Beyond mAbs with TandAbs

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With enhanced efficacy due to a bispecific mode of action and an excellent safety profile partly due to the absence of an Fc region, TandAbs represent a strong front runner in the race to develop alternatives to first-generation antibody therapeutics.

Monoclonal antibodies (mAbs) have become a well-validated and established treatment option for a wide range of indications in oncology, inflammation, infections and autoimmune diseases. However, there is room for improvement in a number of areas such as response rates, molecular size for better tissue penetration and IgG-mediated side effects.

At Affimed, we have developed a novel antibody format, the TandAb®, in order to obtain increased efficacy compared with current therapeutics based on formats such as IgGs, Fabs, single chain antibodies (scFv) and non-IgG scaffolds. TandAbs are tetravalent bispecific antibody constructs comprising two binding sites for each antigen. They bind to target molecules on the surface of, for example, tumour cells and can activate immune effector cells like cytotoxic T-cells or natural killer (NK) cells.

TandAbs possess the same avidity and affinity for each target as an IgG. Combined with their bispecificity, they represent an alternative to first-generation antibody therapeutics. They appear to have a very good safety profile, which is at least partially due to the lack of an Fc-portion. They are also very stable and have a much longer half-life than smaller antibody fragments with molecular weights below the renal clearance threshold, making them a potent alternative to therapeutic mAbs.

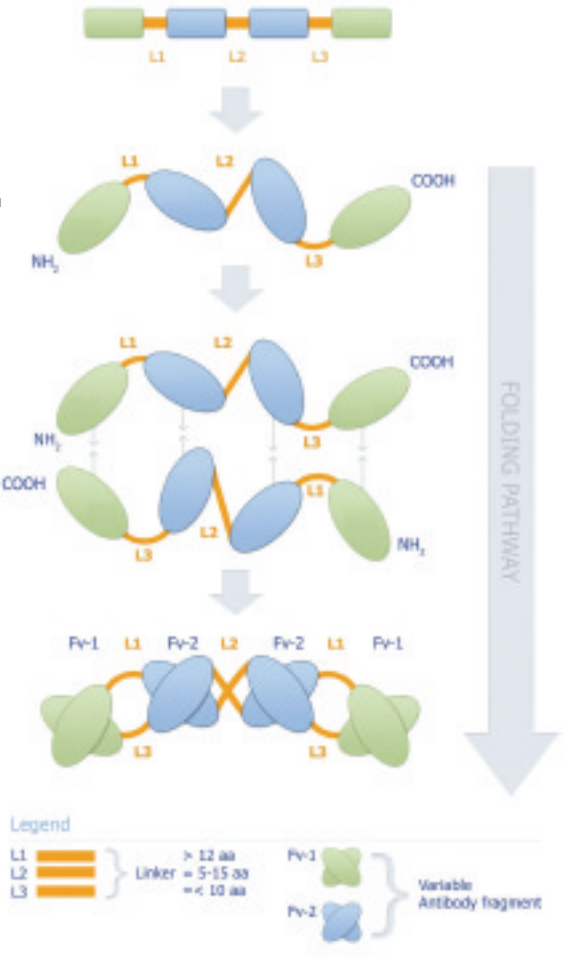


Figure 1: Formation of TandAb antibodies. TandAbs are expressed as a single polypeptide comprising four variable domains connected via amino-acid linkers varying in length. After translation in the endoplasmic reticulum, the monomeric polypeptide pairs head-to-tail with another monomer, forming a functional homodimer TandAb molecule

TandAb PROPERTIES

TandAbs are expressed as a single polypeptide comprising four variable domains connected via amino-acid linkers. After translation, the monomeric polypeptide pairs head-to-tail with another monomer, forming a functional homodimer (see Figure 1). They are highly specific and potent in target killing due to their enhanced antibody-dependent, cell-mediated cytotoxicity; this results from their high affinity and bivalent binding, resulting in the same avidity as IgGs. In contrast to mAbs, when recruiting NK cells as effector cells, they bind equally well to both the low and high affinity alleles of the CD16A receptor, thereby broadening patient responses. Their improved safety profile is probably due to their specific targeting of immune effector cells and the lack of Fc-regions.

Being fully functional without being glycosylated, TandAbs can be produced in prokaryotic and eukaryotic cells. This lack of glycosylation reduces the risk of potential immunogenicity and product inhomogeneity. Furthermore, it could facilitate upstream processing in, for example, *E coli* and be an important factor in reducing the costs of goods (COGs). Stability to freezing and thawing has enabled the development of both frozen and lyophilised formulations. A 15-month stability at 40°C has already been demonstrated for a lyophilised TandAb.

