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Survey Report

Evaluation of genotoxicity testing of FDA approved large molecule therapeutics

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ABSTRACT

Large molecule therapeutics (MW > 1000 daltons) are not expected to enter the cell and thus have reduced potential to interact directly with DNA or related physiological processes. Genotoxicity studies are therefore not relevant and typically not required for large molecule therapeutic candidates. Regulatory guidance supports this approach; however there are examples of marketed large molecule therapeutics where sponsors have conducted genotoxicity studies. A retrospective analysis was performed on genotoxicity studies of United States FDA approved large molecule therapeutics since 1998 identified through the Drugs@FDA website. This information was used to provide a data-driven rationale for genotoxicity evaluations of large molecule therapeutics. Fifty-three of the 99 therapeutics identified were tested for genotoxic potential. None of the therapeutics tested showed a positive outcome in any study except the peptide glucagon (GlucaGen®) showing equivocal *in vitro* results, as stated in the product labeling. Scientific rationale and data from this review indicate that testing of a majority of large molecule modalities do not add value to risk assessment and support current regulatory guidance. Similarly, the data do not support testing of peptides containing only natural amino acids. Peptides containing non-natural amino acids and small molecules in conjugated products may need to be tested.

1. Introduction

Drug substances can be divided into two groups – small molecules and large molecules, with molecular weights typically less than 500 daltons and greater than 1000 daltons, respectively. Small molecule drugs can freely enter the cell and nucleus because of their small size and are therefore evaluated for their potential to cause DNA damage as recommended by the regulatory guidance (ICH S2(R1) (2011)). Small molecule drugs make up the majority of the therapeutic market today. However, due to the potential for high target specificity, fewer side effects, longer half-life, and reduced administration frequency, use of large molecule therapeutics is increasing, especially in the treatment of diseases, such as rheumatoid arthritis, multiple sclerosis, and cancer. Large molecule therapeutics, a class of protein- and peptide-based drugs, are manufactured mainly in genetically engineered cells such as microorganisms, and plant or animal cells. However, some large molecules, such as peptides, can be synthesized chemically and may contain non-natural amino acids or other chemical modifications.

Exposure of patients to therapeutics with potential to cause genetic damage is a public health concern as DNA damage can lead to adverse health consequences. Some mutations in somatic cells have been associated with cancer and other diseases, such as accelerated aging, cardiovascular and neurodegenerative diseases, and immune dysfunction (Altieri et al., 2008; Slatter and Gennery, 2010; Scott et al., 2012). Damage to germ cell DNA can cause infertility, spontaneous abortions, and heritable changes in subsequent generations (Aitken and De Iuliis, 2010). Therefore evaluation of novel pharmaceuticals for potential to induce genetic damage is an important component of preclinical safety studies.

Damage to DNA can manifest in various forms, such as point mutations, strand breaks, formation of adducts, and recombination events. As no single assay is able to detect all types of DNA damage, a test battery has been adopted to evaluate the genotoxic liability of pharmaceutical agents. Genetic toxicology testing is essential for safety evaluation of novel pharmaceuticals (ICH M3(R2), 2009) and the ICH S2(R1) guidance document outlines appropriate *in vitro* and *in vivo* tests. The guidance provides two options for the standard genetic toxicology battery that includes an assessment of mutagenicity in a bacterial reverse mutation assay and

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Table 1
ICH-compliant genetic toxicology test battery.

ICH S2(R1) options	Mutation	*In vitro* mammalian cell assay	*In vivo* test	
			First endpoint	Second endpoint
Option 1	•Bacterial reverse mutation test (471)	•Micronucleus test (487) or •Chromosome aberration test (473) or •Gene mutation test (476[a])	•Erythrocyte micronucleus test (474) or •Bone marrow chromosome aberration test (475)	•Not conducted
Option 2	•Bacterial reverse mutation test (471)	•Not conducted	•Erythrocyte micronucleus test (474)	•Comet assay or •Transgenic rodent somatic and germ cell gene mutation assays (488) or •DNA adduct assays or •Unscheduled DNA synthesis test with mammalian liver cells (486)

Titles of the OECD guidance (1997) documents (with the test number in parenthesis) are given for tests recommended in ICH S2(R1) options. The comet assay guideline was approved recently, but no OECD guideline is available for the DNA adducts endpoint.

