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Concise Review: Extracellular Vesicles Overcoming Limitations of Cell Therapies in Ischemic Stroke

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ABSTRACT

Despite recent advances in stroke therapy, current therapeutic concepts are still limited. Thus, additional therapeutic strategies are in order. In this sense, the transplantation of stem cells has appeared to be an attractive adjuvant tool to help boost the endogenous regenerative capacities of the brain. Although transplantation of stem cells is known to induce beneficial outcome in (preclinical) stroke research, grafted cells do not replace lost tissue directly. Rather, these transplanted cells like neural progenitor cells or mesenchymal stem cells act in an indirect manner, among which the secretion of extracellular vesicles (EVs) appears to be one key factor. Indeed, the application of EVs in preclinical stroke studies suggests a therapeutic role, which appears to be noninferior in comparison to the transplantation of stem cells themselves. In this short review, we highlight some of the recent advances in the field of EVs as a therapeutic means to counter stroke. STEM CELLS TRANSLATIONAL MEDICINE *2017;00:000–000*

SIGNIFICANCE STATEMENT

Despite recent success in therapeutic approaches against stroke, especially in the field of endovascular therapy, additional therapeutic means are still in order. In this sense, the application of extracellular vesicles might be an interesting tool to induce postischemic neuroregeneration, overcoming the limitations and risks of stem cell transplantation themselves.

STATE OF THE ART STROKE TREATMENT

Ischemic stroke treatment currently involves three concepts: The admission of stroke patients to stroke units, the application of thrombolytics, and the recanalization of the occluded vessel by endovascular clot removal [1–4]. With the first stroke units being introduced in the 1990s, stroke management has turned from a purely observational field toward an evidence based therapeutic field. Controlled randomized studies not only demonstrated the utility of the thrombolytic recombinant tissue plasminogen activator to improve stroke outcome when administered intravenously within 4.5 hours after symptom onset [5], but more recently revealed the efficacy of endovascular recanalization therapy [1, 2]. Despite this great success, the majority of patients receive none of the two aforementioned treatments, partially because of narrow time windows or because of significant complication risks. This justifies the need for additional treatments, which alleviate the long-term consequences of a stroke.

POST-STROKE BRAIN REPAIR

With strategies on brain protection having failed in clinics in the 1980s and 1990s, current preclinical research strongly focuses on promoting the regenerative capacities of the ischemic brain. The physiological basis of the latter is the persistence of endogenous neurogenesis in the adult mammalian brain within so called stem cell niches, namely the subventricular zone (SVZ) of the lateral ventricles [6–8] and the subgranular zone of the dentate gyrus [9, 10]. Upon stroke, neural progenitor cells (NPCs) within the SVZ migrate toward the ischemic lesion site where they proliferate [11, 12]. Yet, the stroke-induced promotion of poststroke neurogenesis has restricted functional relevance, as new-born cells show both low survival rates and poorly differentiate into mature neurons [13–15].

In order to use the endogenous regenerative potential of the ischemic brain, two different strategies to manipulate neurogenesis are under investigation: (a) enhancing the resistance of NPCs to delayed degeneration and (b) augmenting the number of NPCs in the ischemic brain tissue. The former can be achieved by the administration of antiapoptotic drugs [14, 16], the latter is thought to be accomplished by stimulating NPC proliferation or by transplantation of exogenous NPCs. Although transplantation of

Table 1. Preclinical studies and clinical trials on systemic poststroke delivery of MSCs and NPCs

