

CEACAM6 CAR-T antitumor efficacy in pancreatic cancer treatment

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INTRODUCTION

Pancreatic cancer is characterized by the lowest survival rate of all cancers in Europe, with estimated over 95,000 deaths every year. With the current therapeutic approaches, the median survival time after the diagnosis is 4.6 months. Immunotherapies with checkpoint inhibitors that have emerged as a novel therapeutic option in many types of malignancies, have shown overall very weak efficacy in pancreatic cancer patients. Therefore, development of effective therapies still remains crucial. Adoptive transfer of T lymphocytes expressing chimeric antigen receptors (CAR) is currently considered the most promising anti-cancer therapeutic available to the patients. To generate the appropriate cell therapy product, T cells are collected from patient peripheral blood and redirected to a specific antigen via viral expression of a CAR. This therapy is currently used in the treatment of acute leukemias and B-cell malignant lymphomas. Clinical trials are ongoing to assess CAR-T effectiveness in other hematological cancers, as well as in solid tumors. The latter, however, remains challenging.

In our approach, we target carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6). CEACAM6 is a cell surface protein that is overexpressed in a wide variety of human cancers, including pancreatic adenocarcinoma, and is associated with tumorigenesis, cancer cell adhesion, invasion and metastasis. CEACAM6 Chimeric Antigen Receptor T-cells (CEACAM6 CAR-T) were generated using a single-domain camelid monoclonal 2A3 antibody against human CEACAM6.

