INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

NONCLINICAL SAFETY EVALUATION OF OLIGONUCLEOTIDE-BASED THERAPEUTICS S13

Draft version
Endorsed on 18 November 2026
Currently under public consultation

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

S13
Document History

Code	History	Date
S13	Endorsement by the Members of the ICH Assembly under *Step 2* and release for public consultation.	18/11/2026

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ICH HARMONISED GUIDELINE
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S13

ICH Consensus Guideline

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LIST OF ABBREVIATIONS

Abbreviation	
ADAR	Adenosine Deaminase Acting on RNA
API	Active Pharmaceutical Ingredient
ASOs	Antisense Oligonucleotides
AUC	Area Under the Curve
CAS9	CRISPR-associated Protein 9
CNS	Central Nervous System
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DART	Developmental and Reproductive Toxicity
DDI	Drug-drug Interactions
DDS	Drug Delivery Systems
EFD	Embryofoetal Development
ePPND	Enhanced Pre- and Postnatal Developmental
FEED	Fertility and Early Embryonic Development
FIH	First-in-Human
GalNAc	N-acetylgalactosamine
GLP	Good Laboratory Practice
gRNA	Guide RNA
hERG	Human Ether-à-go-go-related Gene
HPRT	Hypoxanthine Phosphoribosyltransferase
LNP	Lipid Nanoparticles
MABEL	Minimum Anticipated Biological Effect Level
MALAT1	Metastasis-associated Lung Adenocarcinoma Transcript 1
miRNA	Micro RNA
MoA	Mechanism of Action
mRNA	Messenger RNA
NAMs	New Approach Methodologies
NHPs	Non-human Primates
NOAEL	No Observed Adverse Effect Level
ONTs	Oligonucleotide-based Therapeutics
PAD	Pharmacologically Active Dose
PD	Pharmacodynamics
PK	Pharmacokinetics
PPB	Plasma Protein Binding
PPND	Pre- and Postnatal Development
QSAR	Quantitative Structure-activity Relationship
3Rs	Replacement, Reduction, and Refinement
RISC	RNA-induced silencing complex
RNase H	Ribonuclease H
RNAseq	RNA sequencing
SDLT	Severely Debilitating or Life Threatening
saRNA	Small Activating RNA

Abbreviation

siRNA	Small Interfering RNA
Tg	Transgenic
TK	Toxicokinetic(s)
TLR9	Toll-like Receptor 9
tRNA	Transfer RNA
UV-Vis	Ultraviolet-visible
WOCBP	Women of Childbearing Potential
WoE	Weight of Evidence

1. INTRODUCTION

1.1. Background

Oligonucleotide-based Therapeutics (ONTs) represent a rapidly evolving modality with high potential to address a wide array of medical conditions, rare diseases and personalised treatments. Various regional guidance documents and white papers have been published on the nonclinical safety assessment of ONTs. In the absence of dedicated harmonised guidance, sponsors often referred to ICH M3 for safety evaluations, which does not fully address the unique aspects of ONTs. This ICH Guideline dedicated to ONTs, together with existing ICH Guidelines, provides the basis for a scientifically sound and accepted nonclinical safety evaluation of ONTs. Additionally, it supports the 3Rs principle (replacement, reduction, and refinement) and lays the groundwork for tailored test strategies helping to accelerate patients' access to ONTs.

1.2. Objectives

This Guideline harmonises international standards for nonclinical safety studies of ONTs by considering their specific characteristics, compositions, and Mechanism of Action (MoA). In this Guideline, ONT is defined as the entire product including the oligonucleotide and drug delivery system (DDS) components, if applicable (Section 2.3). ONT-induced toxicities could range from hybridisation-dependent on-target to hybridisation-dependent off-target effects, as well as those induced by chemical components of the complete molecule (1). Emphasis should be given to start with a Weight of Evidence (WoE) approach including the consideration of New Approach Methodologies (NAMs). The specific properties of ONTs may allow for leveraging prior information (Section 2.1), thereby avoiding redundant nonclinical safety assessments and reducing animal use, leading to more efficient development and review processes.

ONTs differ from biopharmaceuticals and small molecules in several safety-relevant features. Adherence to the principles presented in this Guideline is intended to improve the consistency of the nonclinical safety data supporting the efficient development of ONTs.

1.3. Scope

This Guideline is recommending a framework for the nonclinical safety evaluation of ONTs, however, deviations from testing strategies and case-by-case decisions are possible when scientifically justified. ONTs are categorised into classes which are defined by their MoA. The scope of this guideline covers a range of ONT classes including, but not limited to, Antisense Oligonucleotides (ASOs), including Ribonuclease H (RNase H)-dependent, splice switching,

micro (mi)RNA inhibitors (antagomirs), and RNA editing (e.g., Adenosine Deaminase Acting on RNA [ADAR]); small interfering (si)RNAs; miRNA mimics and small activating (sa)RNA. For other ONTs with MoAs that differ from those listed above such as aptamer, decoy, transfer (t)RNA, and CpG ONT(s), this guidance may be relevant to assessing some aspects of nonclinical safety. DDS used to enhance tissue uptake are in scope (Section 2.3). Guide (g)RNA for DNA editing, prophylactic and therapeutic DNA/RNA vaccines and coding messenger (m)RNA used for therapeutic purposes are out of scope.

2. GENERAL CONSIDERATIONS

2.1. Well-characterised ONT

ONTs of a given class and comparable composition (including DDS, Section 2.3) for which adequate prior information is available may be considered well-characterised (Table 1). The well-characterised ONT concept will support a scientifically justified, risk-based nonclinical development strategy to inform study design and potentially avoid the redundant conduct of nonclinical studies, thereby supporting the 3Rs principle.

