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Supplementary Material

Article Title: Inhaled Mebufotenin (GH001) for Adult Patients with Postpartum Depression: A Phase 2a Open-Label Clinical Trial

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LIST OF SUPPLEMENTARY MATERIAL FOR THE ARTICLE

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Supplementary Materials

Supplementary Methods

Prohibited Treatments and Washout

- Monoamine oxidase inhibitor (MAOIs): Patients were required to avoid concomitant administration or presence in the blood of MAOIs, including plant derived MAOIs (such as harmala alkaloids in Ayahuasca or Syrian Rue). Patients were not permitted to use any medication with MAOI activity (such as isocarboxazide, phenelzine, selegiline or tranylcypromine, linezolid, or methylene blue) within 14 days or 5 half-lives (whichever is longer) prior to the first administration and until the end of the trial.
- Serotonergic drugs: Concomitant administration of other agents which, directly or indirectly, act agonistically on serotonergic systems (such as selective serotonin reuptake inhibitors [SSRIs] such as paroxetine or citalopram, a serotonin-norepinephrine reuptake inhibitor [SNRI] such as venlafaxine or duloxetine, L-tryptophan, serotonin [5-HT] or St. John's wort) were prohibited within 14 days or 5 half-lives (whichever was longer, 5 weeks in the case of fluoxetine) prior to the first administration and until the end of the trial.
- Tricyclic antidepressants (TCAs): TCAs such as amitriptyline or imipramine were also contraindicated within 14 days or 5 half-lives (whichever was longer) prior to the first administration and until the end of the trial.
- Psychoactive medication: As a precautionary measure, patients were not permitted to receive psychoactive medication at a defined interval (based on medication half-life) before the dosing day, on the dosing day, and until the end of the trial.
- Dietary supplements: Plant-based or herbal dietary supplements were prohibited.
- Over-the-counter (OTC) medications: OTC medications were not allowed in the 2 weeks prior to the first GH001 administration and until the end of the trial, with the exception of oral

contraceptives, up to 4000 mg paracetamol or 1200 mg ibuprofen per day for a maximum of 3 days and vitamins ad libitum.

Peak Experience Scale

GH001 was administered on a single day as an individualized dosing regimen (IDR). The IDR is based on the Peak Experience Scale (PES)[1], and the administration of a higher dose level is guided by evaluating whether the patient achieved a peak experience (PE) at the previously administered dose. The GH001 IDR comprised of up to three increasing doses of GH001 (6, 12, and 18 mg, administered via the Volcano Medic 2 Vaporization System); if the first dose was well tolerated and no PE was achieved at the first dose level (6 mg), a second higher dose (12 mg) was administered; if the second dose was well tolerated and no PE was achieved with the second higher dose, a third dose (18 mg) was administered.

The achievement of the PE was evaluated using a proprietary questionnaire (the PES) that was completed by the patient once the psychoactive effects had subsided. The PES was developed specifically for the GH001 program and consists of three visual analog scales, each ranging from 0 to 100, summarized as a total average score. The specific three-item features of an intense experience after GH001 administration, i.e., its overall intensity (item one), any feelings of loss of control, to capture the prominent feature of ego dissolution (item two), and its profoundness, to capture how deep and meaningful the experience was (item three). A mean score of ≥75 on the PES has been selected as the threshold determining whether a patient achieved a PE following dosing with GH001, after which no further dose escalation in the GH001 IDR is done.

Supplementary Table 1. Eligibility Criteria

Inclusion Criteria	
Patients were eligible for the trial if all the following criteria were met, unless they fulfilled one or more of the exclusion criteria:	
1	Understands the nature of the clinical trial and has provided signed and dated written informed consent in accordance with local regulations before the conduct of any trial-related procedures.
2	Is female and in the age range between 18 and 45 years (inclusive) at screening.
3	Has a BMI in the range of 18.5 and 40 kg/m^2 (inclusive) at screening.
4	Meets the trial criteria for PPD as assessed by a trial psychiatrist or registered clinical psychologist:

	Diagnosis of Major Depressive Disorder without psychotic features, confirmed by the MINI (v7.0.2), with peri-partum onset that began no earlier than gestation and no later than the first 4 weeks postpartum, and is > 4 weeks postpartum at dosing and ≤12 months postpartum at screening.
	Has a MADRS total score of equal to or greater than 28 at screening and pre-dose on Day 1.
5	Must have either ceased lactating at screening; or, if still lactating or actively breast feeding at screening, must agree to temporarily cease breastfeeding their infant(s) from just prior to receiving study drug on Day 1 through 24 hours post last dose, and to pump and discard all breastmilk during those 24 hours as needed, but need to include a pump/discard at 2.5 hours post last dose and 24 hours post last dose prior to reinitiating breastfeeding.
6	Patients of child-bearing potential must agree to remain completely abstinent (complete avoidance of heterosexual intercourse) or use a highly effective (failure rate <1%), medically accepted contraceptive method for 30 days prior to dosing and for 90 days after GH001 dosing. Highly effective contraception methods include, but are not limited to: bilateral tubal ligation/occlusion, hormone contraceptives that inhibit ovulation, or intrauterine device (including hormone-releasing intrauterine device/systems). Patients must have a negative pregnancy test at screening and on the pre-test day (Day -1).
7	The investigator, after consultation with the patient's GP and/or treating psychiatrist, deems it acceptable, and patient is willing, to delay start of other antidepressant or antianxiety medication until after the end of the trial at Day 8, and patient agrees to keep any psychotherapy unchanged from 7 days prior to dosing on Day 1 until the end of the trial at Day 8.
8	Is able to inhale three liters of air from the test balloon within a single breath at screening and on the pre-test day (Day -1) and prior to first dose (Day 1). An incentive spirometer will be given to patients following screening. They will be encouraged to practice deep inhalations prior to the first dose on the test day (Day 1). Patients failing the test inhalation at screening may have the test inhalation repeated within 14 days of the original screening date.
9	Has a GP or treating psychiatrist and gives permission to contact those physicians for the purpose of discussing medication use, discontinuation, or medication re-initiation or other medical aspects relating to the trial participation and provides their contact details.
10	Is willing and able to comply with all requirements and rules of the trial.
11	Is willing and able to nominate a trusted caregiver that is willing to live with the patient and infant for the duration of the patient's participation in the trial and that is available to take full responsibility for the care and attention (e.g., feeding, changing, etc.) of their infant(s) for the entirety of the test day (Day 1) and for 7 days post last dose, and that is present at screening, provides contact details and consents to being contacted by study staff during the duration of the study. Note: It is expected the mother will continue routine care of their infant(s), but a trusted caregiver must be available as support during the trial period.
Exclusion Criteria	
Patients who met any of the following criteria prior to the first dose of study drug were not eligible for enrollment into the trial:	
1	Has, based on history, psychiatric assessment, and evaluation of the MINI, a current or prior diagnosis of bipolar disorder, a manic or hypomanic episode, a psychotic disorder, MDD or other mood disorder with psychotic features, obsessive compulsive disorder, PTSD, autism spectrum disorder, borderline personality disorder, schizophrenia, delusional disorder, paranoid personality disorder, schizoaffective disorder, clinically significant intellectual disability, or any other psychiatric comorbidity that renders the patient unsuitable for the trial according to the investigator's judgment.
2	Has one or more first or second degree relatives with a current or prior diagnosis of bipolar disorder, psychotic disorder or other mood disorder (including MDD) with psychotic features.
3	Current pregnancy resulting in termination, still-birth, pre-term delivery (before week complete gestational week 37), need for intensive care therapy of mother or intensive care therapy of child of duration >72 hours, or adoption of child away from patient.
4	Has clinically significant premenstrual syndrome or premenstrual dysphoric disorder that renders the patient unsuitable for the study according to the investigator's judgment.
5	Has significant suicide risk as defined by (a) suicidal ideation as endorsed on items 4 or 5 on the C-SSRS within the past year, during the screening period, or at Baseline; or (b) suicidal behaviors within the past year; or (c) clinical assessment of significant suicidal risk during clinical interview; or (d) non-suicidal self-injury within the past.

