

# Are RNAi and miRNA therapeutics truly dead?

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**Only a few years ago pharmaceutical companies were excited about the potential of RNA interference (RNAi). Now, financial volatility and subsequent dissolutions of in-house facilities by pharmaceutical companies have had media channels pronouncing that RNAi therapeutics are dead. However, advances in nanomedicine may now help the vast potential of RNAi therapeutics to be fulfilled.**

## 'Long live the RNAi'

The discovery of RNAi captivated scientists worldwide. This powerful RNA-based mechanism by which cells can carry out sequence-specific post-transcriptional gene silencing has great potential for therapeutic application in diseased cells. Although RNAi works well *in vitro*, it has proved to be somewhat challenging to utilize *in vivo*. The hydrophilicity and large size of siRNAs prohibit them from crossing the cell membrane in the absence of a transfection agent. In fact, naked siRNAs exhibit extremely short half-lives (i.e., minutes), susceptibility to RNases, poor chemical stability, and can induce dissociation of plasmid–DNA complexes. Some siRNA sequences may also induce immunogenicity by nonspecific activation of the immune system through the Toll-like receptor (TLR) pathway. However, chemical modifications to the siRNA backbone, such as introducing 2'-O-methyl or 2'-fluoro into the sugar moiety of selected nucleotides in the sense and antisense strands may allow evasion of the innate immune system, thus protecting siRNA from degradation. These chemical modifications can effectively abrogate TLR interaction while generally preserving silencing activity even if the modifications are extensive [1]. In addition, delivering siRNAs selectively into diseased cells while sparing normal cells has been challenging. All these obstacles have resulted in limited enthusiasm from pharmaceutical companies for investing in RNAi therapeutics.

RNAi garnered a lot of attention when Andrew Fire and Craig Mello won the Nobel Prize in Medicine for their pivotal discovery of double-stranded RNA as the trigger of RNAi in 2006 [2]. This inspired pharmaceutical companies such as Merck, Roche, Pfizer, Novartis, Alnylam Pharmaceuticals, and Takeda to focus on RNAi therapeutics and to

acquire siRNA therapeutic technologies for more than 1 billion US dollars. However, by the end of 2010 this momentum has reversed, followed by the termination of strong partnerships. Merck announced that it was shutting down the San Francisco research laboratory it had acquired as part of its billion US dollar acquisitions. Likewise, Roche and Novartis announced their decisions to abandon the RNAi field. In fact, Novartis announced the shutdown of all its RNAi programs by the end of 2014. The majority of pharmaceutical involvement in RNAi programs already halted in 2010, and has now ended with the failure of Novartis to renew its partnership with Alnylam Pharmaceuticals. In fact, in the past few years, RNAi intellectual property licenses have lost most of their strategic investors because pharma companies based their decisions on sales predictions and therapeutic franchises rather than on knockdown efficiency. Decisions should be made based on scientific understanding of the mechanisms involved and the therapeutic potential that can in turn revolutionize patient care.

## What went wrong?

The prospect of rapid development of drug-specific RNAi for any target gene of choice gained considerable traction and fueled pharmaceutical companies to invest. However, creating a small-molecule drug is challenging. Usually, it takes between 5–7 years to develop a drug candidate that is ready to enter clinical trials (Box 1). Another sobering fact is that less than 10% of the tested molecules in trials will ever reach the market. In fact, the number of drugs that lose their patents exceeds the number of new drugs that are developed and approved each year. Moreover, manufacturing challenges and biocompatibility issues can also cause delays in the clinical translation of drugs.

Most of the traditional methods for RNA delivery do not actually meet the expectation of most companies. In the past few years, nanotechnology researchers largely invested in creating novel and efficient nanovehicles for siRNA and miRNA delivery [3–5]. There is a large projected growth in the usage of nanomaterials for RNAi delivery from 2009 to 2015 (Box 1), in contrast to the delivery of antisense nucleic acids and aptamers. The antisense delivery systems developed in the 1980s, such as Fomivirsen (an antisense phosphorothioate created by Isis) and Pegaptanib (an aptamer created by Eyetech and Pfizer), have not reached the market. By contrast, the global RNAi drug delivery market using nanomaterials is expected to grow to nearly \$24 billion by 2015 at a 5 year compound annual growth rate of 27.9%.

