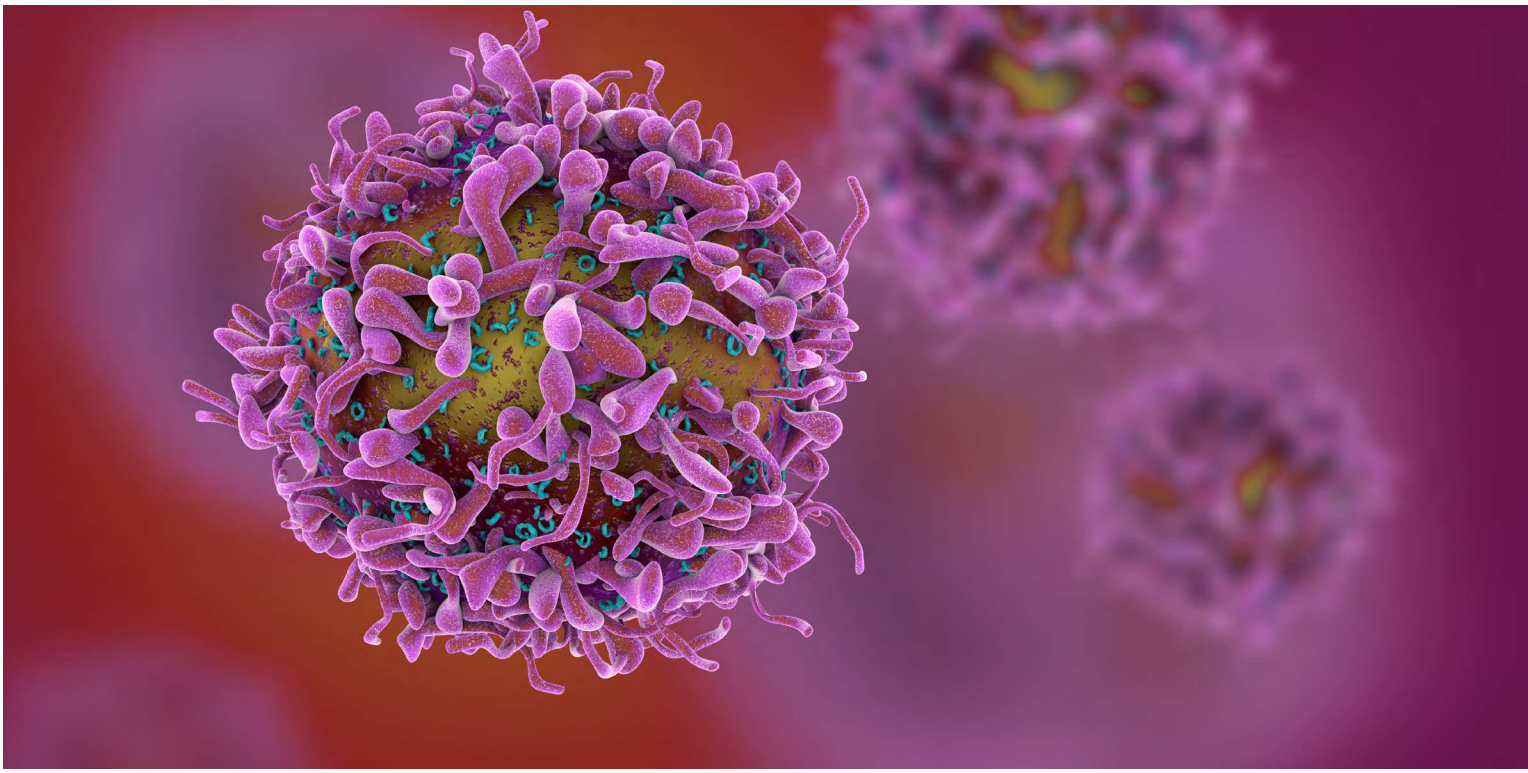
# Immuno-Oncology: Pushing the Frontier of Discovery Through Advanced High Throughput Flow Cytometry



