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REVIEW ARTICLE

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Systemic treatment of recessive dystrophic epidermolysis bullosa with mesenchymal stromal cells: a scoping review of the literature and conclusions for future clinical research

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ABSTRACT

Background: The ability of mesenchymal stromal cells (MSCs) to facilitate regenerative responses in inflamed and injured tissues, coupled with preclinical data suggesting potential to restore defective collagen VII at the dermo-epidermal junction, has raised the hope that MSCs may provide an effective disease-modifying therapy for patients suffering from recessive dystrophic epidermolysis bullosa (RDEB).
Methods: We present a descriptive analysis of the clinical research on systemic MSC administration to RDEB patients available in PubMed, including six early-phase studies and one case report, involving 59 patients who received 1–3 intravenous infusions of MSCs from various sources.
Results: Based on 133 MSC infusions, a total of 44 mostly mild adverse events were reported as definitely, possibly or likely related to the study treatment, only two of which led to treatment discontinuation. Improvements were seen in skin manifestations, disease activity, pain, pruritus and quality of life, with considerable heterogeneity in reported outcome variables and measurement tools between studies, and large inter-patient variability within studies.
Conclusions: Although the current evidence base is limited, reflecting the typical challenges of clinical research in rare diseases, the reported results suggest potential treatment benefits for patients and provide a rationale for continuing to pursue this therapeutic approach.

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Cell therapy; clinical trials; collagen VII; disease-modifying therapy; mesenchymal stromal cells; recessive dystrophic epidermolysis bullosa

Introduction

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare inherited mucocutaneous disorder resulting from biallelic mutations in the *COL7A1* gene encoding for type VII collagen (C7) [1,2]. C7 is a major component of the anchoring fibrils at the dermo-epidermal junction, which are critical for maintaining attachment of the epidermis to the dermis [3,4]. Patients with RDEB suffer from severely compromised cutaneous mechanical stability, which can manifest as blistering, chronic and recurrent wounds, erosions and excessive scarring of the skin, accompanied by severe pruritus, pain and an exceptionally high risk of developing aggressive forms of squamous cell carcinoma [5,6]. In addition, the phenotypic spectrum includes a variety of extracutaneous manifestations such as gastrointestinal, cardiovascular, genitourinary, ocular, and oral involvement and complications [2,7, 8]. Treatment options are mostly limited to comprehensive wound management, trauma prevention, infection control and palliative care of complications [9–11].

Given the significant health impact of RDEB and the severely impaired quality of life, several research groups and clinical teams around the world have focused on developing new and more effective treatments. Broadly speaking, strategies aim to either restore missing C7 through gene correction or protein replacement, or to dampen inflammation with the goal of reducing disease severity, alleviating key symptoms such as itching or pain and slowing damage accumulation and disease progression [12]. Approaches currently under investigation include gene therapy, protein replacement, cell therapy, and pharmacological agents [12–14]. To date, only two medicinal products have been approved by the European Medicines Agency (EMA) and/or the U.S. Food and Drug Administration (FDA) for the treatment of RDEB: the birch triterpene gel Filsuvez® (Oleogel-S10) [15,16] and the viral vector-based gene therapy Vyjuvek™ (beremagene geperpavec) [17], both for topical use.

Mesenchymal stromal cells (MSCs) have gained interest based on the idea that they could combine both C7-restorative and disease-modifying modes of action by expressing the defective protein and secreting factors that alleviate inflammation and facilitate wound healing [14]. Unlike the two topical medicinal products approved so far, MSCs can be administered systemically, with numerous clinical trials across various diseases having demonstrated their favorable safety and tolerability profile [18,19]. This could enable systemic treatment approaches that would target the entire skin, including difficult-to-access wounds, as well as extracutaneous manifestations such as mucosal and organ inflammation and damage. MSCs are widely recognized for their ability to restore homeostasis and facilitate regenerative responses in inflamed and injured tissues including non-healing wounds [20–23], and therapeutic administration of MSCs

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to mouse models of RDEB has resulted in deposition of C7 at the dermo-epithelial junction, de-novo formation of anchoring fibrils and partial reversal of the RDEB phenotype [24–27].

The promising preclinical findings are offset by the typical challenges of clinical research in rare diseases, including small patient populations, highly variable clinical presentation, knowledge gaps in understanding the natural history of the disease, and lack of consensus on appropriate efficacy endpoints. In the light of this situation, this review aims to provide an overview of the available clinical evidence regarding the systemic administration of MSCs for the treatment of RDEB, which may serve as a resource for future research aimed at meeting the urgent needs of patients suffering from this burdensome and devastating disease.

Materials and methods

A literature search was conducted of all articles published in the PubMed database through March 1, 2024, using the search query ("mesenchymal stem cell*"[Title/Abstract] OR "mesenchymal stromal cell*"[Title/Abstract] OR "mesenchymal stem/stromal cell*"[Title/Abstract] OR "Mesenchymal stem cells"[MeSH]) AND ("dystrophic epidermolysis bullosa"[Title/Abstract] OR RDEB[Title/Abstract] OR DEB[Title/Abstract] OR "Epidermolysis bullosa dystrophica"[MeSH]). The query returned a total of 39 records, which were reviewed to identify reports of patients who received systemic transplantation of MSCs for the treatment of RDEB. In order to provide a comprehensive overview of the state of research and to avoid missing any information, not only clinical trials but also case reports were included, as recently proposed for systematic reviews of clinical research in rare diseases [28,29]. Of the initial 39 records, 29 were excluded based on title and abstract review and an additional 3 were excluded based on full-text eligibility assessment (for exclusion reasons see Figure 1). The 7 eligible journal articles were supplemented by 5 additional reports identified through a thorough citation search of literature references and trial registration IDs within the initially identified records, including two journal articles that report on two studies not identified through the PubMed query, and one conference abstract and two publicly available end-of-trial reports that provided additional details on two studies already identified. Together, a total of 12 reports were included in the review (Figure 1).

From the included reports, the following information was extracted: study type and design, MSC type and dosing, number and age of patients, adverse events, and efficacy variables and

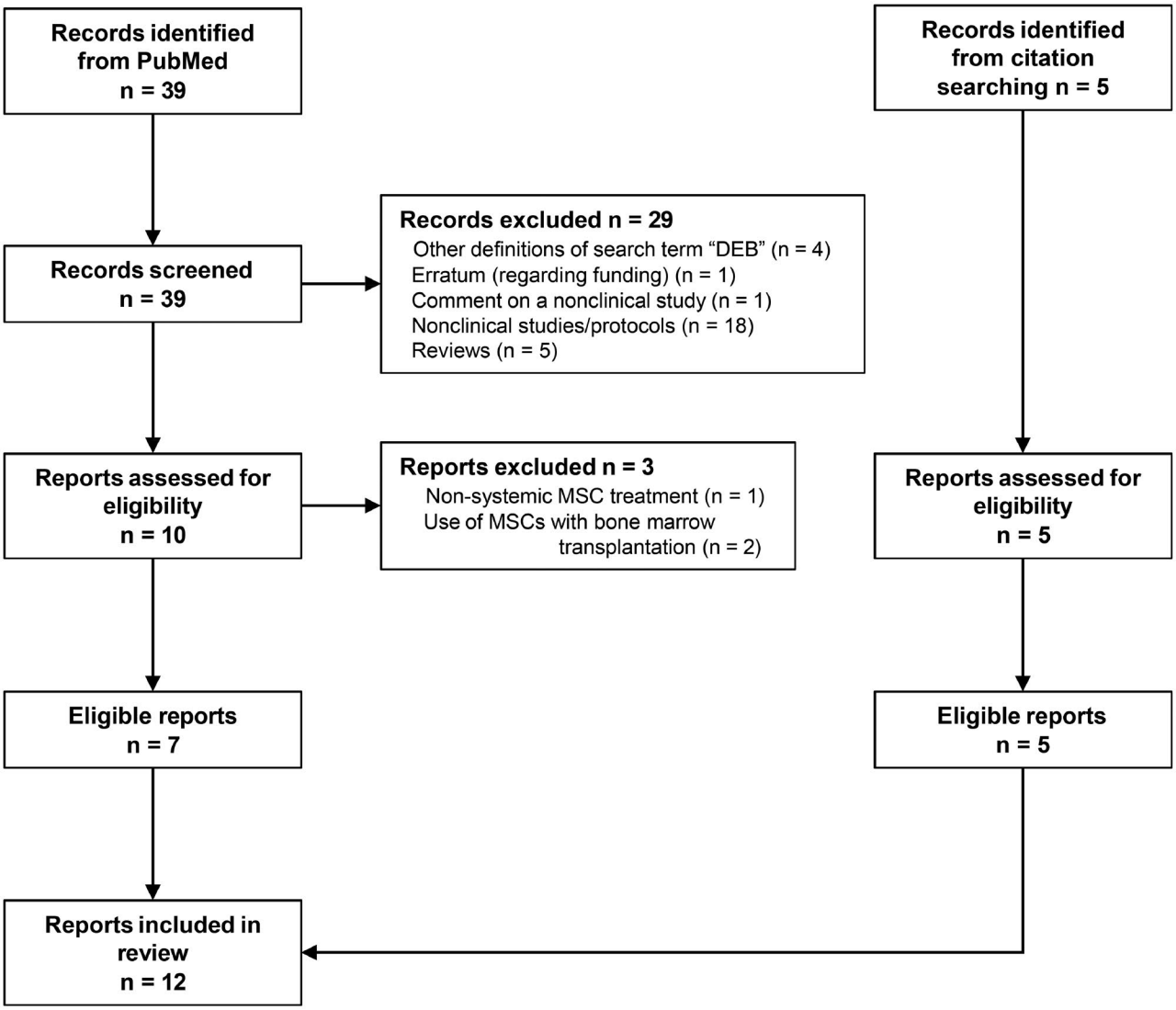

Figure 1. Flow diagram of the literature search and selection

outcomes. Given the small numbers of studies and treated patients in the context of a rare disease, a descriptive comparative analysis of the reports is presented.

Results and discussion

Study designs

The included publications [30–41] report on six early-phase clinical trials in Europe (UK, Germany, Austria, France), Asia (Japan, Republic of Korea), Egypt and the U.S., as well as one patient in Spain who was treated with MSCs under compassionate use. In total, 59 RDEB patients received between one and three intravenous infusions of MSCs derived from a variety of sources. All cell doses were administered on an open-label basis, and none of the trials included a control group (Table 1).

This overall picture is consistent with what is typically observed in clinical trials for rare diseases, which can be summarized as being more likely to be early phase, have smaller sample sizes, recruit to a single arm, and be non-randomized and open label than trials for non-rare diseases [42], reflecting the challenge of recruiting sufficient numbers of patients affected by a rare disease [43]. The conduct of placebo-controlled clinical trials is further hampered by ethical concerns about withholding a potentially effective treatment from patients suffering from a progressive, devastating and potentially life-threatening disease and by the corresponding reluctance of patients to enroll in trials where they may receive a placebo instead of the treatment being studied [43–45].

