Harnessing the Anticancer Activity of the Stapled Peptide ALRN-6924, a Dual Inhibitor of MDMX and MDM2, Using Rational Combination Strategies for Breast Cancer and Other Malignancies

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Background

The purpose was to identify rational anticancer drug combinations with ALRN-6924.

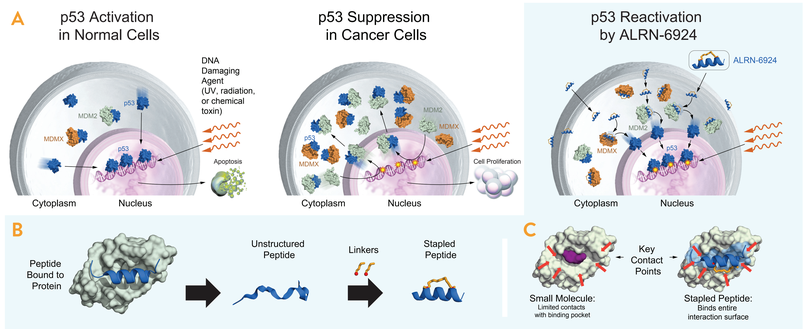
Material and Methods

ALRN-6924 is a cell-permeating, stabilized α-helical peptide that mimics the p53 tumor suppressor protein to disrupt its interactions with both its endogenous inhibitors, MDMX and MDM2. For p53 wild-type tumors, ALRN-6924 can restore p53-dependent tumor suppression leading to antitumor efficacy. ALRN-6924 was tested in combination with 29 drugs for synergistic *in vitro* anticancer activity. Select agents were further evaluated *in vivo*. Drugs listed in Table 1 were assayed in combination with ALRN-6924 using WST-1 and/or CyQUANT cell viability assays in immortalized TP53 wild-type cancer cell lines. Synergy was quantified by the Chou-Talalay combination index (CI) method. *In vivo* ALRN-6924 combinations with palbociclib, abemaciclib, and nab-paclitaxel were tested in the MCF-7 breast cancer model in athymic nude mice.

Results

All the drugs evaluated except dexamethasone were additive or synergistic with ALRN-6924 *in vitro*; no antagonism was observed. Pharmacodynamic biomarkers indicate on-mechanism activity. In MCF-7 xenografts tumor growth inhibition was improved when ALRN-6924 was given in combination with palbociclib, abemaciclib, or nab-paclitaxel compared to single agent therapy. Body weights and mortality data suggest ALRN-6924 and combinations with nab-paclitaxel and palbociclib were tolerated at the doses tested. The combination with abemaciclib was tolerated with interruption and dose-reduction.

Figure 1: The Cell-Permeating Stabilized α-helical Peptide, ALRN-6924, is a First-in-Class Dual Inhibitor of MDMX and MDM2



A) The tumor suppressor p53 is one of the most pursued targets in oncology, playing a central role inducing cell cycle arrest, apoptosis, senescence, autophagy, cellular metabolism and immune surveillance in response to cellular stresses such as DNA damage and oncogenic signals[1]. B) ALRN-6924, a cell-permeating, stapled α-helical peptide that has demonstrated anticancer activity as monotherapy in clinical trials, mimics the p53 tumor suppressor protein to disrupt its interactions with both its endogenous inhibitors, MDMX and MDM2[2,3]. Stapled peptides mimic natural peptide sequences at the interface of protein-protein interactions, displaying a larger surface area of interaction with its target, and providing superior binding properties which reduce off-target effects and the risk of acquiring mutations associated with resistance. C) Furthermore, like natural protein sequences, a peptide can engage with ≥2 targets, e.g. MDMX + MDM2.

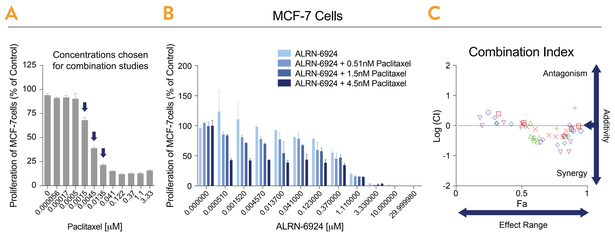
Table 1: ALRN-6924 Synergizes with Commonly Used Pathway-Selective and Chemotherapy Agents

Combination indices for the indicated cell lines and corresponding test article are shown. Cells were dosed with varying concentrations of ALRN-6924, the indicated test article alone, or in combination with ALRN-6924. Cell viability was measured using a standard colorimetric MTT assay. Drug combination index (CI) analysis was conducted following the Chou-Talalay method using CompuSyn. Synergism and antagonism were described based on the CI values according to the Chou and Talalay guidelines.

* *In vitro* CI value at IC_{50} or IC_{90}, average ± SD of ≥ 2 experiments. CI values: 0–0.1, very strong synergism; 0.1–0.3, strong synergism; 0.3–0.7, synergism; 0.7–0.85, moderate synergism; 0.85–0.90, slight synergism; 0.90–1.10, nearly additive; 1.10–1.20, slight antagonism; 1.20–1.45, moderate antagonism; 1.45–3.3, antagonism; 3.3–10, strong antagonism; 10, very strong antagonism). †Single experiment.

