

The Investigational Peptide Drug ALRN-6924, a Dual Inhibitor of MDMX and MDM2, is an Effective Myelopreservation Agent

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Abstract

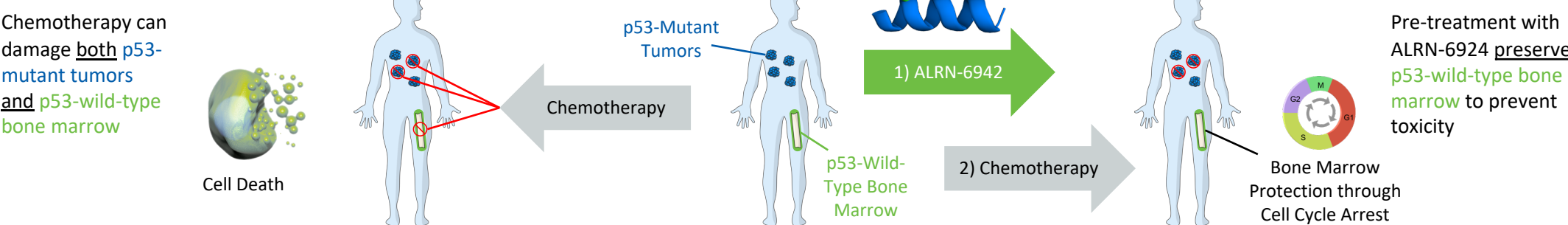
Aim: We investigated whether p53 activation with ALRN-6924 in normal, healthy cells can prevent or reduce chemotherapy-induced hematopoietic and gastrointestinal (GI) toxicity while preserving or enhancing anti-tumor efficacy of chemotherapy in p53-mutant tumors.

Background: ALRN-6924 is a clinical-stage, first-in-class, stabilized cell-permeating alpha-helical peptide that disrupts the interaction of the p53 tumor suppressor protein with its endogenous inhibitors, MDMX and MDM2. For p53 wild-type cells such as normal bone marrow, p53 activation can induce temporary cell cycle arrest, reducing sensitivity to chemotherapy-induced toxicity. For p53-mutant cancer cells, ALRN-6924 has no effect on the cell cycle, leaving them vulnerable to chemotherapy.

Materials and Methods: ALRN-6924-induced cell cycle arrest was measured by flow cytometry in human bone marrow CD34+ cells or MOLM13 cells following incubation with ALRN-6924 for 24 hours. DNA synthesis and DNA content were quantified by flow cytometry using EdU incorporation and Hoechst 33342 staining, respectively, and apoptosis quantified by Annexin-V staining. Gene expression in the bone marrow of ALRN-6924-treated C57BL/6 mice was measured by qRT-PCR, and cell cycle arrest was measured by flow cytometry using EdU incorporation in lineage negative, c-Kit positive hematopoietic stem and progenitor cells. MIC-1, a biomarker of p53 activation, was measured in serum of ALRN-6924-treated mice by quantitative ELISA. Topotecan-induced DNA damage was measured in human bone marrow CD34+ cells by γH2AX incorporation following exposure to vehicle or ALRN-6924 for 24 hours to induce cell cycle arrest, then incubated with topotecan for an additional 24 hours following a wash-out step. Topotecan-induced neutropenia and GI toxicity were measured in female C57BL/6 mice following topotecan treatment on days 1-5 and either ALRN-6924 or vehicle on days 0-4. Female C57BL/6 mice bearing subcutaneous p53-mutant MC38 colon cancer syngeneic tumors and nu/nu mice bearing H69 or H211 small-cell lung cancer xenograft tumors were treated with ALRN-6924, vehicle and topotecan on the same dosing regimen as the toxicity model and followed until tumors reached a model-specific, prespecified endpoint of 1000-2000 mm³.

