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Current Status and Future Perspectives on mRNA Drug Manufacturing

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ABSTRACT

The coronavirus disease of 2019 (COVID-19) pandemic launched an unprecedented global effort to rapidly develop vaccines to stem the spread of the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2). Messenger ribonucleic acid (mRNA) vaccines were developed quickly by companies that were actively developing mRNA therapeutics and vaccines for other indications, leading to two mRNA vaccines being not only the first SARS-CoV-2 vaccines to be approved for emergency use but also the first mRNA drugs to gain emergency use authorisation and to eventually gain full approval. This was possible partly because mRNA sequences can be altered to encode nearly any protein without significantly altering its chemical properties, allowing the drug substance to be a modular component of the drug product. Lipid nanoparticle (LNP) technology required to protect the ribonucleic acid (RNA) and mediate delivery into the cytoplasm of cells is likewise modular, as are technologies and infrastructure required to encapsulate the RNA into the LNP. This enabled the rapid adaptation of the technology to a new target. Upon the coattails of the clinical success of mRNA vaccines, this modularity will pave the way for future RNA medicines for cancer, gene therapy, and RNA engineered cell therapies. In this review, trends in the publication records and clinical trial registrations are tallied to show the sharp intensification in preclinical and clinical research for RNA medicines. Demand for the manufacturing of both the RNA drug substance (DS) and the LNP drug product (DP) has already been strained, causing shortages of the vaccine, and the rise in development and translation of other mRNA drugs in coming years will exacerbate this strain. To estimate demand for DP manufacturing, the dosing requirements for the preclinical and clinical studies of the two approved mRNA vaccines were examined. To understand the current state of mRNA-LNP production, current methods and technologies are reviewed, as are current and announced global capacities for commercial manufacturing. Finally, a vision is rationalised for how emerging technologies such as self-amplifying mRNA, microfluidic production, and trends towards integrated and distributed manufacturing will shape the future of RNA manufacturing and unlock the potential for an RNA medicine revolution.

INTRODUCTION

The COVID-19 pandemic has catapulted messenger RNA (mRNA) vaccines from a relatively niche technology into the mainstream, with several hundred million doses of mRNA vaccines being administered. Due to mRNA's modular nature, companies such as Moderna, BioNTech and CureVac could pivot their mRNA programs for cancer vaccines, protein replacement therapies and other vaccines to respond rapidly to the threat of SARS-CoV-2. This is because mRNA, unlike small molecule and protein-based drug substances, can be tailored to encode almost any protein, while retaining nearly the same chemical characteristics. This allows other crucial technologies, such as nucleoside modifications, lipid nanoparticle (LNP) formulations and manufacturing to remain essentially the same. Since 2012, it had been demonstrated in a pre-clinical proof-of-concept study that mRNA was well suited for rapid pandemic response [1], and the COVID-19 pandemic provided the urgent need to commercialise these technologies.

This modularity and mainstream emergence of mRNA has catalysed an increase in clinical and pre-clinical development of other mRNA-based vaccines and therapeutics. This intensification will further increase the demand for manufacturing the RNA drug substance (DS) and LNP formulated drug product (DP), which has already been strained by global demand for vaccines against SARS-CoV-2. Production setbacks have left the world outside the United States and Europe with shortfalls or delays in receiving ordered doses. Challenges in DS and DP manufacturing present opportunities to develop innovative solutions. This review examines the global demand for clinical and pre-clinical mRNA DS and LNP DP by examining trends in the number of registered clinical trials and publications of pre-clinical development of RNA-based treatments. By also analysing the dosing requirements for representative clinical and pre-clinical studies, an estimate for quantities for formulated RNA is derived. We also examine current RNA DS and LNP DP manufacturing technologies to highlight potential development opportunities. Finally, we examine emerging manufacturing technologies and trends in RNA therapeutics to provide a perspective on the field's future directions.

SECTION 1: DEMAND FOR FORMULATED RNA-LNP DRUG PRODUCT

1.1 mRNA SARS-CoV-2 vaccine preclinical considerations

Both approved mRNA vaccines against SARS-CoV-2 developed by Moderna (mRNA-1273) and Pfizer/BioNTech (BNT162b2) are formulated in LNPs. Unformulated mRNA was found to be swiftly digested by ribonucleases [2] and to cause strong activation of innate immune pathways [3]. LNPs are comprised of four components: an ionisable lipid which is cationic at low pH and near-neutral charge at physiological pH; a zwitterionic phospholipid; cholesterol and a polyethylene-glycol lipid (PEG-lipid) which can be used to control the particle size allowing for sizes of < 100 nm [4] permitting efficient delivery of mRNA into cells [5]. By evaluating the preclinical results published by the two companies, we compare essential factors in their design including the route of delivery, formulation choice and dosage.

1.1.1 Dosage comparisons: BioNTech vs Moderna

In murine immunogenicity trials, Pfizer/BioNTech considered an mRNA dose range of 0.2 – 1 μg, while Moderna explored a range of 0.0025-20 μg. Key details from these studies are summarised in Table 1.1. These studies helped balance high levels of neutralising antibodies and T-cell responses against the potential for adverse drug reaction in mouse models that require low doses and hence minimal raw materials for manufacturing. This helps mitigate against supply chain and production limitations if these studies were conducted in larger species.

Table 1.1 Murine preclinical study parameters for Pfizer/BioNTech and Moderna SARS-CoV-2 Vaccines.

	Pfizer/BioNTech BNT162b2 [6]	**Moderna mRNA-1273 [7]**
Formulation	• ALC-0315 • DSPC • Chol • ALC-0159	• SM-102 • DSPC • Chol • DMG-PEG2k
Route of administration	i.m.	i.m.

Dose volume (μL)	20	50
Amount of mRNA/mouse (μg)	0, 0.2, 1 and 5	0.0025, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5, 5, 10 and 20
Antibody assessment day	7, 14, 21 and 28	14 and 28
# of mice/group (BALB/c strain)	8	10
# of groups	4	14
Mouse strains used	BALB/c	BALB/c; BALB/cJ; C57BL/6J and B6C3F1/J (B6C3F1/J used for immunological and challenge study)
Mouse age	8 – 12 weeks	6 – 8 weeks (immunological study); 16 – 20 weeks (challenge study)
Mouse sex	Female	Female for the immunological study. Male and female for challenge study

ALC-015 (4-hydroxybutyl)azanediyl)bis(hexane-6,1-dihy)bis(2-hexyldecanoate); DSPC (distearoylphosphatidylcholine); chol (cholesterol); ALC-0159 (2-[(polyethylene glycol)-2000]-N2N ditetradecylacetamide); SM-102 (heptadecane-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate); DMG-PEG2K (1-monomethoxypolyethylneglycol-2,3-dimyristylglycerol with polyethylene glycol of average MW 2000)

In mouse models and through to Phase 1, BioNTech compared two candidate vaccines encoding different antigens, which effectively doubles the manufacturing requirements for RNA-LNPs necessary to complete the studies [8,9]. In BALB/c mice, B and T cell responses were investigated after intramuscular injections of 0,2; 1 and 5 μg of either candidate. Similar to BNT162b2, mRNA-1273 encodes the full length of the prefusion spike protein [10]. In 4 different mouse strains, Moderna observed a dose-dependent increase in spike-binding antibodies from 0.0025 – 20 μg BALB/cJ mice were challenged 5 weeks after administering two doses, 3 weeks apart at 0.01, 0.1 and 1 μg, resulting in dose-dependent protection, with complete protection achieved at the 1 μg dose. Interestingly a single dose afforded complete protection using both 1 and 10 μg when challenged after 7 weeks.

1.2 RNA demand from late pre-clinical studies

To evaluate the range of dosage requirements for mRNA vaccines in larger animal models, we reviewed preclinical studies in non-human primates and ferrets. A search of PubMed® with initially set criteria identified 65 results with NHP studies (https://pubmed.ncbi.nlm.nih.gov/?term=mRNA+NHP) and 112 results with ferret studies: (https://pubmed.ncbi.nlm.nih.gov/?term=mRNA+ferrets). All Records (177) were screened based on their title and abstract and of those, 28 full-text articles were assessed for eligibility. Finally, 18 articles were included in the review of preclinical data. Studies eligible for inclusion were studies that reported vaccine effectiveness, mRNA vaccine load, modifications, nanoparticle delivery, route of administration and animal type. Studies that utilised the same vaccine or data source were excluded. These doses are tabulated in Supplementary table 1. The average amount of RNA dosed in each of these studies was 5350 μg.

With this average dosing, we aim to forecast the demand such studies place on RNA production by estimating growth in publications. We examined papers published each year since 1990 that mentioned: “RNA

delivery" and "RNA vaccine" (Figure 1.1). Currently, RNA vaccine publications have grown over 400% in 2021 (427) compared to 2020 (99). The 2020 tally is over 300% of the 2019 count (31). Among papers about "RNA delivery", 2145 were published in 2021, compared to 1901 published in total for 2020. It is clear that RNA vaccines are driving growth in publications in this field, and it is reasonable to expect publication volumes to continue to hold in coming years. If 10% of the approximately 2000 publications involve second species studies, and given the average total RNA dosing above, then 1.1 million µg of formulated mRNA would need to be dosed per year to keep the current pace.

The demand for formulated mRNA could increase further given that RNA doses for such applications like gene replacement therapies tend to be significantly higher than for vaccines. The typical vaccine doses reviewed were in the 10^{-3}mg/kg range, while for protein replacement studies, doses 100-fold higher in the range of 10^{-1} mg/kg are not uncommon [11-14]. Furthermore, studies involving microRNA (miRNA) and small interfering RNA (siRNA) tend to dose in the range of 1 mg/kg [15-17]. Hence, a substantial increase in demand for RNA DS and LNP DP could be on the horizon.

