Conclusions With this set of 7 novel CT-specific TCRs we expand the arsenal of tumor specific TCRs. With this expanding library of TCRs it would be possible to select in future for each cancer patient, based on HLA typing and gene expression, a useful TCR to generate a personalized TCR-gene therapy products. In addition, patients could be treated with multiple TCRs to enhance the efficacy and increase the durability of clinical responses by reducing the likelihood of tumor escape.

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Poster Presentations

P01 Emerging concepts/new agents

P01.01 SAFETY AND EFFICACY STUDY OF PEMBROLIZUMAB IN COMBINATION WITH LENVATINIB IN PARTICIPANTS WITH HEPATOCELLULAR CARCINOMA (HCC) BEFORE LIVER TRANSPLANT AS NEOADJUVANT THERAPY——PLENTY RANDOMIZED CLINICAL TRIAL

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Background Patients with hepatocellular carcinoma (HCC) who exceed standard Milan criteria suffered from high post-transplant recurrence rate.This study will evaluate the safety and efficacy of pembrolizumab in combination with lenvatinib as neoadjuvant therapy in participants with HCC exceeding Milan criteria before liver transplant.

Materials and Methods Participates would be randomly assigned (1:1) to experimental or Comparator/Control by computer-generated allocation based on the envelope method and the hierarchical block randomization method(hierarchy: BCLC stage and AFP level). The envelopes are sealed opaque, and sequentially numbered.Randomization is performed by the trial coordinator.The random number table and the block assignment number table will be kept confidential by the full-time secretary of this project.Center-stratified block-permuted randomization is used in this trial. Then permuted block randomization is used for each stratum with a block size of 4.

Results The initial first patient was recruited in August.2020, the primary hypothesis of this study are that neoadjuvant pembrolizumab plus lenvatinib is superior to regularly waiting in the list with respect to: 1) recurrence-free survival (RFS) as assessed by blinded independent central review (BICR); and 2) Objective Response Rate (ORR).The investigators design a clinical study to explore whether the combination above as a neoadjuvant treatment in patients with advanced HCC before liver transplant could reduce postoperative recurrence and to analyze potential immune biomarker of therapeutic response.

Conclusions The study is still ongoing and the preliminary short term outcome was positive. HCC patients who exceeded milan criteria may benefit from neoadjuvant immunotherapy combined with TKI before liver transplantation.

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P01.02 TLR-MEDIATED SUPPRESSION OF THE CCL22-CCR4 AXIS AS A NEW TARGET FOR TUMOR IMMUNOTHERAPY

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Background Unmethylated CpG-DNA is a potent ligand for the endosomal Toll-like-receptor-9, important for the immune activation to pathogen-associated molecules.[1] CpG and other TLR-ligands show effective immunotherapeutic capacities in cancer treatment by inducing an antitumorigenic immunity.[2] They are able to reduce tumor progression by reduction of intratumoral secretion of the immunoregulating chemokine CCL22[3] and subsequent recruitment of immunosuppressive regulatory T cells (Treg), which express CCR4 the only so far known receptor for CCL22.[4] Our recent work has shown that CCL22 secretion by dendritic cells (DC) in the lymph node, mediates tolerance by inducing DC-Treg contacts.[5] Indeed, in the absence of CCL22, immune responses to vaccination were stronger and resulted in tumor rejection.[6] Therefore, we are aiming to investigate the effects of TLR-ligands on systemic CCL22 levels, elucidating all involved mechanisms to identify new targets for cancer immunotherapy.

Materials and Methods T, B and $CD11c^+$ DCs of wildtype (wt) and $RAG1^{-/-}$ mice were isolated from splenocytes by magnetic-activated cell sorting for *in vitro* assays. Different co-cultures were incubated with CpG and GM-CSF, known as an CCL22 inducer.[5] For *in vivo* experiments, wt mice were treated with CpG, R484 or poly(I:C) alone and in combination with GM-CSF. CCL22-levels in a number of organs were analyzed.

Results Analyzing the different immune cell compartments *in vitro*, we found that DCs in whole splenocytes secrete CCL22 during culture while DC cultured alone showed no CCL22 secretion. When treated with CpG, CCL22-levels were reduced in splenocytes, while it was induced in DC culture alone. The same results were seen when RAG splenocytes, that lack functional B and T cells, were cultured with CpG. CpG treated B cells were able to suppress CCL22 secretion by DC unlike T cells alone. Co-cultures of T and B cells treated with CpG, however, induced the strongest CCL22 suppression in DC. *In vivo*, we could show that all TLR ligands tested reduced CCL22 in a number of organs significantly. Furthermore, CpG showed the strongest suppression of CCL22 even in the presence of the CCL22 inducer GM-CSF.[5]

Conclusions We could show that B cells with T cells mediate CCL22 suppression by TLR ligands. The fact that CpG was able to reduce CCL22 levels even in the presence of the inducer GM-CSF demonstrates the potent CCL22 suppressive capacity of TLR ligands.

REFERENCES

1. O'Neill LA, *et al*. The history of toll-like receptors – redefining innate immunity. *Nat Rev Immunol* 2013;**13**(6):453–60.
2. Rothenfusser S, *et al*. Recent advances in immunostimulatory CpG oligonucleotides. *Curr Opin Mol Ther* 2003;**5**(2):98–106.
3. Wang S, *et al*. Intratumoral injection of a CpG oligonucleotide reverts resistance to PD-1 blockade by expanding multifunctional CD8+ T cells. *Proc Natl Acad Sci U S A* 2016;**113**(46): E7240–E7249.
4. Rapp M, *et al*. CCL22 controls immunity by promoting regulatory T cell communication with dendritic cells in lymph nodes. *J Exp Med* 2019;**216**(5):1170–1181.
5. Piseddu I, *et al*. Constitutive expression of CCL22 is mediated by T cell-derived GM-CSF. *J Immunol* 2020;**205**(8):2056–2065.

