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## 12.04 RE-DIRECTING CAR-T CELLS AGAINST SOLID TUMORS USING T-SIGN-MEDIATED ANTIGEN DELIVERY AND TUMOR MICROENVIRONMENT REPROGRAMMING

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**Background** Chimeric antigen receptor (CAR) T cell therapy has proven highly effective in the treatment of hematologic malignancies, however multiple barriers in the tumor microenvironment (TME) reduce its efficacy against solid tumors. Such barriers include an immunosuppressive TME, poor CAR-T cell trafficking and shortage of highly expressed tumor-specific target antigens. We recently demonstrated that Tumor-Specific Immuno-Gene (T-SIGn) viruses encoding multiple immunostimulatory mediators can reprogram the TME towards a pro-inflammatory phenotype, resulting in a markedly increased therapeutic efficacy of anti-EGFR and anti-HER2 CAR-T cells in an A549 human tumor xenograft and metastasis model.[1] Here we further explored the potential of T-SIGn platform combination with CAR-T cells by developing different T-SIGn viral vectors for the simultaneous tumor-specific expression of CAR-T cell target antigens and immunostimulatory molecules.

**Materials and Methods** We used *in vitro* human tumor cell lines to assess the ability of T-SIGn viruses to induce expression of a range of chimeric CAR-T cell target antigens. Using CD19 as a model antigen, we quantified virus-encoded CD19 expression on tumor cell surface using flow cytometry. *In vivo*, T-SIGn-dependent tumor-specific CD19 expression was assessed by flow cytometry analysis of tumor cell suspensions from A549 subcutaneous lung tumor xenografts in NSG mice.

**Results** In multiple *in vitro* human cell culture models, T-SIGn virus infection led to efficient expression of chimeric CD19 antigen on the tumor cell surface that enabled effective antigen-specific tumor cytotoxicity by anti-CD19 CAR-T cells. T-SIGn viruses with enhanced activity were also successfully generated by encoding immunostimulatory chemokines and cytokines together with chimeric CD19, enabling simultaneous tumor cell-specific CD19 antigen expression and enhancement of CAR-T recruitment and activity. *In vivo*, T-SIGn-dependent CD19 expression was demonstrated in intravenously dosed A549 human tumor xenografts, enabling future studies to optimize T-SIGn co-therapy with CAR-T cells directed against antigens otherwise not expressed in the tumor.

**Conclusions** Together, our data provide a proof of concept that T-SIGn viruses can re-direct CAR-T cells to act against solid tumors by enabling tumor-specific expression of cognate target antigens that are not endogenously expressed by the tumor cells. Further studies are ongoing to explore the full potential of synergistic combination of T-SIGn viruses and T cell therapy against solid tumors.

### REFERENCE

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## 12.05 TUMOR TARGETED INNATE IMMUNE CHECKPOINT BLOCKADE FOR THE TREATMENT OF NEUROBLASTOMA USING BIFUNCTIONAL ANTIBODIES

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**Background** Neuroblastoma is a childhood malignancy characterized by high expression of the disialoganglioside GD2, which is ranked in the top 15 of prioritized cancer antigens. Its prominent tumor specific expression has let to the development of anti-GD2 immunotherapy, resulting in improved patient survival. Some patients, however, still progress highlighting the need for improvement of the existing therapy. Innate immune checkpoints, like CD47, are a relatively new group of potential targets to stimulate the anti-tumor immune response.CD47 is a ubiquitously expressed protein overexpressed on tumor cells and the ligand for the SIRPα receptor expressed on myeloid cells, key effector cells in anti-GD2 based immunotherapy. Binding of CD47 to SIRPα prevents tumor cell phagocytosis and therefore serves as a *don't eat me signal*, providing the tumor with a mechanism to evade destruction and processing by antigen presenting myeloid cells (APCs). Targeting of CD47, however, has proven challenging as its ubiquitous expression on healthy cells forms an antigen sink.

**Approach** To improve anti-GD2 immunotherapy, we developed bifunctional antibodies able to target neuroblastoma and locally interfere with the CD47/SIRPα axis. These bifunctional antibodies recognize GD2 and contain the extracellular SIRPα domain that is able to block CD47.

**Results and Conclusion** *In-vitro*we found that the bifunctional antibody constructs bind tumor cells and block CD47 in a

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

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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 MONOCLONAL ANTIBODY IN COLORECTAL CANCER IMMUNOTHERAPY

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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.

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### 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 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-