PIVAC-18
18TH INTERNATIONAL CONFERENCE ON PROGRESS IN VACCINATION AGAINST CANCER
3rd – 5th of October 2018 - Oslo, Norway
DEAR ATTENDEES

We are delighted to welcome you to the 18th International Conference on Progress in Cancer Vaccination in Oslo!

This is the first PIVAC meeting in Oslo and we hope that the venue provides a successful technical conference as well as some of the culture and scenery of Norway with ample opportunity for collaboration and informal networking.

PIVAC is a highly focused meeting devoted entirely to the development and use of immunotherapies against cancer, in particular cancer vaccines. It reflects the dedicated commitment of the scientific community to the progress of cancer vaccination, the impact that tumour microenvironment and other factors can have on their efficacy and their place in clinical use as monotherapy or in combination with other treatments. This meeting remains one of the few meetings of this size with an impressive speaker list and scientists and clinicians dedicated to this area of research.

We would like to thank the people who have worked with us in planning and organising the scientific program, practicalities, bookings, and supporting social activities and our sponsors who have helped us make this possible. We have a Tannlege Aase & Frue Memorial Lecture and an EACR-sponsored lecture.

Thank you to the entire faculty of the program who gave their time and talent to make this a great scientific conference. We hope you will take every opportunity to broaden your horizons in some of the most important topics in cancer immunotherapy.

We wish you a great PIVAC-18!

On behalf of the organising committee
Sincerely,

Else Marit Inderberg

Organizing committee:
SESSION 1  -  Chair: Mads Hald Andersen

13:30 - 13:45  Welcome
Sigbjørn Smeland, Head of Cancer Division

13:45 - 14:15  Targeting the tumor microenvironment with anti-regulatory T cells
Mads Hald Andersen, Copenhagen University Hospital Herlev, Denmark

14:15 - 14:45  The myeloid intervention in metastatic process
Vincenzo Bronte, University of Verona, Italy

14:45 - 15:15  Proffered papers
Evelina Martinenaite, Center for Cancer Immunotherapy, Herlev Hospital, Denmark: “Targeting arginase in tumor microenvironment”
Anne Marit Sponaas, Norwegian University of Science and Technology, Norway: “PD1 is expressed on exhausted T cells as well as virus specific memory CD8+ T cells in the bone marrow of myeloma patients”

15:15 - 15:30  Coffee Break

15:30 - 16:00  Immunoregulatory circuits in melanoma and their neutralization
Viktor Umansky, Faculty for Biosciences, University of Heidelberg, Germany

16:00 - 16:30  Proffered papers
Panagiotis Christopoulos, Oslo University Hospital, Norway: “Interferons synergize with either TLRs or CD40-induced signaling to efficiently render macrophages tumoricidal in vitro”
Asha Nur Gutale, Oslo University Hospital, Norway: “Immunogenic modulation of A549, a non-small cell lung cancer (NSCLC) cell line”

16:30 - 16:45  Coffee Break

SESSION 2  -  Chair: Sue Oestrand-Rosenberg

16:45 - 17:15  What does tumor innervation mean?
Michael Shurin, University of Pittsburgh, USA

17:15 - 17:45  Soluble-izing and taking a BiTE out of PD-1-mediated immune suppression
Sue Oestrand-Rosenberg, University of Maryland, USA

17:45 - 18:30  Proffered papers
Fakhri Hassouneh, University of Extremadura, Cáceres, Spain: “MicroRNA expression profiling in acute myeloid leukaemia patients and healthy donors according to age”
Nadia Mensali, Oslo University Hospital, Norway: “Antigen-delivery through Invariant chain (CD74) boosts CD8 and CD4 T cell immunity”
Anne Merete Tryggestad, Oslo University Hospital, Norway: “A first in man phase I/II adjuvant dendritic cell vaccine study in high-risk prostate cancer patients following radical surgery reduce the incidence of biochemical relapse”

19:00 - 19:30  Get together - Holmenkollen
SESSION 3  -  Chair: Bjarne Bogen

PRE-CLINICAL

09:00 - 09:30  
CD4+ T cells in the microenvironment and tumour rejection  
Bjarne Bogen, Oslo University Hospital, Norway

09:30 - 10:00  
New approaches against AML, EBV and CMV and preclinical testing in humanized mice  
Renata Stripecke, Hannover Medical School, Germany

10:00 - 10:30  
Proffered papers  
Zuzana Strizova, Charles University in Prague and Motol University, Czech Republic: “TRAIL, FasL, and PECAM-1 expression in T cells and NK cells is upregulated in peritumoral tissue as compared to cells isolated from renal carcinoma and healthy renal tissue”

Marlene Fyrstenberg Laursen, Aalborg University, Department of Health, Science and Technology, Denmark: “Investigations on a Novel Dendritic Cell-Targeted Adjuvant for Anti-Cancer Therapy.”

10:30 - 11:30  
Poster session and coffee

11:30 - 12:00  
The dual role of TAMs as oncogenes and immune suppressants  
Marlies Peeters, Copenhagen University Hospital Herlev, Denmark

12:00 - 12:30  
Vaccination using immunopeptidome-identified antigens for malignant glioma  
Valérie Dutoit, University of Geneva, Switzerland

12:30 - 13:00  
Pre-clinical models for melanoma and osteosarcoma immunotherapy: Dawn of the dogs’ revolution  
Federica Cavallo, University of Torino, Italy

13:00 - 14:00  
Lunch
Activated integrins identify functional antigen-specific CD8+ T cells within minutes after antigen stimulation  
**Cécile Gouttefangeas, University of Tübingen, Germany**

Proffered paper  
**Baiba Olupe, Oslo University Hospital, Norway:** “Lewis lung carcinoma mutanome – a source of neoantigens for cancer vaccine based on Semliki Forest virus vector”

The tumor microenvironment in HPV-induced cancer: implications for immunotherapy  
**Saskia Santegoets, Leiden University Medical Center, Netherlands**

Poster session and coffee

HLA class I loss and cancer immune escape: Rediscovering an old story  
**Federico Garrido, University of Granada, Spain**

Mechanisms and clinical relevance of immune escape of tumors and its role in immunotherapies  
**Barbra Seliger, Martin-Luther-University Halle-Wittenberg, Germany**

Synthetic immune systems to outsmart cancer  
**Carl Figdor, Radboud University Nijmegen Medical Centre, Netherlands**

Conference reception - POSTER PRIZES
Therapeutic HPV16 vaccination is effective as monotherapy in precursormalignt disease, but requires combination treatment in HPV16-induced cancers

Cornelis Melief, Leiden University Medical Center, Netherlands

Complete and long-lasting responses in patients with advanced checkpoint blockade resistant melanoma treated with Adoptive T cell transfer combined with DC vaccination

Rolf Kiessling, Karolinska Institute, Sweden

T Cell Receptor Gene Transfer: How Affinity, Structure, and Crossreactivity Impacts T Cell Function

Michael Nishimura, Loyola University Chicago Stritch School of Medicine, USA

Coffee Break

Immunopeptidomics: Accelerating the development of personalized cancer immunotherapy

Michal Bassani-Sternberg, Ludwig Institute for Cancer Research Lausanne, Switzerland

Boosting immunotherapeutics cells

Pierre Dillard, Oslo University Hospital, Norway

Prognostic significance of immune infiltrates in breast cancer

Costas Baxevanis, St. Savvas Hospital Athens, Greece

Proffered papers

Caroline Laheurte, University Bourgogne Franche-Comte, INSERM UMR1098, France: “Adaptive CD4 Th1 response against telomerase in blood counteracts T-cell exhaustion in non-small-cell lung cancer”

Mia Aaboe Jørgensen, Center for Cancer Immune Therapy, Herlev Hospital, Denmark: “Characterization of immune responses in patients diagnosed with Waldenström’s Macroglobulinemia”

Lunch
SESSION 6 - Chair: Richard Olaussen

14:00 - 14:30  
**Tannlege Olaf Aase og frues Memorial Lecture**  
The Vaccine-Site Microenvironment: Immune activation and regulation at the source  
*Craig Slingluff, University of Virginia School of Medicine, USA*

14:30 - 15:00  
**EACR sponsored Lecture**  
Therapeutic anti-telomerase vaccine targeting CD4 helper T-cells in Advanced Non-Small Cell Lung Cancer. A phase I/II study (UCPVax trial)  
*Olivier Adotévi, University Hospital of Besancon, France*

15:00 - 15:30  
**CMV infection, cancer and survival in the elderly**  
*Graham Pawelec, University of Tübingen, Germany*

15:30 - 15:45  
**Coffee Break**

15:45 - 16:15  
Development, clinical experiences and future role of therapeutic DC vaccines at Oslo University Hospital  
*Gunnar Kvalheim, Oslo University Hospital, Norway*

16:15 - 16:45  
Development and clinical testing of UV1 – a second-generation cancer vaccine targeting the Reverse Transcriptase subunit of human Telomerase (hTERT)  
*Gustav Gaudernack, Ultimovacs AS and University of Oslo, Norway*

SATURDAY 6TH OCTOBER  
Tour of GMP facility Oslo Cancer Cluster  
Social event
Targeting the immunosuppressive tumor microenvironment with anti-regulatory T cells

Mads Hald Andersen
Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark

Equilibrium between immune activation and suppression may be necessary to maintain immune homeostasis, since pro-inflammatory effector T-cells, recently defined as anti-regulatory T cells (anti-Tregs), counteract the functions of regulatory immune cells. Anti-Tregs are naturally occurring T cells that can directly react against regulatory immune cells because they recognize proteins that these targets express, including indoleamine 2,3-dioxygenase (IDO), tryptophan 2,6-dioxygenase, arginase, and programmed death ligand 1 (PD-L1). The activation of such pro-inflammatory effector T-cells offers a novel way to directly target the tumor microenvironment potentially giving them considerable clinical value especially in patients with cancer. Therapeutic vaccination against genetically stable cells with regular HLA expression is an attractive way to directly target immunosuppressive cells in addition to attracting pro-inflammatory cells into the tumor microenvironment. Importantly, vaccination to potentiate anti-Tregs have shown great effect in pre-clinical studies and have proven safe with minimal toxicity in the clinical phase I trials conducted thus far.