6	Has had an inadequate response to an adequate course of electroconvulsive therapy, vagal nerve stimulation, repetitive Transcranial Magnetic or Electrical Stimulation, or deep brain stimulation in the current episode of depression as assessed using the ATHF-SF.
7	Has taken anti-depressive medication (e.g., a SSRI such as paroxetine, fluoxetine (including in combination with olanzapine) or citalopram, a SNRI such as venlafaxine or duloxetine, a TCA such as amitriptyline or imipramine, an antipsychotic such as quetiapine, lithium, an atypical antidepressant such as bupropion or vortioxetine, a NMDA receptor antagonist such as esketamine, a MAOI such as isocarboxazide, phenelzine, selegiline or tranylcypromine within 14 days or 5 half-lives (whichever is longer) prior to dosing (exception: within the last 5 weeks in the case of fluoxetine). Cessation of such medication for the sole purpose of inclusion in this trial is not allowed.
8	Has taken any medication with MAOI activity such as isocarboxazide, phenelzine, selegiline or tranylcypromine, linezolid or methylene blue within 14 days or 5 half-lives (whichever is longer) prior to dosing.
9	Has taken opioids (e.g., oxycodone), or stimulants (e.g., amphetamine) within the last 7 days prior to dosing (or 5 half-lives, whichever is longer).
10	Is taking a total daily dose of benzodiazepines greater than the equivalent of 6 mg/day of lorazepam at screening.
11	Has taken sedatives (e.g., benzodiazepines or non-benzodiazepine sleeping medication [e.g., zolpidem, zaleplon]) within 12 hours prior to dosing.
12	Has taken synthetic or naturally occurring cannabinoids within 7 days prior to dosing.
13	Has taken ayahuasca, kambó, yopo, ibogaine, psilocybin, LSD, mebufotenin (5-methoxy-N,N-dimethyltryptamine [5-MeO-DMT]), DMT, Syrian Rue, ketamine, esketamine, Salvia divinorum or other psychedelic agents or mixtures or dissociative in their synthetic or naturally occurring form within 14 days prior to dosing.
14	Has used synthetic, plant-based, or herbal dietary supplements with known MAOI or antidepressant activity within 14 days prior to dosing.
15	Has received any investigational medication within the last 6 weeks prior to dosing.
16	Has previously experienced a significant adverse reaction to a hallucinogenic or psychedelic drug (e.g., psilocybin, Psilocybe spp. mushrooms, mebufotenin, DMT, ayahuasca, LSD, mescaline) according to the investigator's judgment.
17	Has known allergies or hypersensitivity or any other contraindication to mebufotenin or has diagnosed or suspected genetic monoamine oxidase deficiency or reduced activity, or has one or more immediate family members with diagnosed or suspected genetic monoamine oxidase deficiency or reduced activity (e.g., Norrie disease or Brunner syndrome).
18	Has any current or past clinically significant condition (e.g., severe infection, severe pulmonary disease, uncontrolled hypertension, new onset of hypertensive disorders of pregnancy during pregnancy or in the postnatal period that have not resolved at screening [e.g., gestational hypertension, pre-eclampsia/eclampsia, superimposed pre-eclampsia], uncontrolled diabetes, severe cardiovascular disease, severe hepatic or renal failure, severe brain disorder [including seizure disorder, stroke, dementia, degenerative neurologic diseases, meningitis, encephalitis, and head injury with loss of consciousness]) that may interfere with the interpretation of the trial results, constitutes a health risk for the patient, or that otherwise renders the patient unsuitable for the trial according to the investigator's judgment.
19	Takes any medication or other substance that renders the patient unsuitable for the trial according to the investigator's judgment.
20	Has a clinically significant abnormality in physical examination, vital signs, ECG, or clinical laboratory parameters which renders the patient unsuitable for the trial according to the investigator's judgment.
21	Has a QT interval corrected using Fridericia's formula (QTcF) $\geq$ 470 ms during screening or before dose administration, or history of additional risk factors for torsades des pointes (e.g., heart failure, family history of Long QT Syndrome, or the use of concomitant medications that prolong the QT/QTc interval).
22	Has abnormal thyroid function at screening. Patients that are on thyroid medications are eligible when they are euthyroid at screening.
23	Patient who has a positive pregnancy test at screening or on the pre-test day (Day -1), is pregnant, or plans to become pregnant during the course of the trial and up to 90 days after GH001 dosing.
24	Patients with DSM-5 alcohol use disorder (excluding tobacco and caffeine use disorders) within 6 months prior to screening.

25	If a smoker, is unwilling or unable to abstain from cigarette smoking or vaping on the day of study drug administration (nicotine replacement therapy is permitted).
26	Shows positive alcohol breath test on the pre-test day (Day -1) or on Day 1 prior to the first administration of GH001.
27	Shows a positive drug urine test at screening, on the pre-test day (Day -1) or on Day 1 prior to the first administration of GH001. Patients with a positive drug test at screening may have the test repeated once at a later date. This determination, and the reason for permitting a repeat test, must be recorded in the patient's source documents. A positive repeat drug test or a positive pre administration test is exclusionary. If the urine screen tests positive for benzodiazepines, and the patient took allowed benzodiazepines within the permitted time period as outlined in the exclusion criteria, this will not constitute a screen failure. Details of the benzodiazepine taken, including dose and timing shall be recorded in the source documents.
28	A positive Coronavirus Disease 2019 (COVID-19) test during the screening, on the pre-test day (Day -1) or on Day 1 as confirmed by an antigen or polymerase chain reaction (PCR) test. Patients with a positive test result for COVID-19 during the screening period or on the scheduled trial dosing day may be rescreened, or attend their rescheduled Day -1/Day 1 visit, a minimum of 5 days after initially testing positive, with/without a negative COVID-19 test result, provided that they are clinically recovered, and at the discretion of the investigator.

