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PHASE I STUDIES

Safety, tolerability, and pharmacology of AB928, a novel dual adenosine receptor antagonist, in a randomized, phase 1 study in healthy volunteers

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Summary

Adenosine suppresses antitumor immune responses via A_{2a} and A_{2b} receptors expressed on intratumoral immune cells. This effect is mediated by increased cyclic adenosine 5′-monophosphate (AMP) levels and phosphorylation of cyclic AMP response element binding protein (CREB). We conducted a phase 1, placebo-controlled, single-ascending-dose (SAD) and multiple-ascending-dose (MAD) study to assess the safety, tolerability, pharmacokinetics (PK), including food effect (FE), and pharmacodynamics (PD) of oral AB928, a novel dual $A_{2a}R/A_{2b}R$ antagonist, in healthy volunteers. AB928 doses between 10 and 200 mg once daily and 100 mg twice daily were evaluated. The study enrolled 85 subjects (randomized 3:1, AB928:placebo), 40 each in the SAD and MAD cohorts, and 5 in the FE cohort. AB928 was well tolerated up to the highest dose tested and did not affect any physiologic parameters potentially sensitive to adenosine inhibition. No safety concern was identified. The PK profile of AB928 was linear and dose-proportional, and a clear PK/PD correlation was demonstrated. Significant inhibition of adenosine receptor-mediated phosphorylated CREB was observed at peak plasma concentrations in all dose cohorts and at trough plasma concentrations in the higher-dose cohorts. AB928 plasma levels ≥1 μM were associated with ≥90% adenosine receptor inhibition. In the postprandial state, the rate of AB928 absorption decreased but the extent of absorption was unchanged. Together, these data support further clinical development of oral AB928 in cancer patients.

Keywords AB928 · Adenosine signaling · Adenosine receptor antagonist · Immunotherapy · Oncology · Healthy volunteers

Introduction

Several immunosuppressive mechanisms hinder antitumor immunity. Among them, adenosine signaling has emerged as a key strategy harnessed by tumors to evade immunosurveillance [1].

Adenosine is an immunosuppressive metabolite produced at high levels within the tumor microenvironment [2]. It is mainly generated extracellularly, through sequential dephosphorylation of adenosine triphosphate by the ectonucleotidases CD39 and CD73 [3]. Alternatively, tissue-nonspecific alkaline phosphatase within the tumor can convert adenosine 5′-monophosphate (AMP) to adenosine [4]. High expression of CD73 has been shown to correlate with poor outcomes in tumor types such as triple-negative breast cancer [5] and non-small-cell lung cancer [6]. Extracellular adenosine triggers signaling pathways through 4 distinct G-protein-coupled adenosine receptors: A_1, A_{2a}, A_{2b}, and A_3 [7]. Of these receptor subtypes, the potent immunosuppressive effects of adenosine are mediated by $A_{2a}R$, which is expressed primarily by T cells, and $A_{2b}R$, which is expressed on myeloid cells [8]. $A_{2a}R$ stimulation impairs T cell activation, leading to reduced proliferation and cytokine release [9, 10]. Similarly, adenosine-exposed natural

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killer (NK) cells exhibit reduced cytotoxic activity [11]. Adenosine has also been shown to induce a suppressive phenotype in myeloid cells such as dendritic cells and macrophages, resulting in compromised T cell activation [12] and promotion of a myeloid-derived-suppressor cell-like phenotype [13]. These effects are thought to be mediated primarily through $A_{2b}R$. Alleviation of the suppressive effects of adenosine within tumors results in the restoration of antitumor immune responses in various preclinical models when combined with either anti-programmed cell death-1 (PD-1) or chemotherapy [14–16]. Adenosine receptor inhibition has emerged as an approach for alleviating immune suppression within tumors, although, thus far, only $A_{2a}R$ antagonists initially developed for central nervous system indications are being studied in oncology subjects [17].

AB928 was developed to alleviate immune suppression in the tumor microenvironment by targeting $A_{2a}R$-expressing T and NK cells and myeloid cells, which express both $A_{2a}R$ and $A_{2b}R$ [9, 10, 12]. AB928 inhibits $A_{2a}R$ and $A_{2b}R$ with similar potencies (equilibrium binding constant of 1.4 and 2 nM, respectively) [18]. Consistent with previous reports, AB928 has been shown to result in decreased tumor growth, either alone or in combination with either anti-PD-1 [19] or chemotherapy [20].

Activation of either $A_{2a}R$ or $A_{2b}R$ leads to phosphorylation of the transcription factor cyclic AMP response element binding protein (CREB), which can be detected in circulating leukocytes, allowing for the establishment of pharmacokinetic (PK)/pharmacodynamic (PD) relationships in mice. Moreover, AB928 exhibits concentration-dependent inhibition of phosphorylated CREB (pCREB) in whole blood [21].

Herein, we report results from a first-in-human phase 1, dose-finding study that investigated the safety and tolerability, PK profile, including food effect (FE), and PD profile of AB928 in healthy volunteers.

Subjects and methods

Subjects

Healthy male and female subjects, aged 18 to 55 years, inclusive, who were willing and able to provide informed consent and had a body mass index of 19 to 30 kg/m^2, inclusive, were eligible to participate in the study. Subjects were required to have a negative serologic test for hepatitis B and C and human immunodeficiency virus, and all clinical laboratory tests of blood and urine must have been within the normal range or show no clinically relevant deviations. Female subjects of childbearing potential, with a fertile male sexual partner, were required to use adequate contraception from screening until 90 days after the follow-up visit. All prescribed medication must have been stopped at least 30 days prior to (each) admission to the Clinical Research Center. Exclusion criteria included a history of relevant drug and/or food allergies, history of drug abuse or drug addiction, and positive drug and alcohol screen.

