

# Bringing IO together: how an immunologist and oncologist collaborate in an emerging field

For many years, immunology and oncology operated as separate disciplines within drug discovery. However, recent advances in immuno-oncology (IO) have demonstrated what can be achieved when the two disciplines join forces in developing novel treatments for patients. From checkpoint inhibitors to CAR-T cells and model selection designed to reflect more personalised medicine approaches, immuno-oncology is shifting how both immunologists and oncologists approach drug discovery and development.

In a Q&A, Dr Julia Schüler, DVM, Research Director at Charles River, which specialises in oncology, and Dr Louise Brackenbury, Principal Scientist for Cell Biology at Charles River's newly-acquired KWS BioTest, which specialises in immunology, will provide their unique perspectives on the ways in which IO is reshaping the industry.

## What made you realise that IO was going to have a major impact on your field?

**Louise Brackenbury (LB):** There has been plenty of evidence over the years indicating an important role for the immune system in controlling cancer, however the difficulty had been finding tractable targets. For example, in a 2006 report, Galon and colleagues demonstrated that the density and location of tumour-infiltrating CD3+ and CD45RO+ were a better indicator of patient survival than classical methods<sup>1</sup>.

What really changed everything was when the first few data sets from early clinical trials started to appear, showing the incredible results with Ipilimumab<sup>2</sup> alone and in combination with

Nivolumab<sup>3</sup>. It immediately became clear that enormous progress had been made, and that our ability to manipulate the immune system has provided us with a powerful tool to fight even late-stage and chemotherapy or radiation-resistant cancers.

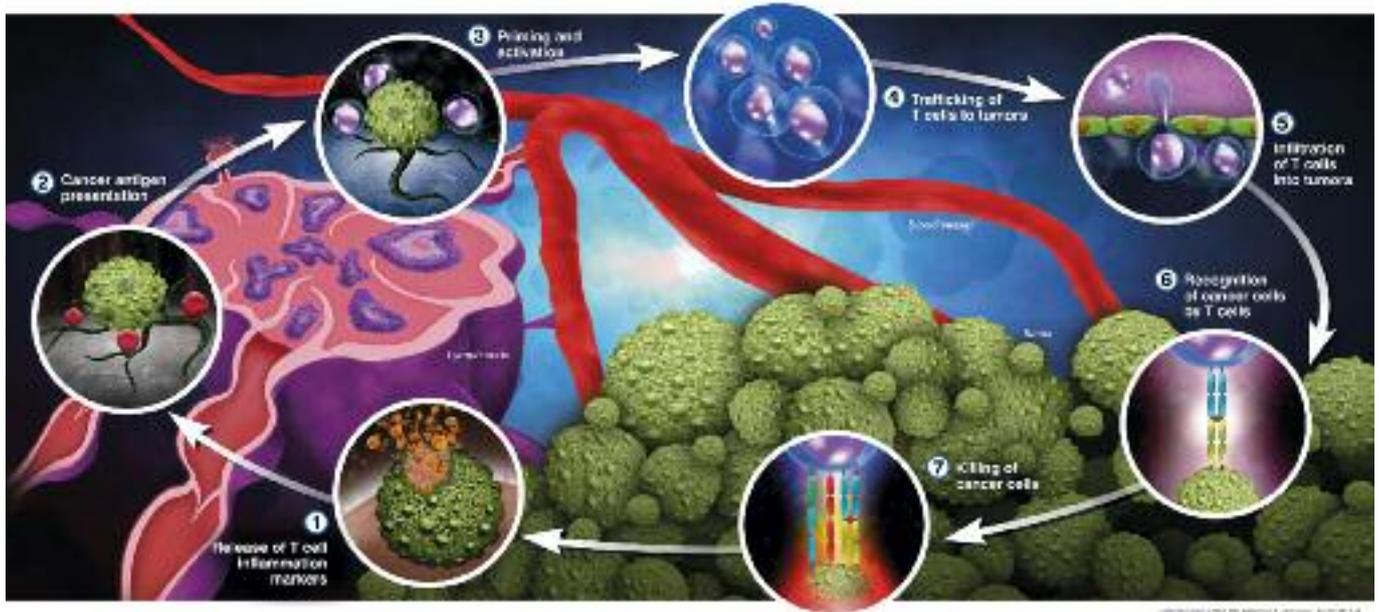
**Julia Schüler (JS):** The complicated cross-talk between cancer cells and the tumour microenvironment (TME) with its different compartments was a research focus in tumour biology for decades. Nevertheless, identifying druggable targets in the TME remained challenging. *Science Magazine's* 'Breakthrough of the Year' in 2013 summarised the impressive results achieved in clinical trials with checkpoint inhibitors, first in late-stage melanoma. For the first time, specifically targeting a non-tumour cell led to significant tumour cell killing across different indications, independent of staging and treatment history.

