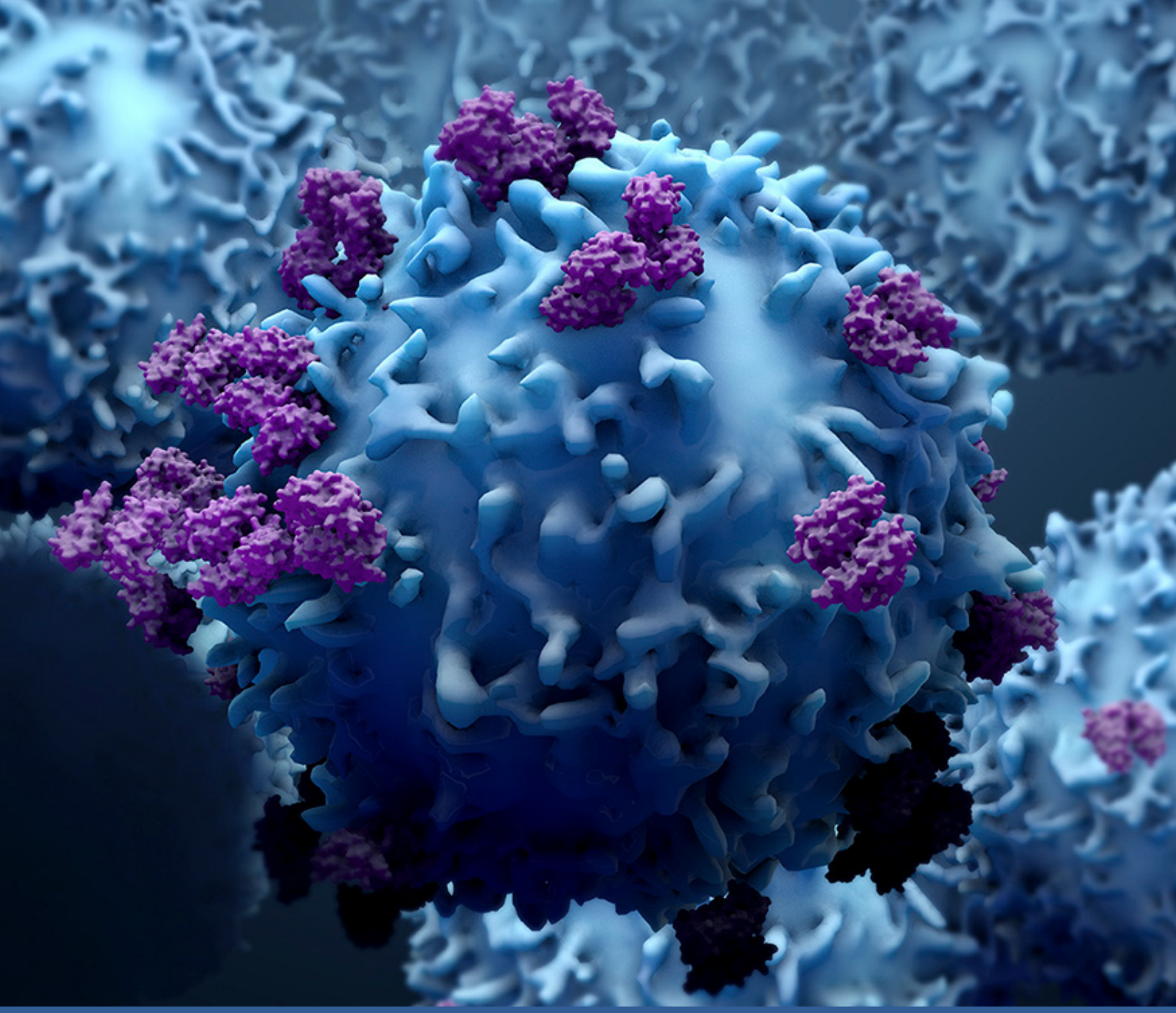

T Cell Exhaustion in Immuno-oncology:

