RCB300 - Tópicos em Biotecnologia III

mRNA vaccines — a new era in vaccinology

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Introduction

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Conventional vaccines

Live attenuated and inactivated pathogens, pathogen subunits

Slow development and large scale production

Unfeffective against non infectious diseases (e.g. cancer)

Nucleic acid therapeutics

First use of IVT mRNA in animals in 1990 testing for protein production

First sign of therapeutic use in 1992 with physiological effect of IVT mRNA transcribed vasopressin in the hypothalamus

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mRNA vaccines

Not infectious or integrating

Easily degraded in vivo

No anti-vector immunity (can be administered repeatedly)

High potential for large scale production

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Basic mRNA vaccine pharmacology

Image Credit: Dmitry Kovalchuk/Shutterstock

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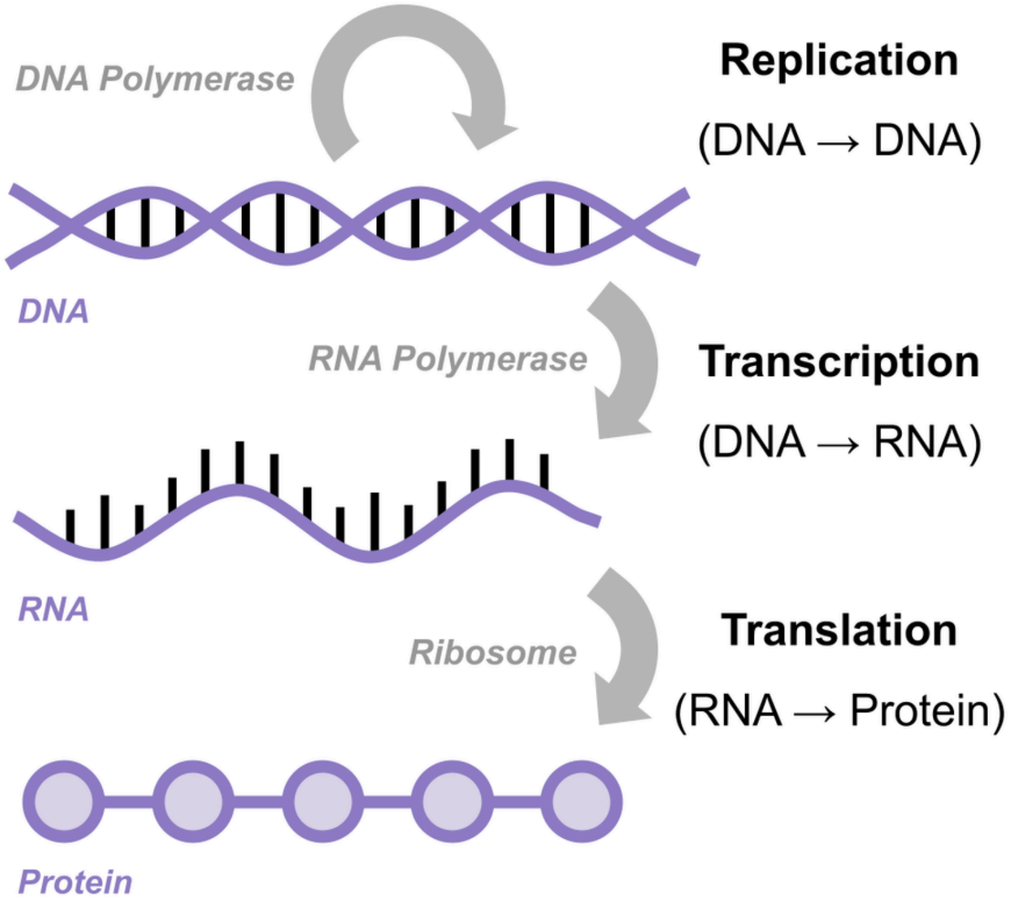
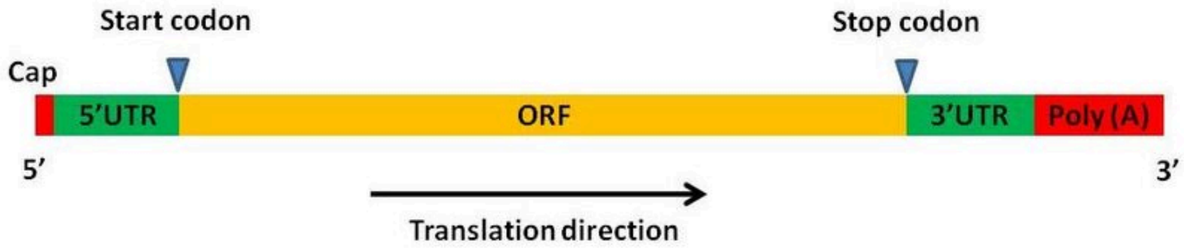
mRNA



