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RESEARCH ARTICLE

Extracellular vesicles as human therapeutics: A scoping review of the literature

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Abstract

Extracellular vesicles (EVs) are released by all cells and contribute to cell-to-cell communication. The capacity of EVs to target specific cells and to efficiently deliver a composite profile of functional molecules have led researchers around the world to hypothesize their potential as therapeutics. While studies of EV treatment in animal models are numerous, their actual clinical benefit in humans has more slowly started to be tested. In this scoping review, we searched PubMed and other databases up to 31 December 2023 and, starting from 13,567 records, we selected 40 pertinent published studies testing EVs as therapeutics in humans.

The analysis of those 40 studies shows that they are all small pilot trials with a large heterogeneity in terms of administration route and target disease. Moreover, the absence of a placebo control in most of the studies, the predominant local application of EV formulations and the inconsistent administration dose metric still impede comparison across studies and firm conclusions about EV safety and efficacy. On the other hand, the recording of some promising outcomes strongly calls out for well-designed larger studies to test EVs as an alternative approach to treat human diseases with no or few therapeutic options.

KEYWORDS
clinical trials, exosomes, extracellular vesicles, therapeutics

1 | INTRODUCTION

Extracellular vesicles (EVs), a heterogeneous group of membrane-surrounded particles released by all cell types, have acquired increasing attention in the last decennia (Couch et al., 2021). EVs are characterized by different biogenesis and release mechanisms which are linked with crucial processes, such as cell activation, senescence and acquisition of effector functions (Estévez-Souto et al., 2022; Picca et al., 2020; van der Grein et al., 2018). While the first studies had hypothesized that EVs would mostly be used by the cell to eliminate disused molecules, EVs are instead now recognized to play a key role in intercellular communication. In particular, EV capacity of transferring a variety of bioactive molecules to recipient cells – including lipids, metabolites, proteins and nucleic acids – is known to affect both physiological and pathological processes (Buzas, 2022; Couch et al., 2021).

Thanks to the described properties, EVs show great potential as therapeutic agents; indeed, numerous preclinical studies have investigated the effects of EVs (from different cell sources) in animal models of neoplastic, cardiovascular, neurodegenerative, and immune diseases, which have been recently reviewed (Wiklander et al., 2019).

Clorinda Fusco, Giusy De Rosa and Ilaria Spatocco contributed equally to this work.

Considering EV biocompatibility and the promising results obtained in animal models, EVs have gained great attention as therapeutics in the human arena and several clinical trials are actually testing their safety and efficacy in a diversified range of conditions. In order to provide a comprehensive update about what has occurred till this point, we have here systematically examined the literature to collect all the studies which used EVs to treat humans and have then used a scoping approach to review those studies independently on EV source, disease treated and study design.

2 | METHODS

2.1 | Literature search and study selection

We first screened Prospero and Cochrane databases to search for possible systematic reviews in preparation, finding no entries. Then, we searched relevant articles in PubMed and Scopus up to 31 December 2023. The strings used for the PubMed search were: ((exosomes) OR (extracellular vesicles)) AND ((therapy) OR (trial) OR (treatment) OR (therapeutic) OR (clinical)) as keywords. The search was limited to the temporal range 1 January 1994–31 December 2023. As example for the search strategy, the strings used in PubMed are attached as Supplementary materials. We also scrutinized the reference lists of previous meta-analyses and included studies, as well as considered non-peer reviewed data in *ClinicalTrials.gov* and in a preprint repository, that is, medRxiv.org. Clinical trials focusing on EVs were extracted from *ClinicalTrials.gov* using the search terms "exosomes" and "extracellular vesicles." We followed the PRISMA checklist to conduct this scoping review (Supplementary materials).

Two investigators (C.Fu. and G.D.R.) independently reviewed the identified abstracts to determine the eligibility of the studies for inclusion in the review. Eligibility criteria for all studies were: (1) treating patients with extracellular vesicles or exosomes; and (2) reporting the disease status in patients who received the treatment. Since we expected small or pilot studies, we opted for including publications which report data from an exiguous number of subjects and no restriction was posed for the comparative treatment nor for the follow-up length. We did not exclude those studies with unclear description of the enrolling criteria and/or the methodology used to isolate/characterize EVs to be transfused. We posed no additional or peculiar exclusion criteria. In the text of the present manuscript, we have adopted the consistent nomenclature of "EVs", independently on the name used in the corresponding studies (exosomes, nanovesicles, *etc*…).

2.2 | Data extraction and quality assessment

All relevant data from the included studies were extracted by three independent reviewers (C.Fu., G.D.R. and I.S.) using a pre-specified, standardized data extraction template in Microsoft Excel (Microsoft, Seattle, WA). In cases of disagreement between the reviewers, the differences were resolved through consultation with two senior team members (F.P. and P.d.C.). Data were finally checked for accuracy by two investigators (E.V. and I.S.). Collected information were study type, number of patients in each group, age and sex of the patients enrolled, comparison treatment, severity of the disease, the origin and preparation methods of EVs. No masking procedure was adopted for either study selection or data extraction.

Two authors (C.Fr. and V.P.) independently assessed the quality of included studies using the Risk of bias 2.0 and the ROBINS-I tools for randomized clinical trials (RCTs) and non-randomized studies, respectively. For the case reports, the same authors used the dedicated JBI critical appraisal checklist. Discrepancies regarding the evaluation of selected items were resolved by a third author (R.L.G.). The protocol was originally registered in osf.io (https://osf.io/c3adg) and then amended to postpone the end date for the search in order to collect the latest literature on the topic.

2.3 | Data analysis

Upon collection of the published studies to be analysed, we recognized the appropriateness of a narrative synthesis without the possibility of an actual meta-analysis. We thus grouped the studies based on the geographic location where they were performed, the type of disease/condition treated, the nature of the EV preparation, EV isolation and characterization methods, administration route and dosing (when available). The descriptive nature of our scoping review aims at generating a collective vision of the state of the art in the field and at identifying the knowledge gaps still present, and not at evaluating the efficacy of EVs for a specific human pathology.

3 | RESULTS

In order to identify all the published studies which used EVs to treat humans, we searched for manuscripts containing the words "extracellular vesicles" or "exosomes" and "trial" or "therapy" or "treatment" or "clinical" in the title and/or the abstract. Upon

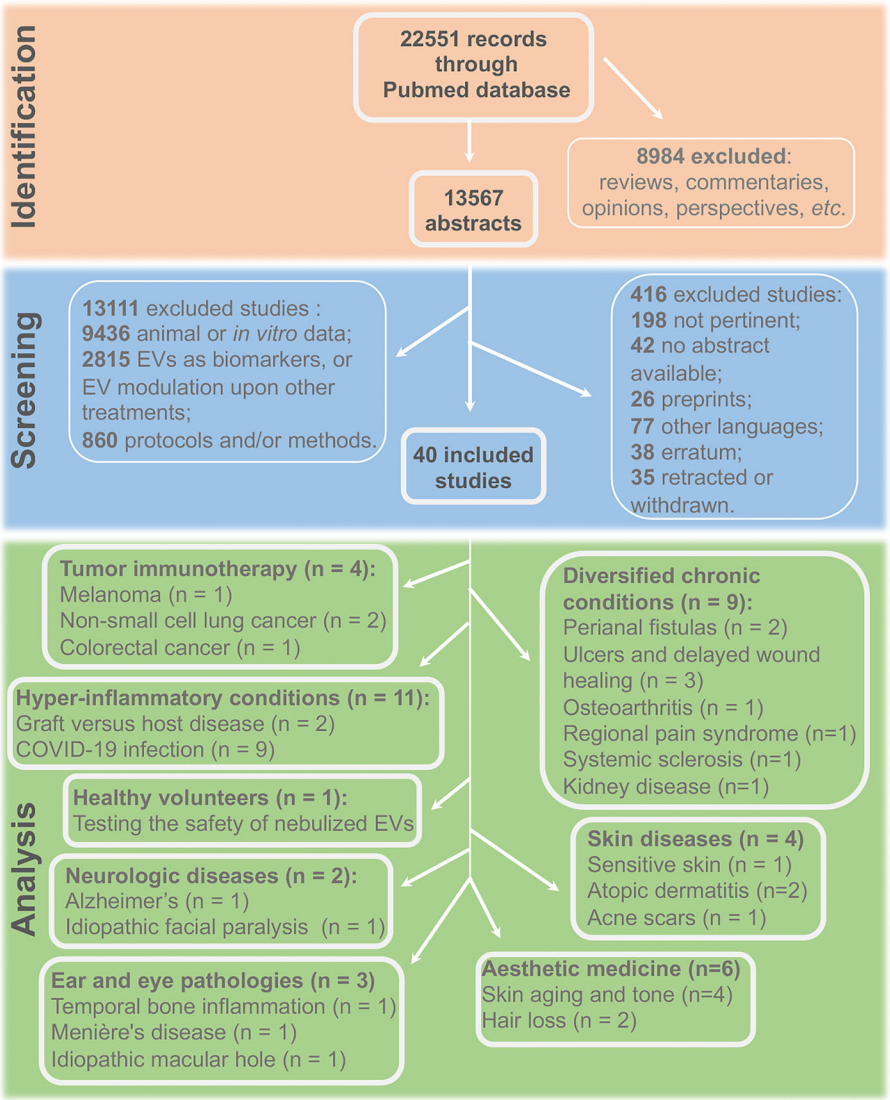

FIGURE 1 Work flow for the scoping review. The three phases of the work (identification, screening and analysis) are differentiated by background colours. In the analysis box, the different studies are subdivided by disease type, a classification also followed in the Results section.

identification of 22,551 records, we selected 13,567 pertinent manuscripts to be evaluated. During the screening phase of the manuscripts, 13,527 were excluded: 198 were not pertinent, 9436 contained animal or in vitro data, 2815 were studies in which EVs were quantified *ex vivo* in search of biomarkers of disease and/or upon other types of treatments, 860 reported protocols and/or methods, 218 were either preprints, erratum, retracted, withdrawn or written in other languages than English. The inclusion flow is presented in Figure 1.

Eventually, 40 studies were included for the review (listed in Table 1), showing a widespread geographic distribution in terms of the research institutions/hospitals coordinating the effort (Figure 2). Overall, we collected data from 605 EV-treated and 133 control subjects (total = 738), with a 53.8% prevalence of female sex (based on 35 studies in which gender distribution was reported) and an average age of 49.8 years (based on 32 studies in which the age mean was reported).

In terms of study types, only eight (20%) were conducted as RCTs, while the other thirty two (80%) were non-randomized studies with different designs, including nine case reports; in 35/40 studies (87.5%), the design did not include a control group, and the treatments were compared with the untreated historical group (26 studies), the other half of the treated body part (e.g., side of the face, six studies) or not compared at all ($n = 3$) (Figure 3a,b). The conditions treated with the EVs are highly heterogeneous and range from cancer ($n = 4$); COVID-19 ($n = 9$); graft versus host disease (GvHD, $n = 2$); neurologic diseases ($n = 2$); ear and eye pathologies ($n = 3$); diversified chronic conditions ($n = 9$, of which $n = 3$ ulcers and delayed wound healing); skin diseases ($n = 4$) and also aesthetic medicine ($n = 6$, i.e., hair regrowth and skin brightness/aging) (Figure 3c).

