

## REVIEW

# Clinical Pharmacology Studies Supporting Oligonucleotide Therapy Development: An Assessment of Therapies Approved and in Development Between 2012 and 2018

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Synthetic nucleotides that utilize RNA-centric pharmacology can target diseases at the RNA level, thus altering protein expression in ways previously inaccessible to small molecules and therapeutic biologics. Recognizing that the unique pharmacology of oligonucleotides may require specific considerations in pre-approval assessment, clinical and nonclinical pharmacology studies being conducted for a selected set of oligonucleotide therapies in a 6-year period were assessed. This investigation focused primarily on the four following areas: (i) drug-drug interaction (DDI) potential, (ii) organ impairment (i.e., renal and hepatic impairment), (iii) immunogenicity, and (iv) cardiac safety. Data were summarized and assessed from 14 Investigational New Drug programs and 7 New Drug Applications submitted to the US Food and Drug Administration (FDA) from the period of January 2012 to August 2018, encompassing 152 unique studies. The assessment of DDI potential was largely consistent with the recommendations of current DDI-relevant guidances. Limited data were available to provide recommendations across organ impairment categories. Limited data on immunogenicity indicate impact on pharmacokinetic, the impact on safety and efficacy, although not extensively evaluated, appeared negligible. Cardiac safety evaluation indicated a potential for discordant translation of risk from nonclinical studies to clinical findings. Continued experience with synthetic oligonucleotide therapies will help inform the development of best practices to support their development and regulatory approval.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Oligonucleotide therapies are an emerging therapeutic modality and limited data are available on how clinical pharmacology studies are supporting the development and approval of oligonucleotide therapies.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This investigation evaluated the clinical pharmacology studies that are being conducted to support the development of oligonucleotide therapies and to inform regulatory decision making. This review assessed studies focused on four topics: (i) drug-drug interaction (DDI) liability, (ii) organ impairment studies, (iii) immunogenicity, and (iv) cardiac safety.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ In this investigation, the majority of oligonucleotides were for rare diseases. Dedicated renal and hepatic

studies are not being routinely conducted during drug development to assess organ impairment resulting in an inability to provide specific recommendations for these patients. In addition, *in vitro* studies appear to be relied on as the primary means to assess DDI liability, whereas immunogenicity and cardiac safety are being assessed using both nonclinical and clinical studies.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study describes the landscape of clinical pharmacology studies performed in the course of oligonucleotide therapeutics development.

Oligonucleotide therapies have emerged as a viable therapeutic modality and the pipeline is growing across multiple therapeutic areas.<sup>1–3</sup> The US Food and Drug Administration

(FDA) has approved 9 RNA-centric oligonucleotide therapies (fomivirsen, mipomersen, eteplirsen, nusinersen, inotersen, patisiran, givosiran, golodirsen, and vitolarsen), 8 of which

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were approved since 2013. Oligonucleotides are distinct from small molecules and biologics in both their physicochemical properties as well as their mechanisms of action. Oligonucleotide therapies are typically synthetically modified, single-stranded or double-stranded RNAs or RNA/DNA hybrids that include antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), microRNAs, and antagomirs. Of particular distinction is that oligonucleotides can be designed to hybridize to complementary targets to modulate specific gene expression across the genome. Additionally, compared with small molecules, oligonucleotides are larger and can be highly negatively charged with low membrane permeability<sup>4</sup> and, unlike therapeutic proteins, which exert their actions extracellularly, oligonucleotides exert their intended pharmacologic effect intracellularly.<sup>4,5</sup> The synthetic modifications to the oligonucleotide backbone, sugar or component nucleobases not only aim to avoid degradation by endogenous nucleases, increase their binding affinity for target sequences, but act to also influence pharmacokinetic (PK) properties, such as tissue half-life, protein binding, and bioavailability of oligonucleotides.<sup>4</sup> With these differences in mechanism and disposition compared with other therapeutic modalities, characterizing the clinical pharmacology of these products in the course of drug development may require special considerations.

