Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Exosomes

Evidence Summary

Exosomes have three major potentials: as biomarkers, therapeutics, and delivery vehicles of therapeutics. Techniques for detection, isolation, and storage are still being optimized.

Neuroprotective Benefit: Exosome cargos have been extensively studied as biomarkers of Alzheimer's disease, but research to utilize exosomes as a therapeutic is still in its infancy.

Aging and related health concerns: A few phase I/II clinical trials have shown anti-tumor responses, though these studies were small and results are still preliminary. Cardioprotective actions have only been examined in laboratory settings.

Safety: So far, small trials in cancer patients have reported mild adverse events, though one patient exhibited grade III liver toxicity. Because there are yet to be identified contents of exosomes, side effects are not entirely predictable.

Availability: Not available commercially.	**Dose**: Varies across and within studies. Median exosomal protein quantity in a lung cancer study was 193 µg per injection.	**Size:** 40-100 nm vesicles
Half-life: Not established and likely depend on preparation; An i.v. formulation may have a half-life of a few minutes to hours.	**BBB**: Penetrant	
Clinical trials: 4 clinical trials testing dendritic cell-derived exosomes for cancer therapy included a total of 86 patients.	**Observational studies**: Only biomarker studies: in AD studies, a total of 1,268 subjects have been examined across 12 studies.	

What is it? Exosomes are extracellular vesicles (40-100 nm in diameter) that are released from cells upon fusion of the multivesicular body (MVB; an intermediate endocytic compartment) with the plasma membrane (Ha et al., 2016). Before release, the vesicles within the MVB are called intraluminal vesicles (ILVs).

Exosomes are composed of various types of proteins, such as major histocompatibility complex (MHC)-II, integrins (adhesion molecules that facilitate cell binding to the extracellular matrix), heat shock proteins (Hsp70, Hsp90), and tetraspanins (CD9, CD63, CD81, and CD82). They are involved in long-range intercellular communication and transmission of macromolecules, including proteins, lipids, mRNAs, miRNAs, and DNA. Membrane proteins on exosomes may target them to specific cells and tissue. They have also been implicated in transmission and spreading of diseases, as they can contain disease-associated cargos. For example, in Parkinson's disease, exosomes can transport misfolded proteins from unhealthy neurons to nearby cells, spreading the disease from cell to cell (Ha et al., 2016). Therefore they also have potential as biomarkers.

Mesenchymal stem cells (MSCs) show therapeutic properties in wound healing, inflammation, hypertension, cardiovascular disease, brain injury, and cancer (Reiner et al., 2017). Recent studies suggest that these benefits are partly mediated by MSC-derived microvesicles/exosomes (Phinney and

Pittenger, 2016). These studies have spurred a new line of research called cell-free regenerative medicine. Other preparations of exosome therapies are also being explored in anti-cancer clinical trials (Edgar, 2016).

Exosomes are also drawing attention as a potential delivery vehicle of therapeutics (Ha et al., 2016). Exosomes can carry a wide variety of molecules such as proteins, lipids, and nucleic acids that are highly unstable on their own. Because exosomes are a natural product of the body, they do not elicit an immune response, making them attractive vehicles. They also cross the blood-brain-barrier through transcytosis (Mora et al., 2016).

Neuroprotective Benefit: Exosome cargos have been extensively studied as biomarkers of Alzheimer's disease, but research to utilize exosomes as a therapeutic is still in its infancy.

Types of evidence:

- 0 meta-analyses
- 0 clinical trials
- 8 observational studies examining exosome contents in healthy adults, people with MCI, and/or those with dementia
- Numerous laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

No studies have tested exosomes as a therapy to prevent dementia or cognitive decline. In a biomarker study of 97 community-dwelling older adults (without MCI), many miRNAs (74) were positively correlated with age (Rani et al., 2017). Cognitive scores were inversely correlated with 13 miRNAs, though the pattern of expression did not match what had previously been described for Alzheimer's disease. Thus these biomarkers may be specific to cognitive decline in normal aging. An miRNA, hsa-let-7g-5p, which is decreased in plasma/serum/blood of Alzheimer's patients, was increased with age in this cohort's plasma samples. Levels of miRNAs widely vary within age groups and within MoCA scores. The roles of individual miRNAs on normal aging, cognitive decline, and Alzheimer's disease are not clearly defined yet.