The myeloid intervention in metastatic process

Vincenzo Bronte
University of Verona, Verona, Italy

NO ABSTRACT

Targeting arginase in tumor microenvironment

Evelina Martinenaite1, Shamaila Munir Ahmad1, Simone Kloch Bendtsen1, Stine Emilie Weis-Banke1, Mia Aaboe Jørgensen1, Inge Marie Svane1, Mads Hald Andersen1
1Center for Cancer Immune Therapy, Herlev Hospital, Herlev, Denmark

Introduction: Cancer progression is associated with an increased immune suppression at the tumor site. Arginase is an enzyme expressed by immune inhibitory cells, such as myeloid derived suppressor cells (MDSCs), reducing arginine availability to the tumor infiltrating immune cells and thus reducing T cell functionality in the tumor milieu. Materials and Methods: We characterized spontaneous immune responses against optimized 38-mer arginase-1-derived peptide in cancer patients and healthy donors using ex vivo and in vitro IFNy ELISPOT and intracellular staining for IFNγ and TNFα. T cell responses were further characterized by combining ELISPOT and magnetic bead sorting of CD4+ and CD8+ memory T cells. The effect of upregulated arginase-1 expression on activation of naturally present arginase-1 specific T cells was investigated by combination of IL-4 stimulation and IFNy ELISPOT. Results and Discussion: We have previously shown that arginase-1 specific T cells can be isolated, expanded and are able to recognize arginase-1 expressing immune cells. In this study, we were further able to demonstrate that T cells recognizing an optimized 38-mer arginase-1 peptide are a natural part of the T cell repertoire, since arginase-1 specific CD4+ and CD8+ memory T cells were found in both healthy donors and cancer patients. We have also shown that arginase-1 specific T cells could be activated by the IL-4-induced upregulation of arginase-1 expression, which suggests their potential role in regulating the immune inhibitory mechanisms. Conclusion: Our study shows that arginase-1 specific CD4+ and CD8+ T cells are a natural part of the immune system, which makes vaccination using arginase-1 derived peptides a promising approach to effectively target arginase producing tumors and inhibitory immune cells such as M2 macrophages and MDSCs in cancer microenvironment.
PD1 is expressed on exhausted T cells as well as virus specific memory CD8+ T cells in the bone marrow of myeloma patients

Anne Marit Sponaas1, Rui Yang1, Even Holth Rustad1, Therese Standal1, Anders Waage1, 2, Tobias S Sløre1, 2, Magne Børset1, 2, and Anders Sundan1
1NTNU, Trondheim, Norway; 2St Olavs Hospital, Trondheim, Norway

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Immunoregulatory circuits in melanoma and their neutralization

Viktor Umansky, Mareike Grees, and Jochen Utikal
Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg and Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany

Despite melanoma immunogenicity and remarkable therapeutic effects of negative immune checkpoint inhibitors, a significant fraction of patients does not respond to current treatments. This could be due to limitations in tumor immunogenicity and profound immunosuppression induced by chronic inflammation in melanoma microenvironment. Moreover, insufficient tumor antigen processing and presentation by dendritic cells (DC) may hamper the development of tumor-specific T cells. Using two genetically engineered mouse melanoma models (RET and BRAFV600E transgenic mice), in which checkpoint inhibitor therapy alone is not efficacious, we applied vaccination with DC expressing multiple chimeric MHC class I receptors. This permits a simultaneous presentation of several melanoma-associated antigens. We found that the DC vaccine significantly improved survival in both transgenic mouse models. CD8 T cells from vaccinated melanoma-bearing mice upregulated activation markers and produced more IFN-γ. Moreover, myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) known to be accumulated in tumor microenvironment were found to express an attenuated immunosuppressive pattern upon the treatment. The combination of DC vaccination with ultra-low doses of paclitaxel or anti-PD-1 antibodies resulted in further prolongation of mouse survival associated with a stronger reduction of MDSC and Treg immunosuppressive phenotype. Our data suggest that a multivalent DC vaccine based on shared tumor antigens induces potent anti-tumor effects and could be combined with checkpoint inhibitors or targeting MDSC to further improve their therapeutic efficiency.
Interferons synergize with either TLRs or CD40-induced signaling to efficiently render macrophages tumoricidal in vitro

Panagiotis Christopoulos¹, Anna Lunde¹, Elisabeth Müller¹, Theodossis Theodossiou², Inger Øynebråten¹, and Alexandre Corthay¹
¹Oslo University Hospital - Rikshospitalet, Oslo, Norway
²Oslo University Hospital - Radium, Oslo, Norway

Introduction: TAMs represent a main component of the tumor-infiltrating leukocytes and therefore re/polarization into their anti-tumor M1 phenotype has raised great interest in cancer immunotherapy. IFNγ and/or LPS have been described as the typical inducers of the classical M1 activation, however less is known about other molecules and their potential functional and phenotypic-induced differences on macrophages activation.

Materials & Methods: LPS, Pam3CSK4, IFNγ, IFNβ and soluble CD40L were used alone or in combinations to activate mouse BMDM. Growth inhibition of LLC cells was investigated using a co-culture in vitro assay. Endocytosis, production of NO and cytokines, expression of cell markers, as well as, the metabolic and intrinsic properties of polarized BMDM were also analyzed.

Results & Discussion: We found that IFNγ induced growth inhibition of cancer cells only when it was used in combination with TLR agonists or sCD40L. Similarly, IFNβ also synergized with TLR ligands for induction of cancer cell growth inhibition. In addition, combinational treatments synergistically upregulated NO, as well as TNFα and IL-12 production in BMDM, whereas IL-10 secretion was suppressed. IFNγ alone or in combination with LPS or Pam3 downregulated experimental endocytosis by macrophages. Furthermore, activated BMDM upregulate CD38, CD40, CD86, MHCII and PD-L1 in different expression patterns depending on the applied stimuli. The mitochondrial respiration was suppressed upon macrophage activation and to the greatest extent following combinational treatments. Finally, proliferation of activated macrophages was negatively associated with NO production whereas apoptosis was not greatly affected by activation. Herein we have shown that TLR agonists, CD40 activation and/or interferons promote distinct functional and phenotypic properties in so-called M1 macrophages.

Conclusions: We conclude that activation of more than one signaling pathway is required to efficiently induce macrophage tumoricidal activity in vitro. Our results point to the potential importance of multiple signal activation in the development of macrophage-mediated cancer immunotherapeutics.

Immunogenic modulation of A549, a non-small cell lung cancer (NSCLC) cell line

Asha Nur Gutale, Justyna Stokowiech, Alexandre Corthay, and Inger Øynebråten
Oslo University Hospital, Rikshospitalet, Oslo, Norway

Background: Most chemotherapeutics are applied with the sole purpose of causing cancer cell death irrespective of the cell death mode. Treatment of cancer cells with immunomodulatory agents can trigger the cells to undergo a type of cell death called immunogenic cell death (ICD). ICD is associated with release of damage-associated molecular patterns (DAMPs) such as HMGB1, a ligand of Toll-like receptor 4 (TLR4) expressed by e.g. antigen-presenting cells. Here, we examined whether chemotherapeutics used in pre-clinical studies and in treatment of human cancers have immunomodulatory effects on a NSCLC cell line.

Aim: Investigate whether the chemotherapeutics bortezomib, mitoxantrone, and salinomycin induce a type of cancer cell death which can be regarded as immunogenic.

Method: The human NSCLC cell line A549 was treated with bortezomib, mitoxantrone, or salinomycin in 24-72 h. Sensitivity to the treatment was determined by trypan blue, and flow cytometry following labelling with annexin V and propidium iodide. Intracellular localization and release of HMGB1 into the culture medium was examined by immunofluorescence microscopy and western blotting.

Results: Treatment with bortezomib or mitoxantrone inhibited the viability of A549 to an extent which correlated with a time dependent increase in the proportion of apoptotic cells. Under normal conditions, HMGB1 is localized in the nucleus. Upon treatment with bortezomib or mitoxantrone, HMGB1 translocated into the cytoplasm and was released into the extracellular space. Salinomycin treatment inhibited A549 proliferation, but did not result in significant cell death. Extracellular HMGB1 was not detected following salinomycin treatment, which is consistent with observed absence of translocation of nuclear HMGB1.

Conclusion: The chemotherapeutics bortezomib and mitoxantrone result in release of HMGB1 from A549, coincident with induction of cell death. The ability of the agents to promote full ICD and elicit an immune response converting cancer cells into an in situ vaccine needs to be explored further.
What does tumor innervation mean?

Michael R. Shurin, Yuri L. Bunimovich, Galina V. Shurin
University of Pittsburgh Medical Center, Departments of Pathology, Immunology and Dermatology, Pittsburgh, PA, USA.

