Silent but deadly delivery

By Michael J. Haas, Senior Writer

A team of U.S. researchers has combined immunostimulatory CpG oligonucleotides with small interfering RNA to attack the ability of cancer cells to avoid immune system detection. The group has shown proof of concept in mice[1] and thinks the approach overcomes key hurdles in using the conjugate's components as stand-alone therapeutics—the low potency of CpG oligonucleotides and the challenges of getting siRNAs exclusively to cells involved in a disease process.

CpG oligonucleotides agonize toll-like receptors (TLRs) and activate immune cells. They are readily internalized by antigen-presenting cells such as dendritic cells (DCs), macrophages and B cells.[2,3] Based on these properties, a group at the **Beckman Research Institute at City of Hope** and colleagues postulated that a CpG oligonucleotide could deliver siRNA cargo specifically to immune cells and bolster their response to tumors.

First, the team conjugated a TLR9-targeting CpG with an siRNA against *signal transducer and activator of transcription 3* (*STAT3*) and showed that TLR9-expressing antigen-presenting cells internalized the conjugate more efficiently than they did unconjugated siRNA targeting *STAT3*.

The team had previously shown that expression of *STAT3* enables tumors to evade host immunity by suppressing immune system responses.[4,5]

In a mouse model of melanoma, peritumoral injections of the conjugate lowered tumor growth compared with that in controls. Systemic injection of the conjugate also reduced the number of lung metastases compared with that in controls.

The *Stat3*-silencing activity of the conjugate enhanced the animals' T helper cell type 1 (Th1) immune responses in several ways, including increased production of DCs with high levels of CD40, CD80 and major histocompatibility complex class II (MHCII), and the recruitment of antigen-specific $CD8^+$ T cells to the tumor microenvironment.

Results showed the conjugate had a dual function as an activator of immune cells and an inhibitor of immunosuppressive mechanisms within those cells, said team member John Rossi, professor of molecular biology at City of Hope (*see* **Figure 1, "The dual effect of a CpG-siRNA conjugate"**).

The team, which also included scientists from **The Johns Hopkins University School of Medicine**, was led by Hua Yu, professor of cancer immunotherapeutics and tumor immunology at City of Hope. Data were published in *Nature Biotechnology*.

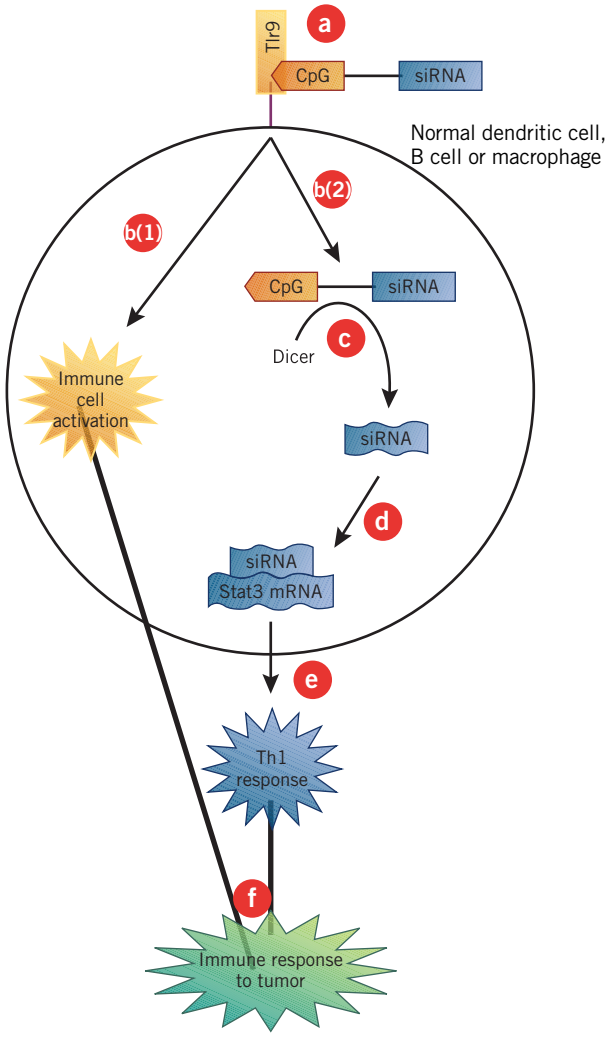

Figure 1. The dual effect of a CpG-siRNA conjugate. Researchers showed that fusing a CpG oligonucleotide with a small interfering RNA molecule targeting *signal transducer and activator of transcription 3* (*Stat3*) led to activation of immune cells and inhibition of immunosuppressive mechanisms within those cells.

