A phase 1, single and multiple ascending dose study of safety, tolerability, pharmacokinetics and pharmacodynamics of AG-519, an allosteric activator of pyruvate kinase-R in healthy subjects

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BACKGROUND

- Pyruvate kinase (PK) deficiency is an inborn error of metabolism resulting in life-long hemolytic anemia associated with serious comorbidities.[1]
- PK deficiency is caused by a functional deficiency of the R-isoform of PK (PK-R) due to mutations in the *PKLR* gene, more than 250 of which have been identified.[2]
- This results in defective glycolysis in red blood cells, and therefore decreased adenosine triphosphate (ATP) and increased 2,3 diphosphoglycerate (2,3-DPG) levels in red blood cells.
- Small molecule allosteric activation of PK-R resulting in increases in ATP and decreases in 2,3-DPG in healthy volunteers has been observed with an earlier molecule (AG-348).[3]
- AG-519 is a potent, highly selective and orally bioavailable second PK-R activator shown preclinically to have no aromatase inhibitory effects that were observed with AG-348, the first small molecule PK-R activator to enter clinical trials.
- Biochemical and preclinical characterisation of AG-519 is described in poster P742 and presentation S830.
- An ongoing phase 1 study in healthy volunteers aims to identify a safe and pharmacodynamically active dose and schedule of AG-519 for subsequent clinical studies in PK deficient patients.

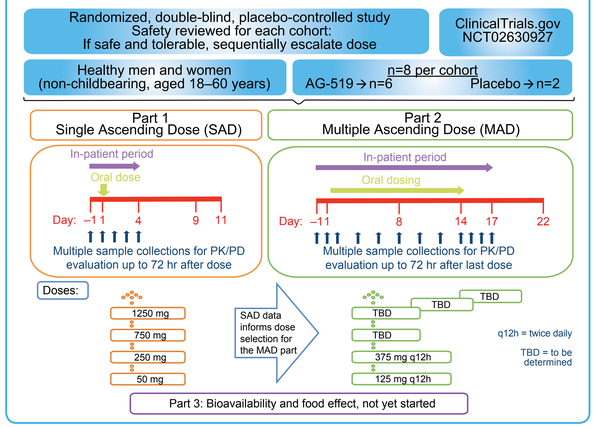
OBJECTIVES

- To report preliminary safety and pharmacokinetic/pharmacodynamic (PK/PD) results of the first-in-human study of AG-519.

METHODS

- Phase 1, single-center, inpatient, randomized, double-blind, placebo-controlled study with single ascending dose (SAD) and multiple ascending dose (MAD) cohorts.
- Doses and treatment schedules are shown in **Figure 1**.

Figure 1. Study design



Participants

- Healthy non-smoking men and women (non-childbearing potential) aged 18 to 60 years who provided written informed consent were eligible.
- Key exclusion criteria included: glucose-6-phosphate-dehydrogenase deficiency, blood donation or blood loss of >400 mL in the previous 3 months.

Assessments

- Safety assessments included monitoring of adverse events (AEs), serious AEs (SAEs), and safety laboratory parameters; results are presented using standard descriptive methods.
- Serial blood sampling was carried out pre-dose and at regular intervals after dosing for PK/PD determination.
- Concentrations of AG-519 in plasma were analyzed by a validated tandem mass spectrometry method.
- ATP and 2,3-DPG concentrations in blood were analyzed using qualified tandem mass spectrometry methods.
- Standard non-compartmental pharmacokinetic parameters were calculated from individual plasma concentration versus time data.

RESULTS

Study status

- As of 29 April 2016, four cohorts from the SAD part and two cohorts from the MAD part have been completed.
- The study is ongoing and the majority of data are still blinded, except:
  - PK/PD data are unblinded for a limited number of external statisticians.
  - Individual subjects may be unblinded for some safety events.

