Rational optimization of proteins as drugs: a new era of 'medicinal biology'

'...biopharmaceuticals are no longer limited to simply mimicking the normal physiological role of a protein...'

David E. Szymkowski, Director of Biotherapeutics, Xencor

In a recent issue of *Drug Discovery Today*, Brown and Superti-Furga [1] explored new strategies to 'rediscover the sweet spot' of drug discovery by challenging the current small-molecule high-throughput screening (HTS) paradigm. Many companies have recently embraced a chemical proteomics approach to identifying high-value drug targets that uses biologically active small molecules as probes for important disease pathways. Although chemical proteomics and genomics methods can be technically challenging, an emphasis on finding 'targets for drugs' rather than on the HTS orthodoxy of 'drugs for targets' has the potential to reduce attrition rates in the industry and fill pipelines with superior small molecules.

Chemical intractability of therapeutically relevant drug target classes

As highlighted by Brown and Superti-Furga [1], chemistry is the limiting factor in identifying promising leads for many classes of drug target. In particular, new discovery biology technologies have generated many novel, clinically relevant targets for which it might be impossible to develop small-molecule leads. For example, all cytokines signal through binding of a protein ligand to a cell-surface receptor; these important targets are considered chemically intractable because of the extensive protein–protein interface that must either be disrupted (to inhibit activity) or mimicked (to stimulate activity) by a small-molecule drug. In contrast to such limitations of medicinal chemistry, Brown and Superti-Furga note that 'the only bright spot (in drug discovery) has been the successful introduction of a range of biological products that bypass the need for small-molecule lead discovery'. Protein drugs, which by definition eliminate the risks associated with a small-molecule lead discovery and medicinal chemistry effort, are therefore better positioned to exploit genomic and proteomic tools than are traditional small-molecule approaches.

Biopharmaceuticals positioned to capitalize on 'omics' technologies

Because genomics, transcriptomics and proteomics technologies identify drug targets and not specific small-molecules, biopharmaceutical drug discovery is uniquely suited to exploit the output of these powerful new tools. Take as an example a soluble protein that is associated with a particular disease and that has been validated in appropriate cell and animal models. An orthodox drug discovery effort would begin with the screening of a small-molecule compound library, followed by extensive 'hit to lead' medicinal chemistry. However, even with convincing biological validation, a chemistry effort on a novel target would not guarantee production of suitable leads for clinical development. There are two lower-risk options to develop a protein-based drug using the same target information. On the one hand, if activity of the target protein alleviates the disease phenotype, then the target itself could be developed into a drug (e.g. erythropoietin, interferons, insulin, Factor VIII and various other growth factors and cytokines). On the other hand, if target activity accentuates the disease phenotype, then a neutralizing antibody or soluble receptor approach is the logical development strategy. Indeed, many successful biopharmaceuticals target disease-related proteins such as tumor necrosis factor (TNF) [Enbrel® (Wyeth; http://www.wyeth.com), Remicade® (Centocor; http://www.centocor.com) and HUMIRA™ (Abbott; http://www.abbott.com)], vascular endothelial growth factor [Avastin™ (Genentech; http://www.gene.com)] and Her2–neu [Herceptin® (Genentech)].

Therapeutic proteins can be developed even when the biology of a target is not well understood. Development of monoclonal antibody (mAb) drugs is one example where correlative biological data (e.g. a proteomics analysis that identifies tumor-specific markers) can be as valuable as causative data (e.g. demonstrating that target activity mediates a disease). This advantage over small-molecule drugs

1359-6446/04/$ – see front matter PII: S1359-6446(03)02957-X

is the result of the unique ability of mAb, through effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), to destroy cells that express the target protein. An excellent example is Rituxan® (Genentech), which is currently the leading antibody therapy with ~US$1 billion in sales for the first half of 2003. Rituxan® binds to CD20 and is thus highly effective against $CD20^+$ B-cell lymphomas; the clinical efficacy of the drug is now known to be partially mediated through ADCC of $CD20^+$ tumor cells [2,3]. Thus, regardless of whether or not discovery biology suggests that a drug should agonize or antagonize the activity of a novel target protein, the path of a biopharmaceutical compound to lead identification rarely requires a comprehensive understanding of the role of the target in disease.

