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The Investigational Chemoprotection Drug ALRN-6924, a Dual Inhibitor of MDMX and MDM2, Shows Potential for Radioprotection

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#P211

Disclosures:
The authors are employees of Aileron Therapeutics, Inc., Boston, MA, USA.
This presentation does not discuss off-label use and/or investigational use of a commercial product/device.

Summary

Aim: We investigated whether p53 activation with ALRN-6924 can prevent toxicities in mouse models of acute radiation injury.

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. In previous studies, ALRN-6924 induced transient, dose-dependent cell cycle arrest in the bone marrow of mice and in healthy human volunteers. In small-cell lung cancer patients and in xenograft tumor-bearing mice receiving topotecan chemotherapy, ALRN-6924 protected bone marrow cells while p53-mutant cancer cells remained susceptible to chemotherapy. Because radiation (like chemotherapy) preferentially affects proliferating cells, we hypothesized that ALRN-6924 may also protect proliferating cells in normal tissues from radiation-induced cellular toxicity.

Methods: Serum levels of macrophage inhibitory cytokine-1 (MIC-1), a biomarker of p53 activation, were measured by ELISA. Cell cycle arrest was measured in the bone marrow of ALRN-6924-treated C57BL/6 mice by flow cytometry using EdU incorporation. Biomarkers of cell proliferation (Ki67), p53-mediated cell cycle arrest (p21), and apoptosis (cleaved PARP) were measured in formalin-fixed mouse bone marrow and GI tract by immunohistochemistry (IHC). C57BL/6 mice (n=7/group) were treated with one or more intravenous 2.4 mg/kg doses of ALRN-6924 at 24, 16, 8, or 1 hour (or combinations thereof) or placebo prior to an abdominally targeted (shielded body) 15 Gy radiation dose and then monitored for body weight (BW). Animal studies were approved by the Institutional Animal Care and Use Committee at Charles River Laboratories, Morrisville, NC.

