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WHO Expert Committee on Biological Standardization

Seventy-seventh report

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WHO Expert Committee on Biological Standardization: seventy-seventh report

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WHO Expert Committee on Biological Standardization

Seventy-seventh meeting held virtually 20 to 24 March 2023

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Abbreviations

ACPA	anti-citrullinated protein antibodies
AG-BRAS	Advisory Group for Blood Regulation, Availability and Safety
ATMP	advanced therapy medicinal product
CCP	cyclic citrullinated peptide
CEPI	Coalition for Epidemic Preparedness Innovations
CI	confidence interval
COVID-19	coronavirus disease 2019
CV	coefficient of variation
DDNS	direct detection by nanopore sequencing
DNA	deoxyribonucleic acid
EDL	WHO Model List of Essential In Vitro Diagnostics
EML	WHO Model List of Essential Medicines
EUL	WHO emergency use listing
FCXM	flow cytometry crossmatch
FVIII	blood coagulation factor VIII
GBS	Group B streptococcus
GBT	WHO global benchmarking tool
GCV	geometric coefficient of variation
GMP	good manufacturing practice(s)
GPLN	Global Polio Laboratory Network
HAV	hepatitis A virus
HBV	hepatitis B virus
HCT	human cells and tissues
HLA	human leukocyte antigen
HPAEC-PAD	high-performance anion exchange chromatography with pulsed amperometric detection
IFU	instructions for use
IgA	immunoglobulin A

IgG	immunoglobulin G
IgM	immunoglobulin M
IPV	inactivated poliomyelitis vaccine
ISTH	International Society of Thrombosis and Haemostasis
IU	International Unit(s)
IUIS	International Union for Immunological Societies
IVD	in vitro diagnostic
JRC	European Commission Joint Research Centre
Lf	limit of flocculation
LNP	lipid nanoparticle
LMIC	low- and middle-income countries
mAb	monoclonal antibody
MenC	meningococcal serogroup C
MERS-CoV	Middle East respiratory syndrome coronavirus
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	messenger RNA
NAT	nucleic acid amplification technique
NIBSC	National Institute for Biological Standards and Control
NRA	national regulatory authority
OPV	oral poliomyelitis vaccine
PCR	polymerase chain reaction
PHEIC	public health emergency of international concern
qNMR	quantitative nuclear magnetic resonance (spectroscopy)
RNA	ribonucleic acid
RRV	Ross River virus
RSS	WHO regulatory systems strengthening
RVF	Rift Valley fever
SAGE	Strategic Advisory Group of Experts
SARS-CoV-1	severe acute respiratory syndrome coronavirus 1
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2

TAG	technical advisory group
tTG	tissue transglutaminase
VEGF	vascular endothelial growth factor
VOC	variant(s) of concern
VZV	varicella zoster virus
WHOCC	WHO collaborating centre
WLA	WHO Listed Authority

1. Introduction

The seventy-seventh meeting of the WHO Expert Committee on Biological Standardization was held virtually from 20 to 24 March 2023. The meeting was opened on behalf of the Director-General of WHO and the Assistant Director-General, Access to Medicines and Health Products, by Dr Clive Ondari, Director, Health Products Policy and Standards. Dr Ondari began by welcoming Committee members, meeting participants and observers. Following the expansion of the Expert Advisory Panel, from which Committee members were selected, Dr Ondari noted that the current 22-member Committee was well balanced in terms of its expertise, and gender and geographical representativeness. In the same year in which WHO would celebrate its 75th anniversary it seemed fitting to note that this Committee was one of its longest-serving advisory bodies.

Reflecting on the characteristically ambitious meeting agenda, Dr Ondari highlighted the vital role of WHO in expanding global access to essential medicines. During recent Executive Board meetings, the Director-General had emphasized the importance of providing WHO evidence-based norms and standards, research data and associated technical support to WHO Member States. In this context, the Executive Board had noted the significance of WHO written standards, including the WHO Guidelines on evaluation of biosimilars which had been adopted on the advice of the Committee at its meeting in April 2022. Other key activities included WHO's continued commitment to support global regulatory system strengthening, and the ongoing assistance being provided to countries producing vaccines and therapeutics against coronavirus disease 2019 (COVID-19). In addition, following the large Mpox outbreak in 2022, WHO had declared a Public Health Emergency of International Concern, with work on the required WHO reference standards promptly being initiated. Dr Ondari further noted that critical technical assistance from WHO continued to be provided to global polio eradication efforts. Dr Ondari then highlighted the important contributions made by WHO in the standardization of vitally needed blood products, with the full support of the Committee and of the Advisory Group for Blood Regulation, Availability and Safety (AG-BRAS). Specific activities in this area included the broadly welcomed revival of the Achilles project which aims to improve blood safety and reduce plasma wastage worldwide.

Dr Ondari concluded by thanking Committee members, the WHO Secretariat and other WHO colleagues, and all meeting participants for their time and invaluable contribution to the current meeting.

Dr Ivana Knezevic, Secretary to the Committee, thanked Dr Ondari for his opening remarks. Welcoming all meeting participants, Dr Knezevic noted that due to the recently increased frequency of such meetings to more flexibly meet the needs of WHO and of custodian laboratories in promptly implementing

new standards, the current 77th meeting of the Committee was taking place during the 75th anniversary of WHO. As the directing and coordinating authority on international health within the United Nations system, WHO would continue to promote the values of the United Nations in line with the principles of respect for human rights, diversity and equity established in its Constitution. Through its activities at headquarters, regional office and country office level, WHO provides leadership on global health matters, shapes the health research agenda, sets norms and standards, articulates evidence-based policy options and provides technical support to countries.

Dr Knezevic reminded participants that the decision-making body of WHO is the annual World Health Assembly attended by delegations from all WHO Member States. Two resolutions adopted by the World Health Assembly in 2014 – namely WHA67.21 on access to biotherapeutic products including biosimilars, and resolution WHA67.20 on regulatory strengthening – remained of particular relevance to the work of the Committee. The reports of the recommendations of the Committee were also submitted to the WHO Executive Board.

Dr Knezevic went on to inform participants that membership of the WHO Expert Advisory Panel on Biological Standardization did not automatically correspond to membership of the Committee, which is assembled each year on the basis of the expertise required to meet the needs of the agenda. Noting that the composition of the Committee had become increasingly dynamic in recent years, Dr Knezevic clarified that this and the continuing expansion of the Expert Advisory Panel was intended to ensure the increasingly broad range of expertise required to address the expanding scope of WHO biological standardization activities.

Dr Knezevic then outlined the meeting procedures and working arrangements. An open information-sharing session involving all participants including non-state actors would be held on Monday 20 March 2023. Committee members, regulatory authority representatives and subject matter experts from governmental organizations would then participate in the main meeting from Monday 20 March to Thursday 23 March 2023. All final decisions and recommendations on the adoption of WHO written standards and the establishment of WHO measurement standards would be made in a closed session held on Friday 24 March attended only by Committee members and WHO staff.

Following the conclusion of the open information-sharing session, the meeting officials were elected. In the absence of dissent, Professor Klaus Cichutek and Dr Salwa Hindawi were elected as Co-chairs. Dr Ian Feavers and Dr Mickey Koh were elected as Rapporteur and Co-rapporteur respectively. Following a brief round of introduction by Committee members, Dr Knezevic presented the declarations of interests completed by Committee members, WHO temporary

advisers and other participants. After evaluation, WHO had concluded that none of the interests declared constituted a significant conflict of interest and that the individuals concerned would be allowed to participate fully in the meeting.

The Committee then adopted the proposed agenda and timetable (WHO/BS/2023.2452).

2. General

2.1 Strategic directions in biological standardization

2.1.1 Vaccines, biotherapeutics, and cell, tissue and gene therapy products: recent and planned activities in biological standardization

Dr Knezevic updated the Committee on recent and planned WHO biological standardization activities in the above areas. Dr Knezevic reminded meeting participants that WHO written standards and WHO measurement standards were based on sound scientific evidence and play an essential role in the development, licensing and lot release of biological products. Acknowledging the vital role of WHO collaborating centres (WHOCCs) in the development of WHO written and measurement standards, and in facilitating their subsequent implementation in countries, Dr Knezevic highlighted in particular the pivotal contribution of the Medicines and Healthcare products Regulatory Agency (MHRA) of the United Kingdom. Dr Knezevic also highlighted the recent successful re-designation of the WHOCC hosted by the Ministry of Food and Drug Safety (MFDS) in the Republic of Korea. Following inevitable delays caused by the COVID-19 pandemic, all WHOCCs were once again submitting their annual reports on schedule. However, due to resource issues arising from the demands of transitioning from virtual to face-to-face meetings, the next meeting of the WHO network of collaborating centres on standardization and regulatory evaluation of vaccines meeting had been postponed until 2024.

With regard to the Committee, the recent shift to biannual meetings now meant that the WHO Secretariat was involved in three parallel meeting activities, namely completion of the formal report of the previous meeting held in October 2022, administration of the current meeting and planning for the next meeting. During an overview of the main outcomes of the previous meeting, Dr Knezevic noted that two WHO written standards had been adopted – the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccine (oral, live, attenuated) and the WHO Global Model Regulatory Framework for medical devices including in vitro diagnostic medical devices. In addition, 24 new or replacement WHO international reference standards had been established, and eight proposals for new standards endorsed by the Committee. Specific topics discussed by the Committee had included standardization issues in relation to the continuing COVID-19 pandemic and challenges related to the nomenclature and assignment of IU to antibody standards for neutralization and binding assays. A WHO document on considerations in developing a global regulatory framework for human cells and tissues and for advanced therapy medicinal products had also been discussed at the previous meeting and would be submitted to the Committee for potential adoption at the current meeting (see section 3.2.1 below).

Dr Knezevic then presented a summary of WHO written standards that had recently been adopted or were currently under consideration. Since 2020, five such WHO documents addressing issues directly relevant to the COVID-19 pandemic had been drafted, subjected to public consultation and proposed for adoption. This included the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases to be considered for adoption at the current meeting (see section 3.1.1 below). In addition, WHO written standards currently under revision included: (a) WHO Guidelines on regulatory preparedness for provision of marketing authorization of human pandemic influenza vaccines in non-vaccine-producing countries; (b) WHO Guidelines on procedures and data requirements for changes to approved vaccines; and (c) WHO Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines (oral).

Dr Knezevic then outlined the broad range of WHO information resources on biological standardization publicly available on the WHO website. These included the formal reports of the Committee published in the WHO Technical Report Series, as well as the executive summaries provided shortly after the conclusion of each Committee meeting. After briefly reviewing a number of relevant past and upcoming consultations, implementation workshops and related events organized by WHO and its external collaborators during 2022–2024, Dr Knezevic concluded by thanking WHO colleagues, all members of WHO drafting and working groups, WHOCC staff and individual experts for their invaluable support and contributions.

Reflecting on standardization challenges in relation to the COVID-19 pandemic, and on the ways in which the pandemic had driven the rapid and unprecedented development of novel prophylactic and therapeutic products, the Committee applauded the efforts of WHO in delivering highly relevant written and measurement standards under considerable time pressures.

2.1.2 Blood products and related in vitro diagnostics: recent and planned activities in biological standardization

Dr Yuyun Maryuningsih began by reviewing the activities of AG-BRAS which had met in person in March 2023. During this meeting, progress made against the 2021–2022 workplan had been discussed and several strategic proposals made. These proposals centred on the harmonization of definitions and terminology around donation, the dissemination and promotion of WHO documents, and the use of the WHO Global Database on Blood Safety to assess the coping capacities of countries in emergency situations. During the same meeting, an update had been provided on the use of COVID-19 convalescent plasma, and round table discussions held on emerging infections and pathogen-

reduction technologies. Discussion of the AG-BRAS 2023–2025 workplan had focused on the revision of WHO guidance on good manufacturing practices for blood establishments, and on the updating of WHO guidance on COVID-19 convalescent plasma. Dr Maryuningsih informed meeting participants that AG-BRAS would be holding a webinar on its activities in mid 2023 and offered to provide the Committee with a progress report at its next meeting in October.

Dr Maryuningsih went on to summarize the progress made in implementing the WHO Action framework to advance universal access to safe, effective and quality-assured blood products 2020–2023 which provides strategic guidance for both WHO activities and worldwide efforts in this area. This had included the development of 10 WHO guidance documents – six of which had now been implemented, with four due to be finalized during 2023. In addition, the recently revived Achilles project had been implemented as part of the WHO Action framework, with activities to date largely focusing on the preparation of pathogen-reduced cryoprecipitate in the Dakar Blood Centre in Senegal. A further Achilles project on contract plasma fractionation had now started in Indonesia, with training provided on strengthening the consolidation of blood services in the country, and on implementing WHO guidance on plasma costing. Dr Maryuningsih also informed the Committee that a proposal to include pathogen-reduced cryoprecipitate on the WHO Model List of Essential Medicines (EML) had been made and would shortly be considered by the WHO Expert Committee on Selection and Use of Essential Medicines.

Following a brief summary of selected upcoming activities of the WHO Blood and other Products of Human Origin (BTT) team, and of the WHO measurement standards relevant to this area that were being proposed for consideration at the current meeting, Dr Maryuningsih invited Dr Cynthia So-Osman to provide an update on the use of convalescent plasma and hyperimmune immunoglobulin products in the treatment of COVID-19. Dr So-Osman began by noting that although the use of such products early in the course of infection had proved effective in the treatment of other diseases, evidence for their efficacy against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remained equivocal. Analysis of emerging data, including systematic reviews, had not demonstrated efficacy in moderate to severe cases of disease – nevertheless, evidence was emerging that in specific circumstances such products could reduce disease severity and mortality in immunocompromised individuals. However, the optimal dose and frequency of administration remain uncertain, with the quantification of "high-titre" products also requiring further consideration.

Dr Cees Smit Sibinga then concluded the update of WHO activities in this area by briefly summarizing the approach taken in developing an upcoming WHO document entitled Guidance on ensuring a sufficient supply of safe blood and blood components during emergencies. Supported by WHO staff, including

regional representatives, a core group had drafted the principal sections of the document, including an introduction and chapters on risk assessment and gap analysis, emergency preparedness, and response and recovery.

While acknowledging the potential utility of convalescent plasma during a pandemic, the Committee expressed concern that the risk of side-effects may outweigh any potential benefits. In addition, the collection of plasma from recovered individuals may impose significant burdens on blood centres without clear evidence of efficacy. However, following clarification of a number of the points raised, the Committee went on to suggest that if robust evidence supporting the use of convalescent plasma or hyperimmune immunoglobulin products were to emerge from the ongoing studies then WHO might consider offering guidance on their use, appropriate titres and likely efficacy against SARS-CoV-2 variants of concern. With regard to WHO efforts to develop guidance on ensuring a safe blood supply, the Committee was assured that once the guidance had been published and disseminated via webinar it would be implemented in line with WHO practice.

2.1.3 Unit assignment to WHO international reference standards for antibodies

Dr Micha Nübling presented an overview of recently discussed issues in relation to the long-recognized challenge of assigning units to WHO international reference standards used to harmonize different types of antibody assay. These issues had once again been highlighted following establishment of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin. This international standard had been assigned an International Unit (IU) for use in virus neutralization assays while, separately, the instructions for use (IFU) also referred to an arbitrary binding antibody unit (BAU) to assist in the comparison of binding antibody assays detecting the same class of antibodies with the same specificity. Although there was good evidence that the standard had been helpful in harmonizing both types of assay, the use of the metrologically unrecognized term BAU to distinguish between the two functions has led to criticism from users and metrology specialists that such an approach was inconsistent and confusing, with potentially adverse impacts on the design and appropriate application of assays.

Dr Nübling reminded meeting participants that at its previous meeting the Committee had recommended that an ad hoc working group be convened to review these and related issues, and to propose potential approaches to the establishment and unit assignment of antibody standards that had potential utility in both neutralization and binding assays. The working group, consisting of 20 participants, had met virtually and had identified a range of options for consideration by the Committee.

After reviewing the options put forward by the working group, a consensus was reached by the Committee that where there was a need to make a clear distinction between different methodologies, such reference standards should be split into two separate materials with unitage (where assigned) in IU. For each material, the standard name itself should clearly indicate the category of assay for which it was intended with the respective IFU reinforcing this distinction. The Committee further recommended that this approach be incorporated into the prospective revision of the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards. The Committee also recommended that the adoption of this approach be underpinned with training for users in the appropriate use of antibody standards.

The Committee then discussed in more detail the conventions to be followed when naming such reference materials. Agreement was reached that to ensure a more consistent approach to the naming of WHO international reference standards for antibodies, the following template should wherever possible be used: WHO International [Standard or Reference Reagent or Reference Panel] for antibodies to [antigen or pathogen] for use in [assay category]. Where required for clarity, additional qualifiers (for example, serum, recombinant, [animal species] or autoantibodies) should be used consistently and be placed in parentheses after the standard name. Accepting that already established standards could only switch to the more standardized nomenclature at replacement, the Committee supported the application of the agreed convention to new or replacement standards with immediate effect.

2.1.4 Development of a Group B streptococcus antibody standard

Group B streptococcus (GBS) can cause severe invasive infections especially in newborns and in elderly and immunocompromised individuals. Given the limitations of antibiotic prophylaxis, WHO identified the development of maternal GBS vaccines as a priority, particularly for use in low- and middle-income countries (LMIC). Polysaccharide-conjugate vaccines based on the most common serotypes have therefore been in clinical development for several years. However, given the relatively low incidence of disease in mothers and newborns, GBS vaccines are likely to be given conditional approval based on surrogates of protection before large clinical studies can demonstrate efficacy. A human antiserum standard would therefore be valuable in harmonizing the quantification of antibody responses and measurement of functional activity during vaccine development and evaluation. Accordingly, in 2017, the Committee had endorsed a proposal to develop a WHO reference standard in this area.

Dr Paul Stickings updated the Committee on the proposed approach to the development of a WHO reference standard based on the generation of an

antiserum with assigned units for immunoglobulin G (IgG) against the six most common serotypes. As the use of GBS serum standards calibrated in μg IgG was well established in the GBS field, the proposed reference standard would be assigned an IgG concentration for each serotype in μg. A collaborative study would be conducted and laboratories with expertise in GBS serology invited to participate. However the proposed approach for value assignment was to use a manufacturer-validated multiplex immunoassay (Luminex) method that had been transferred by the method developer to four other member laboratories of the GASTON consortium, giving a total of five laboratories pre-qualified to perform this method. A panel of human serum samples would also be included in the study to assess commutability.

Acknowledging the global need for GBS vaccines and the importance of reliable antibody standards during their clinical development, the Committee welcomed the initiative while also expressing concerns about the highly assay specific and geographically restricted proposed approach. Previous experience in the development of similar reference standards had highlighted the importance of evaluating candidate materials across a range of different antibody binding assay platforms, especially those based on modified polysaccharide antigens.

The Committee noted that there were precedents for assigning SI units to such a standard for use in antibody binding assays, and for the use of a specified method to assign such units. The Committee recognized that the GASTON multiplex method was likely to be the single largest dataset in the collaborative study and therefore ring-fencing this method for value assignment may be scientifically justified. However, additional methods should be included in the study, where available, and the final decision of the Committee regarding the establishment of a reference material would be made based on the results of the study.

2.2 Cross-cutting activities of other WHO committees and groups

2.2.1 Emergency use listing of COVID-19 vaccines

Dr Carmen Rodriguez Hernandez updated meeting participants on the emergency use listing (EUL) of COVID-19 vaccines. After outlining the eligibility criteria, Dr Rodriguez Hernandez explained that EUL was intended for products used to prevent or treat life-threatening diseases with the potential to cause outbreaks, epidemics or pandemics, and which could be used during a public health emergency of international concern (PHEIC) or other health emergency. In addition, considering a product for EUL even once a PHEIC has been declared over, allows WHO to continue the EUL assessment process, to evaluate expressions of interest based on public health needs and to consider an extension of EUL status or transition to WHO prequalification (PQ).

Currently, nine COVID-19 vaccine candidates were undergoing EUL evaluation, with two of these submissions scheduled for imminent review by the technical advisory groups (TAGs). After briefly setting out the process for prioritizing expressions of interest, Dr Rodriguez Hernandez went on to summarize the 11 COVID-19 vaccines currently under EUL. Based on four manufacturing platforms (messenger RNA, viral vector, inactivated virus and protein subunit), these vaccines covered a range of age indications, shelf-life and storage conditions, with several currently under review for use as boosters or for use in additional age groups.

Dr Rodriguez Hernandez then outlined the three WHO COVID-19 evidence-driven advisory groups that together provide comprehensive recommendations on boosters, variants and vaccine strategies. The TAG on virus evolution tracks the disease epidemiology to determine the impact of emerging variant viruses on transmission, and on the effectiveness of public health measures. The TAG on vaccines determines whether changes to vaccine composition are required, while the Strategic Advisory Group of Experts (SAGE) on Immunization recommends vaccination strategies. It was noted that the WHO position on the use of bivalent COVID-19 vaccines was consistent with the recommendations of both the European Medicines Agency and the US Food and Drug Administration. Dr Rodriguez Hernandez concluded by setting out in detail the triggers, considerations and steps involved in transitioning COVID-19 vaccines from WHO EUL to WHO PQ.

