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| Complete List of Authors: | Petrof, Gabriela; King's College London, St John's Dermatology<br>Lwin, Su; King's College London, St John's Dermatology<br>Martinez-Queipo, Magdalena; King's College London, St John's Dermatology<br>Abdul-Wahab, Alya; King's College London, St John's Dermatology<br>Tso, Simon; King's College London, St John's Dermatology<br>Mellerio, Jemma; King's College London, St John's Dermatology; Great Ormond Street Hospital for Children NHS Foundation Trust, Dermatology<br>Slaper-Cortenbach, Ineke; University Medical Centre Utrecht, Cell Therapy Facility<br>Boelens, Jaap; University Medical Centre Utrecht, Pediatrics<br>Tolar, Jakub; University of Minnesota, Department of Pediatrics<br>Veys, Paul; Great ormond Street Hospital for Children NHS Foundation Trust, Blood and Marrow Transplantation<br>Ofuya, Mercy; King's College London, Health and Social Care Research<br>Peacock, Janet; King's College London, Health and Social Care Research<br>Martinez, Anna; Great Ormond Street Hospital for Children NHS Foundation Trust, Dermatology<br>McGrath, John; St. John's Institute of Dermatology, Genetic Skin Disease Group; |
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# Potential of systemic allogeneic mesenchymal stromal cell therapy for children with recessive dystrophic epidermolysis bullosa

Gabriela Petrof,[1] Su M. Lwin,[1] Magdalena Martinez-Queipo,[1] Alya Abdul-Wahab,[1] Simon Tso,[1] Jemima E. Mellerio,[1,2] Ineke Slaper-Cortenbach,[3] Jaap Jan Boelens,[4] Jakub Tolar,[5,6] Paul Veys,[7] Mercy Ofuya,[8] Janet L. Peacock,[8] Anna E. Martinez,[2] John A. McGrath[1,*]

[1]St John's Institute of Dermatology, King's College London (Guy's Campus), London, United Kingdom

[2]Department of Dermatology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom

[3]Cell Therapy Facility, Department of Clinical Pharmacology, University Medical Centre Utrecht, Utrecht, The Netherlands

[4]Department of Pediatrics, Blood and Marrow Transplantation Program, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands

[5]Department of Pediatrics, Blood and Marrow Transplant, Medical School, University of Minnesota, Minneapolis, Minnesota, USA

[6]Stem Cell Institute, University of Minnesota, Minneapolis, Minnesota, USA

[7]Blood and Marrow Transplantation Department, Great Ormond Street Hospital, London, United Kingdom

[8]Division of Health and Social Care Research, King's College London, United Kingdom

*Correspondence to: John A McGrath, St John's Institute of Dermatology, Floor 9 Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, United Kingdom.

Email: john.mcgrath@kcl.ac.uk

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Abbreviations: RDEB, recessive dystrophic epidermolysis bullosa; BM-MSCs, bone marrow mesenchymal stromal cells; CI, confidence interval.

## SUMMARY

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. 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.

## INTRODUCTION

Epidermolysis bullosa (EB) is a heterogeneous group of inherited disorders characterized by trauma-induced skin blistering and mucosal fragility; approximately 500,000 people worldwide have EB (Fine *et al.,* 2014). One of the most clinically severe subtypes of EB is the recessive dystrophic variant (RDEB) which results from bi-allelic mutations in *COL7A1* leading to reduced or absent basement membrane type VII collagen (C7) and rudimentary or absent anchoring fibrils at the dermal-epidermal junction (DEJ) (Hilal *et al.,* 1993). Poor anchoring fibril function leads to lifelong severe blistering and skin erosions following minor mechanical trauma (Fine, 2013). Currently, there is no effective treatment for RDEB and many individuals develop life-shortening squamous cell carcinomas by the age of 40 years (Fine and Mellerio, 2009a). RDEB is also associated with a considerable health economic burden; for example, wound dressings for a 10-year old child with RDEB have been estimated to cost up to $680 per day (Kirkorian *et al.,* 2014), which equates to >$250,000 per year just for skin care alone.

In the past 5 years, considerable progress has been made in testing innovative treatments for RDEB, including gene, cell, protein, and drug therapy (for review see Hsu *et al.,* 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.,* 2013a), the latter in abstract form only. A clinical trial of *ex vivo COL7A1* gene therapy with grafting of corrected keratinocytes is currently being evaluated (Siprashvili *et al.,* 2014) and criteria to

optimize RDEB subject selection for further clinical trials have been proposed (Gorell *et al*., 2015). From a clinical perspective, it is clear that the most effective therapies for RDEB will need to be given early in life, and probably delivered systemically, in view of the extent of the skin and mucous membrane pathology present in the generalized forms of RDEB.

Our interest is in exploring the potential of intravenously administered MSCs to improve wound healing in RDEB. MSCs represent a heterogeneous collection of connective tissue cells that can undergo self-renewal and also have the capability of differentiating into mesenchymal lineage cell types including bone, cartilage, adipose tissue, and muscle (Caplan, 1991). In addition, MSCs have non-progenitor functions, with roles in immune regulation, cell-growth adjustment, and structural and functional tissue repair (Phinney and Prockop, 2007), including skin wounds (Chen *et al.,* 2008; Prockop 2009; Tolar *et al.,* 2010; Tolar *et al.,* 2011). Nevertheless, tissue repair is not typically associated with the presence of a large number of therapeutic MSCs in the injured tissues, suggesting possible benefits through paracrine secretions or cell-cell contacts that modulate inflammatory and immune responses (Baraniak and McDevitt, 2010). Indeed, the inflammatory microenvironment can regulate the paracrine activity of MSCs and secreted mediators may have a role in the damaged target tissues or organs (Weil et al., 2009; Nauta and Fibbe 2007; Walter *et al.,* 2010; Bianco *et al.,* 2013; Fibbe *et al.,* 2013). In the inflammatory microenvironment, MSCs can produce at least 11 soluble cytokines: TNF-α-stimulated gene-6 (TSG-6), hepatocyte growth factor (HGF), transforming growth factor β (TGF-β), prostaglandin E2 (PGE2), interleukin-6 (IL-6), IL-10, IL-1 receptor antagonist (IL-1RA), inducible NO synthase, indoleamine 2,3-dioxygenase (IDO), galectin-1 (Gal-1), and human leucocyte antigen G (HLA-G) (Pittenger, 2009). This collection of cytokines has the

capacity to suppress inflammation and injury and underscores the widespread therapeutic evaluation of MSCs for damaged tissues and organs, even if the precise mode of repair has still to be defined in detail. Indeed, there are 250 ongoing clinical trials using MSCs for specific disease indications listed on www.clinicaltrials.gov.

