A Double-blind, Placebo-controlled, Randomized, Single Ascending, and Multiple Dose Phase 1 Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Oral Dose Isomyosamine Capsules in Healthy Adult Subjects

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ABSTRACT

Background Aging is tightly linked to chronic disease, frailty, and death. Multi-morbidity, defined as the presence in the same patient of three or more conditions such as neoplastic, cardiovascular, neurodegenerative, metabolic, or autoimmune diseases, becomes more common with age.

Methods The study was performed in a double-blind fashion. Subjects within each dose cohort (Cohorts 1, 2, 3, and 4) were randomly assigned to receive Isomyosamine doses (between 150 mg to 600 mg or placebo) or placebo in a 3:1 ratio (6 active: 2 placebo).

Results Isomyosamine single daily doses each of 150 mg, 300 mg, and 450 mg for 3 days and multiple daily doses of 600 mg for 6 days were safe and well tolerated in healthy subjects. In one dose group, there was a decrease in TNF-α levels found in Isomyosamine treated subjects, but no change in the levels in subjects given placebo. The increase in Isomyosamine exposure was proportional to dose across the dose range of 300 mg to 600 mg when administered as a single dose. There was minimal accumulation of Isomyosamine following 5 days of once daily dosing of Isomyosamine 600 mg. Isomyosamine half-life ranged from approximately 15 minutes to 45 minutes across all doses in the single ascending dose and multiple ascending dose portion of the study. Elimination of Isomyosamine included the renal pathway as a minor route.

Conclusion Isomyosamine will continue to be investigated in phase 2 clinical trials for the treatment of sarcopenia/frailty, hashimoto's thyroiditis and rheumatoid arthritis.

Introduction

Aging is tightly linked to chronic diseases such as neoplastic, cardiovascular,

neurodegenerative, metabolic, or autoimmune diseases, frailty, and death [1, 2]. Preclinical studies have shown that lifespan and health span can be extended by nutritional and pharmacological interventions. The first group includes fasting, overall caloric restraint, restriction of specific nutrients such as the recent branched amino acid [2, 3], or supplementation of micronutrients. A pharmacological approach is, however, desirable considering the low compliance of the general population to dietary changes and the ever-expanding knowledge of the pathways underlying aging [2].

Several drugs have been researched for this indication, such as SIRT activators, polyphenols, metformin, rapamycin, and senolytics, capable of inducing apoptosis in senescent cells, and blockers of telomere shortening [2].

Isomyosamine, an isomer of myosmine, is a synthetic alkaloid derived from tobacco plant, which has been shown to inhibit the production and release of cytokines, including IFN-γ, IL-2, IL-10, and TNF-α, from human peripheral blood mononuclear cells in a dose-dependent manner [4]. TNF-α is the protein in the body that causes inflammation and helps activate the process of aging. In preclinical studies and recently published reports, Isomyosamine could suppress in vitro the release of TNF-α from splenic CD4 T cells and reducing disease incidence and severity in mouse models of autoimmune thyroiditis and multiple sclerosis [2, 4], in the absence of measurable toxicity. These models, based on a relatively short administration (12 weeks for thyroiditis and 3 weeks for experimental autoimmune encephalomyelitis; EAE), included the mechanism of reducing the number of infiltrating Th1 CD4 T cells and follicular B cells 4–5].

Isomyosamine also holds significant promise in other autoimmune conditions such as Rheumatoid Arthritis. Aging brings about a diminished capacity of the tissues to remodel, predisposing to fibrotic diseases like pulmonary, cardiac, and renal fibrosis [6]. Therapies endowed with both anti-inflammatory and anti-fibrotic actions, thus, represent a compelling, synergistic strategy to ameliorate age-related conditions. Aging and age-related diseases represent a therapeutic goal for senolytics and drugs targeting inflammatory or metabolic pathways. In vitro BioMAP profile analysis of Isomyosamine with reference benchmarks has shown that Isomyosamine exhibits similar biological activities to mTOR inhibitors everolimus and sirolimus owing to their largely overlapping mechanisms of action [7].

The ease of Isomyosamine oral dosing is a groundbreaking differentiator compared to currently available TNF-α blockers, all of which require delivery by injection or infusion. No approved TNF inhibitor has ever been dosed orally. The second and third key differentiators are selectivity and low toxicity. Unlike other therapies, Isomyosamine is designed to selectively block TNF-α when it becomes overactivated in autoimmune diseases and cytokine storms, but not to block it from doing its normal job of being a first responder to any routine type of moderate infection. In addition, the drug is not immunosuppressive and has not been shown to cause serious side effects common with traditional therapies that treat inflammation [8]. The current phase 1 study was conducted to further evaluate the safety, tolerability, and pharmacokinetic (PK) of Isomyosamine over the single and multiple dose ranges in healthy male and female adult subjects. The exploratory aims included the quantification of the relationship between plasma concentrations of Isomyosamine and change-from-baseline QTcF, corrected QT interval by Frederica, and to assess the biomarkers, pyridyloxobutyl (POB) adducts in hemoglobin and TNF-α after oral dose of Isomyosamine.

