Rabies: Developing Monoclonal Antibody Cocktails for the Passive Immunization Component of Post-Exposure Prophylaxis

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

July 2021
Clinical/Antimicrobial

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Rabies: Developing Monoclonal Antibody Cocktails for the Passive Immunization Component of Post-Exposure Prophylaxis Guidance for Industry[1]

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I. INTRODUCTION

The purpose of this guidance is to help sponsors in the development of anti-rabies virus monoclonal antibody (mAb) cocktails as an alternative to anti-rabies virus immunoglobulin (RIG) as the passive immunization component of post-exposure prophylaxis (PEP) for the prevention of rabies when given immediately after contact with a rabid or possibly rabid animal. This draft guidance is intended to serve as a focus for continued discussions among the Division of Antivirals, sponsors, the academic community, and the public.[2] This guidance does not address the development of rabies vaccines, products to treat rabies, or mAbs for other indications. The recommendations in this guidance relate to studies to be submitted in support of a biologics license application (BLA) submission under section 351 of the Public Health Service Act (42 U.S.C. § 262) and implementing regulations at 21 CFR part 601.

This guidance does not address general issues of statistical analysis or clinical trial design. Those topics are addressed in the ICH guidances for industry *E9 Statistical Principles for Clinical Trials* (September 1998), *E9(R1) Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials* (May 2021), and *E10 Choice of Control Group and Related Issues in Clinical Trials* (May 2001), respectively.[3]

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way, unless specifically incorporated into a contract. This document is intended only to provide clarity to the public regarding existing requirements under the law. FDA guidance documents, including this guidance, should be viewed only as recommendations, unless

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

[2] FDA encourages sponsors to contact the division to discuss specific issues that arise during the development of rabies mAb cocktails.

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

specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

Rabies has an almost 100% case fatality rate after clinical symptoms develop and there is no proven treatment. However, timely administration of rabies PEP is nearly 100% effective in preventing clinical rabies (WHO 2018). Globally, approximately 20 million people per year receive PEP after potential rabies virus exposure (WHO 2013), including approximately 55,000 people in the United States (Pieracci et al. 2019). Despite available prophylaxis, approximately 59,000 people die from rabies worldwide each year (Hampson et al. 2015, WHO 2018), usually either because PEP was not administered or because PEP was administered incorrectly (WHO 2018).

PEP consists of three components for patients not previously vaccinated against rabies[4]:

1. Thoroughly washing the wound
2. Promptly initiating a rabies vaccine series
3. Promptly administrating RIG in and around the wound
    - In the United States, RIG is recommended in any situation for which PEP is considered appropriate (in patients not previously vaccinated against rabies). Outside the United States, RIG is included for only World Health Organization (WHO) category III exposures, which include any transdermal bites or scratches, contamination of mucous membrane or broken skin with saliva from animal licks, or exposures due to direct contact with bats.

Although thoroughly washing the wound and promptly completing a modern rabies vaccination series alone have been estimated to prevent rabies in approximately 99% of people exposed to rabies virus (WHO 2018), RIG is vital to rabies prevention after more severe exposures (Baltazard and Bahmanyar 1955). RIG is considered particularly important after bites to the head and neck for which it may take less time for the rabies virus to travel from the wound to the brain. People vaccinated with a rabies vaccine series develop rabies virus neutralizing antibodies (RVNAs) >0.5 IU/mL, the level WHO uses as a measure of adequate vaccine response, within 7-14 days (WHO 2018). RIG's chief contribution is providing neutralization activity in the period before the vaccine-induced RVNAs develop.

RIG is produced from the pooled serum of individuals hyperimmunized against the rabies virus, and currently is either of human (HRIG) or equine (ERIG) origin. HRIG and ERIG are considered to have equal effectiveness, but the safety profile of the two products may differ. Only HRIG is commercially available in the United States. Globally, in developing countries where rabies is endemic, ERIG is used more often.

[4] In previously vaccinated individuals, PEP consists of wound washing and an abbreviated vaccine series without RIG. In individuals who have not previously been vaccinated, RIG should be administered concurrently with the first dose of vaccine.

Globally, RIG is used in less than 2% of rabies virus exposures because of several factors, including RIG's dependence on the cold chain and logistical issues such as limited supply. In the United States where RIG is generally available, an alternative to RIG would be useful in case of RIG shortage and to eliminate the theoretical risk of transmission of blood-borne pathogens. For these reasons, mAb cocktails are being developed as an alternative to RIG as the passive component of PEP. WHO has recommended that mAb cocktails contain at least two mAbs that target different, nonoverlapping antigenic sites on the rabies virus envelope G glycoprotein, the protein that is the sole target of the RVNAs elicited by vaccine administration (WHO 2013).

