IMMUNOTHERAPEUTICS the coming of age of cancer immunotherapy as a treatment paradigm

As the oncology drug development landscape has evolved, so too have the processes, methods and equipment used in the fight against cancer. Traditional treatments such as chemotherapy and radiation therapy are still very popular and remain effective methods of fighting the disease as a whole. However, recent research has started to shift away from aggressive indiscriminate treatments towards more targeted therapies.

By Dr Jean Pierre Wery

Modern cancer treatments are becoming increasingly concerned with the development of tailored strategies for subsets of cancer patients (personalised medicine) to significantly improve the opportunity for success during clinical development. These treatments also offer patients greater comfort as many tailored therapies have considerably fewer side-effects, compared to their predecessors.

Research has revealed cancer to be an extremely complex and diverse disease and it is well established that there is no single treatment that can prove effective for all patients, even within a single cancer type or subtype. Attrition rates in oncology are significantly higher than in other therapeutic areas, with only 5% of promising anticancer agents being licensed after successful Phase III trials. The majority of failures are linked to efficacy rather than toxicity and, combined with the continually rising costs of clinical trials, have resulted in an oncology drug development process which is both highly inefficient and enormously costly for pharmaceutical companies. In order to improve the efficiency and cost-effectiveness of developing new therapies, current translational tools need to be enhanced to ensure they are of optimum value for indicating clinical success. Effective preclinical precision profiling screening platforms need to be established which can validate the profile of a prospective drug candidate before entering clinical trials.

Recent years have the seen a considerable increase in the popularity of patient-derived xenograft (PDX) models as a platform for the screening of novel therapeutics for the treatment of cancer. Their use in Phase II-like studies or human surrogate trials has dramatically changed the way compounds are evaluated prior to transitioning into the clinical setting. By establishing deep biological insights into the pharmacological mechanisms of a drug and identifying potential biomarkers important to clinical trial design (ie, patient stratification and study rationale), PDX models provide drug developers with a significantly higher level of confidence for decision-making in the drug discovery process. Recent advances in image guided radiation technology have also enabled the screening of drug candidates for use in drug-irradiation combination therapies, using PDX models to

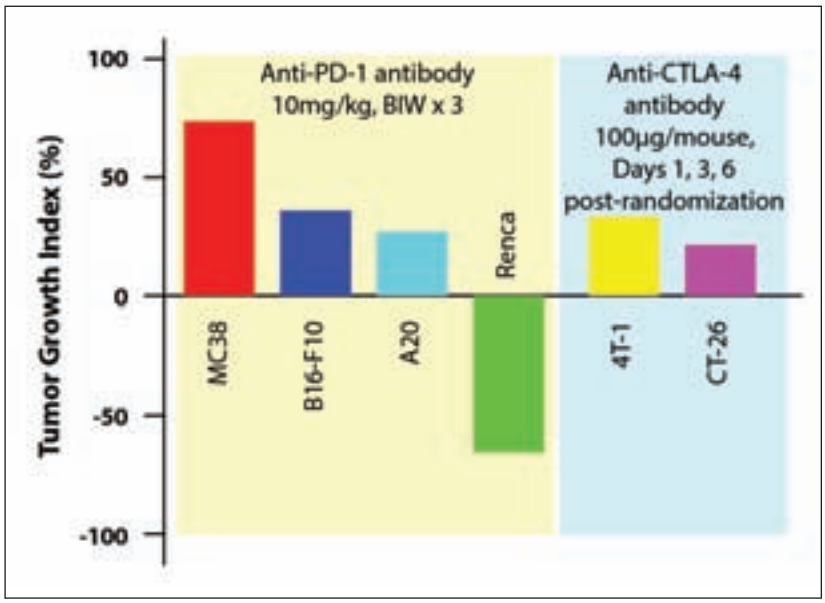

Figure 1
Syngeneic immunotherapy models treatment with anti-PD-1 and anti-CTLA-4. Therapeutic antibodies single agent anti-PD-1 antibody (clone RMP1-14) was tested in the subcutaneous MC38 colon carcinoma, B16-F10 melanoma, A20 B cell lymphoma and Renca renal adenocarcinoma models. Single agent anti-CTLA-4 antibody was tested in the subcutaneous 4T1 breast cancer and CT-26 colon cancer models. Per cent TGI was calculated for each model at termination of the study

show the progression of cancer growth and the effectiveness of the treatment.