[a] Multiple gene mutation assays (MLA, HPRT, gpt) from OECD 476 guideline are included in the ICH S2B guidance (1997); however, only mouse lymphoma assay is included in the ICH S2(R1) guidance document.

genotoxicity evaluation in mammalian cells using *in vitro* and/or *in vivo* tests (Table 1). Depending on the therapeutic indication, a positive outcome in any test can stop the drug development program and additional tests may be required to ensure safety of clinical trial participants.

Large molecule therapeutics are not expected to access the cytoplasm or nucleus like small molecule drugs (Torchilin, 2006) and therefore, genotoxicity studies are not applicable to large molecule therapeutics. The regulatory guidance on preclinical safety evaluation of biotechnology-derived pharmaceutical products (ICH S6, 1997) supports this approach. However, there are many examples of marketed large molecule therapeutics where sponsors have conducted tests for genotoxicity either following the standard ICH test battery or using *in vitro* assays. A survey of genetic toxicity studies conducted on large molecule therapeutic candidates collected from various sponsors was published in 1999 by Gocke et al. We conducted a retrospective evaluation of currently marketed large molecule therapeutics approved by US FDA since 1998 and the information was used to derive a data-driven rationale for the current regulatory recommendations and to identify considerations where genotoxicity assessment may be warranted for large molecule therapeutics.

2. Materials and methods

Novel large molecule therapeutics approved for human use by US FDA since 1998 were identified through Drugs@FDA website. The FDA database provides information on approved products regulated by FDA's Center for Drug Evaluation and Research including product labels and links to pharmacology, medical, chemistry, and summary reviews. However, no information is made available for drug candidates that were submitted for regulatory approval but were either not approved by the agency or the sponsor withdrew the application during the approval process. For the purpose of this review a large molecule therapeutic is defined as a protein- or peptide-based therapeutic administered parenterally and that interacts with a target present on or outside the cell. Based on the size and composition, large molecule therapeutics were divided into subgroups including monoclonal antibody (mAb), fusion protein, antibody drug conjugate (ADC), monoclonal antibody fragment, peptide with fewer than 40 amino acids, medium-sized protein with 40 to 200 amino acids, and large protein with more than 200 amino acids such as enzymes and toxins that are not included in any other groups. Gene therapy or antisense products are not covered in this review as they have different composition (DNA or RNA) and target molecules inside the cell.

The Drugs@FDA website was searched by 'Drug approval reports by month' from January 1998 to October 2013 for relevant large molecule therapeutics approved under a Biological License Application (BLA) or New Drug Application (NDA). Regulatory review documents provide summaries of submitted toxicology studies including reviews on genetic toxicology studies at various levels of detail. These documents were reviewed for information on genetic toxicology studies conducted.

3. Results

A retrospective evaluation was conducted to understand the scope of genetic toxicology studies conducted on US FDA approved large molecule therapeutics. A total of 99 novel large molecule therapeutics approved by US FDA for human use since 1998 were identified. Monoclonal antibodies, peptides, medium and large proteins made up 87% of the 99 approved therapeutics, whereas mAb fragments, ADCs, and fusion proteins made up the remaining 13% (Fig. 1).

Interestingly, more than half (53 of 99) of large molecule therapeutics approved have been tested in at least one genotoxicity assay (Table 2). Of the 53 compounds tested, 32 were evaluated in the standard ICH test battery, 12 were tested in *in vitro* assays only, eight were tested in one *in vitro* and one *in vivo* assay, and one was tested in one *in vivo* study. Number and types of genotoxicity studies conducted on these molecules include: 47 bacterial reverse mutation assays, 39 *in vitro* chromosome aberration assays, 19 mammalian cell gene mutation assays, four *ex vivo* rat liver UDS studies, three *in vivo* chromosome aberration tests, and 40 *in vivo* bone marrow micronucleus tests (Table 2). All *in vivo* studies were conducted by clinically relevant routes for the therapeutic molecules, except for oral administration of DM1, the cytotoxic agent in ado-trastuzumab emtansine, in a rodent micronucleus study. Not surprisingly, none of the large molecule therapeutics tested in the genotoxicity assays showed a positive outcome in any study, except glucagon, a 29 amino acid peptide with molecular weight of 3485 daltons. It was reported to be weakly positive in Ames and chromosome aberration assays. However, its mutagenicity in bacteria was attributed to a feeding effect (false positive due to increase in revertants from histidine and tryptophan released from the test protein) and the borderline increases in the *in vitro* chromosome aberration assay were not reproduced in a repeat