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Developing A General Checklist for The Effective Administration of Extracellular Vesicles in Biomedical and Clinical Research

Azadeh Haghighitalab, Ph.D.[1, 2], Mahboubeh Kazemi Noughabi, Ph.D.[1], Halimeh Hassanzadeh, Ph.D.[1, 2], Faezeh Shekari, Ph.D.[3, 4], Maryam Moghaddam Matin, Ph.D.[2, 5], Rebecca Lim, Ph.D.[6], Hamid Reza Bidkhori, M.D., Ph.D.[6, 7], Ahmad Reza Bahrami, Ph.D.[2, 8*]

1. Stem Cells and Regenerative Medicine Research Group, Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi, Mashhad, Iran
2. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Advanced Therapy Medicinal Product Technology Development Center (ATMP-TDC), Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
6. The Ritchie Centre, Hudson Institute of Medical Research, Clayton, Victoria, Australia
7. Blood Borne Infections Research Center, Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi, Mashhad, Iran
8. Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

The potential application of extracellular vesicles (EVs) in regenerative and personalized medicine has attracted substantial interest in recent years, highlighting the need for standardized protocols for their administration in preclinical and clinical settings. EVs, which play critical roles in intercellular communication and have significant therapeutic potential, have prompted extensive research and advancements in their clinical applications. However, the rapid evolution of this field has also revealed variability in how EVs are isolated, characterized, and used across different studies. Over the past decade, organizations such as the International Society for Extracellular Vesicles (ISEV) and the International Society for Cell and Gene Therapy (ISCT) have actively worked to address these challenges by proposing frameworks for standardizing EV-related research. As the clinical evaluation of therapeutic EVs becomes increasingly commonplace, there is a need for practical guidelines and assessment tools that can aid in evaluating their efficacy and safety. In this context, we propose a comprehensive checklist designed to guide researchers and clinicians in considering critical aspects when designing and conducting biomedical and clinical studies involving EVs. This checklist aims to enhance the standardization of trials and therapeutic procedures, ensuring that clinical reports are prepared with adequate detail. By controlling reproducibility and transparency in research, we believe that our proposed guidelines will contribute significantly to advancing the application of EVs in clinical practice.

Keywords: Biological Products, Extracellular Vesicles, Good Manufacturing Practices, Mesenchymal Stem/Stromal Cells, Translational Medical Research

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Introduction

The use of extracellular vesicles (EVs) is emerging as a new therapeutic modality (1). This growth is reflected in the recent scientometric comprehensive publication (2) which highlights a significant increase in publications focused on clinical applications of EVs. Notably, 94% of relevant publications were released within the last five years. Reaching the plateau in the growth curve of publications indicates the maturation phase of the research on EVs in the benefit of more rigorous and impactful work. This is largely supported by the International Society for Extracellular Vesicles (ISEV) guidelines and publications trying to implement universal approaches for isolation, characterization, and standardization of studies on EVs. However, evidenced by the significant increase in relevant publications and clinical interest, there remains a critical need for standardized guidelines to navigate the challenges associated with their use.

The application of cell-free products, including EVs, is still encumbered by several challenges and technical issues. Although EVs are cell-free products, their properties are

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*Corresponding Address: P.O.Box: 9177948974, Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
Email: ar-bahrami@um.ac.ir

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largely impacted by their parent cell properties and culture conditions (3, 4). Despite the gaps, extensive efforts were put into the clarification of the clinical application of EVs from different aspects. Liu et al. (5) describe advancements in separating EV subpopulations and compare current and emerging isolation methods. A crucial hurdle is the lack of universally accepted standards for EV preparation, particularly under good manufacturing practice (GMP) condition (6-10).

