SUPPLEMENTARY MATERIAL **MS# JID-2015-0220.R1**

Potential of systemic allogeneic mesenchymal stromal cell therapy for children with recessive dystrophic epidermolysis bullosa

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Table S1(A-J). Baseline characteristics of all trial subjects.

Eleven children with RDEB were screened for inclusion into the trial. One child was excluded because of both positive ELISA for C7 antibodies and positive indirect immunofluorescence microscopy (IIF) with binding of the antibodies to the dermal-epidermal junction (DEJ) within the base of salt-split skin. Ten children were enrolled at Great Ormond Street Hospital (London, UK). Participants (5M/5F) had a median age of 4.5 years (range 1–11) and had a genetically confirmed diagnosis of RDEB with partial or complete deficiency of C7 in their skin. Baseline characteristics of the children who participated are listed in individualized sub-tables A-J.

Key for Tables:

BEBSS: Birmingham Epidermolysis Bullosa Severity Score, scale range: 0-100: TBSA: Total Body Surface Area; GSS: Global Severity Score Scale range: 0 – 12; PedsQL^{TM}: Paediatric quality of life questionnaire - parent version: child aged 2-4 years (range:0-84), 5-7 years (range:0-92), and 8-12 years (range:0-92) and child version: child aged 5-7 years (range:0-92) and 8-12 years (range:0-92); Pain scale range: 0-80; Fatigue score scale range: 0-10; Pruritus score scale range: 0- 10. **Child was aged < 6 years at baseline. C7 immunofluorescence: +++ = normal; ++ = slightly reduced; + = reduced; +/- = barely detectable; - = undetectable.

	Subject A
Age (years)	1
Sex	M
Body mass index (kg/m^2)	17
***COL7A1* mutation**	(+/-) c.425A>G, p.Lys142Arg, exon 3; (+/-) c.1939C>G, p.Ser609X, exon 14
Skin C7 protein expression	-
BEBSS	15
BEBSS TBSA (%)	13.5
GSS	10
Blister count	6
Pain score: Child version (≥6 years)	NA
Pain score: Parent version	17
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	3
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	NA
PedsQL score: Parent version	12

Table S1A Baseline characteristics of subject A.

	Subject B
Age (years)	1
Sex	M
Body mass index (kg/m^2)	15
***COL7A1* mutation**	(+/-) c.425A>G, p.Lys142Arg, exon 3; (+/-) IVS5+1G>A
Skin C7 protein expression	+
BEBSS	21
BEBSS TBSA (%)	13
GSS	6
Blister count	1
Pain score: Child version (≥6 years)	NA
Pain score: Parent version	17
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	2
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	NA
PedsQL score: Parent version	NA

Table S1B Baseline characteristics of subject B.

	Subject C
Age (years)	1
Sex	M
Body mass index (kg/m^2)	15
***COL7A1* mutation**	(+/-) c.3840delC, p.Thr1280fsX33, exon 31; (+/-) c.4037delA, p.Lys1346fsX51, exon 34
Skin C7 protein expression	+/-
BEBSS	39
BEBSS TBSA (%)	47
GSS	6
Blister count	3
Pain score: Child version (≥6 years)	NA
Pain score: Parent version	33
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	0
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	NA
PedsQL score: Parent version	NA

Table S2C Baseline characteristics of subject C.

	Subject D
Age (years)	1
Sex	F
Body mass index (kg/m^2)	17
***COL7A1* mutation**	(+/-) c.1573C>T; p.Arg525X exon 12. (+/-) IVS79+1G>C
Skin C7 protein expression	-
BEBSS	18
BEBSS TBSA (%)	12.8
GSS	7
Blister count	2
Pain score: Child version (≥6 years)	NA
Pain score: Parent version	8
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	1
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	NA
PedsQL score: Parent version	30

Table S3D Baseline characteristics of subject D.

	Subject E
Age (years)	4
Sex	M
Body mass index (kg/m^2)	15
***COL7A1* mutation**	(+/-) c.3293delAC, p.Tyr1098fsX1, exon 25; (+/-) c.4894C>T, p.Arg1632X, exon 51
Skin C7 protein expression	-
BEBSS	32
BEBSS TBSA (%)	19
GSS	6
Blister count	6
Pain score: Child version (≥6 years)	NA
Pain score: Parent version	26
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	6
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	NA
PedsQL score: Parent version	39

Table S4E Baseline characteristics of subject E.

	Subject F
Age (years)	7
Sex	F
Body mass index (kg/m^2)	13
***COL7A1* mutation**	(+/-) c.4621delG, p.Gly1541fsX 67, exon 46; other mutation not identified.
Skin C7 protein expression	-
BEBSS	33
BEBSS TBSA (%)	29
GSS	9
Blister count	19
Pain score: Child version (≥6 years)	NA
Pain score: Parent version	22
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	4
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	4
PedsQL score: Parent version	54

Table S5F Baseline characteristics of subject F.

	Subject G
Age (years)	5
Sex	F
Body mass index (kg/m^2)	14
***COL7A1* mutation**	(+/-) c.1732C>T, p.Arg578X, exon 13; (+/-) c.5047C>T, p.Arg1683X, exon 54
Skin C7 protein expression	-
BEBSS	36
BEBSS TBSA (%)	26.5
GSS	6
Blister count	22
Pain score: Child version (≥6 years)	NA**
Pain score: Parent version	28
Fatigue score: Child version (≥6 years)	NA
Fatigue score: Parent version	5
Pruritus score: Child version (≥6 years)	NA
PedsQL score: Child version	44
PedsQL score: Parent version	50

Table S6G Baseline characteristics of subject G.

	Subject H
Age (years)	7
Sex	F
Body mass index (kg/m^2)	12
***COL7A1* mutation**	(+/-) c.409C>T, p.Arg137X, exon 3; (+/-) c.6269delC, p.Pro 2090fsx115, exon 75
Skin C7 protein expression	-
BEBSS	31
BEBSS TBSA (%)	31
GSS	7
Blister count	6
Pain score: Child version (≥6 years)	18
Pain score: Parent version	40
Fatigue score: Child version (≥6 years)	2
Fatigue score: Parent version	5
Pruritus score: Child version (≥6 years)	8
PedsQL score: Child version	32
PedsQL score: Parent version	50

Table S7H Baseline characteristics of subject H.

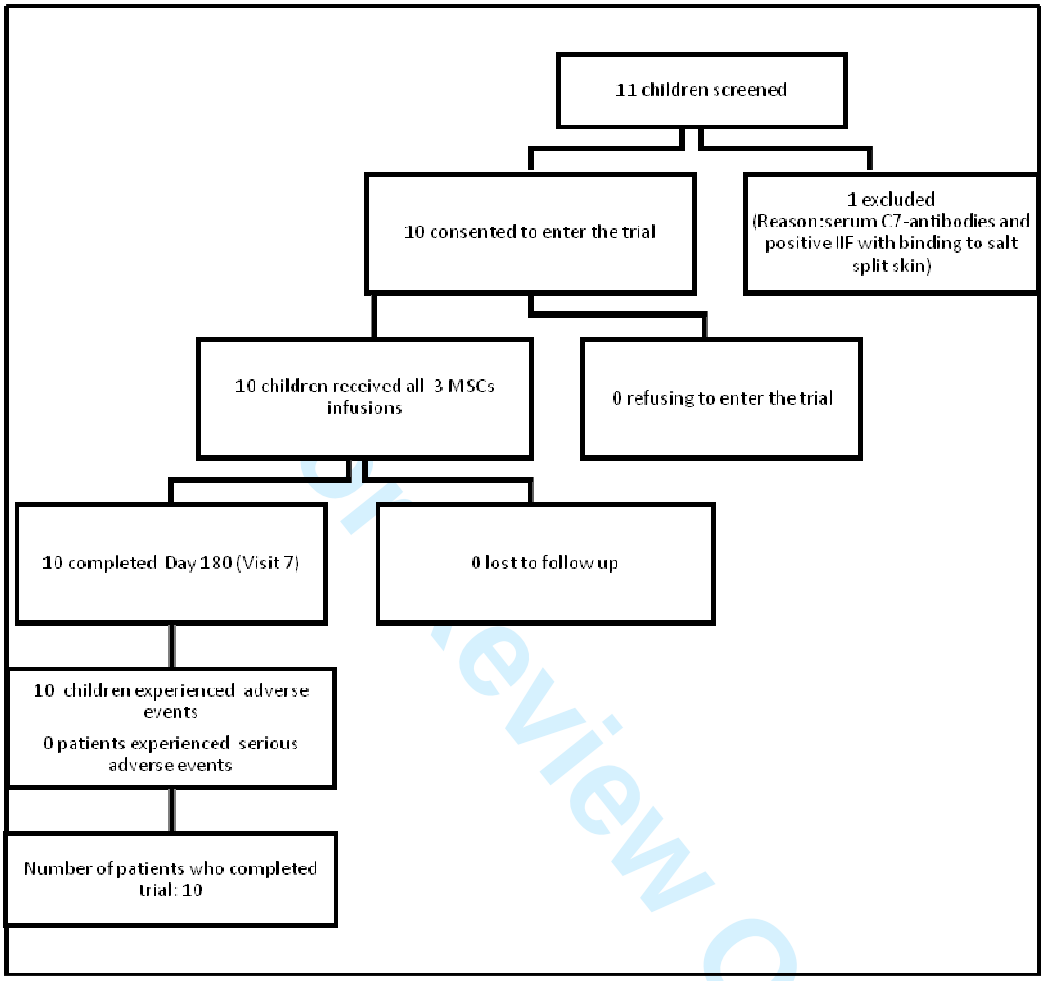
	Subject I
Age (years)	10
Sex	F
Body mass index (kg/m^2)	15
***COL7A1* mutation**	IVS23-2A>G; c.4317delC; p.Pro1441LeufsX271, exon 39
Skin C7 protein expression	-
BEBSS	35
BEBSS TBSA (%)	28
GSS	7
Blister count	5
Pain score: Child version (≥6 years)	34
Pain score: Parent version	19
Fatigue score: Child version (≥6 years)	6
Fatigue score: Parent version	3
Pruritus score: Child version (≥6 years)	8
PedsQL score: Child version	47
PedsQL score: Parent version	59

