IXAKA's versatile platform for the bioproduction & characterization of lentiviral vectors

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Background

- Recent market approval of CAR-T cell therapies and the continuously growing number of gene-based *ex vivo* and *in vivo* therapies evaluated in clinical trials has created a huge constraint on the supply of high quality grade lentiviral vectors (LV) which results in higher manufacturing costs and longer timelines that are not sustainable for early-stage development programs.
- We here report on Ixaka's versatile platform for the manufacturing of high-quality LV material designed to cover customers' needs from R&D *in vitro* and *in vivo* evaluation up to regulatory preclinical studies.

At a glance

- Founded in 2017
- 350 m2 facility based in Paris
- Team of 15 USP, DSP, formulation, QC & QA experts
- Biosafety Level 2 bioproduction suite
- Go-forward lab space design
- 2 LV bioproduction formats: R&D (ultraconcentrated) or pilot-grade quality (Cell suspension in bioreactor)
- LVs available with 2 different VSV-G envelopes
- Proprietary packaging & GOI/envelope expression plasmids
- Several eukaryotic ubiquitous and human-specific promoters available
- Eligible for French Research Tax Credit
- Authorizations to produce Group 2 GMOs
- Quality Management System + Quality by Design in place
- Regulatory guidance expertise
- Bioprocess engineering consulting activity

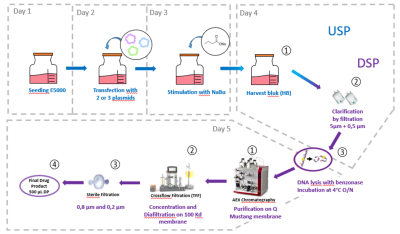
Conclusion

- Platform robustness validated with different GOIs and LV envelopes
- High quality & consistent manufacturing of LV batches in a cost- and time-efficient manner
- Robust and extensive analytical methods for In Process Control and Drug Product characterization
- Ixaka provides an integrated solution to support biotech companies and academic laboratories throughout the development of disruptive gene therapy programs

Outlook

- USP: 10 & 40 L scale-up, optimal plasmid quantity and ratios for cell transfection, the best time for harvest
- DSP: optimization of TFF and chromatography steps
- Process Instrumentation and automation
- Consolidation of analytical package with in-process control
- Implementation of new analytical control for batch release
  - Identity : PCR assay, transgene expression
  - Process residuals : Residual plasmid, PeiPro, Sodium Butyrate
  - Safety : Mycoplasma, adventitious virus, Replication Competent Virus

1- Bioproduction process for Pilot-grade LV batches



Process performance with GFP as GOI

Key figures	GFP env 1	GFP env8
LVs Total Yield	11 %	12 %
HCP (ng/mL) DP	15425	14736
ADN (ng/mL) DP	3118	1677

Impurities Removal (DNA & HCP) > 98%

Fig. 1: Current process allows LV manufacturing within one week. Process is designed for bioreactors up to 2 x 3 L suspension cultures of LV293 producing cell line in chemically-defined medium. Stimulation of LV production is triggered with Sodium Butyrate. After bulk clarification and O/N benzonase treatment, LVs are purified by AEX chromatography, concentrated and formulated by TFF/Diafiltration according to the desired target titer and sterile filtered. Examples of process performance (overall purification yield, residual Host Cell Proteins (HCP) or DNA concentrations in final product) are given with 2 batches of LVs encoding for GFP as Gene of Interest (GOI) with VSV-G envelope protein serotypes 1 and 8.

2- Switching from Erlenmeyer to Bioreactor

Erlenmeyer production

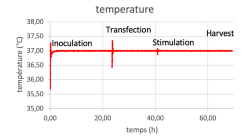
Batch volume : from 25 mL to 3L

No scalability
No In Process control

Bioreactor production

Batch volume : from 1L to 100L+

Linear scalability
In Process Control
- pH
- Temperature
- Disolved Oxygen
- Etc.