Tumor development and progression largely depend on the interactions between the malignant cells and other components of the microenvironment such as fibroblasts, immune and endothelial cells. Neurons are also often identified within solid tumors, but their impact on tumor growth remains poorly understood. Our new data suggest that glial cells, which cover the peripheral nerve axons, can be detected in solid tumors and may play an important role in the formation of the tumor microenvironment by attracting and activating immunosuppressive immune cells. Using both human and murine models, we have recently revealed that cross-talk between malignant cells, peripheral neurons and neuroglial cells plays a role in tumor growth and formation of metastasis in vivo. Our data demonstrate that neuroglial cells can be activated by cancerous cells resulting in up-regulated release of chemokines and attraction and activation of myeloid regulatory cells and thus formation of inflammatory-like immunosuppressive microenvironment. In addition, tumor-activated neurons may also regulate immunomodulatory properties of neuroglial cells and thus participate in shaping specific properties of the tumor microenvironment. Altogether, our new data not only introduce new players in the tumor milieu, but also support a new concept of tumor-neuronal-immune axis controlling activity of immune cells in tumor progression. These data will also lead to the development of a novel antitumor therapeutic strategy: mechanism-based targeting of neuroglial elements in the tumor microenvironment.

Soluble-izing and taking a BiTE out of PD-1-mediated immune suppression

Suzanne Ostrand-Rosenberg¹,², Lucas A. Horn², Nicholas G. Ciavattone³
¹Huntsman Cancer Institute & University of Utah, Salt Lake City, UT, USA
²University of Maryland Baltimore County, Baltimore, MD, USA
³Marlene & Steward Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, MD, USA

Immunotherapies aimed at neutralizing the programmed death-1 (PD-1) pathway have yielded significant therapeutic efficacy in a subset of cancer patients and are now first or second-line therapies for treating patients with several different types of malignancies. However, only a subset of patients responds to antibody therapy with either anti-PD-1 or anti-PD-L1 antibodies. These patients appear to have so-called “hot” tumors containing tumor-reactive T cells. Therefore, checkpoint blockade therapy may be effective in a larger percentage of cancer patients if combined with therapeutics that also activate tumor-reactive T cells. Therefore, we are developing two therapeutics with the potential to activate T cells while simultaneously neutralizing PD-1-mediated immune suppression. One of the therapeutics is a CD3xPD-L1 bispecific T cell engager (BiTE). This BiTE activates and targets both T cells and NKT cells to make them specifically cytotoxic for PD-L1+ tumor cells, despite the presence of myeloid-derived suppressor cells. The CD3xPD-L1 BiTE significantly extends the survival time and maintains activated immune cell levels in humanized NSG mice reconstituted with human PBMC and carrying established metastatic human melanoma tumors. The second therapeutic is a soluble form of the costimulatory molecule CD80 (sCD80). CD80 not only costimulates by binding to T cell-expressed CD28 but inhibits immune suppression by binding to PD-L1 with a similar binding affinity to PD-1. Based on this latter affinity, we have shown that human and mouse sCD80 simultaneously activate their respective species’ T cells via CD28 and prevent T cell anergy by binding tumor cell-expressed PD-L1. Studies with syngeneic mice demonstrate that sCD80 increases tumor-infiltrating T cells and significantly extends survival time. Although sCD80 binds to the coinhibitory receptor CTLA-4, it does not suppress T cell function, leading us to hypothesize that CTLA-4 acts as a decoy receptor for CD80, rather than as a suppressive signaling receptor. These studies suggest that the CD3xPD-L1 BiTE and sCD80 may be efficacious therapeutics either as monotherapies or in combination with other therapies for the treatment of cancer.
Antigen-delivery through Invariant chain (CD74) boosts CD8 and CD4 T cell immunity

Nadia Mensali*, Amalie Grenov ψ‡, Niladri Bhusan Pati§, Pierre Dillard*, Marit Renée Myhre*, Gustav Gaudernack†, Gunnar Kvalheim*, Else Marit Inderberg*, Oddmund Bakke ψ ‡, Sébastien Wälchli*

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†Department of Cancer Immunology, Institute for cancer Research, Oslo University Hospital-Radiumhospitalet, Norway

Eradication of tumours by the immune system relies on efficient activation of a T-cell response. For many years, the main focus has been on CD8+ T cells and the cytotoxic effects they exert. However, growing evidence have demonstrated that stimulation of CD4+ helper T cells is critical for promotion and maintenance of immune memory and long lasting tumour eradication; thus a good vaccine should evoke a two-dimensional T-cell response. We have previously shown that CLIP-modified Invariant chain (Ii) could be used efficiently to load MHC-I molecules with antigenic peptides. We here focus our study on a therapeutically relevant peptide derived from a frameshift mutation in the TGFBRII gene observed in 76% of colorectal cancer patients with microsatellite instability (MSI). This frameshift mutation results in the synthesis of a neo antigen containing overlapping peptides presented by both MHC-I and MHC-II. We took advantage of the fact that we possess TCR specific for three different alleles. We thus generated an Ii construct in which CLIP was exchanged with a long peptide derived from the frameshift. When this construct was electroporated into antigen presenting cells, both MHC-I and -II were loaded as they could be detected by specific TCR. We further analysis the loading of MHC-I and showed that it took place in the endosomal pathway. In addition, we present evidence that antigen presentation after Ii-loading was superior to an ER-targeted minigene construct. Finally, we verified that Ii-expressing dendritic cells could prime CD4+ and CD8+ T cells from a naive population. Taken together, our data show that expanding the CLIP replacement size to cover a larger region of the antigen allows simultaneous loading of cancer specific peptides on MHC-I and MHC-II molecules which leads to activation of both CD8+ and CD4+ T cells. This demonstrates that CLIP replaced Ii constructs fulfill some of the major requirements for an efficient vector for cancer vaccination.

A first in man phase I/II adjuvant dendritic cell vaccine study in high-risk prostate cancer patients following radical surgery

Oslo University Hospital Hospital, Oslo, Norway

Prostate cancer patients diagnosed with high Gleason score (≥ 8) and large tumors (≥T2c) are considered high-risk patients and >50% will develop an early biochemical relapse following radical surgery. Presently, there is no curative therapy available for patients when biochemical relapse occurs. Based on encouraging clinical results from 6 relapsed prostate cancer patients treated under hospital exemption with dendritic cell (DC) vaccines we started an adjuvant, first in man, phase I/II study using autologous, monocyte derived DCs targeting autologous tumor antigens from primary tumor, combined with hTERT and survivin. Twenty patients with pathological stage pT2-pT3b and Gleason score 7b-10 were included in the study. Following surgery prostate specific antigen (PSA) was <0.2 µg/L in all patients. Fifteen patients have received 3-days DCs generated according to our standard protocol with a maturation cocktail composed of GM-CSF, IL-4, TNFα, IL1β and PGE2. Five patients were treated with a TLR 7/8-ligand containing maturation cocktail, resulting in DCs with a polarized release of IL-12p70 and no or low IL-10. The patients received 4 weekly vaccinations, then DTH vaccine at week 8 and thereafter-monthly vaccination the first year, and every third month the second and third year. All patients have completed vaccination. In total 60% of the patients remain without biochemical relapse with a mean observation time of 62 (range 42-89) months. We confirm that the study is feasible and safe. Immune responses in the patients are under investigation. Altogether, our clinical results are promising and the use of adjuvant DC vaccines might become a new approach to prevent biochemical relapse in high-risk prostate cancer patients following radical surgery.
**CD4+ T cells in the microenvironment and tumor rejection**

Bjarne Bogen, Marte Fauskanger, Ole Audun Haabeth, Anders Tveita
University of Oslo and Oslo University Hospital

It has become increasingly clear that CD4+ T cells play an important role in rejection of tumors. Importantly, CD4+ T cells can eliminate tumors even in the absence of CD8+ T cells. The mechanism for this has been studied by our research group for more than two decades, using a mouse multiple myeloma model. The myeloma protein produced by the MM cells is processed by antigen presenting cells and a Variable region peptide [an idiotypic (Id) peptide/neoepitope] is presented on an MHC class II molecule to CD4+ T cells. To study rejection of tumor cells by CD4+ T cells, a TCR-transgenic mouse was made. It was shown tumor-infiltrating macrophages become primed with tumor-specific antigen, enabling stimulation of tumor-infiltrating Th1 cells. The stimulated Th1 cells secrete IFN resulting in macrophage acquisition of an M1 phenotype and an ability to kill tumor cells. Such an indirect killing mechanism implies that tumor cells do not need to express MHC class II molecules to be rejected. To test this, we deleted MHCII in tumor cells by CRISPR/Cas9 technology. Experiments were done both in an MM model, a B lymphoma model and a melanoma model (B16). In either case, MHC class II molecules were not needed for tumor rejection. However, secretion of tumor specific antigen and priming of tumor infiltrating macrophages appeared to be required. Our findings indicate that indirect killing via macrophages is a dominant pathway for CD4+ T cell-mediated killing of tumor cells. Activated macrophages kill tumor cells by an iNOS-dependent mechanism whereby peroxynitrite generated in tumor cells induces apoptosis by the intrinsic pathway.

