Abstracts

PLAP AS TARGET FOR CANCER IMMUNOTHERAPY – DEVELOPMENT AND PRECLINICAL CHARACTERIZATION OF BISPECIFIC MONOCONAL ANTIBODY IN COLORECTAL CANCER IMMUNOTHERAPY

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Poster Presentations

P01 Emerging concepts/new agents

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

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Immunotherapy using T cell-engaging bispecific monoclonal antibodies (BiMabs) 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 isozyme family. PLAP is encoded by the membrane-bound glycoprotein, is one of the four members of cancer-specific antigen on tumor cells, resulting in killing of BiMAbs bind simultaneously to immune effector cells and to a antibodies (BiMAb) is a promising cancer therapy. Such Immunotherapy using T cell-engaging bispecific monoclonal

Results

Conclusion

Disclosure Information


P01.02 SELECTIVE INDUCTION OF CELL DEATH IN JURKAT CELLS WITH RECOMBINANT Fungal lectin CNL

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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 neubulaisis lectin (CNL). We have synthesized recombinant form of CNL and tested its effects on different human cell lines and primary cells used 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-

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tumor antigen dependent manner. We are now evaluating these novel 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

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


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**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

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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. 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 inhibiting the interaction with its ligand PD-L1, but also inhibit tonic signalling. 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.

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**


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

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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...