September 26, 2022

Dockets Management Staff (HFA-305)
Food and Drug Administration (FDA)
5630 Fishers Lane
Room 1061
Rockville, MD 20852
Attn: Docket No. FDA-2022-D-0235

Re: Docket No. FDA-2022-D-0235: Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics

Dear FDA Colleagues:

The Biotechnology Innovation Organization (BIO) thanks the Food and Drug Administration (FDA) for the opportunity to submit comments regarding the Draft Guidance for Industry, **Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics.**

BIO is the world's largest trade association representing biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO's members develop medical products and technologies to treat patients afflicted with serious diseases, to delay the onset of these diseases, or to prevent them in the first place.

BIO strongly agrees with the FDA's assessment of oligonucleotide therapeutics as a rapidly evolving and expanding modality with strong potential to treat both rare and common diseases. BIO also appreciates the Agency's acknowledgement that this modality encompasses a wide range of unique and diverse products with key differences in characteristics such as molecular structure, mechanism of action, and formulation/delivery. This draft guidance provides valuable insight on FDA's expectations around the assessment of oligonucleotide therapeutics and will assist sponsors in planning clinical pharmacology studies with additional clarity. However, BIO has provided a few overarching recommendations below that we believe will increase the utility of the draft guidance for sponsors once finalized.

First, BIO suggests providing further clarification regarding the definition of oligonucleotide therapeutics contained within Lines 32-34 of the guidance. For example, aptamers are oligonucleotides which can bind to a variety of intracellular, extracellular, or cell-surface targets and be used to inhibit protein–protein interactions or assist in targeted

drug delivery.[1-3] However, these products do not appear to be captured within the current definition. Though it may seem a small detail, clear terminology is especially important in such a fast-paced field, and will assist sponsors in interpreting the scope of the guidance and its recommendations. Similarly, BIO requests further clarity regarding FDA's use of the phrase "RNA-centric" in Lines 53-54 of the guidance as it is not clear whether this terminology is referring to the molecular structure of the product (i.e., containing single- or double-stranded RNA) or whether it is referring to the product's mechanism of action.

Secondly, BIO requests that FDA consider adding in subsections within the guidance on the major classes of oligonucleotide therapeutics, such as antisense oligonucleotides (ASOs), microRNA (miRNA), small interfering RNAs (siRNAs), etc. Even if specific recommendations cannot be provided at this time, simply laying out the major classes of therapeutics in development and providing clear terminology and definitions for the terms commonly used to describe the characteristics or functions of different types of oligonucleotides (e.g., gapmers, steric blocking, occupancy-mediated degradation, etc.) would be useful as this field continues to evolve and expand.

Thirdly, it would be useful if FDA noted considerations for different routes of administration (e.g., intravenous vs. intrathecal), particularly given the lower expected systemic circulation following IT administration. Additionally, while plasma is a commonly used matrix for pharmacokinetic (PK) samples, serum has also been used at some companies to characterize systemic PK. We recommend modifying language throughout the document to be more inclusive of other sample matrices (e.g., serum).

Lastly, BIO notes that some of the situations described within the guidance seem hypothetical with no known examples (e.g., "off-target hybridization with CYP enzyme…"). Additional detail regarding the potential for indirect mechanisms of CYP regulation with oligonucleotides would be useful. BIO suggests that the inclusion of published case studies or other references would be useful for providing additional context and assisting sponsors in interpreting the recommendations throughout.

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[1] Keefe AD, Pai S, Ellington A. Aptamers as therapeutics [published correction appears in Nat Rev Drug Discov. 2010 Aug;9(8):660]. *Nat Rev Drug Discov*. 2010;9(7):537-550. doi:10.1038/nrd3141

[2] Kher G, Trehan S, Misra A. Antisense Oligonucleotides and RNA Interference. *Challenges in Delivery of Therapeutic Genomics and Proteomics*. 2011;325-386. doi:10.1016/B978-0-12-384964-9.00007-4

[3] Ni S, Zhuo Z, Pan Y, et al. Recent Progress in Aptamer Discoveries and Modifications for Therapeutic Applications. A*CS Appl Mater Interfaces*. 2021;13(8):9500-9519. doi:10.1021/acsami.0c05750

Additional line-by-line suggestions and points for consideration are included in the table attached.