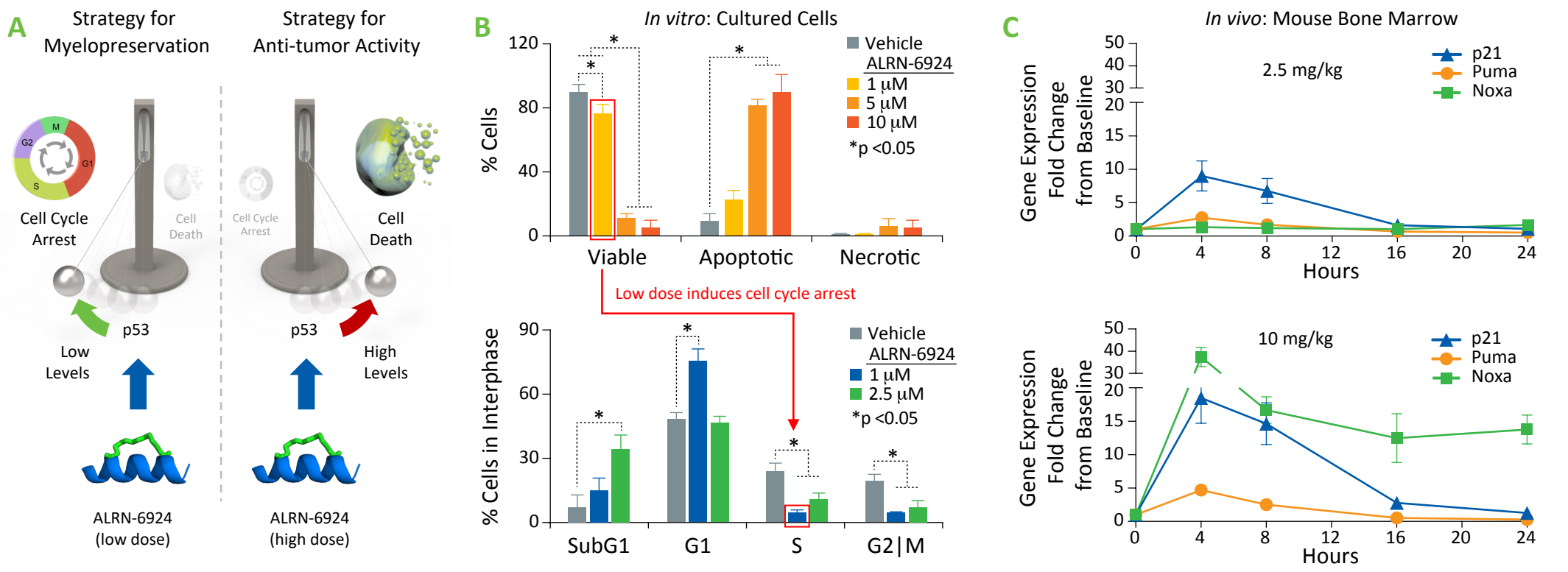
Results: ALRN-6924 induces transient, reversible cell cycle arrest in bone marrow cells *in vitro* and *in vivo*, and protects human bone marrow cells against topotecan-induced DNA damage *ex vivo*. Cell cycle arrest in mouse bone marrow correlated with serum levels of the p53 activation biomarker MIC-1. Consistent with this, activation of p53-target gene and cell cycle arrest marker, p21, but not apoptotic target genes (puma, noxa) correlated with cell cycle arrest at a low dose of ALRN-6924. In a mouse model of topotecan-induced toxicity, ALRN-6924 protected against neutrophil depletion and GI inflammation when daily administration of ALRN-6924 started 24 hours prior to the 1st dose and 30 minutes before each subsequent dose of topotecan. ALRN-6924 does not diminish, but instead enhances topotecan's anti-tumor activity in p53-mutant cancer models. Body weights and mortality data suggest ALRN-6924 and combinations with topotecan were tolerated at the doses tested.

