ANNEX

to Recommendation
of the Eurasian Economic Commission's
Board
date 20 _ No.

GUIDELINES
on Pharmacological Safety Studies of Medicinal Products for Human Use

I. General provisions

1. These Guidelines have been developed to protect clinical study participants and patients receiving registered medicinal products from undesirable effects of medicinal products, and also for rational use of animals and other resources in preclinical and clinical studies.

2. These Guidelines provide definitions, general principles and recommendations for pharmacological safety studies of medicinal products for human use.

3. These Guidelines apply to new chemical compounds and biotechnological medicinal products for human use. When necessary, these Guidelines may apply to registered medicinal products (e.g., due to identification of new adverse events, a change in the patient population, or a new route of administration).

4. When planning pharmacological safety studies and conducting them, you should adhere to a rational approach. The study type and design depend on individual properties and the intended use of the medicinal products. You

should use evidence-based, (preferably) globally recognized methods for studying medicinal products, and use new technologies and methods complying with strict scientific principles.

5. You may include some pharmacological safety endpoints in the design of toxicological, kinetic, clinical studies, etc.; however, the final safety assessment can only be performed in special pharmacological safety studies. Well-planned pharmacological safety studies are able to detect adverse events that may be overlooked during standard toxicological studies.

6. For the purposes of these Guidelines, concepts and terms shall be used with their following meanings:

«pharmacological safety studies» are studies aimed at examining the potential undesirable pharmacodynamic effects of a substance on physiological functions in doses corresponding to the therapeutic range and above, and including three categories of studies: studies of primary pharmacodynamics, studies of secondary pharmacodynamics and pharmacological safety studies;

«studies of primary pharmacodynamics» are studies of the mechanism of action and/or the effects of a substance in relation to its therapeutic target;

«studies of secondary pharmacodynamics» are studies of the mechanism of action and/or the effects of a substance that are not related to its therapeutic target, they are sometimes considered as part of a general pharmacology study of the substance under study.

7. In some cases, information on the primary and secondary pharmacodynamic effects of a substance can contribute to the medicinal product safety assessment and its potential adverse events in humans, therefore, this information shall be examined together with the results of pharmacological safety studies.

II. Planning and organizing pharmacological safety studies

8. The goals of pharmacological safety studies are:

identification of undesirable pharmacodynamic properties of the substance under study that may be significant for human safety;

assessment of undesirable pharmacodynamic and/or pathophysiological effects of the substance under study detected in toxicological and/or clinical studies;

studying the mechanism of observed and/or suspected undesirable pharmacodynamic effects of the substance under study.

In Section 2.4, Module 2 of the registration dossier of the medicinal product, a research plan aimed at achieving the goals stated above should be presented and described in detail.

1. General issues in selecting and designing pharmacological safety studies

9. Since pharmacological effects depend on the properties of a particular substance under study, studies should be selected and planned accordingly, taking into account the following factors (but without limitation):

effects typical for the pharmacological class to which the substance under study belongs, such as the mechanism of action, can result in certain undesirable events (e.g., the arrhythmogenic effect is a common property of antiarrhythmic medicinal products);

adverse events typical for the chemical and pharmacological classes, but not depending on the primary pharmacodynamics (e.g., the ability of antipsychotic medicinal products to prolong the QT interval);

data on receptor binding or effect on enzyme systems, indicating the potential for the development of adverse events;

the results of previous pharmacological safety studies, studies of secondary pharmacodynamics, toxicological studies or clinical applications, requiring further examination in order to identify and characterize the significance of these results for potential adverse reactions in humans.

10. At the early development stages, the data necessary for rational selection and planning of studies in accordance with the provisions of paragraph 9 of these Guidelines (e.g., comparative metabolism) may be lacking, in which case more general approaches to conducting pharmacological safety studies shall be used.

11. Depending on the significance for vital functions, a hierarchy of organ systems can be developed. The most vital organs and systems to be studied in pharmacological safety studies are cardiovascular, respiratory and central nervous systems. Other organ systems, such as the urinary and digestive systems, the functions of which may be temporarily impaired due to undesirable pharmacodynamic effects without causing irreversible harm, do not require immediate studying. When taking into account such factors as a planned clinical study or patient population, you may need to assess pharmacological safety of the impact on other systems (e.g., digestive system in Crohn's disease, kidney function in primary renal arterial hypertension, and the immune system in immunocompromised patients).

2. Test systems

General provisions for selection of test systems

12. To obtain reliable scientific data, you should justify the selection of a suitable animal model or other test systems. Selection factors include:

ability of the model to respond to pharmacodynamic effects;

pharmacokinetic profile, species, breed, gender and age of experimental animals:

susceptibility, sensitivity and reproducibility of the test system;

availability of previously obtained data on the substance under study. When selecting a test system, you should take into account data obtained in humans (e.g., *in vitro* metabolism), if any. The timing of the measurements should be determined by pharmacodynamic and pharmacokinetic properties. You should provide a justification for selecting a particular animal model or test system.

