Nonclinical Safety Assessment of Oligonucleotide-Based Therapeutics

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
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I. INTRODUCTION

The purpose of this guidance is to provide recommendations on approaches for the nonclinical safety evaluation of oligonucleotide-based therapeutics (ONTs) to support clinical development and marketing of these products.

ONTs present unique challenges for nonclinical safety evaluations. Although current guidance for small molecule drugs and therapeutic proteins may mention ONTs, this guidance provides detailed recommendations specific to nonclinical assessment of ONTs that have been developed based on experience to date with this category of products. These recommendations address ONT characteristics that differ from small molecule drugs and therapeutic proteins.

II. SCOPE

This guidance includes recommendations for single-stranded or double-stranded ONTs created synthetically or derived naturally, with native or modified backbone or nucleoside structures that increase or decrease expression and/or function of proteins. Examples of included oligonucleotides are antisense, small interfering RNA, microRNA, transfer RNA, decoys, and aptamers. Immune stimulatory oligonucleotides (e.g., CpG motifs acting via Toll-like receptors) are excluded, as are CBER-regulated products (e.g., DNA/RNA vaccines, virally delivered ONTs, messenger RNA and RNA used for gene editing). An oligonucleotide that is conjugated to other types of molecules (e.g., saccharides, lipids, peptides, antibodies) is included if the oligonucleotide would itself fall within the scope of this guidance.

For ONTs intended to treat cancer, sponsors should consult International Council for Harmonisation (ICH) guidances for industry *S9 Nonclinical Evaluation for Anticancer*

[1] This guidance has been prepared by the Office of New Drugs in the Center for Drug Evaluation and Research at the Food and Drug Administration.

Pharmaceuticals (March 2010) and *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals Questions and Answers* (June 2018) (ICH S9 Q&A)[2] regarding the nonclinical studies recommended to support development of those products. Principles outlined in this guidance may also be relevant to the design and conduct of studies recommended by ICH S9 and ICH S9 Q&A when assessing the nonclinical safety of an ONT for the treatment of cancer.

For ONTs being developed to treat rare diseases, including those that are severely debilitating or life-threatening (SDLT) diseases,[3] sponsors should also consult the guidance for industry *Rare Diseases: Considerations for the Development of Drugs and Biological Products* (December 2023).

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

III. GENERAL CONSIDERATIONS

A. Modes of Activity

ONTs have multiple modes of activity by which they can achieve their intended pharmacological effect. Most commonly, ONTs contain nucleotide base sequences that are complementary to a targeted sequence of an intracellular nucleic acid. The complementary base sequences bind (hybridize) to each other via Watson-Crick-Franklin base pairing. This base sequence-dependent hybridization can then affect the expression of specific genes by various downstream mechanisms (e.g., antisense, silencing). Antisense splice modulator ONTs also act via Watson-Crick-Franklin base pairing to the pre-messenger RNA (mRNA) to affect the inclusion or exclusion of nucleotides from the mature mRNA, thereby changing the level or activity of the encoded protein. Some ONTs (aptamers) are designed to elicit their pharmacological effect by binding to specific proteins or other cellular components in a base sequence-dependent, hybridization-independent manner.

B. Safety Assessments

1. On-Target Effects (Exaggerated Pharmacology)

The potential for adverse effects resulting from on-target activity (exaggerated pharmacology) should be characterized. Generally, this would be assessed empirically in at least one test

[2] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[3] For definition of what constitutes a severely debilitating or life-threatening illness, please see 21 CFR 312.81.

species[4] using the clinical candidate when one or more test species are available that are pharmacologically responsive to the clinical candidate, or by using a test species-active surrogate oligonucleotide.[5] For the purposes of this guidance, the phrase *pharmacologically relevant species* is generally inclusive of both those species that are responsive to the clinical candidate as well as those that are responsive to an appropriately characterized species-selective surrogate oligonucleotide.

For some ONTs, it may not be practical to experimentally assess exaggerated pharmacology in toxicology studies. This scenario could occur, for example, if the targeted element is not present in the genome of the test species (e.g., cryptic splice site, indel, or exogenous target) or if the intended pharmacodynamic (PD) effect (e.g., exclusion of a particular exon) would result in a transcript that fundamentally differs from that produced in the patient population (e.g., if exon exclusion causes a frameshift that results in a transcript that encodes an aberrant amino acid sequence or contains a premature stop codon). In these cases, a literature-based weight-of-evidence (WoE) assessment of the potential adverse effects resulting from exaggerated pharmacology should be provided (e.g., data from genetically modified animal models or data from known human diseases).

