Potency testing approaches: Challenges and Opportunities for mRNA Therapeutics

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Agenda

01. Features of mRNA Therapeutics

02. Potency Testing Strategy

01. Features of mRNA Therapeutics

mRNA technology

Broad mRNA toolkit built out of deep immunological expertise

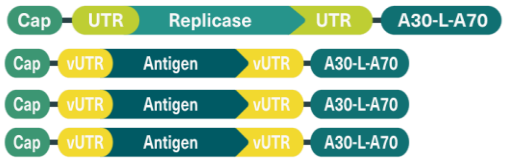
Multiple mRNA formats

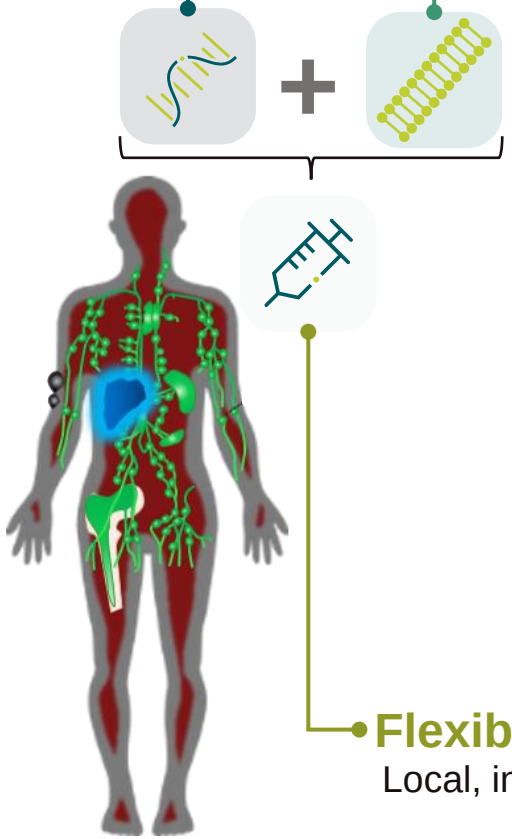
Backbone-optimized
optimized uridine mRNA (uRNA)

Backbone-optimized
nucleoside-modified mRNA (modRNA)

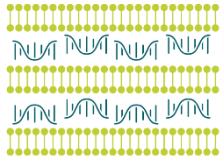
Self-amplifying mRNA (saRNA)

Trans-amplifying mRNA (taRNA)

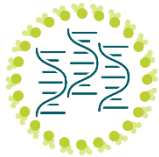




Delivery formulations



Lipoplex (LPX)



Lipid nanoparticles (LNP)

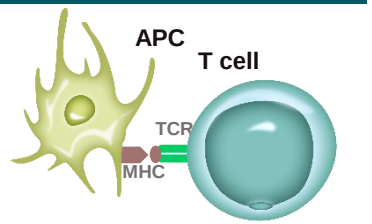
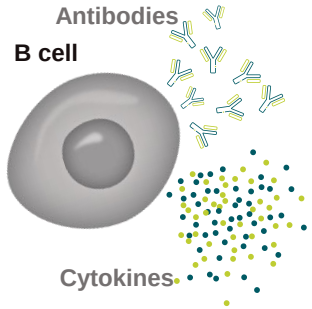
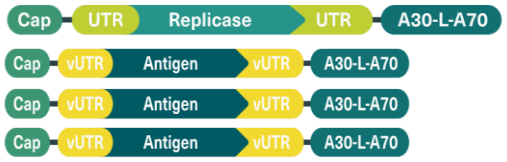
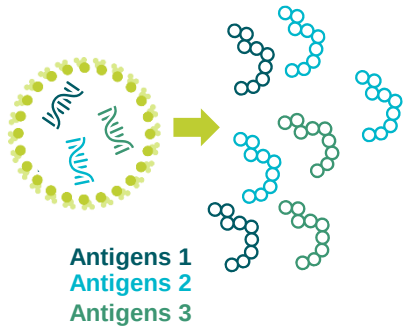
Polyplexes

