

The rise of IMMUNOTHERAPIES *strategies to accelerate their advance into clinical trials*

The striking rise of immunotherapies in the past decade has had a transformative effect on the prospects for the treatment of patients with cancer. Also referred to as biologic therapies, immunotherapies are a new class of drug that harnesses the potential of the body's immune system to target and destroy tumours. With hundreds of antibody-based candidates in the development pipeline, the field represents one of the most promising strategies for cancer treatment. While the promise of biologics is great, so too are the challenges researchers have come up against. The traditional phased approach of drug development considers features of a candidate, such as its pharmacokinetics, sequentially. However, this approach is neither optimal nor sufficiently sophisticated to support the development of immunotherapies, which involves a vast array of combinations of targets, drugs and biomarkers. Researchers have adapted to this challenge by developing novel assays and approaches to accelerate their understanding of the performance of immunotherapies.

Cancer is one of the leading causes of death worldwide, with its prevalence expected to increase by 70% in the next two decades. However, a promising, relatively new, field of cancer therapy is emerging¹. The striking rise of immunotherapies in the past decade has had a transformative effect on the prospects for the treatment of cancer patients with a large number of immunotherapy candidates proceeding to clinical trials, and some already clinically validated. With an estimated market potential of \$34 billion by

2024², immuno-oncology (I-O) represents a rapidly-growing field at the forefront of cancer therapy, attracting much investment from biopharmaceutical companies across the globe.

Immunotherapies: a brief history

Although not until the past decade have we seen significant breakthroughs being made in the field, as far back as the 1890s scientists hypothesised that the stimulation of the immune system could be utilised as a cancer therapy^{3,4}. William B. Coley

By Edward Cribb

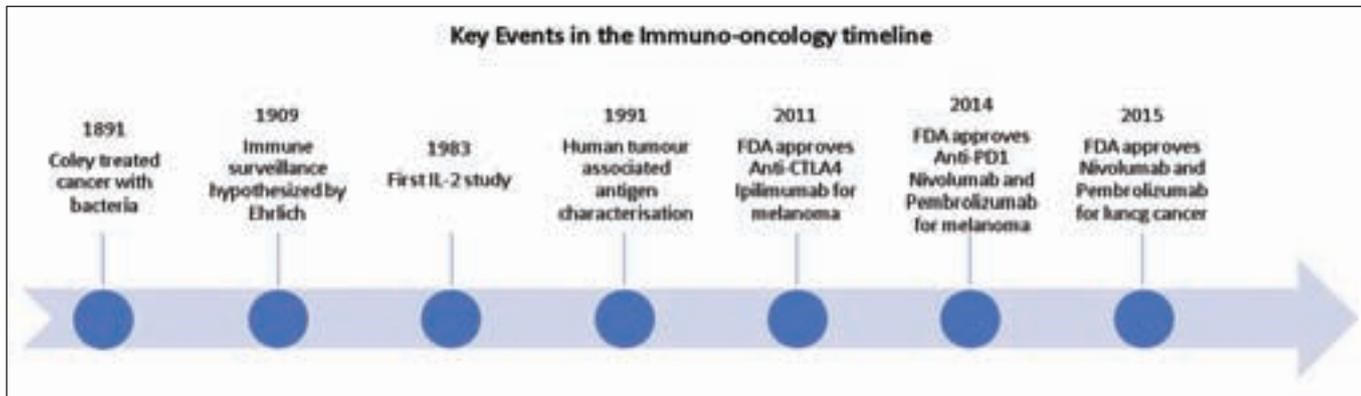


Figure 1
Key events in the immuno-oncology timeline. From Coley's early discoveries through to some of the recent approvals for melanoma and lung cancer. *Timeline adapted from Morrissey et al*

employed a strategy of injecting bacteria into a cancer patient with the goal of stimulating the immune system to attack the tumour³, with this successful strategy earning Coley the title of 'the father of immunotherapy'. We have seen significant progress since Coley's early work (Figure 1) and with a wealth of research now being undertaken in the area of I-O, we are beginning to understand the underlying relationships of cancer cells and lymphocytes⁴. One of the first immunotherapies to be approved was IL-2 therapy, with the hypothesis that IL-2 cytokines would stimulate T-cell activity against the tumour cells. Unfortunately, treatment regimens were not as successful as expected with patients experiencing high levels of toxicity^{4,5}, a hurdle that remains with immunotherapies today.