"Transcription and DNA-Protein Binding - Biological Modeling" Biological Modeling,. Disponível em https://kasvi.com.br/centrifugacao-gradiente-de-densidade/. Acesso em 06/05/2024.

T7, a T3 or Sp6 phage RNA polymerase1



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Virally derived replication machinery

Sukarieh, Rami. Relocalization of eIF4E by its binding partners upon stress.

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Naked mRNA is quickly degraded

Hardly internalized

The solution..?

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(Getty Images/Reprodução)

Recent advances in mRNA vaccine technology

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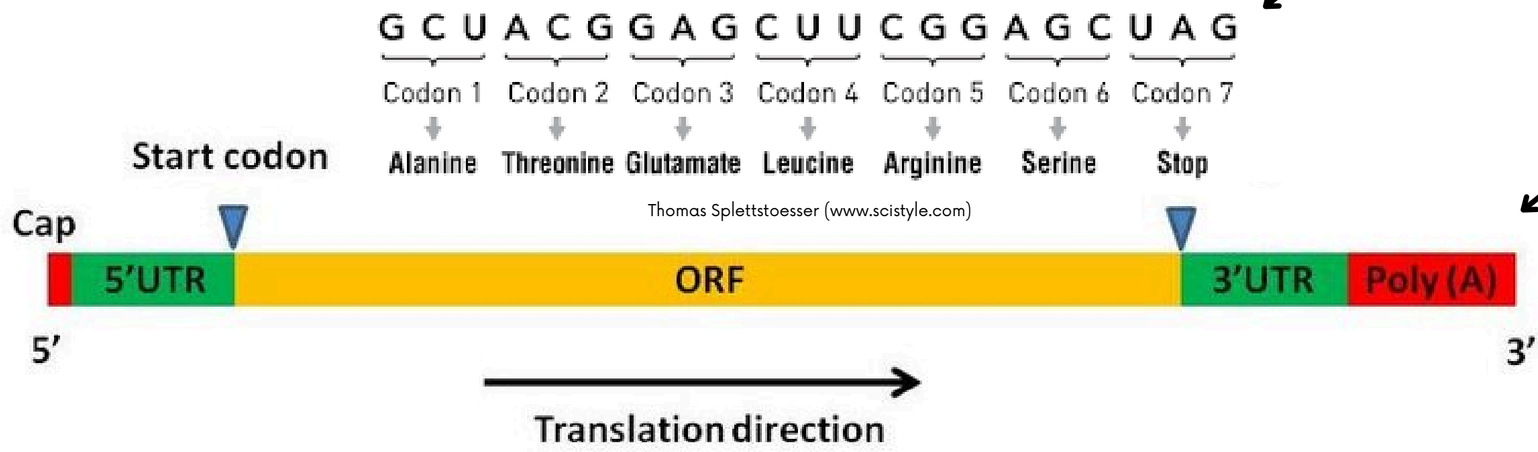
Optimization of mRNA translation and stability

- Vaccinia virus capping enzime
- Sythetic cap
- Cap analogues

- Use of more common codons
- GC enrichment

- Encoding DNA template
- Poli(A) polymerase



Sukarieh, Rami. Relocalization of eIF4E by its binding partners upon stress.

