INCREASED MUTATION BURDEN ALONE IS NOT SUFFICIENT TO INCREASE IMMUNOGENICITY OF DNA MISMATCH REPAIR DEFICIENT TUMORS

Background DNA mismatch repair deficiency (MMRd) drives the accumulation of somatic mutation-derived antigens (neoantigens), which underlies the remarkable response rates of this class of cancer to immune checkpoint blockade (ICB) therapy. Nevertheless, most advanced MMRd tumors do not respond durably to ICB, despite elevated tumor mutation and neoantigen burden. Critical questions remain regarding tumor mutation burden and immunosurveillance in the evolution and therapy resistance of these cancers.

Methods We developed autochthonous mouse models of lung and colon cancer with perturbations of MMR genes (e.g., Msh2) via in vivo CRISPR-Cas9 and conditional knockout (Msh2flox) and characterized their genomic profiles via whole exome and RNA sequencing. We performed randomly-enrolled and blinded preclinical trials of ICB (anti-PD-1/CTLA-4) with and without immunogenic chemotherapy (oxaliplatin/cyclophosphamide). We also established cell lines from these tumors with re-expression of Msh2 to arrest mutagenesis, performed sub-cloning, identified bona fide MHC-I neoepitopes, and tracked tumor growth, response to ICB, and neoantigen-specific T cell responses upon re-transplantation of these lines at different clonal fractions. Finally, we performed a meta-analysis of the predictive power of neoantigen burden and clonality in ICB response of human MMRd gastric and colorectal cancer.

Results As expected, MMRd resulted in elevated mutation and neoantigen burden with signatures consistent with the human disease. Surprisingly, this was not accompanied by an increase in T cell infiltration and the lung and colon cancer models were uniformly non-responsive to ICB, even when combined with immunogenic chemotherapy. Mechanistically, we showed that tumors harbored extensive intratumoral heterogeneity of mutations, which facilitated immune evasion of otherwise immunogenic subclones. Furthermore, neoantigen-specific T cells responses decreased in magnitude and effector differentiation with decreasing neoantigen clonal fraction. Interestingly, we found that immunosurveillance did not alter overall tumor mutation or neoantigen burden but shaped the clonal architecture of neoantigens arising spontaneously during tumorigenesis. Finally, we showed that clonal, but not subclonal, neoantigen burden was predictive of ICB response in clinical trials of MMRd gastric and colorectal cancer.

Conclusions These results provide valuable context for understanding how immunosurveillance shapes the evolution of cancers undergoing sustained mutagenesis, as occurs in human cancer. Our findings point to a critical role of intratumoral heterogeneity in predicting response to immunotherapy and justify deeper clinical exploration of this biomarker in MMRd cancers. Finally, these results have major implications for therapies aimed at deliberately increasing tumor mutation burden. We predict that such therapies would increase intratumoral heterogeneity and undermine anticancer immunity.

Ethics Approval All animal use was approved by the Department of Comparative Medicine (DCM) at MIT and the Institutional Animal Care and Use Committee (IACUC) under protocol number 0714-076-17.