Safety, Tolerance and Pharmacokinetics of HFA-152a in Healthy Volunteers

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SUMMARY

HFA-152a (1, 1-difluoroethane) is being developed as an alternative propellant in pressurized metered dose inhalers (pMDIs). A comprehensive HFA-152a development program is being conducted to support a drug master file suitable for global regulatory submissions. A global regulatory strategy was developed to include analytical assay development, aerosol development, bioanalytical assay development, respiratory sensitization, toxicology (multiple species, acute, sub-chronic, chronic, reproductive and carcinogenicity) to support a US Investigational New Drug (IND) approval to conduct a Phase I propellant-only clinical study.

The Phase I human study was conducted in healthy male volunteers. The volunteers were administered four consecutive doses of 50 µL/actuation from a pMDI within a six-minute timespan which represented the maximum anticipated single dosing session currently utilized in pMDI treatment. Immediately following the last actuation, blood samples were collected for gas chromatography (GC)-headspace analysis for HFA-152a to support pharmacokinetic studies. Additionally, quantitative end points for safety and tolerance were conducted to include pulmonary function testing (PFT), vital signs, taste, clinical chemistry, and clinical observations.

Overall, the data showed that following oral inhalation from a pMDI, HFA-152a was well tolerated, had minimal impact on main aspects of taste scoring and was rapidly cleared from the blood. There were no adverse events during the study. These data support the continued development and utilization of HFA-152a as a safe alternative propellant for use in pMDIs.

INTRODUCTION

pMDIs are under increasing scrutiny due to the environmental impact of the hydrofluoroalkane (HFA) propellants [1]. Currently, HFA 134a and HFA 227ea are the primary propellants utilized globally in pMDIs but because they have high global warming potential (GWP) there is a growing need to transition to low GWP propellants to reduce their global warming contribution [1, 2]. Low GWP propellants including HFA-152a (Koura, UK) and hydrofluorolefin 1234ze(E)

(HFO 1234ze(E)) (Honeywell, USA) are actively being explored to support the development of more environmentally friendly pMDIs [1]. The physicochemical properties of HFA-152a (1,1-difluoroethane), shown in Table 1, compared to existing propellants highlight the similarity of HFA-152a as a potential replacement propellant while lowering the GWP [1, 3]. The GWP of HFA-152a is ~10x lower than HFA-134a and ~24x lower than HFA-227ea [2, 3]. Our industry successfully managed the transition from chlorofluorocarbon (CFC) to hydrofluorocarbon (HFC) propellants in response to the Montreal Protocol [4] and is now transitioning to low GWP propellants to ensure that pMDIs continue to meet patient needs whilst reducing carbon emissions [5].

Table 1. Physicochemical properties for various pMDI propellants.

Propellant	Formula	Boiling point (°C)	Density (g/mL @ 20 °C)	GWP(AR5)
CFC 11	$CFCl_3$	23.7	1.49	4660
CFC 12	CF_2Cl_2	-29.8	1.33	10800
HFA 134a	CF_3 -CFH_2	-26.2	1.23	1300
HFA 227ea	CF_3 -CFH-CF_3	-16.5	1.41	3350
HFA 152a	CF_2H-CH_3	-24.7	0.91	138
HFO 1234ze(E)	CHF=$CHCF_3$	-18.9	1.29	<1

This article will review the HFA-152a development program to support a drug master file suitable for global regulatory submissions. A global regulatory strategy was developed for HFA-152a to include analytical assay development, aerosol development, bioanalytical assay development, respiratory sensitization, toxicology (multiple species, acute, sub-chronic, chronic, reproductive and carcinogenicity) to support a US IND approval to conduct a Phase I propellant-only clinical study.

HFA-152a IND PACKAGE

To conduct the US Phase I clinical trial, an IND package was submitted to, and approved by, the US Food and Drug Administration (FDA). This was a complex program with multiple key milestones/developments required to enable the clinical study.

An analytical assay was required to characterize the aerosol to support each aspect of the enabling pharmacology/toxicology studies and the clinical studies. Based on previous HFA methods, a gas chromatography-flame ionization detection-head space vial (GC-FID-HS) assay was developed. The assay enabled collection of aerosol samples in aerosol sampling bags prior to transferring aliquots into head space autosampler vials. The final good laboratory practice (GLP) validated assay was linear between 25,000 and 300,000 ppm with recovery from inert aerosol bags between 96 and 101%. Critical to the application of the method to the studies was the stability of the samples. The inert bag samples were found to be stable for at least 40 hours. Once transferred to head space auto sampler vials, the vials were stable for three days.

