DTIM

Dutch Tumor Immunology Meeting

Dutch Tumor Immunology Meeting (DTIM) 2018

Breukelen, Thursday June 28 and Friday June 29

GUEST SPEAKERS

Jolanda de Vries
Lana Kandalaft
Jurjen Tel
Lex Eggermont

Sponsors:

KWF KANKER BESTRIJDING

MACS Miltenyi Biotec

Thursday June 28, 2018

09.30-09.55 Registration & Coffee/Tea

09.55-10.00 Welcome

Please Note; Selected abstract-presenters have exactly 15 minutes to present their data, including a few minutes discussion. Please make sure your presentation is on the computer before the session starts.

10.00-11.00 Oral presentation Session 1

Chair: Julien Karrich

Hilma van der Horst (VUMC)
Potent Ex Vivo Anti-Tumor Activity in Relapsed Refractory Multiple Myeloma Using Novel DR5-Specific Antibodies with Enhanced Capacity to Form Hexamers upon Target Binding

César Oyarce Diaz (UMC Groningen)
Metabolic reprogramming of macrophages

Panagiota Bouti (Sanquin)
CD47-SIRPα checkpoint blockade involves kindlin3-dependent enhancement of CD11b/CD18-integrin affinity and cytotoxic synapse formation

Joanna Grabowska (VUMC)
Evaluation of GM3-containing liposomes for antigen targeting to splenic CD169+ macrophages to induce anti-cancer immunity

11.00-11.30 Coffee/Tea Break

11.30-13.00 Oral presentation Session 2

Chair: Rogier Reijmers

Jesper van Eck van der Sluijs (Radboudumc)
Combining Hypomethylating Agents and Dendritic Cell Vaccination to Boost Graft-Versus-Leukemia Immunity in Patients with Acute Myeloid Leukemia

Elisabeth Huijbers (VUMC)
Directing the immune system towards the tumor vasculature by vaccination against extracellular vimentin

Antonius de Waard (Sanquin)
The SPPL3-controlled tumor glycosphingolipid repertoire determines MHC class I functionality

Linda Borst (LUMC)
NKG2A blockade potentiates CD8+ T-cell immunity induced by cancer vaccines

Lisa Holthof (VUMC)
CAR therapy hampered by bone marrow microenvironment in Multiple Myeloma

Maud Plantinga (UMC Utrecht)
Cord-blood stem cell-derived Dendritic cells specifically originate from CD115-expressing precursors

13.00-14.00 Lunch

14.00-14.30 Laptop presentations Session 1

Alsya Affandi (VUMC)
Targeting gangliosides-containing liposomes to human CD169+ antigen presenting cells to induce anti-tumor immune responses

Rosa van Amerongen (LUMC)
Identification of high affinity TCRs directed against the WT1 gene

Saskia van Asten (Sanquin)
Expanded tumor infiltrating lymphocytes upregulate 4-1BB in response to renal cell carcinoma

Rachid Bouzid (Erasmus MC)
Targeting (neo)antigens in pancreatic ductal adenocarcinoma by antigen specific immunotherapy; an immunopeptidomics approach

Jitske van den Bulk (LUMC)
Autologous neo-antigen-specific T cell responses in low mutation burden colorectal cancers

Chih Kit Chung (LUMC)
Thermosensitive hydrogels for CTLA-4 immune checkpoint blocking therapy

Nick van Dijk (NKI)
Neo-adjuvant ipilimumab and nivolumab in high risk resectable bladder urothelial cancer (NABUCCO)

Yusuf Dolen (Radboudumc)
Nanoparticle based iNKT cell vaccines: Optimal administration routes and mode of action

14.30-15.00 Laptop presentations Session 2

Tracy-Jane Eisden (VUMC)
DC-SIGN is an uptake receptor for melanoma-derived autophagosomes in human dendritic cells

Job van Kooten (Erasmus MC)
Adjuvant dendritic cell based immunotherapy after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for peritoneal mesothelioma: Rationale and design of the MESOPEC study

Miranda Meeuwsen (LUMC)
Identification of potent T cell clones recognizing J chain, a promising target for the treatment of Multiple Myeloma

Nadine van Montfoort (LUMC)
Oncolytic viruses, a novel approach to sensitize tumours for cancer immunotherapy

Lotte Mousset (Radboudumc)
Ex vivo AKT-inhibition facilitates generation of polyfunctional stem cell memory-like CD8+ T cells for adoptive immunotherapy

Rogier Reijmers (LUMC)
B cell lineage-specific transcription coactivator BOB1 is indispensable for multiple myeloma cell survival and allows for superior TCR-based targeted therapy

Maud Rijnders (Erasmus MC)
PD-L1 expression according to five monoclonal antibodies in urothelial cell cancer: concordance and clinical implications

15.00-15.30 Laptop presentations Session 3

Lorenzo Spagnuolo (NKI)
Dissecting the synergistic effect of chemotherapy and immunotherapy on anti-tumoral T cell functions in breast cancer

Shabaz Sultan (Radboudumc)
Robust Identification of Immune Cell Subsets in Multiplex Immunohistochemistry

Nadine Grima Sopesens (VUMC)
Proinflammatory activity of vascular targeted therapy against cancer

Marcella Willemsen (AMC)
Prognostic implications of tissue resident memory T cells in human melanoma development

Rosa de Groot (Sanquin)
Effective expansion and reprogramming of tumor infiltrating lymphocytes from non-small cell lung cancers

Marijne Heeren (VUMC)
Indoleamine 2,3-dioxygenase Expression Pattern in the Tumor Microenvironment predicts Clinical Outcome

Julien Karrich (Sanquin)
MISTRG: improved human immune system mouse model to test transplantable T cell therapy against solid tumors

Paul Kemps (LUMC)
Failure to present neo-peptides hampers T cell recognition of neoplastic BRAFV600E expressing Langerhans Cell Histiocytosis Cells

15.30-16.00 Coffee/Tea Break

16.00-17.30 Oral presentation Session 3

Chair: Sonja Buschow

Lotte Mousset (Radboudumc)
Ex vivo AKT-inhibition in CD4+ T cell inhibits memory differentiation and favors skewing towards T helper 2 and regulatory T cells

Dyantha van der Lee (LUMC)
Mutated NPM1 as target for immunotherapy of acute myeloid leukemia

Marieke IJsselsteijn (LUMC)
Next-gen immune profiling as a framework for cancer immunotherapy

Monique van der Kooij (LUMC)
Successful treatment of metastatic melanoma patients with adoptive T cell therapy in combination with low-dose interferon-alpha

Henk-Jan Prins (VUMC)
Generation of universal "off-the-shelf" chimeric antigen receptor (CAR)-engineered T cells in a dish

Leila Akkari (NKI)
Dynamic changes in immune cells post radiotherapy in glioblastoma: Macrophages at play

17.30-18.30 Keynote speaker

Professor Dr. Jolanda de Vries
Radboudumc, Nijmegen

Dendritic cell-based vaccination against cancer

18.30-19.15 PI meeting

18.30-19.15 Drinks (PhD students and postdocs)

19.15-20.30 **Dinner**

20.30-21.30 **Keynote speaker**

Professor Dr. Lana Kandalaft
Ludwig Institute for Cancer Research, Lausanne

Mobilising antitumor immunity: Lessons from ovarian cancer

21.30-00.00 **Networking party**

Friday June 29, 2018

9.00-10.00 Keynote speaker

Dr. Jurjen Tel
Technical University Eindhoven

Single cell analysis reveals functional heterogeneity within plasmacytoid dendritic cells and identifies environmental cues that drive type I IFN production

10.00-11.30 Oral presentation Session 4

Chair: Rieneke van de Ven

Mark Hendriks (UMC Groningen)
Tumor-selective blocking of CD47 'Don't eat Me signaling'

Saskia Santegoeds (LUMC)
The anatomical location determines type of lymphocyte infiltration in tumors of same etiology

Miguel Angel Lopez Venegas (VUMC)
Evaluation of liposomal- and antibody-mediated antigen targeting to DC-SIGN and CD169 on monocyte-derived dendritic cells

Jeroen Slaats (Radboudumc)
Tumor immune escape: unravelling and overcoming the immunosuppressive niches in melanoma

Inge Verbrugge (NKI)
Radiotherapy and cisplatin increase immunotherapy efficacy by enabling local and systemic intratumoral T-cell activity

Elham Beyranvand Nejad (LUMC)
Underlying mechanisms of tumor recurrence after incomplete cancer immunotherapy

11.30-11.50 Coffee/Tea Break

11.50-12.00 AIO Award Ceremony

12.00-13.00 Keynote speaker

Professor Dr. Lex Eggermont
University of Paris Sud

Immunotherapy development in solid tumors: where do lessons from melanoma take us?

13.00-14.00 **Lunch**

Abstracts accepted for oral presentation

Hilma van der Horst (VUMC)

Potent Ex Vivo Anti-Tumor Activity in Relapsed Refractory Multiple Myeloma Using Novel DR5-Specific Antibodies with Enhanced Capacity to Form Hexamers upon Target Binding

Abstract

Hyperclustering of Death Receptor 5 (DR5) after binding of its ligand TRAIL, induces apoptosis. As cancer cells are more sensitive for DR5-mediated apoptosis than nonmalignant cells, targeting DR5 with monoclonal antibodies (mAbs) is considered an appealing therapy for several cancer types. Unfortunately, clinical results have been disappointing so far due to lack of efficacy.

To improve the efficacy of DR5 mAbs we applied the novel HexaBody® technology, which utilizes the concept that, upon target binding, immunoglobulins (IgGs) form hexamers through Fc-Fc interactions. The hexamer formation can be enhanced by introducing specific single point mutations in the Fc domain. Accordingly, we developed the Hx-DR5-01/05 (GEN1029), a mixture of two non-competing DR5-specific antibodies harboring the E430G mutation. Hx-DR5-01/05 induces DR5 agonist activity through hexamer formation, independent of crosslinking.

Here, we tested the efficacy of Hx-DR5-01/05 against a large panel of multiple myeloma (MM) cell lines and primary MM cells derived from patients with newly diagnosed (ND) or relapsed refractory (RR) disease.

Hx-DR5-01/05 induced effective cytotoxicity in MM cell lines, as well as in primary MM cells, whereas its wild-type counterpart did not. Importantly, MM cell lysis levels of 20% or above were observed in a significantly higher fraction of RR patients (74%) compared to ND (30%) patients, indicating the superior therapeutic potential of Hx-DR5-01/05 as compared to conventional DR5 antibodies, especially in relapsed/refractory MM patients.

