The Stapled Peptide ALRN-6924, a Dual Inhibitor of MDMX and MDM2, Displays Immunomodulatory Activity and Enhances Immune Checkpoint Blockade in Syngeneic Mouse Models

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Background

The tumor suppressor p53 is one of the most pursued targets in oncology, playing a central role in inducing cell cycle arrest, apoptosis and senescence in response to cellular stress and oncogenic signals. In addition to its intrinsic anti-tumor activity in cells, p53 activation can induce anti-tumor immunity and plays an important role in the regulation of innate and adaptive immunity. Therefore, p53-reactivating agents in combination with immune checkpoint blockade (ICB) may provide a powerful approach to optimize the body’s immunological response against cancer. ALRN-6924 is an α-helical p53 peptide currently in clinical testing that has demonstrated anti-tumor activity as monotherapy (Merci-Bernstam, et al., ASCO 2017). In this study, we investigated whether p53 reactivation with ALRN-6924 can be leveraged as a new combination partner for ICB.

Methods

Peripheral blood mononuclear cells (PBMCs) were stimulated with ALRN-6924 for 24 hr. Gene expression and cytokine levels were measured using a validated TaqMan assay (ThermoFisher) and the Human XL Cytokine Array Kit (R&D Systems). Immune profiles from pre- and post-treatment tumor biopsy samples were evaluated by Nanostring PanCancer O’Seq and Immune Profiling RNA gene expression panels. Immunoexpression of PD1 was done by flow cytometry. Efficacy and immune cell profile (determined by flow cytometry and IHC) were evaluated in CloudmanS91 and MC38 syngeneic murine tumor models following treatment with ALRN-6924 alone and in combination with anti-PD-1 or anti-PD-L1, including re-challenge studies to test for immunological memory. All in vivo studies were approved by the Institutional Animal Care and Use Committee at Charles River Laboratories, Morrisville, NC.

Results

Ex vivo stimulation of PBMCs with ALRN-6924 promotes transcriptional activation of genes involved in innate and adaptive immunity and the production of pre-immune-stimulating cytokines including IFN-γ, IL-6 and IL-12. mRNA analysis of pre- and post-treatment tumor biopsies from patients treated with ALRN-6924 revealed a differential gene expression pattern consistent with conversion to an inflammatory tumor phenotype. In syngeneic mouse models, ALRN-6924 promotes infiltration of CD8+ T cells, polarization of M1 macrophages in mouse tumors and immunological memory. Moreover, ALRN-6924 synergizes with anti-PD-1 and anti-PD-L1 to induce anti-tumor immunity resulting in an increased number of mice achieving complete regressions (CR), in both p53 wild-type and p53-mutant tumors, compared to single agents.

Conclusions

ALRN-6924 is a first-in-class clinical stage MDMX/2 dual inhibitor displaying promising anti-tumor activity in patients. Recently ALRN-6924 was shown to display strong on-mechanism anti-tumor activity in preclinical models of AML and PTCL. In the current study, reactivation of p53 with ALRN-6924 enhanced the effects of ICB therapy in mice. Furthermore, these studies demonstrate that ALRN-6924 can stimulate immune cells in culture and the recruitment of cytotoxic immune cells to the tumor in vivo. Strikingly, ALRN-6924 enhanced the effects of immune checkpoint inhibitors in both p53 wild-type and mutant models. These results suggest that ALRN-6924 can modulate anti-tumor immunity, possibly via a tumor cell extrinsic mechanism in the tumor microenvironment.

References


Figure 1: ALRN-6924 Induces Tumor-Inflamed Signature in Primary Patient Tumors

Figure 2: ALRN-6924 Stimulates the Production of Immunoeductory Factors ex vivo

Figure 3: ALRN-6924 Promotes Tumor Infiltration of Cytotoxic CD8+ Cells and M1 Macrophages in Both p53 Wild-Type and Mutant Syngeneic Mouse Models

Figure 4: ALRN-6924 Potentiates the Effects of α-PD1 and α-PD-L1 in p53 Mutant Syngeneic Mouse Model (MC38 Colon Carcinoma)

Figure 5: ALRN-6924 potentiates the effects of α-PD1 and α-PD-L1 in p53 Mutant Syngeneic Mouse Model (MC38 Colon Carcinoma)