RESULTS

CEACAM6 CAR expression in transduced T cells

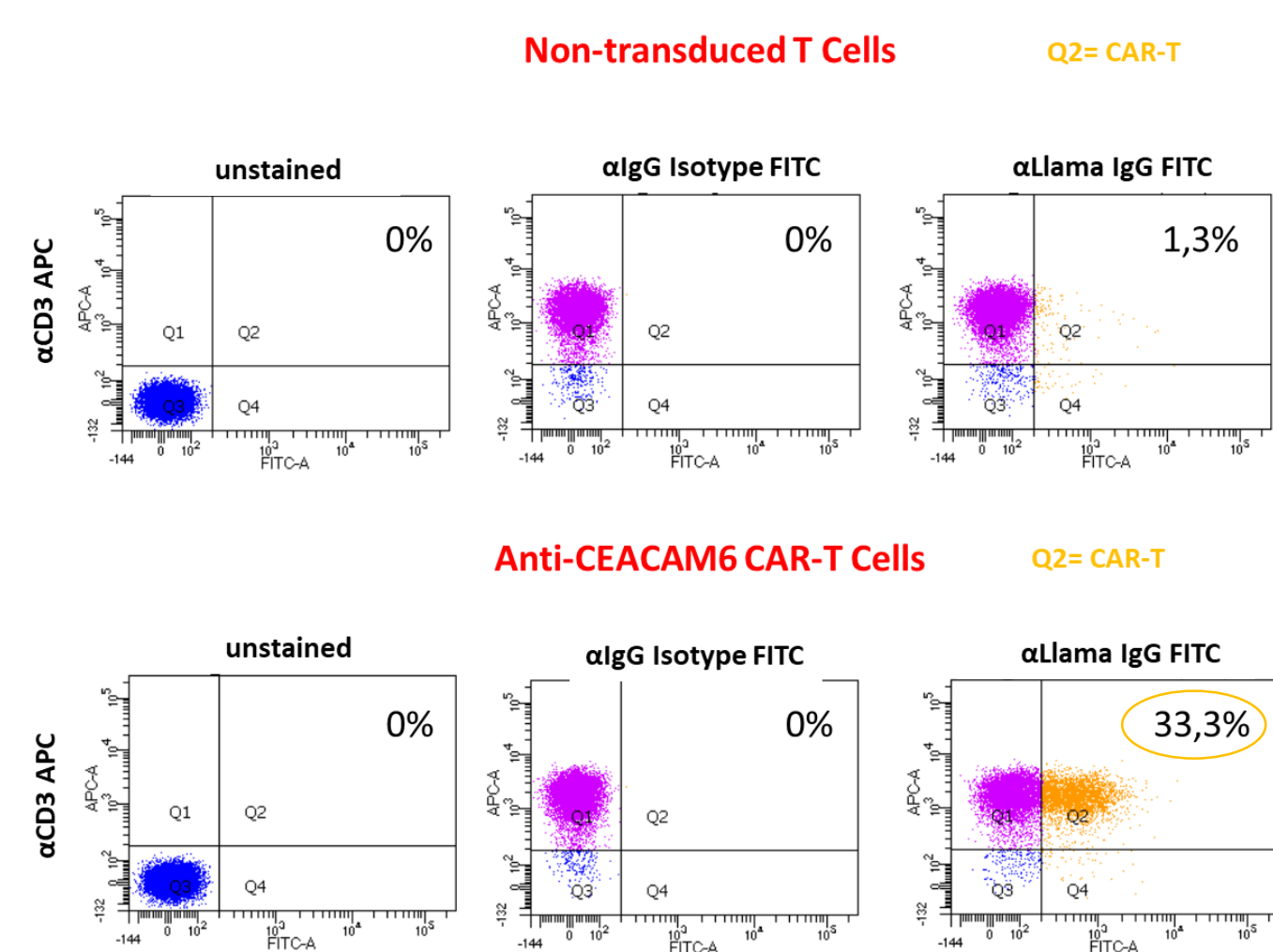


Fig.1. T-cells were activated with CD28/CD3 Dynabeads and expanded in presence of the IL-2. Within 24 hours, cells were transduced with a lentivirus expressing the anti-CEACAM6-CD28-CD3 construct. Flow cytometry was performed after 8 days of cells transduction. Cells were stained with anti-CD3 antibody conjugated to APC to specify T-cells and with anti-llama IgG antibody or its isotype control to evaluate the percentage of cells expressing the 2A3 CEACAM6 antibody. Production efficiency is approximately 30%.

Cytokine release upon CAR-T cells interaction with CEACAM6+ target cells

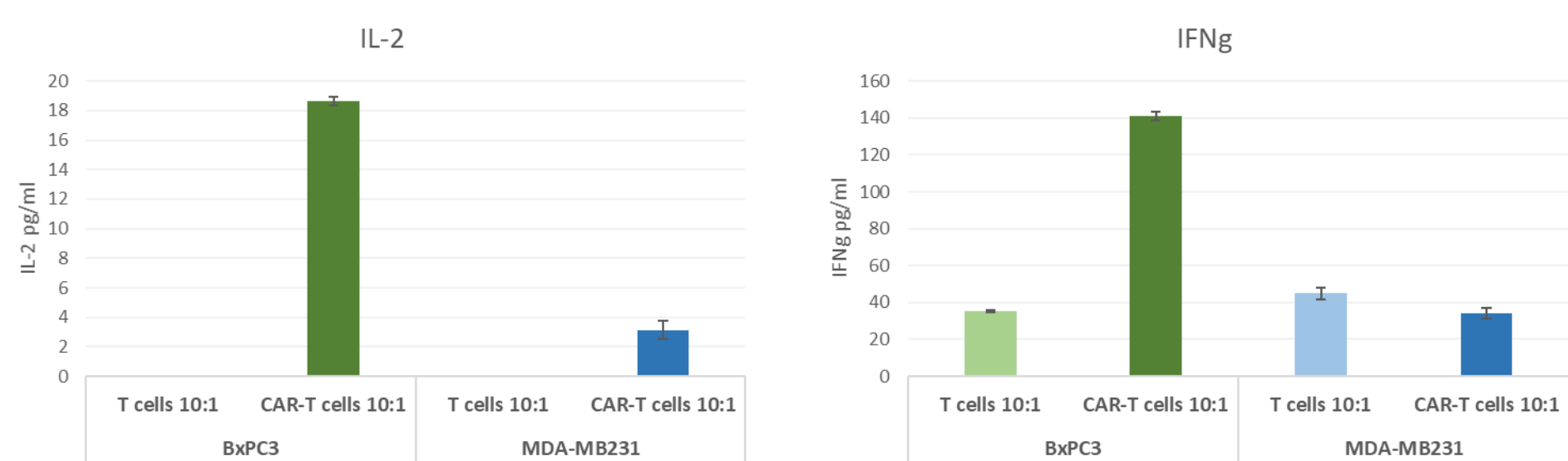


Fig.2. CEACAM6 CAR-T and T-cells were expanded for 14 days before use. BxPC3 (CEACAM6+) and MDA-MB231 (CEACAM6-) cells were seeded on 96-well plate. After 24 hours CAR-T or T-cells were added at a 10:1 (E:T) ratio. Cells were co-cultured for approximately 24 hours before removal of supernatant. IL-2 and IFN γ levels in the medium were determined by ELISA. IL-2 and IFN γ release increases when CEACAM6 CAR-T cells were co-cultured with CEACAM6+ BxPC3 cells.

