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Oligonucleotide-based pharmaceuticals: Non-clinical and clinical safety signals and non-clinical testing strategies

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ABSTRACT

Antisense oligonucleotides, short interfering RNAs (siRNAs) and aptamers are oligonucleotide-based pharmaceuticals with a promising role in targeted therapies. Currently, five oligonucleotide-based pharmaceuticals have achieved marketing authorization in Europe or USA and many more are undergoing clinical testing. However, several safety concerns have been raised in non-clinical and clinical studies. Oligonucleotides share properties with both chemical and biological pharmaceuticals and therefore they pose challenges also from the regulatory point of view. We have analyzed the safety data of oligonucleotides and evaluated the applicability of current non-clinical toxicological guidelines for assessing the safety of oligonucleotide-based pharmaceuticals. Oligonucleotide-based pharmaceuticals display a similar toxicological profile, exerting adverse effects on liver and kidney, evoking hematological alterations, as well as causing immunostimulation and prolonging the coagulation time. It is possible to extrapolate some of these effects from non-clinical studies to humans. However, evaluation strategies for genotoxicity testing of "non-natural" oligonucleotides should be revised. Additionally, the selective use of surrogates and prediction of clinical endpoints for non-clinically observed immunostimulation is complicated by its multiple potential manifestations, demanding improvements in the testing strategies. Utilizing more relevant and mechanistic-based approaches and taking better account of species differences, could possibly improve the prediction of relevant immunological/proinflammatory effects in humans.

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1. Introduction

The term oligonucleotide-based pharmaceuticals refers to antisense oligonucleotides, short interfering RNAs (siRNAs) and aptamers (Cavagnaro et al., 2014). Antisense oligonucleotides are short single-stranded oligonucleotides that typically consist of 13–25 nucleotides, which bind to their complementary mRNA by Watson-Crick base pairing (Fattal and Bochot, 2006). Their binding to mRNA prevents mRNA translation, which subsequently inhibits protein synthesis. Although the prevention of the translation of mRNA can occur through multiple different mechanisms, there are three common routes exploited by therapeutic antisense oligonucleotides: i) mRNA degradation by activating RNase H, ii) translation arrest by forming a steric block to prevent the binding of ribosomes and iii) splicing modulation by exon skipping (Fattal and Bochot, 2006; Bennett and Swayze, 2010; Evers et al., 2015). Exon skipping differs from the other two mechanisms, because it leads to restored protein synthesis (Sierakowska et al., 1996; Van Deutekom et al., 2001).

siRNAs are double-stranded RNAs, that usually consist of around 20 nucleotides (Corey, 2007). Similar to the situation with antisense oligonucleotides, one strand of siRNA binds to its complementary mRNA by Watson-Crick base pairing, while the other strand is

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CHMP, Committee for Medicinal Products for Human Use; CYP, cytochrome P450; EMA, European Medicines Agency; EPAR, European public assessment report; FDA, U.S. Food and Drug Administration; G-CSF, granulocyte colony-stimulating factor; GI, gastrointestinal; IL-6, interleukin-6; IP-10, interferon gamma-inducible protein 10; KC, chemokine (C-X-C motif) ligand 1; MAA, marketing authorization application; MCP-1, monocyte chemotactic protein-1; mRNA, messenger RNA; PEG, polyethylene glycol; PK, pharmacokinetic; PT, prothrombin time; RBC, red blood cell; RISC, RNA-induced silencing complex; RNA, ribonucleic acid; sc, subcutaneous; siRNA, short interfering RNA; SPC, summary of product characteristics; TLR, Toll-like receptor; t1/2, half-life.

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degraded. siRNAs utilize a RNA interference mechanism, which promotes cleavage of the target mRNA by an endogenous enzyme called Argonaut 2 (Elbashir et al., 2001; Meister et al., 2004). siRNA is assembled into a RNA-induced silencing complex (RISC), which is a multiprotein complex, containing Argonaut 2 as well as other proteins (Filipowicz, 2005). One strand of siRNA is degraded, while the other strand stays in RISC and binds to its complementary mRNA, while Argonaut 2 cleaves the target mRNA (Stanton and Colletti, 2010).

Aptamers are single-stranded oligonucleotides that usually consist of 15–45 nucleotides (Bouchard et al., 2010). Aptamers differ from antisense oligonucleotides and siRNAs, because they do not affect the translation of mRNA (Thiel and Giangrande, 2009). Instead, aptamers fold up into specific 3D structures and bind specifically to their target proteins with high affinity (Bouchard et al., 2010). Subsequently, the formed aptamer-protein complex inhibits the protein's biological function (Thiel and Giangrande, 2009).

Natural phosphodiester oligonucleotides are unable to resist metabolism by nucleases; hence, it is challenging to utilize them as pharmaceuticals as such (Bennett and Swayze, 2010). Therefore, chemical modifications have been created to improve the biological stability of oligonucleotides (Juliano et al., 2012). Several similar chemical modifications have been introduced into antisense oligonucleotides, siRNAs and aptamers (Behlke, 2008; Keefe and Cload, 2008; Juliano et al., 2012). The most frequently utilized chemical modifications in oligonucleotides include phosphorothioate backbone modifications, 2′-alkyl derivatization and the synthesis of 2′-fluoro derivatives (Behlke, 2008; Keefe and Cload, 2008; Bennett and Swayze, 2010).

