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Translating Exosome Candidates into Approved Therapies with engEx® Platform Technologies

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Abstract

Exosomes represent one type of extracellular vesicle (EV) generated by all eukaryotic cells. Exosomes are a new modality with tremendous potential to enable delivery of drug substances that are too toxic for free systemic administration, to what were previously thought to be undruggable targets. Early clinical data has revealed the potential efficacy and highlighted the safety of these natural delivery vehicles. Like other next-generation biologics, however, exosomes are complex, and their development and manufacture can be challenging. Therapy developers can now benefit greatly from Lonza's engEx® technology, our experience and deep knowledge regarding exosome process development, optimization, manufacturing, and regulatory understanding gained through interactions with regulatory authorities.

Introduction

The Therapeutic Potential of Exosomes

Targeted delivery of active drug substances for enhanced efficacy and reduced side effects is a major focus of most pharmaceutical companies today. Lipid, polymeric, and chemically modified metal nanoparticles are technologies that are being leveraged to achieve this goal. Exosomes offer a natural alternative with many advantages compared to synthetic approaches.

Exosomes range in diameter from 30 to 120 nm, and encapsulate molecules including DNA, RNA, proteins, and fats, in a lipid bilayer membrane. Exosomes play an important role in intercellular communication, and physiological and pathological processes. The role of exosomes in cell-to-cell communication processes has been documented extensively.[1] Exosomes produced from stem cells may promote anti-inflammatory and regenerative activity, while those from cancer cells may promote tumor metastasis.[2] Exosomes can also act as delivery vehicles for therapeutic cargos. Such cargoes can be attached to the surface or encapsulated within the vesicle lumen. Cargo can include effectors of protein synthesis and gene silencing such as messenger RNA and small interfering RNA respectively, or activators of signaling pathways.

Unlike synthetic nanoparticles, exosomes are recognized as "self" by cells and not as foreign substances. As a result, they do not cause unwanted immune responses (are immune-silent), are rapidly distributed, have a long half-life in circulation, can pass the blood-brain barrier, and are compatible with a broad range of drug substances. Engineered exosomes can also be designed with expanded tissue and cell tropism for effective release of drug substance cargos, and/or designed to overexpress scaffold proteins on the surface or in the lumen to increase the surface area for exogenously loaded cargos.[3]

For these reasons, there is growing interest in the potential of exosomes to serve as targeted drug delivery systems for many types of drug substances, as well as for their use in research and diagnostic applications. Additionally, several pre-clinical studies and early clinical data supporting

both the safety and efficacy of exosome-based therapies derived from several different cell types for the treatment of a host of diseases and disorders are fueling further interest.[3]

Market research estimates the global therapeutic exosome market to be expanding at a compound annual growth rate of 17.4% from $550.6 million in 2022 to $1.99 billion by 2030.[4] Another research projects the market to expand at even higher rates of 27-30% per year.[4,5,6]

Challenges to Therapeutic Exosome Development and Manufacturing

Exosome therapeutics are yet a nascent sector of the pharmaceutical industry, and there is much about exosomes that still must be learned. Perhaps most important is the lack of a deep understanding of the correlations between specific exosome attributes and their functionalities. This has created technical hurdles to effective exosome engineering and served as a barrier to entry into the field.

Loading cargo using general membrane-targeting sequences or internal proteins often results in low expression of the therapeutic payload in exosomes with limited activity. Developing efficient and cost-effective methods for the isolation and purification of desired exosomes from cellular material without altering key characteristics has also been challenging. Ultracentrifugation, precipitation, and size exclusion chromatography when used alone or in combination generally provide exosomes with high levels of impurities. Defining optimal exosome formulations for specific indications and routes of administration has presented other difficulties.