However, with the recent advances in personalised medicine, there is a growing requirement for more relevant models in the preclinical screening of modern drug candidates. By using models which are far more relevant to the clinical situation, in conjunction with precision molecular profiling and data analysis, it is possible to optimise and accelerate personalised therapeutic compounds into the clinic.

Current treatment modalities

There are currently several modalities which can be employed in the treatment of cancer. Their use depends heavily upon the tumour type, progression and even the biology of the patient.

Chemotherapy involves the administration of chemical compounds which are specifically designed to destroy cancer cells. These chemotherapeutics or cytotoxins can be employed either as curative agents for the early stages of the disease, or for palliative treatment if the cancer is in the advanced stages. By killing rapidly dividing cells, cytotoxins attempt to control the growth of the cancer; however, they also have the ability to cause damage to healthy cells undergoing natural division within the body. These compounds can be administered individually as single agents or in combinations to increase effectiveness and overcome the effects of cancer therapeutic resistance.

Radiation therapy is a popular modality for localised cancer treatment and can also be used as a curative measure for early stage disease, palliative treatment for advanced stage cancers and adjuvant therapy for patients following surgical intervention. This treatment involves the use of ionising radiation to damage the DNA of cancerous tissues causing cell death. In an effort to minimise the risks to patients, radiation therapy has evolved and modern day treatments utilise multiple lower intensity beams, angled to meet at the tumour site, ensuring the full dosage is delivered to the cancerous tissue while causing the least damage to the surrounding healthy tissues. The effectiveness of this treatment is highly dependent upon the tumour cell type, as different cancers have different radiosensitivities. Leukaemia, for example, is known to be highly radiosensitive and requires only modest doses of radiation to cause cell death, whereas melanoma is widely considered to be radioresistant.

Both chemo- and radiotherapy are effective methods of preventing the growth of tumours in the initial rounds of treatment; however, both are vulnerable to the effects of treatment resistance. Once the initial rounds of treatment have destroyed the target cancer cells, resistant cells remain and are able to thrive as subsequent rounds of treatment are no longer effective at destroying these cell types. Therefore, in order to combat this problem, combination therapies have been developed where different chemical compounds are administered in subsequent doses in order to kill a greater number of cancerous cells, giving the patient a greater chance of survival. In addition to drug combination therapies, recent developments have seen the rise of drug-radiation combined treatments. The initial round of therapeutics significantly slows progression of the tumour and is then followed by a round of radiation treatment to kill the remaining cancerous cells.

The ideal treatment for any disease is one that can cure or prevent the onset of the disease with the least impact on the quality of life of the patient. Surgery, irradiation and drug treatments in oncology and wider medicine have historically been associated with considerable side-effects. This has fuelled the drive for less invasive, less aggressive treatments. In the case of drug delivery, the search for more advanced drug candidates has led to the development of targeted treatments, which have significantly fewer side-effects than traditional therapies. Targeted agents prevent cancer cells from multiplying by interfering with specific molecules which play a major role in carcinogenesis, or tumour growth. While these biological agents demonstrate effectiveness in stemming tumour growth, they can be combined with cytotoxic compounds to form a more

aggressive treatment with greater capacity for destroying cancer cells.

Introduction to immunotherapy

Many forms of targeted therapies are also examples of immunotherapy, where the body's own immune mechanisms are used to fight cancer. Immunotherapeutic treatments offer a way to increase a patient's comfort while also increasing the treatment efficacy of administered active compounds. The coming of age of immunotherapy as a treatment paradigm for oncology has really been signified by recent fast-track designations and regulatory approvals of immunotherapeutics including the first FDA approvals for anti-PD-1 antibodies in advanced melanoma treatment. PD-1 also known as programmed cell death 1 is a naturally occurring protein which downregulates the immune system and prevents activation of T-cells. The creation of the anti-PD-1 antibody enabled drug developers to design therapeutics which prevent natural suppression of the immune response in the presence of cancers expressing the PD-1 protein. The FDA approvals signal the firm establishment of immunotherapy as an effective treatment paradigm that can bring both potential patient benefits and commercial success for the pharmaceutical industry.