The well-characterised ONT concept is intended to address sequence-independent aspects of nonclinical safety. Hybridisation-dependent on- and off-target effects should be evaluated in accordance with the relevant endpoint-specific sections of this Guideline. An ONT may not be considered well-characterised for all nonclinical endpoints. The amount and type of evidence required may vary depending on the endpoint. Reference to a well-characterised ONT should use a WoE evaluation for each nonclinical endpoint to determine if additional studies are warranted.

For a candidate ONT to be considered well-characterised, sufficient prior nonclinical data should exist from adequately designed and conducted studies that show conclusive and consistent results for the nonclinical endpoints in question (Table 1). Potential sources of nonclinical data include: 1) peer-reviewed publications; 2) data owned by the sponsor; or 3) data available by right of reference. These are referred to as prior information in subsequent sections of this Guideline. Regulatory approval of referenced ONTs is not required. Reference can be made to regional requirements for information from certain sources in a marketing application.

Table 1 General considerations for determining a well-characterised ONT

Factor	General Considerations
Class, Composition and DDS	Relevant attributes include the oligonucleotide class (Section 1.3) and composition including chemical modifications: e.g.,, ribose or other sugar subunits; backbone; base components; number of strands; strand length; and the composition of any DDS.
Relevant Studies	Information on study design should include the species or test system, route of administration, study duration and study endpoints.
Key Data	Key parameters for comparison include endpoint-specific nonclinical safety data and PK or toxicokinetic (TK) profiles (Section 4). Clinical safety and PK data may be supplemental for the assessment.
Data Quality	GLP-compliant studies are preferred where applicable. Relevant non-GLP studies that may provide valuable information include mechanistic and/or investigative studies, and appropriately qualified NAM data.

2.2. New Approach Methodologies (NAMs)

NAMs, as a working definition for the purpose of this guidance, refer broadly to test methods or approaches aimed to progressively replace, reduce, and refine traditional animal studies. These include *in vitro*, *in silico* (including Quantitative Structure-activity Relationship [QSAR] models), *ex vivo*, *in vivo* (e.g., in phylogenetically lower animals) and other approaches, as well as structured combinations of these. NAMs aim to provide data that are predictive of human responses and may inform regulatory decisions.

As is the case for other therapeutic modalities, NAMs for ONTs may be used to:

- Investigate the human relevance or mechanistic basis of findings observed in animal studies, including where species-specific biology limits direct translation to humans;

- Fill data gaps where established animal models are unavailable, insufficiently predictive, or lack biological relevance for the ONT under evaluation; and
- Contribute data to the overall WoE assessment in support of regulatory decision-making.

- Assessment of product-related impurities to address potential safety concerns (e.g., immunotoxicity).

In addition, characteristics of ONTs create unique opportunities for the application of NAMs in nonclinical evaluation. Under these situations, the use of scientifically justified NAMs for specific endpoints should be considered before animal studies are pursued. These include, but are not limited to:

- Evaluation of hybridisation-dependent on-target and off-target effects (Section 3) in human-relevant systems, particularly where the clinical candidate lacks sufficient cross-reactivity in routine nonclinical species.
- Assessment of specific pharmacokinetic and/or toxicological endpoints for ONTs that are considered well-characterised or contain minor compositional differences from well-characterised ONTs, where NAM-derived data may be sufficient to assess the impact of these differences and avoid additional in vivo animal studies.

The use of NAMs to support nonclinical evaluation of ONTs, is encouraged, and should be considered proactively at each stage of the nonclinical program.

When NAMs are employed, the methodology should be characterised to an extent appropriate for the intended context of use, including demonstration of human biological relevance, technical performance (e.g., accuracy, sensitivity, specificity, reproducibility) and known limitations. The appropriate level of methodological rigour and scientific validity required for a NAM is risk-based and should be proportionate with the regulatory weight being placed on the NAM-derived data.

2.3. Drug Delivery Systems (DDS)

Within an ONT class and chemical composition, pharmacological properties are dependent on the route of administration and DDS, including conjugate-based (e.g., N-acetylgalactosamine [GalNAc], monoclonal antibodies) and excipient-based (e.g., Lipid Nanoparticles [LNP]) systems. Toxicology and PK studies should generally evaluate the ONT with its intended DDS rather than individual components. Relevant safety assessment of novel individual DDS components, if considered necessary, should follow respective guidance, e.g., ICH M3 or ICH S6. In some cases, evaluating the separate components might be appropriate, e.g., *in vitro* genotoxicity (Section 7) or

secondary pharmacology (Section 3.3) studies of small molecule-based DDS components. *In vivo* studies resulting in tissue distribution not representative of anticipated clinical situation are discouraged, as they are likely to produce clinically irrelevant safety data.

If lack of species cross-reactivity for a targeting DDS prevents relevant tissue distribution, use of alternative test systems in one species (e.g., animal-specific surrogate DDS) may be considered to assess relevant ONT distribution and potential on-target safety risk with the DDS components. Composition of the animal-specific DDS surrogate should be comparable to the clinical candidate. Lack of cross-species activity also in the oligonucleotide part may warrant the use of a "double-surrogate" (i.e., a combination of two surrogate components) for hazard identification of potential on-target safety concerns (Section 3.1). For ONTs requiring surrogate DDS for *in vivo* studies, the clinical DDS, when relevant (e.g. being a small molecule), should also be evaluated in vivo.

For well-characterised ONTs using comparable route of administration, a reduced level of DDS assessment can be considered, e.g., leveraging prior data on tissue distribution.