## What surprised you when you started to conduct IO studies?

**LB:** Coming from an autoimmune/inflammatory background, I had to get used to the idea that

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Every step of the cancer immunity cycle reveals distinct insights

although we were using the same *in vitro* assays we had developed to model autoimmune disease, there was now a drive to enhance, as opposed to suppress, inflammation with novel IO drugs. In optimising these assays, we often had to dial back the stimulus to open the therapeutic window. In turn this meant we had to completely invert the way we were thinking; for instance, where we had been trying to expand regulatory T cells (Treg), we were now testing therapies aimed at inhibiting the same cell type.

**JS:** Coming from an oncology background, mainly developing targeted therapies, I had to get used to the idea that there were no available biomarkers at the time to aid in selecting the model with the highest potential efficacy. Not only did the *in vivo* model have to be adapted to a new mode of action, it also changed the read-out; besides tumour cell killing, immune cell proliferation, dissemination and differentiation also had to be evaluated in the *in vitro* and *in vivo* models.

**What have you learned from the other discipline (either immunology/ oncology) that you wish you knew earlier on?**

**LB:** It is fascinating and challenging to understand the breadth of mechanisms by which different tumours evade immune responses. For example, cancer cell immune-editing drives the generation of tumour variants resistant to immune cell recognition. The major histocompatibility (MHC) class I pathway mutations can allow cancers to become defective in antigen pre-

sentation to tumour-specific T-cells, and tumour cells can also induce tolerance, apoptosis or exhaustion in tumour-specific effector cells<sup>4-6</sup>.

Secretion of immunosuppressive factors such as prostaglandins, TGFβ and VEGF drive the generation of ‘less effective’ immune cells, including myeloid-derived suppressor cells (MDSCs) and Tregs. While these effects are hurdles, they also represent opportunities for the identification of novel targets. To be involved in a field with such a wealth of novel targets is unprecedented and is driving the huge interest in reprogramming these cell types, or inhibiting their function, to generate robust anti-tumour immunity.

**JS:** It is exciting to learn more about the complex multi-dimensional interactions different immune cell types can perform. To generate a resourceful immune response while maintaining self-tolerance, the immune system is tightly regulated through a combination of stimulatory and inhibitory signals. How to influence this balance in an immuno-oncological context and to understand how many different factors play a role in this complex biological system is an ongoing effort.

**Did you have any misconceptions about the other discipline? What made your thought process change?**

**LB:** It took me a while to realise that the revolution in oncology had started and that there was a key role for conventional chemo or radiotherapeutics, not only in driving tumour cytotoxicity, but also in

enhancing the immune recognition of cancers. Although such cases are relatively rare, local radiotherapy applied to a single lesion in combination with ipilimumab has been shown to induce tumour immunity in non-irradiated metastases, suggesting irradiation of one site can boost systemic immunity (abscopal effect)<sup>7</sup>.

