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To: Director of Prefectural Department of Health

Director of Pharmaceutical Evaluation Division,

Pharmaceutical Safety and Environmental Health Bureau,

Ministry of Health, Labour and Welfare

Guideline for preclinical safety assessment of oligonucleotide therapeutics

Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare, compiled a "Guideline for preclinical safety assessment of oligonucleotide therapeutics" as provided in the attachment. We ask you to inform relevant manufacturers and sellers placed under your administration about this guideline.

This guideline provides basic concepts based on the knowledge of current scientific findings. Therefore, applicants may not necessarily follow the methods described in the guideline strictly as long as their alternative methods are scientifically justified by the advancement of academic knowledge.

＊ This English version of the Japanese Notification is provided for reference purposes only. In the event of any inconsistency between the Japanese original and the English translation, the former shall prevail.

Attachment

Guideline for preclinical safety assessment of oligonucleotide therapeutics

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1. Introduction

1.1 Purpose

The purpose of this guideline is to provide a basic framework recommended for the nonclinical safety evaluation of oligonucleotide preparations (hereafter referred to as ONTs (oligonucleotide therapeutics).

In accordance with the principle of 3Rs (replacement, reduction, or refining the use of animals), this guideline aims to promote the development of ONTs and protect patients from adverse reactions by properly utilizing test animals and other resources without conducting studies that provide little additional value for safety information in humans.

1.2 Background

ONTs have been continuously studied since the late 1970s. The first nucleic acid drugs were approved in the late 1990s, development subsequently progressed, and with the increasing number of applications for ONT marketing approval, much development experience has been accumulated. However, no specific guidelines have been developed to date for the evaluation of the safety of ONTs inside or outside Japan.

The S6(R1) guideline of the International Council for Harmonization of Pharmaceutical Regulations (ICH), "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals"[1)] (hereafter referred to as "ICH S6(R1)) states that "the principles provided by the guidelines can also be applied to oligonucleotides." However, ONTs need to be examined for intra-cellular effects and the effects of chemical modifications that are not generally under consideration for biotechnology-derived pharmaceuticals. Therefore, in evaluating the preclinical safety of ONTs, it is essential to consider not only ICH S6(R1) but also the biological characteristics specific to nucleic acid drugs and other guidelines for chemical synthetic drugs (hereafter referred to as "chemical products").

This guideline presents the basic principles for the nonclinical safety evaluation of ONTs and the considerations for designing each nonclinical safety study.

1.3 Scope

This guideline applies to active ingredients with a linearly linked ribonucleic/deoxyribonucleic acid or artificially modified nucleic acid, binding to a specific nucleotide sequence (hereafter referred to as "hybridization") and causing a biological reaction without synthesizing a new protein. For example, the active ingredients include antisense oligonucleotides, siRNAs, and miRNAs. Most of them are produced by chemical synthesis, using a nucleic acid molecule of a natural type or a chemically modified nucleic acid molecule.

The safety assessment concepts presented in this guideline may also apply to aptamers and decoy nucleic acids.

This guideline does not cover mRNA, RNA used for genome editing, DNA/RNA vaccines, or CpG oligos.

2. Preclinical safety study

Nonclinical safety studies of ONTs include evaluation of the target organ, dose dependency, relationship to exposure, and reversibility to characterize the pharmacological and toxicological properties of the drug.

Nonclinical safety testing, which is usually required for the development of ONTs, must also be performed in accordance with the Nonclinical Practice (GLP) for the safety of pharmaceuticals.

If a nucleic acid drug in development is intended for a life-threatening or serious disease for which there is currently no cure, it may be possible to delay, simplify, or omit particular studies, depending on the individual case. Refer to “Nonclinical Evaluation for Anticancer Pharmaceuticals (ICH S9)”[2)] for applications in advanced cancer.

2.1 Evaluation strategy

Nonclinical safety evaluation of ONTs requires a thorough characterization of each product and consideration of evaluation strategies from a nucleic acid drug-specific perspective. In other words, in many ONTs with the exception of aptamers and decoy nucleic acids, hybridization to the target sequence may result in pharmacological effect (hereafter referred to as "on-target effect"). The on-

target effect may in turn cause “on-target toxicity”, an adverse effect due to exaggerated pharmacology. For evaluation of the on-target toxicity, ICH S6(R1) is considered to be a reference because of high species specificity and target specificity in pharmacological activity.

