Of Mice and Men

Building multifunctional biologics to treat disease

In 1975, Georges Köhler and César Milstein succeeded in making fusions of myeloma cell lines with B cells to create the first murine monoclonal antibody (mAb) in Cambridge, UK. Pioneers, including Sir Gregory Winter, subsequently perfected the techniques to create fully human mAbs. With that, the door was wide open for the exploitation of the enormous potential of mAbs as transformational therapeutic agents, and they now represent the biggest class of therapeutic blockbusters.

In this article, we review some of the antibody-based products under development and explain how our technology platform addresses some of the therapeutic challenges.

It is now universally accepted that human, rather than humanised, chimeric or murine is the goal for mAb-based therapies. Drugs such as the TNF antagonist adalimumab and, more recently, checkpoint antagonists such as ipilimumab (anti-CTLA-4), nivolumab or pembrolizumab (both targeting PD-1) have dramatically improved therapeutic outcomes for important subsets of patients with few or no effective alternatives.

However, the same attributes that make mAbs highly effective as therapeutic molecules under certain circumstances also have a less beneficial flip-side and can present substantial problems for effective treatment in other cases. In particular, mAbs are large molecules (150kDa for the standard IgG format). Therefore, although they avoid rapid first-pass renal clearance, they penetrate very poorly into tissue thereby limiting access to tumours or poorly vascularised tissues. Also, while the fragment crystallisable (Fc) region conveys many functional benefits, there are circumstances under which prolonged systemic exposure to mAb activity or to Fc-mediated effector functions are highly undesirable. Indeed, more recently, it has begun to become apparent that Fc-mediated clearance of mAbs by macrophages can result in detrimentally rapid reduction in therapeutic efficacy.

Combination therapy utilising two or more agents to simultaneously target synergistic pathways is already considered a cornerstone of cancer therapy. The benefits of mAb-based combination therapy are also well known. This was illustrated very clearly by the positive survival impact for advanced melanoma patients who received the combination of ipilimumab plus nivolumab. However, the fact that a substantial proportion of these patients also experienced an increase in treatment-limiting (treatment-related) toxicity proved that simple combinations (e.g. mixtures of two different mono-specific antibodies) are not always the answer. It is increasingly clear that in order, for example, to lift the tail of the cancer survival curve beyond the effect of mere combinations requires the sort of profound effect that can only be achieved by accessing novel biology through the simultaneous engagement of one or more targets by bi-/multi-specific molecules.

> ***"To lift the tail of the cancer survival curve beyond the effect of mere combinations requires accessing novel biology."***

Although the technology for doing so has improved, substantial technical challenges of creating multi-specific mAb formats remain. The standard Y-shaped mAb format is still suboptimal, retaining its large size, an Fc region and a restricted geometry and flexibility, permitting only a limited range of variations in modes of target engagement. Indeed, many of the rather complex solutions to creating molecules for multi-target engagement have been based on bolting additional binding units onto the basic mAb format. These often end up more closely resembling Frankenstein's monster than a therapeutic molecule and with such complex molecules comes a whole host of issues, not least of which is the stability and the chemistry, manufacturing and control (CMC) challenges associated with their manufacture. Such solutions will always be a compromise.

Driven by the desire to step back from and circumvent the constraints inherent in the standard mAb format, a great deal of effort has gone into identifying how best to achieve multi-target engagement for optimal therapeutic benefit. This plays perfectly to the benefits of small modular binding domains. These can broadly be separated into two categories, those based on the more generic non-antibody 'alternative scaffolds' and those based on small antibody binding domains.

The alternative scaffolds are many and varied ranging from Affibodies, Affilins, Anticalins, Atrimers, DARPins, FN3 scaffolds (e.g. Adnectins and Centyrins) to Fynomers, Kunitz domains, Pronectins and OBodies. These domains are mostly derived from intracellular proteins, some non-human, which require synthetic structural modification in order to introduce non-natural sites capable of binding to target. These features substantially elevate a range of potential issues including suboptimal biophysical properties, challenging CMC and higher potential immunogenicity.

Small antibody binding domains have been used to create a variety of different formats (e.g. BiTEs, DARTs TandAbs etc) mostly based on the 30kDa single chain Fv (scFv) subunit which comprises a paired antibody variable heavy and light chain (VH and VL) domain connected as an in-line fusion by a flexible linker. While these formats have had some success (blinatumomab, a BiTE molecule comprising two scFvs – one targeting CD3 and the other CD19 – is now on the market as a second-line treatment for Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukaemia), the technical challenges of working with any domain-based format that requires the pairing of a heavy and light chain for activity are

substantial. Indeed, it is well known that the scFv format is notorious for its instability (exposing hydrophobic surfaces when the VH and VL fall apart), challenging biophysical properties and poor expression yields. As such, the ability to assemble multi-specific molecules comprising more than one scFv subunit merely multiplies the impact of these issues.

