The Patent Landscape of siRNA Nanoparticle Delivery

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Abstract

Alnylam has rights to the fundamental patents claiming siRNA and may be the first to obtain FDA marketing approval. It is not clear at this time whether Alnylam's patents will block other companies from marketing their siRNA therapeutics as they may expire before other siRNA therapeutics receive FDA approval. There have been a large number of patent filings claiming various aspects of nanoparticle delivery of siRNA. None of these patents will block the entire field of siRNA delivery. Companies will likely be able to develop and commercialize their own siRNA drugs with 3' overhangs without fear of third-party patents, so long as marketing occurs after 2021. In addition, companies developing blunt ended siRNA drugs can market at any time with a non-exclusive license to the Carnegie patents.

I. INTRODUCTION

RNA interference (RNAi) is a natural phenomenon for gene silencing. It operates by targeting complementary RNA for destruction. Small interfering RNAs (siRNAs) are small segments of double stranded RNA (dsRNA) that participate in RNA interference and are useful as human therapeutics. siRNA is typically 19-25 base pairs in length, is typically an asymmetric duplex with 3'-overhang of 1-5 nucleotides, sometimes blunt-ended with chemical modifications, and is either processed from longer dsRNAs in the cell or chemically synthesized. The discovery of RNAi led to the award of the Nobel Prize in 2006 for Physiology or Medicine to Andrew Fire and Craig Mellow.

One challenge of siRNA is its delivery to target cells without metabolic clearance and immunogenicity. Effective delivery vehicles include nanoparticle delivery systems. They are colloidal particle carriers of varying architecture and approximately 1-1000 nm in size. Nanoparticles are organic complexes that can be generally categorized to include lipid complexes, conjugated polymers, and cationic polymers, and inorganic complexes that include magnetic nanoparticles, quantum dots, carbon nanotubes, and gold nanoparticles.

Since the discovery of siRNA, many companies have embarked on programs to develop and patent siRNA products, nanoparticle delivery systems and therapeutic methods. This article will

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provide an overview of the patents covering siRNA technology, identify and summarize the different types of nanoparticle delivery systems being patented, summarize the patent litigation to date, and speculate regarding future litigation.

Databases of the U.S. Patent and Trademark Office were searched to identify patents and patent applications that claim RNAi or siRNA. Also searched were patents that claim the nanoparticle delivery of siRNA. The searches revealed that Thermo Fisher (who acquired Dharmacon) and Alnylam were the most prolific patent filers. Alnylam also has the most siRNA candidates in clinical trials and has the only siRNA drug in phase III clinical trials. Alnylam may be the first company to receive FDA approval of an siRNA drug with a commanding patent position.

II. THE FREQUENT FILERS

Our search identified the following companies as the most frequent patent filers on siRNA technology:

Company		**IP landscape**				
Name	Alternate names: former name (chg); subsidiaries (sub); acquisitions (acq)	Number of Issued U.S. Patents				No. of Pending U.S. appl.[1]
		Total No.	Specific siRNA (specific target genes)	General siRNA (chemical structure & modifications)	Delivery Modality including Nanoparticles (NP)	
Thermo Fischer	**Dharmacon** (acq)	**109**	100	9	0	221
Alnylam[2]		**95**	58	25	12	104
Merck	**siRNA Therapeutics** (acq) **(**former **Ribozyme Pharmaceuticals)**	**20**	4	11	5	134
Tekmira	**Inex Pharma-ceuticals** (chg); **Protiva** (acq)	**18**	3	4	11	48
Arrowhead	**Calando** (sub); **Mirus Bio** (acq via Roche)	**18**	2	0	16	29
Novartis		**17**	11	6	0	62
Silence Therapeutics	**Atugen** (acq); **Intradigm** (acq)	**13**	5	3	5	42

[1] No effort was made to identify the applications that have issued into patents.

[2] According to Alnylam's Form 10-K filed at the SEC on 2/19/13, Alnylam's patent estate for RNAi therapeutics includes over 1,800 active cases and over 700 granted or issued patents, of which over 200 are issued or granted in the United States, Europe, and Japan.

Marina Biotech	**mdRNA** (chg); **Cequent** (acq); **Novosom** (acq)	**4**	0	0	4	29
Pfizer	**Wyeth** (acq)	**3**	3	0	0	57
Dicerna		**3**	1	2	0	16
CytRx	**RXi** (sub)	**0**	0	0	0	10

While Thermo Fisher (who acquired Dharmacon and its patent portfolio) is a prolific patent filer, they have focused their business on a line of products for the RNAi researcher, rather than developing products themselves.[3] Alnylam is the most prolific patent filer that is also developing siRNA therapeutics.

