The use of syngeneic models for the discovery of targets for combination therapy and predictive biomarkers of immunotherapy efficacy

Optimal treatment for any disease is one that can cure or prevent spreading with minimal impact on the patient's quality of life. In the case of cancer, therapeutic agents were initially designed to kill rapidly dividing cells. These traditional treatments, namely chemotherapy and radiotherapy, remain the backbone of current therapy. While effective, treatments are often related to severe side-effects and the advance of drug resistance.

By Dr Jean-Pierre Wery

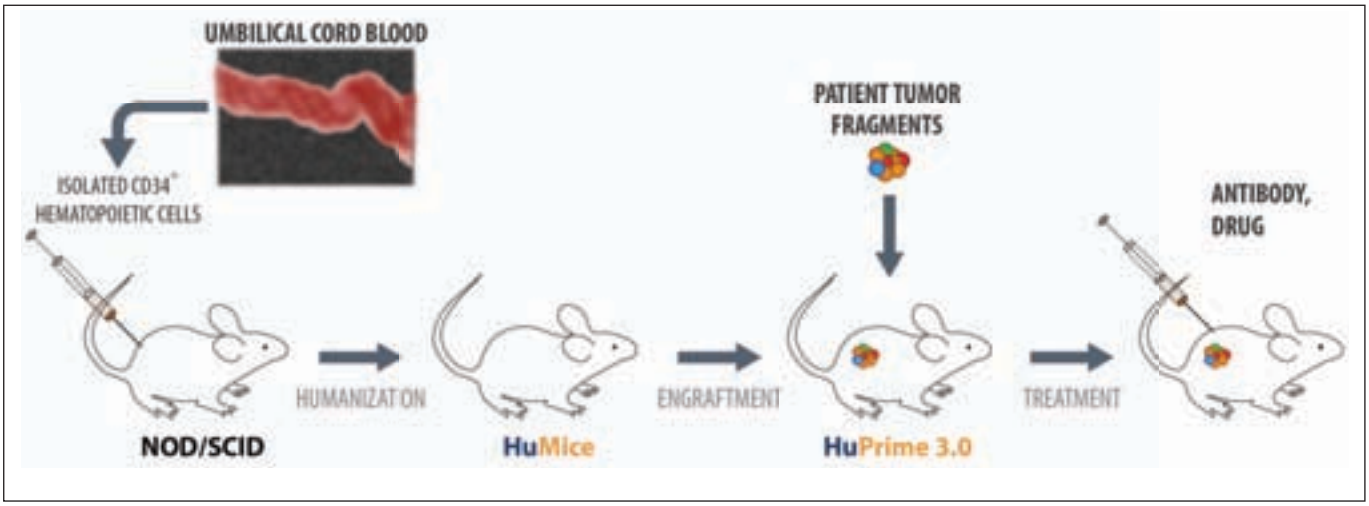
The discovery of crucial molecular pathways that promote tumour growth and maintenance together with the development of drugs that specifically inhibit these pathways has ushered in a new era of cancer medicine, giving rise to new treatment options, including the latest advances in cancer immunotherapy.

An example of advancements in cancer immunotherapy is the use of syngeneic models. Developed 50 years ago as *in vivo* models for oncology drug development, syngeneic models are allografts of murine tumours in mice with a fully competent immune system. Models with a functional immune system are required to drive forward immunotherapy research and to enable the successful transition of immunotherapeutics from the laboratory to the clinic, reducing attrition rates and bringing costs down.

The current situation in therapy

For most cancer types, the best approach to treatment is a combination of surgery, radiotherapy and chemotherapy. Chemotherapy involves the administration of chemical compounds that are specifically designed to destroy cancer cells. By killing rapidly dividing cells, chemotherapeutics attempt to control the growth of the cancer; however, they also have the ability to cause damage to healthy cells. These compounds can be administered individually as single agents or in combinations to increase effectiveness and overcome the effects of drug resistance.

Radiation therapy is utilised to treat localised cancer 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



PDX models, which are derived directly from human cancers, are at the forefront of personalised medicine research, enabling the identification of the right compound for the appropriate patient population before new drugs are tested in the clinic

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.

Both chemo- and radiotherapy are effective methods of preventing the growth of tumours in the initial rounds of treatment; however, they can cause severe side-effects and both are vulnerable to emergence of treatment resistance. Once the initial rounds of treatment have destroyed the target cancer cells, resistant cells may remain which are able to thrive as subsequent rounds of treatment are no longer effective at destroying these cell types. Similarly, targeting one single oncogene at the time can produce rapid responses; however, cancer cells can evolve to overcome the single oncogene inhibition by activating alternative pathways. This generally results in patients relapsing or developing drug resistance. In order to overcome 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. The initial round of therapeutics significantly slows progression of the tumour and is then followed by a round of treatment to kill the remaining 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.

