# Designing a Plug-and-Play System to Produce Engineered Extracellular Vesicles

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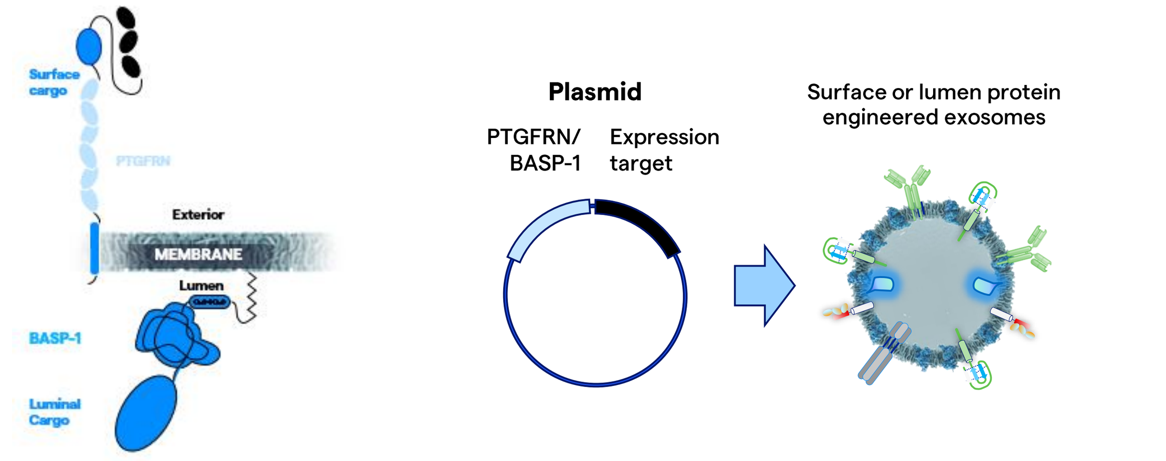
## Introduction

Extracellular vesicles (EVs), including exosomes, are being studied extensively and have emerged as a promising novel technology for the delivery of complex therapeutic payloads such as nucleic acids or proteins. EVs are naturally occurring within the body and have several potential advantages over other delivery vehicles such as LNPs or viral vectors, such as their ability to evade the immune system response and therefore support repeat dosing, as well as their capacity to access biological barriers and deliver a wide range of cargo to specific target tissue types given their tropism profiles. These attributes make EVs favorable candidates to engineer as drug delivery systems.

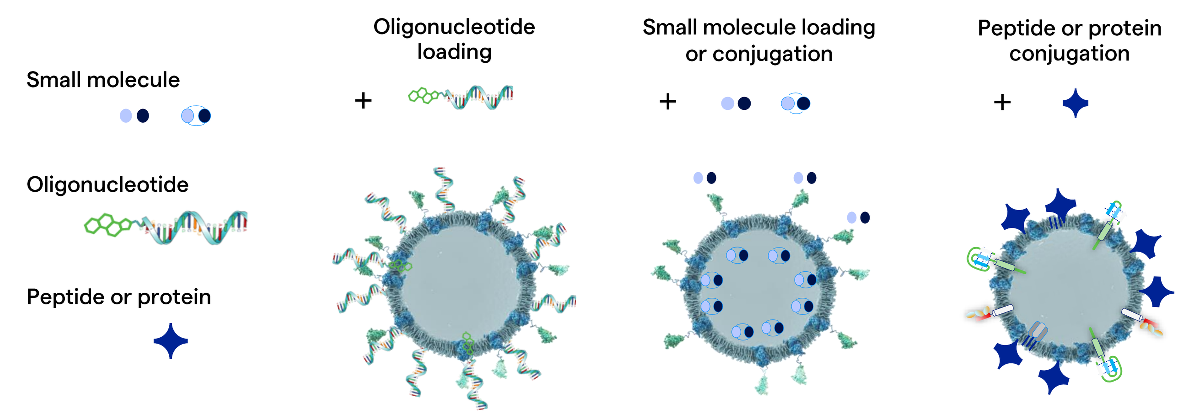
Here, we discuss a plug-and-play platform approach to express biomolecules using engineered EVs. Therapeutic cargo may be loaded onto or within EVs via identified scaffold proteins using novel plasmid design. This concept was explored in the development of therapeutics such as exoSTING®, exoASO-STAT6® and exoIL-12™, which have completed Phase 1 clinical studies within oncology applications. Alternatively, small molecules may be linked to EVs using a proprietary linker technology. A dedicated cell line for EV production was also generated, which resulted in a clone capable of high particle titer, integrity, and purity. These technologies will be made available to the wider scientific community under a research license, or via Lonza's CDMO service offerings.

