

Protocol #0901-966: A Prospective, Randomized, Controlled, Multicenter, Unblinded, Safety and Early Efficacy Trial of ExpressGraft_{Enhance} Skin Tissue Versus Wet to Dry Dressings in the Treatment of Recently Occurring, Non-Infected, Foot Ulcers in Diabetic Patients.

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and Public Health

Focus: Major RAC Questions

- Introduction
- Preclinical comments
- Clinical comments

Stratatech's human skin substitute tissues

- **NIKS® cells** →
- **Genetically enhanced NIKS® cells** →

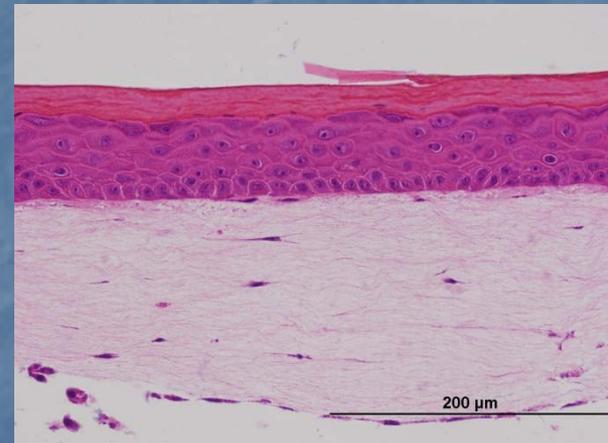
- Proprietary human epidermal progenitors
- FDA CMC testing complete
- Master & working cell banks in place

Epidermal layer:
NIKS® cells
+
Dermal layer:
human fibroblasts
and collagen

Cultured under
proprietary conditions



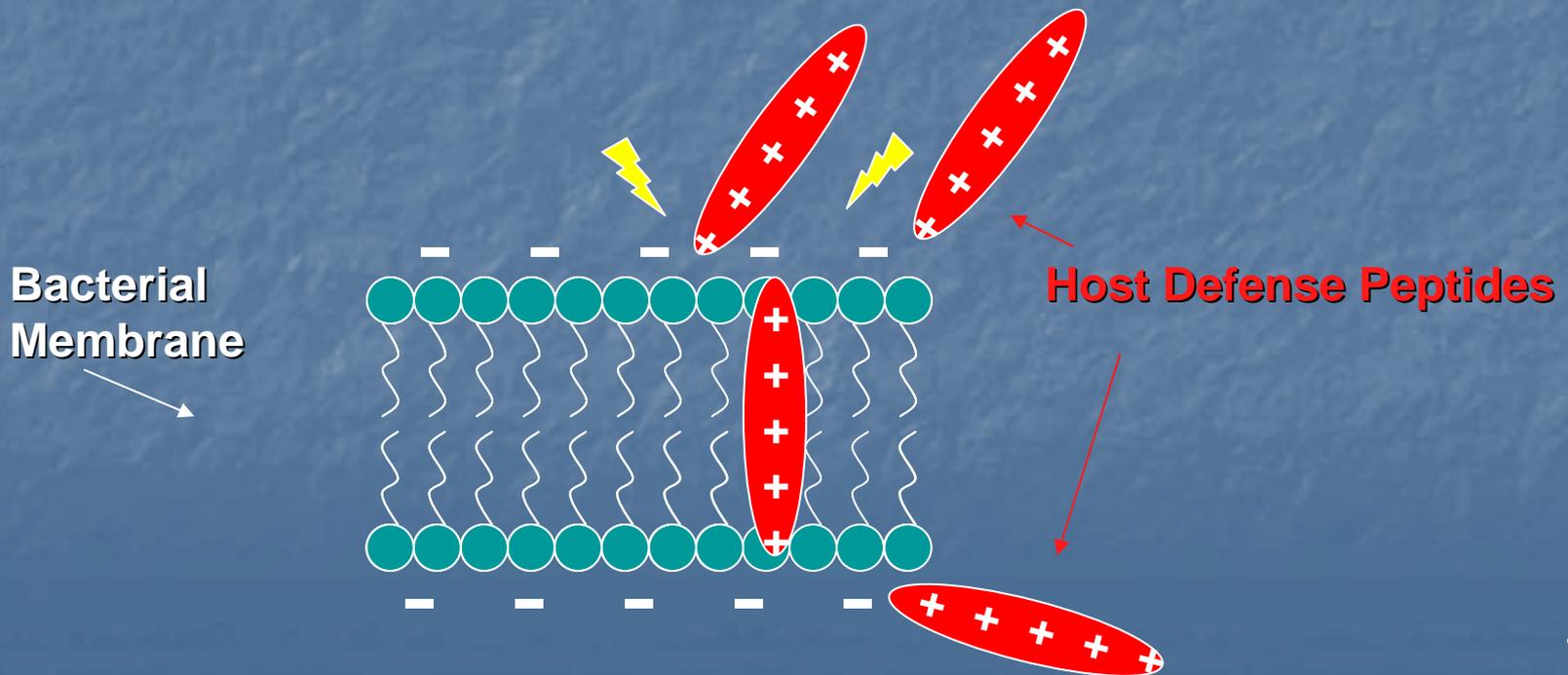
Fully developed multi-layered human skin
Physical barrier present
Biologically active
Strong, durable, suturable



