biodistilled CCR4+ cells reflects on the receptor-ligand interaction. A strongly reduced biotinylation of CCR4+ cells upon addition of an inhibitor indicates an effective inhibitory binding capacity.

**Results** In *vitro* binding assays using APEX proximity labeling were established to create a testing platform for inhibitors of the CCR4-CCL22 axis. For analysis of the murine and human system, CCR4-overexpressing B3Z cells and CCRF-CEM cells that endogenously express CCR4 were used, respectively. In both settings, a strong biotinylation was achieved by adding the CCL22-APEX fusion protein as compared to only APEX as a control for unspecific biotinylation. Addition of two different CCR4 antagonists, C-021 and AZD-2098, lead to significant and dose-dependent reduction of target cell biotinylation. The same assay was conducted using CCR4-transduced primary murine T cells, showing equivalent results. Further, we could demonstrate a decreased biotinylation of cells when recombinant CCL22 had been added, indicating competitive binding inhibition.

**Conclusion** This work reveals a novel application of the APEX proximity labeling system to identify new chemokine-receptor interactions and to screen inhibitors for their binding capacity, facilitating further evaluation of promising inhibitors in functional experiments *in vitro* and *in vivo*. The adaption of the assay protocol for suspension cells and not only adherent cell lines also allows the use of primary murine as well as human cells. Overall, our results present a valuable tool for the evaluation of novel inhibitors of chemokine-receptor axes for immunotherapy of malignant diseases.


**Abstracts**

**P01.05** **MODELLING T CELL MIGRATION IN 3D VASCULAR BEDS IN A HIGH-THROUGHPUT MICROFLUIDIC PLATFORM**

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**Background** Recent clinical success of immune checkpoint inhibitors and chimeric antigen receptor T cells has highly increased the attention for the field of immunotherapy. However, identifying responders to these therapies is challenging underscoring the necessity for translational models that increase understanding of tumor-immune responses.

**Materials and Methods** In the present study, a co-culture system containing immune cells and vasculature was established. Both are essential components of the tumor microenvironment and very often lacking in *in vitro* tumor models, highlighting the added value of our co-culture platform. We focused on optimizing endothelial and CD8+ T cell co-cultures and subsequently assessing T cell migration from the endothelial tubes via endothelial sprouts towards various chemo attractants. In order to generate stratified 3D co-cultures, the Mimetas OrganonPlate Graft containing 64 microfluidic culture units was used. The microfluidic units in this platform are composed of two parallel microfluidics channels and a central chamber. The two parallel microfluidic channels were used for generating parallel endothelial tubules, whilst angiogenic factors (SIP, VEGF, bFGF and PMA) were added to the central chamber of the culture unit resulting in a generation of a gradient and sprouting of the endothelial tubes towards the central chamber. Generated sprouts were stable and perfusable. The central chamber is designed for culturing complex microtissues such as spheroids, organoids and explants.

**Results** Angiogenic endothelial tubules formed vascular beds in presence of added factors within 3–5 days. Once vascular beds were formed, activated and fluorescently labeled CD8+ T cells were loaded in the endothelial tubes and followed in culture for 48 hours. CD8+ T cell migration was observed both via the sprouts as well as by crossing the endothelial barrier, and increased in presence of gradients of CCL2, CCX12 and CCL9. Highest CD8+ T cell numbers were observed in presence of a gradient generated with a mix of these three chemokines.

**Conclusions** Therefore, we present a high throughput co-culture system containing angiogenic endothelial tubules and CD8+ T cells. These co-cultures are highly suitable for studying T cell migration, event which precedes the detection and recognition of antigens at the surface of antigen-presenting cells and for interactions with other cells involved in the immune response. In addition, these co-cultures serve as a platform for understanding the interplay between T cell migration and angiogenesis in the tumor microenvironment. Furthermore, we envision that this model will evolve into an immunocompetent patient-derived tumor model that can be used to study immune responses to tumors.

**Disclosure Information** S. Spelier: Other; Modest; Mimetas. L. de Haan: A. Employment (full or part-time); Significant; Mimetas. J. Suijker: A. Employment (full or part-time); Significant; Mimetas. E. Walinga: Other; Significant; Mimetas. L. van den Broek: A. Employment (full or part-time); Significant; Mimetas. H. Lanz: A. Employment (full or part-time); Significant; Mimetas. J. Joore: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Mimetas. P. Vulto: E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Mimetas. K. Queiroz: A. Employment (full or part-time); Significant; Mimetas.

**P01.06** **DSP216 BLOCKS HLA-G AND CD47 SIGNALING TOWARD IMMUNE CELLS AND REDESIGNS THE IMMUNE SUPPRESSIVE TUMOR MICROENVIRONMENT**

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10.1136/jitc-2022-ITOC9.18

**Background** CD47 checkpoint inhibition has yielded promising results in clinical trials, particularly when combined with other immunotherapeutics. Therefore, we here combined CD47 checkpoint inhibition with checkpoint inhibition of HLA-G using Dual Signaling Protein 216 (DSP216). DSP216 is a new immunotherapeutic composed of sequences selected from the SIRPα and LILRB2 extracellular domains fused to human IgG1. The SIRPα domain of DSP216 binds to and blocks CD47, an important don’t eat me signal, frequently overexpressed on cancer cells. The LILRB2 domain of DSP216 binds to and blocks HLA-G, a pivotal regulator of immune tolerance during pregnancy in placenta that is upregulated in many cancers. HLA-G binds to inhibitory receptors LILRB1 (ILT2) and LILRB2 (ILT4) expressed on macrophages, neutrophils,
dendritic, T, NK and B cells and hereby, suppresses anti-cancer immunity in a multifold manner. Blocking the HLA-G checkpoint reverses this immune suppression and e.g. shift macrophages from a tumor-supportive (M2) towards a tumor-suppressive (M1) phenotype. To avoid off target activity, DSP216 is designed to binds cells in an ‘AND- gate’ fashion, which means that DSP216 should not or minimally bind to cells that express only CD47 or HLA-G, but should bind strongly to cells that express CD47 AND HLA-G due to enhanced avidity. Here, we tested if DSP216 follows this unique binding behavior and if DSP216 can prevent HLA-G mediated polarization of M0 macrophages to M2 macrophages.

