

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 signals¹. 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 immunity². 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 (Meric-Bernstam, et al., ASCO 2017)^{3,4}. In this study, we investigated whether p53 reactivation with ALRN-6924 can be leveraged as a new combination partner for ICB.

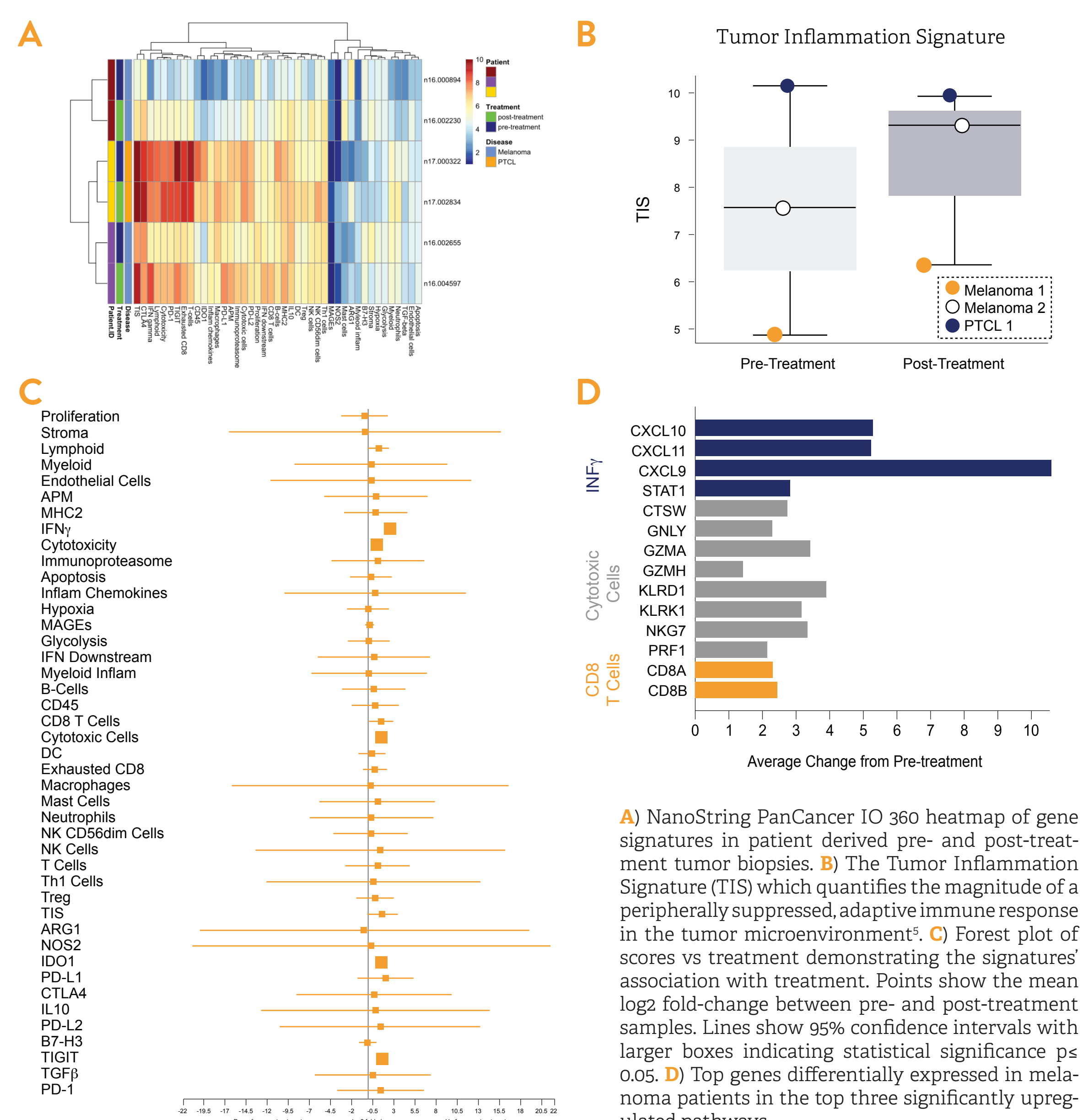
Methods

Peripheral blood mononuclear cells (PBMCs) were stimulated with ALRN-6924 *ex vivo* 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 IO360 and Immune Profiling RNA gene expression panels. Immunophenotyping of PBMCs 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, N.C.