Human research to suggest benefits to patients with dementia:
No studies have tested exosomes as a treatment for dementia. However, numerous studies have examined exosomes as biomarkers of Alzheimer's disease. A table from DeLeo and Ikezu, 2017 summarizes the changes in exosomal protein biomarkers (Table 3) in Alzheimer's disease patients. Many of the biomarker studies in Alzheimer's patients have been led by Goetzl and colleagues.

Neurally-derived exosomes: One of Goetzl's first studies examined neurally-derived exosomes in the plasma of 26 people with Alzheimer's, 16 people with frontotemporal dementia, and 20 patients followed longitudinally, some of whom developed Alzheimer's (Goetzl et al., 2015).

Compared to controls, Alzheimer's patients had significantly higher exosomal levels of cathepsin D (lysosomal protease that cleaves various proteins), lysosome-associated membrane protein 1 (LAMP-1), and ubiquitinylated proteins, and significantly lower levels of Hsp 70. Levels of cathepsin D, LAMP-1, and ubiquitinylated proteins were significantly higher for patients with Alzheimer's than for patients with frontotemporal dementia. Levels of autolysosomal proteins in neurally-derived blood exosomes appear to reflect the pathology of Alzheimer's up to 10 years before clinical onset, based on levels from the longitudinal cohort. Proteolytic dysfunction in Alzheimer's appears to result in an accumulation of cathepsin D-rich autolysosomes.

A subsequent neurally-derived exosome study examined 20 stable MCI subjects, 20 MCI-to-Alzheimer's converters (within 3 years), 10 mild Alzheimer's patients, and 10 controls (Winston et al., 2016). Levels of phospho-T181-tau, phospho-S396-tau, and Aβ1-42 were significantly higher, whereas those of neurogranin and the repressor element 1-silencing transcription factor (REST) were significantly lower in Alzheimer's and MCI-to-Alzheimer's converters compared to cognitively normal controls subjects and stable MCI patients. Abnormal neurally-derived exosome levels of P-tau, Aβ1-42, neurogranin, and REST accurately predicted conversion of MCI to Alzheimer's. REST levels were very low in Alzheimer's and AD-converters compared to controls and stable MCI—there was no overlap in values across AD vs non-AD groups. Neurogranin levels were also very low in Alzheimer's subjects compared to the 3 other groups, with no overlap with MCI and normal controls.

Two other studies examined synaptic proteins in neurally-derived exosomes of Alzheimer's patients. One study showed that exosome levels of synaptophysin, synaptopodin, synaptotagmin-2, and neurogranin were significantly lower in patients with Alzheimer's disease (and also frontotemporal dementia patients) than in controls, but those of growth-associated protein 43 and synapsin 1 were

reduced only in patients with Alzheimer's (Goetzl et al., 2016). Levels of synaptotagmin, synaptophysin, and neurogranin were decreased years before dementia in patients with frontotemporal dementia and Alzheimer's. Interestingly, levels of synaptopodin, synaptotagmin, and synaptophysin, but not of amyloid β-peptide 42 or phospho-T181-tau, were correlated significantly with cognition assessed by mini-mental state examination (MMSE) or AD assessment scale-cognitive subscale (ADAS-cog).

In a follow-up study, neurally-derived exosome levels of glutamate receptor subunit AMPA4 and neurligin 1 (adhesion protein on the postsynaptic membrane) were significantly decreased in Alzheimer's patients and correlated with the extent of cognitive loss (Goetzl et al., 2017). They had a cross-sectional cohort of 28 Alzheimer's patients and 28 controls, along with a longitudinal cohort where 18 Alzheimer's patients were evaluated across time. With this design, they found that in a preclinical period (6-11 years before onset of dementia), levels of AMPA4, neurligin 1, and neurexin 2α (adhesion protein on the presynaptic membrane) were significantly lower than those of controls.

