The Therapeutic Potential of Nanobodies™

The unique features of Nanobodies™ indicate that they should outperform other antibody formats and lead to the development of a number of powerful and innovative therapeutic products.

By Dr Mark Vaeck at Ablynx NV

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Monoclonal antibodies (mAbs) are now an established class of pharmaceuticals with many products approved to treat a wide range of diseases. However, despite clear advantages as therapeutics, conventional antibodies also have significant limitations: they are large and complex proteins that can’t reach targets buried deep in tissues or membranes, and their manufacture in mammalian cell cultures is expensive.

Nanobodies™ are the smallest antigen-binding fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain. Their unique and well-characterised properties enable them to excel conventional therapeutic antibodies in terms of recognising uncommon or hidden epitopes, binding into cavities or active sites of protein targets and exhibiting superior stability. Furthermore, Nanobodies™ have a low immunogenic potential and are easy to manufacture in microbial systems. These favourable biophysical and pharmacological properties, together with the ease of formatting them into multifunctional protein therapeutics, means Nanobodies™ are ideally placed as a new generation of antibody-based therapeutics.

THERAPEUTIC USE OF mAbs

The development of therapeutic monoclonal antibodies (mAbs) over the last 20 years has been a notable success, with several products now approved to treat a range of diseases, including transplant rejection, inflammation, infectious diseases and cancer. With many more candidates in late-phase clinical trials, mAbs have become a well-established class of therapeutic drug.

However, the use of mAbs as therapeutics has its drawbacks. The first mAbs approved by the FDA were unmodified mouse antibodies. Their therapeutic usefulness in humans was severely limited by their xenogeneic nature, which tended to induce strong immune responses. Even the subsequent generations of chimeric or fully humanised mouse antibodies in later years did not completely overcome this inherent problem of immunogenicity. Furthermore, mAbs are relatively unstable compared with small-molecule drugs, which means they can only be administered by injection. Finally, as mAbs require mammalian cell culture for their production, they are expensive to manufacture at large scale.

ANTIBODY DOMAINS

In order to overcome some of these limitations, recent research has focused on strategies to reduce the size and complexity of antibodies whilst preserving their essential therapeutic mode of action. Small antibody fragments, rather than whole antibodies, have been investigated by several research groups in their quest to discover superior drug formats. At Ablynx, we have focused on single immunoglobulin domains with high affinity for the target antigen as a potentially new class of drugs.

Conventional antibodies are composed of two heavy chains and two light chains. Both heavy and light chains need to be associated in a complex heterodimeric structure to allow high-affinity binding to the target antigen. Fragments consisting of the single variable domains of conventional antibody heavy or light chains (VH or VL domains), have been isolated and are sometimes referred to as single-domain antibodies (dAbs) (1). However, these isolated VH or VL domains typically lack the antigen binding capacity of the original antibody. Also, the removal of one domain exposes the hydrophobic interface of the second domain, making the resulting dAbs ‘sticky’ and prone to aggregation (2). Therefore, lengthy and complex protein engineering efforts are required to generate dAbs with sufficient affinity and solubility to be useful as therapeutics.

While such manipulations may achieve some success in resolving the problems with VH and VL domains, nature has provided a simple and ideal solution. Animals belonging to the ‘camelids’, such as camels and llamas, produce in their...
serum fully functional antibodies composed solely of heavy chains (3). Isolation of the variable domains of these camelid heavy-chain antibodies (V_{H\text{H}} domains) results in the generation of fully active, stable and soluble high-affinity antibody domains without the common drawbacks of dAbs (Figure 1). By applying a proprietary cloning and selection procedure (referred to as Nanoclone\textsuperscript{TM} technology), Ablynx is able to perform high-throughput isolation of the genes encoding fully functional antigen-specific camelid V_{H\text{H}} fragments, referred to as Nanobodies\textsuperscript{TM}. These Nanobodies\textsuperscript{TM} can be expressed in engineered bacteria or yeasts for large-scale manufacturing through fermentation, achieving production yields in the gram per litre range (4).

**WHAT IS A NANOBODY\textsuperscript{TM}?**
Nanobodies\textsuperscript{TM} are single antigen-binding domains derived from a functional antibody heavy chain. They contain the unique structural features of the antigen-binding domains of naturally-occurring heavy-chain antibodies that function without a light chain.