The properties of mesenchymal stem cell-derived EVs (MSC-EVs) linked to tissue repair and suppression of innate and adaptive immune cells have been tested in experimental models of allergic, autoimmune and graft-versus-host conditions (Shen et al.,

TABLE 1 Summary of the EV-based clinical studies.

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary end-points	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
1	#14	2005	Escudier B, *et al.*	Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first Phase 1 clinical trial	Metastatic melanoma (stage IIIB and IV)	Clinical trial (Phase 1)	Interventional, single group assignment, open label	N.A.	15	Treatment versus untreated historical group	52.0 (±11.4)	8/7	Intradermal and subcutaneous injection	0.13 versus 0.40E14 (MHC class II molecules); 10 versus 100 micrograms/ml MAGE-3 peptide quantity); four vaccinations at one week interval	Safety and feasibility	Exosomes	Autologous dendritic cells	Ultracentrifugation	ELISA, immunophenotype	https://www.translational-medicine.com/content/3/1/10	10.1186/1479-5876-3-10	Villejuif, France
2	#15	2005	Morse MA, *et al.*	A Phase 1 study of dexosome immunotherapy in patients with advanced non-small cell lung cancer	Non-small cell lung cancer	Clinical trial (Phase 1)	Interventional, single group assignment, open label	N.A.	9	Treatment versus untreated historical group	62.0 (range 44-72)	6/3	Intradermal and subcutaneous injection	3.14E14 /injection for 4 weeks	Safety and feasibility	Exosomes	Autologous dendritic cells	Ultracentrifugation	Not reported	http://www.translationa medicine.com/content/3/1/9	10.1186/1479-5876-3-9	Durham, NC, U.S.A.
3	#18	2016	Besse B, *et al.*	Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC	Non-small cell lung cancer	Clinical trial (Phase 2)	Interventional, single group assignment, open label	NCT01-159288	22	Treatment versus untreated historical group	62.0 (23-79)	7/15	Intradermal injection	1.93 μg for an average of 7 injections	Progression free survival	Small extracellular vesicles	Autologous dendritic cells	Ultrafiltration and ultracentrifugation	FACS, DC Protein Assay	https://doi.org/10.1080/2162402X.2015.1071008	10.1080/2162402X.2015.1071008	Heidelberg, Germany

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TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
4	#19	2008	Dai S, *et al.*	Phase 1 clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer	Colorectal cancer	Clinical trial (Phase 1)	Randomized, parallel assignment	N.A.	40	Treatment versus untreated historical group	52.7 (±8,49)	17/23	Subcutaneous injection	100, 200, 300, and 500 µg	Safety and efficacy	Exosomes	Autologous ascites	Ultracentrifugation	EM, W.B.	https://doi.org/10.1038/mt.2008.1	10.1038/mt.2008.1	Liuzhou, China
5	#20	2014	Kordelas L, *et al.*	MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease	GvHD	Case report	Interventional case report	N.A.	1	Treatment versus untreated historical group	Not reported	0/1	Not reported	1.3–3.5E10 particles/unit (1 unit=EV fraction prepared from supernatants of 4×10E7 MSCs)	Safety and efficacy	Exosomes	Human bone marrow derived mesenchymal stem cells	PEG-based/-ultracentrifugation	NTA, EM, W.B.	http://creativecommons.org/licenses/by-nc-sa/3.0/	10.1038/leu.2014.41	Essen, Germany
6	#23	2022	Zhou T, *et al.*	miR-204-containing exosomes ameliorate GVHD-associated dry eye disease	GvHD-associated dry eye disease	Prospective clinical trial (Phase 1/2)	Interventional, single group assignment, open label	NCT04213248	14	Treatment versus untreated historical group	35.4 (±8,64)	7/7	Eye drops	Not reported	Safety and efficacy	Exosomes	Human umbilical cord-derived mesenchymal stem cells	Ultracentrifugation	NTA, EM, W.B.	https://www.science.org/doi/10.1126/sciadv.abj9617	10.1126/sciadv.abj9617	Guangzhou, China

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7	#27	2020	Sengupta V, *et al.*	Exosomes Derived from Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19	COVID-19: severe	Prospective study	Prospective, non-randomized, open label, cohort study	N.A.	27	Treatment versus untreated historical group	59.0 (range 29-84)	10/17	Intravenous infusion	15 mL	Safety and efficacy	ExoFlo	Human bone marrow-derived mesenchymal stem cells	Not reported	Not reported	https://doi.org/10.1089/scd.2020.0080	10.1089/scd.2020.0080	New York City, NY, U.S.A.
8	#28	2023	Lightner AL, *et al.*	Bone Marrow Mesenchymal Stem Cell-Derived Extracellular Vesicle Infusion for the Treatment of Respiratory Failure From COVID-19: A Randomized, Placebo-Controlled Dosing Clinical Trial	COVID-19: respiratory failure	Prospective clinical trial (Phase 2)	Prospective Phase 2, multicenter, double-anonymiz randomized, placebo-controlled dosing trial	NCT-044-932-42	68	34	Range 18-65	Not reported	Intravenous infusion	High vs. low dose (1.2 and 0.9 trillion EV particles per dose, respectively)	Safety and efficacy	ExoFlo	Human bone marrow derived mesenchymal stem cells	Not reported	Not reported	https://journal.chestnet.org/article/S0012-3692(23)00926-1/fulltext#pag	10.1016/j.chest.2023.06.024	Mesquite, TX, U.S.A.
9	#30	2021	Mitrani MI, *et al.* (a)	Case Report: Administration of Amniotic Fluid-Derived Nanoparticles in Three Severely ill COVID-19 Patients	COVID-19: severe or critical	Case report	Interventional, single group assignment, open label	N.A.	3	Treatment versus untreated historical group	73	2/1	Intravenous infusion	1 mL (nanoparticles concentratio $n = 3.26E11$/mL) for 3\4 doses	Safety, feasibility, and accessibility	Zofin	Human amniotic fluid	Not reported	Not reported	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8010176/	10.3389/fmed.2021.583842	Miami, FL, U.S.A.

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TABLE 1 (Continued)

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10	#31	2021	Bellio MA, *et al.*	Proof-of-concept trial of an amniotic fluid-derived extracellular vesicle biologic for treating high risk patients with mild-to-moderate acute COVID-19 infection	COVID-19: mild-to-moderate	Clinical trial (Phase 1/2)	Proof-of-concept, expanded access trial	NCT-046-57-406	8	Treatment versus untreated historical group	51.9 (± 10.5)	3/5	Intravenous infusion	1 mL on day 0, day 4 and day 8, containing 1-5E11 particles/mL	Safety	Zofin	Human full-term perinatal amniotic fluid	Not reported	Not reported	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8611818/	10.1016/j.bbiosy.2021.100031	Houston, TX, U.S.A.
11	#32	2021	Mitrani MI, *et al.* (b)	Treatment of a COVID-19 long hauler with an amniotic fluid-derived extracellular vesicle biologic	COVID-19: long hauler	Case report	Single patient case report study	N.A.	1	Uncontrolled	55.0	0/1	Intravenous infusion	3.26E11/mL	Safety	Zofin	Human amniotic fluid	Not reported	Not reported	https://doi.org/10.1016/j.rmcr.2021.101502	10.1016/j.rmcr.2021.101502	Miami, FL, U.S.A.
12	#33	2021	Shi M, *et al.*	Preclinical efficacy and clinical safety of clinical-grade nebulized allogenic adipose mesenchymal stromal cells-derived extracellular vesicles	Lung diseases	Pilot trial (safety)	Phase 1 single-arm clinical trial, open label, dose-escalation pilot study, non-randomized, parallel assignment	NCT-043-13-647	24	Treatment versus untreated historical group	(26.2 (± 3.2))	11/13	Nebulization	From 2E8 to 16E8 particles	Safety	Extracellular vesicles	Human adipose-derived mesenchymal stromal cells	Ultracentrifugation	NTA, BA, Immunoblot, TEM	https://doi.org/10.1002/jev2.12134	10.1002/jev2.12134	Shanghai, China

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TABLE 1 (Continued)

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13	#34	2022	Zhu YG, *et al.*	Nebulized exosomes derived from allogenic adipose tissue mesenchymal stromal cells in patients with severe COVID-19: a pilot study	COVID-19: severe or critical	Clinical trial (Phase 2a)	Interventional, single group assignment, open label	NCT-042-76-987	7	Treatment versus untreated historical group	57.0 (IQR,43-70)	3/4	Inhalation	2.0E8 EVs for consecutively 5 days	Safety and efficacy	Exosomes	Human adipose-derived mesenchymal stem cells	Ultracentrifugation	NTA, EM, Immunoblot	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9135389/	10.1186/s13287-022-02900-5	Wuhan, China
14	#35	2022	Chu M, *et al.*	Nebulization Therapy with Umbilical Cord Mesenchymal Stem Cell-Derived Exosomes for COVID-19 Pneumonia	COVID-19: pneumonia	Pilot trial (treatment and management)	Interventional, parallel/-crossover	ChiCTR20-0003-0261	7	39	Range 18–65	3/4	Nebulization	From 7.66e + 0.8 to 7.00e + 0.7 EVs each nebulization	Safety	Exosomes	Human umbilical cord derived-mesenchymal stem cells	Ultrafiltration and ultracentrifugation	NTA, EM, W.B.	https://doi.org/10.1007/s12015-022-10398-w	10.1007/s12015-022-10398-w	Wuxi, P.R. China
15	#36	2023	Zarrabi M, *et al.*	Allogenic mesenchymal stromal cells and their extracellular vesicles in COVID-19 induced ARDS: a randomized controlled trial	COVID-19: severe or critical	Clinical trial (Phase 1/2)	Interventional, randomized controlled, multicentric, placebo-controlled	IRCT-2020-0217-0465-26N2	8	24	49.3 ±-10.8	5/3	Inhalation	One dose of EVs isolated from the 200E6 ± 10% cells	Safety and efficacy	Extracellular vesicles	Human placental and umbilical cord derived-mesenchymal stem cells	Tangential-flow filtration	BA, W.B., DLS, SEM	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10294333/	10.1186/s13287-023-03402-8	Tehran, Iran

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TABLE 1 (Continued)

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16	#37	2022	Gül F, *et al.*	A pilot study for treatment of severe COVID-19 pneumonia by aerosolized formulation of convalescent human immune plasma exosomes (ChipEXO)	COVID-19: pneumonia	Pilot study	Interventional, single-center prospective, controlled, open label, single arm	N.A.	13	Treatment versus untreated historical group	55.9 (± 11.2, range 39-74)	5/8	Jet nebulizer	1-5E10 nanovesicles/5 mL twice a day for five days	Safety	Chip-EXO	Human COVID-19 convalescent plasma-derived exosomes	Ultracentrifugation	NTA, SEM, FTIR	https://doi.org/10.3389/fimmu.2022.963309	10.3389/fimmu.2022.963309	Kayseri, Turkey
17	#41	2023	Xie X, *et al.*	Clinical safety and efficacy of allogenic human adipose mesenchymal stromal cells-derived exosomes in patients with mild to moderate Alzheimer's disease: a Phase I/2 clinical trial	Alzheimer's disease	Clinical trial (Phase 1/2)	Interventional, three-arm, open label, non-randomiz	NCT04388982	9	Treatment versus untreated historical group	≧ 50.0	4/5	Intranasal (spray) administration	Three dosage groups: 2E8 particles/1 mL (Low-dose); 4E8 particles/1 mL (Medium-dose); 8E8 particles/1 mL (High-dose); two times per week for 12 weeks	Safety and efficacy	Exosomes	Human adipose derived mesenchymal stromal cells	PEG-based/-ultracentrifugation	NTA, TEM, W.B.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10582#SP1	10.1136/gpsych-2023-101143	Shanghai, China