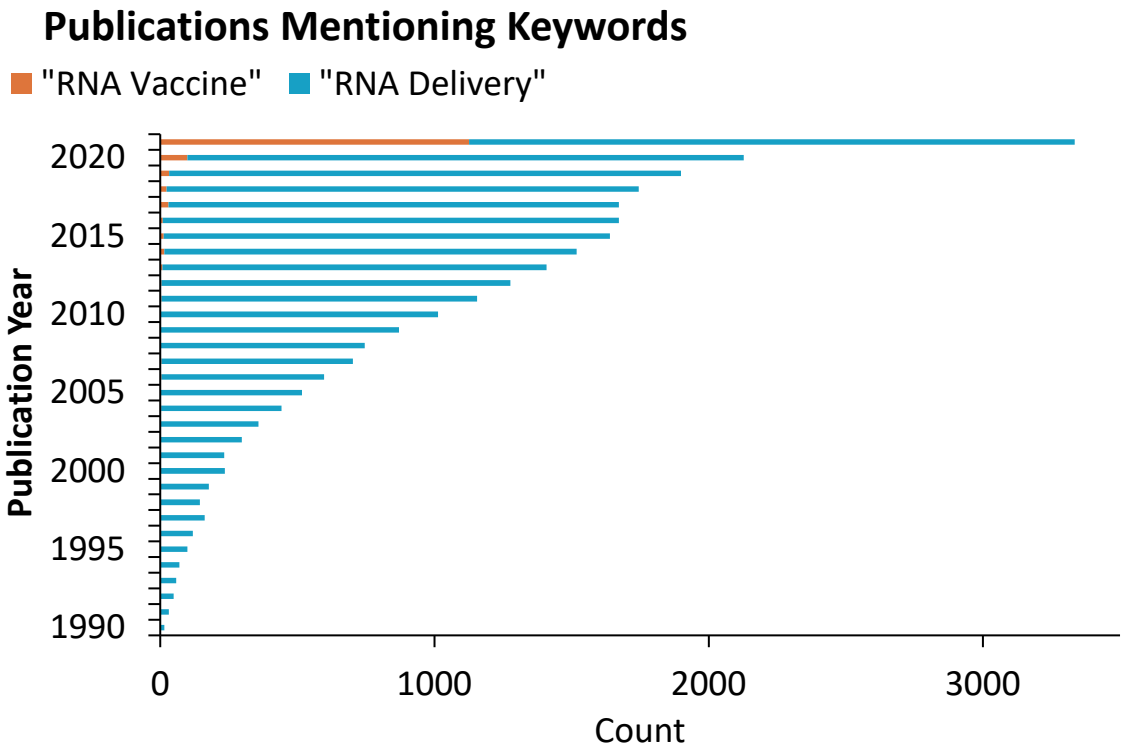

Figure 1.1 Record count for search parameters on PubMed® (https://pubmed.ncbi.nlm.nih.gov) employing publications matching "RNA vaccines" and "RNA delivery" in order of publication date.

1.3 Demand for clinical RNA-LNP production

By far the greatest demand for RNA DS and formulated DP originated from public health programs to vaccinate people against SARS-CoV-2. Moderna and Pfizer/BioNTech have set targets to produce 1 billion and 2

billion doses of their respective vaccines in 2021. At their respective dosing [18 19], this amounts to 100 kg of mRNA in 500 000 L and 60 kg of mRNA in 600 000 L of DP for Moderna and Pfizer/BioNTech respectively. These minimal requirements ignore losses due to process yield and analytical requirements.

An additional demand comes from clinical trials and other RNA medicines. To account for these quantities, we examined the dosing requirements from Phase 1-2 dose-ranging studies conducted by Moderna and Pfizer/BioNTech. While each study required modest doses, the regulatory success of these vaccines has led to an explosion of clinical trials in 2021. It is prudent also to include small interfering RNA and microRNA encapsulated in LNPs, of which one product has already been approved by the FDA (Onpattro, 2018). Additionally, the commercial success of these vaccines will also open doors for other RNA-LNP-enabled modalities, such as gene replacement, CRISPR/Cas9 gene editing and RNA transfected cell therapies that could place a demand on production capacity.

To forecast demand for RNA manufacturing for clinical trials, we searched the clinicaltrials.gov database for studies employing antisense oligonucleotides (ASOs), siRNA, mRNA, saRNA, and mRNA-transfected cell interventions. Observational studies not directly dosing RNA were excluded, along with studies that have been terminated or withdrawn. To date, mRNA has dominated the field as the preferred modality to treat a range of conditions (figure 1.2A; supplementary table 2), namely cancer and now, infectious diseases (figure 1.2B). It is possible to tally these records by study start date to highlight further the influence COVID-19 has had (figure 1.2C). Though there may not be much predictive value in modelling these trends to extrapolate future demand, it is clear that an era of RNA clinical trials is upon us.

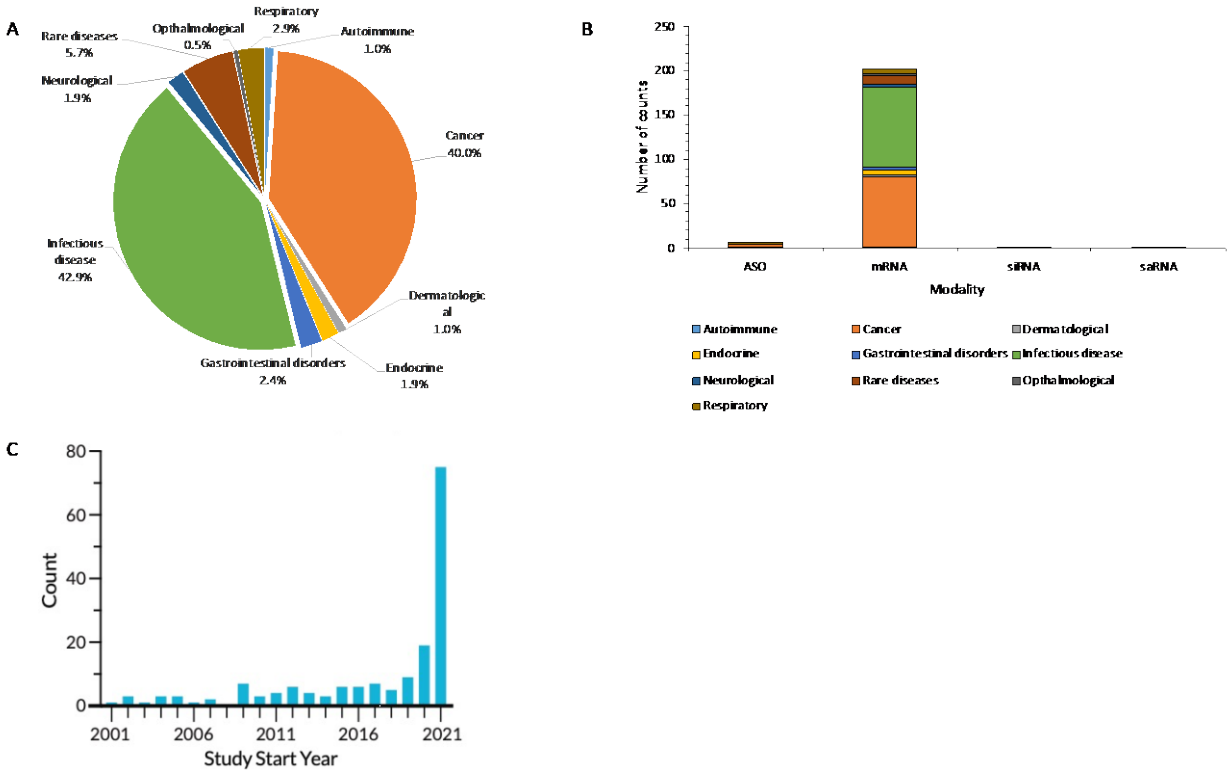

Figure 1.2 Clinical trials of RNA medicines and RNA-transfected cell therapies for (A) disease condition, (B) the modality used for the indicated condition and (C) the number of clinical trials featuring RNA interventions using ASOs, siRNA, mRNA, saRNA and mRNA-transfected cell interventions year-by-year.

To understand the potential impact of these clinical studies on RNA manufacturing, we examined the dosing requirements for 4 published papers [9, 20-22] for phase 1-2 dose-ranging studies for both Moderna and Pfizer/BioNTech SARS-CoV-2 mRNA vaccines. Supplementary table 3 summarises these findings and the mRNA quantities required. From the four studies, the average mRNA usage was 5500 μg and for the 75 trials in 2021, 412 500 μg of RNA would need to be formulated, purified, sterile filtered, and fill-finished. At concentrations of 100 to 200 μg of mRNA per mL, this would amount to 2063 to 4125 mL of DP. While the sheer quantities of these requirements pale in comparison to the commercial needs of vaccinating much of the world's population against a pandemic threat, the challenges lie in producing many different DPs for clinical trials without the economies of scale afforded by commercial production.

SECTION 2: CURRENT MANUFACTURING TECHNOLOGY

2.1 Production of mRNA DS

As a novel therapeutic, the large-scale production of mRNA is challenging and requires manufacturing technologies with approved protocols by regulatory authorities. Currently, large-scale mRNA is produced by either chemical, recombinant or enzyme-based systems. The choice of system is dependent on the characteristics of mRNA being synthesised and the respective clinical application, as discussed below.

2.1.1 mRNA DS Production Platforms

2.1.1.1 Chemical synthesis of RNA

The chemical synthesis of RNA is an automated cyclic process that enables continuous RNA synthesis and is considered a standard and robust method to produce short RNA (< 100 nt) [23]. This method utilises phosphoramidite chemistry and solid-phase support promoting chain elongation from 3′ end to 5′ end [24, 25]. However, the major drawback of solid-phase synthesis is it's 100 nt limit. Longer RNA can only be constructed by ligating smaller RNA using the DNA splint ligation platform [26] which along with the cost of phosphoramidite moieties could hold back the technology for large-scale production.

2.1.1.2 Recombinant Production of RNA

The recombinant production of proteins in a host cell (E. *coli*) is a well-established platform. Recombinant RNA production follows a similar principle; the DNA encoding mRNA of interest is packed into an expression vector and transferred into the host cell for subsequent synthesis of heterologous mRNA and is followed by a series of downstream processing (DSP) steps [27]. However, limitations to producing recombinant RNA include 5′ and 3′

terminal heterogeneity of the transcribed mRNA, degradation of the transcribed mRNA by host nucleases [28] and its inability to incorporate chemically modified ribonucleotides. Utilising technology from Ponchon and Dardel to protect transcribed mRNA from host machinery [29] and innovations from Jacob *et al* to promote highly stable circular mRNA [30], field advancements are being made to tackle these drawbacks. There are a few studies that discuss the lower cost of recombinant RNA production compared to chemical synthesis and in vitro transcription for large-scale production [28, 31]. One approach leverages existing infrastructure for manufacturing recombinant proteins permitting the industry to shift and upgrade the protein manufacturing units which are already in compliance with the Good Manufacturing Practice (GMP) guidelines.

2.1.1.3 Enzymatic synthesis of RNA

2.1.1.3.1 Polymerase chain reaction (PCR) and polymerase chain transcription (PCT)

PCR is an established method for amplifying oligonucleotides (DNA). However, the poor thermostability of RNA polymerases impedes RNA synthesis by PCR. Christopher *et al*. developed a unique double mutant DNA polymerase - TGK polymerase, that is capable of synthesising RNA using ribonucleotides [32] and similarly, Tingjian *et al*. reported the synthesis of RNA using unmodified and 2′- F-modified rNTP by SFM4-3 (DNA polymerase) by PCT [33]. The development of modified and engineered polymerases enabled and advanced efficient RNA production by PCT; however, modified DNA polymerase technology is still in its infancy, and further development is required for its use in large-scale RNA production.

2.1.1.3.2 In vitro Transcription (IVT) of mRNA

IVT (figure 2.1) is considered the gold standard method for the large-scale production of mRNA DS. IVT relies on RNA polymerases derived from bacteriophages which allow robust transcription [34] and facilitates mRNA synthesis using chemically modified ribonucleoside tri-phosphate (rNTPs) with the help of modified RNA polymerases [35, 36]. However, two significant limitations exist. First, a sequence-specific promoter is required by the RNA polymerases to initiate transcription which prevents 5′ user-specific modifications [37]. Secondly, IVT RNA exhibits terminal heterogeneity of transcribed mRNA due to non-specific run-off by RNA polymerase [38].

