

RESEARCH ARTICLE

A systematic review and meta-analysis of clinical trials assessing safety and efficacy of human extracellular vesicle-based therapy

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Abstract

Nowadays, it has become clear that extracellular vesicles (EVs) are not a cellular waste disposal vesicle but are an essential part of an intercellular communication system. Besides the use of EVs in biomarker studies and diagnostics, the potential of EV-therapeutics has been seen by many. They provide unique properties for disease therapy, including strong immune-modulatory actions, the possibility of engineering, low immunogenicity, and the capability of crossing biological barriers. Proof-of-concept of EV-therapeutics for various pathologies has been achieved in preclinical studies. However, clinical trials with EVs have only been emerging slowly. Here, we aim to provide a comprehensive overview of the current state-of-the-art concerning clinical studies using EVs in human therapy. By approaching the current knowledge in a systematic manner, we were able to include 21 reports for meta-analysis of safety and evaluation of efficacy outcomes. Overall, we have shown that EV-based therapy is safe with a low incidence of serious adverse events (SAE; 0.7% (95%-CI: 0.1–5.2%), and adverse events (AE; 4.4% (95%-CI: 0.7–22.2%). Subgroup analysis showed no significant difference in SAE when comparing autologous versus allogeneic administration, as well as engineered versus non-engineered EV products. A significantly higher number of AE was seen in autologous versus allogeneic administration. However, the clinical relevance remains questionable. Evaluation of the clinical outcomes of immunostimulatory, immunosuppressive or regenerative EV-therapies indicated improvement in the majority of treated patients. Despite these promising results, data need to be approached with caution due to a high heterogeneity in the EVs manufacturing methods, study design, and reporting of (S)AE. Overall, we conclude that EV-based therapy is safe and presents a promising opportunity in therapy. More efforts are needed in the standardization and harmonization of reporting of EV isolation and characterization data as well as in the reporting of (S)AE to allow inter-study comparison.

KEYWORDS

clinical studies, exosome, extracellular vesicles, meta-analysis, safety, systematic review, therapy

Mats Van Delen and Judith Derdelinckx contributed equally to this work.

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1 | INTRODUCTION

Extracellular vesicles (EVs) are small membranous structures that are released by almost every cell. EVs consist of a lipid bilayer and carry specific cargo, containing a broad range of bioactive molecules such as nucleic acids, proteins, lipids and metabolites either on the surface or incorporated inside the EVs (Ginini et al., 2022). Historically, the release of EVs was considered solely as a waste-disposal function. However, more recent research shed a new light on the function of EVs being part of a larger intercellular information transfer system (Couch et al., 2021). EV uptake into recipient cells can affect the physiological features of these cells and may alter their phenotypic and functional properties by means of their parental cargo, without the immunogenicity of the parental cell. In addition, EVs can cross biological barriers, such as the blood-brain-barrier (BBB), providing an advantage in reaching the target organ, for instance the central nervous system (CNS) (Simeone et al., 2020). Moreover, EVs can be engineered in various ways to modulate the enclosed cargo or to be used as a drug delivery system (Esmaili et al., 2022; Lennaárd et al., 2021; Zhu et al., 2017). In analogy with cell therapy, engineering of the cell-derived EVs is often used to add or increase the presence of a certain biomolecule in order to enhance its therapeutic potential (Esmaili et al., 2022). Indeed, several non-viral and viral methods have been used to alter the naturally processed cargo of EVs (Lennaárd et al., 2021; Tao et al., 2017). Altogether, these unique features of EVs provide them with a potential therapeutic capacity.

Many preclinical studies have investigated the use of EVs derived from a plethora of parental cell types as therapeutic agent in various disease models (Castilla et al., 2021; Tapparo et al., 2019; Yoo et al., 2022). In a recent systematic review of preclinical studies using EV-based therapeutics, the cellular origin of EVs was mostly either cancer cell-, stem cell- or HEK293 cell-derived (Castilla et al., 2021). Other cellular sources have been used and evaluated in preclinical studies, including dendritic cells (Chen et al., 2018), macrophages (Gong et al., 2019), neuronal cultures (de Rivero Vaccari et al., 2016) and T cells (Fu et al., 2019), but it is clear that the cargo of the EVs and their effect on the recipient cell is largely dependent on the parental cell (Yáñez-Mó et al., 2015). For instance, EVs derived from mesenchymal stromal cells (MSC), a multipotent cell type with immunomodulatory properties, have been shown to prevent and revert lung fibrosis (Mansouri et al., 2019), to ameliorate inflammatory bowel disease (Mao et al., 2017) and to contribute to myocardial repair (Deng et al., 2019), as has also been shown for the parental cells themselves (Eiro et al., 2022; Guo et al., 2020; Moroncini et al., 2018). Hence, depending on their cell of origin, EVs can hold immunomodulatory or regenerative properties (Lemaire et al., 2020). Moreover, these studies indicate that EV-based therapy—from xenogeneic, allogeneic, or autologous origin (Dong et al., 2020; Samuel & Gabrielsson, 2021)—has an enormous potential as therapeutic approach, instigating the start of human clinical studies. Nonetheless, despite promising results in preclinical trials, much remains to be understood about the safety and efficacy of EV-based therapy, and the influence of EV engineering and graft origin on the mode-of-action of EVs.

In this systematic review, we compiled and assessed all current evidence of safety and efficacy of EV-based therapy. Specifically, we included all human studies and clinical trials employing cell-derived EV therapeutics for the treatment of various conditions and diseases. Notably, the current guidelines endorse the use of the term EVs in the absence of subtype-specific markers. This term is generally used throughout the paper, however, when reviewing results of a specific clinical study, the term chosen by the authors (whether it be EVs, exosome, etc.) to refer to the particle was adopted. The incidence of adverse and serious adverse events was statistically evaluated, and the difference in safety profiles between allogeneic and autologous administration in humans, as well as between engineered and non-engineered therapeutic EVs was examined. In addition, we reviewed the clinical outcomes of immunostimulatory, immunosuppressive and regenerative EV therapeutics. Thereby, we aimed to provide a comprehensive overview of the current state-of-the-art concerning the safety and efficacy of EV-based therapy in humans, and to discuss potential implications for further research and clinical use.

2 | METHODS

2.1 | Literature search, selection process and data extraction

Two authors (MvD and JD) systematically and independently searched the following sources in January 2024, including publications until the end of 2023, in a systematic way: (i) Databases of medical literature (MEDLINE via PubMed, Web of Science, SCOPUS, EMBASE) for relevant peer-reviewed articles; and (ii) Websites of clinical trial registrations, including ClinicalTrials.gov and Cochrane Central Register of Controlled Trials (CENTRAL). Details of the search strategy can be found in the Supplemental information (Table S1). For the PubMed search, a filter on clinical studies or clinical trials was applied.

Interventional clinical trials reporting on cell-derived EV treatment were included, irrespective of the clinical trial phase, randomization (randomized, pseudo-randomized and non-randomized) or blinding strategy (single-blinded, double-blinded or unblinded). No restrictions were set on age, ethnicity, or gender regarding the study participants, nor on publication date regarding the manuscript. Only publications in English were included. Studies focusing on the use of EVs as biomarkers, diagnostic studies and/or observational studies were excluded, as well as preclinical or non-human studies, studies using synthetic

