KING'S College LONDON
University of London

Full Study Title:

A prospective phase I/II study to evaluate allogeneic mesenchymal stromal cells for the treatment of skin disease in children with recessive dystrophic epidermolysis bullosa

Study Acronym: EBSTEM

CONFIDENTIAL

End-of-trial Report

Date: 28 September 2015

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Contents

1. Study Synopsis

Title	A prospective phase I/II study to evaluate allogeneic mesenchymal stromal cells for the treatment of skin disease in children with recessive dystrophic epidermolysis bullosa.
Protocol Short Title/Acronym	EBSTEM
Sponsor name	King's College London
Chief Investigator	John A. McGrath
Eudract number	2012-001394-87
REC number	12/LO/1258
Medical condition or disease under investigation	Recessive dystrophic epidermolysis bullosa
Purpose of clinical trial	To assess whether intravenously administered third-party bone marrow-derived mesenchymal stromal cells (MSCs) are safe and have an impact on disease morbidity/severity in children with recessive dystrophic epidermolysis bullosa (RDEB).
Primary objective	To evaluate the safety of allogeneic intravenously administered MSCs in children with RDEB over a 12-month period.
Secondary objective (s)	• Incidence of infusional toxicity. • Increase in C7 deposition at the DEJ post treatment at D0 and D60. • Quantitative analysis of the donor cells

	chimerism at D60. • Improvement of haematological and serological markers of generalised inflammation at D0, D7, D28, D60 and D180 compared to baseline. • Improvement in the clinical appearances of the skin. • Improved quality of life according to validated paediatric QoL scoring systems at screening, D60, D100 and D180. • Pain scoring at screening, D0, D7, D28, D60, D100 and D180. • Reduction in blister occurrence over entire body surface at D0, D7, D28, D60, D100 and D180 as compared to baseline. • Increase in skin strength measured by time to blister formation after skin suction at screening and D100.
Trial Design	Phase I/II, non-randomised, open-label, single-centre, proof-of-concept study.
IMP	Third-party bone marrow-derived mesenchymal stromal cells.
Sample Size	It is anticipated that approximately 15 subjects will be screened for enrolment into the study to obtain 6-10 evaluable subjects. Subjects will be recruited through the Great Ormond Street Investigators' patient database.

Summary of eligibility criteria	**Inclusion Criteria** 1) Subjects who have a diagnosis of recessive dystrophic epidermolysis bullosa (RDEB) characterised by partial or complete C7 deficiency. 2) Subjects who are ≥ 12 months and ≤ 17 years of age at the time of enrolment. 3) Subjects whose responsible 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** Subjects will be excluded from the study if ANY of the following conditions exist: 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 more than 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 b) a

	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.
IMP, dosage and route of administration	Allogeneic bone marrow-derived mesenchymal stromal cells from healthy donors. Dose: 1-3x 10^6 cells/kg via three intravenous administrations at Day 0, Day 7 and Day 28.
Active comparator product(s)	Standard supportive medical care
Maximum duration of study participation	14 months (12 months following the last infusion)
Version and date of final protocol	Version 1.1 14th October 2012
Version and date of protocol amendments	Version 1.2 27 June 2013 Version 2.0 22 July 2013 Version 3.0 12 December 2013 Version 3.1 21 March 2014 Version 3.2 20 June 2014 Version 4.0 01 August 2014

2. Summary of trial outcomes

Individuals with recessive dystrophic epidermolysis bullosa (RDEB) have life-long fragile skin and chronic wounds. RDEB is caused by bi-allelic mutations in COL7A1, leading to a lack of basement membrane type VII collagen (C7). Currently, there is no cure for this condition. We conducted a prospective, phase I/II, open-label study to assess safety of bone marrow mesenchymal stromal cells (BM-MSCs) and their impact on disease severity and quality of life in children with RDEB. The study was conducted at Great Ormond Street Hospital for Children NHS Trust. Ten children were enrolled and each participant received 3 intravenous infusions of BM-MSCs (Day 0, 7 and 28; each dose 1–3 x 10^6 cells/kg). Intravenous BM-MSCs were well tolerated, with no safety concerns. No changes in skin C7 expression were seen. The changes in efficacy outcomes between baseline and 60, 180 days were promising: mean parent-reported pain score (range 0–100) changed from 26.1 (baseline) to 20.6 at 60 days (difference: -5.5; 95% CI: -16.3, 5.3); mean disease severity score changed from 28.3 to 23.1 (-5.2; -10.7, 0.3); mean skin suction blister time was 10.2 mins (baseline) and 11.9 (100 days) (1.7; -0.5, 3.9). Further studies will need to address optimal cell dosage and frequency of re-treatment and to definitively show efficacy.

3. Background

3.1 Epidermolysis bullosa

Epidermolysis bullosa (EB) is a heterogeneous group of inherited disorders characterised by skin blistering and mucosal fragility; approximately 500,000 people worldwide have EB (Fine *et al.,* 2014). One of the most severe subtypes of EB is the recessive dystrophic variant (RDEB) that affects ~800 people in the UK (source www.debra.org.uk). RDEB is caused by bi-allelic loss-of-function mutations in *COL7A1* leading to reduced or absent basement membrane type VII collagen (C7) and poorly formed or absent anchoring fibrils at the junction between the epidermis and dermis (Hilal *et al.,* 1993). Poor anchoring fibril function

leads to lifelong severe blistering and skin erosions following minor mechanical trauma. Currently, there is no effective treatment for RDEB and many individuals develop life-shortening squamous cell carcinomas by the age of 40 years. Total healthcare costs for individuals with severe RDEB living in the UK are estimated to be in excess of £60,000 per year (source www.debra.org.uk), with repeated applications of dressings to large wounds accounting for much of the overall expense.

3.2 Innovative therapies in recessive dystrophic epidermolysis bullosa (RDEB)

In the past 5 years, considerable progress has been made in testing innovative treatments for RDEB, including gene, protein, and drug therapy (Wagner *et al.,* 2010, Uitto *et al.,* 2012, Uitto *et al.,* 2012, El-Darouti *et al.,* 2013, Hovnanian 2013, McElroy *et al.,* 2013, Osborn *et al.,* 2013, Petrof *et al.,* 2013, Tolar and Wagner 2013, Venugopal *et al.,* 2013, Wang *et al.,* 2013, Woodley *et al.,* 2013, 2014). Reported early phase clinical trials include intradermal injections of allogeneic fibroblasts to RDEB wounds (Petrof *et al.,* 2013, Venugopal *et al.,* 2013), as well as whole bone marrow transplantation (BMT) (Wagner *et al.,* 2010). Other published first-in-man studies include intradermal injections of bone marrow-derived mesenchymal stromal cells (BM-MSCs) (Conget *et al.,* 2010), as well as intravenous BM-MSCs in adults with RDEB (El-Darouti *et al.,* 2013). A clinical trial of *ex vivo COL7A1* gene therapy with grafting of corrected keratinocytes is currently being evaluated (Siprashvili *et al.,* 2014). From a clinical perspective, it is clear that the most effective therapies for RDEB need to be given early in life, and probably delivered systemically in view of the extent of any individual's skin and mucous membrane pathology. Nevertheless, a scenario of combination therapies, local and systemic, is highly likely in delivering better clinical care for patients with RDEB in future.