Table S8I Baseline characteristics of subject I.

	Subject J
Age (years)	11
Sex	M
Body mass index (kg/m^2)	14
***COL7A1* mutation**	(+/+) c.7787delG, p.Gly2596fsX34, exon 104
Skin C7 protein expression	-
BEBSS	23
BEBSS TBSA (%)	13
GSS	6
Blister count	2
Pain score: Child version (≥6 years)	8
Pain score: Parent version	14
Fatigue score: Child version (≥6 years)	2
Fatigue score: Parent version	1
Pruritus score: Child version (≥6 years)	4
PedsQL score: Child version	35
PedsQL score: Parent version	41

Table S9J Baseline characteristics of subject J.

Figure S1. Trial profile.



Children were recruited between July and October 2013. All 30 infusions of BM-MSCs were administered by December 2013 and all follow up visits were completed by December 2014. The study was initially designed for the children to be followed up for 24 months after their last infusion of BM-MSCs. Due to lack of serious adverse events observed, however, a substantial protocol amendment approved shortening study completion to 12 months after each subject's last infusion. Safety data were collected for a total of 12 months after the last infusion.

Table S2. Full details of inclusion and exclusion criteria.

Inclusion criteria: 1. Subjects who have a diagnosis of recessive dystrophic epidermolysis bullosa (RDEB) characterized by partial or complete type VII collagen (C7) deficiency. 2. Subjects who are ≥ 12 months and ≤ 17 years of age at the time of enrolment. 3. Subjects whose legal parent/guardian has voluntarily signed and dated an Informed Consent Form (ICF) prior to the first study intervention. Whenever the minor child is able to give consent, the minor's assent will be obtained in addition to the signed consent of the minor's legal guardian.
Exclusion criteria: 1. Subjects who have had other investigational medicinal products within 90 days prior to screening or during the treatment phase. 2. Subjects who have received immunotherapy including oral corticosteroids for ≥ 1 week (intranasal and topical preparations are permitted) or chemotherapy within 60 days of enrolment into this study. 3. Subjects with a known allergy to any of the constituents of the investigational product. 4. Subjects with signs of active infection. 5. Subjects with a medical history or evidence of malignancy, including cutaneous squamous cell carcinoma. 6. Subjects with both a) Positive C7 ELISA and, in addition, b) Positive indirect immunofluorescence (IIF) with binding to the base of salt split skin. 7. Subjects who are pregnant or of child-bearing potential who are not abstinent or practicing an acceptable means of contraception, as determined by the Investigator, for the duration of the treatment phase.

Table S3. Table summarizing the study interventions per visit until Day 180.

VISIT	1	2	3	4	5	6	7
PURPOSE	**up to 4 months prior Day 0**	**Day 0**	**Day 7**	**Day 28**	**Day 60**	**Day 100**	**Day 180**
Patient information and informed consent	X						
Confirmation of consent	X	X	X	X	X	X	X
Inclusion / exclusion	X	X					
Demography	X						
Physical examination	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
DNA analysis	X						
Blood samples	X	X	X	X	X		X
Mesenchymal stromal cells infusion		X	X	X			
Diary card issued[1]	X						
Diary card review		X	X	X	X	X	X
Skin biopsies (historical samples and results may be used for baseline)	X				X		
Disease severity skin score (BEBSS and Global Severity Score)	X				X	X	X
Wound assessment (photographs and blister count)	X	X	X	X	X	X	X
Quality of life questionnaire (PedsQoL)	X				X	X	X
Suction blister time	X					X	
EB pain, sleep and fatigue questionnaire	X	X	X	X	X	X	X
Adverse event assessment	X	X	X	X	X	X	X
Concomitant medication assessment	X	X	X	X	X	X	X

Table S4. Production of BM-MSCs was undertaken according to advanced therapy medicinal product (ATMP) guidelines and the cells were manufactured and expanded according to Good Manufacturing Practice (GMP) regulations. BM-MSCs from the bone marrow of two healthy unrelated donors (male donor aged two years and female donor aged 10 years) were isolated, expanded and packaged at the Cell Therapy Facility at University Medical Centre (UMC) Utrecht, The Netherlands. The cells were screened against an infectious disease panel in accordance with the EU directive 2006/17 (EUD 2006/17/EC). Genomic DNA from both donors was screened for *COL7A1* mutations and none were found.

BM-MSCs from two healthy unrelated donors were manufactured and expanded according to Good Manufacturing Practice (GMP) standards. MSC cell viability and phenotyping were assessed according to the following criteria (based on the minimal criteria for defining MSCs as recommended by the International Society for Cellular Therapy):

- Passage 3
- Cell viability > 70%
- Positive phenotype (≥95%) CD73, CD90, CD105
- Negative phenotype (≤2% positive) CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR

Investigational Medicinal Product components.

Component	**Reference to standards**	**Function**
TC-MSC	In-house testing	Active ingredient
Sterile sodium chloride 0·9%	Registered product for infusion	Filler
Human serum albumin 20%	Registered medicinal product	Source of protein
Dimethyl sulfoxide (DMSO)	GMP-grade	Cryoprotectant

Table S5. Summary of adverse events.

	N	%
Total number of patients in study	10	100
Number of patients who experienced adverse events	10	100
Total number of adverse events reported	163	100
	Number of events	**%**
Intensity		
Mild	101	62.0
Moderate	59	36.0
Severe	3	2.0
Serious		
Yes	0	0.0
Relationship to study drug		
Definitely	32	20.0
Possibly	3	2.5
Likely	1	0.6
Unlikely	4	1.8
Not related	123	75.0
Outcome		
Resolved	153	94.0
Continuing, no further follow up required	10	6.0
Frequency		
Single occurrence	144	88.0
Intermittent	14	9.0
Continuous	5	3.0
Action taken		
None	107	65.0
Required concomitant medication	56	35.0

Table S6. Intensity of adverse events by relationship to MSC infusion.

Intensity	**Relationship to MSC infusion (n (%))**					
	Definitely	**Possibly**	**Likely**	**Unlikely**	**Not related**	**Total**
Mild	18 (18.0)	3 (3.0)	0 (0.0)	3 (3.0)	77 (76.0)	101 (62.0)
Moderate	12 (20.0)	0 (0.0)	1 (1.7)	1 (1.7)	45 (76.0)	59 (36.0)
Severe	2 (67.0)*	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.0)	3 (2.0)
Total	32 (20.0)	3 (1.8)	1 (0.6)	4 (2.5)	123 (75.0)	163 (100)

Values are n(%); MSC: Mesenchymal stromal cells; *The 2 adverse events with severe intensity and definitely related to study drug were dimethyl sulfoxide (DMSO) odor.