Reflecting on the excellent work of the EUL programme during the pandemic, the Committee asked what lessons had been learned regarding the supply of vaccines to LMIC. Dr Rodriguez Hernandez indicated that WHO would need to undertake such an analysis as a collaborative exercise across all relevant WHO departments, with any lessons learned used to improve the response to future public health emergencies.

2.2.2 Meeting of the SAGE on Immunization

The Committee was provided with the agenda of a SAGE on Immunization meeting that was being held concurrently. The meeting had started with reports from the WHO Department of Immunization, Vaccines and Biologicals, and from Gavi, the Vaccine Alliance. These reports had highlighted the priorities for 2023, including the restoration of routine immunization, expansion of malaria vaccination, the re-launching of human papillomavirus vaccination and the integration of COVID-19 vaccination into routine immunization programmes. Following a series of regional reports focusing on the elimination of measles and the challenges faced in delivering measles vaccines globally, an overview had been provided of the WHO approach to partnering with regions and countries to identify priority pathogens for new vaccine development at both regional and global level.

The meeting would then consider disease-specific issues, starting with the strategies and policies required to optimize the global impact of COVID-19 vaccines in the era of Omicron. This would be followed by a review of the status of novel tuberculosis vaccine candidates intended for use in adults and adolescents, and would include information on the tools being developed by WHO to prepare for the introduction of such vaccines in countries with a high burden of disease, along with an overview of the product development plans for M72/AS01E vaccines. On the final day, the SAGE on Immunization would be updated on the current status of the polio eradication programme, and on the implementation of the new eradication strategy, and would be asked to review and consider the endorsement of a number of working group recommendations. These recommendations included restricting the use of Sabin type 2 oral poliomyelitis vaccine (OPV), introducing an additional dose of inactivated poliomyelitis vaccine (IPV) to supplement OPV campaigns in areas with persistent poliovirus transmission, and taking a flexible approach to the use of novel OPV2 for outbreak response. The meeting would then close with an update on the malaria vaccine implementation programme and on the plans for the roll-out of RTS,S/AS01 malaria vaccine, with the SAGE on Immunization providing guidance on implementation.

2.2.3 Meeting of the SAGE on IVDs

The Committee was updated on the outcomes of the 4th meeting of the SAGE on IVDs held in November 2022. This advisory group makes recommendations on policies and strategies related to in vitro diagnostics (IVDs) and to the WHO Model List of Essential In Vitro Diagnostics (EDL). The purpose of the meeting had been to review applications for, and make recommendations on, the updated EDL4, and to discuss current strategies for increasing the availability, accessibility and correct use of IVDs. The meeting had started with an overview of the EDL, which is a policy document based on scientific evidence and consisting of a register of categories of IVD tests along with recommendations on their use (or non-use). Following a summary of the criteria for listing test categories in the EDL and of the process for its updating, attention had turned to the EDL4 planning activities and timeline. A total of 71 candidate tests had previously been identified to inform the EDL call for submissions, with consensus having been reached on 23 high-priority test categories. The EDL4 submissions process had resulted in the addition of nine new IVD categories, editing of two existing entries and the recommendation not to perform typhoid serological tests.

The meeting had then explored the relationship of the EDL to other WHO model or priority lists, with presentations given on the WHO EML, assistive products list and medical devices list (including essential IVDs). The meeting had concluded with a discussion on the specific challenges faced in

developing national EDLs in the WHO South-East Asia Region and the WHO Region of the Americas.

2.2.4 WHO prequalification of biosimilars

Dr Guido Pante summarized a number of recent activities relating to the WHO PQ of biotherapeutics and biosimilars. Noting the considerable difference between the requirements for such products compared with small molecules, Dr Pante explained that rituximab and trastuzumab had been chosen for a prequalification pilot procedure based on disease prevalence, evidence of product efficacy and safety, comparative cost-effectiveness, and the availability of WHO technical guidance on the evaluation of biotherapeutics. Consistent with the prequalification of small molecules, the pilot procedure provided two distinct pathways to prequalification: (a) a full assessment pathway for rituximab or trastuzumab biosimilars that have been registered by a non-stringent regulatory authority based on a reference product approved by a stringent regulatory authority; or (b) an abridged assessment pathway for rituximab or trastuzumab biotherapeutics or their corresponding biosimilars approved by a stringent regulatory authority and marketed in the country of registration.

The results of the pilot project indicated that guidelines and templates produced during the review of 27 dossiers, together with the prequalification of 16 products, provided a sound basis for the prequalification of other biotherapeutics with other indications. Based on this, expressions of interest had been published for products covering a range of therapeutic indications – such products included human insulin and its analogues as well as therapeutics against COVID-19 and Ebola virus disease. Given supply issues, especially but not exclusively in LMIC, an active pharmaceutical ingredient master file (APIMF)-like pathway had been applied to the prequalification of human insulin to ensure its affordability and availability. Related activities included the establishment of an Expert Review Panel – an independent advisory body of technical experts that assesses the quality risks of products that do not meet all stringent requirements, while also providing advice to procurement agencies. Highlighting the global shortage of tuberculin and the development of new in vivo tests, Dr Pante also described plans for the prequalification of the tuberculin skin test for tuberculosis diagnosis based on experience gained from other biotherapeutics and biosimilars.

The Committee sought clarification of the role of the Expert Review Panel and was informed that it offers a service to the procurers of biotherapeutics and biosimilars, including through the provision of advice on product and manufacturer compliance with quality standards and with good manufacturing practices (GMP). As WHO is not a licensing authority, this advisory body does not play a role in the final approval of products, which is the responsibility of the national regulatory authority (NRA). Clarification was also given that in addition

to the prequalification of the biotherapeutics and diagnostic product described in the presentation, the published expressions of interest had also included prophylactic products such as monoclonal antibodies (mAbs).

2.2.5 Update on WHO regulatory systems strengthening activities

Dr Alireza Khadem began his update to the Committee by summarizing the overall maturity levels of regulatory systems in countries, noting that the number of such systems evaluated using the WHO global benchmarking tool (GBT) had steadily increased since 2016. To date, only 30% of countries (n = 57) have well-functioning, integrated regulatory systems that comply with maturity levels three or four, and which therefore meet the expectations set out in resolution WHA 67.20. This represented an increase of seven countries since 2018. Importantly, 34 of the 57 countries are vaccine producers and therefore eligible for EUL or WHO PQ. Dr Khadem reminded meeting participants that the WHO GBT had been revised to include blood in 2019 and then medical devices in 2022.

Dr Khadem went on to set out a number of recent developments in WHO regulatory systems strengthening (RSS) activities, including the adoption of the definition of a "WHO Listed Authority" (WLA) by the WHO Expert Committee on Specifications for Pharmaceutical Preparations in 2020. A WLA is defined as a regulatory authority or a regional regulatory system which has been documented to comply with all the relevant indicators and requirements specified by WHO for the requested scope of listing based on an established benchmarking and performance evaluation process. Two imminent WHO documents – on WLA operational guidance and on performance evaluation – had been developed to support the underlying listing process. Dr Khadem outlined the main elements of the performance evaluation process that has to be met to achieve WLA status. Currently, three risk-based performance evaluation pathways to WLA were being piloted. Next steps would include the establishing of a technical advisory group on WLA, translation of the WLA framework into the six United Nations languages, and the computerization of performance evaluation indicators and tools. Other RSS developments had included the establishment of a voluntary Coalition of Interested Parties Network to promote a unified, strategic and coordinated approach to the strengthening of national and regional regulatory systems, and the development of a global competency framework to support the training and professional development of regulatory staff.

Recognizing the importance of RSS and the value of the WHO GBT in harmonizing the quality-management systems of different authorities, the Committee applauded the excellent progress that had been made under challenging circumstances. Reflecting that regulatory benchmarking and standardization typically focused on quality-management systems and organizations, the Committee asked what could be done to manage the

quality of the output of regulatory systems. Acknowledging the limitations of benchmarking tools, Dr Khadem emphasized the importance of resources and noted that capacity-building in LMIC was a significant output of WHO RSS activities. In addition, assurance was given to the Committee that in promoting good regulatory practice, the WHO GBT incorporated enablers such as regulatory reliance. In addition, despite the already good level of engagement achieved, efforts would continue to be made to promote and provide training on the RSS framework and WHO GBT in LMIC.

3. International Recommendations, Guidelines and other matters related to the manufacture, quality control and evaluation of biological products

3.1 Biotherapeutics other than blood products

3.1.1 Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases

Therapeutic mAb products have become increasingly important for the treatment of a wide range of noncommunicable diseases in areas such as haematology, immunology and oncology, and currently represent the largest class of therapeutic proteins in clinical use. Technological advances in mAb engineering have now led to the development of a wide range of mAbs and related products with potentially significant manufacturing and clinical benefits. However, global access to such products is currently highly limited, with approximately 80% of all mAb therapeutics being sold in North America and Europe. Alongside cost and manufacturing capacities, regulatory challenges have been identified as one of the principal causes of this disparity. In addition, the COVID-19 pandemic had highlighted the potential benefits of such products when rapidly responding to public health emergencies caused by emerging infectious agents, provided that NRAs possessed the required expertise, capacity and regulatory processes to review such products in a timely manner. Although relatively few mAb and related products have to date been approved for the prevention or treatment of infectious diseases, an increasing number of such products were now being developed.

After reflecting on these and other developments during its recent meetings, the Committee had noted the paucity of regulatory advice specifically on the nonclinical and clinical evaluation of mAbs used for pre-exposure prophylaxis, post-exposure prophylaxis and post-infection treatment of infectious diseases. It was recognized that WHO guidance in this area would potentially allow for the clarification of regulatory expectations and improved international regulatory harmonization, thereby improving access to these potentially vital products. A proposal to develop WHO Guidelines for this purpose had therefore been endorsed by the Committee in 2020. The overarching guidance was expected to be broadly applicable to all such mAbs and related products, with subsequent supplementary guidance to be drafted where required on any highly disease-specific regulatory considerations.

The Committee was provided with a detailed overview of the scope, structure and content of the resulting Guidelines document now being proposed for adoption. Following a process of international public consultation and detailed review of relevant existing WHO documents, the current document was

intended to provide flexible guidance on the nonclinical and clinical evaluation of mAbs and related products directed against pathogens or their toxins. Following further clarification of its precise scope, the Committee was provided with a summary of the six major sections of the document. It was anticipated that any disease-specific supplements to the Guidelines would take the form of short companion documents providing additional details and considerations specific to a particular disease.

The Committee proceeded to review the proposed document, taking into account the issues that had been raised during the two rounds of public consultation. The Committee began by congratulating the drafting group on the overall high quality and clarity of the document before suggesting a small number of modifications to the text. During further discussion, refinements were proposed to the title of the document and the wording of the scope was revised. Reflecting on the importance to WHO and custodian laboratories of promoting the use of WHO international reference standards, the Committee also suggested that appropriate text be added highlighting this key aspect.

After due consideration of the modifications made to the text, the Committee recommended that the document WHO/BS/2023.2442 be adopted and annexed to its report (Annex 2).

3.2 Cell, tissue and gene therapy products

3.2.1 Considerations in developing a regulatory framework for human cells and tissues and for advanced therapy medicinal products

Rapid advances in the use of human cells, tissues and gene therapies to treat serious diseases have resulted in a wide range of products that differ considerably in their degree of complexity. These products can broadly be divided into the two categories of human cells and tissues (HCTs) and advanced therapy medicinal products (ATMPs). These hugely diverse product categories pose significant regulatory challenges that could undermine their global accessibility, and appropriate and safe use. While many countries have little or no regulatory framework for HCTs and/or ATMPs, others have established their own national regulatory frameworks and guidance, leading to a lack of global harmonization. World Health Assembly resolution WHA67.20 recognizes the need for increased support and guidance in strengthening national capacities to regulate these increasingly complex biological products.

Following a WHO-led review, the Committee had previously advised that global regulatory convergence in this area would be a vital step in promoting more equitable access to such products, as well as in ensuring their safe and appropriate clinical use. Following subsequent endorsement by the Committee of its proposed development, the above high-level considerations document represents the first step in addressing this issue. The Committee was provided

with an overview of the document background and development process, which had included two rounds of international public consultation. A summary presentation was also given on the structure and content of the document. The document sets out the fundamental principles, concepts and key features of effective regulatory oversight of HCTs and ATMPs, defines key terms, proposes a template for product-categorization decisions and provides an annotated bibliography of useful references and resources.

There was consensus among the Committee that ATMPs should be regulated as medicines and licensed as such, thus requiring stringent regulatory authorization for clinical use. Although ATMPs were being used and tested for use against rare diseases and to meet current unmet needs, they were also increasingly being considered for use in more common clinical conditions such as neurodegenerative and cardiac disorders. It was also agreed that it should be specifically stated in the text that blood transfusion and organ transplantation lay outside the scope of the current document. In addition to the emphasis already placed on the need for stringent manufacturing standards and testing of ATMPs to ensure their safety the Committee highlighted the equally important aspect of addressing the inappropriate clinical use of HCTs and ATMPs of "unproven" efficacy, which can also result in significant patient harm if not supported by adequate nonclinical and clinical evidence. Agreement was reached that the importance of minimizing the risk of introducing unproven therapies for which there was insufficient evidence of efficacy required greater emphasis in the document.

Further discussion then took place with regard to the potentially misleading approach of assigning different risk categories to HCTs and ATMPs. It would be preferable instead to highlight the greater unknown risks associated with ATMPs due to their complex manufacturing processes and novel use. Discussion also took place on the optimum follow-up period required post administration, and whether a defined period could be set given the large degree of variability between different products. In any case, the crucial requirement for post-marketing surveillance and pharmacovigilance should be viewed as an integral aspect of the regulation of ATMPs. The Committee acknowledged that some NRAs may have only limited experience with ATMPs given their wide variety and inherent complexities, and regulatory cooperation, both regionally and internationally and including reliance on other NRAs, would be important and would facilitate harmonization. Such regulatory networks could help to leverage resources more efficiently and enable the sharing of knowledge and experience.

Recognizing the considerable efforts that had been made in its development and after making a number of further minor modifications to the text, the Committee recommended that the document WHO/BS/2023.2441 be adopted and annexed to its report (Annex 3).

4. International reference materials – biotherapeutics other than blood products

4.1 WHO international reference standards for biotherapeutics other than blood products

4.1.1 First WHO International Standard for vascular endothelial growth factor 165

Human vascular endothelial growth factor (VEGF) is a signalling molecule that promotes angiogenesis. It exists in multiple splicing isoforms and is secreted by many cell types including fibroblasts, macrophages, platelets and tumour cells. VEGF165, one of the most abundant isoforms, acts on VEGF receptor 2 (VEGF2) to induce the proliferation and migration of vascular endothelial cells, which is crucial for embryonic development and vascular homeostasis. In addition to promoting angiogenesis, VEGF is also implicated in other biological functions such as blood haemostasis, vasodilation and immunosuppression. The over expression of VEGF results in excess angiogenesis associated with cancers and intraocular diseases. Consequently, anti-angiogenic treatments typically target VEGF using recombinant antibodies (bevacizumab, ranibizumab and brolucizumab) or the fusion protein aflibercept. Several biosimilars have also been developed, some of which are already licensed. Measuring the level of VEGF in patients with cancers or eye disease helps in diagnosis and in assessing response to anti-VEGF medicines. In addition, VEGF165 gene transfer has been explored as a potential angiogenic treatment to promote the formation of new vessels in patients with ischaemic peripheral artery disease.

A WHO international reference reagent for vascular endothelial growth factor 165 was established in 2005. The subsequently increased availability of anti-VEGF biosimilars and the development of gene therapy products have highlighted the importance of having such a WHO reference standard for the harmonization of potency assays for VEGF165 products. As stocks of the current international reference reagent were now depleted, an international collaborative study involving 15 laboratories in eight countries had been conducted to evaluate a potential replacement material. The candidate material (NIBSC code 19/246) consisted of a commercially sourced recombinant human VEGF165 formulated in the same buffer as the current international reference reagent, and had been filled and lyophilized in line with WHO guidance on the preparation of international reference materials.

A range of bioassays and immunoassays had been performed on the candidate material, as well as on the current WHO international reference reagent and in-house reference standards where available. Potency estimates for candidate material 19/246 were calculated relative to the current WHO international reference reagent, with parallel dose–response relationships observed in all

laboratories and assay methods. Good agreement was also observed between laboratories performing bioassays, giving an overall geometric mean potency for candidate material 19/246 of 8778 IU/mL (95% CI = 7781–9902). Data from four laboratories using two different immunoassays gave a lower overall geometric mean potency of 7976 IU/mL (95% CI = 5345–11 902) against the current WHO international reference reagent that was not considered to be significantly lower than the bioassay overall mean. Based on combined data from all assays, it was proposed that candidate material 19/246 be assigned a unitage of 9000 IU/ampoule to maintain continuity with the WHO international reference reagent.

Accelerated degradation studies carried out over 27 months indicated that the candidate material retained its activity at elevated temperatures up to 37 °C, indicating long-term stability. The stability of the material will continue to be monitored for several years. Further studies showed that once reconstituted the candidate material was stable for at least 1 week at 4 °C or at room temperature. Freeze-thaw stability testing indicated that the potency of the candidate material was retained for at least four freeze-thawing cycles.

The Committee felt that this had been an interesting and thorough study, and having considered its report (WHO/BS/2023.2443), and following further clarification of the rationale for the assigned unitage, recommended that the candidate material 19/246 be established as the First WHO International Standard for vascular endothelial growth factor 165 with an assigned unitage of 9000 IU/ampoule.

5. International reference materials – blood products and related substances

5.1 WHO international reference standards for blood products and related substances

5.1.1 Ninth WHO International Standard for blood coagulation factor VIII concentrate

Blood coagulation factor VIII (FVIII) in plasma-derived and recombinant therapeutic concentrates is primarily used to treat the FVIII deficiency haemophilia A. The current WHO international standard for FVIII, established in 2009, is primarily used to support the measurement of product potency (activity) with approximately 1200 ampoules dispatched each year. Stocks of this WHO international standard were now depleted and were expected to be exhausted by the end of 2023.

Following studies on trial fills of a number of therapeutic FVIII concentrates, five candidate replacement materials (three plasma-derived and two recombinant products) had been evaluated in an international collaborative study involving 26 laboratories in 12 countries. The aims of the study had been to: (a) calibrate and assign a unitage to a replacement material; (b) assess the degree of discrepancy between one-stage clotting and chromogenic substrate assays; and (c) assess the differences (in IU) between concentrate and plasma. Using their routine validated methods, most participants performed either a chromogenic method or a one-stage method, with several laboratories performing both assay methods. Intra-laboratory variability expressed as the GCV ranged from 0.6% to 19.0% (with most laboratories below 5%) for assays relative to the current WHO international standard. Inter-laboratory variation was < 3% using the chromogenic method and < 5% using the one-stage method. The largest inter-laboratory variability was observed when the recombinant (rather than plasma-derived) candidate materials were assayed. Of all the candidate materials evaluated, the plasma-derived sample A (NIBSC code 21/142) showed most agreement in terms of the mean values obtained by both methods, as well as the lowest overall inter-laboratory variability for combined estimates (GCV = 2.4%) using both methods. Potential drift in the potency of concentrate compared with plasma was assessed by comparing the mean potencies obtained from assays calibrated either using the current WHO international standard or the earlier sixth WHO international standard. Use of the earlier reference standard was associated with significant differences between methods as well as higher levels of both intra- and inter-laboratory variability. It was concluded that calibration of the replacement material against the sixth WHO international standard would not be appropriate. Accelerated thermal degradation studies carried out over a

relatively short period indicated that overall the candidate material 21/142 was the most stable using either of the two assays.

Commenting on discrepancies noted in stability data generated by the two different assay types, the Committee was informed that no clear explanation could be given but was likely due to inherent differences between the methods. The Committee also noted the limited degradation that had been observed even in the short-term stability studies and was assured that the stability of the candidate material would continue to be monitored over a longer time frame should it be recommended for establishment.

Reflecting on the global use of this WHO reference standard, the Committee expressed concern that most of the collaborative study participants had been in Europe or USA. The Committee reiterated the importance of ensuring geographical representativeness in international collaborative studies wherever possible. The Committee was informed that following a wide call for participants, including through the International Society of Thrombosis and Haemostasis (ISTH), many laboratories had been unable to perform the study assay or had been eliminated based on their response to the pre-study questionnaire. The Committee accepted that it was often challenging finding laboratories both willing and able to participate in such studies, and that this had likely been exacerbated by resource pressures due to the COVID-19 pandemic.