Materials and Methods

This phase 1, double-blind, placebo-controlled, randomized single ascending, and multiple dose study was conducted in healthy adult subjects at a single center. Institutional review board approval was received by ADVARRA, and this trial was performed under IND#138161. The study consisted of 2 parts: single ascending dose (SAD) and multiple ascending dose (MAD). Dosing levels for SAD were 150 mg (Cohort 1), 300 mg (Cohort 2), and 450 mg (Cohort 3) and for MAD was 600 mg (Cohort 4). In each cohort, a total of 8 subjects were randomized to receive either Isomyosamine (N = 6) or placebo (N = 2) for a total of 32 subjects for the entire study. The SAD cohorts consisted of 17 (70.8 %) males and 7 (29.2 %) females) whereas the MAD cohort consisted of 3 (37.5 %) males and 5 (63.5 %) females. The SAD cohorts ages ranged from 22–64 years of age whereas the MAD cohort consisted of ages ranging from 28–50 years of age (▶ **Tables 1** and ▶ **2**). Each subject participated in the study for approximately 8 weeks, including a screening period of up to 30 days, a confinement period in the clinic (3- or 6-days post administration), SAD and MAD respectively, end of study (EOS) on Day 8 or 11, and a telephone follow-up 5 days after EOS. The subjects were administered a single dose or multiple doses of either Isomyosamine or placebo, each subject participated in only one of the four cohorts during the study (▶ **Figs. 1** and **2**). Measurements of QT_C intervals were derived from overread ECGs measured in triplicates by the Investigator at the respective clinical site. Concentrations of Isomyosamine in plasma and urine were determined by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) analytical methods (Keystone Bioanalytical, Inc., North Wales, PA). An LC-MS/MS procedure (M190307.00) has been developed for the quantification of Myridine in K_2EDTA human plasma using Turbo Ionspray LC-MS/MS. In summary, myridine and the internal standard (amphetamine-d5) are isolated from human K_2EDTA plasma by protein precipitation (methanol is used as solvent). After shaking and centrifuging, 20 µL of the supernatant is transferred into a plastic injection vial containing 100 µL of water. The vial is capped and vortexed for 60 seconds. A total of 5–20 µL injection volume is used for LC-MS/MS analysis. The stability parameters include reinjection stability of extracted samples for up to 51.5 hours at 10 °C; refrigeration stability of extracted samples for up to 53.5 hours at 2–8 °C; bench-top stability of unextracted samples for up to 6.9 hours in ice-water; and freeze-thaw stability for up to four freeze-thaw cycles at −70 °C, and long-term storage stability for up to 98 days at −70 °C, which is enough to cover the maximum sample storage period (92 days) [9].

Summary Statistics and Treatment Summarization

Approximately 32 subjects were to be enrolled in this study (▶ **Figs. 1** and ▶ **2**). A total of 4 cohorts with size of 8 (6 active:2 placebo) were explored and considered adequate for characterizing the endpoints planned in this study. No formal sample size calculation was performed for exploratory outcomes as described in the protocol. The sample size is typical for this type of study and was approved by the regulatory agency (FDA) to serve the primary objective of this clinical study (safety and tolerability). Qualitative variables, population size (N for sample size and n for available data) and percentages (of available data for each class of the variable) were presented (▶ **Tables 1** and ▶ **2**). Quantitative non-PK variables were summarized using descriptive statistics, including N, n, mean, standard deviation (SD), coefficient of variation (CV %), median, minimum, and maximum values ▶ **Table 3**). Pharmacokinetic concentrations

▶ **Table 1** Demographics and Baseline Characteristics – Single Ascending Dose (Safety Analysis Set)

		Isomyosamine				
Variable/ Category	**Statistic**	**Placebo (N = 6)**	**150 mg (N = 6)**	**300 mg (N = 6)**	**450 mg (N = 6)**	**All Subjects (N = 24)**
Sex						
Male	n (%)	3 (50.0)	5 (83.3)	4 (66.7)	5 (83.3)	17 (70.8)
Female	n (%)	3 (50.0)	1 (16.7)	2 (33.3)	1 (16.7)	7 (29.2)
Race						
White	n (%)	3 (50.0)	5 (83.3)	6 (100.0)	2 (33.3)	16 (66.7)
Black or African American	n (%)	3 (50.0)	1 (16.7)	0	4 (66.7)	8 (33.3)
Age (years)	n	6	6	6	6	24
	Mean	39.5	48.8	50.7	38.0	44.3
	SD	15.4	10.5	8.6	13.3	12.8
	CV%	39.1	21.4	17.1	35.1	28.8
	Minimum	22	34	34	24	22
	Median	41	53	53	38	46
	Maximum	64	59	58	57	64
WOCBP						
No	n (%)	0	1 (100.0)	2 (100.0)	1 (100.0)	4 (57.1)
Yes	n (%)	3 (100.0)	0	0	0	3 (42.9)
Ethnicity						
Hispanic or Latino	n (%)	3 (50.0)	2 (33.3)	2 (33.3)	1 (16.7)	8 (33.3)
Not Hispanic or Latino	n (%)	3 (50.0)	4 (66.7)	4 (66.7)	5 (83.3)	16 (66.7)
Height (cm)	n	6	6	6	6	24
	Mean	168.98	177.68	174.18	170.18	172.76
	SD	7.91	14.31	6.61	6.22	9.40
	CV%	4.7	8.1	3.8	3.7	5.4
	Minimum	162.6	162.6	162.6	165.1	162.6
	Median	165.1	171.1	174.6	167.7	171.1
	Maximum	180.3	198.1	180.3	177.8	198.1
Weight (kg)	n	6	6	6	6	24
	Mean	81.57	83.43	83.47	74.87	80.83
	SD	12.74	20.46	10.90	9.59	13.60
	CV%	15.6	24.5	13.1	12.8	16.8
	Minimum	63.1	54.1	71.3	64.9	54.1
	Median	78.9	79.6	81.4	74.8	77.8
	Maximum	99.8	114.8	95.8	91.9	114.8
BMI(kg/m **2)	n	6	6	6	6	24
	Mean	28.35	26.17	27.63	25.48	26.91
	SD	2.53	3.43	2.81	2.21	2.84
	CV%	8.9	13.1	10.2	8.7	10.6
	Minimum	23.7	20.6	23.3	23.0	20.6
	Median	28.7	26.6	28.2	25.2	27.1
	Maximum	30.7	31.1	30.8	28.7	31.1

Note(s): BMI = body mass index; CV% = coefficient of variation; SD = standard deviation; WOCBP = woman of childbearing potential. Percentage of woman of childbearing potential are based on the number of female subjects.