Figure 1: Mechanistic Rationale for ALRN-6924 as a Myelopreservation Agent for Chemotherapy of p53-mutant Cancers



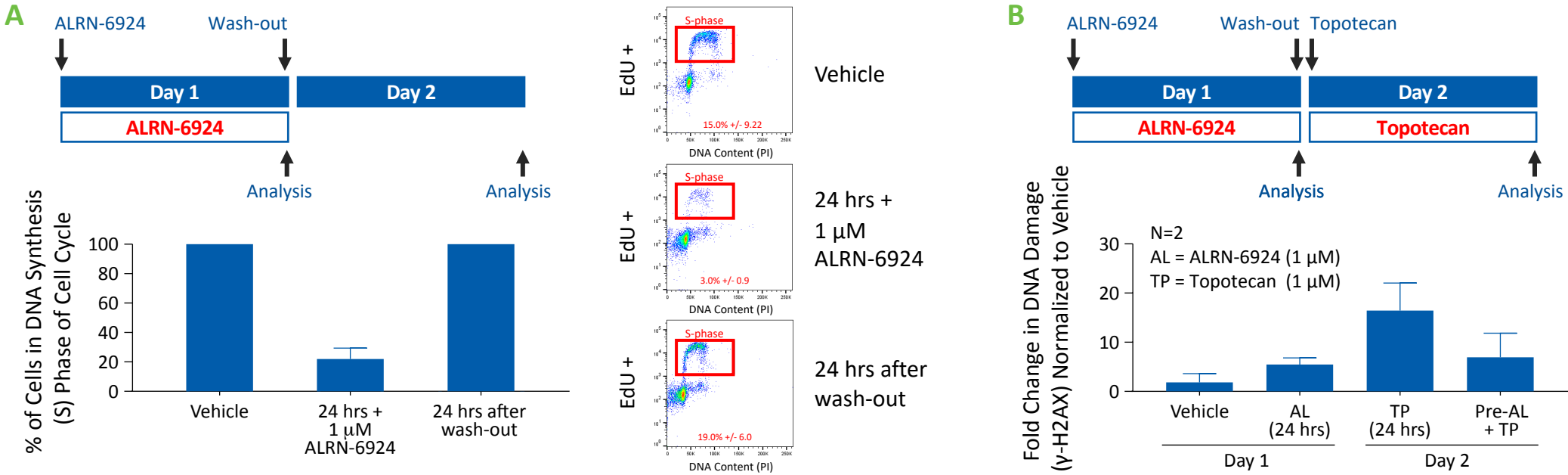
For p53 wild-type cells such as normal bone marrow, p53 activation can induce transient, dose-dependent cell cycle arrest, reducing sensitivity to chemotherapy-induced cellular toxicity. For p53-mutant cancer cells, ALRN-6924 has no effect on the cell cycle, leaving them vulnerable to chemotherapy.

Figure 2: ALRN-6924 Induces Cell Cycle Arrest or Apoptosis in a Dose Dependent Manner



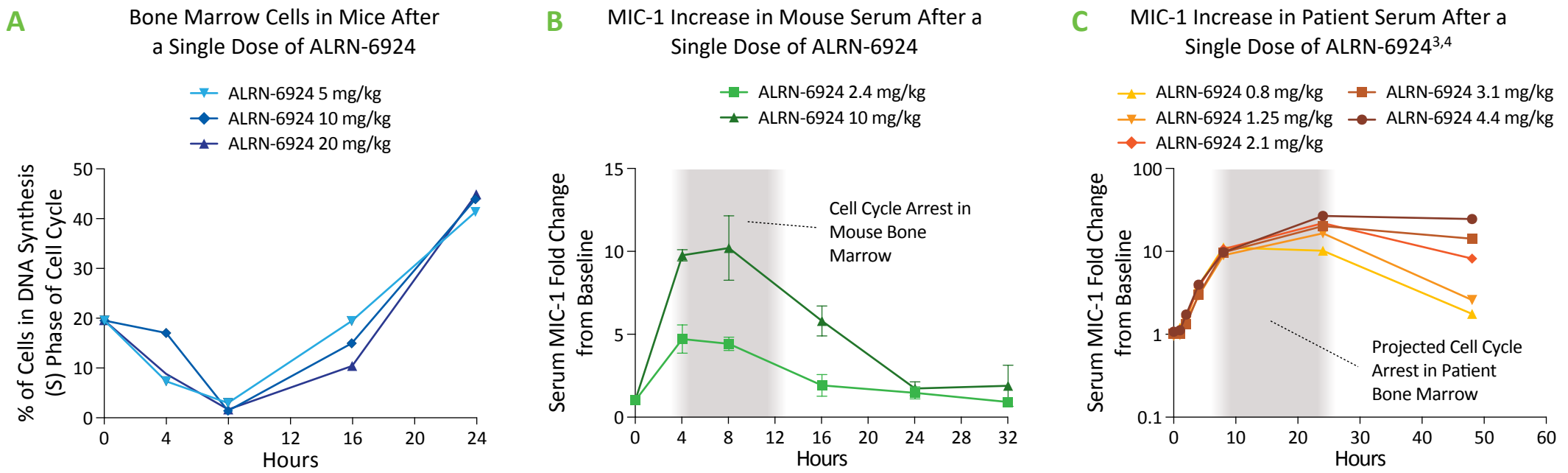
A) ALRN-6924, a cell-permeating, stabilized α-helical peptide that has demonstrated anticancer activity as monotherapy in clinical trials, mimics the p53 tumor suppressor protein to disrupt its interactions with both its endogenous inhibitors, MDMX and MDM2.^{1,4} ALRN-6924 can induce two distinct p53-effects: cell cycle arrest or cell death. Dose-dependent switching from growth arrest to apoptosis is well known in the p53 field.^{5,6} B) Low ALRN-6924 doses induce cell cycle arrest, high doses induce apoptosis in cultured p53-WT MOLM13 cells.⁷ C) Low dose ALRN-6924 (2.5 mg/kg) induces cell cycle arrest marker p21, high dose (10 mg/kg) induces pro-apoptotic markers noxa and puma in mouse bone marrow. ALRN-6924 dosed IV at t=0, then blood, bone marrow, and serum sampled at 4, 8, 16, 24 hours post-dose.

