For decades, basic research and clinical trials have aimed to establish a meaningful cancer immunotherapy. An unprecedented number of medicinal products for cancer immunotherapy are currently being authorised. For this success, the selection of the right molecular targets for immunotherapy has been crucial. Monoclonal antibodies targeting cell surface receptors for growth factors were successfully introduced in clinical routine for the treatment of common solid tumours more than 10 years ago. Since then, however, the focus of cancer immunotherapy has shifted. More recently, many authorized medicines targeting growth factors receptors in solid tumours are no longer based on antibodies, but on small molecule protein kinase inhibitors. Currently, medicines with recent European marketing authorizations for the immunotherapy of cancer come from three major categories: (1) monoclonal antibodies targeting blood cell surface antigens; (2) CAR-T cells for the therapy of haematological neoplasia; and, with broader use including solid tumours, (3) checkpoint inhibitors. For the successful use of many of these medicines, the appropriate companion diagnostics (CDx) are required. Therefore, precision medicine also means selecting precisely the patients who will profit from a specific treatment (using, for example, a CDx). But, how can an informed choice be made with regards to CDx? We will give an overview on the cancer immunotherapies that depend on CDx and outline what to look for when choosing a suitable CDx.

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P05 Reverse translation

**P05.01 PLATELETS ROLE IN IMMUNOTHERAPY RESPONSE IN NON-SMALL CELL LUNG CANCER**

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Background Immunotherapy has revolutionized the therapeutic landscape of non-small cell lung cancer (NSCLC). In particular, therapy based on immune checkpoint inhibitors (ICIs), such as monoclonal antibodies (mAbs) targeting programmed cell death protein 1 (PD-1) pathway, has changed the survival rate of NSCLC patients. However, a subset of patients is responsive to ICIs and another subset develops acquired resistance to ICIs. In the past few decades, increasing studies have highlighted that high platelets (PLTs) count is associated with poor prognosis of NSCLC patients. Therefore, the aim of this research was to investigate the contribution of PLTs in response to immunotherapy.

Materials and Methods Blood samples from advanced (non-resectable, stage IV) NSCLC patients treated with Atezolizumab were collected at the baseline (T0, prior to the first administration) and at disease progression (PD). PLTs were isolated by PLT-rich plasma by centrifugation. PD-L1 and Fc gamma receptors (FcγR) PLTs expression were analyzed, and the release of activated PLTs-associated mediators were measured.

Results We found that PLTs count was higher in NSCLC patients at stage IV than earlier stages. These PLTs showed higher levels of PD-L1 than early stages. Moreover, although not in a statistical manner, ICI-non responder NSCLC patients had slightly higher levels of PD-L1. No differences were found in terms of FcγRIII (CD64), FcγRI (CD32) and FcγRI (CD16) expression on isolated PLTs either at baseline or after treatment. In order to better understand whether drug treatment with atezolizumab could alter PLTs activity, the isolated cells were treated in vitro with the mAb (1 μg/mL) to evaluate the release of mediators associated with PLTs activation. We found that differently than healthy PLTs, the stimulation of PLTs derived from NSCLC non-treated patients with Atezolizumab for 30 minutes induced the release of platelet factor 4 (CXCL4). Similarly, the challenge with Atezolizumab was able to trigger the release of TGF-β from NSCLC-derived PLTs after 5 hours of treatment; nevertheless, we observed that PLTs collected from patients who did not respond to treatment (PD) secreted higher amount of TGF-β at earlier time point (1 hour after Atezolizumab addition).

Conclusions Currently simple and robust biomarkers to predict therapy responses towards ICIs are still missing. Our data suggest that the PLTs expression of PD-L1 at PD may reflect the reduced drug efficacy due to the interaction and binding of mAbs (such as Atezolizumab) to the surface PD-L1 by limiting the drug which is not able to exhibit its pharmacological activity on tumor tissues or innate tumor-infiltrated immune cells.

