ALRN-6924 Induces Cell Cycle Arrest in Bone Marrow Stem Cells and Hair Follicles with Dose-Dependent Degree and Duration of Effects after a Single Infusion in Healthy Volunteers

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Background

ALRN-6924 is a cell-permeating, stabilized alpha-helical peptide that binds to endogenous p53 inhibitors MDMX and MDM2, thereby activating p53 and its transcriptional target p21 to cause cell cycle arrest (CCA). This effect is limited to cells with wild-type, functional p53. In cancer patients with tumors bearing mutated p53, ALRN-6924 treatment selectively induces CCA in normal cells, allowing chemotherapy to selectively target p53-mutant cancer cells that are actively cycling.

Figure 1. Chemoprotection by ALRN-6924

ALRN-6924 temporarily pauses cycling in healthy cells, shielding them from chemotherapy

ALRN-6924 does not interrupt cycling in p53-mutant cancer cells, thus not protecting them from chemotherapy

Healthy cells always have normal p53 and, thus, will be protected from chemotherapy

Cancer cells with mutant p53 will not be protected from chemotherapy

Materials and Methods

A Phase 1 study in heathy volunteers was conducted to evaluate ALRN-6924's pharmacokinetics (PK) and pharmacodynamics (PD). In Part 1 of the study (37 subjects; Voors-Pette et al, ESMO 2021), it was shown that a 1-hour intravenous (IV) ALRN-6924 infusion was safe, well tolerated, and transiently upregulated p21 in human bone marrow (BM) cells with minimal signal for apoptosis. In Part 2 of the study, CCA was directly measured in the BM as well as in hair follicles (HFs) in female subjects across a range of doses by 1-hour IV infusion and 3-minute bolus IV injection.

ALRN-6924 was administered as a single IV infusion or bolus injection at 0.3, 0.6, or 0.9 mg/kg to cohorts of 3 to 9 subjects and compared to placebo. Subjects were evaluated for safety and tolerability. Plasma and serum samples were obtained to determine PK and levels of macrophage inhibitory cytokine-1 (MIC-1), a biomarker of p53 activation. BM was sampled 12 hours post-dose to directly measure CCA by flow cytometry in CD34+, lineage-negative BM stem cells. Occipital scalp skin was sampled by 2 mm punch biopsy for p21 immunohistochemistry in HFs.

Results

As of September 13, 2022, an additional 41 subjects (ages 18-58; 100% female) were evaluated in Part 2. Subjects experienced only mild, transient adverse events (AEs), with nausea/vomiting as the most frequent related AEs. The degree and duration of serum MIC-1 elevation was dose-dependent, indicating more durable p53 activation at higher ALRN-6924 doses. At 12 hours post-dose, the proportion of cycling BM stem cells was significantly reduced at all dose levels. Blinded pathology review suggested ALRN-6924-dependent p21 induction in anagen-phase HFs. Safety profiles, PK and PD were similar for both bolus and infusion.

Figure 2. Study Objectives

Evaluate the safety, tolerability, and PK of ALRN-6924 administered as a 1-hour IV infusion or 3-minute bolus injection
Bolus administration may simplify dosing vs. the current 1-hr infusion clinical regimen

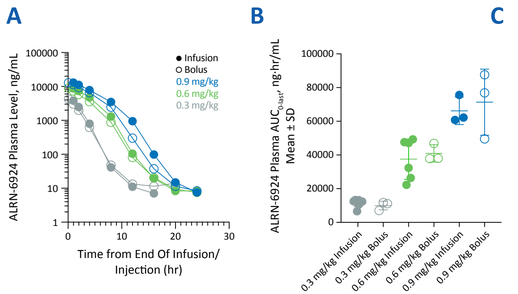
Demonstrate cell cycle arrest in bone marrow stem cells and dose-dependent duration of effect on serum biomarkers
Supports the potential of ALRN-6924 to prevent chemotherapy-induced neutropenia, thrombocytopenia, and anemia; informs optimal chemoprotection dose and regimen

Show activation of p21, a biomarker of cell cycle arrest, in hair follicles of female subjects
Supports the potential of ALRN-6924 to prevent chemotherapy-induced alopecia