**New approaches against AML, EBV and CMV and preclinical testing in humanized mice**

Renata Stripecke
Hannover Medical School, Hannover, Germany

Patients with hematologic malignancies face the risks of disease relapse and viral reactivations after stem cell transplantations. Minimal residual disease, if not controlled by the immune system, can lead to drug resistance and fatal relapse. Human cytomegalovirus (HCMV) and Epstein Barr virus (EBV) reactivations are associated with high morbidity and mortality. Our laboratory explores the targeted genetic manipulation of cellular components of the immune system to lower these risks. We have a long-standing experience with ex vivo genetic reprogramming of monocytes with multicistronic lentiviral vectors, which are capable of differentiating into “SMART” induced dendritic cells (SMART-iDCs) in vivo. The iDCs can be individualized to specific indications through the antigens co-expressed in combination with GM-CSF and IFN-alpha. The validation of SMART-iDCs in mice transplanted with human hematopoietic stem cells (“humanized mice”) showed profound effects in regeneration of lymph nodes and development and maturation of de novo human functional T and B cells. Generation of cryopreserved iDCs under good manufacturing practice was achieved within 24 hours after CD14+ isolation. We are currently exploring fully automated systems for iDC manufacturing in order to enable large-scale distribution for multicenter clinical trials to immunize patients against relapse and HCMV reactivations. As treatment approaches, we are also developing T cells expressing chimeric antigen receptors (CARs) targeted against HCMV and EBV antigens expressed during reactivations. Ultimately, single or combinations of immune therapies, including check-point inhibitors, are preclinically tested in long-term (more than 20 weeks) humanized mice reconstituted with the human immune system.
TRAIL, FasL, and PECAM-1 expression in T cells and NK cells is upregulated in peritumoral tissue as compared to cells isolated from renal carcinoma and healthy renal tissue

Zuzana Strizova¹, Pavla Taborska¹, Dmitry Stakheev¹, Klara Havlova², Stepan Vesely², Jirina Bartunkova¹, and Daniel Smrz¹

¹Department of Immunology, 2nd Faculty of Medicine, Charles University in Prague and Motol University, Czech Republic
²Department of Urology, 2nd Faculty of Medicine, Charles University in Prague and Motol University, Czech Republic

INTRODUCTION: A number of therapeutic strategies are currently tested in clinical trials in patients with renal cell carcinoma (RCC). However, many of those strategies have considerable toxicities and low efficacy, especially in late stages of the disease. Adoptive cell immunotherapy (ACI) that uses ex vivo expanded tumor-infiltrating lymphocytes (TILs) is a promising approach in treatment of various cancers. However, TILs are highly heterogeneous and defining TIL populations with potent cytotoxic and migratory activities within the tumor microenvironment is needed. In our study, we evaluated RCC TILs from 3 different compartments – the tumor, peritumoral tissue and adjacent healthy tissue.

METHODS: A total number of 60 tissue samples from 20 patients with RCC who underwent radical nephrectomy were analyzed in our study. Tissue samples were obtained from the tumor, the peritumoral tissue and the healthy renal tissue. The samples were sliced and enzymatically dissociated into single cell suspensions. The cells were then analyzed by flow cytometry for expression of established markers of lymphocyte cytotoxicity – TRAIL and FasL, and a surrogate marker of lymphocyte migratory activity – PECAM-1. Expression of the markers was next correlated with clinical and histopathological data.

RESULTS: The proportions of NK cells positive for FasL and PECAM-1 were lower in the tumor and the adjacent healthy renal tissue than in the peritumoral tissue (P=0.002). The proportion of FasL+PECAM+ TILs tended to decrease with the dedifferentiation of the tumor. TRAIL was expressed poorly on tumor infiltrating NK cells and T cells as compared to those infiltrating peritumoral and healthy renal tissue.

CONCLUSION: NK and T cells display different migratory/cytotoxic phenotypic patterns based on tumor grading and microenvironmental compartment. Our data suggest that peritumoral TILs express prominent effector phenotype and may therefore represent the population of interest to further investigate with respect to development of TIL expansion protocols.

Investigations on a Novel Dendritic Cell-Targeted Adjuvant for Anti-Cancer Therapy

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Immunotherapy has recently emerged as a promising form of treatment for several kinds of cancer. With the introduction of check-point blockade therapy remarkable results in a subset of patients have been observed. However, some patients do not benefit from this treatment, possibly due to the strength of the immunosuppressive tumor environment in these patients. To circumvent this, finding novel approaches for activation of the immune system is of high priority. Damage-associated molecular patterns (DAMPs) delivered to dendritic cells (DCs) in situ may aid in overcoming the suppressive tumor milieu and lead to DC maturation and activation of an efficient immune response. Recent evidence has shown that the cGAS/STING pathway, which can be stimulated by dsDNA, a potent DAMP, plays an important role in immune activation against cancer in murine tumor models. We have developed a novel DC-targeted adjuvant and tested it on human dendritic cells in vitro. We found that the targeted adjuvant was indeed able to activate human DCs, shown by upregulation of DC maturation markers and an increased ability to activate T cells. We have also shown that the observed maturation of human DCs is indeed dependent on the cGAS/STING pathway.
The dual role of TAMs as oncogenes and immune suppressants

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T cell responses are regulated by integrated signaling of an array of co-stimulatory and co-inhibitory molecules. Breach of co-inhibitory pathways on T cells using blocking antibodies represents a breakthrough in cancer therapy and underscores the potential of characterizing immune regulatory pathways. The TAM family of receptor kinases - Tyro3, Axl, and MerTK - are expressed by cells of the innate immune system and play key roles in promotion of tissue repair and dampening of innate cell activation and the capacity of these cells to elicit T cell responses. TAM are also expressed by cancer cells as oncogenes associated with survival, invasion, chemoresistance, and metastases. We describe that MerTK along with the ligand Pros1 is expressed by CD4 and CD8 T cells upon activation, and that Pros1 delivers a MerTK dependent co-stimulatory signal to the T cell, leading to increased cytokine release and proliferation. We used co-precipitation experiments to demonstrate the MerTK-Pros1 interaction, and also that activated T cells express phosphatidylserine (PtdSer) on the cell surface, which is a prerequisite for optimal MerTK signaling. Strict dependency of MerTK for improved proliferation and cytokine release by Pros1, was demonstrated by siRNA knockdown of MerTK and the use of a specific MerTK inhibitor. Activation of T cells induces a metabolic switch and we next sought to clarify if MerTK stimulation influence T cell metabolism. Our data show that MerTK stimulated CD8 T cells possess considerable mitochondrial spare respiratory capacity (SRC), compared to T cells activated under MerTK blocking conditions, suggesting that MerTK stimulation leads to differentiation of T cells with memory characteristics. This notion was supported by demonstrating that CD45RO+, CCR7+ central memory T cells are preferentially MerTK positive upon activation. In addition, T cells activated under MerTK signaling condition were less prone to activation induced cell upon secondary activation. Moreover, we could show that blocking of MerTK signaling lead to increased mTOR phosphorylation and decreased phosphorylation of STAT5. Cancer cells and innate cells in the tumor micro-environment (TME) express TAM which could lead to ligand competition and consequential inadequate MerTK signaling in the T cell. We therefore studied the impact on Pros1 during expansion of tumor infiltrating lymphocytes (TIL) from melanoma biopsy material, which demonstrated a significantly reduced outgrowth of T cells upon blocking of Pros1. We next demonstrated a positive impact of MerTK signaling on the capacity of tumor specific T cells to control cancer cell growth taking advantage of the xCelligence technique, i.e., blocking of Pros1 significantly diminished cancer cell killing whereas addition of Pros1 improved cytotoxic responses. Our data demonstrate that oncogenes expressed by cancer cells may directly inhibit T-cell responses by ligand depletion in the micro-environment.

Vaccination using immunopeptidome-identified antigens for malignant glioma


The peptidome contains the critical peptides presented at the tumor cell surface having the potential to be recognized by CD8 T lymphocytes. We used peptide elution to identify 10 glioblastoma-associated HLA-A2-restricted antigens that had the characteristics to be highly expressed in tumors, to have very low or absent expression in healthy tissues, and to be immunogenic. These peptides were formulated in the IMA950 multipeptide vaccine that was tested in a phase I/II study in combination with poly-ICLC in patients with newly diagnosed glioblastoma multiforme (n=16) and grade III astrocytoma (n=3, NCT01920191). Two MHC class II-binding peptides were added in order to generate an integrated T cell response. We observed that the IMA950/poly-ICLC vaccine was safe and well tolerated. For the first 6 patients, vaccine-induced CD8 T cell responses were restricted to a single peptide and CD4 responses were not detectable. After optimization of the vaccine formulation, we observed multipeptide CD8 and sustained Th1 CD4 T cell responses. For the entire cohort (n=19), CD8 T cell responses to a single or multiple peptides were observed in 63.2% and 36.8% of patients, respectively. An encouraging median overall survival of 21 months was obtained. These results prompted us to further test this vaccine in combination with an anti-PD1, comparing IMA950/poly-ICLC vs. IMA950/poly-ICLC+anti-PD1 in patients with recurrent GBM (trial starting Q4 2018).
Pre-clinical models for melanoma and osteosarcoma immunotherapy: Dawn of the dogs’ revolution

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Despite several therapeutic improvements, advanced malignant melanoma (MM) and osteosarcoma (OSA) still remain fatal diseases due to recurrences and metastasis, for which standard treatments are not effective. Starting with MM as poster child, checkpoint inhibitors have increased survival but only in a fraction of patients with a pre-existing anti-tumor immune response. This opens up the need to find innovative therapies to “raise the tail” on patients’ survival curves. The development of effective immunotherapeutic strategies depends on the availability of appropriate pre-clinical models. In this context, naturally occurring cancers in companion animals are a great resource. Dogs naturally develop tumors as humans do, in a context of an intact immune system, with strong anatomical and physiological similarities with the human counterpart. In particular some specific canine tumor histotypes, including MM and OSA, are nearly indistinguishable from the human disease; their successful treatment with novel therapies thus carries tremendous translational value.