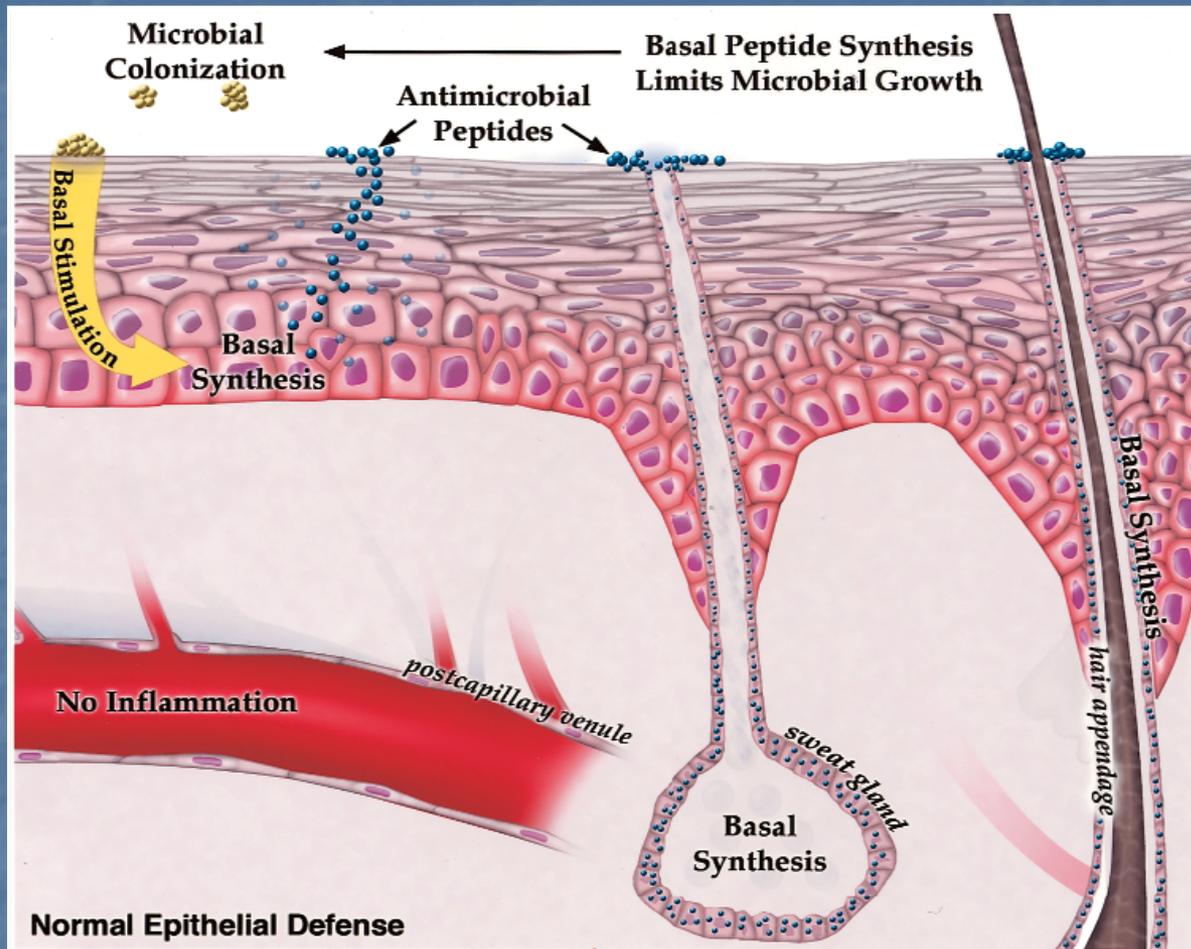
StrataGraft®
ExpressGraft® pipeline

ExpressGraft[®]: cathelicidin

- Broad spectrum and fast acting against Gram positive and Gram negative bacteria, yeast, fungi, viruses
- Produced by phagocytic leukocytes, mucosal epithelium, keratinocytes
- Induced by 1,25-vitamin D3, cytokines, bacterial components, and keratinocyte differentiation
- Suppressed levels in burn wound fluid and diabetic ulcers