Astrocyte-derived exosomes: In a study of astrocyte-derived exosomes, Alzheimer's patients had significantly higher levels of BACE-1 and soluble amyloid precursor protein (APP)-b (soluble peptide) and significantly lower glial-derived neurotrophic factor (GDNF) compared to controls (Goetzl et al., 2016). Differences in levels, however, did not distinguish between Alzheimer's versus controls.

A subsequent study examined complement proteins (involved in the ability of antibodies and phagocytic cells to clear microbes and damaged cells) in astrocyte-derived exosomes in Alzheimer's patients (Goetzl et al., 2018). Alzheimer's patients had significantly higher levels of C1q, C4b, C3d, factor B, factor D, Bb, C3b and C5b-C9 terminal complement complex (TCC) than age-matched controls. Levels of proinflammatory markers, IL-6, TNF-α and IL-1β, were significantly higher for Alzheimer's patients than controls, but there was greater overlap between the two groups than for complement proteins. Complement regulatory proteins CD59, CD46, decay-accelerating factor (DAF) and complement receptor type 1 (CR1), but not factor I, were significantly lower for Alzheimer's patients than controls. It is currently unknown whether A1 and A2, the two subtypes of astrocytes, produce exosomes with different protein compositions.

Exosomal miRNAs: Exosomal miRNAs in biological fluids have emerged as a powerful tool for diagnosis and potential treatment of diseases. A 2016 review on this topic stated that of the miRNAs detected in Alzheimer's patients, miR-9, miR107, and miR-128 are involved in neuronal differentiation, miR-134 is involved in synaptic development and plasticity, and miR-137 is involved in neuronal maturation and dendritic morphogenesis during development (Van Giau and An, 2016). A different 2016 review stated

that of all of the reported changes with miRNAs in Alzheimer's disease, only 6 miRNAs—miR-9, miR-125b, miR-146a, miR-181c, let-7g-5p, and miR-191-5p—were reported by multiple investigators (Kumar and Reddy, 2016). This review discussed 12 studies in which 1268 participants were tested for the expression of circulating miRNAs in different biofluids from Alzheimer's patients, MCI, vascular dementia, and controls. They found that miR-191-5p has the maximum area-under-curve value (0.95) in plasma and serum samples, while smaller area-under-curve values were found for miR-125, miR-181c, miR-191-5p, miR-146a, and miR-9. So far, there is no unanimously identified miRNA that is a precise biomarker for Alzheimer's disease.

In a clinical biomarker study in 35 Alzheimer's and 35 controls, 20 exosomal miRNAs showed significant differences in the Alzheimer's group, among which a panel of 7 miRNAs (miR-342-3p, miR-141-3p, miR-342-5p, miR-23b-3p, miR-338-3p, miR-3613-3p) were highly informative in a machine learning model for predicting Alzheimer's status of individual samples with 83-89% accuracy (Lugli et al., 2015). The authors noted the most interesting single miRNA was miR-342-3p, which was expressed in Alzheimer's patients at about 60% of control levels, highly correlated with other miRNAs that were down-regulated in Alzheimer's, and was also reported to be down-regulated in Alzheimer's in two previous studies.

The specific miRNAs that are detected in biomarker studies appear to vary greatly across studies. Extensive follow-up studies will be required to decipher the roles of miRNAs in Alzheimer's disease and their applicability as a diagnostic tool (Van Giau and An, 2016).

Mechanisms of action for neuroprotection or harm identified from laboratory and clinical research:

Spreading of pathology: Exosomes may be involved in propagating neurodegeneration by spreading disease-associated cargos (Edgar, 2016). Aβ accumulates in the multivesicular bodies within the nerve terminals of Alzheimer's transgenic mice and in the human brain (Liew et al., 2017). An exosomal protein Alix has been associated with senile plaques, strengthening the case for exosomes as mediators of spreading pathology. Exosomal contents can also induce neuronal apoptosis in models of Alzheimer's (Malm et al., 2016). In addition, exosomes secreted from microglia can promote the formation of neurotoxic Aβ and act as a driver in neuronal damage. Based on an endotoxemia study in mice, circulating exosomes may promote neuroinflammation (Li et al., 2018).

