Nucleic acid nanomedicines in Phase II/III clinical trials: translation of nucleic acid therapies for reprogramming cells

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This review presents an integrated analysis of the current-state-of-the-art in nucleic acid nanotherapies and highlights the importance of nanotechnology in the delivery of nucleic acid therapies. While there is no one dominant nanodesign, the diversity of nanodesigns and delivery of different siRNAs, miRNA and DNA to inhibit more than 20 targets in seven disease states in Phase II/III clinical trials reflects the potential of nucleic acid therapies to treat intractable diseases and non-druggable targets. We provide benchmarks to aid in comparing the design, proof-of-concept studies and clinical trials. From this, we demonstrate the importance of generating a strategic framework for integrating clinical ‘wish lists’ for a means to treat intractable diseases with engineering ‘design checklists’ for nucleic acid nanotherapies.

Lay abstract: The application of nanotechnology to medicine – nanomedicine – offers an unprecedented opportunity to improve patient outcomes. This integrated review of emerging clinical successes and failures in Phase II/III clinical trials provides insight into how best to design new technologies for the delivery of nucleic acid therapies.

An integrated analysis of the current-state-of-the-art in nucleic acid nanotherapies is presented with an emphasis on medical perspectives in nanomedicine.

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The application of nanotechnology to medicine – Nanomedicine – offers an unprecedented opportunity to improve patient outcomes through the utilization of ultrasensitive diagnostics and improved, and potentially personalized, therapeutics. It is the collaborative integration of advances in technology, biology and medicine that are empowering these transformative changes. To address the lack of effective or curative-intent therapies for the major intractable or life-threatening diseases such as cancer, chronic kidney, liver, lung and heart disease, nano-based nucleic acid pharmaceuticals – in other words, nanotherapies – are being developed as a new class of potentially curative-intent and course-altering therapies [1–3].

Researchers have identified molecular and epigenetic targets with central roles in the etiology, pathology and progression of many of these major diseases. As a result, nucleic acid therapeutic targets are, in many cases, known *a priori*. Similarly, the fundamental mechanisms of action available through nucleic acid therapies are well known as they harness endogenous mechanisms of RNAi at different entry-points along the RNAi pathway such as dsRNA processed by DICER, short-hairpin loop RNA, siRNA and miRNA – incorporated into the RNA-induced silencing complex (RISC) [4,5], and antisense oligonucleotide DNA processed through RNase-H degradation of DNA–mRNA hybrids [6]. These RNAi mechanisms are robust as they serve as host defense mechanisms against DNA and RNA viruses [7] and, as epigenetic regulators, provide differential activity during the different stages of growth, development, aging and disease. Thus, there is significant potential for nucleic acid therapies to provide a targeted and selective therapeutic benefit. However, the promise of nucleic acid therapies has been slower than expected in coming to fruition as a result of several factors [8], including immunotoxicities, extremely short circulation times ($t_{1/2} \sim 10$ min) of naked nucleic acid therapeutics [9–11], and the risk of carcinogenesis resulting from defunctionalized or replication-deficient viral vector delivery systems [12,13]. Pharmacological challenges also plague the nanodelivery systems used to administer these nucleic acid therapies, including limited target cell uptake, nonspecific release from the nanodelivery system, delivery of functional nucleic acid payloads to the RISC in the cytosol, as well as multiple toxicities such as cytokine release syndrome (CRS) or ‘cytokine storm,’ off-target effects or innate immune responses [14–21]. Nevertheless, the combination of modular nanoplatform designs for targeted and organ-enhanced delivery, and the inherent precision of nucleic acid therapies for currently non-druggable, intractable diseases collectively represent a substantial opportunity for therapeutic advancement. Therefore, nucleic acid nanotherapies provide a logical approach to bring to fruition personalized precision medicine wherein genetic and epigenetic information are translated into safe, target-specific, curative-intent nucleic acid therapies.

2017 progress report & reality checks

There are 26 nanomedicines currently in clinical use [20], including 17 US FDA-approved agents and nine nanopharmaceuticals in Phase III clinical trials (see Figure 1 for a schematic of the most common formulations). These therapies can be classified into four major ‘nano’ categories that carry different payloads spanning chemotherapeutics, antimicrobials, cytokines, enzymes and anesthetics (Table 1). Of note is the lack of even one FDA-approved nanodelivery system for nucleic acid therapies, highlighting the challenge of translating these particular nanomedicines [20]. Nevertheless, there are an increasing number of clinical trials for nucleic acid nanotherapies, mostly in Phase I/ II, with some emerging Phase III studies [14–21]. As seen in cancer, stroke and Alzheimer’s disease, there is a significant discrepancy between preclinical efficacy and successful translation of new nanotherapies to become FDA-approved treatments. In retrospect, multiple Phase III failures of different nucleic acid nanodelivery systems reveal that efficient and safe delivery of nucleic acid therapies *in vivo* is a greater challenge than would be projected from the relative ease with which successful *in vitro* or *in vivo* small-animal transfection studies are performed [14–21]. However, the increasing number of clinical trials investigating nanodelivery of nucleic acid therapies substantiates the clinical (and commercial) potential for nanotechnology as a nonviral means to deliver nucleic acid therapies. Together these efforts provide insight into the identification of the ‘optimal’ nanoplatform as well as clinical strategies most likely to facilitate successful clinical translation, as discussed in the following sections.

The integrated analysis of current nucleic acid delivery systems from both a nanotechnology and medical perspectives provided in this review may assist researchers, clinicians and governmental decision-makers in evaluating

Liposome:

Lipid bilayer encloses an aqueous core

Polyplex:

Cationic polymer + nucleic acid self assemble

Lipoplex:

Cationic lipid forms multiple layers

Lipid nanoparticle:

Mixed lipids + PEGylated-lipids + nucleic acid (DNA, siRNA, miRNA)

Stabilized Nucleic Acid Liposome (SNALP):

Mixed lipids + PEGylated-lipids + nucleic acids

Lipopolyplex:

Liposome + polyplex payload

Multifunctional-siRNA/miRNA nanoconjugates:

Multifunctional moieties conjugated to nucleic acids

Figure 1. Schematic of various nanomedicine designs and structures.

GalNAc: N-acetylated galactosamine; PEG: Polyethylene glycol.

Table 1. Nanomedicines (50–200 nm) in Phase III clinical trials or US FDA-approved.