Immuno-oncology (IO) is increasingly valued as an effective therapy in cancer management. The field encompasses a number of approaches with one common thread: they harness the body's own immune system to fight cancer.[1] Treatments range from cell-based methods, *e.g.*, adoptive cell therapy (ACT) employing immune cells, to antibodies such as checkpoint inhibitors, which were recognized with a Nobel Prize in 2018.[2,3] Advances from bench to bedside have been rapid, culminating in several recent US Food and Drug Administration (FDA) approvals.[4,5] Despite this progress, obstacles remain, for instance the high cost and technical difficulties of phenotyping and purifying immune cells. Perplexingly, some patients show a remarkable treatment response, whereas others do not, and uncovering the mechanistic basis for these differing outcomes is an active area of research which aims to expand general IO efficacy. "Traditional" flow cytometry has proven instrumental for identifying the biology and function of various immune subtypes, but is limited in throughput, slowing analysis and increasing assay volumes and cost. Methods such as ELISpot and ELISA are higher throughput but do not enjoy single-cell resolution nor multiplexing capacity. Addressing the challenges facing IO will require improved technical platforms to accelerate discovery. Advanced high throughput flow cytometry addresses the shortcomings of more traditional methods to stimulate IO research and push the envelope of innovation.

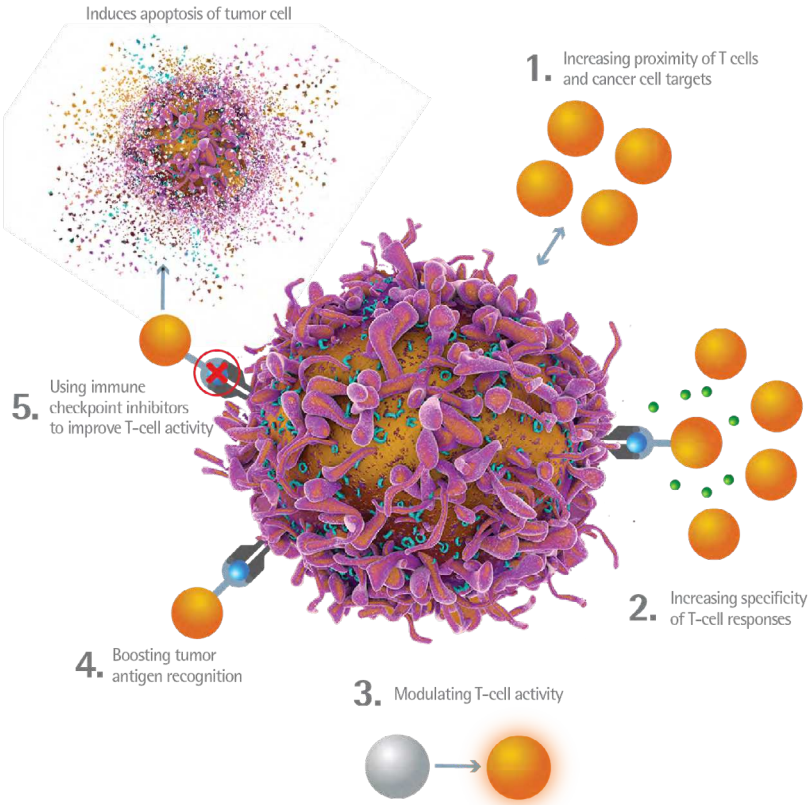



Figure 1: Exploring the different immuno-oncology approaches.

