TRIDs4DEB collaboration: Identification of novel drug discovery starting points for the treatment of recessive dystrophic epidermolysis bullosa

Irene Jover[1], Maria C. Ramos[2], María José Escámez[3], Estrella Lozoya[1], José R. Tormo[2], Ángeles Mencía[3], Marie-Helene Larraufie[1], Cristina Gutierrez[1], Fernando Reyes[2], Vicente García[1], Rosario Cerrato[1], Mercè Pont[1], Carles Puig[1], Miriam Andrés[1], Maribel Crespo[1], Francisca Vicente[2], Nuria Godessart[1], Olga Genilloud[2], Fernando Larcher[3] & Arsenio Nueda[1] (corresponding author Arsenio Nueda, contact e-mail: arsenio.nueda@almirall.com)

[1] R&D Centre, Almirall S.A., Laureà Miró, 08980 Sant Feliu de Llobregat, Barcelona, Spain; [2] Fundación MEDINA, Parque Tecnológico Ciencias de la Salud, Avda. del Conocimiento 34, 18016, Granada, Spain . [3] Department of Bioengineering, Universidad Carlos III de Madrid, 28911 Leganés, Spain.

Background and objective

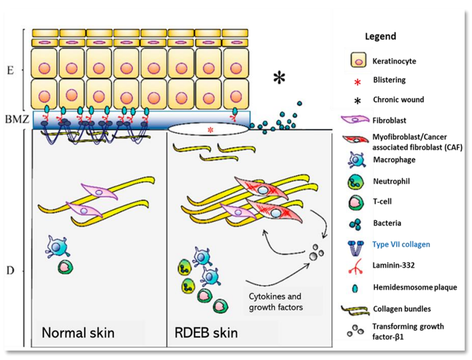


Figure modified from Condorelli et al[1] under the terms of a CC BY license (http://creativecommons.org/licenses/by/4.0/).

Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic disease caused by a deficiency in collagen VII (C7) due to mutations in the COL7A1 gene that lead to disruption of skin and mucosa architecture, blistering, chronic wounds, inflammation, and increased skin cancer risk among other symptoms. RDEB patients have an extremely poor quality of life and often die at young age[2]. One class of mutations in RDEB are premature termination codons (PTC), present in 15-20% of RDEB patients[3].

Objective: Our goal was to identify molecules capable of restoring expression of full length C7 protein in RDEB cells via translational readthrough at PTC mutations or by COL7A1 mRNA upregulation.

TRIDs4DEB: complementary project partners

Universidad Carlos III de Madrid

Fernando Larcher Group. Expertise in generation of cell lines derived from RDEB patient tissue. Organotypic and in vivo models of rare diseases.

MEDINA

Capabilities for HTS and hit characterization. Large microbial extracts natural products library. Track record in discovery of marketed drugs

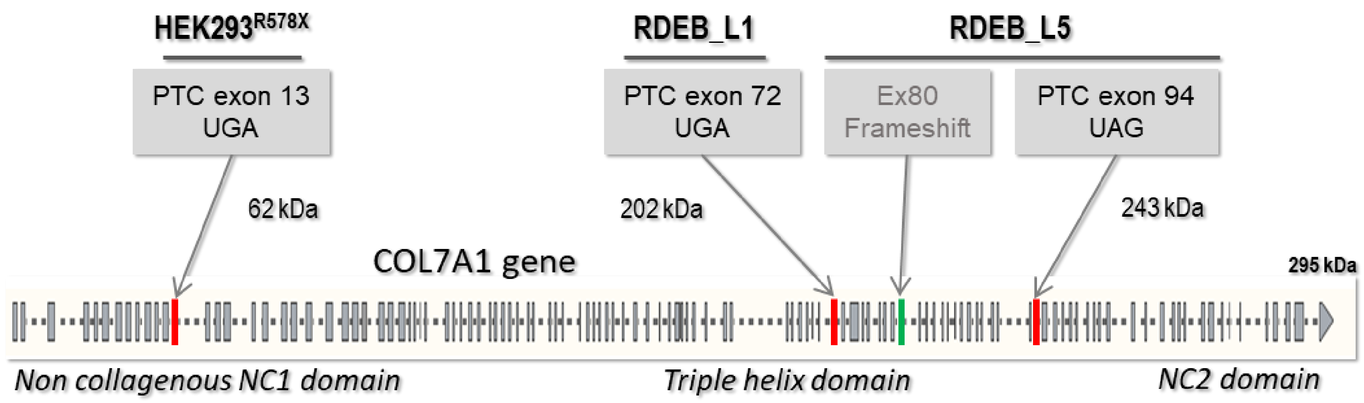
almirall

R&D expertise and capabilities. Small molecule library. Track record bringing drugs to the market.