Although the skin blistering in RDEB is primarily induced by trauma, the failure of wounds to heal quickly and the 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 (Fine and Mellerio, 2009b). 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). 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 6 years of age (Shivaswamy *et al.,* 2009). Thus innovative therapies that reduce skin inflammation in RDEB may potentially have positive clinical benefits in reducing disease burden. Assessing the safety and potential benefit of intravenous infusions of allogeneic BM-MSCs to children with RDEB is the subject of our study.

## RESULTS

### 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 S1 online. 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.

**Clinical safety**

There were a total of 163 adverse events (AEs) full details of which are presented in Tables S2, S3 and S4 online. Initially two serious AEs (SAEs), esophageal 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 odor, although some odor 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 hemodynamic compromise. The mild/moderate AEs included vomiting and pain on swallowing due to esophageal 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.

**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 reactions 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 S5 online). 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.

**Clinical response**

A summary of the clinical secondary outcome measures is shown in Table 2. BEBSS and global severity score (GSS) questionnaires were completed on all 10 participants (Figures S1 and S2 online). 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 S3 online). 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 S4 online). 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 S5 online). 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 (Figure 2). These data are presented in Table S6 online with verbatim accounts recorded in Table S7 online.

Median blister counts at baseline, 60, 180 days were 5.5, 3.5, and 3.5 respectively (Figure S6 online). 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 3.

## 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 well tolerated and 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 recover 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 odor 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; Tolar and Wagner, 2013). 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.



## MATERIALS AND METHODS

Additional methods are provided in the Supplementary Material.

### 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. Full inclusion and exclusion criteria are listed in Supplementary Table S8 online.

**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 S9 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 1–3x$10^6$ 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). Suction blister times were performed at screening and at Day 100 using a negative pressure device (Electronic Diversities, MD, USA).

**Statistics**

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 presented as means, 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). Additional statistical analysis information is detailed in the Supplementary Material.

**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 as detailed in Table S6 online.

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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.

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**Figures and Tables**

**Figure 1.** Trial profile

**Figure 2.** Improved wound healing and reduced skin erythema 8 weeks after the third infusion of BM-MSCs.

**Figure 3**. Suction blister times for each subject at baseline (Day -120) and 100 days after the MSC infusion.

**Table 1**. Baseline characteristics.

**Table 2.** Secondary outcome measures.

**Table 1** Baseline characteristics.

BEBSS: Birmingham Epidermolysis Bullosa Severity Score, scale range: 0-100: TBSA: Total Body Surface Area; 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 | Subject B | Subject C | Subject D | Subject E | Subject F | Subject G | Subject H | Subject I | Subject J |
|---|---|---|---|---|---|---|---|---|---|---|
| **Clinical characteristics** | | | | | | | | | | |
| **Age (years)** | 1 | 1 | 1 | 1 | 4 | 7 | 5 | 7 | 10 | 11 |
| **Sex** | M | M | M | F | M | F | F | F | F | M |
| **Body mass index (kg/m$^2$)** | 17 | 15 | 15 | 17 | 15 | 13 | 14 | 12 | 15 | 14 |
| **Molecular characteristics** | | | | | | | | | | |
| ***COL7A1* DNA mutation** | (+/-) c.425A>G, p.Lys142Arg, exon 3; (+/-) c.1939C>G, p.Ser609X, exon 14 | (+/-) c.425A>G, p.Lys142Arg, exon 3; (+/-) IVS5+1G>A | (+/-) c.3840delC, p.Thr1280fsX 33, exon 31; (+/-) c.4037delA, p.Lys1346fsX 51, exon 34 | (+/-) c.1573C>T; p.Arg525X exon 12. (+/-) IVS79+1G>C | (+/-) c.3293delAC, p.Tyr1098fsX 1, exon 25; (+/-) c.4894C>T, p.Arg1632X, exon 51 | (+/-) c.4621delG, p.Gly1541fsX 67, exon 46 | (+/-) c.1732C>T, p.Arg578X, exon 13; (+/-) c.5047C>T, p.Arg1683X, exon 54 | (+/-) c.409C>T, p.Arg137X, exon 3; (+/-) c.6269delC, exon 75 | IVS23-2A>G; c.4317delC; p.Pro1441Leu fsX271, exon 39 | (+/+) c.7787delG, p.Gly2596fsX 34, exon 104 |
| **Skin C7 protein expression** | - | + | +/- | - | - | - | - | - | - | - |
| **Disease Severity** | | | | | | | | | | |
| **BEBSS** | 15 | 21 | 39 | 18 | 32 | 33 | 36 | 31 | 35 | 23 |
| **BEBSS TBSA (%)** | 13·5 | 13 | 47 | 12·8 | 19 | 29 | 26·5 | 31 | 28 | 13 |
| **Global severity score** | 10 | 6 | 6 | 7 | 6 | 9 | 6 | 7 | 7 | 6 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Blister count** | 6 | 1 | 3 | 2 | 6 | 19 | 22 | 6 | 5 | 2 |
| **Pain sleep and fatigue questionnaire** | | | | | | | | | | |
| **Pain score - Child version (≥6 years)** | NA | NA | NA | NA | NA | NA | NA** | 18 | 34 | 8 |
| **Pain score – Parent version** | 17 | 17 | 33 | 8 | 26 | 22 | 28 | 40 | 19 | 14 |
| **Fatigue score - Child version (≥6 years)** | NA | NA | NA | NA | NA | NA | NA | 2 | 6 | 2 |
| **Fatigue score - Parent version** | 3 | 2 | 0 | 1 | 6 | 4 | 5 | 5 | 3 | 1 |
| **Pruritus score - Child version (≥6 years)** | NA | NA | NA | NA | NA | NA | NA | 8 | 8 | 4 |
| **Quality of life questionnaire** | | | | | | | | | | |
| **PedsQL score (Child version )** | NA | NA | NA | NA | NA | 4 | 44 | 32 | 47 | 35 |
| **PedsQL score (Parent version)** | 12 | NA | NA | 30 | 39 | 54 | 50 | 50 | 59 | 41 |