The regulatory landscape surrounding EVs is evolving, with regulatory bodies emphasizing patient safety. Public safety notifications from the Food and Drug Administration (FDA) (July 22, 2020; https://www.fda.gov/vaccines-blood-biologics/consumers-biologics/consumer-alert-regenerative-medicine-products-including-stem-cells-and-exosomes; December 6, 2019 https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/public-safety-notification-exosome-products), the European Medicines Agency (EMA) Committee for Advanced Therapies (CAT) (April 28, 2020; https://www.ema.europa.eu/en/documents/public-statement/ema-warns-against-using-unproven-cell-based-therapies_en.pdf), and the International Society for Extracellular Vesicles (ISEV) (August 8, 2020; https://www.isev.org/patient-information-and-safety-notice--extracellular-vesicles-exosomes-and-unproven-therapies), all highlight the need for EVs to undergo pre-market review and approval similar to other therapeutic agents, including conventional drugs and cell-based therapies. These warnings underscore the importance of informing patients about the potential risks associated with this evolving therapeutic option.

In a comprehensive systematic review, Van Delen et al. (11) reviewed the safety and efficacy of twenty one EV-based clinical trials, highlighting the challenges of inter-study comparisons due to variations in methodologies. In a scoping review, Rahnama et al. (12) explored the global trend of exosome application in clinical trials, mentioning different critical aspects, including optimization and standardization of EV isolation and characterization, safety, efficacy, and the global market, highlighting the mandates for performing further well-designed robust clinical research.

This study aims to propose a comprehensive questionnaire, referred to as a general checklist (G-check), intended to improve the rigor and validity of clinical studies involving EVs.

G-check aims to address this gap by providing a structured tool for biomedical scientists, clinicians, human research ethics committees, and regulatory bodies. This questionnaire will include various aspects of EV trials, such as general study information, disease etiology and patient demographics, ethical considerations, and specifics about the source and characteristics of EVs used.

What is G-check, why do we suggest it, and who is expected to complete it?

This paper proposes a multi-faceted questionnaire (Table 1) to guide researchers, clinicians, ethics committees, and regulatory bodies in designing and evaluating interventional trials involving EVs. The questionnaire is applicable across all EV classifications (13, 14), including naïve, primed/stimulated cell-derived EVs, and bioengineered/modified EVs. Notably, bioengineered EVs may be categorized as either biotechnological products [akin to non-advanced therapy medicinal products (ATMPs)] or gene therapy medicinal products (GTMPs) under the ATMP umbrella (13-15). Given that EV relevance research is accompanied by numerous technical innovations, supplementary documents (e.g., intellectual property status of novel technologies, patents, and trademarks) would be beneficial to attach to this questionnaire upon need. While a wealth of research exists on the therapeutic potential of EVs, there is a lack of concise and practical clinical practice guidelines. The most relevant resource is the recently released EV checklist (https://ev-zone.org), which serves as a digital tool for standardized reporting of EV research, primarily focused on manuscript preparation (16).

Our proposed questionnaire mostly emphasizes on crucial requirements for conducting clinical trials using EV-based therapeutics. However, it is not intended to replace any of the standard clinical trial protocols or the guidelines set by the FDA and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (https://www.ich.org/page/ich-guidelines), and Good Clinical Practice (GCP) requirements. G-check is not an official guideline or a position paper; rather, it could be considered as a draft for such a piece in the future since it needs support from relevant, internationally reputable scientific associations. Some items (e.g., items 23 and 24) may seem irrelevant at first glance. Still, their consideration may help decide on repeated rounds of future EV- or combinatory cell- and EV-based treatments. It should be noted that questions regarding the immunological aspects of EVs' administration were considered since the application of EVs and the immune cells' function and responses may have some mutual effects (17).

Position papers and guidelines by the International Society for Cell and Gene Therapy (ISCT) Committee on the Ethics of Cell and Gene Therapy (ECGT) and ISEV were reviewed and incorporated into the checklist (18-21). We strongly recommend it to principal investigators, supervisors, and multidisciplinary boards of experts, at the designing, beginning, and recruitment phases of clinical trials. Since some information may be technically essential and confidential, access to completed forms may be restricted to the principal investigator (PI) and representatives of national or international regulatory bodies and/or board members of related companies who have completed the confidentiality forms.