A bioanalytical method was developed to quantify HFA-152a from plasma. This method was validated for mouse, rat, guinea pig, rabbit, canine and human blood. Blood samples were collected (in any species) and transferred to K_3EDTA tubes. Different plasma volumes (mouse – 0.25 mL, rat – 0.5 mL, guinea pig – 1 mL, rabbit – 0.25 mL, canine – 1 mL and human – 1 mL) were transferred to head space autosampler vials for analysis. Each method underwent a complete regulated GLP assay validation to support the defined studies [6]. The human assay range was from 0.216 mg/L to 216 mg/L. Stability issues were observed throughout the bioanalytical

assay development and validation. While HFA-152a is known to be stable in gas form and gas samples were collected and assayed, the samples were not found to be stable beyond 72 hours in any container closure system tested. The instability was initially hypothesized to be caused by HFA-152 leakage from the vials. However, after extensive investigation this was determined not to be the cause and no specific cause was determined. Therefore, all samples were assayed for all studies within 72 hours of collection.

Like HFA-134a and HFA-227, HFA-152a is a gas under standard pressure and temperature conditions. However, the flammability of HFA-152a must also be considered during handling and suitable safety precautions put in place. Non-clinical inhalation exposure systems suitable for flammable materials were developed to conduct pharmacokinetics, respiratory sensitization and toxicology studies. Industry standard rodent nose-only flow-past chambers and large animal face mask exposure systems were developed, characterized, and validated over a wide range of aerosol concentrations of HFA-152a (15,000 ppm to 300,000 ppm) [7]. The systems utilized real time flame ionization detectors to quantify HFA-152a during exposure system operation and the GC-FID-HS as the definitive measurement platform.

In vivo studies included respiratory sensitization studies in guinea pigs and dogs, and non-GLP and GLP toxicology studies in mice, rats, and dogs. Toxicology studies included assessment of acute toxicology, sub chronic and chronic toxicology and preliminary rat/rabbit reproductive toxicology studies. While an extensive description of these studies is outside the scope of this article, HFA-152a was well tolerated in all conditions evaluated, did not induce respiratory sensitivity under any condition evaluated and, in all species, HFA-152a was rapidly cleared from the blood. Taken collectively these data formed the basis for the IND submission to the FDA to support the conduct of a Phase I clinical trial with HFA-152a which focused on safety, tolerability, taste and pharmacokinetics.

HFA-152a PHASE I CLINICAL STUDY

The clinical study was conducted under good clinical practices by the clinical research arm of Lovelace Biomedical Research Institute: Lovelace Scientific Resources under Institutional Review Board (IRB) review and approval.

Eight healthy adult male volunteers between 18 and 60 years old participated in the Phase I study. Major inclusion criteria included good health, no current use of nicotine products and the ability to be properly trained on the use of a pMDI. Key exclusion criteria included history of uncontrolled respiratory disease (chronic obstructive pulmonary disease (COPD), asthma, wheezing, etc.), and abnormal vital signs (heart rate (HR), temperature, respiratory rate (RR), blood pressure (BP) and oxygen saturation (SPO_2)). Table 2 summarizes the study design. The key study objectives were to:

- Assess the taste tolerance.
- Evaluate lung function through Pulmonary Function Testing (PFTs).
- Evaluate vital signs (HR, RR, BP, SpO_2).
- Evaluate clinical chemistry and urine analysis.
- Quantify HFA-152a parent pharmacokinetics in blood.
- Determine the possible presence of metabolites in urine.

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Table 2. Timed schedule of events during dosing.

