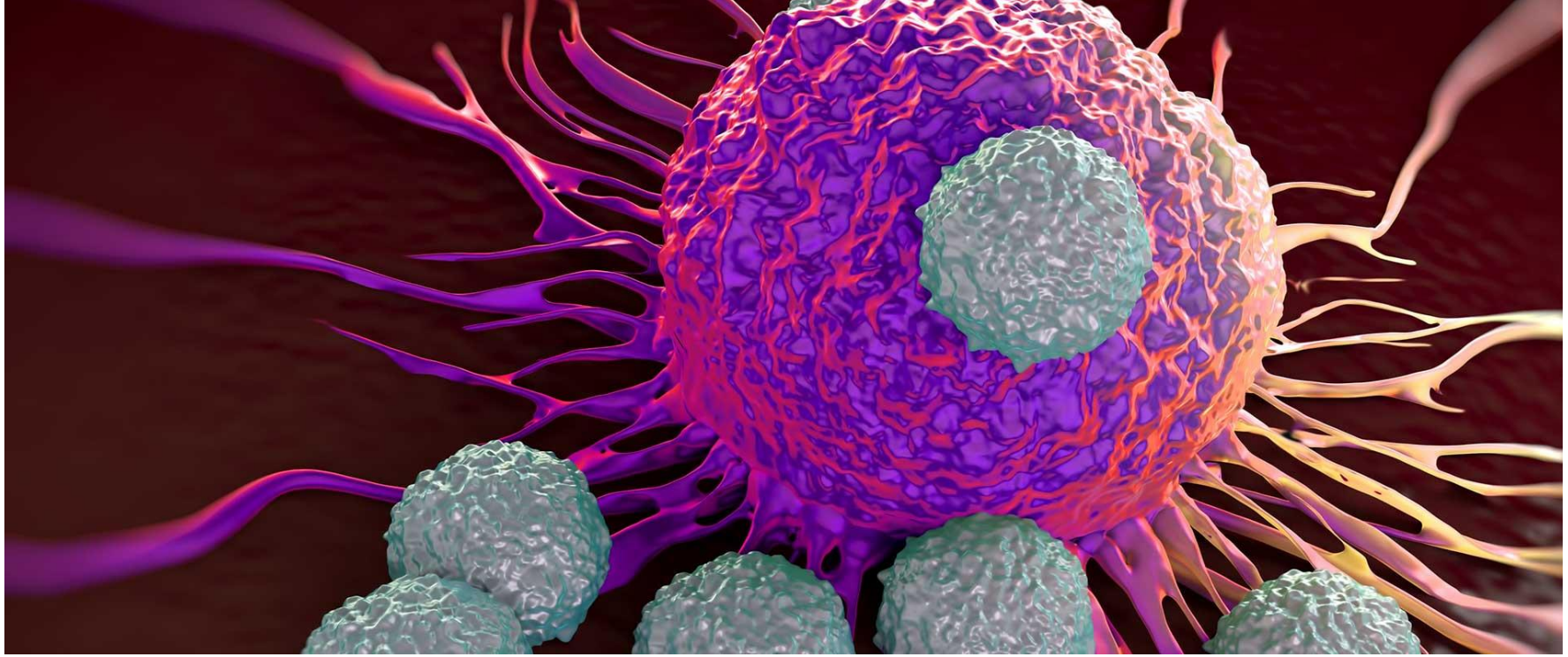

Non-Clinical Testing Methods for Immuno-Oncology Therapies

June 2018 Newsletter

Introduction

Oncologists have long based their treatment options on the three pillars of cancer therapy: chemotherapy, surgery and radiation. However, years of basic and clinical research have led to the emergence of a fourth pillar in cancer care: cancer immunotherapy. Cancer immunotherapy involves the harnessing of the patient's immune system to recognize antigens on cancer cells and specifically destroy tumors or prevent metastasis.

Immuno-oncology research is a rapidly advancing field with incredible potential to transform cancer medicine. Researchers are using cutting-edge immunology findings in order to establish innovative cancer immunotherapies. Researched fields include T cell-, B cell- and NK (Natural Killer) cell-mediated anti-tumor responses, cytokines, co-stimulators, chemotaxis, immune cell migration and immune tolerance. Basic immunology discoveries advance our knowledge of the potential use of certain immune cells and molecules to specifically target tumor cells or instruct the immune system. The immune system plays a critical role in tumor progression. Therefore, it is vital to characterize the potential impact of a drug candidate (antibody or small molecule) on the immune system.

