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Preclinical Data in Cynomolgus Monkeys of ASTX727, a novel oral hypomethylating agent (HMA) composed of low-dose oral decitabine combined with a novel Cytidine Deaminase Inhibitor (CDAi) E7727

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INTRODUCTION

- E7727 is a novel oral cytidine deaminase (CDA) inhibitor
- Decitabine (DAC) is a hypomethylating agent (HMA), administered IV and approved for treatment of myelodysplastic syndromes (MDS) in US. Decitabine is a substrate for CDA and has a short half-life and very low oral bioavailability due to high first-pass effect due to degradation by CDA in GI tract and liver
- Studies presented here were designed to evaluate feasibility of combining the novel CDA inhibitor E7727 with oral decitabine in a new fixed combination oral product ASTX727 to achieve therapeutic exposures and biological activity

METHODS

All studies were conducted in Cynomolgus Monkeys (n=3 per treatment group):

- Pharmacokinetic Study 1: PK of single PO Administration of E7727 followed 1 hr later by PO DAC. DAC was administered as a single, fixed oral dose of 3 mg/kg, alone or 1-hr following single doses of E7727 at 0.1, 0.3, 1, 3, and 10 mg/kg
- Pharmacokinetic Study 2: Effect of Time Delay on PK of Oral Decitabine after Administration of E7727
  - Decitabine was dosed either concomitantly (immediately following E7727 gavage), or with a 30 min, 1 hr, and 2 hr delay. Doses used were 1 mg/kg for E7727 and 3 mg/kg for decitabine
- One-cycle (28-day) PK-PD and Safety Evaluation Study of qdx5 concomitant PO DAC + E7727
  - The objectives of this study were to evaluate pharmacokinetics, pharmacodynamics and safety of the combination treatment of 2 mg/kg decitabine and E7727 at doses of 1, 3 or 6 mg/kg
  - Blood samples for evaluation of hematology and LINE-1 demethylation (measured by pyrosequencing of bisulfite-treated DNA) were collected pre-dose on Days 1 , 8, 11, 15, 22, and 29
  - Safety evaluation endpoints included serum chemistry on Days 8 and 29, hematology, gross necropsy (Day 29) followed by histopathology assessment of the organs of the GI tract, liver and bone marrow

RESULTS

Table 1: Oral Decitabine (DAC) with Varying Doses of E7727 (PK Study 1)

Group	Dose (mg/kg) E7727	Dose (mg/kg) DAC	DAC AUC_{0-t} [b] (ng*hr/mL)	Fold increase AUC_{0-t}	DAC C_{max} [b] (ng/mL)	Fold increase C_{max}
1	0	3	21.7 (8)	1.0	24.4 (7)	1.0
2	0.1	3	138 (43)	6.4	140 (35)	5.7
3	0.3	3	164 (112	7.6	129 (70)	5.3
4	1	3	301 (94)	13.9	281 (102)	11.4
5	3	3	582 (273)	26.8	425 (193)	17.4
6	10	3	1494 (912)	68.9	622 (256)	25.5

a Decitabine administered 1-hr post E7727
b Mean (SD), n = 3 per group

- Following E7727 administration, decitabine exposures increased in dose-dependent manner relative to increasing E7727 doses
- No saturation was evident for the effect of increasing decitabine exposures with increasing E7727 doses

Figure 1: Mean Plasma Concentration-Time Profile for Decitabine (3 mg/kg) Administered 1 hr Post Treatment with Increasing Oral Doses of E7727

