Poster # 1376

Oral Azacitidine and Cedazuridine Approximate Azacitidine Efficacy in a Murine Model

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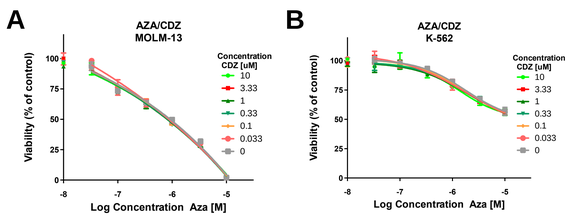
Introduction

DNA methyltransferase inhibitors (DNMTi) improve survival for patients with myelodysplastic syndromes (MDS) and those with acute myeloid leukemia (AML) unable to receive standard cytotoxic chemotherapy, and accordingly, are the backbone of standard of care treatment for these conditions. Standard regimens with DNMTIs, decitabine (DEC) or azacitidine (AZA) include daily subcutaneous (s.c.) or intravenous (i.v.) administration for 5-7 consecutive days (1). Attempts to provide the therapy orally have been limited given rapid clearance of the agents by the enzyme cytidine deaminase (CDA) which is ubiquitous in the gut and liver as part of first-pass metabolism. Recently, cedazuridine (CDZ), an oral inhibitor of CDA, was successfully combined with DEC to approximate the pharmacokinetics of i.v. DEC in patients. To determine if a similar strategy might be feasible in the clinic with AZA, we similarly attempted to increase the bioavailability of oral AZA with CDZ in vivo in a murine cell line model, achieving similar pharmacokinetics and efficacy against human AML cells with parenteral AZA and oral AZA+CDZ (ASTX030). The combination of AZA+CDZ with venetoclax in patient derived xenograft (PDX) emulated responses seen with venetoclax+AZA in the clinic implying a potential all-oral venetoclax based therapy opportunity in myeloid diseases.

Results

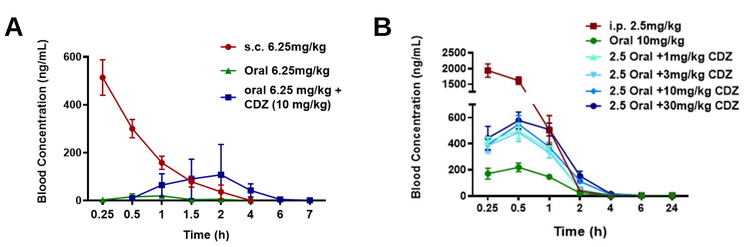
Efficacy of AZA is unchanged by the addition of CDZ in vitro



We first measured GI_{50} in AML cell lines treated with vehicle (DMSO), AZA, and AZA+CDZ combination.

After 72 hours of direct treatment, no differences were noted in viability of cell lines between AZA and AZA+CDZ in vitro, as expected. The GI_{50} of both MOLM-13 (0.815uM) and K562 (>5uM) remained unchanged with the addition of CDZ (A, B). Although cancer cell lines produce CDA, total levels are negligible in comparison to that of the gut and liver. This result led us to more directly pursue an *in vivo* approach to testing the combination of oral AZA and CDZ.

Pharmacokinetics of Oral AZA suggests on-target AUC

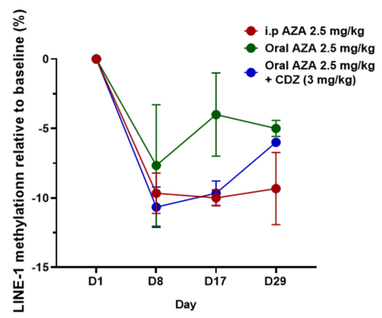


To compare the bioavailability of AZA when dosed orally with CDZ or with traditional parenteral use, we conducted pharmacokinetic studies in both primate and murine models. In male cynomolgus monkeys, dosing with oral AZA alone at 6.25 mg/kg (monkey equivalent dose converted from clinical AZA dose of 75 mg/m^2), resulted in mean systemic AUC_{last} of 18.9±10.1 ng*hr/mL, compared to 369±42 after the same dose was administered s.c. (A). However, when the same oral dose (AZA 6.25 mg/kg) was co-administered with 10 mg/kg CDZ, AZA exposures increased 1634% with AUC_{last} at similar levels, 309±268 ng*hr/mL, as seen with s.c. dosing.