Oligonucleotide-based pharmaceuticals are thought to possess a promising role in targeted therapies, especially in the treatment of genetic diseases, for which there are no or at best, very few treatment options available. The development of oligonucleotide-based pharmaceuticals has progressed rapidly and several have now entered the clinical development phase (www.clinicaltrials.com). In the EU, six pharmaceuticals have gone through the marketing authorization phase (Vitravene SPC, 2002; Macugen EPAR, 2007; Genasense EPAR, 2007; Kynamro EPAR, 2013; Kyndrisa EPAR, 2016; Spinraza EPAR, 2017). However, only three have achieved the marketing authorization (Vitravene SPC, 2002; Macugen EPAR, 2007; Spinraza EPAR, 2017). In the USA, five oligonucleotide-based pharmaceuticals have been granted marketing authorization (Vitravene Pharmacology and Toxicology Review, 1998; Macugen EPAR, 2007; Kynamro EPAR, 2013; Spinraza EPAR, 2017).

Oligonucleotides share properties with both chemical and biological pharmaceuticals (Cavagnaro et al., 2014). For example, they are reminiscent of chemical pharmaceuticals, in the sense that they are manufactured by chemical synthesis resulting in a single entity structure. Conversely, they possess properties resembling the characteristics of biological pharmaceuticals e.g. selected tissue distribution and species specificity. This creates challenges from the regulatory point of view: should non-clinical testing follow the guidelines for chemical or biological pharmaceuticals?

In this paper, we have collected and analyzed the safety data of oligonucleotides, compared their adverse effects in animals and humans. In addition, we have evaluated the applicability of current non-clinical toxicological guidelines for assessing the safety of oligonucleotide-based pharmaceuticals and the potential need for updating the regulatory guidance.

2. Materials and methods

Materials used in this research include the public assessment reports issued by U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), assessment reports by EMA and published literature. Public assessment reports are available at http://www.fda.gov or at http://www.ema.europa.eu for the following products Exondys 51, Genasense, Kynamro, Kyndrisa, Macugen, Spinraza and Vitravene. Published literature searches were conducted using combinations of the following search terms: antisense oligonucleotide, aptamer, oligonucleotide, siRNA, and absorption, delivery, distribution, elimination, excretion, half-life, mechanism of action, metabolism, pharmacokinetic, plasma protein, protein binding, toxicokinetics, and clinical trial, drug, nonclinical, preclinical, therapy, and carcinogenicity, genotoxicity, hepatic, immune system, immunostimulation, kidney, liver, proinflammatory, renal, and adverse effect, safety, toxicity. Databases used in searches included Pubmed, Web of Science, Scopus and Toxline/Toxnet. Most of the searches were conducted between 1.12.2015 and 15.2.2016 with additional searches from 15.9 to 20.10.2016.

The data related to the assessment reports have been anonymized to ensure that confidential information is not being disclosed and that individual products cannot be identified. Work was conducted by analyzing information and parameters from the materials. Findings are presented descriptively and only at the group level as relative proportions of studies conducted with oligonucleotide-based pharmaceuticals at the marketing authorization stage. The number of individual studies with detailed data was restricted; therefore, no statistical analysis was conducted.

3. Results

3.1. Pharmacokinetics

Oligonucleotides are typically administered either via the parenteral routes to achieve systemic effects or locally to the site of action (Vitravene Pharmacology and Toxicology Review, 1998; Genasense EPAR, 2007; Macugen EPAR, 2007; Kynamro EPAR, 2013; Kyndrisa EPAR, 2016; Spinraza Pharmacology and Medical Reviews, 2016). Compared to the literature on antisense oligonucleotides, the amount of pharmacokinetic data related to siRNAs and aptamers is more limited. Antisense oligonucleotides, siRNAs and aptamers exhibit similar pharmacokinetic properties in terms of tissue distribution, metabolism and excretion, but differences in pharmacokinetic parameters also exist (Table 1).

Antisense oligonucleotides are rapidly distributed from plasma to tissues, this is followed by a long elimination phase (Geary et al., 2001; Yu et al., 2004; Genasense EPAR, 2007; Kynamro EPAR, 2013; Kyndrisa EPAR, 2016). First and second generation antisense oligonucleotides are highly bound to plasma proteins (77–99%) (Geary et al., 2001; Yu et al., 2004; Genasense EPAR, 2007; Kynamro EPAR, 2013; Kynamro Pharmacology Review, 2013; Kyndrisa EPAR, 2016). Whereas third generation morpholino oligonucleotides bind less to plasma proteins (<25%). In contrast, virtually no information has been published about the plasma protein binding characteristics of siRNAs and aptamers. Bioavailability after subcutaneous administration of antisense oligonucleotides and aptamers has ranged from 68 to 100% (Genasense EPAR, 2007; Macugen EPAR, 2007; Kynamro EPAR, 2013). The highest tissue concentrations of antisense oligonucleotides, siRNAs and aptamers have been detected in the kidney, liver and spleen (McMahon et al., 2002; Healy et al., 2004; Genasense EPAR, 2007; Macugen EPAR, 2007; Lendvai et al., 2008; Thompson et al., 2012; Kynamro EPAR, 2013; Christensen et al., 2014; Huang et al., 2016; Kyndrisa EPAR, 2016). In addition, antisense oligonucleotides and aptamers have been detected at high concentrations in lymph nodes and bone marrow (Yu et al., 2004; Macugen EPAR, 2007; Lendvai et al., 2008; Kynamro EPAR, 2013; Kyndrisa EPAR, 2016).

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