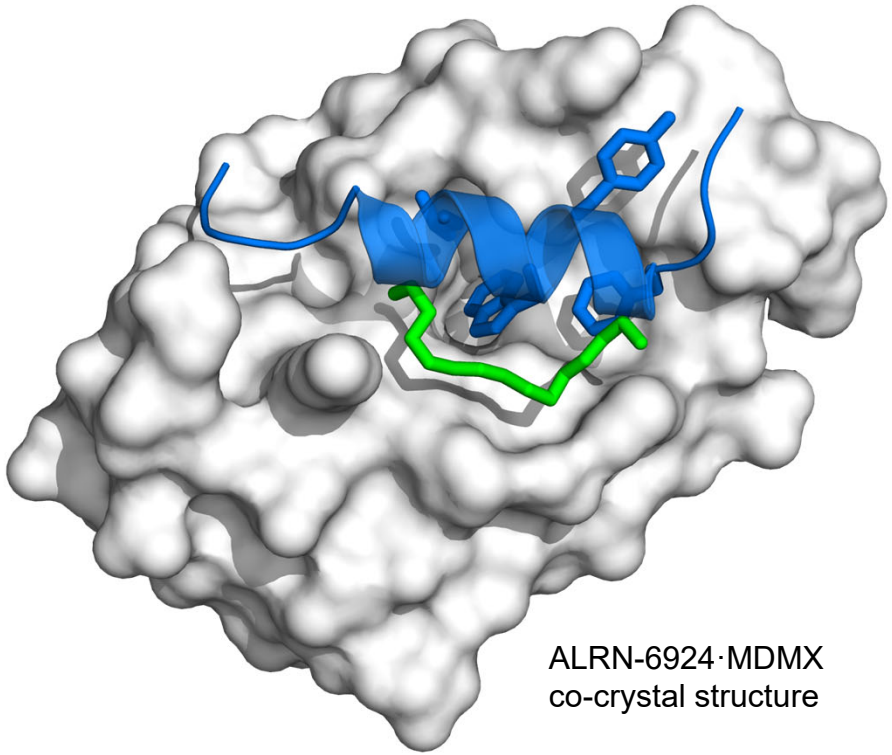
Summary

Results: MIC-1 was elevated in the serum of ALRN-6924-treated mice in a dose-dependent fashion. Repeated doses of ALRN-6924 every 8 hrs yielded sustained MIC-1 elevation, which correlated with increased p21 positivity and reduced Ki67 positivity in the bone marrow, while treatment-dependent changes in cPARP expression were evident, but minimal in magnitude. p21 positivity was increased in jejunum as well. In a nonlethal radiation exposure model, ALRN-6924 yielded significant protection from radiation-induced BW loss in a schedule-dependent manner. Mice receiving one or more doses of ALRN-6924 8 hrs prior to irradiation had an average of 4% BW loss, while placebo-treated mice showed 10% to 15% BW loss five days after irradiation, ($p=0.008$, two-sided t test).

Conclusions:

- ALRN-6924 mitigates body weight loss in a mouse model of acute radiation injury.
- The observed radioprotection effect correlates with pharmacodynamic markers of cell proliferation and cell cycle arrest after one or more doses of ALRN-6924, consistent with previous nonclinical and clinical demonstrations of chemoprotection with ALRN-6924.
- These results provide a rationale to further investigate ALRN-6924 as a radioprotective agent.

Structure and Key Design Properties of ALRN-6924



ALRN-6924·MDMX
co-crystal structure

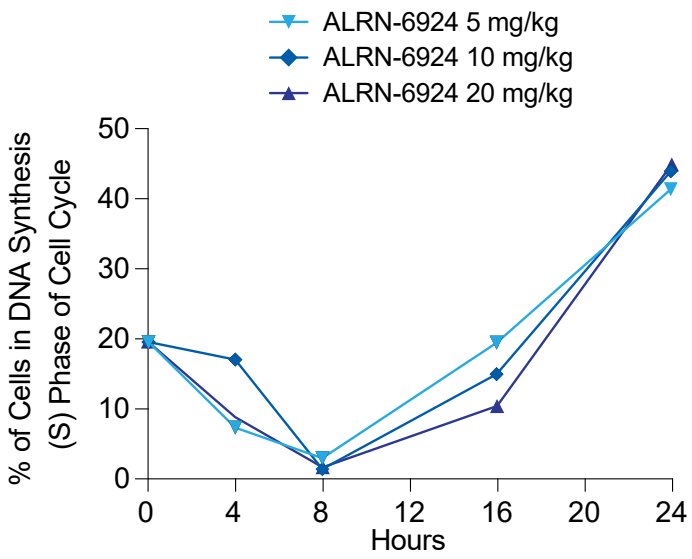
Structure is based on the N-terminal α-helical domain of p53, with a hydrocarbon staple and other chemical modifications to ensure:

- I Protection from proteolytic cleavage
- II Permeation of cell membranes and cell entry
- III High affinity binding to its targets
- IV Preclinical and clinical on-target, on-mechanism effects

ALRN-6924 Induces Cell Cycle Arrest in Mouse Bone Marrow That Correlates With Serum Biomarker MIC-1