Flexible delivery routes

Local, intratumoral, tissue-specific, or systemic

mRNA technology

Each mRNA format is optimized for specific applications

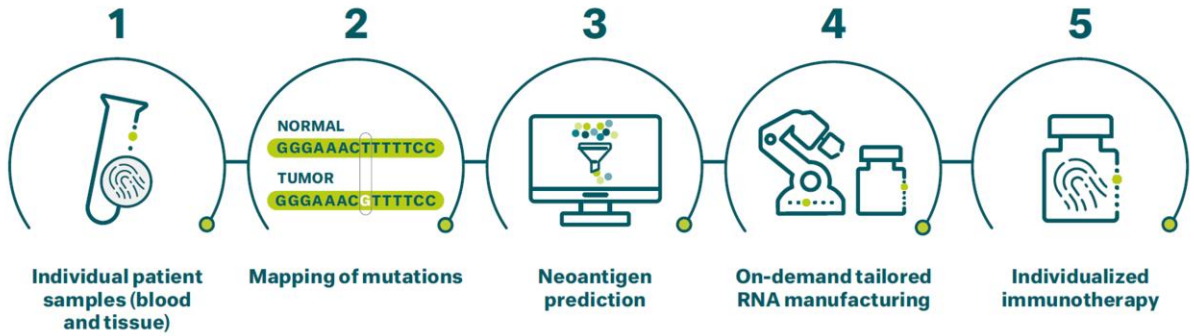
Multiple mRNA formats	Targeted Objectives	Development Platforms
Backbone-optimized uridine mRNA (uRNA) 	Potent T cell response Repeat administration 	Shared antigen mRNA vaccines Individualized neoantigen mRNA vaccines
Backbone-optimized nucleoside-modified mRNA (modRNA) 	Potent B cell response Non-immunogenic vector 	Infectious disease vaccines mRNA-encoded antibodies mRNA-encoded cytokines
Self-amplifying mRNA (saRNA)  Trans-amplifying mRNA (taRNA) 	Sustained expression High potency at low dose Sustained expression High potency at low dose Ability to co-develop multiple antigens 	Infectious disease vaccines

Our investigational mRNA Cancer Immunotherapy Platforms: FixVac and iNeST

FixVac

- **Off-the-shelf mRNA immunotherapy**
- **Targeting a fixed combination of shared antigens**
  - Non-mutated shared antigens shared across patients
  - Applicable for almost all types of tumor antigens

iNeST



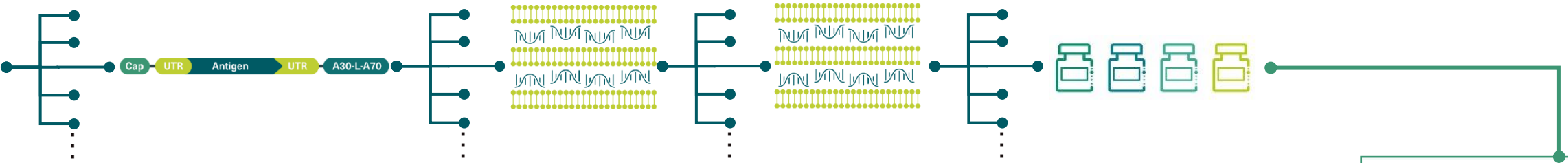
- **Fully individualized mRNA immunotherapy**
- **Targeting 20 neo-antigens unique to each patient**
  - Vast majority of neo-antigens are unique to individual patients
  - Applicable across solid tumor types

Kranz et al., Nature 2016

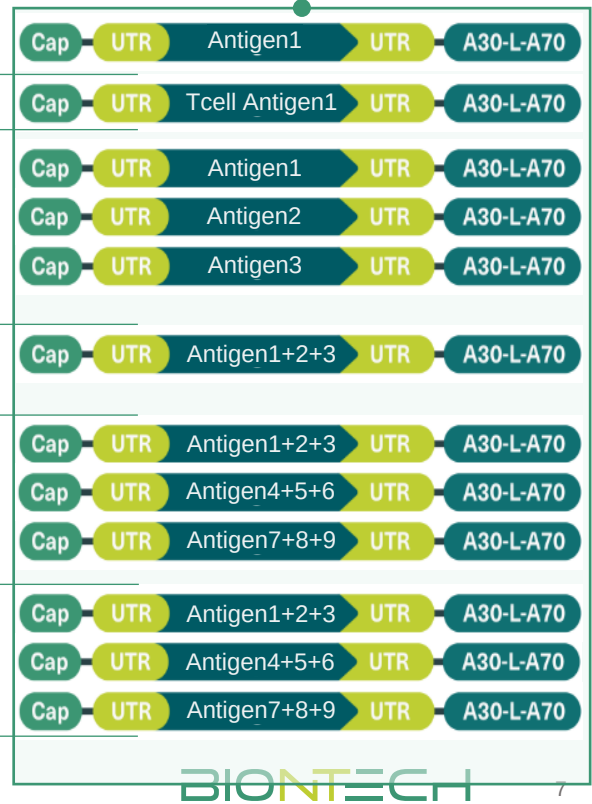
mRNA technology

The four levels of complexity of mRNA vaccines

Multiple mRNA formats + **Multiple delivery formats** + **Targets** = **Combinatory**