A New Approach

In 2023, Lonza acquired assets of Codiak Biosciences, including its novel exosome design, manufacturing technologies and clinical programs. Lonza has integrated Codiak's design and innovative production technologies into its licensing and contract development and manufacturing service offerings. Here, we discuss these technologies, how they overcome existing challenges to therapeutic exosome development and manufacture at scale, and also early clinical evidence demonstrating the ability of exosomes to make targets previously thought to be undruggable accessible.

engEx® Platform Technologies for Therapeutics and Vaccines

The use of a novel purification strategy and protein scaffolds designed to efficiently load various types of drug substances onto the surfaces or into the lumens of exosomes have helped to overcome many of the challenges described (**Figure 1**).[3] A carefully designed linker, meanwhile, allows the attachment of small-molecule payloads to purified exosomes without impacting important EV attributes. These technologies are encompassed in the engEx® platform for production of exosomes for targeted drug and vaccine delivery at therapeutically relevant levels.

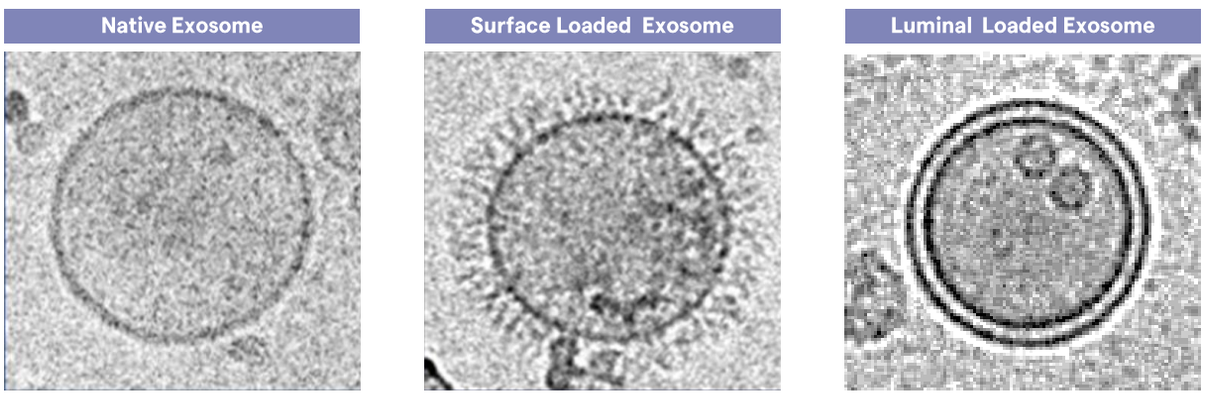

Figure 1. Engineered exosomes with surface and luminal-loaded cargos.

Development

To overcome inconsistent loading and poor expression, proteins preferentially packaged into exosomes with high efficiency were identified.3 This was achieved only after an effective method for producing high-purity exosomes was developed. The process involved successive filtration and ultracentrifugation steps, followed by separation using a discontinuous iodixanol gradient. The desired exosomes were observed in the low-density fractions and largely free of protein-based and other impurities.

In these purified exosomes, two families of proteins (one on the surface, one in the lumen) not previously used for attachment of drug substances were found to be highly abundant. Stable HEK293 cell lines, designed to produce exosomes with overexpressed levels of these proteins and containing green fluorescent protein (GFP) as a surrogate payload, were engineered and various attributes of the exosomes were evaluated. Initial positive results led to further experiments to determine the minimal sequences required for enrichment of the different proteins and binding to different payloads. The result was the identification of two optimal scaffold proteins – PTGFRN for loading drug payloads onto the exosome surface, or BASP1 for the luminal loading of exosomes.

Engineered PTGFRN was found to be effective for surface loading of many different types of bioactive molecules, including antibodies and antibody fragments, cytokines, enzymes, peptides, receptors, and tropism modifiers. The engineered BASP1 protein in the lumen, also via fusion to an N-terminal fragment, was shown to enable loading of cargoes including antigens, adeno-associated viral vectors, peptides, and nucleases and other enzymes (**Figure 2**).

