INTRAVENOUS FIBRONECTIN (FN)-DERIVED cNP8 PEPTIDE PHASE 1 STUDY

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ABSTRACT

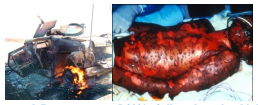
Background: Burns are dynamic injuries characterized by progressive extension of the depth and size of the burn over the course of several days. NeoMatrix Therapeutics (NMT) discovered a natural, partially elastase-resistant, peptide (P46). Next P46 was engineered to a peptide (cNP8) that was completely resistant to elastase. NMT then validated cNP8's ability to speed wound closure with a single intravenous (IV) infusion initiated 8-48h post-burn.

Methods: This Phase 1 clinical trial was a randomized, double-blind, placebo-controlled, single ascending dose study to evaluate the safety, tolerability, and PK profile of cNP8 in healthy male and female subjects, between 18 to 65, that met Inclusion and Exclusion Criteria. Each subject was randomized to receive either a single dose of cNP8 or placebo. Each dose cohort included 6 subjects dosed with cNP8 and 2 subjects dosed with placebo. The first two subjects in each cohort were dosed as sentinel subjects (one with cNP8 and one with placebo) and monitored for adverse events. If no serious events occurred in the acute post-dose period (24h), the remaining 6 subjects were dosed from lowest to highest cNP8 dose (0.001, 0.003, 0.01 and 0.03 mg/kg). Subjects received a single, approximately 30-minute IV infusion of cNP8 or placebo. Samples for serum chemistries and hematology, urinalysis, vital signs, and ECGs were obtained at several time points during the study. Blood samples for cNP8 plasma pharmacokinetic (PK) analysis were collected at pre-dose; 15, 30, and 45 minutes and 1 and 4 hours following the start of study drug infusion. The PK profile of cNP8 was calculated for standard PK parameters using non-compartmental methods and the actual sampling times for each subject. Summary statistics, including number (n), mean, standard deviation, median, and range (minimum, maximum), were tabulated for the observed values; and the change (or percent change) from baseline for all continuous parameters for vital signs, ECG, and laboratory tests were reported.

Results: All 32 subjects who entered the study received study drug, completed the study per protocol, and were included in the safety analysis. There were no deaths, SAEs, or subject discontinuations due to AEs in this study. In total, 7 subjects experienced a total of 9 AEs of which 4 were assessed to be AEs reported on/after initiation of study treatment or any pre-existing AE/condition that worsened in severity following initiation of study treatment. At post-dose timepoints, mean hematology, serum chemistry, and urinalysis parameters remained within reference ranges. After single IV cNP8 infusions of 0.001, 0.003, 0.01, and 0.03 mg/kg, mean concentrations of cNP8 peaked (Cmax) around 30 minutes. After Cmax, rapid clearances were observed across all dose groups, particularly within the first 2 hours.

Conclusion: These results support the favorable safety and PK profiles of cNP8 in healthy subjects.

MISSION: Limit injury progression after burns



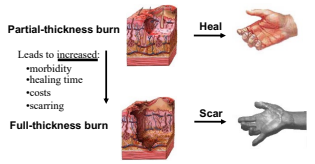
Military need: **Burns represent 5-20% of all combat related injuries**, 20% considered severe or involve >20% of TBSA, resulting in significant morbidity and mortality as well as long-term complications including functional impairment and aesthetic disfigurement

Civilian need: **500,000 burns/yr in USA**, 40,000 require hospitalization, 50% admitted to burn units, 4,000 deaths

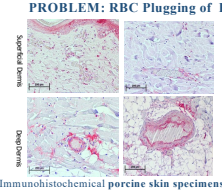
Direct costs: **$2 billion/yr in USA**

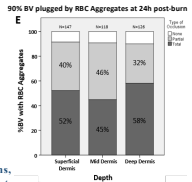
UNMET NEED

Therapy to limit burn injury progression



PROBLEM: RBC Plugging of Peri-burn Blood Vessels

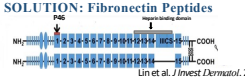




Immunohistochemical **porcine skin specimens**, harvested **at 24h post-burn, had RBC aggregates but no fibrin (red stain) in superficial dermal BV**. However, some fibrin was in perilumina distribution of BV in deep dermis

Asif et al. *Wd Repair Regen*. 2016

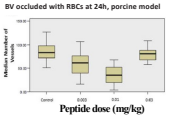
SOLUTION: Fibronectin Peptides

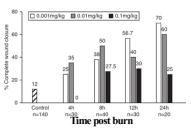


Lin et al. *J Invest Dermatol*. 2014

NMT discovered a 15-residue peptide, P46, in 1st type III repeat of FN. **P46 was redesigned (cNP8)** to resist both serum neutrophil elastase and blood exopeptidases.

