Industry perspective on mRNA technology

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EMA Regulatory and scientific virtual conference on RNA-based medicines

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BIONTECH

Confidential

mRNA technology outlook

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mRNA technology

Broad mRNA toolkit built out of deep immunological expertise

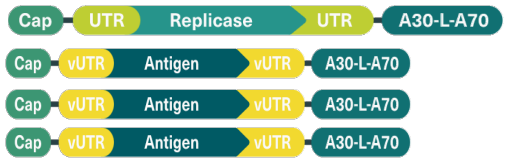
Multiple mRNA formats

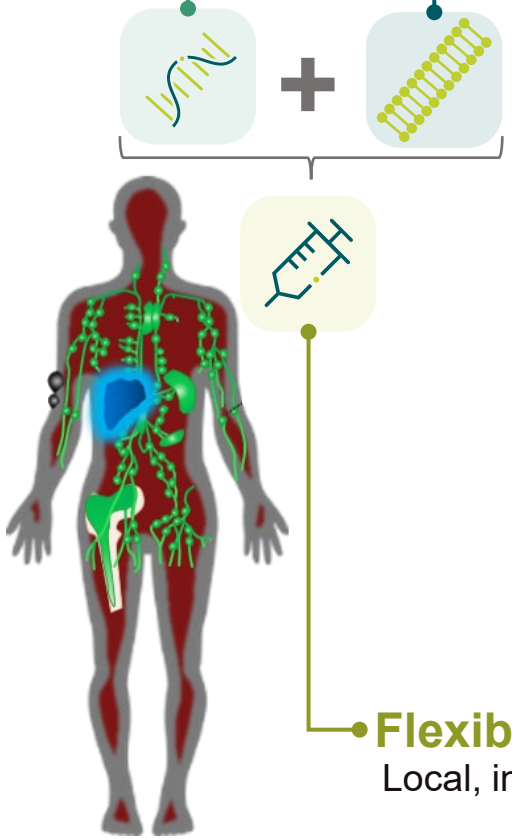
Backbone-optimized
optimized uridine mRNA (uRNA)

Backbone-optimized
nucleoside-modified mRNA (modRNA)

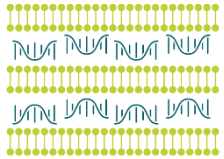
Self-amplifying mRNA (saRNA)

Trans-amplifying mRNA (taRNA)

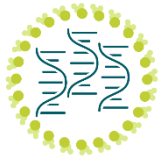




Delivery formulations



Lipoplex (LPX)



Lipid nanoparticles (LNP)

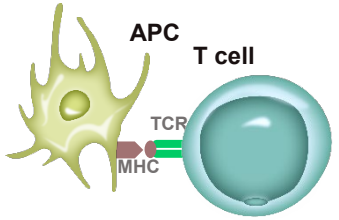
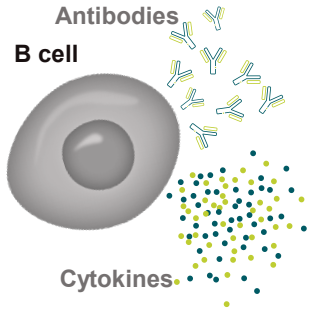
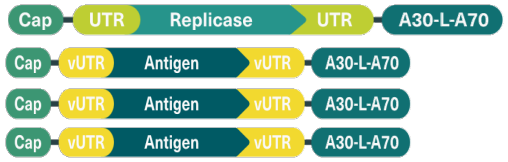
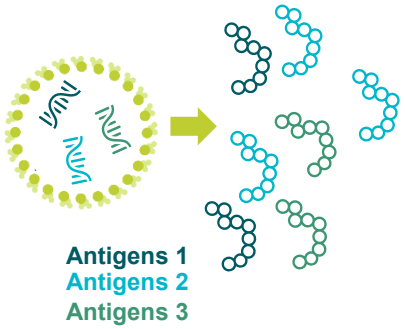
Polyplexes