Species	Cell type	Delivery timing	Key results	References
Mouse	Umbilical cord MSCs	Within 30 minutes	Reduction of brain injury & modulation of TGF expression	[23]
Rat	Adipose-derived MSCs	Within 24 hours	Reduction of brain injury/improved motor coordination	[24]
Rat	Adipose-derived MSCs (i.ventr./i.v./i.a.)	Within 24 hours	Reduction of brain injury/improved motor coordination	[25]
Rat	BM-derived MSCs	Up to 1 month	Increased angiogenesis and better neurological recovery	[26]
Rat	Placenta-derived MSCs	24 hours vs. 8 + 24 hours	increased neurological recovery	[27]
Rat	BM-derived MSCs (i.a.)	d2 and d7	Increased angiogenesis and homing/no effect on neurological recovery	[28]
Rat	BM-derived MSCs	3 hours	Reduction of brain injury/improved functional outcome	[29]
Rat	BM-derived MSCs	24 hours	Increased angiogenesis	[30]
Rat	NPCs (i.a./i.v./i.c.)	24 hours	Migration and distribution patterns depend on delivery routes	[31]
Mouse	NPCs	d7	Reduced brain injury/improved neurological recovery	[32]
Mouse	NPCs	6 hours	Improved neurological recovery	[33]
Mouse	NPCs	Up to 1 month	Reduced brain injury/increased tissue regeneration/ improved functional recovery	[34]
Mouse	NPCs (i.v./i.a./i.s./ i.ventr./i.cort.)	6 hours (i.v.	Sustained reduction of brain injury after systemic transplantation	[35]
Rat	NPCs	24 hours	Reduced tissue injury and better neurological score	[36]
Human Phase II	Adipose-derived MSCs	Within 2 weeks	Recruiting patients	[37]
Human Phase I/II	BM-derived MSCs (i.a.)	Between 5–9 days	No safety concerns/no better outcome after 6 months	[38]
Human	BM-derived MSCs	Within 1 week after randomization	No safety concerns/better outcome for some scores	[39]
Human	BM-derived MSCs	36–133 days poststroke	No safety concerns within 1 year	[40]
Human	BM-derived MSCs	3–12 months poststroke	No safety concerns within 24 weeks	[41]
Human	BM-derived MSCs	3–24 months poststroke	No safety concerns within 24 weeks/improved Barthel index	[42]

This list does not intend to be complete. It reflects a selection of representative studies where MSCs or NPCs have been applied systemically after stroke, that is, intravenously (if not stated otherwise) or intraarterially. Studies using stereotactic transplantation are excluded.
Abbreviations: BM, bone marrow; i.a., intraarterial delivery; i.c., intracisternal delivery; i.cort., intracortical delivery; i.v., intravenous delivery; i.ventr., intraventricular; MSCs, mesenchymal stem cells; NPCs, neural progenitor cells; TGF, transforming growth factor.

stem cells improves poststroke symptoms, grafted stem cells do not replace cells lost in injured tissue. Rather, grafted stem cells act in an indirect manner, very likely by releasing trophic and anti-inflammatory factors that promote the survival, remodeling, and plasticity of the ischemic brain tissue [17–19].

Considering the paracrine nature of stem cell-mediated beneficial effects, the choice of stem cell source might not be essential for achieving recovery-promoting effects of cellular therapeutics. As a matter of fact, in addition to NPCs stem cells derived from various adult tissues have been found to promote restorative effects in the ischemic brain [18, 20–22]. Especially due to their broad availability, their simple handling and their low side effects, bone marrow-derived mesenchymal stem cells (MSCs) became an attractive cell source to treat ischemic stroke in a number of different preclinical models.

Transplantation of MSCs and NPCs After Stroke

Preclinical transplantation studies in a plethora of stroke models using MSCs or NPCs have shown beneficial effects (Table 1) in a large number of different readouts [23, 26–36, 43–45]. NPCs, either administered intracerebrally or systemically, mediate neuroprotection and enhance neurological recovery via stimulation of endogenous angiogenesis and neurogenesis. The mechanisms involved in the process of NPC-induced brain protection and brain regeneration greatly depend on both cell delivery routes and cell delivery timing [34, 35]. For example, acute NPC transplantation reduced neuronal injury and infarct volume, while transplantation at later stages rather modifies poststroke brain regeneration and neuronal plasticity.

