Reducing Toxicity of Immune Therapy Using Aptamer-Targeted Drug Delivery

Eli Gilboa, Alexey Berezhnoy, and Brett Schrand

Abstract

Modulating the function of immune receptors with antibodies is ushering in a new era in cancer immunotherapy. With the notable exception of PD-1 blockade used as monotherapy, immune modulation can be associated with significant toxicities that are expected to escalate with the development of increasingly potent immune therapies. A general way to reduce toxicity is to target immune potentiating drugs to the tumor or immune cells of the patient. This Crossroads article discusses a new class of nucleic acid–based immune-modulatory drugs that are targeted to the tumor or to the immune system by conjugation to oligonucleotide aptamer ligands. Cell-free chemically synthesized short oligonucleotide aptamers represent a novel and emerging platform technology for generating ligands with desired specificity that offer exceptional versatility and feasibility in terms of development, manufacture, and conjugation to an oligonucleotide cargo. In proof-of-concept studies, aptamer ligands were used to target immune-modulatory siRNAs or aptamers to induce neoantigens in the tumor cells, limit costimulation to the tumor lesion, or enhance the persistence of vaccine-induced immunity. Using increasingly relevant murine models, the aptamer-targeted immune-modulatory drugs engendered protective antitumor immunity that was superior to that of current “gold-standard” therapies in terms of efficacy and lack of toxicity or reduced toxicity. To overcome immune exhaustion aptamer targeted siRNA conjugates could be used to downregulate intracellular mediators of exhaustion that integrate signals from multiple inhibitory receptors. Recent advances in aptamer development and second-generation aptamer–drug conjugates suggest that we have only scratched the surface.

Toxicity: A Major Challenge for Immunomodulation

Checkpoint blockade using antibodies to target inhibitory immune receptors like CTLA-4 or PD-1 is heralding the emergence of immune therapy as a major modality in cancer therapy. It is by no means a magic bullet; only a proportion of patients, often less than 50%, respond to therapy (1). Nor is it devoid of toxicity. Mirroring preclinical studies in mice, treatment of patients with ipilimumab, the FDA-approved antibody to CTLA-4, is associated with significant autoimmune toxicities (1, 2), and administration of an agonistic antibody to 4-1BB led to severe hepatic toxicities resulting in several fatalities (3). In contrast, nivolumab and pembrolizumab, two recently FDA-approved antibodies to PD-1, are generally well tolerated, though significant toxicities, including fatalities, were encountered in a small proportion of patients. Yet, when antibodies to PD-1 were used in combination with other immune or targeted therapies the added therapeutic benefit was often offset by increasing toxicity. For example, cotreatment with antibodies to CTLA-4 and PD-1 was accompanied by significant toxicities above what was seen with anti–CTLA-4 alone (4), and coadministration of CTLA-4 antibodies and the BRAF inhibitor vemurafenib elicited unacceptable toxicities (5).

Immune-modulatory drugs can be targeted in vivo by conjugation to a ligand that binds to a receptor expressed on the targeted cell. Monoclonal antibodies are currently the method of choice, yet the development and manufacture of monoclonal antibodies and antibody–drug conjugates remain challenging. Peptides

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doi: 10.1158/2326-6066.CIR-15-0194
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generated by phage display libraries constitute another class of targeting ligands; however, their low affinity and nontrivial drug conjugation protocols may be the reasons why they have not been widely adopted.

Oligonucleotide aptamers represent a novel and emerging platform for generating targeting ligands with desired specificity (9). Aptamers that bind to the desired target can be isolated from a random pool of oligonucleotides in a process known as SELEX (systematic evolution of ligands by exponential enrichment), analogous to the isolation of peptide ligands by phage display (Fig. 1). Current protocols can yield (monovalent) aptamers with low nanomolar to mid-picomolar affinities, superior to those of most bivalent IgG antibodies, and the limit has not yet been reached. Unlike antibodies that are cell-based products, aptamers can be generated in a relatively simple cell-free chemical process, and therefore their development, manufacture, and regulatory approval process will be simpler and less costly. The short nucleic acid–based aptamers are not expected to elicit significant neutralizing immunity that may become an issue, especially with repeated administration of even humanized antibodies. Aptamers have been used mostly to target nucleic acid–based biologic or therapeutic agents, such as siRNAs, microRNAs, or aptamers, and have been evaluated in preclinical in vitro and in vivo murine models for cancer and HIV (10, 11). The use of therapeutic agents in the form of short nucleic acid–based cargo is attractive because conjugation to the targeting ligands, in contrast with conjugation to antibodies or peptides, is a simple procedure involving hybridization of short complementary sequences engineered at the end of the aptamer and its cargo. This Crossroads article focuses on the use of aptamer ligands to target nucleic acid–encoded immune-modulatory agents to disseminated tumor lesions or to the (vaccine-induced) immune cells and use selected examples to highlight the value of targeting and the potential of the aptamer platform.

