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Supplemental Data

Flexible Targeting of ErbB Dimers That Drive Tumorigenesis by Using Genetically Engineered T Cells

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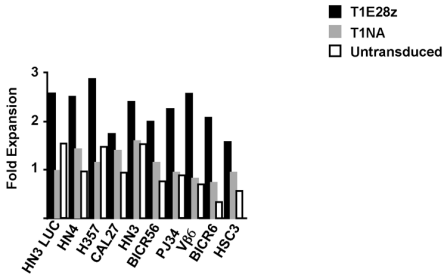
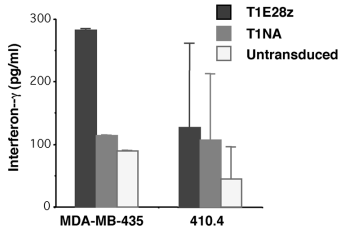
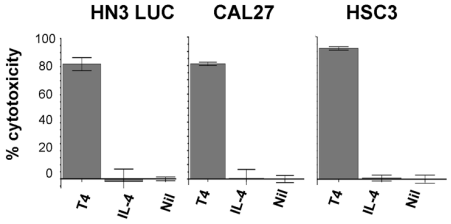


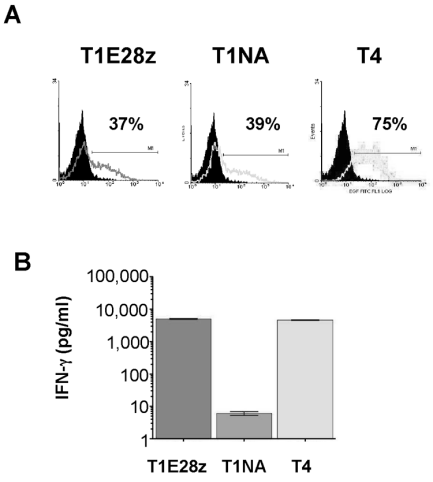
Supplementary Figure 1. Proliferation of T1E28z-engrafted T-cells following activation by head and neck tumor cell monolayers. T-cells engineered to express the indicated CARs were co-cultivated with specified monolayers and propagated in IL-2. Fold T-cell expansion was evaluated after 7 days.



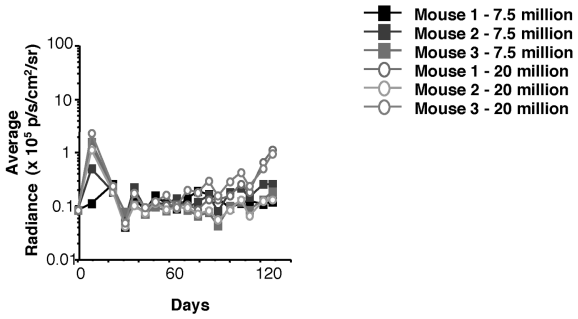
Supplementary Figure 2. T1E28z^{+} T-cells are poorly activated by MDA-MB-435 and 410.4 tumor cell monolayers. Human T-cells engineered to express T1E28z were co-cultivated with ErbB^{lo} 410.4 or ErbB3^{+} ErbB2^{lo} MDA-MB-435 tumor cell monolayers. Untransduced and T1NA-transduced T-cells served as controls. Supernatants were harvested after 72 hours and analyzed for IFN-γ (mean + SD; n=30 (5 independent experiments; 410.4) and n=15 (2 independent experiments; MDA-MB-435)).



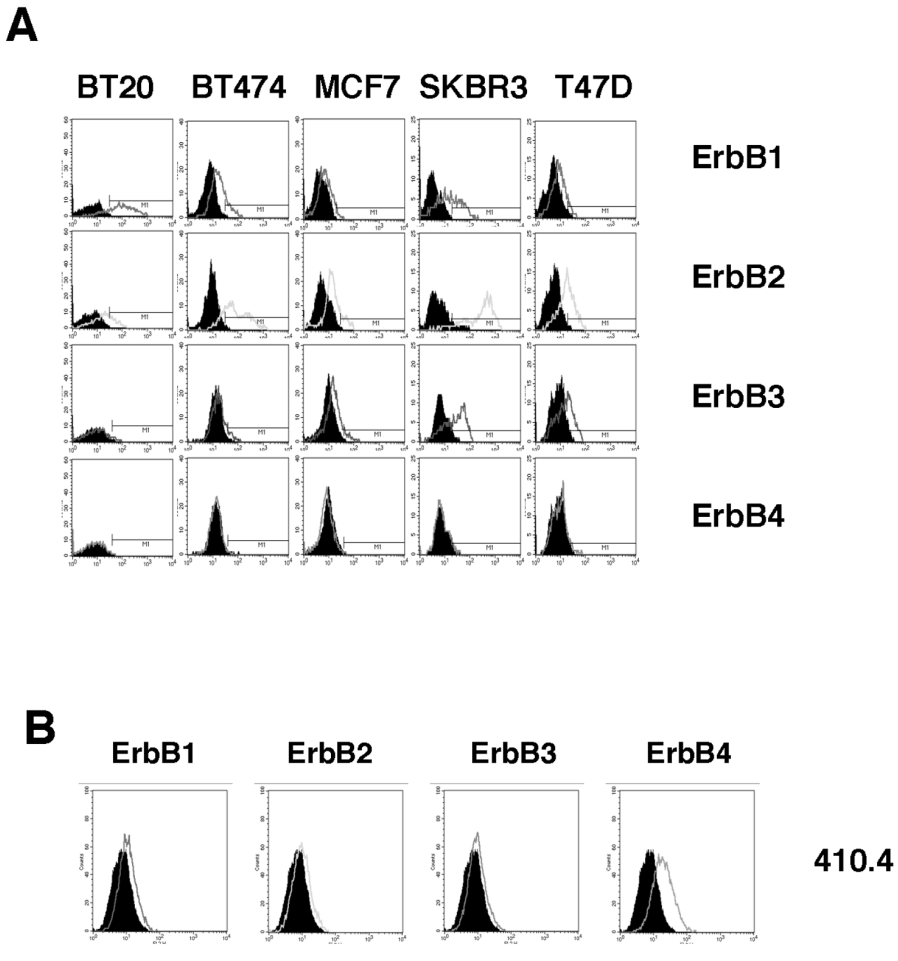
Supplementary Figure 3. T4^{+} T-cells kill head and neck tumor cells. 1 x 10^{6} T4^{+} T-cells were co-cultivated with a confluent monolayer (24-well plate – 1.9cm^{2}) of HN3 luciferase (LUC) and CAL27 cells for 48 hours or HSC monolayers for 72 hours. Cultures were supplemented with IL-4 (30ng/ml). Control cultures were treated with IL-4 alone or with nil. After the specified interval, T-cells were removed and residual viable tumor cells were quantified using XTT (mean + SD, n=2-4 replicates). Percentage lysis was calculated as (100 - cell viability).