Neuroprotective effects of exosomes: Exosomes may also be involved in neuroprotective effects. Exosome secretion from neuronal cells with neutral sphingomyelinase (nSMase2; a sphingolipid-metabolizing enzyme) enhanced Aβ uptake into microglia and significantly decreased extracellular levels

of Aβ (Yuyama et al., 2012). Exosomes may also contain a variety of different molecules that mediate protective effects. Among these is Cystatin C, which is secreted by exosomes (Ghidoni et al., 2011) and has neuroprotective properties (Kaur et al., 2010). Exosomes can also carry neprilysin, which is an Aβ degrading enzyme in the brain (Katsuda et al., 2013). Exosomes secreted from human adipose tissue derived-MSCs are enriched with neprilysin and can decrease both secreted and intracellular Aβ levels. These properties were more pronounced when using exosomes from adipose-derived MSCs compared to those from bone-marrow-derived MSCs.

Potential as a therapeutic for neuroprotection: A study in mice has explored the possibility of using exosomes to target CNS cells. Exosomes were loaded with small interfering RNAs (siRNAs) directed to knock down expression of BACE1, one of the proteases responsible for Aβ biogenesis (Alvarez-Erviti et al. 2011). These engineered exosomes, which were injected intravenously in mice, crossed the blood-brain-barrier, decreased BACE1 mRNA by 60%, and decreased BACE1 protein by 62%. However, effectiveness in Alzheimer's pathology has not been established yet.

In a mouse model of Alzheimer's disease (APP/PS1 mice), injection of exosomes from normoxic MSCs rescued cognition and memory impairment, reduced plaque deposition, and Aβ levels in the brain, decreased activation of astrocytes and microglia, down-regulated proinflammatory cytokines (TNF-α and IL-1β), and up-regulated anti-inflammatory cytokines (IL-4 and -10)(Cui et al., 2017). Interestingly, the group administered exosomes from hypoxic MSC (hypoxia-preconditioned MSC, PC-MSC), which showed even greater improvements in learning and memory, significantly lower plaque deposition and Aβ levels, and significantly increased expression of growth-associated protein 43, synapsin 1, and IL-10. They also showed significantly increased levels of GFAP, TNF-α, IL-1β, and sharp decreases in activation of STAT3 and NF-κB. Notably, exosomes from PC-MSCs increased the level of miR-21 in the brain of Alzheimer's mice and replenishment of miR-21 restored the cognitive deficits in APP/PS1 mice and prevented pathologic features. The mechanism underlying neuroprotective benefits may be due in part to miR-21's ability to restore synaptic dysfunction and inflammatory responses.

In epilepsy: In a mouse model of epilepsy, treatment with A1 exosomes, characterized by robust anti-inflammatory properties, reached the hippocampus within 6 hours and prevented loss of glutamatergic and GABAergic neurons and greatly reduced inflammation in the hippocampus (Long et al., 2017). These effects were coupled with long-term preservation of normal hippocampal neurogenesis and cognitive function.

In diabetes-induced cognitive impairment: In a mouse model of diabetes-induced cognitive impairment, treatment with exosomes from bone marrow-derived MSCs (injected intracerebroventricularly) resulted in improved cognitive functions and histological features similar to those seen with injection of bone marrow-derived MSCs (Nakano et al., 2016). These injected exosomes were internalized into astrocytes and neurons.

In traumatic brain injury: In a proof-of-principle study, an intravenous delivery of MSC-derived exosomes (100 µg protein) improved functional recovery and promoted neuroplasticity in young adult male rats subjected to traumatic brain injury induced by controlled cortical impact (Zhang et al., 2015). Another study also demonstrated that isolated extracellular vesicles from MSCs reduced cognitive impairments in a mouse model of traumatic brain injury (Kim et al., 2016).

