Consideration on mRNA products potency testing

Pawel Widomski

CASSS CMC Strategy Forum Europe

October 17, 2023

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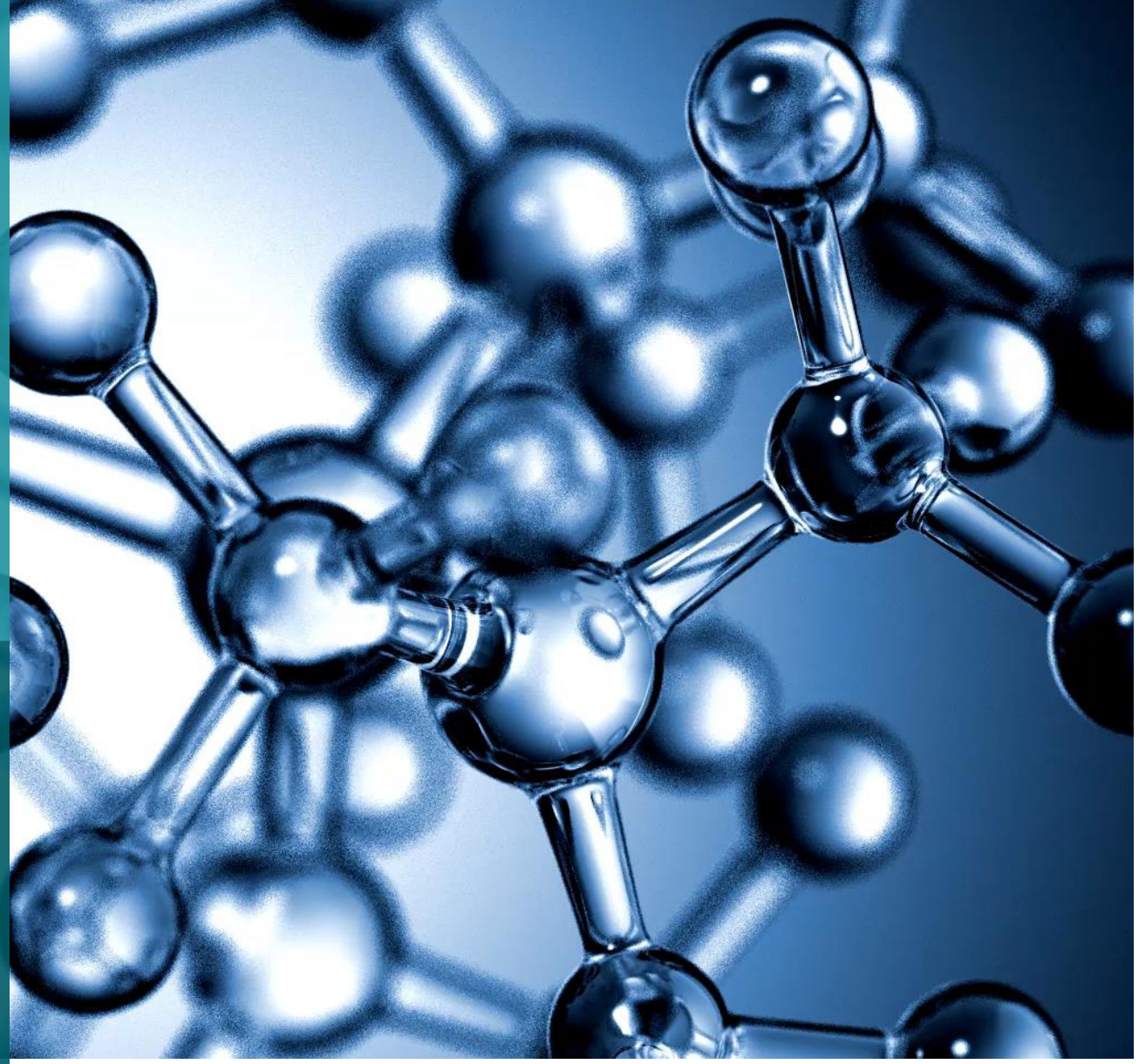
Agenda

