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Safety profile of RNAi nanomedicines[☆]

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ABSTRACT

The emerging class of RNA interference (RNAi) therapeutics is a fundamentally novel approach to treating human disease by enabling the pursuit of molecular targets considered "undruggable" by small molecules and traditional protein therapeutics. A key challenge toward realizing the full potential of this technology is the safe and efficient delivery of siRNA to target tissues. The physical chemical properties of siRNAs preclude passive diffusion across most cell membranes. For systemic administration, novel delivery systems are required to confer "drug-like" pharmacokinetic and pharmacodynamic properties. Engineered nanomaterials and the emerging field of nanomedicine are important drivers of turning the promise of RNAi therapeutics into reality. The current clinical progress of systemically administered siRNA therapeutics is reviewed, with special attention to the toxicity profiles associated with RNAi nanomedicines. As a case study, the preclinical development of ALN-VSP, the first lipid nanoparticle (LNP)-formulated siRNA therapeutic to be tested in cancer patients, is reviewed to broadly highlight some of the preclinical safety challenges and areas of investigation for "next generation" LNP systems.

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Contents

1. Introduction . . . 1730
2. Clinical progress of siRNA nanomedicines . . . 1731
   2.1. SNALP-based delivery . . . 1731
      2.1.1. Hepatocellular targeting . . . 1731
      2.1.2. Tumor tissue targeting . . . 1731
   2.2. RONDEL™ and AtuPlex™ delivery . . . 1732
   2.3. Summary . . . 1732
3. Case study: ALN-VSP preclinical development — a retrospective analysis . . . 1733
   3.1. Background . . . 1733
   3.2. Considerations in safety assessment of LNPs . . . 1733
      3.2.1. Toxicokinetics and distribution . . . 1733
      3.2.2. Size and surface characteristics at physiologic pH . . . 1733
      3.2.3. Toxicology species selection and dosing regimen . . . 1733
      3.2.4. Oligonucleotide "class-based" effects . . . 1733
   3.3. Summary of key safety studies for the ALN-VSP IND application . . . 1733
      3.3.1. Rat toxicology studies . . . 1733
      3.3.2. Non-human primate toxicology studies . . . 1734
      3.3.3. Preclinical summary and impact on phase I trial design . . . 1735
   3.4. Translation of preclinical safety findings to humans . . . 1735
4. Insights for "next generation" nanomedicines . . . 1736
References . . . 1736

1. Introduction

The emerging class of RNA interference (RNAi) therapeutics is a fundamentally novel approach to treating human disease by enabling

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the pursuit of molecular targets considered “undruggable” by small molecules and traditional protein therapeutics. RNAi is a natural cellular mechanism for regulating gene expression. Briefly, long double stranded ribonucleic acid (dsRNA) molecules are processed to small interfering RNA (siRNA, typically staggered duplexes 19–23 bp in length with 2-nucleotide overhangs at the 3′ ends) via the cytoplasmic enzyme Dicer. The siRNA is then bound to the RNA-induced silencing complex (RISC) such that the sense (“passenger”) strand is removed and the antisense (“guide”) strand is retained in the complex. The RISC complex, with guide strand bound, is then able to bind its complementary mRNA and enable cleavage of the mRNA by the endonuclease argonaute-2 (hAgo2) within RISC, ultimately leading to degradation of the target mRNA and reduction in protein expression. The endogenous process of RNAi can be leveraged as an experimental or therapeutic tool by the cytosolic delivery of synthetic siRNAs or expression of short hairpin RNAs (shRNA) with viral vectors. For comprehensive reviews, readers are directed to [1–5].

A key challenge toward realizing the full potential of this technology is the safe and efficient delivery of siRNA to target tissues. The physical–chemical properties of siRNA – namely size (~13 kDa), polyanionic charge, and hydrophilicity – all preclude passive diffusion across most cell membranes. In addition, intravenous injection of naked unmodified siRNA results in rapid renal clearance, degradation by RNAses and potential stimulation of an immune response via recognition by Toll-like receptors (TLRs) [6]. For systemic administration, novel siRNA delivery systems are required to confer “drug-like” properties such as increased circulation time, distribution to target tissues, and effective cytoplasmic delivery to RISC. Engineered nanomaterials and the emerging field of nanomedicine are key drivers of turning the promise of RNAi therapeutics into reality.

While a vast array of engineered nanomaterials are currently being investigated as potential therapeutic tools, only a limited subset of RNAi nanomedicines is currently in human clinical trials. For current reviews of nanomaterials, nanomedicine and nanotoxicology, readers are directed to reviews elsewhere in this issue or [7–10]. Here, the current clinical progress of systemically administered siRNA therapeutics is reviewed, with special attention to the toxicity profile associated with RNAi nanomedicines. In particular, the preclinical development of ALN-VSP, the first lipid nanoparticle (LNP)-formulated siRNA therapeutic to be tested in cancer patients, will be used as a case study example to broadly highlight some of the preclinical safety data related to bringing these novel drug products to human trials. This experience with ALN-VSP also highlights important areas of investigation for “next generation” LNP systems.