Results

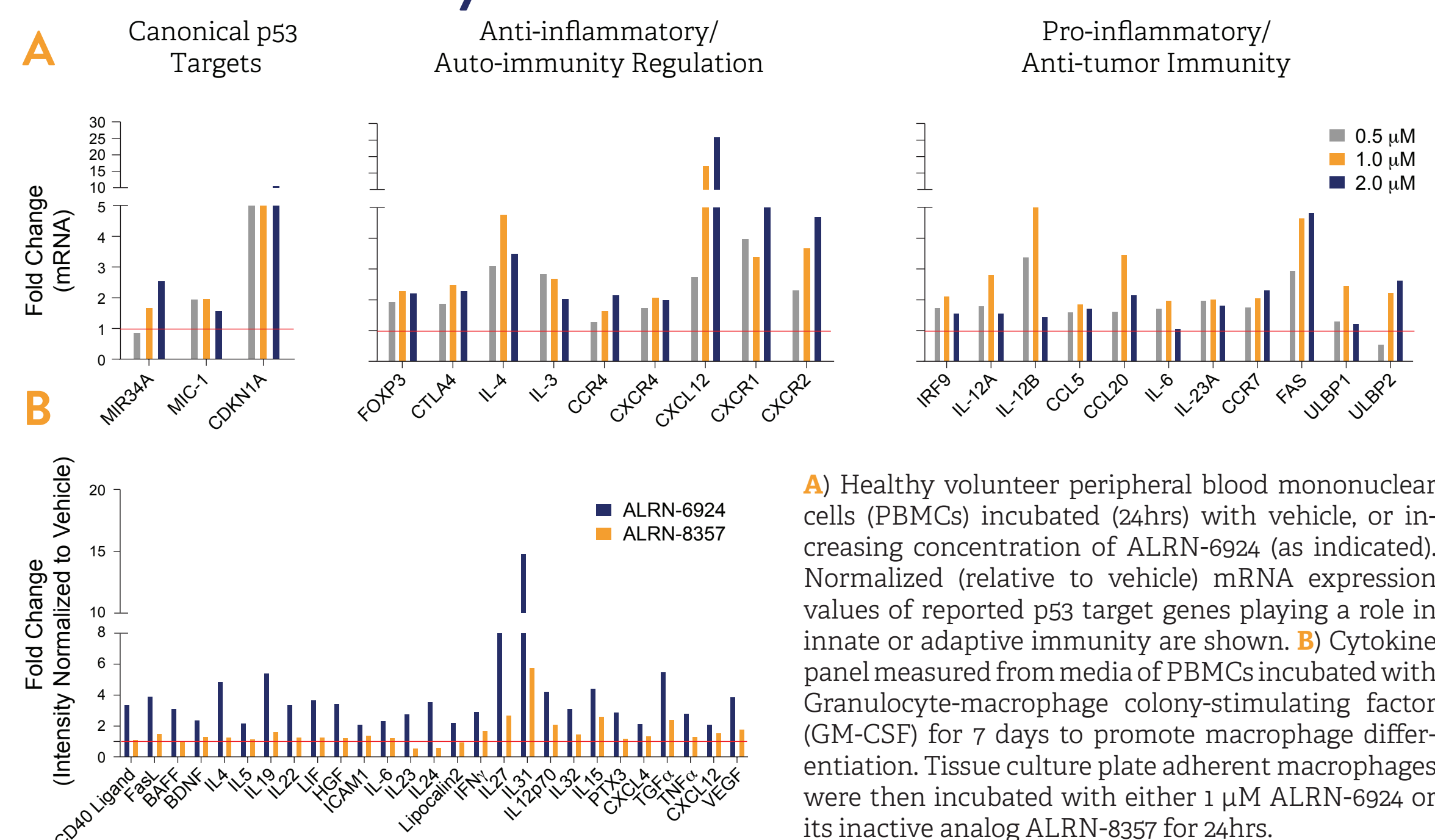
Ex vivo stimulation of PBMCs with ALRN-6924 promotes transcriptional activation of genes involved in innate and adaptive immunity, and the production of immune-stimulating cytokines including INF- γ , IL-6 and IL-12. mRNA analysis of pre- and post-treatment tumor biopsies from patients treated with ALRN-6924 revealed a differential gene expression pattern consistent with conversion to an inflamed 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.