		FDA-approved Targeted v. FDA-approved Non-targeted ■ Ongoing Phase III ■ Failed Phase III – due to lack of efficacy			
Nanomedicines		Liposome ± PEG	PEG-Bioconjugates	Albumin-Nanosphere	Polymeric Micelle
Type of Disease	Cancer	<p>Cisplatin:</p> <ul style="list-style-type: none"> ■ SPI-77 ■ Lipoplatin ■ Aroplatin <p>Cytarabine:</p> <ul style="list-style-type: none"> DepoCyt (lymphoma) <p>Cytarabine + Daunorubicin:</p> <ul style="list-style-type: none"> Vyxeos (AML) <p>Daunorubicin:</p> <ul style="list-style-type: none"> DaunoXome (sarcoma) <p>Doxorubicin:</p> <ul style="list-style-type: none"> Doxil (BRCA, OvCa) Myocet(nonP) (BRCA) ■ ThermoDox <p>Irinotecan:</p> <ul style="list-style-type: none"> Onivyde (mPDAC) ■ Narekt-102(P) <p>Vincristine:</p> <ul style="list-style-type: none"> Marquibo (leukemia) 	<p>Asparaginase:</p> <ul style="list-style-type: none"> Oncospar (ALL) <p>Recombinant granulocyte-colony stimulating factor G (G-CSF):</p> <ul style="list-style-type: none"> Neulasta (neutropenia) Neupogen (neutropenia) 	<p>Paclitaxel:</p> <ul style="list-style-type: none"> Abraxane (mPDAC, BRCA) 	<p>Cisplatin:</p> <ul style="list-style-type: none"> ■ Nanoplatin <p>Paclitaxel:</p> <ul style="list-style-type: none"> ■ Genexol PM ■ Paclical ■ NK-015 <p>Doxorubicin:</p> <ul style="list-style-type: none"> ■ SP1049-C' <p>Docetaxel:</p> <ul style="list-style-type: none"> ■ Bind-014
	Infectious Diseases	<p>Amphotericin:</p> <ul style="list-style-type: none"> Amphotec (fungal infxn) Ambisome (fungal infxn) <p>Cytarabine:</p> <ul style="list-style-type: none"> Depocyt (fungal infxn) 	<p>Interferon-α-2b:</p> <ul style="list-style-type: none"> Pegintron (hepatitis C) <p>Interferon-α-2a:</p> <ul style="list-style-type: none"> Pegasys (hepatitis B or C) 		
	Other		<p>Adenosine Deaminase:</p> <ul style="list-style-type: none"> Adagen (SCID) 		
	Pain-control	<p>Bupivacaine:</p> <ul style="list-style-type: none"> Exparel (pain) 			

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BRCA: Breast cancer; G-CSF: Recombinant granulocyte-colony stimulating factor; infxn: Infection; mPDAC: Metastatic pancreatic adenocarcinoma; OvCa: Ovarian cancer; PEG: polyethylene glycol; SCID: Severe combined immunodeficiency.

the potential of nanomedicines to provide clinical benefit across organ systems and disease states. In particular, we highlight key determinants of efficacy and safety and suggest rationale for why these efficacy/safety determinants may be organ- or tissue-specific, and whether they are differentially affected by the disease or pathophysiological state of the patient. The recognition of the vast spectrum of potential nanotechnology designs/platforms (lipoplexes, liposomes, polyplexes, spherical nucleic acids, DNA origami, nanohybrids, dendrimers, micelles, nanocapsules, dynamic conjugates, nucleic acid nanoconjugates) together with the lessons from documented shortcomings observed in clinical trials [14–21] provides valuable insights into key concepts of material interactions with biological systems and in different disease states at the nanoscale.

These insights will elucidate concepts and design criteria that may assist others in the design of new nanomedicines for the delivery of functionally active nucleic acid therapies, a field that is still in its infancy. Continued study of nucleic acid nanotherapies is warranted due to the potential of nucleic acids to provide curative-intent therapies in almost any disease. For example, identification of a nanoplatform with inherent modular versatility or generality to harness different RNAi targets would be both a basic and clinical breakthrough. Hence, a review of nucleic acid delivery nanotechnologies in current clinical Phase II/III trials is timely.

The goals of this review are, therefore, to: provide a progress report on nucleic acid therapeutics in Phase II or Phase III clinical trials from an integrated clinical and nanodesign perspective; gain insights from both successes and failures of Phase II/III clinical trials that will advance future nucleic acid nanomedicines; and lay out the arguments for and against the paradigm of nanomedicine-mediated nucleic acid delivery to address unmet clinical needs. This review does not cover emerging nucleic acid delivery systems that are still in discovery, preclinical stages or Phase I clinical trials. We begin with an overview of Phase II/III clinical trials of nucleic acid delivery in different organ system diseases to identify the scope of current nucleic acid therapeutics. A matrix analysis of nanotechnology design

Table 2. Overview of nucleic acid nanotherapies in Phase II/III clinical trials by gene target and disease type.

Nucleic Acid Payload	Disease Category						
	Cancer	Infection	Ocular	Renal	Dermal	Metabolic	Genetic Disease
DNA	H19+ tumor cells-DTA: Bladder Ovarian Pancreatic						TTR: ■ Hereditary TTR amyloidosis
	IL-12: Ovarian peritoneal mets						
	wtp53: Glioblastoma Metastatic pancreatic						
siRNA	KRAS ^{G12D} : Local pancreatic	VP24, VP35, Zaire Ebola L-polymerase: ■ ebola virus disease	VEGF-R1: Wet-AMD Choroidal neo-vascularization	p53: Delayed graft function	CTGF: Scars Hyper-trophic scars	ApoB: ■ Hypercholesterolemia	TTR: ■ Hereditary TTR amyloidosis
	PLK1: ■ Liver ■ Liver metastasis Neuroendocrine Colon	All 4 HBV transcripts, cccDNA: ■ HBV-hepatitis	VEGF-A165: Wet-AMD Choroidal neo-vascularization	p53: Acute kidney injury post cardiac surgery		PCSK9: ■ Hypercholesterolemia	GO glycolate oxidase: ■ Primary hyperoxaluria
	PKN3: Metastatic pancreatic	RSV replication, nucleocapsid N-protein: ■ RSV broncho-obliterations	RTP801: Wet AMD Diabetic macular edema				
	Myc: Hepatocellular cancer		Caspase2: Optic neuropathy				
			CTGF: Retinal fibrosis				
miRNA	mir34: Unresectable liver Advanced solid tumors Hematological cancers	anti-miR122: ■ HCV hepatitis		anti-miR21: Alport syndrome		anti-miR103/107: ■ NASH in pre-diabetes and type2 diabetes	

AMD: Age-related macular degeneration; ApoB: Apolipoprotein-B; cccDNA: Covalently closed circular DNA; DTA: Diphtheria toxin-A; HBV: Hepatitis B virus; HCV: Hepatitis C virus; KRAS-G12D: Mutant Kirsten rat sarcoma viral oncogene homolog; Myc: Nuclear phosphoprotein that regulates cell proliferation; NASH: Nonalcoholic steatohepatitis; PCSK9: Proprotein convertase subtilisin/kexin type 9; PDAC: Pancreatic ductal adenocarcinoma; PKN3: Protein kinase N3; RSV: Respiratory syncytial virus; RTP801: Upregulated in retinopathy HIF1a responsive suppressor of mTOR signaling; TTR: Transthyretin; VEGF-R1: VEGF receptor-1.

subclasses and administration routes follows. We then analyze systems that failed in late-stage clinical trials due to safety concerns, followed by those that failed clinical trials due to lack of efficacy. Finally, based on the identified trends and commonalities, we then present a framework that integrates nanotechnology and clinical insights, and that lays an evidence-based argument for the continued development of nanomedicines for nucleic acid therapy.