Antigen-specific Nanobodies\textsuperscript{TM} can easily be derived from the V_{H\text{H}} domains of heavy-chain antibodies that are circulating in the serum of immunised llamas or camels (5). Nanobodies\textsuperscript{TM} are approximately one-tenth the size of conventional antibodies and are encoded by a single gene. In contrast to dAbs derived from unmodified V_{H\text{H}} or V_{L\text{L}} domains of conventional antibodies, Nanobodies\textsuperscript{TM} are inherently soluble and stable, and do not aggregate.

Nanobodies\textsuperscript{TM} naturally have a high homology (approximately 90%) with human V_{H\text{H}} frameworks and, for use as therapeutics, can be further humanised to within 95-99% homology by making a small number of amino-acid substitutions in the framework regions. These humanised Nanobodies\textsuperscript{TM} retain their characteristic stability, solubility and high affinity for the antigen. Moreover, results from primate studies performed by Ablynx have shown that such Nanobodies\textsuperscript{TM} do not elicit any detectable immune response after repeated administration over several months.

Nanobodies\textsuperscript{TM} can also be derived from the V_{H\text{H}} domains of conventional antibodies (from humans, for example) by making specific amino-acid substitutions to provide these unstable V_{H\text{H}} domains with improved stability and solubility. This process is sometimes referred to as ‘camelisation’ (6). However, in contrast to immunising llamas for Nanobody\textsuperscript{TM} generation, the camelisation of V_{H\text{H}} domains is less likely to yield binders with effective affinities for the target antigen.

**ADVANTAGES OVER CONVENTIONAL ANTIBODIES**
The single-domain nature of Nanobodies\textsuperscript{TM} endows them with several unique features compared with conventional antibodies and their fragments. Besides the easy cloning and
The starting point for generating Nanobodies™ is the serum of immunised camelds. Ablynx routinely uses this collection of high-affinity binders from an in vivo matured immune system. Nanobodies™ have other technological and biophysical advantages that enable them to outperform conventional antibodies in many respects.

Although conventional antibodies have several beneficial features — such as their high affinity and selectivity for a target, their ease of discovery and their low inherent toxicity — Nanobodies™ display additional characteristics that make them superior as potential drug molecules.

They have a simple structure and are only one-tenth the size of a conventional antibody. Because they are encoded by a single gene, Nanobodies™ can be engineered into multi-valent and multi-functional formats, which provides great flexibility for the design of different drug formats, including multi-target specificities and tailoring of half-life. Nanobodies™ are highly stable to heat, pH, proteases and protein denaturing agents and, unlike dAbs, they are highly soluble and do not have a tendency to aggregate.

Due to their high solubility and small size, Nanobodies™ are able to engage with epitopes and targets that are hidden or buried in membranes, and cannot be addressed by conventional antibodies (7). Because Nanobodies™ have a somewhat longer and flexible CDR3 loop, they are also capable of binding into the cavities that form the active sites of enzymes. Such cavity-binding Nanobodies™ have been shown to be potent inhibitors of enzymatic activity (8). This property of Nanobodies™ offers the potential to develop novel therapeutics that target enzymes in a highly selective fashion.

Because Nanobodies™ are small proteins, encoded by a single gene and require no post-translational modifications, they can be easily produced in micro-organisms, allowing low-cost manufacture through microbial fermentation. Finally, even though Nanobodies™ are generated from a non-human source, they have a very low immunogenic potential in vivo.

This combination of highly advantageous characteristics means that Nanobodies™ are a promising and novel class of therapeutic proteins that combine the beneficial features of conventional antibodies with many of the desirable properties of small-molecule drugs. This combination of characteristics is unique and is not shared by other single-domain antibodies.

**THERAPEUTIC OPPORTUNITIES**

As demonstrated above, Nanobodies™ are distinguished from other conventional antibody formats by their unique properties of small size, high solubility and intrinsic stability, easy tailoring into pluripotent constructs, recognition of uncommon or hidden epitopes, binding into cavities or active sites of enzymes, receptor clefts, ease and speed of drug discovery, and ease of manufacture. These features indicate that Nanobodies™ should outperform other antibody formats and lead to a number of promising medical applications.