Table 1: Summary of TandAb properties

• Enhanced target killing: antibody-dependent, cell-mediated cytotoxicity (ADCC)
• Excellent safety profile: lack of Fc-mediated side effects
• Same avidity as IgG: bivalent binding for each target
• Favourable half-life: better than other antibody scaffolds
• Production: can be expressed in prokaryotic and eukaryotic cells
• Robust GMP process: established in mammalian cells
• Thermally stable: extrapolated shelf-life of more than two years
• No glycosylation: fewer problems regarding immunogenicity or product homogeneity
• Manifold applications: broad range of potential indications, such as oncology, inflammation, autoimmune diseases, asthma and COPD
• Strong IP situation: patents granted for major world markets including EU, US and Japan

TandAb homodimers have a molecular weight of 100 to 110 kDa, which is far above the renal threshold for first-pass clearance. The estimated half-life of about 23 hours in non-human primates is significantly better than other comparable formats based on antibody binding domains or alternative scaffolds. The properties of TandAbs are summarised in Table 1.

TYPES OF TandAb

There are two modes of action for TandAbs: they can act as a recruiting machinery for immune effector cells, such as T-cells or NK-cells (RECRUIT-TandAb); or they can act via the dual inhibition of two targets such as signalling pathways (BiBLOCK-TandAbs). A third type aimed at further optimising the pharmacokinetic properties (PROLONG-TandAb) has already shown promising results.

RECRUIT-TandAbs

Affimed's experience regarding the activation of cytotoxic immune cells, such as NK-cells and cytotoxic T-cells, and the ability to isolate specific antibodies and generate novel antibody formats was key to the development of RECRUIT-TandAbs. The CD16 receptor is a very strong and potent activator of natural killer cells and is expressed as two isoforms: CD16A on NK-cells (with cytotoxic activity) and CD16B on granulocytes (with uncertain signalling function) that have more than 96 per cent homology. Affimed succeeded in isolating a highly specific human antibody for the activation of the CD16A receptor. Cytotoxic T-cells can be recruited via a similar mechanism of activation, namely generation of a specific antibody against a subunit of the CD3 receptor, an essential part of the T-cell receptor (TCR) complex. Antibodies against CD16A and CD3 comprise the essential effector functions of the RECRUIT-TandAbs (see Figure 2).

In the case of CD16A, the RECRUIT-TandAb binds to a target cell molecule, for example CD30, with two of its binding sites, and to the CD16A receptor with the other two binding sites. This cross-linking event initiates the killing activity of the respective NK-cell. Granules containing cell-lysing components – such as perforin and granzyme – are transported towards the cell membrane of the NK-cell and subsequently secreted in close proximity to the target cell. Perforin causes the formation of aqueous pores in the target cell, thereby facilitating the entry of granzymes and associated components inducing apoptosis or osmotic cell lysis.

A very similar mode of action occurs when using T-cells as immune effector cells. If the TandAb binds with its anti-CD3 binding sites to CD3 on T-cells while simultaneously binding to a molecule on the surface of a tumour cell, the T-cells are activated to induce cell lysis of the targeted tumour cell.

The improved antibody-dependent cell cytotoxicity (ADCC) of RECRUIT-TandAbs opens up the possibility of developing next-generation antibodies based on clinically validated targets. Affimed is developing such candidates based on tetravalent bispecific antibodies targeting CD19 (AFM11, AFM12) and CD30 (AFM13) for lymphomas, or EpCAM (AFM20) and EGFR (AFM21) for solid tumours. Besides cancer, the principle of the RECRUIT-TandAb can be applied to other indications, such as infectious and autoimmune diseases.

BiBLOCK-TandAbs

Signalling pathways often work synergistically to enhance activation or inhibition. Especially in inflammatory diseases and allergic disorders, the synergistic role of chemokine interactions is well established and is currently the focus of new therapeutic approaches. The unique advantage of BiBLOCK-TandAbs is the simultaneous blocking of two targets by two binding sites for each target, thus ensuring high avidity when compared with other bivalent constructs. This mode of action results in a strong blocking or inhibiting signal that is able to intervene, for example, in inflammatory or allergic diseases. This technology platform will facilitate the generation of individually constructed TandAbs against diverse disease targets.