Pharmacological safety studies *in vitro* and *in vivo*

13. It is allowed to use animal models, *ex vivo* and *in vitro* products as test systems. *Ex vivo* and *in vitro* systems include, but without limitation:

isolated organs and tissues;

cell cultures;

cell fragments;

cell organelles;

receptors;

ion channels;

transporters;

enzymes.

In vitro systems may be used in auxiliary studies (for example, to establish the activity profile of the substance under study or to study the mechanism of the effects observed *in vivo*).

14. When conducting *in vivo* studies, it is preferable to use animals without anesthesia. Data obtained from non-immobilized animals that can be used for a long time by telemetry, or to which you can apply other suitable methods intended for conscious animals, or animals adapted to laboratory conditions, are preferred to data obtained from immobilized or non-adapted animals. One of the main conditions when using animals for which no

anesthesia was performed is to prevent animals from developing discomfort and pain.

Experiment design

Sample size and use of controls

15. The size of the experimental group must be sufficient to perform full-scale scientific interpretation of the data obtained. The number of animals or preparations of isolated organs must be sufficient to confirm or exclude the presence of a biologically significant effect of the substance under study. In this case, you should take into account the magnitude of the biological effect which is potentially most likely to be manifested in humans. The experimental study design must include a sufficient number of negative and positive control groups. Well-characterized *in vivo* test systems may require no positive control groups. You should justify the exclusion of control groups from the studies.

Route of administration of the substance under study

16. When conducting studies, it is advisable to use the proposed clinical route of administration of the substance under study. Regardless of the route of administration, the exposure of the substance under study or its main metabolites should be comparable to the values achieved in humans (when available), or exceed them If the substance under study is intended to be administered via several routes (e.g., orally and parenterally), or if significant qualitative and quantitative differences between systemic and local exposure are observed or expected, it is recommended to assess the effects of the substance under study for more than one route of administration.

3. Dose size or concentration of the substance under study. *In vivo* studies

17. Pharmacological safety studies should be planned in such a way as to establish a dose-response relationship for the adverse events observed. Whenever possible, you should examine the dependence of adverse events on time (e.g., the start and duration of the effect). As a rule, you should compare the doses causing adverse events with the doses causing a primary pharmacodynamic effect in the studied animal species or causing the intended therapeutic effect in humans (if possible). The presence of inter-species differences in pharmacodynamic sensitivity has been proven. Therefore, the doses of the substance under study should include and exceed the initial pharmacodynamic and therapeutic ranges. In the absence of adverse events in the study according to the studied pharmacological safety indicators, you must select a dose causing undesirable moderate reactions in this or other studies with the same route of administration and equivalent in duration as the highest dose studied. Such adverse reactions may include dose-limiting pharmacodynamic effects and other toxicity. In practice, some effects occurring in the toxicity range (e.g., tremors and fasciculations when recording an electrocardiogram) can distort interpretation of the results, and also limit the dose of the substance under study. Studying one group with a limiting dose according to the above scheme may be sufficient when there are no adverse events at the pharmacological safety endpoints in experimental animals.

In vitro studies

18. *In vitro* studies should be planned in such a way as to establish the «concentration – response» relationship for the substance under study. You should select such a range of concentrations of the substance under study as

to increase the probability of detecting a response on the test system used. The physico-chemical properties of the substance under study and other factors specific to the method may affect the upper limit of the indicated range. When there's no effect, the selected concentration range should be justified.

4. Duration of studies

19. Pharmacological safety studies are usually carried out with a single administration of the substance under study. In case that pharmacodynamic effects occur after a certain period of treatment, or when the results of preclinical studies with repeated (multiple) administration of the substance under study or the results of using the substance under study in humans raise concerns about its pharmacological safety, you must select the duration of pharmacological safety studies in such a way as to take into account the possibility of detecting such effects during the experiment.

5. Studying metabolites, isomers and finished medicinal products

20. During pharmacological safety studies, in general, you should examine every basic substance and their main metabolites achieving or able to achieve systemic exposure in humans. The main metabolites are often assessed as part of studying the basic material in animals. If it turns out that the main metabolites are absent in humans or formed in animals in relatively low concentrations only, the effect of such metabolites on the pharmacological safety endpoints should be studied. Besides, if it is known that human metabolites contribute significantly to the pharmacological effect of the medicinal product, such active metabolites should be studied. In case that metabolites have not been adequately studied during *in vivo* studies of

the basic compound, they may be studied using *in vitro* systems, for practical reasons.

21. If the medicinal product is a mixture of isomers of the substance under study, each individual isomer should be examined *in vitro* or *in vivo*.

22. Pharmacological safety studies of a finished medicinal product are only carried out in case of a significant change in its composition, which modifies the pharmacokinetics and/or pharmacodynamics of the active substance compared with the previously studied composition (that is, due to active excipients, such as penetration enhancers, liposomes and other changes, e.g., polymorphism).

6. Core battery of pharmacological safety studies

23. The purpose of the core battery of pharmacological safety studies is to study the effect of the substance under study on the vital functions of the human body. In this regard, the cardiovascular, respiratory and central nervous systems, as a rule, are considered vital systems of organs requiring examination within the core battery of pharmacological safety studies. In some cases, based on scientific evidence, the core battery of pharmacological safety studies should include additional studies listed in subsection 7, section II of these Guidelines, or, subject to the conditions described in subsection 8, Section II of these Guidelines, you may refrain from carrying out the core battery of pharmacological safety studies.