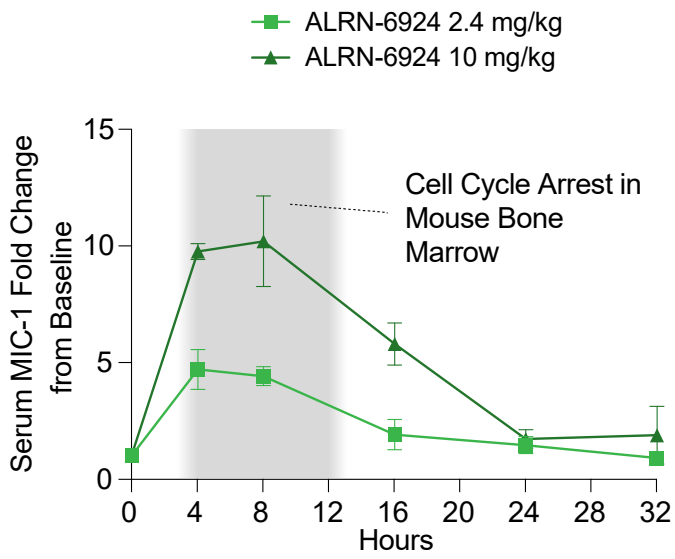
A

Bone Marrow Cells (Lin$^-$, Kit$^+$) in Mice After a Single Dose of ALRN-6924



B

MIC-1 Increase in Mouse Serum After a Single Dose of ALRN-6924

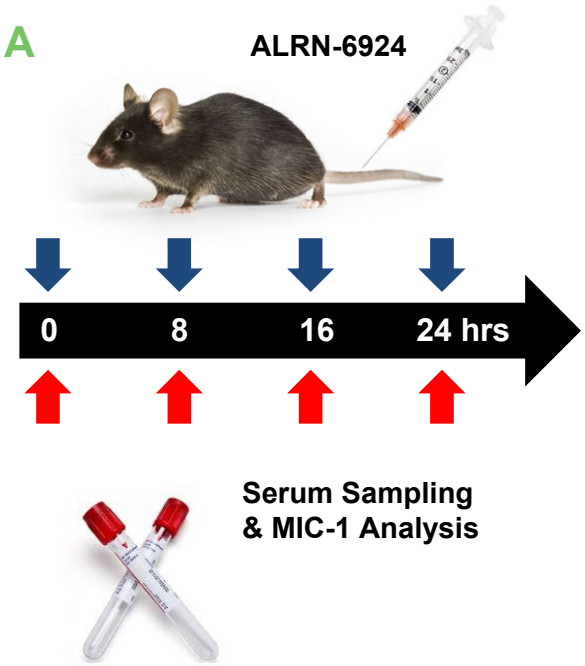


A) Cell cycle arrest in the bone marrow of ALRN-6924-treated C57BL/6 mice reached a maximum at 8 hrs after treatment in lineage-negative, c-Kit positive hematopoietic stem and progenitor cells.

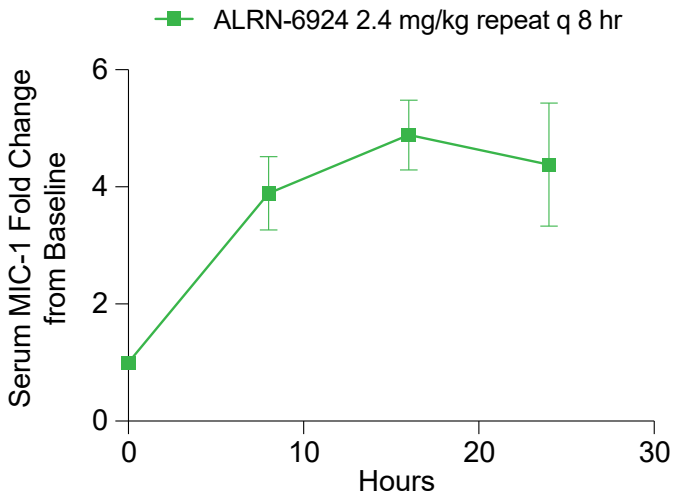
B) Levels of serum MIC-1, a biomarker of p53 activation, peak between 4 and 8 hours after treatment with ALRN-6924 *in vivo*, which correlates with cell cycle arrest in bone marrow.

