ALRN-6924, a Dual Inhibitor of MDMX and MDM2 that Causes Minimal Thrombocytopenia in Patients, Disrupts Different Stages of Thrombopoiesis Compared to MDM2-only Inhibition

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Abstract

Background: ALRN-6924 is a stabilized, cell-permeating a-helical peptide that disrupts interactions of the p53 tumor suppressor protein with its endogenous inhibitors, MDMX and MDM2. ALRN-6924 can thereby restore p53-dependent anti-cancer activity in p53 wild type tumors. In an ongoing ALRN-6924 clinical trial with solid tumor and lymphoma patients enrolled as of 13 August 2018 there have been 1/2 dose events (AEs) of Grade 3/4 thrombocytopenia (TCP), respectively, at doses that yield objective responses (NCITC202646311-3, 7).

Aim: To explore the differences in TCP grades and frequencies from published solid tumor trials for ALRN-6924 and MDM22, we investigated the effects of dual MDMX/MDM2 inhibition vs. MDM2-only inhibition during different stages of megakaryocyte maturation.

Methods: Clonogenicity was measured in megakaryocyte (MK), erythroblast (Ei), and granulocyte/macrophage (GM) progenitor colony-forming unit (CFU) assays. Human bone marrow CD34+ cells were seeded in methylcellulose semi-solid media containing IL-3 and IL-6; and TPO. Epo or GM-CSF together with ALRN-6924, dasatinib, or controls. CFU-MK, CFU-Ei, and CFU-GM colonies were measured after 14 days of culture. Megakaryocyte maturation was modeled in vitro using a 14-day liquid culture assay. Human bone marrow CD34+ cells were cultured in liquid medium containing SCF and TPO. Megakaryocyte maturation was assessed by measuring the expression of CD41 and CD42. To assess the effects of ALRN-6924 and RG7387 and the platelet development, drugs were added to the culture at different stages during the 14-day period of megakaryocyte maturation in vitro. DNA synthesis was measured by BrdU, and apoptosis by measuring Annexin V and propidium iodiine staining. Healthy C57B6 mice treated with ALRN-6924 3x/week were monitored for blood counts 2x/week; hematopoietic stem and progenitor cells in bone marrow were measured at 4 weeks.

Results: In vitro, ALRN-6924 disrupted MK colony formation more potently than RG7387, with IC50 of 0.024 and 0.12 μM, respectively, while their effects were comparable in CFU-Ei and CFU-GM assays with IC50 of 0.12 μM. Conversely, RG7387 disrupted megakaryopoiesis by inducing apoptosis and cell cycle arrest in maturing MK 10-fold more potently than ALRN-6924. Consistent with these findings, ALRN-6924 induced minimal effects on platelet counts in mice at doses that are effective in xenotransplanted and syngeneic tumor models, despite a significant decrease in MK and erythroid progenitors, while immature hematopoietic stem and progenitor cells numbers remained unaltered.

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

Table 1: MDM2/MDM2 Drugs: Phase 1 All-comers Trials

<table>
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<tr>
<th>Drug</th>
<th>% p53 wild type tumors + lymphomas</th>
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Figure 2: Ex vivo Megakaryocyte Development Assay

Figure 3: Selective MDM2 Inhibition Potently Disrupts DNA Replication and Induces Apoptosis During Megakaryocyte Development, Abrogates Platelet Production Ex vivo

Figure 4: ALRN-6924 Potently Disrupts Proliferation of Immature Megakaryocyte Progenitors (CFU-MK) Compared to MDM2 Selective Inhibitor but Demonstrates Equotent Activity Against Erythroid (CFU-E) and Granulocyte-Monocyte (CFU-GM) Progenitors

Figure 5: ALRN-6924 Reduces the Frequency of Megakaryocyte and Erythroid Progenitors (MEP) but Does Not Induce Thrombocytopenia in C57BL/6 Mice

Conclusions

• ALRN-6924 potentially inhibits the dormancy capacity of megakaryocyte progenitors in MK-CFU assays but is less potent during the process of megakaryocyte maturation than the MDM2-only inhibitor RG7387. Similarly, mice treated with ALRN-6924 showed a statistically significant reduction in MIP frequency but did not develop thrombocytopenia.

• These findings suggest that the disruption of later stages of megakaryocyte maturation with MDM2-selective inhibitors more severely affects platelet production, ultimately resulting in thrombocytopenia. However, inhibition of less mature megakaryocyte progenitors, prior to entering terminal maturation, appears to be better tolerated. One explanation may be that ALRN-6924 triggers compensatory mechanisms resulting in increased output of intermediate maturation of megakaryocytes or alternative stem/progenitor differentiation pathways bypassing phenotypic MEPs.

• These results may explain the differences in TCP grades and frequencies from published solid tumor trials for ALRN-6924 vs. MDM2-only inhibitors, and suggests that MDM2 in bone marrow cells, where MDM2 mRNA expression is the highest of all 37 normal tissues reported in the Protein Atlas, may contribute to these differential effects on bone marrow toxicity. Furthermore, these studies suggest functional differences between MDM2 and MDM2 during megakaryopoiesis requiring further characterization.

• In collaboration with Pfizer, ALRN-624 is currently being tested in combination with palbociclib in MDM2-amplified cancers (NCITC202646311), and another Phase 1b/2 clinical trial is being planned to evaluate ALRN-624 as a myeloproliferative agent to protect against chemotherapy-induced toxicity.

References

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