A key advantage for IVT is that the process parameters for the mRNA manufacturing process are independent of the sequence of template DNA, allowing a generalised production process to be designed to produce any mRNA of interest, potentially allowing for accelerated regulatory approvals manufacturing novel DSs. However, consumables such as enzymes, cap analogues and rNTPs elevate the costs. Additionally, RNA polymerases are highly susceptible to oxidative inactivation leading to fragmentation of mRNA products [39]. Altogether, with imperative improvements, IVT is the current state-of-the-art technology that offers cell-free, cost-effective, straightforward, coherent large-scale production of clinical use mRNA. As the demand for GMP mRNA increases, bio manufacturers are experiencing rapid demand for mRNA produced to GMP specifications with companies such as Aldevron investing in new GMP manufacturing sites[40]. China's footprint within mRNA vaccines for COVID-19 is continually growing with one of the highest numbers of registered COVID-19 vaccine trials in the last two years (69 registered clinical vaccine trials and 6 approved [41], date of access: 22nd of January 2022). To support this rapid growth of mRNA technology within China, GenScript ProBio announced the opening of a GMP

Manufacturing site in Zhenjiang, Jiangsu Province, which would be China's largest commercial GMP facility which would double the companies production capacities from this facility alone [42].

2.1.2 Purification of mRNA DS

Purification of mRNA DS is vital to achieving biologically active and therapeutically administrable mRNA. The mRNA produced at lab scale can be purified using DNases to remove DNA and subsequently isolated by lithium chloride precipitation [43]. However, multiple sophisticated DSP steps need to be employed to purify clinical-grade mRNA at a large scale. Chromatography is considered a standard DSP method used by the biopharmaceutical industry for purification processes and is widely accepted for its easy adaptability, scalability and economic feasibility. Size exclusion chromatography (SEC) also offers robust purification of mRNA [44,45]; however, it is confined by its inability to separate similar-sized RNA.

Similarly, ion-exchange chromatography, affinity chromatography, ion-pair reverse-phase chromatography facilitates efficient large-scale RNA purification with enhanced yield [46,47]. Tangential flow filtration (TFF) aids in removing smaller contaminants and concentrates the DS, along with precipitation reactions, facilitating expediated purification of mRNA [48]. Nonetheless, the cost and efficiency of the DSP are highly dependent on the product-specific purification process designed by the individual manufacturer.

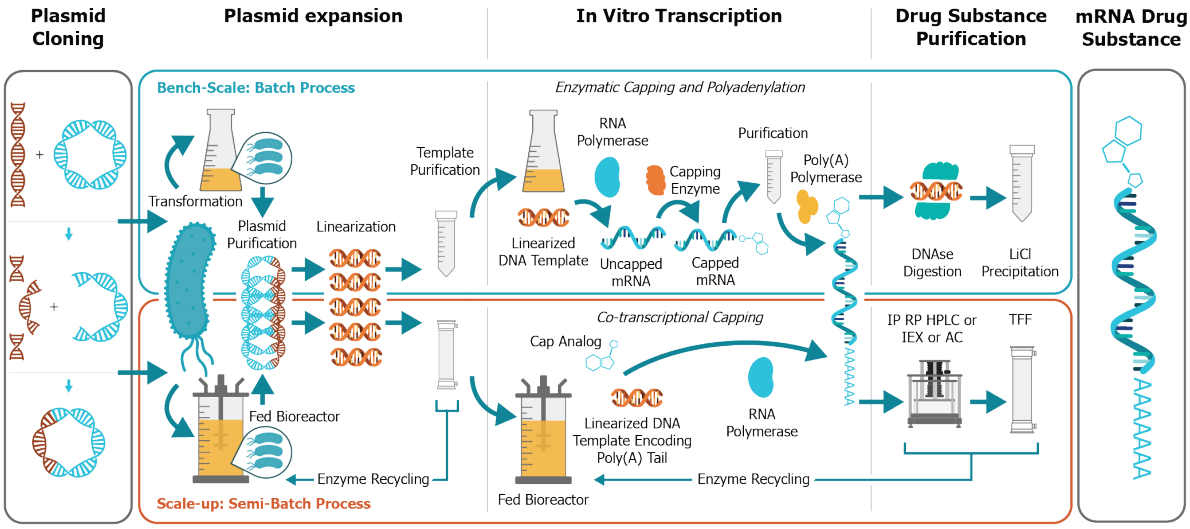

Figure 2.1 Process of IVT and DS purification and upstream processes to produce the DNA template. Acronyms: IP RP HPLC = Ion-pair reverse phased high-performance liquid chromatography; IEX = Ion exchange chromatography; AC = Affinity chromatography; TFF = Tangential Flow Filtration.

2.2 Challenges and opportunities for RNA-LNP DP Formulation

Formulating the RNA DS into an LNP DP is critical for efficacy because charged nucleic acids have a low probability of spontaneously crossing a lipid bilayer into the cytoplasm where they are biologically active [49]. Current

methods involve continuous self-assembly of LNPs by precipitation from ethanol upon mixing with nucleic acids in aqueous buffer resulting in nucleic acid encapsulation [49, 50]. Initial reports were not specific about the methodology of mixing [49] but mixing by T-junction has been cited as a scalable approach [1, 50]. To ensure electrostatic loading, the aqueous phase containing RNA is pH-buffered below the apparent pKa of the ionisable lipid. The mechanism of intracellular nucleic acid delivery changed: whereas cationic lipoplexes disrupt cell membranes to allow nucleic acids into the cytoplasm, ionisable LNPs containing cholesterol enters cells through an endogenous receptor-mediated endocytosis pathway and mediate the release of nucleic acids from the endosome upon acidification [51]. These LNPs were found to be more potent for in vivo small interfering RNA (siRNA) activity than those produced by the pre-formed vesicle method [52]. Lipid film hydration has been a traditional method for the production of LNPs where hydration of the film using an aqueous buffer with nucleic acid was used to passively encapsulate the therapeutic agent which often results in large heterogeneous vesicles (> 100 nm) which would require downstream size reduction methods such as sonication to reduce vesicle sizes [53, 54]. The lack of control, reproducibility and requirement of additional downstream processes limits the scale-up potential of the method.

For nearly a decade, microfluidic mixing methods have been reported in the literature to formulate RNA-LNPs for cancer therapeutics [15, 55, 56], protein replacement therapies [57-59], gene editing [60-62] and RNA vaccines [63-66]. Microfluidics enables non-turbulent mixing of the aqueous and organic phases to control self-assembly. The technology has been demonstrated to be scalable from microlitres, enabling bench-scale formulation development [67], to litres where integration with at-line analytical methods enables an important step towards an integrated manufacturing suite [68]. Historically, the staggered herringbone mixer (SHM) has been widely used utilising typical flow rates in tens of mL/min, which is an order of magnitude faster than typical hydrodynamic flow focusing devices [69]. However, the structure of the staggered herringbone chevrons adds multi-dimensional dependencies and practical limitations making it hard to achieve throughput speeds required to GMP specifications [70] which results in multiple SHM mixers needing to be arrayed in parallel to achieve higher throughput while retaining the same critical quality attributes such as size, PDI and encapsulation efficiency [15, 71, 72]. Innovations in microfluidic mixers have introduced next-generation toroidal mixers (TrM) that retain non-turbulent advective mixing of SHM, but enable single-mixer flow rates an order of magnitude greater (200 mL/min vs 12 mL/min) by increasing mixer dimensions while maintaining critical quality attributes of RNA-LNP [54, 73]. This retains compatibility with discovery and preclinical scale production and eases process scale-up [54, 67, 70, 74]. The details of how mRNA-1273 and BNT162b2 were produced for preclinical and clinical development have not been clarified, however; Moderna has published numerous studies utilising microfluidic SHM technology [11, 12, 75] and ethanol-drop nanoprecipitation [76, 77] and publications describing the non-human primate studies of BNT162b2 indicate LNPs were formed by transfer of an ethanolic solution of lipids into an aqueous buffer by diafiltration [6, 78].

SECTION 3: FUTURE PERSPECTIVES

3.1 self-amplifying RNA: a platform to reduce the RNA manufacturing burden

Like mRNA, self-amplifying RNA (saRNA) contains a 5' cap, a poly-A tail, and 5' and 3' untranslated regions but also encodes non-structural proteins that form a replicase to enable amplification upon intracellular delivery [79]. There are limited studies directly comparing mRNA and saRNA vaccines using the same formulation; however, we have compiled a direct comparative study between mRNA and saRNA using the same formulation in table 3.1. In a study by Vogel *et al*. [80] and Brito *et al*. [81], it was found that saRNA was at least 64-fold more potent than mRNA. Likewise, when directly investigating SARS-CoV-2 formulations we directly compared mRNA and saRNA with the same formulation used in preclinical/clinical trials (supplementary table 4) and found saRNA to be 6 – 100 fold times more potent than mRNA [21, 22, 82-86].

To quantify the scale-up volume requirements of mRNA vaccines compared to saRNA vaccines, it was assumed that the IVT of mRNA and saRNA results in the same mass yield from an equivalent volume and encapsulation efficiencies [82]. Following particle production, both mRNA and saRNA were presumed to result in the same purification efficiency and that the yield scales linearly with production volume. Based on the studies shown above, saRNA production requires 6- to 100-fold less RNA, and because of the nitrogen/phosphate (N/P) ratio is held constant, 6- to 100- fold less lipids. Thus, in the context of a pandemic, it would theoretically be possible to make 6- to 100-times more doses of a vaccine with the same batch volume and subsequently have a lower cost of and time required for production. To emphasise the advantages of saRNA vaccines, we compared the scale-up volume requirements for the Pfizer/BioNTech mRNA vaccine and a saRNA vaccine. Each Pfizer/BioNTech COVID-19 vaccine dose contains 0.3 mL and is currently priced at $19.50 USD. Assuming the entire population consists of 7.7 billion people, and each person receives two doses, a total of 15.4 billion doses or 4.5 million litres of the vaccine is required. In perspective, 4.5 million litres would fill 2 Olympic sized swimming pools (2500 m^3), whereas saRNA vaccines, which we assume are 100-fold more potent, would only require 0.02 Olympic sized swimming pools. Assuming that production costs, which account for materials, RNA and LNP production, scale linearly, vaccinating the entire population with Pfizer/BioNTech's mRNA vaccine would cost approximately 150 billion USD. In contrast, with saRNA, it would only cost approximately 1.5 billion USD. Ultimately, using saRNA for vaccine production would likely greatly reduce production and cost burden

3.2 – Perspectives on Evolving Commercial Manufacturing Model for RNA-LNP

Currently, large, centralised manufacturing is the status quo for therapeutic development. As drug manufacturing must meet high quality and safety standards, each site must have the knowledge, skilled workforce and infrastructure available to meet these requirements. As the development of RNA intensifies, so too will the trend towards more personalised medicines tailored to specific disease genotypes. This trend permits treatments that are effective for smaller fractions of the population, and conceivably treatments for individuals.