Project partially funded by CDTI

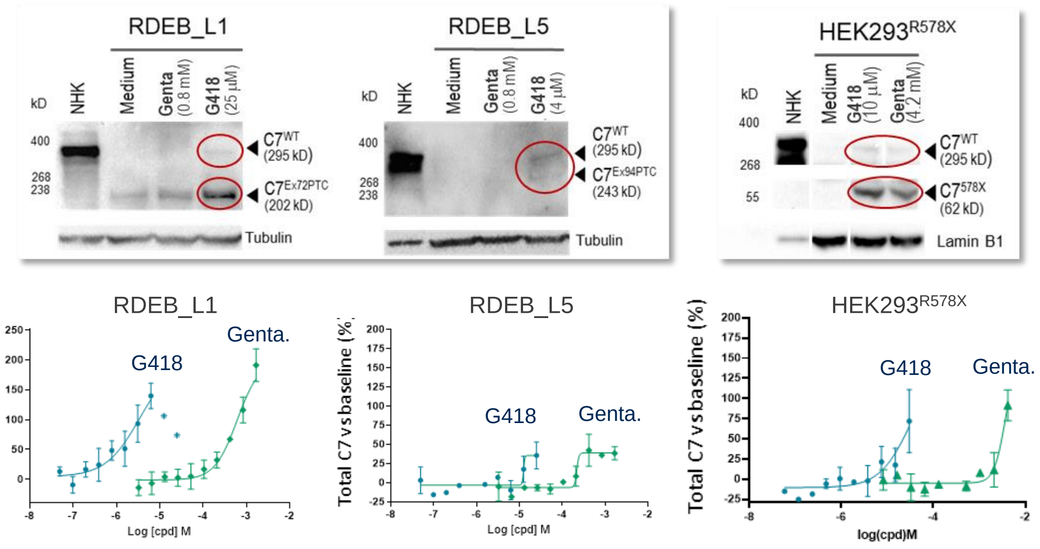
GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN CDTI INNOVACIÓN

Generation of RDEB patient-derived and recombinant cell lines



Keratinocytes from 2 patients* with PTC mutations in the COL7A1 gene were used to generate immortalized cell lines RDEB_L1 and L5. A cell line containing a recombinant C7 PTC mutation known[4] to respond to reference readthrough drugs G418 and gentamicin, was also generated.

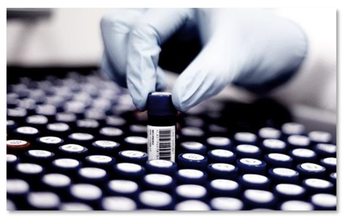
Characterization of patient and recombinant cell lines



An AlphaLISA assay was developed for quantitative detection of C7 protein. Cell lines were validated with readthrough agents in western blot and in the more sensitive AlphaLISA assay, detecting full length and truncated C7.

Phenotypic HTS screen: 3 chemical series identified

8,273 compounds selected from **Almirall library**

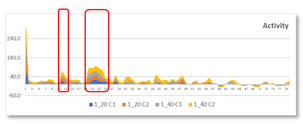


20,160 extracts selected from **Fundación Medina library**

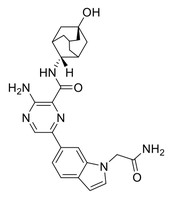
Physiological stimulus: **TGFβ**

RDEB_L1 and L5 patient-derived cell lines

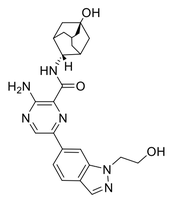
Gain-of-function endpoint: **COLVII protein detection**