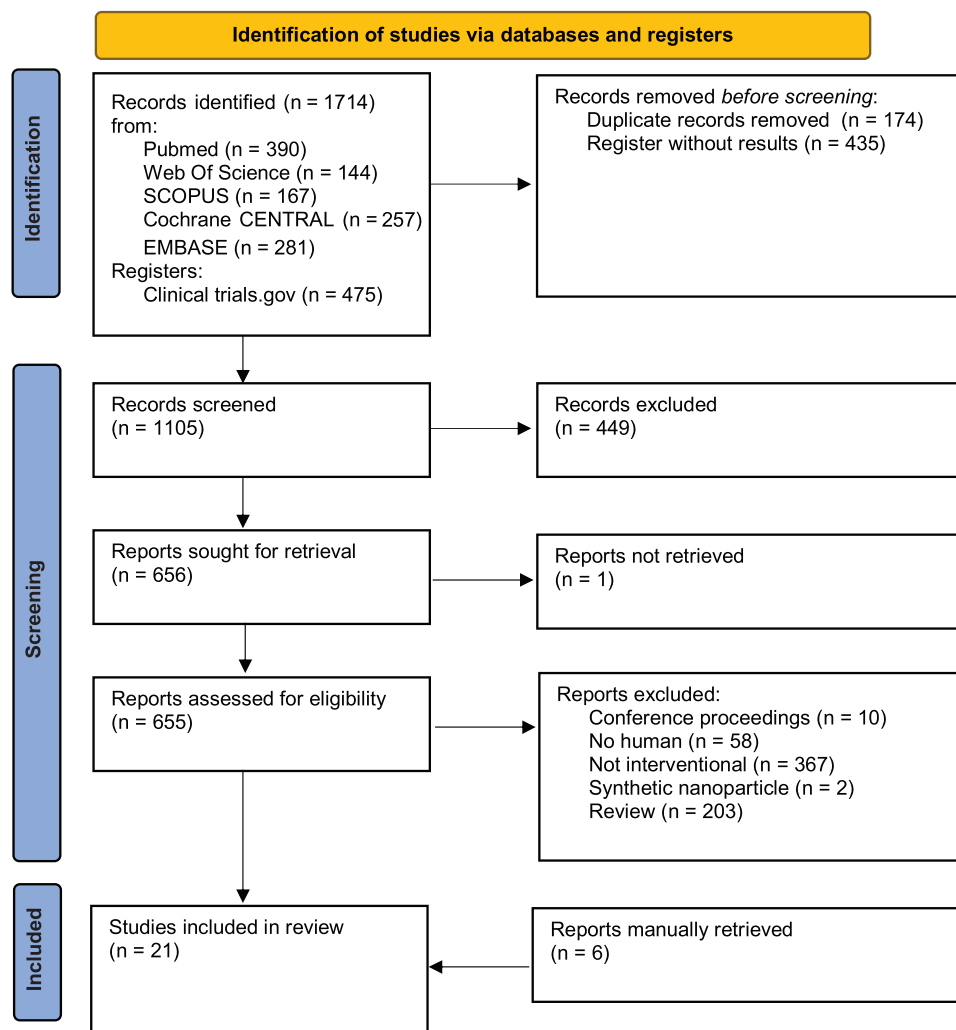


FIGURE 1 PRISMA flowchart, visualizing the literature research process.

nanoparticles, systematic reviews, case reports and retrospective studies. Studies were also excluded in case of overlapping data with other studies, or in case no information on EV source, characterization and dose was provided by the authors.

The PRISMA guidelines were used in the implementation of the literature review process (Page et al., 2021). A PRISMA flow diagram was used to visualize the number of in- and excluded studies and the reason for exclusion (Figure 1). Duplicate records from different databases were removed before screening. The initial selection was based on title, abstract and keywords, followed by the final selection based on the full text. References were imported in EndNote (Clarivate, version 8.2). Where available, the following information was extracted from the full-text articles: (i) General information (author, title, source, publication date, language, duplicate publications, sponsor type, enrolment dates, clinical study registration number); (ii) Study characteristics (study design, duration of follow-up, sample size); (iii) Participant characteristics (gender, age, ethnicity, number of participants recruited, disease and stage); (iv) Intervention and control condition (cell type, route of administration, dose and frequency, comparator if a control group is included); (v) Outcomes [AEs, including type, severity and assessment of causality, immunological outcome measures, clinical outcome measures (stable disease, disease worsening or disease improvement)]. Disagreements in selected records between reviewers (MvD and JD) were solved by a consensus meeting after reading the full-text article or, if needed, by a third reviewer (NC). In case the clinical trials were registered, but no publication of results was found, the principal investigator(s) was (were) contacted.

2.2 | Safety and efficacy outcome parameters

The primary outcome was the proportion of patients with minimally 1 serious adverse event (SAE). Additional variables included (i) the proportion of patients with minimally one adverse event (AE), excluding injection site reactions, (ii) EV-specific study

design: type of disease, immunocompatibility (autologous vs. allogeneic), EV dose, and EV source; (iii) clinical outcomes: proportion of patients who showed a clinical response (stable or improved disease status), no clinical response or deteriorated clinical response; (iv) engineering of EV: proportion of studies working with engineered EVs (either by modification of parental cell, or modification of the EV itself), and nature of the modification; and (v) routes of administration. For the classification of adverse events as SAE or AE, the definition in the original manuscript was used.

2.3 | Study risk of bias (quality) assessment

Risk of bias was assessed by ROBINS-I tool for non-randomized trials (Sterne et al., 2016) or Cochrane's Risk of Bias tool (Rob2) (Sterne et al., 2019) for randomized trials. Risk of bias was graded as low, moderate or high risk. Results from the risk of bias assessment were visualized using the robvis tool (McGuinness & Higgins, 2021).

2.4 | Data synthesis and statistical analysis

Categorical data were summarized as numbers, ratio's and/or percentages calculated in Microsoft Excel 16.80 and visualised using pie charts or bar graphs using Datawrapper. Overall proportions and corresponding 95% confidence intervals were calculated using a random effects meta-analysis for proportions. Heterogeneity between studies was further explored by subgroup analyses. Subgroup differences were evaluated by the between subgroups heterogeneity statistic in the random effects meta-analysis. Heterogeneity was quantified using the I^2 statistic, which describes the percentage of variation in study estimates across studies that is due to heterogeneity rather than chance. Forest plots were used to visualize the results. All analyses were performed in R version 4.1.3. Any p -value < 0.05 was considered statistically significant.

2.5 | Registration

The protocol for this systematic review was pre-registered on PROSPERO with registration number CRD42021287150.

3 | RESULTS

3.1 | Search results

We followed the approach as laid out in the PRISM flow diagram (Figure 1) to enable a systematic search of relevant databases and registers, and selected interventional clinical studies that complied with the predefined inclusion and exclusion criteria. In total, we screened 1714 unique records, 21 of which were finally included in the meta-analysis. These records included data from clinical studies evaluating safety and/or efficacy of EV therapy for the treatment of cancer ($n = 7$, Besse et al., 2015; Dai et al., 2008; Dong et al., 2022; Escudier et al., 2005; Gao et al., 2019, 2020; Morse et al., 2005), COVID-19 ($n = 5$, Chu et al., 2022; Shapira et al., 2022; Shi et al., 2021; Zarrabi et al., 2023; Zhu et al., 2022), dry-eye disease ($n = 1$, Zhou et al., 2022), chronic kidney disease ($n = 1$, Nassar et al., 2016), fistula ($n = 2$, Nazari et al., 2022; Pak et al., 2023), stroke ($n = 1$, Dehghani et al., 2022), wound repair ($n = 1$, skin sensitivity ($n = 1$, Ye et al., 2022), refractory macular holes ($n = 1$, Zhang et al., 2018) and venous ulcer ($n = 1$, Gibello et al., 2023), and this in phase I ($n = 12$, Chu et al., 2022; Dai et al., 2008; Escudier et al., 2005; Gao et al., 2020; Gibello et al., 2023; Johnson et al., 2023; Morse et al., 2005; Nazari et al., 2022; Pak et al., 2023; Shi et al., 2021; Ye et al., 2022; Zhang et al., 2018), phase II ($n = 5$, Besse et al., 2015; Dong et al., 2022; Guo et al., 2019; Zarrabi et al., 2023; Zhu et al., 2022) or mixed phase ($n = 4$, Dehghani et al., 2022; Nassar et al., 2016; Shapira et al., 2022; Zhou et al., 2022) studies. In one study, only healthy volunteers were included. An overview of the study characteristics can be found in Table 1.

Risk of bias was assessed by ROBINS-I tool for non-randomized trials (Sterne et al., 2016) or Cochrane's Risk of Bias tool (Rob2) (Sterne et al., 2019) for randomized trials. Risk of bias was graded as low, moderate or high risk, as visualized in Figures S1 and S2. Risk of bias was considered low in 11 studies, moderate in seven studies and severe in three studies. A severe risk of bias was mainly associated with risk of bias due to confounding by co-interventions that were not controlled for.

3.2 | Isolation, characterization and dosage determination of the EV product

Proper isolation and characterization of the EVs is essential for the formulation of the therapeutic product. When choosing the isolation technique, it is important to consider both the specificity of the method (i.e., how efficient the method is in separat-

TABLE 1 Overview of the interventional clinical studies on EV-based therapy included in the meta-analysis.

