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Cancer immunology

Maarten Nijen Twilhaar, PhD student

Incorporation of Toll-like receptor ligands and inflammasome stimuli in GM3 liposomes to induce dendritic cell maturation and T cell responses

1Nijen Twilhaar MK, 2Czentner L, 1Bouma RG, 1Olesek K, 1Grabowska J, 1Wang AZ, 1Affandi AJ, 1Belt SC, 1Kalay H, 2van Nostrum CF, 1van Kooyk Y, 2-4Strom G and 1den Haan JMM

1 Department of Molecular Cell Biology and Immunology, Cancer Center Amsterdam, Amsterdam Infection and Immunity Institute, Amsterdam University Medical Center, Vrije Universiteit Amsterdam, 1081 HZ Amsterdam, The Netherlands.
2 Department of Pharmaceutics, Faculty of Science, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands;
3 Department of Biomaterials Science and Technology, Faculty of Science and Technology, University of Twente, 7522 NB Enschede, The Netherlands
4 Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore

Cancer vaccination aims to activate immunity towards cancer cells and can be achieved by delivery of cancer antigens together with immune stimulatory adjuvants to antigen presenting cells (APC). APC maturation and antigen processing is a subsequent prerequisite for T cell priming and anti-tumor immunity. In order to specifically target APC, nanoparticles, such as liposomes, can be used for the delivery of antigen and adjuvant. We have previously shown that liposomal inclusion of the ganglioside GM3, an endogenous ligand for CD169, led to robust uptake by CD169-expressing APC and resulted in strong immune responses when supplemented with a soluble adjuvant. To minimize the adverse effects related to a soluble adjuvant, immune stimulatory molecules can be incorporated in liposomes to achieve targeted delivery of both antigen and adjuvant. In this study, we incorporated TLR4 (MPLA) or TLR7/8 (3M-052) ligands in combination with inflammasome stimuli, 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC) or muramyl dipeptide (MDP), into GM3 liposomes. Incorporation of TLR and inflammasome ligands did not interfere with the uptake of GM3 liposomes by CD169-expressing cells. GM3 liposomes containing a TLR ligand efficiently matured human and mouse dendritic cells in vitro and in vivo, while inclusion of PGPC or MDP had minor effects on maturation. Immunization with MPLA-containing GM3 liposomes containing an immunogenic synthetic long peptide stimulated CD4+ and CD8+ T cell responses, but additional incorporation of either PGPC or MDP did not translate into stronger immune responses. In conclusion, our study indicates that TLRL-containing GM3 liposomes are effective vectors to induce DC maturation and T cell priming and thus provide guidance for further selection of liposomal components to optimally stimulate anti-cancer immune responses.

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Cancer immunology
Dennis Gout, PhD student

Fusion of TNF-α to TrisomAb potentiates neutrophil-mediated anti-tumor effects

1,2,3Gout DY, 1,2,3Duru G, 1Tuk CW, 1,2,3Sewnath CAN, 1,2,3Gruijs M, 1,2,3Heemskerk N, 1,4van Egmond M

1Department of Molecular Cell Biology and Immunology, Amsterdam UMC, Amsterdam, The Netherlands
2Cancer Center Amsterdam, Amsterdam UMC, Amsterdam, The Netherlands
3Amsterdam Institute for Infection and Immunity, Amsterdam, The Netherlands
4Department of Surgery, Amsterdam UMC, Amsterdam, The Netherlands

Recruitment and activation of neutrophils, in addition to macrophages and NK cells, through treatment with TrisomAb, an IgG1-based bi-specific antibody, significantly decreased tumor growth in an aggressive B16F10 murine melanoma model. Additionally, TNF-α is a potent attractor and activator of innate and adaptive immune cells. However, systemic TNF-α treatment in cancer patients led to dose-limiting toxicity, limiting its efficacy despite promising anti-tumor results. This project aims to bolster the anti-tumor effects of TrisomAb through the fusion of TNF-α to its C-terminus.
After SDS-PAGE confirmed successful generation of the immunocytokine (TrisomAb-TNF), binding affinity of TrisomAb-TNF for FcαRI, FcɣRIII and a tumor antigen (EGFR) were shown to be comparable to the TrisomAb original via surface plasmon resonance and functional binding assays. Additionally, in vitro tumor killing via FcɣRIII was shown to be unimpeded in ADCP assays using macrophages, while TrisomAb-TNF treatment increased tumor killing in neutrophil ADCC assays. Following these promising results, we are now investigating the in vitro effects of TrisomAb-TNF on the adaptive immune system, as well as the effects on in vivo tumor infiltration by immune cells.
Concluding, TrisomAb-TNF shows potential to surpass TrisomAb in its anti-tumor effects and to induce an enduring adaptive immune response.

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Cancer immunology
Aru Wang, PhD student

Development of a versatile nanocancer vaccine format

1,2,3Aru Z. Wang, 1,2,3Priscilla D. A.M. Heijnen, 2,4Janneke Maaskant, 1,2,3Katarina Olesek, 5Johan M.S. van der Schoot, 5Martijn Verdoes, 6Ferenc A. Scheeren, 1,2,3Hendrik Brink, 1,2,3Alsya J. Affandi, 1,2,3Hakan Kalay, 2,4Coen P. Kuijl, 1,2,3Joke M.M. d

1Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Molecular Cell Biology and Immunology, Boelelaan 1117, Amsterdam, The Netherlands, 2Cancer Center Amsterdam, Cancer Biology and Immunology program, Amsterdam, The Netherlands, 3Amsterdam institute for Infection and Immunity, Cancer immunology program, Amsterdam, The Netherlands, 4Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Medical Microbiology and Infection Control, Boelelaan [1117], Amsterdam, The Netherlands, 5Department of Tumour Immunology, Radboud Institute for Molecular Life Sciences, Institute for Chemical Immunology, Nijmegen, The Netherlands, 6Department of Medical Oncology, Leiden University Medical Center, Leiden, The Netherlands.

Recent cancer immunotherapies have improved the outlook of many cancer types, however, the majority of patients still does not benefit from these new therapies. The clinical response of cancer patients towards these therapies is correlated with the presence of activated immune cells in the tumor. Cancer vaccines can be utilized to increase tumor-specific immune responses in non-responding patients and should contain antigens specifically expressed in the tumor. Patient-specific neoantigens form an attractive type of cancer antigen as there will be no tolerance to these antigens, but this also necessitates the production of patient-specific cancer vaccines. Our aim is to develop a versatile cancer vaccine format in which patient-specific tumor antigens can be conjugated by a proximity-based sortase A reaction to antibodies specific for dendritic cells to stimulate immune responses.
We used the CRISPR/HDR platform to switch hybridoma's secreting rat IgG2a specific for mouse DEC205 or control rat IgG2a to produce mouse heavy chain IgG2a antibodies with a sortase A recognition motif followed by a linker and a SpyTag. The 'switched' DEC205 and control mouse IgG2a antibodies were produced and the binding to CHO cells expressing DEC205 was confirmed. In addition, we produced recombinant protein that consists of Sortase A linked to a SpyCatcher protein. Binding of the SortaseA-SpyCatcher to the 'switched' antibodies will enable efficient proximity-based sortase A mediated ligation of neoantigens. We detected antibody-Spytag binding to SpyCatcher protein by SDS page gel and ELISA and will further optimize this. In future experiments, we will ligate Alexa-647 and OVA peptides to the switched antibodies and test these for binding and the induction of immune responses. We expect that this novel method of proximity-based sortase mediated antigen ligation will enable fast and efficient production of patient-specific cancer vaccines.

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Cancer immunology
Gizem Duru, PhD student

Activating tumor endothelial cells to increase immune infiltration and cancer immunotherapy

1,2,3Duru G, 1,2,3Gout D, 1,2,3,4Van Egmond M, 1,2,3Heemskerk N

1Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Molecular Cell Biology and Immunology, Boelelaan 1117, Amsterdam, The Netherlands. 2Cancer Center Amsterdam, Cancer Biology and Immunology program, Amsterdam, the Netherlands. 3Amsterdam institute for Infection and Immunity, Cancer immunology program, Amsterdam, the Netherlands. 4Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Surgery, De Boelelaan 1117, Amsterdam, Netherlands.