The development pathway for rabies mAb cocktails is challenging because of many complicating factors including the following:

- Without RIG, wound washing and rabies vaccination by themselves are ~99% effective at preventing clinical rabies. Complete PEP with RIG increases this rate to ~99.9%, but the exact contribution of RIG to the effectiveness of PEP is unknown. Consequently, trial sizes required to power for noninferiority versus RIG with mortality as an endpoint are infeasible, even if a noninferiority margin could be determined, whereas placebo-controlled trials would likely be considered unacceptable based on expert input. These topics were discussed during an FDA public workshop and advisory committee meeting.[5]

- Multiple factors affect the risk of rabies development after potential exposure through an animal bite, which makes comparison to a historical control challenging. Whether the bite was from a rabid animal is usually not known, and the likelihood of the animal being rabid varies widely by location. Other factors include the location of the bite on the body, number and depth of bites, viral inoculum in the saliva of the biting animal, type of rabies vaccine used as part of PEP, host factors, and the time interval between the bite and initiation of PEP.

- Selecting an appropriate dose for the mAb cocktail is challenging, as too high a dose could interfere with the vaccine response and thus increase the risk of developing rabies.

- The mAb cocktails are qualitatively different from HRIG preparations, so they will have a different development pathway. A chief concern with mAb cocktails is diminished breadth of activity and durability against different rabies virus strains, as mAb cocktails could contain as few as two antibodies compared with polyclonal RIG. RVNA levels, which have been used as an endpoint in many HRIG trials, do not measure breadth of activity. For new HRIG preparations standardized to the same potency as a marketed HRIG product, and with similar RVNA profiles, it was reasonable to assume that these new products would likely have similar efficacy and breadth of activity to the marketed HRIG product. This assumption cannot be extrapolated to mAb cocktails.

[5] Materials for the 2017 workshop *Developing Rabies Monoclonal Antibody Products as a Component of Rabies Post-exposure Prophylaxis* are available at https://www.fda.gov/drugs/news-events-human-drugs/developing-rabies-monoclonal-antibody-products-component-rabies-post-exposure-prophylaxis. Materials for the 2019 Antimicrobial Drugs Advisory Committee are available at https://www.fda.gov/advisory-committees/advisory-committee-calendar/april-25-2019-antimicrobial-drugs-advisory-committee-meeting-announcement-04252019-04252019.

Because of the unique complexities of drug development for rabies mAb cocktails, FDA convened discussions with multiple stakeholders, including a public workshop in 2017[6] and an advisory committee meeting in 2019.[7] These discussions helped FDA formulate recommended regulatory pathways for rabies mAb cocktail development. At these discussions there was consensus that superiority trials of mAb cocktails versus placebo, for the passive PEP component, are likely to be considered unacceptable and that adequately powered noninferiority trials of mAb cocktail versus RIG are not logistically feasible. In addition, there was agreement that surrogate endpoints of protection are not established for the passive component of PEP. Therefore, FDA is recommending an approach combining nonclinical and clinical data to demonstrate substantial evidence of effectiveness for rabies mAb cocktails.

III. DEVELOPMENT PROGRAM

A. General Considerations

Development of mAbs for use in rabies PEP requires careful balancing and integrated assessment of data from nonclinical studies, healthy volunteer clinical trials, and clinical trials enrolling persons with known or suspected rabies exposure. Because adverse outcomes from decreased performance of the passive component of PEP can be lethal but rare and difficult to attribute causally, sponsors should consider other available types of data at each step in the development sequence. Some of these interrelationships will be emphasized in the following sections.

1 Nonclinical Virology Development Considerations

a. Epitope mapping

Sponsors should characterize the epitope of each mAb, including identifying amino acids critical for neutralization (e.g., contact residues). These studies should include selecting and characterizing neutralization-resistant variants in cell culture, ideally using multiple resistant variants that were independently selected from antigenically diverse viruses. Sponsors should determine the frequency of amino acid polymorphisms at critical amino acid positions in circulating rabies virus strains.

b. Antiviral activity in cell culture

The neutralizing activity of the mAb cocktail, the individual mAb constituents of the cocktail, and an HRIG comparator should be evaluated in cell culture against a panel of rabies virus strains representative of the antigenic diversity of circulating strains. The panel should include strains from multiple host species (e.g., bats, dogs, foxes, raccoons, skunks) and from multiple locations (i.e., the United States and areas in Asia and Africa where rabies is endemic). In addition, the panel should include strains with polymorphisms at amino acid positions critical for

[6] See footnote 5.

[7] See footnote 5.

neutralization by each mAb. The results of the neutralization assays should be reported as the 50% effective concentration (i.e., EC_{50} values reported as ng/mL and/or International Units [IU]/mL). Ideally, the mAb cocktail will demonstrate a breadth of neutralizing activity that is at least as broad as that of HRIG. Sponsors should consider evaluating potential Fc-mediated mechanisms of antiviral activity (e.g., antibody-dependent cellular cytotoxicity), if applicable.

c. Animal challenge studies

Animal models of rabies PEP (e.g., hamster, dog) should demonstrate that the mAb cocktail at the to-be-marketed concentration and dose is superior to placebo and similar to or better than HRIG in reducing mortality.[8] These animal challenge studies should test various concentrations and doses of the mAb cocktail and be conducted both with and without a concomitant rabies vaccine. Studies comparing the effects of the mAb cocktail and HRIG on vaccine response in the animal models should be completed, and sponsors should consider a comparison of the prophylactic windows of the mAb cocktail and HRIG. Selecting rabies virus challenge strains should depend on human exposure risks (e.g., dog and bat strains) and susceptibility of the mAbs based on cell culture data; ideally, these studies will include challenge strains that are among the least susceptible to neutralization in cell culture to increase confidence that reductions in mortality with the challenge strains could be extrapolated to other, more susceptible strains.

