The effect of aspartate β-hydroxylase inhibition on cancer immunotherapy

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Background: Aspartate β-hydroxylase (ASPH) is overexpressed in 70-90% of various solid tumors, contributing to tumor cell proliferation, migration, and invasion. In addition, recombinant ASPH inhibits natural killer (NK) cells, but an effect on other immune cells has not been described. Small molecule inhibitors have been developed to target tumors overexpressing ASPH. In this study, we combined ASPH inhibition with immunotherapy to enhance cancer therapy and seek out affected immune cells.

Methods: C57BL/6 mice were inoculated with TC-1/A9 tumor cells characterized by reversible MHC class I downregulation and treated with the ASPH inhibitor MO-I-1151 in combination with the ODN1826 oligonucleotide application or DNA vaccination to assess the effect of ASPH inhibition on innate and adaptive immunity, respectively. Induced immune responses were analyzed by *in vivo* depletion of immune cell subpopulations and *in vitro* examination of splenocytes using T-cell proliferation assay and IFN-γ ELISPOT assay. Tumor-infiltrating immune cells were analyzed by flow cytometry and single-cell RNA sequencing (scRNA-seq).

Results: The ASPH inhibitor alone or combined with ODN1826 did not reduce tumor growth, but ASPH inhibition significantly enhanced the anti-tumor effect induced by DNA vaccination. *In vivo* depletion suggested an important anti-tumor role of $CD8^+$ T cells, supported by a significant increase of activated $CD8^+$ T cells in the ELISPOT assay. Flow cytometry did not show increased infiltration with $CD8^+$ T cells after combined therapy with DNA vaccination and ASPH inhibition, but regulatory T lymphocytes were significantly decreased. These results were confirmed by scRNA-seq, which also showed higher activation of γδ T cells, NK cells, and some myeloid cells after combined therapy.

Conclusions: These results suggest that ASPH inhibition may stimulate anti-tumor T cell-mediated adaptive immunity and activate various cells of innate immunity. Further studies should analyze ASPH expression in immune cells and elucidate the mechanisms involved in ASPH effects.