



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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Development of COVID-19 therapies: Nonclinical testing considerations

Paul Baldrick

Clinical Development & Commercialisation Services, Labcorp Drug Development Inc. (formerly Covance), Harrogate, North Yorkshire, HG3 1PY, United Kingdom

ARTICLE INFO

Handling Editor: Dr. Martin Van den berg

Keywords:
 COVID-19
 SARS-CoV-2
 Vaccine
 Monoclonal antibody
 Small molecule
 Nonclinical
 Toxicity

ABSTRACT

Therapies have been developed in the last couple of years to allow vaccination against, or treatment of patients with, COVID-19 using pathways such as Emergency Use Authorization (EUA) in the USA and Conditional Marketing Authorization (CMA) in the EU and UK. However, nonclinical studies were performed to allow such authorization and these were reviewed for 6 vaccines, 7 biological (monoclonal antibodies [mAbs]) and 4 small molecule therapies to examine whether the number and types of studies normally needed for regulatory agency authorization have been reduced. Results showed that the short answer is generally no. Thus, a battery of immunogenicity/efficacy or related pharmacology/biological activity studies showing utility against SARS-CoV-2 were performed as well as general toxicity studies across all 3 compound classes along with pharmacokinetic studies for mAbs and small molecules and, reproduction toxicity testing for vaccines and small molecules; additionally, genotoxicity testing occurred for small molecules. What was different from conventional, lengthy drug development, was that for vaccines and small molecules, leverage to existing platform technology or data available for other development programs, respectively, occurred. Recognition that mAbs can target the spike protein leading to neutralization allowed rapid development into clinical candidates.

1. Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2 virus). Despite being a completely new disease in 2019, a number of therapies have been developed in the last couple of years to allow vaccination against, or treatment of patients with, COVID-19. Although specifically for treatment against the SARS-CoV-2 virus, available vaccines were developed from knowledge of existing platforms used for other vaccine development (whether successful or not) allowing for rapid assessment of nonclinical safety and clinical efficacy. For biologicals such as monoclonal antibodies (mAbs), development to allow targeting against the spike protein receptor binding domain (RBD) has involved more investigation of nonclinical efficacy and safety as well as clinical efficacy in the treatment of SARS-CoV-2. For available small chemically synthesized molecules, assessment has largely involved leveraging from nonclinical information generated in investigating other indications or from established use of marketed drugs with demonstration of clinical efficacy in the treatment of SARS-CoV-2. This paper examines the published nonclinical packages of studies across vaccine, mAb and small molecule drugs currently approved for use in the United States of America (USA), European Union (EU) and the United Kingdom (UK) (these regions were selected due to accessibility of published

information), with the goal of analyzing whether the number and types of studies normally needed for regulatory agency authorization for patient use have been reduced. It should be noted that although the examined vaccines and drugs have been authorized for use, full marketing approval has not been granted. In the USA, Emergency Use Authorization (EUA) has occurred as the Food and Drug Administration (FDA) “*may authorize unapproved medical products or unapproved uses of approved medical products to be used in an emergency to diagnose, treat, or prevent serious or life-threatening diseases or conditions ... when certain criteria are met, including there are no adequate, approved, and available alternatives*” (FDA, 2022a). Similarly, in the EU, Conditional Marketing Authorization (CMA) has occurred with the European Medicines Agency (EMA) indicating that “*In the interest of public health, applicants may be granted a conditional marketing authorization for such medicines on less comprehensive clinical data than normally required, where the benefit of immediate availability of the medicine outweighs the risk inherent in the fact that additional data are still required*” (EMA, 2022a). In the UK, use of CMA has also occurred, with the Medicines and Healthcare products Regulatory Agency (MHRA) highlighting that “*The scheme has the same eligibility criteria as the EU scheme and is intended for medicinal products that fulfill an unmet medical need. Examples would be for serious and life-threatening diseases where no satisfactory treatment methods are available or where the product offers a major therapeutic advantage*” MHRA

E-mail address: paul.baldrick@covance.com.

<https://doi.org/10.1016/j.yrtph.2022.105189>

Received 21 March 2022; Received in revised form 8 May 2022; Accepted 17 May 2022

Available online 21 May 2022

0273-2300/Crown Copyright © 2022 Published by Elsevier Inc. All rights reserved.

(2020).

2. Materials and methods

Currently available vaccines to allow vaccination against COVID-19 and mAbs or small molecules to allow treatment of patients with COVID-19 were determined as given in Tables 1–3. Website searching involved use of the term “authorized products for COVID-19” in the USA, EMA and UK. Furthermore, using relevant websites (as given in the Reference section), nonclinical packages of studies supporting their authorization were reviewed and presented in these Tables. The study types were then compared to the testing expectations as given in regulatory guidance.

Regulatory guidance is available to support the development of new vaccines, biologicals such as mAbs and small molecules and is discussed below.

2.1. Vaccines

Nonclinical testing expectations for vaccines include appropriate pharmacodynamic studies to assess immunogenicity in a relevant species and a (usually repeat dose) general toxicity testing (usually in one species and commonly in rat or rabbit which has been shown to be immunologically relevant by raising antibodies to the vaccine) with the number of doses administered equal to or one more (N+1 rule) than the doses proposed in humans (WHO, 2005). Further testing considerations are for local tolerance (injection site reactivity), although commonly assessed as part of the repeat dose toxicity study nowadays and reproduction toxicity testing if the target population for the vaccine includes pregnant women and women of childbearing potential. As per regulatory guidance, the latter study often takes the form of use of one species that develops an immune response to vaccine antigen, with dosing prior to mating and throughout pregnancy using episodic doses and fetal examination should occur after Caesarian sectioning as well as birth to weaning evaluation of pups after littering (FDA, 2006). For adjuvanted vaccines (to enhance the antigenic immune response), additional testing requirements can occur (WHO, 2005; WHO, 2013). Thus, for adjuvants in general, additional studies will involve inclusion of adjuvant and full vaccine in immunogenicity testing to demonstrate the utility of its inclusion, while for novel adjuvants, stand-alone adjuvant groups in general and/or reproduction toxicity testing or adjuvant only toxicity studies need to occur as does a battery of genotoxicity testing with the adjuvant. Kinetic evaluation of the adjuvant including biodistribution is also needed. Recently, FDA has issued guidance specifically around development of vaccines to prevent COVID-19 (FDA, 2020a). This guidance largely follows established vaccine testing requirements in that “For a COVID-19 vaccine candidate consisting of a novel product type and for which no prior nonclinical and clinical data are available, nonclinical safety studies will be required prior to proceeding to FIH clinical trials” as follows:

- Efficacy: “The functional activity of immune responses should be evaluated *in vitro* in neutralization assays using either wild-type virus or pseudovirion virus” and “Immunogenicity studies in animal models responsive to the selected COVID-19 vaccine antigen should be conducted to evaluate the immunologic properties of the COVID-19 vaccine candidate”
- Biodistribution: “studies in an animal species should be considered if the vaccine construct is novel in nature and there are no existing biodistribution data from the platform technology”
- Toxicology: “studies should be completed and analysed prior to initiation of FIH clinical trials” and “Data from toxicity studies may be submitted as unaudited final draft toxicologic reports to accelerate proceeding to FIH clinical trials with COVID-19 vaccine candidates. The final, fully quality-assured reports should be available to FDA within 120 days of the start of the FIH clinical trial”

Table 1

Nonclinical testing package for vaccines used against COVID-19.

| Vaccine and use | Nonclinical studies | Reference |
|---|---|--|
| COVID-19 Vaccine AstraZeneca (ChAdOx1-S [recombinant]) or Vaxzevria in UK) is a recombinant replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 spike glycoprotein and containing $\geq 2.5 \times 10^8$ infectious units in a formulation of established excipients given as a 0.5 mL IM dose | <ul style="list-style-type: none"> • <i>In vitro</i> functional activity and examination of immunogenicity in mice and pigs as well as efficacy/immunogenicity testing ferrets and in rhesus monkeys (challenged with SARS-CoV-2) occurred; no evidence for concern for VAERD was seen • Safety pharmacology testing involved a cardiovascular and respiratory IM study in mice, while examination of CNS function was included in the repeat-dose toxicity study in mice; no effects were seen • Various biodistribution studies (including examination for viral shedding) in mice with the same or similar vector platform (and/or another viral insert) and a single dose IM biodistribution study in mice with COVID-19 Vaccine AstraZeneca (ongoing at time of initial authorization) • Toxicity testing comprised 3 GLP studies in mice with 2 IM doses, 14 days apart with a 13-day recovery period using the same or similar vector platform (and/or another viral insert) which showed low toxicity, with no other relevant effects than those related to a normal immune response and a GLP study in mice using 3 IM doses (control and one dose level of COVID-19 Vaccine AstraZeneca), 2 weeks apart with a 28-day recovery period. Findings were non-adverse and related to inflammatory reaction to the vaccine (slightly higher body temperature as well as changes in a few hematology and plasma chemistry parameters; raised spleen weights and dose site inflammation were seen); antibodies against the spike-glycoprotein were raised • Preliminary and main development and reproduction toxicity studies in mice with IM doses given 13 days prior to pairing and again on GD 6 or on GD 6 and again on GD 15 with one dose level and controls showed no adverse findings (the main study in mice was ongoing at time of initial authorization); an immune response was demonstrated in dams and seropositivity was measured in fetuses and pups | EMA (2021c); MHRA (2021a) |
| COVID-19 Vaccine Janssen (Ad26.COV2.S) is a monovalent, recombinant, replication-incompetent adenovirus type 26 | <ul style="list-style-type: none"> • <i>In vitro</i> functional activity and examination of immunogenicity in mice and New Zealand White rabbits occurred as well as efficacy/ | EMA (2021d); FDA (2021a); MHRA (2021b) |