(plasma only) and parameters (plasma and urine) were summarized using descriptive statistics, including N, n, arithmetic mean, SD, CV%, median, minimum, and maximum. In general, the tables, figures, and listings were presented separately by study part (SAD/MAD), if not otherwise specified. Non-PK data were presented for each treatment group with placebo subjects from all treatment groups combined into a single, overall placebo group. Non-PK data for all active-treated or all subjects combined were also presented when appropriate. Pharmacokinetic data were presented for active treatment groups. Geometric mean and geometric CV% were additionally included for PK parameters, where applicable. In contrast,

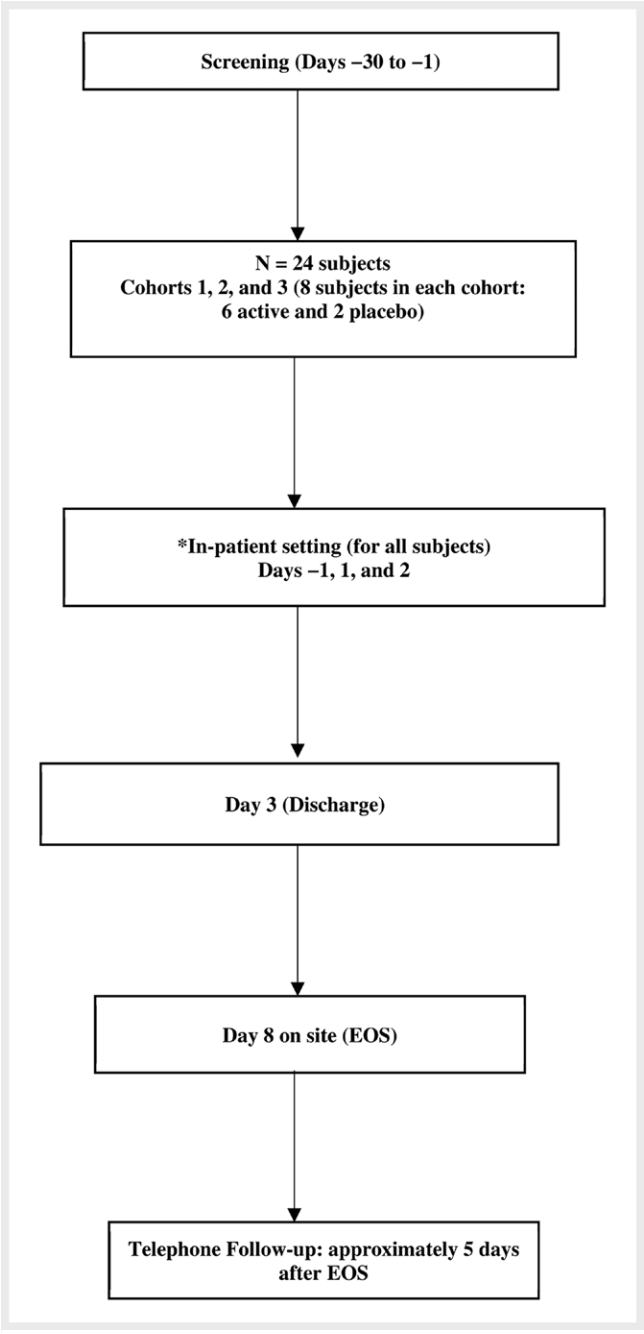
▶ **Table 2** Demographics and Baseline Characteristics – Multiple Dose (Safety Analysis Set)

		Isomyosamine		
Variable/ Category	**Statistic**	**Placebo(N = 2)**	**600 mg (N = 6)**	**All Subjects (N = 8)**
Sex				
Male	n (%)	0	3 (50.0)	3 (37.5)
Female	n (%)	2 (100.0)	3 (50.0)	5 (62.5)
Race				
White	n (%)	1 (50.0)	4 (66.7)	5 (62.5)
Black or African American	n (%)	1 (50.0)	2 (33.3)	3 (37.5)
Age (years)	n	2	6	8
	Mean	ND	39.2	38.4
	SD	ND	10.2	8.7
	CV%	ND	26.0	22.8
	Minimum	36	28	28
	Median	ND	39	36
	Maximum	36	50	50
WOCBP				
No	n (%)	0	0	0
Yes	n (%)	2 (100.0)	3 (100.0)	5 (100.0)
Ethnicity				
Hispanic or Latino	n (%)	0	3 (50.0)	3 (37.5)
Not Hispanic or Latino	n (%)	2 (100.0)	3 (50.0)	5 (62.5)
Height (cm)	n	2	6	8
	Mean	ND	169.50	169.99
	SD	ND	8.53	7.30
	CV%	ND	5.0	4.3
	Minimum	170.2	160.0	160.0
	Median	ND	168.1	170.2
	Maximum	172.7	182.9	182.9
Weight (kg)	n	2	6	8
	Mean	ND	67.98	70.43
	SD	ND	8.65	10.58
	CV%	ND	12.7	15.0
	Minimum	66.2	56.5	56.5
	Median	ND	66.2	67.3
	Maximum	89.3	80.6	89.3
BMI(kg/m * * 2)	n	2	6	8
	Mean	ND	23.77	24.36
	SD	ND	4.63	4.47
	CV%	ND	19.5	18.3
	Minimum	22.7	18.2	18.2
	Median	ND	23.6	24.0
	Maximum	29.6	30.4	30.4