References	Clinical trial phase	Disease	N° of patients	N° in control group	Human cell source	Immunocompatibility	EV engineering	Route of Administration	Isolation technique	Dosage
Morse et al. (2005)	I	Unresectable pretreated stage III or IV NSCLC	9	N/A	Dendritic cell	Autologous	Yes, passively loaded via incubation of the parental cell or exosomes with MAGE-A3, -A4, -A10, and MAGE-3DPO4 peptides.	One dose given as 2 injections: 10% of volume intradermal, 90% subcutaneous	Density gradient ultracentrifugation	Number of MHC molecules per ml
Escudier et al. (2005)	I	Stage IIIB and IV melanoma	15	N/A	dendritic cell	Autologous	Yes, pulsed with MAGE3	10% of volume intradermal, 90% subcutaneous	Density gradient ultracentrifugation—diafiltration	Number of MHC molecules per ml
Besse et al. (2015)	II	NSCLC	22	NA	dendritic cell	Autologous	Yes, tumor antigen-loaded (PR5 pan-DR, MAGE-3 DP04, MAGE-1 A2, MAGE-3 A2, NY-ESO-1 A2 and MART-1 A2)	Intradermal	Density gradient ultracentrifugation—diafiltration	Number of MHC molecules per ml
Guo et al. (2019)	II	Malignant pleural effusion	11	N/A	tumor cell	Autologous	Yes, MTX packaging	Intrapleural infusion	Differential ultracentrifugation	Number of particles
Dong et al. (2022)	II	Non-squamous non-small cell lung cancer	40	39	tumor cell	Autologous	Yes, MTX packaging	Intrapleural infusion	Differential ultracentrifugation	Number of particles
Gao et al. (2020)	I	Advanced bile duct cancer	20	N/A	HL-60 cell line	Allogeneic	Yes, MTX packaging	Infusion into the bile-duct lumen	Differential ultracentrifugation	Number of particles
Dai et al. (2008)	I	Stage III and IV colorectal cancer	40	N/A	ascites fluid	Autologous	No	Subcutaneously	Density gradient ultracentrifugation	Total protein
Shi et al. (2021)	I	Healthy volunteers	24	N/A	MSC	Allogeneic	No	Inhalation (nebulized)	Differential ultracentrifugation	Number of particles
Zhu et al. (2022)	IIa	Severe COVID-19-related pneumonia	7	N/A	MSC	Allogeneic	No	Inhalation (nebulized)	Differential ultracentrifugation	Number of particles

(Continues)

TABLE 1 (Continued)

References	Clinical trial phase	Disease	N° of patients	N° in control group	Human cell source	Immunocompatibility	EV engineering	Route of Administration	Isolation technique	Dosage
Chu et al. (2022)	I	COVID-19	7	N/A	MSC	Allogeneic	No	Inhalation (nebulized)	Precipitation	Cell equivalent
Shapira et al. (2022)	I and II	COVID-19	35	N/A	HEK-293 cell line	Allogeneic	Yes, CD24 overexpression on membrane	Inhalation (nebulized)	Precipitation	Number of particles
Zarrabi et al. (2023)	II	COVID-19-induced ARDS	8	24	MSC	Allogeneic	No	One group intravenously, one group combined intravenously and by inhalation	Tangential flow filtration	Cell equivalent
Zhou et al. (2022)	I and II	cGVHD-associated dry eye disease	14	N/A	MSC	Allogeneic	No	Topical (ocular)	Differential ultracentrifugation	Total protein
Nassar et al. (2016)	II and III	Stages III and IV chronic kidney disease	20	20	MSC	Allogeneic	No	One intravenous, 1 intra-arterial	Differential ultracentrifugation	Number of particles
Nazari et al. (2022)	I	Perianal fistula in Crohn's disease	5	N/A	MSC	Allogeneic	No	Local injection of the fistula tract	Differential ultracentrifugation	Total protein
Pak et al. (2023)	I	Perianal fistula in non-Crohn's disease	1	11	MSC	Allogeneic	No	Local injection of the fistula tract	Differential ultracentrifugation	Number of particles
Dehghani et al. (2022)	I and II	Cerebrovascular disorders, malignant middle cerebral artery infarct	5	N/A	MSC	Allogeneic	Yes, transfected with miR-124	Intraparenchymal	Differential ultracentrifugation	Total protein
Johnson et al. (2023)	I	Delayed wound healing	11	11	Platelet	Allogeneic	No	Subcutaneous	Ligand-based exosome affinity purification — diafiltration	Total protein
Ye et al. (2022)	I	Female sensitive skin	22	N/A	MSC	Allogeneic	No	Topical	Differential ultracentrifugation	N/A
Zhang et al. (2018)	I	Macular holes	5	N/A	MSC	Allogeneic	No	Intravitreal	Differential ultracentrifugation	Total protein
Gibello et al. (2023)	I	Chronic venous ulcers	4	5	Serum-derived	Autologous	No	Topical	Precipitation	Number of particles

Abbreviations: N°, number; EV, extracellular vesicle; N/A, not applicable; NSCLC, non-small cell lung cancer; MSC, mesenchymal stem cell; CD, cluster of differentiation; cGVHD, chronic graft-versus-host disease; MTX, methotrexate; COVID-19, Coronavirus disease 2019; HEK-293, human embryonic kidney cells; MAGE, melanoma antigen gene.

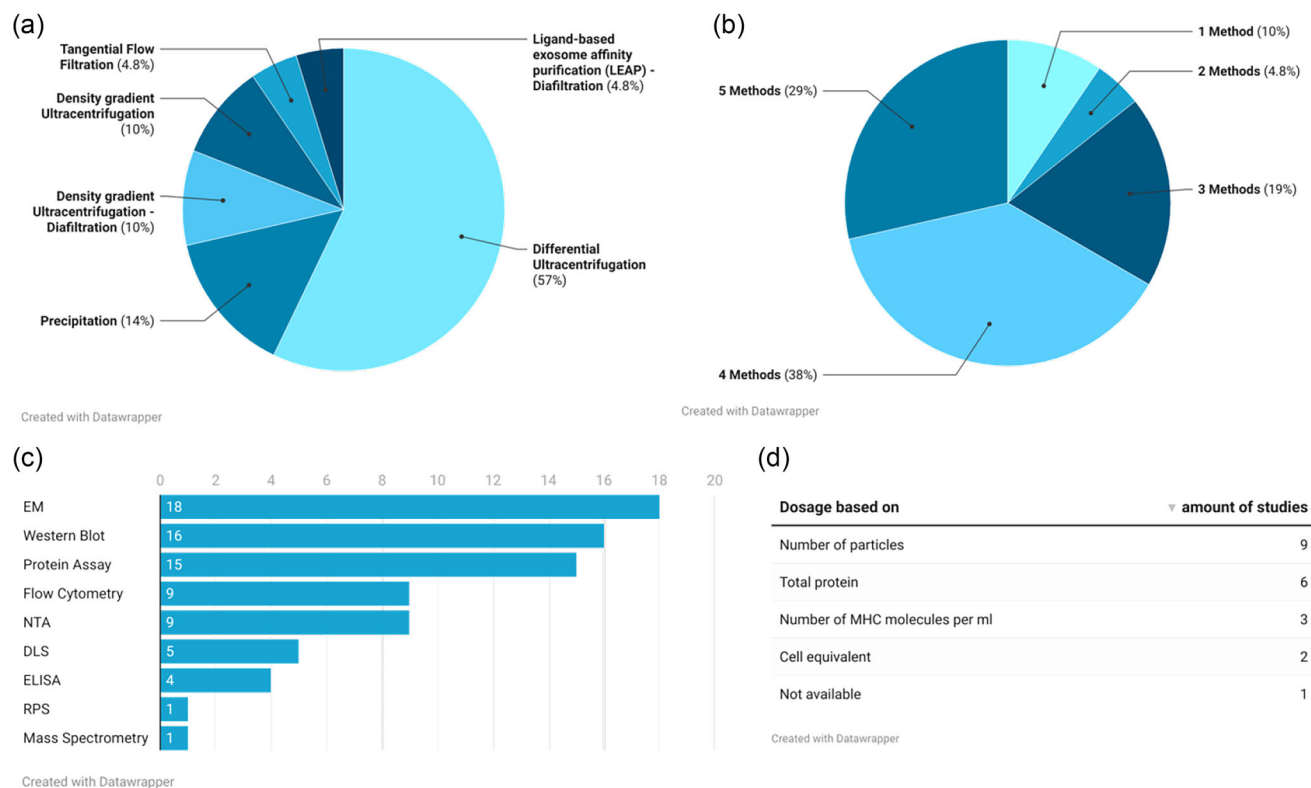


FIGURE 2 Isolation and characterization of therapeutic EV in clinical trials. (a) Isolation techniques used in the included clinical trials ($n = 21$) with percentages in brackets, (b) number of methods used for the characterization of the EV product with percentages in brackets, (c) different methods used for the characterization of the EV product with number of studies using the method in bars and (d) table of the parameter on which dosage of the EV product is used. Abbreviations used: electron microscopy (EM), nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), enzyme-linked immunosorbent assay (ELISA), resistance pulse sensing (RPS), major histocompatibility complex (MHC).