Sincerely,

/s/
Rachel Coe
Manager, Science and Regulatory Affairs
Biotechnology Innovation Organization

SPECIFIC COMMENTS:

LINE NUMBER	TEXT	ISSUE & PROPOSED CHANGE
I. INTRODUCTION		
Lines 33-35	"Oligonucleotide therapeutics include a wide variety of synthetically modified RNA or RNA/DNA hybrids that are specifically designed to bind to a target RNA sequence to alter RNA and/or protein expression. Even within the therapeutic modality, oligonucleotide therapeutics can differ in several ways, including but not limited to…"	BIO suggests that the current terminology regarding gene/protein expression is not correct and can lead to misunderstandings. Suggest: "Oligonucleotide therapeutics include a wide variety of synthetically modified RNA or RNA/DNA hybrids that are specifically designed to bind to a target RNA sequence to alter ~~RNA and/or protein expression~~ RNA expression and/or protein production. Even within the therapeutic modality, oligonucleotide therapeutics can differ in several ways, including but not limited to…"
Line 54	This guidance generally applies to oligonucleotide therapeutics that use an RNA-centric mechanism of action.	Per overarching comments, RNA-centric is a vague term. BIO suggests further clarity be provided.
Lines 63-65	"Oligonucleotide therapeutics that use mechanisms of action such as direct modulation of proteins (e.g., aptamers) or immunostimulation (e.g., TLR9 agonists) are beyond the scope of this guidance."	BIO requests clarification on the exclusion of the direct modulation of proteins (aptamers) and immunostimulatory oligonucleotides. We urge FDA to reconsider this decision as further clarity on these topics would be valuable to sponsors and it is unclear why these mechanisms are beyond this guidance. If FDA's position regarding the exclusion of these therapeutic types, it would be useful if the rationale for this exclusion were briefly outlined in the introductory section to help with understanding. We also suggest clearly specifying that replicons are also out of scope for this guidance.
III. CLINICAL PHARMACOLOGY CONSIDERATIONS		
Line 80	"These drugs have longer tissue and pharmacodynamic half-lives."	Longer pharmacodynamic half-life is not unique to oligonucleotide therapeutics. It is possible that this may reflect the characteristics of proteins with slow turnover rates. For increased accuracy and

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		specificity to oligonucleotide therapeutics, we suggest clarifying that the sustained pharmacodynamic responses are *due* to the longer tissue half-life. Suggest: "These drugs have longer tissue half-life ~~and pharmacodynamic half-lives~~, leading to sustained pharmacodynamic responses"
Lines 87-90	"Therefore, in multiple-dose studies, sponsors should include an assessment of appropriate pharmacodynamic biomarkers (e.g., target mRNA, target protein, or a downstream biomarker that reflects modulation of the target protein) or consider other response measures."	Sometimes, validated pharmacodynamic biomarkers or other measures are not available for target diseases. We suggest adding "if feasible." Suggest: "Therefore, in multiple-dose studies, sponsors should include an assessment of appropriate pharmacodynamic biomarkers (e.g., target mRNA, target protein, or a downstream biomarker that reflects modulation of the target protein) or consider other response measures if feasible."
Lines 92-94	"…pharmacodynamic endpoints should be discussed with the appropriate FDA review staff, especially in cases where the pharmacodynamic endpoints might not directly reflect target knockdown (e.g., cerebrospinal fluid for central nervous system targets)."	BIO notes that it is not clear how the provided example of cerebrospinal fluid illustrates a pharmacodynamic endpoint that might not directly reflect central nervous system (CNS) targets.
Lines 96-101	"Oligonucleotide therapeutics have certain unique characteristics compared to small molecule or biological products (e.g., chemistry, structure, sites of action, pharmacokinetic disposition, pharmacodynamics). Therefore, sponsors should consult Sections II.A. to II.D. below for considerations when characterizing QTc interval prolongation, performing immunogenicity risk assessment, assessing the impact of hepatic and renal impairment, and determining the potential for drug-drug interactions during oligonucleotide therapeutic development."	While topics such as QT prolongation, and renal and hepatic impairment studies are important aspects of oligonucleotide development, a position on whether preclinical and human clinical absorption, distribution, metabolism, and excretion (ADME) and quantitative whole-body autoradiography (QWBA) studies are conducted should also be discussed or at least mentioned as an individual section or topic. BIO recommends adding a paragraph (or separate section) on the relevance of evaluating oligonucleotides in preclinical ADME/QWBA studies to evaluate the distribution into various tissues and organs (i.e.,