Study design

This was a randomized, double-blind, placebo-controlled, single-ascending-dose (SAD) and multiple-ascending-dose (MAD) study. Healthy subjects were randomly assigned (3:1) to receive AB928 ($n = 6$) or matching placebo ($n = 2$) in each of the SAD cohorts (single oral dose of 10, 25, 75, or 150 mg or 2 doses of AB928 100 mg, 12 h apart) and MAD cohorts (10, 25, 75, 150, or 200 mg/day for 4 days). All study drug doses were administered in a fasted state except for MAD 200 mg, which was given in a fed state (standard high-fat breakfast). The MAD part of the study was started once the third SAD cohort was completed and a decision was made to escalate to the fourth dose cohort. Dose escalation was based on available safety, PK, and PD data. The study also included a FE cohort, in which subjects received 2 doses of AB928 100 mg. The 100-mg dose was selected based on available data from the SAD and MAD cohorts. Subjects fasted for the first dose of AB928 on Day 1. After a brief wash-out period, subjects received the second AB928 dose after a standard high-fat breakfast on Day 7.

Subjects were observed in the Clinical Research Center on Days 1–2 (SAD), Days 1–4 (MAD), and Days 1–2 and 7–8 (FE), and they were discharged on Day 3 (SAD), Day 5 (MAD), and Days 3 and 9 (FE). Follow-up visits occurred on Days 7–10 (SAD), Days 9–12 (MAD), and Days 13–16 (FE).

Assessments

This study assessed the safety, tolerability, PK, and PD of AB928 versus placebo. The incidence, severity, and causal relationship of treatment-emergent adverse events (TEAEs) were monitored alongside dose-limiting or intolerable adverse events (AEs), abnormal laboratory findings (clinical chemistry, hematology, and urinalysis), and clinically relevant changes in vital signs, 12-lead electrocardiogram, telemetry, physical examination, neurological examination, body sway test, Bond and Lader Visual Analog Scale of Mood and Alertness, and Digit Symbol Substitution Test.

Blood samples for PK and PD analyses were collected throughout the study at scheduled visits. AB928 plasma concentrations were determined using liquid chromatography/tandem mass spectrometry. Whole blood from AB928- or placebo-treated subjects was stimulated ex vivo with the synthetic adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA). Multi-color phosphoflow cytometry was used to assess phosphorylated CREB levels in $CD8^+$ cells in whole blood. Antibodies recognizing rabbit anti-human pCREB and mouse anti-human CD8 (clone RPA-T8) were obtained from Cell

Signaling Technologies (Beverly, MA, USA) and BD Biosciences (San Diego, CA, USA), respectively. At each PD time point, the baseline level of pCREB in each subject was determined from samples without NECA and expressed as the geometric mean of the fluorescent signal generated from the pCREB antibody staining in $CD8^+$ cells. Elevated pCREB signals resulting from stimulation with NECA were assessed similarly. The responses of an individual subject at PD time points following dosing were then compared with the subject's response at the pre-dose time point. The response to NECA stimulation at the pre-dose time point was taken to represent the maximal assay response in the absence of AB928. The percentage by which the signal was inhibited following AB928 dosing was then calculated by normalizing against the NECA response seen at the pre-dose time point. Placebo subjects were also included in the analysis to determine biological and technical assay variation as PD sampling occurred over the course of several days.

Statistical analysis

The sample size was customary for phase 1 studies evaluating safety, PK, and/or PD parameters and was not based on formal hypothesis tests. For each of the SAD and MAD study parts, placebo subjects from each cohort were pooled into a single placebo group. For all continuous variables, summary statistics included number, mean, median, standard deviation, minimum, and maximum. PK parameters were determined using serum concentrations with Phoenix® WinNonlin® version 6.3 or higher (Pharsight, Inc.) and SAS® version 9.4 for dose-proportionality assessment. The incidence of TEAEs were classified by system organ class and preferred term according to the Medical Dictionary for Regulatory Activities (version 20.1), the relationship to study drug, and the severity for each dose.

Results

Subject disposition and characteristics

Eight-five subjects participated in the study between October 2017 and March 2018 (40 subjects each in the SAD and MAD cohorts and 5 subjects in the FE cohort). All 85 subjects completed the study as per protocol, with all included in the safety analysis set. The 65 subjects who were randomized to active treatment (30 subjects in each of the SAD and MAD cohorts and 5 subjects in the FE cohort) were included in the PK analysis set. Baseline demographics were similar among cohorts in the SAD and MAD parts of the study (Table 1).

Safety and tolerability

No deaths or other serious adverse events (SAEs) occurred, and no subject discontinued study drug because of an AE in any part of the study (Table 2). Except for a tonsillitis-related clinically significant laboratory abnormality in the SAD 10-mg cohort, there were no trends or other clinically significant changes from baseline in laboratory parameters, vital signs, electrocardiogram recordings, or any of the other safety assessments in the study.

SAD cohorts Eighteen of 30 subjects (60%) who received AB928 and 6 of 10 subjects (60.0%) who received placebo experienced at least 1 TEAE. The most common AEs (≥10% of subjects) reported with AB928 (vs placebo) were nausea (13% vs 10%), pollakiuria (13% vs 0%), abdominal pain (10% vs 10%), and headache (10% vs 30%). All events were of Grade 1 or 2 intensity, and the vast majority resolved (~96%); none of the ongoing/resolving events were considered to be treatment-related. AEs determined to be related to study drug by the investigator were reported in 16 of 30 subjects (53%)who received AB928 and in 4 of 10 subjects (40%) who received placebo. The most common treatment-related AEs (≥10% of subjects) reported with AB928 (vs placebo) were nausea (13% vs 10%) and headache (10% vs 20%) (Table 3). One subject in the 10-mg cohort had a clinically significant change from baseline in safety laboratory results (C-reactive protein increase from 1 mg/L at screening to 25.2 mg/L at follow-up visit). This abnormality was considered related to a tonsillitis event, which resolved.