2. Off-Target Hybridization-Dependent Effects

Given their ability to form Watson-Crick-Franklin base pairs, ONTs have the potential to bind to nontargeted RNA or DNA sequences that share partial or complete complementarity with the base sequence present in the ONT, which can lead to adverse effects in humans that might not be detected in toxicology studies. Therefore, sequence-dependent off-target assessments should be conducted for ONTs using appropriate in silico and in vitro methodologies to identify potential off-target hybridization.

The following points should be considered when designing sequence-dependent off-target assessments:

- Several different types of ONTs exist (e.g., antisense oligonucleotides, small interfering RNAs, aptamers). The criteria for defining high-risk off-target binding and sensitivity to mismatches are expected to differ for each ONT type. Appropriate criteria should be provided and justified for the applicable type of ONT drug product.

- All elements of the ONT drug product and its predicted metabolites that are available for off-target hybridization should be assessed, including both the sense and the antisense strands, overlapping ends, etc.

[4] FDA supports the principles of the 3Rs (replace/reduce/refine) for animal use in testing when feasible. FDA encourages sponsors to consult with review divisions when considering a nonanimal testing method believed to be suitable, adequate, and feasible. FDA will consider whether the alternative method is adequate to meet the nonclinical regulatory need.

[5] A *surrogate oligonucleotide* has comparable chemistry, length, formulation, and modifications as the clinical candidate ONT, but with a base sequence that recapitulates the intended pharmacological activity in the test species.

- Potential hybridization to the transcriptome and nuclear and mitochondrial genome should be addressed.

- The in silico methods used, including the criteria for defining potential off-target binding, should be justified.

- The importance of any potential off-target hybridization identified in silico should be further investigated in in vitro assays (e.g., cell-based analysis, RNA-seq). Scientific justification for the assays selected should be provided.

- Information from human genetic diseases and animal models (e.g., mouse knockouts) can be used as part of a WoE assessment to understand potential consequences of off-target effects.

- Relevance of toxicology animal species for assessing off-target effects should be considered by determining whether the human off-target site is conserved in the animal.

- Additional factors, such as temporal and cell-type-specific expression, pharmacokinetic (PK) properties, and hybridization-dependent binding efficiency may be considered in the overall risk assessment of potential off-target effects.

Careful assessment and clinical monitoring should be performed for any potentially human-relevant off-target hybridization-dependent hazards identified, especially those that could not be evaluated in the animal toxicology studies.

3. Off-Target Hybridization-Independent Effects

ONTs intended to act through binding to cellular nucleic acids can also interact with proteins or other cellular components directly. Although these interactions do not involve nucleic acid base pairing, the interactions can still be sequence-dependent. In addition, ONTs such as aptamers, which are not designed to interact directly with other nucleic acids, may also exhibit off-target non-hybridization-dependent effects.

Unlike off-target hybridization-dependent effects, off-target hybridization-independent effects are not as amenable to predictive toxicology approaches because of the complexity of the interaction of oligonucleotides with non-nucleic acid targets. Consequently, empirical toxicity assessment of each new clinical candidate ONT is important. This assessment generally follows that described in ICH guidance for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, Questions and Answers (R2)* (February 2013) for small molecule drugs, with some modifications as described below.

IV. PHARMACOLOGY

A. Primary and Secondary Pharmacology

Primary pharmacology studies should investigate the mode of action and/or effects of an ONT in relation to its desired therapeutic target. These studies may also provide information about the duration of effect and can contribute to dose selection for both nonclinical and clinical studies.

Some ONTs may be highly specific for human targets and consequently show no pharmacological activity in nonhuman species. In these cases, pharmacology information may be obtained from the use of a test species-active surrogate oligonucleotide. In vitro studies using human cells or genetically modified animals expressing the human target or in which the target is knocked out can also provide information about potential biological effects from the ONT.

Nonclinical pharmacology data may also be helpful in generating supportive efficacy information (on a case-by-case basis) and in establishing the prospect of direct benefit in support of clinical investigations involving children, especially for conditions that occur only in children.[6]

Typical small molecule secondary pharmacology studies (e.g., receptor, ion channel and enzyme binding and functional screens) are generally not warranted for ONTs whose pharmacological mode of action is through nucleic acid hybridization. If unexpected toxicities are observed in animal toxicity studies or clinical trials, a secondary pharmacology assessment may help elucidate the cause of the toxicity. Secondary pharmacology studies may be appropriate for ONTs with mechanisms other than nucleic acid hybridization (e.g., aptamers), as there may be the potential for activity at unintended targets, particularly for those that bear structural similarity to the target. For certain classes of ONT, additional directed secondary pharmacology assessments may be appropriate based on identified class liabilities (e.g., complement activation by 2'-MOE- phosphorothioate antisense oligonucleotides).