We have recently shown that canine MM and OSA highly express the Chondroitin Sulfate Proteoglycan 4 (CSPG4), similarly to human counterparts. CSPG4 is a highly glycosylated transmembrane proteoglycan playing a central role in pathways regulating cancer progression and metastasization. The immune-targeting of CSPG4 by means of DNA vaccination could therefore represent an interesting opportunity to fight against CSPG4+ tumors. To test this hypothesis, we performed a veterinary trial in which client-owned dogs with surgically resected CSPG4+ MM were vaccinated with a plasmid coding for CSPG4. Adjuvant CSPG4 vaccination was effective in significantly prolonging the survival of canine patients as compared to conventionally treated controls, mainly thanks to the induction of anti-CSPG4 antibodies. A veterinary trial to investigate the efficacy of this adjuvant treatment also for canine OSA patients is ongoing.

Activated integrins identify functional antigen-specific CD8+ T cells within minutes after antigen stimulation

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Rapid beta2-integrin activation upon T-cell receptor stimulation is essential for the formation of stable contacts between T-lymphocytes and their targets, and for effective effector function. We have established a simple, rapid, and sensitive flow cytometry-based assay for assessing antigen-specific T cells using fluorescent multimers of intercellular adhesion molecule (mICAM-1) that specifically bind to activated β2-integrins. This assay is applicable for monitoring of a broad range of virus-, tumor- and vaccine-specific CD8+ T cells either ex vivo or after in vitro expansion. mICAM-1 binding correlates well with classical pMHC multimer staining, but, notably, it identifies the subset of antigen-specific CD8+ T cells with immediate and high functionality, i.e., expressing high levels of cytotoxic markers and cytokines. In addition, the assay is suitable for isolating viable antigen-reacting T cells that can be further expanded or cloned in vitro.

Altogether, and compared to the methods currently available, staining of activated β2-integrins presents the unique advantage of requiring activation times of only several minutes, therefore delivering functional information nearly reflecting the in vivo situation. Hence, the mICAM-1 assay is utmost suitable for rapid and precise assessment of functional antigen-specific T-cell responses including for monitoring patient samples during cancer vaccine studies.
Lewis lung carcinoma mutanome – a source of neoantigens for cancer vaccine based on Semliki Forest virus vector

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Introduction
Neoantigens arise from tumor-specific mutations and are promising targets for cancer vaccination based on neoepitope recognition by T-cells. Encoding neoantigens in a Semliki Forest virus (SFV) vector may improve the immunogenicity of the cancer vaccine. Characterizing cancer cell mutanome is the first step towards finding potential neoepitope sequences for encoding in a vector.

Material and Method
Lewis lung carcinoma (LLC) is a mouse model for lung cancer. LLC cell exome was sequenced in parallel with an exome of normal tissue sample from a syngeneic C57BL/6 mouse and sequencing reads were aligned to the mouse reference genome. Unique sequence alterations within the exome of LLC were identified and annotated. Protein-altering mutations that lead to exchange, insertion, or deletion of one or more amino acids and created new amino acid sequences due to premature start codon or due to shift of reading frame were prioritized.

Results and Discussion
LLC cells are highly mutated with almost 86000 unique sequence alterations, out of which 10 % change the amino acid sequence of proteins. The most frequent type of protein-altering variant (> 90 %) was a missense point mutation leading to a one-amino-acid change. Another type of missense mutation was giving rise to a new start codon in the 5’ untranslated region of a transcript, thus creating a unique peptide sequence with a high immunogenic potential. Short protein-altering insertions and deletions (indels) comprised 1.6 % for in-frame indels and 5 % for frameshift indels, which may give rise to completely unique and highly immunogenic neoepitopes. Next, peptide immunogenicity will be validated in silico and the most immunogenic neoantigens will be encoded in a recombinant SFV for use in anti-tumor vaccination in LLC model.

Conclusion
LLC cells contain tumor-specific protein-altering mutations and therefore LLC tumor model has a potential for testing neoantigen-encoding SFV vaccine.

The tumor microenvironment in HPV-induced cancer: implications for immunotherapy

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Although it is well established that the tumor immune microenvironment plays an important role in therapy responsiveness and survival, the impact of the microenvironment of the original tissue on the shape of this tumor microenvironment (TME) is largely unknown. Perhaps because primary tumors of the same type can have different mutations and activated oncogenic pathways, some of which are known to impact the constitution of the tumor microenvironment. To study the potential impact of the original tissue on the intratumoral immune contexture, we applied high-dimensional single-cell mass cytometry (CyTOF) analysis and functional studies on immune cell populations of human papillomavirus (HPV)-induced primary tumors of the cervix (CxCa) and oropharynx (OPSCC), two tumors that share the same virus-driven oncogenic pathway but arise in different anatomical locations. Our data shows that despite the same etiology of these tumors, the composition and functionality of their lymphocytic infiltrate substantially differed. CxCa displayed a 3-fold lower CD4:CD8 ratio, contained more activated CD8+CD103+CD161+ effector T cells and less CD4+CD161+ effector memory T cells than OPSCC. CD161+ effector cells produced the highest cytokine levels among tumor-specific T-cells. Differences in CD4+ T cell infiltration between CxCa and OPSCC were reflected in the detection rate of intratumoral HPV-specific CD4+ T cells and in their impact on OPSCC and CxCa survival. In conclusion, the strong differences in lymphocytic infiltrate between oncogenic HPV-driven primary CxCa and OPSCC indicate a role for the originating tissue in shaping the immune contexture. Moreover, the problem of CD4+ T-cell attraction in CxCa should form a point of focus for future immunotherapeutic approaches aiming to treat progressive CxCa.
HLA Class I loss and cancer immune escape: rediscovering an old story

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Recent publications by different groups are reporting the relevance of HLA class I losses in different tumors including the responsible molecular mechanisms such as LOH in chromosome 6 in lung cancer and β2 microglobuline mutations in relapsed melanoma metastatic lesions after immunotherapy. Our laboratory has been HLA-typing tumor tissues for a long time and found that indeed this is a frequent phenomenon. Primary tumors are HLA-I positive at early stages. T lymphocytes infiltrate tumor tissues, recognize and destroy HLA class I positive cancer cells (permissive phase I). This phase can end with the total destruction of the tumor. Alternatively, HLA-I negative tumors cell variants can emerge. At this phase, tumors are heterogeneous for HLA-I expression. HLA class-I loss variants are immunoselected in vivo by antitumor CTLs and escape T cell recognition and destruction during the natural history of tumor development. At the end, tumors are uniformly HLA class-I negative with T lymphocytes and other mononuclear cells surrounding the tumor nest (encapsulated phase II) (1). The transition from phase I to phase II will probably last for a short period of time. Both reversible (“soft”) and irreversible (“hard”) defects of HLA class I have been described in solid tumors and in cancer cell lines. Immunotherapy is effective in eliminating HLA-I positive tumor cells and HLA deficient tumor cells with reversible/“soft” molecular lesions. Tumour cells with loss or downregulation of HLA-I antigens due to irreversible/“hard” lesions escape therapy-induced T cell mediated immune attack and produce new distant tumor lesions. We believe that the reexpression and enhancement of tumor HLA-I expression is responsible for the tumor regression observed in patients under different immunotherapy protocols. We recommend monitoring the HLA class I tumor profile before, during and after cancer immunotherapy.

Mechanisms of immune escape of tumors and its role in immunotherapies

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Despite the successful implementation of immunotherapies, there are challenges for these therapeutic strategies in solid tumors as a result of the immune suppressive nature of the tumor microenvironment as well as the immune escape mechanisms of tumor cells. While several tumor infiltrating immune cell subpopulations can foster tumor development, exhibit immune suppressive activity and decrease the efficacy of effector immune cells, tumor cells have developed multiple mechanisms to escape immune recognition and to modulate immune cell function. These include in particular abnormalities in the classical HLA class I antigen, the interferon (IFN) signal transduction pathways and overexpression of non-classical HLA antigens as well as of immune checkpoint molecules. This has been recently shown to be crucial for the efficacy of immunotherapies against solid tumors and might be associated with the development of resistances to these treatment options. Thus, a deeper understanding of the key immune players and regulatory pathways of tumor cells involved in the complexity and dynamic interaction between tumor and immune cells might be important for the identification of prognostic factors and reversion of the immune escape processes thereby boosting immune responses against cancer cells and avoiding immune resistances in order to improve the efficacy of immunotherapies. Reversion of tumor immune escape is not only mediated by IFNs, but also by epigenetic drugs, proteoglycans, and anti-oxidant substances as well as microRNAs and RNA-binding molecules.
Synthetic immune systems to outsmart cancer

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During the past decade we have extensively explored dendritic cell based cancer vaccines. Dendritic cells (DC) isolated from a patient are loaded with tumor antigen and immune modulators to activate dendritic cells to optimize antigen presentation and T cell stimulation. We now know that this form of immunotherapy is safe and more recently we have also started to use natural DC circulating in the blood instead of monocyte derived DC. In particular myeloid DC and plasmacytoid DC are a powerful combination, now being tested in a phase III trial. Because with current DC based vaccinations a new vaccine must be generated for each patient, we have initiated studies to look for alternatives, where we either can target DC in vivo or even replace DC by the generation of ‘synthetic DC’. During my talk I will elaborate on these novel cancer vaccine developments and on the idea to design ‘synthetic lymph nodes’ for local cancer treatment.

 Therapeutic HPV16 vaccination is effective as monotherapy in pre-malignant disease, but requires combination treatment in HPV16-induced cancers

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Background:
We have studied the T cell response to and the clinical activity of a therapeutic vaccine against HPV16 E6/E7. This vaccine, called ISA101, consists of overlapping long peptides (25-35-mers) of the HPV16 oncoproteins E6 and E7.

Methods:
The peptides were formulated in Montanide ISA-51 adjuvant and injected sc at doses of 20-300 μg.