Three distinct phenotypic immune profiles can be observed in solid tumors: 1) cancers with immune cells inside the tumor (referred to as 'hot' tumors), 2) cancers with immune cells at the invasive margin ('excluded' tumors), and tumors lacking the presence of immune cells ('desert' tumors). Whereas 'hot' tumors respond best to immunotherapy, efficacy can be hampered by an immunosuppressive environment. Furthermore, immunotherapeutic responses in 'excluded' and 'desert' tumors are limited due to lack of immune cell infiltration. To overcome immunosuppression and the biological barrier for tumoricidal immune cells and improve the efficacy of immunotherapy, we have generated a tumor targeting bispecific antibody that is fused to TNF (TrisomAb-TNF). TrisomAb is a bispecific antibody format that targets EGFR+ tumors and recruits innate immune cells that express FcαRI. TrisomAb shows superior anti-tumor activity compared to conventional IgG, but efficacy in vivo is partly hampered due to limited immune cell infiltration. TNF is a pro-inflammatory cytokine well known to increase the exposure of cell adhesion molecules (CAMs) and leukocyte activating chemokines on endothelial cells. In this project, we therefore investigate the effects of TrisomAb-TNF on endothelial cells and leukocyte transendothelial migration in tumors. We analyzed ICAM-1 expression on TrisomAb-TNF stimulated Human Umbilical Cord Endothelial Cells (HUVEC) using flow cytometry. ICAM-1 surface expression of HUVEC treated with TrisomAb-TNF was comparable to TNF. TrisomAb alone did not increase ICAM-1 on HUVEC. Furthermore, in a live cell imaging model for leukocyte transendothelial migration, we examined how TrisomAb-TNF affected neutrophil-endothelial adhesion and transendothelial migration under shear flow conditions. HUVECs were grown in ibidi 6 µ-Slide channels until a full monolayer was established and subsequently stimulated with TNF, TrisomAb or TrisomAb- TNF. High numbers of adherent and transmigrated neutrophils were observed in the flow channels treated with TrisomAb-TNF and TNF, whereas neutrophil adhesion and transmigration in unstimulated or TrisomAb conditions was absent or very low. Thus, our data indicates that TrisomAb-TNF is functionally capable to increase neutrophil transendothelial migration, in part through increased endothelial ICAM-1 surface expression. As such, TrisomAb-TNF has the potential to increase intratumoral neutrophil infiltration and therapeutic efficacy of TrisomAb in pre-clinical tumor mouse models.

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Cancer immunology
Celine Sewnath, PhD student

Neutrophil-mediated tumor cell killing induces uptake of antigens and dendritic cell maturation

123C.A.N. Sewnath, 1,2,3,4M. van Egmond

1Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Molecular Cell Biology and Immunology, Boelelaan 1117, Amsterdam, The Netherlands, 2Cancer Center Amsterdam, Cancer Biology and Immunology program, Amsterdam, the Netherlands, 3Amsterdam institute for Infection and Immunity, Cancer Immunology program, Amsterdam, the Netherlands, 4Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Surgery, Netherlands

Immunotherapy is a promising strategy for cancer treatment. Unfortunately, many tumors have an immunosuppressive microenvironment, precluding the induction of long-term adaptive immune responses. This immunosuppressive tumor environment can be infiltrated with various immune cells that can secrete anti-inflammatory mediators thereby preventing tumor cell killing by other immune cells. Recently, we developed a bi-specific antibody called TrisomAb, which consists of three elements. It can target tumor-associated antigens (binding to tumor cells) and FcαRI (binding of neutrophils) with a functional IgG Fc tail (binding of NK cells and macrophages). Our goal is to investigate if adaptive immune responses can be induced using TrisomAb. Therefore, co-cultures with neutrophils, dendritic cells, tumor cells and TrisomAb were performed. Uptake and activation measurement was done by flow cytometry. Furthermore, the cytokine profile of co-cultures were assessed by ELISA. Our data showed that TrisomAb recruited and activated neutrophils, which induced tumor cell killing. Co-culture of tumor cells with dendritic cells, neutrophils and TrisomAb resulted in release of tumor antigens by neutrophils and enhanced antigen uptake by dendritic cells, as well as secretion of pro-inflammatory cytokines (i.e. IL-12p40) that are involved in induction of T cell responses. Taken together, neutrophil-mediated killing via TrisomAb leads to tumor cell antigen uptake by dendritic cells. Moreover, tumor antigen uptake by dendritic cells results in activation and subsequent secretion of factors that could lead to induction of adaptive immune responses after treatment with TrisomAb.

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Cancer immunology
Nordin Zandhuis, PhD student

ZFP36L2 REGULATES CYTOKINE PRODUCTION IN CD8+ T CELLS IN A CONTEXT-DEPENDENT MANNER

N.D. Zandhuis1,2, A. Guislain1,2, S. Engels1,2, M. Turner3, M. Wolkers1,2

1Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, The Netherlands
2Oncode Institute, Utrecht, The Netherlands
3Department of Immunology, The Babraham Institute, Cambridge, UK

CD8+ T cells kill target cells by releasing cytotoxic molecules and inflammatory cytokines, such as TNF and IFNγ. The magnitude and duration of cytokine production is defined by post-transcriptional regulation, which is by and large driven by so-called RNA-binding proteins. In T cells however, it is not well-defined which RNA-binding proteins are key in this regulatory process. Here, we found that the RNA-binding protein ZFP36L2 regulates cytokine production in CD8+ T cells in a context-dependent manner. Specifically, we observed increased frequencies of IFNγ producing T cells, but not of TNF and IL-2, during T cell priming of ZFP36L2-deficient murine T cells. This was concomitant with decreased proliferation of ZFP36L2-deficient naïve T cells. Conversely, cytokine production of effector T cells was identical between WT and ZFP36L2-deficient CD8+ T cells when reactivated in vitro and ex vivo. Strikingly, during prolonged exposure to tumour cells in vitro or within the tumour microenvironment in vivo, ZFP36L2-deficient CD8+ T cells maintained substantially higher percentages of IFNγ-producing cells, even though ZFP36L2-deficient CD8+ T cells displayed a similar “exhaustion” marker expression profile. Together these results indicate that ZFP36L2 is directly regulating cytokine production in CD8+ T cells in a context-dependent manner.

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Cancer immunology
Paula Martinez Sanz, PhD student

Characterizing humanized MISTRG mice as a preclinical model to study neutrophil-mediated immunotherapy in cancer

1 Martínez-Sanz, P., 1 Hoogenboezem M., 2 Laurant. A, 2 Amsen D., 3 Tytgat G.A.M., 1 Matlung H.L., 1,4 Kuijpers T.W., 2 Karrich J.J.

1 Dept. of Molecular Hematology, Sanquin Research, Amsterdam, the Netherlands, 2 Dept. of Hematopoiesis, Sanquin Research, Amsterdam the Netherlands, 3 Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands, 4 Dept. of Pediatric Immunology, Rheumatology and Infectious Diseases, Emma Children's Hospital, Amsterdam UMC, Amsterdam, the Netherlands

The MISTRG mice are considered an improved humanized mouse model as compared to other conventional humanized mice. They have been genetically modified to develop a proper human myeloid compartment, making them a unique and very suitable model to study the innate immune system in vivo. Despite being nicely represented, the neutrophil population in the humanized MISTRG mice seems to be retained in the bone marrow and does not mobilize into circulation. Here, we aim at characterizing the human neutrophil population further so as to establish a model in which human neutrophil biology and their contribution in various immune processes can be studied in vivo, including thioglycolate-induced peritonitis and tumor infiltration. We successfully isolated human bone marrow neutrophils in humanized MISTRG mice. By magnetic sorting with CD15 beads, we confirmed all neutrophil maturation stages from promyelocytes (CD11b-CD16-) to end-stage segmented cells (CD11b+CD16+) to be present in the bone marrow and found normal functionality (i.e. degranulation, reactive oxygen species (ROS) production, adhesion and antibody-dependent cellular cytotoxicity towards antibody-opsonized tumor cells) ex vivo. Flow cytometry-based assays allowed us to differentiate the contribution of each of the neutrophil bone marrow subpopulations on their ability to produce intracellular ROS and to degranulate, which positively correlated with the maturation state of the cell. In addition, we managed to induce the release of the mature and segmented human neutrophils with phenotype CD11b+CD16+ into the periphery of humanized MISTRG mice in response to two well-established neutrophil-mobilizing agents (i.e. G-CSF and/or Plerixafor). Last, we detected extravascular infiltration of human neutrophils in response to acute (thioglycolate-induced peritonitis) as well as to chronic (tumor) inflammation. Altogether, these data qualifies the humanized MISTRG mice as the most relevant in vivo model to study neutrophil-mediated immunotherapy in cancer.

