Full Study Title:

A phase I/II study evaluating allogeneic mesenchymal stromal cells in adults with recessive dystrophic epidermolysis bullosa

Study Acronym: ADSTEM

CONFIDENTIAL

End-of-trial Report

Date: 29/05/2018

EudraCT: 2014-004500-30

<table>
<tr>
<td>Sponsor

King’s College London
Strand, London. WC2R 2LS
Contact: Jackie Pullen
Deputy Director & Quality Manager
King’s Health Partners Clinical Trials Office, 16th Floor Tower Wing, Guy’s Hospital, Great Maze Pond, London. SE1 9RT
Tel : +44 (0) 207 188 5732
Fax: +44 (0) 207 188 8330
Email: jackie.pullen@kcl.ac.uk</td>
<td>Chief Investigator

Professor John A McGrath
Mary Dunill Chair in Cutaneous Medicine and Consultant Dermatologist
Department of Genetics and Molecular Medicine, St John’s Institute of Dermatology, King’s College London, 9th Floor Tower Wing, Guy’s Hospital, Great Maze Pond, London. SE1 9RT
Tel: +44 (0) 207 188 6409
Fax: +44 (0) 207 188 8050
Email: john.mcgrath@kcl.ac.uk</td>
</tr>
<tr>
<td>Principal Investigator

Professor Jemima Mellerio
Consultant in Dermatology St John’s Institute of Dermatology
Guy’s and St Thomas’ NHS Foundation Trust, Westminster Bridge Road, London SE1 7EH
Tel: +44(0)207188 6399
Fax: +44 (0) 207188 6379
Email: jemima.mellerio@gstt.nhs.uk</td>
<td rowspan="2">Co-Investigators

Dr Ellie Rashidghamat,
Clinical Research Fellow
Department of Genetics and Molecular Medicine, St John’s Institute of Dermatology, 9th Floor Tower Wing, Guy’s Hospital, Great Maze Pond, London, SE1 9RT
Tel: +44 (0) 207188 6353
Fax: +44 (0) 207188 8050
Email: ellie.rashid@kcl.ac.uk

Dr Gabriela Petrof
Clinical Research Fellow
Department of Genetics and Molecular Medicine, St John’s Institute of Dermatology, 9th Floor Tower Wing, Guy’s Hospital, Great Maze Pond, London, SE1 9RT
Tel: +44 (0) 207188 6353
Fax: +44 (0) 207188 8050
Email: gabriela.petrof@kcl.ac.uk

Dr Su M Lwin
Clinical Research Fellow
Department of Genetics and Molecular Medicine, St John’s Institute of Dermatology, 9th Floor Tower Wing, Guy’s Hospital, Great Maze Pond, London, SE1 9RT
Tel: +44 (0) 207188 6353
Fax: +44 (0) 207188 8050
Email: su.m.lwin@kcl.ac.uk</td>
</tr>
<tr>
<td>Co-Investigators

Professor Francesco Dazzi
Professor of Regenerative and Haematological Medicine, Kings College London, 16th Floor Tower Wing,
Guys Hospital, Great Maze Pond, SE1 9RT
Email: Francesco.dazzi@gstt.nhs.uk

Dr Emma Wedgeworth
Consultant in Dermatology St John’s Institute of Dermatology
Guy’s and St Thomas‟ NHS Foundation Trust, Westminster Bridge Road, London SE1 7EH
Tel: +44(0)207188 6399
Fax: +44 (0) 207188 6379
Email: emma.wedgeworth@gstt.nhs.uk</td>
</tr>
</table>

<table>
<tr>
<td>Trial Statistician

Dr Salma Ayis
Senior Lecturer in Medical Statistics
School of Population Health & Environmental Sciences
Faculty of Life Sciences and Medicine
King’s College London
4th Floor Addison House, Room 4.07, Guy's Campus London SE1 1UL, UK
Tel. +44 (0) 207 848 8222 (work)
Fax: +44 (0) 207 848 6620 (work)</td>
<td>Trial Management

Sonia Serrano
Clinical Trial Manager
NIHR Biomedical Research Centre
Guy’s and St Thomas’ NHS Foundation Trust
16th Floor, Tower Wing, Guy’s Hospital, Great Maze Pond, London, SE1 9RT
sonia.serrano@gstt.nhs.uk;
Phone: +44 (0) 20 7188 7188 ext. 53369
Fax: +44 (0) 20 7188 3472</td>
</tr>
<tr>
<td>Laboratories

The Robin Eady National Diagnostic Epidermolysis Bullosa laboratory, VIAPATH, St Thomas‟ Hospital, London, UK
Telephone: +44 (0) 207 188 7229

Immunofluorescence laboratory, St John‟s Institute of Dermatology, St Thomas‟ Hospital, Westminster Bridge Road, London SE1 7EH
Telephone: +44 (0) 207 188 6364

VIAPATH Pathology Services, GSTT
St Thomas‟ Hospital, Westminster Bridge Road, London SE1 7EH
+44 (0) 207188 7188</td>
<td rowspan="2">Trial Steering Committee

Dr Dusko Ilic, Committee Independent Chair (KCL), Reader in Stem Cell Sciences, Division of Women’s Health, King’s College London

Prof. Marcel Jonkman, TSC member University of Groningen, Professor and chair of Dermatology, University Medical Center Groningen Department of Dermatology, room 2.081, University Medical Center, Groningen

Dr Anna Martinez, TSC member GOSH, Consultant in paediatric dermatology, Great Ormond Street Hospital NHS Foundation Trust

Professor John A. McGrath
St John’s Institute of Dermatology, King’s College London

Ellie Rashidghamat
St John’s Institute of Dermatology, King’s College London

Salma Ayis
Department of Primary Care and Public Health Sciences, King's College London</td>
</tr>
<tr>
<td>Trial Monitoring and Pharmacovigilance

Clinical Research Associate, King’s Health Partners Clinical Trials Office, 16th Floor Tower Wing, Guys Hospital, Great Maze Pond, SE1 9RT

Funding source

Dystrophic Epidermolysis Bullosa Research Association, UK</td>
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</table>

Table of contents:

1. Study Synopsis

Title	A Phase I/II study evaluating allogenic mesenchymal stromal cells in adults with recessive dystrophic epidermolysis bullosa
Protocol Short Title/Acronym	ADSTEM
Sponsor name	King's College London
Chief Investigator	John A. McGrath
Eudract number	2014-004500-30
REC number	15/NE/0006
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 severity in RDEB
Primary objective	To evaluate the safety of allogeneic intravenously administered MSCs in adults with RDEB over a 8 or a 12-month period
Secondary objective (s)	1. Presence of new type VII collagen at the dermal-epidermal junction post treatment on, Day 28, Day 60, and Month 6. 2. Changes in general markers of inflammation at Day 14, Day 28, Day 60, Day 100, Month 6 (for all patients) and Month 12 (for the first eight eligible patients) or Month 8 (for the last two eligible patients) compared to baseline. 3. Changes in specific markers of inflammation on Day 14, Day 28, Day 60 and Month 6 compared to baseline using ELISA and LUMINEX platforms

	Specific inflammatory markers include: HMGB-1, TNF α, IFN γ, IL-10, IL-17A, IL1 β, MMP-2, MMP-9, MMP-11 and TIMP-1. 4. Changes in the clinical appearance of the skin. 5. Change in BEBSS and EBDASI scores at Day 28, Day 60, Day 100, Month 6 (for all patients) and Month 12 (for the first eight eligible patients) or Month 8 (for the last two eligible patients) compared to baseline. 6. Change in Quality of Life Score using the QOLEB questionnaire at Day 28, Day 60, Day 100, Month 6 (for all patients) and Month 12 (for the first eight eligible patients) or Month 8 (for the last two eligible patients) 7. Change in pruritus score using the Leuven Itch Scale (LIS) at Day 28, Day 60, Day 100, Month 6 (for all patients) and Month 12 (for the first eight eligible patients) or Month 8 (for the last two eligible patients) compared to baseline. 8. Quantification of total blister numbers over the entire body surface area at Day 28, Day 60, Day 100, Month 6 (for all patients) and Month 12 (for the first eight eligible patients) or Month 8 (for the last two eligible patients) compared to baseline. 9. Increase in the skin strength measured by time to blister formation after negative pressure skin suction test at Day 28, Day 60, Day 100, Month 6 (for all patients) and Month 12 (for the first eight eligible patients) or Month 8 (for the last two eligible patients) compared to baseline.
Trial Design	Phase I/II, non-randomised, open-label, single-centre.
Sample Size	10 Patients
Summary of inclusion criteria	**Inclusion Criteria** 1) Individuals with a diagnosis of RDEB confirmed by DNA analysis. 2) Individuals ≥ 18 years and ≤ 65 years of age, both male and female

	3) Individuals that have voluntarily signed and dated an informed consent form (ICF) prior to the first study intervention.
IMP, dosage and route of administration	Allogeneic bone marrow-derived mesenchymal stromal cells from healthy donors. Dose: 2-4x 10^6 cells/kg via two intravenous administrations at Day 0 and Day 14.
Active comparator product(s)	Standard supportive medical care
Maximum duration of study participation	8 visits over 12 months are planned for the first eight eligible patients, and over 8 months for the last two eligible patients.
Version and date of final protocol	Version 5.0 13th February 2017
Version and date of protocol amendments	Version 1.0 – 4th December 2014 Version 2.0 – 21st January 2015 Version 2.1 – 25th September 2015 Version 3.0 – 5th October 2015 Version 4.0 – 14th December 2015 Version 4.1 – 29th June 2016 Version 4.2 – 7th November 2016 Version 5.0 – 13th February 2017 Version 5.1 – 6th July 2017

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 whether intravenously administered third-party bone marrow-derived mesenchymal stromal cells (MSCs) are safe and have an impact on disease severity in RDEB in adults.

The study was conducted at Guy's & St Thomas Hospital NHS Trust. Ten adults were enrolled and all ten received the first infusion (Day 0) and nine participants received the second infusion of BM-MSCs (Day 14; each dose 2–4 x 10^6 cells/kg).

Clinical burden of RDEB improved in 8 subjects with a decrease in disease activity at day 28 and day 60 post-MSCs compared to baseline for the BEBSS, EBDASI activity and the QOLEB scores. Leuven Itch Score subscales of frequency, severity and consequences of itch showed a significant reduction at days 28 and 60 post MSCs. In serum, levels of HMGB1, a potential biomarker, showed a reduction following infusion of MSCs at day 28 and day 60 compared to baseline.

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 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 adults with RDEB is the subject of the current study.

4. Materials and Methods

4.1 Study protocol and participant eligibility

This non-randomised, open-label phase I/II trial was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA), with EudraCT number: 2014-004500-30. The North East - York Research Ethics Committee provided Ethics approval. Adults of either sex above 18 years of age with the inherited severe fragility disorder, recessive dystrophic epidermolysis bullosa (RDEB) were eligible to take part. Written informed consent of the participant was obtained.

Below is the inclusion and exclusion criteria used for the trial:

Inclusion Criteria

1) Individuals with a diagnosis of RDEB confirmed by DNA analysis.

2) Individuals ≥ 18 years and ≤ 65 years of age, both male and female

3) Individuals that have voluntarily signed and dated an informed consent form (ICF) prior to the first study intervention.

Exclusion Criteria

Subjects were excluded from the study if ANY of the following conditions existed:

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).

3) Subjects with a known allergy to any of the constituents of the investigational product.

4) Subjects with a medical history or evidence of malignancy, including cutaneous squamous cell carcinoma.

5) 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. Abstinence is defined as refraining from heterosexual intercourse during the trial period, in line with the preferred and usual lifestyle of the subject.

6) Subjects with both a) positive C7 ELISA and b) a positive indirect immunofluorescence (IIF) with binding to the base of salt split skin.

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 14, Day 28, Day 60 and Day 100, Month 6 and Month 8 or 12 included full blood count, renal and liver profile, C-reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR)

The Medicine for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 definitions were used for the safety aspects. All adverse events (AEs) and serious adverse events (SAEs) were documented in the medical notes and recorded in the eCRF. However, medical events that were expected as part of the natural disease course in RDEB were identified and listed in the protocol and not required to be recorded in the eCRF or reported to the sponsor, unless the use of the IMP resulted in a prolongation of existing hospitalization. Unscheduled and/or emergency hospitalisations not expected due to the natural course of the disease were reported via the sponsor's normal SAE reporting practice and recorded in the study electronic database. This also applied to other important medical events as assessed by the CI.

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 three healthy unrelated donors 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).

4.2.3 Dose of BM-MSCs and infusion schedule

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 Centre Utrecht as part of the protocol: 'Treatment of steroid resistant grade II to IV acute GvHD by infusion of mesenchymal stem cells expanded with human plasma and platelet lysate; a phase I/II study (UMC Utrecht; study NL13729.000.07). The dose and frequency of infusions were endorsed by the trial advisory board. Each participant in the trial received two separate intravenous infusions of same donor BM-MSCs on Day 0 and day 14 at a dose of 2-4 x 10^6 cells / kg. The infusions were given as a day-case procedure; vital signs were checked prior to the administration of MSCs and at 15, 30, 45 and 60 minutes after administration was complete. No HLA-typing or subject conditioning was performed on any of the recipients of the MSCs.

5. Study objectives

The primary objective was to assess safety. Secondary objectives were to assess efficacy on clinical responses, to identify the best cohort of individuals to target for future trials and therapies, to improve understanding of in vivo and in vitro responsiveness to MSCs, to identify candidate molecules germane to activating MSCs and making them clinically more potent, independently of the permissive conditions of the patient, and to assess the impact of MSC infusions on reducing disease morbidity/severity. We assessed participants by conducting 8 visits over 8 or 12 months, with infusions on Days 0 and Day 14. Clinical assessment and photographs were undertaken for all participants to provide clinical evidence of overall skin condition and wound healing. The Birmingham Epidermolysis Bullosa Severity Score (BEBSS), an Epidermolysis Bullosa Activity and Scarring Index (EBDASI), Leuven itch score, and Quality of Life questionnaires were completed to assess clinical responses. Blister counts and clinical photographs were completed by the patients during dressing changes and the data and images were reviewed at each visit.

5.1 Blood and skin profiling

Blood samples for haematology and biochemistry were taken at all study visits and analysed at the VIAPATH pathology laboratories, St Thomas' Hospital. All subjects were tested for serology at baseline. Subjects whom had never a DNA sample analysed for the *COL7A1* mutation, provided one at baseline to assess eligibility and this was tested at The National

Diagnostic EB laboratory at St Thomas' Hospital. Serum immunofluorescence (indirect IMF) for antibodies against C7 was performed in blood samples at screening, D14, D28, D60 and Month 6 at the Immunofluorescence laboratory at St Thomas' Hospital.
Skin biopsies were taken under local anaesthetic at screening for direct immunofluorescence (DIF) for C7, electron microscopy (EM) and gene expression analysis (RNA-seq). This was also performed at D28, D60 and M6.

Suction blister induction times were performed at each visit except on the days of mesenchymal stromal cell infusion. This was performed on the same site of the same limb, as resistance to blister will vary according to anatomical location. This metric was performed using a negative pressure device (Electronic Diversities, MD, USA). The blisters were created through the use of 3 mm custom-made suction chambers that are attached to the patient's skin. Once the chamber was secured to the patient's skin, the device was turned on at a pressure of 15 mmHg. 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 patients.

6. Statistical analysis

RDEB is a rare disease and so a large study was not feasible. All study participants were included in all analyses unless they had withdrawn consent. For patients who dropped out prior to visit 7 (Month 6), efforts were made to try to replace withdrawn patients. Descriptive statistics such as means, standard deviations (SDs), minimum, maximum, frequencies and proportions, as appropriate were presented. No hypothesis tests were performed. When all patients completed visit 8, final analysis of the collected data was performed and the results are to be published in a peer-reviewed journal.

For secondary outcome measures, differences in means between baseline and subsequent visits and 95% confidence interval were estimated. As the distribution of differences is often not normal, therefore do not meet the requirement for the application of a t-test, the p-values based on the non-parametric sign-rank test were provided alongside the p-values from the t-test. The p-values were however exploratory and were not meant to be used for hypothesis testing. Profiles of measurements for each participant over the 8 visits were displayed graphically. Box plots were used to present the medians, together with the upper and lower quartile range, maximum and minimum measure at each visit.