24. If you exclude a single test(s) or a study of certain organs, systems or functions from the core battery of pharmacological safety studies, you should provide a justification based on scientific data.

Central nervous system

25. The effect of the substance under study on the central nervous system should be properly studied, assessing the motor activity, change in behavior, coordination of movements, sensory reflexes (motor reflexes) and body temperature of the test system (study subject) (e.g., use a battery of functional observation tests, a modified Irwin test or other tests).

Cardiovascular system

26. You must properly study the effect of the substance under study on the cardiovascular system, assess the blood pressure, heart rate and electrocardiogram, as well as data obtained *in vivo, in vitro* and/or *ex vivo*, including methods for assessing repolarization and conduction disorders in the myocardium (including in accordance with the Annex to these Guidelines).

Respiratory system

27. You must properly study the effect of the substance under study on the respiratory system, assess the respiratory rate and other respiration function parameters (e.g., respiratory volume or hemoglobin oxygen saturation). In order to assess the respiratory function, clinical observation of laboratory animals, as a rule, appears insufficient, therefore, using an appropriate methodology, you must perform quantitative measurement of these parameters.

7. Subsequent and additional pharmacological safety studies

28. Based on the pharmacological properties and chemical class of the substance under study, you can assume the possibility of the development of

adverse events. Besides, according to the results of the core battery of pharmacological safety studies, clinical studies, pharmacovigilance, *in vitro* and *in vivo* experimental studies or due to scientific literature, additional concerns may arise. If such adverse events cause a concern for human safety, they should be properly studied as part of subsequent or additional pharmacological safety studies.

Follow-up studies of the core battery of pharmacological safety studies

29. Follow-up studies of the core battery of pharmacological safety studies are intended to provide a deeper understanding of the results of the core battery of studies of vital functions or obtain additional data. Paragraphs 30–37 provide a list of studies aimed at further examination of these systems with respect to potential adverse pharmacodynamic effects. This list is not complete or mandatory, the studies described should be selected individually after taking into account factors such as previously obtained preclinical and clinical data. In some cases, it is advisable to study such phenomena within the framework of other preclinical and/or clinical studies.

Central nervous system studies

30. You should explore behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory and/or electrophysiological studies, etc.

Cardiovascular system studies

31. You must study cardiac output, ventricular contractility, vascular resistance, the effect of endogenous and/or exogenous substances on the cardiovascular system, etc.

Respiratory system studies

32. You should study respiratory tract resistance, lung tissue elasticity, pulmonary arterial pressure, blood gases, blood pH, etc.

Additional pharmacological safety studies

33. Additional pharmacological safety studies are carried out to assess potential adverse pharmacodynamic effects on the part of functions of organ systems not studied in the core battery of studies or toxicity studies with repeated (multiple) administration of the substance under study, when there is a reason for such an assessment.

Urinary system studies

34. You must study the effect of the substance under study on the performance of kidneys (e.g., study the urine volume and density, osmolality, pH, water-electrolyte balance, urine protein content, urine cytology, and blood biochemistry parameters (blood urea nitrogen, creatinine and plasma proteins)).

Autonomic nervous system studies

35. You should study the effect of the substance under study on the autonomic nervous system (e.g., binding of the substance under study to the autonomic nervous system receptors, the functional response to the use of agonists or antagonists *in vitro* or *in vivo*, direct stimulation of autonomic nerves and measurement of the cardiovascular system reaction, assessment of baroreflexes , heart rate variation).

Digestive system studies

36. You should study the effect of the substance under study on the digestive system (e.g., gastric secretion, potential for damage to the gastrointestinal tract, bile secretion, *in vivo* transit duration, *in vitro* ileum contractility, pH measurement and food retention in the stomach).

Studies of other organ systems

37. When there are grounds, you should assess the effect of the substance under study on the organ systems not studied earlier (e.g., conducting a study on the potential for development of medicinal product dependence, muscle, immune and endocrinous function).

8. Conditions under which additional pharmacological safety studies shall not be conducted

38. If the pharmacological properties of a substance under study for topical use are well characterized, and its low systemic exposure or distribution to other organs and tissues is established, then you may choose not to conduct additional pharmacological safety studies.

39. Additional pharmacological safety studies may not be required until the first administration of cytotoxic medicinal products intended for treatment of patients with terminal forms of cancer. The results of additional pharmacological safety studies of cytotoxic medicinal products with a new mechanism of action may be of value.

40. For biotechnological medicinal products with a high specificity for the target receptor, it is sufficient to assess the pharmacological safety endpoints within the framework of toxicological and/or pharmacodynamic studies, therefore, you may reduce or exclude the pharmacological safety research program for such medicinal products.

41. With respect to biotechnological medicinal products, that are a new therapeutic class, and/or similar medicinal products without a high specificity for the target receptor, more detailed pharmacological safety studies should be considered.

42. There are other exceptions not requiring pharmacological safety studies (e.g., a new salt with the same pharmacokinetic and pharmacodynamic properties).

