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CHMP SWP REFLECTION PAPER ON THE ASSESSMENT OF THE GENOTOXIC POTENTIAL OF ANTISENSE OLIGODEOXYNUCLEOTIDES

According to the ICH S6 Guideline "Preclinical Safety Evaluation of Biotechnology-Derived Pharamceuticals", the range and type of genotoxicity studies routinely conducted for pharmaceuticals are usually not applicable to biotechnology-derived pharamaceuticals. Such studies including newly developed systems should be performed only in those cases where there is cause for concern.

With regard to oligodeoxynucleotides at least two issues need to be assessed which may indicate a cause for concern, i.e. (a) degradation products of the phosphorothioate oligodeoxynucleotides being nucleotide analogues might lead to mispairing and thus induction of point mutation when integrated into newly synthesized DNA and (b) site-specific mutations might be induced by triplex formation of the oligodeoxynucleotides with the DNA fiber.

Incorporation of phosphorothioate nucleotides into DNA

In phosphorothioate oligodeoxynucleotides the internucleotide linkages have been modified relative to normal DNA by replacing a non-bridging oxygen in the phosphate linkage for a sulfur, which confers higher resistance to nuclease degradation. Although the major metabolic pathways eliminate nucleotides through catabolism, it is possible that phosporothioate mononucleotides may be substrates for various kinases, and thus be incorporated in the triphosphate nucleotide pool. As part of this pool, nucleotide thiotrisposphates could be incorporated into newly synthesized DNA with the consequence that binding to complementary nucleotides occurs with reduced fidelity thus leading to mispairing and eventually point mutations.

The potential of inducing mutations by incorporation of phosphorothioate nucleotides into DNA should be accessible with sufficient sensitivity to standard mutagenicity testing (as has been shown with other nucleotide analogues). Data from regulatory submissions are available on genotoxicity testing of a 21mer phosphorothioate oligodeoxynucleotide from a previous submission in a centralized procedure which include the following tests:

- Ames test
- Mouse lymphoma assay
- Chromosome aberration test with CHO cells
- Mouse bone marrow mircronucleus test (i.v. appl.)

All tests were negative. The important question of cellular uptake of the polyanionic and water soluble phosphorothioate oligonucleotide was also addressed in these studies. Sufficient direct and indirect evidence for entering of the oligonucleotides into target cells was provided including dose-related cytotoxicity in mammalian cell tests, oligonucleotide antibody staining in CHO cells, and receptor mediated endocytosis in mammalian cells. Thus, the negative results were considered to be valid findings indicating that phosphorothioate nucleotides (as potential degradation products/metabolites from phosphorothioate oligodeoxynucleotides) are unlikely to pose a genotoxic hazard.

In view of these data further studies to assess the mutagenic potential of phosphorothioate nucleotides from other phosphorothioate oligodeoxynucleotides are deemed not necessary.

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DNA triplex formation

Triple helices can be formed when oligonucleotides bind sequence-specifically to the duplex DNA fiber. As a consequence of triple helix formation induction of mutations were observed in mammalian cells treated with oligonucleotides (Wang et al., Science 271, 1996, 802-805). An oligonucleotide consisting of 30 nucleotides generated mutations in the target gene at a frequency of 0.27%, 13 times above the spontaneous background in the assay. Shorter oligonucleotides (20 and 10 nucleotides) were less effective in producing mutations. As a possible mechanism it is proposed that the triple helix blocks transcription at the site, triggering gratuitous and potentially error-prone repair. With regard to the therapeutic use of antisense oligonucleotide the possibility of site-directed mutagenesis, i.e. induction of mutation in that gene coding for the mRNA target of the antisense product, should be taken into consideration.

Testing for site-directed mutagenesis is not accessible to standard mutagenicity assays since the models used are based on specific response genes, either for reverse mutations (bacterial strains used in Ames test) or forward mutations (HPRT or TK mammalian cell models). An appropriate approach for the detection of induced mutations in a specific gene is the investigation of restriction fragment length polymorphism. Other PCR-based techniques may be also useful.

Summary and recommendations

Available data from standard genotoxicity testing with a phosporothioate oligonucleotide were found to be negative and thus provide sufficient evidence that phosphorothioate nucleotides (as potential degradation products/metabolites from the phosphorothioate oligodeoxynucleotides) are unlikely to pose a genotoxic hazard via incorporation into newly synthesized DNA. However, another cause for concern refers to binding of oligonucleotides to the DNA fiber which may lead to triplex formation and eventually induction of site-specific mutations (Wang et al 1997). The possibility of formation of triple helices of the antisense product with the DNA fiber and the potentially resulting consequences should be discussed. If an experimental approach for testing of this possibility is deemed necessary the application of standard mutagenicity tests is considered inappropriate. Instead, techniques such as the restriction fragment length polymorphism assay could be used for detecting gene-specific mutagenesis.