1) mRNA technology outlook
2) mRNA potency testing



mRNA technology outlook

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mRNA technology

Broad mRNA toolkit built out of deep immunological expertise

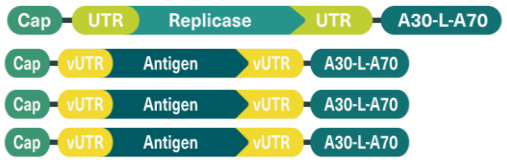
Multiple mRNA formats

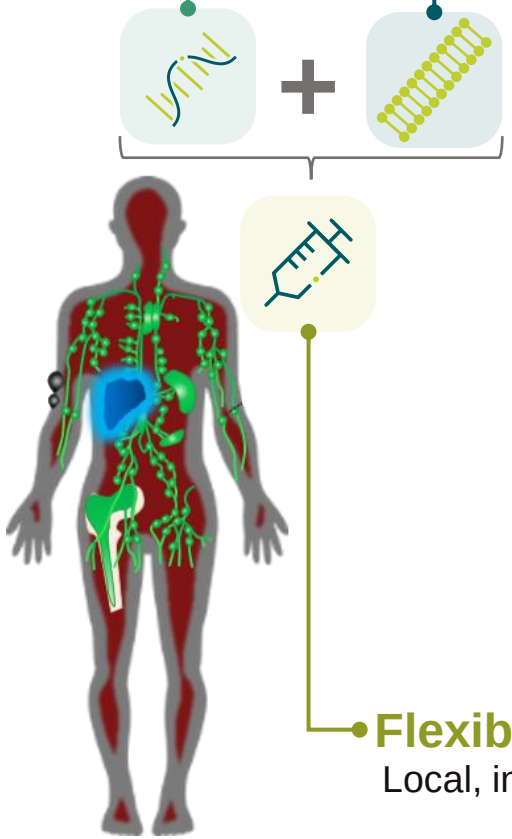
Backbone-optimized
optimized uridine mRNA (uRNA)

Backbone-optimized
nucleoside-modified mRNA (modRNA)

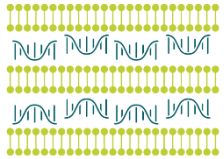
Self-amplifying mRNA (saRNA)

Trans-amplifying mRNA (taRNA)

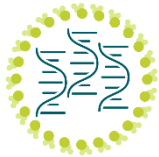




Delivery formulations



Lipoplex (LPX)



Lipid nanoparticles (LNP)

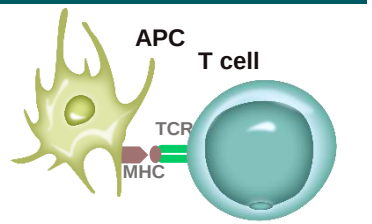
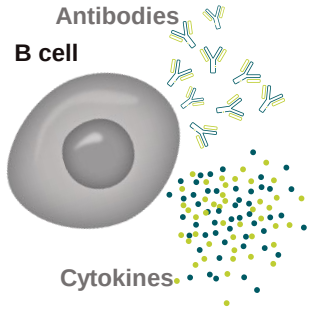
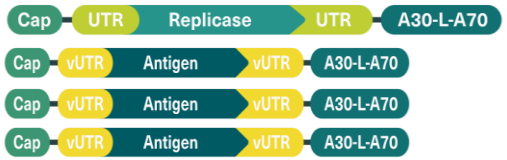
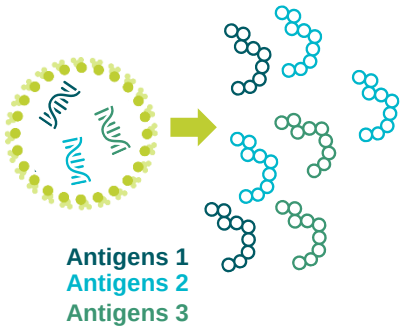
Polyplexes