3.3 Mesenchymal stromal cells (MSC) in RDEB

MSCs represent a heterogeneous collection of mostly non-progenitor connective tissue cells that are structurally and functionally different from self-renewing stem cells and progenitors. Initially considered to be a population of stromal cells supporting and organising parenchymal frameworks, several studies have identified important roles for MSCs in modulating tissue inflammation and promoting tissue repair, including skin wounds (Chen *et al.,* 2008, Prockop 2009, Tolar *et al.,* 2010, Tolar *et al.,* 2011). Indeed, there are 250 ongoing clinical trials using MSCs for specific disease indications on www.clinicaltrials.gov. Precisely how MSCs impact on the process of tissue repair is not fully known, although immunomodulatory changes (T-cells, dendritic cells), a stimulatory paracrine function, and local immunosuppressive changes, have been observed (Nauta and Fibbe 2007, Walter *et al.,* 2010, Bianco *et al.,* 2013, Fibbe *et al.,* 2013). Moreover, within murine bone marrow, a sub-population of MSCs (still heterogeneous but positive for platelet-derived growth factor receptor alpha, PDGFRα), has been shown to contribute directly to epithelial repair in skin (Tamai *et al.,* 2011).

Although the skin blistering in RDEB is primarily induced by trauma, the failure of wounds to heal quickly and their tendency for the repair process to break down due to further mechanical injury and secondary bacterial skin infections, typically leads to acute and chronic inflammation in the skin. Transcriptomic studies in RDEB wounds have identified elevated levels of pro-inflammatory cytokines and matrix metalloproteinases, enzymes that breakdown collagen and elastic tissue in skin (Nagy *et al.,* 2011, Petrof *et al.,* 2013). Clinically, prolonged skin inflammation leads to scarring, contractures and an increased risk of developing squamous cell carcinomas, particularly in areas of chronic inflammation even as young as age six (Shivaswamy *et al.,* 2009). Thus innovative therapies that reduce skin inflammation in RDEB potentially may have positive clinical benefits in reducing disease

burden. Thus, assessing the safety and potential benefit of repeated intravenous infusions of allogeneic BM-MSCs to children with RDEB is the subject of the current study.

4. Materials and Methods

4.1 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 and site-specific approval (Ref:12/LO/1258) for Great Ormond Street Hospital. The trial is registered 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, characterised 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. Full inclusion and exclusion criteria are listed in the Appendix.

4.2 Study procedures

4.2.1 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 and liver profile. Serious adverse events were defined as any adverse event that results in death, is life-threatening, required hospitalisation or prolongation of existing hospitalisation, resulted in persistent or significant disability or incapacity. Later in the trial, we added a substantial amendment to the protocol whereby a number of expected adverse events (AEs) and serious adverse events (SAEs) that do not require reporting. These include AEs and SAEs as a result of

venesection, cannulation, skin biopsy or hospitalisations expected to take place as a result of disease progression in children with RDEB.

Adverse event as a result of venesection and cannulation include:

i) Mild bruising at site of needle puncture

Adverse event as a result of the 4mm skin biopsy include:

i) Mild bruising at the site of the skin biopsy

ii) Cutaneous skin infection requiring oral course of antibiotics

iii) A small scar will result after each skin biopsy, resembling an old chickenpox scar.

All hospitalizations that are expected to take place as a result of disease progression will not be reported, including any planned elective surgeries. This may include but not limited to:

- Skin
    - Skin infection
    - Review of a wound
- Teeth
    - Dental extractions/ abscess
- Hand
    - hand surgery
    - De-gloving injury
    - OT review and splints
- Transfusions
- Overnight stay for reviews
- Blood monitoring, routine blood tests
- Corneal abrasions

- Eye Infections
- Gastrointestinal problems
    - Dysphagia, Oesohageal stricture and dilation
    - Gastrostomy insertion, leakage or blockage/ jejunal tube insertion leaking/ blockage
    - NG tube insertion
    - Constipation
- Vertebral or other fractures
- iv pamidronate
- contactures requiring physiotherapy
- Hydrotherapy
- ENT
    - Tonsillitis
    - otis externa
    - otis media
- Pain
    - pain assessment for acute or chronic pain

Thus, any hospitalization not associated with the use of the IMP will not be reported, unless the use of the IMP results in a prolongation of existing hospitalization. Unscheduled and/or emergency hospitalizations not expected due to the natural course of the disease will be reported via the sponsor's normal SAE reporting practice.

4.2.2 Production of MSCs

Production of BM-MSCs was subject 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 ten years) were isolated, cultured 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). DNA from both donors was screened for *COL7A1* mutations and none were found.

4.2.3 Dose of BM-MSCs and infusion schedule

Each child in the trial received three 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 a day-case procedures; premedication with chlorphenamine was given 30 min before administration of the cells. Cryopreserved cells were thawed and immediately infused over 10 minutes via a peripheral cannula. No HLA-typing was performed on any of the recipients of the MSCs. 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.

5. 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 and then 2 further safety visits up to 12 months after the last infusion. Structured phone interviews to obtain qualitative data were held at 9 months. 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 assessment and photographs 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 Paediatric Quality of Life (Paeds QoL) assessment, were completed as per protocol. Blister counts and clinical photographs were done by the parents during dressing changes and the data and images were reviewed during each visit.

5.1 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). 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).

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.

6. Statistical analysis

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).

6.1 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.

7. Results

7.1 Study design and participant characteristics

Following regulatory and ethics approvals, children with RDEB were invited to participate (Figure 1). 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 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 are listed in Table 1 and details of the trial assessment time-points and metrics are given in Table 3. The dose of MSCs for this study was chosen based on safety and efficacy data from previous clinical trials with intravenous MSCs, predominantly for steroid resistant graft-versus-host disease. Of note, MSCs have been administered previously in varying doses and regimens ranging from 1-9 x 10^6 cells/kg in either single or repeated infusions. The dosing regimen used in this trial was based on a regimen implemented at the University Medical Center Utrecht (UMC Utrecht; study NL13729.000.07). The dose and frequency of infusions were endorsed by the trial advisory board. 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, and positive outcomes noted by the children and their parents, 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. All children completed the trial.