The Committee considered the report of the study (WHO/BS/2023.2444) and, noting ISTH support for this reference standard, recommended that the candidate material 21/142 be established as the Ninth WHO International Standard for blood coagulation factor VIII concentrate with an assigned unitage of 9.5 IU/ampoule.

6. International reference materials – in vitro diagnostics

6.1 WHO international reference standards for in vitro diagnostics

6.1.1 WHO international reference reagents for antibodies to human leukocyte antigen

The presence of donor-specific antibodies to human leukocyte antigen (HLA) in transplant recipients can lead to hyperacute rejection thus making prospective testing for such antibodies in potential recipients a crucial step in ensuring successful transplantation. Recent developments in solid phase assay systems (such as flowcytometric and bead-based assays) have allowed for the far more sensitive detection of pre-sensitization in potential transplant recipients compared to the conventional complement-dependent cytotoxicity assay for antibody detection. To support assay validation, monitor trends and allow for the setting of acceptance criteria for sensitivity, anti-HLA run controls with different levels of alloreactivity have been developed and manufactured at MHRA as CE-IVD reagents for over 20 years.

Following endorsement by the Committee in 2022 of a proposal to provide such run controls as WHO international reference reagents to promote their global accessibility, an international collaborative study involving 21 laboratories in eight countries had been conducted to assess the suitability four candidate materials for use as run controls in flow cytometry crossmatch (FCXM) and Luminex (LX) bead-based assays. Two of the candidate materials (NIBSC codes 10/142 and 17/212) were intended for use as high and low background anti-HLA negative controls respectively, while candidate materials 17/238 and 21/378 were intended for use as strong and weak positive anti-HLA controls respectively for alloantibody characterization. Most study participants correctly identified candidate materials 10/142 and 17/212 as negative in both FCXM and LX assays. Most study participants also correctly identified candidate materials 17/238 and 21/378 as positive samples – though there was some disagreement as to whether candidate material 21/378 was strongly or weakly positive. This variation was considered to be due to laboratory-specific cut-off and assay threshold criteria, and it was concluded that the use of this material in combination with candidate material 17/238 would help harmonize such variations. The candidate materials were intended to serve as qualitative positive/negative controls rather than quantitative standards and therefore no unitage was proposed.

Post-production accelerated thermal degradation studies had been carried out at a range of temperatures up to 37 °C – however, the data did not fit the Arrhenius equation model and so the annual loss of activity could not be reliably estimated. MHRA monitors the real-time stability of anti-HLA antibody preparations that it produces by performing FCXM assays annually. Based on

real-time stability data for candidate material 10/142 a shelf-life of 10 years from the date of lyophilization has previously been assigned to these preparations to meet CE-marking requirements. However, as WHO international reference reagents, the standards would not be assigned an expiry date but instead their stability would be monitored in real time at the recommended storage temperature. Evidence obtained from previous testing indicates that these freeze-dried preparations have good long-term stability.

Noting that regional variations in donor HLA haplotype were to be expected, the Committee expressed concern that the collaborative study laboratories represented only a very narrow geographical distribution, and queried whether the results of the study could be extrapolated to populations worldwide. The Committee was reassured by evidence indicating that where the candidate materials had been used more widely, outside the current collaborative study, no issues had been reported. It was further speculated that any such issues may be identified by local proficiency programmes. Nevertheless, the Committee suggested that a cautionary note on the geographical limitations of the collaborative study be included in the IFU. The Committee went on to query whether the collaborative study had demonstrated sufficient agreement between participants and was assured that more than 90% of the laboratories had returned concordant results for the positive and negative samples. Less concordant results were only evident for the weakly positive sample 21/378, where differences between participant assay sensitivity, cut-off and threshold criteria were probably most critical.

Having received reassurance regarding the above matters, and having considered the report of the study (WHO/BS/2023.2445), the Committee recommended that the following four candidate materials be established as WHO international reference reagents for antibodies to human leukocyte antigen without assigned unitage:

- Candidate material 10/142 – negative plasma for antibodies to HLA
- Candidate material 17/212 – negative serum for antibodies to HLA
- Candidate material 17/238 – strong positive plasma for antibodies to HLA
- Candidate material 21/378 – weak positive plasma for antibodies to HLA.

6.1.2 First WHO International Standard for antibodies to citrullinated peptide/protein

Rheumatoid arthritis is an autoimmune disease characterized by chronic, erosive polyarthritis. Elevated levels of rheumatoid factor and various autoantibodies in serum and synovial fluid are markers of the autoimmune response. Along with

rheumatoid factor, autoantibodies known as anti-citrullinated protein antibodies (ACPA) are considered valuable markers of disease prognosis. The detection of ACPA depends on antibody binding to citrullinated peptide fragments, with various diagnostic assay kits having been developed based on a combination of synthetic citrullinated peptides. The use of different assay formats and synthetic antigen targets has led to high inter-assay variability and poor quantitative agreement between diagnostic laboratories. Although run controls and calibrators are provided with commercial kits, they are based on arbitrary units with no traceability to a common standard, and thus the quantitative values obtained vary significantly between kits. Although the need to standardize ACPA assays has been widely recognized, including by the International Union for Immunological Societies (IUIS), no preparation has yet been adopted as a reference standard for calibrating commercial assays.

Stocks of the historically used WHO international standard and other international reference materials produced in the 1960s were now depleted and their ACPA content unknown. In order to evaluate a new candidate material for its suitability to serve as a WHO international standard in ACPA assays, an international collaborative study had been conducted involving 27 laboratories in 14 countries. The candidate material (NIBSC code 18/204) had been commercially sourced, and consisted of a defibrinated plasma pool derived from five individuals diagnosed with rheumatoid arthritis. Participant laboratories used their in-house ACPA kits and assay formats to evaluate the candidate material 18/204, a comparator sample and an IUIS ACPA standard, along with serum samples obtained from individual patients to assess commutability.

Since the early 2000s, a range of ACPA assays have been developed. Although based on a similar methodology, the target antigen used by successive assay generations has been incrementally refined to improve specificity and sensitivity. A total of 19 different methods were used by participants with most being of the second-generation (anti-CCP2) type which uses cyclic citrullinated peptides to improve epitope presentation. Study results showed good agreement between the potency estimates for the coded duplicate samples, regardless of the assay used, with most intra-laboratory GCVs = < 10%. There was, however, a considerable spread in reported potencies across laboratories for all sample types (GCV = > 175%) highlighting the need for standardization. When expressed relative to candidate material 18/204, the inter-laboratory GCV for potency estimates of the comparator sample was reduced from 185% to 57%. The overall geometric mean potency of the candidate material 18/204 was 264 IU/mL (GCV = 175%). The calibration of samples against the candidate material resulted in a marked reduction in inter-laboratory variability, with most anti-CCP2 methods aligning. In addition, the candidate material proved to be commutable with clinical samples in 6 of the 8 different anti-CCP2 assay methods used. With regard to the other methods used, improved alignment was noted. Accelerated

thermal degradation studies carried out over 13 months indicated no significant loss of activity at elevated temperatures. The data also indicated that candidate material 18/204 would be sufficiently stable at 20 °C for shipment at ambient temperature and for long-term storage at −20 °C.

Commenting on the large spread of values, even when calculated relative to candidate material 18/204, the Committee acknowledged that the standardization of autoantibody measurement was challenging. The Committee was however satisfied that the prospective standard would be useful given the clear relative harmonization of assay data. The Committee considered the report of the study (WHO/BS/2023.2446) and recommended that the candidate material 18/204 be established as the First WHO International Standard for antibodies to citrullinated peptide/protein with an assigned unitage of 260 IU/ampoule.

6.1.3 Fifth WHO International Standard for hepatitis B virus DNA for NAT-based assays

Despite the existence of effective vaccines and antiviral therapies, hepatitis B virus (HBV) remains a significant public health problem worldwide. HBV is transmitted in blood and bodily fluids, making accurate and sensitive detection of its presence crucial in assuring the safety of blood and blood products. Nucleic acid amplification technique (NAT)-based assays have been used for blood screening since the 1990s. Such assays are also routinely used in the diagnosis and management of HBV infection, particularly in monitoring the response of chronically infected patients to antiviral therapy. A range of both commercial and laboratory-developed NAT-based assays are currently in use calibrated against the WHO international standard, which was first established in 1999 and which has now been replaced three times, most recently in 2016.

Stocks of the current Fourth WHO International Standard for hepatitis B virus DNA for NAT-based assays were now depleted and an international collaborative study involving 10 laboratories in seven countries had therefore been conducted to evaluate a candidate material for its suitability to serve as a replacement WHO international standard. The candidate material (NIBSC code 22/120) consisted of lyophilized human plasma containing HBV and was derived from a stock of Eurohep R1 reference material. Using a range of commercial real-time PCR assays, the candidate material had been evaluated alongside the current WHO international standard, the candidate liquid bulk, a secondary reference reagent and two HBV-positive plasma samples (genotype A and genotype E). Twelve data sets had been returned for analysis. Overall mean potency estimates for the current WHO international standard and for candidate material 22/120 were 6.00 and 6.01 $\log_{10}$ IU/mL respectively. Based on the overall mean of all assay methods relative to the current WHO international standard, a potency estimate of 5.99 $\log_{10}$ IU/mL was estimated

for candidate material 22/120. Agreement between the results obtained by different laboratories was improved when potency was expressed relative to the current WHO international standard, with inter-laboratory variability higher than intra-laboratory variability, underscoring the continued need for the standardization of HBV NAT-based assays and for accurate calibration using a WHO international standard. Accelerated thermal degradation studies indicted no drop in potency after 3 months, making it impossible to apply the Arrhenius model to estimate the stability during long-term storage at −20 °C. Although stability testing will continue, the initial data, together with experience gained with the previous international standards, indicated adequate stability.

Noting that no laboratory performing the commonly used Novartis HBV NAT-based assay had participated in the collaborative study, the Committee acknowledged the challenge of covering all commercially available NAT-based assays, and agreed that the omission was unlikely to have significantly impacted the study outcome. The Committee went on to discuss the relevance of the global distribution of HBV genotypes, which had not been addressed by the study. Although the A2 genotype on which the proposed standard was based was prevalent in Europe, other genotypes were common in other regions. However, as NAT-based assays used PCR primers to conserved sequences in the viral genome, variations in the genotype would not be expected to affect the utility of the proposed replacement standard in harmonizing assay results worldwide. With regard to assay sensitivity in detecting other genotypes, especially in window-period blood donations, the Committee was assured that the potential impact of genotypic variation would be taken into account when assessing the next replacement standard. In the meantime, it may be prudent to review available data from the collaborative study that had supported the establishment of the First WHO International Reference Panel for hepatitis B genotypes for NAT-based assays in 2009.

Noting that the issues raised had not caused any concerns during the use of previous iterations of the proposed international standard, the Committee considered the report of the study (WHO/BS/2023.2447) and recommended that the candidate material 22/120 be established as the Fifth WHO International Standard for hepatitis B virus DNA for NAT-based assays with an assigned unitage of 5.69 $\log_{10}$ IU/vial.

6.2 Proposed new projects and updates – in vitro diagnostics

6.2.1 Proposed First WHO International Standard for antibodies to tissue transglutaminase

Coeliac disease is a long-term autoimmune disorder primarily affecting the small intestine, and is a major public health problem worldwide. Analysis suggests that its incidence has increased by an average of 7.5% per year in recent decades,

with the highest incidences observed in females and children. The pooled global prevalence of coeliac disease has been estimated to be 1.4%. Diagnosis is typically based on a combination of coeliac-specific serology and duodenal biopsy findings. Critical biomarkers include IgA and IgG autoantibodies against tissue transglutaminase (tTG) in human serum, and variations in their levels are used as part of disease management.

Although commercially available diagnostic test kits are available to measure the levels of these autoantibodies, the controls and calibrators supplied with such kits are assigned arbitrary units (usually U/mL). Consequently, the results obtained from different test kits are not comparable and positive/negative threshold values vary significantly. Moreover, several studies have shown that the manufacturer-recommended assay cut-offs are not optimal for the majority of these assays. Calibration against an international standard would improve comparability between different tTG autoantibody tests, and improve disease monitoring. It was anticipated that such an international standard would be used by diagnostic kit manufacturers to calibrate their internal standards, and by research institutions.

A candidate material based on serum obtained from the plasmapheresis of a single patient with coeliac disease had been commercially sourced. Working with MHRA, the European Commission Joint Research Centre (JRC) intends to establish this material as a working standard to be made available from its catalogue – however, a proportion of the material would also be assessed for its suitability to serve as an international standard with assigned units in IU/vial. The international standard would then be used to calibrate regional, national and other secondary standards, while the working standard would be used by diagnostic kit manufacturers and clinical laboratories for test calibration, as well as by research institutions and external quality assurance schemes. MHRA and JRC would then coordinate distribution to ensure that both standards were exhausted at the same time to facilitate replacement and ensure unit alignment. An international collaborative study would be conducted in two parts: (a) evaluation of the suitability of the candidate material and assignment of unitage; and (b) assessment of commutability of the candidate material with individual patient samples.

Having been assured that the collaborative study would involve most kit manufacturers globally and would include other laboratories, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a First WHO International Standard for antibodies to tissue transglutaminase.

7. International reference materials – standards for use in high-throughput sequencing technologies

7.1 Proposed new projects and updates – standards for use in high-throughput sequencing technologies

7.1.1 Proposed WHO International Reference Reagent for control of RNA extraction, PCR and nanopore sequencing for direct detection of poliovirus

One major objective of the WHO Polio Eradication Strategy 2022–2026 is to improve the detection of, and response to, polioviruses through sensitive surveillance. As a global specialized laboratory for poliovirus detection, research and development, MHRA plays an important role in supporting the Global Polio Laboratory Network (GPLN) that conducts routine surveillance for polioviruses in clinical and environmental samples. To support the endgame of poliovirus eradication, rapid and direct detection methods are required to allow for rapid responses to outbreaks and to eliminate the risks associated with the cell culturing of polioviruses.

Although various molecular diagnostic methods exist for poliovirus, the nanopore sequencing of such viruses is relatively new. By using a nested polymerase chain reaction (PCR) approach to sequence the VP1 PCR product (commonly used by the GLPN for the genetic characterization of polioviruses) following the direct detection by nanopore sequencing (DDNS) protocol, vaccine-derived and wild-type polioviruses can be identified within 3 days of sample receipt. Recent DDNS protocol training activities had highlighted the need for a positive control for the RNA extraction, PCR and nanopore sequencing steps. Such a positive control would support method development and could be used as a run control in the DDNS protocol. As there was currently no positive control that could be used to test the complete workflow, starting from RNA extraction up to sequence generation, it was proposed that a WHO international reference reagent be developed to meet this need.

The WHO international reference reagent would be based on Coxsackievirus A20 isolates grown in cell culture provided by MHRA, and would be assessed for suitability in a collaborative study involving several GPLN laboratories, as well as other clinical laboratories and research organizations that detect poliovirus on a regular basis. The collaborative study would include DDNS protocol and Sanger sequencing methods, with stool-based or sewage-based sample formulations used to assess the effect of the matrix on extraction. With the sourcing of material now complete, it was anticipated that the report of the collaborative study would be submitted to the Committee for its consideration in October 2024.

Having clarified that the number of collaborative study participants would be limited because DDNS was a relatively new approach, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a WHO International Reference Reagent for control of RNA extraction, PCR and nanopore sequencing for direct detection of poliovirus.

8. International reference materials – standards for use in public health emergencies

8.1 WHO international reference standards for use in public health emergencies

8.1.1 Expansion of the First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern

The First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern (VOC) was established in 2022 and is used to facilitate serological assay development. At its establishment, the three-member panel consisted of pooled convalescent plasma or sera obtained from unvaccinated individuals infected with either an early 2020 SARS-CoV-2 isolate, an Alpha variant or a Delta variant. In order to expand the panel to include antibodies to Gamma and Omicron variants, an international collaborative study had been conducted involving nine laboratories in seven countries. Participant laboratories had assessed the suitability of two candidate materials for use in serological assays detecting antibodies to SARS-CoV-2. One of the candidate materials (NIBSC code 22/126) was a pool of plasma obtained from six donors in Brazil and collected at the peak of disease caused by the Gamma variant in 2021. The other candidate material (NIBSC code 22/128) was a pool of convalescent plasma obtained from nine donors in South Africa who had been infected with sequence-confirmed Omicron variant B.1.1.529. The specifications of the freeze-dried candidate materials were consistent with those recommended for WHO reference materials.

To ensure continuity with the 2022 collaborative study used to establish the panel, the candidate materials were assessed along with two members of the current panel (NIBSC codes 21/296 and 21/300), the Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code 21/340) and the First WHO International Standard for antibodies to SARS-CoV-2 variants of concern (NIBSC code 21/338). The materials were evaluated using a range of neutralization assays, with their reactivity in binding assays primarily targeting IgG responses to the spike, receptor binding domain or nucleoprotein also investigated. Study results indicated that both candidate materials performed well in all assays, with good agreement observed among laboratories in ranking the samples. The neutralization titre of the candidate Gamma material was found to be comparable to that of the Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin in assays using early 2020 isolates, with good activity also observed against the VOC, including Omicron BA.1 and BA.2. The candidate Omicron material exhibited the lowest potency of all the collaborative study samples against the early 2020 isolates but was among the highest titre samples against the Omicron isolates. Based on the results from a small number

of laboratories, a similar pattern of responses had been observed for the binding antibodies to the spike and receptor binding domain. Accelerated thermal degradation studies indicated that both candidate materials were sufficiently stable to serve as international standards, with data indicating no loss of potency for at least 2 weeks at temperatures up to 45 °C. Although long-term stability could not be predicted by applying the Arrhenius model, the data suggest that the candidate materials would be suitable for storage at 20 °C and could be shipped at ambient temperature.

Reflecting on the challenge of keeping pace with the rapid evolution of SARS-CoV-2 variants, the Committee applauded the work that had been done, and was assured that the candidate materials would cover the currently predominant BA.4 and BA.5 variants. The Committee considered the report of the study (WHO/BS/2023.2450) and recommended that the candidate materials 22/126 and 22/128 be added to the First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern without assigned unitage.

9. International reference materials – vaccines and related substances

9.1 WHO international reference standards for vaccines and related substances

9.1.1 Second WHO International Standard for meningococcal serogroup C polysaccharide

The bacterium *Neisseria meningitidis* (meningococcus) is an important cause of meningitis and bacteraemia worldwide. Highly effective vaccines based on the meningococcal capsular polysaccharide are widely available as monovalent and multivalent formulations and offer protection against serogroups A, C, W and Y. The measurement of both total polysaccharide content and unconjugated polysaccharide content is crucial in assessing vaccine potency and quality. Following the approval of the first monovalent meningococcal serogroup C (MenC) conjugate vaccine more than 20 years ago, the First WHO International Standard for meningococcal serogroup C polysaccharide was established in 2011.

Since that time, steady demand had resulted in the depletion of this WHO international standard and in 2022 the Committee had endorsed a proposal to evaluate a manufacturer-donated material for its suitability to serve as a replacement international standard. In recent years, several such bacterial polysaccharide standards had been established with one key issue being the way in which SI units were assigned to this group of standards. The most recent such standards had been assigned SI units based on quantitative nuclear magnetic resonance (qNMR) spectroscopy as the primary method. To reduce the relatively high degree of uncertainty of measurement associated with this technique it had further been proposed that study laboratories performing qNMR spectroscopy would be provided with a comprehensive protocol for performing the analysis along with a certified reference material to reduce inter-laboratory variability. As part of the study design, resorcinol assays would also be used to ensure continuity with the current WHO international standard.

An international collaborative study involving 20 laboratories in 11 countries had been conducted using various assay methods including qNMR spectroscopy, resorcinol assays, high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and immunoassays. The candidate material (NIBSC code 20/314) was filled and freeze-dried in glass ampoules. Study results showed that intra-laboratory variability was lowest using qNMR (median coefficient of variation (CV) = 1.4%), with both resorcinol and HPAEC-PAD methods being more variable, (median CV = 4.1% and 4.2% respectively). Inter-laboratory variability was also lowest for qNMR (CV = 3.5%), highest for the HPAEC-PAD method (CV = 9.6 %) and intermediate the resorcinol assay (CV = 6.3 %). The robust mean

MenC polysaccharide content of candidate material 20/314 estimated by qNMR was 964.6 µg/ampoule, with no significant difference obtained using the other assay types. Using the data obtained by qNMR a combined standard uncertainty of 1.12% was calculated corresponding to an expanded uncertainty of ± 23.7 µg/ampoule (k = 2.20). It was anticipated that vaccine manufacturers and control laboratories would need to assess the impact of changing to the replacement standard, with such impact likely to depend on assay method, which standard had previously been used and the product specifications being tested. Real-time stability studies of the candidate material stored at −20 °C performed 12 months after production indicated that the material was stable with respect to both MenC polysaccharide content and molecular size. Similarly, reconstituted material stored at −20 °C also proved to be stable for at least 11 months. Modelling based on molecular sizing data using the Arrhenius equation indicated a rate of change of 0.015% per month.