(continued on next page)

Table 1 (continued)

| Vaccine and use | Nonclinical studies | Reference |
|---|---|---|
| <p>vectored vaccine encoding the SARS-CoV-2 spike protein and containing not less than 8.92 log₁₀ infectious units in a formulation of established excipients given as a 0.5 mL IM dose</p> | <p>immunogenicity testing in Syrian golden hamsters and rhesus monkeys (challenged with SARS-CoV-2); risk for VAERD was considered low based on measurement of Th1 skewing of the immune response confirmed in mice, rabbits and monkeys and from assessments of lung histopathology in hamster and monkeys</p> <ul style="list-style-type: none"> • Biodistribution was examined in 2 IM studies in rabbits with Ad26 vector vaccines with other antigens • Toxicity testing was performed in a GLP study in rabbits (shown to be a relevant species by eliciting an immune response to antigen contained in vector) injected IM at one dose level on 3 occasions with a 2-week interval period followed up with a 3-week recovery period. Findings included transient inflammation consistent with an immunologic response to vaccination (including increases in plasma proteins as well as white blood cell counts increased lymphoid cellularity of germinal centers in lymph nodes and spleen), which were all partially or completely reversible after recovery period; findings were considered mild transient effects as expected from a local/general inflammatory reaction subsequent to vaccination • In a combined embryo-fetal and pre- and postnatal GLP study, rabbits were given a one dose level IM injection 7 days prior to mating followed by 2 vaccinations on GD 6 and GD 20 with a subgroup of females necropsied on GD29 for uterine and fetal examination and a subgroup allowed to give birth for offspring survival and development evaluation through to lactation day 28. There were no study findings confirming evidence of no impaired female fertility and or harmful effects with respect to reproductive toxicity; parent animals and fetuses showed SARS-CoV-2.S protein-specific antibody titers • Spike protein expression in a mammalian cell population <i>in vitro</i> was demonstrated. Pharmacology studies comprised immunogenicity testing in mice and rats and efficacy/immunogenicity testing in rhesus monkeys (after infectious SARS-CoV-2 challenge); no evidence of | <p>EMA (2021e); FDA (2021b); MHRA (2021c)</p> |
| <p>COVID-19 Vaccine, mRNA (Comirnaty in US and EU or BNT162b2 in UK) contains 30 µg of nucleoside-modified messenger RNA (mRNA) encoding the viral spike glycoprotein of SARS-CoV-2 that is formulated in 4 lipids (lipid nanoparticles</p> | | |

Table 1 (continued)

| Vaccine and use | Nonclinical studies | Reference |
|---|--|-----------|
| <p>[LNP]) given as a 0.3 mL IM dose; 2 of the lipids (ALC-0315 and ALC-0159) were considered novel excipients</p> | <p>VAERD was seen in the latter study</p> <ul style="list-style-type: none"> • Biodistribution testing occurred using expressing RNA as a surrogate reporter in mice (IV) and rats (IM). In addition, the biodistribution and metabolism of ALC-0315 and ALC-0159 occurred and in an IV study in rats following administration of expressing RNA encapsulated in LNPs made with radio-labeled lipid markers. The metabolism of ALC-0315 and ALC-0159 was also evaluated <i>in vitro</i> using blood, liver microsomes, S9 fractions and hepatocytes • Repeat-dose GLP toxicity studies with COVID-19 Vaccine, mRNA at the clinical dose or a related vaccine (different codon optimization) administered by IM injection to rats (shown to be immunologically relevant from generation of SARS-CoV-2 antibodies) once every week for a total of 3 doses over 17 days with 3 weeks of recovery was tolerated without evidence of systemic toxicity. Key (reversible) findings were site edema and erythema/histopathological changes plus increases in white blood cells and acute phase reactants and decreased albumin:globulin ratios and enlarged spleen and/or draining and inguinal lymph nodes which is considered an inflammatory response plus enlarged liver and portal vacuolation; the latter finding may be linked to LNP, primarily ALC-0315 accumulation in liver • In a combined fertility and developmental reproduction toxicity study, IM administration of COVID-19 Vaccine, mRNA occurred to female rats 21 and 14 days before the start of mating and on GD9 and GD20 at the human clinical dose; one subgroup was terminated at GD21 and another (litter) group was terminated at postnatal day 21. Other than some changes in body weight and food consumption and effects localized to the injection site, there were no effects on mating performance, fertility or any ovarian or uterine parameters nor on embryo-fetal or postnatal survival, growth or development in the offspring. SARS-CoV-2 neutralizing antibody titers were measured in females, fetuses and offspring | |

(continued on next page)

Table 1 (continued)

| Vaccine and use | Nonclinical studies | Reference |
|--|--|---|
| COVID-19 Vaccine Moderna (Spikevax in US) is a single-stranded, 5'-capped messenger RNA (mRNA) encoding the pre-fusion stabilized spike glycoprotein of SARS-CoV-2 that is formulated in lipids (LNP) given as a 0.5 mL IM dose; the LNP constituent SM-102 was considered a novel excipient | <ul style="list-style-type: none"> Immunogenicity evaluation (innate immune system) following IM dosing occurred in mice with LNP-formulated luciferase RNA with chemokine and cytokine measurement and the same assessment using human peripheral blood monocytes <i>In vitro</i> functional activity and pharmacology studies which comprised immunogenicity and efficacy (with challenge from the SARS-CoV-2 virus) testing in mice, Syrian golden hamsters and rhesus monkeys; assessment for potential risk for VAERD from examination of T helper responses showed no concerns Biodistribution with a related vaccine construct was examined in rats following IM dosing. Although the novel excipient SM-102 was not examined, biodistribution testing for the related SM-86 analogue occurred Toxicity testing comprised a limited non-GLP study with IM dosing in rats (using 3 dose levels, given 3 weeks apart) with COVID-19 Vaccine Moderna (with antibodies to the spike protein demonstrated) plus 6 GLP studies with related LNP-mRNA compounds with 3 or 4 doses given by IM injection to rats over 4 or 6 weeks with a 2-week recovery period. Many of the reversible findings were related to an inflammatory response and/or resulting stress following LNP-mRNA administration (although increased eosinophil count, fibrinogen and activated partial thromboplastin time were considered potentially clinically relevant) A GLP reproductive and developmental toxicology study was performed in female rats at the clinical dose with IM dosing twice before mating and twice during mating (28 and 14 days prior to mating and on GD1 and GD13). No effects on female fertility, embryo-fetal or post-natal survival, growth or development in F1 offspring occurred (non-adverse effects were limited to an increase in number of fetuses with common skeletal variations of 1 or more rib nodules and 1 or more wavy ribs, with no effect on viability and growth of F1 generation pups); a SARS-CoV-2 antibody response was in dams, fetuses and offspring Genotoxicity testing included a bacterial reverse mutation | <p>EMA (2021f); FDA (2021c); MHRA (2021d)</p> |

Table 1 (continued)

| Vaccine and use | Nonclinical studies | Reference |
|---|---|--------------------|
| Nuvaxovid is a full-length SARS-CoV-2 recombinant spike protein (5 mg) formulated adjuvanted with Matrix-M (containing Fraction-A and Fraction-C of Quillaja saponaria Molina extract) in a formulation of known excipients given as a 0.5 mL IM dose | <p>test and <i>in vitro</i> micronucleus test with SM-102 which showed no genotoxic activity, a single dose study of NPI luciferase mRNA in SM-102-containing LNPs to rats which showed no dose-dependent change in polychromatic erythrocyte count. Increases in micronucleated erythrocytes were seen in a micronucleus study in rat after IV dosing with a related vaccine construct but proposed non-genotoxic effects causing this finding included hyperthermia, disturbance of erythropoiesis and increase and inflammation of spleen, which could affect clearance of micronucleated cells from blood</p> <ul style="list-style-type: none"> Studies in mice were performed to optimize the protein construct based upon the confirmation of the protein and the involvement of an adjuvant with further examination of immunogenicity in baboons as well as immunogenicity/efficacy studies in hamsters, cynomolgus monkeys and rhesus monkeys (following SARS-CoV-2 infection) [monkey studies ongoing at time of initial authorization]; no evidence of VAERD following exposure to SARS-CoV-2 virus was seen Biodistribution study in mice (specially to evaluate the Matrix-M1 adjuvant) was in planning at time of initial authorization A repeat-dose GLP-compliant study with Nuvaxovid in rabbits was performed, with or without the adjuvant Matrix-M1 given IM on 4 occasions (Day 1, 8, 15 and 36). The presence of Matrix-M1 adjuvant significantly enhanced anti-spike IgG responses. Findings consistent with an immune stimulation occurred including increased plasma proteins and inflammation at the injection sites which recovery except for the latter reactions. In addition, 6 GLP-compliant repeated dose toxicity studies in rats and rabbits with other viral glycoproteins in combination with Matrix-M1, or Matrix-M1 alone, showed findings of local injection site inflammation, reversible enlargement of the lymph nodes draining the injection sites and chemical markers of inflammation, which were generally reversible A GLP developmental and reproduction toxicity study in | <p>EMA (2022b)</p> |

