Monitoring the balance between immune repression and stimulation in cancer immunotherapy

Cancer is still one of the main causes of death worldwide, yet recent strides towards more effective treatments have been made in the form of new cancer immunotherapies. By weaponising the body's own immune defences, these drugs have shown great promise in clinical trials and are now experiencing significant commercial success. As cancer affects the efficient function of the immune system by disrupting the careful balance between immune repression/tolerance and stimulation, current immunotherapies powerfully work to restore this balance. However, the downside is that this can potentially lead to serious side-effects such as inflammatory disorders and autoimmune diseases. Using autoantibodies as biomarkers could help to minimise these issues by making it easier to predict patient responses and select suitable patients for each therapy, as well as by offering a tool for detecting immune-related Adverse Events (irAEs) as early as possible and ideally before clinical symptoms occur.

By Dr Peter Schulz-Knappe and Dr Georg Lautscham

Although much progress has been made in the battle against cancer in recent decades, it is still the cause of one in every four deaths in the US; a statistic that is similarly replicated across Europe and other regions of the developed world[1]. As intense research has begun to reveal how the immune system naturally works to suppress cancer initiation, progression and metastasis, new insights into how cancer can counteract these immunological defences have been revealed. Based on these fundamental scientific findings, ground-breaking immunotherapies have been developed that reinforce our natural immune defences against cancer[2].

As well as providing cancer patients with additional, and often better, treatment options, immunotherapies have also stimulated a booming immuno-oncology market, offering significant commercial incentives. Indeed, a recent report by Research and Markets estimates this area will be worth around $14 billion in 2019, rising to more than $34 billion by 2024[3]. As cancer immunotherapies are increasingly being used to treat patients, and commercial investment and sales revenues

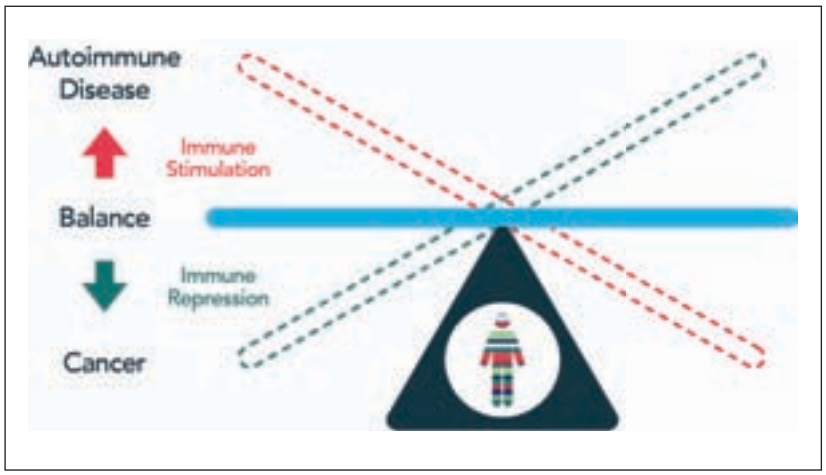

Figure 1
Stimulating the immune system to fight cancer involves monitoring the careful balance between immune repression and stimulation to detect side-effects such as the formation of autoimmune diseases

continue to rise, there is a crucial need for a better understanding of how they work. This includes exploring the potential risks involved, as well as finding new ways to improve patient outcomes through a precision medicine approach; for example, by developing and using new biomarkers for better therapeutic response prediction and ongoing treatment monitoring[4].

A careful balance

Cancer immunotherapies have been largely developed based on our growing knowledge of how the immune system naturally acts to repress tumour formation, as well as the methods that cancerous cells use to suppress or hide from the immune system in order to evade detection and attack[5,6]. Thus, immunotherapies weaponise the body's own defences against tumours by manipulating the system in two subtly distinct ways: firstly, they can directly strengthen or activate immune responsiveness against the cancer cells (active immunotherapies) or secondly, they can try to overcome the tumour's immuno-suppressive tactics so that the immune system can recognise and destroy the cancer cells (passive immunotherapies).

However, the immune system is complex, so any factors that alter its behaviour can have unexpected consequences. For example, while immunotherapies can successfully fight cancer, if they overstimulate the immune system or cause it to target healthy, normal cells, they can trigger immune-related Adverse Events (irAEs) such as severe inflammatory responses and autoimmune diseases.