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TABLE 1 (Continued)

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18	#43	2023	Dresch-nack PA, Belshaku I	Treatment of idiopathic facial paralysis (Bell's Palsy) and secondary facial paralysis with extra-cellular vesicles: a pilot safety study	Idiopathic facial paraly-sis (Bell's Palsy)	Pilot trial (safety)	Interventi-onal, non-randomized	IRCM-2021–304	7	Treatment versus untreated histori-cal group	Range 32–61	5/2	Intra-venous infusion/-injection into the affected area (with ultra-sound guid-ance)	13 cc intra-venously and 2 cc directly into tissue around the facial nerve on visits during weeks l, 2, and 4	Safety	ExoFlo	Human bone marrow-derived mes-enchy-mal stem cells	Not reported	Not reported	https://bmcneurol.biomedcentral.com/articles/10.1186/s12883-023-03400-6	10.1186/s12883-023-03400-6	Elmsford, NY, U.S.A.
19	#44	2021	Vozel D, *et al.*	Autologous Platelet- and Extra-cellular Vesicle-Rich Plasma Is an Effective Treatment Modality for Chronic Postopera-tive Temporal Bone Cavity Inflamma-tion: Random-ized Controlled Clinical Trial	Temporal Bone Cavity Inflam-mation	Clinical trial (Phase N.A.)	Interven-tional, prospec-tive ran-domized con-trolled, parallel assign-ment	NCT0-4281-901	11	11	patients: 47.0 (± 18.0); con-trol group: 52.0 (± 24.0)	4/18	Direct admin-istra-tion in mas-toid cavity	2.1 mL of autolo-gous platelet-derived and extracel-lular vesicle-rich plasma (504 × 10E9/L platelets and EVs in PVRP) at the day of recruit-ment, and 1 month later	Efficacy	Nano-sized mem-brane vesi-cles	Plasma	2-step centrifu-gation*	FACS, immuno labeling, EM*	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC82944	10.3389/fbioe.2021.677541.	Ljubljana, Slovenia

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TABLE 1 (Continued)

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20	#46	2021	Warnecke A, *et al.*	First-in-human intracochlear application of human stromal cell-derived extracellular vesicles	Hearing loss (Menière's disease)	Case report	Interventional case report	N.A.	1 (one ear)	1 (the other ear)	55.0	0/1	Intracochlear application	From 20 to 40 µL corresponding to a dose of 2E9 to 4E9 EVs	Safety and feasibility	Extracellular vesicles	Human umbilical cord-derived mesenchymal stem cells	Tangential-flow filtration and ultracentrifugation	NTA, FACS, EM	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8178433/	10.1002/jev2.12094	Salzburg, Austria
21	#48	2018	Zhang X, *et al.*	Effects of mesenchymal stem cells and their exosomes on the healing of large and refractory macular holes	Large and refractory macular holes	Pilot trial	An in vivo study	N.A.	7	Treatment versus untreated historical group	67.1 (+/-6.1)	5/2	Intravitreal injection	20/50 µg	Safety and efficacy	Exosomes	Human umbilical cord-derived mesenchymal stem cells	Ultracentrifugation	BCA, EM. W.B.	https://link.springer.com/article/10.1007/s00417-018-4097-3	10.1007/s00417-018-4097-3	Tianjin, China
22	#52	2022	Nazari H, *et al.*	Evaluating the safety and efficacy of mesenchymal stem cell-derived exosomes for treatment of refractory perianal fistula in IBD patients: clinical trial Phase 1	Perianal fistula (Crohn's disease-associated)	Clinical trial (Phase 1/2)	Prospective clinical trial, open label	NCT05499156	5	Treatment versus untreated historical group	35.4 (± 8.7)	2/3	Injection into thre tissue surrounding the fistula tract	5 mL of the exosome solution (quantity not revealed)	Safety and efficacy	Exosomes	Human umbilical cord-derived mesenchymal stem cells	Ultracentrifugation	DLS, FACS, W.B., EM	https://doi.org/10.1093/gastro/goac075	10.1093/gastro/goac075	Tehran, Iran

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TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
23	#53	2023	Pak H, *et al.*	Safety and efficacy of injection of human placenta mesenchymal stem cells derived exosomes for treatment of complex perianal fistula in non-Crohn's cases: Clinical trial Phase 1	Perianal fistula (not Crohn's disease-associated)	Clinical trial (Phase 1)	Interventional, single arm	NCT-05-402-748	11	Treatment versus untreated historical group	43.3 (±7.7)	3/8	Injection along the fistula tract	0.5E10 EVs every week, for three weeks	Safety and efficacy	Exosomes	Human placenta-derived mesenchymal stem cells	Ultracentrifugation	BA, DLS, W.B., EM	https://doi.org/10.1111/jgh.16110	10.1111/jgh.16110	Tehran, Iran
24	#55	2022	Messa GE, *et al.*	Treatment of a recurrent ischial ulcer with injected exosomes	Ischial ulcers	Case report	Interventional case report	N.A.	1	Treatment versus untreated historical group	38.0	0/1	Injection into the base and walls of the wound	1 cc of exosomes (quantity not revealed)	Efficacy	ExoFlo	Human bone marrow derived mesenchymal stem cells	Not reported	Not reported	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC92383	10.1093/jscr/rjac271	New Orleans, LA, U.S.A.
25	#56	2023	Johnson J, *et al.*	First-in-human clinical trial of allogeneic, platelet-derived extracellular vesicles as a potential therapeutic for delayed wound healing	Delayed wound healing	Clinical trial (Phase 1)	Double-blind, placebo-controlled, prospective, randomized clinical trial	ACTR-N126-200-0094-4932	11	A wound in the other arm assigned to receive the placebo formulation	29.0 ±10.4	3/8	Subcutaneous injection	A single dose (100 μg in 340 μL)	Safety	Extracellular vesicles	Human activated platelets	Ligand-based Exosome Affinity Purification (LEAP) chromatography	BA, NTA, Cryo-TEM, W.B., MS	https://onlinelibrary.wiley.com/doi/10.1002/jev2.12332	10.1002/jev2.12332	Parkville, VIC, Australia

(Continues)

TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary end-points	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
26	#57	2023	Gibello L, *et al.*	First pilot case-control interventional study using autologous extracellular vesicles to treat chronic venous ulcers unresponsive to conventional treatments	Chronic venous ulcers	Pilot study	Pilot case-control interventional study	CS2/109-5/00-904-91	4	5 (Standard of care on a smaller surface area)	77.0 (range 18-85)	3/1	Multilayer bandage (peri-wound injection)	Six doses of active s-EVs	Efficacy	Extracellular vesicles	Serum	Ultracentrifugation	ELISA, FACS, TEM, W.B.	https://doi.org/10.1016/j.phrs.2023.106718	10.1016/j.phrs.2023.106718	Turin, Italy
27	#58	2016	Nassar W, *et al.*	Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases	Chronic kidney disease	Pilot study (Phase 2/3)	Randomized, placebo-controlled Phase 2/3 clinical pilot study	N.A.	20	20	Group A 32.1 (±9.2); Group B 34.2 (± 6.2); range 26-44	10/10	Intravenous infusion and intra-arterial injection	Two doses (1E10 p/g)	Safety	Extracellular vesicles	Human umbilical cord-derived mesenchymal stem cells	Ultracentrifugation	Proteomic array, FACS, TEM	https://doi.org/10.1186/s40824-016-0068-0	10.1186/s40824-016-0068-0	Cairo, Egypt

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TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
28	#60	2021	Gupta A, *et al.*	Cell-free stem cell-derived extract formulation for treatment of knee osteoarthritis: study protocol for a preliminary non-randomized, open-label, multi-center feasibility and safety study	Knee osteoarthritis (grade II/III)	Prospective study (early Phase 1)	Interventional, single group assignment, open label	NCT-049-717-98	12	Treatment versus untreated historical group	Not reported	Not reported	Intra-rticular injection	Not reported	Safety	Exosomes	Human progenitor endothelial stem cells	Not reported	Not reported	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8377854/?report=classic	10.1186/s13018-021-02672-3	Laredo, TX, U.S.A.
29	#62	2023	Assar S, *et al.*	Improvement in the clinical manifestations of interstitial lung disease following treatment with placental mesenchymal stromal cell extracellular vesicles in a patient with systemic sclerosis: A case report	Complicated Systemic sclerosis: interstitial lung disease	Case report	Case report	N.A.	1	Treatment versus untreated historical group	55.0	1/0	Intravenous infusion	Three doses (0.8–1.2E9 particles/kg) on three consecutive days	Safety and efficacy	Extracellular vesicles	Human placental mesenchymal stromal cell	Not reported	Not reported	https://doi.org/10.1016/j.rmcr.2023.101923	10.1016/j.rmcr.2023101923	Kermanshah, Iran

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TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary end-points	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
30	#63	2023	Paicius R, *et al.*	Safety and Efficacy of Intravenous ExoFlo in the Treatment of Complex Regional Pain Syndrome	Complex Regional Pain Syndrome	Pilot trial (safety and efficacy)	Prospective, pilot study	N.A.	10	Uncontrolled	55.8 (range 40-80)	7/3	Intravenous infusion	15 mL ExoFlo, on day one and day 4	Safety	ExoFlo	Human bone marrow-derived mesenchymal stem cells	Not reported	Not reported	https://www.painphysicianjournal.com/current/pdf?article=Nzc3MQ%3D%3D&journal=156	Not reported	Newport Beach, CA, U.S.A.
31	#64	2022	Ye C, *et al.*	hMSC exosomes as a novel treatment for female sensitive skin: An in vivo study	Sensitive skin	Clinical trial	An in vivo study	N.A.	22	Treatment versus untreated historical group	40.0 (± 8.1)	22/0	Cream	1 ml/ twice a day for 28 day	Safety and efficacy	Exosomes	Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells	Ultracentrifugation	EM, W.B.	https://doi.org/10.3389/fbioe.2022.1053679	10.3389/fbioe.2022.1053679	Guangdong, China
32	#67	2021	Park KY, *et al.*	Exosomes derived from human adipose tissue-derived mesenchymal stem cells for the treatment of dupilumab-related facial redness in patients with atopic dermatitis: A report of two cases	Dupilumab-related facial redness	Case report	Two-cases report	N.A.	2	Treatment versus untreated historical group	28 and 33 years	0/2	Topical (transdermal electroporation)	1 ml	Safety and efficacy	Exosomes	Human adipose tissue-derived mesenchymal stem cells	ExoSCRT technology, Tangential-flow filtration	NTA, Cryo-TEM, W.B., FACS	https://doi.org/10.1111/jocd.14153	10.1111/jocd.14153	Seoul, Korea