B. Safety Pharmacology

Safety pharmacology evaluations of ONTs should address effects of the drug substance on vital functions. The cardiovascular, respiratory, and central nervous systems are usually considered the vital organ systems that should be studied.[7] Pharmacologically relevant species should be used; however, if there are no pharmacologically relevant species (as defined in section II.B.1 of this guidance), an assessment should still be conducted to assess off-target effects. Safety pharmacology evaluations can be conducted as stand-alone studies or as part of single- or repeat-dose toxicity studies when appropriate and feasible. If effects on the pharmacological target (or hybridization-dependent off-target) could potentially impact safety pharmacology endpoints,

[6] For additional information, see the draft guidance for industry, sponsors, and IRBs *Ethical Considerations for Clinical Investigations of Medical Products Involving Children* (September 2022). When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[7] See the ICH guidance for industry *S7A Safety Pharmacology Studies for Human Pharmaceuticals* (July 2001).

then it may be appropriate to conduct safety pharmacology assessments when PD effects are maximal, as well as at the time of peak drug concentration (T_{max}).

Generally, the in vitro human ether-a-go-go-related gene (hERG) assay is not warranted for ONTs belonging to a well-characterized ONT class[8] that has demonstrated negligible ability to inhibit this ion channel. If the target biology or off-target hybridization-dependent activity suggests the potential for affecting hERG expression or function, or if the ONT is known to accumulate in the heart, then a hERG assay may be appropriate. If a hERG assay is conducted, it is important to consider the time required to see an ONT-mediated change in protein level/function in the assay cell line when designing the protocol.[9] In vivo cardiovascular safety pharmacology assessments should be conducted for all ONTs before clinical administration.

In addition, for ONTs delivered directly to an organ system (e.g., via inhalation, intrathecal administration), more extensive assessment of effects on that organ system may be warranted. Conversely, traditional safety pharmacology studies may not be warranted for ONTs that are delivered locally (e.g., intravitreally or topically) for well-characterized ONT classes for which systemic exposure has been demonstrated to be negligible.

Further guidance on the design of safety pharmacology studies and recommended endpoints to be assessed can be found in ICH 7A and the ICH guidances *S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals* (October 2005) and *E14 and S7B Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential--Questions and Answers* (August 2022).

V. PHARMACOKINETICS

As for other investigational drugs, the nonclinical absorption, distribution, metabolism, and excretion parameters of an ONT should be understood to inform the safety assessment to support clinical trials.

Before human clinical trials are initiated, systemic exposure data in the species used for repeated-dose toxicity studies should be available. This should include exposure to conjugated moieties and components of the formulation (e.g., components of lipid nanoparticles) when appropriate. The ONT should generally be delivered via the intended clinical route. Scientific justification should be provided if a different route is used.

[8] Well-characterized ONT classes, for the purposes of this guidance, include those classes for which one or more products with the same chemical characteristics have been the subject of approval/licensure and for which substantial nonclinical safety data are (1) publicly available in the literature or otherwise characterizable as generally accepted scientific knowledge, (2) owned by the sponsor, or (3) available by right of reference. It is possible that an ONT class may be well-characterized for some, but not all, nonclinical endpoints (e.g., carcinogenicity). A class, in this context, refers to ONTs that have conserved backbone, ribose (or other subunit), targeting or other chemical modifications, differing from one another only in base sequence.

[9] Qu, Y, R Kirby, R Davies, A Jinat, S Stabilini, B Wu, L Yu, B Gao, and HM Vargas, 2023, Time Is a Critical Factor When Evaluating Oligonucleotide Therapeutics in hERG Assays, Nucleic Acid Ther, 33(2):132–140.

In vitro plasma protein binding data for animals and humans should be evaluated before initiating human clinical trials. For well-characterized classes of ONTs, for which interspecies differences in plasma protein binding are demonstrated to be minimal and independent of base sequence, it may be appropriate to rely on class data.