The Committee was in agreement that qNMR, as a primary measurement method, was the appropriate way to assign units to polysaccharide reference standards. This approach was also scientifically justified as it had been associated with the lowest CV in the study data. However, although the unitage would be assigned by qNMR, laboratories using the standard were likely to use other methods, with in-house standards calibrated against the WHO international standard. The Committee therefore advised that the IFU should contain sufficient information to support users establishing the necessary conversion factors. Although manufacturers were highly experienced in conducting impact assessments and applying conversion factors as necessary, other users may require more technical support. The Committee considered the report of the study (WHO/BS/2023.2448) and recommended that the candidate material 20/314 be established as the Second WHO International Standard for meningococcal serogroup C polysaccharide with an assigned unitage of 0.965 ± 0.024 mg/ampoule. The Committee further recommended that the nomenclature used for all such meningococcal polysaccharide standards should be consistent on the WHO and MHRA websites.

9.1.2 First WHO International Standard for antibodies to Rift Valley fever virus for neutralization assays (human plasma); and First WHO International Standard for antibodies to Rift Valley fever virus for binding assays (human plasma)

Rift Valley fever (RVF) is a zoonotic viral infection transmitted to humans through direct or indirect exposure to blood, bodily fluids and other tissues of infected animals, or through the bite of infected mosquitoes. Immunoassays detecting antibodies to RVF virus are typically used for diagnosis. Overall mortality is estimated to be less than 3% with the majority of human cases being mild.

However, in a small proportion of cases, more severe symptoms can develop, including eye disease (potentially resulting in blindness), meningoencephalitis, hepatitis and haemorrhagic fever. Although RVF is endemic in sub-Saharan Africa, outbreaks have also been recorded elsewhere in Africa and in Arabia, and the RVF virus has been identified by the WHO R&D Blueprint for action to prevent epidemics as a top 10 priority pathogen due to its outbreak potential. Although there are currently no specific treatments or licensed RVF vaccines, several vaccine candidates are in development and WHO proposed requirements for RVF vaccines (inactivated) have been published.

Recognizing the need to facilitate the standardization of the serological assays required to develop RVF vaccines and therapeutics, the Committee had endorsed a proposal in 2019 to develop a WHO international standard for antibodies to RVF virus. An international collaborative study involving 19 laboratories in 10 countries had now been conducted to evaluate a candidate material (NIBSC code 22/104) in a wide range of neutralization and binding assays. The candidate material comprised a lyophilized pool of plasma obtained from seven Ugandan donors who had recovered from RVF. The candidate material was assessed as part of a blinded sample panel which also included pools of convalescent sera of differing antibody titres obtained from Kenyan donors, two convalescent plasma donations from Tunisia and the liquid bulk form which the candidate material had been derived.

Study results indicated that the candidate material in both its final lyophilized formulation and liquid bulk form produced the highest detected potency in all neutralization assays. Similarly, both materials ranked as the highest-titre IgG samples detected by all participants using quantitative binding methods that targeted either specific domains of the glycoprotein or the whole virus. Expressing the neutralizing and binding antibody titres obtained relative to the candidate material 22/104 reduced inter-laboratory variation. Although quantitative methods calibrated to the proposed WHO international standard would be vital in supporting vaccine development, most antibody binding methods used in the collaborative study targeted the whole virus or the nucleoprotein and provided a qualitative result. Similarly, the study assays specifically detecting IgM responses were qualitative. Overall, there was good agreement in scoring a sample as either positive or negative for the antigen targeted. Although the results of the first 6 months of an accelerated thermal degradation study were too variable to fit the Arrhenius model, and thus to predict its long-term stability, the data suggested that the candidate material 22/104 would be sufficiently stable to serve as an international standard and to be shipped at ambient temperature.

The Committee considered the report of the study (WHO/BS/2023.2449) in the context of its earlier decisions on the nomenclature and assignment of

units to different types of antibody standards (see section 2.1.3 above). The Committee agreed that the study data supported the assignment of IU to two separate reference standards: one for use in neutralization assays and the other for use in antibody binding assays. The Committee therefore recommended that the candidate material 22/104 be established as both the First WHO International Standard for antibodies to Rift Valley Fever Virus for neutralization assays with an assigned unitage of 250 IU/ampoule, and as the First WHO International Standard for antibodies to Rift Valley Fever Virus for binding assays with an assigned unitage of 250 IU/ampoule (specific to anti-glycoprotein IgG). The Committee further recommended that the IFU for the latter material should recommend that users specify the antigenic region of the glycoprotein targeted by their method.

9.2 Proposed new projects and updates – vaccines and related substances

9.2.1 Proposed WHO International Reference Reagent for diphtheria antitoxin for use in flocculation test (equine)

Diphtheria vaccines are one of the most widely used and successful human vaccines, and form an essential component of the primary immunization schedule of children, as well as being used for the reinforcement of immunity in adults and adolescents. Measurement of the limit of flocculation (Lf) content is important in the production of diphtheria and diphtheria toxoid-containing glycoconjugate vaccines as such products are formulated based on the Lf content of the bulk purified toxoid. In addition, diphtheria toxoid used in the production of vaccines for human use has to meet minimum requirements for antigenic purity, expressed as the Lf content per mg of protein nitrogen.

The flocculation test is an antibody binding assay that requires the use of a reference material to ensure its standardization. The current non-WHO reference reagent (NIBSC code 63/007) consisted of a lyophilized hyperimmune equine serum and had been established in 1963 as the Fourth British Reference preparation. Following high levels of demand, stocks of this material were now completely depleted. It was envisaged that the current level of demand of around 300 ampoules per year would continue due to the use of the flocculation test by vaccine manufacturers. Despite a number of challenges, sufficient equine diphtheria antitoxin had now been purchased to produce around 2000 ampoules of a replacement reference material with an approximate potency of 1250 IU/mL. It was proposed that the candidate material be calibrated in an international collaborative study involving laboratories using the WHO recommended flocculation (Ramon) method. Stability studies would then be carried out following establishment. Experience with similar reference standards indicated that the candidate material would be stable even at elevated temperatures for

many years. It was envisaged that the results of the collaborative study would be submitted for consideration by the Committee in October 2024.

Noting that the proposed collaborative study was similar to that recently conducted to support establishment of the current WHO international reference reagent for tetanus antitoxin, and recognizing the urgent need for this reference material, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a First WHO International Reference Reagent for diphtheria antitoxin for use in flocculation test (equine).

9.2.2 Proposed First WHO International Standard for antibodies to gonococcus (serum)

Neisseria gonorrhoeae (gonococcus) is the causative agent of gonorrhoea and a major cause of sexually transmitted infections worldwide, with more than 80 million cases reported in 2020. Previous infection with gonococcus does not confer protective immunity and re-infection is common among at-risk populations. WHO has set a target of reducing the incidence of gonorrhoea by 90% by 2030 and, despite being treatable with antibiotics, effective vaccines will be required if this is to be achieved – in large part due to the increasing emergence of antimicrobial resistance. Nevertheless, despite decades of research efforts, there is currently no approved vaccine, with vaccine development currently at the preclinical or early clinical stage. Recent renewed enthusiasm for vaccine development has been driven by the observation that implementation of a meningococcal outer membrane vesicle vaccine in New Zealand in 2004 also conferred cross-protection against gonococcal infection with an estimated effectiveness of 30%.

As serological assays are essential for evaluating immune responses to candidate vaccines, the development of a WHO international standard for antibodies to gonococcus was being proposed. Such an international reference material would enable laboratories to set up and monitor such assays, thus potentially allowing for the harmonization of antibody response measurements between different laboratories and clinical trials. An international collaborative study would be carried out to assess the suitability of a candidate material derived from pooled human serum donated by immunized volunteers. It was envisaged that the results of the collaborative study would be submitted for consideration by the Committee during 2026–2027.

Noting that gonorrhoea vaccine development was at a relatively early stage, the Committee welcomed the timely opportunity to consider this proposal, even if it may have to change over time to cover emerging candidate vaccines. Although evidence that the meningococcal outer membrane vesicle vaccine provides some protection against gonorrhoea was encouraging, the Committee reflected on the challenges in developing a gonorrhoea vaccine and in sourcing and developing the proposed reference standard. These challenges included

the extreme antigenic variability of the gonococcus, and the implications of this on the antigenic coverage of both prospective vaccines and the proposed international standard. In addition, there was a lack of evidence of immunity in recovering patients, and a need to develop correlates of protection. Despite these and other challenges, the Committee regarded this as an important project and endorsed the proposal (WHO/BS/2023.2451) to develop a First WHO International Standard for antibodies to gonococcus (serum).

While acknowledging that the development of WHO written standards needed to be matched to WHO priorities and resources, the Committee noted that in addition to the licensed meningococcal vaccines now used worldwide, gonorrhoea was one of an increasing number of bacterial diseases for which outer membrane vesicle vaccines were being developed. Such developments might benefit from the availability of a WHO written standard specifically on this type of vaccine.

9.2.3 Proposed WHO International Reference Reagent for messenger RNA lipid nanoparticles

Messenger RNA (mRNA) encapsulated in lipid nanoparticles (LNPs) has been the production platform underlying the most widely used COVID-19 vaccines. This in turn has reinforced the perceived utility of the approach in the development of a wider range of safe and effective products. Numerous such mRNA-LNP products are now in clinical development for the prevention or treatment of other infectious diseases, cancer, and genetic disorders. The characterization and routine testing of these products requires the development of analytical methods and it was envisaged that the development of a robust, stable and well-characterized reference material would support method development, validation and control. The proposed WHO international reference reagent: (a) would have a nominal mRNA content and would serve as an assay control; (b) is not intended to be used as a calibrant or to define any regulatory parameter; and (c) would be based on an LNP-encapsulated generic mRNA target so as to be product agnostic. Intended to serve as a control material during assay development, troubleshooting and routine performance monitoring, the anticipated users of the proposed reference standard would include manufacturing laboratories, contract research organizations, academic laboratories and national control laboratories. An international collaborative study would be carried out to assess the suitability of the candidate material to serve as a reference standard in different analytical methods including separation techniques, encapsulation and expression assays. It was envisaged that the results of the collaborative study would be submitted for consideration by the Committee in 2024.

Acknowledging the success of vaccines based on LNP-encapsulated mRNA and the potential for future developments in this field, the Committee commended this initiative to support the standardization of the analytical

methods that would be used to characterize and control such products. However, concern was also expressed that the utility of a reference reagent based on a generic mRNA target would be limited given the importance of mRNA sequence specificity in product testing. Nevertheless, the Committee accepted that the proposed reference standard was intended to support the development of analytical methods rather than regulatory decisions, and that not all analytical tests were based on the mRNA sequence. It also noted that MHRA and other organizations had highlighted that such a control material would have been useful when setting up methods for testing COVID-19 vaccines, and considered that the project was likely to proceed regardless of WHO endorsement. After careful consideration, the Committee decided not to endorse the proposal (WHO/BS/2023.2451) to develop a First WHO International Reference Reagent for messenger RNA lipid nanoparticles but instead endorsed a pilot study to explore the likely levels of demand and usage of such a reference standard with a range of stakeholders, including biotechnology companies, vaccine developers and manufacturers, and national control laboratories.

9.2.4 Proposed First WHO International Standard for antibodies to Mpox virus

First recognized in 1970, Mpox (formerly monkeypox) is endemic to Central and West Africa, with occasional outbreaks linked to travel or the export of animals from endemic areas. The causative mpox virus is related to the other pathogenic orthopoxviruses, namely smallpox and cowpox. Following a large outbreak of Mpox starting in May 2022, WHO declared a public health emergency of international concern. Currently available smallpox vaccines offer a degree of protection against Mpox, with a reported efficacy of 85% based on observational studies. Currently three smallpox-based vaccines were approved in certain countries for the prevention of Mpox in at-risk populations, along with a single antiviral drug. New vaccines were now in preclinical development, including mRNA vaccines based on a horsepox virus.

Establishing a WHO international standard for Mpox antibodies would support the development and harmonization of the serological assays needed to monitor vaccine-induced immune responses, new treatments and disease epidemiology. In 2022, MHRA, in partnership with CEPI, produced a research reagent (NIBSC code 22/218) based on a pool of convalescent plasma from 100 individuals that could potentially serve as a WHO international standard. Initial results from a pilot study involving five laboratories had indicated that the material would be suitable for use in both neutralization and antibody binding assays. However, issues arising from the proposal include a lack of commercially available assays, complications in the interpretation of results due to cross-reactivity between different orthopoxviruses, declining incidence of Mpox

worldwide and poor uptake of the research reagent. If endorsed, the proposed collaborative study would be based on the information collected through the pilot study – though additional samples would be needed, including plasma or serum obtained from vaccinees. It was envisaged that the results of the collaborative study would be submitted for consideration by the Committee in October 2024.

Reflecting on the significance of Mpox, the Committee agreed that initiation of the collaborative study should not be deferred because of the recent decrease in global disease incidence as Mpox outbreaks would continue to sporadically occur. Having being assured that the collaborative study design would take into account the range of Mpox virus variants, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a First WHO International Standard for antibodies to Mpox virus.

9.2.5 Proposed Third WHO International Standard for antibodies to hepatitis B virus surface antigen

Hepatitis B is a potentially life-threatening liver disease caused by the hepatitis B virus (HBV). HBV infection is a significant health problem worldwide and can lead to chronic infection that increases the risk of mortality from cirrhosis and liver cancer. Disease burden is highest in the Western Pacific and Africa. In highly endemic areas, HBV is mostly spread from mother to child at birth or through horizontal transmission. Transmission can also occur through needlestick injury, tattooing, piercing and exposure to infected blood and bodily fluids. Highly effective hepatitis B vaccines are available and are typically administered to infants in combination with other paediatric vaccines. While infection acquired in adulthood leads to chronic hepatitis in less than 5% of cases, infection in infancy and early childhood leads to chronic hepatitis in about 95% of cases. In addition to its use in standardizing the evaluation of vaccine potency, the WHO international standard is also needed to monitor the antibody content of immunoglobulin therapies and to calibrate controls for diagnostic kit evaluation.

Based on current levels of demand, the current WHO international standard (NIBSC code 07/164) would likely be depleted by the middle of 2024. Although a bulk immunoglobulin would be the preferred material for the proposed replacement, plasma or serum may also be suitable source materials as they are commutable and similar to clinical samples. It was envisaged that the results of the proposed collaborative study would be submitted for consideration by the Committee in 2024.

Noting that the highest burden of HBV infection was in the Western Pacific and Africa, the Committee strongly recommended that the collaborative study be sufficiently representative and include laboratories from regions with high levels of infection. Acknowledging the importance of this reference

standard, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a Third WHO International Standard for antibodies to hepatitis B virus surface antigen.

9.2.6 Proposed Third WHO International Standard for antibodies to hepatitis A virus

Hepatitis A virus (HAV) causes an inflammatory disease of the liver and is usually spread by ingestion of contaminated food or water. HAV infections are common in LMIC with poor sanitary conditions and hygiene practices, with sporadic outbreaks occurring worldwide. Epidemics related to contaminated food or water can quickly arise and can be prolonged, affecting communities for months through person-to-person transmission. The symptoms of hepatitis A vary widely, with adults exhibiting symptoms more often than children, and with disease severity tending to be higher in older age groups. HAV persists in the environment and is not necessarily inactivated by food production processes. A number of effective hepatitis A vaccines are licensed worldwide as either single or combination formulations, with immunoglobulin therapies also available.

HAV antibody reference standards are used to harmonize the assays used to assess immunity to infection and vaccination responses, monitor antibody levels in immunoglobulin preparations, and calibrate controls for diagnostic kits. Stocks of the current WHO international standard (NIBSC code 97/646) were now running low and were predicted to be depleted by the end of 2024. An international collaborative study involving 6–10 laboratories was being proposed to calibrate a replacement material. A donated liquid bulk (16% immunoglobulin) would be used as the source material to produce a minimum of 5000 ampoules. Anticipated users included clinical and public health laboratories, vaccine manufacturers, assay kit manufacturers and research laboratories. It was envisaged that the results of the proposed collaborative study would be submitted for consideration by the Committee in 2024.

Noting that the proposal appeared to be clear and straightforward, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a Third WHO International Standard for antibodies to hepatitis A virus.

9.2.7 Proposed Second WHO International Standard for antibodies to varicella zoster virus

Varicella zoster virus (VZV) causes varicella (chickenpox) and in 10–20% of cases can remain latent in neural ganglia, with subsequent reactivation resulting in herpes zoster (shingles). Varicella is characterized by an itchy rash, most often starting on the scalp and face, accompanied by fever and malaise. After gradually spreading to the trunk and other extremities, the rash typically heals in 2–3 weeks, but complications such as pneumonia or encephalitis can occasionally occur

– sometimes with serious or fatal consequences. Safe and effective attenuated varicella vaccines are available worldwide, including in combined formulations with measles-mumps-rubella (MMR) vaccines. A vaccine formulation containing higher levels of the virus has also been developed for the prevention of shingles in the elderly, while immunoglobulin therapy is used in immunosuppressed patients to control infection. Although diagnosis is typically based on signs and symptoms, antibody-based diagnostic kits are also used.

The current WHO international standard (NIBSC code W1044) is primarily used by clinical and public health laboratories, vaccine manufacturers, assay kit manufacturers and research organizations in assays measuring VZV antibody levels and in the calibration of diagnostic kits. Stocks of this international standard were now low and predicted to be depleted by the end of 2024. An international collaborative study, involving 6–10 laboratories, was being proposed to calibrate a replacement standard intended to last for around 10 years. Although a bulk immunoglobulin would be the preferred material for the proposed replacement, plasma or serum may also be suitable source materials as they are commutable and similar to clinical samples. Sourcing sufficient reactive material to produce enough standard to last for about a decade was recognized as a potential challenge. It was envisaged that the results of the proposed collaborative study would be submitted for consideration by the Committee in 2024.

Noting that the collaborative study design was likely to be similar to that used for the replacement of the HBV and HAV antibody standards discussed above (see sections 9.2.5 and 9.2.6), the Committee encouraged the study organizers to ensure that participant laboratories represented as many regions as possible. After due consideration, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a Second WHO International Standard for antibodies to varicella zoster virus.

9.2.8 Proposed Fourth WHO International Standard for inactivated poliomyelitis vaccine

Poliomyelitis (polio) is a highly infectious disease caused by poliovirus that can result in total paralysis and death. Polio largely affects children under 5 years of age but is a threat to any unprotected individual. The three poliovirus serotypes are all transmitted by person-to-person spread mainly through the faecal-oral route. There is no treatment for polio but the global implementation of highly effective vaccines has brought the disease to the brink of eradication. As a result of the WHO Global Polio Eradication Initiative, cases of disease caused by wild-type poliovirus have decreased by 99% since 1988 with only serotype 1 still circulating in two countries. Inactivated poliomyelitis vaccine (IPV) will be an essential element in completing the global eradication of the disease, and its

use has been steadily increasing with many products prequalified by WHO and licensed worldwide.

The WHO international standard is used primarily by manufacturers and national control laboratories to calibrate secondary reference standards and harmonize bioassays that measure the D-antigen content of IPV derived from wild-type poliovirus strains. Based on anticipated demand, the current standard would be depleted by 2026 and a proposal for its replacement was presented to the Committee. An international collaborative study involving at least 10 laboratories would be carried out to calibrate a prospective replacement WHO international standard. Two candidate materials consisting of commercial lots of IPV had been donated by European vaccine manufacturers and would be evaluated for their reactivity and specificity. It was envisaged that the results of the proposed collaborative study would be submitted for consideration by the Committee in 2024.

Noting that this appeared to be a straightforward replacement of an existing international standard, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a Fourth WHO International Standard for inactivated poliomyelitis vaccine.

9.2.9 Proposed WHO International Reference Reagent for antibodies to Ross River virus

Ross River virus (RRV) is a zoonotic virus transmitted by a variety of mosquito vectors and is the cause of Ross River fever. Ross River fever is the most common vector-borne disease in Australia but has also been reported in the Pacific Islands and among travellers returning from endemic regions. Ross River fever is characterized by rash, fatigue and polyarthralgia that can last for weeks, months or even longer – though RRV infection is estimated to be asymptomatic in approximately 30% of cases. Diagnosis is based on serum IgG evaluation, with currently no treatment or licensed vaccine available. As infections can be mild or asymptomatic, the epidemiology of RRV infection is not well understood and standardized assays are thus important for serosurveillance. Such assays will also be crucially important for the clinical development of treatments and vaccines.