César Oyarce Díaz (UMC Groningen)

Metabolic reprogramming of macrophages

Abstract

Cervical and ovarian cancer are still major causes of death. In both, a high infiltration of tumour-associated macrophages (M2-like macrophages) is associated with a poor prognosis. Therefore, these immunosuppressive cells could be considered as targets for therapy intervention in the context of immunotherapies. Since phenotype/activity of M2 macrophages are related to their metabolic profile, we studied if drug-induced manipulation of metabolic pathways can either prevent M2 polarization or polarize M2-like macrophages into a more M1-like phenotype with anti-tumoral activity.

Several candidate drugs were selected based on their described effect(s) on specific metabolic pathways. Some of the drugs inhibiting glutaminase, PPAR-γ and carnitine palmitoyltransferase-1 prevented IL-4-mediated M2 polarization as based on a decrease in Arg-1 expression and an increase in glycolysis (feature of M1-like macrophages). Furthermore, two of the drugs targeting carnitine palmitoyltransferase-1 and the electron transport chain strongly enhanced IFN-γ-mediated macrophage RE-polarization from M2 to M1. Both drugs induced a collapse of mitochondrial respiration, skewing the metabolism from OXPHOS (an M2-like feature) to glycolysis.

Coculture studies of macrophages and TC1 cells demonstrated that most of the drugs that prevented M2-polarization inhibited M2-induced tumour cell proliferation or even strongly increased macrophage-mediated tumour cell death.

We showed that by altering macrophages metabolism we can prevent their M2-polarization or improve M2-to-M1 RE-polarization, and thus increase antitumor activity. Our results strongly suggest that by drug-induced modulation of the metabolism of macrophages a pro-

inflammatory tumour environment can be created that likely can augment the efficacy of cancer immunotherapies.

Panagiota Bouti (Sanquin)
CD47-SIRPα checkpoint blockade involves kindlin3-dependent enhancement of CD11b/CD18-integrin affinity and cytotoxic synapse formation
Abstract
Recently, we established that neutrophils kill antibody-opsonized tumour cells by a novel cytotoxic process that we have termed trogoptosis. This previously unknown killing mechanism involves trogocytosis (from Greek trogo, gnaw), where fragments of target cell membrane are actively taken up by the neutrophil, thereby disrupting the target cell plasma membrane and killing the cancer cells. Trogocytosis and subsequent killing is strictly dependent on antibody-opsonization of the tumour cells, neutrophil Fcγ-receptor signalling and CD11b/CD18 integrin-dependent cytotoxic synapse formation. Furthermore, it is promoted by CD47-SIRPα checkpoint inhibition. Here, we present evidence that CD47-SIRPα interactions act by controlling the initial stage of the killing process i.e. the CD11b/CD18-dependent cytotoxic synapse formation. In particular, CD47-SIRPα interactions negatively regulated the CD11b/CD18 inside-out activation that occurred as a consequence of Fc-receptor signalling in neutrophils. Moreover, the inhibitory effect acted via the integrin-associated protein kindlin-3, as demonstrated, amongst other things, by using neutrophils from rare LAD-III patients that have mutations in FERMT3 and lack kindlin-3 expression. Collectively, these findings demonstrate that CD47-SIRPα interactions control a kindlin-3-dependent pathway of CD11b/CD18-integrin activation, and that targeting the CD47-SIRPα checkpoint primarily improves integrin activation, and therefore also the resultant cytotoxic synapse formation, trogocytosis and killing during neutrophil ADCC towards cancer cells.

Joanna Grabowska (VUMC)
Evaluation of GM3-containing liposomes for antigen targeting to splenic CD169+ macrophages to induce anti-cancer immunity
Abstract
Liposomes are an attractive antigen delivery system and have been successfully used for vaccination strategies. We previously have shown that antibody-mediated antigen (Ag) targeting to CD169+ macrophages stimulates superior Ag-specific CD8 T cell responses. CD169, also known as sialoadhesin or siglec-1, is a sialic-acid binding lectin which has been described to bind to ganglioside GM3. Here we tested GM3-containing liposomes for their binding and uptake by murine CD169+ macrophages and their capacity to induce immune responses. As expected, CD169+ macrophages strongly and specifically bound GM3 liposomes, while macrophages from sialoadhesin knock-in mice bearing a mutation in the CD169 ligand binding pocket were incapable of binding GM3 liposomes. After intravenous administration, GM3/ovalbumin-containing liposomes specifically bound to CD169+ macrophages in a sialic acid dependent manner and stimulated ovalbumin-specific CD8 and CD4 T cell and B cell responses. Surprisingly, liposomes without GM3 also stimulated immune responses, while not binding to CD169+ macrophages. In our current studies we will also evaluate liposomes that contain the ganglioside GM1, that does not bind CD169, for their capacity to evoke an immune response.

Jesper van Eck van der Sluijs (Radboudumc)
Combining Hypomethylating Agents and Dendritic Cell Vaccination to Boost Graft-Versus-Leukemia Immunity in Patients with Acute Myeloid Leukemia
Abstract
Allogeneic hematopoietic stem cell transplantation (alloSCT) can be curative for patients with haematological malignancies. However, relapse remains a major problem, illustrating the necessity for the development of novel treatment approaches targeting tumor cells and inducing graft-versus-tumor (GVT) immunity. In this respect, there is a rational for combined treatment of hypomethylating agents (HMAs) with dendritic cells (DC) vaccinations. HMAs are well-tolerated and exert direct anti-proliferative and pro-apoptotic effects on tumor cells, while vaccination with naturally occurring myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) to boost GVT immunity are highly attractive. Here, we show increased diversity and frequency of TAA-specific T cell responses post-alloSCT in acute myeloid leukemia (AML) patients treated with HMA decitabine as compared to patients receiving standard conditioning regimen. Furthermore, in vitro HMA decitabine-primed NK cells show an increased killing capacity of THP-1 and primary AML blasts of patients. Adoptive transfer of NK cells in combination with decitabine treatment resulted in superior THP-1 control in vivo, enhanced expression co-stimulatory molecules KIR and NKp44 and increased expression of perforin, TRAIL and TNF-a. To further boost GVT immunity, DC vaccination is a promising strategy. We present an ex vivo GMP-compliant culture protocol for generating DC subsets from CD34+ hematopoietic stem and progenitor cells (HSPCs) of alloSCT donor origin. High numbers of BDCA1+ mDCs and pDCs could be generated, sufficient for multiple vaccination cycles. mDCs efficiently prime and induce expansion of naïve T cells, while pDCs are superior in activating NK cells. We anticipate that combined administration of HMA decitabine and HSPC-derived DC vaccination will boost GVT immunity and induce long-lasting tumor control.

Elisabeth Huijbers (VUMC)
Directing the immune system towards the tumor vasculature by vaccination against extracellular vimentin
Abstract
Angiostatic therapy has shown promise in the clinic, but its contribution to patient survival has been limited until now. Therefore, we have developed a vaccination strategy, which has the potential to improve angiostatic therapy in a safe way and to be a preferred therapy for combination with conventional effective anti-cancer strategies. To identify suitable targets for vaccination against the tumor vasculature we performed suppression subtractive hybridization on human patient material and an RNA sequencing screen in the pool of mouse embryo-specific genes. Thereby, we identified a number of novel tumor endothelial markers, which are selectively overexpressed in tumor endothelial cells compared to normal healthy adult endothelium and are therefore ideal targets for vaccination.
One of these markers, the cytoskeletal protein vimentin, was found to externalize from tumor endothelial cells, while expression in all other cells in the body is exclusively intracellular. This makes extracellular vimentin (eVim) a really specific target for therapy. eVim is overexpressed in the vasculature of different solid tumors, but not present in normal healthy tissue. Indeed, therapeutic targeting in tumor bearing mice by a monoclonal antibody resulted in efficient inhibition of tumor growth. With an antibody-inducing vaccine targeting eVim proof-of-concept was obtained in preclinical models for colon carcinoma and melanoma. No toxicity was observed after vaccination. Directing the patient's own immune system towards the tumor endothelial cells or to factors secreted by these cells is an attractive approach and can be developed as an efficient and safe treatment for cancer.

Antonius de Waard (Sanquin)
The SPPL3-controlled tumor glycosphingolipid repertoire determines MHC class I functionality
Abstract
MHC class I (MHC-I) molecules are key regulators of T cell activation. Processes catalyzing functional MHC-I antigen presentation are therefore often dysregulated in cancer. To identify such processes, we performed a state-of-the-art genome-wide screen using haploid genetics. We identified the intramembrane protease SPPL3 as a novel positive regulator of antigen presentation as validated by a reduction of CD8+ T cell responses against CRISPR/Cas9 engineered SPPL3 knockout (KO) cells. Additional genome-wide screens revealed that SPPL3 controls enzymes of the glycosphingolipid (GSL) synthesis. Indeed, mass spectrometry of the GSLs showed that the repertoire is completely altered in SPPL3 KO cells. Moreover, systematically knocking out enzymes in the GSL synthesis pathway enabled us to create a map of the GSL repertoire affecting MHC-I. In combination with the mass spectrometry data, we discovered that lacto- and neolactoseries GSLs generated by the enzyme B3GNT5, hinder antibody and receptor association with MHC-I. We determined that SPPL3 controls this process by proteolytically inactivating B3GNT5 and therefore (neo-)lactoseries GSLs are only present in the absence of SPPL3. Screening a panel of diverse tumor cell lines revealed that GSLs impair MHC-I in AML and several other types of tumors, suggesting that GSLs may be involved in escape from T cell surveillance.
In conclusion, GSL regulation by SPPL3 is a novel regulatory mechanism of functional MHC-I antigen presentation. Targeting GSL synthesis may therefore constitute a novel mode of immunotherapy for cancer.