		Time (Minutes)										
		Pre-Test	0	Immediately Post	10	20	30	45	60	120	240	360
Procedures	Blood Collection PK Samples	X		X	X	X	X	X	X	X	X	X
	Urine Collection	X							X			
	Pulmonary Function Testing	X							X			
	Direct Physical Effects of MDI Use			X			X					
	Direct Taste Effects of MDI Use			X			X					
	Adverse Events/ Vital Signs		X	X	X	X	X	X	X	X	X	X

HFA-152a (Koura) was filled into pMDI canisters fitted with a 50 μL metering valve. Volunteers were administered four actuations within six minutes. The time of the last dose was used as the immediate time point for all collections and in all analysis. Prior to enrollment, the volunteers were trained on proper utilization of the pMDI. This included comfortably emptying of their entire lungs, inhaling in a controlled manner over three seconds to fill their lungs with actuation of the pMDI at the start of inhalation, followed by a 10-second breath hold. HFA-152a dosing was observed for each dose for each volunteer to confirm compliance with proper technique.

Demographics

Eight male participants were enrolled in this study. The average age was 44.6 years old (range 30 to 60). Summary demographics are included in Table 3. All participants completed both visits for this study. Dose delivery was completed by all subjects. All study events were completed without any serious adverse events.

Table 3. Summary of patient demographics.

ID	Gender	Age	Race	Ethnicity	Education
LSR2846001	Male	30	Native Indian	Hispanic	Master's Degree
LSR2846002	Male	59	Caucasian	Non-Hispanic	Bachelor's Degree
LSR2846003	Male	60	Caucasian	Hispanic	Master's Degree
LSR2846004	Male	39	Caucasian	Hispanic	Bachelor's Degree
LSR2846005	Male	54	Native Indian	Non-Hispanic	Associates Degree
LSR2846006	Male	35	Caucasian	Non-Hispanic	Some College
LSR2846007	Male	31	Caucasian	Non-Hispanic	Bachelor's Degree
LSR2846008	Male	49	Caucasian	Unanswered	Associates Degree

Taste assessment

Each volunteer evaluated the taste immediately post dose and again 30 minutes post dose. The evaluation included quantification of the following aspects of taste: bad, good, bitter, sweet, salty, metallic and cold with results presented on a scale of 1–10 scale (10 being the most severe). The results are summarized in Tables 4A and 4B. One of the eight participants indicated a slight bad taste (0.8/10; 10 being the most severe) immediately post dosing. Zero participants indicated a bad taste 30 minutes post dosing. Similarly, two participants indicated a slight metallic taste (normalized score: 1.6/10 and 0.2/10 respectively) immediately post dosing. Zero participants indicated a metallic taste at 30 minutes post dosing. Six participants indicated a slight cold taste (overall average 1.4/10) immediately post dosing. One participant indicated a slight cold taste (0.7/10) 30 minutes post dosing. These results indicated that the taste of HFA-152a was not a clinical concern.

Table 4A. Taste summary – immediately post dose.

ID	Bad	Good	Bitter	Sweet	Salty	Metallic	Cold
LSR2846001	0.8	0.0	0.0	0.0	0.0	1.6	5.7
LSR2846002	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846003	0.0	0.0	0.0	0.0	0.0	0.0	0.9
LSR2846004	0.0	0.0	0.0	0.0	0.0	0.0	0.6
LSR2846005	0.0	0.0	0.0	0.0	0.0	0.2	0.0
LSR2846006	0.0	0.0	0.0	0.0	0.0	0.0	1.9
LSR2846007	0.0	0.0	0.0	0.0	0.0	0.0	0.6
LSR2846008	0.0	0.0	0.0	0.0	0.0	0.0	1.1
Average	0.1	0	0	0	0	0.2	1.4

All scores normalized to a scale of 1 – 10; 10 being the most severe.

Table 4B. Taste summary – 30 minutes post dose.

ID	Bad	Good	Bitter	Sweet	Salty	Metallic	Cold
LSR2846001	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846002	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846003	0.0	0.0	0.0	0.0	0.0	0.0	0.7
LSR2846004	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846005	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846006	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846007	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSR2846008	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average	0	0	0	0	0	0	0.1

All scores normalized to a scale of 1–10; 10 being the most severe.

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Pulmonary function testing

PFT was performed immediately post dosing and at 60 minutes post dosing. The key PFT (forced expiratory volume in 1 second, FEV_1) results are summarized in Table 5. The observed differences in FEV_1 were considered minimal and not clinically relevant.

Table 5. FEV_1 summary results.

