Introduction to biotech drugs

**Abstract**

This article provides an overview of drugs produced by biotechnology, often called biotech drugs. When these drugs were first submitted to the regulatory authorities in the 1980s, it was recognised at an early stage that new approaches were needed for their development programmes. Challenges specific to biotech drugs include their potential for immunogenicity, the need for novel bioanalytical assays and the requirement to use animal models in preclinical studies in which the drug is pharmacologically active. These issues, among others, present regulatory challenges that are unique to large molecules.

**Definition and manufacturing**

Biotech is the term used for biotechnology or products produced by biotechnology. These drugs are also called biologicals, biotech drugs, biological drugs or biopharmaceuticals. True biotech products are manufactured in live biological systems known as expression systems. They are generated using recombinant DNA technology, and the expression cells are amplified in bacterial or mammalian cell culture. Ultimately the desired product is produced in large scale fermentation vessels. The end stage fermentation product, a heterogeneous mixture, is then purified in a series of processing steps to remove extraneous host cell and other contaminants to produce the drug product. Some of the newest production systems now being used are transgenic animals (eg, goats, cattle) to serve as bioreactors.1

During the manufacturing process, many immunoassays and bioassays must be developed. These are quantitative assays specifically designed for each step in quality control, product characterisation, stability testing, comparability analysis, in-process controls, and the potency or functional activity of the drug candidate.2 The latter, measured by a potency or bioassay, is required by regulatory authorities to establish the biological activity of each drug lot, and must be finalised prior to marketing approval. Thus, the manufacturing process and its accompanying immunoassays and bioassays are unique to drug and sponsor and essentially define the marketed drug. This is the crux of the debate over biosimilars (ie, generic biopharmaceuticals): copies of biotech drugs can be produced but structurally complex biological drugs won’t be identical copies, at least with existing technology. Attempts to produce generic versions of biotech products are therefore referred to as biosimilars in an attempt to recognise the structural differences that may exist between the original and biosimilar product.

**Physical characteristics**

Biopharmaceuticals are physically very different from small molecule drugs. The latter are generally sized at 1 kDa or less and the former are generally greater than 30 kDa.3 We call them high molecular weight drugs as opposed to low molecular weight drugs (ie, small molecule or traditional drugs). Examples of biotech products are monofunctional antibodies (ie, one target), bifunctional antibodies (ie, two targets), antibody fragments, peptides, fusion proteins, plasma derived proteins, growth factors, cytokines, various ligands and receptors, vaccines, nucleic acids, gene therapies, cell therapies, and engineered or modified live (or dead) tissues. Note that this is not an exhaustive list. There are unknown types and numbers of biotech products that failed at some stage of development. For reasons of confidentiality we will only know about these drugs if they are brought back into development.

**Route of administration**

The route of administration of a biotech drug is very different from a traditional drug taken as a pill or capsule, and each drug is developed with a unique route of administration. These drugs are mainly given intravenously, subcutaneously or intramuscularly. There are also biotech drugs given to patients by intrathecal, intraarticular, intravitreal and inhalation routes. They cannot be given orally because they would be degraded in the gastrointestinal tract. However, the delivery of biotech drugs orally is an active area of research.

Most biotech drugs are given in the clinic but for some chronic indications the trend is to develop subcutaneous versions so that they can be self-administered at home with an auto injector device. For example, many biotech drugs indicated for rheumatoid arthritis (RA) now have auto injectors. Rebif (interferon beta-1a), a biotech drug used to treat multiple sclerosis, is also available with an auto injector device.

**Immunogenicity**

A unique characteristic of biotech drugs is that the immunogenicity of the drug is always evaluated with immunoassays both in animal toxicity studies and in clinical trials. Although biotech drugs are not always immunogenic, we tend to assume they will be because the human immune system is designed to generate antibodies to foreign or non-self biological molecules, particularly foreign proteins. We usually look for non-specific antibodies (those that bind to any part of the drug) and neutralising antibodies to the drug which bind to the ‘business end’ (ie, the

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part that binds to its target). Neutralising antibodies are more important to us because they can diminish or negate the effect of the drug and alter its pharmacokinetics (eg, increase clearance) and pharmacodynamics. Neutralising antibodies can diminish or eliminate the drug’s therapeutic effect. Self-generated antibodies to a biotech drug have been known to react with the endogenous version of the protein and have serious clinical consequences (eg, Epogen (epoetin alpha)). In a preclinical animal toxicity study, neutralising antibodies can obviate any toxicities and complicate interpretation of the study results. In animal studies we can sometimes ‘dose through’ the antibody effect, a phenomenon that also occurs in humans. This means antibodies are generated and then diminish with time and continued dosing. For some marketed drugs, there is an inverse relationship between dose and antibody generation to the drug. Note that non-neutralising and neutralising antibodies can be generated to a biotech drug with no clinical consequences (for example see the prescribing information for Intron A (interferon alpha-2b)). Also, although animals may generate antibodies to biotech drugs in preclinical experiments, this result cannot be automatically extrapolated to humans.