At ITR, we measure and characterize these different aspects of the immune system in response to various cancer immunotherapies. In this newsletter, we highlight different techniques that we use to assess immunotoxicity as well as immune system functionality in response to different immuno-oncology-based therapies. These regulatory processes, which are essential to drive preclinical decisions, include:

- T Cell-Dependent Antibody Response (TDAR) Assay
- Cytokine Response Assay
- Immunophenotyping Assay

I. T Cell-Dependent Antibody Response (TDAR) Assay

The continuous emergence of novel immunomodulatory therapeutics has brought more attention in the past decades to the evaluation of potential adverse effects on the immune system. A functional evaluation, such as the TDAR assay is considered the most appropriate method to address immunotoxicity in a non-clinical setting. TDAR is a measure of immune function that is dependent upon the effectiveness of multiple immune processes, including antigen uptake and presentation, T cell help, B cell activation and antibody production. We established this assay at our facility both for juvenile as well as adult rats. We are also currently establishing the TDAR assay in Cynomolgus monkey. Below is a representation of the study design (Fig. 1) along with our results (Fig. 2) for the TDAR assay in adult rats. This assay consists of evaluating the primary TDAR to Keyhole Limpet Hemocyanin (KLH) in immunocompetent or immunosuppressed by Cyclophosphamide (CPA) Sprague-Dawley rats for subsequent use in regulatory immunotoxicity studies. For both the adult and juvenile rats, the immunization scheme results in a robust antibody response, while treatment with an effective and well-tolerated dose of the positive control prior to and following immunization demonstrates the ability to detect a statistically significant immunosuppression.

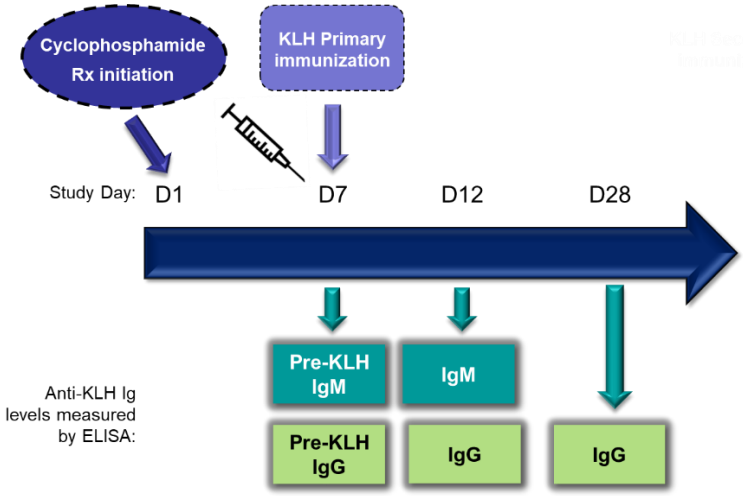

Figure 1:

Study design for T Cell-Dependent Antibody Response (TDAR) assay

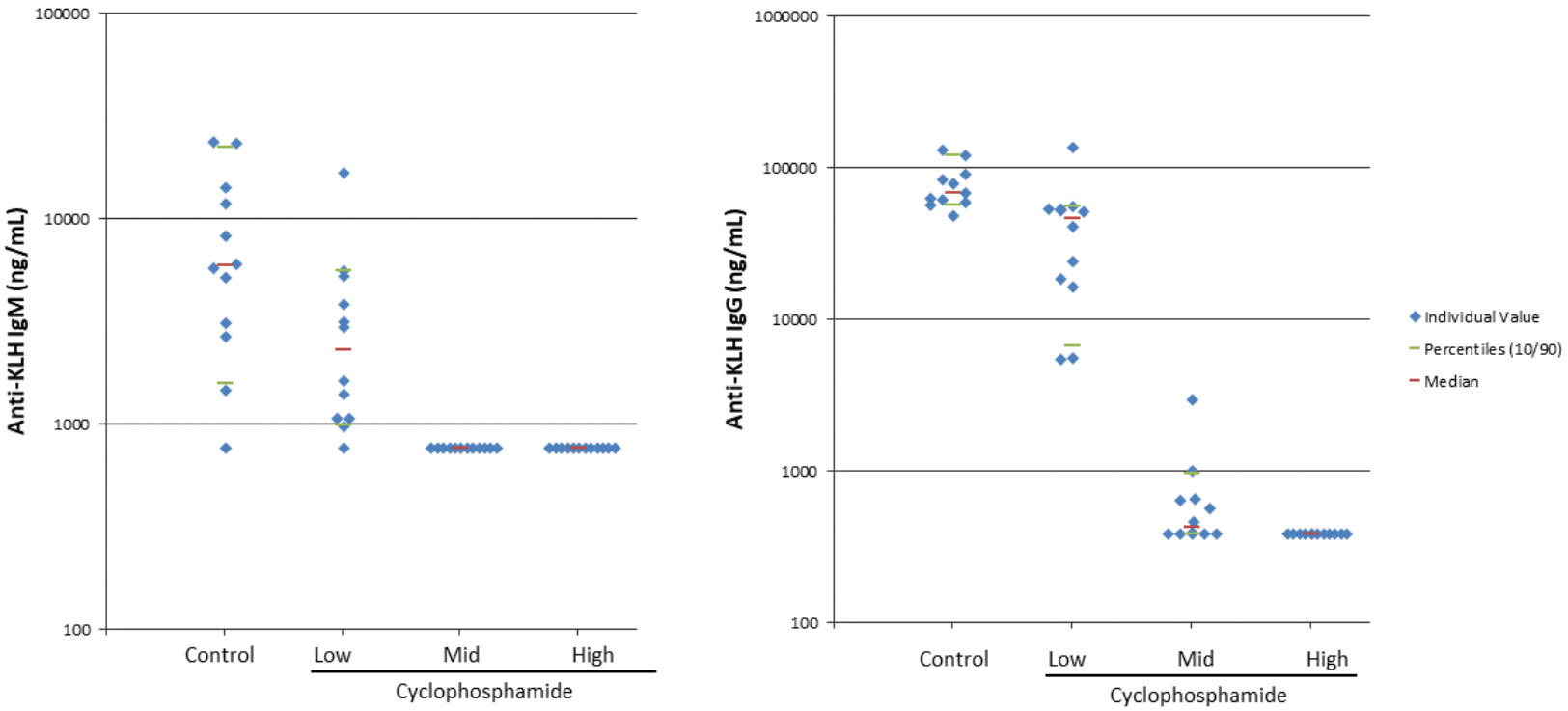

Figure 2:

Concentration of anti-KLH IgM (Day 12) and IgG (Day 28) following daily administration of Cyclophosphamide at low, mid and high dose levels

II. Cytokine Response Assay

Cytokines are small secreted proteins that are important for cell signaling and are released by different cells, in particular by cells of the immune system. Cytokines have several key roles such as stimulation of immune cell expansion or inhibition of cancer cell proliferation. Cytokines may include chemokines, interferons (IFN), interleukins (IL) and tumor necrosis factors (TNF), all of which have different effects. IL-2 was one of the first cytokines to be approved for cancer treatment specifically for metastatic melanoma and metastatic renal cancer. IFNs have potent anti-proliferative properties and can activate the anti-tumor activity of multiple immune system cells. IFN-α was also another early immuno-oncology therapy approved in the 1980s for treatment of hairy cell leukemia and is now used as part of treatment protocols for a variety of cancers. IFN-α activates multiple responses in dendritic cells and cytotoxic T cells that lead to durable anti-tumor responses and is now being explored as a combination therapy with other cancer treatments.

At ITR, we utilize a Cytometric Bead Array (CBA) method, which measures various cytokines such as the ILs (IL-2, IL-4, IL-5 and IL-6), TNF and IFN (IFN-ɣ). We perform this using flow cytometry. These multi-parametric flow cytometry panels provide a snapshot of the cytokine expression in a single assay. These cytokine panels could be customized based upon a sponsor's request and are available for different animal species including mice, rats, rabbits, dogs and monkeys.

