mRNA : Definition, Delivery & Applications

Guidelines

OZ BIOSCIENCES
The art of delivery systems

This application note will mainly focus on synthetic mRNA. The tools developed by OZ Biosciences to synthesize high quality mRNA encoding various of-the-shelf or on-demand custom genes and carrier for in-vitro, ex-vivo and in-vivo delivery will be reviewed.

www.ozbiosciences.com

RNA therapeutics is booming and the mRNA field is generating huge expectations. In such a way, microRNA (miRNA), small interfering RNA (siRNA), messenger RNA (mRNA), long non-coding RNA (lncRNA), self-amplifying RNA (sa RNA) and genome editing systems containing RNA components like guide RNA (gRNA), represent useful tools in research and clinics.

As represented in figure 1 below, qualities mRNA holding for best results (I) as well as vehicles needed to deliver the RNA (II) will be discussed. In part (III) of this application note, the broad potential and numerous applications of mRNA in both research labs and clinics will also be presented.

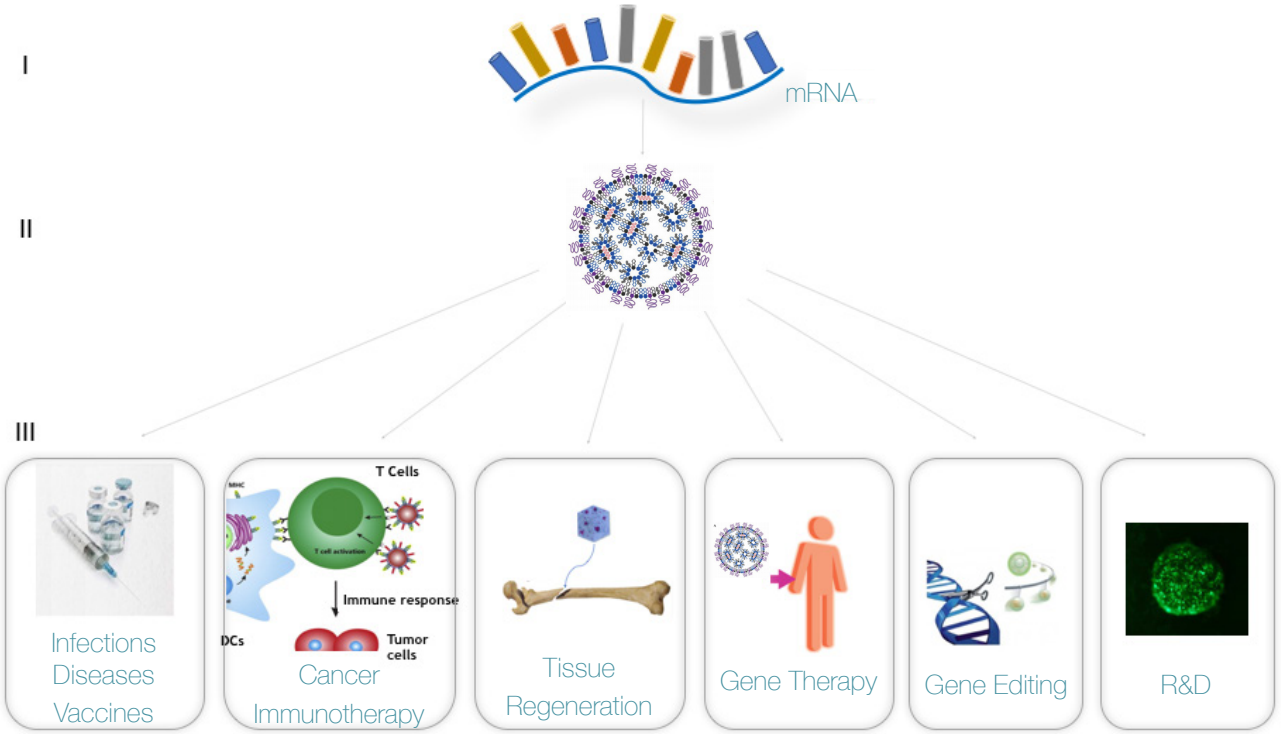


Figure 1 Scheme representing mRNA formulation into LNPs and their applications

The mRNA plays a key role in the 'central dogma' of molecular biology, which deals with the transfer of sequence information from DNA to RNA to protein.

OZ Biosciences provides a broad portfolio of mRNA:

- **OVA**
- **Spike SARS-Cov2**
- **mCherry**
- **F-Luc**
- **Tomato**
- **GFP**
- **CRE**
- **Cas9**
- **β-Gal**

RNAs STRUCTURE AND SYNTHESIS

Mature mRNA produced in higher eukaryotes is composed of an open reading frame (ORF) region that encodes the sequence to be translated, flanked by five-prime (5′) and three-prime (3′) untranslated region (UTR), and further stabilized by 5'cap and 3′ poly (A) tails. Indeed, pre-messenger RNAs (pre-mRNAs) must undergo several modifications to be protected from 5'-3' exonucleases, be transported from the nucleus to the cytoplasm, and translated. The first modification to occur is the addition of a guanosine nucleotide to the 5' terminal nucleotide with a particular 5'-5' linkage (capG). The inverted nucleotide is then methylated at the N7 position by an RNA guanine-N7 methyltransferase which forms the minimal cap structure (cap0) found in lower eukaryotes. The roles of the N7-methylation in stability, splicing, polyadenylation, mRNA export, and translation have been well defined and characterized for many years (Decroly E et al., 2011). Higher eukaryotes can further modify this minimal cap structure with the addition of a methyl group on the ribose 2'-O position of the first transcribed nucleotide (cap1) and sometimes at the adjoining nucleotide (cap2). Cap1 is central to the non-self-discrimination of innate immune response against foreign RNA (Daffis S et al., 2010). The poly (A) tail in 3' protects the mRNA molecule from enzymatic degradation in the cytoplasm and helps in transcription termination, export of the mRNA from the nucleus and translation.

