Safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple doses of AG-519, an allosteric activator of pyruvate kinase-R in healthy subjects

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BACKGROUND

- Pyruvate kinase (PK) deficiency is a congenital hemolytic anemia caused by deficiency of the glycolytic enzyme red cell PK (PK-R) due to mutations in the *PKLR* gene.
- PK catalyzes the last enzymatic step in the glycolytic pathway and is the main source of adenosine triphosphate (ATP) production in red blood cells.
- *PKLR* mutations lead to defective proteins that are associated with reduced ATP levels and premature hemolysis of red cells.
- Small-molecule allosteric activation of PK-R resulting in increases in ATP and decreases in 2,3-diphosphoglycerate (2,3-DPG) in healthy volunteers, and rapid and sustained increases in hemoglobin in some patients with PK deficiency, has been observed with an earlier molecule, AG-348, the first small-molecule PK-R activator to enter clinical trials.[1,2]
- AG-519 is the second small-molecule PK-R activator to enter clinical trials. AG-519 is a potent, highly selective, and orally bioavailable PK-R activator devoid of the aromatase inhibitory effects that were observed with AG-348.

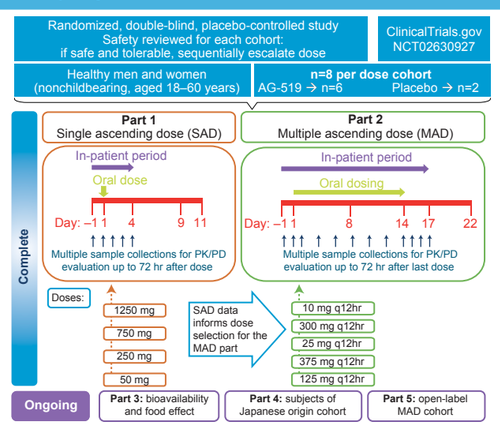
OBJECTIVE

- To report unblinded safety and pharmacokinetic/pharmacodynamic (PK/PD) results from the single ascending dose (SAD) and multiple ascending dose (MAD) cohorts of the first-in-human study of AG-519.

METHODS

- Phase 1, single-center, in-patient, randomized, double-blind, placebo-controlled study with SAD and MAD cohorts.
- Doses and treatment schedules are shown in **Figure 1**.

Figure 1. Study design



Participants

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

Assessments

- Safety assessments included monitoring of treatment-emergent adverse events (AEs), serious AEs (SAEs), and safety laboratory parameters; results are summarized by AG-519 dose and by pooling placebo-treated subjects using descriptive statistics.
- Serial blood sampling predose 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 noncompartmental pharmacokinetic parameters were calculated from individual plasma concentration versus time data.

RESULTS

Study status

- Completed Parts 1 and 2 (four SAD and five MAD cohorts).
- Part 1 and the first two cohorts of Part 2 have been previously reported.[3]
- The study is ongoing and final data are not yet available for the bioavailability and food effect study (Part 3), subjects of Japanese origin cohort (Part 4), or open-label MAD cohort (Part 5).

Subject disposition and characteristics

- SAD: all 32 enrolled subjects completed the study.
- MAD: 37 of 40 enrolled subjects completed the study.
  - Three subjects in the placebo group withdrew from the study early for personal reasons; one received all planned doses, one received doses through Day 13, and one received doses through Day 11.

Table 1. Demographics and baseline characteristics

Characteristic	SAD		MAD	
	Placebo n=8	Pooled AG-519 n=24	Placebo n=10	Pooled AG-519 n=30
Men, n (%)	7 (87.5)	20 (83.3)	7 (70)	28 (93.3)
Age in years, mean (range)	37.8 (18–58)	41.5 (19–58)	42.8 (18–57)	38.4 (19–59)
Body mass index kg/m^2, mean (range)	24.8 (21.9–27.4)	26.8 (19.3–31.1)	27.7 (24.8–30.9)	26.2 (20.6–31.3)
Race, n (%)				
White	8 (100)	21 (87.5)	9 (90)	27 (90)
Black	0	3 (12.5)	0	1 (3.3)
Asian	0	0	1 (10)	0
Other	0	0	0	2 (6.7)
Ethnicity, n (%)				
Not Hispanic or Latino	8 (100)	24 (100)	10 (100)	30 (100)

Safety

- Similar frequency of AEs in placebo- and AG-519–treated subjects.
- All AEs were mild or moderate (grade 1 or 2) in severity, the most common being headache (**Table 2**).
- No apparent dose relationship with regard to the overall incidence of AEs or most common AEs (**Table 3**).
- As previously reported, there was one case of grade 2 thrombocytopenia in a subject receiving 375 mg AG-519 q12hr, which resolved spontaneously within 7 days after the last dose.
- One subject who received 125 mg AG-519 q12hr experienced three AEs (all grade 1) of skin flushing in response to sunlight.
- After data cutoff, one ongoing SAE of drug-related cholestatic hepatitis was reported (bioavailability and food effect study; 300 mg). This event is being further evaluated.