**Receptor Modulation with Aptamer-Targeted Aptamer Conjugates**

To explore the use of aptamer ligands as an alternative to immune-modulatory antibodies, in collaboration with the Sullenger laboratory, we developed blocking CTLA-4 aptamers (12), agonistic 4-1BB aptamers (13), and agonistic OX40 (14).
aptamers, and have shown that in transplantable murine tumor models on a molar basis they were at least as potent as corresponding antibodies.

4-1BB is a major costimulatory receptor transiently upregulated on activated CD8\(^+\) T cells (3). In mice, systemic administration of agonistic 4-1BB antibodies potentiates antitumor immunity, but also elicits CD8\(^+\) T-cell hyperplasia, organ-wide inflammatory responses, and liver damage (15, 16), and in cancer patients severe liver toxicity (3). With the aim of reducing toxicity, an agonistic bivalent 4-1BB aptamer was targeted to the tumor lesions by conjugation to a second aptamer that binds to a product expressed preferentially on tumor cells. In murine tumor models, the tumor-targeted 4-1BB aptamer exhibited a superior therapeutic index compared with an agonistic nontargeted 4-1BB antibody (17).

Given that most receptors internalize upon ligand binding, and thereby will reduce the availability of the 4-1BB aptamer ligand to the tumor-infiltrating T cells, in a subsequent iteration the 4-1BB aptamer was targeted to products secreted into the tumor stroma by conjugation to corresponding aptamers. The premise was that the tumor-infiltrating T cells will encounter and respond to the immune-modulatory ligand before their engagement of the MHC–peptide complex on the tumor cell, and thereby obviate its premature internalization by the tumor cell. Another advantage of targeting immune modulation to the tumor stroma is that, unlike tumor-specific products like PSMA or Her-2, many stroma-secreted products, like VEGF, osteopontin, tenasin-C, metalloproteases, and others, are expressed by virtually all tumor lesions. Under scoring both the potency and broad applicability of stroma-targeted 4-1BB costimulation in preclinical murine tumor models, systemic administration of VEGF-targeted 4-1BB aptamer conjugates engendered potent antitumor immunity against multiple tumors of distinct origin in subcutaneous, postsurgical lung metastasis, methylcholanthrene-induced fibrosarcoma, and oncogene-induced autochthonous glioma models (18). Suggestive of the potency of aptamer-targeted immune modulation, the antitumor effects seen with the VEGF-targeted 4-1BB conjugates were substantially more pronounced than what has been reported in the literature. Consistent with the underlying premise of tumor targeting, the VEGF-targeted 4-1BB aptamer conjugates exhibited a significantly higher therapeutic index than an agonistic 4-1BB antibody; on a molar basis, one tenth the dose of aptamer conjugate elicited comparable antitumor effects without evidence of toxicity, whereas treatment with a therapeutic dose of antibody was associated with immune anomalies and liver toxicity (18).

Ongoing studies using second-generation aptamers, nanoparticle-encapsulated formulations, and multivalent aptamer scaffolds, promise to further enhance their bioactivity and therapeutic value. Tumor stroma–targeted immune modulation with such bi-specific aptamer conjugates could, therefore, represent a next generation of broadly applicable, less costly drugs with significantly reduced toxicity.

Prolonging Immune Responses with Aptamer–siRNA Conjugates

Preclinical studies and clinical trials have shown that, in the setting of chronic infection and cancer, persistence of the immune response, and not its initial magnitude generated shortly after vaccination, correlates with protective immunity (19), supporting an argument that a weak yet long-lasting immune response could be clinically more effective than a potent but short-lived immune response. Strategies that promote the persistence of antitumor immunity will therefore be an important component of successful immune therapy.

