We need to talk about flow cytometry

This technology has vast potential to help patients but remains underutilized and underappreciated. The tools are there. What's lacking are harmonized controls, regulatory guidelines and database options for sharing validated tests.

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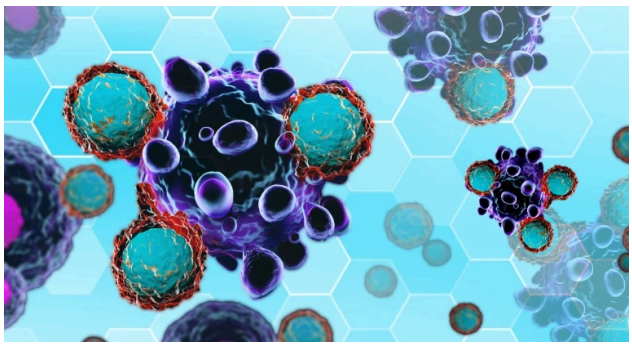


Flow cytometry can be a vital tool in CAR T cell therapy, used in lymphocyte collection, in vivo monitoring and functional evaluation of infused cells. *Credit: Slingshot Biosciences, Inc.*

It may surprise many to hear that some of today's most advanced treatments, such as cell therapies for cancer, diabetes, and autoimmunity, depend on flow cytometry — a 50-year old technology.

Chimeric antigen receptor (CAR) therapy, for instance, involves removing a patient's cells, reprogramming and reinfusing them into the patient, to seek and destroy the cancer. And the role of flow cytometry? "It is how you identify the cells that will go in the product," says Therese Choquette, head of analytical sciences at Switzerland-based Tigen Pharma.

Although flow cytometry is dealing with modern, complex biomedical challenges — from drug development to patient diagnosis and monitoring — regulatory approval has not kept pace with technical advances. Most flow cytometry assays are lab-developed tests (LDTs) — designed, manufactured and validated within a single laboratory. Currently, there are limited

database options for sharing flow cytometry assay insights, and the lack of FDA-standardized controls makes test development difficult. This is a stark contrast to fields like clinical chemistry, where methods are tightly regulated and harmonized using controls. “Guidelines available today for validation of analytical methods are not adapted to cell therapy,” Choquette says. “So, you need to be really innovative.”

As a result, Choquette and her colleagues must not only develop new assays for every cell type, but they also need to create a way to test assay accuracy. With more specific regulatory guidelines and harmonized controls, this process could be streamlined, speeding up the development process and improving time-to-market for novel therapies.

It’s not just researchers’ time and money at stake: without more streamlined approaches to standardization for flow cytometry assays, there’s a risk that patients will miss out on potentially life-saving therapies.

What is flow cytometry?

The first commercial flow cytometer was developed as a research tool in the 1960s, and many clinicians still think of it as a research tool.

“Flow cytometry gives clinically important and actionable results,” says Oral Alpan, an allergist and immunologist and founder of the biotech Amerimmune. “But the community has to be educated on flow cytometry’s use in clinical medicine.”

In a flow cytometer, cells suspended in a solution move, one by one, through laser light. Surrounding detectors collect the reflected light, deriving information about the cells. In a sample prepared with, for instance, a fluorescently labelled antibody, cell-surface markers can be analysed for diagnosis and disease phenotyping — flow cytometry can count cancer-induced changes in white blood cells and cell-surface receptors associated with cancer’s growth, and much more.

Oncology is the most fertile ground for novel applications of flow cytometry. As Hemant Mishra, principal scientist at Caribou Biosciences, wrote[1] in a review of flow cytometry in immunotherapy: “In addition to diagnosis, there are several other applications of flow cytometry in the management of various cancers which include treatment monitoring, or even selecting a personalized precision-based immunotherapy in synch with advanced genetic tests to increase the chances of favourable prognosis and complete remission.”

Andy Rawstron, consultant clinical scientist at HMDS (Haematology Malignancy Diagnostic Service) in Leeds, United Kingdom, agrees: “We have recently focused on developing high-sensitivity tests to detect very low levels of residual disease across a broader spectrum of blood cancers.” These tests can detect some types of lymphoma at 10–100 times lower levels than existing methods.

The number of molecular targets that can be tracked in modern cytometers increases as the technology matures. “We can now identify which people with early-stage blood cancer are most at risk of infection or having a sub-optimal treatment response,” Rawstron says.

Beyond oncology

But flow cytometry is not limited to cancer care and can be used to personalize treatment for many conditions. “An increasing number of small molecule inhibitors and therapeutic antibodies effectively target autoimmune disorders,” says Rawstron. Some treatments need a companion diagnostic to confirm the drug's suitability for the patient. If this involves, for example, monitoring a gene for a specific cell-surface marker, flow cytometry can detect it.

