Phase 1, Open-label, Exposure-Escalation study to Evaluate the Safety and Tolerability of Nebulised HS4-20 in healthy volunteers

Clinical Study Report:	HSM4/cdv/2000-01
Name of Test Drug/ Investigational Product:	HS4-20
Indication Studied:	Healthy Volunteers
Study Duration:	5yrs
Dose:	HS4-20 500ppm via nebuliser
Planned Exposures:	30secs, 1 min, 5 mins, 10 mins, 15 mins, 20 mins, 30 mins, 45mins, 90mins.
Administered Exposures:	30secs, 1 min, 5 mins, 10 mins, 15 mins, 20 mins, 30 mins, 45mins, 90mins.
Phase:	1
Date:	Aug 2020
Study Start Date:	Jul 2015
Study End Date:	Jun 2020
Sponsor:	Hypo-stream ltd 31A Charnham Street Hungerford Berkshire RG17 0EG

Medical Monitor: Myles Dakin
Hypo-stream ltd
31A Charnham Street
Hungerford
Berkshire
RG17 0EJ

Sponsor Signatory/ Contact Person Thomas Kenny
Hypo-stream ltd
31A Charnham Street
Hungerford
Berkshire
RG17 0EJ

1 Signature page

We have read this report and confirm that to the best of our knowledge it accurately describes the conduct and the results of the study.

Mr Myles Dakin Principle Clinical Investigator Hypo-Stream ltd	Date
Dr Thomas Kenny Chief Medical Officer Hypo-Stream Ltd	Date
Professor Richard Aspinall Chief Scientific Officer Hypo-Stream Ltd	Date

2 List of Abbreviations/ Definitions of Terms

ADR	adverse drug reaction
AE	adverse event
CRF	Case report form
DMC	Data Monitoring Committee
ICF	Informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IP	Investigation product
OTC	over-the-counter
PI	Principle Investigator
Serious Burns	>20% TBSA
TBSA	Total Body Surface Area

3 Ethics

3.1 Ethical Conduct of the study

This study was to be conducted in accordance with the following:

ICH Guidance for Industry E6 Good Clinical Practice (ICH-GCP)

Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, October 2000, with clarification on Paragraph 29 by 53rd WMA General Assembly, Washington, October 2000)

Specifically, this study was to be based on adequately performed laboratory and animal experimentation. The study was conducted by scientifically and medically qualified persons. The benefits of the study were to be in proportion to the risks and the rights and welfare of the subjects were to be respected. The physicians conducting the study were to assure that the hazards not outweigh the potential benefits. Each subject or his/her legally authorized representative was to provide written, informed consent before any study-related tests or evaluations were to be performed.

3.2 Subject Information and Consent

A properly written and executed ICF, in compliance with the Declaration of Helsinki, ICH-GCP (Section 4.8), and other applicable local regulations, was to be obtained for each subject prior to entering the subject into the study. The PI was to prepare the ICF and was to provide the documents to the Sponsor for approval. A copy of the approved ICF and, if applicable, a copy of the approved subject information sheet, minor assent form, parental ICF for studies involving minors, and all ICFs translated to a language other than the native language of the

clinical site were also to be received by the Sponsor or its designee prior to the delivery of study drug.
Subjects under the age of 18 years were to provide written assent (if required), and his/her legally authorized representative (parent or legal guardian) was to provide written informed consent for such subjects. The PI was to provide copies of the signed ICF to each subject (or the subject's legally authorized representative) and was to maintain the original in the subject's record file.