- one RNA (one antigen) in one LPX
- one RNA (one T-cell string) in one LPX
- Mix of different LPX (one antigen per RNA)
- Different antigens on one RNA in one LPX
- Mix of different LPX (multiple antigens on one RNA)
- Multiple RNAs in one LPX
- Fully individualized products



02. Potency Testing Strategy

Current situation

- Since the application of mRNA technology is relatively new, **regulatory guidelines** and **industry standards are still evolving**
- There is a need for continuous dialogue between industry and regulators to address arising questions.

- **Several initiatives are currently ongoing** to discuss and harmonize not only analytic activities/procedures and quality control (including potency) for mRNA vaccines e.g.:
  - Ph. Eur. Commission established a new working party on mRNA vaccines EDQM[1]
  - United States Pharmacopeia – National Formulary; USP **draft** guidiance[2] on the analytical procedures for mRNA vaccines (2nd version):

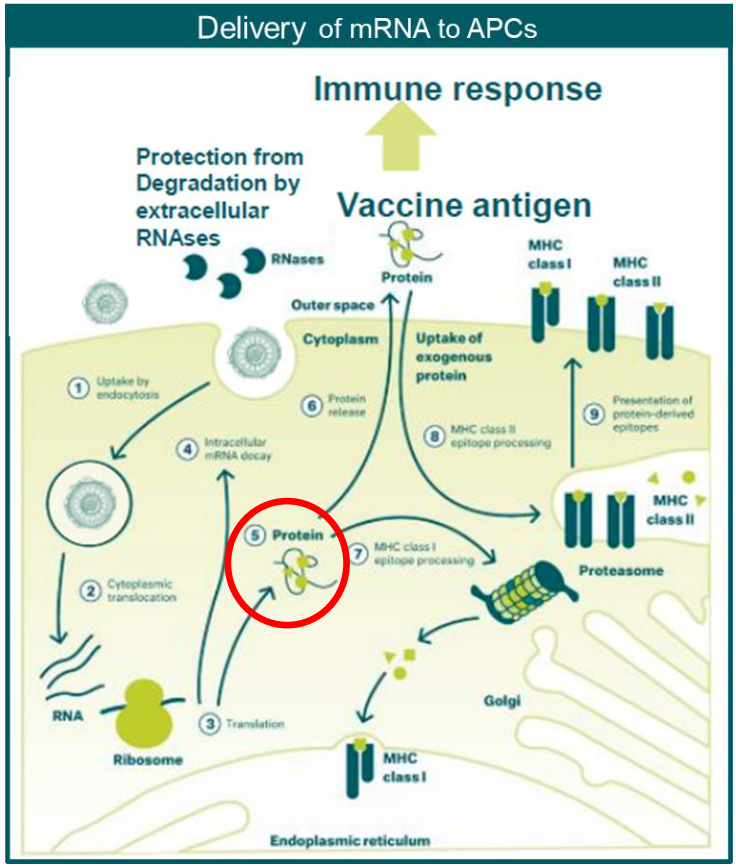


Table 3. Characterization and release testing for mRNA Drug Product

Quality	Attribute	Method
Potency	Expression of target protein	Cell-based assay

1: https://www.edqm.eu/en/-/ph.-eur.-commission-establishes-a-new-working-party-on-mrna-vaccines
2: https://www.uspnf.com/notices/analytical-procedures-mrna-vaccines-20230428

Potency of mRNA products

Potency definition (ICH Q6b):

