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Messenger RNA: The Inexpensive Biopharmaceutical

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***Abstract*—In contrast to the prejudices plaguing the use of mRNA as a drug, we now know that the large scale production of mRNA under GMP conditions is in reality relatively easy and robust, and therefore inexpensive, when compared to the production of other biopharmaceutics such as peptides, proteins, modified microorganisms (i.e., bacteria or viruses), and cells. It actually is the opposite of the situation in laboratories. Messenger RNA can be used for vaccination and gene therapy, thereby recapitulating the usage of all other biologic medical products (even cell-based therapies because mRNA can be used to reprogram cells in vivo or to stimulate their proliferation). This, combined with its versatile use for vaccination and gene therapy, lends mRNA promise as a superlative biopharmaceutical.**

Keywords—mRNA, GMP, vaccine, Gene Therapy

Main Text

Since the discovery by Wolff *et al*. in 1990 [1] that the injection of naked nucleic acids in the form of plasmid DNA (pDNA) or messenger RNA (mRNA) in animals results in protein expression, primarily pDNA-based methods have been used in pre-clinical studies. Until the publication of an mRNA-based vaccine by Hoerr *et al.* [2] and the subsequent founding of the first company dedicated to the development of injectable mRNA formulations - CureVac GmbH (Tuebingen, Germany) - in 2000, only four publications had reported the therapeutic utilization of direct administration of formulated mRNA: in 1992, Jirikowski et al. used mRNA for gene therapy [3], and shortly thereafter, Martinon *et al*., Conry *et al*. and Qiu *et al*. reported the use of mRNA for vaccination [4-6]. The relative lack of effort dedicated to the pre-clinical evaluation of injectable mRNA-based therapies has been ascribed to (i) the cost of mRNA production in laboratories and (ii) prejudice associated with the supposed fragility of mRNA, as detailed below.

(i) Working with mRNA in laboratories requires dedicated space, material, solutions and cautious handling techniques (*e.g.,* working with gloves). In addition, producing 1 microgram of capped mRNA in a laboratory cost approximately $1.50 (available kits costing approximately $450 provide reagents to produce approximately 300 micrograms of capped mRNA). Accordingly, mRNA-based therapeutic strategies requiring small amounts of mRNA, such as *in vitro* transfection of dendritic cells (DCs) [5 micrograms mRNA used to transfect DCs for one vaccine dose in mice [7]], to make vaccines have been preferred over direct injection [routinely 30 to 50 micrograms of mRNA for one vaccine dose in mice [2, 8]]. Under this scenario, the cost of mRNA vaccination (prime plus one boost) using direct injection is approximately $100 per mouse.

(ii) Messenger RNA may be degraded during production, storage or formulation due to ubiquitous RNases that sometimes contaminate laboratory labware, equipment and products. Researchers are taught to assess the integrity of RNA (*e.g.,* by gel electrophoresis), to keep it on ice during experiments, and to maintain samples below -20°C for long-term storage.

However, these points are moot when mRNA to be used as an Active Pharmaceutical Ingredient (API) is manufactured under Good Manufacturing Practice (GMP) conditions:

(i) Whatever the pharmaceutical product, its manufacturing requires dedicated clean room facilities, a sophisticated quality management system, and a dedicated team of trained personnel. As a GMP requisite, surfaces are ultraclean, materials are single use or washed in a monitored way, and the reagents used for production are highly pure. Thus, all surfaces and materials are free of RNase (and other pernicious enzymes such as DNase and proteases). Trained personnel work in gloves and additional protections, such as protective clothing, masks, and protective hair nets. The air in the rooms is filtered and is maintained at a constant temperature and humidity. The implementation, running costs and maintenance of clean room facilities is estimated account for more than 95% of the costs of any GMP production. Materials required for the specific product being manufactured have a minimal impact on the cost of the final pharmaceutical biological product, whether it is pDNA, protein, peptide, oligonucleotide, modified microorganism, cells, or mRNA. Messenger RNA production, as opposed to peptide or oligonucleotide productions, requires few toxic solvents, which are costly to store, use, and dispose of and does not require sophisticated synthesizers which may be costly in term of maintenance. As opposed to pDNA, cells, or modified microorganisms, no cell culture