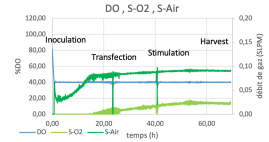
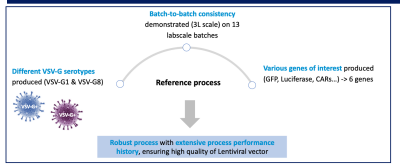
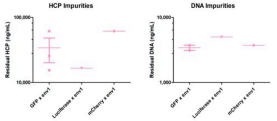


Fig.2 : New production process allows In Process Monitoring. Switching from Erlenmeyer to bioreactor enables robust and controlled production process. Instrumentation allows In line measurements, data extraction and analysis leading to process regulation and automation. Therefore, process repeatability and standardization are improved. Metabolite analysis (Data not shown) further increases process understanding and enables process optimization and implementation of new culture modes.

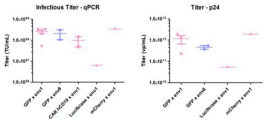
3- A robust reference process validated with different GOIs & envelopes



DP Impurities



Consistent DP Titers across analytical methods



DP size

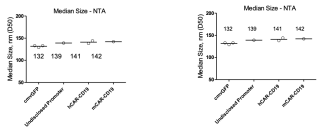


Fig. 4: Batch-to-batch consistency of 13 pilot grade LV bioproduction campaigns.
Results for infectious/physical titers and residual HCP and DNA impurities are given for LV batches produced in-house and encoding for various GOIs with VSV-G envelope protein (2 serotypes available).

4- Biophysical methods correlation and size distribution on LV batches

Fig. 5: Biophysical methods correlation and size distribution analysis. In order to optimize physical titer determination, we have been developing various biophysical quantitative methods to characterize the lentivector batches. These methods give orthogonal characterization of the product. Good correlation between methods is shown on the bulk and the purified product with the Videodrop/Neville technique. Hence, these methods show robustness and repeatability. NTA characterization completes the analysis with particle size distribution and mean size distribution.

5- Extensive & orthogonal characterization of LV Drug Product

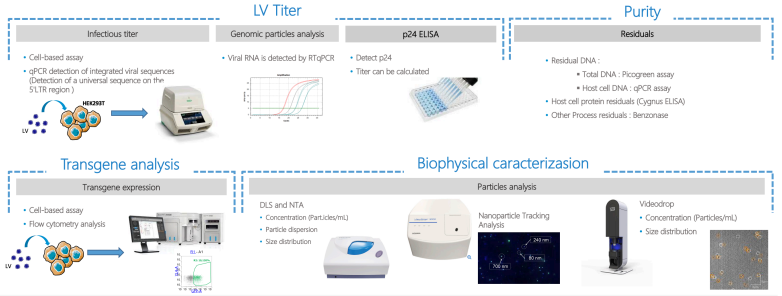
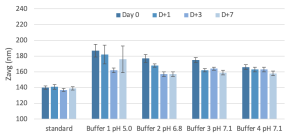


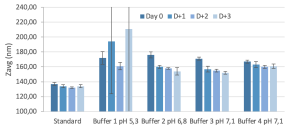
Fig. 3: Ixaka's analytical platform.
LVs batches undergo on-site characterization with complementary methods for infectious/physical titer determination, quantification of impurities and identity/integrity checks. The whole analytical package requires 5 days and results are compiled in certificates of analysis for each batch. RTqPCR, Videodrop and p24 are used for in-process control.

6- Drug Product Formulation and stability evaluation

Temperature Stress Test at 25°C



Temperature Stress Test at 40°C



Long-term stability at -80°C

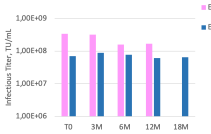


Fig. 6: Drug Product formulation and stability evaluation.
Our teams includes formulation experts working on product formulation and product stability. Various stress conditions (Physical and Chemical stress) can be performed and analyzed with the help of DLS and NTA. Information on concentration, size distribution and zeta potential evolution helps to determine the right conditions to formulate the drug product.