Safety, Tolerability and Acceptability Study of Needle-Free Injection System Vs. Conventional Hypodermic Needle

Clinical Study Report

Title: An open label study to investigate safety, tolerability and acceptability of Needle Free Injection System (NFIS) in healthy volunteers in comparison to conventional needle-based system.

Principal Investigator: Dr. Almas Pathan

Sponsor: IntegriMedical

Sponsor Authorized Signatory: Scott McFarland

Date Study Initiated: 20 Jan 2021

Date Study Completed: 01 Nov 2021

Date of Report: 14th March 2022

Version: 1.0

Prepared By: Jehangir Clinical Development Centre Pvt. Ltd.

This study was performed in compliance with ICH E6R2 "Guidance on Good Clinical Practice", Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

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1. Title

An open label study to investigate safety, tolerability and acceptability of needle free injection system in healthy volunteers in comparison to conventional needle-based system.

Name of investigational medical device: Needle Free Injection System

Indication Studied: The present study is the first in human assessment of IM-NFIS that compares safety, tolerability and acceptability of needle free injection system in healthy volunteers with conventional hypodermic needle-based system at 5 different sites of administration (forearm, abdomen, thigh, buttocks and arm).

Name of the Sponsor: IntegriMedical.

Protocol identification: IM/NFIS/01, Version 3.0

Study Initiation Date: 20 Jan 2021

Date of early study termination, if any: Not Applicable

Study Completion date (last patient completed): 01 Nov 2021

Name and affiliation of Principal Investigator: Dr. Almas Pathan, Jehangir Clinical Development Centre Pvt Ltd, Jehangir Hospital Premises, 32 Sassoon Road, Pune 411001, Maharashtra, India

This study was performed in compliance with ICH E6R2 “Guidance on Good Clinical Practice”, Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

Date of Clinical Study Report: 14th March 2022

2. List of Abbreviations of Terms

Abbreviations	Full Name
AE	Adverse Event
CRF	Case Report Form
CRO	Contract Research Organization
ICF	Informed Consent Form
ICH-GCP	International conference of Harmonization – Good Clinical Practice
ICMR	Indian Council of Medical Research Ethical Guidelines for Biomedical Research on Human Subjects
IEC	Institutional Ethics Committee
IMD	Investigational Medical Device
IRB	Institutional Review Board
MGRS	Multicenter Growth Reference Study
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WHO	World Health Organization

3. Ethics

3.1 Institutional Ethics Committee (IEC)

The protocol and consent form was reviewed and approved by the Institutional Ethics Committee of JCDC. The EC is registered with the CDSCO (Registration No.-ECR/352/tnst/MW2013/RR-19 and accredited by Association for the Accreditation of Human Research Protection Program(AAHRPP). The Ethics Committee is accredited by National Accreditation Board for Hospitals and Health Care Providers (NABH) (Certificate No. EC-CT-2018-0023).

3.2 Ethical Conduct of the Study

This study was performed in compliance with ICH E6R2 "Guidance on Good Clinical Practice", Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

3.3 Patient Information and Consent

The informed consent was obtained from the subject/LAR of the subject by the Principal Investigator. Subject/LAR of the subject provided written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, risks, burdens and benefits of treatment. Personal data from subjects enrolled in this study were limited to those necessary to investigate the safety and tolerability of the investigational study device used in this study.