7.2 Clinical safety

There were a total of 163 adverse events (AEs), full details of which are presented in Tables 5, 6 and 7 in the Appendix. Initially two serious AEs (SAEs), oesophageal dilatation and skin infection, were reported but were subsequently downgraded in line with the current protocol (version 4.0, 1st August 2014) as they were considered to be complications of RDEB and not the cell infusions. Seventy-eight percent (127/163) of AEs were either unlikely or not related to the BM-MSC infusion, which were consistent with complications related to RDEB. With regard to the severity of AEs that were definitely, possibly or likely to be related to the MSC

infusions, 21/36 (58%) were mild, 13/36 (36%) were moderate, and 2/36 (6%) were severe, of which the two severe cases were DMSO odour, although some odour was noted following 28 of the 30 infusions and lasted for up to 48 hours. Mild nausea occurred during two infusions, abdominal pain and bradycardia were observed during two other infusions; all these AEs resolved within 15 minutes without treatment or haemodynamic compromise. The mild/moderate AEs included vomiting and pain on swallowing due to oesophageal strictures, corneal abrasions, recurrent spontaneous and trauma-induced blistering, wound infections and age-related accidental injuries. No AEs resulted in either discontinuation or reduction in the dose of the study drug. The intravenous administrations of BM-MSCs, including cannulation, were well tolerated. Likewise, the suction blister device and procedures caused no concerns or sequelae for the children.

7.3 Laboratory safety

Laboratory safety assessments did not reveal any adverse impact of the BM-MSCs on renal, liver or bone marrow function. We did not identify any rash or signs of allergic reaction during the infusions. Anti-C7 antibodies were detected by serum ELISA at baseline in 9/10 participants but none of these positive sera showed binding to the DEJ by IIF. Following MSCs, there were no changes in these ELISA or IIF data (Table 8). Skin biopsies revealed no increase in C7 deposition and no new formation of anchoring fibrils at Day 60 when compared to baseline. FISH analysis of skin specimens from four children who received sex-mismatched BM-MSCs taken on Day 60 did not show evidence of donor cell chimerism for sex-mismatched donor cells.

7.4 Clinical response

A summary of the clinical secondary outcome measures is shown in Table 9. BEBSS and global severity score (GSS) questionnaires were completed on all 10 participants (Figures 2 and 3). Pain, fatigue and pruritus scores were completed independently in separate

questionnaires for children over 6 years old (n=3) as well as by the parents. Mean parent-reported pain score was lower at 60 days than at baseline (difference in means: -5.5 points; 95% CI -16.3, 5.3); similar changes were seen at day 180 (difference in means -3.0 (-14.7, 8.7) (Figure 4). Change in mean disease severity (total BEBSS) was -5.2 points (95% CI -10.7, 0.3) and change in mean BEBSS total body surface area (TBSA%) was -5.9 points from baseline to Day 60 (-15.3, 3·5); similar changes were seen to 180 days for both BEBSS measures (Figure 5). Mean global severity score was 7.0 at baseline and 4.6 at Day 60 (mean difference: -2.4 (95% CI: -3.4, -1.4). Corresponding mean change at day 180 was -1.6 (-3.0, -0.24).

Mean quality of life score (higher is worse) reported by parents was 41·9 at baseline and 37·5 at Day 60 (difference: -4.4; 95% CI: -8.1, -0.7) and 39·0 at Day 180 (difference: -2.9; 95% CI: -7·5, 1·8) (Figure 6). Qualitative data (telephone interviews 9 months after the infusions) revealed positive impressions for better wound healing in all 10 subjects and for a lessening in skin redness in 9/10 (Table 10). Verbatim qualitative data is presented in Table 11.

Median blister counts at baseline, 60 and 180 days were 5.5, 3.5 and 3.5 respectively (Figure 9). Mean suction blister times were 10.2 at baseline and 11.9 at Day 100 (difference: 1.7; 95% CI: -0.5, 3.9); individual data are shown in Figure 9.

8. Discussion

We report a clinical trial of intravenous infusions of BM-MSCs in children with RDEB. Availability of BM-MSCs as a pre-manufactured, quality-controlled product without the need for HLA matching makes it a safe therapeutic option for children with this severe genetic skin condition. The administration of 1-3 million cells/kg in 3 infusions over 30 days was very well

tolerated, without significant AEs. Children (>6 years of age) and their parents reported increased speed of wound healing, reduction in blister numbers, reduction in pruritus, increased skin resistance to trauma and reduced pain during dressing changes. All of the parents reported improvement of their children's skin disease, more evident after the second or third infusions, and typically starting in the week following the second infusion. The degree and duration of clinical improvement was variable, usually ranging from 3-6 months after the first infusion, although the benefits in one child persisted for 12 months.

No increase in C7 deposition or the formation of new anchoring fibrils was seen at Day 60 after the first infusion. Thus there is no evidence to indicate that allogeneic MSCs directly restore the inherent skin pathology in RDEB. The mechanism of action through which the MSCs improve wound healing in RDEB is not known but the benefits appear to be indirect and trophic in nature. Conceptually, the anti-inflammatory effects of systemic MSC therapy may have clinical benefits in terms of better wound healing and less scarring, findings supported by other studies in RDEB that showed the helpful anti-inflammatory actions of ciclosporin and mycophenolate mofetil in RDEB (Del-Rio, 1993; El-Darouti et al., 2013b), notwithstanding the potential longer term implications of increased skin malignancy with those drugs, a complication not reported for MSCs.

The natural history of generalized RDEB is one of progressively worsening blistering, scarring and contractures; spontaneous improvement is very rare and limited to cases of bullous disease of the newborn, or subjects with atypical COL7A1 mutations that lead to leaky splice sites or in-frame exon skipping, or individuals who develop skin patches of revertant mosaicism, none of which were present in our trial participants. In this early phase trial safety was the primary outcome, therefore, it was not powered to determine efficacy and to demonstrate benefit. The changes observed in pain scores, BEBSS and BEBSS TBSA, while not conclusively indicating benefit, are promising and the results will inform the design of a definitive trial. With regard to qualitative data and potential clinical impact, parents noted

significant reduction in pruritus, and pain reductions that allowed children to bathe and perform other activities previously unthinkable due to painful wounds. Increased energy levels and improved appetites were also evident. The parents perceived skin redness, itching, skin resilience, wound healing and pain control were the key areas of noticeable change to their children's disease. Although healing of individual wounds can occur spontaneously in RDEB, in our study there was clinical improvement of the whole body surface area as well as objective increased suction blister times signifying increased skin resilience in 8/10 children. The rate of wound healing improved with chronically ulcerated areas of skin beginning to show signs of healing, often for the first time in months or years. The general improvement in skin condition, together with increase in skin resilience to trauma, enabled the children to participate more fully in play and family life.

The small sample size and the lack of a control group are limitations to this study. RDEB is a rare genetic skin disease with an incidence of 1 in 17,000 live births and therefore an underpowered study was justified with the trend of the results presented being more helpful in data interpretation of secondary outcome measures compared to absolute p-values. Inclusion of a control group raised both ethical and practical concerns: it was considered unethical for children to participate in a study in which they would receive a non-active substance and be subjected to skin biopsies and multiple blood tests. Moreover, the preservative in the BM-MSCs is dimethyl sulfoxide (DMSO), which produces an easily detectable odour shortly after infusion.

