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

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Aim: We investigated whether p53 activation with ALRN-6924 can prevent or reduce chemotherapy-induced hematopoietic 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 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.

Materials and methods: 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. DNA synthesis and DNA content were quantified by flow cytometry using EdU incorporation and Hoechst 33342 staining, respectively. 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, Kit positive hematopoietic stem and progenitor cells. Topotecan-induced DNA damage was measured in human bone marrow CD34+ cells by H2γX 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 was 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 syngeneic tumors were treated with ALRN-6924, vehicle and topotecan on the same dosing regimen and followed until tumors reached >1000mm^3.

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*. In a mouse model of topotecan-induced neutropenia, ALRN-6924 protected against neutrophil depletion when daily administration started 24 hours prior to the 1st dose and 30 minutes before each subsequent

dose of topotecan. ALRN-6924 does not diminish topotecan's anti-tumor activity in the p53-mutant MC38 syngeneic mouse cancer model, with the ALRN-6924 + topotecan combination yielding modest enhancement of survival. Body weights and mortality data suggest ALRN-6924 and combinations with topotecan were tolerated at the doses tested.

Conclusions: ALRN-6924 reduces chemotherapy-induced hematopoietic toxicity in healthy human bone marrow cells *ex vivo* and in mouse models of topotecan-induced neutropenia *in vivo*, while preserving or enhancing anti-tumor efficacy in p53-mutant tumors when administered intravenously prior to chemotherapy. 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.