

Sharing Life Sciences Innovations

## Immunotherapy: a cure for cancer?



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# BIOVOX WHITE PAPER

## IMMUNOTHERAPY: A CURE FOR CANCER?

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## INTRODUCTION

For the first time in history a cure for cancer is within reach. Treating cancers that were previously considered untreatable and significantly prolonging life is the common goal of the experts featured in this white paper.

BioVox and Turnstone invited 8 immunotherapy experts to discuss the opportunities and challenges of immunotherapy for cancer treatment.

Join us and learn from the experts!

*'The staggering clinical responses are driving immunotherapy forward.'*

David Gilham, Celyad

*'Our common challenge for the coming years is for immunotherapy to become the first line treatment.'*

Gregory Driessens, iTeos Therapeutics

*'We will really make a difference with immunotherapeutics. But can we continue to help patients with the available budgets? A significant discussion on pricing will be due soon.'*

Dirk Reyn, eTheRNA Immunotherapies

*'The immunotherapy field is crowded. Soon progress will max out. As a company, you've got to think about this next level.'*

Anush Suri, Janssen Immunosciences



Ann Van Gysel – CEO Turnstone Communications

*'Personalized diagnostics are going to be important to follow the mutations in the tumors, anticipate and jump ahead.'*

Geert Cauwenbergh, RXi Pharmaceuticals

*'Several immunotherapy players should change the way they look at IP.'*

Caroline Pallard, NLO

*'Our pioneering research in cancer vaccines created a wealth of information. A deeper understanding in this evolving field will tell if there will also be a place for preventative approaches in the future.'*

Jamila Louahed, GSK

We also take a closer look into immunotherapy innovations in academia. A special insight features the projects of the Ghent University Cancer Research Institute.

Moderated by  
Lenny Van Steenhuyse  
Jef Van der Borght  
Ann Van Gysel

## IMMUNOTHERAPY: LIFE-AND-DEATH INNOVATIONS FOR CANCER TREATMENT

In recent years, significant scientific breakthroughs have opened the door to harnessing the immune system to fight cancer. For the first time in history, this brings a cure within reach. However, there are still some scientific and business hurdles to overcome. We have brought together experts from companies developing diverse immunotherapeutic technologies: **Anish Suri** (Senior Director of Janssen Immunosciences, Janssen Research & Development, LLC), **Geert Cauwenbergh** (President and CEO of RXi Pharmaceuticals), **Gregory Driessens** (Head of In Vivo Pharmacology at iTeos Therapeutics), **David Gilham** (Vice President of Research and Development at Celyad), **Caroline Pallard** (European Patent Attorney at NLO), and **Dirk Reyn** (CEO of eTheRNA Immunotherapies). They revealed why immunotherapy is more than just the latest hype and what it will take to bring these potentially lifesaving treatments to the patient.



### Helping the immune system destroy cancer cells

Immunotherapies can be broadly defined as treatments that support the body's own immune system in fighting cancer cells. However, this definition is somewhat inclusive. Certain traditional therapies, such as radio- or chemotherapy, may also fulfill these criteria, as they can result in an immunogenic cell death. True immunotherapies can be classified into three types: products that stimulate the immunity, that remove certain inhibitions of the immune system, or that influence the tumor

environment. This can be achieved using a range of agents, from small molecules to oligonucleotides, antibodies, or even cell therapies.

Our round table participants represent a diverse field of immunotherapeutic companies. The first is **eTheRNA**, a Belgian mRNA-based immunotherapy company whose TriMix technology delivers (as a cell therapy or through direct injection) key immune modulators to the dendritic cell (DC), a pivotal cell in the immune system. **Celyad** is a Belgian company that is developing next-generation cell-based immunotherapies that rely on chimeric antigen receptor (CAR) natural killer (NK) T cells. **Mirimmune**, from the USA, uses a cell therapy approach and was acquired by **RXi Pharmaceuticals**, applying their patented technology to bring RNAi into the cell. **iTeos Therapeutics** is a spin-off of the Ludwig Cancer Institute and has small molecule inhibitor programs and antibodies to target the tumor micro-environment as well as a platform to discover new targets of interest. Johnson & Johnson (J&J) has recently launched **Janssen Immunosciences**, a strategic department that focuses on leveraging the immune system in many diseases, including oncology. Finally, **NLO's** expertise could give us insight into the intellectual property (IP) challenges of this emerging field.

## Bringing a cure for cancer within reach

### A paradigm shift in cancer treatment

Established therapies lead to a prolongation of survival, but most often there is no cure. Exceptions include surgery that is performed before the cancer has spread, but cures are rare and limited to certain cancer types. Now, with immunotherapy, the potential exists for treatment of cancers that were previously considered untreatable, significantly prolonging life. There is hope that this could transform old-fashioned chemotherapy agents from first line treatments to a last resort, considering their side effect profile.

*Gregory Driessens, Head of In Vivo Pharmacology at iTeos Therapeutics*



At the moment, immunotherapy is still the second or third line of treatment for many cancers, and the challenge will be to become the first line of treatment. Selecting the right patient for the treatment will be one of the main challenges for the coming years.

More on iTeos Therapeutics on page 22

Immunotherapy can be a significant accelerator toward reaching that goal. Research into how the immune system can be leveraged to fight cancer has been ongoing for decades. The field began drawing mainstream attention after the discovery of checkpoint inhibitors, which are now seen as the poster children of the area. Cancer cells can hide from the immune system by posing as normal cells. The presence of certain proteins — called checkpoints — on the cell surface puts a brake on the immune system. Drugs that can inhibit this recognition, typically antibodies, release the brake and allow T cells to attack the tumor. A first step in reversing the order of treatments has already been taken, with the first line approval for checkpoint inhibitors in lung cancer.

The most remarkable results were obtained in melanoma, with the checkpoint inhibitors ipilimumab (a CTLA-4 inhibitor), and pembrolizumab and nivolumab (PD-1 inhibitors). For example, a treatment with ipilimumab could achieve a complete response in 15 – 20% of melanoma patients. Terminally ill

patients that received this therapy were in sustained remission afterward. These patients, who would normally have had only months to live, are currently alive after 10 years. While discussing a cure for cancer was always farfetched, it is no longer considered as unrealistic since the publication of these results.

Different immunotherapy approaches often show a synergistic effect, making combination therapies the new standard. For example, the combination of ipilimumab with nivolumab could increase the response rate in melanoma patients to the 40 – 50% range.

Nevertheless, activating the immune system more strongly is not without risk; in some of the early trials, 1% of the patients died because their immune system derailed. Improved knowledge on how to use these agents has diminished the risk of severe side effects. However, the acceptable benefit-risk ratio for cancer treatments is an additional ethical factor that has to be included in the equation for new innovative therapies. Historically, established methods, such as surgery or chemotherapy, have faced these considerations as well.

*David Gilham, Vice President of R&D at Celyad*



Treatment-related toxicity is an issue for all early phase clinical trials, and immuno-oncology is no exception. However, the risk-benefit balance is always considered — thus, while there are well-publicized toxicity issues in the CD19 CART cell therapy, the staggering clinical response rate in patients with advanced treatment resistance to the disease are driving the therapy forward. Clearly, more research is ongoing to identify the root causes of such toxicity and further improve safety profiles, but the clinical responses in these therapies are raising challenges in clinical trial design to support the desire of patients to bring such effective therapies into the market as soon as possible.

More on Celyad on page 18

### **Tackling the great diversity of tumors**

Every type of cancer is different, and a range of therapies must be developed to be able to help all patients. In the initial phase, most of the companies in the field started by working on melanoma because it is a known immunogenic cancer. While the tumor reacts well, and the populations and risk factors are clear, the number of melanoma patients is smaller than for many other cancers. In the next wave, companies started targeting tumors that are more prevalent, such as lung, renal, bladder, and breast cancer. Checkpoint therapy was particularly successful in lung cancer, a very large indication, in both second and first line treatment. Immunotherapy is now even the first line of treatment in patients with metastatic disease.

There remains a great deal of research to be done, even in melanoma, which is the cancer type in which the best response has been achieved. Despite the major breakthroughs with current checkpoints that prolong the life of half the skin cancer patients who receive the treatment, the disease continues to progress in the other half. It is also time to tackle the biggest challenges and start working on the cancers that are more difficult to treat, such as pancreatic cancer. Perhaps small biotech companies could drive this next wave of immunotherapy research. The big pharmaceutical companies more carefully balance risk and reward and are therefore less prone to accept this challenge. At the same time, as there is currently no treatment for pancreatic cancer, any innovation that can benefit the patient will be eagerly adopted in clinical practice.

## More than just a hype

Just as in any innovative field, immunotherapy is currently going through a bubble that will eventually burst. However, the bubble is believed to be longer lasting and the negative effect will be less severe because its results are a matter of life and death. In fact, because of the diversity of immunotherapeutic approaches, the field has been described as a foam, made up of different bubbles. This makes a complete collapse very unlikely. When the initial hype fades, the field will still be pushed forward by some of the early clinical successes and those products that are already on the market. The coming years will also reveal the durability of the clinical responses that are now seen. True long-term cures will be a significant improvement that will keep investors on board.

*Anish Suri, Senior Director of Janssen Immunosciences*



You need these early wins to drive the enthusiasm and energy, but a byproduct of that always is that the field gets crowded. That's nothing against this value proposition, far from it. It just means that after a while, progress will max out. As a company, you've got to think about the next level.

More on Janssen Immunosciences on page 24

As it is human nature to want to be where the action is, the field has become very crowded. There has been an emergence of many small biotech firms, but large pharmaceutical companies are also moving into the space. Despite the available array of immunotherapeutic approaches, many companies limit themselves to creating new checkpoint inhibitors and testing out combinations. While there is still a long way to go, eventually this type of research will lead to a plateau in benefit for the patient. In parallel, companies are looking for new approaches that take innovation to the next level. The strategic thinking behind this next version of immunotherapy will be key for companies to differentiate themselves.

## The future of immunotherapy

### Innovation through collaboration

Despite the progress that has been made, the immune system and immunotherapy still remain something of a black box. Somewhat more unique to this field is that all stakeholders are combining their efforts at the moment. Academia, industry, and regulatory authorities are working hand-in-hand to expedite progress.





The reason for this willingness to cooperate is twofold. First of all, it is a unique opportunity to defeat a disease that has a devastating impact on patients worldwide. A clear example of that impact is that regulatory authorities are more prepared to maintain an open mind. They accept that the mechanism of action may sometimes be difficult to describe. However, they work hard to protect the patient by trying to understand the field and being as informed as possible concerning trial design. The overwhelming indications of effectiveness also result in a large number of fast-track designations for novel immunotherapeutics. Second, the companies are willing to collaborate because they are aware both that they face a significant challenge that is difficult to take on alone, with new insights emerging daily, and that the payoff will be large enough to share. Many international consortia have been set up around different topics. One recent example is the consortium TESLA that has been created to validate different approaches for the identification of personalized cancer antigens, called neoepitopes. The participants know that if the scientific challenges that these consortia tackle are cracked, the patient will win, and ultimately so will they.