4. Investigator and Study Administrative Structure

Principal Investigator: Dr. Almas Pathan

Sponsor: IntegriMedical

Clinical Laboratory: Jehangir Hospital

5. Introduction and Background Information

Drug delivery is an important technology in the healthcare sector that uses different systems or approaches to deliver any pharmaceutical to achieve its intended therapeutic effect (1). Drug delivery involves different routes of administration which includes but is not limited to parenteral, inhalation, transdermal, oral etc. Certain pharmaceuticals cannot be delivered orally due to susceptibility to enzymatic degradation and poor absorption due to their molecular size. Such pharmaceuticals are administered through the parenteral route by using hypodermic needle and a syringe. The use of hypodermic needles is very common and the oldest way to overcome the physical barrier. Ideally, a solution of a drug is forced under piston stress straight into the bloodstream or exact tissue. This necessitates skin perforation using a needle, which is associated with trauma and pain. To overcome these drawbacks, other alternative methods have been investigated like jet injections, dermabrasion, thermal ablation, laser, tape stripping, etc. (2) Reduction of the pain and time of injections may lead to improved patient satisfaction and compliance, as well as reduced anxiety in populations of patients who require frequent or ongoing injections to treat their medical conditions. A needle-free delivery system offers the potential to address such issues. They may enhance safety, improve dosing accuracy, and increase patient compliance, particularly in self-administration settings. The needle free injection technology does not involve the use of needles for delivery of pharmaceutical and instead is delivered via a high-pressure stream of liquid which penetrates the site of injection (3). The needle free injection technology has been reported to overcome some of the risks of needles including reduced risk of needle stick injury, eliminated risk of disease transmission from reused needles, reduce scar tissue at the injection site caused by needle damage to the tissue, easier self-administration, etc. The working principle of needle free injection works on different technologies including spring system, gas propelled system, etc. (4) The newly designed needle free injection systems have overcome most of the risks posed by needles by incorporating disposable cartridges to avoid infection, introducing adjustable parameters selected according to skin site properties and thickness as well as the desired depth level intended to deliver the medication. IntegriMedical® Needle Free Injection System (IM-NFIS) is intended to deliver drugs and biologics through intradermal, intramuscular, or subcutaneous sites. Typical doses range from 0.1 ml to 0.5 ml and are delivered to various injection depths. The energy for the device comes from compressed spring which when released propels the plunger forward delivering the medication at high speed thus penetrating the skin.

6. Study objectives

6.1 Primary Objectives

• To investigate safety of needle free injection system

6.2 Secondary Objectives

• To understand the acceptability and tolerability of needle free injection system

6.3 Primary endpoints

• Injection site reactions as assessed according to the toxicity scale provided by US FDA guidance with grading 0-4

6.4 Secondary endpoints

• Pain assessment using 100-mm VAS scores (0 mm = no pain at all; 100 mm = a lot of pain) immediately after each administration (before needle removal)

• Acceptability of needle free injection using a questionnaire

7. Investigational Plan

7.1 Overall Study Design

This was a 5-day open label study to investigate safety, tolerability and acceptability of needle free injection system in 30 healthy volunteers (5 cohorts with 6 subjects in each cohort) in comparison to conventional needle-based system. Prospective healthy volunteers were identified for the study by the study investigator/study team after the screening procedure and qualifying the study in-/exclusion criteria. All study procedures began only after obtaining signed informed consent from the subjects/legally acceptable representatives (LARs). Subjects were randomized for the five sites of injection (forearm, abdomen, thigh, buttocks and arm). Each subject acted as a test (Saline delivery through Needle free injection) and control arm (Saline delivery through Hypodermic needle) for the allocated site of injection. Each site was divided into areas for receiving test and control product as given below:

Cohort 1

Forearm Right: Saline delivery through Needle free injection system

Forearm Left: Saline delivery through Hypodermic needle

Cohort 2

Abdomen area divided into two halves,

Right Half: Saline delivery through Needle free injection system

Left Half: Saline delivery through Hypodermic needle

Cohort 3

Thigh Right: Saline delivery through Needle free injection system

Thigh Left: Saline delivery through Hypodermic needle

Cohort 4

Buttocks side Right: Saline delivery through Needle free injection system

Buttocks side Left: Saline delivery through Hypodermic needle

Cohort 5

Arm Right: Saline delivery through Needle free injection system

Arm Left: Saline delivery through Hypodermic needle

Each participant received two injections (once for the test device and second time for the control device) within an interval of 5-10 minutes in between (5). Participants were evaluated for site reactions, pain level and acceptability separately after each injection for Needle free injection system and conventional hypodermic needle.

The study included a screening period (0 day) and a 4-day study period. The study included 5 time points: Visit 1/ Time point 1 (Baseline/screening visit/Day 0), Visit 2/ Time point 2 (at day 1 from baseline/Enrolment/Administration of product using needle free injection and hypodermic needle), Time point 3 (at day 2 from baseline/Telephonic follow-up), Time point 4 (at day 3 from baseline/Telephonic follow-up) and Time point 5 (at day 4 from baseline/Telephonic follow-up/EOS).

At visit 1 following laboratory investigations were performed for screening of participant:

Complete blood count

Urine pregnancy

Serum creatinine

Chest X ray

Subjects were randomized as per site of injection at Visit 2 during enrolment. Following the randomization each participant in cohort 2 to 5 received known 0.5 ml volume of saline using a needle free injection system at the designated site and areas of abdomen, thigh, buttocks and arm. Participants in cohort 1 received 0.1ml volume of saline using a needle free injection system in the designated forearm. Within a time interval of 5-10 minutes, participants received second injection of known volume

of saline (0.5 ml volume for cohorts 2 to 5 and 0.1 ml volume for cohort 1) using conventional hypodermic needle at the designated site and area.

