Characterization of a pharmaceutical lentiviral vector by orthogonal particle analysis techniques

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Purpose

Lentiviral vector (LVV) products are used to generate therapeutic chimeric antigen receptor (CAR) T cells. Virus particle concentrations (total, infectious, functional) need to be well characterized to ensure consistent potency, quality, and safety of LVV products. Besides the intended nanometer sized virus particles, particulate impurities of similar and larger (up to µm) size may also be present due to process- or product-related impurities. As a result, pharmaceutically relevant samples are highly heterogeneous in terms of particle size, concentration, and composition. In our study, we evaluate the usefulness of multiple orthogonal or complementary particle characterization techniques (NTA, DLS, ILM, MRPS) for a comprehensive characterization of LVV products from different manufacturing processes.

Materials and methods

LVV products from a suspension and an adherent manufacturing process were diluted in 25 mM HEPES (pH 7.0) with different dilution factors (200x for material 1 in ILM, 50x for material 2 in ILM, at least 100x for material 1 in NTA, at least 50x for material 2 in NTA) and analyzed by following orthogonal particle characterization techniques.

Nanoparticle tracking analysis (NTA)

- NanoSight NS300 (Malvern Instruments)
- Particle detection (size, concentration) based on light scattering and tracking of Brownian motion
- Light source: laser (488 nm CW)
- Size range: 10 nm – 1000 nm (sample and system configuration dependent)
- Concentration range: 10^6 – 10^9 particles/ml

Dynamic light scattering (DLS)

- Zetasizer Nano S (Malvern Instruments)
- Light source: He-Ne laser (633 nm)
- Size range: 0.3 nm – 10 µm
- Bulk analysis method (qualitative information only)

Interferometric light microscopy (ILM)

- Videodrop (Myriade)
- Particle detection based on light scattering and interference, tracking of Brownian motion
- Light source: LED (450 nm)
- Size range: 80 nm – 500 nm (biological particles)
- Concentration range: 10^8 – 10^{10} particles/ml

Microfluidic resistive pulse sensing (MRPS)

- nCS1 (Spectradyne)
- Particle detection based on Coulter principle (conductivity of sample required)
- Size range: 50 nm – 10 µm (cartridge dependent)
- Concentration range: 10^4 – $5x10^{11}$ particles/ml (cartridge dependent)

DLS and MRPS analysis of LVV products

DLS analysis of LVV products in several dilutions gave a mean Z-average of 755 nm for material 1 (suspension production process) and 89 nm for material 2 (adherent production process). However, both materials showed high polydispersity of the samples (Figure 1) which limited further interpretation of DLS results.

MRPS analysis of 100 nm polystyrene beads diluted in 25 mM HEPES (LVV sample dilution buffer) indicated too little sample conductivity, while dilution in 1x PBS led to expected results (Figure 2). Measurement of LVV samples by MRPS showed blockage of the system and requires further sample preparation besides adjustment of conductivity (experiments ongoing).

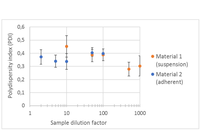


Figure 1: DLS analysis of diluted LVV samples from two different production processes (suspension and adherent).

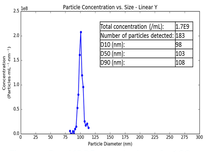
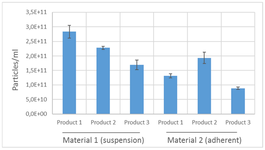


Figure 2: MRPS analysis of 100 nm polystyrene beads (CPC100, Izon) diluted 10,000-fold in 1x PBS (TS-300 cartridge).

Analysis of LVV products by ILM and NTA

ILM

Analysis of different LVV products of material 1 resulted in particle concentrations between $2x10^{11}$ and $3x10^{11}$ particles/ml whereas particle concentrations of LVV products of material 2 were generally lower with $9x10^{10}$ - $1x10^{11}$ particles/ml (Figure 3, left). Mode diameters of the same samples tended to be higher for material 1 (158 – 202 nm) than for material 2 (128 – 140 nm) (Figure 3, right), which indicates the presence of additional nm-sized impurities in material 1. Filtration (0.2 µm PES) of material 1 samples led to a reduction of the particle concentration by ~50% and a reduction in mode diameter from ~200 nm to ~160 nm (Figure 4) reflecting the removal of larger particulate impurities.



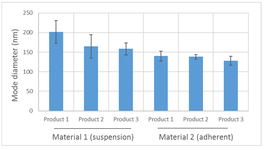


Figure 3: ILM analysis of six different LVV products from two different production processes. Particle concentrations are corrected by dilution factors.

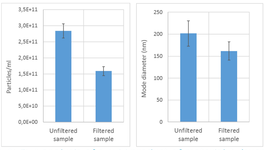
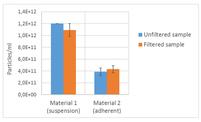


Figure 4: ILM analysis of a LVV product of material 1 (suspension process) before and after sample filtration with a 0.2 µm PES filter. Particle concentrations are corrected by dilution factor.

NTA

The analysis of LVV products from two different production processes by NTA showed lower particle concentrations (Figure 5, left) and slightly lower mode diameters for material 2 than material 1 (Figure 5, right). Sample filtrations with 0.2 µm PES filters did not have a major impact on particle concentrations or mode diameters indicating that NTA parameters were appropriately adjusted to predominantly detect particles smaller than 200 nm.



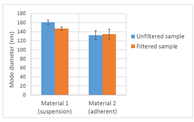


Figure 5: NTA analysis of two different LVV products from different production processes before and after sample filtration with a 0.2 µm PES filter. Particle concentrations are corrected by dilution factors.

Comparison of ILM, NTA and ELISA results

LVV particle concentrations determined by ILM were similar to physical titers by p24 ELISA for material 1 (Table 1) and material 2 (Table 2); NTA values were slightly higher. Mode diameters by ILM and NTA agreed well for both materials (Table 1 and 2).

Table 1: Average particle concentrations and mode diameters for material 1 (suspension process).

Technique	ILM	NTA	ELISA
Particle concentration (p/ml)	$3x10^{11}$	$1 x10^{12}$	$2x10^{11}$
Mode diameter (nm)	167	161	n/a

Table 2: Average particle concentrations and mode diameters for material 2 (adhorent procese).

Technique	ILM	NTA	ELISA
Particle concentration (p/ml)	$1x10^{11}$	$4x10^{11}$	$1x10^{11}$
Mode diameter (nm)	139	132	n/a

Conclusions

- NTA was confirmed as a valuable but low-throughput quantitative analysis tool for LVV products of different manufacturing processes.
- ILM proved suitable for fast LVV quantification agreeing well with ELISA-based viral particle concentrations, which are considered widely as reference values in LVV titration.
- In summary, ILM enables quick and simple measurement of LVV particle size and concentration, while NTA provides more optimization possibilities tailored to sample properties such as temperature control or a fluorescence detection mode.

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