While uncontrolled study designs may be appropriate to generate evidence of the safety and potential efficacy of a drug in early clinical development, regulatory authorities will generally require randomized controlled trials as a product progresses and eventually reaches the marketing authorization application stage, although some flexibility may be provided in the area of orphan drugs [46,47]. In fact, approximately one-quarter to one-third of the pivotal trials submitted to support the approval of orphan drugs authorized in the EU or in the U.S. used non-controlled designs [48–50]. In cases where the gold standard of randomized placebo-controlled trials is not affordable, investigators, in close consultation with the regulatory authorities, may use external controls from other clinical trials or real-world data representing the natural history of the disease without intervention or with standard of care [46,47]. However, such approaches are particularly challenging in RDEB due to the extremely limited availability of external control and natural-history data. Alternatively, modified study designs that retain the advantages of placebo-controlled trials while minimizing placebo exposure, such as crossover designs or unequal randomization ratios, may be considered [47]. The birch triterpene gel Filsuvez (Oleogel-S10) gained approval on the basis of a randomized controlled trial [51,52] in which the offer of an open-label extension may have facilitated the willingness to enroll in a placebo-controlled trial [53]

Table 1. Clinical studies of systemic MSC application to treat RDEB: Designs, interventions and participants.

	El-Darouti et al. [30,31]	Petrof et al. [32,33]	Rashidghamat et al. [34,35]	Fujita et al. [36]	Maseda et al. [37]	Lee et al. [38]	Kiritsi et al. [39–41]
Study IDs	None reported	EudraCT2012-001394-87; ISRCTN46615946; EBSTEM	NCT02323789; EudraCT2014-004500-30; ADSTEM	jRCT1080224498; JapicCTI-184563	n.a.	NCT04520022	NCT03529877; EudraCT 2018-001009-98
Study type	Interventional, "pilot study"	Interventional, phase I/II	Interventional, phase I/II	Interventional, phase I/II, "pivlot study"	Compassionate use	Interventional, phase I/IIa	Interventional, phase I/IIa
Single-/multi-center?	Not specified	Single-center	Single-center	Two-center	Single-center	Single-center	Multi-center
Countries	Egypt	UK	UK	Japan	Spain	Republic of Korea	Germany, Austria, France, UK, U.S.
Placebo control?	No[a]	No	No	No	No	No	No
Blinded?	No[a]	No	No	No	No	No	No
Cell type	Allogeneic BM-MSCs	Allogeneic BM-MSCs	Allogeneic BM-MSCs	Allogeneic BM-MSC-derived SSEA-3$^+$ Muse cells	Allogeneic AT-MSCs	Allogeneic UCB-MSCs	Allogeneic ABCB5$^+$ skin-derived MSCs
Cell dose	2×10^6/kg +/− 5 mg/kg/d cyclosporine	$1–3\times10^6$/kg	$2–4\times10^6$/kg	$3.25–3.55\times10^5$/kg (1.5×10^7/patient)	1×10^6/kg	$1–2\times10^6$/kg (pediatric) or 3×10^6/kg (adult)	2×10^6/kg
Application route	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous	intravenous
Treatment schedule	D0	D0, D7, D28	D0, D14	D0	D0, D21, D42	D0, D14, D28	D0, D17, D35
Number of patients	14	10	10	2[b]	1	6[c]	16
Age of patients	13 pediatric (1–12 years); 1 adult (20 years)	1–11 years	26–44 years	22–26 years	17 years	2 pediatric (8–13 years); 4 adult (21–60 years)	9 pediatric (4–13 years); 7 adult (20–36 years)

[a]Trial was placebo-controlled and blinded for concomitant cyclosporine administration, but open-label regarding MSC application.
[b]Three further patients treated in this trial presented with the dominant form of DEB (DDEB).
[c]One of these patients received the study treatment under an expanded access pathway.

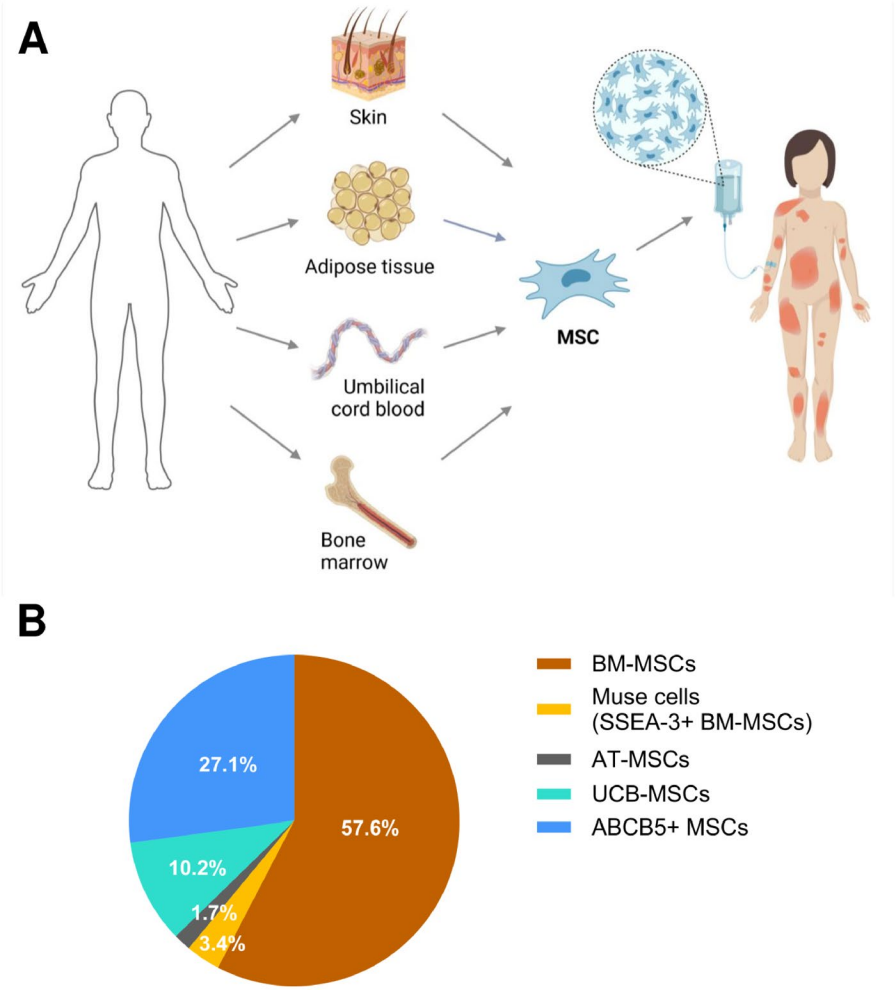

Figure 2. MSC types studied for intravenous treatment of RDEB in humans. (A) Tissue sources of the MSC products. Created with BioRender.com. (B) Percentage distribution of RDEB patients by infused MSC type. Total number of patients = 59

MSC types

All MSC preparations were of allogeneic origin. Source tissues included bone marrow (BM), adipose tissue (AT), umbilical cord blood (UCB) and skin (Table 1; Figure 2(A)). BM-MSCs were the most frequently administered cell type, followed by skin-derived MSCs expressing the ATP-binding cassette superfamily member ABCB5 (ABCB5$^+$ MSCs) and UCB-MSCs (Figure 2(B)).

BM-MSCs

The majority of patients (34, 57.6%) were treated with BM-MSCs. Initially, the rationale for using BM-MSCs to treat RDEB was based on the idea of potentially increasing C7 skin levels in the recipient. *In vitro*, BM-MSCs express C7 at lower or similar levels to healthy fibroblasts [25,26], but significantly increased *COL7A1* transcription and/or C7 protein expression when incubated in the presence of cytokines such as transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α or cocultured with fibroblasts [24,54]. In addition, mechanistic studies suggested that BM-MSC exosomes can increase C7 levels by delivering C7 alpha chain-encoding mRNAs to recipient fibroblasts and by facilitating C7 transport within the extracellular space toward the basement membrane [55]. *In vivo*, intradermal injection of BM-MSCs into various models of RDEB resulted in production and deposition of C7 at the dermo-epidermal junction, *de-novo* formation of anchoring fibrils, facilitation of wound healing, improved skin integrity, and partial reversal of the RDEB phenotype [24–26]. Similar, albeit transient, benefits were also observed in two human patients with severe generalized RDEB following intradermal injection of BM-MSCs [56]. In addition to C7 restoration, the positive effects of BM-MSCs on RDEB skin wound healing were also attributed to the MSCs' anti-inflammatory properties, which induced a shift of the inflammatory state of the wound and granulation tissue formation toward the physiological situation [25]. However, the need for multiple and repeated injections into the inflamed, scarred and often painful skin of RDEB patients is a major drawback of intradermal treatment approaches. In addition, RDEB is not a skin-limited disease, but involves multiple extracutaneous sites that are inaccessible for local injections. This has led clinical researchers to investigate the intravenous route.

El-Darouti et al. [30] infused freshly isolated MSCs derived from bone marrow aspirate of one healthy parent of each patient, whereas Petrof et al. [32] and Rashidghamat et al. [34] used ex-vivo expanded BM-MSCs from healthy unrelated donors manufactured according to Good Manufacturing Practice (GMP) standards. Petrof et al. [32] reported on the cells' viability and the phenotype according to the minimal criteria for defining MSCs as recommended by the International Society for Cellular Therapy [57] of the cells administered.

Muse cells

Multilineage-differentiating stress-enduring (Muse) cells represent small MSC subpopulations expressing, in addition to classical MSC markers, the pluripotent surface marker stage-specific embryonic antigen 3 (SSEA-3) [58]. Intravenous injection of Muse cells isolated from human BM-MSCs into healthy mice resulted in accelerated healing of full-thickness excisional wounds associated with linear deposition of human C7 in the dermal basement membrane zone [59]. Provided as a clinical-grade cell product (nafimestrocel, CL2020; Life Science Institute, Tokyo, Japan), intravenous BM-MSC-derived Muse cells were investigated in a pilot study in 2 human RDEB patients (3.4% of total patients included in this review) [36]

AT-MSCs

AT-MSCs were administered to 1 RDEB patient (1.7%) on a compassionate use basis [37]. The investigators based their rationale on the reported benefits of using BM-MSCs in RDEB [30,32,34], the ease of obtaining AT-MSCs compared to BM-MSCs, and the overall similar therapeutic potential between BM-MSCs and AT-MSCs [60]. The AT-MSCs were derived from lipoaspirate of an unrelated donor, expanded and isolated from the stromal fraction according to GMP regulations. Viability and immunophenotype were reported for each of the three final cell products infused.