Flexible delivery routes

Local, intratumoral, tissue-specific, or systemic

mRNA technology

Each mRNA format is optimized for specific applications

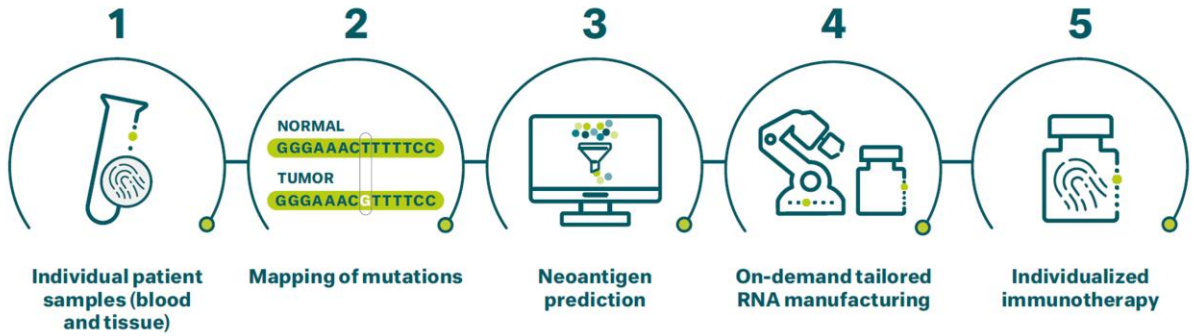
Multiple mRNA formats	Targeted application	Platforms
Backbone-optimized uridine mRNA (uRNA) 	Potent T cell response Repeat administration 	Shared antigen mRNA vaccines Individualized neoantigen mRNA vaccines
Backbone-optimized nucleoside-modified mRNA (modRNA) 	Potent B cell response Non-immunogenic 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 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

Multi-antigen approach for cancer treatment

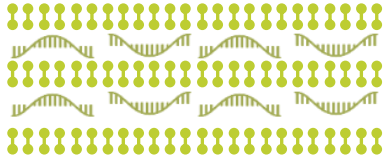
Vaccine backbone with shared antigens

Backbone-optimized uridine mRNA (uRNA)

Multi-antigen approach tailored to each indication

+

Lipoplex



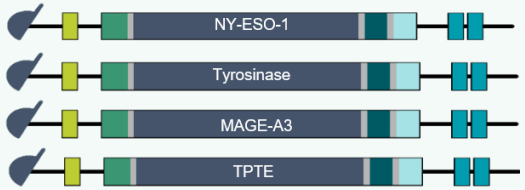
RNA-LPX formulation (IV)

=

FixVac

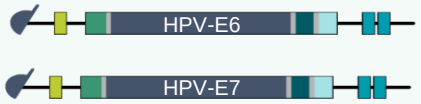
Fixed vaccine combination against shared tumor-associated antigens

Melanoma



BNT111 encodes 4 tumor-associated antigens covering >90% of patients with cutaneous melanoma

HPV16+ HNSCC

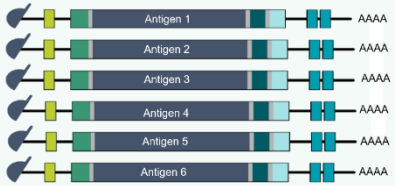


BNT113 encodes 2 oncoproteins exclusively expressed in pre-malignant and malignant tissue

Prostate cancer

BNT112 encodes 5 related antigens specific to prostate cancer

NSCLC



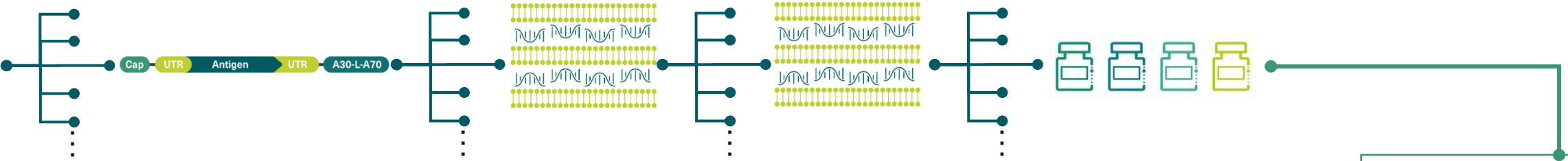
BNT116 encodes 6 different NSCLC tumor-associated antigens

HNSCC, head and neck squamous-cell carcinoma; HPV, human papilloma virus; NSCLC, non-small-cell lung cancer.