Repeated Doses of ALRN-6924 Every 8 Hrs Yield Sustained MIC-1 Elevation

A



B



A) C57BL/6 mice (n=20) were administered 2.4 mg/kg ALRN-6924 every 8 hrs with n=5 sampled per time point for serum MIC-1 analysis

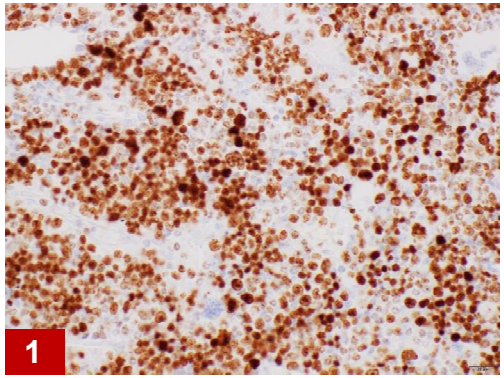
B) Murine MIC-1 levels demonstrated sustained elevation following repeated dosing every 8 hrs

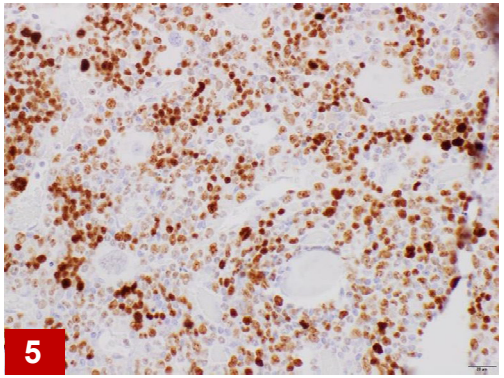
Multiple ALRN-6924 Dose Regimens Were Evaluated for Bone Marrow and GI Tract Cell Cycle Arrest, Cell Proliferation, and Apoptosis Biomarkers

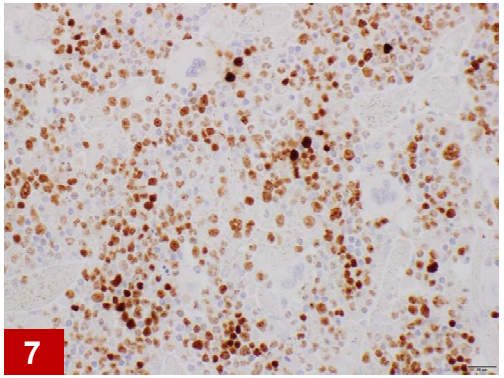
		Dosing Relative to Sampling					
Group	Treatment	-24	-16	-8	-1	0	hr
1	Vehicle						
2	ALRN-6924 2.4mg/kg						
3	ALRN-6924 2.4mg/kg						
4	ALRN-6924 2.4mg/kg						
5	ALRN-6924 2.4mg/kg						
6	ALRN-6924 2.4mg/kg						
7	ALRN-6924 2.4mg/kg						

C57BL/6 mice (n=3/group) were treated with one or more doses/schedules of ALRN-6924 prior to collection of femur and jejunum for IHC analysis of cell proliferation (Ki67), p53-mediated cell cycle arrest (p21), and apoptosis (cPARP) biomarkers. Pathology analysis was conducted in a blinded fashion.

Bone Marrow Staining for Cell Proliferation Marker Ki67 Was Lowest for | -8 hr | and | -16, -8 hr | Schedules