MAD cohorts Twenty-seven of 30 subjects (90%) who received AB928 and 8 of 10 subjects (80%) who received placebo experienced at least 1 TEAE. The most common AEs (≥10% of subjects) reported with AB928 (vs placebo) were headache (27% vs 30%), abdominal pain (23% vs 20%), nausea, dizziness (17% vs 10% each), abdominal distension, vessel puncture site bruise (10% vs 10% each), constipation, palpitations, and hot flush (10% vs 0% each). All events were of Grade 1 or 2 intensity and most resolved (~93%); none of the ongoing/resolving events were considered to be treatment-related. AEs determined to be related to study drug by the investigator were reported in 15 of 30 subjects (50%) who received AB928 and in 5 of 10 subjects (50%) who received placebo. The most common treatment-related AEs (≥10% of subjects) reported with AB928 (vs placebo) were abdominal pain (20% vs 10%), nausea (17% vs 10%), dizziness and headache (10% vs 10% each), and abdominal distension and constipation (10% vs 0% each) (Table 3).

FE cohort Four of 5 subjects (80%) experienced at least 1 TEAE. The most common AE (>1 subject) was headache (3 subjects), which occurred in 1 subject in the fasted group

Table 1 Demographic and other baseline characteristics in healthy subjects

Characteristic	SAD cohorts (single dose)						MAD cohorts (qd dose × 4)						FE cohort (single dose) AB928
	Placebo $N = 10$	AB928					Placebo $N = 10$	AB928					
		10 mg $N = 6$	25 mg $N = 6$	75 mg $N = 6$	150 mg $N = 6$	100 mg q12h $N = 6$		10 mg $N = 6$	25 mg $N = 6$	75 mg $N = 6$	150 mg $N = 6$	200 mg fed $N = 6$	100 mg fasted-fed $N = 5$
Age, years													
Mean (SD)	26 (7)	27 (8)	24 (3)	28 (13)	23 (2)	26 (9)	28 (13)	29 (10)	24 (3)	27 (13)	38 (14)	29 (13)	33 (13)
Median	25	25	25	23	22	24	22	27	24	22	38	25	26
Min-Max	19–43	18–40	19–27	19–54	20–27	18–40	18–54	21–48	19–27	18–51	22–53	21–55	23–54
Sex, n (%)													
Male	6 (60)	2 (33)	3 (50)	3 (50)	2 (33)	3 (50)	1 (10)	4 (67)	2 (33)	0	3 (50)	1 (17)	2 (40)
Female	4 (40)	4 (67)	3 (50)	3 (50)	4 (67)	3 (50)	9 (90)	2 (33)	4 (67)	6 (100)	3 (50)	5 (83)	3 (60)
Race, n (%)													
White	10 (100)	5 (83)	6 (100)	6 (100)	5 (83)	5 (83)	7 (70)	6 (100)	6 (100)	6 (100)	5 (83)	5 (83)	5 (100)
Black or African American	0	1 (17)	0	0	0	0	1 (10)	0	0	0	0	0	0
American Indian or Alaska Native	0	0	0	0	0	0	1 (10)	0	0	0	0	0	0
Multiple	0	0	0	0	1 (17)[a]	1 (17)[b]	1 (10)	0	0	0	1 (17)[a]	1 (17)[a]	0
Ethnicity, n (%)													
Not Hispanic or Latino	10 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	9 (90)	6 (100)	6 (100)	6 (100)	5 (83)	6 (100)	5 (100)
Hispanic or Latino	0	0	0	0	0	0	1 (10.0)	0	0	0	1 (17)	0	0
BMI, kg/m^2													
Mean (SD)	22.7 (2.4)	23.4 (1.7)	22.5 (2.0)	23.6 (2.2)	22.2 (3.0)	24.8 (2.2)	24.0 (2.6)	24.3 (2.9)	22.1 (1.9)	23.2 (1.9)	23.1 (1.6)	24.8 (1.0)	23.9 (1.7)
Median	23.2	23.2	22.4	23.1	21.2	25.3	22.9	24.5	22.3	22.9	23.5	24.7	23.7
Min-Max	19.3–26.9	21.3–26.4	19.8–25.9	21.6–27.5	20.0–28.1	20.5–26.9	21.6–29.9	21.1–28.0	19.0–24.3	20.6–26.1	20.0–24.6	23.7–26.5	22.4–26.7

BMI, body mass index; *FE*, food effect; *MAD*, multiple-ascending dose; *Max*, maximum; *Min*, minimum; *q12h*, every 12 h; *qd*, once daily; *SAD*, single-ascending dose; *SD*, standard deviation

