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.


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**Materials and Methods** Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donor buffy-coats by density gradient centrifugation using Ficoll-Paque PLUS and activated with a polymeric nanomatrix agonist for CD3 and CD28 T cell receptors, in IL-2-containing culture medium. Activated PBMCs were transduced with produced CAR-encoding lentivirus - transient transfection of 293ET cells with different ratios of CAR-transgene, packaging, rev and envelope plasmids - either in flat- or round-bottom 96-well plates, in the presence or absence of the activation reagent. Transduction efficacy in T cells was assessed by both tdTomato fluorescence (reporter gene) and CAR surface expression through flow cytometry.

**Results** The transgene: packaging:rev:envelope plasmid ratio enabling the highest transduction rate - 50% of both tdTomato and surface CAR expression - was chosen for further CAR-transductions. The presence of T-cell-activating nanomatrix in transduction-media has demonstrated to influence CAR-transduction efficacy. Moreover, differences in tdTomato fluorescence levels between T cells transduced in flat- or round-bottom microwells were also observed.

**Conclusions** 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**


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**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 (TAA)s.1 Despite highly successful against hematological malignancies, CAR-T cells efficacy against solid tumors has been limited, owing to lack of accessible TAA 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.