Figure 3: ALRN-6924 Protects Healthy Human Bone Marrow CD34+ Cells Against Topotecan-induced DNA Damage



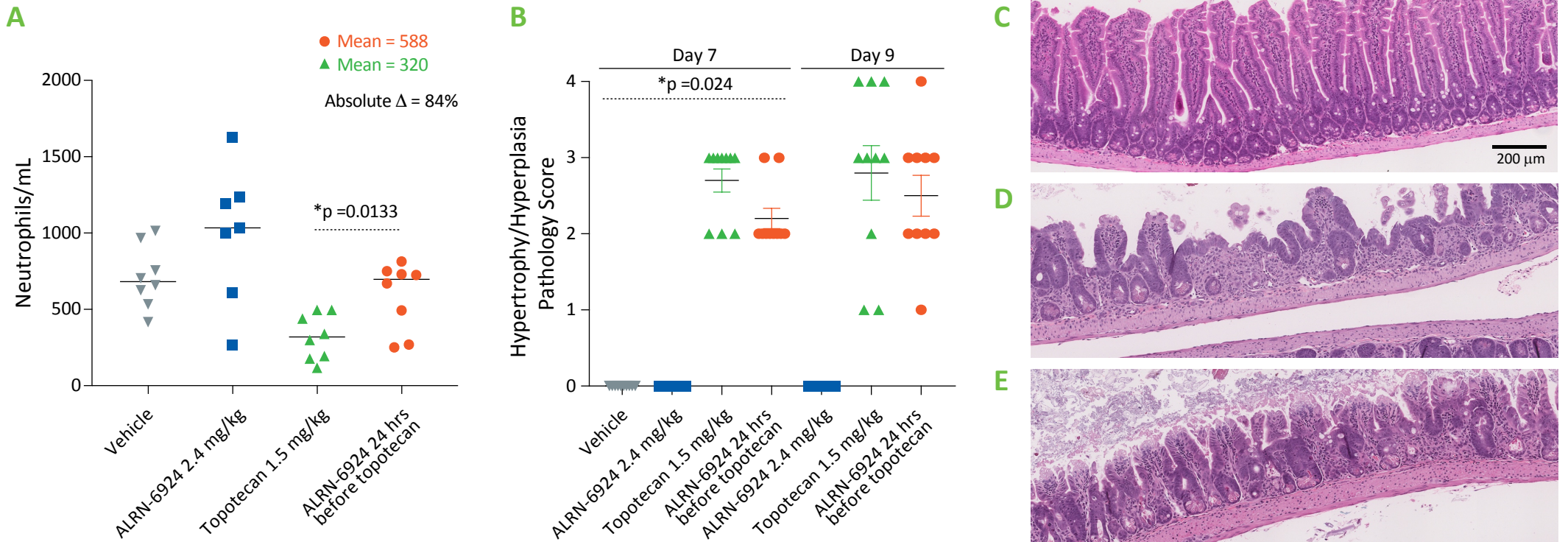
A) ALRN-6924 induces reversible cell cycle arrest in healthy human bone marrow cells. ALRN-6924-induced cell cycle arrest was measured by flow cytometry in human bone marrow CD34+ cells following incubation with ALRN-6924 *ex vivo* for 24 hours. B) ALRN-6924 protects healthy human bone marrow cells against topotecan-induced DNA damage *in vitro*. Topotecan-induced DNA damage was measured in human bone marrow CD34+ cells by γH2AX incorporation following exposure to vehicle or ALRN-6924 for 24 hours to induce cell cycle arrest, then incubated with topotecan for an additional 24 hours following a wash-out step.