**Table 2** 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.

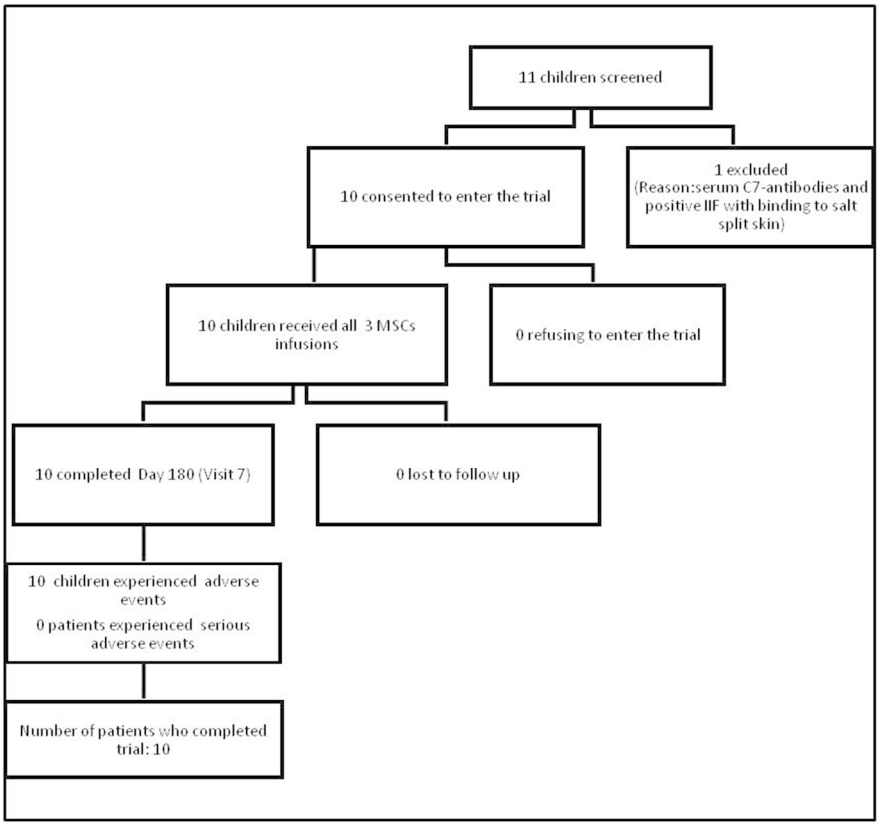



Figure 1. Trial profile
167x146mm (300 x 300 DPI)

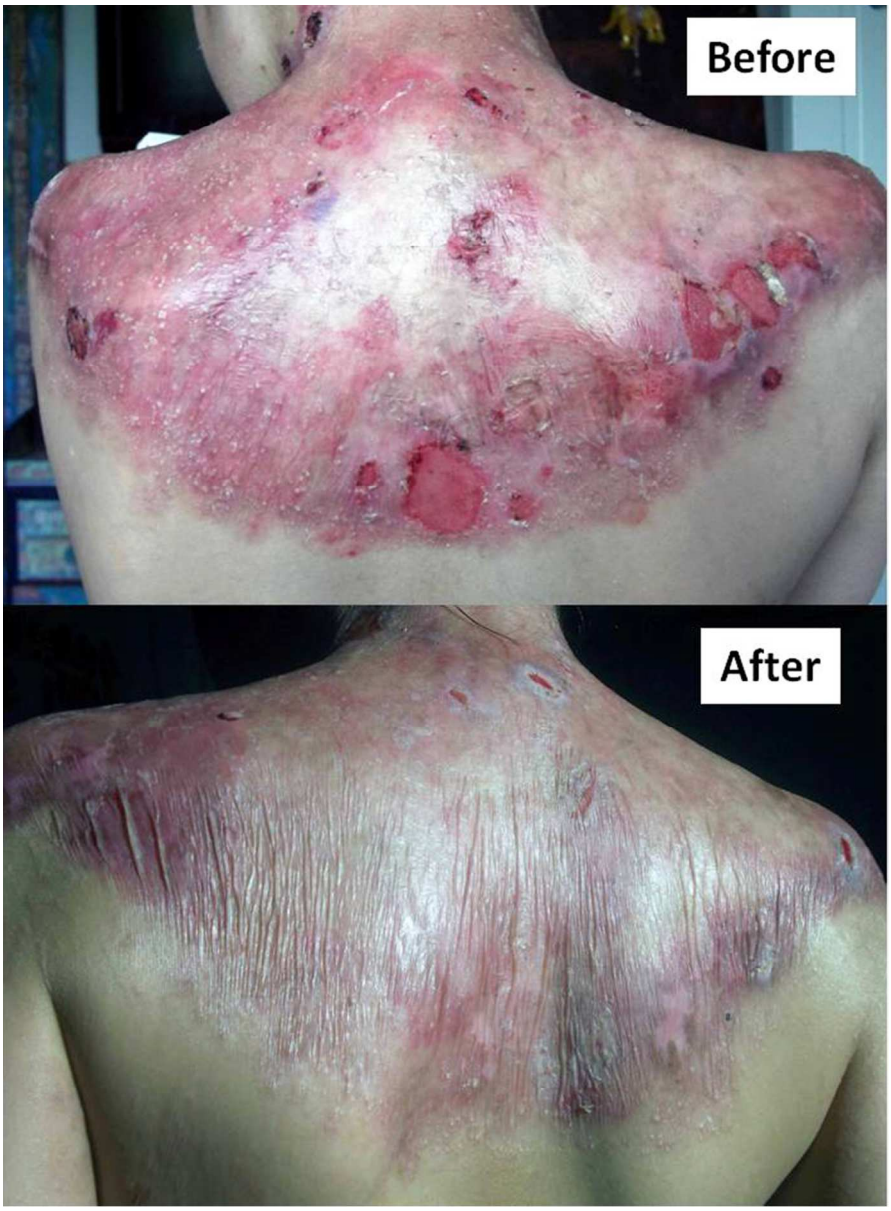



Figure 2. Improved wound healing and reduced skin erythema 8 weeks after the third infusion of BM-MSCs.
119x161mm (300 x 300 DPI)

