Gentamicin Therapy Induced Functional Type VII Collagen in RDEB Patients Harboring Nonsense Mutations

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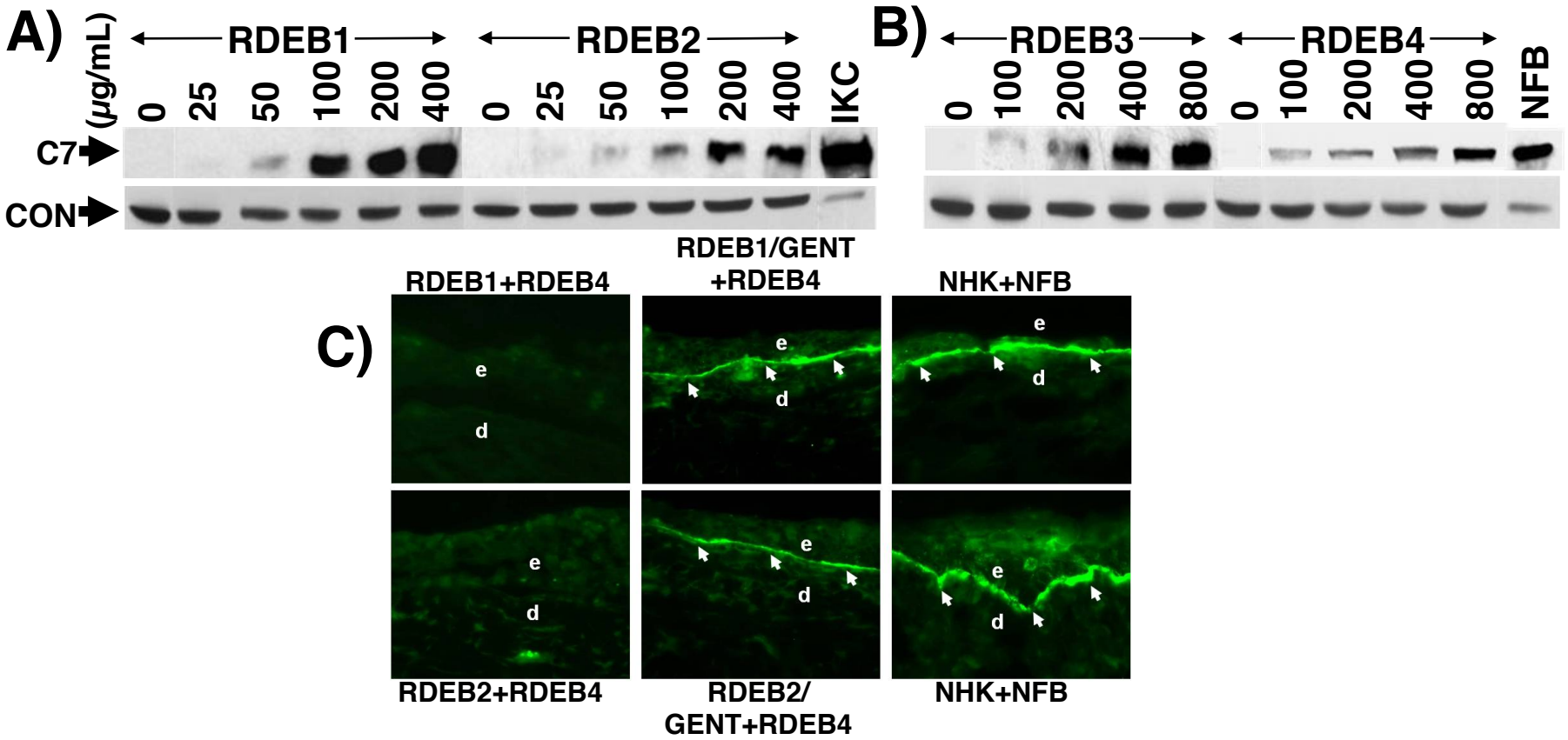
Conflicts of Interests

We have no Conflict of Interest.

Gentamicin Induced Functional Full-length Type VII Collagen in RDEB Cells



RDEB1: R578X/Q906X; RDEB2: Q251X/Q251X; RDEB3:R578X/R578X; RDEB4:R613X/R1683X

Cogan et al, Mol. Ther. 2013

OBJECTIVES

- **The primary objectives for this double-blind, placebo-controlled study were:**

  1) The detection of new type VII collagen and anchoring fibrils in the patients' dermal-epidermal junction.