## engEx® Technologies to Engineer Exosomes

Cell Engineering



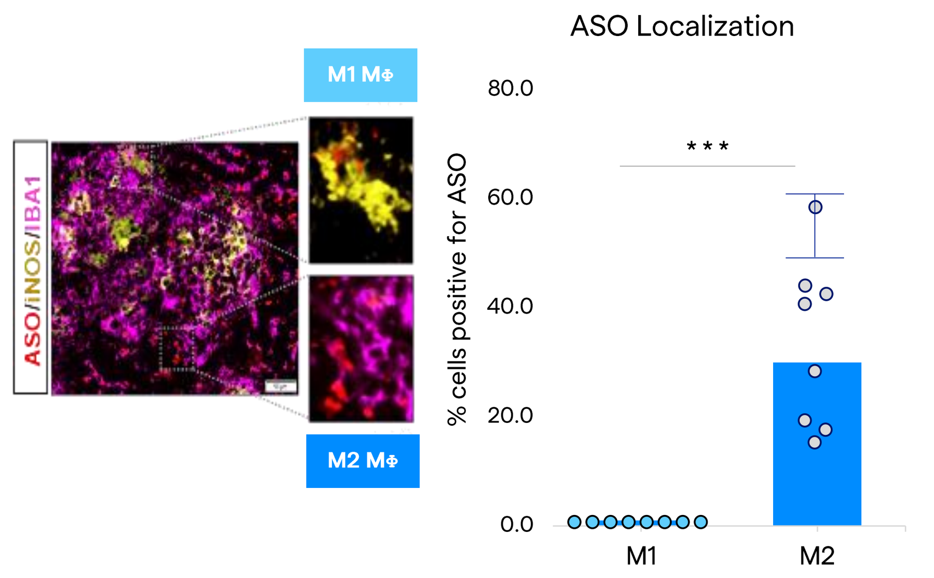

Post-Purification Loading



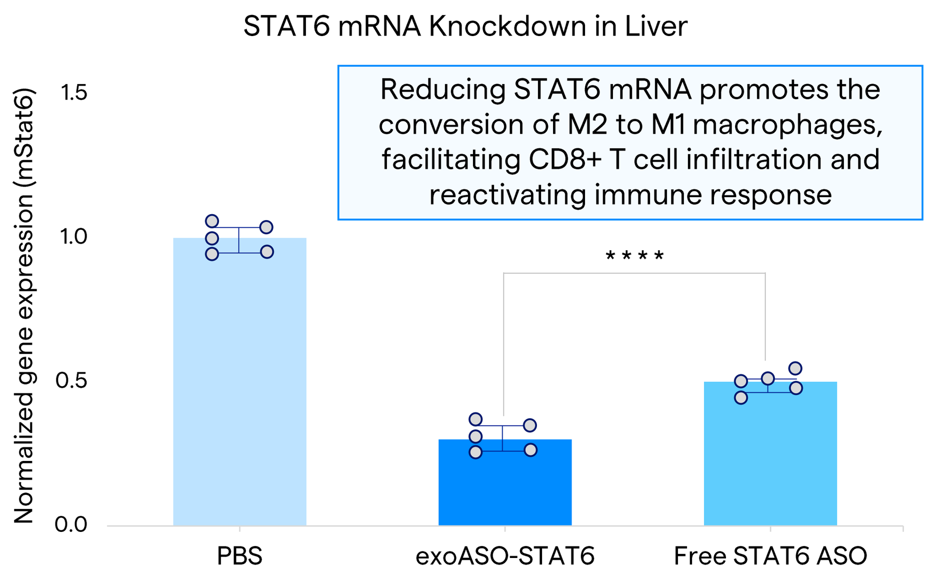

**Completed Phase 1 clinical studies :**

- exoASO-STAT6®
  - Payload: ASO
  - Route: Intra-venous
  - Indications: Gastric, Hepato-cellular Carcinoma
- exoIL-12™
  - Payload: Endo. IL-12
  - Route: Intra-tumoral
  - Indication: Cutaneous T-Cell Lymphoma
- ExoSTING®
  - Payload: STING CDN
  - Route: Intra-tumoral
  - Indications: Chondrosarcoma, Cervical Cancer