There are three main groups of immunotherapy used to treat cancer: cell-based therapies, antibody therapies and cytokine therapies. They all exploit the fact that cancer cells often have subtly different molecules on their surface that can be detected by the immune system. These molecules, known as cancer antigens, are most commonly proteins but also include other molecules such as carbohydrates. Immunotherapy is used to provoke the immune system into attacking the tumour cells by using these cancer antigens as targets.

Cell-based therapies, also known as cancer vaccines, usually involve the removal of immune cells from someone with cancer, either from the blood or from a tumour. Immune cells specific for the tumour will be activated, grown and returned to the person with cancer where the immune cells provoke an immune response against the cancer. Cell types that can be used in this way are natural killer cells, lymphokine-activated killer cells, cytotoxic T cells and dendritic cells. The only cell-based therapy currently approved for use is Dendreon's Provenge, which is used for the treatment of prostate cancer.

Antibody therapies are currently the most successful form of immunotherapy, with many approved treatments for a wide range of cancers. Antibodies are proteins produced by the immune system that bind to a target antigen on the surface of a cell. In normal physiology they are used by the immune system to fight pathogens. Each antibody is specific to one or a few proteins and those that bind to cancer antigens are used in the treatment of cancer. Cell surface receptors are common targets for antibody therapies and include the epidermal growth factor receptor and HER2. Once bound to a cancer antigen, antibodies can induce antibody-dependent cell-mediated cytotoxicity, activate the complement system, prevent a receptor interacting with its ligand or deliver a payload of chemotherapy or radiation, all of which can lead to cell death. There are 12 antibodies currently approved for the treatment of cancer, including: Cetuximab for colon cancer, Ibritumomab tiuxetan approved for non-Hodgkin's lymphoma and Rituximab for B-type white blood cell-related cancers.

Cytokines are proteins that function as molecular messengers to allow cells of the immune system to communicate and co-ordinate responses to antigens. Many cellular signalling mechanisms involve direct cell to cell contact, however cytokine secretion allows for more rapid signal propagation between immune cells. By characterising cytokines, researchers hope to take advantage of their vast signalling capacities to develop more effective cancer treatments.

Challenges

While immunotherapy demonstrates an extremely promising treatment option for cancer patients, advancements in the field have inevitably uncovered subsequent challenges and barriers to further development. One of the most significant challenges is ascertaining why some patients and diseases benefit from these treatments while others do not. Researchers still do not know how to maximise the benefits from immunotherapeutic agents, eg through combination therapy approaches or between targeting different immune checkpoints.

Efforts to reveal this information are currently being limited by a distinct lack of experimental immunotherapy models which feature a functioning immune system, which would provide an invaluable tool in answering these questions and developing better treatments. At present, the majority of experimental cancer models comprise human tumours grown in immunocompromised mice, derived from either *in vitro* immortalised cancer cells (cell line derived xenografts (CDX) or patient tumours (PDX)). These immunocompromised surrogates have historically been useful in studying the effects of cytotoxins on the development of human

