Research Strategy: Fredrik Y Frejd

Scaffolding as a strategy for developing protein drugs

Protein drugs have had a tremendous impact on our society in recent decades. Drugs such as insulin and growth hormone, blood coagulation factors and enzyme replacement therapies have prolonged life expectancy and increased the quality of life for millions of individuals. The development of human or humanised monoclonal antibodies has raised the potential of protein-based drugs to new heights. Today these represent a substantial drug class offering treatments in major areas such as oncology and inflammatory diseases but also, to a lesser extent, in other areas including respiratory and metabolic diseases and recently, diseases of the central nervous system including Alzheimer's disease.

Essential for this success is the ability to generate drugs with very high target recognition, selectivity, and binding strength. In oncology, the inbuilt effector functions of these drugs that can mediate tumour cell killing is an advantage. However, antibody-based drugs also come with challenges: large multi-domain glycosylated proteins penetrate tissue more slowly than smaller proteins. They are difficult to administer subcutaneously at high doses and they are costly to produce. What's more, they have a defined geometry hindering certain binding formats.

To overcome these potential limitations, other protein structures – so-called alternative scaffolds for affinity recognition – have been investigated for their ability to harbour large molecular diversity while retaining the main advantages of antibodies. These advantages are high selectivity and binding strength as well as favourable drug-like properties in a defined basic shape. Two themes for these scaffolds have emerged. The first are proteins that mimic the basic antibody immunoglobulin fold with a beta-sandwich fold and loops and the second is other structures of proteins[1, 2]. The antibody mimic approach was initially very successful with emerging scaffolds such as adnectins or tetranectin-based domains. After some initial clinical tests the main activities within this class are those of the single domain antibody, also referred to as nanobodies. Initially spearheaded by companies such as Domantis and Ablynx, the technology is now widely available in both academic settings and in industry.

The second type of structure is a more diverse collection of scaffolds. Clinical activity to date confirms an interest in exploring not only subcutaneous or intravenous administration, but also intraocular administration, inhalation and even oral applications. Companies such as Pieris are probing their anticalin technology in inhalation while Molecular Partners has conducted Phase 3 trials with the intraocular VEGF blocking DARPin abicipar. The clinically most advanced active scaffold platform is currently in Phase 3 clinical trials and is based on technology developed by our company in Sweden, Affibody AB. Being one of the smallest scaffolds available, amenable to both chemical synthesis and recombinant manufacture, Affibody molecules will be described below as an example of how protein scaffolding can be a tool for overcoming the limitations of antibody drug development.

The Affibody scaffold is based on a small, very robust triple helical protein fold, stabilised by a hydrophobic core and composed of only 58 amino acids allowing peptide synthesis as well as recombinant production. The unique scaffold stability can accommodate randomisation of more than 20% of the total protein sequence. This means that large molecular libraries can be generated. High affinity binders are enriched and isolated by biopanning selection procedures e.g. phage display[3]. The increasing body of evidence verifies the utility of the Affibody scaffold for creating affinity proteins overcoming limitations of antibodies[4], including tumour-targeting Affibody molecules[5], bispecific protein constructs, biotechnological tools, and selective inflammation blocking[6].

Theranostic precision medicine scaffold

Theranostics is a term to describe a highly efficacious oncology treatment strategy where diagnostic biomarkers are paired with therapeutic agents to identify and target cancer cells for destruction. The prototypic approach is utilising a tumour targeting agent, first armed with a diagnostic radionuclide for imaging detection and characterisation of the tumour followed by a version armed with a therapeutic radionuclide. A radioactive dose is delivered to the tumour while sparing normal tissues. The concept was recently rejuvenated with the approval of two therapy products from Novartis. One is the somatostatin receptor specific Lutathera for the treatment of gastroenteropancreatic neuroendocrine cancers and imaging using Octreoscan and similar tracers, and the other the PSMA specific Pluvicto in prostate cancers with imaging using Locametz among other tracers. Breast cancer is, however, an area where less progress has been made[7].

Metastatic breast cancer is the most advanced stage of breast cancer. It is estimated that there are more than 168,000 women living with metastatic breast cancer in the US today. Many women have estrogen receptor positive breast cancer which often can be treated with hormone therapy. A challenge, however, is to know which women have tumours that are ER positive. Traditionally, tumour receptor status is determined by biopsy and immunohistochemistry. It is a method that can bring a lot of information, but it is invasive, limited to the one tumour lesion that is biopsied, and it can suffer from sampling errors.

A precision medicine tool in this context is the estrogen receptor specific radiotracer Cerianna. By offering women imaging with Cerianna, receptor status can be shown in all metastatic lesions, guiding subsequent treatment decisions. Another highly important receptor expressed in breast cancer is human epidermal growth factor receptor 2 (HER2). Overexpression of HER2 has been associated with poor prognosis but patients are today treated with HER2 targeted

treatments with good outcomes. A major challenge is, however, to understand HER2 status in women presenting or relapsing with metastatic disease. Emerging evidence now shows that there is substantial heterogeneity of the disease, and that HER2 status in a patient can change as the disease progresses[8].

There are monoclonal antibodies available for HER2, but a major limitation for imaging is their large size and therefore slow blood kinetics. It is just not feasible to use antibodies for imaging on the same day as tracer administration in a hospital routine. Instead, imaging must be performed several days after administration of the antibody[9]. The successful examples of diagnostic imaging are small molecules or peptides, which clear rapidly from circulation thus providing an excellent tumour to blood contrast.