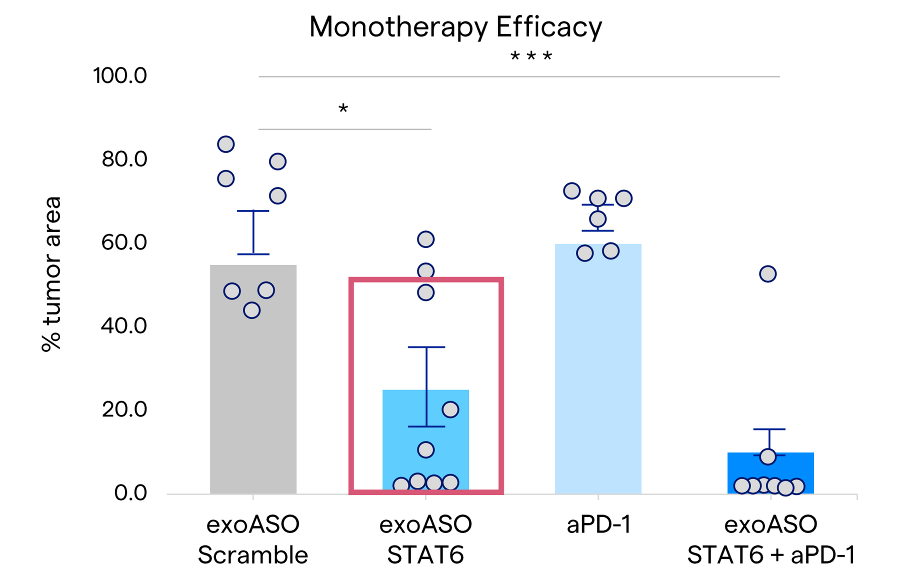
## exoASO-STAT6®: Targeted Nucleic Acid Delivery



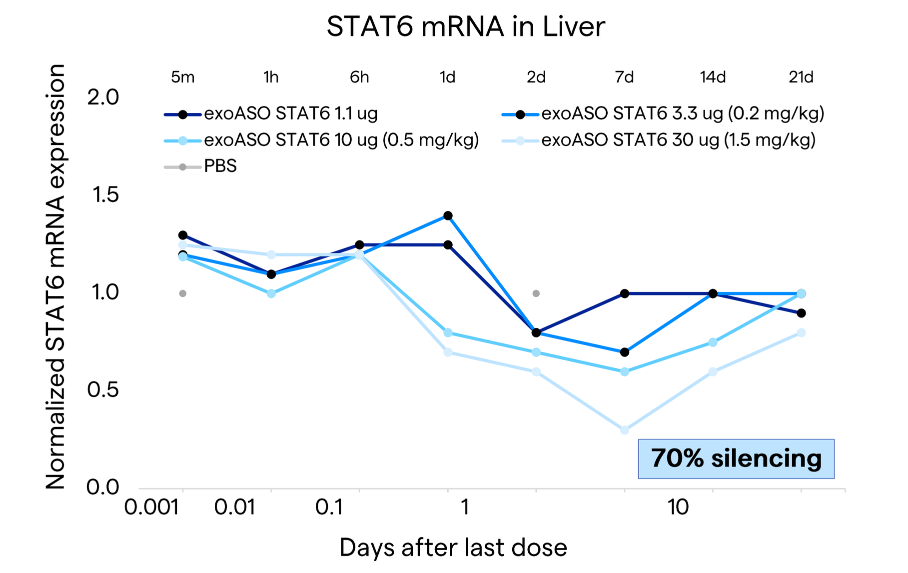

Selective delivery to tumor-associated M2 macrophages (Mϕ)