As immunotherapies progress, several modalities within the scope of I-O have been identified and developed, and these can be split into two distinct categories – passive therapies and active therapies. Active therapies include cancer vaccines and oncolytic viruses, while passive therapies include immunomodulatory monoclonal antibodies, with the most well-known examples being checkpoint inhibitors⁴. Tumours are able to avoid attack from the immune system by hijacking normal immune-suppressive pathways, which in healthy tissue would provide protection against infection but not destroy otherwise healthy tissue³. Upregulation of negative regulators of the immune system can allow malignant cells to defeat the immune surveillance system, promoting the formation and progression of tumours³. As immunomodulatory monoclonal antibodies (mAbs), checkpoint inhibitors act by blocking receptors on cells that inactivate host T-cell migration. With hundreds of antibody-based candidates in the development pipeline, the field of I-O represents one of the most promising strategies for cancer treatment, includ-

ing, for example, the treatment of lung cancer and melanoma. There are already several FDA-approved immunotherapy agents for melanoma and lung cancer, such as Nivolumab and Pembrolizumab, which are anti-PD1 checkpoint inhibitors; and Ipilimumab, an anti-CTLA-4 checkpoint inhibitor^{3,14,15}.

Preclinical safety assessments of novel immunotherapies

While the promise of biologics is great, so too are the challenges researchers have come up against. The traditional phased approach of drug development considers features of a candidate sequentially. However, this approach is neither optimal nor sufficiently sophisticated to support the development of immunotherapies, which involves a vast array of combinations of targets, drugs and biomarkers. Taking immunomodulatory monoclonal antibodies (mAbs) as an example, the objective of preclinical studies is to define the toxicological profile of the mAb. This can include the pharmacological profile, identification of starting dose for human studies and profiling the bio-distribution, on-off target binding and overall toxicity of the molecule³. Unique challenges arise for immunotherapies as these agents are interacting with the tumour microenvironment, and animal models often do not mimic the exact environment found in humans, along with differences in immune response. The importance of assessing off-tumour binding in normal tissues is paramount. Since immunotherapies are, in one way or another, stimulating the immune system to identify and attack cancer cells, so too are normal, otherwise healthy tissues open to attack. This undesirable side-effect of immunotherapies has been well documented, as has the extent to which these effects can cause serious autoimmune complications. A significant proportion of patients receiving

Ipilimumab experience autoimmune-like symptoms and require close monitoring throughout the treatment regime⁹.

Assessing antibody binding using tissue cross-reactivity studies

Researchers have adapted to this challenge by developing novel assays and approaches to accelerate their understanding of the performance of immunotherapies. One such approach is the tissue cross-reactivity study, which provides valuable insights into the likely *in vivo* response of a novel antibody or antibody-like molecular structure prior to commencing expensive clinical trials. Such studies enable a detailed assessment *in vitro* of the binding of a test article across a panel of 33 or more organs and across a variety of species. By considering the candidate's profile of on- and off-target binding, scientists gain an understanding of the propensity of the structure to bind, with high specificity, to target healthy organs in which binding is undesired. In turn, this increases confidence in the candidate's performance, provides evidence-based safety and risk data, and accelerates its advance into clinical trials.

There is no prescriptive structure for the design of a tissue cross-reactivity study, with specific study goals dictating the overall design. Regulatory requirements will dictate whether the study requires a panel of full-face tissues or whether a tissue micro array (which comprises a single slide with multiple cores of tissue from the identified 33 organ normal tissue panel) can be used. A regulatory-compliant GLP tissue cross-reactivity study can be split into two distinct phases; a non-GLP immunohistochemistry (IHC) method development and validation phase, followed by a GLP tissue cross-reactivity phase. The non-GLP phase involves full and extensive IHC method development including testing and optimising parameters for test article titration, antigen retrieval, blocking, amplification and detection reagents. This is followed by an assessment of specificity, linearity, reproducibility and a background staining assessment on a limited tissue panel from a single donor. An experienced study pathologist is often required to review the validation slides and final optimal method to add a seal of approval before proceeding into the GLP tissue cross-reactivity phase.

Once a robust IHC method is validated, the method is applied across a panel of at least 33 tissues. Guidelines from the FDA recommend a panel of 33 tissues from three unrelated adult donors. For researchers seeking to gain regulatory approval in multiple jurisdictions, it is advised to assess

binding across an extended panel of 38 tissues, covering both FDA and EMA recommendations⁷. Although these tissue panels are recommended, additional tissues from target organs specific to the test article can be included depending on particular toxicity concerns and the mode of action. At least two concentrations of the test article are recommended, while three concentrations (the optimum concentration identified in the validation phase, and also a sub-optimal and supra-optimal concentration) provides further information and ensures tissues of varying target expression can be assessed.

