967 UNFAVORABLE NEOANTIGEN ARCHITECTURES BLUNT ANTI-TUMOR T CELL RESPONSES IN A MOUSE MODEL OF LUNG ADENOCARCINOMA

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Background Intra-tumoral heterogeneity (ITH) can limit effective anti-tumor immune responses despite the presence of immunogenic neoantigens. Yet, there is little mechanistic insight into how ITH blunts anti-tumor T cell responses or how clonal and sub-clonal neoantigens define immunologically unfavorable neoantigen architectures, preventing the rational design of neoantigen-targeting immunotherapy approaches for heterogenous tumors.

Methods Using lentiviral transduction, we expressed single or multiple neoantigens with different levels of immunogenicity in a transplantable, syngeneic mouse model of lung adenocarcinoma (driven by an oncogenic $Kras^{G12D}$ mutation and a deletion of p53). Modeling ITH-associated clonal and sub-clonal neoantigen expression, we systematically analyzed the impact of neoantigen architectures on neoantigen-specific T cell responses and overall immune-mediated tumor control.

Results Clonal co-expression of multiple neoantigens by tumor cells had distinct, converse effects on neoantigen-specific T cell responses dependent on neoantigen immunogenicity. Clonal expression of a poorly immunogenic (weak) neoantigen together with a highly immunogenic (strong) neoantigen, resulted in stronger T cell responses toward both antigens. The synergistic effect extended to increased neoantigen-specific in-vivo killing capacity and improved tumor control. In contrast, clonal co-expression of multiple strong neoantigens had detrimental effects on neoantigen-specific T cell responses, with immunogenic competition weakening the response against each individual neoantigen. Analysis of cross-presenting dendritic cells (cDC1) in the tumor-draining lymph node implicated differential T cell priming as a pivotal mechanism for these converse effects. When modeling sub-clonal tumors, the observed synergistic effects on T cell responses were less profound. Expression of multiple strong neoantigens on the other hand continued to impair anti-tumor immunity. Unfavorable neoantigen architectures were able to hinder immune-mediated tumor control even in tumors with a highly immunogenic clonal neoantigen trunk.

Conclusions Neoantigen architecture and ITH can be beneficial or detrimental for T cell responses. In tumors with high ITH, synergistic effects are reduced while detrimental effects may persist, providing a rationale for the higher immunogenicity of clonal tumors compared to their sub-clonal counterparts. These findings have strong implications for the design of immunotherapy approaches, including but not limited to cancer vaccines.

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Ethics Approval All mouse experiments were approved by MIT's Committee on Animal Care (CAC) – DHHS Animal Welfare Assurance # D16-00078

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