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 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

Multi-antigen approach for cancer treatment

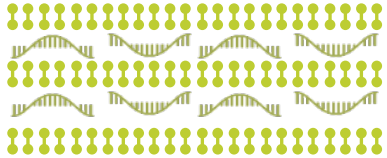
Vaccine backbone with shared antigens

Backbone-optimized uridine mRNA (uRNA)

Multi-antigen approach
tailored to each indication

+

Lipoplex



RNA-LPX formulation (IV)

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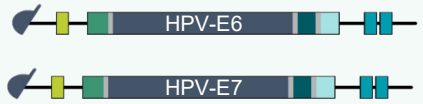
FixVac

Fixed vaccine combination against shared tumor-associated antigens

Melanoma

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

HPV16+ HNSCC

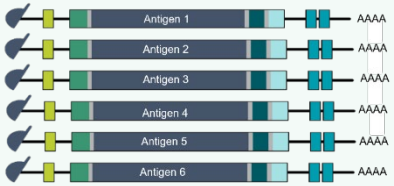


BNT113 encodes 2 oncoproteins exclusively expressed in pre-malignant and malignant tissue

Prostate cancer

BNT112 encodes 5 related antigens specific to prostate cancer

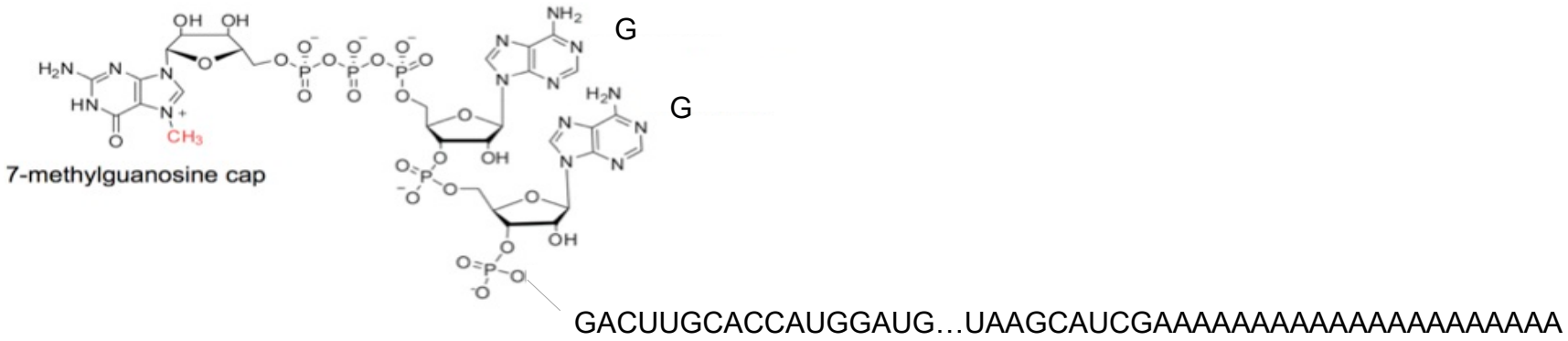
NSCLC



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 vs. oligonucleotides



mRNA contains:

- Several hundreds up to few thousand nucleotides in length
- 2'-OH (i.e., ribose) from end to end
- Mostly non-modified bases (i.e., A, G, C and U) with limited set of modified bases (e.g., N1-methyl-pseudouridine)
- Cap - specific structure (m7GpppG) at the 5' end
  - required for stability and to be recognized by the cell as mRNA
- Poly(A)-tail at the 3' end
  - required for stability and to be recognized by the cell as mRNA

Considerations on platform concept

The class of mRNA-based products

- We define the **class** of mRNA-based products as using exogenous mRNA to express a pharmaceutically active protein
- Each **group** / **mRNA platform** within this **class** uses a specific **mRNA format** combined with a certain **formulation**;
  - thus, within each **group** we would like to use **prior knowledge** gained from our experience using the same type of **mRNA format** and **formulation**;
- In addition, there are some aspects that are common to all **groups** / **mRNA platforms** and thus apply to the whole **class** of mRNA-based therapeutics and vaccines.

