# Self-replicating RNA therapeutics for Off-the-Shelf Precision Immunotherapy targeting acquired resistance mutations in low TMB tumors

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## Abstract

**Background**
Keynote-942 (AACR 2023; ASCO 2023) represents the first clinical success of a personalized cancer vaccine. mRNA-4157 is a personalized neoantigen vaccine (Moderna) that, when combined with pembrolizumab, demonstrated prolongation of RFS and DMFS when given as adjuvant therapy for high-risk melanoma following complete resection. These findings demonstrate that the immune system can be successfully primed to target cancer neoantigens for clinical benefit; however, individualized vaccines are costly in time and manufacturing, limiting generalizability.
**Methods**
An alternative approach is to focus on shared antigens that arise across a large frequency of patients, enabling an 'o!-the-shelf' targeted immunotherapy. Success factors for such an approach include identification of shared (neo)antigens that are: (1) present in large numbers of patients, (2) specific to tumors, and (3) under evolutionary pressure to be retained. Acquired resistance mutations (ARM) fulfill these criteria as they: (1) arise in the majority of patients undergoing targeted therapy, (2) are specific or over-represented in tumors, (3) are retained as a result of the active and ongoing selective pressure from the targeted therapeutic, (4) have clear prognostic value as they drive treatment resistance, and (5) have not been clinically responsive to existing checkpoint inhibitors. Thus, ARMs are ideal as targets for innovative approaches to cancer immunotherapy.
**Results**
Replicate Bioscience has developed a precision immunotherapy (PIO), RBI-1000, targeting ARM in ER+ breast cancer. RBI-1000 is a self-replicating RNA (16,000 bases on a novel alphaviral vector encoded in a lipid nanoparticle) encoding multiple on-target and bypass mutations that arise as patients are on 1L endocrine therapy (e.g., ESR1m+). Immune responses, inclusive of both antibodies and T cells, elicited by RBI-1000 leads to control and elimination of tumor cells expressing ARM in preclinical models. Coupling targeted therapy SOC and PIO (i.e. estrogen blockade and RBI-1000) generates a synthetic immune lethal state for the tumor: if the tumor retains endogenous ESR1, it is subject to the targeted therapy, while if it develops an ARM, it is now eliminated by RBI-1000. RBI-3000, our EGFRm PIO and second oncology therapeutic, targets the known resistance mutations, and primary mutations that are less responsive to tyrosine kinase inhibitor (TKI) therapy and will be administered in combination with SOC TKIs. The coupling of targeted and PIO therapies is anticipated to better address clinical need in the metastatic setting, with targeted therapy replacing surgical resection to address tumor bulk.
**Conclusions**
PIO is a novel approach to cancer immunotherapy that is widely applicable to any cancer with characterized ARM.

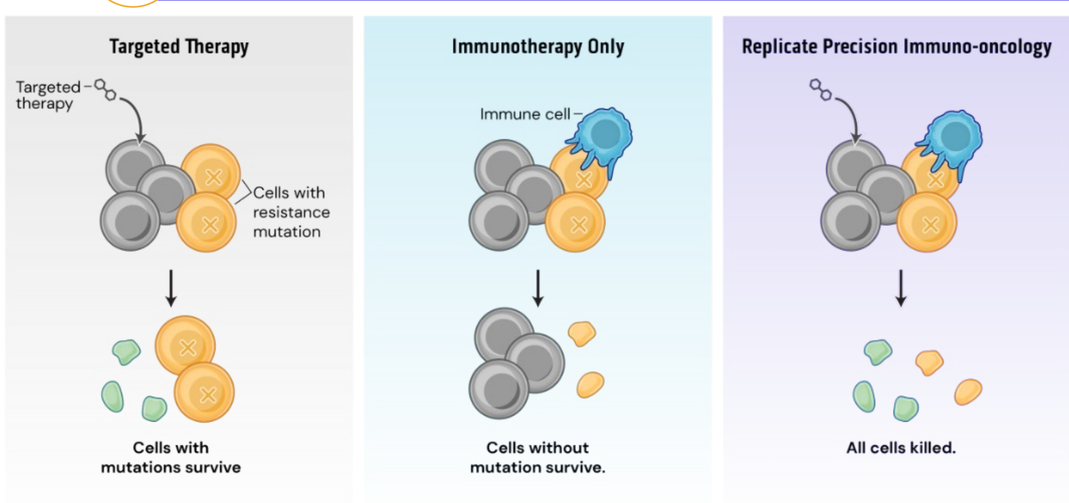
## **Four key development challenges** for precision immuno-therapeutic design