Figure 4: ALRN-6924 Induces Transient, Reversible Cell Cycle Arrest in Mouse Bone Marrow Cells *in vivo*, which Correlates with Serum Levels of MIC-1, a Biomarker for p53 Activation



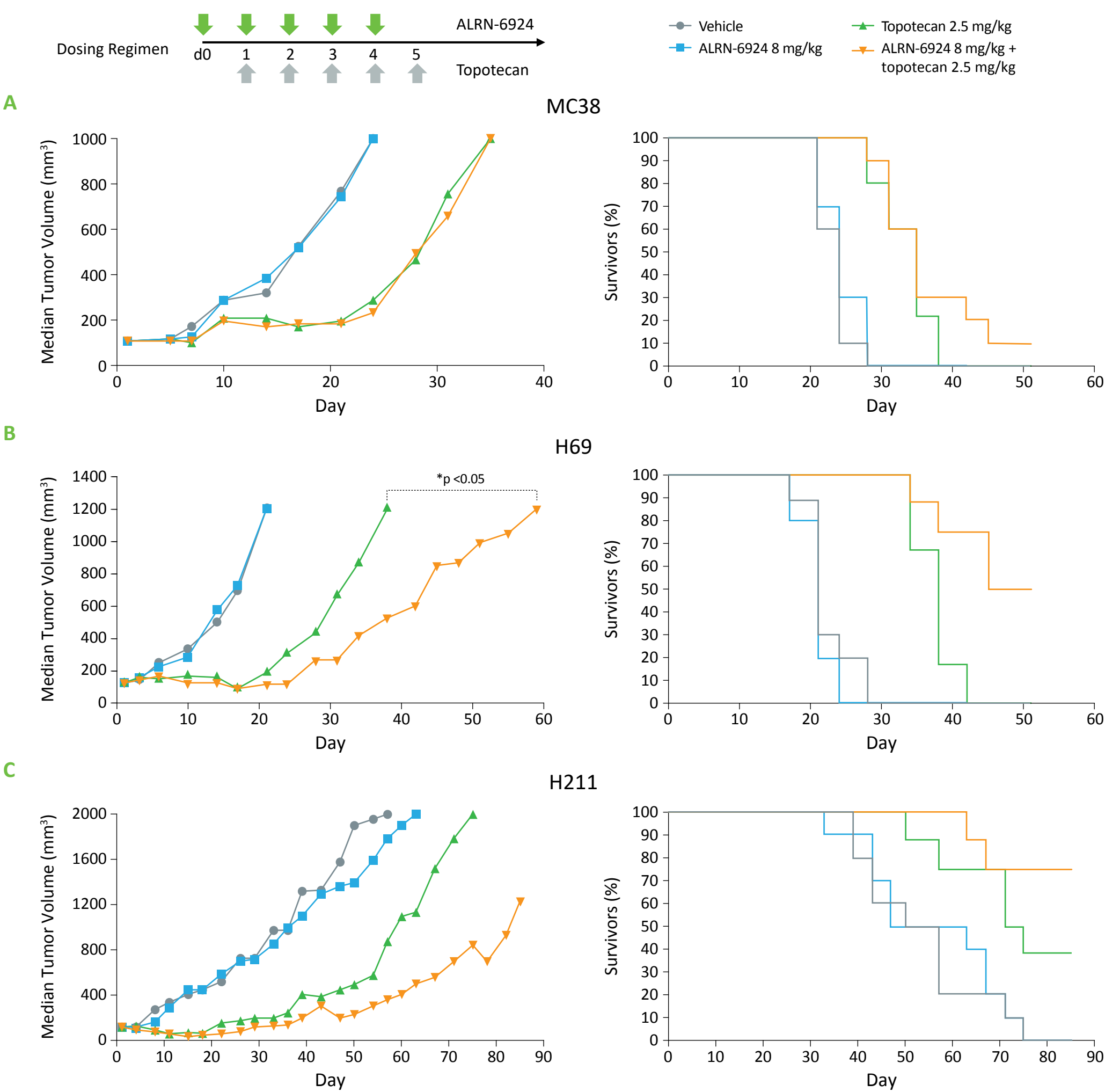
A) Cell cycle arrest in the bone marrow of ALRN-6924-treated C57BL/6 mice was measured by flow cytometry using EdU incorporation in lineage negative, c-Kit positive hematopoietic stem and progenitor cells. B) Murine MIC-1 levels peak between 4 and 8 hours post treatment with ALRN-6924 *in vivo*. MIC-1 levels in the serum of ALRN-6924-treated C57BL/6 mice were measured by ELISA. ALRN-6924-induced MIC-1 expression correlates with cell cycle inhibition *in vivo* and reflects the 2-hour plasma half-life of ALRN-6924 in mouse. C) MIC-1 levels in ALRN-6924 dose-escalation patients^{3,4} predict cell cycle arrest in patient bone marrow.