7. Results

7.1 Participant characteristics

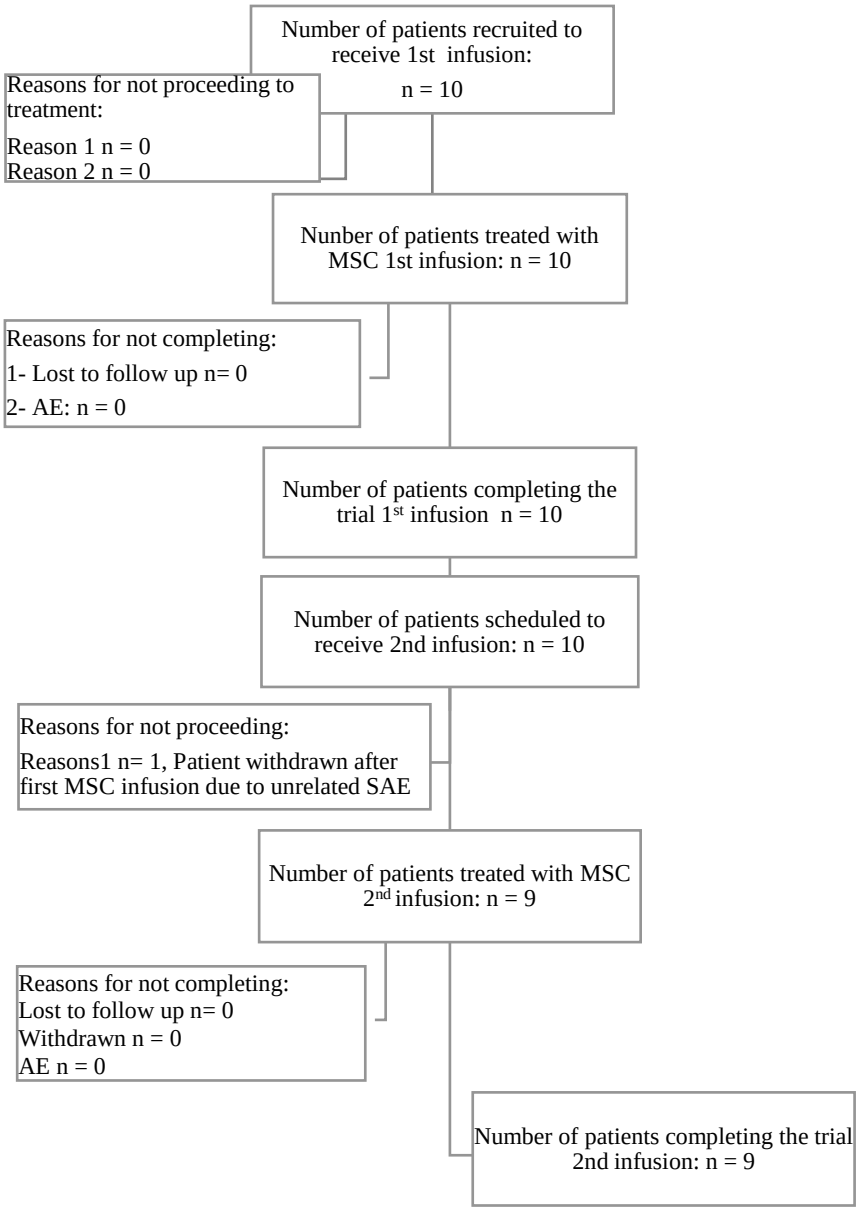
Following regulatory and ethics approvals, adults with RDEB were invited to participate. Twelve adults with RDEB were screened for inclusion into the trial. Two adults were excluded because one of them was diagnosed with squamous cell carcinoma (SCC) during screening, and the other patient withdrew consent after screening. Ten adults were enrolled at Guy's & St Thomas Hospital (Figure 2). Participants had a median age of 34.9 years (range 26–44) and had a genetically confirmed diagnosis of RDEB. Baseline characteristics of the adults are listed in Table 3 and details of the trial assessment time-points and metrics are also given in Table 3.

Table 3. Demographic and clinical details at screening (Visit 1)

Patient ID	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Age (Year)	31	29	27	31	35	44	26	55	43	27	35	36
Sex	Male	Female	Female	Female	Female	Male	Male	Male	Female	Female	Male	Male
Ethnicity	Greek	White - British	Asian	White - Britis	White - Britis	White - Britis	White - Britis	White - Britis	White European	White - Britis	White - Britis	Polish - White
Height (CM)	174	160		153	157	176	120	168	163	160	176	177
Weight (kg)	73	57	52.2	41.3	43.9	61	22.1	84.7	68	56.7	80	85.3
BMI (kg/m2)	24.11	22.27		17.64	17.81	19.69	15.35	30.01	25.59	22.15	25.83	27.23
QoL EBS	31	35		18	34	24	28	5	19	23	26	13
EBDASI	199	254		148	227	167	296	72	52	71	88	32
BEBBS	48	72		29	76	69.5	89	14.25	13.5	21.75	19	6.13
Blister count	13	13		6	12	9	1	0	17	11	2	2
Vitals												
Systolic Blood	102	105	107	111	105	138	92	151	118	128	136	141
Diastolic Blood	63	69	70	65	67	80	64	93	50	67	89	86
Heart rate (pp)	90	102	77	92	90	89	133	72	67	72	57	79
Respiratory rate	13	12	13	14		16	24		12	12	16	18
Pulse oximetry	97		99	99	100	97	100	98	100	100	95	96
Temperature (C^0)	36.8	35.9	37	37.3	37	36.3		36.4	36.5	36.3	36.6	36.6

Patient ID	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
Laboratory tests												
CRP (mg/L)	151	139	97	13	93	73	198	15	2	12	6	2
ESR (mm/hr)	101	116		34	102	90	115	37	8	25	5	2
Haemoglobin (g/dL)	10.2	8.8	8.5	11.1	8.8	11.5	8.9	13.2	13	12	12.8	14.9
White cell count	19.4	11	6.6	6.2	12.3	12.6	12.2	7.5	5.6	9.3	11.7	7.8
Creatinine (µmol/L)	105	78	41	50	53	73	49	96	64	59	53	90

Figure 2 Trial Final Flowchart



7.2 Clinical safety

The safety data showed no serious AE among participants. It is worth noting however that a zero-event rate in just 9 patients is compatible with an upper 95% confidence interval of over 46%. There were 9 adverse events (AEs) experienced by 3 patients (AEs; Table 4 (b)) One patient (ID4) had a sore throat and a runny nose, another (ID6) had a skin infection affecting his back, loose and frequent stools, nightmares and vomiting. A third (ID9) had a hyperkeratotic nodule right lower leg, ear infection, generally felt under the weather, had an

infected blister right leg, and chest infection. None of the AEs were related to the intervention, and all resolved before the end of the study.

Table 4 (b). Serious adverse events and adverse reactions, by patient, date, and relation to intervention

Patient ID	Event	Event Type*	Start date	End date	Related to IMP	Outcome
02	Deterioration of renal function	AE (Severe)	15/08/2015	11/09/2015	Not related	Ongoing at the end of the study
04	Sore throat	AE (Mild)	05/10/2015	09/10/2015	Not related	Resolved
04	Sore throat and runny nose	AE (Mild)	18/12/2015	28/12/2015	Not related	Resolved
05						
06	Skin infection affecting back	AE (Mild)	16/12/2015	03/01/2016	Not related	Resolved
06	Loose and frequent stools, known history of IBS	AE (Mild)	12/01 /2016	15/01/ 2016	Not related	Resolved
06	Nightmares	AE (Mild)	30/12/2015	13/01/2016	Not related	Resolved
06	Vomiting	AE (Mild)	18/04/2016	21/04/2016	Not related	Resolved
09	Hyperkeratotic nodule right lower leg	AE (Mild)	10/06/2016	06/12/2016	Not related	Resolved with sequelae
09	Ear infection and generally feeling under the weather and infected blister right leg	AE (Mild)	23/06/2016	29/06/2016	Not related	Resolved
09	Chest infection	AE (Mild)	Unknown	03/08/2016	Not related	Resolved

Note. *Differences were calculated as Baseline measure minus other subsequent measurements. Negative values indicate an increase after the baseline visit. CI: Confidence interval*

7.3 Laboratory safety

7.3.1 General Inflammatory Markers

General inflammatory markers including Creatinine level, Albumin, C-reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), Haemoglobin, and White Cell Count (WCC) did not show clear changes over time. Variations across patients were observed, with most variations seen in WCC. The profiles of these are displayed in Figures (2.1) (a, b, c, d, e and f). Averages

(median) of the general inflammatory markers were displayed by the Box plots, Figures (2.2) (a, b, c, d, e and f).

Figure 2.1 (a) Creatinine

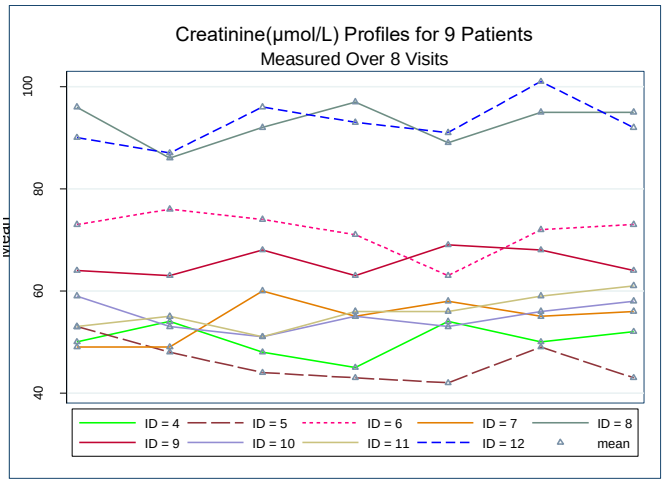


Figure 2.2 (a) Creatinine

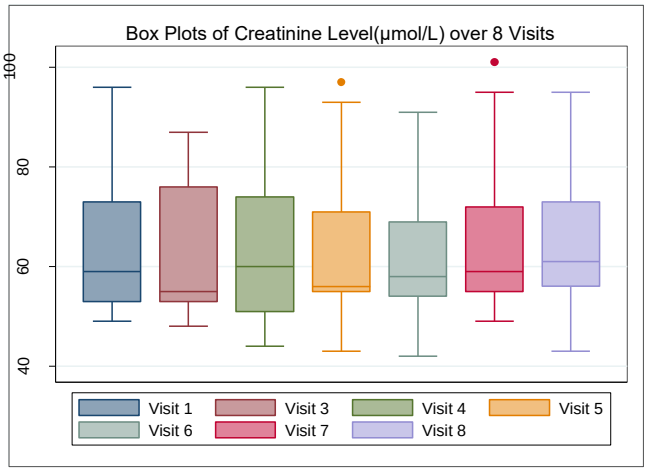


Figure 2.1 (b) Albumin

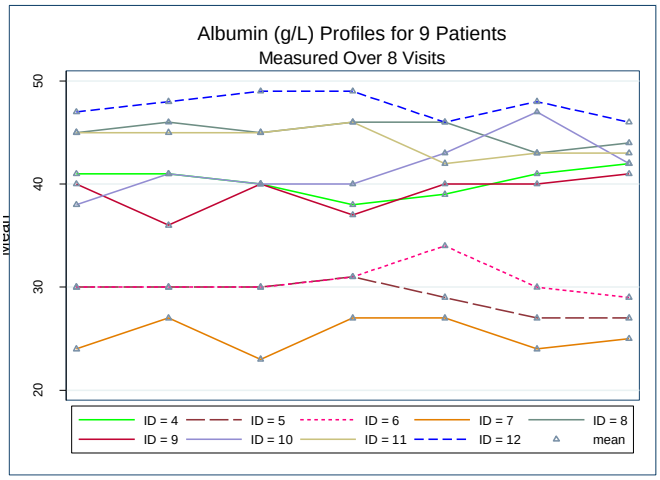


Figure 2.2 (b) Albumin

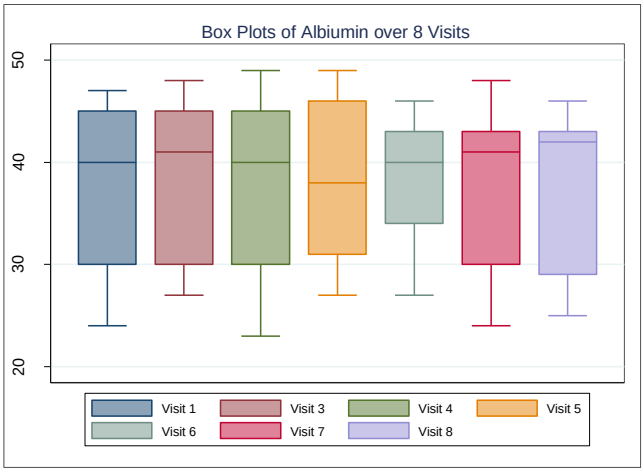


Figure 2.1 (c) C-reactive Protein (CRP) (mg/L)

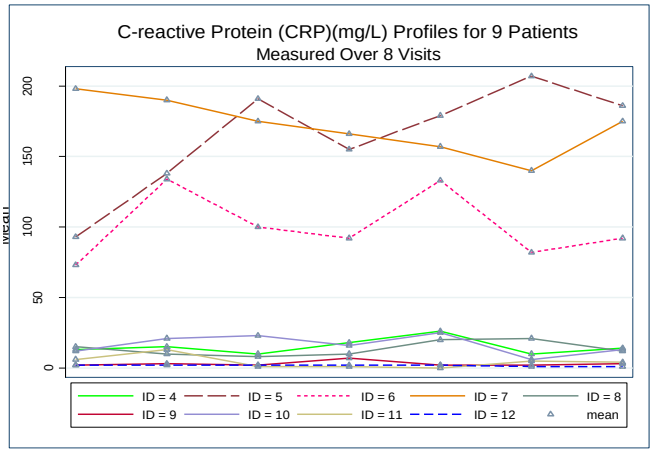


Figure 2.2 (c) C-reactive Protein (CRP) (mg/L)

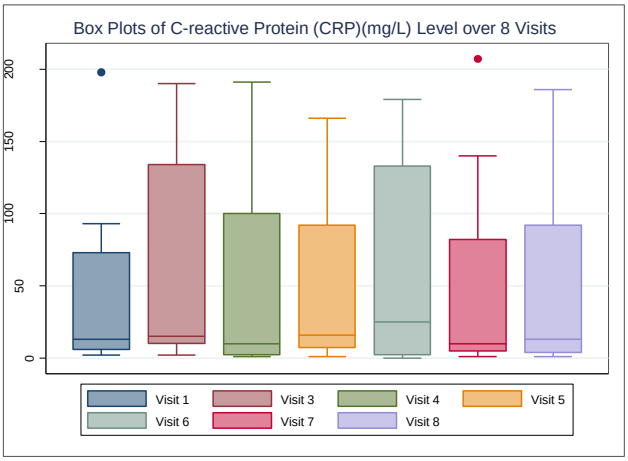


Figure 2.1 (d) Erythrocyte Sedimentation Rate (ESR) (mm/hr)

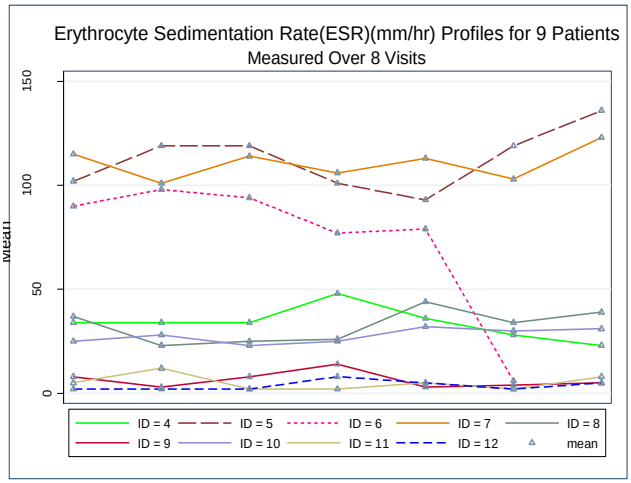


Figure 2.2 (d) Erythrocyte Sedimentation Rate (ESR) (mm/hr)

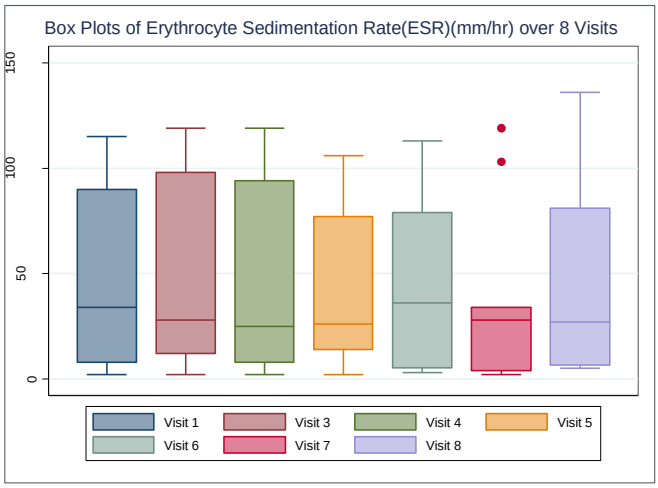


Figure 2.1 (e) Haemoglobin (g/dL)

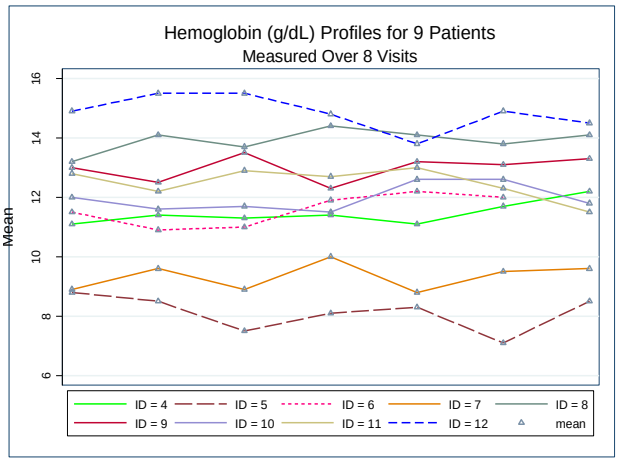


Figure 2.2 (e) Haemoglobin (g/dL)