2. Early-Phase Clinical Development Considerations

Trials in healthy subjects not exposed to rabies virus should evaluate the pharmacokinetics, RVNA levels, and initial safety and tolerability of the mAb cocktail versus HRIG both when administered alone and when administered with a rabies vaccine series.

A dose-ranging trial of the mAb cocktail versus HRIG in the absence of a rabies vaccine in healthy volunteers should include both intramuscular and subcutaneous administration to reflect how these products could be administered for PEP. Blood samples should be collected at multiple time points to accurately capture the peak RVNA levels and the RVNA concentration-time profile and to fully characterize the pharmacokinetic profile of each mAb. Important endpoints include demonstration of the following for the doses of the mAb cocktail chosen for further development:

- Similar or higher RVNA levels (in IU/mL) for the mAb cocktail versus HRIG at each of multiple time points through Day 14 (i.e. throughout the earliest time period when passive antibodies may be the principal contributor to neutralizing activity, as well as the period from Day 7 to Day 14 when vaccine-induced RVNAs would be expected to become apparent in most people with vaccine coadministration).

A second trial in healthy volunteers should compare various doses of the mAb cocktail versus HRIG versus placebo when administered in combination with a rabies vaccine series. If various

[8] We support the principles of the 3Rs, to reduce, refine, and replace animal use in testing when feasible. FDA encourages sponsors to consult with us if they wish to use a nonanimal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method.

rabies vaccines and routes of vaccine administration (intramuscular or intradermal) are expected to be used in the phase 3 trials, each of these rabies vaccines and routes of vaccine administration should be tested with the mAb cocktail in the phase 1 healthy volunteer trials to assess for acceptable levels of vaccine interference. If FDA-approved rabies vaccines will not be used in the phase 3 trials, the potential for interference with FDA-approved rabies vaccines should be evaluated in healthy volunteer trials. Important endpoints in the healthy volunteer trials in which the mAb cocktail or HRIG is administered with a rabies vaccine series include demonstration of the following for the dose of mAb cocktail chosen for further development in trials in potentially rabies-exposed subjects:

- Comparable RVNA levels for the mAb cocktail versus HRIG at earlier time points (up to 7 days), before RVNAs produced by vaccine would be expected to predominate—There is no established protective threshold at early time points, but HRIG is considered to be effective.

- Comparable vaccine interference to that observed with HRIG—The proportion of subjects with RVNA levels ≥0.5 IU/mL at Day 14 was used to measure vaccine interference for a recently FDA-approved HRIG product. However, if the mAb cocktails alone increase RVNA levels to ≥0.5 IU/mL at Day 14 and later, there could be complete interference with vaccine response, which would not be detected using this method. In this situation, vaccine interference could be measured by assessing the proportion of subjects with RVNA levels ≥0.5 IU/mL at a later time point when the mAb contribution to the RVNA levels would be expected to be much less than 0.5 IU/mL.

- Comparable Day 14 RVNA geometric mean titers for the mAb cocktail versus the HRIG groups, acknowledging that these RVNAs would be a combination of vaccine-induced RVNA and RVNA from passive immunization with mAb cocktail or HRIG—Based on the pathophysiology of rabies virus infection, total RVNA at this time point would be important for rabies virus neutralization regardless of the RVNA source.

3. Efficacy Considerations

A traditional approval can potentially be based on a multicenter clinical trial enrolling subjects with suspected rabies exposure, if those trial results are supported by evidence from the cell culture, animal model data, and healthy volunteer data described above. Initial BLA submissions for rabies mAb cocktails could be submitted for either a second-line or a first-line indication depending on the number of subjects enrolled and the level of efficacy demonstrated, as described in more detail in section III. B. Discussions in this guidance assume a trial to support a second-line indication would be performed first, before proceeding to a larger trial to support advancing to a first-line indication.

In either scenario, because diminished efficacy of rabies mAb cocktails could result in death, rabies mAb cocktail development should proceed in a stepwise fashion to minimize risk to trial subjects. The mAbs initially chosen for cocktail development should be complementary in terms of neutralization activity and have activity against a diverse panel of rabies virus strains. Broad coverage is particularly important for development in the United States, where rabies deaths have

been reported from domestic exposures (predominantly due to bat, raccoon, fox, and skunk strains) and exposures during international travel due to canine strains (Pieracci et al. 2019). In addition, mAb choice should consider the amino acid sequence and whether any residues in the complementarity-determining regions could undergo posttranslational modifications that might affect antigen binding. After sponsors have chosen mAbs, data should be obtained from cell culture activity studies, animal challenge studies, toxicology studies, and clinical trials in healthy volunteers not exposed to rabies virus (both with and without rabies vaccine). These data can inform dose selection and provide support for antiviral activity and breadth of coverage. The next step is a clinical trial of the mAb cocktail versus RIG, in combination with wound washing and a rabies vaccine series, in potentially rabies virus-exposed subjects.

