Background Cholangiocarcinoma (CCA) is a biliary epithelial tumor with poor prognosis, for which there are not effective therapeutic options in advanced stages. Innovative therapeutic strategies are highly needed. Cellular immunotherapy holds great promise for the treatment of solid tumors. Among various cellular immunotherapy approaches, our group contributed to the development and testing of Cytokine Induced Killer lymphocytes (CIK) endowed with intrinsic MHC-unrestricted tumor killing activity. Recently, it was reported that CIK may be engineered with Chimeric Antigen Receptors (CARs) generating immune effectors with double antitumor potentiality, strategically important to counteract the frequent heterogeneity of tumor antigen expression. To this end, in the context of CCA mesothelin (MSLN) is emerging as a promising CAR target.

Here, we hypothesized to generate a novel and effective cell therapy strategy against CCA redirecting patient-derived CIK, with a CAR against MSLN acting as relevant CCA target.

Methods MSLN-CAR.CIK were generated from patients’ PBMC and were transduced with a lentiviral vector encoding the second generation anti-MSLN CAR including the co-stimulatory domain 4BB. CAR expression and extended phenotype of mature CAR-CIK were assessed by flow cytometry. The expression of MSLN, were assessed in CCA cell lines and CCA surgical specimens. In parallel, target molecules recognized by CIK (MICA/B, ULBPs) were evaluated.

Tumor killing in 2D models was evaluated at different effector:target ratio by flow-cytometry and bioluminescence-based essays. In order to increase the complexity of CCA models 3D tumor spheroids were generated from different CCA cells bearing a reporter gene (RFP) and co-incubated with effector cells at ratio 2:1. Fluorescence images were acquired at different times using fluorescence microscopy.

Results We successfully generated MSLN-CAR.CIK from peripheral blood of tumor patients ($n=5$) (figure 1A). CAR.CIK immunophenotype was comparable to unmodified controls (NTD.CIK): (42±5)% CAR+, (49±8)% CD3+CD56+, (77±6)% CD3+CD56+ and (83±7)% NKG2D+. We found high (>90%) membrane expression of MSLN in 6/7 CCA cell lines with high (>90%) membrane expression of MSLN in 6/7 CCA cell lines that were all effectively killed by MSLN-CAR.CIK in 2D assays. The observed tumor lysis was significantly enhanced ($n=12$) compared to NTD.CIK: 80% vs 30% (E/T 2:5:1), 70% vs 20% (E/T 1:2, $p<0.0001$) (figure 1B). The intense activity, along with tumor infiltration, by MSLN-CAR.CIK was observed also against CCA 3D spheroids.

Conclusions We report that MSLN-CAR.CIK effectively targets CCA cells in both 2D assays as well as in 3D models. Our findings provide translational bases to support clinical cellular immunotherapy studies with MSLN-CAR.CIK in the challenging field of advanced CCA.

REFERENCES