ing EVs from non-EV contaminants) and the efficiency of the method (i.e., how well can the method recover EVs in the final product). We checked all included studies for the methods used to isolate and characterize the used EV product. In the included studies, a total of six different isolation techniques is used (Figure 2a). Notably, most studies (12/21, 57%) used differential ultracentrifugation as a method to isolate EVs in the therapeutic product (Figure 2a). Other isolation methods used were sucrose density gradient ultracentrifugation ($n = 2$), sucrose density gradient ultracentrifugation followed by diafiltration ($n = 2$), precipitation ($n = 3$, of which two followed by ultracentrifugation and one followed by diafiltration), tangential flow filtration ($n = 1$), and ligand-based affinity purification followed by diafiltration ($n = 1$) (Figure 2a).

Following isolation, characterization of the EVs is essential to support claims on its functional aspects. In addition, every investigational medicinal product (IMP) needs to be characterized for identity, purity, and potency of the product (European Medicines Agency, 2022). The choice of characterization techniques is thus important for the results to provide an answer on the required characteristics. While most studies used either three ($n = 4$), four ($n = 8$) or five different methods ($n = 6$) to characterize the used EVs, three studies only used one ($n = 2$) or two ($n = 1$) methods (Figure 2b). Electron microscopy ($n = 18$), western blot ($n = 16$) and total protein quantification ($n = 15$) were used most often in the 21 included studies (Figure 2c). Also flow cytometry ($n = 9$, both single-vesicle as well as bead-based), nanoparticle tracking analysis (NTA, $n = 9$), dynamic light scattering (DLS, $n = 5$) and ELISA ($n = 4$) were seen in multiple studies. Resistive pulse sensing (RPS) and mass spectrometry were both only used once in a recent study (Johnson et al., 2023). Indeed, electron microscopy is often used to analyze morphology, while some studies also employed this technique for size determination. Nanoparticle tracking analysis, dynamic light scattering, and flow cytometry are used to determine concentration and/or size. Western blot, flow cytometry and ELISA were mainly used to verify the presence of both EV-associated, as well as engineering-associated markers. In addition, western blot was often used to analyze the presence of non-EV-associated markers to verify the purity of the isolation process and final product.

Dosage of the EVs is important for inter-study comparison. However, the reported dosage used in the included studies is often based on different key characteristics (Figure 2d). Several reporting parameters were used to support the administered dose. Most often, dosage was based on quantification of the number of particles in the EV product ($n = 9$) or total protein in the EV sample ($n = 6$). In earlier studies ($n = 3$), also the number of Major Histocompatibility Complex (MHC) molecules was used to determine the dose of the final product, whereas more recent studies used the cell equivalent, that is, EVs

produced by a certain number of cells ($n = 2$). Only one study did not disclose the dosage used in the human experiments (Ye et al., 2022).

In conclusion, there is a wide range in both the reported isolation as well as characterization methods used for EVs in the included clinical trials, albeit that differential ultracentrifugation, western blot and electron microscopy were among the most frequently used. Determination of the dosage is based upon different factors throughout the included studies.

3.3 | Meta-analysis of safety and efficacy outcomes in clinical EV therapy studies

3.3.1 | Safety of EV-based therapies

Serious adverse events (SAE)

Following the literature search, 21 studies were included in the meta-analysis. Combined, these studies report on the safety of EV administration in 335 patients. An SAE was observed in 6/335 patients, resulting in a low overall frequency of 0.7% (95%-CI: 0.1–5.2%), as visualized in Figure 3a. There was a low level of heterogeneity between the studies ($I^2 = 0\%$, 95%-CI 0–47%). The most frequent SAE was liver dysfunction ($n = 2$, Besse et al., 2015; Dong et al., 2022), followed by pyrexia ($n = 1$, Dong et al., 2022), vomiting ($n = 1$, Dong et al., 2022) and an acute asthmatic exacerbation ($n = 1$, Shapira et al., 2022).

In a subgroup analysis, the occurrence of SAE was compared following the administration of autologous (2.0%, 95%-CI 0.3–3.9%; $n = 7$ studies) versus allogeneic EVs (0.5%, 95%-CI 0.1–3.6%; $n = 14$ studies), which was not statistically significant ($P = 0.35$; Figure 3b). Similarly, there was no difference in the occurrence of SAE ($P = 0.99$) following administration of engineered (3.2%, 95%-CI 0.9–11.2%, $n = 8$ studies) versus non-engineered EVs (0.0%, 95%-CI 0.0–100.0%, $n = 13$ studies) (Figure 3c).

Adverse events (AE)

Similarly, 19 studies reported an overall frequency of 4.4% for the occurrence of AE (95%-CI: 0.7–22.2%); Figure 4a). However, a large heterogeneity between studies ($I^2 = 60\%$, 95%-CI 34–76%) was demonstrated.

There was a higher occurrence of adverse events following the administration of autologous (34.0%; 95%-CI 14.4–61.2%; $n = 5$ studies) versus allogeneic EV (0.9%, 95%-CI 0.0–18.0%; $n = 14$ studies; Figure 4b; $P = 0.019$), although there was again large heterogeneity between the studies. Similarly, a statistically significant difference in the frequency of AE ($P = 0.0027$) could be detected following administration of engineered (46.8%, 95%-CI 23.8–71.3%, $n = 7$ studies) versus non-engineered EVs (1.5%, 95%-CI 0.1–14.8%, $n = 12$ studies) (Figure 4c).

3.3.2 | Proof-of-concept of EV-based therapies

Depending on the disease being treated, the EVs have different desired effects. In the discussion of clinical outcomes, all included clinical trials were categorized based on the intended purpose of the treatment. This purpose was defined as either immunostimulatory ($n = 7$), immunosuppressive ($n = 9$) or regenerative ($n = 4$).

EVs with immunostimulatory capacity for the treatment of cancer

From our search, two phase I trials were identified investigating the safety and feasibility of the use of dendritic cell (DC)-derived exosomes loaded with tumor-associated peptides for the treatment of stage III/IV non-small cell lung cancer (NSCLC) (Morse et al., 2005) and stage III/IV metastatic melanoma (Escudier et al., 2005). The EVs were charged with the several melanoma-associated antigen (MAGE) A3-, A4- or A10-derived peptides either directly via passive incubation of the EVs or indirectly by peptide pulsing of the parental cells (Escudier et al., 2005, Morse et al., 2005). The loading method used differed per specific peptide and between the cohorts. While the safety of the treatment with EVs was demonstrated in both studies as evidenced by the absence of SAE and low occurrence of grade I/II AE, the studies also included an efficacy outcome as secondary endpoint. In both studies, the therapeutic exosomes were administered in two injections given intradermally (10% of the formulation) and subcutaneously (90% of the formulation) weekly over the course of 4 weeks. Morse et al. (2005) showed the development of a delayed type hypersensitivity (DTH) response in three out of nine patients upon rechallenge with MAGE peptides, indicative for the presence of a systemic immune response directed against the tumor antigen. Besides, preliminary immunological blood analysis suggested an increase of MAGE-specific cells in one patient out of nine patients, an increase of CD4+CD25+ T cells in two patients and an increase of natural killer (NK) cell activity in two patients. While no conclusion can be made on disease stability post-treatment, the time until progressive disease varied among cohorts treated with different peptide loading techniques and peptide concentration (Morse et al., 2005). Escudier et al. (2005) reported a clinical response in four out of 15 treated patients (one partial remission, one minor response, and two stable disease). While immunomonitoring of lymphocyte subsets did not show any alteration of immune cell subsets, an enhanced NK cell activity was observed in the four responding patients (Chaput et al., 2006; Escudier et al., 2005). In a follow-up phase II trial with DC-derived exosomes for the treatment of NSCLC,