Table 2. Summary of AEs

AEs	SAD		MAD	
	Placebo n=8	Pooled AG-519 n=24	Placebo n=10	Pooled AG-519 n=30
Subjects experiencing any AE, n (%)	2 (25)	8 (33.3)	5 (50)	17 (56.7)
Grade 1–2	2 (25)	8 (33.3)	5 (50)	17 (56.7)
Grade ≥3	0	0	0	0
Subjects experiencing any SAE, n (%)	0	0	0	0
Subjects experiencing any AE leading to discontinuation, n (%)	0	0	0	0
Most common AEs (≥2 subjects in pooled AG-519 group), n (%)				
Headache	1 (12.5)	3 (12.5)	2 (20)	9 (30)
Nasopharyngitis	0	3 (12.5)	0	2 (6.7)
Diarrhea	0	1 (4.2)	0	2 (6.7)
Rash	0	0	0	2 (6.7)
Subjects experiencing any treatment-related AE, n (%)[a]	0	2 (8.3)	0	4 (13.3)
Treatment-related AEs occurring in ≥2 subjects	0	0	0	0

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

Table 3. Summary of AEs by dose in MAD study (Part 2)

AEs	Placebo n=10	AG-519 10 mg n=6	AG-519 25 mg n=6	AG-519 125 mg n=6	AG-519 300 mg n=6	AG-519 375 mg n=6	Pooled AG-519 n=30
Subjects experiencing any AE, n (%)	5 (50)	4 (66.7)	2 (33.3)	4 (66.7)	3 (50)	4 (66.7)	17 (56.7)
Most common AEs (≥2 subjects in pooled AG-519 group), n (%)							
Headache	2 (20)	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)	3 (50)	9 (30)
Nasopharyngitis	0	0	0	1 (16.7)	0	1 (16.7)	2 (6.7)
Diarrhea	0	2 (33.3)	0	0	0	0	2 (6.7)
Rash	0	2 (33.3)	0	0	0	0	2 (6.7)
Subjects experiencing any treatment-related AE, n (%)[a]	0	0	0	2 (33.3)	1 (16.7)	1 (16.7)	4 (13.3)

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

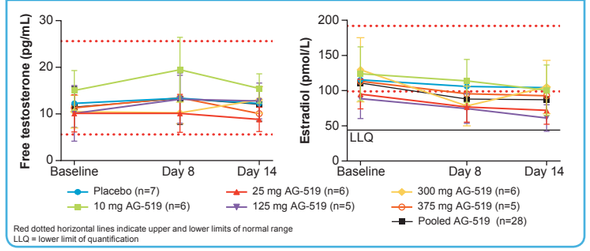
Hormone assessments

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

Pharmacokinetics

- Variability of pharmacokinetic parameters was moderate.
- Exposure to AG-519, as measured by area under the concentration versus time curve (AUC), increased in a dose-proportional, or slightly greater than dose-proportional, manner following a single dose (**Table 4**).
- Absorption was rapid (median time of maximum observed concentration [t_{max}] ranged from 0.5 to 1.0 hr) (**Table 4**).

Figure 2. Mean (SD) free testosterone and serum estradiol in male subjects in MAD study



Red dotted horizontal lines indicate upper and lower limits of normal range
LLQ = lower limit of quantification

Table 4. 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 $t_{½}$ (hr)	CL/F (L/hr)	Vz/F (L)
50	214 (43)	1.0 (1.0, 2.0)	617 (33)	903 (NC)[a]	29.4 (NC)[a]	55.4 (NC)[a]	2348 (NC)[a]
250	1433 (34)	0.5 (0.5, 2.0)	3661 (35)	3883 (36)	30.1 (16)	64.4 (36)	2768 (48)
750	5010 (31)	1.0 (1.0, 2.0)	17,636 (34)	18,541 (33)	25.4 (18)	40.5 (33)	1461 (51)
1250	7045 (27)	1.0 (0.5, 3.0)	27,954 (17)	29,734 (17)	22.3 (12)	42.0 (17)	1343 (23)

Data shown as geometric mean (geometric mean CV), except $t_{½}$, which is mean (CV), and t_{max}, which is median (minimum, maximum)
n=6 for each dose level except [a]n=2
$AUC_{0–12hr}$ = AUC from 0 to 12 hr; $AUC_{0–\infty}$ = AUC from 0 extrapolated to infinity; CL/F = apparent clearance; C_{max} = maximum concentration; CV = coefficient of variation; NC = not calculated due to insufficient quantifiable concentration versus time data; $t_{½}$ = apparent terminal elimination half-life; Vz/F = apparent volume of distribution

- AG-519 had a rapid distribution or elimination phase during the 12 hr after dosing and low concentrations after 24 hr (**Figure 3**).
- The pharmacokinetic results of the MAD study were consistent with those of the SAD study (**Table 5**).
- The accumulation index, defined as Day 14 $AUC_{0–12}$ divided by Dose 1 $AUC_{0–12}$, ranged from 1.22 at 10 mg q12hr to 1.47 at 125 mg q12hr, and was consistent with an effective half-life of ~4–6 hr.