Review of Phase II/III clinical trials of nucleic acid nanotherapies by organ-specific diseases

Several nucleic acid nanotherapies are under development to address an array of nucleic acid targets underlying organ-specific diseases (Table 2 & Supplementary Table 1). The breadth of scope is shown by the 30 targets spanning three nucleic acid therapy classes – DNA, siRNA and miRNA – in 27 different indications across seven disease categories. Interestingly, the majority of nucleic acid nanotherapies delivered to date are siRNA, with 19 siRNA payloads being tested across seven disease categories (Table 2). Importantly, these siRNAs target distinct genetic pathways implicated in different diseases, such as oncogenic signaling proteins (currently undruggable KRAS^{G12D} and polo-like kinase1), senescence (p53), apoptosis (caspase2), angiogenesis (VEGF-R1 and VEGF-165) promalignant proliferation (Myc, PKN3), vital transcripts of Ebola, hepatitis B and respiratory syncytial viruses, fibrosis (CTGF), hypercholesterolemia (ApoB, PCSK9), familial amyloidosis (transthyretin [TTR]) and hyperoxaluria (glycolate oxidase).

The spectrum of pathogenic siRNA targets reflects the multitarget versatility inherent in nucleic acid therapies and the potential of nanotechnology to capitalize on this versatility. This spectrum is also reflected in the miRNA nanotherapies spanning different miRNAs involved in cancer (miR34), Alport syndrome (miR121), hepatitis C (miR122) and nonalcoholic steatotic hepatitis (miR103/107; Table 2). Reaching Phase II/III clinical trials provides evidence that these nanodesigns (Tables 2 & Table 3) have attained key efficacy benchmarks for nucleic acid therapy. These benchmarks represent major scientific milestones and include: increased circulation time of siRNAs/miRNAs/DNAs to facilitate target tissue accumulation; sufficient protection of the siRNA/miRNA/DNA

Table 3. Representative nucleic acid nanocarriers in Phase II/III clinical trials.

Targeted v. Non-targeted ■ Terminated Due to Toxicity ■ Terminated Due to Non-Efficacy ■ Ongoing Phase III							
Route of Administration	Nanocarriers	Polyplex	Liposome	LNP / SNALP	PLGA-NP	Lipoplex / Polyconjugate	Multi-functionalized Nucleic Acid

	Local	<u>H19-specific PEI polyplex:</u> <ul style="list-style-type: none">• Intratumoral – PDAC• Intravesical – non-muscle bladder cancer FDA-fast track• Intraperitoneal – ovarian cancer			<u>PLGA-NP scaffold:</u> <ul style="list-style-type: none">• Intratumoral – PDAC	<u>PEI-PEG-cholesterol:</u> <ul style="list-style-type: none">• Intraperitoneal – ovarian cancer	<u>End-capped siRNA:</u> <ul style="list-style-type: none">■ Aerosolized – RSV broncho-obliterans■ Intravitreal – wet AMD■ 2'-O-methyl protected RNA:<ul style="list-style-type: none">• Intravitreal – wet AMD■ Self-delivering siRNA:<ul style="list-style-type: none">• Intravitreal – retinal fibrosis• Intravitreal – optic neuropathy• Intradermal – hypertrophic scars <u>Multi-functionalized siRNA nanoconjugate:</u> <ul style="list-style-type: none">■ Delayed graft kidney function• Acute kidney injury cardiac surgery
	Intravenous (bolus or infusion)		<u>DOTAP-DOPE + anti-Transferrin scAb:</u> <ul style="list-style-type: none">• Metastatic PDAC• Glioblastoma <u>Liposome-MRX-34:</u> <ul style="list-style-type: none">■ Liver cancer■ Advanced cancer■ Lymphomas	<u>SNALP:</u> <ul style="list-style-type: none">• TTR-amyloidosis• Liver cancer/metastasis• Hepatitis-B• Hepatitis-C■ Ebola infection■ Hypercholesterolemia <u>LNP:</u> <ul style="list-style-type: none">■ TTR-FAP■ Liver cancer■ Primary hyperoxaluria		<u>Lamellar lipoplex:</u> <ul style="list-style-type: none">• Advanced PDAC <u>Dynamic Poly-Conjugate+cholesterol:</u> <ul style="list-style-type: none">■ Hepatitis-B	
Subcutaneous						<u>mExo-ASO(DNA):</u> <ul style="list-style-type: none">■ TTR-amyloidosis <u>GalNAc-anti-miRNA:</u> <ul style="list-style-type: none">• NASH• Alport syndrome■ HCV hepatitis <u>GalNAc3-siRNA:</u> <ul style="list-style-type: none">• Primary hyperoxaluria• Hypercholesterolemia■ TTR-FAC <u>GalFAC:</u> <ul style="list-style-type: none">• Primary hyperoxaluria	

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BRCA: Breast cancer; G-CSF: Recombinant granulocyte-colony stimulating factor; infxn: Infection; mPDAC: Metastatic pancreatic adenocarcinoma; OvCa: Ovarian cancer; PEG: Polyethylene glycol; SCID: Severe combined immunodeficiency.

ALL: Acute lymphoblastic leukemia; **AML:** Acute myeloid leukemia; **BRCA:** Breast cancer; **G-CSF:** Recombinant granulocyte-colony stimulating factor; **infxn:** Infection; **mpDAC:** Metastatic pancreatic adenocarcinoma; **OvCa:** Ovarian cancer; **PEG:** Polyethylene glycol; **SCID:** Severe combined immunodeficiency.

payload from serum and tissue nucleases; effective biodistribution of nucleic acid therapy; uptake of therapeutic nucleic acids into the target cells; and successful trafficking to the cytoplasm and uptake into RISC (siRNA/miRNA), DICER (dsRNA) or into the nucleus (DNA) [8]. Given that the PD1/PD-L1 inhibitor immunotherapy took 20 years to reach the clinic with multiple Phase III trials still ongoing, the relatively rapid jump to Phase II/III clinical trials for nucleic acid nanotechnologies within a decade is a promising portent of future success.

A matrix analysis of nanodesign subclasses & administration route in Phase II/III clinical trials

A review of nanotherapies in Phase II/III clinical trials shows that there is no singular nanodesign with an advantage over others (Table 3, Figure 1). The only two ongoing Phase III clinical trials use either a lipid nanoparticle (LNP) or a multifunctionalized siRNA-nanoconjugate (Supplementary Table 1). While both nanotechnologies deliver siRNA, the spectrum of nucleic acid deliverables is large and includes DNA, miRNA or siRNA (Table 3). In Phase II clinical trials, a number of different nanotechnologies are represented, including polyplex, liposome, LNPs and stabilized nucleic acid lipid particles (SNALP), biodegradable poly(lactide-co-glycolic) acid nanoparticles (PLGA-NPs), lipoplex hybrids, dynamic polyconjugates and nucleic acid nanoconjugates (Table 3, Figure 1). The compositional and structural diversity of these systems exceeds that of the current FDA-approved nanomedicines (Table 1) and this is, perhaps, a result of the aim to meet greater challenges in delivery of functionally intact nucleic acid therapies as opposed to drugs, biologics or small molecules.

