intratumoral TLR3 expression and T-cell infiltration is currently analyzed.

Results Global deficiency of murine Tlr3, or TRIF, induced significantly increased tumor formation, associated with increased morbidity indicating a tumor suppressive role. Coherently, TLR3 expression is highly significantly decreased in human colorectal cancer compared to normal mucosa, significantly correlated with poor survival. TLR3 deficient cell lines show reduced migration and slightly declined proliferation suggesting an oncogenic role on the cell-autonomous level. Nevertheless, gene expression analysis revealed that the dsRNA induced expression of T-cell attracting cytokines CXCL10 and CXCL11 in colon cancer cell lines is exclusively dependent on TLR3. These chemokines were shown to favor a TH1-type antitumoral response.

Conclusions TLR3 favors tumor suppression in vivo, presumably resulting from non-cell-autonomous factors such as the production of CXCL10 and 11 and resulting T-cell infiltration. This may outweigh the putative cell-autonomous oncogenic functions of TLR3 deficiency.


P09.12 BIFUNCTIONAL SIRPα-CD123 FUSION ANTIBODY FOR THE ELIMINATION OF ACUTE MYELOID LEUKEMIA STEM CELLS

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Background Despite advances in the development of novel strategies against acute myeloid leukemia (AML), treatment options are limited and most patients relapse. Relapse occurs due to the persistence of chemotherapy-resistant leukemic stem cells (LSCs), which re-initiate the outgrowth of the disease, highlighting the need of targeting LSCs to improve patient survival. LSCs are characterized by the expression of the interleukin-3 receptor α, also known as CD123. CD123 is expressed on AML blasts and LSCs, and shows a moderate expression on normal hematopoietic stem cells, claiming CD123 as a suitable target antigen. CD47 is a ubiquitously expressed immune checkpoint upregulated on LSCs where it acts as an immune escape mechanism. CD47 transmits a ‘don’t eat me’ signal upon its interaction with the signal regulatory protein alpha (SIRPα) receptor on macrophages, thus inhibiting phagocytosis. In order to efficiently eliminate LSCs, we have designed a bifunctional antibody that specifically targets CD123 and simultaneously blocks CD47. Importantly, our strategy restricts the benefits of the CD47 blockade to CD123+ AML cells. Thus, we hypothesize a lower risk for on-target off-leukemia toxicity.

Materials and Methods The bifunctional SIRPα-CD123 antibody was generated by fusing an extracellular domain of the SIRPα receptor, which functions as the CD47 blocking domain, to the CD123 antibody. The biological activity of the SIRPα-CD123 antibody was examined using AML-derived MOLM-13 cells, primary AML patient material and patient-derived xenografted (PDX) AML cells with NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice.

Results The SIRPα fusion improved the binding of the bifunctional SIRPα-CD123 antibody to AML cells compared to a conventional CD123 antibody. The SIRPα-CD123 antibody enhanced the elimination of the AML-derived MOLM-13 cells by antibody-dependendent cellular cytotoxicity via NK cells. Additionally, the cytotoxicity was confirmed using primary patient-derived AML cells. Furthermore, an improved cytotoxicity towards leukemia initiating AML PDX cells was observed with the SIRPα-CD123 antibody using luciferase bioluminescence in vivo imaging. With regards to the inhibition of CD47 signaling, we were able to show a blockade of CD47 on CD123+CD47+ cells by the SIRPα-CD123 antibody. Correspondingly, a significant increase in phagocytosis of primary patient-derived AML cells mediated by monocyte-derived macrophages was observed in both allogeneic and autologous setting. We were also able to show a preferential binding to MOLM-13 in the presence of a 20-fold excess of red blood cells indicating a potential low on-target off-leukemia toxicity.

Conclusions The bifunctional SIRPα-CD123 fusion antibodies target the CD123+CD47+ cells and stimulate their phagocytosis by blocking the inhibitory CD47 signal. The dual mode of action of the SIRPα-CD123 has the potential to deplete the AML LSCs through NK cell cytotoxicity and macrophage-mediated phagocytosis while restricting the CD47 related on-target off-leukemia toxicity.