UCB-MSCs

Six RDEB patients (10.2%) were treated with infusions of UCB-MSCs [38]. While, to our knowledge, UCB-MSCs have not been preclinically studied for potential therapeutic efficacy in RDEB, the investigators referred to studies of UCB-derived unrestricted somatic stem cells (USSCs), which were considered putative MSC progenitors within the UCB [61]. UCB-USSCs, upon systemic administration to C7 null (*Col7a1*$^{-/-}$) mice, migrated to skin blisters, induced a change in wound macrophages from a CD206^{-} to the anti-inflammatory M2a (CD206^{+}) phenotype, ameliorated the blistering phenotype, and prolonged the life span of the animals [62]. Histology showed an overall improvement in the dermo-epidermal adhesion and *de novo* C7 expression in the basement membrane zone [62]. In addition, UCB-USSCs effectively suppressed excessive TGF-β signaling in the paw skin of C7 null mouse, suggesting that the cells may be able to suppress TGF-β signaling-induced fibrosis in RDEB [63]. This hypothesis was recently supported in the C7-hypomorphic mouse model of RDEB, which recapitulates the fibrotic disease progression of RDEB, in which systemic administration of UCB-USSCs attenuated paw inflammation and digit mutilation through the induction of anti-inflammatory macrophages and increased skin interleukin 1 receptor antagonist (IL-1RA)/interleukin (IL)-1α ratios [64]

The UCB-MSCs studied in human patients were manufactured and expanded under GMP conditions [38]. The authors stated that the cells were confirmed to meet the quality control criteria approved by the South Korean Ministry of Food and Drug Safety; however, data on the characteristics of the cells were not provided.

ABCB5+ MSCs

ABCB5^{+} MSCs were infused to 16 RDEB patients (27.1%) [39]. Compared to BM-MSCs, skin-derived ABCB5^{+} MSCs have shown a higher basal expression of C7 protein [65]. In addition, comparative transcriptional analysis revealed increased expression of *VCAM1* [encoding vascular cell adhesion molecule 1 (VCAM-1)] and several homeobox (Hox) genes, particularly *HOXA3* in ABCB5^{+} MSCs compared to BM-MSCs [65]. Given the importance of VCAM-1 in homing to the perivascular niche [66,67] and of the transcription factor *HOXA3* in coordinating wound healing [68–70], these observations suggest that ABCB5^{+} MSCs have advantageous capabilities for skin homing and facilitating wound healing [65].

In vivo, intravenously transplanted mouse ABCB5^{+} MSCs persisted in mouse skin across fully allogeneic barriers for at least 17 days [71]. In a wounded NSG mouse model, human ABCB5^{+} MSCs homed to full-thickness dorsal skin wounds after intravenous injection and persisted for at least 14 days with significantly superior engraftment capacity compared to intravenously transplanted human BM-MSCs [65]. Wound healing properties of ABCB5^{+} MSCs have been confirmed in a chronic wound model [72] and in clinical trials in treatment-refractory chronic venous ulcers [73,74]. The observed effects were attributed to IL-1RA released by the MSCs, which shifted the prevalence of proinflammatory M1 macrophages to anti-inflammatory, repair-promoting M2 macrophages in the wound tissue [72]. In a *Col7a1*$^{-/-}$ mouse model of RDEB, intravenously administered human ABCB5^{+} MSCs reduced RDEB pathology and markedly prolonged the lifespan of the animals by significantly reducing skin infiltration with pro-inflammatory macrophages [27]

ABCB5^{+} MSCs were isolated from *ex vivo* expanded primary cultures derived from human skin and delivered as a well-characterized and standardized GMP-compliant advanced-therapy medicinal product (ATMP) [75,76]. Product release data were reported for each cell batch infused into RDEB patients, including vitality, viability and biological functionality as demonstrated by potency assays testing the cells' immunomodulatory, pro-angiogenic and endothelial differentiation capacity [39].

Cell doses and dosing schedules

Except for the two patients treated with Muse cells, all patients received cells in the millions-per-kilogram range common for MSC infusions, with dosing frequencies ranging from one to three infusions at intervals of 7 to 21 days (Table 1). In contrast, Muse cells were administered once at a roughly 10-fold lower cell dose [36] (Table 1).

None of the identified trials investigated more than one dose or dosing regimen in parallel. This is another typical consequence of limited patient numbers due to the low prevalences of rare diseases, which is reflected in observations that approximately 35% and 45% of orphan drugs approved in the EU and in the U.S., respectively, have received marketing authorization without having included a dose-finding study in their drug development program [77,78]. On the other hand, in the field of MSC therapy development, there is clear need to identify the most effective, yet safe cell dose, since increasing evidence suggests that MSC exposure-response curves often follow an inverted U-shape, with MSC doses not only below but also above an effective dose range being less effective [79]. In addition to conventional dose-finding studies, other alternative data sources can provide valuable information on exposure-response relationships that can help optimize doses and regimens for rare indications, including data from the use of the product in other patient populations [78]. In fact, in the BM-MSC trials by Petrof et al. [32] and Rashidghamat et al. [34], the selection of MSC dose and infusion schedule was based on previous clinical trials of intravenously infused BM-MSCs, primarily for the treatment of graft-versus-host disease [33,35]. A certain degree of transferability of effective intravenous MSC dose levels between different patient populations is supported by a systematic meta-analysis of clinical trials registered in the U.S. National Institutes of Health clinical trial registry (http://ClinicalTrials.gov), which indicated a relatively narrow dose range of minimally effective doses of

intravenously infused MSCs between 70×10^6 and 190×10^6 cells for a 70 kg patient (equivalent to 1×10^6 to 2.7×10^6 cells/kg) across multiple indications [79]. From a retrospective perspective, the doses used in the RDEB trials (except for the Muse cell study) (Table 1) were within or slightly above this range. While the authors of the Muse cell study [36] did not comment on their dose selection, it has been suggested that a higher homing efficiency of the Muse cells to injured or damaged tissues as compared to non-SSEA-3-sorted MSCs would allow for lower cell doses [80], which was supported by pilot studies in which intravenous infusion of 1.5×10^7 Muse cells per patient showed beneficial effects in patients suffering from cardiovascular or neurodegenerative conditions [81–83].

As with dose levels, none of the studies compared more than one dose regimen in parallel. Given the short half-life of intravenously infused MSCs in the recipient, it is now generally accepted that multiple infusions are superior to a single infusion, as not only the strength but also the duration of exposure to the infused MSCs is critical for the MSCs to unfold their effect, particularly in the treatment of chronic, degenerative diseases [79,84,85]. This means that the benefit of an MSC therapy is likely to be underestimated if cells are administered only once [84,85]. Moreover, in RDEB even lifelong treatment with disease-modifying, symptom-relieving therapies such as MSC infusions will be required as long as curative therapies that can permanently reconstitute the deficient C7 are not available. Therefore, it will be important to determine the optimal intervals between MSC infusions and whether they can be extended over time for maintenance therapy. In any case, investigators must be aware that proceeding to phase 3 trials with an incorrect or non-optimal dose or dose regimen may result in the failure of therapies that would otherwise be effective [77,78]. With this in mind, the authors of all the studies of intravenous MSCs in RDEB unanimously stated that further studies are needed to determine the optimal dosage and dosing schedule for their product [30,32,34,36–39].

Sample sizes

The number of participants per study ranged from 1 to 16 (median 10) (Table 1). This is lower than what has been observed for phase II rare disease trials in general, according to an aggregate analysis of EU and/or U.S. interventional rare disease trials registered on clinicaltrials.gov through September 2015 [86]. Specifically, for the Orphanet prevalence class of 1–9 per million, where the published prevalence estimates for RDEB fall ([87–97], Table 2), median actual and median expected sample sizes of phase II clinical trials across all indications were 22 and 38, respectively [86]. On the other hand, the sample sizes of the phase I/II trials of the approved RDEB treatments Oleogel-S10/Filsuvez ($n=10$ [98]) and beremagene geperpavec/Vyjuvek ($n=12$ [99]) show that early-phase trials with sample sizes more similar to the MSC studies can lead to product process. Nevertheless, a common, inherent challenge in the analysis of clinical trial data in rare diseases, including RDEB, is their complex and heterogeneous pathology involving multiple organ systems [44], resulting in high inter-individual variability in clinical outcomes, which, combined with small sample sizes, can drastically reduce the statistical power of a trial [43].

One strategy to overcome recruitment barriers may be to use multicenter collaborations, when possible across multiple countries [42]. However, most of the studies of MSC in RDEB were conducted at a single site, with only the largest ($n=16$) trial of ABCB5$^+$ MSCs [39] involving multiple (>2) study sites and multiple countries (Table 1). This suggests that there is significant untapped potential to increase the number of patients recruited into rare disease trials [42,86]. Looking again at the two medicinal products approved by the EMA and/or FDA for the treatment of RDEB, while the early-phase trials, conducted in 10 and 12 patients, were single-center studies [98,99], the pivotal trials that paved the way for marketing approval of Oleogel-S10/Filsuvez [52] and beremagene geperpavec/Vyjuvek [100] were multicenter studies that were able to enroll 175 (out of a total of 223 EB patients) and 31 RDEB patients, respectively.

Table 2. Prevalence estimates of RDEB based on published data.

Country	Date of survey	Prevalence, Patients per 1 million population
England & Wales [87]	22 Apr 2021	3.3
Scotland [88]	1 May 1992	2.2[a]
Northern Ireland [89]	31 Jan 1991	1.5[b]
Germany [90]	15 May 2021	5.9[c]
The Netherlands [91]	31 Dec 2018	2.7[d]
Romania [92]	31 Dec 2023	2.4
Slovenia [93]	2020	2.8[e]
U.S. [94]	1990	0.9
	Jan 2002	1.4
Chile [95]	31 Dec 2023	3.7[f]
Australia [96]	Sep 2016	1.9[g]
New Zealand [96]	Sep 2016	0.6[h]
Japan [97]	1994	1.6

[a]Reported as localized RDEB 1.0, RDEB inversa 0.4, RDEB Hallopeau–Siemens 0.8.
[b]Reported as 3.0 for DEB, with 50% of DEB patients being affected by RDEB.
[c]Reported as 12.16 for DEB, with 48.4% of DEB patients being affected by RDEB.
[d]Reported as 8.3 for DEB, with 32.9% of DEB patients being affected by RDEB.
[e]Reported as 20 for EB (all subtypes), with 13.8% of EB patients being affected by RDEB.
[f]Reported as 5.5 for DEB, with 68% of DEB patients being affected by RDEB.
[g]Reported as 45 per 24.2 millions.
[h]Reported as 3 per 4.73 millions.