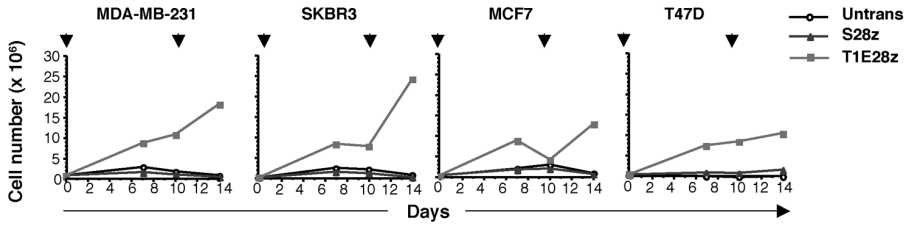
Supplementary Figure 4. In-vitro characterization of adoptively transferred T-cells. (A) Human T-cells were engineered to express T1E28z, T1NA or the T4 combination. $T4^+$ T-cells were expanded in IL-4 for 3 weeks while $T1E28z^+$ and $T1NA^+$ T-cells were propagated in IL-2. Cell surface CAR expression was detected using an anti-EGF monoclonal antibody. (B) Functional activity of CAR-engrafted T-cells was tested prior to adoptive transfer by co-cultivation of 1 x 10^6 T-cells with a confluent well of HN3 tumor cells (24-well plate – 1.9cm^2). Supernatants were harvested after 72 hours and analyzed for IFN-γ by ELISA (mean + SD of triplicate cultures).



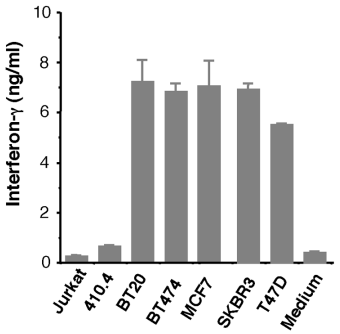
Supplementary Figure 5. Sub-group analysis of immunotherapy using $T4^+$ T-cells. $T4^+$ T-cells were infused IP at a dose of 7.5 x 10^6 or 20 x 10^6 cells in mice bearing an established HN3 LUC xenograft. Data shown are bioluminescence emissions from each of 3 mice per administered T-cell dose.



Supplementary Figure 6. ErbB expression by human breast cancer and murine mammary carcinoma cell lines. (A) Cell surface expression of ErbB1, ErbB2, ErbB3 and ErbB4 was analyzed by flow cytometry using ErbB-specific monoclonal antibodies. Filled histograms indicate staining with a matched isotype control antibody. (B) ErbB expression by 410.4 murine mammary carcinoma cells was analyzed after intracellular staining with polyclonal anti-ErbB antisera. Filled histograms show non-specific staining by control antiserum.



Supplementary Figure 7. Proliferation of T1E28z$^+$ T-cells following activation by breast cancer cell monolayers. Human T-cells engineered to express T1E28z were co-cultivated with the indicated cancer cell monolayers. Comparison was made with untransduced and control (S28z)-transduced T-cells. After 24 hours, IL-2 (100U/ml) was added. T-cells were enumerated at the indicated intervals. Stimulation on a confluent monolayer was performed at time points indicated by the overhead arrows.



Supplementary Figure 8. Interferon-γ production by T4$^+$ T-cells when stimulated by breast cancer cell monolayers. Human T-cells were engineered to express T4 and were expanded for 2 weeks using IL-4. Cells were then cultured with the indicated breast cancer cell monolayers. Jurkat suspension cells, 410.4 cell monolayers and medium alone served as negative control stimuli. Supernatants were harvested after 72 hours and analyzed for IFN-γ. Similar results were obtained in 2 independent experiments.