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TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
33	#68	2023	Han HS, *et al.*	Adipose-derived stem cell exosomes for treatment of dupilumab-related facial redness in patients with atopic dermatitis	Dupilumab-related facial redness	Prospective study	Prospective pilot study	N.A.	20	Uncontrolled	≧ 18	Not reported	Topical	20 mg (970,000 ppm)	Safety and efficacy	Exosomes	Human adipose tissue-derived mesenchymal stem cells	ExoSCRT technology, Tangential-flow filtration	NTA, Cryo-TEM, W.B., FACS	https://doi.org/10.1080/09546634.2023.2220444	10.1080/09546634.2023.2220444	Seoul, Korea
34	#69	2020	Kwon HH, *et al.*	Combination Treatment with Human Adipose Tissue Stem Cell-derived Exosomes and Fractional CO2 Laser for Acne Scars: A 12-week Prospective, Double-blind, Randomized, Split-face Study	Acne Scars	Clinical study	Prospective, double-blind, randomized, split-face study	N.A.	25 (one side of the face)	25 (the other side of the face)	35.6 (± 8.2, range19-54)	7/18	Gel solution	One dose at 9.78E10 particles/ml (on day 1) and 1.63E10 particles/ml (on subsequent days)	Safety and efficacy	Exosomes	Human adipose tissue-derived stem cells	Tangential-flow filtration	NTA, EM, FACS	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9309815	10.2340/00015555-3666	Hwaseong, Korea

(Continues)

TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
35	#70	2021	Jang B, *et al.*	Extracellular Vesicles from Korean Codium fragile and Sargassum fusiforme Negatively Regulate Melanin Synthesis	Skin quality	Clinical study	An in vivo study	N.A.	21 (one side of the face)	21 (the other side of the face)	46.0 (range 20-50)	Not reported	Skin patch and cream	5 μg/mL EVs (once a day for 4 weeks)	Not reported	Nanosized membrane vesicles	Codium fragile and Sargassum fusiforme	Ultracentrifugation	DLS, EM	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8560586/	10.14348/molcells.2021.2167	Seoul, Korea
36	#71	2023	Chernoff G	Combining topical dermal infused exosomes with injected calcium hydroxylapatite for enhanced tissue biostimulation	Skin quality	Pilot study	Prospective Phase 1, non-randomiz	ICSS-2022-007	30	Treatment versus untreated historical group	Range 34-72	Not repor	Topical dermal infusion	1 cc, containing 1 million of exosomes	Safety and efficacy	Exosomes	Human placental mesenchymal stem cells	Not reported	Not reported	https://onlinelibra wiley.com/doi/10.1111/jocd.15695	10.1111/jocd.15695	Indianapolis, IN, U.S.A.
37	#72	2022	Proffer SL, *et al.*	Efficacy and Tolerability of Topical Platelet Exosomes for Skin Rejuvenation: Six-Week Results	Facial skin aging	Prospective study	Single-arm, non-randomized, longitudinal study	N.A.	56	Treatment versus untreated historical group	54.0 (±11, range 40-80)	48/8	Topical	Twice a day for 6-weeks (quantity not revealed)	Safety and efficacy	Exosomes	Human platelets	Not reported	Not reported	https://doi.org/10.1093/asj/sjac149	10.1093/asj/sjac149	Rochester, MN, U.S.A.

(Continues)

TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
38	#73	2023	Park GH, *et al.*	Efficacy of combined treatment with human adipose tissue stem cell-derived exosome-containing solution and microneedling for facial skin aging: A 12-week prospective, randomized, split-face study	Facial skin aging	Prospective study	Prospective, randomized, split-face, comparative study	N.A.	28 (one side of the face)	28 (the other side of the face)	54.0 (±7.8, range 43–66)	20/8	Topical and microneedling	5E9 particles in a 2mL solution	Safety and efficacy	Exosomes	Human adipose tissue-derived mesenchymal stem cells	Not reported	Not reported	https://doi.org/10.1111/jocd.15872	10.1111/jocd.15872	Seoul, Korea
39	#74	2022	Sasaki GH	Clinical Use of Extracellular Vesicles in the Management of Male and Female Pattern Hair Loss: A Preliminary Retrospective Institutional Review Board Safety and Efficacy Study	Hair Loss	Retrospective clinical case study	Retrospective, open label	N.A.	31	Treatment versus untreated historical group	F:62.9 (range, 28-87) M:43.3 years (range, 27-76)	22/9	Intradermal injection	1 to 8 mL (quantity not revealed)	Tolerability and Safety	XoFlo	Human bone marrow derived mesenchymal stem cells	Not reported	Not reported	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC93426	10.1093/asjof/ojac0	Loma Linda, CA, U.S.A.

(Continues)

TABLE 1 (Continued)

Study n.	Reference	Year	Authors	Publication Title	General pathology	Study type	Description	Trial Registration	EV treated group (n)	Control group (n)	Age of enrolled patients (years, mean+/-)	Sex (F/M)	EV route of administration	EV doses	Primary endpoints	EV nomenclature	EV biological source	EV isolation	EV characterization	Publication url	Publication doi	Last author's geographic affiliation
40	#75	2023	Norooznezhad AH, *et al.*	Treatment of persistent chemotherapy-induced hair loss (Alopecia) with human mesenchymal stromal cells exosome enriched extracellular vesicles: A case report	Chemotherapy-related hair loss (Alopecia)	Case report	Case report	N.A.	1	Treatment versus untreated historical group	36.0	1/0	Subcutaneous injection	Three sessions (140–160 μg; 2.5–3.2E10 particles every four weeks	Safety and efficacy	Extracellular vesicles	Human placental mesenchymal stromal cells	Not reported	Not reported	https://doi.org/10.1016/j.heliyon.2023.e15165	10.1016/j.heliyon.2023.e15165	Kermanshah, Iran

Notes: Summary of the relevant data extracted from the 40 manuscripts (authors and title reported on the left) included in the scoping review, with the relative information pertaining to EVs (e.g., cell source, isolation method and characterization) and to their clinical use (e.g., patients' characteristics, EV dosage, and the endpoints for the study).

Abbreviations: BA, Bradford colorimetric assay; BCA, Bicinchoninic acid protein assay; DC protein assay, Detergent compatible protein assay; DLS, Dynamic light scattering; ELISA, Enzyme Linked ImmunoSorbent Assay; EM, Electron microscopy; FACS, Flow cytometry; FTIR, Fourier-transform infrared spectroscopy; MS, mass spectrometry; NTA, Nanoparticle tracking analysis; TEM, Transmission Electron microscopy; WB, Western blotting.

FIGURE 2 Geography of EV-based clinical studies. World map highlighting the geographic locations (colour code refers to macro-areas, as indicated) where the identified 40 studies were coordinated (based on the last author's affiliation). Several of the studies were multicentric, which is not reported in the figure.

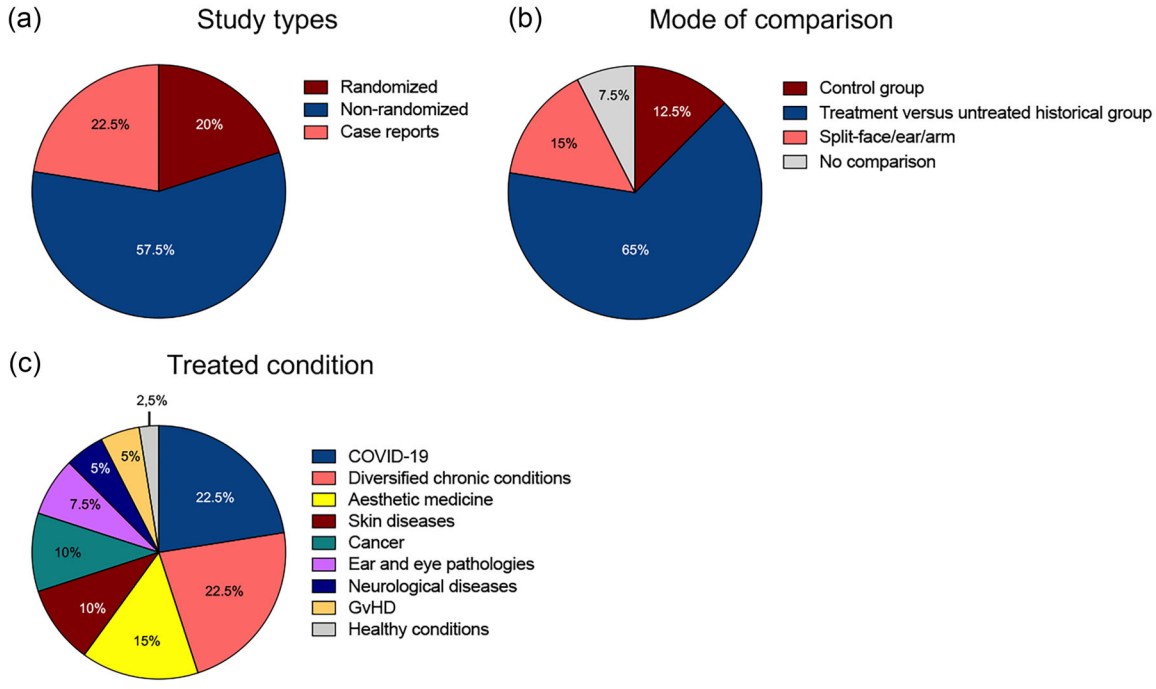

FIGURE 3 Main characteristics of the EV-based clinical studies. Pie charts showing the distribution, among the 40 selected publications, of clinical study type (a); the mode of comparison (b) and the disease treated (c). GvHD, graft versus host disease; TBC, temporal bone cavity.