Results:
In patients with high grade vulvar intraepithelial neoplasia (VIN), vaccine-induced clinical responses were observed in 18 of 34 (53%; 95% CI, 35.1–70.2) patients at 3 months and in 15 of 29 (52%; 95%CI, 32.5–70.6) patients, 8 of whom displayed a complete histologic response, at 12 months after the last vaccination. All patients displayed vaccine-induced T-cell responses, which were significantly stronger in patients with complete responses. Importantly, viral clearance occurred in all but one of the patients with complete histologic clearance. In patients with advanced HPV16+ cervical cancer or incurable HPV16+ oropharyngeal cancer (OPC), vaccine monotherapy did not induce robust immune responses or clinical responses. However, timed vaccination with ISA101 during standard of care chemotherapy in relapsed cervical cancer patients was associated with restoration of robust T cell responses to the vaccine and a correlation was found between the strength of vaccine-induced T cell responses and overall survival. In 22 patients with incurable OPC, ISA vaccination combined with anti-PD-1 checkpoint blockade (nivolumab) was associated with approximately twice the overall response rate (36%) of that reported in patients treated with nivolumab monotherapy (16%). Moreover the median overall survival was 17.5 months, approximately twice that of HPV16+ OPC patients treated with nivolumab alone (9 months).

Conclusion:
Therapeutic vaccine monotherapy is effective in premalignant disease. The reason for the need to co-treat in cancer as opposed to pre-malignant disease is the hostility of the cancer micro-environment towards T cells.

References:
⁴Massarelli et al. JAMA Oncology, in press.
Complete and long-lasting responses in patients with advanced checkpoint blockade resistant melanoma treated with Adoptive T cell transfer combined with DC vaccination

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Purpose: Checkpoint blockade (CPB) has revolutionized therapy of metastatic melanoma (MM), but less than half of patients experience durable responses why there is a need for other therapies. The aim of this trial was to investigate the toxicity and feasibility of a treatment where transfer of autologous tumor infiltrating lymphocytes (TIL) was combined with autologous dendritic cell (DC) vaccination in patients with stage III/IV melanoma failing on CPB.

Experimental design: A two-armed phase I trial was performed with 5 patients assigned to each cohort (A or B) with autologous TIL without (A) or with (B) autologous tumor loaded DC. Patients were pre-treated with a precondition regimen prior to TIL transfer. Administration of TIL was followed by IL-2 administration (100000 U/Kg q8hx14).

Radiological evaluation (CT/PET) was performed to evaluate clinical responses according to RECIST criteria.

Results: In cohort A, all treated patients showed mixed response or stable disease, but none durable. In cohort B, 2 patients responded with complete responses (CR) still ongoing (> 18 mo), and 2 showed PR’s, of which 1 is still ongoing (> 21 mo) with only one small bone-lesion remaining, while the other had a short response (< 4 mo). One patient died early during treatment course and therefore could not receive DC vaccinations. Long-lasting persistency of several of the injected TIL derived T cell clones was demonstrated in blood and skin of the treated patients.

Conclusions: TIL therapy combined with DC vaccination can result in complete clinical responses in patients who have failed on CPB.

T Cell Receptor Gene Transfer: How Affinity, Structure, and Crossreactivity Impacts T Cell Function

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Adoptive T cell transfer using T cell receptor (TCR) gene modified T cells has had success in treating solid tumors and hematologic malignancies. However, due the limited number of TCR's for clinical use, there are very few malignancies that can be treated with TCR gene modified T cells. There are two key hurdles to identifying promising TCR's for use in patients has been good targets that are not expressed on normal tissues and low TCR affinity. Several groups have used methods to enhance the affinity of a TCR to improve the targeting of tumors. One concern is that the TCR modifications could lead to crossreactivities with normal cells and tissues. Indeed, there have been cases of serious adverse events using affinity enhanced TCR gene modified T cells. We have studied how TCR affinity impacts T cell crossreactivity and function of TCR gene modified T cells. We have taken a different approach by making structure guided modifications to a TCR which targets MART-1 presented by HLA-A2. The functional phenotypes and crossreactivity was measured using a panel of previously described MART-1 homologs derived from both self and non-self proteins. The impact of the structural changes to the TCR on T cell function and crossreactivity will be discussed.
Immunopeptidomics: Accelerating the development of personalized cancer immunotherapy

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The remarkable clinical efficacy of the immune checkpoint blockade therapies has motivated researchers to discover immunogenic epitopes and exploit them for personalized vaccines. Mutated human leukocyte antigen binding peptides (HLA-p) are currently the leading targets for T-cell recognition of cancer cells. Most studies attempt to identify neoantigens based on predicted affinity to HLA molecules. We have shown that the direct identification of tissue derived neoantigens by mass spectrometry is becoming feasible; however, methodologies for purification of HLA-p for mass-spectrometry analysis have been a major limitation. We have recently designed a novel high-throughput, reproducible and sensitive method for sequential immuno-affinity purification of HLA-I and -II peptides that is suitable for both cell lines and tissues and for drug-screening assays.

The massive amount of HLAp data we acquire while hunting down the neo-antigens is highly valuable. We have compiled a large immunopeptidomics database across dozens of cell types and HLA allotypes. First, we have shown that by taking advantage of co-occurring HLA-I alleles across dozens of immunopeptidomics datasets we can rapidly and accurately identify HLA-I binding motifs. Consequently, training HLA-I ligand predictors on refined motifs significantly improves the identification of neoantigens. Second, our database captures the global nature of the in vivo peptidome averaged over many HLA alleles, and therefore, reflects the propensity of peptides to be presented on HLA complexes, which is complementary to the existing neoantigen prediction features. We have shown as a proof of concept that our immunopeptidomics MS-based features improved neoantigen prioritization by up to 50%. Overall, immunopeptidomics facilitates direct identification of neoantigens and it can also improve the prediction of clinically relevant neoantigens for personalized anti-cancer vaccines.

Boosting immunotherapeutic cells

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Immunotherapy is an emerging strategy to stop the progression and eventually eradicate tumors. The principle relies on exploiting the patient’s immune system to recognize cancer. An antigen receptor specific for a cancer marker can be genetically produced and can be transferred into effector cells of the patient, providing him/her with an anti-cancer effector cell. This process is called effector cell redirection, and is mainly performed on T cells. The manufacture of redirected T-cells is a long process and currently involves the use of expensive cytokines, growth factors and labour-intensive cell growing over several weeks. It is highly costly, indeed, the two T cell-based products available today are sold between 380,000 and 450,000 USD per patient. Moreover, the preparation time, although fast in consideration to the effort, lasts between 6 to 8 weeks which is sometimes longer than the life expectancy of a patient (e.g. pancreatic cancer patient). In addition, this process is not optimal in regards to the quality of the prepared cells. Indeed, a lot of the patients enrolled for ACT have already been through a series of heavy treatments, such as chemotherapy, which can affect the numbers and quality of the effector cells used. There is a clear need for improvement and we and others have proposed solutions to improve the fitness of the injected T cell product.

We herein present an original method based on the modulation of the respiratory chain of the effector cells (using Antimycin A (AA) or Rotenone (R)) for immunotherapeutics cell culture. A growing number of studies are pointing out the importance of the metabolism in effector cells’ ability to discriminate and eradicate tumor cells. Especially, Reactive Oxygen Species (ROS) are thought to be an important player in the sensitivity and specificity of the TCR activation process. We then selected a wide range of molecules thought to increase ROS production in effector cells and were able to identify AA and Rot as able not only to improve killing efficiency of different effector cells cocultured with different tumor models but also to significantly increase their specificity. This activity was conserved in xenograft in vivo models. Additionally, by studying toxicity of those molecules in prolonged culture conditions, we were able to demonstrate that these molecules yield higher effector cell density thus permitting to drastically diminish the need for medium. These findings represent significant advantages both for the efficacy of the therapeutic product as well as the manufacturing process which is logistically heavy and expensive.
Prognostic significance of immune infiltrates in breast cancer

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In breast cancer patients (BCa) with different molecular subtypes both stromal as well as intratumoral TILs are equally predictive for clinical outcome. Notwithstanding the general consensus that TILs have a prognostic value in BCa, there remain several issues which hinder their broad application as biomarkers in the routine setting. These include the identification of immune cell populations with the most clinical relevance, their distribution in specific tumor regions and the mode of their evaluation (separate or combined). Recently published and ongoing studies in our laboratory have demonstrated differential densities of CD8+ and CD163+ immune infiltrates in the invasive margin (IM) and tumor center (TC) in BCa with significant prognostic value. Moreover, we demonstrated that the combined evaluation of CD8+ or CD163+ immune cell densities in IM and TC allows better stratification and improves the prognostic value of TNM staging in BCa (favorable combined immune signature; FCIS). Focusing on the TIL population with suppressor function, the FoxP3+ cells, we also observed maximum OS when differential densities of FoxP3+ cells (i.e., low in the TC and high in IM) were combined with inverse densities of CD8+ cells (i.e., high in the TC and low in IM). These results may have an impact regarding the functional role of FoxP3+ cells intratumorally, although attention must be given to the fact that FoxP3 is a common marker for activated T cells and therefore multiplexed immunohistochemistry should be applied for their precise identification. We also show the prognostic value of peritumoral tertiary lymphoid structures (TLS) adjacent or distal to the tumor (aTLS and dTLS, respectively). aTLS combined with FCIS has an even better improved prediction of clinical outcomes, defining a reinforced FCIS (RFCIS).