(which requires complex media and incubators) or cell banking (which requires -80°C refrigerators and eventually liquid nitrogen) is required to produce mRNA under GMP conditions. Those features make mRNA synthesis fast (the transcription and purification of mRNA can be achieved within a day, while cell culture is a lengthy process) and reliable (transcription is robust, while cell growth or chemical synthesis have variable parameters that are hard to control and standardize). In conclusion, mRNA production under GMP conditions is in reality easier and faster, and therefore less expensive, than the production of other biological drugs such as peptides, proteins, pDNA, cells, and modified microorganisms.

(ii) RNA at neutral or acidic pH is a very stable molecule (unless it is designed to be active as a ribozyme). This is one of the reasons why RNA is often referred to as the polymer at the basis of life [9]. It naturally appears fragile when it is in an RNase-containing solution; even DNA appears fragile in DNase containing solutions, proteins and peptides appear fragile in protease containing solutions, and recombinant microorganisms and cells appear fragile in solutions containing detergents. Thus, the notion that mRNA is more fragile than other biologicals is only true under laboratory conditions in which RNases are abundant contaminants of labware and solutions. In fact, GMP mRNA is more stable than pDNA, proteins, cells and microorganisms: mRNA can be boiled, frozen, lyophilized and re-dissolved several times without alteration.

To obtain mRNA, a DNA template is required (Figure 1). It is usually pDNA (although as an alternative PCR products are used). Messenger RNA therefore requires an additional step beyond plasmid mRNA production. Thus, it may be assumed that mRNA will cost more time and money than pDNA to produce. However, under GMP conditions, this assumption is again false. In GMP mRNA production, pDNA can be used as a starting material (together with nucleotides, a cap analog, enzymes and chemicals). Thus, pDNA must meet the standards determined by the Qualified Person and the relevant authorities [*e.g.,* identity by pDNA sequence, purity by analysis of the structure (i.e., supercoiled versus relaxed circle versus linear), quantification of contaminants (*e.g.,* LPS and chromosomal DNA) and concentration by spectrophotometry]. This documented starting material is easily obtained from several companies dedicated to offering pDNA production (*e.g.,* Plasmid Factory in Germany). Because DNA (pDNA as well as contaminating genomic DNA) is destroyed after transcription, the specifications for pDNA are those established for ingredients that are not part of the final API. In addition, the requisite amount of pDNA is relatively low (1 mg of template pDNA yields approximately 20 mg of capped mRNA), and potential contaminants (*e.g.,* LPS) are diluted 20-fold prior to transcription (documented pDNA stocks are typically maintained at 1 mg/ml and used for transcription at 0.05 mg/ml) and further eliminated by mRNA purification.

In addition, mRNA has advantages that make it a surprisingly an easy and consequently relatively inexpensive biologic medical product (depicted in Table 1: “The benefits and difficulties of the production of biopharmaceuticals under GMP conditions”):

- Upscaling: A transcription reaction produces the same final concentration of mRNA whether it is performed in a 10 microliter or 10 milliliter volume. Upscaling the transcription reaction to a volume of a liter or more should not pose any problems. In contrast, producing for example pharmaceutical grade pDNA on a large scale requires the pelleting of grams of bacteria and lysing them while avoiding excessive sheering forces that break genomic DNA, leading to contamination.
- Using established conditions to produce a GMP molecule with a different sequence: Every mRNA molecule consists of A, C, G and U residues (and eventual modifications). Thus, the final molecule will always be soluble and stable at neutral pH and will not present any unpredictable behavior. Thus, established methods (i.e., protocols, in-process and end-product controls) can be used for the production of any mRNA under GMP conditions, independent of the sequence. In contrast, production of any new peptide or protein requires the re-optimization of all production steps: synthesis, purification and storage, as well as the eventual re-validation of in process and end product controls (certain protein or peptide sequences may interfere with these tests).
- Lyophilization/resolubilization: mRNA can be lyophilized and resuspended immediately in water-based solutions regardless of its sequence. In contrast, microorganisms most often cannot be lyophilized, and peptides or proteins may require peculiar solvents depending on sequence.
- Storage and temperature stability: mRNA in solution can be stored for weeks at room temperature as long as it is pure (i.e., free of RNase contamination) and in a neutral or acidic solution. Lyophilized mRNA can be stored for months at room temperature. That is why mRNA is championed as a suitable vector for vaccination and gene therapy in poor countries (a two million Euro prize was awarded by the EU for an mRNA vaccine developed by CureVac in an effort to stimulate innovative solutions for vaccine transportation and storage to locations where a cold chain cannot be guaranteed; efforts are also supported by the Bill & Melinda Gates Foundation). Messenger RNA can also be incubated at 95°C to kill possible viral contaminants. On the contrary, plasmid DNA, proteins, microorganisms, and cells are denatured by heating and damaged by long-term storage in solution at room temperature.

