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Review

Nanomedicinal delivery approaches for therapeutic siRNA

Michael Keller*

Novartis Pharma AG, Technical R&D, CH-4057 Basel, Switzerland

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ABSTRACT

RNA interference (RNAi) has been named as "breakthrough technology" in 2002 by Science magazine. In a short timespan this technology has conquered life sciences and numerous therapeutic approaches and is now well underway to become an important pilier of novel class of RNA based therapeutics. This mini-review focuses on nanomedicinal delivery approaches for siRNA that have shown promise with small molecules, and have recently been applied with the aim to deliver siRNA to humans for treatment of disease.

RNA interference (RNAi) has become the darling of life science research and pharmaceutical development soon after reports of gene interference by nucleotides in plants surfaced (Napoli et al., 1990; Vanderkrol et al., 1990). The identification of a catalytic mechanism behind RNA interference based on work carried out on *Caenorhabditis elegans* (Fire et al., 1998), the discovery of short antisense RNAs complementary to the targeted mRNA (Hamilton and Baulcombe, 1999), and the description of synthetic duplexes of 21-nucleotide RNAs that mediate gene silencing in cultured mammalian cells (Elbashir et al., 2001a,b) subsequently triggered a biotechnology revolution of immense dimensions.

RNAi has since become a routine tool for researchers working on target validation, gene function/pathway analysis (Cherry, 2008; Muller-Hartmann et al., 2007) and RNA based therapeutics (De Souza et al., 2009; Wagner, 2008). Its importance to life sciences is underlined with the award of the Nobel price in Physiology in 2006 to Andrew Fire and Greg Mello for their discovery of RNA interference—gene silencing by double-stranded RNA.

From a therapeutic perspective, encouraging preclinical and clinical developments are reported regularly. RNA *per se* is labile due to its molecular structure and its biological role as a transient mediator of information. As a result, RNA drug delivery systems (DDS) have taken an important place from the start of therapeutics development projects in order to provide protection against degradation and to increase intracellular uptake.

In particular, liposomal siRNA delivery approaches have been at the forefront of successful *in vivo* studies. Liposomal nanoparticles (LNs) – also dubbed liposomal nanomedicines – have diameters centered ~100 nm (Fenske and Cullis, 2008). With small hydrophobic drugs they have recently demonstrated great promise to offer new treatments in cancer therapy, vaccine development and cholesterol management. Numerous liposomal drug formulations are in preclinical development: vincristine, vinorelbine and topotecan, for instance, are investigated for cancer treatment and are based on sphingosomal formulations (1), while diaminocyclohexane form of platinum (2) or a temperature sensitive doxorubicin LN (3) are also investigated for cancer treatment. A number of LNs have received approval for commercialization and include amphothericin B (*e.g.* AmBisome®, Abelcet®) to treat systemic fungal infections, or doxorubicin (*e.g.* Doxil®, Myocet®) and daunorubicin (DaunoXome®) to treat cancer. Disappointingly, one such liposomal nanomedicine approach aiming at increasing the effect of cis-platin in inoperable head and neck cancer patients showed promise in the preclinical phase but failed to demonstrate efficacy in a PI/II clinical trial (Harrington et al., 2000, 2001).

As a novel technology, siRNA has first progressed into clinics using naked siRNA. This limits the application range to mainly direct injection (*e.g.* intravitreal) or topical administration. One leading siRNA company, Sirna-Merck, has developed Sirna-027, a chemically optimized, short interfering RNA (siRNA) currently moving into Phase II development for the treatment of the wet-form of age related macular degeneration (AMD) as part of a broad collaboration with Allergan, Inc. in the area of ophthalmic diseases (4). Another leading siRNA company, Alnylam, has developed ALN-

* Tel.: +41 61 696 7051; fax: +41 61 696 70 56.
E-mail address: michael-1.keller@novartis.com.

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RSV01, for the treatment of RSV infection targeting the nucleocapsid "N" gene of the RSV genome, a gene that is required for the replication of the RSV, and has shown promise in a Phase II GEMINI study in adults experimentally infected with RSV (5). Other companies and academic institution also pursue clinical programs: Opko Health is pursuing a clinical study to compare the safety and effectiveness of bevasiranib given either every 8 weeks or every 12 weeks after an initial pre-treatment with three injections of Lucentis® (ranibizumab) compared to Lucentis® given every 4 weeks to people with wet AMD. Quark pharmaceuticals have two siRNA drugs, RTP801i-14 (AMD) and I5NP (acute kidney injury) in clinical phases. I5NP is a siRNA that is systemically administered and was licensed from Silence Therapeutics that also developed the AtuPlex technology, a liposomal siRNA DDS with increased systemic delivery to the endothelium, liver and tumors (Aleku et al., 2008). Other clinical trials are recruiting and can be followed in reference as the above mentioned (6).

Interestingly, siRNA nanomedicines have already progressed towards clinical trials. Historically, DOTAP mediated delivery of siRNA to mice can be found in 2003, already emphasizing the observation of sequence related immune stimulation as adverse effects that need serious attention (Sioud and Sorensen, 2003). Such putative immune stimulatory motifs (*e.g.* 5′-UGUGU-3′) were later identified (Judge et al., 2005); abrogation of immune stimulation was then demonstrated by using chemically modified siRNAs (Judge and MacLachlan, 2008; Judge et al., 2006; Morrissey et al., 2005). Important milestones using sub-100 nm liposomal nanomedicines were reported with the stable nucleic acid lipid particles (SNALP) technology on non-human primates. ApoB-specific siRNAs were encapsulated in SNALPs and administered intravenously to cynomolgus monkeys at doses of 1 or 2.5 mg/kg. A single siRNA injection resulted in dose-dependent silencing of APOB messenger RNA expression in the liver 48 h after administration, with maximal silencing of >90% (Zimmermann et al., 2006). This technology has meanwhile been filed for Investigational New Drug (IND) with the United States Food and Drug Administration (FDA) seeking approval to begin a human clinical trials which would be the first systemically delivered siRNA to go into clinical trials (5).

In summary, RNA interference – having already revolutionized life sciences in only a few years since its discovery – is now well underway to become an important novel class of therapeutics that might as well transform the pipelines of big pharmaceutical companies from small molecular drugs and antibodies towards RNA based drugs.

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