SPOT LIGHT

A Quick Pivot to COVID-19 Research with CyTOF

LORI TURNER, PHD

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Moving forward with mass cytometry in the midst of the pandemic

Lori Turner, PhD, a postdoc in the NIHR Cambridge BRC Cell Phenotyping Hub and the Department of Psychiatry at the University of Cambridge, had been using the Maxpar® Direct™ Immune Profiling Assay™ in a potentially groundbreaking search for treatments for depression that act through the immune system.

But with the onset of the COVID-19 pandemic, the multi-site Antidepressant Trial With P2X7 Antagonist JNJ-54175446 (ATP), the first clinical study by the Wellcome Trust Consortium, came to a halt in March 2020.

While the team waited for the ATP trial to restart, its members decided to repurpose their entire workflow to do a COVID-19 study. “We had everything sitting there ready to go. We thought, this is a perfect opportunity to immunophenotype COVID-19,” says Turner.

The team got back to work using that same workflow staining, fixing and freezing COVID-19 samples and then thawing them later for analysis. The ability to work with frozen samples was a defining factor of the new study, since more samples were coming in than could be analyzed fresh. The team also purified white blood cells and performed immunophenotyping on them.

The study gave Turner a unique opportunity to compare flow versus mass cytometry data for quite a large number of patients, since their new analysis used both. She found that across the various types of immune cells – naive B cells, memory B cells, gamma delta T cells, MAIT cells and CD4 naive central memory cells, to name a few – there was “a remarkable correlation” in the data. “I was really impressed to see that,” says Turner.

Moving forward, Turner is considering using the workflow in other studies as well, especially as things have changed with the pandemic. “We are not recalling patients into the hospital, and so we’re planning lots of multi-site studies in the future,” she notes. By validating the workflow for general use, it will become very easy for the lab to adopt quickly. “You add the whole blood to the [Maxpar Direct Assay] tube, stain it for 30 minutes, add a stabilizer for 10 minutes and then stick it at –80 °C. Or stick it on dry ice and ship it to a central site where samples can be analyzed.” With several trials using the workflow in planning stages, Turner thinks it’s going to make a lot of their work much simpler going forward.

Listen to Lori Turner’s story :
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The influence of inflammation on mental health

How a new clinical trial using CyTOF® could change treatment options for depression

As an immunologist with a background in host-pathogen interactions, Turner explores immune system function and dysregulation in various psychiatric disorders.

> **"With depression ... we see a spectrum of symptoms. We believe that different symptoms correlate with numbers of certain immune cells and changes occurring in these cells."**

Before ATP was put on hold, Turner was coordinating the biomarker studies in the clinical trial as part of the Wellcome Trust Consortium for the Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA). Rather than target the nervous system, as do many current medicines for depression and neurodegeneration, the NIMA consortium focused on the immune system. Several studies have already linked the immune system to psychiatric and neurodegenerative disorders, revealing increased levels of inflammation in patients with depression[1,2] and Alzheimer's disease[3]. By bringing together academia and pharmaceutical companies, NIMA investigates whether targeting the immune system could become an effective alternative treatment.

Immune cell biomarkers in depression

Certain immune cell subsets are increased in depression while others are decreased, and these changes relate to the level of severity of specific symptoms. Turner focuses on whether new treatments could influence these changes and subsequently affect symptoms. "With depression, some people feel anxiety or fatigue for example, and some people don't – we see a spectrum of symptoms. We believe that different symptoms correlate with numbers of certain immune cells and changes occurring in these cells. This is what we've set out to identify," explains Turner.

In the Phase 2 clinical trial, the research team intends to test an anti-inflammatory that crosses the blood-brain barrier to block activity of the P2X7 receptor, present on various immune cells and linked to stress-related depression. The group will perform immune profiling by mass cytometry to monitor the immune system before, during and after treatment. With any observed response, further

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study could identify immune biomarkers related to that response. Patients will also complete additional blood tests, questionnaires and magnetic resonance imaging brain scans throughout the trial to more comprehensively understand anti-inflammatory effects on the immune system and the brain.

Neuro-immunophenotyping with CyTOF

Turner came into mass cytometry through her work with the flow cytometry core facility at Cambridge. While her early research used flow cytometry, her more recent work at the Cambridge Biomedical Research Centre (BRC), part of the National Institute for Health Research (NIHR), requires larger panels that can only be analyzed by mass cytometry. In the first phases of the study, Turner directed preclinical work to assess depressed participants and healthy controls by mass cytometry, using the Standard BioTools™ customizable human immune monitoring kit. Initial data focused on the P2X7 receptor and potential associated biomarkers.

For the clinical trial, the team moved to the Maxpar Direct Immune Profiling Assay. "Since standardization is extremely important for a multi-site clinical trial, we needed a pre-validated, as-is kit that included the markers we were focused on and that could be used across multiple sites since the trial will be carried out across five centers in the UK," says Turner. "The idea of being able to add a whole blood sample directly to a pre-prepared assay tube, reducing site-to-site variation, was a huge factor for us. And the inclusion of Maxpar Pathsetter™ software allows us to quickly generate a report that goes into our online database, which is then shared with the clinical team for complete quality control."

As the trial moves forward, the group is optimistic about the opportunity to identify related biomarkers that indicate response to the treatment. Good results could then initiate an expanded trial with more and different types of participants. Turner notes that the Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter software can evolve with the study, allowing addition of new markers to investigate other aspects of immune cell subsets. For a list of this and other clinical trials employing mass cytometry, go to clinicaltrials.gov.

Learn more about the award-winning Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter software.

Read multi-site study publications:

Bagwell, C.B. et al. "Multi-site reproducibility of a human immunophenotyping assay in whole blood and peripheral blood mononuclear cells preparations using CyTOF technology coupled with Maxpar Pathsetter, an automated data analysis system." *Cytometry Part B Clinical Cytometry* 98 (2019): 146–160.

Geanon, D. et al. "A streamlined CyTOF workflow to facilitate standardized multi-site immune profiling of COVID-19 patients." *medRxiv* (2020): doi.org/10.1101/2020.06.26.20141341.

> "Since standardization is extremely important for a multi-site clinical trial, we needed a pre-validated, as-is kit that included the markers we were focused on and that could be used across multiple sites ...

View results and download white paper:
Deep Immune Profiling with the Maxpar Direct Immune Profiling System

Download poster:
The Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter Analysis

References:

1. Chamberlain, S.R. et al. “Treatment-resistant depression and peripheral C-reactive protein.” *British Journal of Psychiatry* 214 (2019): 11–19.

2. Lynall, M-E. et al. “Peripheral blood cell immunophenotyping reveals distinct subgroups of inflamed depression.” *bioRxiv* (2019): doi:10.1101/706309.

3. Hakobyan, S. et al. “Complement biomarkers as predictors of disease progression in Alzheimer’s disease.” *Journal of Alzheimer’s Disease* 54 (2016): 707–716.

Learn more about the award-winning Maxpar Direct Immune Profiling Assay and Maxpar Pathsetter software:
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