Aside from this trial, the only other study reporting both cutaneous and systemic positive outcomes for RDEB has been the report of whole BMT following myeloablation (Wagner *et al.,* 2010). However, there was a high mortality rate of >20% in that cohort. Reduced intensity conditioning regimens for BMT are being studied in other clinical trials although detailed safety and efficacy data for those treatments have not yet been published. There

were no safety concerns in the use of allogeneic BM-MSCs in children with RDEB in our trial and there were suggestions of clinical benefit. Infusion of allogeneic BM-MSCs is not a cure for RDEB but such intervention appears to provide a safe and potentially disease-modifying treatment until such a time that more curative therapies are developed.

Although further studies exploring the trophic benefits of allogeneic MSCs in ameliorating the clinical severity of RDEB are planned, other recent data have demonstrated that BM-MSCs contain a sub-population of cells that include epithelial progenitors capable of differentiation into keratinocytes (Tamai et al., 2011). These MSCs are platelet-derived growth factor receptor alpha (PDGFR-α) positive and are recruited to damaged skin by release of high mobility group box 1 (HMGB1) from hypoxic keratinocytes in RDEB blister roofs, with involvement of a stromal derived factor 1 alpha (SDF1-α) / C-X-C chemokine receptor type 4 (CXCR-4) signaling axis (Iinuma et al., 2015). Other studies have investigated pre-conditioning of MSCs for potential clinical benefit in RDEB. Notably, exposure of MSCs to TGF-β, TNF-α or SDF1-α has been shown to upregulate COL7A1 expression and C7 protein secretion in a time and concentration-dependent manner (Perdoni et al., 2014). Moreover, these cytokines also lead to increased MSC production of the anti-inflammatory protein TSG-6 that has already been implicated in the indirect trophic benefits of allogeneic MSCs (Pittenger, 2009). Thus future clinical trials are likely to assess systemic delivery of COL7A1-supplemented autologous RDEB MSCs, with possible pre-conditioning. In the interim, our current trial indicates that intravenous injections of allogeneic unmatched BM-MSCs, without any pre-conditioning, are both safe and appear to improve some of the clinical manifestations of RDEB.

9. References

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10. Appendix

Table 1(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/m²)	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 1A Baseline characteristics of subject A.

	Subject B
Age (years)	1
Sex	M
Body mass index (kg/m²)	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 1B 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 1C 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 1D 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 1E 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 1F 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 1G 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 1H Baseline characteristics of subject H.

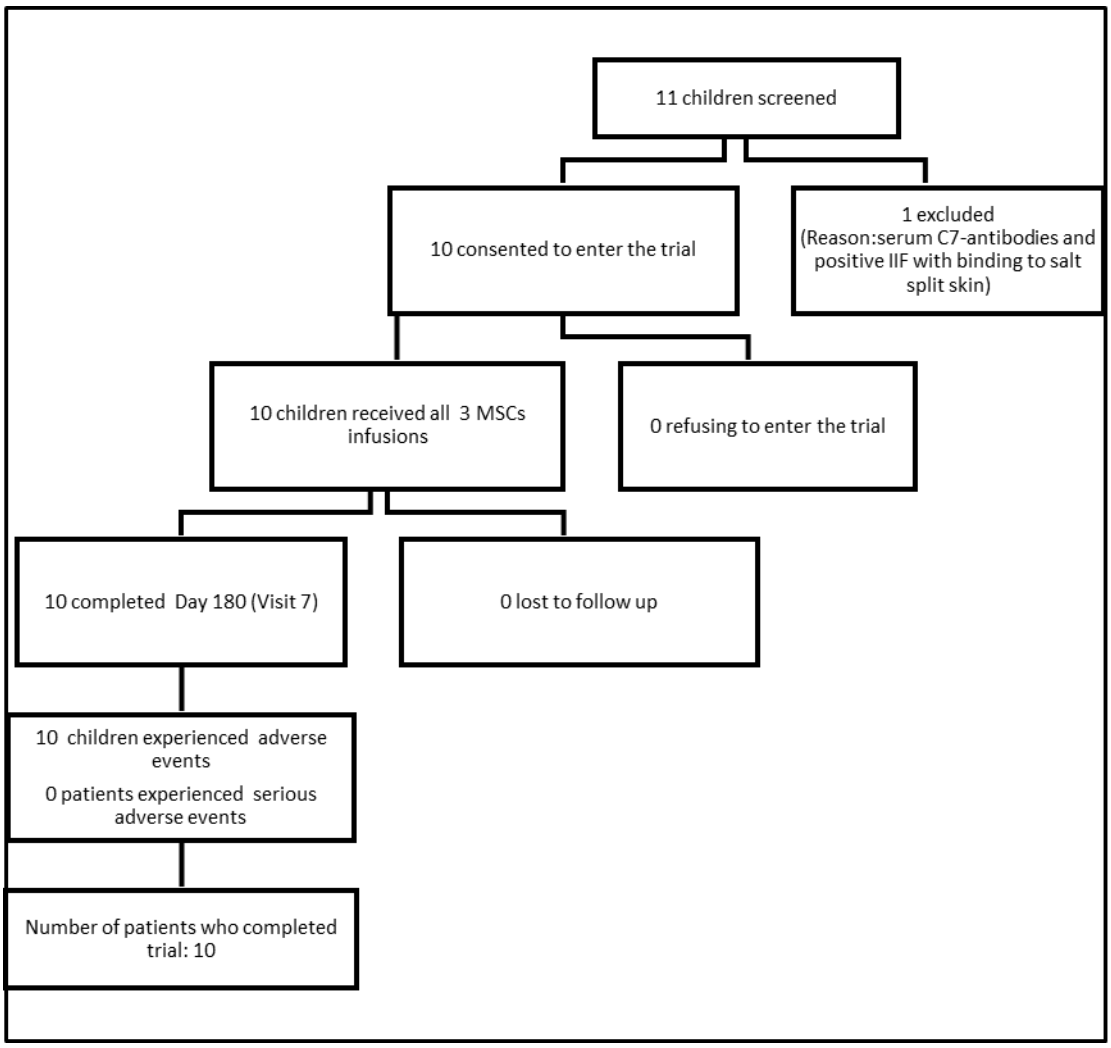
	Subject I
Age (years)	10
Sex	F
Body mass index (kg/m^2)	15
***COL7A1* mutation**	IVS23-2A>G; c.4317delC; p.Pro1441LeufsX271, exon 39
Skin C7 protein expression	-
BEBSS	35
BEBSS TBSA (%)	28
GSS	7
Blister count	5
Pain score: Child version (≥6 years)	34
Pain score: Parent version	19
Fatigue score: Child 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 1I 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 1J Baseline characteristics of subject J.