  2) Safety parameters such as gentamicin-induced ototoxicity or nephrotoxicity and antibodies to type VII collagen.

- **A secondary objective was assessment of wound closure and new blister formation in two equal-sized, erosive Wounds treated topically.**

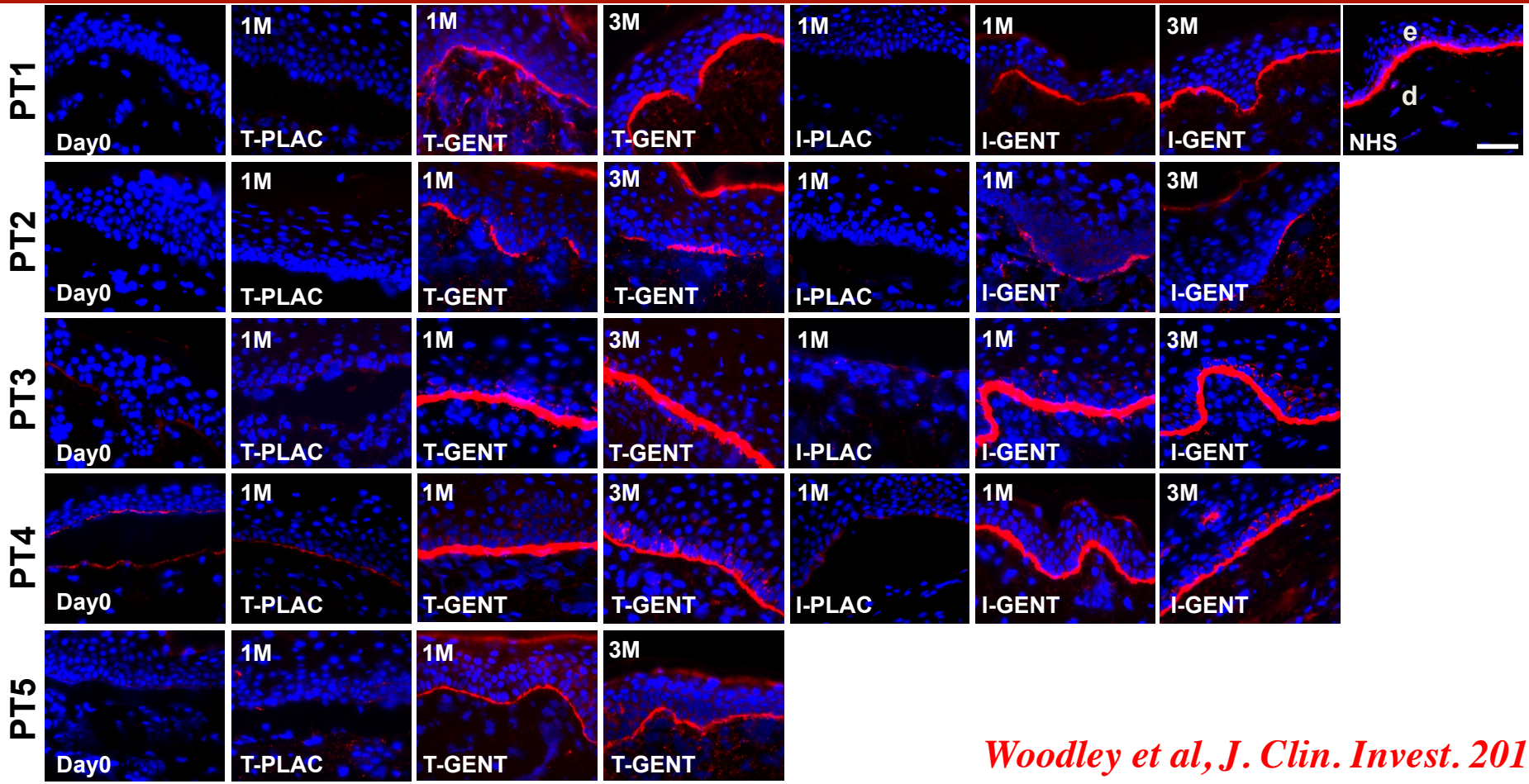
Study Protocol

- Two equal-sized wounds were treated TID with 0.1% gentamicin sulfate ointment or placebo vehicle for 2 weeks.
- Two Test Sites of intact skin received intradermal injections of gentamicin (8 mgs) or placebo for two days.
- Test Site biopsy specimens were evaluated for C7 and anchoring fibrils at day 0, months 1 and 3.
- Safety parameters (CBC, CMP, creatinine clearance, audiometry and anti-C7 antibodies) were evaluated at Day 0, months 1 and 3.
- Weekly telephone interviews were conducted to assess patient safety and the status of the Test Sites.
- Weekly patient photographs of the Test Sites and patients' daily diaries were submitted to USC.

Summary of the Mutations, C7 Expression and AFs of the Five RDEB Patients

ID	PT1	PT2	PT3	PT4	PT5	NHS
Sex	Male	Male	Female	Female	Male	
Age, y	29	16	31	26	8	
Allele1 / Allele2	**R578X** / V168GfsX12	**R578X** / **R578X**	**R2814X** / IVS17-2delA	**R236X** / IVS85-1G>A	**R613X** / **R1683X**	- / -
C7 at DEJ %	2.0	0	4.5	11.6	0.8	100
AFs	0	0	0	+	0	+++++