It is not feasible to adequately power a clinical trial to demonstrate noninferiority of mAb cocktails versus RIG, both in combination with rabies vaccine and wound washing, for an endpoint of rabies-free survival. The exact contribution of the passive immunization component of PEP is unknown but is believed to be very small compared with the contribution of wound washing and administration of a rabies vaccine. In addition, patients presenting with WHO category III rabies virus exposures will be highly heterogenous with regard to their actual risk of developing clinical rabies in the absence of the mAb cocktail or RIG. It is also not feasible to adequately power a clinical trial to demonstrate superiority of mAb cocktails versus RIG because PEP including RIG is nearly 100% effective.

Consequently, evaluation of efficacy will rely on a clinical trial demonstrating an acceptable rabies-free survival rate in subjects presenting with WHO category III rabies virus exposures in rabies-endemic countries[9] who receive the mAb cocktail in place of RIG as part of PEP. However, a double-blinded, randomized, active-controlled design comparing the mAb cocktail with RIG, both in combination with wound washing and rabies vaccine, is still recommended to adequately characterize safety and to confirm comparable early RVNA levels and vaccine interference when the mAb cocktail or RIG are administered in and around the wound. In addition, including an active control would serve as a point of reference in the event of PEP failures to better determine if the failures were due to decreased efficacy of the mAb cocktail versus unforeseen factors such as an unexpectedly low vaccine response or a novel viral strain.

4. Safety Considerations

Generating a robust safety database from adequately blinded, well-controlled human trials in appropriate populations is important because of the wide variety of affected populations and possible exposures that would qualify for PEP. An application for a new mAb cocktail for the passive immunization component of PEP should include safety data from at least 1,000 subjects

[9] For the purposes of this guidance document, *rabies-endemic countries* are considered to be countries in which rabies circulates in the dog population and dog bites are known to pose a meaningful risk of rabies transmission and death for humans. Reasons for recommending that substantial proportions of clinical trials be conducted in such rabies-endemic countries include the following: (1) canine rabies is critically important to the total global burden of human rabies exposures in need of PEP and (2) assumptions and estimates regarding likelihood of human rabies deaths after an exposure with receipt of PEP are based mostly on experience with dog bites in rabies-endemic countries, so interpretation of trial results may be subject to more uncertainty of expected outcomes after other types of known or suspected rabies exposures.

who received the mAb cocktail dose proposed for marketing. A safety database larger than 1,000 subjects may be necessary if significant safety signals are identified in development. This total can include healthy subjects from the phase 1 trials as well as potentially rabies virus-exposed subjects in both rabies-endemic countries and non-rabies-endemic countries. If the mAb cocktail is already approved in other countries, and there are postmarketing data that are well-characterized in terms of number of patients dosed, number of rabies deaths, and serious adverse events, these data may be considered for use as part of the safety database if the Agency agrees.

B. Phase 3 Efficacy Trial Considerations

With the exception of section III. B. 9. d, the following sections describe Agency recommendations for a trial designed to support a second-line indication.

1. Trial Design, Including Randomization, Stratification, and Blinding

The trial should be a multicenter, double-blind, randomized controlled trial of the mAb cocktail versus RIG, each in combination with thorough wound washing and rabies vaccine series, in subjects with WHO category III rabies virus exposure. FDA recommends 1:1 randomization for the clinical trial to support licensure. The trial should be designed such that at least 750 subjects with WHO category III exposure in rabies-endemic countries are treated with PEP including the mAb cocktail and followed for at least one year to demonstrate a rabies-free survival rate >99.5%[10]. This means that the trial should enroll at least 1,500 subjects with WHO category III exposure in rabies-endemic countries, with additional enrollment in non-rabies-endemic countries for an adequate safety evaluation.

Stratification should be considered for factors influencing the risk of rabies development, such as the time interval between exposure and randomization (≤ or >24 hours), the location of the bite or bites (above versus below the neck), and the number of bites. Sponsors should carefully document all components of PEP for all enrolled cases. If any subject develops rabies, review of the PEP administration for that case should be conducted and documented in a blinded fashion by experts unaware of the subject's treatment assignment.

2. Trial Population and Location

To draw conclusions about mAb cocktail efficacy from clinical trial survival results, the trial should predominantly enroll subjects in rabies-endemic countries. When a patient presents for rabies PEP, it is generally not known whether the exposure was from a rabid animal. This is also expected to be the case in a clinical trial. The likelihood that the exposure was from a rabid animal varies widely by location, with the risk being much higher in rabies-endemic countries. FDA prefers that the trial enroll subjects in several rabies-endemic countries with different endemic rabies virus strains. However, FDA encourages sponsors to include some trial sites in

[10] The 2019 advisory committee concurred that approval based on lack of rabies mortality in a trial that randomizes at least 750 subjects to receive the mAb cocktail as part of PEP would be sufficient for a second-line indication in situations where HRIG is not available because survival with PEP including RIG is estimated to be >99.9%.

the United States and other non-rabies-endemic countries to allow for safety evaluation in a broad population.