In general, the PKs of small molecules and therapeutic proteins are influenced by factors, including their physicochemical properties and formulation, as well as intrinsic and extrinsic patient factors.<sup>6,7</sup> These factors are routinely considered both during drug development and regulatory review and may be evaluated in dedicated clinical and nonclinical studies. Several regulatory guidance documents provide recommendations for the development of small molecules and/or biologics, such as evaluating the effects of drug-drug interactions (DDI)<sup>8–10</sup> and organ impairment<sup>11,12</sup> on PKs, assessing immunogenicity of a product and its consequences,<sup>13,14</sup> and evaluating the QT/QTc interval prolongation potential.<sup>15</sup> Because oligonucleotide therapies are distinct from small molecules and therapeutic proteins, the range of studies needed to support approval may differ from what is generally required of small molecules and biologics.

Given the limited regulatory experience with only a few oligonucleotide therapies approved (mostly for rare diseases), and lack of guidance specific to this product category, we sought to understand the nature of studies that characterize the clinical pharmacology of oligonucleotide therapies. This was accomplished by surveying approved and investigational oligonucleotide therapeutic programs to identify general program characteristics as well as (i) *in vitro* and clinical studies evaluating DDI potential, (ii) impact of organ impairment on PKs, (iii) studies assessing immunogenicity potential, and (iv) nonclinical and clinical studies designed to evaluate cardiac safety. The evaluation of DDI interaction potential, impact of organ impairment on PKs, assessment of immunogenicity potential, and the evaluation of nonclinical and clinical cardiac safety were selected because these are typical clinical pharmacology studies conducted during the course of drug development and regulatory guidance documents focus on these investigations for small molecules and biologics; the

FDA also published a request for public comment in the Federal Register to collect input on these specific areas.<sup>16</sup>

## METHODS

### Data sources: Study data extraction

Synthetic oligonucleotide drug development programs were identified through a search of archived correspondence between the FDA and drug developers using an internal FDA informatics platform. Search terms included: oligonucleotide, antisense, and siRNA. Retrieved documents were manually reviewed to identify programs that reached the milestone of an end-of-phase II meeting or submitting a complete New Drug Application (NDA) between January 1, 2012, and August 31, 2018. Although there are other oligonucleotides with non-nucleotide targets, such as aptamers and those that are utilized for immunostimulation (e.g., toll-like receptor agonists), herein, we limited inclusion to oligonucleotides designed to modulate the activity of nucleotide targets.

For the investigational programs reaching the end-of-phase II milestone or having NDAs submitted/approved, program-level characteristics such as the type of oligonucleotide (ASO vs. siRNA), indication, route(s) of administration, phase of study, and NDA submission were recorded.

For investigational programs reaching end-of-phase II, Investigator's Brochures were obtained. For programs that had reached the NDA submission milestone, Common Technical Documents, drug labeling at the time of approval, clinical pharmacology review documents, and approval letters, when applicable, were obtained. Retrieved documents were then systematically reviewed to obtain the following information on all ongoing, planned, or completed clinical studies: study phase as reported by the drug developer, status of the study as indicated in the source documents (planned, ongoing, completed, or terminated), study population (healthy vs. patient population), route of administration, dosing regimen, and unit of dosing (fixed vs. weight based). Dosing regimen was classified as a single dose if the study subjects received single administration of the study drug, multiple dose if trial subjects received more than one dose of the study drug, as repeated administration of one dose or multiple administration of multiple doses and combination if the trial utilized single and multiple dosing strategies.

Studies evaluating the effects of clinical and *in vitro* DDI, renal impairment, hepatic impairment, immunogenicity, and nonclinical and clinical cardiac safety studies were specifically identified. Where available, the results of such studies were recorded as reported.

### Statistics

Formal hypothesis testing was not performed. Descriptive statistics are provided.

## RESULTS

### Program characteristics

In this investigation, a total of 81 distinct synthetic oligonucleotide programs were identified and 21 (14 Investigational New Drugs, and 7 NDAs) drug development programs were selected, as described in the Methods section. The 21 development programs were composed of 13 antisense oligonucleotides and eight siRNAs and were being developed

for 15 unique molecular targets. Of the seven NDAs, five were ultimately approved. All approved NDAs were for orphan status, 80% (4/5) were granted both priority review and fast track status, and one program was granted accelerated approval.