Immunosassays must be developed by the sponsor to detect antibodies to the drug candidate in the serum of animals and humans. A screening assay is usually first developed to determine if antibodies are present and, if positive, additional assays are done for confirmation of antibody presence and to determine if they are neutralising.

Species specificity
Another term that is frequently bandied about in the biotech drug world is ‘species specificity’. This means that you can’t take your drug candidate and inject it into a rodent or nonrodent species in your toxicology studies and assume this is going to be acceptable to the regulatory agencies. In the 1980s when the interferons were being developed, companies assumed they could use a small molecule drug development programme for any new biotech drug. They injected rodents with recombinant interferons and nothing happened, not because they were not toxic, but because they have little or no pharmacological activity in rodents. The receptors either don’t exist or are different enough from the human receptors to cause poor binding of the drug to its putative receptor. To evaluate the toxicity of biologics, the drug candidate must be shown to have pharmacological activity in the animal model chosen for preclinical studies. This has necessitated using primate models for many biopharmaceuticals, because biotech drug receptors are not necessarily found in lower species.

But what if the drug candidate only binds to the human or chimpanzee receptor? These types of drugs are more challenging to develop. Some biotech drugs have been tested in chimpanzees but the data obtained are limited to pharmacokinetic and clinical chemistry, because necropsies are no longer permitted. In fact, experimentation with chimpanzees is now banned in many regions, notably the EU.

One approach for these drug programmes is to develop the analogous molecule in a lower species. An anti-TNF mouse version of Remicade (infliximab) (eg, cV1c) was developed and used for repeat dose and reproductive toxicology studies in mice. Another example is Raptiva (efalizumab), for which a surrogate antibody that binds CD11a in mice was developed (muM17) and used in repeat dose toxicology, reproductive toxicology, and toxicokinetic preclinical studies.

The ICH (International Conference on Harmonisation) guidance (ICH S6) for preclinical development of biopharmaceuticals states that safety evaluation should normally include two relevant species (ie, two species in which the drug is pharmacologically active). However, when only one relevant species can be identified (or when the biological activity of the biopharmaceutical is well understood) it is acceptable to do toxicity testing in only one species. Note that the regulatory authorities will require studies demonstrating degree of binding of the drug to its receptor or epitope in various species to support the choice of species for toxicity testing.

Preclinical studies
Another difference between small molecule drugs and large molecule drugs is that the traditional toxicological studies designed for chemicals do not always work well for biotech drugs. The two-year rodent bioassay or carcinogenicity study, a classic study used to evaluate carcinogenicity of chemicals, small molecule drugs, and food additives, is never required by regulators for biotech drugs. The reasoning is that protein drugs do not cross the nuclear membrane and interact with DNA. And, even if they did, there are technical problems with this study because many biologics are not pharmacologically active in rats and mice. Alternatively, if they do have pharmacological activity you may encounter immunogenicity issues. Also, there may be technical problems with dosing rodents with large molecules repeatedly for the lifetime of the animal. If a biotech drug in development has proliferation as a mechanism of action there are alternative approaches to glean information on the mitotic characteristics of the drug on normal cells and tumour cells such as cellular proliferation assays, short term carcinogenicity studies using transgenic animal models, assessment of the biopharmaceutical’s receptor on tumour cells, and xenograft studies (effects of drug on tumours implanted in mice). Sponsors have also looked for oncological diseases in chronic repeat dose toxicity studies.

Other studies not required by regulatory authorities are genotoxicity studies for similar reasons (no putative interaction of the drug with DNA). Also, most genotoxicity studies rely on cytotoxicity for validity; an effect that is not feasible for a biologic drug because of their large size. For many biotech drugs, genetic toxicology studies were done, especially for the early drugs and mostly results were negative, spurious, or false positives. A positive result for a biotech drug in a genotoxicity battery of tests may be due only to exaggerated pharmacological response—a common result of any toxicity study for a biopharmaceutical. An exception to this rule is if the drug has a free linker or is an immunoconjugate (eg, has a small molecule drug attached to it). Note that before the ICH S6 document was published, stating that genotoxicity tests were not required, many biotech drugs were subjected to the standard battery of tests. This is reflected in the labelling of many of the early drugs.