2. Clinical progress of siRNA nanomedicines

2.1. SNALP-based delivery

Formulation of siRNA in lipid nanoparticles is one of the most widely used strategies for in vivo systemic delivery to target tissues. The stable nucleic acid lipid particle (SNALP) is comprised of four different lipids — an ionizable lipid (DLinDMA) that is cationic at low pH, a neutral helper lipid, cholesterol, and a diffusible polyethylene glycol (PEG)-lipid. The particle is approximately 80 nm in diameter and is charge-neutral at physiologic pH. During formulation, the ionizable lipid serves to condense lipid with the anionic siRNA during particle formation [11]. When positively charged under increasingly acidic endosomal conditions, the ionizable lipid also mediates the fusion of SNALP with the endosomal membrane enabling release of siRNA into the cytoplasm [12]. The PEG-lipid stabilizes the particle and reduces aggregation during formulation, and subsequently provides a neutral hydrophilic exterior that improves pharmacokinetic properties.

Biodistribution studies in rodent and non-human primate models have shown that SNALP predominantly distributes to the liver and spleen following IV administration, likely due both to a) the fenestrated endothelium in those organs that normally filters macromolecules up to 100 nm in size, and b) the mononuclear phagocytic system (MPS) which actively removes microbes and particles (both self and non-self) from the circulation. As described by Akinc et al. [12], the uptake of SNALP into hepatocytes appears to be dependent on binding of the particle to endogenous apolipoprotein E (ApoE), which in turn promotes binding and endocytic internalization via the low-density lipoprotein receptor (LDLR) and perhaps other LDLR family members expressed on the surface of hepatocytes. Interestingly, this ApoE-dependent mechanism does not appear to be required for LNPs that contain a cationic lipid (permanently charged at physiologic pH). Proof of concept studies of pharmacologic mechanism (target mRNA inhibition, protein knockdown, and efficacy) have been previously described in several rodent and non-human primate models with SNALPs [13–15].

2.1.1. Hepatocellular targeting

To date, two clinical programs have been initiated using SNALP-siRNA formulations (Table 1). Tekmira Pharmaceuticals recently completed a phase I single-dose study of SNALP-ApoB in adult volunteers with elevated LDL cholesterol. ApoB is predominantly expressed in the liver and jejunum and is essential for the assembly and secretion of VLDL and LDL [14]. Seventeen subjects received a single dose of SNALP-ApoB (dose escalation across 7 dose levels). There was no evidence of liver toxicity (anticipated as the potential dose-limiting toxicity based on preclinical studies). One (of two) subjects at the highest dose experienced flu-like symptoms consistent with immune system stimulation, and the decision was made to conclude the trial [16].

Alnylam Pharmaceuticals has similarly advanced ALN-TTR01, which employs the SNALP technology described above and targets hepatocyte production of both mutant and wild-type TTR to treat TTR amyloidosis (ATTR). Three ATTR syndromes have been described: familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC) — both caused by autosomal dominant mutations in TTR; and senile systemic amyloidosis (SSA) cause by wild-type TTR [17]. A placebo-controlled, single dose-escalation phase I trial of ALN-TTR01 was recently completed in patients with ATTR. ALN-TTR01 was administered as a 15-minute IV infusion to 31 patients (23 with study drug and 8 with placebo) within a dose range of 0.01 to 1.0 mg/kg (based on siRNA). Treatment was well tolerated with no significant increases in liver function tests. Infusion-related reactions were noted in 3 of 23 patients at ≥0.4 mg/kg; all responded to slowing of the infusion rate and all continued on study. Minimal and transient elevations of serum cytokines IL-6, IP-10 and IL-1ra were noted in two patients at the highest dose of 1 mg/kg (as anticipated from preclinical and NHP studies). Lowering of serum TTR, the expected pharmacodynamic effect of ALN-TTR01, was observed at 1 mg/kg [18].

2.1.2. Tumor tissue targeting

Lipid nanoparticles, cyclodextrin-based nanoparticles, and lipoplex-siRNA have also successfully advanced into clinical development for delivery of siRNA to tumors (Table 1). Similar to hepatocellular targeting via fenestrated endothelium, delivery to tumor tissue is also in part predicated on the morphologically and functionally abnormal (“leaky”) vasculature and dysfunctional lymphatic drainage common to many solid tumors [7,19]. This so called enhanced permeability and retention (EPR) effect is a passive delivery strategy best exemplified by traditional liposomal oncology agents like Doxil® (liposomal doxorubicin). The surface pegylation of the liposome (“stealth” liposome) decreases plasma clearance and uptake by phagocytic cells and essentially increases local tumor tissue residence time, thereby increasing local exposure to doxorubicin cargo. It should be noted that while the EPR effect is an effective strategy for the local delivery of lipophilic small molecule drugs, simply increasing residence time in the extracellular space of tumor tissue may not be sufficient for cytoplasmic delivery of siRNA macromolecules;

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