

In Situ immunization by bispecific antibody targeted T cell therapy in breast cancer

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Metastatic breast cancer remains an incurable disease. Most patients experience objective treatment responses associated with palliation of symptoms; however, progression inevitably occurs. Thus, there is an urgent need for innovative therapeutic strategies that may halt disease progression. Adoptive T-cell therapy is a potent and promising treatment option with the potential to harness the intrinsic antitumor power of the immune system and provide long-term immunity against cancer.

The potential of our immune system's remarkable specificity, potency, and memory has come to fruition as a promising new era of cancer immunotherapeutics unfolds. Although it has long been known that immune manipulation using a "vaccination strategy" provides life-long protection against fatal infections, exploration of means to bend the immune system to fight cancer has only begun over the past 2 decades. Nonetheless, dramatic progress in both discovery and technology has led to rapid development and clinical translation of immune based therapies. With recent approvals of a prostate cancer vaccine (Provenge[®]), immune check point blockers (Yervoy[®], Keytruda[®], Opdivo[®]) and a bispecific antibody (Blinicyto[®]), the landscape of immunotherapy has taken a leap over conventional approaches. Furthermore, the ability of adoptive T-cell therapy to render complete remission in relapsed/refractory lymphoid malignancies is an extraordinary milestone in the fight against the cancer.^{1,2} The concept of Paul Ehrlich's "magic bullet," which once referred to monoclonal antibodies that selectively target affected cells without causing toxic side effects, is no longer confined to just monoclonal antibodies, as novel forms of immunotherapy

similarly display selective targeting with reduced side effects.

A number of chimeric antigen receptor (CAR)- and T cell receptor (TCR)-T cell therapies are being evaluated for treatment of solid malignancies, although it remains to be seen if such engineered T cells will function effectively in immunosuppressive tumor microenvironments.³ Apart from monoclonal antibodies for breast cancer, there are currently no other FDA approved immunotherapeutic options available. Although advances in treatment have improved survival outcomes for patients with metastatic breast cancer, progression of disease is inevitable and the frequency of durable responses is low.⁴ A number of HER2-targeted therapies (*trastuzumab* [Herceptin[®]], *pertuzumab* [Perjeta[®]] and *Ado-trastuzumab emtansine* [T-DM1] an antibody-drug conjugate) are approved for HER2 3+ expressing breast cancer; however, 75–80% patients who have 0–2+ HER2 expression or patients who develop resistance to HER2 targeted therapies need non-toxic therapies aimed at improving progression-free and overall survival.

Our previous studies showed that anti-CD3 x anti-HER2 bispecific antibody

[HER2Bi]-armed activated T cells (HER2 BATs) are highly cytotoxic to a breast cancer cell line (MCF-7) that expresses only a few HER2/*neu* receptors and is considered negative by IHC.⁵ The markedly increased cytotoxic capacity of HER2 BATs toward very low level expressing HER2/*neu* targets provides a novel and unique strategy to treat women with HER2/*neu* negative (0–2+ expression) tumors. Our strategy utilizes the high-affinity humanized anti-HER2 antibody, trastuzumab, to redirect the non-MHC restricted, perforin/granzyme mediated cytotoxicity of activated T cells to both high and low HER2-expressing targets by arming activated T cells *ex-vivo* with HER2Bi. In mice, co-injections of HER2 BATs with PC-3 prostate cancer cells in a Winn assay completely prevented development of tumors, while intravenous injections of HER2 BATs significantly delayed growth of established PC-3 tumors compared to mice that received activated T cells or vehicle alone ($P < 0.001$).⁶ We have shown that targeting HER2 in non-overexpressing (i.e., 0–2+) tumor cells is feasible and shows promising results in patients with metastatic disease beyond first line therapy. Evidence of clinical and

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immunologic responses in women with HER2 0/1+ status in our Phase I trials suggests a therapeutic benefit even in the absence of HER2 over-expression and provides encouraging results in HER2 low-negative patients treated with armed activated T cells.⁷ The median overall survival (OS) was 36.2 months for all 23 patients (22 evaluable and 1 non-evaluable = 23), 57.4 months for the HER2 3+ patients, and 27.4 months for the HER2 0–2+ patients. BATs infusions induced endogenous cytotoxic T-cell and immunokine responses that persisted up to 4 months.⁷ Our findings showed that cellular immune responses develop and may augment immune based killing of tumors even in patients who were progressing.

One explanation for the encouraging OS in HER2 0–2+ patients could be a polyclonal immune responses that may target residual chemotherapy resistant HER2 positive “cancer stem-like cells” (CSC) that exhibit self-renewal properties and are responsible for cancer relapse. Prior studies also suggest that anti-HER2 reagents may be effective against HER2 positive CSC in tumors that are primarily HER2 negative.⁸

Arming activated T cells with low levels of HER2Bi bridges the effectors and targets resulting in tumor cell lysis, T helper type 1 (Th₁) cytokine/chemokine release, and induction of long-term immune responses without causing the severe cytokine release syndrome seen with anti-

