Outline

I. Individualized cancer vaccines

II. Personalized RNA mutanome vaccine manufacture

III. Regulatory considerations

In principle, all tumors harbor mutations...
Tumor Immunotherapies

Immune response → Tumor responds → Survival of the patient

Vaccine

RNAs Peptides Antigen-loaded Dendritic cells

mRNA Therapeutics

Therapeutic messenger (m)RNAs are used to introduce the genetic information for a protein, encoded by the respective mRNA, into a cell of interest. So, while the mRNA is the active pharmaceutical ingredient (API), the active principle is (generally) the translated protein.
mRNA Structure and Optimization

Protein expression from an mRNA is dependent on

- its stability (how long is the mRNA present in the cell)
- its translational efficiency (how much protein is made from one mRNA)

These in turn are defined by the structural characteristics of the mRNA, i.e.:

- 5’ cap
- 5’ UTR
- open reading frame
- 3’ UTR
- poly(A)

Advantages of mRNA

- No integration into the genome
- Needs only to reach the cytoplasm, not the nucleus
- Transient expression of the encoded antigen
- Degraded into nucleotides, i.e. no toxic metabolites
- Antigen delivery independent of HLA-haplotype
- Induction of CD8+ and CD4+ T cell responses
- If desired, RNA acts as its own adjuvant (recognition by toll-like receptors)
- Relatively simple production process independent of the sequence
- Possibility to introduce new functionalities through sequence modifications and chemically modified building blocks
Direct application of mRNA

RNA „vaccine“ (local application of RNA into lymph node or systemically after formulation with liposomes targeting dendritic cells)

• Induction and expansion of antigen-specific T cells
• Systemic distribution
• Anti-tumoral effects

Mechanism of Action
**Induction of anti-tumor immunity with mRNA**

**Recent publication of first-in-human results for personalized cancer vaccines**

**Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer**

- Sahin et al., Nature 547:222

**An immunogenic personal neoantigen vaccine for patients with melanoma**

- Ott and Hu et al., Nature 547:217

Support for clinical translation of vaccine mechanism of action

- Clinical feasibility
- Preliminary safety and tolerability
- Preliminary evidence of disease control

**Neo-epitope specific T-cell responses detected in blood and tumor**

**Killing of autologous tumor cells mediated by neo-epitope-specific TCR**
Outline

I. Individualized cancer vaccines

II. Personalized RNA mutanome vaccine manufacture

III. Regulatory considerations

Personalized Cancer Vaccine Manufacturing

- Supply Chain involves clinical sites and clinical operations
- Process starts with collection of whole blood and tumor tissue for DNA and RNA preparation and sequencing (Target Selection Process)
- After neoepitope selection, the patient-specific RNA cancer vaccine is manufactured
 Manufacture of Individualized Cancer Vaccine

- Fully individualized vaccine – custom-made for each patient and specific for their tumor
- On demand production
- Suitable for potentially all tumor types
- Induction of immune responses with high tumor specificity

Computational Medicine

IVAC® MUTANOME Technology Platform

Use patient tumor-specific mutations

For a selective activation of the immune system by custom-made RNA vaccines
RNA Manufacturing: *In vitro transcription*

- Transcribed region
- Linear template DNA
- Transcription using T7 RNA pol., cap (r), ATP, GTP, CTP, UTP
- *In vitro* transcribed mRNA (> 500 copies per template DNA) with a yield of > 5 mg/ml
- Raw reaction mixture with mRNA and impurities (T7 RNA pol., remaining cap and NTPs, hydrolyzed DNA, ...)
- Completely cell-free process (but some of the starting materials come from *E. coli*).

**mRNA purification**

Removal of:

- Process-related impurities (e.g. unconsumed NTPs, T7 RNA pol., template DNA)
- Product-related impurities (e.g. break off transcripts, side products)

Challenges:

- Highly charged and dynamic molecule
- RNA vs. DNA – removing the chemical cousin
- Scalability of the purification process
Integrity of purified mRNA

QC testing should follow principles of ICH guidelines.