Abbreviations: AFHF-SF = Antidepressant Treatment History Form: Short Form; BMI = Body mass index; C-SSRS = Columbia-Suicide Severity Rating Scale; DMT = N,N-dimethyltryptamine; DSM-5 = Diagnostic and Statistical Manual of Mental Disorders; ECG = Electrocardiogram; GP = General practitioner; LSD = Lysergic acid diethylamide; MADRS = Montgomery–Åsberg Depression Rating Scale; MAOI = Monoamine oxidase inhibitor; MDD = Major depressive disorder; MINI = Mini-International Neuropsychiatric Interview; NMDA = N-methyl-D-aspartate; PPD = Postpartum depression; PTSD = Post-traumatic stress disorder; SNRI = Serotonin and norepinephrine reuptake inhibitor; SSRI = Selective serotonin reuptake inhibitor; TCA = Tricyclic antidepressant.

Supplementary Table 2. Schedule of Assessments

Assessment	Description	Schedule
Efficacy		
MADRS[2]	10-item clinician-rated scale; symptoms are rated on a 7-point scale from 0 (no symptoms) to 6 (severe symptoms); overall scoring range: 0–60, with higher scores indicating more severe depression; administered using the structured interview guide for the MADRS.	Baseline, Day 1 (2 hours post-last IDR dose), Day 2, Day 8
CGI-S[3]	Single 7-point scale from 1 (normal) to 7 (extremely ill); the CGI-S assessment was conducted after the MADRS assessment; administered by a psychiatrist or registered clinical psychologist with adequate experience with patients with MDD.	Baseline, Day 1 (2 hours post-last IDR dose), Day 2, Day 8
BIMF[4]	20-item self-reported patient centered tool assessing postpartum maternal functioning, which was designed to measure functioning in the year after childbirth; each item is rated on a 7-point scale from 0-6 as follows: 0. Strongly disagree; 1. Disagree, 2. Somewhat disagree; 3. Neutral; 4. Somewhat agree; 5. Agree; 6. Strongly agree; total score range: 0-120, with higher scores indicating greater severity of functional impairment.	Baseline Day 8

Assessment	Description	Schedule
Safety		
TEAEs	AEs were coded using Medical Dictionary of Regulatory Activity (Version 26.0).	Baseline, Day 1, Day 2, Day 8
Vital signs	Vital signs were measured pre-dose (before first IDR dose only), after any PsE had fully subsided, and 60 minutes after each IDR dose; heart rate and SpO_2 were monitored continuously during the acute psychoactive phase; measurements were recorded at 5, 10, 15, 20, 25, and 30 minutes after each IDR dose.	Baseline, Day 1 (after each IDR dose and at discharge), Day 2, Day 8
ECG	Standard 12-lead electrocardiogram; variables included heart rate, RR, QT, PR, and QRS intervals and QT interval corrected using Fridericia's formula.	Baseline, Day 1 (at discharge), Day 2, Day 8
Clinical laboratory tests	Included hematology, clinical chemistry, thyroid function test, and urine samples for laboratory safety and to assess for drugs of abuse or alcohol.	Baseline and Day 8
Spirometry	Pulmonary function test; measures FVC, FEV_1, FEV_1/FVC ratio.	Baseline, Day 1 (post-each IDR dose and at discharge), Day 2, Day 8
MOAA/S[5]	6-point scale assessing level of sedation, with a score of 5 ("responds readily to name spoken in normal tone") to 0 ("no response after painful trapezius squeeze"); administered by a physician.	Baseline, Day 1 (after each IDR dose, 60 minutes after final dose, and at discharge)
BPRS[6]	18-item clinician-rated scale assessing psychiatric symptoms; items rated on a 7-point Likert scale from 1 (not present) to 7 (extremely severe); total score range: 18–126, higher scores indicate greater severity; covers areas like anxiety, depression, hostility, hallucinations, disorientation; administered via semi-structured interview by trained clinicians.	Baseline, Day 1 (at discharge), Day 2, Day 8
C-SSRS[7]	Detailed questionnaire assessing suicidal behavior and ideation, with five questions addressing each; administered by a psychiatrist or licensed clinical psychologist; baseline/screening version was used at screening; Since Last Visit was used at other time points.	Baseline, Day 1 (at discharge), Day 2, Day 8
CADSS[8]	19-item subjective scale administered by a psychiatrist or licensed clinical psychologist; each item scored 0 (not at all) to 4 (extremely; total score: 0–76) based on the patient's described experience	Baseline, Day 1 (at discharge), Day 2, Day 8
CADR	Proprietary structured discharge assessment administered by a physician to ensure that the patient was without hallucinations and that any AEs had subsided.	Day 1 (at discharge)
Cognitive assessments[9]	Cognition was assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB). The test order in CANTAB is VRM (immediate recall), RVP, SWM, DSST, and VRM (delayed recall).	Baseline, Day 1 (at discharge), Day 2, Day 8
Psychoactive Effects		
PES[1]	Three visual analog scales (0–100) for assessing intensity, feeling of loss of control, and profoundness during a psychoactive experience; PES score was the average of the three scale scores; a mean score of 75 or greater was the threshold for determining that a patient achieved a Peak Experience.	Day 1 (after each IDR dose)

