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AUTHOR'S VIEW

CD4$^+$ Th1 to the rescue in HER-2+ breast cancer

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ABSTRACT

HER2 overexpression leads to downregulation of MHC class-I. CD4$^+$ Th1 cytokines, IFNγ and TNFα, and monoclonal antibodies, trastuzumab and pertuzumab, restore MHC class-I expression, and enable CD8$^+$ recognition and cytolysis. Restoration of the anti-HER2 CD4$^+$ Th1 immune response in combination with HER2 targeted therapy appear to be critical to successful anti-HER2 CD8$^+$ immunotherapy.

Abbreviations: CD, cluster of differentiation; CTL, cytotoxic T lymphocyte; DTH, delayed type hypersensitivity; E:T, effector cell to target cell ratio; HER, human epidermal growth factor receptor; HLA, human leukocyte antigen; IFN, interferon; MCF, mean channel fluorescence; MHC, major histocompatibility complex; PD-L1, programmed death-ligand-1; Th1, type 1 T-helper; TNF, tumor necrosis factor.

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T-cell antitumor responses are associated with improved outcomes in HER2 breast cancer. Increased lymphocytic infiltrate and overexpression of lymphocyte-associated genes in HER2^{pos} tumors correlate with prolonged distant metastasis free survival and decreased recurrence rates.[1] Furthermore, there is a strong association between immune gene expression and recurrence free survival following treatment with adjuvant trastuzumab, suggesting that the subset of HER2 positive tumors with a high-level of immunologic activity are better situated to benefit from treatment with adjuvant trastuzumab.[2]

However, attempts to boost cellular immunity have produced disappointing results. For example, HER2$_{369–377}$ (KIFGSLAFL; E75), the most widely studied HER2 immunogenic peptide, has been identified endogenously in breast and ovarian cancers. Vaccination with HLA-A2-restricted peptide 369–377 generates HER2$_{369–377}$ reactive CD8$^+$ T-cells; paradoxically, patients with HER2^{low}-expressing tumors mount a more robust immunologic response after vaccination than patients with HER2^{high}-expressing tumors—patients with HER2^{low}-expressing tumors demonstrate a larger DTH response, a more sustained specific CTL response, and a more prolonged disease free survival.[3] Additionally, others have shown that immunization results in peptide-specific CTLs that fail to recognize HLA-A2^{pos} HER2^{pos} tumor cells,[4] spurring the controversy as to whether this epitope is processed and presented by HER2 expressing tumors.

We further explored this complex interaction between CD8$^+$ T-cells and HER2^{pos} tumor cells. Overexpression of HER2 has been shown to downregulate MHC class-I.[5] The resulting decrease of MHC class-I may explain why peptide-specific CTLs generated by HER2$_{369–377}$ vaccination fail to recognize the HLA-A2^{pos} HER2^{pos} tumors. Additionally, the magnitude of HER2 expression resulting in MHC class-I downregulation may explain the differential response to HER2^{low} and HER2^{high} expressing cells—CD8$^+$ T-cells recognize HER2^{low} tumor cells with sustained MHC class-I expression, but not HER2^{high} tumor cells with deficient MHC class-I expression. In our recent manuscript, we confirmed that MHC class-I expression is maintained on HER2^{low} cell lines, but is severely diminished on HER2^{high} cell lines. Furthermore, we demonstrated that HER2$_{369–377}$–specific CD8$^+$ T-cells recognize the epitope HER2$_{369–377}$ on the HER2^{low}/MHC class-I expressing tumor cells, but not the stealth HER2^{high}/MHC class-I deficient tumor cells.[6]

We then investigated strategies to restore MHC class-I expression using CD4$^+$ Th1 cytokines, IFNγ and TNFα, and HER2 targeted tyrosine kinase inhibitors, trastuzumab and pertuzumab. The CD4$^+$ cytokine, IFNγ, has been shown to induce MHC class-I expression in HER2 overexpressing tumors in murine models.[7] Similarly, we found that all HER2 expressing cell lines treated with IFNγ/TNFα significantly increase MHC class-I expression. However, dual treatment with IFNγ/TNFα was only able to significantly increase anti-HER2 CD8$^+$ mediated recognition and cell lysis in HER2^{intermediate} cell lines, but not HER2^{high} cell lines.[6]

In contrast, while treatment with monoclonal antibodies alone, trastuzumab, pertuzumab, or both trastuzumab and pertuzumab, had little effect on MHC class-I expression or CD8$^+$ mediated cytotoxicity, the combination of a monoclonal antibody and IFNγ/TNFα dramatically increased MHC class-I expression on all HER2 expressing cell lines, and was significantly better than Th1 cytokine treatment (IFNγ/TNFα) in HER2^{high} cell lines. Similarly, only the combination of a monoclonal antibody and IFNγ/TNFα rendered the HER2^{high} cells susceptible to CD8$^+$ mediated recognition and lysis.[6] Consistent with the clinical benefit seen following treatment with dual