When advancing into preclinical and clinical development, biopharmaceuticals have additional advantages over small-molecule drugs. For example, protein drugs are often more selective and safer to use than small molecules and can be developed with smaller, shorter and cheaper clinical trials [4]. As a consequence, biopharmaceuticals represent a steadily growing percentage of new drugs that have been approved as the number of new small molecules approved has decreased; proteins represented 12.5% of 40 FDA approvals in 1999 and 34.6% of only 26 approvals in 2002 [5–7]. According to the FDA (http://www.fda.gov/cder/biologics/biologics_table.htm), there are ~44 protein drugs approved in the US, although not all are novel therapeutics (e.g. the list includes interferons from different manufacturers and pegylated versions of existing drugs). This wealth of new protein drugs resulted in a strong 28% annual growth over the past five years, which translates into a global biopharmaceuticals market of ~US$32.4 billion in 2002 [7].

Developing better biopharmaceuticals: 'natural products' versus rational protein engineering

Analogous to small-molecule drugs, biopharmaceuticals have advanced through three stages of development, but on a shorter timescale. First-generation small-molecule drugs were isolated and used in their natural form (e.g. as unmodified compounds isolated from plant extracts). Next, improved drugs were created through relatively simple changes to the active natural parent; the synthesis of aspirin (acetylsalicylic acid) from the natural product salicylic acid is perhaps the earliest example of this approach. Most recently, the coupling of sophisticated medicinal chemistry with a greater understanding of target biology has led to the development of highly selective small-molecule inhibitors with improved therapeutic activities (e.g. selective cyclooxygenase 2 inhibitors were developed from an understanding of the molecular targets of aspirin). Analogously, for the first generation of biopharmaceuticals, many unmodified natural proteins were developed into drugs. Insulin, erythropoietin, interferons, granulocyte-colony-stimulating factor (G-CSF) and Factor VIII are just a few examples of human proteins that are now produced recombinantly as drugs with effectively unaltered protein sequences. Through simple modifications, second-generation versions of several of these protein drugs have been produced. For example, a hyperglycosylated version of erythropoietin [Aranesp® (Amgen; http://www.amgen.com)] and pegylated versions of interferon-α (IFN-α) [Pegasys® (Roche; http://www.roche.com)] and G-CSF (Neupogen®; Amgen) that have improved pharmacokinetics over the unmodified protein are now on the market. However, such modifications can also result in a dramatic loss in drug potency, as seen with pegylated IFN-α [8].

Unfortunately, native proteins that have evolved for optimized activity *in vivo* are often not optimized with regard to drug properties. Therefore, biopharmaceuticals are now entering the third stage of product development in which true lead optimization to modulate the physical, chemical and biological properties of proteins is possible. Rather than simply mimicking the physiological role of a native protein, or blocking that role with an antibody, future protein drugs will be engineered to fit specific clinical criteria, including reduced immunogenicity, altered substrate specificity and differential activity against multiple natural receptors. New tools are being developed to rationally engineer proteins and transform a suboptimal native protein into a successful drug that has improved recombinant expression, solubility, stability, pharmacokinetics, safety, selectivity and potency. To engineer native proteins into improved drugs, companies such as Applied Molecular Evolution (http://www.amevolution.com), Maxygen (http://www.maxygen.com), and Xencor (http://www.xencor.com) have taken diverse approaches that include combinatorial mutagenesis, gene shuffling and computational structure-based protein design. New ways to engineer, produce and screen protein libraries, particularly when library design is based on an understanding of protein structure and function, have made true lead optimization of biopharmaceuticals possible. In the same way that medicinal chemistry uses synthetic modifications to explore a structure–activity relationship for a set of small molecules, protein drugs can now be improved through rational 'medicinal biology', either by optimizing the target protein itself or an antagonizing antibody [9]. Probably the most sophisticated biopharmaceutical design effort to date resulted in the 2003 launch of the selective growth hormone receptor (GHR) antagonist Somavert® (Pfizer; http://www.pfizer.com) [10]. Somavert®

was engineered with eight amino acid differences from the natural growth hormone agonist to create a GHR-selective antagonist that neither activates nor antagonizes the prolactin receptor, thereby giving rise to a better safety profile [11]. Another example of the use of structure-based medicinal biology to generate a protein with a new therapeutic mechanism of action is an engineered version of human TNF that sequesters subunits of the pro-inflammatory native cytokine into inactive heterotrimeric complexes [12]. Such examples demonstrate that biopharmaceuticals are no longer limited to simply mimicking the normal physiological role of a protein – improved drugs with novel therapeutic activities can now be created through rational protein design.