To date, the majority of nanodesigns for nucleic acid nanodelivery systems in Phase II/III are nontargeted lipid-based systems, such as liposomes (hollow, liquid-core, lipid-shell), LNPs (colloidal, solid-core, lipid-shell) and lipoplexes (lipid-polymer complexes). Lipid-based nanodesigns, especially those with diameters of ≤ 100 nm localize to the liver by 'default' via accumulation in Kupffer cells or hepatocytes (Table 3). This is likely due, in part, to the relatively large (150–200 nm) sinusoidal endothelium fenestrations that allow passage of these small particles and, subsequent, exposure to the Kupffer cells - cells of the reticuloendothelial system that are responsible for clearing colloids from the circulatory system. Interestingly, most of these nucleic acid nanodelivery systems are nontargeted (Table 3). While nanoformulations of 'traditional' drugs are designed to avoid liver accumulation in order to achieve wider tissue biodistribution, nucleic acid nanotherapies targeting hepatocyte genetic targets leverage this organ-specific accumulation to their advantage. Passing through the 150–200-nm fenestrated sinusoidal endothelial openings in the liver, these nucleic acid nanotherapies are exposed to both Kupffer macrophage cells lining the hepatic sinusoids and plates of hepatocytes as they drain toward the hepatic central veins, thereby providing a mechanism for direct exposure to, and uptake by, hepatocytes and Kupffer cells and subsequent clearance through the bile secretion system. This mechanism is responsible for successful delivery of RNAi payloads to the liver but, based on the relatively high doses of nucleic acid required, is an inefficient delivery mechanism. Nevertheless, given the central role of the liver as a vital organ for metabolism, detoxification and factor production, hepatocyte-targeted nucleic acid therapies impact not just liver-specific diseases, such as hepatic cancer, hypercholesterolemia and viral hepatitis, but also systemic diseases such as familial amyloidosis and hyperoxaluria that express culprit mutant genes in the liver including TTR and glycolate oxidase, respectively (Tables 2 & Table 3).

Similarly, the localization of intravenously administered siRNA nanoconjugates to the kidney shows that 'default' kidney localization is also a viable method for achieving RNAi delivery when the target pathology is in the kidney. The 6–10 nm fenestrations of the kidney entrap any nanodelivery system with a size greater than 10 nm. One example of successful delivery to the kidney is in delayed kidney graft function and acute kidney injury (Tables 2 & Table 3). It can be deduced that kidney localization of siRNA nanoconjugates does not worsen kidney graft function or acute kidney injury but, rather, alleviates them. This observed efficacy reiterates the potential of nucleic acid nanotherapies to address intractable diseases via nonobvious, novel therapeutic paradigms at the nanoscale.

Interestingly, these nanotechnologies are delivered through nine different administration routes (Table 3). By using these different administration routes, many of the limitations of early first-generation nucleic acid nanotherapeutics can be overcome. For example, delivery via the locoregional intravesical/bladder or intraperitoneal route of polyethylenimine (PEI)-DNA polyplexes overrides the systemic toxicity of branched PEI. This results in effective delivery of a plasmid DNA expression vector for diphtheria toxin driven by tumor-cell specific H19 promoter to nonmuscle bladder cancer and ovarian peritoneal cancer. The feasibility of intravitreal administration is also demonstrated with no safety concerns in Phase III clinical trials (Table 4); however, the intravitreal administration of anti-VEGF 'naked' siRNAs for wet aging-associated macular degeneration attained no target-specific efficacy (Table 5). Negative efficacy results in the Phase III trial with naked anti-VEGF siRNA (Bevasiranib) highlight the

Table 4. Nucleic acid nanocarriers that failed clinical trials due to toxicity.

Nanocarrier	Payload	Safety and Toxicity Results	Efficacy Results	Status
<ul style="list-style-type: none"> • Type of carrier • Targeting moiety • Target organ • Administration route 	<ul style="list-style-type: none"> • Dose • Disease 			
1. mrX34 <ul style="list-style-type: none"> • liposome (120nm) • non-targeted • liver • intravenous 	Anti-miR34* <ul style="list-style-type: none"> • 70 mg/m² • unresectable liver cancer or metastasis 	<ul style="list-style-type: none"> • 5 immune related severe adverse events • severe grade 4 cytokine storm • 3 of 5 affected patients died 	None reported	Phase I Clinical Trial Terminated IND placed on clinical hold by FDA
2. Revusiran <ul style="list-style-type: none"> • GalNAc3-siRNA • targeted-ASGPR1 • liver • subcutaneous 	siRNA*-TTR <ul style="list-style-type: none"> • 500 mg/dose • hATTR-cardiomyopathy 	<ul style="list-style-type: none"> • Increased mortality in treated (18) group vs placebo (8) 	☒ At 6 months, 92.7% TTR knockdown wt = mutant; stable 6-minute walk distance vs FAC historic decline of 20-24 meters after 6 months	Phase III Clinical Trial Terminated
3. ARC-520 <ul style="list-style-type: none"> • polyconjugate • targeted-cholesterol • liver • intravenous 	siRNA-HBV cccDNA <ul style="list-style-type: none"> • 2-4 mg/kg • HBV-hepatitis 	<ul style="list-style-type: none"> • FDA placed on hold due to primate death at a very high dose (NOTE: dose higher than clinical study) 	☒ Single dose reduced HBV surface antigen 60-80%	Phase IIb Clinical Trial On Hold IND placed on clinical hold by FDA
4. RG-101 <ul style="list-style-type: none"> • GalNAc3-miRNA • targeted-ASGPR1 • liver • subcutaneous 	Anti-miR122 <ul style="list-style-type: none"> • 4 mg/kg • HCV-hepatitis 	<ul style="list-style-type: none"> • FDA placed on clinical hold due to 2 patients developing jaundice (however, both cases were complex: #1: HCV + ESRD; #2: HCV + CAD + Type2 Diabetes) 	☒ 15 of 28 patients with HCV RNA levels below level of quantitation (BLOQ); 12 of the 15 were Target Not Detected. Cohort included difficult to treat genotypes: liver fibrosis; viral relapse after prior IFN-containing regimen.	Phase II Clinical Trial On Hold IND placed on clinical hold by FDA
5. TKM-080301 <ul style="list-style-type: none"> • SNALP • non-targeted • liver • intravenous 	siRNA : PLK1 <ul style="list-style-type: none"> • 0.9 mg/kg • advanced liver cancer or metastasis 	<ul style="list-style-type: none"> • severe cytokine storm with grade 3 thrombocytopenia, hypoxia and dyspnea • to avoid cytokine storms, dose reduced to 0.75 mg/kg for extension efficacy study: 33% rigors, 25% fever, 13% infusion reaction. 	☒ 51% of patients with stable disease; 22% with partial response; tumor density reduction of up to 59% was observed.	Phase I/II Clinical Trial Company looking for partner

Clinical trial information is given as per nanotherapy listed in Supplementary Table 1.