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Cancer immunology
Leonie Behrens, PhD student

Unravelling the method of action of the innate immune checkpoint CD47-SIRPα

1 2 3 Behrens LM
4 van den Berg TK
1 2 3 5 van Egmond M

1 Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Molecular Cell Biology and Immunology, Amsterdam, the Netherlands
2 Cancer Center Amsterdam, Cancer Biology and Immunology program, Amsterdam, the Netherlands
3 Amsterdam Institute for Infection and Immunity, Cancer Immunology program, Amsterdam, the Netherlands
4 Byondis B.V., Department of Immuno-Oncology, Nijmegen, the Netherlands
5 Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Surgery, Cancer Center Amsterdam, Amsterdam, the Netherlands

In the last decades, different methods have been developed to harness the immune system for the treatment of cancer. One way to effectively stimulate an anti-tumor response is by the use of tumor-specific monoclonal antibodies (mAbs). These mAbs specifically bind to tumor antigens and are recognized by different immune cells. Neutrophils have been shown to kill antibody-opsonized tumor cells through a process called trogocytosis. During this process, the neutrophils phagocytose small pieces of the tumor cell cytosol, eventually inducing a necrotic type of cancer cell death, named trogoptosis. A way for tumor cells to evade the immune system is through the expression of checkpoint molecules. CD47-SIRPα is an innate immune checkpoint axis, where CD47 is expressed on virtually all cells, while SIRPα is expressed on myeloid cells, such as neutrophils and macrophages. Tumor cells often overexpress CD47 and thereby prevent tumor elimination by these myeloid cells. Blocking the interaction between CD47 and SIRPα has been shown to potentiate tumor cell killing of antibody-opsonized tumor cells. It is currently still unknown how SIRPα signaling prevents neutrophil-mediated tumor cell killing. However, it has been shown that SIRPα signaling controls Kindlin3-dependent CD11b/CD18 integrin activation. Thus, during my PhD I will investigate the method-of-action of CD47-SIRPα signaling in neutrophils. In addition, we will be studying the effect of blocking the CD47-SIRPα axis in combination with other immunotherapies to potentiate tumor cell killing.

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Cancer immunology
Tara Muijlwijk, PhD student

Attraction of immune cells by head and neck cancer cell lines and tumor specimen-conditioned media

1Muijlwijk T, 2Remkes R, 3Fritz L, 4Poell JB, 5Leemans CR, 6Brakenhoff RH, 7van de Ven R

Amsterdam UMC, location VUmc Department of Otolaryngology / head and neck surgery, Cancer Center Amsterdam

Background: Head and neck squamous cell carcinomas (HNSCC) are classified in human papillomavirus (HPV)-positive and HPV-negative tumors. In general, HPV-negative HNSCC are characterized by many chromosomal gains and losses. Previously, we and others identified a HPV-negative subgroup with few or absent copy number alterations (CNA-silent) and a different mutational profile. Generally, tumors with low copy number changes have been associated with high immune infiltration scores, but for HNSCC such data are lacking. In this study we aim to investigate immune cell attraction characteristics of various subclasses of HNSCC using functional assays.
Methods: Eight HNSCC cell lines and 13 conditioned media of HNSCC biopsies were used to characterize their ability to attract immune cell subsets in a transwell migration system. Chemokines were quantified using the Olink Target 96 Immuno-Oncology panel.
Results: Most cell lines induced migration of monocytes, B cells and CD4+ T-cells whereas they did not induce CD8+ T-cell or cDC1 migration. Tumor-conditioned media did also not attract CD8+ T-cells while these did induce potent monocyte and cDC1 migration. High levels of MCP-3, MCP-4, CXCL5, CCL2 and CCL20 correlated with cDC1 migration.
Conclusions: Immune cell migration patterns did not differ in relation to tumor sites, genetic profile or HPV-status. Specific chemokines present in tumor-conditioned media are not produced by HNSCC cell lines and may explain the difference in cDC1 migration. The noted lack of CD8+ T-cell attraction may explain why PD-1 inhibitors are effective in only a minority of HNSCC patients. Our data emphasizes the need to improve CD8+ T-cell attraction in HNSCC.

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Cancer immunology
Thomas van Ee, PhD student

Mechanisms of immune evasion in with KRAS-mutant lung adenocarcinoma: A role of MAPK-pathway activation

1van Ee TJ, 2Naves D, 3van Maldegem F, 4Böttger F, 5Jimenez C, 6Gorris M, 7de Vries J, 8Fransen M, 9de Gruijl TD, 10Veltman J, 11Bahce I, 12Radonic T, 13van Kooyk Y

1dept. of Molecular Cell Biology and Immunology
2dept. of Pathology
Only first author, how am I supposed to link affiliations per author using this format?

There is emerging evidence of modulatory ability of (in)activated oncogenic pathways in shaping antitumor immune responses. Synergistic efficacy of KRAS-G12C inhibitor AMG510 with anti-PD-1 immune checkpoint blockade (ICB) demonstrated to reverse the immunosuppressive tumor microenvironment. We hypothesized that activation of RAS-signaling pathway, not necessarily KRAS-mutation status, induces a distinct immune evasion pattern.
Sixty-four clinically annotated surgically resected LADC were analyzed using multiplex immunohistochemistry (mIHC) panels and scanned with Vectra® Polaris™ to quantify lymphocytes (CD3, FoxP3, CD20, CD45ro, CD8) and myeloid cells (HLA-DR, CD83, CD68, CD14, CD163). Subset of 21 patients was profiled with liquid chromatography tandem-mass spectrometry proteomics. Granulocyte neutrophils and immunohistochemistry for PD-(L)1, MAPK and p-ERK were semi-quantitatively assessed.
Median follow-up period was 34 months (IQR: 19-60). Thirty-four out of 64 patients (53%) had high MAPK-pathway activation and significantly shorter recurrence-free survival (RFS) compared to low MAPK-activation. Interestingly, MAPK-activation was not always associated with KRAS-mutation (14/26 KRASMut,20/38 wildtype). Unbiased gene-set enrichment analysis of proteomics was performed for MAPK-high versus MAPK-low patients. mIHC showed increased infiltration of M2 macrophages around the tumors of MAPK-high cases compared with MAPK-low cases. Of these, only MAPK-high tumors were strongly infiltrated by T-helper (CD3+ FoxP3- CD8-) and T-regulatory (CD3+ FoxP3+) cells.
MAPK-pathway activation was associated with poor RFS in curatively treated LADC patients. Coordinated infiltration of T-helper and T-regulatory cells, M2 macrophage border and exclusion of cytotoxic T-cells in MAPK-high tumors might contribute to its underlying immune-suppressed state. Our data open the possibility of novel combination therapies, including RAS-inhibitors and different ICBs, outside the KRAS-mutant group.

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Cancer immunology
Fabio Balzarini, PhD student

MICROBIAL-GLYCOENGINEERING APPROACH TO IMMUNOTHERAPY

1_Balzarini F, 2_Bruijns C.M. S, 3_Silipo A, 4_ Chiodo F, 5_Van Kooyk Y

1_Department of Molecular Cell Biology and Immunology, Amsterdam University Medical Center, Amsterdam Institute for Infection and Immunity, 2_Vrije Universiteit Amsterdam, Amsterdam Netherlands. 3_Centro Nazionale delle Ricerche (CNR), 4_Universita`degli studi Federico II, Napoli, Italy. 5_Finlay Institute, Havana, Cuba. 6_Marie Skłodowska-Curie Actions, International Training Network Glytunes, European Union.