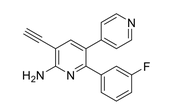
Compound 1



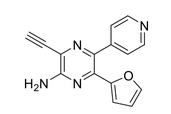
Compound 3



Compound 7



Compound 8



FM #1

FM #2

Origin: Kinase inhibitors program

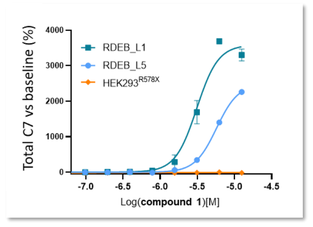
C7 (AlphaLISA)	Cpd 1	Cdp 3
EC_{50} [µM]	3	1.9

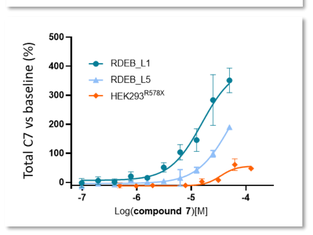
Origin: GPCR antagonists program

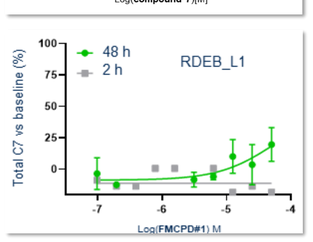
C7 (AlphaLISA)	Cpd 7	Cpd 8
EC_{50} [µM]	17	40

Origin: known antibiotic.

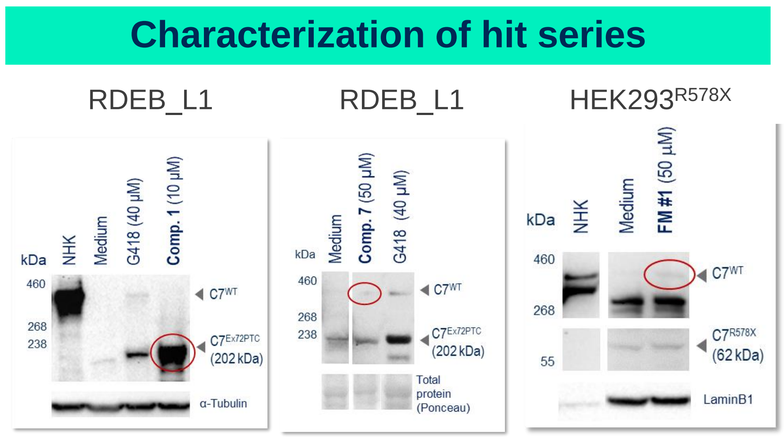
C7 (AlphaLISA)	FM#1	FM#2
EC_{50} [µM]	>50	42







Characterization of hit series

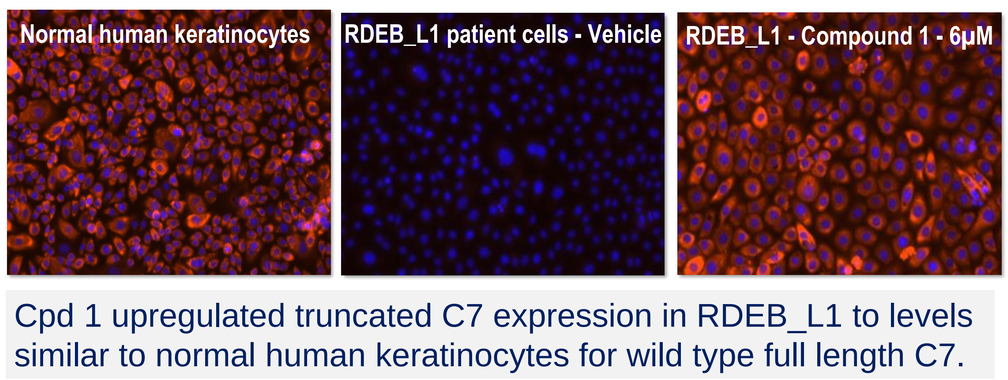


Compound 1 upregulated truncated C7 protein levels. Compound 7 & FM #1 compound induced partial expression of full length C7 indicating a translational readthrough activity in these hits.