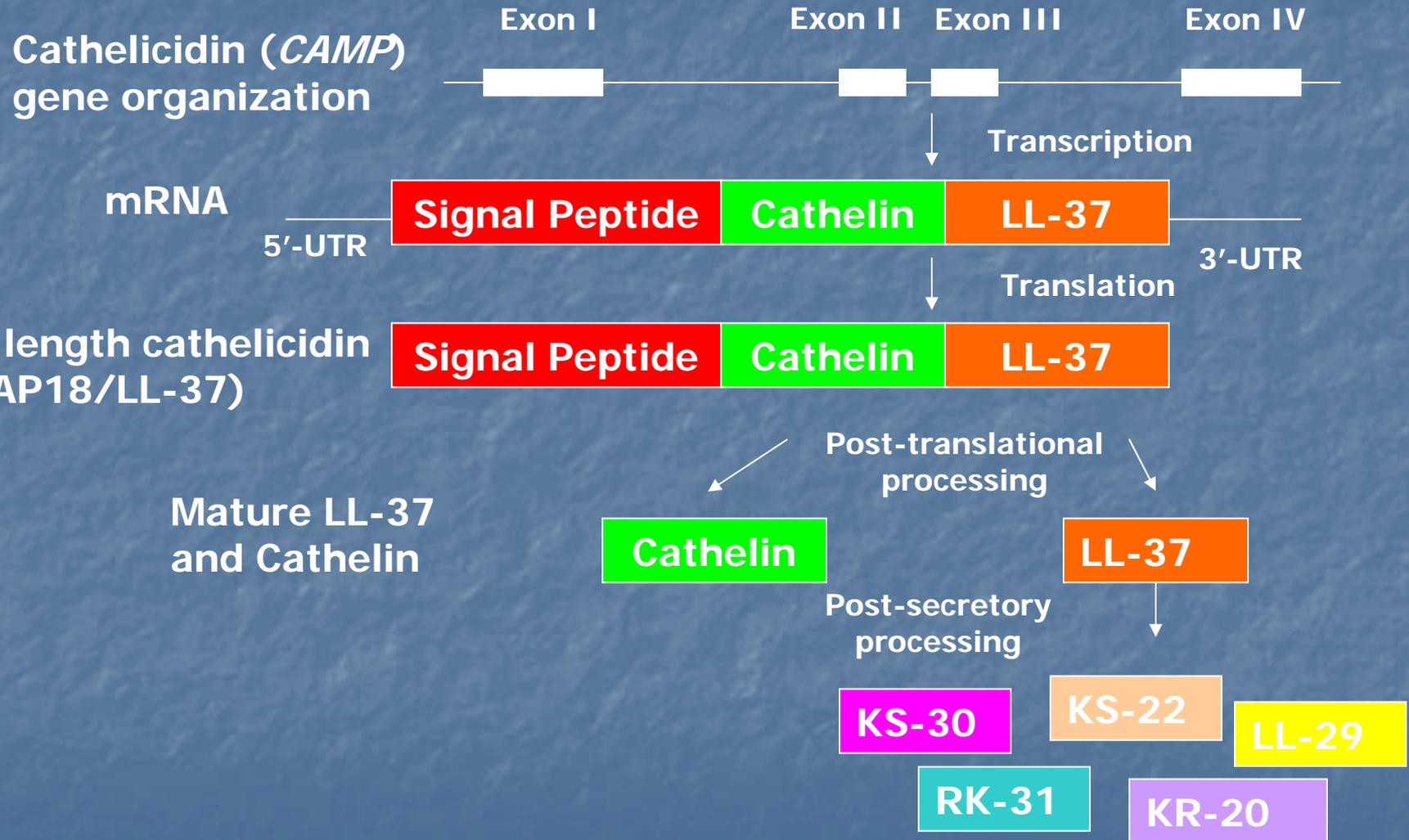
Cathelicidin in human tissues



J Allergy Clin Immunol. 110(6):823-31, 2002

Normal flora chronically stimulate epithelial surfaces to express host defense peptides.

Post-translation modifications activate human cathelicidin



Biological activities of LL-37

(Adapted from Nijnik and Hancock, *Current Opinions in Hematology* 16:41, 2009)

Activity	Description	Citation
Microbicidal activity	Acts on broad spectrum pathogens	Bowdish et al, J Leukoc Biol, 77:451, 2005; Durr, et al., BBA 1758:1408, 2006
Inhibits biofilm formation	Inhibits <i>P. aeruginosa</i> biofilms	Overhage et al., Infect Immun 76:4176, 2008
Induces virulence genes	Induces group A <i>Streptococcus</i> resistance to opsonophagocytic killing by leukocytes	Gryllos et al., PNAS 105:16755, 2008
Chemotaxis	Acts as chemokine for neutrophils, monocytes, mast cells, T cells	Soehnlein et al., Blood 112:1461, 2008
Mast cell degranulation	Release histamines, prostaglandins from mast cells	Niyonsaba et al., Eur. J. Immunol 31:1066, 2001
Induction of immune mediators	Induces chemokines in monocytes, IL-8 in keratinocytes	Braff et al., J. Immunol. 174:4271, 2005
Regulation of inflammation	Suppresses LPS-induced inflammatory cytokines	Lande et al., Nature 449:564, 2007
Apoptosis	Induces neutrophil secondary necrosis	Zhang et al., J. Leuko. Biol. 84:780, 2008
Wound healing	Promotes keratinocyte migration and wound healing	Carretero et al., J. Invest. Dermatol. 128:223, 2008
Angiogenesis	Promotes vascularization by effects on vascular endothelium	Kuczulla et al., J. Clin. Invest. 111:1665, 2003