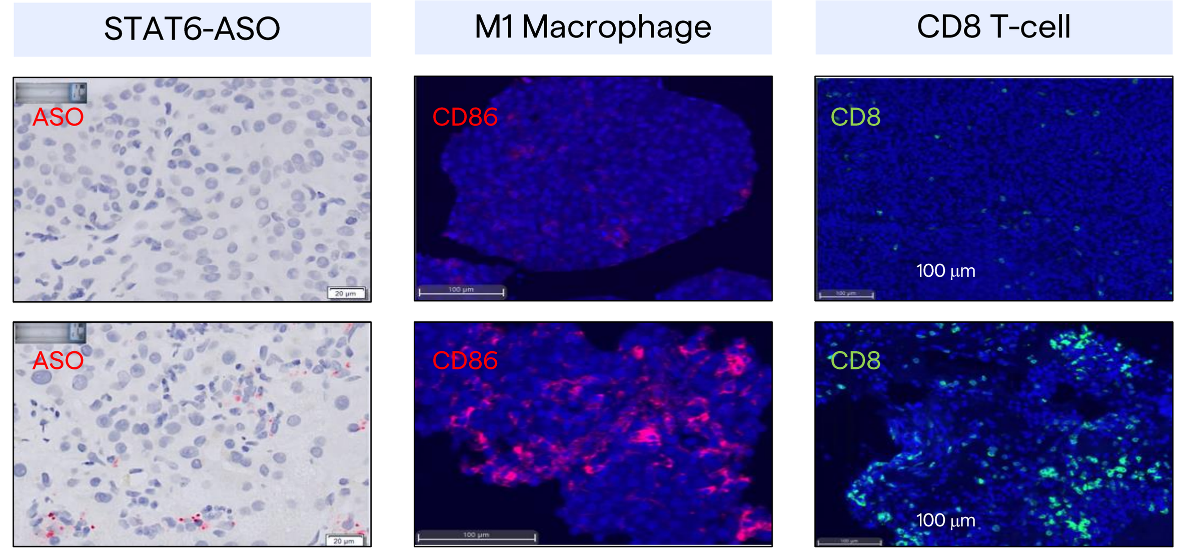

80% silencing of STAT6 mRNA observed




Potent monotherapy activity observed as a single agent or as part of a combination treatment

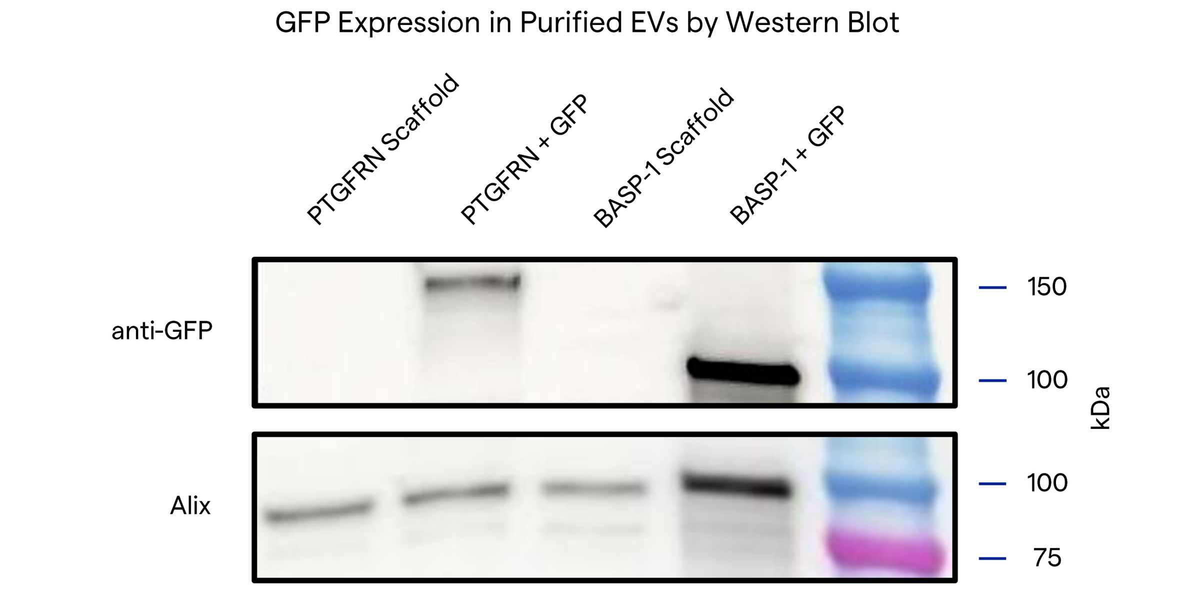



Dose-proportional STAT6 knockdown for one week




Robust reprogramming of tumor micro-environment observed from **Phase 1** clinical studies for exoASO-STAT6® therapy in **75%** of the patients with hepatocellular carcinoma as seen from histopathological analysis of tumor biopsy samples