RNA can be synthesized in vitro by the so-called in vitro transcription (IVT) technique (FIG2). Linearized plasmid DNA, PCR products or cDNA can be used as templates for transcription if they contain a double-stranded RNA polymerase promoter region in the correct orientation with consensus promoter sequences of different RNA Polymerases T7, T3 or SP6. RNA will be synthesized by action of a T7 polymerase. Please, note that Cap1 can be added during the IVT (Cap analog) or added enzymatically after initial IVT (Fig 2_1*). In a similar manner, poly(A) tail can be encoded in the DNA template or added by enzymatic method after IVT (Fig 2_2*). Enzymatic addition of the cap can be accomplished using guanylyl transferase and 2′-O-methyltransferase to yield a Cap 1 (N7MeGpppN2′-OMe) structure, while the poly-A tail can be obtained through enzymatic addition via poly-A polymerase. The common size of IVT mRNAs ranges between 500 to 5000 nucleotides, although smaller or larger RNAs can be synthesized upon protocol adaptation.

Most of mRNAs from OZ Biosciences are codon optimized. In fact, codon optimization is an option when designing custom RNA.

www.ozbiosciences.com

RNAs STRUCTURE AND SYNTHESIS

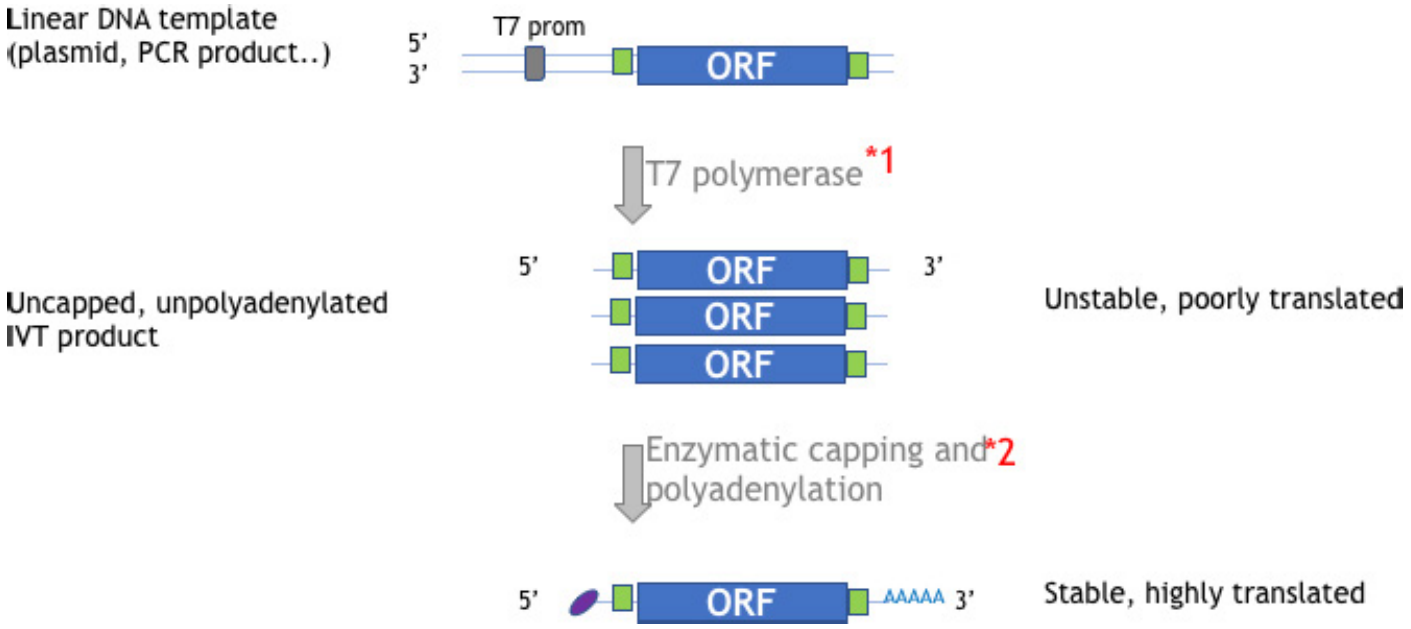


Figure 2 Scheme of synthetic RNA production by in vitro transcription

Due to the propensity of cells to limit the duration of expression of mRNAs, only a transient and low level of expression can be achieved in vivo, necessitating the administration of high doses of mRNA. Stability, durability and expression levels of mRNA-based vaccines have been achieved through the optimization of specific sequence elements and synthesis of self-replicating RNAs (saRNAs or Replicons). Indeed, gene expression upon RNA transfection in mammalian hosts can be significantly enhanced by substituting rare codons with more frequent ones (Levy JP et al., 1996). As a result, the strategy of using synonymous codons while maintaining the original protein sequence proved to be particularly successful and nowadays most of the DNA templates are codon optimized for a specific host.