Linda Borst (LUMC)
NKG2A blockade potentiates CD8+ T-cell immunity induced by cancer vaccines
Abstract
The inhibitory receptor CD94-NKG2A is expressed on subsets of NK cells and CD8+ T cells. Its ligand, the non-classical MHC class I molecule HLA-E, is commonly over-expressed in human cancers. We previously demonstrated that high HLA-E expression in ovarian carcinoma and NSCLC may neutralize the survival benefit of T cell infiltration, suggesting NKG2A could represent an immune checkpoint. Here, we demonstrate that the presence of an immune response, in TIL samples obtained from human HNC biopsies in patients with measurable immune reactivity to HPV16-viral antigens and in tumor mouse models upon immune therapy, increased the frequencies of NKG2A+-positive CD8+ TILs. Expression of Qa-1, the mouse homologue of HLA-E, on tumor cells also increased after immunotherapy. In vitro studies revealed that this increase was mediated by T cell-derived IFNγ. In three different mouse tumor models, efficacy of immunotherapy was higher in Qa-1b deficient tumors compared to WT tumors, indicating that the Qa-1b – NKG2A axis limits efficacy of immunotherapy. Intratumoral analysis in different tumor models revealed that the frequency of CD45+ cells was increased in the Qa-1b deficient tumors which was due to the increase of CD8+ T cells. In the absence of ligand on the tumor we saw that the frequency of CD8+ T cells expressing NKG2A was increased and the expression level enhanced. Asides, we also observed that in the Qa-1b deficient tumors the percentage of PD-1, TIM3 and Lag3 positive CD8+ T cells was enhanced. In conclusion, NKG2A is enriched on CD8+ TILs and functions as an immune checkpoint that restrains therapeutic efficacy of cancer immunotherapy.

Lisa Holthof (VUMC)
CAR therapy hampered by bone marrow microenvironment in Multiple Myeloma
Abstract
The complex crosstalk between multiple myeloma (MM) cells and its bone marrow microenvironment plays an important role in MM progression and the development of drug- and immune resistance. Earlier, we have shown that bone marrow mesenchymal stromal cells (MSCs) protects MM cells from lysis mediated by CD4 T cells, CD8 T cells and by NK cells. A promising novel strategy for cancer immunotherapy includes chimeric antigen receptor-transduced T cells (CAR T cells). Therefore, we are now addressing the question whether the stromal cell-MM cell interactions can also influence CAR T cell-mediated lysis of MM cells. To this end we measure the survival of luciferase-transduced MM cells that were cultured in the presence versus absence of MSCs and treated with second generation CAR T cells having different antigen specificities (CD38, BCMA, CD138), different affinities (CD38 CARs) and different costimulatory domains (CD28 or 41BB). So far, we observed enhanced MM cell survival in the presence of MSCs upon treatment with CD38 and CD138 CAR T cells but not with BCMA CAR T cells. The CD38 CAR affinity seems also to influence the susceptibility to microenvironment induced resistance. Current efforts are focusing on understanding the impact of the CAR costimulatory domain and on the possibility to modulate the microenvironment-induced immune resistance to CAR T cells towards the improvement of the efficacy of CAR T cell therapy.

Maud Plantinga (UMC Utrecht)
Cord-blood stem cell-derived Dendritic cells specifically originate from CD115-expressing precursors
Abstract
Dendritic cells (DCs) are professional antigen presenting cells, which instruct both the innate and the adaptive immune system. Once mature, they provide all necessary signals to activate and prime naïve T-cells. These characteristics makes them excellent candidates for vaccination. Intervention strategies aiming at manipulation of DCs require in-depth insights in their development and functional properties. DCs can be generated from CD34+-cells. CB is a good source to provide CD34+-stem cells for the generation of Cord blood (CB)-derived DC. We demonstrate that CD34+ CB -derived stem cells develop in several types of myeloid progenitor cells. Recent advances in state-of-the-art techniques led to significant insights into DC ontogeny. Isolation of the DC progenitor cells by flow cytometry, enables precise characterization, using RNA sequencing, to clear which progenitor drives the development of DCs. Here we show that CD115+-progenitor cells are responsible for the generation of the CB-DCs. Sorted CD115+-cells specifically generate DCs after 7 days of differentiation. Functional assays demonstrate that CD115-derived DCs are highly mature upon stimulation, migrate efficiently and possess a high capacity to stimulate tumor-antigen-specific T-cells. Gene set enrichment analysis displayed an enriched conventional DC profile within the CD115-derived DCs compared to CB monocyte-derived DC. The discovery of a committed DC precursor in the CB-derived stem cell culture further enables optimized utilization of DC-based vaccine to provide powerful anti-tumor activity and long-term memory-immunity, e.g. in combination with hematopoietic cell transplantation (HCT) from the same CB graft to overcome relapse in refractory cancer patients.

Lotte Mousset (Radboudumc)
Ex vivo AKT-inhibition in CD4+ T cell inhibits memory differentiation and favors skewing towards T helper 2 and regulatory T cells
Abstract
Ex vivo AKT-inhibition is a promising strategy for the improvement of adoptive T cell therapy by promoting early memory-like CD8+ T cells with superior expansion capacity and functionality. Recently, this strategy is also gaining interest in the chimeric antigen receptor (CAR) T cell. As CAR T cells are most often generated from unselected peripheral blood lymphocytes (PBLs), it is of great importance to know the effect of AKT-inhibition on the other and most prominent T cell population in this material: the CD4+ T cells. Here, we studied the effect of AKT-inhibition on CD4+ T cell memory differentiation and subset skewing. We observed that inhibition of AKT during T cell activation resulted in early memory CD4+ T cells, based on retained expression of CD62L, CCR7 and CXCR4 while this was lost on control cells. Moreover, we determined expression of extracellular markers, intracellular transcription factors and cytokine production to define the subsets T helper (Th)1, Th2, Th17 and Treg. Though Th17 cells were absent in our model, AKT-inhibited cells showed increased CCR4 and GATA3 expression and more IL4 production, indicating skewing towards Th2 cells. Additionally, elevated CD25 and FOXP3 expression and increased IL10 production indicated skewing towards (Tregs). Moreover, skewing towards Th1 cells was reduced as cells expressed less CXCR3 and IFNγ production was reduced. Importantly, these effects on CD4 functionality were still present after one week re-stimulation without AKT-inhibitor, indicating a permanent effect. Together, this shows that AKT-inhibition preserves CD4+ T cell memory differentiation, though induces skewing towards more Th2, and Tregs, while reducing the Th1 population. These finding should be taken in consideration when generating AKT-inhibited early memory T cells from total PBLs.

Dyantha van der Lee (LUMC)
Mutated NPM1 as target for immunotherapy of acute myeloid leukemia
Abstract
The most frequent subtype of acute myeloid leukemia (AML) is defined by mutations in the nucleophosmin (NPM1) gene. Mutated NPM1 is an attractive target for immunotherapy, since it is an essential driver gene and 4 base pair frameshift insertions occur in the same region in 30% of AML, resulting in a novel C-terminal alternative reading frame of 11 amino acids. By searching in the HLA class I ligandome of primary AML, we identified multiple peptides derived from mutated NPM1. For one of these peptides, i.e. HLA-A*02:01-presented CLAVEEVSL, we searched for specific T-cells in healthy individuals using peptide-MHC tetramers. Tetramer-positive CD8 T-cell clones were isolated and analyzed for reactivity against primary AML with mutated NPM1. From one selected clone with superior anti-tumor reactivity, we isolated the T-cell receptor (TCR) and demonstrated specific recognition and lysis of HLA-A*02:01-positive AML with mutated NPM1 in vitro after retroviral transfer to CD8 and CD4 T-cells. Anti-tumor efficacy of TCR-transduced CD8 and CD4 T-cells was confirmed in a mouse model engrafted with a human AML cell line expressing mutated NPM1. These data show that mutated NPM1-derived peptides are presented on AML and that CLAVEEVSL is a neoantigen that can be efficiently targeted on AML with mutated NPM1 by TCR gene transfer in a co-receptor independent fashion. Immunotherapy targeting mutated NPM1 may therefore contribute to treatment of AML.

Marieke IJsselsteijn (LUMC)
Next-gen immune profiling as a framework for cancer immunotherapy
Abstract
Immunotherapy has emerged as one of the most promising treatments for cancer. However, a substantial portion of patients are not responsive to current immunotherapies. Not only the mutational burden of tumours, but also the quality and quantity of tumour-infiltrating immune cells are an important prognostic indicator in several cancer types, including colorectal cancer. Multiplex immunophenotyping technologies are essential to unravel the complexity of anti-tumour immune responses. Most techniques are held back by the lack of spatial context, limitations in the number of targets that can be visualised simultaneously, and/or cumbersome protocols. Imaging mass cytometry overcomes these limitations by allowing for the visualisation of up to 40 markers using metal-conjugated antibodies combined with spatial information. We developed a 35 marker immune cell panel for formalin-fixed paraffin embedded tissue, enabling the discovery of novel immune subsets, with potential clinical relevance. In order to validate findings in an high-throughput setting, we developed a Tyramide signal amplification (TSA)-free method for the simultaneous detection of 7 cellular targets by immunofluorescence. Using this method we designed a T-cell (CD3, CD8, FOXP3, CD45RO, GZMB, Keratin and DAPI) and a myeloid panel (PDL1, CD68, HLA-DR, CD163, CD11c, Keratin and DAPI) which allow for extensive cancer immunophenotyping. Combining the here described methods for deep cancer immunophenotyping will accelerate the identification of immune biomarkers with clinical relevance. Furthermore, this framework supports the discovery of previously unappreciated immune cell subsets, involved in anti-tumour immunity.

Monique van der Kooij (LUMC)
Successful treatment of metastatic melanoma patients with adoptive T cell therapy in combination with low-dose interferon-alpha
Abstract
Between 2012 and 2017 twenty-two patients with progressive metastatic melanoma were treated with adoptive T cell therapy in combination with low-dose interferon-alpha (IFNa) in our phase I/II trial. Our primary objective was to evaluate the safety of this treatment combination. Therefore, three cohorts of patients were infused with increasing dosages ranging from 1 to 10 x 10^8 T cells per infusion, with three infusions per cycle and a maximum of two cycles. Secondary objectives were to monitor clinical benefit according to the response evaluation criteria in solid tumors (RECIST) and to assess and validate immunological parameters that were reported to correlate with response to therapy in our pilot cohort (Verdegaal, 2011).
No treatment-related serious adverse events were observed. Four patients did not complete treatment due to fast disease progression. Six out of 18 evaluable patients (33,3%) had clinical benefit recorded as stable disease (SD). Importantly, most of these patients had progressive disease after one or more lines of (immuno)therapy at inclusion. The patient with durable ongoing SD displays vitiligo as an on-target adverse event. Interestingly, clinical benefit correlated with the pre-conditioning effect of low-dose IFNa on both the PBMCs composition and total blood count of the patients. No major phenotypical or functional differences were observed between T cells administered to patients that experienced clinical benefit and patients that did not. In depth phenotypical characterization of pre- and post-conditioning blood samples is ongoing and preliminary data will be presented.
In summary, we conclude that treatment with the combination of T cells and low-dose IFNa is safe and effective and that clinical benefit correlates with the effectiveness of low-dose IFNa conditioning.