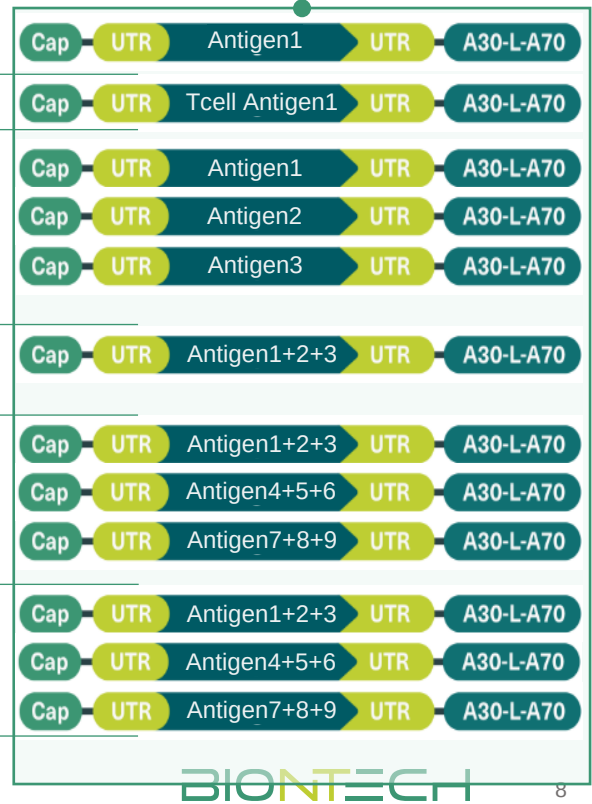
mRNA technology

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



mRNA potency testing

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Potency of mRNA products

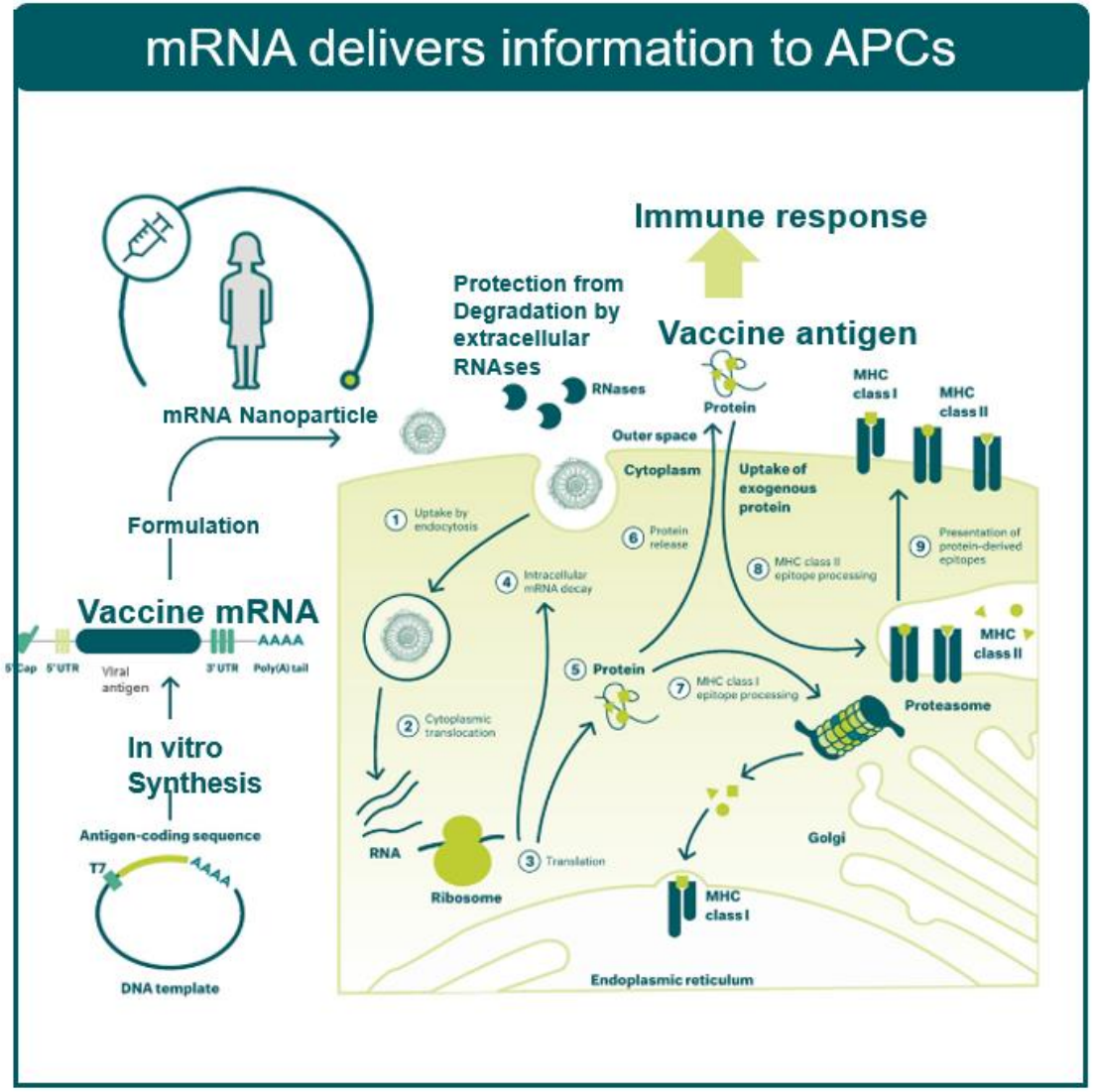
Potency definition (ICH Q6b):

