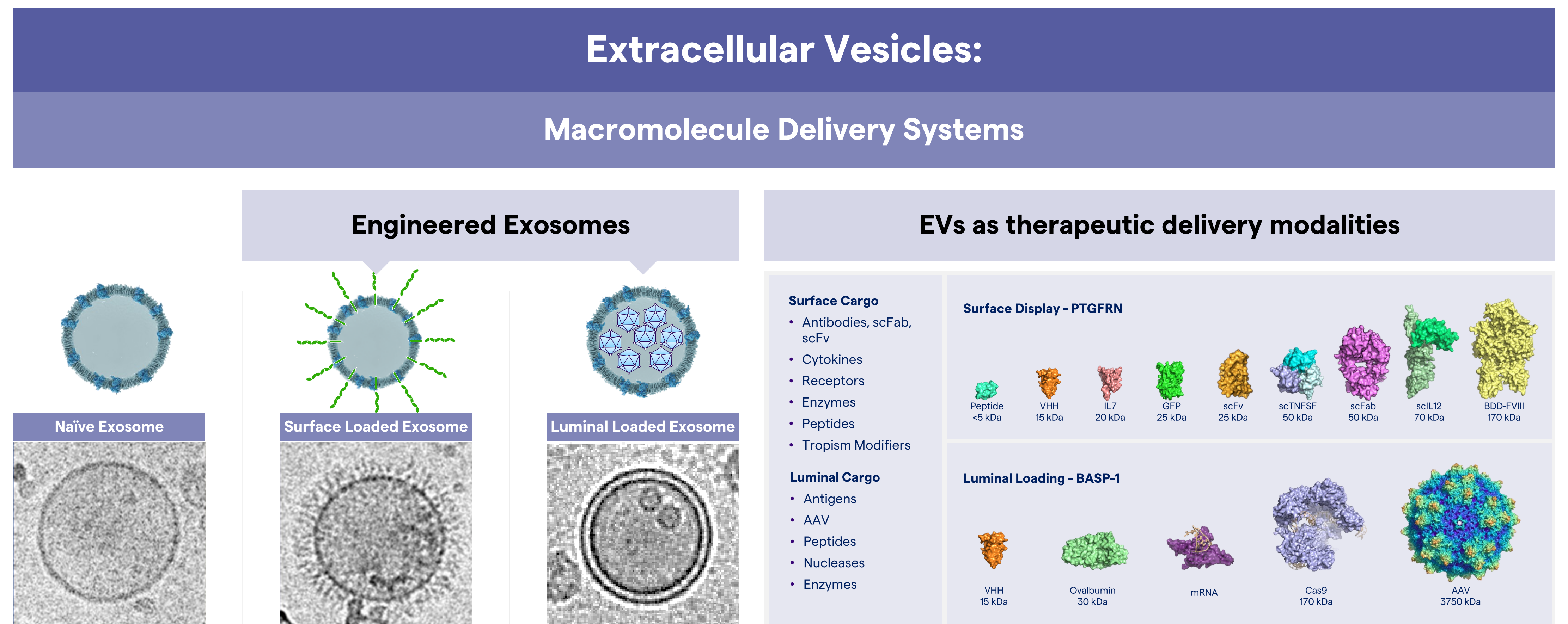# Extracellular Vesicles:
Engineering new therapeutic delivery modalities