Understanding this Delicate Balancing Act

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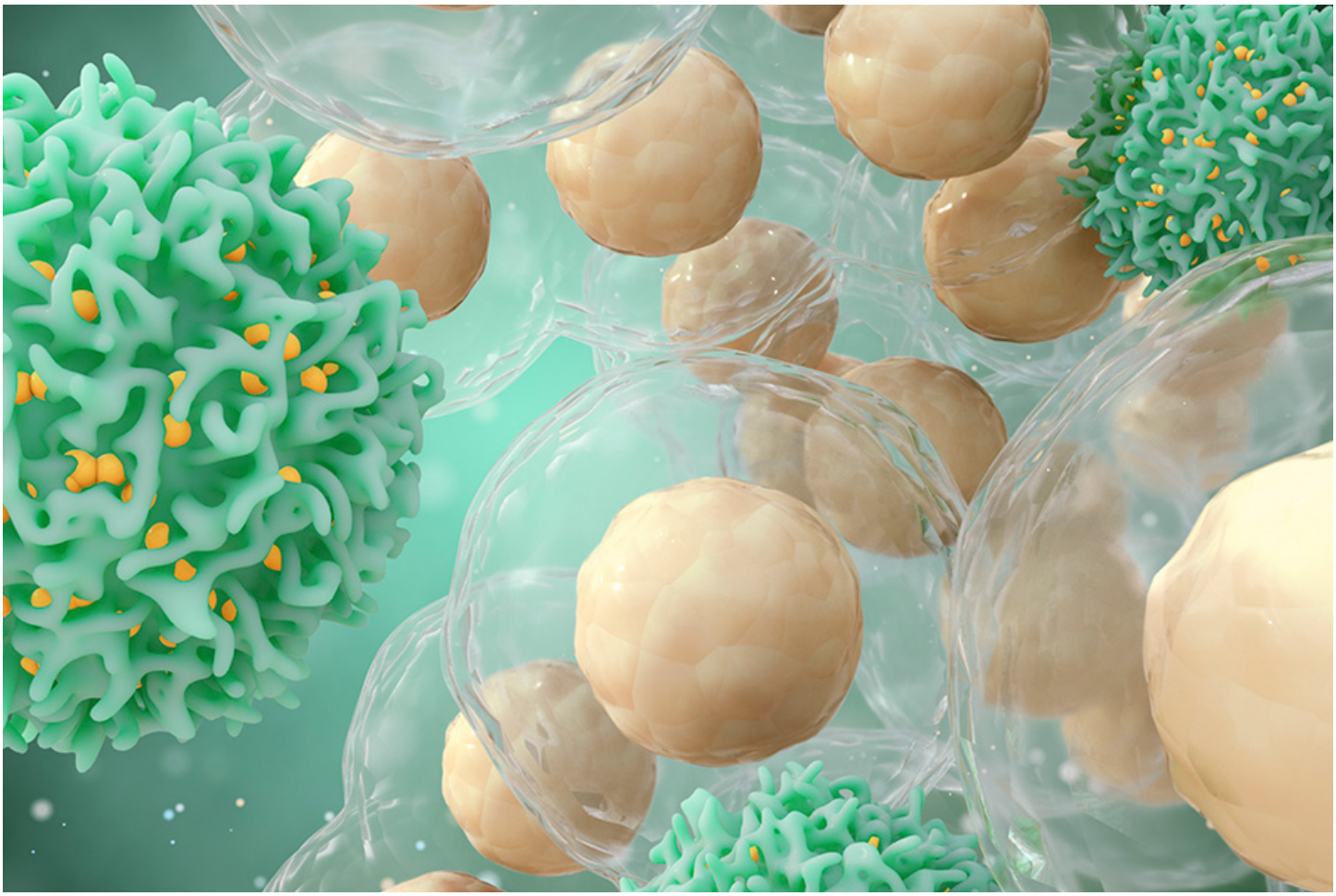
We are in an era of unprecedented advances in immune-based therapies and personalized medicine for the treatment of a wide array of cancers. These cutting-edge therapies have been informed by basic immunology research that has been driven by flow cytometry technology. Our understanding of the molecular mechanisms associated with T cell exhaustion have been pivotal to the development of many of these therapies, but this research has also revealed that reversing exhaustion can have unexpected consequences.