4 Investigators and Administrative Structure

The study was conducted at 1 site in the United Kingdom. The site, site addresses, and curriculum vitae of the PIs who participated in the study are provided in the Appendix (Section 13)

5 Introduction

M3 is the name given to the physiologic aqueous chlorine based upon the endogenous hypo-halide produced during the respiratory burst by the myeloperoxidase membrane enzyme in neutrophils and inflammatory cells.
Hypo-Stream have defined a clinically useful composition as an aqueous solution and developed clinical utility as a prospective surgical site wash, a drench-wash for full thickness burns treatment and for treatment of non-healing wounds (either post-surgical, as a consequence of radiotherapy or venous leg ulcers).
Phase II clinical studies of topical application, surgical lavage, drench-washes with HS3-1001 (data on file) have shown clinical efficacy, and an excellent safety profile and tolerability. HS3-1001 has EMA Orphan class designation as an aqueous drench-wash for full-thickness burns greater than 20% TBSA.
Data from the Phase II study in full thickness burns demonstrated that HS3-1001 has efficacy in clearing colonisation by a broad spectrum of pathogens, including AMR species. It also demonstrated efficacy in clearing infection and colonisation by gram negative bacteria, specifically Pseudomonas sp. as well as reversing / ameliorating the systemic inflammatory response mediated by elevated serum Interleukin 6 (Il-6). This global, systemic acute phase inflammatory response is seen in various disease processes and is historically defined by what is diagnosed as the initiating disease; i.e. sepsis, acute respiratory distress syndrome, cytokine storm etc.
50% of patient admitted with serious burns also have inhalation burns, where the pseudomonas colonisation and infection are highly correlated with elevated serum Il-6 and high mortality levels. With Pseudomonas sp. implicated in deterioration secondary to inhalation burns and mechanical ventilation (Gellatly and Hancock, 2013).
The Hypo-Stream clinical development team identified the potential for a nebulised M3 to be reformulated for administration to the respiratory system and potentially to the pulmonary air-spaces. If the clinical efficacy as an antimicrobial and anti-inflammatory demonstrated in the phase II clinical treatment of patients with severe burns (catastrophic injury to

epithelium), was reproduced in the respiratory epithelium this would be highly valuable in treating this unmet need and potentially save lives and reduce lung function morbidity.
On the basis of the safety and clinical efficacy data the clinical development team at Hypo-Stream began a programme of evaluation of a re-formulated M3 for respiratory – pulmonary administration (HS4-20).
The component oxidizing agents present in unbuffered dilute sodium hypochlorite in saline solutions have previously been shown to have antibacterial properties in isolation of each other(Sakarya et al., 2014) as well as the ability to induce the proliferation of keratinocytes through their action on intermediates in the nuclear factor-kappa B pathway (Leung et al., 2013, Thomas et al., 2009, Winter et al., 2008).

6 Study Objectives

6.1 Primary Objectives

The primary objective of the study was to assess the safety and tolerability of escalating exposure to nebulised HS4-20 in Healthy Volunteers.

7 Investigational Plan

7.1 Overall Study Plan

This study was designed as a Phase 1, open-label, exposure-escalation study in approximately 18 healthy volunteers who were 2 to 80 years old. Three exposure cohorts were planned.
Cohort one consisted of 2 adults

> Initially a single healthy volunteer. Inhalation of of HS4-20 500ppm via nebuliser. Monitoring of Oxygen saturation, pulse rate, blood pressure and performance of coordination tests. Inhalation programme 30 seconds followed by 30-minute observation. 1 minute followed by 30-minute observation. The observation periods were kept at 30-minutes and the period of administration was increased to 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes. On a subsequent day the administration period was increased to 45 minutes and 90 minutes.
> This programme was repeated with a further healthy volunteer after a seven-day observation period on the first volunteer.
> Both volunteers were followed up for 6 months, with no further inhalations and monitored for adverse reactions and any longer-term effects.

Cohort two consisted of cohort one plus an additional 4 adults

> The programme was then extended to a further group of healthy volunteers and following the induction phase of administrations for 30secs, 1 minute, 5 minutes, 10 minutes and then 15 minutes, a daily administration time of 20 minutes was recommended based upon the assumption of the Il-6 neutralisation time of 15 minutes with the 500ppm HS4-20 seen in the mechanistic study(data on file) plus a circulation time of 4-5 minutes for blood circulation around the body.