Immunotherapy

Taking advantage of a patient's own immune system has shown remarkable results. There are three main types of immunotherapy, which includes cell-based therapies, cytokine therapies and antibody therapies, including the popular checkpoint inhibitors. Cell-based therapies, also known as 'cancer vaccines', usually involve the *ex vivo* expansion of immune cells from cancer patients, which will be activated and infused back, where they are expected to induce a strong immune response against cancer. This approach, known as adoptive cell transfer (ACT), has been restricted to small clinical trials so far; however, treatments using these engineered immune cells have generated some remarkable responses in patients with acute lymphoblastic leukaemia (ALL) or lymphoma. The first and only therapeutic cancer vaccine currently approved by the FDA is Dendreon's Provenge, used for the treatment of prostate cancer[1].

Cytokines are compounds produced during the inflammatory response and there has been evidence of antitumour effects. IL-2, for example, was shown to be able to stimulate T-cells proliferation promoting their antitumour response. However, the high dose required was associated with strong side-effects and cytokines are nowadays rarely used as standalone treatment.

Antibody therapies, such as the anti-PD-1, anti-CTLA-4 and anti-PD-L1 antibodies, are currently the most successful form of immunotherapy. Programmed Cell Death 1 (PD-1), Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA-4) and Programmed Death-Ligand 1 (PD-L1) are endogenous proteins that naturally down-regulate activated immune cells to prevent autoimmune diseases.

The use of an antibody that binds to these proteins prevents this negative feedback and has been exploited for keeping the immune system alert to fight cancer.

The most palpable issue surrounding the development and application of immunotherapy is the necessity to find a balance between the higher costs of immunotherapy versus standard therapies and its superior efficacy. This is currently the most significant drawback of immunotherapy and one of the main reasons why there has been some hesitation around using immunotherapy as a first line treatment.

Although immunotherapy research is fast moving, scientists are still looking into how to maximise the benefits from immunotherapeutic agents, as cancer is a complex disease with extremely diverse histopathology and heterogeneous pathogenic mechanisms. While immunotherapy demonstrated to be an extremely promising treatment option for cancer patients, advancements in the field have inevitably uncovered subsequent challenges and barriers to further development. Researchers still do not know how to maximise the benefits from immunotherapeutic agents, for example, 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.

Syngeneic models

Immunocompromised models, such as cell line-derived xenografts (CDX) or patient-derived xenografts (PDX), are extremely useful tools for studying the physiopathology of human tumours and investigating the response to anti-cancer agents in a live host. However, to study the effects of immunotherapy it is necessary for the host to have a functional immune system. Experimental mouse tumour models have provided key mechanistic insights into host antitumour immune responses, and these have guided the development

of novel treatment strategies. To accelerate the translation of these findings into clinical benefits, investigators need to gain a better understanding of the strengths and limitations of different mouse models as tools for deciphering human antitumour immune responses.

Following the success of new immunotherapy agents, such as checkpoint inhibitors, syngeneic models have resumed centre stage. Syngeneic models are murine tumours grown in the same strain of mice in which the tumour originated. In these models the recipient mouse has a fully functional immune system which provides an effective approach for studying how immunotherapy performs. These models offer several undeniable advantages. They are relatively inexpensive, reproducible, there is a strong baseline of drug response data, and they come in a wide variety of tumour types. They are also readily available so studies are easily conducted with statistically meaningful numbers of mice per group.

Because syngeneic models retain an intact immune system, they are considered particularly relevant models to study immunologically-based therapies, used either as single agents or in combination with other drugs that modulate the immune system's ability to identify and destroy cancer cells[2].

Although we have developed a greater understanding of the immunological make up of tumours and their responsiveness to checkpoint inhibitors, it is evident there is still more to learn. Questions are starting to be asked around how to best position the available preclinical models for drug development and how they can help identify biomarkers of response in the patient population. Biomarkers identification in the case of immunotherapeutics provides a big challenge as the immune response to cancer involves several different cell types and mechanisms, with a variety of spatial and temporal manifestations. Analysis of peripheral blood and serum following immunotherapy remains the standard approach, but the importance of obtaining tissue to study the immune response at the site of the tumour is becoming increasingly evident.

Syngeneic tumour models have long been used in cancer research. The recent clinical success of the anti-CTLA4 and anti-PD1 antibodies has encouraged interest in the use of syngeneic models to evaluate cancer immunotherapeutics. Researchers discovered that many of the canonical signalling pathways inhibitors already marketed or still under development can interact with the immune environment, thus synergising with cancer immunotherapies.