Figure 5: ALRN-6924 Protects Against Neutrophil Depletion and GI Toxicity when Administered Prior to Topotecan



A) Topotecan-induced neutropenia was measured in female C57BL/6 mice following topotecan treatment on days 1-5 and either ALRN-6924 or vehicle on days 0-4. B) ALRN-6924 protects against topotecan-induced GI toxicity in when administered prior to topotecan. C) GI tract in vehicle-treated mouse. D) GI tract in topotecan-treated mouse shows marked epithelium hypertrophy/hyperplasia; moderate expansion of lamina propria, moderate crypt loss. E) GI tract in topotecan-treated mouse with ALRN-6924 pre-treatment shows less extensive hypertrophy/hyperplasia; mild crypt loss.

Figure 6: ALRN-6924 Does Not Diminish, and Moderately Enhances, Topotecan's Anti-Tumor Activity in p53-Mutant Mouse Cancer Models



C57BL/6 mice (n=10) bearing established syngeneic MC38 colon cancer tumors (A) or athymic nu/nu mice bearing established H69 (B) or H211 (C) small-cell lung cancer xenograft tumors were treated with topotecan on days 1-5 and either ALRN-6924 or vehicle on days 0-4. Studies were approved by the Institutional Animal Care and Use Committee at Charles River Laboratories, Morrisville, N.C. The combination of ALRN-6924 with topotecan yielded enhancement of survival in these p53-mutant models, possibly due to p53-mediated immune activation.⁴

Conclusions

ALRN-6924 reduces chemotherapy-induced hematopoietic toxicity in healthy human bone marrow cells *ex vivo* and in mouse models of topotecan-induced neutropenia and GI toxicity *in vivo*, while enhancing anti-tumor efficacy in p53-mutant tumors when administered intravenously prior to topotecan chemotherapy. Cell cycle arrest in mouse bone marrow correlated with serum levels of the p53 activation biomarker MIC-1 and cell cycle arrest marker, p21. These results support the first ALRN-6924 clinical trial for myelopreservation in topotecan-treated small-cell lung cancer patients (NCT04022876). Additional studies are underway to support ALRN-6924 as a tumor type-agnostic myelopreservation agent for cancer patients with tumors bearing p53 mutations who are treated with chemotherapy.

References

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