Moreover, especially during a pandemic, supply chain challenges can occur due to lockdowns; closures of upstream processes, reduction of the workforce due to health concerns and travel can lead to severe disruptions of

the worldwide supply of necessary drugs in addition to the shortage of components like vials, flow filters and syringes all represented scale-up challenges from the unprecedented vaccine production demand [87]. Figure 3.1 shows a map of publicly announced facilities involved in manufacturing Moderna's and Pfizer/BioNTech's SARS-CoV2 mRNA vaccines. Both vaccines rely on a network of different facilities located in the US and continental Europe. With production restricted to these two regions, the global supply of these vaccines was distributed from relatively few fill/finish sites across international borders. In response, several countries including Australia, Canada, China, Singapore, South Africa and South Korea, have announced plans and partnerships to enable domestic manufacturing of mRNA drugs (figure 3.2).

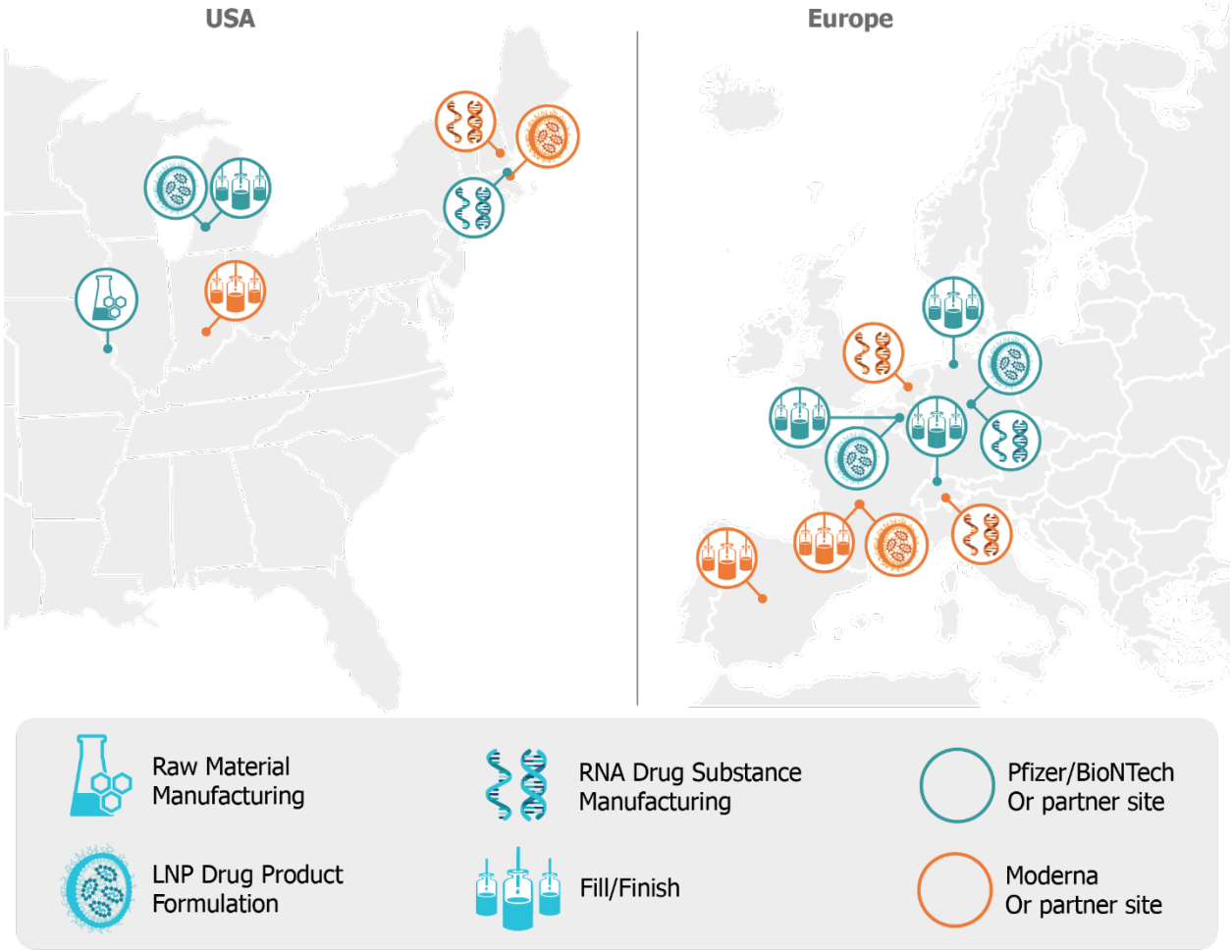

Figure 3.1 Current manufacturing sites for Covid-19 mRNA Vaccines. Functions such as DS manufacturing, formulation and fill/finish are often performed in separate facilities requiring shipment of bulk intermediate products. Details of each site are provided in supplementary table 5.

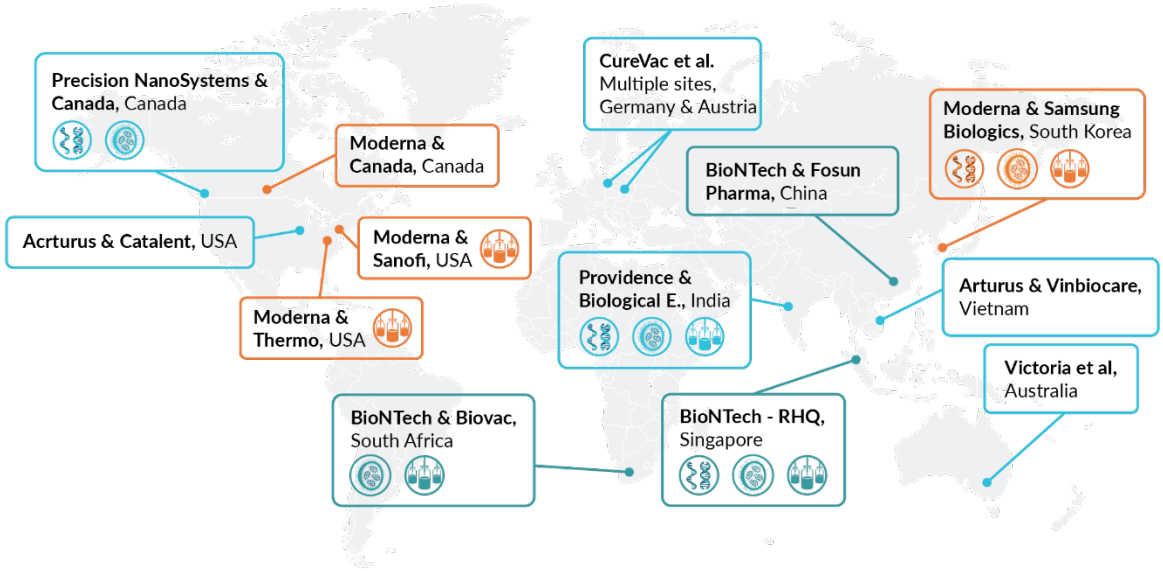

Figure 3.2 Additional mRNA Manufacturing Capacity Announced Globally. This map highlights numerous partnerships and programs that have been announced to build mRNA manufacturing sites in various locations globally. These actions indicate a shift from centralised manufacturing towards decentralised manufacturing models. See supplementary table 6 for more details of these announced sites.

A decentralised approach to manufacturing would allow drug manufacturers to produce drugs to meet the specific needs of the local region or ideally individuals. This model - termed precision medicine – would incorporate a specific patient's genes and lifestyle into the treatment to enhance safety and efficacy while reducing side effects. While current manufacturing is not suited for regional production, smaller, decentralised manufacturing sites would allow for a more personalised approach to therapeutic development. This could be achievable for RNA-based drugs where a recent study by Kis et al. showed that the RNA vaccine production process could be two to three orders of magnitude smaller than conventional vaccine production processes [87].

Despite the potential benefits of having multiple decentralised manufacturing sites, the approach would require significant changes to the regulatory framework. Currently, safety is assessed based on statistical information from large numbers of trial participants. With a more personalised approach, it is unlikely that the administration pool would be high enough to meet the same standards. Moreover, only a handful of companies worldwide obtain the knowledge and intellectual property to produce RNA-based medicines, and a decentralised model could be limited by the regional availability of a trained workforce and knowledge distribution. Additionally, regional vaccine

manufacturing centres' initial cost is higher than one large centre as each manufacturing line requires a complete set of equipment including analytical, downstream, and fill & finish capacities, which are usually shared in a centralised model.

Without the economies of scale afforded by centralised manufacturing, decentralised facilities can be more economical by producing multiple DPs. This approach leverages modular technologies, processes, and procedures common across several DPs, thus sharing the cost of infrastructure, raw materials, human resources, and development. mRNA therapeutics are particularly well suited for this model because the sequence of mRNA nucleotides can be altered substantially while maintaining the overall chemical properties of the DS. Figure 3.3 illustrates how a variety of RNA DSs can be manufactured using shared resources to produce a spectrum of RNA DPs spanning a variety of indications.

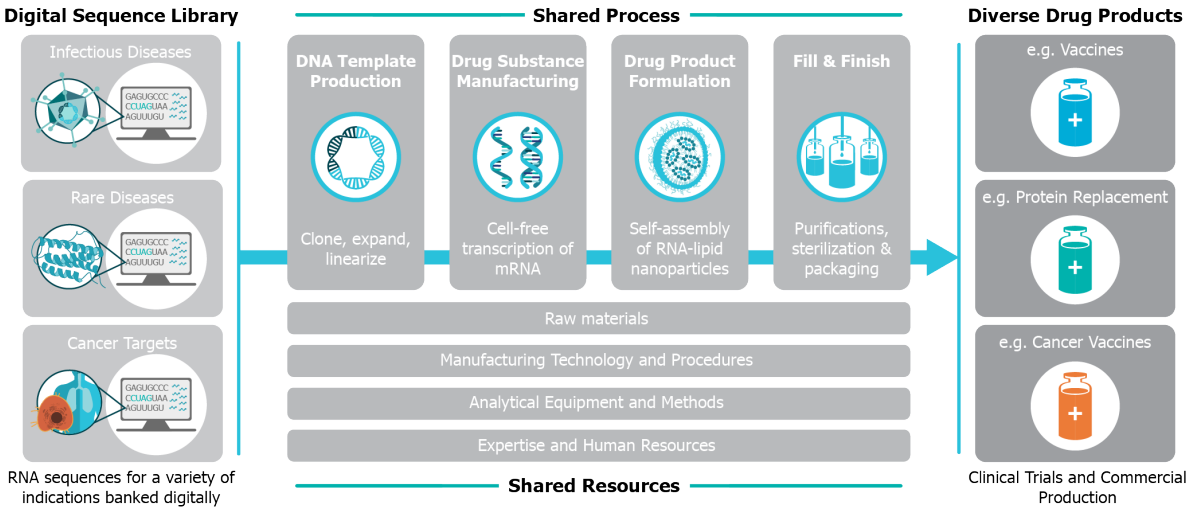

Figure 3.3 Multiproduct Manufacturing for RNA Medicine: Schematic diagram illustrating a typical manufacturing process applicable to various RNA therapeutics, indicating shared processes, technology and resources. Resource and cost-sharing contribute to reduced costs as more RNA therapies emerge, making individualised therapies more feasible. Facilities in place or being built for RNA vaccines can produce other RNA drugs, contributing to production capacity for other gene and cell therapies.