In male NSG-SGM3 mice, dose-normalized systemic exposures ($AUC_{0\text{-}inf}$, ng*hr/mL divided by dose, mg/kg) were 26 and 647 after single 10 mg/kg oral and 2.5 mg/kg i.p. AZA dose. When given orally at 2.5 mg/kg in combination with CDZ at increasing doses from 1 to 30 mg/kg, AZA dose-normalized AUC increased dose-dependently to 224-364, an 8.6 to 14-fold increased over AZA p.o. alone (B). These data show that the addition of CDZ to oral AZA leads to similar pharmacokinetics as parenteral dosing in both primate and murine models.

LINE-1 Assay reveals effectivity of oral 5'Azacitidine and CDZ

To verify that oral administration of AZA did not affect its efficacy toward global methylation patterns on DNA, we tested the blood from mice treated with both i.p. and oral regimens of AZA and CDZ with a LINE-1 assay measuring levels of hypomethylated residues both pre and post treatment (2).

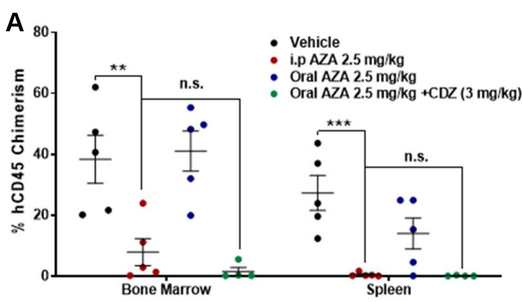


Oral AZA+CDZ resulted in similar demethylation as i.p. AZA, whereas oral AZA alone was unable to replicate the DNA demethylation induced by i.p. AZA in the LINE-1 assay. The transient hypomethylating effect induced by oral AZA alone quickly receded.

Addition of CDZ to AZA allows for effective Oral AZA dosing in an *in vivo* murine model

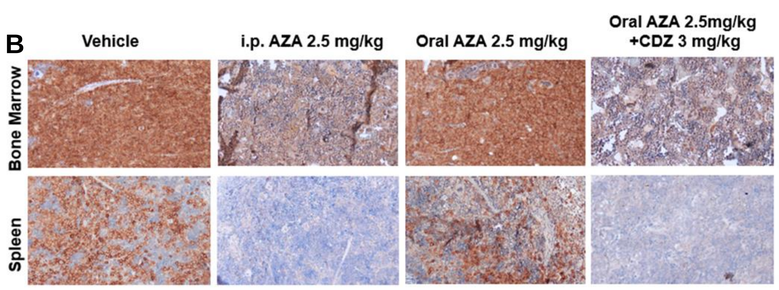
Following pharmacokinetic and pharmacodynamic assessment of oral AZA dosed with CDZ, we then used this regimen in an *in vivo* human cell line derived xenograft transplantation experiment (CDX). For this we studied CDZ, AZA, and the AZA+CDZ combination in a systemic AML MOLM-13 in NSGS mice. During treatment, the kinetics of the MOLM-13 human cell line expansion was defined by detection of human CD45$^+$ cells in the blood as detected by flow cytometry. At approximately three weeks after transplant, vehicle mice treated only with CDZ (30mg/kg) became moribund, and all experimental groups were sacrificed for analysis of chimerism.

In the CDX, as expected, i.p. AZA significantly decreased leukemic expansion in the bone marrow and spleen whereas CDZ alone had no effect on leukemic expansion in the CDX, in the marrow and spleen (A). Likewise, single agent oral AZA alone failed to decrease AML expansion in both the bone marrow and spleen of treated mice; however, the addition of CDZ to oral AZA led to significant decreases in AML in both bone marrow and spleen, showing scant CD45+ cells.