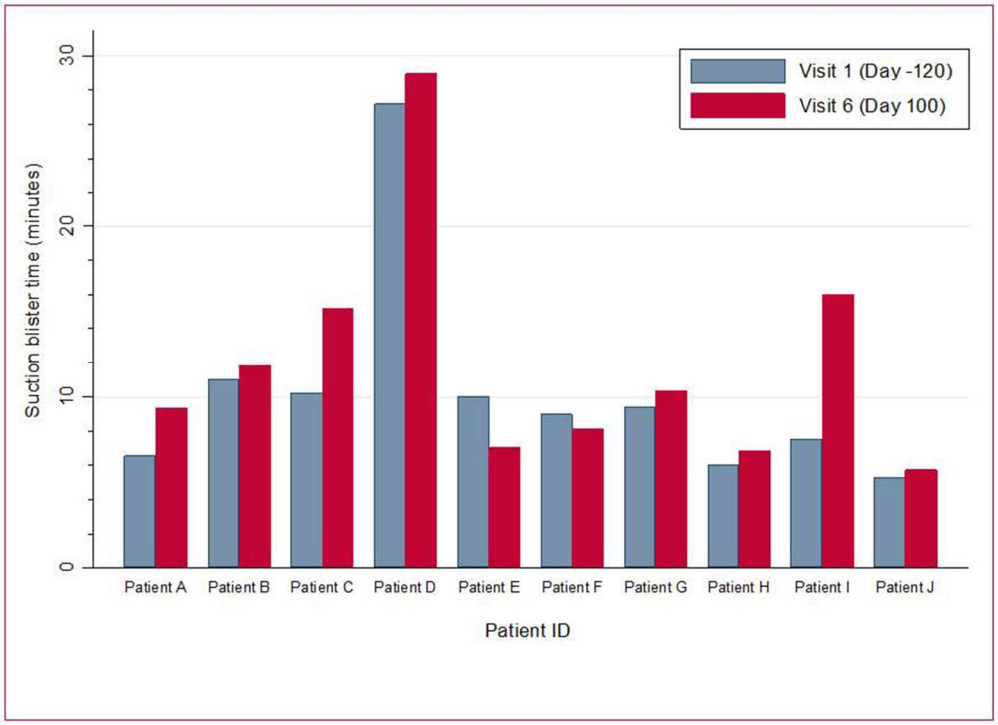



Figure 3. Suction blister times for each subject at baseline (Day -120) and 100 days after the MSC infusion.
148x112mm (300 x 300 DPI)

**SUPPLEMENTARY MATERIAL**

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

Gabriela Petrof,[1] Su M. Lwin,[1] Magdalena Martinez-Queipo,[1] Alya Abdul-Wahab,[1] Simon Tso,[1] Jemima E. Mellerio,[1,2] Ineke Slaper-Cortenbach,[3] Jaap Jan Boelens,[4] Jakub Tolar,[5,6] Paul Veys,[7] Mercy Ofuya,[8] Janet L. Peacock,[8] Anna E. Martinez,[2] John A. McGrath[1,*]

