A Phase 1 Study of the Dual MDMX/MDM2 Inhibitor, ALRN 6924, in Healthy Volunteers

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#1791P

Summary

Background: ALRN-6924 is a cell-permeating, stabilized alpha-helical peptide that that binds with high affinity to endogenous p53 inhibitors MDM2 and MDMX. Treatment with ALRN-6924 initiates p53 transcriptional activity, leading to cell cycle arrest. This effect is limited to cells with wild-type, functional p53; therefore, for cancer patients with tumors harboring mutated p53, pre-treatment with ALRN-6924 selectively induces cell cycle arrest in normal cells, thus allowing chemotherapy to preferentially target p53-mutant cancer cells that are actively cycling.

Materials and Methods: A three-part Phase 1 study in heathy volunteers is being conducted to evaluate ALRN-6924 pharmacokinetics (PK) and pharmacodynamics (PD). The objectives are as follows: In Part 1, to determine a dose of ALRN-6924 that initiates p53-mediated transcriptional regulation and yields transient cell cycle arrest via p21 induction in human bone marrow with minimal signal for apoptosis. In Part 2, to determine the time to onset, magnitude, and duration of bone marrow PD effects. In Part 3, to determine a repeat-dose regimen that extends the duration of cell cycle arrest in the bone marrow for chemoprotection from longer acting chemotherapies. Subjects are being evaluated for safety and tolerability; blood, skin, and bone marrow samples are obtained for PD evaluation.

Results: At the time of abstract submission, a total of 37 subjects (females and males aged 18-65) were enrolled and evaluated in Parts 1 and 2 of the trial. In Part 1, a total of 14 subjects (6 placebo, 4 each at 0.3 and 0.6 mg/kg of ALRN-6924) received one IV infusion of study drug, and samples were obtained 8 hours post dosing. Immunohistochemistry (IHC) analysis showed that both dose levels showed robust induction of p21, a p53-regulated mediator of cell cycle arrest, in bone marrow cells, with minimal signal for apoptosis compared to placebo. In Part 2, 23 subjects allocated to 8 groups received a one 0.3 mg/kg infusion of ALRN-6924. Samples were obtained at 4, 8, 12, 16, 20, 24, 36, and 48 hr post-infusion. The 0.3 mg/kg dose showed excellent tolerability: subjects experienced only mild, transient AEs. Robust p21 induction was observed in bone marrow cells, with peak expression between 4 hr and 16 hr following ALRN-6924 administration.

Figure 1: Study Schema - Clinical Study to Evaluate ALRN-6924 in Healthy Human Volunteers

PART 1 — **Determination of Optimal Dose**
3 groups of subjects were treated with a single dose of placebo (n=6), ALRN-6924 0.3 mg/kg (n=4), or ALRN-6924 0.6 mg/kg (n=4)

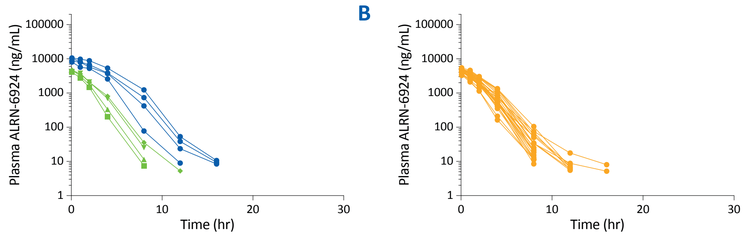
PART 2 — **Kinetics of Pharmacodynamic Effects in the Bone Marrow**
23 subjects were treated with a single dose of 0.3 mg/kg ALRN-6924, with bone marrow sampled from **3** subjects each at successive time points 4-48 hrs post-infusion

PART 3 — **Confirmation of a Universal Treatment Schedule for ALRN-6924**
16 subjects were treated in groups of **4**, each receiving 1, 2, 3, or 4 doses of ALRN-6924, with bone marrow sampled 18 hrs after each dose

Table 1: Population Characteristics

Parameter		Part 1: Placebo N=6	Part 1: ALRN-6924 0.3 mg/kg (N=4)	Part 1: ALRN-6924 0.6 mg/kg (N=4)	Part 2: ALRN-6924 0.3 mg/kg (N=23)	Total ALRN-6924 0.3 mg/kg (N=27)
AGE (median)		30	26	23	29	29
GENDER	Male	2	1	2	10	11
	Female	4	3	2	13	16
RACE	Asian	0	0	0	1	1
	Black	1	0	1	1	1
	White	5	4	3	21	25
BASELINE HEIGHT (median, cm)		168	178	179	174	174
BASELINE WEIGHT (median, kg)		71	73	67	72	72
BODY MASS INDEX (median, kg/m²)		23	23	22	24	23

Figure 2: ALRN-6924 Plasma Pharmacokinetics



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PK Attribute (% CV)	Part 1 n=4 at 0.3 mg/kg	Part 1 n=4 at 0.6 mg/kg	Part 2 n=23 at 0.3 mg/kg
$t_{½}$ (hr)	1.6 (20)	3.2 (24)	1.0 (27)
C_{max} (µg/mL)	4.5 (7)	9.5 (14)	4.5 (14)
AUC_{all} (µg·hr/mL)	9.6 (19)	36 (27)	12.1 (22)

A) Part 1: Plasma pharmacokinetics at 0.3 and 0.6 mg/kg (n=4 each) shows dose-dependent exposure increase. B) Part 2: low patient-to-patient variability across n=23 subjects. C) Slower clearance (longer $t_{½}$) at higher ALRN-6924 doses. Dose-proportional C_{max} and more-than-proportional AUC_{all}.

