Doubling the BET

By Joanne Kotz, Senior Editor

Less than seven weeks after a group of academics reported the first potent bromodomain inhibitor[1] and showed the molecule's efficacy in cancer, a separate group of researchers at **GlaxoSmithKline plc** and **The Rockefeller University** has generated a selective bromodomain inhibitor that has therapeutic activity in preclinical models of sepsis and endotoxic shock.[2]

Proteins that contain bromodomains provide a scaffold for the assembly of chromatin complexes that modulate gene expression, including increasing the expression of inflammatory genes.

In a paper published this past September in *Nature*, a team from the **Dana-Farber Cancer Institute** and the **Structural Genomics Consortium** reported the generation of JQ1, a selective inhibitor of the bromodomain and extra terminal domain (BET) family, which is one of the six families of bromodomain-containing proteins. The compound caused tumor regression in a mouse xenograft model of a rare cancer driven by a translocation of a BET family member called bromodomain containing 4 (BRD4).[3]

Now, a team led by Alexander Tarakhovsky, professor of immunology at Rockefeller University, and Kevin Lee, VP and head of GlaxoSmithKline's EpiNova DPU in the Immuno-Inflammation Centre of Excellence for Drug Discovery, has identified another benzodiazepine-based compound called I-BET (GSK525762A). It inhibited substrate binding to BET family members and did not interact with other families of bromodomain-containing proteins.

The researchers first characterized the effects of I-BET in activated macrophages. In bone marrow–derived macrophages treated with the general immune stimulant lipopolysaccharide (LPS), I-BET lowered expression of a subset of LPS-inducible genes compared with vehicle control. In contrast, I-BET treatment of the same cells in the absence of LPS stimulation had minimal effects on gene transcription.

The team next tested the effects of I-BET in mouse models of bacterial-induced inflammation. I-BET cured mice with LPS-induced endotoxic shock, whereas none of the vehicle-treated mice survived. In mice with established bacteria-induced sepsis, I-BET increased overall survival compared with vehicle control.

Data were published in *Nature*.

"The I-BET compounds were shown to exhibit a unique anti-inflammatory profile and so have the potential to provide a completely novel treatment paradigm for immune-inflammatory conditions," said Lee.

"Small molecule regulation of chromatin architecture through the targeting of bromodomains has now emerged as an interesting new approach in anticancer and anti-inflammatory drug discovery," said Stephen Shuttleworth, CSO of **Karus Therapeutics Ltd.** "The team at EpiNova is at the forefront of this area."

Karus is developing a series of oral, selective histone deacetylase 6 (HDAC6) inhibitors that display potent efficacy in preclinical models of rheumatoid arthritis. The company aims to start Phase I testing in 2011.

HDACs, like proteins with bromodomains, are part of a cell's histone acetylation machinery.

Sorting out selectivity

I-BET suppressed some proinflammatory chemokines and cytokines, including IL-6 and IL-1β, but had no effect on others such as tumor necrosis factor (TNF). Thus, a key next step will be to uncover the full subset of inflammatory genes that are suppressed by I-BET under different conditions.

Wayne Hancock, professor of pathology and laboratory medicine at the **University of Pennsylvania**, noted that in macrophages, I-BET selectively affected the genes that become active in the later stages of the inflammatory cascade, whereas housekeeping genes and early proinflammatory genes were not affected by the inhibitor.

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—Kevin Lee, GlaxoSmithKline plc

Hancock wanted to see whether the LPS-induced changes in macrophages are duplicated in the mouse models in which I-BET showed therapeutic benefit. He also suggested assessing the effects of I-BET on T cell–dependent immunity because modulating T cells in addition to macrophages could expand the potential clinical impact of these inhibitors.

"The binary nature of the transcriptional response of the reported BET inhibitor is striking," said Robert Sims, director of biology at epigenetics company **Constellation Pharmaceuticals Inc.** "The transcription of some genes is dramatically decreased by BET bromodomain inhibition, while others are not affected in the slightest."

The real question, said Sims, is whether there are "selective effects that define relevant therapeutic opportunities."

One way to go about answering the question, he said, is to explore the genes modulated by BET inhibitors in response to different types of inflammatory stimuli.

Beyond the shock factor

BET inhibitors may have efficacy in inflammatory diseases beyond sepsis and endotoxic shock. Based on the disclosed macrophage data, Shuttleworth said, "it is reasonable to assume that this compound [I-BET] would display efficacy in a number of immune-inflammatory disease settings, including rheumatoid arthritis, lupus and inflammatory bowel disease, and in organ transplant rejection. Multiple sclerosis is also a possibility, assuming that the compound can penetrate the blood brain barrier effectively."

Sims had a more measured view of a BET inhibitor's chances in chronic inflammatory diseases. Because the paper only reported the effects of the BET inhibitor in sepsis and toxic shock models, which involve rapid inflammatory responses, Sims said it is unknown whether chronically stimulated inflammatory genes would also be sensitive to suppression by I-BET.

In addition, he said, potential toxicity with long-term BET inhibition could limit the use of BET inhibitors to acute inflammatory conditions.

Tarakhovsky said ongoing work in his laboratory includes assessing the impact of I-BET on stimulated B cells, T cells and neurons. He said I-BET selectively represses gene expression in each cell type but noted that the genes affected are very different in each cell type.

Meanwhile, "GSK has made a significant investment in establishing leadership in the bromodomain arena," Lee told *SciBX*. "Numerous programs are underway across a range of therapeutic areas," including investigating the potential of BET inhibitors in a wide variety of immune-inflammatory diseases.

GSK declined to disclose the patent or licensing status of the reported findings.

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Contact: Alexander Tarakhovsky, The Rockefeller University, New York, N.Y.
e-mail: tarakho@rockefeller.edu
Contact: Kevin Lee, GlaxoSmithKline Medicines Research Centre, Stevenage, U.K.
e-mail: Kevin.2.Lee@gsk.com
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COMPANIES AND INSTITUTIONS MENTIONED

Constellation Pharmaceuticals Inc., Cambridge, Mass.
Dana-Farber Cancer Institute, Boston, Mass.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Karus Therapeutics Ltd., Highfield, U.K.
The Rockefeller University, New York, N.Y.
Structural Genomics Consortium, Oxford, U.K.
University of Pennsylvania, Philadelphia, Pa.