Figure 1. 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 2. 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 3. 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	X	X	X	X	X	X	X

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**
questionnaire							
Adverse event assessment	X	X	X	X	X	X	X
Concomitant medication assessment	X	X	X	X	X	X	X

Table 4. 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 5. 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 6. 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 7. 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	Oesophageal 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 8. 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	–	–	–

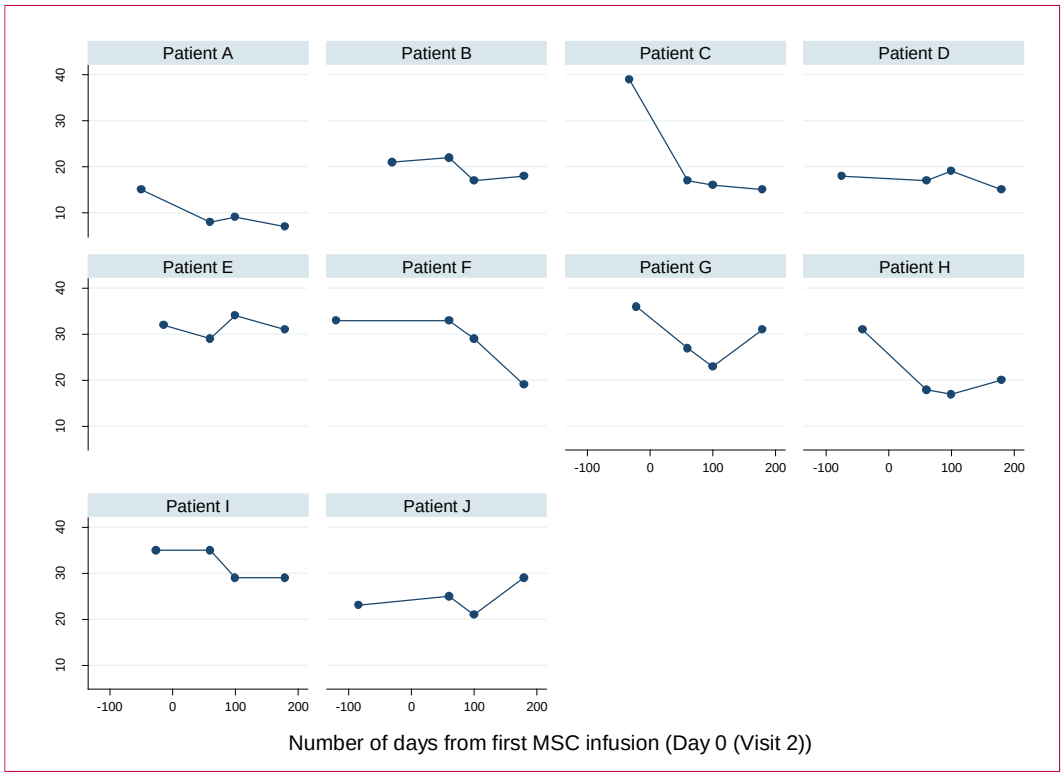
The negative cut-off values were: BP180 antibody <20 U; BP230 antibody <10 U; C7 antibody <6 U.

Table 9. 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™: Pediatric quality of life; * PedsQL™ child version for children over 5 years; ** PedsQL™ parent version for children over 2 years; §Child version of the Pain sleep and fatigue questionnaire for children > 6 years.

Figure 2. 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).



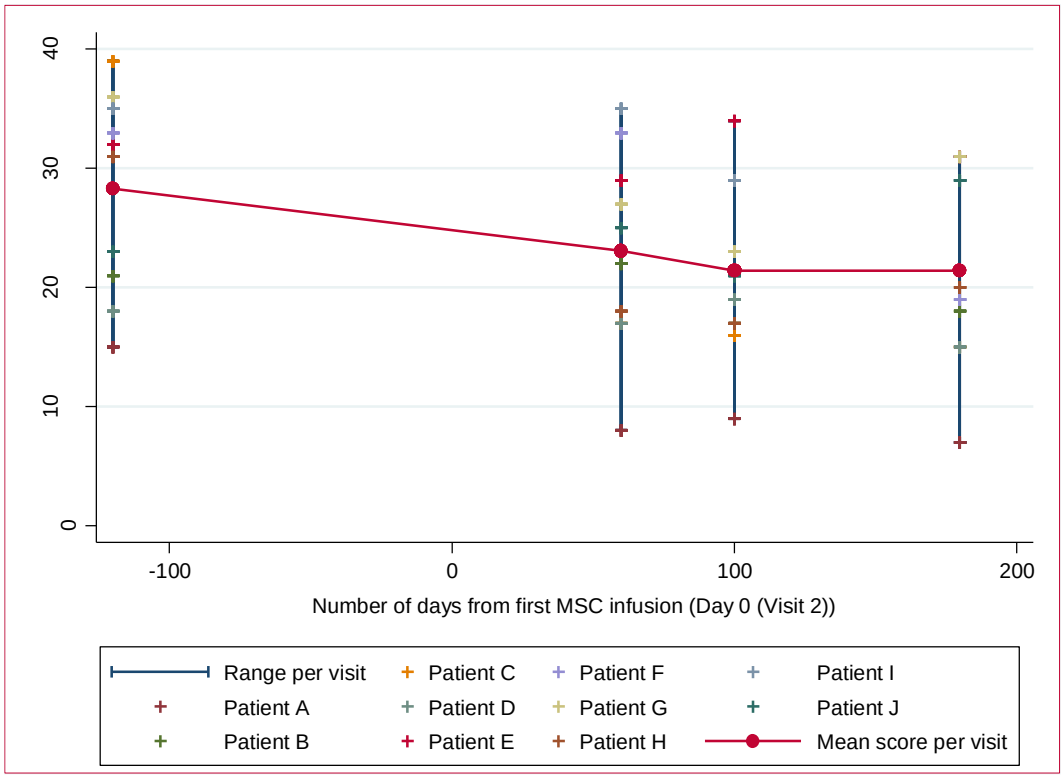
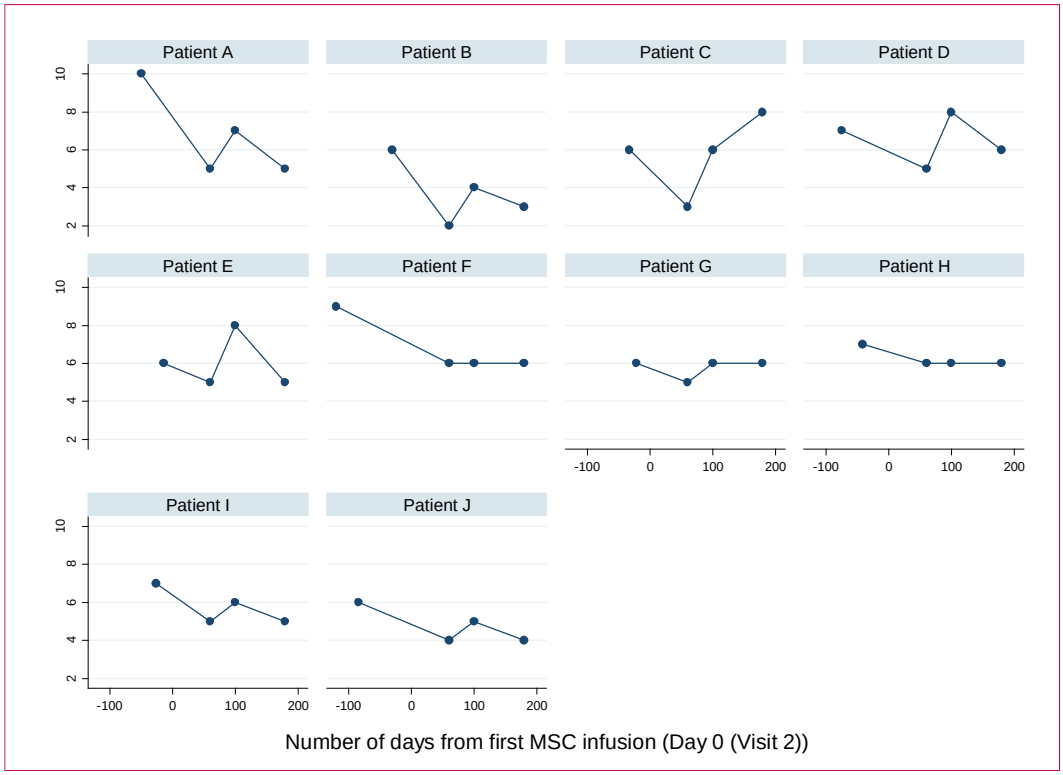