**Figure S1.** 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).

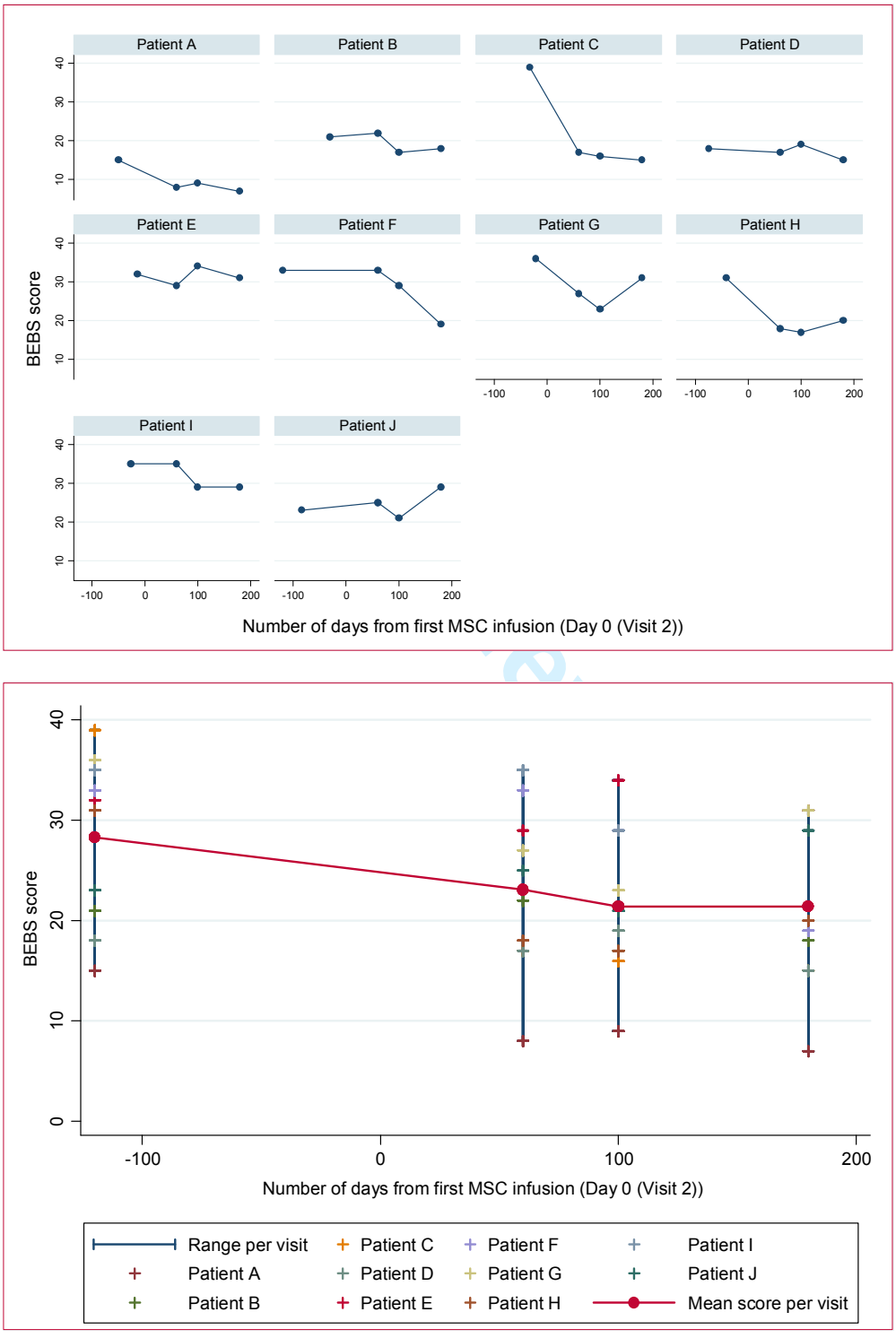


**Figure S2.** Global Severity Scores for each patient (N=10) by number of days from first MSC infusion (top); distribution of global severity scores, with means and range per visit by number of days from first MSC infusion (N=10) (bottom).

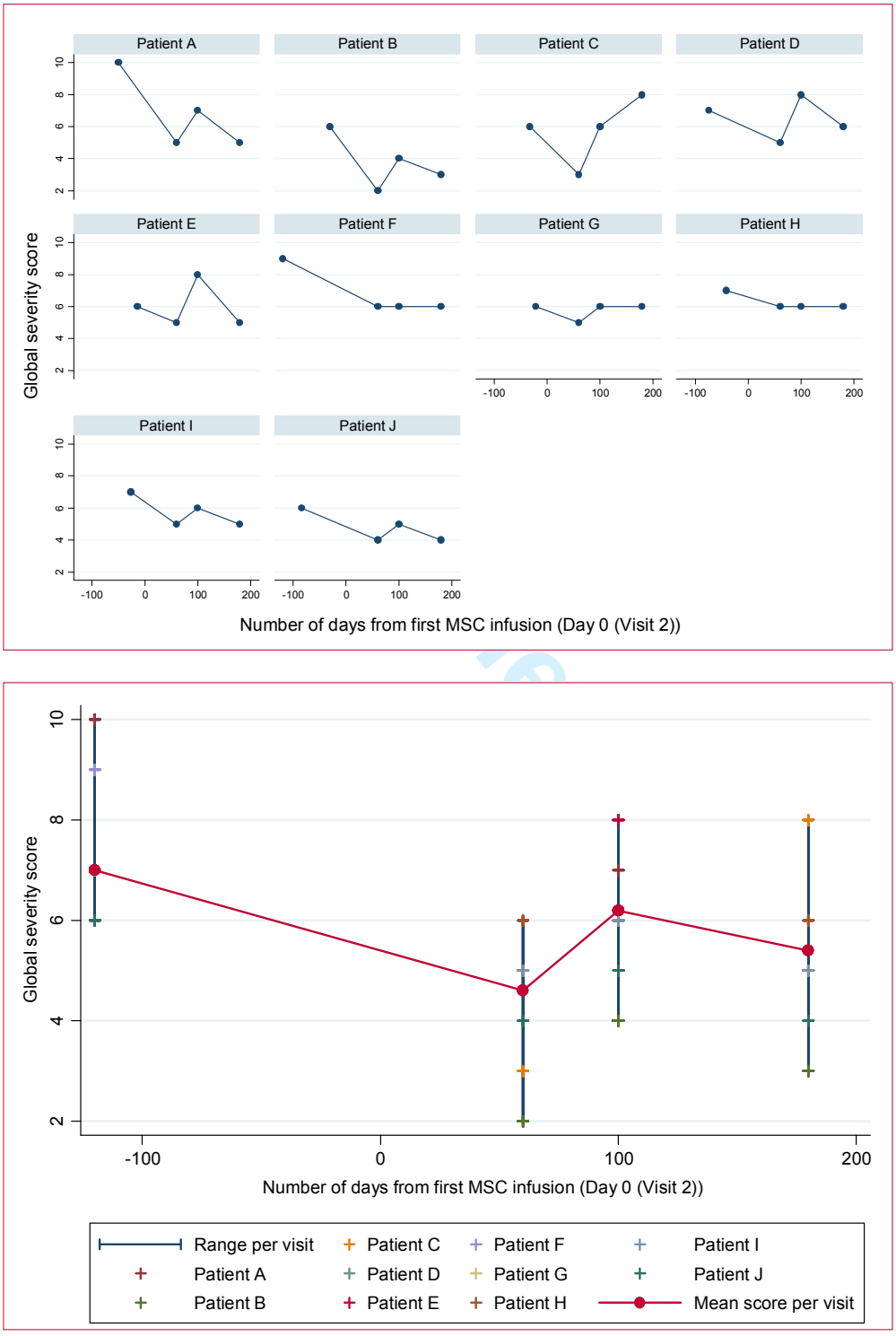
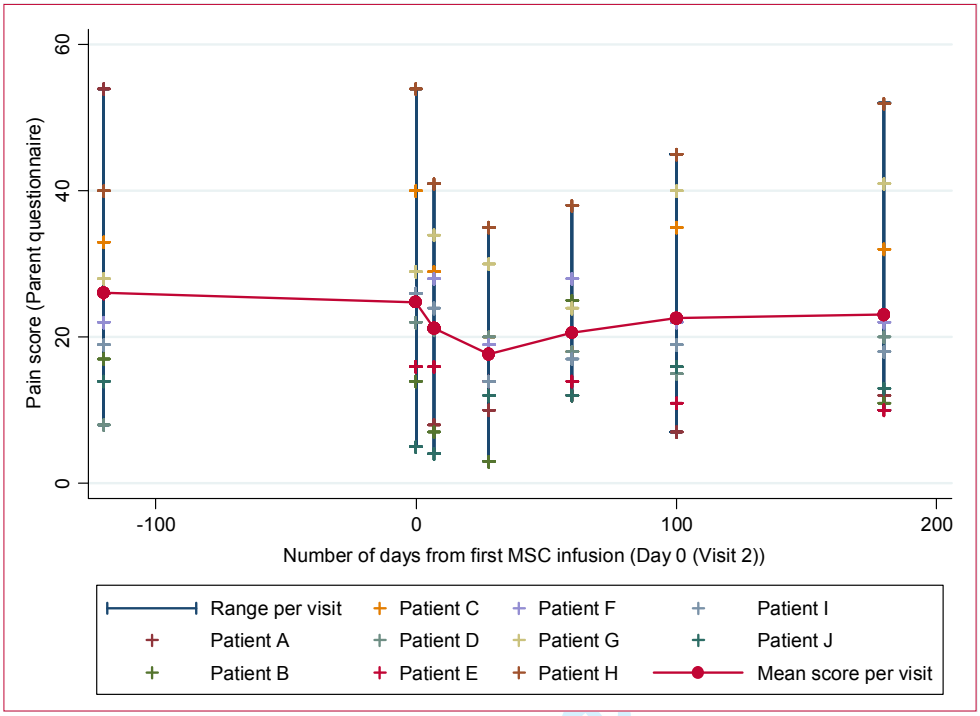
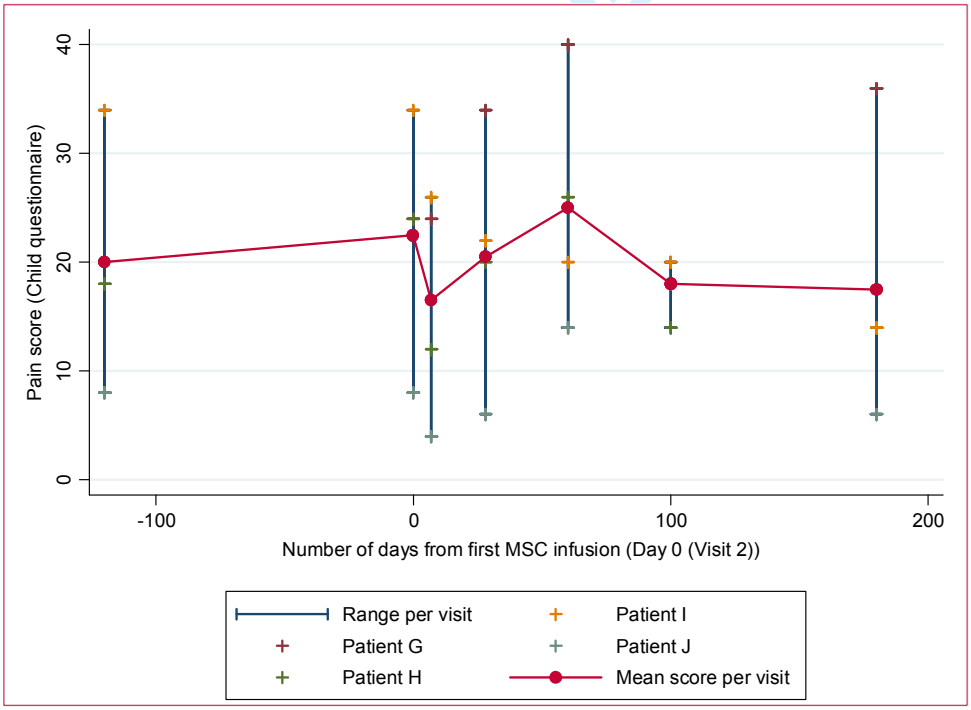