### Investigating cancer prevention

In theory, it is definitely possible to go one step further and use immunotherapy treatments to bring cancer from a manageable to a preventable condition. Similar to vaccination against infectious diseases, the immune system could be primed to recognize specific cancer antigens. Once the antigen specificity is in place, an immunotherapy treatment would then provide a very powerful solution to eradicate cancer cells when needed or when they reappear. However, a true preventative approach has a number of drawbacks.

To investigate the feasibility of such an approach, trials could be performed for cancers with a known genetic component and a very unfavorable outcome. One example is certain types of breast cancer, where people who have been tested and shown to possess genetic risk factors, sometimes take radical preventative measures, such as mastectomy and ovariectomy. The question is whether solid trial data would convince those at risk to go for a less radical immunotherapeutic treatment instead. In line with available immunotherapeutics for treatment of metastatic disease, the price could be high. It remains a challenging discussion whether and how much either the patients or the insurance providers would be willing to pay for treatments for a disease that might never present itself. Furthermore, as with any treatment, immunotherapeutics may lead to side effects. Are patients willing to risk these immediate drawbacks for hypothetical future risks?

Other types of prevention, however, can be envisioned. For example, to prevent cancer recurrence in melanoma patients where the tumor has been surgically removed, an additional treatment with checkpoint inhibitors and/or immune activating products could be given. Preliminary data investigating this approach has been very encouraging.

Another route could target lifestyle modification. Preliminary data shows that changes in the microbiome can influence the effectiveness of checkpoint blockade therapy. Drugs or lifestyle changes could thus precede the actual treatment and make patients become responders to a certain drug.

*Anish Suri, Senior Director of Janssen Immunosciences*



Microbiome-based approaches can either sustain or enhance the right antitumor environment. We know now, for example, that some microbial species will produce short chain fatty acids that are very beneficial for regulatory T cells in autoimmune diseases, particularly in cases of inflammatory bowel disease. Some of the early therapies that we are testing at Janssen are focused on that and are going into the clinic in the near future. Similarly, for example, in colorectal cancer, there may be some species that exacerbate the early incidences of the adenoma. This is another way of thinking about lifestyle modification or the preventative landscape.

More on Janssen Immunosciences on page 24

### The right treatment for the right patient

Many companies in the immunotherapy space have started developing and marketing new products without fully understanding which tumor targets would offer the most benefit. Therefore, a concerted effort is needed to understand the diversity of tumor antigens. At the moment, a great deal of effort is put into DNA sequencing, but there's a whole realm of complexity beyond that, with RNA transcriptomics and proteomics. This remains to be explored to bring insight to a level that allows for true personalized cancer treatments.

*Geert Cauwenbergh, CEO of RXi Pharmaceuticals*



I think personalized diagnostics are going to be important. A tumor is a living thing, and it does whatever it takes to survive! So it's going to use tricks in order to do that. Following what kinds of mutations happen in tumor tissues is necessary to anticipate and jump ahead, and that's where personalized diagnostics are going to help and may actually reduce the cost.

More on RXi Pharmaceuticals on page 26

With the current state-of-the-art treatments in melanoma, about 50% of the patients achieve a long-term remission. One way to improve the benefit-risk profile of new immunotherapies is having good biomarkers to predict which patients will respond. All companies — from small biotech firms to big pharma — are looking into that question. Identifying responders for their drug allows them to have higher success rates in their clinical trials and build a more favorable value proposition and better health economic story. Being able to couple a drug to a specific diagnostic tool could also make the resulting IP stronger. Finally, the availability of responder criteria is an important asset in negotiations with potential partners for combination treatments.

In addition to predicting short-term responders, one also wants to be able to understand long-term treatment efficacy. For this, a better understanding of T cell profiles in patients is an essential monitoring tool during treatment. Afterward, it is equally important to have long-term immunomonitoring tools to check if patients are in a sustained state of remission. While such tools have been developed in the academic space, they are difficult to commercialize.

Many believe that in a clinical setting, personalized cancer treatment may be the ultimate goal, but it is likely to become a part of the physician's armamentarium. The treatment will always go through an initial period where the available general methods need to be used from a price value perspective and to keep the patient's disease under control while the personalized treatment is being developed.

### Combination therapy is the next big thing

There is a consensus in the field that combinations of different immunotherapies will be the optimal way to treat patients. By going from a single checkpoint inhibitor to a combination, the response rate in melanoma patients could be increased from 20 to 50%. Similarly, promising results were obtained by combining mRNA-based immune activating therapies and checkpoints. This understanding that combinations of immunotherapies work synergistically drastically impacts the way companies develop their products. Traditionally, a product was developed and approved, and combinations were tested afterward. Now, the partners have to be picked earlier on in the process, which is inherently more risky and also challenging in terms of partnering, value creation towards investors, pricing, reimbursement, and, last but not least, IP.

*Gregory Driessens, Head of In Vivo Pharmacology at iTeos Therapeutics*



In immunotherapeutic development, you're no longer the main decider because combinations will be very powerful. You can have a very good product, but for the optimal combination, you will be dependent on a partner. That makes the equation a bit more complicated compared to a monotherapy treatment where you can decide your entire marketing strategy by yourself.

More on iTeos Therapeutics on page 22

## A unique situation for intellectual property (IP)

### Evolving toward a new model

The success of combination treatments means that to get a new therapeutic to the patient, IP from different parties may have to be cross-licensed. For the traditionally monopolistic IP models in pharma, this is a significant challenge. In contrast to electronics, where 10,000 patents can be combined to create a single smartphone, cross-licensing is not part of the normal business strategy in pharma. The product cycles and development paths in electronics are also very different. A great deal of money has typically been invested to develop a new treatment for a given issue in the health care field.

*Caroline Pallard, European Patent Attorney at NLO*



For immunotherapeutic innovations, several players should change the way they look at IP. The first question start-ups get asked by potential investors is if they have freedom to operate. Investors don't want to have to take a license from a third party because it introduces uncertainty and drives up the costs. This could mean the end of many innovative and potentially lifesaving programs. Start-ups forget that you don't per se need a patent to commercialize an invention, but you need it to get access to other patents. For innovation in the sector, a fresh look is needed at the way patents are dealt with, and companies will have to become more open in terms of sharing IP for a fee.

However, there are reasons to believe that people will be more open to collaborative efforts in immunotherapy because the potential profits are so substantial that there will be more than enough to share. In other areas where the potential revenues are smaller, this discussion is more difficult. In addition, from an ethical point of view, immunotherapeutic solutions can drive the science significantly forward and offer a shot at curing cancer. Companies realize that battling each other aggressively over IP would not benefit the public image of the pharmaceutical industry.

### How to claim territory in an emerging field

The immunotherapy space is very crowded, and there are a large number of parties claiming IP. Only the true pioneers in a given field may be allowed a quite broad scope of protection for their product. Other parties will subsequently try to optimize this technology, which will be seen as a series of incremental inventions that are granted an increasingly narrower scope of protection.

*Caroline Pallard, European Patent Attorney at NLO*



The optimal strategy for an immunotherapeutics company is to file when you have at least a proof of concept with in vitro data. At this stage, it's better to draft something that's as broad as possible. Then you can back up the application with new data during the priority year. Some think that clinical studies are just meant to demonstrate that your product is doing what it's supposed to do. They forget that you may also optimize anything that will help build up the protection of your product, like an improved formulation or a frequency of administration. After approval, when you find new applications for your product, you have to develop an IP strategy per product and indication. The timing for the first patent filing is essential: it's not good to file too early, but obviously, when you file too late, you're done. You don't know in advance how much time you have.

There is, however, an inherent risk that these dominant patents are defined so broadly that they slow down innovation in other areas, as researchers investigating unrelated immunotherapeutic strategies might end up infringing the patented technology. For combination therapies, patenting a specific combination remains challenging even if there is great deal of data showing a synergistic effect.

*Geert Cauwenbergh, CEO of RXi Pharmaceuticals*



Our core patent is valid until 2029. It describes how we brought all aspects of our technology together, and it's very well defined. Everybody who wants to get into that space needs a license until 2029 to get access to this technology, and then they can take a composition of matter. That is one of the reasons why we are giving licenses in fields that we are not immediately interested in or not capable of entering because we don't have the money. We're giving licenses in exchange for equity or money. That is probably how other people should do it, at least if they have a "blocking" patent.

More on RXi Pharmaceuticals on page 26

## Bringing the solutions to the patient

In addition to the need for alternative research and IP strategies created by the success of combination therapies, there are a number of other specific challenges to overcome to make these new immunotherapeutics available in the clinic.

### Breaking out of the black box

Despite decades of research, all the intricacies of the highly complex human immune response have not yet been unraveled. Some successful targets and strategies have been identified over the years, but aspects of the immune system remain a black box. While this lack of knowledge is the biggest hurdle, it is also the biggest enabler of progress. It forces all those involved to think creatively to bring the required solutions. The rapid progress that is being achieved makes it more challenging for the companies to define the right questions to answer in their development plan.

*Dirk Reyn, CEO of eTheRNA Immunotherapies*



The field and knowledge are changing very quickly. If you look at all the publications and trial results that have come out over the last year, there is so much new information to consider. This forces all of us to critically evaluate and adapt our development plans and protocols in a somewhat continuous way because of new evidence that appears. I think it makes it intellectually more challenging and enjoyable, but it's new for many of us.

More on eTheRNA Immunotherapies on page 20

The key will be to further unravel the science behind cancer development. To cure cancer, over 200 types of tumors need to be addressed. The very promising initial results must be expanded from their niche and small cancer areas to broader mainstream cancer therapy.

### Taking the bad with the good

Just as in traditional cancer treatments, most immunotherapeutics are not without side effects. Indeed, for most, there is an inherent link between efficacy and risk. The most notorious example is the neurotoxicity and cytokine storms induced by CART cell therapy. In this therapy, T cells from the patient are isolated from the blood and altered in vitro to target cancer cells. Afterward, they are infused back into the bloodstream, where they quickly and completely wipe out a tumor. Their unseen cancer-killing potential also comes with a high risk of severe side effects or even death when the immune system sometimes derails. A great deal of effort is going into making these therapies safer. They are still extremely new, and the safety profile will improve as the mechanisms of action are better understood. This is not specific to cell therapies; it is an evolution that all therapies go through.

Cell therapies have a different kind of toxicity than typically considered for small molecules. In small molecules, toxicity is most often directly linked to the compound itself and can be measured in animal studies. In cell therapy, compounds are used in minute concentrations in vitro to reprogram or alter the behavior of human cells. Once these cells are administered to the patient, side effects may still occur, which is a form of cellular toxicity. Because of this fundamental difference, the books on toxicology have to be rewritten in terms of the toxicity studies that need to be done during clinical development and many of the traditional models are not helpful.

