Multi-functional, Fibronectin (FN)-derived Peptides, cP12 and cNP8, for Burns and Acute Traumatic Wound Therapy

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Abstract

BACKGROUND: Over 400,000 burn patients present to emergency departments in the US every year. Of these about 30,000 patients are admitted to one of the 128 burn centers. Burns are dynamic injuries characterized by progressive extension of tissue injury depth over several days post-burn. This process is termed burn conversion occurs when the dermal injury converts partial dermal thickness destruction to full-thickness dermal destruction. Burn conversion has a significant impact in short-term care (increased infection, fluid imbalance, and skin grafts) as well as prolonged hospitalization, and long term sequelae such as increased scarring, scar contracture, rehabilitation, inability to return to service, and reduced quality of life. Despite huge advances in our understanding of wound healing at cell and molecular levels and how they can go awry, no cost effective, transformative therapy has reached the market for burn conversion, an unmet need.

A 14-residue peptide (P12) was derived from FN and cyclized (cP12) to prevent digestion by exopeptidases found normally in blood and tissue fluids. An intravenous (IV) dose of 0.01mg/kg cP12 infused within 1-4h post-burn in a porcine burn model appeared to speed wound closure (funded by the Armed Forces Institute of Regenerative Medicine, AFIRM, W81XWH-08-2-0034). Using a dose range, one log lower to one log higher than 0.01mg/kg, we confirmed 0.01mg/kg cP12 is the optimal IV dose at 1-4h post-burn that speeds burn wound closure 14d post-burn (70% vs 20% control). These data were submitted to the IND (funded by JWMRP, W81XWH-15-C-0043). We believe that vasodilation of the peri-burn microvasculature is the mechanism of action for cP12 burn therapy (see **Figures 5** and **6**). A Phase 1 Clinical was successfully completed in 2019 (Military Burn Research Program, MBRP, W81XWH-18-2-0059).

cP12 milestones

1. Orphan Drug designation 2011
2. IND accepted 2017
3. Fast Track designation 2019.
4. Successful completion of a Phase 1 Clinical Trial 2019
5. Phase 2a Trial Synopsis to be resubmitted to the IND 2022
6. Posed to conduct a Phase 2a Clinical Trial

cP12 is extremely sensitive to human neutrophil elastase (HNE), an endopeptidase abundant in wounds beginning about 4 days post tissue damage. Thus, cP12 is minimally active as a topical therapy by 4 days post injury (funded by Joint Warfighter Medical Research Program, JWMRP, W81XWH-14-1-0100).

Subsequently, NeoMatrix Therapeutics (NMT) discovered another peptide (P46) in the FN first type III repeat. P46, a 15-residue peptide, is substantially resistant to elastase (J Invest Dermatol 138:2480-2483, 2018). NMT engineered several peptides from P46 to resist high concentrations of human neutrophil elastase during a 37°C incubation for 24 hours. After cyclization, a biologically active, engineered peptide, designated cNP8 was chosen for further study. cNP8 speeds healing, and reduces scarring when given IV at 0.001-0.01mg/kg from 8-24h post-burn (J Invest Dermatol 140:1480-1483, 2020) (funded by JWMRP, W81XWH-15-C-0043). Given that cNP8 is 67% homologous to cP12 with similar molecular weights (1736 and 1763, respectively) and with similar isoelectric points (~11.5), we surmise that blood levels in pigs and humans are about the same for 0.01mg/kg doses, i.e 100nM to 100pM over 1 hour post-infusions (results pending). Since cNP8 contains an RWRPK sequence that strongly vasodilates the microvasculature at low levels, we posit that this is also the mechanism of action for cNP8 IV therapy (see **Figure 5**).

In contrast to IV therapy, we believe that **topical cP12 and cNP8** promote wound healing through fibroblast survival and growth at 1μM - 100μM as has been shown for both **cP12** (*J Invest Dermatol* 134:1119-1127, 2014; *J Invest Dermatol* 134:921-929, 2014) and **cNP8** (*J Invest Dermatol* 138:2480-2483, 2018; *J Invest Dermatol* 140:1480-1483, 2020). At μM doses cP12 also promotes angiogenesis for P12/cP12 (Poster MHSRS-22-04958). Given the sequence similarities of cNP8 to cP12, we propose that cNP8 will also stimulate angiogenesis at μM doses.

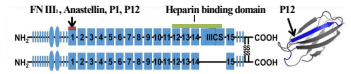
cNP8 milestones

1. Preclinical safety and efficacy complete for IV therapy
2. Manufacturing cNP8 Drug Product complete
3. Stability of cNP8 Drug Product ongoing
4. cNP8 for IV therapy IND submission scheduled for late 2022.
5. cNP8 for topical therapy awarded 2022 by Joint Warfighter Medical Research Program (JWMRP).