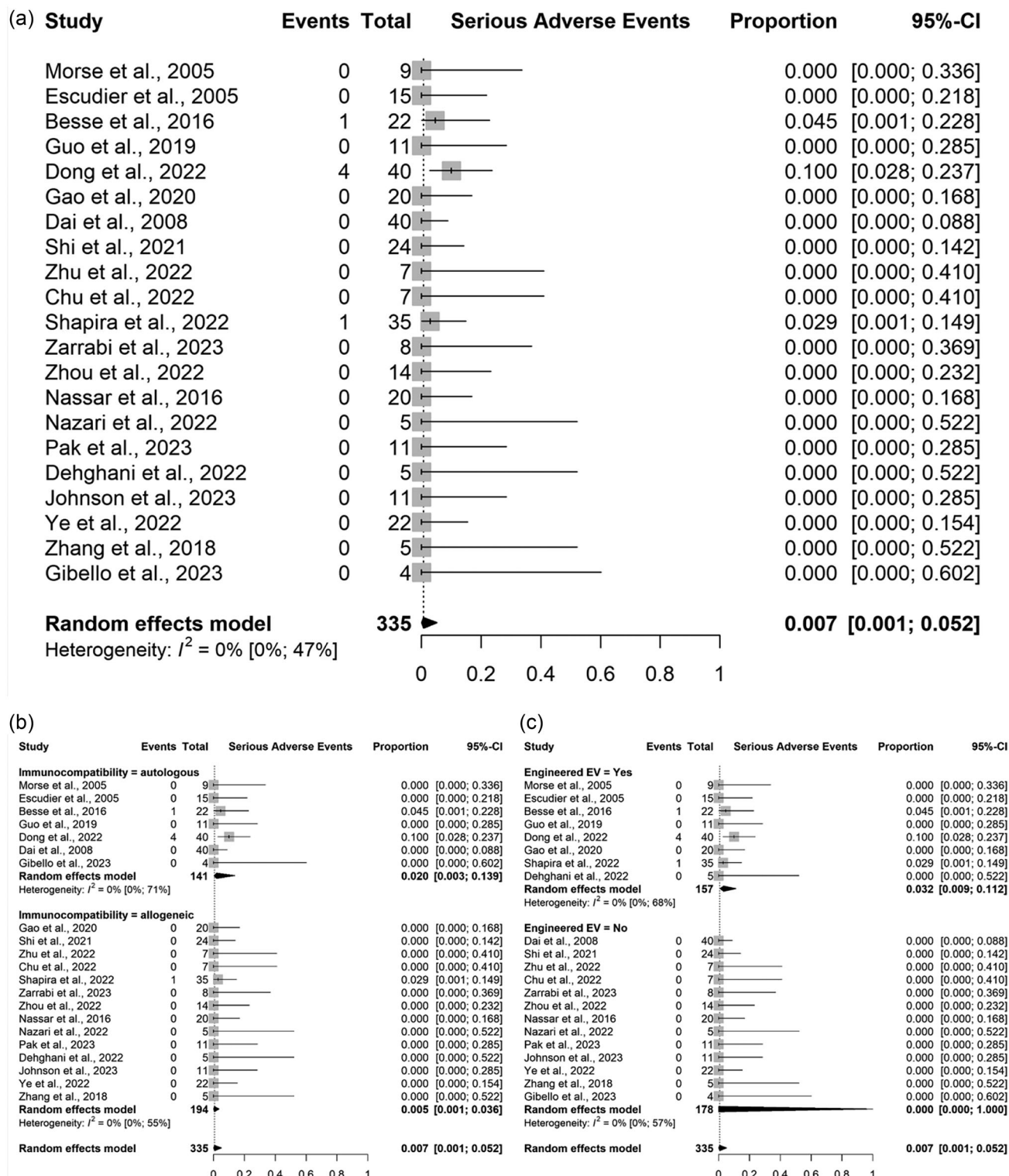


FIGURE 3 Serious adverse events (SAE). For studies with a control group, only SAE reported in the experimental arms were used for the meta-analysis. Per study, the number of events is shown, as well as the total number of study participants. Next, the proportion of serious adverse events was visualized per study by the middle of a grey box showing the proportion, with the 95%-CI visualized by horizontal lines. (a) The overall frequency of serious adverse events was 0.7% (95%-CI: 0.1–5.2%), as indicated by the black diamond and horizontal lines at the bottom of the forest plot. (b) Subgroup analysis of the proportion of serious adverse events following the administration of allogeneic versus autologous EV. (c) Subgroup analysis of the proportion of serious adverse events following the administration of engineered versus non-engineered EV.

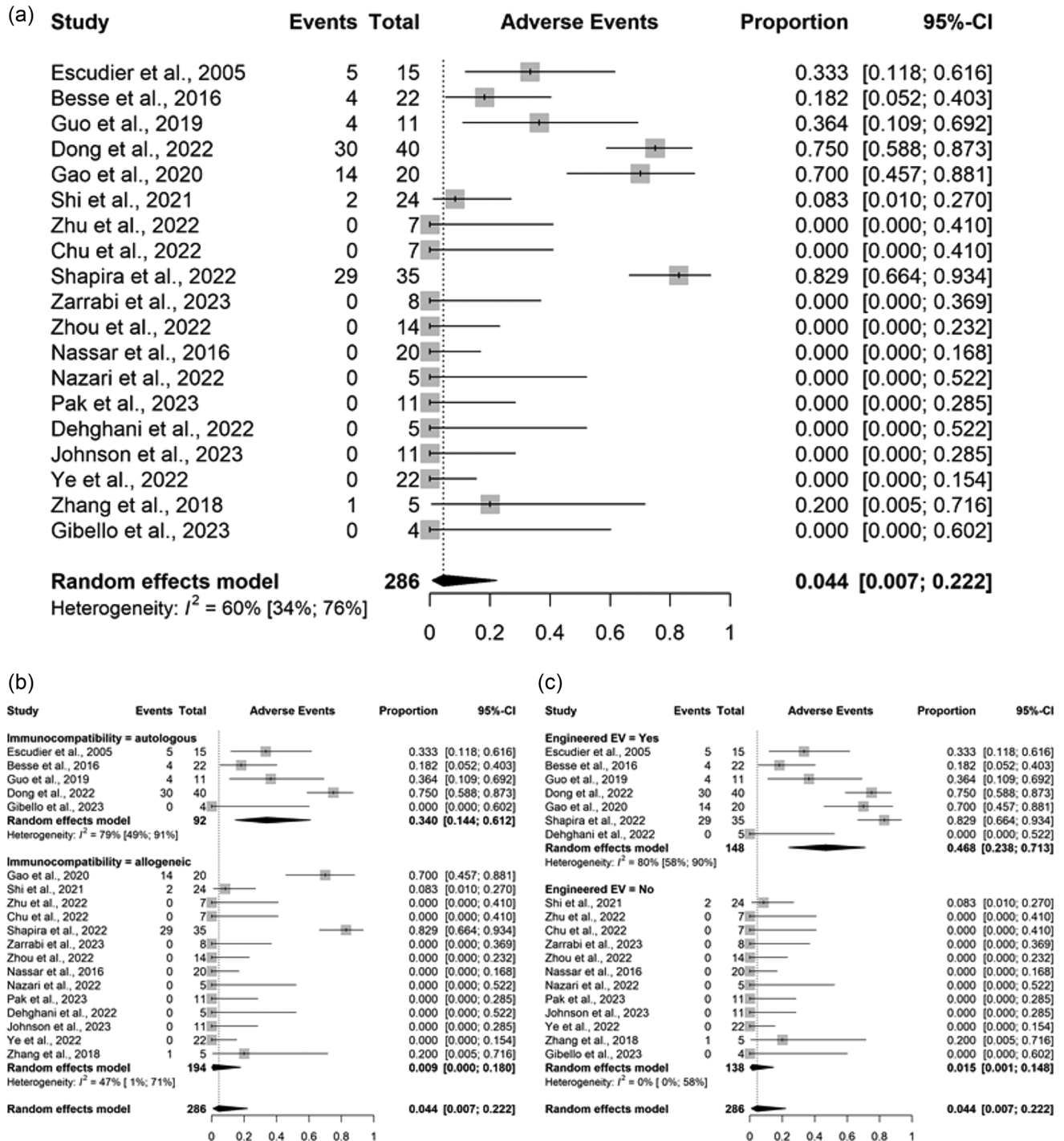


FIGURE 4 Adverse events (AE). For studies with a control group, only AE reported in the experimental arms were used for the meta-analysis. Studies that reported injection-site reactions as adverse events were not included in this analysis ($n = 2$). Per study, the number of events is shown, as well as the total number of study participants. Next, the proportion of adverse events was visualized per study by the middle of a grey box showing the proportion, with the 95%-CI visualized by horizontal lines. (a) The overall frequency of adverse events was 4.4% (95%-CI: 0.7–22.2%) as indicated by the black diamond and horizontal lines at the bottom of the forest plot. (b) Subgroup analysis of the proportion of serious adverse events following the administration of allogeneic versus autologous EV. (c) Subgroup analysis of the proportion of serious adverse events following the administration of engineered versus non-engineered EV.