Participants reported the pain level separately after each injection. Pain assessment was done using a VAS score (6). Investigator also performed an assessment of injection sites at 2 min and between 20 and 30 min following each injection. Injection site reactions were assessed according to the toxicity scale provided by FDA guidance with grading 0-4 (7). Participants were also trained to measure the local site reactions. Photos of injection site were taken by the principal investigator at 2 min and between 20 and 30 min following each injection for record purpose. For visits 3 to 5 participants were requested to report the local site reactions and systemic reactions telephonically and send the photos of injection site to the Principal Investigators. Participants complaining of site reactions were called at the site for further evaluation. Study coordinators masked the identity of the participants. Acceptability questionnaire (8) was completed by the participant before leaving from the study site. A follow phone contact was made with the participant at 24hr, 48hr and 72 h after the injections to assess for injection site reactions and adverse events.

Inclusion/Exclusion Criteria

Inclusion Criteria

- Male or female in the age group 18 to 45 years both inclusive
- Able and willing to sign the informed consent form
- Physical examination without clinically significant findings
- Hemoglobin in the opinion of a PI as clinically not significant
- WBC and differential in the opinion of a PI as clinically not significant
- No history of liver disorders in past 3 months
- No history of kidney disorders in past 3 months
- No history of cardiovascular disorders in past 3 months
- No history of neurological disorders in past 3 months
- Negative human chorionic gonadotropin (beta-HCG) pregnancy test (urine) on day of enrollment
- In good general health without clinically significant medical history and based on clinical judgement of principal investigator

Exclusion criteria

- Breast-feeding women
- More than 10 days of systemic immunosuppressive medications or cytotoxic medications within the 4 weeks prior to enrollment or any within the 14 days prior to enrollment
- Blood products within 16 weeks prior to enrollment
- Bleeding disorder history (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM, SC injection or blood draws
- Investigational research products within 4 weeks prior to enrollment or planning to receive investigational products while on the study
- Asthma that is not well controlled
- Diabetes mellitus (type I or II)
- Evidence of autoimmune disease or immunodeficiency
- Idiopathic urticaria within the past year
- Hypertension that is not well controlled
- Malignancy that is active or history of malignancy
- Any medical, psychiatric, social condition, occupational reason or other responsibility that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a volunteer s ability to give informed consent

Table 1: Schedule of Assessments

Procedures	**Day 0+3 days/ Screening/ /Visit 1/Time point 1**	**Day 1/ Eligibility/ Enrolment/ /Administration of product using needle free injection and hypodermic needle/ Visit 2/ Time point 2**	**Day 2/ Telephonic follow-up/ Time point 3**	**Day 3/ Telephonic follow-up/ Time point 4**	**Day 4/ Telephonic follow-up/ Time point 5/EOS**
Informed consent	X				
Demographics	X				
Medical history	X				
Prior medication (if any)	X				
Current/concomitant medication	X				
General Physical examination, including height, weight, BMI BP, pulse	X				
Laboratory Investigations					
CBC	X				
Urine pregnancy		X			
Serum creatinine	X				
Chest X ray	X				
Eligibility		X			
Randomization		X			
Product administration		X			
AE/SAE: Local and Systemic		X	X*	X*	X*
Pain assessment using VAS scale		X			
Acceptability using questionnaire		X			

*AE/SAE assessment only for those parameters which are mentioned in Table 1 and 3 will be done telephonically using FDA toxicity scale and photo of the injection site will be send to the Principal Investigator.

7.2 Treatment

7.2.1 Treatments Administered and Identity of Investigational Product(s)

Investigational medical device - IntegriMedical® Needle Free Injection System (IM-NFIS)

Mode of administration – Five sites of injection (forearm, abdomen, thigh, arms and buttocks) for intra dermal, intramuscular and subcutaneous route.

Administration schedule – Subjects were randomized for the five sites of injection (forearm, abdomen, thigh, arms and buttocks). Each subject acted as a test (Saline delivery through Needle free injection) and control arm (Saline delivery through Hypodermic needle) for the allocated site of injection. Each site was divided into areas for receiving test and control product as given below:
Forearm Right: Saline delivery through Needle free injection system

Forearm Left: Saline delivery through Hypodermic needle

Abdomen area divided into two quadrants,

Quadrant Right: Saline delivery through Needle free injection system

Quadrant Left: Saline delivery through Hypodermic needle

Thigh Right: Saline delivery through Needle free injection system

Thigh Left: Saline delivery through Hypodermic needle

Buttocks side Right: Saline delivery through Needle free injection system

Buttocks side Left: Saline delivery through Hypodermic needle

Arm Right: Saline delivery through Needle free injection system

Arm Left: Saline delivery through Hypodermic needle

7.2.2 Method of Assigning Subjects to Treatment Groups

Subjects were randomized for the five sites of injection (forearm, abdomen, thigh, arms and buttocks). Each subject acted as a test (Saline delivery through Needle free injection) and control arm (Saline delivery through Hypodermic needle) for the allocated site of injection. within an interval of 5-10 minutes in between. After each injection participants were evaluated for site

reactions, pain level and acceptability of Needle free injection system in comparison to conventional hypodermic needle.

