tumor antigen dependent manner. We are now evaluating these noval constructs for their ability to induce phagocytosis in different APCs. In addition we are looking into the effects these antibodies have on cytokine expression and expression of other immune regulatory markers. In-vivo we are using SPECT/CT and biodistribution analysis to determine their tumor targeting ability. Ultimately, we would like to show whether tumor targeted inhibition of the CD47-SIRPa axis using the bifunctional antibodies results in improved anti-tumor immunity

Disclosure Information F. Schuurmans: None. **A. Wittner:** None. **R.J.E. van den Bijgaart:** None. **S. Tahk:** None. **S. Heskamp:** None. **K. Hopfner:** None. **G.J. Adema:** None.

Poster Presentations

P01 Emerging concepts/new agents

P01.01 PLAP AS TARGET FOR CANCER IMMUNOTHERAPY – DEVELOPMENT AND PRECLINICAL CHARACTERIZATION OF BISPECIFIC MONOCLONAL ANTIBODY IN COLORECTAL CANCER IMMUNOTHERAPY

[1]NS Alrishedan*, [1]W Bodmer, [1]V Liebe lastun, [2]V Golubovskaya, [2]J Wu, [3]A Bransi, [3]P Umana, [3]C Klein, [3]R Mateus Seidl. *[1]Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK; [2]ProMab Biotechnologies, California USA, CA, USA; [3]Roche Glycart AG, Switzerland, Switzerland*

10.1136/jitc-2022-ITOC9.13

Immunotherapy using T cell-engaging bispecific monoclonal antibodies (BiMAb) is a promising cancer therapy. Such BiMAbs bind simultaneously to immune effector cells and to a cancer-specific antigen on tumor cells, resulting in killing of the latter. Placental alkaline phosphatase (PLAP), a plasma membrane-bound glycoprotein, is one of the four members of alkaline phosphatase isozyme family. PLAP is encoded by the *ALPP* gene. PLAP is expressed in placenta and has not been detected in other normal tissues. It has been shown that PLAP is released into the serum of patients with PLAP expressing tumours such as testis tumours. When PLAP is expressed ectopically in cancers, such as ovarian or colon carcinomas, it is essentially cancer specific and so an excellent target for immune based antibody therapy .The focus of my work is on colorectal cancer (CRC) and will mainly be based on the use of a well characterised panel of over 100 colorectal cancer derived cell lines about 20% of which express PLAP at the mRNA and protein levels . The cell lines are good representatives of primary tumors to use for in vitro preclinical testing of a new immunotherapeutic PLAP x CD3 BiMAb being developed for treatment of CRC. Worldwide, colorectal cancer has one of the highest cancer incidences and in the United States is the third cause of mortality in cancer patients. This emphasises the need to find novel effective treatments for colon cancer We found that a CD3 x PLAP BiMAb induced specific killing of PLAP-positive colorectal cancer cell lines, using peripheral blood mononuclear cells (PBMCs) as source of T cells, and that the killing depends on PLAP expression. The expression of PLAP in our cells varies and there is heterogeneity of PLAP expression within cell lines. However, we found that the effect of CD3 x PLAP BiMAb treatment was extended to PLAP-negative cells, when co-cultured with PLAP-positive ones, indicating a bystander effect. The bystander effect on PLAP-negative cells is only visible after 48 hours of treatment, suggesting an indirect killing mechanism. The important to study bystander killing to target cancer cells immune killing escape.To investigate further the mechanism of bystander killing, our early findings suggest CD3 x PLAP BiMAb activated T cells induce killing on bystander killing. We are studying the effect of the T cells subtypes and of the soluble factors secreted by the activated T cells.

Disclosure Information N.S. Alrishedan: None. **W. Bodmer:** F. Consultant/Advisory Board; Modest; ProMab Biotechnologies. **V. Liebe lastun:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Roche Glycart AG , Switzerland. **V. Golubovskaya:** A. Employment (full or part-time); Modest; ProMab Biotechnologies. **J. Wu:** A. Employment (full or part-time); Modest; ProMab Biotechnologies. **A. Bransi:** A. Employment (full or part-time); Modest; Roche Glycart AG, , Switzerland. **P. Umana:** A. Employment (full or part-time); Modest; Roche Glycart AG, , Switzerland. **C. Klein:** A. Employment (full or part-time); Modest; Roche Glycart AG, , Switzerland. **R. Mateus Seidl:** A. Employment (full or part-time); Modest; Roche Glycart AG, , Switzerland.