6. Anz D, *et al*. Suppression of intratumoral CCL22 by type i interferon inhibits migration of regulatory T cells and blocks cancer progression. *Cancer Res* 2015;**75**(21):4483–93.

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P01.03 TARGETING DIACLYGLYCEROL KINASE ALPHA AND ZETA BY SELF DELIVERING RNAI TO OPTIMIZE TLYMPHOCYTES FOR ADOPTIVE THERAPY OF SOLID TUMORS

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Background Evidence indicates that diacylglycerol kinases (DGK) are promising targets for the optimization of T cell activity, for example in the setting of adoptive cell therapy (ACT). The tumor microenvironment (TME) of human renal cell carcinoma (RCC) is an immunosuppressive setting where T and NK cell functionality is blocked. DGK–α is a negative regulator of TCR signaling, functioning by metabolizing diacylglycerol to phosphatidic acid and thereby limiting the activation of MAPK/ERK1/2 signaling pathway. DGK-α is found increased in tumor-infiltrating lymphocytes (TIL) from RCC patients and also in adoptively transferred T cells after infiltrating into the TME.[1] We previously reported that inhibition of DGK-α restored functionality of unresponsive CD8 T cells and NK cells from RCC-TIL. Other studies demonstrated that knockdown or pharmacologic inhibition of DGK-α and DGK-ζ alone or together increased target cell killing and cytokine production, and protected T cells from inhibitory factors in the TME.[2] However, there are no inhibitors for DGK-ζ and available DGK-α inhibitors have undesired pharmacokinetic/pharmacodynamic properties and are highly toxic precluding their clinical application. Here, we present data using a novel RNA interference (RNAi) technology that can specifically target each DGK isoform.

Materials and Methods INTASYL™ compounds incorporate drug-like properties into RNAi, resulting not only in enhanced cellular uptake in the presence of serum but also eliminating the need for further transfection reagents. Toxicity of compounds applied alone or in combination was assessed by 7-AAD flow cytometry analysis and WST assay. Silencing of mRNA and protein was analyzed by RT-qPCR and SimpleWestern. Downstream signaling pathways and T cell function were analyzed to demonstrate pharmacological efficacy.

Results Two DGK-ζ compounds and one DGK-α compound were analyzed using Jurkat T cells and primary human TCR-transduced T cells. No effects were seen on cell viability for the compounds applied alone or in combination. On-target knockdown was achieved in Jurkat T cells evidenced by RT-qPCR and SimpleWestern. Silencing of mRNA and protein occurred quickly after 24h, peaked between 48h and 72h and lasted at least for 96h. Stimulation under DGK-targeting INTASYL treatment resulted in enhanced levels of phosphorylated ERK1/2 and enhanced secretion of IL-2.

Conclusions INTASYL™ self-delivering RNAi compounds represent a promising approach to target intracellular immune checkpoints such as DGKs. The good toxicity profile allows for combined application of several compounds enabling targeting of multiple checkpoints, which likely is necessary to counteract the complex and heterogeneous inhibitory influences of the TME. The technology enables the anti-tumor activity of T and NK cells for immunotherapy, and can be used in ACT and direct therapeutic applications towards the TME.

REFERENCES

1. Moon EK, Wang L-C, Dolfi DV, Wilson CB, Ranganathan R, Sun J, *et al*. Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor-transduced human T cells in solid tumors. *Clin Cancer Res* 2014;**20**(16):4262–73.
2. Jung I-Y, Kim Y-Y, Yu H-S, Lee M, Kim S, Lee J. CRISPR/Cas9-mediated knockout of DGK improves antitumor activities of human T cells. *Cancer Res* 2018;**78**(16):4692–703.

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P01.04 LENTIVIRAL PROTEIN VPX DELIVERY SYSTEMS AS POTENTIAL WEAPONS TO IMPROVE CYTARABINE TREATMENT RESPONSE AGAINST ACUTE MYELOID LEUKEMIA

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Background Acute myeloid leukemia (AML) is an aggressive cancer of the blood, where malignant myeloid blasts accumulate in the bone marrow. One of the challenges of effective AML treatment is resistance to cytarabine (or ara-C), a standard AML chemotherapeutic drug used in front-line treatment today. In 2017, Schneider *et al.* reported the dNTPase sterile alpha motif and HD-domain-containing protein 1 (SAMHD1) to be a targetable biomarker for ara-C treatment response.[1] The intracellular triphosphorylated active form of ara-C, ara-CTP, was recognized as a substrate by SAMHD1 and is hydrolyzed back to ara-C. This led to a decrease in the amount of ara-CTP within the cells and consequently reduced cytotoxicity.[1] SAMHD1 can be targeted by the lentiviral accessory protein Vpx for proteasomal degradation by interacting with the proteasomal degradation complex and SAMHD1. This study aims to use Vpx to target SAMHD1 in AML cells to improve ara-C sensitivity.

Materials and Methods In order to manipulate SAMDH1 levels using Vpx, different Vpx delivery systems were developed. These are virus-like particles (VLPs) packaged with different homologs of Vpx from Simian Immunodeficiency Viruses (SIV) and HIV-2, and cell-penetrating peptides (CPPs) bound to either a 67 amino acid truncated SIVmac Vpx (67aaVpx) or to the WT full-length form. Two different CPPs were used in the synthesis: TAT and CPP44. The latter