Establishment of a research facility for the production, concentration and purification of viral vectors and Virus-Like Particles (VLPs) for the application in gene therapy, vaccine and antibody development

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Aim: Reducing Production Costs of 2nd Generation Biologics

The ongoing development of active substances produced in bacteria, yeast, insect or even mammalian cells has revolutionized the pharma market in the past four decades. The 1st generation biologics where mostly monoclonal antibodies and therapeutic proteins such as hormones and cytokines. Currently, more complex biologics path new ways in vaccine development and somatic gene therapy. These 2nd generation active substances are replication-incompetent particles engineered from a variety of different parental viruses. Particularly, vectors and Virus-Like Particles (VLPs) derived from membrane enveloped species such as retroviruses are promising candidates for innovative therapeutic approaches in the battle against cancer and infectious diseases (see fig. 1). Since the production costs are comparably high, processes need to be streamlined to enhance productivity and reduce product loss to provide a broad population with stateof- the-art medication at affordable prices.

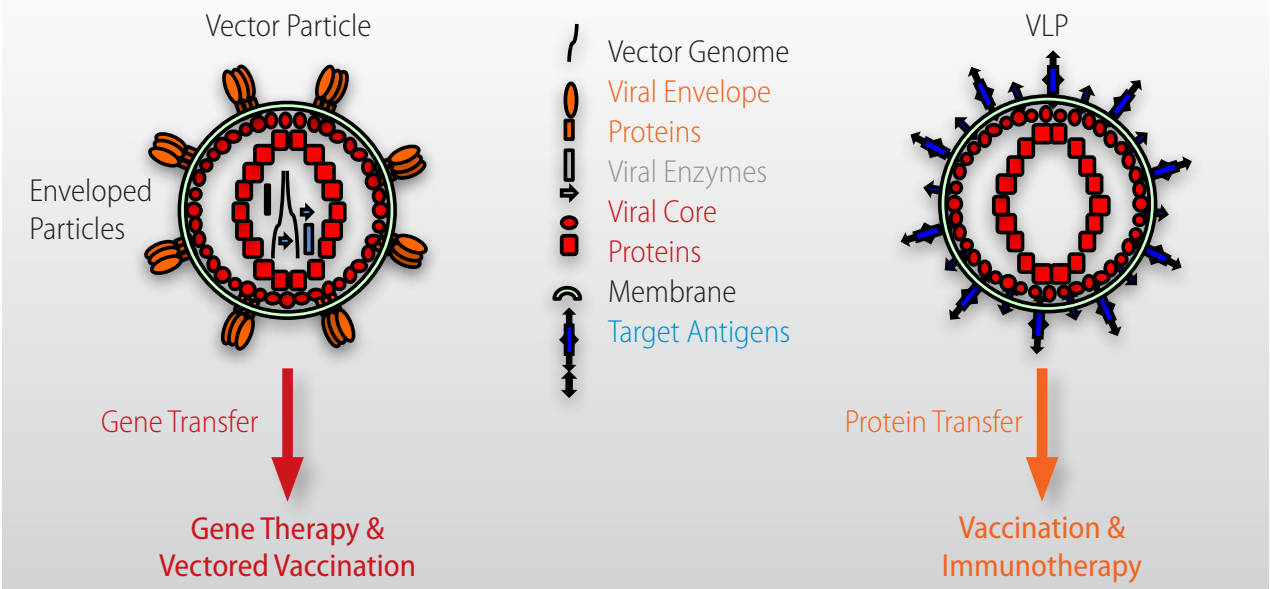

Figure 1: Structural composition and applications of viral vectors and VLPs

Step 1: CLD – Cell Line Development achieving higher Cell Productivity

To decrease production costs for 2nd generation biologics, higher cell productivities will have to be achieved. Consequently, both parameters of the producer cell system will be optimized. Different parental mammalian cell lines from a variety of donor species will be screened for their basal productivity using standardized expression vectors encompassing easy-to-quantify reporter genes. Subsequently, the cell lines qualified will be utilized to identify the best vector expression system and expression cassette design. Advanced techniques such as transposon vector technology will be employed. Upon selection of the most efficient donor cell and expression system, highly productive viral packaging cell lines will be established (see fig. 2 for schematic illustration of the production flow).

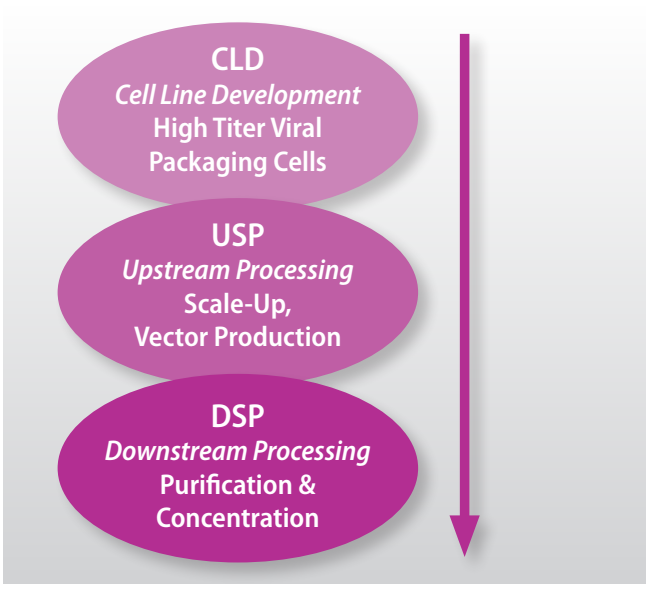

Figure 2: Production flow

Step 2: USP – Upstream Processing and Scale-Up of Production

Production will be scaled up to volumes of up to 10 liters employing single-use technology and devices such as shaker and spinner flasks and cell bags. Defined serum-free cultivation media will be screened for further productivity optimization shedding a first light on the results to be expected in industrial production scenarios.

Read Out: Analysis of Product Recovery and Integrity

– Western blot-analysis and ELISA of viral protein quantity
– Protein gel staining to assess vector purity and composition
– Electron microscopy & dynamic light scattering to detect aggregation
– Easy-to-measure biological activity by titration of transduction-competent particles (fig. 3)

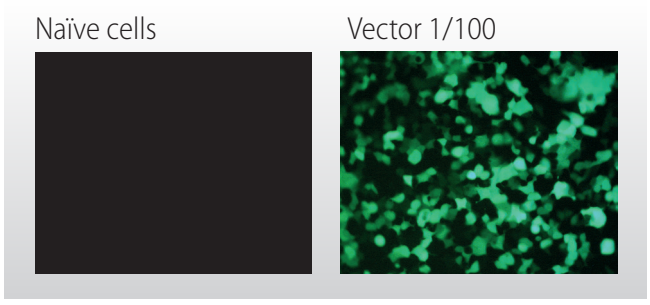

Figure 3: Transduced target cells expressing the reporter gene egfp.

Step 3: DSP – Downstream Processing enhancing Product Recovery

Current industrial production and downstream processes suffer a product loss of up to 80 % mostly attributed to inefficient product recovery during purification or the loss of the products biological activity during concentration. Thus, a range of purification and concentration techniques will be rigorously assessed. Amongst others these are micro-, ultra- and nanofiltration using dead-end and cross-flow approaches, modern adsorber membranes, ion-exchange and size exclusion chromatography.

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