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

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Abstract

The objective was to determine whether p53 activation with ALRN-6924 in normal, healthy cells can prevent or reduce chemotherapy-induced hematopoietic and gastrointestinal (GI) toxicity while preserving anti-tumor efficacy of chemotherapy in p53-mutant tumors.

Background: ALRN-6924 is a clinical-stage, first-in-class, stapled alpha-helical peptide that disrupts the interaction of the p53 tumor suppressor protein with its endogenous partners, MDMX and MDM2. For p53 wild-type tumor models, p53 activation can reduce transient cellular 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 cellular cycle arrest was measured by flow cytometry in human bone marrow 53+ cells or MOLM13 cells following incubation with ALRN 6924 for 24 hours. DNA synthesis and DNA content were quantified by flow cytometry using PI incorporation and HeLa 3G24 staining, respectively, and apoptosis quantified by Annexin V staining. Gene expression in the bone marrow of ALRN-6924-treated C57BL6 mice was measured by qRT-PCR, and cell cycle arrest was measured by flow cytometry using PI incorporation in in vitro experiments. p53-positive hematopoietic progenitor cell (HPC) proliferation and maximal percentage of p53-HPCs 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 PARP incorporation following exposure to vehicle or ALRN-6924 for 24 hours in a reversible cell cycle, then measured in female C57BL6 mice following topotecan treatment on days 5 and 6. Female C57BL6 mice bearing substantial 53+ murine MOLM13 xenografts were treated with ALRN-6924, vehicle and topotecan on the same dose regimen as the toxicity model and followed until tumors reached a model-specific, predetermined endpoint of 3000 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 activator biocytin 1 nt 5. Consistent with this, subcutaneous administration of ALRN-6924 to BALB/c mice induced a transient increase in p53 targets, such as g-ras, correlated with cell cycle arrest at a dose of ALRN-6924. In a mouse model of topotecan-induced toxicity, ALRN-6924 protected against neutrophil depletion and GI inflammation daily administration of ALRN-6924 started 24 hours after 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. Myelopreservation with 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