Topical & Intradermal Gentamicin Induced New C7



Woodley et al, J. Clin. Invest. 2017

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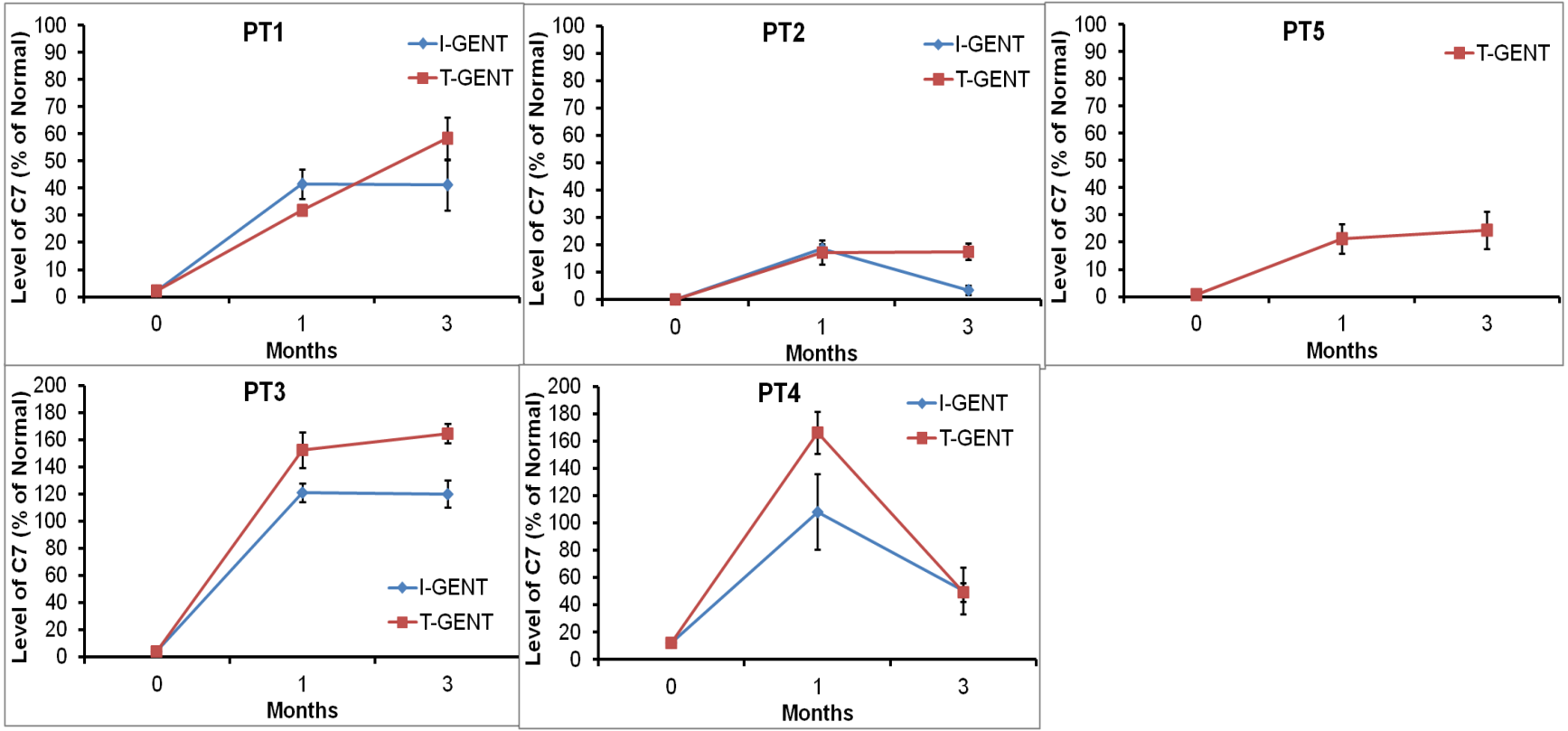