QC testing of RNA should include integrity

-> more than 90% in the main peak

Quality Control Testing

Quality attributes of mRNA mutanome vaccines need to be prospectively defined, as are conventional therapeutics.

Testing each batch of mRNA and each batch of RNA-Lipoplex should follow principles of ICH Q6B, and include tests such as integrity, appearance, bioburden, and potency.
RNA lipoplex preparation

RNA-lipoplex nanoparticles assembled from RNA drug substance and liposomes in a single-use fluid-path system


Outline

I. Individualized cancer vaccines

II. Personalized RNA mutanome vaccine manufacture

III. Regulatory considerations
Batch Release Data are not available for initial IND/CTA

In general, regulatory expectations are that DS and DP batch release data are provided in the initial IND or CTA.

However, batch release data are not available for initial CTA for an individualized product because there are no DP batches manufactured until after patients are enrolled and their custom batches are manufactured and tested.

Therefore, representative batch data can be provided in IND or CTA to show the testing results.

Regulatory guidelines for mRNA

- There are no specific guidelines for mRNA in the EU (and the same holds true for the US). In any case, the general documents like the ICH guidelines on specifications and stability testing and impurities apply.

- The general requirements for manufacturing of mRNA are specified by the guidelines for (current) Good Manufacturing Practice.
  - EC Consultation Document: Good Manufacturing Practice for Advanced Therapy Medicinal Products
  - General observation: While CMC reviewers are open to innovative products and approaches, inspectors tend to take conventional viewpoint.
**GMP Manufacturing**

- Traceability of all materials (especially the critical starting materials, e.g. NTPs, cap analog, DNA template)
- Supplier qualification
- Formal process development
- Training of personnel
- Suitable clean rooms
- Batch release with predefined specifications

---

**Overview of RNA regulatory environment**

**The European Regulatory Environment of RNA-Based Vaccines**


**Abstract**

A variety of different mRNA-based drugs are currently in development. This became possible, since major breakthroughs in RNA research during the last decades allowed impressive improvements of translation, stability, and delivery of mRNA. This article focuses on antigen-encoding RNA-based vaccines that are...

**Classification of mRNA therapeutics**

In the EU, mRNA is classified as “Gene Therapy Medicinal Product” based on EU Directive 2001/83/EC:

a) it contains an active substance, which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; and

b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence

(except for RNA vaccines against infectious diseases and for chemically synthesized RNAs like siRNAs)

**In the US, mRNA is considered Gene Therapy, based on FDA definitions:**

Human gene therapy is the administration of genetic material to modify or manipulate the expression of a gene product or to alter the biological properties of living cells for therapeutic use

---

**Regulatory guidelines for mRNA in EU**

- Accordingly, EU Directive 2001/83/EG defines the general requirements with respect to quality and preclinical as well as clinical studies for authorization of mRNA. This has been amended by EU Directive 2009/1290/EG for “Advanced Therapy Medicinal Products” (ATMPs).

- With the classification as “Gene Therapy Medicinal Product”, the EMA draft “Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products” CAT/80183/2014 (replacing the “Note for guidance on the quality, preclinical and clinical aspects of gene therapy medicinal products” CPMB/BWP/3088/99) applies for mRNA.
Regulatory guidelines for mRNA in EU

- Similarly, the EMA “Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products” CHMP/GTWP/125459/2006 should be consulted.

- For cancer immunotherapy, further requirements for preclinical and clinical studies are given in the EMA “Guideline on the evaluation of anticancer medicinal products in man” CHMP/205/95/Rev.4 as well as “ICH guideline S9 on nonclinical evaluation for anticancer pharmaceuticals” CHMP/ICH/646107/2008.

FDA guidelines for mRNA


- FDA Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines (2011)


Acknowledgements

**BioNTech AG:**
- Andreas Kuhn
- Heinrich Haas
- Sebastian Hörner
- Björn Kloke
- Felicitas Müller
- Uğur Sahin

**Genentech/Roche:**
- Rainer Müller
- Maryse Gueguen
- Paul McDonald
- Manuel Mercier
- Emel Akkurt
- Don Low
- Leslie Kulakow
- Todd Renshaw