Table 1. Population Characteristics

Parameter		Placebo N=14	IV Infusion ALRN-6924 0.3 mg/kg N=9	IV Infusion ALRN-6924 0.6 mg/kg N=6	IV Infusion ALRN-6924 0.9 mg/kg N=3	Bolus Injection ALRN-6924 0.3 mg/kg N=3	Bolus Injection ALRN-6924 0.6 mg/kg N=3	Bolus Injection ALRN-6924 0.9 mg/kg N=3	Overall N=41
AGE (median)		24	27	30	36	25	23	23	24
GENDER	Female	— *all female subjects* —							
RACE	Asian	1	0	0	0	0	0	0	1
	White	12	9	5	3	3	2	3	37
	Other	1	0	1	0	0	1	0	3
BASELINE HEIGHT (median, cm)		171	174	167	167	178	170	176	171
BASELINE WEIGHT (median, kg)		65	69	65	63	81	69	78	67
BODY MASS INDEX (median, kg/m²)		23	23	24	21	25	23	25	23

Figure 3. ALRN-6924 Plasma PK Shows Similar Exposure for 1-hr IV Infusion and 3-minute IV Bolus Injection



Dose	Route	C_{max} µg/mL	AUC_{last} µg·hr/mL	$t_{½}$ hr
0.9 mg/kg	Infusion	12.9 (9.9)	66.2 (12)	2.0 (5.1)
	Bolus	13.0 (16)	71.3 (27)	1.7 (5.1)
0.6 mg/kg	Infusion	9.0 (20)	37.5 (32)	2.0 (30)
	Bolus	8.8 (4.5)	40.9 (13)	1.9 (15)
0.3 mg/kg	Infusion	4.2 (2.2)	11.6 (12)	2.4 (93)
	Bolus	4.0 (20)	10.0 (20)	2.4 (74)

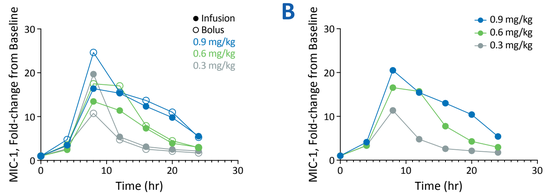
A) ALRN-6924 plasma pharmacokinetics show a similar plasma clearance profile by infusion and bolus dosing. Symbols at each time point are mean values across all subjects in the indicated dose cohort. (B, C) ALRN-6924 exhibits dose-proportional C_{max} and more-than-proportional AUC_{last}. Symbols are shown by subject; tabulated values are shown as Mean (%CV).

Table 2. Adverse Events Were Primarily Grade 1; No Grade ≥3 Events Were Reported; No SAEs or AEs Led to Discontinuation of Study Participation‡

Parameter		Placebo N=14	IV Infusion ALRN-6924 0.3 mg/kg N=9	IV Infusion ALRN-6924 0.6 mg/kg N=6	IV Infusion ALRN-6924 0.9 mg/kg N=3	Bolus Injection ALRN-6924 0.3 mg/kg N=3	Bolus Injection ALRN-6924 0.6 mg/kg N=3	Bolus Injection ALRN-6924 0.9 mg/kg N=3	All ALRN-6924-treated subjects N=27
TEAEs Occurring in >10% of Subjects									
ANY TEAE		8 (57)	8 (89)	6 (100)	3 (100)	3 (100)	2 (67)	3 (100)	25 (93)
NAUSEA	Grade 1	1 (7)	6 (67)	3 (50)	3 (100)	3 (100)	1 (33)	0	16 (59)
VOMITING	Grade 1	0	3 (33)	2 (33)	2 (67)	2 (67)	1 (33)	0	10 (37)
	Grade 2	0	0	0	0	0	0	3 (100)	3 (11)
BIOPSY SITE PAIN	Grade 1	6 (43)	0	1 (17)	0	3 (100)	1 (33)	0	5 (19)
HEADACHE	Grade 1	1 (7)	2 (22)	1 (17)	0	0	0	0	3 (11)
SAEs Occurring in Any Subject									
ANY SAE		0	0	0	0	0	0	0	0

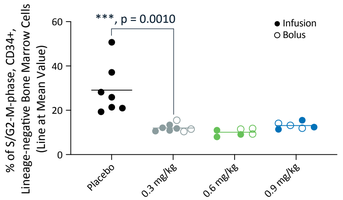
‡*Note*: Prophylactic antiemetics were not administered in this study. Antiemetics are mandated prior to chemotherapy/ALRN-6924 dosing in current ALRN-6924 chemoprotection clinical trials.