In vitro assessment of metabolites is not considered to be essential for support of early clinical development, since ONTs are catabolized by highly conserved endonucleases and exonucleases, rather than being metabolized by cytochrome P450-containing enzymes. Consequently, ONTs have a low potential for generating toxicologically relevant human-disproportionate metabolites. Catabolism of ONTs results in chain-shortened oligonucleotides whose toxicity profile broadly overlaps with that of the parent ONT. The potential for chain-shortened oligonucleotides to exhibit novel hybridization-dependent off-target activity should be assessed as described in section II.B.2., above. For ONTs employing novel components (e.g., backbone chemistry, ribose modifications, linkers, targeting moieties, formulation components), an in vitro assessment of the metabolites of these components should generally be provided to support initiation of human clinical trials.

ICH M3(R2) recommends that further information on PK parameters (e.g., absorption, distribution, metabolism, and excretion (ADME)) in test species and in vitro biochemical information relevant to potential drug interactions should be available before exposing large numbers of human subjects or administering for long duration (generally before phase 3). These data can be used to compare human and animal metabolites to determine whether any additional testing is warranted. For well-characterized ONT classes, it may be appropriate to rely on class data for some parameters (e.g., distribution and excretion).

If an ONT contains novel chemistry or modifications, data from empirical, product-specific characterizations should be provided to support clinical development, following the timing provided in ICH M3(R2). For in vivo ADME assessments, the route of administration should be relevant to the intended clinical route of administration. For conjugated or formulated ONTs, the clinical drug product or comparable formulation should be tested. Any differences between the clinical formulation and the formulation used in the nonclinical assessment should be identified and justified.

Full ADME characterization of a species-specific surrogate is generally not warranted and is not an appropriate substitute for assessing ADME of the clinical candidate ONT. Confirmation of animal exposure to a surrogate is generally best demonstrated by assessment of PD activity, rather than PK/toxicokinetic parameters.

VI. GENERAL TOXICITY STUDIES

A. Species Selection, Study Design, Duration and Timing of Submission

Toxicity studies of ONTs should generally be conducted in two species: one rodent and one nonrodent (see note 1 regarding selection of nonrodent species). Generally, at least one species

should be pharmacologically relevant. Testing of the clinical candidate in two species to identify non-hybridization-dependent effects is still recommended even if only one or neither species is pharmacologically relevant. In cases where a surrogate molecule is used, animal studies should typically include some groups treated with the clinical candidate and an additional group (or groups) treated with the surrogate. The use of a surrogate oligonucleotide to assess the impact of exaggerated pharmacology may not be practical under some circumstances (see section II.B.1., above).

The design of toxicity studies for ONTs, including endpoints assessed, are generally similar to those for small molecule drugs. Criteria for high doses used for ONTs in toxicity studies are similar to those described in ICH M3(R2).

Appropriate dosing frequency and duration of the toxicity study should consider aspects of drug distribution and half-life, as well as the clinical dosing regimen. The half-life of ONTs can be shorter in animals than humans; consequently, dosing in animals can be more frequent than in humans to ensure sufficient drug exposure. However, more frequent dosing in shorter duration nonclinical studies is generally not sufficient to address effects from a similar number of doses in clinical trials of longer duration. Given the generally prolonged half-life of ONTs at the site of action (in tissues), the onset of PD activity may be delayed, and the duration of the PD effect of an ONT may be prolonged. Consequently, parameters such as distribution phase plasma exposure may not correlate to PD activity. In such cases, PD biomarkers may provide information useful for designing appropriate dosing frequency in toxicity studies.

The duration of chronic toxicity studies should generally be consistent with the recommendations in ICH M3(R2); for example, chronic nonrodent toxicity studies should generally incorporate a dosing period of 9 months or longer. This also applies to ONTs that are conjugated to polypeptides of greater than 40 amino acids and therefore considered to be biological products.[10] Although designated a biological product, the pharmacological and toxicological activity of the conjugated ONT will typically be associated with the oligonucleotide component, which has a long tissue half-life.

Assessment of reversibility of toxicity is generally recommended and may include incorporation of groups with a terminal nondosing period.[11] However, for oligonucleotides with prolonged tissue half-life (and consequently prolonged duration of effect), inclusion of a nondosing period of a sufficient duration to assess recovery may not be feasible.

The timing of report submission and the duration of the dosing phase of the toxicity studies needed to support different phases of clinical development should generally be consistent with the recommendations in ICH M3(R2). Modifications to these recommendations may be

[10] An alpha amino acid polymer with a specific, defined sequence that is greater than 40 amino acids in size is a biological product and is subject to the provisions of section 351 of the Public Health Service Act regarding the requirements for supporting a marketing application (see 21 CFR 600.3(h)(6) and the final rule, "Definition of the Term 'Biological Product'" 85 FR 10057, February 21, 2020).

[11] See ICH M3(R2).

appropriate for ONTs intended to treat cancer, SDLT hematologic disorders, or rare SDLT indications.