Table S7. Adverse events (AEs) by system organ class and relationship to MSC infusion.

			Relationship to MSC infusion					
System organ class	**Adverse event**	**No. of patients**	**Definitely**	**Possibly**	**Likely**	**Unlikely**	**Not related**	**No. of AEs**
Ear, Nose and Throat	Epistaxis	1	0	0	0	0	1	1
	Sore throat	3	0	0	0	0	3	3
Eyes	Conjunctivitis	1	0	0	0	0	1	1
	Corneal abraision	4	0	0	0	0	20	20
	Sore eyes	1	0	0	0	0	3	3
Dermatological	Skin/mucosal blisters/wounds	9	0	2	0	0	16	16
	Dry skin	2	0	0	0	0	2	2
	Fine hair growth	1	0	1	0	0	0	1
	Milia	1	0	0	0	1	0	1
	Pruritus	4	0	0	1	1	2	4
	Rash	2	0	0	0	1	3	4
Lymph nodes	Lymphadenopathy	1	0	0	0	0	1	1
Gastrointestinal	Abdominal pain	1	1	0	0	0	0	1
	Reflux	1	0	0	0	0	1	1
	Constipation	2	0	0	0	0	2	2
	Diarrhea	5	0	0	0	0	9	9
	Increased appetite	2	0	0	0	1	2	2
	Nausea	2	2	0	0	0	3	3
	Vomiting	5	0	0	0	0	6	6
Respiratory	Cough	3	0	0	0	0	4	4
Cardiovascular	Bradycardia	1	1	0	0	0	0	1
Genitourinary	Oliguria	1	0	0	0	0	1	1
Musculoskeletal	Joint pain	1	0	0	0	0	1	1
Infectious	Fever	2	0	0	0	0	2	2
	Respiratory tract infections	5	0	0	0	0	10	10
	Skin infections	5	0	0	0	0	7	7
	Urinary tract infections	1	0	0	0	0	1	1
DMSO odor	DMSO odor	10	28	0	0	0	0	28
Mood	Irritability	1	0	0	0	0	1	1
Procedures	Esophageal dilatation	4	0	0	0	0	4	4
	Routine surgical procedure related to complications of EB	1	0	0	0	0	1	1
	Dental procedure	1	0	0	0	0	1	1
Accidental injuries	Accidental injuries	5	0	0	0	0	18	18
Total no. of patients in study		10						
Total no. of patients with AEs		10						163

Table S8. Summary of anti-BP180, anti-BP-230 and anti-C7 antibody levels (in units) in the sera of the children.

Patient ID	Pre-treatment (screening)			Post-treatment (Day 60)		
	BP180	**BP230**	**C7**	**B180**	**BP230**	**C7**
A	42	29	13	27	34	13
B	68	66	35	58	50	23
C	32	32	15	54	31	11
D	97	68	24	97	97	28
E	2	2	1	2	3	1
F	45	48	10	42	40	13
G	60	41	29	52	50	17
H	42	28	16	51	48	19
I	28	28	4	32	29	4
J	70	47	20	48	46	18
005–excluded	132	94	52	–	–	–

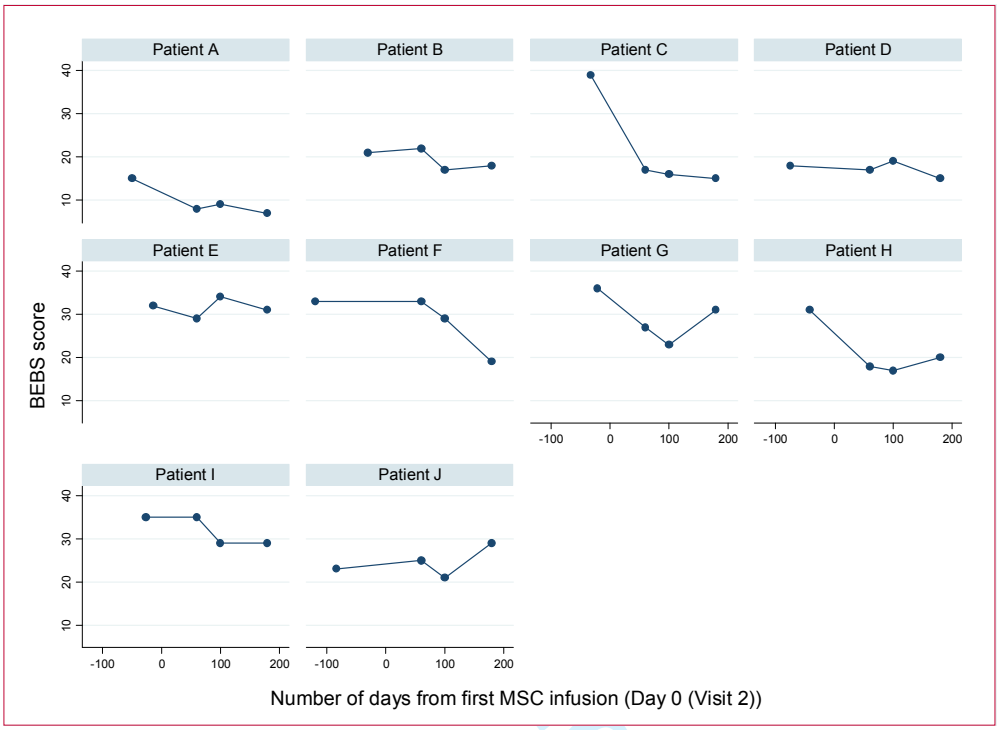
The negative cut-off values were: BP180 antibody <20 U; BP230 antibody <10 U; C7 antibody <6 U.

Table S9. Secondary outcome measures.

Outcome	N	Baseline$^{\phi}$ Mean (SD)	Day 60 Mean (SD)	Mean difference Day 60-Baseline$^{\phi}$ (95% CI)	Day 180 Mean (SD)	Mean difference Day 180-Baseline$^{\phi}$ (95% CI)
Pain, sleep and fatigue questionnaire						
Pain score (Child version)§	3	20·0 (13·1)	20·0 (5·1)	0·0 (-30·2, 30·2)	11·3 (4·6)	-8·7 (-33·2, 15·8)
Pain score (Parent version)	10	26·1 (13·5)	20·6 (8·2)	-5·5 (-16·3, 5·3)	23·1 (12·9)	-3·0 (-14·7, 8·7)
Fatigue score (Child version)§	3	3·7 (2·1)	3·0 (1)	-0·6 (-4·5, 3·1)	2·3 (0·6)	-1·3 (-5·1, 2·5)
Fatigue score (Parent version)	10	3.0 (2)	3·2 (1·7)	0·2 (-1·5, 1·9)	3·9 (1·7)	0·9 (-0·5, 2·3)
Pruritus (Child version)§	3	6·7 (2·3)	5·3(1·2)	-1·3 (-4·2, 1·5)	5·3 (1·2)	-1·3 (-4·2, 1·5)
Severity						
BEBSS	10	28·3 (8·3)	23·1 (8·3)	-5·2 (-10·7, 0·3)	21·4 (8·2)	-6·9 (-12·7, -1·1)
BEBSS TBSA (%)	10	23·3 (11·2)	17·4 (6·9)	-5·9 (-15·3, 3·5)	14·4 (8·4)	-8·9 (-18·9, 1·1)
Global severity score	10	7·0 (1·4)	4·6 (1·3)	-2·4 (-3·4, -1·4)	5·4 (1·3)	-1·6 (-2·96, -0·24)
Quality of life questionnaire						
PedsQL score (Child version)*	5	32·4 (17·0)	27·2 (12·5)	-5·2 (-25·6, 15·2)	29·6 (4·4)	-2·8 (-18·6, 13·0)
PedsQL score (Parent version)**	8	41·9 (15·2)	37·5 (15·3)	-4·4 (-8.1, -0·7)	39·0 (14·5)	-2·9 (-7·5, 1·8)
		Baseline$^{\phi}$ Median (IQR)	**Day 60 Median (IQR)**	**Day 180 Median (IQR)**		
Blister count	10	5·5 (2·0, 6·0)	3·5 (1·0, 7·0)	3·5 (3·0, 7·0)		
		Baseline$^{\phi}$ Mean (SD)	**Day 100 Mean (SD)**	**Mean difference Day 100-Baseline$^{\phi}$ (95% CI)**		
Suction blister time (minutes)	10	10·2 (6·3)	11·9 (6·9)	1·7 (-0·5, 3·9)		

Footnote: ϕ Baseline is Day -120 (Visit 1); SD: Standard deviation; IQR: Interquartile range; CI: Confidence interval; BEBS: Birmingham Epidermolysis Bullosa Severity; TBSA: Total body surface area; PedsQL^{TM}: Pediatric quality of life; * PedsQL^{TM} child version for children over 5 years; ** PedsQL^{TM} parent version for children over 2 years; §Child version of the Pain sleep and fatigue questionnaire for children > 6 years.

