

The messenger's great message for vaccination

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Poly ribonucleic acid (RNA) is the only polymer capable to recapitulate all processes of life: containment of genetic information, enzymatic activities and capacity to create defined 3D structures. Since it has a remarkable chemical stability (at neutral or acidic pH) and can be modified to enhance/reduce particular features (e.g., stability in biological RNase containing milieus or recognition by immune sensors), it is a particularly versatile and ideal active pharmaceutical ingredient. However, the utilization of RNA as a gene vehicle (messenger RNA, mRNA) for therapy has only recently been exploited. Within this scope, mRNA-based vaccines designed to trigger anti-cancer, anti-virus or anti-allergy immune responses have been developed. Modifications of mRNA vectors and implementation of adequate formulations have allowed to turn this natural superlative biological molecule into a safe active pharmaceutical ingredient that can virtually address any medical need including vaccination or immunotherapy. This is the newest great message delivered by this messenger.

Antigen-specific cytotoxic T-lymphocytes can prevent or cure infections as well as cancers. Nucleic acid-based vaccines are more efficacious than protein formulations in triggering cytotoxic T cells [1] and are also not hindered by production issues (on the contrary, recombinant proteins may be hard to produce on a large scale and GMP conditions). Among genetic vaccination methods, messenger RNA (mRNA)-based formulations have been the most recent ones. Nowadays, this may appear astonishing because it is being recognized more and more that this genetic vehicle is the most appropriate to safely immunize. Indeed, recombinant mRNA allows strong (high amount of protein translated from optimized mRNA), immunogenic (RNA is a natural danger signal for immunity) and transient (especially non-replicative mRNA is virtually totally eliminated within a maximum of a few days) expression of a unique antigen (recent review by Sahin *et al.* [2]). Meanwhile, other formats (e.g., plasmid DNA, recombinant viruses, recombinant bacteria) may be associated with persistence that is a major risk issue in gene therapy, as well as a turn down of

adaptive immunity (persisting antigens may induce some tolerance) or priming of anti-vehicle rather than anti-targeted antigen immunity. Being biased by textbook knowledge (RNA is depicted as a very fragile molecule) and limited by financial resources (production of mRNA in a laboratory is more expensive than production of plasmid DNA or recombinant viruses/bacteria) and in spite of early evidences from Wolf *et al.* [3] that directly injected naked mRNA can be taken up and translated, the scientific community focused on DNA–viruses–bacteria-based formulations for gene therapy should it be vaccination or non-immunogenic gene replacement. Few reports on the potential of mRNA for gene therapy had been published [4–7] up to the seminal article by Gilboa's team disclosing vaccination by adoptive transfer of mRNA-transfected dendritic cells (DCs) [8]. Gilboa's group has performed the first clinical studies related to this technology and established a dedicated company: Merix Bioscience (now Argos Therapeutic: www.argostherapeutics.com). Pivotal clinical studies are ongoing. Many research and clinical groups have

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followed by establishing the methods (culture of DCs or other antigen-presenting cells [APCs], production of mRNA, transfection of APCs) for performing preclinical and clinical evaluations of this vaccine format. DC-based vaccines were then 'en vogue': protein-, peptide-, pDNA- and mRNA-pulsed cells were evaluated and optimized for vaccination. This strategy has dominated the area of mRNA-vaccination for over 10 years. It is now accepted that direct injection of naked or encapsulated (cationic polymers, lipids and virus shelves) mRNA (simple or replicative) is a practical approach that can be optimized to generate immune responses, competing or even outcompeting (e.g., injection of naked mRNA in lymph nodes [9]) with the one induced by mRNA-transfected DCs in preclinical models. Through ongoing clinical studies, doses of few hundred micrograms of recombinant mRNA per treatment day are administered to patients. The use of replicative mRNA may allow lowering the required amount of nucleic acid [10]. Replicative mRNA codes for two proteins: an RNA replicase complex that produces from the template 'genomic' recombinant mRNA a large amount of antigen-coding 'sub-genomic' mRNA. As a result, very low doses of mRNA (eventually packed in nanoparticles, liposomes or capsid) can be used to induce an immune response *in vivo* in mice. The theoretical possibility that such replicons would associate with endogenous (onco) genes or viruses by, for example, trans-splicing has to be taken into account to establish safety endpoint of clinical studies. In addition, as any individual may have been infected with alpha-viruses, such as Sindbis, the pre-existing anti-replicase T-cell response may be immunodominant and decrease the effective T-cell response against the targeted antigen. However, should early clinical studies indicate the safety and efficacy of this vaccine format, it could be particularly adapted for anti-viral vaccination of large populations by injections of encapsulated or naked replicative mRNA.