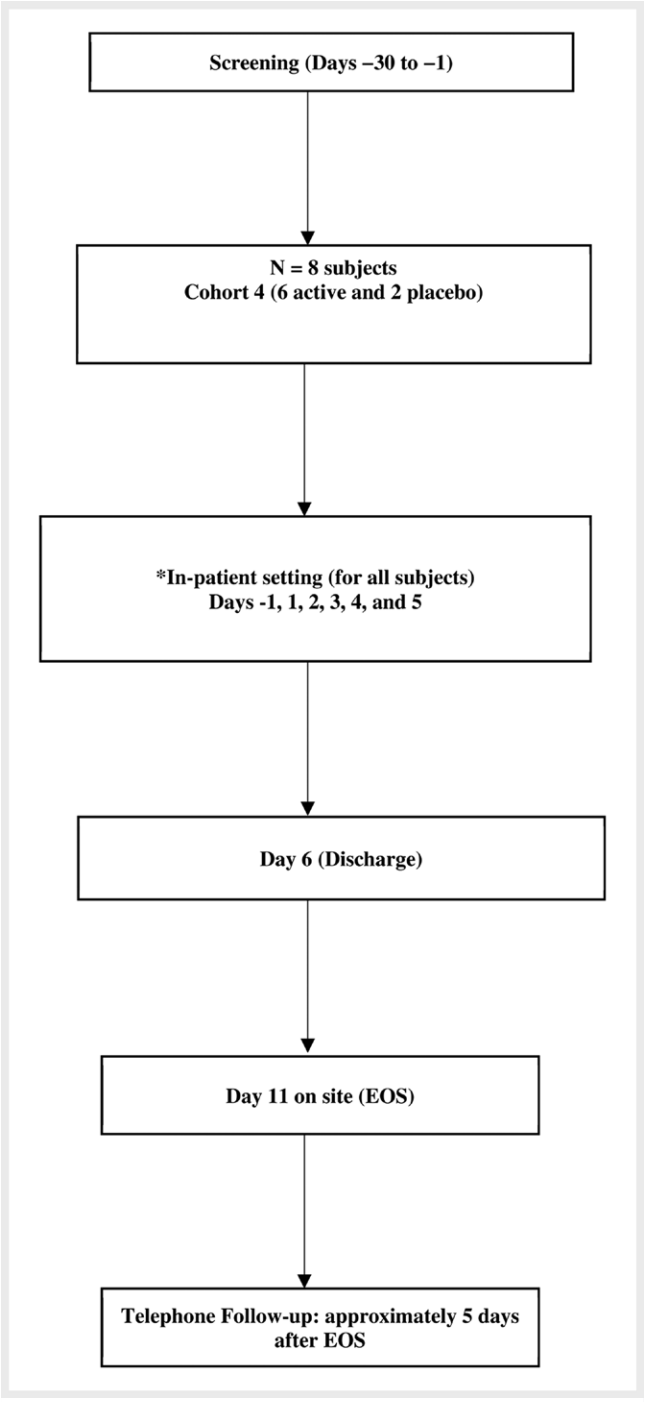
Note(s): BMI = body mass index; CV% = coefficient of variation; SD = standard deviation; WOCBP = woman of childbearing potential; ND = not determined. Percentage of woman of childbearing potential are based on the number of female subjects.

t_{max} was only summarized using N, n, median, minimum, and maximum.

Pharmacokinetic Analyses: Plasma PK parameters were calculated for Isomyosamine for each treatment using noncompartmental methods. Isomyosamine plasma concentrations and PK parameters were listed and summarized using descriptive statistics (▶ **Figs. 3** and ▶ **4**). Area under the plasma concentration-time curve (AUC) was quantified from time zero extrapolated to infinity, last, and 24 hours (ng * h/mL). AUC was calculated by linear up/log down trapezoidal summation for zero to last timepoint. AUC for zero to infinity was calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quan-



▶ **Fig. 1** Study Schema – Single Dose.



▶ **Fig. 2** Study Schema – Multiple Dose.

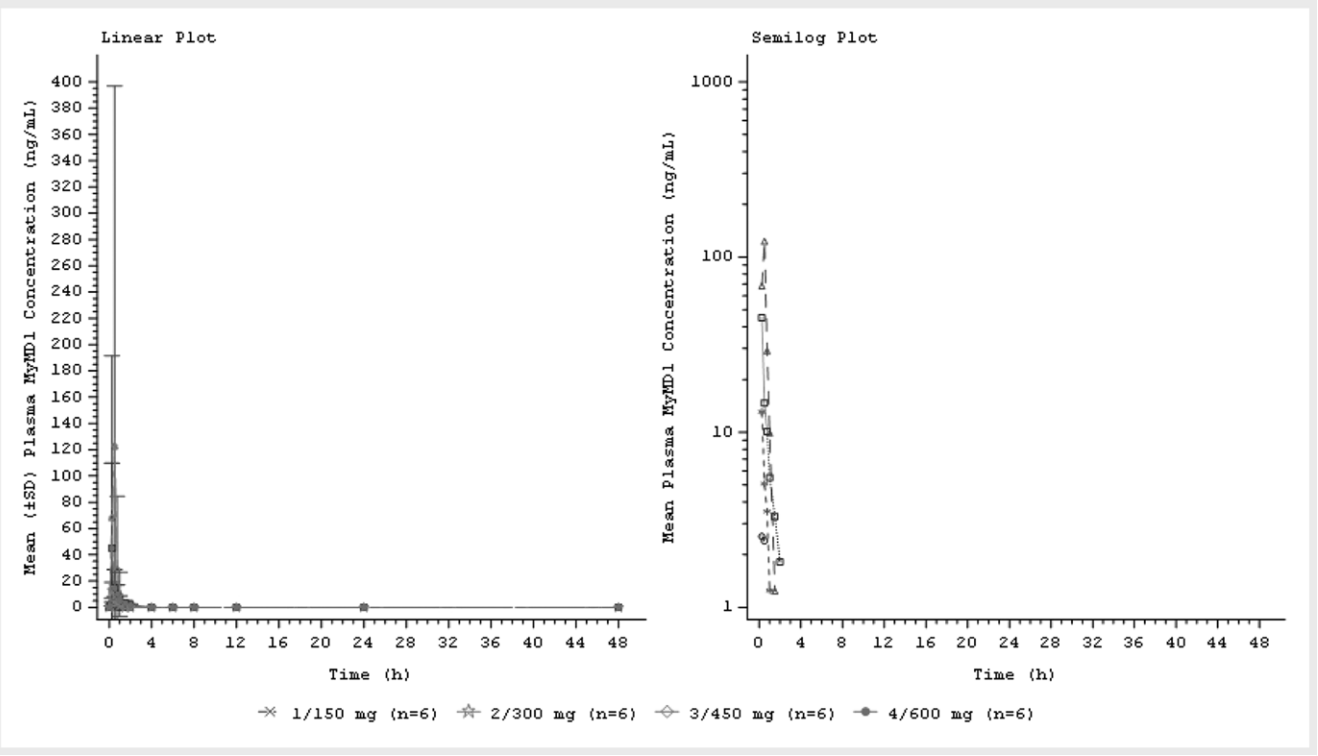
tifiable observed concentration divvied by the elimination rate constant. AUC for zero to 24 hours was calculated by linear up/log down trapezoidal summation and extrapolated/interpolated to the nominal time of 24 hours. Concentration-QTc Analysis: The relationship between plasma concentration of Isomyosamine and change-from-baseline QT interval corrected using Fridericia's formula (QTcF) was investigated by mixed-effects modeling (▶ **Fig 5**). The software use to the complete the mixed modeling for Concentration-QTc analysis (as well as all summaries, listings, and graphs) for this study us SAS Version 9 (SAS Institute, Cary NC).