## Adoptive Cell Therapy

ACT (also known as adoptive cell transfer) is a cell-based method that recruits a patient's own immune cells to identify cancer biomarkers. This increases the targeting accuracy and consequently treatment efficacy. A number of ACTs are in preclinical and clinical stages of development, including tumor-infiltrating lymphocytes (TILs),[6] T-cell receptors (TCRs),[6] chimeric antigen receptor T cells (CAR-Ts),[7] and personalized adoptive cellular therapy targeting MDS stem cell neoantigens (PACTN).[8] Broadly speaking, the approaches may be classified as either native (*i.e.*, not genetically modified) and engineered (*i.e.*, genetically modified).

### Tumor-infiltrating Lymphocytes

Tumor-infiltrating lymphocytes (TILs) are lymphocytes that have penetrated into a patient's tumor and possess cancer-killing capacity. They are harvested from dissociated patient tumor tissue, expanded *ex vivo*, and adoptively reinfused into the patient. They are not genetically manipulated, but naturally recognize specific antigens, *e.g.*, melanocyte differentiation antigens (MDA) from melanomas. The technique was pioneered by Rosenberg for melanoma patients and significantly improved the objective response rate.[9] It is presently being evaluated for other solid tumors in clinical trials, including colon, breast, ovarian, prostate, thyroid, and myeloma.[6]

Despite demonstrated efficacy in around 50% of cases, widespread adoption of TILs faces several obstacles, such as the need for accessible tumor tissue, complexity and time of the procedure, intensive chemotherapeutic treatment to achieve lymphodepletion, and the heterogeneity and immunosuppressive nature of the tumor microenvironment.[10,11] Additionally, TIL efficacy may be mediated by a subgroup of memory T cells,[12] suggesting that enrichment in this population may improve treatment outcome.

Since patient biopsy samples are difficult to obtain, they are precious and generally small, and produce few TILs. Therefore, TIL research can be facilitated by discovery on a miniaturized scale. Furthermore, determining the most effective TIL subset for *ex vivo* expansion relies on both phenotypic (*i.e.*, CD receptor expression) and functional (*i.e.*, effector cytokine production) characterization. Unfortunately, conventional assays need to be performed in tandem or serially on multiple patient-derived TILs, which wastes time and consumes scarce samples. In marked contrast, using an advanced high throughput flow cytometry platform such as the iQue3 which is capable of multiplexed phenotypic and functional characterization, aids in the faster selection of the most promising TIL subpopulation for *ex vivo* expansion.[13] Furthermore, the entire experimental process can be streamlined by applying the same format directly from 96- and 384-well plates, saving on time and consuming smaller amounts of the valuable patient-derived TIL sample. This accelerates the overall process, and shortens the time needed for TIL therapy, which will improve patient outcomes.

### CAR-Ts

CAR-Ts are engineered T cells that have been genetically modified to express chimeric receptors (CAR) that combine antigen-binding and T cell activating functions.[14] The CAR is designed to target a specific antigen, *e.g.*, CD19 in hematological malignancies,[7] increasing the CAR-Ts ability to identify and hone in on cancer cells. Once CAR-Ts bind to antigen-expressing tumor cells, they are

"The iQue has greatly facilitated our work in the development of a personalized immunotherapy for AML and MDS patients."
- Alison Tarke, Research Scientist, PersImmune.

activated, proliferate and switch to a cytotoxic cancer-killing mode. CAR-Ts can be developed from a patient's T cells, expanded *ex vivo*, and autologously reinfused, although allogenic CAR-Ts are also possible. Although CAR-Ts have shown remarkable response rates, particularly in some hematological malignancies for which it is FDA approved, trials are on-going for solid tumors (colorectal cancer, breast cancer, gastric cancer, adenocarcinoma).[15] Their resounding success is offset by some challenges, including lower or uncertain efficacy in solid tumors, off-target effects and associated toxicity, and high manufacturing costs.