2.4. Good Laboratory Practice

Pivotal toxicity studies are expected to be performed in compliance with Good Laboratory Practice (GLP). It is recognised that studies employing specialised test systems which are often needed for ONTs may not be able to comply fully with GLP. Areas of non-compliance in pivotal toxicology studies should be identified and any impact be evaluated relative to the overall safety assessment. When studies do not meet full GLP compliance, these data from these studies may still be used to support clinical trials and marketing authorisations.

2.5. Severely Debilitating or Life-threatening (SDLT) Diseases

In alignment with ICH M3 principles, ONTs being developed for severely debilitating or life-threatening diseases that lack effective treatment options may warrant flexible testing approaches on a case-by-case basis to expedite drug development and respective guidance should be consulted. For these situations, and particularly for individualised ONTs (single or few patients), the nonclinical program may be abbreviated, deferred, or supplemented with targeted studies as scientifically justified. This flexibility may be particularly applicable but not limited to ONTs with well-characterised class and composition. Timely engagement with regulatory authorities is encouraged.

3. PHARMACOLOGY

3.1. On-target Effects

For on-target effects, studies in at least one pharmacologically active species should be conducted to understand the pharmacodynamics (PD) of an ONT. These studies can help define dose levels, frequency for the toxicology program and should be completed before First-in-Human (FIH) trials. It is possible to utilise *in vitro* methods or other NAMs to generate this data, however, given the unique *in vivo* dynamics of ONTs (e.g., distribution, prolonged tissue half-life), such approaches should be scientifically justified.

Given the specificity of many ONTs to human sequences, the use of animal-specific surrogates or transgenic models may be needed to understand on-target effects across the pharmacology and toxicology program. In some cases, there may be no *in vivo* systems that express the target of an ONT, such as disease-specific sequences. In these situations, *in vitro* systems derived from human tissue or manufactured to express the human target may be utilised.

3.1.1. Use of Surrogate Molecules and Transgenic Models

Use of animal-specific surrogates should generally be limited to rodents. The surrogate should be applied at a single dose level and minimal dosing frequency that represents the highest intended clinical level of target modulation in a relevant tissue. They should be designed to reproduce the human distribution profile in the relevant animal model, considering the similarity of composition to the clinical candidate, including the option to use an animal active DDS, creating a "double-surrogate". Research-grade material and a minimal level of characterisation of the surrogate molecule to demonstrate it is fit for purpose is sufficient. Alternatively, use of a transgenic model expressing the human target may be considered. If a transgenic model is used for nonclinical safety assessments, its relevance to the endpoints being assessed should be demonstrated (e.g., background pathology, reproductive performance). In studies where a surrogate is used, the clinical candidate should still be tested for other ONT-related toxicities. If adequate information about the pharmacological target exists to characterise any potential hazard, specific safety assessments with surrogates or transgenic animals may not be needed.

3.2. Hybridisation-dependent Off-target Effects

ONTs have the potential to bind to unintended nucleic acids that share partial or complete complementarity with the base sequence present in the ONT, also known as hybridisation-dependent off-target effects. This may lead to effects in humans that can be difficult

to assess in animal studies due to species-specific sequence differences. Therefore, hybridisation-dependent off-target assessments should be conducted using appropriate *in silico* and *in vitro* methodologies in human systems. The degree and type of assessment depends on the likelihood of potential adverse off-target effects and characteristics of the ONT class. Evaluation is generally not necessary for ONT classes that do not act *via* sequence-dependent binding to nucleic acids (e.g., aptamers, CpG ONTs).

Off-target identification and risk assessment of an ONT should be conducted prior to FIH clinical trials and should be sufficient for marketing authorisation. The following points should be considered when designing hybridisation-dependent off-target assessments (2):

Off-target identification

- An *in silico* assessment of potential hybridisation to the full human transcriptome (if intended to bind RNA), as well as an *in vitro* human cell-based screen (e.g., RNA sequencing) should be conducted. The *in silico* sequence alignment criteria and the *in vitro* experimental systems and methods should be scientifically justified and tailored to the ONT mechanism and properties.
- The full-length nucleic acid component of the drug product that is available for off-target hybridisation should be assessed. If certain components are considered unlikely to cause off-target hybridisation effects, their exclusion from analysis should be scientifically justified (e.g., low probability of RNA-induced silencing complex [RISC] incorporation of an siRNA sense strand).

Risk assessment

- Potential off-targets identified in these screens should be experimentally investigated and their biological relevance to human safety evaluated.
    - Assessing the likelihood of relevant off-target activity should focus on tissues and cell types showing relevant ONT activity (Section 4) and an *in vitro* margin assessment relative to on-target.
    - Assessment of potential consequences of off-targets with relevant activity should include information from location of expression and biological role of the off-target relative to tissue distribution and activity of the ONT. In general human off-target effects are not adequately evaluated in *in vivo* toxicology studies due to the frequent lack of sequence conservation of the off-target in the toxicology species. However,

potential consequences of human off-target effects, if modulated to relevant levels in the animal, may be informative in the overall risk assessment.

3.3. Secondary Pharmacology

Typical small molecule secondary pharmacology assessments (e.g., receptor, ion channel, and enzyme binding and functional screens) are generally not warranted for the oligonucleotide part of the ONT if the pharmacological MoA is through nucleic acid hybridisation. Small-molecule-like components of novel DDS may warrant secondary pharmacology assessment. Such studies may also be appropriate for ONTs with mechanisms other than nucleic acid hybridisation (e.g., aptamers), or as follow-up studies for any ONT with unexpected toxicities.