9. Periods of pharmacological safety studies with regard to clinical development

43. When planning a pharmacological safety study program, the conditions specified in paragraphs 38-42 of these Guidelines should be taken into account, where no additional pharmacological safety studies shall be carried out, to determine the need for particular studies.

Studies to be carried out before the first administration of the substance under study to humans

44. Before the first administration to humans, you should investigate the effect of the active substance under study on the functions listed in the core battery of pharmacological safety studies. You should conduct all subsequent and additional studies, the need for which has been identified. Data from well-planned and well-conducted toxicological studies examining pharmacological safety endpoints may reduce or eliminate the need for separate pharmacological safety studies.

Studies conducted during clinical development

45. In order to clarify the observed or suspected adverse events in animals or humans during clinical development, additional studies may be required.

Studies conducted before registration of the medicinal product

46. Before registration of a medicinal product, you should assess its effect on the systems listed in subsection 7, section II of these Guidelines; the absence of the need for such studies should be justified. Data available from well-planned and well-conducted toxicological studies investigating the pharmacological safety endpoints, as well as clinical research data, can contribute to this assessment and replace pharmacological safety studies.

10. Compliance with the Good Laboratory Practice Rules of the Eurasian Economic Union

47. Preclinical pharmacological safety studies are conducted in accordance with the Good Laboratory Practice Rules. Due to the unique design and practical features of some pharmacological safety studies, it is not always possible to ensure their compliance with the Good Laboratory Practice Rules. Even if the Good Laboratory Practice Rules are not followed, you must ensure a high quality of the data obtained and integrity of pharmacological safety studies. If a study does not comply with the Good Laboratory Practice Rules, you must provide for a possibility to recreate the study by properly maintaining research documentation and archiving data. You must properly justify each study (or part of a study) that does not comply with the Good Laboratory Practice Rules, and describe its potential impact on the assessment of pharmacological safety endpoints.

48. The core battery of pharmacological safety studies is usually conducted in accordance with the Good Laboratory Practice Rules. Subsequent and additional studies should, as much as possible, comply with the Good Laboratory Practice Rules. A pharmacological safety study may be part of toxicological studies, in which case the latter should be carried out in accordance with the Good Laboratory Practice Rules.

49. Studies of primary pharmacodynamics may not comply with the Good Laboratory Practice Rules. Studies of secondary pharmacodynamics, in general, may not comply with the Good Laboratory Practice Rules. The results of studies of secondary pharmacodynamics conducted when selecting a compound can be taken into account when assessing pharmacological safety. When there are no grounds for a negative assessment of the quality of the studies conducted (e.g., the pharmacological safety endpoints not achieved, the chemical or therapeutic class results not achieved), there is no need to repeat the studies, complying with the Good Laboratory Practice Rules. In some cases, the results of studies of secondary pharmacodynamics can contribute the most to assessing the safety of potential adverse events in humans, such studies are usually conducted in accordance with the Good Laboratory Practice Rules.

ANNEX

to the Guidelines on Pharmacological
Safety Studies of Medicinal Products
for Human Use

INSTRUCTIONS
on preclinical assessment of the ability of the substance under study to cause a slowdown in ventricular repolarization (prolong the QT interval)

I. General provisions

1. Assessment of the effect of medicinal products on ventricular repolarization and arrhythmogenic risk is the subject of active research at the preclinical and clinical stages.

2. This document describes a strategy of preclinical studies on assessing the potential of the active substance under study to slow down ventricular repolarization. This annex contains information about preclinical analyses and integrated risk assessment.

3. The QT interval (time from the beginning of the QRS complex to the end of the T wave) on the electrocardiogram (ECG) is a measure of ventricular depolarization and repolarization duration. Prolonged QT interval can be congenital or acquired (e.g., drug-mediated). When ventricular repolarization is slowed down and the QT interval is prolonged, the risk of ventricular tachyarrhythmias, including pirouette tachycardia, increases, especially when combined with other risk factors (e.g. hypokalemia, organic heart diseases, bradycardia). In this regard, much attention is paid to the

potential arrhythmogenic effects of medicinal products associated with a prolonged QT interval.

4. Ventricular repolarization, as determined by the duration of the myocardial action potential, is a complex physiological process. This is a cumulative result of operation of many membrane ion channels and carriers. Under physiological conditions, the functions of these ion channels and carriers are highly interdependent. The activity of each ion channel or carrier is affected by many factors, including, but not limited to, intracellular and extracellular ion concentration, membrane potential, intercellular electrical interaction, heart rate, and autonomic nervous system activity. Other important factors include metabolic state (e.g., acid-base balance), location and type of the cardiomyocyte. The action potential of human ventricles consists of five successive phases:

phase 0: increase in the action potential is mainly due to the fast-transient incoming current Na^+ (I_{Na}) via the Na+-channels;

phase 1: the action potential stops rowing, and the phase of early repolarization occurs due to inactivation of Na+-channels and the transient output current K^+ (I_{to}) via K^+-channels;

phase 2: the action potential plateau reflects the balance between the incoming Ca^{2+} current (I_{Ca}) via L-type Ca^{2+}-channels and the output repolarizing K+-currents;

phase 3: steady downward motion of the action potential and the late repolarization phase occur due to output current K^+ (I_{Kr} and I_{Ks}) via the K^+-channels of delayed rectification;

phase 4: the resting potential is maintained by the incoming rectifying K^+-current (I_{Ki}).