P09.13 OPTIMIZATION OF A GMP-GRADE LARGE-SCALE EXPANSION PROTOCOL FOR CYTOKINE-INDUCED KILLER CELLS USING GAS-PERMEABLE STATIC CULTURE FLASKS

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Background Cytokine-Induced Killer (CIK) cells are ex vivo expanded T cells with NK cell phenotype. They express both CD3 and CD56 antigens, and exert a potent antitumor activity against a variety of tumors. Several clinical trials demonstrated the safety and the feasibility of CIK cell therapy, with very low side effects and minimal graft-versus-host toxicity. In this study, we developed a GMP-compliant protocol for robust large-scale expansion of CIK cells using G-Rex® gas-permeable static culture flasks.

Materials and Methods CIK cells were obtained by stimulating healthy donor PBMCs with GMP-grade IFN-γ, IL-2 and CD3 mAbs, and were cultured in G-Rex® or G-Rex®6M well
plates. CIK cells in G-Rex®6 were split only once at day 7 to reduce cell density, whereas the number of CIK cells cultured in G-Rex®6M was not adjusted. In both culture conditions, fresh IL-2 was provided every 3–4 days. We compared these two culture protocols with the culture in standard flasks. Phenotype was analyzed by flow cytometry and cytotoxicity was assessed against several tumor cell lines by calcine-release assay.

**Results** CIK cells cultured in G-Rex®6 well plates showed an outstanding cell expansion compared to G-Rex®6M well plates or standard culture flasks, with a 400-fold expansion and a mean of 10^9 total cells obtained per single well in 14 days, starting from just 2.5 × 10^6 cells per well. Moreover, the cultures in G-Rex®6 were characterized by a higher percentage of CD3+CD56+ cells, as compared to G-Rex®6M or standard culture flasks. Cells cultured in all devices had a comparable expression of NKG2D, NKP30, NKP44, 2B4 receptors. Importantly, CIK cells expanded in G-Rex®6 were as cytotoxic as cells expanded in standard culture flasks. Conversely, CIK cells cultured in G-Rex®6M showed a remarkable reduction of cytotoxicity against tumor cell targets, thus suggesting that cell density during expansion could affect CIK cell activity.

**Conclusions** We propose a GMP-compliant protocol for robust large-scale production of CIK cells. G-Rex® system allows to obtain large amounts of CIK cells highly enriched in the CD3+CD56+ subset and endowed with high cytotoxic activity; this can be accomplished with just a single cell culture split at day 7, which dramatically reduces the culture manipulation as compared to the standard culture flasks. Notably, this strategy can be further and easily scalable to produce CIK cells for clinical immunotherapy applications.

**Disclosure Information**

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**P09.14** BLOCKING COUNTERREGULATION OF UNFOLDED PROTEIN RESPONSE BY TARGETED PROTEIN SYNTHESIS INHIBITION PRODUCES HIGHLY SYNERGISTIC CELL DEATH IN SEVERAL CANCER ENTITIES

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**Background** Because tumor cells have high proliferation rates the demand for energy on the one hand and proteins on the other hand is high. In line, protein folding machinery of the ER is heavily used. 2-Deoxyglucose (2-DG) not only blocks energy synthesis by inhibiting glycolysis but also blocks synthesis of mannosyl leading to impaired N-linked glycosylation, which dramatically reduces the culture manipulation as compared to the standard culture flasks. Notably, this strategy can be further and easily scalable to produce CIK cells for clinical immunotherapy applications.

**Disclosure Information**

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**P09.15** TARGETING THE STROMA TO ENHANCE EFFECTOR MEMORY T CELL INFILTRATION AND ANTI-TUMOR RESPONSE TO ANTI-PD1 ANTIBODY IN PANCREATIC DUCTAL ADENOCARCINOMA

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**Background** Pancreatic ductal adenocarcinoma (PDAC) is resistant to immune checkpoint inhibition. One of the major resistance mechanisms is attributed to myeloid cells as an immunosuppressive element within the stroma of PDAC. It has been reported that focal adhesion kinase inhibitor (FAK) can suppress immunosuppressive myeloid cells such as tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSC), consequently sensitizing tumor to anti-PD1 antibody in mouse models of PDAC. Our group has previously shown in a murine model that targeting the stroma via PEGylated recombinant human hyaluronidase (PEGPH20) enhanced the anti-tumor activity of the whole cell vaccine (GVAX) by targeting CXCR4-expressing myeloid cells and led to an increase in infiltration of CCR7+ effector memory T cell subsets. Here, we evaluate the hypothesis that FAK expressing myeloid cell subsets modulate T cell infiltration in human pancreatic ductal adenocarcinoma.