For other types of drugs, compound toxicity remains a possibility, but the time frame for possible side effects has significantly changed. With oligonucleotide-based technologies like mRNA, the coding sequence is brought into the cell and is only active for a short time. The mRNA is transcribed into a well-known protein in the cell that exerts its action while the original compound disappears. Here, toxicity has traditionally been much less of an issue, except for vehicle toxicity induced by some of the carriers used to protect the oligonucleotides. Antibodies stay active for a longer time; so more care has to be taken.

### Is there money for innovative research?

Many companies in the immunotherapy space are academic spin-offs or small biotech firms. They rely on external (venture capital) funding and investments to develop promising products from an idea to a market-ready drug. Finding those types of investors is currently even more grueling. After the crisis in 2008, there has been a consolidation of venture funds, especially in Europe. At the same time as the number of funds became more limited, a surplus of high-quality research was performed. All the major VC funds currently may receive up to 500 applications per year. Thus, more than ever, companies that want to raise money have to stand out and check all the boxes. There is also a trend amongst VC funds toward funding more mature innovations, increasing the length of the valley of death. It is a real risk that this evolution will prevent lifesaving treatments from reaching the clinic.

In the US, raising money is further hampered by the fact that investors ask for increasingly large returns and guarantees. On the other hand, the ticket sizes are often larger. Once an investor is found, it is feasible to raise 15 million dollars with a single investor in a series A round. In Europe, a large number of people need to be brought to the table to obtain a similar investment.



### New business models for production and distribution

Once products are approved, they need to be mass-produced. For some immunotherapy treatments, this may also create a significant hurdle. For small molecule synthesis and antibody production, the processes are well established and the market offers a choice of approved facilities. For oligonucleotides, for instance, the situation is more complex. Today, there are only a limited number of manufacturing plants worldwide. Oligonucleotide companies are thus faced with an oligopoly that keeps the prices high and hampers innovation. Once more production plants are able to supply to therapeutic companies, the prices will come down rapidly.

Production is most complex for cell therapies. An autologous treatment requires blood to be drawn from the patient, cells to be isolated and processed in the lab, and finally re-administered to the patient by infiltration. All these steps need to be performed in a limited time frame at high standards, requiring specialized labs in the vicinity. Making cell therapies broadly available would thus depend upon the creation of a network of cell biology labs that follow standardized methods. This requires a significant investment for most companies, making it more difficult to build a solid business case at the moment. Despite the incredible scientific results obtained with, for example, CAR T cells, this remains a major drawback.

To make cell-based therapies work, the current health care system may need to adapt and new business models must be created. A potential solution may be the introduction of GMP cell biology labs in major hospitals in different countries that are capable of handling different standard protocols. In this scenario, companies will need to do business in a completely different manner and work more closely with hospitals that produce their products. Consequently, university hospitals will become the pharmacies of the future that distribute the available cancer cell therapies. This alternative form of business development will also disrupt the balance in the traditional triad (doctor, patient, and company) in favor of patient empowerment.

*David Gilham, Vice President of R&D at Celyad*



The challenges for cell therapy are high, but if the clinical responses are strong enough, they are worth meeting. Companies are preparing to bring their first products to the patient worldwide. Two strategies have been devised: centralized production, where cells are shipped in and out, or localized production areas close to the patient. For administration, a distribution system is already in place in the form of cell transplantation centers in hospitals. These are accepted as a standard therapeutic approach. Working with the infrastructure that's already present makes it slightly less daunting.

More on Celyad on page 18

## Who will pay for these treatments?

Only a few immunotherapies are currently commercially available. In the traditional pricing models utilized by big pharmaceutical companies, the price of life is one of the determining factors. Because of their lifesaving potential, the price was thus set very high when marketing the first checkpoint inhibitors.

Under the new healthcare pact, the Belgian government has recently freed up an additional yearly budget of € 300 million to reimburse innovative therapies. While this seems like a large sum, in practice, it translates to only a limited number of cancer patients who can receive the checkpoint inhibitor treatment at the current price levels.

Within these assumptions, the entire incremental budget may be spent on a monotherapy for a single large cancer indication. However, there is a whole wave of innovative medicines coming with a profound impact on many other cancer types. Furthermore, these will probably be administered as combination treatments, multiplying their health economic impact. It is clear that the pressure on the health care budget will continue to grow in the coming years. While being able to cure cancer will lower the costs for palliative treatment and hospices, this will not be sufficient.



*Dirk Reyn, CEO eTheRNA Immunotherapies*



I think immunotherapy is going to really put more stress on the question if we can continue to help patients with the available budgetary means. It's the price of life. One can really make a difference with immunotherapeutics right now, but in the health economic prospect, we're going to hit a wall, and we don't know where the door is yet. The budgetary system of communicating vessels between generics and innovation is running out of water. I think this is a more substantial item than saving amounts in other budgets to pay for this type of innovation. Immunotherapy is likely to create a significant discussion in the pharma industry on how these drugs need to be priced in the future because the old model of benchmarking traditional cancer therapy that prolongs life for several months versus products that may provide a cure is not sustainable.

More on eTheRNA Immunotherapies on page 20

Immunotherapy is one of those treatments that can shift an overhaul of not only reimbursement but also the entire health care system to much higher on the agenda. Former initiatives like promoting the use of generics will no longer be sufficient to cover the additional costs for the range of therapies that is coming. The system of how hospitals and physicians are paid and how the pharmaceutical industry works will come under significant stress due to the wave of new cancer medication. All stakeholders know that more money will have to be freed up to make it happen, and more people start to realize that everybody will have to contribute.

Eventually, many believe that market forces will ensure that prices will come down. At the moment, pioneering companies have to charge enough to recoup their massive R&D costs and fund new innovations. Production costs will also drop thanks to process improvements and greater production volumes. Similarly, chemotherapy or even penicillin were astonishingly expensive when they first came out, but both are now affordable to all patients.

If the prices will not go down, reimbursement will not be able to follow suit, and we'll end up in a system of two-speed medicine. Only the privileged few will be able to afford a lifesaving treatment. This is already the case in the US.

Governments have attempted to patch up the current system and force the next generation to handle the overhaul of the reimbursement system. This seems no longer possible and the early successes of immunotherapeutics are accelerating progress.

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### Celyad

Celyad, based in Mont-Saint-Guibert (Belgium), was originally established in 2004 as a company active in regenerative cell therapy. However, two years ago, Celyad decided to move into immuno-oncology. The company has licensed technology from Dartmouth College in the US that is based on CAR T cells expressing an NK cell receptor. A phase I clinical trial to evaluate the safety of this treatment started in January 2017.

#### Celyad's CAR T cells in a nutshell

T cells eliminate infected cells and cells under stress, but they also have a central role in targeting cancer. The difficulty for T cells when fighting cancers is that tumor cells develop many systems to avoid the immune response. Celyad is now engineering these T cells by introducing a gene that encodes a cell surface receptor that enhances the capability of T cells to recognize tumor target proteins. More specifically, the first receptor Celyad is working with is the NK cell receptor NKG2D. NKG2D recognizes up to eight different ligands, of which at least one is expressed on 80% of tumors, including hematological as well as solid tumors.

The NKG2D receptor is expressed in fusion with a T cell receptor activating domain called CD3 $\zeta$ . When NKG2D binds the target ligand, the T cell becomes operational because of the fusion with CD3 $\zeta$  that drives activation. These cells are referred to as NKR-2 CAR T cells (in brief, NKR-2 T cells).

Overexpression of NKG2D is preferred in T cells rather than NK cells because NK cells possess a great deal of inhibitory receptors, making it more difficult to trigger them against individual targets. Additionally, the methods to isolate T cells from a patient's blood and multiply them to large numbers before reinfusion are well described, while clinical manipulation of NK cells remains challenging.



# Celyad



#### THINK without chemotherapy

When Celyad licensed the NKR-2 technology, a small clinical trial was being carried out at the Dana-Farber Cancer Institute in Boston. **David Gilham, Vice President of R&D at Celyad**, says: "Twelve patients have been treated with a very low dose of cells, starting from 1 million and going up to 30 million NKR-2 T cells. There was no evidence of toxicity, no cytokine release syndrome or anything else adverse due to the NKR-2 T cells. The trial also gave us some indications about the potential clinical activity. Actually, one acute myeloid leukemia (AML) patient treated at the highest dose 7 months ago had his blood parameters normalized with no subsequent therapy."

This pilot study led to a trial that Celyad is completely sponsoring and initiating, called Therapeutic Immunotherapy Using NKR-2 T cells (THINK). This is a multinational trial, involving three centers in Belgium and three centers in the US, and it is planned to treat around 100 patients across seven different cancer indications.

***“A major difference concerning this trial compared to most CAR T cell trials is that patients do not undergo preconditioning with toxic chemotherapy.”***

Gilham explains: “The reasons to use preconditioning chemotherapy prior to receiving cell therapy are numerous. It creates space for the T cells so that they can expand when they get into the patient and can generate as many T cells possible to target the tumors. However, in our trial, chemotherapy might reduce the activity of the NKR-2 T cells because they not only have a direct antitumor effect but also might induce other aspects of the immune system. Because our patients do not receive chemotherapy, they also don’t have to be hospitalized for an extensive period during the treatment, which is a great advantage for the patients.”

The trial is split into two arms, one treating hematological tumors (i.e., AML and multiple myeloma) and the other solid tumors (more specifically pancreatic, ovarian, bladder, colorectal, and triple-negative breast cancer). The patients will be treated in a dose escalation manner. A first cohort of patients receives 300 million cells per dose, with each dose administered three times, 2 weeks apart. Then, the same is done for 1 billion, and 3 billion cells per dose. When the maximum tolerated dose is established, it will be tested against the different types of tumors. “This phase I trial is so extensive because of the broad targeting capacity of NKG2D. The main readout is safety, but we’re hoping to see some suggestions and observations concerning the activity, giving us directions to go into a further clinical testing. Initial exploratory results are expected in Q4 2017,” adds Gilham.

## Monopoly on NK receptors

When asked about the competitors in the field, Gilham is not worried: “Other companies that work in the CAR T cell field, such as Novartis and Kite Pharma, target CD19 or B cell specific malignancies. We are working in non-B cell hematologic and solid tumors, where there are a limited number of early competitive trials at the time being, using targets which are present in a more limited number of cancer types. But there is an increasing level of academic and early commercial activity in the area. However, there are no other companies that are directly exploiting NK receptors in a CAR T cell format as far as we are aware, so I think we can say with some certainty that we are the world leaders in this specific area.”

