched in pro-angiogenic factors and in the macrophage-attractant CCL2, favoring *in vitro* monocyte recruitment to the tumor. *In vivo*, FDC significantly increased FL cell lines tumorigenicity ($p<0.001$), being these FL-FDC co-xenografts highly infiltrated with mouse M2 macrophages ($CD206^{+}$). Remarkably, macrophage depletion with liposomal clodronate decreased tumor growth, supporting the contribution of macrophages to FL progression.

In vitro, primary cultures of FL cells induce monocyte differentiation into a M2-like macrophage phenotype ($CD163^{high}$ $CLEC4A^{high}$ $CD86^{low}$). Moreover, FL viability was significantly increased in FL-M2 co-cultures ($p<0.01$), and gene sets related to migration, adhesion and invasion were enriched in FL cells. The chemokine analysis of co-culture supernatants indicated an enrichment of CXCL12, CCL2, CCL3, and CCL18 known to recruit lymphocytes, macrophages and several immunosuppressive cells, together with MMP9 related to matrix degradation and invasiveness.

We then analyzed the expression of the M2-marker CD163 and CSF1-R in FL tumor biopsies form the Hospital Clinic (Barcelona). While the expression of CD163 did not correlate with clinical parameters, we found a strong correlation of CSF1-R expression with the histological grade ($p<0.001$), thus representing a good therapeutic target. In this regard, we have found that the CSF1-R inhibitor Pexidartinib (PLX-3397) impedes monocyte differentiation, decreases M2 macrophage adhesion and switches the macrophage polarization towards a M1 phenotype. PLX-3397 hampers the pro-survival effect provide by M2 to FL primary cells, supporting a role for this receptor in FL-M2 crosstalk. *In vivo* PLX-3397 experiments are under way, both as monotherapy and in combination with the standard of care Rituximab (anti-CD20 mAb).

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Using functional genetic screens to understand cancer immune evasion

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With the emergence of checkpoint inhibition as an effective cancer therapy, there is also a growing need to identify new biomarkers of response as well as novel therapeutic targets. Functional CRISPR screens have proven to be an important tool for identifying key genes in complex biological processes. Here we use an *in vitro*and *in vivo*CRISPR screening strategy to better understand how tumors evade the immune system.

We collected pre and post treatment biopsies from melanoma patients treated with an anti PD-1 inhibitor. Upon performing whole exome sequencing on biopsies from patients who progressed on therapy, we identified a list of candidate genes. We included genes that fell into one of the three categories: were mutated in more than one patient, were mutated only at relapse and had mutations in both alleles or had a heterozygous mutation at baseline and homozygous on progression. After generating a target gene list, we constructed a focused CRISPR library consisting of multiple gRNAs targeting each of those genes. We have used this library to perform a genetic screen in melanoma cells co-cultured with matched T-cells that recognize and eliminate the melanoma cells.

In addition, we also generated an adenovirus transformed mouse embryonic fibroblast cell line, which does not form tumors in the immunocompetent mice due to T-cell killing. When we knocked out B2M in the adenovirus transformed cells to rescue the effect of T-cell killing, we surprisingly did not observe tumor growth. We are now investigating the role of innate immune system in preventing tumor growth. We can then use this system to perform an *in vivo*CRISPR screen, investigating which genes are crucial for immune evasion. Next, we can cross validate hits from both platforms to identify robust hits that can potentially serve as predictive biomarkers for immunotherapy response.

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Macrophage-derived Granulin drives resistance to immune checkpoint inhibition in metastatic pancreatic cancer

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The ability of disseminated cancer cells to evade the immune response is a critical step for efficient metastatic progression. Protection against an immune attack is often provided by the tumour microenvironment that suppresses and/or excludes cytotoxic $CD8^+$ T cells. Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive metastatic disease with unmet needs, yet the immuno-protective role of the metastatic tumour microenvironment in pancreatic cancer is not completely understood. In this study, we find that macrophage-derived granulin contributes to cytotoxic $CD8^+$ T cell exclusion in metastatic livers. Mechanistically, we find that granulin expression by macrophages is induced in response to colony stimulating factor-1. Genetic depletion of granulin reduces the formation of a fibrotic stroma, thereby allowing T cell entry at the metastatic site. While metastatic PDAC tumours are largely resistant to anti-PD-1 therapy, blockade of PD-1 in granulin depleted tumours restored the anti-tumour immune defence and dramatically decreased metastatic tumour burden. These findings suggest that targeting granulin may serve as a potential therapeutic strategy to restore $CD8^+$ T cell infiltration in metastatic PDAC, thereby converting PDAC metastatic tumours, which are refractory to immune checkpoint inhibitors, into tumours that respond to immune checkpoint inhibition therapies.