This white paper will give an overview of how T cell exhaustion develops and what molecular strategies can be used to overcome this state in the context of cancer immunotherapy. Flow cytometry applications for basic and clinical research are also highlighted.

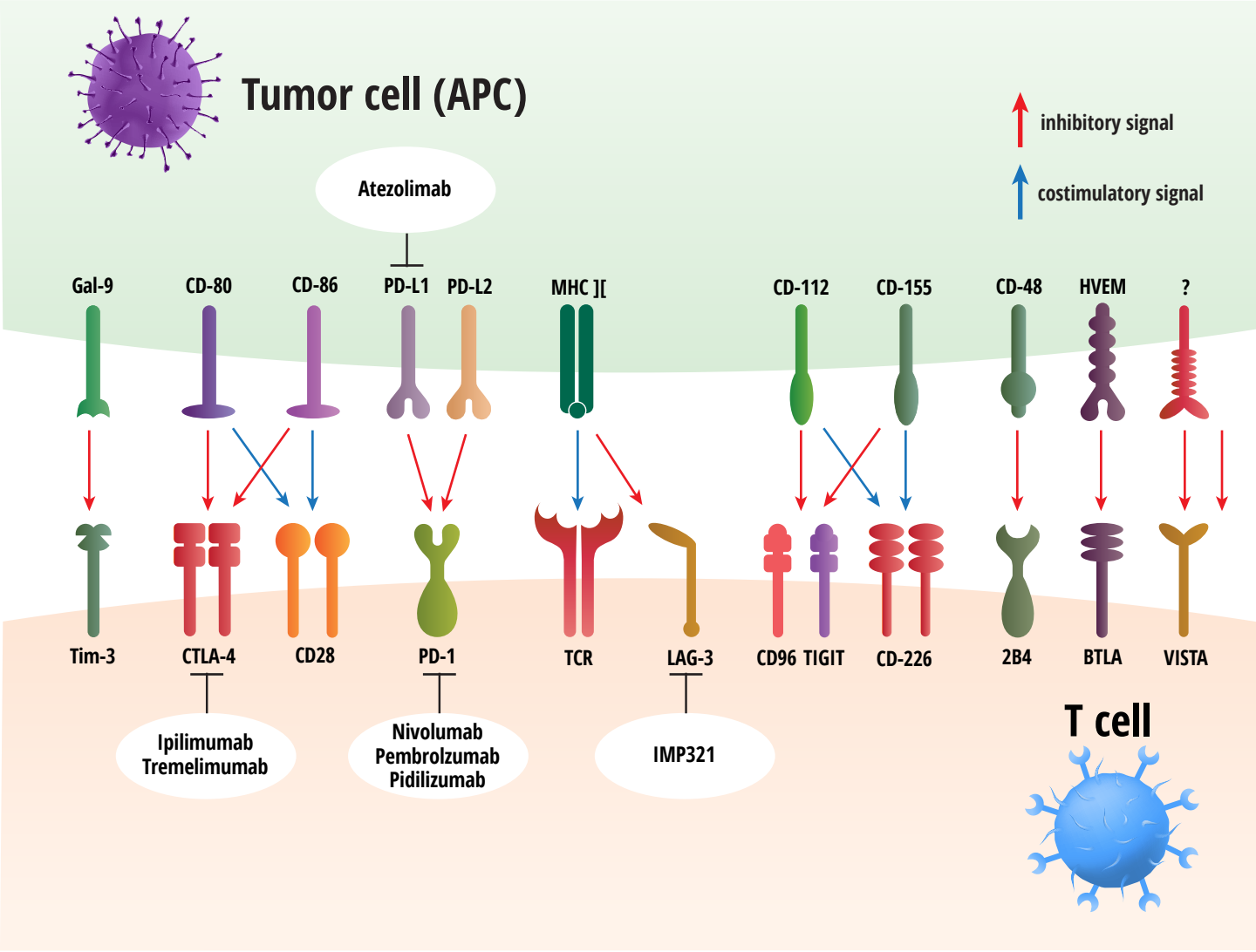
What is T Cell Exhaustion?