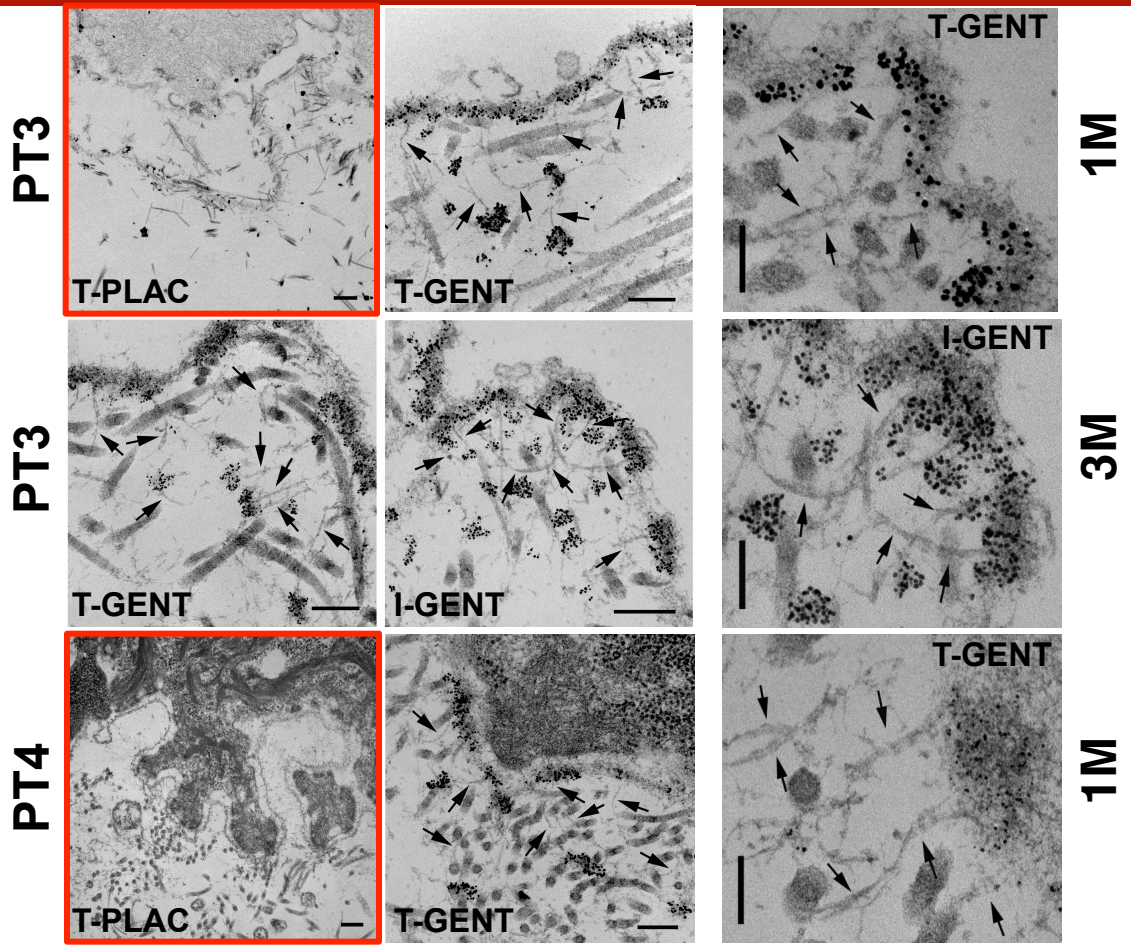
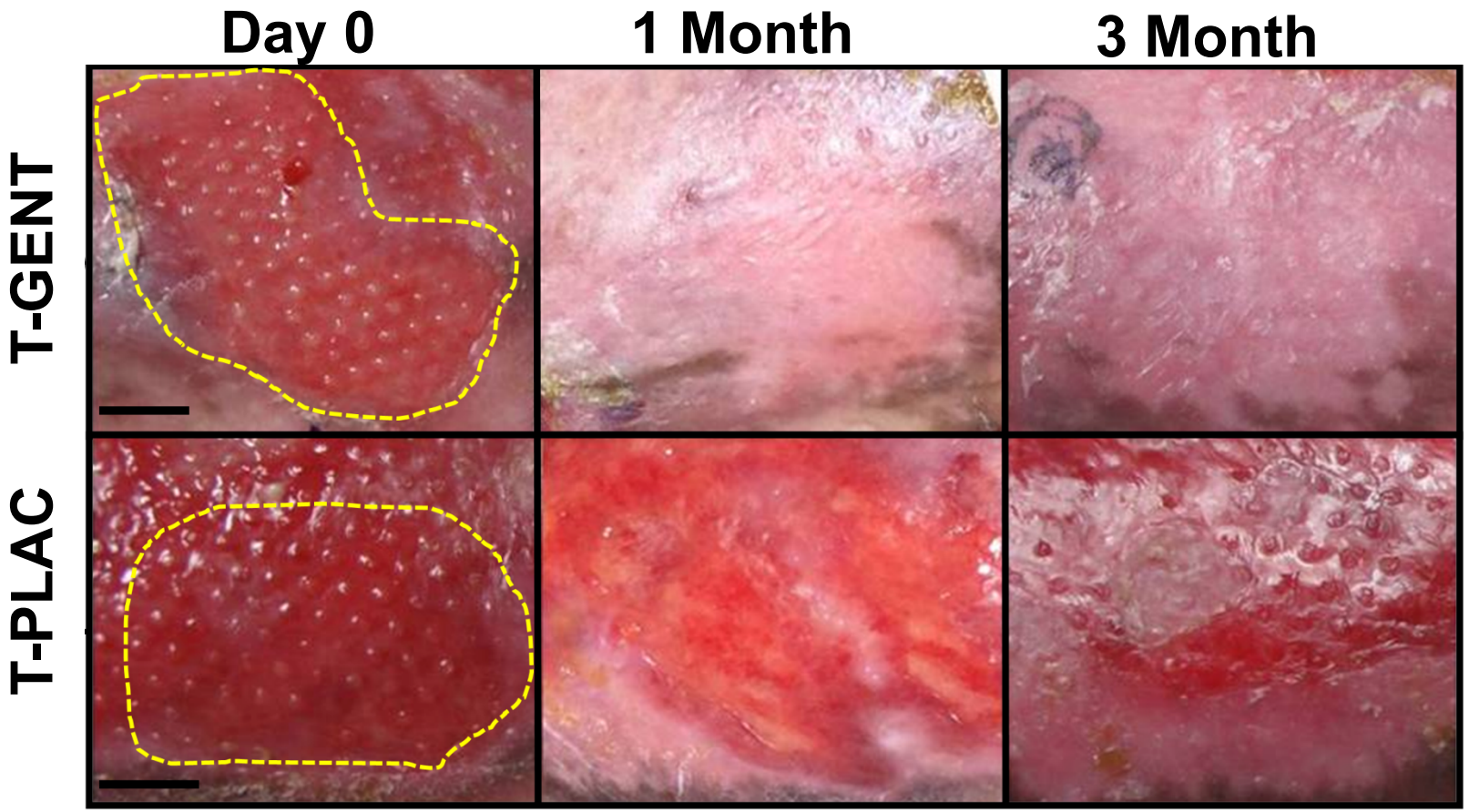