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

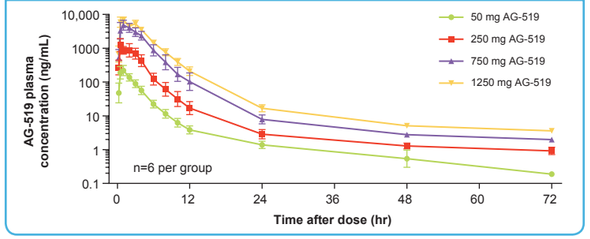


Table 5. Pharmacokinetic parameter values of AG-519 following multiple oral doses

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 $t_{½}$ Day 14 (hr)
10	31.3 (35)	40 (38)	112 (34)	135 (25)	NC	NC	NC
25	98.3 (38)	126 (35)	323 (37)	454 (34)	50.1 (27)[a]	3728 (35)[a]	51.9 (13)[a]
125	498 (58)	728 (60)	1578 (36)	2235 (40)	55.9 (40)	5389 (38)	55.5 (21)
300	1352 (58)	2010 (38)	5062 (36)	7270 (29)	41.3 (29)	3179 (43)	53.8 (14)
375	1967 (44)	3000 (45)	7467 (23)	9651 (16)	38.5 (18)[a]	2438 (16)[a]	44.3 (18)[a]

Data shown as geometric mean (geometric mean CV), except $t_{½}$, which is mean (CV). Day 14 is after the last dose
n=6 for each dose level except [a]n=5

Pharmacodynamics

- A dose-dependent decrease in blood 2,3-DPG concentration was observed following a single AG-519 dose, reaching minimum levels after 24 hr and remaining decreased after ~72 hr (**Figure 4**).
- A dose-dependent decrease in blood 2,3-DPG concentration was observed following multiple doses of AG-519, reaching minimum levels before the morning dose on Day 4 and remaining decreased 72 hr after the final dose (**Figure 5**).
- There were minimal increases in blood ATP levels after a single dose of AG-519.
- A dose-dependent increase in blood ATP concentration was observed following multiple doses of AG-519, reaching maximum levels before the morning dose on Day 12 and persisting through 72 hr after the final dose (**Figure 6**).
- Pharmacodynamic results on Day 14 following multiple doses of AG-519 showed a mean decrease of up to 61% in blood 2,3-DPG levels from prestudy baseline at a dose of 300 mg q12hr and a mean increase of up to 63% in blood ATP levels from prestudy baseline at a dose of 125 mg q12hr. By contrast, healthy volunteers receiving placebo showed minimal changes in 2,3-DPG or ATP levels.

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

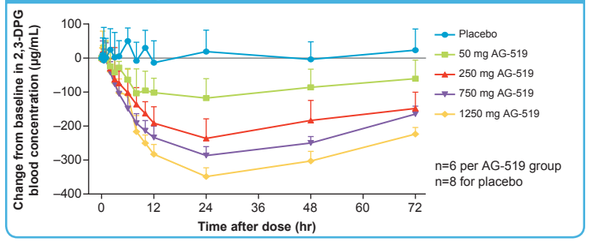


Figure 5. Mean (+SD) change in blood concentration versus time profiles of 2,3-DPG following multiple oral doses of AG-519

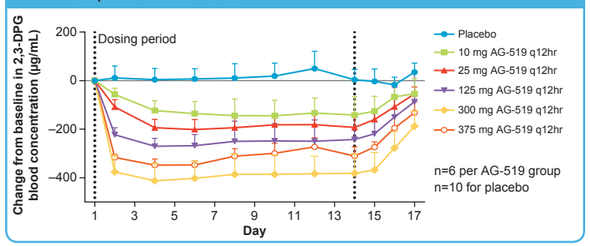
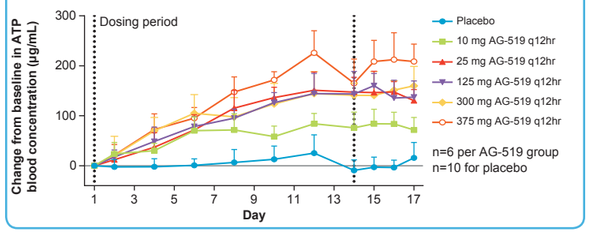


Figure 6. Mean (+SD) change in blood concentration versus time profiles of ATP following multiple oral doses of AG-519



CONCLUSIONS

- AG-519 is well tolerated in healthy subjects at doses ranging from 10 to 375 mg q12hr for 14 days.
- 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 pharmacodynamic effect of AG-519.
- 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.

Discussion

- The PK/PD data from healthy subjects will inform dose selection for potential studies of AG-519 in patients with PK deficiency.
- The 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 402 on December 4, 2016).

Acknowledgments

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Disclosures

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