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THE PRIMARY CRISPR DIRECTIVE – THE NEXT FRONTIER IN CELL SCREENING

A conversation with **NICOLA MCCARTHY**, Screening Business Unit Manager, Horizon Discovery Group

In drug discovery, companies use CRISPR-based cell screens to look for signs of drug resistance and sensitivity, find targets for synthetic lethality, and decipher complex phenotypes. The cells used are stable, predictable and easy to handle. However, these 'cell lines' have adapted to survive in culture, which can cause them to differentiate from the human tissues they are supposed to represent. Primary cells taken directly from people, for example T cells from blood donors, are more physiologically relevant than their cell line counterparts. However, using them for screening has proven difficult: it is hard to keep them alive long enough for the editing and in vitro analysis and the screening techniques applied to cell lines will not always work for primary cells. After several years of research, Horizon Discovery has developed a CRISPR screening technique that works in primary T cells.

Why is it important to focus on primary cells?
Our customers asked us to develop CRISPR screens that use primary cells to improve their drug discovery outcomes. Because primary cells are closer to a patient's cells, the screens can identify targets that could be more relevant to the clinic. By contrast, screens of continuously cultured cancer cell lines that have mutated and adapted to grow in culture medium might give skewed results compared with healthy tissue.

What has changed in primary T cell work?
Our work in primary T cells has been helped by advances in the cell therapies that have now made it into patients. Companies and researchers developing adoptive T cell and CAR-T cell therapies have found ways to keep autologous and allogeneic cells alive in culture long enough for manipulation, expansion and delivery to the patient. These technologies and techniques that allow cell therapies to move from bench to bedside are now returning to the bench and being used in new applications for in vitro screening. The biggest changes have been in the improvements in culture media and the commercial availability and ease of use of the antibodies required to activate the T cells as part of the screening process.

What was the development process for these screens?
When I started working on CRISPR-Cas9 screening with the immunology team three years ago, we started with primary T cells. We made this choice because our CRISPR libraries are lentivirus-based, and we knew that this cell type could be infected with a lentivirus; we believed this would be a straightforward way to begin the project. But it turned out to be harder than we thought.

For some reason – and we are still not clear why – delivering Cas9 to primary T cells using a lentivirus vector was really hard. Other groups have found this challenging as well. Further work showed that we could deliver Cas9 mRNA using electroporation, and so we created a dual system, using both the lentivirus and the electroporation technology as a workaround. All in all, it took two and a half years of research. In January 2019, Horizon Discovery's Screening Unit extended its CRISPR screening service to include ex vivo T lymphocytes for immunology-based research in drug discovery.

PRIMARY CELLS PROVIDE A SURROGATE MODEL THAT IS ONE STEP CLOSER TO THE CLINIC

How did you validate your approach?
To check that our primary cell screen is not missing anything that screens in cancer cell lines can find, we carried out a proof-of-principle screen in primary T cells, duplicating published work that had been conducted in 2015 in Jurkat cells — a leukaemia T cell line. We used a library targeting genes that regulate metabolism and asked the same question as the published paper: which genes regulate sensitivity to the drug phenformin? Our primary T cell screen found the same published target, GOT1, and we also found something new — additional genes that the screen in the Jurkat T cell line missed and we are working to validate them. Because primary cell screens use cells that more closely mimic the cells in the body, these additional targets could be more relevant, potentially reducing failures at the translation between bench and bedside.

Where can the primary T cell-based CRISPR screens help?
These screens will be useful in immuno-oncology, where pharma and biotech companies can use them to select candidates for development, for example finding drugs that will stop the tumour microenvironment 'switching off' T cells, or ensuring that T cells survive and remain active for longer. Drug developers can also use the screens to assess their existing pipelines and verify the mechanism of action of molecules in preclinical and clinical studies.

What's in the future for these types of CRISPR screens?
We want to extend this screening approach to other immune cells, such as B cells, macrophages and natural killer cells. As well as looking at mechanisms to upregulate the immune system, the screens could also pick out ways to turn the immune system down in autoimmune disease.

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