There is great excitement and enthusiasm in immuno-oncology, with new and established companies entering the field. Gilham replies: “For Celyad, we clearly hope to eventually show strong clinical responses with NKR-2. Ideally, of course, we hope this will be seen at a stage that will allow us to discuss with the regulatory authorities optimal routes toward a licensed product that we hope will offer a major benefit for patients with advanced cancer. In this field, CD19 CAR T cell therapy sets the benchmark with the possibility of getting an effective product to the market in 1 to 2 years, and we truly hope that NKR-2 will be the first such impactful product that is able to target hematological and solid cancers.”

Celyad

+32 10 39 41 00  
info@celyad.com

David Gilham  
Vice president of R&D

www.celyad.com

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### eTheRNA Immunotherapies

With its expertise in mRNA production and knowledge of the biology of dendritic cells, eTheRNA has developed TriMix, a new mRNA technology platform. TriMix consists of a cocktail of three mRNAs, which pushes dendritic cells beyond their limits and helps them raise an immune response against cancer antigens. With TriMix, eTheRNA believes it has developed an incredibly versatile tool for cancer immunotherapy.

The origins of eTheRNA lie with the Vrij Universiteit Brussel (VUB) and its lab for Molecular and Cellular Therapy, headed by **Prof. Kris Thielemans**. Calling on Prof. Thielemans' expertise in mRNA production, the VUB spun off eTheRNA as an mRNA production facility, serving academic institutions and small to mid-cap pharma companies. To this day, eTheRNA remains one of the only four GMP-accredited mRNA production units worldwide.

Since its foundation in 2013, eTheRNA has undergone a true transformation from production company to development biotech focused on oncology. In addition to mRNA production, Prof. Thielemans also set his knowledge of dendritic cells and their potential in cancer therapy to work. After testing the ability of different constructs and mixes of mRNAs to potentiate and activate dendritic cells, he found a unique mix of three mRNAs capable of just that. With its unique TriMix technology, the company set out to test this mRNA cocktail as a cancer therapy and eTheRNA 2.0 was born.

#### Recipe of the secret mix

As the first rationally designed platform to stimulate the immune system, TriMix facilitates the three steps in which dendritic cells assert their function:

1. Dendritic cell activation, mediated by a molecular danger signal (e.g. DAMPs, PAMPs, ...)
2. Dendritic cell maturation, mediated by support signals from T helper cells ( $T_h$  or  $CD4^+$  T cells)
3. Activation of cytotoxic ( $CD8^+$ ) T cells by dendritic cells

The danger signal required for dendritic cell activation in the first step is simulated by expression of constitutive active Toll-like receptor 4 (caTLR4) mRNA. The second step, interaction with  $T_h$  cells, is mediated by mRNA of CD40 ligand (CD40L). Finally, interaction with  $CD8^+$  T cells is enforced with mRNA of CD70. This unique combination of mRNA of caTLR4, CD40L and CD70 constitutes eTheRNA's TriMix technology.

#### Cells or naked mRNAs?

Initially, to establish a proof of concept, TriMix was used to develop an **ex vivo product**. In this procedure, dendritic cells are extracted from a cancer patient's blood and treated with the mix of mRNAs to prime them for therapy. Afterwards, the enhanced dendritic cells are readministered to the patient where they activate  $CD8^+$  T cells and stimulate them to destroy cancerous cells.



This strategy was clinically validated, in collaboration with Dr. B. Neyns at the University Hospital UZ Brussels, in four studies in patients with stage III to stage IV melanoma. These patients had previously gone through many different treatment options unsuccessfully and had very low life expectancies. eTheRNA's CEO Dirk Reyn elaborates on the remarkable outcomes:

"We observed a 2-5% complete response in patients treated with the checkpoint inhibitor ipilimumab (Yervoy) in the VUB internal access program, which was completely in line with the data published by BMS. When combined with TriMix however, this percentage jumped to 20%. Similar beneficial effects were seen in patients where the primary melanoma tumor had been removed surgically. While 30% of the patients remained cancer-free after one year, 60% of patients receiving TriMix remained protected over a one year period."

While the ex vivo cellular product has proven its merits in the clinic, eTheRNA is pursuing a different route with TriMix. Instead of treating isolated dendritic cells from the bloodstream, TriMix can also be directly injected into the neighborhood of dendritic cells to achieve the same effect in a much more patient-convenient, efficient and cost-effective way. This **in vivo approach** might offer several advantages for eTheRNA's technology and is supported by an extensive set of preclinical data.

"The in vivo approach in which we directly inject our mRNA mix is a very scalable product," explains Reyn. "TriMix is produced enzymatically and can be shipped easily to any location. This way, we can make TriMix a globally available, off-the-shelf and convenient to administer product. While the ex vivo approach for TriMix is without any doubt valuable, its applicability is more limited and we intend to develop the product for very personalized treatment based upon tumor profiling."

## Where to put the needle

With the four studies in melanoma patients having demonstrated the efficacy of the ex vivo product, eTheRNA now hopes to repeat this success with the in vivo product. To this end, three new studies will be initiated over the course of 2017, with another to follow in 2018.

When directly injecting TriMix into patients, two interesting options present themselves. The TriMix can either be injected directly into the tumor (intratumoral administration) or into the lymph nodes (intranodal administration). This choice may also determine whether tumor associated antigens (TAAs), or rather their mRNAs, should be added to TriMix. As TAAs are readily available in the tumor environment, adding them to the mix when administering directly into the tumor is believed to have little added value. This is not the case for injection into the lymph nodes, where tumor-specific material is absent and adding TAAs will help the dendritic cells to prime the cytotoxic T cells for the tumor type in question.

"Recently, data has been published showing the unique effects of intratumoral administration of different cancer drugs," Reyn says. "Scientists are now realizing this could add a new dimension to cancer treatment that hadn't been tested before. Of course, this requires the tumor to be accessible, which isn't the case for all patients."

In the coming years, eTheRNA will focus on demonstrating the efficacy of its in vivo product in both melanoma and triple negative breast cancer. Once this has been established, the company hopes to expand the applicability of this new platform technology to various other indications.

eTheRNA immunotherapies

[www.etherna.be](http://www.etherna.be)

+32 3 369 17 40  
[info@etherna.be](mailto:info@etherna.be)

Dirk Reyn  
CEO  
+32 475 96 52 57 37

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### iTeos Therapeutics

iTeos Therapeutics started in 2012 as a spin-off from the Ludwig Institute for Cancer Research (LICR), one of the top three cancer institutes in the world, and the Université Catholique de Louvain (UCL). In 2014, they licensed rights to preclinical compounds targeting IDO1 and TDO2 to Pfizer, providing the company with the necessary resources to expand from 7 to 40 people and develop a pipeline of five proprietary programs for hot (inflamed) and cold (not inflamed) tumors. Since then, Pfizer and iTeos have moved one IDO1 program into the clinic and expect to add programs in 2018.

#### A first clinical trial with IDO1

The indoleamine 2,3-dioxygenase (IDO1) mechanism was initially discovered by LICR in Brussels. It is one of the main mechanisms used by cancer cells to escape an immune response. IDO1 is an enzyme that degrades tryptophan into kynurenine, resulting in the inactivation of T cells. It is the same mechanism that occurs in pregnancy to allow the baby to develop without a reaction from the immune system. "If you manage to inhibit this mechanism, the tumor becomes visible again for the immune system and can be attacked," says **Michel Detheux, CEO of iTeos**. "IDO1 is a cornerstone strategy to develop novel immunotherapies."

*"We are a spin-off of one of the most successful and well-known cancer research institutes in the world, and only two years after the start-up, we signed a licensing agreement with one of the top three pharma companies in the world."*

In September 2016, iTeos and Pfizer started a clinical trial with IDO1 inhibitors. "The drug candidate is able to pass through the blood-brain barrier. Hence, it is being tested in patients with glioblastoma. The first results are to be expected in 2017," explains Detheux.



#### A2A receptor antagonists: even more promising?

In addition to IDO1 inhibitors, iTeos is focusing on a number of other programs, including adenosine A<sub>2A</sub> receptor antagonists. Many different types of tumors produce high levels of adenosine within the tumor micro-environment. Adenosine modulates the immune response in such a way that tumor cells are no longer attacked. Detheux adds: "I believe that these A<sub>2A</sub> antagonists are even more promising than the IDO1 inhibitors. We are developing this program independently and plan a first clinical trial early in 2018."

Other companies, such as Heptares (UK), Palobiofarma (Spain), Juno Therapeutics (Seattle, US), Corvus Pharmaceuticals (CA, US) and Arcus Biosciences (CA, US), also focus on A<sub>2A</sub> receptors as a possible route to fight cancer. Detheux comments: "Indeed, but we believe we have the best-in-class

approach to designing a compound for application in immuno-oncology, which is superior to that of our competitors.”

iTeos also has three other proprietary programs underway:

- TIGIT inhibitory antibodies (candidate selection 2017)
- Galectin-3 inhibitory antibodies (lead identification 2017) and
- STING agonists for cold tumors (proof of concept for targeted delivery in late 2017)

*“I want to stress that cancer immunotherapy is revolutionary and that more than half the trials in oncology are currently dedicated to immunotherapy.”*

## Collaborations leading to an IPO

iTeos has received a number of public grants for early-stage discovery, including € 1.6 million through a BioWin project called IT-Targets, shared with ChemCom S.A., ImmunXperts S.A., the de Duve Institute, and Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM). This project will focus on G-protein coupled receptors (GPCRs), which will be selected by profiling the most important immune cell types purified from clinical samples. Detheux comments: “GPCRs have been underexploited in oncology. We want to identify and validate novel GPCR targets for cancer immunotherapy treatment.”

*“We are building a unique mix of expertise in tumor immunology and translational medicine to develop new immuno-oncology drugs.”*

iTeos recently announced a collaboration with Cristal Therapeutics, a Dutch expert in nanotechnology, to develop a program targeting cold tumors. They are also collaborating with Adimab, an expert in antibody development. “They are the perfect partner to fully develop our antibody programs,” comments Detheux.

“The strategy of iTeos,” continues Detheux, “is to identify partners that could be investors but also pharmaceutical partners and who will support the development of iTeos as a sustainable company in cancer immunotherapy. We want to be able to fund best-in-class as well as first-in-class programs in the long term, with the goal of achieving clinical proof of concept.”

*“We manage our resources very carefully. We invested € 4.4 million to get one program in the clinic and to develop a pipeline with five proprietary programs only four years after we were founded.”*

“We are currently working on a series C fundraising to move our other programs into the clinic, and if everything goes as expected, we should be ready for an IPO in 2019 or 2020. This will be in Europe or the US, depending on the progress of our programs and the location of our investors,” concludes Detheux.

iTeos Therapeutics

+32 71 91 99 33  
info@iteostherapeutics.com

Michel Detheux  
CEO

www.iteostherapeutics.com

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### Janssen Immunosciences

The Janssen Pharmaceutical Companies of Johnson & Johnson have a strong tradition in immunotherapy. The company recognizes the importance of the immune system and founded a dedicated research institute last year within Janssen R&D, called Janssen Immunosciences. Here, researchers look at the different aspects of the immune system and emerging technologies to address novel challenges in various disease areas. These range from conventional autoimmune diseases to the immune mechanisms in neuroinflammation and immuno-oncology. Janssen Immunosciences exists in addition to the established therapeutic areas and harnesses emerging science, expertise, and innovation to drive novel concepts and create value for the stakeholders.