Naïve T cells have the potential to be activated in response to infection and can rapidly proliferate and differentiate into a variety of effector T cell subsets. Activation can be triggered by exposure to antigens from infectious agents or vaccines, and $CD4^+$ and $CD8^+$ T cells are armed with an arsenal of molecular weapons that make them suited to fight infection, or later, to transition into memory T cells. The majority of effector CD8+ T cells die off during the contraction phase of T cell proliferation when antigen exposure or infection diminishes, but a subset of these cells express CD127 and can differentiate into memory T cells that produce IL-2, IFN-γ, and TNF-α, and can expand upon re-exposure to the same antigen[1].



If antigen persists, such as during chronic infection or from exposure to a tumor antigen, effector $CD8^+$ T cells continue to be stimulated and lose effector function, thus entering a state of unresponsiveness or "exhaustion."[2] $CD4^+$ T cells can also become exhausted, particularly during chronic viral infections, and produce lower levels of TNF-α and IFN-γ and higher levels of IL-10 and IL-21, which may contribute to CD8+ T cell exhaustion[3]. These $CD4^+$ or $CD8^+$ T cells display changes in their transcriptional programs, which leads to overexpression of multiple inhibitory receptors, most notably, PD-1, CTLA-4, Lag-3, and Tim-3[3,4].

Under normal conditions, these inhibitory receptors are critical checkpoints of T cell function that can protect against autoimmunity[5]. In exhausted T cells, overexpression of these receptors and engagement with multiple ligands on antigen-presenting cells trigger multiple intracellular signaling pathways that alter expression of transcription factors, including T-bet, NFAT, and EOMES. The combination of changes in transcription factor expression in the context of chronic antigen exposure leads to a shift toward exhaustion[8], including changes in cytokine expression[6], functional impairment[7], metabolic change[8], and an inability to proliferate or persist[9]. This contributes to the overall failure of the immune system in chronic infections like HIV and for the unchecked proliferation of tumor cells. *(Fig.1)*