"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)

- A variety of **Modes of Action (MoAs)** of mRNA products are possible.

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



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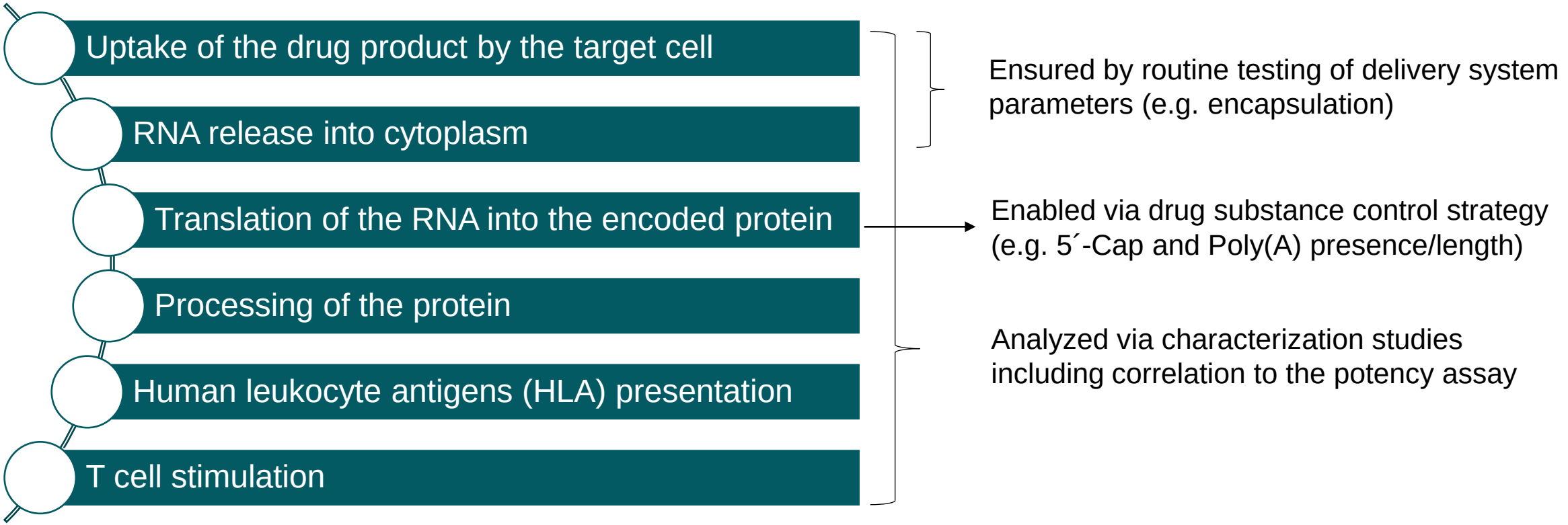
Example of Mode of Action