After 1 week of observed administration. The healthy volunteers self-administered during a three-month observation period and followed up over a period of 2 years and monitored for adverse reactions and any longer-term effects.

Cohort three consisted of 12 adults and children

The programme was then extended to a further group of healthy volunteers at the extremes of age and following the induction phase of administrations for 5 minutes, 10 minutes and then 15 minutes, a daily administration time of 20 minutes. The healthy volunteers self-administered during a three-month observation period and followed up over a period of three months.

7.2 Discussion regarding Study Design

Study HSM4/cdv/2000-01 was designed as a first-in-humans Phase 1, open-label, exposure-escalation study to assess the safety and tolerability of nebulised HS4-20.

7.3 Selection of Population

7.3.1 Inclusion Criteria

- Provided written, signed informed consent, or, in the case of participants under the age of 18, written informed consent by a parent or legal guardian, after the nature of the study had been explained, and prior to any research-related procedures.
- Agreed to comply with all study procedures.
- Were in generally good health as evidenced by physical examination.

7.3.2 Exclusion Criteria

- Donated blood or plasma within 30 days prior to the study drug administration.
- Used any over-the-counter (OTC) medication, including vitamins, within 7 days prior to the administration of study drug, without evaluation and approval by the investigator.
- Used any prescription medication within 14 days prior to the administration of study drug without evaluation and approval by the investigator.
- Any uncontrolled pulmonary condition

7.4 Treatments

Subjects (or their legally authorized representative) could have withdrawn their consent to participate in the study at any time without prejudice. The investigator was to withdraw from the study any subject who requested to be withdrawn. A subject's participation in the study was to be discontinued at any time at the discretion of the investigator and in accordance with his/her clinical judgment.

If a subject failed to return for scheduled visits, a documented effort was to be made to determine the reason. If the subject could not be reached by telephone within 7 days, a

certified letter was to be sent to the subject (or the subject's legally authorized representative, if appropriate) requesting contact with the investigator. This information was to be recorded in the study records.

Cohort one

Inhalation of HS4-20 500ppm via nebuliser.
Inhalation programme 30 seconds followed by 30-minute observation.
1 minute followed by 30-minute observation.
The observation periods were kept at 30-minutes and the period of administration was increased to 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes.
On a subsequent day the administration period was increased to 45 minutes and 90 minutes.
After a further seven-day observation period the same schedule was repeated on the second volunteer.

Cohort two

induction phase of administrations for 30secs, 1 minute, 5 minutes, 10 minutes and then 15 minutes, each followed by a 30 minute observation period.
Then from day 2, a daily administration time of 20 minutes

Cohort three

Induction phase of administrations for 5 minutes, 10 minutes and then 15 minutes, each followed by a 30min observation phase
Then from day 2, a daily administration time of 20 minutes.

HS4-20 was supplied as a sterile aqueous solution. HS4-20 was nebulised at a concentration of 500ppm.

This was to be an open-label study; no randomization was to be used in assigning subjects to each cohort.

All prescription and OTC medications taken by a subject for 30 days before Screening were to be recorded on the designated CRF. The investigator could have prescribed additional medications during the study as long as the prescribed medication was not prohibited by the protocol. In the event of an emergency, any needed medications could have been prescribed without prior approval but the Sponsor's medical monitor must have been notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study were to be recorded on the CRF.

Use of any other investigational product or investigational medical device was to be prohibited within 30 days before Screening and until all scheduled study assessments were completed.

OTC medications and vitamins were to be prohibited within 7 days before Day 1 (study administration) and until all scheduled study assessments were completed without evaluation and approval by the investigator.
Use of any prescription medication was to be prohibited within 14 days before Day 1 and until all scheduled study assessments were completed without evaluation and approval by the investigator.
All used and unused drug containers were to be kept by the investigator. The quantity dispensed, returned, used, lost, etc., was to be recorded on the dispensing log provided for the study.
The PI was to be responsible for maintaining accurate records (including dates and quantities) of study drug received, subjects to whom study drug was dispensed (subject-by-subject dose specific accounting), and study drug lost or accidentally or deliberately destroyed. The investigator was to retain all unused or expired study supplies until the study monitor confirmed the accountability data.
Unused study drug could have been destroyed on site, per the site's standard operating procedures, but only after the Sponsor granted approval for drug destruction. The monitor was to account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site was to be documented. Documentation was to be provided to the Sponsor and was to be retained in the investigator's study files. If a site was unable to destroy study drug appropriately, the site could have returned unused study drug to the Sponsor upon request. The return of study drug or study drug materials was to be accounted for by the Sponsor.