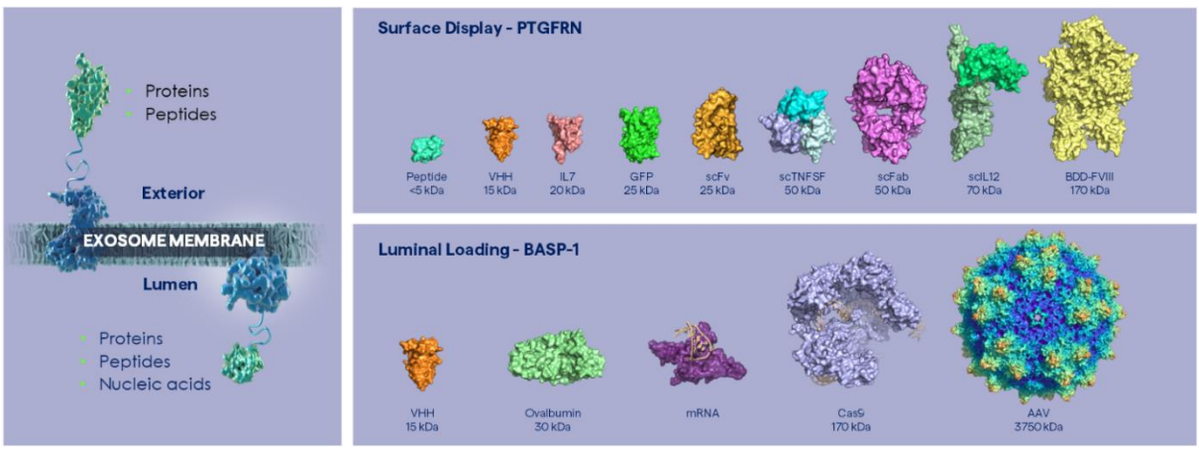

Figure 2. Therapeutic payloads ranging across different types of biomolecules and sizes may be surface-displayed or luminally loaded onto exosomes.

Notably, in addition to exosomes generated using HEK293 cells, exosomes with overexpressed PTGFRN or BASP1 proteins scaffolds were also obtained from transiently transfected Chinese hamster ovary (CHO) cells. Furthermore, exosomes isolated from a range of different cell types were demonstrated to contain endogenous PTGFRN and BASP1 proteins. These results suggest that use of these two proteins scaffolds should have broad applicability across many different types of cells.

The engEx® platform encompasses technologies for the development of stable cell lines that generate exosomes containing overexpressed PTGFRN and/or BASP1 fused to specific drug substances.

High Internal and External Payload Densities

In addition to affording control over the loading of cargos to exosomes, a key advantage of the engEx® platform is the high level of scaffold protein overexpression, which results in a much greater surface area to which payloads can be attached, whether on the surface or in the lumen of the exosome. The ability to generate exosomes with high payload densities, combined with the high productivity of the cell lines, reduces the cost of goods for these therapeutics.

Post-Production Modification Option

One limitation of the use of scaffold proteins to luminally or surface-load payloads to exosomes is the inability to use small-molecule drug substances. This issue has been overcome through the development of a unique linker technology that does not affect important exosome attributes (**Figure 3**). Extensive screening of many different chemically complementary linkers was performed to identify those with the greatest potential, resulting in the selection of an azo derivative for the engEx® platform.