B. First-in-Human Dose Selection

The first-in-human dose in healthy volunteers should be selected using the general principles outlined in the guidance for industry *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers* (July 2005). In general, a reasonable starting dose can be defined as one that is expected to result in no more than one-tenth of the exposure observed at the no observed adverse effect level in the most relevant (or more sensitive) species and is typically estimated by calculating a human equivalent dose of the animal dose normalized to body surface area. For severe, irreversible, or unmonitorable toxicity it may be more appropriate to use a larger safety margin. Alternative approaches to calculation of the human equivalent dose may be appropriate for some classes of ONTs or routes of administration (e.g., intrathecal dose normalized by cerebrospinal fluid volume) with scientific justification.

Sponsors should consult ICH S9 for appropriate starting dose guidance for ONTs intended to treat cancer.

VII. GENOTOXICITY STUDIES

Genotoxicity testing of ONTs composed exclusively of natural nucleic acids is not necessary. ONTs containing non-native (1) nucleic acids, (2) backbone structures, or (3) other structures, such as conjugates or linkers, should be assessed for genotoxic potential.

Consistent with published literature,[12] ONTs reviewed by CDER have not exhibited genotoxic activity in the standard test battery recommended in the ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012). Therefore, an assessment for an ONT belonging to a class that is well-characterized for genotoxicity may be informed by the characteristics of the class, provided that the size, structure, modifications, and impurity profile are consistent with those of the class, with the only difference being the base sequence.

For those ONTs warranting an empirical assessment of genotoxicity, the test batteries recommended in ICH S2(R1) are generally considered appropriate, provided data are available to support cellular uptake of the ONT into the cells analyzed. The literature suggests that some of the bacterial strains used in the bacterial mutagenesis assay do not adequately take up some ONTs under standard culture conditions.[13] In cases where the ONT is not taken up by the

[12] Berman, CL, SA Barros, SM Galloway, P Kasper, FB Oleson, CC Priestley, KS Sweder, MJ Schlosser, and Z Sobol, 2016, OSWG Recommendations for Genotoxicity Testing of Novel Oligonucleotide-Based Therapeutics, Nucleic Acid Ther, 26(2):73–85.

[13] Ibid.

bacterial strains used in the Ames assay, consideration should be given to using a mammalian in vitro cell gene mutation assay.

VIII. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES

The assessment of developmental and reproductive toxicity (DART) for ONTs should be conducted in accordance with the ICH guidance for industry *S5(R3) Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals* (May 2021). Typically, this assessment relies on animal studies that assess effects on fertility and early embryonic development, embryofetal development (EFD), and pre- and postnatal development (PPND) endpoints. Fertility and early embryonic development and PPND studies are routinely conducted in rodents, whereas two species, typically rodents and rabbits, are used in EFD studies. There can be cases where the WoE from existing data (e.g., mechanism of action, phenotypic data from genetically modified animals, class effects, general toxicology study results, preliminary EFD study results, human genetic data) can indicate an obvious adverse effect of an ONT on fertility or pregnancy outcome. In these instances, these data may provide adequate information to communicate the risk to reproduction and embryofetal development, and no additional nonclinical studies are warranted.

Although doses and the dosing regimen should generally be selected based on the criteria identified in ICH S5(R3), there can be product- or class-specific attributes of an ONT that may necessitate modifications to the standard DART study designs. In particular, some ONT classes are rapidly cleared from circulation, while having extended tissue residence times and protracted PD effects, which allows for infrequent administration in the clinic. This can pose challenges in the design of the dosing regimen of the DART studies, particularly EFD studies, because the objective of the EFD study is to achieve adequate exposure to both the PD effects and the chemical structure at each stage of organogenesis. Consequently, the dosing regimen for DART studies of ONTs with protracted PK/PD effects is often designed to achieve a balance between ensuring exposure at all stages of development and avoiding clinically irrelevant maternal toxicity resulting from excessive drug exposure or accumulation at the sites of distribution (see note 2). For such products, consultation with the FDA review division regarding appropriate study design is recommended. The approach taken should be scientifically justified.

The effect of the intended pharmacological activity of the ONT on DART endpoints should be assessed. If the clinical candidate is not pharmacologically active in the routine species, an appropriate species-specific surrogate molecule that is active in rodents or rabbits should be used, provided there is sufficient characterization of the model to ensure pharmacological relevance. A typical study design might employ three dose levels of the clinical candidate and a single dose level of the surrogate, though other study designs can be used with justification. The surrogate should generally be dosed at a level that results in the maximal PD effect in the dam. Exposures to the surrogate that exceed the maximal PD effect are not relevant to the safety assessment because these represent off-target activities of an oligonucleotide that will not be administered to humans. Other bases for dose selection for the surrogate (e.g., maximum tolerated dose) may be appropriate with justification.