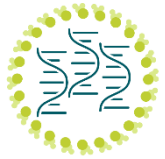
BioNTech's prior knowledge in mRNA therapeutics and vaccines

Modular Concept of the fundamental CMC elements

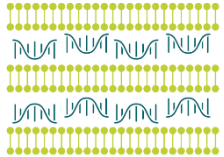
modRNA

- The **modRNA**-based vaccine Comirnaty (BNT162b2) has been **extensively administered to humans** worldwide and demonstrated **favorable safety and reactogenicity** in adults and adolescence as well as pediatric populations.
- Furthermore, **modRNA**-LNP platforms used for therapeutic protein expression **are being assessed in BioNTechs immune oncology clinical programs** using multiple treatment cycles and dose ranges.

LNP



- During BNT162 program development BioNTech has collected clinical data on **modRNA**, as well as **uRNA** platforms.



LPX

- The safety and reactogenicity of **uRNA** platform vaccines with **LPX** formulation have been explored in RNA based cancer immunotherapy over the past decade.

uRNA

Generic CMC aspects for the class of mRNA-based products

All mRNA-based therapeutics and vaccines are chemically similar, and all are manufactured by a cell-free enzymatic process, commonly called *in vitro* transcription, that uses a DNA template, an RNA polymerase and nucleoside triphosphate building blocks; thus, there are some **generic CMC aspects applying to all**; examples include:

- The **identity of the mRNA**, i.e., its sequence, is defined by the DNA template; this is well documented in the scientific literature and has been verified by BioNTech for all mRNA types used until now → this **prior knowledge** should be considered, e.g., for identity testing.
- The **same set of starting and auxiliary materials** is used → this **prior knowledge** should be considered, e.g., for definition of the specifications and quality of these materials.
- The **main degradation pathway for mRNA** is hydrolysis of the phosphate-ribose backbone; based on BioNTech experience and as published in the scientific literature, this happens within a certain range in a statistical manner per nucleotide and time → this **prior knowledge** should be considered, e.g., for setting the required stability studies and initial assignment of shelf-lives.

Example for an mRNA platform: Cancer vaccines

- As initially developed for our first clinical trial in oncology, this uses **non-modified mRNA** (i.e., containing A, C, G, and U) with a **modified cap0**-structure as well as **5' and 3' UTR** sequences and a **poly(A)-tail** developed for high efficacy as the **mRNA format.**
- For mRNA-based cancer vaccines, corresponding **tumor-associated antigens are encoded on the mRNA**; this can be one mRNA with only one antigen (**monovalent**) or multiple mRNAs with one antigen each (**multivalent**).
- For each new cancer vaccine sequence, only the **open reading frame is changed**.
- As of now, this platform has been applied for **more than 30 antigens**; the corresponding mRNAs range from less than 1000 to up to 3000 nucleotides with a certain variation in the relative sequence composition (ratio of A/C/G/U)
- Essentially the **same process** is used for all these mRNAs (buffer composition, concentration of enzymes, reaction temperature, …), with only a few process parameters directly impacted by mRNA length and sequence varied based on pre-defined rules developed over time

➢ Thus, this **mRNA platform** corresponds to an **mRNA sequence space** for the specific **mRNA format** with a corresponding **process space.**

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Concept for a formulation platform approach

To enable faster development of new candidates within one **platform**, we propose a concept for product development covering a defined formulation with the corresponding formulation and **process space.**

Based on the available CMC data, BioNTech considers the LPX and LNP formulations as **development platform formulations.**

- The LPX platform formulation is used in multiple programs. The process and product data show, that this platform can be used for mRNAs of different length without impacting the quality parameter.
- The LNP platform, considering the robustness of the process and consistency of the quality at different scales, the manufacturing process can be described as very well understood. Therefore, the BNT162 formulations (concentrate and ready to use) can be manufactured using the same process for different mRNAs. No adaptation of process parameter and specifications are required.