CD3-based BiAbs. *In vitro*, HER2 BATs divide, maintain surface HER2Bi, and mediate perforin/granzyme cytotoxic activity for at least 336 hours during successive rounds of exposure to tumor cells.⁹ We have shown enhanced BAT-mediated killing of tumor cells and, in the presence of the resultant Th₁ cytokine-enriched microenvironment, a significant reduction in regulatory T cells (CD4⁺/CD25^{hi}/CD127^{lo}), granulocytic CD14⁺/HLA-DR⁺/CD11b⁺/CD33⁺ leukocytes and monocytic CD14⁺/HLA-DR⁺/CD11b⁺/CD33⁺ myeloid-derived suppressor cell (MDSC) populations as compared to control culture conditions. Furthermore, BATs inhibited MDSC differentiation and attenuated MDSC suppressive activity through downregulation of COX2, PGE₂ and ARG1, the latter of which was potentiated in the presence of Th₁ cytokines.¹⁰

Our working hypothesis is that HER2 BATs infusions not only provide immediate tumor cell killing but also modify the tumor microenvironment through release of Th₁ cytokines and chemokines. HER2 BATs mediated tumor cell lysis may create “fertile” ground for *in situ* immunization through the inhibition of Tregs and MDSC populations and the recruitment and activation of endogenous immune cells that result in antigen/epitope spreading (Fig. 1). These changes may induce the development of long-term tumor specific memory T cells as well as a shift in the phenotype of tumor-associated macrophages from an M2 to an M1 phenotype that increases sensitivity to chemotherapy.

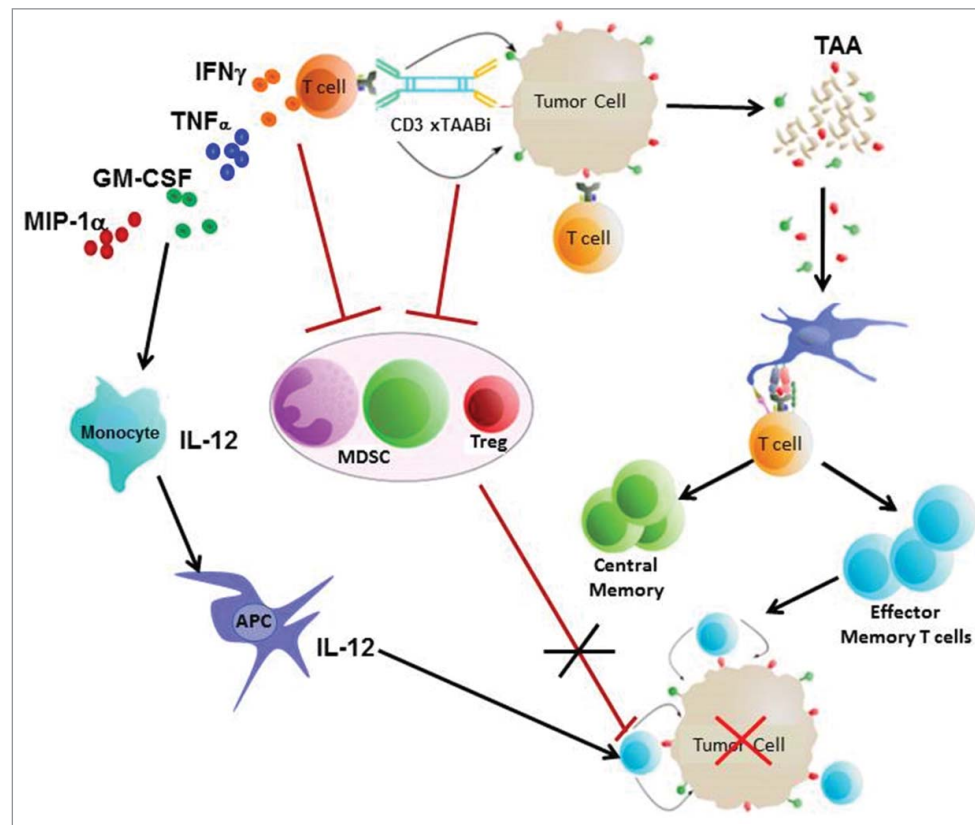


Figure 1. Modulation of the tumor microenvironment and BAT infusion-induced endogenous immunity. Tumor antigens/epitopes are released during bispecific antibody-armed activated T cell (BAT)-mediated killing of tumor cells. Cytokines and chemokines (IFN γ , TNF α , GM-CSF, MIP-1) are secreted as a function of BAT-mediated killing of tumor cells that inhibit immune suppressor cells (T_{regs} and MDSCs), recruit endogenous immune cells and induce maturation and activation of dendritic cells (DCs). Antigen presentation to naïve T cells by tumor-antigen primed DCs, and the interleukin 12 (IL-12) and T helper type 1 (Th₁) cytokine-enriched milieu stimulate proliferation and differentiation of T cells and B cells that can lead to tumor-specific effector and central memory cell development and antitumor immunity. GM-CSF/CSF2, granulocyte-macrophage colony stimulating factor; IFN γ , interferon γ ; MDSC, myeloid-derived suppressor cell; MIP1, macrophage inflammatory protein 1; TNF α , tumor necrosis factor α ; T_{reg}, regulatory T cell.

Disclosure of Potential Conflicts of Interest

L.G.L is co-founder of Trans-target Inc. and A.T. has no conflicts of interest.

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