Age distribution

Depending on the subtype, RDEB is associated with significant premature mortality, most commonly due to life-threatening disease complications such as sepsis, failure to thrive, organ (most common respiratory, cardiac, renal) failure, and cutaneous squamous cell carcinoma (cSCC) [101–106]. Accordingly, the majority (61%-72%) of living RDEB patients are in their first two decades of life, while only 1%-3% of RDEB patients are 50 years of age or older [106,107]. Because the symptoms, complications, and therapeutic needs of RDEB patients vary with age [105,106,108,109], the potential benefits of a therapeutic approach should be evaluated in all affected age groups.

The age distribution of the RDEB patients treated with MSCs is shown in Figure 3(A); for comparison, the real-world distribution as recorded in the U.S. and Canada [106] is shown in the first (left) bar of the figure. The first two BM-MSC trials [30,32] treated predominantly young pediatric patients. The third BM-MSC trial [34] specifically enrolled adult patients to address the need for data in adults. While only individual RDEB patients have been treated with Muse cells or AT-MSCs, the UCB-MSC and ABCB5$^+$ MSC trials covered a wide range of age cohorts, with the ABCB5$^+$ MSC trial most closely resembling the real-world age distribution.

The youngest RDEB patients treated with MSCs were 9 children aged 1–2 years who had participated in one of the BM-MSC trials [30,32] (Figure 3(B)), while none of the MSC studies treated patients under the age of 1 year. However, although RDEB symptoms increase with age, serious complications can develop even at a young age. For example, children with RDEB have a high cumulative risk of growth retardation due to poor nutrient intake as a

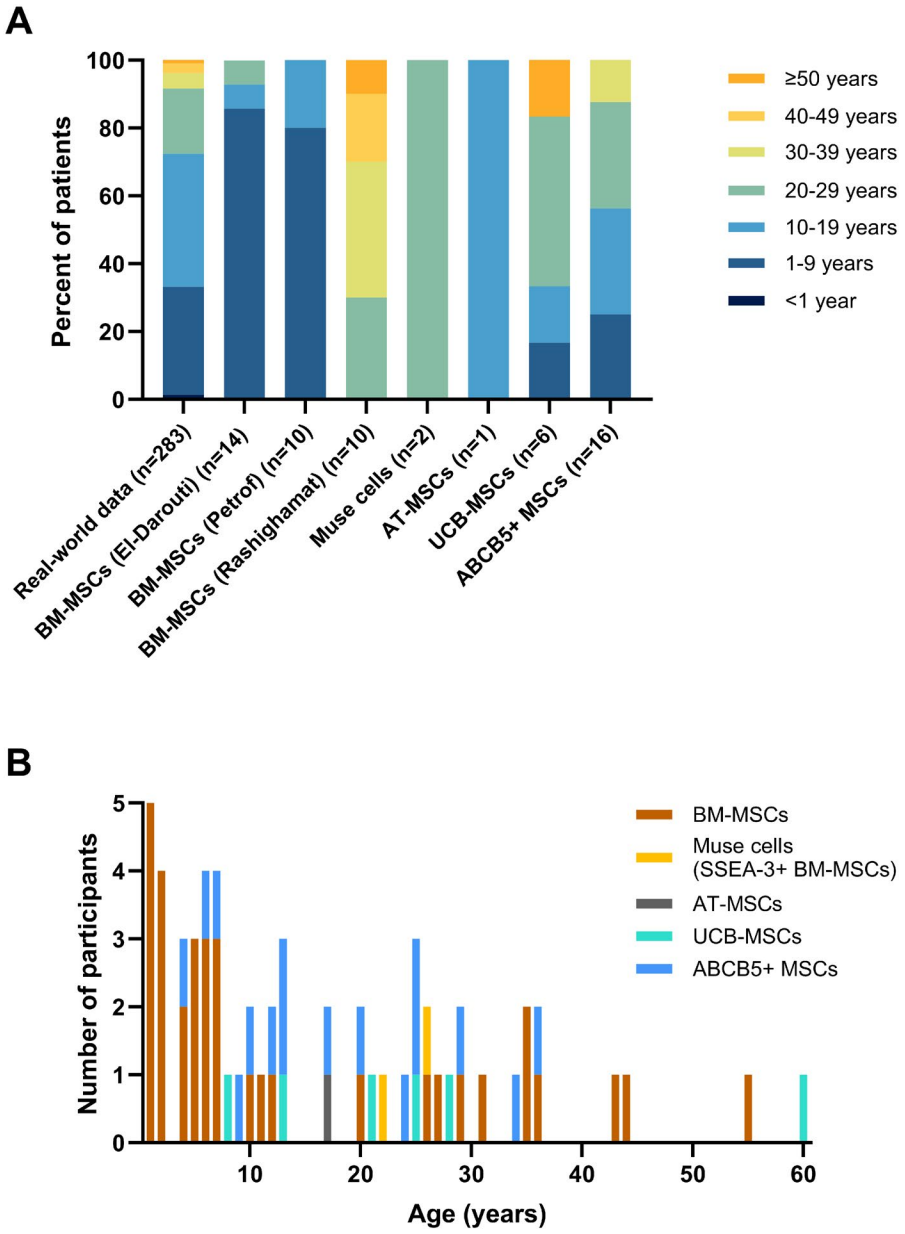

Figure 3. Age of RDEB patients treated with MSCs. (A) Age distribution of the study populations compared to the real-world distribution (left bar). Real-world data are from an observational study of 283 RDEB patients in U.S. and Canada enrolled in the Epidermolysis Bullosa clinical characterization and outcomes database between 2011 and 2017 [106]. (B) Number of patients by age and type of MSCs administered

result of severe oral and esophageal disease activity and scarring, which frequently necessitates gastrostomy tube feeding, sometimes as early as infancy [110]. Indeed, data from a large cohort study of showed that 17% of RDEB patients with known age at first gastrostomy tube placement had their first placement in the first year of life [106]. In addition, approximately 20% and 39% of RDEB patients who underwent esophageal dilatation for dysphagia due to esophageal strictures had their first esophageal dilatation within the first 3 and 5 years of life, respectively [106,111], and 16% of RDEB patients with known age at first hand surgery to correct deformities such as contractures or pseudosyndactyly had their first hand surgery within the first 5 years of life [106]. Given this situation, it seems reasonable to initiate systemic, disease-modifying therapies such as MSC infusions early in life with the goal of mitigating or delaying damage accumulation by reducing inflammatory disease activity before functional damage or other potentially irreversible complications have developed [112]. Since extrapolation of safety and efficacy data from older patients would not be appropriate in this scenario, studies in patients in the first year of life are desired in accordance with ethical requirements and regulatory recommendations [113,114].

Safety

The safety data derived from the reports on the use of MSCs in RDEB patients include data from 3 DDEB patients each receiving one dose of Muse cells, as the Muse cells study was conducted in both RDEB and DDEB patients and safety data were reported without distinguishing between the two subtypes of DEB [36]. Thus, safety data are available for 62 patients exposed to a total number of 133 intravenous doses of MSCs (Table 3).

A total of 44 adverse events (AEs) were reported as definitely, possibly or likely related to the study treatment, of which 26 (59%)

were classified as mild, 13 (30%) as moderate, and 4 (9%) as severe (for one AE, the grade of severity was not specified [38]) (Figure 4(A)). Two of the four AEs classified as severe were dimethyl sulfoxide (DMSO) odor [32], while the remaining two were hypersensitivity reactions that occurred during the second cell infusion of ABCB5$^+$ MSCs [39]. These hypersensitivity reactions were the only two treatment-related AEs that were considered serious and led to discontinuation of study treatment. Outcome was reported for 43 of the 44 AEs; all resolved without sequelae (Table 3).

Approximately two-thirds of the treatment-related AEs were DMSO odor (Figure 4(B)), all reported in one study (pediatric BM-MSC trial [32]) (Table 3). DMSO odor is a common reaction to cryopreserved cell therapy products that contain DMSO as cryoprotectant. It manifests as a garlic-like odor, caused by the DMSO metabolite dimethyl sulfide, which is excreted in the breath, urine, feces, and through the skin for up to 48 h after infusion [115]. Compared to freshly isolated, non-cryopreserved cells, such as the BM-MSCs administered by El-Darouti et al. [30], the use of cryopreserved cells, as was the case in the other studies [32,34,36,37,39] (the UCB-MSC study did not provide information on whether the cells were cryopreserved or not [38]), has important advantages: While fresh cells are only viable for several hours to a few days after harvest, cryopreservation allows sufficient time for rigorous quality control testing, enables storage and off-the-shelf availability, and increases the geographical reach of viable cells by creating a larger window of time in which the cells can be shipped from the manufacturing site to the site of clinical use [116]. A drawback is the potential side effects of DMSO, the current gold standard cryoprotectant used in cell cryopreservation [116]. In addition to DMSO odor, gastrointestinal and cardiovascular reactions have also been associated with DMSO [115], so the few gastrointestinal (2 mild nausea, 1 abdominal pain) and cardiovascular (1 bradycardia) AEs that occurred transiently during BM-MSC infusions in the pediatric trial [32] could also be due to DMSO.

The traditional strategy to reduce or prevent potential DMSO-related side effects is to remove DMSO by repeated centrifugation and washing cycles between cell thawing and infusion [115,116], which was part of the manufacturing process for ABCB5$^+$ MSCs [75]. However, post-thaw washing or alternative (non-centrifugation) methods [115] to remove DMSO from a thawed cell therapy product are labor-intensive and typically must be performed at the manufacturer's facility; so the cells will be delivered in a thawed state. The increased logistical challenges associated with shipping thawed cells pose a significant barrier to the use of cell therapies, particularly in rare disease trials where study populations are geographically dispersed. Of note, Rashidghamat et al. [34], who administered an analogous bedside-thawed, DMSO-containing BM-MSC product as studied by Petrof et al. [32] in children to an adult study population, did not observe any treatment-related AEs [34].

Table 3. Clinical studies of systemic MSC application to treat RDEB: Patient exposure and treatment-related AEs.