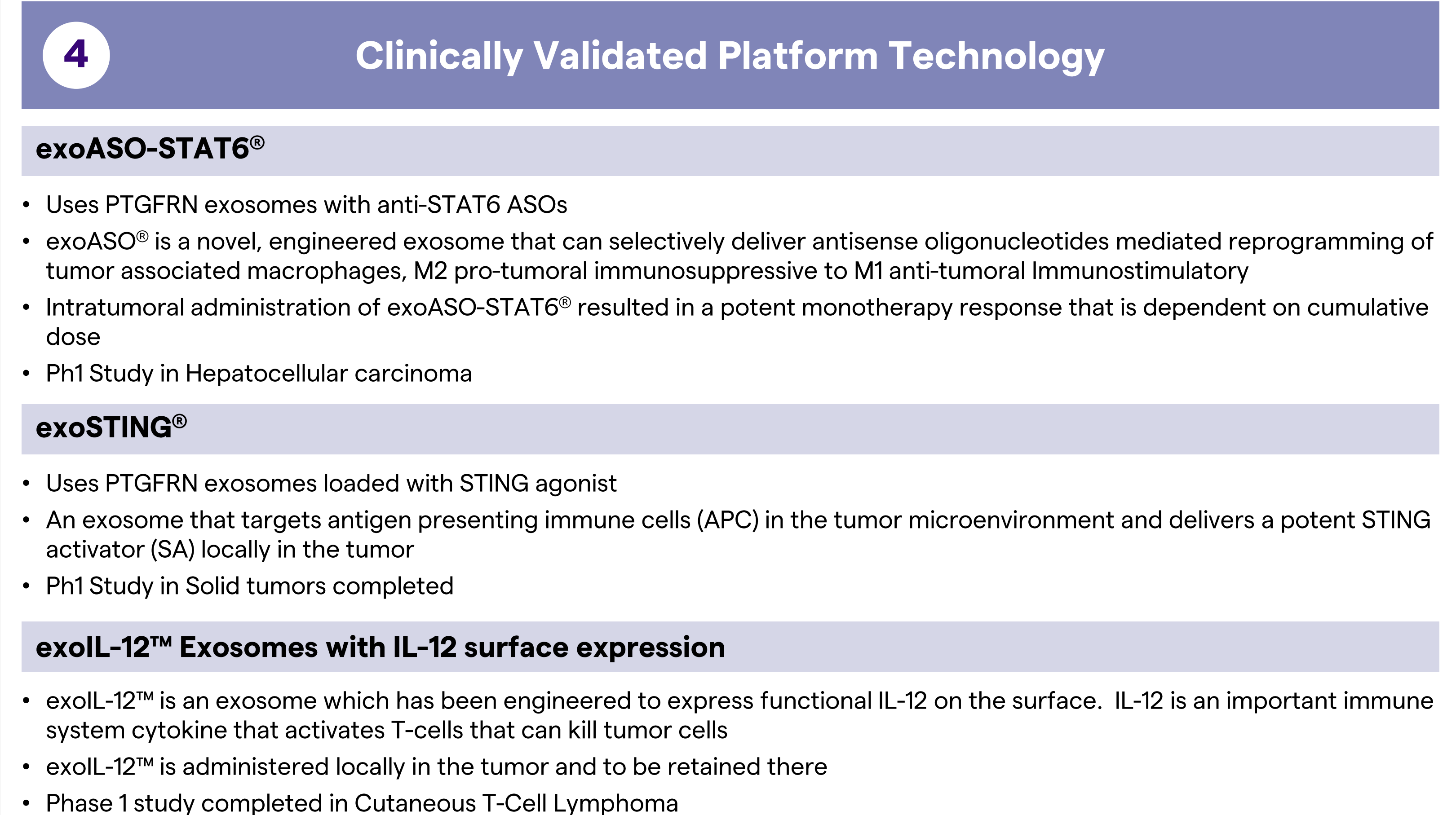