CEACAM6 CAR-T mediated cell cytotoxicity

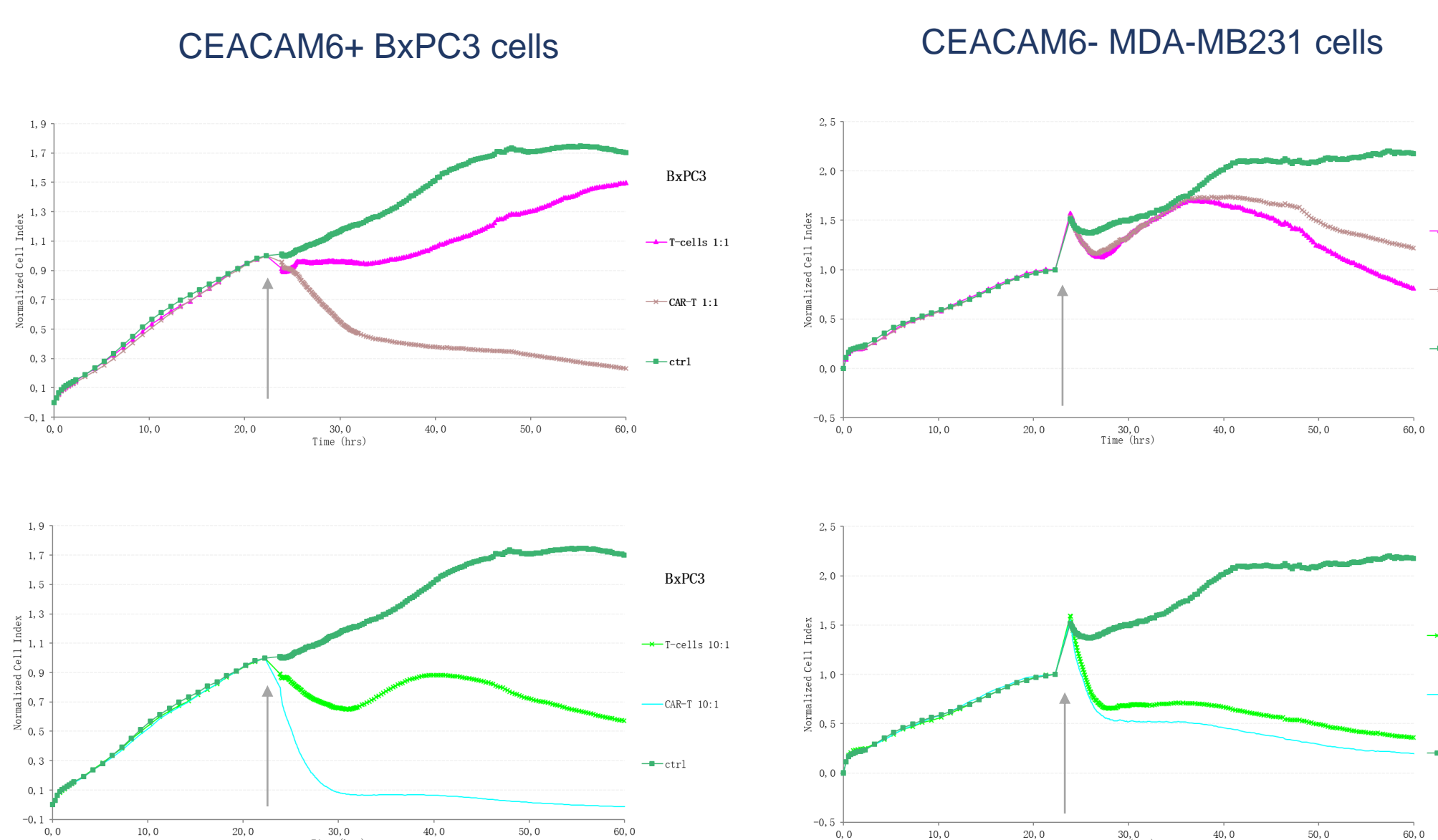


Fig.3. CEACAM6 CAR-T and T-cells were expanded for 8 days before use. Target cells: BxPC3 and MDA-MB231 were seeded on 96-well plate and incubated for 24 hours before addition of effector cells: CAR-T or unmodified T cells 1:1 or 10:1 (E:T) ratio. Cell viability was evaluated using the Real Time Cell Analysis (RTCA) system from ACEA Biosciences. CEACAM6 CAR-T cells in contrast to unmodified T-cells significantly decreased the viability of CEACAM6+ BxPC3 cells. Effect of CEACAM6 CAR-T cells on CEACAM6 negative MDA-MB231 cells was similar to unmodified T-cells.