Another superlative feature of mRNA (and of nucleic acid-based drugs in general) is that it can encode several proteins or peptides (i.e., epitopes). To this end, the mRNA coding sequence can contain Internal Ribosome Entry Site (IRES) or code for a single chain protein in which subunits are linked by inert linkers (*e.g.,* GGGGS sequences). Thus, one

mRNA can replace several peptides (in the case of vaccinations) or protein chains (in the case of gene therapy), making mRNA less expensive than individual protein or peptide pharmaceutical components. The company BioNTech (Mainz, Germany) is a leader in the design and utilization of mRNA-encoded polyepitopes that can be used as individualized anti-cancer vaccines [10]. Thanks to its versatility [11-14] and inexpensive production features, the mRNA format allows such a design and production of vaccines that are made for a single individual. Further, mRNA libraries reflecting transcripts from tumor cells or malignant tissues can be used to vaccinate against cancer [15, 16].

Four companies have implemented large scale GMP production of mRNA: BioNTech (www.biontech.de: "actual production of up to grams of mRNA"), CureVac (www.curevac.com: "possible production capacity of up to 3.5 million doses p.a. at current site"), EtheRNA (www.etherna.be: "mid-size mRNA production facility") and ModeRNA (www.modernatx.com: production of "dozens to hundreds of unique mRNA constructs per week").

The first humans injected with naked mRNA were melanoma patients participating to a monocentric phase I/II study that we implemented at the University Hospital of Tuebingen, Germany [16]. We initiated thereafter few more investigator-driven monocentric studies [17-19] that have further documented the safety and efficacy (immunogenicity) of different formulations of mRNA given intra-dermally. CureVac has initiated company-sponsored (multicentric) clinical studies that further validated the safety and efficacy of intra-dermal mRNA-based vaccine formulations [20]. BioNTech's team and affiliates have further recently reported that systemic (intra-venous) injection of liposome encapsulated mRNA is a very potent method to vaccinate cancer patients [21].

Conclusion

In conclusion, the large scale production of mRNA under GMP conditions is in reality relatively easy and robust, and therefore inexpensive, when compared to the production of other biopharmaceutics such as peptides, proteins, modified microorganisms (i.e., bacteria or viruses), and cells. It actually is the opposite of the situation in laboratories. Messenger RNA can be used for vaccination and gene therapy, thereby recapitulating the usage of all other biologic medical products (even cell-based therapies because mRNA can be used to reprogram cells *in vivo* or to stimulate their proliferation). Millions of mRNA-vaccine doses can be readily produced by dedicated companies and the utilization of self-amplifying mRNA [22] would allow to use very low vaccine dose turning mRNA into for example prophylactic vaccines for a given viral threat within short time and at low cost. In contrast to the prejudices plaguing the use of mRNA as a drug, we now know that the production of mRNA as an API is relatively inexpensive and robust. This, combined with its versatile use for vaccination and gene therapy, lends mRNA promise as a superlative biopharmaceutical.

References

1.Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A*, et al.* Direct gene transfer into mouse muscle in vivo. *Science* 1990,**247**:1465-1468.