Likewise, the transplantation of MSCs, which have been administered systemically in the majority of studies, revealed promising effects in experimental stroke models. MSC transplantation was found to reduce neuronal injury and infarct volume, increase angiogenesis and neurogenesis, and improve neurological recovery. Although a majority of studies has been performed on BM-derived MSCs, some studies imply the application of adipose-derived MSCs which might appear to be an attractive cell type as well [24, 25], since the latter is easy to obtain. Due to their beneficial effects in the preclinical models, controlled randomized clinical trials (Table 1) using MSCs (and to a lesser extent NPCs as well) for stroke treatment have been started [38–40, 46]. Although patient recruitment is so far low, which precluded more

final conclusions from these studies, some studies reported beneficial outcomes after MSC transplantation. Of note, no clinically relevant side effects within the observation periods of maximal 5 years have been observed.

At the mechanistic level, it was initially proposed that applied MSCs enter the damaged tissue and replace lost cell types. However, in preclinical stroke as well as in other disease models, MSCs are hardly detected in affected tissues [47–52]. Most of systemically applied MSCs get trapped within the lungs [53, 54]. Due to these observations, the initial idea that MSCs can replace cells in affected tissues or directly interact with target cells became challenged, and the hypothesis emerged that MSCs effectively act in a paracrine rather than a cellular manner [55].

Structure of Extracellular Vesicles and Biological Properties

Whereas early studies proposing a paracrine mode of action of administered MSCs claimed that soluble factors, such as growth factors or cytokines, mediate the stem cells' beneficial therapeutic effects [47]; more recent data qualified extracellular vesicles (EVs) as the critical agents [56]. Indeed, MSC-derived EVs (MSC-EVs) mediating therapeutic activities have been documented in a variety of different preclinical models and in a GvHD patient as well [49, 56–59].

EVs are released by almost all cell types and are detected as membrane-surrounded vesicles in all body fluids [60]. According to their origin, different EV types can be discriminated [61]. Exosomes are derivatives of the late endosomal compartment and have diameters of 70–150 nm. They correspond to intraluminal vesicles (ILVs) that are formed by the inward budding of the limiting membrane of sorting and late endosomes. The ILV containing endosomes are called multivesicular bodies (MVBs) or multivesicular endosomes. At the example of maturing reticulocytes, it has been shown that MVBs can fuse with the plasma membrane and release their ILVs as exosomes into the extracellular compartment [62–64]. In contrast, microvesicles (MVs), which have diameters of 100–1,000 nm, are formed as bud offs of the plasma membrane; together with apoptotic bodies which have said sizes of 500 nm to several micrometers, exosomes and MVs form the most prominent EV subtypes [65].

EVs contain specific molecular signatures reflecting their cell of origin [60, 66, 67]. Apart from lipids and proteins, metabolites and nucleic acids are recovered in prepared EV fractions [68–70]. A proportion of EVs might contain molecules that cells cannot metabolize, which are released into the extracellular environment for further processing. Other EVs seem to be assembled in a tailored manner to act as intercellular communication vehicles mediating complex signal exchanges between cells within and between different organs [60, 61, 71].

Preclinical Studies Using EVs in Animal Models Unrelated to Ischemia

In recent years, EVs have made a tremendous progress in biomedical research. At first, EVs were considered as debris. In 1996, however, Raposo and colleagues showed that B cells release MHC-II containing EVs which can activate T cells [72]. Yet, until the finding that EVs contain RNAs, in 2006 and 2007 [68, 70], EV research was sparse. Thereafter, the EV field started to grow exponentially. Positive therapeutic effects of MSC-EVs were reported for the first time in 2009; the group of Giovanni Cammussi described EV-mediated therapeutic activities in a kidney failure model [59]. In 2010, the group of Sai Kiang Lim and Dominque de Kleijn discovered cardioprotective activities in their MSC-EV fractions [49]. We were the first group who applied MSC-EVs to a human patient in an individual treatment attempt. We applied an allogeneic MSC-EV fraction to a steroid refractory Graft-versus-Host Disease patient, who failed to react on several second side strategies. Remarkably, the clinical symptoms declined during and after the 2-week lasting MSC-EV therapy significantly, without revealing any side effects [57]. Meanwhile, EVs have been applied to several preclinical diseases models unrelated to ischemia, with some of them mentioned in Table 2.