CEACAM6 CAR-T antitumor efficacy in BxPC3 xenograft model

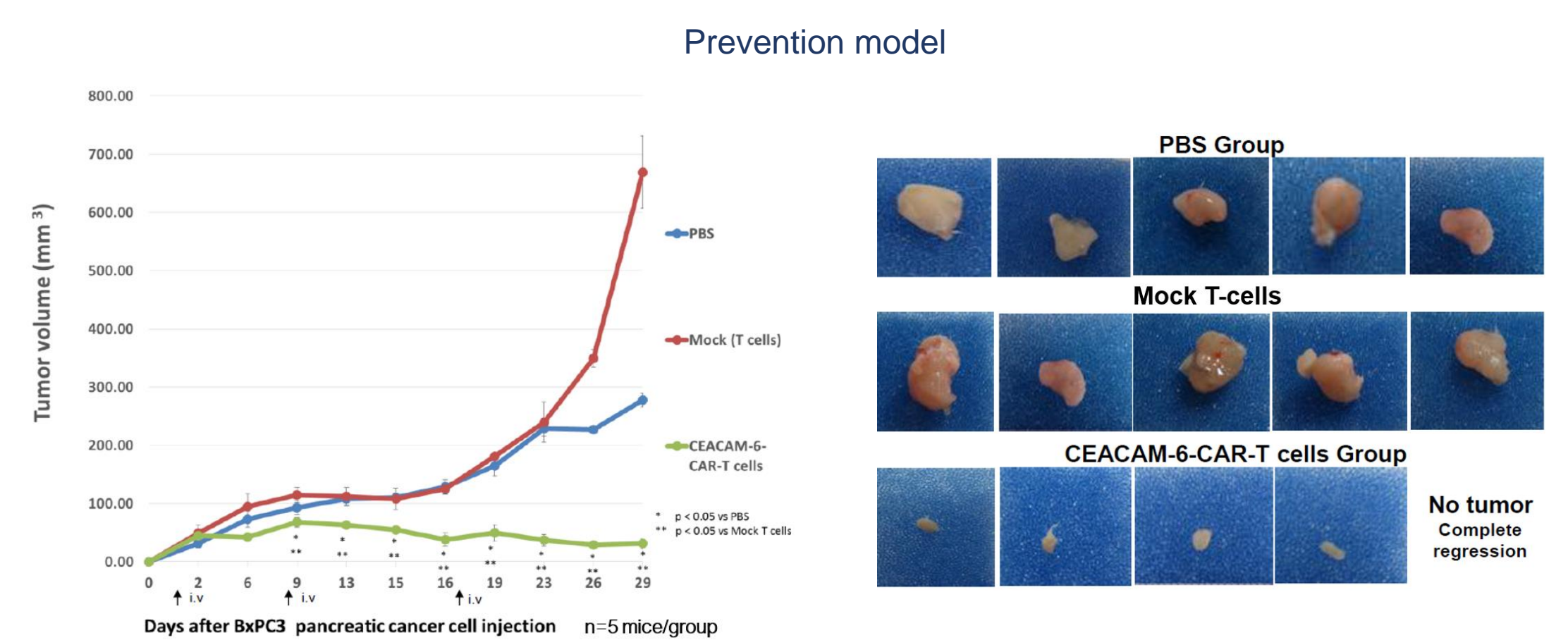


Fig.4. BxPC3 cells (2×10^6 cells/mouse) were injected subcutaneously into the hind flank of CIEA NOG female mice. Mice were treated with either PBS, untransduced T cells (mock T cells) or CEACAM6 CAR-T cells intravenously into the tail vein on days 1, 8 and 15 after tumor injections. Tumor size was measured with calipers and tumor volume was calculated using the formula $(\text{length} \times \text{width}^2)/2$. Treatment with CEACAM6 CAR-T cells significantly decreased the growth of the BxPC3 xenografts (ANOVA performed on day 30 data shows $p=0.0212$ for PBS vs CAR-T cells and $p<0.0001$ for mock T cells vs CAR-T cells). Right panel shows tumors excised at the end of the study.