While at this stage of RNA preparation, the molecules are fully mature, rather stable and translatable, mRNAs are still immunogenic. Altering the mRNA's base composition is an established method that consists in reducing immunogenicity for mRNA therapeutics. Since uridine-rich sequences of RNA trigger the innate immune response, depleting uridine triphosphate from the final mRNA sequence can improve evasion. Substituting a U-containing codon during sequence optimization for one without U will result in the same downstream protein while reducing the mRNA immunogenicity.

While mRNA from our catalog are either unmodified or 100% moU, custom mRNAs from OZ Biosciences can be synthesized with the modified nucleotides of your choice.

RNAs STRUCTURE AND SYNTHESIS

Similarly, modified nucleoside triphosphates (NTPs) such as pseudouridine triphosphate, thiouridine triphosphate, N1-methylpseudouridine triphosphate, and 5-methoxyuridine (moU) triphosphate can be substituted for the uridine triphosphate base to evade innate immune detection. Indeed, replacement of only 25% of uridine and cytidine with 2-thiouridine and 5-methyl-cytidine synergistically decreased mRNA binding to pattern recognition receptors, such as TLR3, TLR7, TLR8 and RIG-I, in human peripheral blood mononuclear cells (PBMCs) (Kormann M. S. D et al., 2011). In addition, double stranded RNA (dsRNA) impurities formed during transcription significantly contribute to the innate immune activity of mRNA therapeutics. Purification of IVT mRNA by means of high-performance liquid chromatography (HPLC), or alternative simplified methods using cellulose columns or selective digestion of dsRNA fragments using RNAse III have been established and proven to greatly reduce immune reaction (Kariko K et al., 2011) (Baiersdörfer M et al., 2019). At OZ Biosciences, our mRNA are synthesized by IVT using linearized DNA template and cap1 is added by enzymatic mean, while polyA is already encoded within the template, insuring a determined A length.

Development of self-amplifying RNAs (saRNAs) has allowed enhanced expression using a lower dose of injected RNA. As a matter of fact, saRNAs are longer (~10 kb) and encode factors to promote amplification of the RNA within the host cell. saRNAs hold a genetic replication machinery derived from positive-stranded mRNA viruses, most commonly from alphaviruses such as Sindbis and Semliki-Forest viruses. Generally, the ORF encoding viral structural proteins is replaced by the selected transcript of interest, and the viral RNA-dependent RNA polymerase is retained to direct cytoplasmic amplification of the replicon construct. As a result of their self-replicative activity, saRNAs can be delivered at lower concentrations than conventional mRNA vaccines to achieve a comparable antigen expression (Vogel Ab et al., 2018).

Although cells can uptake some naked RNAs and chemically modified RNAs, the use of naked RNA presents important limitations: nucleic acids have unfavorable pharmacokinetics and pharmacodynamics when administered without a delivery agent, which leads to low dose-response and toxicity. As a result, appropriate delivery methods are still essential for both enabling systemic applications of RNA therapeutics, and further maximizing their therapeutic window. mRNA transfection is rapidly emerging as a promising method for nucleic acid-based therapy and offers an attractive substitute to plasmid DNA.

Viral and non-viral mRNA delivery methods have already proven their efficiency in vitro and in vivo. Both of these methods consist in engineering safe and efficient nanocarriers that contain the desired exogenous nucleic acid to be administered systemically or locally to a patient.

OZ Biosciences revolutionizes Polyfection with the design and synthesis of patented Cationic **H**ydroxylated **A**mphiphilic **M**ulti-block **P**olymers (***CHAMP***) which are **biocompatible**, **cleavable**, **pH-responsive** and **bi-functional**.

Helix-IN

www.ozbiosciences.com

RNA DELIVERY VEHICLES

Viral and non-viral methods differ by their properties:

Viral vector

This approach is based on the use of modified viruses, essentially retroviruses, lentiviruses, adenoviruses and adeno-associated viruses (AAV), to carry into their own viral genome the desired nucleic acid sequence.

Genetically modified viruses (viral vectors) have been used to efficiently deliver nucleic acid into cell cytoplasm (Kay MA et al., 2001). Indeed, for a long time, the most effective way to deliver gene-based therapeutics to human cells was to use a virus that had been modified to carry medicinal cargo rather than harmful, self-replicating genes. This method is referred to as viral gene delivery. Continuous researches and development in virus engineering have propelled the number of viral vector-based Human clinical trials for rare genetic diseases or acquired single gene disorders and lead to EMA approved and marketed virus-based therapies Glybera (uniQure) and Strimvelis (Orchard Therapeutics). Three additional US FDA approved cell therapies retroviral vector-based Yescarta™ (Gilead) lentiviral-based Kymriah™ (Novartis) and AAV-2-based gene therapy Luxturna™ (Sparks Therapeutics) have reached the market since 2017. Nonetheless, due to their manufacturing costs, the biological risk associated to their interaction with the hosting cell and the potential hazards involved in their manipulation, researches focused on the development and optimization of synthetic alternatives to viruses for the delivery of nucleic acid. Over the last 20 years, non-viral gene delivery has become popular due to enhanced safety profiles, lower rates of adverse immunogenic reactions and ease of manufacturing.

Non-viral vector

This approah is based on the engineering of nanocarriers capable of interacting with nucleic acids in order to protect them from degradation and to condense them into nanosized complexes that can be more easily internalized by cells.
Non-viral gene delivery reagents are designed to execute 4 functions:

1. Reversibly bind nucleic acids
2. Mask their negative electrostatic charges
3. Favor the interaction with the cellular surface
4. Help the nucleic acid to follow the correct intracellular path to reach its target.