Therefore, in order to protect cancer patients from these harmful side-effects, it is crucial to develop immunotherapeutic strategies that consider the delicate balance between immune repression and immune stimulation (**Figure 1**). On the one hand, if the immune system is being repressed this can lead to cancer growth, while on the other hand, too much immune stimulation can lead to the unwanted destruction of normal cells.

In an ideal world, the best immunotherapies would be those that destroy tumours and restore a healthy status. However, given that these treatments boost immune system activity by design, it is likely that most immunotherapies will tend towards over-stimulating the system in many patients. As such, the best route forward would be to use these therapies in conjunction with diagnostic tools that can monitor the activity of the immune system in every individual patient in response to treatment, so that corrective measures can be taken if efficacy is low or irAEs are likely to occur.

Effectively monitoring and maintaining this balance will require detailed knowledge of how both passive and active immunotherapies modulate the immune system to target cancer cells, with each approach having subtle differences when it comes to mode of action and the specific risks involved. Below we explore each therapy type in more detail.

Active immunotherapies

Active immunotherapies involve directly stimulating the natural immune system to target tumours using inflammatory factors such as cytokines or 'therapeutic cancer vaccines'. So far, research has shown that these can be effective in reinforcing the immune system to eliminate cancer cells. For example, therapeutic cancer vaccines have been shown to improve the prognosis of patients with certain types of cancers, including prostate, breast, lung, pancreatic, colorectal and blood cancers[7]. Given this progress, it is perhaps no surprise that the US Food and Drug Agency (FDA) has already approved some cancer vaccines for clinical use, such as sipuleucel-T (Provenge, Valeant Pharmaceuticals) for use in some men with metastatic prostate cancer[8].

Cancer vaccines are based on the premise that proteins secreted by the tumour cells or expressed on the tumour surface, called tumour-associated antigens (TAAs), can be recognised by the immune system. To create a specific anti-cancer vaccine, TAAs are first extracted from the patient's tumour, to be subsequently transferred into a suitable delivery vector, and then injected back into the patient. This stimulates a heightened immune response specifically against the cancer cells. Costimulatory adjuvants are also injected into the patient along with the vaccine to ensure a robust and sustained immune response (**Figure 2**).

However, due to the complex nature of the immune system, with its multitude of interacting cells, hormones and cytokines, it can be difficult to create a cancer vaccine that has a sufficient response profile. Vaccines can also disrupt the delicate balance of the immune system, especially when using additional adjuvants. Although rare, in the worst cases this can cause severe inflammatory responses in the patient. Such irAEs tend to be more of a risk when using passive immunotherapies such as checkpoint inhibitors[9], which we shall explore in more detail next.

Passive immunotherapies

Growing tumours can evade recognition and destruction by the immune system by expressing or secreting inhibitory factors, which inactivate the function of powerful, cancer-eliminating cytotoxic T-lymphocytes, sending them into a state of anergy. Passive immunotherapies usually involve combating these immuno-suppressive effects in some way. Checkpoint inhibition is the most promising approach and offers significant hope to cancer patients. This involves developing highly specific monoclonal antibodies targeting checkpoint proteins on cytotoxic T-lymphocytes, which averts the immunosuppressive effects of cancer cells and consequently stimulates the immune system to begin recognising and destroying them[10].

Early clinical results using checkpoint inhibitors show even greater promise than that seen for cancer vaccines, with trials suggesting that they can substantially improve the prognosis of patients with cancers including advanced melanoma[11], non-small cell lung cancer[12] and bladder cancer[13]. These highly- positive results have led to rapid FDA approval of some checkpoint inhibitor drugs, including ipilimumab (Yervoy, BMS) and nivolumab (Opdivo, BMS), as well as pembrolizumab (Keytruda, Merck). As their clinical use continues to rise, they are currently undergoing a dramatic growth period in sales revenue[14] (**Figure 3**). Investment into clinical studies is also exploding. Around 250 studies are currently being performed on up to 45 different checkpoint inhibitors globally, and this surge in research is set to continue with more than 10 new checkpoint inhibitors and agonists currently entering clinical trials and many more in early development[15].