III. THE FUNDAMENTAL siRNA PATENTS

Our searches identified three patent families claiming fundamental aspects of RNA interference (see following chart). The first patent family was filed by Fire and Mello of the Carnegie Institution of Washington. The second and third patent families were filed by Thomas Tuschl of the Max-Planck-Gesellschaft zur Foerderung de Wissenschaften e.V and others from MIT, the Whitehead Institute for Biomedical Research, and the University of Massachusetts.

Patent family	Assignee	Granted Patents	Claim Scope	Filing date	Issue Date	Term	Notes
"Carnegie" patents	Carnegie Institution of Washington	**6,506,559** **7,560,438**	Broad; genetic inhibition by dsRNA without length limitation	12/18/1998 10/30/2002	1/14/2003 7/14/2009	12/18/2018 12/18/2018	Broadly available via non-exclusive licensing
"Tuschl I" patents	Max Planck; MIT; Whitehead; UMass	**8,420,391** **8,552,171**	isolated dsRNA of about 21-23 nucleotides ('171); method of producing knockdown organism thereby ('391)	10/4/2010 10/4/2010	4/26/2013 10/8/2013	3/30/2021 3/30/2021	Alnylam has non-exclusive rights from three co-owners; UMass has licensed its interest separately to third parties
"Tuschl II" patents	Max Planck; MIT; Whitehead; UMass	**7,056,704** **7,078,196**	Method of preparing dsRNA of 19-25 bp with 3' overhang of 1-5 nucleotides ('704, '196); dsRNA with each strand of 19-23 nt and 3'	4/27/2004 4/27/2004	6/6/2006 7/18/2006	11/29/2021 11/29/2021	Licensed exclusively to Alnylam, subject to the right of UMass to sublicense the US Tuschl

[3] "About Dharmacon," accessed December 17, 2013 at http://www.thermoscientificbio.com/about-dharmacon/.

"Tuschl II" patents		**8,362,231** **8,372,968**	overhang of 1-3 nt ('231); dsRNA with each strand of 19-25 nt and 3' overhang of 1-5 nt ('968);	1/6/ 2010 8/7/ 2009	1/29/ 2013 2/12/ 2013	3/30/ 2021 3/30/ 2021	II patent family to Merck or Merck & Co., Inc. Alnylam has sub-licensed to third parties

The Carnegie patents, while important, are available on a non-exclusive licensing basis and expire relatively early (2018). Thus, the Carnegie patents will likely not block third parties from marking siRNA therapeutics in the United States.

The Tuschl II patents claim short double stranded RNA molecules and at least one 3'-overhang. These characteristics are required by most siRNA therapeutics. The Tuschl patents are reportedly licensed exclusively to Alnylam.[4] Thus, parties other than Alnylam and its sublicensees are blocked from developing siRNA therapeutics with 3' overhangs until after 2021. However, most siRNA therapeutics are in an early stage of development and most drugs never obtain FDA approval. Therefore, it is not clear at this time whether the Tuschl II patents will block companies from marketing siRNA therapeutics.

Other patents that may be of importance are the so-called "Zamore Design Rule" patents owned by the University of Massachusetts Medical School and licensed to Silence Therapeutics. These patents reportedly allow one to design dsRNAi agents having decreased off-target silencing through certain structural modifications and are said to be fundamental to the creation of RNA-based therapeutics.[5] However, Alnylam has downplayed the importance of these patents.[6] It is not clear whether these patents will block any companies from marketing siRNA therapeutics.

IV. COMPANIES WITH siRNA THERAPEUTICS/NANOPARTICLES

The following companies were identified as having at some point siRNA therapeutics and/or nanoparticle carriers in development. The patent estates and license agreements are complex and brief details are provided in the notes below. For more information, the reader should consult the SEC filings of the respective companies.

[4] *See*, Alnylam's Form 10-K filed at the SEC on 2/19/13.

[5] The Zamore Design Rule Patents are U.S. Pat. Nos. 7,459,547, 7,732,593, 7,772,203 and 7,750,144. http://www.eurekalert.org/pub_releases/2011-12/uomm-upo121211.php.

[6] http://www.genomeweb.com/rnai/alnylam-execs-downplay-importance-silence-therapeutics-zamore-ip.