The trial should start by enrolling adults with wounds considered lower risk for rabies development in the absence of RIG (such as wounds in the lower extremities). Adolescents (for the purposes of this guidance, defined as pediatric subjects 12 years and older) may be included with adults from trial initiation, particularly if enrollment occurs at sites where RIG is otherwise not available. If a prespecified interim analysis finds no reason to stop the trial, the trial should be expanded to enroll subjects with higher risk WHO category III exposures. The trial should also be expanded to include pediatric subjects younger than adolescents (i.e., less than 12 years old) after the prespecified interim analysis, as approximately 40% of rabies cases occur in children (WHO 2018). Available data can be leveraged for initial pediatric dosing, with pharmacokinetic and RVNA sampling in the initial pediatric cohort for dose confirmation. Sponsors are encouraged to engage in early discussions with the Agency about the appropriate time for including pediatric clinical trial subjects depending on available information from their development program.[11]

Enrolling a variety of subjects of different races, ethnicities, sex, and ages and with different comorbidities is particularly important for a trial evaluating mAb cocktails for rabies PEP because rabies PEP is needed by every segment of the population exposed to a rabid animal. In addition, host factors such as age or genetic variations could influence the response to the rabies vaccine and by extension vaccine interference.

3. Entry Criteria

Promptly administering PEP is critical for reducing the risk of clinical rabies disease. Consequently, trial entry criteria should be limited to factors that can be assessed in a short period of time (less than one hour). Entry criteria should clearly define the types of exposures, including the allowable animals causing the exposure. Baseline factors that are considered important but which cannot be ascertained in this short time frame, such as evidence of previous rabies vaccine administration, can be used to exclude subjects from the intention-to-treat (ITT) population if clearly defined in the protocol.

Passive immunization with RIG or a mAb cocktail may provide the most added benefit in subjects who present later after exposure. Consequently, rabies-free survival in these subjects would best support the efficacy of the mAb cocktail, but enrollment of these subjects would be associated with the most risk if the mAb cocktail is less effective than RIG. It would be reasonable to limit trial entry to subjects who present within two to three days of rabies virus exposure to balance the risk of treatment delay with the need for informative rabies-free survival data.

[11] FDA regulations at 21 CFR Part 50, subpart D, contain additional safeguards for children enrolled in clinical investigations. Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects may involve children as set forth in 21 CFR 50.52.

374 *4. Dose Selection*
375
376 Sponsors should select the dose for the phase 3 trial based on data from the nonclinical studies
377 and the phase 1 trials in healthy volunteers. The selected dose should be high enough that it
378 provides comparable breadth of neutralizing activity to HRIG in cell culture activity studies,
379 similar reductions in mortality to HRIG in animal challenge studies, similar or higher RVNA
380 levels through Day 14 compared with HRIG in phase 1 clinical trials without vaccine, and
381 comparable early RVNA levels (up to Day 7) compared with HRIG in phase 1 clinical trials with
382 vaccine. However, the selected dose should be low enough that it provides similar or lower
383 levels of vaccine interference to HRIG in the phase 1 clinical trials with vaccine.
384
385 *5. Use of Active Comparators*
386
387 For approval considerations in the United States, because mAb cocktails may be used in place of
388 HRIG, sponsors should use HRIG as the comparator in enough subjects to allow for a sufficient
389 safety comparison. However, in trials in rabies-endemic countries, comparisons evaluating
390 rabies-free survival could be done using either HRIG or ERIG as the active comparator. The
391 choice of comparator at different study sites should consider local standard of care as well as
392 input from local regulatory authorities and stakeholders. Sponsors are encouraged to discuss the
393 choice of active comparator at different study sites with the Agency early in the planning stages
394 of clinical trials.
395
396 *6. Efficacy Endpoints*
397
398 The following endpoints are recommended as evidence of efficacy:
399
400 1. Comparable RVNA levels for the mAb cocktail versus RIG recipients at early time points
401 (up to 7 days), before RVNAs produced by vaccine predominate.
402
403 2. Comparable vaccine interference for the mAb cocktail versus RIG recipients. Vaccine
404 interference can be assessed by the proportion of subjects who develop vaccine-induced
405 RVNAs ≥0.5 IU/mL, the threshold used by WHO as a measure of adequate vaccine
406 response.
407
408 i. For mAb cocktail products that lead to RVNA levels much lower than 0.5 IU/mL
409 when administered alone, vaccine interference can be measured at Day 14 or Day 28.
410
411 ii. For mAb cocktail products that result in RVNA levels close to or above 0.5 IU/mL
412 when administered alone, vaccine interference should be measured at later time points
413 when the mAb cocktail's contribution to the RVNA levels are expected to be much
414 less than 0.5 IU/mL (after five half-lives).
415
416 3. Absence of rabies mortality through at least one year after PEP initiation. The
417 occurrence of one or more rabies deaths would raise significant review concerns.
418
419