The availability of several plasmapheresis donations presented an opportunity to prepare an international reference reagent for antibodies to RRV. RRV antibodies in these source materials had been identified using antibody binding assays and confirmed by microneutralization assays. Approximately 3000 vials of lyophilized candidate material had been prepared using plasma pooled from the antibody-positive donors. Potential users of the reference material included research laboratories, organizations developing Ross River fever vaccines, IVD manufacturers and clinical laboratories. An international collaborative study involving laboratories representing these potential users was

being proposed to assess the potency and specificity of the candidate material.

Noting the similarities of RRV to other emerging arboviruses such as chikungunya virus and Zika virus, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a WHO International Reference Reagent for antibodies to Ross River virus.

9.2.10 Proposed First WHO International Standard for antibodies to SARS-CoV-1

Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) is the coronavirus that caused the 2002–2004 SARS outbreak and is listed as a priority pathogen by the WHO R&D Blueprint. The outbreak started in South-East Asia and had an overall case fatality rate of 9% – though with markedly higher fatality rates among those over 60 years of age. The pandemic potential of the virus, which evidence suggests was of zoonotic origin, was demonstrated by the detection of cases in numerous countries across five continents in a space of several weeks. The isolation of closely related coronaviruses from potential reservoir and intermediary host species means that the threat of future outbreaks, including pandemic outbreaks, remains. Following the COVID-19 pandemic, efforts have refocused on the development of pan-coronavirus vaccines and therapeutics, with CEPI currently funding 13 such potential vaccine developments.

WHO international standards for antibodies to MERS-CoV (responsible for Middle East respiratory syndrome) and to SARS-CoV-2 (responsible for the continuing COVID-19 pandemic) have already been established. An international standard for antibodies to SARS-CoV-1 would facilitate vaccine development and regulatory approval, as well as help define immunological correlates of protection. It was anticipated that such a reference standard would be used by national control laboratories, public health laboratories, vaccine and therapeutic antibody manufacturers, assay kit producers and research laboratories.

The candidate material would be produced from source material donated by NIH (USA) (500 mL of purified IgG manufactured under GMP for therapeutic use) which had been prepared from pooled convalescent plasma collected from individuals in Hong Kong during the 2002–2004 SARS outbreak. The proposed collaborative study would involve 15–20 laboratories worldwide using a range of serological assays for the detection of SARS-CoV-1 antibodies. The laboratories would include representatives of the various prospective users of the reference standard. To evaluate the performance of the candidate material and assess its commutability, the study panel would also include convalescent serum/plasma samples obtained from other convalescent individuals and encompassing a range of antibody titres. As the candidate material is a therapeutic intravenous immunoglobulin there may be issues with regard to its commutability and

different formulations of the material would be investigated. It was envisaged that the results of the proposed collaborative study would be submitted for consideration by the Committee in October 2024 or early 2025.

Highlighting the importance of the proposed reference standard in the context of pandemic preparedness, and following due consideration of the issues raised, the Committee endorsed the proposal (WHO/BS/2023.2451) to develop a First WHO International Standard for antibodies to SARS-CoV-1.

Annex 1

WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products

WHO Recommendations, Guidelines and other documents are intended to provide guidance to those responsible for the development and manufacture of biological products as well as to others who may have to decide upon appropriate methods of assay and control to ensure that such products are safe, reliable and potent. WHO Recommendations (previously called Requirements) and Guidelines are scientific and advisory in nature but may be adopted by an NRA as national requirements or used as the basis of such requirements.

Recommendations and guidance on biological products are formulated by international groups of experts and published in the WHO Technical Report Series[6] as listed below. A historical list of Requirements and other sets of Recommendations is available on request from the World Health Organization, 20 avenue Appia, 1211 Geneva 27, Switzerland.

Reports of the WHO Expert Committee on Biological Standardization published in the WHO Technical Report Series can be purchased from:

WHO Press
World Health Organization
20 avenue Appia, 1211 Geneva 27
Switzerland
Email: bookorders@who.int
Website: www.who.int/bookorders

Individual Recommendations and Guidelines and other documents may be obtained free of charge as offprints by writing to:

Technical Standards and Specifications unit
Department of Health Product Policy and Standards
Access to Medicines and Health Products
World Health Organization
20 avenue Appia, 1211 Geneva 27
Switzerland

[6] Abbreviated in the following pages to "TRS".

Recommendations, Guidelines and other documents	Reference
Animal cells, use of, as in vitro substrates for the production of biologicals	Revised 2010, TRS 978 (2013)
BCG vaccines (dried)	Revised 2011, TRS 979 (2013)
Biological products: good manufacturing practices	Revised 2015, TRS 999 (2016)
Biological standardization and control: a scientific review commissioned by the UK National Biological Standards Board (1997)	Unpublished document WHO/BLG/97.1
Biological substances: International Standards and Reference Reagents	Revised 2004, TRS 932 (2006)
Biosimilars, evaluation of	Revised 2022, TRS 1043 (2022)
Biotherapeutic products, changes to approved biotherapeutic products: procedures and data requirements	Adopted 2017, TRS 1011 (2018)
Biotherapeutic protein products prepared by recombinant DNA technology	Revised 2013, TRS 987 (2014); Addendum 2015, TRS 999 (2016)
Blood, blood components and plasma derivatives: collection, processing and quality control	Revised 1992, TRS 840 (1994)
Blood and blood components: management as essential medicines	Adopted 2016, TRS 1004 (2017)
Blood components and plasma: estimation of residual risk of HIV, HBV or HCV infections	Adopted 2016, TRS 1004 (2017)
Blood establishments: good manufacturing practices	Adopted 2010, TRS 961 (2011)
Blood plasma (human) for fractionation	Adopted 2005, TRS 941 (2007)
Blood plasma products (human): viral inactivation and removal procedures	Adopted 2001, TRS 924 (2004)
Blood regulatory systems, assessment criteria for national	Adopted 2011, TRS 979 (2013)
Cholera vaccines (inactivated, oral)	Adopted 2001, TRS 924 (2004)
Dengue tetravalent vaccines (live, attenuated)	Revised 2011, TRS 979 (2013)
Diphtheria, tetanus, pertussis (whole cell), and combined (DTwP) vaccines	Revised 2012, TRS 980 (2014)

Recommendations, Guidelines and other documents	Reference
Diphtheria vaccines (adsorbed)	Revised 2012, TRS 980 (2014)
DNA vaccines, plasmid	Revised 2020, TRS 1028 (2021)
Ebola vaccines	Adopted 2017, TRS 1011 (2018)
Enterovirus 71 vaccines (inactivated)	Adopted 2020, TRS 1030 (2021)
Haemophilus influenzae type b conjugate vaccines	Revised 1998, TRS 897 (2000)
Haemorrhagic fever with renal syndrome (HFRS) vaccines (inactivated)	Adopted 1993, TRS 848 (1994)
Hepatitis A vaccines (inactivated)	Adopted 1994, TRS 858 (1995)
Hepatitis B vaccines prepared from plasma	Revised 1994, TRS 858 (1996)
Hepatitis B vaccines (recombinant)	Revised 2010, TRS 978 (2013)
Hepatitis E vaccines (recombinant)	Adopted 2018, TRS 1016 (2019)
Human cells and tissues and advanced therapy medicinal products, regulatory considerations	Adopted 2023, TRS 1048 (2023)
Human immunodeficiency virus rapid diagnostic tests for professional use and/or self-testing Technical Specifications Series for WHO Prequalification – Diagnostic Assessment	Adopted 2017, TRS 1011 (2018)
Human interferons prepared from lymphoblastoid cells	Adopted 1988, TRS 786 (1989)
Influenza vaccines (inactivated)	Revised 2003, TRS 927 (2005)
Influenza vaccines (inactivated): labelling information for use in pregnant women	Addendum 2016, TRS 1004 (2017) to Annex 3, TRS 927 (2005)
Influenza vaccines (live)	Revised 2009, TRS 977 (2013)
Influenza vaccines, human, pandemic: regulatory preparedness	Adopted 2007, TRS 963 (2011)
Influenza vaccines, human, pandemic: regulatory preparedness in non-vaccine-producing countries	Adopted 2016, TRS 1004 (2017)
Influenza vaccines, human, pandemic: safe development and production	Adopted 2018, TRS 1016 (2019)

Recommendations, Guidelines and other documents	Reference
In vitro diagnostics (WHO-prequalified), collaborative procedure between WHO and NRAs for assessment and accelerated national registration	Adopted 2020, TRS 1030 (2021)
In vitro diagnostic medical devices, establishing stability of, Technical Guidance Series for WHO Prequalification – Diagnostic Assessment	Adopted 2017, TRS 1011 (2018)
Japanese encephalitis vaccines (inactivated) for human use	Revised 2007, TRS 963 (2011)
Japanese encephalitis vaccines (live, attenuated) for human use	Revised 2012, TRS 980 (2014)
Louse-borne human typhus vaccines (live)	Adopted 1982, TRS 687 (1983)
Malaria vaccines (recombinant)	Adopted 2012, TRS 980 (2014)
Measles, mumps and rubella vaccines and combined vaccines (live)	Adopted 1992, TRS 840 (1994); Note 1993 TRS 848 (1994)
Medical devices including in vitro diagnostic medical devices, WHO Global Model Regulatory Framework, WHO Medical device technical series	Revised 2022, TRS 1045 (2023)
Meningococcal polysaccharide vaccines	Adopted 1975, TRS 594 (1976); Addendum 1980, TRS 658 (1981); Amendment 1999, TRS 904 (2002)
Meningococcal A conjugate vaccines	Adopted 2006, TRS 962 (2011)
Meningococcal C conjugate vaccines	Adopted 2001, TRS 924 (2004); Addendum (revised) 2007, TRS 963 (2011)
Monoclonal antibodies as similar biotherapeutic products	Adopted 2016, TRS 1004 (2017)
Monoclonal antibodies against infectious diseases nonclinical and clinical evaluation	Adopted 2023, TRS 1048 (2023)
Monoclonal antibodies, production and quality control	Revised 2022, TRS 1043 (2022)
Papillomavirus vaccines (human, recombinant, virus-like particle)	Revised 2015, TRS 999 (2016)

Recommendations, Guidelines and other documents	Reference
Pertussis vaccines (acellular)	Revised 2011, TRS 979 (2013)
Pertussis vaccines (whole-cell)	Revised 2005, TRS 941 (2007)
Pharmaceutical products, storage and transport of time- and temperature-sensitive	Adopted 2010, TRS 961 (2011)
Pneumococcal conjugate vaccines	Revised 2009, TRS 977 (2013)
Poliomyelitis vaccines (inactivated)	Revised 2014, TRS 993 (2015); Amendment 2019, TRS 1024 (2020)
Poliomyelitis vaccines (oral)	Revised 2022, TRS 1045 (2023)
Poliomyelitis vaccines: safe production and quality control	Revised 2018, TRS 1016 (2019) Amendment 2020, TRS 1028 (2021)
Quality assurance for biological products, guidelines for national authorities	Adopted 1991, TRS 822 (1992)
Rabies vaccines for human use (inactivated) produced in cell substrates and embryonated eggs	Revised 2005, TRS 941 (2007)
Reference materials, secondary: for antibody testing	Adopted 2022, TRS 1043 (2022)
Reference materials, secondary: for NAT-based and antigen assays: calibration against WHO International Standards	Adopted 2016, TRS 1004 (2017)
Regulation and licensing of biological products in countries with newly developing regulatory authorities	Adopted 1994, TRS 858 (1995)
Regulatory risk evaluation on finding an adventitious agent in a marketed vaccine: scientific principles	Adopted 2014, TRS 993 (2015)
Respiratory syncytial virus vaccines	Adopted 2019, TRS 1024 (2020)
RNA vaccines, messenger, for prevention of infectious diseases	Adopted 2021, TRS 1039 (2022)
Rotavirus vaccines (live, attenuated, oral)	Adopted 2005, TRS 941 (2007)
Smallpox vaccines	Revised 2003, TRS 926 (2004)
Snake antivenom immunoglobulins	Revised 2016, TRS 1004 (2017)

Recommendations, Guidelines and other documents	Reference
Sterility of biological substances	Revised 1973, TRS 530 (1973); Amendment 1995, TRS 872 (1998)
Synthetic peptide vaccines	Adopted 1997, TRS 889 (1999)
Tetanus vaccines (adsorbed)	Revised 2012, TRS 980 (2014)
Thiomersal for vaccines: regulatory expectations for elimination, reduction or replacement	Adopted 2003, TRS 926 (2004)
Thromboplastins and plasma used to control oral anticoagulant therapy	Revised 2011, TRS 979 (2013)
Tick-borne encephalitis vaccines (inactivated)	Adopted 1997, TRS 889 (1999)
Transmissible spongiform encephalopathies in relation to biological and pharmaceutical products[7]	Revised 2005, WHO (2006)
Tuberculins	Revised 1985, TRS 745 (1987)
Typhoid vaccines, conjugated	Revised 2020, TRS 1030 (2021)
Typhoid vaccines (live, attenuated, Ty21a, oral)	Adopted 1983, TRS 700 (1984)
Typhoid vaccines, Vi polysaccharide	Adopted 1992, TRS 840 (1994)
Vaccines, changes to approved vaccines: procedures and data requirements	Adopted 2014, TRS 993 (2015)
Vaccines, clinical evaluation: regulatory expectations	Revised 2016, TRS 1004 (2017)
Vaccines, regulatory considerations: use of human challenge trials	Adopted 2016, TRS 1004 (2017)
Vaccines, lot release	Adopted 2010, TRS 978 (2013)
Vaccines, nonclinical evaluation	Adopted 2003, TRS 927 (2005)
Vaccines, nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines	Adopted 2013, TRS 987 (2014)
Vaccines, prequalification procedure	Adopted 2010, TRS 978 (2013)
Vaccines, stability evaluation	Adopted 2006, TRS 962 (2011)
Vaccines, stability evaluation for use under extended controlled temperature conditions	Adopted 2015, TRS 999 (2016)

[7] Available online at: https://apps.who.int/iris/bitstream/handle/10665/68932/a85721.pdf?sequence=1

Recommendations, Guidelines and other documents	Reference
Varicella vaccines (live)	Revised 1993, TRS 848 (1994)
Yellow fever vaccines (live, attenuated)	Revised 2010, TRS 978 (2013) Amendment 2021, TRS 1039 (2022)
Yellow fever vaccines, laboratories approved by WHO for the production of	Revised 1995, TRS 872 (1998)
Yellow fever virus, production and testing of WHO primary seed lot 213-77 and reference batch 168-736	Adopted 1985, TRS 745 (1987)

Annex 2

Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases

Guidelines published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, these WHO Guidelines may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to these Guidelines are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the guidance set out below.

Abbreviations

ADA	anti-drug antibody
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
ADE	antibody-dependent enhancement (of disease)
ADR	adverse drug reaction
CDC	complement-dependent cytotoxicity
COVID-19	coronavirus disease 2019
Fab	fragment antigen-binding (region)
Fc	fragment crystallizable (region)
FIH	first-in-human
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
mAb	monoclonal antibody
MED	minimum effective dose
NRA	national regulatory authority
PD	pharmacodynamic(s)
PEP	post-exposure prophylaxis
PK	pharmacokinetic(s)
PrEP	pre-exposure prophylaxis
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TK	toxicokinetic(s)

1. Introduction

Monoclonal antibodies (mAbs) represent the largest class of therapeutic proteins in clinical use. However, the majority of currently marketed mAbs are used for the treatment of noncommunicable diseases such as cancer or autoimmune disorders. Although only a small number of mAbs have to date been licensed to treat or prevent infectious diseases, the number of such products in development is growing (*1*–*4*).

Advances in recombinant biotechnology and protein chemistry, combined with a greater understanding of mAb structure and function, have led to growing interest in recombinant varieties of mAbs such as chimeric mAbs, mAb fragments, single domain mAbs and multispecific mAbs. These mAb variants may offer significant production, formulation and clinical advantages, including improved production yields, greater stability, the potential for alternative routes of administration, multiple antigen targeting, prolonged half-lives, increased bioavailability, enhanced functional activity and/or altered tissue penetration. These technological advances in mAb engineering have led to the revision of the WHO nomenclature system to accommodate the growing diversity of mAb products (*5*).

Due to their established history of safe use, the rapid onset of their clinical effect and the relatively short time required to bring them to production, mAbs potentially offer a real-time response to emerging infectious diseases. As a result, they are considered to be a high priority for development due to their potential impact during public health emergencies such as coronavirus disease 2019 (COVID-19) (*6*), and in the treatment of chronic infectious diseases such as acquired immunodeficiency syndrome (AIDS). However, this will require that national regulatory authorities (NRAs) have the expertise, capacity and regulatory processes in place needed to review mAb products under such circumstances.

In 2020, the WHO Expert Committee on Biological Standardization discussed the advances being made in mAb engineering and production technologies, and the growing importance of such products in the management of infectious diseases (*6*). The Committee noted that although a range of WHO documents relevant to mAbs had already been published, these focused primarily on their use as biotherapeutics for noncommunicable diseases, with little guidance provided on nonclinical or clinical evaluation specific to pre-exposure prophylaxis (PrEP), post-exposure prophylaxis (PEP) or to post-infection treatment with mAbs. It was envisaged that the provision of such guidance on the evaluation of mAbs against communicable diseases would help to clarify regulatory expectations during their development and licensure processes, facilitate international regulatory harmonization efforts and thus improve access to such products. The Committee therefore endorsed a proposal to develop a

WHO Guidelines document that would be broadly applicable to mAb products intended for prophylaxis and/or treatment of infectious diseases.

Following a detailed review of existing WHO documents to identify where additional guidance and clarity was required on the nonclinical and clinical evaluation of pathogen-directed mAbs and related biological products, the current WHO Guidelines document was developed through a process of international and public consultation. The Guidelines should be read in conjunction with the relevant sections of the existing WHO documents referenced throughout the text. A number of non-WHO documents are also cited where these may provide additional supporting information. In addition, a number of considerations regarding nonclinical and clinical approaches to abbreviated regulatory submissions for mAbs are provided in the Appendix to these Guidelines. It is envisaged that, where required, individual supplements to the Guidelines may also be developed in the future on disease-specific regulatory considerations for these products.

2. Purpose and scope

These Guidelines are intended to provide guidance to NRAs, sponsors, manufacturers and investigators on the nonclinical and clinical evaluation of mAbs directed against the antigens of invading pathogens or their toxins, and which are used specifically in the pre- and post-exposure prevention or treatment of human infectious diseases. It should be noted that the general principles outlined in the document would also apply to mAbs that target endogenous human proteins with the intention of preventing or treating infections (for example, a mAb to a cell surface receptor that prevents viral entry to the cell) – however, such products may require additional nonclinical and clinical studies depending on the protein target(s). Immunomodulatory antibodies are not within the scope of these Guidelines as they are not directed against the infectious agent itself, or against a toxin antigen, but towards components of the host immune response, such as T-cells or cytokines.

The guidance provided is intended to apply to mAbs regardless of their isotype, as well as to other recombinant mAb-related antigen-binding proteins based on an immunoglobulin scaffold. These products can include, but are not limited to:

- antibody fragments, such as single-chain variable fragments and fragment antigen-binding (Fab) fragments;
- single domain antibodies;
- bispecific or multispecific antibodies;
- mAbs or related antibody proteins which have been chemically modified, such as through conjugation;

- mAbs which have been modified (such as through sequence substitutions, additions, and/or altered glycosylation) for the purposes of extending the half-life, or reducing or enhancing the effector function; and
- multiple mAb substances co-formulated within a final product ("antibody cocktail").

It should be noted that for the purposes of this document the term "monoclonal antibody" or "mAb" is used to encompass the breadth of the substances and products listed above, unless otherwise stated.

Small recombinant proteins intended to mimic mAb binding activity, but which have little or no immunoglobulin structure (for example, DARPins, affimers and anticalins), can differ significantly from mAbs in their pharmacological profiles (for example, in their bioavailability, pharmacokinetics (PK) and/or distribution) as well as in their formulation. With regard to convalescent serum immunoglobulins, although the basic principles for evaluating their pathogen-directed effects are similar to those described in the current document, there will be differences in their nonclinical and clinical evaluation, and such products would need to comply with regulations for testing blood-derived products. As a result, only parts of this document may be applicable to both small recombinant mAb mimetic proteins and pathogen-specific plasma-derived immunoglobulins, and sponsors of such products are encouraged to consult with the NRA on possible additional requirements. Similarly, these Guidelines do not apply to nucleic-acid-based platforms which use DNA, RNA or viral vector technology to deliver genetic sequences that encode for mAb production in vivo following administration. Such products face their own unique regulatory challenges that are best addressed in separate guidance.

Guidance on the production and quality control aspects of mAbs is provided in the WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use (*7*). These Guidelines take into account the extensive technological advances made in the field of mAb manufacturing since the original murine-hybridoma-derived mAbs were produced in the 1970s. Such advances include greatly improved production and purification methods, conjugation technologies, mAb fragments and mAbs derived from plant-based production systems. For mAb products developed as biosimilars, reference should be made to the WHO Guidelines on evaluation of biosimilars (*8*).