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



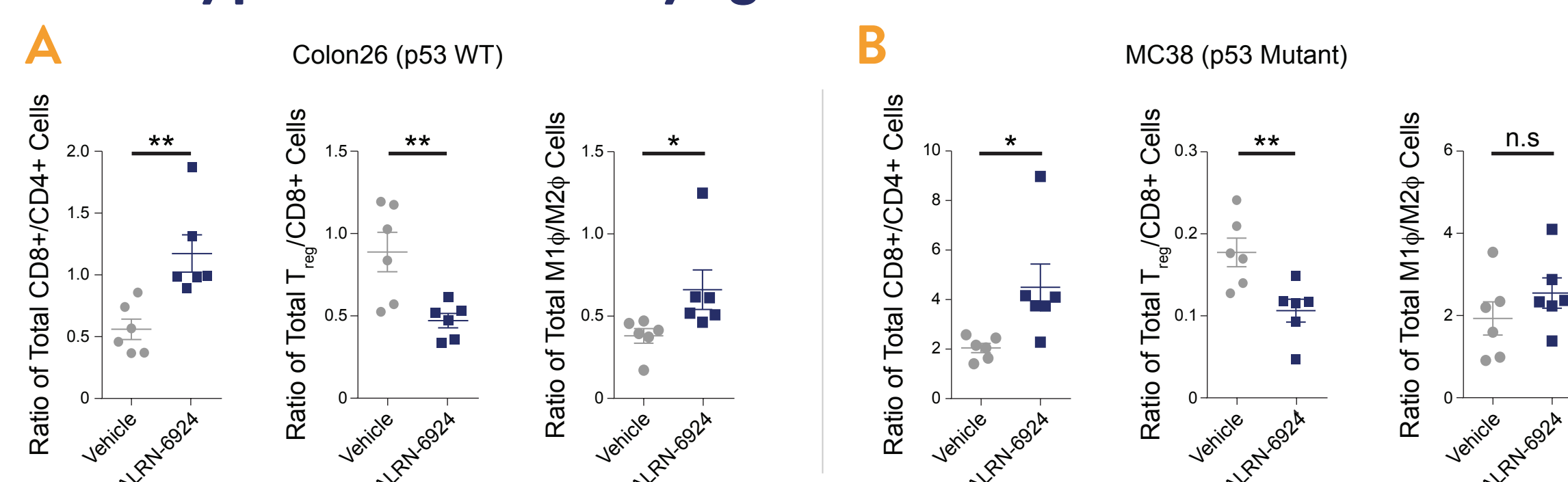
A) NanoString PanCancer IO 360 heatmap of gene signatures in patient derived pre- and post-treatment tumor biopsies. **B)** The Tumor Inflammation Signature (TIS) which quantifies the magnitude of a peripherally suppressed, adaptive immune response in the tumor microenvironment⁵. **C)** Forest plot of scores vs treatment demonstrating the signatures' association with treatment. Points show the mean log₂ fold-change between pre- and post-treatment samples. Lines show 95% confidence intervals with larger boxes indicating statistical significance $p < 0.05$. **D)** Top genes differentially expressed in melanoma patients in the top three significantly upregulated pathways.

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



A) Healthy volunteer peripheral blood mononuclear cells (PBMCs) incubated (24hrs) with vehicle, or increasing concentration of ALRN-6924 (as indicated). Normalized (relative to vehicle) mRNA expression values of reported p53 target genes playing a role in innate or adaptive immunity are shown. **B)** Cytokine panel measured from media of PBMCs incubated with Granulocyte-macrophage colony-stimulating factor (GM-CSF) for 7 days to promote macrophage differentiation. Tissue culture plate adherent macrophages were then incubated with either 1 μ M ALRN-6924 or its inactive analog ALRN-8357 for 24hrs.

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



A) Flow Cytometry analysis of tumor cell infiltrates in Colon26 and **B)** MC38 murine syngeneic mouse models in BALB/c and C57BL/6 background, respectively. Data is presented as a ratio representing total cell counts where * $p \leq 0.05$ and ** $p \leq 0.01$. Tumors (80 - 120 mm³) were treated with ALRN-6924 (20 mg/kg) administered intravenously twice weekly for two weeks (days 1, 4, 8, 11). Tumors were harvested on day 14.

