Monoclonal antibodies are currently the most successful class of therapeutic agents. However, the conventional immunoglobulin (Ig) format is not always optimally suited to meet clinical demands. First, immunological effector functions mediated by the Fc region can evoke undesired side effects. Second, poor tissue penetration due to the large molecular size hampers successful treatment of solid tumors. Also, the long circulation in blood resulting from both the large size and FcRn-mediated endosomal recycling is unfavorable both for therapies that require flexible adjustment of dosing and for in vivo imaging applications. Finally, due to their complex biomolecular architecture, including four polypeptide chains with around 1500 amino acids and at least two glycosylation sites, the production of full size antibodies is costly and requires mammalian expression systems.

As a consequence, during the last two decades more than 50 alternative types of binding proteins have been proposed with the intention to overcome some of the inherent limitations of antibodies. However, only a minority of these ‘alternative scaffolds’ have reached the clinic so far, which can be seen as the ultimate success in pharmaceutical biotechnology. According to recent reviews, ten drug candidates based on seven different protein scaffolds have been tested in clinical trials while one biological received market approval (the engineered Kunitz domain/protease inhibitor ecallantide) [1,2]. At present, the biopharmaceutical development is dominated by the following protein scaffolds:

- Affibodies based on the Z-domain of Staphylococcal protein A [3];
- Adnectins (previously called monobodies) derived from the 10th extracellular domain of human fibronectin III (Fn3) [4];
- Anticalins, obtained by engineering the natural binding site of the cup-shaped human lipocalins [5];
- Avimers, based on a multimerized engineered LDLR-A module [6];
- DARPinS, designed ankyrin repeat proteins based on a consensus scaffold [7];
- Fynomers, engineered from the SH3-domain of the human Fyn tyrosine kinase and applied as fusion protein with a monoclonal antibody [8].

Neglecting business aspects such as access to capital, competitive landscape or intellectual property, the chance of a biopharmaceutical to reach the clinic or, eventually, the market is primarily dependent on the so-called developability of the molecule [9]. To this end, three major factors need to be considered: manufacturing, safety, and pharmacology (including biological activity). All three together ultimately determine the overall chance of success to develop a biological drug. In principle, for most of these parameters the non-Ig scaffolds should offer advantages if compared with classical antibodies and, therefore, present a worthwhile alternative.

Engineered protein scaffolds are generally easier and more cost-efficient to manufacture.
in prokaryotic hosts for several reasons: usually, they contain no or only few disulfide bridges and they lack complex posttranslational modifications (PTMs) such as glycosylation. In addition, they are significantly smaller than antibodies and monomeric, which does not only considerably facilitate downstream processing and quality control but, in principle, might even allow full chemical synthesis. Furthermore, when choosing a microbial expression system such as Escherichia coli or yeast, the threat of viral contamination can largely be ignored during processing as no animal cells are involved; on the other hand, endotoxin removal must be carefully monitored in case of a bacterial host cell.

One potential caveat of engineered scaffolds is the lack of platform downstream processes since an established affinity matrix like, for example, immobilized protein A, which simplifies the first capture step during antibody purification, is often not available. Initial capture might also be complicated when intracellular expression is performed (in E. coli) and the protein of interest must be separated from abundant host proteins and other cell constituents. In some rare cases, such as for Affibody molecules, their stability may be utilized for enrichment under denaturing conditions (e.g., elevated temperature, extreme pH) that precipitate or degrade most of the host cell proteins. Alternatively, bacterial [10] and yeast expression systems that allow highly efficient secretion are available today, offering a preferred route for some of the non-Ig protein scaffolds.

Physical stability of the biological can be improved by protein engineering, which is particularly advanced for some types of protein scaffolds, whereas in the case of antibodies the current rules for ‘humanization’ allow only moderate alterations to their framework and constant regions. PTM, which might give rise to a wide range of isoforms that have to be separated in a tedious downstream process, can either be eliminated by site-directed mutagenesis of the non-Ig scaffold, may simply be omitted through choice of a prokaryotic expression system or is absent from the beginning. Most of the alternative scaffolds do not require PTM for activity; therefore, product homogeneity can be generally achieved easier. The absence of unwanted glycosylation can also reduce the risk of hypersensitivity in patients or suppress the tendency to form aggregates.

The second parameter for developability, safety, is primarily related to immunogenicity and target specificity. With just few exceptions, for example, Anticalins [5], most of the engineered scaffolds are not of human origin and, thus, unknown to the body. This can quickly lead to neutralizing antibodies or, in the worst case, cause allergic reactions. However, as clinical data so far have proven, the level of immunogenicity even for the non-endogenous protein scaffolds is usually low, most likely due to their small size, compact structure as well as low aggregation propensity. Additionally, critical T-cell epitopes can be removed by protein engineering. Off-target interactions have not been reported so far, but corresponding side effects could limit the therapeutic window with regard to applied dose and frequency of administration.

The third crucial aspect of developability concerns pharmacology, in particular bioavailability and pharmacokinetics. While the small size of most alternative binding proteins is generally considered a benefit with regard to high specific activity and good tissue penetration, the resulting fast excretion via kidney filtration usually also constitutes a disadvantage. Nevertheless, today there are several options available to increase plasma half-life either by chemically or genetically conjugating a larger molecule (polymer) or by implementing FcRn-mediated endosomal recycling via fusion with Fc or albumin.

For the site-specific chemical conjugation of PEG, radioactive labels or chemical toxins, the presence of a free Cys residue is helpful and can easily be engineered into most of the non-Ig scaffolds. While PEGylation leads to retarded renal filtration via a molecular size effect and has become the gold standard for plasma half-life extension during the last decades, alternative technologies were recently proposed; PASylation, for example, utilizes a genetically encoded polypeptide made of the natural amino acids Pro, Ala and/or Ser and provides the same biophysical properties as PEG but, in contrast, allows cheaper manufacturing and is biodegradable [11].

In principle, non-Ig scaffolds also offer additional modes of action compared with antibodies. This is partly related to their differently shaped binding interface, which can address target epitopes not easily accessible by conventional Igs. In the context of the most advanced scaffolds mentioned further above, one can distinguish between binding via flat surfaces, cavities or protruding loops, for example, to reach sterically hidden clefts or to tightly embrace small hapten compounds [12]. Furthermore, due to their small size, scaffolds are ideal components for the design of fusion proteins, where two unrelated proteins are genetically joined to combine the functionalities of both parent proteins in a single polypeptide chain. Therefore, bispecific fusion proteins enable identical biodistribution for both protein components and simplify manufacturing [13]. Finally, the small size and robust nature of alternative binding proteins opens novel routes to administration, for example, via transdermal delivery or inhalation.
In conclusion, the growing number of clinical programs clearly indicates that non-Ig protein scaffolds have emerged beyond the stage of academic research. However, it also seems that this area of discovery approaches consolidation since the majority of ≥50 scaffolds that were proposed in the scientific literature have never delivered more than an initial example. For the more promising members of this class on the route toward novel biopharmaceutical drugs major tasks have been mastered, including the challenges arising during bioprocess development. Forthcoming data both from advanced clinical studies, and also from the laboratory design of innovative multifunctional formats, will show soon whether engineered protein scaffolds really live up to expectations.

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