(continued on next page)

Table 1 (continued)

| Vaccine and use | Nonclinical studies | Reference |
|--|--|--------------|
| COVID-19 Vaccine Valneva consists of inactivated whole virus particles of SARS-CoV-2 (with no less than 25 Antigen Units) adjuvanted with CpG 1018 in combination with aluminium hydroxide given as a 0.5 mL IM dose | <p>rats with or without the adjuvant Matrix-M1 (administered 27 and 13 days prior to cohabitation and on GD 7 and GD15) (which was preceded by a pilot study) showed no adverse findings; dams, fetuses and offspring all showed anti-spike IgG titers</p> <ul style="list-style-type: none"> • Testing of the adjuvant Matrix-M1 in GLP-compliant bacterial reverse mutation and mammalian cell micronucleus assays confirmed the absence of any indication for a genotoxic potential • Published details currently limited • In toxicity testing, IM administration on 3 occasions at 2 weekly intervals to rats was well tolerated. All observations were considered physiological or immunological responses to the vaccine • Testing in a reproductive toxicity study in female rats did not affect reproductive parameters, delivery or fetal development. The vaccine was administered by IM injection twice prior to mating and on GD6 | MHRA (2022a) |

IM intramuscular; GLP Good Laboratory Practice; VAERD vaccine-associated enhanced respiratory disease; GD Gestation day.

- Reproduction toxicology: “prior to enrolling pregnant women and women of childbearing potential who are not actively avoiding pregnancy in clinical trials, sponsors conduct developmental and reproductive toxicity (DART) studies with their respective COVID-19 vaccine candidate”
- Vaccine-associated Enhanced Respiratory Disease: the FDA guidance also discusses the need to evaluate for a theoretical risk for COVID-19 vaccine-associated enhanced respiratory disease (VAERD) and “To support proceeding to FIH clinical trials, sponsors should conduct studies characterizing the vaccine-induced immune response in animal models evaluating immune markers of potential ERD outcomes. These should include assessments of functional immune responses (e.g., neutralizing antibody) versus total antibody responses and Th1/Th2 balance in animals vaccinated with clinically relevant doses of the COVID-19 vaccine candidate”

For quicker development of vaccines, recent guidance endorses use of already established platform technology as given in FDA’s “Development and Licensure of Vaccines to Prevent COVID-19” (FDA, 2020). Platform technology is one that has standardised components which are consistent across target vaccines such that the only change is in antigen or nucleic acid sequence for antigen expression (Bennet et al., 2020). The FDA guidance states that “it may not be necessary to perform nonclinical safety studies prior to FIH clinical trials if the COVID-19 vaccine candidate is made using a platform technology utilized to manufacture a licensed vaccine or other previously studied investigational vaccines and is sufficiently characterized, it may be possible to use toxicology data (e.g., data from repeat dose toxicity studies, biodistribution studies) and clinical data accrued with other products using the same platform to support FIH clinical trials for that COVID-19 vaccine candidate. Vaccine manufacturers should summarize the findings and provide a rationale if considering using these data in lieu of performing nonclinical safety studies”. However, it has

Table 2

Nonclinical testing package for mAbs used for treatment of COVID-19.

| mAb and use | Nonclinical studies | Reference |
|---|--|--|
| Casirivimab/imdevimab (REGEN-COV in US and Ronapreve in EU) are recombinant human IgG1 mAbs that target the RBD of the spike protein of SARS-CoV-2 administered together as a single IV infusion over a minimum of 60 min | <ul style="list-style-type: none"> • Casirivimab and imdevimab were assessed in studies of epitope mapping, binding, neutralization (including alpha and beta variants), effector function, resistance and ADE of infection. <i>In vivo</i> activity was assessed in hamster and rhesus macaque models of SARS-CoV-2 infection • Casirivimab and imdevimab were evaluated either alone or administered together in single dose PK work and in a GLP 4-week repeat-dose toxicology study in cynomolgus monkeys with an 8-week recovery using both IV and SC dosing. No adverse findings were observed (minor, non-adverse liver enzyme changes were observed with no histopathology correlates) • GLP tissue cross-reactivity studies were conducted in both normal adult and select fetal human and cynomolgus monkey tissues; no binding of clinical concern was observed with either casirivimab or imdevimab in either species | EMA (2021g); FDA (2020c); MHRA (2021e) |
| Tixagevimab/cilgavimabare (Evusheld) are neutralizing IgG1 mAbs that bind to distinct, non-overlapping epitopes within RBD of the spike protein of SARS-CoV-2 administered as 2 separate consecutive IM injections | <ul style="list-style-type: none"> • Tixagevimab and cilgavimabare were assessed in binding, anti-viral and neutralizing (including alpha, beta, gamma and delta variants [plus omicron variant to support UK authorization]) assays, in Syrian hamster plus rhesus monkey models of SARS-CoV-2 infection and a cynomolgus monkey model of SARS-CoV-2 challenge plus cellular and humoral immune responses testing in mice and cynomolgus monkeys. Assessment included examination for ADCC, ADCP, ADNKA and ADCD activity; the potential for ADE of infection was examined cellular and as part of the monkey model • In a toxicology study in cynomolgus monkeys, tixagevimab and cilgavimab had no adverse effects when administered via IM injection • In tissue cross-reactivity studies with tixagevimab and cilgavimab using human adult and fetal tissues, no binding of clinical concern was detected | FDA (2021d); MHRA (2022b) |
| Bamlanivimab/etesevimab are recombinant neutralizing human IgG1κ mAbs to the spike protein of SARS-CoV-2 (bind to | <ul style="list-style-type: none"> • Bamlanivimab and etesevimab were assessed in studies of epitope mapping, binding, neutralization (including alpha and beta | EMA (2021h); FDA (2021e) |

(continued on next page)

Table 2 (continued)

| mAb and use | Nonclinical studies | Reference |
|---|--|-----------------------------|
| different but overlapping epitopes in the RBD) administered together as a single IV infusion | <p>variants, with gamma variant ongoing at time of authorization), effector function, resistance and ADE of infection (cell culture and in an African green monkey model of SARS-CoV-2 infection). Antiviral activity was assessed for both mAbs in a rhesus macaque prophylaxis model and as a treatment with etesevimab only</p> <ul style="list-style-type: none"> • Combined toxicology work was not performed but a 3-week rat study with bamlanivimab and a 3-week cynomolgus monkey study with etesevimab showed no adverse effects when administered IV. Non-adverse increases in neutrophils were observed in rats dosed with bamlanivimab • In tissue cross reactivity studies using human adult and fetal tissues as well as cynomolgus monkey tissue, no binding of clinical concern was detected for bamlanivimab or etesevimab | |
| Sotrovimab (Xevudy in EU) is a human IgG1κ mAb targeting the spike protein RBD of SARS-CoV-2 administered as an IV infusion over 30 min | <ul style="list-style-type: none"> • Virology evaluation included epitope mapping, binding and neutralization (including alpha, beta and gamma variants) assays, effector function studies Fc-dependent mechanisms of action, NK-cell mediated killing and monocyte-mediated phagocytosis and ADE of infection examination in the SARS-CoV-2 infected Syrian golden hamster model • Sotrovimab was evaluated in cynomolgus monkeys in a single dose IV PK study, a single dose IV radiolabeled material study (including lung and other tissues of respiratory tract distribution) and a GLP 2-week repeat dose toxicology study with a 105-day recovery period using IV dosing. No adverse, drug-related findings were observed and non-adverse findings included injection site reactions, a decrease in lymphocytes in male animals that correlated with a decrease in thymus weight and thymic involution and an increase in heart weights in females without correlative gross or histopathology findings and presence of anti-drug antibodies • GLP tissue cross-reactivity studies were conducted using adult human and cynomolgus monkey tissues; no binding of clinical concern was observed | EMA (2021i); FDA (2021f) |

Table 2 (continued)

| mAb and use | Nonclinical studies | Reference |
|--|---|-------------|
| Regdanvimab (Regkirona) is a recombinant human IgG1 mAb that binds to the RBD of the spike protein of SARS-CoV-2 and is administered as a single IV infusion | <ul style="list-style-type: none"> • A non-GLP protein microarray study evaluating potential binding to 66 human embryofetal proteins was conducted. Proteins evaluated were secreted or membrane bound proteins expressed in embryofetal tissue that had little or no expression in adult tissues. No binding of clinical concern was observed • No injection site reactions were noted following a single IM injection to minipigs • Studies included epitope mapping, binding and neutralizing (including alpha, beta, gamma and delta variants) activity and examination of Fc-mediated function for ADCC and CDC, ADCP, ADNP and NDNKA along with <i>in vivo</i> efficacy testing against SARS-CoV-2 infection in transgenic mice (human angiotensin-converting enzyme 2 [hACE2] expressing), Syrian hamsters, ferrets and rhesus monkeys; ADE was assessed <i>in vitro</i> and in hACE2 transgenic mice • Single IP dose Syrian hamster PK study • Toxicity studies via IV dosing of 2 weeks or 3 weeks with 10-day recovery period duration in cynomolgus monkeys; non-adverse clinical pathology and histopathology findings (sinusoidal increased cell of liver, increased cellularity of bone marrow and thymic atrophy) were seen in one high dose animal in the 2-week study • Tissue cross-reactivity studies were conducted on human (adult, fetal and neonatal) and cynomolgus (adult) tissues with specific positive staining seen in meningeal arachnoid cap cells in brain and spinal cord in tissues | EMA (2021j) |
| Bebtelovimab is a recombinant neutralizing human IgG1κ mAb to the spike protein of SARS-CoV-2 administered as a single IV injection over at least 30 s | <ul style="list-style-type: none"> • Assessed in studies of epitope mapping, binding, neutralization (including alpha, beta, gamma, delta and omicron variants), effector function, resistance and ADE of infection. Bebtelovimab was also assessed for activity in a hamster model of SARS-CoV-2 infection and for ADE in an African green monkey model • Single dose PK studies in rats and cynomolgus monkeys and in a 3-week IV rat toxicity study, bebtelovimab had no adverse effects; a 3-week SC rat study showed non-adverse changes in hematology parameters | FDA (2022d) |