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		whether this is required or not). In addition, clear guidance indicating that conduct of a human clinical ADME study for oligonucleotides is not recommended and the rationale (e.g., ethical reasons or otherwise) for this position would be helpful.
Lines 103-106	"Specific considerations should be given to the chemistry (e.g., backbone modification, conjugation), drug target, plasma protein binding, and route of administration as these factors determine the distribution of the oligonucleotide therapeutic to the liver, kidneys, and other tissues as well as determine the exposure (local or systemic) to the drug."	As the PK of oligonucleotides is transient and short-lived with high variability and does not closely reflect its more durable target tissue distribution and pharmacodynamic (PD)/efficacy/safety profiles (lines 85-87), we believe it is important to acknowledge that bridging formulations or devices based on a traditional PK endpoint for bioequivalence (BE) studies may not be the most appropriate approach. In certain cases, PD could also be used as a primary endpoint (where available) to optimize drug development. We recommend the guidance allow for the flexibility to use PD as a primary endpoint instead of or in addition to a traditional PK endpoint for BE studies to bridge formulations and/or devices where appropriate. We further recommend the guidance state that FDA and sponsors should discuss the most appropriate approach in the context of the specific oligonucleotide development program.
Lines 103-106	"Specific considerations should be given to the chemistry (e.g., backbone modification, conjugation), drug target, plasma protein binding, and route of administration as these factors determine the distribution of the oligonucleotide therapeutic to the liver, kidneys, and other determine the exposure (local or systemic) to the drug."	For GalNAc conjugated and LNP formulated siRNAs and GalNAc conjugated ASOs, there is no scientific evidence that plasma protein binding determines distribution, clearance or PK/PD effects. This has recently been discussed by Humphreys S et al. NAR: 50(11): 6020–6037, 2022. We propose to remove plasma protein binding from this sentence or modify as this may be different for the lipidated siRNAs. Suggest: Specific considerations should be given to the chemistry (e.g., backbone modification, conjugation), drug target, plasma protein binding where relevant and route of administration as these factors determine the distribution of the oligonucleotide therapeutic to the liver,

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		kidneys, and other determine the exposure (local or systemic) to the drug.”
Lines 108-110	“Additionally, appropriate bioanalytical methods should be used to characterize the parent oligonucleotide and any relevant metabolites, including chain-shortened metabolites.”	BIO requests further clarification on FDA's definition of "metabolite" … Perhaps a suggestion could be something like the following, “Any short-chained product of primary active species (e.g., linked to targeting moiety) with potential pharmacological relevance." For siRNA chain-shortened metabolites that are formed from the sense and antisense strand via exo and endonuclease activity, n-1, n-2, n-x metabolites of the 5 prime and 3 prime ends of the antisense strand can retain pharmacological activity. Which criteria should be used to quantify these chain-shortened metabolites? Typically, there is a disconnect between plasma PK of drug related material and target organ PK of drug related material. If the definition suggested above is used, measuring the circulating levels of metabolites in this case would not be relevant. Also, in studies of the tissue metabolites of GalNAc targeted ASOs, the conjugate is rapidly cleaved within 24hr to leave the parent/primary metabolite which is retained in the tissues for several weeks before returning to the circulation. We suggest that the Bioanalysis assay should simultaneously measure both molecules.
Section II-A : Characterizing QTc Interval Prolongation and Proarrhythmic Potential		
Lines 114-128	General comment to the section	Guidance on the timing of evaluation of potential QTc interval prolongation and proarrhythmic potential of the drug in relation to dosing of drug would be valuable to sponsors e.g., should the evaluation be at maximal exposure and/or maximal pharmacodynamic effect? And would there be certain cases where one timing would be preferred over the other?

