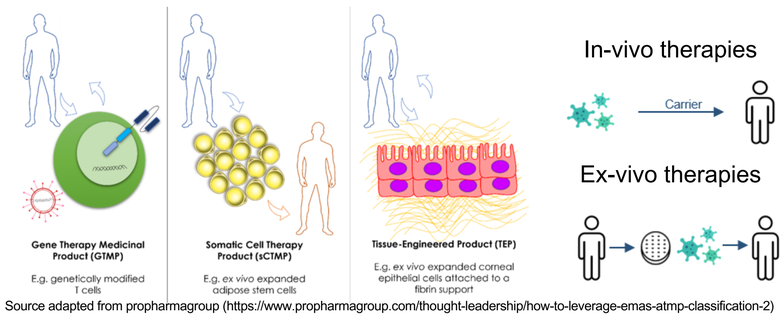
FINDING THE RIGHT PATH FOR CELL AND GENE THERAPY ANALYTICS (AND MORE)

Authors: Stefano Baila, Nicolo Sacchetti, Luigi Barbarossa, Simone Bertolacci, Luca Benedan and Claudia Benati

1 Introduction

Eurofins, a well-known first-class BioPharmaceutical outsourcing services partner, has a strong presence, among other, in the CGTs segment. Here we will present the ongoing efforts of the Biopharma Product Testing (BPT) division to establish analytical platforms to characterize, monitor and release viral vectors, as well as gene-edited cells. The primary objective is to ensure the safety of these products, by developing reliable and fit-for-the-purpose analytical solutions that could not only accelerate the manufacturing and release of viral vector-based therapies, but also reduce costs and conserve valuable resources. Furthermore, the insights gained from this exercise can be extrapolated to establish similar analytical platforms for other critical parameters, such as identity, purity, and potency, ensuring the overall safety and efficacy of cell and gene therapies. Specifically, in the poster we will provide some examples of analytical platforms developed for LVV based gene therapies.
The proposed platforms align with Eurofins' commitment to promote the sustainability and affordability of CGTs, which are central topics for the industry. Going beyond BPT activities, we will explore the identified opportunities of industrialization and innovation to foster CGTs development according to "the Eurofins way" approach, driven by our testing-for-life philosophy.

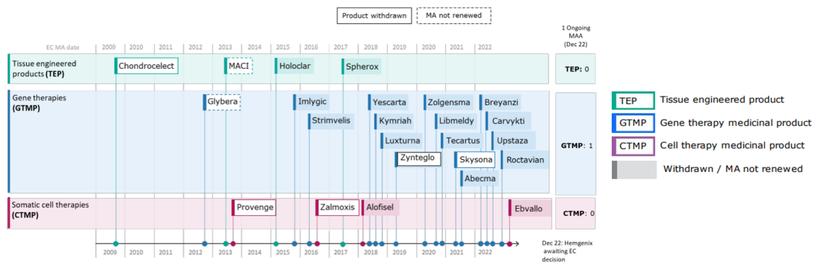
2 ATMPs overview



Source adapted from propharmagroup (https://www.propharmagroup.com/thought-leadership/how-to-leverage-emas-atmp-classification-2)

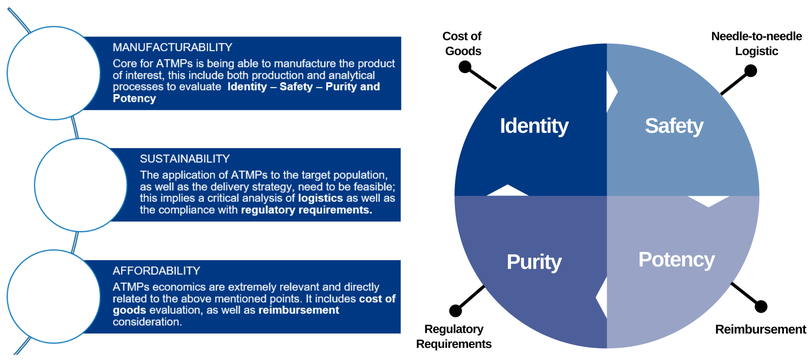
ATMPs are classified into three main groups:
(i) Gene therapy medicinal products (GTMPs) - Depending on whether the gene modifications are made in the laboratory or directly on the patient, these are defined as **ex-vivo or in-vivo**, (ii) Cell Therapy Medicinal Products (CTMPs) and (iii) Tissue Engineered Products (TEPs) – they can be tailor-made for the individual patient (**autologous**: cells from patient are collected, treated, expanded and re-introduced into the same patient) or manufactured for larger population (**allogenic**: cells from healthy donors are collected, treated, expanded and introduced into multiple end patients).
Like all medicines, ATMPs, both for clinical trials and for commercial use, must align with the regulations related to Good Manufacturing Practice (GMP).