exosomes were loaded by peptide pulsing of the parental cell with MAGE peptides, Melanoma Antigen Recognized by T cells (MART1/Melan-A) and Epstein-Barr Virus (EBV) peptides. In this study, 22 patients were treated by intradermal injection of exosomes. The treatment regime consisted of weekly, bi-weekly or 3-weekly vaccination phases separated by disease follow-up stages (Besse et al., 2015). This study did not meet its primary endpoint defined as 50% progression-free survival (PFS) in patients 4 months post-chemotherapy, but instead reached a 32% PFS rate (seven patients) with an overall median PFS of 2.2 months (22 patients). No change in the tumor antigen-specific T cell response was observed in these patients following EV treatment, albeit that an enhanced NK cell activity was observed in some patients similar to what was seen during the phase I trials mentioned above (Escudier et al., 2005; Morse et al., 2005).

Tumor cell-derived EVs, loaded with methotrexate (MTX) by incubation of the parental cells, were used in three of the total included studies to treat patients with lung cancer-associated malignant pleural effusion (MPE) (Dong et al., 2022, Guo et al., 2019) and cholangiocarcinoma (Gao et al., 2020). A pilot clinical trial by Guo et al. (2019) treated 11 patients with six intrapleural infusion of tumor cell-derived EVs on alternating days. The study reported an objective clinical response and symptomatic improvement in 10 out of 11 patients (four complete responses and six partial responses). In addition, the study reported a decrease in pleural effusion volume in nine of 11 patients, as evidenced by not needing further pleural drainage and a significant decrease in tumor cells in pleural effusion. Analysis of the immune profile revealed changes in the tumor-infiltrating lymphocyte compartment during treatment, but post-treatment alterations were not investigated. In a second trial using MTX-loaded EVs, Dong et al. (2022) assessed the efficacy to treat MPE in NSCLC by six intrapleural infusions every other day in a placebo-controlled study. In the study, no significant difference was seen in the number of AE ($p = 0.4647$), seven SAE were recorded (three in the treatment group, four in placebo group) of which one SAE (grade 3 hepatic dysfunction) was designated as drug-related SAE. Patients ($n = 40$) who received treatment had an objective response rate (ORR) for MPE of 82.50% with 10 patients going into complete remission, 23 into partial remission and one stable disease. This 82.50% ORR in the MTX-EV treated group was a significant increase as compared to the saline placebo-controlled group which had an ORR of 59.97% (six complete remission, 17 partial remission and eight stable disease). However, no significant difference in the median PFS between the treated group (6.4 months; 95% CI 4.5–12.3) and the placebo group (7.3 months; 95% CI 6.1–10.4) was observed (Dong et al., 2022). Besides MPE, MTX-loaded EVs were also used to treat extrahepatic cholangiocarcinoma by Gao et al. (2020). MTX-loaded EVs were administered by percutaneous transhepatic biliary drainage in which tumor-derived EVs were injected in the bile-duct lumen. Treatment regime consisted of daily injection over the course of seven days. Here, 25% (five out of 20 patients) showed relieve of the bile-duct obstruction as assessed by bile-duct radiography. In addition, evaluation of the stool color changed from a pale, clayed color to yellow in line with the treatment results. Analysis of the bile draining before and after treatment revealed an increase in neutrophils upon treatment with MTX-loaded EVs and an increase in neutrophil-associated tissue-degrading enzymes potentially shedding more light on the mode-of-action of these EVs in cholangiocarcinoma (Gao et al., 2020).

Lastly, in a study by Dai et al. (2008) autologous exosomes derived from ascites fluid were used to treat patients with stage III/IV colorectal cancer. Patients received subcutaneous injection weekly over the course of 4 weeks. Of the 40 patients included in this study, none of the patients treated with the exosomes alone ($n = 22$) showed a therapeutic response. When GM-CSF was used as an adjuvant ($n = 18$), one patient presented with stable disease and one patient with minor response. Nonetheless, patients treated in the cohort with the highest and second highest dose ($n = 20$) showed systemic antitumor immunity regardless of the use of GM-CSF as an adjuvant as demonstrated by DTH. Using GM-CSF as an adjuvant favored patients in the cohorts treated with lower doses of exosomes to elicit systemic antitumor responses. Despite the study's conclusion that this therapy was safe and feasible, no follow-up phases were started.

EVs with immunosuppressive capacity

The safety and feasibility of EVs to downmodulate the immune system was evaluated in eight of the included clinical trials. The diseases treated comprised coronavirus disease 2019 (COVID-19, $n = 5$), dry eye disease ($n = 1$), chronic kidney disease ($n = 1$) and perianal fistula ($n = 2$).

During the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, several therapeutic approaches were evaluated to provide a therapeutic solution for (severe) ongoing COVID-19. Among others, several groups evaluated the use of EVs for the treatment of COVID-19. Despite the numerous trials registered that are also finished, only few published their data (already) or provided sufficient evidence of the safety and efficacy concerning their EV product. We found five trials meeting the predetermined criteria for inclusion (Chu et al., 2022; Shapira et al., 2022; Shi et al., 2021; Zarrabi et al., 2023; Zhu et al., 2022). A phase I trial by Shi et al. (2021), the MEXVT study, evaluated the use of allogeneic adipose tissue MSC-derived EVs for the treatment of acute respiratory distress syndrome (ARDS). In this study, 24 healthy volunteers were treated with a single dose administered via nebulization. Following favorable safety results (Shi et al., 2021), a phase II trial was initiated and seven patients with severe COVID-19 related pneumonia were treated with MSC-EVs daily for 5 days (Zhu et al., 2022). Given the small sample size, it is difficult to draw firm conclusions regarding the efficacy of the MSC-EVs, but several trends were observed. The number of lymphocytes increased in all seven patients, while several inflammation biomarkers, including C reactive protein (CRP, six out of seven), interleukin-6 (five out of seven) and lactate dehydrogenase (six out of seven) showed a decreasing trend. Clinical improvement was observed as demonstrated by resolution of pulmonary lesions in all patients, improved chest CT image scores

and two patients being able to switch from a high-flow oxygen nasal cannula at the start of treatment to a standard oxygen nasal cannula (Zhu et al., 2022). Chu et al. (2022) evaluated the treatment of seven COVID-19 patients with umbilical cord-derived MSC-EVs via nebulization. Similar to the results above, this study showed a decreasing trend for the CRP levels in five out of seven patients with severe or mild COVID-19 treated with MSC-EVs, and chest CT images indicated absorption of pulmonary lesions in several patients (Chu et al., 2022).

While the previous studies show promising results, only a single arm was included in the clinical trial design. In a recent phase II randomized controlled trial for the treatment of COVID-19 by Zarrabi et al. (2023), the use of MSC-derived EVs was compared to MSC therapy and a control group of COVID-19 patients. First, MSC were administered intravenously to all patients in the intervention group ($n = 19$). Subsequently, a second intervention consisting of intravenously administered MSC ($n = 11$) or inhalation of MSC-EVs ($n = 8$) took place. A mortality rate of eight patients in the control group ($n = 24$) and three patients in the MSC-treated group was reported, while no patients died in the MSC-EV treated cohort. Interestingly, immunological analysis showed the downregulation of inflammatory markers IL-6, IFN- γ and CRP in patients in the MSC-EV intervention group, as compared to the group treated with MSC alone and the control cohort. A decrease in TNF- α was observed in the MSC-EV treated group compared with the control cohort, albeit that downmodulation of TNF- α was even more prominent in the MSC alone-treated patients.

