A self-replicating RNA precision medicine approach to therapeutic protein delivery of narrow therapeutic index biomolecules

Zelanna Goldberg[1], Christian Maine[1], Gabrielle P. Dailey[2], Christine Domingo[1], Gaelle Picarda[1], Hunter Little[1], Annie Chou[1], Jessica Sparks[1], Darina Spasova[1], Shigeki Miyake-Stoner[1], Christopher A. Rabiola[2], Erika J. Crosby[2], Zachary C. Hartman[2], Herbert K. Lyerly[2], Nathaniel Wang[1], Parinaz Aliahmad[1]

[1]Replicate Bioscience Inc., [2]Duke University

Abstract #2732

Abstract

Protein drug replacement using nucleic acid technologies has been a sought-after solution for *in situ* production of proteins with poor half-lives and challenging manufacturability. Linear mRNA approaches have failed clinically in this technological use case due to low levels of protein expression and poor durability. Self-replicating RNA (srRNA) is a new technology for expression of biotherapeutics combining the advantages of fully synthetic drug products with higher peak levels of protein expression with active expression persisting out to 49 days. RBI-2000 is a novel srRNA encapsulated in a lipid nanoparticle and encoding two distinct proteins on the same strand of RNA. The first protein is a pro-inflammatory multimeric cytokine to promote *de novo* immune cell generation and infiltration. The second molecule is a single chain high affinity protein antagonist of downstream mediators of the inflammasome to prevent sterile inflammation, aberrant angiogenesis, and tumor invasiveness. We selected a novel alphaviral vector which demonstrated an enhanced peak level of protein expression vs. the prototypical vector that has been used in other RNA products. These encoded proteins elicited a pharmacodynamic response that had no evidence of decay in the first 7 days following a single treatment down to doses of 0.01 mcg. Using an implanted MC38 tumor model, tumor control was obtained at the lowest, single dose tested at 0.1 mcg of RBI-2000. When tested in combination with a checkpoint inhibitor (CPI), a single dose of the CPI and a single dose of RBI-2000 at 10 mcg resulted in 80% tumor control in the mice (8/10 mice). Tumor re-challenge of the mice at a site distal to the original implantation showed 100% protection. Collectively, these data demonstrate the feasibility of novel srRNA vectors as a protein drug replacement approach including for the expression of complex, multimeric cocktails of proteins.

Introduction

Messenger RNA has proven successful for use in simple, single protein vaccine applications in which a short burst of moderate protein expression allows priming of an effective immune response. However, the limitations of this short pulse of protein expression preclude its wider application in other indications in which higher and durable protein expression is required. Self-replicating RNA (srRNA) is a next generation RNA technology that improves both the peak and durability of protein expression due to its ability to replicate inside the host cell. Improving protein expression by orders of magnitude positions srRNA as an ideal platform for applications such as delivery of biotherapeutics for oncology, autoimmunity and rare disease.

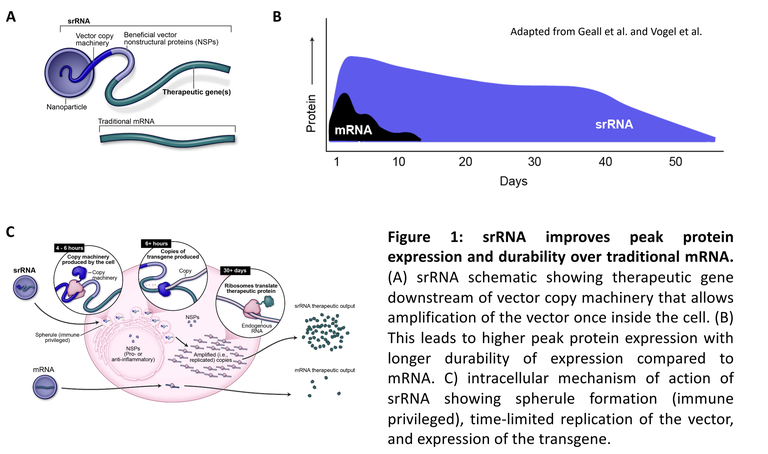

Figure 1: srRNA improves peak protein expression and durability over traditional mRNA. (A) srRNA schematic showing therapeutic gene downstream of vector copy machinery that allows amplification of the vector once inside the cell. (B) This leads to higher peak protein expression with longer durability of expression compared to mRNA. C) intracellular mechanism of action of srRNA showing spherule formation (immune privileged), time-limited replication of the vector, and expression of the transgene.