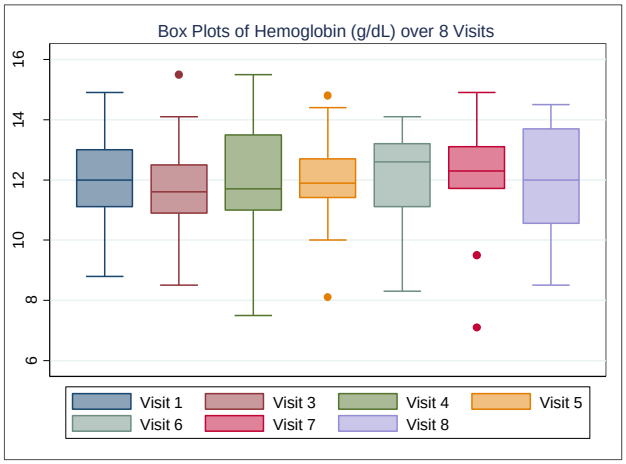


Figure 2.1 (f) White Cell Count (WCC)

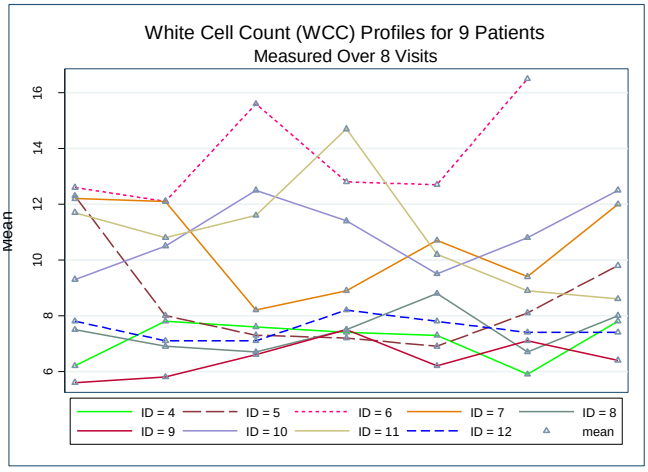
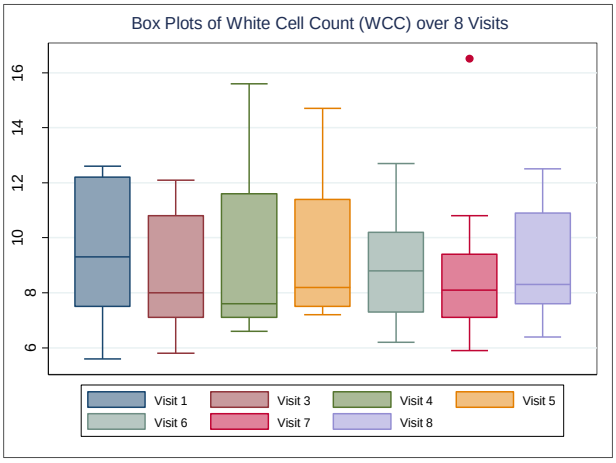


Figure 2.2 (f) White Cell Count (WCC)



Differences between baseline and day 28, and between baseline and day 60, and 95% confidence intervals and p values were given in Table 2.2.

Table 2.2. Mean differences [95% confidence intervals (CI)] between measures taken at baseline and 2 subsequent visits for general inflammatory factors

	Comparison visits	Mean Difference	[95% CI]		p value (t-test)	p value (signrank)
Creatinine Level(µmol/L)	Visit 1 Versus Visit 4	0.33	-4.69	5.36	0.882	0.8124
	Visit 1 Versus Visit 5	1.00	-2.77	4.77	0.557	0.635
Albumin	Visit 1 Versus Visit 4	-0.22	-1.06	0.62	0.559	0.7915
	Visit 1 Versus Visit 5	-0.56	-2.19	1.08	0.456	0.4352
C-reactive Protein (CRP) (mg/L)	Visit 1 Versus Visit 4	-10.89	-38.06	16.29	0.383	0.8111
	Visit 1 Versus Visit 5	-5.89	-25.17	13.39	0.501	0.5494
Haemoglobin (g/dL)	Visit 1 Versus Visit 4	0.02	-0.44	0.49	0.915	0.7209
	Visit 1 Versus Visit 5	-0.10	-0.65	0.45	0.685	0.8588
White Cell Count (WCC)	Visit 1 Versus Visit 4	0.22	-1.95	2.39	0.819	0.9528
	Visit 1 Versus Visit 5	-0.04	-2.07	1.98	0.961	0.5529

Note. **Note**. *Differences were calculated as Baseline measure minus other subsequent measurements. Negative values indicate an increase after the baseline visit. CI: Confidence interval*

7.3.2 Specific Inflammatory Markers

High Mobility Group Box-1 (HMGB-1) was remarkably lower at day 28 and day 60 than baseline, the mean decrease at the two times respectively was: 4.86 (95%CI: 0.36 to 9.35) and 7.19 (95%CI: 1.26 to 13.11), and this potential biomarker remained low at month 6 where the last measurement was taken. Table 2.3.

The profiles of HMGB-1 for the 9 participants, show similar trends in general, with a sharp drop that remains stable over the observation period. (Figure 3.1a). Average estimates have similarly shown a decrease in the median HMGB-1 over time. (Figure 3.2a).

Figure 3.1 (a) High Mobility Group Box-1 (HMGB-1)

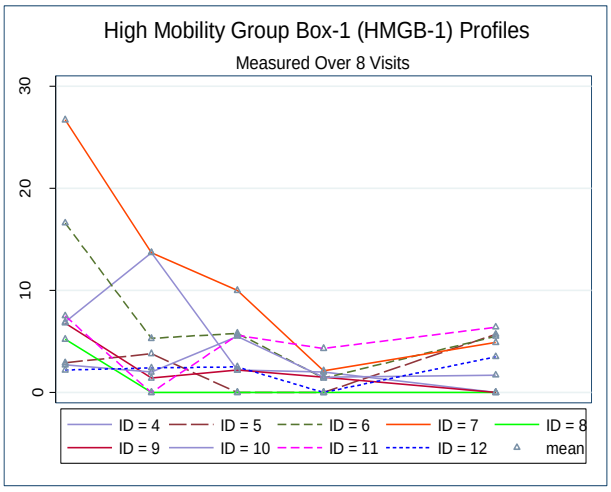
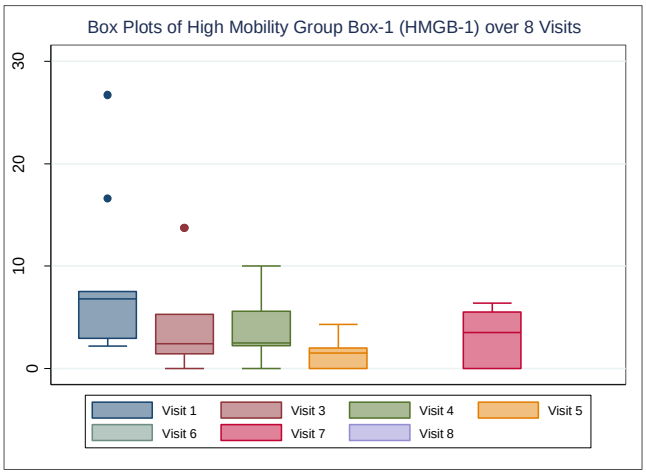


Figure 3.2 (a) High Mobility Group Box-1 (HMGB-1)



Mean TNFα, decreased at days 28 and day 60 compared to baseline, by 2.27 (95%CI: -5.34 to 9.88), and 5.73 (95%CI: -2.74 to 14.20) at the two-time points respectively. Table 2.3.

The profiles of individual patients have similarly shown a modest decrease overall, with exception of one patient (ID10) who has shown fluctuations and a striking increase at month 6. Figure 3.1 (b) The box plots on the other hand have similarly shown a decrease in median over time. Figure 3.2 (b)

Figure 3.1 (b) Tumour Necrosis Factor Alpha (TNF-α)

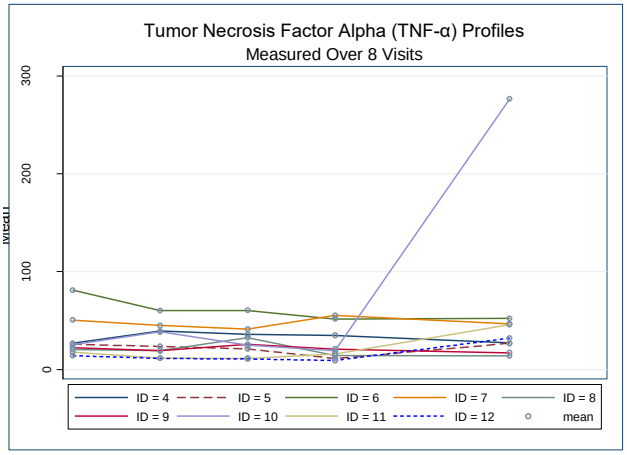
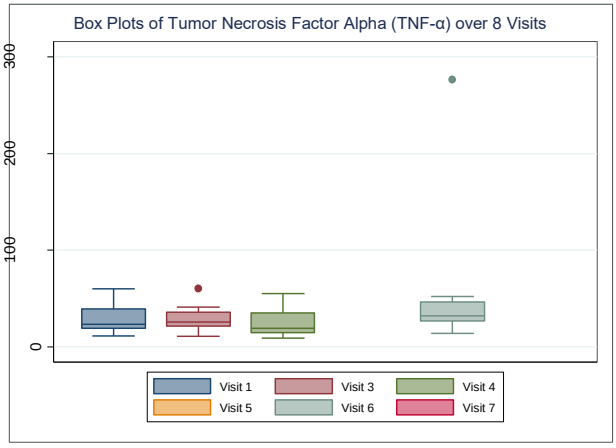


Figure 3.2 (b) Tumour Necrosis Factor Alpha (TNF-α)



Interferon Gamma (IFN-γ) measures were slightly lower at day 28 and day 60, the mean difference from baseline was 32.03 (95% CI: 29.15 to 93.22) and 60.44 (60.95 to 181.84) respectively. The profiles of individual patients, reflect the very small decrease, bearing in mind very small units of measurement. An exception was one patient (ID10) that started at a higher level than the other participants, and showed a drop at day 28, and day 60 followed by an increase. Figure 3.1 (c). The Box plots, highlight the outlying observations for one patient (ID10) and a relatively stable median over time. Figure 3.2 (c)

Figure 3.1 (c) Interferon Gamma (IFN-γ)

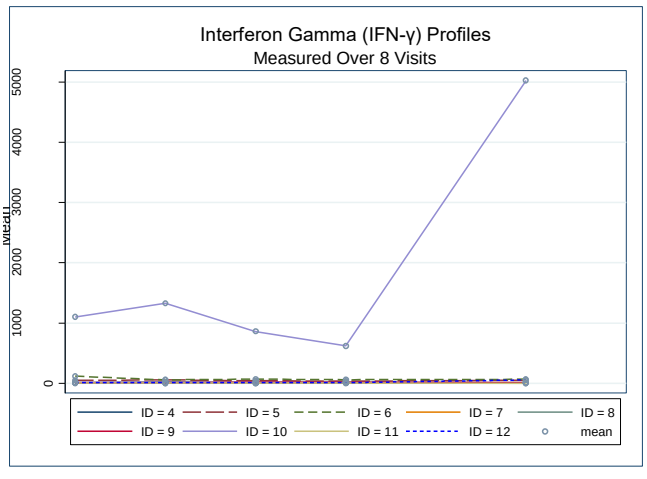
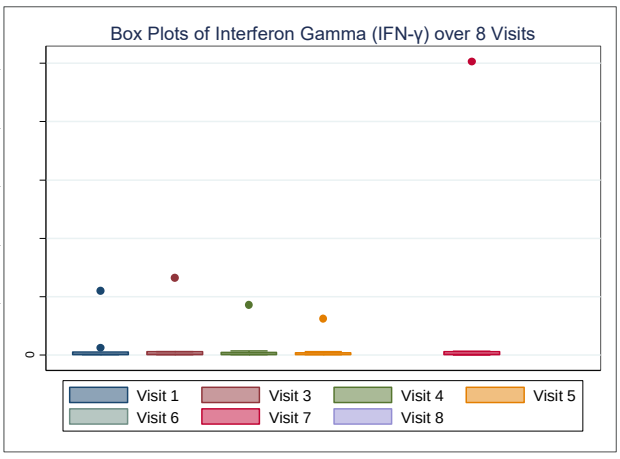


Figure 3.2 (c) Interferon Gamma (IFN-γ)



IL-17A, was lower at day 28 and day 60 compared to baseline. The mean difference at the two visits respectively was: 5.89 (95% CI: -5.88 to 17.66) and 28.36 (95%CI: -18.01 to 74.72). The profiles show similar trends across patients with the exception of one patient (ID10) who

has shown a dramatic increase at month 6 (visit 7) (Figure 3.1 (d)). The average estimates displayed by the Box plots, Figure 3.2 (d), have shown minimal change over time, highlighted the small units used, and the outlying observations for one patient.

Figure 3.1 (d) Interleukin-17A (IL-71A)

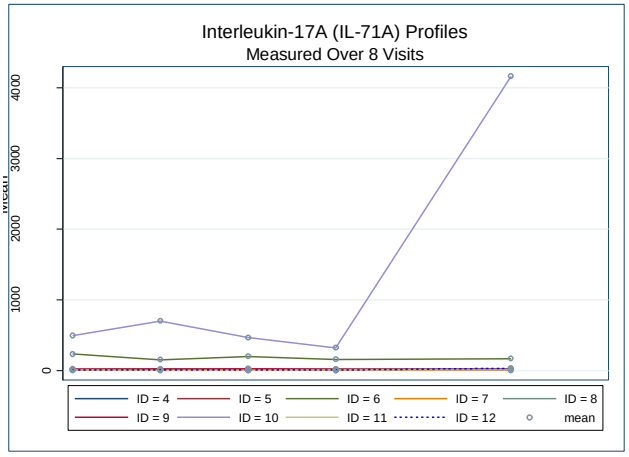
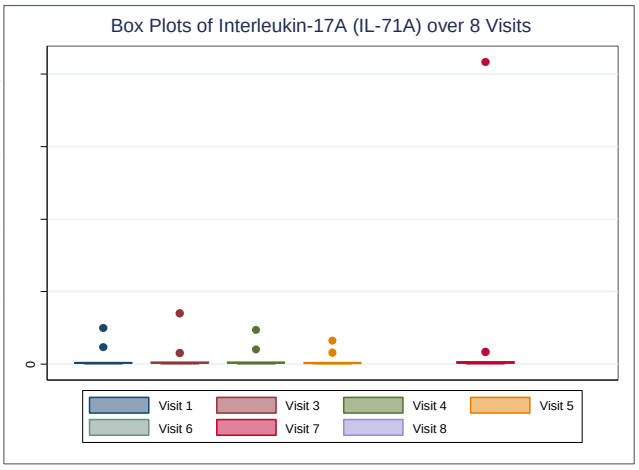


Figure 3.2 (d) Interleukin-17A (IL-71A)



Interleukin-1 (IL-1) similarly decreased at days 28 and day 60 compared to baseline, differences were 1.38 (95% CI: -0.27 to 3.03) and 2.53 (95% CI: 0.06 to 5.01) respectively. Table 2.3. Profiles of individual patients are displayed in Figure 3.1 (e) and box plots in Figure 3.2 (e).