Safety Analysis: All safety data were listed by subject. Treatment-emergent adverse events (AEs) were summarized for each treatment by system organ class, preferred term, maximum intensity, and relationship to test article (▶ **Tables 4** and ▶ **5**). There were no statistical comparisons between the treatment groups for safety data.

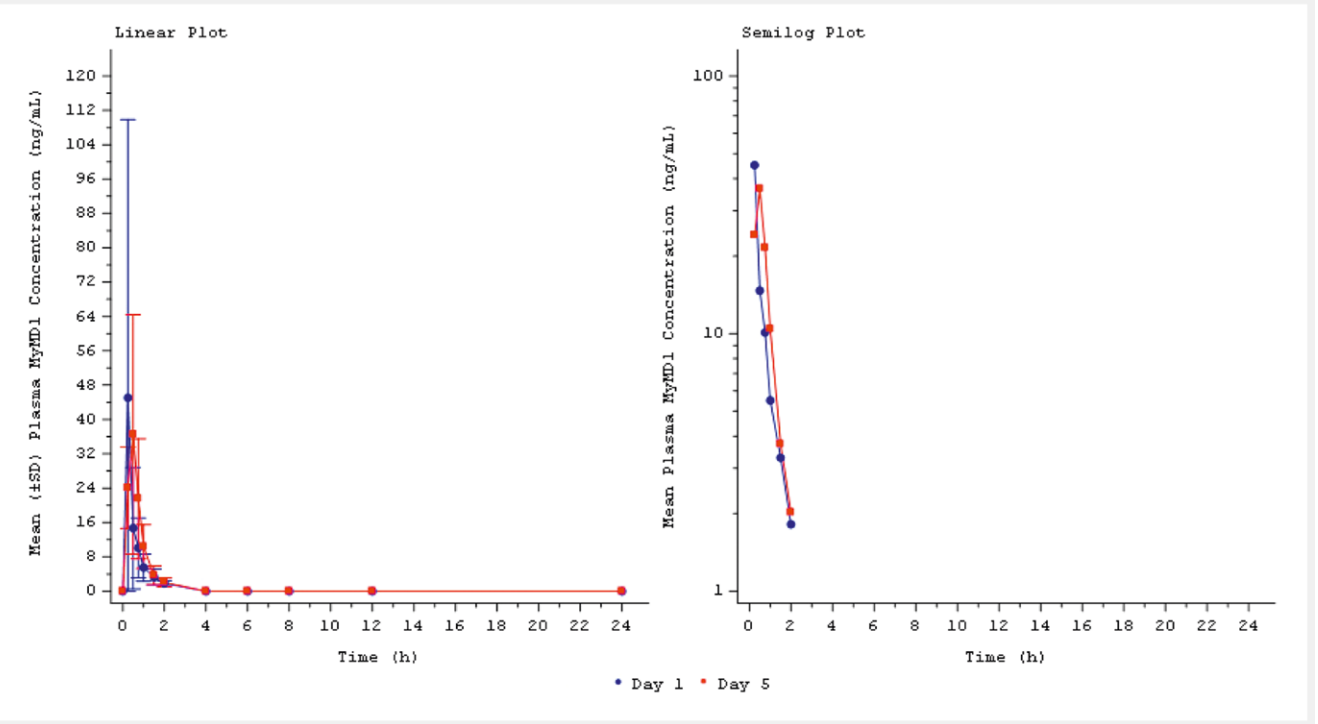
▶ **Table 3** Descriptive Statistics for Isomyosamine Plasma Pharmacokinetic Parameters by Cohort and Dose – SAD and MAD (Pharmacokinetic Analysis Set)