G-check for clinical administration of EVs is a questionnaire of 100 questions composed of five parts, which are conceptually focused on:

A. General information regarding the study and the PI/s (10 items), B. Disease and patient (18 items), C. Type of study and ethical issues (24 items), D. Cell/source of EVs (16 items), and E. EVs (32 items).

Key lines (37 lines) and lines that seem obligatory (17 lines) are highlighted in light- and dark-grey, respectively. Some items are general and have common answers for all patients (93 lines), while others, indicated by bold underlined numbers, are patient-specific (7 lines). To improve the clarity, schematic representations with clear and detailed legends can be used instead of text for items 64, 66, 67, and 71. Filling out and the archival of this questionnaire for each EV-based therapy case would be beneficial in terms of clarifying and effective documentation of the process. This would also streamline the scientific and ethical evaluation of clinical studies by pertinent committees.

Does G-check need periodic corrections and updates?

To ensure the highest standards in stem cell and EV/ exosome research, we invite a diverse panel of experts from biomedical sciences and members of the international community actively involved in clinical applications of EVs to critically review and update the G-check (version 1.0) while citing this original version properly. This collaborative effort will prioritize patient-centric questions encompassing pathobiological, demographic, and molecular biological aspects. Beyond evaluating EV quality and processing, assessing the impact of interfering molecular pathways under various physiological and pathological conditions is crucial. We anticipate developing source-specific versions of the G-check, informed by previous and future recommendations from the ISEV, to enhance its practical applicability and facilitate its widespread adoption in the field.

What is the most expected and close-to-implementation utilization of G-check?

G-check is designed as a general platform to facilitate the community by offering exclusive versions of the questionnaire based on the application area, while also ensuring consistency across different applications. Current evidence suggests that G-check is particularly well-suited for evaluating EVs derived from primary (naïve cells directly isolated from tissue), conditioned (cells exposed to specific conditions or stimuli to be equipped with desirable characteristics) (22), or genetically modified/ manipulated mesenchymal stem/stromal cells (MSCs) (23). This preference arises from the extensive safety and efficacy evaluations conducted in numerous translational research studies and ongoing clinical trials (24-26). It seems that the amount of scientific data that exists in this field, in addition to the efforts that have been made in the past for proceeding with the MSC-based cell therapy procedures manufacturing, standardization, and commercialization, facilitate and promote the utilization of their EVs in comparison to other available sources (27). Among the various sources of EVs explored for therapeutic applications, those originating from MSCs are increasingly favored. This preference comes from their remarkable regenerative properties and their ability to modulate immune responses effectively. MSC-derived EVs possess a distinct cargo enriched with essential growth factors, cytokines, and RNA molecules, which is vital in promoting tissue repair and managing inflammation. Such a unique composition not only holds promise for addressing a broad spectrum of health conditions - ranging from cardiovascular diseases to neurological disorders - but also enhances safety by decreasing adverse immune reactions. Moreover, these vesicles facilitate intercellular communication and support cell survival in challenging environments, further reinforcing their status for innovative therapies (28). In brief, MSCs are a prominent focus in exosome-related studies, not only due to their exceptional capacity for producing significant amounts of EVs (29-31) but also because of their advanced stage in clinical translation and commercialization, positioning them as a promising avenue for developing innovative exosome-based therapies.

The FDA landmark approval of Ryoncil, an allogeneic bone marrow-derived MSC product, in December 2024 marks a pivotal moment for the field of MSC therapy. This achievement, celebrated by the ISCT, is poised to significantly invigorate research and development efforts across diverse therapeutic areas by fostering renewed enthusiasm and attracting substantial investment (32). In a recently published study, Figueroa-Valdés et al. (33) described the establishment of clinical-grade EVs from umbilical cord mesenchymal stromal cells from pre-clinical mouse studies to first-in-human intra-articular administration in the context of osteoarthritis.