Figure 3.1 (e) Interleukin-1 (IL-1)

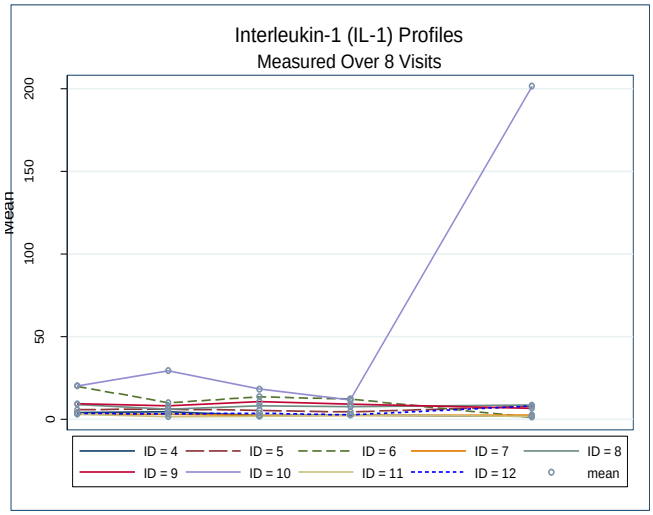
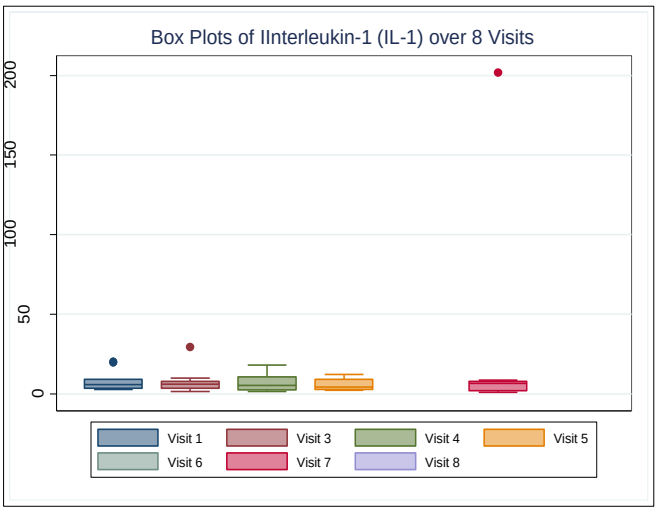


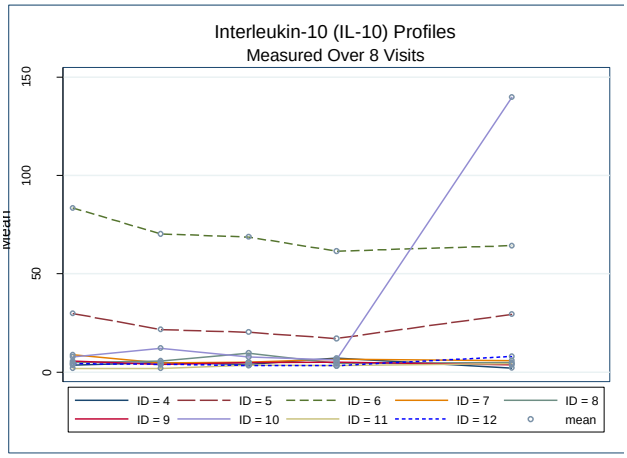
Figure 3.2 (e) Interleukin-1 (IL-1)



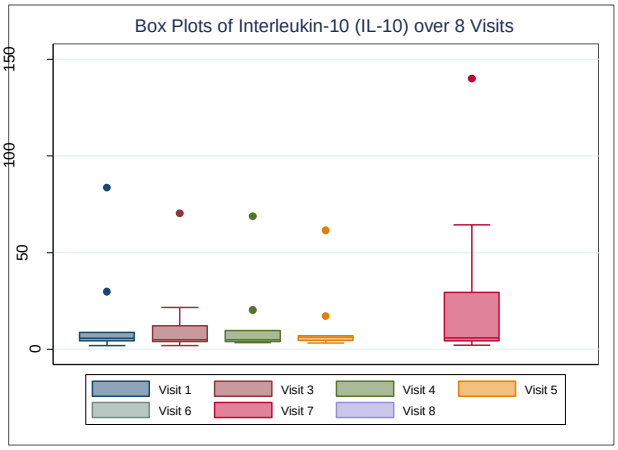
Interleukin-1 (IL-10) mean estimate, was lower by 2.53 (95% CI: -2.13 to 7.20) at day 28 and by 3.96 (95% CI: -2.29 to 10.20) at day 60, compared to baseline. One patient (ID10) continued to have dramatically high measure at month 6, unlike the others. Another patient (ID6) has higher baseline measure than others, decreased at day 28, and day 60, then remain stable,

while a third (ID5) started from a moderate value, dropped slightly at day 28 and day 60, then increased slightly at month 6. The rest of the patients, followed a similar pattern. Figure 3.1 (f), display the individual profiles. Figure 3.2 (f) highlights some outliers, mostly due to two patients having higher measures than others.

Figure 3.1 (f) Interleukin-10 (IL-10)



3.2 (f) Interleukin-10 (IL-10)



Matrix metalloproteinase-2 (MMP-2), (MMP-9) and (MMP-11) estimates have shown wide variations within patient and between patients. Overall a decrease was observed, and the largest average decrease was shown by MMP-9, where changes between baseline and day 28, and between baseline and day 60 respectively were: 4099.08 (-2998.62 to 11196.77) and 3522.03 (-629.91 to 7673.98). The corresponding figures for MMP-11 were: 17.02 (-58 to 92.17) and 11.23 (-74.0 to 96.46) where the lowest change among the 3 markers was observed. The profiles of individual patients were displayed, in Figure 3.1 (g), (h) and (i). The box plots, show a decreasing trend of medians over time, for MMP-2 and MMP-11, while fluctuations were observed for MMP-9. Figure 3.2 (g), (h) and (i).

Figure 3.1 (g) Matrix Metalloproteinase-2 (MMP-2)

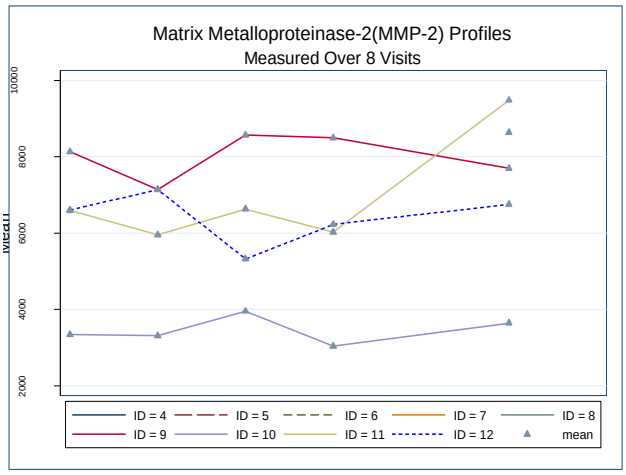


Figure 3.2 (g) Matrix Metalloproteinase-2 (MMP-2)

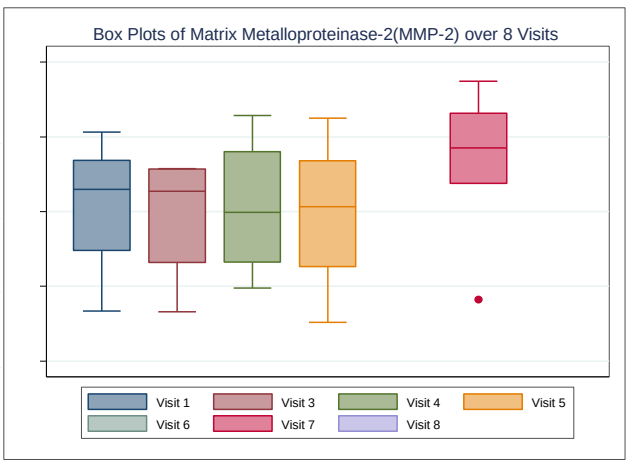


Figure 3.1 (h) Matrix Metalloproteinase-9 (MMP-9)

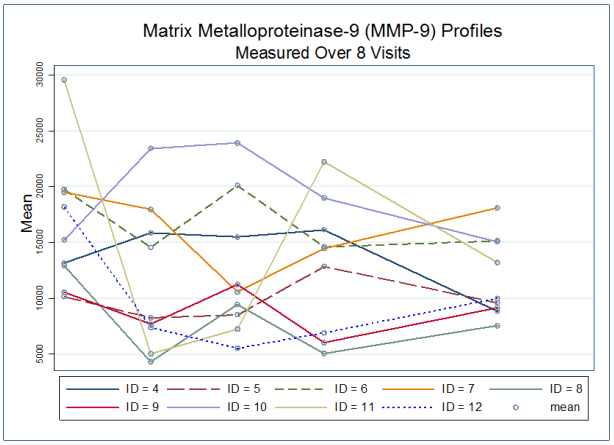


Figure 3.2 (h) Matrix Metalloproteinase-9 (MMP-9)

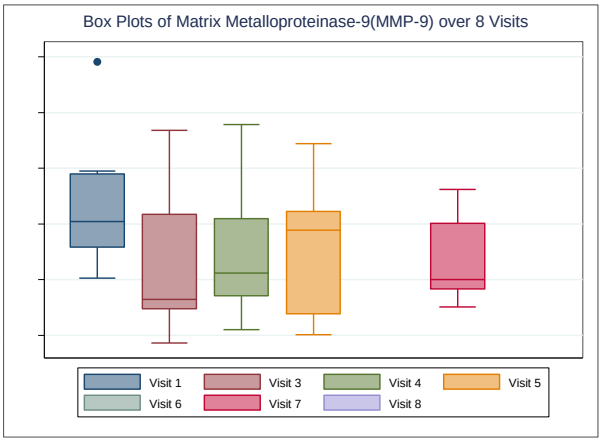


Figure 3.1 (i) Matrix Metalloproteinase-11 (MMP-11)

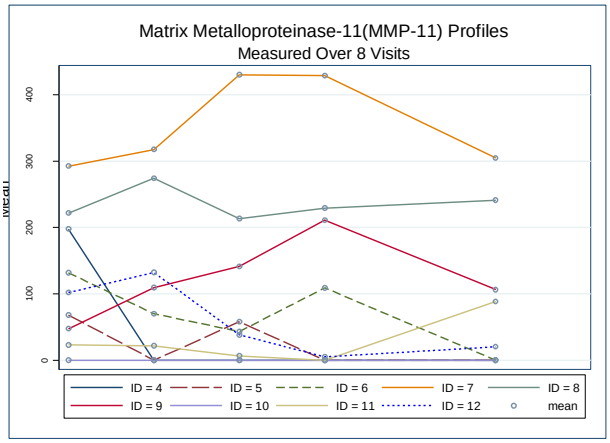
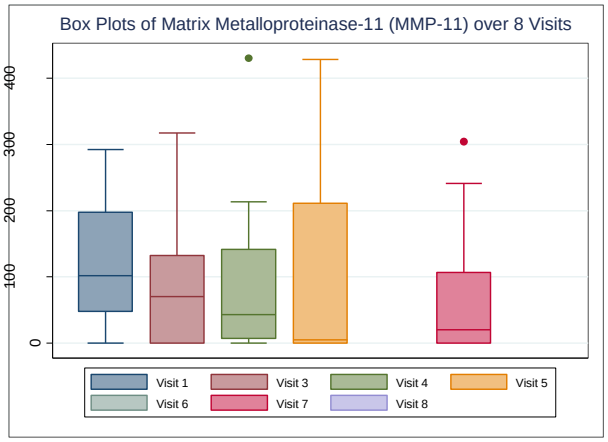


Figure 3.2 (i) Matrix Metalloproteinas-11 (MMP-11)



Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) varied across patients; with 5 patients showing stable levels over time, two patients (ID6 and ID7) had an increase in scores at visits 4 and 5, followed by a decrease that then remained stable. One patient (ID5) showed a decrease that then remained stable. Another (ID8) showed a slight increase followed by a dramatic drop. Individual profiles were displayed in Figure 3.1 (j) and medians, upper and lower quartiles were summarised in Figure 3.2 (j)

Figure 3.1 (j) Tissue Inhibitor of Metalloproteinases-1 (TIMP-1)

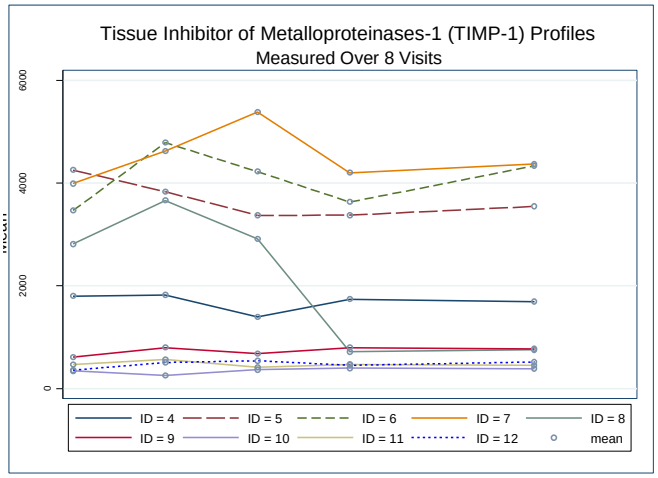


Figure 3.2 (j) Tissue Inhibitor of Metalloproteinases-1 (TIMP-1)

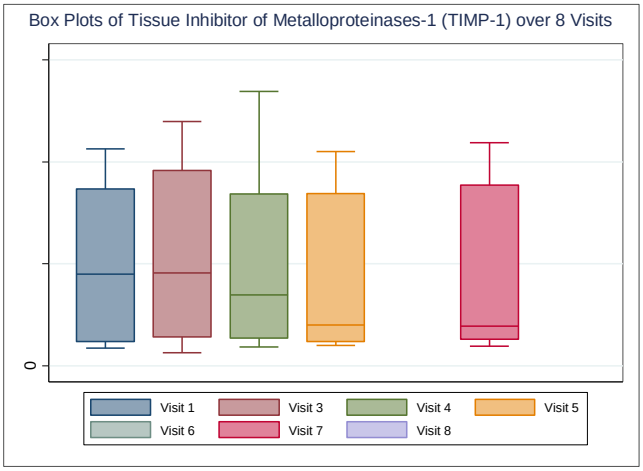


Table 2.3. Mean differences [95% confidence intervals (CI)] between measures taken at baseline and 2 subsequent visits for specific inflammatory factors

	Comparison visits	Mean Difference	[95% CI]		p value (t-test)	p value (signrank)
High Mobility Group Box-1 (HMGB-1)	Visit 1 Versus Visit 4	4.86	0.36	9.35	0.038	0.0284
	Visit 1 Versus Visit 5	7.19	1.26	13.11	0.023	0.0077
Tumour Necrosis Factor Alpha (TNF-α)	Visit 1 Versus Visit 4	2.27	-5.34	9.88	0.512	0.5147
	Visit 1 Versus Visit 5	5.73	-2.74	14.20	0.157	0.1386
Interferon Gamma (IFN-γ)	Visit 1 Versus Visit 4	32.03	-29.15	93.22	0.262	0.2604
	Visit 1 Versus Visit 5	60.44	-60.95	181.84	0.284	0.3743
Interleukin-10 (IL-10)	Visit 1 Versus Visit 4	2.53	-2.13	7.20	0.246	0.3424
	Visit 1 Versus Visit 5	3.96	-2.29	10.20	0.182	0.1731
Interleukin-17A (IL-71A)	Visit 1 Versus Visit 4	5.89	-5.88	17.66	0.282	0.3428
	Visit 1 Versus Visit 5	28.36	-18.01	74.72	0.196	0.2135

Interleukin-1 (IL-1)	Visit 1 Versus Visit 4	1.38	-0.27	3.03	0.090	0.0506
	Visit 1 Versus Visit 5	2.53	0.06	5.01	0.046	0.0076
Matrix Metalloproteinase-2 (MMP-2)	Visit 1 Versus Visit 4	48.00	-1316.58	1412.58	0.918	0.715
	Visit 1 Versus Visit 5	221.23	-422.86	865.31	0.354	0.2733
Matrix Metalloproteinase-9 (MMP-9)	Visit 1 Versus Visit 4	4099.08	-2998.62	11196.77	0.220	0.2604
	Visit 1 Versus Visit 5	3522.03	-629.91	7673.98	0.086	0.0506
Matrix Metalloproteinase-11(MMP-11)	Visit 1 Versus Visit 4	17.02	-58.13	92.17	0.616	0.4061
	Visit 1 Versus Visit 5	11.23	-74.00	96.46	0.769	0.5529
Tissue Inhibitor of Metalloproteinases-1 (TIMP-1)	Visit 1 Versus Visit 4	-129.88	-626.72	366.97	0.563	0.4413
	Visit 1 Versus Visit 5	258.12	-330.09	846.34	0.341	0.7671

Note. *Differences were calculated as Baseline measure minus other subsequent measurements. Negative values indicate an increase after the baseline visit. CI: Confidence interval*

7.4 Clinical response

7.4.1 Profiles of Quality of Life (QoL) Assessed by The Quality of Life in Epidermolysis Bullosa score (QOLEB) Over 8 Visits

The Quality of Life in Epidermolysis Bullosa Score (QOLEB) was completed by 8 participants at day 28, day 60, day 100, and months 6, and by all 9 participants at baseline and the last measurement, month 12 (or month 8 for 2 patients). Mean drop in scores at day 28 and day 60, was: 1.89 (95% CI: -0.87 to 4.65) and 3.13 (95%CI: -0.26 to 6.51) lower than baseline. The profiles over time show wide variations between participants while, within participant scores seem relatively stable over time with the exception of two cases where either the scores increased slightly after day 60 (ID6) or dropped and remained low after day 28 (ID10). (Figure 4.1). The box plots indicate a decrease in median over time that remain so after day 60. Figure 4.2.