Early preclinical studies may employ a non-GLP study utilising tissue micro arrays (TMAs) containing an FDA-recommended tissue panel. This allows researchers to gain an insight into on-off target binding at an early stage, and can help direct development programmes to address undesired off-target binding. Furthermore, the use of TMAs is a considerably more economic approach for early preclinical studies, and is becoming increasingly popular to assess the safety profile early on in the development pipeline. However, for regulatory submission and advance into clinical trials, a GLP study comprising a full tissue panel is required.

Several important elements should be considered when conducting GLP-compliant tissue cross-reactivity studies. Firstly, the experience of the scientists in performing tissue cross-reactivity assays with novel test articles, which often require extensive immunohistochemistry protocol development and validation. Some biopharmaceutical companies choose to subcontract GLP tissue cross-reactivity studies to specialist contract research organisations, in order to gain access to their expertise in conducting these specialist studies, and to ensure robust data suitable for regulatory submission.

Secondly, the sourcing and quality of the tissue panel used within the study. It is important to ensure that the frozen tissue is sourced with fully-informed patient consent, and the tissues are handled carefully prior to freezing to ensure preservation of antigenicity and morphology. Since all data output is reliant on the expression of the test article in the tissue sections, it is critical that the tissue panel is of a high quality with no areas of necrosis or autolysis on any tissue. One or two tissue integrity biomarkers can be included as part of the tissue cross-reactivity study to demonstrate that the study materials meet these stringent quality criteria. A single section from each tissue is stained with routine tissue architecture biomarkers such as vimentin or cytokeratin, or as a multiplexed dual stain, which are complementary and provide broad staining over the majority of tissue architecture.

References

- 1 World Health Organisation (2017). Cancer Fact Sheet. Accessed 22 May 2017: <http://www.who.int/mediacentre/factsheets/fs297/en/>.
- 2 Global data (2016). Immunology Strategic Insight: Multi-indication and Market Size Analysis. Accessed 22 May 2017: <https://www.globaldata.com/store/report/gdhc057poa-immunology-strategic-insight-multi-indication-and-market-size-analysis/>.
- 3 Morrissey, KM et al (2016). Immunotherapy and Novel Combinations in Oncology: Current Landscape, Challenges and Opportunities. *Clin Transl Sci* 9: 89-104.
- 4 Kamta, J et al (2017). Advancing Cancer Therapy with Present and Emerging Immuno-Oncology Approaches. *Frontiers in Oncology*, 7: 64.
- 5 Rosenberg, SA (2014). IL-2: The First Effective Immunotherapy for Human Cancer. *Immunol*, 192 (12) 5451-5458.
- 6 Stroncek, DF (2017). Systematic evaluation of immune regulation and modulation. *Journal of Immunotherapy of Cancer*, 5:21.
- 7 Leach, MW et al (2010). Use of Tissue Cross-reactivity Studies in the Development of Antibody-based Biopharmaceuticals: History, Experience, Methodology, and Future Directions. *Toxicologic Pathology*, 38: 1138-1166.
- 8 Antonia, SJ et al (2014). Immunology Combinations: A Review of Clinical Experience and Future Prospects. *Clin Cancer Res*. 20(24).
- 9 Weber, JS et al (2015). Toxicities of Immunotherapy for the Practitioner. *Journal of Clinical Oncology* 33(18).
- 10 Kontermann, RE, Brinkmann, U (2015). Bispecific antibodies. *Drug Discovery Today* 20 (7): 838-847.

Continued on page 54

Continued from page 53

- 11** Spiess, C et al (2015). Alternative molecular formats and therapeutic applications for bispecific antibodies. *Molecular Immunology* 2(A): 95-106.
- 12** Topp, MS et al (2011). Targeted Therapy With the T-Cell-Engaging Antibody Blinatumomab of Chemotherapy-Refractory Minimal Residual Disease in B-Lineage Acute Lymphoblastic Leukemia Patients Results in High Response Rate and Prolonged Leukemia-Free Survival. *Journal of Clinical Oncology* 28 (18): 2493-2498.
- 13** Zhang, X et al (2017). The development of bispecific antibodies and their applications in tumor immune escape. *Experimental Hematology & Oncology* 6:12.
- 14** Schwab, CL et al (2014). Past, present and future targets for immunotherapy in ovarian cancer. *Immunotherapy* 6(12): 1279-1293.
- 15** Zavala, VA, Kalergis, AM (2015). New clinical advances in immunotherapy for the treatment of solid tumours. *Immunology* 145(2): 182-201.
- 16** Assal, A et al (2015). Emerging targets in cancer immunotherapy: beyond CTLA-4 and PD-1. *Immunotherapy* 7(11): 1169-1186.

