Hyper-Interferon Sensitive influenza induces adaptive immune responses and overcomes resistance to anti-PD-1 in murine NSCLC

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Despite recent advances in immunotherapy, many patients with NSCLC fail to respond to immune checkpoint inhibitors (ICI) or acquire resistance after an initial response. Exclusion of T cells from the tumor or the presence of a dysfunctional T cell compartment within the tumor microenvironment (TME) constitute two central hallmarks of resistance to ICI. Seminal studies have identified that loss of LKB1 in *KRAS*-mutant NSCLC drives resistance to ICI, possibly through the suppression of STING which results in dysregulation of the interferon (IFN) signaling. Because of the critical function of host IFN signaling in activation of anti-tumor adaptive immune responses, treatment strategies that leverage the IFN pathway hold promise for combating immune resistance.

*In situ* vaccination (ISV) with immune stimulating viruses has emerged as a potential strategy to overcome immune resistance by directly ameliorating the immunosuppressive TME and promoting host anti-tumor immune activation. Utilizing a high-throughput, genome-wide approach, we recently engineered a hyper–interferon-sensitive (HIS) virus as a vaccine candidate by incorporating multiple interferon (IFN)-sensitive mutations into the influenza A genome. HIS virus induced robust IFN responses in human and murine NSCLCs *in vitro*, which was superior to wild-type (WT) influenza. While HIS and WT viruses had similar replication capacity in IFNAR-/- mice, a ~3-log reduction in viral titers was observed in the lungs of immunocompetent mice treated with HIS compared to WT, consistent with IFN-mediated abrogation of HIS replication. ISV with HIS demonstrated superior efficacy compared to WT virus in multiple syngeneic murine models of NSCLC with known driver mutations (K, KP, KPL) and varying mutational burden. Flow phenotyping and single cell RNA-sequencing studies revealed that HIS induced host adaptive immune responses. The efficacy of HIS was depended on local IFN signaling and endogenous T lymphocytes. HIS ISV synergized with anti-PD-1 to overcome resistance in murine NSCLCs. Successful combination therapy with HIS ISV and anti-PD-1 resulted in improved overall survival and the establishment of enduring systemic tumor-specific immune memory. These studies present compelling evidence supporting the clinical translation of HIS virotherapy as a novel ‘off-the-shelf’ strategy to combat resistance to immunotherapy in patients with NSCLC.