(continued on next page)

Table 2 (continued)

| mAb and use | Nonclinical studies | Reference |
|---|---|--------------------------------|
| Tocilizumab (Actemra) is a recombinant humanized mAb that selectively binds to both soluble and membrane-bound human interleukin (IL)-6 receptors (sIL-6R and mIL-6R) and subsequently inhibits IL-6-mediated signaling through these receptors with a single 60 min IV infusion (or second dose if needed) | <ul style="list-style-type: none"> In tissue cross reactivity studies using human adult and fetal tissues, no binding of clinical concern was detected Package of nonclinical used for marketing of tocilizumab for rheumatoid arthritis indications referenced and included: <ul style="list-style-type: none"> Biological activity/ pharmacology testing including binding to IL-6 receptors and subsequent inhibition and IL-6 neutralization; ADCC and CDC activity was evaluated as was tissue cross reactivity. <i>In vivo</i> testing included a collagen-induced cynomolgus arthritis model, wild type mouse model of amyloidosis and transgenic human IL-6-expressing mouse model PK studies in cynomolgus monkeys Pivotal toxicity work was once-a-week dosing 6-month IV study with 2 months' recovery in cynomolgus monkeys with showed higher dose reversible granulomas in liver and irreversible degeneration of skeletal muscle as major treatment-related toxicities An embryofetal development IV study in cynomolgus monkeys with tocilizumab showed an increase in the incidence of abortion/ embryo-fetal deaths but no evidence of teratogenicity plus no adverse findings were seen in fertility and early embryonic development a peri-postnatal IV studies in mice using a murine analogue of tocilizumab | BLA 125276 (2009); FDA (2021g) |

IV intravenous; IM intramuscular; SC subcutaneous; PK pharmacokinetics; GLP Good Laboratory Practice; ADE antibody dependent enhancement; ADCC antibody-dependent cell-mediated cytotoxicity; ADCP antibody-dependent cellular phagocytosis; ADNKA antibody-dependent natural killer cell activation; ADCD antibody-dependent complement deposition; CDC complement-dependent cytotoxicity; ADNP antibody-dependent neutrophil-mediated phagocytosis.

been pointed out that the reality is that for vaccine candidates that are based on existing platforms, but for which changes have been made to target specific antigens, additional, limited nonclinical studies assessing immunogenicity and safety of specific antigen may be required and submitted in parallel with preliminary clinical trials (Bennet et al., 2020).

2.2. Biological drugs

Nonclinical testing expectations for biological drugs such as mAbs include key studies of primary pharmacology/biological activity *in vitro* and *in vivo*, repeat dose general toxicity and reproductive performance and developmental toxicity (Baldrick, 2017; ICH S6(R1), 2011). Guidance indicates the need for 2 species (rodent and non-rodent) for general

Table 3

Nonclinical testing package for small molecules used for treatment of COVID-19.

| Small molecule and use | Nonclinical studies | Reference |
|--|--|-------------------------|
| Remdesivir (Veklury) is a SARS-CoV-2 nucleotide analogue RNA polymerase inhibitor prodrug (with activity from its nucleoside triphosphate metabolite) administered by IV infusion over 30–120 min for 3 days | In addition to new pharmacology/biological action studies, the package of nonclinical studies used for earlier investigation for other indications were referenced and included: <ul style="list-style-type: none"> <i>In vitro</i> antiviral activity and replication inhibition studies occurred as did resistance profiling in cell culture and in a mouse model. Remdesivir showed antiviral activity in SARS-CoV-2-infected rhesus monkeys. Various cytotoxicity studies were performed Safety pharmacology core battery was performed including hERG channel inhibition potential, cardiovascular assessment in cynomolgus monkeys plus neurological evaluation and respiratory function evaluation in rats; no notable findings were seen Formation to active metabolite demonstrated in various cell types including lung; PK studies were performed in rat and monkey (including level in peripheral blood mononuclear cells), plasma protein binding, plasma stability and blood/plasma concentration studies occurred, <i>in vitro</i> metabolism was examined in hepatocyte while <i>in vivo</i> metabolism, distribution and excretion work occurred in species including rat and monkey using radiolabeled remdesivir Toxicity testing included 2- and 4-week GLP IV studies in rats and cynomolgus monkeys as well as a 7-day IV study in rhesus monkeys and a 7-day IM study in cynomolgus monkeys. In rats, clinical pathology and microscopic findings indicative of kidney injury and/or dysfunction were seen but not in cynomolgus monkeys (non-toxic dose levels were used); 7-day IV study in rhesus monkeys resulted in increased mean urea nitrogen and increased mean creatinine, renal tubular atrophy and basophilia and casts and 7-day IM study in cynomolgus monkeys showed adverse kidney changes. Findings were generally reversible Reproduction toxicity battery of fertility and early embryonic toxicity study in rats, embryo-fetal development study in rats and rabbits and pre- and post-natal development study in rats: main findings were reduction in number of corpora lutea, | EMA (2020); FDA (2022e) |

(continued on next page)

Table 3 (continued)

| Small molecule and use | Nonclinical studies | Reference |
|---|---|--|
| Molnupiravir (Lagevrio in EU and UK) is 5'-isobutyrate prodrug of antiviral ribonucleoside analogue N-hydroxycytidine (NHC) that inhibits SARS-CoV-2 replication by causing accumulation of nucleotide changes in viral RNA and is taken orally every 12 h for 5 days | implantation sites and viable embryos, and lower mean ovary and uterus/cervix/oviduct weights in fertility and early embryonic toxicity study along with maternal toxicity in this and rabbit embryo-fetal development study | |
| | <ul style="list-style-type: none"> Remdesivir was not genotoxic in bacterial mutagenicity, chromosome aberration using human peripheral blood lymphocytes and <i>in vivo</i> rat micronucleus assays | |
| | In addition to new pharmacology/biological action studies, the package of nonclinical studies used for earlier investigation for other indications were referenced and included: <ul style="list-style-type: none"> Antiviral activity in cell culture against variety of viruses with resistance and cross-resistance evaluation including use of a SARS-CoV-2 replicon-based phenotypic assay as well as cytotoxicity and off-target (mainly with NHC) plus anti-SARS-CoV-2 activity in a humanized mouse (implanted subcutaneously with human lung tissue), Syrian hamster and ferret models with molnupiravir Secondary pharmacology panel for off-target effects Safety pharmacology core battery including hERG channel inhibition potential, cardiovascular assessment in dog plus central nervous system (CNS) and respiratory studies in rats; no findings were noted ADME package of studies including drug-drug interaction evaluation Pivotal oral toxicity study of 28 days or 3 months in rat and dogs with molnupiravir; in 28-day dog study, bone marrow changes affecting all hematopoietic cell lines and causing subsequent hematological abnormalities including severe thrombocytopenia was seen and in 3-month rat study, abnormal bone (growth plate) and cartilage formation were noted (not seen in 28-day study giving similar exposure but in older rats) Reproductive toxicity studies included fertility in rats, embryo-fetal development studies in rats and rabbits and a pre- and postnatal development study in rats with molnupiravir; in embryo-fetal development, delayed and incomplete ossification in fetuses was noted along with (in the presence of maternal toxicity) high dose embryo- | EMA (2022c); FDA (2021h); MHRA (2021f) |