Assessment	Description	Schedule
MEQ30[10]	30-item questionnaire comprising four factors: mystical, positive mood, transcendence of time and space, and ineffability; each item is rated on a 6-point scale from 0 (none, not at all) to 5 (extreme, more than ever before in my life and stronger than 4); total score is calculated as the average of all non-missing item scores for a scoring range of 0–5.	Day 1 (after each IDR dose)
CEQ[11]	26-item tool comprising 7 factors (grief, fear, death, insanity, isolation, physical distress, and paranoia); each item is scored from 0 (none, not at all) to 5 (extreme, more than ever before in my life); total score was calculated as the average of transformed item scores for a scoring range of 0–5.	Day 1 (after each IDR dose)
Duration of PsE	Physician evaluation.	Day 1 (after each IDR dose)
Pharmacokinetics		
Breastmilk sampling	To assess the level of mebufotenin and its metabolites bufotenin and 5-MIAA in breastmilk samples.	Baseline, Day 1 (<2.5 hours post-dose), Day 2, Day 8

Abbreviations: BIMF = Barkin Index of Maternal Functioning; BPRS = Brief Psychiatric Rating Scale; CADR = Clinical Assessment of Discharge Readiness; CADSS = Clinician Administered Dissociative States Scale; CEQ = Challenging Experience Questionnaire; CGI-S = Clinical Global Impression Severity Scale; C-SSRS = Columbia-Suicide Severity Rating Scale; DSST = Digit symbol substitution test; ECG = Electrocardiogram; FEV_1 = Forced expiratory volume in one second; FVC = Forced vital capacity; HAM-D-17 = Hamilton Rating Scale for Depression; IDR = Individualized dosing regimen; MADRS = Montgomery–Åsberg Depression Rating Scale; MEQ30 = Mystical Experience Questionnaire (30-item); MDD = Major depressive disorder; MOAA/S = Modified Observer's Assessment of Alertness and Sedation scale; PES = Peak Experience Scale; PR = Pulse rate; PsE = Psychoactive effects; RR = Respiratory rate; RVP = Rapid visual information processing; SpO_2 = Oxygen saturation; SWM = Spatial working memory; TEAE = Treatment-emergent adverse events; VRM = Verbal recognition memory.

Supplementary Results

Supplementary Table 3. Patient Disposition

Parameter, n (%)	**GH001 (N = 10)**
Patient Screening, n	
Screened	24
Screening failed	14
Reason for screening failure	
Failed to meet eligibility criteria	10
Out of window screening	2
Withdrawal by patient	1
Lost to follow-up	1
Patients receiving IDR doses	
Dose 1 (6 mg)	1 (10.0)
Dose 2 (6 + 12 mg)	7 (70.0)
Dose 3 (6 + 12 + 18 mg)	2 (20.0)

Supplementary Table 4: Summary of Barkin Index of Maternal Functioning at Baseline and Day 8

	Score Range	Baseline		Day 8	
		n	Mean (SD)	n	Mean (SD)
Total Score	0-120	9	69.7 (14.8)	9	100.7 (10.6)
Self care	0-18	9	6.9 (2.1)	9	12.1 (4.6)
Infant care	0-18	9	11.2 (0.7)	9	11.2 (1.0)
Mother-child interaction	0-18	9	10.6 (3.5)	9	15.8 (1.4)
Psychological well-being	0-18	9	31.7 (7.9)	9	49.0 (7.2)
Social support	0-18	9	10.2 (4.2)	9	15.4 (2.1)
Management	0-12	9	20.2 (6.5)	9	29.2 (4.2)
Adjustment	0-18	9	5.8 (2.2)	9	9.9 (1.5)

Abbreviations: SD = Standard deviation.

Supplementary References

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