NMT Peptides contain RWRPK & dilate cutaneous arterioles



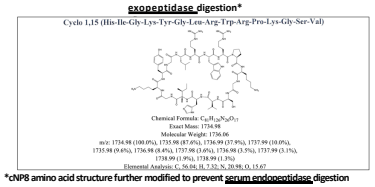


Preclinical Safety Studies

Preclinical Safety Studies were conducted by **Charles River Laboratories**, a Good Laboratory Practice (GLP) laboratory, for rat and dog toxicopharmacology and for genetic toxicity studies. All studies were coordinated and evaluated by **Reno and Associates.**

1. **Single-dose Toxicity on Rats**
2. **Single-dose Toxicity on Dogs**
3. **Two GLP-compliant in vitro genetic toxicity studies**

Figure 1. cNP8 structure cyclized to prevent blood exopeptidase digestion*



*cNP8 amino acid structure further modified to prevent serum endopeptidase digestion

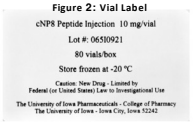
Table 1. Composition of "cNP8 Peptide Injection"

Name of Ingredient	Quality	Function	Quantity per mL
cNP8	GMP	Active	10 mg
Sodium Hydroxide	NF	pH Adjustment	as needed
Glacial Acetic Acid	USP	pH Adjustment	as needed
Nitrogen	NF	Inert Overlay	N/A
Sterile Water for Injection	USP	Solvent	1 mL *q.s. (Quantum sufficit)*
Total			**1 mL**

Table 2: Container Closure System for "cNP8 Peptide Injection"

Component	Description	Supplier
Glass vial	5-mL Type I clear glass tubing vial with 20-mm opening	West Pharmaceutical Services, Inc. (Exton, PA)
Stopper	20-mm fluorotec coated chlorobutyl rubber lyophilization stopper	West Pharmaceutical Services, Inc. (Exton, PA)
Crimped cap	20-mm aluminum seal with plastic flip-off cap	West Pharmaceutical Services, Inc. (Exton, PA)

Figure 2: Vial Label



Study Design of cNP8 Clinical Trial

Primary Objective: To assess the safety and tolerability of single ascending intravenous (IV) doses of cNP8 in healthy subjects.
Secondary Objective: To investigate the pharmacokinetic (PK) profile of cNP8 after single ascending IV doses in healthy subjects.

AIM 1. Develop and Validate Bioanalytic Assay for cNP8 in human plasma
AIM 2. IRB and HRPO Submission and Approval
AIM 3. Initiate First in Human Study to assesses safety and tolerability of IV cNP8, and to assess cNP8 plasma pharmacokinetics (PK).
AIM 4. Submit Case Report Form (Briefing Book); Request and Attend End of Phase 1 (EOP1) meeting with FDA receiving Guidance on CRF and proposed Phase 2a CT synopsis

Study Design of cNP8 Clinical Trial and Results

Randomized double-blind, placebo-controlled, single ascending dose (SAD) study to evaluate the safety, tolerability, and PK profile of cNP8 in healthy male and female subjects.

Each dose cohort included 6 subjects dosed with cNP8 and 2 subjects dosed with placebo.

As this is a first-in-human study, the first two subjects in each cohort were dosed as sentinel subjects (one with cNP8 and one with placebo)

If no serious or severe events occurred in the acute post-dose period (24 hours post dose), the remaining 6 subjects in the cohort were dosed.

Table 3. Planned Increasing Dose Levels

Cohort	Group	Dose Level (mg/kg)	Number of Subjects: cNP8	Placebo	Total
1	Sentinel	0.001	1	1	2
			5	1	6
2	Sentinel	0.003	1	1	2
			5	1	6
3	Sentinel	0.01	1	1	2
			5	1	6
4	Sentinel	0.03	1	1	2
			5	1	6
Total			24	8	32

Subject Inclusion/Exclusion Criteria:

1. Individuals were eligible for the study if they **met all inclusion** and no exclusion criteria.
2. The criteria were assessed at the screening visit (within 30 days prior to administration of study drug on Day 1).
3. Continued subject eligibility verified on Day -1 upon admission.

Selection and Timing of Dose for Each Subject:

1. Subjects fasted from all food and drink (except for water) for 10 hours prior to study drug (or placebo) administration
2. Study drug or placebo infused over a period of approximately 30 minutes, and subjects continued fasting for 4 hours after the start of the infusion.
3. Study drug infusion occurred in the morning of Day 1.