7.5 Safety and Tolerability Variables

Safety was to be assessed by examining the incidence of all treatment-emergent adverse events (hereafter referred to as AEs) reported during the study period and clinically significant changes in vital signs.
Vital signs were to be measured after resting for 5 minutes and included seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in mmHg, heart rate in beats per minute, respiration rate in breaths per minute and oxygen saturation.
According to the ICH definition, an adverse event (or adverse experience) is "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the IP."
An adverse drug reaction (ADR) is described by the ICH as "all noxious and unintended responses to a medicinal product related to any dose." This means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, ie, the relationship cannot be ruled out.

An AE could have included intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma). Whenever possible, it was preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.
The reporting period for nonserious AEs was to be the period from the first administration of study drug through Week 26 or early termination. If a nonserious AE remained unresolved at the conclusion of the study, the PI and Sponsor's medical monitor were to make a joint clinical assessment as to whether continued follow-up of the AE was warranted and the results of this assessment were to be documented. Resolution was to be defined as the return to baseline (Screening) status or stabilization of the condition with the expectation that it remained chronic.
The investigator was to assess AEs for severity, for relationship to study drug, and as to whether the event met one or more of the definitions of an SAE.
The investigator was to determine the severity of each AE and was to record it on the source documents and AE CRF using the categories defined below.

7.6 Statistical Plan

Due to the exploratory nature of this study and small sample size overall, no formal statistical tests were to be performed for inference. Inferential statistics were to be used for descriptive purposes only. Unless specified otherwise, all summaries were to be comprised of standard descriptive statistics. Standard descriptive statistics were to include the following:

- For continuous parameters: number of observations (or subjects), mean, standard deviation, median, minimum, and maximum
- For categorical parameters: frequency and percent of observations in each category

Both efficacy and safety data were to be summarized by individual cohort and all cohorts combined.

7.6.1 Determination of Sample size

A total of 18 subjects, treated in 3 cohorts, were planned for this study. A total cohort size of 18 subjects equally balanced across genders and with a wide age range, was deemed sufficient to meet the objectives of this study.
No formal sample size calculations or power determinations were to be conducted.

7.6.2 Planned Analysis

All subjects who received any amount of study drug and had post dose safety data were to be included in the safety analyses.

7.6.3 Handling of Drop out and missing data

All analyses were to be presented based on observed data only.

7.6.4 Data Monitoring Committee

A DMC was to be used in this study to monitor the safety of subjects in this first clinical study of HS4-20. Planned reviews were to occur after the completion of the induction phase of each cohort and at the end of each cohort's dosing and follow-up periods.

7.6.5 Safety Analyses

Safety was to be assessed by examining the incidence of all AEs reported during the study period and clinically significant changes in vital signs.

Only AEs reported during the study period were to be included in AE summaries. An AE was to be defined as any AE of new occurrence, increased in frequency, or worsened in severity following study drug administration. If the onset of an AE was missing and the AE resolution was either after the dose date or missing, then the AE was to be considered treatment emergent. Any AEs judged by the investigator as possibly or probably related to study drug were to be considered drug related. If relationship to study drug was missing, the AE was to be considered drug related.

The incidence and severity of AEs for all AE and all drug-related AEs was to be summarized by system organ class (SOC), preferred term, and severity. For those AEs that occurred more than once during the study, the maximum severity was to be used to summarize the AEs by severity. Subjects who reported multiple events that coded to a common preferred term or SOC were to be counted only once per preferred term or SOC.