The dialogue between the innate and the adaptive branches of the immune system is critical for protection against infections, as well tumor, autoimmune, allergic and inflammatory diseases.
In past decade, our understanding of the activation of the innate immune system through pattern recognition receptor (PRR) improved a lot and it helped to better understand the mechanisms to drive immune responses and to increase and adjuvate the outcome of vaccination and immunotherapy. PRR, like C-type Lectin (CLR), I-type Lectin (ITL, also called Siglecs), Toll-like Receptor (TLR) and Nod-like receptors (NLR), recognize molecular motives present in the environment and induce antigen presentation, signal transduction and production of effector molecules in innate immune cells.
Most of the studies have been done by triggering a single PRR with a specific synthetic (or isolated) molecular pattern but pathological conditions are much more complex and there are always multiple activations of different PRRs from different molecular patterns or a single complex molecule that is able to trigger different PRRs.
Since glycosylation emerged to be a remarkable biomolecule modification with immune-modulating effect, mediated by CLR and Siglecs, by using glycosylated microbial molecules and particles as PRR ligands (PRRLs), like capsular polysaccharide (CPS) and outer-membrane vesicles (OMV) isolated from bacteria, we will improve vaccine formulations, with the ultimate aim to test them in tumor mouse models.
We included these PRRL in different engineered formulations to create a Microbial Adjuvanted Glycosylated Molecules (MAGMs) by absorbing them on Aluminium Hydroxide (Alum) or by encapsulation in PLGA nanoparticles. MAGM help to induce a multiple PRR targeting that was tested in human monocyte and in mice bone marrow derive dendritic cells (BMDC) to understand the series of immune responses. As results, the outcome generated after MAGM stimulation is dependent by the activities of CTL and Siglecs which enhance APC targeting and avoid possible side effects of the vaccination strategy.

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Cancer immunology
Kelly Boelaars, PhD student

Stromal derived sialic acids induce immune suppression in the tumor microenvironment of pancreatic cancer

Boelaars K, van Kooyk Y

Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Molecular Cell Biology and Immunology, Cancer Center Amsterdam, Amsterdam Infection and Immunity Institute, Amsterdam, Netherlands

Pancreatic ductal adenocarcinoma (PDAC) is characterized by abundant desmoplastic stroma that drives tumor progression and immune evasion. Identification of immune inhibitory mechanisms induced by stromal cells is keys to our understanding of immune evasion in PDAC. Sialic acid containing glycans are known to suppress immune cells via interaction with Siglec receptors, and are overexpressed on PDAC tumor cells. It is currently unknown whether stromal cells express sialic acids and whether the sialic acid/Siglec axis contributes immune suppression.
Here, we show that PDAC stromal cells drive differentiation of monocytes to suppressive macrophages, a process triggered by Siglec receptors on monocytes. The PDAC stroma abundantly expresses sialic acids, which serve as ligands for Siglec receptors. Removal of sialic acid on stellate cells reduces the differentiation to suppressive macrophages. Our data show a novel mechanisms of stromal mediated immune suppression that may provide a new target for immunotherapy in PDAC.

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Cancer immunology
Magali Coccimiglio, PhD student

REDUCTION OF SURFACE SIALIC ACID AS A POTENTIAL TOOL AGAINST CANCER

1Magali Coccimiglio, 2Kelly Boelaars, 3Thomas Boltje, 4Fabrizio Chiodo, 5Yvette van Kooyk

1Dept. of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands; Amsterdam Infection & Immunity Institute, Amsterdam, the Netherlands; Cancer Center Amsterdam, the Netherlands
2Dept. of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands; Amsterdam Infection & Immunity Institute, Amsterdam, the Netherlands; Cancer Center Amsterdam, the Netherlands
3Institute for Molecules and Materials, Radboud University, Nijmegen, the Netherlands
4 Dept. of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands; Institute of Biomolecular Chemistry, National research Council (CNR-ICB), Naples, Italy
5Dept. of Molecular Cell Biology and Immunology, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands; Amsterdam Infection & Immunity Institute, Amsterdam, the Netherlands; Cancer Center Amsterdam, the Netherlands

During tumorigenesis, changes in glycosylation occur, leading to the aberrant expression of different surface glycans on tumor cells. Particularly, hypersialylation has been identified as a major hallmark in many cancer types. Sialic acid expression has been related to tumor growth and metastasis. Moreover, sialic acids can be recognized by Sialic acid binding immunoglobulin type lectins (Siglecs) on immune cells. As most Siglecs have immune inhibitory potential and are expressed on a variety of immune cells, sialic acids are considered immune checkpoints that control the suppressive state of the tumor immune microenvironment, and could determine immunotherapy effectiveness.
To control sialylation in tumor development, we used a global sialyltransferases inhibitor (P-FNANA), to reduce surface sialic acids on different tumor cell lines. We characterized the expression of sialic acid ligands for different Siglecs and studied their expression upon treatment with P-FNANA using flow cytometry. Furthermore, we evaluated whether cell migration in vitro is affected by the reduction of surface sialic acids.
We demonstrated that P-FNANA efficiently reduces the expression of Siglecs' ligands on tumor cells, which could enhance anti-tumor immune responses. In conclusion, the sialylation pathway on tumor cells can be impaired, making it a potential target for combinatorial therapies against cancer.

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Cancer immunology
Hester Heimans, PhD student

The role of tissue resident memory T cells in solid tumors

1Heimans H, 2Verkerk T, 3Kragten N, 4Rus T, 5Griffioen L, 6van der Burg S, 7van Hall T, 8van Gisbergen K

1dept. of Hematopoiesis, Sanquin 2dept. of Hematopoiesis, Sanquin 3dept. of Hematopoiesis, Sanquin 4dept. of Hematopoiesis, Sanquin 5dept. of Medical Oncology, LUMC, 6dept. of Medical Oncology, LUMC, 7dept. of Medical Oncology, LUMC, 8dept. of Hematopoiesis, Sanquin

CD8 T cells are an essential component of the immune system to fight cancer. In particular, tissue resident memory T cells (Trm) have been suggested to represent a potent CD8 T cell subset to counter tumor growth in various solid cancers, since their presence at the tumor site has been linked to improved disease outcome in patients.
Trm have been previously identified in the epithelia of the skin, small intestine and lungs where they occupy strategic positions to encounter invading pathogens. We found that Trm specifically upregulate the transcription factor Hobit in pathogen-specific Trm marking the separation of Trm from circulating memory T cell lineages. Besides Trm, tumors harbor exhausted CD8 T cells but the developmental pathway of this subset is unknown. We aim to investigate how Trm separate from exhausted CD8 T cells in a tumor setting. To identify Trm in tumors, we developed a Hobit reporter mouse which enables tracing and manipulation of Trm through the Trm-restricted transcription factor Hobit. We observed the presence of CD8 T cells that displayed a Trm phenotype, including upregulation of Hobit in a pancreatic tumor model. Our findings indicate that Trm may separate from other tumor-residing T cells after upregulation of Hobit expression.
Currently, therapies employing expanded tumor infiltrating lymphocytes or inhibitory immune checkpoint antibodies are used to harness the immune system for anticancer responses. A better understanding of the developmental pathway of tumor Trm may improve these therapies through the recruitment of these CD8 T cells with potent anti-tumor activity.