ExpressGraft_{Enhance} tissue

UbC Pro	Bsd	Inv Pro	hCAP-18	globin
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Mammalian hCAP-18/LL-37 expression fragment

- Funded in partnership with NIDDK
- Stably transfected, clonal cell line
- ~20 copies stably integrated on chr 19q
- Non-tumorigenic, karyotypically stable
- Master and working cGMP cell banks
- hCAP-18/LL-37 concentration in tissue
 - By volume: 1.8 μ M (9 μ g/ml)
 - By surface area: 227 ng/cm²

Preclinical comments

- Biological activities ExpressGraft_{Enhance}
 - ExpressGraft_{Enhance} tissue viability
 - hCAP-18/LL-37 in circulation
- Neutrophils and ExpressGraft_{Enhance} tissue
 - Secondary necrosis
- Antimicrobial activity against DFU pathogens
 - *Acinetobacter baumannii*
 - Group A *Streptococcus*

Viability of ExpressGraft_{Enhance} tissue

- Lot release testing
 - Ensures tissue is viable and metabolically active
 - Comparable to StrataGraft[®] tissue
- Nude mouse grafting experiments
 - Viable human keratinocytes in engrafted StrataGraft[®] tissue
 - Normal morphology, MHC expression for > 3 months
- Human trial of StrataGraft[®] tissue
 - Tissue viable 1 week after placement in wound bed
 - Viability was more consistent than cadaver allograft
- Viability of ExpressGraft_{Enhance} tissue
 - In the context of diabetic foot ulcer (DFU) environment remains to be determined

Preclinical evaluation: circulating levels of transgene-derived hCAP-18/LL-37

Proposed Preclinical Subchronic Toxicity and Biodistribution Study

- 24 nude mice will be grafted with ~25% TBSA of ExpressGraft_{Enhance} tissue.
- Study endpoints at 1, 2, and 8 weeks after engraftment of ExpressGraft_{Enhance} tissue.
- Necropsy to identify local or systemic toxicity.
- Quantify serum hCAP-18/LL-37 levels by ELISA.
- Detection of transgene DNA by PCR in grafted area and distant organs.

Clinical evaluation: circulating levels of transgene-derived hCAP-18/LL-37

- Serum level of endogenous hCAP-18/LL-37 is 1,200 ng/ml
- ExpressGraft_{Enhance} tissue contains 227 ng/cm²
 - Maximum exposure from single application
 - < 0.09% TBSA, 16.3 cm² = 3,700 ng
 - 3,700 ng of hCAP-18/LL-37 would increase serum levels by only ~ 1 ng/ml, which is ~ 0.1% increase over baseline.
- Proposed clinical study
 - Patient serum samples will be collected for determination of serum hCAP-18/LL-37 levels by ELISA.

Preclinical comments

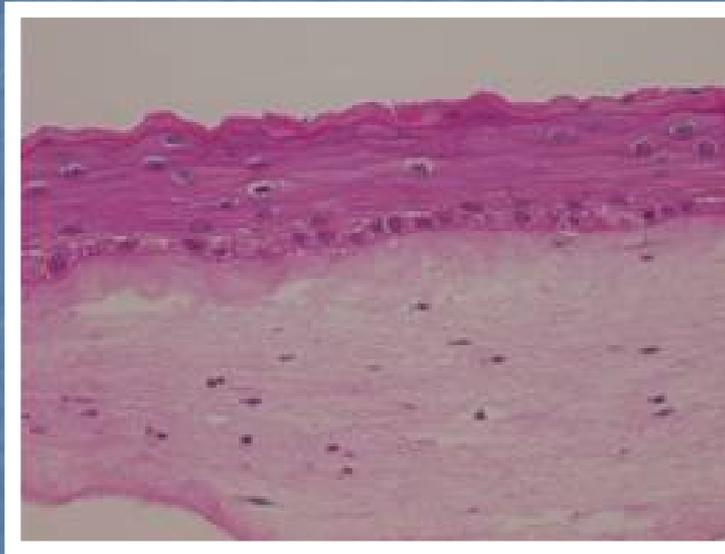
- Biological activities ExpressGraft_{Enhance}
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LL-37 and neutrophils