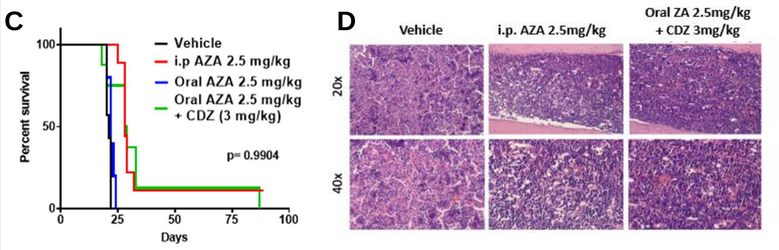


Differences between the oral AZA+CDZ group and traditional AZA i.p. dosing revealed no significant differences in either bone marrow or splenic tissue.
*P < .05, **P < .01, ***P <.001.

Immunohistochemical staining for human CD45 further revealed decreased expansion of AML in both bone marrow and spleen of i.p. AZA and oral AZA+CDZ treated mice (B).



As previously noted, mice treated with CDZ (vehicle) alone died within 21 days of MOLM-13 cell transplant. Kaplan-Meier survival analysis revealed both i.p. AZA and oral AZA+CDZ led to a significantly increased lifespan against vehicle (vehicle vs i.p. AZA and Oral AZA+CDZ, P<.0001 and P<.001) with no significant difference in survival between i.p. AZA and oral AZA+CDZ treated mice (C).

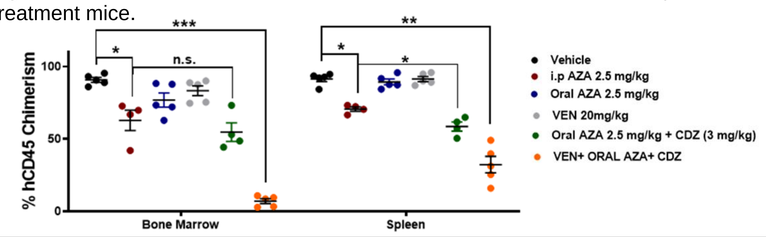


Throughout the duration of the experiments, mice in treatment groups had no significant weight loss, with no group losing more than 20% in body weight (data not shown). After tissues were harvested from experimental mice, bone marrows stained with H&E were reviewed for toxicity induced changes in architecture, hypocellularity or dysplasia. Pathology analysis revealed no significant bone marrow toxicity in treated mice (D), suggesting that AZA preferentially affected transplanted MOLM-13 AML cells at doses given. Further, no unforeseen effects of CDZ on normal murine bone marrow were noted.

Oral 5'Azacitidine shows efficacy in a PDX model of AML

After validating the safety and efficacy of oral AZA in a CDX model of AML, we sought to further validate this combination in a PDX primary patent sample model of AML. NSGS mice were transplanted with primary AML cells and after 35 days, engrafted mice were randomized to receive 2.5 mg/kg i.p. AZA, 2.5 mg/kg oral AZA, 3 mg/kg CDZ + 2.5 mg/kg oral AZA or CDZ alone as vehicle at 30 mg/kg. All oral AZA and CDZ was administered via oral gavage daily for 7 consecutive days; i.p. therapy was similarly given for 7 consecutive days. At approximately seven weeks after transplant, mice treated with CDZ or oral AZA alone became moribund, and all experimental groups were sacrificed for analysis of chimerism.

While oral AZA alone was unable to significantly decrease AML expansion in the bone marrow and the spleen, the addition of CDZ to oral AZA led to significant decreases in AML in both bone marrow and spleen, along with those of i.p treatments. The addition of oral AZA+CDZ to VEN treatment resulted in significant decreases of tumor burden in both the bone marrow and spleen of treatment mice.



Conclusions

- In pK analysis, the azacitidine AUC achieved with AZA+CDZ (ASTX030) was dose-dependent and similar between oral AZA+CDZ and i.p. AZA.
- Decreases in LINE-1 methylation with oral AZA+CDZ were comparable to those seen with intraperitoneal i.p. AZA.
- The addition of VEN to AZA+CDZ resulted in significant decreases in tumor expansion in an AML CDX and PDX, suggesting a potential all-oral administration of this emerging standard of care.

References

1. Lyons RM, et al. J Clin Oncol. 2009;27(11):1850-6.
2. Yang AS, et al. Cancer Res. 2006;66(10):5495-503.

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