A self-replicating RNA Precision Immunotherapeutic for Overcoming Resistance to Endocrine Therapy in ER+BC

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Abstract

Advanced or metastatic ER+HER2- breast cancer (ER+BC) is an incurable disease. Standard 1L therapy utilizes endocrine blockade with a nonsteroidal aromatase inhibitor (NSAI) combined with a CDK4/6 inhibitor (CDK4/6i). While most patients respond and disease remains controlled for over 2 years (mPFS ~25-28 months), virtually all patients progress over time, requiring more toxic therapies with decreasing periods of disease control. Prolonging the time that the tumor remains under endocrine control is therefore of significant medical value. The most common molecular drivers of endocrine resistance are well characterized and include mutations in ESR1 and bypass mechanisms in select signaling pathways. RBI-1000 is a self-replicating RNA (srRNA) precision immunotherapeutic to generate robust immunity directed against acquired resistance mutations that develop ER+ BC in response to endocrine therapy (ET). RBI-1000 includes 6 on-target mutations within the estrogen receptor ligand binding domain, and bypass mutations either in the form of activating mutations in PI3K kinase or amplifications of HER2/HER3. Precision immunotherapy (PIO), combined with SOC targeted therapy, educates the patient's immune system to eliminate tumor cells that express acquired resistance mutations. Thus, the tumor is in a lose-lose situation where it is either eliminated by the targeted therapy, or if it develops acquired resistance mutations to the targeted therapy, it is killed by the PIO. Specifically for endocrine resistance, RBI-1000 PIO offers an attractive therapeutic approach to prevent/delay endocrine resistance specifically, by generating an effective immune response against those tumor cells that express the resistance-associated molecules that arise while on ET+CDK4/6i. The ability to encode multiple targets in a single therapeutic srRNA molecule, alongside the prolonged expression of srRNA in host cells (>49 days) creates a versatile and powerful cancer drug development platform. srRNA precision immunotherapeutics are delivered intramuscular (IM – i.e., a jab in the non-dominant deltoid) and generate an effective immune response via the same pathways and mechanisms as a traditional vaccine. We have demonstrated that this srRNA encapsulated in a lipid nanoparticle primes polyfunctional CD4 and CD8 T cells and antibody responses, leading to tumor growth inhibition and improved survival in a mouse model expressing the targeted acquired resistance mutations. Priming of T cells against acquired mutations is also confirmed in human HLA-transgenic mice. The immune cell-mediated elimination of clones expressing the acquired resistance mutations is predicted to prolong endocrine control of ER+BC, in an analogous manner to small molecule or monoclonal antibody targeted therapies, but with a more favorable dosing and adverse event profile due to precise immunologic targeting and no DDI. RBI-1000 is anticipated to enter clinical studies in the first half of 2024.

Introduction

Replicate's **precision immuno-oncology (PIO)** drug development engine leverages the advantages of self-replicating RNA (srRNA) to improve drug development (Figure 1) of agents targeting acquired resistance mutations. 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.

Synthetic Immune Lethality with multitargeting srRNA

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 and identified a lead in approximately 6 months. (Figure 3).

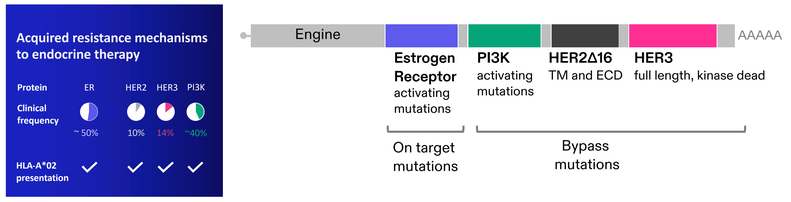

Figure 3: Structure of RBI-1000 cassette. RBI-1000 contains high frequency acquired resistance mutations found in ER+ breast cancer. Specifically, RBI-1000 contains most frequent activating mutations in ESR1 (6) mutations and PI3 kinase (4 mutations), a truncated HER2 gene, and full length, kinase-dead 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.