Cohort/Dose Statistic	$AUC_{(0\text{-inf})}$ (ng·h/mL)	$AUC_{(0\text{-last})}$ (ng·h/mL)	$AUC_{(0-24)}$ (ng·h/mL)	C_{max} (ng/mL)	t_{max}[a] (h)	$t_{1/2}$ (h)	CL/F (L/h)	V_z/F (L)	C_{avg} (ng/mL)	FI (%)	RAUC	RC_{max}	LI
SAD Cohorts Cohort 1/150 mg													
n	0	5	5	5	5	0	0	0	–	–	–	–	–
Geo Mean	NC	5.213	6.491	2.9019	1.02	NC	NC	NC	–	–	–	–	–
Geo CV%	NC	160.3	183.1	45.0	(0.48, 8.00)	NC	NC	NC	–	–	–	–	–
Cohort 2/300 mg													
n	3	6	6	6	6	3	3	3	–	–	–	–	–
Geo Mean	7.175	6.246	6.521	13.3595	0.25	0.3971	41810	23960	–	–	–	–	–
Geo CV%	14.2	21.4	20.5	32.7	(0.25, 0.50)	61.9	14.2	81.0	–	–	–	–	–
Cohort 3/450 mg													
n	5	6	6	6	6	5	5	5	–	–	–	–	–
Geo Mean	20.04	14.49	15.19	26.0582	0.25	0.2226	22450	7211	–	–	–	–	–
Geo CV%	358.0	362.4	342.5	486.3	(0.25, 0.55)	24.0	358.0	445.0	–	–	–	–	–
MAD Cohort													
Cohort 4/600 mg (Day 1)													
n	5	6	6	6	6	5	5	5	–	–	–	–	–
Geo Mean	19.74	15.50	17.60	26.7205	0.26	0.7145	30390	31320	–	–	–	–	–
Geo CV%	83.7	90.5	81.7	142.4	(0.25, 0.52)	60.5	83.7	179.0	–	–	–	–	–
Cohort 4/600 mg (Day 5)													
n	0	6	6	6	6	4	6	4	6	6	6	6	5
Geo Mean	NC	25.32	27.12	32.0875	0.50	0.5485	22130	18110	1.130	2840	1.541	1.201	1.272
Geo CV%	NC	50.1	48.3	64.4	(0.25, 0.50)	89.2	48.3	98.2	48.3	40.1	97.5	225.0	92.7

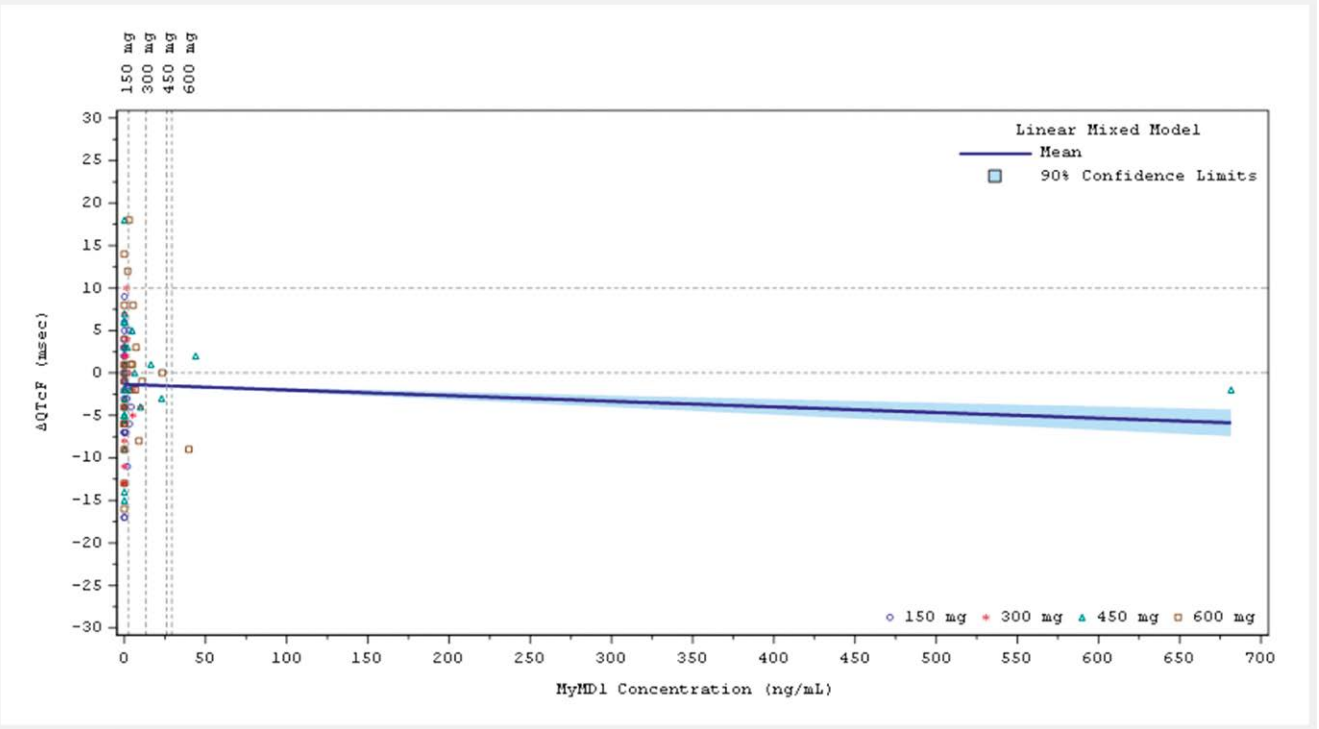
[a] Median (minimum, maximum) are displayed for t_{max}. Note(s): CV% = coefficient of variation; Geo = geometric; NC = not calculated; SD = standard deviation.



▶ **Fig. 3** Mean (±SD) Single-dose Isomyosamine Plasma Concentrations versus Scheduled Time by Dose – (SAD and MAD Day 1) (Pharmacokinetic Analysis Set).



▶ **Fig. 4** Mean (±SD) Single-dose and Multiple-dose Isomyosamine Plasma Concentrations versus Scheduled Time (MAD Day 1 and Day 5) (Pharmacokinetic Analysis Set).