P01.02 SELECTIVE INDUCTION OF CELL DEATH IN JURKAT CELLS WITH RECOMBINANT FUNGAL LECTIN CNL

[1]MM Perisic Nanut*, [1]S Žurga, [1]Š Konjar, [1]M Prunk, [2]J Kos, [1]J Sabotič. *[1]Institute Jozef Stefan, Ljubljana, Slovenia; [2]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia*

10.1136/jitc-2022-ITOC9.14

Background Due to their capacity to specifically recognize subtle alterations in glycoproteins on the cell surface lectins are increasingly being used in diagnostic (for identification of malignant or premalignant cells) and therapeutic purposes (for targeted drug delivery).

Materials and Methods We have previously isolated and biochemically characterized a fungal lectin Clitocybe nebularis lectin (CNL) . We have synthetized recombinant form of CNL and tested its effects on different human cell lines and primary cells using various biochemical and molecular biological assays.

Results CNL is a GalNAcβ1–4GlcNAc-binding lectin that shows an antiproliferative effect solely on the leukemic Jurkat T cells. Furthermore, recombinant CNL treated Jurkat T cells exhibited archetypal features of early apoptosis, homotypic agglutination but lacked the activation of initiating and executing caspases since none of the features of CNL-induced cell death was effectively blocked with the pan-caspase inhibitor or different peptidase inhibitors. In addition, CNL binding induced Jurkat cells to release the endogenous damage-associated molecular pattern molecule high-mobility group box 1 (HMGB1), which is typically associated with necroptosis. We have identified CD45 and CD43 cell surface glycoproteins as main binding targets on the cell surface of Jurkat cells. However, the blockade of CD45 phosphatase activity failed to block either CNL-induced homotypic agglutination or cell death. Remarkably, a plant lectin, *Wisteria floribunda* agglutinin (WFA), which shows similar specificity in ligand binding, showed less selective cytotoxicity and induced cell death in Jurkat cells, Tall-104 acute lymphoblastic leukemia, and Hut-

87 cutaneous T-cell lymphoma cell lines with similar uncharacteristic features.

Conclusions Selective targeting of Jurkat T cells may reflect potential applicability of CNL in novel strategies for treating and/or detecting acute T cell leukemia. [Perišić Nanut, M, Žurga, S, Konjar, Š, Prunk, M, Kos, J, Sabotič, J. The fungal Clitocybe nebularis lectin binds distinct cell surface glycoprotein receptors to induce cell death selectively in Jurkat cells. FASEB J. 2022; 36: e22215. doi:10.1096/fj.202101056RR]

Disclosure Information M.M. Perisic Nanut: None. **S. Žurga:** None. **Š. Konjar:** None. **M. Prunk:** None. **J. Kos:** None. **J. Sabotič:** None.

P01.03 RNAI MEDIATED PD-1 KNOCKDOWN INDUCES A TCF-1 POSITIVE POPULATION IN ACTIVATED HUMAN CD8 T CELLS WITH STEM-LIKE ASSOCIATED MARKER PROFILE

[1]AS Herbstritt*, [2]M Maxwell, [2]D Yan, [2]B Cuiffo, [2]J Cardia, [2]S Zhou, [2]SP Fricker, [1]E Noessner. *[1]Helmholtz Zentrum Munich, Munich, Germany; [2]Phio Pharmaceuticals, Marlborough, MA, USA*

10.1136/jitc-2022-ITOC9.15

Background Adoptive Transfer of antigen specific T cells (ATT) is a powerful tool in the treatment of cancer. However, there are still hurdles to satisfactory efficacy.[1] One of them is the upregulation of immune-inhibitory receptors like programmed cell death protein 1 (PD-1). Silencing PD-1 at the mRNA level would not only prevent expression and therefore the inhibitory interaction with its ligand PD-L1, but also inhibit tonic signalling.[2] This should increase proliferation, cytotoxicity, cytokine production and metabolic activity via the AKT pathway. Another well-known hurdle to ATT efficacy is the poor persistence of effector T cells in patients. Stem-like memory subsets of CD8 T cells such as those marked by TCF-1 expression may therefore represent an advantageous effector population for ATT, as they show longer persistence, higher proliferative activity, responsiveness to checkpoint inhibitors and the ability to differentiate into new effector T cells.[3] Increasing the proportion of this population is thought to be beneficial in anti-tumor therapy. Here, we present data showing that specific downregulation of PD-1 using a novel RNA interference (RNAi) technology increases the frequency of a CD8 T cell population with a stem-like associated marker profile.