Company		**Product Pipeline**			**Notes**
Name (location)	Alternate names: former name (chg); subsidiaries (sub); acquisitions (acq)	Product Name	Develop-ment Stage (PC, PI, PII, PIII)[7]	Category: siRNA trigger; delivery modality including nanoparticle (NP)	
Alnylam Pharma-ceuticals (Cambridge, MA, USA)	None	**ALN-TTR02 ("Patisiran")** **ALN-PCS02** **ALN-VSP02** **ALN-TMP** **ALN-TTRsc** **ALN-AT3** **ALN-AS1** **ALN-PCSsc** **ALN-CC5** **ALN-AAT** **(ALN-RSV01)**[8]	PIII PI PI PC PII PI PC PC PC PC (PII)	siRNA trigger with NP (**SNALP**) siRNA trigger with NP (**GalNAc-siRNA**) (naked siRNA)	Alnylam is a non-exclusive licensee for therapeutic purposes of Carnegie and Tuschl I patents and an exclusive licensee for therapeutic purpose of Tuschl II patents[9]
Merck & Co. (Whitehouse Station, NJ, USA)	**siRNA Therapeutics** (acq) **(**formerly **Ribozyme Pharmaceuticals)** (San Francisco, CA, USA)	N/A			Shut down SF site (siRNA Therapeutics) in July 2011

[7] PC, Pre-Clinical; PI, Phase I; PII, Phase II; and PIII, Phase III Clinical Trials.

[8] ALN-RSV01 is unformulated naked siRNA that is inhaled, but was included in the table for comprehensive coverage of Alnylam's siRNA product portfolio.

[9] *See* Alnylam's Form 10-K filed at the SEC on 2/19/13.

Tekmira Pharma-ceuticals **(Burnaby, BC, Canada)**	**Inex Pharma-ceuticals** (chg); **Protiva Biotherapeutics** (acq) (Seattle, WA, USA)	**TKM-PLK1 (TKM-080301)** **TKM-Ebola** **TKM-HBV** **TKM-ALDH2**[10] **TKM-Marburg** stable nucleic acid-lipid particle (**SNALP**)[11]	PII PC PC PC PC	siRNA trigger with NP (**SNALP**) NP (liposome)	SNALP technology was exclusively licensed to Alnylam, which was subject of recent litigations
Arrowhead Research Co. (Pasadena, CA, USA)	**Calando Pharma-ceuticals** (sub); **Mirus Bio** (acq via **Roche Madison** (Madison, WI, USA)	**CALAA-01**[12] **ARC-520** Dynamic PolyConjugate (**DPC**)[13] RNAi/Oligonucleotide Nanoparticle Delivery (**RONDEL**) lipid nanoparticle **(LNP)**	PI PI	siRNA trigger with NP (**RONDEL**) siRNA trigger with NP (**DPC**) NP (polymer) NP (polymer) NP (liposome)	The Tuschl I and II patents were reportedly licensed to Arrowhead. In addition, DPC was licensed to Alnylam for a single RNAi product.[14]
Novartis AG (Basel, Switzerland)		N/A	N/A	N/A	Ended 5-year RNAi alliance with Alnylam in 2010

[10] "Tekmira reportedly has an exclusive license from Alnylam Pharmaceuticals, Inc. under its InterfeRx™ program to develop TKM-ALDH2 for the treatment of alcohol dependence." Tekmira Press Release of March 1, 2012 (http://investor.tekmirapharm.com/releasedetail.cfm?ReleaseID=653067).

[11] Tekmira's patents include U.S. Patent Nos. 8,283,333, 8,058,069, 7,982,027, 7,901,708, and 7,799,565, according to Form 20-F/A filed at the SEC on 10/30/2013.

[12] Further development of RONDEL and CALAA-01 has reportedly been suspended as the Company focuses on its DPC delivery platform. Arrowhead's Form 10-K filed at the SEC on 12/18/13.

[13] *See* US Patent No. 8,501,930.

[14] *See* Arrowhead's Form 10-K filed at the SEC on 12/18/13.

Silence Therapeutics (London, UK)	**Atugen AG** (acq) (Berlin, Germany); **Intradigm Co.** (acq) (Palo Alto, CA, USA)	**Atu027** (AtuPLEX-PKN3) **Atu111** (DACC-Ang2) **AtuRNAi** **AtuPLEX**[15] **DACC** **DBTC**	PIIb PC	siRNA trigger with NP (**AtuPLEX**) siRNA trigger with NP (**DACC**) siRNA trigger NP (liposome)	AtuRNAi is a blunt-ended siRNA molecule that may not be covered by the Tuschl II patents; Silence has reportedly in-licensed Carnegie patents, and exclusively licensed siRNA design rules by Phillip Zamore of Univ. of Massachusetts[16]
Marina Biotech (Bothell, WA, USA)	**mdRNA** (chg); **Cequent Pharmaceutical** (acq) (Cambridge, MA, USA); **Novosom AG** (acq) (Halle, Germany)	Unlocked Nucleobase Analog (**UNA**)-containing siRNA **(UsiRNA)**[17] **SAMRTICLES®**[18] **DiLA²** (Histidine-containing Di-alkylated Amino Acid)[19]		siRNA trigger NP (liposome) NP (liposome)	Carnegie patents licensed to Marina; UNA technology licensed non-exclusively to Tekmira in 2012[20]; Arcturus Therapeutics acquired Marina's UNA IP portfolio in Aug. 2013[21]

[15] *See* U.S. Patents Nos. 8,017,804 and 8,357,722.