Increasing CAR-T specificity for cancer cells by identifying more selective antigen-binding CARs would help mitigate off-target reactions. "Tunable" CAR-Ts that moderate activity against antigens, such as HER-2, which are present on both cancer cells and healthy cells could decrease off-target side effects.[16] Killing specificity may also be boosted by enhancing CAR affinity for tumor-specific antigens on cancer cells. Killing efficiency may be improved by concurrent checkpoint blockade to prevent CAR-T cell exhaustion.[17] Although T-cells have been the bastion of CAR-T research, alternative immune cells for CAR applications, such as γδ T, natural killer, NKT, and cytokine-induced killer cells, may prove more efficacious against solid tumors.[18]

All these possible solutions require large-scale screening of potential CAR-Ts to evaluate their cancer killing capacity. The process involves comprehensive characterization of CAR-T surface activation markers, effector cytokine profiling (*e.g.*, IFNγ and TNFα), population expansion, and central memory or stem cell markers.[19] Expression of surface immune checkpoint inhibitor is also necessary for evaluating CAR-T cell exhaustion. The conventional workflow aliquots the original cancer-exposed CAR-T sample to profile each parameter separately, tediously combining all the data from distinct assays into the final analysis. These limitations can be addressed using an advanced high throughput flow cytometry platform such as the iQue3 which can concurrently evaluate CAR-T surface expression (activation and inhibition), cytokine production, and expansion all within the same sample. The data obtained can be analyzed using the integrated ForeCyt software which simplifies data analysis. Additionally, iQue3's patented sampling enables assays with smaller sample size and reagent volume requirements, increasing the throughput of CAR-T discovery, far outpacing the conventional workflow in speed and ease.

"We'll be using the iQue at the front end of our process to characterize our screened library outputs and it will replace and extend our current phage ELISA. This approach will scale our project throughput in a way that would have been impossible using our current methods."

**Checkpoint inhibitors**

Immune checkpoint receptors are a class of surface protein molecules that modulate an inhibitory effect on immune cells.[20] They include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death-ligand 1 (PD-L1), which is a PD-1 ligand. Under normal physiological conditions, checkpoint receptors prevent immune system overactivity, counteracting autoimmunity. However, they are also expressed by immune and tumor cells in cancer patients,[21] where they create an immunosuppressive environment. Checkpoint blockade works by administering antibodies against CTLA-4, PD-1, or PD-L1, which bind and block the immune checkpoint, lifting the brake on immune cell activation. The immune system is thus able to mount an anti-tumor response. In clinical trials, antibody checkpoint inhibitors achieved extraordinary tumor shrinkage and durable remission in multiple types of cancer, hematological and solid.[22-24] Their impressive effect has led to multiple FDA approvals and adoption as front-line care in certain cancers, such as non-small cell lung carcinoma.[25]

Unfortunately, checkpoint blockade can trigger adverse immune reactions, and although it is very successful in some patients, it is fruitless in others or produces a heterogenous response.[21,26] Its therapeutic efficacy may also be blunted by the development of resistance within the tumor microenvironment. Blocking alternative inhibitory checkpoint receptors with novel antibodies may lead to more effective treatments with fewer side effects. Identification of biomarkers, including immune markers, may also help identify patients likely to respond to checkpoint blockade. These possible solutions necessitate generating new specific antibodies against alternative checkpoint receptors and testing their ability to trigger immune activation upon exposure to cancer cells. Advanced high throughput flow cytometry can uncover the status of the tumor immune microenvironment, to understand the biology in tumors that do not respond or those that develop resistance.

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- Matt Johnson, Chief Technology Officer, Avacta.

The efficacy of newly identified checkpoint blockade antibodies can be conveniently assessed using multiplexed assay formats for immune cell surface receptor expression, cytokine production, and immune expansion. Thus, boosting the discovery rate, accelerating development of novel checkpoint inhibitors.

The progress so far in preclinical and clinical testing of IO has shown its tremendous promise, motivating further research to overcome its current limitations. Utilizing advanced high throughput flow cytometry platforms such as the iQue3 will accelerate progress within the field through multiparametric analysis, increasing throughput and cost-effectiveness.


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