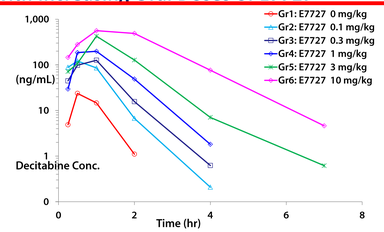
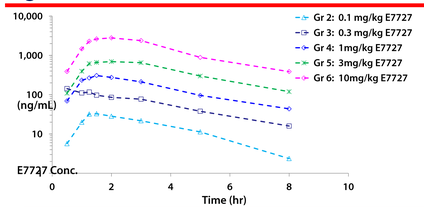
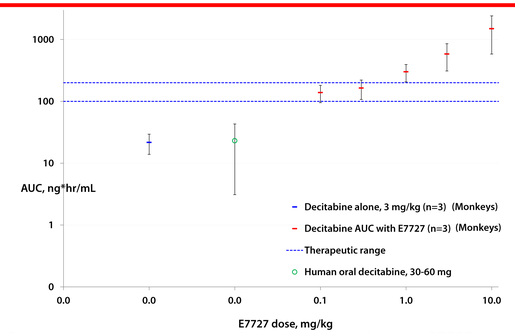


Figure 2: Mean Plasma Concentration-Time Profile for E7727 Administered Orally



- Pharmacokinetics of E7727 showed dose proportional increase in both C_{max} and AUC_{0-t} with no saturation observed.
- Tmax was in the range of 1.5-2.2 hours and elimination half-life (T1/2) ranged 2.1-4.5 hours.

Figure 3: Decitabine AUC increase when dosed with CDA inhibitor E7727 in Monkeys



- Decitabine oral AUC exposures in clinical therapeutic range were achieved at E7727 doses of 0.1-1.0 mg/kg when decitabine was dosed 1 hr following E7727

Table 2: Effect of Time Lag on Pharmacokinetics of Oral Decitabine after Administration of E7727 in Male Cynomolgus Monkeys (PK Study 2)

Group / Time-lag[a]	Dose, mg/kg E7727	Dose, mg/kg DAC	DAC AUC[b] ng*hr/mL	AUC_DDI fold increase	DAC C_{max} [b] ng/mL	C_{max}_DDI fold increase
Decitabine alone[c]	0	3	21.7	1.0	24.4	1.0
1 / concomitant	1	3	139	6.4	94.6	3.9
2 / 30 min	1	3	151	7.0	164	6.7
3 / 1 hr	1	3	327	15.1	270	11.1
4 / 2 hr	1	3	369	17.0	321	13.2

a Time lag refers to the delay before dosing with decitabine after E7727 administration
b Mean of n = 3 per group
c From previous PK study 1

PK / PD and Safety Evaluation Study in Cynomolgus Monkeys following Concomitant Repeat-Dose (Daily X5) Oral Administration of E7727 and Decitabine

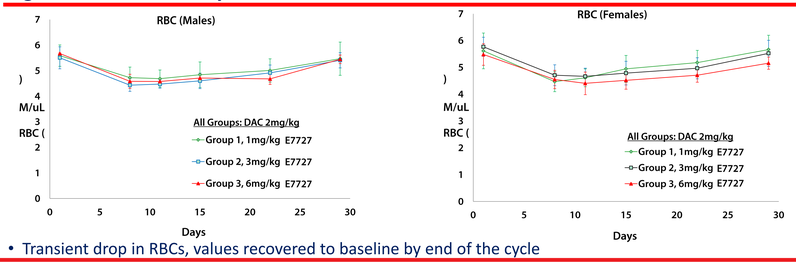
Table 3: Decitabine (2 mg/kg) PK parameters on Day 5 after concomitant administration with E7727

Group (sex)	E7727 dose mg/kg	Decitabine C_{max} (ng/mL)	Decitabine T_{max} (hr)	Decitabine $T_{½}$ (hr)	Decitabine AUC_{last} (ng*hr/mL)
1 (F)	1	236 (97)	1.3 (0.3)	0.9 (0.2)	316 (228)
1 (M)	1	83.2 (16)	1.3 (0.3)	0.8 (0.3)	155 (54)
2 (F)	3	272 (103)	1.7 (0.3)	0.7 (0.1)	420 (135)
2 (M)	3	340 (221)	1.7 (0.3)	0.7 (0.1)	402 (207)
3 (F)	6	337 (161)	1.5 (0.5)	0.8 (0.1)	540 (123)
3 (M)	6	398 (225)	1.0 (0.5)	0.9 (0.2)	567 (440)

Data are Mean (SD)