Subject disposition and characteristics

- SAD: All 32 subjects enrolled as of 29 April 2016 completed the study.
- MAD: Of 16 subjects enrolled as of 29 April 2016, 14 completed the study.
  - Two subjects withdrew consent for personal reasons; one from the 375 mg q12h cohort who received all planned doses, and one from the 125 mg q12h cohort who missed the last two doses.

Table 1. Demographic/baseline characteristics

Characteristic	SAD (n=32)	MAD (n=16)
Women/men, n (%)	5/27 (16/84)	3/13 (19/81)
Age in years, mean (range)	40.6 (18–58)	39.8 (18–57)
Body mass index kg/m^2, mean (SD)	26.05 (3.16)	25.74 (3.09)
Race, n (%)		
White	29 (91)	13 (81)
Black	3 (9)	1 (6)
Asian	–	1 (6)
Other	–	1 (6)
Ethnicity, n (%)		
Not Hispanic or Latino	32 (100)	16 (100)

Preliminary safety

- All AEs were mild or moderate (grade 1 or 2) in severity, the most common being headache (**Table 2**).
- There were no dose-limiting toxicities.
- There was one clinically significant change in laboratory parameters:
  - One subject (receiving AG-519 375 mg q12h) experienced AEs of bleeding gums and low blood platelet count (defined as clinically significant thrombocytopenia) on Day 13–14 (Grade 2, with a nadir of 53 x 10^9 platelets/L) where Day 14 was the last dose. Platelet levels started to recover within 5 days of the last dose and returned to normal levels 7 days after the last dose.
- One subject (in the placebo/AG-519 125 mg q12h cohort) experienced three AEs (all Grade 1) of skin flushing in response to sunlight.
- No SAEs have been reported to date.

Table 2. Blinded summary of AEs

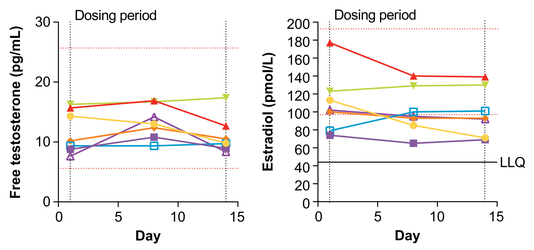
Adverse event	SAD (n=32)	MAD (n=16)
Subjects experiencing any AE, n (%)	10 (31)	11 (69)
Grade ≥3	0	0
Subjects experiencing any SAE, n (%)	0	0
Subjects experiencing any AE leading to discontinuation, n (%)	0	0
Most common AEs (≥2 subjects in either group), n (%)		
Headache	4 (13)	3 (19)
Nasopharyngitis	3 (9)	2 (13)
Subjects experiencing any treatment-related AE[a], n (%)	2 (6)	3 (19)
Treatment-related AEs occurring in ≥2 subjects	0	0

[a]Judged possibly or probably related to treatment
AEs were graded using National Cancer Institute Common Terminology Criteria for Adverse Events, Version 4.03

Preliminary hormone assessments

- Analysis of free testosterone and estradiol in MAD cohorts 1 and 2 indicated the absence of aromatase-inhibitory activity, as expected (**Figure 2**).

Figure 2. Levels of serum estradiol and free testosterone in individual male subjects in MAD cohort 2 (375 mg BID)



LLQ, lower limit of quantification; red dotted horizontal lines indicate upper and lower limits of normal range.

Preliminary pharmacokinetics

- Variability of pharmacokinetic parameters was moderate.
- Exposure to AG-519, as measured by area under the concentration × time curve (AUC), increased in a dose-proportional, or slightly greater than dose-proportional, manner following a single dose (**Table 3**).
- Absorption was rapid (median time of maximum observed concentration [T_{max}] ranged from 0.5–1.0 hr), but was slightly prolonged at higher doses (**Table 3**).