[a] White and Black or African American

[b] White and American Indian or Alaska Native and Asian

Table 2 Overall summary of treatment-emergent adverse events

Subjects with	SAD cohorts (single dose)						MAD cohorts (qd dose × 4)						FE cohort (single dose)	
	Placebo $N = 10$	AB928					Placebo $N = 10$	AB928					AB928	
	n (%)	10 mg $N = 6$ *n* (%)	25 mg $N = 6$ *n* (%)	75 mg $N = 6$ *n* (%)	150 mg $N = 6$ *n* (%)	100 mg q12h $N = 6$ *n* (%)	*n* (%)	10 mg $N = 6$ *n* (%)	25 mg $N = 6$ *n* (%)	75 mg $N = 6$ *n* (%)	150 mg $N = 6$ *n* (%)	200 mg fed $N = 6$ *n* (%)	100 mg fasted $N = 5$ *n* (%)	100 mg fed $N = 5$ *n* (%)
Any AE	6 (60)	3 (50)	1 (17)	4 (67)	6 (100)	4 (67)	8 (80)	3 (50)	6 (100)	6 (100)	6 (100)	6 (100)	4 (80)	4 (80)
Treatment-related AE	4 (40)	2 (33)	1 (17)	3 (50)	6 (100)	4 (67)	5 (50)	1 (17)	4 (67)	4 (67)	3 (50)	3 (50)	1 (20)	0
Grade 1 AE	4 (40)	2 (33)	1 (17)	3 (50)	5 (83)	3 (50)	8 (80)	3 (50)	6 (100)	6 (100)	5 (83)	4 (67)	4 (80)	4 (80)
Grade 2 AE	2 (20)	1 (17)	0	1 (17)	1 (17)	1 (17)	0	0	0	0	1 (17)	2 (33)	0	0
Treatment-related Grade 1AE	3 (30)	1 (17)	1 (17)	3 (50)	5 (83)	3 (50)	5 (50)	1 (17)	4 (67)	4 (67)	3 (50)	2 (33)	1 (20)	0
Treatment-related Grade 2 AE	1 (10)	1 (17)	0	0	1 (17)	1 (17)	0	0	0	0	0	1 (17)	0	0
Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discontinuation due to AE	0	0	0	0	0	0	0	0	0	0	0	0	0	0

AE, adverse event; *FE*, food effect; *MAD*, multiple-ascending dose; *q12h*, every 12 h; *qd*, once daily; *SAD*, single-ascending dose; *SAE*, serious adverse event

and in 2 subjects in the fed group. All events were of Grade 1 intensity and all resolved. One AE (eructation), which occurred in the 100-mg fasted group, was considered related to study drug by the investigator.

Pharmacokinetic evaluation

SAD cohorts The mean plasma concentration-time profiles for AB928 following administration of single oral doses are illustrated in Fig. 1a. AB928 was rapidly absorbed, with mean time to maximum plasma concentration (T_{max}) values ranging from 1.5 to 2.3 h at the 10- to 150-mg dose levels (Table 4). After single oral doses, AB928 exposure was dose proportional with a slope of 1.031 (90% confidence interval [CI]:0.936, 1.126) for maximum plasma concentration (C_{max}), 1.101 (1.002, 1.201) for area under the concentration-time curve from time 0 to the last measurable point, and 1.111 (0.995, 1.226) for area under the concentration-time curve from time 0 to infinity ($AUC_{0\text{-inf}}$).

MAD cohorts The mean plasma concentration-time profiles for AB928 following administration of multiple oral doses are illustrated in Fig. 1b. AB928 was rapidly absorbed in the fasted state, with mean T_{max} values ranging from 1.00 to 2.00 h (Table 4). The mean T_{max} was prolonged to 5.17 h at 200 mg administered with a standard high-fat meal. After multiple oral doses, AB928 exposure was slightly less than dose proportional with a slope of 0.931 (90% CI: 0.857, 1.005) for C_{max} and 0.936 (0.849, 1.023) for area under the concentration-time curve from time 0 to 24 h on Day 1 and 0.939 (0.869, 1.009) and 0.935 (0.839, 1.031), respectively, on Day 4.

FE cohort The mean plasma concentration-time profiles for AB928 following administration of a single oral dose (100 mg) in a fasted and fed state are illustrated in Fig. 1c. The mean T_{max} of AB928 was prolonged from 1.60 to 3.40 h when the fasted versus fed state was compared (Table 4). After a standard high-fat meal, the mean C_{max} was reduced from 1172 to 794 ng/mL. The mean $AUC_{0\text{-inf}}$ values were 15,980 and 16,324 ng·h/mL for the fasted and fed states, respectively, indicating a similar total exposure between the 2 groups. These results show that food affected the rate, but not the extent of AB928 absorption.

Pharmacodynamic evaluation

The inhibition of $A_{2a}R$-mediated effects by AB928 was determined on $CD8^+$ T cells in whole blood by the decrease in pCREB levels following stimulation ex vivo with NECA. To determine the inhibition of pCREB due to AB928, the responses of an individual subject at PD time points post-dose were compared to that subject's response pre-dose. The

Table 3 Summary of study drug-related treatment-emergent adverse events (in >1 subject in either total group)