3.4. Safety Pharmacology

Safety pharmacology evaluation of an ONT should use a scientifically justified, risk-based WoE approach to determine the need, scope, and test system for assessment of potential adverse effects on vital organ functions prior to FIH clinical trials. Where an ONT is considered well characterised for the specific safety pharmacology endpoint being considered, relevant existing data may be leveraged to inform the need, scope, and study design of additional assessment (Section 2.1). The assessment of cardiovascular, respiratory, and central nervous system functions should be considered in accordance with ICH S7A.

A pharmacologically active species should be used to assess impacts of on-target or exaggerated pharmacology when feasible for safety pharmacology endpoints. Where no pharmacologically active species is available, an animal-specific surrogate with relevant biodistribution (Section 3.1.1), or another fit-for-purpose test system (e.g., NAM, Section 2.2) may be used for hazard identification.

Safety pharmacology endpoints should be incorporated into general toxicology studies when feasible. Assessment in a standalone study, NAM, or other fit-for-purpose approach should be scientifically justified. The timing of in vivo assessments should reflect relevant tissue exposure and/or PD effects rather than plasma exposure alone. For ONTs delivered directly or reaching relevant exposure or productive uptake in a vital organ system assessment should be tailored to further characterize potential risk in the vital organ system. Conversely, for locally administered ONTs with negligible systemic exposure and no plausible systemic risk, dedicated safety pharmacology assessment in other organs may not be warranted.

Cardiovascular safety assessment for ONTs should in general follow ICH S7B and ICH E14/S7B Q&As. For systemically administered ONTs with unknown risk, a sensitive nonclinical *in vivo* QTc assessment is recommended as the initial assessment; the *in vitro* human ether-à-go-go-related gene (hERG) assay may not be informative for ONTs and is generally not recommended as a routine first test. The need for additional nonclinical assessment beyond an *in vivo* QTc assessment should be determined from the totality of evidence, including assay relevance, exposure, mechanism, and relevant prior data. Positive *in vivo* findings may warrant qualified alternative *in vitro* assays or other fit-for-purpose follow-up to characterise the finding.

4. PHARMACOKINETICS

Nonclinical PK analysis of ONTs should follow the principles outlined in ICH M3, ICH S3A, and ICH S3B. Within an ONT class and chemical composition, PK properties are dependent on the route of administration and DDS. ONTs typically show rapid plasma clearance, specific tissue distribution with prolonged tissue exposure, and a disconnect between plasma PK and tissue PD effect. Both PK and PD assessments are important for linking exposure to functional activity. Prior data from a comparable ONT and administration route can be leveraged for modelling of exposure and distribution to allow reduced level of PK assessment in subsequent studies provided the model adequately describes the PK in those following studies.

4.1. Pharmacokinetic/Toxicokinetic Profiles

Pharmacokinetic/toxicokinetic (PK/TK) profiles of the ONT should be adequately characterised in the species used in the nonclinical development program, using the intended clinical route of administration when possible. Integration of systemic PK/TK profiles with other relevant data is described in the section covering general toxicology studies (Section 5). PK/TK information from non-oligonucleotide components of the DDS, e.g., conjugate ligand or LNP excipients, is not needed unless they constitute a potential safety concern on their own. To minimise animal use, microsampling (ICH S3A Q&A) should be used when feasible.

4.1.1. Pharmacokinetic/Toxicokinetic Profiles of surrogates

Animal-specific surrogate can be used for hazard identification of potential on-target safety concerns (Section 3.1). If adequate PD effect has been demonstrated in surrogate-treated animals, PK/TK assessment of the surrogate is not required as these data are not used for quantitative risk assessments or defining safety margins for the candidate ONT.

4.2. Tissue Distribution

Tissue distribution should be characterised using appropriate approaches, e.g., tissue homogenate-based methods to generate quantitative exposure data. When evaluating distribution of a novel DDS, an oligonucleotide targeting a broadly expressed transcript (e.g., Metastasis-associated Lung Adenocarcinoma Transcript 1 [MALAT1] or Hypoxanthine Phosphoribosyltransferase [HPRT]) may be used to evaluate the tissue distribution and productive uptake of the oligonucleotide. These data may support assessment of potential distribution of an ONT with the comparable DDS. Specific PK/PD effects may need confirmation in relevant tissues for each clinical candidate, using an animal-specific surrogate for the therapeutic target if necessary (Section 3.1.1).

4.3. Metabolism

Metabolism of the oligonucleotide component of an ONT primarily involves cleavage by nucleases that show high degree of species conservation. The resulting chain shortened metabolites therefore have a low risk of being disproportionate human metabolites. Relevant species differences as defined by ICH M3 should be assessed in plasma and urine at steady state before exposing large numbers of human subjects, generally before Phase III. The type and potential biological activity of chain-shortened metabolites may differ between ONT classes (e.g., siRNA and RNase H ASOs). For individual unique and disproportionate human oligonucleotide metabolites exceeding 10% of total drug-related exposure, further safety assessment may be warranted. For any human metabolite exceeding 10% of total drug-related exposure, hybridisation-dependent off-target assessment (Section 3.2) should be considered.

Studies and timing to assess metabolism of novel DDS components (e.g.,, linker/ligand) should follow the respective guidelines for that modality, e.g., ICH M3 for small molecule and ICH S6 for biotechnology-based components.

4.4. Plasma Protein Binding

Data on plasma protein binding (PPB) is not used for ONT safety assessment and is generally not required for interpretation of toxicology studies. Exceptions may include specific *in vitro* assays or evaluation of unexpected in vivo observations where PPB may be an important parameter.

4.5. Drug-drug Interaction

The oligonucleotide part of an ONT is generally metabolised by nucleases, where the risk for drug-drug interactions (DDI) as a substrate is low with no requirement for nonclinical DDI assessment.