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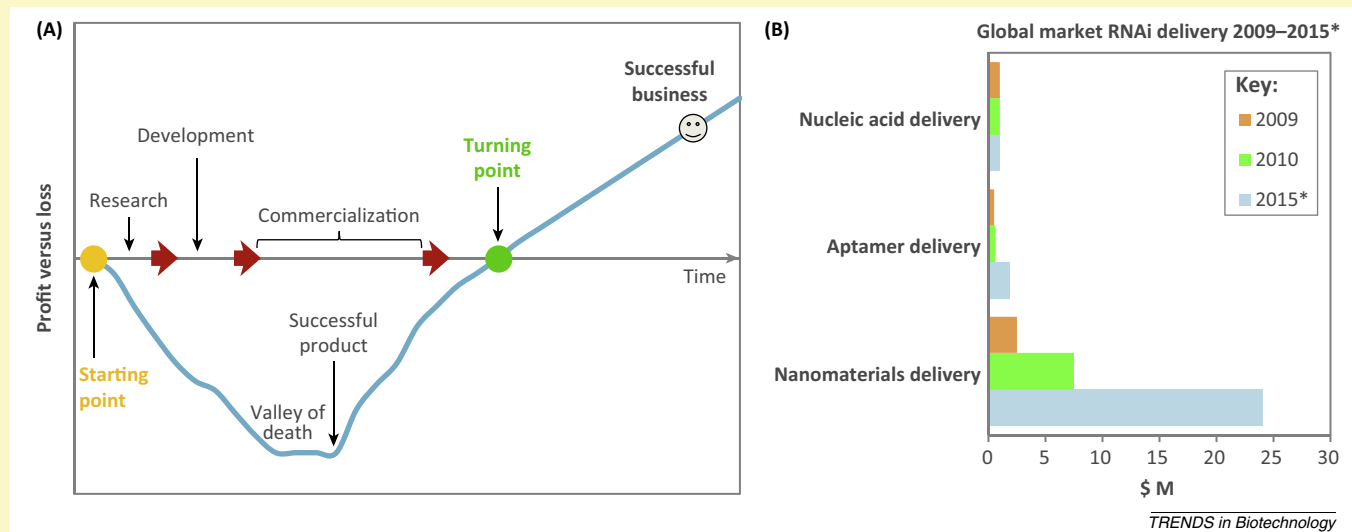
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**Box 1. Overcoming the Valley of Death in product commercialization.**

The most difficult period in the lifetime of a product is conventionally viewed as a 'Valley of Death' in business development (Figure 1A). It is here that many 'good' scientific ideas, technologies, and new products and processes die. This is the time when its manufacturer must identify and eliminate any limitation, optimize manufacturing processes, minimize production costs, and prepare for commercialization process. The turnover of the 'commercialization' or the proof of

concept occurs only after spending huge amounts of money and time. If a specific product is able to go through the 'Valley of Death' with success (which usually overlaps with a Phase II clinical trial, typically requiring ~20–100 million dollars) the probability for reaching the market is higher. This process may take as long as 20 years. However, the market for RNAi drug delivery, especially through nanoparticles, looks promising (Figure 1B).



**Figure 1.** (A) The story of a successful product/business: profit/loss 'Valley of Death' diagram as a function of time, with typical failure versus successful development stages. (B) The global RNAi drug delivery market for nucleic acid, aptamer, and nanomaterials delivery technologies between 2009 and 2015 (\*projection). Source: BCC Research.

**Will Nanotechnology motivate companies to invest?**

Although it may perhaps be considered fragile progress, huge strides were made in creating nanomaterials to avoid off-target effects subsequent to either systemic or intratumoral administration of RNAi therapeutics, especially in murine models [6]. In addition, the IC<sub>50</sub> value for RNAi delivery systems has been reduced by 99% in only the past 5 years following the introduction of nanodevices. These include lipidoids [7], stable nucleic acid lipid particles (SNALP), and ionizable cationic lipid 1,2-dilinoleoyloxy-3-dimethylaminopropane (DLinDMA), which have superior delivery capacity. siRNA delivered with DLinDMA, produced by Alnylam Pharmaceuticals, has *in vivo* activity at siRNA doses as low as 0.01 mg/kg in rodents and 0.1 mg/kg in nonhuman primates [8].