AEs that were assessed by the investigator as possibly or probably related to study drug were to be summarized by SOC, preferred term, and maximum severity. Drug-related AEs were to be summarized similarly to AEs.

7.7 Changes in conduct of the study or panned analysis

Of the 18 subjects enrolled in the study all met the eligibility criteria as defined by the protocol and received treatment as appropriate to the escalation schedule appropriate to their cohort.

8 Study Subjects

Characteristic	Cohort 1	Cohort 2	Cohort 3	Combined
n	2	4	12	18
Gender				
Male	2(100%)	3(75%)	4(33%)	9(50%)
Female	0(0%)	1(25%)	8(66%)	9(50%)
Age at baseline				
Mean(SD)	53(4.2)	47.75(7.3)	43.9(24.7)	45.7(20.6)
Median	53	47.5	41	42
Min, Max	50,56	41, 55	3, 80	3, 80

9 Safety Evaluations

9.1 Extent of Exposure

All subjects were exposure to HS4-20 according to their cohort.

9.2 Adverse Events

4 subjects reported adverse events during the initial parts of induction phase of treatment (cohorts one and two, mild cough or feeling of wanting to cough), not experienced on subsequent exposure. Patients in cohort three were warned of this possibility and none reported it.

1 subject with underlying asthma report a sensation similar to asthma symptoms, no wheeze, no decrease in PEFR, treatment was paused for 1 week and re-introduced with no recurrence of sensation.

All subjects experienced increased expectoration after dosing.

No other adverse events occurred during the 1hour period immediately following treatment.

No adverse events occurred during the follow up periods that were thought to be related to treatment.

9.3 Listing of Serious Adverse Events

There were no SAEs during the duration of the treatment or follow phases of this study.

10 Discussion and overall Conclusions

Results for the Phase 1, Open-label, Exposure-Escalation study to Evaluate the Safety and Tolerability of Nebulised HS4-20 in healthy volunteers.

Headline results:

i. Sample size n= 18
ii. Serious Adverse events – 0
iii. Adverse events during induction – 4 (all grade 1 events)
iv. Adverse events during or in the immediate 1 h period following treatment – 18 (all grade 1 event)
v. Other Adverse events thought to be related to treatment - 0
vi. Age range 3-80 yrs
vii. Longest period of continuous administration - 90 mins
viii. Mode period of continuous administration - 20 minutes
ix. Maximum duration of repeated daily 20 minutes administration – over 4 years

11 Tables and Figures

11.1 Adverse events

Adverse Event	**N**
Mild cough/sensation of wanting to cough	4
Increase in expectoration	18
Asthma like sensation	1

12 References

Gellatly, S. L. & Hancock, R. E. 2013. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. *Pathog Dis,* 67**,** 159-73.

Leung, T. H., Zhang, L. F., Wang, J., Ning, S., Knox, S. J. & Kim, S. K. 2013. Topical hypochlorite ameliorates NF-κB-mediated skin diseases in mice. *J Clin Invest,* 123**,** 5361-70.

Sakarya, S., Gunay, N., Karakulak, M., Ozturk, B. & Ertugrul, B. 2014. Hypochlorous Acid: an ideal wound care agent with powerful microbicidal, antibiofilm, and wound healing potency. *Wounds,* 26**,** 342-50.

Thomas, G. W., Rael, L. T., Bar-Or, R., Shimonkevitz, R., Mains, C. W., Slone, D. S., Craun, M. L. & Bar-Or, D. 2009. Mechanisms of delayed wound healing by commonly used antiseptics. *J Trauma,* 66**,** 82-90; discussion 90-1.

Winter, J., Ilbert, M., Graf, P. C., Ozcelik, D. & Jakob, U. 2008. Bleach activates a redox-regulated chaperone by oxidative protein unfolding. *Cell,* 135**,** 691-701.

13 Appendices

13.1 Site Address

Unit B9
Melbourn Science Park
Melbourn
Cambridgeshire SG8 6HB