→ **Each formulation** platform provides data to reduce or waive **process characterization** and reduced **process validation** for new mRNA products using the same formulation.

→ **Each formulation** platform provides data, supporting **shelf life** of new mRNA products using the same formulation complemented by **confirmatory stability studies**.

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Concept for process validation for one platform

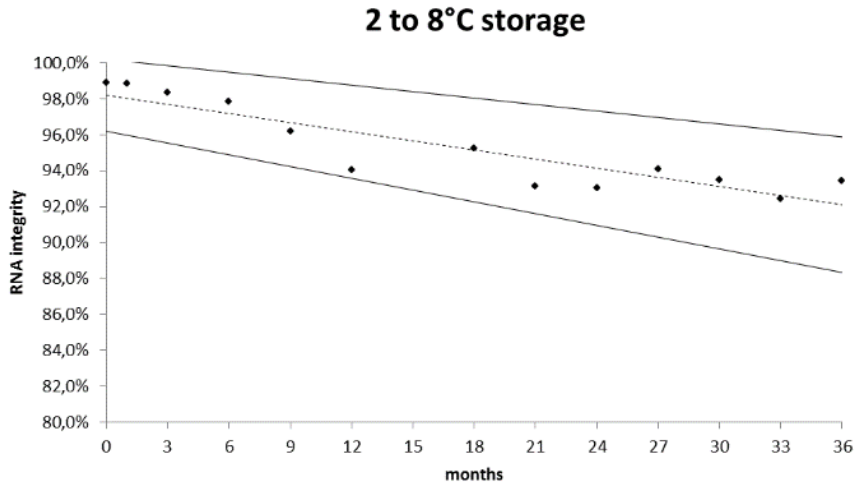
To enable faster development of new candidates within one **platform**, we propose a concept for process validation covering

- ❑ a certain **mRNA sequence space** with the corresponding **process space**
  - For this a set of mRNA sequences will be defined that cover the **mRNA sequence space** (shortest and longest sequence, high and low end of nucleotide distribution)
  - For these sequences, one validation run each will be performed using the corresponding sequence-dependent process parameters within the pre-defined **process space**
  - After successful validation, all new sequences for the same RNA platform falling within this **mRNA sequence and process space** could be manufactured without further process validation

- ❑ reduced **process validation/verification** for new LNP-based mRNA products based on LNP platform data
  - for the same formulation but different mRNA construct PPQ/confirmatory run approach leveraging prior manufacturing experience and comparability approach.

Stability concept for one platform

- The main degradation pathway for RNA is hydrolysis of the phosphate-ribose backbone.
- Within a defined **mRNA sequence space** and a specific chemical environment (buffer composition and pH, RNA concentration and for the drug product identity and concentration of the lipids), this happens with an average degradation rate.
- Based on a dataset from the first sequences within one platform, this allows assignment of an initial shelf-life for each new sequence as long as it is within the sequence space.



- **Shelf life** of new mRNA products leveraged from available **platform data** using the same formulation, complemented by **confirmatory stability studies**.