Figure 4: ALRN-6924 Potentiates the Effects of α -PD1 and Promotes Immunologic Memory in p53 WT Syngeneic Tumor Model (CloudmanS91 Melanoma)

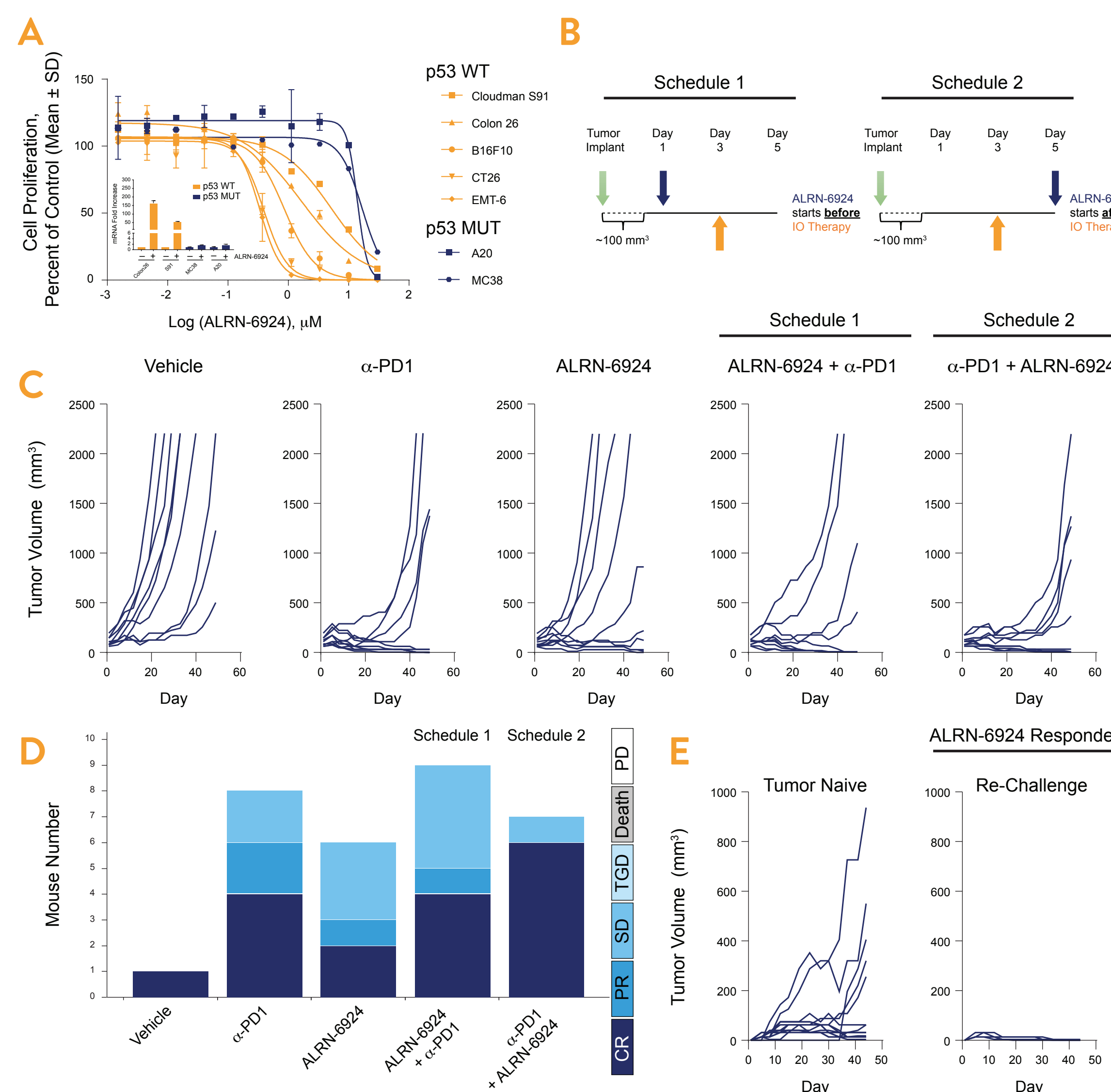
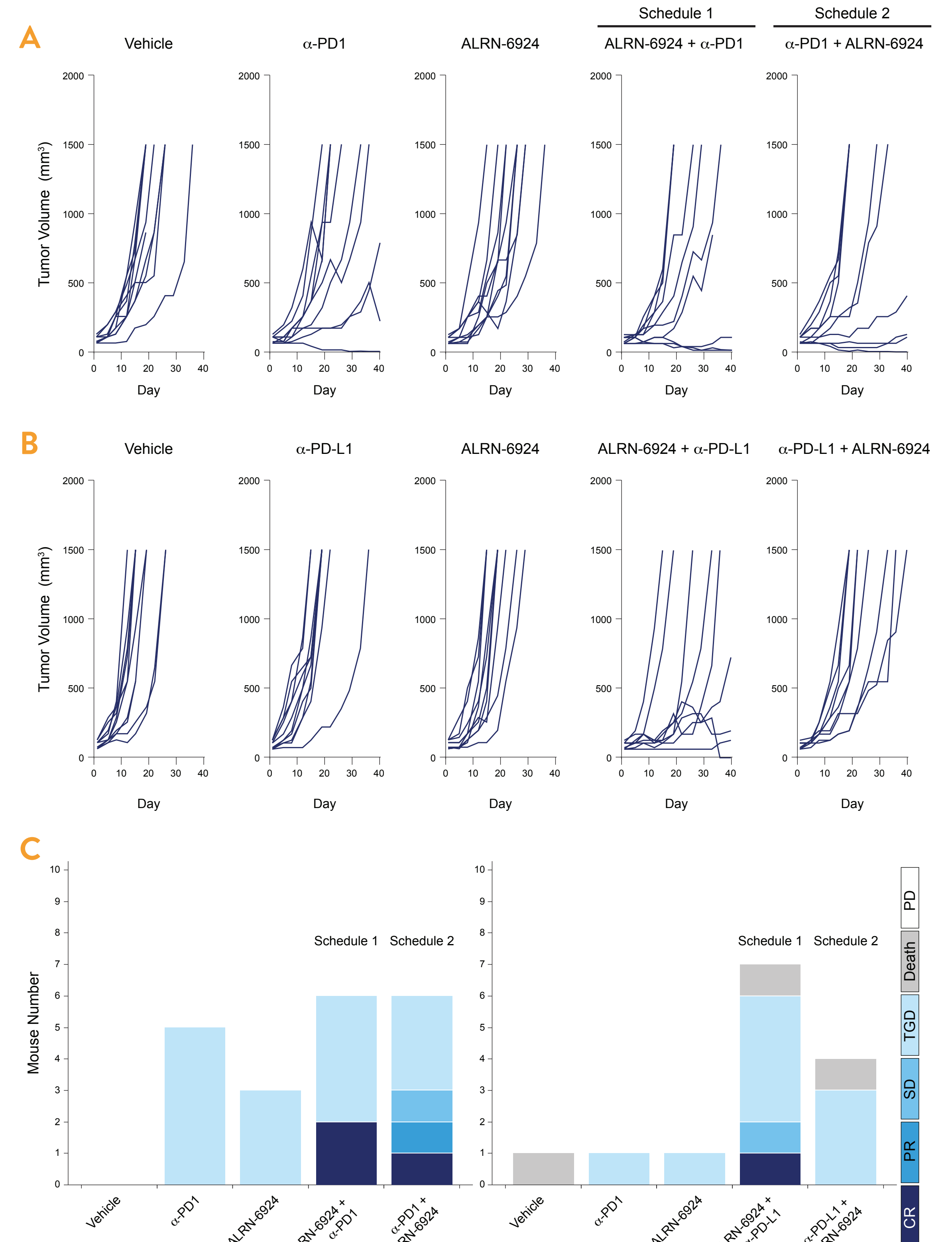


Figure 5: ALRN-6924 potentiates the effects of α - PD1 and α -PD-L1 in p53 Mutant Syngeneic Mouse Model (MC38 Colon Carcinoma)



Conclusions

ALRN-6924 is a first-in-class clinical stage MDMX/2 dual inhibitor displaying promising anti-tumor activity in patients^{3,4}. Recently, ALRN-6924 was shown to display strong on-mechanism anti-tumor activity in preclinical models of AML⁶ and PTCL⁷. 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

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