Approach

RBI-2000 is the first srRNA to encode multiple biotherapeutic proteins on a single vector backbone. Both molecules have anti-tumor properties but targeting different aspects of the tumor immunity cycle to achieve optimal synergy. Molecule 1 encodes is a high potency agonist that drives T cell activation, differentiation and effector function. This is paired with molecule 2 which acts on the immunosuppressive tumor microenvironment. Molecule 2 is an antagonist of an inflammatory mediator that promotes angiogenesis, supports suppressive myeloid populations and leads to increased tumor invasiveness. The combination of both molecules will act to create a permissive tumor microenvironment for newly primed T cells to enter and perform their anti-tumor functions. Clinical PK/PD analysis and proof-of-concept reads through to alternative biotherapeutic molecule delivery for oncology or other indications.

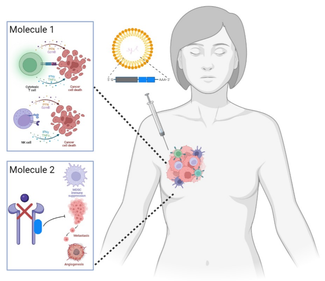

Figure 2: RBI-2000 encodes multiple biotherapeutic molecules in a single drug product for tumor immunotherapy. Molecule 1 is a high potency agonist that drives immune cell activation and recruitment to the TME. Molecule 2 reverses inflammation and prevents angiogenesis and tumor invasiveness. This is the first demonstration of using srRNA to express multiple biotherapeutic molecules. Clinical PK/PD reads through to alternative biotherapeutic molecule combinations and multimeric proteins (e.g. mAbs) in various disease types.

Results

The most commonly used srRNA vector in the field is derived from the alphaviral family member Venezuelan equine encephalitis virus (VEE). We mined the diversity of the alphavirus family to screen alternative vectors to VEE and compare the effect on protein expression and immunogenicity. Our data suggests that there is no one-size-fits-all approach to srRNA drug development and that different viral family members will bring different properties depending on the protein insert. Our approach is tailored towards each transgene and therapeutic indication to identify the lead drug product candidate. Identification of the lead for RBI-2000 is shown in figure 3 following *in vivo* screening of multiple vectors.

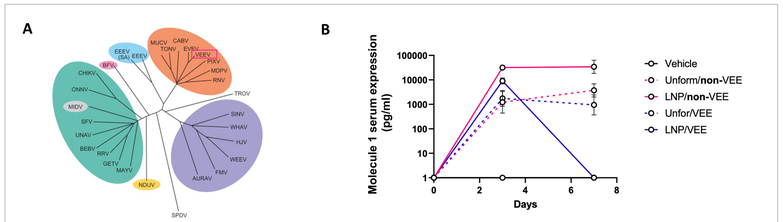

Figure 3: Screening multiple alphavirus srRNA vectors identifies lead candidate for RBI-2000 A) Diversity of the alphavirus family indicating the most commonly used srRNA vector in the field, VEE B) Serum concentration of molecule 1 following a single dose of RBI-2000 in mice. Different alphaviral vectors were compared with and without LNP formulation

We measured the pharmacokinetic and pharmacodynamic activity of RBI-2000 in vivo (Figure 4). Following a single intramuscular injection into mice we measured the serum concentrations of molecule 1 and 2 on day 3. Both molecules are expressed to high levels. Molecule 1 is bioactive in these mice with expression of a downstream pharmacodynamic molecule measurable in the serum at day 3 and expression remained stable at day 7 in all doses tested.

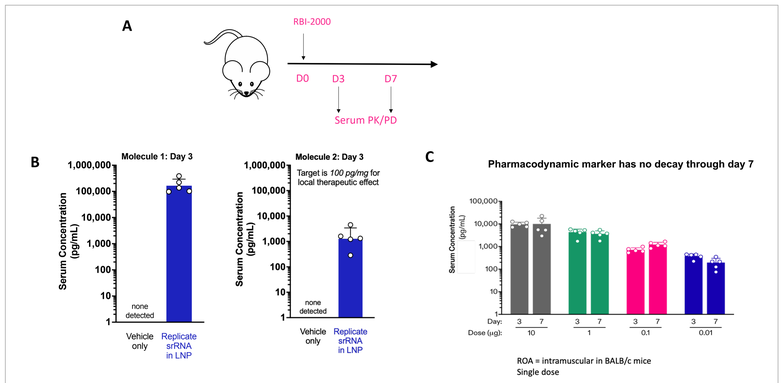

Figure 4: RBI-2000 expresses high levels of encoded biotherapeutic proteins which results in sustained pharmacodynamic marker expression. A) PK/PD analysis of RBI-2000 following a single intramuscular injection into naïve Balb/c mice. B) Serum concentration of both biotherapeutic proteins C) Dose dependent pharmacodynamic marker expression in the serum of mice following RBI-2000 dose with no decay by day 7

RBI-2000 was tested in the mouse syngeneic tumor model MC38. Following implantation of the tumor cells subcutaneously, tumors were measured and mice were randomized and enrolled onto the study when they entered the 30-80mm^3 range. A single dose of RBI-2000 was administered intratumorally and tumor volumes were measured over time. Some groups received RBI-2000 in combination with anti-PD-1 mAb (Figure 5A). Tumor growth inhibition is observed for RBI-2000 monotherapy and improved with the combination of anti-PD-1 mAb (Figure 5B). Complete responders were rechallenged with MC38 at day 70. All RBI-2000 treated mice that responded to the primary tumor rejected the secondary MC38 challenge demonstrating robust immunological memory (Figure 5C).