7.2.3 Selection of volume of saline administered

Following the randomization each participant in cohort 2 to 5 received known 0.5 ml volume of saline using a needle free injection system at the designated site and areas of abdomen, thigh, buttocks and arm. Participants in cohort 1 received 0.1ml volume of saline using a needle free injection system in the designated forearm. Within a time interval of 5-10 minutes, participants received second injection of known volume of saline (0.5 ml volume for cohorts 2 to 5 and 0.1 ml volume for cohort 1) using conventional hypodermic needle at the designated site and area.

7.2.4 Blinding (If Applicable)

Not Applicable

7.3 Analysis of Safety and tolerability Measurements

Safety evaluation includes assessment of Injection site reactions as assessed according to the toxicity scale provided by US FDA guidance with grading 0-4. Acceptability and Tolerability was determined using a questionnaire and a VAS score respectively.

7.4 Data Quality Assurance

A representative of the independent quality assurance team at JCDC monitored the study to assess the compliance with approved protocol and ICH-GCP guidelines and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

7.5 Statistical Analysis

Statistical analysis was performed using the SPSS Version 20 software. All available data was used in the analyses.

7.6 Protocol Deviations

There were no protocol deviations noted in the conduct of the study. All 30 volunteers complied to the various trial related procedures and the study was conducted in compliance with the study protocol.

8. Subject Disposition

8.1 Study Subjects

A total of 30 healthy volunteers providing consent and found eligible for participation in the study were enrolled in the five-day study (5 cohorts and 6 subjects in each cohort). Subjects in the first 4 cohorts (forearm, abdomen, buttock, and thigh) were enrolled and completed the study during

the last week of January 2021. Volunteers in the fifth cohort (arm) were studied in the last week of October 2021. All 30 volunteers successfully completed the stipulated five-day study period. Data generated on these 30 healthy volunteers who received both the intervention and control injections form the basis of this report.

8.2 Demographics

The demographic and patient characteristics of the study subjects are summarized in Table 2. The mean age of the 30 male subjects enrolled in the study was 26.2 years (median 22.5 years; range 18 to 43 years). Mean weight was 61.6 Kg (SD 11.6 kg) and mean height was 169.2 cm (SD 7.6 cm). All except one subject were non-smokers and non-alcoholics. Subject-wise listing of demographic characteristics are tabulated in Appendix A.

Table 2: Demographic characteristics of the 30 subjects at baseline

Demographic characteristic		Mean (SD) / No. (%) (N = 30)	
Age (years)	n	30	
	Mean	26.2	
	SD	8.5	
	Median	22.5	
	Min	18	
	Max	43	
Age group	18 - 20 years	13	**43.3%**
	21 - 30 years	9	**30.0%**
	31 years or above	8	**26.7%**
Gender	Female	0	**00.0%**
	Male	30	**100.0%**
Ethnicity	Indian	30	**100.0%**
Race	Asian	30	**100.0%**
Weight (Kg)	n	30	
	Mean	61.6	
	SD	11.6	
	Median	61.5	
	Min	44	
	Max	89	
Height (cm)	n	30	
	Mean	169.2	
	SD	7.6	
	Median	170.0	
	Min	146	
	Max	181	
Smokers	No	29	**96.7%**
	Yes	1	**03.3%**
Alcoholic	No	29	**96.7%**
	Yes	1	**03.3%**

8.3 Past and Current Medical History

None of the study subjects reported any past / current medical history (Appendix B).

8.4 Vital Signs

Vital signs of the study subjects at screening are summarized in Table 3. Subject-wise listings are tabulated in Appendix C. The study subjects had 'normal' body temperature, heart rate, respiratory rate, and blood pressure at the time of screening.