ASGPR1: Liver-specific asialoglycoprotein receptor-1; **CAD:** Coronary artery disease; **cccDNA:** Covalently closed circular DNA; **ESRD:** End-stage renal disease; **GalNAc3:** Trivalent *N*-Acetyl galactosamine nanoconjugate; **hATTR:** Hereditary transthyretin-mediated amyloidosis; **HBV:** Hepatitis B virus; **HCV:** Hepatitis C-virus; **IFN:** Interferon; **PLK1:** Polo-like kinase-1; **SNALP:** Stabilized nucleic acid lipid nanoparticle; **TTR:** Transthyretin.

insufficiency of naked nucleic acid therapies and the need for nanodelivery systems to attain intracellular delivery of functionally intact nucleic acids to be effective (Table 5).

The subcutaneous (subQ) administration route demonstrates feasibility and versatility with multifunctionalized antisense oligonucleotide (ssDNA), anti-miRNA and siRNA payloads. The successful knockdowns of multiple gene targets in different gene networks: TTR, glycolate oxidase, apolipoprotein B, proprotein convertase subtilisin/kexin type 9 (PCSK9), polo-like kinase-1 (PLK1), Myc, as well as hepatitis viral gene transcripts (Tables 2 & Table 3) showcase the breadth of the potential of this approach. However, as shown in Table 4, the subQ route for administration of nucleic acids using a trivalent *N*-acetylated galactosamine (GalNAc3)-TTR-siRNA attains molecular knockdown and provides a clinical convenience, but does not prevent cytokine storm risk in Phase III clinical trials in familial amyloidotic cardiomyopathy patients.

Analysis of safety failures of nucleic acid nanotherapies in clinical trials

Review of the four siRNA nanotherapies and one miRNA nanotherapy (mrX34) that failed in Phase II/III clinical trials due to safety concerns demonstrates that all five nucleic acid nanodelivery systems involve hepatic uptake resulting from either targeted accumulation (three out of five) or nontargeted default-liver localization (two out of five; Table 4). Notably, toxicities are observed in both miRNA and siRNA systems, both lipid-based and nonlipid-based nanodesigns administered via subQ and intravenous administration routes and in different diseases: liver cancer, hepatitis and familial TTR-mediated amyloidosis.

More specifically, of the four Phase II/III studies terminated or placed on hold due to FDA-safety concerns, three involved targeted liver-specific nanoconjugate designs. One nanopatform, ARC520, is a cholesterol-targeted polyconjugate administered intravenously to deliver siRNA-targeting hepatitis B viral cccDNA in patients with chronic hepatitis B (Table 4& Supplementary Table 1). ARC-520 was placed on hold due to a late death, albeit in a high-test dose primate, during the Phase II clinical trial. The cause of death is not reported to date. The other two liver-specific nanoconjugates targeted the hepatic-specific asialoglycoprotein receptor via a GalNAc3 moiety conjugated to a stabilized siRNA. One nanopatform delivered siRNA to inhibit TTR in familial amyloidosis cardiomyopathy patients (Revusiran – Alnylam, Inc.), and the other delivers miR-122 in patients with

Table 5. Nucleic acid nanocarriers that failed Phase II/III clinical trials due to lack of efficacy.

Nanocarrier • Type of carrier • Targeting moiety • Target organ • Administration route	Payload • Dose • Disease	Safety and Toxicity Results	Efficacy Results	Status
1. DCR-MYC • LNP • non-targeted • liver • intravenous	siRNA:myc • liver cancer	☑ Well tolerated	No efficacy; evidence of delivery but knockdown less than projected; no clinical activity	Phase I/II Clinical Trial Discontinued by Dicerna
2. ALN-RSV01 • mFxn-dsRNA • non-targeted • bronchi • aerosolized	siRNA: RSV nucleocapsid N protein • RSV bronchiolitis	☑ Well tolerated	No efficacy; missed primary endpoints, including: decrease in viral parameters, symptom scores, bronchiolitis obliterans	Phase IIB Clinical Trial Discontinued by Anylam
3. TKM-130803 • SNALP • non-targeted • liver • intravenous	siRNA: Ebola VP24, VP35, L-polymerase • ebola virus disease	☑ Well tolerated; only 1/56 patients experienced an infusion reaction	No efficacy; reached futility endpoint	Phase II Clinical Trial Terminated by Arbutus
4. AGN211745 (sirna-027) • end-protected shRNA • Non-targeted • eye • intravitreal	shRNA: VEGFR1 • age-related wet AMD	Some safety concerns	No efficacy	Phase II Clinical Trial Terminated
5. Bevasiranib (Cand5) • siRNA naked • non-targeted • eye • intravitreal	siRNA: VEGF-A 165 • age-related wet AMD choroidal neovascularization	☑ No safety concerns	No efficacy	Phase III Clinical Trial Terminated

Clinical trial information is given as per nanotherapy listed in Supplementary Table 1.

LNP: Lipid nanoparticle; mFxn: Multifunctionalized; RSV: Respiratory syncytial virus; SNALP: Stabilized nucleic acid lipid nanoparticle; VEGFR1: VEGF receptor-1.

chronic hepatitis C (RG-101). Both are administered subcutaneously. Notably, the cause of increased deaths in the Revusiran cohort (500 mg/dose) has not been reported. RG-101 was placed on hold due to two test patients developing jaundice but no deaths (Table 4). The fourth siRNA nanotherapy terminated in Phase I/IIa comprised a nontargeted lipid-based nanodesign, SNALP, delivering siRNA to inhibit PLK1 in patients with advanced liver cancer, TKM-080301 (Arbutus, Inc.). A Grade 3 CRS (with worst being Grade 4) was observed with a 0.9 mg/kg of TKM-080301; whereas no cytokine storms were observed with lower dose at 0.75 mg/kg (Table 4). The fifth nanopatform terminated due to safety concerns is a nontargeted liposomal nanotechnology combining PEGylated, neutral and cationic lipids to deliver miRNA-34, MRX34 (MirnaTherapeutics). However, MRX34 clinical trial was terminated in Phase I and the Investigational New Drug Application (IND) placed on clinical hold by the FDA due to five immune-related severe adverse events with severe Grade 4 (worst level) CRS, resulting in three deaths among the five CRS-affected patients. It should be noted that the Phase I study was designed to identify the maximum tolerated dose and dose-limiting toxicity at the end of one treatment cycle in 3–6 liver cancer patients (NCT01829971, ClinicalTrials.gov).

While the ‘default’ liver uptake is leveraged to deliver nucleic acid therapies to the liver, the toxicities observed including CRS or cytokine storm in Phase II/III studies, as well as in a Phase I study that was designed to identify the maximum tolerated dose and dose-limiting toxicity, highlight the liver’s complex roles in immune response and acute phase response. The observation of cytokine storm or CRS [22] across multiple distinct targeted and nontargeted nanodesigns with different payloads and administration routes, but with the shared commonality of liver as the site of action, collectively suggests a hyperactive response to the liver-localized or liver-targeted nucleic acid nanodelivery system. While definitive studies need to be performed, a putative hyperactive CRS response is concordant with a ‘primed hepatic immune system’ to be expected in the presence of chronic intractable diseases with chronic low grade, if not overt, inflammation as occurs in viral hepatitis, familial amyloidotic cardiomyopathy and cancer.