Figure 4.1 Profiles of Quality of Life (QoL) Assessed by The Epidermolysis Bullosa Simplex (EBS) Over 8 Visits

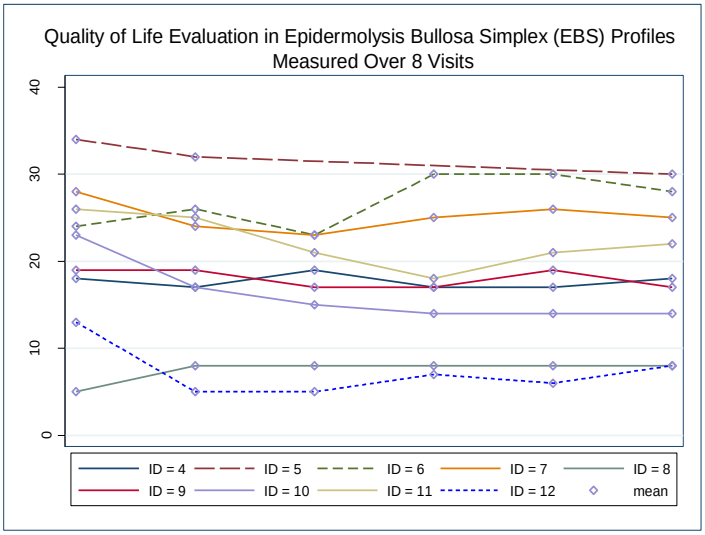
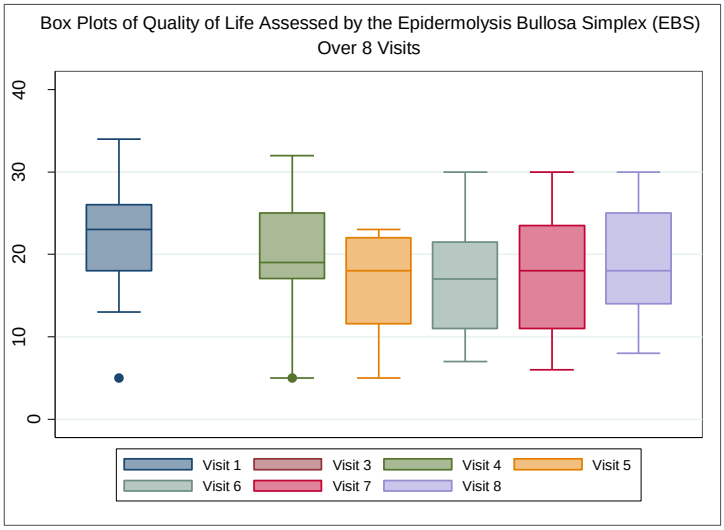


Figure 4.2 Box Plots of Quality of Life (QoL) Assessed by The Epidermolysis Bullosa Simplex (EBS) over 8 Visits



Birmingham Epidermolysis Bullosa Severity Score (BEBSS) decreased slightly at day 28 and day 60, the mean change was: 0.33 (95% CI: -0.3 to 0.97) and 1.61 (95% CI: -0.05 to 3.27) for the two visits respectively. Table 2.4.

Table 2.4. Mean differences [95% confidence intervals (CI)] between measures taken at baseline and 2 subsequent visits for quality of life measures

	Comparison visits	Mean Difference	[95% CI]		p value (t-test)	p value (signrank)
Quality of Life Evaluation in Epidermolysis Bullosa (QOLEB)	Visit 1 Versus Visit 4	1.89	-0.87	4.65	0.153	0.1716
	Visit 1 Versus Visit 5	3.13	-0.26	6.51	0.066	0.0789

Note. *Differences were calculated as Baseline measure minus other subsequent measurments. Negative values indicate an increase after the baseline visit. CI: Confidence interval*

7.4.2 Profiles of Birmingham epidermolysis bullosa severity score (BEBSS) and the Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI) Over 8 Visits

The scores' profiles over visits show variations across participants, while these were relatively stable within patient. The medians similarly show minimal decrease over time. Figure 5.1 (a) and Figure 5.2 (a)

Figure 5.1 (a) Birmingham epidermolysis bullosa severity score (BEBSS)

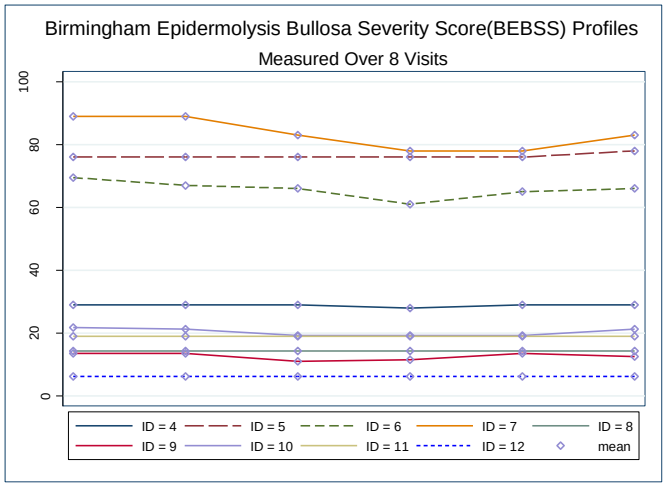
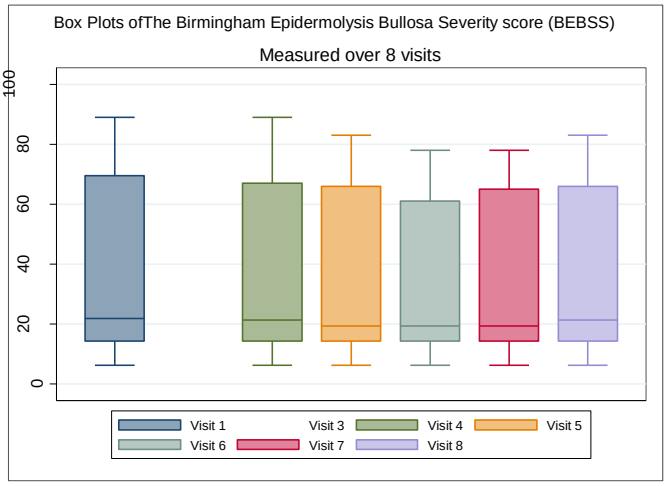


Figure 5.2 (a) Birmingham epidermolysis bullosa severity score (BEBSS)



The Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI) scores overall, have shown minimal changes over time. Between patients' variations were wide, and these were minimal within patient. The profiles, show stable scores over time. Figure 5.1 (b)

Figure 5.1 (b) The Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI)

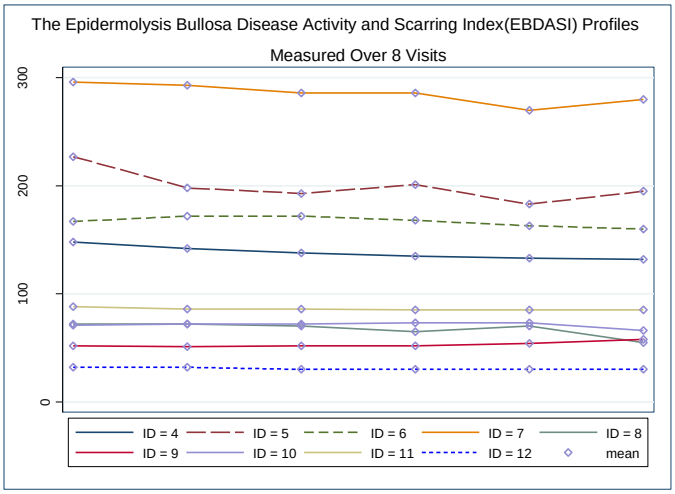
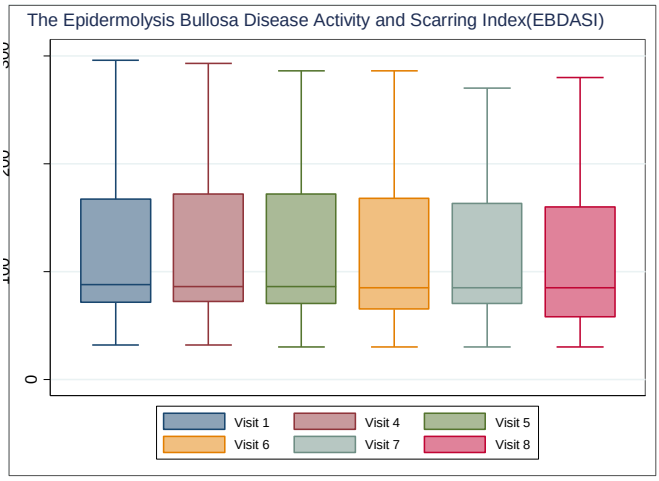


Figure 5.2 (b) The Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI)



The subscales on the other hand are different. The activity subscale on average decreased by 4.89 (95% CI: -2.42 to 12.20) and 7.0 (95% CI: -1.59 to 15.59) at days 28 and day 60 respectively. Table 2.5

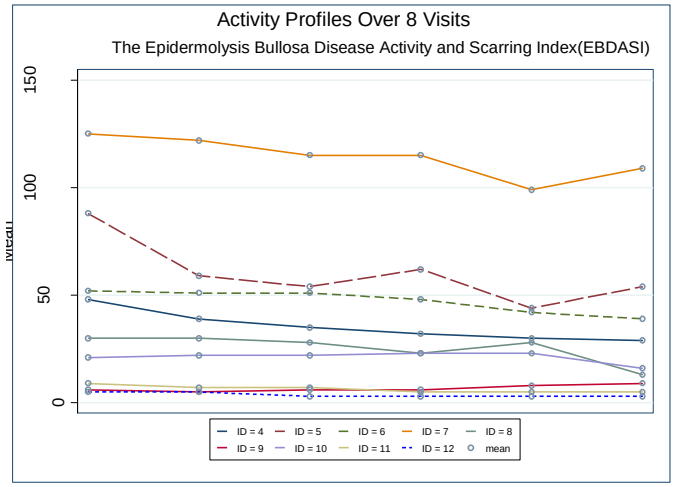
Table 2.5. Mean differences [95% confidence intervals (CI)] between measures taken at baseline and 2 subsequent visits

	Comparison visits	Mean Difference	[95% CI]		p value (t-test)	p value (signrank)
Birmingham Epidermolysis Bullosa Severity Score(BEBSS)	Visit 1 Versus Visit 4	0.33	-0.30	0.97	0.262	0.158
	Visit 1 Versus Visit 5	1.61	-0.05	3.27	0.056	0.0477
The Epidermolysis Bullosa Disease Activity and Scarring Index(EBDASI)	Visit 1 Versus Visit 4	3.89	-3.71	11.48	0.272	0.2091
	Visit 1 Versus Visit 5	6.00	-2.89	14.89	0.158	0.0951
EBDASI-Activity	Visit 1 Versus Visit 4	4.89	-2.42	12.20	0.161	0.0414
	Visit 1 Versus Visit 5	7.00	-1.59	15.59	0.097	0.0201
EBDASI- Severity	Visit 1 Versus Visit 4	-1.00	-2.63	0.63	0.195	0.158
	Visit 1 Versus Visit 5	-1.00	-2.63	0.63	0.195	0.158

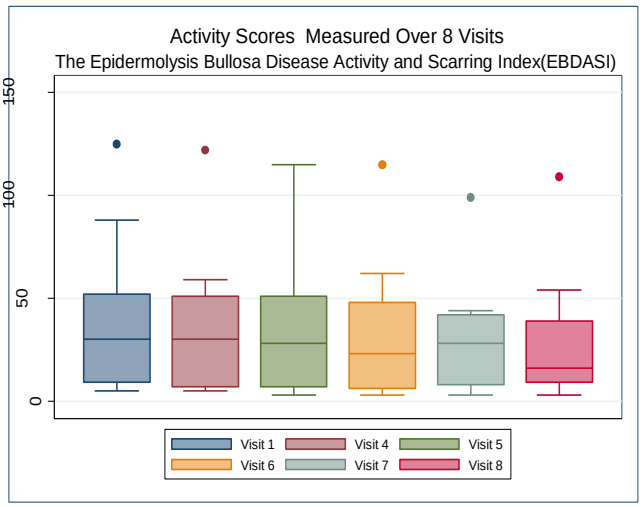
Note. *Differences were calculated as Baseline measure minus other subsequent measurements. Negative values indicate increase after the baseline visit.*
CI: Confidence interval

The profiles for the subscales are displayed in Figures 5.1 (c.1) and (c.2) and median scores by Figures 5.2 (c.1) and (c.2).

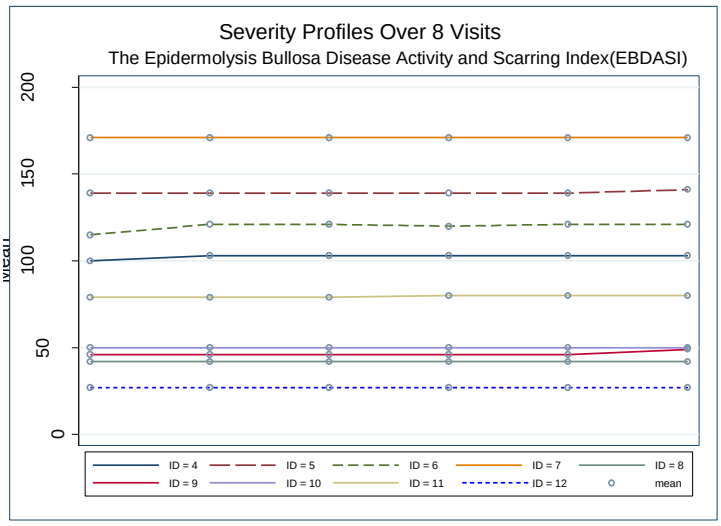
Figures 5.1 (c.1) Activity Scores Profiles (EBDASI)



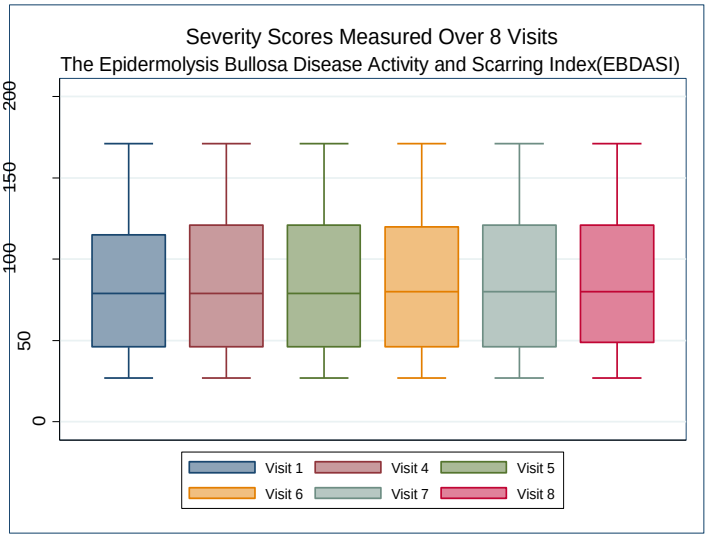
Figures 5.2 (c.1) Activity Scores of (EBDASI)



Figures 5.1 (c.2) Severity Scores Profiles (EBDASI)



Figures 5.2 (c.2) Severity Scores of (EBDASI)



7.4.3 Total Blister Count and Suction Blister Time Profiles

Total blister count over the entire body surface area has shown a decrease on average at day 28, and day 60 compared to baseline. The average decrease was 2.78 (95% CI: -1.67 to 7.22) at day 28, and 2.88 (95% CI: -2.01 to 7.76) at day 60. Table 2.6.