The company's oncology research keeps a strategic focus on three key diseases (hematological malignancies, lung cancer, and prostate cancer), allowing them to develop a deeper knowledge and understanding. At the moment, it already has a couple of successful antibody-based drugs available to patients and physicians, such as daratumumab, which can induce apoptosis of multiple myeloma cells by targeting the CD38 molecule that is highly expressed on the cell surface. Furthermore, an early pipeline of classical immunomodulators is being developed that can induce or enhance a robust antitumor response, including novel checkpoint inhibitors but also vaccination approaches.

After the initial success with checkpoint inhibitors, the field has seen a flurry of activity and may even be crowded. "I don't think you can just keep on going and develop antibodies for checkpoint blockade molecules for clinical evaluation without a clear understanding of what kinds of patients are best suited for benefit," **Anish Suri, Senior Director of Janssen Immunosciences**, says. "I don't think the industry can sustain that kind of a paradigm from the perspective of expected patient benefit or the costs of drug development," he clarifies. "These types of treatments will reach a plateau in efficacy, and it's up to us to break through this ceiling by developing the next generation of immunotherapeutics, driven by an increase in depth of disease understanding."

#### Understanding the specificity of the immune response: implications for opportunities in vaccination or lifestyle alterations for cancer prevention

Suri believes very strongly in a vaccination approach where the specificity is first set in place by priming with an antigen, followed by releasing the brakes on the immune system with checkpoint inhibitors. "That's an example of one," he explains, "but you can also think about the use of oncolytic viruses, which could provide other ways of dealing with the tumors and making them immunogenic to enhance antitumor immunity." There is a finite amount of diversity that individuals can bear or display, so understanding the available repertoire is essential. This is a first step toward creating a robust, sustained response. "These things could make it from a manageable disease into a preventable condition," Suri says. "These are concepts that we may not have challenged ourselves with a decade ago, but with the



promising data from the initial success of checkpoint blockade therapies, it is well worth thinking about." In fact, a near-term focus of Janssen Immunosciences in Beerse, Belgium is to develop emerging science and capabilities to delineate the diversity and specificity of the immune repertoire in both health and disease.

Recent data has also demonstrated the potential influence of the microbiome on the immune response against cancers in the context of immunotherapeutic treatment efficacy. For example, a correlation has been found between the presence of certain bacteria in the gut and the response to checkpoint blockade therapy. This could not only be used as a predictive biomarker for patient stratification but perhaps also as a future therapeutic approach. Using early lifestyle changes to alter the microbiome and thus create or maintain or influence the protective immune repertoire may potentially support new strategies that progress the field in a very different way.

"Microbiome approaches could either sustain or enhance the right antitumor environment," Suri explains. "This could be both at the level of sculpting the adaptive immune repertoire and also by means of other components or microbial derivatives that sustain or inhibit tumor growth because of factors that are produced or not. We know now, for example, that some microbial species will produce short chain fatty acids that are very beneficial for regulatory T cells in autoimmune diseases, particularly in the case of inflammatory bowel diseases," he continues. "Some of the early therapies that we are testing at Janssen are focused on that and are going into the clinic in the near future. Similarly, for example, in colorectal cancer, there may be some species that exacerbate the early incidences of the adenoma. This is another way of thinking about lifestyle modification or the preventative landscape."

The promise for immunotherapy is vast, because you can see it being active on its own; you can see it being anchored as a vaccination strategy if you know the antigens; you can see it being anchored in combination approaches with radiation and chemotherapy, depending on what those manipulations add; and then, from a microbial interface, there are opportunities as well. So, clearly, for all these spaces, the anchor becomes the immune system. This underlying common denominator is the strategic reason for us to have created Janssen Immunosciences. The immune component is very visible not only in immuno-oncology but also in metabolic diseases or neuro-inflammation. When we're in a situation like this, you can't boil the ocean, so you have to pick and choose what you do and how you extract value in the short term and continue to develop the space in the long term.

– Anish Suri, Senior Director Janssen Immunosciences

## Setting up a global network to harness innovations and spark collaborations for progress

Janssen also shows a healthy self-awareness. "Janssen and J&J look at the world with very open eyes," Suri confirms. "Of course, there's a lot of internal effort, but we're smart enough to know we cannot capture everything internally."

Janssen has set up four Innovation Centers worldwide, where early emerging science from academic groups, start-ups, and small biotech firms is developed and supported to obtain proof of concept. Other support mechanisms have been created in the form of JLABS, workplaces that incubate small companies, or JLINX, which brings ideas together with investment capital. "We were the pioneers to set up this framework to harness global innovation, and that has worked out very well in our favor," Suri says. "A lot of the deals we have done, including some that have gone to the clinic, have come through these networks."

Janssen Immunosciences

[www.janssen.com/immunology/](http://www.janssen.com/immunology/)

Brian Kenney  
+1 215 620 0111  
[bkenney1@its.jnj.com](mailto:bkenney1@its.jnj.com)

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### RXi Pharmaceuticals

RXi Pharmaceuticals was co-founded by Craig Mello, PhD, the co-recipient of the 2006 Nobel Prize in Medicine for the discovery of RNA interference (RNAi). To advance the use of RNAi into therapeutics, RXi has developed a novel and proprietary self-delivering RNAi platform, termed sd-rxRNA<sup>®</sup>, that has many advantages over its competitors in the RNAi space. A robust pipeline and extensive patent estate provide for the development and commercialization of advanced RNAi therapeutics across numerous therapeutic areas.

The company's sd-rxRNA technology has demonstrated very efficient cellular uptake in many cell types, including cells of the skin and eye and also T cells, to name but a few. Current clinical programs include two phase II trials in dermatology, a phase I/2 trial in ophthalmology, and R&D activities (because of a recent acquisition) in the area of cell-based immuno-oncology.

#### RNAi: a recap

RNAi compounds are oligonucleotides that can be used to very specifically reduce the level of proteins that are undesirable or produced in excessive amounts. For cell-based immuno-oncology, RNAi compounds may be extremely useful for immune checkpoint modulation. A checkpoint is a protein on an immune cell that reduces its ability to destroy tumor cells. "RXi's goal is to reduce the mRNA coding for a specific checkpoint using its sd-rxRNA platform, leading to a reduction of the targeted checkpoint and allowing the immune system to carry out its normal function of killing cancer cells," explains **Dr. Geert Cauwenbergh, President and CEO of RXi Pharmaceuticals**. "With our technology, multiple checkpoints can be targeted at the same time. Whereas the cumulative toxicity of multiple antibodies can be significant, RNAi compounds may not lead to that same problem."

*"Results to date have demonstrated that our sd-rxRNA platform is uniquely suited for immune checkpoint modulation in cellular immuno-oncology therapies, such as CAR T cells. The targeted knockdown is achieved quickly and is very potent."*

#### Let's put RNAi in CAR T cells!

**MirImmune**, a company that was active in the field of cell-based cancer immunotherapy very early on, grasped the potential of this technology. They received a license from RXi to use their innovative RNAi compounds in cell-based immunotherapy to treat cancer. Within 18 months, MirImmune identified potent RNAi compounds against six different checkpoints, some of them extracellular and others intracellular.

*"Several RNAi compounds targeting different checkpoints can even be used at the same time for the same cells, be it extra- or intracellular, maintaining each compound's effectiveness without diminishing cell viability."*



**RXi Pharmaceuticals**  
Next Generation in RNAi<sup>®</sup>



## First in vivo tests

MirlImmune tested the effect of anti-PD-1 RNAi compounds in a mouse model for ovarian cancer. (PD-1 is a well-known immune checkpoint.) They transfected Meso CART cells (T cells engineered to target mesothelin, which is overexpressed on many solid tumors) with an anti-PD-1 sd-rxRNA compound. Then they injected these engineered CART cells into human ovarian cancer tumors that had been implanted in mice and observed the animals for 1 month. The results were quite remarkable. In mice treated with the CART cells modified with anti-PD-1 sd-rxRNA, there was a significant reduction of tumor growth compared to untreated tumors. Treatment with non-modified CART cells as a control did not significantly reduce tumor growth. Moreover, at the end of the study, the reduction of PD-1 protein in the modified CART cells isolated from the mice was still close to 100%, indicating a potentially long-lasting effect.

The anti-PD1-sd-rxRNA was also tested for activity in tumor-infiltrating lymphocytes (TILs). The potential of TILs transfected with the anti-PD-1 compound to kill melanoma cells was evaluated in vitro. Two different dose levels were tested and compared with a PD-1 antibody. The antibody and the lower RNAi dose appeared to have about an equipotent killing effect on tumor cells. The killing activity of TILs treated with the higher dose of sd-rxRNA on the melanoma cells was substantially increased compared to the lower dose and the antibody.

*“If we can demonstrate that we can block a checkpoint in T cells for 3 months, the average lifetime of a T cell, we might be able to replace antibodies with RNAi compounds.”*

## When will we know if it works in humans?

“Our intent is to start a clinical trial as soon as possible, and we are working to optimize RNAi compounds as we speak,” says Cauwenbergh. “We would like to collaborate with an institute in China that is very active in cell therapy. In the US, we plan to collaborate with a prominent cancer center in Boston and other leading academic centers. In one of our programs, we will be focusing on the improvement of standard of care cell therapies that are already approved by the FDA. The only thing we have to do is introduce our checkpoint inhibitor to the existing ex vivo cell treatment protocol. If all goes well, the first clinical entry could be happening within the next 2 years.”

*“Conventional chemotherapy may become a last instead of a first resort.”*

RXi Pharmaceuticals has a \$ 15 million market cap, but Cauwenbergh would not even sell it for double the price: “I don’t want our RNAi technology to be used for only one specific aspect of human disease. It has potential in so many therapeutic areas. I want to make sure that it will be used to its full potential, and this sentiment is behind our ongoing expansion into cancer immunotherapy and to other disease areas in the near-term future.”

“The nice thing about working in this space,” concludes Cauwenbergh, “is that the FDA, academia, and industry really work hand-in-hand. They know that the road isn’t straight. It’s like being an explorer discovering a new continent. We don’t know what to expect and what rules and regulations are appropriate.