MSCs versus MSC-derived exosomes: The protective effects of MSC-derived exosomes vs MSCs themselves were tested by Doeppner et al. (2015) in a mouse model of focal cerebral ischemia. They showed that MSC-derived exosomes do not perform inferiorly to MSCs and are able to promote neuronal recovery and angiogenesis, modulate post-stroke immune responses, and prevent post-ischemic immunosuppression.

APOE4 interactions: None available.

Aging and related health concerns: A few phase I/II clinical trials have shown anti-tumor responses, though these studies were small and results are still preliminary. Cardioprotective actions have only been examined in laboratory settings.

Types of evidence:

- 4 clinical trials in cancers
- Numerous laboratory studies

Cancers: ONLY PHASE I/II CLINICAL DATA. A 2016 review of 4 clinical trials testing dendritic cell-derived exosomes for cancer therapy in advanced malignancies reported that the therapy is feasible and generally safe (Pitt et al., 2016). There have been 2 trials (phase I and II) in advanced non-small-cell lung cancer, 1 phase I trial in metastatic melanoma, and 1 phase I trial in advanced colorectal cancer. Treatments involved weekly injections of exosomes derived from dendritic cells or ascites for 4 weeks. A total of 86 patients across 4 trials were enrolled.

In the phase I study in advanced colorectal cancer, 40 people were enrolled and they received autologous ascites-derived exosomes (Aex; 100-500 µg) or Aex plus granulocyte-macrophage colony-stimulating factor (Dai et al., 2008). Aex plus granulocyte-macrophage colony-stimulating factor induced a beneficial tumor antigen (carcinoembryonic antigen)-specific antitumor cytotoxic T lymphocyte response at a higher incidence (10/13 patients) compared to Aex alone (2/10 patients).

The phase II clinical trial in 22 patients with non-small cell lung cancer reported that IFN-gamma-dendritic cell-derived exosomes loaded with MHC class I- and class II-restricted antigens resulted in progression-free survival for over 4 months in 7 patients (32%); however, the primary endpoint (50% of patients with progression-free survival for 4+ months) was not reached (Besse et al., 2015). The median exosomal protein quantity per injection was 193 µg. An increase in NKp30(natural cytotoxicity receptor)-dependent natural killer cell functions were seen in a fraction of patients with defective NKp30 expression. This phase II trial, while not reaching the primary endpoint, confirmed the capacity of dendritic cell-derived exosome therapy to boost the natural killer cell arm of antitumor immunity in patients with advanced non-small-cell lung cancer. This therapy may be beneficial in patients harboring NKp30-specific functional defects that occur in some cancers, such as gastrointestinal stromal tumors, neuroblastoma, chronic lymphocytic leukemia, and non-small-cell lung cancer.

In summary, exosome therapy produced encouraging stimulatory effects on natural killer cells, though T cell responses appeared to be lacking. Future studies are needed to fully understand the components of dendritic cell-derived exosomes (e.g., mRNAs, miRs, cytokines, lipid mediators) and their functions on immune cells. In addition, future efforts are needed to standardize procedures involved in exosome isolation, storage, cargo loading, quality control, and efficacy evaluation to optimize clinical trials and treatments (Wang et al., 2016).

Cardiovascular: POTENTIAL BENEFIT BASED ON PRECLINICAL DATA. Benefits of stem cell-derived exosomes have not been tested in heart patients yet. Based on a 2017 review of key publications from ClinicalTrials.gov, stem cell-based transplantation is one of the most promising treatments for damaged myocardial tissue from ischemia (Yu et al., 2017). Therapies involved different kinds of stem cells, including MSCs, embryonic stem cells, iPSCs, and cardiac stem cells. However, not all trials showed benefit and exploring the therapeutic effects of stem cell-derived exosomes may hold greater promise.