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Expanded NK cells (eNK) in combination with daratumumab for the treatment of multiple myeloma

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Natural killer cells are part of our immune defense against transformed cells and are a promising option for use as an adoptive cellular therapy against cancer. To increase their therapeutic efficiency, human allogenic NK cells should be expanded and activated before its clinical use. The purpose of this study was to test the efficacy of expanded natural killer cells (eNKs) as a treatment against multiple myeloma (MM) cell lines and plasma cells from bone marrow aspirates of MM patients. We produced expanded allogenic NK cells using activation with feeder cells and cytokines. The cytotoxic effect of eNKs was studied alone and in combination with daratumumab, an anti-CD38 monoclonal antibody currently used in second line MM therapy. eNK cells were cytotoxic against MM cell lines, inducing a 20-70% specific cell death, depending on the cell line. In the case of MM patient samples, when eNKs were harvested during the optimum expansion period, they produced a cytotoxic effect against the MM cells. Daratumumab alone was also effective against some of the patient samples. However, when combined, eNKs and daratumumab produced a greater cytotoxicity than when they were used separately. These data indicate that the combination of eNK cells with daratumumab could be a promising approach for the treatment of MM.

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Immunogenicity and treatment of multiple myeloma

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Understanding cell death mechanisms is pivotal to design effective approaches for cancer treatment. Recent introduction of the concept of immunogenic cell death (ICD), as well as the current availability of immunotherapy for multiple myeloma (MM), has outlined the need to improve current immunotherapeutic protocols to take advantage of the immunomodulatory potential that ICD inducers have on the tumour itself and its microenvironment.

In the present study, we have examined the immunogenic potential and cell death mechanism exerted by new anti-myeloma drugs, such as the proteasome inhibitor carfilzomib, as well as its combination with endoplasmic reticulum (ER) stressors (chloroquine and DBeQ). Interestingly, chloroquine and DBeQ remarkably potentiated apoptosis induced by carfilzomib in MM cell lines and also in CD38+ bone marrow mononuclear cells (BMMC) isolated from MM patients. Moreover, the role of ER stress and the implication of caspases in triggering cell death has been evaluated. Concerning the immunogenicity of cell death, we have assessed the plasma membrane exposure of different ER chaperones. In particular, calreticulin (CRT), heat-shock protein 70 (Hsp70) and BiP levels were increased on the surface of MM dying cells. Furthermore, the implication of caspases and autophagy to the immunogenicity of cell death, particularly, to the translocation of CRT were investigated. Since accumulating clinical evidence indicates that CRT exposure may have a potential prognostic value in different human cancers, we also assessed the presence of ecto-CRT in BMMCs of MM patients, as well as its possible relationship with the BM immune profile ($CD4^+$/$CD8^+$ T cells, NK cells, Tregs, etc). Finally, given the positive impact that ICD inducers have on dendritic cell (DC) biology and its possible application in developing better DC anticancer vaccines, we also tested the capacity MM-treated cells to induce DCs maturation.

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Lung fibroblasts co-evolve during breast cancer pulmonary metastasis to acquire metastases-promoting transcriptional programs

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Mortality from breast cancer is almost exclusively a result of tumor metastasis, and lungs are one of the main sites of metastatic relapse. Formation of a hospitable microenvironment in distant organs is required for the establishment of metastases. Cancer-associated fibroblasts (CAFs) are prominent players in the microenvironment of many primary tumors, including breast cancer. However, the role of CAFs in the formation of a permissive metastatic niche is still largely unresolved. To characterize the dynamic changes in CAFs during the formation of lung metastases, we combined transgenic mice that develop autochthonous mammary tumors followed by spontaneous lung metastasis (MMTV-PyMT) with reporter mice in which all fibroblasts are fluorescently labeled (Col1α-YFP), thus enabling isolation of lung fibroblasts in an unbiased manner. We isolated fibroblasts from normal mice, or from mice with micro- or macro-metastases and profiled their transcriptome by RNA-Seq. Data analysis with multiple bioinformatics tools revealed that fibroblasts in the lung metastatic niche are transcriptionally dynamic and plastic, indicating that fibroblasts co-evolve during the process of pulmonary metastases formation. Characterization of the most prominent transcriptional programs indicated that the main tasks operative in metastases-associated fibroblasts include extracellular matrix remodeling, stress response and shaping the inflammatory microenvironment at the metastatic niche. Furthermore, we found that Myc is a prominent transcription regulator in fibroblasts during the process of metastases formation. Deepening our understanding of the functional contribution of stromal pathways to breast cancer metastasis is the key to developing therapeutic approaches that may prevent metastatic relapse.