	El-Darouti et al. [30,31]	Petrof et al. [32,33]	Rashidghamat et al. [34,35]	Fujita et al. [36]	Maseda et al. [37]	Lee et al. [38]	Kiritsi et al. [39]
Patient exposure							
Cell type	BM-MSCs	BM-MSCs	BM-MSCs	Muse cells	AT-MSCs	UCB-MSCs	ABCB5$^+$ MSCs
Cell dose	2×10^6/kg	$1–3\times10^6$/kg	$2–4\times10^6$/kg	$1.98–3.55\times10^5$/kg[a]	1×10^6/kg	$1–2\times10^6$/kg (pediatric) or 3×10^6/kg (adult)	2×10^6/kg
Planned number of doses per patient	1	3	2	1	3	3	3
Total number of exposed patients	14	10	10	5[b]	1	6	16
Total number of doses	14[c]	30	19[d]	5[a]	3	18	44[e]
Treatment-related AEs[f]							
Total number	0[g]	36[h]	0	1	3	1	3
Description (number of events)	n.a.	DMSO odor (28), nausea (2), abdominal pain (1), bradycardia (1), skin/mucosal blisters/wounds (2), fine hair growth (1), pruritus (1)	n.a.	Paresthesia (1)	Infusion-related reaction (3)	Acute gastritis (1)	Hypersensitivity reaction (2), lymphadenopathy (1)
Intensity (number of events)	n.a.	Mild (21), moderate (13), severe (2)[i]	n.a.	Mild (1)	Mild (3)	Not specified	Mild (1), severe (2)[j]
Number of serious AEs	0	0	0	0	0	0	2
Number of AEs resulting in treatment discontinuation	0	0	0	0	0	0	2
Outcomes	n.a.	Resolved without sequelae	n.a.	Resolved without sequelae	Resolved without sequelae	Not indicated	Resolved without sequelae

[a]Including 3 doses administered to DDEB patients.
[b]Including 3 DDEB patients.
[c]Of these, 7 doses were given to patients concomitantly treated with 5 mg/kg/d cyclosporine.
[d]One patient was withdrawn after the first MSC infusion due to an unrelated serious AE (deterioration of known renal impairment at the time of blood sampling before the first infusion).
[e]Two patients were withdrawn during the second MSC infusion due to hypersensitivity reactions to the cell product.
[f]Including AEs categorized as definitely, possibly or likely treatment-related.
[g]Reported as "No major side effects were reported.".
[h]Of these, 28 were DMSO odor.
[i]Both events classified as severe were DMSO odor.
[j]Including 1 mild lymphadenopathy and 2 severe hypersensitivity reactions.

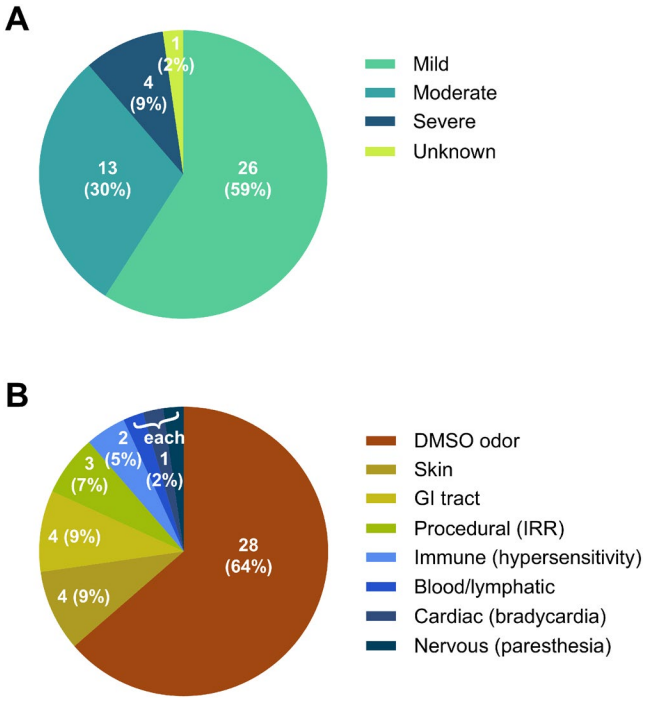

Figure 4. Treatment-related AEs ($n = 44$) reported from treatment of 62 patients (59 RDEB, 3 DDEB) receiving a total of 133 infusions of MSCs. (A) Number (% of total AEs) of AEs by severity grade. (B) Number (% of total AEs) of AEs by system organ class. DMSO odor could not be assigned to any system organ class and is displayed as a separate category. IRR, infusion-related reaction.

Infusion-related AEs were also observed with AT-MSCs [37] and ABCB5$^+$ MSCs [39]. In the case of AT-MSCs, all three infusions were associated with mild infusion-related reactions that manifested as fever with forehead flushing, dyspnea and chills, or vomiting [37]. All these symptoms have repeatedly been reported with infusions of DMSO-containing cell preparations [115]. Unfortunately, while the AT-MSC case report indicated that the cells had indeed been cryopreserved with DMSO, it was not specified whether a post-thawing procedure was performed to deplete the cryoprotectant [37]. The two reactions to the ABCB5$^+$ MSC product were considered more likely to be due to immunologic sensitization, because the product was DMSO-depleted [75] and both events occurred during the second cell infusion after a well-tolerated first infusion [39]. However, because the presence of alloantibodies was not determined in the two patients affected, it remains unclear whether these reactions were truly immune responses to the MSCs. While both events were successfully managed and resolved without sequelae on the day they occurred, the patients were excluded from the third MSC infusion [39]. In future trials of intravenous MSCs, premedication with antihistamines should minimize the risk of infusion-related reactions, whether due to nonimmune (e.g., DMSO-induced) histamine release [115] or immunological sensitization.

Another caveat that has been raised in the context of cell therapy for RDEB is the concern that MSCs may, at least in theory, increase the inherent predisposition of RDEB patients to develop cSCC [117,118]. In the adult BM-MSC trial [34], two of the ten participants developed an cSCC 6 to 7 months after the first MSC infusion. Given that the development of cSCC is a common complication of RDEB in adults [104,119], the two cases were not considered related to treatment. In principle, MSCs can home to tumor microenvironments, where they can both suppress and promote tumor growth [120–123]. However, to date, neither systematic preclinical safety studies [76,124–127] nor clinical trials [19,120] have observed cancer formation from experimentally or therapeutically administered MSCs. Given that MSC therapy is expected to have a normalizing effect on the disturbances in skin tissue homeostasis and integrity [24–27,59,62–64], which in turn are considered to be permissive for cSCC formation in RDEB [6,128–132], it has been speculated that long-term treatment with MSCs may even reduce the risk of developing cSCCs [63]. However, as long as it cannot be ruled out with certainty that MSCs may promote tumor growth of cSCC in RDEB, MSC treatment of patients with cSCC should be carefully avoided, which was ensured by appropriate exclusion criteria in the MSC studies reviewed here [32,34,38,39]. In addition, to safely administer MSC therapies to RDEB patients, any potential for malignant transformation of the MSCs themselves must be ruled out by strict cell quality control measures. For ABCB5$^+$ MSCs, measures implemented in the manufacturing process to identify any signals of non-physiological cell behavior during cell expansion have been reported, including mandatory in-process controls for cell morphology, contact inhibition, time between passages, cell cycle phase distribution and aneuploidies [73]. Undoubtedly, even with such measures, patients need to be carefully monitored for potential malignancy development before and during cell therapy.

Overall, the study results suggest a favorable safety profile of MSC therapies and support the conduct of follow-up studies, although the data are far too limited to make a conclusive safety assessment. This is another common challenge for orphan drug development programs: Even at the approval stage, the number of patients treated will not allow reliable detection of uncommon or rare adverse reactions [133,134], and post-approval studies are usually required to provide the opportunity to detect potentially unknown adverse reactions over time in a larger and broader patient population [135,136].

Efficacy

Outcomes

The complex pathology and phenotypic heterogeneity of RDEB is reflected by a great diversity of outcomes reported from clinical trials, which complicates the comparison and interpretation of clinical trial results and thus represents a major challenge in the field of clinical research and therapy development [137]. This is evident in the studies of MSCs in RDEB, where there is considerable heterogeneity in both the outcome domains assessed and the instruments used for measurement (Table 4): While all MSC studies measured outcomes of cutaneous manifestations and biochemical/histological markers, six of the seven studies scored overall disease severity and symptoms (pain, pruritus), three studies reported on quality of life, and one study assessed outcomes related to physical and psychological functioning and resource use. Moreover, even where the same instruments were used, comparisons between studies are further complicated by differences in the time points at which these outcomes were measured (Table 4).

Cutaneous manifestations

Cutaneous wounds are among the most distressing manifestations of RDEB because they can be painful and itchy; require extensive daily wound care that is time-consuming, costly, and often dependent on assistance; result in fibrotic changes, scarring and mutilation; and place patients at increased risk for infection and life-threatening cSCCs [138]. Wound healing is therefore considered to be one of the most clinically important outcomes of RDEB

Table 4. Clinical studies of systemic MSC application to treat RDEB: Reported efficacy outcomes.

			Time points of measurement						
Outcome area[a]	Outcome domain	Outcome	El-Darouti et al. [30,31]	Petrof et al. [32,33]	Rashidghamat et al. [34,35]	Fujita et al. [36]	Maseda et al. [37]	Lee et al. [38]	Kiritsi et al. [39–41]
Cutaneous manifestations	Blister / wound healing	Total blister count	n.d.	BL, D0, D7, D28, D60, D100, M6	BL, D0, D14, D28, D60, D100, M6, M8/M12[b]	n.d.	n.d.	BL, D0, D14, D28, D56, D112, W24	n.d.
		Ratio of blister area to BSA						BL, D56, D112, W24	
		Rate of blister healing	BL, W12	n.d.	n.d.	n.d.		n.d.	n.d.
		Proportion of healed wounds	n.d.	n.d.	n.d.	n.d.		n.d.	D17, D35, W12
		Time to wound closure	n.d.	n.d.	n.d.	n.d.		n.d.	W12
		Proportion of durably healed wounds	n.d.	n.d.	n.d.	n.d.		n.d.	W12
		Change in target ulcer size	n.d.	n.d.	n.d.	D0, W2, W4, W8, W12, W20, W28, W36, W44, M12		n.d.	n.d.
		Parent perception	n.d.	M10	n.d.	n.d.		n.d.	n.d.
	Blister / wound formation	Rate of new blister / wound formation	BL, W12	n.d.	n.d.	n.d.	n.d.	n.d.	D17, D35, W12
	Total skin involvement	TBSA affected by RDEB	BL, W12	BL, D60, D100, M6	n.d.	n.d.	BL, M6, M12, Y2	BL, D56, D112, W24	n.d.
	Skin resistance	Suction blister induction time	n.d.	BL, D100	D0, D28, D60, D100, M6, M8/M12[b]	n.d.	n.d.	n.d.	n.d.
		Parent perception of skin resistance		M10	n.d.				
	Skin redness	Parent perception of skin redness	n.d.	M10	n.d.	n.d.	n.d.	n.d.	n.d.
Clinical assessment	Overall disease severity	BEBSS	n.d.	BL, D60, D100, M6	BL, D28, D60, D100, M6, M8/M12[b]	n.d.	BL, D21, D42, M2, M3, M6, M9, M12, Y2	BL, D56, D112, W24	n.d.
		EBDASI		n.d.	BL, D28, D60, D100, M6, M8/M12[b]		BL, D21, D42, M2, M3, M6, M9, M12, Y2	n.d.	D0, D17, D35, W12
		iscorEB		n.d.	n.d.		n.d.	n.d.	D0, D17, D35, W12
		Global severity score		BL, D60, D100, M6	n.d.		n.d.	BL, D56, D112, W24	n.d.
Symptoms	Pruritus	Itching score	n.d.	BL, D0, D7, D28, D60, D100, M6	BL, D28, D60, D100, M6, M8/M12[b]	D0, W2, W4, W8, W12, W20, W28, W36, W44, M12	BL, D21, D42, M2, M3, M6, M9, M12, Y2	BL, D0, D14, D28, D56, D112, W24	D0, D17, D35, W12
		Parent perception of child's itch		M10	n.d.	n.d.	n.d.	n.d.	n.d.
	Pain	Pain score	n.d.	BL, D0, D7, D28, D60, D100, M6	n.d.	D0, W2, W4, W8, W12, W20, W28, W36, W44, M12	BL, D21, D42, M2, M3, M6, M9, M12, Y2	BL, D0, D14, D28, D56, D112, W24	D0, D17, D35, W12
		Parent perception of child's pain		M10	n.d.	n.d.	n.d.	n.d.	n.d.
Quality of life (QoL)	EB-specific QoL	QoLEB	n.d.	n.d.	D0, D28, D60, D100, M6, M8/M12[b]	n.d.	n.d.	BL, D56, D112, W24	D0, D17, D35, W12
	General health-related QoL	PedsQL™	n.d.	BL, D60, D100, M6	n.d.	n.d.	n.d.	n.d.	n.d.
		EuroQoL-5D		n.d.		n.d.	BL, D21, D42, M2, M3, M6, M9, M12, Y2		
		Not specified		n.d.		Not specified	n.d.		