Figure S2. Birmingham Epidermolysis Bullosa Severity Scores (BEBSS) (Moss *et al.*, 2009) for each patient (N=10) by number of days from first MSC infusion (top); distribution of BEBSS, with means and range per visit by number of days from first MSC infusion (N=10) (bottom).



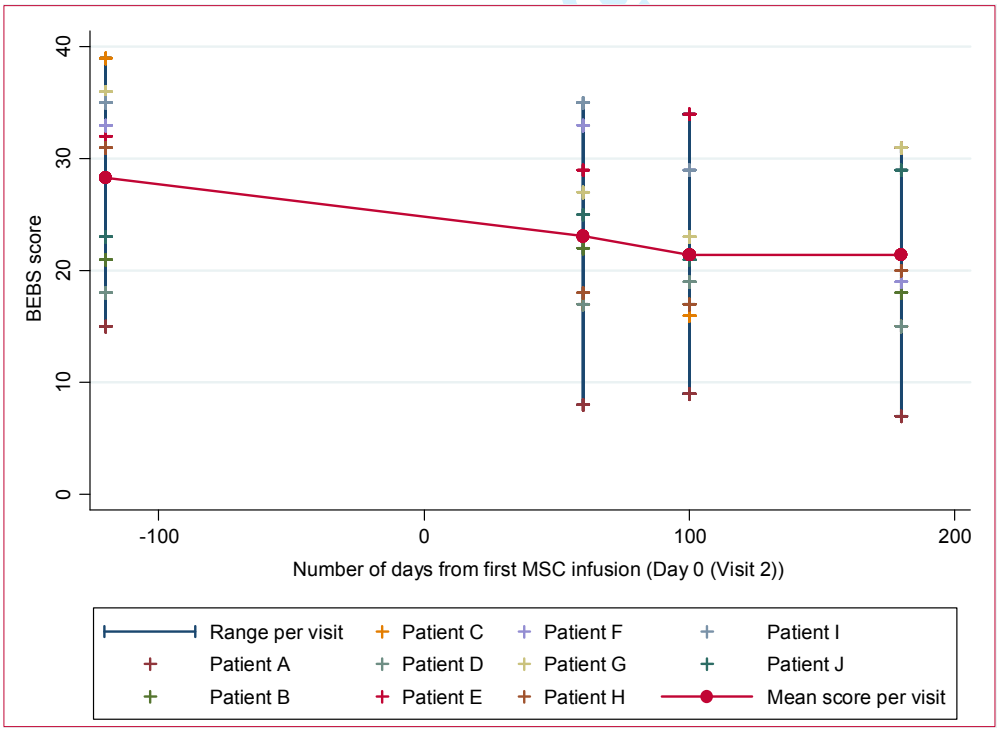
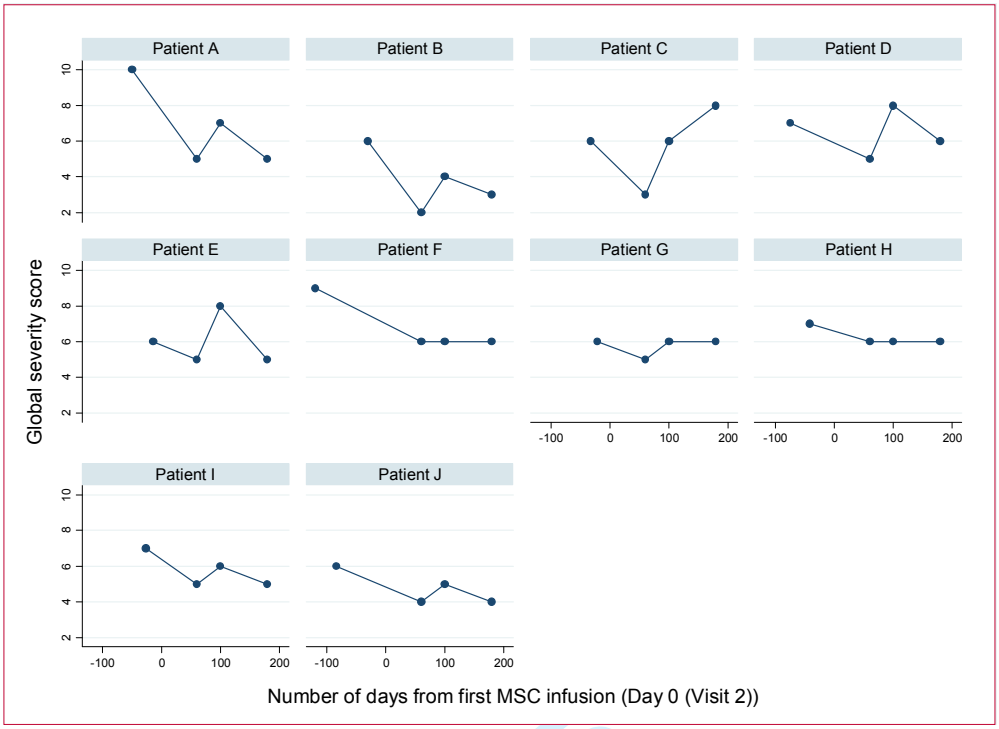


Figure S3. Global Severity Scores for each patient (N=10) by number of days from first MSC infusion (top); distribution of global severity scores, with means and range per visit by number of days from first MSC infusion (N=10) (bottom).