Approved ATMPs in EU



Overview of the approved ATMPs in EU (Source EMA/CAT/50775/2023)- From 2018 GTMPs lead the approved ATMPs: of 16 approved products 14 are GTMPs, of which 9 are ex-vivo gene edited products. Sustainability and Affordability are key factors: for economical reasons about 40% of the approved products were withdrawn (or at risk to be).

Manufacturability – Sustainability – Affordability



Eurofins BPT offers a complete testing solution for ATMPs

We provide testing support at all phases of product develpoment directly from the sponsor of their CMOs:

- Raw Material
- Cell banks
- Viral banks
- Plasmid vectors
- Bulk vector harvest
- Drug substance/drug product

3 The Platform approach

Compendial vs non-compendial tests

Compendial tests	Non-compendial tests
Methods described in Eur.Ph./USP compendial monographsand general chapters	Methods not described in Eur.Ph./USP compendial monographs
Suitability of the methods shall be verified under actual conditions of use	Full validation of the methods is required

The ideal journey to method validation

"The goal of development is to obtain an analytical procedure fit for its intended purpose: to measure an attribute or attributes of the analyzed material with the needed specificity/selectivity, accuracy and/or precision over the reportable range." ICH Q14

	Early Development	GLP	GMP (Phase I & II)	GMP (Phase III+)
Method Standard	Solid, well-understood method	Scientifically sound method	Minimally qualified method	Fully validated method
Analytic Features	Build robustness into the method	Establish results for appropriate validation elements	Limited precision and robustness studies	Extensive evaluation precisi0on of and robustness
Validation Requirements	Not a requirement, unless requested by a client	Not a requirement, unless requested by a study director	Only required for safety methods	Required
Documentation Requirements	Good documentation practices	Good documentation practices, usually no written protocol or report	Written protocol and report with acceptance criteria by Phase II	Full validation protocol and report

Platform Development and Validation

- A platform analytical procedure can be defined as a multi-product method, suitable to test quality attributes of different products, without significant change to its operational conditions, system suitability and reporting structure [draft ICH Q2(R2) and ICH Q14]
- The platform methods are set-up and validated (green boxes) making the methods immediately available unless minor suitability activities are required (only in case of new matrices).

Method set-up
- Testing the feasibility of the method
- Optimization of the protocol and experimental condition
- Investigation of different parameter, which include robustness, limit of detection and limit of quantification
- SST (system suitability test) is established

Method Validation
- Parameters to be evaluated: specificity, linearity, accuracy, precision detection limit, limit of quantification and robustness.
- If a validated standard is not available, during the validation is possible to qualify the custom standard

Matrix Suitability
- Absence of inhibitory substances must be demonstrated
- Sample pretreatment steps may be necessary
- Test of 3 different batches of the given product is recommended

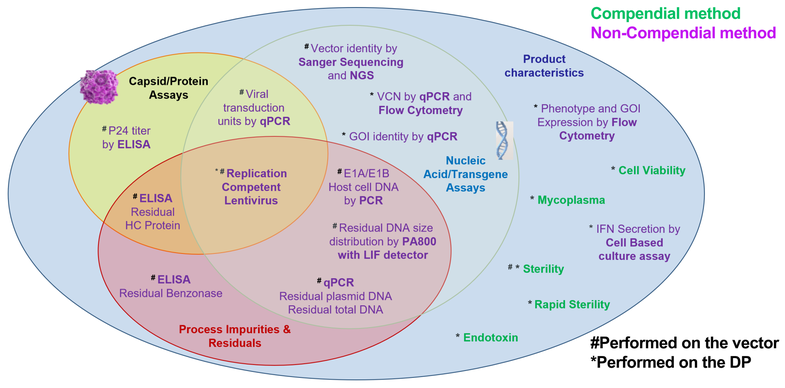
Routine analysis
- Positive and negative controls (Internal and External)
- SST (system suitability test) is verified

Parameters evaluated during Platform validation

		Qualitative test	Quantitative test
Parameter	Specificity	+	+
	Working range Suitability of calibration model Lower range limit verification	 - + (LOD)	 + + (LOQ/LOD)
	Accuracy	-	+
	Precision Repeatability Intermediate precision	 - -	 + +
	Robustness*	+	+

*Robustness may be conducted during development

Ex vivo gene therapy: an example of analytical panel



Platform set-up for a qualitative test (RCL)

Culture the cells for 14 days and collect multiple timepoints → DNA extraction is performed by using commercial kit based on silica columns with high affinity for DNA → Amplification of extracted DNA with specific primers is performed to reveal the presence of the target nucleic acid → Presence or absence of target nucleic acid and system suitability criteria are evaluated using dedicated software