However, as with cancer vaccines, checkpoint inhibitors also have significant downsides. Firstly, even for the most promising treatments, less than 30% of patients respond due to their high specificity; checkpoint inhibitors only work when they are able to block the specific target molecule that the cancer cell is using to mask itself from the cytotoxic T-lymphocytes. For example, some cancer cells express Programmed Death-Ligand 1 (PD-L1), which binds to the Programmed Death 1 (PD-1) receptor on T-lymphocytes and stops the cancer cell from being detected and destroyed. To combat this, monoclonal antibodies targeting PD-L1 or PD-1 can be used as they bind to these factors and stop this protein complex from forming (effectively blocking the molecular trick used by the tumour to avoid detection).

This mechanism underpins how a number of checkpoint inhibitor drugs work to combat cancer's immunosuppressive effects. These include the FDA-approved nivolumab and pembrolizumab, mentioned previously, as well as others that are rapidly following their lead and currently in clinical trials, such as avelumab (Merck, Pfizer) and atezolizumab (TECENTRIQ, Roche). However,

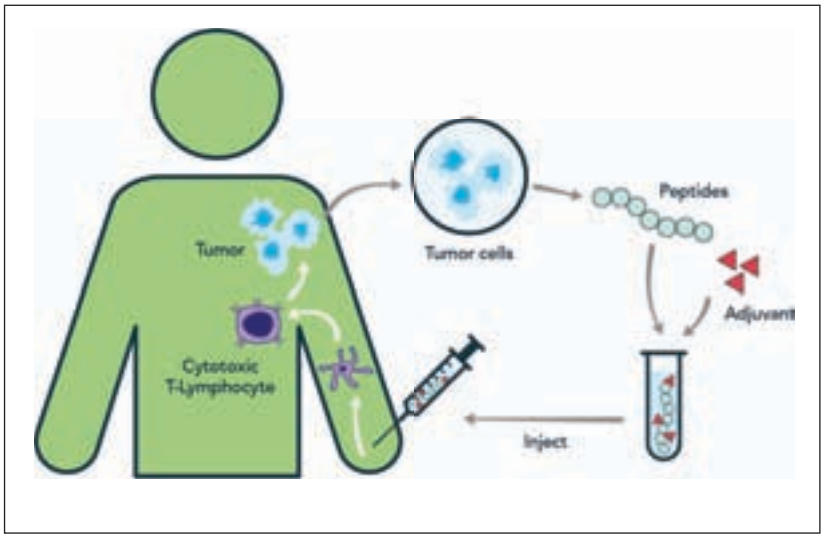

Figure 2
A simple diagram showing how many cancer vaccines work

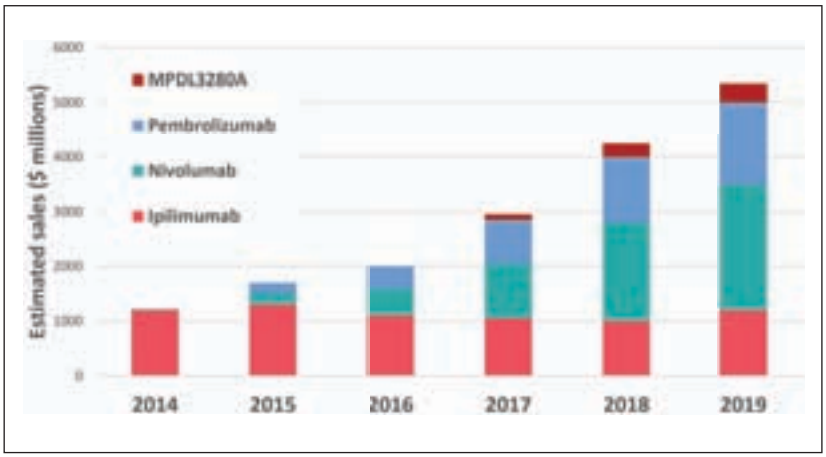

Figure 3
Estimated major market sales of selected immune checkpoint inhibitors from 2014 to 2019 (US, France, Germany, Italy, UK, and Japan). *Adapted from Webster, 2014*[13]