5. The action potential can be prolonged due to reduced inactivation of the incoming Na+- or Ca2+-currents, increased activation of the Ca2+-

current, or inhibition of one or several outgoing K+-currents. The rapidly and slowly activated components of the delayed rectification potassium current (I_{Kr} and I_{Ks}) apparently play the most important role in determining the action potential duration and, accordingly, the QT interval. The hERG gene (human ether-à-go-go--related-gene) and the KvLQT1 gene encode the pore-forming proteins KCNH2 and KCNQ1, which are believed to represent the α-subunit of human potassium channels responsible for I_{Kr} and I_{Ks}, respectively. These α-subunit proteins can form hetero-oligomeric complexes with auxiliary β-subunits (i.e., products of the MiRP and MinK genes), which apparently modulate the bandwidth properties of the channel proteins. The most common mechanism for prolonging the QT interval with medicines is inhibition of the delayed rectification potassium current, responsible for I_{Kr}.

6. This document applies to new chemical compounds for human use and to registered medicinal products (e.g., when adverse clinical events, a new patient population, or a new route of administration result in a risk of such an adverse effect which has not been studied before). The main document describes the conditions under which studies are not required.

7. *In vitro* I_{Kr}- and *in vivo* QT analyzes described in paragraphs 16, 17 of this Annex, conducted for the purpose of medicinal product registration, should be carried out in accordance with the Good Laboratory Practice Rules. The follow-up studies described in paragraphs 20-22 of this Annex should, as much as possible, comply with the Good Laboratory Practice Rules.

8. Since *in vitro* and *in vivo* analyses are complementary approaches, you should perform both types of analysis.

9. The experimental approach and risk signs must be tailored to the substance under study, depending on its profiles of pharmacodynamics, pharmacokinetics and safety.

II. Planning and organizing studies of the effect on ventricular repolarization

10. Study goals include:

establishing the ability of the substance under study and its metabolites to slow down ventricular repolarization on a test system;

correlation of the degree of ventricular repolarization slowdown with the concentrations of the substance under study and its metabolites.

11. The study results should be used for:

clarifying the mechanism of action of the substance under study;

assessing the risk of ventricular repolarization slowdown and QT interval prolongation in humans while considering it in combination with other data.

Principles for selecting and planning pharmacological safety studies of medicinal products for human use

12. Preclinical methodology helps find out the following:

ion currents measured in isolated animals or human cardiomyocytes, cultures of heart cell lines and heterologous expressing systems of cloned human ion channels;

action potential parameters in isolated heart preparations or certain electrophysiological parameters characterizing the duration of the action potential in anesthetized animals;

electrocardiogram parameters measured in conscious or anesthetized animals;

arrhythmogenic effects measured in isolated heart preparations or in animals.

These four functional levels can be studied by using *in vitro* and/or *in vivo* methods. Data from the functional levels listed above are deemed useful and complementary.

13. *In vitro* electrophysiological studies allow for examining potential cellular mechanisms that are not apparent from *in vivo* data. Changes in other cardiovascular parameters or impact on several ion channels can complicate data interpretation. This problem can be solved with the help of complementary assessments on other systems. Despite the fact that repolarization slowdown can occur due to modulation of several types of ion channels, I_{Kr} inhibition is the most common mechanism for implementing medicine-induced QT interval prolongation in humans.

14. *In vivo* models with a full set of molecular, biochemical and physiological systems can also be informative in terms of human response to the substance under study. Carefully planned and properly conducted *in vivo* studies enable you to assess the basic material and metabolites and to assess the safety margins. *In vivo* ECG assessment provides information on the conduction properties and extracardiac effects (e.g., tonus of the autonomic nervous system). Studies of the action potential parameters make it possible to obtain information on integral activity of several ion channels of the heart.

Strategy for preclinical pharmacological safety studies of medicinal products for human use

15. The overall preclinical study strategy for assessing the risk of ventricular repolarization slowdown and QT interval prolongation is presented in Figure 1.