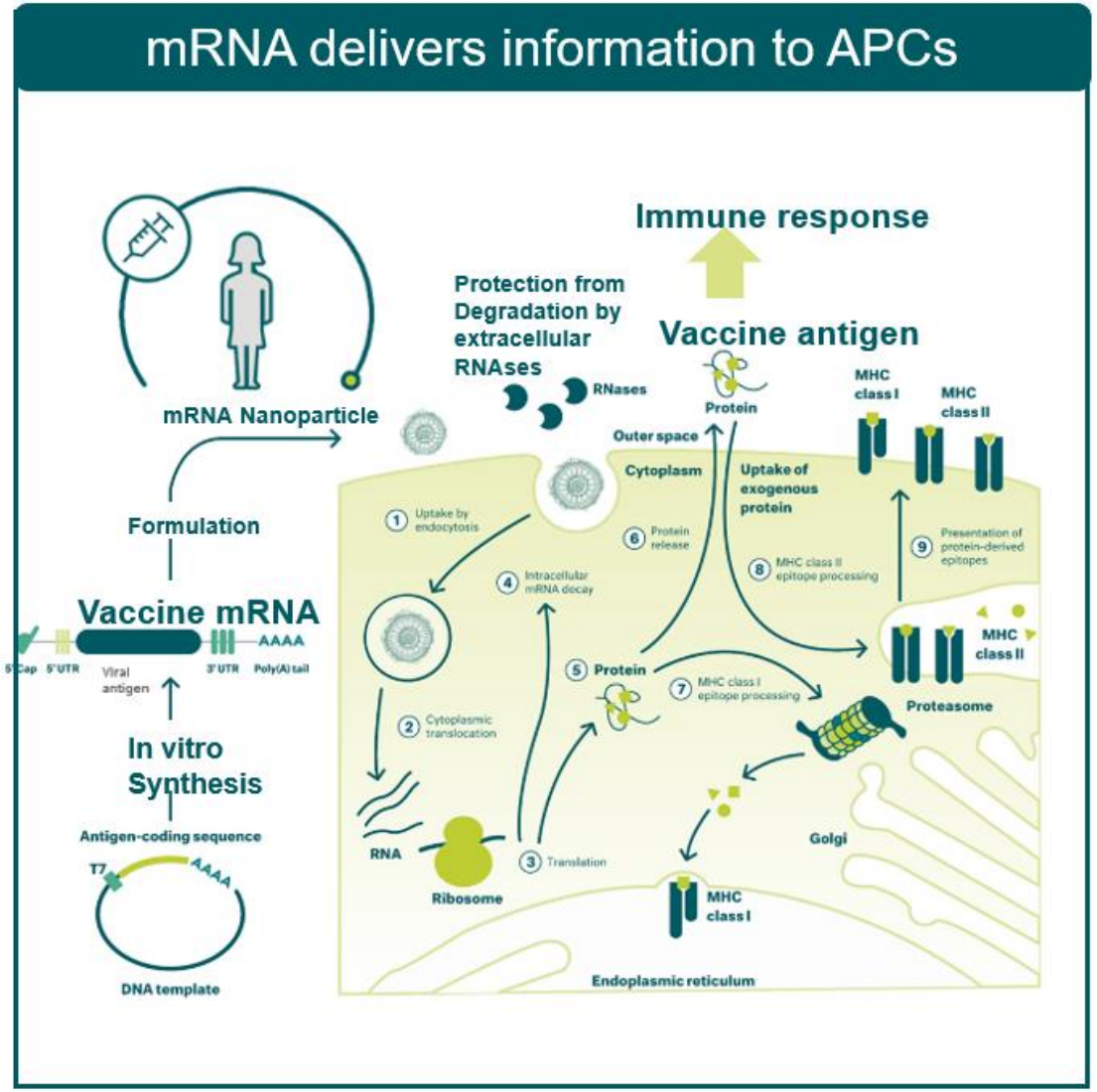
"The measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties."

- **Biological activity** of **mRNA product(s)** is a complex function of final drug product properties, including:
  - delivery to target cells with suitable delivery system
  - **translation** of the mRNA-encoded protein(s)

- **mRNA** is defined as **biological substance**, therefore potency testing at release and during stability is expected by regulators.

- The potency is the result of a variety of **different CQAs**

- Different **Mode of Actions (MoA)** and **mechanisms of efficacy contribute to the potency**