Henk-Jan Prins (VUMC)
Generation of universal “off-the-shelf” chimeric antigen receptor (CAR)-engineered T cells in a dish
Abstract
Having rapid access to “off-the-shelf” T cell products, which can be applied across histocompatibility limitations, would greatly benefit the broader applicability of adoptive T cell therapy. To achieve this goal, we set out to use induced pluripotent stem cells (iPSC) as an unlimited source of T cells and as an ideal platform to generate safe and efficient “off-the-shelf” chimeric antigen receptor (CAR) T cells via CRISPR/Cas9-mediated genetic editing of their T cell receptor and the HLA molecules. We recently reported that engineering of T cell-derived iPSC (TiPSC) with CARs can be an efficient strategy to concomitantly harness the unlimited availability of iPSCs and direct the specificity and functional potential of TiPSC-derived T cells. Towards our ultimate goal, in this study we aimed to i) generate TiPSC-derived T cells with a doxycycline (dox) inducible CAR (iCAR) which also lack the expression of the endogenous T cell receptor (TCR-/-iCAR TiPSC); ii) silence the HLA class I expression in order to prevent their rejection by T-cells and iii) protect the TiPSC from NK cell attack by expressing the HLA-E on the cell surface.
We successfully generated mature CD8 single positive T cells expressing a CAR upon dox treatment and no TCR on their surface. The in vitro generated TCR-/- iCAR-T cells were able to expand and elicit significant specific tumor cell lysis upon stimulation with tumor cells. In addition we have generated b2m-/- HLA-E+ TCR-/- iCAR-TiPSC and differentiated them into CD8αα T cells. We demonstrated that elimination of HLA class I inhibited lysis by alloreactive T cells and HLA-E expression inhibited NK cell cytotoxicity. Our results provide the basis for future translational use of synthetic iPSC-derived T cells for immunotherapy.

Leila Akkari (NKI)
Dynamic changes in immune cells post radiotherapy in glioblastoma: Macrophages at play
Abstract
Eighty percent of tumors that develop in the central nervous system are malignant gliomas, with over half being glioblastoma multiforme (GBM), the most aggressive form of this disease. Even following treatment with standard of care therapy, the overall 5-year survival rate of patients diagnosed with GBM is less than 5%. In GBM patients and mouse models of the disease, the major non-cancerous cell type in the glioma microenvironment is tumor-associated macrophages/microglia (TAMMs). We previously used an inhibitor of colony stimulating factor 1 receptor (CSF-1R), BLZ945, to target macrophages in a mouse model of gliomagenesis. CSF-1R inhibition dramatically improves survival in a long-term intervention trial and markedly regresses established high-grade lesions. To determine the translational clinical potential for CSF-1R inhibition, we designed preclinical trials in combination with radiotherapy and examined how re-educating TAMMs could increase radiation efficacy. Our data support a progressive change in macrophage education in the course of therapeutic response underlying their pro-tumorigenic functions. In recurrent irradiated tumors, the ratio and signatures of macrophages infiltrating from the periphery and tissue-resident microglia are significantly altered, suggesting that changes in TAMM sub-populations favor glioma recurrence. Incorporating long-term treatment with BLZ945 in tumors treated with fractionated radiation led to a block in tumor recurrence, indicating that reversing cancer and therapy-induced macrophage programming has the potential to inhibit disease relapse. Together these results identify critical roles for CSF1R-dependent TAMMs in blunting the response to standard of care treatment, and promoting glioma recurrence.

Mark Hendriks (UMC Groningen)
Tumor-selective blocking of CD47 'Don't eat Me signaling'
Abstract
CD47 is a ubiquitously expressed cell surface glycoprotein that serves to prevent phagocytosis of healthy 'self' cells by interacting with SIRPα expressed on different types of phagocytes. Intriguingly, a majority of malignancies overexpresses CD47 presumably to evade phagocytic elimination and prevent immunogenic processing of tumor antigens. Currently, a phase I dose escalation trial using a humanized anti-CD47 mAb is recruiting patients with refractory hematological malignancies (NCT02678338). However, the clinical efficacy of current CD47-blocking antibodies is potentially limited by a massive 'sink' formed by CD47 expression on a broad variety of normal (blood) cells. Moreover, lack of tumor selectivity of current CD47-blocking agents may induce immunologic adverse effects when applied in patients. To address these issues, we constructed bispecific antibody (bsAb) EGFRxCD47 to direct CD47-blockade to EGFR-expressing cancer cells and to promote tumor-selective phagocytosis. Our preliminary results demonstrate cooperative binding activity of EGFRxCD47 towards EGFR+/CD47+ cancer cells, which translated into enhanced tumor cell phagocytosis in an EGFR-directed manner. We are currently studying whether EGFR-directed phagocytosis of cancer cells by bsAb EGFRxCD47 promotes tumor antigen processing by dendritic cells.

Saskia Santegoets (LUMC)
The anatomical location determines type of lymphocyte infiltration in tumors of same etiology
Abstract
The tumor immune microenvironment (TME) determines clinical outcome. Whether the original tissue in which a primary tumor develops influences this microenvironment is not well understood. We applied mass cytometry and functional studies to analyze human papillomavirus (HPV)-induced primary tumors of the cervix (CxCa) and oropharynx (OPSCC), two types of tumors arising in distinct anatomical locations but sharing the same etiology. Clear phenotypic differences between immune cells infiltrating the TME of CxCa and OPSCC tumors were found. Whereas HPV+ OPSCC tumors were strongly infiltrated with IgM+, non-class switched B cells and CD4+ T cells, HPV+ CxCa tumors were strongly infiltrated with CD8+ T cells. The CD4:CD8 ratio was 2.5x higher in OPSCC than CxCa and both T cell subset frequencies were close to those found in the tissue of origin. PBMC analysis revealed a high level of comparability between CxCa and OPSCC patients. Subsequent unsupervised hierarchical clustering through the CITRUS algorithm led to the identification of distinctive tumor-specific populations of CD161+ effector memory CD4+ T cells and CD103+ tissue-resident effector CD8+ T cells, both with a highly activated CD38+, HLA-DR+, and/or PD-1+ phenotype. CD161+ CD4+ T cells produced the highest cytokine levels and their numbers correlated with the detection of intratumoral HPV-specific CD4+ T cells. Interestingly, CxCa were often less infiltrated with HPV-specific CD4+ T cells despite their presence in metastatic CxCa lymph nodes. The differences in CD4+ T cell infiltration (total and HPV-specific) may explain why (HPV-specific) CD4+ T cells have an impact on survival in OPSCC but not CxCa. In conclusion, the anatomical location has an impact on a tumor's immune contexture and this bears consequences on clinical outcome.

Miguel Angel Lopez Venegas (VUMC)
Evaluation of liposomal- and antibody-mediated antigen targeting to DC-SIGN and CD169 on monocyte-derived dendritic cells
Abstract
Dendritic Cell Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) and CD169/Sialic acid binding immunoglobulin type lectin I (siglec-1), are lectin receptors expressed by macrophages in secondary lymphoid organs and are implicated in antigen uptake. Our aim is to compare the efficacy of these lectin receptors with regard to antigen uptake and cross-presentation by monocyte-derived DCs (moDCs).
Fluorescent DC-SIGN and CD169-specific antibodies or liposomes containing the DC-SIGN and CD169 specific ligands Lewis Y and monosialodihexosylganglioside (GM3) were used to target gp100 melanoma antigen to moDCs. Binding and uptake of our targeting strategies was investigated by flow cytometry, while imaging flow cytometry was employed to study antigen routing. The determination of gp100 cross-presentation was assessed by coculturing targeted moDCs with gp100 specific HLA-A201-restricted T cells and analyzing IFNγ production.
Our preliminary data show that liposome and antibody targeting of gp100 to DCSIGN and CD169 lead to effective binding, but also suggests that DCSIGN and CD169 lectin receptors may have a differential capacity to endocytose and to stimulate cross-presentation of antigens. Our studies will help to determine which lectin receptor is the most efficient to target antigens to for the activation of anti-melanoma T cell responses.

Jeroen Slaats (Radboudumc)
Tumor immune escape: unravelling and overcoming the immunosuppressive niches in melanoma
Abstract
Immunotherapeutic strategies have reported success in enhancing antitumor immunity. However, long-term favorable clinical outcomes remain scarce due to the ability of tumors to foster an immunosuppressive microenvironment. Intravital melanoma imaging reveals that tumor immune suppression takes place within well-defined microenvironmental niches. This project aims to characterize these niches by multi-scale cellular and molecular profiling, and functionally explore immunosuppressive niches to enhance T cell effector functions. Intravital melanoma imaging of intradermal B16F10 melanoma tumors shows a suppressed anti-tumor immune response at the tumor rim, as evidenced by high numbers of cytotoxic T lymphocytes (CTLs) that fail to effectively eradicate tumor cells. In contrast, the invasion zone displays robust CTL-mediated tumor cell killing. To reveal the landscape of immunosuppressive mechanisms in invasive B16F10 tumors, a protocol has been developed to excise defined tumor subregions (core, rim, and invasion zone) for single cell RNA sequencing. In addition, immunohistochemical characterization of the tumor microenvironment reveals the immune contexture in immunosuppressive subregions and the effect of adoptive T cell therapy hereon. Using a candidate approach, adenosine revealed itself as potent suppressor of tumor cell killing by CTLs, and this effect can be partially reverted by adenosine 2A receptor antagonism. Overall, this research project combines spatiotemporal visualization, molecular mapping, and functional analyses to overcome the mechanistic knowledge gap on intratumoral heterogeneity of the antitumor immune response.