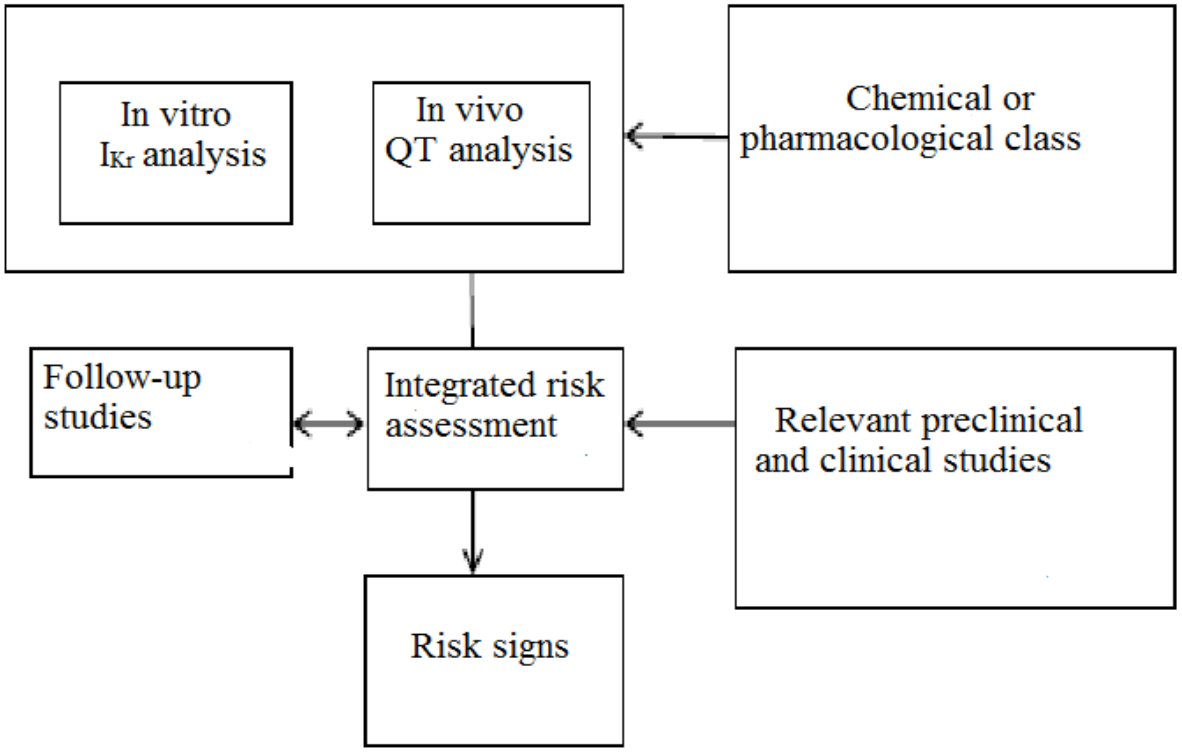

Fig. 1 Strategy for preclinical pharmacological safety studies of medicinal products

In vitro I_{Kr} study

16. *In vivo* I_{Kr} analysis assesses the effect on ion current through a native or expressing I_{Kr}-channel protein, e.g., encoded by the hERG gene (paragraphs 29-34 of this Annex).

In vivo QT analysis

17. *In vivo* QT analysis measures ventricular repolarization indicators, such as the QT interval (paragraphs 35-40 of this Annex). This analysis can be planned in such a way as to achieve the goals of both the core battery of pharmacological safety studies (cardiovascular study from the core battery) and the study goals according to this Annex. This will enable reduction of the use of animals and other resources in the experiment.

Chemical or pharmacological class of the substance under study

18. You must consider whether the substance under study belongs to the chemical or pharmacological class, some members of which demonstrated the ability to prolong the QT interval in humans (e.g., antipsychotics, H_1-histamine antagonists, fluoroquinolones). This factor must be taken into account (when it matters) when selecting a reference compound(s), and included in the integrated risk assessment.

Relevant preclinical and clinical data

19. Additional data required for an integrated risk assessment include the results of:

pharmacodynamic studies;

toxicological or safety studies;

pharmacokinetic studies, including plasma concentrations of the basic substance and metabolites (including human data, when available);

drug interaction studies;

tissue distribution and cumulation studies;

post-registration studies.

Follow-up studies

20. Follow-up studies are aimed at obtaining a deeper understanding or additional knowledge about the ability of the substance under study to slow down ventricular repolarization and prolong the QT interval in humans. Such studies may provide additional data about activity, mechanism of action, inclination of the "dose - response" curve or response amount. Follow-up studies are aimed at solving specific problems; therefore, various *in vivo* and *in vitro* study designs are applicable.

21. If the results of various preclinical studies are not consistent and/or the results of clinical studies differ from those of preclinical studies, a retrospective assessment and follow-up preclinical studies may cast light on the discrepancies that emerged. The results of follow-up studies may be an essential component of an integrated risk assessment.

22. When selecting and planning follow-up studies, besides relevant preclinical and clinical data, you should consider the following:

performing ventricular repolarization analyses measuring action potential parameters on isolated heart preparations (paragraphs 29-34 of this Annex);

using specific electrophysiological parameters characterizing the action potential duration in anesthetized animals (paragraphs 35-40 of this Annex)

repeated (multiple) administration of the substance under study;

selecting species and sex of animals;

using metabolic inducers and inhibitors; using associated positive control substances and reference compounds (see paragraphs 27-28 of this Annex);

inhibition of other channels not assessed earlier;

measuring electrophysiological parameters at several time points;

distorting effects in conscious animals that limit data interpretation, such as effects on the heart rhythm or autonomic tonus induced by the substance under study, or toxicity such as tremors, convulsions or vomiting.