[16] Silence Therapeutics, *Intellectual Property*, at http://silence-therapeutics.com/collaborations/current-partnerships/#!/platform-technologies/intellectual/; *Silence Therapeutics Announces Issuance of New U.S. Patent Broadly Covering Methods for Enhancing Silencing Activity of RNAi Therapeutics* (June 9, 2010), at http://silence-therapeutics.com/wp-content/uploads/2012/01/release_100609.pdf.

[17] *See* U.S. Patent No. 8,314,227.

[18] *See* U.S. Patent No. 7,371,404.

[19] *See* U.S. Patent Nos. 8,501,824, and 7,959,505.

[20] http://www.sec.gov/Archives/edgar/data/1447028/000117184312004316/newsrelease.htm.

[21] http://www.marketwired.com/press-release/marina-biotech-monetizes-una-intellectual-property-estate-through-agreement-with-arcturus-pinksheets-mrna-1820724.htm.

Pfizer, Inc. (New York, NY, USA)	**Wyeth** Pharmaceuticals (acq) (Collegeville, PA, USA)	N/A	N/A	N/A	discontinued RNAi program in Feb. 2011
Dicerna Pharma-ceuticals (Watertown, MA, USA)	none	**DCR-M1711** **DCR-0114** **DCR-0729** Dicer Substrate siRNA (**DsiRNA**)[22] **EnCore NP**	PC PC PC	siRNA trigger **(DsiRNAi)** with NP (**EnCore)** siRNA trigger NP (liposome)	
CytRx Co. (Los Angeles, CA, USA)	**Rxi Pharma-ceuticals** (sub) (Worcester, MA, USA)	**RXI-109** **PVR** **RXI-209** **sd-rxRNA (self-delivering RNAi drug)**	PII PC PC	**sd-rxRNA** based siRNA trigger siRNA trigger	

The term "siRNA trigger" indicates small double-stranded RNA molecules of various designs and compositions which guide the RNA interference (RNAi) pathway via cleavage and degradation of its cognate mRNA targets in a sequence-specific manner. The most classical form of siRNA trigger is the siRNA design based on the "**Tuschl II**" patent family. These patents claim small dsRNA of 19-25 base pairs that contain 3'-overhang of 1-5 nucleotides on one or both strands. This patent family is exclusively licensed to Alnylam, which in turn grants exclusive or non-exclusive sublicenses to other companies such as Tekmira for RNAi therapeutics directed to specific gene targets.[23]

A second siRNA trigger is Unlocked Nucleobase Analog (UNA)-containing siRNA (**UsiRNA**) developed by Marina Biotech.[24] It is a duplex siRNA that is modified with non-nucleotide acyclic monomers termed UNA. Optionally, UsiRNA may also contain conformationally restricted nucleotides (CRN) that minimize the potential off-target effects by either strand of the siRNA duplex. This reportedly decreases the potential for cytokine induction, and provides protection from nuclease degradation.[25]

A third siRNA trigger is Dicer Substrate siRNA (**DsiRNA),** co-invented by John Rossi of the Beckman Research Institute of the City of Hope, CA and Mark Behlke of Integrated DNA Technologies (IDT),[26] and developed by Dicerna Pharmaceuticals.[27] It is a longer, 25-30 bp

[22] *See* U.S. Patent No. 8,084,599.

[23] Alnylam's Form 10-K for the fiscal year 2012, pg. 25.

[24] Marina has assigned its UNA technology to Arcturus Therapeutics, Inc. in August 2013. http://finance.yahoo.com/news/marina-biotech-monetizes-una-intellectual-110000866.html.

[25] http://www.marinabio.com/usinra_technology.

[26] http://www.dicerna.com/about-advisory.php.

asymmetric dsRNA containing a single 2-nt 3'-overhang and a blunt end, that induces preferential processing by Dicer of only one siRNA strand (the "guide" strand) and reduction of off-target effects compared with classical siRNAs of smaller, conventional size (19-25 bp).

AtuRNAi is a proprietary siRNA platform developed by Silence Therapeutics that differs in design from the Tuschl-type molecules - a blunt-ended siRNA molecule without the conventional 3'-overhang.[28] **AtuRNAi** is stabilized against nuclease degradation by modification with 2′-*O*-methyl (2′-OMe) ribonucleotides.[29]

Several types of siRNA delivery/carrier modalities, including various types of nanoparticles (NPs), are currently utilized in clinical trials. The following is a brief survey summarizing the NP-based siRNA delivery modalities. Delivery modalities that employ naked siRNA[30] (e.g., Quark Pharmaceuticals, Opko Health, and Sylentis), longer short-hairpin RNA (shRNA) (e.g., Gradalis's pbi-shRNA in bilamellar invaginated vesicle lipoplex (BIV-L)[31]), and other non-NP based vehicles (e.g., Silenseed's LODER polymer[32] and Marina Biotech/Cequent's tkRNAi[33]) are excluded from this survey.

