From the ashes of a failed pain drug, a new therapeutic path emerges

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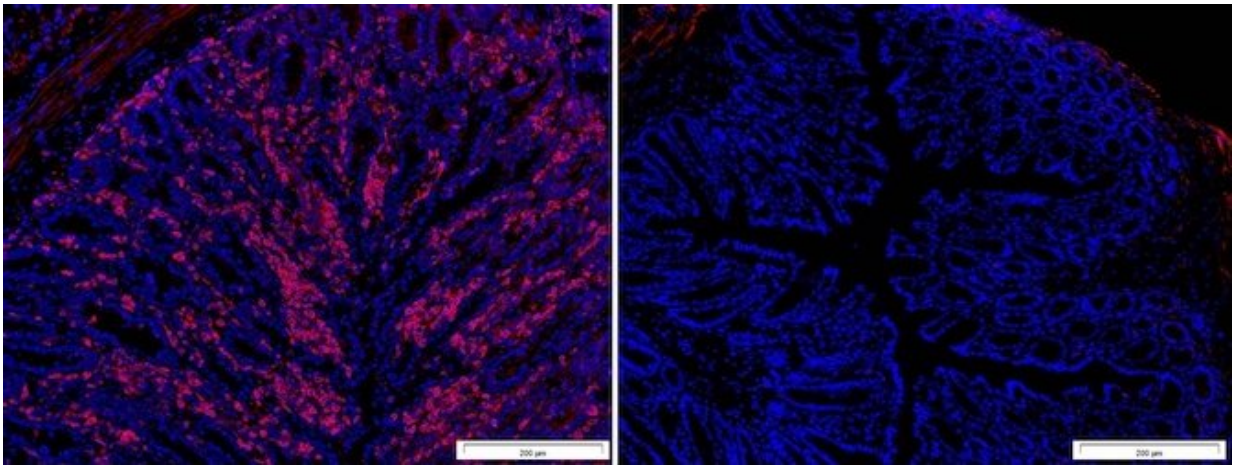

In colitis, an infiltration of activated T cells (shown in red on the left) is clearly visible in the colon. By blocking the BH4 pathway (shown at right), the researchers were able to deeply suppress T cell function. Credit: Woolf Lab/Boston Childrens Hospital/IMBA

In 2013, renowned Boston Children's Hospital pain researcher Clifford Woolf, MB, BCh, Ph.D., and chemist Kai Johnsson, Ph.D., his fellow co-founder at Quartet Medicine, believed they held the key to non-narcotic pain relief. Woolf had shown that tetrahydrobioptrin—a protein also known as BH4—is a primary natural modulator of neuropathic and inflammatory pain sensitivity. Quartet was founded on the premise that inhibiting BH4 production could prevent the progression of acute pain to

chronic pain in millions of patients, without threat of addiction or tolerance.

With solid human genetic data and chemical biology, plus $17 million in series A funding, Quartet looked primed for success. But in the summer of 2017, toxicology studies of the company's lead candidate revealed neurologic side effects. Hope for the promising pain drug cratered, taking Quartet with it.

Now, however, a surprising discovery about BH4 will likely rekindle interest in the once-promising pathway and could have profound implications for treating autoimmunity and cancer. In today's Nature, Woolf and his team at Boston Children's Hospital, together with immunologists from the Institute of Molecular Biotechnology (IMBA) in Vienna report that BH4 also functions as a kind of immunological thermostat in the body, raising and lowering the activity levels of T cells.

In animal models of autoimmune disease and human cell lines, the researchers were able to inhibit T cell proliferation by blockading the BH4 pathway pharmacologically. In models of cancer, they were able to enhance T cell responses by elevating BH4 levels.

"By targeting BH4, we are able to suppress T cell activity in inflammatory conditions and increase their activity in the case of cancer," says Woolf, director of the F.M. Kirby Neurobiology Center at Boston Children's, who co-led the study. "The ability to target the same pathway in opposite directions is significant and represents a whole new therapeutic approach."

An immunological thermostat

Specifically, the researchers found that BH4 regulates the balance of available iron for mitochondria. To transition to an activated state, T

cells need higher levels of mitochondrial energy; to produce it, mitochondria need higher levels of iron. When T cells are under pressure, the body produces more BH4, increasing the supply of available iron, allowing the cells to divide and activate. When BH4 levels are low, mitochondria can't get the iron they need and T cell activity is suppressed. In the case of cancer, the study revealed that a metabolite produced by tumors works to block BH4, inhibiting T cell activation and cancer surveillance. It also showed that this response could be countered by augmenting BH4.

"The beauty of it is that the effect is upstream of specific types of T cell function," says Woolf. "Most drugs being developed now to treat autoimmune conditions are targeting specific kinds of T cells. This covers them all."

The team found that the BH4 pathway is only active in cases of infection or when proliferation needs to occur—and is not required for the normal formation of T cells.

Finally, the paper reports the development of a highly potent small molecule, QM385, that inhibits the BH4 pathway, blocking T cell proliferation and autoimmunity.