During the set-up the following conditions are evaluated:
- Optimization of PCR conditions
- First indication of detection limit, primer and probe specificity and method robustness
- Data collection with the aim of best designing the validation protocol and establishing the acceptance criteria

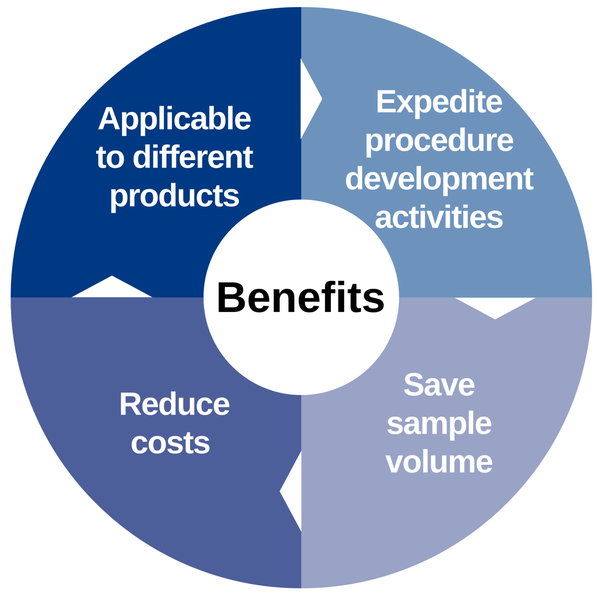
Platform set-up for a quantitative test (VCN)

Sample from client → DNA extraction is performed by using commercial kit based on silica columns with high affinity for DNA → Amplification of extracted DNA with specific primers is performed to reveal the presence of the target nucleic acid → Presence or absence of target nucleic acid and system suitability criteria are evaluated using dedicated software

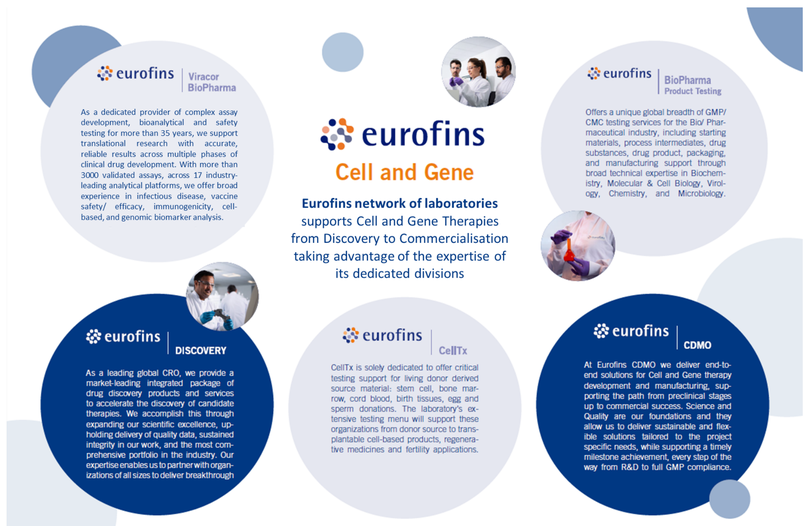
During the set-up the following conditions are evaluated:
•Optimization of PCR conditions
•First indication of limit of detection, accuracy, precision, linearity, primer and probe specificity and method robustness
•Data collection with the aim of best designing the validation protocol and establishing the acceptance criteria

[1] Parameter applicable to qualitative and quantitative tests
[2] Parameter applicable to quantitative tests

Platform benefits



4 Our BioPharmaceutical Services



5 Conclusion

- Quality control testing plays a key role in determining the sustainability and manufacturability of Cell and Gene therapeutics, which are core element for the industry.
- The development of validated analytical methods typically require large efforts both in terms of budget and of timelines, and often consume large amounts of precious clinical batches/samples.
- The platform approach allows us to provide pre-validated analytical methods that only require reduced qualification activities, allowing for considerable savings in terms of timeline, costs and material requirements, while ensuring high quality and reliable data from the early phases of drug development.
- This exercise will also provide us the foundations to further apply the Platform approach to the manufacturing activities further contributing to cell and gene market sustainability and affordability #theEurofinsway.

Acknowledgements: Valentina Curella, Marta Vismara, Beatrice Botrugno, Francesca dell'Angelo, Simona Faraci and Ginevra Sofia Totolo.

eurofins | BioPharma Product Testing

Eurofins Biolab SRL
Via Bruno Buozzi, 2 Vimodrone (MI)
InfoFarma@eurofins.com
www.eurofins.it

Poster

Scientific articles & Whitepapers