EDITORIAL

Self-replicating messenger RNA based cancer immunotherapy

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In 1989, the Nobel Prize in chemistry was awarded to Thomas Cech for his findings that RNA in living cells not only act to encode information enabling the production of specific proteins from DNA, but its three-dimensional structure enables it to serve as a catalyst [1]. His discovery gave rise to an expanding appreciation of the roles of RNA in biology, and a variety of technology advances have enabled the design of RNA molecules to function as potential medicines inhibiting or facilitating a variety of biologic processes. In addition to these novel capabilities, the central role of RNA in the biology of normal protein production created opportunities exploiting this role for gene therapy or therapeutic protein production. For example, rather than delivering DNA into cells to produce a novel protein following integration, transcription, and translation of messenger RNA (mRNA), one could introduce mRNA directly into cells where it would be translated in the cytoplasm to produce the protein of interest. Although transient, direct delivery of mRNA circumvents the need to enter the nucleus which avoids the risk of genomic integration in the production of many functional proteins or peptides. This is especially important in creating vaccine targeting oncogenes, in addition to exploiting the role of some mRNA in stimulating the immune system via toll-like receptors (TLRs).

The use of mRNA as a therapeutic was initially thought to be limited due to its susceptibility to enzymatic degradation and hydrolysis. Nonetheless, 50 micrograms of mRNA in liposomes could be injected into mice to elicit antibody responses [2]. As an alternative to direct in vivo mRNA delivery, naked mRNA was introduced in vitro into phagocytic dendritic cells (DC) to generate protein antigens to be presented as a component of a cellular vaccine targeting cancer [3]. The ability of naked mRNA to be taken up by DC and produce the encoded antigen was demonstrated to stimulate both antibody and cellular responses that had antitumor effects in murine and clinical studies. Nonetheless, this method required the mRNA to be loaded ex vivo into DC which served as a carrier for delivery to the patient. Enhancing the delivery of naked mRNA into cells was subsequently demonstrated, and additional opportunities were explored to improve the ability to efficiently deliver mRNA for therapeutic purposes using both cellular and acellular techniques.

Advanced acellular methods to deliver mRNA therapeutics included developing increasingly sophisticated lipid containing microvesicles or nanoparticles (LNP). Cationic lipids, ionizable lipids and other types of lipid have been explored for both in vitro and in vivo mRNA delivery, and current lipid nanoparticles have been investigated and successfully used clinically for the delivery of mRNA. Indeed, mRNA packaged into LNPs formed the basis for successful vaccines generated to prevent illness from COVID19 [4]. In addition to LNPs, more complex carriers, such as recombinant viral like particles were developed and scaled for clinical use, adding additional capabilities to mRNA delivery. Although mRNA delivery using LNPs has proven to be clinically useful, there remain significant limitations, such as the need for considerable amount of mRNA/lipid to elicit immune responses. Strategies to increase delivery of nucleic acids in vivo include improved lipid macrovesicles, and alternatives including electroporation and sonoporation [5, 6].

Improvements in the utility of mRNA to deliver proteins as either antigens within vaccines or therapeutic proteins by either enhancing or diminishing its innate immune reactivity were also evolving. For example, the immune engagement of mRNA which was potentially beneficial for vaccines/immune therapy was seen as a barrier to therapeutic protein delivery. Because it was known that eukayotic RNA contains many types of chemical modifications, and various viruses and bacteria modify their RNA to evade immune recognition, directed chemical modifications of mRNA were made to improve the stability and function of the mRNA, while reducing its ability to activate innate immune responses. In addition, the translational capacity of mRNA has been improved through both nucleotide substitutions as well as targeted modifications of the protective 5′-cap and poly(A) tail, reducing mRNA degradation. Indeed, chemical modifications of mRNA continue to be developed to optimize the uses of mRNA as a therapeutic.