Promising delivery modalities are polyfection when using polymeric nanoparticles and lipofection when based on lipid systems.

Cationic polymers

Cationic polymers are attractive biomaterials for the complexation of nucleic acids, such as DNA and siRNAs (Mainini F et al., 2020) (Zhang P et al., 2017) . Polyethyleneimine (PEI) is one of the most studied, constructed from repeating units of amino functions attached to an aliphatic ethylene (-CH2CH2-) spacer, which is responsible for high levels of aqueous solubility and also for pH buffering capacity in the endosomal/lysosomal pathway.

OZ Biosciences's main expertise is the synthesis of patented cationic lipid-based transfection reagents.

Lullaby

DreamFect Gold

DreamFect

HYPE-293

HYPE-CHO

RmesFect

OZ Biosciences also offers a **custom LNPs** formulation service with nucleic acids or drug.

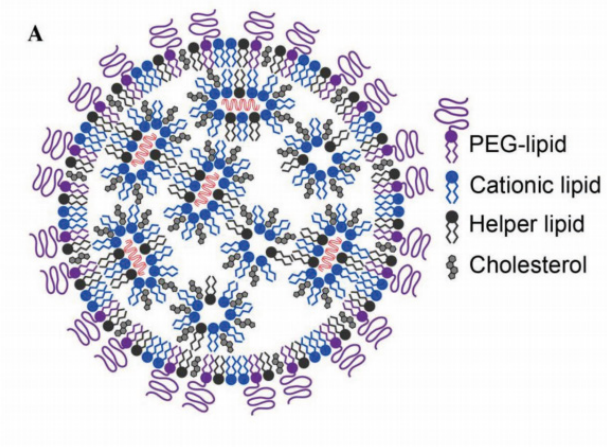


Figure 3. Scheme of LNP-containing mRNA from (Evers MJW et al., 2018)

RNA DELIVERY VEHICLES

Lipid systems

Lipid-based delivery systems include liposomes, micelles, nanoliposomes, nanoemulsions, nanostructured lipid carriers (NLCs) and lipid nanoparticles (LNPs). A number of liposomes are on the market, and many more are in the pipeline. Lipidic nanoparticles are the first nanomedicine carrier system to make the transition from concept to clinical application, and they are now an established technology platform with considerable clinical acceptance. We'll focus on LNPs as they are the most popular non-viral gene cargo system. Lipidic nanoparticles are the first nanomedicine delivery system to make the transition from concept to clinical application, and they are now an established technology platform with considerable clinical acceptance. We'll focus on LNPs as they are the most popular non-viral gene delivery system.

LNPs (Lipid nanoparticles) are liposome-like structures especially geared towards encapsulating a broad variety of nucleic acids (RNA and DNA); they are the most clinically advanced non-viral gene delivery system. While traditional liposomes include one or more lipid bilayers surrounding an aqueous core, only some LNPs present a contiguous bilayer that would qualify them as lipid vesicles or liposomes. Other LNPs adopt a micelle-like structure, encapsulating drug molecules in a non-aqueous core. The lipidic membrane can be positively, neutral or negatively charged, depending on the phospholipid composition. Generally, LNPs are composed of a complexing aminated lipid (either ionizable or cationic), a phospholipid helper, cholesterol, a poly (ethyleneglycol)-lipid conjugate and the therapeutic nucleic acid. Cationic lipids are usually amphiphilic molecules, comprising a positively-charged head group, generally based on amine groups, and a hydrocarbon chain or cholesterol derivate, attached via a linker (e.g. glycerol). The positively charged head group of the lipid can electrostatically interact with negatively charged nucleic acids and allow their entrapment in a lipid-based nanoparticle. Cationic lipids including 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), N-[1-(2,3-dioleoyloxy) Propyl]-N,N,N-trimethylammonium chloride (DOTMA) have been used in combination with neutral lipids such as cholesterol (Chol), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DSPE) to form LNPs.

PEGylated phospholipids are used in many lipid-based drug carriers primarily because they offer what is known as a stealth effect to the drug product as it circulates within the body.The human immune system is driven to protect the body from any foreign object, and medicinal nanoparticles are no exception. To promote delivery efficiency and to increase the circulation time for cargo molecules to reach intended diseases sites, PEG is added to shield these nanoparticles by preventing blood plasma proteins from absorbing into the liposome surface, increasing bloodstream circulation lifetime. To formulate these lipid-based nanovectors, we applied scalable manufacturing methods (microfluidic mixers or microfluidization). Microfluidic mixers are efficient systems based on mechanics of fluids, capable of producing synthetic particles with consistent size and biophysical properties. Resulting formulations are characterized in terms of hydrodynamic size (Z-average diameter, the intensity weighted mean hydrodynamic size), polydispersity index (PDI) and zeta potential (ZP) by dynamic light scattering (DLS) as well as nucleic acid entrapment loading. Until recently, the use of LNPs in registered clinical trials was mainly restricted to siRNA delivery. In 2021, mRNA formulation in LNPs is approved by FDA and administered in humans. The best examples are the mRNA vaccines for SARS-Cov2 that are already widely distributed (Moderna, or Pfizer/BioNtech vaccines).

RNA can be applied for a wide variety of purposes that range from R&D to the most recent human therapies.