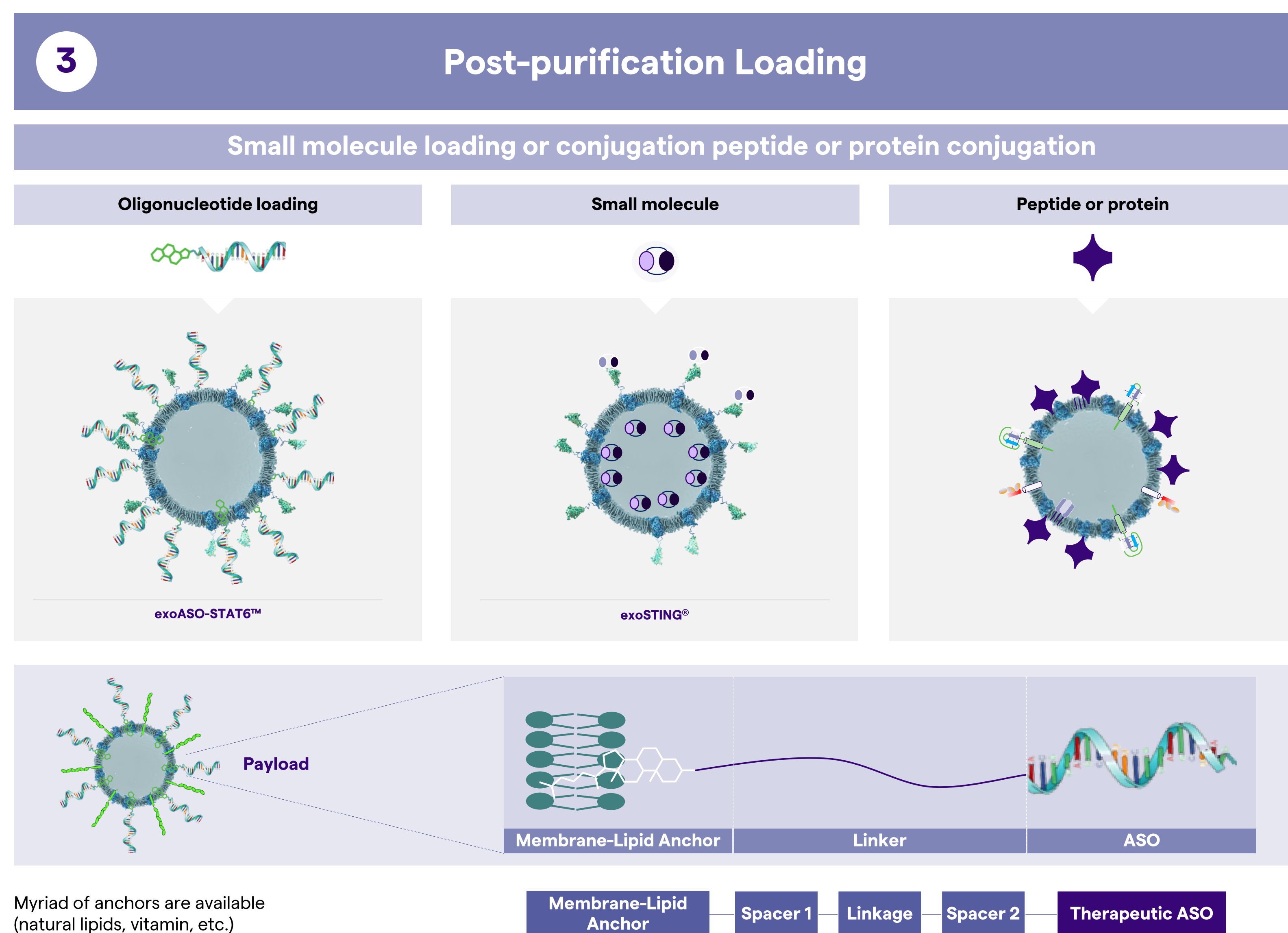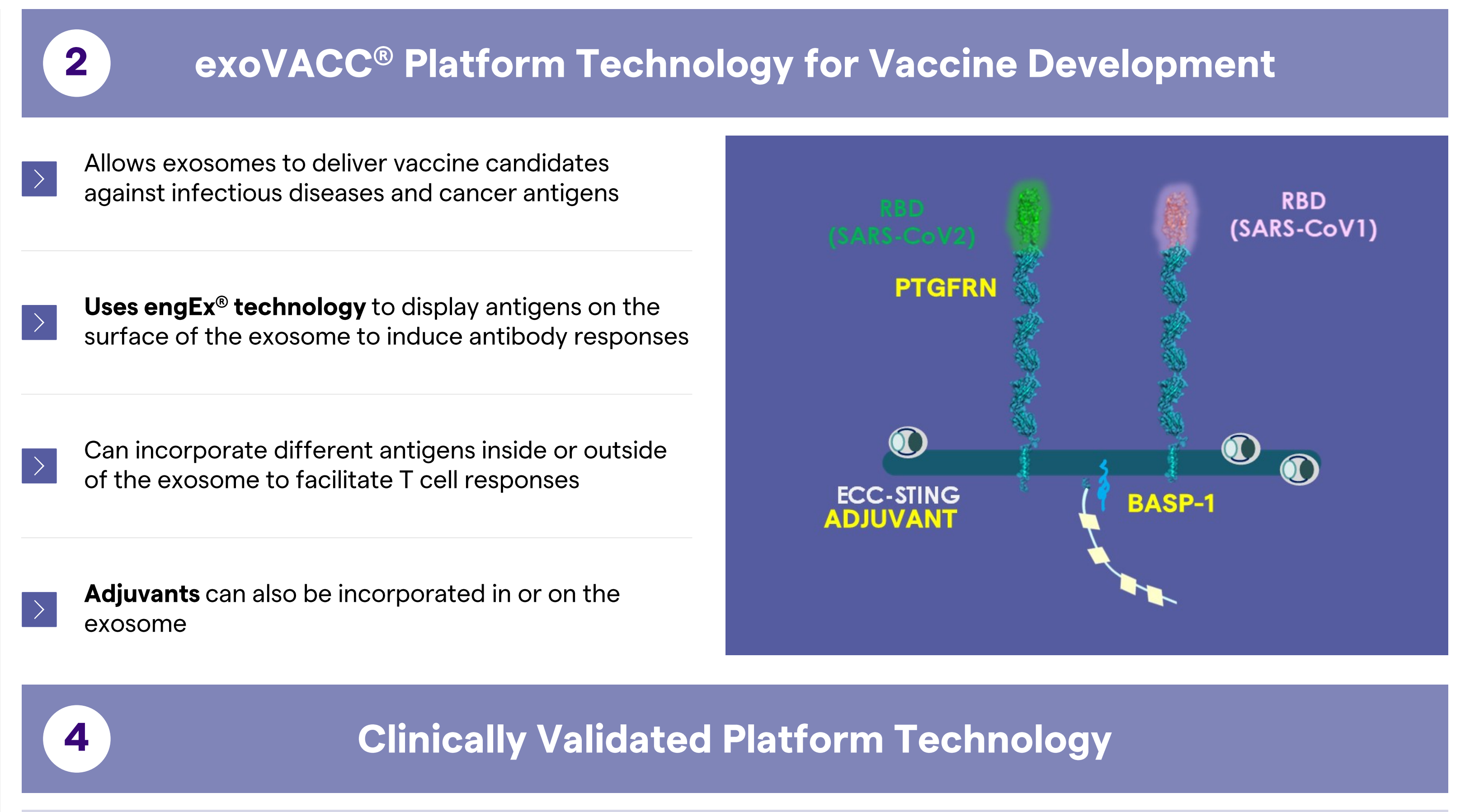