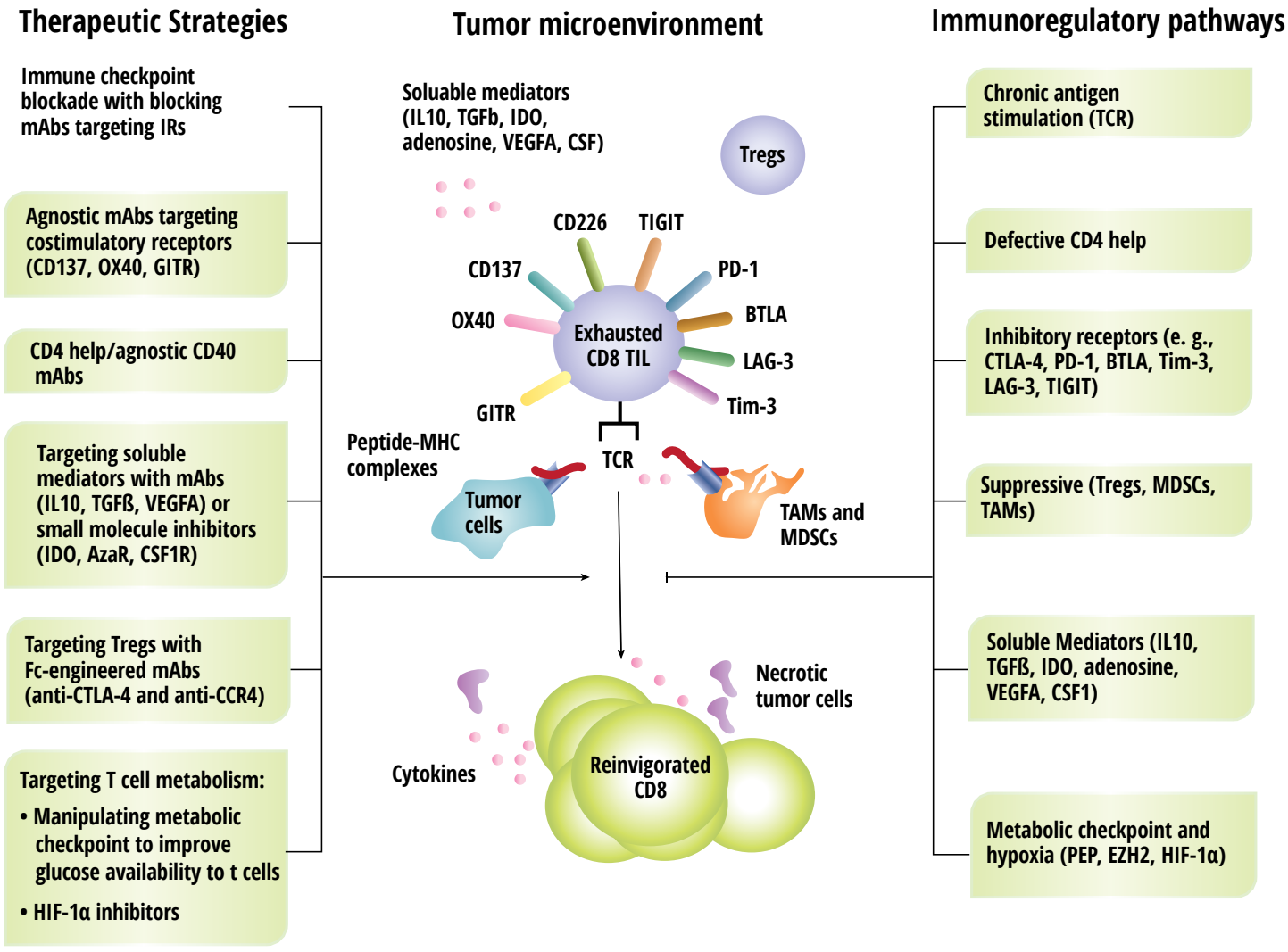
Published in Cell Communication and Signaling 2016 ***T cell exhaustion: from pathophysiological basics to tumor immunotherapy***
Kemal Catakovic, Eckhard Klieser, Daniel Neureiter, Roland Geisberger

Inhibitory/costimulatory receptors and their corresponding ligands. Schematic overview of inhibitory/costimulatory receptors expressed by T cells interacting with their counterpart on antigen-presenting cells (APCs) or tumor cells. Additionally, various blocking antibodies against inhibitory receptors or their ligands in clinical trials are depicted with the aim of reversing T cell exhaustion.