MedDRA preferred term	SAD cohorts (single dose)							MAD cohorts (qd dose × 4)						
	Placebo $N=10$ n (%)	AB928						Placebo $N=10$ n (%)	AB928					
		10 mg $N=6$ n (%)	25 mg $N=6$ n (%)	75 mg $N=6$ n (%)	150 mg $N=6$ n (%)	100 mg q12h $N=6$ n (%)	Total $N=30$ n (%)		10 mg $N=6$ n (%)	25 mg $N=6$ n (%)	75 mg $N=6$ n (%)	150 mg $N=6$ n (%)	200 mg fed $N=6$ n (%)	Total $N=30$ n (%)
Nausea	1 (10)	0	0	2 (33)	0	2 (33)	4 (13)	1 (10)	0	1 (17)	2 (33)	1 (17)	1 (17)	5 (17)
Abdominal pain	1 (10)	0	0	0	0	2 (33)	2 (7)	1 (10)	1 (17)	2 (33)	1 (17)	1 (17)	1 (17)	6 (20)
Headache	2 (20)	0	0	0	2 (33)	1 (17)	3 (10)	1 (10)	0	0	0	1 (17)	2 (33)	3 (10)
Abdominal distension	1 (10)	0	1 (17)	0	0	0	1 (3)	0	0	1 (17)	1 (17)	0	1 (17)	3 (10)
Constipation	0	1 (17)	0	0	0	0	1 (3)	0	1 (17)	0	1 (17)	0	1 (17)	3 (10)
Chest pain	0	0	0	1 (17)	0	1 (17)	2 (7)	0	0	1 (17)	0	0	0	2 (7)
Dizziness	0	0	0	0	0	0	0	1 (10)	0	1 (17)	1 (17)	1 (17)	0	3 (10)
Feeling jittery	0	0	0	0	2 (33)	0	2 (7)	0	0	0	0	0	0	0
Insomnia	0	0	0	0	2 (33)	0	2 (7)	0	0	0	0	0	0	0
Pollakiuria	0	0	0	0	4 (68)	0	4 (13)	0	0	0	0	0	0	0
Diarrhea	1 (10)	0	0	0	0	0	0	1 (10)	0	0	0	2 (33)	0	2 (7)
Palpitations	0	0	0	0	0	0	0	0	0	0	0	0	2 (33)	2 (7)
Hot flush	0	0	0	1 (17)	0	0	1 (3.3)	0	0	0	0	0	2 (33)	2 (7)

MAD, multiple-ascending dose; *MedDRA*, Medical Dictionary for Regulatory Activities; *q12h*, every 12 h; *qd*, once daily; *SAD*, single-ascending dose

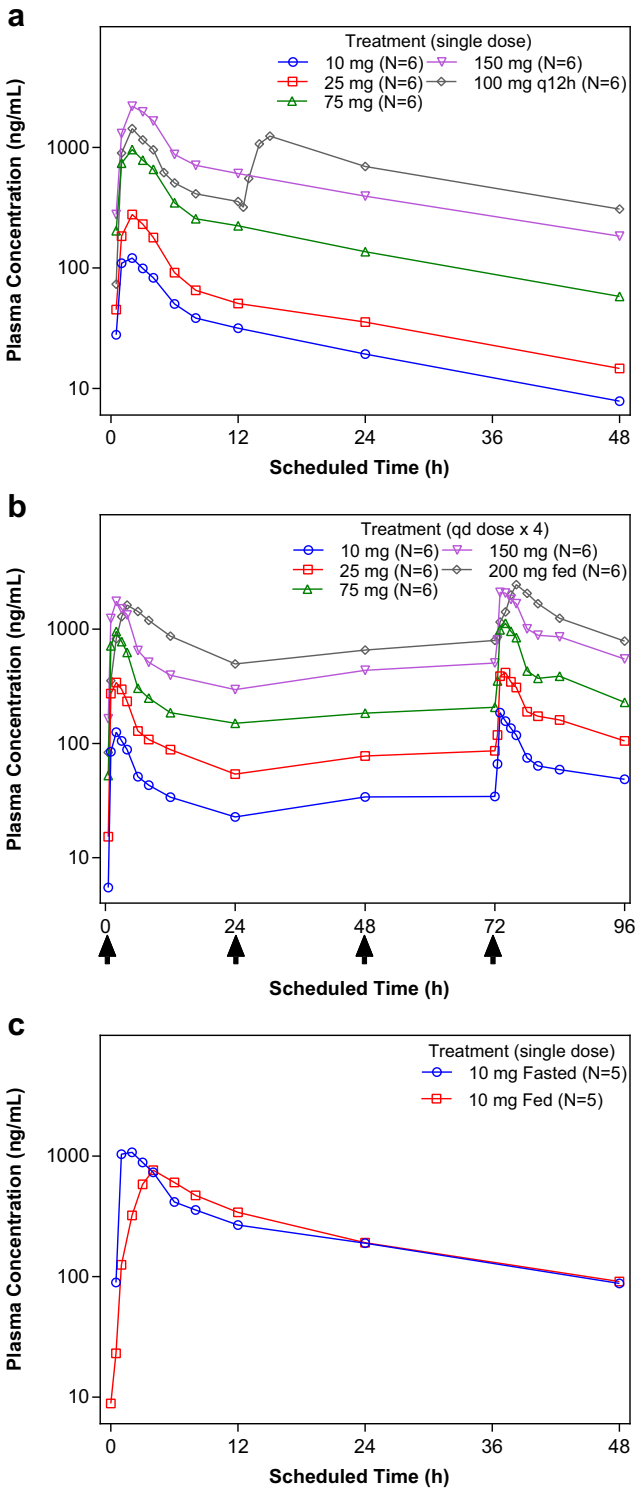

Fig. 1 Mean plasma concentrations of AB928 over time (semi-logarithmic scales). (**a**) Single-ascending-dose cohorts. (**b**) Multiple-ascending-dose cohorts. (**c**) Food effect cohort. On the multiple-ascending-dose graph, the x-axis reflects time from the first dose. Arrows indicate AB928 dosing; on Days 2 and 3, PK levels were only determined from pre-dose samples. *q12h*, every 12 h; *qd*, once daily

pre-dose response was taken to represent the maximal assay response in the absence of AB928. NECA (5 μM) induced a significant, and maximal, increase in pCREB (Fig. 2) in peripheral blood $CD8^+$ T cells, with elevations being observed in all subjects prior to dosing. Placebo-treated subjects were also included to enable assessment of biological and technical assay variation as PD sampling occurred over the course of several days. This variation in placebo-treated subjects is also indicated in Fig. 3.