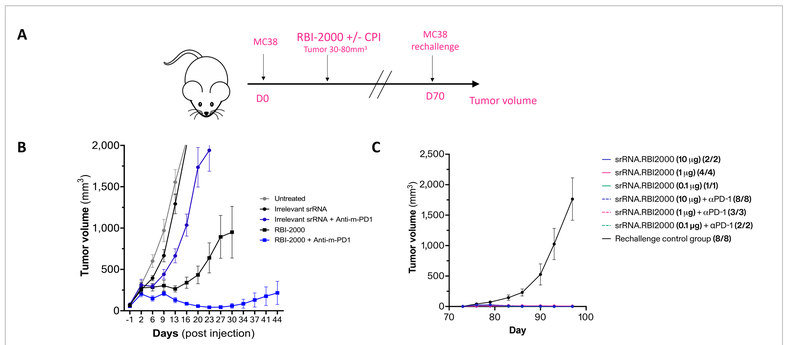

Figure 5: *RBI-2000 leads to tumor growth inhibition and long-lasting memory responses in mouse tumor model.* A) a single dose of RBI-2000 was given to tumor bearing mice with or without anti-PD-1 (D3-6, 8-12) and tumor volume was measured over time. B) shows significant tumor growth inhibition using RBI-2000 as a monotherapy (10 mcg) or combination therapy (10 mcg + anti-PD-1). C) following tumor clearance in complete responders, mice were rechallenged with MC38 on the opposite flank. 100% of the primary tumor complete responders rejected the secondary implant.

RBI-2000 was tested for anti-tumor efficacy in a cold, CPI refractory, mouse syngeneic tumor model. Following implantation of B16F10 cells subcutaneously, tumors were measured and once in range mice were randomized and enrolled onto the study (Figure 6). Mice were treated with a single intratumoral dose of RBI-2000 with or without anti-PD-1 mAb. Tumor growth inhibition demonstrated for all doses of RBI-2000 as a monotherapy. The 10ug RBI-2000 was also paired with anti-PD-1 mAb and tested as a combination therapy and showed no improvement to RBI-2000 alone. This is in contrast with the MC38 tumor model results show in Figure 5. RBI-2000 appears to drive anti-tumor responses alone in CPI refractory, cold tumors suggesting a mechanistic difference depending on the tumor type.

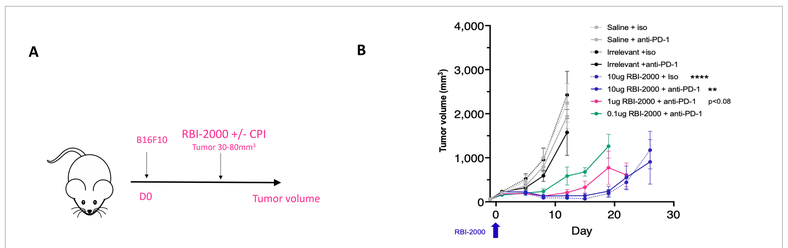

Figure 6: RBI-2000 inhibits growth of cold tumor model as a monotherapy. A) B16F10 tumor bearing mice were randomized and treated with RBI-2000 with or without anti-PD-1 mAb therapy (D3-6, 8-12). B) RBI-2000 shows a dose-dependent tumor growth inhibition with significant inhibition at 10ug doses either as a monotherapy or combination therapy.

Discussion

- srRNA is a next generation RNA platform that delivers higher protein expression for a longer length of time enabling its use for a wider range of therapeutic applications than traditional mRNA.
- RBI-2000 is an immunotherapeutic oncology drug that encodes 2 molecules on a single RNA backbone that act synergistically to reduce the immunosuppression in the TME and drive potent anti-tumor T cell responses.
- Pharmacokinetic analysis in vivo shows high expression both molecules following a single dose and durable expression of a pharmacodynamic marker down to 0.01ug doses.
- RBI-2000 synergizes with checkpoint blockade to inhibit the growth of established tumors in a mouse tumor model, resulting in durable anti-tumor memory following rechallenge in complete responders.
- RBI-2000 is able to drive a potent anti-tumor immune response in a checkpoint blockade refractory, cold tumor model as a monotherapy.

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