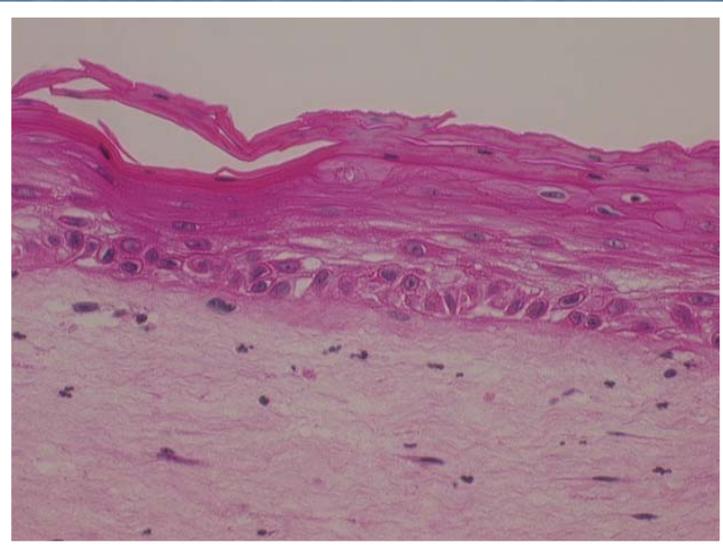
- LL-37 is chemotactic for neutrophils and induces secondary necrosis of annexin V-positive neutrophils *in vitro*.
 - Secondary necrosis of apoptotic cells occurs in settings where normal clearance mechanisms of apoptotic cell fragments are overwhelmed or deficient.
 - Necrosis of neutrophils could potentially affect viability of adjacent tissue and wound healing.
- No difference in neutrophil infiltration with ExpressGraft_{Enhance} compared to StrataGraft®
 - Diabetic rat model
 - Murine burn model of infection

LL-37 and neutrophils

StrataGraft®



ExpressGraft_{Enhance}



Tissue analyzed after removal from murine burn model of infection

Preclinical comments

- Biological activities ExpressGraft_{Enhance}
 - ExpressGraft_{Enhance} tissue viability
 - LL-37 in circulation
- Neutrophils and ExpressGraft_{Enhance} tissue
 - Secondary necrosis
- Antimicrobial activity against DFU pathogens
 - *Acinetobacter baumannii*
 - Group A *Streptococcus*

ExpressGraft_{Enhance} is antimicrobial against *Acinetobacter baumannii* in vivo

- Clinical

- A

- A

- a

- Murine

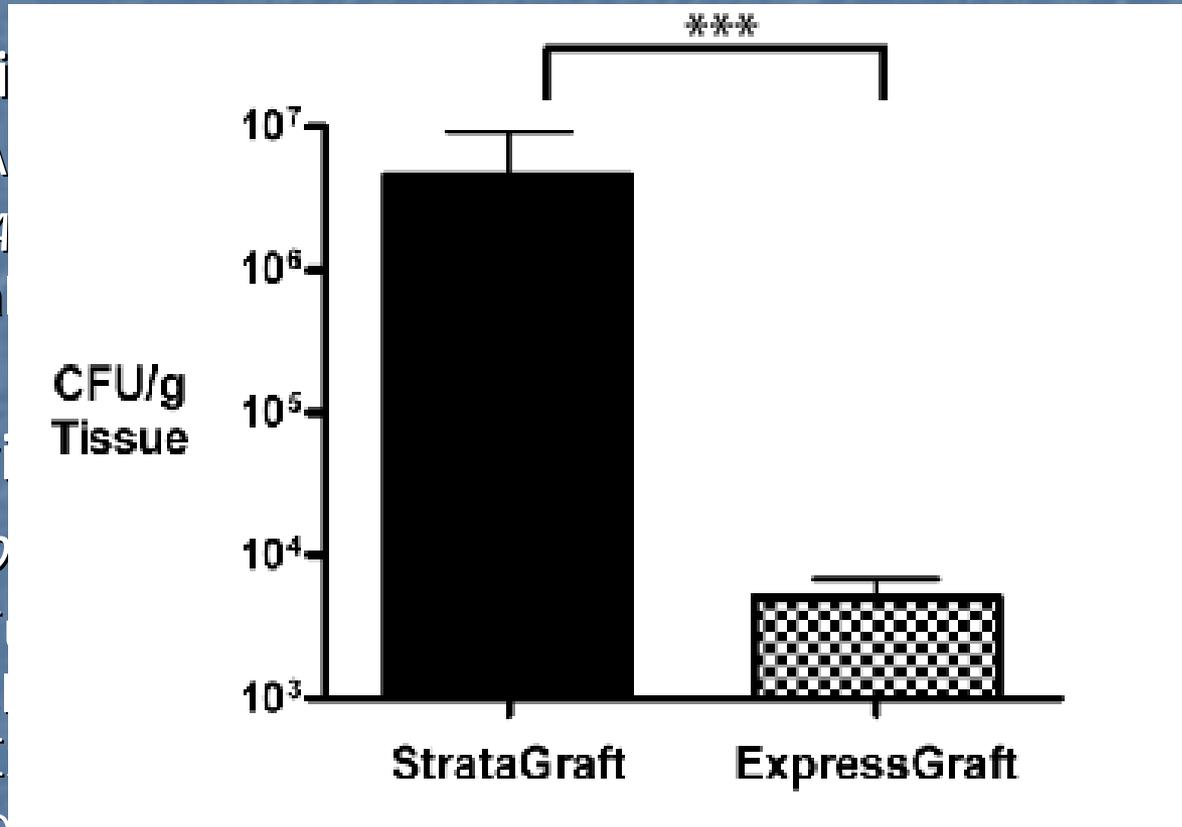
- *A. b*

- F

- c

- E

- Quantitative bacterial cultures after 72 hr



s (Ge et

resistant

Molecular Therapy, 2009

Group A *Streptococcus*

- Asymptomatic colonization
 - Oropharynx of 15-20% of children
- Self-limited infections
 - Streptococcal pharyngitis
- Invasive infections
 - Cellulitis
 - Necrotizing fasciitis
- Streptococcal toxic shock syndrome
- Diabetic foot ulcers (DFU)
 - Not common pathogen in DFU
 - Identified in only 6 of 812 wound cultures (<1%), Ge et al., 2002