The most common siRNA delivery platform is stable nucleic acid–lipid particles (**SNALP**) developed originally by Tekmira. **SNALP** is out-licensed and used as a delivery platform for three of Alnylam's products in human clinical trials (**ALN-TTR02, ALN-PCS02, and ALN-VSP02)**. **SNALP** is a lipid nanoparticle (LNP)-based siRNA trigger formulation that comprises ionizable lipids, shielding lipids (e.g., polyethylene glycol (PEG)), cholesterol, and endogenous or exogenous targeting ligands such as ApoE lipoprotein.[34] It is a mono-lamellar (single lipid bilayer) liposome neutrally charged at physiological pH and stabilized by PEG that encapsulates the siRNA trigger.[35] **SNALP** is reportedly suitable for delivery to various tissue and cell types.

First generation **SNALP** contained the ionizable cationic lipid DLinDMA (1,2-dilinoleyloxy-3-dimethylaminopropane)[36] and was used in **ALN-VSP02** and **ALN-TTR01**.[37] Second generation **SNALPs**, **DLin-KC2-DMA** and **DLin-MC3-DMA**, were generated by a structure-activity relationship (SAR) study that optimized the pKa of the ionizable amino lipid head groups of the cationic lipid

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[27] http://www.dicerna.com/approach-about-dicer.php.

[28] Czauderna F. *et al*. Functional studies of the PI(3)-kinase signaling pathway employing synthetic and expressed siRNA. *Nucleic Acids Res.* 31(2):670-82 (2003).

[29] http://silence-therapeutics.com/platform-technologies/rnai-platform.

[30] Thakur A. *et al*. Strategies for ocular siRNA delivery: Potential and limitations of non-viral nanocarriers. *J Biol. Eng*. 6(1):7 (2012).

[31] http://mywebsitepronto.com/clients/gradalis/pipeline/pbi-shstmn1-lp; Rao, D.D. *et al*. Enhanced target gene knockdown by a bifunctional shRNA: a novel approach of RNA interference. *Cancer Gene Ther.* 17:780–791 (2010).

[32] http://silenseed.com/page/221.

[33] http://www.marinabio.com/tkrnai_platform.

[34] Kanasty, R. *et al*. Delivery materials for siRNA therapeutics. *Nature Mater.* 12, 967–977 (2013).

[35] Haussecker, D. The business of RNAi therapeutics in 2012. *Mol Ther - Nucleic acids*. 1:e8 (2012).

[36] Morrissey, D.V. *et al*. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. *Nat. Biotechnol.* 23(8):1002-7 (2005).

[37] http://www.alnylam.com/capella/wp-content/uploads/2012/02/ALNY-2012-AsiaTIDES-Delivery-Update.pdf.

moiety.[38] These second generation **SNALPs** were used in **ALN-TTR02** and **ALN-PCS02**.[40] A third generation **SNALP** named **reLNP** (rapidly eliminated LNP), represented by L319, was created by Alnylam. **reLNP** reportedly provides biodegradability to the existing **SNALP** platforms and rapid elimination from plasma and tissue.[39]

Another type of liposomal siRNA delivery platform is **AtuPLEX** developed by Silence Therapeutics (which acquired Atugen). **AtuPLEX** is a multi-lamellar (multiple lipid bilayer) and positively-charged siRNA-lipoplex that combines siRNA with three-lipid liposomes. The liposomes contain Silence's proprietary cationic lipids AtuFect01,[40] co-lipids (fusogenic or stabilizing), and PEGylated lipids, to form a nanoparticle structure with siRNA embedded within multiple lipid bilayers of the particle. The **AtuPLEX** system is designed to deliver siRNA to vascular endothelial cells of various organs, and is used in **Atu027**, Silence's lead oncology candidate currently in Phase IIa trials.

Silence Therapeutics employs two additional lipid nanoparticle delivery platforms that are related to **AtuPLEX** but have different target tissue specificity. **DACC** is a proprietary lipid delivery system that includes AtuFect01 and is used to embed siRNAs into a multiple lipid bilayer structure.[41] While closely related to the **AtuPLEX** system, **DACC** has significantly different biopharmaceutical properties, and delivers siRNA to the pulmonary vascular endothelium. The **DACC** delivery system is incorporated in **Atu111**, Silence's preclinical development candidate for the treatment of acute lung injury. **DBTC** is a proprietary lipid delivery system that delivers siRNA to hepatocytes and the hepatic vascular system of the liver parenchyma, rather than merely targeting liver hepatocytes.[43]