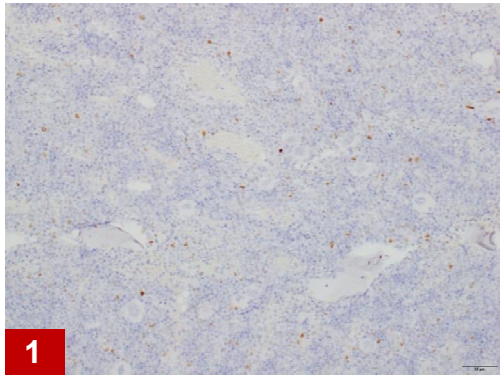


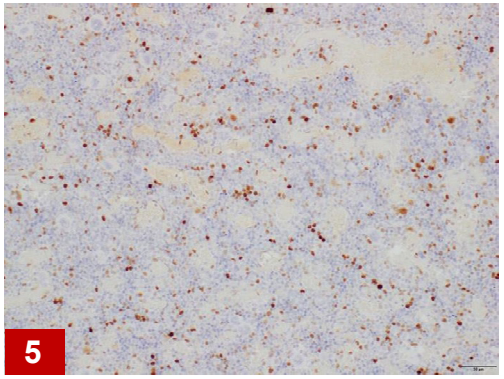


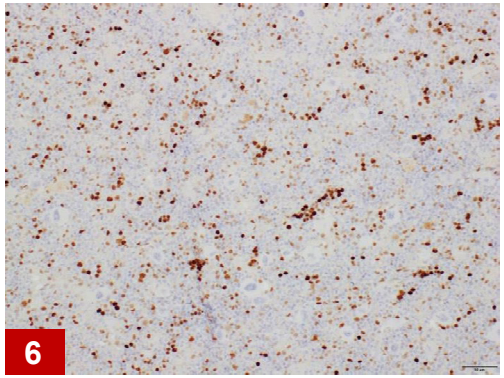


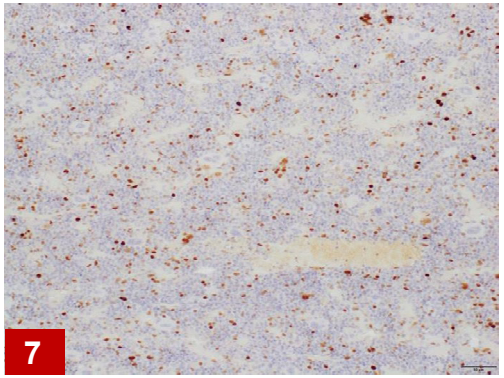
Group	Treatment	Dosing Relative to Sampling: -24 hr	-16 hr	-8 hr	-1 hr	0 hr
1	Vehicle	■			■	■
2	ALRN-6924 2.4mg/kg	■			■	■
3	ALRN-6924 2.4mg/kg	■				■
4	ALRN-6924 2.4mg/kg		■			■
5	ALRN-6924 2.4mg/kg			■		■
6	ALRN-6924 2.4mg/kg	■		■		■
7	ALRN-6924 2.4mg/kg		■	■		■

Bone Marrow Staining for Cell Cycle Arrest Marker p21 Was Highest for | -8 hr |, | -24, -8 hr |, | -16, -8 hr | Schedules