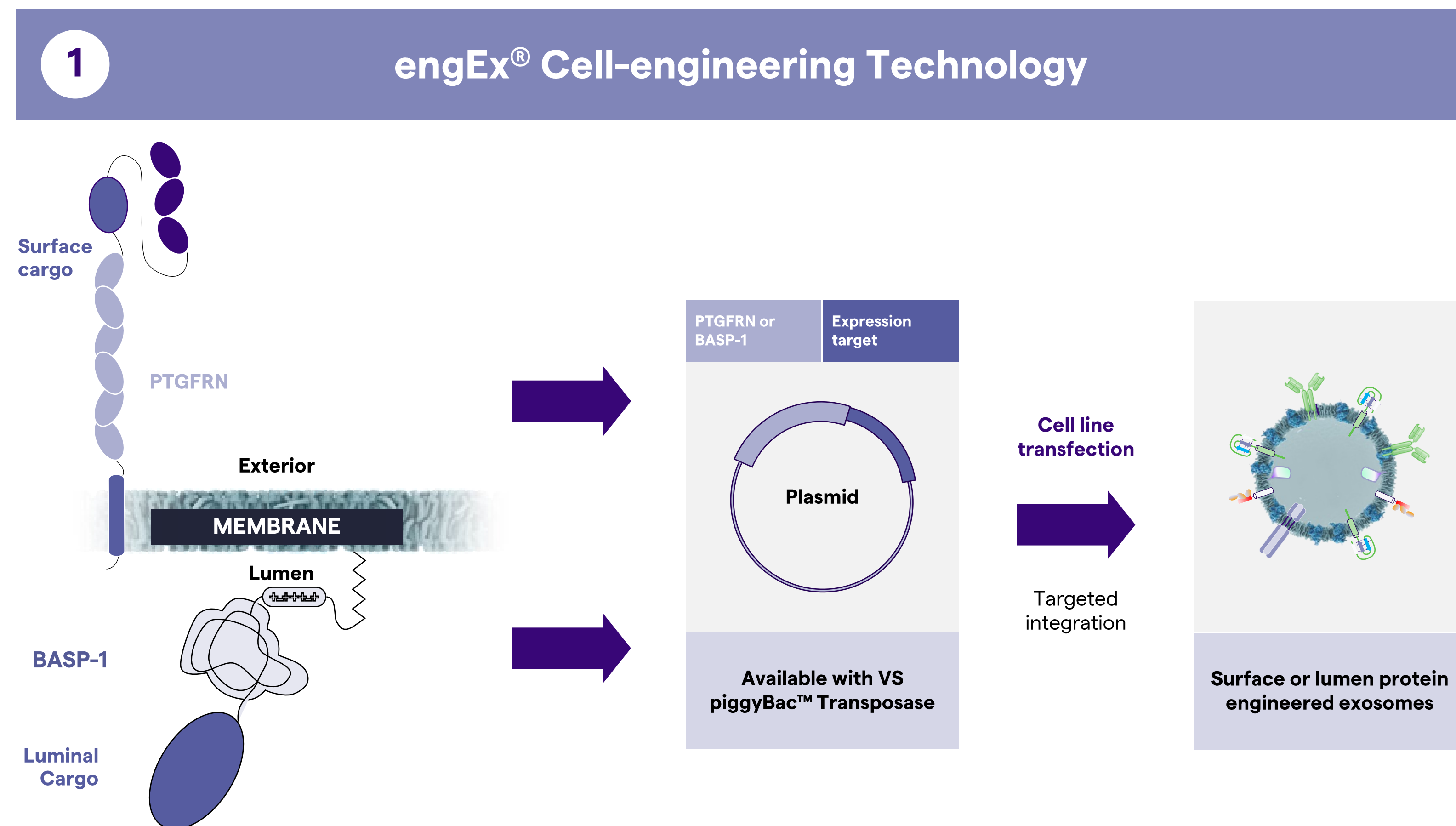## Innovate together with Lonza in Your Lab®