Figure 3. Global Severity Scores for each patient (N=10) by number of days from first MSC infusion (top); distribution of global severity scores, with means and range per visit by number of days from first MSC infusion (N=10) (bottom).



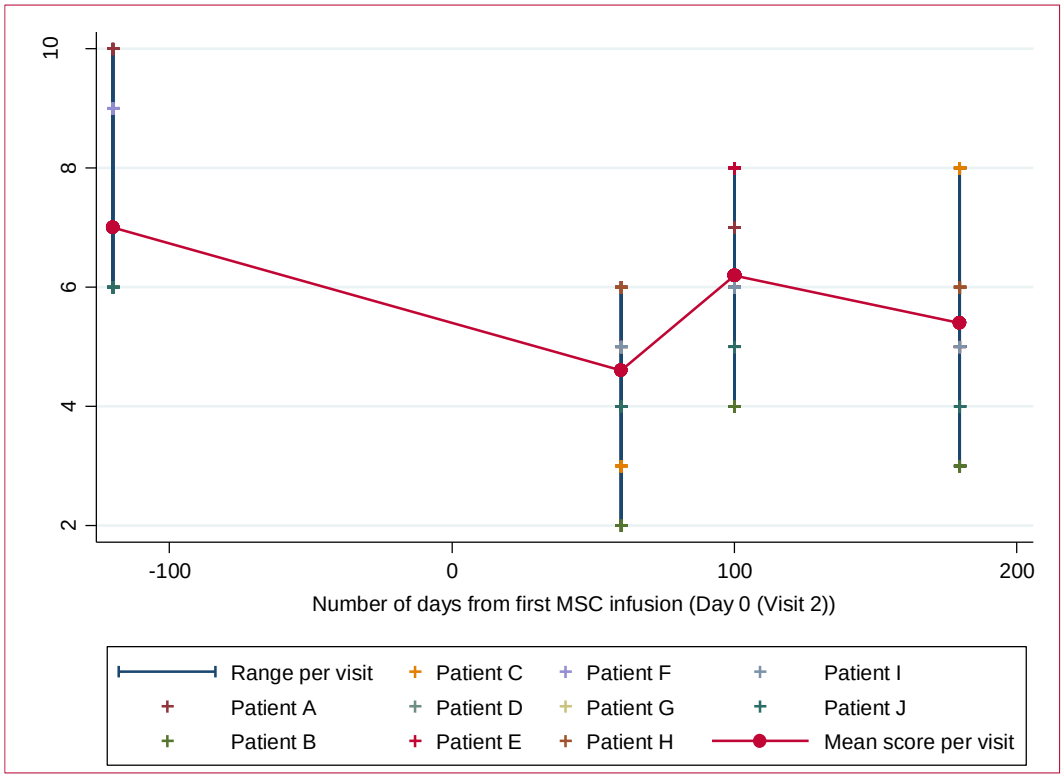
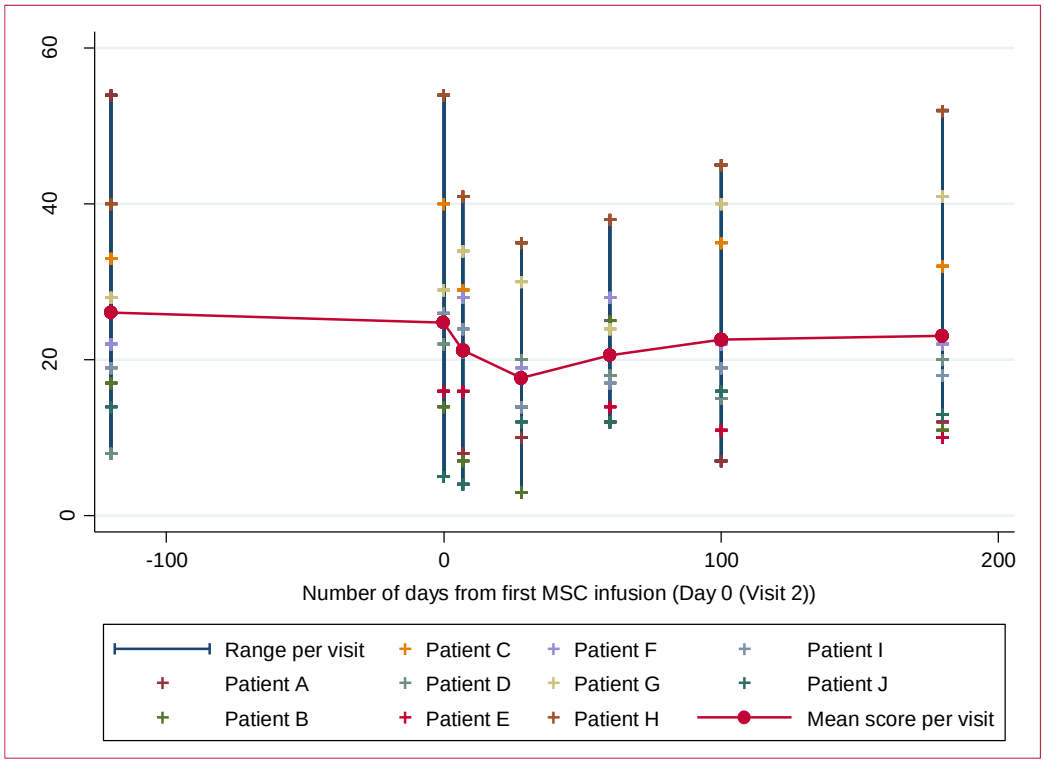
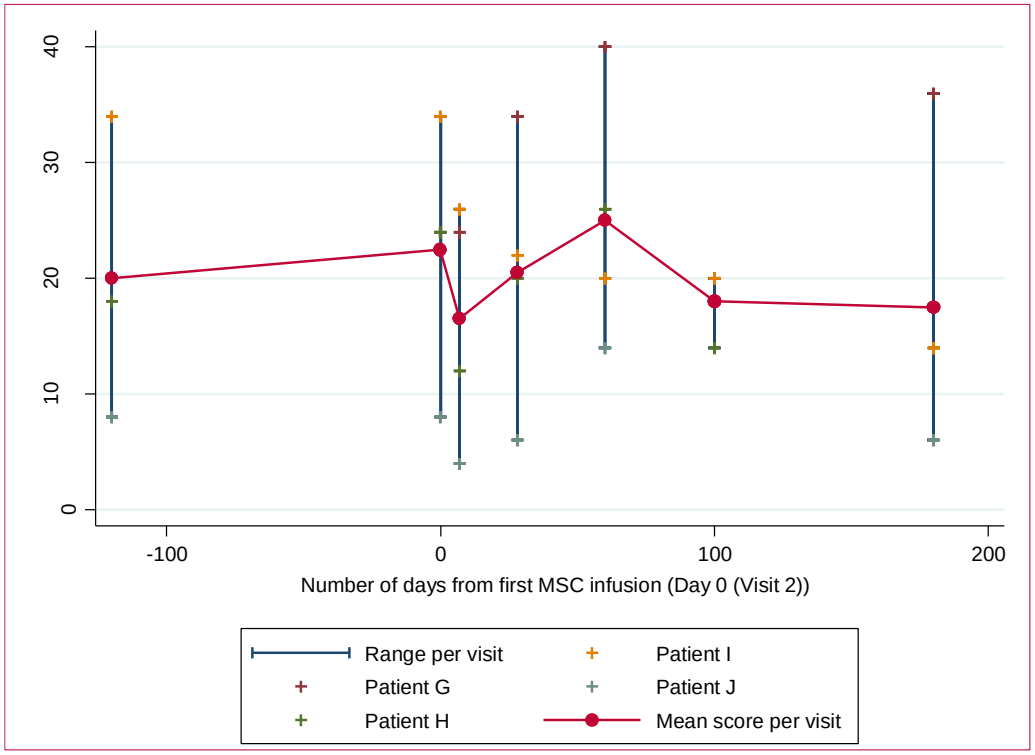