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Metabolic crosstalk in the TME guides T cell differentiation and the anti-tumour response

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Introduction: Tumour infiltrating lymphocytes (TIL's) are capable of mounting an anti-tumour response. TIL's, however encounter a myriad of suppressive elements in the tumour microenvironment (TME), such as regulatory immune cells, soluble factors released by tumour cells and the presence of inhibitory molecules on the surface of tumour cells. Metabolic competition within the TME results in the modulation of immunometabolism, which may skew the T-cell response to a pro-tumour like phenotype resulting in exhaustion and failure to carry out effector functions. Understanding the impact of elements within the TME is vital to improving responses to new therapies such as immunotherapy.

Aim: To evaluate the impact of nutrient deprivation and hypoxia, common elements of the TME on T-cell differentiation and effector subsets.

Methods: A human $CD4^+$ T-cell line (Jurkat) was cultured in media simulating aspects of the TME. Nutrient deprivation was simulated using glucose and glutamine free media, Severe hypoxia (0.5% O_2) was induced using the Don Whitely Hypoxystation . T cells were analysed by Flow Cytometry and effector subset differentiation was evaluated by transcription factors expression (T-bet, EOMES, FOXP3, GATA3, RORyt).

Results: T-cells cultured in conditions of hypoxia and nutrient deprivation had altered expression of transcription factors suggesting that nutrient deprivation within the TME can affect TIL subsets and potentially plasticity.

Discussion: This study demonstrates that metabolic competition within the TME polarises T-cell responses by directly guiding the differentiation of distinct effector subtypes. Therefore, targeting T cell metabolism represents a promising immunomodulatory therapy for use in combination with immunotherapy

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Comparative immune profiling of an aggressive 4T1-based versus a non-aggressive Py230-based intraductal model for triple-negative breast cancer

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The intraductal model for breast cancer allows to investigate the human disease process from early ductal carcinoma *in situ* (DCIS) to late-stage invasive carcinoma (IC). However, detailed knowledge on the immunological changes associated with intraductal tumor growth is currently lacking. Our study aimed to characterize the tumor microenvironment upon DCIS to IC progression in the immunocompetent intraductal model, focusing on triple-negative breast cancer (TNBC). Therefore, BALB/c-derived 4T1 and C57BL/6-derived Py230 mammary tumor cell lines were intraductally inoculated in a syngeneic background and tumor growth was monitored weekly for 6 weeks. The 4T1 cells grew aggressively and invaded the ductal barrier more rapidly than the less invasive Py230 cells. The aggressive tumor progression in the 4T1-based model was accompanied by enhanced production of two immune-related biomarkers chitinase 3-like 1 and lipocalin 2 as well as by more severe splenomegaly compared to the Py230-based model. Flow cytometric and immunohistochemical analysis identified that the ductal breakthrough of both mammary tumor cell lines was associated with primary tumor infiltration of immune cells, including macrophages, neutrophils and T-cells. Furthermore, 4T1 primary tumors showed a more inflammatory and active tumor immune microenvironment based on cytokine profiles and the expression of granzyme B (biomarker for active cytotoxic T-cells) compared to Py230 primary tumors. Our results compared for the first time the immunological changes associated with aggressive versus non-aggressive triple-negative mammary tumor progression in the intraductal TNBC model, providing key information for its future use as immunotherapeutic screening tool.

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Influence of mesenchymal stem cells, isolated and cultured from glioblastoma multiforme samples on Tregs and Th17 isolated from healthy donors

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Glioblastoma multiforme (GBM) is one of the most aggressive tumors of the central neural system. In agreement with the data in the literature, our previous results described the presence of mesenchymal stem cells (GB-MSC) in the tumor microenvironment. MSC have proven immunosuppressive properties but there is no description of the action of GB-MSC on the lymphocyte populations.

The aim of our study was to isolate and culture GB-MSC and to investigate their influence (by secretory factors or by cellular contact) on two T helper subpopulations – Th17 and Treg. For this purpose, we isolated peripheral blood mononuclear cells (PBMCs) from healthy donors and cultured them with supernatant from GB-MSC. Also, we co-cultured PBMC with isolated GB-MSC.