mRNA potency testing

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Potency of mRNA products

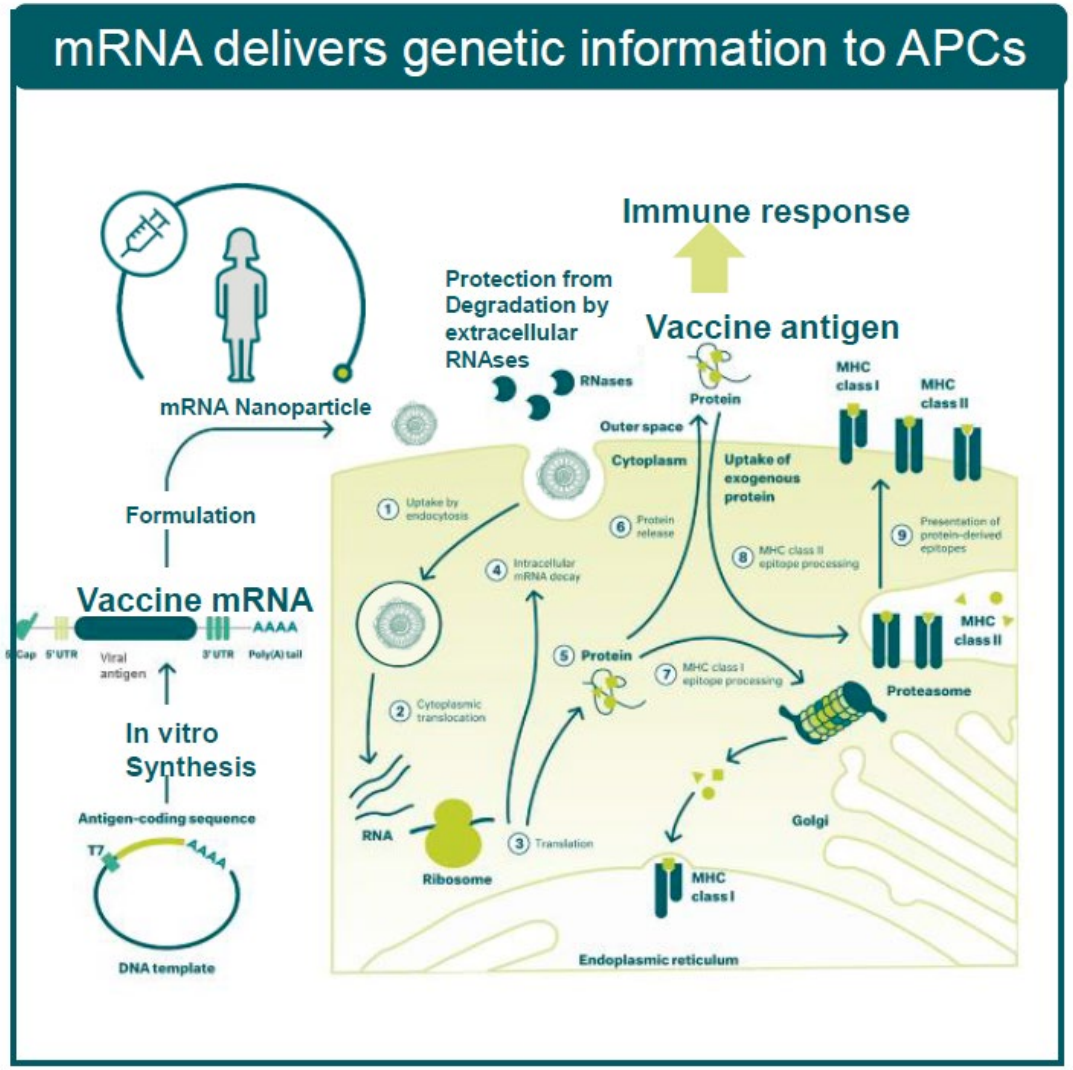
Potency definition (ICH Q6b):

"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)

