Enhancing HER2/neu Specific T-cell Therapy by Targeting the Tumor Endothelium

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ABSTRACT
Success of adoptive T cell therapy for cancer can be hindered by the tumor stroma, which consists of factors that not only promote tumor growth but also prevent egress of tumor-specific T cells into the tumor parenchyma. The role of the tumor endothelium in regulating T cell homing, adhesion and transendothelial migration has gained increasing interest in cancer immunotherapy, specifically for prostate cancer; endothelins (ET) and their receptors can mediate tumor growth, angiogenesis and metastasis. The purpose of this study was to determine if, in breast cancer, targeting endothelin-1 (via endothelin B receptor (ETRB) blockade) might abrogate the endothelial barrier and increase T cell homing to tumors, thereby augmenting the efficacy of HER2/neu antigen specific Type 1 helper (Th1) cell therapy of spontaneous HER2/neu mammary tumors. By reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC), we found overexpression of ETRB in peritumoral stroma of spontaneous mammary tumors derived from HER2/neu transgenic mice. We treated tumor-bearing mice with HER2/neu specific T cells alone or HER2/neu specific T cells + BQ-788, a specific ETRB inhibitor peptide. The addition decreased tumor growth by 84%. Thus, in this model of HER2/neu mediated breast cancer, targeting the tumor endothelium can significantly enhance the clinical efficacy of HER2/neu specific Th1 cells. Studies are underway to evaluate the biological mechanisms induced by endothelin targeting that cooperate with T cell therapy in the resulting significant success of the combined treatment. The data provide a rationale for combining tumor antigen specific T cell therapy with endothelin blockade to induce an effective antitumor response in breast cancer.

BACKGROUND
• Treatment with HER2/neu specific Th1 cells can significantly induce regression of neu+ spontaneous mouse mammary tumors in neu-tg mice (80% growth inhibition versus control) but is not curative.

• Lack of neu specific Th1 cells observed in the treated tumor may be due to ineffective T cell egress and penetration into tumor parenchyma.

• Endothelin receptors A and B interact with endothelin, a peptide produced by various cell types, to regulate the constriction or dilation of blood vessels and therefore may impact T cell egress into tumors.

• Endothelin receptor B blockade on tumor stroma can augment T cell homing to tumors in vivo (Buckanovich, et al., Nature Medicine, 2008).

RESULTS
Figure 1. Spontaneous breast tumors from neu-transgenic mice express endothelin receptor A and receptor B mRNA. qPCR assay was used to assess expression levels. Controls included mouse lung (high expressor) and mouse ovarian tumor cell lines designated Id8 and Id8-VEGF.

Figure 2. Spontaneous breast tumors from neu-transgenic mice express endothelin receptor B protein. Immunohistochemistry reveals ETRB expression (arrows) in the peritumoral stroma, 100x (B). No expression seen in negative control (no primary antibody), 100x (A).

Figure 3. Inhibition of breast cancer by neu specific T cells in vivo can be enhanced by endothelin receptor B neutralization. HER2-specific T cells (1.5 x 10^6) were injected i.v. on days 4, 6 and 8 into mice bearing spontaneous neu+ mammary tumors along with PBS as control vehicle (black square; n=1), or with 300 μg ETRB antagonist (blue, n=3) or combined ETRB/ETRA antagonists (green, n=3) i.v. on day 3 followed by 3 treatment cycles.

Figure 4. Long term follow-up of endothelin receptor B antagonist treated tumors reveals immune escape. Tumors in Figure 3 were monitored for tumor growth out to day 60. Shown are p101 Tc treated tumor (n=1) and the individual p101 Tc+ETRB antagonist treated tumors (n=3). In the latter group, tumor regression occurs rapidly during the first 20 days, followed by a stagnant period (day 20-40) and eventual escape (day 40-60).

CONCLUSIONS
• ETRB and ETRB are expressed in spontaneous breast tumors of neu+tg mice and can serve as potential targets for immune manipulation.

• Neutralization of ETRB or ETRB/ETRA along with neu-targeted T cell therapy augments tumor inhibition in vivo. This likely occurs via the facilitation of enhanced T cell egress into tumors. Future directions include investigations into mechanisms.

• Combining tumor antigen specific T cell therapy with endothelin blockade may represent an effective immunotherapeutic approach for breast cancer treatment. However, the resulting immune escape observed in treated tumors suggests the need to evaluate prolonged treatment cycles.