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Enhancing Immuno-Oncology with RNA Interference

by Dr. Alexey Eliseev, RXi | [Email the author](#)

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The “War on Cancer” declared by the National Cancer Act of 1971 proceeded through a series of evolutionary developments in chemotherapy, radiation treatments and targeted therapies, but until recently was only marginally successful in extending the lives of late-stage cancer patients. A major breakthrough has been achieved over the last decade with the development of immunotherapeutic treatments that empower a patient’s own immune cells to destroy tumors. Two main success stories in immunotherapy are immune checkpoint modulation by monoclonal antibodies, such as anti-PD-1, and adoptive cell transfer of modified immune cells, such as CAR T cells.

CAR T cells targeting B lymphocyte’s CD19 receptor have shown remarkable success in the treatment of hematologic malignancies, leading to multi-year remissions in large subsets of late-stage patients. The results of clinical trials have been so convincing that Big Pharma companies such as Novartis, Celgene and Amgen, among others, have now entered the field of cell-based autologous treatments—a move that will require major adaptation of the currently existing pharma business model. However, CAR T and other types of treatment based on patients’ T cells and NK cells have so far been much less successful in the treatment of solid tumors. One of the major issues in solid tumors is their immunosuppressive microenvironment. This suggests that a combination of the two approaches, immune checkpoint inhibition and adoptive cell transfer, could potentially lead to effective treatment for a broad range of cancers.

The need for checkpoint modulation in CAR T therapies has been well recognized by the medical and scientific communities. A potentially powerful approach is combinations of CAR T with monoclonal antibody drugs—for example, targeting the PD-1/PD-L1 checkpoint axis. Three such antibody drugs (Opdivo and Keytruda for PD-1 and Tecentriq for PD-L1) have already received FDA approvals. Some combinations have been studied in various animal models, and

one of them is currently in a clinical trial by Kite Pharma (<https://clinicaltrials.gov/ct2/show/NCT02926833>).

An alternative approach to targeting immunosuppressive mechanisms is *ex-vivo* treatment of therapeutic cells to eliminate the expression of checkpoint proteins. Because the production of most types of therapeutic immune cells already involves some form of gene editing, an appealing idea is to additionally edit the cell's genome to knock out particular checkpoint genes. The advent of CRISPR/Cas9 technology offers a relatively simple knockout process. In fact, the first ever use of CRISPR in humans was recently made in a Chinese clinical study where PD-1-knockout autologous lymphocytes were given to patients with non-small cell lung cancer (<https://clinicaltrials.gov/ct2/show/NCT02793856>). A more sophisticated engineering of CAR T cells, also involving a PD-1 knockout, was recently described by the group of Carl June. The corresponding product is expected to enter clinical trials this year.

RNA interference offers a more nuanced alternative to gene editing for checkpoint modulation in therapeutic immune cells. Unlike genetic knockouts, the silencing effect by interfering RNA is transient in nature. As such, it avoids long-term issues inherent for the engineered T cells that live and proliferate in the body for a long time and may lead to potentially dangerous side effects.

The use of RNAi to improve the properties of therapeutic immune cells has been explored by several groups. As demonstrated recently in a clinical study, siRNA can be used to target intracellular regulators of immune response (<https://clinicaltrials.gov/ct2/show/NCT02166255>). This offers a potential advantage over monoclonal antibodies that block only extracellular targets.

From the therapeutic perspective, any technology supplementing already complicated *ex-vivo* treatment of autologous cells should introduce minimal disturbance in the cell treatment protocol. But most RNAi delivery techniques either involve the use of toxic (*e.g.* lipid-based) chemical compositions or physical techniques, such as electroporation, that may be destructive for the therapeutic cells. In particular, T cells commonly used for adoptive transfer are notoriously difficult to transfect with siRNA compounds. Even optimized electroporation techniques at best provide about 50-percent transfection efficiency and 70-percent viability, and improving one of these parameters degrades the other.