Table 2.6 Mean differences [95% confidence intervals (CI)] between measures taken at baseline and 2 subsequent visits for blister assessments

	Comparison visits	Mean Difference	[95% CI]		p value (t-test)	p value (signrank)
Total Blister Count	Visit 1 Versus Visit 4	2.78	-1.67	7.22	0.188	0.4011
	Visit 1 Versus Visit 5	2.88	-2.01	7.76	0.207	0.2258
Suction blister time	Visit 1 Versus Visit 4	-10.11	-184.63	164.40	0.897	0.9528
	Visit 1 Versus Visit 5	134.00	-53.24	321.24	0.138	0.1731

The profiles show wide variations across patients, for example one patient (ID5) had a sharp drop at day 28, followed by an increase at day 60, followed by a drop in subsequent visits. In two patients (ID9 and ID10) the blister number decreased at day 28 and day 60, then fluctuates slightly. Figure 6.1 (a)

The box plots, show a decrease in median at all visits that follow the baseline. The decrease was clearer at day 28 and day 60, and four relatively higher values were highlighted. Figure 6.2 (a)

Figure 6.1. Total Blister Count and Suction Blister Time Profiles

6.1 (a) Blister Count Profiles

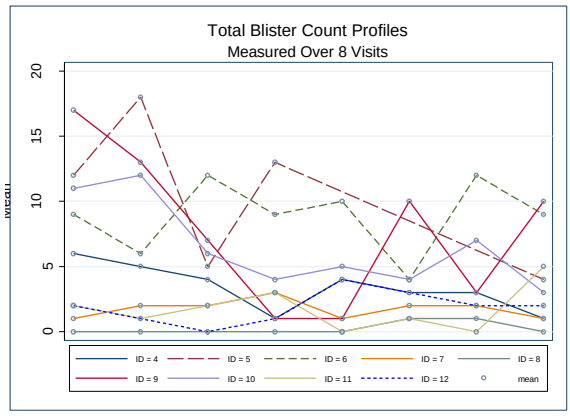
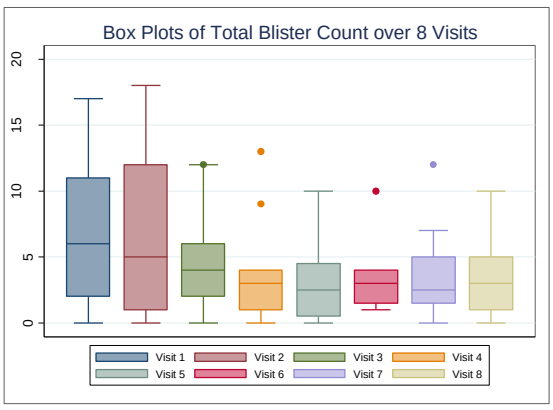


Figure 6.2 (a) Total Blister count



Suction blister time on average was slightly longer at day 28 compared to baseline, with an average difference of 10.11 (95% CI: -164.40 to 184.63) seconds. Wide variations across patients and within patient over time observed. The lowest suction blister time was observed in 4 patients (ID4, ID5, ID6 and ID7), moderate time in patients (ID8, ID10, ID11 and ID12)

while one patient (ID9) has the longest time that also fluctuates between visits. Figure 6.1 (b). The median suction time, was lowest at day 14 (visit 3), highest at day 28, and moderate fluctuation was observed after. Figure 6.2 (b)

Figure 6.1 (b) Suction Blister Time

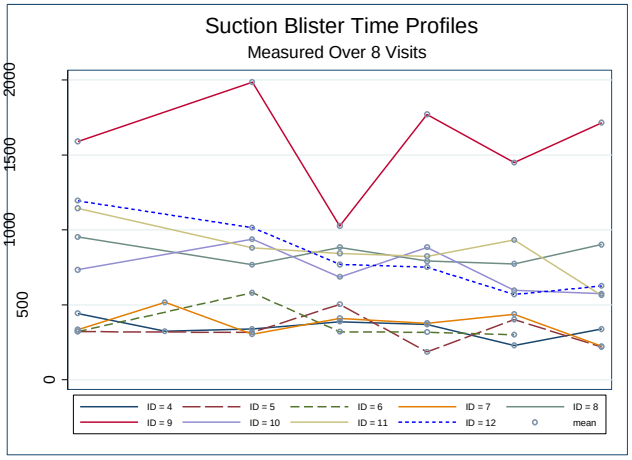
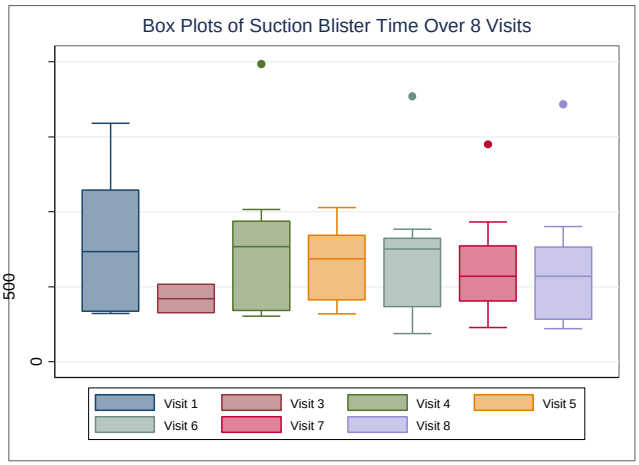


Figure 6.2 (b) Suction Blister Time



7.4.4 Leuven Itch Scale (LIS) profiles

Change in pruritus score was assessed using the Leuven Itch Scale (LIS). A drop in itch frequency was observed, at days 28, 60, 100 and month 6. The mean decrease at these times respectively was: 13.89(95% CI: 3.76 to 24.02), 18.75 (95% CI: 9.07 to 28.43), 15.63 (95% CI: 4.81 to 26.44), and 12.50 (95% CI: 1.33 to 23.67). Table 3.

The general trend was a decrease at day 28 and day 60 that remain stable to month 6 for most patients, followed by an increase in the last visit, month 8/12. Figure 7.1 (a). The Box plots, similarly have shown a decrease followed by an increase in the final visit. Figure 7.2 (a).

Figure 7.1 (a) Frequency

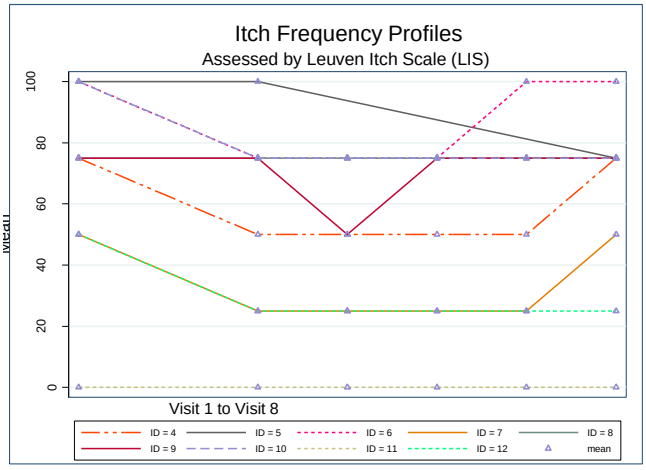
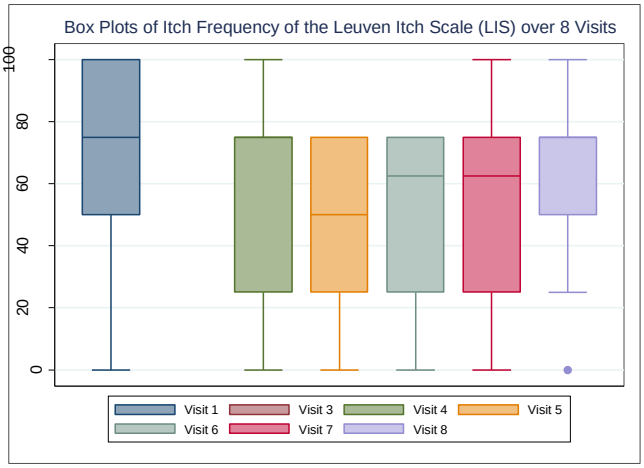


Figure 7.2 (a) Frequency



Wide variations observed in duration, as most estimates were zero however, the profiles have no clear meaningful interpretation. Figure 7.1 (b) and 7.2 (b).

Figure 7.1 (b) Duration

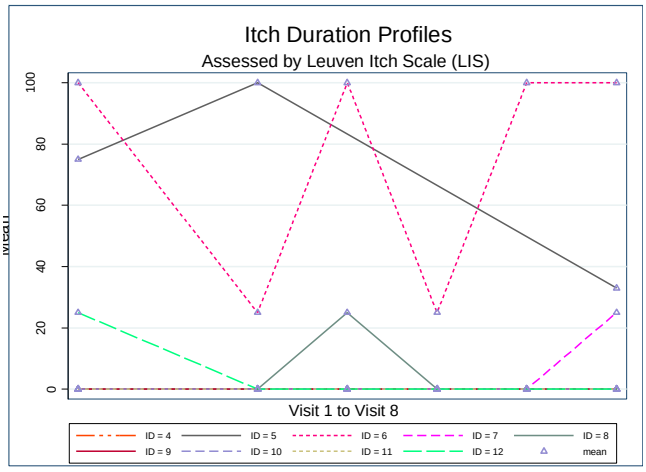
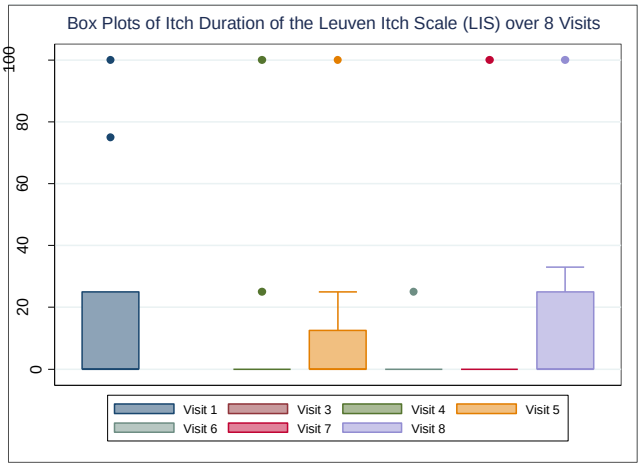


Figure 7.2 (b) Duration



Itch severity scores, have shown a drop, with a mean difference of 15.44 (95% CI: 4.47 to 26.42) at day 28, and 15.16 (95% CI: -1.47 to 32.05) at day 60 lower than baseline. Table 3.

Some patients seem to maintain reasonably low levels afterwards while fluctuations were also observed, and two patients (ID4 and ID9) showed the most dramatic changes over time. Figure 7.1 (c). The box plots, show a decrease in median at day 28 and day 60, followed by a gradual increase at subsequent visits. Figure 7.2 (c).

Figure 7.1 (c) Severity

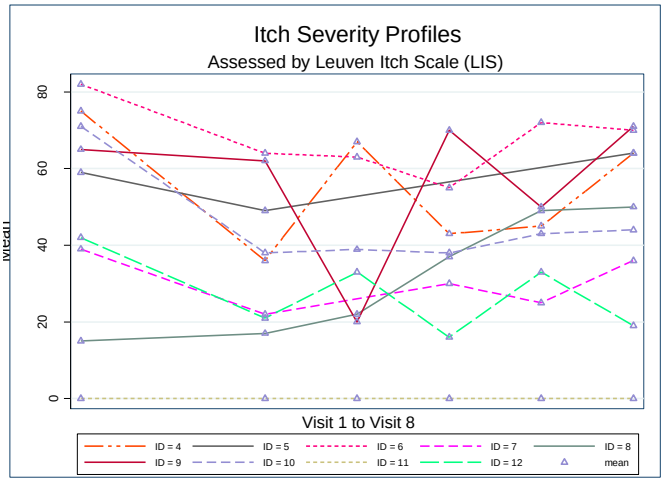
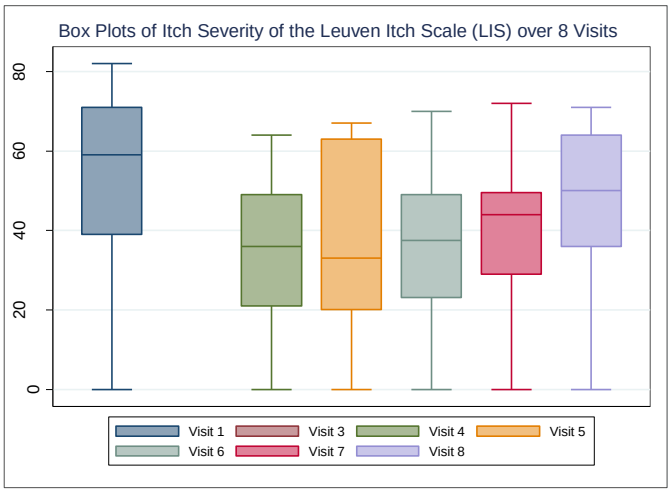


Figure 7.2 (c) Severity



The consequences of itch similarly decreased, the mean difference from baseline was 12.64(95% CI: 0.40 to 24.88), 17.21 (95%CI: 6.40 to 28.01), 14.26 (95% CI: 4.51 to 24.0) and 10.95 (95% CI: 0.78 to 21.12) at day 28, day 60, day 100 and month 6 respectively. (Table 3).

The pattern of the profiles and medians over time was like these seen in the severity score. Figure 7.1 (d), Figure 7.2 (d)

Figure 7.1 (d) Consequences

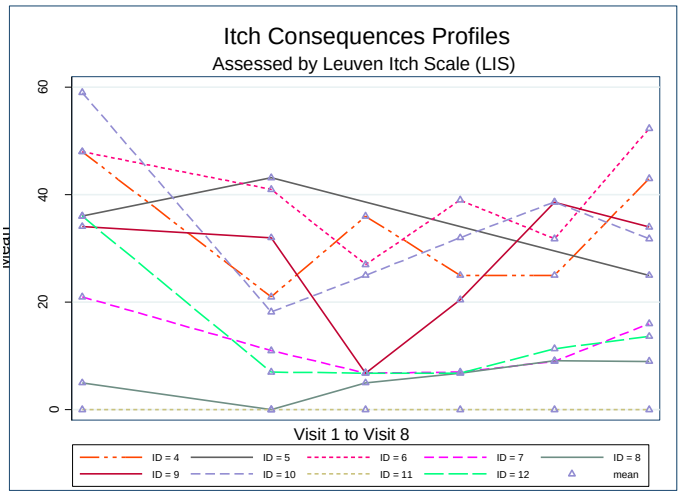
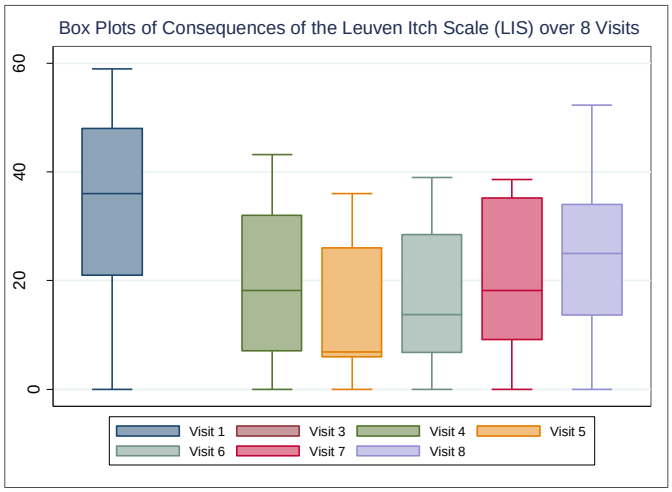


Figure 7.2 (d) Consequences



Distress scores similarly dropped at day 28 and day 60, the difference was 17.11 (95% CI: -3.68 to 37.91) and 26.84 (2.71 to 50.97) respectively. Some variations across patients were observed. Figure 7.1 (e).

Figure 7.1 (e) Distress

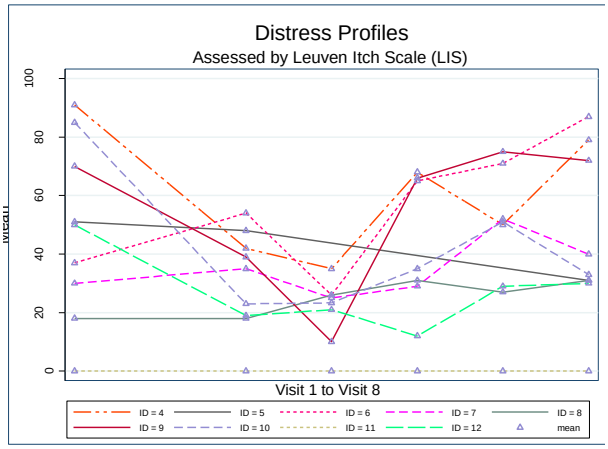
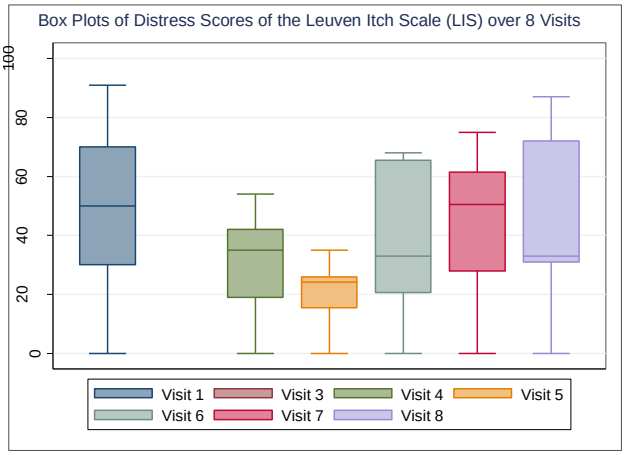


Figure 7.2 (e) Distress



The box plots, show a decrease in average followed by an increase in day 100, and month 6, followed by a decrease in month 8/12.

Body surface area affected by itch has shown a decrease over time in general, but there was an increase of 0.83 (95% CI: -7.03 to 8.70) in the mean difference between baseline and day 28, while at day 60, day 100, and month 6, respectively, the difference was: 2.44 (95% CI: -6.65 to 11.52), 3.31 (95% CI: -0.47 to 7.10) and 5.81 (95% CI: -1.79 to 13.42), lower than baseline. The profiles show wide variations across patients Figure 7.1 (f).