*May cause structural, kinetic and translational changes

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Modulation of immunogenicity

Exogenous mRNA is inherently immunostimulatory

Causes DC maturation and robust B and T responses

May inhibit antigen expression

Weak vaccination effect

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Modulation of immunogenicity

Purification of single strand mRNA

- Lowers aberrant dsRNA PAMP recognition
- Reverse-phase fast protein liquid chromatography (FPLC), High-performance liquid chromatography (HPLC)

Incorporation of naturally ocurring modified nucleosides

- Lowers inherent mRNA PAMP recognition
- Pseudouridine, 1-methylpseudouridine

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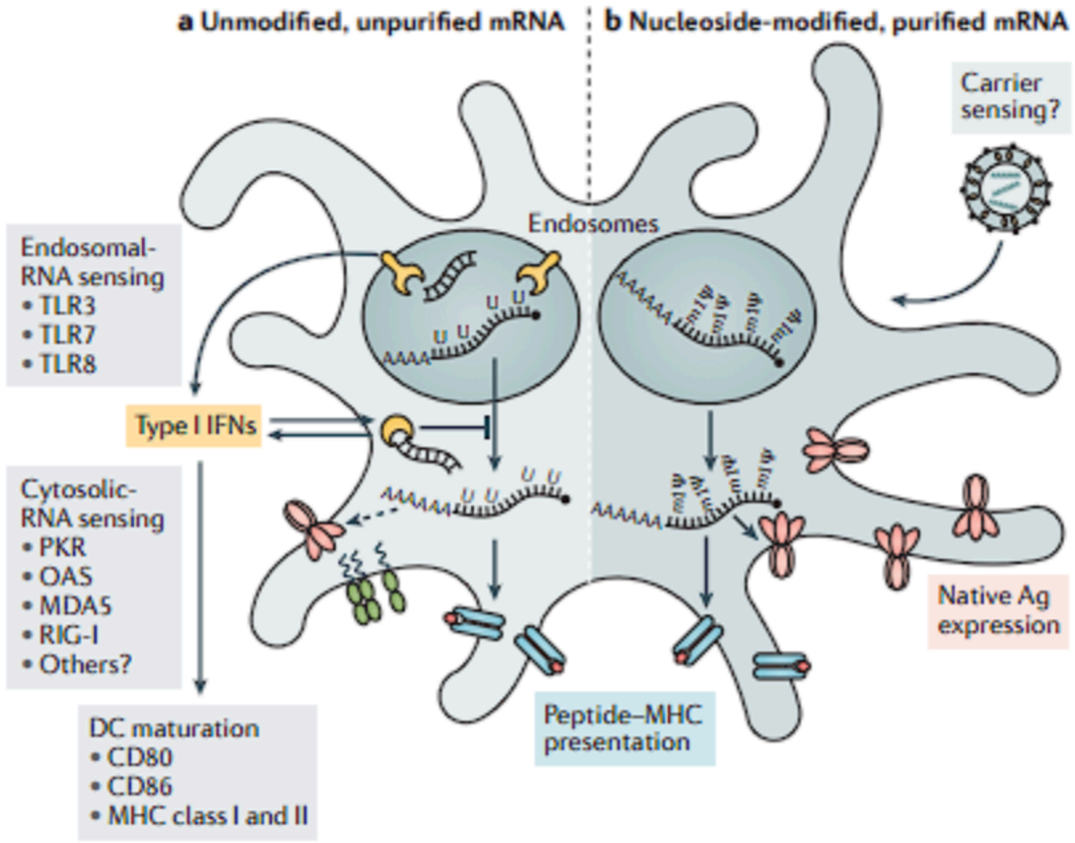
Modulation of immunogenicity



Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018 Apr;17(4):261-279. doi: 10.1038/nrd.2017.243. Epub 2018 Jan 12. PMID: 29326426; PMCID: PMC5906799.

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Modulation of immunogenicity

Unmodified, non-purified mRNA yielded more robust protein production in HeLa cells than nucleoside-modified mRNA

- Variations in RNA sequence optimization
- Stringency of mRNA purification to remove dsRNA contaminants
- Level of innate immune sensing in the targeted cell types

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Modulation of immunogenicity

Adjuvants

MF59 (Novartis)

Cationic nanoemulsion

Used in self-replicating RNA vaccines

Increased immunogenicity and effectiveness

TriMix

CD70, CD40L and active TLR7 mRNA

Used in naked, unpurified, unmodified mRNA

Increased DC maturation and cytotoxic T lymphocyte response in cancer

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Modulation of immunogenicity

Vaccine platforms

RNActive (CureVac AG)

Codelivered RNA complexed with protamine

Used in naked, unmodified, sequence optimized mRNA

TLR7 and TLR8 signaling (IFN-1, inflamatory cytokines)