Unintended effects other than the on-target effects (hereafter referred to as "off-target toxicity") may be toxicities caused by hybridization to sequences that are similar but not identical to the target (hereafter referred to as "hybridization-dependent off-target toxicity") or by the structural, physical, and chemical properties of ONTs without hybridization (hereafter referred to as "hybridization-independent off-target toxicity"). Human and animal genomic sequences differ regarding this hybridization-dependent off-target toxicity, so instead of pursuing human safety in animal tests, this guideline recommends in silico analysis using DNA/RNA information and in vitro gene expression analysis using human cells. In addition, because hybridization-independent off-target toxicity of ONTs is caused by the physical properties (e.g., chemical modifications), similar to toxicities of chemically synthesized drugs, the ONT toxicity should be evaluated in reference to preclinical safety guidelines for chemicals. Furthermore, common toxicities (class effects) are known to be caused by a certain category of ONTs which have similar structure and physical and chemical properties. Thus, information on class effects can be a useful reference for ONTs in development.

2.2 Animal species

Nonclinical safety studies of ONTs, like other medical drugs and biopharmaceuticals, should be evaluated in two species.

The off-target toxicity of the investigational ONT should be assessed in rodents and non-rodents. The on-target toxicity can be also evaluated if either or both of the species used are responsive to the pharmacological effect of ONT. If the ONT does not demonstrate pharmacological activity in an available animal species, the on-target toxicity of the investigational ONT could be evaluated in a toxicity study by using a homologous nucleic acid (hereafter referred to as a "surrogate") that produces the target pharmacological effect in the animal species used in that toxicity study. The surrogate toxicity study is generally conducted in only one animal species.

2.3 Study design for repeated-dose toxicity studies

2.3.1 Dose selection

1) High dose selection

It is necessary to set doses with consideration for both on-target and off-target toxicities of the nucleic acid drug. For on-target toxicity, a PK-PD approach will be used in reference to ICH S6(R1) to determine a high dose that maximizes the intended pharmacological effect in the nonclinical species. For off-target toxicity, researchers can set (i) maximum tolerance (MTD), (ii) saturation of exposures, (iii) maximum feasible dose (MFD), (iv) 50-fold margin of clinical exposure, or (v) limited dose (1000 mg/kg) as a target with reference to "Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (hereafter referred to as ICH M3(R2)"[3].

The highest dose should be used for the high-dose group in repeated-dose toxicity studies unless there is evidence to select a lower dose that will provide the maximum pharmacological effect or that will meet one or more of the criteria (i) – (iv) as described above.

2) Dose levels

For dose selection, it is desirable to have a 3-stage treatment group in order to monitor any potential dose-response relationship. When evaluating surrogates, it is not necessary to conduct an independent study, but one additional group should be available to confirm the effects of excessive pharmacological effects due to surrogates.

2.3.2 Administration route and frequency of administration

The intended clinical route of administration should be used, and the dosing frequency should be selected with consideration for the anticipated human pharmacokinetic profile. When using a route other than the clinical application route, the suitability of this alternate route must be justified.

2.3.3 Study period

ICH M3(R2)[3] will be used as a reference for the duration of repeated-dose toxicology studies.

2.3.4 Reversibility

For the assessment of reversibility, "Questions & Answers for ICH M3(R2) Guideline (M3(R2)

Q&As(R2))"[4] will be helpful.

2.4 Study timing

ICH M3(R2)[3] will be used as a reference for the timing of preclinical safety studies of ONTs.

2.5 Metabolites/degradation products

Safety is not considered to be a particular concern for naturally occurring nucleic acid components degraded by nucleases. However, metabolites and degradation products containing chemically modified moieties need to be evaluated for non-clinical safety in accordance with ICH M3(R2)[3], similar to conventional chemical products.

2.6 Impurities

Impurities derived from ONTs can be classified into categories including oligonucleotide-related substances, organic small-molecule impurities, residual solvents, and elemental impurities, with reference to "Items to Consider in Assuring and Evaluating the Quality of Oligonucleotide Therapeutics"[5]. In the ONT manufacturing process, it is necessary to reduce the impurities as much as possible, and to evaluate the safety in humans based on information such as the quality of the ONT and the results of non-clinical studies and published data.

