

biotinylated 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 nonspecific biotinylation. Addition of two different CCR4 antagonists, C-021 and AZD-2098, led 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.

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

S Spelier, L de Haan*, J Suijker, E Walinga, L van den Broek, H Lanz, J Joore, P Vulto, K Queiroz. *Mimetas, Oegstgeest, Netherlands*

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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 OrganoPlate Graft containing 64 microfluidic culture units was used. The microfluidic units in this platform are composed of two parallel microfluidic channels and a central chamber. The two parallel microfluidic channels were used for generating parallel endothelial tubules, whilst angiogenic factors (S1P, 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 tubules 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, CCX112 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.

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

¹U Jacob*, ²L Tamir, ²M Abdeen, ¹GA Huls, ²A Chajut, ¹E Bremer. ¹UMCG, Groningen, Netherlands; ²KAHR medical, Jerusalem, Israel

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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,