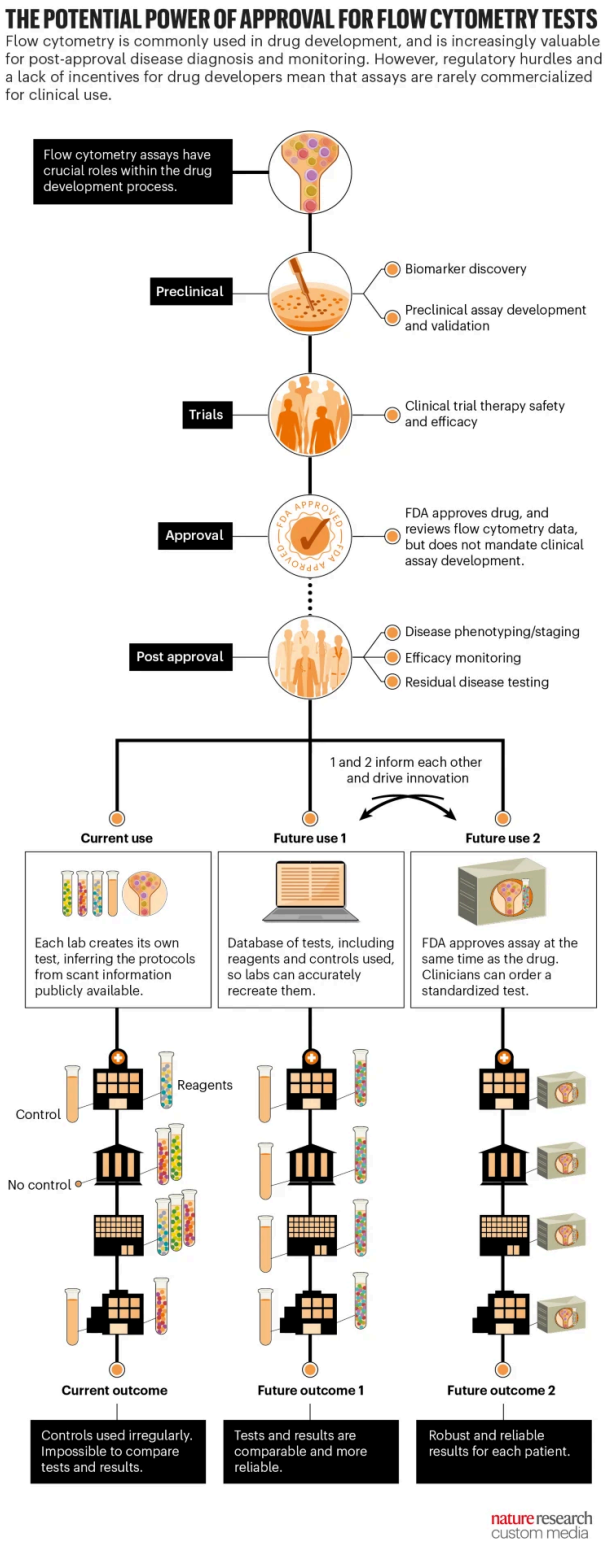
In 2015, Rawstron and colleagues used flow cytometry to assess methotrexate treatment outcomes in rheumatoid arthritis[2]. Methotrexate does not produce a response if it doesn’t eliminate enough malignant B-cells. Flow cytometry can count these cells. If the technology sees that the methotrexate didn’t adequately reduce a patient’s load of B-cells, an extra dose of the drug “produced both better depletion and clinical responses than placebo with no worsening of safety,” Rawstron’s team reported.

Existing tests can also be applied to more diseases. Alpan describes the basophil activation test (BAT), which uses flow cytometry to detect surface changes in these immune cells after exposure to an allergen[3]. “First reported 32 years ago, it has still not been FDA-approved, because there was no push by industry to commercialize it or validate it clinically,” Alpan says. He and his colleagues have used the test to predict a range of allergies to foods, including peanuts[4].

CAR cells are also expected to be used outside of oncology. The standard CAR approach uses T cells, which are primed to fight infections. With the right gene editing, they can be engineered to tackle anything from heart failure to fungal infections[5].

For these burgeoning applications, flow cytometry provides a window into cell populations. For the technology to be broadly adopted in the clinical space, however, assays need to be validated, using controls to separate the intended target from background signals, and for reproducibility, at scale. [see 'The potential power of approval for flow cytometry tests']

The challenge is that there are few off-the-shelf controls. “A major issue for flow cytometry is the lack of cellular standards with known expression of the proteins of interest,” says Rawstron.



Overcoming obstacles

Slowly, flow cytometry advocates are addressing these issues. Alpan and his colleagues are working toward FDA approval of their BAT test[6]. They recommend engaging with regulators to discuss a test's technical features, such as the instruments and the reagents to be used, during a trial's planning stage. Hopefully, this can reduce some of the challenges raised in later regulatory steps. The FDA also plan to tighten oversight of LDTs to make them safer and more accurate.

Choquette is hopeful that results can be made more reproducible between labs using synthetic controls. “Slingshot Biosciences provides standardized sets of T cell mimics that have the same properties as the cells you are working on,” she explains. “This helps with the validation.”

Slingshot is continuing to expand its offering of control-cell types, labelled with a broad selection of markers for many uses. The company produces synthetic white blood cells to replace their biological counterpart, which have notoriously poor stability and consistency, with a robust product for pre-clinical and commercial applications.

Good controls are an important part of the flow cytometry conversation, but not the only one. Better education for clinicians on the technology’s use will also be important: from development, to manufacturing, to diagnostics. As Choquette says, “Every new product is always about the patients and the hope that it gives them.”

For more information on Slingshot Bio’s synthetic cell technology, visit their website.

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