While all previous COVID-19 studies made use of a non-engineered EV therapeutic, Shapira et al. (2022) evaluated the use of an engineered CD24-enriched Hek-293 cell line-derived EV product as a potential therapeutic for COVID-19 in a phase Ib/Ia clinical trial with 35 patients. CD24 has been reported to be able to discriminate between damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). Hence, enabling the cell to downregulate the response to DAMPs by suppressing the NF- κ B pathway without altering the viral clearance induced by PAMPs recognition (Chen et al., 2009; Fang et al., 2010; Shapira et al., 2022). Notwithstanding the absence of a placebo-controlled group, the investigators reported an improvement of the respiratory rate, a decrease of CRP levels in the blood, improvement in chest CT images, as well as improvement in symptoms (Shapira et al., 2022), in accordance with the beforementioned studies.

Besides their applicability for the treatment of COVID-19, umbilical cord MSC-derived EVs were also tested in a single-arm clinical trial with 14 patients with graft-versus-host disease (GVHD)-associated dry eye disease (Zhou et al., 2022). EVs were administered via topical application in an eye droplet formulation. Treatment led to the relief of symptoms such as burn, redness, sting etc. and the improvement of several other clinical indicators, such as decrease of the ocular surface disease index, longer tear-film breakup time and increased tear secretion (Zhou et al., 2022). In addition, mouse studies suggested an immunological response by inducing a transition in macrophages from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype. However, these immunological results were not validated in patients. More studies are needed to assess the efficacy and confirm these initial results against a control group.

In a placebo-controlled trial by Nassar et al. (2016), patients with stage III/IV chronic kidney disease were treated with umbilical cord MSC-EVs for their anti-inflammatory and anti-fibrotic function to improve renal function and attenuate disease progression. The intervention group consisted of 20 patients who received treatment with umbilical cord MSC-EVs, while 20 patients were allocated to the placebo-controlled group. EVs were administered by two injections, first intravenous followed a week later by injection in the intra-renal arteries. Proof of concept of EV efficacy was assessed as a secondary endpoint as the duplication of the estimated glomerular filtration rate (eGFR) or a fifty percent reduction of creatine levels in the serum. Patients in the treatment group showed a significantly improved eGFR and significantly decreased serum creatine levels from baseline to week 12, while patients in the control group did not display any significant alterations in both clinical parameters. However, the improvement in eGFR and decrease in serum creatine levels did not meet its predefined endpoints as specified in the secondary outcomes. While these results show MSC-derived EVs were able to significantly improve kidney function compared to the placebo-controlled group, investigators reported these changes to be transient throughout the study period of 1 year. In addition, treatment regimen consisted of 2 injections which was noted by the study to be needed since the response to treatment was heterogenous in time with some patients showing a response after the first dose, while others only did after the second dose (Nassar et al., 2016).

Lastly, two trials evaluated the use of MSC-derived EVs for the treatment of perianal fistula in patients with (Nazari et al., 2022) and without Crohn's disease (Pak et al., 2023). A prospective phase I trial by Nazari et al. (2022) used a single injection of umbilical cord-derived MSC-EVs in the tissue surrounding the tract for the treatment of inflammatory bowel disease (IBD)-related perianal fistula in five patients. During the follow-up, no (serious) adverse events were reported and laboratory analysis did not show any abnormal results. Evaluation of efficacy outcomes included solely clinical outcomes showing a response in four out of five patients, including complete healing in three patients and clinical improvement (including reduction in discharge and clearing of skin irritation) in one patient. In a following phase I trial by Pak et al. (2023) evaluating the use of MSC-EVs for the treatment of non-Crohn's disease patients with perianal fistula, 11 patients were included receiving treatment with placenta-derived MSC-EVs. Patients were evaluated for safety of the treatment, as well as quality of life (QoL) questionnaires and physical examination of the healing process. Overall, the mean QoL increased significantly from 37 ± 10.47 before intervention to 54.78 ± 17.60 after intervention with seven out of 11 patients showing an increase. A total of nine patients showed partial ($n = 5$) or complete ($n = 4$) healing during follow-up. While the investigators report these results to be in line with MSC treatment of perianal fistula, no

comparison group was included. Nonetheless, the favorable safety results and data from clinical outcomes could indicate its potential as a new therapeutic over the current treatments.

The use of EVs for regenerative purposes

Despite numerous technological advances, there is still an urgent need for new therapeutic strategies in the field of regenerative medicine. In this systematic review, five studies were included evaluating the use of therapeutic EVs for the purpose of regeneration in stroke (Dehghani et al., 2022), wound healing (Johnson et al., 2023), sensitive skin (Ye et al., 2022), the healing of refractory macular holes (Zhang et al., 2018), and chronic venous ulcers (Gibello et al., 2023).

In a prospective phase I clinical trial by Dehghani et al. (2022), the use of allogeneic MSC-derived EVs was evaluated for the treatment post decompressive craniectomy in patients with malignant middle cerebral artery infarct. Treatment was administered via a single dose intraparenchymal injection. Improvements were seen in four out of five patients on several clinical measures, such as the modified Rankin Scale, NIH Stroke Scale and CRP. However, since this trial had a single-arm study design with a focus on safety and feasibility, these efficacy results are not reliable enough to discuss the effectiveness of the treatment (Dehghani et al., 2022). No follow-up studies on the products were found.

In the included study, two trials evaluated the use of EVs for the regeneration and healing of skin disease. In the Plexoval II study (Johnson et al., 2023), 11 healthy controls were given a skin biopsy wound in each upper arm of which one was randomly assigned to the treated group, and one to the placebo group. The treatment regime consisted of a subcutaneous injection of allogeneic platelet-derived EVs, whereas the placebo wound got equivalent volume of isotonic solution. Favorable safety data were reported. However, no difference was observed in mean healing time of the EV-treated wound as compared to the placebo-treated wound with all wounds fully closed at day 30 of the follow-up (Johnson et al., 2023). Second, Ye et al. (2022) evaluated the use of umbilical cord-derived MSC-EVs for the treatment of diagnosed sensitive skin in women. Important to note is that the authors did not disclose the dosage used in human subjects, however an in vitro dose-escalation experiment using a fibroblast scratch assay was used. Evaluation of the treatment consisted of safety and clinical outcomes, no (serious) adverse events were reported. While no individual patient data was included, the authors reported a significant decrease in objective (roughness, scales, erythema) and subjective (tension, burning, itching) symptoms. Several clinical skin indices were included, but the only significant improvements compared to baseline were seen in sebum (at day 14), a* value correlating with erythema of the skin (days 7, 14 and 28), and pH value (day 28). Similarly, the lactic acid stinging test score improved significantly at days 14 and 28 of the study.

Zhang et al. (2018) evaluated the use of either intravitreal injection of MSC or MSC-derived EVs in a single-arm clinical trial for the treatment of large and refractory macular holes in seven pilot cases of which five patients were treated with the MSC-derived EVs and two with MSC. Closure of the macular hole was observed in four out of five patients treated with MSC-EVs and in two out of two patients treated with MSC, which was accompanied by improvement of the best-corrected visual acuity score (Zhang et al., 2018). Notably, one patient treated with MSC developed a fibrotic membrane on the retina that had to be removed. Despite these promising results, we did not find any follow-up trials.

Lastly, Gibello et al. (2023) used serum-derived extracellular vesicles in a case-controlled pilot study in four patients with chronic venous ulcers (CVU). EVs were topically administered in six repetitive doses over a course of 2 weeks. Treatment was evaluated by safety follow-up and the ulcer was evaluated by clinical and morphological parameters until 30 days after the last EV treatment. Analysis of the CVU median surface area showed a significant reduction after 30-day follow-up when comparing the EV-treated ulcer compared to the ulcer receiving standard-of-care. In addition, histological analysis of a 4-mm punch biopsy showed an increase in fibrosis and microvascular proliferation in EV-treated ulcers compared to non-EV treated ulcers.

