

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

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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 TLR-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.

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

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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 Fc α RI, Fc γ RIII 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 Fc γ RIII 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.

Development of a versatile nanocancer vaccine format

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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.

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*

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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 Fc α RI. 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.

Neutrophil-mediated tumor cell killing induces uptake of antigens and dendritic cell maturation*123C.A.N. Sewnath, 1,2,3,4M. van Egmond*

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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 Fc α RI (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.

A novel cancer immunotherapy; vaccination against tumor vascular extracellular vimentin*Huijbers EJM, Van Beijnum JR, Van Loon K, Griffioen AW*Angiogenesis Laboratory, Cancer Center Amsterdam, Department of Medical Oncology, VU
University Medical Center, Amsterdam UMC, Amsterdam, The Netherlands**Background**

Angiogenesis, the development of neovasculature, is required to sustain tumor growth and metastasis of solid tumors. The tumor neovasculature expresses specific markers, which are selectively overexpressed in tumor endothelial cells compared to normal healthy adult endothelium and are therefore ideal targets for vaccination.

Methods

One of these tumor vascular markers is the extracellular cytoskeletal protein vimentin. This marker is externalized from tumor endothelial cells, while expression in all other cells in the body is exclusively intracellular. Extracellular vimentin (eVim) is pro-angiogenic and functionally mimics vascular endothelial growth factor (VEGF) action, while concomitantly acting as inhibitor of leukocyte-endothelial interactions, thereby hampering leukocyte infiltration into the tumor. eVim is overexpressed in the vasculature of different solid tumors, but not present in normal healthy tissue.

Results

Using iBoost, our proprietary conjugate vaccine technology for induction of efficient antibody responses against self-antigens, we were able to generate strong eVim specific humoral immune responses, resulting in inhibition of tumor growth in preclinical models without affecting the normal healthy vasculature. Furthermore, in an ongoing clinical study in client owned dogs with spontaneous bladder cancer our eVim vaccine shows effective and safe inhibition of angiogenesis and tumor growth.

Conclusions

Targeting of extracellular vimentin by vaccination therefore presents a promising antiangiogenic immunotherapy strategy that is currently translated into clinical testing.

Expression of neolacto-series glycosphingolipids by tumors impairs anti-tumor responses by immune cells.

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The transcriptional signature of neolacto-series glycosphingolipid (nsGSL) expression highly associates with patient survival in a number of cancers, such as glioma. We recently identified that nsGSLs on such tumor cells negatively affect immune cell activation in vitro (Jongsma et al., Immunity 2021). However, the mechanism underlying this immune suppression is unknown.

We discovered in a flow cytometry approach with barcoded cell lines that nsGSLs sterically shield several, but not all, immune cell surface receptors. In depth analyses of shielded receptor properties revealed that they have significantly shorter extracellular domains compared to non-shielded receptors, which may relate to the limited extracellular length of nsGSLs. Secondly, using genome editing and pharmacological inhibitors, we found that negatively charged sialic acids of nsGSLs likely interact with positively charged amino acids of shielded proteins. This interaction inhibited antibody binding to surface receptors, which was highly dependent on affinity as we established with a well-characterized antibody panel against CD147. Consequently, low-affinity interactions of the central immune receptors HLA class I and CD47 with their ligands LIR-1, KIR2DL2 (HLA class I), and SIRP- α (CD47) were largely impaired by nsGSLs. Moreover, killing of nsGSL overexpressing tumor cells by effector cells such as neutrophils, NK cells and gamma delta T cells was significantly reduced.

Overall our data strongly indicate that expression of nsGSLs by tumor cells prevents productive communication towards immune cells through charge-based shielding of short receptors from their low affinity ligands. Because the GSL synthesis pathway is safely targeted in lysosomal storage diseases, our data warrant investigations on the efficacy of GSL synthesis inhibition to treat patients with nsGSL-rich tumors.