▶ **Fig. 5** Change-from-baseline QTcF versus MYMD1 Plasma Concentration with Population Regression Line and 90 % Confidence Band - Day 1 (Concentration- QTc and Pharmacokinetic Analysis Set).

▶ **Table 4** Overall Summary of All Treatment-emergent Adverse Events – Single Ascending Dose (Safety Analysis Set)

	Isomyosamine				
	Placebo (N = 6)	**150 mg (N = 6)**	**350 mg (N = 6)**	**450 mg (N = 6)**	**All Active (N = 18)**
TEAS	0	1 (16.7 %)	0	0	1 (5.6 %)
Related TEAs	0	0	0	0	0
Severe TEAs	0	0	0	0	0
Serious TEAs	0	0	0	0	0
TEAEs leading to discontinuation	0	0	0	0	0
TEAEs leading to death	0	0	0	0	0

Note(s): TEAE = treatment-emergent adverse event. A subject experiencing multiple occurrences of an adverse event in a category was counted, at most, once for that category for each treatment and once for "All Active".

Analysis Sets

All Subjects Enrolled Analysis Set: All subjects signed the informed consent form; this set was used primarily for subject accounting purposes. Safety Analysis Set: The safety analysis set contained all subjects randomly assigned to study treatment and who took at least 1 dose of study treatment and were analyzed according to the actual treatment received. Subjects in this population were used for all demographics, safety, and dosing summaries. Biomarker, PK listings, PK parameter derivations, and plots of individual concentration-time data were also based on the Safety Analysis Set. Pharmacokinetic Analysis Set: This set consisted of all subjects who took at least one dose of active drug (Isomyosamine) and had at least 1 quantifiable concentration collected post dose without events (protocol deviations) thought to significantly affect the PK sample. Subjects were analyzed according to actual treatment received. The PK summaries were based on this set. All the protocol deviations reported in SAD part were either study procedures criteria or laboratory assessment criteria and all were minor and were not expected to have any impact on the study outcome. No protocol deviations were reported in MAD part. There was no COVID-19 impact on the protocol deviations. Biomarker Analysis Set: This set consisted of all subjects who had at least 1 quantifiable concentration collected post dose without deviations or events that significantly affect the biomarker endpoint. Subjects were analyzed according to actual treatment received. Concentration-QTc (C-QTc) Analysis Set: This set consisted of all subjects who took at least one dose of

▶ **Table 5** Overall Summary of All Treatment-emergent Adverse Events – Multiple Ascending Dose (Safety Analysis Set)

Isomyosamine		
	Placebo (N = 2)	**600 mg (N = 6)**
TEAS	1 (50 %)	3 (50 %)
Related TEAs	0	3 (50 %)
Severe TEAs	0	0
Serious TEAs	0	0
TEAEs leading to discontinuation	0	0
TEAEs leading to death	0	0

Note(s): TEAE = treatment-emergent adverse event. A subject experiencing multiple occurrences of an adverse event in a category was counted, at most, once for that category for each treatment and once for "All Active".

active drug (Isomyosamine) and had at least 1 evaluable baseline and 1 evaluable post dose electrocardiogram (ECG) endpoint (QTc) without protocol deviations or events that would affect the C-QTc analysis. The C-QTc analyses were based on this set.

Results

Disposition and Demography

A total of 32 subjects were enrolled across four cohorts. Twenty-four subjects on Isomyosamine (six per cohort) and eight subjects on placebo (2 per cohort) were randomized in the SAD (Cohorts 1–3) and MAD (Cohort 4). Of the 24 subjects randomized in SAD part, 23 (95.8 %) completed the study. One subject was unable to complete the follow-up phone call. All the 8 subjects randomized in the MAD part completed the study.

In SAD part, most of the subjects were white and not Hispanic or Latino (16 [66.7 %]) and males (17 [70.8 %]). In MAD part, most of the subjects were white and not Hispanic or Latino (5 [62.5 %]) and females (5 [62.5 %]; (▶ **Tables 1** and ▶ **2**).

Pharmacokinetic Results

Absorption of Isomyosamine was rapid following a single dose administered in both the SAD and MAD portions of the study. Following a single 150 mg dose of Isomyosamine, median t_{max} was approximately 1 hour. Median t_{max} following single doses of 300 mg to 600 mg, was approximately 15 minutes. Over the SAD Isomyosamine dose range of 150 mg to MAD 600 mg (4-fold increase in dose), geometric mean $AUC_{(0\text{-last})}$ and C_{max} values increased approximately 3-fold and 9-fold, respectively. A 2-fold increase in dose from 300 mg to 600 mg resulted in an approximate 2.5-fold and 2-fold increase in geometric $AUC_{(0\text{-last})}$ and C_{max}, respectively. Isomyosamine geometric mean half-life following a single dose in both the SAD and MAD portions of the study ranged from approximately 15 minutes to 45 minutes following doses from 300 mg to 600 mg. In the MAD portion of the study, 600 mg administered once daily for 5 days resulted in an approximate 20 % to 50 % rate of accumulation relative to exposure following a single 600 mg dose (based on geometric mean accumulation ratios for C_{max} and AUC, respectively). Following Isomyosamine 600 mg administered once daily for 5 days, the half-life was approximately 30 minutes. The linearity index (LI) was just slightly larger than unity providing evidence. Isomyosamine PK is time independent. In the SAD portion of the study, less than 0.02 % of a Isomyosamine dose was excreted in urine following a single dose of 150 mg to 450 mg. No subject receiving a single dose of Isomyosamine of 150 mg to 450 mg had N-nitrosonornicotine detected in urine and only three subjects had 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) nicotine-derived nitrosamine ketone detected in urine following a single dose of Ismoyosamine. These quantifiable levels of NNAL nicotine-derived nitrosamine ketone were observed from 32 hours to 48 hours post dose only. Placebo subjects were not part of this analysis. In the MAD portion of the study, less than 0.03 % of the 600 mg Isomyosamine single dose was excreted in the urine on Day 1 and less than 0.02 % of the 600 mg Isomyosamine daily dose was excreted on Day 5. No subject had N-nitrosonornicotine detected in urine on Day 1 or Day 5. Only one subject had NNAL nicotine-derived nitrosamine ketone detected in urine following a single dose on Day 1 and no subjects had detectable levels on Day 5.