RONDEL (RNAi/Oligonucleotide Nanoparticle Delivery) platform[42] is used in Arrowhead Research's product **CALAA-01**, currently in Phase I clinical trials for treating solid tumors. **CALAA-01** is a non-liposomal polymer-based nanoparticle re-optimized for *in vivo* siRNA delivery.[43] **RONDEL** has four components that self-assemble into nanoparticles, consisting of (i) siRNA strands, (ii) cyclodextrin-containing polymers (CDPs), (iii) polythethylene glycol (PEG) as steric stabilization agents, and (iv) human transferrin (Tf). Tf is a targeting ligand for binding to transferrin receptors (TfR) that are typically upregulated on cancer cells.[44] CDPs are linear polycationic oliogomers containing positively charged amidine groups alternating with sugar (cyclodextrin) moieties. The positively charged CDP polymer associates with the negatively charged backbone of siRNAs to form nanoparticles less than 100 nm in diameter, with the siRNA at their

[38] Semple S.C. *et al*. Rational design of cationic lipids for siRNA delivery. *Nat Biotechnol*. 28(2):172-6 (2010); Jayaraman, M. *et al*. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing *in vivo*. *Angew. Chem. Int. Ed. Engl*. 51(34):8529–8533 (2012).

[39] Maier, M.A *et al*. Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Mol. Ther.* 21:1570–1578 (2013).

[40] http://silence-therapeutics.com/platform-technologies/#!/platform-technologies/delivery-platform.

[41] http://silence-therapeutics.com/2011/09/30/silence-therapeutics-signs-sirna-delivery-collaboration-with-top-10-pharma-company.

[42] http://www.arrowheadresearch.com/technology/rondel.

[43] Davis, M. E. *et al*. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* 464, 1067–1070 (2010).

[44] Davis, M. E. The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic. *Mol. Pharm.* 6, 659–668 (2009).

cores and cyclodextrin groups on their surfaces.[45] Components (iii) and (iv) associate non-covalently with the hydrophobic cores in the CDP via a hydrophobic adamantine group covalently bound to one end of the PEG. The resulting complex is an siRNA containing nanoparticle coated with PEG (stabilizer) and PEG-targeting ligands (TfR).[46]

Dynamic PolyConjugates (**DPC**) is another class of non-liposomal, polymer conjugate-based siRNA delivery platform first reported in 2007.[47] Arrowhead Research acquired **DPC** from Roche Madison, which had acquired it from Mirus Bio.[48] **DPC**s are small nanoparticles, 5-20 nm in size, containing the amphipathic endosomolytic polymer poly(butyl amino vinyl ether) (PBAVE), to which shielding agents (e.g., PEG) and targeting ligands are reversibly attached, and siRNA attached via a hydrolysable disulfide linker.[49] While the membrane disrupting PBAVE polymer is masked by the PEG side chains, the PEG and targeting ligands are released in the acidic environment of the endosome to trigger endosomal release.[50] Once in the reducing environment of the cytoplasm, the disulfide linkage is cleaved to release the siRNA.

An improved, newer generation **DPC** system is employed in Arrowhead's **ARC-520**, currently in Phase I clinical trials for the treatment of HBV infection.[51] Atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) are employed to produce polymers that are reportedly homogeneous and amenable to large scale manufacturing.[52] siRNA is not attached to the **DPC** polymer. Instead, a siRNA-cholesterol conjugate is co-injected with a melittin-like peptide that has reversibly masked endosomolytic properties, similar to the PBAVE polymer.[53]

Another example of a conjugate-based siRNA delivery system is **GalNAc-siRNA**, a liver-targeted triantennary siRNA conjugate used in several drug candidates by Alnylam (**ALN-TTRsc, ALN-AT3, and ALN-PCSsc)**.[54] In **GalNAc-siRNA**, the 3'-terminus of the sense strand of chemically modified siRNA is conjugated with the carbohydrate N-acetylgalactosamine (GalNAc) via a triantennary spacer. **GalNAc-siRNA** provides targeted delivery to hepatocytes via subcutaneous administration.[37] GalNAc ligand binds with high affinity to the asialoglycoprotein receptor (ASGPR) that is highly expressed on hepatocytes, and is taken up by clathrin-mediated endocytosis to release the siRNA in the cytoplasm.

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[45] http://www.arrowheadresearch.com/technology/rondel.

[46] Kanasty, R. *et al*. Delivery materials for siRNA therapeutics. *Nature Mater.* 12, 967–977 (2013).

[47] Rozema D.B. *et al.* Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. *Proc. Natl Acad. Sci. USA* 104:12982-12987 (2007).

[48] http://www.biotechprofiles.com/companyprofile/ArrowheadMadison.aspx.

[49] http://www.arrowheadresearch.com/technology/dynamic-polyconjugates.