Cancer and Exhaustion

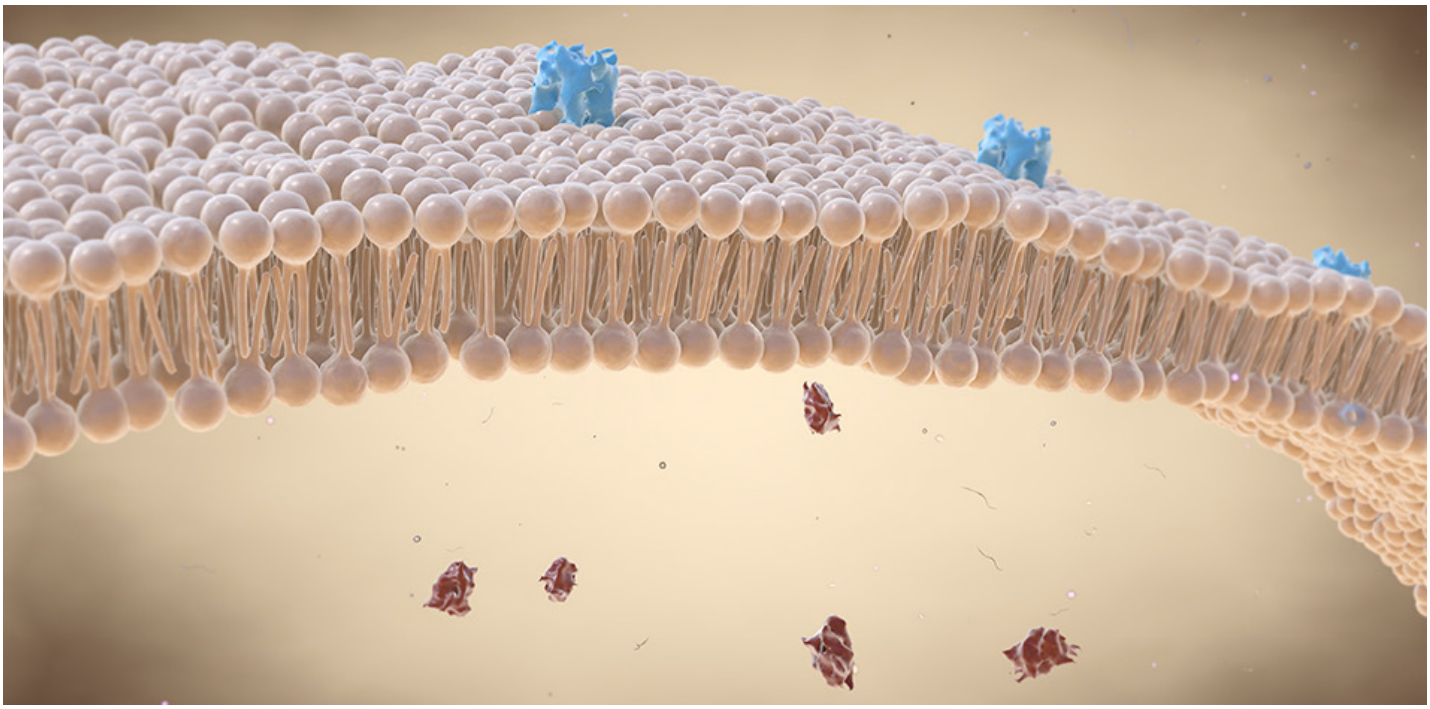
In the tumor microenvironment (TME), T cell exhaustion is triggered by chronic tumor antigen exposure. Exhausted T cells in tumors, also defined as tumor-infiltrating lymphocytes (TILs), express higher levels of inhibitory receptors that can bind to their respective ligands expressed on tumor cells or antigen-presenting cells, which contributes to impaired anti-tumor responses[10,11,12]. As an area of intense research driven by breakthroughs in flow cytometry techniques, several studies have shown that T cell exhaustion induced by tumor antigens may be different than that triggered by chronic viral infection[13]. The TME appears to play a critical role in driving TIL dysfunction, including production of immunosuppressive soluble mediators by myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages[14,15]. *(Fig.2)*

Fig. 2. Immunoregulatory pathways in the TME and strategies to reverse tumor-induced T-cell exhaustion