Table 3: Subject characteristics at baseline - Vital signs

Vital signs		Mean (SD) / No. (%)	Normal	Abnormal, clinically not significant	Abnormal, clinically significant	Not done
		(N = 30)	Mean SD/No (%)			
Temperature (axillary) (^{0} F)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	97.7				
	SD	0.7				
	Median	97.8				
	Min	96.2				
	Max	98.8				
Heart rate (beats / min)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	79.6				
	SD	6.5				
	Median	78.0				
	Min	70				
	Max	98				
Respiratory rate (breath / min)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	16.6				
	SD	1.8				
	Median	17.5				
	Min	12				
	Max	20				
Systolic BP (mm / Hg)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	116.6				
	SD	10.6				
	Median	113.0				
	Min	100				
	Max	140				
Diastolic BP (mm / Hg)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	75.0				
	SD	7.3				
	Median	75.0				
	Min	60				
	Max	88				

8.5 General and Systemic Examination

General and systemic examination data of the study subjects at screening are summarized in Table 4. Subject-wise listings are tabulated in Appendix D. None of the subjects had any complications. All subjects in both the groups were 'normal' with respect to general appearance, head, ENT, eyes, skin, neck, abdomen, cardiovascular, respiratory, musculoskeletal, neurological, and lymphatic systems.

Table 4: Subject characteristics at baseline – General and systemic examination

	Mean (SD) / No. (%) (N = 30)							
Physical examination	**Normal**		**Abnormal, clinically not significant**		**Abnormal, clinically significant**		**Not done**	
General appearance	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Head	30	**100%**	0	**0%**	0	**0%**	0	**0%**
ENT	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Eyes	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Skin	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Neck	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Abdomen	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Cardiovascular system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Respiratory system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Musculoskeletal system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Neurological system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Lymphatic system	30	**100%**	0	**0%**	0	**0%**	0	**0%**

8.6 Prior and Current Medications

None of the study subjects reported any past / current medical history (Appendix E).

8.7 Inclusion Criteria and Exclusion Criteria

Subject-wise, details of inclusion criteria and exclusion criteria data are listed in Appendix F and G respectively.

8.8 Study Subjects' Conclusion

All the 30 male adult healthy volunteers recruited for the study had 'normal' findings at screening with respect to anthropometric parameters, vital signs, and physical examination. None of them had any medical history and were not on any concomitant medications in the past and at the time of enrolment into this clinical study.

9. Safety Evaluation (Results and Discussion)

9.1 Administration of Study Products & Time to Assessments

Injection Sites

All 30 subjects received both injections. Subjects were first administered saline with needle free injection (NF Injection) system followed by conventional hypodermic needle injection (CHN Injection). NF injection was given in the right side and the CHN injection on the left side. Five injection sites were used - forearm, abdomen, buttock, thigh, and arm, six subjects in each group.

Time to Pain Assessment Using VAS Score Post Injections

Post NF injection, VAS pain score was recorded within 1 min for all subjects (Table 5). In the case of CHN injection, VAS pain score was recorded within 2 min for 28 subjects; for remaining 2 subjects the measurement was completed in 3 min.

Time to FDA Toxicity Assessments Post Injections (02 min & 20-30 min)

FDA toxicity assessments were done within 02 min for all subjects post NF injection and CHN injection. Toxicity assessments were repeated post 20-30 min of each injection; median time was 28 min post NF injection and 27 min post CHN injection (Table 5). Individual subject-wise details of actual time of each of these assessments showing compliance to protocol are presented in Appendix H and I, for the NF injection and CHN injection, respectively.

Table 5: Time to VAS pain assessment and FDA toxicity assessments

		NF Injection (N = 30)	CHN Injection (N = 30)
Time to VAS pain score assessment (min) post injection	Median	1	2
	Min	1	1
	Max	1	3
Time to FDA toxicity assessment (02 min) post injection	Median	2	2
	Min	2	2
	Max	2	2
Time to FDA toxicity assessment (20-30 min) post injection	Median	28	27
	Min	20	20
	Max	30	30

9.2 FDA Toxicity Scale Assessments

9.2.1 Local Reactions (2 min and 20-30 min post injections)

Data on local reactions at 2 min and at 20-30 min following NF injection and CHN injection are summarized in the following Table 6. Post 2 min, one subject (receiving NF injection in Arm) and three subjects post CHN injection (two in Arm and one in Abdomen) reported Grade 1 pain (does not interfere with activity). Grade 1 (mild discomfort to touch) tenderness was reported by two each, NF injection (both Forearm) and CHN injection (one Forearm and one Abdomen) subjects. None reported erythemia / redness or induration. At 20-30 min post injections no local reaction was reported in for both injection methods. Subject-wise listings are provided in Appendix J-M.

At 2 min post injection, 29 subjects receiving NF injection reported no pain compared with 27 in CHN injection group. However, the higher number in NF injection group is not statistically significant (P = 0.3006). Tenderness, redness, and induration was reported by equal number of subjects in both groups at post 2 min and post 20-30 min.