Figure 3.3 represents an RNA drug foundry, which is analogous to semiconductor foundries that revolutionised the computer and integrated electronics industry. Initially, computer processor designs were associated with proprietary manufacturing processes developed for a specific plan with production limited to a small number of manufacturers. The introduction of semiconductor foundries that employed standardised manufacturing processes, suitable for producing any number of circuit designs decoupled chip design from manufacturing capabilities and infrastructure. This democratised semiconductor chip design enabled fabless semiconductor companies leading to an explosion of innovation and market competition [88]. The concept of an RNA medicine

foundry, enabled by modular manufacturing technologies such as single-use bioreactors, microfluidics, and in-line analytical capabilities represents a potential for democratising the design of future RNA medicines.

3.3 Major bottlenecks for the near future

The acceleration of mRNA therapeutics has been remarkable due to COVID-19 with Moderna entering a clinical trial 63 days after the SARS-CoV-2 genome sequences were published[89]. Despite the rapid developmental process, companies have experienced challenges in the mass production of vaccines to meet global demand. In this context, providing an ample supply of lipids that have the leading role in delivering mRNA into the body is challenging. For example, the Pfizer-BioNTech vaccine needs almost 1.5 tonnes of total lipid for 1 billion people (2 billion vaccine doses), representing a significant increase in lipid mass production to meet global vaccine demand. In particular, proprietary ionisable cationic and PEG lipids have complex synthetic steps and need specialised manufacturing expertise. Globally, there are few places to produce lipids and Pfizer had a lipid-supply agreement for 5-years with Croda, and BioNTech agreed with Evonik and Merck KGaA while Corden Pharma supplies lipids to Moderna [90]. mRNA production is another major hurdle; for example, Pfizer-BioNTech, Moderna and CureVac vaccines need 60kg, 200 kg, 24 kg mRNA, respectively, to vaccinate 1 billion people. A study about the techno-economic feasibility of producing mRNA vaccines showed that annual manufacturing amounts of vaccines are substantially dependent on RNA dose and they gave a striking example: the time to produce 8 billion vaccine doses can be decreased from 2.6 months to 8 days by reducing the dose from 1 μg to 0.1 μg. This dose reduction can be achieved with saRNA vaccines that can provide immune responses with a lower dose than mRNA dose.

CONCLUSION

The modularity of RNA drugs enabled rapid adaptation of the technology to develop vaccines to combat the COVID-19 pandemic. This modularity will likewise bring about the RNA revolution in the biopharmaceutical industry, as vaccine advancements are applied to accelerate the development of other RNA-enabled gene and cell therapies. Publications and clinical trial registrations rose sharply in 2021, creating demand for RNA DS and LNP DP manufacturing already strained to supply SARS-CoV-2 vaccines globally. With these and future manufacturing challenges come opportunities for innovations and new technologies.

Technologies such as saRNA reduce dosing by up to 100-fold, thus expanding the manufacturing capacity of existing infrastructure, and allowing new infrastructure to be built at smaller scales. Smaller-scale production is conducive to integrating DP manufacturing, DS formulation and fill/finish activities into a single suite, in contrast to the currently fragmented model requiring shipment of bulk intermediates between states and countries. Additionally, microfluidic technology, already established for preclinical development of RNA-LNPs, has recently seen the launch of commercially available next-generation mixers allowing throughput from a single mixer to be scaled to suit all stages of drug development.

The trend towards integrating manufacturing steps aligns with announcements from numerous countries to acquire domestic RNA drug manufacturing capacity in response to shortages of SARS-CoV-2 vaccines being manufactured only in a few jurisdictions. These facilities can produce the drugs the local population needs and allow vaccines targeting regional variants to be rapidly developed and produced in pandemic situations. Economies of scale from these smaller facilities can be achieved through a multiproduct manufacturing model, where sequences for various RNA DSs for gene silencing, protein replacement, vaccines, and RNA engineered cell therapies are stored digitally, and manufactured on demand. Such a model would also improve the economics of producing drugs for rare diseases, representing an essential step towards realising a future with truly individualised RNA medicines.

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Supporting Information.

Supplementary Table 1: Examination of mRNA vaccine studies against various infectious.

Supplementary Table 2: Clinical trials of RNA medicines and RNA-transfected cell therapies for (A) disease condition, (B) the modality used for the indicated condition.

Supplementary Table 3: Dosing requirements for mRNA-1273 and BNT162b1/2.

Supplementary Table 4: A question of potency: A direct head-to-head comparative study mRNA and saRNA with the same formulation.

Supplementary Table 5: Details of COVID-19 mRNA vaccine manufacturing sites.

Supplementary Table 6: Details of Additional mRNA Manufacturing Sites Announced Globally.

REFERENCES

1. Geall, A. J.; Verma, A.; Otten, G. R.; Shaw, C. A.; Hekele, A.; Banerjee, K.; Cu, Y.; Beard, C. W.; Brito, L. A.; Krucker, T.; O'Hagan, D. T.; Singh, M.; Mason, P. W.; Valiante, N. M.; Dormitzer, P. R.; Barnett, S. W.; Rappuoli, R.; Ulmer, J. B.; Mandl, C. W., Nonviral delivery of self-amplifying RNA vaccines. *Proceedings of the National Academy of Sciences* **2012,** *109* (36), 14604.
2. Whitehead, K. A.; Dahlman, J. E.; Langer, R. S.; Anderson, D. G., Silencing or stimulation? siRNA delivery and the immune system. *Annu Rev Chem Biomol Eng* **2011,** *2*, 77-96.
3. Lutz, J.; Lazzaro, S.; Habbeddine, M.; Schmidt, K. E.; Baumhof, P.; Mui, B. L.; Tam, Y. K.; Madden, T. D.; Hope, M. J.; Heidenreich, R.; Fotin-Mleczek, M., Unmodified mRNA in LNPs constitutes a competitive technology for prophylactic vaccines. *NPJ Vaccines* **2017,** *2*, 29.
4. Evers, M.; Kulkarni, J.; van der Meel, R.; Cullis, P.; Vader, P.; Schiffelers, R., State-of-the-Art Design and Rapid-Mixing Production Techniques of Lipid Nanoparticles for Nucleic Acid Delivery. *Small Methods* **2017,** *2*.
5. Pardi, N.; Tuyishime, S.; Muramatsu, H.; Kariko, K.; Mui, B. L.; Tam, Y. K.; Madden, T. D.; Hope, M. J.; Weissman, D., Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *Journal of Controlled Release* **2015,** *217*, 345-351.
6. Vogel, A. B.; Kanevsky, I.; Che, Y.; Swanson, K. A.; Muik, A.; Vormehr, M.; Kranz, L. M.; Walzer, K. C.; Hein, S.; Guler, A.; Loschko, J.; Maddur, M. S.; Ota-Setlik, A.; Tompkins, K.; Cole, J.; Lui, B. G.; Ziegenhals, T.; Plaschke, A.; Eisel, D.; Dany, S. C.; Fesser, S.; Erbar, S.; Bates, F.; Schneider, D.; Jesionek, B.; Sanger, B.; Wallisch, A. K.; Feuchter, Y.; Junginger, H.; Krumm, S. A.; Heinen, A. P.; Adams-Quack, P.; Schlereth, J.; Schille, S.; Kroner, C.; de la Caridad Guimil Garcia, R.; Hiller, T.; Fischer, L.; Sellers, R. S.; Choudhary, S.; Gonzalez, O.; Vascotto, F.; Gutman, M. R.; Fontenot, J. A.; Hall-Ursone, S.; Brasky, K.; Griffor, M. C.; Han, S.; Su, A. A. H.; Lees, J. A.; Nedoma, N. L.; Mashalidis, E. H.; Sahasrabudhe, P. V.; Tan, C. Y.; Pavliakova, D.; Singh, G.; Fontes-Garfias, C.; Pride, M.; Scully, I. L.; Ciolino, T.; Obregon, J.; Gazi, M.; Carrion, R., Jr.; Alfson, K. J.; Kalina, W. V.; Kaushal, D.; Shi, P. Y.; Klamp, T.; Rosenbaum, C.; Kuhn, A. N.; Tureci, O.; Dormitzer, P. R.; Jansen, K. U.; Sahin, U., BNT162b vaccines protect rhesus macaques from SARS-CoV-2. *Nature* **2021,** *592* (7853), 283-289.
7. Corbett, K. S.; Edwards, D. K.; Leist, S. R.; Abiona, O. M.; Boyoglu-Barnum, S.; Gillespie, R. A.; Himansu, S.; Schäfer, A.; Ziwawo, C. T.; DiPiazza, A. T.; Dinnon, K. H.; Elbashir, S. M.; Shaw, C. A.; Woods, A.; Fritch, E. J.; Martinez, D. R.; Bock, K. W.; Minai, M.; Nagata, B. M.; Hutchinson, G. B.; Wu, K.; Henry, C.; Bahl, K.; Garcia-Dominguez, D.; Ma, L.; Renzi, I.; Kong, W.-P.; Schmidt, S. D.; Wang, L.; Zhang, Y.; Phung, E.; Chang, L. A.; Loomis, R. J.; Altaras, N. E.; Narayanan, E.; Metkar, M.; Presnyak, V.; Liu, C.; Louder, M. K.; Shi, W.; Leung, K.; Yang, E. S.; West, A.; Gully, K. L.; Stevens, L. J.; Wang, N.; Wrapp, D.; Doria-Rose, N. A.; Stewart-Jones, G.; Bennett, H.; Alvarado, G. S.; Nason, M. C.; Ruckwardt, T. J.; McLellan, J. S.; Denison, M. R.; Chappell, J. D.; Moore, I. N.; Morabito, K. M.; Mascola, J. R.; Baric, R. S.; Carfi, A.; Graham, B. S., SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* **2020,** *586* (7830), 567-571.
8. He, Y.; Zhou, Y.; Liu, S.; Kou, Z.; Li, W.; Farzan, M.; Jiang, S., Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. *Biochem Biophys Res Commun* **2004,** *324* (2), 773-81.
9. Walsh, E. E.; Frenck, R. W., Jr.; Falsey, A. R.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Neuzil, K.; Mulligan, M. J.; Bailey, R.; Swanson, K. A.; Li, P.; Koury, K.; Kalina, W.; Cooper, D.; Fontes-Garfias, C.; Shi, P. Y.; Türeci, Ö.; Tompkins, K. R.; Lyke, K. E.; Raabe, V.; Dormitzer, P. R.; Jansen, K. U.; Şahin, U.; Gruber, W. C., Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N Engl J Med* **2020,** *383* (25), 2439-2450.
10. Moderna Safety and Immunogenicity Study of 2019-nCoV Vaccine (mRNA-1273) for Prophylaxis of SARS-CoV-2 Infection (COVID-19). https://clinicaltrials.gov/ct2/show/NCT04283461. Accessed: January 6, 2022.
11. Sedic, M.; Senn, J. J.; Lynn, A.; Laska, M.; Smith, M.; Platz, S. J.; Bolen, J.; Hoge, S.; Bulychev, A.; Jacquinet, E.; Bartlett, V.; Smith, P. F., Safety Evaluation of Lipid Nanoparticle-Formulated Modified mRNA in the Sprague-Dawley Rat and Cynomolgus Monkey. *Vet Pathol* **2018,** *55* (2), 341-354.
12. Sabnis, S.; Kumarasinghe, E. S.; Salerno, T.; Mihai, C.; Ketova, T.; Senn, J. J.; Lynn, A.; Bulychev, A.; McFadyen, I.; Chan, J.; Almarsson, Ö.; Stanton, M. G.; Benenato, K. E., A Novel Amino Lipid Series for

mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates. *Mol Ther* **2018,** *26* (6), 1509-1519.
13. Jiang, L.; Berraondo, P.; Jericó, D.; Guey, L. T.; Sampedro, A.; Frassetto, A.; Benenato, K. E.; Burke, K.; Santamaría, E.; Alegre, M.; Pejenaute, Á.; Kalariya, M.; Butcher, W.; Park, J.-S.; Zhu, X.; Sabnis, S.; Kumarasinghe, E. S.; Salerno, T.; Kenney, M.; Lukacs, C. M.; Ávila, M. A.; Martini, P. G. V.; Fontanellas, A., Systemic messenger RNA as an etiological treatment for acute intermittent porphyria. *Nature Medicine* **2018,** *24* (12), 1899-1909.
14. Zhu, X.; Yin, L.; Theisen, M.; Zhuo, J.; Siddiqui, S.; Levy, B.; Presnyak, V.; Frassetto, A.; Milton, J.; Salerno, T.; Benenato, K. E.; Milano, J.; Lynn, A.; Sabnis, S.; Burke, K.; Besin, G.; Lukacs, C. M.; Guey, L. T.; Finn, P. F.; Martini, P. G. V., Systemic mRNA Therapy for the Treatment of Fabry Disease: Preclinical Studies in Wild-Type Mice, Fabry Mouse Model, and Wild-Type Non-human Primates. *The American Journal of Human Genetics* **2019,** *104* (4), 625-637.
15. Yaghi, N. K.; Wei, J.; Hashimoto, Y.; Kong, L.-Y.; Gabrusiewicz, K.; Nduom, E. K.; Ling, X.; Huang, N.; Zhou, S.; Kerrigan, B. C. P.; Levine, J. M.; Fajt, V. R.; Levine, G.; Porter, B. F.; Marcusson, E. G.; Tachikawa, K.; Chivukula, P.; Webb, D. C.; Payne, J. E.; Heimberger, A. B., Immune modulatory nanoparticle therapeutics for intracerebral glioma. *Neuro-Oncology* **2017,** *19* (3), 372-382.
16. Novobrantseva, T. I.; Borodovsky, A.; Wong, J.; Klebanov, B.; Zafari, M.; Yucius, K.; Querbes, W.; Ge, P.; Ruda, V. M.; Milstein, S.; Speciner, L.; Duncan, R.; Barros, S.; Basha, G.; Cullis, P.; Akinc, A.; Donahoe, J. S.; Narayanannair Jayaprakash, K.; Jayaraman, M.; Bogorad, R. L.; Love, K.; Whitehead, K.; Levins, C.; Manoharan, M.; Swirski, F. K.; Weissleder, R.; Langer, R.; Anderson, D. G.; de Fougerolles, A.; Nahrendorf, M.; Koteliansky, V., Systemic RNAi-mediated Gene Silencing in Nonhuman Primate and Rodent Myeloid Cells. *Molecular Therapy - Nucleic Acids* **2012,** *1*.
17. Zimmermann, T. S.; Lee, A. C. H.; Akinc, A.; Bramlage, B.; Bumcrot, D.; Fedoruk, M. N.; Harborth, J.; Heyes, J. A.; Jeffs, L. B.; John, M.; Judge, A. D.; Lam, K.; McClintock, K.; Nechev, L. V.; Palmer, L. R.; Racie, T.; Röhl, I.; Seiffert, S.; Shanmugam, S.; Sood, V.; Soutschek, J.; Toudjarska, I.; Wheat, A. J.; Yaworski, E.; Zedalis, W.; Koteliansky, V.; Manoharan, M.; Vornlocher, H.-P.; MacLachlan, I., RNAi-mediated gene silencing in non-human primates. *Nature* **2006,** *441* (7089), 111-114.
18. FDA *Fact sheet for healthcare providers administering vaccine (vaccination providers) emergency use authoriszation (EUA) of the Moderna COVID-19 vaccine to prevent coronavirus disease 2019 (COVID-19)*; 2021.
19. FDA *Fact sheet for healthcare providers administering vaccine (vaccination providers) emergency use authoriszation (EUA) of the Pfizer-BioNTech COVID-19 vaccine to prevent coronavirus disease 2019 (COVID-19)*; 2021.
20. Sahin, U.; Muik, A.; Derhovanessian, E.; Vogler, I.; Kranz, L. M.; Vormehr, M.; Baum, A.; Pascal, K.; Quandt, J.; Maurus, D.; Brachtendorf, S.; Lörks, V.; Sikorski, J.; Hilker, R.; Becker, D.; Eller, A.-K.; Grützner, J.; Boesler, C.; Rosenbaum, C.; Kühnle, M.-C.; Luxemburger, U.; Kemmer-Brück, A.; Langer, D.; Bexon, M.; Bolte, S.; Karikó, K.; Palanche, T.; Fischer, B.; Schultz, A.; Shi, P.-Y.; Fontes-Garfias, C.; Perez, J. L.; Swanson, K. A.; Loschko, J.; Scully, I. L.; Cutler, M.; Kalina, W.; Kyratsous, C. A.; Cooper, D.; Dormitzer, P. R.; Jansen, K. U.; Türeci, Ö., COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature* **2020,** *586* (7830), 594-599.
21. Jackson, L. A.; Anderson, E. J.; Rouphael, N. G.; Roberts, P. C.; Makhene, M.; Coler, R. N.; McCullough, M. P.; Chappell, J. D.; Denison, M. R.; Stevens, L. J.; Pruijssers, A. J.; McDermott, A.; Flach, B.; Doria-Rose, N. A.; Corbett, K. S.; Morabito, K. M.; O'Dell, S.; Schmidt, S. D.; Swanson, P. A.; Padilla, M.; Mascola, J. R.; Neuzil, K. M.; Bennett, H.; Sun, W.; Peters, E.; Makowski, M.; Albert, J.; Cross, K.; Buchanan, W.; Pikaart-Tautges, R.; Ledgerwood, J. E.; Graham, B. S.; Beigel, J. H., An mRNA Vaccine against SARS-CoV-2 — Preliminary Report. *New England Journal of Medicine* **2020,** *383* (20), 1920-1931.
22. Mulligan, M. J.; Lyke, K. E.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Neuzil, K.; Raabe, V.; Bailey, R.; Swanson, K. A.; Li, P.; Koury, K.; Kalina, W.; Cooper, D.; Fontes-Garfias, C.; Shi, P.-Y.; Türeci, Ö.; Tompkins, K. R.; Walsh, E. E.; Frenck, R.; Falsey, A. R.; Dormitzer, P. R.; Gruber, W. C.; Şahin, U.; Jansen, K. U., Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* **2020,** *586* (7830), 589-593.
23. Caruthers, M. H. J. B. S. T., A brief review of DNA and RNA chemical synthesis. **2011,** *39* (2), 575-580.
24. Scaringe, S. A.; Francklyn, C.; Usman, N. J. N. a. r., Chemical synthesis of biologically active oligoribonucleotides using β-cyanoethyl protected ribonucleoside phosphoramidites. **1990,** *18* (18), 5433-5441.
25. Usman, N.; Cedergren, R. J. T. i. b. s., Exploiting the chemical synthesis of RNA. **1992,** *17* (9), 334-339.