Despite these advancements, the key challenges in EV production, including heterogeneity and scalability, must be addressed for effective manufacturing. Proposed solutions highlight the importance of standardization and quality control in EV production. Moreover, EVs from engineered cell lines are particularly advantageous, as they facilitate scaling and reproducibility. Establishing optimized protocols the EV isolation and storage is essential for ensuring consistency and quality. This involves standardizing reagents, selecting appropriate storage containers, and outlining specific storage requirements. In addition, it is key to apply robust quality management systems and utilize state-of-the-art facilities that comply with GMP, with a primary focus on ensuring the safety of both donors and patients (34, 35). Upon addressing these issues, the clinical translation of EV-based therapies would be successfully realized.

Table 1: The general checklist (G-check) for the administration of EVs in biomedical research applications and clinical trials (version 1.0)

	A. General information (10 items)	
1	Title of the study	
2	Main purpose	
3	Hypothesis	
4	Contact information of the principal investigator Name: Affiliation: Address: Postal code: Email: Tel: Pager: Fax:	
5	Ethical approval Name of the ethical committee that approved the study: Code/ID: Date of issue: IND number (available in the USA) or the equivalent code from other national approval systems:	
6	Multicenter study? Local or international study? Lead PI: Lead site: Who holds the primary approval?	☐ Yes ☐ No
7	Contact information of the primary coordinator of the study Name: Affiliation: Address: Postcode: Email: Tel: Pager: Fax:	
8	Will the study include the application of the cells in combination with EVs? Please describe.	☐ Yes ☐ No

Table 1: Continued

	A. General information (10 items)	
9	Describe all interventions and follow-up procedures considered for the current study in separate paragraphs. ▪ **Interventions** ▪ **Follow-ups**	
10	Does the principal investigator have direct control over the study's accuracy, the quality of the products, and the proper and timely follow-up? Please mention the follow-up duration and relevant details.	☐ Yes ☐ No
	B. Information about disease and patient (18 items)	
11	Disease name (indication), also known as	
12	Is it an auto-immune disease?	☐ Yes ☐ No
13	Have valid pre-clinical studies confirmed the use of this method in treating the disease?	☐ Yes ☐ No
14	Has this method been used for the disease in previous validated and registered clinical trials?	☐ Yes ☐ No
15	Does the disease have a genetic origin?	☐ Yes ☐ No
16	Is it necessary to ask the patient about the history of a particular disease in their family members? Please describe the rationale.	☐ Yes ☐ No
17	Do inflammatory events play a vital role in the pathology or progression of the disease?	☐ Yes ☐ No
18	Will the patient's inflammatory status (acute vs. chronic inflammation) be evaluated based on the standard quantitative assays before therapy administration?	☐ Yes ☐ No
19	Is the effectiveness of EVs in treating the disease or dampening the symptoms limited to their use over time or at a specific stage of the disease?	☐ Yes ☐ No
20	Is there any standard of care for the individuals introduced to the EV-therapy procedure? If yes, please describe.	☐ Yes ☐ No
21	Does the patient experience recurrent disease or intermittent attacks?	☐ Yes ☐ No
22	Does the patient have an underlying disease that may interfere with the treatment method? If yes, please describe.	☐ Yes ☐ No
23	Has the patient received any cellular product or cell-based therapeutic method previously? If yes, please describe.	☐ Yes ☐ No
24	Has the patient had a previous organ transplantation? If yes, please describe.	☐ Yes ☐ No
25	All defined inclusion criteria for the patient were reviewed by more than one health care professional (Min: 2 independent individuals).	☐
26	Does the inclusion criteria include quantitative criteria in addition to qualitative ones?	☐ Yes ☐ No

