Therapeutic Fc-fusion proteins and peptides as successful alternatives to antibodies

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Therapeutic antibodies have captured substantial attention due to the relatively high rate at which these products reach marketing approval, and the subsequent commercial success they frequently achieve. In the 2000s, a total of 20 antibodies (18 full-length IgG and 2 Fab) were approved by the Food and Drug Administration (FDA) or European Medicines Agency (EMA). In the 2010s to date, an additional three antibodies (denosumab, belimumab, ipilimumab) have been approved and one antibody-drug conjugate (brentuximab vedotin) is undergoing regulatory review and may be approved in the US by August 30, 2011. However, a less heralded group of antibody-based therapeutics comprising proteins or peptides fused with an Fc is following the success of classical antibodies.

A total of six Fc-fusion products have been approved, and one (aflibercept) is undergoing regulatory review in the US, European Union and Japan, with approval in the US possible by August 20, 2011 (Table 1). These immunoglobulin-derived products are thus more plentiful on the market than antigen-binding fragments (three approved products; abciximab, ranibizumab, certolizumab pegol), radioimmunoconjugates (two approved products; tositumomab-1131, ibritumomab tiuxetan), bispecific antibodies (one approved product; catumaxomab) and antibody-drug conjugates (gemtuzumab ozogamicin, which was approved in the US in 2000 but withdrawn from the market in 2010). Importantly, 2010 global sales for etanercept, the most commercially successful Fc-fusion protein at USD $7.3 billion, were superior to those of the most successful IgG, such as bevacizumab ($6.9 billion), rituximab ($6.8 billion) or infliximab ($6.5 billion).

The approval and commercial success of the Fc-fusion products indicates that the Fc part of the immunoglobulin molecule is as important as the antigen-binding region.

Examination of the therapeutic antibodies and derivatives that are approved (or in review as of July 2011) indicates that there are numerous options to modulate protein targets. Tumor necrosis factor (TNF) can be efficiently complexed by full-length antibodies (infliximab, adalimumab, golimumab), pegylated Fab (certolizumab pegol) or Fc-fusion protein (etanercept). This is also true for vascular endothelial growth factor A (VEGFA), which is modulated by three marketed products, the full-length humanized antibody (bevacizumab), the affinity-matured Fab derived from bevacizumab (ranibizumab) and the Fc-fusion protein “VEGF-Trap” (aflibercept). A third example is IL1, which can be sequestered by a fusion protein (rilonacept), a human antibody (canakinumab) or the IL-1 antagonist anakinra (recombinant human IL-1 receptor antagonist that must be administered daily because of its rapid clearance in vivo). Among the other targets successfully modulated by marketed Fc-fusion proteins or peptides are CD2 (aflacept), CD80/86 (abatacept, belatacept) and thrombopoietin receptor (romiplostim).

The primary reason for fusion of a binding moiety with Fc is half-life extension. Many biologically active proteins and peptides have very short serum half-lives due to fast renal clearance, which limits their exposure in the target tissue and, consequently, their pharmacological effects. The Fc domain prolongs the serum half-life of antibodies and Fc-fusion proteins due to pH-dependent binding to the neonatal Fc receptor (FcRn), which salvages the protein from being degraded in endosomes. As an additional benefit, the Fc portion of Fc-fusion proteins allows easier expression and protein A-affinity purification, which confers practical advantages in the development of antibody and Fc-fusion therapeutics.

As a variation on the theme, Centocor is developing the MIMETIBODY™ platform to extend the half-life of different peptides while retaining pharmacological activities similar to their parent peptides. In an initial proof of the concept, CNTO 528, a 20 amino acid erythropoietin (EPO) mimetic peptide identified from phage libraries, is being investigated. CNTO 528 can bind and activate the Epo receptor in vitro and in vivo, and was shown to be well-tolerated in a Phase 1 clinical study.