Adaptive CD4 Th1 response against telomerase in blood counteracts T-cell exhaustion in non-small-cell lung cancer

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Introduction: The IFN-γ+ CD4 Th1 response plays a critical role in anticancer immunity and has been extensively studied from tumor microenvironment in many cancers. Exhausted T cells, which express co-inhibitory receptors such as PD-1, TIM-3, are characterized by the loss of most of antitumor T cell functions. Here we investigate the clinical significance of circulating anti-tumor CD4 Th1 response and exhausted T cells in non-small-cell lung cancer (NSCLC).

Methods: 170 naïve-treatment NSCLC patients were enrolled in this study. The antitumor adaptive Th1 response was assessed by IFN-γ ELISPOT assay in blood lymphocytes using a mixture of eight highly promiscuous and Th1-polarized HLA class II-restricted epitopes from telomerase (TERT). PD-1 and TIM-3 were used to characterize exhausted T cell by flow cytometry.

Results: The presence of anti-TERT Th1 response was detected in 59/170 pts (35%). NSCLC patient showed high level of PD-1+ and/or TIM-3+ exhausted T cells. We found an opposite link between anti-TERT Th1 and exhausted T-cells in NSCLC patients. In contrast to exhausted CD8 T cells, the presence of anti-TERT Th1 response was associated with a low rate of exhausted CD4 T-cells. NSCLC dissemination is associated with high T-cell exhaustion status but low anti-TERT Th1 response. Our results indicated that exhausted CD4 T cell and anti-TERT Th1 response display opposite prognosis value in NSCLC. While high level of anti-TERT Th1 cells play a protective role, hyper-exhausted PD-1+TIM-3+ CD4-T cells negatively affect patients’ survival. By combining these two prognostic factors, we stratified patients in distinct group. Patients with anti-TERT Th1high/CD4-PD-1-TIM-3low immune profile had better overall survival than anti-TERT Th1low/CD4-PD-1-TIM-3high group (median OS : not reached versus 4 months respectively p<0.0001).

Conclusion: Thus, high circulating anti-TERT Th1 response plays a strong protective role by counteracting the poor prognosis associated to hyper-exhausted CD4 T cells in NSCLC. Our study provides a dynamic blood-based immunomonitoring tool allow patients stratification.
Characterization of immune responses in patients diagnosed with Waldenström’s Macroglobulinemia

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Introduction: Therapeutic cancer vaccinations aim at enhancing anti-cancer immunity. The present study aims at characterizing immune responses in the hematological malignancy Waldenström’s Macroglobulinemia (WM) in order to identify attractive targets for therapeutic peptide vaccinations.

Materials and Methods: Healthy donors and WM patients were screened for IFNγ responses against epitopes derived from the cancer neoantigen MYD88 L265P and immune regulators such as Arginase 1 and PD-L1 using IFNγ ELISPOT. To characterize the immune responses a MYD88 L265P-specific T cell culture was generated and tested using intracellular IFNγ and TNFα staining and cytotoxicity assays.

Results: Both WM patients and, unexpectedly, healthy donors display in vitro immune responses towards the MYD88 L265P peptide when analyzed with ELISPOT. Furthermore, in vitro immune responses were detected towards epitopes derived from Arginase 1 and PD-L1 in WM patients.

Discussion: It should be determined whether the cancer neoantigen MYD88 L265P-specific immune responses identified in healthy donors are naïve or memory responses, as the occurrence of memory T cell responses in healthy individuals would imply, that the individual has acquired the mutations earlier in life, but the immune system has been able to clear the mutant cells.

Conclusion: We identified naturally occurring T cells specific towards the neoantigen MYD88 L265P and immune regulators such as Arginase 1 and PD-L1 in WM patients. These targets may hold a potential for cancer immune therapy in WM patients.

The Vaccine-Site Microenvironment: Immune activation and regulation at the source

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The effects of cancer vaccine adjuvants are mediated locally; however, little is known about changes induced in the vaccine site microenvironment (VSME) by commonly used adjuvants, including incomplete Freund’s adjuvant (IFA) and toll-like receptor (TLR) agonists. To understand these effects, we biopsied VSMEs from clinical trial patients and evaluated cellular infiltrates and gene expression signatures. Murine studies have raised concerns that IFA may have negative effects, and instead highlighted the value of TLR agonists combined with CD40 ligation. However, in humans, we find that peptide vaccines with a TLR agonist alone are weakly immunogenic and that adding IFA to TLR agonists consistently enhances the magnitude and durability of the T cell responses to vaccines. We have also found that repeat vaccination with peptides in IFA can induce tertiary lymphoid structures in the VSME and strong T cell responses. Gene expression analyses of VSME reveal that vaccination with peptides in IFA dramatically increases expression of both CD40L and CD40, as well as markers of dendritic cell (DC) activation and maturation (CD80, CD83, CD86). However, expression of CD70 and IL12 are not convincingly enhanced, suggesting that additional DC activation signals are needed. Repeat vaccination with peptides in IFA enhances Tbet (TBX21) expression and decreases Th2 (GATA3) and Th17 (RORC) expression, suggesting a Th1-dominant VSME. In contrast, the TLR3 agonist increased GATA3 and RORC. These findings suggest that IFA supports a VSME more favorable for inducing T cell responses than a TLR agonist alone, but that additional DC-activating signals may further enhance immunogenicity.
Therapeutic anti-telomerase vaccine targeting CD4 helper T-cells in Advanced Non-Small Cell Lung Cancer. A phase I/II study (UCPVax trial)


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Background: There are compelling evidences for designing cancer vaccine specifically to induce CD4 Th1 response. UCPVax is a vaccine targeting telomerase (TERT) selected to stimulate CD4 T-cell response. In preclinical model, UCPVax triggered potent anti-TERT Th1 response in vivo and eradicated tumor growth.

Method: UCPVax is composed of two promiscuous HLA-DR-binding peptides derived from TERT called UCP2 and UCP4. Peptides were emulsified in Montanide ISA 51VG and injected subcutaneously in separate sites. Continuous Reassessment Method (CRML) dose escalation design was used and three doses levels of 0.25-, 0.5-, and 1mg was evaluated. Primary objectives included safety, tolerability and dose limiting toxicities to identify the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D). Secondary objectives included immune responses according to the dose level and antitumor activity. Maximal number of patients is planned to be 18 and 54 for phase 1 and 2 respectively.

Results: The study started at March 2016, twelve patients with refractory/progressive NSCLC were enrolled in two centers in France (Besançon and Paris). They were treated in escalating dose at three-dose levels (0.25mg, 0.5mg and 1mg). Patients received a number of 12 to 40 injections of UCPVax. Any dose limiting toxicity was observed and no sign of severe treatment or immune-related adverse events were observed; no MTD was defined. The main side effects reported were grade 1 or 2, asthenia, local reaction at the injection site and flu-like symptoms. Five patients experienced stable disease and clinical benefit. For three of them, the treatment was extended beyond three-month duration, up to nine months for two patients. Immune monitoring revealed that most patients mounted strong CD4 and CD8 T-cells against TERT at least after three injections.

Conclusion: Results from this study indicate that UCPVax was safe, well tolerated and immunogenic at the doses and schedule tested. The results obtained encourage a future evaluation of UCPVax in other malignancies, either as monotherapy or in combination immune checkpoint inhibitors. (NCT2818426)

CMV infection, cancer and survival in the elderly

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Immune control of autochthonous cancer in the current era of immunomodulatory antibody therapy has shown that the immune system can be a powerful constraint on tumour growth, in which CD8+ T cells have a major role. However, solid cancers are diseases of older people, and hallmarks of an ageing immune system include decreasing amounts of peripheral CD8+ naïve T cells and often increasing amounts of late-stage differentiated CD8+ memory T cells (LSD cells). In industrialized nations, the proportion of the population infected with CMV increases with age, and this parallels the accumulation of LSD cells which is much less marked in CMV-seronegatives. However, low amounts of CD8+ naïve cells are also seen in CMV-seronegatives, showing that this virus is not also responsible for this age-associated difference. Is the occurrence of cancer and response to therapy different in the elderly and in CMV-infected or non-infected patients? It is regularly assumed that immunity against cancer is compromised in the elderly but in fact clinical experience thus far, including our own, suggests that older cancer patients (at least melanoma patients) respond to immunomodulatory antibody therapy (at least CTLA-4 and PD-1-directed) just as well as or better than younger patients. CMV infection, the frequency of which increases with age, may actually have a beneficial influence on this and be one mechanism by which older patients have a better clinical outcome. Recent findings suggest that many TILs are specific for viruses like CMV and EBV, rather than tumour antigens, potentially explaining some of these results.
Development, clinical experiences and future role of therapeutic DC vaccines at Oslo University Hospital

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Our therapeutic DC vaccine program started in 2000 and since then more than 250 patients have been included in different DCs trials. Initially patients with metastatic melanoma and prostate cancer were treated and in contrast to many others Oslo decided to use DCs transfected with tumor mRNA. The rational for tumor mRNA combine the immune stimulatory capacity of DCs with the broad antigen repertoire of tumor cells, which reduce the risk of tumor escape by the use of a wide spectrum of antigens, including individual antigens unique to each patient, recruit both CD4+ and CD8+ T cells and is feasible in rare cancer forms. In the first studies we found that RNA/DC-vaccine production was feasible, well tolerated, mounted specific immune responses in >50% of patients and clinical effects was related to immune responses. Intradermal injection turned out to become superior to intranodal injection and since then all our DC vaccines were administered intradermally. During these years we tested out clinically DCs produced in 7 days, 5 days and 3 days as well as different DC maturation methods. Experiences from these trials will be presented. DC vaccines on patients with metastatic disease show only a modest clinical effect which is not durable. Therefore, over the last years we changed our strategy to use DC vaccines only as adjuvant therapy preventing recurrence of the disease. Some clinical experiences will be given of 3 different adjuvant DC clinical trials: 1) High risk prostate cancer patients following radical surgery, 2) patients with Glioblastoma Multiforme following radical surgery and chemoradiotherapy and 3) AML patients following induction therapy. We conclude that therapeutic DC vaccines are still an attractive therapeutic strategy and combinations with checkpoint inhibitors, radiotherapy and chemotherapy remains to be tested in the clinic.