26. Kershaw, C. J.; O'Keefe, R. T., Splint ligation of RNA with T4 DNA ligase. In *Recombinant and In Vitro RNA Synthesis*, Springer: 2013; pp 257-269.
27. Ponchon, L.; Beauvais, G.; Nonin-Lecomte, S.; Dardel, F. J. N. p., A generic protocol for the expression and purification of recombinant RNA in Escherichia coli using a tRNA scaffold. **2009,** *4* (6), 947-959.
28. Ponchon, L.; Dardel, F. J. M., Large scale expression and purification of recombinant RNA in Escherichia coli. **2011,** *54* (2), 267-273.
29. Ponchon, L.; Dardel, F. J. N. m., Recombinant RNA technology: the tRNA scaffold. **2007,** *4* (7), 571-576.
30. Litke, J. L.; Jaffrey, S. R. J. N. b., Highly efficient expression of circular RNA aptamers in cells using autocatalytic transcripts. **2019,** *37* (6), 667-675.
31. Stepanov, V. G.; Fox, G. E., In Vivo Production of Small Recombinant RNAs Embedded in 5S rRNA-Derived Protective Scaffold. In *RNA Scaffolds*, Springer: 2021; pp 75-97.
32. Cozens, C.; Pinheiro, V. B.; Vaisman, A.; Woodgate, R.; Holliger, P. J. P. o. t. N. A. o. S., A short adaptive path from DNA to RNA polymerases. **2012,** *109* (21), 8067-8072.
33. Chen, T.; Romesberg, F. E. J. J. o. t. A. C. S., Polymerase chain transcription: exponential synthesis of RNA and modified RNA. **2017,** *139* (29), 9949-9954.
34. Krieg, P. A.; Melton, D. J. M. i. e., [25] In vitro RNA synthesis with SP6 RNA polymerase. **1987,** *155*, 397-415.
35. Pardi, N.; Muramatsu, H.; Weissman, D.; Karikó, K. J. S. m. R.; modulation, c. m., In vitro transcription of long RNA containing modified nucleosides. **2013**, 29-42.
36. Ibach, J.; Dietrich, L.; Koopmans, K. R.; Nöbel, N.; Skoupi, M.; Brakmann, S. J. J. o. b., Identification of a T7 RNA polymerase variant that permits the enzymatic synthesis of fully 2′-O-methyl-modified RNA. **2013,** *167* (3), 287-295.
37. Beckert, B.; Masquida, B., Synthesis of RNA by in vitro transcription. In *Rna*, Springer: 2011; pp 29-41.
38. Pleiss, J. A.; Derrick, M. L.; Uhlenbeck, O. C. J. R., T7 RNA polymerase produces 5′ end heterogeneity during in vitro transcription from certain templates. **1998,** *4* (10), 1313-1317.
39. Rosa, S. S.; Prazeres, D. M.; Azevedo, A. M.; Marques, M. P. J. V., mRNA vaccines manufacturing: Challenges and bottlenecks. **2021**.
40. Aldevron Aldevron's Second GMP Manufacturing Facility Buildout Reaches Major Milestone.
41. TrackVaccines Vaccination rates, approvals and trials by country V.
42. GenScriptProBio China's Largest Commercial GMP Plasmid Manufacturing Facility is Put into Operation, GenScript ProBio Expanding Manufacturing Capacity Again.
43. Cathala, G.; SAVOURET, J.-F.; Mendez, B.; West, B. L.; Karin, M.; Martial, J. A.; Baxter, J. D. J. D., A method for isolation of intact, translationally active ribonucleic acid. **1983,** *2* (4), 329-335.
44. Lukavsky, P. J.; Puglisi, J. D. J. R., Large-scale preparation and purification of polyacrylamide-free RNA oligonucleotides. **2004,** *10* (5), 889-893.
45. Kim, I.; Mckenna, S. A.; Puglisi, E. V.; Puglisi, J. D. J. R., Rapid purification of RNAs using fast performance liquid chromatography (FPLC). **2007,** *13* (2), 289-294.
46. Edelmann, F. T.; Niedner, A.; Niessing, D. J. M., Production of pure and functional RNA for in vitro reconstitution experiments. **2014,** *65* (3), 333-341.
47. Weissman, D.; Pardi, N.; Muramatsu, H.; Karikó, K., HPLC purification of in vitro transcribed long RNA. In *Synthetic messenger RNA and cell metabolism modulation*, Springer: 2013; pp 43-54.
48. Heartlein, M.; DeRosa, F.; Dias, A.; Karve, S., Methods for purification of messenger RNA. Google Patents: 2018.
49. Semple, S. C.; Klimuk, S. K.; Harasym, T. O.; Dos Santos, N.; Ansell, S. M.; Wong, K. F.; Maurer, N.; Stark, H.; Cullis, P. R.; Hope, M. J.; Scherrer, P., Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionizable aminolipids: formation of novel small multilamellar vesicle structures. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2001,** *1510* (1), 152-166.
50. Jeffs, L. B.; Palmer, L. R.; Ambegia, E. G.; Giesbrecht, C.; Ewanick, S.; MacLachlan, I., A Scalable, Extrusion-Free Method for Efficient Liposomal Encapsulation of Plasmid DNA. *Pharmaceutical Research* **2005,** *22* (3), 362-372.
51. Cullis, P. R.; Hope, M. J., Lipid Nanoparticle Systems for Enabling Gene Therapies. *Molecular Therapy* **2017,** *25* (7), 1467-1475.
52. Semple, S. C.; Akinc, A.; Chen, J.; Sandhu, A. P.; Mui, B. L.; Cho, C. K.; Sah, D. W. Y.; Stebbing, D.; Crosley, E. J.; Yaworski, E.; Hafez, I. M.; Dorkin, J. R.; Qin, J.; Lam, K.; Rajeev, K. G.; Wong, K. F.; Jeffs, L.

B.; Nechev, L.; Eisenhardt, M. L.; Jayaraman, M.; Kazem, M.; Maier, M. A.; Srinivasulu, M.; Weinstein, M. J.; Chen, Q.; Alvarez, R.; Barros, S. A.; De, S.; Klimuk, S. K.; Borland, T.; Kosovrasti, V.; Cantley, W. L.; Tam, Y. K.; Manoharan, M.; Ciufolini, M. A.; Tracy, M. A.; de Fougerolles, A.; MacLachlan, I.; Cullis, P. R.; Madden, T. D.; Hope, M. J., Rational design of cationic lipids for siRNA delivery. *Nature Biotechnology* **2010,** *28* (2), 172-176.
53. Maclachlan, I., Liposomal formulations for nucleic acid delivery. 2007; pp 237-270.
54. Roces, C. B.; Lou, G.; Jain, N.; Abraham, S.; Thomas, A.; Halbert, G. W.; Perrie, Y., Manufacturing Considerations for the Development of Lipid Nanoparticles Using Microfluidics. *Pharmaceutics* **2020,** *12* (11).
55. Ramishetti, S.; Hazan-Halevy, I.; Palakuri, R.; Chatterjee, S.; Naidu Gonna, S.; Dammes, N.; Freilich, I.; Kolik Shmuel, L.; Danino, D.; Peer, D., A Combinatorial Library of Lipid Nanoparticles for RNA Delivery to Leukocytes. *Advanced Materials* **2020,** *32* (12), 1906128.
56. Mohanty, S.; Jyotsana, N.; Sharma, A.; Kloos, A.; Gabdoulline, R.; Othman, B.; Lai, C. K.; Schottmann, R.; Mandhania, M.; Schmoellerl, J.; Grebien, F.; Ramsay, E.; Thomas, A.; Vornlocher, H.-P.; Ganser, A.; Thol, F.; Heuser, M., Targeted Inhibition of the NUP98-NSD1 Fusion Oncogene in Acute Myeloid Leukemia. *Cancers* **2020,** *12* (10).
57. Nabhan, J. F.; Wood, K. M.; Rao, V. P.; Morin, J.; Bhamidipaty, S.; LaBranche, T. P.; Gooch, R. L.; Bozal, F.; Bulawa, C. E.; Guild, B. C., Intrathecal delivery of frataxin mRNA encapsulated in lipid nanoparticles to dorsal root ganglia as a potential therapeutic for Friedreich's ataxia. *Scientific Reports* **2016,** *6* (1), 20019.
58. An, D.; Schneller, J. L.; Frassetto, A.; Liang, S.; Zhu, X.; Park, J.-S.; Theisen, M.; Hong, S.-J.; Zhou, J.; Rajendran, R.; Levy, B.; Howell, R.; Besin, G.; Presnyak, V.; Sabnis, S.; Murphy-Benenato, K. E.; Kumarasinghe, E. S.; Salerno, T.; Mihai, C.; Lukacs, C. M.; Chandler, R. J.; Guey, L. T.; Venditti, C. P.; Martini, P. G. V., Systemic Messenger RNA Therapy as a Treatment for Methylmalonic Acidemia. *Cell Reports* **2017,** *21* (12), 3548-3558.
59. Ramaswamy, S.; Tonnu, N.; Tachikawa, K.; Limphong, P.; Vega, J. B.; Karmali, P. P.; Chivukula, P.; Verma, I. M., Systemic delivery of factor IX messenger RNA for protein replacement therapy. *Proceedings of the National Academy of Sciences* **2017,** *114* (10), E1941.
60. Miller, J. B.; Zhang, S.; Kos, P.; Xiong, H.; Zhou, K.; Perelman, S. S.; Zhu, H.; Siegwart, D. J., Non-Viral CRISPR/Cas Gene Editing In Vitro and In Vivo Enabled by Synthetic Nanoparticle Co-Delivery of Cas9 mRNA and sgRNA. *Angewandte Chemie International Edition* **2017,** *56* (4), 1059-1063.
61. Finn, J. D.; Smith, A. R.; Patel, M. C.; Shaw, L.; Youniss, M. R.; van Heteren, J.; Dirstine, T.; Ciullo, C.; Lescarbeau, R.; Seitzer, J.; Shah, R. R.; Shah, A.; Ling, D.; Growe, J.; Pink, M.; Rohde, E.; Wood, K. M.; Salomon, W. E.; Harrington, W. F.; Dombrowski, C.; Strapps, W. R.; Chang, Y.; Morrissey, D. V., A Single Administration of CRISPR/Cas9 Lipid Nanoparticles Achieves Robust and Persistent In Vivo Genome Editing. *Cell Reports* **2018,** *22* (9), 2227-2235.
62. Rosenblum, D.; Gutkin, A.; Kedmi, R.; Ramishetti, S.; Veiga, N.; Jacobi, A. M.; Schubert, M. S.; Friedmann-Morvinski, D.; Cohen, Z. R.; Behlke, M. A.; Lieberman, J.; Peer, D., CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. *Science Advances* **2020,** *6* (47), eabc9450.
63. Bahl, K.; Senn, J. J.; Yuzhakov, O.; Bulychev, A.; Brito, L. A.; Hassett, K. J.; Laska, M. E.; Smith, M.; Almarsson, Ö.; Thompson, J.; Ribeiro, A.; Watson, M.; Zaks, T.; Ciaramella, G., Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. *Molecular Therapy* **2017,** *25* (6), 1316-1327.
64. Patel, S.; Ashwanikumar, N.; Robinson, E.; DuRoss, A.; Sun, C.; Murphy-Benenato, K. E.; Mihai, C.; Almarsson, Ö.; Sahay, G., Boosting Intracellular Delivery of Lipid Nanoparticle-Encapsulated mRNA. *Nano Letters* **2017,** *17* (9), 5711-5718.
65. Richner, J. M.; Jagger, B. W.; Shan, C.; Fontes, C. R.; Dowd, K. A.; Cao, B.; Himansu, S.; Caine, E. A.; Nunes, B. T. D.; Medeiros, D. B. A.; Muruato, A. E.; Foreman, B. M.; Luo, H.; Wang, T.; Barrett, A. D.; Weaver, S. C.; Vasconcelos, P. F. C.; Rossi, S. L.; Ciaramella, G.; Mysorekar, I. U.; Pierson, T. C.; Shi, P.-Y.; Diamond, M. S., Vaccine Mediated Protection Against Zika Virus-Induced Congenital Disease. *Cell* **2017,** *170* (2), 273-283.e12.
66. Hassett, K. J.; Benenato, K. E.; Jacquinet, E.; Lee, A.; Woods, A.; Yuzhakov, O.; Himansu, S.; Deterling, J.; Geilich, B. M.; Ketova, T.; Mihai, C.; Lynn, A.; McFadyen, I.; Moore, M. J.; Senn, J. J.; Stanton, M. G.; Almarsson, Ö.; Ciaramella, G.; Brito, L. A., Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. *Mol Ther Nucleic Acids* **2019,** *15*, 1-11.