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Cancer immunology
Dimitri Lindijer, PhD student

The Glyco-Code in Pancreatic Cancer: Immune Suppressive Signatures, Novel Opportunities for Diagnosis and Therapy

1Lindijer DV, 1Boelaars K, 1van Ee TJ, 1van Vliet SJ, 1Rodriguez E, 1van Kooyk Y

1Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Molecular Cell Biology and Immunology, Amsterdam Institute for Infection and Immunology, Cancer Center Amsterdam, Boelelaan 1117, Amsterdam, The Netherlands

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive, difficult to treat, cancer with a 5-year overall survival of 9.3%. Novel immunotherapies have provided little improvement due to high immune tolerance of the tumor microenvironment (TME). Glycosylation is a metabolic process that regulates immune tolerance based on recognition by immune cell expressed glycan binding receptors, such as C-type lectins, galectins and Siglecs. The exact contribution of various glycan structures in creating the immune suppressive microenvironment remain unclear.
Recently, we defined two subtypes in PDAC based on glycosylation signatures, namely the fucosylated and basal subtype [1]. The fucosylated subtype is characterized by high expression levels of fucosylated O-glycosylation structures able to bind DC-SIGN, while the basal subtype lacks those and showed increased expression levels of galectins. Interestingly, these subtypes are related to an epithelial and mesenchymal status, respectively. Also we demonstrate that sialic acids on both subtypes of PDAC regulate myeloid cell differentiation via immune inhibitory receptors Siglec-7 and Siglec-9 expressed on myeloid cells [2].
Our research indicates an important role for the glyco-code in modulating anti-tumor immunity in pancreatic cancer. To further explore the effect of tumor glycosylation, we create gene knock-outs of fucosylation and sialylation pathway in PDAC tumor cells and perform 3D co-culture experiments with these tumor cell glyco-variants together with stromal and immune cells. Our 3D spheroid model showed macrophage infiltration only when PDAC tumor cells are co-cultured with pancreatic fibroblasts. This model together with a new organ-on-a-chip model [3] will help us to further unravel the contribution of the tumor glyco-code on migration, infiltration and differentiation of immune cells in the TME of PDAC.
References
1. Rodriguez E, Boelaars K, Brown K, Madunić K, van Ee T, Dijk F, Verheij J, Li RJE, Schetters STT, Meijer LL, Le Large TYS, Driehuis E, Clevers H, Bruijns SCM, O'Toole T, van Vliet SJ, Bijlsma MF, Wuhrer M, Kazemier G, Giovannetti E, Garcia-Vallejo JJ, van Kooyk Y. Analysis of the glyco-code in pancreatic ductal adenocarcinoma identifies glycan-mediated immune regulatory circuits. Commun Biol. 2022 Jan 11;5(1):41. doi: 10.1038/s42003-021-02934-0. PMID: 35017635; PMCID: PMC8752754.
2. Rodriguez E, Boelaars K, Brown K, Eveline Li RJ, Kruijssen L, Bruijns SCM, van Ee T, Schetters STT, Crommentuijn MHW, van der Horst JC, van Grieken NCT, van Vliet SJ, Kazemier G, Giovannetti E, Garcia-Vallejo JJ, van Kooyk Y. Sialic acids in pancreatic cancer cells drive tumour-associated macrophage differentiation via the Siglec receptors Siglec-7 and Siglec-9. Nat Commun. 2021 Feb 24;12(1):1270. doi: 10.1038/s41467-021-21550-4. PMID: 33627655; PMCID: PMC7904912.
3. Firatligil-Yildirir B, Bati-Ayaz G, Tahmaz I, Bilgen M, Pesen-Okvur D, Yalcin-Ozuysal O. On-chip determination of tissue-specific metastatic potential of breast cancer cells. Biotechnol Bioeng. 2021 Oct;118(10):3799-3810. doi: 10.1002/bit.27855. Epub 2021 Jun 21. PMID: 34110014.

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Cancer immunology
Julien J. Karrich, Postdoc

Humanized mice as a pre-clinical model to improve immunotherapy of human cancer

1Karrich JJ, 1Babala N, 1Laurent A, 1Bovens A, 1Castenmiller S, 1De Groot R, 2Hoogenboezem M, 3Rongvaux A, 4Schumacher TN, 1Wolkers MC, 1Amsen D.

1Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands. 2Research Facility, Sanquin Amsterdam, Amsterdam, the Netherlands. 3Program in Immunology, Fred Hutchinson Cancer Research Center. 4Division of Tumor Biology and Immunology, The Netherlands Cancer Institute-Antoni van Leeuwenhoek, Amsterdam, The Netherlands.

INTRODUCTION
T cells are powerful weapons in the fight against cancer, as evidenced by the success obtained with checkpoint blockade or treatment with adoptively transferred tumor infiltrating lymphocytes (TILs). Nonetheless, the majority of cancer patients is not cured by such therapies. Explanations include limited T cell persistence and/or tumor specificity of infused T cells in-vivo, inability to home into the tumor, induction of T cell exhaustion as well as the influence of tolerogenic factors within the tumor micro-environment. Feverish attempts are made to test different combination therapies to improve treatment success. The number of possible variations is however prohibitively large. It is therefore essential that we base treatment innovations on deep understanding of the mechanisms controlling T cell immunity in vivo. Although insights can be obtained from studying the murine immune system, these cannot always be translated to humans, given the existence of differences between the immune systems of these species. Study of the human immune system is impeded by the requirement to perform experiments in vitro, which do not approximate the complexity of the immune system and also fail to accurately mimic metabolic conditions in vivo. We, therefore, set out to design a human in vivo model to study human adoptive T cell therapy of cancer.
METHODS
To do so, we made use of the newly developed immunodeficient MISTRG mouse model (Rongvaux et al., Nature Biotechnology 2014), which expresses strategically-chosen human versions of critical immune response genes, including GMCSF/IL3, MCSF, Thrombopoietin and SIRP□. These mice allow development of a functional human immune system upon engraftment with human CD34+ stem cells. Importantly, compared to traditional NSG models, the human immune compartment in reconstituted MISTRG mice includes lymphocytes, as well as myeloid and NK cells at close to physiological levels.
RESULTS
We showed that human melanoma tumor cell lines, as well as patient derived Non-Small Cell Lung Carcinoma tumors efficiently grew in “humanized” MISTRG animals. In fact, engraftment was more robust in humanized MISTRG mice than in MISTRG mice lacking a human immune system. Furthermore, engrafted human tumors became vascularized and consistently exhibited human immune infiltration with B, NK, myeloid, and conventional CD4+ and CD8+, as well as regulatory T cells. The composition of the human immune infiltrate varied between tumor types (regardless of stem cell donor). Some included strong infiltration by both B and T cells, while others contained T cells but no B cells, and yet other tumors featured a near absence of human immune infiltrates. Strikingly, CD8+ T cells infiltrating in tumors exhibited ted hallmarks of activation and exhaustion, suggesting tumor reactivity. With a protocol adapted from human TIL therapy studies, we could expand these cells in vitro.
Finally, we successfully performed adoptive T cell transfer experiments in which syngeneic human MISTRG CD8+ T cells, expressing tumor-specific TCRs, were infused into humanized MISTRG tumor-bearing recipients. Surprisingly, such tumor-specific CD8 T cells failed to effectively infiltrate

established tumors in humanized MISTRG mice, despite the presence of a clear “endogenous” human CD8 T cell infiltrate.

CONCLUSIONS

All in all, we have established a pre-clinical murine model to study and thereby improve human T cell therapy of cancer. The model will be used to study how adoptively transferred T cells are best targeted into tumors as well as to test and develop methods to prevent exhaustion of human tumor reactive T cells.

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Cancer immunology
Eleonora Nardini, PhD student

Induction of tolerance to therapeutic factor VIII in HA by modification with α2,3 sialic acid

1 Nardini E, 1 Peterse E, 1 Li RJ E, 1 van Kooyk Y

1 Amsterdam UMC, location Vrije Universiteit Amsterdam, Molecular Cell Biology and Immunology, Amsterdam institute for Infection and Immunity, De Boelelaan 1117, Amsterdam, The Netherlands

Our awareness of the importance of glycosylation in the regulation of the immune system has greatly broadened the way see autoimmune diseases. There is now growing evidence that sialic acid–terminating glycans act as “SMAPs”, thus contributing to the maintenance of peripheral tolerance. Indeed, dendritic cells (DCs) are equipped with inhibitory Siglecs receptors that promote CD4+ T cell skewing to Tregs at the expenses of effector T cells.
Thinking out of the box, sialylation could prove useful also in the treatment of hemophilia A (HA), where the major complication is the development of anti-FVIII neutralizing antibodies. Finding novel approaches to tackle FVIII immunogenicity is therefore an urgent concern.
Here, we aim to modify immunodominant peptides from FVIII with an α2,3-sialic acid to induce preventive or therapeutic antigen-specific tolerance.
Based on the predicted secondary structure and promiscuity of binding to diverse HLA haplotypes, one sequence was selected to be conjugated to α2-3 sialic acid. In vitro, our data show that the sialylated sequence efficiently binds to Siglec-9, Siglec-3 and Siglec-1, which are all immune suppressive receptors expressed on DCs. Upon triggering of these receptors, monocyte derived DCs express an anti-inflammatory cytokine profile, thus indicating the induction of a tolerogenic phenotype.
Altogether, our data show promising results to use sialylated immunodominant sequences from highly immunogenic antigens as a strategy to prevent aberrant immune responses.