While a neoepitope exposed on a putative protein corona on each NP has been considered a key factor in immune activation *in vivo* by nanoparticles [23], this review of current Phase II/III studies for nucleic acid nanotherapies suggests that the liver's role as an immunologically complex organ [24] needs to be factored in at the design stage. This is concordant with the fact that different cells in the liver (macrophage Kupffer cells [25], liver sinusoidal endothelial cells [25] and hepatocytes [26]) are all capable of producing IL-6, the key cytokine upregulated in CRS cytokine storms [22]. The presence of IL-6 receptor on IL-6-producing hepatocytes and T/B cells sets up autocrine and paracrine feedback loops, which then amplify the cytokine cascades in CRS 'cytokine storms.' This relationship provides a putative common mechanism for different nanodesigns localized to the liver and associated with CRS cytokine storms in clinical trials (Table 4). While a nanoparticle-specific or patient-specific protein corona [23] could be involved, the direct activation of the liver immune system, especially if primed by low-grade chronic inflammation in intractable diseases, would better explain the differential CRS toxicity in some but not all patients. This is particularly true given that a nanodesign-driven neoepitope immune activation is expected to be more generalized.

Additionally, just as dose–response effects are observed in CRS detected in the clinical trial of TKM-080301, the occurrence of severe CRS in some but not all Phase II/III patients tested with the identical nanodelivery system and payload, respectively, suggests that disease-stage severity and/or co-morbidities likely play a determining role in vulnerability to development of cytokine storm responses. Likewise, although nucleic acids – dsDNA or ssRNA – are expected to trigger TLR-mediated innate immune responses similar to viral dsDNA and ssRNA [27], the observed occurrences of severe CRS in a minority of patients also support the notion that disease-stage severity and co-morbidities are the key determining factors, and that nucleic acid nanotherapy designs can be successful in attenuating the innate immune response to nucleic acid payloads. This is similar to the increased risk for CRS resulting from adoptive T-cell immunotherapy or CAR-T immunotherapies in cancer patients with greater tumor burden [22]. Therefore, just as CRS is managed to avoid life-threatening complications in adoptive T-cell therapy in cancer, we suggest the same clinical paradigm has the potential to also be applied to nucleic acid nanodelivery systems, thereby facilitating the safe use of the therapy and avoiding automatic termination of late-stage clinical trials. Corresponding dose–response studies of efficacy coupled with dose–response effects on hepatic immune activation should help identify the optimal dose and therapeutic index for liver-homing or liver-targeted nanotechnologies.

Analysis of efficacy failures of nucleic acid nanotherapies in Phase II/III clinical trials

Just as the review of safety failures in Phase II/III clinical trials is critical to the development of nanomedicines, a review of efficacy failures in Phase II/III clinical trials is also critical. As shown in Table 5, comparative analysis of Phase II/III studies shows that naked nucleic acid therapies (naked siRNA) or end-protected dsRNAs, even if delivered directly to the target tissue via injection, is frequently insufficient to achieve efficacy. This may be due to lack of uptake in the target cell, degradation in the ECM or in the cytoplasm among other factors. This observation reiterates the need for nano-encapsulated or nano-conjugated delivery systems that can deliver functional nucleic acid payloads to the terminal intracellular sites, including RISC for siRNA/miRNA, cytoplasmic RNA for antisense oligonucleotides and nuclear transcriptional machinery for DNA payloads. However, also shown in Table 5, two lipid-based nontargeted NPs for hepatic delivery in Phase II/III failed to meet efficacy milestones, along with two cap-protected RNA payloads, despite demonstrating preclinical efficacy. Intriguingly, all Phase II/III studies that were discontinued – due to missed efficacy end points or terminated due to futility – involved nontargeted nanodesigns.

Nevertheless, further trend analyses of efficacy failures (Table 5) also reveal key insights applicable to the development of nucleic acid nanotherapies, as follows.

Feasibility of aerosolized administration route

Aerosolized drug delivery is relatively well tolerated – given its wide-spread use to treat asthma – opening an avenue for locoregional delivery for nucleic acids. However, while demonstrated safety of aerosolized delivery of thymidine-capped dsRNA is a step forward (ALN-RSV01), the efficacy failure of this aerosolized T-capped dsRNA (ALN-RSV01) to decrease viral titers in Rous sarcoma virus (RSV) infections indicates potential multifactorial issues for aerosolized nanodelivery systems that need to first be addressed. These may span a broad spectrum of delivery issues, including physical failure to reach infected epithelia in lower bronchi and bronchioles, especially in areas with RSV-induced bronchiolar obstruction; poor uptake by infected alveolar epithelia; the inability to override ongoing RNAi usurped by RSV via miRNAs by siRNA panel; and, more broadly, the need for improved

nanodelivery systems. Despite these setbacks, we suggest that successful delivery via the aerosolized route may be useful for other indications, such as delivery of RNAi nanomedicines for pulmonary diseases.

The use of nanodelivery systems for infectious diseases

Analysis of [Tables 5](#) and [Table 2](#) shows that even with identical nanodesigns, two nucleic acid therapies may exhibit different efficacy outcomes for liver infections: Tekmira Pharmaceuticals Corp.'s Hepatitis B virus (TKM-HBV; TKM-001467) demonstrated efficacy in Phase IIb at 0.4 mg/kg dose in contrast to their Ebola virus (TKM-130803) which did not, although both were well tolerated. While TKM-HBV demonstrates feasibility of halting chronic infections, this 'twin-like NP study' suggests that selection of targets maybe more critical than the vehicle, or that the composition and resulting physicochemical properties of the combined nucleic acid/vehicle carrier plays a more critical role than would otherwise be expected.

The failure of siRNA therapy in Ebola virus disease is likely multifactorial. Although this therapy worked in nonhuman primate studies with recombinant Ebola viral proteins, nanodelivery to the liver did not work in Phase II for systemic Ebola virus disease, since Ebola virus infects not just the liver but also the lung, spleen, and kidney, and causes increased permeability of the systemic vascular endothelium [28]. In contrast, HBV is primarily a hepatic infection. Moreover, since Ebola virus proteins VP30, VP35 and VP40 are proven suppressors of RNA silencing (SRSs) [29], the TKM-Ebola, which inhibits only VP35, VP24 and L-polymerase, does not suppress VP30 and VP40. Thus, it may be expected that this therapy provides insufficient override of the Ebola virus, thus explaining the observed nonefficacy in the Phase II trial of TKM-130803 for Ebola. Unlike TKM-HBV, which inhibits all HBV transcripts, TKM-Ebola targeted only three of the seven EBV transcripts, which suggests that nonefficacy of TKM-Ebola is likely due to EBV escape or resistance since only three of seven EBV transcripts were halted.

Altogether, these nonefficacy Phase II/III studies reiterate the challenges in translating an effective nanodelivery system to the clinic, and that selection of the target(s) for the nucleic acid nanotherapy is a critical step in the process.