3 categories of mRNAs are available on OZ Biosciences catalog:

- **Reporter gene mRNAs**: ideal controls to study transfection efficiency

- **Genome editing mRNAs**: Cas9 mRNA for CRISPR Genome editing

-**Vaccine/Antigen mRNAs**: optimal as controls for immunization or vaccine studies

RNA APPLICATIONS

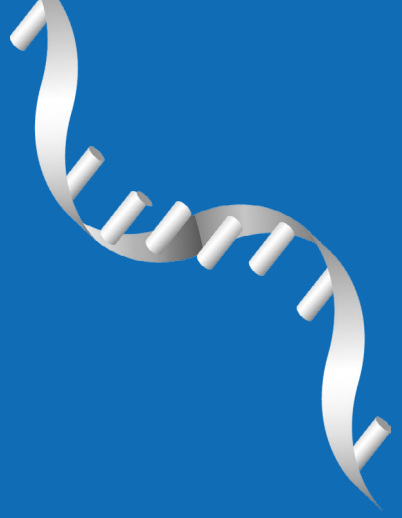
Vaccines

Vaccinology is shifting toward synthetic RNA platforms which allow for rapid, scalable, and cell-free manufacturing of prophylactic and therapeutic vaccines. The potential advantages of mRNA as a vaccine range from the discovery of immunogens to rapid response manufacturing. In contrast to plasmid DNA (pDNA) vaccines, mRNA does not need to be transported across the nuclear membrane and it can directly be translated in the cytosol, so that any potential genomic integration is avoided (Luo D et al., 2000). The core principle behind mRNA as a technology for vaccination is to deliver the transcript of interest, encoding one or more immunogen(s), into the host cell cytoplasm where expression generates translated protein(s) to be within the membrane, secreted or intracellularly located. Due to the propensity of cells to limit the duration of expression of mRNAs, only a transient and low level of expression can be achieved in vivo, necessitating the administration of high doses of mRNA. Notably, improvements in the stability, durability and expression levels of mRNA-based vaccines have been achieved through the optimization of specific sequence elements and use of Replicon-based vaccines (see section I). With the maturation of scale-up manufacturing, mRNA vaccines have supreme advantages over other vaccine techniques due to the rapid production and large-scale deployment. For both types of RNA, clinical batches can be generated within weeks after the availability of a sequence encoding the immunogen (Pardi N et al., 2018). There are currently two different types of synthetic RNA vaccines: conventional mRNA and self-amplifying RNA (saRNA). Use of conventional mRNA strategies (also referred to as non-replicating or non-amplifying mRNA) against infectious diseases and cancers has been investigated in several preclinical and clinical trials (Pardi N et al., 2018).The best examples are the mRNA vaccines for SARS-Cov2 that are already widely distributed (Moderna, or Pfizer/BioNtech vaccines).

Cancer Immunotherapies

The development and application of mRNA therapeutics for treatment of cancer have boomed in the past years. The generation of prophylactic vaccines against hepatitis B virus (HBV) and human papillomavirus (HPV), which are causes of liver and cervical cancers respectively, have been successful because their circumvents three major challenges facing the development of therapeutic cancer vaccines:
- low immunogenicity;
- established disease burden;
- the immunosuppressive tumor microenvironment.

Those approaches explore the use of mRNA to reshape the tumor micro environment (TME) by delivering mRNA encoding stimulatory molecules, blockade of inhibitory molecules and immune checkpoint blockade to restore immunological fitness at the tumor site. The immunosuppressive TME is a major obstacle in cancer immunotherapy. Still, much of the work on therapeutic cancer vaccines has taken aim at tumor-associated antigens (TAAs), which are aberrantly expressed self-antigens (neoantigens). Neoantigens, which arise from mutated proteins in cancer cells, are truly cancer-specific and can be highly immunogenic, though the vast majority are unique to each patient's cancer and thus require the development of personalized therapies. As mRNA therapeutics have a flexible production process, this patient immunization can be done on a personalized level.



OZ Biosciences is involved in two EU research funding projects:

1- The cmRNA European project for which chemically modified mRNAs encoding bone healing proteins have to be delivered into cells (MSCs) in vitro or directly on site within a matrix (GAM).

2- A novel modified natural killer (NK) cell immunotherapy for the treatment of solid tumours.

www.ozbiosciences.com

RNA APPLICATIONS

Naked RNA or protected mRNA can be injected at various sites directly into the tumor, intramuscular (IM), intradermal (ID) and intravenous (IV) but also by inhalation. Preclinical models have demonstrated that the vaccine encoding tumor-specific antigens promotes an anti-tumor immunity and prevents multiple tumors, including melanoma, hepatocellular carcinoma, colorectal cancer, gastrointestinal cancer, and pancreatic adenocarcinoma. For instance, a single intratumoral dose of murine IL12 mRNA induced dose-dependent expression of the encoded protein in syngeneic tumor-bearing mice. Similarly, MEDI1191 (Moderna), an human IL12 RNA in phase I for treatment of solid tumors, induces robust, dose-dependent hIL12p70 protein production in all patient tumor-derived models tested, including 2 melanoma, 2 HNSCC PDX models, 7 colorectal and endometrial cancer patient tumor slice cultures (Hewitt SL et al., 2020). mRNA vaccine candidates have also been tested in human clinical trials using either whole tumor cell transcriptome to target metastatic melanoma, or specific TAAs to target metastatic melanoma and renal cell carcinoma, eliciting tumor antigen-specific antibody and T cell responses. More recently, trials targeting prostate and non-small cell lung cancer have shown mRNA vaccines to be safe, well tolerated and immunogenic.Chimeric antigen receptor (CAR)-T or -NK cells represent an exciting new opportunity in cancer treatment. In CAR-T or CAR-NK therapy, a patient's T or NK cells are genetically modified in a laboratory before being returned to the patient, where they kill targeted cancer cells. In a recent study published in Cancers, authors described a method using mRNA transfection to generate CAR-T cells targeting melanomas at the clinical scale, under full GMP compliance (Wiesinger M et al., 2019). Authors report an injectable nanocarrier that delivers in vitro-transcribed CAR or TCR mRNA for transiently reprograming of circulating T cells to recognize disease-relevant antigens. In mouse models of human leukemia, prostate cancer and hepatitis B-induced hepatocellular carcinoma. Repeated infusions of these polymer nanocarriers induced sufficient host T cells expressing tumor-specific CARs or virus-specific TCRs to cause disease regression at similar levels to bolus infusions of ex-vivo engineered lymphocytes. So far, non-replicating mRNAs (naked or formulated with lipid, peptide or polymers) are mostly investigated in clinical trials for cancer treatment (Miao L et al., 2021). However, self-amplifying mRNAs have gained extensive attention and are being evaluated in both cancer and infectious diseases due to long-lasting efficacy and lower required dosages.