LEAD PRODUCTS

TandAbs are widely applicable and show promising therapeutic benefits in a broad range of diseases. Our lead compound, AFM13, successfully completed preclinical and toxicity studies in 2010, followed by IND and IMPD filings to treat Hodgkin's lymphoma. Orphan drug designation was granted in 2009 for both the US and Europe. The bispecific tetravalent TandAb antibody

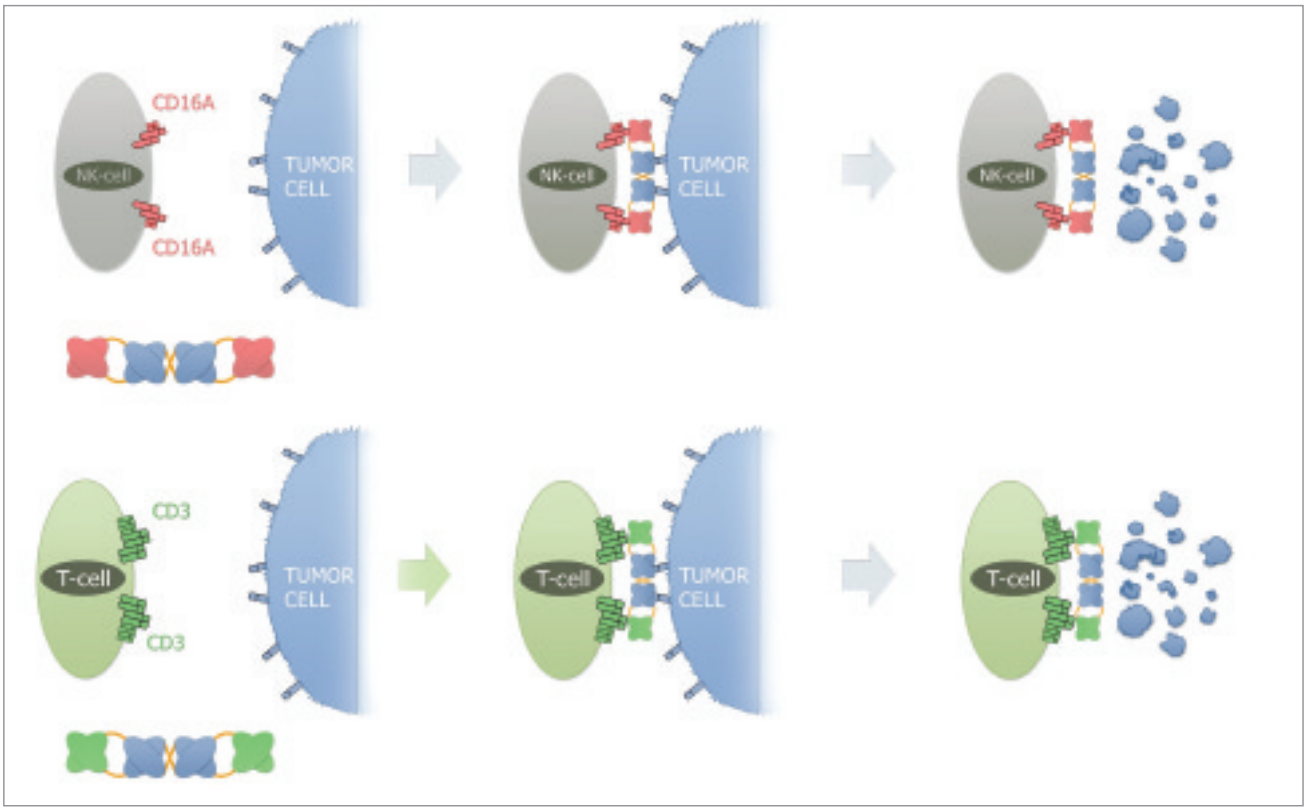
targeting CD30 and CD16A began clinical trials in 2010. The Phase 1 repeat dose escalation study is enrolling approximately 40 Hodgkin's lymphoma patients through two centres in Europe and the US. The first patients have now been treated with AFM13, receiving a total of four doses in weekly intervals. In the initial dose groups the antibody has been well tolerated. Final data are expected by the second quarter of 2012.

AFM11 and AFM12 are in preclinical development for the treatment of non-Hodgkin's lymphoma (NHL). AFM11 binds to the molecular targets on cancer (CD19) and cytotoxic T cells (CD3), while AFM12 binds to CD19 and to CD16A (the receptor on NK cells). AFM11 is expected to enter clinical trials in 2012.

An Anti-EpCAM TandAb, AFM20, is being developed as a potent alternative to existing mAbs for the treatment of solid tumours. EpCAM (epithelial cell adhesion molecule), a pan-epithelial differentiation antigen, is involved in homotypic cell-cell interactions. EpCAM is expressed on the basolateral cell membrane of virtually all simple epithelia and is over-expressed in their derived tumours. Therefore, EpCAM can be regarded as a pan-carcinoma marker and represents an important target for the immunotherapy of epithelial carcinomas, such as breast, lung, colon and prostate. Preclinical data indicate that AFM20 is more potent than the respective human anti-EpCAM IgG. The favourable *in vitro* properties indicate that this product could provide a highly attractive therapeutic alternative to existing antibodies in solid tumour therapy.