For the 21 programs included, 152 unique clinical studies were identified. Of the 152 studies, 48 studies were phase I, 43 were phase II (including phase I/II), and 43 were phase III (including phase II/III). An additional 4 studies were open label extension studies, and study phase was not specified in 14 studies (Table 1). Studies in healthy subjects represented 20% (30/152) of the studies reviewed, whereas 80% (122/152) of the studies were in patients. Of the studies in healthy subjects, 93% (28/30) were reported as phase I and studies in patients were spread across the other reported phases of development. Over 40% (20/48) of these studies enrolled the target population in phase I studies.

These oligonucleotide therapies represented six distinct routes of administration. Subcutaneous (s.c.) administration was the most common route of administration (47%) followed by intravenous (i.v.) administration (36%), and other (14%; e.g., intrathecal, rectal, intramuscular, and oral). Multiple routes of administration were used for 24% of the oligonucleotide therapeutics. The majority (73%) of all studies used multiple dose regimens, 18% used single dose regimens, and 9% explored both single and multiple dose regimens. Both fixed and body weight-based dosing strategies were explored. Fixed dosing was utilized in 63% of the studies, whereas 35% of the studies utilized weight-based dosing, and 2% evaluated both fixed and weight-based dosing.

### Drug-drug interaction

Seven dedicated DDI studies evaluating the DDI potential were identified (3 Investigational New Drugs and 1 NDA). Of these, six studies evaluated the drug interaction potential

of the drug as a perpetrator and one study evaluated the interaction potential as both a perpetrator and victim. Four studies had results submitted at the time of analysis. Of these, one was considered positive, wherein the oligonucleotide was considered a perpetrator.

For the five approved NDAs, one evaluated DDI in two dedicated *in vivo* studies before marketing. Three NDAs utilized population PK analysis to evaluate the potential for DDI. No specific recommendation regarding DDI was included in the labeling for any of the five NDAs (Table 2). At the time of approval, there were no postmarketing requirements or commitments to study drug interactions.

### Renal impairment

Three dedicated renal impairment studies were identified in our investigation of these 21 programs. A single renal impairment study demonstrated an increase in AUC of ~ 1.5-fold, 1.7-fold, and a 2-fold for mild, moderate, and severe renal impairment, respectively. The other two studies did not demonstrate an effect of renal impairment.

For the five approved NDAs, three programs used a population PK approach to evaluate the effect of renal impairment, and evaluated patients with normal function, mild, and moderate renal impairment. Specific recommendations regarding renal subgroups were included in labeling for three NDAs (Table 2). The percentage of the investigational drug renally excreted unchanged was 5% for 4 NDAs and 65% for one (eteplirsen) NDA. At the time of approval, there were no postmarketing requirements or commitments to study the impact of renal impairment.

### Hepatic impairment

Only one dedicated hepatic impairment study was identified as ongoing at the time of this analysis. For the five

**Table 1 Summary of study design features by phase of development**

		Reported phase of development (N = 152)					Total
		1	2 <sup>a</sup>	3 <sup>b</sup>	OLE	NS	
Unique studies		48	43	43	4	14	152
Route of administration	i.v.	18	13	14	0	10	55
	s.c.	20	21	24	4	2	72
	Other (i.t., p.r., i.m., p.o.)	6	7	5	0	2	20
	Multiple	4	1	0	0	0	5
Study status	Planned	1	0	1	0	0	2
	Ongoing	3	12	24	3	0	42
	Completed	44	29	16	1	14	104
	Terminated	0	2	2	0	0	4
Study population	Healthy	28	0	0	0	2	30
	Patients	20	43	43	4	12	122
Dose regimen	Single dose	21	4	2	0	1	28
	Multiple dose	18	39	41	4	12	111
	Combination	9	3	0	0	1	13
Unit of dosing	mg	32	26	32	4	3	97
	mg/kg	14	17	11	0	11	53
	Multiple	2	0	0	0	0	2

OLE, open label extension; NS, not specified.

<sup>a</sup>Includes studies reported as phase I/II. <sup>b</sup>Includes studies reported as phase II/III.