Table 1: Continued

	B. Information about disease and patient (18 items)	
27	Does the study use internationally accepted standard criteria to track the safety (phase I)/ safety and efficacy (phase II/ III) of the treatment strategy?	☐ Yes ☐ No
28	Will a set of qualitative and quantitative parameters be applied to track the effects of the treatment schedule? Please describe all primary and secondary outcome measurements.	☐ Yes ☐ No
	C. Type of study and ethical issues (24 items)	
29	Interventional study	☐ Yes ☐ No
30	What type of scientific study is this? Please specify.	
31	The phase of the study	
32	Number of participants	
33	National/international registration code of the trial	
34	Is there any conflict of interest for members of the research team? Please describe.	☐ Yes ☐ No
35	Which demographic data will be collected during the study? ☐ Age ☐ Ethnicity ☐ Gender/biological sex ☐ Genotype ☐ Social history ☐ Others, please describe.	
36	Do data from previous interventional studies support the safety of the method?	☐ Yes ☐ No
37	Do data from previous interventional studies support the efficacy of the method?	☐ Yes ☐ No
38	Will there be a public call to participate in the study, or is there another way to identify participants? If no, please describe.	☐ Yes ☐ No
39	Will the study be conducted on people of a particular gender or race? If yes, please explain the reason.	☐ Yes ☐ No
40	Will the patient receive standard medication while using this treatment? Provide a comprehensive report of the medications received by the patients.	☐ Yes ☐ No
41	An appropriate method is envisaged for the confidential recording of research data.	☐

Table 1: Continued

	C. Type of study and ethical issues (24 items)	
42	A suitable method for recording and archiving data has been considered so that the results can be scientifically and explicitly proven based on classified data and can be presented to legal observers at any time.	☐
43	Informed consent has been obtained from patients or their legal representatives.	☐
44	If a person refuses to participate in the study at any time, the patient will not be deprived of continuing treatment with standard methods.	☐
45	Are all interventions and follow-up procedures free of charge for the patients?	☐ Yes ☐ No
46	Is there any financial relationship between doctors and patients?	☐ Yes ☐ No
47	Is the patient categorized as a member of a vulnerable group? ☐ Children ☐ Elderly ☐ Mentally retarded ☐ Prisoners ☐ Addicted ☐ Illiterate ☐ Others. If yes, explain the urgent need to do research on these people.	☐ Yes ☐ No
48	Proper control groups are considered in the study. Please describe all control groups.	☐
49	Will the trial include an arm of patients treated with one of the existing standards of care?	☐ Yes ☐ No
50	Data related to any participant will not be excluded from the study, and the PI confirms honest reporting of possible undesirable data or events.	☐
51	As soon as observing an unusual event or the occurrence of unexpected symptoms, even in one patient, it will be reported, and the study will be paused to investigate the cause/causes of this event.	☐
52	Compensation for any damage to the patient resulting from participating in this study will be the responsibility of the lead PI.	☐
	D. Cell/Source of EVs (16 items)	
53	Please indicate which types of eukaryotic or prokaryotic sources will be applied for EV isolation. ☐ Prokaryotic ☐ Eukaryotic ☐ Single cell eukaryotic microorganisms/yeast ☐ Eukaryotic-Plant cells ☐ Eukaryotic-body fluids ☐ Eukaryotic-*ex vivo* tissue sample ☐ Eukaryotic-primary culture ☐ Eukaryotic-cell line (master cell bank) ☐ Eukaryotic-cell line (working cell bank)	

Table 1: Continued

	D. Cell/Source of EVs (16 items)	
54	If EVs will be isolated from any source other than primary or developed cell cultures (e.g., body fluids or tissue samples), please specify all relevant information regarding the below criteria. -Primary source and its initial characteristics: - Collection tube and considerations: - Pre-EV isolation storage condition: - Donor-relevant data (as far as they are available): Ethnicity: Gender/biological sex: Age: Genotype: Social history: Medication intake: Nutritional status: Metabolic parameters: Pregnancy/other complications: Possible consideration of circadian rhythms:	
55	Please indicate, Source and tissue origin of EV-producing cells: Passage number: Detailed method of passaging: Recovery time (if applicable): Initial cell seeding density: Doubling time: The volume of collected conditioned media: Data regarding sterility test for media (mycoplasma, bacteria, viruses, fungi, and…):	
56	Is the source autologous or allogenic?	
57	Is it possible to use autologous cells/source (or due to the genetic origin of the disease, allogenic ones are preferred)? Please specify if autologous cells are genetically edited before administration as the EV-producing source.	☐ Yes ☐ No ☐ Not confirmed
58	Is HLA-typing performed in the case of allogeneic cells/source? If not, please explain the reason.	☐ Yes ☐ No
59	Will cells be applied in addition to their EVs during the process? If yes, specify the type of cell, dose, and route of administration.	☐ Yes ☐ No
60	Is a detailed GMP-compliant process defined for the isolation/preparation of producer cells?	☐ Yes ☐ No