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Cancer immunology
Adrien Laurent, PhD student

A CD4+ T cell help quality control checkpoint in CD8 T cells depends on Notch receptors

*#1 Laurent ARG, #1 Babala N, #1 Karrich JJ, 1 Bovens A, *2 Borst J, *1 Amsen D*

(1) Department of Hematopoiesis, Sanquin Research, Amsterdam, the Netherlands. Landsteiner Laboratory, Amsterdam UMC, University of Amsterdam, the Netherlands.
(2) Oncode Institute, Utrecht, the Netherlands. Leiden University Medical School, the Netherlands.
# These authors contributed equally * These authors contributed equally

One of the eponymous helper functions of CD4+ T cells is to promote activation of CD8+ T cells. Such help is critical for responses against cellular antigens, such as those associated with cancer. Without help from CD4+ T cells, cellular antigens elicit CD8+ T cell responses that are numerically and functionally feeble and short-lived. Help from CD4+ T cells is communicated to CD8+ T cells via XCR1+BATF+ dendritic cells (DC) during priming in lymph nodes. Recognition of cognate antigen induces expression of CD40 ligand on CD4+ T cells, which subsequently triggers CD40 on the presenting DC and thereby "licenses" these cells to optimally activate CD8+ T cells. The efferent signals provided by such licensed DC are incompletely known. One signal is mediated by CD27, which activates part of the CD4+ T cell help program in CD8+ T cells. Here we show that Notch also controls critical aspects of CD4+ T cell help. Ligation of CD40 induces expression of ligands for Notch on XCR1+BATF+ DC. Using a DNA vaccine encoding an MHC-I restricted epitope alone or in combination with MHC-II restricted epitopes, we document that expression of Notch1/2 receptors is necessary in CD8+ T cells for CD4+ T cell help-dependent proliferation as well as to acquire full effector function and migratory abilities. Moreover, effector CD8+ T cells elicited without help or Notch, highly express inhibitory receptors, such as PD1 or BTLA. RNA sequencing showed that Notch controls a large portion of the genetic program induced by CD4+ T cell help in CD8+ T cells. The Notch-dependent transcriptome only partially overlapped with the CD27-dependent transcriptome, showing that these two receptors make complementary contributions to the CD4+ T cell help program in CD8+ T cells. Correspondingly, CD27 agonist treatment failed to rescue the defective responses made by Notch1/2-deficient CD8+ T cells. Deliberate activation of CD27 did partially restore proliferation of Notch1/2-deficient CD8+ T cells or of wild type CD8+ T cells activated in the absence of CD4+ T cells help. Such cells, however, could not leave the lymph nodes draining the vaccination site. Our results therefore reveal the existence of a Notch-dependent quality control mechanism to ensure that CD8+ T cells remain in lymph nodes until they have received the full complement of CD4+ T cell help signals.

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Cancer immunology
Joris Bos, PhD student

Anti-PD1, Capecitabine, and Oxaliplatin for the first-line treatment of dMMR esophagogastric cancer (AuspiCiOus-dMMR): a proof-of-principle study

1Bos J, 2Haj Mohammad N, 3Derks S, 4Laarhoven van HWM

1dept. of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam, The Netherlands; Oncode Institute, Utrecht, The Netherlands
2dept. of Medical Oncology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
3dept. of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, VU University, The Netherlands; Oncode Institute, Utrecht, The Netherlands
4dept. of Medical Oncology, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam, The Netherlands

Doublet cytotoxic treatment demonstrates survival benefit over single drug therapies in patients with advanced esophagogastric cancer (EGC). Previous clinical data suggests an improved survival outcome in patients with microsatellite instability (MSI) treated with PD-1 inhibitor monotherapy or in combination with chemotherapy in first line palliative treatment. The positive effect of immunotherapy was only seen after a couple of months of treatment. This suggests that the patient might benefit from a short course of chemotherapy at the start of treatment. However, reports on the effects of cytotoxic treatment in MSI-high tumors range from beneficial to detrimental. Thus, the value of cytotoxic treatment as well as the effect on the tumor immune microenvironment (TME) in MSI-high tumors is unclear. Therefore, in AuspiCious-dMMR we will study the effect of sequential treatment in patients with mismatch repair deficient (dMMR) EGC on the tumor immune microenvironment and, more specifically, the impact on the infiltration of cytotoxic T cells, before and after a short course of chemotherapy, and during treatment with PD-1 inhibition. Before treatment, after chemotherapy and after two cycles of immunotherapy fresh tumor biopsies are collected to study the primary outcome (T cell infiltration and the interferon-γ signature) as well as translational research purposes (immune profiling and organoid culturing). Blood is collected at the same timepoints for phenotyping of circulating immune cells, determination of cytokine profile, and circulating tumor DNA. Also, fecal samples are collected to determine changes in the intestinal microbiome.

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Cancer immunology
Aram de Haas, PhD student

Functional consequences of two lipooligosaccharide variants of Burkholderia cenocepacia carrying different glycan epitopes on dendritic cell function

Aram de Haas 1, Sven Bruijns 1, Flaviana Di Lorenzo 2, Antonio Molinaro 2, Fabrizio Chiodo 1, Yvette van Kooyk 1

1 Department of Molecular Cell Biology and Immunology, Amsterdam University Medical Center, Cancer Center Amsterdam, Amsterdam Institute for Infection and Immunity, Vrije Universiteit Amsterdam, Amsterdam, Netherlands
2 Department of Chemical Sciences, University of Naples Federico II, Napoli, Italy

Dendritic cells (DCs) express various classes of receptors that aid in their antigen presenting role, such as C-type lectin receptors (CLRs) and Toll-like receptors (TLRs). Lipooligosaccharides (LOS) derived from bacteria can have CLR binding as well as TLR activating moieties, among other things via the various glycans of which the LOS is composed. To determine how the glycan structures of LOS molecules contribute to targeting and/or activating properties, we used two LOS variants derived from Burkholderia cepacia mutants, which only differ in the presence (WaaF) or absence (WaaC) of one additional heptose monosaccharide in the core structure of the LOS.
Incorporated into liposomes, both WaaF and WaaC retain their relative targeting and activating properties, as is observed in moDC binding, maturation and cytokine data, without reducing cell viability. The role of CLR DC-SIGN in these processes was resolved by blocking the DC-SIGN receptor, leading to reduced maturation and cytokine production of the moDCs. The innate immunological effects, such as moDC activation and cytokine production, that LOS has also translated to increased IL-17 production by memory CD4+ T cells after they were co-cultured with WaaF, instead of WaaC, liposome pulsed moDCs.
We show that subtle glycan differences on the LOS structures have detrimental effects on moDCs. Because the WaaF LOS structure targets both CLRs and activates TLRs this LOS structure is an interesting vaccine adjuvant candidate, for instance when incorporated in liposomal based vaccine platforms.

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Cancer immunology
Tynisha Rafael, PhD student

IL-10 expression rate and cellular origin in the tumor microenvironment of penile cancer

1Rafael TS, 2Michielon E, 3de Kok M, 4Bekers E, 5van der Poel HG, 6Brouwer OR, 7Fehres CM, 8de Gruijl TD, 9Jordanova ES.

1Dept. of Medical Oncology, 2Dept. of Molecular Cell Biology and Immunology, 3Dept. of Molecular Cell Biology and Immunology, 4Dept. of Pathology, 5Dept. of Urology, 6Dept. of Urology, 7Dept. of Rheumatology, 8Dept. of Medical Oncology, 9Dept. of Dept. of Obstetrics and Gynaecology.