Table 4. Summary of All Subjects

Category	Pooled Placebo N=8 n (%)	cNP8 0.001 mg/kg N=6 n (%)	cNP8 0.003 mg/kg N=6 n (%)	cNP8 0.01 mg/kg N=6 n (%)	cNP8 0.03 mg/kg N=6 n (%)	cNP8 Pooled Active N=24 n (%)	Overall N=32 n (%)
Number of subjects who had an AE	1 (12.5)	2 (33.3)	2 (33.3)	0	2 (33.3)	6 (25.0)	7 (21.9)
Number of subjects who had a serious AE	0	0	0	0	0	0	0
Number of subjects who had a TEAE	1 (12.5)	1 (16.7)	1 (16.7)	0	1 (16.7)	3 (12.5)	4 (12.5)
Number of subjects who had a serious TEAE	0	0	0	0	0	0	0
Number of subjects who had a TEAE	0	1 (16.7)	1 (16.7)	0	0	2 (8.3)	2 (6.3)
Number of subjects who had a treatment-related serious TEAE	0	0	0	0	0	0	0
Number of subjects who had a TEAE leading to study discontinuation	0	0	0	0	0	0	0
Number of subjects who had a TEAE leading to death	0	0	0	0	0	0	0

Abbreviation: AE = adverse event; TEAE = treatment-emergent adverse event; TRAE = treatment-related AE

Figure 3
Mean (± SD) Plasma Concentration by Time: Linear Scale (PK Population)

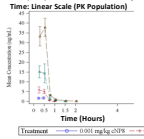
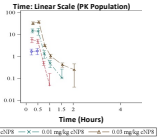


Figure 4
Mean (± SD) Plasma Concentration by Time: Linear Scale (PK Population)



Conclusions

1. Fibronectin has been shown to play a vital role in wound healing and is deficient in burn patients' wounds and blood. cNP8 is a novel bioactive peptide that is bioengineered from fibronectin. It is being developed for treatment to improve wound healing of acute, deep, second degree burns in hospitalized patients.

2. Study NMT-cNP8-101 was a Phase 1, single-center, randomized, double-blind, placebo-controlled, first-in-human, single-ascending dose (SAD) study to evaluate the safety, tolerability, and PK profile of cNP8 in 32 healthy male and female subjects. The study consisted of a screening period, inpatient dosing and observation period, and safety follow-up period. Four dosing cohorts were evaluated: 0.001, 0.003, 0.01, and 0.03 mg/kg. Cohorts were studied sequentially starting with the lowest cNP8 dose.

3. Safety - Single-ascending IV doses of cNP8 (0.001, 0.003, 0.01, and 0.03 mg/kg) were well tolerated and exhibited a favorable safety profile in healthy adult male and female subjects.
No deaths, SAEs, or subject discontinuations due to AEs occurred.

4. After single IV cNP8 infusions of 0.001, 0.003, 0.01, and 0.03 mg/kg, mean concentrations of cNP8 peaked (Cmax) around end of infusion.
After Cmax, **rapid clearances** were observed across all dose groups, particularly within the first 2 hours.

REFERENCES

1. Asif B, Abdul R, Fenner J, Lin F, Hirth DA, Hassani J, McClain SA, Singer AJ, Tonnesen, MG, Clark RAF. Blood vessel occlusion in periburn tissue is secondary to erythrocyte aggregation and mitigated by a fibronectin-derived peptide that limits burn injury progression. *Wd Rep Regen*, 24:501-513, 2016.

2. Frame MD, Lin F, Dewar AM, Clark RAF. Vasoactive effect of fibronectin-derived Epiviosamine-1 and related peptides in quiescent and stress models. *Microcirculation* 24:e12369, 2017, DOI 10.1111/micc.12369, PMC5542862,

3. Clark RAF, Fenner J, Sasson A, McClain SA, Singer AJ, Tonnesen MG. Blood vessel occlusion with erythrocyte aggregates causes burn injury progression - microvasculature dilation as a possible therapy. *Exp Dermatol* 27:625-629, 2018.

4. Lin K, Lin F, Clark RA. Elastase Digestion of fibronectin releases an epiviosamine peptide with fibroblast growth and survival activity. *J Invest Dermatol* 138:2480-2483, 2018.

5. Lin F, Prasad A, Weber-Fishkin S, Clark RA. Engineered Fibronectin Peptide is Resistant to Elastase Digestion, Speeds Healing, and Attenuates Scarring in Porcine Burns. *J Invest Dermatol* 140:1480-1483, 2020.

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