Yoshimoto and colleagues tracked tumour antigen-specific CD8+ T-cell responses before, during and after chemoradiotherapy, and showed that, at least in a proportion of patients, CD8+ T-cell anti-tumour immunity was enhanced<sup>8</sup>. There is now an increasing body of data to suggest that radio and chemotherapy play a dual role in both direct tumour cytotoxicity and enhancing immune function.

**JS:** I originally underestimated the influence of immunology, beyond direct manipulation of the immune system, through different modes of action. The induction of immunogenic cell death by well-established compounds, like oxaliplatin, on top of its cytotoxic effect, made me aware of the fact that many, if not all, of the standard of care therapies in oncology have to be re-evaluated in the light of possible immunogenic components of their activity profile. This will help to identify new treatment options and therapeutic areas.

### **With the rise of IO, how have changes in the clinical oncology landscape affected preclinical model development?**

**LB:** Pre-clinical testing of chemo and radiotherapies was historically carried out using xenograft models, in which the immune system is largely lacking. In IO, there is a requirement to have an intact immune system in place and either syngeneic or humanised patient-derived xenograft (PDX) models have become more commonly used. While syngeneic models are suitable for many small molecule programmes, a lack of species cross-reactivity limits their use for the development of many biologics.

To overcome this, humanised transgenic mouse models may be generated so that an anti-human antibody may be used in a murine model. When developing such models, it is critical to ensure that the biology of the target is recapitulated, not just confirm expression. An alternative is to perform pre-clinical testing using PDX in animals with a reconstituted human immune system. This can be a very powerful approach, particularly when screening against a broad range of PDX. A different school of thought revolved around the acceptance that animal models may not be informative, and the rapid growth of complex *in vitro* 3D culture systems has allowed a

major shift towards a lot of pre-clinical drug discovery in the IO space being carried out primarily using *in vitro* human assay systems. Such complex multi-cellular systems can model the tumour microenvironment well, and in co-culturing with immune cells *in vitro*, you can monitor the effects of novel biologics in the tumour microenvironment.

**JS:** Preclinical cancer research relies strongly on animal models, often based on human tumour cells transplanted into immunocompromised mice. The major readout of PDX models is delay of cancer growth or tumour shrinkage, known as tumour growth inhibition (TGI), which is broadly accepted proof of efficacy for cytotoxic and cytostatic therapies.

IO compounds, however, require additional read-outs beyond TGI. Conventional PDX models can only partially reflect the role of the TME (vasculature, tumour-stroma interaction, tumour immunology), since human tumour cells are interacting with a microenvironment of mouse origin. This gap is likely to contribute to the high failure rate of new cancer drugs in early clinical development, often not meeting their primary endpoints while preclinical data has been encouraging. It is now recognised that the severely-impaired rodent host immune system is not always sufficiently reflective of certain aspects of host-tumour immune interaction. Thus, beside new models recapitulating the crosstalk between tumour and immune system, additional readouts are under development that can help to understand preclinical data and have the potential to identify translational biomarkers.

### **How has the advent of personalised medicine affected IO?**

**LB:** Personalised medicine has had an incredible impact, particularly with the use of CAR-T cell therapy in hematological malignancies, and by expanding tumour-infiltrating lymphocytes (TILs) from an individual's tumour *in vitro* with IL-2, and then reinfusing. Such therapies mean that patients can benefit from highly-specific treatments, which provide the potential for far greater success than the current standard of care alternatives.

However, due to their cost and complexity, these regimes are only available to a select few. Moving forward, the challenge will be to make these therapies accessible to all. There has been a lot of interest in trying to develop 'off the shelf' versions, applicable to multiple recipients at a reduced cost, but this remains elusive to date.

The generation of bi-specific antibodies, which

bring immune effectors and tumour targets in close proximity also show promise, but each individual's tumour requires costly screening to identify a tumour-specific antigen. This model is also dependent on the recruitment of a functional, non-senescent immune cell, although this may be overcome by co-administration of other therapeutics, such as ipilimumab or nivolumab, or immuno-stimulatory treatments targeting co-stimulatory molecules, such as OX40. What is most exciting is that personalised approaches are being adopted in cancer treatment. With the discovery of better biomarkers, lower-cost personalised treatments are more likely.