Cathelicidins and Group A *Streptococcus*

- Cathelicidin deficient mice (Nizet et al., 2001)
 - Larger wounds in response to Group A Strep
- Transgenic mice with cathelicidin targeted to skin (Lee et al., 2005)
 - Smaller ulcers after subdermal Group A Strep injection
 - 60% fewer surviving bacteria
- Antimicrobial activity against Group A Strep (Dorschner et al., 2001)
 - 1-2 μM LL-37 inhibits growth *in vitro*
 - ExpressGraft_{Enhance} tissue contains 1.8 μM hCAP-18/LL-37

LL-37 and Group A Strep virulence genes

- Bioactive small molecules exhibit hormesis
 - Many classes of bioactive molecules represented
 - Subinhibitory concentrations of antibiotics are potent modulators of bacterial transcription (Yim et al., 2006)
- Sub-inhibitory concentrations of LL-37 (100 nM) *in vitro* (Gryllos, 2008)
 - Induced expression of virulence factors
 - Increased resistance to phagocytic killing by leukocytes
- hCAP-18/LL-37 in ExpressGraft_{Enhance} is 1.8 μM
 - MIC for Group A Strep is 1-2 μM
 - Human sweat is $\sim 1 \mu\text{M}$
- Pre-IND FDA discussion included augmenting pre-clinical program
 - In vitro studies on Group A Strep
 - Animal model

Clinical comments

- Modified clinical trial design
- Immunogenicity of ExpressGraft_{Enhance} tissue
 - Mixed lymphocyte response (MLR) assay
 - Panel reactive antibodies
- Stopping rules

StrataGraft[®] clinical trial overview

Phase I/IIa clinical trial – complex skin defects (15 patients with $\geq 5\%$ TBSA)

- Unmodified NIKS[®] cells
- Funded in partnership with NIAMS
- Compare to standard of care (cadaver allograft) prior to autograft
- Evaluate safety of StrataGraft[®] skin tissue
 - Dose escalation
 - Early efficacy

Primary endpoints met

- Autograft take @ 2 wks post placement
- Wound appearance, graft take

Results

- No product-related AE or SAE
- *In vitro* immunology assessments show no difference from control
 - Mixed lymphocyte response
 - Cytotoxicity
 - Panel reactive antibodies
- *In vivo* immunology assessments showed mild inflammatory infiltrate in both groups

StrataGraft[®] Trial Demographics

	Age (yr)	Gender	% TBSA	MOI	%TBSA
	64	M	17.5	MVC - burn	0.3
	65	F	6	Thermal burn	0.3
	52	M	16	Thermal burn	0.3
	21	M	13	Thermal burn	0.3
	40	M	5	MVC	0.6
	52	F	8	Necrotizing Fasciitis	0.6
	66	M	25	Explosion - burn	0.6
	46	F	15	Necrotizing Fasciitis	0.9
	29	M	73	Explosion - burn	0.9
	38	M	12	Explosion - burn	0.9
	48	F	13	Thermal burn	0.9
	20	M	17.5	Electrical burn	1.2
	34	M	42.5	Explosion - burn	1.5
	38	M	31	Thermal burn	1.5
	38	M	41.5	Thermal burn	1.5
mean ± sd	43.4 ± 14.7	73.3% M 26.7% F	22.4 ± 18.2		0.82 ± 0.45 23