Example of Mode of Action for an mRNA-based cancer immunotherapy.



Quality attributes potentially impacting potency

Antigen translation depends on:

Material	CQA	Scope of testing
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	**dsRNA**	❑ Control the level of dsRNA • Controlling the level of dsRNA in in vitro transcribed mRNA is important to limit induction of cytokines.
DS, DP	**RNA integrity**	❑ Determination of the intact RNA and detection of potential degradation products
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 particle size

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

mRNA characterisation studies

Structural and functional attributes confirmed by mRNA characterisation:

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** such as Human leukocyte antigens (HLA) presentation and T cell stimulation will be evaluated using clinical samples (GCLP studies).

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):

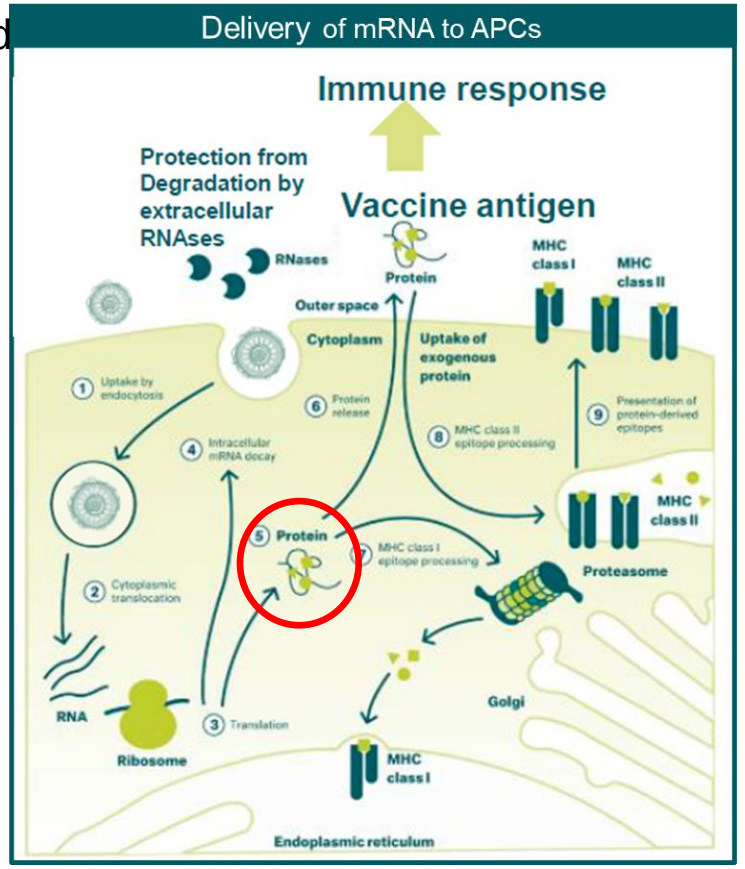


Table 3. Characterization and release testing for mRNA Drug Product

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

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Challenges on potency testing of mRNA products

- The potency concept ideally applicable to all DP combinations.
- Potential lack of specific detection antibodies (e.g., T cell antigens per se are not optimal targets to induce 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.
- Setting a clinically meaningful acceptance criterion for a potency assay (in particular: for patient individualized products).