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that is expressed by a variety of cells, including but not limited to tumor cells, macrophages and B cells. In several human papillomavirus induced cancers, including penile cancer, comprehensive analysis of IL-10 in situ is lacking. The aim of this study is to investigate the presence and role of IL-10 producing B cells and other IL-10 producing cells in the tumor microenvironment of penile cancer. Primary tumor material from penile cancer patients (n=145) were stained using a novel dual in situ hybridization and immunohistochemistry (IHC) workflow. IL-10 RNA probe in combination with CD19/cytokeratin/DAPI multiplex IHC was performed on tissue microarray sections. A quantitative scoring algorithm for determining IL-10 status was set using the cut-off points 1, 4, 10 and 15 dots/cell. IL-10 mRNA was detected in tumor cells, various stromal cells and B cells. Both single punctate dots and clusters of IL-10 mRNA molecules were observed. Moreover, patients with high expression of IL-10 (>15 dots/cell) in tumor had larger tumors (p=0.015) and a worse survival (p=0.028). B cells, irrespective of IL10 expression, were linked to improved survival (p=0.026) and smaller tumor size (p=0.035). Altogether, these findings demonstrate the importance of assessing IL-10 in situ; IL-10 expression in tumor cells was associated with poor survival. On the other hand, B cells presence in general was linked to good prognostic parameters, irrespective of IL-10 expression.

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Cancer immunology
Francesca Favaro, PhD student

TRAIL receptors participate in pro-tumorigenic IL-8 secretion in non-small cell lung carcinoma

1-2-3-4Favaro F, 1Luciano F, 1Moreno-Caceres J, 1Hernandez-Madrigal M, 2-3-4Montironi C, 2-3-4Both D, 2-3-4Eldering E, 1Muñoz-Pinedo C

1Preclinical and Experimental Research in Thoracic Tumors (PReTT), Molecular Mechanisms and Experimental Therapy in Oncology Program (Oncobell), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, 08908 Barcelona, Spain.
2Amsterdam UMC location University of Amsterdam, Dept of Experimental Immunology, Meibergdreef 9, Amsterdam, the Netherlands
3Amsterdam institute for Infection and Immunity, Cancer Immunology, Amsterdam, the Netherlands
4Cancer Center Amsterdam, Cancer Biology, Amsterdam, the Netherlands

Interleukin 8 (IL-8/CXCL8) is a pro-angiogenic and inflammatory chemokine that plays a role in cancer development. Non-small cell lung carcinoma (NSCLC) produce high amounts of IL-8, and this is associated with poor prognosis and resistance to chemo- radio- and immunotherapy. The signaling pathways that promote IL-8 release by lung cancer are still poorly characterized.
We show here that IL-8 release is controlled by TRAIL receptor 1 (DR4) and 2 (DR5) in both squamous and adenocarcinoma NSCLC cell lines, regardless of their mutational status. NSCLC constitutively secrete IL-8, which in culture was further increased by growing the cells in glucose starvation. In A549 cells, constitutive IL-8 production was dependent both on NF-κB and ERK-MAPK pathways. We observed that DR4 and DR5, known regulators of these signalling pathways, participated in constitutive IL-8 secretion. TRADD and RIPK1, key components of the TRAIL receptor cytoplasmic complex (DISC/FADDosome), were also implicated in the production of IL-8. Via protein analysis we discovered that DR4 controlled NFκB activity, whereas DR5 regulated ERK-MAPK pathway. Analysis of mRNA expression data from publically available patients data indicated that IL-8 expression levels correlated independently with TRAIL, DR4 and DR5 mRNA. DR4 and DR5 expression also correlated with markers of angiogenesis and neutrophil infiltration in lung squamous carcinoma and adenocarcinoma. These results suggest that TRAIL receptors should be inhibited rather than further activated in NSCLC patients.

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Cancer immunology
Chiara Montironi, PhD student

Leukemic cells suppress CD4+ T cell function by inducing pseudohypoxia and autocrine purinergic signaling

1,2Montironi C, 1,2,3Jacobs C, 1,2Cretenet G, 1,2,3Peters FS, 1,2,4Bins A, 5,6Schomakers B, 5,6van Weeghel M, 7Jongejans A, 2,3Kater AP, 1,2,3Simon-Molas H and 1,2Eldering E.

1 Department of Experimental Immunology, Amsterdam UMC, University of Amsterdam, the Netherlands
2 Amsterdam Institute of Infection and Immunity, Cancer Center Amsterdam, and Lymphoma and Myeloma Center (LYMMCARE), Amsterdam, the Netherlands.
3 Department of Hematology, Amsterdam UMC, University of Amsterdam, the Netherlands
4 Department of Medical Oncology, Amsterdam UMC, University of Amsterdam, The Netherlands
5 Laboratory Genetic Metabolic Diseases, Amsterdam UMC-AMC, University of Amsterdam, Amsterdam Gastroenterology and Metabolism, Amsterdam Cardiovascular Sciences, Amsterdam, the Netherlands.
6 Core Facility Metabolomics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
7 Department of Clinical Epidemiology and Biostatistics, Amsterdam UMC, University of Amsterdam, The Netherlands

Dysfunction in the T-cell compartment is a prominent feature of indolent non-Hodgkin's lymphomas, where it hinders the efficacy of T-cell based immunotherapy. Normally, T cells rely on metabolic switches to initiate an immune response, namely a rapid shift to glycolysis accompanied by amplification of OXPHOS capacity. Given the link between T-cell metabolism and function, we hypothesized that altered metabolism induced by malignant cells is at the basis of T-cell dysfunction. T cells derived from healthy donors were co-cultured with malignant B-cell lines from different origin and analyzed by flow cytometry, transcriptomics and metabolomics.
Exposure to the cell line PGA-1, derived from chronic lymphocytic leukemia (CLL), resulted in defective activation, proliferation and effector function of immune T cells, coupled with inability to enhance mitochondrial cellular metabolism. Leukemic cells induced an hypoxic gene signature on CD4+ T cells, driven by the stabilization of HIF1α, which skewed the fate of pyruvate to lactate, instead of entering the Krebs cycle. HIF1α stabilization occurred in normoxic condition due to so-called pseudohypoxia, caused by the imbalance of the metabolites α-ketoglutarate and succinate. The hypoxic and glycolytic phenotype acquired by T cells triggered the purinergic signaling pathway in an autocrine manner and augmented extracellular adenosine concentration. This dampened TCR signaling, as confirmed by reduced phospho-S6. Importantly, specific blocking of adenosine receptors A2AR/A2BR partially restored T cell activation and p-S6.
In conclusion, this novel mechanistic insight suggests that modulation of hypoxia and purinergic pathway might be an efficient strategy to overcome T cell dysfunction in cancer.

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Cancer immunology
Demi Both, PhD student

No role of death receptors in regulating cell death upon endoplasmic reticulum stress in B cell malignancies

1,2,3,4Demi Both, 1,2,3,4,5Francesca Favaro, 1,2Ingrid Derks, 5Cristina Muñoz-Pinedo, 1,2,3,4Eric Eldering

1Departments of Experimental Immunology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands. 2Lymphoma and Myeloma Center Amsterdam, LYMMCARE, The Netherlands. 3Cancer Center Amsterdam. 4Amsterdam Infection & Immunity Institute, Amsterdam. 5Institut d'Investigació Biomèdica de Bellvitge - IDIBELL, L'Hospitalet de Llobregat, Spain.

Impairments in protein folding in the endoplasmic reticulum (ER) lead to a condition called ER-stress, which can proceed to trigger apoptosis. Since B cells have high immunoglobulin production, studying ER stress-mediated cell death could provide novel targets for treatment. There is controversy concerning involvement of the death receptor (DR)4 and DR5-caspase-8 –Bid pathway in ER stress mediated cell death, and this axis has not been fully studied in B cell malignancies. We address this here by creating a series of CRISPR KOs along the proposed pathway in different B-cell malignancy cell lines. Unexpectedly, although DR4 and/or DR5 are essential for killing via TRAIL, they are dispensable for ER-stress induced-cell death, mediated via thapsigargin, tunicamycin, brefeldin or bortezomib. The caspase-8–Bid pathway was activated upon ER-stress, but this was DR4/5 independent and rather a result of the feedback loop from mitochondrial-induced apoptosis via Bax/Bak activation.
In conclusion, we show that there is no role for DRs in ER stress-mediated cell death in B-cell malignancies as it is demonstrated in solid tumors. Consequently, combined activation of the two cell-death pathways could have a synergistic effect. We are currently investigating this interesting option to effectively kill malignant B-cells.