**Table 2** Summary of specific clinical pharmacology-relevant information for the five approved oligonucleotide therapeutics

Clinical pharmacology information of interest	Drug (year of approval)				
	Mipomersen (2013)	Eteplirsen (2016)	Nusinersen (2016)	Patisiran (2018)	Inotersen (2018)
Route of administration	s.c.	i.v.	i.t.	i.v.	s.c.
DDI					
<i>In vitro</i> CYP or transporter study performed	Yes	Yes	Yes	Yes	Yes
Dedicated human <i>in vivo</i> DDI study performed	Yes <sup>a</sup>	No	No	No	No
Population PK approach utilized	Yes	No	No	Yes	Yes
Actionable recommendation in labeling <sup>b</sup>	No	No	No	No	No
HI					
Dedicated HI study performed	No	No	No	No	No
Population PK approach utilized	No	No	No	Yes	Yes
Relevant labeling language	Contra-indicated for moderate and severe hepatic impairment	Not studied	None	No dose adjustment for mild HI; others not studied	No dose adjustment for mild HI; others not studied
RI					
Dedicated RI study performed	No	No	No	No	No
Population PK approach utilized	Yes	No	No	Yes	Yes
Relevant labeling language	Not recommended for severe RI or on dialysis	Not studied	None	No dose adjustment for mild or moderate RI; others not studied	No dose adjustment for mild or moderate RI; others not studied
Immunogenicity					
Impact of ADAs on PK evaluated	Yes	Not evaluated	Not evaluated	Yes	Not interpretable
Actionable recommendation in labeling <sup>b</sup>	No	No	No	No	No
Cardiac safety					
<i>In vitro</i> hERG assay performed	Yes	No	No	No	Yes
Dedicated TQT study performed	Yes	No	No	No	No
ECG-monitoring performed in clinical studies	Yes	Yes	Yes	Yes	Yes
Actionable recommendation in labeling <sup>b</sup>	No	No	No	No	No

ADA, anti-drug antibody; DDI, drug-drug interaction; ECG, electrocardiogram; hERG, human ether-a-go-go; HI, hepatic impairment; PK, pharmacokinetic; RI, renal impairment; TQT, thorough QT study.

Information is from the original approved labeling and clinical pharmacology reviews available from Drugs@FDA and other regulatory documents.

<sup>a</sup>Two dedicated DDI studies (simvastatin and ezetimibe, and warfarin) performed. <sup>b</sup>Actionable recommendation in labeling = dosage modification.

approved NDAs, none performed a dedicated hepatic impairment study (either in patients or volunteers). Two NDAs used a population PK approach to evaluate the effect of hepatic impairment in patients with normal function and mild impairment. Specific recommendations regarding hepatic subgroups were included in labeling for three of the five approved NDAs (Table 2). At the time of approval, there were no postmarketing requirements or commitments to study the impact of hepatic impairment.

### Immunogenicity

Anti-drug antibodies (ADAs) were evaluated in 29% (44) of the 152 studies. Of the 44 studies, 55% (24) evaluated the investigational drug via the s.c. route, 27% (12) via the i.v. route, and 18% (8) via other routes. Of the 44 studies, 44% (20) reported

the presence of ADAs. Of the 20 studies that reported the presence of ADAs, 60% (12) evaluated the investigational drug via the s.c. route, 20% (4) via the i.v. route, and 20% (4) via other routes. The range of subjects that were ADA positive was ~ 1–68%. ADAs were transient in some individuals within these studies. Of the five approved NDAs, three evaluated the impact of immunogenicity on the PK, two of which reported an increase in trough concentration ( $C_{trough}$ ). Limited data was available on the impact of immunogenicity on safety and efficacy; two NDAs did not evaluate the impact, whereas three NDAs reported no impact on safety and efficacy. No specific recommendation regarding immunogenicity was included in the labeling for any of the five NDAs (Table 2). At the time of approval, three NDAs had postmarketing requirements to evaluate the impact of immunogenicity.

