CELL & GENE THERAPY INSIGHTS

Supplementary Data

Viral vector manufacturing: how to address current and future demands?

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DISCLAIMER

While working on this article, we noticed that detailed information about GMP manufacturing protocols at scale is often not available publicly. We have cited relevant literature where possible, but information has also been obtained through discussions with experts and presentations at the ECI workshop ("Developing a toolkit to engineer viral vector manufacturing and next generation gene therapies"), ECI conference and other relevant meetings. However, ultimately it is hard to establish a true comparison considering the many differing analytical procedures and the lack of standardized vector types which incur substantial differences in vector products.

GENERAL ASSUMPTIONS

- An average recovery of infectious titer post-downstream processing of 25% was assumed for all production technologies for LV
- An average recovery of physical titer post-downstream processing of 50% was assumed for all production technologies for AAV
- 20% extra virus was assumed to be needed for QC for both AAV and LV
- If a dose range was cited in the literature, the average dose was assumed to calculate annual requirements. For example: single dose of 1 to 2.5 x 10^8 viable transduced T cells will be averaged as 1.3x10^8 viable transduced T cells

INDICATION SPECIFIC ASSUMPTIONS

B-cell lymphoma (LV)

- MOI of 4 was assumed following ECI pre-conference workshop discussions
- An annual patient treatment population of 5,000 was assumed based on predictions of the 2 currently available commercial products

- A dose of 1.75 x 10^8 viable transduced T cells (weight independent) was assumed based on the average adult dose range commercially available of 1-2.5 x 10^8 viable transduced T cells

β-thalassemia (LV)

- MOI of 100 was assumed based on preclinical trial literature [56] and ECI pre-conference workshop discussions (discussions ranged between 50 and 200)
- β-thalassemia is an ultra-rare disease, therefore a patient population of 10 patients per year was assumed based on the national USA birth rate and a prevalence of 1:100,000 [57]
- A dose of 7.1×10^6 CD34+ cells/kg was assumed and 70kg average patient weight [58]

Age-Related Macular Degeneration (LV)

- Annual dose requirement based on disease prevalence of 17% of over-55 year olds [59], population size and aiming for a potential uptake of 25% of new cases each year. 10,000 total assumed patients per year.
- A dose of 8 × 10^5 TU per eye [60] and treatment of both eyes was assumed

Leber's congenital amaurosis (AAV2)

- Patient population is based on prevalence of 1:50,000 births, assuming 80 patients per year [61]
- 1.5 × 10^{11} vg per eye based on publicly available dosage [5]. Treatment of one eye only was assumed

Lipoprotein lipase deficiency (AAV1)

- Dose of 1 × 10^{12} gc/kg based on publicly available data [62,63]
- As this is an ultra-rare disease a patient population of 25 per year in North America and Europe was assumed based on i) average prevalence of 1 in 1,000,000 and annual birth rate and ii) existing market. These estimates were based on an internal study performed at the National Research Council Canada

Spinal muscular atrophy (or SMA, AAV9)

- Dosage of 1 × 10^{14} vg/kg assuming patients were 6 kg (this is administered early in life, when weights are low) [64]
- Assuming 1,000 patients per year [1]

Duchenne muscular dystrophy (or DMD, AAV9)

- Dosage of 1 × 10^{15} vg per patient [3] assuming patients are 20kg (clinical trials are enrolling adolescents and children) [65]
- The patient population assumed was 500,000 per year [3]

TECHNOLOGY SPECIFIC ASSUMPTIONS

- All titers stated here are titers assumed for the harvest (i.e. pre-DSP and pre-QC).

10-layer Cell Factory (CF-10):

- It was assumed that 4.7 × 10^{14} vg/m^2 AAV vector could be produced from a 10-layer Cell Factory [66]
- It was assumed that 1.32 × 10^{11} TU m^{-2} LV can be produced from a 10-layer Cell Factory [1]
- The area of a CF-10 was assumed to be 0.632 m^2

Stirred tank reactors (STRs)

- 5 x 10^{13} vg/L based on references as average productivity of the range found for suspension cells in the literature for AAV [16,33]
- It was assumed that 1 × 10^{10} TU L^{-1} of LV can be produced from a stirred tank bioreactor [9]
- For both LV and AAV, the volumes of STRs were assumed to range from 1 L to 2,000 L

Fixed bed reactors (FBRs)

- The area of the FBRs were based on iCELLis® nano and 500 surface areas, for both low and high compaction. These ranged from 0.53 to 2.65 m^2 and 0.8 to 4 m^2 for the iCELLis® nano and 66 to 333 m^2 and 100 to 500 m^2 for the iCELLis® 500 [24]
- Based on expert input, we have only displayed surface areas covered for low compaction rates up to 333 m^2 as we suspect it is the largest surface area currently used for GMP manufacturing [1]
- It was assumed that (2.2×10^{14}) vg/m^2 AAV vector could be produced from FBR iCELLis® bioreactors [66]
- For LV, it was assumed that 6×10^{10} TU/m^2 can be produced from an iCELLis 500 bioreactor [23]

Improved STR

- For both AAV and LV, 10-fold higher yield was assumed compared to STR
- For LV, 3 harvests were assumed (i.e. 2,000 L x 3) and only one for AAV (2,000 L)