Inge Verbrugge (NKI)
Radiotherapy and cisplatin increase immunotherapy efficacy by enabling local and systemic intratumoral T-cell activity

Abstract
To improve the success of cancer immunotherapy, PD-1 blockade must be combined with rationally selected additional treatments that enable cytotoxic T cells (CTLs) to eradicate primary tumors and metastases. Radiotherapy and chemotherapy are cytotoxic, but can also modulate immune responses and therefore serve as attractive partners for immunotherapy combinations. However, there is very little information available on the mechanistic basis for success or failure of (chemo)-radio-immunotherapy ((C)(R)IT) combinations, especially in models for metastatic disease.
Here, we demonstrate in a mouse model, one requirement for therapeutic success is de novo induction of a tumor-specific CTL response. This requirement can be met by targeting the CD137 costimulatory receptor. A second requirement is that the tumor micro-environment (TME) must permit CTL activity. Our study reveals that conventional DNA-damaging anti-cancer regimens can serve this purpose. In the context of CD137/PD-1-targeting immunotherapy, radiotherapy created a 'CTL-permissive' TME that was associated with CTL-extrinsic, rather than CTL-intrinsic transcriptomic alterations. Cisplatin functionally mimicked the effect of radiotherapy on a non-irradiated tumor in the same mouse and improved CTL-dependent tumor control.
In conclusion, to achieve control of disseminated disease, (radio-)immunotherapy approaches should not only result in enhanced T cell priming, but also sustain T cell activity in the tumor micro-environment, which can be achieved by modulating T cell intrinsic or extrinsic parameters. Rational approaches to achieve the latter may include low-dose cisplatin, particularly in a setting where PD-1 blockade by mAbs is insufficiently effective.

Elham Beyranvand Nejad (LUMC)
Underlying mechanisms of tumor recurrence after incomplete cancer immunotherapy

Abstract
Cancer vaccines aim to induce specific T cell responses directed against tumor cells. Previously, we have shown therapeutic efficacy of vaccination with synthetic long peptide vaccines in mouse tumor models and in patients with HPV-induced neoplastic lesions. However, under less optimal vaccine conditions the SLP-vaccinated mice display tumor recurrences despite the initially dramatic T-cell mediated regression induced by the vaccine. Here, we investigated the underlying mechanisms focusing on the tumor microenvironment. Our data showed that recurrent tumors displayed a significantly reduced leukocyte infiltration when compared to primary tumors. In particular, the number of infiltrating CD8 T cells was much lower. This was accompanied by lower levels of chemokine receptors including CXCR3 on the T cells, and a reduced CXCL9 and CXCL10 production by intratumoral immune cells. Moreover, although these T cells are capable of producing inflammatory cytokines, the cytotoxic capacity to kill tumor cells was inferior compared to the T cells at the time of regression. Notably, we did not observe any difference in the expression of T cell inhibitory molecules such as PD-1, Tim3 and LAG3 at the time of relapse. Vaccination of mice that were injected with recurrent tumor cells did not result in tumor regression, despite the fact that the systemic CD8 T cell response was amplified systemically following boost SLP vaccination at the time of recurrence and that these T cells showed an elevated effector phenotype. Together, that the observed defective T cell infiltration and increasing intrinsic tumor resistance to killing occurs suggests that incomplete cancer immunotherapy leads to immune selection and tumor escape. On-going work exploring immune selection and tumor heterogeneity by using RNA seq analysis and cell barcoding will be discussed.

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Abstracts accepted for laptop presentation

Alsya Affandi (VUMC)
Targeting gangliosides-containing liposomes to human CD169+ antigen presenting cells to induce anti-tumor immune responses
Abstract
Pancreatic cancer forms a major cause of cancer related deaths with a very short mean overall survival of just 6-12 months. Our previous work has already demonstrated that CD169+ macrophages can stimulate superior immune responses including helper and cytotoxic T cell responses. Using liposomes containing CD169-binding gangliosides, we hypothesized that these liposomes could be used to target and deliver antigens to human CD169+ antigen-presenting-cells (APCs).
We observed that liposomes containing GM3, GD3, GM1, GD1a, GT1b, could efficiently bind and were taken up by CD169-overexpressing THP1 cells and human monocyte–derived dendritic cells (moDCs). moDC liposome binding and uptake could be further enhanced by IFN-α-induced CD169 upregulation, and blocked using neutralizing α-CD169 antibody. Furthermore, ganglioside-containing liposome were taken up by human peripheral blood CD169+ monocytes, CD169+ CD123+ and CD169+CD11c+ DCs, and splenic macrophages. The levels of uptake were associated with CD169 expression.
To conclude, several ganglioside-containing liposomes bind to human CD169 and could potentially be used to target CD169+ APCs. Ongoing studies evaluate their intracellular trafficking using imaging flow cytometry and whether these liposomes can be used to induce pancreatic tumor antigen-specific T cell responses.

Rosa van Amerongen (LUMC)
Identification of high affinity TCRs directed against the WT1 gene
Abstract
The Wilms' tumor gene 1 (WT1) is widely over-expressed in a broad range of cancers. Using T-cell receptor (TCR)-based therapies, WT1-expressing tumor cells could be targeted. Several research groups have been searching for WT1-specific TCRs; however, low affinity and limited efficacy have been common. We aim to identify more potent high affinity T-cells by searching within the allo-HLA repertoire. Nonetheless, high affinity T-cells can increase toxicity risks as well. Especially since WT1 shows expression in several healthy tissues, potential toxicities need to be explored. In PBMCs of healthy individuals, we have searched for WT1-specific CD8+ T-cells, using 8 different WT1-specific peptide-MHC tetramers. These peptides are presented by HLA-A*01:01, HLA-A*02:01, HLA-A*24:01 or HLA-B*35:01 and were selected based on literature and HLA-peptide elution data of primary AML material. Initially, over 4,800 tetramer positive CD8+ T-cell clones were collected via single-cell sorting. Specific recognition of peptide loaded target cells, as well as target cells transduced with the WT1 gene were analysed and in total, 54 T-cell clones were selected for further screenings. Using cell panels consisting of different malignant cell types, efficacy screenings were conducted. Altogether, high affinity WT1-specific T-cell clones were identified for 3 different peptides, recognizing both tumor cell lines and primary tumor material. Safety screenings are planned, to investigate reactivity against a broad panel of healthy cell types with quantified WT1 gene expression levels.

Saskia van Asten (Sanquin)
Expanded tumor infiltrating lymphocytes upregulate 4-1BB in response to renal cell carcinoma
Abstract
The transfer of autologous tumor infiltrating lymphocytes (TILs) is a promising therapy for solid tumors. Patients suffering from renal cell carcinoma (RCC) respond to immunotherapy such as high dose IL-2, albeit with severe toxicities. TIL therapy may therefore be more suitable to treat this tumor type. Previously we successfully expanded tumor reactive TILs from non-small cell lung cancer (NSCLC). Here we cultured TILs from 16 RCC patients. The proliferation rates were lower compared to what we previously found for non-small cell lung cancer (NSCLC) arguing for the presence of suppressive tumor derived factors.
T cells of 11 out of 16 patients showed clear upregulation of the activation marker 4-1BB upon exposure to tumor digests and no or lower reactivity to healthy kidney tissue from the same patient. The tumor-specific upregulation of other activation markers (CD40L, CD107a, PD1) was only found in a subset of patients. Surprisingly, tumor-specific activation was rarely accompanied by the production of IFN-γ, TNF-α or IL-2, while these cytokines were abundantly expressed by expanded NSCLC-derived TILs. This suggests that tumor-reactive 4-1BB positive T cells produce another yet to be defined cytokine profile. To define the tumor microenvironment and its effects on TILs, we are currently correlating the tumor reactivity with a comparative analysis of lymphoid infiltrates to the corresponding healthy kidney tissue. In conclusion, expanded T cells from RCC are tumor reactive as evidenced by 4-1BB upregulation, but the TIL expansion conditions may require further optimization. RCC is thus a conceivable candidate for the application of TIL therapy.

Rachid Bouzid (Erasmus MC)
Targeting (neo)antigens in pancreatic ductal adenocarcinoma by antigen specific immunotherapy; an immunopeptidomics approach
Abstract
Pancreatic ductal adenocarcinoma (PDAC) is a form of cancer with a poor prognosis. 4,1 per 100.000 cases were estimated every year in 2012 and the incidence is rising fast. Patients rarely survive longer than 5 years (<5%) and surgery is the only curative treatment. However, only 10-15% of newly diagnosed cases are deemed eligible for surgery and of these patients, 80% will relapse. Thus, novel curative therapies are urgently needed and immunotherapy, including immune checkpoint inhibition, has sparked some hope. However, CTLA-4 blockade fails to induce anti-tumor responses in advanced pancreatic cancer patients and similarly treatment with PD-(L)1 blockers has been disappointing. Possibly, limited efficacy resulted from a lack of pre-existing immune responses against tumor antigens, despite the fact that PDAC is associated with both tumor antigens and recurrent mutations, which may be a source of neoepitopes. A major hurdle could be ineffective presentation of these (neo)antigens to the immune system. Antigen specific immunotherapies based on the ex vivo or in vivo loading of prevalent (neo)antigens to dendritic cells (DCs) have the potential to induce or boost PDAC directed immune responses. Such therapy could be already effective alone by inducing anti-tumor T cells but could also render patients responsive to checkpoint inhibition or treatment modalities targeting the tumor micro environment, thereby further sustaining induced anti-tumor responses. We aim to identify high potential (neo)antigens for PDAC DC-based immunotherapy by combining in silico prediction and a human leukocyte antigen (HLA) peptidomics approach. For the latter, we use mass spectrometry to verify (neo)antigen presentation on PDAC cell- and antigen-fed DCs. Currently we are setting up this study and our first results will be presented.

Jitske van den Bulk (LUMC)
Autologous neo-antigen-specific T cell responses in low mutation burden colorectal cancers
Abstract
Innovative treatment options are required to improve cure rates in advanced colorectal cancer patients. Immune checkpoint blockade therapy (anti-PD-1) was shown to be effective in colorectal cancers with high mutation burden (e.g. mismatch repair deficient) as anti-tumour reactivity is largely explained by the recognition of somatically mutated antigens (neo-antigens). No immunotherapeutic strategies are currently available for patients diagnosed with low mutation burden CRC, while they could greatly benefit from the induction of immune responses. We hypothesized that if autologous neo-antigen-reactive T cells are present in such patients, they might benefit from specific immunotherapeutic interventions that stimulate neo-antigen recognition.
In order to detect neo-antigens, whole exome and RNA next-generation sequencing were performed in cancer and healthy tissues from colorectal cancer patients. Corresponding peptides were synthesized and tested for their ability to induce immune cell activation in lymphocytes isolated from the tumour tissue and from peripheral blood. Neo-antigen-specific T cell responses were identified against 5 out of 39 neo-antigens corresponding to 35 somatic mutations that were expressed in the tumour tissue from a CRC patient.
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 autologous T cells in a mismatch repair proficient, low mutation burden CRC patient. This finding supports the widespread evaluation of the potential to employ neo-antigen-targeted therapies to improve the treatment of colorectal cancer patients.