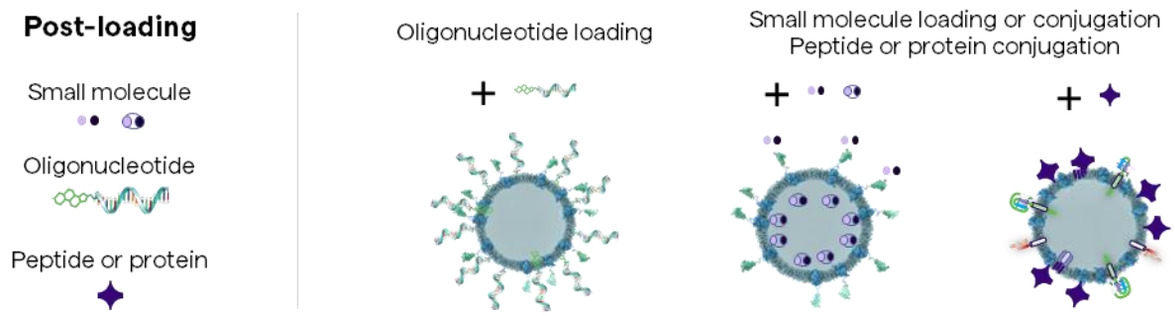

Figure 3. engEx® platform tools using a unique linker technology for post-purification loading.

Modification of exosomes post-purification avoids the need to invest in extensive cell-line engineering efforts to develop cell lines capable of producing exosomes with small molecules (e.g., nucleic acids such as microRNA and small interfering RNA) fused to their surfaces. Attachment of the linker is performed using simple, industry-established protocols, allowing for the rapid screening of numerous sequence variations of a given nucleotide.

Furthermore, the linker technology can be used with exosomes containing loaded bioactive molecules. For instance, a loaded protein or antibody fragment can be used to target delivery of the nucleic acid payload to a specific type of cell. As such, these exosomes could serve as alternatives to antibody-drug conjugates.

Documented Delivery to Target Cells
Exosomes engineered using engEx® technology to overexpress internal or external scaffold proteins that are fused to many different bioactive molecules and/or linked to nucleotides have been shown to deliver their payloads in a targeted manner to different cell types, including immune (dendritic, T, B, natural killer, and Kupffer) and tumor cells, retinal ganglia, astrocytes, pneumocytes (Types 1 and 2), and liver sinusoidal endothelium cells.

Vaccine Applications
The engEx® platform can be used to generate not only exosome-based therapeutics, but also vaccine candidates against infectious diseases and cancer antigens. The exoVACC® program employs engEx® technology to display antigens on the surfaces or in the lumens of the exosome to induce T-cell responses. Activity can be boosted by incorporating adjuvants in or on the exosome, including via post-purification linkage.

Safety and Tolerability
A key advantage of exosomes as drug-delivery vehicles is their attractive safety profile, which results from the fact that these nanoparticles are a natural biological component within living organisms. Increased immune silencing leads to reduced toxicity and greater tolerability than synthetic drug-delivery solutions such as lipid nanoparticles.

Early clinical data (vide infra) clearly demonstrates the safety of exosomes. Attached drug substances, that when administered systemically cause significant side effects (e.g., interleukin 12

and STING agonists), can be safely given to patients when loaded into or onto exosomes. In addition, because engEx® technology enables production of exosome nanoparticles with higher loading levels, it is possible to safely administer higher doses for increased potency.

Manufacturing Solutions
As a novel modality with candidates still in early clinical development, little information is available on large-scale production of exosomes in compliance with cGMP requirements. Lonza therefore invested heavily in development of a proprietary, cost-effective platform process for the production of engEx® exosomes that can be scaled all the way to commercial.

While exosomes differ from recombinant proteins, many of the technologies used for antibody manufacturing can be adapted for exosome production. The upstream process leverages perfusion technology, enabling continuous exosome production and capture. The downstream purification process incorporates unit operations specifically designed to remove troublesome by-products and process-related impurities to afford high-quality exosomes.

Using the current process, which has been demonstrated at the 1000-liter scale, multiple successful GMP manufacturing campaigns for early-phase clinical candidates have been completed. The stability of the obtained therapeutic candidates using the engEx® platform technology has also been demonstrated.

Present efforts at Lonza are focused on further improving the applicability of the existing platform process for larger-scale GMP production and on delivering a fully continuous process from exosome generation through fill/finish.