ID	Pre-FEV_1 (liter)	Post-FEV_1 (liter)	Percent change (%)
LSR2846001	3.84	3.73	-3
LSR2846002	3.04	3.08	2
LSR2846003	3.53	3.64	3
LSR2846004	3.45	3.31	-4
LSR2846005	3.2	3.11	-3
LSR2846006	3.52	3.5	-1
LSR2846007	4.42	4.35	-2
LSR2846008	4.72	4.67	-1

Vital signs and clinical chemistry, hematology and urinalysis

Vital signs (heart rate, temperature, respiratory rate, blood pressure and oxygen saturation) were measured at each time point blood was collected. Standard ranges included HR: 50–100 beats per minute, temperature: 97.0–99.1˚F, respiratory rate: 10–20 beats per minute, blood pressure: 80/50 –160/100 mm/Hg, and oxygen saturation: 94–100%. All vital signs were reviewed by trained medical staff during the study and after the study to assess impact of HFA-152a. No vital signs at any time point were outside of the protocol defined acceptable ranges. Blood and urine were collected prior to the first dose and 60 minutes post dosing for analysis. No correlations or adverse results were quantified in the clinical chemistry, hematology or urinalysis. Further, no adverse events were noted during aspect of this study.

HFA-152a plasma pharmacokinetics

Blood samples were collected at 10, 20, 30, 45, 60, 120, 240 and 360 minutes post the last dose. The blood was collected in K_3EDTA tubes and immediately transferred into head space vials. Blood samples were assayed with a fully validated GC-FID-HS method with a working range of 0.216 to 216 mg/L. All analysis runs included matrix-based standards and QCs with standard performance metrics to release results. Urine samples were snap frozen at -20˚C and saved for GC-FID-HS analysis. Plasma and urine samples were also assayed via nuclear magnetic resonance (NMR) to explore for potential metabolites. The NMR samples were assayed with ^{19}F NMR on a Bruker Avance III 500 NMR spectrometer. Chemical shifts were referenced to $CFCl_3$ and $CDCl_3$.

The sample analysis results are summarized in Table 6 and graphically in Figure 1. NMR analysis of all plasma and urine samples showed presence of HFA-152a but no metabolites in any samples from any matrix at any timepoint.

Table 6. HFA-152a blood concentration results.

ID	Pre	Immediate	10 Min	20 Min	30 Min	45 Min	60 Min	120 Min	240 Min	360 min
LSR2846001	BQL	0.319	BQL*	BQL*	BQL*	BQL*	BQL*	BQL*	BQL*	BQL*
LSR2846002	BQL	0.336	0.136	BQL	BQL	BQL	BQL	BQL	BQL	BQL
LSR2846003	BQL	NS	0.416	0.23	0.121	BQL	BQL	BQL	BQL	BQL
LSR2846004	BQL	1.863	0.22	0.117	BQL	BQL	BQL	BQL	BQL	BQL
LSR2846005	BQL	0.694	0.267	0.158	BQL	BQL	BQL	BQL	BQL	BQL
LSR2846006	BQL	BQL	0.512	0.134	BQL	BQL	BQL	BQL	BQL	BQL
LSR2846007	BQL	0.884	0.236	0.124	BQL	BQL	BQL	BQL	BQL	BQL
LSR2846008	BQL	0.138	0.465	0.218	BQL	BQL	BQL	BQL	BQL	BQL
Average	BQL	0.319	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

BQL < 0.108 mg/L for all samples except noted below. *BQL < 0.216 mg/L. NS: No sample.

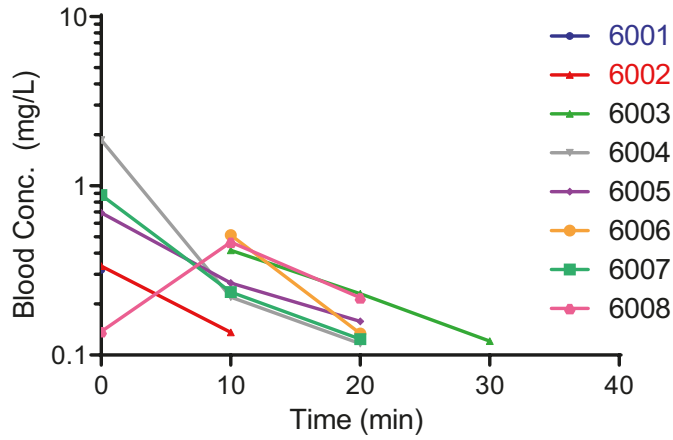

Figure 1. Concentration vs. time graph for all eight participants.