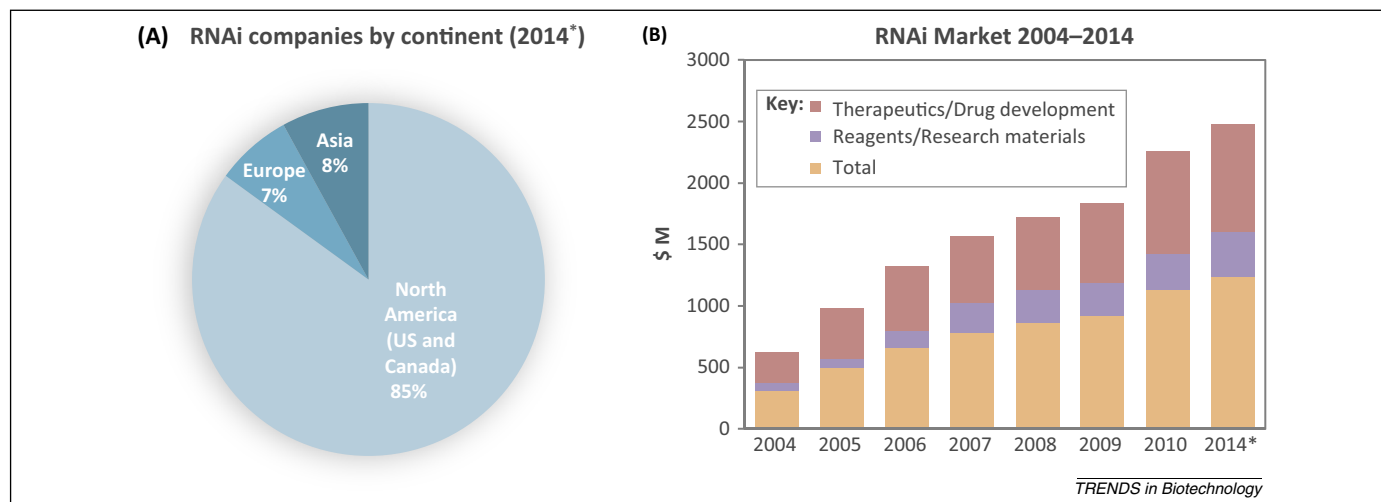
Lipid and polymer-based delivery systems have enabled RNAi and microRNA technologies to reach the market. Roche's Tekmira liposomal delivery technology shows unprecedented efficiency in animal challenge models for Ebola. This technology rapidly produced a wealth of important clinical data. The lipoplex delivery technology of Silence Therapeutics also combines siRNA with developed lipid moieties containing cationic lipids, co-lipids (fusogenic or stabilizing), or PEGylated lipids. This company produces several nanoparticle constructs with different pharmacodynamic properties, enabling functional delivery to various cell types upon systemic administration. This type of technology appears to be a pleasant surprise in terms of safety and early efficacy data.

Rosetta Genomics had also brought to market a miRNA-based cancer diagnostic service for healthcare providers and their patients, in particular for lung and kidney cancers. Rosetta Genomics is a leader in platform technologies for the identification, extraction, quantification, and analysis of miRNAs from a wide range of sample types, covering a large portion of known human and viral miRNAs.

Alnylam Pharmaceuticals focused on delivery of RNAi therapeutics, producing lipid nanoparticles that encapsulate the siRNA molecule [9], and thereby enabling systemic delivery through intravenous drug administration. Studies with lipid nanoparticle-based RNAi therapeutics demonstrated potent, rapid, and durable target gene silencing in preclinical as well as in clinical studies [10–13]. Further, lipid nanoparticle-based RNAi therapeutics were found to be generally well tolerated in the clinical studies conducted to date. More recently, the company began the development of a sugar molecule, 'GalNac', for conjugation to siRNAs to simplify subcutaneous administration.