(*Continued*)

Table 4. Continued.

Outcome area[a]	Outcome domain	Outcome	Time points of measurement: El-Darouti et al. [30,31]	Petrof et al. [32,33]	Rashidghamat et al. [34,35]	Fujita et al. [36]	Maseda et al. [37]	Lee et al. [38]	Kiritsi et al. [39–41]
Biochemical / histologic markers	Expression of C7 at the dermo-epithelial junction	Increase in C7 fluorescence intensity vs. BL	n.d.	BL, D60	D28, D60, M6	W4[c]	n.d.	BL, D56	n.d.
	Anchoring fibrils (AFs)	Number of AFs	BL, W12	BL, D60	BL, D28, D60, M6	W4[c]	n.d.	BL, D56.	n.d.
		AF structure and distribution	n.d.	n.d.	BL, D28, D60, M6	n.d.		BL, D56	
	Immune cell infiltration of the skin	Number of total macrophages ($CD68^+$)	n.d.	n.d.	n.d.	n.d.	n.d.	BL, D56	n.d.
		Number of M2 macrophages ($CD206^+$)						BL, D56	
		Number of mast cells ($c\text{-}Kit^+$)						BL, D56	
	Circulating inflammatory markers	General markers of inflammation	Time points not specified[d]	n.d.	BL, D14, D28, D60, D100, M6, M8/M12[b,e]	n.d.	BL, D21, D42, M2, M3, M6, M9, M12, Y2[f]	BL, D0, D14, D28, D56, W24[d]	n.d.
		Acute-phase reactants	n.d.		BL, D14, D28, D60, D100, M6, M8/M12[b,g]		BL, D21, D42, M2, M3, M6, M9, M12, Y2[h]	BL, D0, D14, D28, D56, W24[i]	n.d.
		Cytokines	n.d.		BL, D14, D28, D60, M6[j]		BL, D21, D42, M2, M3, M6, M9, M12, Y2[k]	BL, D0, D14, D28, D56, W24[l])	D0, D17; D35, W12[m]
		Neuropeptides	n.d.		n.d.		n.d.	BL, D0, D14, D28, D56, W24[n]	n.d.
		HMGB-1	n.d.		BL, D14, D28, D60, M6		n.d.	n.d.	D0, D17, D35, W12
Functioning	Sleep	Sleep score	n.d.	BL, D0, D7, D28, D60, D100, M6	n.d.	n.d.	n.d.	n.d.	n.d.
	Energy level	Fatigue score	n.d.	BL, D0, D7, D28, D60, D100, M6	n.d.	n.d.	n.d.	n.d.	n.d.
	Family life	Parent perception of family life	n.d.	M10	n.d.	n.d.	n.d.	n.d.	n.d.
Resource use	Use of healthcare resources	Parent perception of healthcare resource use	n.d.	M10	n.d.	n.d.	n.d.	n.d.	n.d.
Other	Outlook for the future	Parent outlook for the future	n.d.	M10	n.d.	n.d.	n.d.	n.d.	n.d.

[a]Based on outcome areas identified by Korte et al. [137] in a systematic review of reported outcomes in published studies in EB from 1991 to 2021.
[b]M12 for the first 8 participants; M8 for the last 2 participants.
[c]n = 1.
[d]Complete blood count.
[e]Erythrocyte sedimentation rate, white blood cell count, creatinine.
[f]White blood cell count.
[g]CRP, albumin.
[h]C-reactive protein, fibrinogen, prealbumin, retinol transporter protein.
[i]C-reactive protein.
[j]TNF-α, IFN-γ, IL-1, IL-10, IL-17A, MMP-2, MMP-9, MMP-11, TIMP-1.
[k]IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-13, IL-15, IL-17, MCP-1/CCL2, sCD40L, TNF-α, VEGF, fractalkine, TGF-β.
[l]IL-1β, IL-6.
[m]Profile of 80 cytokines as targeted by a commercial array (Human Cytokine Antibody Array – Membrane, ab133998, Abcam).
[n]Substance P, calcitonin gene-related peptide.
BL: baseline (value recorded at screening or taken from patient history); D: day; M: month; n.d.: not determined; Y: year.

treatment from a patient [138,139], pathophysiological [140] and regulatory perspective [141]. However, the healing characteristics of RDEB wounds differ significantly from those of other chronic wound etiologies, exhibiting wide inter- and even intra-patient variability creating an unpredictable pattern of wound healing and recurrence [142]. Studies investigating the natural history of RDEB wounds have defined two distinct wound types: recurrent wounds, which heal within an average of 6 weeks and reopen within an average of 3 weeks, and chronic wounds, which may not heal for years [143] but can also be very dynamic in nature [144]. This points to a potential limitation of outcomes that measure healing of a randomly selected target wound, which is that a single wound may not be a true reflection of the wound-healing efficacy of an intervention, as target wounds may spontaneously heal while new wounds may develop [142]. Given this, most of the studies of MSCs in RDEB have measured outcomes related to the patient's overall wound burden (Table 4).

Using BM-MSCs, Petrof et al. and Rashidghamat et al. achieved an average 36% reduction from baseline in total blister count (i.e., including blisters present at baseline and blisters developed after baseline) on day 60 (32 days after the last infusion) and day 28 (14 days after the last infusion), respectively, which persisted at least until day 180 and day 60, respectively [32,34,35]. In the UCB-MSC trial, the mean total blister count decreased by 52% on day 56 (28 days after the last infusion) and even by 70% on day 168 (140 days after the last infusion). However, comparing these results between studies should be done with caution, as all three studies reported a high degree of variability both between and within patients.

Unlike the above studies, the BM-MSC trial by El-Darouti et al. [30] and the ABCB5$^+$ MSC trial [40,41] analyzed skin lesions separately for those present at baseline and those that developed after baseline. For blisters present at baseline, El-Darouti et al. [30] found that the healing rate increased from slow (7–11 days) to moderate (3–5 days) in 11 patients and rapid (2 days) in 3 patients at week 12 (12 weeks after BM-MSC infusion). In addition, a marked slowing of post-baseline blistering was observed. Accompanying daily cyclosporine, which was administered to increase survival and prolong persistence of the transplanted MSCs in the host, had no additional effect on blister healing and formation kinetics as compared to BM-MSC infusion alone [30]. In the ABCB5$^+$ MSC trial, 65% of open wounds present at baseline were closed by week 12 (49 days after the last infusion), with 74% of wounds that had closed on day 17 and/or day 35 remaining closed for at least 7 or as many as 9.5 weeks after closure [40], which is well above the average time to re-opening of 3 weeks reported in natural history studies [143]. There was also a marked slowing of new wound formation [41]. This trial also looked at the healing of the newly formed wounds and found that during MSC therapy, these wounds healed even faster and a greater portion of healed wounds remained stably closed compared to the wounds present at baseline [41].

Only one study [36] evaluated healing of selected target wounds. The aforementioned risk of inadvertently selecting wounds that would heal spontaneously was addressed by selecting more than one target wound (two to four) per patient and by including only refractory and recurrent ulcers that had been present for more than 4 weeks. In this study, the two RDEB patients had an average wound size reduction of 100% and 60%, respectively, at 4 weeks after infusion of Muse cells [36].

In addition to or instead of outcome measures assessing blister and wound healing and formation, four studies evaluated the overall cutaneous manifestations of RDEB by assessing the total body surface area (TBSA) affected by RDEB lesions (Table 4). All of these studies reported a reduction in mean affected TBSA, which was most pronounced in the BM-MSC trial by El-Darouti et al. (66% at baseline vs. 25% at 12 weeks, as calculated from their Table 1 [30]), followed by the AT-MSC case report (23% at baseline vs. 12% at 6 months [37]), the BM-MSC trial by Petrof et al. (23% at baseline vs. 14% at 6 months [32]) and the UCB-MSC trial (16% at baseline vs. 11% at day 56 [38]). It is unclear whether the results are comparable because the studies did not specify the types of skin changes included, nor the method and therefore accuracy of TBSA calculation. Nevertheless, as the latter three of the four studies also assessed the overall disease severity using the Birmingham Epidermolysis Bullosa Severity Score (BEBSS), which includes the assessment of TBSA, it is likely that they reported TBSA measurements according to the BEBSS definitions [145], as was indicated for the BM-MSC trial by Petrof et al. [32].

Clinical assessment

While skin fragility is the most prominent feature of the disease, RDEB is not limited to the skin. In addition to skin blisters, wounds and scarring, the phenotypic spectrum includes a variety of extracutaneous manifestations such as gastrointestinal, cardiovascular, genitourinary, ocular, and oral involvement and complications [2,7,8,108]. To quantify the overall clinical severity of EB, specific scoring instruments have been developed that cover important manifestations and provide a more holistic picture of disease impact. At least one of these instruments, most commonly the BEBSS and/or the Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI), was used to assess treatment efficacy in five of the seven studies of MSCs in RDEB (Table 4).