Table 6: Summary of local reactions post injections (2 min and 20-30 min)

Signs & Symptoms		NF Injection At 2 min (N = 30)	CHN Injection At 2 min (N = 30)	P Value	NF Injection At 20 - 30 min (N = 30)	CHN Injection At 20 - 30 min (N = 30)	P Value
Pain	**No**	29	27	>0.2	30	30	>0.2
	Yes	1	3		0	0	
	Grade 1	1	3		NA	NA	
Tenderness	**No**	28	28	>0.2	30	30	>0.2
	Yes	2	2		0	0	
	Grade 1	2	2		NA	NA	
Erythema / Redness	**No**	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
	Size	NA	NA		NA	NA	
Induration	**No**	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
	Size	NA	NA		NA	NA	

9.2.2 Vital Signs (2 min and 20-30 min post injections)

Vital signs including body temperature, heart rate, blood pressure, and respiratory rate were measured after administration of NF injection and CHN injection post 2 min and post 20-30 min. Table 7 below summarizes the data for both these groups at two time points defined. Appendix N-Q lists the subject specific vital data points.

Mean vital signs parameters at 2 min post NF injection were not statistically (P >0.2; paired t-test) different from similar measurements taken post CHN injection. This conclusion was valid across both groups at 20-30 min post injections also.

Table 7: Summary of vital signs post injections (2 min and 20-30 min)

Vitals		NF Injection At 2 min (N = 30)	CHN Injection At 2 min (N = 30)	P Value	NF Injection At 20 - 30 min (N = 30)	CHN Injection At 20 - 30 min (N = 30)	P Value
Body temperature (in F)	n	30	30		30	30	
	Mean	97.3	97.3	>0.2	97.2	97.2	>0.2
	SD	0.84	0.70		0.72	0.66	
	Median	97.5	97.2		97.2	97.2	
	Min	95.2	96.2		95.4	95.2	
	Max	98.6	98.9		98.4	98.4	
Fever	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Heart rate (bpm)	n	30	30		30	30	
	Mean	80.3	78.4	0.2	77.8	79.6	0.2
	SD	7.30	6.79		7.34	7.07	
	Median	80.5	80.0		77.5	78.0	
	Min	62	62		62	63	
	Max	91	90		95	90	
Tachycardia	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Bradycardia	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Blood pressure – Systolic (mm / Hg)	n	30	30		30	30	
	Mean	115.3	115.3	>0.2	116.9	118.5	>0.2
	SD	9.87	11.70		10.64	10.44	
	Median	114.0	112.5		117.5	118.0	
	Min	100	100		100	96	
	Max	140	144		140	140	
Blood pressure – Diastolic (mm / Hg)	n	30	30		30	30	
	Mean	75.2	74.5	>0.2	73.7	73.3	>0.2
	SD	8.02	7.96		7.65	7.95	
	Median	75.5	75.0		74.5	72.0	
	Min	57	56		60	60	
	Max	86	88		88	88	
Hypertension (Systolic)	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Hypertension (Diastolic)	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Hypotension (Systolic)	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Respiratory rate (per min)	n	30	30		30	30	
	Mean	16.4	16.0	0.2	16.5	15.9	0.1
	SD	1.54	1.71		1.72	1.62	
	Median	16	16		16	16	
	Min	14	12		14	14	
	Max	20	18		20	20	

9.2.3 Systemic Examination (2 min and 20-30 min post injections)

Systemic examination carried out for after NF and CHN injections administration at 2 min and 20-30 min. The data summarized in Table 8 demonstrates that none of the subjects in both injection types reported any difficulties. Subject wise data for all the parameters are listed in Appendix R-U.

Systemic examination parameters (nausea, diarrhea, headache, fatigue, and myalgia) at 2 min post NF injection were not statistically (P >0.2; chi-square test) different from similar measurements taken post CHN injection. This conclusion was valid across both groups at 20-30 min post injections also.

Table 8: Systemic examination post injections (2 min and 20-30 min)

Parameters		NF Injection At 2 min (N = 30)	CHN Injection At 2 min (N = 30)	P Value	NF Injection At 20 - 30 min (N = 30)	CHN Injection At 20 - 30 min (N = 30)	P Value
Nausea / Vomiting	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Diarrhoea	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Headache	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Fatigue	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Myalgia	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	

9.2.4 Local Reactions (24-, 48- and 72-hours post injections)

All 30 study subjects (who received both NF and CHN injections) were telephonically contacted post 24, 48, and 72 hours of injections. Data on signs and symptoms reported by them are tabulated in Table 9 below. Subject-wise (and day-wise) listings for individual signs and symptoms are

included in Appendix V-X. None of the subjects reported any kind of complaints on all the three instances of telephonic follow up.

