Targeting acquired resistance mutations in tumors using self-replicating RNA

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Abstract

Drug resistance remains the major driving factor behind the clinical failure of targeted therapeutics. Current precision oncology approaches to clinically address acquired resistance mutations have increasingly long and complex drug development timelines and burdensome toxicities. To address these issues, we have designed a novel approach to target acquired resistance mutations using self-replicating RNA (srRNA). In ER+ breast cancer, acquired resistance mutations develop in the ligand binding domain of the estrogen receptor (ESR1) rendering them insensitive to endocrine therapies. Even in a heterogenous tumor environment, removal of cells harboring acquired resistance mutations will drive sensitivity of the remaining cells to concurrently administered standard-of-care endocrine therapy. This creates a lose-lose situation for individual tumor cells due to competing selective pressures: cells without acquired resistance mutations are removed via endocrine therapy, whereas mutated cells are removed by the immune system lengthening the time patients can effectively be on endocrine therapy. We have demonstrated that this srRNA vaccine can induce robust immune responses to targeted acquired resistance mutations and result in tumor control in mouse models. Based on these results, we will advance this candidate into clinical testing.

Introduction

Drug resistance is a major limiting factor in the control of cancer. Both intrinsic and acquired resistance have been described as mechanisms that drive treatment failure and tumor progression. Replicate's **precision immuno-oncology (PIO)** approach leverages the advantages of self-replicating RNA (srRNA) to improve drug development (Figure 1) of agents targeting acquired resistance mutations. Traditional small molecule and mAb approaches in precision oncology suffer from lengthy and expensive development times with potential for high overlapping toxicity when combining drugs. The **Replicate PIO approach** is rapid, requiring only the sequence of the mutation, and does not rely on screening large libraries. Our srRNA vectors encode inserts containing multiple mutations allowing a combination of targets with a single drug product. srRNA has advantages over traditional mRNA vectors by generating robust, high quality, and durable $CD8^+$ and $CD4^+$ T cell and antibody responses.

	REPLICATE's Precision Immuno-oncology (PIO)	Precision oncology (small molecule, mAb)	Immuno-oncology (Checkpoint inhibitor, TIL)
Targeting **AcqMUT**	**Sequence itself sufficient** for drug (encoded directly into RNA)	Lengthy screening of chemical libraries (Lead ID, LO, LLO)	Not applicable
Targeting **multiple AcqMUT**	**Multi-targeting easy** (can encode multiple mutations in RNA)	Candidates oft screened in parallel (may be infeasible for late line)	Not applicable
Combination strategy	**Multi-targeted single agent**; dose intervals make combos easier	Layering, often overlapping toxicity	Layering toxicity
Off target **toxicity**	**Low expected reactogenicity**	Always present; often limiting	GI, lung, liver; often fatal
Total development **timeline / costs**	**Fast** and **low-moderate**	Variable	Lengthy and high

Figure 1: Replicate Bioscience Precision Immuno-oncology approach is superior to traditional small molecule and mAb approaches to cancer therapy. srRNA allows quick, multi-target drug development with lower cost of goods than traditional small molecule or antibody-based approaches. Lower doses of srRNA can elicit similar or improved levels of immune response compared mRNA therapeutics which lowers the chance of reactogenicity.

The mechanism underlying the Replicate PIO approach is termed **synthetic immune lethality** (Figure 2). In the absence of a selective pressure, tumors will edit out tumor cells bearing mutations recognized by the immune system via immunosurveillance. To address this, **synthetic immune lethality** forces the tumor into a lose-lose scenario by combining the active selective pressure exerted by standard of care (SOC) targeted therapy with our **PIO** directed towards resistance mechanisms induced by SOC. We leveraged this approach to treat ER+ breast cancer where resistance develops to SOC anti-estrogen therapy. By encoding the most common mutations/pathways of resistance to anti-estrogen therapies we combine our srRNA PIO with SOC and force the tumor into elimination by synthetic immune lethality.

