plates. CIK cells in G-Rex6[®] 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 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 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 A. Ventura: None. P. Palmerini: None. A. Dalla Pietà: None. R. Sommaggio: None. G. Astori: None. K. Chieragato: None. M. Tisi: None. C. Visco: None. O. Perbellini: None. M. Ruggeri: None. E. Cappuzzello: None. A. Rosato: None.

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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10.1136/jitc-2020-ITOC7.114

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.

Disclosure Information F. Gsottberger: None. C. Meier: None. S. Petkovic: None. L. Mellenthin: None. M. Krumbholz: None. M. Metzler: None. A. Mackensen: None. F. Müller: None.

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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10.1136/jitc-2020-ITOC7.115

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