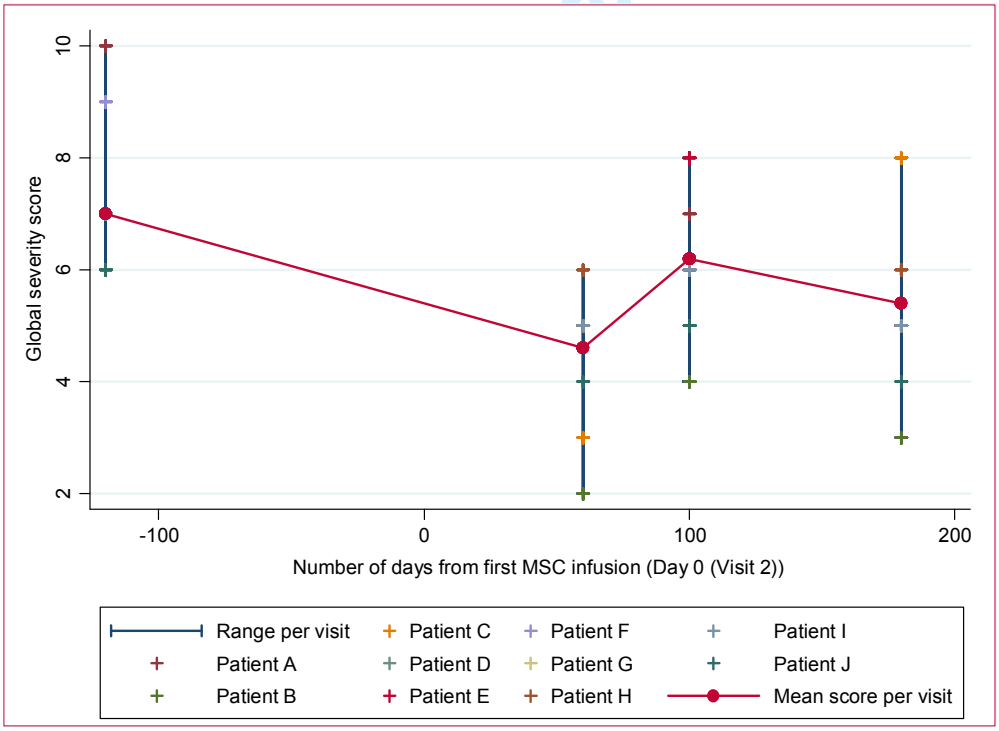
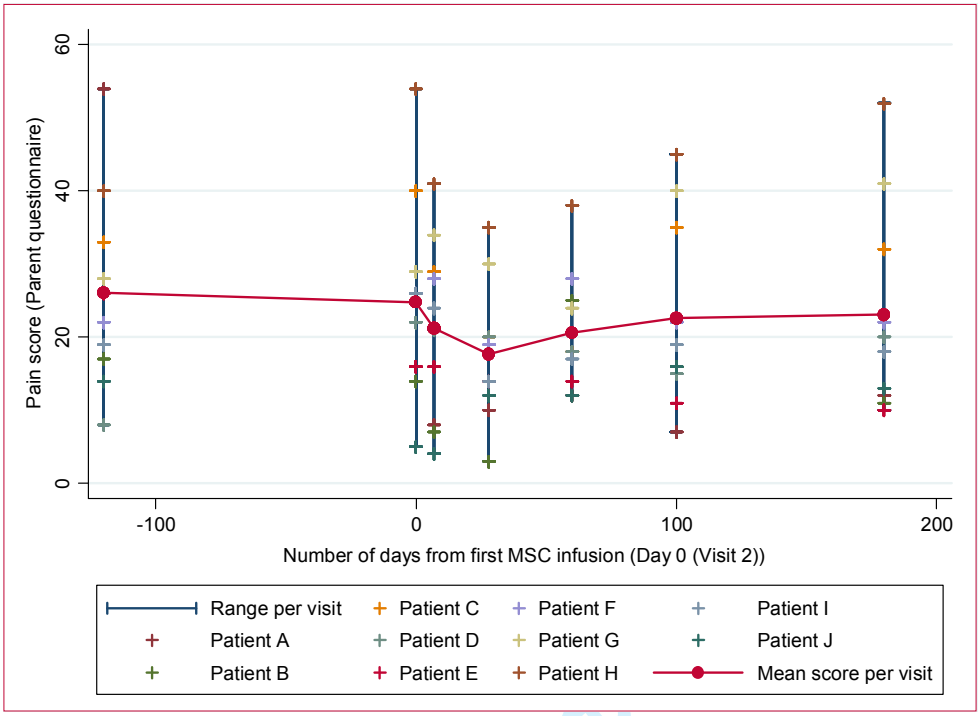
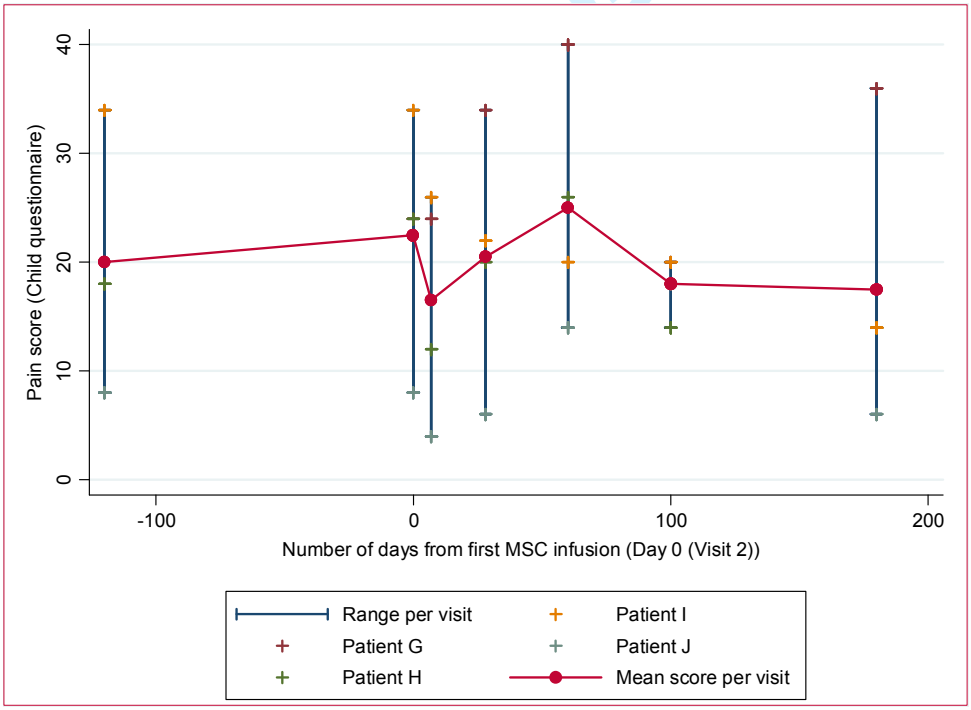


Figure S4. Parent and child versions of pain scores from Pain, Sleep and Fatigue Questionnaire. Top = parent: Graph showing distribution of scores with means and range by number of days from first MSC infusion (N=10). Bottom = child: Graph showing distribution of scores with means and range by number of days from first MSC infusion (N=4).





*Patient G was < 6 years at baseline and so was not eligible to complete the questionnaire at visit 1 but completed it at subsequent visits.

Figure S5. Percentage total body surface area (TBSA) affected by epidermolysis bullosa (EB) calculated from BEBSS for each patient (N=10) by number of days from first MSC infusion.

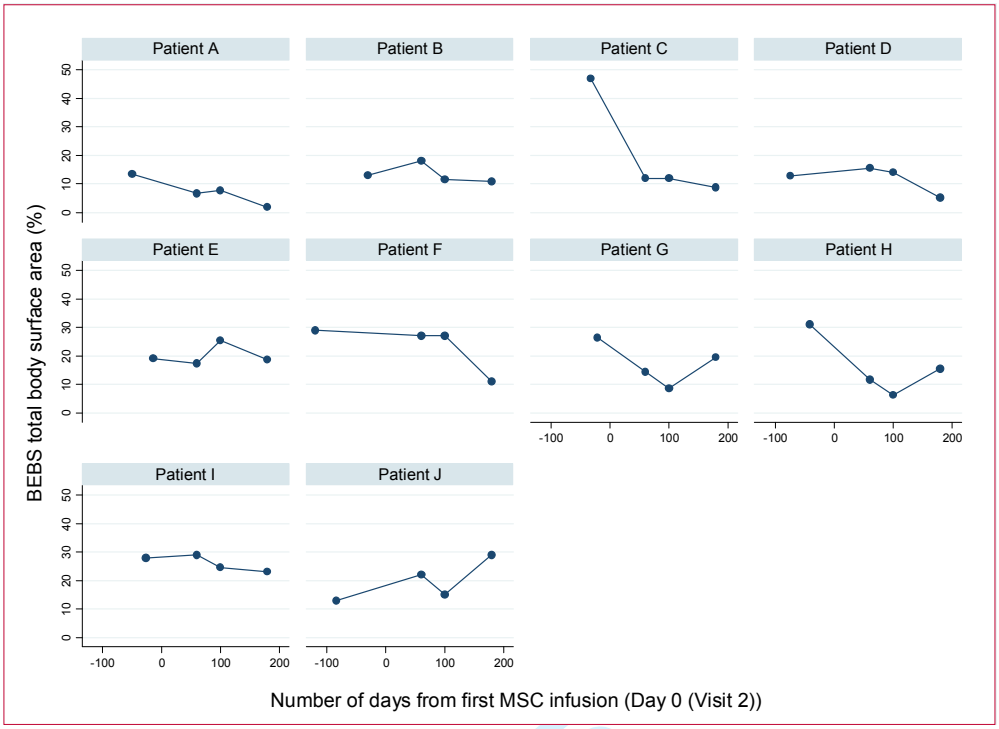
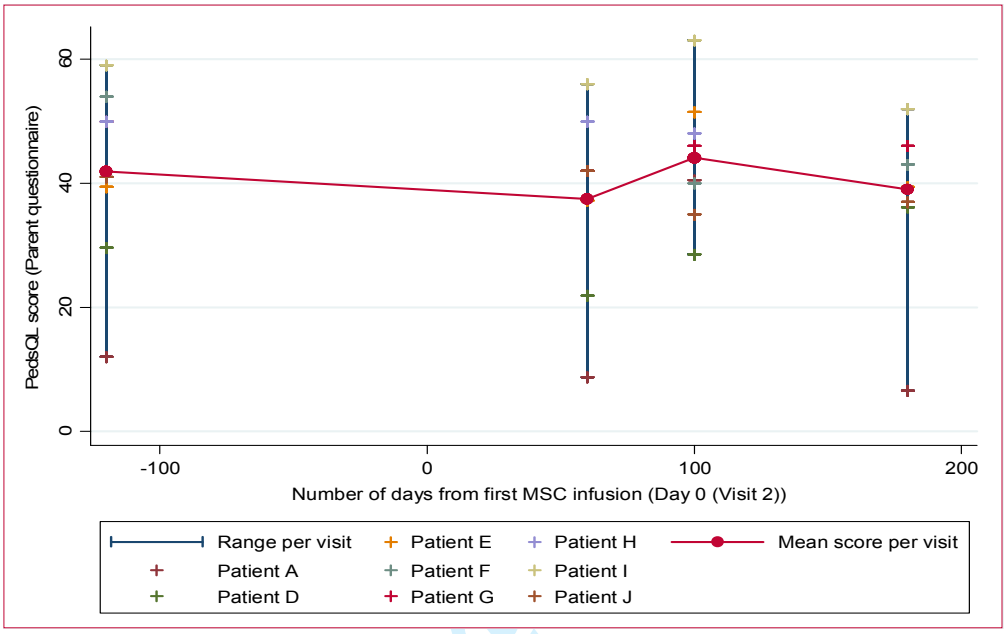


