

**N-GLYCOSYLATION INHIBITION HINDERS  
IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT  
CELLS IMPROVING CAR T CELL EFFICACY**

<sup>1</sup>Camilla Sirini\*, <sup>1</sup>Beatrice Greco, <sup>1</sup>Chiara Balestrieri, <sup>1</sup>Barbara Camisa, <sup>1</sup>Elena Tiziano, <sup>1</sup>Laura Falcone, <sup>1</sup>Rita El Khoury, <sup>1</sup>Oronzina Botrugno, <sup>1</sup>Giovanni Tonon, <sup>1</sup>Miriam Redegalli, <sup>1</sup>Federica Pedica, <sup>1</sup>Alice Bergamini, <sup>2</sup>Fabio Ciceri, <sup>1</sup>Chiara Bonini, <sup>1</sup>Monica Casucci. <sup>1</sup>IRCCS San Raffaele Scientific Institute, Milan, Italy; <sup>2</sup>Hematology and Bone Marrow Transplant Unit – San Raffaele Hospital, Milan, Italy

**Background** Adoptive transfer of CAR T cells demonstrated impressive results against B-cell malignancies, but still limited efficacy against solid tumors. In this context, multiple challenges need to be overcome, including poor tumor recognition and strong immunosuppression within the tumor microenvironment (TME). Our Unit has recently reported that pharmacological inhibition of N-glycan synthesis in cancer cells increases CAR T cell efficacy by improving tumor recognition and preventing T cell exhaustion. In this project, we investigated the role of N-glycosylation blockade on TME cells in the context of colorectal cancer (CRC) and pancreatic adenocarcinoma (PDAC)-derived liver metastases and CEA-specific CAR T cell therapy.

**Methods** To understand the effect of N-glycosylation blockade on TME cells (both M2-macrophages, M2-M and Hepatic stellate cells, HepSCs), we analyzed the phenotypic and transcriptional profile and we performed in vitro functional assays, such as tripartite co-cultures, suppressive assays and released-cytokines analysis. Moreover, to evaluate the effect of N-glycosylation inhibition on TME cells in vivo, we exploited immunodeficient mice reconstituted with a human immune system (huSGM3), engrafted intra-hepatically with tumor cells and treated with CEA CAR T cells.

**Results** In vitro studies revealed that N-glycosylation inhibition abolishes the ability of both TME cells to restrain T cell proliferation and increases the elimination of cancer cell lines and patient-derived tumor organoids (PDOs from CRC-liver metastases). Interestingly, these effects were associated with profound phenotypic and transcriptional changes in M2-M and HepSCs. In particular, the treatment was able to inhibit M2-polarization in terms of surface markers expression, IL-10 secretion and gene expression profile, and was shown to hinder the activation of HepSCs and inhibit the PD-1/PDL-1 axis. Interestingly, in the in vivo model, the presence of human immune cells supports CAR T cell responses and helps recreate an immune TME more representative of the human disease. Importantly, using these mice we observed that N-glycosylation inhibition increases CEA CAR T cell antitumor activity, in terms of survival, and this is associated with the downregulation of immunosuppressive genes in tumor-infiltrating human immune cells.

**Conclusions** Overall, these data suggest that blocking N-glycosylation can help overcome multiple barriers that currently limit CAR T cell efficacy in solid tumors, acting not only on tumor cells, but also on immunosuppressive tumor microenvironment cells.

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