7. Trial Procedures and Timing of Assessments

The trial should follow subjects for at least one year to monitor for rabies deaths. Descriptive details about the exposure should be recorded and should include whether the bite was provoked, the number of bites, location and depth of the bites (including pictures of the bites), the time interval between the exposure and PEP initiation, and the species or type of animal involved in the exposure. Sponsors should make reasonable efforts to ascertain and record the rabies status of the animal involved in the exposure, as this data is critical to analysis of benefit. In addition, sponsors should prospectively assess whether PEP was administered promptly and correctly and record this at the time PEP is administered.

8. Endpoint Adjudication

The trial should include a plan for a thorough, unbiased, blinded adjudication of any deaths.

9. Statistical Considerations

For considerations regarding statistical analysis methods, sponsors should refer to the FDA guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products* (May 1998).

a. Analysis populations

In general, the primary efficacy analysis should include all subjects who are randomized and receive any part of the assigned therapy during the trial. However, if subjects are excluded from the ITT population based on previous rabies vaccine administration or other baseline factors that could not be ascertained during screening, a modified ITT population can be considered for the primary efficacy analysis. Sponsors can use a per-protocol population, which may be affected by post-randomization exclusions, as a secondary efficacy population.

b. Efficacy analyses

The preferred co-primary endpoints for the phase 3 trial are described above in section III. B. 6. The following are recommendations for analyzing the primary efficacy endpoints:

- For early RVNA levels, sponsors should justify criteria for comparability and choice of specific time points before trial initiation.

- For vaccine interference, a noninferiority margin of at most 10%[12] for the proportion of subjects with RVNA levels ≥0.5 IU/mL is generally clinically acceptable. However,

[12] Studies of vaccine response after PEP regimens containing HRIG plus vaccine show a very high proportion of subjects with RVNA levels ≥0.5 IU/mL at Day 14. For example, in the efficacy analysis population of a study in which 116 subjects were randomized to receive one of two HRIG products plus vaccine, all 59 subjects who received the first HRIG product had RVNA ≥0.5 IU/mL at Day 14 (100%, exact 95% CI 93.9-100%); 56/57 receiving the second HRIG product had RVNA ≥0.5 IU/mL at Day 14 (98.2%, exact 95% CI 90.6-100%) (Matson et al. 2020).

sponsors should discuss their choice of noninferiority margin with the Agency before trial initiation.

- A BLA submission for a second-line indication should be supported by a clinical trial demonstrating >99.5% rabies-free survival among subjects with WHO category III exposure in rabies-endemic countries treated with the mAb cocktail as part of PEP [13]. This means the lower bound of the 95% confidence interval for the rabies-free survival would be >99.5% (using the Clopper-Pearson method). A threshold of rabies-free survival of >99.5% was chosen because it is higher than the ~99% estimated rabies-free survival with wound washing and rabies vaccine alone (without RIG) but would not require trial sizes that may be prohibitively large.

- Sponsors should perform the primary efficacy endpoints analyses within important subgroups based on demographic and baseline characteristics (e.g., sex, race, age, renal impairment, hepatic impairment, time interval between exposure and randomization (≤24 hours or >24 hours), the location of the bite or bites (above versus below the neck), and the number of bites). The purpose of these analyses is to explore the consistency of the primary efficacy endpoint results across these subgroups.

c. Handling of missing data

Sponsors should make every attempt to limit discontinuation of subjects from the trial. When the loss is unavoidable, sponsors should explain the causes of missing data and attempt to determine the final status of a subject who does not complete the protocol. Analyses excluding subjects with missing data or other posttreatment outcomes can be biased because subjects who do not complete the trial may differ substantially in both measured and unmeasured ways compared with subjects who remain in the trial. The primary method of handling missing data in the analysis should be prespecified in the protocol or the statistical analysis plan. Sensitivity analyses should demonstrate that the primary analysis results are robust to the assumptions regarding missing data.

d. Statistical considerations for a trial to support a first-line indication

To expand from a second-line to a first-line indication, applicants may conduct an additional clinical trial or may potentially use pooled data from several trials, data available from other countries in which the mAb cocktail was previously approved, or information from a registry after discussion with the Agency. As previously discussed in section III. A. 3., data from a clinical trial supporting a first-line indication can be submitted either in a supplemental BLA after initial approval or in the original BLA. This trial should include data from at least 6,000 subjects receiving the mAb cocktail as part of PEP after WHO category III rabies virus exposure in rabies-endemic countries. Because survival with PEP including RIG is estimated to be >99.9%, expanding to a first-line indication would require submission of additional clinical data demonstrating >99.9% rabies-free survival among subjects with WHO category III exposure in

[13] This guidance assumes a single multicenter trial would be conducted, but applicants may also potentially use pooled data from several trials if the total number of subjects meets the described requirements.

rabies-endemic countries treated with the mAb cocktail as part of PEP. If the true rabies-free survival rate of PEP containing the mAb cocktail is 99.99%, enrollment of at least 6,000 subjects provides at least 80% power to demonstrate a survival rate >99.9%.