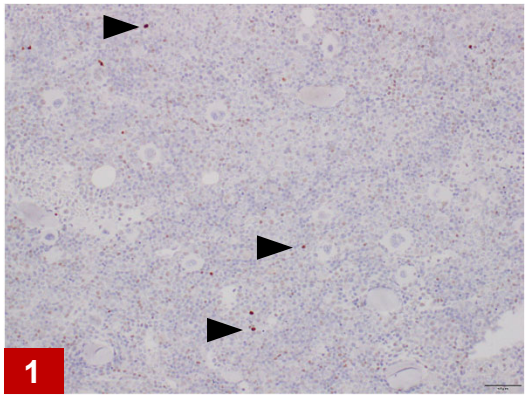




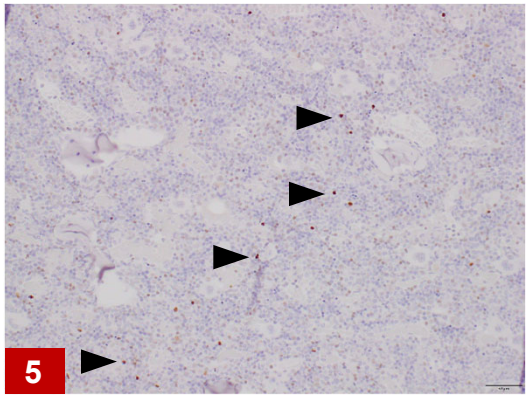
Group	Treatment	Dosing Relative to Sampling: -24 hr	-16 hr	-8 hr	-1 hr	0 hr
1	Vehicle	■			■	■
2	ALRN-6924 2.4mg/kg	■			■	■
3	ALRN-6924 2.4mg/kg	■				■
4	ALRN-6924 2.4mg/kg		■			■
5	ALRN-6924 2.4mg/kg			■		■
6	ALRN-6924 2.4mg/kg	■		■		■
7	ALRN-6924 2.4mg/kg		■	■		■

Sparse Staining for cPARP in Bone Marrow with Treatment-dependent Trends for | -8 hr | and | -16, -8 hr | Schedules

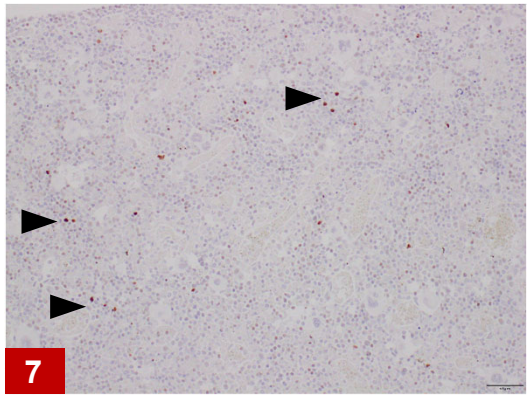
Group 1: Vehicle



Group 5: | -8 hr |

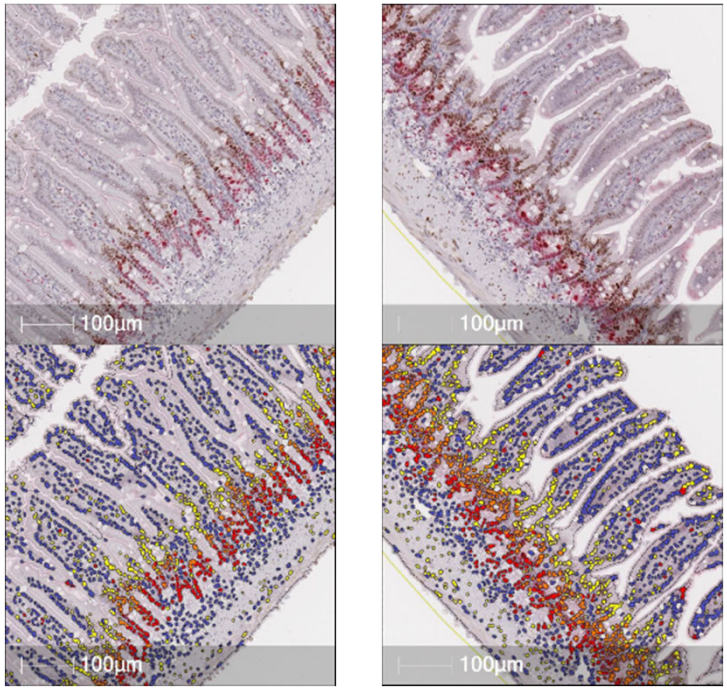


Group 7: | -16, -8 hr |



ALRN-6924 Upregulates p21, a Biomarker of p53-mediated Cell Cycle Arrest, in the GI Tract of Mice

Group 1: Vehicle | **Group 7: | -16, -8 hr |**



Dual Chromogen Staining
Brown: p21-positive
Red: Ki67-positive

False Color Image
Blue: Negative nuclei
Yellow: p21-positive
Orange: p21 + Ki67
Red: Ki67-positive

Formalin-fixed jejunum were co-stained for p21 (cell cycle arrest) and Ki67 (proliferation) and analyzed by multicolor chromogenic IHC.