Novel non-oligonucleotide DDS components should follow modality-specific guidelines for nonclinical DDI assessment (Section 2.3).

5. GENERAL TOXICOLOGY STUDIES

5.1. Single Dose Toxicology Studies

Single dose toxicity studies are generally not recommended, when acute toxicity can be evaluated in repeated dose studies.

5.2. Repeat Dose Toxicology Studies

5.2.1. Novel ONTs

For the evaluation of novel ONTs, it is recommended to use two species (rodent and nonrodent), where at least one species should show pharmacological activity with the clinical candidate or with the use of a surrogate, (Section 3.1.1) if feasible. If neither species is pharmacologically relevant and a surrogate is not feasible, testing the clinical ONT in two species is still recommended to evaluate compound toxicities. In this case, the risk related to the target biology should be assessed based on a WoE approach.

Options for reduced chronic toxicity testing of novel ONTs may be considered when the following criteria are met: 1) The ONT with a novel composition belongs to an established class (e.g., ASOs or siRNAs), 2) when subchronic studies for these ONTs do not reveal unexpected adverse toxicities and the outcome of future chronic studies would be considered predictable, and 3) the PK/TK profile is similar to well-characterised ONTs. In this case, a chronic study in a single species for up to 6 months (also for non-rodent species) may be considered sufficient. A reduced testing approach may be supplemented by the use of NAMs if applicable. High dose levels in pivotal toxicology studies should be justified based on multiple considerations, including the maximal feasible or maximal tolerated dose and, where feasible, exposure margins of up to 25-fold over the maximum anticipated human exposure.

5.2.2. Well-characterised ONTs

When an ONT under development is well-characterised for general toxicology (Section 2.1), an adapted approach could be considered. A single species 3-month subchronic study may be sufficient, when the animal toxicities are well understood and predictable. In these cases, a reduced number of dosing groups may also be considered. A decision on the need for a chronic study (where a 6-months duration is considered sufficient, also for non-rodent) should be based on a WoE evaluation, considering the available data on the well-characterised nature of the ONT candidate

and the findings in the subchronic study. Where delayed toxicity is expected, a WoE alone might not be sufficient and would generally require a chronic study with a duration in line with ICH M3. In the event that novel, unexpected adverse toxicities are observed in the subchronic study, that have not been observed with these well-characterised ONTs previously (e.g., new target organ or different toxic effect in known target organ), consideration should be given to conducting chronic studies in one or two species, depending on the nature of the toxicity and human relevance. High dose levels in pivotal toxicology studies should be justified based on the same considerations as for novel ONTs, and where feasible, exposure margins of up to 10-fold over the maximum anticipated human exposure.

5.2.3. General Study Design Aspects

For single species general toxicity programs, the most sensitive species should be chosen if the toxicities observed in previous studies may have human relevance. Where possible the lowest species should be considered, which may require the need for a surrogate ONT to induce clinically relevant PD activity in relevant target tissue (Section 3.1.1), along with the clinical candidate.

For plasma exposure, the comparison should take into account differences in dosing frequency between the animal study and patients, where a cumulative area under the curve (AUC) approach may be used. Comparison based on plasma exposure may also be appropriate for the determination of safety margins. For local administration it may be appropriate to extrapolate based on the volume of the specific organ (e.g., cerebrospinal fluid or eye fluid).

The duration of tissue exposure and PD effects are often sustained for ONTs (Section 4) which should be reflected in the study design. The duration of dosing in humans should be supported by the duration of dosing in toxicology studies. The day of the last dose defines the study duration. For dosing duration for oncology products, reference is made to ICH S9 and Q&A.

Repeated dosing frequency should be equal to or exceed the anticipated dosing frequency in the clinical trials. However, possible differences in PD effect duration and/or tissue half-life between animals and humans should be taken into account in order to ensure sufficient coverage of tissue exposure and/or PD effect in the animals.

A recovery group may be informative to understand the potential reversibility of adverse toxicological effects. Full recovery of the PD effect and elimination of tissue exposure may not occur at the end of the recovery phase. Therefore, the duration of the recovery period should be based on any prior knowledge regarding trends in recovery of toxicity. Such a period may not be

needed in all studies. Recovery groups should be reduced to a minimum, particularly for well-characterised ONTs.

5.2.4. Considerations for Drug Delivery System Species Cross-reactivity

Studies where the DDS (e.g., monoclonal antibodies) is not cross-reactive in the animal species under investigation are likely to yield safety profiles that are irrelevant to humans owing to significantly different distribution profiles. A single species toxicology program is recommended when there is one cross-reactive species or no cross-reactive species, where in the latter case a surrogate DDS needs to be considered in that single species if feasible. When the DDS is cross-reactive in both rodent and non-rodent species, a 2-species toxicology program is recommended for novel ONTs.

5.3. Local Tolerance

In most cases, the potential adverse effects of local administration can be evaluated in general toxicology studies, thus obviating the need for standalone local tolerance studies.

6. FIRST-IN-HUMAN (FIH) DOSE SELECTION

The FIH dose should be selected using the general principles outlined in ICH M3, ICH S9 and ICH S9 Q&A for oncology indications, and appropriate regional guidance. In general, a starting dose can be defined as one that is predicted to achieve exposure below that observed at the NOAEL in the most sensitive species or the more human-relevant species when clinical relevance of the observed toxicity is known. An appropriate safety factor is dependent on indication, previous experience with similar products of which the safety profile is known, and whether identified risks are clinically monitorable. A cumulative AUC approach described in Section 5.2 should be used. The dose level can be estimated by calculating a human equivalent dose of the animal dose normalised to body weight, body surface area, or other factors (e.g., volume at site of local delivery), when scientifically justified. Alternatively, an approach using the pharmacologically active dose (PAD) or minimal anticipated biological effect level (MABEL) can be applied. Local guidance documents are available on when these approaches are appropriate. Examples to use the PAD or MABEL approach include, but are not limited to, situations where ONTs target immunostimulatory pathways, or when target biology is disconnected between the animal models and humans, or when there is no biomarker for PD available. Other modelling approach may also be considered, as well as scaling factors based on previous experience. For severe, irreversible, or unmonitorable toxicity, it may be more appropriate to use a larger safety factor.