Integrated risk assessment

23. Integrated risk assessment is an assessment of the results of preclinical studies, including the results of follow-up studies and other relevant data. Integrated risk assessment must be scientific and tailored to the substance under study. Such an assessment can help in planning clinical

studies and interpreting their results. When available, integrated risk assessment should be included in the researcher's brochure and preclinical review in module 2 of the registration dossier of the medicinal product. Depending on the stage of medicinal product development, the integrated risk assessment should also take into account:

analysis sensitivity and specificity;

activity of the substance under study in relation to the reference compound (compounds);

relationship between exposures related to the repolarization effect and exposures of the substance under study providing a primary pharmacodynamic effect on preclinical experimental animal species or the proposed therapeutic effect on humans;

contribution of metabolites to the QT interval prolongation, as well as metabolic differences between humans and animals.

Proof of risk

24. Proof of risk is a general conclusion based on the results of an integrated risk assessment of the ability of the substance under study to slow down ventricular repolarization and prolong the QT interval in humans.

Periods of preclinical studies and integrated risk assessment in relation to clinical development

25. You should provide for conducting preclinical studies aimed at assessing the risk of ventricular repolarization slowdown and QT interval prolongation before the first administration of the substance under study to a human. Their results as part of the integrated risk assessment can justify planning and interpretation of the results of follow-up clinical studies.

3. Test systems

Selecting test systems

26. This section provides an overview of the methodologies currently used to assess the potential of a substance under study to slow down ventricular repolarization and prolong the QT interval. When selecting most suitable test systems, you must take into account the following:

scientific credibility and stability of the analytical methodology and experimental endpoints;

standardization of analyses and medicinal products;

reproducibility of results;

relevance of endpoints or analysis parameters for assessing risk to humans.

Use of positive control substances and reference compounds

27. In order to demonstrate *in vitro* response of ion channel preparations and action potential duration analyses, you must use the submaximal effective concentration of the positive control substance and include it in each study. In case of *in vivo* studies, positive control substances must be used to validate and determine the test system sensitivity, but you don't need to include them in each study.

28. With regard to substances under study belonging to the chemical or pharmacological class associated with QT interval prolongation in humans, you should provide for the use of an accompanying reference compound(s) (belonging to the same chemical and pharmacological class of substances) during *in vitro* and *in vivo* studies, in order to facilitate ranking the activity of the substance under study in relation to its comparators.

Electrophysiological *in vitro* studies

29. Electrophysiological *in vitro* studies can provide valuable data on the effect of the substance under study on the action potential duration and/or ionic currents in the heart. These studies play an important role in assessing the potential for QT interval prolongation and in clarifying the cellular mechanisms affecting repolarization. Electrophysiological *in vitro* studies use single cells (e.g., heterologous expressing systems, disaggregated cardiomyocytes), or multicellular preparations (e.g., Purkinje fiber, papillary muscle, trabeculae, perfused myocardium, intact heart). Heterologous expressing systems (human ion channel proteins are expressed on non-cardiac cell lines) are used to assess the effect of the substance under study on a specific ion channel. Compared with expressing systems, disaggregated myocytes cause more technical difficulties, but they allow you to assess the impact on the action potential duration and ion currents. Despite the fact that preparations of individual cells are more fragile, they feature minimized diffusion barriers at the action site. Multicellular preparations are stable test systems for studying the action potential duration. Analysis of the parameters of each action potential phase, such as V_{max} for phase 0 (I_{Na}), APD_{30} or APD_{40} for phase 2 (I_{Ca}) and «triangulation" for phase 3 (I_K), allows us to study the impact on certain channels responsible for these phases. Besides, some parameters obtained from the Langendorff preparation help obtain data on arrhythmogenic risk.

30. Tissue and cell preparations for *in vitro* analyses are obtained from various laboratory animal species, including rabbits, ferrets, guinea pigs, dogs, pigs, and sometimes from humans. Repolarization ionic mechanisms in adult rats and mice differ from the ionic mechanisms of larger animal species, including humans (the main ionic currents controlling repolarization in adult rats and mice are I_{to}), therefore, using tissues of these species is unacceptable.

When selecting a test system, you must take into account the inter-species differences, in particular, which ion channels of the heart contribute to the heart repolarization and the action potential duration. When using native tissues or heart cells, you must take into account the properties and the source of the medicinal product, since ion channel distribution depends on cell location and type.

31. The concentrations of the substance under study during *in vitro* studies should encompass a wide range, covering the expected maximum therapeutic plasma concentration and going beyond it. Increasing concentrations are studied until the properties of the concentration-response curve are identified or until the physical and chemical effects start limiting the concentration. Ideally, the exposure duration should be sufficient for obtaining balanced electrophysiological effects, unless the viability of the cell or tissue preparation impedes it. You must specify the exposure duration. In order to determine sensitivity of *in vitro* analytical systems, appropriate positive control substances should be used.

32. Factors capable of distorting or limiting the interpretation of *in vitro* electrophysiological studies include:

testing high concentrations of the substance under study may be hampered by its low solubility in aqueous physiological saline solutions;

adsorption to glass or plastic or nonspecific binding to the matrix under study can reduce concentration of the substance under study in an incubation or perfusion solution;

concentrations of the substance under study may be limited by its cytotoxic or physical and chemical properties violating the cell membrane integrity, which prevents from reaching electrophysiological endpoints;

heart cells and tissues feature low metabolic capabilities for metabolism of medicinal products, therefore, *in vitro* studies using the basic material do

not allow for obtaining data on the effect of metabolites. If preclinical or clinical *in vivo* studies reveal QT interval prolongation that is inconsistent with the results of *in vitro* studies using the basic material, you should consider conducting studies of metabolites using *in vitro* test systems.