Summary – the future for biopharmaceuticals lies in medicinal biology

Although still in its infancy compared to small-molecule medicinal chemistry, biopharmaceutical medicinal biology shows great potential to repopulate the drug development pipelines of the pharmaceutical industry. Successful biopharmaceutical companies are beginning to surpass the sales of small-molecule companies. For example, in 2002, Amgen reported sales of US$5 billion, which exceeds those of established pharmas such as Bayer (http://www.bayer.com) and Schering AG (http://www.schering.de). Even Roche, which has been in the small-molecule business for over 100 years, now has eight proteins among its top 15 products, which account for ~60% of its US$5 billion sales in the first half of 2003 (http://www.roche.com/home/investor/inv-finance/inv-reports/inv-reports-2003-half-year.htm). Despite limitations, for example, a lack of oral bioavailability and potential immunogenicity [13,14], protein drugs have become successful contributors to the pharmaceutical armamentarium, and can be as efficacious (and profitable) as small-molecule drugs. Our nascent ability to rationally design native proteins into better drugs opens up the same opportunities for biopharmaceuticals that medicinal chemistry did for small-molecule pharmaceuticals. Just as most companies today optimize small-molecule natural products and HTS hits through extensive medicinal chemistry, biopharmaceutical companies can now also optimize the native forms of enzymes, cytokines or antibodies through structure-based medicinal biology to generate superior protein drugs.

References

1 Brown, D. and Superti-Furga, G. (2003) Rediscovering the sweet spot in drug discovery. *Drug Discov. Today* 8, 1067–1077

2 Cartron, G. *et al.* (2002) Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood* 99, 754–758

3 Weng, W-K. and Levy, R. (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J. Clin. Oncol.* 21, 3940–3947

4 Reichert, J.M. (2001) Clinical development of therapeutic medicines: a biopharmaceutical versus pharmaceutical product comparison. *Drug Inf. J.* 35, 337–346

5 Drews, J. (2003) Strategic trends in the drug industry. *Drug Discov. Today* 8, 411–420

6 Reichert, J.M. (2003) Trends in development and approval times for new therapeutics in the United States. *Nat. Rev. Drug Discov.* 2, 695–702

7 Bibby, K. *et al.* (2003) Biopharmaceuticals – moving to centre stage. *Biopeople North American Biotechnology Industry and Supplier's Guide* 3–11

8 Bailon, P. *et al.* (2001) Rational design of a potent, long-lasting form of interferon: a 40 kDa branched polyethylene glycol-conjugated interferon alpha-2a for the treatment of hepatitis C. *Bioconjugate Chem.* 12, 195–202

9 Marshall, S.A. *et al.* (2003) Rational design and engineering of therapeutic proteins. *Drug Discov. Today* 8, 212–221

10 Thorner, M.O. *et al.* (1999) Growth hormone (GH) receptor blockade with a PEG-modified GH (B2036-PEG) lowers serum insulin-like growth factor-I but does not acutely stimulate serum GH. *J. Clin. Endocrinol. Metab.* 84, 2098–2103

11 Goffin, V. *et al.* (1999) The human growth hormone antagonist B2036 does not interact with the prolactin receptor. *Endocrinology* 140, 3853–3856

12 Steed, P.M. *et al.* (2003) Inactivation of TNF signaling by rationally designed dominant-negative TNF variants. *Science* 301, 1895–1898

13 Koren, E. *et al.* (2002) Immune responses to therapeutic proteins in humans – clinical significance, assessment and prediction. *Curr. Pharm. Biotechnol.* 3, 349–360

14 Chirino, A.J. *et al.* (2004) Minimizing the immunogenicity of protein therapeutics: from preclinical screening to rational design. *Drug Discov. Today* 9, 82–98

David E. Szymkowski
Xencor
111 West Lemon Avenue
Monrovia
CA 91016, USA
e-mail: david.szymkowski@xencor.com