Table 1: Continued

	D. Cell/Source of EVs (16 items)	
61	Producer cells were fully characterized or their identity confirmed. Please describe.	☐
62	Which medium will be applied during cell culture? Please indicate the type, source, and concentration of all cell-culture supplements or pH buffering agent/s that will be applied during the process.	
63	Is the culture condition designed to be xeno-free and/or endotoxin-free? Please describe.	☐ Yes ☐ No
64	Are large-scale (3D) cell culture methods applied during the process? If yes, please describe the entire process.	☐ Yes ☐ No
65	Is monitoring the metabolic status of the producer cells required during the cell culture procedure?	☐ Yes ☐ No
66	Are the cells treated or modified during the process? If yes, please describe the entire process. Do the priming or modification methods pass the GMP and GCP criteria? Please describe.	☐ Yes ☐ No
67	Will the application of viral-based methods accompany these modifications? If yes, please describe the entire process.	☐ Yes ☐ No
68	Are there any safety or ethical concerns over modifications? Please explain if yes.	☐ Yes ☐ No
	E. Extracellular vesicles/exosomes (32 items)	
69	What type of EV/EV subtypes will be applied during the process? Please write the exact name (based on the updated terms and definitions of the ISEV) and size range. Which medium/solution will be used for the storage of EVs?	
70	Which type of conditioned medium harvest is considered to isolate EVs: ☐ Single harvest ☐ Multiple harvest ☐ Continuous harvest Please determine the complete or partial collection of the conditioned medium and pooling strategy if applicable.	
71	The isolation process will be performed based on the following method: ☐ Ultracentrifugation ☐ Sucrose gradient centrifugation ☐ Polymer precipitation ☐ Tangential flow filtration (TFF) ☐ HPLC ☐ Microfluidics ☐ Commercial method ☐ Others, please describe. Please describe the detailed protocol (Washing steps, Filtration, centrifugation speed, duration, rotor type if applicable, and any extra purification steps, etc.) Please specify if EVs are produced by these methods in GMP-grade facilities.	
72	Which medium will be applied during the collection of the conditioned medium, and the storage of EVs, respectively? Is the collecting medium considered to be an EV-depleted medium? Please describe.	