The toxicology studies developed for chemicals, drugs, and food additives have been modified for biologic drugs. Safety pharmacology is generally built into the repeat dose studies. Reproductive toxicology studies are done in cynomolgus monkeys if feasible and if the drug is pharmacologically active in this species (instead of the classic rat model). Single dose studies using highly exaggerated human doses
are often not feasible for large molecules and thus are not always done for biopharmaceuticals. Examples of marketed biotech drugs for which no single dose studies were submitted to FDA are Avastin (bevacizumab), Enbrel (etanercept), and Simulect (basiliximab).

A study that is unique to antibody drugs is the ‘tissue cross-reactivity’ study, also called an ‘immunohistochemistry’ study. Sponsors have to do tissue cross-reactivity studies for all antibody products submitted to regulatory authorities. These studies are de facto for antibody drugs but are also done for fusion protein drugs to study nontarget binding in tissues and to support the selection of species for toxicology studies. The US FDA antibody guidance document³ states that animal and human tissues should be fixed and stained after exposure with the antibody drug candidate. Staining appears at sites of antibody binding and can be very specific, nonexistent, or ubiquitous. The goal is to determine if the drug candidate will bind to other sites than its target. Off target binding is a safety concern.

**Pharmacokinetics**

The pharmacokinetics of biotech drugs are very different from small molecule drugs. Whereas the half-lives of small molecule drugs are minutes or hours, the half-lives of biologicals, antibody drugs for example, are days or weeks; others have short half-lives but prolonged pharmacological effects (eg, EpoGEN (epoetin alfa)). In the small molecule world, regulators look for safety problems around the time of C max in animals and in humans. But, for biotech products, we are more concerned about total exposure (AUC) instead of C max because these drugs will exhibit toxicities at any part of the time versus concentration curves. The absorption and distribution of biotech drugs can be more complicated than small molecule drugs, which is reflected in the pharmacokinetics. The physical structure of a biotech drug such as an antibody, helps define the pK profile. For example, antibody drugs that are engineered as fragments will have relatively short half-lives compared with intact antibody drugs.⁴

**Examples of biotech drugs and their targets**

Antibody drugs are the largest class of biotech drugs and oncology and autoimmune diseases are the most prevalent indications. These drugs have a range of highly selective targets. Herceptin (trastuzumab) targets the HER2 receptor on breast cancer tumours. Rituxan (rituximab) targets the CD20 ligand (antigen) on plasma cells (B cells) and kills the B cell population, both malignant and normal cells. It does not eradicate very immature (progenitor) B cells so this cell population regenerates after the end of the therapy cycle. Avastin (bevacizumab) is an antibody drug that binds to VEGF, blocking a protein needed for growth of blood vessels to tumours (angiogenesis). Remicade (infliximab) binds to tumour necrosis factor (TNF) and blocks an immunostimulatory pathway implicated in RA. TNF-α, and TNF-β, inducers of inflammatory cytokines, are examples of therapeutic targets for biotech drugs. Similarly, other cytokines such as interleukins are also targets for biotech drug development.

**Regulatory guidance for biotech drug development**

There are regulatory guidelines that are unique to biopharmaceuticals; most address manufacturing and preclinical studies. For preclinical development of biotech drugs, see the ICH S6 document. This was written by an expert committee of the International Conference for Harmonization by representatives of the US, EU, and Japan in the 1990s. A reassessment of this document began in 2008 by an ICH expert committee, and is expected to be finalised in a couple of years.
Biotech drugs have been under development for 25 years but this is still a new and evolving field. The first generation of drugs is being replaced by second and third generation drugs. The future of biotech drugs includes easier and more comfortable routes of administration for patients and caregivers, new and improved targets, and more sophisticated engineering.

**Conclusion**

Biotech drugs are now a popular forum. The briefing covered a broad range of topic areas involved and summarised the case law associated with each area. This allowed attendees to clearly understand the current thinking associated with these critical areas of development.

The Association of the British Pharmaceutical Industry (ABPI) held its latest in a series of breakfast briefings at its offices in Whitehall, London in November. The hour-long presentation, by Marjan Noor and David Nickless from international law firm Howrey LLP, considered whether EU law was keeping up with the development of biotech and biological products.

Biologics now account for an increasing share of most pharma companies’ product pipeline. However, to a greater extent than their chemical counterparts, they give rise to many unresolved legal difficulties. For example, how much utility must a patent disclose for an isolated gene sequence before the sequence is deserving of patent monopoly? Are biologics appropriately regulated with innovator data adequately protected and does regulation of biosimilars go far enough? On biotech inventions in the wider sense, where must the line be drawn on morality?

The briefing covered the critical areas of biologics development associated with legal difficulties including the role of patents in biologicals, the European regulation of biologicals (including data exclusivity issues and SmPC protection), the biosimilars approach and morality of biotech inventions.

### References