It is expected that both a rodent and a nonrodent (typically the rabbit) will be used in the in vivo EFD assessment of ONTs, with at least one of these species evaluating the intended pharmacology. If neither the rat nor the rabbit is a relevant species, and generation of a clinically relevant surrogate for these species is not feasible (e.g., target is present only in primates), consideration can be given to assessment of EFD endpoints in the rat and nonhuman primate, preferably through conduct of an enhanced PPND study, if the nonhuman primate is pharmacologically responsive to the ONT. Generally, however, consistent with the approach for small molecule drugs, nonhuman primates are considered to be a nonroutine test species for DART assessment for including ONTs and should not be used without scientific justification.

Measurement in the fetus of the ONT level in a known organ of distribution (e.g., liver) can be useful in assessing whether there was fetal exposure to the drug. For ONTs that act through down modulation of mRNA, and that are expected to be pharmacologically active, measuring levels of the target mRNA(s) can help in establishing whether fetal exposures were associated with pharmacological activity.

ICH M3(R2) should be consulted regarding the timing of submission of DART study reports as it relates to supporting enrollment into clinical trials of women of childbearing potential or pregnant women as well as submission of the marketing application. For ONTs intended to treat cancer, SDLT hematologic disorders, or rare SDLT indications, the relevant guidance should be consulted.[14]

IX. CARCINOGENICITY STUDIES

Sponsors should follow ICH guideline *S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals* (March 1996) with regard to determining whether a carcinogenicity assessment is warranted for a particular development program. As ONTs are generally developed to treat chronic conditions, a carcinogenicity assessment will be expected for most programs. ICH M3(R2) should guide the timing of submission of completed in vivo carcinogenicity studies.

The ICH guidance for industry *S1B Testing for Carcinogenicity of Pharmaceuticals* (July 1997) should be consulted regarding appropriate approaches for assessing human carcinogenicity risk. The conduct of in vivo carcinogenicity studies in two species is one approach to assessing this risk and would generally be appropriate for ONTs in a class with limited data regarding class liability for carcinogenicity risk. For ONTs that are members of a class that have been well characterized for carcinogenicity risk, it can be appropriate to conduct a 2-year carcinogenicity study in a single species. Additionally, as described in the ICH guidance for industry *S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals* (November 2022), the use of a WoE approach for concluding that a rat carcinogenicity study would not add value to the carcinogenicity assessment is also available to those ONT programs with data that can address

[14] See ICH S9, ICH S9 Q&A, and the guidances for industry *Oncology Pharmaceuticals: Reproductive Toxicity Testing and Labeling Recommendations* (May 2019), *Severely Debilitating or Life-Threatening Hematologic Disorders: Nonclinical Development of Pharmaceuticals* (March 2019), and *Rare Diseases: Considerations for the Development of Drugs and Biological Products*.

all the factors described in that guidance, including a completed chronic toxicity study in the rat. Note that, as indicated in ICH S1B(R1), a mouse study (2-year or 6-month transgenic) would still be expected for these programs.

The ICH guidance for industry *S1C(R2) Dose Selection for Carcinogenicity Studies* (September 2008) and ICH S1B (for exposure-based dose selection in the rasH2-Tg mouse) should be consulted regarding appropriate approaches for dose selection for carcinogenicity studies. For ONTs that are intended to be dosed infrequently in patients, a limit dose can be defined as 1,500 mg/kg/administration, rather than 1,500 mg/kg/day, as long as dosing in the animals is at least as frequent as that used in patients, and the animals achieve at least tenfold the human exposure.

The potential for the intended pharmacology of the ONT to increase tumor risk should be addressed. If two in vivo carcinogenicity studies are conducted, the effect of the intended pharmacology should be assessed in at least one species. If the clinical candidate lacks pharmacological activity in rodents, a species-specific surrogate oligonucleotide can be used to assess tumor risk associated with the intended pharmacology. Ideally, dose selection for the surrogate would be based on achieving a maximal PD effect; however, other valid dose selection strategies identified in ICH S1C(R2) and ICH S1B(R1) may also be used. If a surrogate is used to address the effect of intended pharmacology, both the surrogate and the clinical candidate should be used in the carcinogenicity study.