An anti-EGFR TandAb is being developed as a new approach for the treatment of breast and colorectal carcinomas. EGFR is over-expressed on the cell surface of many tumours and is one of the most important targets to have been addressed by small molecule and

Figure 2: RECRUIT TandAbs and their mode of action. The RECRUIT-TandAb is able to strongly activate immune effector cells such as natural killer cells (NK-cells) and T-cells. For the recruitment of NK-cells, Affimed has developed a highly specific and potent antibody against the CD16A receptor, and for the recruitment of T-cells, an antibody with high affinity against the CD3 receptor. The RECRUIT-TandAb binds to target cell molecules, for example CD30, with two of its binding sites, and to the CD16A receptor with the other two binding sites. This cross-linking event initiates the killing activity of the respective NK-cell through ADCC. This ADCC is much more efficient than that induced by IgGs or modified IgGs, as shown by internal experiments (data not shown). A very similar mode of action occurs when using T-cells as immune effector cells. CD3 is part of the T-cell receptor complex (TCR). If the TandAb binds with its anti-CD3 binding sites to T-cells while simultaneously binding to a molecules on the surface of a tumour cell, the T-cells are activated to induce cell lysis of the targeted tumour cell (ADCC).



mAb strategies. Alternative approaches are being developed using anti-EGFR immunotoxin conjugates, but there is still a need for a therapeutic molecule that ensures better efficacy and safety than current treatments. Affimed has isolated a fully human high-affinity antibody that specifically binds to EGFR on the cell surface. The RECRUIT-TandAb AFM21 combines this human antibody with antibody recruiting immune effector cells, such as NK-cells or T-cells. Preclinical studies are currently in progress.

Affimed's AFM15 programme targets T-cell-mediated autoimmune and inflammatory diseases, such as rheumatoid arthritis, moderate-to-severe psoriasis, inflammatory bowel disease and Type I diabetes. AFM15 is an anti-CD3 product that has been developed in bivalent and tetravalent formats. CD3 is a clinically validated target for autoimmune and inflammatory diseases and, by blocking CD3, AFM15 down-modulates the T-cell receptor complex, leading to an immune regulation. The absence of an Fc-portion eliminates possible cross-linking of T-cells to Fc receptor positive cells, thus preventing T-cell proliferation and cytokine release syndrome. Preclinical *in vitro* and *in vivo* data have demonstrated the strong immunosuppressive activity of AFM15 and reduced amounts of cytokine release. In addition to triggering fewer side effects, potential immunogenicity is reduced due to the absence of constant domains. AFM15 will be partnered or co-developed with a pharmaceutical or biotech partner for further preclinical and clinical development.

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A synergistic effect can be obtained by blocking two different chemokines or chemokine receptors. Affimed's AFM19 is a BiBLOCK-TandAb that simultaneously blocks two major chemokine signalling pathways to combat hyper-responsive immune reactions. Chemokines play a major role in the mediation of pathological pathways in atopic diseases like asthma, allergies and inflammation. While some chemokines are considered homeostatic and are involved in controlling the migration of cells during normal processes of tissue maintenance or development, other chemokines are pro-inflammatory. Besides their participation in signalling cascades that generate responses like chemotaxis, degranulation, release of superoxide anions and changes in the avidity of cell adhesion molecules, chemokines can also induce high levels of IgE and affect smooth muscle cells in blood vessels and airways. Preliminary data indicate that AFM19 strongly binds to the selected targets and effectively inhibits the signalling pathways.

CONCLUSION

TandAb technology represents a versatile platform for the development of novel immune therapeutics for various diseases. Bispecificity and bivalency for each target is a potent combination for either recruiting and activating immune effector cells to kill tumour cells (RECRUIT-TandAb); suppression of immune effector functions (PROLONG-TandAb); or inhibition of signaling pathways by blocking chemokines or cytokines and their receptors (BiBLOCK-TandAb). Due to the lack of immunoglobulin constant regions, the TandAb has a good safety profile with a reduced risk of triggering an immune response and a lower immunogenic potential. The recruit technology implementing either T-cells or NK-cells has proved to be highly efficient in TandAb-directed cell lysis. In the case of recruitment of NK-cells via specific CD16A targeting, a broader patient response can be expected in contrast to mAbs since TandAbs bind equally well to the high and low affinity CD16A receptor alleles. Pharmacokinetic data from non-human primates indicate a longer half-life compared with other recombinant antibody fragments or alternative scaffolds.

In addition to the successful *in vitro* and *in vivo* proof of concept studies, clinical data with the AFM13 RECRUIT-TandAb will be available during the course of 2011 – and this could further validate the TandAb technology as a very promising approach for bringing innovative therapeutic antibodies from the bench to the bedside.