[50] Kanasty, R. *et al*. Delivery materials for siRNA therapeutics. *Nature Mater.* 12, 967–977 (2013).

[51] http://www.arrowheadresearch.com/press-releases/arrowhead-data-demonstrates-rnai-candidate-arc-520-silences-hepatitis-b-virus.

[52] http://www.arrowheadresearch.com/sites/default/files/press_releases/pdf/Arrowhead-Research-Corporation_-_DPC-Technology-White-Paper-November2011.pdf.

[53] Wooddell, C. I. *et al.* Hepatocyte-targeted RNAi therapeutics for the treatment of chronic hepatitis B virus infection. *Mol. Ther.* 21, 973–985 (2013).

[54] http://phx.corporate-ir.net/phoenix.zhtml?c=148005&p=irol-newsArticle&ID=1886030&highlight.

SMARTICLES and **DiLA²** are amphoteric liposomes used by **Marina Biotech** in several of their preclinical candidates.[55] They are pH dependent charge-transitioning particles that provide siRNA to cells by local or systemic administration.[56] **SMARTICLES** are stable in blood and distribute in the same manner as conventional liposomes but become positively charged when they cross cell membranes, leading to delivery of the siRNA within sites of inflammation, tumors, liver, and spleen.[57] **SMARTICLES** were used by ProNAi Therapeutics in its recent Phase I clinical trials of PNT2258, an anti-Bcl-2 cancer drug.[58]

DiLA² (Di-alkylated Amino Acids) is a platform for creating liposome formulations from dialkylated amino acids for delivery of UsiRNA (see above). **DiLA²** reportedly allows one to modify key aspects of the delivery system such as charge, linker and acyl chains to optimize the properties of the liposome. For example, **DiLA²** allows one to optimize delivery to a target tissue of interest, and permits inclusion of peptides to improve a variety of delivery characteristics such as encapsulation of nanoparticles, cellular uptake, endosomal release and cell/tissue targeting.[59]

EnCore is a nanoparticle system developed by Dicerna Pharmaceuticals for delivery of DsiRNA (see above) to the liver and solid tumors. **EnCore** contains a lipid-dsiRNA core surrounded by an envelope of a different lipid mixture that mediates accumulation, internalization, and release of siRNA payload into the target cell.[60] This sub-structured particle is designed for preferential accumulation in tumors and reportedly provides high levels of DsiRNA.[61]

Though not NP-based, **sd-rxRNA** (self-delivering RNAi compounds) developed by RXi Pharmaceuticals is noteworthy. **sd-rxRNA** is employed in **RXI-109** in Phase II clinical trials for dermal scarring.[62] **sd-rxRNA** is an alternative approach to delivery in which drug-like properties are built into the RNAi compound itself. **sd-rxRNA** contains a single-stranded phosphorothioate region, a short duplex region, and various nuclease-stabilizing and lipophilic chemical modifications, to reportedly provide efficient spontaneous uptake in multiple cell types.[63]

V. siRNA LITIGATIONS

There were two litigations involving Carnegie patents. *Ali v. Carnegie Institution of Washington,* (U.S. District Court Oregon, 2012) was filed by a *pro se* plaintiff and voluntarily dismissed in September 2012. The other litigation, *Cold Spring Harbor Laboratory v. Ropes & Gray* (U.S. District Court Massachusetts, 2011), was a patent malpractice case and not substantively related to the siRNA patents. This case was dismissed for lack of subject matter jurisdiction.

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[55] http://www.marinabio.com/development_pipeline.

[56] http://finance.yahoo.com/news/marina-biotech-announces-licensee-pronai-113000463.html.

[57] http://www.investorvillage.com/mbthread.asp?mb=11383&tid=4769035&showall=1.

[58] http://www.otcmarkets.com/stock/MRNA/news/Marina-Biotech-Announces-that-Licensee-ProNAi-Therapeutics-Reported-Phase-1-Study-Results-using-SMARTICLES--174--Nucleic-Acid-Delivery-Technology?id=55879&b=y.

[59] http://www.marinabio.com/dila2_delivery_technology; Adami, R.C. *et al*. An amino acid-based amphoteric liposomal delivery system for systemic administration of siRNA. *Mol. Ther.* 19:1141–1151 (2011).

[60] http://www.dicerna.com/approach-about-lnp.php.

[61] http://www.dicerna.com/pdf/ILCA%202012%20-%20Delivery%20Data%20-%20Poster%20P-004.pdf.

[62] http://www.rxipharma.com/rxi-109.

[63] http://www.rxipharma.com/sd-rxrna.

