Leveraging Novel Therapeutics and Diagnostic Assays

Immunotherapy has been the focus of medical research for many years, but as scientists continue to experiment with and learn about the form of therapy, it is becoming apparent we may not have even scratched the surface of its potential

Marisa Pearce at MolecularMD, an ICON company

Immunotherapy is far from a novel concept, and its history is intriguing. It all began in 1891 when Dr William B Coley attempted to creatively harness the patient's immune system. William achieved durable complete remission in several types of malignancies to include sarcoma, lymphoma, and testicular carcinoma by injecting mixtures of live and attenuated bacteria into patients' tumours. Needless to say, we have come a long way.

The idea of using immunotherapy in cancer reemerged in 1957 when Lewis Thomas and Sir Frank Macfarlane Burnet first proposed their theory of cancer immunosurveillance. They postulated that lymphocytes acted as sentinels to identify and eliminate somatic cells that were transformed by spontaneous mutations. In 1976, interleukin 2 (IL2)'s clinical utility was demonstrated as an immunotherapeutic agent and achieved FDA approval for its use in combatting metastatic kidney and melanoma cancers. Other advancements included the use of antibody therapies – most notably, rituximab, which was approved by the FDA in 1997 to target non-Hodgkin's lymphoma. Rituximab binds to CD20 on the surface of immature B cells, subsequently targeting them for elimination by natural killer (NK) cells.

T Cell Research

Most recently, James P Allison was named a recipient of the 2018 Nobel Prize in Physiology or Medicine for the culmination of his research identifying the T cell receptor (TCR), the part of a T cell that binds to antigen and functions as the T cell's ignition switch. James' concept of checkpoint blockade to treat cancer was based on his focused career on the study of the basic biology of T cells. The first example of this treatment approach used antibodies to block the action of CTLA-4, which acts as a 'brake' on T cell activation. Release of this brake enables a powerful immune response to the tumour cells. The first drug in this new call of cancer therapies, ipilimumab, was approved for late-stage melanoma by the FDA in 2011. Since 2014, a rapid succession of other checkpoint inhibitors targeted to programmed death-ligand 1 (PD-L1) or programmed cell death protein 1 (PD-1) have been approved, including pembrolizumab (Keytruda) and nivolumab (Opdivo) for varied lymphomas and solid tumours, atezolizumab (Tecentriq) for bladder cancer, avelumab (Bavencio) for metastatic Merkel cell carcinoma, and durvalumab (Imfinzi) for bladder and non-small cell lung cancer.

Adoptive Cell Therapy

Adoptive cell therapy is another approach for harnessing the power of the immune system to target cancer cells. For chimeric antigen receptor (CAR)-T therapies, a patient's T cells are isolated and then engineered to express a CAR on its surface. The receptor enables the T cells to identify and destroy cancer cells that express a specific target molecule. Tisagenlecleucel (Kymriah) was the first FDA-approved CAR-T therapy for treatment of relapsed or refractory B cell precursor acute lymphoblastic leukaemia, quickly followed by Axicatagene cilleucel (Yescarta) for large B cell lymphoma patients who have failed conventional treatment. Ongoing trials progressing into Phase 2 are evaluating anti-B cell maturation antigen (BCMA) CAR-T therapy in late-stage relapsed/refractory multiple myeloma patients. These types of studies suggest the potential for CAR-T therapies to move beyond lymphoma and leukaemia and towards other haematological cancers (1). In addition to CAR-T therapies, TCR-engineered T cells are a novel option for adoptive cell

> **The ideal biomarker test accurately matches drugs with patients who will benefit from them**

types of sporadic cancer also exhibit MSI caused by somatic hypermethylation of the MLH1 promoter (1). Gold standards include IHC and polymerase chain reaction (PCR)-based testing such as the Promega MSI System.

Interestingly, a variety of MSI-associated cancers show significant responses to anti-PD-1 inhibitors in patients who have failed conventional therapy (2). Patients with somatic hypermutation and neoepitope formation have favourable responses to immunotherapy. Studies have shown the correlation between MSI and an elevated rate of lymphocytic infiltration relative to tumours without MSI (3).