Results

Figure 1

Bioactive peptides are nested within the 1st type three repeat of FN



Lin et al. *J Invest Dermatol*. 134:1119-1127, 2014

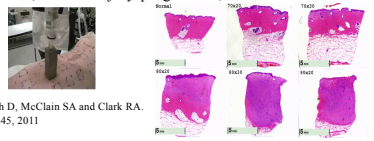
Table 1

P12 is cryptic within the 1st type three repeat of FN (FNIII$_1$) and cNP8 was engineered from FNIII$_1$

Peptide	Sequence
FNIII$_1$	GPVEVFITETPSQPNSHPIQWNAPQ*PSHISKYILRWRPK*NSV *GRWKEAT*IPGHLNSYTIAGLKPGVVYEGQLISIQQYGHQEVT RFDFTTTSTSTPVTSNTVTGETTPF
To cyclize, cP12 has a peptide-bond connecting its terminal ends.	*PSHISKYILRWRPK* *JID*. 134:1119-1127, 2014
To cyclize, the engineered cNP8 has a peptide-bond connecting its terminal ends	*HIGKYGLRWRPKGSV* *JID* 140:1480-1483, 2020

Figure 2

Porcine, vertical-injury-progression, burn model



Singer AJ, Hirth D, McClain SA and Clark RA. *JBCR* 32:638-645, 2011

Over a decade ago, we developed a porcine model that mimic burn conversion in humans, i.e. conversion of a deep dermal burn to a full thickness burn. Using an aluminum bar we found that an 80° C bar held on the back of 25-30kg outbred Yorkshire pig caused a deep dermal burn that often (70%) became full thickness. Using this model we found that red blood cell aggregates in the peri-burn area, not microthrombi, appeared to cause burn conversion, (see **Figure 4**)

Figure 3

Discovery Paradigm

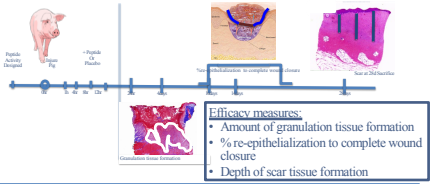
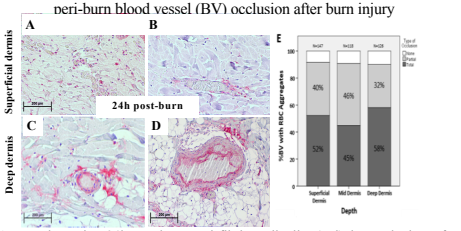


Figure 4

Red blood cells (RBC) aggregates, not microthrombi, are the main cause of peri-burn blood vessel (BV) occlusion after burn injury

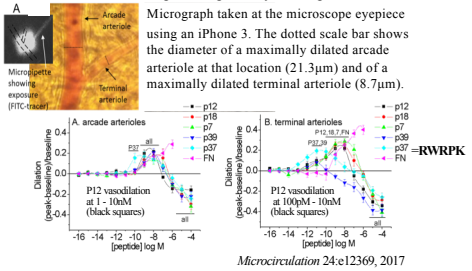


A-E. In burn sites 24h post-burn, anti-fibrin antibodies (red) detected wisps of fibrin around blood vessels (BVs) and occasional as strands along the lumina periphery. However, BVs appeared to be occluded by RBCs aggregates. By 48h most BVs were still occluded with RBC aggregates (not shown).
Wd Repair Regen. 24:501-513, 2016

Results

Figure 5

P12 and its fragments vasodilate arcade and terminal arterioles of the hamster cheek pouch optimally at 100pM to 10nM

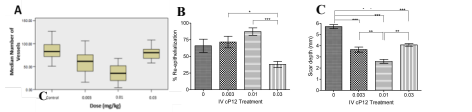


Micrograph taken at the microscope eyepiece using an iPhone 3. The dotted scale bar shows the diameter of a maximally dilated arcade arteriole at that location (21.3μm) and of a maximally dilated terminal arteriole (8.7μm).