Woodley et al, J. Clin. Invest. 2017

Gentamicin Induced New Anchoring Fibrils



Topical Gentamicin Improved Wound Closure



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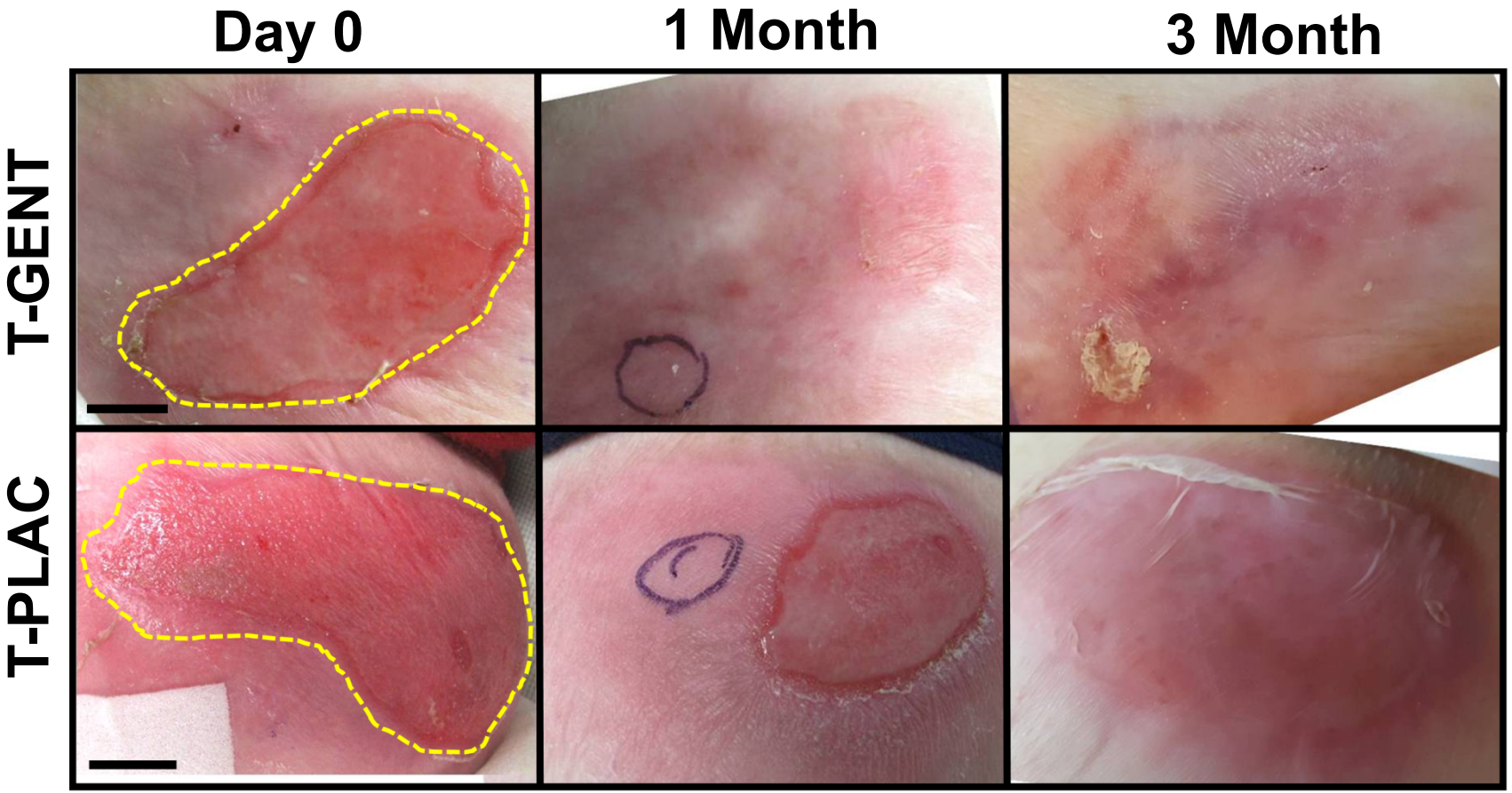


Characteristics and Clinical Assessment of the Wounds Treated with Topically in the Five Study Patients