Tested against cancer and infectious diseases

RNAdjuvant (CureVac AG)

Unmodified single strand RNA stabilizes with cationic carrier peptide

Used in non-mRNA vaccines

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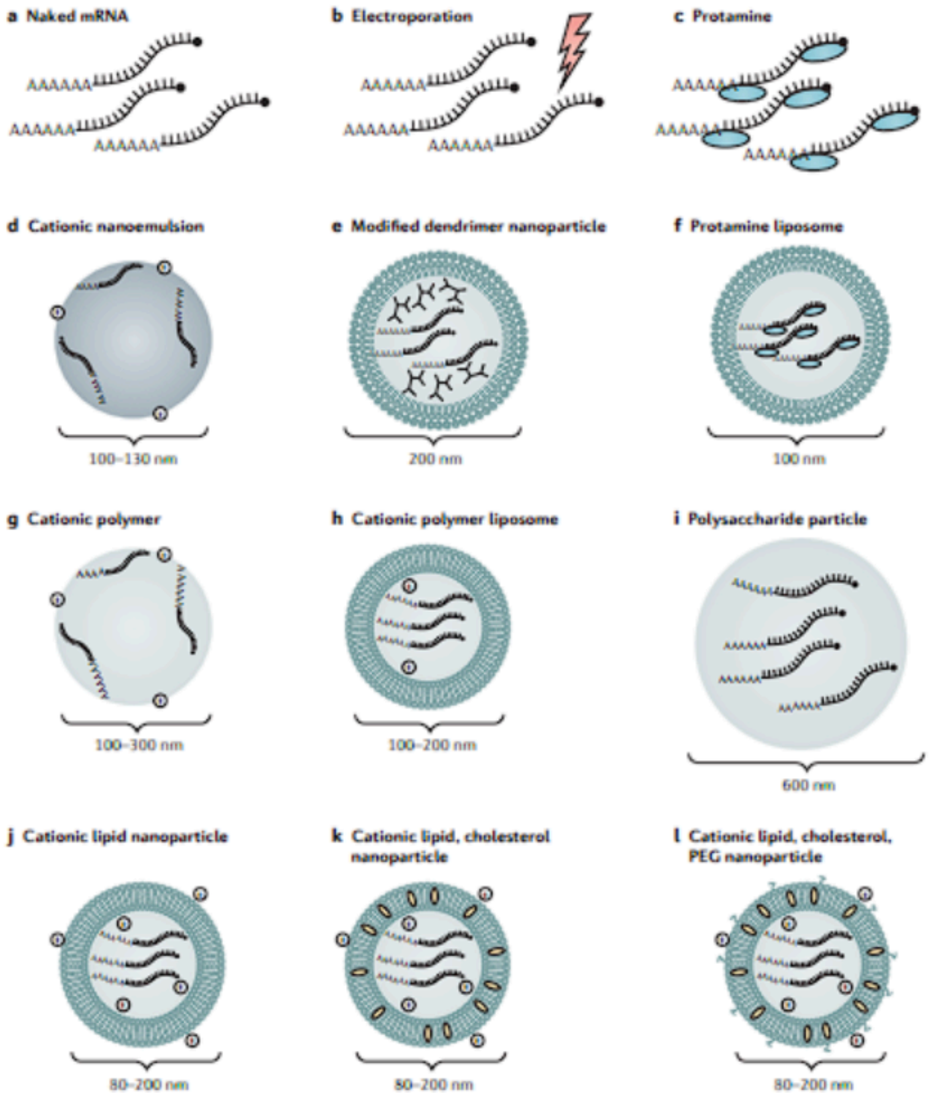
Progress in mRNA delivery



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Progress in mRNA delivery

Ex vivo loading onto DCs

Allows precise control of the cellular target

High transfection efficiency

Strong cell-mediated immune response

Expensive and labor intensive

Direct parenteral injection

Must penetrate lipid membrane

Cell type dependent uptake

Physicochemical properties may interfere

Low specificity

Rapid and cost-effective

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Progress in mRNA delivery

Complex with gold particles

Used in conjunction with gene gun

In vivo electroporation for increased efficacy

May increase cell death

Restricts access to target cells and tissues

Protamine

Protects from RNAses degradation

Limited protein expression and efficacy

Solved by RNActive vaccine platform

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Progress in mRNA delivery

Cationic lipid and polymers

Highly efficient transfection reagents

Developed for siRNA administration

Limited in vivo efficacy

High toxicity

Lipid nanoparticles

Most appealing and commonly used

Ionizable catiotinic lipid promotes self assembly into virus-like particles that release mRNA onto the cytoplasm