Lymph node stromal cells have the toolbox to control anti-tumor immunity

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Tumors have developed several strategies to evade anti-tumor immunity including impaired T cell responses. Tissue-draining lymph nodes (LN) are essential for initiation of efficient adaptive immune responses. LN stromal cells (LNSCs) organize and preserve the LN architecture and facilitate immune cell activation. Our lab has previously shown that LNSCs play an important role in peripheral tolerance (Baptista et al. 2014, Nadafi et al. 2020). LNSCs present self-antigens in MHC-I or MHC-II, which leads to the generation and maintenance of regulatory T cells (Tregs). As most tumor-antigens are unaltered or mutated self-antigens, we hypothesize that LNSCs present tumor-antigens as self-antigens in the context of key immune regulatory factors resulting in impaired anti-tumor immune responses. We find that primary human LNSCs stimulated with melanoma-conditioned media or after co-culture with melanoma tumor cells have increased tumor-antigen mRNA expression. This correlates with increased HLA-DR cell surface expression. Expression of other cell surface molecules involved in modulation of T-cell responses (CD80, CD86, CD54/ICAM-1, CD58/LFA-3, PD-L1) is also increased in melanoma-stimulated LNSCs, indicating that presence of melanoma results in an activated profile of LNSCs. Furthermore, mRNA levels of inhibitory T-cell regulators IDO1 and TGFB1 are increased in melanoma-stimulated LNSCs. These data indicate that LNSCs have the machinery to regulate T-cell responses. Their immunoregulatory toolbox is altered in cancer, which may result in evasion of anti-tumor immunity. Understanding tumor-derived changes in LNSCs at the molecular and cellular level will aid in unravelling the role of LNSCs in anti-tumor immunity and contribute to development of new immunotherapeutic strategies.

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

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CD8+ T cells kill target cells by releasing cytotoxic molecules and inflammatory cytokines, such as TNF and IFN γ . 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 IFN γ 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 IFN γ -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.

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

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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.

Unravelling the method of action of the innate immune checkpoint CD47-SIRP α *1 2 3 Behrens LM**4 van den Berg TK**1 2 3 5 van Egmond M*

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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-SIRP α is an innate immune checkpoint axis, where CD47 is expressed on virtually all cells, while SIRP α 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 SIRP α has been shown to potentiate tumor cell killing of antibody-opsonized tumor cells. It is currently still unknown how SIRP α signaling prevents neutrophil-mediated tumor cell killing. However, it has been shown that SIRP α signaling controls Kindlin3-dependent CD11b/CD18 integrin activation. Thus, during my PhD I will investigate the method-of-action of CD47-SIRP α signaling in neutrophils. In addition, we will be studying the effect of blocking the CD47-SIRP α axis in combination with other immunotherapies to potentiate tumor cell killing.

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

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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.

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

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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.

How to prevent T cells from getting tired of fighting cancer?

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Cytotoxic CD8⁺ T cells (CTLs) are essential for efficient anti-tumor immunity. Adoptive therapies using tumor-reactive CTLs have resulted in durable remission in human cancer patients. However, CTLs in the tumor microenvironment almost always lose their function in a process termed exhaustion, contributing to the failure of T cell based therapies in many patients with solid tumors.

Understanding the mechanisms controlling exhaustion is, therefore, essential for rational design of new treatments against cancer. Exhaustion is generally believed to result from excessive and repeated stimulation of T cells. We have found an alternative explanation for the development of this dysfunctional state, namely the activation of CD8⁺ T cells in the absence of the full complement of signals required to license robust durable responses. In particular, our results show that signaling via Notch receptors operates already during priming of CD8⁺ T cells to prevent the development of exhaustion. This protective role of Notch can be harnessed to improve adoptive T cell therapies of cancer. Constitutive activation of Notch maintains functional properties, such as production of IFN γ , TNF and Granzyme B, in adoptively transferred tumor-reactive T cells that infiltrated a model melanoma in mice. Single cell transcriptome analysis showed that Notch paradoxically promotes general progression through the 4 developmental stages of exhaustion that have been defined. Nonetheless, Notch maintains these cells in a functional and highly proliferative state. Notch therefore uncouples the acquisition of much of the signature exhaustion transcriptome from loss of function and proliferative capacity. Importantly, constitutive activation of Notch in adoptively transferred T cells results in better control of tumor growth *in vivo*. These results suggest that Notch can be used in the clinic. To avoid potential off-target toxicity, we designed a version of Notch, a Chimeric Notch Receptor (CNR), in which activity is strictly regulated by tumor antigen. Triggering of the CNR by heterologous ligand induces Notch signaling *in vitro*. Preliminary *in vivo* results indicate that the CNR is able to control T cell exhaustion in mouse intratumoral T cells. These proof of principle studies suggest novel possibilities to enhance CTL responses for the treatment of cancer in human patients.