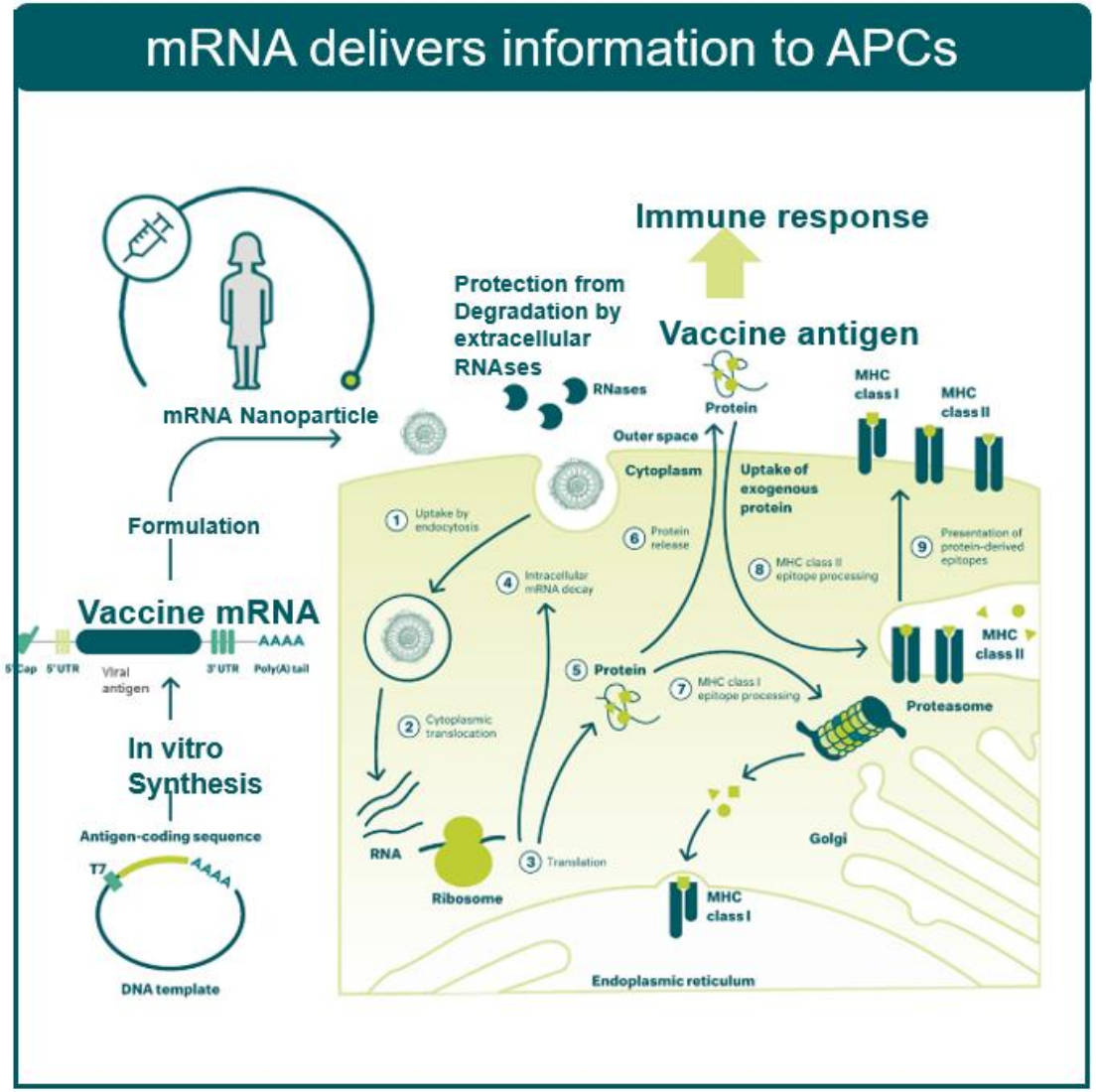
Example of Mode of Action and mechanism of Efficacy

Mode of Action

1) Encapsulation and Lipid particle size
2) Encapsulation
3) RNA Integrity, 5´Cap and Poly(A) tail