Microcirculation 24:e12369, 2017

Figure 6

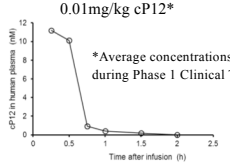
Cyclized P12 (cP12) reduces RBC occlusion of periburn BV, speeds healing, and attenuates scarring in porcine burns



A. After optimal cP12 IV infusion (0.01mg/kg) at 4h post-burn, RBC aggregates occluding BVs are markedly reduced at 24h post-burn; **B.** At 14d post-burn, re-epithelialization is significantly increased; and **C.** At 28d scarring is significantly reduced. Thus, decrease in RBC aggregates correlates with an increase in re-epithelialization and decrease in scarring. *Wd Repair Regen.* 24:501-513, 2016

Figure 7

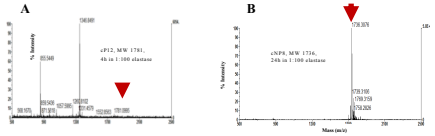
Human Pharmacokinetics after 30min intravenous (IV) infusion of 0.01mg/kg cP12*



After a 30min IV infusion of 0.01mg/kg cP12, plasma cP12 levels ranged from 430pM to 11nM over a 1hour post-infusion time in normal healthy adult humans (Phase 1 Clinical Study) and juvenile Yorkshire pigs (not shown). Thus, the optimal cP12 dose that reduces burn microvasculature occlusion with aggregated RBCs at 24 hours post-burn, that speeds re-epithelialization at 14d post-burn in the porcine burn model results from a cP12 plasma concentration that also causes microvascular BV dilation in the hamster cheek pouch model. Thus, we believe the primary mechanism of cP12 IV infusion is dilation of the peri-burn microvascular BVs, which clears RBCs aggregates.

Figure 8

cP12 is elastase sensitive while cNP8 is elastase resistant



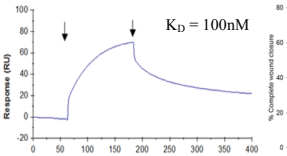
A. cP12 is completely digested by 1:100 human neutrophil elastase in PBS at 37°C for 4h. This endopeptidase is abundant in wounds by 4h post-injury. **B.** cNP8 resists 1:100 elastase in PBS at 37°C for 24h.
J Invest Dermatol 140:1480-1483, 2020

Results

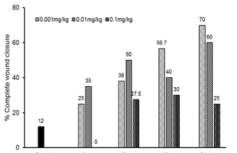
Figure 9

Cyclized NP8 (cNP8) binds PDGF-BB and speeds wound closure in porcine burns when infused 4-24h post-burn

A. cNP8 binds PDGF-BB



B. cNP8 promotes wound closure

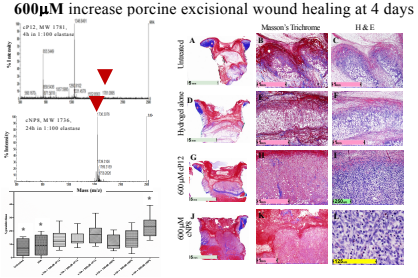


A precursor of cNP8 was discovered in an elastase digest of FNIII1(*J Invest Dermatol* 138:2480-2483, 2018). We used amino acid substitution to make cNP8 completely elastase resistant (see **Figure 8B**) and reference below). **A.** Using plasma resonance technology we found cNP8 binds PDGF-BB similarly to cP12. **B.** In addition, cNP8 promoted 14d wound closure best when administered by IV infusion over 30min beginning 8-24h post-burn*.

* *J Invest Dermatol* 140:1480-1483, 2020

Figure 10

Topical cP12 (elastase sensitive) and cNP8 (elastase resistant) at **100 - 600μM** increase porcine excisional wound healing at 4 days



Peptide diffusion from HA into wounds over 12h would include wound concentrations in 10 – 100μM range consistent with active *in vitro* concentration range (10 – 100μM). *Wd Repair Regen* 27:634-649, 2019

Clinical Use of cP12 and cNP8

Dose Range	Mechanism	Administration	Clinical Benefit
1μM - 100μM	Cell survival & growth	Topical cNP8	Speed healing
100nM - 10μM or higher	Bacteriostatic - Bacterial killing	Topical cP12 or cNP8	Decrease infection
10pM - 10nM	MicroBV dilation & tissue survival	IV infusion cP12 or cNP8	Tissue salvage

Summary and Conclusion

1. FN peptides cP12 at 0.01mg/kg or cNP8 at 0.001-0.01mg/kg, infused IV into porcine burn models within 1-4h and 8-24h post-burn, respectively, speed wound closure. cP12 clears RBC aggregates from peri-burn microvasculature. Since the sequence of cNP8 is similar to cP12, including presence of the vasoactive RWRPK peptide, we posit cNP8 has the same ability, as cP12, to clear aggregated RBCs from peri-burn BVs.
2. When cP12 or cNP8 are applied topically to wounds at 100 to 600μM, they promote healing likely by increasing angiogenesis and mesenchymal cell survival and growth at tissue concentrations of 10 to 30μM after 24h diffusion. cNP8 is more robust for topical therapy secondary to its resistance to elastase.

Acknowledgements

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