Tregs in the lymphocyte population were tested based on the CD4, CD25 and FoxP3 markers, whereas Th17 were detected based on the expression of markers CD3, CD4, CD161, and CD196.

The classical Tregs - CD4+CD25+FoxP3+, showed a significant increase in both experimental settings. The results we obtained also demonstrate a statistically significant increase in the so-called "mysterious population" of T helpers - CD4+CD25-FoxP3+ cells, upon exposure to GB-MSC supernatants. Contrary, Th17 showed a significant decrease in the lymphocyte population when were co-cultured with GB-MSC cell cultures.

Conclusion: Based on our results we can conclude that GB-MSC participate actively in the regulation of the T helper populations and exert well established for other MSC types immunosuppressive functions by secretory factors and /or by cellular contact.

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Study of “inmunocheckpoint” genes expression in histological variants of colorectal carcinoma

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Since the discovery of “inmunocheckpoints” proteins and their role in cancer, there have been a great development of immunotherapy therapies for treating cancer patients. One of these therapies is the use of anti “inmunocheckpoints” strategies as anti CTLA4 or anti PD-L1 treatments. In colorectal cancer, only a small percentage of patients get a benefit from these therapies.

Conventional carcinomas (CCs) which represent most colorectal carcinomas (CRCs) have no alteration in microsatellite instability (MSI) and are classified as microsatellite stable tumors (MSS) while tumors with high-grade of microsatellite instability (MSI-H) has been associated with proximal-located sporadic CRC showing MLH1 promoter methylation and BRAF mutation. MSI-H tumors present more lymphocytic infiltrate than CCs. MSI-H tumors are considered as one end-point of the so-called serrated polyp pathway. In contrast, another CRC from this pathway, the serrated adenocarcinoma (SAC), which is more frequently KRAS mutated, usually microsatellite stable (MSS) and has no lymphocytic infiltrate, is associated with a bad prognosis.

Patients with MSI-H tumors have been reported to get some benefits from immunotherapy treatments while CC and SACs patients do not obtain any benefits. These differences are believed to be due to the differences in the microsatellite instability (MSS or MSI tumors).

The aim of this study is to determine the “inmunocheckpoint” genes expression in the different subtypes of colorectal carcinomas.

Results: After analyzing PD-L1 and CTLA4 expression in CC, SAC and MSI-H tumors (11 CC, 19 SAC, 9 MSI-H), CTLA4 expression is higher in SAC than in CC or MSI-H tumors (p=0,44) while the difference in PD-L1 expression is not statistically significant among the tumors (p=0,906). The difference between MSS and MSI-H tumors was also analyzed but no statistically differences were found (CTLA4 p=0,317; PD-L1 p=0,460).

These results suggest that other factors may be decisive for the immunotherapy results in colorectal patients.

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A novel combination immunotherapy for pancreatic cancer: IL-15 and anti-CD40 stimulation join hands to raise the stakes

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Background: Pancreatic cancer (PC) is the 3rd deadliest cancer worldwide with the lowest 5-year survival of all cancers. Therapeutic improvements have barely been made over the last decade. Within the tumour microenvironment, tackling the stromal shield is needed to overcome treatment resistance. CD40 stimulation has already demonstrated moderate anti-tumour responses in PC, including some anti-stroma effects. We have shown that interleukin (IL)-15 stimulated NK cells are capable of tackling both tumour as well as the surrounding desmoplastic stroma. Therefore, we explored a novel combination immunotherapy consisting of an agonistic anti-CD40 monoclonal antibody and IL-15 in two mouse models of PC.

Methods: C57BL/6 mice bearing Panc02 or KPC tumours were treated over a two-week period with IL-15 and/or anti-CD40. Tumour kinetics and survival were monitored. Tumour infiltrating lymphocytes (TIL) were characterised using multicolour flow cytometry and immunohistochemistry. Experiments depleting different immune cell populations were performed. Re-challenge experiments were executed to check immune memory induction.

Results: Combination treatment of IL-15 and anti-CD40 caused distinct reduction of tumour growth rates in comparison with single agent treatments. Mice receiving the combination treatment showed significantly increased survival, with 60-80% of the mice becoming completely tumour free. Characterisation of TIL revealed that the combination caused increased amounts of infiltrating CD8+ T cells, NK cells and neutrophils while T regulatory cells were decreased. Depletion of CD8+ T cells and NK cells confirmed that both immune cells are mechanistically involved. Re-challenge experiments showed induction of immune memory by IL-15 but anti-CD40.