Figure S6. Parent version of pediatric quality of life scores (PedsQL) showing distribution of scores with means and range by number of days from first MSC infusion (N=8)*
*PedsQL parent version can only be completed for children over 2 years.



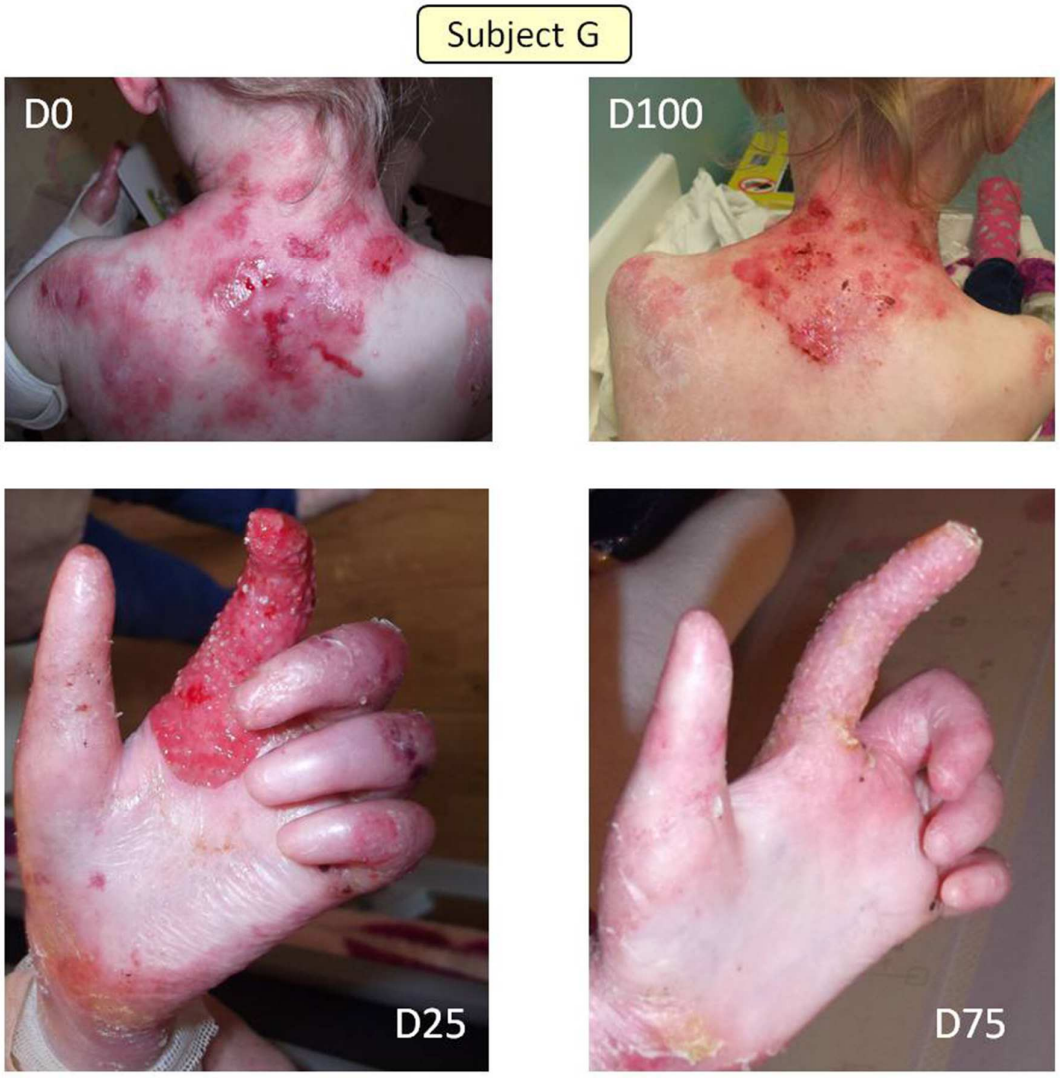


Figure S7. Clinical appearances in Subject G following BM-MSCs.

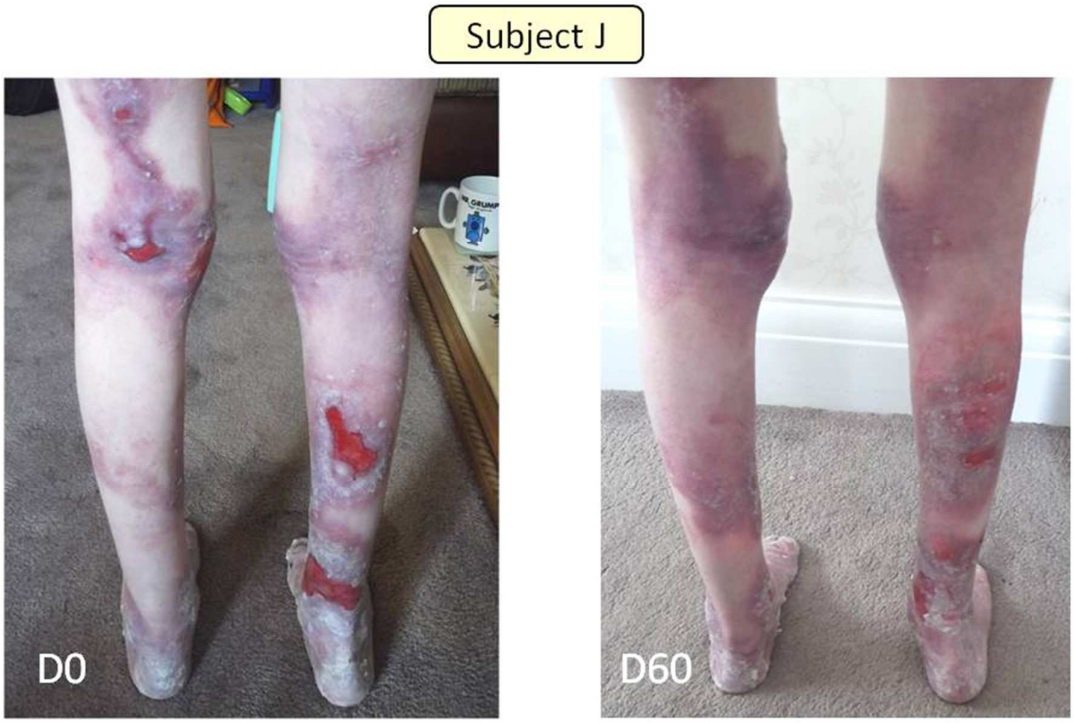


Figure S8. Clinical appearances in Subject J following BM-MSCs.

Table S10. Qualitative data analysis.