### 1 Target selection given tumor heterogeneity
(tumor specificity; prevalence in patients; evolutionary pressure to retain targets)

| | Tumor-associated antigens | | Acquired resistance mutations | Tumor-specific antigens | | |
|---|---|---|---|---|---|---|
| | Overexpressed proteins, differentiation antigens | Cancer testis antigens | | Oncoviral antigens | Shared neoantigens | Personalized neoantigens |
| **Tumor specificity** | Variable | Moderate | High | IDEAL | | |
| **Prevalence in multiple patients** | High | | IDEAL | High | | Very low |
| **Evolutionary pressure to retain** | Variable | | IDEAL | Low | Variable | Very low |

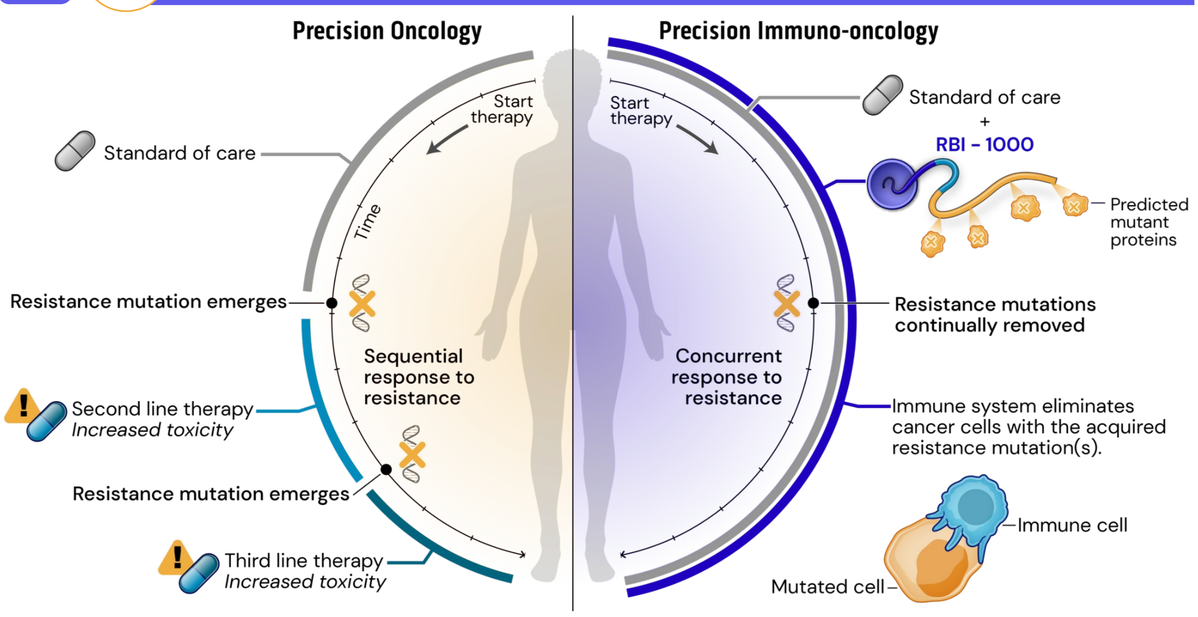
Antigen selection for tumor immunotherapies is key for priming durable immune responses against stable targets:
- **Tumor associated antigens** are highly prevalent but may lack tumor specificity and may have variable evolutionary stability.
- **Neoantigens** have ideal tumor specificity but personalized neoAg have low prevalence and minimal selective pressure to be retained.
- **Shared neontigens** have higher prevalence but have a passive evolutionary pressure to be retained, since the mutation may not longer be required, or a compensatory mutation may easily arise.
- **Acquired resistance mutations** (ARM) are a class of antigens which are tumor specific, widely prevalent and have an active evolutionary pressure to be retained in the presence of SOC therapy.

