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mRNA 2.0:

Developing xRNA Medicines as the Next Big Therapeutic Modality.

Summary

Rajiv Datar, PhD, EDP (MIT)

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A. mRNA 2.0: Design and Integration of Novel RNA Sequences and its Delivery Around a Common Platform *sans* PEGylation or Lipid Nanoparticles.

Messenger ribonucleic acid (mRNA) therapy represents a new and novel approach for treating a wide range of diseases, encompassing both immune-related and non-immune conditions. Distinct characteristics of mRNA drugs are demanded in different therapeutic indications. Compared to recombinant protein drugs, mRNA drugs have a dramatically shorter production cycle and are not limited to short peptide sequences. mRNA sequences can easily deliver numerous tumor antigen fragments simultaneously, thereby increasing the diversity of antigenic epitopes. Conventional linear non-replicating mRNA is characterized by a short half-life. Two innovative mRNA structures have been developed with longer half-lives, namely self-amplifying mRNA (saRNA) and circular mRNA (circRNA) [1]:

- The half-lives of mRNA and miRNA in whole blood were calculated to be 16.4 h and 16.42 ± 4.2 h, respectively, indicating that the half-lives of mRNA were **similar** to those of miRNA; and
- The half-lives of circRNA and lncRNA (long non-coding RNA) in whole blood were 24.56 ± 5.2 h and 17.46 ± 3.0 h, respectively.

However, an important key point to note is this: separate from the fact that (a) circRNA and saRNA are not only large and complicated molecules in themselves, but also to produce at scale, the *translated therapeutic* will continue to demonstrate *short* in vivo half-lives; and (b) it is not at all clear what the long-term impact of a continuously present RNA will have on cytotoxicity – conventional mRNAs have evolved with short half-lives for a reason!

Endpoints News broke the news last week on the stealth Chinese biotech RiboX starting a first-in-human clinical trial (CT) with circular RNA (circRNA) and claiming a single dose over 4 weeks, beating the US groups who've poured 10x more money and are still not there!

A former Moderna CSO - currently an informal advisor to us – expected circRNA to be in the 3-4 weeks range and self-amplifying RNA (saRNA) to be about 2-3 weeks. The former time range may have just been confirmed by RiboX, with the latter being confirmed in a Q&A with Verve Therapeutics last year at an ASGCT conference where the presenter indicated their saRNA would be dosed about every 2 weeks.

Our data has shown a single dose of miRNA to last **5+ weeks** in the novel and patented, self-assembling Surface Fill Hydrogel Nanoparticle (SFH-NP) controlled release vehicle, without PEGylation or Lipid Nanoparticles, which is better than RiboX's circRNA at 4 weeks.

By design, our CLADed-mRNAs have similar half-lives to conventional mRNAs, but they translate into therapeutics with short or long (ie, tunable) half-lives. Furthermore, the CLAD-mRNAs can be prepared as "linear mRNA," "circRNA," or even as "saRNA." And, when integrated as cargo with the SFH-NP controlled-release vehicle, it has the potential to fine tune dosing from 1 week to 8 weeks or more, reducing dosing-frequency combinations by 10- to 1,000 fold!

[1] Wang and Liu, Nat Sci Rep 12, 7259 (2022).

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B. Example of Nanoparticle-Hydrogel Composite for *Nucleic Acid Molecule Delivery*

Mesothelioma is an aggressive cancer covering anatomic surfaces (e.g. lining of the lungs, heart, abdomen, etc.) that resists multi-modality therapies. Regional recurrence of mesothelioma from residual tumor cells prevents long-term benefits after surgical resection. Furthermore, there is no clinical consensus on intracavitary adjuvants that are effective in extending the tumor reduction effect of surgery.

A new technology and platform fulfills this unmet clinical need by providing a local regional therapeutic platform to shuttle cancer-specific *microRNA*, thereby circumventing systemic administration challenges. This technology showcases nanoparticles comprised of microRNA bound to disordered peptides that are embedded in a hydrogel engineered from self-assembling β-hairpin peptides. The nanoparticle hydrogel composition is a shear-thinning composite, capable of being syringe-injected or sprayed onto body cavities harboring mesothelioma xenografts. This biodegradable material can be fine-tuned by choice of self-assembling peptides in the gel matrix, of disordered peptides, and of microRNA to produce an optimal anti-cancer effect with a time-released delivery profile. After administration of a single application, this hydrogel composite produced a durable pre-clinical response in multiple xenograft cancer models. In principle, this localized regional treatment strategy could be applied to other surface cancers.

Competitive Advantages:

- Biodegradable and biocompatible material that minimizes cytotoxic side effect in vitro and in vivo.
- Can be fine-tuned by choice of self-assembling peptides in the gel matrix, of disordered peptides in the nanoparticles, and of nucleic acids to produce an optimal therapeutic effect with time-released delivery.
- Both syringe-injectable and sprayable to effectively cover complex tissue surface topology.
- Only needing a single administration should reduce clinical trial, manufacturing, and commercialization costs.
- Only needing a single administration should improve patient compliance for future applications.