The extent of adenosine receptor inhibition by AB928 on peripheral blood $CD8^+$ T cells showed that doses ≥75 mg once daily resulted in significant target inhibition throughout the dosing period. At 150 mg once daily, target coverage was maintained throughout the dosing period (≥90% at trough, 24 h following the Day 4 dose; Figs. 2 and 3a, and Table 5), whereas lower doses produced lower levels of receptor blockade. A twice-daily dose of 100 mg AB928 evaluated in the SAD part of the study also provided effectively complete inhibition at trough plasma concentrations (88%; Table 5). AB928 plasma levels ≥1 μM resulted in maximal (≥90%) $A_{2a}R$ inhibition (Fig. 3b).

Discussion

We report on the first-in-human, placebo-controlled evaluation of AB928, an orally bioavailable, selective dual $A_{2a}R$/$A_{2b}R$ antagonist with demonstrated immunomodulatory activity. In this study, single- and multiple-ascending doses were administered to healthy adult subjects to characterize the safety, tolerability, PK, including FE, and PD profiles of AB928 to inform selection of the starting dose of AB928 for clinical trials in cancer patients.

AB928 was well tolerated when administered as a single oral dose (10, 25, 75, 150 mg, or 100 mg every 12 h) or multiple oral doses (10, 25, 75, 150, or 200 [fed] mg once daily for 4 days) and did not affect any physiologic parameters potentially sensitive to adenosine inhibition. No AEs prevented escalation to higher doses. The overall AE profiles of AB928 and placebo were generally similar. All AEs were reported as Grade 1 or 2 in intensity, and none of the events were serious or resulted in study drug discontinuation. All events resolved, except for a few Grade 1 events in the SAD (~4%) and MAD (~7%) cohorts. None of the ongoing events were deemed to be treatment-related by the investigator. The most common treatment-related AEs (≥10% of subjects) reported with AB928 were nausea and headache in the SAD cohorts and abdominal pain, nausea, dizziness, headache, abdominal distension, and constipation in the MAD cohorts. None of these events were dose-dependent.

The observed PK profile of AB928 was linear in the dose range of 10 to 150 mg. AB928 was rapidly absorbed under fasted conditions. In a postprandial state, the rate of AB928 absorption (C_{max}) decreased but the extent of AB928

Table 4 Summary of mean (SD) AB928 PK parameters and dose proportionality assessment

PK parameter	SAD cohorts (single dose)						MAD cohorts (qd dose × 4)						FE cohort (single dose)	
	AB928					Dose Proportionality[a]	AB928					Dose Proportionality[b]	AB928	
	10 mg $N=6$	25 mg $N=6$	75 mg $N=6$	150 mg $N=6$	100 mg q12h $N=6$	Slope (90% CI)	10 mg $N=6$	25 mg $N=6$	75 mg $N=6$	150 mg $N=6$	200 mg fed $N=6$	Slope (90% CI)	100 mg fasted $N=5$	100 mg fed $N=5$
DAY 1														
C_{max} (ng/mL)	140 (23.6)	332 (121)	1054 (284)	2300 (600)	1752 (652)	1.031 (0.936–1.126)	145 (30.7)	386 (63.0)	977 (242)	1883 (455)	1795 (413)	0.931 (0.857–1.005)	1172 (225)	794 (111)
T_{max} (h)	1.50 (0.57)	1.50 (0.55)	1.68 (0.52)	2.33 (0.52)	8.00 (6.95)	NA	1.67 (0.82)	1.83 (0.75)	2.00 (0.01)	1.83 (0.75)	5.17 (1.83)	NA	1.60 (0.55)	3.40 (0.55)
AUC_{0-24} (ng·h/mL)	1041 (198)	2090 (589)	7616 (971)	19,676 (5207)	19,624 (5990)	NA	1132 (365)	2876 (404)	7217 (1898)	14,888 (3824)	21,612 (4547)	0.936 (0.849–1.023)	9218 (1406)	8903 (772)
AUC_{0-last} (ng·h/mL)	1360 (280)	2750 (890)	9841 (1287)	26,555 (7110)	31,952 (10251)	1.101 (1.002–1.201[c])	NA	NA	NA	NA	NA	NA	12,621 (2808)	12,365 (2114)
AUC_{0-inf} (ng·h/mL)	1626 (451)	3400 (1369)	11,487 (1645)	33,080 (9967)	NA	1.111 (0.995–1.226)	NA	NA	NA	NA	NA	NA	15,980 (4772)	16,324 (6938)
$t_{1/2}$ (h)	19.4 (6.34)	22.0 (9.24)	19.1 (3.83)	22.1 (4.18)	NA	NA	NA	NA	NA	NA	NA	NA	23.2 (7.23)	21.5 (12.3)
DAY 4														
C_{max} (ng/mL)	NA	NA	NA	NA	NA	NA	196 (72.9)	450 (37.7)	1216 (269)	2432 (255)	2568 (292)	0.939 (0.869–1.009)	NA	NA
T_{max} (h)	NA	NA	NA	NA	NA	NA	1.00 (0.00)	1.50 (0.55)	1.67 (0.51)	1.67 (1.21)	4.00 (1.10)	NA	NA	NA
AUC_{0-24} (ng·h/mL)	NA	NA	NA	NA	NA	NA	1859 (665)	4420 (517)	11,182 (3211)	24,128 (6374)	32,764 (5133)	0.935 (0.839–1.031)	NA	NA
Rac AUC_{0-24}(ng·h/mL)	NA	NA	NA	NA	NA	NA	1.62 (0.15)	1.55 (0.18)	1.56 (0.29)	1.64 (0.22)	1.54 (0.21)	NA	NA	NA
$t_{1/2}$ (h)	NA	NA	NA	NA	NA	NA	44.8 (13.8)	34.2 (15.3)	32.8 (18.5)	27.1 (7.74)	40.2 (21.7)	NA	NA	NA