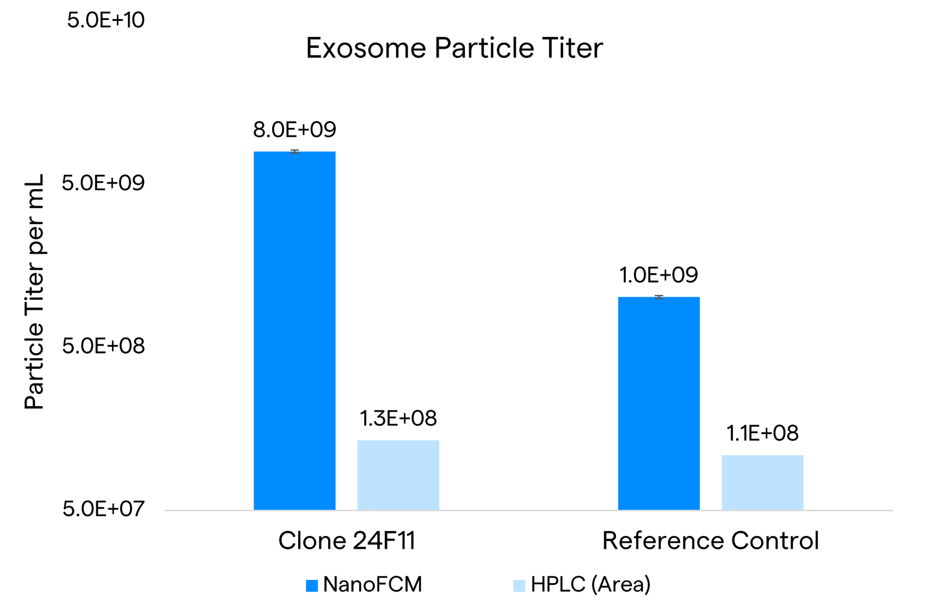
## Platform Development: Plasmids and Cell Line



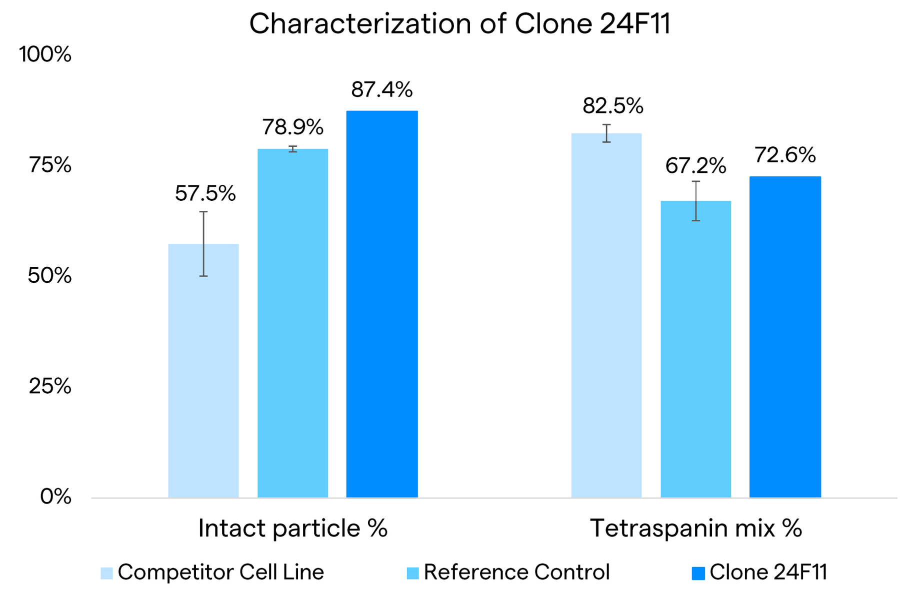

| Expected Molecular Weight (kDa) | |
|---|---|
| GFP | 27 |
| BASP-1 | 65 |
| PTGFRN | 96 |
| BASP-1-GFP Fusion | 92 |
| PTGFRN-GFP Fusion | 123 |
| Alix (Reference) | 95 |

* Western blot cropped to display relevant samples.

Vectors designed to express scaffold-cargo fusion proteins : Cargo loading in EVs successful




Clone 24F11 : A high-productivity dedicated cell line for EV production




High particle integrity and purity observed resulting in superior exosome quality

## Conclusions

1. engEx® technologies may be used to engineer exosomes to link or load therapeutic payload such as proteins, nucleic acids or small molecules to their surfaces or lumens. This technology forms the basis for clinical programs using engineered EVs that have shown promise in clinical trial.
2. Partnering with Lonza allows access to our expertise and technologies via our EV platform that will help improve the manufacturing productivity and scalability of EV-based gene therapies, which could potentially accelerate the time to clinic and market for these life changing treatments.

For more details and to access Lonza's EV platform, contact us at **licensing@lonza.com**