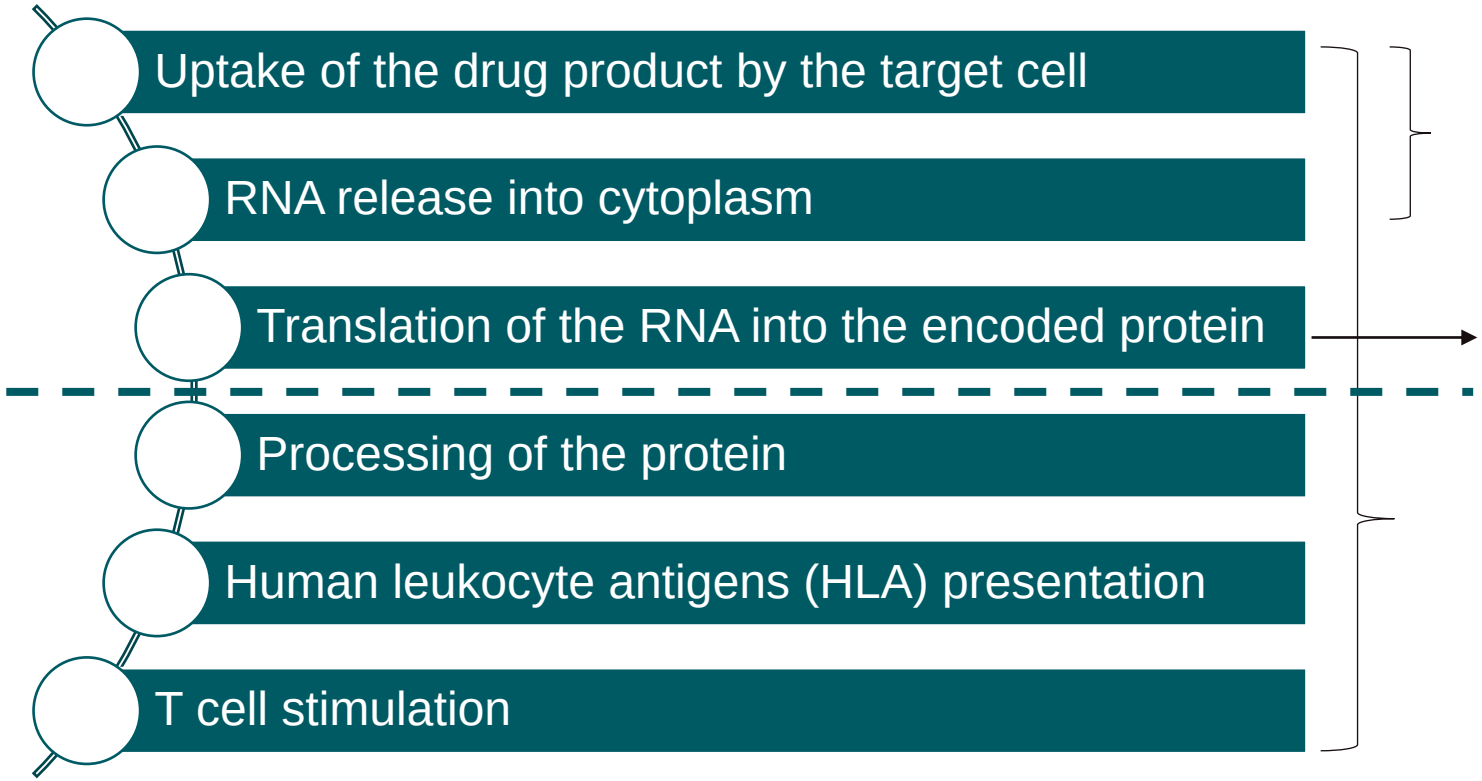
Mechanism of Efficacy

4) Intrinsic cellular mechanisms covered by clinical studies and/or characterization data



Example of Mode of Action and mechanism of Efficacy

Example of Mode of Action and mechanism of efficacy for an mRNA-based cancer immunotherapy



Ensured by routine testing of delivery system parameters (encapsulation and Lipid particle size)

Enabled via drug substance control strategy (RNA integrity, 5´-Cap and Poly(A) presence/length)

Analyzed via clinical activity/data and/or characterization studies including correlation to the potency assay

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Quality attributes potentially ensuring potency

Antigen translation depends on:

Material	CQA	Scope of testing
DS	**dsRNA**	❑ Control the level of dsRNA • Controlling the level of dsRNA in in vitro transcribed mRNA is important to limit induction of cytokines.
DS	**5´-Cap**	❑ Determination of relative amount of 5'-capped RNA species in drug substance • The presence of the appropriate 5'-cap protects the mRNA thereby helping to ensure mRNA translation.
DS	**Poly(A) tail**	❑ Determination of presence and/or length of the poly(A) tail • Presence of the poly(A) tail protects the RNA thereby helping to ensure translation.
DS, DP	**RNA integrity**	❑ Determination of the intact RNA and detection of potential degradation products
DS,DP	**RNA content**	❑ Determination of concentration • Ensures delivery of correct amount of RNA
DP	**RNA encapsulation / free RNA ***	❑ Determination of free and total RNA • Proper encapsulation ensures delivery of the RNA and improve the chances of transfection.
DP	**Particle size**	❑ Determination of free and total RNA • Control of particle size ensures delivery of the RNA

* Dependent on the product, one or the other quality attribute to be assessed

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DS	**P**	slation.
DS, DP	**R**	
DS,DP	**R**	• Ensures delivery of correct amount of RNA
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DP	**Particle size**	❑ Determination of free and total RNA • Control of particle size ensures delivery of the RNA

Potency is not a specific attribute rather a combination of CQAs which enable the different mode of actions and the mechanism of efficacy

* Dependent on the product, one or the other quality attribute to be assessed

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mRNA attributes covered in characterization studies

Structural and functional attributes confirmed by mRNA characterization:

Attribute	Scope of testing
Primary Structure	Expected RNA sequence verified (e.g., sequencing or fingerprinting)
Poly(A)-tail	Presence and length of Poly(A)-tail
5‘-Cap Structure	5‘capping structure and 5‘-end profile confirmed
High Order Structure (HOS)	The type of HOS confirmed by spectoscopic analysis
Drug Substance Activity	Size and identity of translated protein (after DS in vitro translation) confirmed by Western blot analysis

Other:

- **Drug Product Activity:** In Vitro Expression of DS formulated in drug product determined by suitable cell-based or cell-free techniques
- **Further parts of MoA and mechanism of efficacy** such as Human leukocyte antigens (HLA) presentation and T cell stimulation will be evaluated using clinical samples (GCLP studies) and the clinical studies itself.