### 2 Taking advantage of immune surveillance and pruning
(directed immunotherapy targeted against mutations that have an active selective pressure)



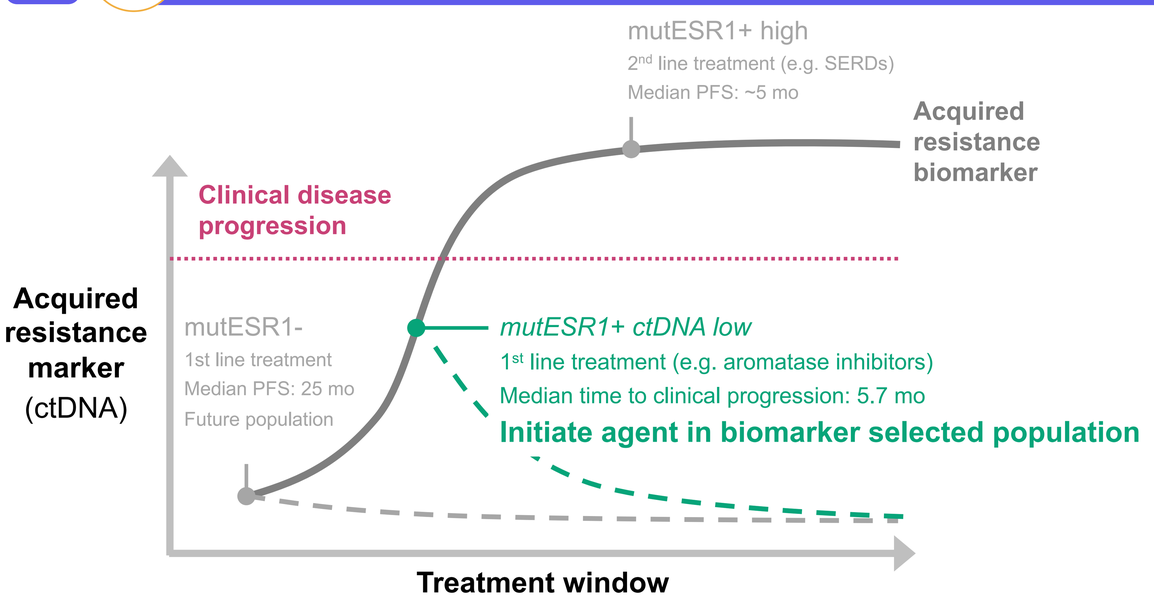

In several low TMB tumors oncogenic signaling pathways have been successfully targeted by small molecules therapeutics. This treatment results in a selective pressure, and the tumor will mutate to acquire resistance to the targeted therapeutic (left panel).
Directed immunotherapies alone may successfully clear cells bearing targeted mutations but are inactive against the non-ARM tumor cells (middle panel).
In **Synthetic Immune** Lethality, the approved targeted therapy acts to prune and debulk the tumor systemically (in the absence of surgical intervention) and exerts and active selective pressure to retain the acquired resistance mutations while our concomitantly administered directed immunotherapy induces ARM-specific T-cells to eradicate the remainder of mutant clones either in the primary tumor or metastasis.