Enter self-deliverable RNAi technology (sd-rxRNA), which differ from natural and most synthetic RNA interference molecules in that they are chemically modified to allow for an easy internalization of the compounds by most types of cells and silencing of the targeted genes. While still at an early stage, RNAi and specifically sd-rxRNA offer quite unprecedented flexibility in targeting immunosuppressive

pathways. One of the unique features of the technology is that it can modulate multiple checkpoint genes in a single therapeutic treatment. Furthermore, the choice of targets for RNAi is not limited to those expressed on the immune cell surface.

Going forward, the RNAi “cocktail” approach lends itself to highly customized and potentially personalized cell-based immuno-oncology treatments. The recently coined word “immunome” reflects a growing body of information about different immunological mechanisms operating, in particular, in different cancer types and even different patients. It is reasonable to suggest that sd-rxRNA technology, due to its simple technological implementation and flexibility, could be used to treat therapeutic cells with customized RNAi cocktails for individualized tumor- and patient-specific therapies.

An intriguing and open question is whether transient silencing can offer therapeutic benefits over permanent knockouts of immunosuppressive genes by CRISPR or similar techniques. Of course, it is impossible to answer this question definitively until clinical trials show long-term safety implications of in-body proliferation of immune cells that permanently lack control mechanism, such as PD-1. It is likely that each technology will find its niche based on a balance between long term efficacy and safety required for a particular oncology indication. But the ease of sd-rxRNA technology implementation in adoptive cell transfer combined with its flexibility and the lack of potential safety concerns provides it with a unique edge for future therapeutic uses in immuno-oncology.

Alexey Eliseev, PhD, is the chief business officer of [RXi Pharmaceuticals](#), where he is leading the business development initiatives for the Company's immunotherapy program. Dr. Eliseev is a highly accomplished leader with over 20 years of experience in academia, biotechnology industry and venture capital, and most recently was the founder and CEO of MirImmune Inc.

What RXi Pharmaceuticals is working on

The sd-rxRNA technology introduced above was originally developed by RXi Pharmaceuticals for direct *in-vivo* applications. Some sd-rxRNA products are currently in clinical trials by RXi in dermatology and ophthalmology applications. Recently, with the acquisition of startup company MirImmune, RXi has started developing sd-rxRNA for *ex-vivo* treatment of therapeutic cells to silence checkpoint genes and thus expand the therapeutic applicability of the corresponding cell treatments.

Ex-vivo applications are an area where there are competitive advantages of sd-rxRNA technology over “traditional” RNAi that require vehicles or physical techniques for delivery. The transfection of cells and the silencing of targets by sd-rxRNA are achieved with close to 100-percent efficiency while maintaining nearly complete cell viability. This remarkable efficiency has been demonstrated in a variety of therapeutic cell types, such as native human T cells, including tumor infiltrating lymphocytes, CAR T cells and NK cells, among others. Initial in-vivo studies with the cells treated by sd-rxRNA suggest that the knockdown effect can last for weeks, thus providing sufficient duration of checkpoint silencing for therapeutic efficacy.

Some proof-of-concept results were obtained with CAR T cells targeting mesothelin, an antigen selectively expressed in a number of tumor types, including pancreatic and ovarian cancers. Meso-CAR T cells have been tested in the clinic numerous times, but so far their therapeutic efficacy has been limited. It is likely that immunosuppression via various checkpoint pathways is one of the main impediments to the efficacy of these solid-tumor targeting CAR T cells. In their initial studies, MirImmune (now RXi) silenced the PD-1 gene in meso-CAR T cells by *ex-vivo* treatment with the corresponding sd-rxRNA. The subsequent administration of the PD-1-silenced CAR T to a xenograft mouse model of ovarian cancer significantly slowed down the tumor growth, while the untreated CAR T cells were ineffective.

Early results by RXi show that up to four (and likely more) checkpoint genes, both extra- and intracellular, can be silenced when the cells are treated with a “cocktail” of sd-rxRNA compounds. The observed silencing by the mixture is as efficient as by the treatment with any individual sd-rxRNA. The silencing of multiple genes is an area of potential advantage over checkpoint targeting by monoclonal antibodies. Indeed, *in-vivo* combinations of multiple antibodies with cellular treatments will likely lead to high systemic toxicities, let alone prohibitive costs of combining multiple biologic treatments.