Conclusion: To our knowledge, this is the first study demonstrating that combination of IL-15 and anti-CD40 exhibits a profound anti-tumour response in two mouse models of PC resulting in prolonged survival and even total eradication of >60% of PC. These data provide a solid proof of principle to advance with this combination strategy to a clinical trial.

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Understanding the tumour cell-immune cell relationship in the glioblastoma microenvironment

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Immunotherapy is a promising therapy for cancer, including difficult to treat cancers such as glioblastoma (GBM), the most common and aggressive form of brain cancer in adults. However, immunosuppression in GBM is a major problem in developing specific immunotherapies. Moreover, current clinical studies demonstrate non-uniform efficacies among different patients and types of GBM. As immunosuppression may be influenced by GBM molecular characteristics, it is likely that different types of GBM have different immunological profiles. Therefore, it is important to investigate the immunological profiles in different types of GBM to more effectively rationalise new immune-therapeutic strategies. This study aims to identify the types, number, and cell-to-cell localisation of specific immune cells, and to characterize the function and potential role of the immune cells via transcriptomic profiling. Immuno-profiling was performed using multiplex immunohistochemistry with two antibody panels. The first panel included CD3 (T cells), CD68 (macrophages), CD11c (dendritic cells), CD20 (B cells), and TMEM119 (microglia), and the second panel included antibodies specific for T cell lineage, such as CD8 (cytotoxic T cells), CD4 (helper T cells), and FOXP3 (regulatory T cells). My data suggests high infiltration of innate immune cells in the GBM microenvironment, including dendritic cells, macrophages, and microglia, and low numbers of effector T cells, which are mostly localized around the vasculature. Interestingly, a high number of Nestin^{+} cells were also seen around this area, suggesting a possible immunosuppressive interaction between glioma stem-like cells and tumour infiltrating lymphocytes. Future research will focus on comparing the immunological profiles of different types of GBM and deciphering the lineage of GBM infiltrating immune cells through deconvolution bioinformatic analysis and single-cell RNA sequencing.

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Coordinated signals from PARP-1 and PARP-2 promotes an immune response to tumours

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Abstract withheld at the author's request

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Immune-mediated negative selection removes neoantigens in highly infiltrated tumors

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Cancer is an evolutionary process where somatic mutations interplay with the environment allowing for adaptation and drift. The role of the immune system has for long time been recognized in cancer, but whether tumors primarily grow by adapting to immune-mediated negative selection or by acquisition of immune-escape phenotypes remains an open question. In recent years, next generation sequencing technologies have allowed us to explore the imprinted signatures of positive and negative selection in the cancer genome. Here, we study the extent of immunoediting associated to negative selection of antigen-presenting clones versus acquisition of novel immuno-suppressive phenotypes. We classified more than 7000 tumors from TCGA as hot or cold using pathology-based or RNA-seq based mesurements. Then, we compare the extent of immune-mediated negative selection using dN/dS of clonal versus subclonal neoantigens in immune-hot and immune-cold regions. We found that the extent of immunoediting was tissue specific and associated to the amount of lymphocyte infiltration.

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Novel BAFF-receptor antibody to natively folder and glycosylated recombinant protein eliminates drug resistant B cell malignancies in vivo

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Rituximab is a proven therapeutics for lymphoma, yet primary or acquired resistance occurs in relatively high rates. B cell activating factor receptor (BAFF-R/TNFRSF13C) is a tumor necrosis factor receptor superfamily member specifically involved in B lymphocyte development and mature B cell survival. We developed novel therapeutic antibodies, C55 and C90, against BAFF-R for the purpose of treating Rituximab-resistant patients. The mAbs bound with high affinity to human B cell lymphoma, and were proven to be specific to B-cell-containing organs such as tonsil and spleen demonstrated by immunohistochemistry. C55 and C90 were able to elicit ADCC in primary human lymphomas: they efficiently killed tumor cells derived from patients with lymphoma and chronic lymphocytic leukemia (CLL) (n=8). Notably, 5 of these primary lymphomas were from patients who had relapsed after rituximab treatment. We next determined the activity of C55 and C90 in animal models of drug resistant lymphoma in NOD *scid* gamma (NSG) mice model, and observed remarkable *in vivo* anti-tumor effects of both antibodies that lasted beyond 90 days. Our *in vitro* and *in vivo* results strongly support the translational nature of our novel BAFF-R specific antibodies. One of the antibodies, C90, went through intensive antibody engineering to improve its manufacturability and is currently in cell line development.

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