A non-compartmental analysis (NCA) was performed for each participant on the concentration vs. time data. In this NCA, all below quantification limit (BQL) samples were treated as missing and each patient was modeled individually. Based on the limited data due to rapid clearance, only time to maximum concentration (T_{max}), maximum concentration (C_{max}), AUC (area under the concentration vs. time curve) when at least three data points were present, and mean residence time (MRT) were determined. MRT represented the average time a molecule resides in the body and was determined to be a more appropriate reflection of the clearance than half-life for these data. These results are shown in Table 7. Note that for any participant that did not have three concentration values the AUC was not calculated. These values were treated as missing when the average was determined for the study AUC.

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Table 7. Non-compartmental analysis results.

ID	T_{max} (min)	C_{max} (mg/L)	AUC* (min*mg/L)	MRT (min)
LSR2846001	0.00	0.319	NC**	NC**
LSR2846002	0.00	0.336	NC**	4.26
LSR2846003	10.0	0.416	6.92	15.6
LSR2846004	0.00	1.86	9.32	5.29
LSR2846005	0.00	0.694	6.55	7.50
LSR2846006	10.0	0.512	NC**	12.1
LSR2846007	0.00	0.884	6.65	6.69
LSR2846008	10.0	0.465	6.28	11.2
Average	3.75	0.69	7.14	8.95

*AUC was only calculated for participants that had three or more concentration values. NC values were treated as missing in calculation of study AUC average. **NC = Not calculated.

The NCA performed on the HFA-152a concentration vs. time profile showed rapid absorption, with all participants except one having their T_{max} as the first assayed time point. All participants had low exposure (AUC) with an average AUC of 7.14 min*mg/L. The more standard measure of clearance, i.e., half-life, was not able to be calculated as there were too few points in the apparent terminal clearance phase. Therefore, the MRT was determined for each participant. The average MRT was 8.95 min, which showed a rapid clearance from the blood. No metabolites were detected in any sample via NMR.

CONCLUSIONS

The primary objective of this study was to assess the safety and tolerability of HFA-152a following oral inhalation from a pMDI. In advance of this clinical study, a complete non-clinical program was conducted to evaluate the safety, tolerance and pharmacokinetics in a range of different species to support an IND.

Eight volunteers were administered four consecutive doses of 50 μL/actuation from a pMDI within a six-minute timespan. The quantitative end points to assess safety and tolerability included pulmonary function testing and vital signs. Concurrently, taste and clinical observations were performed. Blood samples were collected to quantify HFA-152a via a GC headspace method. The analysis of the PFTs for each participant prior to dose delivery and 60 minutes post-delivery showed no change in FEV_1. All vital signs at all time points pre and post dosing were all within the normal, expected range. Therefore, it was determined that none of these endpoints showed a clinically relevant adverse impact on the safety or tolerability of HFA-152a following oral inhalation from a pMDI.

Self-rated assessment of taste showed that one of the eight participants indicated a slight bad taste immediately post dosing and zero participants indicated a bad taste 30 minutes post dosing. Similarly, two participants indicated a slight metallic taste with no one reporting metallic taste at 30 minutes post dosing. Six participants indicated a slight cold taste immediately post dosing and one participant at 30 minutes post dosing. These results indicated that the taste of HFA-152a was not a clinical concern.

The pharmacokinetic NCA on HFA-152a following oral inhalation showed rapid absorption, with all participants except one having their T_{max} as the first assayed time point. The average systemic AUC was low (7.14 min*mg/L) and the clearance was rapid with an average MRT of 8.95 min. No metabolites were detected in any sample via NMR.

Overall the data showed that following oral inhalation from a pMDI, HFA-152a was well tolerated, had minimal impact on main aspects of taste scoring and was rapidly cleared from the blood. There were no metabolites detected in urine by NMR analysis. There were no adverse events during the study. These data support the continued development and utilization of HFA-152a as a safe alternative propellent in pMDIs with a significant potential to reduce the global warming impact of pMDIs.

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