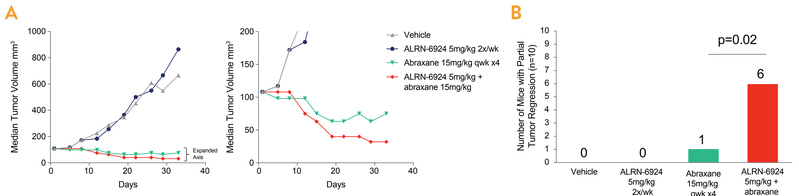
Cancer	Cell line	Drug	CI*
Breast Cancer	MCF-7	palbociclib	0.45 ± 0.17
		abemaciclib	0.57 ± 0.23
		ribociclib	0.39 ± 0.08
		paclitaxel	0.80 ± 0.05
		docetaxel	0.37 ± 0.02
		eribulin	0.66 ± 0.36
		everolimus	0.59 ± 0.28
		fulvestrant	0.41 ± 0.17‡
		carboplatin	0.49 ± 0.04
		gemcitabine	0.58 ± 0.05
		dexamethasone	No change
Colon Cancer	HCT-116	5-FU	0.07 ± 0.03
Melanoma	A375	trametinib	0.41 ± 0.12
		dabrafenib	0.33 ± 0.15
		vemurafenib	0.48 ± 0.20
	MEL-JUSO	binimetinib	0.32 ± 0.10
		pimasertib	0.39 ± 0.08
		selumetinib	0.49 ± 0.08
T Cell Lymphoma	MOLT-3	romidepsin	0.38 ± 0.00
B Cell Lymphoma	DOHH-2	vincristine	0.09 ± 0.06
		cyclophosphamide	0.11 ± 0.13
		rituximab	0.40†
		doxorubicin	0.04†
	JVM2	ibrutinib	0.59 ± 0.27
Acute Leukemia	MV-4-11	cytarabine/Ara-C	0.98 ±0.02
		azacitidine	0.49 ± 0.32
		decitabine	1.08 ± 0.07
		midostaurin	0.83 ± 0.04
		venetoclax	0.20 ± 0.03

Figure 2: ALRN-6924 Displays Additive to Synergistic Antitumor Activity in Combination with Paclitaxel *in vitro*



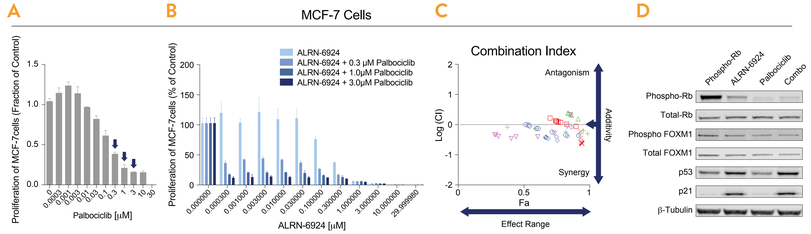
A) Cell viability in response to varying concentrations of paclitaxel (blue arrows denote concentrations chosen for combination studies). B) Proliferation of MCF-7 cells treated with the indicated dose of paclitaxel and varying concentrations of ALRN-6924. C) Combination indices for the drug combination of ALRN-6924 and paclitaxel were determined as described in Table 1.

Figure 3: ALRN-6924 Enhances the Antitumor Activity of Nab-Paclitaxel in the MCF-7 Breast Cancer Xenograft Model



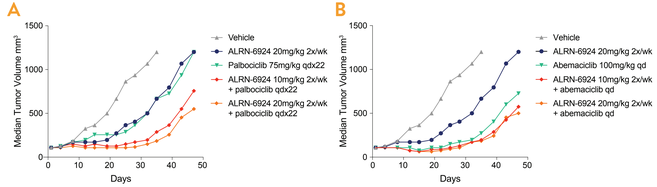
A) Athymic nude mice with established tumors (n = 10 per group) were treated for 4 weeks with ALRN-6924 twice-weekly alone or in combination with weekly doses of nab-paclitaxel. Compounds were co-administered intravenously at the indicated doses. B) Objective tumor responses on d32 (partial regression = 3 consecutive measurements <50% of starting volume). Studies were approved by the Institutional Animal Care and Use committee at Charles River Laboratories, Morrisville, N.C

Figure 4: ALRN-6924 Displays Additive to Synergistic Antitumor Activity in Combination with Palbociclib *in vitro*



A) Cell viability in response to varying concentrations of palbociclib (blue arrows denote concentrations chosen for combination studies). B) Proliferation of MCF-7 cells treated with the indicated dose of palbociclib and varying concentrations of ALRN-6924. C) Combination indices for the drug combination of ALRN-6924 and palbociclib were determined as described in Table 1. D) Protein expression of pharmacodynamic biomarkers demonstrating on-mechanism activity of test articles as single agents and in combination.

Figure 5: ALRN-6924 Increases Progression Free Survival in Combination with Palbociclib and Abemaciclib in the MCF-7 Breast Cancer Xenograft Model Compared to Single Agents



A) Athymic nude mice with established tumors were treated with ALRN-6924 alone (intravenously twice weekly for 3 weeks) or in combination with palbociclib (orally daily for 22 days) or B) abemaciclib (orally daily - 100mg/kg. Dosing was stopped on day 14 due to weight loss. After a brief treatment holiday, dosing resumed on day 18 at a lower dose – 75mg/kg for 5 days.) Studies were approved by the Institutional Animal Care and Use committee at Charles River Laboratories, Morrisville, N.C.

Conclusions

ALRN-6924 is a first-in-class clinical stage MDMX/2 dual inhibitor displaying promising anticancer activity in patients[2,3]. Recently, ALRN-6924 was shown to display strong on-mechanism anti-tumor activity in preclinical models of AML[4] and PTCL[5]. In the current study, we demonstrate that ALRN-6924 can be rationally combined with pathway-selective and chemotherapy agents. These results, plus promising ALRN-6924 safety and antitumor activity as a monotherapy[3], support the development of combination regimens for breast cancer and other malignancies.

References

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