Lonza Cell & Gene

Introduction

Extracellular Vesicles (EVs), including exosomes, are emerging as promising natural nano-scale platforms for delivering nucleic acids, proteins, and small molecules due to their immune silencing properties. However, precision EV engineering has been challenging to date. Such technologies have also been largely inaccessible for a broader community due to technical complexities. Here, we discuss materials and methods to express biomolecules on EVs.

Protein scaffolds, including Prostaglandin F2 receptor negative regulator (PTGFRN) and Brain acid soluble protein 1 (BASP1) were developed and clinically proven to effectively load therapeutic proteins on the surface or lumen of EVs. Chemical linkers were developed to link nucleic acids or small molecules to the EV membrane while preserving their bioactivity and potency. Technologies described here will be available to Pharma, Biotech and Academic communities under a Research License for broader access. They will also be available via contract development and manufacturing services for producing engineered EVs.

Extracellular Vesicles:
Macromolecule Delivery Systems

Engineered Exosomes

Naïve Exosome

Surface Loaded Exosome

Luminal Loaded Exosome

EVs as therapeutic delivery modalities

Surface Cargo
- Antibodies, scFab, scFv
- Cytokines
- Receptors
- Enzymes
- Peptides
- Tropism Modifiers

Luminal Cargo
- Antigens
- AAV
- Peptides
- Nucleases
- Enzymes

Surface Display - PTGFRN

| Peptide <5 kDa | VHH 15 kDa | IL7 20 kDa | GFP 25 kDa | scFv 25 kDa | scTNFSF 50 kDa | scFab 50 kDa | scIL12 70 kDa | BDD-FVIII 170 kDa |

Luminal Loading - BASP-1

| VHH 15 kDa | Ovalbumin 30 kDa | mRNA | Cas9 170 kDa | AAV 3750 kDa |

engEx® Exosome Linking and Loading Technologies

1 engEx® Cell-engineering Technology

Surface cargo

PTGFRN

Exterior

MEMBRANE

Lumen

BASP-1

Luminal Cargo

PTGFRN or BASP-1 / Expression target

Plasmid

Cell line transfection

Targeted integration

Available with VS piggyBac™ Transposase

Surface or lumen protein engineered exosomes

2 exoVACC® Platform Technology for Vaccine Development

- Allows exosomes to deliver vaccine candidates against infectious diseases and cancer antigens

- **Uses engEx® technology** to display antigens on the surface of the exosome to induce antibody responses

- Can incorporate different antigens inside or outside of the exosome to facilitate T cell responses

- **Adjuvants** can also be incorporated in or on the exosome

RBD (SARS-CoV2)

RBD (SARS-CoV1)

PTGFRN

ECC-STING ADJUVANT

BASP-1

4 Clinically Validated Platform Technology

exoASO-STAT6®

- Uses PTGFRN exosomes with anti-STAT6 ASOs
- exoASO® is a novel, engineered exosome that can selectively deliver antisense oligonucleotides mediated reprogramming of tumor associated macrophages, M2 pro-tumoral immunosuppressive to M1 anti-tumoral Immunostimulatory
- Intratumoral administration of exoASO-STAT6® resulted in a potent monotherapy response that is dependent on cumulative dose
- Ph1 Study in Hepatocellular carcinoma

exoSTING®

- Uses PTGFRN exosomes loaded with STING agonist
- An exosome that targets antigen presenting immune cells (APC) in the tumor microenvironment and delivers a potent STING activator (SA) locally in the tumor
- Ph1 Study in Solid tumors completed

exoIL-12™ Exosomes with IL-12 surface expression

- exoIL-12™ is an exosome which has been engineered to express functional IL-12 on the surface. IL-12 is an important immune system cytokine that activates T-cells that can kill tumor cells
- exoIL-12™ is administered locally in the tumor and to be retained there
- Phase 1 study completed in Cutaneous T-Cell Lymphoma

3 Post-purification Loading

Small molecule loading or conjugation peptide or protein conjugation

Oligonucleotide loading	Small molecule	Peptide or protein

exoASO-STAT6™

exoSTING®

Payload

Membrane-Lipid Anchor | Linker | ASO

| Membrane-Lipid Anchor | Spacer 1 | Linkage | Spacer 2 | Therapeutic ASO |

Myriad of anchors are available (natural lipids, vitamin, etc.)

Conclusion

- engEx® protein scaffolds can be used to effectively load therapeutic proteins on the surface or lumen of the EVs. Chemical linkers are also available that can link nucleic acids or small molecules to the EV membrane.

- Gene therapy developers can now benefit greatly from not only the technologies independently, but also our experience and deep knowledge regarding EV process development, optimization, manufacturing and regulatory expertise through interactions with the FDA.

- Partnering with Lonza allows access to technologies and expertise that will help improve the manufacturing productivity and scalability of EV-based gene therapies and could potentially accelerate time to clinic and market for these life changing treatments.

Lonza in Your Lab®

Research Licenses
- Covers all pre-clinical work
- Enables creating an unlimited number of cell lines*
- A complete package including access to relevant plasmids and an extensive know how with expert support.

Commercial Licenses
- Flexible approaches to commercial terms
- A portable system for work in-house or at partners and a range of service providers
- Favourable terms for manufacturing at Lonza*

*Subject to the terms and conditions of the license agreement

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