INDUCING MISMATCH REPAIR DEFICIENCY IN COMBINATION WITH ANTI-CTLA4 THERAPY IS HIGHLY EFFECTIVE AGAINST NON-IMMUNOGENIC NEUROBLASTOMA TUMORS

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Background Despite rigorous multimodal therapy, recurrence is common among high-risk neuroblastoma patients. Neuroblastoma is a poorly immunogenic tumor with low tumor mutational burden (TMB). Currently, immunotherapy with immune checkpoint inhibitors (ICIs) are not approved for neuroblastoma. Novel strategies to sensitize neuroblastoma to ICIs are urgently needed. We have induced mismatch repair (MMR) deficiency in mouse neuro-2a tumors and show that these tumors become highly immunogenic and responsive to anti-CTLA4 but not anti-PD1 therapy.

Methods The MMR gene *Mlh1* was knocked out of neuro-2a and B16F10 cells, and clones were selected. MMR-deficient (dMMR) and -proficient (pMMR) neuroblastoma tumors were grown in immunocompetent and immunodeficient animals. Tumors were harvested, and tumor-infiltrating lymphocytes (TILs) were analyzed by flow cytometry. Neuro-2a tumor-bearing mice were treated with anti-PD1, anti-CTLA4, or a combination of both antibodies. NK cells were depleted in mice treated with anti-CTLA4 using the anti-asialo GM1 antibody to examine their role in the efficacy of anti-CTLA4 treatment. dMMR B16F10 melanoma tumor-bearing mice were treated with anti-PD1 followed by the analysis of TILs. Publicly available TARGET and TCGA databases were mined to examine the effect of T-cell infiltration on neuroblastoma and melanoma patient's survival, respectively.

Results We show that high-risk neuroblastoma and melanoma patients with tumors containing high levels of T-cell and memory T-cell-related genes have improved survival. Additionally, inducing MMR deficiency in neuro-2a cells renders these tumors immunogenic, with high T-cell infiltration, and inhibits tumor growth in mice in an immune-dependent manner. We also show that dMMR neuroblastoma tumors are highly sensitive to anti-CTLA4 but not anti-PD1 treatment. Anti-CTLA4 therapy cured most tumor-bearing mice, inducing immune memory, epitope spreading, and increased tumor-specific T-cells in cured animals. Notably, the effect of anti-CTLA4 therapy was independent of NK cells. In neuroblastoma tumors with induced MMR deficiency, anti-PD1 treatment antagonized anti-CTLA4 by upregulating inhibitory molecules on T-cells and increasing the level of dysfunctional TILs. Interestingly, anti-PD1 therapy was effective against high TMB-background melanoma tumors with induced MMR deficiency. Our data suggests that this effect relied on lowering T-cell inhibitory molecules rather than increasing T-cell infiltration into tumors.

Conclusions Inducing MMR deficiency in low and high TMB-background tumors leads to distinct responses to anti-PD1 therapy, with low levels of T-cell exhaustion being a positive indicator of response. Anti-CTLA4 therapy in combination with induced MMR deficiency is a novel strategy for treating low TMB-background high-risk neuroblastoma.

Ethics Approval This study was approved by the Animal Care Committee at Western University; approval number 2017-030.

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