4 | DISCUSSION AND CONCLUSION

In recent years, the field of EV research has increased exponentially and a clear evolution towards translation of in vitro experiments to preclinical research and clinical studies is seen. The role of EVs in intercellular communication and their ability to carry parental cell-derived cargo, as well as engineered cargo, provide a unique opportunity in diagnostics and therapeutics (Kodam & Ullah, 2021). In the present study, we performed a meta-analysis of the safety of EV-based therapies in humans over various diseases observed in current clinical studies. In addition, preliminary efficacy data were evaluated and summarized where possible. To our knowledge, this study is the first comprehensive overview of safety and efficacy data in EV-based therapeutic studies in a systematic review.

Our meta-analysis shows that EV-based therapy is safe and well tolerated as evidenced by the overall low frequency of (serious) adverse events. Only six SAE were reported in a total of 335 patients being liver dysfunction ($n = 2$), followed by pyrexia ($n = 1$), vomiting ($n = 1$) and an acute asthmatic exacerbation ($n = 1$). Nonetheless, a larger interstudy variation was observed in the occurrence of AE compared to SAE. This heterogeneity can be caused by differences in the reporting of AE. Indeed, in the included studies, different reporting guidelines were used, such as the WHO toxicity criteria, Common Terminology Criteria for Adverse Events version 3 (CTCAEv3), version 4 (CTCAEv4) and version 5 (CTCAEv5), while other studies did not mention

the used reporting guideline. Despite the existence of several reporting guidelines on the occurrence of AE in clinical studies, several publications highlight the variety and underreporting of AE (Maggi et al., 2014; Phillips et al., 2019). This heterogeneity in reporting of AE is also noticeable when considering injection site reactions. Out of the 21 studies, only two studies included extensive injection site reactions such as redness, pain during puncture among others as AE. As mentioned above, these two studies were not included in the formal analysis of AE since this would cause heterogeneity in the dataset and would result in skewing of the data. Interestingly, no significant difference was observed in the occurrence of SAE when comparing administration of allogeneic versus autologous EVs. A significant difference was observed in the occurrence of AE with more AE reported in the use of autologous administration. Although the clinical relevance of this finding remains questionable, it must be noted that a potential bias due to engineering could be present since all studies using autologous administration in this analysis made use of engineered EVs. Nevertheless, since these results suggest allogeneic administration of EVs to be at least as safe as autologous administration, using allogeneic EVs as a therapeutic opens the way to an off-the-shelf approach. Such an approach would provide great advantages over autologous therapy since it could be readily available should a patient need it, lower individual costs per patient and result in less variability in the product (Depil et al., 2020; Mason & Dunnill, 2009). However, disadvantages of allogeneic EVs as therapy must be considered too, such as the possibility of a more rapid clearance of allogeneic EVs as compared to autologous EVs (Witwer & Wolfram, 2021). While it provides an interesting opportunity to the field, more research is needed to the effects of allogeneic EV therapy.

The therapeutic effect of EVs is largely dependent on the cargo associated with the EVs (Murphy et al., 2019). However, this cargo can be heterogenous since the cargo of EVs derived from an identical parental cell can vary depending on cell culture parameters (Shekari et al., 2023; Sherman et al., 2021). Enriching or adding a certain bioactive molecule to the EVs cargo could be an interesting strategy to enhance the therapeutic effect. In this study, no statistically significant difference was observed on the occurrence of SAE when comparing the therapeutic use of EVs that were engineered versus non-engineered EVs. In contrast, the occurrence of AE did show a significant difference. However, it must be noted that in the non-engineered studies 10 out of 12 trials reported 0 adverse events, which could be attributed to the underreporting of AE as noted above. Our results suggest that engineering of EVs do not induce more SAE as compared to non-engineered EVs, and that engineering of EVs can be used to redirect or enhance the EVs therapeutic potential. Several methods exist for the loading of EVs, future research could elucidate whether certain engineering methods are safer and more fit to preserve the EVs' integrity and enhance their mode-of-action. Altogether, these results showed that EV-based therapy is safe and feasible in patients. However, there are some important considerations in interpreting these results. For instance, we observed a high variability in methods used to isolate the final EV products. Differential ultracentrifugation, considered the 'gold standard' for EV isolation, is still used most often and provides an intermediate recovery and specificity (Théry et al., 2018). Despite its ease, clinical translation of this technique to large-scale manufacturing is not optimal regarding batch-to-batch consistency (Ghodasara et al., 2023). Our analysis also showed that most trials employed established characterization techniques such as western blot, total protein quantification and electron microscopy for the characterization of the EV product followed by flow cytometry and nanoparticle tracking analysis. These techniques can be used to assess identity and purity of the therapeutic product regarding GMP-compliance. However, the assessment of potency prior to administration was often not reported.

Over the last years, the International Society for Extracellular Vesicles (ISEV) released the Minimal Information on Studies with Extracellular Vesicles (MISEV) guidelines describing and suggesting several techniques in designing EV studies and reporting (Welsh et al., 2024). Despite these guidelines, first published in 2014 (Lötvall et al., 2014), only three studies published after this date reported the use of these guidelines in the characterization or experimental design of the EVs. Indeed, the reporting on the isolation and characterization of EV products in the included studies was immensely diverse making inter-study comparison difficult. For instance, the measure used to identify the number/amount of EVs administered or the markers used to determine the purity of the EV-product differed between studies, underlining the urgent need of standardization efforts in the EV field (Soekmadji et al., 2020; Witwer et al., 2021). This would enable further analysis into correlations between manufacturing parameters (such as isolation method, purity and EV dosage) and the incidence of adverse events, as well as the efficacy of the EV treatment.

All studies taken together, a positive clinical response or amelioration of clinical symptoms were reported in 168 out of a total of 335 patients, with 36 of the remaining patients without clinical response showing improvement in immunological responses. These results should be interpreted with caution since efficacy and clinical outcome data were in most studies purely based on effect measurements and not on comparison to controls. In addition, the effect of confounding factors due to previous treatments or the effect of the route-of-administration cannot be evaluated at this stage. This is also reflected in the risk-of-bias assessment which indicate a serious risk of bias in three of the included studies due to co-interventions that were not controlled for. Further, it is not clear whether a single-dose administration or repeated administration has an influence on the therapeutic effect of EVs. Also in cell therapy, this question remains subject to discussion as preclinical studies indicate a beneficial effect of repeated administration, but human clinical trials remain unclear (Vrtovec & Bolli, 2019). In our study, most included trials used repeated administration instead of a single dose. Lastly, although endpoints were described for proof-of-concept of the EVs in the included studies, most studies did not include a control group. More research and randomized placebo-controlled trials are necessary before conclusions can be drawn on the efficacy of EV-based therapeutics.

In conclusion, we have shown that EV-based therapeutics is generally safe in patients as demonstrated by a low frequency of (serious) adverse events. Moreover, this systematic review suggests that immunocompatibility of the EV therapeutic has no significant effect on the risk of serious adverse events. Lastly, this study shows that engineering of EVs may influence the occurrence of adverse events, but not serious adverse events, in patients treated with an EV-based therapeutic. The current state-of-the-art is too preliminary to draw any general conclusions on the efficacy of such therapies. It is important to note that, despite considerable advancements in the field of EVs, several major hurdles to clinical translation such as standardization of isolation and characterization, as well as reporting these parameters is important upon further development in this field to ensure reproducibility and reliability. Such efforts will not only improve scientific practices, but also ensure smooth clinical translation to the patients. These hurdles could be addressed by both regulatory agencies (e.g., the requirement of clinical trials to report (S)AE according to an international-agreed-upon reporting guideline) as well as international societies (could include inter-society publishing of 'good-practices' and the requirement by society-associated journals to report data according to publicly available standardized reporting guidelines, for example, MISEV2023 (Welsh et al., 2024) and researchers themselves (e.g., disclosing sufficient experimental details of upstream and downstream manufacturing procedures to ensure reproducibility).

AUTHOR CONTRIBUTIONS

Mats Van Delen: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing—original draft; writing—review and editing. **Judith Derdelinckx:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; visualization; writing—original draft; writing—review and editing. **Kristien Wouters:** Formal analysis; methodology; software; visualization; writing—review and editing. **Inge Nelissen:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing—review and editing. **Nathalie Cools:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the fundings in this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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