Tissue Regeneration

Regenerative medicine is a branch of modern medicine that seeks to develop new methods for replacing, repairing, or healing damaged cells, tissues, and organs. mRNAs have been used for differentiation and trans-differentiation into a variety of cell types including neurons (Goparaju SR et al., 2017), β-cells (Koblas T et al., 2016), endothelial progenitors (Van Pham P et al., 2017), cardiovascular cells (Zangi L et al., 2013), and myogenic cells (Warren L et al., 2010). However, the majority of studies using cmRNA for cell differentiation and tissue engineering have been performed on bone, and to a lesser extent, on heart regeneration. There are two main methods of gene transfer used for tissue regeneration:

1. Cells of interest such as mesenchymal stem cells (MSCs) are first transfected with nucleotide molecules in vitro and subsequently transplanted into defect sites;
2. Gene activated matrices (GAM), comprising scaffolds decorated with complexes containing target genes are directly transplanted into defect sites and serve as support to recruit MSCs in vivo. The latter method is more commonly used for mRNA-based therapy in bone regeneration (Balmayor ER et al., 2016). Thanks to the expertise, OZ Biosciences makes polymer and lipids in house and takes partinvolved in European consortium on the topic.

For specific use, Oz Biosciences offers a custom mRNA synthesis service including:

- Gene synthesis cloning and DNA template production
- MRNA synthesis by in vitro transcription
- Purification and quality control

RNA APPLICATIONS

Recent publications have demonstrated successful **bone regeneration** following the application of cmRNAs encoding BMP2 in femur (ZS, 2016), and calvarial (Elangovan S et al., 2015) bone defect models. Delivering hBMP-2 cmRNA to a defected femur in a rat model results in new bone tissue formation as early as 2 weeks after application of very low doses. Overall, those studies demonstrate the feasibility and therapeutic potential of a new cmRNA-based gene therapy strategy-that is safe and efficient. When applied clinically, this approach could overcome BMP-2 growth factor associated limitations in bone regeneration.

When the reprogramming of cells or the regeneration of tissue needs a relatively long time-course, the ideal biomaterials for prolonged delivery of mRNA should be determined to compensate for the transient effect of mRNA and to mitigate the need for multiple transfections. Overall, the accumulating knowledge of enhanced mRNA synthesis, modification, delivery and targeting should overcome these obstacles and fulfill promise of a bright future for translation of cmRNA toward clinical applications (Badieyan ZS et al., 2019).

Gene Therapy

The initial concept of gene therapy is adding a new gene or replacing a faulty gene by safely delivering genetic materials to the target cells. After delivering genetic materials, missing or defective proteins can be produced in the target cells. Various proteins can be supplemented by the cellular translational process to regenerate defected tissues. To achieve this goal, the recombinant plasmid DNA (pDNA) technique was first introduced to express target proteins in cells. There were several obstacles for the delivery of pDNA into cells including poor cellular uptake, poor endosomal escape, and inefficient translocation into the nucleus. Many approaches aimed to overcome these barriers, which included viral and non-viral delivery systems. In general, the viral vectors are powerful protein expression systems. However, intrinsic cytotoxicity and immunogenicity of viral systems have limited their wide use in clinical applications. In contrast, non-viral systems relieved some safety concerns on cellular cytotoxicity and immunogenicity by utilizing biocompatible lipids and polymers.

OZ Biosciences offers **Cas9 protein**, **Cas9 mRNA** and their respective delivery carrier (**ProDeliverIN CRISPR** ; **RmesFect™ CRISPR; PolyMag CRISPR**).

ProDeliverIN CRISPR kit is a transfection reagent optimized for recombinant Cas9 protein delivery or Cas9/gRNA RNP complexes.

RmesFect™ CRISPR transfection Reagent is specifically designed for mRNA/gRNA transfection with high efficiency and low toxicity.