Example: **BNT162b4**,
a prophylactic vaccine candidate:

The vaccine candidate is composed of mRNA encoding for highly-conserved T-cell antigens from SARS-CoV-2 non-spike proteins that are highly conserved across a broad range of SARS-CoV-2 variants.

Purpose: Enhancing and broadening T-cell immunity and potentially extending durability of protection.

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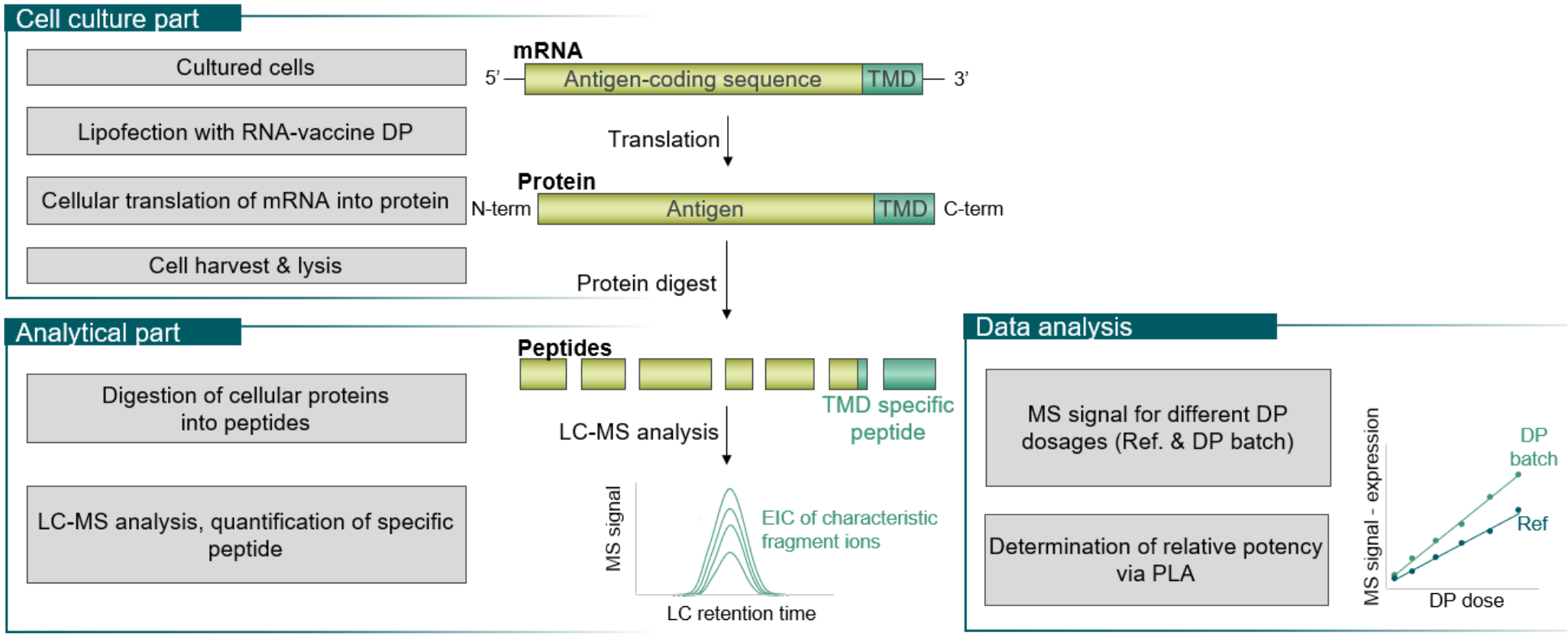
Outlook

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.

Under development: Antibody-independent potency testing concept



EIC: Extracted ion chromatogram; PLA: Software tool

THANK YOU

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