Figure 4. Parent and child versions of pain scores from Pain, Sleep and Fatigue Questionnaire. Top = parent: Graph showing distribution of scores with means and range by number of days from first MSC infusion (N=10). Bottom = child: Graph showing distribution of scores with means and range by number 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 5. 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.

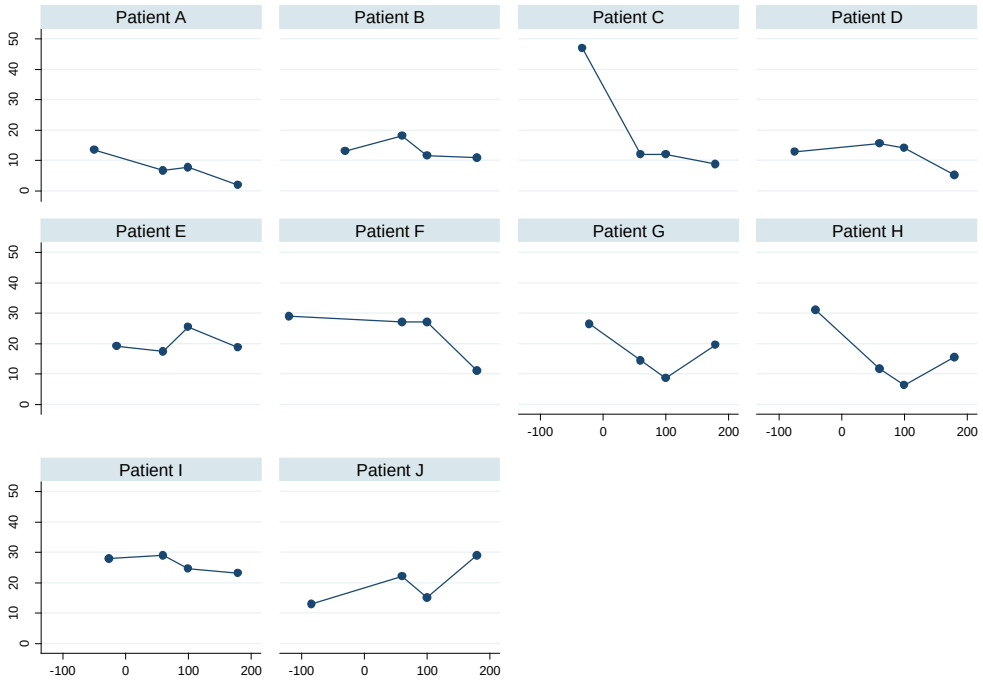
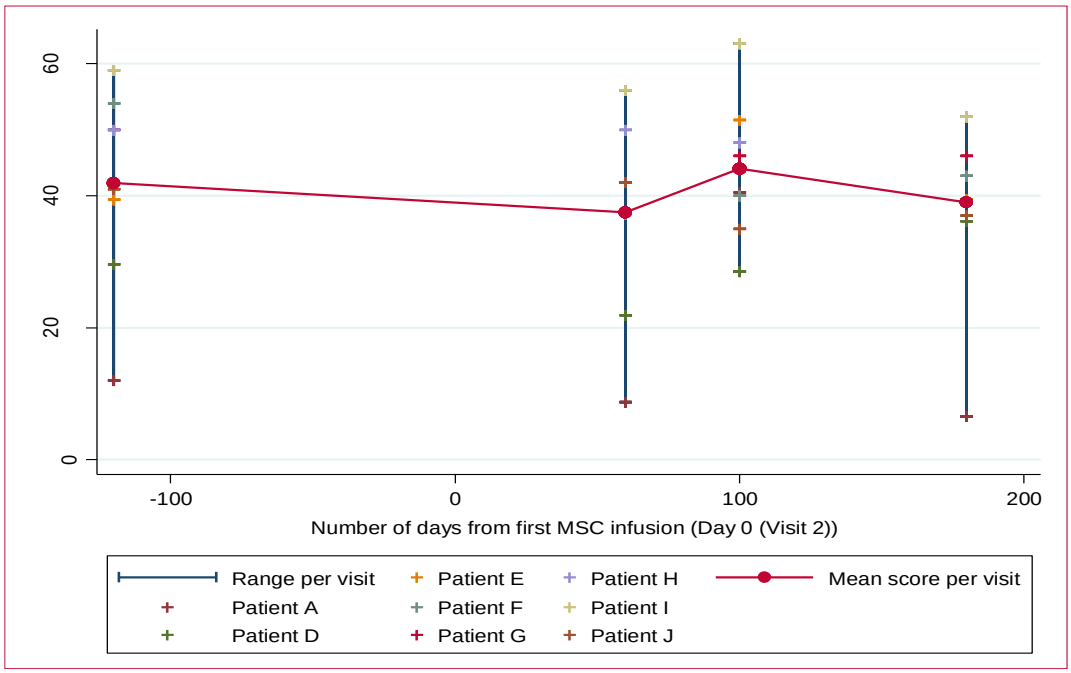


Figure 6. Parent version of pediatric quality of life scores (PedsQL) showing distribution of scores with means and range by number of days from first MSC infusion (N=8)*
*PedsQL parent version can only be completed for children over 2 years.