The therapeutic benefit of EVs has been analyzed in various disease conditions, including inflammatory processes and cancer models. Similar to stem cells derived from different tissues, stem cell derived EVs exert multiple effects on different target cells. Similar to stem cells derived from different tissue sources inducing a variety of actions in biological tissues, EVs depending on their stem cell source have multiple effects on target cells, which may show overlaps, but also differences between cell sources. The latter is vital in understanding the different beneficial effects that EVs can yield. As such, EVs from a certain cell might show beneficial effects in a variety of malignant diseases like hepatocellular carcinoma, gastric cancer or brain tumor, but not be equivalent in their cellular actions. Although a direct comparison between these studies is not eligible due to different study designs, EVs might either have a direct impact on tumor formation or enhance sensitivity to chemotherapy [75, 84, 86, 96]. Similar evidence for overlapping effects of EVs came from studies in inflammatory/infectious conditions, such as arthritis, hepatitis C, HIV, and sepsis [74, 87, 93, 98, 99]. One has to stress that several observations are still limited to in vitro research only. Particularly important from the authors' point of view, EVs have successfully been used in preclinical neurodegenerative disease models, such as amyotrophic lateral sclerosis and Parkinson's disease, as well as in myasthenia gravis where EVs were found to modulate inflammatory responses and cell survival [73, 82, 88]. Further evidence for a role of EVs in modulating inflammatory responses and tissue regeneration was found in animal models of traumatic brain injury and skin wounds [91, 92].

Preclinical Studies Using EVs in Animal Models Associated with Ischemia

More recent studies identified the therapeutic efficacy of EVs in experimental conditions mimicking peripheral limb, heart or brain ischemia, that is, in models of peripheral occlusive artery disease, myocardial infarction and stroke (Table 3). For myocardial ischemia, the therapeutic efficacy of EVs has been shown in a large number of in vitro and in vivo studies [49, 101–111]. Thus, EVs from various cell sources including MSCs and embryonic stem cells, promoted cellular survival, reduction of infarct size, and stimulated myocardial remodeling and angiogenesis. Of note, these EV actions were associated with functional recovery evaluated by ejection fraction.

To the best of the authors' knowledge, six different studies have examined effects of EVs in ischemic stroke models, most in rats and one in mice [112–114, 116–118]. In the first rat study,

Table 2. Therapeutic application of EVs in preclinical disease models unrelated to ischemia