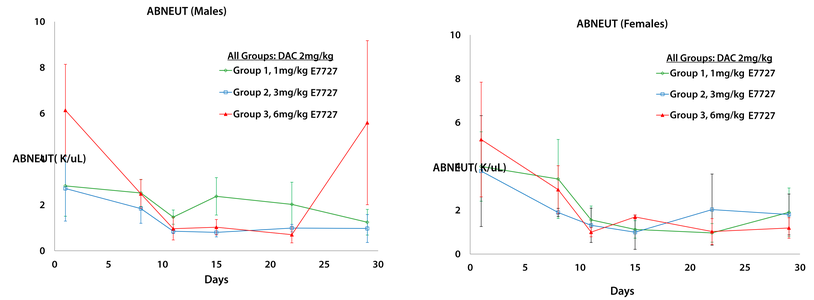
- Pharmacokinetic exposures for decitabine were similar between males and females
- Dose-dependent increase in decitabine C_{max} and AUC was evident
- Systemic AUC exposures for the 3 mg/kg E7727 dose group were ~ 3.6-fold higher than is reported in the clinic for 20 mg/m^2 IV infusion (comparison based on Decitabine PI)

Figure 4a: Effect of Daily X5 Treatment of oral E7727+Decitabine on RBC



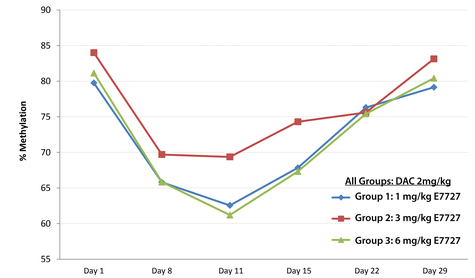
- Transient drop in RBCs, values recovered to baseline by end of the cycle

Figure 4b: Effect of Daily X5 Treatment of oral E7727+Decitabine on Neutrophils



- Neutropenia post treatment evident for all groups

Figure 5: LINE-1 Demethylation in Blood after Daily X5 Treatment of PO Decitabine (2 mg/kg qdx5) with E7727 in Cynomolgus Monkeys



- Significant LINE-1 demethylation (range -17.4 / -24.5%) observed for all E7727+DAC dose levels

Safety Evaluation of Oral E7727+Decitabine Treatment in One Cycle Study (Daily X5, Followed by 23-Day Recovery) in Cynomolgus Monkeys

- Treatment of daily X5 with oral E7727+decitabine, followed by 23 days was well tolerated in monkeys using doses of 2 mg/kg for decitabine (24 mg/m2 human equivalent dose (HED)) and 1, 3 and 6 mg/kg of E7727 (12, 36 and 72 mg/m2 HED)
- Hematology evaluation revealed changes (neutropenia) consistent with the pharmacological action of decitabine
- Serum clinical chemistry panel showed all parameters on Days 8 and 29 within historical range for cynomolgus monkeys
- Gross necropsy and histopathology evaluation (GI and liver) showed no treatment-related findings

SUMMARY & CONCLUSIONS

- Preclinical oral administration of decitabine and CDA inhibitor E7727 resulted in significantly improved decitabine exposures in cynomolgus monkeys
- Daily X5 treatment with oral decitabine+E7727, dosed concomitantly, delivered decitabine systemic exposures similar or higher than decitabine AUC after decitabine IV 1-hr infusion (20 mg/m2)
- Oral DAC (2 mg/kg QD x5)+ E7727 (1, 3 or 6 mg/kg QD x5) induced potent LINE-1 demethylation
- Hematological changes (decrease) were observed in RBCs and neutrophils consistent with the mechanism of action of decitabine
- One cycle treatment of oral E7727+decitabine was well tolerated in cynomolgus monkeys with no noteworthy findings in the GI or liver
- Data from these studies support the initiation of clinical First in Human (FIH) trial of ASTX727, a novel oral HMA combining oral decitabine with the CDA inhibitor E7727

COI Discloser: All authors are employees of Astex Pharmaceuticals

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Poster can be downloaded from www.astx.com