### Cardiac safety

Electrocardiogram (ECG) monitoring was performed in 66% (100) of the 152 studies. Of these, 4% (4) were thorough-QT studies. Specifically, of the five approved NDAs, one NDA (mipomersen) performed a thorough-QT study, whereas all performed ECG-monitoring in the drug development program. However, no specific recommendations were made regarding cardiac safety monitoring for any of the five NDAs (Table 2). At the time of approval, there were no postmarketing requirements to evaluate cardiac safety.

### Relevant nonclinical studies supporting DDI and cardiac safety assessment

Our review of nonclinical cardiac safety studies performed for the 21 programs identified 52 *in vitro* DDI studies evaluating CYP and/or transporters and 23 nonclinical cardiac safety studies (Table 3). Of the 52 *in vitro* DDI studies, 8 studies evaluated the drug as a DDI victim, 36 evaluated its role as a perpetrator, and 8 studies evaluated its role as both victim and perpetrator. Of the 52 DDI studies, 40 evaluated the potential for CYP-mediated DDI and 12 evaluated the potential for transporter-mediated DDI. DDI potential of the oligonucleotide as a victim was identified in only one study and as a perpetrator in four studies. Of the four instances where the drug acted as a perpetrator, two impacted the expression of both CYP1A2 and CYP2C8 in liver microsomes and hepatocytes, and one impacted the expression of only CYP1A2. The only instance of the oligonucleotide as a victim was mediated by a transporter. The most commonly used test systems in the evaluation of CYP induction were human hepatocytes, in CYP inhibition were human microsomes, and in the evaluation of the investigational drug as a CYP substrate, human microsomes (Table 4). The most commonly used test systems that were specified in evaluation of transporter-mediated DDI were mammalian cell lines or a combination of mammalian cell line and Sf9 vesicles (Table 4). All of the five approved NDAs evaluated the drug using *in vitro* DDI studies (Table 2).

Of the 23 nonclinical cardiac safety assessments, 6 evaluated the potential for *in vitro* human ether-a-go-go (hERG) inhibition and 16 evaluated effect of the drug on ECG parameters in animal studies (Table 3). None of the studies reported significant inhibition of hERG or an impact on ECG parameters. Two of the five approved NDAs evaluated the drug's potential for hERG interaction *in vitro* (Table 2).

**Table 3 Summary and distribution of results by type of nonclinical study**

Study category	Evaluation type	Number of studies	Positive results
DDI (n = 52)	Victim	8	1
	Perpetrator	36 <sup>a</sup>	4
	Victim and Perpetrator	8	0
Cardiac safety (n = 23)	hERG	6	0
	ECG	17	0

DDI, drug-drug interaction; ECG, electrocardiogram; hERG, human ether-à-go-go.

<sup>a</sup>Includes one ongoing study, hence unknown outcome.

### DISCUSSION

This investigation sought to further understand the types of clinical pharmacology studies that were conducted to support the development of oligonucleotide therapies entering late stages of development and marketing applications. Specifically, this investigation focused on considerations to evaluate DDI potential, effect of organ impairment (i.e., renal and hepatic impairment), impact of immunogenicity, and cardiac safety. Overall, DDI potential was predominantly assessed using *in vitro* methodologies, whereas there was a paucity of data for many clinical pharmacology assessments, especially organ impairment. Immunogenicity data only had a minor impact on PK, and *in vitro* cardiac safety may not translate to clinical findings. Many of the drugs have been studied for rare diseases and this may have influenced the range of clinical pharmacology assessments utilized across the 21 programs. Additionally, many of the programs in this investigation utilized one of the FDA's expedited programs for serious conditions. Additional data in larger populations are needed to better assess what clinical pharmacology studies are necessary for the evaluation of synthetic oligonucleotides.

#### Program characteristics

The data compiled in this investigation were informative on aspects of studies conducted during oligonucleotide development. For instance, we evaluated various dosage regimens and routes of administration. Multiple-dose regimens were used in 73% of the studies. In addition, both fixed-dose and weight-based dosing strategies were used. Subcutaneous and i.v. dosing accounted for over 80% of the routes of administration. However, other routes of administration, including intrathecal and rectal administration, were also utilized. Distinct delivery and dosing considerations may impact the clinical pharmacology assessment of the investigational drug. As such, the clinical pharmacology strategy used in each program will be, in part, influenced by dosing regimens and routes of administration.