The duration of dosing in the FIH trial should be supported by an equal duration in animal studies. In cases of novel ONTs with a lack of knowledge on their toxicological profile, or when concerns exist around delayed toxicities, adding a recovery period after the last dose in the animal study may be considered; however, it does not allow for extending the duration of dosing in the FIH trial. The duration of the recovery period does not need to extend until PD has returned to baseline, it should be an acceptable duration that allows for characterisation of the potential delayed toxicity.

7. GENOTOXICITY STUDIES

Genotoxicity risk evaluation for ONTs should be conducted in an integrated framework, taking into account chemical composition, historical knowledge and empirical testing, MoA (class, on/off-target biology) and ONT-specific aspects.

7.1. Novel ONTs

For ONTs containing compositions of non-natural nucleic acids and/or other chemical structures (i.e., small molecule-based DDS), an experimental evaluation of genotoxicity should be made. The tests outlined in ICH S2 are generally considered appropriate for ONTs and this guidance should be followed. For *in vitro* testing, the concentrations achieved should align with the limits outlined in ICH S2, i.e., be the assay-specific highest test concentration or limited by solubility/cytotoxicity. For *in vivo* testing, reference is made to ICH S2 for dose selection and with regard to confirming exposure in the tissue in which genotoxicity assessment is made (which would include bone marrow). However, it may be possible to leverage existing tissue exposure data for comparable ONTs, supplemented by an assessment of blood or plasma levels of the candidate in the *in vivo* genotoxicity test.

It is recommended to test the intact ONT, which would include the oligonucleotide with any DDS if used. If the ONT contains a component that carries no genotoxicity risk (e.g., a protein/monoclonal antibody targeting moiety), then it may be more appropriate to test the oligonucleotide with the attached linker component. Testing of individual components may also be appropriate to help elucidate a positive/equivocal result in a genotoxicity test.

Genotoxicity testing of ONTs composed exclusively of naturally occurring nucleic acids is not necessary, unless the primary pharmacological target, ONT class or any confirmed hybridisation-dependent off-target(s) carry a potential genotoxicity risk (Section 3 and Section 7.3). The standard ICH S2 test battery may not be appropriate and the sponsor should scientifically justify the approach.

7.2. Well-Characterised ONTs

Where an ONT is composed entirely of chemical structures that have been well characterised for genotoxicity, differing in sequence, length and/or modification combination (i.e., ribose, base, backbone, DDS) only, experimental evaluation of the ONT is not warranted (Section 2.1). There is no scientific basis for the exact number of compounds required to support applicability of well-characterised for genotoxicity. However, considering that industry (3) and regulatory experience (6) indicate that ONTs have not exhibited genotoxicity in the standard ICH S2 test battery to date, if negative results are obtained for two or more oligonucleotides with the same modification(s), the chemical structure(s) can be assumed to have a low genotoxicity concern. Where an ONT contains nucleotide modifications considered well-characterised and a small molecule DDS previously demonstrated as negative for genotoxicity in a single assessment only, further testing would not be expected.

7.3. Mechanism of Action-Specific Principles

If an ONT constitutes a novel class (i.e., new MoA), any potential risk of genotoxicity associated with this should be considered and testing conducted if appropriate. This includes, where relevant, mechanisms affecting DNA repair, genome stability, chromosomal segregation, replication stress, or other pathways associated with maintenance of genomic integrity. Similarly, where the pharmacological target carries a potential genotoxicity risk, this should be considered in the overall risk assessment, taking into account available evidence from human genetics, target biology, animal models, published literature, and other relevant data as part of the evaluation. It may be necessary to exclude such risks using an appropriate test or assessment, which should be scientifically justified. Findings from hybridisation-dependent off-target assessments (Section 3.2) should also be incorporated into the overall evaluation of genotoxicity risk, where applicable. Where genotoxicity is observed, mechanistic investigations should be conducted to determine whether a threshold-based mechanism is supported. The outcome of these investigations should inform the overall risk assessment and any safety margin.

7.4. Triplex Risk

It is acknowledged that there is a theoretical potential for ONTs to interact directly with double-stranded genomic DNA, in a sequence-dependent manner through triple helix (triplex) formation, to produce site-specific mutations. Stringent sequence criteria are necessary for triplex formation, which are not typically compatible with ONT design (e.g., ≥ 12 polypurine or polypyrimidine

stretches, with a very low tolerance for mismatches [4; 5]). The risk of triplex formation is further minimised for ONTs with poor nuclear distribution.

The potential for an ONT to cause triplex formation should be addressed by considering its sequence (including length of polypurine/pyrimidine stretches and mismatches) and the risk assessment documented. Where it is not possible to avoid a potential triplex-forming sequence in an ONT it may be necessary to assess the risk experimentally, using scientifically justified approaches.

8. CARCINOGENICITY STUDIES

Sponsors should follow ICH S1A and ICH S9 with regard to determining whether a carcinogenicity assessment is warranted for a particular development program. ICH M3 and ICH S9 Q&A should guide the timing of submission of completed *in vivo* carcinogenicity studies. ICH S1B and Addendum and ICH S6 may be consulted regarding applicable approaches and principles for assessing human carcinogenicity risk.