A meta-analysis of 6 preclinical studies in ischemia/reperfusion injury confirmed the therapeutic potential of MSC-derived exosomes in improving heart function (Zhang et al., 2016). Numerous other

studies have shown that MSC-derived exosomes exhibit cardio-protective activity and are efficacious in animal models of myocardial infarction, stroke, perinatal hypoxic-ischemic brain injury, and hind-limb ischemia (reviewed in Phinney and Pittenger, 2016). However, further mechanistic studies are required for validation and optimization of this approach for cardiac regeneration. Studies suggest that miRNAs contained in MSC-derived exosomes reduce cardiac fibrosis following myocardial infarction.

Safety: So far, small trials in cancer patients have reported mild adverse events, though one patient exhibited grade III liver toxicity. Because there are yet to be identified contents of exosomes, side effects are not entirely predictable.

Types of evidence:

- 4 clinical trials
- Numerous reviews
- Numerous laboratory studies

Safety data from clinical trials: In the phase I study in 40 patients with advanced colorectal cancer, both the autologous ascites-derived exosomes (Aex; 100-500 µg) alone or the combination of Aex plus granulocyte-macrophage colony-stimulating factor (GM-CSF) were safe and well-tolerated (Dai et al., 2008). The most frequently reported adverse events causally related to the use of Aex or Aex plus GM-CSF were mild (grade I-II) in severity and included injection site reactions (37 events) including erythema, pruritus, or pain, fatigue (3 patients), fever (1 patient), and nausea (1 patient). There were no significant hepatic, renal, pulmonary, cardiac, hematologic, or neurologic toxicities attributable to the treatments. No clinical manifestations of autoimmune reactions were observed and no significant changes in temperature and blood pressure were recorded. However, in a phase II clinical trial in 22 patients with non-small-cell lung cancer, one patient exhibited grade III hepatotoxicity (Besse et al., 2015). Other clinical trials using exosome-based therapies in cancer patients reported adverse events that were mild (under grade II)(Pitt et al., 2016; Wang et al., 2016)..

Preclinical safety studies: A safety study in rabbits, guinea pigs, and rats reported that exosomes from human umbilical cord MSCs (400 µg diluted in 200 µl phosphate-buffered saline) infused intravenously were well-tolerated with no adverse effects on liver or renal function (Sun et al., 2016). However, there has been a study reporting that exosomes derived from bone marrow MSCs promote tumor growth in a mouse xenograft model (Zhu et al., 2012). These exosomes enhanced vascular endothelial growth factor expression in tumor cells (by activating the ERK1/2 pathway). Also, cancer cell-derived cytokines can

induce MSCs to secrete soluble factors, which then promote tumor growth in a paracrine manner (Liew et al., 2017).

Inherent properties of exosomes: Exosomes are likely to be safer than cell-based therapies, which have inherent risks such as occlusion of microvasculature or unregulated growth of transplanted cells (Xiong et al., 2017). An important advantage of exosomes over other nanoparticles (e.g., liposomes) is their lack of accumulation in the liver, which is usually responsible for unwanted side effects and toxicity (Cardoso et al., 2016).

The precise content of MSC-exosomes remains largely unknown because these vesicles contain many molecules that are yet to be identified (Liew et al., 2017). This may lead to the possible concurrent transport of these unidentified and potentially harmful biomolecules to the recipient cells and cause unwanted side effects. Therefore, a more in-depth and rigorous characterization of the contents of these vesicles is needed to ensure their safety.

Transfer of pathology: While exosome therapies are being developed and tested in cancer patients, depending on the origins of exosomes, they may be involved in cancer development, progression, metastasis, and drug resistance through promotion of carcinogenesis and tumor growth, angiogenesis, modulation of tumor microenvironment, modulation of immune responses, and induction of mechanisms involved in drug resistance (Mora et al., 2016). Exosomes from cancer cells can transport oncogenes and onco-miRNAs to other cells.

Other theoretical risks: There are other potential concerns such as off-target effects, biodistribution and pharmacokinetic profiles, allergic reactions, and enrichment of harmful substances in exosome-based products purified from natural sources (Reiner et al., 2017).