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Cancer immunology
Armando van Bruggen, Postdoc

Chronic lymphocytic leukemia actively induces T-cell dysfunction by contact-dependent signaling via CD24 and CD52

1,2Jaco A. C. van Bruggen, 1,2F.S. Peters, 1,2G. Cretenet, 3,4J. Joseph Melenhorst, 1,2Eric Eldering, 1,2Arnon P. Kater

1Department of Hematology, Cancer Center Amsterdam, Lymphoma and Myeloma Center Amsterdam, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands;
2Department of Experimental Immunology, Amsterdam Infection & Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, Netherlands;
3Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA.
4Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, PA, USA.

Introduction
Success rates of autologous T cell-based therapies, such as CAR-T cell therapy, in chronic lymphocytic leukemia (CLL) have been suboptimal and correlate with failure of activation and proliferation of T cells in vitro and in vivo. Previous data showing that impaired CD8 T-cell activation, proliferation and metabolic reprogramming could be restored by purifying CLL T cells via cell-sorting (van Bruggen et al., Blood, 2019) indicating that an as yet unknown, CLL-derived factor is responsible for acquired T-cell dysfunction. In this study we aim to elucidate the mechanistic basis of CLL-mediated T-cell dysfunction.
Results
Dynamic analysis of αCD3/CD28 stimulated autologous T cells in presence of CLL cells over a period of 9 days revealed that T-cell activation (CD25, CD71, CD95 and PD-1) in CLL is in fact not impaired but occurs in a delayed fashion. CLL T cells reached peak activation after 5-6 days in contrast to 2-3 days for age-matched healthy donors. This delayed T cell receptor-induced T cell activation was largely normalized with tumor cell depletion by flow-sorting prior to activation. Accordingly, in absence versus presence of autologous CLL cells, CAR-T cells derived from CLL patients showed enhanced proliferation, cytokine production and cytotoxicity, indicating potential clinical relevance. These findings show that T cells in CLL are not (terminally) exhausted but that a CLL-derived factor interferes with proper T-cell activation, leading to a delay in activation and impaired proliferation and cytotoxicity. We attempted to identify the mechanism of action in which CLL cells induce T cell dysfunction and whether these suppressive effects are mediated through a soluble factor secreted by CLL cells or by contact-dependent mechanisms.
Previous studies have shown that CD40 activation of CLL cells results in increased expression of key surface-expressed adhesion and costimulatory molecules, but also in alterations of immune-modulatory cytokines secretion. This model was therefore used to decipher mechanisms of CLL-mediated T cell dysfunction. CD40-activation of CLL cells resulted in improved T-cell activation and proliferation upon αCD3/CD28 stimulation in a contact-dependent manner (based on trans-well experiments).
Several clinically approved kinase inhibitors were tested to identify signaling cascades involved in CD40-mediated alleviation of T-cell dysfunction. Only pre-treatment of CLL cells with the SRC-inhibitor dasatinib (100nM) abrogated the enhanced T-cell activation induced by CD40-activated CLL cells. Additional control experiments excluded direct effects of dasatinib on T cell function. Dasatinib did not reduce expression of co-stimulatory markers on CD40-activated CLL cells, indicating that lack of co-stimulation was not the sole explanation for CLL-mediated T cell dysfunction. RNA sequencing of CD40-stimulated CLL cells treated with or without dasatinib and filtered for membrane-bound factors revealed the Sialic acid-binding Ig-like lectin 10 (Siglec-10) ligands CD24 and CD52 as potential candidates responsible for inhibiting T-cell function in CLL, which we confirmed at the protein level.

We also found increased expression of Siglec-10 on CLL T cells, suggesting a role for Siglec-10 ligation in inhibition of the TCR signaling cascade. Indeed, inhibition of Siglec-10 ligation by blocking CD24, and CD52 antibodies subsequently improved T-cell activation despite presence of CLL cells.

Conclusion

These results demonstrate that T cells derived from CLL patients are not terminally dysfunctional and can be revived. Our observations indicate that CLL cells actively suppress (CAR) T-cell function in a contact-dependent fashion through CD24- and CD52-mediated Siglec-10 ligation. These proteins might represent targets for therapeutic intervention aimed at enhancing T-cell function in CLL.

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Cancer immunology
Karoline Kielbassa, PhD student

Ibrutinib treatment interrupts TLR9-induced CD40 upregulation in the lymph node thereby sensitizing CLL cells to venetoclax

1,3,4,5Kielbassa K, 1,3,4,5Haselager MV, 1,2Bax D, 1,2Driel van BF, 1,2Dubois J, 6Levin MD, 6Westerweel PE, 7Svanberg R, 7,8Niemann CU, 2,3,4,5Kater AP, 1,3,4,5Eldering E

1,2Departments of Experimental Immunology and Hematology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
3Lymphoma and Myeloma Center Amsterdam, LYMMCARE, The Netherlands
4Cancer Center Amsterdam. 5Amsterdam Infection & Immunity Institute, Amsterdam. 6Department of Internal Medicine, Albert Schweitzer hopsital, Dordrecht. 7Rigshospitalet, Department of Hematology, Copenhagen, Denmark. 8Copenhagen University, Department of Clinical Medicine, Copenhagen, Denmark.

In the lymph node (LN) microenvironment, chronic lymphocytic leukemia (CLL) cells receive costimulatory signals, which induce anti-apoptotic Bcl-2 proteins and thereby increase drug resistance. Treatment with the BTK inhibitor ibrutinib forces CLL cells from the LN to the peripheral blood, where they become fully dependent on Bcl-2. Since in vitro studies showed that CD40 signaling is involved in the resistance to the Bcl-2 inhibitor venetoclax, it is important to understand the effects of ibrutinib treatment on venetoclax sensitivity and CD40 signaling.
Upon prolonged ibrutinib treatment, CLL cells lose their sensitivity to CD40 signaling, thereby decreasing resistance to venetoclax. Impaired CD40 activation after ibrutinib treatment was confirmed by a reduced induction of Bcl-2 proteins, CD40 protein expression and its activation marker CD95.
A key finding was that TLR9 stimulation via CpG led to increased CD40 protein induction specifically in the LN, while B cell receptor (BCR) stimulation had no effect on CD40 expression. Recent studies showed TLR9 activation upon recognition of unmethylated mitochondrial DNA in CLL cells, providing an alternative to bacterial CpG-DNA in vivo.
Our data indicate that ibrutinib potentially interrupts TLR9-induced CD40 upregulation, which normally primes CLL cells in the LN for drug resistance, revealing novel therapeutic possibilities.

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Cancer immunology
Alsya Affandi, Postdoc

Establishment of rapid and high-throughput DC-T cell priming assays for validation of anti-cancer nanovaccines

1Affandi AJ, 1den Hollander F, 1Olesek K, 1den Haan JMM

1dept. of Molecular Cell Biology & Immunology

Dendritic cells (DCs) are a group of antigen-presenting cells (APCs) that are specialized in processing and presenting antigen to T cells, and in cancer, they play a crucial role in eliciting anti-tumor cytotoxic CD8+ T cell responses. DC-based therapies have generated a significant interest for the development of therapeutic anti-cancer vaccine. The goal of in situ DC-targeting technologies is to efficiently deliver tumor antigens (ag) directly to DCs, for example using antibodies or ligands that bind to DC-specific receptors.
However, due to low percentages of human primary DCs and ag-specific naive T cells, testing these nanovaccines often rely on using monocyte-derived DCs and TCR-transduced antigen-specific T cell clones, which does not truly reflect priming of naïve T cells by primary DCs in vivo. Here, we aim to establish a simple, rapid (10d), and high-throughput assay, designed to rapidly prime naive ag-specific T cells using whole PBMCs (Bozkus 2021) which relies on both primary DCs and naïve T cells, thereby fully recapitulating DC priming of ag-specific T cells in vivo. Using 18-color spectral flow cytometry to immunophenotype DCs, we found that mainly CD1c+ DC (DC2), were present and activated after 24h culture, prior to antigen exposure. After addition of peptides, adjuvant, and a further 9 day T cell expansion, we were able to detect ag-specific CD8+ T cells specific for melanoma-associated antigen MART-1 (n=3), based on the production of IFNγ and TNFα. Similar findings were found using influenza peptide. Our ongoing experiment investigates whether our DC-targeting nanovaccine platform can also effectively prime tumor ag-specific T cells ex vivo. This fast and robust method will enable us to test our DC-targeting nanovaccines in a highly efficient manner.