**Figure S3.** Parent and child versions of pain scores from Pain, Sleep and Fatigue Questionnaire. Top = parent: Graph showing distribution of scores with means and range by number of days from first MSC infusion (N=10). Bottom = child: Graph showing distribution of scores with means and range by number 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 S4**. 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.

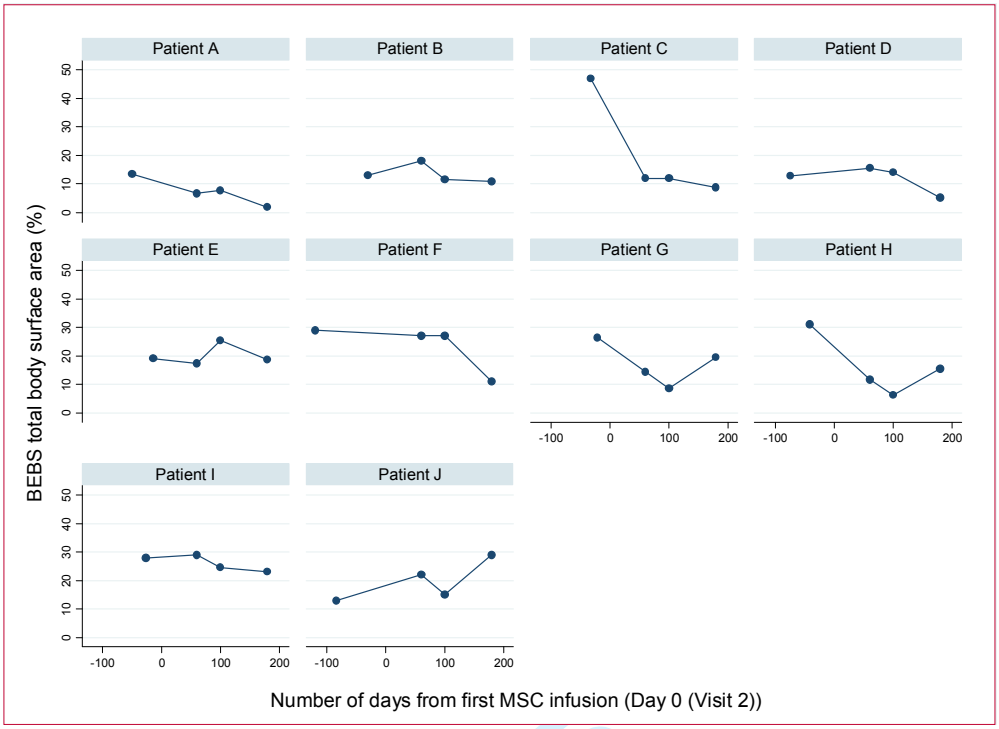


**Figure S5.** Parent version of pediatric quality of life scores (PedsQL) showing distribution of scores with means and range by number of days from first MSC infusion (N=8)*
*PedsQL parent version can only be completed for children over 2 years.