Concentration-QTcF Results

A non-significant exposure-response relationship was observed between Isomyosamine concentration (p value for slope = 0.3523 and the corresponding 90 % CI included zero) over the Isomyosamine dose range evaluated and change-from-baseline QTcF. The magnitude of the slope combined with its direction (slope estimate = 0.008469) would not be indicative of a cause for concern. Changes predicted in QTcF at geometric mean C_{max} values (range of −1.54 msec to -−1.34 msec) were small and negative for all Isomyosamine doses; upper 90 % confidence limits ranged from 0.07 msec to 0.26 msec.

Safety Results

Isomyosamine single doses each of 150 mg, 300 mg, and 450 mg and multiple doses of 600 mg were safe and well tolerated in healthy subjects. There were no new or unexpected safety findings reported in this study.

- No AE leading to discontinuation, severe AEs, serious AEs, or deaths were reported during the study.
- One subject in 150 mg group (SAD) had mild headache, 1 subject in the placebo group (MAD) had moderate vulvovaginal mycotic infection and 3 subjects in 600 mg group (MAD) had mild AEs of dysgeusia.
- Analyses of laboratory parameters, vital sign, ECG, and physical findings did not reveal any clinically relevant effect of Isomyosamine.
- In one dose group (150 mg), there was a decrease in TNF-α levels found in Isomyosamine treated subjects, but no change in the levels in subjects given placebo (p = 0.05).

Biomarker Results

In both SAD and MAD parts, minor fluctuations were reported in cytosine, guanine, deoxyguanosine, thymidine, butanone, and TNF-α values, but due to the small sample size, there are no conclusions that can be ascertained. One dose group treated with

Isomyosamine had a decrease in TNF- α, with no change reported in levels in subjects receiving placebo.

Discussion and Conclusion

This report details the phase 1 clinical trial as part of the clinical development program of Isomyosamine to evaluate the safety, tolerability, and pharmacokinetic (PK) profile over the single and multiple dose ranges in healthy male and female adult subjects. Isomyosamine single doses each of 150 mg, 300 mg, and 450 mg and multiple doses of 600 mg were safe and well tolerated in healthy subjects. The increase in Isomyosamine exposure was proportional to dose across the dose range of 300 mg to 600 mg when administered as a single dose. There was minimal accumulation of Isomyosamine following 5 days of once daily dosing of Isomyosamine 600 mg. Isomyosamine half-life ranged from approximately 15 minutes to 45 minutes across all doses in the SAD and MAD portion of the study and the renal pathway is a minor route of elimination for Isomyosamine. A non-significant exposure-response relationship was observed between Isomyosamine concentration (p value for slope = 0.3523 and the corresponding 90 % CI included zero) over the Isomyosamine dose range evaluated and change-from-baseline QTcF. The magnitude of the slope combined with its direction (slope estimate = − 0.008469) would not be indicative of a cause for concern. Changes predicted in QTcF at geometric mean Cmax values (range of −1.54 msec to −1.34 msec) were small and negative for all Isomyosamine doses; upper 90 % confidence limits ranged from 0.07 msec to 0.26 msec. There was no significant relationship between Isomyosamine concentration and change-from-baseline QTcF. Changes predicted in QTcF at geometric mean C_{max} values (range of −1.54 msec to −1.34 msec) were small and negative for all Isomyosamine doses.

Limitations and Future Research

Common in Phase 1 clinical trials and per protocol, this study included a small sample size limiting the ability to have adequate power to analyze exploratory endpoints. Additionally, the population consisted of more white and male subjects, however, these characteristics were well-balanced across cohorts. The sample size was adequate for the purpose of determining safety and tolerability in humans to permit continuation by regulatory authorities for the clinical development of the novel therapeutic compound.

Isomyosamine has been investigated in vitro and in animal models suggesting various mechanisms and potential indications for development. A study utilizing a mice model of spontaneous thyroiditis suggests that Isomyosamine ameliorates thyroiditis acting on specific lymphoid subsets via the reduction of Th1 responses and TNF-a release [4]. Another study in mice and in vitro showed improvement of the course of experimental autoimmune encephalomyelitis (EAE) induced by immunization with myelin oligodendrocyte glycoprotein and suppressed activation of effector T cells without causing global immunosuppression or toxicity [5]. Lastly, most recently, a researchers in vitro and in vivo concluded that Isomysoamine possessed anti-proliferative, anti-inflammatory, and anti-fibrotic properties that were more inhibitive than rapamycin and resulted in improved mice health span characteristics (milder body weight loss, greater muscle strength, and slower progression to frailty) [7]. The prior in vitro and in vivo research in combination with the current trial in humans lays the foundation for future support and testing of Isomyosamine as a potential treatment for autoimmune diseases. Isomyosamine is now under investigation in a phase 2 trial targeting the sarcopenia/frailty as the indication with future plans to perform clinical trials for other indications, specifically treatment of hashimoto's thyroiditis and rheumatoid arthritis.

In conclusion, Isomysoamine single doses each of 150 mg, 300 mg, and 450 mg, and multiple doses of 600 mg were safe and well tolerated in a healthy population supporting the advancement of clinical development of this compound for various autoimmune diseases.

Conflicts of Interest

Drs. Brager, Chapman, and Kaplin are employees of MyMD Pharmaceuticals, Inc.

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