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P06 Cell therapy in solid tumors

**P06.01 ROR1-CAR T-CELLS AS NOVEL TREATMENT STRATEGY FOR ANAPLASTIC THYROID CARCINOMA**

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Background Anaplastic thyroid carcinoma (ATC) and poorly differentiated thyroid carcinoma (PDTC) are rare thyroid malignancies with an extremely poor prognosis. Current therapies include operation, radiochemotherapy and kinase inhibitors, but finally most patients relapse and require further treatments. Thus, new therapeutic strategies are needed. CAR-T (chimeric antigen receptor) therapy is a modern approach to manipulate and target the patient’s own T cells against a specific marker on tumor cells. The Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1) is an essential transmembrane receptor during embryonic development and is associated with the non-canonical Wnt signaling pathway. ROR1 is barely expressed in adult healthy tissue, but can be re-upregulated on specific tumor cell types. In this study we investigated ROR1 as a potential target for CAR T-cell therapies in thyroid carcinoma.

Materials and Methods Initially, the expression of the ROR1 receptor was determined in primary ATC/PDTC and compared to non-malignant thyroid tissue. ROR1 mRNA expression on ATC cell lines was correlated with ROR1 surface expression.

Disclosure Information None.
as measured by flow cytometry. The efficiency of ROR1 directed CAR T-cell lysis was tested by co-incubating ATC cells together with ROR1-CAR T-cells in 2D cultures and 3D spheroid models and untransduced T-cells served as controls. To exclude cell line-based effects and to prove that the CAR T-cells specifically target ROR1-expressing cells, CRISPR/Cas9 was used to knock out ROR1 in three ROR1-positive ATC cell lines. Cytobead arrays were used to determine CAR T-cell activity.

Results Our data demonstrates an overexpression of ROR1 in ATC/PDTC compared to non-malignant thyroid tissue. In addition, three different ATC cell lines showed overexpression of ROR1, and we could show that pre-treatment with kinase inhibitors like Lenvatinib or Sorafenib does not affect ROR1 surface expression. ROR1-positive ATC cell lines were efficiently lysed by ROR1-CAR T-cells in different 2D- and 3D cell culture models, while untransduced T-cells had no effect. The cytokine profile including IFN-γ, TNF-α and GM-CSF showed a specific activation of ROR1-CAR T-cells in the presence of ROR1 positive ATC cell lines. ROR1 knock out ATC cells were not affected by ROR1-CARTs and did not induce cytokine activation, thus proofing specificity for the target. Animal models are ongoing and will be finalized at presentation.

Conclusions In summary our data proof ROR1 as a viable and specific target for several types of thyroid carcinoma which is the basis for the design of a clinical phase I trial using ROR1-CAR T-cells in metastasized ATC and PDTC patients.


Materials and Methods Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donoruffy-coats by density gradient centrifugation using Ficoll-Paque PLUS and activated with a polymeric nanomatrix agonist for CD3 and CD28 receptor. Transduction media has demonstrated to influence CAR-transduction efficacy. Moreover, differences in transduction-media have demonstrated to influence CAR-transduction efficacy. Overall, the present work provides insight on relevant experimental parameters that impact anti-nucleolin CAR-transduction in human T cells.

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References


Background Triple-negative breast cancer (TNBC) is an aggressive cancer with low survival rates, still seeking for efficient targeted therapies. Genetic modification of human T cells to express chimeric antigen receptors (CAR-T cells) redirects T cells activity towards pre-established tumor-associated antigens (TAAs).1. Despite highly successful against hematological malignancies, CAR-T cells efficacy against solid tumors has been limited, owing to lack of accessible TAAs and tumor microenvironment (TME)-mediated immunosuppression.2 In this context, nucleolin, a membrane-nucleus shuttling phosphoprotein, has been demonstrated to be overexpressed on both TNBC cancer and endothelial cells from angiogenic blood vessels,3 emerging as an accessible target. In this respect, this work focuses on the first steps of anti-nucleolin CAR-T cells manufacturing, aiming at optimizing lentiviral-based CAR-transduction conditions.

Background CAR-T cell-based therapies hold great promise for neuroblastoma, because of its potential for targeted effects and already promising results in adult solid cancers. However, neuroblastoma tumour cells can escape T cell mediated killing by inhibition of T cells through secretion of immunosuppressive factors. Here, we have identified Macrophage Migration Inhibitory Factor (MIF) and Midkine (MDK) by scRNA-seq analysis to have a negative effect on the cytotoxicity of T cells. Next to that, we have found both MIF and MDK in high abundance in the secretome of neuroblastoma patient derived organoids. These factors have a suppressive effect on T cells and blocking their function might increase the efficacy of T cell based therapies for neuroblastoma.

Materials and Methods To study immunoregulatory interactions in neuroblastoma, single-cell RNA-sequencing of 25 tumours