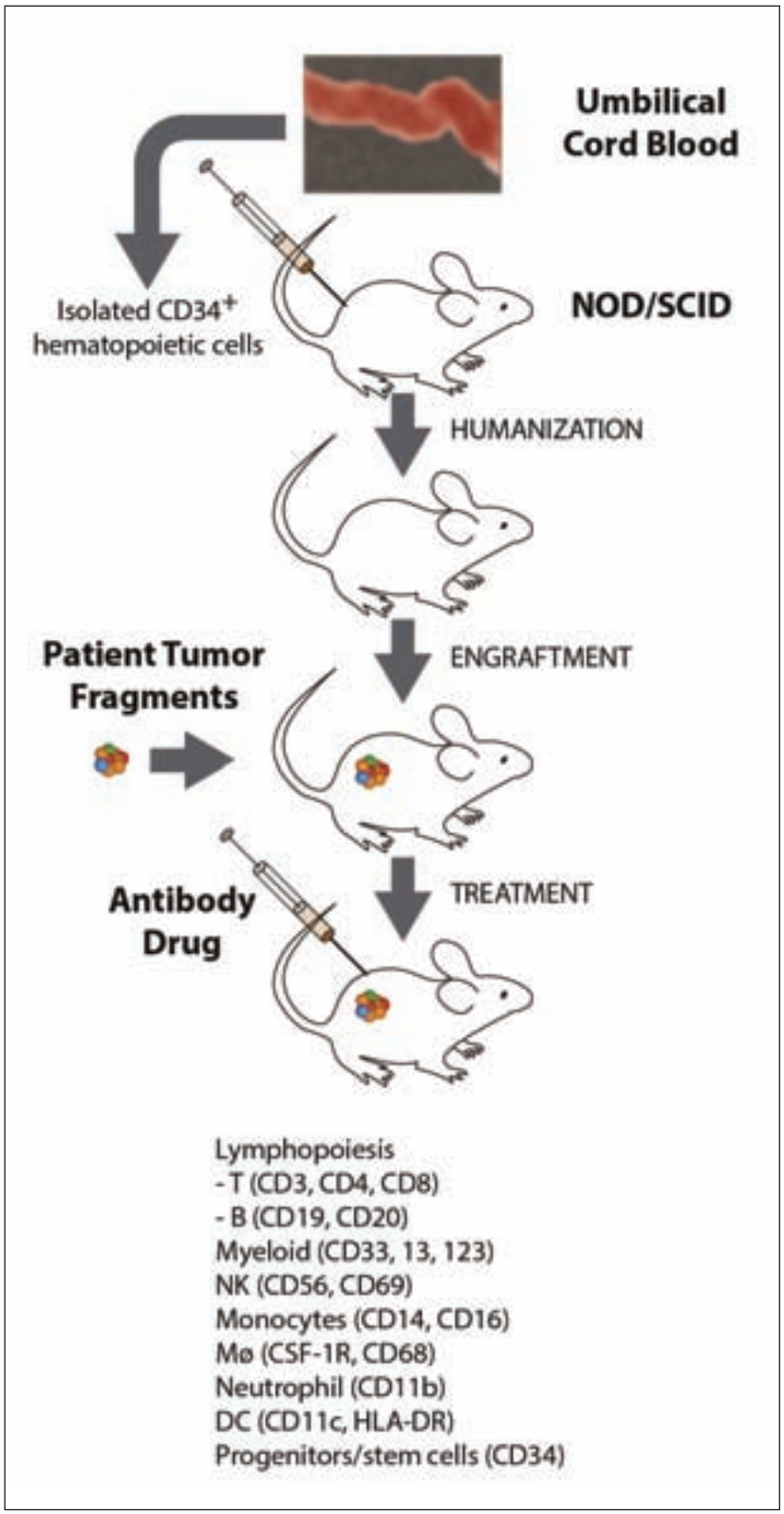

Figure 2: The process for developing patient-relevant surrogate models to be used in pre-clinical drug candidate trials. Humanised mice are developed through inoculating human hematopoietic cells into immunocompromised mice, then through combination with PDX models the function of the target and the immune response can be evaluated

tumours in a live animal host. However, to study the effects of immunotherapeutics, it is necessary for the host to have a functional immune system.

At present syngeneic and genetically engineered mutant mouse (GEMM) models with functional murine immunity are available and widely used in immunotherapy research. Syngeneic or allograft transplantation models are created when tumour tissues derived from a genetic line of mice are transplanted into a host that shares that genetic background. This is particularly advantageous as it prevents the tumour from being rejected. **Figure 1** shows how immunotherapeutic antibodies tested against the immune checkpoint proteins PD-1 and CTLA-4 (a similar protein which also downregulates the immune response) in a range of syngeneic models across a variety of cancer types. A large variation in tumour growth inhibition (TGI) was observed across models, highlighting the importance of selecting the appropriate model for the evaluation of single agent or combination regimen cancer immunotherapeutics.