Table 9: Summary of local reactions post injections (24, 48 and 72 hours)

Signs & Symptoms		At 24 hours (N = 30)	At 48 hours (N = 30)	At 72 hours (N = 30)
Pain	No	30	30	30
	Yes	0	0	0
Tenderness	No	30	30	30
	Yes	0	0	0
Erythema/Redness	No	30	30	30
	Yes	0	0	0
Induration/Swelling	No	30	30	30
	Yes	0	0	0

9.2.5 Systemic Examination (24-, 48- and 72-hours post injections)

Systemic examination carried out for all subjects telephonically post 24, 48, and 72 hours after injections. The data summarized in Table 10 demonstrates that none of the subjects reported any difficulties. Subject wise data for all the parameters are listed in Appendix Y, Z, and AA.

Table 10: Systemic examination post injections (24, 48 and 72 hours)

Parameters		At 24 hours (N = 30)	At 48 hours (N = 30)	At 72 hours (N = 30)
Nausea / Vomiting	No	30	30	30
	Yes	0	0	0
Diarrhoea	No	30	30	30
	Yes	0	0	0
Headache	No	30	30	30
	Yes	0	0	0
Fatigue	No	30	30	30
	Yes	0	0	0
Myalgia	No	30	30	30
	Yes	0	0	0

9.3 VAS Pain Assessment Score (2 min post injections)

Pain score was assessed within 2 min following the NF injection and CHN injection. 76.7% of the subjects reported no pain post NF injection compared with 30.0% in the CHN injection recipients (Table 11). The percentage of those who reported no pain post NF injection (77%) was significantly higher as compared with CHN injection group (P <0.01; Chi square test). Mean pain score for the NF injection was 0.23 and for CHN injection it was reported as 1.07. The lower pain score post NF injection as compared with CHN injection was statistically significant (P <0.01; paired-t test for comparison of 2 means). Hence, tolerability of NFIS was proven through this study. Individual pain scores are listed in Appendix AB.

Table 11: VAS pain score assessment following NF and CHN injections

Pain score		**NF Injection (N = 30)**		**CHN Injection (N = 30)**		**P Value**
		Number (%) of subjects				
None	(0)	23	**76.7%**	9	**30.0 %**	**P <0.01**
Mild	(1, 2, or 3)	7	**23.3%**	21	**70.0 %**	
Moderate	(4, 5, or 6)	0	**0.0%**	0	**0.0%**	
Severe	(7, 8, 9, or 10)	0	**0.0%**	0	**0.0%**	
	n	30		30		
	Mean	0.23		1.07		P <0.01
	SD	0.43		1.01		
	Median	0.00		1.00		
	Min	0.00		0.00		
	Max	1.00		3.00		

9.4 FDA Toxicity Scale Assessment Conclusion

Toxicity assessments were carried out on all 30 subjects post 2 min and 20-30 min administration of needle free injection system (NF injection) and followed by conventional hypodermic needle injection (CHN injection). These assessments did not highlight any safety concern. Only one subject complained of pain post NF injection after 2 min, three following CHN injection. Tenderness was reported by two subjects for both injection types after 2 min. No other local reactions were noted. Vitals remained stable post NF injection and systemic examination did not highlight any complaints. Toxicity assessments carried out telephonically post 24, 48, and 72 hours did not bring out any complaints. None of the subjects reported any specific adverse events

following the administration of injections during the entire planned follow up period. VAS pain assessment scores demonstrated that the NF injection induced (statistically) significantly lower pain scores as compared with CHN injection. NF injection was well tolerated as that of the convention injection (CHN injection). Hence, tolerability of NFIS was proven through this study.

9.5 Acceptability Assessments

Acceptability Questionnaire Responses Analysis

Acceptability questionnaire was administered to the study subjects post administration of NF injection and CHN injection. Responses given by the subjects separately for the two injections are tabulated in Table 12. Individual subject responses are listed in Appendix AC and AD. The NF injection was generally acceptable with many questions responded as 'not at all' by all subjects.

Significantly higher percentage (90%) responded that they did not feel anxious about receiving the NF injection as compared with 43.3% with the CHN injection ($P < 0.01$; 2x2 chi-square test with continuity correction). All the subjects (100%) were not bothered by pain during the NF injection as compared with 43.3% in the CHN injection ($P < 0.01$; 2x2 chi-square test with continuity correction).