To address the fundamental inefficiency of mRNA delivery, an additional strategy was to exploit self-replicating RNA, which would allow small amounts of delivered mRNA to be amplified within cells to clinically useful levels. Self-replicating RNA vectors are typically derived from positive strand RNA viruses and have unique features. The RNA is directly released in the cytoplasm, where it creates an immune privileged compartment called a spherule on cytoplasmic membranes such as the endoplasmic reticulum. Here, it is subjected to extensive amplification by using the host cells endogenous machinery to create mRNA copies indistinguishable from those normally produced within the cell. srRNA vectors are generated by removal of the structural proteins and replacement with heterologous genes of interest. The process is also inherently safe and self-limiting. First, the replication occurs exclusively in the cytoplasm and the vector, or the gene of interest, will not be integrated into the host cell genome. Second, the replication machinery is only actively expressed from the vector for approximately 6 h, after which the machinery is subject to degradation according to the protein's normal half-life. The expression of the target protein from a viral subgenomic promoter generates large amounts of recombinant protein, which can act as antigen for vaccine development or the clinically relevant expression of a therapeutic protein.

The first clinical application of a srRNA vector targeting cancer utilized an alphavirus vector, packaged in a viral capsid provided in trans and to create single cycle virus-like replicon particles (VRP). In contrast to mRNA, VRPs were highly efficient in transfecting DCs resulting in significant levels of antigen expression in vitro. VRPs could be repeatedly administered to patients with metastatic cancer expressing the tumor antigen (CEA) and could overcome high titers of neutralizing antibodies and elevated Treg levels to induce clinically relevant CEA-specific T cell and

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antibody responses [7]. Some advanced cancer patients treated with VRP had long term survival benefits [8].

A more contemporary development has been fully synthetic srRNAs where the srRNA vectors are delivered using non-viral delivery methods such as LNPs, which confer multiple benefits. Operationally, a fully synthetic system simplifies manufacturing lowering cost of goods and accelerating deployment. Biologically, lack of a viral shell results in no/ lower anti-vector immunity allowing repeated dosing and the ability to encode multiple large genes of interest, which normally were limited by the packaging capacity of the viral particle. In this edition of Cancer Gene Therapy, investigators review recent developments in srRNA technology and its current clinical applications, highlighting the advantages of these approaches currently, and the long-term potential to leverage these advantages to improve both preventive strategies and therapeutics against a variety of diseases.

In this issue, Lundstrom provides an overview of the history, current status, and future directions using self-replicating RNA viruses [9]. Aliahmad et al describes next generation synthetic self-replicating RNA vaccines which are currently being evaluated [10]. In addition, they argue that a new drug development approach leveraging panels of customizable, synthetic srRNA vectors can be engineered for the specific features that will be required for clinical success. Daily et al provide a review of the features of srRNA vaccines that can be applied for cancer vaccines, and Morse et al review the clinical trials involving srRNA vaccines targeting cancer [11, 12]. The compilation of these reviews highlight many of the capacities and opportunities of srRNA as a cancer immunotherapy platform and provide insight into the potential future use of these technologies, which includes the concept of disease interception. For example, the use of potent srRNA vaccines will enable systemic immune responses to antigens generated by therapeutic resistance mechanisms. In one example, resistance to forms of endocrine therapy in breast cancer is related to the somatic mutations found in the estrogen receptor (ER) alpha. By directing the immune system against mutant ERalpha, malignant cells expressing mutant ERalpha and resistant to endocrine therapy will be targeted and eliminated by systemic B and T cell responses. The immune mediated elimination of mutant ERalpha expressing clones, in turn, will lead to a tumor expressing wild type ERalpha, and a clinical return to sensitivity to endocrine therapy. This concept, so called immune synthetic lethality, could be applied to a variety of therapeutic interventions in which known resistance mechanisms occur in a predictable fashion. Overall, the promise of srRNA will be applied to these and other novel approaches to target cancer. The delivery of srRNA to conduct additional tasks within cells, including gene editing, also holds great promise, and will be addressed in future issues.

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COMPETING INTERESTS

HKL is on the board of directors and has equity in Oncosec, is on the scientific advisory board and has equity in Replicate.