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Challenges on potency testing of mRNA products

- Certain T cell antigens are per se are not optimal targets to induce antibodies. Potential lack of specific detection antibodies to quantify each translated antigen in a potency assay
- Generation of detection antibodies is challenging, which limits the accelerated development option offered by mRNA technology.
- Potential cross-reactivity of antibodies to detect multi-construct products may impact the potency assay.
- There is a need for highly sensitive techniques for more potent vaccines with potentially lower dosage.
- Especially for patient individualized products a specific potency assay will become a challenge

New control strategy concepts and solutions are needed to overcome these challenges **without negatively** impacting **safety, quality and efficacy** of the product

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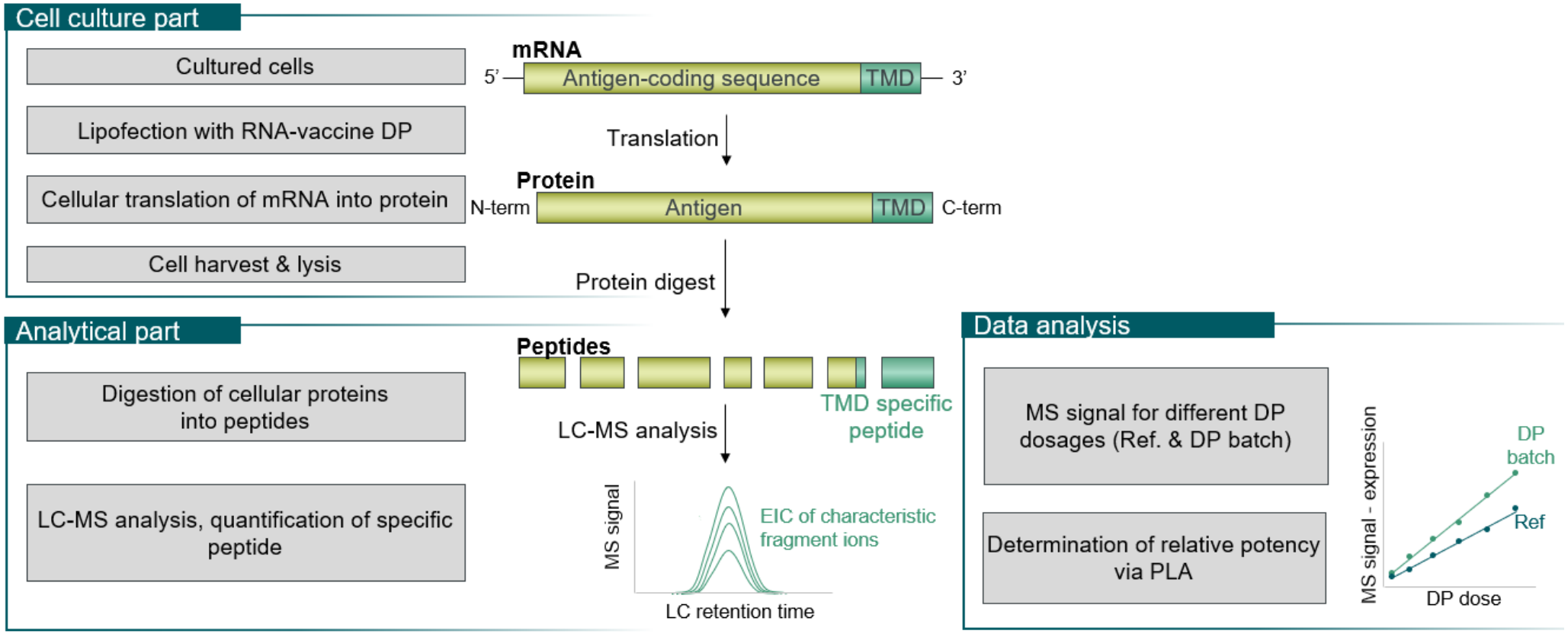
How do we potencially overcome these challanges?