PolyMag CRISPR kit is the only magnet assisted transfection reagent optimized to deliver high level of plasmid DNA and/or mRNA expressing Cas9 and guide RNA (gRNA).

www.ozbiosciences.com

RNA APPLICATIONS

2 examples of gene therapy using either siRNA-LNP or mRNA LNP.

siRNA-LNP drug in clinic

In patients with hereditary transthyretin (hATTR) amyloidosis, a protein called transthyretin which is defective and breaks easily circulates in the blood. The broken protein forms amyloid deposits in tissues and organs around the body, including around the nerves, where it interferes with their normal functions. A small synthetic interfering RNA (siRNA) formulated in LNP (ONPATTROTM) has been designed to attach to and block the genetic material of the cell responsible for producing transthyretin. Then, this reduces the production of defective transthyretin, thereby reducing the formation of amyloids and relieving the symptoms of hATTR amyloidosis. **ONPATTRO (patisiran) was approved by the FDA in 2018 and was the first LNP-based drug in its kind to reach the market.**

mRNA-LNP in preclinical phase

The promise of gene therapy for the treatment of cystic fibrosis has yet to be fully clinically realized despite years of efforts toward correcting the underlying genetic defect in the cystic fibrosis transmembrane conductance regulator (CFTR). mRNA therapy via nanoparticle delivery represents a powerful technology for the transfer of genetic material to cells with large, widespread populations, such as airway epithelia. Researchers have deployed a clinically relevant lipid-based nanoparticle (LNP) for the packaging and delivery of large chemically modified CFTR mRNA (cmCFTR) to patient-derived bronchial epithelial cells. This is resulting in an increase in membrane-localized CFTR and a rescue of its primary function as a chloride channel. Furthermore, nasal application of LNP-cmCFTR restored CFTR-mediated chloride secretion to conductive airway epithelia in CFTR knockouts mice for at least 14 days. On day 3 post-transfection, CFTR activity peaked, recovering up to 55% of the net chloride efflux, characteristic of healthy mice. This magnitude of response is superior to liposomal CFTR DNA delivery and is comparable to outcomes observed in the currently approved drug ivacaftor. LNP-cmRNA-based systems represent a powerful platform technology for correction of cystic fibrosis and other monogenic disorders (Robinson E et al., 2018).

Gene Editing

Genome editing" or "Genome engineering" gives the ability to introduce a variety of genetic alterations (deletion, insertion…) into mammalians cells. During the past decade, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were the tools of choice for genome editing technologies until the very recent discovery of CRISPR/Cas9 technology that have revolutionized the field. Successful CRISPR/Cas9 genome editing can be performed through diverse approaches (plasmids, mRNA, nuclease, viral delivery). Accordingly, efficient nucleic acid delivery (transfection or transduction) represents a critical step for genome editing experiments.

Several studies and patents have shown that the use of bioreducible LNPs for the co-delivery of Cas9 mRNA and gRNA demonstrat highly efficient in vitro genome editing, as well as rapid knockdown of the target gene (Qiu M et al., 2021). LNP systems containing optimized ionizable cationic lipids can exhibit remarkable in vivo potency at low doses (i.e. 0.02 mg siRNA/kg body weight for silencing hepatocytes target genes in rodents following intravenous injection).

OZ Biosciences provides the most common reporter mRNAs and the rising generations of research reagents based on molecular delivery systems to serve and assist the life science community in its mission.

Transfection Solutions

In vivo Delivery

Transduction Enhancers

High Quality mRNAs

Vaccine Adjuvants

Cellular Assay Kits

R&D

Applications of RNA in R&D labs are wide. From gene silencing, to gene overexpression, RNA probes, labelling, RNA pull down, cell tracing, and cell reprogramming; RNA has its own role. Gene silencing effect constitutes a very helpful tool to study gene's function and a promising approach for new therapeutic treatments. Short RNA duplexes (siRNA: small interfering RNA, shRNA: small hairpin RNA and dsRNA: double strand RNA) are extremely selective by interacting and inducing the degradation of their specific mRNA targets and thereby inhibit the resulting protein production. mRNA transfection of reporter genes (e.g. green (GFP) or yellow (YFP) fluorescent proteins, lacZ, alkaline phosphatase or firefly luciferase), represents a highly effective tool for the evaluation and tracing of viable cells by fluorescence microscopy and flow cytometry (Fig 4).

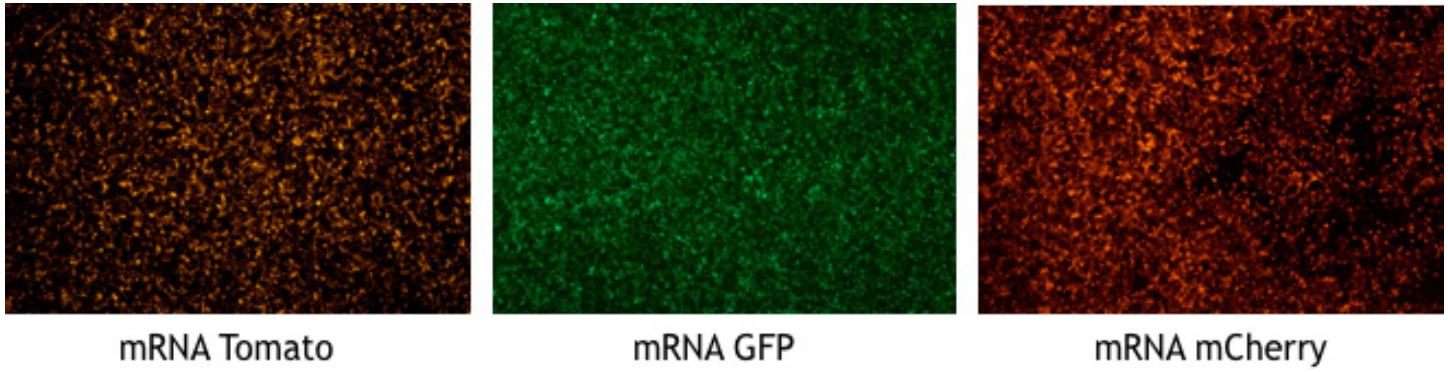


Figure 4. Expression of fluorescent proteins upon transfection of mRNA with Rmesfect transfection reagent (TR) using a ratio TR(µl):RNA(µg) of 3.5:1.