A WoE approach can be applied to assess the carcinogenic potential of ONTs. The assessment should thoroughly address seven factors including 1) on-target pharmacology, 2) ONT-specific attributes (chemical composition, mechanism of action, and route of administration), considering available data from other ONTs with shared characteristics, including those that are well-characterised for relevant endpoints, 3) off-target effects, 4) histopathology findings, 5) genotoxicity, 6) hormonal effects, and 7) immune perturbation. General toxicology study data from any rodent and/or non-rodent studies at least 3 months in duration may be used to inform the carcinogenicity assessment.

Following the WoE assessment, there may be justification that human carcinogenic risk is sufficiently characterized such that additional animal data is not warranted. In cases where insufficient information is available to determine carcinogenic potential, a bioassay in a single rodent species is sufficient, based on minimal disposition and metabolic differences expected between species. The sponsor should justify the rodent model used based on feasibility, prior data, knowledge of the target biology, and consideration of the pharmacological relevance of the clinical candidate and/or surrogate ONT. For novel ONTs, the 2-year bioassay, in either the rat or mouse, is considered more appropriate for human carcinogenicity risk assessment rather than the 6-month Tg rasH2 mouse model. If there is uncertainty regarding carcinogenic potential based on target biology, evaluation in a pharmacologically relevant rodent species or by use of a rodent surrogate

would be appropriate. Both the surrogate and the clinical candidate should be included in the carcinogenicity study to differentiate between compound- and on-target-mediated effects. For programs where a surrogate assessment is not considered feasible, sponsors should summarise available information on the expected carcinogenic risk from intended pharmacology (e.g.,, phenotypic data from humans or genetically modified animal models for that target) in the carcinogenicity assessment.

ICH S1B and ICH S1C should be consulted regarding approaches for dose selection for carcinogenicity studies. A limit dose can be defined per administration, as long as dosing in the animals is at least as frequent as that used in patients, and the appropriate multiple of human exposure is achieved (e.g., based on plasma exposure or other relevant comparison). The relative tissue half-life of the ONT in the animals and humans (the latter being based on modelling) should be considered, and dosing frequency adjusted accordingly.

8.1. Considerations for DDS Species Cross-reactivity

Where there is a lack of relevant oligonucleotide distribution due to limited DDS cross-reactivity in rodent species, the scientific justification of an experimental carcinogenicity assessment should be evaluated using the factors mentioned above. It is possible, due to lack of cross-reactivity and feasibility, a carcinogenicity bioassay may not be appropriate. In such cases, additional studies to address specific concerns on carcinogenicity risk may be used on a case-by-case basis.

9. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES

Nonclinical evaluation of developmental and reproductive toxicity (DART) of ONTs should be conducted in agreement with ICH S5 with respect to the required DART endpoints. ICH M3 should be consulted regarding the timing of submission of DART study reports as it relates to supporting enrolment of Women of Childbearing Potential (WOCBP) or pregnant women in clinical trials as well as submission of the marketing application.

DART assessments should include a pharmacologically relevant species. If the ONT is not pharmacologically active in a routine nonclinical species, a surrogate molecule (Section 3.1.1) should be tested together with the clinical candidate in one species in all DART studies. For EFD studies, the clinical candidate should be tested for compound-toxicity in two species, typically rodents and rabbits. For ONTs that are well-characterised (Section 2.1) for EFD endpoints, the single pharmacologically responsive species can be considered for testing the clinical candidate and, if required, the surrogate molecule to assess pharmacology-related effects.

Non-human primates (NHPs) are considered a non-routine species. Their use in DART studies should be considered when NHPs are the only pharmacologically relevant species, alternative testing strategies (e.g., surrogates, transgenic animals) are not suitable and a WoE approach is insufficient to inform on the risk. In that case, ICH S6 can be followed for guidance on timing for DART assessment in NHPs. In advance of an ePPND study, a rodent EFD study to assess compound toxicity of the clinical candidate may be conducted according to the timelines outlined in ICH M3 to support region-specific enrolment of WOCBP in clinical trials.

Although doses and the dosing regimen should generally be selected based on the criteria identified in ICH S5, ONT specific properties (e.g., delayed onset of pharmacology) may warrant modifications to the standard DART study designs. A combined FEED/EFD study may be appropriate for ONTs with such delayed PD effects, as dosing is initiated appropriately before fertilisation. In EFD studies, ONT exposure should be sufficient over the entire duration of organogenesis, which may require alternative dosing strategies (e.g.,, fractionated dosing; staggered cohort-based dosing), while limiting tissue accumulation, exaggerated PD and maternal toxicity. The suitability of longer dosing intervals in FEED studies will likely require different dosing regimens for both segments of FEED/EFD studies.

DART studies may be omitted partially or entirely if: 1) maternal systemic exposure is shown to be negligible by the route of administration (e.g.,, intravitreal), and the risk of indirect effects (e.g.,, maternal toxicity) on the foetus is low, 2) existing data (e.g.,, phenotypic data from genetically modified animals, general toxicology study results, preliminary EFD study results, human genetic data) suggest an adverse effect on reproduction or development, or 3) the chemical toxicity of the ONT is well-characterised for DART endpoints and the on-target pharmacology is proven to not affect reproduction and development. For instance, this may apply to derivatives (e.g.,, minor sequence variants, change to a well-characterised or more specific DDS) of previously characterised products. Any omission or modification of DART studies should be scientifically justified on a case-by-case basis.