Alternative detection systems and assays for potency measurement should be considered, especially if the production of antibodies against the target / POI (protein of interest) is challenging.

e.g. Antibody-independent potency testing concept



EIC: Extracted ion chromatogram; PLA: Software tool

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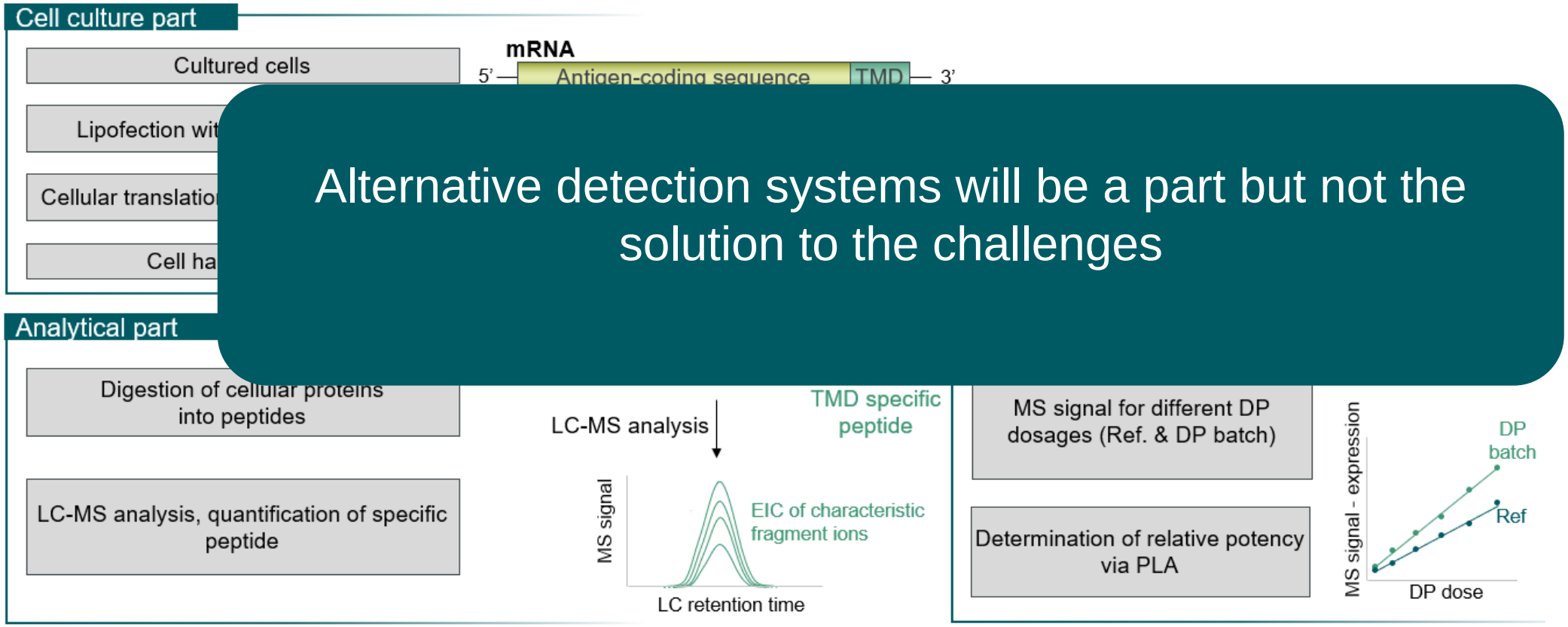

EIC: Extracted ion chromatogram; PLA: Software tool

Potential solutions for potency testing

1) There will be **no “one fits all products solution”** for potency testing

2) Alternative detection systems and assays for potency measurement will be part of the solution

3) Tight control via DS and DP control strategy of mode of action impacting CQAs (very sensitive measures – prior knowledge)

4) overarching platform data package (prior knowledge on process performance, product quality and stability)

5) Supportive characterization data on different level of the MOA and mechanism of efficacy including clinical data

6) Adaptation of current potency testing concepts for individualized products

Proposal: Adaptation of current potency control strategy concepts for individualized products

Case study on potency assay testing for individualized cancer vaccines

- **Key differences between fixed target and individualized drugs**
  - **Advantage:** the number of manufactures batches manufactured during clinical development (order of magnitude higher for individualized drugs) → results in much more prior knowledge
  - **Challenge:** High number of patient specific targets

Possible solution

- **Ongoing validation approach**
  - Ongoing validation for potency testing is repeated in a defined time interval (“process is the product”)
  - Potency testing via bracketing approach, which reflects the diversity of patient specific constructs/targets

Thank you

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