Oral Presentations

10 Young researcher session

10.01 EFFECTIVE SOLID TUMOR THERAPY THROUGH ENHANCED RECRUITMENT AND IMMUNE SUPPRESSION SHIELDED T CELLS

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Background CAR T cell therapy remains ineffective in solid tumors. Scarce T cell infiltration and T cell suppression at the tumor site are two notable therapy limitations. T regulatory (Treg) cells are capable of suppressing effective anti-tumor responses through inhibitory factors such as transforming growth factor β (TGF- β). Treg cells expressing the C-C chemokine receptor 8 (CCR8) have been found to accumulate and to correlate with poor prognosis in breast cancer. We postulated that CCR8 could be exploited to redirect effector T cells to the tumor site while a dominant-negative TGF- β receptor 2 (DNR) can simultaneously shield them from TGF- β .

Materials and Methods CCR8 and DNR can be expressed in murine and human T cells upon retroviral transduction. T cell receptor (TCR) and chimeric antigen receptor (CAR) antigen specific models in murine and human systems were utilized. qPCR, IF microscopy, ELISA and the cancer genome atlas (TCGA) database were used in the steps of ligand identification and hypothesis generation. We employed flow cytometry and multi-photon intra-vital microscopy to interrogate infiltration, proliferation and phenotype of T cell products. Mechanistically, CRISPR was used to dissect the role of the CCL1-CCR8 positive feedback loop in T cell therapy.

Results We identified that in an in vivo pancreatic murine model of cancer, the CCR8 gene was upregulated in tumor infiltrated lymphocytes compared to T cells that accumulated in the spleen. In this same tumor model, CCL1 could be detected in tumor explants. We identified that this secreted CCL1 from activated effector T cells potentiates a feedback loop for CCR8⁺ T cell recruitment to the tumor site. The introduction of CCR8 and DNR receptors in primary T cells improved migration towards CCL1 and improved proliferation capacity in the presence of TGF- β . Besides these effects, these receptors did not further impact effector and memory phenotype or secretome of T cell products. The CCR8-driven sustained and improved infiltration synergized with TGF- β -shielding conferred by the DNR for improved therapeutic efficacy, allowing tumor rejection in models that are otherwise completely resistant to CAR T cell therapy.

Conclusions We conclude that the combination of CCR8- and DNR-transduction into antigen-specific T cells can exploit two critical biological axes to render T cell therapy effective in solid tumors such as pancreatic cancer. Beyond TGF- β , relieving other immunosuppressive axes may further sustain a CCL1 feedback loop mechanism to improve anti-tumoral

function of CCR8+ ACT. This therapeutic approach could be extended to other Treg-rich solid tumor entities where limited infiltration into the tumor and intra-tumoral T cell proliferation prevent therapeutic success. Furthermore, the CCL1-CCR8 axis heralds the potential to be used as a target to improve the efficacy of immunotherapies beyond ACT.

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10.02 GENOMIC HLA HOMOZYGOSITY IS FREQUENT IN ESOPHAGEAL ADENOCARCINOMA AND RELATED TO LOW IMMUNOGENICITY

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Background Classical human leukocyte antigen (HLA) class I molecules are expressed by most somatic cells and present peptides to cytotoxic T cells. The HLA-genotype of an individual contains up to six different HLA-I molecules and defines the repertoire of peptides that can be presented to cytotoxic T cells. Homozygosity for one or more HLA-loci could translate in a smaller repertoire of tumour neoantigens possibly presented to cytotoxic T cells in an individual and potentially predispose such individuals with a disadvantage to fight a nascent tumour.

Material and Methods High-resolution HLA-genotyping from germline normal DNA of 80 esophago-gastric adenocarcinoma (EGA) patients was performed with the NGS method by Illumina. Whole exome sequencing (WES) was performed on tumor tissue and normal peripheral blood cells (n=39). The data were processed, and non-synonymous mutations were called. The amount of potential high-affinity binders derived from 10 cancer testis antigens (CTAs) frequently expressed in EGA and non-synonymous mutations obtained from WES data were determined using an in-silico approach for MHC-binding (IEDB.org). RNA-extraction and gene expression profiling were performed using the NanoString technology.

Results We compared the frequency of HLA homozygosity in EGA patients to an HLA-matched reference population derived from a large cohort of bone marrow donors (n=7.615 out of 615.017 donors). We demonstrate that EGA patients are more likely to be homozygous for at least one HLA-I gene than the control population. In EGA patients, 35% of HLA-A, -B, and -C alleles were homozygous in comparison with 19% of HLA alleles among the HLA-matched general population. This difference corresponded to an odds ratio (OR) for homozygosity of 2.282 (95% confidence interval (CI) 1.442-3.615, p<0.001). The odds ratios for homozygosity at HLA-A (OR=1.885, CI=1.111-3.236, p<0.05), HLA-B (OR=3.045, CI=1.346-6.499, p<0.05) and HLA-C (OR=2.170, CI=1.445-3.579, p<0.05) were significantly different. We then aimed to estimate the influence of HLA-homozygosity in the context of tumour immune surveillance. Predictions by IEDB analysis resource tool indeed showed a reduced repertoire of high and moderate-affinity MHC-