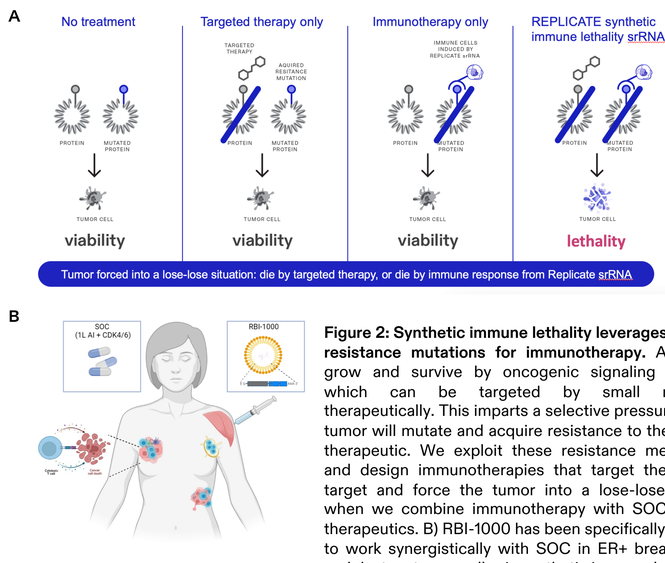

Figure 2: Synthetic immune lethality leverages acquired resistance mutations for immunotherapy. A) Tumors grow and survive by oncogenic signaling pathways which can be targeted by small molecules therapeutically. This imparts a selective pressure and the tumor will mutate and acquire resistance to the targeted therapeutic. We exploit these resistance mechanisms and design immunotherapies that target the mutated target and force the tumor into a lose-lose situation when we combine immunotherapy with SOC targeted therapeutics. B) RBI-1000 has been specifically designed to work synergistically with SOC in ER+ breast cancer and destroy tumor cells via synthetic immune lethality

Approach

RBI-1000 is designed to exploit clinically characterized pervasive and predictable mutations and amplifications arising in therapy resistant ER+ BC. RBI-1000 immune targets ESR1, PIK3CA/PI3K, ERBB2/HER2, and ERBB3/HER3, which have all been clinically associated with resistance to endocrine therapy. Using a proprietary screening approach, we empirically tested ~60 constructs to optimize to combine all 4 antigen targets into a self-replicating RNA vector. The approach allowed us to identify a lead in approximately 6 months. The lead cassette contains the most common activating mutations in ESR1 (6 mutations) and PI3 kinase (4 mutations). HER2 is encoded as a truncated version and HER3 is a full-length kinase-dead variant. Each of the encoded antigens have demonstrated HLA-A2 binding epitopes (Figure 3).

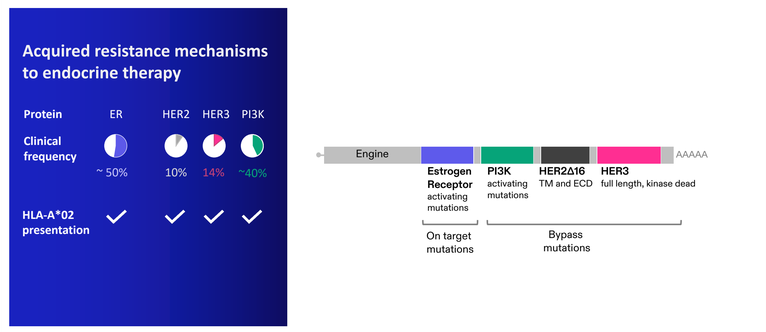

Figure 3: Structure of RBI-1000 cassette. RBI-1000 contains high frequency acquired resistance mutations found in ER+ breast cancer. Specifically, RBI-1000 contains most frequent activating mutations in ESR1 (6) mutations and PI3 kinase (4 mutations), a truncated HER2 gene, and full length, kinase-dead HER3. The lead cassette was selected following an empirical screen of ~60 different designs which test order and spacing using a combination of IRES and 2A elements. Numerous lines of evidence, by us and others, point to HLA-A2 restricted epitopes being present in all of these antigens. There is expected to be broad HLA reactivity for these encoded mutations in humans.

Results

The lead RBI-1000 cassette was cloned into our library of novel srRNA vectors and a clinical candidate was identified using an in vivo immunogenicity screen. Multiple srRNA vectors were used in combination with different lipid nanoparticle (LNP) formulations to screen through 13 unique drug-product candidates (Figure 4). Mice received 2 doses of each srRNA candidate and T cell function was measured by IFN_γ ELISpot. Recall responses ex vivo to mutant ESR1 peptides, HER2 and HER3 peptide libraries were used to identify the lead drug-product candidate. Candidates were additionally counter-screened against wild-type reactivity. Strong immunogenicity due to the srRNA platform was demonstrated against all antigen targets.