With regard to the biological activity of the Fc portion, improved knowledge of Fc receptors present on immune cells allows tailored engagement of associated effector functions, such as antibody-dependent cell-mediated cytoxicity (ADCC), complement-dependent cytotoxicity (CDC) or phagocytosis, by modulation of the binding affinities to Fc receptors through mutations or glyco-engineering. ADCC and CDC are important effector functions, especially for anti-cancer IgG1 antibodies that are designed to selectively destroy tumor cells. The presence of a bisecting N-acetylgalactosamine associated with depletion in fucose residues (e.g., by genetic knockdown of alpha-1,6-fucosyltransferase) from oligosaccharides in the conserved attachment region to Fc receptors result in an increase of ADCC up to 100 fold in vitro. ADCC was also shown to be enhanced for non-fucosylated IgG4 through improved FcyRIII binding, as well as for Fc-fusion proteins...
manufacturing of HD203, an etanercept biosimilar, which is soon to be studied in a Phase 3 trial (NCT01270997) in Korea that will evaluate the equivalence of efficacy and safety of HD203 compared to etanercept in patients on a treatment regimen for rheumatoid arthritis.

Advances in science and technology, including protein engineering and design, cell line development and bioprocessing, have provided access to an unprecedented array of new types of antibody-based therapeutics such as the Fc-fusion proteins. The commercial success of the first generation molecules has fueled interest and the dedication of resources, to the development of second- and third-generation versions. Physicians and patients look forward to the potential benefits these products may bring.

Because first generation antibody (rituximab, infliximab, trastuzumab, cetuximab) and the Fc-fusion protein (etanercept) blockbusters will lose patent protection soon, these products have become targets for biosimilar companies. This fact is illustrated by the recent $720 million licensing deal between Merck Bioventures and South Korea-based Hanwha Chemical for a biosimilar product, as well as by the announcements from Sandoz and many other companies detailing their biosimilar development plans. In its deal with Hanwha, Merck has agreed to take on development and manufacturing of HD203, an etanercept biosimilar, which is soon to be studied in a Phase 3 trial (NCT01270997) in Korea that will evaluate the equivalence of efficacy and safety of HD203 compared to etanercept in patients on a treatment regimen for rheumatoid arthritis.

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Table 1. Marketed Fc-fusion proteins

| International non-proprietary (trade) name | Description | Mode of action | Year/indication of first US approval |
|-------------------------------------------|-------------|---------------|------------------------------------|
| Etanercept (Enbrel)’ | 75 kDa soluble extracellular domain (ECD) of tumor necrosis factor (TNF) receptor II fused to human IgG1 Fc | Binds membrane-bound and soluble forms of TNF, thereby reducing concentrations of inflammatory cytokines | 1998/rheumatoid arthritis |
| Alefacept (Amevive)’ | First ECD of lymphocyte function-associated antigen 3 (LFA-3) fused to human IgG1 Fc | Binds CD2; blocks the interactions between LFA on APCs with CD2 on T cells, thereby inhibiting T-cell activation | 2003/plaque psoriasis |
| Abatacept (Orencia)’ | ECD of human cytotoxic T lymphocyte associated molecule-4 (CTLA-4) fused to human IgG1 Fc | Blocks the interactions between CD80 or CD86 on APCs and CD28 on T cells, thereby inhibiting T-cell activation | 2005/rheumatoid arthritis |
| Rilonacept (Arcalyt)’ | Two chains, each comprising the C-terminus of the IL-1R accessory protein ligand binding region fused to the N-terminus of the IL-1RI ECD, fused to human IgG1 Fc | Binds IL-1, thereby preventing interaction with endogenous cell-surface receptors | 2008/plaque psoriasis |
| Romiplostim (Nplate)’ | Peptide thrombopoietin (TPO) mimetic fused to the C-terminus of aglycosylated human IgG1 Fc; produced in E. Coli | Binds and agonizes the TPO receptor; Fc functionality minimized due to lack of glycosylation | 2008/thrombocytopenia |
| Belatacept (Nulojix)’ | ECD of CTLA-4 fused to human IgG1 Fc; differs from abatacept by two amino acid substitutions (L104E, A29Y) in the CTLA-4 region | Blocks the interactions between CD80 or CD86 on APCs and CD28 on T cells, thereby inhibiting T-cell activation | 2011/prophylaxis of organ rejection in adult kidney transplant recipients |
| Aflibercept (Eylea™) | ECDs of VEGF receptors 1 and 2 fused to human IgG1 Fc | Binds all forms of VEGF-A, as well as placental growth factor, thereby inhibiting angiogenesis | PDUFA date August 20, 2011/ Undergoing review as a treatment for wet age-related macular degeneration as of July 2011 |