I.P. vs I.V. CAR-T Treatment For Epithelial Ovarian Cancer

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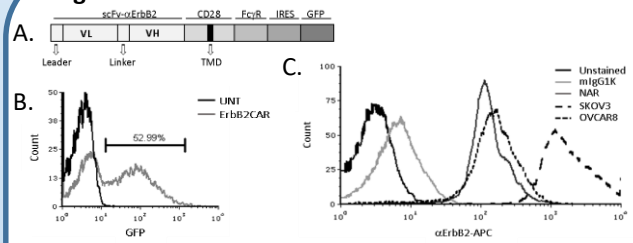
Abstract

Epithelial ovarian cancer is often diagnosed at advanced stages due to intraperitoneal spread. While significant progress in surgical and chemotherapeutic treatments for EOC, the survival rates for this disease have only modestly improved. Chimeric antigen receptor (CAR) T cells enable T cells to directly bind tumor-associated antigens in an MHC-independent manner inducing tumor rejection. While CAR-T cell therapy shows great promise in hematological malignancies, its implication for solid tumors is far more difficult. Innovative approaches are therefore needed to increase the specificity of CAR modified T cells exclusively against tumors. The aim of this study was to assess the efficacy and safety of I.P. (intra peritoneal) *versus* I.V. (intra venous) CAR-T cell therapy in ovarian mouse model.

We have constructed CAR targeting ErbB2 (ErbB2CAR) that is overexpressed in epithelial ovarian cancers. It is composed an external anti-ErbB2-specific scFv followed by internal activating domain of human FcγR domain and human CD28 costimulatory domain. We evaluated the functionality of ErbB2CAR *in-vitro*, and found that the ErbB2CAR secreted IFNγ (500-40,000pg/ml) and showed high killing potential after stimulation with ovarian cell lines (OVCAR8, SKOV3 or NAR). In *in vivo* assay, we have found that treatment with *I.P.* injection of ErbB2CAR cells to treat tumor bearing mice with NAR-LUC lead to remission and increased survival compared to the *I.V.* treatment. Moreover, we have found that *I.P.* ErbB2CAR cells were less circulating thus safer to non-tumor tissues compared to the I.V. treatment.

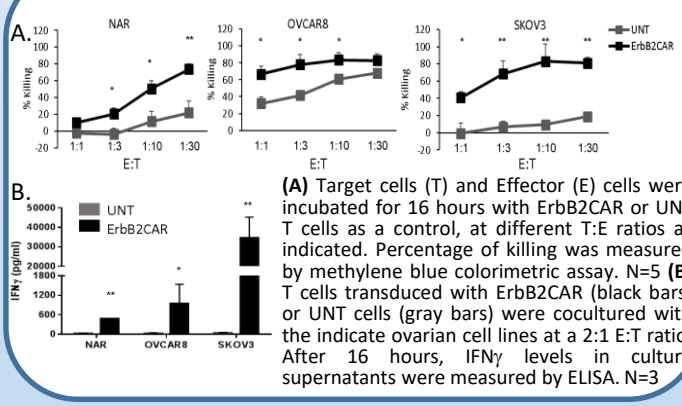
Results

Figure 1: *In-vitro* ErbB2-CAR characterization:



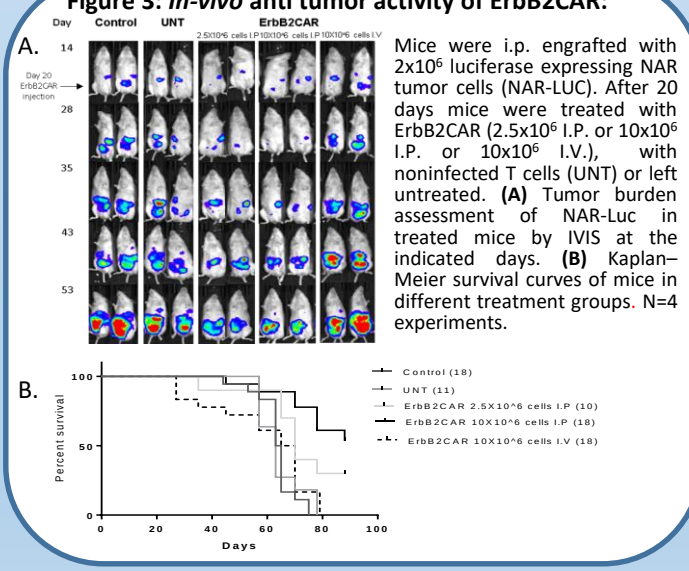
(A) ErbB2CAR construct is comprised of an scFv specific for ErbB2 followed by the costimulation domain of CD28 and activation domain from FcγR. Follwed by GFP protein. **(B)** Flow cytometry analysis of lymphocytes transduced with ErbB2CAR-GFP using Retroviral infection **(C)** Expression of ErbB2 antigen on human ovarian cancer cell lines NAR, OVCAR8 and SKOV3. Isotype control-mouse IgG1-APC.