Table 1: Continued

	E. Extracellular vesicles/exosomes (32 items)	
73	EV preparation/characterization guidelines followed in this study and detection limit of different assays in addition to positive or negative controls will be reported whenever possible.	☐
74	Will any toxicology studies, including genotoxicity, tumorigenicity, reproductive toxicity, developmental toxicity, or immunotoxicity, be conducted on the EV preparations? Please describe.	☐ Yes ☐ No
75	Are preparation/characterization steps (including morphological evaluations, particle size measurements, protein content assessments, EV markers, etc.) documented?	☐ Yes ☐ No
76	Electron or atomic force microscopy will be applied during the characterization steps.	☐ Yes ☐ No
77	Nano tracking analysis (NTA) or any other clinically accepted method will be applied to quantify the exact amount of the vesicles before administration.	☐
78	Which method will be used to determine the protein, lipid, or nucleic acid content of EVs? Proteins: Lipids: Nucleic acids: Others, please describe:	
79	EVs will be exposed to any enzymatic or non-enzymatic treatments. If yes, please describe.	☐ Yes ☐ No
80	Will vesicles be evaluated to be negative for non-specific EV markers?	☐ Yes ☐ No
81	In addition to the EV-specific markers, the presence of some cell-specific markers will also be evaluated in the final EV preparation. Please describe.	☐ Yes ☐ No
82	Are EV preparations checked for tissue factor activity?	☐ Yes ☐ No
83	Will any potency assay be performed before the administration of EVs? If yes, please describe.	☐ Yes ☐ No
84	Will EVs be loaded with particular contents? If yes, please describe.	☐ Yes ☐ No
85	Will EVs be produced by primed/genetically modified cells? If yes, please describe.	☐ Yes ☐ No
86	Is a specific method or compound used to induce particle production by the cells? If yes, please describe.	☐ Yes ☐ No
87	Has this method (Q. 86) been clinically approved?	☐ Yes ☐ No
88	Will the final product be assessed for cellular component and residue contamination?	☐ Yes ☐ No
89	Will the final product be tested for any viral residue?	☐ Yes ☐ No
90	Will the final preparation be evaluated to be endotoxin- and pathogen-free?	☐ Yes ☐ No
91	Will the final preparation be evaluated to be free from chemical contamination?	☐ Yes ☐ No
92	Will the final preparation be evaluated regarding the presence of soluble proteins and non-EV particles? Please describe.	☐ Yes ☐ No

Table 1: Continued

	E. Extracellular vesicles/exosomes (32 items)	
93	EVs will be prepared freshly, or will they be stored before their administration? In the case of storage, please specify the items below. Temperature: Duration: Freezing media contents: Possible initial snap freezing: Storage vessel composition/make up: Number of possible freeze-thaw rounds: Pre- and post-storage comparative quality check analyses: Concentration and number of EVs before and after freezing Sterility studies before and after freezing (endotoxin, bacteria, fungi, mycoplasma, and…) Please specify if stability studies were performed at different time points.	
94	Are EVs stained/labeled, lyophilized, or modified by any other strategy following their isolation steps? ☐ Stained/labeled Lyophilized ☐ Others. Please describe.	☐ Yes ☐ No
95	Are EVs produced in the same place where they should be applied to the patient? If it is essential to transport EVs, which conditions are mandatory? Temperature: Humidity: Light/dark consideration: Others, please describe:	☐ Yes ☐ No
96	Will the quality and stability of EVs be evaluated following these modifications?	☐
97	Will EVs be applied with additional compounds, such as hydrogels or as encapsulated particles? If yes, please describe.	☐ Yes ☐ No
98	Was the utilization of these compounds previously approved?	☐ Yes ☐ No
99	EVs will be administered (Route of administration) ☐ Systemically ☐ Locally ☐ Inhalation Please describe the detailed protocol.	
100	Please indicate the dose and time intervals of EV administration Dose: Time intervals: Complementary Notes:	

EVs; Extracellular vesicles, GMP; Good manufacturing practice, HLA; Human leukocyte antigen, HPLC; High-performance liquid chromatography, IND; Investigational new drug application, ISEV; International society for extracellular vesicles, NTA; Nanoparticle tracking analysis, PI; Principal investigator, and TFF; Tangential flow filtration.

The growing interest in the therapeutic potential of EVs within regenerative and personalized medicine underscores the necessity for standardized administration protocols. The variability in EV isolation, characterization, and application highlights significant challenges that need to be addressed for reliable clinical outcomes. By proposing a comprehensive checklist, this initiative aims to provide researchers and clinicians with essential guidelines to enhance trial standardization and improve the reproducibility and transparency of EV-related studies. Ultimately, these efforts are crucial for advancing the safe and effective application of EVs in clinical practice, facilitating their translation from laboratory research to therapeutic interventions.

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Authors' Contributions

A.R.B., A.H.; Conceptualization. A.H., M.K.N.; Writing-original draft. H.H., F.Sh., M.M.M., R.L., H.R.B.; Writing-review and editing. All authors approved the final version of the manuscript.

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