DIRNANO

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mRNA cancer vaccines

Messenger ribonucleic acid (mRNA) vaccines are based on synthetized mRNA molecules that represent an intermediate which provides instructions for the ribosomes in the cell cytoplasm to translate protein-encoding deoxyribonucleic acid (DNA) into targeted peptides/proteins (1). The synthesized mRNA is a product of *in vitro* transcription reaction (IVT) in which phage polymerases, such as T3, T7 or Sp6, synthesize mRNA from a linear DNA template by incorporating nucleotide triphosphates (NTPs).
Following this, the DNA template is degraded and mRNA capped so it can be efficiently translated *in vivo*. As such, the resulting mRNA product resembles naturally occurring mRNA molecules in eukaryotic cells (2). The mRNA contains the open reading frame (ORF), 5' and 3' untranslated regions (UTRs), 5'cap and a polyadenylic tail (poly A tail) (3).

The mechanism of action of mRNA cancer vaccines is based on the delivery of a tumor associated antigen (TAA) and/or tumor specific antigen (TSA), which is coded within the mRNA that, after reaching the cytosol of specific antigen presenting cells, is being translated to a protein of interest which will eventually be presented within the MHC I and/or II complex. The presented antigen is further recognized by lymphocytes and this further leads to initiation of a cascade that will result in the antigen-specific anti-tumor immune response (4).

These vaccines could be classified in two groups: non-replicating (or conventional) and self-amplifying. What differs between these two types is that the latter, beside the antigen of interest, encodes also for the viral replicating machinery. Currently, the non-replicating ones are prevalently used for cancer treatment while the self-amplifying ones are designed for infectious diseases treatment (5). The mRNA cancer vaccines can be delivered through various routes, including standard ones such as intramuscular, subcutaneous, intranasal, intradermal, and other less frequent such as intranodal, intratumoral

or intrasplenic (6).

HISTORY

The development of mRNA cancer vaccines started in the 90s with a first publication by Conry and colleagues where they used a naked mRNA (mRNA without any carrier) targeting human carcinoembryonic antigen (CEA) and were able to observe elevated antibody levels in immunized animals (7).
Various studies followed, however due to instability of the early mRNA vaccine formulations, they induced high innate immunogenicity and could not be successfully delivered to target tissues and cells. Consequently, for decades, research in this field was diverted towards development of protein-and DNA-based vaccines.

IMPROVEMENTS

A lot of effort has been invested to surpass these limitations. The inherited innate immunogenicity of the mRNA was drastically reduced, and thus translatability improved, through utilization of nucleoside modification and/or incorporation of naturally-occurring chemically modified nucleosides, and improvements in the purification process resulting in highly successful removal of double stranded RNA (8,9).
Furthermore, development of more efficient capping systems further improved translational stability and efficiency of IVT mRNAs (8).

Another crucial improvements was the development of efficient delivery vehicles, such as lipid nanoparticles (LNPs) (9). LNPs are cationic carriers that have been approved by the Federal Drug Agency (FDA) as the most efficient IVT mRNA delivery vehicles and are, therefore, most widely used. These nanoparticles can pack and condense negatively charged mRNA through interactions with positively charged lipids, resulting in the formation of lipoplexes which prevent mRNA degradation and promote delivery to target cells. Besides providing protection, these carriers promote cellular uptake of mRNA and its delivery to the translational machinery due to their physico-chemical properties, that allow their interaction with cell membranes. All this improves the translation efficiency of the mRNAs (10).

All these improvements led the FDA to grant emergency approval for the use of two mRNA vaccines, Pfizer BioNTech/BNT162b2 and Moderna/mRNA-1273, against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Furthermore, in 2023, the Nobel prize in Physiology or Medicine was awarded to mRNA vaccine technology pioneers, Dr. Katalin Karikó and Dr. Drew Weissman (11–13). Due to the tremendous success of mRNA vaccines in the fight against SARS-COVID-19, a great amount of research is now directed to the use of these therapeutics to fight infectious diseases, but also cancer.

ADVANTAGES OF mRNA CANCER VACCINES

Several features of these vaccines make them highly promising and currently advantageous as compared to other types. For instance, since these vaccines are delivered and processed in the cell cytoplasm, they do overcome

complications related to delivery into the nucleus which are encountered with DNA vaccines. Also, since no viral vectors are used, any potential risk of accidental insertional mutagenesis is prevented (14). Furthermore, the mRNA vaccines induce a rapid and transient protein expression as compared to plasmid DNA or viral vectors, reducing the possibility of potential auto-immune complications. The immunogenicity and expression of the mRNA vaccines can be optimized, which further improves their safety (15).

A very important feature of the mRNA vaccines is their rapid translatability, ease of modification and adaptation to new targets, such as in the case of patients suffering from malignant disease where the tumor can change rapidly and the targeted antigen may need to be replaced. Or in case of inter-individual heterogeneity of cancer patients, where this can be determinant for the success of immunization (16). As compared to other vaccination platforms, such are standard peptide vaccines, mRNA vaccines can be designed and produced in a labor-saving and relatively simple way. They are easy, inexpensive and can be produced in large quantities due to the high yields of the IVT reaction (17).