Early Clinical Validation

Early clinical studies have demonstrated the ability of exosome candidates produced using engEx® technology to make the undruggable druggable. Two of the candidates, **exoSTING®** and **exoIL-12™**, make it possible to treat patients with molecules that when administered on their own show significant systemic toxic side effects. A third, **exoASO-STAT6®**, addresses a target previously thought not to be targetable. All have been shown to have excellent safety and tolerability, resulting from use of the body's natural messaging system for delivering effective drug substances in a stealth-like manner.

The first two candidates for delivery of interleukin-12 (IL-12) and a STING agonist were initially developed for intratumoral delivery. While tumor targeting was necessary for the latter, this conservative approach allowed for initial investigation of safety and efficacy for the former. Studies on intravenous (IV) delivery of the IL-12 candidate were then initiated given the excitement created by its initial clinical results. Preliminary data on systemic delivery supporting some level of efficacy without toxic side effects has generated more interest.

Overall, it was found that repeat dosing of engEx® engineered therapeutic exosomes is well tolerated in cancer patients with no treatment-related adverse events. In addition, pharmacokinetic (PK)/pharmacodynamic (PD) relationships for the candidates clearly established

remarkable translatability from mouse to non-human primates to man, enabling dose modeling to minimize the number of dose-escalation cohorts required in future studies. The observed clinical activity also demonstrated dose-dependent PD effects consistent with the proposed mechanisms of action.

Taken together, this clinical data provides validation of exosome therapeutics and strongly reinforces the potential of the engEx® platform technology.

exoIL-12™
In patients with cutaneous T-cell lymphoma (CTCL), T-cells develop abnormalities that make them attack the skin. IL-12 is a potent, pro-inflammatory type 1 cytokine that has been shown to exhibit excellent anti-tumor efficacy in preclinical models, but results in unacceptable side effects in humans. exoIL-12™ is an engEx® exosome engineered to express functional IL-12 on its surface. In patients with CTCL, exoIL-12™ demonstrated improved safety vs. recombinant IL-12, providing local retention and lack of systemic exposure to the cytokine. Deep and durable effects on injected and abscopal lesions were observed due to potent and prolonged activation of T and NK cells in the tumors, confirming anti-tumor activity.

Two CTCL patients each received 20 injections (6 µg) to multiple lesions over a period of > 6 months, and 14 healthy volunteers were administered single doses between 0.3-12 µg. No treatment-related or unrelated Grade 3 or higher adverse events occurred. Phase 1 studies have been completed.

exoSTING®
Development of immunotherapies for the treatment of solid tumors has been challenging due to the ability of these tumors to leverage the tumor microenvironment against anti-cancer agents. exoSTING® exosomes are engineered to express cGAS/stimulator of interferon genes (STING) agonists on their surfaces via fusion to the PTGFRN scaffold protein. CDK-002 is an exoSTING® exosome that targets antigen presenting immune cells (APCs) in the tumor microenvironment of solid tumors and delivers a potent STING activator (SA) locally in the tumor.

In a completed Phase 1 study, the exoSTING® exosome was shown to exhibit 100-fold improved potency versus the free SA without any systemic exposure to the SA, and consequently a dramatically improved safety profile. Dose-dependent pharmacology was observed along with innate and adaptive immune activation, T-cell expansion/preservation, and local and distal tumor responses, with confirmation of anti-tumor activity.

exoASO-STAT6®
Checkpoint inhibitors have experienced great success in cancer therapy, but their effectiveness in many patients is limited due to immunosuppressive tumor-associated macrophages (TAMs) with an M2 phenotype. One approach to overcoming this problem is through reprogramming of TAMs toward a proinflammatory M1 phenotype.

engEx® exosomes containing the azo linker (fused to the PTGFRN scaffold protein) for delivery of oligonucleotides have been labeled as exoASO® exosomes. exoASO-STAT6® is a novel engEx® exosome that selectively delivers antisense oligonucleotides to mediate reprogramming of TAMs

from the M2 to the M1 phenotype. The oligonucleotide linked to the exosomes targets the transcription factor signal transducer and activator of transcription (STAT) 6 (STAT6), which has traditionally been considered undruggable.