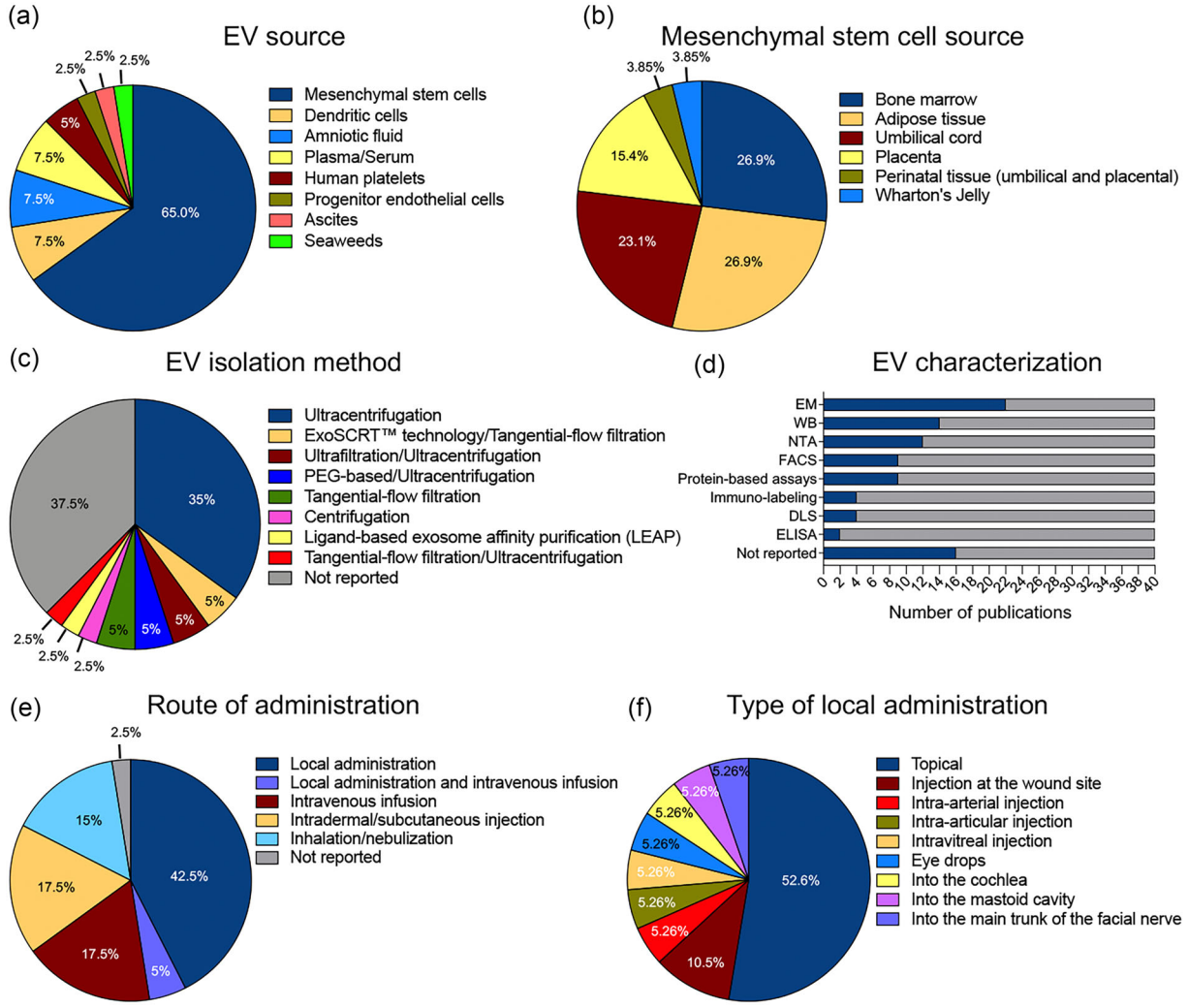

FIGURE 4 EV cell source, isolation, characterization and administration in the EV-based clinical studies. (a,b) Pie charts showing the relative quantities of studies making use of specific biological sources for the EV preparations (a) and the relative origin of mesenchymal stem cells for the specific studies using those cells as EV source (b). (c) Pie chart showing the relative quantities of studies making use of specific isolation procedures for the EV preparations. (d) Contingency stacked bar graph showing the relative quantities of studies making use of specific characterization methods for the EV preparations. (e,f) Pie charts showing the relative quantities of studies making use of specific administration routes for EVs in the clinics (e) and the relative subtype of local administration (f). BA, Bradford colorimetric assay; BCA, Bicinchoninic acid protein assay; DC protein assay, Detergent compatible protein assay; DLS, Dynamic light scattering; ELISA, Enzyme Linked ImmunoSorbent Assay; EM, Electron microscopy; FACS, Flow cytometry; FTIR, Fourier-transform infrared spectroscopy; MS, mass spectrometry; NTA, Nanoparticle tracking analysis; TEM, Transmission Electron microscopy; WB, Western blotting.

2021). Indeed, among the studies evaluated, those making use of MSC-EVs to achieve immunoregulatory effects are the most represented (26/40 studies, 65%, Figure 4a). In particular, the most common sources of MSCs are the bone marrow and the adipose tissue (seven studies each); perinatal tissues also represent a relevant source of MSCs in the selected studies (12 studies), despite being stratified according to the precise derivation (umbilical cord, placenta or Wharton's Jelly) (Figure 4b). Among the isolation methods to purify/enrich the EVs prior to the clinical application, ultracentrifugation is the most used (14/40, 35%, by itself; other 5/40, 12.5%, in combination with other techniques); to be noted, 15/40 studies did not report the EV purification process utilized (Figure 4c). In terms of EV characterization, the most frequent one was electron microscopy (utilized in 22/40 studies), followed by Western blotting (utilized in 14/40 studies) and nanoparticle tracking analysis (NTA, utilized in 12 studies) (Figure 4d). In half of the cases (50%), EVs were characterized by at least three independent techniques; notably, though, in 16/40 (40%) studies, no EV characterization was reported (Figure 4d). When investigating the chosen EV route of administration, the most frequent one was local (utilized in almost half of the studies, 19/40), followed by intravenous infusion and intradermal/subcutaneous injection (seven studies each), and inhalation/nebulization ($n = 6$ studies) (Figure 4e). Regarding local administration, ten studies used a topical one (by means of either cream, skin patch, or gel), and five studies used injection at various sites (wound site, intra-articular, intra-arterial, intravitreal); one study used eye drops, two studies administrated the EVs into the ear (i.e., into the cochlea and into the mastoid cavity) and one into the main trunk of the facial nerve (Figure 4f).

The complete information about the selected studies (disease treated, study design and endpoints, EV types and used nomenclature, EV isolation and characterization and other relevant clinical and experimental details) is summarized in Table 1 and the risk of bias analysis reported in Table S1. Before evaluating them in more details, the studies have been subdivided based on the pathological frame in which EVs were utilized.

4 | CANCER IMMUNOTHERAPY

4.1 | Metastatic melanoma

The oldest studies selected in the present review present data on the clinical testing the EVs in the field of cancer immunotherapy; this approach, generally aimed at strengthening or inducing host antitumor immune response, is now an established therapeutic option adding to the traditional chemo- and radiotherapy to treat both haematological and solid malignancies (Esfahani et al., 2020). In particular, the adoptive transfer of autologous dendritic cells (DC) pulsed with tumour peptides to drive anti-tumour specific T cell responses, approved by the Food and Drug Administration (FDA) in 2010, was demonstrated to prolong survival of metastatic castration-resistant prostate cancer patients (Chang et al., 2023; Kantoff et al., 2010) The possible development of an alternative vaccination strategy, based on tumour antigen-presenting EVs of DC origin, was suggested by a milestone paper published at the end of last century which demonstrated that the EVs released by cancer peptide-pulsed DCs could prime cytotoxic T cells and induce the rejection of established tumours in mice (Zitvogel et al., 1998). EVs were hypothesized to have the advantage of expressing high levels of antigens, in combination with costimulatory molecules; as a matter of fact, DC-derived EVs were characterized at the molecular level and the procedures for their purification and storage optimized (Lamparski et al., 2002; Théry et al., 2001). Those preliminary studies led to a Phase 1 clinical trial, conducted from 2000 to 2002 in Villejuif, France, and published in 2005. Epitopes of the melanoma antigen gene (MAGE-3 peptides) were selected to pulse autologous DCs as tumour antigens, since they show high grade of specificity; then fifteen metastatic melanoma patients (bearing MAGE-3 positive tumours) received four vaccinations with DC-derived EVs (study n.1, Table 1) (Escudier et al., 2005). The study demonstrated the feasibility of large scale exosome production and safety of their inoculation in human subjects: indeed, as for the primary endpoint, EVs did not show toxicity in any of the treated patients. As for the secondary endpoints (specific anti-MAGE T cell responses and clinical outcome), only one objective response was recorded according to the RECIST (Response Evaluation Criteria in Solid Tumours) criteria and specific $CD4^+$ and $CD8^+$ T cells generated by the exosome vaccines were not detected in any of the patients (Escudier et al., 2005). Authors suggested that DC derived-exosomes may be specifically endowed with NK cell stimulatory capacity *in vivo*, although relative data were not reported in that original work nor subsequent studies have appeared in support of this hypothesis in metastatic patients.

4.2 | Non-small cell lung cancer

In the same year (2005), a parallel clinical trial, performed at the Duke University Medical Centre, used autologous DC-EVs as therapeutics to boost immunity toward non-small cell lung cancer (NSCLC) (study n.2, Table 1) (Morse et al., 2005). Similarly to the above study, after *in vitro* DC generation, exosomes released by those cells (named DEX) were isolated and loaded with several MAGE peptides. MAGE antigens, in fact, are expressed also by several other tumours beside melanoma, including NSCLC (Gotoh et al., 1998). Nine NSCLC patients completed a therapy of four DEX doses at weekly intervals. All the tested formulations were well tolerated with only mild adverse events, such as injection site reactions, arm pain and flu like illness, while delayed type hypersensitivity (DTH) against MAGE peptides was detected in one third of the patients. Despite the production of the DEX vaccine was confirmed to be feasible and DEX therapy was well tolerated in advanced lung cancer patients, long term stability of disease and activation of immune effectors related to DEX vaccination were detected in only few individuals (Morse et al., 2005).

In summary, the first two Phase 1 clinical trials aimed at vaccinating cancer patients with peptide-pulsed DEX have proven their safety but have failed to show relevant immunizing capacity. Starting from those discouraging reports, researchers have strived to develop second-generation DEX with enhanced immune-stimulatory properties. In this context, it was unveiled that the cytokine interferon-γ (IFN-γ) specifically and efficiently induces DCs to release DEX expressing several markers (e.g., CD80, CD86), with potent $CD8^+$ T-cell-triggering potential in vitro and *in vivo* (Viaud et al., 2011). In a Phase 2 clinical trial, the primary objective was hence to investigate whether that second generation manufactured vaccine (IFN-γ-DEX loaded with MHC class I- and class II-restricted cancer antigens) could improve the rate of progression-free survival (PFS): in details, the primary endpoint of the study was to observe at least 50% of patients with PFS at 4 months after chemotherapy cessation in unresectable NSCLC patients ($n = 22$). This Phase 2 trial confirmed the capacity of DEX to boost the NK cell arm of antitumor immunity in patients with advanced NSCLC; nonetheless, the primary endpoint was not reached (study n.3, Table 1) (Besse et al., 2016).

4.3 | Colorectal cancer

A Phase 1 clinical trial conducted in China and published in 2008 tested the usage of ascites-derived exosomes (denominated AEX) in combination with the granulocyte–macrophage colony-stimulating factor (GM-CSF) in the immunotherapy of 40 colorectal cancer patients (20 received only AEX, 20 AEX + GM-CSF); there was not a control group not receiving AEX. The therapy was safe and well tolerated, but AEX, bearing the carcinoembryonic antigen, were able to induce antitumor cytotoxic T lymphocyte (CTL) response only in combination with GM-CSF (study n.4, Table 1) Dai et al., 2008).

5 | HYPER-INFLAMMATORY CONDITIONS

5.1 | Graft versus host disease

In a study published 10 years ago, EVs released by MSCs were used as an experimental approach to treat the therapy-refractory graft versus host disease (G*v*HD) in one patient, upon her agreement and the approval by the Legal Department of the University Hospital Essen, in Germany (study n.5, Table 1) (Kordelas et al., 2014). To reduce the risk of unexpected reactions of the patient's immune response on MSC-EV administration, before the treatment, investigators decided to test the immunemodulatory impact of the EVs on the peripheral blood mononucleated cells (PBMCs) of the patient in a mixed lymphocyte reaction experiment: in the presence of the EVs, the effector activities of the PBMCs, that is, cytokine release, were actually decreased. The treatment dose was then decided based on the relative application regime of MSCs usually administered in G*v*HD patients; EVs obtained from the supernatant of 4×10^7 MSCs were defined as 1 unit per body weight of that patient. Soon after the start of exosome infusion, the intestinal, cutaneous and mucosal symptoms associated to G*v*HD significantly improved, allowing a reduction in steroid dosage. The patient eventually died of pneumonia several months after the treatment; still, authors of the study concluded that the MSC-EV therapy may turn out to be a safe strategy to treat subjects with therapy-refractory GvHD but also other hyper-inflammatory conditions (Kordelas et al., 2014). Nonetheless, no specific clinical trials have been published since that study to confirm that hypothesis.