Chih Kit Chung (LUMC)
Thermosensitive hydrogels for CTLA-4 immune checkpoint blocking therapy
Abstract
CTLA-4 immune checkpoint blockade represents an attractive cancer treatment modality, but has limitations owing to the induction of adverse side effects. When dosed systemically, CTLA-4 blocking antibodies spread rapidly in circulation and often trigger systemic side effects. Local and sustained immune checkpoint blockade potentially limits these side effects, without compromising therapeutic efficacy. Slow release drug delivery systems are promising candidates for reducing side effects and our current research is centered on injectable poloxamer hydrogels. These hydrogels are biodegradable and could extend antibody release in vivo. Henceforth, we incorporated CTLA-4 blocking antibody in poloxamer hydrogels and performed various physico-chemical and biological characterizations. We next evaluated the effects of hydrogel mediated CTLA-4 blockade on tumor growth inhibition in mice. Hydrogels with poloxamer concentrations ranging from 17 to 25% display a time-to-gelation ranging from 20 to 30 seconds at 37 degrees celsius. A 20% poloxamer formulation showed sustained antibody release in vitro over 1 week. Hydrogels delivering antibodies were then injected in mice and several days later, blood was drawn for serum antibody levels determination. Mice receiving human IgG and anti-CTLA-4 antibody display lower serum antibody levels in contrast to mice receiving these antibodies in PBS. Next the effect on tumor growth inhibition was studied in BALB/c mice bearing CT26 colon carcinoma tumors. Mice receiving anti-CTLA-4 via hydrogels had a comparable tumor growth inhibition rate as mice receiving anti-CTLA-4 in PBS and Immune Freund's Adjuvant. Post-mortal analysis revealed that the hydrogel depot was cleared and no sign of inflammation was observed. In summary, poloxamer hydrogels represent a promising platform for CTLA-4 blocking therapy.

Nick van Dijk (NKI)
Neo-adjuvant ipilimumab and nivolumab in high risk resectable bladder urothelial cancer (NABUCCO)
Abstract
Although muscle-invasive urothelial cancer (UC) can be cured by surgery, recurrence rates are high. Despite impressive response rates to neo-adjuvant cisplatin-based chemotherapy, the absolute overall survival benefit is only 5%. Immunotherapy targeting the PD-1/PD-L1-axis has shown promising activity in UC, particularly when combined with anti-CTLA-4, and patients with lymph node only disease treated with frontline immunotherapy appear to benefit most. Since responses to immunotherapy often appear to be durable, neo-adjuvant immunotherapy may improve prognosis, particularly for high risk N+ disease.
This is a single-arm phase 1B trial to establish whether sequenced pre-operative ipilimumab and nivolumab is safe in high risk UC, defined as upper/lower tract cT3-4aN0 OR ≥T1, cN+ OR ≥T1, any N, resectable retroperitoneal lymph node metastasis. Patients are eligible if they are ≥ 18 years with WHO performance 0-1. Patients must be cisplatin ineligible or refuse cisplatin-based chemo with no previous treatment with PD-(L)1 and CTLA-4 immunotherapy. To mitigate the risk of immune-related toxicity, patients are treated with a mitigated schedule (based on Meerveld-Eggink et al., Ann Oncol 2017): ipi 3 mg/kg (day 1), ipi 3 mg/kg + nivo 1 mg/kg (day 22) and nivo 3 mg/kg (day 43) followed by radical cystectomy or nefro/ureterectomy (day 57-71) with appropriate LN dissection. Six patients will undergo a re-TUR for in-depth analysis of T cell infiltrates. The primary endpoint of this trial is the percentage of patients having surgery <12 weeks after study enrollment. Secondary endpoints are efficacy (pCR) and translational. In total 24 patients will be included. At the time of abstract submission, 6 patients were included.
Clinical trial identification: NCT03387761.

Yusuf Dolen (Radboudumc)
Nanoparticle based iNKT cell vaccines: Optimal administration routes and mode of action
Abstract
Within the last couple of years, several groups demonstrated enhanced anti-tumor T cell responses by co-delivery of an iNKT cell agonist next to a protein antigen. Similarly, we demonstrated enhanced CD8 T cell responses by encapsulating both components within PLGA nanoparticles. In this study, we aim to compare different administration routes for these vaccines by means of cytotoxic T cell and antibody responses as well as their overall effect on tumour growth.
In vivo imaging demonstrated that 200 nm nanoparticles mainly localize in the spleen after intravenous (iv) administration. This was not observed by subcutaneous, intranodal or intramuscular administration. In parallel, iNKT cell activation in spleen could only be detected after iv injection, all other routes could activate iNKT cells in draining LNs in a lesser extent. High IgG1 and IgG2c levels were observed after iv injection, indicating that B cells might get more iNKT cell help in the spleen. Similarly, the highest cytotoxic T cell response was observed after iv route. Accordingly, B16.ova tumour growth was controlled longer by iv administration whereas only a short-term delay was observed by subcutaneous or intranodal injections.
These results indicate that the largest numbers of iNKT cells and T cells, which are present in spleen, are accessible only via iv administration of nanoparticles. Additionally, the dominance of iNKT1 cell subset in spleen favour a Th1 response after iv vaccination. Depending on the similarity of mouse and human iNKT cell distributions, we speculate that it would be more advantageous to perform vaccinations through iv vaccination in a clinical setting.

Tracy-Jane Eisden (VUMC)
DC-SIGN is an uptake receptor for melanoma-derived autophagosomes in human dendritic cells

Abstract
Cross-presentation of tumor-associated antigens (TAAs) by dendritic cells (DCs) is critical for the induction of anti-melanoma cytotoxic T cells. However, melanoma-derived TAAs contribute to an immune suppressed tumor microenvironment and thereby halt DC maturation. We hypothesize that melanoma-derived autophagosomes contain a broader spectrum of TAAs as well as damage associated molecular patterns that could potentially break tolerance and induce anti-tumor T-cell responses.
We isolated autophagosomes from the human melanoma cell line SK-MEL-28 by mild sonication followed by differential centrifugation. Immunoblotting analysis showed that the isolated fraction was positive for the autophagosomal markers LC3-II, Atg5 and Atg16L1. Furthermore, high-resolution flow cytometry analysis confirmed enrichment for LC3-II positive vesicles. To determine the autophagosome binding receptors, isolated autophagosomes were labelled with a fluorescent dye and subsequently targeted to monocyte-derived DCs. Notably, blocking experiments showed that the C-type lectin receptor DC-SIGN is involved in the binding and uptake of the autophagosomes by DCs. Binding experiments in DC-SIGN-transfectants corroborated the role of DC-SIGN as an autophagosome targeting receptor. Furthermore, DCs incubated with autophagosomes upregulated the maturation markers CD80, CD86, CD40 and CD70, which suggests that melanoma-derived autophagosomes are immunogenic. In addition, our preliminary data show that these autophagosomes may have the potential to evoke an anti-melanoma cytotoxic T cell response.

Job van Kooten (Erasmus MC)
Adjuvant dendritic cell based immunotherapy after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for peritoneal mesothelioma: Rationale and design of the MESOPEC study

Abstract
Malignant peritoneal mesothelioma (MPM) is an uncommon but aggressive neoplasm, with low survival rates even after palliative surgery and/or systemic chemotherapy. Recent advances in treatment strategies focusing on cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have drastically improved the median survival, but recurrence rates are high. Current systemic chemotherapy in the adjuvant setting is of limited efficacy, while dendritic cell based immunotherapy (DCBI) has yielded promising results. The goal of this study is to assess the feasibility and safety of administering DCBI after CRS-HIPEC in patients with MPM. Secondary objective is the determination of an immunological response against the tumor as result of the adjuvant therapy.
We will conduct a single-center phase II study. The study population will consist of adult patients with a histologically confirmed diagnosis of MPM. 4 to 6 weeks before CRS-HIPEC a leukapheresis will be performed of which the monocytes will be used for differentiation to dendritic cells(DCs). These DCs will be pulsed with PheraLys, a tumor cell lysate derived from 5 well-characterized cell lines from patients with malignant mesothelioma. The tumor lysate-pulsed autologous DCs(MesoPher) are re-injected 8-10 weeks after surgery, 3 times every two weeks. After the third injection with MesoPher, revaccinations to boost the immune system are given after 3 and 6 months. Inclusions started March 2018, and we expect to have enrolled al patients by March 2020. Results are expected in September 2020.
The MESOPEC study will determine if administering DBCI in patients with MPM after CRS-HIPEC is feasible and safe. This study could be the first step for new treatment strategies that will show prolonged survival with limited adverse effects in patients.

Miranda Meeuwsen (LUMC)
Identification of potent T cell clones recognizing J chain, a promising target for the treatment of Multiple Myeloma
Abstract
CD19 targeting CAR T-cell therapies have shown great promise for the treatment of multiple B-cell malignancies, but cannot be used to treat multiple myeloma (MM) due to absence of this antigen. In-house microarray data shows that joining chain (J chain) is highly expressed in four out of five primary MM samples whereas no expression is observed in healthy tissues, which makes J chain an interesting target for the treatment of MM. As a result of the intracellular localization, a TCR based approach is needed to target J chain. An HLA-A*01:01 restricted peptide derived from J chain was eluted from a MM cell line and identified by mass spectrometry. With this peptide, peptide MHC (pMHC) tetramers were generated. HLA-A*01:01 negative buffy coats were stained with pMHC-tetramer, tetramer+ CD8+ T cells were single cell sorted, clonally expanded and screened for recognition of J chain peptide loaded HLA-A*01:01+ K562 cells. Peptide specific clones were selected of which four T cell clones showed good recognition of HLA-A*01:01+ MM cell lines. EBV-LCLs and ALL cell lines express J chain 10 fold lower than primary MM. Despite the relatively low expression, multiple EBV-LCL's and ALL cell lines were recognized by all four T cell clones. Recognition was highly specific for the target peptide, since no recognition of HLA-A*01:01 negative target cells and J chain negative HLA-A*01:01 positive target cells was observed. To conclude, we identified four HLA-A*01:01 J chain restricted T cell clones that potently recognized multiple MM cell lines. Target cells with low J chain expression were also recognized indicating that the TCRs are of high affinity and might be promising for TCR gene transfer therapy of MM.