**Materials and Methods**

Binding of DSP216 to HLA-G\(^+\) CD47\(^+\) cancer cells was tested by flow cytometry. The binding was blocked with CD47 and HLA-G blocking antibodies to test if the binding was specific. Undesired binding of DSP216 to red blood cells (RBCs) and PBMCs was tested by flow cytometry. The effect of DSP216 on the polarization of M0 macrophages co-cultured with HLA-G\(^+\) CD47\(^+\) cancer cells was tested by monitoring the expression of CD163, CD206 and HLA-DR by flow cytometry.

**Results**

DSP216 bound dose-dependently to HLA-G\(^+\) CD47\(^+\) cancer cells and blocking the binding with HLA-G or CD47 blocking antibodies showed that binding was specific to CD47 and HLA-G. When DSP216 was mixed with RBCs and PBMCs, it didn’t bind to single positive CD47\(^+\) RBCs and bound very weakly to CD47\(^+\) PBMCs. These experiments show that there is indeed strong binding through enhanced avidity when both targets, HLA-G and CD47, are present and weak binding if CD47 but no HLA-G is expressed. In co-culture of M0 macrophages with HLA-G\(^+\) CD47\(^+\) cancer cells, DSP216 prevented HLA-G induced upregulation of the M2 specific markers CD163 and CD206 and induced expression of the M1 specific marker HLA-DR. As tumor associated macrophages (TAMs) with an M2 like phenotype play an important role in the creation of an immuno-suppressive tumor microenvironment, this is a first indication that DSP216 can inverse an immune suppressive tumor microenvironment.

**Conclusions**

DSP216 binds exclusively to HLA-G\(^+\) CD47\(^+\) cells and not to single positive cells, like RBCs and PBMCs. DSP216 prevents HLA-G mediated polarization of M0 macrophages to M2 macrophages and instead promotes polarization to M1 macrophages. DSP216 is a novel bifunctional Fc-fusion therapeutic that has the potential to reverse tumor suppressive signaling by tumor associated macrophages and unleash the anti-tumor activity of various cells from innate and adaptive immune response.

**Disclosure Information**

L.J. Jacob: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; KAHR medical. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; KAHR medical. F. Consultant/Advisory Board; Significant; KAHR medical.

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**Abstracts**

**P01.07** GALECTIN 9 DEPENDENT IMMUNE EVASION MACHINERY AS A POTENTIAL TARGET FOR PERSONALISED IMMUNOTHERAPY OF CANCER

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**Background**

Cancers affect a large number of people worldwide and sometimes have mortality rates of over 90% (pancreatic ductal adenocarcinoma (PDAC), high grade glioblastomas). Currently, immunotherapeutic strategies fail in number of cancer cases due to a lack of understanding of the molecular mechanisms operated by these cancers to evade immune surveillance. Recent evidence demonstrated that the protein galectin-9 is highly expressed in majority of cancer cells. Importantly, this protein is a crucial part of the immune evasion machinery operated by human malignant tumours. Understanding the biochemistry of this immune evasion machinery is required for designing highly efficient and affordable personalised targeted immunotherapy of human cancers.

**Materials and Methods**

We used, glioblastoma, PDAC, breast and renal cancer cell lines as cancer cell models. Jurkat T lymphocytes, TALL-104 cytotoxic T cells and primary human lymphocytes, TALL-104 cytotoxic T cells and primary human lymphocytes were employed for immune evasion studies. C57 BL16 mice were used for in vivo studies. Western blot analysis, on-cell and in-cell Western, ELISA, qRT-PCR, chromatin immunoprecipitation (ChIP) assays as well as a variety of biochemical tests were used to conduct the studies.

**Results**

We found that T cells trigger galectin-9 expression and secretion (in most cases requires Tim-3 protein (T cell immunoglobulin and mucine domain containing protein 3)) in a variety of human cancer cells including but not limited to glioblastoma, PDAC, breast and renal cancers. Galectin-9 triggers exhaustion and often death of cytotoxic T cells. Other immune checkpoint proteins including VISTA (V-domain Ig-containing suppressor of T cell activation) and PD-L1 (ligand of programmed cell death protein 1) promote apoptotic death of cytotoxic T cells induced by galectin-9. Activity of T helpers, required to induce cytotoxic T lymphocytes is also suppressed by galectin-9 in cooperation with VISTA and PD-L1. We discovered that expression of all these immune checkpoint proteins is differentially controlled by the autocrine transforming growth factor beta type 1 (TGF-β)-Smaβ3 pathway as well as several types of interferons.

**Conclusions**

The immune evasion machinery triggered by TGF-β-Smaβ3 and interferon pathways and operated by galectin-9, Tim-3, VISTA and PD-L1 should be considered as a potential target for personalised immunotherapy of cancer. Importantly, blood and tumour sample tests could help mapping the exact immune evasion networks employed by each tumour and the pathways responsible to produce respective immune checkpoint proteins. As such, personalised targeted