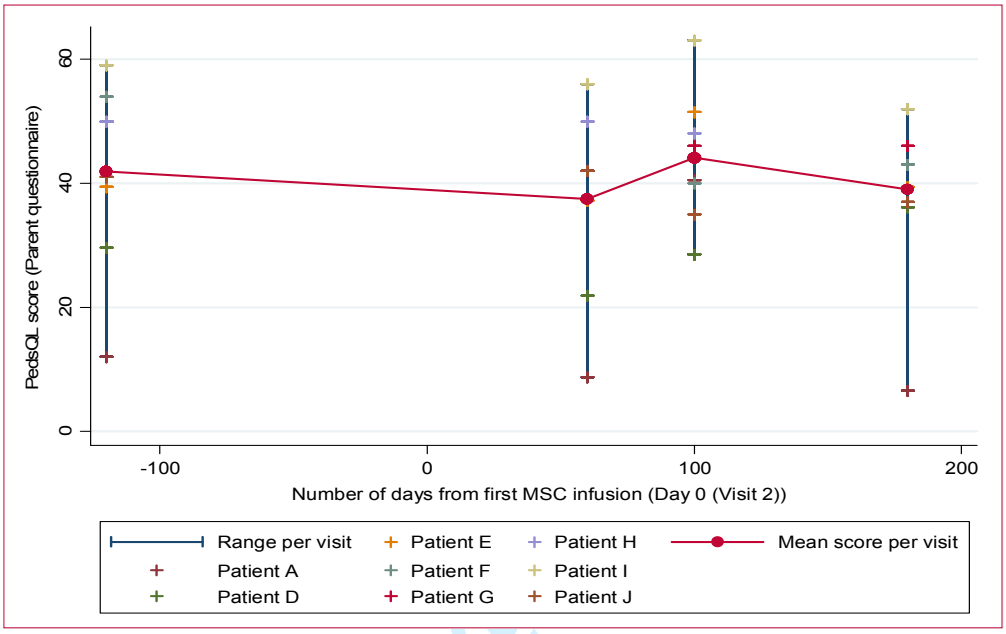


**Figure S6.** Distribution of blister count for each patient (N=10) by number of days from first MSC infusion (top); distribution of blister count with means and range per visit by number of days from first MSC infusion (N=10) (bottom).

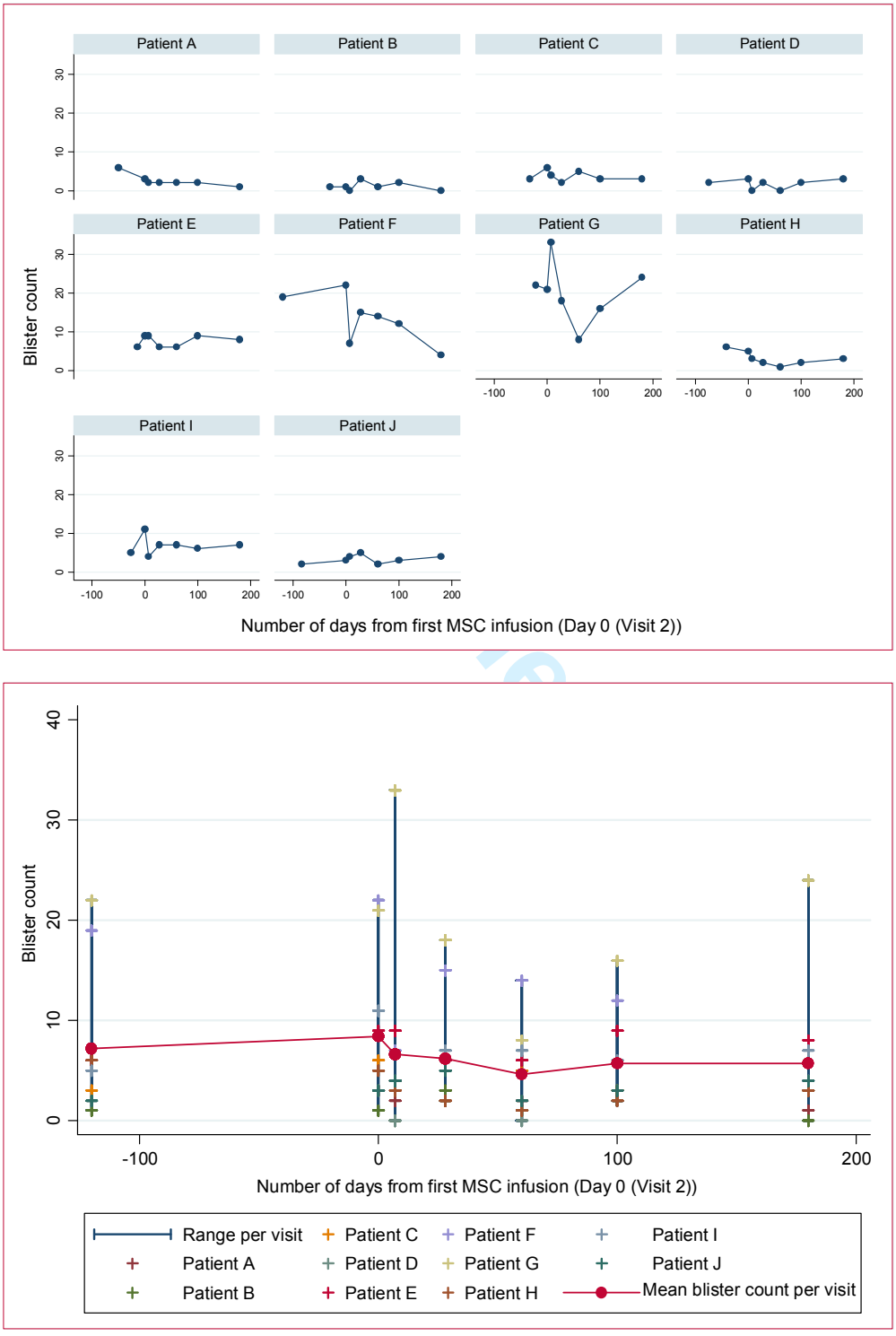


**Table S1.** Table summarizing the study interventions per visit until Day 180.

| **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 |

| 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** |
| 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 S2.** 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 S3.** 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 S4.** Adverse event by system organ class and relationship to MSC infusion.

