Shortening the Timeline from Customer into Clinics: Development of a Downstream Processing Platform for Adeno-associated virus (AAV)

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1. Background

Many diseases are based on a genetic defect and are curable in the rarest cases. Gene therapies based on viral vectors offer the possibility of minimizing or completely eliminating the symptoms of the gene defect. The gene therapeutics approved to date are among the most expensive drugs in the world (1). This poster presents investigations to derive an economic and scalable method for purification of AAV.

2. Development Strategy

Platform approach

Inefficient manufacturing processes and the in part small effected patient populations result in high manufacturing costs, which pose an economic challenge to the manufacturing companies (2). One way to reduce production costs is to develop a platform technology for the purification of AAV particles. Standardization should ensure the reproducibility, scalability and economic efficiency of the process.

Steps

- a generic DSP platform for AAV was established in different steps (Fig. 1)

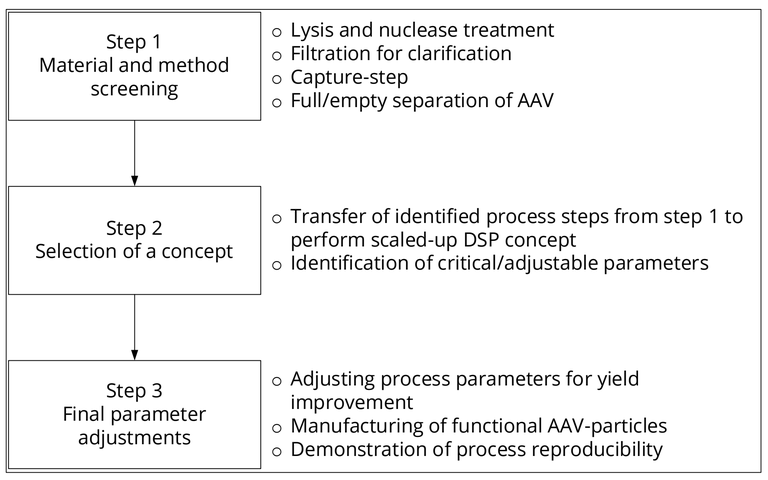

Fig.1: **Flow-scheme of steps for Downstream Processing Platform generation:** From the initial screening phase for suitable materials and methods the DSP concept was derived. Critical process parameters were adjusted in the last step.

3. Challenges

- Development of a generic platform for a wide range of AAV serotypes or surface modified subtypes
- Different approaches described for efficient AAV purification (e.g. AIC, TFF, HIC and CEX, see Fig. 2)
- Separation of full/empty AAV-particles (based on differences in surface properties caused by different DNA load) to ensure safety of end product (Fig. 3)
- High concentrations must be achieved for therapeutic applications to keep the dosis volume low

Method	AIC	HIC/CEX	TFF
Process steps	1	2	1
Preparation step after clarification	no	yes	no
Process time for processing 1L clarified harvest	400 - 500 min	HIC: 270 min CEX: 270 min	400 - 500 min
Protein reduction [LRV]	3,0	> 1,96	1,74
DNA reduction [LRV]	1,78	1,12	0,26
Yield capsid	> 65 %	< 30 %	10 - 25 %
Neutralization step after process step	yes	yes	no
End concentration	> 1,0E+13 cp/mL	> 1,0E+12 cp/mL	> 1,0E+12 cp/mL

Fig.2: **Decision matrix for capture-step:** Different strategies for AAV purification were compared towards process relevant criteria and evaluated according to showing good (green), neutral (yellow) or poor (red) performance.

Fig.3: **Flow chart AAV analytics via SEC-MALS/UV for empty/full ratio determination.** A precise method for full/empty separation was implemented at IDT. Full/empty ratio can be analyzed by both UV absorption and multi-angle light scattering (MALS) independently. In addition, analysis via MALS is suitable for determination of diverse titer values (e.g. capsid or viral genome titer).