Theme	**The impact of the clinical trial has on a child with RDEB**					**The wider impact of the clinical trial**		
Sub-theme	Wound healing	Skin redness	Pruritus	Skin resilience	Pain control	Parents' future outlook	Quality of family life	Utilization of healthcare resources
Perceived positive impact	10/10	9/10	5/10	5/10	5/10	10/10	9/10	4/10
No noticeable impact	0/10	1/10	1/10	3/10	1/10	0/10	0/10	1/10
Perceived negative impact	0/10	0/10	4/10	0/10	0/10	0/10	0/10	0/10
Did not comment	0/10	0/10	0/10	2/10	4/10	0/10	1/10	5/10

Table S11. Verbatim qualitative data.

Semi-structured telephone interviews were conducted with the parents of all trial participants at 9 months after the last MSC infusion. The parents recalled their experience of caring for their children with RDEB prior to and during the clinical trial. The rate of wound healing improved with chronically ulcerated areas of skin beginning to heal up. The general improvement to skin condition, together with increase in skin resilience in trauma, enabled the children to participate more fully in play and family life. One parent reported a one-fifth reduction in the child's oral morphine analgesia requirement.

"There was an improvement in the colour of her skin and we noticed how quickly everything healed. I am sure [name of patient] was in less pain. [name of patient] was more able to cope with her [sibling] being rougher with [name of patient]. We had to reduce the oramorph by a fifth before the bandage changes. I am sure she was experiencing less pain. [name of patient]'s skin was more resistant so she was more prepared to let her sister fling her about the room, you know, like big sisters do. Or maybe it was because she was in less pain. [the skin] could bump but not blister. Or if her sister was doing 'row row row', it would leave finger marks on her [previously before the clinical trial], but not [now, during the clinical trial]. [name of patient's sibling] was just braver, more able to exist as a functional sister. It was very important for us that [name of sibling] was able to interact with her more like normal siblings. It makes you realize how many times you say stop, don't do that, how you are always on edge"

Some parents reported a reduction in the amount of the time required to provide skin care for their children. The amount of dressings required has also reduced. A parent reported about 50% reduction in dressings.

One parent described he often need to return home to assist with his child's skin care prior to the clinical trial. During the clinical trial he saw a reduction in unscheduled absence from work as his child's skin condition improved. One parent reported that the improvement to her child's skin condition was one of the key factors that enabled her to take up part-time employment after the clinical trial commenced.

"[I took time off work] 4 or 5 times a month. I have to change a shift, ring a colleague and disrupt a shift. I haven't taken any days off [since the clinical trial started]. You can see the difference."

The improvement to the children's RDEB has led to improved quality of family life with two families reporting they went abroad for holidays and one family reporting regular visits to the zoo since the clinical trial began, which they would not have otherwise done if their children's skin condition did not improve.

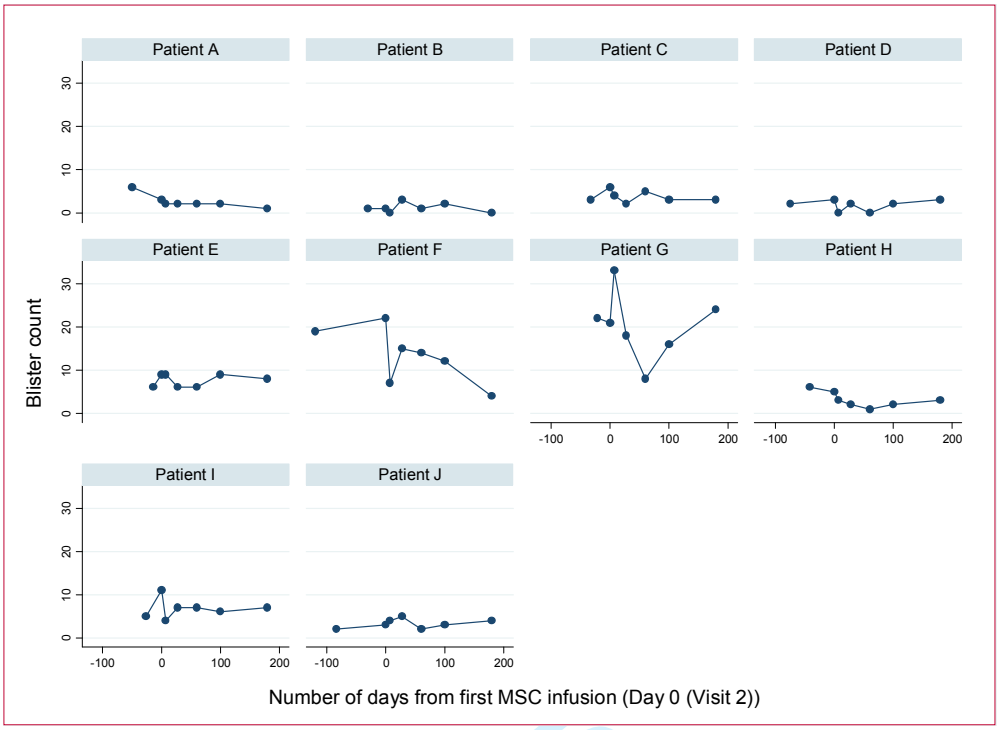
"As you can imagine, his skin was all healed up. We were able to put him in the water. Every single day, he was in the ocean. We had to do the dressings everything but the difference was that he can do that and he didn't feel pain. [He had] some areas with little blisters. He was very happy to be in the water. That's why we'd try what we can to go on holiday again. [the

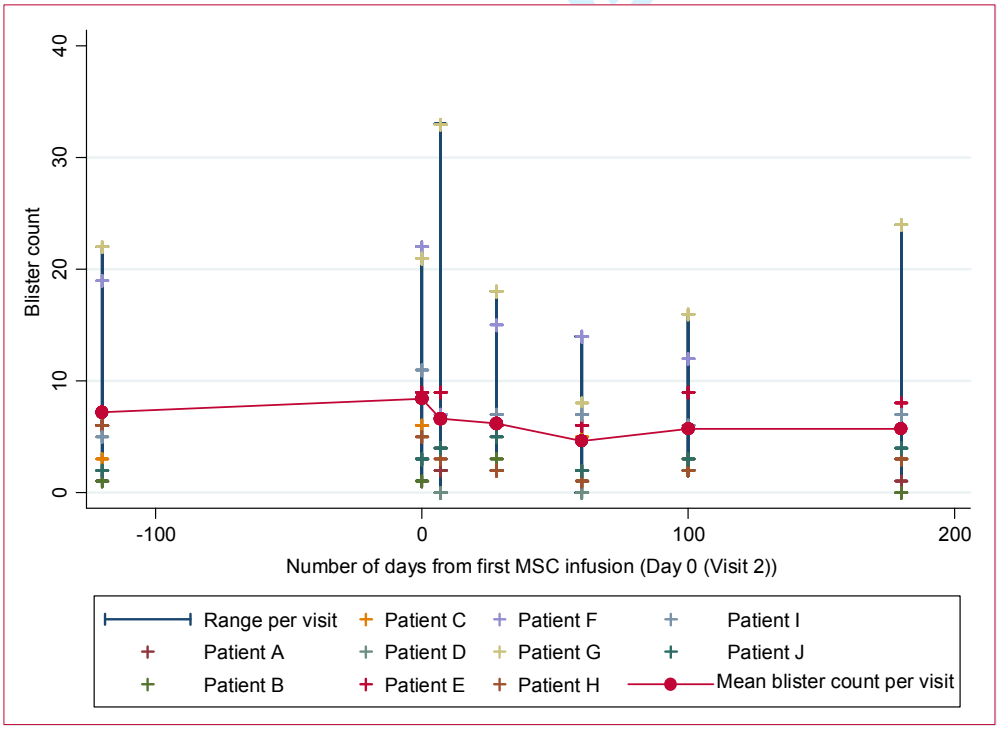
clinical trial made a] big difference for him."

The parents of all the children had a more positive outlook for the future of their child with the parents of one child stated that the improvement to their child's RDEB condition was a contributing factor to their decision to have another child.

"Before we even had [name of child] we wanted 3 or 4 children–it was never an option to have just 1 child. If things had been really bad with [name of child], like she wasn't going to walk, I don't think we would have had another child. It's very difficult to know. The fact that we made the decision to have the second one [child] was because of the hope we had from the trial and it certainly has contributed to our decision."