Intriguingly, Meizhang Li et al., have reported an increase of small-sized EVs shuttling programmed death ligand-1 (PD-L1) in plasma samples from G*v*HD patients upon infusion with human umbilical cord-derived Wharton's jelly MSCs (clinical trial NCT03158896), compared with baseline (Li et al., 2021). Mechanistically, the interaction between PD-L1 and PD-1 is hypothesized to induce anergy, exhaustion or apoptosis to establish donor's T cell tolerance and prevent GvHD (Fujiwara et al., 2014). This study was not included in the present scoping review, since it reports the observational quantification of EVs in plasma samples of patients treated with MSCs themselves, not the therapeutic use of EVs; still, the paper describes a relevant EV-dependent phenomenon, which may contribute to the immune suppressive phenotype of infused MSCs, strengthening the therapeutic potential of MSC-EVs in G*v*HD (Li et al., 2021).

In a prospective clinical trial conducted at the State Key Laboratory of Ophthalmology, in Guangzhou, China, human umbilical cord MSC-EVs were used to treat refractory G*v*HD-associated dry eye disease, which causes intolerable pain and visual impairment (study n.6, Table 1) (Zhou et al., 2022). Topically administered as eye drops to 14 G*v*HD patients four times a day per eye for 2 weeks, MSC-EVs were able to reduce the inflammatory damage of the ocular surface and accelerate corneal epithelial recovery. Differently from artificial tears, which are unable to hamper inflammation and thus cannot prevent dry eye disease progression, MSC-EVs exerted their therapeutic effect by suppressing the inflammatory injuries and restoring the ocular homeostasis, without any relevant systemic complication, although their long-term efficacy and safety remain to be evaluated (Zhou et al., 2022).

5.2 | Coronavirus disease (COVID)-19

Infection with severe acute respiratory syndrome coronavirus (SARS-CoV)-2, the cause of worldwide COVID-19 outbreak, can be mild or asymptomatic, but also cause severe and critical pneumonia and acute respiratory distress syndrome (ARDS) (Chen et al., 2020; Guan et al., 2020; Huang et al., 2020). Interstitial pneumonia is frequently associated with massive release of cytokines ("cytokine storm"), now recognized as a major COVID-19 pathogenetic factor able to aggravate the ARDS and organ failure, leading to fatal outcome (Chen et al., 2020; Guan et al., 2020; Huang et al., 2020).

Ten studies (five in the USA, three in China, and two in Iran and Turkey), published in the years 2020–2023, have tested different types of EV formulations as novel interventions to suppress the uncontrolled and excessive immune response and protect COVID-19 patients from systemic organ damage. The first prospective nonrandomized study addressed the safety and efficacy of bone marrow MSC-EVs (name of the product: ExoFlo) as treatment for 24 subjects with COVID-19-related respiratory distress syndrome via intravenous injection. No adverse events were reported, and patients' oxygenation improved, together with

a discrete reduction of C-reactive protein and D-dimer (study n.7, Table 1) (Sengupta, Sengupta, Lazo, Woods, et al., 2020). After that, a prospective Phase 2 multicentre (five sites across the USA), double-blind, randomized, placebo-controlled study tested the safety and efficacy of ExoFlo in reducing mortality of patients with moderate-to-severe COVID-related ARDS. Again, no treatment-related adverse events were reported, ventilation-free days increased and mortality (at day 60) decreased upon treatment with ExoFlo as compared to placebo (study n.8, Table 1) (Lightner et al., 2023).

Other three studies made use of a recently developed derivative of the human amniotic fluid, named Zofin, which contains EVs released by the perinatal tissues (Del Rivero et al., 2022). In the first of these three American studies, Zofin (three to four doses in the order of 10^{11} particles) was compassionately administered to three critically ill patients suffering from severe, multi-organ complications, upon more than 40 days of hospitalization and in concomitance with ongoing authorized standard of care available at that time (supplemental oxygen, anti-inflammatories, antibiotics, antiviral medication, and other medications required to manage their multiorgan failure symptoms). The patients showed no adverse events associated with the therapy and experienced respiratory and general clinical status improvements, including acute delirium resolution and decrease of inflammatory biomarkers (study n.9, Table 1) (Mitrani et al., 2021a). Since the compassionate use of Zofin in critical COVID-19 conditions resulted safe during that first study, the preparation was then tested on high risk patients ($n = 8$) with mild-to-moderate infection as a proof-of-concept, expanded access trial. Consistently, patients experienced no serious adverse events; moreover, all COVID-19-associated symptoms resolved or became stable as indicated by chest x-ray reports, inflammatory biomarkers quantification and normalization of lymphocyte count throughout the study period (study n.10, Table 1) (Bellio et al., 2021). COVID-19 requires medical care not only in the acute infection phase, but also afterwards, since mental fog, tachycardia, and extreme fatigue have been described post-COVID-19 infection, decreasing the quality of life of long-term symptomatic individuals ("long haulers"). In the third study regarding the therapeutic use of Zofin, the preparation was used in a single long hauler patient case experiencing prolonged shortness of breath and respiratory impairment; keeping in mind the limited information that can be gained from a single case report, the treatment was followed by respiratory improvements as testified by chest X ray images and oxygen saturation measurement (study n.11, Table 1) (Mitrani et al., 2021b). It is important to highlight here that Zofin is an acellular therapeutic manufactured to retain over 300 naturally occurring growth factors, cytokines, and chemokines as well as more than 100 unique microRNAs, in addition to EVs (Del Rivero et al., 2022). Hence, the described anti-inflammatory properties of Zofin in COVID-19 patients cannot be univocally reconducted to the sole action of the EV component.

While both ExoFlo and Zofin were administered intravenously, one study explored the safety of nebulized human adipose-derived MSC-EVs (quantitative range: $2–16\times10^8$ particles) in healthy volunteers ($n = 24$). The good tolerability of MSC-EV nebulization suggested this route of administration as a promising therapeutic strategy for diseases affecting the lungs, such as COVID-19 (study n.12, Table 1) (Shi et al., 2021). Indeed, one subsequent study reported the results of a preliminary Phase 2a single-arm, open-labelled, interventional trial aimed at testing the safety and efficiency of aerosol inhalation of human adipose-derived MSC-EVs (up to a total amount of 2×10^8 EVs for five consecutive days) in seven patients with severe COVID-19 related pneumonia enrolled at Jinyintan Hospital, Wuhan, China, in March 2020. While a slight increase of peripheral blood circulating lymphocytes was reported, no adverse events were indeed recorded, as well as different degrees of resolution of pulmonary lesions after aerosol inhalation (study n.13, Table 1) (Zhu et al., 2022). Another pilot trial tested the safety and efficacy outcomes of a nebulization therapy with umbilical cord-derived MSC-EVs on seven patients with COVID-19 pneumonia. The treatment did not induce allergic reactions while it did promote the absorption of pulmonary lesions and reduced the duration of hospitalization in case of mild pneumonia (study n.14, Table 1) (Chu et al., 2022). In a parallel randomized controlled, multicentric, placebo-controlled study performed in Iran, the treatment with MSCs followed by their released EVs showed the capability to reduce the circulating levels of inflammatory markers in COVID-19 patients with ARDS, and possibly decrease respiratory failure and death in those subjects (study n.15, Table 1) (Zarrabi et al., 2023).

Finally, a single-centre prospective, single arm interventional study has tested the safety and efficacy of ChipEXO, a product derived from convalescent plasma of COVID-19 patients, in patients with severe COVID-19 pneumonia at risk of respiratory failure via jet nebulization, as an add-on to ongoing conventional COVID-19 treatment. During the 5 days of treatment, all oxygenation parameters improved and inflammatory markers decreased, with 11 out of 13 treated subjects recovering without any sequelae to lungs or other organs (study n.16, Table 1) (Gul et al., 2022).

5.3 | Neurologic diseases

Alzheimer's disease (AD) is among those human pathologies for which there are no effective treatments able to slow or reverse disease progression (EclinicalMedicine, 2021). In preclinical animal models of AD, the capability of MSC-EVs to reach the brain upon either intracerebroventricular or systemic injections has been connected with their effect of cognitive impairment amelioration (Cui et al., 2018; Wang et al., 2018). Based on these reports, a Phase 1/2 clinical trial was conducted to explore the safety and efficacy of allogenic human adipose tissue-derived MSCs-EVs intranasally administrated to patients with mild to moderate AD, divided into three dosage groups (see Table 1 for details). While no effect was reported concerning amyloid or tau deposi-

tion among the three groups, the medium-dose arm showed lower hippocampal volume shrinking, and decreased disease scores associated with cognitive scales, warranting for further larger clinical trials (study n.17, Table 1) (Xie et al., 2023).

Another neurological condition with highly debilitating outcomes is the paralysis of the facial nerve, with Bell's palsy being a common mononeuropathy with persistent symptomatology. After a first case study suggesting the opportunity to treat Bell's palsy with adipose-derived stem cells (Ahn et al., 2023), one pilot study sought to determine whether a 4-week treatment with MSC-EVs (injected into the area of the main trunk of the facial nerve on the affected side) was able to restore facial nerve function in seven patients with idiopathic facial paralysis, unable to return to normal upon diversified therapeutic approaches. No adverse effects were observed in the study and authors report a progression of independent motion of the affected eyelid, brow motion, and commissure for all the treated patients (study n.18, Table 1) (Dreschnack & Belshaku, 2023).

5.4 | Ear and eye pathologies

A randomized controlled clinical trial was started between March and September 2019 at the University Medical Centre of Ljubljana, Slovenia, with the aim of evaluating the efficacy of an autologous blood-derived product called PVRP (for platelet- and extracellular vesicle-rich plasma, known to attain favourable immune, haemostatic and regenerative effects), for the treatment of chronically inflamed post-surgical temporal bone cavities (study n.19, Table 1) (Vozel et al., 2021). In details, a radical cavity is produced by the removal of the posterior external ear canal wall during a cholesteatoma surgery and leads to inflammation, tissue granulation and multiple suppurative periods in some of the cases, significantly worsening the patients' quality of life (Pareschi et al., 2019). In the study, PVRP was administered to chronically inflamed radical cavities via PVRP-soaked ear wicks, and resulted, compared to standard therapy, in higher percentage of patients reaching symptom-free conditions at 12 weeks post treatment (Vozel et al., 2021). Another study described the first intracochlear application of EVs (released from human umbilical cord-derived MSCs) in a 55-year-old patient (on a 'named patient basis'), suffering from hearing loss caused by Menière's disease. The use of MSC-EVs as a novel adjuvant therapeutic approach prior to the insertion of a cochlear implant is intended to curb the inflammatory reaction usually occurring upon implantation and the promising results of the study have certainly paved the way to subsequent controlled clinical trials (study n.20, Table 1) (Warnecke et al., 2021).

Idiopathic macular hole (MH) is one of the most common causes of visual impairment, particularly in individuals with myopia; while small recent-onset MH measuring (≤400 μm) is successfully managed by pars plana vitrectomy, larger and long-standing MHs are less likely to close with the standard procedure (Ezra et al., 2004). In one pilot study performed at the Eye Institute & School of Optometry and Ophthalmology in Tianjin, China, either human umbilical cord-derived MSCs ($n = 2$ patients) or MSC-EVs ($n = 5$ patients) were tested to assess their efficacy in promoting the healing of large and refractory MHs upon intravitreal injection (study n.21, Table 1) (Zhang et al., 2018). In preclinical studies, authors had found that MSC-EVs exerted equal therapeutic effects of MSCs in protecting the retina from the injury, either laser-induced or experimental autoimmune uveitis-dependent (Bai et al., 2017; Yu et al., 2016). Furthermore, they had fluorescently tracked injected EVs and observed quick infiltration of the mouse retina, advocating against the possibility of EVs drifting away after local injection (Yu et al., 2016). Since the first patient of the trial, who received 50 μg of MSC-EVs, experienced moderate inflammation in the anterior chamber of the eye, which necessitated the application of steroid eyedrops, the MSC-EV dose was reduced to 20 μg thereafter, with no remarkable inflammation in any of the treated patients (Zhang et al., 2018). Followed up to 3 years after treatment, they neither showed long-term side effects of MSC-EVs, nor evidence of overt inflammation nor teratoma development. In terms of efficacy, authors found that four of the five MHs closed successfully with MSC-EVs therapy, with three patients achieving a satisfactory improvement in visual acuity (Zhang et al., 2018). The absence of a control group does not allow to draw solid conclusions; moreover, specific toxicity studies are warranted in larger trials with MSC-EVs.