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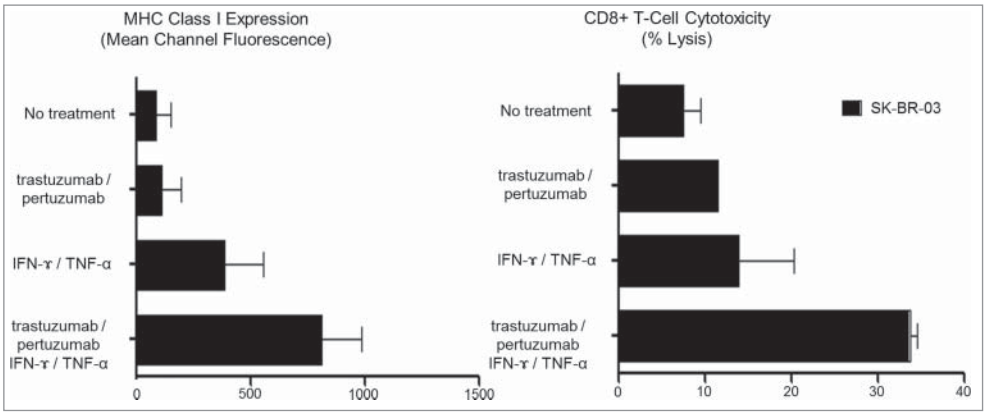

Figure 1. The effect of $CD4^+$ Th1 cytokines (IFNγ/TNFα) and HER2 targeted monoclonal antibodies (Trastuzumab/Pertuzumab) on MHC class-I expression and $CD8^+$ T-cell mediated cytotoxicity in SK-BR-3 cells (E:T = 10:1). Both HLA-A2 MHC class I expression and $CD8^+$ cytotoxicity remained low following treatment with Trastuzumab alone (96.4 ± 70.6 MCF, 11.7%), Pertuzumab alone (103.4 ± 81.6 MCF, 9.7%), and Trastuzumab/Pertuzumab (113.8 ± 83.2 MCF, 11.5%), at levels similar to MHC class I expression without treatment (87.1 ± 64.9 MCF, 9.5%). HLA-A2 MHC class-I expression and $CD8^+$ cytotoxicity both increased following treatment with IFNγ/TNFα (386.6 ± 171.4 MCF, 20.4%), IFNγ/TNFα and Trastuzumab (512.9 ± 95.1 MCF, 28.9%), IFNγ/TNFα and Pertuzumab (486.3 ± 166.7 MCF, 28.6%), with the greatest increase following treatment with IFNγ/TNFα and Trastuzumab/Pertuzumab (813.3 ± 117.8 MCF, 34.6%).

monoclonal antibodies, trastuzumab and pertuzumab, treatment with IFNγ/TNFα and both trastuzumab and pertuzumab increased MHC class-I expression and $CD8^+$ mediated cytotoxicity more than IFNγ/TNFα or IFNγ/TNFα and either trastuzumab or pertuzumab (Fig. 1).

Just as HER2 cancer cells lack expression of MHC class-I, they also lack expression of programmed death-ligand-1 (PD-L-1). In addition to inducing MHC class-I expression, the $CD4^+$ cytokine, IFNγ, is also known to induce PD-L-1 expression on breast cancer cells with low endogenous PD-L-1 expression.[8] We showed that IFNγ upregulated PD-L1 expression in all HER2 expressing cell lines, and that combined IFNγ/TNFα treatment further enhanced PD-L1 expression. Upregulation of PD-L-1 would facilitate the use of PD-1/PD-L-1 inhibition in the treatment of HER2 breast cancer.[6]

$CD4^+$ T-cells have long been recognized as a critical aide to the survival and function of $CD8^+$ T-cells; our work has shown that $CD4^+$ T-cells further facilitate $CD8^+$ cytotoxicity by modifying the tumor environment. Unfortunately, we have previously shown that there is an early and progressive loss of anti-HER2 $CD4^+$ Th1 response in breast tumorigenesis—healthy patients have a strong anti-HER2 $CD4^+$ Th1 immune response that is decreased in patients with ductal carcinoma in situ and nearly absent in patients with invasive breast cancer.[9] We also showed that the magnitude of the immune response correlates with clinical outcomes—a depressed anti-HER2 $CD4^+$ Th1 immune response correlates with an increased risk of recurrence, and a robust response correlates with an increased rate of pathologic complete response following neoadjuvant chemotherapy. Although this deficit does not appear to be corrected by surgery, radiation, chemotherapy, or HER2 targeted monoclonal antibodies, we showed that it can be corrected by HER2 peptide pulsed DC1 vaccination resulting in long term maintenance of anti-HER2 immune response.[9,10]

These results support the multivalent targeting of HER2. Anti-HER2 Th1 cells together with HER2 directed antibodies may enhance the tumoricidal effects of anti-HER2 $CD8^+$ T-cells and facilitate the potential use of checkpoint inhibitors in the treatment of $HER2^{pos}$ breast cancer. Consideration should be given to restoring the anti-HER2 Th1 in the design of HER2 directed therapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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