Table 3 (continued)

| Small molecule and use | Nonclinical studies | Reference |
|---|---|-----------------------------|
| Paxlovid (nirmatrelvir = SARS-CoV-2 main protease inhibitor co-packaged with already approved ritonavir = HIV-1 protease inhibitor [as a pharmacokinetic enhancer]) given orally twice daily for 5 days | fetal lethality and teratogenicity and reduced fetal growth in rats and reduced fetal body weights [Based on general and reproduction toxicity study findings, molnupiravir was not authorized for use in patients less than 18 years old (juvenile toxicity study in rats was planned to further examine bone/cartilage formation-related findings)] | |
| | <ul style="list-style-type: none"> Molnupiravir and its metabolite NHC were positive for mutagenicity in <i>in vitro</i> Ames assays, molnupiravir gave a negative result in <i>in vitro</i> and <i>in vivo</i> rat micronucleus tests, an equivocal result in a rat Pig-a assay but was negative in transgenic Big Blue® rat assay (Based on the weight of evidence and expert input as well as the short-term use, risk of mutagenicity following treatment was considered to be low; however, a transgenic rodent male germ cell mutagenicity assay is planned) Virology-related studies with nirmatrelvir included binding and anti-viral activity (ritonavir had no activity against SARS-CoV-2), resistance development and cross-resistance using cell cultures as well as protease activity and cytotoxicity measurement. Antiviral activity was assessed in a mouse model of SARS-CoV-2 infection. <i>In vitro</i> off-target testing was performed Core battery safety pharmacology testing including hERG channel inhibition potential, cardiovascular assessment in cynomolgus monkeys plus CNS and respiratory studies in rats (with additional ion channel evaluation and guinea pig isolated Langendorff-perfused heart model and rat isolated ascending aorta tissue studies) with nirmatrelvir: some minor effects were seen in CNS, respiratory and cardiovascular parameters but are monitorable in clinic and had no correlating findings were seen in toxicity testing ADME with nirmatrelvir included single dose PK studies in rats and monkeys, plasma protein binding, liver microsome and hepatocyte studies (and <i>in vivo</i> metabolite examination in rats and monkeys) along with evaluation of excretion in rats and monkeys | EMA (2021k); FDA (2021i) |

(continued on next page)

Table 3 (continued)

| Small molecule and use | Nonclinical studies | Reference |
|--|--|---|
| Baricitinib (Olumiant) is a Janus kinase (JAK) inhibitor (with an action in inhibiting intracellular signaling pathways associated with cytokine receptor activation) with once daily oral use for up to 14 days | <ul style="list-style-type: none"> • Toxicology testing comprised 4-day range-finders plus 2-week and 4-week GLP studies in rats and cynomolgus monkeys with nirmatrelvir. 2-week toxicity studies produced recoverable hematology and coagulation findings in rats and increased fibrinogen in cynomolgus monkeys that had no clinical or microscopic correlations in rats and were therefore not considered adverse; recoverable hepatocellular hypertrophy consistent with microsomal enzyme induction and associated with thyroid follicular cell hypertrophy were seen in rats and considered non-adverse. 4-week studies confirmed a lack of toxicity • Other than some minor bodyweight changes, no reproduction toxicity was seen in studies of fertility and early embryo developmental in rats, embryo and fetal developmental effects in rats and rabbits and pre- and postnatal developmental in rats with nirmatrelvir (embryo-fetal effects were reported in studies with ritonavir but mainly at maternally toxic dose levels and at multiples of use in Paxlovid) • Bacterial reverse mutation, <i>in vitro</i> micronucleus and rat micronucleus assays with nirmatrelvir were all negative <p>The package of nonclinical used for marketing of baricitinib for rheumatoid arthritis were referenced and included:</p> <ul style="list-style-type: none"> • Pharmacology package with inhibition of relevant signaling pathway and cytokine-stimulated cell preparations and <i>in vivo</i> studies (e.g., rat adjuvant arthritis model) as well as secondary pharmacology and a core battery of safety pharmacology studies (hERG assay, cardiovascular function in dogs plus CNS and respiratory studies in rats; minor high dose effects were seen in the latter 2 studies) • Full battery of adsorption, distribution, metabolism and excretion studies • Full battery of toxicity studies including pivotal oral studies of up to 6 months in rats and 9 months in dogs which identified immunosuppressive effects (e.g., decreased peripheral blood leukocytes and lymphoid depletion/atrophy in bone marrow and spleen, lymph nodes) in both species and gastrointestinal tract (inflammation, | <p>FDA (2020d); NDA 207924 (2018)</p> |

Table 3 (continued)

| Small molecule and use | Nonclinical studies | Reference |
|------------------------|---|-----------|
| | <p>infiltrates) and liver (infiltrates/inflammation, bile duct hyperplasia) in dogs. Safety margins were established</p> <ul style="list-style-type: none"> • Baricitinib produced negative results in a battery of genotoxicity assays and was negative in a 2-year carcinogenicity study in rats and a 26-week study in Tg.rasH2 mice • Fertility and early embryonic development study in rats showed fertility was reduced and maintenance of pregnancy was adversely affected. In embryofetal development studies, baricitinib was teratogenic in both rats and rabbits; findings included skeletal malformations such as bent limb bones and rib anomalies. In a pre- and postnatal development study in rats, findings in offspring included decreased survival and body weight, increased incidence of malrotated forelimbs and immune suppression. Safety margins were established | |

IV intravenous; GLP Good Laboratory Practice.

toxicity testing but in the case of mAbs, it often involves use of the nonhuman primate only (as shown to be biologically relevant through sequence homology, species cross-reactivity and target binding, which are not often observed in rodents). Safety pharmacology evaluation (to investigate potential undesirable pharmacological effects from assessment of central nervous, respiratory and cardiovascular system function) is usually incorporated into toxicity studies and a detailed examination for immunogenicity/immunotoxicity (for example, antibody formation, immune system cell population effects or induced antibody response) may also be included. As mAbs are given parenterally, dose site irritation is a consideration but potential effects can be evaluated in toxicity studies obviating the need for separate local tolerance work. Consistent with the protein nature of mAbs, *in vitro* absorption, distribution, metabolism and excretion (ADME) and genotoxicity studies are not performed; a stand-alone pharmacokinetic (PK) study is usually performed in the biologically relevant animal species. Secondary pharmacology assessment (in the form of tissue cross reactivity testing to identify any off-target binding preferably in human tissues), sometimes referred to as “Other toxicity testing”, is also a standard requirement. Reproductive toxicity testing can range from a full battery of fertility, embryo-fetal development and pre/post-natal development testing (using one or more biologically relevant species), although for mAbs testing can be reduced to performance of an “enhanced” pre- and post-natal development design in non-human primates (NHPs), along with a paper risk assessment addressing any other potential reproductive safety concerns. Carcinogenicity bioassays are not necessary for mAbs, although again a paper assessment of any tumorigenic potential may still be needed.

Recently, to assist in early regulatory agency interaction through a pre-Investigational New Drug Application (IND) consultation, FDA have issued a guidance entitled “General Considerations for Pre-IND Meeting Requests for COVID-19 Related Drugs and Biological Products” which endorses use of Coronavirus Treatment Acceleration Program (CTAP) (FDA, 2020b). It is stated that “FDA may exercise some flexibility in the types and amount of data necessary to support drug development for

treatment or prevention of COVID-19, but for proposals to proceed — when involving unapproved drugs, new doses or formulations of an approved drug, or new routes of administration (e.g., inhalation) that have never been administered to humans — typically nonclinical *in vivo* data will be needed to determine the risks of the drugs and to support safe starting doses in humans”. To support the pre-IND meeting request, there needs to be “A summary of the available nonclinical pharmacology and toxicology data” highlighting that “Pivotal nonclinical safety studies should be conducted according to good laboratory practices (GLPs)”. Specifically, to support First-In-Human (FIH) clinical testing for biological products, it is recommended that “A battery of nonclinical studies to support a FIH trial should include assessment of applicable safety pharmacology studies (e.g., cardiovascular, respiratory, and central nervous system assessments) but can be incorporated into the general toxicology study. In general, *in vivo* studies should be conducted, and will include one general toxicology study in a relevant species and a tissue cross-reactivity assay in human tissues when indicated and technically feasible. When indicated, sponsors should consider studies that assess enhanced potential for toxicity in an animal model of infection. The drug product that is used in the definitive pharmacology and toxicology studies should be comparable to the product proposed for the initial clinical studies”. Of interest, there is no requirement for reproduction toxicity testing.

2.3. Small molecules

Nonclinical testing expectations for small molecule drugs include key studies of *in vitro* and *in vivo* primary pharmacology, secondary pharmacology (to evaluate for any signal from non-target interaction in a panel of ion channels, receptors and enzymes), safety pharmacology (“core battery” of studies as mentioned earlier), *in vitro* and *in vivo* ADME, repeat dose (2 species) general toxicity, reproductive performance and developmental toxicity plus genotoxicity (usually a bacterial reverse mutation [Ames] assay and an *in vitro* mammalian cell assay along with a rodent bone marrow micronucleus test) Baldrick (2017); ICH M3(R2) (2009). Generally, carcinogenicity studies are needed in 2 rodent species for drugs with >6 months or frequent intermittent clinical use.

Recently, as mentioned earlier, FDA have issued a guidance entitled “General Considerations for Pre-IND Meeting Requests for COVID-19 Related Drugs and Biological Products” which includes reference to small molecule development (FDA, 2020b). As well as the flexible approach described earlier, it is stated that for small molecule drugs “A battery of nonclinical studies to support a first-in-human (FIH) trial should include assessment of standard safety pharmacology studies (e.g., cardiovascular, respiratory, and central nervous system assessments) but can be incorporated into general toxicology studies. In general, FDA expects a pre-IND meeting request for a small molecule drug to include data from general toxicology studies in two species (at least one nonrodent) and genetic toxicology, including an Ames reverse mutation assay and a second *in vitro* assessment. The drug substance used in the toxicology studies should be identical to that proposed for clinical investigation”. However, contingency is also given by FDA for already approved drugs for use against COVID-19, for example, antivirals, where it is stated that “For approved drugs, reference to FDA-approved labeling may suffice in some cases”, with the need to “cross-reference any other new drug application (NDA), biologics license application (BLA), or IND for the drug”. However, there is the expectation of at least some new data (*in vitro* and/or *in vivo*) to show potential antiviral activity against SARS-CoV-2.