2.6.1 Oligonucleotide-related substance

Oligonucleotide-related substances exhibit similar physicochemical properties and cannot be separated individually from related substances, making it difficult in many cases to qualify the oligonucleotide-related substances based on "Impurities in New Drug Substances Q3A(R2)" (hereafter referred to as "ICH Q3A")[6]. Therefore, the safety of oligonucleotide-related substance in ONTs should be evaluated based on the results of preclinical safety studies (e.g., impurity profiles, toxicity profiles) of the drug substance or drug product.

If a mismatch sequence was included in the in silico analysis of the hybridization-dependent off-target genes, it is assumed that the hybridization-dependent off-target genes of major

oligonucleotide-related substances were also investigated. If each oligonucleotide-related substance is assumed to be present at a level sufficiently lower than the active ingredient, it is meaningless to evaluate off-target toxicity due to hybridization for those substances.

2.6.2 Other

Based on the impurity profile of the drug substance or drug product, the safety of small-molecule impurities should be evaluated with reference to ICH Q3A, "Impurities in New Drug Substances (ICH Q3B(R2))"[7] and "Assessment and Control of DNA Reactivity (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risks (ICH M7)"[8]. Residual solvents should be assessed with reference to the "Impurities: Guideline for Residual Solvents (ICH Q3C)"[9], and elemental impurities should be assessed with reference to the "Guideline for Elemental Impurities (ICH Q3D(R1))"[10].

2.7 Drug delivery system (DDS) formulation

For DDS formulations such as conjugates and liposomes, it is essential to evaluate the safety of the formulation. The safety of carrier materials (e.g., proteins, lipids, sugars, etc.), should be assessed by appropriate toxicity tests, depending on the level of concern and using the available information.

3. Individual studies

3.1 Toxicokinetics and pharmacokinetics

The assessment of systemic exposure in toxicity studies for ONTs should be conducted in accordance with "Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (ICH S3A)"[11].

3.2 Safety pharmacology

Undesirable pharmacodynamic effects on key physiological functions (central nervous system, cardiovascular system and respiratory system) should be evaluated with reference to "Safety

Pharmacology Studies for Human Pharmaceuticals (ICH S7A)"[12], but do not necessarily require independent safety pharmacology studies. Since it is unlikely that ONTs act on ion channels such as hERG channels, in vitro evaluations such as hERG studies are less meaningful. However, in vitro evaluation may be considered to further investigate findings such as cardiovascular assessment in an in vivo setting.

3.3 Single-dose toxicity

If acute toxicity information is available from dose escalation studies, short-term dose-ranging studies, pharmacology studies, or repeated-dose toxicity studies, independent single-dose toxicity studies are not recommended.

3.4 Repeated-dose toxicity

These studies will be conducted to assess (1) the accumulation of ONTs in specific organs following repeated doses and (2) the evolution of the toxicity profile. Refer to ICH M3 (R2) [3,4] for the recommended duration and timing of the study.

3.5 Genotoxicity

Genotoxicity testing of nucleic acid drugs consisting of only native nucleic acids is not necessary.

For the evaluation of chemically modified nucleic acid drugs, refer to "Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (ICH S2 (R1))"[13].

3.6 Reproductive and developmental toxicity

Reproductive and developmental toxicity (DART) studies should, in principle, be conducted in accordance with "Revision of S5 Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals (ICH S5 (R3))"[14], after giving due consideration to the clinical application and the target disease. Rodents are usually used in fertility studies and pre-postnatal development studies. Rodents and rabbits are usually used in embryo-fetal development (EFD) studies. When a clinical

candidate is not pharmacologically active in these species, it is not necessary to conduct EFD studies with clinical candidates in non-human primates (NHP). Instead, assessment with surrogates in species commonly used in EFD studies (rodents and rabbits) is acceptable due to the advantages of such species for DART testing [14]. For the timing of DART studies, see the ICH M3(R2) guideline [3].

When the weight of evidence (e.g., mechanism of action, phenotypic data from genetically modified animals, class effects) suggests an obvious adverse effect on fertility or pregnancy outcome, these data can provide adequate information to communicate the risk for reproduction, and no additional preclinical studies are warranted.