Figure 4. Serum MIC-1, a Biomarker of p53 Activation, is Transiently Elevated Following a Single Dose of ALRN-6924, with Dose-dependent Degree and Duration of Effect



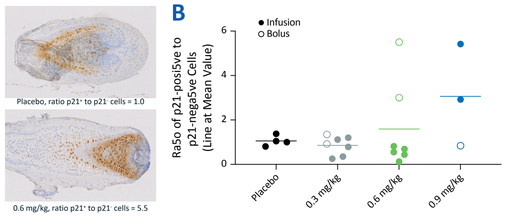
A) Higher ALRN-6924 doses yield more durable p53 activation, with similar results for 1-hour IV infusion and 3-minute bolus IV injection. B) For clarity, combined results for patients receiving either an infusion or bolus are shown by dose level. Symbols at each time point are mean values across all subjects in the indicated dose cohort.

Figure 5. A Single ALRN-6924 Dose Significantly Reduces the Proportion of Bone Marrow Stem Cells Undergoing Cell Division



Bone marrow was sampled 12 hrs post-dose for analysis by flow cytometry. Placebo-treated subjects have a mean of 29% CD34+, lineage-negative stem cells in the S/G2-M-phase of the cell cycle, consistent with independent reports.[2, 3] Patients treated with ≥0.3 mg/kg ALRN-6924 by infusion or bolus injection have >50% fewer dividing cells than of placebo values. Results are shown for 27 subjects yielding adequate BM samples for analysis. Significance by two-tailed t-test.

Figure 6. Analysis of Hair Follicles 12 hrs Post-dose Suggests Dose-dependent Elevation in p21 Protein Expression, a Biomarker of p53-induced Cell Cycle Arrest



Occipital scalp skin was sampled 12 hrs post-dose by 2 mm punch biopsy. A) Micrographs of p21 staining in a placebo-treated subject (top) vs. 0.6 mg/kg subject (bottom). B) Ratio of p21-positive to p21-negative hair matrix cells in anagen-phase HFs was determined by blinded pathology review. Average staining for 21 subjects yielding measurable HFs is shown by dose level, combining bolus- and infusion-dosed subjects. (Note: 2 mm punch biopsies yielded 0-2 measurable HFs per subject.)

Conclusions

ALRN-6924 shows a favorable safety profile for use as a selective chemoprotection agent. Cell cycle arrest measured in bone marrow supports prevention of chemotherapy-induced neutropenia, thrombocytopenia, and anemia, while hair follicle results support the potential of ALRN-6924 to prevent chemotherapy-induced alopecia. Safety profiles, PK and PD were similar for both bolus and infusion, supporting the use of a 3-minute IV bolus that may simplify administration vs. a 1-hr infusion. Prolonged elevation of serum MIC-1 levels at higher ALRN-6924 doses is indicative of prolonged cell cycle arrest in bone marrow and other tissues, and therefore prolonged chemoprotection. The dose-dependent degree and duration of effect supports development of a universal treatment schedule for broad use across cancer indications and types of chemotherapy.

References

1. Voors-Pette, et al. A Phase 1 study of the dual MDMX/MDM2 inhibitor, ALRN 6924, in healthy volunteers. ESMO Congress, 2021.
2. Zhang S., et al. Study of different phases in growth cycle of human bone marrow cells and their growth speed, using rat-human cell hybridization, premature chromosome condensation, and sister chromatid differentiation techniques. *Exp Hematol.* 16(3):221-5, 1988.
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Acknowledgements

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