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		We also recommend FDA call out the importance of concentration-QT analysis from early phase studies as an option to characterizing QTc interval prolongation and proarrhythmic potential.
Lines 121-128	"An assessment of QT prolongation risk and a proposed QT assessment plan should be submitted for all oligonucleotide therapeutic development programs as outlined in the FDA guidance entitled E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs (October 2012) and the E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs Questions and Answers (R3) (June 2017). All proposals in the QT assessment plan should be adequately justified and discussed with the Agency. The timing and extent of the clinical QT assessment depend upon the overall benefit/risk profile of the oligonucleotide therapeutic."	BIO suggests that the draft guidance be updated to reference and align with the recommendations put forward in March 2022, by the ICH E14/S7B Q&A Implementation Working Group. This guidance enables the integration of nonclinical (S7B) core assays and a first-in-human QT assay (E14[R3]) to assess QTc interval risk. BIO also notes that the addition of one or more additional references in this section would be useful as well. Further clarification on whether FDA expects clinical assessments to follow the approach for typical small molecules or traditional biologicals (e.g., monoclonal antibodies), would be useful. These two classes are much different in assessing QT prolongation risks. Thus, clarity would help Sponsors to develop adequate clinical pharmacology plans. Rationale and criteria for each scenario will be helpful as well. BIO also notes that the mainstream modalities of oligonucleotides are low risk for hERG and to date, the QTc prolongation and proarrhythmic risk of oligonucleotides has proven to be low in nonclinical and clinical development. Further clarification from FDA on when these assessments are relevant and scientifically justified would be useful. We note that Dr. Hobart Rogers' presentation from a recent joint FDA and DIA workshop indicated that for "QT prolongation, in vitro assessment is recommended, followed by ECG monitoring in pivotal trials." However, recent publications have stated that in vitro studies are not useful for assessing hERG risk. Does the Agency recommend conducting in vitro evaluations such as hERG assessments? Currently, it appears that sponsors follow different in vitro approaches without clear guidance/rationale. Ultimately, sponsors are committed to

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		providing this information and conducting Qt prolongation studies in vivo.
Section III-B : Performing Immunogenicity Risk Assessments		
General Comment on Section		Given that PK for certain oligonucleotides (e.g., siRNAs) is short-lived and not necessarily reflective of long duration of pharmacodynamic action and efficacy, the guidance may benefit on some specific text around more relevance assessing immunogenicity impact on PD rather than only on PK.
Lines 141-154	"The clinical immunogenicity assessment for an oligonucleotide therapeutic should follow a risk-based approach and be included in a product-specific immunogenicity risk assessment as outlined in the FDA guidance entitled Immunogenicity Assessment for Therapeutic Protein Products (August 2014)."	The referenced guidance discusses the utility of nonclinical studies (in vitro and animal) in measuring cytokine release and immunogenicity (pages 32-35). Suggest expanding this passage to include nonclinical risk-based assessments as well, so that it is aligned with the guidance and recognizes the importance of nonclinical studies in a risk-based approach in preparing for clinical studies. We recognize that, depending on the modifications and backbone, oligonucleotides can result in a considerable degree of anti-drug antibodies (ADA) in nonhuman primates (NHP). Although the translation of oligonucleotide ADA in vivo to human patients is unclear, nonclinical studies remain valuable and new chemistries could have an impact on PK/PD. Suggest: "The clinical and nonclinical immunogenicity assessment for an oligonucleotide therapeutic should follow a risk-based approach and be included"
Lines 157-160	"As determined by the immunogenicity risk assessment it may be appropriate to develop multiple immunogenicity assays to measure immune responses to the different components of an oligonucleotide therapeutic, such as the carrier component (e.g., PEGylated lipid nanparticles) and	The current version suggests that an anti-sense oligonucleotide always has multiple components. A single immunogenicity assay may be appropriate if this is not the case. For clarity, suggest revising as follows: Suggest: "As determined by the immunogenicity risk assessment, in cases where the anti-sense oligonucleotide includes a carrier