Table 2: Adverse Events: All Events Were of Grade 1 Severity; No SAEs or AEs Led to Discontinuation of Study Participation

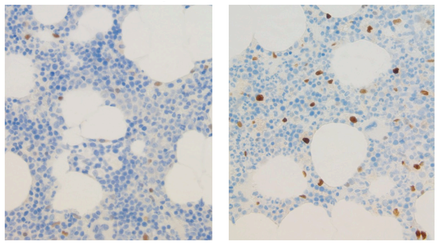
Parameter	Part 1: Placebo (N=6)	Part 1: ALRN-6924 0.3 mg/kg (N=4)	Part 1: ALRN-6924 0.6 mg/kg (N=4)	Part 2: ALRN-6924 0.3 mg/kg (N=23)	Total ALRN-6924 0.3 mg/kg (N=27)
TEAEs Occurring in >10% of Subjects					
ANY TEAE	3 (50)	2 (50)	4 (100)	21 (91)	23 (85)
NAUSEA	0	1 (25)	3 (75)	5 (22)	6 (22)
VOMITING	0	1 (25)	1 (25)	2 (9)	3 (11)
BIOPSY SITE PAIN	3 (50)	0	0	11 (48)	11 (41)
FATIGUE	0	0	1 (25)	1 (4)	1 (4)
HEADACHE	0	0	2 (50)	9 (39)	9 (33)
DIZZINESS	0	1 (25)	1 (25)	3 (13)	4 (15)
SAEs Occurring in >10% of Subjects					
ANY SAE	0	0	0	0	0

Following poster submission, a "totals" column mistakenly combining placebo- and ALRN-6924-treated subjects was removed

Figure 3: Both 0.3 and 0.6 mg/kg ALRN-6924 Doses Induce p21 (a Biomarker of p53-induced Cell Cycle Arrest) in 3-7% of Bone Marrow Cells†, but Only 0.6 mg/kg Induces Minimal Levels of Cleaved-PARP (a Marker of Apoptosis) in <1% of Cells

Subject	Group	p21, % at Differential Intensities† 0	1+	2+	3+	cPARP‡ # cells per HPF
1	Plac.	100	0	0	0	2
2	"	100	0	0	0	1
3	"	100	0	0	0	19
4	"	100	0	0	0	12
5	"	100	0	0	0	19
6	"	100	0	0	0	21
7	0.3 mg/kg	94	0	2	4	19
8	"	96	0	2	2	21
9	"	96	0	1	3	7
10	"	95	0	1	4	85
11	0.6 mg/kg	94	0	1	5	17
12	"	93	0	2	5	61
13	"	97	0	3	0	70
14	"	95	3	2	0	96

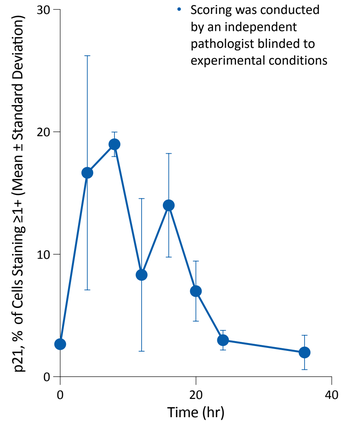
Representative p21 Staining



†Note: bone marrow is largely composed of quiescent stem cells and mature, nonproliferating cells; only 5% to 20% of cells are actively cycling.[1,2]

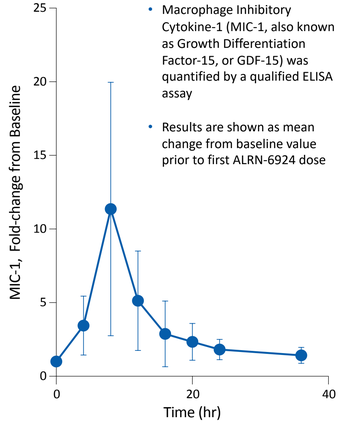
‡Due to low % of cells with positive staining, results are shown as actual number of cells per high-power field (HPF). Scoring in bone marrow core biopsies sampled 8 hrs post-dose was conducted by a pathologist blinded to experimental conditions.

Figure 4: IHC Analysis in Bone Marrow Shows p21 Protein Expression, a Biomarker of p53-induced Cell Cycle Arrest, is Transiently Elevated Up To 20 hrs Following a Single 0.3 mg/kg Dose of ALRN-6924



- Scoring was conducted by an independent pathologist blinded to experimental conditions

Figure 5: Serum MIC-1, a Biomarker of p53 Activation, is Transiently Elevated Up To 20 hrs Following a Single 0.3 mg/kg Dose of ALRN-6924



- Macrophage Inhibitory Cytokine-1 (MIC-1, also known as Growth Differentiation Factor-15, or GDF-15) was quantified by a qualified ELISA assay
- Results are shown as mean change from baseline value prior to first ALRN-6924 dose

Conclusions

- ALRN-6924 is a potent, specific agonist of wild-type p53, as evidenced by induction of p21 and MIC-1, which are p53-dependent
- This study in healthy human volunteers (Parts 1 and 2 completed to date) is consistent with prior PK-PD results from non-clinical species and confirms the mechanism of action of ALRN-6924 in human
- The time course of p21 and MIC-1 pharmacodynamics are similar, indicating both biomarkers are activated via the same mechanism with return to baseline levels of cell cycling in normal tissues following ALRN-6924 administration to achieve transient chemoprotection
- The time to onset, duration, and magnitude of PD effects inform dosing schedules to be used in clinical trials for selective chemoprotection in TP53-mutated cancer patients
- The healthy volunteer study of ALRN-6924 is ongoing, with the ultimate objective to develop a universal regimen (dose/schedule) that could be applied broadly across different cancer indications and types of chemotherapy

References

1. 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.
2. Morrison S.J. and Weissman I.L. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity.* 1(8):661-73, 1994.

Acknowledgments

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