Sources and dosing:

Sources: Exosomes are pooled from cellular supernatant or body fluids, then isolated by differential centrifugation; therefore, these preparations represent enrichment and not purification of exosomes alone (Edgar, 2016). This is because there are other types of microvesicles that are roughly the same size as exosomes (40-100 nm diameter), including apoptotic bodies and ectosomes, derived from cells undergoing apoptosis and plasma membrane shedding, respectively. In addition, there are no exclusive markers for intraluminal vesicles or multivesicular bodies as they undergo continuous maturation, during which they gain and lose proteins.

Challenges in isolation/purification: The International Society for Extracellular Vesicles and the Society for Clinical Research and Translation of Extracellular Vesicles Singapore (SOCRATES) convened a workshop to discuss best-practice models for the therapeutic use of exosomes (Reiner et al., 2017). They note that stringent, harsh purification procedures required to obtain a pure product could result in loss of function, through damage to exosome-intrinsic effectors or loss of extrinsic, loosely associated factors that act with exosomes to exert function. Purity may be achieved at the expense of scalability, yield, cost, and therapeutic potency. Exosome isolation kits are easy to use and highly scalable but can co-precipitate other large complexes and thus not to be exosome-specific. The degree of purity will need to be balanced with the desirable potency, reproducibility, and stability measures. The authors also recommend endotoxin testing of the end-product to ensure the absence of microbial contamination. For clinical studies, it is critical to have a reproducible and GMP-compliant method with a reproducibility metric (e.g., molecular fingerprinting). Standardization of cell culture conditions is key; however, in the MSC field, no standardized cell culture conditions have been defined.

Exosomes cannot be visualized in most microscopic platforms, and therefore electron microscopic (EM) approaches (e.g., whole mount EM, scanning EM, transmission EM, and electron tomography) are required (Mora et al., 2016). The best method of defining exosomes biochemically is likely through a combination of markers, including tetraspanins, Alix, and others, while excluding resident plasma membrane proteins. Current exosome counting methods do not necessarily distinguish exosomes from other particles. Single exosome-based analysis by fluorescent labeling and advanced flow cytometry may be an up-and-coming technology to solve this problem (Reiner et al., 2017).

Quality control of exosomes: Based on discussions from the Workshop held by the International Society for Extracellular Vesicles and the Society for Clinical Research and Translation of Extracellular Vesicles Singapore (SOCRATES), fingerprinting assays provide quality control and establish batch-to-batch consistency by examining a narrowly defined set of molecular markers of exosome therapy that are expected to be present, absent, or to reach a specific threshold (Reiner et al., 2017). Combined with other characteristics such as size range, molecular markers should be measured at different stages of production and across batches. Fingerprinting assays have yet to be standardized and will likely be specific to each production process and clinical indication. It is also critical to identify and validate parameters of potency assays for specific clinical indications.

Special challenges include defining the active ingredient or excipients of exosome therapy as it is not clear in many cases whether the lipid membrane, the internal content, or a combination is required for the therapeutic benefit.

Customizing exosomes: Currently, electroporation is the best way for loading siRNAs, miRNAs, and small DNA fragments into exosomes. However, this process often induces aggregation and degradation of these materials (Kooijmans et al., 2013). Nucleic acid loading can also have low efficiency (as low as 0.05%)(Reiner et al., 2017). An improved method for sensitive cargo loading, such as siRNAs and mRNAs, is needed (Wang et al., 2016).

Routes of administration: In a study in a mouse model of cancer, intravenous injection of exosomes derived from immature dendritic cells resulted in a successful delivery of doxorubicin (cancer drug) to tumor tissues (Tian et al., 2014). The half-life after systemic injection of exosomes ranges from a few minutes to a few hours. With oral administration, exosomes are exposed to extreme pHs though they may have therapeutic effects on the intestinal luminal epithelial surface rather than non-GI tissues. In a study in mouse models of brain inflammation (multiple sclerosis and brain tumor), intranasal administration of exosomes successfully transferred curcumin and a Stat 3 inhibitor (involved in signal transduction and transcription) to inflamed areas of the brain (Zhuang et al., 2011). These exosomes were selectively taken up by microglial cells and subsequently induced apoptosis of these cells and inhibited tumor growth.

