Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biologicals 75 (2022) 1–2

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

An overview of non-clinical safety studies in current Turkish regulations for the development of COVID-19 vaccines

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Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has become a pandemic [1] and the search for an effective vaccine against COVID-19 all over the world resulted in 284 COVID-19 vaccine candidates in development as of August 2021 of which 110 are at clinical trials stage [2].

In order to guide the vaccine development studies including those against SARS-CoV-2, the national regulatory authority of Turkey, Turkish Medicines and Medical Devices Agency (TITCK), has published a table indicating the regulatory authority requirements for the transition of viral vaccine candidates to clinical trials in September 2020, and then revised the table in December 2020 [3]. During that course, TITCK has also published a new guidance document entitled "The Guideline on the Non-Clinical Evaluation of Human Vaccines" in October 2020 which has been revised recently in May 2021 [4]. The table for the requirements for the transition of viral vaccine candidates to clinical trials is a legally non-binding guidance summarizing the non-clinical studies that must be completed prior to clinical studies in humans [3], while the guidelines for the non-clinical evaluation of human vaccines contains general principles regarding the non-clinical studies before and during clinical trials [4].

Both documents [3,4] are based on and almost in complete accordance with the current World Health Organization (WHO) guideline on nonclinical evaluation of vaccines [5], in terms of quality evaluation and, *in vitro* and *in vivo* non-clinical studies. WHO guideline, currently in force, on nonclinical evaluation of vaccines states that, in general, one relevant animal species is sufficient for use in toxicity studies to support initiation of clinical trials and two or more species may only be necessary under some conditions, such as when the mechanism of protection is not well understood or when there are species-specific or strain-specific differences in the pharmacodynamic effects of the vaccine candidate [5]. Although the newly published TITCK regulatory guidelines are in full-agreement with these recommendations, they both state that the repeated-dose toxicity studies should be performed in two mammalian species, at least one of which is a non-rodent [3,4], in contrary to WHO guideline which does not recommend the conduct of the repeated dose toxicity studies in two species as far as the required conditions are met [5].

The only internationally-agreed-upon guidance strongly endorsing the conduct of repeated dose toxicity studies in two mammalian species is the International Council for Harmonisation's (ICH) M3(R2) guideline on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals [6]. However, this recommendation in ICH M3(R2) is mostly valid for conventional drugs and does not include preventive vaccines. As a matter of fact, the recommendations for both the dose administered (the clinical application dose that maximizes the animal's exposure to the vaccine candidate and induces an immune response *vs.* low, medium and high doses) and the number and the frequency of doses (equal to or greater than the recommended number of human doses, usually n+1 *vs.* multiple doses administered on a timely schedule) for the conduct of repeated dose toxicity studies for preventive vaccines recommended in WHO guideline on nonclinical evaluation of vaccines [5] are quite different that those recommended in ICH M3(R2) for the conventional small molecule drugs [6]. Furthermore, ICH M3(R2) rather represents the existing consensus regarding the type and duration of non-clinical safety studies and their timing to support the conduct of human clinical trials and marketing authorization for both small and large molecules [6].

Regulatory requirements for non-clinical studies for the development of protective vaccines were first published in 1997 [7] in the European Medicines Agency's (EMA) Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines guideline, where it was stated that a study on repeated dose toxicity in one animal species is normally requested for vaccines that will require multiple doses in clinical setting [8]. Later, the WHO guideline on nonclinical evaluation of vaccines published in 2005 [5] has been recognized by both EMA and US Food and Drug Administration (FDA).

In July 2010, the global regulatory environment and the actual perspectives from the EMA, FDA, WHO and Japan on regulatory toxicology and risk assessment processes for vaccine development, mainly focusing on preventative vaccines and common issues and current regulatory challenges related to nonclinical toxicity testing were compared and described in more detail than in published guidelines, in a workshop

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https://doi.org/10.1016/j.biologicals.2022.01.006
Received 12 September 2021; Accepted 12 January 2022
Available online 14 January 2022

organized during the XII international Congress of Toxicology held in Barcelona [9]. It was concluded that one relevant species is in general, sufficient to conduct toxicity studies for preventive vaccine products and the two species approach applies to new adjuvant for human vaccines [9], as stated in the EMA guideline on adjuvants in vaccines for human use, published in 2005 [10]. At this point, it should be noted that the two species approach outlined in the 2005 EMA adjuvants' guideline covers only the vaccines containing new adjuvants [10]. On the other hand, in the 2014 WHO guideline on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines, it is stated that use of a single species is generally acceptable, and one properly designed, conducted and interpreted repeated dose toxicity study in one relevant species should be sufficient when no major safety signals are revealed in the study results [11].

Considering the source and production methods of preventive vaccines developed against COVID-19, one might think that they should be considered as biotechnology-derived products and accordingly, two species approach recommended for the biotechnology-derived products in ICH S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals guideline [12] should apply for these vaccines, as well. Nevertheless, conventional bacterial or viral vaccines are not within the scope of the ICH S6 guideline [12]. Furthermore, unlike conventional small molecular drugs, neither vaccines nor the biotechnology-derived products are metabolized by the cytochrome P450 enzymes. Therefore, species relevance for small molecule drugs which is based on a comparison to human metabolite profiling in liver microsomes and/or hepatocytes across a number of test species does not apply for vaccines, so that, there is no need for conducting the non-clinical safety studies in both a rodent and a non-rodent to assure that major human metabolites and the parent molecule are present and, therefore, qualified by at least one or both of species studied. Instead, for both biologics and vaccines, pharmacological activity is the main determinant of species relevance forming the basis of the non-clinical toxicology program and associated regulatory strategy [13–16]. In accordance, the use of one species for all general toxicity studies for biologics is justified when there is only one relevant species, i.e., the biologic candidate is pharmacologically active in only one species [7,12]. Similarly, one relevant animal species is generally sufficient for the toxicology program of the candidate vaccines provided that the relevance of the animal species is based on the immunogenicity or efficacy of the vaccine in the selected species, by fulfilling the required criteria such as the demonstration of immune response following immunisation (humoral and/or cellular) that is similar to the expected response in humans after vaccination, a similar immunological effect to any adjuvant used in the product, and susceptibility of selected species to the pathogen, reflecting the course of infection in man [7,17].

In conclusion, compliance with global guidances decreases interregional differences in nonclinical safety requirements, promotes the timely conduct of clinical trials, decreases overall development costs, and reduces animal use according to the 3Rs: initiative of reduce, refine, and replace [14,18,19]. Despite almost complete accordance with the current WHO guideline [5], newly published TITCK guidelines for non-clinical evaluation of vaccines clearly state that repeated dose toxicity studies should be conducted in two species, although a repeated dose toxicity study performed in a single relevant species, with fulfillment of the conditions specified in both TITCK and WHO guidelines, would be sufficient for the conduct of clinical phase studies without the need for a second repeat dose toxicity study in an additional species. Besides that, since there is no explanation as to whether the word should used in the statement indicates an absolute requirement or a recommendation, it may easily be perceived as an obligation by the national vaccine developers and therefore, may encumber them in the global competition for the development of effective and safe vaccines against COVID-19 with delaying the timely conduct of clinical trials and increasing the overall development costs. Furthermore, it may pose additional problems since it is inconsistent with the 3R principles. Therefore, it should be removed from the current TITCK guidelines for non-clinical evaluation of vaccines.

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