Hiding in plain sight

Shane Cronin, a post-doc researcher from Ireland, arrived in the Woolf lab in 2006. He had trained in Vienna with noted immunologist Josef Penninger, MD, Ph.D. at IMBA, and now planned to shift his focus to the neurobiology of pain.

"I wanted to leave immunology behind," says Cronin, lead author of the study. "Fat chance."

Woolf's team had just had its first major BH4 publication, which characterized the pathway as a key modulator of pain. To identify compounds that inhibit the expression of BH4, Woolf devised a drug screen using GFP fluorescent mice and asked Cronin to oversee the project.

The screen yielded plenty of hits—and for Cronin, an odd sense of déjà vu. The results pointed to the same compounds Cronin had used in his previous immunology lab to regulate T cell function.

"First I thought, okay, this is a bit of a quip, but it became very specific very quickly and I knew what I was seeing," says Cronin. But just to be sure, he reviewed existing literature on BH4, and used reagents and technology from a neighboring immunology lab to confirm his initial finding. Woolf was intrigued and encouraged Cronin to keep exploring. But Cronin had a problem: at the time, Woolf's lab lacked the tools and equipment for studying T cells.

When an opportunity to move back to Vienna presented itself, Cronin saw his chance. Penninger agreed to accept Cronin back into his lab at IMBA and threw his full support and knowledge behind the project. Cronin now had access to the resources and experience of one of Europe's leading immunology labs.

"And like that, it just worked out," says Cronin.

'Binary' therapeutic potential

Together, Penninger, Woolf, Cronin and the other members of the BH4 group, spent the next eight years extending their finding into models of immune-related diseases—contact dermatitis, multiple sclerosis, colitis—and finally cancer.

"There was no magic moment—just eight years of collaborative effort, putting together a puzzle, taking it apart, starting again," says Cronin. "But I guess that's the beauty of science—starting with a 'that's odd' moment and finding something incredible."

Working with Penninger, who co-led the study with Woolf, Cronin probed the binary therapeutic potential of BH4. If T cells proliferated in immune-related diseases, he wondered, what about cancer, where the same cells are often suppressed? Penninger and Cronin were able to boost BH4 levels in several mouse models of cancer, and the effect was immediate. Tumors shrank and the metastatic spread all but ceased.

"As a trained immunologist who was involved in defining some of the paradigmatic T cell activation pathways, I had this idea that I basically knew it all and what was left to discover would only be details," says Penninger, who now leads the Life Sciences Institute of the University of British Columbia, Vancouver. "It was like opening an entirely new door in T cell biology—a door we can now rationally close to treat autoimmunity or keep open for T cells to kill cancer."

Which brings us back to Quartet.

Building on a 'successful failure'

In August of 2017, as the company neared completion of its initial IND, leadership received some troubling news. A preclinical study revealed that although the BH4-inhibiting pain drug was "on target," it was also crossing the blood brain barrier at higher than expected levels. Because BH4 also plays an essential role in the production of key neurotransmitters, the team worried that that BH4 inhibition would reduce or prevent certain nerve signals. Ultimately, the decision was made to bring Quartet to a close.

In a blog post, Quartet chairman and founding investor Bruce Booth eulogized the company, hailing it as a "successful failure." The company's three-year investment had characterized the BH4 pathway in vivo in various pain models, developed and tested more than 1,500 potential BH4 inhibitors and produced a vast amount of data.

Those data can now be used to advance the new discovery toward the clinic. Woolf believes that clinical testing for immune-related diseases could begin in as early as 18 months.

"It's unusual to start out with lots of chemistry, lots of knowledge. Normally, you've just got interesting biology and you have to build a startup from there," Woolf says. "Because of the fruits of Quartet's chemistry and data, we're nearly ready to go."

Keeping an open mind

Initial targets of interest for this BH4 inhibitor could include atopic dermatitis, psoriasis, systemic lupus erythematosus, polyarthritis and inflammatory bowel disease. On the oncology side, the team is starting more or less from scratch.

"We're seeing great biological effect in terms of tumor suppression, but we still need to identify an effective pharmacological way to achieve this and address the full safety issues," says Woolf.

The team is exploring compounds to augment BH4 in cancer patients, with the hope they could one day be used either alone or in combination with other therapies, such as immune checkpoint inhibitors. Although it's still early, Woolf and Penninger are excited about the potential applicability of the technique, and a bit in awe of how it all came together.

"It's just strange," says Woolf. "I'm a neurobiologist—I never expected to be working in immunology. But these days, I guess we all try to avoid locking ourselves into silos."

"There are many interesting discoveries to be made at the intersections and borders of fields if one keeps an open mind and is willing to follow what nature tells us," says Penninger.

More information: Shane J. F. Cronin et al, The metabolite BH4 controls T cell proliferation in autoimmunity and cancer, *Nature* (2018). DOI: 10.1038/s41586-018-0701-2

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