Exosome delivery to the brain: Successful delivery of exosomes to the brain has been demonstrated by targeting the acetylcholine receptor through fusion of exosome membrane protein, LAMP-2b, with the neuron-specific RVG peptide (Alvarez-Erviti et al., 2011). Instead of docking to neurons, specific delivery of exosomes can also be achieved through targeting non-neuronal resident cells in the brain. For example, the fusion of exosome membrane proteins to peptides that recognize the gap junction protein, connexin 43, which is highly expressed in astrocytes, might enhance exosome delivery to the brain (Liew et al., 2017). As discussed above, intra-nasal administration has been shown to rapidly deliver exosomes to the brain, and the repeated administration of these vesicles continued to exert a therapeutic effect without causing toxicity or behavioral abnormalities in the mice (Zhuang et al., 2011).

Biobanking exosomes: It may be too early to effectively biobank exosomes. Currently, there are no strictly defined conditions for isolating or storing exosomes (Mora et al., 2016). Optimal time, temperature, storage period, freezing-thaw cycles, thawing conditions, or other storage variables have not been thoroughly evaluated yet. Kalra and colleagues examined the stability of exosomes at different temperatures and discovered that although at 90 days all samples were stable, there was an advantage in storing exosomes at -80˚C (Kalra et al., 2013). However, this may vary across exosomes derived from different sources or based on different isolation protocols. In addition, the particular lipid composition

of exosomes may have an impact on optimal cryopreservation procedures. There is currently no specific information regarding the effect of anticoagulants in the collection and storage of exosomes.

Specific markers in/on exosomes may be used in the future to obtain a patient "Exogram", a signature profile of each person's exosomes, to precisely diagnose and/or monitor treatment responses as part of a precision medicine effort (Mora et al., 2016).

ExoCarta: ExoCarta.org is an online database of proteins, lipids, and RNAs identified in published and unpublished studies of exosomes (people can submit their datasets). You can run queries or browse by species (e.g., homo sapiens), though it is not designed for disease/state characterization. As of 2/15/18, there are 9,769 proteins, 1,116 lipids, 3,408 mRNAs, and 2,838 miRNAs associated with exosomes.

Research underway:

Clinical Trials: There are 23 clinical trials involving exosomes on ClinicalTrials.gov, but most are using exosomes as an outcome measure, analytic tool, or biomarker. There are 3 that are testing exosome preparations as interventions. One study is testing allogenic MSC-derived exosome (200 µg total protein of exosome transfected by miR-124, delivered stereotaxically through the skull) in patients with acute ischemic stroke (NCT03384433). It is an open-label study that is estimated to be completed in January 2019. Another open-label clinical trial is evaluating the effects of exosomes derived from the participants' plasma to treat intractable cutaneous ulcers (e.g., rheumatic disease, peripheral arterial disease, chronic venous insufficiency, decubitus or burns)(NCT02565264). The treatment will be daily for 28 days. It is scheduled to be completed in March 2018. The third study is a phase I trial to test the ability of plant exosomes in delivering curcumin to malignant colon tissue as well as to normal colon tissue (NCT01294072). Newly diagnosed colon cancer patients who are undergoing surgery will be enrolled. This study is scheduled to be completed in December 2020.

Gaps in knowledge and future directions: It is worrisome that clinical trials on exosomes have started before a consensus definition on exosomes has been achieved (Mora et al., 2016). There are numerous gaps in knowledge that need to be addressed, such as the consensus isolation techniques that preserve biological characteristics, and understanding the functional proteins and subgroups. We need a standardized protocol to assay the identities of exosome contents and their potencies to determine appropriate dosing (Phinney and Pittenger, 2016). Also, methods of storage and recovery of these products that maintain exosome potency are needed. Therapeutic efficacy needs to be evaluated in well-controlled, appropriately powered clinical trials.

Search terms:

Pubmed, Google: exosomes

- + cognitive, +Alzheimer's, + clinical trial, + ApoE4, + peripheral neuropathy, + cardiovascular, + cancer, + safety

ClinicalTrials.gov: exosomes (23)