In vehicle-treated mice, Ki67 staining is marked in crypt cells, with mild p21 staining evident in rapidly cycling, transit-amplifying cells that differentiate as they migrate out of the crypts onto the villi.

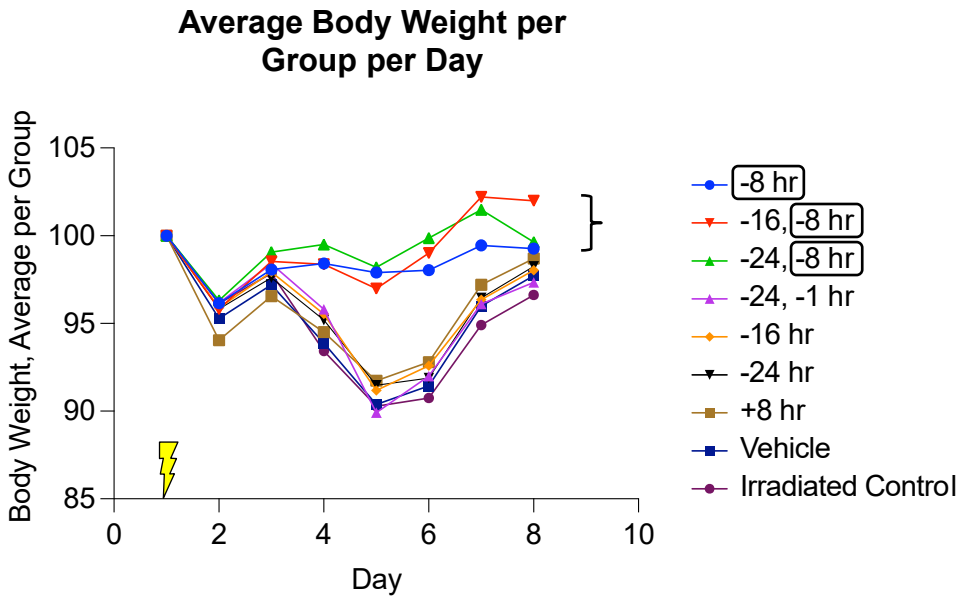
Treatment with ALRN-6924 yields markedly more p21 staining in rapidly cycling, transit-amplifying cells than in placebo-treated mice.

Multiple ALRN-6924 Dose Regimens Were Evaluated for Reduction in Radiation-induced Mortality and BW Loss

		Dosing Relative to Irradiation						
Group	Treatment	-24	-16	-8	-1	0	8	hr
1	Irradiated Control							
2	Vehicle							
3	ALRN-6924 2.4mg/kg							
4	ALRN-6924 2.4mg/kg							
5	ALRN-6924 2.4mg/kg							
6	ALRN-6924 2.4mg/kg							
7	ALRN-6924 2.4mg/kg							
8	ALRN-6924 2.4mg/kg							
9	ALRN-6924 2.4mg/kg							

C57BL/6 mice (n=7/group) were exposed to a single dose of abdominally-targeted radiation at 15 Gy following one or more doses/schedules of ALRN-6924 and then monitored for body weight.

ALRN-6924 Protects Mice from Radiation-Induced Toxicity, with | -8 hr |, | -24, -8 hr |, | -16, -8 hr | Schedules Best



Body weight loss is significantly improved for mice receiving one or more ALRN-6924 doses at 8 hrs prior to irradiation

Conclusions

- ALRN-6924 mitigates body weight loss in a mouse model of acute radiation injury.
- The observed radioprotection effect correlates with pharmacodynamic markers of cell proliferation and cell cycle arrest after one or more doses of ALRN-6924, consistent with previous demonstrations of chemoprotection with ALRN-6924.
- These results provide a rationale to further investigate ALRN-6924 as a radioprotective agent.

Acknowledgments: We thank Inotiv for IHC analysis, Charles River Laboratories for *in vivo* studies, and Eric Smith for graphical assistance.