3.7 Carcinogenicity

Regarding the need to conduct carcinogenicity studies, "Need for Carcinogenicity Studies of Pharmaceuticals (ICH S1A)"[15], "Testing for Carcinogenicity of Pharmaceuticals (ICH S1B)"[16], and "Dose Selection for Carcinogenicity Studies of Pharmaceuticals (ICH S1C(R2))"[17] are used as references. When there are concerns about carcinogenicity due to the mechanism of action (e.g., immunosuppression), and when a concern about carcinogenicity is supported by genotoxicity studies, repeated-dose toxicity studies, or an off-target toxicity assessment due to hybridization, it is difficult to rule out such concerns through non-clinical safety studies. Instead, risk should be communicated appropriately with consideration for clinical risks and benefits.

3.8 Local tolerance

If it is possible to assess the site of administration in a repeated-dose toxicity study etc., it is not necessary to perform an independent local irritation study.

3.9 Immunotoxicity studies

It is not recommended that independent immunotoxicity studies be conducted if it is possible to assess the results of repeated-dose toxicity studies, etc. Refer to "Immunotoxicity Studies for Human Pharmaceuticals (ICH S8)"[18], where appropriate.

3.10 Photosafety

For chemically modified nucleic acids, photosafety should be assessed with reference to "Photosafety Evaluation of Pharmaceuticals (ICH S10)"[19] if there are particular photosafety concerns.

4. References

1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, ICH S6(R1) (implemented by MHLW/PMDA, Japan, dated March 23, 2012; PFSB/ELD Notification No. 0323-1)
2) Nonclinical Evaluation for Anticancer Pharmaceuticals, ICH S9 (implemented by MHLW/PMDA, Japan, dated June 4, 2010; PFSB/ELD Notification No. 0604-1)
3) Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, ICH M3(R2) (implemented by MHLW/PMDA, Japan, dated February 19, 2010; PFSB/ELD Notification No. 0219-4)
4) Questions & Answers: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, ICH M3(R2) Q&As (R2) (implemented by MHLW/PMDA, Japan, dated August 16, 2012: PFSB/ELD Administrative Notice)
5) Items to Consider in Assuring and Evaluating the Quality of Oligonucleotide Therapeutics (PSEHB/PELD Notification No. 0927-3, dated September 27, 2018)
6) Impurities in New Drug Substances, ICH Q3A(R2) (implemented by MHLW/PMDA, Japan, dated December 16, 2002/December 4, 2006: PFSB/ELD Notification No. 1216001, 1204001)
7) Impurities in New Drug Substances, ICH Q3B(R2) (implemented by MHLW/PMDA, Japan, dated June 24, 2003/July 3, 2006: PFSB/ELD Notification No. 0624001, 0703004)
8) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risks, ICH M7 (implemented by MHLW/PMDA, Japan, dated

November 10, 2015: PSEHB/PELD Notification No. 1110-3)

9) Impurities: Guideline for Residual Solvents, ICH Q3C (implemented by MHW/PMDA, Japan, dated March 30, 1998: PFSB/ELD Notification No. 307)

10) Guideline for Elemental Impurities, ICH Q3D(R1) (date of Step4: 22 March 2019)

11) Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies, ICH S3A (implemented by MHW, Japan, dated July 2, 1996: PAB/PCD Notification No. 443)

12) Safety Pharmacology Studies for Human Pharmaceuticals, ICH S7A (implemented by MHLW/PMDEC, Japan, dated June 21, 2001: PFSB/ELD Notification No. 902)

13) Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, ICH S2(R1) (implemented by MHLW/PMDA, Japan, dated September 20, 2012: PFSB/ELD Notification No. 0920-2)

14) Revision of S5 Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals, ICH S5(R3) (date of Step 4: 18 February 2020)

15) Need for Carcinogenicity Studies of Pharmaceuticals, ICH S1A (implemented by MHW/PMDEC, Japan, dated April 14, 1997: PAB/PCD Notification No. 315)

16) Testing for Carcinogenicity of Pharmaceuticals, ICH S1B (implemented by MHW, Japan, dated July 9, 1998: PMSB/ELD Notification No. 548)

17) Dose Selection for Carcinogenicity Studies of Pharmaceuticals, ICH S1C(R2) (implemented by MHLW/PMDA, Japan, dated November 27, 2008: PFSB/ELD Notification No. 1127001)

18) Immunotoxicity Studies for Human Pharmaceuticals, ICH S8 (implemented by MHLW/PMDA, Japan, dated April 18, 2006: PFSB/ELD Notification No. 0418001)

19) Photosafety Evaluation of Pharmaceuticals, ICH S10 (implemented by MHLW/PMDA, Japan, dated May 21, 2014: PFSB/ELD Notification No. 0521-1)