#### DDI potential

The DDI potential of oligonucleotide therapies was evaluated primarily via *in vitro* studies. These findings are consistent with literature noting that oligonucleotides have a limited potential to be victims of CYP-mediated interactions.<sup>17</sup> Of the four *in vitro* studies that had positive findings of the oligonucleotide acting as a perpetrator, three studies identified CYP1A2 and two studies identified CYP2C8 as the mediating enzymes. The structural similarities between antisense oligonucleotides and the prototypical substrates of these particular enzymes (i.e., the similarity between the purine nucleic acids of antisense oligonucleotides and the heterocyclic amine substrates of CYP1A2, and the similarity between the large anionic substrates of CYP2C8 and the polyanionic profile of antisense oligonucleotides), have been proposed as an explanation for the observation.<sup>18,19</sup>

The results of the seven dedicated DDI studies in this investigation demonstrated that oligonucleotides, in certain circumstances, can be perpetrators of CYP-mediated DDIs based on the mechanisms of interaction (e.g., involvement



**Table 4 Summary of CYP-mediated and transporter-mediated DDI evaluation by test system**

	DDI evaluation type (n)	Evaluation system (n)
CYP (n = 40)	CYP induction (15)	Human hepatocytes (13), other microsomes (1), not specified (1)
	CYP inhibition (19)	Human hepatocytes (5), human microsomes (11) other microsomes (1) multispecies S9 (1), and not specified (1)
	CYP substrate (6)	Human microsomes (3), multispecies microsomes (2), multispecies S9 (1)
Transporter (n = 12)	Transporter substrate (2)	Mammalian cell lines (1), Sf9 vesicles (1)
	Transporter inhibition (2)	Mammalian cell lines and Sf9 vesicles (1)
	Transporter substrate and inhibition (8)	Mammalian cell lines (2), mammalian cell lines and Sf9 vesicles (1), not specified (5)

Multispecies microsome = microsomes from animal species including human microsome, other microsome = non-human species microsome. DDI, drug-drug interaction.

in the heme-synthesis pathway<sup>20</sup> or immune-related upregulation). Only a single dedicated DDI study evaluated the potential of an oligonucleotide as a DDI victim and did not result in a positive finding.

Current guidance on CYP450 enzyme-mediated and transporter-mediated DDI recommends *in vitro* DDI assessments to determine DDI potential of the investigational drug as a DDI victim and perpetrator. These data can be used to determine the need for clinical DDI studies. For *in vitro* DDIs, recommended approaches include using microsomal systems, recombinant human CYP enzymes, hepatocytes, and *in vitro* transporter systems.<sup>9</sup> The literature evidence of system-dependent and backbone-dependent outcomes<sup>18,19</sup> suggests that additional considerations may be required for oligonucleotide therapies. For clinical DDIs, recommended approaches include dedicated standalone or nested DDI studies, and cocktail approaches. A risk-based approach is recommended to determine the type of DDI evaluation that may be required.<sup>8</sup>

### Organ impairment

In this investigation, a limited number of organ impairment studies were identified. Dedicated studies to evaluate the effect of renal function were conducted for three programs, and the effect of hepatic function for one program. The lack of dedicated studies characterizing renal or hepatic impairment may again be related to the nature of the target indications. Although regulatory requirements do not necessarily change for rare diseases, challenges of patient availability and available populations to assess organ impairment may be limited for study. Several programs utilized population PK to evaluate organ function as a covariate. However, eligibility criteria in studies used in population PK studies may have limited the enrollment of patients with varying organ function, thereby

limiting the utility of this approach. This lack of information limits the ability to provide dosing recommendations for patients with impaired organ function.