Toxicokinetic evaluation should be performed in accordance with ICH S5. The probability of adverse developmental events due to exposing the offspring to the ONT *via* maternal milk is considered very low (due to low ONT levels in milk and low oral bioavailability) and in general, the evaluation of lactational exposure may not be necessary.

9.1. Juvenile Animal Studies

For the need for juvenile toxicology studies, the same considerations as outlined in ICH S11 apply to ONTs. For the study design, ONT-specific aspects, such as use of a surrogate and dosing frequency, are described in other sections of this guideline (Section 3.1.1).

10. IMMUNOTOXICITY STUDIES

The assessment of immunotoxicity of ONTs should follow the principles of ICH S8, applying a risk-based approach informed by the class and composition. Some types of ONTs are known to have the potential to engage and activate components of the innate immune system (e.g.,, nucleic acid-sensing pathogen-associated molecular pattern receptors, macrophages and histiocytes, and complement pathways), in addition to intended on-target pharmacological effects.

For ONTs with a potential immunostimulatory risk, human *in vitro* assessments (e.g.,, cytokine release tests with peripheral blood mononuclear cells or whole blood) may have utility in hazard identification. Standalone, dedicated *in vivo* immunotoxicity assessments are not typically warranted. However, assessment of ONT-induced effects on the immune system (e.g.,, cytokines, complement factors) in general toxicology studies may provide useful context for understanding potential observations seen with classes of ONTs that are known to engage the immune system or other classes that are not well-characterised in this context.

ONTs may provoke the production of anti-drug antibodies. Sponsors should consult ICH S6 for when a nonclinical assessment of anti-drug immunogenicity is warranted. The potential for immunogenicity against an ONT to adversely affect patient safety or efficacy is best characterised during clinical testing.

11. PHOTOSAFETY STUDIES

Although ONTs are excluded from the scope of ICH S10, the guideline may still be referenced for photosafety evaluation of ONTs. Initial photosafety evaluation for ONTs should be conducted in an integrated framework, considering the absorption of light within the ultraviolet-visible range of concern (i.e., 290–700 nm), biodistribution, and clinical or nonclinical findings indicative of photosafety concern. The ONT (including DDS) should be assessed for light absorption, noting that components known to lack phototoxic potential (e.g.,, monoclonal antibodies) can be excluded from the evaluation. If a photosafety concern was identified, measurements of individual components may be warranted. If the light absorption is determined to be comparable to natural nucleic acid bases, then an additional assessment is not warranted. If these assessments indicate a

photosafety concern, further evaluation should be conducted with reference to ICH S10. Data from other ONTs with comparable chemical modifications can be leveraged. If such data allows for an adequate photosafety assessment, no further evaluation is needed.

12. NONCLINICAL ABUSE LIABILITY

Dedicated nonclinical abuse liability studies are generally not warranted for ONTs. Abuse liability should be considered when there is a plausible rapid-onset reward or reinforcement mechanism, or other relevant evidence of CNS safety concern associated with the ONT. ICH M3 should be referenced for points to consider in the initial assessment of abuse potential. Where abuse liability studies are considered necessary, the rationale and study design should be scientifically justified.

13. OTHER TOXICITY STUDIES

13.1. Impurities/Degradants

Impurities derived from ONTs can be classified into two broad categories: 1) product-related impurities and 2) process-related impurities (e.g.,, organic small-molecule impurities, residual solvents, elemental impurities).

13.1.1. Product-related Impurities

Product-related impurities are structurally related to the parent oligonucleotide and to one another. Available methods and technologies make it challenging or impossible to separate individual impurities of similar mass or similar chemical structures. This in turn makes it difficult in many cases to qualify the oligonucleotide-related substances in a manner consistent with current ICH small molecule drug impurities guidance. While there is an expectation to characterise oligonucleotide-related impurities as thoroughly as is technically feasible, in cases where resolution of unique impurities is not possible, impurities may be grouped based on structural similarities.

Differences in impurity profile between marketed drug batches and the drug batches investigated during development should be evaluated using a risk-based approach. Existing data, including the impurity profiles of batches used in prior nonclinical and clinical studies, published knowledge for the same ONT class and composition, and *in vivo* metabolite data should be fully leveraged. The safety thresholds proposed by the sponsor for oligonucleotide-related impurities can be supported with peer-reviewed publications and should be scientifically justified. Although definitive toxicology studies conducted to enable clinical development will provide important

data for oligonucleotide-related impurity qualification, dedicated *in vivo* qualification studies are generally discouraged.

Although it is theoretically possible for oligonucleotide-related impurities to exert hybridisation-dependent off-target effects, the low levels at which individual impurities are typically present are unlikely to manifest a relevant biological effect. Additionally, the *in silico* off-target analyses routinely conducted on the parent sequence are considered relevant for key impurity groups (e.g.,, N-1, N+1). Therefore, *in silico* predictions and follow-up *in vitro* analyses to specifically address hybridisation-mediated off-target effects of impurities are generally not warranted.

In circumstances where an individual impurity is at an unusually high level and possesses structural features expected to alter safety, these impurities may need further consideration, which could include prior knowledge, *in silico* or *in vitro* characterisation, including NAMs (Section 2.2).

13.1.2. Process-related Impurities

For process-related impurities, existing guidance addresses regulatory expectations for the control, identification, characterisation, and qualification of other impurities related to synthesis of ONTs. Note that even though the ICH guidance referenced below may have been written with ONTs outside their scope, process-related ONT impurities are applicable in the following guidance. Sponsors are referred to ICH Q3A, ICH Q3B, and ICH M7. Residual solvents should be assessed with reference to ICH Q3C. Elemental impurities should be assessed with reference to ICH Q3D. Extractables and leachables should be assessed with reference to ICH Q3E.

14. REFERENCES

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