While MSI-guided immunotherapy serves as a practical surrogate, it may be displaced by mutational burden and immunogenic neoepitope analyses. The use of next-generation sequencing (NGS) has been evaluated for the correlation between tumour mutation burden (TMB), MSI, and PD-L1 with a large study cohort. The overlap between TMB, MSI, and PD-L1 differed among cancer types. However, MSI high (MSI-H) status can be determined by NGS across cancer types, and offers distinct data for treatment decisions regarding immune checkpoint inhibitors (4).

Understanding TMB

The latest approach in the evolving field of immunoncology biomarkers is TMB. Data has accumulated that suggests TMB is an independent predictor for patient stratification and response to immunotherapies, such as checkpoint inhibitors. "The methods used so far to assess tumour mutation burden in clinical studies have included whole exome sequencing and several laboratory-developed, targeted next generation sequencing panels," said Cindy Spittle PhD. "But in order to fully determine the value of TMB as a predictive biomarker, a standardised panel, workflow and data analysis pipeline for TMB assessment is needed."

Clinical Testing

Practical considerations such as sample quantity and quality are key for the selection of an assay suitable for clinical research studies and later as a diagnostic. Access to tissue and the process by which it is processed is challenging, given many of the specimens are from cancer types with limited material beyond the primary biopsy or are derived from formalin-fixed paraffin-embedded tissues. TMB methods that require less

input material are preferential for routine clinical testing, as well as those that can leverage modified workflows to account for poor quality DNA due to deamination during tissue fixation.

The development of tumour-specific TMB range and cut-off criteria is needed to drive its use for precision medicine. Preliminary data have shown that gastrointestinal (GI) tumours with MSI have a higher cut-off and broader range, than for example, cervical or uterine MSI-positive cancers (see Figure 1, page 80).

Microsatellite stable (less than or equal to one of five loci) and MSI (greater than or equal to two or more of five loci) are detected on the Promega Microsatellite Instability 1.2 Analysis System.

TMB can range by tumour type. While the majority of samples were found to harbour less than 125 variants/MB, an initial study cohort ranged from 10 to 2,779 variants/MB. This variance has been observed in a number of other mutational burden studies and will require larger datasets to determine appropriate diagnostic cut-offs.

Tumour Infiltrating Lymphyocytes

Infiltration of immune cells, particularly infiltration of anti-tumour type 1 lymphocytes, has predicted improved prognosis in many different tumour types, including colon, ovarian, lung, and breast cancer. Recent evidence has emerged that tumour infiltrating lymphocytes (TILs) present in these cancers prior to treatment can predict response to therapy and improved prognosis, including the amount of lymphocytic infiltration and the phenotype of the infiltration (3). Methods include the analysis of PD-L1 and infiltrating immune cells such as CD3, CD8, and CD163. However, IHC scoring and classification can be complicated by the definition of positivity and intratumoural heterogeneity.

A more comprehensive assessment of the tumour and its microenvironment could include whole transcriptome sequencing for gene expression profiling in addition to whole exome sequencing for mutational burden. Again, the amount and quality of tissue is relevant to clinical testing and creates restraints on these types of comprehensive analyses. Targeted NGS panels or multiplexed real-time quantitative PCR assays can be used to interrogate the most relevant genes involved in tumour-immune cell interactions.