In basic research, fluorescently labeled nucleic acids are widely used in bulk and single-molecule experiments to elucidate fundamental processes, such as folding, replication, transcription and translation. In cells, fluorescent labeling is needed to visualize the subcellular localization of nucleic acids, most importantly mRNAs. Fluorescent RNAs can also be generated by IVT: 5-(3-Aminoallyl)-uridine-5'-triphosphate labeled with Cy3 (Cy3-UTPs) for instance can replace UTP during in vitro transcription and be used as a substrate for T7 RNA polymerase to generate labeled probes. The resulting assembled mRNA will emit fluorescence. **For that matter, OZ Biosciences offers mRNAs labeling upon request.**

References:

Badieyan ZS et al. (2019). Concise Review: Application of Chemically. Stem Cells Translational Medicine, 8:833-843.
Baiersdörfer M et al. (2019). A Facile Method for the Removal of dsRNA Contaminant from In Vitro-Transcribed mRNA . Mol Ther Nucleic Acids, 15:26-35.
Balmayor ER et al. (2016). Chemically modified RNA induces osteogenesis of stem cells and human tissue explants as well as accelerates bone healing in rats. Biomaterials, 131-146.
Daffis S et al. (2010). 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members . Nature, 468(7322):452-6.
Decroly E et al. (2011). Conventional and unconventional mechanisms for capping viral mRNA . Nat Rev Microbiol, 10(1):51-65.
Elangovan S et al. (2015). Chemically modified RNA activated matrices enhance bone regeneration. Journal of Controlled Release, 28:22-28.
Evers MJW et al. (2018). State-of-the-Art Design and Rapid-Mixing Production Techniques of Lipid Nanoparticles for Nucleic Acid Delivery. small methods, 1700375.
Goparaju SR et al. (2017). Rapid differentiation of human pluripotent stem cells into functional neurons by mRNAs encoding transcription factors . Sci Rep, 7:42367.
Hewitt SL et al. (2020). Intratumoral IL12 mRNA Therapy Promotes TH1 Transformation of the Tumor Microenvironment. Clin Cancer Res, 26:1–15.
Kariko K et al. (2011). Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA . Nucleic Acid Res, 39(21):e142.
Kay MA et al. (2001). Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics . Nat Med, (1):33-40.
Koblas T et al. (2016). Reprogramming of Pancreatic Exocrine Cells AR42J Into Insulin-producing Cells Using mRNAs for Pdx1, Ngn3, and MafA Transcription Factors. Molecular Therapy Nucleic Acids, 5:e320.
Kormann M. S. D et al. (2011). Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. nature Biotechnology, 29, pages 154–157 .
Levy JP et al. (1996). Retroviral transfer and expression of a humanized, red-shifter green fluorescent protein gene into human tumor cells. Nat. Biotechnol, 14:610-614.
Luo D et al. (2000). Synthetic DNA delivery systems. Nat Biotechnol, (1):33-7.
Mainini F et al. (2020). Lipid and Polymer-Based Nanoparticle siRNA Delivery Systems for Cancer Therapy . Molecules, 25(11):2692.
Miao L et al. (2021). mRNA vaccine for cancer immunotherapy. Mol Cancer, 20:41.
Pardi N et al. (2018). mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov, (4):261-279.
Qiu M et al. (2021). Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific in vivo genome editing of Angptl3. PNAS, 118 (10).
Robinson E et al. (2018). Lipid Nanoparticle-Delivered Chemically Modified mRNA Restores Chloride Secretion in Cystic Fibrosis. Mol Ther, 8:2034-2046.
Van Pham P et al. (2017). Production of endothelial progenitor cells from skin fibroblasts by direct reprogramming for clinical usages. In Vitro Cell. Dev.Biol.-Animal, 53:207-216.
Vogel Ab et al. (2018). Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses. Mol Ther., 26(2):446-455.
Warren L et al. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA . Cell Stem Cell, 5:618-30.
Wiesinger M et al. (2019). Clinical-Scale Production of CAR-T Cells for the Treatment of Melanoma Patients by mRNA Transfection of a CSPG4-Specific CAR under Full GMP Compliance . Cancers, 11(8):1198.
Zangi L et al. (2013). Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. Nat Biotechnol, 31, 898–907.
Zhang P et al. (2017). History of Polymeric Gene Delivery Systems . Top Curr Chem (Cham). , 375(2):26.
ZS, B. (2016). Transcript-activated collagen matrix as sustained mRNA delivery system for bone regeneration. Journal of Controlled Release, 239:137-148.

Europe / Asia OFFICE
OZ Biosciences SAS
Parc Scientifique de Luminy, zone entreprise,
case 922, 13288 Marseille Cedex 09,
FRANCE
Ph : +33 486 94 85 16
Fax : +33 486 94 85 16
E-mail: contact@ozbiosciences.com

OZ BIOSCIENCES
The art of delivery systems

US OFFICE
OZ Biosciences Inc.
4901 Morena Boulevard, Suite 901
San Diego, CA 92117,
USA
Ph : +1 858 246 7840
Fax : +1 855 631 0626
E-mail : contactusa@ozbiosciences.com

www.ozbiosciences.com