The fate of antigen-stimulated T cells is regulated by a balance of intracellular mediators that promote the differentiation of the activated T cells to become short-lived effector cells or long-lasting memory cells. Seminal studies using pharmacologic inhibitors of mediators of effector differentiation, such as the mTOR inhibitor rapamycin or the GSK3\(\beta\) inhibitor TWS119, led to enhanced memory responses and protective immunity (reviewed in ref. 20). Nonetheless, given the broad distribution of their targets, pharmacologic agents can exhibit undesirable immune-suppressive and nonimmune effects. For example, rapamycin inhibition of mTOR promotes the development of immunosuppressive regulatory Foxp3\(^+\) CD4\(^+\) T cells and polarizes dendritic cells (DC) to become tolerogenic antigen-presenting cells, raising significant challenges in translating these findings to human patients.

To promote the generation of long-lasting memory CD8\(^+\) T-cell responses, our group developed a method to limit the inhibition of mTOR activity to CD8\(^+\) T cells, with the use of 4-1BB aptamer-targeted RPTOR siRNA conjugates (21). 4-1BB is transiently upregulated on activated CD8\(^+\) T cells, and RPTOR is an essential component of mTOR complex 1 (mTORC1), but not mTOR complex 2 (mTORC2). Systemic administration of the 4-1BB aptamer–RPTOR siRNA conjugates to mice via the tail vein down-regulated mTORC1, but not mTORC2, activity in circulating 4-1BB–expressing CD8\(^+\) T cells, led to the generation of potent CD8\(^+\) T-cell responses, and enhanced vaccine-induced protective immunity in tumor-bearing mice. In contrast, although nontargeted administration of the general mTOR inhibitor rapamycin also enhanced antigen-activated CD8\(^+\) T-cell memory, both mTORC1 and mTORC2 were downregulated in the CD8\(^+\) T cells, the cytotoxic effector functions of the reactivated memory cells were reduced, alloreactivity of splenic DCs was diminished, and mice failed to reject a tumor challenge (21).

Enhancing CD8\(^+\) T-cell memory and protective immunity in vivo is predicated on the efficient delivery of siRNA to a sizable proportion of the circulating CD8\(^+\) T cells. Yet, unlike delivery to hepatocytes or tumors, delivery of siRNA to normal or malignant hematopoietic cells has been notoriously difficult and inefficient (22). Systemic administration of the 4-1BB aptamer-targeted RPTOR siRNA conjugates via the tail vein was remarkably efficient, leading to the downregulation of RPTOR RNA and mTORC1 activity in more than 60% of the circulating 4-1BB CD8\(^+\) T cells (21). Preliminary studies suggest that siRNA knockdown of CD25, Axin-1, Smad3, or Smad4 in CD8\(^+\) T cells is equally efficient (E. Gilboa, unpublished data).

In summary, 4-1BB aptamer–targeted siRNA delivery to activated CD8\(^+\) T cells in vivo was efficient and specific, resulting in enhanced immune memory and protective antitumor immunity that were superior to that of nontargeted administration of a pharmacologic drug, rapamycin. Future studies will determine whether these conclusions can be extended to other targeting aptamers, other hematopoietic subsets, and eventually to human patients.

Improving on PD-1 Blockade and Immune Modulation

PD-1 blockade is arguably the flagship of immune therapy that has resulted in unprecedented clinical responses with
minimal toxicities (as monotherapy). But it is not a "magic bullet"; only a proportion of patients exhibit significant and durable responses (1). Failure to respond has been attributed to the absence of a preexisting immune response at the tumor site (23), or to the dysfunctional state of tumor-infiltrating T cells that have upregulated multiple inhibitory receptors in addition to PD-1 (24). The two approaches discussed below showcase how the aptamer platform can provide unique solutions to these challenges.

**Sensitizing the tumor to immune recognition**

Expression of PD-L1 in the patient's tumor, often triggered by an intratumoral immune response, has been suggested as a biomarker for responsiveness to PD-1 blockade therapy (25). But it is far from a perfect marker. Some patients whose tumors were PD-L1 negative responded to PD-1 blockade therapy, whereas some patients whose tumors expressed PD-L1 did not respond. In a recent study, Rizvi and colleagues (26) showed that expression of neoantigens in the tumor lesions is a better predictor, and therefore a better biomarker, for selecting patients to undergo PD-1 blockade therapy. This makes sense because neoantigens are the most potent tumor rejection antigens capable of activating immune cells, especially under adverse conditions.