Monitoring Replication-Competent Viral Vector Systems

Viral vectors are a common tool to introduce novel or recombinant genes into cell therapy products for treatment of human diseases. They are used to introduce genes into mature T cells to generate immunity to cancer through the delivery of CARs and/or cloned T cell receptors (5). Self-inactivating lentiviral vectors have recently been used in multiple clinical trials to introduce genes into haematopoietic stem cells to correct primary immunodeficiencies and haemoglobinopathies. Early studies identified issues with recombination leading to lymphomas in primate models. This risk now is extremely low with more recently engineered viral vector systems (6). Even though the risk of developing secondary malignancies is low, health authorities in the US and Europe require long-term follow-up studies of cell and gene therapies using viral vectors. All cell- and gene-based products modified by viral vectors and intended for patient use must be tested for replication-competent lentiviral (RCL) or replication-competent retroviral (RCR) prior to treatment of the patient (7). Cell-based assays have been used, however, many Phase 2/3 clinical trials with novel cellular therapies require infusion of fresh cells, which does not allow completion of a full cell-based RCL/RCR assay (up to six weeks) prior to infusion. Quantitative PCR-based assays provide a quick alternative for the rapid assessment of RCL/RCR in products intended for infusion. Using the same methodology, patients should also be followed for replication-competent sequences as part of the long-term follow-up protocol.

Vector Copy Number Analysis

With the advent of adoptive cell therapy, a number of targetable biomarkers has emerged. The first biomarker target was CD19, a B cell marker expressed highly on malignant cells. Emerging biomarkers include BCMA, PSCA, CD33, CD5, ROR1, CD123, CD133, Her2, Muc1, EpCam, among others (8). IHC or RT-qPCR can be used to identify tumours that express the molecule that is being targeted by the CAR-T or TCR, providing a novel genomics-oriented approach to patient selection. Patients expressing the target of interest, such as BCMA, can be enrolled in the clinical trial. Following infusion, patients are monitored for persistence of recombinant T cells and drug/dose response. Droplet digital PCR qPCR-based assays are frequently used to quantify the vector copy number (VCN) of the endogenous target sequences. The VCN assays are used for pharmacokinetic (PK) measurements, a novel DNA-based assay to monitor drug levels as opposed to typical PK testing performed via high-performance liquid chromatography or mass spectrometry-based measurements of a small molecule drug.

Looking Forward

Patient selection and monitoring is critical at all stages of the drug development and approval process. The ideal biomarker test accurately matches drugs with patients who will benefit from them. In some cases, this can be accomplished by one biomarker, including T cell receptor therapies, CAR-T, and antibody-based therapies. However, biomarkers for drugs that target checkpoint pathways are more complicated. New programs incorporate different immunotherapies in combination trials. Biomarker selection for immunotherapies is as unique as the immunotherapies and cancers they treat and will require customised approaches for their identification.

References
1. Marshall HT and Djamgoz MBA, Immuno-oncology: Emerging targets and combination therapies, *Front Oncol*: 2018
2. Visit: www.ebiomedicine.com/article/S2352-3964(19)30019-2/pdf
3. Umar A *et al*, Revised bethesda guidelines for hereditary nonpolyposis colorectal cancer (lynch syndrome) and microsatellite instability, *J Natl Cancer Inst* 96(4): pp261-8, 2004
4. Dudley JC *et al*, Microsatellite instability as a biomarker for PD-1 blockade, *Clin Cancer Res* 22(4): pp813-70, 2016
5. Teng MW *et al*, Classifying cancers based on T-cell infiltration and PD-L1, *Cancer Res* 75(11): pp2,139-45: 2015
6. Vanderwalde A *et al*, Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients, *Cancer Med* 7(3): pp746-56, 2018
7. Visit: www.nature.com/articles/s41375-018-0106-0
8. Visit: jeccr.biomedcentral.com/articles/10.1186/s13046-018-0817-0

About the author

Marisa Pearce is Scientific Affairs Liaison at MolecularMD, an ICON company, and has over 10 years of experience in oncology biomarker test development. She has been involved in the planning and design of molecular tests across the continuum from translational research to clinical deployment. These efforts include research use only assays for solid tumour profiling to the commercialisation of companion diagnostics for minimal residual disease monitoring. Marisa's diverse background enables her to serve as subject matter expert on the selection and use of a variety of technologies for biomarker assay development. At MolecularMD, an ICON plc company, biopharma clients have access to a fit-for-purpose, platform-agnostic approach leveraging technology platforms such as NGS, droplet digital PCR, and multiplexed IHC for the codevelopment of clinical trial assays and/or downstream diagnostics aligned with the therapeutic program objectives. Email: mpearce@molecularmd.com