3. Terminology

The following definitions apply to the terms as used in these WHO Guidelines. These terms may have different meanings in other contexts.

Antibody cocktails: see **co-formulated mAbs**.

Antibody-dependent cellular cytotoxicity (ADCC): an effector function of the immune response in which fragment crystallizable (Fc) receptor-bearing effector cells can recognize and lyse an antibody-coated target cell expressing pathogen-derived antigens on their surface. Also called antibody-dependent cell-mediated cytotoxicity.

Antibody-dependent cellular phagocytosis (ADCP): an effector function of the immune response in which Fc receptor-bearing macrophages, or other phagocytic cells, phagocytose an antibody-coated target cell or microorganism.

Antibody-dependent enhancement (ADE): a phenomenon that occurs when antibodies promote, rather than inhibit, the infectivity of a microorganism and may occur through a number of possible mechanisms. Also called "antibody-dependent disease enhancement".

Antibody mimetic proteins: peptides or proteins that are not structurally related to antibodies but which recognize and bind to specific antigens. Such proteins usually have a molar mass of 3–20 kDa. Also called "antibody mimetics".

Anti-drug antibodies (ADAs): host antibodies that are capable of binding to the administered mAb therapeutic. This may or may not inactivate the administered mAb and/or induce serious adverse effects (see also **neutralizing antibodies** below).

Biological activity: the ability or capacity of a mAb to elicit a defined biological effect in vitro (for example, in cultured cells, bacteria or viruses) or in vivo (that is, in animal models and/or in humans).

Co-formulated mAbs: a final product formulated to contain two or more mAbs, mAb conjugates and/or mAb fragments, each of which recognizes a different epitope or antigen. These may also be referred to as "antibody cocktails", "antibody mixtures", "pooled antibody products" or "oligoclonal products". Co-formulated mAbs are not the same as individual mAb products that may later be co-administered during treatment.

Complement-dependent cytotoxicity (CDC): an immune response in which an antibody–antigen complex activates complement and induces the formation of a terminal lytic complex that is inserted into a cell membrane, resulting in lysis and cell death.

Effector function: the capacity of the **fragment crystallizable (Fc) region** of a mAb, following binding to the antigen, to engage with elements of the immune system through interactions with Fc receptors to generate functional responses. Effector functions include ADCC, ADCP and opsonization.

Fragment antigen-binding (Fab) region: a region on an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy and the light chain.

Fragment crystallizable (Fc) region: the tail region of an antibody derived from the second and third constant domains of the two heavy chains. This region is responsible for the effector functions of antibodies through its interactions with cell surface Fc receptors and some proteins of the complement system; however, such effector functions require the Fc region to be appropriately glycosylated.

Human challenge trial: a clinical trial involving the inoculation of healthy volunteers with a challenge agent before or after the administration of an investigational product.

Immunoconjugates: antibodies conjugated to a second molecule. Such molecules may include a toxin, anti-infective agent (antibiotic, antiviral or antifungal), radioisotope, label or non-bioactive compound. Immunoconjugates may be used in diagnosis and in targeted immunotherapy. Also called "antibody-drug conjugates".

Neutralizing antibodies: can refer to antibodies which neutralize the infecting organism or toxin by preventing it from binding to and/or infecting the host cell. However, neutralizing antibodies may also refer to antibodies formed against the mAb product that render it inactive against its intended target (see also **anti-drug antibodies (ADAs)** above).

Opsonization: the effector function in which antibodies bind to the surface of the antigen rendering it more readily identified and engulfed by phagocytic cells (for example, macrophages) for destruction.

Platform technology: an existing technology, or group of technologies, that are applied to the development and/or production of similar mAb products by a manufacturer. A given manufacturer might have one or more platforms on which they will develop various mAbs. A platform would be considered when the elements of the manufacturing methods and/or processes, the mAb protein scaffold, and the compliance with good manufacturing practices are unchanged. The experience and knowledge gained, data generated (on manufacturing, control and stability), and the validation of unchanged methods can all be used as supportive data for the more rapid assessment and development of a new mAb product candidate that fits within the boundaries of the platform.

Prophylaxis: a passive immunization or treatment intended to prevent an infection or an infectious disease, and given either as pre-exposure prophylaxis (PrEP) or post-exposure prophylaxis (PEP).

Therapeutic index: ratio of the median toxic dose to the median effective dose (TD_{50}:ED_{50}).

Therapeutic window: the range of mAb dosage, or its concentration in blood, that is sufficient to provide an effective response without significant adverse effect.

4. General considerations

The administration of antibodies for the prevention or treatment of infectious diseases is not a new concept. Human convalescent and immune animal sera were first used during the late 19th century as immunotherapies against both bacterial and viral infections (*9–12*). Human and equine plasma-derived immunoglobulins (such as anti-rabies, anti-hepatitis B and anti-tetanus immunoglobulins) continue to be used (*12*), with some included in the current WHO Model List of Essential Medicines. However, serum products can face issues of standardization, safety, supply and access (*13*). The introduction of mAb products offers the advantages of a more reliable and larger commercial supply, along with the potential for products that have better consistency between lots, are safer, can be engineered to have longer half-lives and which offer greater specificity and functionality than immune antisera and polyclonal antibodies (*9*, *10*, *12*, *14*).

The mAb bioengineering and production technologies now available also potentially allow for the rapid development of new products directed against emerging infectious diseases for which there are no available vaccines or therapeutics. Passive immunization through the administration of mAbs can provide rapid and direct benefits in preventing or treating an infectious disease – unlike active immunization through vaccination which may take weeks and may require multiple doses for the emergence of a protective effect. This is particularly important: (a) for immunocompromised individuals and those who cannot be vaccinated due to contraindications; (b) for those who are working or living in zones of high transmission during rapidly evolving epidemics or pandemics; and/or (c) when vaccination or other antimicrobial agents may not yet be available. As a result, mAbs are becoming an important addition to the repertoire of therapeutic and prophylactic products for infectious diseases, along with preventive vaccines and small-molecule antimicrobials (*10*, *12*, *15*, *16*).

Anti-infective mAbs

Currently, anti-infective mAbs are mostly full-length immunoglobulin G (typically abbreviated to "IgG") molecules – though immunoglobulin A (IgA) and immunoglobulin M (IgM) mAb isotypes are also under investigation. These mAbs can act directly by neutralizing the pathogen and inhibiting its ability to bind to human cell receptors, with fragment crystallizable (Fc)-receptor-dependent uptake by Kupffer cells and sinusoidal endothelial cells in the liver removing the toxin, bacterium, virus or other pathogen from the bloodstream (*17*). Such products may also act through their Fc-mediated effector function mechanism by stimulating immune responses such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP),

complement-dependent cytotoxicity (CDC) or opsonophagocytosis. Due to this potential range of functions, an understanding of the mechanism(s) of action of the mAb is crucial in evaluating its activity in both nonclinical and clinical studies.

Along with understanding the intended mechanism of action of mAbs, it is also important to characterize their physicochemical properties, which may include their size and charge variants, post-translational modifications, conjugations, hydrophobicity, potential for aggregation, glycosylation patterns or C-terminal heterogeneity (*7*). All of these biochemical properties can significantly impact upon mAb half-life, tissue distribution, stability, susceptibility to enzyme degradation, excretion, and their pharmacological and/or reactogenic potential. For example, engineering amino acid changes in the Fc region of mAbs can lead to longer half-lives, as well as to enhanced or decreased effector functions such as their interactions with host Fc receptors or proteins of the complement system (*18–20*). Differences in post-translational glycosylation can also lead to functional changes and alterations to half-life (*21*, *22*). Therefore, each individual mAb product may present a unique biochemical and biophysical profile which should be taken into consideration during their evaluation. Nevertheless, due to structural similarities among mAb products, the knowledge and technological experience of a manufacturer may be used to develop platform manufacturing processes that could be applicable to other mAbs produced by the same manufacturer using the same technologies and processes (*7*). Information from other manufacturers, or the products of other production processes, would not necessarily be supportive of such a proposal. Careful consideration is required in this regard, and an individual case-by-case approach may be justified but should be discussed and agreed with the relevant NRA(s).

Monoclonal antibody delivery

The biodistribution and ability of a mAb to reach site(s) of pathogen activity are other important considerations during product development. The physicochemical properties of the mAb, along with its formulation and route of administration, will all influence the compartments which it can access. To date, most mAbs have been administered by the intravenous route, often in specialized health-care settings and with administration times ranging from 30 minutes to several hours. However, considerable attention is now being given to the subcutaneous or intramuscular administration of highly concentrated mAbs which can be administered in only a few minutes. Other alternative mAb delivery routes are also being explored, including nasal, inhaled, oral, intraocular, intrathecal and dermal routes, some of which are of particular interest for the administration of mAbs directed against infectious diseases. Specifications, formulations and safety issues for mAbs delivered by these alternative routes may

differ from those for products to be administered by the intravenous route due to issues related to immunoglobulin concentration, viscosity, aggregation and stability – and this will need to be borne in mind during both nonclinical and clinical evaluation (*23*). In recent years, several mAb fragments and small mAb mimetic proteins based on non-immunoglobulin scaffolds have been generated using affinity selection technology. These highly engineered proteins are significantly smaller than full-length mAbs and have physicochemical properties which may be designed to influence their bioavailability and tissue penetration range. Despite offering several advantages, such mAb fragments and mimetic proteins may have reduced half-lives and are usually unable to elicit effector functions such as ADCC or CDC.

Potential adverse effects

Two potential adverse impacts of mAbs should be assessed throughout the product development programme, and should be monitored following their marketing approval – namely the emergence of antimicrobial resistance, and antibody-dependent enhancement (ADE) of disease. These effects may have significant impacts on product efficacy and safety, and should be considered during benefit–risk and/or safety assessments of mAb products to infectious diseases.

As has been observed with small-molecule antimicrobials, selection for resistance of the infecting pathogen to the mAb may occur and should be monitored for throughout the product life-cycle. For example, bacteria can be induced to produce antibody-degrading proteinases (*24–26*) or changes to the target antigen can occur through natural mutagenic selection processes – either of which could reduce the efficacy of mAb therapies (*24*, *27*, *28*). Similarly, the emergence of multiple strains and escape mutants among viruses can lead to new variants that may evade mAb therapies, for example through alteration of the antigenic structure of an epidemic pathogen in real time (*29–31*).

The potential emergence of organisms resistant to mAbs necessitates rational drug design approaches including the exploration of mAbs that target highly conserved antigens or epitopes, the combination of a mAb with one or more small-molecule drugs, or the use of co-formulated mAbs (antibody cocktails) that contain mAbs targeting separate antigens or epitopes (*16*, *29*, *32*, *33*). The development of bispecific mAbs through bioengineering to combine the epitope specificities of two antibodies and simultaneously interact with different antigens or epitopes is also being explored (*34*).

ADE is also an important aspect to consider as part of the nonclinical and clinical programmes of any mAb against infectious diseases, particularly if the functions of the epitope are not clearly understood. Disease enhancement may occur through facilitation of the pathogen life-cycle (for example, by easing

viral entry into a cell, promoting replication in target cells or facilitating cell-to-cell transmission) or through the enhancement of physiological responses (for example, complement activation). In the case of the former, antibody-mediated enhancement is classically defined as Fcγ-receptor–mediated enhanced disease, which may occur in the presence of non-neutralizing antibodies, sub-neutralizing antibody concentrations or low-affinity antibodies. Although ADE is more classically observed with viral infections (*35*–*37*), the ADE of bacterial infections has also been reported (*38*–*40*) and may be linked to antibody isotype and glycosylation patterns (*24*).

The assessment of potential ADE can be difficult during nonclinical and clinical development programmes as its mechanisms are not always fully understood, and may or may not translate between its nonclinical observation and occurrence, or risk, in the clinic. Cell culture methods may provide an effective model in which to explore the potential mechanisms of ADE but may not be predictive of clinical outcome, and detecting its impact in clinical studies might be difficult if its occurrence is rare (*37*, *41*).

Regulatory considerations

The nonclinical and clinical sections of these Guidelines describe a traditional path for evaluating the safety and efficacy of mAbs against infectious diseases and are likely to apply to the majority of products developed. However, a benefit–risk assessment of some epidemiological circumstances may warrant, or require, that the sponsor and NRA consider alternative approaches to evaluating product safety and efficacy, while balancing the regulatory requirements for safety and efficacy against ensuring product accessibility during a time of critical need. Such circumstances may occur, for example, for rare or neglected diseases, localized and/or short-lived outbreaks, an infection with a high fatality rate or during a public health emergency. Consideration of any alternative nonclinical and/or clinical plan will require good communication with the NRA, with discussions occurring as early as possible during product development. NRAs are encouraged to use good regulatory reliance practices and other collaborative approaches with regulatory partners when assessing submissions for mAb products for which alternative review strategies may be warranted.

In the case of the rapid development of products against a priority pathogen, such as during a public health emergency, consideration may be given to abbreviating the nonclinical and/or clinical requirements by deferring or omitting certain studies in order to expedite product development and regulatory evaluation. However, the benefit–risk ratio of such an approach must always be considered and early consultation with the NRA is strongly advised under such circumstances. Further discussion of this topic is provided in the Appendix to these Guidelines.

Standards and other reference materials

Standards and other reference materials play a vital part in the quality control and regulatory authorization processes of all biological products, including mAbs. Where they are available, such materials may be included in antigen quantification or bactericidal assays, or used in the determining of antibody concentrations or in methods for monitoring serological end-points. The standardization of assay methods used to support the nonclinical and clinical evaluation of mAbs will also be important in ensuring the comparability of laboratory results within and between countries, and between different clinical trials.

WHO international standards, reference reagents and other reference materials are the primary standards in use worldwide, and when available should be included in bioassays. In addition, NRAs and manufacturers should establish secondary (regional, national), working standards for use in assays supporting nonclinical and clinical studies, as well as for the purpose of testing mAb quality on a lot-to-lot basis (*7*).

5. Nonclinical evaluation

This section sets out a flexible approach to the nonclinical evaluation of mAbs intended for use in the prevention or treatment of infectious disease. The approach includes the use of both in vitro and in vivo (animal) studies. The guidance provided is intended to be complementary to, and should be read in conjunction with, Part B and Appendix 5 of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*) and the WHO guidelines on nonclinical evaluation of vaccines (*43*). Additional guidance can be found in section 5 of the WHO Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products (SBPs) (*27*), as well as in the WHO Guidelines on procedures and data requirements for changes to approved biotherapeutic products (*28*). ICH guidance on the preclinical safety evaluation of biotechnology-derived pharmaceuticals (*44*) and on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals (*45*) should also be consulted, along with any relevant guidance from NRAs.

The initial discovery and characterization of a mAb typically involves the assessment of numerous mAb candidates in a variety of assays that evaluate their effectiveness in pathogen or toxin neutralization and determine their likely mechanism(s) of action. Although these tests will generally be performed using research materials, subsequent mechanistic and efficacy studies should be carried out using clinically relevant mAb product lots if possible. Where this is not feasible, the lots studied should be comparable with respect to their physicochemical characterization data, biological activity, stability and

formulation (*7*). Such studies may include preliminary in vitro or animal tests performed with mAb product lots produced by a polyclonal cell population expressing the mAb as the first step in isolating a stable, high-expressing clone for the final manufacturing step. The continued comparability of the test material should be demonstrated whenever a new or modified manufacturing process is used or when other significant changes in the product or its formulation are made in an ongoing development programme. Comparability can be evaluated based on biochemical and biological characterization (that is, identity, purity, stability and potency) (*7*, *27*). In some cases, additional studies may be needed (for example, nonclinical pharmacokinetic (PK) studies, pharmacodynamic (PD) studies and/or toxicology studies). The scientific rationale for the approach taken should be provided. It should be noted that the mAb product lots used in pivotal nonclinical studies must adequately represent the quality and formulation intended for use in subsequent clinical investigations.

Pivotal nonclinical toxicity studies should comply with good laboratory practices (*44*, *46*). Data integrity should be maintained where internal standard operating procedures are not used, such as for dose-ranging studies or early toxicity studies that are not compliant with good laboratory practices.

All studies conducted in animals should follow the 3Rs principles ("Replace, Reduce, Refine") and minimize the use of animals in research. Although animal study end-points need to best reflect those expected during clinical evaluation, such studies should terminate as early as possible to minimize suffering, particularly in the case of studies in which animals are infected. Where available, consideration should be given to the use of validated alternative in vitro methods for toxicological evaluation.

5.1 General considerations in nonclinical evaluation

The primary objectives of both in vitro and animal nonclinical studies are to define the pharmacological and toxicological effects of investigational products prior to the initiation of human studies (*43*). This will involve:

- Functional characterization of the product, such as its ability to prevent disease, reduce pathogen load, impair toxin activity, promote pathogen clearance from the blood and tissues, improve clinical signs, prevent or reduce weight loss, or reduce severity of infection.
- Identification of possible toxicities, their potential for reversibility and likelihood of potential adverse or undesirable effects.
- Identification of a safe starting dose for first-in-human (FIH) studies and of safe dose escalation when possible.

There are several important factors to consider when designing nonclinical studies for mAbs intended to prevent or treat a human infectious disease. Knowledge of the mAb target antigen of the infecting pathogen and its biology is expected, as is characterization of the binding site/epitope and evaluation of the specificity and selectivity of the mAb to the pathogen. Unwanted and unexpected cross-reactivity with animal or human cells and/or tissues need to be explored. In addition, naturally occurring changes to the antigen (that is, through antigenic drift or shift) may occur through the course of some epidemics and result in reduced affinity of the mAb to the target antigen. The potential for such reduced affinity through epitope mutation should therefore be considered and prospectively evaluated, if relevant, before a mAb is committed to clinical study, and should be monitored by the sponsor (for example, through in vitro tests using antigens derived from circulating and emerging strains).

Nonclinical study design should be guided by, and tailored to, the type of data needed, and by whether it is a PK, PD or safety study. Data derived from PD, PK and short-term toxicity studies help to approximate the FIH dose and dosing margins. PD studies in animals help to define the lower range of the efficacious therapeutic dose (for example, minimum effective dose) whereas short-term toxicity studies provide an indication of the upper range for a safe FIH dose. PK studies provide information on the blood concentration–time profile of the mAb following administration that can help refine the therapeutic dose range. In some cases, PK data may also provide an estimate of the lower dose range for use in FIH studies where PD data are not available. In vitro and modelling studies for mAbs for which there are sufficient data and experience may be acceptable alternatives for estimating FIH doses, but this should be discussed with the NRA in advance. In vitro and modelling studies for estimating FIH doses may not be sufficient for novel mAb products for which there is limited experience.

The selection of a suitable animal species for use in evaluating mAbs against an infectious disease could prove challenging, and may not necessarily be the same species across the different study types. Scientific justification should be provided for the animal species selected for use in each study and should take into account the likely suitability of the resulting data in guiding human clinical studies. Selection of the animal species, and the potential to combine end-points within one study, should be discussed with the NRA. This is particularly important where established animal models of infection do not exist, are not relevant to human physiology or do not reflect the pathology of the infection in humans.

The nature of the mAb product itself should also inform species selection since this may also influence the study results. Although the target antigen for anti-infective mAbs is unique to the infecting pathogen, regardless of the host, the subsequent response by the host to the mAb-bound pathogen can vary significantly in nonclinical studies depending on the host species and on

the species from which the mAb has been derived. For example, the use of a humanized mAb in a mouse model would not necessarily predict the activity or safety of the same humanized mAb in humans. For this reason, understanding the impact of host species and mAb differences will be crucial in the preclinical development programme and in the translation of nonclinical data to the clinical situation.

The induction of anti-drug antibodies (ADAs) is species specific, and their occurrence in animal studies is generally not relevant in terms of predicting the potential immunogenicity of mAb products in humans. Nevertheless, the detection of ADAs in animals may provide some insight as to potential complications, particularly for mAb-related products, and may also assist in the interpretation of data derived from animal toxicity studies. For example, ADA formation can increase the clearance of the mAb and impact its PK and/or toxicokinetics (TK), which in turn can reduce its pharmacological and/or toxicological effects. The induction of ADAs could also result in other pharmacological and/or toxicological changes including the emergence of new toxic effects. Therefore, all such PK and TK effects of ADA formation should be considered (see sections 5.3 and 5.4 below).

In addition, consideration should be given to situations where the mechanism of action of the mAb involves a secondary response such as ADCC, ADCP or CDC, which may vary greatly depending on antibody Fc and animal model Fc receptors. Such pharmacological properties, and whether or not they are species specific, should be considered when interpreting exposure–response relationships, PK parameters and tissue toxicity in animal studies. The degree of similarity of the animal infection model to human infection must also be taken into consideration.

In all animal studies it is important to sequence, characterize and standardize the pathogen challenge strain and its dose on administration. Where the passage of pathogenic strains may lead to the development of variants – as for example in the case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – it is vital to use challenge material at defined and standardized passage levels (*47*). It may also be informative to genotype pathogens isolated from animals that succumb to infection despite mAb exposure in order to assess whether the susceptibility to such infection correlated with antigenic drift or shift in the pathogen.