CLINICAL STUDIES

Although no cancer mRNA vaccines are yet FDA approved, highly promising results have been obtained in several clinical trials, including different tumor types (18).

One of the very promising mRNA cancer vaccines is mRNA 4157 containing information for up to 34 tumor neoantigens is developed by Moderna. This vaccine in provided great results recently in phase 2b clinical trial (NCT03897881) where it was tested in combination with immunecheckpoint therapy (ICI) pembrolizumab in 154 III/IV stage melanoma patients. The combination of vaccine and pembrolizumab resulted in 44% increase in relapse-free survival of these patients and significantly improved results obtained with pembrolizumab only (19).

Another promising one is the mRNA cancer vaccine developed by BioNTech named BNT116 encoding for up to 6 tumor antigens that is in Phase 2 clinical trial (NCT05557591) in combination with ICI Cemiplimab targeting non-small cell lung carcinoma (NSCLC) (20). The same company developed mRNA cancer vaccine encoding for up to 20 tumor neoantigens and tested it in Phase 1 clinical trial (NCT04161755) in combination with ICI atezolizumab and combination of chemotherapy (mFOLFIRINOX) (21). The vaccine was able to successfully stimulate antigen specific T cell response against several tumor antigens and prolong survival of patients. BioNTech is developing several other mRNA cancer vaccines targeting different tumors antigens in various tumor types such as melanoma, bladder, colorectal cancer, etc (22). Besides, there are currently 55 clinical trials involving mRNA cancer vaccines, thus witnessing the high interest in this promising field (search terms used: cancer; mRNA cancer vaccine; ClinicalTrials database accessed on 31 October 2023) (23).

ABOUT AUTHOR

Within the DIRNANO Consortium, I work in Spain where I am doing my Ph.D. studies at Fundación Rioja Salud, specifically at the Center for Biomedical

Research of La Rioja (CIBIR), within the Angiogenesis Research Group led by Dr. Alfredo Martínez. My main duties within the Consortium are to test *in vivo* cancer vaccine candidates developed by other consortium members.
In addition, my Ph.D. Project is focused on the development of mRNA cancer vaccines targeting one of the crucial regulators of tumor related angiogenesis, called adrenomedullin (AM). AM is a small 52 amino-acid peptide that acts as an autocrine/paracrine growth factor and promotes tumor cell survival, metastasis and immune-evasion, thus representing an important target for anti-cancer therapy. My construct is based on a gene fusion of adrenomedullin and an specific sequence of Keyhole Limpet Hemocyanin (KLH), a molecule having carrier and adjuvant properties. The resulting mRNA is encapsulated within LNPs and tested in cancer mouse models.

DIRNANO NEWS

Article titled “Structure-Guided Approach for the Development of MUC1-Glycopeptide-Based Cancer Vaccines with Predictable Responses” has been recently published in the Journal of American Chemical Society Au (JACS Au) (doi: 10.1021/jacsau.3c00587). This article is a result of the fruitful collaboration between several members of DIRNANO, including 4 ESRs.

Review was published by the University of Newcastle, laboratory of Prof. Moein Moghimi (University of Newcastle) with the title ”Activation of the complement system by nanoparticles and strategies for complement inhibition” (24).

Ander Eguskiza Bilbao (ESR9) just finished his secondment at Fundación Rioja Salud in the group of Dr. Alfredo Martínez, where he got introduced to basics of in vivo work and got familiar with several *in vivo* models used in cancer research.

Marek Feith (ESR8) is currently completing his secondment in Logroño, also in the group of Dr. Alfredo Martínez, focusing his research activities on the metabolic response of breast cancer cell lines to the combined treatment with nanoparticles containing photosensitizer and ferroptosis-inducing drugs.

The Winter School will be held in Enschede from the 5th to the 8th of December 2023, with LipoCoat hosting this event.

NEWS IN THE FIELD

The Nobel prize for 2023 in the field of Physiology and Medicine was awarded to Dr Katalin Karikó and Dr. Drew Weissman for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19. Their discoveries dramatically accelerated the vaccinology field and development of mRNA nano-therapeutics for various diseases, predominantly infectious diseases and cancer (13).

World Congress on Advanced Materials and Nanotechnology will be organized in Vancouver, Canada, from 27th to 28th of November.

SFNano Conference will be organized in Montpellier, France from 4th to 6th December.

International Conference on Nanoscience, Nanotechnology & Advanced Material (IC2NAM) will be organized in Zurich, Switzerland from 16th to 17th of December.

Zhou et al. recently published an article focusing on manganese-enriched zinc peroxide functional nanoparticles for potentiating cancer immunotherapy (25).

Moderna, one of the major players in the mRNA therapeutics field, struck a deal with Immatics, a German pharmaceutical company, to advance development of mRNA cancer vaccines and also test their mRNA cancer vaccine in combination with Immatics cancer therapy named IMA203 (26).

Stay tuned and follow all updates in our website and in our LinkedIn page!

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