Analysis of nanotechnology & clinical medicine insights

Nanomedicine has finally reached the convergence of scientific opportunity and recalcitrant medical challenges. Now, the potential of nanomedicines to aid and even, in some diseases, offer durable cures, is both exciting and worthy of significant continued effort. Importantly, some nanodesigns demonstrate efficacy in multiple disease applications, albeit also exhibiting failures due to toxicity or nonefficacy in others ([Table 1 to 4](#)). These successful multiapplication nanodesigns comprise nontargeted nanoplateforms: PEI-polyplex for bladder, pancreatic ductal adenocarcinoma and ovarian cancers; SNALP, for liver cancer, hepatitis B, hepatitis C, TTR-mediated familial amyloidosis; and liposomes for metastatic pancreatic ductal adenocarcinoma and glioblastoma. Similarly, emerging targeted nanodesigns exhibit multiapplication successes, including GalNAc3-siRNA for primary hyperoxaluria, hypercholesterolemia; and GalNAc-miRNA for nonalcoholic steatotic hepatitis and Alport syndrome. Notably, the multiple successes span different disease categories reiterating the potential broad impact of nanotherapies.

The modular potential of nucleic acid nanodelivery systems for combinatorial payloads, targeting moieties, microenvironment-stimuli response and modulation, among others, may benefit from a design framework guided by clinically oriented efficacy and safety parameters from the outset. Such a rubric is best designed through integrating the experience of both nanoengineering and medicine collaborators. More specifically, nanoengineering, clinical and translational medicine collaborators can establish metrics of nanodesigns (size, charge, surface properties, intracellular capabilities) for intended clinical applications from the outset, matched with translational guideposts (efficacy and safety parameters) on nanomedicine performance in disease-specific settings. These can be integrated into a disease-specific roadmap that outlines: known or projected disease-specific nanomedicine interactions, disease-specific considerations in nanoparticle-biological system interactions regarding *in vivo* cell reprogramming and epigenetic-resetting, and validated *in vitro* and *in vivo* preclinical testing parameters that are predictive of clinical efficacy and safety.

Altogether, consideration of the above in the advancement of nanomedicines is best integrated into an actionable medical 'wish list' and corresponding actionable nanoengineering 'checklist' in order to guide the study of disease-specific nucleic acid nanomedicines along the research-development continuum. Below, we provide a 'first draft' of what these wish lists and checklists may look like.

Box 1. miRNAs validated to be nodal in different disease categories**Cancer**

- Metastasis: miR-9, miR-212
- EMT: miR-155; decreased miR-200 family, miR-192
- Apoptosis resistance: miR-21
- Oncogenesis: miR-122, miR-148a, miR-363 Tumor suppressive miRNAs: miR-15a, miR-26, miR-29, miR-30 and let-7 families, miR3

Fibrosis

- miR-20a, miR-21, miR-27a, miR-27b, miR-29a, miR-29c, miR-92a, miR-122, miR-146a, miR-155, miR-221, miR-222, and miR-224

Heart failure

- miR-101-a
- Decreased in border zone of MI: miR-29a, miR-29b, miR-29c miR-149
- Decreased in human cardiac hypertrophy: miR-30, miR-133
- Canine atrial fibrosis: ↓miR-133, miR-590

Chronic kidney disease

- Human: miR-let7b, let7f miR-16, miR-18a, miR-27a, miR-30d increased: miR-21 (i.e., needs antagomirs)

Chronic liver disease

- Advanced F3-4 stage fibrosis: miR-182, miR199a-5p, miR-200a-5p and miR-183
- Early stage F1-F2: miR-148-5p, miR-1260b, miR-122-3p and miR-378i

Chronic lung disease

- Anti-fibrotic miRNAs: (induction reduced fibrosis – idiopathic pulmonary fibrosis, silicosis) miR-26a miR-486-5p miR-489

A nanomedicine ‘medical wish list’ for nucleic acid therapies will encompass the following Identification of unmet clinical needs with suboptimal or no therapy

Molecular basis of disease: clarification of clinical unmet need; identification of cell targets; prioritization of payload(s) to deliver, or imaging to be attained or both; and identification of disease progression and treatment response biomarkers or surrogate markers.

For example, hepatic fibrosis associated with persistent inflammation is a high unmet need as hepatic fibrosis is progressive, leading to chronic liver disease and failure. Disease progression is further complicated by the vital role of the liver in inflammation, immune response, detoxification and metabolism. Thus, for therapy to be successful, antifibrosis therapy would *a priori* require targeting of RNAi payloads to profibrotic pathways in hepatocytes and hepatic stellate cells (e.g., profibrotic miRNAs, chronic liver disease miRNAs), as well as RNAi payloads to attenuate proinflammatory pathways in Kupffer cells and hepatocytes (Box 1). Strategic aligning of preclinical and clinical studies could be facilitated by biomarkers for chronic liver disease/fibrosis such as circulating levels of ECM proteins (hyaluronic acid), matrix metalloproteases (MMPs), MMP inhibitors (tissue inhibitors of MMPs), as well as fragments of procollagen III [30]. Intuitively, the complexity of chronic intractable diseases will most likely be better addressed by the modular versatility inherent in nanodelivery systems for nucleic acid therapies, as evidenced by the fact that to date, there is no curative-intent ‘traditional’ drug for chronic liver disease.

Limitations of current clinical therapies/interventions: identification of standard-of-care limitations; and analysis of Phase II/III clinical trial failures of traditional or nanotherapeutics.

Identification of disease-specific co-morbidities that affect nanomedicine efficacy & safety

Potential confounders of safety, such as (*list not exhaustive*): chronic low-grade inflammation: cytokines and inflammatory cell levels; alterations in circulating proteins that could affect the biocompatibility of nanomedicines; and presence of primed biological first responders: mononuclear phagocyte system, innate immune system, complement system, endothelial activation and hemostasis/coagulation system.

Potential confounders of efficacy, such as (*list not exhaustive*): changes in extracellular matrix in disease-target tissue; status of microvascular health, endothelial activation; and metabolic imbalance, liver function and kidney function.

Prioritization of potential mechanism-based deliverables

Nodal epigenetic RNAi deliverables: miRNA mimics or anti-miRNAs; dsRNA or siRNA; and antisense oligonucleotides ssDNA.

While siRNAs and antisense oligonucleotide ssDNAs are gene specific, therapeutic miRNA deliverables require prevalidation for selection. A search of the literature reveals that key or nodal miRNAs are identified in different disease categories (). As shown for siRNA, the potential for reversing these epigenetic changes at the miRNA level is feasible. Identifying which ones or which combinations to restore and at what time point will be important and potentially high impact.

A nanomedicine 'nanotechnology checklist' for nucleic acid therapies will encompass the following

Optimal design to attain efficacy & safety

Regardless of nanodesign and payload, a nucleic acid nanotherapy must meet criteria for payload protection, payload delivery to target site and safety (Figure 1). Based on the analysis of current nanotherapies in Phase II/III clinical trials, the balanced attainment of all three are necessary for the optimal nanodesign.