Not far behind are RXi Pharmaceuticals, who are focused on discovering, developing, and commercializing innovative therapies based on next-generation RNAi platforms. RXi's first RNAi product candidate, RXI-109, entered into RXi's first human clinical trial in 2012 for dermal applications.

A joint effort from all these companies may revive and stabilize the enthusiasm and investment in the field of RNAi. Although RNAi was somehow abandoned, new means for efficacious delivery of siRNA have led companies such as



**Figure 1.** Global analysis on the RNAi market for 2014. **(A)** RNAi companies by continent, and **(B)** RNAi market between 2004 and 2014. The 2014\* values are projections. Source: Bioworld Publishing Group.

Alnylan Pharmaceuticals and Silence Therapeutics to bring RNAi back to the front page with strong long-term investments.

### The RNAi industry is 'back in business'

Although no RNAi nano-drug has reached the marketplace, despite the billion dollar investments, there is still potential for pharmaceutical companies to leverage RNAi therapies to revolutionize patient care. Most of the negative evaluations regarding RNAi in the industry will likely dissipate once market indicators suggest development and progress (Figure 1).

Pharmaceutical companies have not abandoned RNAi altogether, and should exploit the toolkit available to materials scientists for revitalizing the highly promising field of nucleic acid delivery. This would allow companies such as Alnylan Pharmaceuticals and Genzyme, who have hundreds of scientists who have been developing RNAi therapeutics against numerous targets, to turn this investment into profit, while allowing translational products to reach the market and improve patient health. However, we cannot forget that this will take time. We should remember that monoclonal antibodies took more than 20 years for FDA approval before blockbusters such as Avastin or Erbitux reached the market.

Hopefully, in the long run, cancer therapy using RNAi, and especially miRNA nano-platforms, will become common practice in treating a range of diseases, in particular those associated with oncogenesis and infectious disease. Extremely innovative and exciting ongoing work from the combined efforts of researchers from various disciplines, including chemistry, biology, and materials science, as well as by clinicians and engineers, is creating new solutions that will hopefully make pharma companies reinvest in the field.

Successful products such as siRNA lipid-based nanoparticles (ALN-VSP02) that target KSP and VEGF for the treatment of solid tumors, or ALN-PCS02, which targets PCSK9 for hypercholesterolemia therapy, have showed success in clinical trials and are ready to reach the market. Mipomersen (previously promoted as the first RNAi drug), an antisense therapeutic that targets the mRNA for apolipoprotein B, became a success story as a cholesterol-reducing drug candidate. The locked nucleic

acid (LNA)-based drug candidate Miravirsin, developed by Santaris Pharma, has also become a huge success as a potent and selective inhibitor of miR-122, a liver-specific microRNA that hepatitis C virus requires for replication.

### Concluding remarks and future perspectives

Today's delivery capabilities already provide several high-quality RNAi therapeutic opportunities, especially by exploiting advances in the nanotechnology field. Access to and testing of these technologies, which are already used for tissue engineering and drug delivery, is crucial for successful RNAi therapies. Systemic RNAi delivery should now be replaced with smart delivery systems that enable local [14], targeted, and selective uptake of such potent molecules, while increasing their stability and residence time at the site of interest. Toxicity and immunogenicity associated with systemic RNAi administration can be minimized by exploiting targeted and selective delivery vehicles that enable effective and confined drug concentration with long retention of the therapeutic agent at the site of interest. This approach has already shown promising outcomes, as demonstrated by RNAi companies including Rosetta Genomics and Alnylan Pharmaceuticals, whereas systemic delivery strategies are perhaps more distant goals. In fact, Alnylan Pharmaceuticals is leading the translation of RNAi as a new class of innovative medicines, with a core focus on RNAi therapeutics for the treatment of serious and life-threatening diseases.

Combination products that enable smart delivery of RNAi, together with existing potent chemotherapeutic drugs, may present the next revolution in cancer therapy.

### Disclaimer statement

The opinions expressed and arguments employed herein are those of the authors and do not reflect the official views of the companies involved.

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