AUC_{0-24}, area under the concentration-time curve from time 0 to 24 h; *AUC_{0-inf}*, area under the concentration-time curve from time 0 to infinity; *AUC_{0-last}*, area under the concentration-time curve from time 0 to last measurable time point; *CI*, confidence interval; *C_{max}*, maximum plasma concentration; *FE*, food effect; *MAD*, multiple-ascending dose; *NA*, not applicable; *q12h*, every 12 h; *qd*, once daily; *Rac*, accumulation ratio; *SAD*, single-ascending dose; *SD*, standard deviation; *$t_{1/2}$*, terminal half-life; *T_{max}*, time to maximum plasma concentration

[a] Dose level for SAD Cohort 5 (100 mg q12h) is not included

[b] Dose level for MAD Cohort 5 (200 mg qd fed) is not included

[c] 90% CI does not contain 1: deviation from dose proportionality

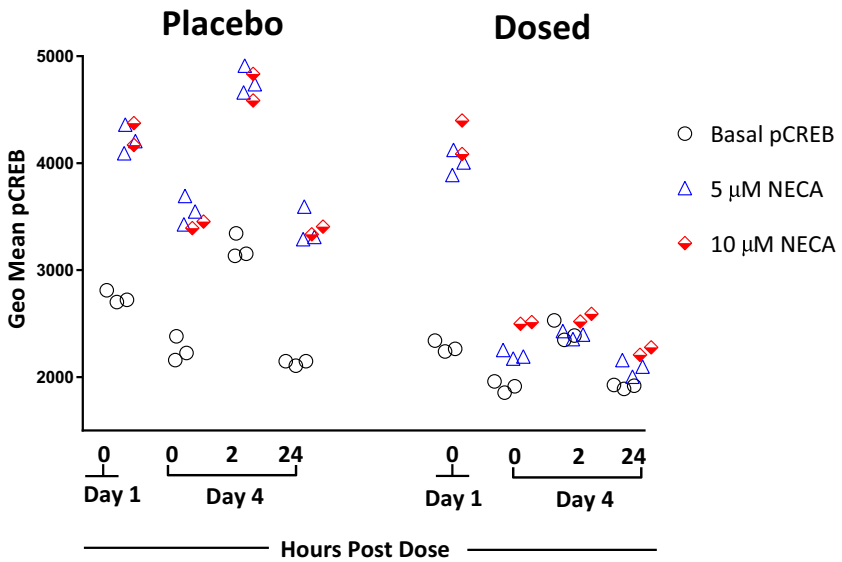

Fig. 2 PD results from placebo and dosed subjects following stimulation with 5 and 10 μM NECA in the 150 mg once-daily AB928 cohort. These raw data, collected over the course of 4 days, are representative of data across all cohorts in which a maximal pCREB response was observed at pre-dose (or in placebo-treated subjects at all PD time points) following stimulation with 5 μM NECA. The raw data from the dosed subject are representative of the maximal inhibition observed in the presence of 5 μM NECA following 4 once-daily doses with 150 mg AB928. This raw data illustrates that while the absolute geometric mean may vary over the course of sampling, the assay window between baseline and NECA stimulation remains quite consistent in placebo-treated subjects. *NECA*, 5'-N-ethylcarboxamidoadenosine; *pCREB*, phosphorylated cyclic adenosine monophosphate response element binding protein; *PD*, pharmacodynamics

absorption (AUC) was unchanged. AB928 exhibited a long $t_{1/2}$ of approximately 20 h across all dose cohorts. As expected, AB928 exposure increased following multiple doses, with a mean accumulation ratio of approximately 1.6 across dose cohorts. The PK profile of AB928 supports once-daily dosing of AB928 in cancer patients.

The pCREB whole blood assay used to measure target engagement demonstrated robust PK/PD correlations with AB928. Dosing regimens with AB928 were identified that can provide maximal adenosine receptor blockade assessed as a function of NECA-stimulated pCREB induction in peripheral blood $CD8^+$ T cells. Previous experiments conducted in vitro indicated that NECA is significantly more potent than adenosine at inducing CREB phosphorylation in human blood. Our PD analysis focused on stimulation with 5 μM NECA under physiologically relevant conditions. We believe that this provided adenosine receptor activation comparable to what might be expected from significantly higher intratumoral adenosine concentrations. In response to the 5 μM NECA challenge, 4 consecutive once-daily doses of 75 mg AB928 approached our target of ≥90% inhibition (average, 76%) at trough. Complete inhibition of NECA-stimulated pCREB induction was observed at trough in the subsequent MAD cohorts of 150 mg and 200 mg AB928. In healthy volunteers, the PK/PD correlations support that doses greater than 75 mg and up to 150 mg once daily provide maximal inhibition at trough. A twice-daily dose of 100 mg AB928 was also evaluated in the SAD part of the study, and this dosing regimen also provided effectively complete inhibition at trough (88%).

In conclusion, AB928 was well tolerated and demonstrated predictable PK and robust PD activity supportive of once-daily dosing regimens for maximal adenosine receptor inhibition. No safety concern was identified up to the highest dose tested in each study part. Across the single and multiple dose levels assessed, the PK profile of AB928 was linear and dose-proportional, and a clear PK/PD correlation was demonstrated. AB928 plasma levels ≥1 μM were associated with ≥90% adenosine receptor inhibition. This study in healthy volunteers provides an important understanding of the safety, PK, and PD of AB928 and allows more accurate dose modeling and exposure prediction for evaluation in oncology indications. Clinical development continues with phase 1/1b trials of AB928 in combination with other immunotherapy agents or chemotherapy in patients with breast or ovarian cancer, gastroesophageal or colorectal cancer, lung cancer, and advanced malignancies. The rationale for these combinations is supported by high expression of the adenosine-producing enzymes CD73 and/or tissue-nonspecific alkaline phosphatase in these tumor types and the preclinical data that we, and others, have generated demonstrating beneficial activity when combining adenosinergic pathway inhibitors (such as $A_{2a}R/A_{2b}R$ antagonists or CD73 inhibitors) with a range of clinically relevant interventions affecting a broad array of different immune cell types [6, 13, 16, 20].