5.2 Pharmacodynamics and biological activity

5.2.1 In vitro studies

Biological activity may be evaluated using in vitro assays to determine which effects of the product may be related to clinical activity. Several concentrations of the product should be tested during in vitro pharmacology studies. If a

mAb fragment or immunoconjugate is used it should be tested in that form. Appropriate newer assay technologies should be employed as they become available and validated.

In vitro studies for demonstrating mechanism of action can include assays that characterize the binding site(s), binding affinity to the exogenous target, infecting organism or bacterial toxin, mechanism of pathogen inactivation/destruction (for example, bactericidal, opsonophagocytic or neutralizing activity that includes effects on variants) and effector function of the mAb. Structural biology approaches can also be used to map the mAb-antigen complexes at the atomic level. In vitro studies may also help to evaluate the impact of: (a) antigenic variations such as those which occur naturally through genetic drift or shift; (b) bacterial capsule switching; and (c) escape mutations in the pathogen. These antigenic variants may be isolated in the laboratory or derived from clinical isolates. The sponsor should also consider the potential for cross-resistance with other marketed antibodies/drugs.

5.2.1.1 Cell culture studies

Cell culture models can be invaluable tools for the early screening of mAb product candidates, for assessing the effects of mAbs against pathogens of interest and for exploring mAb mechanism(s) of action. Cell culture systems are an integral component of the in vitro assessment of mAb neutralizing activity and antibody effector functions such as ADCC, ADCP or opsonization. However, cell culture systems may not have been established for all infectious agents, particularly during the early stages of a pandemic or when a pathogen is recalcitrant to cell culture methodologies or environments. Where cell culture models do exist, care should be taken to ensure that the environmental conditions are suitable for maintaining proper functionality of the mAb, and to minimize the interference of assay reagents. The use of tissues or cells from different species in cell culture models may also provide insight into the most relevant animal model to use in PD studies.

For co-formulated mAbs, the neutralizing activity of each of the constituent mAbs should be tested and any potential synergistic or antagonistic effect of the combination determined.

5.2.1.2 Tissue cross-reactivity studies

The non-target tissue binding of mAbs may have serious consequences, particularly when certain immunoconjugates are used. Therefore, cross-reactivity studies should usually be conducted prior to FIH studies to detect any non-target tissue binding or other cross-reactions.

Any unintended reactivity of an investigational mAb with human tissue should be determined using a frozen panel of tissues or representative cell

cultures (*44*). Several concentrations of the candidate product should be tested as the ability to detect cross-reactions may depend on the concentration of the mAb. The NRA should be consulted on the requirements for the human tissue panel. Likewise, the possibility of evaluating off-target reactivity with human proteins using a validated cell and/or protein microarray assay should be discussed with the NRA. When cross-reactivity signals are detected, studies should be expanded to more tissues. Although the use of animal tissues may help interpret some findings from animal studies, tissue cross-reactivity testing in a full panel of animal tissues is not recommended (*44*).

5.2.2 Animal studies

In vivo animal PD studies are important in understanding the biological activity of the mAb in a living system. As animal PD studies are also used for the approximation of FIH doses they should be conducted where possible. However, as the requirements for PD studies to be conducted on mAbs against infectious diseases may vary between countries, and as in vitro and/or modelling studies may be acceptable alternatives for mAbs with sufficient associated data and experience, this should be discussed with the NRA as early as possible in the mAb development pathway. PD studies should be based on assays that ensure that the mAb is functional against the targeted infectious agent. However, classic PD/PK assessment may be of limited relevance in animal models. For most pathogens there will be a wealth of knowledge and experience of relevant assays amassed from work on the disease and its prevention. Existing knowledge of natural and/or vaccine-induced immunity may also provide additional insights during the nonclinical evaluation of the mAb product under development.

An attempt should be made to study the dose-dependence of PD effects when an animal model for the infection is available. The use of a broad range of doses, including high doses, may allow for better prediction of the therapeutic index. When two or more mAbs are co-formulated in the final product, only the intended combination should be evaluated in animals. The PD of each individual mAb and its co-formulation should be evaluated in vitro.

For proof-of-concept studies demonstrating anti-pathogen activity, preference should be given to studying the mAb in a model in which the infection in the animal is similar to that in humans. Consideration should be given to establishing how similar the infection is in the chosen animal model to human infection and disease. Due to the wide range of mAbs and infectious diseases that fall within the scope of the current document, the choice of animal species should be decided on a case-by-case basis and a scientific rationale justifying the model selected should be provided.

Animal studies may be useful in evaluating the proof of concept or providing evidence of potential efficacy, and (where relevant) in identifying the potential therapeutic window. Studies of mAbs intended for prophylaxis will

be designed differently from those of therapeutic mAbs and, where possible, should be based on relevant experience from studies of the infectious disease and pathogen in question. Candidate mAb products should be assessed with the view to establishing the most effective treatment protocol.

Where animal models of the infection do not exist or are not available for use due to supply or ethical reasons, alternative approaches will need to be justified and the NRA consulted. Supporting evidence of the functionality of the mAb might then be derived from human convalescent serum in which serum antibodies could, for example, recognize similar antigens and neutralize or remove the infecting agent.

5.2.3 Safety pharmacology

The purpose of a safety pharmacology study is to investigate the functional effects of the candidate mAb product on vital functions and major physiological systems. These usually include the cardiovascular, respiratory and central nervous systems. However, in accordance with ICH guidance (*44*, *48*), safety pharmacology studies might not be necessary – though a justification for their omission should be provided. Investigations of cardiovascular, respiratory and central nervous system parameters could instead be incorporated into the design of toxicity studies.

The tissue distribution of the mAb may be influenced by a number of its physicochemical properties (for example, molecular size and glycosylation) and by its source or formulation. Therefore, such factors should be taken into consideration when assessing the impact of the product on vital functions and physiological systems.

5.3 Pharmacokinetics and toxicokinetics

PK and TK studies are undertaken in order to understand exposure in animals, to allow animal-to-human extrapolation and to predict margins of safety for clinical trials based on exposure. Additional guidance can be found in section B.3 of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*). Although PK and TK evaluations may be integrated into broader pharmacology and/or toxicology studies, there may be limitations when interpreting PK and/or TK data due to the lack of a relevant animal model when the mAb is directed against an infecting agent.

PK and TK study design, and the interpretation of PK and TK data, should also take into consideration the nature of the mAb or immunoconjugate, its stability, ability to bind serum proteins, the presence or absence of the infection, and/or target antigen expression and level in the recipient animal model, as well as the route of administration (see also section 5.1 above).

5.3.1 Assays

Selecting the assay for use in PK and TK studies needs careful case-by-case consideration and the scientific rationale should be provided. The assay format should, preferably, be the same for animal and human studies, using validated techniques that are appropriate for the matrix and species. The possible influence of plasma-binding proteins and/or antibodies in plasma/serum on the performance of the chosen assay should be investigated and taken into consideration.

Product-specific assays should:

- cover the pharmacological/toxicological or PK aspects;
- represent and/or predict the clinical situation;
- broadly cover all functional aspects (for example, half-life); and
- be tailored to the product and be fully justified.

5.3.2 Other considerations

- ***Absorption***: absorption studies are not required for intravenously administered mAbs. However, for mAbs administered via other routes (for example, intramuscular or subcutaneous) an evaluation of absorption and bioavailability should be conducted before the start of human Phase I studies.
- ***Distribution***: should be investigated as appropriate and the physicochemical and kinetic properties of the mAb taken into consideration, along with the fact that its distribution will vary depending on the route of administration. Although mAbs may initially be confined to the vascular system, they may subsequently distribute to the extravascular space as a result of various factors, including bulk flow and active transport.
- ***Metabolism***: classic biotransformation studies, as performed for pharmaceuticals, are not needed for mAbs. However, conjugated mAbs would require an understanding of the metabolic fate of the conjugated molecule following its deconjugation.
- ***Elimination***: information on clearance/elimination in relevant animal models should be available prior to clinical studies in order to predict margins of safety based on exposure and dose. For an immunoconjugate, information on the elimination of the conjugated molecule should also be available.

5.4 Toxicology studies

Due to the wide range of mAbs and infectious diseases that fall within the scope of the current document, the choice of animal model and toxicological studies should be decided on a case-by-case basis and justified. When animal models of the disease are used for proof-of-concept studies, a toxicological assessment can be included to provide additional information on any potential target-associated toxicity. Where this is not feasible, appropriate risk mitigation strategies should be considered and discussed with the NRA.

For mAbs that show off-target binding to human tissues and/or produce toxicity in animal studies, additional toxicological testing may be justified.

A published review of the nonclinical safety evaluation of therapeutic antibodies highlights important considerations in planning a nonclinical programme, the types of nonclinical safety studies needed and a general timeline for their conduct in relation to clinical trials (*49*).

5.4.1 General considerations

A short-term repeat-dose toxicity study that investigates more than one dose level should be performed. For mAbs intended for multiple dosing during prophylactic treatment or during the course of infection, the dosing regimen investigated should reflect the dosing used in the worst-case clinical scenario. The study recovery period should be justified and may need to reflect the length of elimination time of the mAb (for example, 5 half-lives). Justification should be provided when a single-dose toxicity study is proposed (for example, for mAbs with a long half-life). The selection of species should also be justified by the sponsor.

Toxicity testing requirements should be discussed with the NRA. Ideally, testing should be conducted in healthy animals to allow for clearer interpretation of toxicity in the absence of disease, and to represent healthy subjects administered the mAb for prophylactic purposes. Testing should be performed in both male and female animals and at a stage in their development that reflects the most sensitive in the proposed target human population (for example, young, middle-aged or elderly). The number of animals tested may vary depending on whether the study is conducted in rodent or non-rodent species. Likewise, the route of administration of the mAb product should reflect the intended route of its administration in clinical studies. When two or more mAbs are co-formulated, or otherwise developed to be used in combination, testing should be conducted on the combined mAbs. Any adverse responses noted may warrant further evaluation of each mAb individually. For immunoconjugate products, nonclinical safety studies should be performed on the immunoconjugate. In addition, the safety of the conjugate molecule (that is,

the "payload") should be understood and acceptable; otherwise further studies may be required and conducted according to appropriate guidance.

The potential development of ADAs may complicate the study and interpretation of the toxicology effects observed in animals and should be considered if immune-mediated reactions occur (*44*). The predictive values of repeated-dose studies for potential outcomes in humans should take the formation of ADAs and associated immunogenicity issues into account and may be discussed with the NRA. It should also be taken into account that infectious diseases in humans may not require repeated long-term treatment with mAbs and, therefore, the risk of inducing an anti-mAb immune response in the clinic may be reduced.

Local tolerance should be evaluated according to established methods (for example, evaluation of erythema/eschar and oedema). If feasible, the potential local adverse effects of the product can be evaluated in the toxicity studies, thus obviating the need for separate local tolerance studies.

5.4.2 Genotoxicity and carcinogenicity

Genotoxicity and carcinogenicity studies are generally not applicable to mAbs (*42*). However, such studies may be required for an immunoconjugate and should be considered on a case-by-case basis.

5.4.3 Developmental and reproductive toxicity

Developmental and reproductive toxicity studies may not be necessary for a mAb targeting an infectious agent (that is, a non-human antigen) but this requirement may vary by country and should be discussed with the NRA in advance. National guidelines may or may not be aligned with other guidelines in which additional considerations on the requirements for developmental and reproductive toxicity studies are discussed – see for example ICH S6(R1) (*44*). An NRA may require developmental and reproductive toxicity studies for mAbs intended for administration in women of childbearing potential – particularly if the product is an immunoconjugate or non-traditional mAb protein for which there is little clinical experience.

When conducted, the specific study design and dosing schedule may be modified on the basis of issues related, for example, to species specificity, immunogenicity, biological activity and/or a long elimination half-life. The species-specific profile of embryo-fetal exposure during gestation should also be considered when interpreting results. High molecular weight proteins (> 5 kDa) do not cross the placenta by simple diffusion. For antibodies with a molecular weight as high as 150 kDa, there exists a specific transport mechanism involving the neonatal Fc receptor which determines fetal exposure, with the expression of this receptor varying across species. In humans and non-human primates,

immunoglobulin G placental transfer is low in the period of organogenesis and begins to increase in the early second trimester, reaching its highest levels late in the third trimester. Further discussion of this can be found in the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*). The results of any prenatal and postnatal developmental studies should be submitted as part of the application for marketing approval. Evaluation of potential effects of the product on female and male fertility, when appropriate, should also be completed before the start of Phase III trials.

5.5 Additional considerations in nonclinical evaluation

- ***Antibody-dependent enhancement (ADE)***: the potential for ADE should primarily be evaluated through in vitro mechanistic studies. A dedicated animal study for ADE assessment is not warranted – though the potential for ADE may be assessed as part of the PD/proof-of-concept study if an animal model of the disease is available (37).
- ***Impurities***: safety concerns may arise as a result of the presence of impurities in the final product. These impurities may be product-related (for example, mAb molecular variants, aggregates or fragments) with properties not comparable to the desired product, or process-related (for example, media components or host cell proteins). There are potential risks associated with host cell contaminants, whether derived from bacterial, yeast, insect, plant or mammalian cells. The presence of cellular host contaminants can result in allergic reactions and other immunopathological effects. Adverse effects associated with nucleic acid contaminants are theoretical, but include potential integration into the host genome. However, it is preferable to rely on quality control and manufacturing processes to minimize the amount of impurities present rather than to establish a nonclinical testing programme to evaluate their potential effects. Additional information can be found in Part A of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*).
- ***Ecotoxicity/environmental fate***: mAbs are generally not considered to be a particular hazard for the environment and are expected to be fully metabolized via catabolic pathways, with negligible renal excretion. No special precautions are expected in terms of product use and disposal. Nevertheless, for some chemically modified or conjugated mAbs, a full environmental risk evaluation should be undertaken, unless otherwise justified.

- ***Anaphylaxis***: although uncommon in humans, the intravenous injection of protein-based products such as mAbs can lead to various hypersensitivity-type reactions ranging from mild to severe – the molecular mechanisms of which may differ and are mostly unknown. Similar hypersensitivity and infusion reactions may also be observed during animal studies but these may not be reflective of a risk of such reactions occurring in humans. The results of guinea-pig anaphylaxis tests, which are generally positive for protein products, are usually not predictive of reactions in humans and should not be conducted.
- ***Immunotoxicity studies***: are generally not required but should be considered if any adverse effects of mAbs on the immune system were noted during PD or toxicity studies and which resulted in potential decreased host resistance to infectious agents (*42*).

6. Clinical evaluation

The guidance provided in this section on the clinical evaluation of mAbs for potential use in the prevention or treatment of infectious disease is intended to be complementary to Part C of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*) and section 6 of the WHO Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products (SBPs) (*27*). The WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*50*) may also provide useful information, particularly when considering the clinical trial design for mAbs intended for prophylactic use.

All clinical trials must be conducted under the principles of good clinical practice (*47*, *51*). Additional guidance on the implementation of good clinical practice principles can be found in the WHO Handbook for good clinical research practice (GCP) (*52*).

In some cases, clinical development could proceed by combining Phase I and Phase II, or Phase II and III, studies into Phase I/II, Phase II/III or platform studies. Although the nuances of such combined or platform clinical study designs are not specifically addressed here, the principles outlined below remain applicable.

6.1 General considerations in clinical evaluation

Each infectious disease has unique characteristics depending on the nature of both the invading microorganism and the host. Infectious diseases can be categorized by:

- microorganism type (bacterial, viral, fungal or parasitic), serotype or variant;
- the minimum infective dose;
- site of infection (for example, lung, urinary tract, bone or skin);
- host factors (such as prior infection, and whether they are immunocompromised, newborn, pregnant or elderly); and
- epidemiological features (for example, nosocomial, foodborne or waterborne, sexually transmitted, seasonal or geographically restricted).

Indications for the prophylaxis or treatment of infectious diseases are usually defined by the nature of the infectious process and/or symptoms of the disease. Each infectious disease also needs to be considered in terms of its severity, stage of pathogenesis (colonization, tissue invasion, latency and dissemination), rate of replication/multiplication, and the acute and chronic clinical phases of the disease. Participants enrolled in clinical trials must be appropriately identified according to these variables.

Clinical trial design and site selection for evaluating mAbs against infectious diseases must also reflect the epidemiological status of the pathogen. Clinical trial size and duration can vary depending on the biological half-life of the mAb product, whether the pathogen is in circulation and the number of people at risk within a community. The circulation of pathogen serotypes, subtypes or variants should also be noted, particularly if the mAb has differing affinities to each. For some highly lethal pathogens (for example, anthrax bacterium or rabies virus) it may not be ethical or feasible to conduct clinical safety and efficacy trials. In such cases, product safety and efficacy would need to be estimated from animal models of the disease and from safety and PK studies in healthy, uninfected volunteers. Discussions with the NRA are crucial when considering approaches to the evaluation of mAbs against such diseases.

Clinical evaluation and product development programmes for mAbs against infectious diseases should be specific and take into consideration whether the product to be evaluated is intended to be used as a prophylactic (PrEP and/or PEP), as a therapeutic or both. If a new mAb is to be evaluated for its ability to prevent an infection, then the goal of prophylaxis should be clearly stated in the protocol. Primary prophylaxis (that is, prevention of the acquisition of an infectious organism or of the development of an invasive infection of an organism already present in a patient) should be distinguished from secondary prophylaxis (that is, prevention of the reactivation of an infectious agent already harboured by a patient subsequent to a primary infection).

The activity of a treatment may be muted for those infectious diseases in which the symptoms appear or remain after the pathogen load has peaked

(for example, as observed for COVID-19 following infection with SARS-CoV-2, or whooping cough following infection with *Bordetella pertussis*). This may have a significant influence on the clinical development of a mAb intended for PEP or therapy, especially with regard to the timing of product administration, and the selection of end-points and timing of their assessment. In such cases, rapid point-of-care diagnostics may be important in the evaluation and ultimate use of the intervention. An understanding of the epidemiology, pathology and transmission of the infecting agent may inform the introduction and use of the treatment prior to the emergence of clinical symptoms or diagnosis. Such rapid initiation of the therapy should be considered among those at greater risk of infection and/or at risk of developing a serious illness.

In general, participants in clinical trials of therapeutic products should be representative of the population targeted for eventual product use. Because of the functionality of the mAb, healthy volunteers may not be suitable candidates for therapeutic trials – but may be appropriate for prophylactic studies. Healthy volunteers may also provide useful data on product safety, PK and potential for ADA induction. Therefore, the nature of the mAb, the target antigen and the proposed clinical application should all be considered before deciding to enrol healthy volunteers in a trial.

Sponsors and investigators should carefully consider the clinical benefits against the risks for mAbs intended to be administered as a single dose, multiple doses in a single course or multiple courses of therapy. Repeat administration of the mAb may alter its safety and activity profiles. Changes in antigen modulation by the mAb and immune responses to the mAb may prevent extrapolation of single-dose data to multiple-dose schedules. Furthermore, where there is an ADA response against the mAb product, repeated administration may lead to loss of therapeutic benefit and potential toxicity. In addition, participants with known hypersensitivity to proteins or other components contained within the clinical trial materials, or with a history of relevant allergies, should be excluded from product development clinical studies.

6.1.1 Inclusion and exclusion criteria

Establishing the inclusion and exclusion criteria for subjects of any clinical trial requires careful consideration. The criteria will be product dependent and should be based on a risk assessment which takes into consideration the nonclinical study results, any prior clinical experience with the same or similar mAb of the same class and/or target antigen/epitope, the product dose and dosage, knowledge gained from PK and PD studies and the type of infectious disease. Both inclusion and exclusion criteria should be rational and scientifically justified in the clinical trial application.

In general, as product development advances through clinical studies the exclusion criteria should diminish to broaden the range of study subjects, and to include subjects from the intended target population. FIH studies would thus have the most conservative criteria for subjects, with broadening of the inclusion criteria during Phase Ib and II trials. Modelling from PK and PD study data may help to generate dosing information for expanding inclusion to certain subgroups in larger Phase III trials. Open-label safety studies might also be considered with a special population subset during Phase III or post-licensure studies in order to obtain additional safety information to supplement the product indications. Additional guidance on special populations is provided in section 6.6 below.

6.2 Phase I studies

Phase I and FIH trials are conducted to determine the initial safety and tolerability of the investigational product following completion of the essential nonclinical studies. Clinical experience has demonstrated that most humanized mAbs are, in general, well tolerated. However, mAb fragments, single domain and bispecific mAbs, and chemically modified and/or conjugated mAbs may have little or no clinical background information. Therefore, the safety assessment will be key when planning FIH trials for such products.