3. Results

The nonclinical testing packages for 6 vaccines used against COVID-19 is shown in Table 1. Testing was consistent with guideline recommendations described earlier and with key evaluation of *in vivo* efficacy, general toxicity and reproduction toxicity. Given the choice of possible models for investigation of immunogenicity and/or efficacy against

SARS-CoV-2 infection, a different use of species occurred for 5 vaccines (data for one vaccine are currently not published). Thus, immunogenicity testing itself tended to occur in mice (usually Balb/c), although 3 vaccines also utilized rats, rabbits or pigs, while immunogenicity/efficacy evaluation used rhesus monkeys in 5 cases, with 3 vaccines also using Syrian golden hamsters and single vaccines testing in ferrets and cynomolgus monkeys as well. Vaccines were evaluated for VAERD with no evidence for an exacerbation of respiratory disease, measured indirectly from Th1 skewing or from lung histopathology in efficacy models. It is unclear why safety pharmacology testing (a cardiovascular and respiratory study in mice) occurred for one vaccine but this may have occurred to satisfy a specific requirement for development of vaccines in Japan, where guidance requires strong justification for not performing stand-alone safety pharmacology studies (Sun et al., 2012). Although not necessarily a feature of vaccine development, some form of bio-distribution evaluation occurred among the vaccines looking at distribution after IM dosing and in 3 cases involved reference to studies with a related platform or vaccine construct. Additionally, specific bio-distribution testing occurred for novel excipient constituents for 2 vaccines and was planned at the time of authorization for the vaccine containing a novel adjuvant. General toxicity testing with the vaccine took the form of a repeat dose study (ranging from IM injection on 2 to 4 occasions, weekly, every 2 weeks or every 3 weeks) in one species (3 vaccines used rats, 2 vaccines used rabbits and one vaccine used mice, which were confirmed as relevant species by generating an antibody response with generation of SARS-CoV-2 antibodies). One dose level (identified as the clinical dose) and control were usually used (although more than one level was used for a couple of the vaccines) and studies had an off-dose recovery period of up to 4 weeks. Reference to other toxicity testing with related platform or vaccine construct occurred for 3 vaccines. All 6 vaccines were well tolerated in toxicity studies but showed a consistent pattern of non-adverse and generally reversible inflammatory reactions to the vaccine, which usually included some or all of - increases in plasma proteins as well as white blood cell counts, raised spleen weights, increased lymphoid cellularity of germinal centers in lymph nodes and spleen and dose site inflammation. Reproduction toxicity testing occurred for all 6 vaccines and assessed fertility, embryo-fetal and post-natal development with use of the same species used for general toxicity studies. Typically, vaccine was IM dosed on one (2 vaccines) or 2 occasions at one dose level (plus control) prior to mating and twice (one vaccine was only dosed once) during gestation (days of dosing differed across the vaccines). No adverse findings were seen and studies reported SARS-CoV-2 neutralizing antibody titers in parent animals, fetuses and offspring. As per regulatory guidance, genotoxicity testing occurred for the novel excipient used in one vaccine, with such testing also performed for the novel adjuvant in another vaccine.

The nonclinical testing package for mAbs used for treatment after COVID-19 is shown in Table 2. All but one of them had no previous authorized use, while one (tocilizumab) already had marketing approval for another indication and reference was made to the existing nonclinical studies (it was therefore excluded for comparison of studies used for authorization of the remaining 6 products). In addition, 3 products involved use of a “cocktail” of 2 mAbs together (binding to different epitopes of the RBD of SARS-CoV-2). Overall, testing was consistent with guideline recommendations described earlier and with key evaluation of *in vitro* and *in vivo* biological activity, general toxicity and tissue cross reactivity studies to support clinical use. Initial testing for the 6 mAbs products included key evaluation of binding, anti-viral and neutralizing assays across some or all of the World Health Organization’s current SARS-CoV-2 “Variants of Concern”, namely alpha (B.1.1.7), beta (B.1.351), gamma (P.1), delta (B.1.617.2) and omicron (B.1.1529) (WHO, 2022), although, based on known variants at the time of development, only the most recently approved mAb (bebtelovimab) included the omicron variant in the neutralizing assay battery (the significance of this situation will be discussed later). The 3 products involving use of 2

mAbs tended to be evaluated either alone or administered together. As for vaccine testing described earlier, *in vivo* biological activity testing against SARS-CoV-2 infection showed a different use of species, with 2 mAb products and one mAb product using hamsters and rhesus monkeys, respectively as the sole test species of SARS-CoV-2 infection. For 3 other mAb products testing involved hamsters and rhesus monkeys, hamsters, rhesus monkeys and cynomolgus monkeys or, hamster, rhesus monkeys, human angiotensin-converting enzyme 2 [hACE2] expressing transgenic mice and ferrets. As part of the biological activity battery, assessment for antibody-dependent enhancement (ADE) of infection occurred. It is reported that data from the study of SARS-CoV and other respiratory viruses suggest that anti-SARS-CoV-2 antibodies could exacerbate COVID-19 through ADE, either by enhanced antibody-mediated virus uptake into Fc gamma receptor IIa (FcγRIIa)-expressing phagocytic cells leading to increased viral infection and replication, or by excessive antibody Fc-mediated effector functions or immune complex formation causing enhanced inflammation and immunopathology (Lee et al., 2020). Both ADE pathways can occur when non-neutralizing antibodies or antibodies at sub-neutralizing levels bind to viral antigens without blocking or clearing infection. ADE can be measured in several ways, including *in vitro* assays, immunopathology or lung pathology. A combination of testing *in vitro* and in the animal models of SARS-CoV-2 infection used for the 6 mAb products was reported (for 2 mAb products, an African green monkey model was also used). Single dose pharmacokinetic (PK) studies occurred for 4 of the 6 mAb products (presumably kinetics for the other 2 mAb products was assessed as part of the general toxicity work). General toxicity testing took the form of a repeat dose study (with dose route reflecting that to be used in the clinic [IV for 5 mAb products and IM for one mAb product] and for up to 4 weeks) in the cynomolgus monkey on all occasions. For the 3 mAb products that involved 2 separate mAbs, approaches of testing of the individual components only or either alone and administered together were used. All of the mAbs were well tolerated with no adverse findings seen. As per regulatory guidance, tissue cross reactivity testing (generally in human adult and fetal tissue) was performed for all the 6 mAb products, with no binding of clinical concern seen.

The nonclinical testing package for the 4 small molecules used for treatment after COVID-19 is shown in Table 3. The pathway to authorization appears to have been different from the vaccines and mAbs described above as the compounds had been in development in the past or had marketing approval for other indications. Reference was made to the existing nonclinical studies as well as performance of new pharmacology/biological activity studies for the 3 antiviral products. The latter studies included antiviral testing using *in vitro* assays as well as administration in mouse (2 products) or rhesus monkey (one product) models of SARS-CoV-2 infection. Remaining nonclinical testing was of a conventional nature for a small molecule and included safety pharmacology, ADME, 2 species general toxicity, reproduction toxicity and genotoxicity studies. Furthermore, a much more extensive package of studies was available for the 4th product, the janus kinase inhibitor baricitinib to support its marketed use for rheumatoid arthritis (due to the chronic nature of this disease, the package included chronic duration toxicity studies and carcinogenicity testing).

4. Discussion and conclusions

As a result of EUA in the US and CMA in the EU/MHRA, a number of vaccines, mAbs and small molecules have recently been authorized for use against COVID-19. The nonclinical data packages supporting authorization have been reviewed.

A first area of discussion is the status for continual use of mAbs for treatment of COVID-19. As shown in Table 2, initial testing included evaluation of binding, anti-viral and neutralizing assays across some or all of the World Health Organization's current SARS-CoV-2 "Variants of Concern". In January 2022, FDA published a press release with revision

to the EUA for 2 of the mAbs products examined in this review, namely the combination products bamlanivimab/etesevimab and casirivimab/imdevimab (FDA, 2022b). It was stated that "Because data show these treatments are highly unlikely to be active against the omicron variant, which is circulating at a very high frequency throughout the United States, these treatments are not authorized for use in any U.S. states, territories, and jurisdictions at this time. In the future, if patients in certain geographic regions are likely to be infected or exposed to a variant that is susceptible to these treatments, then use of these treatments may be authorized in these regions". It can only be assumed that both these mAb products (which were originally not tested against the omicron variant) showed reduced and/or short-acting viral neutralization activity when subsequently tested. Currently, other mAb products examined in this review are still authorized in the US and there has been no announcement from EMA on bamlanivimab/etesevimab and casirivimab/imdevimab use. Although relating to vaccines and not mAbs, the situation in the latter region is also complicated as at an earlier date to the FDA's public statement (early 2021), EMA issued a "Reflection paper on the regulatory requirements for vaccines intended to provide protection against variant strain(s) of SARS-CoV-2" which is applicable where the parent vaccine has been granted marketing authorization (Marketing Authorization Holders [MAHs]) in the EU (EMA, 2021a). This document states that for "There is no requirement to conduct any further *in-vitro* or *in-vivo* nonclinical testing to support the development of variant vaccines. If MAHs choose to conduct such studies they will be viewed as supportive of the clinical data". However, another EMA document "Procedural guidance for variant strain(s) update to vaccines intended for protection against Human coronavirus", issued in late 2021 gives some leeway for further testing (EMA, 2021b). It outlines the process for marketing authorization (a variation) when there is a change in authorized human coronavirus vaccines composition so as to protect against new or multiple variant strain(s) "provided the technological platform of the vaccine remains similar". Such changes include "replacement or addition of a serotype, strain, antigen or coding sequence or combination of serotypes, strains, antigens or coding sequences". Overall, the future will certainly lie in providing robust multi-SARS-CoV-2 variant neutralization activity for mAb candidates. The future may also involve use of bispecific mAbs. As an example, it has been shown that various investigational bispecifics (which cross-linked adjacent spike proteins of the virus), showed strong neutralization activity against various SARS-CoV-2 variants as well as efficacy in a hamster model of SARS-CoV-2 infection (Cho et al., 2021).