Polyethylene glycol increases half-life of formulations

Cholestherol helps stabilization

Phospholipids help maintain bilayer structure

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mRNA vaccines against infectious diseases

Vaccine Development

mRNA vaccines will fulfil many aspects of an ideal clinical vaccine:

- Favourable safety profile in animals;
- Versatile and rapid to design for emerging infectious diseases;
- Amenable to scalable good manufacturing practice (GMP) production.

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Potent immune response

Several formats of mRNA vaccines induce:

- Strong CD8+ T cell responses, likely owing to the efficient presentation of endogenously produced antigens on MHC class I molecules;
- Potent CD4+ T cell responses.

Two major types of RNA vaccine have been utilized against infectious pathogens:

- Self-amplifying or replicon RNA vaccines;

- Non-replicating mRNA vaccines.

1. Ex vivo loading of DCs;
2. Direct in vivo injection into a variety of anatomical sites.

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Self-amplifying mRNA vaccines

Based on an alphavirus genome, where the genes encoding the RNA replication machinery are intact but the genes encoding the structural proteins are replaced with the antigen of interest.

Protective immunization in preclinical studies against multiple infectious diseases including influenza, RSV, Rabies, Ebola, and HIV-1.

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Dendritic cell mRNA vaccines

Development of infectious disease vaccines using this approach has been mainly limited to a therapeutic vaccine for HIV-1.

This intervention proved to be safe and elicited antigen-specific CD4+ and CD8+ T cell responses, but no clinical benefit was observed.

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Direct injection of non-replicating mRNA vaccines

Works demonstrated that intradermally administered uncomplexed mRNA encoding various influenza virus antigens combined with a **protamine-complexed RNA adjuvant** was immunogenic in multiple animal models.

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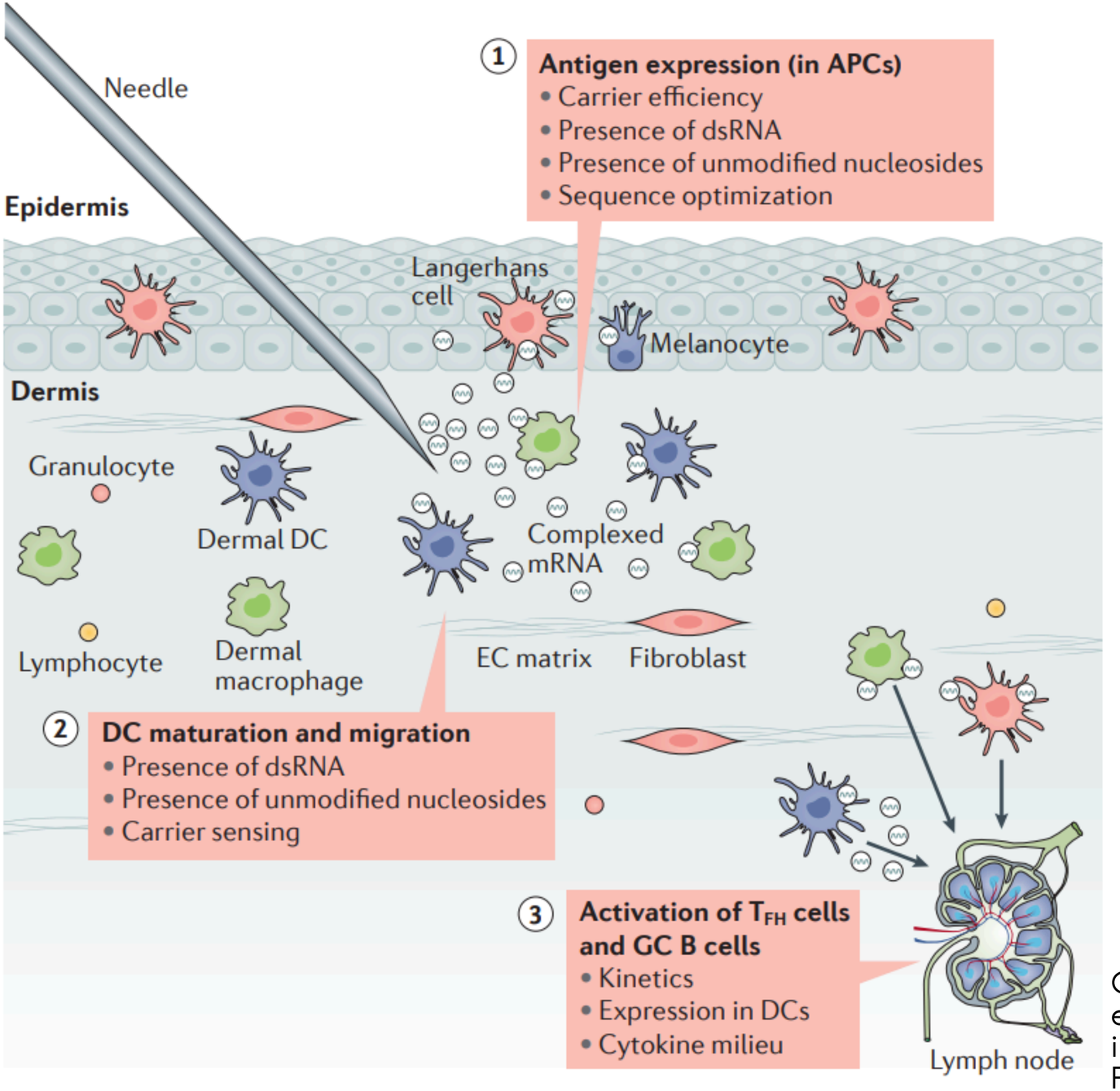

