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/m²/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 (⁸⁹Zr-atezolizumab) positron emission tomography (PET) CT (for EAC, ESCC or GEJ cancers) or MRI (for RC) before (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 a 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

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