A small number of groups have opted to focus on a single antibody-derived domain from which to create therapeutic molecules. At only 13-15kDa in size, these are roughly half the size of the scFv molecules and do away with the need to pair a heavy and light chain. Domantis (now GlaxoSmithKline Plc) utilised a single human VH3 germline framework from which to build a naïve *in vitro* library for phage-based drug discovery. The VH3 germline used as the basis for the library was known to be the most stable as an isolated VH domain. However, this approach is yet to derive a marketed product, potentially due to limitations in diversity or stability of the *in vitro* derived and synthetically randomised domains.

Others have adopted an *in vivo* approach. The camelids have developed a specialised set of VH domains (termed VHH domains) which have an essential set of so-called 'camelising' mutations in framework region 2 (FR2). These mutations prevent the pairing of the VHH domains with the light chains that are also present as part of the same immune response. Although other parts of the camelid VHH can be 'humanised' these camelising mutations must remain.

In order to cope with having to include this protection from contaminating light chains, the camelid single domains have had to compromise their structural diversity. All evolved from a single VH3 germline which has subsequently developed into a limited number of related subfamilies. However, it is notable almost certainly in light of their *in vivo* route of generation, camelid-derived multi-valent/multi-specific molecules (termed 'Nanobodies' by Ablynx) have made substantial in-roads into the clinic.

Importantly, therefore, the *in vivo*-derived camelid VHH domains have made excellent progress, validating the single antibody variable domain as an attractive starting point for creating a new class of efficacious therapeutics.

For reasons of safety and immunogenicity, therapeutic mAbs developed as drugs transitioned from being murine in sequence to fully human. Historically, about 70% of those human mAbs on the market today have been derived *in vivo* from a transgenic mouse. It is therefore logical that the desired way forward for development of the next generation of antibody single domain-based therapeutics, is the creation of *in vivo*-derived fully human VH domains using a transgenic mouse.

We believe that Crescendo Biologics has solved this technically challenging task by creating stable, fully human VH domains (about 13kDa 'Humabody VH') using a proprietary transgenic mouse devoid of any antibody light chains. *In vivo* maturation using the normal immunological machinery (VDJ recombination, somatic hypermutation etc) optimises the potency of our Humabody molecules, and develops superior biophysical properties. The mice act as natural filters for VH domains from any of the human germline families (not just the VH3 family) that are stable in the absence of a partner light chain. Low affinity, unstable or aggregating variants are effectively filtered out, while stable VH domains optimised further *in vivo*.

Crescendo Biologics Ltd

Founded: 2009

Located: Babraham Research Campus, Cambridge, UK

Based on IP from the Babraham Institute

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Created using constructs built to contain more than 20 V-genes covering all the human germline families, the Crescendo mouse delivers a diverse array of Humabody building blocks that can be configured into an almost limitless range of multifunctional molecules that are robust and easy to express at very high yields in simple microbial systems. They can be formulated for a range of different routes of administration and due to their small size, can penetrate rapidly into and accumulate efficiently in tissues. Their systemic exposure can also be modulated by use of a serum albumin-binding binding domain enabling prolonged systemic exposure or rapid clearance from systemic circulation, each of which can drive up the therapeutic index depending on the mode of action being deployed.

Thus, the Humabody format enables the assembly of just what is required in a molecule to achieve maximal therapeutic benefit from optimal target engagement with no extraneous components. This ability to derive formats that are optimally configured for therapeutic efficacy, capable of accessing novel biology which is unachievable using regular mAbs, makes it a possible for Crescendo to create molecules built to:

- engage two or more targets on the same cell (e.g. PD-1xLAG-3 bispecific driving dual-checkpoint blockade in highly exhausted T cells expressing both targets);
- simultaneously engage single targets from different angles through multiple epitopes (e.g. biparatopic targeting of a tumour-specific marker such as PSMA with a payload-bearing format to drive internalisation); and
- deliver highly targeted T cell co-stimulation (e.g. a bispecific molecule that specifically targets local T cell costimulatory activity only in the tumour microenvironment whilst minimising systemic, non-specific activation)

Such small, versatile, single VH domain building blocks permit the rapid exploration of a vast range of 3D format space to identify optimal therapeutic solutions. This fully modular plug and play approach lacks the constraints of traditional mAbs and enables a radical rethink of how a molecule can be assembled to deliver enhanced therapeutic benefit across a wide range of indications. Through judicious choice of target and format, we believe that this can deliver the potential for substantial therapeutic impact, including the potential to raise the tail on the cancer survival curve.

This article was written by Brian McGuinness, head of new product and business opportunities, and Peter Pack, chief executive, of Crescendo Biologics Ltd in the UK.