Disease condition	In vitro/in vivo	EV source	Key results	References
Amyotrophic lateral sclerosis	In vitro	Adipose-derived stem cells	Alleviation of SOD1 and mitochondrial dysfunction	[73]
Hepatitis C	In vitro	Umbilical MSCs	Antiviral activity by microRNA transport	[74]
Cancer therapy	In vivo (mice)	Modified melanoma cells	Suppression of tumor growth	[75]
Osteochondral disease	In vivo (rats)	Embryonic MSCs	Increased cartilage repair	[76]
Head and neck cancer cells	In vitro	(Ir)radiated head and neck cancer cells	Increased survival of irradiated tumor cells	[77]
Chemotherapy-induced POF	In vitro/in vivo (mice)	Amniotic fluid stem cells	Prevention of ovarian follicular atresia	[78]
Diabetic nephropathy	In vivo (rats)	Human urine-derived stem cells	Increased cell survival/vascular regeneration	[79]
Osteoporosis	In vitro/in vivo (rats)	Human-induced pluripotent stem cell-derived MSCs	Enhanced bone regeneration	[80]
Endothelial regeneration	In vitro	EPCs	Increased re-endothelialization	[81]
Myasthenia gravis	In vivo (rats)	Atorvastatin-modified BM-derived DCs	Suppression of immune responses	[82]
Traumatic brain injury	In vivo (mice)	MSCs	Reduced inflammation and cognitive impairment	[83]
Hepatocellular carcinoma	In vitro/in vivo (rats)	Modified adipose tissue-derived MSCs	Increased sensitivity to chemotherapy	[84]
Experimental colitis	In vivo (rats)	MSCs	Attenuation of inflammation	[85]
Gastric cancer	In vitro	MSCs	Increased drug resistance	[86]
Arthritis	In vivo (mice)	Bovine milk	Diminished cartilage pathology/reduced inflammation	[87]
Parkinson's disease	In vitro	Dental pulp stem cells	Reduced apoptosis	[88]
Carrageenan-induced inflammation	In vivo (mice)	Human dental pulp stem cells	Suppressed inflammation	[89]
Skin burn	In vitro/in vivo (rats)	Human umbilical cord MSCs	Increased angiogenesis in wounded tissue	[90]
Cutaneous wounds	In vivo (rats)	Human induced pluripotent stem cell-derived MSCs	Promotion of collagen synthesis and angiogenesis	[91]
Traumatic brain injury	In vivo (rats)	MSCs	Enhanced neurological recovery/increased angiogenesis and neurogenesis	[92]
HIV infection	In vitro	Breast milk	Inhibition of infection of monocyte-derived DCs	[93]
Endotoxin-induced lung injury	In vivo (mice)	MSCs	Reduced inflammatory response	[94]
Cisplatin-induced kidney injury	In vitro/in vivo (rats)	Human umbilical cord MSCs	Reduced cell injury/increased cell proliferation	[95]
Brain tumor	In vivo (rats)	MSCs	Reduced glioma growth	[96]
Liver fibrosis	In vitro	Human umbilical cord MSCs	Reduced liver fibrosis	[97]
Sepsis	In vivo (rats)	DCs	Decreased release of cytokines/reduced mortality	[98]
Arthritis	In vivo (mice)	Modified DCs	Anti-inflammatory actions	[99]

This list does not intend to be complete. It reflects a selection of studies based on their influences on the development of this field.
Abbreviations: ALS, amyotrophic lateral sclerosis; BM, bone marrow; CTx, chemotherapy; DCs, dendritic cells; EPCs, Endothelial progenitor cells; HIV, human immunodeficiency virus; MSCs, mesenchymal stem cells; POF, premature ovarian failure; SOD1, superoxide dismutase.

Chopp and colleagues [113] intravenously applied MSC-EVs in a model of transient intraluminal middle cerebral artery occlusion. EVs were administered via tail vein injection at 24 hours post-stroke. The authors observed a significant reduction of brain injury and neurological impairment that was associated with enhanced postischemic neurogenesis. In the hitherto only mouse study, we studied effects of MSC-derived EVs in transient intraluminal middle cerebral artery occlusion. Using the polyethylene glycol (PEG) method EVs were enriched from MSC conditioned media. MSCs were raised from BM samples of two healthy bone marrow donors; as serum supplement 10% human platelet lysate was used [119, 120]. MSC-EVs were administered at days 1, 3, and 5 poststroke. The treatment enhanced neurological recovery and increased endogenous neurogenesis and angiogenesis, at the same time reversing stroke-induced peripheral immunosuppression. In a head-to-head comparison, the therapeutic potential of MSC-EVs was comparable to that of the transplanted MSCs from which the MSC-EVs were derived [112].

A more recent rat study examined the effects of MSCs combined with MSC-EVs [114], demonstrating that combined MSC

Table 3. Therapeutic application of EVs in preclinical disease models associated with ischemia

