

UNLEASHING AN APPROACH TO EXPAND ANTIGEN-SPECIFIC IMMUNE RESPONSE VIA SYNERGISTIC INTEGRATION OF PHOTOTHERMAL THERAPY AND FLA-B-HER2 VACCINATION IN A DD-HER2/NEU MICE ORTHOTOPIC BREAST CANCER MODEL

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Background Immunotherapy, including therapeutic cancer vaccines (TCVs), is an emerging approach in breast cancer treatment. Combining TCVs with other modalities can potentially improve clinical outcomes.¹ Photothermal therapy (PTT) is a minimally invasive modality using laser-generated hyperthermia to kill cancer cells. Previous studies showed that the Toll-like receptor 5 (TLR5) agonist flagellin acts as an effective adjuvant, inducing cell-mediated immunity (CMI) when combined with TAA peptide epitopes.² This study aimed to combine the flagellin-adjuvanted peptide vaccine (FlaB-Her2 Vax), targeting Her2/Neu66–74 (p66, CTYVPANASL) epitope, with PTT to induce an anti-tumor immune response in breast cancer. Nanoparticles (NPs) containing near-infrared (NIR) photothermal agent indocyanine green (ICG), cyclin arginine-glycine-aspartate (cRGD) peptide, and flagellin adjuvant were prepared for PTT optimization. The potentiation of FlaB-Her2 Vax with optimized PTT was evaluated in a mice abscopal model of DD-her2/neu breast cancer. The study also analyzed flagellin's effect on modulating the tumor microenvironment (TME) during PTT therapy.

Methods Primary tumors were treated with an 808nm laser at 50°C and 2W for 5 minutes to optimize PTT. FlaB-Her2 Vax was administered via peritumoral injection before and after PTT to prime and boost the immune system. Survival and tumor growth in primary and abscopal sites were assessed. Effector cytokines promoting CD8+ T cell activation in the spleen and antigen-presenting cell activation in tumor tissue and tumor-draining lymph nodes (TDLNs) were evaluated. Additionally, flagellin's TME-modulating effect was investigated through peritumoral injection in tumor-bearing mice.

Results The combination therapy (PTT+FlaB-Her2 Vax) significantly improved overall survival compared to PTT alone or FlaB-Her2 Vax alone. Combination therapy and FlaB peritumor treatment induced immunogenic cell death and a subsequent tumor-specific immune response, demonstrating a significant anti-tumor immune response. Combination therapy enhanced infiltration of tumor antigen-reactive CD8+ T cells and accumulation of migratory CD103+ dendritic cells (DCs) secreting CXCL10, facilitating tumor antigen cross-presentation in the TME. Furthermore, PD-1-targeting checkpoint inhibitor therapy significantly enhanced the therapeutic benefits of PTT combined with FlaB-Her2 Vax, dependent on CD8+ T cells.

Conclusions FlaB adjuvanted-tumor-specific peptide vaccines effectively induced tumor-specific T, B cells, and DCs, promoting tumor antigen cross-presentation in the TME. The combination of local PTT and TCV induced systemic immunity against established tumors and metastases in an aggressive breast cancer model. This approach holds potential as a clinical method for HER2-positive breast cancer patients.

Acknowledgements The authors are grateful to Ms. Myeung Suk Kim and Youn Suhk Lee for their excellent assistance in animal experiments analyses.

REFERENCES

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Ethics Approval All animal experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Chonnam National University (CNU IACUC-H-2021–50) and performed in accordance with the relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.0855>