Preclinical studies of exoASO-STAT6® exosomes showed single agent anti-tumor activity in models of aggressive hepatocellular carcinoma (HCC). Intratumoral administration of exoASO-STAT6® resulted in a potent monotherapy response that was dependent on cumulative dose. A Phase 1 clinical trial of systemically administered (IV) exoASO-STAT6® in patients with advanced HCC and patients with liver metastases from primary gastric cancer or colorectal cancer, where high STAT6 transcript levels correlate with poor prognosis for patients was initiated based on these results.

Exosome and Extracellular Vesicle Expertise at Lonza

Lonza is the first contract development and manufacturing organization to build strong capabilities supporting exosome-based therapeutics. The company currently has functional exosome development at Walkersville-Shady Grove and has undertaken technology transfer to its Houston facilities. The acquisition of Codiak Biosciences added an exosome manufacturing facility in Massachusetts, along with the cell line engineering and manufacturing technologies, underlying knowledge and experience associated with the engEx® exosome platform. In addition, Lonza added a 500L perfusion bioreactor and downstream process with fully scalable unit operations for the production of exosome drug substances and drug products.

Beyond these capacity increases, Lonza is improving the cell line engineering of engEx® exosomes leveraging VS piggyBac™ transposase technology and our proprietary HEK293 cell lines to increase exosome design, expression, and tropism. Lonza's VS piggyBac™ transposon technology can be leveraged to further increase the natural targeting ability of exosomes. During cell engineering, DNA is preferentially inserted into stable regions of the cell genome that are associated with highly expressed genes. As a result, exosomes with specific traits can be generated in high titers. We are also optimizing both the upstream and downstream bioprocesses to strengthen and scale the engEx® manufacturing platform, enabling cost-effective, large-scale GMP production of therapeutic exosomes. Our manufacturing platform is supported by our cutting-edge characterization toolbox, including single-exosome analysis through nanoflow cytometry, which ensures precise analysis of EV products.

Lonza aims to be the top provider of end-to-end services for exosome-based therapies. Our deep understanding of exosome quality and impurity profiles allows us to produce potent, highly controlled exosomes that ensure precise delivery and maintain safety, even with complex drug substances. We are committed to expanding our development and manufacturing solutions to support clients in creating naïve and engineered exosomes with tissue-specific targeting for regenerative, antiviral, and therapeutic applications.

Helping Translate Exosome Candidates into Approved Therapies

Exosomes are cell-derived nanoparticles that can act as a carrier of naturally occurring biomolecules with therapeutic benefit as well as enable safe delivery of drug substances too toxic for free systemic administration. Early clinical data has revealed the potential efficacy and

highlighted the safety of these natural delivery vehicles. Like other next-generation biologics, however, exosomes are complex, and their development and manufacturing can be challenging.

Lonza can simplify the development process. Therapy developers can now benefit greatly from not only engEx® technologies independently, but also our experience and deep knowledge regarding process development, optimization, manufacturing of exosomes from multiple cell sources (e.g. exosomes from mesenchymal stem cells) and regulatory expertise through interactions with regulatory authorities.

Customers looking to benefit from Lonza's engEx® exosome platform have several options. Through licensing agreements, they can access engEx® tools and technologies needed to create their own cell lines and generate engineered exosomes in their laboratories. Once this early work is completed, customers can continue to advance their projects in-house, or leverage Lonza's process development and large-scale manufacturing capabilities. Alternatively, given a payload and drug target, Lonza can develop a cell line that will highly express exosomes with maximal loading and potency, and cost-effectively produce those exosomes using our perfusion-based process from research to commercial scale.

Partnering with Lonza not only allows access to technologies and expertise that will help improve the manufacturing productivity and scalability of exosome-based therapeutics, but can potentially speed the path to clinic and market for these novel life-changing treatments.

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