Figure 7.1 (f) Surface Area

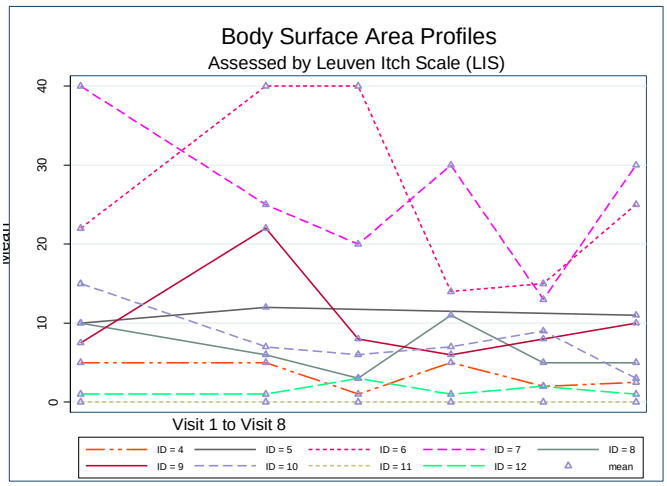


Figure 7.2 (f) Surface Area

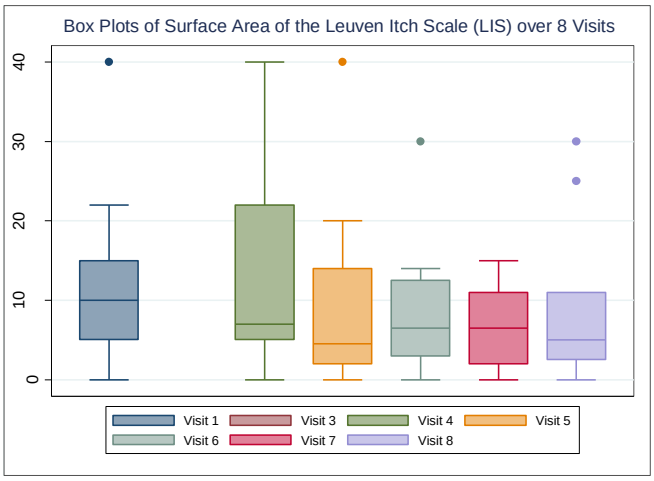


Table 3. Mean differences [95% confidence intervals (CI)] between measures taken at baseline and 4 subsequent visits, on Leuven Itch Scale (LIS) 6 domains

	Comparison visits	**Mean Difference**	**[95% CI]**		P value	P value
Frequency	Visit 1 Versus Visit 4	13.89	3.76	24.02	0.013	0.025
	Visit 1 Versus Visit 5	18.75	9.07	28.43	0.003	0.014
	Visit 1 Versus Visit 6	15.63	4.81	26.44	0.011	0.025
	Visit 1 Versus Visit 7	12.50	1.33	23.67	0.033	0.046
Duration						
	Visit 1 Versus Visit 4	8.33	-13.15	29.82	0.397	0.518
	Visit 1 Versus Visit 5	0.00	-11.17	11.17	1.000	1.000
	Visit 1 Versus Visit 6	12.50	-9.84	34.84	0.228	0.158
	Visit 1 Versus Visit 7	3.13	-4.26	10.51	0.351	0.317
Severity						
	Visit 1 Versus Visit 4	15.44	4.47	26.42	0.012	0.018
	Visit 1 Versus Visit 5	15.16	-1.74	32.05	0.071	0.051
	Visit 1 Versus Visit 6	12.50	-4.39	29.39	0.124	0.107
	Visit 1 Versus Visit 7	9.00	-7.70	25.70	0.243	0.182
Consequences						
	Visit 1 Versus Visit 4	12.64	0.40	24.88	0.045	0.044
	Visit 1 Versus Visit 5	17.21	6.40	28.01	0.007	0.019
	Visit 1 Versus Visit 6	14.26	4.51	24.00	0.011	0.030
	Visit 1 Versus Visit 7	10.95	0.78	21.12	0.038	0.079
Distress						
	Visit 1 Versus Visit 4	17.11	-3.68	37.91	0.094	0.151
	Visit 1 Versus Visit 5	26.84	2.71	50.97	0.034	0.042
	Visit 1 Versus Visit 6	9.38	-12.38	31.13	0.342	0.292
	Visit 1 Versus Visit 7	3.25	-18.96	25.46	0.740	0.888
Surface Area						
	Visit 1 Versus Visit 4	-0.83	-8.70	7.03	0.813	0.952
	Visit 1 Versus Visit 5	2.44	-6.65	11.52	0.546	0.440
	Visit 1 Versus Visit 6	3.31	-0.47	7.10	0.077	0.110
	Visit 1 Versus Visit 7	5.81	-1.79	13.42	0.114	0.079

Legend: For all box plots, Q3 is the upper quartile, below which 75% of the data falls. It represents the upper side of the rectangle. Q1 is the lower quartile, below which 25% of data falls. It represents the lower side of the rectangle. Interquartile range IQR is the difference between Q3 and Q1. Outliers, are values that are either larger than the Q3 by at least 1.5 times the IQR or smaller than Q1 by at least 1.5 times the IQR. The box plots on the other hand show lower medians at day 28, and day 60, compared to baseline, that increased at day 100 and month 6, and decreased at month 8/12.

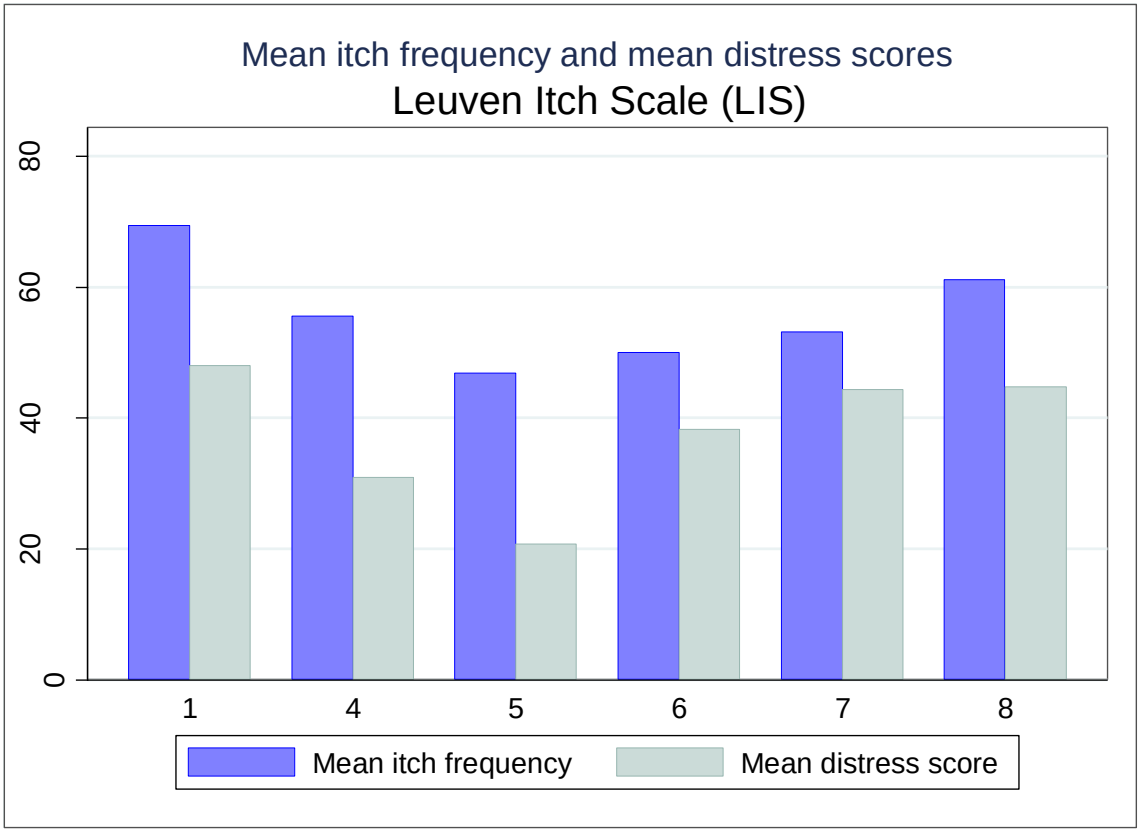
8. Discussion

We conducted a phase I/II open label clinical trial giving two infusions of bone marrow-derived allogeneic mesenchymal stromal cells (MSCs; 2-4 x 10^6 cells/kg) 2 weeks apart to 10 adults with RDEB without subject conditioning or HLA typing. No serious adverse events were reported up to 12 months post-MSCs. The clinical burden of RDEB improved in 8 subjects with a decrease in disease activity at day 28 and day 60 post-MSCs compared to baseline for the BEBSS, EBDASI activity and the QOLEB scores. Leuven Itch Score subscales of frequency, severity and consequences of itch showed a clear reduction at days 28 and 60 post MSCs. In serum, HMGB1 levels were reduced after MSCs at day 28 and day 60 compared to baseline.

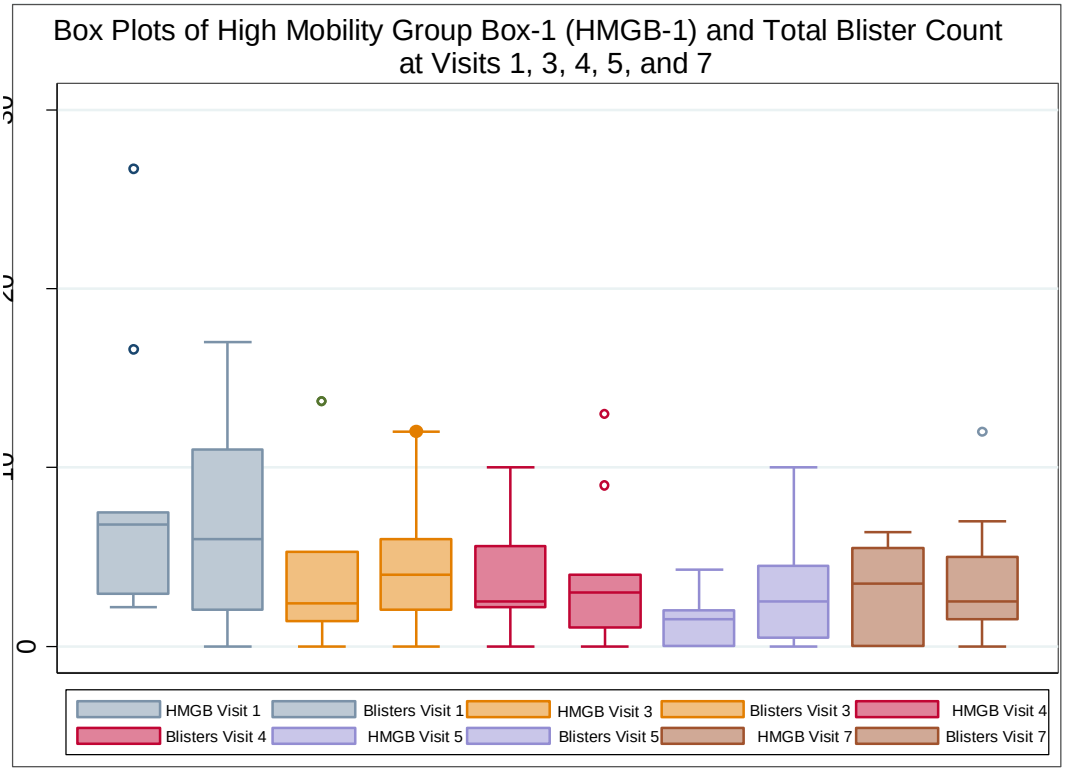
Note. Other relations of interest were observed and graphically illustrated, see Additional Figures (A-H)

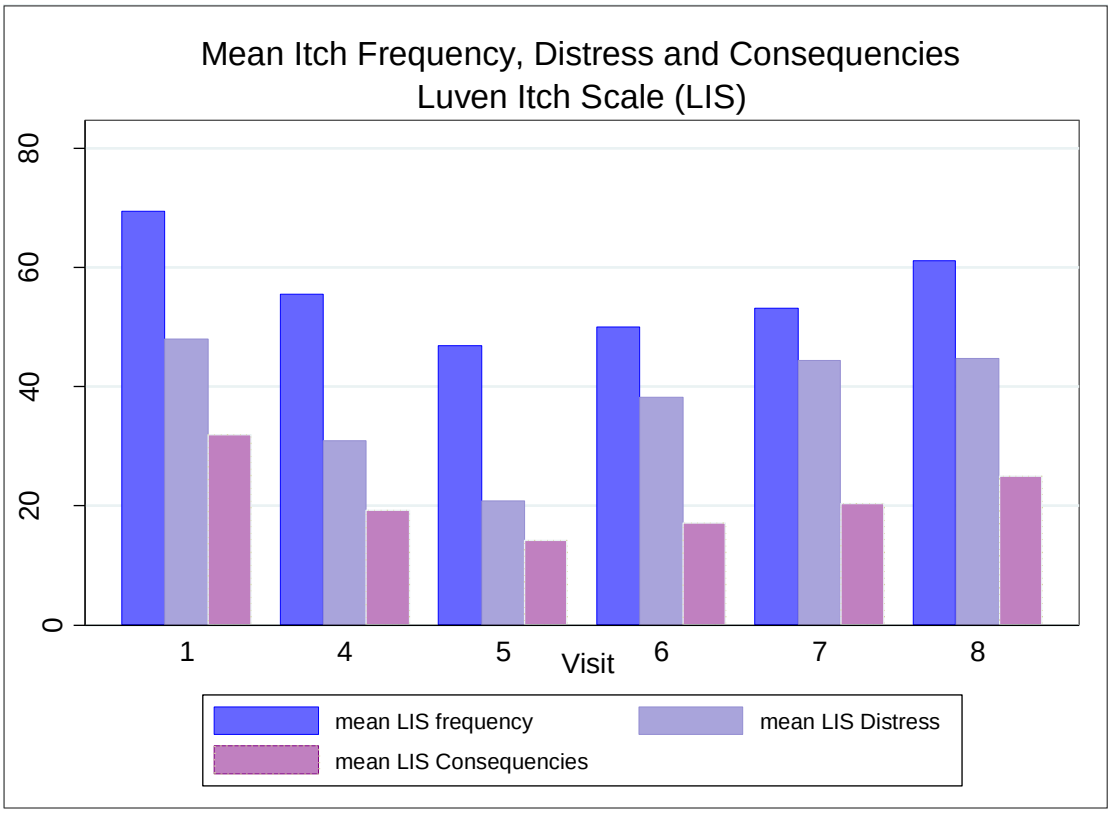
Additional figures

(A)

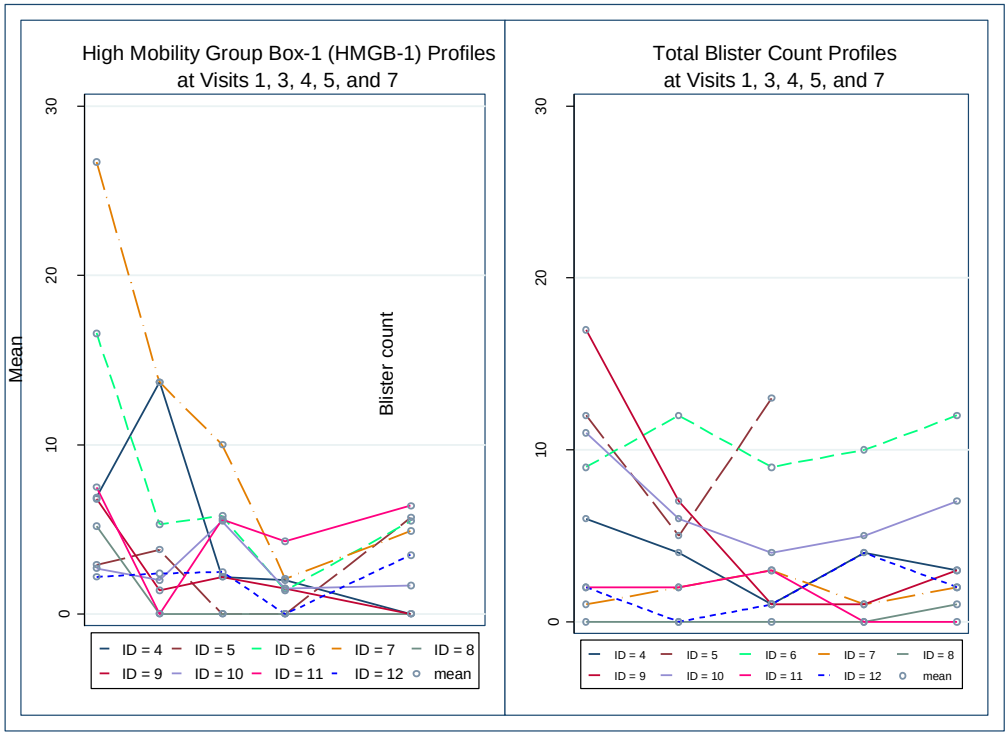


(B)

