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P04.02 DIVERSITY OF CD4+ BLOOD T-CELL CLONALITY PREDICTS LONGER SURVIVAL WITH CTLA4 OR PD-1 CHECKPOINT INHIBITION IN ADVANCED MELANOMA

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Background T cells play a central role in tumor immunity. In principle, T cell requires antigen recognition by T-cell receptor (TCR) to gain effector function. Antigen-driven activation leads to clonal T-cell expansion with generation of progeny cells that all express the same chronotypic TCR. This makes TCR analysis a useful tool to comprehensively and individually understand antigen-specific T-cell responses. Indeed, we previously showed that the TCR repertoires of $CD8^+$ T cells but not $CD4^+$ T cells are restricted with many clones in the blood of psoriasis patients. Together with the strong genetic association to HLA-C*06:02 causing an autoimmune $CD8^+$ T-cell response against melanocytes in psoriasis, our results from TCR analyses clearly indicate an autoimmune pathogenesis of psoriasis.

Patients and Methods Here, we utilize our expertise to understand how anti-tumor T-cell responses affect clinical responses and immune-related adverse events (irAEs) in therapeutic checkpoint inhibitions. We analyzed melanoma patients upon the therapeutic blockade of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or programmed cell death 1 (PD-1) using TCR Vβ-gene spectratyping.

Results Surprisingly, we observed variable levels of restriction in $CD4^+$ and extensive restrictions in $CD8^+$ T-cell repertoires in the blood of melanoma patients compared to healthy controls. This indicates the presence of a substantial numbers of $CD4^+$ and $CD8^+$ T-cell clones in the blood prior to the initiation of immunotherapy. The clones detected in the blood were enriched in tumor-infiltrating lymphocytes (TILs). This suggests that melanoma-reactive T-cell clones circulate more frequently in melanoma patients, although it is generally assumed that tumor-specific T-cell clones are only detectable in TILs. Greater diversification particularly in $CD4^+$ blood T-cell clones before immunotherapy correlated with long-term survival after CTLA4 or PD-1 inhibition. In patients who developed severe immune-related adverse events (irAEs) during CTLA4 blockade, we detected newly expanded blood T-cell clones, suggesting that newly emerged T-cell responses contributed to these irAEs.

Conclusions Our data demonstrate that the diversity of T-cell clones in the circulation may reflect the anti-melanoma responses. This study provides a rationale for predicting clinical responses to checkpoint inhibitors using patient's blood, and also emphasizes importance of $CD4^+$ T cell-mediated anti-tumor immunity in melanoma.

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P04.03 EXPRESSION PROFILES OF IMMUNE MARKERS AS PREDICTORS OF SURVIVAL IN SURGICALLY-TREATED NSCLC

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Background Surgery is the treatment of choice for early and for some locally advanced non-small cell lung cancer (NSCLC). Ipsilateral hilar and mediastinal lymph nodes are generally removed at the time of tumor resection. There is now increased awareness about the physiological role of lymph nodes in cancer. We investigated the expression profiles of immune-related markers in matched tumor tissue, affected and unaffected N1 and N2 lymph nodes in patients with NSCLC and their relation to survival.

Materials and Methods Internal hospital databases were screened for surgically-treated NSCLC patients with documented relapse or long-term disease-free survival (defined as 3 years). Data on patients' age, sex, surgery, (neo)adjuvant therapy, tumor characteristics and time and location of relapse was extracted. FFPE tissue blocks of primary tumor, affected and unaffected lymph nodes were collected. mRNA was extracted from these tissues and expression profiling of 751 immune-related genes was performed using the PanCancer IO 360 panel by NanoString Technologies. **Results**

A total of 754 NSCLC patients were screened. Of these, 71 patients showed long-term disease-free survival and 80 patients had local or systemic relapse within 3 years after surgery. Expression profiles of immune-related genes in tumor and lymph node immune populations differed between patients with and without 3-year disease-free survival.

Conclusions Expression profiles of immune-related genes differ between patients with and without relapse. Our findings show that differences in expression profiles of immune-related genes in tumor and lymph nodes should be taken into account when assessing patient prognosis.

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P04.04 PROGRAMMED DEATH-LIGAND 1 POSITRON EMISSION TOMOGRAPHY IMAGING DURING NEOADJUVANT (CHEMO)RADIOTHERAPY IN ESOPHAGEAL AND RECTAL CANCER (PETNEC): A PROSPECTIVE NON-RANDOMIZED OPEN-LABEL SINGLE-CENTER PILOT STUDY

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Background Immune checkpoint inhibitors (ICI), such as atezolizumab (anti-programmed death-ligand 1; PD-L1), have been proven to be an effective strategy in solid cancers. However, the overall response rate to ICI is currently limited to an intrinsically responsive tumor immune microenvironment (TIME) or depended on an appropriate foregone immune stimulus, such as radiotherapy. The concept of combining radiotherapy with ICI is currently investigated in a variety of solid cancers. However, little data is known on the expression dynamics of immune checkpoint ligands, such as PD-L1, during neoadjuvant chemoradiotherapy (CRT) or short-course preoperative radiotherapy (SCPRT) in human solid malignancies.

