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 H2yX 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 >1000mm³.

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.