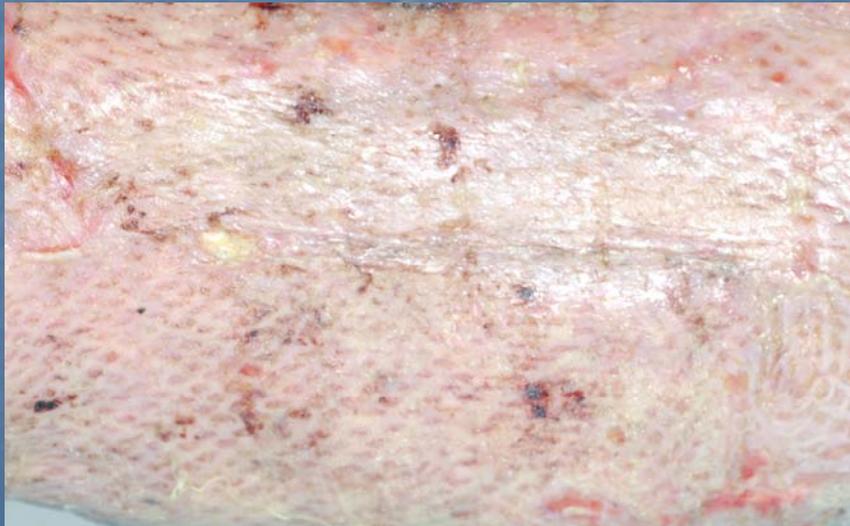
StrataGraft[®] clinical trial results



Cadaver allograft, day 7



StrataGraft[®], day 7



Cadaver allograft, 2 weeks p STSG



StrataGraft[®], 2 weeks p STSG

Modified ExpressGraft_{Enhance} trial design

- Patient cohort 1- patient safety
 - Single application of ExpressGraft_{Enhance}
 - Interim safety report and review
- Patient cohort 2- safety and early efficacy
 - Multiple applications of ExpressGraft_{Enhance}

Draft informed consent

- Patients must provide own informed consent
- Wording of patient sample storage has been modified to be more clearly defined
- Potential patient risk associated with exposure to cellular components have been more clearly stated
- Finalized following NIH, FDA and IRB input

Clinical comments

- Modified clinical trial design
- Immunogenicity of ExpressGraft_{Enhance} tissue
 - Mixed lymphocyte response (MLR) assay
 - Panel reactive antibodies
- Stopping rules

Immunogenicity of ExpressGraft_{Enhance}

- ExpressGraft_{Enhance}
 - hCAP-18/LL-37 expressed by human keratinocytes
 - Allogeneic tissue
- Expect ExpressGraft_{Enhance} will be replaced by the patient keratinocytes
 - Bioengineered cell-based skin therapies do not elicit acute allogeneic responses but are replaced by autologous cells
 - Therapeutic delivery of hCAP-18/LL-37 would be limited temporally
- Three methods used to assess immunogenicity of StrataGraft[®] are proposed for ExpressGraft_{Enhance}
 - Proliferation assay (mixed leukocyte reaction)
 - Cytotoxicity assay
 - Panel reactive antibodies

NIKS[®] cells express MHC class I but not co-stimulatory molecules

- *In vitro* data with high doses of IFN- γ
 - No detectable B7-1 or B7-2
- *In vivo* clinical trial data from StrataGraft[®]
 - No upregulation of MHC class II
 - No marked inflammatory infiltrate
 - T cells (CD3)
 - B cells (CD20)
 - Langerhans cells (CD1a)
 - No evidence of sensitization in patient PBMC
 - *In vitro* proliferation assay
 - Cytotoxicity assay
 - No evidence of antibody generation in response to NIKS cells

Proliferation Assay (MLR)

- Patient PBMC mixed with irradiated:
 - NIKS^{hCAP-18/LL-37} cells
 - Allogeneic PBMC
 - Autologous PBMC
 - Or medium alone
- Proliferative response of patient PBMC based on ³H-thymidine uptake
- Baseline values can be compared to those obtained after ExpressGraft_{Enhance} exposure and examined for enhanced proliferative responses to the NIKS^{hCAP-18/LL-37} cells

Panel Reactive Antibodies (PRA)

- Assesses allosensitization to ExpressGraft_{Enhance}
- Data from the clinical trial of StrataGraft[®] show:
 - One patient of 15 developed an antibody response to an allele on NIKS[®] cells
 - Reactivity against only one of the alleles expressed on NIKS[®] cells
 - Also developed reactivity toward additional alleles **not** expressed on NIKS[®] cells
 - Patient in low dose cohort
 - Patient received multiple blood transfusions
 - This coincidental reactivity is not indicative of enhanced sensitization to NIKS[®] cells

Clinical comments

- Clinical trial design
 - Patient eligibility
 - Informed consent
- Immunogenicity of ExpressGraft_{Enhance} tissue
 - Mixed lymphocyte response (MLR) assay
 - Panel reactive antibodies
- Stopping rules

Modified stopping rules

- Patient enrollment will be stopped and enrolled study subjects may discontinue study treatment if the following AEs are determined to be related to ExpressGraft_{Enhance} skin tissue exposure.
 - Serious skin infection requiring hospitalization and intravenous antibiotic
 - Grade 4 necrotic tissue or gangrenous tissue
 - Acute serious hypersensitivity reaction
 - Systemic responses such as anaphylaxis or sudden acute unrelievable pain
 - Amputation or death

Modified stopping rules

- After treatment of the initial patient cohort enrollment will not continue until a comprehensive safety review is completed and approved by the safety monitoring committees (e.g., IRBs, FDA, NIH RAC)