Materials and Methods This is a prospective non-randomized open-label single-center investigator-initiated pilot study (NCT no. NCT04564482). Patients with either rectal cancer (RC), oesophageal adenocarcinoma (EAC), gastroesophageal junction (GEJ) cancer or oesophageal squamous cell carcinoma (ESCC), whom are assigned by the routine multidisciplinary tumor board (MDT) to receive a standard neoadjuvant CRT/SCPRT, will be enrolled. Standard neoadjuvant regimens include CRT (50 Gy in 2 Gy fractions over 25 working days + capecitabine 1650 mg/m^2/d PO) or SCPRT (25 Gy in 5 Gy fractions over 5 working days) for RC patients and CRT according to the CROSS protocol (41.4 Gy in 1.8 Gy fractions over 23 working days + carboplatin AUC of 2 mg/ml/min + paclitaxel 50 mg/m2 IV Q1W) for patients with EAC, ESCC or GEJ cancer. Patients will receive a PD-L1 (^{89}Zr-atezolizumab) positron emission tomography (PET) CT (for EAC, ESCC or GEJ cancers) or MRI (for RC) bevor (day 0) and during neoadjuvant treatment (day 10-14).

Results The primary endpoint of this pilot study is the none-invasive assessment of PD-L1 expression dynamics during neoadjuvant CRT/SCPRT by a PD-L1 PET imaging approach. Secondary objectives are the correlation between PD-L1 PET expression dynamics and radiographic as well as pathological therapy response.

Conclusions This is the first in human study, which assesses PD-L1 expression dynamics during different neoadjuvant radiotherapeutic regimens. A detailed understanding of the impact of radiotherapy on PD-L1 expression, monitored by an none-invasive PET imaging approach, allows the application of radiotherapy as part of a novel immunotherapeutic concept.

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P05 'Lost in Translation'?

P05.01 ORGANOID-SPECIFIC OPTIMIZATION OF KILLING ASSAYS TO TEST NOVEL IMMUNOTHERAPIES IN A HIGH-THROUGHPUT SYSTEM

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Background The immunotherapeutic drug dinutuximab, which binds to disialoganglioside (GD2) and activates natural killer (NK) cells, is part of the standard regimen in high-risk neuroblastoma (NB) patients. However, dinutuximab only results in tumor reduction in a subset of patients, and survival rates of high-risk neuroblastoma patients are below 60%. Novel immunotherapies are therefore needed. Current in vitro models lack the ability to study novel immunotherapies with high-throughput screening (HTS). We aimed to optimize NB organoid-lymphocyte cocultures for HTS, and possibly personalized testing, of novel antibody-mediated and cellular immunotherapies.

Materials and Methods Two patient-derived organoids (691B: GD2$^+$MHC-I$^-$ and 691T: GD2$^-$MHC-I$^+$) were transduced with an endogenous luciferase construct to use D-luciferin-induced bioluminescence as readout for cell growth. The growth rate, optimal seeding density and optimal pre-culture time per organoid were determined by density curves, and the number of needed cells was downscaled to facilitate HTS. After pre-culture, the luciferase-transduced organoids were co-cultured with primary PBMCs from healthy donors, PRAME-TCR transduced T cells or CAR-T cells.[1] Several effector:target (E:T) ratios and timepoints were tested to identify the optimal window for read-out of dinutuximab-induced antibody-dependent cytotoxicity (ADCC) and T-cell mediated cytotoxicity. The required number of immune cells per ratio was calculated based on the expansion rate of organoid cells after 48 and 72 hours.

Results The density screens showed an optimal seeding density of 5000-10.000 organoid cells per well, yielding a high luminescence signal while minimizing the number of cells needed. Already at the lowest E:T ratio (1:3), we observed killing of the MHC-I expressing 691T organoid, likely based on allogeneic recognition of the organoids by T cells. The killing efficacy increased with higher E:T ratios and co-culture time. Pre-culturing of organoids for 72 hours before addition of effector cells resulted in formation of larger 3D spheres, which reduced the killing efficacy for all E:T ratios. ADCC effects of dinutuximab were studied in GD2$^+$MHC-I$^-$ 691B organoids. Addition of dinutuximab resulted in 25% increase of killing after 24 hours and reached up to 70% increase after 72 hours for 10:1 and 20:1 E:T ratios. Higher E:T ratios were likely needed because NK cells make up a smaller proportion of PBMCs than T cells. Dinutuximab did not increase killing of the GD2$^-$ organoid, confirming specificity of the antibody. T cell mediated killing was almost 100% for MHC-I$^+$691T organoids after 24 hours of culturing with PRAME-TCR transduced T cells and CAR-T cells at a 1:3 E:T ratio, showing the high anti-tumor cytotoxicity of these cells and potential for HTS at very low E:T ratios.

Conclusions We have developed a robust in vitro bioluminescence-based organoid/lymphocyte co-culture assay with a low cell input, to facilitate high-throughput screening of novel antibody-based or cellular immunotherapies, possibly combined with chemotherapeutic or targeted compounds. In the future this method may be applied for personalized drug screens.

REFERENCE

1. Avital L Amir, *et al*. PRAME-Specific Allo-HLA-restricted T cells with potent antitumor reactivity useful for therapeutic T-cell receptor gene transfer. *Clin Cancer Res* 2011.

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