Immunotherapy-specific combination strategies

Combination approaches targeting more than one regulatory node of the immune system at a time have a strong potential for immunotherapy-specific combination strategies. Moreover, the new immunotherapies are also attractive combination partners for existing standard-of-care treatment options, including chemotherapy and radiation, which until recently were considered incompatible. It is now clear, however, that many standard-of-care agents have positive effects on the tumour's immunological environment. For example, both chemotherapy and radiation, by inducing DNA damage, have been shown to render tumour cells more readily recognisable by the immune system.

Targeting immune checkpoints such as PD1/PDL-1 and CTLA-4 has achieved noteworthy benefit in multiple cancers by blocking immunoinhibitory signals and enabling patients to produce an effective antitumour response. Anti-PD-1/PDL-1 and CTLA-4 antibodies work by preventing the down regulation of the immune system by tumour cells and were initially tested as monotherapy, showing promising results in clinical trials. However, a large ongoing study on patients with advanced metastatic solid tumours demonstrated the antitumour efficacy of these checkpoint inhibitors could be enhanced by combining their administration, as this allows to target two different checkpoints at the same time.

Combination of radiotherapy with immunotherapy has shown some promising results. Radiotherapy alone may not be sufficient to trigger anti-tumour immune responses, especially in poorly immunogenic cancers. Thus, the combination of radiotherapy with immune modulators may have the capability to escalate anti-tumour responses to a level that could suppress or eliminate cancer. Combination of an anti-CTLA-4 antibody with radiotherapy were tested on patients with recurring metastatic prostate cancer, showing signs of activity that encouraged further investigation.

A recent study at Crown Bioscience demonstrated the combination outcome of irradiation and immunotherapy in a preclinical model of syngeneic breast cancer. Tumour-bearing mice were irradiated using the SARRP allowing a more accurate treatment schedule, with planned protocols similar to those utilised in the clinic. Radiotherapy was combined with the anti-CTLA-4 antibody to investigate the outcome of this combination strategy. While immunotherapy alone had no statistically significant impact on tumour growth, radiotherapy as a single agent did reduce tumour size. However,

the addition of immunotherapy to radiotherapy exerted an additive effect on tumour growth inhibition over single agent therapy alone and resulted in higher levels of tumour infiltrating immune cells, which are generally associated with better prognosis in tumour bearing mice undergoing this type of treatment. In a different study, investigators tested the *in vivo* response to different checkpoint inhibitors of a range of syngeneic models and sequenced and immuno-profiled syngeneic model-derived tumours in an attempt to discover a genetic signature predictive of response and to better inform the selection of models for immunotherapy studies. They found a defined set of genes as potential predictive biomarkers of response for immunotherapy.

Conclusion

The recent clinical success of cancer immunotherapies has renewed attention in a field that holds great promise for transforming the treatment landscape by harnessing the patient's own immune system to fight cancer. Numerous immunotherapeutic strategies are being developed to elicit an antitumour immune response, the most successful being the blockage of immune cells inhibition by the tumour using specific antibodies. Interestingly, combination strategies utilising two antibodies targeting different inhibitory nodes have shown the most promising outcomes. Combining immunotherapy with conventional standard of care agents could also result in more stable and durable antitumour responses since chemo and radiotherapy could sensitise cancer cells making them more susceptible to immunotherapy. In addition, response identified through preclinical models can be used in predicting patient response in the clinic allowing informed insights and decisions to be made.

Although immunotherapy appears to be a promising treatment option for cancer patients, it is unclear as to why some patients benefit from these treatments and others do not. There is, therefore, a distinct need for more accurate research models.

Models can transform cancer treatment and provide treatments for cancer forms that historically had very poor survival rates. Syngeneic models have aided guiding the selection of the most appropriate combination approaches of immunotherapy with conventional cancer treatment that currently hold the promise of becoming the most effective strategy for improving cancer patient survival.

The use of immunocompetent models, such as syngeneic models, is essential when studying the effect of immunotherapeutic agents. These models are driving immunotherapy research and are providing important insights into the efficacy of new compounds and the development of more effective combination strategies. Furthermore, the right preclinical models can open opportunities for repositioning agents that may have failed or where patients have relapsed.

The next generation of immunotherapy models will harness the full function of the human immune system against human tumours, to provide information on how the human immune response affects tumour growth, and will allow the evaluation of novel immunotherapeutics. Humanised mice have been developed through inoculating human hematopoietic cells into immunocompromised mice, enabling indication selection and responder population identification across a range of cancer types within this platform. DDW

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