After the demonstration by Hoerr *et al.* that injection of naked or protamine-complexed mRNA can induce on its own an adaptive immune response in mice [11], an innovative and ambitious endeavor initiated in 1999 by Dr. Hoerr, and involving the author as well as Dr. Von der Mulbe, Profs. Rammensee and Jung from the University of Tübingen, Germany, led to the setting up of the first company dedicated to the development of vaccines consisting of directly injected recombinant mRNA: CureVac GmbH [12]. An immediate challenge for the company has been the implementation of pharmaceutical production of mRNA. Indeed, for the transfected DC approach, cells were the vaccine component to qualify, GMP quality mRNA was not required for early clinical studies (mRNA was even not firmly established as being a component of a DC vaccine, as transfected cells were eventually matured *in vitro* before injection, thus having possibly fully degraded the transfected recombinant mRNA). Starting from scratch, as there were no example or guidelines worldwide for the validation of mRNA as a drug, we set up an entire pharmaceutical facility dedicated to the production of mRNA as a drug and validation process, as well as end-product controls (reviews by Pascolo [13–15]). Although most investments in the field at that time were made in DC- or DNA-based

vaccines, visionary private investors realized the great potential of off-the-shelf mRNA formulations and the total private equity support garnered by CureVac from 1999 to 2014 is over US\$ 135 million. The year 2014 has been pivotal, as the company disclosed the first deals (down payments, milestone payments and royalties) with large pharmaceutical industries: a license to Sanofi-Aventis for anti-viral vaccines and a license to Boehringer Ingelheim for an anti-lung cancer vaccine. Many improvements made in the mRNA-based vaccine formulations have been disclosed and involved the optimization of the mRNA (review by Sahin *et al.* [2]), its formulation (e.g., the role of calcium [16], injection in lymph nodes [9], encapsulation in liposomes that can protect against degradation and deliver to secondary lymphoid organs [17], Gene Gun delivery [6,18]), stability/translation efficacy (e.g., *cis*-stabilizing sequences, codon optimization and improved 5' cap structures [19]), purification (high pressure liquid chromatography purified RNA being better than unpurified mRNA [16]) and adjuvants (e.g., GM-CSF [20], protamine-RNA particles [21] and radiotherapy [22]). Those improvements are associated with new intellectual property that fostered the development of more biotechnology companies dedicated to the development of vaccines based on off-the-shelf mRNA formulations:

- BioNTech AG in Germany through its Ribological® RNA technology and Ribotransporter mRNA formulations implements and evaluates intralymph node injections of optimized naked mRNA [9], as well as systemic injections of encapsulated mRNA, which can direct the nucleic acid to APCs located in secondary lymphoid organs, such as spleen [17]. In addition, using full gene sequencing and dedicated algorithms, the company is developing mutanome-based mRNA vaccines to vaccinate against personalized tumor mutations in cancer patients ([23] is the first-in-human study evaluating a personalized vaccination targeting the mutanome in patients with advanced melanoma).
- eTherRNA uses an mRNA cocktail consisting of one transcripts coding for an antigen and three transcripts (so-called 'Trimix') coding for immunostimulating proteins: CD70, activated TLR4 and CD40L [24]. The Trimix cocktail can also be used as an indirect vaccine because it stimulates anti-cancer immunity by modifying *in situ* after intratumor delivery cancer-associated APCs in a way that boosts their natural capacity to stimulate/prime anti-cancer T cells.

Meanwhile, large pharmaceutical industries have started making alliances with these biotechnology companies, and Novartis has created its own mRNA-vaccine department focusing on the use of replicative mRNA.