5.5 | Fistulas, ulcers and delayed wound healing

Crohn's disease (CD), a chronic immune-mediated inflammatory disorder affecting the gastrointestinal tract, may present intestinal stricture and fistula complications. In subjects with severe colon and rectum inflammation, perianal fistulas are the most prevalent and affect up to 40% of Crohn's patients (Schwartz et al., 2019). At the University of Medical Sciences, in Tehran, Iran, a Phase 1 clinical trial has aimed to assess the safety of administering EVs derived from human umbilical cord MSCs to patients with complex perianal fistulas associated with CD ($n = 5$). Patients did not experience any short- or long-term adverse events and neither leucocytosis nor abnormal liver function were reported (study n.22, Table 1) (Nazari et al., 2022). Regarding efficacy, at the 6-month follow-up, four patients (80%) had responded to treatment. Skin inflammation was resolved in all patients, thus alleviating burning sensation; nonetheless, the treatment was inefficacious in the presence of fibrotic tissue, suggesting that EVs are unable to penetrate it and hence unsuitable for chronic conditions characterized by significant fibrosis (Nazari et al., 2022). In a similar study from the same research group, EVs derived from placental MSCs were injected in 11 patients presenting with complex perianal fistulas not associated to CD. The success rate (meaning significant improvement or complete resolution) was

high (10/11 patients) and none of the subjects showed any acute or latent allergic reaction or injection related complications, demonstrating safety and satisfactory therapeutic effect of those EVs for the specific condition (study n.23, Table 1) (Pak et al., 2023). Notably, EV success rate in these Iranian studies was significantly higher compared to that reported in a Spanish study which evaluated the compassionate injection of either stromal vascular fraction, autologous expanded adipose-derived, or allogenic adipose-derived stem cells for complex perianal fistula, in which healing was reported in 24/45 (46.2%) patients (Herreros et al., 2019).

At the Department of Surgery, Louisiana State University Health Sciences Centre in New Orleans, the first use of MSC-EVs in the reconstruction of pressure ischial ulcer was reported in a patient who presented a persistent wound despite several months of appropriate care and antibiotic therapy. The treatment resulted to be successful, since, after six subcutaneous EV injections over 8 weeks, the ulcer was completely healed, calling for additional studies of this promising therapeutic approach (study n.24, Table 1) (Messa et al., 2022).

In Melbourne, Australia, a specific chromatographic approach (Ligand-based Exosome Affinity Purification, LEAP) was tested for the isolation of activated platelet-derived EVs transporting biological factors with regenerative properties, such as insulin growth factor (IGF) and transforming growth factor beta (TGF-ß) (Johnson et al., 2023). In a double-blind, placebo-controlled, Phase 1 clinical trial, the authors of the study have assessed the safety of those EVs in treating wounds in healthy volunteer adults, demonstrating safety and tolerability of injected LEAP-purified EVs. Since the process of wound healing was normal in those subjects, the EV treatment did not modify the recovery time and further studies will have to assess therapeutic efficacy in patients with delayed or disrupted wound healing (study n.25, Table 1) (Johnson et al., 2023). On the other hand, a pilot case-control study performed in Turin, Italy, investigated the potential of autologous serum-derived EVs (enriched in TGF-β1) in improving the healing process in patients with chronic venous ulcers unresponsive to conventional treatments. EV-treated lesions (three times a week, for 2 weeks) showed increased granulation tissue compared to untreated ones; more evident sloughy tissue reduction; larger microvascular proliferation areas in the regenerated tissue (study n.26, Table 1) (Gibello et al., 2023).

5.6 | Other diversified chronic conditions

In 2016, a publication reported the results of treating twenty chronic kidney disease patients with umbilical cord blood-derived MSC-EVs: compared with a placebo treated group, those individuals exhibited significant improvement of kidney function, as shown by several kidney-related parameters, such as glomerular filtration rate, blood creatinine and urea levels, and urine albumin-creatinine ratio. Moreover, EV-treated patients exhibited significant increase in plasma TGF-β1, and IL-10 and significant decrease of TNF-α, suggesting amelioration of the overall inflammatory state (study n.27, Table 1) (Nassar et al., 2016).

Osteoarthritis (OA) is the most common form of chronic arthritis, in which structural changes of hyaline articular cartilage, subchondral bone, ligaments, capsule, synovium, muscles, and periarticular changes occur. OA mostly occurs at the level of hands, hips, and knees; it can cause pain and stiffness, and reduce function, sensibly decreasing the quality of life (Glyn-Jones et al., 2015). One trial, selected in the present scoping review, will eventually evaluate the treatment of severe OA patients by using novel source of biologics, that is, the cell-free extract from human progenitor endothelial stem cells (study n.28, Table 1) (Gupta et al., 2021). The biologically relevant elements of regenerative medicine, that is, growth factors, cytokines, hyaluronic acid, and EVs have been previously found in large quantities by the same authors in that cell-free extract (Chan et al., 2013).

Another report investigated the treatment efficacy of placental MSC-EVs (eight doses) in a 55-year-old woman with a 10 years history of severe systemic sclerosis, complicated by interstitial lung disease. The patient showed significant improvement in her clinical symptoms starting a month after the first dose: reduced fibrosis, dyspnoea, cough and supplemental oxygen need, calling for further investigation and clinical trials for a condition with no current approved or golden standard treatment (study n.29, Table 1) (Assar et al., 2023).

Finally, ExoFlo (the same product tested in COVID-19 patients, see above), was also tested on 10 subjects with complex regional pain syndrome, an extremely painful inflammation-driven disorder. Besides reported as safe, the product resulted in a significant clinical improvement (including scale of pain, questionnaires, and dynamometer testing) across the patient group, notwithstanding the study limitation of a control lack (study n.30, Table 1) (Paicius et al., 2023).

5.7 | Skin diseases

Four of the evaluated publications describe the topical application of EVs for the treatment of dermatologic conditions. The first study, conducted in China, enrolled 22 female subjects with sensitive skin to be treated with EVs released by primary human MSCs (study n.31, Table 1) (Ye et al., 2022). Skin sensitivity, including atopic dermatitis and rosacea, is a condition of subjective cutaneous hyper-reactivity to environmental factors such as cold, heat, and wind (Berardesca et al., 2013); its mean prevalence among Chinese women reaches 15%, detrimentally influencing life quality of millions of individuals (Xu et al., 2013). MSC-

EVs were reported to improve scores of objective symptoms (e.g., roughness and erythema), and subjective symptoms, such as tension, burning, or itching, upon treatment (Ye et al., 2022). Other two of those studies were performed in Korea and aimed at treating individuals with facial redness subsequent to a specific biological treatment for atopic dermatitis (Dupilumab). A total of twenty-two patients with atopic dermatitis and refractory Dupilumab-related facial redness were successfully treated with electroporation-assisted topical application of human adipose tissue-derived MSC-EVs, leading to marked improvement in erythematous facial lesions (studies n.32 and 33, Table 1) (Park et al., 2022; Han et al., 2023). The last study was also conducted in Korea and reported the results of a 12-week prospective, double-blind, randomized, "split-face" trial, which evaluated the safety and clinical efficacy of adipose tissue stem cell-derived EVs as an adjuvant therapy for atrophic acne scars. In particular, 25 patients received fractional CO_2 laser to the whole face; post-laser treatment, for each patient, one side of the face was treated with adipose tissue stem cell-derived EV gel and the other side was treated with a control gel. Final follow-up evaluation revealed that EV application achieved a significant improvement compared with the control sides, characterized by a milder treatment-related erythema and shorter post-treatment downtime (study n.34, Table 1) (Kwon et al., 2020).

5.8 | Aesthetic medicine

Six of the included studies for the present scoping review concerned the use of EVs for aesthetic reasons. One study has examined the potential of EVs from seaweeds (i.e., *Codium fragile* and *Sargassum fusiforme*) as cosmetic agents. Beside an *in vitro* analysis of melanin synthesis using a three-dimensional model of human epidermis, the application of a prototype cream containing *Codium fragile*-derived EVs was tested in a skin whitening efficacy test (study n.35, Table 1) (Jang et al., 2021). In particular, 21 healthy women were enrolled in the study at the Korea Institute of Dermatological Sciences and the product was applied to half of the participant's face (a placebo cream was applied on the other half) once a day for 4 weeks. By a spectrophotometer evaluation, the EV cream was reported to enhance skin brightness (Jang et al., 2021).

Other three studies regarded skin rejuvenation. In one of those, carried out in Indiana, U.S.A., topical absorption of placental MSC-EVs was tested as a skin bio-stimulation to reduce wrinkles, pores, oiliness and unwanted pigment in 40 enrolled individuals. Either alone, or mixed with ancillary ingredients (such as botulinum toxin and hyaluronic acid dermal filler), EVs were reported to improve the tone and texture of the skin (study n.36, Table 1) (Chernoff, 2023). In the second study, an allogeneic human EV product derived from leukocyte-reduced apheresed platelets was tested in a prospective, single-arm, non-randomized, longitudinal study and reported to not only be well-tolerated, and well-liked by participants but to also objectively reduce redness and wrinkles, while augmenting luminosity and skin colour evenness (study n.37, Table 1) (Proffer et al., 2022). In the third of those studies, adipose tissue-derived MSC-EVs were evaluated in the treatment of facial skin aging in a 12-week, prospective, randomized, split-face, comparative study, which showed EV capability to reduce wrinkles and augment skin elasticity, hydration, and pigmentation (study n.38, Table 1) (Park et al., 2023).

The last two studies regarded hair loss. The first was a preliminary retrospective open-label one, testing the intradermal injection of a single treatment with human bone marrow-derived MSC-EVs in the management of hair loss in 22 female and nine male subjects at early stages of alopecia or in remission from previous treatments. The author reported no adverse reactions, and clinical efficacy was suggested by growth responses over the next 6 months from the treatment (study n.39, Table 1) (Sasaki, 2022). In the second study, the subcutaneous scalp injection of placental MSC-EVs (every 4 weeks for three continuous months) in a 36-year-old woman with persistent chemotherapy-induced alopecia induced complete regrowth of terminal hair (study n.40, Table 1) (Norooznezhad et al., 2023).