The trial to support a first-line indication should be a randomized controlled trial to make the efficacy data more interpretable and to allow for a comparative safety evaluation. Trial randomization should be preferably 3:1 (enrolling 8,000 subjects total), or at most no greater than a 6:1 ratio (enrolling 7,000 subjects total), of mAb cocktail versus the RIG comparator, both in combination with wound washing and vaccine. The primary endpoint for a trial to expand from a second-line to a first-line indication should be rabies-free survival through at least one year after PEP initiation[14]. The lower bound of the 95% confidence interval (using the Clopper-Pearson method) for rabies-free survival will be used to evaluate whether the survival rate is >99.9%.

10. Risk-Benefit Considerations

The benefit of a mAb cocktail for use in place of RIG is different in the United States than in rabies-endemic countries where RIG is not readily available. In the United States, except for several brief shortages, HRIG has been readily available. HRIG is believed to be highly effective and has an excellent safety profile. Consequently, for FDA approval, a mAb cocktail should have a safety profile similar to HRIG's as well as efficacy similar to HRIG's. In addition to an imbalance in rabies-free survival, any nonclinical or clinical data for the mAb cocktail that suggest new safety signals or issues that could decrease efficacy compared with HRIG could result in an unfavorable benefit-risk assessment. Issues that could decrease efficacy include but are not limited to a shorter half-life or lower peak RVNA levels from the mAb cocktail alone that might result in a gap in RVNA coverage before the vaccine response manifests, higher rates of vaccine interference, or cell culture studies indicating decreased coverage of different rabies virus strains.

C. Other Considerations

1. Nonclinical Safety Considerations

The nonclinical safety assessment for the development of anti-rabies virus mAb cocktails should follow approaches outlined in the ICH guidance for industry *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (May 2012).

For mAbs directed against rabies virus, sponsors can conduct toxicology studies in one species, as specified in ICH S6(R1). For species selection for the nonclinical safety assessment, ICH S6(R1) notes that tissue cross reactivity (TCR) studies employing immunohistochemical techniques can be used by comparing tissue binding profiles between human and animal tissues when a pharmacologically relevant species cannot be identified by other approaches. FDA recommends conducting a good laboratory practice compliant TCR study using a panel of 32

[14] If an applicant wishes to submit a BLA with data supporting a first-line indication without first submitting data to support a second-line indication, FDA recommends contacting the Agency early in development to discuss specifics of clinical trial design.

human tissues. For the list of tissues and detailed technical information about immunohistochemistry studies, sponsors should refer to the guidance for industry *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* (February 1997). Sponsors should also consider alternative technologies, such as those employing protein microarrays, to evaluate *off-target* binding, but these technologies cannot replace the TCR study using immunohistochemical techniques unless appropriately justified. Although mAbs could be evaluated separately, it is typically sufficient to conduct the TCR study with the mAb cocktail at the intended clinical ratio.

If no off-target binding of significant clinical concern is observed in the TCR and/or alternative studies using human tissues/proteins (e.g., no or only minimal cytoplasmic binding observed), then conducting a short duration repeat-dose toxicology study (e.g., 3 week) in a single species should be sufficient. Although rats have typically been used in this scenario, sponsors can select the species of their choice with justification. Alternatively, if the mAbs bind to human tissues in the TCR study, sponsors should evaluate mAb binding to tissues from the nonclinical species to be used for toxicology testing. As stated in ICH S6(R1), evaluating select animal tissues can also provide information on the extrapolation of toxicity observed. Sponsors should conduct a TCR study using select tissues from several candidate species and include animal tissues that correspond to those where human tissue binding was observed. Typically, sponsors can select a single species for toxicology testing in this scenario. Although sponsors can select any animal species that demonstrates similar binding to that seen in human tissues, FDA strongly recommends that sponsors discuss species selection with the Agency to facilitate a final determination before initiating the toxicology study. The amount of clinical concern of any off-target human tissue/protein binding is determined on a case-by-case basis. When binding of potential clinical concern is observed (e.g., cell membrane binding), the Agency may recommend additional studies to help inform the potential clinical relevance of the findings.

The design of the repeat-dose toxicology study should follow existing guidance found in ICH S6(R1). For rabies mAbs, sponsors should consider the following:

- A good laboratory practice compliant repeat-dose toxicology study of at least 3 weeks in duration (i.e., 3 weeks of treatment) that includes all standard toxicity endpoints including toxicokinetic analysis is recommended.

- Including a recovery group with a treatment-free period of approximately 5 half-lives following the last mAb administration is recommended.

- The route of administration in the toxicology studies should be the same as that planned for clinical trials in healthy subjects, typically intramuscular.