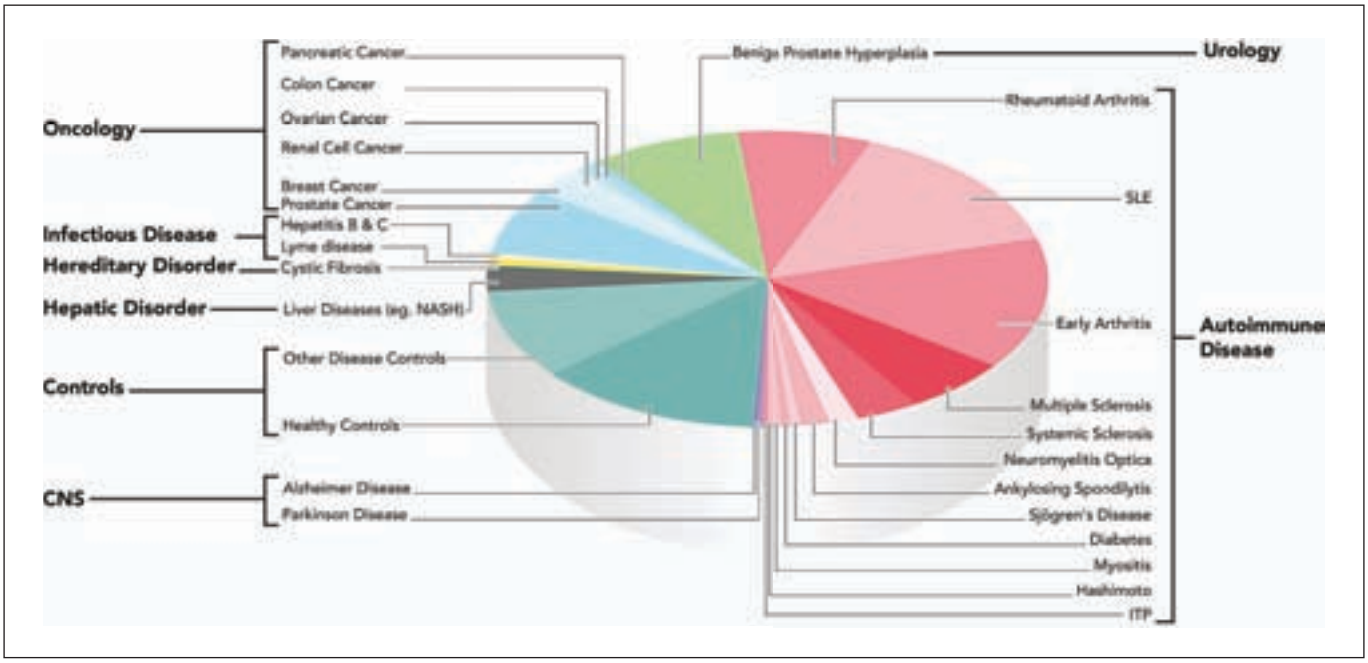

Figure 4: Protagen's autoantibody database includes more than 80 million data points derived from more 14,000 patients

the PD-L1/PD-1 masking mechanism is just one tactic used by cancer cells, which can also adopt numerous other immunosuppressive approaches that take advantage of the natural immune checkpoint system. Whatever the molecular basis, manipulating or blocking these interactions can help to overcome many of the suppression tactics used by cancer cells.

While many checkpoint inhibitor drugs are currently on the market and in development, a number of the checkpoint receptors, agonists and antagonists used by cancer cells to escape the immune system have yet to be effectively drugged. As it stands, patients with these tumours will not respond to the checkpoint inhibitors that are currently available and may require a different treatment altogether.

Secondly, as checkpoint inhibitors strongly activate the immune systems of patients, they can frequently lead to irAEs, such as the pathological joint effects observed in rheumatoid arthritis, as well as a potentially devastating range of dermatologic, gastrointestinal, hepatic, endocrine and other inflammatory problems. The irAEs caused by checkpoint inhibitors can thus greatly impact patients' health and quality of life. As such, there is a significant need to better understand the mode of action of common checkpoint inhibitors if we are to improve efficacy and reduce the impact of irAEs.

Using biomarkers to monitor the immune system

In order to be effective, both passive and active immunotherapeutic strategies must be designed to consider the delicate balance between adequately stimulating the immune system to strictly detect and destroy cancer cells, while preventing unwanted harmful irAEs. One way to monitor and regulate this careful balance is through using blood-borne biomarkers such as cytokines, cellular components and autoantibodies as biomarkers to better predict patient response, select suitable patients for treatment and detect irAEs early on before clinical symptoms occur[4,16].