| | | | Relationship to MSC infusion | | | | | |
|---|---|---|---|---|---|---|---|---|
| **System organ class** | **Adverse event** | **Number of patients** | **Definitely** | **Possibly** | **Likely** | **Unlikely** | **Not related** | **Total number of events (N)** |
| **Total number of patients in study**<br><br>**Patients who experienced adverse events** | | **10**<br><br>**10** | | | | | | **163** |
| Ear, Nose and Throat | Total in class | 4 | 0 | 0 | 0 | 0 | 4 | 4 |
| | Epistaxis | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Sore throat | 3 | 0 | 0 | 0 | 0 | 3 | 3 |
| Eyes | Total in class | 5 | 0 | 0 | 0 | 0 | 24 | 24 |
| | Conjunctivitis | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Corneal abrasion | 4 | 0 | 0 | 0 | 0 | 20 | 20 |
| | Sore eyes | 1 | 0 | 0 | 0 | 0 | 3 | 3 |
| Dermatological | Total in class | 8 | 0 | 3 | 1 | 3 | 23 | 30 |
| | Spontaneous | 7 | 0 | 2 | 0 | 0 | 12 | 14 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | skin/mucosal blisters and wounds | | | | | | |
| | Trauma induced skin/mucosal blisters and wounds | 2 | 0 | 0 | 0 | 4 | 4 |
| | Dry skin | 2 | 0 | 0 | 0 | 2 | 2 |
| | Fine hair growth | 1 | 0 | 1 | 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 | Total in class | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Lymphadenopathy | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Gastrointestinal | Total in class | 9 | 3 | 0 | 0 | 1 | 20 | 24 |
| | Abdominal pain | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| | Gastro-esophageal 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 | 1 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Nausea | 2 | 2 | 0 | 0 | 0 | 1 | 3 |
| | Vomiting | 5 | 0 | 0 | 0 | 0 | 6 | 6 |
| Respiratory | Total in class | 3 | 0 | 0 | 0 | 0 | 4 | 4 |
| | Cough | 3 | 0 | 0 | 0 | 0 | 4 | 4 |
| Cardiovascular | Total in class | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| | Bradycardia | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| Genitourinary system | Total in class | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Reduced urine output | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Musculoskeletal | Total in class | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Joint pain | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Infections | Total in class | 8 | 0 | 0 | 0 | 0 | 20 | 20 |
| | Fever | 2 | 0 | 0 | 0 | 0 | 2 | 2 |
| | Respiratory tract infections | 5 | 0 | 0 | 0 | 0 | 10 | 10 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Skin infection | 5 | 0 | 0 | 0 | 0 | 7 | 7 |
| | Urinary tract infection | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Medical and surgical procedures | Total in class | 5 | 0 | 0 | 0 | 0 | 6 | 6 |
| | 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 | Total in class | 5 | 0 | 0 | 0 | 0 | 18 | 18 |
| | Accidental injuries | 5 | 0 | 0 | 0 | 0 | 18 | 18 |
| DMSO odor | Total in class | 10 | 28 | 0 | 0 | 0 | 0 | 28 |
| | DMSO odor | 10 | 28 | 0 | 0 | 0 | 0 | 28 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mood | Total in class | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| | Irritability | 1 | 0 | 0 | 0 | 0 | 1 | 1 |

MSC: Mesenchymal stromal cells; EB: Epidermolysis Bullosa; DMSO: Dimethyl sulfoxide .

**Table S5.** 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 | – | – | – |

**Table S6.** 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 S7:** 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."*

**Table S8.** Full details of inclusion and exclusion criteria.

| |
|---|
| **Inclusion criteria:**<br>1. Subjects who have a diagnosis of recessive dystrophic epidermolysis bullosa (RDEB) characterized by partial or complete type VII collagen (C7) deficiency.<br>2. Subjects who are ≥ 12 months and ≤ 17 years of age at the time of enrolment.<br>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:**<br>1. Subjects who have had other investigational medicinal products within 90 days prior to screening or during the treatment phase.<br>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.<br>3. Subjects with a known allergy to any of the constituents of the investigational product.<br>4. Subjects with signs of active infection.<br>5. Subjects with a medical history or evidence of malignancy, including cutaneous squamous cell carcinoma.<br>6. Subjects with both<br>a) Positive C7 ELISA and, in addition,<br>b) Positive indirect immunofluorescence (IIF) with binding to the base of salt split skin.<br>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 S9.** Additional information on the bone marrow-derived mesenchymal stromal cells (BM-MSCs) (top); Composition of the IMP (bottom).

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

| **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 |

## SUPPLEMENTARY METHODS

### Details of the statistical analysis methods

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).

**Suction blisters**

Suction blister time was measured using a two-chamber negative pressure device with three 3mm orifices (NP2 model, Electronic Diversities, Finksburg, 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).

**Preparation of skin biopsy specimens for fluorescence *in situ* hybridization (FISH) analysis**

The skin samples were transported in 10% neutral buffered formalin. Within 48 hours tissue was immersed in sequential 70%, 100% ethanol, xylene and paraffin wax using an automated tissue processor. Subsequently, it was embedded in paraffin wax, allowed to cool at room temperature and was stored at room temperature. Paraffin-embedded samples were cut at 5μm using a Leica Microtome RM 2125. Sections were picked up on silane coated slides.

The paraffin-embedded sections were de-waxed by incubation in xylene for 5minutes at room temperature and then through graded ethanol immersions (of 2 minutes each), beginning with 100%, followed by 90%, 70% and finally 50% ethanol. Slides were washed in water and then immersed in Mayer's hematoxylin for 3 minutes, and then washed and immersed in 0·5% eosin Y (VWR) staining solution for 3 minutes. Sections were mounted by covering with 22x50mm coverslips (VWR) using DPX in xylene mountant and then visualised.

**Indirect immunofluorescence and ELISA for C7, BP180 and BP230 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, BP230 and the anti-NC1and NC2 domain of C7.



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