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	oligonucleotides conjugated to protein targeting ligands (e.g., Fab fragments)."	component (e.g., PEGylated lipid nanoparticles) or is conjugated (e.g., to a protein targeting ligand, such as Fab fragments), it may be appropriate to develop multiple immunogenicity assays."
Lines 162-164	"In addition, the mechanism of action of some oligonucleotide therapeutics generates a modified protein (e.g., splice-altering, exon-skipping oligonucleotide therapeutics); in such cases, the sponsor should consider an immunogenicity assay measuring antibodies to the modified protein."	If a modified protein is made, there may be a risk for antibodies or cellular responses that could recognize the modification. The risk of antibodies or cellular responses could be influenced by whether the protein is secreted or expressed intracellularly.
Lines 166-169	"Additionally, unwanted innate immune activation should also be measured when appropriate (e.g., oligonucleotide therapeutic-induced cytokine release, presence of sequences that are known to be immunogenic in humans such as GU, CpG or 5'-P, presence of natural nucleosides with 2'-deoxy, 2'-OH or unmethylated C)."	BIO suggests referencing the FDA guidance "Immunogenicity Assessment for Therapeutic Protein Products" here as well. Specifically, from the guidance, we suggest including references that mention the utility of evaluating cytokine and/or compliment activation in nonclinical toxicity studies (i.e., IND enabling NHP study). Oligonucleotides can be immunoreactive and it is possible to begin characterizing the degree of effect in nonclinical safety studies before first in human studies as part of a risk-based approach. However, BIO also notes that the term "immunogenic" is imprecise within the context of cytokine induction, as it implies induction of a specific immune response, rather than activation of nucleic acid-sensing receptors of the innate immune system. Also, saying natural DNA (2'-deoxy) is immunogenic seems to be a stretch as the siRNA field accepts DNA in the modified siRNA. We suggest providing references or modifying this statement to provide further clarity. Suggest: "Additionally, unwanted innate immune activation should also be measured when appropriate (e.g., oligonucleotide therapeutic-induced cytokine release, presence of sequences that are known to be ~~immunogenic~~ immunostimulatory in humans such as GU, CpG or 5'-P,

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		presence of natural nucleosides with 2'-deoxy, 2'-OH or unmethylated C).
Line 176-177	"Of note, as determined by the immunogenicity risk assessment, it may be adequate to bank samples in early development (e.g., Phase 1/ First-in-human studies) for later testing if there is new evidence of altered pharmacodynamics, or immune-mediated adverse events."	As it may not be known at the time what may occur in later studies, it would be useful for FDA to detail a standard battery of tests to be performed in early studies for these oligos at a minimum. Additionally, certain samples have stability issues with long-term storage (e.g., plasma for cytokine analyses). Suggest: "Of note, as determined by the immunogenicity risk assessment, it may be adequate to bank samples in early development (e.g., Phase 1/ First-in-human studies) for later testing if there is new evidence of altered pharmacokinetics, pharmacodynamics, or immune-mediated adverse events. Consideration should be given to which samples can be stored indefinitely for later testing and which parameters need to be measured at the time they were taken even if only to be reviewed in the context of data from future studies."
Lines 183-185	"In certain circumstances, the FDA could also recommend assessing for nucleotide sequence-specific antibodies and/or bioactivity (e.g., neutralization, enhancement). Any recommendations for these assays will be informed by clinical concerns, such as oligonucleotide sequence cross-reactivity..."	In these sentences the term "cross-reactivity" indicates Ab binding to native oligos rather than the drug. It is not clear how Abs in the extracellular space would cross-react with native oligonucleotide sequences in the intracellular compartment. We suggest providing examples or removing the term "cross-reactivity".
Section III-C : Characterizing the Impact of Organ Impairment on Pharmacokinetics, Pharmacodynamics, and Safety		
General Comment		BIO notes that plasma protein binding is notably omitted from this paragraph. It would be helpful if the guidance specifically mentioned that plasma protein binding assessment on plasma from renal or hepatic impaired subjects is not required in line with the lack of relevance of plasma protein binding for PK of oligonucleotides and as discussed in Humphreys S et al. NAR: 50(11): 6020–6037, 2022