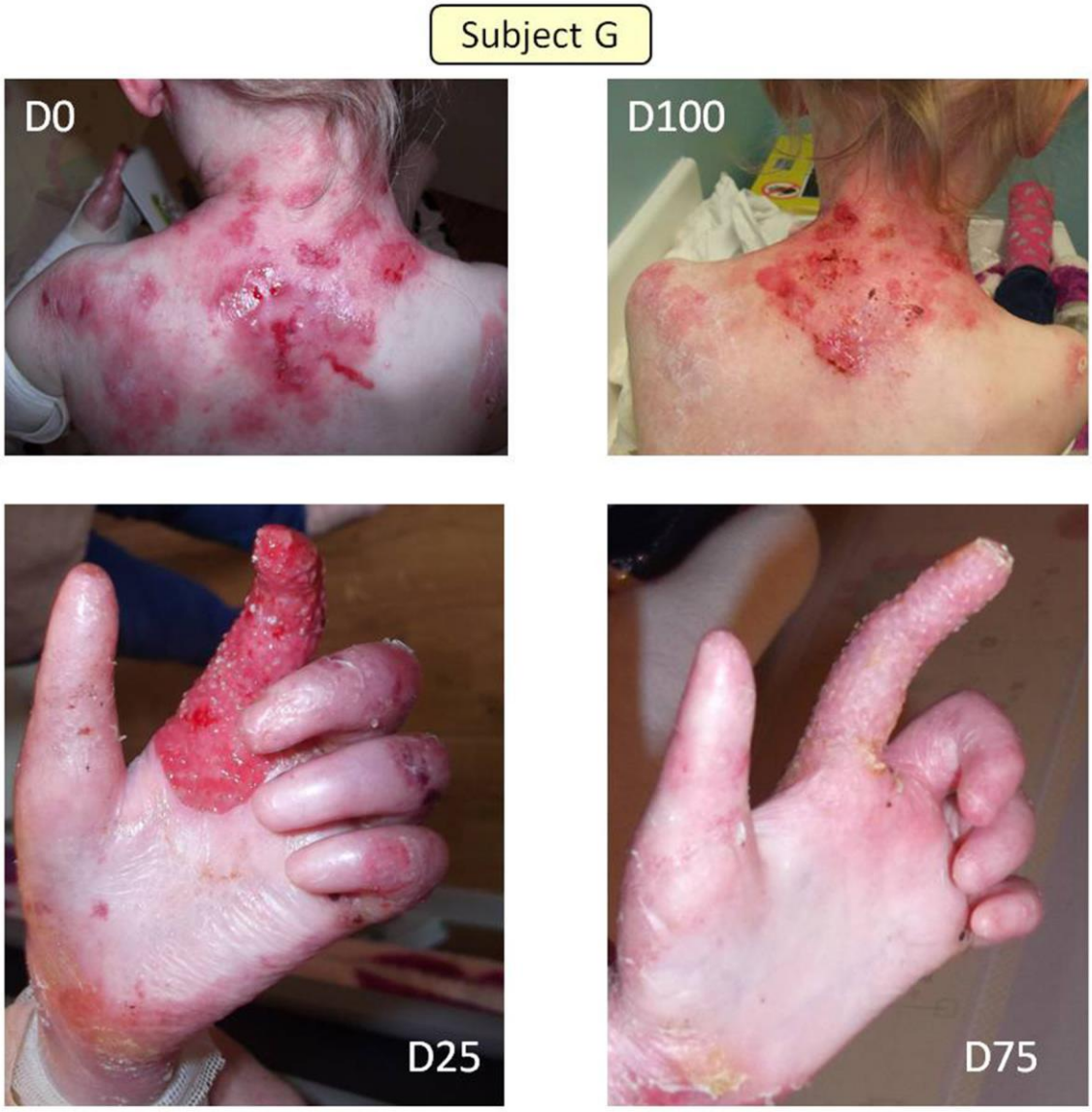


Figure 7. Clinical appearances in Subject G following BM-MSCs.

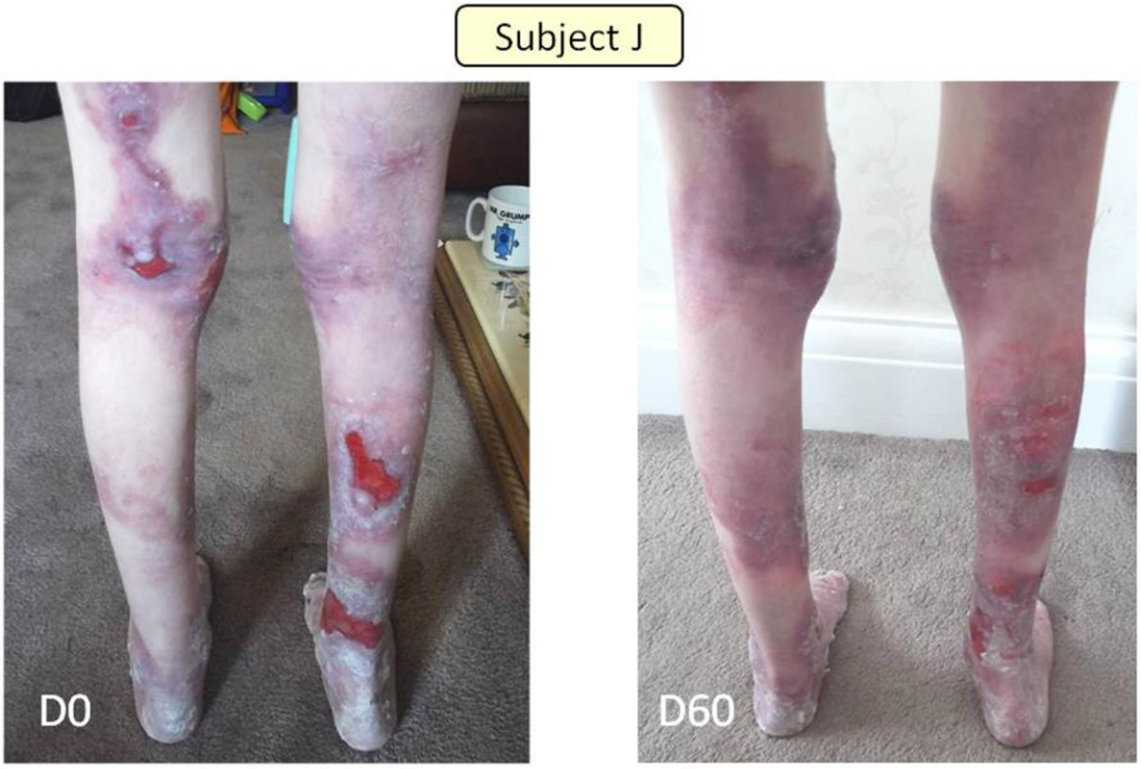


Figure 8. Clinical appearances in Subject J following BM-MSCs.

Table 10. 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 11. 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 9. 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).