binders (both CTA-derived and mutation-derived peptides) in the homozygous cohort. Our findings demonstrate a reduced amount of potentially immunogenic peptides in EGA patients with HLA-homozygosity for at least one locus, which may result in impaired cancer immunosurveillance. In line with this observation, we also found increased levels of CTA expression in homozygous compared to heterozygous patients. After artificial modification of the genotype of homozygous patients to a heterozygous genotype, the set of predicted good-binding peptides was comparable to the heterozygous cohort.

Conclusion Our results highlight the effect of HLA-I homozygosity on the immunopeptidome as important prerequisite of anti-tumor immunity. The high frequency of genomic HLA-I homozygosity observed in the EGA cohort may reflect an increased cancer risk for these patients. Together with previous reports demonstrating reduced survival after checkpoint therapy, our study suggests consideration of germ-line HLA-homozygosity for the design and interpretation of immunotherapeutic trials.

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10.03 INTERLEUKIN-22 REGULATES ANTI-TUMOR IMMUNITY IN MOUSE MODELS OF LUNG AND BREAST CARCINOMA

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Background High expression of CD155 (poliovirus receptor, PVR) is associated with a poor prognosis of lung adenocarcinoma (LUAD) and triple-negative breast cancer (TNBC) patients. When overexpressed, this molecule inhibits the anti-tumor function of NK and cytotoxic T cells through binding to its inhibitory co-receptors TIGIT and CD96, and downregulation of stimulatory CD226 (DNAM-1). However, the exact mechanism of CD155 overexpression on the tumor cells remains unclear. Here we demonstrate that interleukin-22 (IL-22), a cytokine known to promote cancer progression, induces upregulation of CD155 on tumor cells in mouse models of breast and lung cancer and may, thus, inhibit antitumor immunity and promote lung metastasis.

Materials and Methods To study the influence of IL-22 on antitumor immunity, we utilize IL-22-deficient animals in syngeneic mouse models of metastatic breast and lung cancer. For this purpose, we generated tumor cells deficient in IL-22

receptor (IL-22R) or in CD155 and tumor cells, that constantly express CD155 independent of its natural regulation. Here, we determine the incidence of metastasis and antitumor NK and T cell responses in the lung, the primary site of metastasis.

Results We demonstrate that murine cancer cells upregulate CD155 surface expression upon treatment with recombinant IL-22, whereas this effect is abolished in the absence of IL-22R. Furthermore, IL-22-deficient animals have a lower metastatic burden in the lung and demonstrate a dramatic increase in IFN- γ production in NK, and, to a lower extent, cytotoxic T cells. Moreover, this effect is reversed when CD155 is expressed on the tumor cells independent of its natural regulation, which enables lung metastases in IL-22 deficient animals. Phenotypically, NK cells in IL-22 knockout mice have a higher expression of co-stimulatory receptor CD226, which is linked to the antitumor potential of these cells.

Conclusions Here we demonstrate a novel pathway of cytokine-mediated cancer progression, where IL-22 is capable of inducing CD155 on the tumor cells and, therefore, promotes an immunosuppressive tumor microenvironment. This highlights the potential of IL-22 as a target for immunotherapy considering the complexity of the CD155-dependent immunoregulatory network.

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10.04 A LIBRARY OF NOVEL CANCER TESTIS SPECIFIC T-CELL RECEPTORS FOR T-CELL RECEPTOR GENE THERAPY

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Background The positive clinical effect of T-cell receptor (TCR) gene therapy on tumor regression has previously been demonstrated by NY-ESO-1 TCR-gene therapy. To seriously increase the number of cancer patients that can be treated with TCR-gene therapy we aim to identify a novel set of high-affinity Cancer Testis (CT) specific TCRs targeting different CT-antigens in a variety of prevalent HLA-class I alleles.

Materials and Methods In this study, we selected by bioinformatic tools the most promising CT-genes to target, and from these genes we identified by HLA-peptidomics the naturally processed and presented HLA-class I peptides. With these peptides HLA-tetramers were generated, and by MACS enrichment and single cell sorting CT-specific CD8⁺ T-cell clones were selected from the allo-HLA repertoire of healthy donors. By performing several different functional assays the high function avidity CT-clones with a safe recognition pattern were selected. To evaluate the potential for clinical application in TCR-gene therapy, TCRs were sequenced, and transferred into peripheral blood derived CD8⁺ T cells.

Results In total we identified, 7 novel CT-specific TCRs that effectively target MAGE-A1, MAGE-A3, MAGE-A6 and MAGE-A9 expressing tumors cells in the context of HLA-A1, -A2, -A3, -B7, -C7 and -B35.