Materials and Methods INTASYL™ compounds incorporate drug-like properties into RNAi, resulting not only in enhanced cellular uptake but also eliminates the need for transfection reagents. TCR53-transduced T cells, suitable for ATT, were incubated with PD-1 targeting INTASYL compound PH-762 for 24h. As controls, cells were either treated with a non-targeting compound (NTC) or left untreated (UTC). Following PH-762 loading, T cells were co-cultured with the autologous tumor cell line RCC-53 for 96h. PD-1 knockdown efficacy was assessed along with other markers of interest via flow cytometry before and after co-culture.

Results PH-762 treatment reduced PD-1 surface expression in TCR53 T cells after 24h by ~50% compared to UTC or NTC. PH-762 mediated PD-1 silencing increased the subset of TCF-1 positive T cells at 24h post compound treatment and continued through 96h of co-culture with tumor cells. The TCF-1 positive cells expressed stem-like markers including higher expression levels of CD127 and CCR7 together with CD95 and lower levels of perforin.

Conclusions Increasing the proportion of stem-like CD8 T cells holds promise for optimizing ATT. PD-1 knockdown in TCR53 CD8 T cells for ATT by PH-762 induced the emergence of a T cell population expressing stem-like phenotypic markers including TCF-1. Further experiments are underway to assess the effects of the induced stem-like properties on a functional level, including proliferative activity and effector cell differentiation.

REFERENCES

1. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J* 2021;**11**(4):69.
2. Fernandes RA, Su L, Nishiga Y, *et al*. Immune receptor inhibition through enforced phosphatase recruitment. *Nature* 2020;**586**(7831):779–84.
3. Siddiqui I, Schaeuble K, Chennupati V, *et al*. Intratumoral Tcf1+PD-1+CD8+ T Cells with Stem-like Properties Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy. *Immunity* 2019;**50**(1):195–211.e10.

Disclosure Information A.S. Herbstritt: C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals. **M. Maxwell:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **D. Yan:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **B. Cuiffo:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **J. Cardia:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **S. Zhou:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **S.P. Fricker:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **E. Noessner:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals.

P01.04 APEX-MEDIATED BIOTINYLATION AS A POTENT TOOL FOR EVALUATION OF CHEMOKINE-RECEPTOR INTERACTIONS AND RESPECTIVE BINDING INHIBITORS

[1]A Beimert*, [1]C Perleberg, [1]B Meyer, [1]B Kühnemuth, [1]K Schnell, [1]P Layritz, [1]J Grün, [1]J Gärtig, [1,2]I Piseddu, [1]K Bahner, [1]S Endres, [1,2]D Anz. *[1]Division of Clinical Pharmacology, Department of Internal Medicine IV, University Hospital Munich, LMU, Munich, Germany; [2]Department of Internal Medicine II, University Hospital Munich, LMU, Munich, Germany*

10.1136/jitc-2022-ITOC9.16

Background The immunosuppressive tumor microenvironment (TME), which is strongly shaped by regulatory T cells (Treg), represents a major drawback for anti-cancer immunity and cancer immunotherapy. The migration of Treg into the TME is mediated by the CC chemokine receptor 4 (CCR4) whose main ligand, CCL22, is overexpressed in many tumor entities and is associated with unfavorable prognosis. Therefore, therapeutic blockade of the CCR4-CCL22 axis to suppress Treg migration is a promising strategy to overcome tumor-derived immune suppression. To study such chemokine-receptor interactions and to assess the binding capacity of potential inhibitors, we aimed to establish a screening tool using APEX-mediated biotinylation.

Materials and Methods In the presence of hydrogen peroxide, the enzyme ascorbate peroxidase (APEX) oxidates biotin-phenol to short-lived, highly reactive radicals that biotinylate structures in close proximity. Using a streptavidin-linked fluorophore that strongly binds biotin, the biotinylated structures can subsequently be analyzed and quantified via flow cytometry. To analyze the binding capacity of inhibitors of the CCR4-CCL22 axis, a CCL22-APEX fusion protein was created that binds to CCR4-expressing cells. Thus, the level of