Commercial Applications:

- Platform for delivery of nucleic acid molecules.
- Could be used for treatment of all surface tumors including, but not limited to, all anatomic locations of mesothelioma, metastatic tumors involving pleural surfaces (e.g. lung, breast, colon, renal, esophageal, thymic/ thymoma, etc), and/ or metastatic tumors involving peritoneal surfaces (e.g. ovarian cancer) Specific nucleic acids could be selected and loaded into the hydrogel for use in treating these diverse types of malignancies.

C. Example of Peptide Hydrogels for *Rate-Controlled Delivery of Therapeutics*

Hydrogels represent an attractive controlled drug-delivery system that have been used in various clinical applications, such as: tissue engineering for wound healing, surgical procedures, pain management, cardiology, and oncology. High-water content of hydrogels confers tissue-like physical properties and the crosslinked fibrillar network enables encapsulation of labile small molecule drugs, peptides, proteins, nucleic acids, proteins, nanoparticles, or cells. The porosity of the mesh-like network contributes to enhanced protection and controlled release of therapeutics compared with the rapid clearance and degradation of some proteins observed using conventional drug-delivery methods. Although all hydrogel platforms provide spatial and temporal control over the release of therapeutics, the current standard requires designing a unique hydrogel for a select therapeutic agent for a specific application. This one therapeutic agent-one gel model adds significant research and regulatory burden.

This example illustrates the development of a novel syringe-injectable/sprayable hydrogel platform that can deliver a variety of different therapeutic agents. This hydrogel can be used to deliver *small molecules, peptides, proteins, nucleic acids, nanoparticles, or cells*. Further, this hydrogel has been engineered to be compatible with a tunable protein delivery system and platform that enables the release of different kinds of proteins based on the electrostatic interaction between the fusion sequence engineered directly at the amino- or carboxy-terminus of proteins and the anionic fibrillar network of the hydrogel. In a proof-of-concept study, a cytokine protein, IL-7, a therapeutic agent critical for improving T cell development for immunotherapy in cancer, sepsis, and HIV-infected patients was successfully delivered. Administration of a *single dose* of IL-7 encapsulated within the hydrogel exhibited comparable development of T cell populations compared to soluble IL-7 added every 3 days in vitro and to daily subcutaneous IL-7 injections for greater than 30 days in vivo. Thus, this hydrogel platform can substantially reduce the amount of protein needed to attain desired endpoints and potentially reduce patient burden for targeted therapies.

Furthermore, the hydrogel platform can be used to culture cells in 2D and 3D environments and deliver these cells as potential therapeutic agents. Murine studies have shown the ability to culture and deliver fluorescently labeled human dermal fibroblasts encapsulated in the hydrogel at high cellular concentrations. Stem and cancer cells can also be grown within the 3D hydrogel environment; instead of the commonly used murine-derived Matrigel reagent and subsequently these cells may be injected in vivo, even into larger scale animal models such as non-human primates. These proof-of-concept studies suggest that the hydrogel platform can be used to encapsulate an appropriate number of cells of interest over time in vivo for therapeutic applications. Overall, the hydrogel platform is a promising system that is applicable for numerous non-clinical and clinical applications.

Competitive Advantages:

- Rate-controlled release of therapeutic drugs including small molecules, nucleic acids, peptides, proteins, or nanoparticles: therapeutic drugs encapsulated in the anionic hydrogel platform.
- Rate-controlled release of therapeutic whole cells: such as stem cells, T cells, CAR T cells for cancer therapy and/or fibroblasts.
- T cell enhancer for in vitro and in clinical setting: stimulating T cells in cultures, essential in adoptive T cell therapy, cancer and HIV-immunotherapy, boosting T cell levels in sepsis patients, and modulating T cell populations in autoimmune diseases.
- Delivers locally or systemically a variety of different therapeutic drugs including small molecule, peptides, proteins, nucleic acids, nanoparticles, or cells.
- Reduces the amount of drugs or cells needed to attain desirable clinical results.
- Reduces the patient burden from multiple injections and travel time to the clinic.
- Offers targeted syringe or catheter injectable delivery or sprayable delivery.
- Is a tunable delivery platform that can attenuate peptide or protein release rate within hydrogel.

Commercial Applications:

- Cancer – especially those involving therapeutic whole cells – such as stem cells, T cells, CAR T cells for cancer therapy and/or fibroblasts.
- Sepsis – especially when boosting T cell levels is effective.
- HIV.
- Autoimmune disorders – especially to modulate T cell populations.
- Transplantation.
- Delivering viruses for gene therapy or oncolytic viruses for cancer therapy.

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