33. When developing new technologies for analyzing potassium channels that can be used for preliminary screening of substances under study in order to identify leading candidates, the consistency of new and standard technologies should be confirmed before these new technologies are used for examination and registration of a medicinal product.

34. You may use competitive binding protocols in which substances under study are tested for their ability to replace the radiolabeled hERG channel blocker in a hERG-expressing cell line. At the same time, competition for the binding sites with radioligands prevents obtaining information on the agonistic or antagonistic effects of the substance under study on I_{Kr}. Moreover, this analysis will not detect substances under study that bind to hERG at sites other than radioligand binding sites. Based on these potential limitations, such analysis is not considered a replacement for the potential (voltage) fixation analyses described in paragraphs 29-33 of this Annex.

Electrophysiological *in vivo* studies

35. Models with intact animals make it possible to study ventricular repolarization or arrhythmias associated with it, where the integral effect of the entire complex of ion channels and cell types is assessed. Besides, animals demonstrate potential neuronal and hormonal impacts on the pharmacodynamic effect of medicines.

36. The QT interval length on an electrocardiogram is the most commonly used endpoint measuring the effect of the substance under study

on ventricular repolarization. In specialized electrophysiological studies, data on ventricular repolarization (e.g., duration of the monophasic action potential and effective refractory period) can also be obtained with *in vivo* models. At the same time, you can assess additional safety parameters of interest, including arterial blood pressure, heart rate, PR interval, QRS complex width, and arrhythmias.

37. The QT interval and the heart rate feature an inverse non-linear relationship varying between species and between individuals of the same species. Thus, a change in the heart rate affects the QT interval, which may distort the assessment of the effect of the substance under study on ventricular repolarization and on the QT interval. There are two important situations when there is heart rate variability between individuals: first, due to the difference in the autonomic tonus, and, second, due to the impact produced by the substance under study on the heart rate. In this regard, when interpreting data from *in vivo* test systems, you must take into account the concurrent changes in the heart rate. Ideally, data on the QT interval obtained after administration of the substance under study should be compared with control and baseline data at comparable heart rates. If the heart rate variability is not conditioned by the substance under study, it can be reduced by acclimatization or by using animal models with anesthesia. If the effects are due to the substance under study, the most common approach is to correct the QT interval for the heart rate (QTc) using Bazett's formula, Fridericia formula and other approaches. You should justify your selection of a formula for correcting heart rate with data obtained from the test system. If the heart rate differences between intervention and control are significant, the correcting formulae may not be effective for assessing the risk of QT interval prolongation. An alternative approach is maintaining a constant heart rate using an artificial pacemaker. Sometimes it is more appropriate to analyze the

QT/RR ratio, including correcting the QT interval using formulae for individual experimental animals.

38. Laboratory animal species used during electrophysiological *in vivo* studies include dogs, monkeys, pigs, rabbits, ferrets and guinea pigs. Repolarization ionic mechanisms in adult rats and mice differ from the ionic mechanisms of larger animal species and humans (the main ionic currents controlling repolarization in adult rats and mice are I_{to}), therefore, using rats and mice is not acceptable. You must select most suitable *in vivo* test systems and justify your decision.

39. The dose range must be consistent with the range used in the core battery of pharmacological safety studies and, in all realistic cases, cover or exceed the expected exposure in humans. The dose range may be limited by animal intolerance to the substance under study (e.g., vomiting, tremors, or hyperactivity). When conducting studies aimed at correlating the degree of ventricular repolarization deceleration with the concentrations of the basic substance under study and its metabolites, you may use controlled exposure with continuous intravenous infusion. Monitoring the exposure of the substance under study and metabolites provides an opportunity to interpret the data on the "dose-response" and "concentration-response" relationships and to plan follow-up studies, when appropriate.

40. When conducting studies and interpreting the results, take into account the following factors:

data collection and analysis method;

sensitivity and reproducibility of the test systems;

dosing period and measured points;

heart rate and other effects

distorting QT interval data interpretation;

inter-species and gender differences (e.g., cardiac electrophysiology, hemodynamics or metabolism of medicinal products);

the ability of medicinal products affecting several ion channels to give a complex form of the "dose-response" relationship in an experiment that can be hard to interpret.

Simulation of pathological conditions and arrhythmias

41. The exact relationship between the ventricular repolarization slowdown induced by the substance under study and the risk of arrhythmia is unknown. It is advisable to directly study the arrhythmogenic risk of medicinal products prolonging the QT interval. When assessing arrhythmogenic action, you may use animal models and arrhythmogenic activity indicators (e.g., electrical instability, temporal and/or spatial refractoriness dispersion, inverse use-dependence, changes in the action potential configuration). We encourage interested parties to develop the models described and evaluate their suitability for determining the risk to humans.