III. Immunophenotyping Assay

Immunophenotyping is a technique used to identify different cell lineages through the use of antibodies that specifically identify antigens or markers on the cell surface or in its cytoplasm or nucleus. Some antigens are lineage-specific and are thereby found on one cell type whereas others are found on various cells. Few antibodies are completely lineage-specific. The term “immunophenotyping” is most frequently used in reference to phenotyping hematopoietic cells but this technique can be and is also used to identify cells of non-hematopoietic origin.

At ITR, we typically use immunophenotyping using various activation/proliferation markers mainly to identify the following hematopoietic cell populations:

- T Lymphocytes:
  - Helper T Lymphocytes
  - Cytotoxic T Lymphocytes: o Naïve o Effector Memory o Central Memory o Stem Cell Memory
  - Regulatory T Cells
- B lymphocytes
- Natural Killer Cells
- Monocytes:
  - Classical
  - Activated

Fig. 3 shows an example of immunophenotypic analysis of monkey whole blood leukocytes.

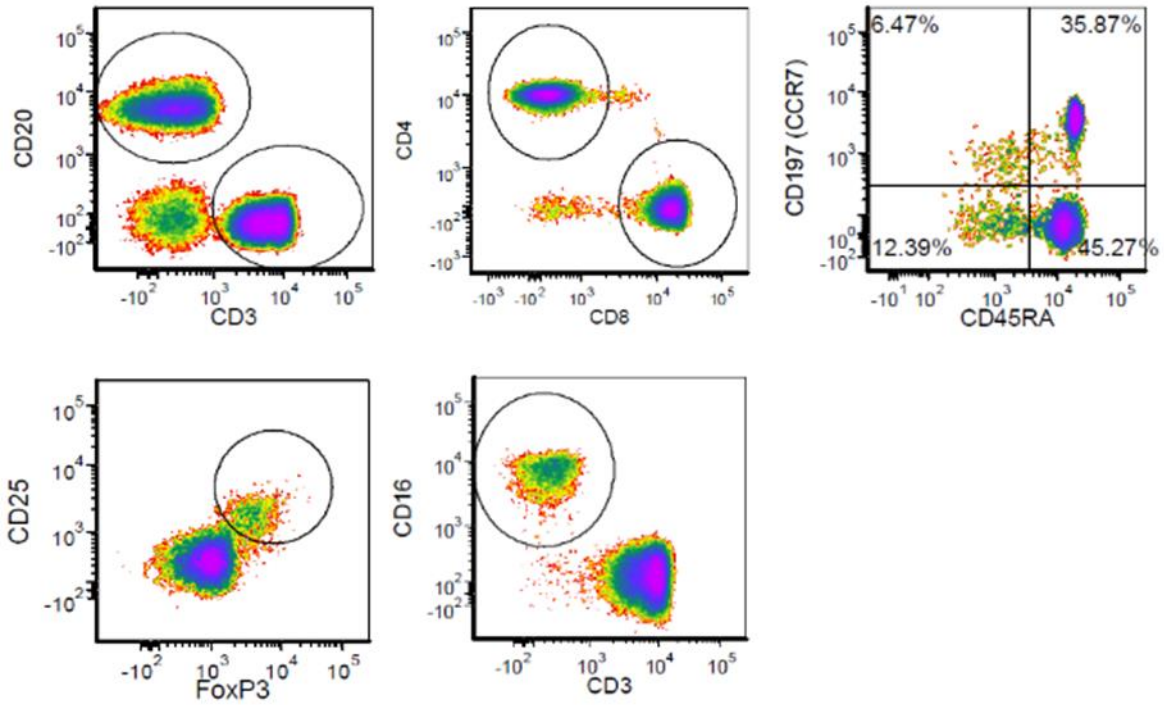

Figure 3:

Immunophenotypic analysis of Cynomolgus whole blood leukocytes. B cells (CD20+); total T cells (CD3+); helper T cells (CD4+); cytotoxic T cells (CD8+), Naïve/Memory T cells (CD197/CD45RA), regulatory T cells (CD25+FoxP3+), Natural Killer cells (CD3-CD16+)

Conclusion

Immuno-oncology is changing the nature of cancer treatment and new therapies will be coming to market in the coming years. It is essential to be able to test the effect of these drugs in a non-clinical setting. At ITR, we have established various techniques some of which we discuss in this newsletter. All of these methods will be essential to the advancement of future therapies.