Figure S9. Distribution of blister count for each patient (N=10) by number of days from first MSC infusion (top); distribution of blister count with means and range per visit by number of days from first MSC infusion (N=10) (bottom).





SUPPLEMENTARY METHODS

Study protocol and participant eligibility

This open-label phase I/II trial was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA), with EudraCT number: 2012-001394-87. The UK National Research Ethics Committee London-Bloomsbury provided ethics approval (Ref:12/LO/1258). The trial is registered prospectively with controlled-trials.com ISRCTN46615946. Children of either sex, aged ≥ 12 months and ≤ 17 years were eligible to take part. Children had a diagnosis of RDEB, characterized by partial or complete absence of C7. Written informed consent of the parents and written informed assent from the child (if over 5 years old) was obtained.

Safety assessments

The safety and tolerability of BM-MSCs were assessed by monitoring the occurrence of adverse events identified during the infusions by vital sign measurements, physical examinations and standard laboratory tests. Laboratory tests performed at screening, Day 0, Day 7, Day 28, Day 60 and Day 180 included full blood count, renal liver profiles and inflammatory markers. Serious adverse events were defined as any adverse event that results in death, is life-threatening, required hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity.

Production of MSCs

Production of BM-MSCs was undertaken according to advanced therapy medicinal product (ATMP) guidelines and the cells were manufactured and expanded according to Good Manufacturing Practice (GMP) regulations. Further details of the cells are presented in Table S3 online. BM-MSCs from the bone marrow of two healthy unrelated donors (male donor

aged two years and female donor aged 10 years) were isolated, expanded and packaged at the Cell Therapy Facility at University Medical Centre (UMC) Utrecht, The Netherlands. The cells were screened against an infectious disease panel in accordance with the EU directive 2006/17 (EUD 2006/17/EC). Genomic DNA from both donors was screened for *COL7A1* mutations and none were found.

Dose of BM-MSCs and infusion schedule

Each child in the trial received 3 separate intravenous infusions of same donor BM-MSCs on Day 0, 7, and 28, at a dose of 1–3x10^6 cells / kg. The infusions were done as day-case procedures; premedication with chlorphenamine was given 30 min before administration of the cells. On the day of infusion, cryopreserved cells were transported in liquid nitrogen, thawed in a 37 degrees water bath and immediately infused over 10 minutes via a peripheral cannula. Vital signs (blood pressure, respiratory rate, heart rate, pulse oximetry and temperature) were checked before administration of the cells and thereafter every 15 minutes for one hour after the infusion and on discharge. Skin biopsies obtained for previous diagnostic testing (as part of routine clinical care) were used as baseline samples for direct immunofluorescence microscopy (DIF) for C7 and transmission electron microscopy (TEM) for anchoring fibrils.

Study objectives

The primary objective was to assess safety. Secondary objectives were to assess efficacy on clinical and functional outcomes, as well as skin pathology. We assessed participants by conducting 6 follow up visits over 6 months (after the infusions) and then 2 further safety assessments (one physical, one by telephone) up to 12 months after the last infusion. Structured phone interviews to obtain qualitative data were held at month 9. Skin samples

were analysed by DIF and TEM at screening and at Day 60 at the National Diagnostic Epidermolysis Bullosa Laboratory at St Thomas' Hospital (Viapath, London, UK). Clinical assessments were undertaken for all participants at each visit. The Birmingham Epidermolysis Bullosa Severity Score (BEBSS), a Global Severity and Improvement Score (GSIS) questionnaire, a Pain Sleep and Fatigue assessment, and a Pediatric Quality of Life (PedsQL^{TM}) assessment, were completed as per protocol. Blister counts and clinical photographs were performed by the parents during dressing changes and the data and images were reviewed during each visit by GP, MMQ or SML.

Blood and skin profiling

Blood samples for hematology and biochemistry were taken and analyzed at screening, Day 0, Day 7, Day 28, Day 60 and Day 180 at the Great Ormond Street Hospital pathology laboratories. Sera were analysed for C7 antibodies by indirect IIF and ELISA at screening and Day 60 at the Immunodermatology Laboratory at St Thomas' Hospital (Viapath, London, UK). For cases in which the BM-MSC donor cells were sex-mismatched (4/10), quantitative donor analysis using fluorescence in situ hybridization (FISH) was performed on tissue sections (Department of Cytogenetics, Guy's Hospital) using previously published techniques (Neat *et al*., 2013). Suction blister times were performed at screening and at Day 100 using a negative pressure device (Electronic Diversities, MD, USA). The Negative Pressure Cutaneous Suction System is a self-contained instrument package. The blisters are created through the use of suction chambers that are attached to the patient's skin. Briefly, the numbered chambers are connected to the appropriate chamber control channel. Once the chamber is secured to the patient's skin, the device is turned on at a pressure of 12–15 mmHg. This pressure creates a suction blister in a healthy person in 60 minutes. The application of negative pressure from the instrument console, to the chamber interior, causes the patient's

skin to be gently drawn through the openings in the orifice plate approximately the size of the opening(s) in the orifice plate. The procedure caused no discomfort to the children and the discomfort was minimal to the parents. A video of how the procedure is performed has been published previously (Tolar and Wagner, 2013). Unwounded, non-scarred skin on the anterior thigh was used for all suction blister measurements.

Details of the statistical analysis methods

RDEB is a rare disease and so a large study is not feasible. To primarily assess safety, this study sought to recruit 10 children. Assuming that no serious adverse events were observed then the 95% CI around this estimate would be 0 to 31%.

The mean changes in efficacy measures (such as pain score, BEBSS) were estimated using the paired t method. This method requires that the *changes* (not the values at the individual time points) follow a Normal distribution, which was observed here. Results are therefore presented as means and estimated mean differences between time points and 95% confidence intervals. As this is an early phase trial no significance tests were conducted and so no p values are given. Analyses were performed using the Stata statistical software (StataCorp. 2013, version 13.0).

The scales of the pediatric quality of life questionnaire (PedsQL) differed depending on the age of the child, and ranged from either 0–84 (aged 2–4 years) or from 0–92 (aged 5–13 years). In order to make the scales comparable across all children, the scores for the younger children (ranged 0–84) were rescaled to 0–92 by multiplying by 92/84 (Varni *et al.*, 1999; 2002; 2003).

For the child version of the Pain Sleep and Fatigue Questionnaire, only patients aged >6 years were eligible to complete these. Children who had completed the questionnaire for all the seven visits were included in the analysis (n=3/10). One patient did not complete the questionnaire at visit 1 (baseline) but completed it at subsequent visits.

Trends in outcomes over time were plotted for the individual patients to show the extent of any variability between them. This is considered more informative than plotting means over all patients at each time point since these can obscure important differences between patients and provide a misleading picture of the trends. All analyses were performed using Stata version 13·0 statistical software (StataCorp. 2013).

Qualitative analysis

Semi-structured telephone interviews were conducted with the parents of all trial participants at 9 months after the last infusion of BM-MSCs. The parents were asked standardized questions to explore their perception of their children's participation in this clinical trial and the impact of the BM-MSCs on both the children and family as a whole. The parents were invited to comment on their respective telephone interview transcript as part of the respondent validation process. The transcripts were analyzed using content analysis that enables the conversion of textual data into numerical data.

ELISA for BP180, and BP230 and C7 antibodies

Anti-BP180, anti-BP230 and anti-C7 antibodies were measured using the MESACUP ELISA kits (MBL, Japan) according to the manufacturer's instructions. The kits measure antibodies

against BP180 (NC16a domain), BP230 (-N and –C domains) and C7 (NC1and -NC2 domains).

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AUTHOR CONTRIBUTIONS

All authors participated in design of the protocol and interpretation of the results of the trial. JAM served as Chief Investigator and AEM and Principal investigator. The academic investigators: GP, SML, MMQ, AAW, ST, JEM, AEM, and JAM had a leading role in the trial design, trial conduct, protocol amendments and data collection. GP, MMQ, AEM and SML were responsible for screening of participants and conduct of follow up visits. Data analysis was performed by King's College London medical statisticians (JLP and MO). Qualitative analysis was performed by ST and MMQ. Data interpretation and submission for publication were performed by JAM, GP, MMQ, AEM, JEM, and SML. All authors had complete access to the data and mutually made the decision to submit for publication.