Acknowledgements

NIH Recombinant DNA Advisory Committee Members and Staff

John Centanni, MS, Associate Director Regulatory Affairs, Stratatech

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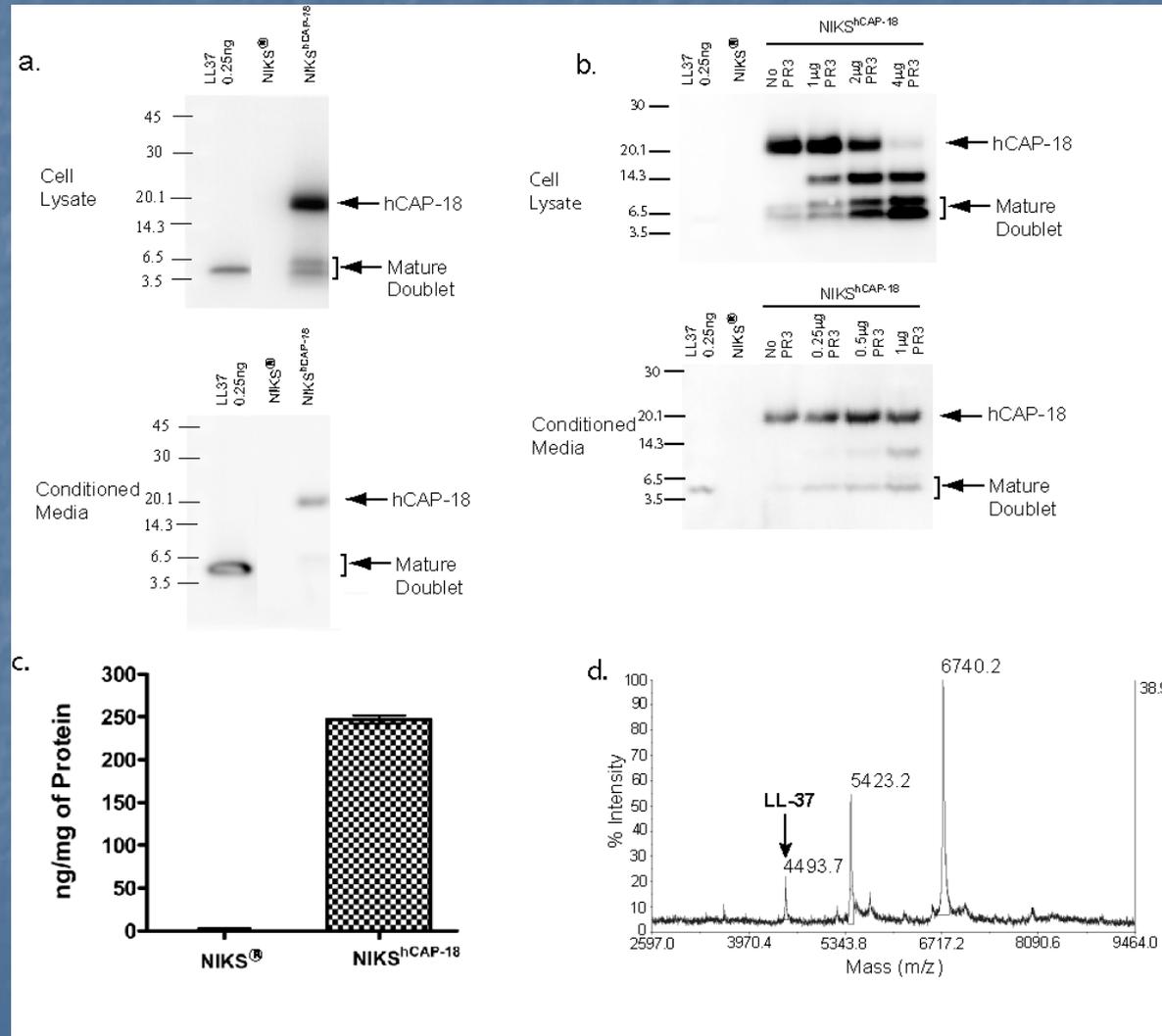
Discussion of Protocol #0901-966 :

A Prospective, Randomized, Controlled, Multicenter, Unblinded, Safety and Early Efficacy Trial of ExpressGraft_{Enhance} Skin Tissue Versus Wet to Dry Dressings in the Treatment of Recently Occurring, Non-Infected, Foot Ulcers in Diabetic Patients.

Clinical classification of a diabetic foot infection

Clinical manifestations of infection	Infection severity	PEDIS grade
Wound lacking purulence or any manifestations of inflammation	Uninfected	1
Presence of ≥ 2 manifestations of inflammation (purulence, or erythema, pain, tenderness, warmth, on induration), but any cellulitis/erythema extends ≤ 2 cm around the ulcer, and infection is limited to the skin or superficial subcutaneous tissues; no other local complications or systemic illness.	Mild	2
Infection (as above) in a patient who is systematically well and metabolically stable but which has ≥ 1 of the following characteristics: cellulitis extending > 2 cm, lymphangitic streaking, spread beneath the superficial fascia, deep-tissue abscess, gangrene, and involvement of muscle, tendon, joint or bone.	Moderate	3
Infection in a patient with systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, severe hyperglycemia, or azotemia)	Severe	4

hCAP18 is processed to active forms (LL37)



Clinical trial flow chart