There were two litigations involving Tuschl patents. *Max-Planck-Gesellschaft v. Whitehead Institute* (U.S. District Court Massachusetts, 2009), famously known as the "RNAi litigation," was a dispute over the IP ownership to the Tuschl II patents and business transactions that allegedly violated joint agreements between the parties. The plaintiffs (who included Alnylam) asserted that Whitehead incorporated the Tuschl II patents' inventive subject matter into a patent application in an earlier patent family (the "Tuschl I" patents), that Whitehead has been prosecuting as a non-exclusively assignee. This litigation was settled in March 2011 before trial on the condition that Max Planck control the future prosecution of the Tuschl I and Tuschl II patent families in the United States. *University of Utah v. Max-Planck-Gesellschaft* (U.S. District Court Massachusetts, 2011) is currently ongoing. In this litigation, Utah is challenging the inventorship of the Tuschl II patents. Utah is asserting that professor Brenda Bass should have been listed as the sole or one of the inventors of the Tuschl patents. Utah alleges that Bass first conceived of the 2-nt 3'-overhang, the key feature of the Tuschl II patents, and communicated that conception to the other inventors. Should Utah be successful in this litigation, they will become the sole or co-owner of the Tuschl II patents. In that case, Utah will be free to grant additional licenses to the Tuschl II patents. It is expected that this case will be litigated vigorously, if not settled, since it affects the rights to the basic patents covering siRNA therapeutics and held exclusively by Alnylam.

Tekmira v. Alnylam (Massachusetts Superior Court, 2011) was a litigation relating to the siRNA delivery technology. Tekmira asserted that Alnylam misappropriated confidential information including trade secrets by disclosing it to a third party and incorporating it into Alnylam's own patent filings. This litigation settled in November 2012 with a restructuring of the licensing agreements and relationship between the parties.

VI. FUTURE LITIGATION?

Patent protection of siRNA therapeutics is critical for establishing exclusivity in the marketplace. However, patents will not stop a competitor from testing a patented drug in the clinic. Under U.S. law, there is no infringement if the patented drug is used to obtain information for submission to the U.S. Food and Drug Administration.[64] Thus, even in the face of broad dominating patents, companies are free to make and test their drugs in the clinic. They need only be concerned about such patents if they remain in force at the time of marketing.

After 2021, litigation concerning siRNA and nanoparticle patents will be unlikely, as no company will have a dominating position. Each company developing an siRNA drug can develop its own nanoparticle delivery system and sell it, so long as the particular delivery system does not infringe third party patents in force at the time.

It is not clear at this time whether there will be infringement litigation concerning the Carnegie and Tuschl patents, since most therapeutics never receive FDA approval and most siRNA therapeutics are in an early stage of development. Should companies obtain marketing approval after 2021, these patents will have expired.[65] If early approval is anticipated, rather than litigation, what is more likely is that requests for Inter Partes Review (IPR) of the Carnegie and Tuschl patents will be filed. If successful, the IPRs will clear the way for siRNA therapeutics to be marketed prior

[64] *See,* 35 U.S.C. § 271(e)(1).

[65] While it is possible that Alnylam may obtain marketing approval by 2021 and obtain patent term extension (PTE) for delays in obtaining regulatory approval, the scope of the patent term extension is limited to the approved product, an approved use of the product, or a method of manufacturing the product, if claimed. Thus, a PTE will not affect a competitor from marketing a product other than the approved product subject to the PTE.

to 2021. The advantage of filing an IPR is that validity of the patent is determined on a preponderance of the evidence standard. This is a much lower standard than the clear and convincing evidence standard required in district court litigation.[66] Tellingly, IPRs have been instituted in 83% of IRPs that have been filed as of the end of 2013.

Further, it is unlikely that generic pharmaceutical companies will mount an ANDA challenge to the Carnegie and Tuschl patents. Assuming that Alnylam obtains marketing approval by 2016, which appears unlikely due to the time required for clinical trials and delays in regulatory approval, Alnylam will enjoy a five-year period of "data exclusivity." During this time period, generic pharmaceutical companies are not allowed to rely on Alnylam's clinical data in support of their drug applications. This exclusivity will then expire the same year that the Tuschl II patents expire. If marketing approval comes at a later time, even with patent term extension for delays in obtaining FDA approval, the period of data exclusivity is likely to extend beyond the patent terms. Thus, ANDA litigation concerning the Carnegie and Tuschl patents appears unlikely.

VII. CONCLUSION

By far, Alnylam is the leader in bringing siRNA therapeutics to the market with 6 drugs in clinical trials and one in phase III. It is notable that Alnylam advanced ALN-TTR02 from phase I to phase III trials in less than 2 years, and may be on a fast tract for approval. Given that Alnylam has licenses to the fundamental Carnegie and Tuschl siRNA patents, Alnylam will have a commanding position in the field should they be the first to obtain FDA approval.

[66] There are also disadvantages to filing an IPR including drastic estoppel provisions that bar one from asserting in litigation validity defenses that could have been brought in a previously filed IPR.