4. DSP Concept

The DSP concept comprising of lysis and enzymatic treatment for DNA reduction, followed by clarification filtration. Afterwards Affinity interaction chromatography (AIC) for capturing is performed. Enrichment of full AAV's is performed by Anion exchange chromatography (AEX). The last steps are the final concentration and diafiltration into formulation buffer and sterile filtration.

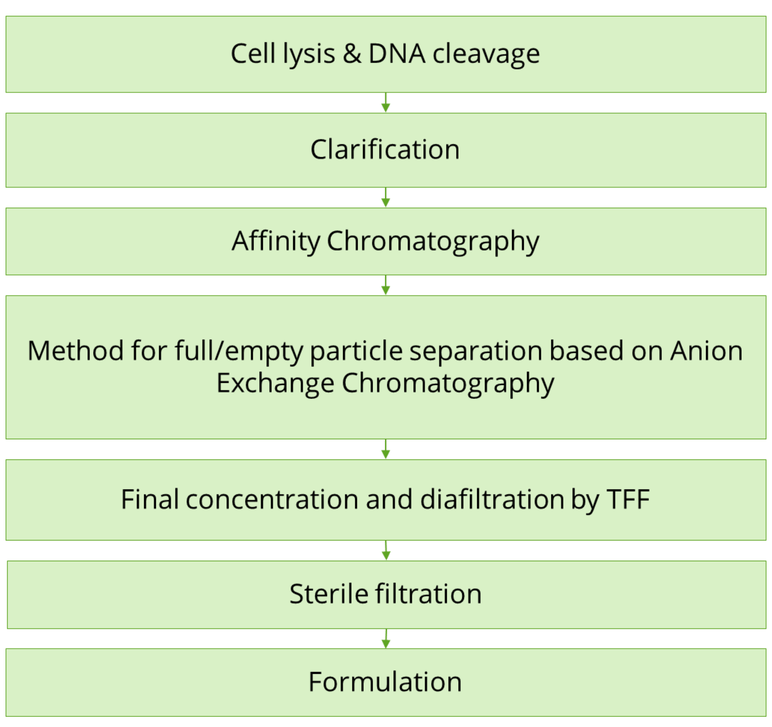

Figure 4: **Illustration of a typical AAV platform manufacturing approach established at IDT.**

5. Optimization

The initial concept run revealed critical process parameters, which were evaluated and adjusted. In coorperation with supplier, the optimal performance was achieved. Adjustments were e.g.:

- Equilibration strategy for filter
- Elution buffer (pH and buffer components) at AIC was changed
- Change of cut-off to 100 kDa size at TFF step

The combination of all adjustments lead to a significant increase of step and overall process yield (Fig. 5).

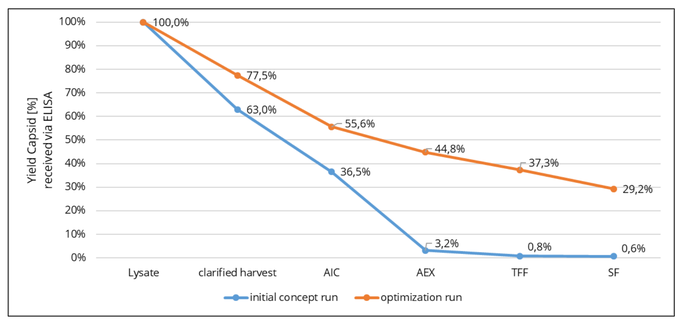

Fig.5: **Improvement of AAV yield by adjustment of critical process parameters.** Performance of 1st concept run showed different aspects for improvement. These aspect were tested in an optimization run and showed clear yield increase.

Conclusion

IDT has developed a purification platform for AAV based on sophisticated experimental data and a multi-criteria decision matrix.

The key to success was the detailed investigation on each process steps. This intense study revealed valuable insights into AAV-specific challenges and enabled the well-informed selection of the most suitable purification technologies and strategy.

The established DSP strategy serves as a ready to use platform for AAV manufacturing. Due to the generic approach, several serotypes and surface modifications of AAV are manufacturable. The standardizing of the material and process concept leads to minimum timelines into clinics and production costs while high process understanding ensures process reliability.

Inquiries are welcome

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References

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