Biodistribution of injected mRNA and intracellular fate (including counterproductive effects, such as eventual general inhibition of translation induced by the presence of foreign RNA within cells) are being intensively studied. Through a better knowledge of those parameters, improved (sequence/base modifications, formulation and protection against RNases) mRNA with higher bioavailability and translation efficacy as well as mRNA vaccine/drug combinations aiming at improved

priming of an immune response can be developed and tested. They will be superlative mRNA vaccine formulations or protocols of the future.

mRNA-based vaccines in clinical development are designed to trigger anti-viral and anti-cancer immunity. One immunotherapeutic area that requires a safe and versatile vaccine format, such as direct injection of mRNA, is desensitization against allergies. It is routinely performed by frequent intradermal injections of allergens (alternatively, a few intralymph node injections of the allergen can be used [25]). It allows desensitization in a number of patients. It requires the adequate allergen of a pharmaceutical quality. Should the allergen not be available, desensitization cannot be envisioned. mRNA can be produced to encode any protein allergen. An important feature of an mRNA-based vaccine is safety and that is the most relevant characteristic when anti-allergy immunotherapy is concerned. Accordingly, it could be shown that the injection of allergen encoding mRNA can prevent or treat allergy in animal models [26,27]. No doubt that anti-allergy mRNA-based formulations will attract large investments guaranteeing the development, besides anti-viral and anti-cancer vaccines, of anti-allergy immunotherapeutic products based on transcripts.

Although potential hurdles such as implementation of GMP production and evidence of functionality in humans have been overcome within the last 8 years and clinical testing of mRNA formulations is being broadened worldwide (several multicentric studies are ongoing in USA, Europe and Switzerland), further understanding and optimization of this vaccine format may help in improving its efficacy. Delivery of mRNA into adequate APCs as obtained, for example, through intravenous injection of liposomal mRNA formulations efficiently triggers innate and adaptive immunity [17]. Activation of innate cells principally through engagement of Toll-like receptors, such as TLR-3, TLR-7 and TLR-8, and eventually of non-immune cells through the activation of RIG-1 and MDA5 is evidenced by the production of IFN- α . These interferons are on their own potent anti-cancer and anti-viral proteins and help in activating efficacious T cells. However, they can be toxic and prevent translation. Thus, high induction of IFN- α may be of therapeutic relevance to treat ongoing infections or established tumors but may antagonize functionality (reduced antigen expression) and prevent clinically (because of possible side effects) the use of mRNA-based vaccines dedicated to prophylaxes of cancer, virus infections or treatment of allergies. Introducing a certain amount of non-immunostimulating mRNA in the formulations (e.g., mRNA totally or partially containing pseudouridines instead of uridines) may be an adequate solution to lower Type I IFN production and thereby reduce side effects. Dose/schedule finding, determination of the ideal injection site (e.g., intradermal or intravenous) and optimal formulation (triggering TLRs or not) must be determined for each

mRNA vaccine formulation and related to the life-threatening grade of the treated disease: treatment of advanced cancer can be done for each patient in a hospitalized surrounding where severe side effects of an injected efficacious drug can be controlled, while broad prophylactic vaccination of healthy people or desensitization of allergy patients require very safe formulations easily administered in ambulant settings. Thus, validation, optimization and implementation of mRNA-based vaccines, which is a relatively recent technology and will hopefully soon result in the release of anti-cancer immunotherapeutic products, also will require further scientific, medical and financial supports to fulfill its great potential and become the solution to many medical indications: cancer, infections and allergies. All tested approaches (DC, intralymph node, intraskin, etc.) may have advantages and disadvantages most adapted to address one or the other targeted clinical condition.

Compared to other vaccine formats, off-the-shelf mRNA-based formulations have not yet triggered the interest they deserve as illustrated by the still relatively low number of dedicated publications. However, it slowly federates more and more researchers and more and more companies. Prejudice over the costs and stability of mRNA in a laboratory surrounding may be one of the reasons of the late and slow development of off-the-shelf mRNA-based therapeutic approaches. Not all scientists involved in the development of vaccines are aware that there is no correlation between laboratory and GMP costs of reagents. Thus, although laboratory use of recombinant mRNA is more costly than the use of DNA, viruses, bacteria, cells or peptides, pharmaceutical grade biological products have similar prices that are associated to validation costs and not to the price of required goods. There are three available GMP production facilities for mRNA in Europe: CureVac and BioNTech (through its filial Eufets) in Germany and eTheRNA in Belgium. The ongoing efforts to validate mRNA-based vaccine drugs for several indications and the intrinsic fantastic potential of mRNA to safely and efficaciously address a broad range of diseases (also including non-immunogenic gene therapy for gene complementation or cell reprogramming or genome modifications) will probably stimulate the involvement of more research teams and implementation of more companies. mRNA-based off-the-shelf therapeutic products addressing cancers, infections, allergies and genetic diseases are going to be the efficacious and safe medical drugs of the future.

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