**JS:** Personalised medicine in the light of IO is mostly defined by designing the drug (cells, compound or vaccine) specifically targeted against the individual tumour. As IO is based on the idea that the body fights its own cancer, personalised medicine is a fundamental part of this treatment strategy. This requires that the individual cancer is profoundly characterised to identify its unique weaknesses and druggable targets. This elaborate approach implies the possibility of a growing knowledge about tumour biology which might help us to identify possible treatment strategies tackling common characteristics of specific tumour subtypes in the next five to 10 years. In parallel, the development of urgently-needed predictive biomarkers will be facilitated.

### **What are the biggest opportunities for IO to improve patient outcomes?**

**LB:** Tumors have been classified as being immunologically 'hot' (eg melanoma) or 'cold' (eg prostate cancer), based on the degree and type of immune infiltrate. 'Hot' tumours are therefore relatively highly immunogenic, but to counteract this the tumour has developed a range of mechanisms to subvert this response. Checkpoint inhibitors work relatively well in the context of a 'hot' tumor as they can reverse at least some of the immunosuppressive mechanisms to unleash the existing T-cell response, but they can be less effective in combating immunologically 'cold' tumors, to which a T-cell response may never have developed. Immunogenic cell death (ICD) is a form of apoptosis caused by chemotherapy agents such as oxaliplatin or radiotherapy<sup>9</sup>. It is characterised by the detection of danger-associated molecular patterns (DAMPs) including extracellular ATP and surface-exposed calreticulin by tumour cells after induction of endoplasmic reticulum stress. Such DAMPs drive dendritic cell activation and promote the development of an anti-tumour CTL response. The careful timing and use of combinations of

chemoradiotherapy or oncolytic viruses in combination with checkpoint inhibitors may render 'cold' tumours more immunogenic, and there is a great opportunity for IO to improve patient outcomes in both 'hot' and 'cold' settings.

**JS:** The deeper understanding of how fully-approved therapies influence the patient's immune system opens the possibility for an endless number of combination approaches. Well-known treatments can be repurposed in combination, leading to increased patient benefits in a timely and safe manner. The possibility of targeting immune cells or other parts of the TME can prevent the development of acquired resistance against targeted therapies. Beyond that, it becomes possible to treat a tumour already heavily pretreated as the mode of action of a checkpoint inhibitor is largely independent of the altered signalling pathways within the tumour cell.

The possibility of influencing tumour growth via the microbiome is another opportunity to improve a patient's outcome. Preclinical data is promising, but clearly indicate that like other strategies in IO, the mechanism of action is not fully understood. Finally, the knowledge around the importance of the tumour-stroma crosstalk for drug sensitivity as well as resistance is becoming more and more obvious. Drugs targeting stromal cells and other compartments of the TME beyond TILs are currently under investigation.

### **What are the biggest challenges faced by the field of IO right now?**

**LB:** In many ways, I think one of the biggest challenges to those in the IO field is to determine which combinations of checkpoint inhibitors are best for each cancer indication, and for the individual. The success of ipilimumab, pembrolizumab, nivolumab and avelumab has meant that an unprecedented amount of money has been put into identifying novel checkpoint pathways and immune stimulatory modulators. There are currently 636 registered clinical trials involving nivolumab alone.