*“We are progressing carefully, and together we’re building the plane while we’re flying it.”*

RXi pharmaceuticals

+1 508 929 3640

Geert Cauwenbergh  
Dr. Med. Sc. President and CEO  
gcauwenbergh@rxipharma.com

www.rxipharma.com

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### ImmunXperts

Although immunotherapy in cancer is booming, it isn't by any measure an easy field to navigate. Many oncology ventures willing to explore this new terrain find themselves quickly lost in an array of immune cell types, epitopes, and antibodies, or simply do not have the capacity to take on the immense workload. To compensate for this, Sofie Pattijn and two colleagues founded ImmunXperts as a development partner for companies focused on immunotherapy. But ImmunXperts is definitely not your "classic" CRO.

Every new immunotherapy drug has already gone through an intense preclinical development stage before being evaluated in clinical trials. During this phase, in vitro assays are used to characterize and compare drug candidates. By measuring and observing their effect on immune cells, the more promising and less risk-bearing drug candidates can be selected, which drastically increases their chance of passing in vivo tests and early clinical trials later on. Because of this, immunological in vitro tests can generate valuable information. Sadly, designing, implementing, and performing them can be a most difficult task.

**Sofie Pattijn, CTO at ImmunXperts:** "When the breakthrough of checkpoint inhibitors proved that the immune system is able to have a profound impact on cancer, oncologists became convinced that this could be the cancer therapy of the future. In response, many companies tried to develop immunological assays in-house, without much success. This caused the notion that these tests didn't work or were too difficult to perform to grow in the market. We decided to step in and put our expertise in this field to work via ImmunXperts."

*"Many companies in the field had communicated the need for a flexible development partner. There's a clear demand for immunological knowledge in the oncology space and that's where our expertise can make a difference."*

### Exactly the right test

When considering ImmunXperts, "custom" is the word to keep in mind. The company designs in vitro tests specifically tailored to a client's drug in development. Take, for instance, checkpoint inhibitors: many of these molecules are currently in development, but there isn't one assay that fits them all. Each of the inhibitors requires tailor-made in vitro assays to generate meaningful data. These can all be tested on a myriad of immune cell types or in a broad range of different cancers. The sheer amount of possible combinations makes it quite clear why custom assays are needed.

**Thibault Jonckheere, CEO at ImmunXperts:** "Internally we have developed a qualitative and reliable platform of assays, which we then customize and optimize depending of our client's needs. Take a

ImmunXperts



mixed lymphocyte reaction (MLR) assay, for example. We can offer this test in over 30 forms, with different subpopulations of blood cells, working with healthy or sick patient cells, and so on. Our strength lies in carrying out an assay in a way that answers our customers' questions. We either develop these tests with our customers and afterwards transfer it to them or we run the assay for them.

## Constantly moving forward

While training your customers to do the services you offer them seems like a controversial business plan, that's exactly what ImmunXperts does. As companies don't have the time, resources, and expertise to develop these assays and stay up to date with the fast-moving technology available, they turn to ImmunXperts.

"We know that providing and passing on know-how to customers is a unique CRO model, yet we see the enormous added value we can bring to drug developers," says Pattijn. "We see ourselves as the interim immunology department of our clients, and educating them is an integral part of what we do. In doing so, we develop long-standing relationships with our customers and continue to provide anything from advice to materials, even after a project has finished."



"I think this mode of operating says a lot about our company culture," adds Jonckheere. "As a close partner, we wish to share our knowledge with as much people as possible. This is also a learning experience for us while at the same time forcing us to keep moving forward. We're constantly on the lookout for better or newer tests. In that perspective, we're not a CRO but more of a mobile development team!"

ImmunXperts

[www.immunxperts.com](http://www.immunxperts.com)

+32 7 196 01 33  
[info@immunxperts.com](mailto:info@immunxperts.com)

Thibault Jonckheere, CEO  
[thibault.jonckheere@immunxperts.com](mailto:thibault.jonckheere@immunxperts.com)  
Sofie Pattijn, CTO  
[sofie.pattijn@immunxperts.com](mailto:sofie.pattijn@immunxperts.com)

## IMMUNOTHERAPY COMPANIES IN THE PICTURE

### PDC\*line Pharma

Founded as a spin-off from the French Blood Bank, PDC\*line Pharma is focused on developing immunotherapeutic vaccines. These vaccines contain their proprietary and unique plasmacytoid dendritic cell (PDC) line, whose remarkable characteristics make it excellently suited for immunotherapy. By boosting the immune system with their vaccine in combination with checkpoint inhibitors, PDC\*line Pharma aims to help the currently large group of non-responders to immunotherapy.

Joel Plumas, head of R&D at the French Blood Bank in Grenoble, has studied PDCs for nearly 20 years. Together with Laurent Levy, he decided to found PDC\*line Pharma. By the end of 2015, they offered **Eric Halioua (serial entrepreneur and former CEO of Promethera Biosciences, Myosix and Murigenitics)** to join and scale up the company. The brand-new CEO proposed to develop the company in Belgium, with its rich biotech ecosystem and well-known cell therapy expertise. Today, PDC\*line Pharma is operational from its headquarters in Liège as well as from a small research team in Grenoble. The company combines 14 researchers with a seasoned management team and has raised more than €6 million in equity and loans since its foundation.



**PDC\*line**  
pharma  
ADVANCED CANCER  
VACCINES

#### A cell line with a dual nature

Central to PDC\*line Pharma's technology is a remarkable cell line of plasmacytoid dendritic cells, named the "PDC\*line." While PDCs are one of the rarer cell types in the blood, the cell line that PDC\*line Pharma relies on is of leukemia origin. The company leverages several advantages of the cell line, attributed to both its plasmacytoid and tumorigenic nature, for immunotherapeutic vaccines.

"The potency of our cell line is extremely high," explains Eric Halioua. "PDC\*line is much more potent to prime and boost antitumor-specific cytotoxic T cells, than conventional vaccines, and improves the response to checkpoint inhibitors. When comparing the capacity for antigen presentation with both allogenic and autologous myeloid DCs, we found a 20- to 200-fold increase in CD8<sup>+</sup> T cell expansion. This shows that our cell line is very effective in inducing immunity. A second benefit is that our cells do not elicit any allogeneic response, meaning that our cell line will not be rejected by the treated patient. This allows us to develop a very scalable, off-the-shelf, and standardized product. Our cell line is



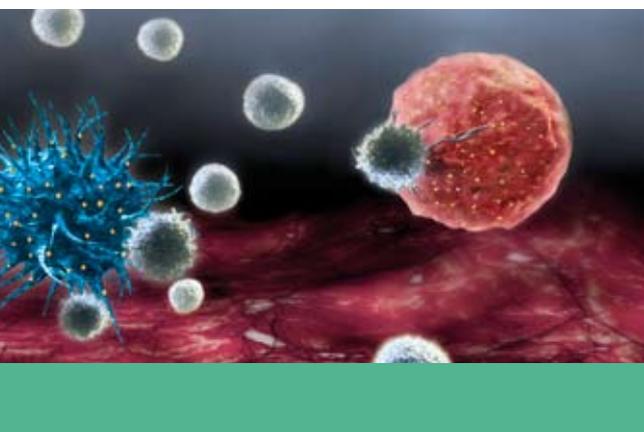
GMP-approved, and the PDCs are already mature. We can grow them in large volumes in suspension, which also lowers costs. These characteristics give our cell line a significant competitive edge.”

PDC\*line Pharma’s cell line is also capable of crossing the endothelium of the blood vessels, thanks to the adhesion molecule L-selectin. This makes intravenous administration a viable strategy for their therapeutic vaccine. The absence of PD-1, PD-L1 and PD-L2 from PDC\*line further prevents tumor-induced immune suppression, a frequent hurdle in cancer immunotherapy.

## Path to the clinic

A first clinical trial in melanoma patients has already been completed, with results expected by mid-2017. In this trial, the PDCs were primed with four different cancer antigens, providing a broad basis for tumor recognition. “For our upcoming trial in lung cancer, we will go over six different tumor antigens,” adds Halioua enthusiastically.

After first explorations in melanoma and lung cancer, subsequent trials will explore the combination of PDC vaccines with an anti-PD-1 checkpoint inhibitor. As with many in the field, PDC\*line Pharma strongly believes in a combinatorial approach of immune stimulation and blocking inhibitory signals from the tumor. Checkpoint inhibitors were quite successful in the latter goal, but only in a small subset of patients. By using PDC\*line to stimulate the immune system and increase T cell infiltration into the tumor, non-responders to checkpoint inhibitors might become susceptible to this kind of therapy.



## Supported by the experts

To support the clinical trials, PDC\*line Pharma is looking to complete a financing round in 2017. Multiple investors have already reached out to the young company, a testament to their technology’s potential.

“In our previous financing round, we were able to convince a set of very knowledgeable and experienced individuals to invest in our company,” confirms Halioua. “Both our management team, board of directors, and investors include former GSK Vaccine leaders and key experts in therapeutic vaccines. To us, this commitment is a very important validation of our scientific and strategic position.”

PDC\*line Pharma

[www.pdc-line-pharma.com](http://www.pdc-line-pharma.com)

Eric Halioua  
President & Chief Executive Officer  
+32 474 05 78 66  
[e.halioua@pdc-line-pharma.com](mailto:e.halioua@pdc-line-pharma.com)

## GSK: LESSONS LEARNED FROM THE MAGE-A3 PHASE III TRIAL

For more than 20 years, GSK was at the forefront of cancer vaccine development. Building on the results of the Brussels branch of Ludwig Cancer Research on one of the first cancer antigens, MAGE-A3, a treatment was envisioned that could prevent relapse after surgery. Unfortunately, after going through the entire process of clinical research, the compound failed to show efficacy in phase III trials, and the program was put on hold. While no treatment could be brought to the patient, Jamila Louahed, Vice President of Vaccine R&D and Fernando Ulloa Montoya, Innovation Project Leader Data Sciences and Clinical Systems at GSK Biologicals, look back on the program as a positive learning experience that has led to new insights for the field.

### MAGE-A3 to target cancer cells

The MAGE-A3 protein was an interesting antigen for vaccine research as it could selectively target cancer cells. Its gene is expressed during embryogenesis and switched off at birth, except in some cells in the testes that are unable to present antigens to the immune system. So, while the protein is not present in healthy cells, demethylation processes reactivate the gene during cancer progression in various cancers, including non-small-cell lung cancer (NSCLC) and melanoma. Therefore, MAGE-A3 can be regarded as a strictly tumor-specific antigen.

### Leading science

After many years of R&D, the Vaccines Business Unit of GSK started two Phase III trials in 2006 to assess the efficacy of their MAGE-A3 cancer immunotherapeutic in surgically resected NSCLC and in resected stage III melanoma. The efficacy was assessed both in the overall population and in patients with a potentially predictive immune gene signature. Most trials in cancer drug development focus on metastatic tumors. In contrast, GSK consciously chose to test its vaccine in adjuvant settings. "We recruited patients that had their tumor surgically resected and used the vaccine to prevent the cancer from coming back. This is a true immunization approach in line with what prophylactic vaccines have done in infectious diseases," Louahed says.