Figure 2: *In-vitro* activity of ErbB2CAR T cells:



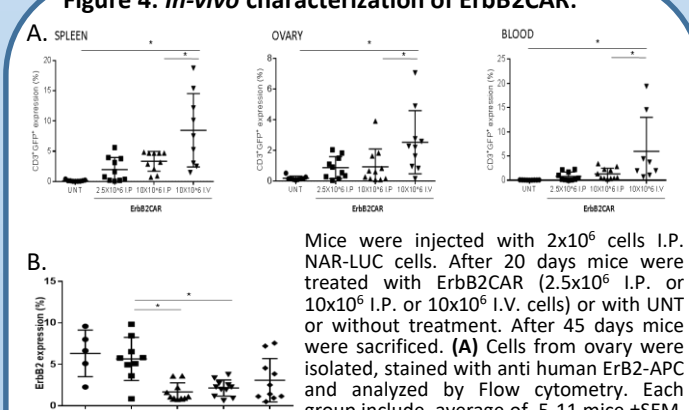
(A) Target cells (T) and Effector (E) cells were incubated for 16 hours with ErbB2CAR or UNT T cells as a control, at different T:E ratios as indicated. Percentage of killing was measured by methylene blue colorimetric assay. N=5 **(B)** T cells transduced with ErbB2CAR (black bars) or UNT cells (gray bars) were cocultured with the indicate ovarian cell lines at a 2:1 E:T ratio. After 16 hours, IFNγ levels in culture supernatants were measured by ELISA. N=3

Figure 3: *In-vivo* anti tumor activity of ErbB2CAR:



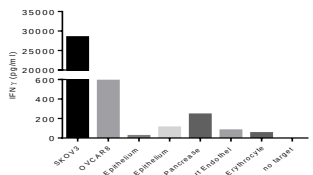
Mice were i.p. engrafted with $2x10^6$ luciferase expressing NAR tumor cells (NAR-LUC). After 20 days mice were treated with ErbB2CAR ($2.5x10^6$ I.P. or $10x10^6$ I.P. or $10x10^6$ I.V.), with noninfected T cells (UNT) or left untreated. **(A)** Tumor burden assessment of NAR-Luc in treated mice by IVIS at the indicated days. **(B)** Kaplan–Meier survival curves of mice in different treatment groups. N=4 experiments.

Figure 4: *In-vivo* characterization of ErbB2CAR:



Mice were injected with $2x10^6$ cells I.P. NAR-LUC cells. After 20 days mice were treated with ErbB2CAR ($2.5x10^6$ I.P. or $10x10^6$ I.P. or $10x10^6$ I.V. cells) or with UNT or without treatment. After 45 days mice were sacrificed. **(A)** Cells from ovary were isolated, stained with anti human ErB2-APC and analyzed by Flow cytometry. Each group include average of 5-11 mice ±SEM. * P<0.05 1-Way ANOVA test. **(B)** Cells from spleen ovary and blood were collected and stained with anti human CD3 and analyzed by Flow cytometry. Each group include average of 8-11 mice ±SEM. * P<0.05 One-Way ANOVA test.

Figure 5: Safety analysis of ErbB2CAR.



Primary cells from different tissues of healthy human donors were co-cultured with lymphocyte T cells transduced with ErbB2CAR at a 2:1 E:T ratio for 24 hours. Supernatants were collected, and secreted IFN-γ was measured by ELISA. For positive control we used SKOV3 and OVCAR8 cells.

Conclusion:

Our results are a proof of concept which demonstrates the advantages of CAR T cells administered *I.P.* and offers a safe strategy with has a clinical potential for the treatment of ovarian cancer.