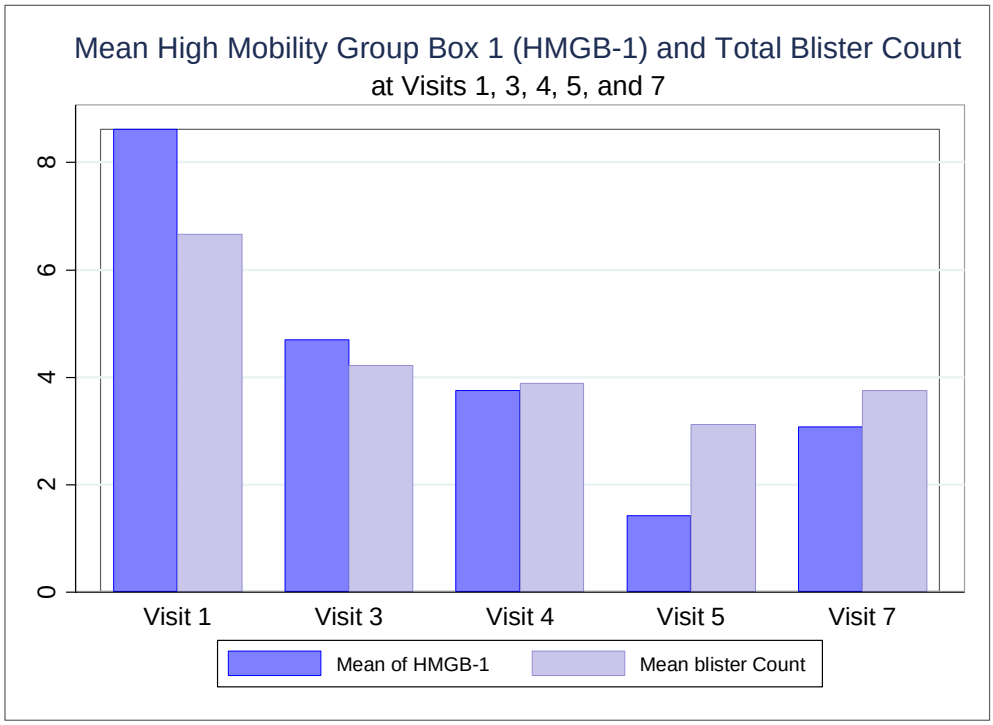

Box Plots of High Mobility Group Box-1 (HMGB-1) and Total Blister Count
at Visits 1, 3, 4, 5, and 7
0
HMGB Visit 1
Blisters Visit 1
HMGB Visit 3
Blisters Visit 3
HMGB Visit 4
Blisters Visit 4
HMGB Visit 5
Blisters Visit 5
HMGB Visit 7
Blisters Visit 7


Mean Itch Frequency, Distress and Consequencies
Luven Itch Scale (LIS)
0
20
40
60
80
1
4
5
6
7
8
Visit
mean LIS frequency
mean LIS Distress
mean LIS Consequencies

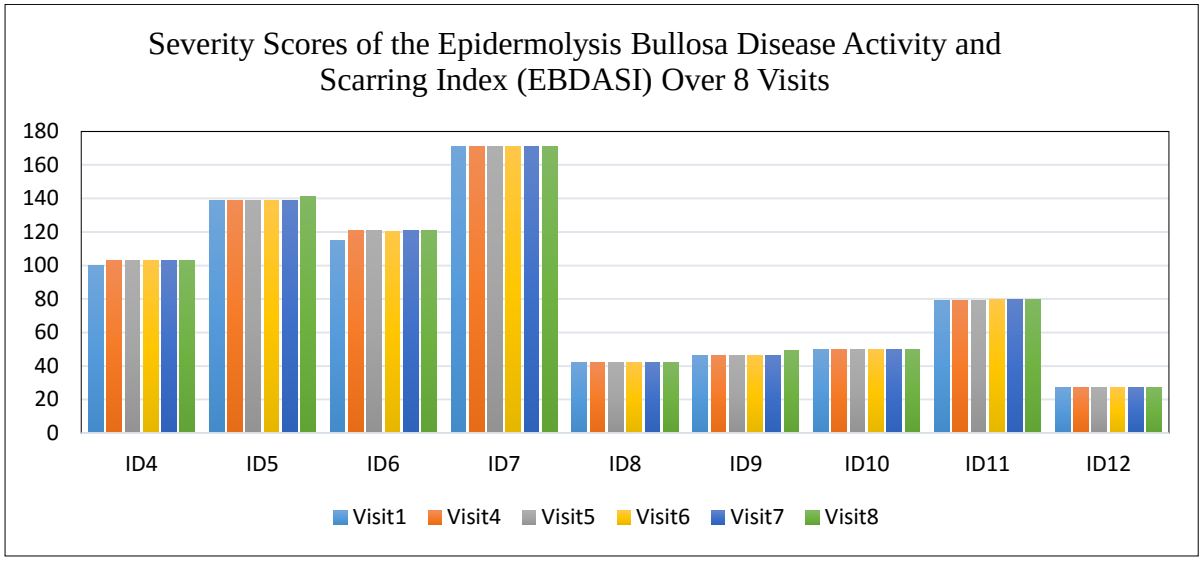
(D)


High Mobility Group Box-1 (HMGB-1) Profiles
at Visits 1, 3, 4, 5, and 7
Mean
Blister count
0
10
20
30
ID = 4
ID = 5
ID = 6
ID = 7
ID = 8
ID = 9
ID = 10
ID = 11
ID = 12
mean
Total Blister Count Profiles
at Visits 1, 3, 4, 5, and 7

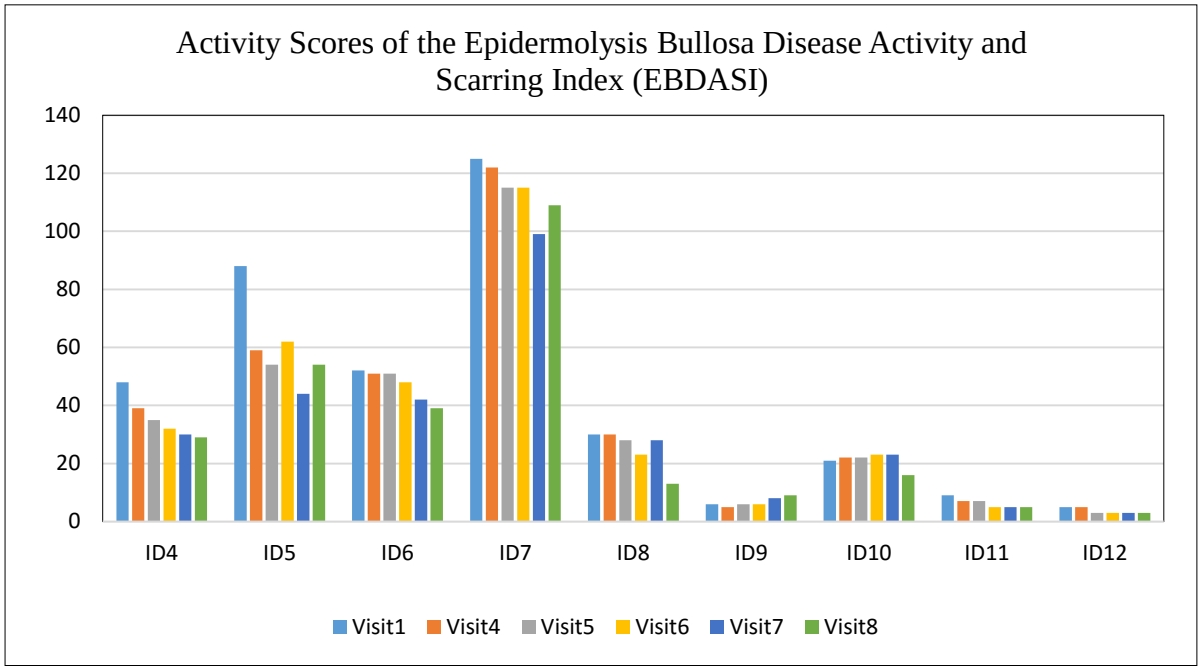
(E)


Mean High Mobility Group Box 1 (HMGB-1) and Total Blister Count
at Visits 1, 3, 4, 5, and 7
0
2
4
6
8
Visit 1
Visit 3
Visit 4
Visit 5
Visit 7
Mean of HMGB-1
Mean blister Count

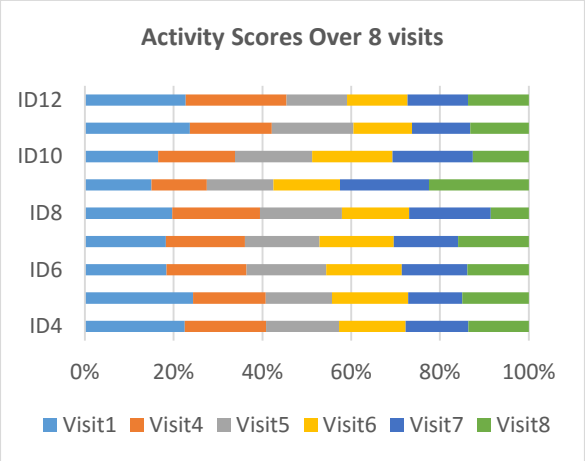
(F)

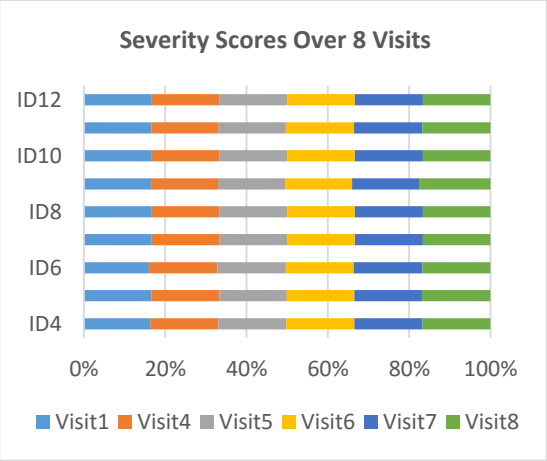

Severity Scores of the Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI) Over 8 Visits
180
160
140
120
100
80
60
40
20
0
ID4
ID5
ID6
ID7
ID8
ID9
ID10
ID11
ID12
Visit1
Visit4
Visit5
Visit6
Visit7
Visit8

(G)


Activity Scores of the Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI)
140
120
100
80
60
40
20
0
ID4
ID5
ID6
ID7
ID8
ID9
ID10
ID11
ID12
Visit1
Visit4
Visit5
Visit6
Visit7
Visit8

(H)





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10. Appendix

Appendix 1. Baseline patient characteristics and clinical data for recruited patients

Patient 04	Demographic data	
	Age	31
	Gender	Female
	Weight	41.3
	Clinical data	
	Partial or complete loss of type 7 collagen	Complete loss of collagen 7
	Mutation	c.1732C>T, p.Arg578X, exon 13; c.7786delG, p.Gly2596ValfsX33, exon 104
Patient 05	Demographic data	
	Age	35
	Gender	Female
	Weight	43.9
	Clinical data	
	Partial or complete loss of type 7 collagen	Complete absence of type VII collagen staining
	Mutation	c.1732C>T;p.Arg578* ; c.7474C>T; p.Arg2492*
Patient 06	Demographic data	
	Age	44
	Gender	Male
	Weight	61
	Clinical data	
	Partial or complete loss of type 7 collagen	Partial reduction in the intensity and pattern of type VII collagen labelling
	Mutation	c.1732C>T; p.Arg578X; IVS20+2T>C
Patient 07	Demographic data	
	Age	26
	Gender	Male
	Weight	22.1
	Clinical data	
	Partial or complete loss of type 7 collagen	Absent type VII collagen
	Mutation	c.186delG; p.Gly62fsX39; IVS79+1G>C
Patient 08	Demographic data	
	Age	55
	Gender	Male
	Weight	84.7
	Clinical data	
	Partial or complete loss of type 7 collagen	Linear and bright and of similar intensity to control
	Mutation	c.5047 C>T, p.R1683X, exon 54; c.5720/21 GA>AT, p.G1907D, exon 68
Patient 09	Demographic data	
	Age	43
	Gender	Female
	Weight	68
	Clinical data	
	Partial or complete loss of type 7 collagen	Linear and bright and of similar intensity to control
	Mutation	p.Gly2213Arg; p.Arg2791Pro
Patient 10	Demographic data	
	Age	27
	Gender	Female
	Weight	56.7
	Clinical data	

	Partial or complete loss of type 7 collagen	Partial reduction in the intensity and pattern of type VII collagen labelling
	Mutation	p.Arg2069Cys in exon 74; c.5669InsG in exon 67
Patient 11	Demographic data	
	Age	35
	Gender	Male
	Weight	80
	Clinical data	
	Partial or complete loss of type 7 collagen	Linear and bright and of similar intensity to control
	Mutation	c.5047C>T,p.R1683X, exon 54; c.5869C>T, p.R1957W, exon 71
Patient 12	Demographic data	
	Age	36
	Gender	Male
	Weight	85.3
	Clinical data	
	Partial or complete loss of type 7 collagen	Linear and bright and of similar intensity to control
	Mutation	(+/+) c.6205C>T, p.Arg2069Cys, exon 74

Appendix 2: Table summarizing the study interventions per visit until M8/12

Study Visit	Screening	V2 D0	V3 D14	V4 D28	V5 D60	V6 D100	V7 M6	V8 M8 or M12
	(<200 day)		±3D	±7D	±14D	±14D	±21D	±28D
Informed consent	X	X	X	X	X	X	X	X
Screening	X							
Assessment of eligibility	X							
Skin biopsy for RNA sequencing	X			X	X			
Skin biopsy for IMF	X			X	X		X	
Skin biopsy for EM	X			X	X		X	
Blood for gene expression	X		X	X	X		X	
Blood for protein expression	X		X	X	X		X	
HLA typing	X							
Infection screen	X							
Standard FBC, U&Es, LFTs, CRP and ESR	X	X	X	X	X	X	X	X
Indirect IMF	X		X	X	X		X	
Physical examination and	X	X	X	X	X	X	X	X
Medical history	X							
Concomitant medication review	X	X	X	X	X	X	X	X
BEBSS and EBDASI	X			X	X	X	X	X
Leuven itch score	X			X	X	X	X	X
Quality of Life Questionnaires	X			X	X	X	X	X
Blister Count	X	X	X	X	X	X	X	X
Photography	X	X	X	X	X	X	X	X
Suction blister time assessment	X			X	X	X	X	X
Patient diary card issued/reviewed	X	X	X	X	X	X	X	X
Infusion of Mesechymal Stromal Cells		X	X					
Adverse events documentation		X	X	X	X	X	X	X

Appendix 3 - List of study visits completed by date and reason for withdrawal

Patient ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Patient 01	12/06/2015		Failed screening – developed SCC					
Patient 02	22/06/2015	13/08/2016	Discontinued after 1st infusion due to SAE					
Patient 03	23/06/2015		Withdrew consent					
Patient 04	01/07/2015	20/10/2015	04/11/2015	16/11/2015	21/12/2015	28/01/2016	04/05/2016	03/10/2016
Patient 05	17/11/2015	01/02/2016	15/02/2016	29/02/2016	29/03/2016	04/05/2016	10/08/2016	08/02/2017
Patient 06	11/08/2015	16/12/2015	30/12/2015	15/01/2016	26/02/2016	17/05/2016	15/06/2016	21/06/2017
Patient 07	02/09/2015	09/11/2015	23/11/2015	01/12/2015	04/01/2016	02/03/2016	16/05/2016	07/11/2016
Patient 08	16/10/2015	17/02/2016	01/03/2016	23/03/2016	21/04/2016	06/06/2016	26/08/2016	20/01/2017
Patient 09	30/03/2016	27/04/2016	11/05/2016	24/05/2016	04/07/2016	03/08/2016	17/10/2016	10/05/2017
Patient 10	23/05/2016	03/08/2016	17/08/2016	31/08/2016	04/10/2016	09/11/2016	31/01/2017	12/07/2017
Patient 11	25/05/2016	18/11/2016	30/11/2016	19/12/2016	17/01/2017	27/02/2017	11/05/2017	05/07/2017
Patient 12	18/07/2016	17/11/2016	01/12/2016	15/12/2016	16/01/2017	15/02/2017	25/05/2017	07/07/2017

Appendix 4: Mesenchymal Stromal Cells (MSC) intended and actual infusions per patient by visit and date

Patient ID	Intended MSC infusion	Actual MSC infusion	Date of consent	Date of 1st MSC infusion	Date of 2nd MSC infusion
Patient 4	2	2	01/07/2015	20/10/2015	04/11/2015
Patient 5	2	2	10/08/2015	01/02/2016	15/02/2016
Patient 6	2	2	11/08/2015	16/12/2015	30/12/2015
Patient 7	2	2	02/09/2015	09/11/2015	23/11/2015
Patient 8	2	2	16/10/2015	17/02/2016	01/03/2016
Patient 9	2	2	30/03/2016	27/04/2016	11/05/2016
Patient 10	2	2	23/05/2016	03/08/2016	17/08/2016
Patient 11	2	2	25/05/2016	18/11/2016	30/11/2016
Patient 12	2	2	18/07/2016	17/11/2016	01/12/2016

Appendix 5. Production of BM-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. BM-MSCs from the bone marrow of three healthy unrelated donors 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 all three donors was screened for *COL7A1* mutations and none were found.

BM-MSCs from three 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