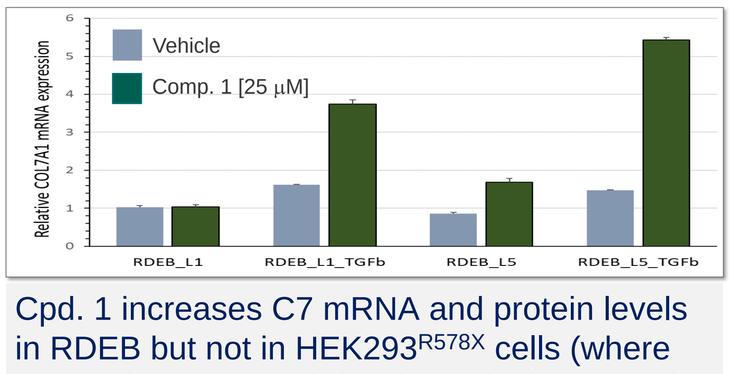
HTS with 2 RDEB patient-derived cell lines and a library of 8,273 small molecules and 20,160 extracts. Fractionation - purification of active extracts to identify natural product hits

3 chemical series: 2 from Almirall library, and 1 from Fundación Medina library **were identified**. Hits from Almirall library were legacy compounds from previous drug discovery programs. FM#1 is a known antibiotic with no reported translational readthrough activity. FM#2 is a related molecule isolated from the same extract.

Compound 1 characterization

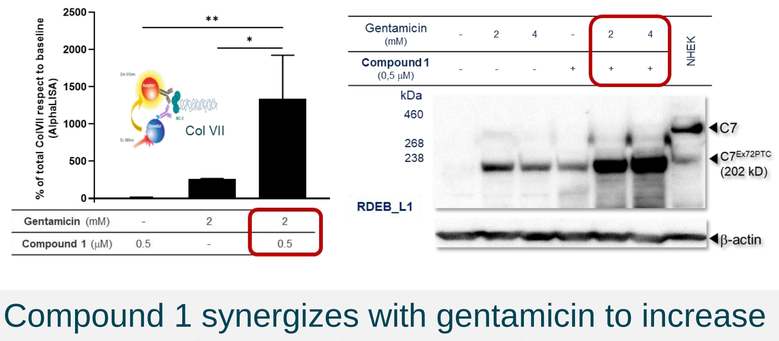


Cpd 1 upregulated truncated C7 expression in RDEB_L1 to levels similar to normal human keratinocytes for wild type full length C7.



Cpd. 1 increases C7 mRNA and protein levels in RDEB but not in HEK293^{R578X} cells (where expression of C7 is driven by a viral promoter) suggesting a promoter or lineage-specific effect.

Synergy of compound 1 and gentamicin



Compound 1 synergizes with gentamicin to increase expression of truncated C7 protein in RDEB_L1 cells

Conclusions and Future Perspectives

Using patient-derived keratinocytes and a library of 8,273 small molecules and 20,160 microbial extracts evaluated in a phenotypic screen interrogating C7 levels, we identified 3 chemical series. 2 showed PTC readthrough activity, and 1 upregulated truncated C7 expression, showing synergistic activity when combined with reference readthrough drug gentamicin. These compounds represent potential starting points for novel systemic therapies that could complement topical RDEB treatments. TRIDs4DEB was a collaboration between Almirall, UC3M and Fundación MEDINA with partial financial support from the Centre for the Development of Technology and Innovation (CDTI) of Spain's Ministry of Science. A patent application[5] has been submitted. A scientific publication is in preparation.

*RDEB patient keratinocytes were isolated from surplus skin samples sourced for diagnostic purposes after obtaining Ethical Committee approval and patient informed consent (HULP: PI-3911, CEI Hospital Universitario de la Paz, Madrid).

References: [1] Condorelli et al. (2020) Int J Mol Sci 20; [2] Rashidghamat & McGrath (2017) Intractable Rare Dis Res. 6; [3] Hou PC et al (2023) Ther Clin Risk Manag. 19; [4] Cogan et al. (2014) Mol. Ther. 22. [5] European patent application EP23382842.

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