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		BIO also requests additional guidance on what actions or specific evaluations the Sponsor could consider when evaluating the degree of portal hypertension and shunting of blood flow around the liver.
Line 195-197	"These early assessments, along with safety and tolerability information, should be used to inform the enrollment of subjects with a full range of hepatic and/or renal function in the late-phase trials."	The guidance should give room to exclude certain subjects with some renal/hepatic impairment based on the disease, benefit/risk assessment. Also, modeling and simulation can be used to waive the enrollment of some degree of renal/hepatic impairment into the studies.
Lines 203-206	"When the oligonucleotide therapeutic is not predominantly renally cleared or does not target the liver, the sponsor should enroll subjects with a full range of renal or hepatic function, respectively, in late-phase trials based on information from nonclinical studies and early clinical experience."	BIO notes that this sentence does not cover the cases when the oligonucleotide therapeutic is not liver targeting but predominantly cleared by liver (for example, CNS targeting). We suggest providing guidance for this scenario. It may also be difficult to find patients with severe renal impairment to enroll in the clinical study. This text suggests that the sponsor must actively seek to recruit patients with a full range of renal or hepatic function in the trial *even if not consistent with the target patient population*. Additionally, the word "full" connotes "complete" or "healthy" in this section. Overall, we suggest modifying the terminology here and emphasizing that each scenario should take into account the indication/disease population and benefit/risk assessment. Suggest: "When the oligonucleotide therapeutic is not predominantly renally cleared or does not target the liver based on information from nonclinical trials and early clinical experience, the sponsor should enroll the target patient population in late-phase clinical trials irrespective of the extent of the patient's renal or hepatic impairment."
Lines 211-212	"In such situations, different strategies can be used to study the impact of renal impairment on response and drug exposures."	Clarify that this evaluation may not require a separate renal or hepatic impairment study. Suggest: "In such situations, different strategies can be used to study the impact of renal impairment on response and drug exposures, such

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		as evaluation from disease population in late-stage clinical trials, instead of a dedicated clinical pharmacology study."
Lines 215-216	"When applicable, this study should be a multiple-dose study to enable adequate characterization of pharmacodynamic effects."	Given that many oligonucleotides have very long, durable PD effects and dosing can be infrequent (e.g., every 3 to 6 months) it may be infeasible to require a multiple dose renal or hepatic impairment study and to evaluate PD effects over a long duration. If the achievement of steady state PK and/or PD is needed, this will be infeasible for many oligonucleotides. We suggest FDA specify which scenarios a multiple dose study would reasonably be expected (e.g., where multiple doses are necessary to collect adequate safety and PK information in the population of interest).
Lines 220-222	"When the oligonucleotide therapeutic targets the liver, the sponsor can consider alternative approaches that allow for sequential or adaptive enrollment starting in early phase studies of tolerability, safety, and pharmacodynamics.[8]"	This paragraph describes the therapeutics targeting liver, but the citation (reference 8) mentions evaluating patients with renal impairment. As subsequent sentences (line 222-225) discuss the hepatic function and its impairment, it is likely that the reference 8 may not be the right citation in this paragraph. If reference 8 is an appropriate citation, further elaboration on this is necessary to enhance the understanding of this paragraph.
Lines 223-224	"The sponsor should consider the degree of portal hypertension and shunting of blood flow around the liver in these studies."	This sentence may refer to including subjects with portal hypertension or with shunting of blood flow around the liver. BIO requests FDA provide detailed guidance on how to identify these subjects and how many should be included.
Section III-D : Considerations for Assessing Drug Interactions		
Section III-D (1) Pharmacokinetic Interactions with Cytochrome P450 Enzymes and Transporters		
Line 250-252	"If the oligonucleotide therapeutic undergoes substantial renal active secretion as an unchanged drug, it could be	BIO notes that this sentence is duplicative and redundant as Lines 248-250 state: ".....or renal uptake or efflux transporters such as OAT1,