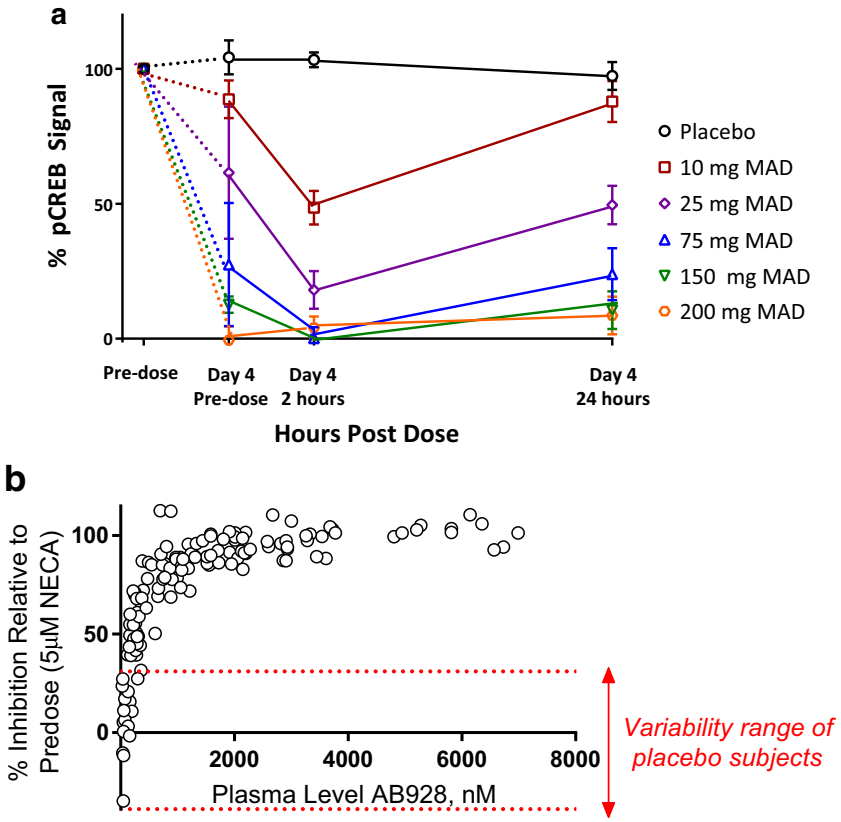

Fig. 3 Inhibition of adenosine receptor activation by AB928 on CD8$^+$T cells. (**a**) Percentage of pCREB remaining following challenge with 5 μM NECA in subjects after 4 once-daily doses of AB928; and (**b**) the PK/PD correlation in AB928-dosed subjects measured by pCREB inhibition. The dashed red line indicates the range of variation that was observed in the placebo-treated subjects, inclusive of single data points. *$A_{2a}R$*, adenosine 2a receptor; *MAD*, multiple-ascending dose; *NECA*, 5'-N-ethylcarboxamidoadenosine; *pCREB*, phosphorylated cyclic adenosine monophosphate response element binding protein; *PD*, pharmacodynamic; *PK*, pharmacokinetics

Table 5 Relationship between mean AB928 plasma levels and pharmacodynamic endpoints

Dose cohort by study part	2 Hours Post Dose		24 Hours Post Dose	
	AB928 Plasma Concentration (ng/mL)	% Inhibition of 5 μM NECA	AB928 Plasma Concentration (ng/mL)	% Inhibition of 5 μM NECA
SAD cohorts				
10 mg single dose	121	63%	19.9	6%[a]
25 mg single dose	292	ND	41.0	ND
75 mg single dose	993	≥90%	138	63%
150 mg single dose	2272	100%	404	≥90%
100 mg twice (12 h apart)	1527	≥90%	745	88%[b]
MAD cohorts				
10 mg qd (Day 4)	163	51%	53.4	12%[a]
25 mg qd (Day 4)	420	82%	108	51%
75 mg qd (Day 4)	1148	100%	261	76%
150 mg qd (Day 4)	2113	100%	566	≥90%
200 mg qd fed (Day 4)	1517	≥90%	819	≥90%

MAD, multiple-ascending dose; *ND*, not determined; *NECA*, 5'-N-ethylcarboxamidoadenosine; *qd*, once daily; *SAD*, single-ascending dose

[a] Some or all dosed subjects were within the range of variation observed in placebo subjects (−39 to 30%)

[b] Data collected 12 h following the second daily dose

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Compliance with ethical standards

Conflict of interest LS, LJ, ML, DA, JJ, AR, JT, JP, MW, and JK are employees of Arcus Biosciences, Inc., and RT and GA are employees of PRA Health Sciences.

Ethical approval This study was conducted in the Netherlands in accordance with the ethical principles that have their origin in the Declaration of Helsinki,and complied with the International Conference on Harmonization E6 Guideline for Good Clinical Practice and the European Union CTD Directive, as incorporated into Dutch Law. The protocol was approved by the institutional review board. The data were analyzed by PRA Health Sciences (safety and PK) and Arcus Biosciences, Inc. (PD).

Informed consent Informed consent was obtained from all individual participants included in the study.

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