In particular, the CpG oligonucleotide component [**a**] binds toll-like receptor 9 (Tlr9) on normal dendritic cells (DCs), B cells and macrophages to activate the cells [**b(1)**] and promote internalization of the conjugate [**b(2)**].

Within the cell, the Dicer enzyme cleaves the siRNA from CpG [**c**], allowing the siRNA to bind and silence Stat3 mRNA [**d**], upregulating the cell's ability to initiate a T helper cell type 1 (Th1) immune response [**e**].

Together, immune cell activation and Th1 response promote an immune response to tumors [**f**], leading to a decrease in tumor growth.

"This manuscript is an exciting demonstration of a novel, combined immunotherapy approach that activates the innate immune

system through TLR9 while also inactivating *STAT3*, one of the counter-regulatory mechanisms that may normally prevent the full benefit of TLR9 activation," said Arthur Krieg, CSO of **Pfizer Inc.**'s Research Technology Center. "The study provides evidence that this strategy may enhance the antitumor effect of TLR9 therapy and provides a rationale to advance this approach toward human clinical trials."

Krieg was a founder and former CSO of Coley Pharmaceutical Group, which Pfizer acquired in 2008 for $230 million. He invented CPG 7909 (PF-3512676), a CpG nucleotide targeting TLR9 that has completed Phase I and Phase I/II trials to treat cancer.

Yu declined to say whether her team's study used CPG 7909.

Antonin de Fougerolles, VP of research, immunology, metabolic and viral disease at **Alnylam Pharmaceuticals Inc.**, agreed that the results provide proof of concept for conjugation-based approaches to siRNA delivery.

"We at Alnylam evaluate and research a variety of different siRNA delivery technologies and have a long-standing effort on conjugate-based approaches," he said.

de Fougerolles did express concerns about the safety of targeting TLRs to deliver siRNA cargo. "Incorporating a TLR9 agonist has efficacy advantages in the context of developing an oncology therapeutic but also has potential for negatively impacting general tolerability" of siRNA therapeutics because systemic administration of a TLR inhibitor could have serious side effects, he said.

He suggested that a locally administered conjugate was likely to be better tolerated, but added that "it will be important to evaluate the advantages and disadvantages in the context of the overall therapeutic window and the disease" with preclinical safety studies.

Yu acknowledged that "not much is known about the safety or toxicity of this conjugate" but told *SciBX* that mice injected with up to 100 µg of the conjugate had no side effects. "We're doing safety studies now, but we don't anticipate any major issues" resulting from the siRNA or CpG parts of the conjugate, she said.

Added Rossi: "In the literature I have seen no evidence that synthetic, unconjugated siRNAs have any toxicity—except that some may stimulate TLRs, which is what we want in this case."

Yu also noted that CpG oligonucleotides have been tested in the clinic, and "while they have not been very potent, they have not shown toxicity either."

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> ***—Arthur Krieg, Pfizer Inc.***

Expanding the platform

Although the City of Hope team's focus is on targeting normal immune cells with the conjugate to treat solid tumors, the team also thinks the compound could target cancer cells to treat hematological malignancies such as multiple myeloma (MM) and leukemia.

"One thing that we discussed in the paper is that TLR9 is often overexpressed by malignant B cells," said Yu. "So we could use this conjugate to target cancer cells" with other types of siRNA to kill tumor cells and/or possibly convert certain malignant B cells into normal, antigen-presenting cells, she said.

The team also plans to explore the applicability of its technology as an siRNA delivery platform to treat indications other than cancer, including inflammation and autoimmunity.

City of Hope has filed multiple patent applications covering the findings and is seeking out-licensing and a range of partnering opportunities, said Brian Clark, director of the institution's Office of Technology Licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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The Johns Hopkins University School of Medicine, Baltimore, Md.
Pfizer Inc. (NYSE:PFE), New York, N.Y.