Table 3. Pharmacokinetic parameter values of AG-519 following a single oral dose

Dose, mg	C_{max}, ng/mL	T_{max}, hr	AUC_{0-12}, ng/mL•hr	$AUC_{0-\infty}$, ng/mL•hr	Terminal $t_{1/2}$, hr	Cl/F, L/hr	Vz/F, L
50	229 (91)	1.0 (1.0, 2.0)	627 (186)	NC	NC	NC	NC
250	1505 (559)	0.5 (0.5, 2.0)	3721 (1238)	3929 (1304)	30 (5)	72 (25)	3220 (1361)
750	5208 (1620)	1.0 (1.0, 2.0)	17956 (5542)	18641 (5624)	25 (5)	46 (16)	1734 (851)
1250	7240 (1786)	1.0 (0.5, 3.0)	27676 (4711)	29043 (5003)	22 (3)	46 (8)	1481 (302)

Mean (standard deviation) except T_{max} which is median (minimum, maximum)
N=6 for each dose level
AUC_{0-12} = AUC from 0 to 12 hr; $AUC_{0-\infty}$ = AUC from 0 extrapolated to infinity; Cl/F = apparent clearance; C_{max} = maximum concentration; $t_{1/2}$ = apparent terminal elimination half-life; T_{max} = time of maximum observed concentration; Vz/F = apparent volume of distribution; NC, not calculated due to insufficient quantifiable concentration-time data

- AG-519 had a rapid distribution or elimination phase during the 12 hr after dosing (**Figure 3**).
- The pharmacokinetic results for cohorts 1 and 2 of the MAD study were consistent with those of the SAD study (**Table 4**).
- The accumulation index, defined as Day 14 AUC_{0-12} divided by Dose 1 AUC_{0-12}, ranged from 1.30 at 375 mg q12h to 1.46 at 125 mg q12h, and was consistent with an effective half-life of approximately 6 hr.

Figure 3. Mean (SD) plasma concentration-time profiles of AG-519 following a single oral dose

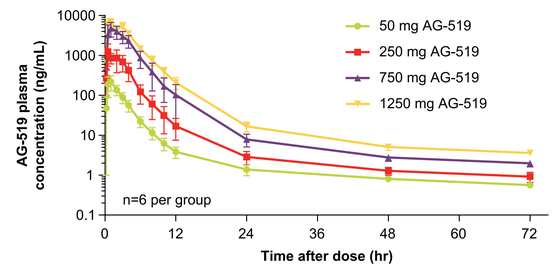


Table 4. Pharmacokinetic parameter values of AG-519 following single and multiple oral doses (cohorts 1 and 2 only)

Dose, mg	C_{max} Dose 1, ng/mL	C_{max} Day 14, ng/mL	AUC_{0-12} Dose 1, ng/mL•hr	AUC_{0-12} Day 14, ng/mL•hr	Cl/F Day 14, L/hr	Vz/F Day 14, L	Terminal $t_{1/2}$ Day 14, hr
125 q12hr	561 (304)	832 (501)	1493 (443)	2179 (838)	64 (20)	5011 (1711)	56 (12)
375 q12hr	2118 (910)	3225 (1268)	7042 (1685)	9142 (1414)	42 (7)	3030 (1005)	50 (12)

Mean (standard deviation). Day 14 is after the last dose
N=6, except n=5 or 6 for Day 14 parameters subject to confirmation once study is unblinded
AUC_{0-12} = AUC from 0 to 12 hr; C_{max} = maximum concentration; Cl/F = apparent clearance; $t_{1/2}$ = apparent terminal elimination half-life; Vz/F = apparent volume of distribution

Preliminary pharmacodynamics

- A dose-dependent decrease in blood 2,3-DPG concentration was observed following a single AG-519 dose (up to ~43% decrease), reaching minimum levels after 24 hr and remaining decreased after ~72 hr (**Figure 4**).
- The maximum decrease in 2,3-DPG blood levels in the 375 mg q12h dose level of the MAD study was similar to that achieved by the highest single dose of 1250 mg, with minimum levels (up to ~47% decrease) reached ~72 hr after the first dose (**Figure 5**).