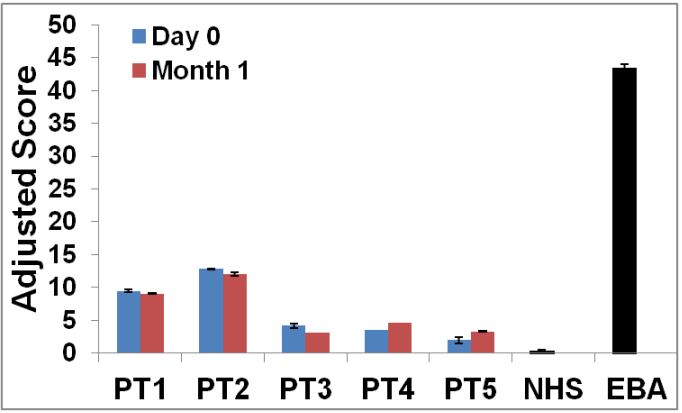
ID		PT1		PT2		PT3		PT4		PT5	
Treatment		GENT	PLAC	GENT	PLAC	GENT	PLAC	GENT	PLAC	GENT	PLAC
Location		Left Shoulder	Right Shoulder	Lower Abdomen	Right Thigh	Right Thigh	Right Thigh	Left Shoulder	Left Back	Upper Left Arm	Upper Right Arm
Treated Area		13.0	11.3	16.1	16.3	11.0	11.1	4.1	3.8	4.5	4.5
Wound History		> 3 yr	> 3 yr	1-3 yr	1-3 yr	> 3 yr	> 3 yr	1-3 yr	< 1 yr	< 1 yr	< 1 yr
Wound Closure	1M	+++	+	+++	+	+++	+	+	+++	+++	++
	3M	+++	+++	+++	+	+++	+	+	+	+++	++
Blister Events	0-1M	0	1	0	1	0	1	1	0	0	1
	0-3M	1	3	1	3	1	4	1	1	0	2

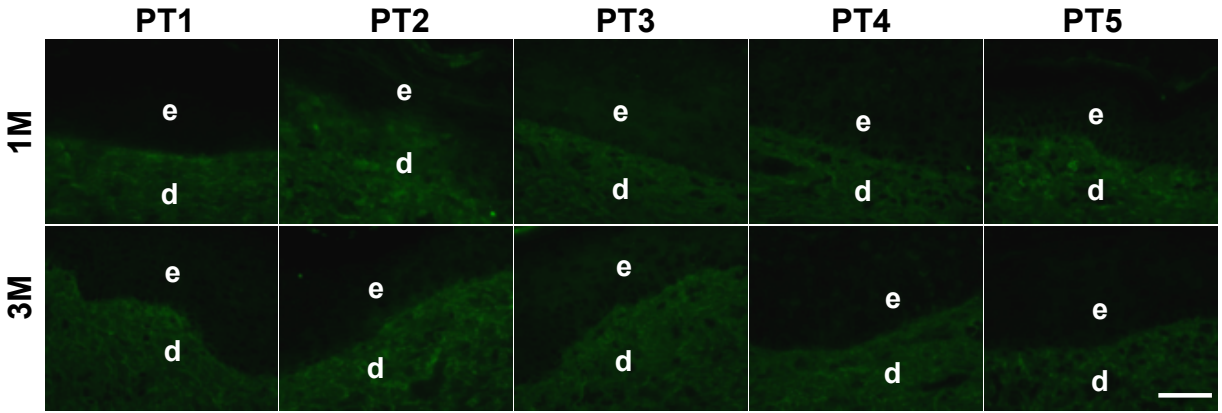
Woodley et al, J. Clin. Invest. 2017

Assessment of Safety

Safety Parameters:

complete blood counts; blood urea nitrogen; calculated creatinine clearance; liver function tests; pure-tone audiometry.





Conclusions and Implication

- Gentamicin administration created new full-length C7 and AFs in the DEJ of skin of RDEB patients with nonsense mutations.
- The restoration of C7 varied between 20% and 165% of the C7 expression in normal human skin and was durable for at least three months.
- Topical gentamicin corrected dermal-epidermal separation, improved wound closure and reduced new blister formation.
- There were no untoward side effects.
- Induction of new C7 did not generate new anti-C7 autoantibodies.

Gentamicin-mediated nonsense-suppression therapy may provide a novel, low cost, non-invasive, readily available therapy for RDEB patients harboring nonsense mutations.

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Patients and Their Families

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NIAMS

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