67. Thomas, A.; M. Garg, S.; De Souza, R. A. G.; Ouellet, E.; Tharmarajah, G.; Reichert, D.; Ordobadi, M.; Ip, S.; Ramsay, E. C., Microfluidic Production and Application of Lipid Nanoparticles for Nucleic Acid Transfection. In *Multiple Myeloma: Methods and Protocols*, Heuck, C.; Weinhold, N., Eds. Springer New York: New York, NY, 2018; pp 193-203.
68. Forbes, N.; Hussain, M. T.; Briuglia, M. L.; Edwards, D. P.; Horst, J. H. t.; Szita, N.; Perrie, Y., Rapid and scale-independent microfluidic manufacture of liposomes entrapping protein incorporating in-line purification and at-line size monitoring. *International Journal of Pharmaceutics* **2019,** *556*, 68-81.
69. Karnik, R.; Gu, F.; Basto, P.; Cannizzaro, C.; Dean, L.; Kyei-Manu, W.; Langer, R.; Farokhzad, O. C., Microfluidic platform for controlled synthesis of polymeric nanoparticles. *Nano Lett* **2008,** *8* (9), 2906-12.
70. Webb, C.; Forbes, N.; Roces, C. B.; Anderluzzi, G.; Lou, G.; Abraham, S.; Ingalls, L.; Marshall, K.; Leaver, T. J.; Watts, J. A.; Aylott, J. W.; Perrie, Y., Using microfluidics for scalable manufacturing of nanomedicines from bench to GMP: A case study using protein-loaded liposomes. *International Journal of Pharmaceutics* **2020,** *582*, 119266.
71. Belliveau, N. M.; Huft, J.; Lin, P. J.; Chen, S.; Leung, A. K.; Leaver, T. J.; Wild, A. W.; Lee, J. B.; Taylor, R. J.; Tam, Y. K.; Hansen, C. L.; Cullis, P. R., Microfluidic Synthesis of Highly Potent Limit-size Lipid Nanoparticles for In Vivo Delivery of siRNA. *Mol Ther Nucleic Acids* **2012,** *1* (8), e37.
72. Shepherd, S. J.; Warzecha, C. C.; Yadavali, S.; El-Mayta, R.; Alameh, M. G.; Wang, L.; Weissman, D.; Wilson, J. M.; Issadore, D.; Mitchell, M. J., Scalable mRNA and siRNA Lipid Nanoparticle Production Using a Parallelized Microfluidic Device. *Nano Lett* **2021,** *21* (13), 5671-5680.
73. Lou, G.; Anderluzzi, G.; Schmidt, S. T.; Woods, S.; Gallorini, S.; Brazzoli, M.; Giusti, F.; Ferlenghi, I.; Johnson, R. N.; Roberts, C. W.; O'Hagan, D. T.; Baudner, B. C.; Perrie, Y., Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: The impact of cationic lipid selection. *Journal of Controlled Release* **2020,** *325*, 370-379.
74. Fleck, D.; Phu, L.; Verschueren, E.; Hinkle, T.; Reichelt, M.; Bhangale, T.; Haley, B.; Wang, Y.; Graham, R.; Kirkpatrick, D. S.; Sheng, M.; Bingol, B., PTCD1 Is Required for Mitochondrial Oxidative-Phosphorylation: Possible Genetic Association with Alzheimer's Disease. *The Journal of Neuroscience* **2019,** *39* (24), 4636.
75. Liang, F.; Lindgren, G.; Lin, A.; Thompson, E. A.; Ols, S.; Rohss, J.; John, S.; Hassett, K.; Yuzhakov, O.; Bahl, K.; Brito, L. A.; Salter, H.; Ciaramella, G.; Lore, K., Efficient Targeting and Activation of Antigen-Presenting Cells In Vivo after Modified mRNA Vaccine Administration in Rhesus Macaques. *Mol Ther* **2017,** *25* (12), 2635-2647.
76. Corbett, K. S.; Flynn, B.; Foulds, K. E.; Francica, J. R.; Boyoglu-Barnum, S.; Werner, A. P.; Flach, B.; O'Connell, S.; Bock, K. W.; Minai, M.; Nagata, B. M.; Andersen, H.; Martinez, D. R.; Noe, A. T.; Douek, N.; Donaldson, M. M.; Nji, N. N.; Alvarado, G. S.; Edwards, D. K.; Flebbe, D. R.; Lamb, E.; Doria-Rose, N. A.; Lin, B. C.; Louder, M. K.; O'Dell, S.; Schmidt, S. D.; Phung, E.; Chang, L. A.; Yap, C.; Todd, J. M.; Pessaint, L.; Van Ry, A.; Browne, S.; Greenhouse, J.; Putman-Taylor, T.; Strasbaugh, A.; Campbell, T. A.; Cook, A.; Dodson, A.; Steingrebe, K.; Shi, W.; Zhang, Y.; Abiona, O. M.; Wang, L.; Pegu, A.; Yang, E. S.; Leung, K.; Zhou, T.; Teng, I. T.; Widge, A.; Gordon, I.; Novik, L.; Gillespie, R. A.; Loomis, R. J.; Moliva, J. I.; Stewart-Jones, G.; Himansu, S.; Kong, W. P.; Nason, M. C.; Morabito, K. M.; Ruckwardt, T. J.; Ledgerwood, J. E.; Gaudinski, M. R.; Kwong, P. D.; Mascola, J. R.; Carfi, A.; Lewis, M. G.; Baric, R. S.; McDermott, A.; Moore, I. N.; Sullivan, N. J.; Roederer, M.; Seder, R. A.; Graham, B. S., Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. *N Engl J Med* **2020,** *383* (16), 1544-1555.
77. Hassett, K. J.; Benenato, K. E.; Jacquinet, E.; Lee, A.; Woods, A.; Yuzhakov, O.; Himansu, S.; Deterling, J.; Geilich, B. M.; Ketova, T.; Mihai, C.; Lynn, A.; McFadyen, I.; Moore, M. J.; Senn, J. J.; Stanton, M. G.; Almarsson, O.; Ciaramella, G.; Brito, L. A., Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. *Mol Ther Nucleic Acids* **2019,** *15*, 1-11.
78. Maier, M. A.; Jayaraman, M.; Matsuda, S.; Liu, J.; Barros, S.; Querbes, W.; Tam, Y. K.; Ansell, S. M.; Kumar, V.; Qin, J.; Zhang, X.; Wang, Q.; Panesar, S.; Hutabarat, R.; Carioto, M.; Hettinger, J.; Kandasamy, P.; Butler, D.; Rajeev, K. G.; Pang, B.; Charisse, K.; Fitzgerald, K.; Mui, B. L.; Du, X.; Cullis, P.; Madden, T. D.; Hope, M. J.; Manoharan, M.; Akinc, A., Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Mol Ther* **2013,** *21* (8), 1570-8.
79. Herweijer, H.; Wolff, J. A., Self-amplifying vectors for gene delivery. *Advanced Drug Delivery Reviews* **1997,** *27* (1), 5-16.

80. Vogel, A. B.; Lambert, L.; Kinnear, E.; Busse, D.; Erbar, S.; Reuter, K. C.; Wicke, L.; Perkovic, M.; Beissert, T.; Haas, H.; Reece, S. T.; Sahin, U.; Tregoning, J. S., Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses. *Molecular Therapy* **2018,** *26* (2), 446-455.
81. Brito, L. A.; Chan, M.; Shaw, C. A.; Hekele, A.; Carsillo, T.; Schaefer, M.; Archer, J.; Seubert, A.; Otten, G. R.; Beard, C. W.; Dey, A. K.; Lilja, A.; Valiante, N. M.; Mason, P. W.; Mandl, C. W.; Barnett, S. W.; Dormitzer, P. R.; Ulmer, J. B.; Singh, M.; O'Hagan, D. T.; Geall, A. J., A Cationic Nanoemulsion for the Delivery of Next-generation RNA Vaccines. *Molecular Therapy* **2014,** *22* (12), 2118-2129.
82. McKay, P. F.; Hu, K.; Blakney, A. K.; Samnuan, K.; Brown, J. C.; Penn, R.; Zhou, J.; Bouton, C. R.; Rogers, P.; Polra, K.; Lin, P. J. C.; Barbosa, C.; Tam, Y. K.; Barclay, W. S.; Shattock, R. J., Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. *Nature Communications* **2020,** *11* (1), 3523.
83. Pollock, K.; Cheeseman, H.; Szubert, A.; Libri, V.; Boffito, M.; Owen, D.; Bern, H.; O'Hara, J.; McFarlane, L.; Lemm, N.-M.; McKay, P.; Rampling, T.; Yim, Y. T.; Milinkovic, A.; Kingsley, C.; Cole, T.; Fagerbrink, S.; Aban, M.; Tanaka, M.; Mehdipour, S.; Robbins, A.; Budd, W.; Faust, S.; Hassanin, H.; Cosgrove, C.; Winston, A.; Fidler, S.; Dunn, D.; McCormack, S.; Shattock, R. J., Safety and Immunogenicity of a Self-Amplifying RNA Vaccine Against COVID-19: COVAC1, a Phase I, Dose-Ranging Trial. SSRN: 2021.
84. Vabret, N., Preclinical data from SARS-CoV-2 mRNA vaccine. *Nature Reviews Immunology* **2020,** *20* (8), 461-461.
85. Vogel, A. B.; Kanevsky, I.; Che, Y.; Swanson, K. A.; Muik, A.; Vormehr, M.; Kranz, L. M.; Walzer, K. C.; Hein, S.; Güler, A.; Loschko, J.; Maddur, M. S.; Tompkins, K.; Cole, J.; Lui, B. G.; Ziegenhals, T.; Plaschke, A.; Eisel, D.; Dany, S. C.; Fesser, S.; Erbar, S.; Bates, F.; Schneider, D.; Jesionek, B.; Sänger, B.; Wallisch, A.-K.; Feuchter, Y.; Junginger, H.; Krumm, S. A.; Heinen, A. P.; Adams-Quack, P.; Schlereth, J.; Kröner, C.; Hall-Ursone, S.; Brasky, K.; Griffor, M. C.; Han, S.; Lees, J. A.; Mashalidis, E. H.; Sahasrabudhe, P. V.; Tan, C. Y.; Pavliakova, D.; Singh, G.; Fontes-Garfias, C.; Pride, M.; Scully, I. L.; Ciolino, T.; Obregon, J.; Gazi, M.; Carrion, R.; Alfson, K. J.; Kalina, W. V.; Kaushal, D.; Shi, P.-Y.; Klamp, T.; Rosenbaum, C.; Kuhn, A. N.; Türeci, Ö.; Dormitzer, P. R.; Jansen, K. U.; Sahin, U., A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and prevents lung infection in non-human primates. **2020**.
86. Rauch, S.; Roth, N.; Schwendt, K.; Fotin-Mleczek, M.; Mueller, S. O.; Petsch, B., mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents. *bioRxiv* **2020**, 2020.10.23.351775.
87. Kis, Z.; Kontoravdi, C.; Dey, A. K.; Shattock, R.; Shah, N., Rapid development and deployment of high-volume vaccines for pandemic response. *Journal of Advanced Manufacturing and Processing* **2020,** *2* (3), e10060.
88. Liu, M., Taiwan and the foundry model. *Nature Electronics* **2021,** *4* (5), 318-320.
89. Kyriakidis, N. C.; López-Cortés, A.; González, E. V.; Grimaldos, A. B.; Prado, E. O., SARS-CoV-2 vaccines strategies: a comprehensive review of phase 3 candidates. *NPJ Vaccines* **2021,** *6* (1), 28.
90. Challener, C. A., Meeting the Demand for Lipid Excipients. *BioPharm International* **2021,** *34* (6), 18-20.

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