The BEBSS [145] scores 11 items, including TBSA, involvement of nails, mouth, eyes, larynx and esophagus, scarring of hands, skin cancer, chronic wounds, alopecia, and nutritional compromise. In contrast to a pediatric population treated with BM-MSCs, which achieved a reduction in mean BEBSS by approximately 5 points on day 60 and 7 points on day 180 [32], an analogous BM-MSC preparation produced only minimal changes in adults [34]. It would be important to find out whether the BEBSS actually responds better to MSC therapy in children than in adults. This would support the idea of starting MSC therapy for RDEB in childhood, before difficult-to-treat damage and complications have accumulated. However, there were additional differences between the two trials, including different cell dose and number of cell infusions (Table 1) and time points of outcome measurement (Table 4), which may have contributed to the different BEBSS results. In addition, the BEBSS was highly variable between patients [34], making it even more difficult to compare results between studies. All this is also true for the case report of the use of AT-MSCs [37] and the study of UCB-MSCs [38], which also showed some reduction in BEBSS, most pronounced in the patient treated with AT-MSCs.

It is important to note that instruments scoring the overall clinical severity of RDEB combine several symptoms and complications, so that any change in the overall score does not necessarily represent the intent of the treatment [137]. In particular, disease-modifying therapy approaches such as MSC infusions target potentially modifiable disease activity but are not expected to alleviate chronic damage components. Therefore, overall disease severity scores may not be sensitive enough to reliably detect changes with treatment [145]. To mitigate this limitation, the EBDASI distinguishes between ongoing, treatable disease activity and accumulated, irreversible damage by quantifying the severity of involvement of the skin, scalp, mucous membranes, nails, and

other epithelialized surfaces separately in terms of activity (EBDASI activity) and damage (EBDASI damage) [146].

All three studies that used the EBDASI as an efficacy outcome tool, i.e., the BM-MSC trial in adults [34], the case report of the use of AT-MSCs [37] and the trial of ABCB5$^+$ MSCs [39] (Table 4), observed some degree of improvement in the EBDASI activity subscore. With ABCB5$^+$ MSCs, the improvement was clinically meaningful (according to the MCID of a 9-point decrease defined by Jain et al. [147]) in 36% of patients [39]. The greatest reduction in the EBDASI activity subscore was reported for the patient treated with AT-MSCs [37]. However, whether this effect is reproducible in further patients remains to be determined. In contrast, virtually no changes in the EBDASI damage subscore were observed in any of these MSC studies [34,37,39]. While it is indeed not expected that disease-modifying treatment approaches such as MSC infusions can reduce preexisting damage and thus reduce the EBDASI damage subscore, it was hypothesized that such treatments could result in slowing or preventing further accumulation of damage as measured by the EBDASI damage subscore [148]. This would mean that even a non-increase in the EBDASI damage subscore could already represent a treatment effect. In fact, real-world data have indicated that the overall disease severity trajectory of an RDEB patient, as captured by the EBDASI, remains stable or shows a gradual increase in the absence of intervention [149]. However, the database is limited, and further research into the natural history of the disease severity scores is needed to determine whether an improvement (or no change) is indeed indicative of a treatment effect.

Symptoms

From a patient perspective, pruritus and pain are consistently ranked as two of the three most frequent and most bothersome symptoms of RDEB, along with skin lesions/blisters [150]. Severely impacting the quality of life, recalcitrant pruritus and pain are key determinants of the burden of having EB and are the symptoms assigned with the highest importance for need for symptom reduction [109,139,151,152].

Pruritus was assessed in all studies except the BM-MSC study by El-Darouti et al. [30] (Table 4). Four studies used unidimensional tools, measuring itch intensity using a categorical 0-10 numerical rating scale (NRS) or a continuous 10-cm visual analog scale (VAS), ranging from "no itch" to "worst itch imaginable". BM-MSC and UBC-MSC infusions achieved similar mean reductions in itch intensity scores of 21% on day 60 and 28% on day 56, respectively, which were maintained until day 180 and day 156, respectively [32,38]. Following ABCB5$^+$ MSC infusion, the greatest reduction in itch intensity, 38%, was observed on day 35 [39]. In contrast, no reduction in itch intensity was observed with Muse cells [36].

While unidimensional scales such as the NRS and the VAS are simple and efficient tools for measuring subjective itch intensity [153], they have potential limitations: because these instruments require patients to report itch intensity over a set period of time, typically 24 h, they are susceptible to environmental and psychosocial confounders present at the time of recording [153,154]. In addition, some patients may report average versus peak itch intensity, which can lead to different levels of sensitivity and specificity of itch measurement [153]. Most importantly, unidimensional scales capture only one aspect (such as intensity) of the complex and multifactorial pathology of itch and thus provide an incomplete picture of itch sensation [155]. In contrast, multidimensional tools provide a more comprehensive rating of the itch impact [153,154]. The BM-MSC trial by Rashidghamat et al. [34] and the AT-MSC case report [37] used the Leuven Itch Scale (LIS), which captures, over the preceding month, different aspects of the itch symptom, translated into subscale scores on six dimensions: itch frequency, severity, duration, distress, consequences and surface [156]. Unfortunately, while the LIS has been evaluated in a prospective registry study of the natural history of RDEB in 50 individuals [109], it has not been validated for use in individuals under the age of 18 years [156]. In adult patients, Rashidghamat et al. observed significant reductions in itch frequency, severity and consequences on days 28 and 60 following BM-MSC infusions, whereas no changes in itch duration, distress and surface were achieved [34]. The 17-year-old patient treated with AT-MSCs reported improvements in all six itch dimensions, with the greatest reductions being seen on day 100 [37].

Pain was assessed in five of the seven studies (Table 4), four of which measured pain intensity using unidimensional tools such as a continuous 10-cm VAS or a categorical 0-10 NRS ranging from "no pain" to "worst pain imaginable". The observed effects of the cell treatments on pain intensity varied considerably between studies, both in magnitude and duration of change in pain intensity scores. With the single infusion of Muse cells, there was only a transient significant reduction in mean pain intensity score at 2 weeks, which returned to baseline levels by week 8. Longer-lasting effects were achieved with three infusions of ABCB5$^+$ MSCs (12% reduction on day 17 and 24% reduction on day 35 and at week 12) [39] and UCB-MSCs [40% reduction (from 7.5 to 4.5) on day 56 and 25% reduction (from 7.5 to 5.5) on day 168] [38]. The patient treated with AT-MSCs experienced a steady decrease in pain intensity starting immediately after the third infusion, with pain intensity reaching its lowest level at 9 months (0.5, representing a 93% reduction from a baseline of 7.5) [37].

As with pruritus, the unidimensional measurement of pain intensity has several shortcomings. For example, studies have suggested that pain is not a linear phenomenon, so it cannot necessarily be assumed that, e.g., a score of 5 accurately reflects twice the intensity of 2.5 [157]. The main limitation of single-item pain intensity scales in complex diseases such as RDEB is that they do not assess the full range of individual pain qualities, including acute and chronic, nociceptive and neuropathic pain resulting from many sources such as skin blisters, chronic wounds and ulcerative lesions, dressing changes and bathing, surgery, dental and periodontal disease, dysphagia and constipation, joint contractures, and corneal abrasions [158,159]. Therefore, multidimensional, disease-specific validated pain assessment is warranted [137], as recommended by regulatory authorities, particularly for trials in chronic pain [160]. As no such validated EB-specific instrument was available at the time of their trial, Petrof et al. [32] used a non-validated EB-specific questionnaire developed by the pediatric psychologist consultant Christina Liossi at Great Ormond Street Hospital for Children (London, UK). The questionnaire scored the intensity of pain rated by the patients' parents for different sources of pain such as the skin, muscles, bones, mouth, teeth, eyes, bowel opening, urination, and dressing changes, reported as a total score (range 0–80) [161]. Using this instrument, a 21% (from 26.1 to 20.6) reduction in pain intensity on day 60 and an 11% (from 26.1 to 23.1) reduction in pain intensity on day 180 were observed with BM-MSC infusions in children [32]. It would be interesting to see if and to what extent the cell treatment affected the pain of the different sources differently.

Quality of life

Patients with RDEB suffer from itching, pain, chronic inflammation, fibrotic changes and anemia, all of which affect quality of life by

interfering with a wide range of daily activities, including bathing and showering, eating, sleeping, writing, shopping, and participating in sports and other recreational activities [138,162]. Quality of life was assessed in in all studies except the BM-MSC study by El-Darouti et al. [30] (Table 4). In two studies, generic instruments were used: the Pediatric Quality of Life Inventory™ (PedsQL™) [163] in the BM-MSC trial in children [32] and the EuroQoL (EQ)-5D [164] in the patient treated with AT-MSCs [37]. Three studies, the BM-MSC trial in adults [34], the UCB-MSC trial [38], and the trial of ABCB5$^+$ MSCs [39], used the EB-specific Quality of Life in Epidermolysis Bullosa (QoLEB) questionnaire [165], whereas the publication of the Muse cell study does not specify the QoL instrument [36].

The results are difficult to compare across studies because not only were different instruments used, but different measures (absolute score points, absolute change from baseline, percentage change from baseline) were reported. Except for the Muse cell study, where no change was observed [36], all studies showed some improvement in quality of life [32,34,37–39], with the most pronounced improvements seen in the patient treated with AT-MSCs [37]; however, where significance levels were reported, the changes were not statistically significant with wide variability between participants [34,39]. The observed lack of response of the QoLEB score to MSC therapy was thought to be possibly due to the fact that the QoLEB score covers a number of aspects related to accumulated scarring and damage, such as the ability to move, write and eat, which are not the primary target of disease-modifying therapy approaches such as MSC infusions [39].

Biochemical/histologic markers

Given the complex nature and the heterogeneity of phenotypes in RDEB, researchers are searching for biochemical and/or histologic markers as objective, quantifiable indicators of disease severity and response to an intervention. Parameters evaluated in the MSC studies that could serve as biomarkers in RDEB include molecular and histologic markers of skin integrity (C7 expression at the dermo-epithelial junction, numbers and structure of anchoring fibrils) and of local (skin immune cell infiltration) and systemic (circulating inflammatory markers) inflammation (Table 4).

In the studies that followed C7 expression at the dermo-epithelial junction (BM-MSC trials in children [32] and adults [34], Muse cell study [36], and UCB-MSC trial [38]), increases in C7 fluorescence intensity from baseline were found only in exceptional cases (1 patient each in the BM-MSC trial in adults [34] and in the UCB-MSC trial [38]). Mostly negative results were also seen for the presence and structure of anchoring fibrils at the dermo-epithelial junction: Only the BM-MSC study by El-Darouti et al. reported increased numbers of anchoring fibrils after cell treatment [30], whereas in the studies on BM-MSCs by Petrof et al. [32] and Rashidghamat et al. [34], Muse cells [36] and UCB-MSCs [38] no numerical or morphological changes in anchoring fibrils were observed. Given these results, C7 expression and the presence of anchoring fibrils at the dermo-epithelial junction are unlikely to be appropriate markers of response to treatment with intravenous MSCs. This is supported by a preclinical study of BM-MSCs injected intradermally into *Col7a1*-hypomorphic mice, which indicated that high local MSC concentrations are required for effective C7 restoration and *de novo* formation of (immature) anchoring fibrils, which may not reliably be achieved by systemic MSC infusions [25]. Even MSCs engineered to overexpress C7, which successfully rescued the RDEB phenotype in a human:murine chimeric RDEB model through de-novo formation of mature anchoring fibrils when injected intradermally, failed to do so when injected intravenously [166]. Overall, it seems to be emerging that the observed benefit of systemic MSC therapy in RDEB is primarily related to immunomodulatory, anti-inflammatory, anti-fibrotic and trophic effects [27,62,64,65,118].