The present review showed that a variety of animal species (including mice, hamster, ferrets and various NHP strains) have been used to examine immunogenicity/efficacy against SARS-CoV-2 infection for authorized vaccines and mAb products (as well as to a lesser degree for the authorized small molecules), although the jury is still out on how well findings translate to the human situation. In a review of currently available SARS-CoV-2 respiratory disease animal models, the ability to replicate human symptoms of fever, respiratory distress, mortality, decreased appetite, weight loss, viral replication in airways and pneumonia was compared to various models/species (Bennet et al., 2020). These included hACE2 transgenic mice (had mortality, weight loss, viral replication in airways and pneumonia markers), mouse-adapted SARS-CoV-2 model (had mortality, decreased appetite, weight loss, viral replication in airways and pneumonia markers), hamster (had weight loss, viral replication in airways and pneumonia markers), ferret (had fever, decreased appetite, viral replication in airways and pneumonia (equivocal) markers and NHP (macaque) (had decreased appetite, viral replication in airways and pneumonia markers). Thus, none of the current models replicates the full human disease. Another issue is that even when a model has been developed, supply issues can occur. Thus, although studies showed that hACE2 transgenic mice could mimic the human clinical features after viral infection, this model (due to structural differences between mouse and human ACE2 receptors, which SARS-CoV-2 uses to enter cells, coronaviruses do not infect wild type mice and rats and this transgenic mouse strain was developed to express

the human variant of ACE2), is only available at a few commercial breeders, resulting in limited availability (Genzel et al., 2020). This may be the reason why this model was used for testing in only one of the authorized products reviewed (regdanvimab). Finally, another confounding factor for efficacy (and toxicity) testing is the current shortage of NHPs (particular sexually mature animals) due to the substantial increase in demand for testing of new COVID-19 candidates. FDA have involved themselves by issuing a guidance that strongly supports use of species other than NHPs, if possible and “when scientifically justified”, in general toxicity and reproduction toxicity testing for small molecule and biological product drug development programs (FDA, 2022c).

With regard to general toxicity testing, all 6 vaccines were well tolerated (supporting clinical entry) but showed evidence of an inflammatory response, which was consistent with that known of this type of treatment. Indeed, a review of 30 repeat dose toxicity studies with a variety of vaccines, generally in rat or rabbit and usually by IM injection, all showed signs of enhanced acute and/or chronic inflammation at the dose site compared with that seen in control animals, often accompanied by changes in draining lymph nodes and the spleen (lymphoid hyperplasia and/or increased weight) (Baldrick, 2016). Other associated signs of a response to vaccine dosing were altered clinical pathology parameters (commonly raised blood neutrophil count and altered globulin level). Supporting use in a female clinical population, reproduction toxicity testing with the 6 vaccines showed no adverse effects. Confirming that available regulatory guidelines only give broad recommendations for general toxicity testing, a number of study design differences occurred in the testing packages for the vaccines including species used, dosing occasion and frequency, number of doses and duration of recovery period. Similarly, for reproduction toxicity testing, each vaccine was dosed on different days prior to mating and during gestation. However, these differences did not affect the overall safety assessment. General toxicity testing of the 6 mAb products were well tolerated with no adverse findings seen. The use of healthy monkeys to assess toxicity raises an interesting question as to relevance, as the animals have no viral target. It will be interesting to see if future development of viral targeted therapies such as these utilize healthy animals with no target to bind. Lack of off-target effects was confirmed by data showing no binding of clinical concern in tissue cross reactivity testing. Authorization of the mAbs occurred without the need for reproduction toxicity testing, which as described earlier (FDA, 2020a), is not a regulatory guidance requirement. The 6 mAb products showed no adverse effects in reproductive organs in toxicity testing and a lack of cross-reactivity with reproductive or fetal tissues in the tissue cross-reactivity studies. General toxicity and reproduction toxicity testing as well as genotoxicity studies established the safety profile of the 4 small molecules used for treatment of COVID-19, supporting safe clinical use.

A noted aim of this review was to analyse whether the number and types of studies normally needed for regulatory agency authorization of vaccines, biologicals (mAbs) and small molecules for patient use have been reduced. The short answer is generally no. Thus, a battery of immunogenicity/efficacy or related pharmacology/biological activity studies showing utility against SARS-CoV-2 were performed as well as general toxicity studies across all 3 compound classes along with PK studies for mAbs and small molecules and, reproduction toxicity testing for vaccines and small molecules; additionally, genotoxicity testing occurred for small molecules. What was different from conventional, lengthy drug development, was that for vaccines, existing platform technology had already been developed which was modified to allow use of SARS-CoV-2 spike protein and in some cases, regulatory agency acceptance that some of the nonclinical work was still ongoing or planned. Recognition that mAbs can target the RBD of the spike protein leading to neutralization allowed rapid development into clinical candidates which received authorization (EUA/CMA). Generally, with the small molecules for treatment of COVID-19, most of the nonclinical package of studies were in place as used to investigate potential use for

other disease indications. The utility of toxicity testing in supporting a lack of effects from activation of the immune system from vaccine use and in identifying any target organs/safety signals for clinical use with small molecules was confirmed. However, the utility of performing toxicity studies with mAbs in healthy animals which have no target remains an open question.

Declaration of conflicting interests

No potential conflicts were declared.

Funding

No financial support for the research, authorship and/or publication of this article was declared.

Funding body information

No funding body was involved in supporting this work.

CRedit authorship contribution statement

Paul Baldrick: Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Baldrick, P., 2016. Dose site reactions and related findings after vaccine administration in safety studies. *J. Appl. Toxicol.* 36 (8), 980–990. <https://doi.org/10.1002/jat.3314>.
- Baldrick, P., 2017. Getting a molecule into the clinic: nonclinical testing and starting dose considerations. *Regul. Toxicol. Pharmacol.* 89, 95–100. <https://doi.org/10.1016/j.yrtph.2017.07.027>.
- Bennet, B.M., Wolf, J., Laureano, R., Sellers, R.S., 2020. Review of current vaccine development strategies to prevent coronavirus disease 2019 (COVID-19). *Toxicol. Pathol.* 48 (7), 800–809. <https://doi.org/10.1177/0192623320959090>.
- BLA 125276, 2009. Tocilizumab (Actemra) FDA pharmacology review. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/125276s000PharmR.pdf. Accessed March 2022.
- Cho, H., Gonzales-Wartz, K.K., Huang, D., Yuan, M., Peterson, M., et al., 2021. Ultrapotent Bispecific Antibodies Neutralize Emerging SARS-CoV-2 Variants. <https://doi.org/10.1101/2021.04.01.437942> bioRxiv [Preprint] 2021.04.01.437942.
- EMA, 2020. European public assessment report for conditional marketing authorisation (CMA): Remdesivir. https://www.ema.europa.eu/en/documents/assessment-report/veklury-epar-public-assessment-report_en.pdf. Assessed March 2022.
- EMA, 2021a. Reflection paper on the regulatory requirements for vaccines intended to provide protection against variant strain(s) of SARS-CoV-2. https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-regulatory-requirements-vaccines-intended-provide-protection-against-variant_en.pdf. Assessed March 2022.
- EMA, 2021b. Procedural guidance for variant strain(s) update to vaccines intended for protection against Human coronavirus. In: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/procedural-guidance-variant-strains-update-vaccines-intended-protection-against-human-coronavirus_en.pdf. Assessed March 2022.
- EMA, 2021c. Assessment report for conditional marketing authorisation (CMA): COVID-19 vaccine AstraZeneca [COVID-19 vaccine (ChAdOx1-S [recombinant])]. https://www.ema.europa.eu/en/documents/assessment-report/vaxzevria-previously-covid-19-vaccine-astrazeneca-epar-public-assessment-report_en.pdf. Assessed March 2022.
- EMA, 2021d. Assessment report for conditional marketing authorisation (CMA): COVID-19 vaccine Janssen. https://www.ema.europa.eu/en/documents/assessment-report/covid-19-vaccine-janssen-epar-public-assessment-report_en.pdf. Assessed March 2022.
- EMA, 2021e. Assessment report for conditional marketing authorisation (CMA): comirnaty [COVID-19 mRNA vaccine (nucleoside-modified)]. https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf. Assessed March 2022.
- EMA, 2021f. Assessment report for conditional marketing authorisation (CMA): COVID-19 vaccine Moderna. https://www.ema.europa.eu/en/documents/assessment-report/covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf. Assessed March 2022.