Looking forward, therapies targeting a range of other molecules such as TIM3, LAG3, IDO, CD39, CD73 or arginase will come online. As these new therapies reach the market, the potential combinations will increase exponentially, along with the costs, which will eventually be passed on to the patient. We are likely to see increases in the response rates of patients in combination trials, but the key challenge will be in identifying biomarkers that can enable the right combinations to be chosen for each patient's tumour. When you factor in

potential combinations with cancer vaccines and different chemotherapeutic agents, this task seems particularly daunting. Further, although efficacious, CAR-T cells along with other immune modulatory therapies can be associated with significant immune toxicity. Managing the balance between efficacy and toxicity remains a challenge and as patient survival increases following successful treatment, there will also be a need to understand more about how IO therapies affect the immune system long-term.

**JS:** Many of the challenges in IO go back to the rapid development the field experienced in the last few years. The high number of compounds entering clinical trials, without any reliable biomarker for patient stratification, leads to results that are difficult to interpret. Many of those compounds entered clinical trials lacking complete pre-clinical data packages. This exaggerates the problem as many observations were completely unexpected, especially regarding side-effects.

The delicately-tuned immune system in a patient suffering from cancer is difficult to control and the toxicities are completely different from those of targeted therapies or cytostatics. In contrast to the latter, side-effects in IO seem only partly dose-dependent. The design of beneficial combination therapies for defined indications or even individual patients is challenging. The lack of predictive biomarkers and the fragmented understanding of the biology behind it lead to disappointing results in clinical trials.

### How do you anticipate IO drug discovery and development changing in the next five years?

**LB:** Looking towards the future, cancer therapies will become more specific and targeted to minimise off-target effects. Macrophage-targeted compounds, such as CSF1 modulators are likely to result in specific depletion of MDSCs and other myeloid populations while leaving the lymphocytic compartment intact, whereas Treg-targeted biologics, such as anti-TIGIT or anti-GITR antibodies, may specifically deplete this population through antibody-dependent cellular cytotoxicity. This will ultimately allow a more robust anti-tumour immune response. Our increasing ability to manipulate genetic information and introduce or delete specific genes using CRISPR/Cas9 technology gives us a unique opportunity, perhaps in combination with immunomodulators, to induce neoantigen-specific CTL responses while preventing or reversing exhaustion of the expanded T-cells.

High-throughput gene sequencing, in combination with proteomic approaches, will allow for more specific analysis of tumour biopsies and determine which combination of IO modulators are most appropriate for the patient at that time. As tumour escape mutants evolve and the therapy loses effectiveness, this process could be repeated to identify novel neoantigen targets. Finally, through the use of high content analysis, the biomarker field will catch up, enabling better patient stratification and targeting of what will become a suite of immunotherapy combinations to the specific needs of the patient. This is an exciting time to be involved in IO drug discovery and I believe that this field offers real potential to enhance long-term survival rates, particularly in hard-to-treat cancers.

**JS:** IO drug discovery will broaden by manipulating not only T-cells in well-described ways, but by targeting myeloid populations to trigger tumour shrinkage. New advents like the microbiome and the combination of treating different parts of the TME are evolving tremendously and are rapidly moving toward innovative therapeutic strategies that could translate into tangible benefit for cancer patients. The emerging field of cell-based therapies will have a remarkable impact on the FDA approval policies in oncology. In the past, approvals were typically restricted to compounds, but the field will be expanded to include medical devices which enable the production of personalised cell-based therapies. This will have an impact on drug development strategies in the pre-clinical space as well as in clinical trials. **DDW**

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*Dr Julia Schüler studied veterinary medicine at the Freie Universität Berlin, Germany and at the Tierspital Zürich, Switzerland, where she received her DVM. She then joined the lab of Professor Dr Heiner Fiebig, founder of Oncotest GmbH, where she worked on orthotopic implantation of solid cancer PDX in immunocompromised mice. After receiving her PhD from the University of Berlin, Dr Schüler worked in the field of innate immunology at the Max-Planck Institute for Immunobiology, Freiburg.*

*Dr Louise Brackenbury received her PhD in viral immunology from UCL, prior to two postdoctoral fellowships at Imperial College and Bristol University. She joined KWS BioTest in 2012 before being acquired by Charles River in early 2018.*

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