Based on this hypothesis, dermal immune cell infiltration was evaluated in the UCB-MSC trial [38]: While treatment did not affect the density of CD68$^+$ total macrophages, it significantly increased the proportion of CD206$^+$ anti-inflammatory, repair-promoting M2 macrophages from baseline to day 56 after the first cell infusion. In contrast, skin infiltration with c-Kit$^+$ mast cells was significantly reduced on day 56 [38]. While induction of anti-inflammatory macrophages in the skin has also been demonstrated in mouse models of RDEB after systemic treatment with UCB-USSCs and ABCB5$^+$ MSCs [27,62,64], it remains to be determined whether the changes in dermal cell infiltration observed in the UCB-MSC trial [38] can be reproducibly achieved after MSC infusion in human RDEB patients.

With respect to circulating inflammatory markers (Table 4), no changes in general inflammatory markers [30,34,37] or in acute phase reactants [34,37] were observed as a result of MSC infusions. An exception was the patient treated with AT-MSCs, who presented at baseline with elevated levels of the acute-phase reactants C-reactive protein, fibrinogen and prealbumin, and decreased levels of retinol transporter protein, all of which normalized after MSC infusion [37]

Serum cytokine levels were investigated in the BM-MSC trial in adults [34], in the patient treated with AT-MSCs [37], and in the UCB-MSC [38] and ABCB5$^+$ MSC [39] trials (Table 4). With the exception of the patient treated with AT-MSCs, who showed a decrease in serum TGF-β levels after the third MSC infusion [37], no significant changes in the serum levels of a wide range of cytokines were observed as a result of MSC treatment, including the cytokines that have been found altered in RDEB patients or observed to be correlated with disease severity as measured by the BEBSS or TBSA affected by wounds ([167–173], Table 5). Taken together, the studies did not identify a cytokine that could be used to predict or monitor the success of treatment of RDEB with MSCs.

Another molecule that has been proposed as a serum biomarker of RDEB disease severity is the alarmin high-mobility group box 1 HMGB-1, as its levels have been found to be elevated in RDEB patients [173–175] and positively correlated with the BEBSS [175] (Table 5). An association between HMGB-1 serum concentrations and RDEB disease severity was also observed in the ABCB5$^+$ MSC trial, where the baseline levels were positively correlated with the EBDASI [39]. In the studies that monitored serum HMGB-1 levels, namely the BM-MSC trial in adults and the ABCB5$^+$ MSC trial, decreases in circulating HMGB-1 after cell infusion were more pronounced in the patients with higher baseline levels [34,39]. Further research is needed to understand if and for which patient groups HMGB-1 could serve as a disease biomarker in RDEB.

Conclusions and outlook

The urgent need to develop more effective treatments for RDEB is complicated by the challenges typically associated with clinical research in rare diseases. These include small and geographically dispersed patient populations, wide variability in symptoms and manifestations, knowledge gaps in understanding the natural course of the disease, and lack of consensus on appropriate outcome measures. As a result, the current evidence base for the use of allogeneic MSC infusions for the systemic treatment of RDEB is limited by

Table 5. Serum factors reported to be elevated or decreased in RDEB patients[a] and/or positively or negatively correlated with RDEB disease severity.

		Positively correlated with				Negatively correlated with	
Factor	Elevated in RDEB patients	BEBSS	EBDASI	TBSA affected by wounds	Decreased in RDEB patients	BEBSS	TBSA affected by wounds
IL-1β	[167,168]						
IL-2	[168]						
IL-6	[167–172]	[167–169]		[170]			
IL-6/IL-10 ratio	[169]	[169]					
IL-12		[167]					
TGF-β	[170]						
TNF-β	[167,168]						
IFN-γ	[167,168,171]						
CXCL12	[173]			[173]			
HMGB-1	[173–175]	[175]	[39]				
IL-10					[169]	[169]	
TNF-α					[167]		
CCL21					[173]		
CCL27							[173]

The numbers refer to the publications in which the observations were published.
[a]The study by Annichiarico et al. [167] did not differentiate between recessive and dominant DEB. The study by Nguyen et al. [171] included patients with other DEB subtypes in addition to RDEB patients (9 RDEB, 1 DDEB, 2 DEB pruriginosa, 2 DEB with unknown inheritance).

a relative paucity of published clinical trials, which all have small sample sizes and uncontrolled designs and are therefore likely to have low statistical power [43]. Efficacy assessments were typically based on changes in outcome parameters from baseline, which must be viewed in the context that manifestations in RDEB do not develop in a linear and uniform manner. Instead, the clinical picture is made up of acute symptoms that may flare up and down ("activity") and accumulating, chronically progressing components ("damage"). Therefore, for some parameters, such as wound closure, an observed improvement may not necessarily be due to treatment because a certain proportion of wounds will close on their own; for other parameters, even no change from baseline (and thus no worsening) may indicate a treatment success.

Even with careful consideration of these circumstances, it appears reasonable to conclude that infusions of allogeneic MSCs as an emerging approach for the systemic treatment of RDEB have demonstrated a favorable safety and tolerability profile, and the reported clinical outcomes suggest potential treatment benefits for patients. Signs of improvement were seen particularly in skin manifestations, overall disease activity, pruritus, pain and quality of life, and it appears that the potential of MSC infusions lies primarily in modifying the disease through immunomodulatory, tissue homeostasis-restoring pathways rather than in restoring C7 and forming de-novo anchoring fibrils at the dermo-epidermal junction. Together, the reported safety and efficacy results provide a rationale for pursuing and further developing this therapeutic approach.

Future studies should aim for larger sample sizes and improved, preferably placebo-controlled designs to increase the statistical power of the studies and the significance of the observations. Two ongoing randomized, placebo-controlled trials, a two-center trial investigating umbilical cord tissue-derived MSCs (UC-MSCs) in an estimated 36 children [176] and an international phase-3 trial further investigating ABCB5$^+$ MSCs in an estimated 74 children and adults [177], use cross-over designs and open-label extensions to allow all participants access to the investigational product. This not only increases the statistical power with the same number of participants but may also increase the willingness of patients to consent to a placebo-controlled trial.

In order to accelerate the clinical translation of novel treatments for EB, harmonization of outcomes and outcome measurement tools is considered an important prerequisite [137]. This goal is being pursued by the COSEB (Core Outcome Sets for Epidermolysis Bullosa) initiative, a global group of stakeholders working together to establish a consensus-based core set of outcomes for each major EB type [178]. By ensuring that the most relevant outcomes are measured and reported consistently across clinical trials [178,179], this is expected to allow a more accurate and robust comparison of the benefits of different treatment approaches, which will help to increase the efficiency of clinical research to identify the most effective MSC types/products, doses and dosing schedules.

Particular care must be taken when selecting the outcome parameter to be used as the primary efficacy endpoint of a clinical trial. Especially when seeking regulatory approval for a particular drug product, it is important to select an outcome parameter that most rapidly, sensitively, and reproducibly reflects the primary mode of action of the product. For example, EB disease severity scoring systems such as BEBSS and EBDASI are valuable and reliable tools for providing a holistic picture of an individual patient's current disease burden, monitoring long-term disease progression, and optimizing management in clinical practice [147]. By quantifying the natural variability between patients, they can also aid in pathogenesis research [145]. However, holistic disease scoring systems are likely to capture certain disease characteristics that are not targeted by the (primary) mode of action of a particular therapy approach, which may limit their responsiveness to a particular investigational therapy being evaluated in a clinical trial. For example, MSC therapies are not expected to directly affect fibrotic damage parameters captured by EB disease severity scores, and potential indirect effects by preventing or slowing further damage accumulation would require more time than typically covered by efficacy follow-up periods of clinical trials. Therefore, disease severity scores, especially when evaluating their subscores, can provide valuable insight into potential changes in multiple aspects of the disease with an investigational therapy being studied in a clinical trial. However, when it comes to pivotal trials, a more narrowly defined, MSC therapy-specific measure used as the primary endpoint may increase the power of the trial to detect a difference versus placebo.

One of the most clinically meaningful outcomes in RDEB [138,139,141], and a particularly robust measure [180], is wound healing. For systemic therapy approaches such as intravenous MSC infusions, it would be ideal to assess a patient's total wound

burden. However, full-body examinations can be very stressful for people with RDEB and may not always be possible. Alternatively, healing of individual target wounds may be evaluated, as was done in the Muse cell study [36]. Given the highly dynamic and variable pattern of wound development and healing in RDEB [142–144], careful definition of appropriate selection and stratification criteria is essential to select target wounds that are unlikely to heal spontaneously and to ensure comparability between treatment and control groups. In addition to evaluating the effect of an investigational product on the wound healing process, it is desirable to evaluate the effect on the durability of the wound closure achieved, but surprisingly this is rarely done. Of the MSC trials in RDEB, only the ABCB5$^+$ MSC trial reported such data [40].

Last but not least, the success of any MSC-based treatment approach will strongly depend on the homogeneity and quality of the cell product. Products containing living cells are inherently prone to heterogeneity, as gene and protein expression profiles can vary widely depending on variations in donor characteristics as well as methods of cell expansion, cell isolation, and product formulation [181–184]. Therefore, it is critical to define inclusion/exclusion criteria for cell donors, apply strict definitions of all production steps and in-process controls, and provide comprehensive characterization of the product including marker expression/identity, purity, viability and biological functionality/potency of the cells, as has been reported for the ABCB5$^+$ MSC-based product [39,75,185]. In addition, for the use of MSCs to treat RDEB in clinical practice, it will be essential to upscale the cell manufacturing processes from manual handling of two-dimensional culture to volumetric scalable cell production based on three-dimensional culture systems that allow a constant and reliable delivery of the required cell numbers.

Disclosure statement

ENR and KD are employees of RHEACELL GmbH & Co. KG. MHF is inventor or co-inventor of U.S. and international patents assigned to Brigham and Women's Hospital and/or Boston Children's Hospital, licensed to RHEACELL. He holds stock in RHEACELL and serves as a scientific advisor to RHEACELL. MAK is CSO of RHEACELL.

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