Precision Cell Identification

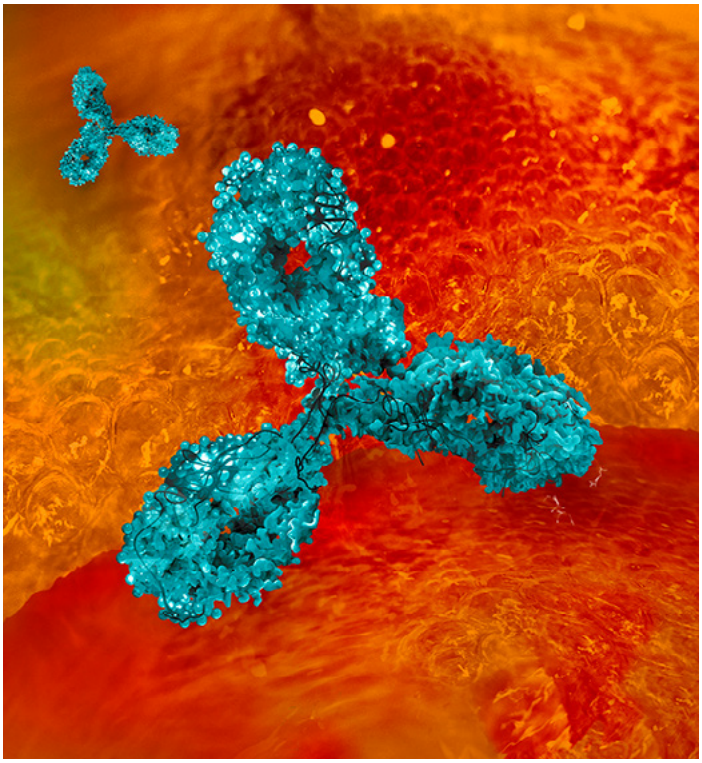
Flow cytometry has been instrumental to understanding T cell exhaustion and for monitoring treatments that can reverse this state. Exhausted T cells are a heterogeneous population. Identification of these cells requires rigorous flow cytometry protocols that can discern up to 20 surface and intracellular markers and discriminate exhausted T cells from other subsets with similar phenotypes, including regulatory T cells, follicular helper T cells, and effector memory T cells[16]. Both CD4+ and $CD8^+$ T cells express high levels of multiple inhibitory receptors, including PD-1, LAG-3, CD160, 2B4, and TIGIT, and evaluation of their expression levels, as well as levels of inflammatory and immunosuppressive cytokines, is critical to gauging their level of exhaustion. T-bet and EOMES are also key markers, as different levels of these transcription factors are associated with subsets of exhausted T cells that respond differently to immunotherapy. Flow cytometry advances, particularly in the expansion of the number of colors that can be monitored, has not only advanced basic research in T cell exhaustion, but is instrumental to preclinical and clinical evaluation of biologic candidates that can reverse exhaustion.



Blockade Breakthroughs

The prodigious body of basic research that has emerged from T cell exhaustion studies has revolutionized the field of targeted immunotherapy. Blockade of these inhibitory receptors can restore function to exhausted T cells, and this concept was first described with the blockade of PD-1, which restored T cell function during chronic viral infection or improved anti-tumor responses[18,19]. This “checkpoint blockade” was first tested clinically as a cancer treatment for the development of anti-CTLA-4 and anti-PD-1 monoclonal antibodies[20,21].

With the approval of these therapies by the FDA in recent years, checkpoint blockade has yielded impressive results for the treatment of previously devastating cancers including metastatic melanoma and non-small cell lung cancer. Clinical flow cytometry has been essential to preclinical and clinical studies that monitor immune function and treatment efficacy. The widespread use of checkpoint blockade has also revealed the limitations of this approach, particularly the emergence of adverse events associated with reversing T cell exhaustion or the development of treatment-refractory tumors[22]. Current clinical trials are exploring the effectiveness of combining different checkpoint inhibitors or using them with other treatment modalities to enhance the effectiveness of this therapeutic approach and limit adverse events.



A Bright Future

The discovery T cell exhaustion and the development of checkpoint inhibitors has greatly advanced the field of immuno-oncology and continues to drive basic research and the preclinical development. Flow cytometry technology has also propelled this research through the development of hardware and reagents that can detect more colors and improved data analysis methods, like t-SNE, that enable the visualization of high-dimensional datasets. The impact of this research was further validated by the 2018 Nobel Prize in Physiology or Medicine that honored James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation[23]."

At Flowmetric, we are at the forefront of preclinical and clinical flow cytometry research as a contract research organization. We work with industry and academic partners to integrate breakthroughs in basic research with the search for better biologics. We have many years of experience developing validated flow cytometry protocols that can monitor multiple cell subsets concurrently in a sample, including the evaluation of T cell exhaustion in the context of checkpoint blockade. Beyond protocol development, we have expertise in preclinical toxicology and safety evaluation and data analysis. The future is bright for this and other therapies that can redirect the immune system to respond in a therapeutic manner. Let us help you be part of the revolution.

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