Figure 4. Mean (+SD) change in blood concentration-time profiles of 2,3-DPG following a single oral dose of AG-519

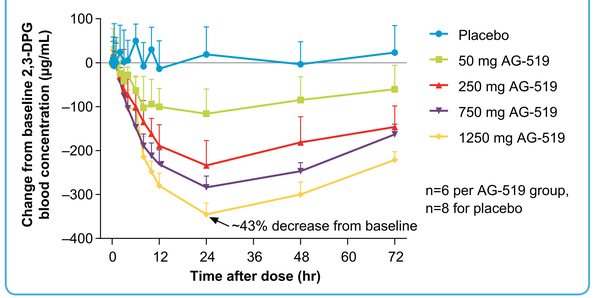
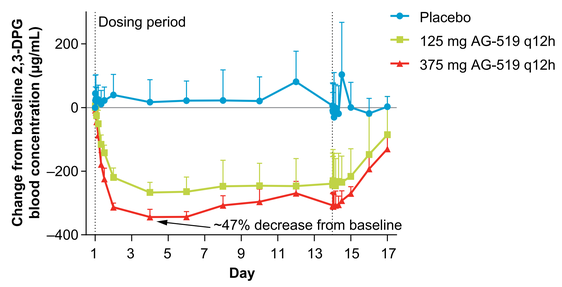
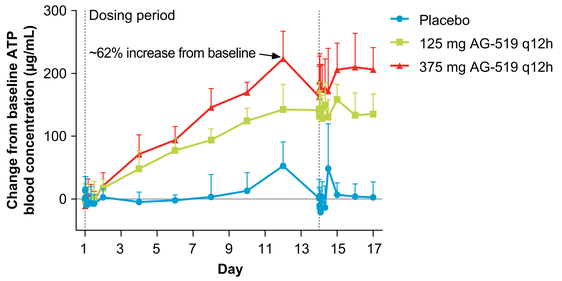


Figure 5. Preliminary mean (+SD) change in blood concentration-time profiles of 2,3-DPG following multiple oral doses of AG-519 (cohorts 1 and 2 only)



- There were minimal increases in blood ATP levels in the SAD study; ~24% increases were observed 72 hr after the highest AG-519 dose of 1250 mg.
- There were substantial increases (~62%) in ATP levels in cohorts 1 and 2 of the MAD study, with levels peaking at Day 12 and persisting through Day 17 (**Figure 6**).

Figure 6. Preliminary mean (+SD) change in blood concentration-time profiles of ATP following multiple oral doses of AG-519 (cohorts 1 and 2 only)



CONCLUSIONS

- AG-519 has a favorable safety profile to date and was well tolerated in healthy subjects receiving a single dose up to 1250 mg, or 375 mg q12h for 14 days.
- As expected, AG-519 does not demonstrate the inhibition of aromatase previously observed with AG-348.
- AG-519 also demonstrated a favorable pharmacokinetic profile.
- The robust dose-dependent changes in ATP and 2,3-DPG blood levels are consistent with increased activity of PK-R, the expected PD effect of AG-519.
- The potency and activity of AG-519 as an activator of both wild-type and mutant forms of PK-R is similar to that of AG-348, a PK-R activator currently in phase 2 testing in patients with PK deficiency (NCT02476916; oral presentation S466 on 11 June).
- These data support the hypothesis that AG-519 may be able to enhance glycolytic activity in red cells of patients with PK deficiency to address the underlying cause of the disease.
- The PK/PD data from healthy subjects will inform dose selection for potential studies of AG-519 in patients with PK deficiency.

Acknowledgments

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References

1. Grace R et al. *Am J Hematol* 2015;90:825–30. **2.** Canu G et al. *Blood Cell Mol Dis* 2016;57:100–09. **3.** Yang H et al. 20th EHA Congress, 2015, Abstract S138.

Disclosures

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