Considerations for effectiveness of a directly injected mRNA vaccine.
Pardi et al.

mRNA cancer vaccines

Cancer vaccines can be designed to target tumour-associated antigens that are preferentially expressed in cancerous cells: **growth-associated factors**, or **antigens that are unique to malignant cells owing to somatic mutation.**

Most cancer vaccines are **therapeutic**, rather than prophylactic, and **seek to stimulate cell-mediated responses, such as those from CTLs**, that are capable of clearing or reducing tumour burden.

DC mRNA cancer vaccines

As DCs are central players in initiating antigen-specific immune responses, it seemed logical to utilize them for cancer immunotherapy.

Several studies demonstrated that electroporation of DCs with mRNAs encoding costimulatory molecules resulted in a substantial increase in the immune stimulatory activity of DCs.

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Direct injection of mRNA cancer vaccines

Intranodal

Direct mRNA injection into secondary lymphoid tissue.

Intranasal

Needle-free, noninvasive manner of delivery that enables rapid antigen uptake by DCs.

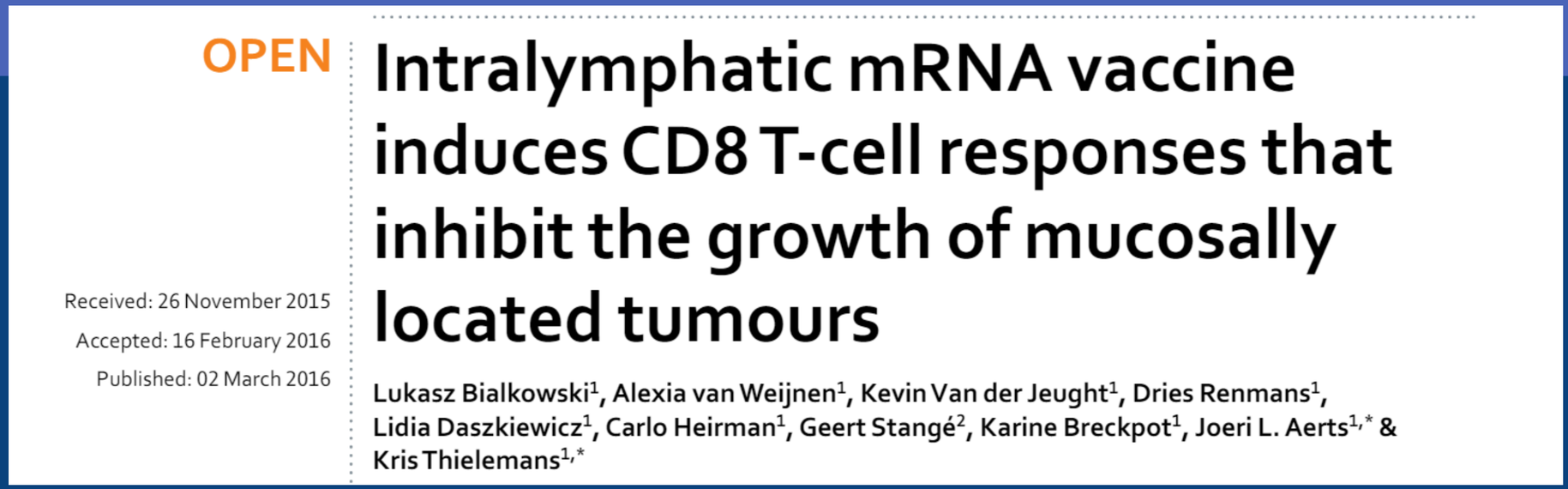
Intratumoural

Useful approach that offers the advantage of rapid and specific activation of tumour-resident T cells.

Systemic

Not common owing to concerns about aggregation with serum proteins and rapid extracellular mRNA degradation.

The combination of mRNA vaccination with adjunctive therapies, such as traditional chemotherapy, radiotherapy and immune checkpoint inhibitors, has increased the beneficial outcome of vaccination in some preclinical studies.



OPEN

Intralymphatic mRNA vaccine induces CD8 T-cell responses that inhibit the growth of mucosally located tumours

Received: 26 November 2015
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Lukasz Bialkowski[1], Alexia van Weijnen[1], Kevin Van der Jeught[1], Dries Renmans[1], Lidia Daszkiewicz[1], Carlo Heirman[1], Geert Stangé[2], Karine Breckpot[1], Joeri L. Aerts[1,*] & Kris Thielemans[1,*]

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Therapeutic considerations and challenges

Good manufactoring practice production

Regulatory aspects

Safety

Good manufactoring practice production

Advances

