The Stapled Peptide ALRN-6924, a Dual Inhibitor of MDMX and MDM2, and the CDK4/6 Inhibitors Palbociclib, Ribociclib, or Abemaciclib Synergistically Enhance Each Other’s in vitro and in vivo Anticancer Activity

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Background
ALRN-6924 is a cell-permeating, stapled α-helical peptide that disrupts the interaction of the p53 tumor suppressor protein and its inhibitors, MDMX and MDM2. Reactivation of p53 with ALRN-6924 in TP53-wild type tumors triggers cell cycle arrest and apoptosis resulting in enhanced efficacy. Clinical evaluation of ALRN-6924 (a) reduces aerobic cell growth arrest via the interleaved RB pathway and co-amplification of MDM2 and CDK4 (both on chromosome 12q) is a known emerging strategy; suggesting that combinations of ALRN-6924 and CDK4i may be synergistic. This study evaluated the antitumor efficacy and pharmacodynamics (PD) of ALRN-6924 combined with palbociclib, ribociclib, or abemaciclib.

Material and Methods
ALRN-6924 was tested in combination with palbociclib or abemaciclib in HCC-36 breast cancer cell lines and MDMX- and MDM2-co-amplified SJSA-1 tumor cell lines using WST-1 cell viability assays. Synergy was quantified by the Chou-Talalay combination index method. Single-agent and combination were evaluated in cell culture using assays for apoptosis (Annexin V, caspase3/7, proliferation (EdU), senescence [Giemsa stain], colony growth [Giemsa], and Western blot analysis of p53, p21, phospho-Rb, FOXM1, and phospho-FOX), and p21 mRNA. In vivo combinations with palbociclib, ribociclib, or abemaciclib were tested in athymic nude mice HCC-36, SJSA-1 xenograft models.

Results
ALRN-6924 combinations with palbociclib or abemaciclib display synthetic synergy in vitro antiproliferative activity in HCC-36 and SJSA-1 cells. ALRN-6924 induced senescence in vitro as a monotherapy and in combination with CDK4i. Western blot assays show that ALRN-6924/palbociclib combination triggers sustained chromatin biomarker activation, vs. treatment of E2F1 mRNA are sustained after wash-out in combination, but not in single-agent treated cells. MCF-7 and SJSA-1 tumors growth inhibition in combination with ALRN-6924/palbociclib combination and the killing of single agent is additive. SJSA-1 xenografts show that ALRN-6924/palbociclib combinations inhibit SJSA-1 tumor cell proliferation and senescence. Body weights and tumor sizes data show the combination of ALRN-6924 with palbociclib or ribociclib, 5 mg/kg/day was well tolerated, the combination with abemaciclib 100 mg/kg/day was tolerated with interruption and dose reductions. No pharamcokinetic (PK) or drug-drug interactions were noted in nude mice due to different modes of metabolism for ALRN-6924.

Conclusions
This study demonstrates that ALRN-6924 and CDK4i combinations show synergetic activity. PD biomarkers indicate on mechanisms of synergy that is sustained after wash-out. In vivo efficacy biomarkers, PK, and tolerability results, plus clinical evidence that the most frequents and concerning safety issues for CDK4/6i’s (inconsistent, leukopenia, elevation in transaminases) combined with ALRN-6924 reported safety profile support the development of combination regimens for leukemia and other malignancies. A Phase II trial to evaluate the combination of ALRN-6924 and palbociclib in MDMX and MDM2/CDK4i-compiled cancer will start enrolling patients in the first quarter of 2019 (ClinicalTrials.gov identifier NCT-02264613).

References

Figure 2: ALRN-6924 in Combination with CDK4/6i’s Displays Synergistic Anti-proliferative Activity in vitro

Figure 3: ALRN-6924 in Combination with Palbociclib Displays Synthetic Cell Cycle Inhibition and Induces Apoptosis

Figure 4: The Combination of ALRN-6924 and Palbociclib Triggers Sustained Activation of the RB Tumor Suppressor Pathway

Figure 5: ALRN-6924 Induces Senescence in vitro as a Monotherapy and in Combination with CDK4/6i’s

Figure 6: No Pharmacokinetic Drug-Drug Interactions Are Observed in nude Mice

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