Autoantibodies are often produced during a patient's natural immune response against TAAs, or after the administration of a cancer vaccine[17]. They also play a key role in the incidence of autoimmune disease, the likes of which can be triggered by most cancer immunotherapies, including checkpoint inhibitors[9]. Thus, profiling a cancer patient's autoantibody signature could be a powerful tool for shaping therapeutic strategies during clinical trials and routine clinical use.

For example, before trials even begin, autoantibody profiling could provide hints about the subtype of cancer from which a particular patient is suffering, predict their risk of experiencing irAEs, as well as classify them into potential 'responders' and 'non-responders', enabling the informed selec-

tion of suitable patients for trials and more effective treatments when the therapy reaches the clinic[18,19]. In addition, autoantibodies could show whether the vaccine or checkpoint inhibitors are inducing the desired immune response against the target, thereby tracking the effectiveness of the therapy. On top of this, autoantibodies can enable the prediction and monitoring of irAEs to indicate whether a patient's immune system has been successfully activated by the treatment, as well as to alert clinical staff so they can potentially prevent any unwanted long-term harm to patients.

A new autoantibody profiling tool for cancer immunotherapies

Autoantibodies are already established biomarkers for the diagnosis, prognosis and patient stratification of autoimmune diseases, but they have not yet been systematically analysed in cancer patients undergoing immunotherapies. To help support these efforts and fill these knowledge gaps, Protagen has developed a powerful autoantibody biomarker development engine called SeroTag®, which can detect autoantibodies in patients' serum samples with high sensitivity and specificity. The data can also be cross-referenced with the company's autoantibody profile database, which includes more than 80 million data points derived from more than 14,000 patient samples (**Figure 4**), to quickly define multi-marker panels as well as disease- and/or pathway-specific signatures.

The company is already proactively working to bring this approach to the field of cancer immunotherapy and recently announced two research collaborations, one with the US National Cancer Institute (NCI) and the other with the German National Center for Tumor Diseases (NCT). Both programmes will utilise SeroTag® to identify new autoantibody biomarker signatures that can be used to predict therapy response and detect irAEs in patients treated with checkpoint inhibitors, therapeutic vaccines or combination therapies.

The NCT study will explore autoantibody profiles in patients with advanced melanoma. There is a clear need to improve our knowledge in this area, as more than half of patients with metastasised melanoma see no long-term benefit when given current checkpoint inhibitors[20], so the efficacy of these drugs needs to be improved. In addition, a recent meta-analysis of 81 articles (including a total of 1,265 melanoma patients from 22 clinical trials and suffering from a variety of cancers, including melanoma) showed an overall incidence of irAEs in 72% of patients treated with the checkpoint inhibitor ipilimumab, with 24% of these being high-grade adverse events[21]. This is where using autoantibody profiling could really help, by identifying those patients most likely to benefit from therapy (thereby improving efficacy rates), and detecting the irAEs experienced by those undergoing therapy as early as possible, before they have a chance to cause serious damage.

Conclusion

Immunotherapies are poised to lead the way in providing better cancer treatments and even have the potential to cure cancer. As their use (and the revenue they generate) continues to grow, it is now more crucial than ever to ensure that they can be effectively employed in the clinic with maximum efficacy and minimal side-effects.

Autoantibodies as biomarkers are already showing great promise in monitoring the careful balance between effective and overzealous immune system activity. Through profiling patients' autoantibody signatures, it would be possible to precisely pre-select suitable patients, predict disease incidence, measure treatment progress and monitor risks throughout clinical trials and into wider clinical use.

Using technologies such as SeroTag®, autoantibody profiling will play an important role in supporting the future development and use of treatment strategies that have been carefully designed to boost efficacy while minimising the impact of inflammatory side-effects. DDW

Dr Peter Schulz-Knappe *joined Protagen in 2010 as Chief Scientific Officer. He trained as a medical doctor before working as a cell biologist, and brings more than 25 years of protein and peptide biochemistry experience, coupled with biomarker discovery and development expertise. He has more than 15 years of management experience in R&D and served as both CEO and CSO at BioVisioN and as CSO at Proteome Sciences.*

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