Interestingly, comparative analysis of different multifunctionalized siRNA or miRNA nanoconjugates with different outcomes (e.g., safe and effective vs efficacious but unsafe vs safe but nonefficacious [Table 5]) reveals that the effective dose of nucleic acid nanotherapy used in clinical trials tends to be predictive of efficacy and safety. Higher dose levels (≥ 5 mg/kg) are associated with nonsafety, while too low a dose is nonefficacious but safe. The exceptions are the efficacious and safe 10 mg/kg used in delayed graft function in kidney transplant patients which are likely due, in retrospect, to immunosuppressive therapy given to kidney transplant patients. Similarly, the high doses used in locoregional delivery, 5 and 10 mg/kg are effective and safe as they are administered intradermally into collagen-rich target scar tissue. Notably, even at high dose of 5 mg/kg, 'naked siRNA' administered intravitreally was not effective, thus reiterating the need for a nanodelivery system. This trend remains to be tested with more nanotherapies in the future, and in the interim, can serve as a guide for efficacy and safety projections.

Testing of efficacy & safety in the context of disease severity & co-morbidities

Many late-stage clinical trial failures indicate a discordance of preclinical and Phase I safety studies, which are typically performed in healthy volunteers, and Phase II/III efficacy/safety studies that are performed in the presence of disease. To address this problem, preclinical testing and characterization of nanoparticle pharmacological properties (biodistribution, clearance, efficacy, toxicity, off-target effects, etc.) should be performed in the milieu of disease co-morbidities – and these simulated in *in vitro* and *in vivo* systems. To be predictive, experimental testing systems should simulate paradigms of disease co-morbidities, such as low-grade cytokine elevation, which lowers the threshold for cytokine storm syndromes; disease-associated metabolic imbalances, which predispose patients to nonimmune toxicities; and disease-associated perturbations in microvascular dysfunction, endothelial activation and/or renal/hepatic clearance, which individually and collectively may decrease the therapeutic index, thereby leading to late-stage Phase III toxicity and/or nonefficacy. Similarly, the preclinical testing of potential off-target effects of RNAi deliverables, which could contribute to toxicity, is best evaluated in the complex milieu of disease pathogenesis with co-morbidities simulated in preclinical *in vitro* and *in vivo* models. The need for improved *in vitro* and *in vivo* models can be attested to by any who attend basic and clinical research conferences and workshops – at least one session of nearly all events is dedicated to a discussion of the need for and strategies to develop improved *in vitro* and *in vivo* models to more accurately predict human responses.

For example, testing of nanotherapies should be done in advanced-stage xenograft tumor models. More specifically, xenograft models of metastasis using therapy-resistant tumor-initiating cells or cancer stem cells, such as pancreatic peritoneal metastasis [31,32] or metastatic lung and liver xenograft models [33,34], should provide more disease context, relevant co-morbidities and *in vivo* recapitulation of inflammation all seen in Phase III clinical trials, rather than heterotopic or orthotopic primary tumors. Additionally, the testing of integrated efficacy and safety is best measured in survival studies in cancer models. The use of tumor volume changes has already been shown to translate poorly to preclinical efficacy.

In the context of single-mutation diseases, testing in the corresponding genetically engineered mouse model should be performed at an advanced stage to recapitulate the patients who will form the majority of the Phase II/III clinical trials. To test both efficacy and safety in the advanced disease stage are essential. Correlation of preclinical

with clinical efficacy may be facilitated by the use of biomarkers that are relevant to advanced- or late-stage disease patients.

Importantly, testing in older animal also adds a clinically realistic benchmark, since chronic inflammation increases with age and contributes to, and often exacerbates, most diseases. *A priori*, while these models are more costly at the preclinical stage, cost-effectiveness is recovered, if not amplified, through a higher proportion of successful Phase III clinical trials. Testing in these ‘tough’ models that recapitulate the full breath and complexities of human diseases may help to eliminate nanotherapies that are vulnerable late-stage clinical trial failures.

Testing of potential innate immune response(s) to the nanodelivery system & its components

Based on the observation that Phase II/III studies are terminated due to cytokine storms or CRS which were not detected in earlier preclinical studies or in Phase I clinical trials, testing for risk of cytokine storms triggered by nucleic acid nanodelivery designs would lead to invaluable information when incorporated into nanotechnology lead selection parameters during research development. Additionally, Phase I/II studies in patients with the intended disease application could be more informative than Phase I in normal volunteers.

A Nanomedicine ‘Integrated Development-Regulatory Process’ for nucleic acid therapies will encompass the following. Equally important to elucidating and aligning the ‘Medical Wish Lists’ and ‘Engineering Checklists’ is the need to integrate the government (e.g., EU, FDA, etc.) filing process into the nanomedicine development process. Because of the central role of industry in the clinical development phase, the clinical trials database needs to record the key features of nanoparticles (size, charge, composition and payload), dose and results, especially of efficacy and safety, in electronic databases (e.g., ClinicalTrials.gov) as a prerequisite for regulatory review and processing, with links to publications if any. This will be critical to advancing the field and will facilitate development. Currently, information gathering suffers from nonuniform sources of information spanning publications, patent publications, government databases, equity research analysis, company pipeline descriptions and investor statements. On a practical note, once intellectual property (IP) is protected, the shared information will accelerate the advance of nanomedicine development as a therapeutic class fulfilling its potential, especially in intractable diseases with no current therapies.

Conclusion

This integrated medical and nanotechnology review of the nucleic acid nanotherapies in Phase II/III clinical trials of patients with intractable diseases reaffirms the potential of nucleic acid therapeutic approach and the importance of nanodelivery systems. Objective medical assessment of Phase II/III clinical trial failures in safety or efficacy in some nanodesigns, as well as in some, but not all, patients per nanodesign, provide new perspectives to improve current nanodesigns, and current preclinical testing and clinical trial designs. Molecular medicine advances in the identification of novel molecular epigenetic and genetic targets, coupled with evidence of emerging successes of nucleic acid nanodelivery systems in multiple intractable diseases, despite some Phase II/III setbacks, reinforce the mandate to advance nucleic acid nanotherapies. This review documents that while we are ‘not there yet,’ we are ‘getting there’ and, more importantly, we are learning ever more ‘how to get there’ because we ‘need to get there.’

Future perspective

The identification of preclinical testing paradigms that increase the translational success of nanotherapies will accelerate the development of nucleic acid nanomedicines. With each FDA approval will come a more well-defined understanding of the nanodesigns and components necessary to successfully fulfill the paradigms nanomedicine-based delivery. As we decipher this ‘translational code,’ a vast array of ever increasing versatility and design will become available to both the researcher and the clinician. Solving this preclinical-to-clinical translational bottleneck will be a watershed moment in the nanomedicine.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/nnm-2018-0122

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Executive summary

- Review recent clinical progress in nucleic acid nanotherapies.
- Discuss the failures in both safety and efficacy in Phase II/III clinical trials and potential underlying causes.
- Describe the delivery of different siRNAs, miRNA and DNA with various nanotechnology-based systems (nanoparticle/nanoassembly).
- Contextualize the findings and provide a framework for integrating clinical 'wish lists' for a means to treat intractable diseases with engineering 'design checklists' for nucleic acid nanotherapies.

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