It is recognized that there are scenarios under which an in vivo assessment of the carcinogenicity of the pharmacological activity of a surrogate oligonucleotide may not be warranted, including when (1) the pathway being perturbed is well understood and is known to contribute to tumorigenesis, (2) the surrogate oligonucleotide would affect wild type rodents in a manner that does not recapitulate the intended pharmacology (e.g., for some splice-modulating ONTs that target pre-mRNA to alter the nucleic acids that are included in the mature mRNA, thereby affecting reading frame), or (3) the target does not exist in rodents (e.g., for viral or bacterial targets or human genes for which there is no rodent ortholog). For programs with these characteristics, a 2-year study in a single rodent species is still generally expected to assess the tumorigenic potential of hybridization-independent off-target effects of the clinical candidate. The sponsor should also summarize available information regarding the expected risk of exaggerated pharmacology on tumorigenicity (e.g., data from genetically modified animal models, data from known human diseases).

There is the potential for human-specific, off-target hybridization-dependent activity that could affect carcinogenic risk (e.g., the ONT or a metabolite affects the activity of an unintended target with a known role in carcinogenesis). This type of hazard should be addressed through a WoE assessment, clinical monitoring, and appropriate communication of potential risks via informed consent and product labeling, as animal studies are likely to be uninformative in assessing this risk.

X. LOCAL TOLERANCE

Local tolerance can generally be assessed as part of single- or repeated-dose toxicity studies. The local toxicity of ONTs with complex vehicles should be assessed by testing the entire clinical formulation in toxicity studies if feasible.

XI. IMMUNOTOXICITY

The assessment of immunotoxicity of ONTs should follow ICH and FDA guidance on this topic.[15] Many types of ONTs are known to engage and activate components of the innate immune system (e.g., macrophages, histiocytes, complement). Although stand-alone, dedicated immunotoxicity assessments are not typically warranted, assessment of ONT-induced effects on the immune system (e.g., cytokines, complement) in general toxicity studies can provide useful context for understanding observations seen in these studies.

ONTs may provoke the production of antidrug antibodies. Sponsors should consult the ICH guidance for industry *S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (May 2012) regarding when a nonclinical assessment of antidrug immunogenicity is warranted. The potential for immunogenicity against an ONT to adversely affect patient safety or efficacy is best characterized during clinical testing.

XII. PHOTOSAFETY

The ICH guidance for industry *S10 Photosafety Evaluation of Pharmaceuticals* (January 2015) should be consulted for recommendations on photosafety assessment. If ultraviolet-visible light absorption of an ONT is determined to be solely due to natural nucleic acid bases, then additional photosafety assessment is not warranted.

XIII. OTHER TOXICITY STUDIES

A. Formulations, Targeting Moieties

In general, ONTs should be tested in in vivo nonclinical studies using the same drug delivery formulation and method as intended in humans. In some circumstances, toxicity of novel excipients may also be addressed by studies of the excipients alone. For guidance regarding the safety evaluation of excipients, please see the guidance for industry *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients* (May 2005). If an ONT is conjugated to another molecule such as a peptide or carbohydrate component (e.g., GalNAc), then the conjugated ONT should be assessed in nonclinical studies.

[15] See the ICH guidance for industry *S8 Immunotoxicity Studies for Human Pharmaceuticals* (April 2006) and the guidance for industry *Nonclinical Evaluation of the Immunotoxic Potential of Pharmaceuticals* (June 2023).

Sponsors may be able to use nonclinical data from an unconjugated ONT with the same chemistry and nucleotide sequence to support some aspects of the nonclinical assessment when conjugated to a well-characterized moiety. The acceptability of such data will depend on whether adequate doses were used in assessing the unconjugated ONT and whether a maximum pharmacological response was achieved (if relevant). Reliance on data from a previously developed unconjugated ONT would generally not be adequate to support the safety of ONTs conjugated to novel structures for which there is limited experience.

It is recognized that certain targeting moieties (e.g., antibodies) may confer additional species specificity to the ONT in addition to those related to the oligonucleotide itself. Decisions regarding species selection and the design of appropriate surrogate molecules will need to be carefully considered in these cases. Consultation with the appropriate FDA review division is recommended.

B. Impurities/Degradants

As for other synthetically derived drug products, impurity levels in ONT drug products should be adequately controlled and demonstrated to be present at levels that do not raise safety concerns. Impurities derived from ONTs can be classified into two broad categories: (1) oligonucleotide-related substances and (2) other impurities (e.g., organic small-molecule impurities, residual solvents, elemental impurities). In the ONT manufacturing process, impurities should be reduced to the lowest practical level and evaluated for safety in humans based on the level of the impurity, existing data about the impurity (including published data), and the results of nonclinical toxicology studies in which the animals are exposed to the impurity.