Bi-specific and bi-functional antibodies have specific applications in the treatment of cancer by delivering toxic payloads or binding T-cells to tumours. The single-domain nature of Nanobodies™, their solubility and robustness, make them ideal for such uses. Using a bifunctional fusion between a cancer-targeting Nanobody™ and a pro-drug converting enzyme, researchers have demonstrated the complete elimination of established human adenocarcinoma grafts in nude mice (9).

Because of their inherent stability to extremes of pH and the presence of proteases, Nanobodies™ can survive the harsh conditions in the gut system. This creates opportunities for orally delivered Nanobodies™ to treat disorders of the gastro-intestinal tract. Ablynx has obtained proof-of-concept in animal models for the successful treatment of inflammatory bowel disease with orally administered Nanobodies™.

The delivery of therapeutics across the blood brain barrier (BBB) remains a major challenge in the treatment of neurological diseases and cancers of the brain. Recently, Nanobodies™ have been identified that efficiently transmigrate across the BBB epithelium (10). Using these Nanobodies™ for selective delivery of therapeutic drugs (either biopharmaceuticals or chemical conjugates) will open up new avenues for the treatment of brain diseases.

Because of their exceptional stability and solubility, Nanobodies™ can be formulated for injection as well as for oral administration. Ablynx has preclinical Nanobody™ development programmes in rheumatoid arthritis, inflammatory bowel disease and thrombosis. The company also has several discovery programmes in the areas of oncology and CNS.

Nanobodies™ are also expected to have a future as a tool for diagnosis — particularly of cancer. Their superior penetration potential, high-affinity target binding and fast clearance from the circulation represent an ideal basis for the development of imaging products.

**NANOCLONE™ DISCOVERY PLATFORM**

Nanoclone™ is an integrated suite of technologies that enables the rapid discovery of large numbers of high-affinity Nanobodies™ against a variety of therapeutic targets. This platform, which was developed at Ablynx as an industrialised process, represents a powerful drug discovery engine.

The starting point for generating Nanobodies™ is the serum of immunised camelds. Ablynx routinely uses
llamas, which are immunised with low doses of human disease targets. The antigen-primed lymphocytes, and more particularly the B cells, are isolated from the serum of the llamas and serve as the starting material for Nanobody™ gene cloning. Because Nanobodies™ are simple, small protein domains encoded by single genes that are expressed abundantly in the antigen-primed B cells of immunised llamas, their cloning from individual B cells is straightforward.

By applying the Nanoclone™ technology, Ablynx is able to directly clone Nanobodies™ from large numbers of target-specific B cells, without the generation and screening of complex gene expression libraries, as might be required for dAbs or other conventional antibody fragments. Since Nanoclone™ also uses the natural immune system of a hyper-immunised animal, no further in vitro affinity maturation is required to generate sub-nanomolar affinities. Individual Nanobodies™ are easily expressed in bacterial cells and can immediately be screened for functional activity in a bioassay.

IP AND PATENT POSITION
Ablynx holds the dominant patent position in the field of Nanobodies™; it has exclusive rights to more than 50 granted patents and pending patent applications, including an exclusive license to the patents covering the basic structure, composition, preparation and uses of Nanobodies™ (the ‘Hamers patents’). All products, including therapeutics, that contain Nanobodies™ are covered by these patents, irrespective of their species of origin. As a result of its exclusive patent rights, Ablynx is the only company in the world capable of commercialising healthcare products based on Nanobodies™.

In addition, the Nanoclone™ procedure does not rely on third-party patents describing the use of expression libraries and phage display. Finally, the unique structural properties of Nanobodies™ means that they do not rely on patents that typically affect the generation, production or use of conventional antibodies. For example, the absence of an antibody light chain in Nanobodies™ means that they do not rely on patents describing the co-expression of heavy and light chains (such as the ‘Cabilly’ patents, for example).

Furthermore, humanised Nanobodies™ do not require methods of humanisation such as CDR grafting or veneering.

CONCLUSION
Conventional antibodies have been approved for a wide range of therapeutic and diagnostic applications, but have certain inherent limitations as a therapeutic class. Nanobodies™ represent a novel class of therapeutic drugs that combine the beneficial properties of conventional antibodies with advantageous characteristics of small molecule drugs. Their unique features – superior stability and solubility, their high affinity for the target, their cavity binding property and their ease of manufacture – offer a promising avenue for the development of powerful and innovative therapeutic products.

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References