Nadine van Montfoort (LUMC)
Oncolytic viruses, a novel approach to sensitize tumours for cancer immunotherapy
Abstract
Cancer immunotherapy is particularly effective in patients with immune-active tumours. Strategies to convert immune-silent to immune-active tumours are desperately needed to broaden the number of patients that benefit from immunotherapies. Oncolytic viruses (OV) are an emerging class of cancer therapeutics that combine direct killing of tumour cells with promoting an adaptive anti-tumour immune responses. In the current study we explored whether OV can represent a useful strategy to make immunologically cold tumours hot.
Oncolytic reovirus, one of the lead candidates for clinical development, was used as a prototype oncolytic virus in a transplantable pancreatic cancer mouse model, named KPC3, which is a typical example of a cold tumour. In vitro studies demonstrated that KPC3 cells are susceptible to reovirus replication and oncolysis. In vivo dose-response studies indicated that intra-tumoural injection of reovirus leads to localized replication only in the tumour. Replication was accompanied by reduced tumour growth and a fast induction of an inflammatory gene signature, including upregulation of T cell-attracting chemokines. Seven days after reovirus injection, enhanced numbers of intra-tumoural T cells, skewing of intra-tumoural T cells towards CD8+ T cells and enhanced systemic activation of T cells was observed. Moreover, pre-treatment with reovirus enhanced efficacy of agonistic CD40 antibody therapy.
Oncolytic reovirus induces an immune-recruiting inflammatory response in the tumour microenvironment that has the potency to ignite an immune-promoting profile favourable for complementary cancer immunotherapy. These findings warrant the development of effective combinations of OV with other immunotherapeutic strategies.

Lotte Mousset (Radboudumc)
Ex vivo AKT-inhibition facilitates generation of polyfunctional stem cell memory-like CD8+ T cells for adoptive immunotherapy
Abstract
Adoptive T cell therapy has shown clinical potential for patients with cancer, though effective treatment is dependent on longevity and potency of tumor-reactive T cells. Previously, we showed that ex vivo inhibition of AKT using the research compound Akt-inhibitor VIII retained differentiation and improved functionality of minor histocompatibility antigen (MiHA)-specific CD8+ T cells. Here, we compared a panel of clinically applicable AKT-inhibitors with an allosteric or adenosine triphosphate-competitive mode of action. We analyzed phenotype, functionality, metabolism and transcriptome of AKT-inhibited CD8+ T cells using different T cell activation models. Most inhibitors facilitated T cell expansion while preserving an early memory phenotype, reflected by maintenance of CD62L, CCR7 and CXCR4 expression. Moreover, transcriptome profiling revealed that AKT-inhibited CD8+ T cells clustered closely to naturally occurring stem cell-memory CD8+ T cells, while control T cells resembled effector-memory T cells. Interestingly, AKT-inhibited CD8+ T cells showed enrichment of hypoxia-associated genes, which was consistent with enhanced glycolytic function. Notably, AKT-inhibition during MiHA-specific CD8+ T cell priming uncoupled preservation of early memory differentiation from ex vivo expansion. Furthermore, AKT-inhibited MiHA-specific CD8+ T cells showed increased polyfunctionality with co-secretion of IFN-γ and IL-2 upon antigen recall. Together, these data demonstrate that AKT-inhibitors with different modality of action promote the ex vivo generation of stem cell memory-like CD8+ T cells with a unique metabolic profile and retained polyfunctionality. Akt-inhibitor VIII and GDC-0068 outperformed other inhibitors, and are therefore promising candidates for ex vivo generation of superior tumor-reactive T cells for adoptive immunotherapy.

Rogier Reijmers (LUMC)
B cell lineage-specific transcription coactivator BOB1 is indispensable for multiple myeloma cell survival and allows for superior TCR-based targeted therapy
Abstract
Although still incurable, much progress has been made in the treatment of multiple myeloma (MM). Recent advances in immunotherapy have contributed substantially, of which the most promising, T cells modified to express a chimeric antigen receptor (CAR) directed against B cell maturation antigen (BCMA). Another approach is introducing a transgenic T cell receptor (TCR) into cytotoxic T cells. Recently, for MM, we successfully demonstrated in vivo efficacy of a transgenic TCR targeting the transcription coactivator octamer binding protein-1 (BOB1) in the context of HLA-B*07.02. BOB1 is a B cell lineage specific protein that is highly expressed in all B cell malignancies, including MM. Like rituximab (anti-CD20) treatment, targeting BOB1 will only affect the B cell lineage, which makes it attractive for immunotherapy with high on-target and low off-tumor effects. This prompted us to further explore the significance of BOB1 in MM. To this end, we applied CRISPR/Cas9 to disrupt BOB1 expression in several MM cell lines. Remarkably, upon single-cell sorting and DNA sequencing, all targeted clones revealed in-frame deletions only. Moreover, using the TIDE algorithm for easy detection of predominant types of indel mutations in a targeted pool revealed mostly (>85%) wild type sequences only, while generally a mere 5% is expected for any irrelevant gene. We are currently extending these findings to other B cell malignancies and study the functionality of the in-frame mutated BOB1 variants on target gene expression, and study the effect on in vitro and in vivo growth. Together, these data suggest that BOB1 is indispensable for myeloma cell survival, which identifies BOB1 as a superior target for TCR-based immunotherapy.

Maud Rijnders (Erasmus MC)
PD-L1 expression according to five monoclonal antibodies in urothelial cell cancer: concordance and clinical implications
Abstract
High PD-L1 expression is frequently applied as an inclusion criterion or stratification factor in clinical trials on immune checkpoint inhibitors (ICIs). However, the predictive value of PD-L1 in urothelial cell cancer (UCC) shows conflicting results, which may be confounded by the use of different PD-L1 companion diagnostics. The objective of this study was to accurately compare PD-L1 expression of five commercially available PD-L1 antibodies in UCC patients.
Tissue Microarrays (TMA) containing samples of 139 muscle-invasive UCC patients (≥pT2) were stained with the anti-PD-L1 antibodies 22C3, 28-8, SP142, SP263 and E1L3N on the Ventana Benchmark (SP142, SP263) and DAKO platforms (22C3, 28-8, E1L3N). PD-L1 expression was manually scored on tumor cells and infiltrating immune cells according to corresponding assay specifications used in clinical trials.
PD-L1 expression was found to be positive in 20% (SP263), 21% (SP142), 23% (28-8), 27% (22C3), and 27% (E1L3N) of cases. No relations between clinicopathologic parameters and PD-L1 expression were observed. Concordance in treatment-determining score varied from 72% to 90% and was lowest for E1L3N (mean 75%). Considering only companion diagnostic tests 22C3, 28-8, SP142 and SP263, PD-L1 status was concordant in 78% of patients. When one test result was discordant (n=15; 11%), SP142 (n=7) and 28-8 (n=5) were most likely different.
Agreement of PD-L1 assessment is good with similar PD-L1 status by four antibodies used in companion diagnostic tests. Therefore, application of different companion PD-L1 antibodies and platforms may have limited effects on therapeutic decision making in ICI treatment.

Lorenzo Spagnuolo (NKI)
Dissecting the synergistic effect of chemotherapy and immunotherapy on anti-tumoral T cell functions in breast cancer
Abstract
Immunotherapies targeting immune checkpoint molecules proved effective for the treatment of several solid tumors, including melanoma and lung cancer. However, the majority of breast cancer patients does not respond to immune checkpoint inhibition, highlighting the need for new immunomodulatory approaches. The combination of immuno- and conventional therapies is a promising strategy to elicit effective anti-tumor immunity. Our aim is to characterize the effect of de novo mammary tumors on T-cell functionality, and to assess whether effective anti-tumor immunity can be induced by combining immunotherapy and chemotherapy.
Mammary tumors in K14cre;Cdh1F/F;Trp53F/F (KEP) mice, a spontaneous tumor model that resembles human ILC, show a decreased infiltration of CD8+ and CD4+ T cells, and increased percentage of T regulatory cells, compared to tumor-free mammary glands. Tumor-infiltrating CD8+ T cells have an increased expression of multiple inhibitory receptors, and decreased IFNg production, compared to peripheral CD8+ T cells of KEP mice. However, in vitro exposure of sorted tumor-derived CD8+ T cells to IL-15 or IL-2 augments their capacity to produce IFNg, suggesting that their dysfunctional state is not terminal. Treatment of KEP mice with a-CTLA4 and a-PD1 does not affect tumor growth, but a synergistic therapeutic benefit is seen when they are combined with cisplatin, in a CD8+ T-cell dependent mechanism. Our results indicate that tumor-infiltrating CD8+ T cells display features of dysfunctional T cells, but this state is partially reversible. Nonetheless, only the combination with chemotherapy and immunotherapy is able to elicit an efficient CD8+ T-cell response. We are now dissecting the mechanisms underlying the synergistic response, in order to ultimately contribute to the design of new immunomodulatory strategies for breast cancer.

Shabaz Sultan (Radboudumc)
Robust Identification of Immune Cell Subsets in Multiplex Immunohistochemistry
Abstract
Immunohistochemistry (IHC) allows imaging of tumour-infiltrating immune cells (TILs) within tumour microenvironments. TIL presence and localization is thought to be relevant for predictive and prognostic purposes in immunotherapy. However TILs come in many different subsets, which might have fundamentally different functions. Therefore it is important to be able to differentiate different types of TILs in IHC data.We designed a six marker multiplex IHC panel of immune cell markers (CD3, CD8, FOXP3, CD45RO, CD56) and a tumour marker. Reliable phenotyping based on multiplex IHC poses several challenges, compared to better established cell phenotyping methodologies such as flow cytometry. Particularly, IHC-based phenotyping requires a cell segmentation step, which brings in significant uncertainty. Segmentation errors can bias cell population counts and muddle phenotype discrimination. Here, we aim to develop robust methodology for multiplex IHC-based immune cell quantification that acknowledges this uncertainty. We base this method on both unsupervised clustering and supervised classification algorithms. To validate our multiplex IHC phenotyping results, we split PBMC populations from human donors in two, embedding one half in pellets for multiplex IHC imaging and evaluating the other half using the same immune cell markers analysed with flow cytometry. We show that our multiplex IHC phenotyping analysis is able to distinguish phenotype clusters, which correspond to cell populations identified in flow cytometry data. To our knowledge, this is the first direct validation of multiplex IHC phenotyping. Our results will be of interest to all researchers who are using multiplex IHC to image the tumour microenvironment.