"This means we chose the hard way to do the clinical development," she continues. "Working in adjuvant settings complicated the trials. A bigger sample size was required as the patients also experienced benefit from the surgery itself. Furthermore, they needed to be followed up for longer to detect significant differences with the placebo control." While in metastatic settings, the outcomes can be evaluated faster, it took GSK about 7 years to do the first evaluation.





In setting up the trial, the company was also well aware of the importance of patient selection. While MAGE-A3 is present in a broad range of tumor types, the gene is not reactivated in every patient. All patients were screened for MAGE-A3 expression in the tumor to make them eligible for the trials. "This added a lot of complexity to patient enrolment but also created a hurdle of meeting all the regulatory requirements for a companion diagnostic that could be linked with treatment," Louahed explains. "However, it was really acknowledged by the field that we have done the right thing."

The lung trial (MAGRIT) is the largest therapeutic trial ever done in the adjuvant setting of NSCLC. While MAGE-A3 immunotherapeutic failed to demonstrate increased survival in patients post-surgery, the studies provide a large body of information on disease progression and clinical outcomes in the adjuvant setting of NSCLC and melanoma. While not meeting the primary endpoint was disappointing, the study also provided insights into clinical characteristics, cancer recurrence, and survival in patients with thoracic surgery and chemotherapy.

### The importance of translational research

GSK also made sure to emphasize the importance of understanding the factors affecting the response to the MAGE-A3 immunotherapeutic by including translational research from early clinical trials. By looking at what happened in the tumor before treatment, the company showed that not all cancer patients were alike. Different tumor features would affect whether the patient would respond or not.



The most important finding indicated that whether tumors expressed certain immune related genes associated with immune cells infiltration seemed to be a predictive factor. Later, this observation was also associated with other immuno-oncology treatments. Furthermore, the experiments revealed that a vaccination approach is unlikely to work by itself and will also require a form of immunomodulation that not only induces the right immune response but will also inhibit cancer immune resistance mechanisms.

The decision to emphasize translational research not only proved to be insightful for the design of a clinical trial but also for the immuno-oncology field in general. This strong emphasis on translational research was maintained in the Phase III studies. An important finding in the adjuvant melanoma study was that although the tumor immune-related signature could not select a subpopulation benefitting from the treatment, it was found to be a very strong predictor of disease outcome, independent of treatment. This result was prospectively validated using a novel approach with an in vitro companion diagnostic (IVD) assay developed for clinical application. "In this regard, despite the lack of treatment effect in the overall study, external melanoma clinicians recognized that the finding and rigorous prospective validation of the prognostic immune gene signature, could give an important additional tool for clinical decision making in this setting. It was previously thought that all patients in this melanoma population were equally at high risk of relapse," said Fernando Ulloa-Montoya who was the scientific lead for translational research and biomarkers in the Cancer Immunotherapeutics program.

“GSK was also pioneering in the field of personalized medicine and biomarker IVD development. This was the first time that a multi-gene expression signature aimed to be prospectively validated as a predictor of treatment response in large Phase III studies. A novel approach allowing optimization and validation of this signature within these studies was discussed with regulatory authorities, implemented and completed. The demonstration of feasibility of this approach is a step forward in personalized medicine and it offers an option for complex biomarker development and validation in immuno-oncology or in other fields” Ulloa-Montoya explained.

## A future for cancer prevention?

Thanks to the robust trial design, the negative results could be attributed to a lack of clinical efficacy of the MAGE-A3 immunotherapeutic. This was very disappointing, but the company stands behind its decisions. “Although they did not meet the primary endpoint, these Phase III trials remain a landmark study in the field of oncology.

“After decades of slow progress, the immuno-oncology field has recently made important strides. The recent success of immuno-modulators and the encouraging results obtained with other immunotherapeutic approaches have clearly demonstrated that unlocking and boosting an individual’s immune system is a key requirement in fighting cancer. However, this is only the beginning, and a clear and exciting path of opportunities and next generation immuno-oncology therapies and strategies is rapidly emerging,” Louahed says.

Based on the negative phase III results, a business perspective necessitated suspending the cancer vaccine program. Some of the patients that showed a clinical benefit during the trials are still being followed up. GSK continues to contemplate the field and has acquired new technology platforms that could be used to obtain a better anti-cancer immune response. Once there is more evidence about these in the clinic, or new combinations are discovered that could be meaningful in an early setting, a return to cancer vaccines is not out of the question.

In the meantime, GSK has not abandoned the field of (immuno-) oncology. The GSK pharma division is building the ‘next generation’ of immunotherapies intending to widen the range of cancer patients who benefit. Among them, different antibodies, such as an OX40, ICOS agonist and TCR cell-based therapies are in clinical development. Despite the initial setback, the belief in immuno-oncology is still present. A deeper understanding in this relatively young and evolving field will tell if there will also be a place for preventative approaches in the future.

“This study is proof of the importance of innovative science,” Louahed says. “Despite not meeting its primary endpoint, the wealth of information we have been able to gather from this study has been vital in helping us assess the path forward, and that is the only way we will ever make progress in this new and complex area of science”.

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[www.gsk.com](http://www.gsk.com)

Jamila Louahed  
VP Head Research and Development center, Belgium  
+32 2 656 57 36  
[jamila.x.louahed@gsk.com](mailto:jamila.x.louahed@gsk.com)

# IMMUNOTHERAPY IN ACADEMIA

## State-of-the-art

It is clear that immunotherapy is becoming a major strategy in the treatment of solid and hematological malignancies. Immune checkpoint inhibitors, DC-based tumor vaccinations, and engineered T cell based therapies have shown impressive results in a wide range of cancers.

### Checkpoint inhibitors

Monoclonal antibodies targeting tumor and T cell surface antigens have become the standard of care in various solid and hematological malignancies. Research is now focusing on defining new targets and increasing response rates while limiting side effects. In addition, bispecific antibodies (BiTes), which target both surface antigens on the tumor and the T cells (CD3) and thereby engage and trigger T cells, are entering clinical care and being optimized further.

The anti-CTLA4 antibody ipilimumab was the first checkpoint inhibitor to become FDA-approved for the treatment of metastatic melanoma after demonstrating a clear survival benefit compared to classical chemotherapy. Greater clinical activity has also been observed with the anti-PD1 inhibitors nivolumab and pembrolizumab in several malignancies, such as melanoma, non-small cell lung carcinoma (NSCLC), renal cell carcinoma, bladder cancer, and classical Hodgkin lymphoma. The PD-L1 (Programmed Death Ligand 1) inhibitors atezolizumab, durvalumab, and avelumab are emerging as immunotherapeutic options in the treatment of patients with NSCLC, bladder cancer, and stage IV Merkel cell carcinoma. Several clinical trials are currently ongoing, investigating the value of checkpoint inhibitors in various tumor types.

Despite these encouraging results, the majority of patients do not respond to immunotherapy. One strategy to increase response rates is to combine different immunotherapeutic agents. When administering ipilimumab with nivolumab, an improved overall response rate has been reached in metastatic melanoma patients, albeit with a notable risk of serious immune-related adverse events. Further tweaking the dose regimens in these combinations has allowed for significant improvements in tolerability while enabling unprecedented objective responses in treatment-naïve NSCLC and even chemorefractory small-cell lung cancer.

In addition to the identification of efficient combination strategies, research worldwide is focusing on the definition of biomarkers. One of the potential markers is PD-L1 expression in tumor tissue; the hypothesis is that the higher the expression, the better response to anti-PD1/anti-PD-L1 agents. This has been most clearly demonstrated in lung and bladder cancer. In other tumors, such as melanoma, renal cell cancer, and Merkel cell carcinoma, the relation is less clear. Responses to immunotherapy have also been observed in PD-L1-negative patients, and PD-L1 expression may vary within one tumor and/or according to the staining technique, making its use as a biomarker difficult in routine practice. Moreover, it has been shown to change over time, influenced by the treatments administered. Nevertheless, immunotherapy tends to give higher responses in tumors with a high mutational burden, which is illustrated by the responses to immunotherapy in colorectal cancers that are microsatellite instability high (MSI-H).

### Cancer vaccination

In contrast to checkpoint inhibitors, cancer vaccines have failed to deliver major clinical breakthroughs in oncology thus far. However, it is expected that the field of cancer vaccination will be revitalized very soon thanks to (1) the ability to rationally combine a vaccine with checkpoint inhibition and (2) the identification of patient-specific cancer neoantigens predicted by deep sequencing of the tumor's mutanome. In preclinical models, neoantigen-based cancer vaccines have demonstrated tumor control rates equivalent to checkpoint inhibition alone, and this is increasingly attracting attention from big pharma.

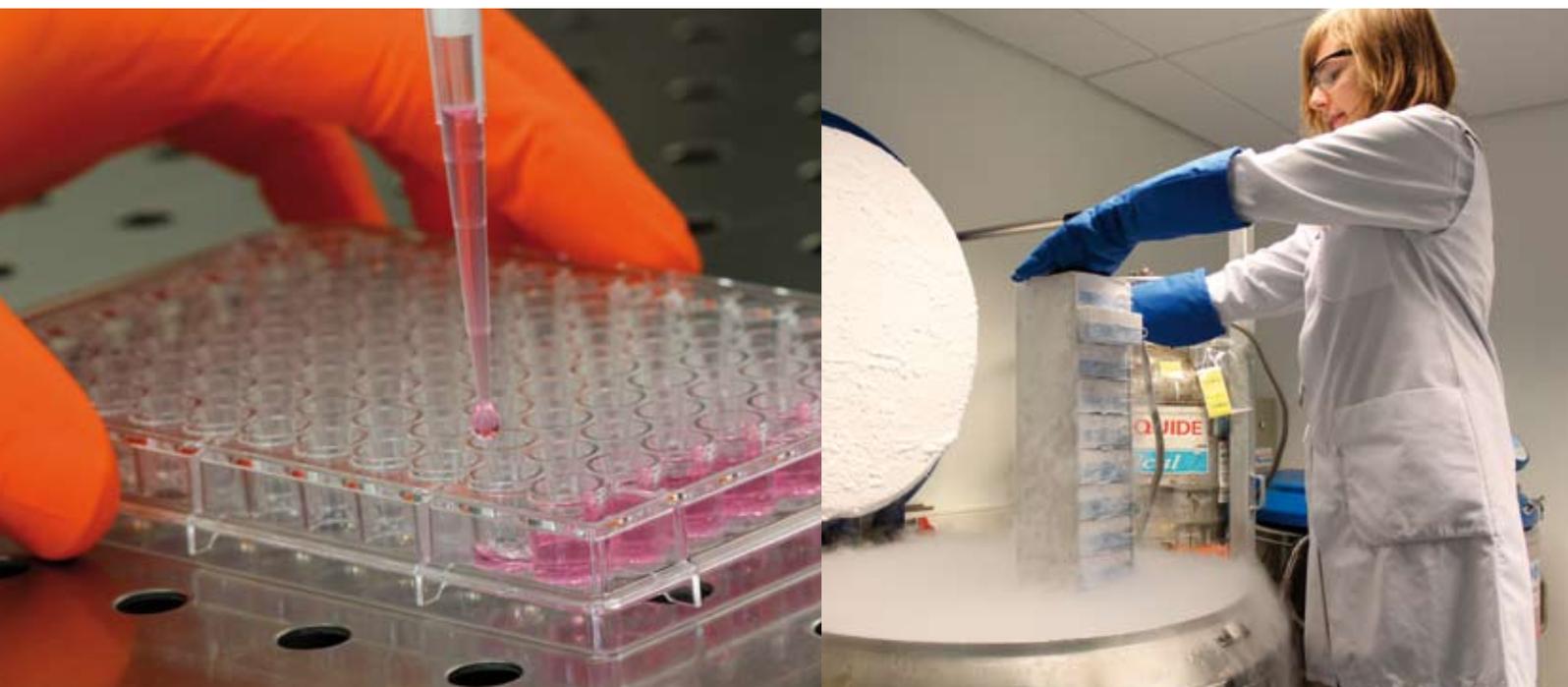
In contrast to synthetic vaccines, cellular vaccines are still being actively developed. Sipuleucel-T (Provenge®, Dendreon Corp.) was the first vaccine similar to DCs to show a survival benefit in castrate-resistant prostate cancer. With its whole-tumor mRNA-loaded DC vaccine, **Argos Therapeutics** has advanced its own whole-tumor mutanome-targeted DC vaccine (Arcelys platform) well into phase III in renal cell cancer. Other companies see clear opportunities in developing individualized cancer therapies based on autologous DC vaccination as well (including **Northwest Biotherapeutics** and **CiMAAS**). Moreover, different research teams and companies (e.g., **CureVac AG**, **BioNTech AG**, and **Moderna Therapeutics**) are currently evaluating mRNA cancer vaccines after direct injection in patients.