Oligonucleotide therapies typically distribute to the liver and/or kidneys, so understanding the implications of organ impairment on the various oligonucleotide platforms is important. In general, renal and hepatic impairment guidances<sup>11,12</sup> are mostly aimed at the development and regulatory assessment of small molecules. These studies typically rely on single dose evaluations in subjects with varying organ function facilitating PK bridging to subjects with normal renal function, and, consequently, offering the ability to provide appropriate recommendations. Taking the distribution and excretion pathways of the specific oligonucleotide therapy into consideration will be essential to determine how the impact of organ impairment should be assessed during drug development. For example, many oligonucleotides and their metabolites are excreted renally,<sup>21</sup> current guidance on evaluating the PK in patients with impaired renal function details the circumstances in which renal impairment study might be indicated based on the fraction eliminated unchanged in urine.<sup>12</sup> Oligonucleotide therapies that are targeted to the liver (e.g., GalNAc-linked) might warrant dedicated hepatic impairment studies.

### Immunogenicity

During development of oligonucleotide therapeutics, the presence of ADAs and their impact on safety, efficacy, and PKs are typically evaluated. In this investigation, 20 studies identified the presence of ADAs. There was a wide range of ADA titers in subjects positive for ADAs, and, in some cases, these ADAs were transient. The clinical significance of ADAs on safety and efficacy was inconclusive in these studies. Two of the five approved NDAs did note a small increase in  $C_{trough}$ , however, there were no actionable recommendations as a result. Further studies are needed to evaluate the association of ADAs with safety, efficacy, and PKs. Some of this information is anticipated to come from the postmarketing requirements that have been issued for the three NDAs to evaluate the impact of immunogenicity.

It is important to note that the detection of ADAs is highly dependent on the sensitivity and specificity of the assay being used.<sup>14</sup> This may explain the large variability in ADAs detected in the programs that identified positive ADAs. Because of their intracellular site of action, assays to evaluate neutralizing antibodies cannot be readily developed.

### Cardiac safety

Typically ranging between 6 and 13 kilodalton, oligonucleotides are, on average, larger than small molecules but much smaller than therapeutic biologics. Some studies suggest that some oligonucleotides are too large and hydrophobic to interact with hERG channels,<sup>22</sup> however, clinical findings indicate that oligonucleotides may still cause abnormalities in cardiac electrophysiology.<sup>23,24</sup> All 23 of the nonclinical cardiac studies (6 hERG and 17 ECG) identified in this investigation were negative. Of the five approved NDAs, two (inotersen, nusinersen) report some

cardiac electrophysiology abnormalities in their FDA-approved labeling. The labeling for inotersen mentions that 5.4% of inotersen-treated patients had evidence of QRS widening on ECG compared with only 1.7% of placebo-treated subjects.<sup>24</sup> In the sham-controlled portion of the nusinersen studies 2.4% of subjects had changes in QTcF values > 500 ms and change from baseline values >60 ms were observed in a small (2.4%) number of subjects.<sup>23</sup> Inotersen had a negative hERG study, however, its labeling reflects the aforementioned QRS widening and indicates that *in vitro* cardiac safety may not translate to clinical findings. Given these signals, additional data may be needed to guide best practices regarding the assessment of cardiac safety with oligonucleotides, as the current nonclinical assays may not predict cardiac safety.

## CONCLUSION

The findings of this investigation summarize the types of clinical pharmacology assessments conducted with oligonucleotides from 2012 to 2018. Many of the programs were for rare diseases or conditions, and although the clinical pharmacology study requirements are not different for rare diseases, often the nature of the indication (e.g., pediatric) may limit the number of patients available for study and the potential toxicity may not be suitable to allow evaluation in healthy subjects. In this investigation, the assessment of DDI potential was largely consistent with the systematic and risk-based recommendations of current DDI-relevant guidances. Clinical data are limited with respect to effects of DDI and organ impairment and collecting data through population PKs or dedicated studies may be appropriate to inform dosing in special populations. Similarly, data related to the clinical effects of immunogenicity on PKs are limited, but indicate some impact on PKs ( $C_{\text{trough}}$ ), however, effects on safety or efficacy are inconclusive due to limited data. Findings from clinical cardiac safety evaluation indicate a potential for discordance with nonclinical studies. Nonetheless, as oligonucleotide therapies are developed for broader populations and common diseases, a risk-based approach to requiring routine intrinsic and extrinsic factor studies seems prudent. Continued collective experience with this therapeutic modality will help inform best practices for future drug development and regulatory assessment of synthetic oligonucleotide therapies.

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