5.9 | Unpublished studies registered in *ClinicalTrials.gov*

We also screened *ClinicalTrials.gov* to search for registered studies using the same criteria as above. Among the resulting 478 outputs, 68 studies effectively were planned to treat a condition or a disease through the administration of EVs or to assess their safety in humans, while the remaining entries were related to the use of EVs for diagnostic or other purposes. Of those 68 entries, 10 were already published studies and are part of the 40 studies summarized in the previous paragraphs. Among the remaining 58 entries (summarized in Table S2), 15 studies had COVID-19 or long-COVID-19 as the condition to be targeted, while 14 were related to other infectious diseases or immunological conditions, including respiratory distress of multiple aetiologies. Four studies intended to treat either wounds or injuries, five were designed to test the effect of EVs against cardiovascular diseases (CVDs), three against liver cirrhosis or failure, three against oncological conditions, four for age-related diseases or skin aging, and 10 targeted other, peculiar diseases or explored safety in healthy volunteers. Most studies were focused on diseases with few therapeutic options. For instance, among those targeting CVDs, one study aims to explore the efficacy of allogenic MSC-EVs in patients with acute ischemic stroke while another will evaluate the effect of a human-derived EV formulation in patients subjected to coronary stent implantation, two acute conditions that can be treated only through fibrinolytic therapy or endovascular procedures

to minimize ischemia-dependent damage. Similarly, one study aims at targeting heart failure with reduced ejection fraction, a disease with increasing therapeutic options but still characterized by unmet needs (Ceriello et al., 2021).

Among the 58 studies, 29 were active and/or recruiting, six were completed or terminated, and the remaining were all inactive (not recruiting, suspended, withdrawn, or with unknown status). Among those completed or terminated early, only three posted results. One of them (NCT03857841-terminated) only enrolled two patients before the study was stopped by the sponsor due to a business decision. Another study evaluated the safety and efficacy of aerosol inhalation of MSC-EVs (NCT04491240) and enrolled 30 subjects with COVID-19 (20 of them treated with inhaled EVs, 10 with placebo). No adverse event (neither severe nor moderate) in the 30 days following discharge was observed, while efficacy measures were unchanged. These data were not linked to a publication. Another study (NCT04313647) tested essentially the same approach (safety of inhaled MSC-EVs) in healthy volunteers ($n = 24$ with increasing concentration of exosomes), reporting only two non-serious adverse events up to 7 days after nebulization. These preliminary results were published as a part of a larger mechanistic paper in animal models (Shi et al., 2021), while the same authors then performed a pilot efficacy study using the same preparation and design, and the results were presented in one of the papers commented in the previous sections (Zhu et al., 2022).

Overall, most studies registered in *ClinicalTrials.gov* and testing the efficacy or safety of EV-based preparations in humans are still underway or are inactive. Among those concluded or terminated and posting unpublished results, two out of three were developed to test non-intravenous administration (inhalation studies), while the remaining one only enrolled two subjects. Much of the interest was in targeting COVID-19, which reflects the rush - widely characterizing the research in the last years - to find efficacious therapies for this condition. Reasons for the lack of study completion for those currently inactive are unclear in most of the cases.

6 | DISCUSSION

Although still in the early stages, pilot studies testing the safety and efficacy of EV-based therapies are providing preliminary insights into the translation of EVs from preclinical models to human applications, also highlighting the hardest challenges ahead of us.

The characteristics and properties emerged from preclinical studies and attributed to EV-based therapeutics are reflected in those first human studies. For instance, MSC-EVs are held to display anti-inflammatory, immunomodulatory, and tissue-regenerative effects, allowing their application in a wide range of diseases and conditions. As a result of this background knowledge, almost two-thirds of the collected studies tested the effect of MSC-EVs in a variety of conditions, all with a known immunological/inflammatory component, or in the field of regenerative medicine. However, most of those studies were small pilot trials with no placebo control, impeding any conclusion relative to the efficacy of the tested approach. In addition, given that most of the studies aimed at targeting diseases with a predominantly local damage (e.g., ulcers and fistulas), the route of administration was mostly a local application. Only nine studies (including five studies regarding the treatment of COVID-19 subjects) tested the intravenous infusion of EVs (of which two administered EVs also by local route), hampering any firm interpretation relative to both the safety and the specificity of EV formulations. This latter aspect is somehow surprising since targeted delivery was a putative strength of EVs in preclinical studies. Indeed, EVs can shuttle molecules which augment specific tissue targeting, thus enhancing the therapeutic efficacy while minimizing off-target effects. However, none of the studies here selected have shown results with modified EVs and all the published results concern non-engineered EVs. Thus, whether EVs can be specifically delivered to one tissue/organ in humans cannot be established with current knowledge.

Basic research into EVs has long been held back by technological hurdles. Despite the substantial advancements in separating procedures and characterization techniques, investigators must remain cautious when attributing specific functions to EVs, especially since recent data demonstrate the presence of extracellular non-EV particles in the exosome size range. The promiscuity of the EV isolate has not been resolved before going into the clinics, especially considering those studies in which the presence of many other components (cytokines, hormones, growth factors) is recognized from the beginning. As a relevant example, "Zofin" composition, derived from perinatal tissue paracrine factors, contains two fractions believed to contribute to its therapeutic mechanism of action: i) EVs and ii) soluble proteins/extracellular matrix components (Bellio et al., 2021; Mitrani et al., 2021a, 2021b). In principle, this aspect does not represent an obstacle to clinical translation since a specific preparation must demonstrate safety and efficacy independently of its components. On the other hand, it limits knowledge about EVs and hampers a wide diffusion of this approach, possibly limiting also the independent reproduction of the results. Indeed, establishing the exact mediator of the effect is essential to allow independent researchers to reproduce the approach and validate the findings. Alternatively, specific formulations, especially those patented, might represent unique products with a restricted access, following the existing marketing rule for common drugs.

In general, the available results do not help to clarify the issues identified in preclinical studies and raised in recent perspectives regarding EV-based therapies (Lee et al., 2023; Shekari et al., 2023; Thakur, 2023). In particular, meta-analyses on the efficacy of MSC-EV therapy in animal models of disease found that dose-response and biodistribution studies were infrequently conducted, although they are essential to design optimal EV dosing in sight of clinical translation (Tieu et al., 2021, 2020). Moreover, pre-

clinical studies have not yet succeeded in implementing standardized guidelines on how EV dose should be actually measured (i.e., protein content, particle numbers, RNA quantity?) and few of those studies strictly analysed whether the overexpression of one bioactive molecule is more effective than others (Roefs et al., 2020; Yang et al., 2019). A multidisciplinary workshop, recently convened by the National Heart, Lung and Blood Institute, helped identifying (i) limited knowledge on EV heterogeneity, (ii) poorly understood pharmacokinetics or pharmacodynamics *in vivo* and (iii) low batch to batch reproducibility among the most critical knowledge gaps in the field and recognized the quantitative/qualitative analysis of EV-based therapeutics and the development of novel strategies for scaling-up EV production as potential solutions for advancing translation of therapeutic EVs into the clinic (Li et al., 2023). Lastly, it has been recognized how preclinical EV research is still too vague in identifying and reporting the potential risk of bias in study design (Tieu et al., 2021, 2020). A systematic review and meta-analysis of animal studies on EV efficacy in wound healing and skin regeneration unveiled how risk of bias was uncertain for most studies due to insufficient reporting, to the point that reproducibility and comprehensive evaluation of evidence is still hampered by a general lack of transparency (Al-Masawa et al., 2022). Nonetheless, animal studies remain irreplaceable for dissecting exogenous EV biodistribution *in vivo*, as systematically reviewed by Kang et al. (2021); in particular, one robust and sensitive assay allows to track the uptake of EV-associated RNA cargo into specific tissues upon intravenous administration and may become instrumental for the precise design of human therapeutics (Ciullo et al., 2022). On the other hand, we must remain cautious since the methodologies of EV tracking for imaging purposes was demonstrated to actually alter and potentially compromise EV pathophysiological distribution (Lázaro-Ibáñez et al., 2021). The field of EVs as therapeutics should provide and follow guidelines similarly to the process of synthetic liposome development, starting from the promotion of animal study standardization (Herrmann et al., 2021).

In conclusion, further knowledge is necessary to address the following aspects:

1. Identification of optimal cellular sources for specific disease conditions, including non-human sources.
2. Optimization of methods for isolating and characterizing EVs.
3. Standardization of large-scale production of EVs in compliance with Current Good Manufacturing Practice regulations and consideration of regulatory factors.
4. Development of efficient procedures for storing EVs while maintaining optimal quality, as using fresh preparations is impractical. Of note, the freezing procedure has been recently demonstrated to dramatically impact EV integrity, purity and molecular phenotype (Gelibter et al., 2022).
5. Establishment of dosing regimens (the appropriate quantity but also the number of necessary repeated administrations) to enhance therapeutic effectiveness while avoiding undesired toxicity and serious side effects.
6. Identification of the most efficient route of administration to minimize EV doses and prevent adverse systemic side effects.
7. Understanding the biodistribution and clearance of EVs *in vivo*.
8. Overcoming limitations caused by the absence of placebo controls and the use of open-label administration in EV therapy, which both make it challenging to establish robust efficacy proof. Additionally, differentiating the efficacy derived solely from EVs when combined with ongoing care is problematic.
9. Careful evaluation of the long-term risks associated with EV treatment. Patients receiving EV therapies should undergo thorough monitoring for several years to detect any unwanted immunological events and/or tumour formation.

Above all, if the community aims at learning and advancing from published work, the full disclosure of isolation methods and/or biological characteristics of the EV-related product tested in clinical studies is not only helpful, it is the only acceptable option. In a commentary paper, Lim *et al.* solicited the authors of one of the studies described here (Sengupta, Sengupta, Lazo, Woods, et al., 2020) to provide the lacking details of the utilized product ExoFlo, such as its precise biological nature, the compliance to good manufacturing practices, the actual dose (Lim et al., 2020). Independently of the content of the clarifying manuscript which was subsequently published by the authors of the ExoFlo study in response to Lim *et al.* solicitation (Sengupta, Sengupta, Lazo, Hicok, et al., 2020), this back and forth discussion has clearly proven the urgency felt by researchers in the field to access the necessary information in order to assess the rational and medical value of the EV clinical use.

The research field of EVs as potential therapeutics has made substantial advancements over the past two decennia. However, it is important to consider the findings of the published studies, systematically reviewed here, as exploratory rather than conclusive. Indeed, the studies collected and analysed are all small pilot studies with a large heterogeneity in terms of EV source, administration route and target diseases. Furthermore, several of those studies demonstrate insufficient detailing of the study design and poor characterization and/or description of the preparation. While there have been some promising outcomes, multiple challenges must be tackled to ensure the successful translation of EV-based therapies. While at this stage safety and efficacy of EV formulations cannot be firmly established for none of the treated human conditions, ongoing and future studies, if well-designed, will help in understanding whether EVs represent a possible approach to treat a range of diseases with no or few therapeutic options.

AUTHOR CONTRIBUTIONS

Paola de Candia conceived the idea and Francesco Prattichizzo prepared the draft of the protocol. Paola de Candia and Francesco Prattichizzo contributed to study design and data interpretation and wrote the manuscript. Clorinda Fusco, Giusy De Rosa and Ilaria Spatocco performed data collection and selection, and participated in manuscript preparation. Elisabetta Vitiello and Ilaria Spatocco checked the accuracy of the collected information and prepared the figures. Rosalba La Grotta, Chiara Frigè, and Valeria Pellegrini assessed the quality and risk of bias of included studies and helped with the preparation of the tables. Claudio Procaccini, Giuseppe Matarese, and Roberto Furlan reviewed the manuscript for intellectual and methodological content. All authors approved the final version and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data extracted from included studies, and all relevant information can be found in the paper.

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SUPPORTING INFORMATION

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