Nadine Grima Sopesens (VUMC)
Proinflammatory activity of vascular targeted therapy against cancer
Abstract
We have shown that (i) tumor endothelial cells lack sufficient expression of adhesion molecules and that (ii) one of the mechanisms of enhanced immunity after exposure to angiostatic drugs is the induction of adhesion molecule expression in the tumor vasculature. We now demonstrate that sunitinib, a widely used drug for the treatment of e.g. renal cell cancer (RCC), significantly augments endothelial intercellular adhesion molecule-1 (ICAM-1) expression, the most important adhesion molecule for leukocyte extravasation and infiltration of the tumor tissue. Other angiostatic targeted compounds, such as axitinib, erlotinib and crenolanib, showed similar results, further confirming the induction of endothelial adhesiveness by angiostasis. The induction of endothelial ICAM-1 has functional impact on the extravasation of leukocytes, as preliminary data show that sunitinib enhanced the number of transmigrated leukocytes, mainly CD3+ lymphocytes, in a trans-endothelial migration assay.
To investigate whether the above mentioned results have impact on leukocyte infiltration in human cancer, tumor tissues of phase II trials of VEGF pathway targeted therapy, given prior to cytoreductive surgery, were used to quantify leukocyte infiltration. We observed a coincident pro-inflammatory effect of this treatment. Sunitinib as well as bevacizumab pretreatment resulted in a significant enhancement of leukocyte infiltration into the tumor. This was observed for all tested leukocyte subsets, such as (cytotoxic) T cells, neutrophils and macrophages. In addition, RCC tumors were found to be massively infiltrated by macrophages, most of which expressed the M2 phenotype (CD163).
This study contributes to the important concept of anti-vascular therapy to boost immunity and the expected benefit of combining immunotherapy approaches with vascular targeted strategies.

Marcella Willemsen (AMC)
Prognostic implications of tissue resident memory T cells in human melanoma development
Abstract
Tissue-resident memory T (TRM) cells permanently reside in epithelial barrier tissues and can respond rapidly upon reinfection. Recently, expression of the retention integrin very late antigen (VLA)-1, by vaccine-induced T cells was found to correlate with longer patient survival in melanoma. More interestingly, VLA-1 was frequently co-expressed with tissue residence markers, such as CD69 and CD103. Furthermore, CD103-dependent TRM cells seem to play a key role in sustaining immunity to melanoma, indicating a crucial function for TRM cells in antitumor immunity. Yet, its role in melanoma development remains unknown and might be relevant, for there are numerous neoplastic lesions in the skin that rarely become overt cancers. This research, therefore, aimed to identify the prognostic relevance of skin resident memory T cells in human melanomagenesis.
Healthy skin, nevus naevocellulares, dysplastic nevus, lentigo maligna, lentigo maligna melanoma, nodular primary melanoma, superficially spreading primary melanoma and cutaneous metastatic melanoma (all n=7) were analyzed by immunohistochemistry for the presence of TRM cells. The prognostic significance of CD103 and VLA-1 expression on TRM cells was also investigated.
The presence of TRM cells may serve as prognostic marker for disease progression. Furthermore, intratumoral TRM cells, especially in primary melanoma, may potentially be a good effector in cancer immunotherapies.

Rosa de Groot (Sanquin)
Effective expansion and reprogramming of tumor infiltrating lymphocytes from non-small cell lung cancers
Abstract
Non-small cell lung cancer (NSCLC), the second most occurring type of cancer, is highly recurrent with limited 5-year survival. NSCLCs contain high numbers of T cells, and although suggestive of tumor reactivity, these tumor infiltrating lymphocytes (TILs) are unable to eradicate the cancer. Here we investigated whether TILs from NSCLC can be reprogrammed and used for autologous T cell therapy. TILs were isolated from tumor tissues and reprogrammed via in vitro activation using a standard TIL expansion protocol. Strikingly, the vast majority of expanded TILs from primary NSCLC tumor tissues (stage I-IIIA) contained tumor reactive T cells; TILs from 13/17 donors (76,5%) produced inflammatory cytokines in response to autologous tumor digests, but not in response to distal non-tumorous lung tissues. Tumor responses ranged from 1%-35% of cytokine-producing TILs, which correlated with expression of activation markers CD137(4-1BB) and CD154(CD40L) on tumor-reactive CD8+ and CD4+ T cells, respectively. Furthermore, TIL cultures with high cytokine responses also contained poly-functional T cells that produced IFN-γ together with TNF-α and/or IL-2. Importantly, increased percentages of CD103+/CD69+ CD8+ T cells, CD279(PD1) high CD4+ T cells and CD25+/Foxp3+ T cells, within the tumor infiltrates compared to autologous lung infiltrates, were strong predictors of the intensity of tumor-reactivity in expanded TILs. Collectively, our data show that expansion and reprogramming of TILs from NSCLC to obtain tumor reactive T cells, is effective, strongly suggesting that autologous T cell therapy should be considered to treat NSCLC patients.

Marijne Heeren (VUMC)
Indoleamine 2,3-dioxygenase Expression Pattern in the Tumor Microenvironment predicts Clinical Outcome

Abstract
Indoleamine 2,3-dioxygenase (IDO) can act as immunoregulator by inhibiting T cells via the degradation of tryptophan (trp) into kynurenine (kyn). The kyn/trp-ratio in serum is a prognostic factor for cervical cancer patients, however, information about the relationship between serum levels and IDO expression in the tumor microenvironment is lacking. IDO expression was studied in 71 cervical cancer samples by immunohistochemistry, and the link between kyn/trp-ratio in serum, clinicopathological characteristics, and the presence of T cells (CD8, Ki67, and FoxP3) in tumors were examined. In addition, we studied IDO1 and IFNG gene expression using RNAseq data from 144 cervical tumor samples published by The Cancer Genome Atlas (TCGA).
We demonstrate that patchy tumor IDO expression is associated with an increased systemic kyn/trp ratio in cervical cancer (P=0.009), whereas marginal tumor expression at the interface with the stroma is linked to improved disease-free and disease-specific survival (DFS: P=0.017, DSS: P=0.043). The latter may be related to T cell infiltration and localized IFNγ-release inducing IDO expression. Indeed, TCGA analysis revealed a positive correlation between IDO1 and IFNG mRNA expression levels (P<0.001) and a significant association with improved DFS for high IDO1 and IFNG levels.
Our data indicate that the serum kyn/trp-ratio and IDO expression in primary tumors are not clear-cut biomarkers for prognosis and stratification of patients with cervical cancer for clinical trials implementing IDO inhibitors. Rather, a marginal IDO expression pattern in the tumor dominantly predicts favorable outcome, which appears to be related to IFNγ-release in the cervical tumor microenvironment.

Julien Karrich (Sanquin)
MISTRG: improved human immune system mouse model to test transplantable T cell therapy against solid tumors

Abstract
Transfusion of in-vitro expanded T cells isolated from tumors (Tumor-Infiltrating lymphocytes or TILs), back into patients is a promising form of therapy for yet untreatable types of cancer, such as melanoma. Although significant progress has been achieved in optimizing in-vitro TIL cultures, many parameters affecting T cell responses against tumors cannot be approximated in-vitro. Therefore, we aim to establish and characterize a human immune system mouse model to study TIL responses to tumors in vivo. To this end, we are using the newly developed “MISTRG” mouse model, which allows superior development of a human immune system, which includes both lymphocytes as well as functional myeloid and NK cells at close to physiological levels. Importantly, these mice allow establishment of human tumors that are histologically similar to human tumors in terms of vascularization and infiltration of myeloid cells. This model therefore more reliably mimics human tumors than traditional models using NSG mice. We will use this new model to engraft patient-derived non-small cell lung carcinoma (NSCLC) tumor cells and subsequently test ex-vivo expanded TILs in an autologous setup. To do so, tumor cells are isolated isolated directly from patient biopsies and expanded ex-vivo as organoid culture, cell line culture, or in-vivo in a xenograft model. We will use this model to study parameters that determine the outcome of TIL therapy and to develop and test different treatment modalities.

Paul Kemps (LUMC)

Failure to present neo-peptides hampers T cell recognition of neoplastic BRAFV600E expressing Langerhans Cell Histiocytosis Cells

Abstract

Langerhans Cell Histiocytosis (LCH) is a rare neoplastic disorder of myeloid origin, characterized by CD1A+ CD207+ histiocytes (LCH-cells). These LCH-cells universally express constitutively phosphorylated ERK, mostly as the result of single somatic mutations in genes confined to the MAPK signalling pathway. Moreover, the LCH-cells are typically accompanied by a diverse inflammatory infiltrate, often including T cells. It is, however, unclear whether (neo-)peptides derived from the (mutated) MAPK proteins can be recognized by these T-cells and thereby trigger an anti-tumor immune response.

We quantified CD8+ T cells in first disease onset FFPE biopsies of n=157 LCH-patients and correlated these findings to their mutational status, Human Leukocyte Antigen (HLA) genotype and event-free survival (EFS). In addition, we explored NetMHC and IEDB prediction algorithms to identify strong HLA class I binding (neo-)peptides derived from mutated MAPK genes identified in patients from our cohort.

No significant EFS advantage was detected for patients with high lesional CD8+ T-cell infiltration. Furthermore, only a single 11-mer neo-peptide derived from the BRAFV600E protein was predicted to be naturally processed and to bind strongly to HLA class I molecules, being HLA-A*11:01 and (to a lesser extent) HLA-A*03:01. In vitro binding assays confirmed these predictions. Surprisingly, we could however not detect this 11-mer neo-peptide in the HLA peptidome eluted from HLA-A*11:01 bearing BRAFV600E transduced EBV-transformed B-cells (EBV-LCL). This absence of neo-peptides could explain why HLA-A*11:01 (and/or HLA-A*03:01) genotype in n=52 BRAFV600E mutated LCH-patients was not correlated with superior EFS. Therefore, we conclude that BRAFV600E neo-peptide-specific T-cells are highly unlikely to be involved in the regression of LCH lesions.

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