- Rapid and simple production compared to traditional vaccine platforms
- Possible standardization for diverse immunogens: suitable for emerging diseases
- Enzymes and components can be obtained from commercial suppliers, avoiding safety concerns

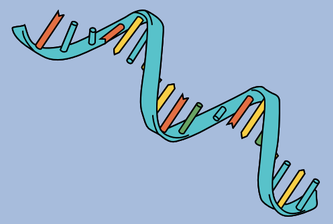
Challenges

- Sequence properties, like extreme length, may pose challenges
- Availability of GMP-grade components, though some are limited or costly
- Development of stable formulations for varied storage conditions
- Improvement in stability: nanoparticle packaging or RNase inhibitor co-formulation

Regulatory aspects

- **Lack of specific guidance** from FDA or EMA for mRNA vaccines
- Regulators have accepted approaches for demonstrating safety and efficacy
- Principles from DNA vaccines and gene therapy likely **applicable to mRNA**
- **Specific guidance expected** as mRNA vaccines gain prominence

Safety



- mRNA production **mitigates** common vaccine risks
- Theoretical risks like infection or DNA integration are not a concern
- mRNA vaccines generally considered **safe**
- Variability in **site/systemic reactions** among mRNA platforms: inflammation, biodistribution, stimulation of auto-reactive antibodies...
- Some mRNA platforms induce potent interferon responses
- Extracellular RNA may affect cell permeability and blood clotting, warranting safety evaluation in diverse delivery systems

Conclusion

mRNA vaccines are experiencing a burst in basic and clinical research

Preclinical and clinical reports showing the efficacy of these platforms

Two recent clinical reports: lower immunogenicity and side effects

Change of expression and immunostimulatory profiles

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Conclusion

Further research is needed

Recent advances in understanding and reducing the innate immune sensing of mRNA

Fast progress: innate immune sensing of RNA and in vivo delivery methods

Investment in mRNA vaccine companies

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Conclusion

The future of mRNA vaccines is extremely bright, and the clinical data and resources provided by companies and other institutions are likely to substantially build on and invigorate basic research into mRNA-based therapeutics.

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Thank you!