Genetically-engineered mice produce spontaneous tumours following somatic activation of an oncogene, and/or inactivation of a tumour suppressor gene. Both of these model types have associated benefits and limitations with respect to clinical relevance and utility and there exists an unmet need for new models with complete immunocompetency to help drive oncology research forward.

Driving immunotherapeutics in oncology forward

To complement syngeneic and GEMM models with functional murine immunity, recent research has led to the development of a collection of allografts of spontaneous murine tumours studied in mice with complete immunocompetency. These tumours have never been manipulated or adapted to grow *in vitro* and cover a wide diversity of cancer types, enabling preclinical research into specific pathways and the discovery of new predictive biomarkers for targeted immunotherapy agents.

The next generation of immunotherapy models will also harness the full function of the human immune system against human tumours, to provide information on how the human immune response affects tumour growth, as well as information on evaluation of novel immunotherapeutics. Humanised mice have been developed through inoculating human hematopoietic cells (from cord blood stem cells) into immunocompromised mice. These mice can then be uniquely combined with PDX models – the most predictive type of preclinical xenograft model available – which not only

evaluates the function of the target, but also the immune response in models that preserve the genomic integrity and heterogeneity seen in patients (shown in **Figure 2**). This should enable indication selection and responder population analysis across a range of cancer types within this platform.

It is also possible to generate models of transient human immunity (developed by mixing peripheral blood mononucleated cells with xenograft models), to provide a simple alternative to the full stem cell reconstitution approach.

One of the most exciting trends emerging in 2014 was the use of image guided irradiation in preclinical testing. While image guided radiation therapy has been available in the clinic for some time, it has only been in recent years that the technology has been adapted for use on small animal surrogates. By using this new technology in conjunction with a PDX model preclinical trial, it is possible to assess the effectiveness of drug-radiation combination therapies, closely mimicking contemporary human disease and current treatment regimens, facilitating a rapid, higher throughput evaluation of potential radiosensitisers for cancer treatment.

Conclusion

The successes of immunotherapy research and agents such as anti-PD-1 antibodies have been established along with the significant benefits offered to the patient. However, progress in the field has been hindered through a lack of experimental immunotherapy models with a functioning immune system. The continued development of immunotherapies depends highly upon the availability of clinically relevant screening platforms which can indicate the effectiveness of treatments across a wide range of cancer types.

Due to the diversity of cancer, a large collection of relevant surrogate models is required. Current models of murine immunity including syngeneic and GEMM models can be used to interrogate novel immune treatments through activating the mouse immune system, and to interrogate the complete process of cancer progression and to assess where stimulating the immune system is most beneficial, respectively. Newly developing platforms comprising allografts of spontaneous murine tumours, studied in mice with complete immunocompetency combine the improved predictive power of GEMM models with an operational simplicity, consistency and robust growth for pharmacology research.

Platforms of human immunity are being developed to enable immunotherapeutics to be evaluated using highly predictive PDX models (that preserve the genomic integrity and heterogeneity seen in patients) within humanised mice. Reconstitution of a human hematopoietic system within immunocompromised mice through engraftment of human cord blood $CD34^+$ cells offers a unique opportunity to study immunotherapeutics within a human tumour microenvironment. In addition, by combining models of transient human immunity developed by mixing PBMCs with xenograft models, it is possible to provide a simple alternative to the full stem cell reconstitution approach for immunotherapy research. DDW

Prior to joining CrownBio, ***Dr Jean Pierre Wery*** *was Chief Scientific Officer at Monarch Life Sciences, a company dedicated to the discovery and development of protein biomarkers. Prior to joining Monarch, Dr Wery spent three years at Vitae Pharmaceuticals, Inc where he was VP of Computational Drug Discovery. Before joining Vitae he worked for 12 years at Eli Lilly and Company in various scientific and management positions. Dr Wery received his BS and PhD in Physics from the University of Liege, Belgium. Following his PhD, he did postdoctoral studies at Purdue University with Professor Jack Johnson. Dr Wery has authored more than 50 abstracts and publications.*