### 3 Debulking tumor to stack deck in favor of immune system
(removing tumor bulk with targeted therapy; immune system targets residual cells)



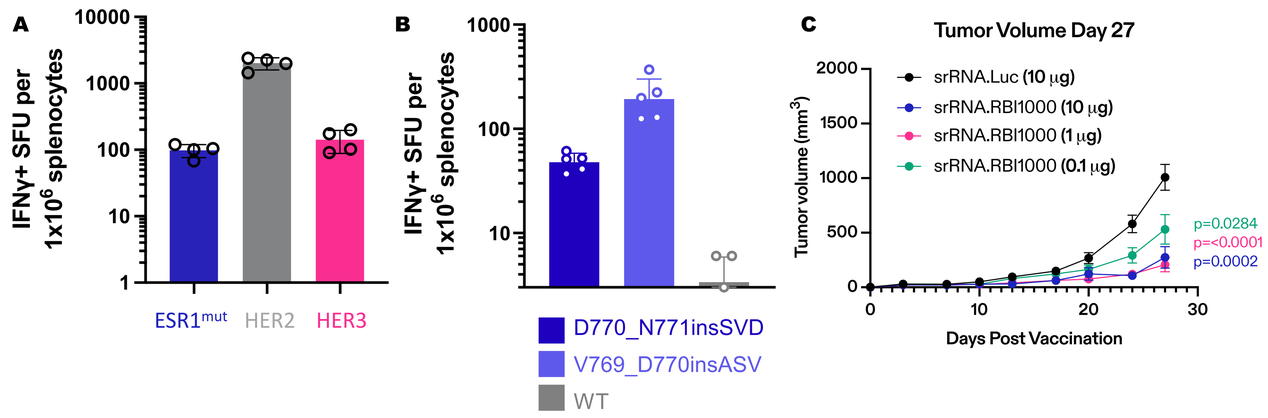

Replicate's **Precision Immuno-Oncology (PIO)** approach is a forced coupling of the targeted therapy MOA with the immunotherapy (srRNA "vaccine").
Recent clinical data has indicated that cancer vaccines can be effective attacking residual tumor cells following tumor debulking (e.g. via physical resection or a chemical therapy).
Our products include multiple common acquired resistance mutations allowing it to be an off-the-shelf agent that can be paired with tumor debulking agents (including systemic agents such as TKIs, hormonal therapies, CDK4/6 inhibitors, etc.).

### 4 Cost and time efficient trials to clinical POC
(clinical vs immunologic endpoint selection; new combos beyond CPI)




The use of ctDNA allows selection of patient populations with emerging molecular resistance prior to clinical disease progression that will most likely benefit from PIO in first line therapy. ctDNA monitoring over time following treatment provides a surrogate marker for early signs of efficacy. The combination of patient selection and immunological monitoring by ctDNA results in improved patient selection and more efficient clinical trials.

## srRNA primes T cell responses target acquired resistance mutations in breast and lung cancer




**Preclinical modeling successfully shows immunogenicity and anti-tumor efficacy by targeting common acquired resistance mutations found in ER+ breast cancer and lung cancer.** A) HLA-A2 transgenic mice received 2 doses of srRNA encoding ARM from ER+BC and spleens were analyzed by IFN$_\gamma$ ELISpot. We successfully measured T cell responses to mutant ESR1 peptides, HER2 and HER3. B) A similar approach targeted common ARM in lung cancer by encoding EGFR exon 20 insert 1&2 in srRNA and immunizing mice. IFN$_\gamma$ ELISpot shows priming of T cells against both targets with no cross reactivity to a wild type version of EGFR. C) srRNA targeting HER2 can prime T cells in tumor bearing mice resulting in tumor growth inhibition demonstrating anti-tumor efficacy at 0.1μg doses of srRNA

## CONCLUSIONS

| | 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 |

- Precision Immuno-oncology (PIO) is advantaged over traditional small molecule and antibody approaches for tumor immunotherapy
- Utilizing our clinical next-generation srRNA platform we can design 1 drug product targeting multiple acquired resistance mutations
- Replicate's srRNA platform has demonstrated clinical safety which will allow combinations without the layering of toxicity associated with small molecule and traditional IO agents
- Next-generation srRNA has fast development times, from target to clinic in 2 years, with low development costs