Unfortunately, it has proven difficult to generate high affinity protein binders from these classes in general. Instead, protein scaffolding has allowed the generation of the Affibody molecule ABY-025, a small, chemically synthesized HER2 targeting agent with very high binding affinity for HER2. The tracer was shown to locate tumour metastases in patients as early as 2010[10]. It was found to be the first report on clinical imaging data obtained with a non-immunoglobulin-based scaffold protein. A larger study concluded that images can be generated within hours after administration, and that ABY-025 accurately quantifies whole-body HER2-receptor status in metastatic breast cancer, resulting in changed treatment in approximately 20% of the patients[8].

A recent subsequent study reported that imaging with ABY-025 could predict metabolic response in HER2-positive breast cancer and can be useful as an adjunct diagnostic tool as it appears to provide an estimate of the HER2 expression required to induce metabolic remission by targeted therapies[11]. A number of solid tumours express low levels of HER2 and treatment benefit has been documented with the highly potent HER2 specific ADC-drug Enhertu even in hard-to-treat patients[12]. In a study presented at the ESMO congress in 2023, the ability of ABY-025 to also detect HER2 low metastatic lesions was reported, which potentially broadens the scope of ABY-025 to detect treatable HER2-expression in patients with different types of solid tumours[13]. The ABY-025 molecule is now being transformed into a therapeutic agent to create a theranostic pair for treatment of HER2-expressing cancers. Preclinical work suggests therapeutic potential and the agent is currently in IND-enabling development[14].

Formatting for IL-17 driven diseases

Auto-inflammatory activity is a major driver of diseases such as rheumatoid arthritis and psoriasis. The cytokine IL-17 is an important component acting on a variety of cells. Monoclonal antibodies that block IL-17 such as Cosentyx, Taltz and Bimzelx have proven efficacious in several indications including psoriasis, psoriatic arthritis, ankylosing spondylitis and non-radiographic spondyloarthritis. The results are encouraging, but arguably there is still room to increase the therapeutic efficacy with more potent drugs. IL-17 is presented in several variants, the best known being IL-17 AA homodimer, IL-17AF heterodimer or IL-17FF homodimer.

Whereas Cosentyx blocks IL-17A, Bimzelx blocks both IL-17A and IL-17F to inhibit the cytokine pathway more completely. It has been argued that the desired mechanism of action may be blocking IL-17A only, and that blocking the IL-17F component may result in an increased incidence of fungal infections, as IL-17 signaling on epithelial cells is important for physiologic regulation of mucosal immunity and barrier defences[15].

The strategy for a better tailored drug should therefore incorporate better affinity, more complete blocking, absolute affinity for IL-17 A and not F and ideally also be able to dose higher than current drugs using SC administration do. For an antibody, it is sterically difficult to distinguish between the IL-17 AA homodimer over the IL-17AF heterodimer. Improving the monovalent affinity to be better than the 2 pM of Taltz is also challenging, it is already a very good affinity.

The Affibody scaffold approach has however proven that it is possible to overcome these limitations[6, 16]. Our lead drug candidate izokibep consists of two IL-17A binding Affibody molecules connected via a linker to provide simultaneous cooperative binding only for IL-17 AA homodimers. The affinity was 10,000-fold higher for IL-17AA versus IL-17AF and was numerically very good, KD 0.3 pM. It blocked IL-17AA efficiently as shown in cell and animal models.

In the linker connecting the two IL-17A binding Affibody molecules, an albumin binding domain was inserted, resulting in long plasma exposure by binding to endogenous plasma albumin and accumulation at sites of inflammation and very good skin exposure. In an early clinical study in psoriasis patients, therapeutic effect could be seen already at a low single 2 mg dose. The therapeutic efficacy increased with higher dose and repeated administrations, up to seven biweekly doses of 40 mg where all patients at week 12 had an absolute PASI of less than 1.5[16]. The results were confirmed and expanded in a Phase 2 randomised placebo controlled multicenter study. 109 patients with moderate to severe plaque psoriasis were randomised to receive either placebo, 2, 20, 80 or 160 mg every two weeks for 12 weeks when the primary endpoint was assessed with up to 71% of patients achieving PASI90. Izokibep dosing was continued at the higher dose groups and maintained the benign safety profile and high efficacy for up to three years[6].

Recent results from a Phase 2 trial in psoriatic arthritis further support the efficacy and safety of izokibep in IL-17 mediated disease. Especially, the high efficacy results on enthesitis, which is inflammation in a difficult to reach site where the tendon inserts to the bone, raise the confidence that izokibep by virtue of smaller size and albumin association, may have an advantage in this disease as compared to monoclonal antibodies. Izokibep is now in Phase 3 clinical development in several indications.

Conclusion

The clinical results that are currently underway in various clinical fields suggest that protein scaffolds have an important role to play and fill a current void in precision medicine. These small protein drugs combine the advantages of monoclonal antibodies with molecular properties that make them easier to manufacture, to concentrate to higher doses, to format fit for target modulation and to reach hard-to-treat tissues that are too poorly vascularised for

antibodies to penetrate efficiently. Based on the clinical development results published so far, there is great potential that they will provide an important tool to overcome the limitations of antibody-based drugs. The Affibody scaffold, a small and versatile protein, offers attractive platform solutions for targeted cancer precision imaging, therapy, and selective cytokine blockade in autoimmune diseases.

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