Commentary: Mark Vaeck

A new technology for targeting proteins inside cells

Many of the most interesting disease targets for new drug development are located inside the cells of our body. They comprise a wide variety of well-known but complex proteins that regulate intracellular processes and that, when mutated or overexpressed, can cause major diseases like cancer, inflammation or neurodegeneration. Unfortunately the majority of these intracellular targets are 'undruggable' by conventional small chemical drugs because their functionally relevant domains lack the hydrophobic pockets that are required for small molecule binding. Moreover, these targets cannot be addressed by currently available biological-like antibodies since they lack the ability to penetrate membranes to reach the inside of cells.

Over the past decade our Belgium-based company Complix NV has developed a preclinical portfolio of biologics directed against intracellular targets that we believe represents a novel approach for treating cancer and other important diseases. In our lead programme we are currently selecting a development candidate with a view to bringing our first product into the clinic by the end of 2020. In this article we explain how we arrived at this point in the company's development.

From the time we started the company in 2008, our mission has been to develop a new drug modality that combines the beneficial properties of antibodies, namely high affinity and specificity, with the cell penetration capacity of small chemicals. Done successfully, this could make a vast and entirely unexploited target universe accessible to drug development. Our technology platform is based on a novel small protein scaffold which goes by the invented name Alphabody. This combines the first letter of the Greek alphabet with a shortened version of the word 'antibody.' The Alphabody has been designed to access the most desirable but intractable intracellular proteins that are implicated in disease.[1]

The early years

During the first years of the company's history we spent considerable time and effort to fine-tune and perfect the Alphabody's drug-like properties including its stability in the circulation of blood, in cells and tissues and its manufacturability by fermentation in *Escherichia coli*, the standard production micro-organism. We also designed the Alphabodies to be non-immunogenic and most importantly, endowed them with efficient cell penetration capacity. By screening and testing hundreds of different amino acid sequence motifs embedded in the Alphabody structure, we were able to select the optimal motifs for promoting cell membrane penetration, both *in vitro*, as well as *in vivo* in animal disease models.

These sequence motifs usually contain a mixture of hydrophobic and cationic residues, and enable the Alphabody to pass efficiently through the cell membrane bilayer to reach the cytosolic space. The uptake process by the cell penetrating Alphabodies, or CPABs, is not receptor mediated and therefore does not result in endosomal entrapment. More recently we also characterised CPAB motifs that mediate intranuclear penetration, giving them the capacity to reach intranuclear targets. Additionally, we established that CPAB concentrations in the cytosol as well as in the cell nucleus can reach therapeutically relevant levels, or in the low microM range.

At the same time we invested considerably in building an efficient and reliable drug discovery engine. Our goal was to establish a discovery process that would enable us to address multiple complex disease targets in parallel, and develop functionally active CPABs in a competitive timeframe. Given their unique coil structure of three alpha helices, Alphabodies can support an unlimited random variation in the alpha-helical sequences displayed on their outside surface without losing their stability. This means that using phage display as a tool, a great diversity of Alphabody binders can be created in which the target binding motifs are displayed either on the outside of the helical structures, which we call 'helix binders,' or within the grooves located between two helices, or 'groove binders.' Biopanning on a particular target with these huge 'helix libraries' or 'groove libraries' is the first step in the discovery process of new target-binding Alphabodies. Often this process yields a large panel, usually several hundreds, of nanoM range binders which are analysed and clustered according to their binding motif sequences.

Based on these analyses we then create smaller dedicated affinity maturation libraries which typically can yield sub-nanoM binders to the target. Using this efficient process we have not yet encountered any target structures against which we could not generate high-affinity binders within less than six months. In some exceptional cases, like in our programme targeting the Mcl-1 protein, we succeeded in designing high-affinity binders *in silico* based on the known three-dimensional structure of the target. Yet most of the time we used a combination of library screening with rational design approaches.

In order to create a viable drug modality, we also needed to equip the Alphabody with a property that provides it with a sufficiently long half-life in the circulation. Alphabodies are small, non-glycosylated proteins with a molecular weight in the range of 8,000 to 15,000 dalton. Hence they are rapidly excreted *in vivo* through the renal excretion system and have a half-life of less than half an hour in rodents. Our scientists designed another small sequence motif that they integrated into the Alphabody structure and which exerts serum albumin binding. By engineering different affinities for albumin they could also vary the half-life extension profile of the CPABs.

Alphabodies equipped with such an albumin binding motif consequently exhibited a much longer half-life (many hours in mice), and most importantly, demonstrated a significantly superior biodistribution profile and clearly enhanced concentrations in various tissues and organs. In particular,

we could now detect microM range concentrations of CPABs in tumour tissue of mice engrafted with human tumour cell lines. At this stage we had established the design of a novel biological scaffold that could integrate all of the essential properties for a truly therapeutic modality: high affinity and selective target binding, stability, manufacturability and half-life extension resulting in appropriate biodistribution. All of these properties can be integrated into a single small CPAB format, which is made possible by the particular versatility of the Alphabody scaffold, but which would be very difficult to achieve with any other protein or peptide format.