- Dose selection should be justified according to ICH S6(R1) (i.e., the high dose should provide product exposure approximately 10 times greater than the maximal anticipated clinical exposure).

- The same ratio of rabies mAbs selected for clinical administration should typically be administered in the toxicology study.

- The drug substance or substances used in the toxicology study (i.e., toxicology lot material) should be sufficiently representative of the good manufacturing practice-grade clinical material.

- The intended clinical formulation should be administered in the toxicology study.

- As discussed in ICH S6(R1), measurement of anti-drug antibodies should be conducted as specified. Sponsors should collect appropriate samples during the study (e.g., at the end of both the treatment and the recovery periods) for possible anti-drug antibody analysis to help interpret the toxicology study results.

- Local tolerance assessments should be included as part of the repeat-dose toxicology study.

Chronic repeat-dose, genotoxicity, and carcinogenicity studies are not necessary. To inform potential reproductive and developmental effects, sponsors should conduct a TCR study using human fetal tissues or studies using alternative protein interaction technologies, with appropriate justification. If no specific concerns are identified in the repeat-dose toxicology and TCR studies, developmental and reproductive toxicology studies are not necessary.

ICH S6(R1) states that, when animal models of disease are used to evaluate proof of principle, safety assessments can be included in the evaluation to provide information on potential target-associated safety aspects. Thus, FDA encourages sponsors to collect safety information of rabies mAbs in the animal challenge studies, as feasible.

2. Chemistry, Manufacturing, and Controls Considerations

Sponsors should develop cocktails of at least two monoclonal antibodies that recognize distinct, nonoverlapping conserved epitopes of rabies virus glycoprotein. All mAbs in the cocktail should be broadly neutralizing against rabies virus strains from multiple animal species and from multiple locations (see section III. A. 1. a.). Combining the individual mAbs to make the cocktail may occur either at the formulated drug substance step in manufacturing or during drug product manufacturing.

a. Candidate selection

During the candidate selection stage of development, FDA recommends that sponsors assess the variable (V) region amino acid sequences of the mAb candidates for potential sites of posttranslational modifications that could affect binding to the antigen. Such posttranslational modifications include but are not limited to deamidation, oxidation, V-region glycosylation or glycation. If any final candidates have amino acid residues prone to a posttranslational modification that could result in reduced potency of the product, these primary amino acid sequences should be engineered out of the sequence, provided that the amino acid is not crucial for binding specificity. If the specific amino acid residue is crucial for activity of the mAb, formulation and forced degradation studies should be performed early in development to

638 determine levels of the posttranslational modification that may be present without a reduction in
639 potency.
640
641 b. Control strategy: potency assays
642
643 Potency for individual mAb drug substances and the mAb cocktail (either formulated drug
644 substance or drug product) typically include a Binding enzyme-linked immunosorbent assay
645 (ELISA) and a rapid fluorescent focus inhibition test (RFFIT). Potency results for the Binding
646 ELISAs are reported as a percentage of the reference standard. Potency results for the RFFIT
647 assay are typically reported as IU per mL. For the RFFIT assay, the reference standard should be
648 an international standard, such as the WHO International Standard Anti-Rabies Immunoglobulin,
649 also known as SRIG, or the U.S. Standard Rabies Immunoglobulin. Alternatively, an in-house
650 reference standard may be qualified against one of the international reference standards.
651 Sponsors should justify how the RFFIT potency results are reported and the chosen reference
652 standard. Potency of the individual mAbs based on the RFFIT assay should be considered when
653 determining the ratio for combining the mAbs. The advantage of using an international
654 reference standard is that the potency of each mAb can be determined relative to the same
655 standard.
656
657 c. Control strategy: ratio of mAbs in cocktail
658
659 The ratio of the individual mAbs in the cocktail may be based on mass or potency. Each mAb in
660 the cocktail may have different potency in the RFFIT assay, which may be more apparent when
661 using an international reference standard. Sponsors should justify the ratio and develop an assay
662 that can demonstrate lot-to-lot consistency.
663
664 *3. Labeling Considerations*
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666 To support the approval of a mAb cocktail as the passive component of PEP for the prevention
667 of rabies, sponsors should demonstrate that the mAb cocktail has neutralizing activity equal to or
668 superior to HRIG against a breadth of rabies virus strains found in the United States (bat, fox,
669 skunk, and raccoon strains) and from international exposures in returning travelers (primarily
670 dog strains). FDA does not recommend limiting the indication to only a subset of rabies virus
671 strains because the rabies virus strain would not be known at the time PEP is administered, and
672 the species of animal that bites a patient will not necessarily correlate with the lineage of the
673 rabies virus strain (Ma et al. 2018).
674
675 *4. Postmarketing Considerations*
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677 A plan should exist to monitor for rabies deaths as well as safety concerns that may emerge with
678 use of the mAb cocktail in the postmarketing setting. In addition, sponsors should have a plan
679 and infrastructure to surveil new rabies virus strains and assess activity of the mAb cocktail
680 against these new strains, which should be discussed with the Agency during product
681 development.
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