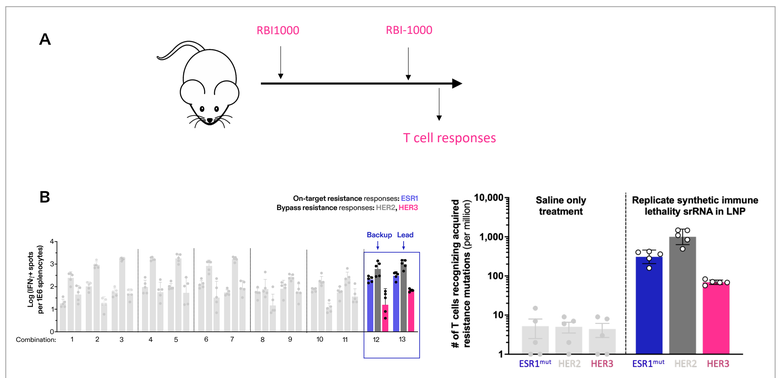

Figure 4: In vivo vector and LNP screening determines optimal drug product for RBI-1000. To identify RBI-1000, the 13 candidates with unique vector and LNP combination were in naïve mice. A) Mice received 2 doses of srRNA and spleens were analyzed by IFN_γ ELISpot. B) shows T cell responses to mutant ESR1 peptides, HER2 and HER3 overlapping libraries. A lead and backup candidate was selected for RBI-1000

RBI-1000 was assessed in a tumor efficacy study to determine whether it primes T cells of sufficient magnitude and quality to control a mouse tumor. Due to the lack of mutant ESR1 syngeneic tumor models, we chose to assess anti-tumor function in a HER2+ tumor model (Figure 5). Tumor-bearing mice received 2 doses of RBI-1000 resulting in significant tumor growth inhibition down to a 0.1 µg dose, with saturation of tumor efficacy at 1 µg. T cell analysis in these mice confirmed the expansion of polyfunctional $CD4^+$ and $CD8^+$ anti-tumor T cells at all doses tested. In this experiment we demonstrate the advantage of srRNA over traditional mRNA which often biases towards $CD4^+$ T cell responses. Additionally, independent of tumor model, we demonstrate tumor control at substantially lower doses than competitive srRNA approaches (10 µg) or linear mRNA approaches (40 µg)

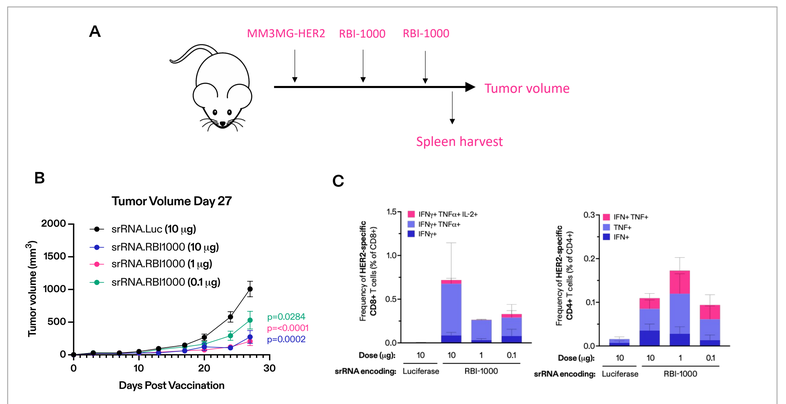

Figure 5: RBI-1000 primes anti-tumor T cell responses resulting in significant tumor growth inhibition in vivo. Tumor volumes in mice implanted with a MM3MG-HER2 stably transfected cell line (A), stably expressing human HER2, at Day 0 and treated with two administrations of RBI-1000 or a control srRNA (srRNA.Luc) at Day 3 and 17. B) Tumor volumes are shown up to Day 27, which was the last day with no deaths (euthanasia was requested at tumor volumes > 2000 mm^3) in the study. C) Splenocytes were harvested and restimulated with HER2 peptide library on day 27 and intracellular cytokine staining was performed. Means and standard errors are displayed. Tumor volume statistics displayed are 2way ANOVA tests of RBI-1000-treated mice compared to irrelevant control srRNA-treated mice. Actual p values are specified.

Discussion

- **Precision immuno-oncology (PIO)** allows quick, cost-effective, and safe drug development for combination tumor therapeutics over traditional small molecule oncology approaches
- To enable **PIO**, Replicate is targeting acquired resistance mutations using **synthetic immune lethality**: our PIO is used in combination with SOC targeted therapeutics
- Replicate has built a library of novel srRNA vectors; our srRNA vectors are advantaged compared to competitive linear mRNA or srRNA approaches in terms of dose and elicited immune responses
- RBI-1000 targets frequent acquired resistance mutations and bypass mechanisms involved in progression of ER+ breast cancer following SOC anti-estrogen therapies
- RBI-1000 induces robust immunogenicity to acquired resistance targets and T cell induced by this therapy can successfully inhibit tumor growth in a mouse tumor model
- RBI-1000 will be advanced into the clinic in ER+ breast cancer patients in 2024 for validation of PIO and the synthetic immune lethality approach to tumor immunotherapy

References

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