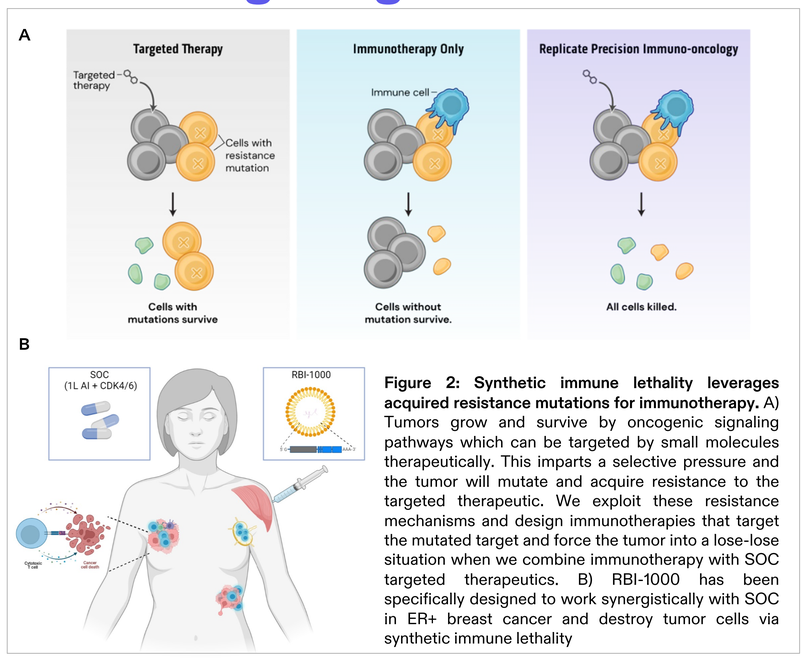

Figure 2: Synthetic immune lethality leverages acquired resistance mutations for immunotherapy. A) Tumors grow and survive by oncogenic signaling pathways which can be targeted by small molecules therapeutically. This imparts a selective pressure and the tumor will mutate and acquire resistance to the 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

Results

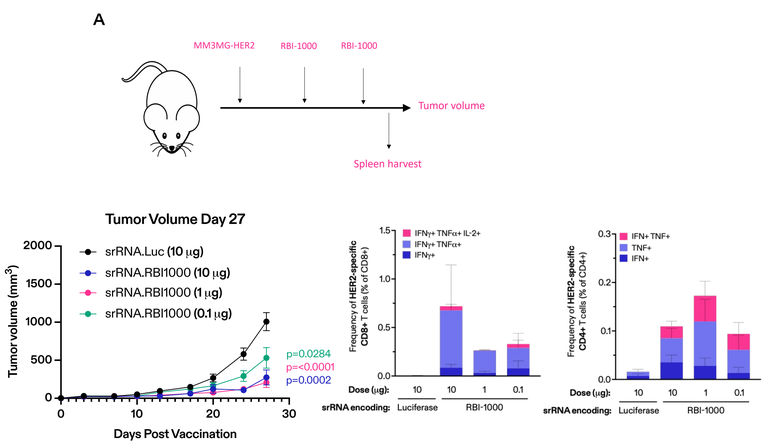

Figure 5: RBI-1000 primes anti-tumor T cell responses resulting in significant tumor growth inhibition in vivo. Tumor volumes in mice implanted with a MM3MG-HER2 stably transfected cell line (A), stably expressing human HER2, at Day 0 and treated with two administrations of RBI-1000 or a control srRNA (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.

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 on both Balb/c and a human HLA transgenic background.

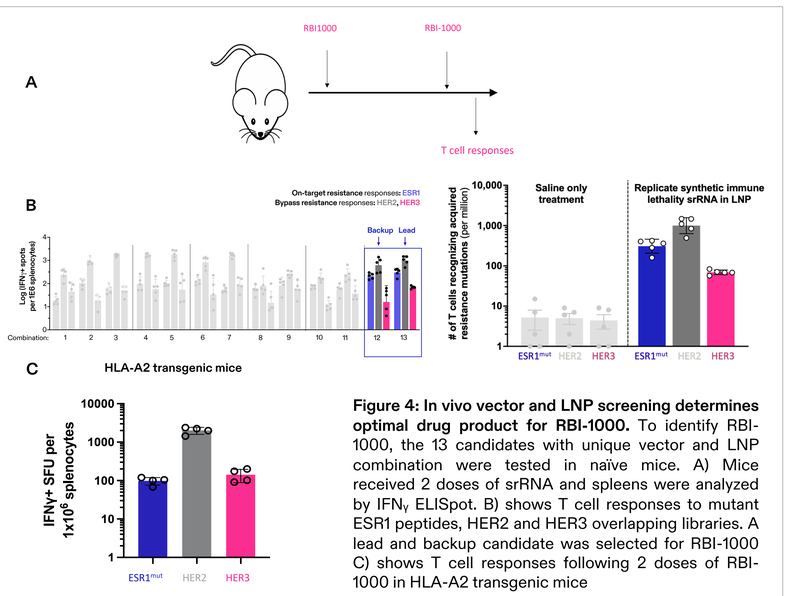

Figure 4: In vivo vector and LNP screening determines optimal drug product for RBI-1000. To identify RBI-1000, the 13 candidates with unique vector and LNP combination were tested in naïve mice. A) Mice received 2 doses of srRNA and spleens were analyzed by IFN_γ ELISpot. B) shows T cell responses to mutant ESR1 peptides, HER2 and HER3 overlapping libraries. A lead and backup candidate was selected for RBI-1000 C) shows T cell responses following 2 doses of RBI-1000 in HLA-A2 transgenic mice

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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- Vogel et al. 2018 Mol Ther
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www.replicatebioscience.com