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## PRODUCT R&D

# DOWNSTREAM WITHOUT A NET

By Stephen Parmley, Senior Writer

**ModiQuest B.V.** has discovered that its therapeutic mAb against citrullinated histones has the potential to treat autoimmune diseases by inhibiting the formation of neutrophil extracellular traps that play a role in the pathogenesis of autoimmunity. Because it acts on pathways downstream of PAD, the mAb may have fewer side effects than PAD inhibitors already in development.

Last month, ModiQuest spun out Citryll B.V. as a single-asset company to develop the lead therapeutic anti-citrullinated protein antibody (tACPA) for inflammatory diseases such as rheumatoid arthritis (RA).

“Our therapeutic tACPA antibodies have an advantage over small molecule PAD inhibitors because they don’t have a wide tissue distribution like small molecules and they don’t inhibit the normal function of PAD enzymes, and so have a decreased likelihood for adverse effects,” Citryll CEO Helmuth van Es said.

The five members of the PAD family of proteins sit atop a pathological cascade that drives RA and other autoimmune diseases. Various PADs, including **PAD4**, modify histones and other proteins by replacing positively charged arginine residues with neutral citrulline residues, which alters the tertiary structure of the protein and exposes inflammatory neo-epitopes that can make the proteins autoantigenic.

In neutrophils, activation of **PAD4** can lead to hypercitrullination of nuclear chromatin, which results in the formation of neutrophil extracellular traps (NETs) that under normal circumstances eliminate infectious pathogens from circulation. But dysregulated formation and release of NETs also results in citrullinated proteins entering the circulation, where they can be seen as autoantibodies and trigger an inflammatory response. NET dysregulation has been linked to several human autoimmune diseases, including RA and systemic lupus erythematosus (SLE).

“Citrullinated proteins are released into circulation and at some point tolerance against these autoantigens is broken and rheumatoid arthritis develops,” said van Es. “Normally PADs are not extracellular, but when you have tissue damage they can leak out of the cell and then you get a whole cascade that slowly builds up over the years in RA patients.”

In the environment of inflamed joints, extracellular PADs become highly active and citrullinate proteins such as fibrinogen, which contributes to an enhanced local inflammatory response.

However, citrullination of proteins by PADs is also important to normal cellular functions such as skin keratinization, insulation of neurons, and gene regulation. On that basis, researchers at ModiQuest reasoned that targeting the citrullinated autoantigens could be a safer alternative than pan-inhibition of PADs.

At least two companies, **Padlock Therapeutics Inc.** and its partner **Evotec AG**, are developing PAD inhibitors that could treat autoimmune diseases by blocking NET formation. Padlock is currently focused on **PAD2** and **PAD4** because of their disease associations but has not ruled out developing pan-PAD inhibitors. The company declined a request to comment.

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Helmuth van Es, Citryll B.V.

### MODIQUEST'S QUEST

ModiQuest did not set out to develop therapeutic mAbs against citrullinated proteins for autoimmune disease. Instead, the company began by studying the functions of mAbs that are naturally found in RA patients.

van Es said the mAbs arise prior to disease onset in RA, making them useful diagnostic markers, and ModiQuest wanted to determine whether the mAbs contributed to disease pathology or were a by-product of it.

In 2013, a ModiQuest team identified and cloned multiple antibodies against citrullinated proteins from RA patients and tested them in mouse models of acute RA. Surprisingly, the team found that several of the mAbs did not exacerbate disease in the models, but instead treated it.

The team also showed that combining one of these therapeutic mAbs — now dubbed tACPAs — with dexamethasone reduced

inflammation and flares in a mouse model of acute RA when administered after disease onset.

The researchers also mapped the epitope recognized by the lead tACPA to a peptide sequence in the N-terminus of histone 2A, a site that **PAD4** citrullinates. The mAb did not stain tissue arrays from healthy volunteers but did stain healthy granulocytes and macrophages, indicating the compound might have minimal off-target effects. However, ModiQuest did not determine the antibodies' full mechanism of action in that study.

The company reported the data in the *Journal of Clinical & Cellular Immunology*.

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In May, ModiQuest researchers reported that the lead tACPA inhibited NET formation. In a human neutrophil-based assay of calcium ionophore-induced NET formation, the lead tACPA decreased NET formation compared with a control antibody. The company presented the data at the 2015 Protein and Antibody Engineering Summit (PEGS).

van Es told BioCentury that the tACPA inhibits formation of NET lattices, which prevents the release of new autoantigens and proinflammatory factors. In addition, he said, tACPAs could work in concert with macrophages to enhance clearance of NETs, NET remnants, and the toxic citrullinated histones from tissues and circulation.

Those additional effects would give a tACPA more than just a safety advantage over PAD inhibitors, said van Es. "We have in our hands an antibody that can inhibit NETosis, that binds citrullinated epitopes on histones, and could likely interfere with

the toxicity of histones, since circulating histones contribute to a number of different disease phenotypes as well."

Although Citryll has not selected the lead indication for the tACPA, the company will continue testing the mAb in RA models and will compare it to JAK and PAD inhibitors, van Es said.

"There is a whole bunch of diseases where we think inhibition of NETs could be therapeutic," such as idiopathic pulmonary fibrosis (IPF), where NETs play a role in fibrosis formation, he said. "We have preliminary *in vivo* data for IPF as well as colitis. We are also developing biomarker assays based on the literature and our own findings that should allow us to measure NET components in the sera of RA patients and select the right patient population for tACPA treatment."

According to van Es, about 15% of RA patients have antibodies that activate **PAD4**, resulting in severe disease. These antibodies would be markers for patients most likely to benefit from tACPA.

Having funded the project using internal ModiQuest funds, van Es said Citryll is now actively seeking a partner.

"Since we are a small company, we need to be very focused on one disease," he said. "We are looking for a development partner because they might have more insights into progressing the molecule in other diseases."

ModiQuest has multiple pending and issued patents covering the tACPA antibodies and their therapeutic uses in RA and pulmonary fibrosis. Citryll has an exclusive option to license the IP portfolio. **■**

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#### COMPANIES AND INSTITUTIONS MENTIONED

Citryll B.V., Oss, the Netherlands  
Evotec AG (Xetra:EVT), Hamburg, Germany  
ModiQuest B.V., Oss, the Netherlands  
Padlock Therapeutics Inc., Cambridge, Mass.

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#### TARGETS AND COMPOUNDS

JAK - JAK kinase  
PAD (PAD1) - peptidyl arginine deiminase  
PAD2 (PAD12) - peptidyl arginine deiminase type II  
PAD4 (PAD14) - peptidyl arginine deiminase type IV

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#### REFERENCES

Chirivi, R., et al. "Anti-citrullinated protein antibodies as novel therapeutic drugs in rheumatoid arthritis." *Journal of Clinical & Cellular Immunology* (2013)  
Martz, L. "Padlock's keys to academia." *BioCentury Innovations* (2015)  
Rhodes, J. "Unlocking PADs." *BioCentury* (2015)

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