MICROBIAL-GLYCOENGINEERING APPROACH TO IMMUNOTHERAPY

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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.

Stromal derived sialic acids induce immune suppression in the tumor microenvironment of pancreatic cancer*Boelaars K, van Kooyk Y*

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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.

REDUCTION OF SURFACE SIALIC ACID AS A POTENTIAL TOOL AGAINST CANCER

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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.

Wil bij nader inzien geen evt. presentatie geven vanwege te weinig relevante data

The role of tissue resident memory T cells in solid tumors

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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.

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

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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-Yildirim B, Bati-Ayaz G, Tahmaz I, Bilgen M, Pesen-Okvur D, Yalcin-Ozuyal 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.

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

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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 GM-CSF/IL3, M-CSF, Thrombopoietin and SIRP α . 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 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.

Induction of tolerance to therapeutic factor VIII in HA by modification with α 2,3 sialic acid

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Our awareness of the importance of glycosylation in the regulation of the immune system has greatly broadened the way we 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 expense 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 α 2,3-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 α 2-3 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.

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*

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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.

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*

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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.

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

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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.

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

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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.

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

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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- κ B 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 NF κ B 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.

Targeting macrophages metabolism to prevent EMT: clues to reduce cancer therapy resistance

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Despite major advances the treatment of esophageal adenocarcinoma (EAC), the outcome remains poor. This is due to the development of therapy resistance mechanism such as the epithelial-mesenchymal transition (EMT). Tumor-associated macrophages promote EMT in several cancers; however, whether intratumoral macrophages promotes therapy resistance in EAC is still unknown. Here we show that targeting macrophage metabolism reduces macrophage-mediated EMT in EAC cells. Macrophages gene signature was increased in resections from EAC patients after neo-adjuvant chemoradiotherapy, as compared with biopsies samples. Moreover, EMT related genes strongly correlated with macrophages geneset expression in the same tissue. Particularly, pro-tumor macrophages CD163 gene expression strongly correlated with mesenchymal markers ZEB1, Vimentin (VIM) and N-cadherin, which was further confirm by IHC. To functionally demonstrate that macrophages promote EMT in EAC cells we performed in vitro coculture experiments. Macrophages and EAC cells were then brought to a single-cell suspension, mixed in a 5:1 macrophage:EAC cells ratio, and cultured in Matrigel for 5 days. Macrophage-induced EMT was determined by multicolor flow cytometry. Anti-tumor M1 macrophages promoted an epithelial phenotype (increased E-cadh and EpCAM expression in EAC cells) whereas pro-tumor M2 macrophages strongly induced a mesenchymal phenotype (increased N-cadh and CXCR4 expression in EAC cells). Furthermore, preventing lactate transport (using Syrosingopine and AZD3965) and glutaminolysis (with Telaglenastat) prevented pro-tumor macrophage-mediated reduction in E-cadherin in in EAC organoids. Combined, these data shows that inhibiting macrophage metabolism impairs macrophage-mediated EMT. This has the potential to enhance the effect of currently used treatments and improve the outcome of esophageal cancer therapy

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

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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 HIF1 α , which skewed the fate of pyruvate to lactate, instead of entering the Krebs cycle. HIF1 α 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.

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

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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.

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

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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 α CD3/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 α CD3/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.

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

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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.

Establishment of rapid and high-throughput DC-T cell priming assays for validation of anti-cancer nanovaccines*1Affandi AJ, 1den Hollander F, 1Oleseck K, 1den Haan JMM*

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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 naive 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 IFN γ and TNF α . 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.