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	important to evaluate whether an oligonucleotide is a substrate of renal transporters in vitro"	OAT3, OCT2, MATE1, and 249 MATE2/K are generally not anticipated to have a significant impact on the pharmacokinetics of oligonucleotide therapeutics."
Lines 257-260	Refer to the FDA guidance entitled In vitro Drug Interaction studies ...for general considerations when conducting in vitro experiments and interpreting data.	The referenced guidance is developed for typical small molecules for which the plasma total/unbound Cmax or hepatic inlet concentration is relevant for contextualization of which concentration range of test compound needs to be evaluated or for the interpretation of drug-drug interaction (DDI) risk as perpetrator. For oligonucleotides, there is a disconnect between plasma PK and liver/kidney PK so the hepatic inlet concentration or plasma Cmax seems irrelevant to assess DDI risk for liver and kidney efflux transporters. The referenced guidance also specifies criteria on when to test metabolites for DDI potential. For oligonucleotides based on current scientific knowledge there is no concern for off target DDI potential of metabolites.
Lines 256-267	Based on current experience, oligonucleotide therapeutics either do not modulate or minimally modulate the major CYP enzymes and drug transporters. However, an overall recommendation for specific types of oligonucleotide therapeutics (e.g., based on chemistry or delivery strategies) cannot be provided at this time. The sponsor should provide adequate justification if in vitro assessments of oligonucleotide perpetrators in drug-drug interactions are not conducted."	We commend the Agency's decision that sponsors can provide justification not to conduct in vitro drug interaction studies as a perpetrator. In assessing the effect of oligonucleotide therapeutics on enzymes and transporters, the selection of the test system will be critical. It should be realized that in in vitro cell models most of the oligonucleotide drug molecules will be trapped in endosomes and lysosomes and therefore will not have access to the active side of metabolic enzymes or transporters. Incorporating this point in the guidance will be helpful.
Lines 270-273	"The potential of an oligonucleotide therapeutic to modulate CYP enzymes or transporters to modulate CYP enzymes or transporters directly (e.g., via off-target	BIO notes that the *potential* for an oligonucleotide therapeutic to modulate CYP enzymes or transporters should be *considered*, not

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	hybridization with CYP enzymes or transporter mRNA transcripts) or indirectly (e.g., by interfering with the synthesis or degradation of heme or by modulating cytokines) should be evaluated."	evaluated as this connotates that a more in-depth evaluation is always justified. The indirect effect on CYPs will be depending on the target of the oligonucleotide drug such as in the case of for instance a drug affecting heme synthesis. Further assessment of indirect effects on CYP enzymes or transporters may be warranted for ASOs that target certain cytokines (e.g., IL-6) that are known to modulate CYP expression. BIO suggests modifying the last part of this sentence to note that this evaluation could be case by case depending on the target biology. Experimentation investigations should be driven by a risk assessment of the potential for the oligonucleotide therapeutic to have a direct or indirect effect on CYPs. Furthermore, the potential that oligonucleotide drugs will down-regulate CYPs or transporters due to cross-hybridization is extremely low. In selecting sequences for oligonucleotide drugs care is taken to ensure specificity for the target of interest. Certain nuclear transcription factors such as pregnane X receptor and constitutive androstane receptor are known to directly modulate the expression of various P450 isoforms. Finally, it would be useful if the Agency provided further clarification in terms of risk assessment towards "direct" and "indirect" modulation effect. Due to lack of information on in vitro in vivo correlation (IVIVC), there is no effective in vitro system/tool to investigate clinical DDI potential for "indirect" modulation to CYP enzyme and transporters by oligonucleotide therapeutics. Suggest: "The potential of an oligonucleotide therapeutic to modulate CYP enzymes or transporters to modulate CYP enzymes or transporters directly (e.g., via off-target hybridization with CYP enzymes or transporter mRNA transcripts) or indirectly (e.g., by

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		interfering with the synthesis or degradation of heme or by modulating cytokines or transcription factors)) should be ~~evaluated~~ considered on a case-by-case, taking into account the target biology."
Section III-D (2) Pharmacodynamic Interactions		
	Alternative mechanisms for DDIs for siRNA	BIO notes that the draft guidance doesn't discuss the (theoretical) potential for DDIs due to competition with the ASGPR receptor (for GalNAc conjugated oligonucleotides), other uptake mechanisms, or for binding to the AGO-2 protein (part of the RISC complex). We suggest that if the therapeutic uses active receptor transport, sponsors may need to assess potential for an effect on the uptake of other saturating molecules using the same active transport.