- report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2021g. Assessment report: casirivimab and imdevimab. In: https://www.ema.europa.eu/en/documents/referral/regn-cov2-antibody-combination-casirivimab/imdevimab-covid19-article-53-procedure-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2021h. Assessment report: bamlanivimab and etesevimab (2021). In: https://www.ema.europa.eu/en/documents/referral/eli-lilly-company-limited-antibody-combination-bamlanivimab/etesevimab-covid19-article-53-procedure-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2021i. Assessment report: sotrovimab. In: https://www.ema.europa.eu/en/documents/referral/sotrovimab-also-known-vir-7831-gsk4182136-covid19-article-53-procedure-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2021j. Assessment report: regdanvimab. https://www.ema.europa.eu/en/documents/assessment-report/regkirona-epar-public-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2021k. Assessment report: Paxlovid. In: https://www.ema.europa.eu/en/documents/referral/paxlovid-pf-07321332-ritonavir-covid-19-article-53-procedure-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2022a. Conditional marketing authorization. <https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/conditional-marketing-authorisation>. Accessed March 2022.
- EMA, 2022b. Assessment report for conditional marketing authorisation (CMA): nuvaxovid [COVID-19 vaccine (recombinant, adjuvanted)]. https://www.ema.europa.eu/en/documents/assessment-report/nuvaxovid-epar-public-assessment-report_en.pdf. Accessed March 2022.
- EMA, 2022c. Assessment report: Molnupiravir. In: https://www.ema.europa.eu/en/documents/referral/lagevrio-also-known-molnupiravir-mk-4482-covid-19-article-53-procedure-assessment-report_en.pdf. Accessed March 2022.
- FDA, 2006. Guidance for industry: considerations for developmental toxicity studies for preventive and therapeutic vaccines for infectious disease indications. <https://www.fda.gov/files/vaccines,%20blood%20%26%20biologics/published/Guidance-for-Industry-Considerations-for-Developmental-Toxicity-Studies-for-Preventive-and-Therapeutic-Vaccines-for-Infectious-Disease-Indications.pdf>. Accessed March 2022.
- FDA, 2020a. Guidance for industry: development and licensure of vaccines to prevent COVID-19. <https://www.fda.gov/media/139638/download>. Accessed March 2022.
- FDA, 2020b. Guidance for industry and investigators: COVID-19 public health emergency: general considerations for pre-IND meeting requests for COVID-19 related drugs and biological products. <https://www.fda.gov/media/137927/download>. Accessed March 2022.
- FDA, 2020c. Emergency use authorization (EUA): casirivimab and imdevimab. <https://www.fda.gov/media/144468/download>. Accessed March 2022.
- FDA, 2020d. Emergency use authorization (EUA): baricitinib. <https://www.fda.gov/media/144473/download>. Accessed March 2022.
- FDA, 2021a. Emergency use authorization (EUA) for Janssen COVID-19 vaccine (Ad26.COV2.S). <https://www.fda.gov/media/146338/download>. Accessed March 2022.
- FDA, 2021b. Summary basis for regulatory action for COVID-19 vaccine, mRNA (COMIRNATY). <https://www.fda.gov/media/151733/download>. Accessed March 2022.
- FDA, 2021c. Summary basis for regulatory action for COVID-19 vaccine, mRNA (SPIKEVAX). <https://www.fda.gov/media/155931/download>. Accessed March 2022.
- FDA, 2021d. Emergency use authorization (EUA): Tixagevimab and cilgavimabare. <https://www.fda.gov/media/155107/download>. Accessed March 2022.
- FDA, 2021e. Emergency use authorization (EUA): bamlanivimab and etesevimab. <https://www.fda.gov/media/146255/download>. Accessed March 2022.
- FDA, 2021f. Emergency use authorization (EUA): sotrovimab. <https://www.fda.gov/media/150130/download>. Accessed March 2022.
- FDA, 2021g. Emergency use authorization (EUA): tocilizumab. <https://www.fda.gov/media/150748/download>. Accessed March 2022.
- FDA, 2021h. Emergency use authorization (EUA): Molnupiravir. <https://www.fda.gov/media/155241/download>. Accessed March 2022.
- FDA, 2021i. Emergency use authorization (EUA): Paxlovid. <https://www.fda.gov/media/155194/download>. Accessed March 2022.
- FDA, 2022a. Emergency use authorization. <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization>. Accessed March 2022.
- FDA, 2022b. FDA statement: coronavirus (COVID-19) update: FDA limits use of certain monoclonal antibodies to treat COVID-19 due to the omicron variant. <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-limits-use-certain-monoclonal-antibodies-treat-covid-19-due-omicron>. Accessed March 2022.
- FDA, 2022c. Guidance for industry: nonclinical considerations for Mitigating nonhuman primate supply constraints arising from the COVID-19 pandemic. <https://www.fda.gov/media/155950/download>. Accessed March 2022.
- FDA, 2022d. Emergency use authorization (EUA): bebtelovimab. <https://www.fda.gov/media/156396/download>. Accessed March 2022.
- FDA, 2022e. Emergency use authorization (EUA): Remdesivir. <https://www.fda.gov/media/155772/download>. Accessed March 2022.
- Genzel, L., Adan, R., Berns, A., van den Beucken, J.J.J.P., Blokland, A., et al., 2020. How the COVID-19 pandemic highlights the necessity of animal research. *Curr. Biol.* 30 (18), R1014–R1018. <https://doi.org/10.1016/j.cub.2020.08.030>.
- ICH M3(R2), 2009. ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for Pharmaceuticals. https://database.ich.org/sites/default/files/M3_R2_Guideline.pdf. Accessed March 2022.
- ICH S6(R1), 2011. International conference on harmonisation: Preclinical safety evaluation of biotechnology-derived Pharmaceuticals. https://database.ich.org/sites/default/files/S6_R1_Guideline_0.pdf. Accessed March 2022.
- Lee, W.S., Wheatley, A.K., Kent, S.J., DeKosky, B.J., 2020. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. *Nat. Microbiol.* 5 (10), 1185–1191. <https://doi.org/10.1038/s41564-020-00789-5>.
- MHRA, 2020. Conditional Marketing Authorisations, exceptional circumstances Marketing Authorisations and national scientific advice. <https://www.gov.uk/guidance/conditional-marketing-authorisations-exceptional-circumstances-marketing-authorisations-and-national-scientific-advice>. Accessed March 2022.
- MHRA, 2021a. Public assessment report for conditional marketing authorisation (CMA): Vaxzevria (previously COVID-19 vaccine AstraZeneca, suspension for injection). https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1003840/CMA_UKPAR_COVID_19_Vaccine_AstraZeneca_PAR_16.07.2021.pdf. Accessed March 2022.
- MHRA, 2021b. Public assessment report for conditional marketing authorisation (CMA): COVID-19 vaccine Janssen suspension for injection (COVID-19 vaccine (Ad26.COV2.S [recombinant])). https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/996096/COVID19_Vaccine_Janssen_suspension_for_injection_UKPAR.pdf. Accessed March 2022.
- MHRA, 2021c. Public assessment report for conditional marketing authorisation (CMA): COVID-19 mRNA vaccine BNT162b2 (BNT162b2 RNA). https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/997584/COVID-19_mRNA_Vaccine_BNT162b2_UKPAR_PFIZER_BIONTECH_ext_of_indication_11.6.2021.pdf. Accessed March 2022.
- MHRA, 2021d. Public assessment report for conditional marketing authorisation (CMA): COVID-19 vaccine Moderna, 0.20 mg/mL dispersion for injection. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/977367/UKPAR_COVID_19_Vaccine_Moderna_07.04.2021_CMA_Reliance_PAR_-_final.pdf. Accessed March 2022.
- MHRA, 2021e. UK public assessment report for conditional marketing authorisation (CMA): casirivimab and imdevimab. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1034672/PAR_Ronapreve_P_LBG_00031_0925_11.11.2021.pdf. Accessed March 2022.
- MHRA, 2021f. UK public assessment report for conditional marketing authorisation (CMA): Molnupiravir. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1042560/Lagevrio_Public_Assessment_Report.pdf. Accessed March 2022.
- MHRA, 2022b. Summary of product characteristics for Evusheld. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1061470/gb-spc-Evusheld.pdf. Accessed March 2022.
- MHRA, 2022a. Summary of product characteristics for COVID-19 vaccine Valneva suspension for injection. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1069169/COVID-19_Vaccine_Valneva_SmPC_-_final.pdf. Accessed April 2022.
- NDA 207924, 2018. FDA pharmacology review for baricitinib (Olumiant). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/207924Orig1s000PharmR.pdf. Accessed March 2022.
- Sun, Y., Gruber, M., Matsumoto, M., 2012. Overview of global regulatory toxicology requirements for vaccines and adjuvants. *J. Pharmacol. Toxicol. Methods* 65 (2), 49–57. <https://doi.org/10.1016/j.vascn.2012.01.002>.
- WHO, 2005. World Health Organization: guidelines on nonclinical evaluation of vaccines. https://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf. Accessed March 2022.
- WHO, 2013. World Health Organization: guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. https://www.who.int/biologicals/areas/vaccines/ADJUVANTS_Post_ECBS_edited_clean_Guidelines_NCE_Adjuvant_Final_17122013_WEB.pdf. Accessed March 2022.
- WHO, 2022. Tracking SARS-CoV-2 variants. <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>. Accessed March 2022.