Table 12: Acceptability responses given by subjects' post NF and CHN injections

Question		NF Injection (N = 30)		CHN Injection (N = 30)		P Value
		Number (%) of subjects				
1. Just before your injection, did you feel anxious about receiving your injection?	Not at all	27	**90.0%**	13	**43.3%**	<0.01
	A little	3	**10.0%**	14	**46.7%**	
	Moderately	0	**0%**	3	**10%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
2. How bothered were you by pain during the injection?	Not at all	30	**100%**	13	**43.3%**	<0.01
	A little	0	**0%**	16	**53.3%**	
	Moderately	0	**0%**	1	**3.3%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
3. How bothered were you by redness at the injection site?	Not at all	30	**100%**	29	**96.7%**	>0.2
	A little	0	**0%**	1	**3.3%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
4. How bothered were you by swelling at the injection site?	Not at all	30	**100%**	29	**96.7%**	
	A little	0	**0%**	1	**3.3%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
5. How bothered were you by itching at the injection site?	Not at all	30	**100%**	29	**96.7%**	>0.2
	A little	0	**0%**	1	**3.3%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
6. How bothered were you by hardening (a bump) at the injection site?	Not at all	30	**100%**	28	**93.4%**	>0.2
	A little	0	**0%**	2	**6.6%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
7. How bothered were you by bruising at the injection site?	Not at all	30	**100%**	28	**93.4%**	>0.2
	A little	0	**0%**	2	**6.6%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
8. How acceptable was/were your local reaction(s)?	Totally acceptable	27	**90.0%**	21	**70.0%**	0.1
	Very acceptable	3	**10.0%**	5	**16.7%**	
	Moderately acceptable	0	**0%**	4	**13.3%**	
	A little acceptable	0	**0%**	0	**0%**	
	Not at all acceptable	0	**0%**	0	**0%**	
9. How acceptable was your pain?	Totally acceptable	28	**93.3%**	19	**63.3%**	0.01
	Very acceptable	2	**06.7%**	10	**33.3%**	
	Moderately acceptable	0	**0%**	1	**3.3%**	
	A little acceptable	0	**0%**	0	**0%**	
	Not at all acceptable	0	**0%**	0	**0%**	
10. How satisfied were you with the injection system that was used to administer the product?	Very satisfied	24	**80%**	17	**56.7%**	0.1
	Satisfied	6	**20%**	13	**43.3%**	
	Neither satisfied nor dissatisfied	0	**0%**	0	**0%**	
	Dissatisfied	0	**0%**	0	**0%**	
	Very dissatisfied	0	**0%**	0	**0%**	

9.6 Acceptability Conclusions

Needle free injection system was well accepted. None of the subjects receiving this injection complained of pain, redness, swelling, itching, hardening, and bruising at the injection site. More than 90% of the respondents indicated that the local reaction and pain was totally acceptable. NF injection had a significantly higher satisfaction percentage compared with the CHN injection administration.

9.7 Overall Conclusion

Findings of the study state that there is no significant difference in terms of tenderness, redness, and induration for 2 groups. Thus, the study concludes that NFIS is well tolerated just like CHN. Also, mean vital signs parameters and systemic examination parameters (nausea, diarrhea, headache, fatigue, and myalgia) at 2 min post NF injection were not statistically different from similar measurements taken post CHN injection. This finding was valid across both groups at 20-30 min post injections also. None of the subjects reported any kind of complaints on all the three instances of telephonic follow up. This indicates NFIS to be similar to CHN with respect to safety of device. The percentage of those who reported no pain post NF injection (77%) was significantly higher as compared with CHN injection group ($P < 0.01$; Chi square test). Further, significantly higher percentage (90%) responded that they did not feel anxious about receiving the NF injection as compared with 43.3% with the CHN injection. All the subjects (100%) were not bothered by pain during the NF injection as compared with 43.3% in the CHN injection ($P < 0.01$; 2x2 chi-square test with continuity correction). Hence, acceptability of NFIS was proven through this study.

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11. Signature Page

Clinical Study Report Prepared By:	
I have prepared and read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study	
Senior Medical Writer	Name: Dr. Arati Ranade Jehangir Clinical Development Center, Pvt. Ltd. Jehangir Hospital Premises, 32, Sasoon Road, Pune 411001 A N Ranade Date and Signature: 14-Mar-2022
Bio- Statistician	Name: Dr. B. Kishore Kumar B. Kishore Kumar Date and Signature: 14-Mar-2022
Clinical Study Report Approved By:	
I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.	
Sponsor Representative	Name: Address: Date and Signature:
Investigator	Name: Dr. Almas Pathan Jehangir Clinical Development Center, Pvt. Ltd. Jehangir Hospital Premises, 32, Sasoon Road, Pune 411001 Date and Signature: 14/MAR/2022