The question remains, however, of what to do with the majority of patients that will be excluded from therapy because their tumors express few or no neoantigens (27). Because the work of Rizvi and others has provided strong, if circumstantial, evidence that neoantigens play a pivotal role in sensitizing tumors for immune recognition, the answer would be to generate neoantigens in the patient's disseminated tumor lesions (with emphasis on "disseminated"), provided it can be done in a clinically feasible, broadly applicable, and cost-effective manner. Several years ago, we described an approach to do just that by inhibiting a process in the tumor cells called nonsense-mediated mRNA decay (NMD; ref. 28). NMD is an evolutionary conserved mRNA surveillance mechanism in eukaryotic cells that detects and eliminates mRNAs encoding "defective products." The hypothesis was that by inhibiting the NMD process such "defective products" would accumulate in the cell and function as potent neoantigens to which the immune system was not tolerized. NMD was inhibited by knockdown of essential NMD factors like SMG-1 or Upf-2 using siRNAs that were targeted to the tumor cells by conjugation to a tumor-specific aptamer. Targeting was essential to avoid NMD downregulation in normal cells that could potentially elicit an autoimmune inferno. In subcutaneous and metastatic murine tumor models tumor-targeted NMD inhibition led to significant inhibition of tumor growth that was superior to that of vaccination with a best-in-class conventional vaccine (28). Increasing the neoantigen content of disseminating lesions may, therefore, become an important component of treatments to enhance the effectiveness of immune-potentiating drugs, not limited to PD-1 blockade.
Reversing immune dysfunction by targeting intracellular mediators of exhaustion

A second reason why some patients may not respond to PD-1 blockade therapy is that the tumor-infiltrating T cells become progressively exhausted, upregulating multiple inhibitory receptors in addition to PD-1, like LAG-3, TIM-3, CD200, or TIGIT (24). Treatment with two or more blocking antibodies is, however, becoming challenging, cost prohibitive, and increasingly impractical. The approach, which, at present, is little more than an idea, is to target intracellular mediators of exhaustion that function downstream to and integrate signals from multiple inhibitory receptors (Fig. 2). A growing list of such candidates have been described (29, 31–37). For example, partial inhibition of Blimp-1 in exhausted T cells led to the downregulation of multiple inhibitory receptors and prevented exhaustion (29). Intracellular mediators of exhaustion can be readily downregulated with siRNA that is targeted to the patient’s exhausted T cells with aptamers that bind to PD-1 or other inhibitory receptors expressed on the exhausted T cells (regardless of whether the aptamer does or does not inhibit its function). The success of this approach will be critically dependent on the ability to deliver the siRNA to a significant proportion of the patient’s exhausted T cells. The preclinical murine studies to promote immune memory discussed above and in ref. (21) suggest that aptamer-targeted siRNA delivery is uniquely capable of doing that.

Conclusions

This Crossroads article introduces a distinctive class of nucleic acid–based immune-modulatory drugs that are targeted to the tumor or to the immune system with oligonucleotide aptamer ligands. Aptamer-targeted nucleic acid therapeutics offer an emerging platform that is uniquely capable of doing that, and prevented exhaustion (29). Intracellular mediators of exhaustion can be readily downregulated with siRNA that is targeted to the patient’s exhausted T cells with aptamers that bind to PD-1 or other inhibitory receptors expressed on the exhausted T cells (regardless of whether the aptamer does or does not inhibit its function). The success of this approach will be critically dependent on the ability to deliver the siRNA to a significant proportion of the patient’s exhausted T cells. The preclinical murine studies to promote immune memory discussed above and in ref. (21) suggest that aptamer-targeted siRNA delivery is uniquely capable of doing that.

References


Aptamer-Targeted Cancer Immunotherapy

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by a bequest from the Dodson Estate and the Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, and a grant (KGO0348) from the Susan G. Komen for the Cure of Breast Cancer Foundation (E. Gilboa).

Published online November 5, 2015.


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