Therapeutical model

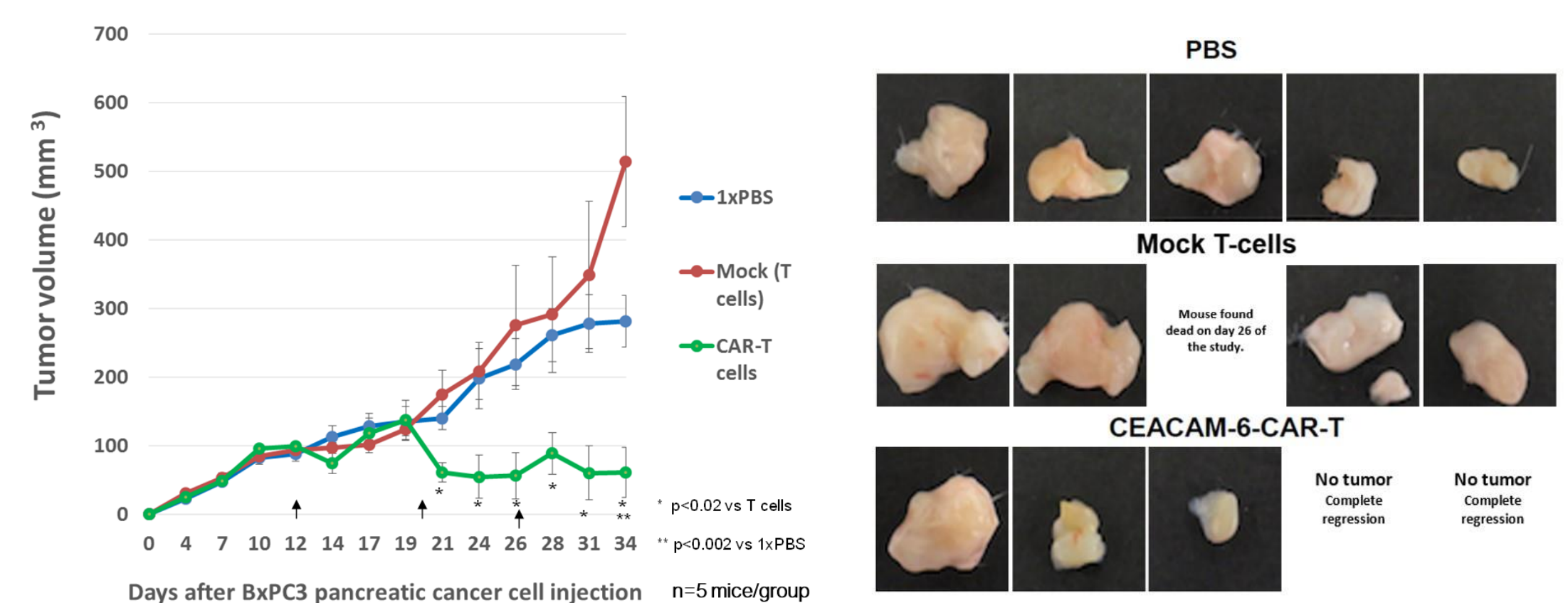


Fig.5. CEACAM-6 CAR-T cells significantly decreased BxPC3 xenograft tumor growth *in vivo*. Arrows indicate day of injection (days 12, 20, 26). The p-values were calculated and on day 34, the difference was significant with $p<0.002$ in CAR-T group versus PBS and $p<0.02$ in CAR-T group versus Control Mock T cell group. Right panel shows tumors excised at the end of the study. A significant reduction in tumor volume was observed in CEACAM-6 CAR-T treated mice and tumors in 2 animals were completely eliminated.

CONCLUSIONS

- CAR-T cells targeting CEACAM6 antigen are highly effective in reducing cell viability of the CEACAM6-expressing pancreatic cancer cell line BxPC3 *in vitro*
- CEACAM6 CAR-T cells significantly reduce the growth of the BxPC3 tumor *in vivo* both when used in a prevention model and in a model where treatment was initiated after the tumor had been established
- Camelid single chain antibodies can be easily adopted for use in CAR-T therapies

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