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

Results Global deficiency of murine Tlr3, or TRIF, induced significantly increased digestive 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.

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

[1]S Tahk*, [1]SM Schmitt, [2,3]B Vick, [4]C Augsberger, [1]L Pascual Ponce, [2,3,5]I Jeremias, [6]G Wittmann, [4,3,7]M Subklewe, [1]NC Fenn, [1]K Hopfner. *[1]Department of Biochemistry and Gene Center, Ludwig-Maximilians-Universität München, Munich, Germany; [2]Research Unit Apoptosis in Hematopoietic Stem Cells, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany; [3]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), partner site Munich, Heidelberg, Germany; [4]Laboratory of Translational Cancer Immunology, Ludwig-Maximilians-Universität München, Munich, Germany; [5]Department of Oncology, Dr von Haunersches Kinderspital, Ludwig Maximilians-Universität München, Munich, Germany; [6]Department of Transfusion Medicine, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany; [7]Department of Hematology and Oncology, Medizinische Klinik und Poliklinik III, Klinikum der Universität München, Munich, Germany*

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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-Prkdc^{scid} IL2rg^{tm1Wjl}/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-dependent 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 allogenic 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.

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

[1]A Ventura*, [1]P Palmerini, [1]A Dalla Pietà, [2]R Sommaggio, [3]G Astori, [3]K Chieregato, [4]M Tisi, [5]C Visco, [4]O Perbellini, [4]M Ruggeri, [1]E Cappuzzello, [1,2]A Rosato. *[1]University of Padua, Padua, Italy; [2]Veneto Institute of Oncology IOV – IRCCS, Padua, Italy; [3]Advanced Cellular Therapy Laboratory, Department of Hematology, Vicenza Hospital, Vicenza, Italy; [4]Hematology Department, San Bortolo Hospital, Vicenza, Italy; [5]Department of Medicine, Section of Hematology, University of Verona, Verona, Italy*

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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-Rex6® or G-Rex®6M well

plates. CIK cells in G-Rex6® were split only once at day 7 to reduce cell density, whereas the number of CIK cells culterd 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 calcein-release assay.

Results CIK cells cultured in G-Rex6® 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-Rex6® were characterized by an 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.

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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

F Gsottberger*, C Meier, S Petkovic, L Mellenthin, M Krumbholz, M Metzler, A Mackensen, F Müller. *University Hospital of Erlangen, Erlangen, Germany*

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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, accumulation of misfolded proteins, and increased unfolded protein response (UPR). However, due to compensatory events, UPR-induced apoptosis is hampered. Therefore, we combined 2-DG with targeted protein synthesis inhibition by immunotoxins, consisting of an antibody and pseudomonas exotoxin, to enhance UPR mediated cell death.

Materials and Methods Established cell lines and patient-derived B-ALL samples were treated *in vitro* with various protein synthesis inhibitors and UPR-inducers. Drug synergy was determined mathematically as fold-increase over additivity. Biochemical studies were performed using western blots. *In vivo* enhancement was tested using systemic xenograft models.

Results The combination of Moxetumomab and 2-DG achieved a two to nine-fold synergy *in vitro*. Synergy was abrogated by the addition of Mannose suggesting UPR as cause of synergistic cell death. Similarly, Moxetumomab enhanced UPR-inducers Bortezomib and tunicamycin and protein synthesis inhibition by cycloheximide and puromycin enhanced 2-DG suggesting a conserved mechanism. Using HB21, an immunotoxin targeting human transferrin-receptor, breast cancer, hepatocellular carcinoma, and glioblastoma were sensitized to 2-DG induced cell death. Biochemically, 2-DG increased XBP-1-cleavage, expression of pro-apoptotic CHOP and of anti-apoptotic BIP. Moxetumomab, however, blocked the upregulation of BIP while maintaining CHOP correlating with synergistic increase in PARP-cleavage and apoptosis. In two systemic mouse models, bone marrow (BM) lymphoma infiltration was not reduced by 2-DG or tunicamycin alone but was reduced after treatment with Moxetumomab alone by 5-fold in the JeKo-1 and by 16-fold in the Ramos model, respectively. The combination of Moxetumomab and 2-DG achieved a three-fold synergy in the JeKo-1 model and achieved MRD-negative BM status in the Ramos model. Against patient-derived B-ALL of the Burkitt's type, 2-DG and Moxetumomab were up to 5-fold more active *in vitro* and up to 7-fold more active in mouse xenografts *in vivo*.

Conclusions Cell death after persisting unfolded protein response is synergistically enhanced by tumor-cell specific inhibition of protein synthesis against four distinct tumor entities at physiologically achievable concentrations. Our approach of immunotoxin-induced targeted protein synthesis inhibition opens a novel, so far undescribed therapeutic window which may warrant clinical evaluation.

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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

A Osipov*, L Zheng. *Johns Hopkins University, Baltimore, MD, USA*

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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 (FAKi) 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