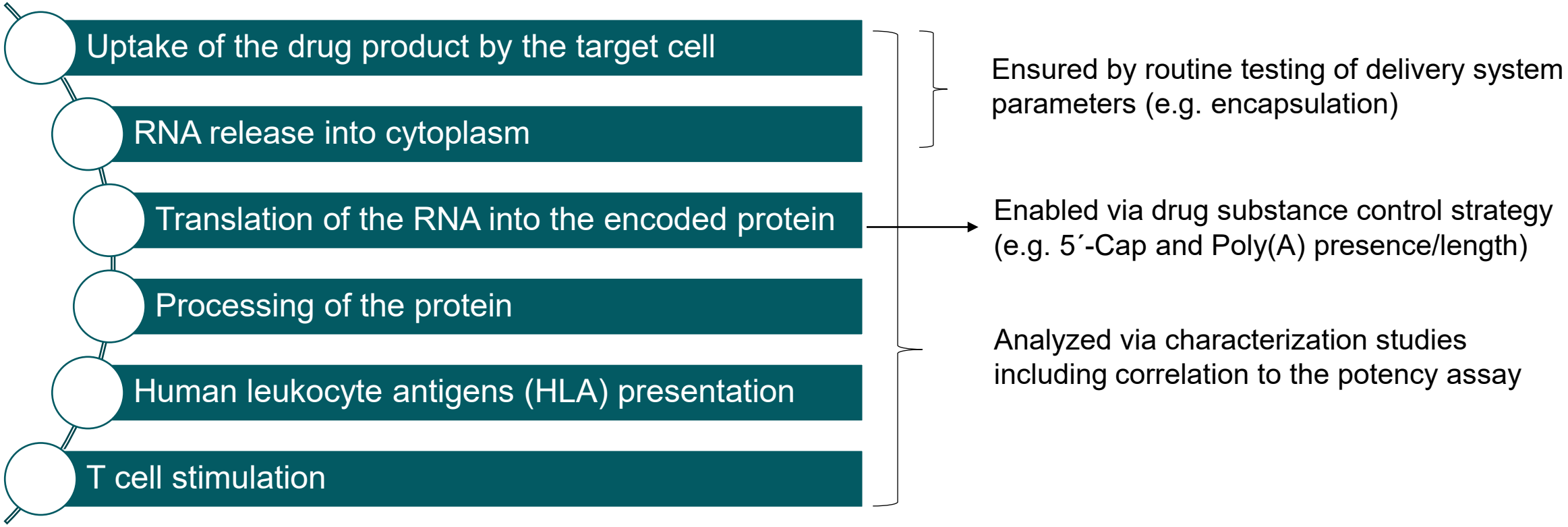
- A variety of **Modes of Action (MoAs)** of mRNA products are possible.

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



Challenges on potency testing of mRNA products

Example of Mode of Action for an mRNA-based cancer vaccine initiating immune response.



Problem statement:
What is the relevant part of the **Mode of Action of mRNA based products** that has to be shown for:
i) **release and stability testing** ii) **characterization**?

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mRNA 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 lenght 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

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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 Biological Activity	Size and identity of translated protein (after DS in vitro translation) confirmed by Western blot analysis
Drug Product Biological 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).	

Challenges on potency testing of mRNA products

- The need of highly sensitive techniques for more potent vaccines with potentially lower doses.
- Potential lack of specific detection antibodies (e.g., variability of B cell antigens, T cell antigens per se not optimal targets to induce antibodies) to quantify each translated antigen in a potency assay.
- Difficulty to generate the detection antibodies limits the accelerated development option offered by mRNA technology.
- Setting a clinically meaningful acceptance criterion for a potency assay (in particular: for patient individualized products).
- Potential cross-reactivity of antibodies to detect multi-construct products may impact the potency assay.

Example: **BNT162b4**,
a prophylatic vaccine:

The vaccine 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.

Call for dialogue

In the light of fast developing mRNA technology bringing various opportunities to address most pressing health challenges and unmet medical need, there is a need for **continuous dialogue between industry and regulators** to address arising questions:

- What is/are the appropriate part(s) of **Mode of Action** to be routinely controlled for various mRNA multi-antigen constructs or multi-construct mixtures present in a single drug product?
- How to reasonably address the absence of a specific antibody against each of the antigens in a multi-antigen construct or multi-construct mixtures?
- If and what combination of the biophysical approaches can be considered to determine/monitor structural and functional attributes correlated to potency?
- Can a matrix of functional attributes (e.g., translatability, mRNA integrity) serve as **surrogate for potency** testing?
- Can potency evaluation in characterization studies be a substitute for routine release and stability testing?

THANK YOU

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