1. Oligonucleotide-Related Impurities

Oligonucleotide-related impurities (e.g., n+1, n-1 sequences) exhibit similar physicochemical properties to one another and to the ONT and cannot practically be separated individually from related substances, making it difficult in many cases to qualify the oligonucleotide-related substances in a manner consistent with current ICH small molecule drug impurities guidance.[16] Therefore, the primary basis for establishing the safety of oligonucleotide-related impurities in ONTs is by qualification of the impurities in the nonclinical safety studies during the testing of the drug substance or drug product. Toxicity testing of ONTs enriched with particular impurities or classes of impurity, or the testing of an isolated impurity, may be useful in qualifying impurities that are not present at sufficiently high levels in the nonclinical drug batches. It may also be possible to rely on existing data regarding impurities commonly encountered in oligonucleotide synthesis from oligonucleotides of the same class (and synthetic method) containing similar or higher levels of the impurities.

As each individual oligonucleotide-related impurity is typically present at a level that is negligible compared with the level of the active pharmacological ingredient, it is generally unnecessary to evaluate off-target toxicity due to hybridization for those impurities.

[16] See the ICH guidance for industry *Q3A(R2) Impurities in New Drug Substances* (June 2008).

Degradation products or impurities that are also significant metabolites present in animal and/or human studies are generally considered qualified.

2. Other Impurities

Existing guidance adequately addresses regulatory expectations for the control, identification, characterization, and qualification of other impurities (generally process-related impurities) related to synthesis of ONTs. Sponsors are referred to ICH Q3A(R2) and the ICH guidances for industry *Q3B(R2) Impurities in New Drug Products* (August 2006) and *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (July 2023). Residual solvents should be assessed with reference to the ICH guidance for industry *Q3C(R8) Impurities: Guidance for Residual Solvents* (December 2021). Elemental impurities should be assessed with reference to the ICH guidance for industry *Q3D(R2) Elemental Impurities* (September 2022).

C. Juvenile Animal Studies

When evaluating the need for juvenile animal studies for ONTs being developed for pediatric indications, sponsors should follow the recommendations provided in the ICH guidance for industry *S11 Nonclinical Safety Testing in Support of Development of Pediatric Pharmaceuticals* (May 2021).

XIV. NOTES

Note 1. Historically, nonhuman primates have typically been used as the nonrodent species for the general toxicological assessment of oligonucleotides, given the high degree of conservation of nucleic acid base sequences between humans and nonhuman primates. Consequently, there is a relatively rich understanding of the effects of various classes of oligonucleotides in nonhuman primates. Nonetheless, consistent with the 3Rs, sponsors are encouraged to consider the use of phylogenetically lower nonrodent species when scientifically appropriate.

Note 2. There are multiple factors that should be considered when designing the dosing regimen for EFD studies for ONTs. Briefly, such factors include the PK/PD profile, degree of structural and chemical novelty, and tolerability in pregnant females of the test species (e.g., as established in a preliminary EFD study).[17] More typical (e.g., daily) dosing regimens may be appropriate for oligonucleotides: (1) with a short PK/PD profile, requiring frequent clinical dosing (i.e., more often than once a week); (2) with a chemical structure for which there are only limited data; or (3) that exhibit relatively low maternal toxicity following repeated administration, regardless of the clinical dosing regimen. If maternal toxicity precludes daily or alternative daily dosing, consideration can be given to the use of multiple cohorts with staggered dosing initiation such that each cohort receives the full target dose (as determined by the criteria in ICH S5(R3)) at the clinical dosing interval. In this manner each cohort is dosed on a different gestation day (or range

[17] Cavagnaro, J, C Berman, D Kornbrust, T White, S Campion, and S Henry, 2014, Considerations for Assessment of Reproductive and Developmental Toxicity of Oligonucleotide-Based Therapeutics, Nucleic Acid Ther, 24(5):313–325

of days), thus ensuring assessment of the effect of exposure across organogenesis. To avoid excessive maternal toxicity, it may also be acceptable to split a weekly (or longer duration) target dose into daily or alternate daily doses, such that the aggregate dose administered reaches the target dose. To support such split dosing, it is important to understand whether the lower achieved peak drug concentration (C_{max}) can affect the total aggregate accumulation of oligonucleotide in the target organs, the distribution to new target organs, or the ability of the oligonucleotide to partition into the placenta or conceptus.