2.Hoerr I, Obst R, Rammensee HG, Jung G. In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies. *Eur J Immunol* 2000,**30**:1-7.

3.Jirikowski GF, Sanna PP, Maciejewski-Lenoir D, Bloom FE. Reversal of diabetes insipidus in Brattleboro rats: intrahypothalamic injection of vasopressin mRNA. *Science* 1992,**255**:996-998.

4.Conry RM, LoBuglio AF, Wright M, Sumerel L, Pike MJ, Johanning F*, et al.* Characterization of a messenger RNA polynucleotide vaccine vector. *Cancer Res* 1995,**55**:1397-1400.

5.Martinon F, Krishnan S, Lenzen G, Magne R, Gomard E, Guillet JG*, et al.* Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. *Eur J Immunol* 1993,**23**:1719-1722.

6.Qiu P, Ziegelhoffer P, Sun J, Yang NS. Gene gun delivery of mRNA in situ results in efficient transgene expression and genetic immunization. *Gene Ther* 1996,**3**:262-268.

7.Boczkowski D, Nair SK, Snyder D, Gilboa E. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. *J Exp Med* 1996,**184**:465-472.

8.Carralot JP, Probst J, Hoerr I, Scheel B, Teufel R, Jung G*, et al.* Polarization of immunity induced by direct injection of naked sequence-stabilized mRNA vaccines. *Cell Mol Life Sci* 2004,**61**:2418-2424.

9.Gilbert W. Origin of life: The RNA world. *Nature* 1986,**319**.

10.Kreiter S, Vormehr M, van de Roemer N, Diken M, Lower M, Diekmann J*, et al.* Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature* 2015,**520**:692-696.

11.Pascolo S. Messenger RNA-based vaccines. *Expert Opin Biol Ther* 2004,**4**:1285-1294.

12.Pascolo S. Vaccination with messenger RNA. *Methods Mol Med* 2006,**127**:23-40.

13.Pascolo S. Vaccination with messenger RNA (mRNA). *Handb Exp Pharmacol* 2008:221-235.

14.Sahin U, Kariko K, Tureci O. mRNA-based therapeutics - developing a new class of drugs. *Nat Rev Drug Discov* 2014,**13**:759-780.

15.Carralot JP, Weide B, Schoor O, Probst J, Scheel B, Teufel R*, et al.* Production and characterization of amplified tumor-derived cRNA libraries to be used as vaccines against metastatic melanomas. *Genet Vaccines Ther* 2005,**3**:6.

16.Weide B, Carralot JP, Reese A, Scheel B, Eigentler TK, Hoerr I*, et al.* Results of the first phase I/II clinical vaccination trial with direct injection of mRNA. *J Immunother* 2008,**31**:180-188.

17.Rittig SM, Haentschel M, Weimer KJ, Heine A, Muller MR, Brugger W*, et al.* Intradermal vaccinations with RNA coding for TAA generate CD8+ and CD4+ immune responses and induce clinical benefit in vaccinated patients. *Mol Ther* 2011,**19**:990-999.

18.Rittig SM, Haentschel M, Weimer KJ, Heine A, Muller MR, Brugger W*, et al.* Long-term survival correlates with immunological responses in renal cell carcinoma patients treated with mRNA-based immunotherapy. *Oncoimmunology* 2016,**5**:e1108511.

19.Weide B, Pascolo S, Scheel B, Derhovanessian E, Pflugfelder A, Eigentler TK*, et al.* Direct injection of protamine-protected mRNA: results of a phase 1/2 vaccination trial in metastatic melanoma patients. *J Immunother* 2009,**32**:498-507.

20.Kubler H, Scheel B, Gnad-Vogt U, Miller K, Schultze-Seemann W, Vom Dorp F*, et al.* Self-adjuvanted mRNA vaccination in advanced prostate cancer patients: a first-in-man phase I/IIa study. *J Immunother Cancer* 2015,**3**:26.

