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 tumors such as testis tumours. When PLAP is expressed in 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.

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 synthesized 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 executory caspases since none of the features of CNL-induced cell death was effectively blocked with the pan-caspase inhibitor or different peptidease 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. 

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**RESEARCH HIGHLIGHTS**

**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


**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 inhibit the inhibitory interaction with its ligand PD-L1, but also inhibit tonic signaling. 2. This should increase proliferation, cytotoxicity, cytokine production and metabolic activity via the AKT pathway. Another well-knonwn 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 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. PH-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**


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

1. A. Beimert*, C. Perleberg, B. Meyer, B. Kühnemuth, K. Schnell, P. Layritz, J. Grüner, J. Görtz, I. Piseddu, K. Bahner, S. Endres, D. Anz, 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.

**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) oxidizes 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