Disease condition	In vitro/in vivo	EV source/EV isolation	Key results	References
Limb ischemia	In vivo (mice)	Human-induced pluripotent stem cell-derived MSCs/UC	Promotion of angiogenesis	[100]
Myocardial ischemia[a]	In vitro	MSCs/Exo-Quick	Increased survival of cardiomyctes	[101]
Myocardial ischemia	In vivo (rats)	MSCs/Exo-Quick	Increased angiogenesis/ reduced inflammation	[102]
Myocardial ischemia	In vivo (rats)	Umbilical cord MSCs/UC	Improved systolic function	[103]
Myocardial ischemia	In vitro/in vivo (mice)	Cardiac fibroblast-derived iPS cells/UC	Increased myocardial survival	[104]
Myocardial ischemia	In vivo (rats)	Embryonic stem cells/UC	Increased myocardial regeneration	[105]
Myocardial ischemia[b]	In vitro (rats)	Coronary perfusates after remote pre-conditioning/UC	Reduction of infarct size	[106]
Myocardial ischemia	In vitro/in vivo (rats)	Plasma from rats and humans/UC	Cardioprotection	[107]
Myocardial ischemia	In vitro	GATA-4 overexpressing MSCs/UC	Cardioprotection	[108]
Myocardial ischemia	In vitro/in vivo (rats)	MSCs/UC	Increased angiogenesis/systolic function	[109]
Myocardial ischemia	In vivo (mice)	MSCs/HPLC	Reduced infarct size	[110]
Myocardial ischemia	In vitro/in vivo (mice)	Cardiac progenitor cells/UC	Increased survival of cardiomyocytes	[111]
Myocardial ischemia	In vivo (mice)	Human embryonic stem cell-derived MSCs/HPLC	Reduction of infarct size	[49]
Stroke	In vivo (mice)	MSCs/PEG	Neurological recovery/increased angiogenesis and neurogenesis/reversal of peripheral postischemic immunosuppression	[112]
Stroke	In vivo (rats)	MSCs/UC	Enhanced neurological recovery/angiogenesis and neurogenesis	[113]
Stroke	In vivo (rats)	Adipose derived MSCs/UC	Reduction of infarct volume/increased neurological recovery	[114]
Stroke	In vivo (rats)	Adipose derived MSCs/miRCURY	Increased functional recovery/ neuroplasticity/white matter repair	[115]
Stroke	In vivo (rats)	MSCs/UC	Enhanced neuroplasticity/increased neurological recovery	[116]
Stroke	In vitro/in vivo (rats)	miR-133b-overexpressing MSCs/UC	Secondary EV release by astrocytes/increased neural plasticity and neurological recovery	[117]
Stroke	In vivo (mice)	Embryonic stem cells/UC	Reduction of poststroke inflammation/ restoration of neurovascular unit	[118]

[a]EVs administered in a prophylactic manner, that is, prior to ischemia.
[b]EVs were given as coronary perfusates from rats exposed ischemic pre-conditioning.
Abbreviations: HPLC, high performance liquid chromatography; iPS, induced pluripotent stem cells; MSCs, mesenchymal stem cells; PEG, polyethylene glycol; UC, ultracentrifugation.

and MSC-EV delivery was superior in terms of brain protection and neurological recovery when compared with MSC transplantation or EV injection only. These studies raised the question of how therapeutic effects of EVs may be boosted by loading naïve EVs with biologically active molecules such as noncoding RNAs, which by means of EVs may safely be transported to target tissues [121]. In rats exposed to transient middle cerebral artery occlusion, increased neural plasticity and neurological recovery were noted after delivery of EVs obtained from miR-133b overexpressing MSCs when compared with EVs obtained from naïve MSCs [117]. In vitro experiments using oxygen-glucose-deprivation suggested that the enhanced action of miR-133b containing EVs may be due to stimulation of secondary EV release from astrocytes [117]. In another study, EVs harvested from MSCs transfected with a miR-17-92 cluster plasmid induced better neurological recovery when compared with EVs derived from naïve MSCs [116]. These observations stress the heterogeneity of EV actions depending on the loading of EVs with survival and plasticity promoting molecules.