Finally we wanted to establish *in vivo* proof-of-concept with our CPAB platform, a prerequisite to stimulating interest from big pharma companies. We therefore selected Mcl-1 as the target for our lead programme. When we started the programme in 2013, Mcl-1 had been established as a scientifically validated cancer target, an important regulator of apoptosis and overexpressed in several types of cancer. No anti-Mcl-1 inhibitors were known to be in clinical development and Mcl-1 was recognised as an interesting but challenging drug target.

Tumour growth inhibition

By using a rational structure-based design approach, taking into account the fact that the ligand for Mcl-1 has a clearly defined helical structure that could be engrafted onto our Alphabody structure, we succeeded in generating high-affinity, sub-nanoM binders to Mcl-1 that exhibited substantial *in vitro* potency and induced apoptosis-mediated cell death in a range of Mcl-1 dependent cancer cell lines. Most importantly, when we equipped our lead anti-Mcl-1 CPABs with our half-life extension technology, we observed significant tumour growth inhibition, or a 50% to 60% tumour volume reduction, in mouse models with engrafted Mcl-1 dependent cancers, without any indication of toxicity in the animals. However, after all this work, we learned that at least two other Mcl-1 programmes had entered the clinic making Mcl-1 a less obvious candidate for us to pursue as a lead programme. The two other projects were being run by Amgen Inc and by a partnership between Laboratoires Servier and Novartis.

At this stage, and given the highly competitive environment in which we operate, we sought to secure a partnership to leverage our platform. This we did in 2015 when Merck & Co Inc expressed an interest in our CPAB platform for oncology applications. After a detailed due diligence, the company signed an agreement with us in December of that year. Under the deal, Merck obtained exclusive rights to two cancer targets that they had selected and which were not subject to ongoing programmes at Complix.

We would discover CPABs against these targets and develop these up to proof-of-concept in animal models while Merck would take on further drug development and pay royalties to Complix on sales of any marketed products in the future. As part of the deal, Merck is fully funding the research activities and Complix received an upfront payment and is eligible for success fees and development milestones for a total potential value of $280 million. Complix retains rights to its own technology and IP and is free to work on any other disease targets. This deal provided us with significant cash and a validation of the platform, whilst ensuring our rights to ongoing programmes and to all potential new target programmes outside the two that Merck had selected.

We reached the point, less than two years ago, where we had developed a discovery engine that could generate novel CPAB leads against any intracellular targets in a competitive period of time. The whole process from obtaining target material for biopanning until the generation of functionally active CPABs typically takes about 18 months. Hence we were ready to start building a broad and valuable product pipeline.

Focus on oncology

We decided to focus on oncology as our therapeutic area of choice, despite the intense competition. Indeed while there have been significant advances in cancer research and some high-profile breakthroughs such as the novel immuno-therapies, a significant proportion of cancer patients still have less than satisfactory medicines.

Having taken this decision, we embarked on a broad and systematic analysis of the cancer target space and made a selection of potentially interesting intracellular targets based on a number of criteria including scientific validation, cytosolic or nuclear presence, abundance and turnover, structural features, competition in the marketplace and most importantly, the inability of conventional small molecule drugs to modulate these targets. We brought these down to a shortlist of about 30 and then selected six targets as active programmes. These include transcription factors or components of transcription factors, enzymes, proteins regulating the cell cycle and intranuclear targets. All of these are technically challenging but exciting targets. Any one of these programmes could yield a drug with first-in-class properties.

An example of a programme with considerable potential is one in which CPABs are being developed to act on the downstream part of the Wnt signalling cascade. We have confirmed that our CPABs modulate a factor that is part of the 'enhanceosome' that drives the transcription of Wnt target genes. This target is known to be overexpressed in several types of cancer and is also suspected to play a role in other important diseases with high medical need like fibrosis.

In summary, we are now progressing an exciting pipeline of CPAB based compounds against important intracellular cancer targets with the potential to yield more than a few first-in-class products, and a lead programme that is expected to reach the clinic in the second half of 2020. We believe that the CPAB platform has a unique ability to generate novel breakthrough cancer therapeutics based on their ability to interact with molecular targets that are part of essential oncogenic pathways. This in turn holds promise for improved treatments for cancer patients.

Reference:

1. Vaeck, Mark, Alphabodies – working inside the cell, *MedNous*, June 2014.

This article was written by Dr Mark Vaeck, a co-founder and Chief Executive Officer of Complix NV.