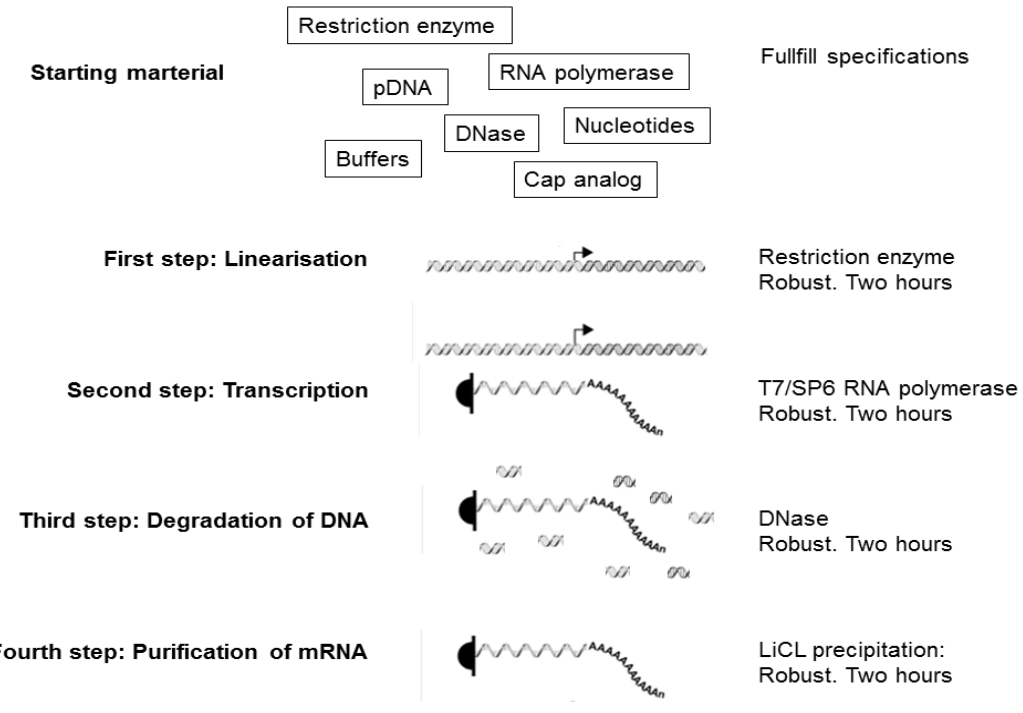
21.Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC*, et al.* Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* 2016,**534**:396-401.

22.Brito LA, Kommareddy S, Maione D, Uematsu Y, Giovani C, Berlanda Scorza F*, et al.* Self-amplifying mRNA vaccines. *Adv Genet* 2015,**89**:179-233.

Figures

The four steps of production are presented which can lead from starting material to final mRNA using plasmid DNA as starting material (PCR products can also be used). In step 1, the plasmid is presented with the relevant gene (to be transcribed) in dark grey under the control of a T7 or SP6 promoter (symbolized by the arrow). On the right is indicated the method and the minimum time required for each step. Although in research laboratories the whole process can be done in one day, in GMP conditions, in process controls in-between production steps make the manufacturing running over a few days. In addition, end product controls have to be performed for the release of GMP mRNA. The word “Robust” indicates that the step is processive, can be easily upscale and is reproducible from batch to batch. Final precipitation by Lithium Chloride may be replaced or followed by a more stringent purification step using for example HPLC-based methods.

Figure 1: Production of mRNA.



Tables

Table 1: The benefits and difficulties of the production of biopharmaceuticals under GMP conditions

	Upscaling	Re-using established GMP conditions	Solubility in water	Stability	Lyophilisation
Peptide	😐	😐	😐	😐	😐
Plasmid DNA	😐	🙂	🙂	😐	🙂
Recombinant microorganisms	😐	😐	😐	😐	☹
mRNA	🙂	🙂	🙂	🙂	🙂
Proteins	😐	😐	😐	😐	😐
Cells	😐	😐	☹	☹	☹

🙂 Easy / guarantied

😐 Eventually uneasy / not guaranteed, depending on sequence/organism/cell

☹ Difficult / not possible