

## NOVEL CELLULAR IMMUNOTHERAPY WITH ANTI-MESOTHELIN CAR-KILLER LYMPHOCYTES AGAINST ADVANCED CHOLANGIOCARCINOMA

<sup>1,2</sup>Annamaria Massa\*, <sup>3</sup>Valeria Leuci, <sup>2</sup>Federica Galvagno, <sup>2</sup>Chiara Donini, <sup>2</sup>Francesca Vita, <sup>2</sup>Ramona Rotolo, <sup>2</sup>Alessia Proment, <sup>4</sup>Letizia Vitali, <sup>3</sup>Elisabetta Fenocchio, <sup>2</sup>Elisa Vigna, <sup>3</sup>Giuliana Cavalloni, <sup>5</sup>Massimo Aglietta, <sup>2</sup>Dario Sangiolo. <sup>1</sup>University of Turin, Orbassano (Torino), Italy; <sup>2</sup>University of Turin, Turin, Italy; <sup>3</sup>Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy; <sup>4</sup>University of Turin, Candiolo, Italy; <sup>5</sup>Università di Turin, Turin, Italy

**Background** Cholangiocarcinoma (CCA) is a biliary epithelial tumor with poor prognosis, for which there are not effective therapeutic options in advanced stages.<sup>1</sup> Innovative therapeutic strategies are highly needed. Cellular immunotherapy holds great promise for the treatment of solid tumors.<sup>2</sup> 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.<sup>3</sup> Recently, it was reported that CIK may be engineered with Chimeric Antigen Receptors (CARs) generating immune effectors with double antitumor potentiality,<sup>4,5</sup> 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.<sup>6,7</sup>

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 for the second generation anti-MSLN CAR including the co-stimulatory domain 4-1BB. 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 assays. 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+CD8+ and (83±7)% NKG2D+. We found 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.

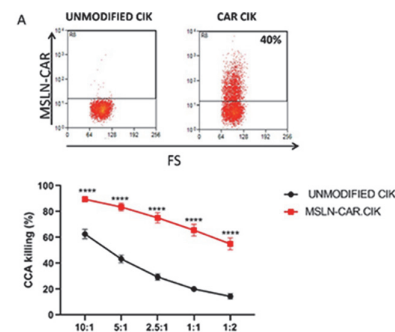
**Conclusions** We report that MSLN-CAR.CIK effectively targets CCA cells in both 2D assays as well as in 3D models. Our findings provide translational bases to support clinical cellular immunotherapy studies with MSLN-CAR.CIK in the challenging field of advanced CCA.

## REFERENCES

1. Brindley PJ, Bachini M, Ilyas SI, Khan SA, Loukas A, Sirica AE, Teh BT, Wongkham S, Gores GJ. Cholangiocarcinoma. *Nat Rev Dis Primers*. 2021 Sep 9;7(1):65.

- Menon S, Shin S, Dy G. Advances in Cancer Immunotherapy in Solid Tumors. *Cancers (Basel)*. 2016 Nov 24;8(12):106.
- Giraudo L, Gammaitoni L, Cangemi M, Rotolo R, Aglietta M, Sangiolo D. Cytokine-induced killer cells as immunotherapy for solid tumors: current evidence and perspectives. *Immunotherapy*. 2015;7(9):999–1010.
- Leuci V, Donini C, Grignani G, Rotolo R, Mesiano G, Fiorino E, Gammaitoni L, D'Ambrosio L, Merlini A, Landoni E, Medico E, Capellero S, Giraudo L, Cattaneo G, Iaia I, Pignochino Y, Basirico M, Vigna E, Pisacane A, Fagioli F, Ferrone S, Aglietta M, Dotti G, Sangiolo D. CSPG4-Specific CAR.CIK Lymphocytes as a Novel Therapy for the Treatment of Multiple Soft-Tissue Sarcoma Histotypes. *Clin Cancer Res*. 2020 Dec 1;26(23):6321–6334.
- Leuci V, Casucci GM, Grignani G, Rotolo R, Rossotti U, Vigna E, Gammaitoni L, Mesiano G, Fiorino E, Donini C, Pisacane A, Ambrosio LD, Pignochino Y, Aglietta M, Bondanza A, Sangiolo D. CD44v6 as innovative sarcoma target for CAR-redirection CIK cells. *Oncoimmunology*. 2018 Feb 15;7(5):e1423167.
- Mandrekar P, Cardinale V. Periostin and mesothelin: Potential predictors of malignant progression in intrahepatic cholangiocarcinoma. *Hepatol Commun*. 2018 Apr 25;2(5):481–483.
- Yu L, Feng M, Kim H, Phung Y, Kleiner DE, Gores GJ, Qian M, Wang XW, Ho M. Mesothelin as a potential therapeutic target in human cholangiocarcinoma. *J Cancer*. 2010 Oct 1;1:141–9.

**Ethics Approval** We received approval for collection of patient samples and the associated informed consent document from the Institutional Review Board (IRB) per Declaration of Helsinki guidelines (Prot. Number 225/2015).



**Figure 1.** Generation and anti-CCA activity of CAR.MSLN CIK. A) Representative flow-cytometry showing that patient-derived MSLN-CAR.CIK. B) in vitro MSLN-CAR.CIK intensely killed CCA cells even at very low effector/target ratio (p<0.0001 as compared with controls).

**Abstract 190 Figure 1** Generation and anti-CCA activity of CAR.MSLN CIK. A) Representative flow-cytometry showing that patient-derived MSLN-CAR.CIK. B) in vitro MSLN-CAR.CIK intensely killed CCA cells even at very low effector/target ratio (p<0.0001 as compared with controls)

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0190>