Development and clinical testing of UV1 – a second-generation cancer vaccine targeting the Reverse Transcriptase subunit of human Telomerase (hTERT)

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Telomerase represent a near universal target for a cancer vaccine since it is highly expressed in 85-90% of all cancers, and only weakly in normal tissue. Patients with objective clinical responses after vaccination provide a unique opportunity to identify the immunological characteristics underlying tumour regression. We screened a hTERT peptide library using blood samples from cancer patients experiencing long-term survival following vaccination with different first-generation hTERT vaccines GV1001 and hTERT mRNA loaded DCs). The library consisted of long, overlapping hTERT peptides, and immune responses were measured in terms of proliferation of CD4+ T- cells. Strong proliferation responses were observed against several novel hTERT epitopes not present in the vaccines given. Based on these data, three hTERT peptides shown to elicit strong proliferation responses (Th1 type) across several long-term survivors were selected as components in the UV1 vaccine. UV1 is thus the first cancer vaccine based on epitope spreading data, and patient data associating UV-1 responses with survival benefit. UV1 contains multiple epitopes capable of a broad population coverage. Both Th and CD8 cells recognize UV1 epitopes and cloned T cells are multifunctional.

UV1 has recently been investigated in three phase I/IIa trials where UV1 is given as intradermal injections with GM-CSF as adjuvant. In melanoma [EudraCT No. 2013-005582-39] UV1 is given in combination with Iplimumab. In NSCLC [EudraCT No. 2012-001852-20] patients with stage IIIb-IV disease receive UV1 as monotherapy. Patients with newly diagnosed, androgen sensitive, metastatic prostate cancer [EudraCT No. 2012-002411-26] receive UV1 concomitant with androgen therapy. Immunological and clinical data from all trials are now available and all point in the same direction. A high number of immune responders are observed in the 3 trials (69-100% in evaluable patients), thus ensuring an excellent population coverage in a non-HLA typed patient population. The immune responses are long lasting and diverse, reflecting the matching of HLA diversity and the diversity of epitopes in UV-1. Survival is associated with immune responses in all 3 patient groups and a clear positive signal is obtained in all 3 trials when compared to matching historical data. Patients in all groups are being followed up for 5-10 years. In the UV-1/Iplimumab combination trial a synergistic effect was observed resulting in more rapid Th1 responses, requiring as little as only 1 week of vaccination. With this combination treatment, 2 year survival was 75% and 3 year survival 67%. These data demonstrate that providing a Signal 1 stimulus (UV-1), concomitantly with an release from Signal 2 blockade (by Iplimumab) may bring out the true clinical potential of a cancer vaccine such as UV-1 and encourages further clinical trials aimed at simultaneous stimulation of Signal 1 and 2.
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 lower 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. 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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- POSTERS -

A Universal Killer T-Cell for Adoptive Cell Therapy of Cancer

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T cell-mediated immunotherapy of cancer has achieved remarkable results in hard-to-beat cancers. The main challenge of adoptive T-cell transfer (ACT) is its labour intensive and costly production and logistics as well as its dependency on the quality of the patient’s T cells. To overcome these hurdles we have designed a universal cell line for TCR expression by modifying the FDA-approved NK cell line, NK-92. Advantages of using this cell line is that it is easy to expand and can readily be genetically engineered. However, tumour cell recognition and killing by NK-92 is not antigen specific. This can be controlled by introducing an antigen receptor, such as a chimeric antigen receptor (CAR) or, as in the current work, a TCR. We herein present evidence that NK-92 cells are as specific and potent as redirected T cells to kill target cells.

Finally, encouraging in vivo data showed that mice receiving UK92 cells expressing a therapeutic TCR experienced reduction in tumor load and enhanced survival compared with control mice. If confirmed, the use of UK92 as a universal cell line might pave the way to truly off-the-shelf therapeutic effector cells for cancer immunotherapy and leading to drastic reduction of cell production time, logistic and cost.
T-cell based immunotherapy of cancer

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T-cell based immunotherapy represents an attractive strategy for the treatment of cancer. Whereas cellular anti-tumour immune responses have typically been attributed to CD8 T cells, CD4 T cells play a critical role in tumor elimination and the priming and maintenance of CD8 T-cell responses. We have isolated CD4 T cells reactive against tumour antigens from patients who experienced clinical benefit from treatment with cancer vaccines targeting universal tumor antigens and frequent neoantigens. Strong T-cell responses correlated with enhanced survival and tumour regression in such late stage cancer patients. These HLA class II-restricted T-cell clones recognised target cells loaded with long peptides or protein and some CD4 T-cell clones could also directly recognize tumour.

TCRs were expressed in expanded donor T cells by mRNA electroporation or retroviral transduction. They were found to be functional in both CD8+ and CD4+ T cells producing cytokines such as TNF-α and IFN-γ with the capacity of target cell killing. We also show preliminary in vivo data for one of our broadly applicable TCRs recognizing a universal antigen, hTERT, presented on one of the most frequent HLA alleles, HLA-DP4. We believe that combining HLA class I- and class II-restricted TCRs for T-cell redirection may provide a more potent therapeutic effect in adoptive T cell therapy. HLA class II-restricted TCRs may additionally have direct therapeutic value both in haematopoietic malignancies and in melanoma where tumour cells frequently express HLA class II. Importantly, CD4 T cells and HLA class II TCRs therefore have the potential to orchestrate broad and long-lasting immune responses that enable cancer control.

Combinatorial IGK-CD19 CAR primarily targets IgK+ malignant B-cells and is less prone to serum IgG inhibition

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The first Chimeric antigen receptor (CAR) T-cell therapies have been approved for treatment of B-cell malignancies. This is mainly due to the success of CAR T cells targeting B-lymphocyte antigen CD19, which has led to astonishing results in clinical trials. Since CD19 is a general B-cell antigen, CAR-T cells eliminate all B-lineage cells, including non-malignant B cells. Therefore, the patients suffer from impaired humoral immune response, increasing susceptibility to severe infections. Since B-cell lymphoma and chronic lymphocytic leukemia cells have a clonally restricted expression of Immunoglobulin (Ig) light chains, either Ig-kappa or Ig-lambda, Ig-kappa positive tumor cells can be targeted while sparing normal Ig-lambda positive B-cells. In this respect, we isolated the sequence encoding the antigen-binding parts of an anti-Ig kappa antibody and designed a second-generation CAR construct (IGK CAR). Initial studies using single chain variable fragment (scFv) fused to human IgG confirmed the specific binding to Ig-kappa positive target cells. Expression of IGK CAR in expanded peripheral blood T cells and subsequent testing of the CAR T cells in various in vitro assay with target cells, demonstrated cytokine production and potent killing of Ig-kappa expressing B-cell lines such as BL-41, whereas no response was observed against Ig lambda positive B-cell lines such as Granta-519. We compared IGK CAR with a clinical CD19 CAR (fmc63) and observed similar potency in target cell killing. Previous reports have showed that the presence of free immunoglobulins present in human serum could inhibit IGK CAR T cells, and our tests confirmed this. To improve IGK CAR T cells in the presence of IgGs while maintaining the specificity, we utilized a combinatorial CAR system, where the signaling domains were split. Our design demonstrated efficient killing of Ig-kappa+ cells and were less sensitive to free IgG as compared to IGK CAR T cells. Additionally, we observed a trade-off between specificity and cytotoxic potential. Increasing one individual component of the combinatorial system made the cells less prone to serum IgG inhibition but demonstrated somewhat higher cytotoxic activity against Ig-kappa negative targets. Our fully adjustable design therefore brings another perspective to the field by regulating the individual expression levels according to the treatment needs. Hence, enabling T cells to be either more aggressive or specific depending on the treatment efficiency and on the on-target toxicity in patients. Taken together, our in vitro data demonstrate that IGK-CD19 CAR combination is as potent as IGK CAR T cells or CD19 CAR T cells, and provides an alternative by combining their benefits into one design and thus reduces on-target toxicity.

CD4 T-cell based immunotherapy of cancer

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Use of a stimulation cocktail of OK432, TLR7/8 ligand and PGE2 to improve dendritic cell-based cancer immunotherapy

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Dendritic cells (DC) have been used in cancer immunotherapy trials for several decades, but it is clear from the rather disappointing clinical results that this approach needs improvement. One major issue with the cells used previously was the lack of IL-12 production due to the addition of PGE2 to the maturation cocktail. In this study we generated monocyte-derived DC in a short 3 day protocol and used a penicillin-killed S. pyogenes preparation (OK432), a TLR7/8 agonist and reduced amounts of PGE2. Phenotype, migratory and T-cell stimulatory capacity were analyzed. The OK432 cocktail stimulated DC showed a mature phenotype with upregulation of CD83, CD80, CD86, HLA-DR and CCR7. The OK432-cocktail treated cells showed some migratory capacity in a chemotaxis assay towards CCL19 and were able to induce antigen-specific T cells. Moreover, they secreted substantial amounts of IL-12p70. We therefore propose the OK432 cocktail as an interesting alternative to current maturation cocktails used in DC-based immunotherapy.
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