Thirdly, having a study pathologist that is experienced in evaluating slides from tissue cross-reactivity studies is another key consideration, to ensure that the data output is accurate and reliable. A peer review of pathology data, often from a subset of study slides, adds to the integrity of study data and reduces any bias.

A look to the future

There is still huge scope within the area of I-O, and scientists are experimenting with novel antibody structures and ways to enhance immune cell recruitment and target cancer cells¹¹. Bispecific antibodies are of particular interest to scientists, as these structures have the ability to bind to two different surface receptors with a high level of specificity, allowing a direct connection between a tumour cell and a T-cell¹³. Bispecific T-Cell engager (BiTE) technology has produced several FDA-approved therapies such as blinatumomab, for the treatment of chemotherapy-resistant acute lymphocytic leukaemia, with a whole host in clinical development¹⁰. BiTEs are small molecules consisting of the variable regions only, which are connected by a flexible linker peptide¹⁰. They present as a potent molecule which can be used in low concentrations but also have a short serum half-life which is due to their structure. Preclinical safety assessments for these antibody structures will also require an on-off target binding assessment via tissue cross-reactivity studies, along with other pharmacological assessments. The varying formats, structures and pharmacokinetic profiles of these molecules will allow clinicians to have a greater choice when it comes to therapies and treatment regimes, allowing a more personalised approach in the future.

In addition to novel constructs, scientists have turned to combination therapies to help improve treatment response rates. CTLA-4 and PD-1 inhibitors are currently used as individual therapies in the treatment of advanced melanoma and lung cancer, and when administered in combination a greater efficacy can be achieved in a broader population of patients^{3,8,16}. A new challenge is presented in the safety considerations of these novel immunotherapies as scientists explore combination therapies to improve efficacy. This adds to the complexity of safety assessment assay designs which must take into consideration the non-standard response kinetics and potential autoimmune side effects when combining two or more agents that actively stimulate the immune system. Furthermore, the sequence in which these agents are administered can have differing effects and out-

comes. This presents an opportunity to develop predictive and prognostic biomarkers that can aid in patient selection and direct therapy to the patients who will benefit the most, along with the prediction of immunotherapy toxicity^{6,9}. As yet validated biomarkers with predictive and prognostic value do not exist and is another area of active research⁹. Until these biomarkers are developed, it is important to understand the safety profile and off-target binding toxicities of these agents, particularly when used in combination therapies.

Conclusion

The landscape of cancer treatment has changed dramatically over the last two decades. As our understanding of new immunotherapies progresses, and the way we target the treatment of cancer adapts, so too must the way we assess the safety profile of these agents. There are still many challenges to overcome as we explore the possibilities of novel antibody constructs, along with mono- and combination immunotherapy. As we increase our understanding of this novel therapy for cancer, there also becomes a requirement for predictive and prognostic biomarkers that can aid in patient selection and direct therapy to the patients who will benefit the most⁶. Until these biomarkers are developed, and a personalised treatment regime can be implemented, it is important to understand the safety profile and off-target binding toxicities of these therapies, particularly when used in combination. Compared to a clinical trial, the investment required in a preclinical tissue cross-reactivity study is modest, and provides valuable information that enables scientists to make evidence-based decisions on whether to progress a candidate into clinical trials and how clinical investigations should be directed. It may still be too early in the development of immunotherapies to know the best practice for complete risk assessment in preclinical studies. However, evaluation of antibody tissue cross-reactivity in a panel of 33 or 38 organs is likely to remain a critical element of preclinical safety assessment for this new age of immunotherapies, playing a vital role in accelerating new candidates into human clinical trials. **DDW**

Edward Cribb is Client Services Manager at Propath UK, one of Europe's leading specialists in GLP- and GCP-compliant molecular pathology and histopathology services. With more than 10 years' experience in molecular pathology and histopathology, Ed works closely with biopharmaceutical companies to help develop research strategies to achieve their study objectives.