Clinical Studies Using EVs in Humans

Despite an increasing body of evidence demonstrating that EVs might serve as biomarkers for stroke outcome [122], there is currently no study in which EVs (and especially MSC-EVs) have therapeutically been administered to human stroke patients. According to the promising data obtained in a variety of different animal models and the very promising result of the individual treatment attempt of a GvHD patient with MSC-EVs, a number of groups now try to translate EVs into the clinics. As EVs are novel biological agents and MSC-EVs are not considered as Advanced Therapy Medicinal Products (ATMP), they provide a new class of biologicals, for whose production no concrete rules have been defined by the FDA or any other national regulatory agency, yet. To this end, experts in the field have summarized in an International Society of Extracellular Vesicles (ISEV) position paper the different therapeutic EV-application fields, discussed their regulatory status and recommended requirements to be fulfilled to translate EVs as therapeutic agents into the clinics [56].

Current Limitations and Benefits of EV-Based Treatment Paradigms

Despite their different origin and their different proposed sizes, EV subtypes cannot be discriminated during isolation until now. Thus, the ISEV agreed in 2014 to name fractions proposed to contain exosomes, MVs, apoptotic bodies and/or other EV types appropriately as EV fractions [123]. Since EV fractions contain a heterogeneous mix of different EV types, care has to be taken, of how EVs are purified and characterized. As such, the application of differential centrifugation (i.e., ultracentrifugation) is hampered by a low EV output due to restricted sample volumes in comparison to other techniques like size exclusion chromatography [124]. In this sense, the recently identified observation of low density lipoprotein contamination after EV enrichment might pose a problem for the evaluation of past and future work when dealing with mechanistic approaches [125]. On the contrary, for pure therapeutic applications, contaminations might be tolerated. Despite a plethora of different enrichment techniques available, ultracentrifugation, however, remains to be the gold standard for EV enrichment, albeit other techniques such as PEG isolation provide some advantages (own unpublished observation). Consequently, the ISEV has released consensus recommendations on EV purification and characterization [123]. Still, several studies do not follow these recommendations, making it difficult to compare research outcomes. To increase the reliability of the data and to promote standardization in the field the EV-TRACK consortium was formed which defined several criteria to score EV-based studies that will hopefully be followed in the future [126].

Furthermore, caution has to be taken when interpreting studies from both the stem cell and the EV field. Comorbidities and comedications, for instance, might modulate experimental outcomes. As such recommendations—especially from the cardiologic field—have been made in order to overcome typical pitfalls of cell-based therapies [127–129]. The latter emphasize the necessity of selecting the appropriate cell type or components of the secretome depending on the endpoint chosen and the definition of the application mode, including the amount of applications, the application timing and the delivery routes, to name but a few.

As EVs lack nuclei they cannot self-replicate and thus in contrast to cells do not contain any endogenous tumorigenic potential. In addition, EVs are easier to handle and, due to their small size, they can be sterilized by filtration [56]. Thus, EV-based therapeutics provide several advantages over cellular therapeutics, resulting in a competition between several research groups to produce MSC-EVs for the clinical setting. There are several challenges connected to this issue. On the one hand, large volumes have to be processed under good medical practice compliant conditions to obtain sufficient material to treat a patient. Then, as MSCs provide a heterogeneous cell entity, MSC-EV fractions may show varying therapeutic activities as well. Indeed, the authors detected significant differences in the cytokine profile of independent MSC-EV preparations during their own research activities [57].

Conclusion

The application of stem cell derived EVs, especially that of MSC-EVs, offers a great opportunity for adjuvant stroke treatment. For now, EVs appear to be safe in mammals and potentially also in man, thus avoiding putative side effects that are inherent to stem cell transplantation such as malignant stem cell transformation. Besides, tissue engineering techniques allow the usage of EVs as potent carriers for bioactive molecules, which may be used for overcoming tissue barriers such as the blood-brain barrier for targeting distinct cell populations [56]. Yet, fundamental questions as to their exact mode of action and their optimal enrichment, characterization, and storage have to be answered to optimize them for the clinical setting [56].

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Author Contributions

T.R.D., M.B., D.M.H., and B.G.: manuscript writing, final approval of the manuscript.

Disclosure of Potential Conflicts of Interest

The authors declare to have no conflict of interest.

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