### T cell based cancer therapies

Despite stimulation, the immune system remains inefficient against many tumors, as high-affinity T cells that recognize the tumor cells are lacking due to negative selection in the thymus soon after birth. Researchers worldwide have therefore sought ways to generate T cells with a new specificity, recognizing tumor cells with high affinity. Both T cell receptors (TCR) and CAR are used to transfer tumor specificity to these T cells.

CART cell therapy is currently the most successful of the two strategies and represents a completely novel cancer treatment: fully patient tailored, targeted, and carrying a possible cure for otherwise palliative cancer patients, even those with a high tumor burden. The biggest successes have been made using the CD19/20 CART cells for lymphoid malignancies. However, this promising cancer therapy still faces some important challenges. First, the extreme power of these cells also results in considerable, possibly life-threatening, toxicities. The pathophysiology of these toxicities, especially the neurotoxicity, needs to be unraveled to permit better treatment or prevention. In addition, safety switches, such as split receptors or suicide genes, need to be built in. Second, the longevity of the T cells is crucial for clinical efficacy and needs to be optimized. Third, to be able to treat a wide range of both hematological and solid malignancies, good tumor-specific antigens need to be identified and targeted with optimized CART constructs.



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Finally, the fact that the powerful T cells encounter an immunosuppressing micro-environment needs to be addressed; combining the therapy with other forms of immunotherapy, such as checkpoint inhibitors and cancer vaccinations, will hopefully lead to improved and more durable effects.

## Immunotherapy at CRIG-ION - future perspectives

### The state of the art and current needs

Despite these very positive evolutions, responses in immunotherapy remain limited to specific subgroups of patients, and there are currently no markers available to pre-identify these responders. The detection of molecular signatures that can predict response to immunotherapy or the detection of other immune strategies that can further increase responses would be of great value to further optimize the clinical results of immunotherapy in cancer. In addition, immunotherapies are currently directed toward specific malignancies (such as melanoma, lung cancer, prostate cancer, renal cell carcinoma, bladder cancer, classical Hodgkin lymphoma, Merkel cell carcinoma, and lymphoid malignancies), and a great deal of research is still needed to target other malignancies (e.g., breast cancer).



### Biomarker studies: predicting/monitoring response

CRIG-ION conducts research to define signatures that could aid in predicting the response to immunotherapy. Monitoring the dynamics of immunoregulatory blood cells during immunotherapy has the potential to deliver novel, minimally invasive biomarkers. Researchers from CRIG-ION demonstrated that the expression of indoleamine-2,3-dioxygenase (IDO) early in the disease course of melanoma is an independent prognostic factor reflecting a state of immune intolerance that remains consistent throughout the disease course. IDO expression in several other cancer types has been associated with a negative prognosis. Therefore, IDO might be a new point of attack to restore adequate anti-tumoral immunity response. **Ghent University Hospital** is taking part in clinical trials with IDO inhibitors. Furthermore, assessment of tumor-infiltrating lymphocytes (TILs) has emerged as a prognostic biomarker in several solid tumors, and their prognostic role in squamous cell carcinoma of the head and neck is also being explored. The innate immunity biomarkers chitinase 3-like 1 and lipocalin-2 are being studied in the context of breast cancer metastasis.

### Novel immunotherapeutic strategies

In this research context, the use of in vivo models enables optimal treatment strategies, sequences, and doses to be explored quickly before a specific protocol for human clinical trials is designed. CRIG-ION presents a number of different in vivo models and will further develop other preclinical models. There is, for instance, an immunocompetent mouse model for triple-negative breast cancer and lung cancer. Additionally, dogs with spontaneous tumors are also used by CRIG-ION researchers to evaluate novel cancer (immuno-)therapies.

### Cell-based (immuno-) therapies

Aside from the immune checkpoint blockade molecules, another immunotherapeutic strategy that is advancing toward clinical practice is cell-based therapy. Vaccination strategies with DCs and adoptive T cell transfer based on TILs and peripheral blood lymphocytes (that are not genetically manipulated) have been explored primarily in melanoma and lung cancer, but responses are variable. Ghent University Hospital has a production facility for such cell-based immunotherapeutic strategies. Techniques to fine-tune these labor-intensive procedures and to optimize the responses are the subject of current research. CRIG-ION is developing a DC vaccine targeting the patient's whole tumor mutanome. Preclinical development has now reached certain important milestones, and the produc-

tion method is currently being translated into a GMP-compliant protocol. Approval by regulatory authorities and the start of the first human trial for non-small cell lung cancer is scheduled for Q4 2017. As the source of the antigen is mRNA amplified from the patient's own tumor, this vaccination approach can be extended to other types of cancer as well. More fundamentally oriented researchers within CRIG-ION are focusing on the design of synthetic mRNAs to increase the efficiency of mRNA-based cancer vaccines and immunotherapeutics.

One of the new revolutions in the domain of genetically manipulated T cell-based therapy is the CAR T cell therapy; this is producing very strong responses to one specific tumor antigen and is currently in a clinical trial for patients (children) with B-lymphoid malignancies at Ghent University Hospital. CAR T cells directed against either CD19 or CD20 have shown a very significant activity against lymphoid malignancies, even in the case of high tumor burden. However, myeloid malignancies, such as AML, are less obvious targets for CAR T cells; all membrane markers present on AML cells are also present on normal myeloid cells, leading to significant toxicities. Therefore, CRIG-ION is developing a new T cell based therapy.



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### Combination strategies

Combination strategies with existing immunotherapies (anti-CTLA4 and anti-PD1) have demonstrated superior effect in melanoma patients at the cost of an increased prevalence of serious immune-related adverse events. Combination with other established anti-cancer strategies, such as chemotherapy, targeted agents, and radiotherapy will also be investigated. CRIG-ION focuses on a possible activating effect of radiotherapy on immunotherapy with investigator-initiated trials in several cancer types in humans. Radiotherapy-induced cell death can stimulate a tumor-specific immune response by the massive exposure of tumor antigens to the immune system and the release of danger-associated molecules, exerting an endogenous "immuno-adjuvant" effect. In advanced canine cancer patients, CRIG-ION researchers demonstrated that IL-12 gene therapy in combination with metronomic chemotherapy triggered an influx of immune cells in the tumor, decreased angiogenesis, and resulted in an increase of the body weight.

### Alternative delivery systems

Alternative advanced delivery systems, such as using lipid nanoparticles and microbubbles that may stably deliver mRNA to immune cells in vivo (and not after leukapheresis in the lab), are being explored at CRIG-ION. Covalent anchoring of small interfering RNA (siRNA) (specifically silencing tumor-promoting genes) nanomedicines to the surface of tumor-migrating cytotoxic T cells and the use of stem cells to produce more tumor-specific immune cells are being explored as new cell-based immunotherapeutic strategies. CRIG-ION also investigates how polymer nanotechnology could help in activating the immune system against cancer via vaccine nanoparticles with molecular adjuvants for immune cell targeting and applying polymer chemistry to target cancer cells with specific stimuli to enhance their immunogenicity and alleviate their immune-suppressing action.

### Novel targets for immunotherapy

As mentioned, IDO might be a new therapeutic target to reverse acquired immune tolerance, and its inhibitors are currently being tested in clinical trials at CRIG-ION along with other new targets for immunotherapeutic approaches. These involve the reversal of an installed immune tolerance (e.g., IDO inhibitors) or the activation of immunostimulatory



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molecules such as the BH7 family (e.g., CD28, CD80, CD86) and the tumor necrosis factor (TNF) family (e.g., GITR, OX40, CD40). Research into unraveling the molecular signaling mechanisms in cancer cells and immune cells controlling effector and immune suppressive regulatory T cell responses could also identify new targets for therapy. For example, MALT1 protease expression in cancer cells not only plays a direct role in tumor cell proliferation but is also involved in TCR signaling and regulatory T cell development; small compound MALT1 inhibitors are therefore being developed and characterized as an interesting “dual hit” anti-cancer approach.

### **Broadening the indications for immunotherapy**

Lastly, CRIG-ION and Ghent University Hospital are taking part in several industry- and academia- initiated clinical trials testing existing immunotherapies in many different cancer subtypes, such as hepatocellular carcinoma and bladder carcinoma.

In conclusion, immunotherapy has changed the field of cancer treatment dramatically and holds promise for many new indications in cancer therapeutics. At this point, however, a large number of new research questions arise relating to how to further optimize these results and broaden the indications. Research at CRIG-ION focuses on these questions.

Medical Research Building 2, UZ Gent  
De Pintelaan 185  
9000 Gent

[CRIG@UGent.be](mailto:CRIG@UGent.be)  
[ION@uzgent.be](mailto:ION@uzgent.be)

Pieter Rondou, PhD  
CRIG coordinator  
+32 9 332 24 53

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