ANTI-CTLA-4 THERAPY DEPLETES TREGS AND EXPANDS ICOS+ T-CELLS IN NEUROBLASTOMA TUMORS WITH INDUCED DNA MISMATCH REPAIR DEFICIENCY

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Background Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) highly expressed on regulatory T-cells (Tregs) inhibit the activation of pro-inflammatory T-cells responsible for eliminating cancer cells. Anti-CTLA-4 can enhance T-cell activation by increasing CD28 co-stimulatory signaling through CTLA-4 blockade or depletion of Tregs by Fc-dependent effector mechanisms. Strategies to improve its therapeutic efficacy are needed as patient response rates to anti-CTLA-4 are low. Response to anti-CTLA-4 has been positively correlated with tumor mutation burden (TMB). Defects in the DNA mismatch repair (MMR) pathway can increase TMB and the production of neoantigens that promote anti-tumor immune responses. Here we investigate the underlying mechanism(s) to which induced MMR deficiency in an immunologically-cold and low TMB tumor model can enhance the therapeutic effect of anti-CTLA-4. We hypothesize that induced MMR deficiency in tumors enhances anti-CTLA-4-mediated Treg depletion and increases the infiltration and activation of effector T-cells.

Methods MMR deficiency was induced in a syngeneic murine neuro-2a neuroblastoma cell line by knocking-out MLH1 expression using CRISPR-Cas9. Wildtype MMR-proficient (pMMR) or induced MMR-deficient (idMMR) neuro-2a cells were inoculated into immunocompetent A/J mice and treated with anti-CTLA-4. Tumors were immunophenotyped by flow cytometry and mixed-lymphocyte reaction assays were used to examine the effects of MMR deficiency and anti-CTLA-4 on T-cell activation and proliferation.

Results Induced MMR deficiency in neuroblastoma tumors enhances the anti-tumor immune response induced by anti-CTLA-4. MMR deficiency in neuroblastoma tumors promoted anti-CTLA-4-mediated Treg depletion and increased intratumoral CD3+ T-cells. idMMR neuroblastoma tumors had an increase of ICOS+ T-cells compared to pMMR tumors. In addition, ICOS+ T-cells were increased further with anti-CTLA-4 treatment.

Conclusions Our data show that inducing MMR deficiency in low TMB and immune-cold neuroblastoma tumors can enhance the anti-tumor effect of anti-CTLA-4 by increasing T-cell activation and depletion of Tregs. By understanding the underlying mechanism(s) of anti-CTLA-4 in idMMR tumors, it may justify targeting the MMR pathway to improve the response to immune checkpoint inhibitors in patients with immunologically-cold and/or low TMB tumors that are refractory to immunotherapy. Future studies will assess how inducing MMR deficiency alters the tumor microenvironment to enable anti-CTLA-4-mediated Treg depletion and the significance of ICOS+ T-cells in the efficacy of anti-CTLA-4 therapy in this setting.