Initial studies of a therapeutic mAb in Phase I are generally single-dose escalation studies. Along with investigating product safety, the goal of Phase I clinical studies for mAb products should be to determine the minimum effective dose (MED) to be further pursued in Phase II trials. The MED can be considered to be the lowest mAb dose that provides an observable beneficial effect, and is usually defined by its pharmacokinetic or pharmacodynamic measurements (for example, degree of antigen binding or as determined during nonclinical studies) and, where appropriate, by the tolerability of the product (for example, the maximally tolerated dose). However, in the case of an unconjugated mAb, studies to identify the maximally tolerated dose may not be necessary.

Initial safety and tolerability studies at different doses may be conducted in healthy volunteers, where appropriate, to determine the mAb safety profile and potential physiological responses. Subjects with infections might also be considered, where appropriate, to obtain early PK/PD and safety data for mAbs intended for a treatment indication. However, the inclusion of infected patients in FIH and Phase I studies should be discussed with the NRA. If the product is intended to be given for an infectious disease in the elderly, in children or in other specific groups, safety and tolerability data may be required within those populations. However, this would also depend on a benefit–risk assessment and the type of infectious disease, as well as the extent of clinical familiarity with the mAb. In some cases, it may be more appropriate to start Phase I trials in young, healthy subjects and then consider expanding the investigations in later

(Phase Ib) trials to broader age ranges and/or other specific populations. The expectations and requirements for safety and tolerability studies conducted in special populations should be discussed with the NRA.

Traditionally, the starting dose for FIH studies is based on the safety and toxicity information derived from testing in a relevant animal model. For biological therapeutics such as mAbs other approaches may be considered, and may be necessary, particularly if no relevant animal model of the infectious disease exists. As the effect of a mAb is often species specific and is targeting a non-native antigen, it may be more appropriate to base the FIH doses on a minimal anticipated biological effect level, the MED or possibly on predictive computer simulation and modelling.

When extrapolating from animal doses to human doses, information on the dose required for prevention or treatment of the infection may be of great value. The target dose in humans, or concentration range, should be based on both in vitro studies in which the mAb-antigen activity has been measured and studies of a relevant animal model if available. If animal models of the disease are judged to be impossible or of no relevance, and the initial in vivo studies are to be performed in humans, then testing should begin at a low dose based on extrapolation from in vitro tissue culture studies and/or from information gathered in clinical trials of a similar mAb. However, in such cases the toxicity studies in animals would be important for providing supportive safety information prior to FIH administration. In all such cases, the NRA should be consulted.

If use of a multiple-dose mAb regimen is anticipated, then multiple-dose schedules should be explored after basic data on toxicity, peak levels, clearance, distribution and biological effects are available from single-dose studies. Multiple-dose studies may also be assessed as part of Phase IV trials, and following marketing authorization, if the indication is to be expanded later from single-dose. The time required for recovery from the biological effects of single doses should also be well understood prior to initiation of multiple-dose regimens. The rationale for dosing schedules should be provided and should take into account dose tolerance, available PK and PD data in humans, and relevant animal models of safety and efficacy. PK studies to determine the relationships between human ADA titres and circulating antigen levels and organ distribution, clearance and toxicity may be necessary.

Before undertaking the repeated administration of conjugated antibodies, all organ toxicities and pathology resulting from single-dose administration should be characterized. The timing of recovery from all toxic effects should be determined. Intra-patient dose escalation may be appropriate if no toxicity is seen at the initial dose levels or if it is possible to use initial safe "test" doses and if cumulative toxicity is deemed unlikely.

6.3 Clinical pharmacology

6.3.1 Pharmacogenomics

Pharmacogenomics have little impact on mAbs directed towards antigen epitopes on infectious organisms except, perhaps, in individuals who may develop ADAs.

6.3.2 Pharmacokinetics

The PK profile is an essential part of the basic description of a prophylactic or therapeutic product and should always be investigated. PK studies should be performed for the intended dose range and route(s) of administration. Additional information may be found in section C.2.3 of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*).

The design of PK studies should take into consideration:

- the structure of the product (for example, whether it is a whole mAb, Fab or immunoconjugate) and its route of administration (for example, subcutaneous, intravenous or intramuscular);
- the potential impact of age, sex, immune status, weight and body mass index, as well as other physiological or disease status aspects which may impact the PK of a mAb;
- determination of plasma concentration profiles, distribution, clearance and elimination of the mAb;
- determination of doses for further study based on dose-concentration effect relationship and correlation with desired concentrations estimated from in vitro studies;
- determination of the organs and sites in which the mAb is distributed (including sites of infection), metabolized and eliminated;
- relationships between the elimination rate/disposition and the route of administration;
- relationship between the elimination rate and the antigen load;
- presence and load of a circulating antigen; and
- presence and nature of ADAs.

Multiple-dose PK studies may not be required if the mAb is intended to be given only in a single dose. However, they should be conducted when multiple-dose strategies are to be implemented as part of product development. The dose proportionality should be evaluated in single-dose or multiple-dose studies and the clinical consequences discussed. Time-dependent changes in PK parameters may occur during multiple-dose treatment, either due to elimination or due to formation of ADAs. The effect of ADAs on PK should be evaluated, preferably by

ensuring that sampling times for PK and ADAs coincide. The clinical relevance of ADAs for PK/PD should also be discussed. It is recommended that PK should be determined at several dose levels on several occasions during long-term studies, particularly if the mAb has been modified to extend its half-life.

In some cases, dedicated PK studies may not have been performed for the approval of some mAbs. Instead, population PK data from long-term trials could have been considered and used to establish the PK profile and the impact of other factors (based on sparse PK samples in clinical trials). The use of population PK and modelling/simulation applications may be acceptable to NRAs as a tool in guiding drug development.

As with all pharmaceuticals, one potential limitation of mAbs used for the treatment of infections is the unknown distribution of the passively infused mAb into tissues affected by the disease. The mAb isotype, its subclass and glycosylation pattern may significantly impact upon its bioavailability at the site of infection. Although similar limitations may also apply to mAb fragments, their smaller molecular size may permit greater tissue penetration than full-sized mAb products, albeit at the cost of more rapid clearance.

For conjugated mAb products, PK studies should consider both the intact substance as well as its components following deconjugation in vivo. For the development of co-formulated mAb products for infectious diseases, the intended combination of substances should be evaluated in PK/PD studies and early clinical trials. The PK of the individual mAb substances should also be analysed, if feasible.

6.3.3 Pharmacodynamics

The bioanalytical sampling necessary for PD studies (for example, for viral load or colony forming units) is usually conducted throughout the clinical development programme depending on the outcomes. The potential PD mechanism of action will largely depend on the nature of the antigen target, its role in the pathogenesis of the infecting organism, and the mAb isotype and structure (that is, whether it is an intact mAb or mAb fragment, conjugated or bispecific).

PD are usually investigated in the context of combined PK/PD studies. Such studies may provide useful information on the relationship between dose/exposure and effect, particularly if performed at different dose levels.

6.3.4 PK/PD relationships

The relationship between the administered dose, serum concentration and PD response (PK/PD relationship) and antigen load should be evaluated as part of the mAb development programme. PK and safety can initially be assessed in healthy volunteers. The PK, combined with nonclinical PD target levels, should

guide the doses to be evaluated in infected subjects. If feasible, markers for both mAb activity and safety should be measured, preferably in the same study. Such studies may involve the ex vivo assessment of the neutralizing activity in serum collected at different time points following mAb administration.

Therapeutic mAbs often demonstrate nonlinear PK, where the area under the curve (AUC) is not proportional to the dose administered. The extent of such nonlinearity can depend upon the total body load of the target antigen, the accessibility of the target antigen to the mAb, mAb–antigen affinity and mAb dose(s). Antibacterial mAbs may also exhibit PK properties which reflect target-mediated drug disposition due to opsonophagocytosis or through the formation of antibody–toxin complexes. This may potentially lead to complicated tissue distribution patterns during bacterial infections.

MAbs that have been modified to provide extended half-lives allow for less-frequent dosing and longer-term prophylaxis against an infection. However, the high affinity of such mAbs and the involvement of the host immune system in their pharmacological actions may lead to complex and nonlinear PK and PD.

6.4 Efficacy – Phase II and III studies

The clinical trial design of Phase II and III studies for efficacy determination will depend on whether the mAb is intended as a prophylactic or therapeutic product. Clinical trials for prophylactic mAbs may have much in common with those used to assess vaccine efficacy in that the clinical evaluation would primarily focus on disease prevention. However, the onset of mAb activity would be more rapid than that of vaccines and the duration of effect may be shorter.

The efficacy of a mAb should be evaluated in terms of its ability to prevent the disease, prevent disease progression (that is, prevent deterioration in overall clinical status, hospitalization or death) and/or reduce clinically relevant end-points following diagnosis. Depending on the type of infection, efficacy might also include the ability to eliminate the pathogen, or reduce its shedding, from the body. An emphasis should be placed on designing randomized controlled trials that take into account the intended target population, the selected clinical end-point(s), and case definitions and detection. The stage of infection in a participant when entering a clinical trial (that is, the clinical starting point) may also influence efficacy outcomes, and it will be important to establish clinical criteria or clinical markers for entering the study. For example, anti-SARS-CoV-2 mAbs were generally found to be more effective when administered early to patients with symptomatic COVID-19 and prior to hospitalization (*53*, *54*). The local epidemiology of circulating pathogen strains or variants may also affect efficacy outcomes, particularly if the mAb has different binding affinities to such variants or binds targets which are not universally present in all strains of the pathogen. For this reason, sequencing of the infecting pathogen or identification of the strain or variant present in clinical study samples may be needed.

Along with the primary clinical outcomes, biomarkers may provide useful secondary and complementary information for consideration and analysis. Biomarkers may include pathogen burden (for example, viral load, colony forming units or antigens linked to chronic parasitic infections) or host-response factors (for example, CD4 T-cell levels) that can be shown to be relevant to the pathophysiology and/or recovery from an infection. Such biomarkers may be considered once identified and once the assays for their detection have been validated – however, their selection should be discussed with the NRA. Further discussion of biomarker evaluation processes and steps to follow are outlined in section C3.3 of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*).

Some mAbs may be intended for the treatment of rare infectious diseases for which the target population is very small. Consequently, trials that are considered confirmatory for rare disease indications are often based on a limited number of subjects. While such studies must still be designed with the rigour of traditional trials and should be conducted to a high standard in order to provide reliable and valid data for assessing product efficacy and safety, some flexibility is needed with regard to the statistical methods to be used. Single-arm studies (for example, in which reduction of clinical symptoms and/or viral load are evaluated) may sometimes be justified when there is no known effective therapeutic product, and standard of care is only supportive – however, this approach should be discussed with the NRA in advance.

The selection of an appropriate comparator for use in efficacy trials will also require careful consideration. A double-blind trial design should be used in efficacy studies intended to prevent or treat infections. An appropriate comparator would be an approved mAb to the pathogen or small-molecule antimicrobial agent – however, a placebo control may be considered when no known agent is effective or when the natural history of the untreated infectious disease is relatively benign or self limiting. Any other current standard of care practices for the prevention or treatment of the infection must be provided to all participants regardless of the treatment arm. For example, this might include routine counselling and advice on prevention of infection transmission, provision of hydration and electrolyte solutions during episodes of diarrhoea and use of anti-inflammatory medications.

6.4.1 Clinical end-points

The selection of both primary and secondary end-points for mAbs intended to treat infectious diseases can be difficult as they may not necessarily include the reduction or elimination of an infecting pathogen, will likely be product specific and will also depend on the prophylactic or therapeutic indication for the mAb product. End-points are usually explored and clarified during Phase II trials – however, the end-points selected may change over time with increased

knowledge and clinical experience with the mAb, and/or may even differ between countries. In all cases, both the primary and secondary end-points will need to be established before initiating Phase III studies.

End-points selected for efficacy studies should be as clinically meaningful and patient oriented as possible, and able to demonstrate a benefit relative to an appropriate comparator (which may be an active therapeutic or placebo) or, if available, the current standard of care. There should be sufficient supporting evidence that the primary end-point can provide a valid and reliable measure of clinically relevant treatment or prophylactic benefit in the targeted patient population. Laboratory-confirmed case ascertainment is encouraged, even if conducted in a sub-population. It is important to discuss the proposed end-point(s) with the NRA early in the trial design process. In some cases, a biomarker (for example, CD4 levels in the case of infection with human immunodeficiency virus) might be considered acceptable as a study end-point in chronic infections when based on biological plausibility and the mechanism of action of the product. However, the selection of surrogate end-points may have limited value if their predictive capacity is not well established, or if used for acute infectious diseases in which relevant clinical outcomes can be readily measured.

The timing of product administration relative to the start of infection is also important in relation to expected outcomes and clinical end-points, and should be pre-specified and standardized where possible. For some infections, it may be difficult to demonstrate benefit in patients with more severe or advanced disease.

The end-points chosen should be able to distinguish between the mAb product under investigation and the comparator, and to account for confounders which may be related to immune responses or immune status (for example, following vaccination or prior exposure to the infection). It may be permissible to combine the results obtained for patients who have received no prior therapy with those for patients who have received other anti-infective therapies, but this should be pre-specified in the protocol and a rationale provided as to why no differences in outcomes are expected between the two groups. If such clinical designs are being considered, it is advisable to also incorporate appropriate statistical considerations, including hierarchical testing strategies. If a pre-specified subset analysis demonstrates no difference between outcomes in the two groups (that is, no influence of prior therapy) then the results obtained for each group could be combined.

6.4.2 Phase II studies

Phase II studies provide the first evaluation of the activity and potency of a mAb product in patients. These studies aim to determine the correct dosage, identify

common short-term side-effects and determine the best regimen and clinical measures to be used in subsequent pivotal clinical trials.

Comparative randomized Phase II trials are generally preferred for demonstrating that the mAb interacts correctly with its target, and in turn alters the progress of the infectious disease or its symptoms. These trials may involve placebo and/or active comparator agents such as antibiotics or antivirals. In studies into the prevention or treatment of infectious diseases, placebo controls may only ethically be considered within most study populations when no known effective agent has full regulatory approval, or when the natural history of the untreated disease is relatively benign or self limiting. If used, the placebo should, whenever possible, be identical in appearance to the study drug.

Phase II studies usually explore a variety of possible end-points. Defining clinically meaningful end-points in protocols will lend greater credibility and validity to the study. The timing of clinical end-point determination for trials of a prophylactic or therapeutic mAb needs specific consideration. For a therapeutic product, both clinical variables (for example, resolution of symptoms) and laboratory results showing a decrease in infectious viral/bacterial load can be considered as end-points.

If the mAb product shows a promising clinically relevant end-point in Phase II trials for a serious or life-threatening condition for which no other treatment option exists, or is intended for use during a public health emergency, then approval based on limited data may be possible, with further confirmatory efficacy data to be provided through post-marketing studies. Further discussion of this issue is provided in the Appendix to these Guidelines.

6.4.3 Phase III studies

Controlled Phase III clinical studies are designed to evaluate the benefit of the mAb in a patient population that is either at risk of acquiring the infection or which has a confirmed diagnosis of the infection. These studies are conducted to establish efficacy at the chosen dose(s) and dosing regimen against the primary and secondary end-points established during Phase II studies, and to further evaluate product safety and monitor its potential side-effects.

Specific decisions on the size of the study group will depend on factors which may include: (a) the magnitude of the effects of interest (the end-points) in comparison to the active comparator or placebo; (b) the incidence of the infectious disease within the community at the time of the clinical study; (c) the characteristics of the study population; and (d) the study design. Confirmatory Phase III clinical studies must be adequately sized and powered to meet the primary end-points, and to accord with the statistical analysis plan.

As a general principle, two confirmatory studies are preferred which demonstrate that the results can be replicated in relevant and diverse populations. In some cases, one well-controlled pivotal Phase III study with statistically

compelling and clinically relevant results could be sufficient for product marketing authorization. However, such results should be supported by the mechanism of action, Phase II study results and any complementary information obtained from other trials with the same mAb product that might help to define the target populations and indications. In other cases, a second confirmatory study may be necessary to demonstrate that the results can be replicated. The requirements for both single and repeat studies should be discussed with the NRA.

6.4.4 Human challenge trials

Human challenge trials are clinical trials in which participants are intentionally challenged with an infectious agent in order to evaluate the efficacy of a prophylactic or therapeutic pathogen-directed mAb. Such trials have proven useful in the clinical evaluation of some vaccines and may provide similar clinical support for mAbs against some infectious diseases, particularly where there are insufficient cases within a population to conduct large Phase III studies or to provide support for an emergency use authorization, or when animal models are not available (*55*). The use of human challenge trials in the clinical development plan should be discussed with the NRA in advance for consideration and feedback regarding their potential role.

The use of such trials requires a strong and thorough risk assessment and ethical evaluation prior to commencement. For infections with lower risk (such as those with low mortality, an acute onset which can be readily and objectively detected, or an absence of any indication of long-term or late-onset harm) and/or for which efficacious treatments exist, a human challenge trial may be feasible. However, for infections associated with high fatality rates and/or in the absence of an effective treatment, this approach is not recommended. To reduce the risks associated with the infection, it may be possible to use less-virulent or attenuated strains of the disease agent, but if so the binding affinity of the mAb to the strain in comparison to the wild-type organism should be determined and the results included in the submission. Regardless of the pathogen used in human challenge trials, it is important that they are well characterized and that a standardized challenge strain and dose are used throughout.

Additional information on human challenge trials is provided in WHO Human challenge trials for vaccine development: regulatory considerations (*55*). Guidance on the ethical considerations of such studies is provided in WHO guidance on the ethical conduct of controlled human infection studies (*56*).

6.5 Safety

The continual evaluation of mAb product safety is an important component within all phases of clinical studies. Although mAbs generally have a very good safety profile, each product is unique and should be considered independently.

Animal testing conducted during nonclinical development may not reveal all adverse events that might occur in humans – the lack of a safety signal in animals does not exclude the potential for safety issues in humans. Therefore, FIH studies should include risk-mitigation and risk-management strategies which may include the use of well-spaced and gradual dose-escalation, ad hoc review of emerging data and stop criteria. For mAbs against infectious diseases, attention should be given to potential hypersensitivity, autoimmune and immune-complex issues, and to the potential for ADE – though potential problems of this nature should have been ruled out as far as possible during nonclinical evaluation (*42*).

Safety data should be obtained from a sufficient number of subjects during the clinical trials to characterize and quantify the product safety profile, which can include the type, frequency and severity of adverse drug reactions (ADRs). In some cases, it may be possible to consider safety data from multiple clinical studies if both the product tested and the study conditions are sufficiently similar.

To assess potential changes in the ADR profile over time and to capture potentially delayed ADRs, the safety evaluation should continue for a reasonable length of time following product administration, taking into account the intended duration of the mAb activity and its half-life. However, rare adverse events are unlikely to be detected at this stage of product development and evaluation.

In the case of mAbs conjugated to a toxin, undesired tissue targeting and toxin release due to degradation are major safety concerns. Therefore, patients receiving such conjugated mAbs should be monitored more frequently for potential toxic effects.

General guidance on safety as well as on required cardiac studies is provided in the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*). Additional requirements for mAb product safety evaluation should be discussed with the NRA.

6.5.1 Reactogenicity

The reactogenicity of a prophylactic or therapeutic mAb can be a significant problem and should be monitored in all phases of clinical development. Immune responses to the mAb can vary greatly among subjects and may have little or no clinical effect, or may interfere significantly with the safety and/or activity of the product. Therefore, the monitoring of ADA titres and of immune activity is of great importance in evaluating the safety and activity of mAbs and in designing protocols involving their repeat administration (*57*).

Product reactogenicity can be influenced by patient, disease and product factors. Patient-related factors that might predispose an individual

to a particular type of immune response include their genetic makeup, pre-existing immunity, immune status and history of immunomodulating therapy. Treatment-related factors include dosing schedule and route of administration. Product-related factors that may influence the likelihood of an immune response include the similarity of the mAb to endogenous human immunoglobulins, the manufacturing process, and product post-translational modifications, formulation and stability characteristics.

Developing assays to test for ADAs can be methodologically challenging as standard assay formats involving anti-immunoglobulin reagents are not appropriate for this product class. Depending on the mAb construct, assays for ADAs will need to be developed that can distinguish them from the administered mAb product.

6.6 Special populations

As in any clinical development programme, studies in special populations would be expected where relevant to the indications. This may include, for example, in the elderly or children who may be more susceptible to the disease (such as COVID-19 or respiratory syncytial virus, respectively). Therefore, it is important to define both the nature of the infectious disease in these special populations and the features of the population which make them unique. In all cases, the inclusion of special populations in clinical studies should be discussed with the NRA.

6.6.1 Paediatric population and children

The extent of safety studies needed in children will depend on whether or not extrapolation from adults and children of other age groups is possible. Some mAbs may be designed for use in children from the beginning of product development, such as those targeting diseases which pose a greater risk to newborns, infants and/or children. Evaluation should be carried out in the appropriate age group, and it is usually recommended to begin with older children before extending the trial to younger children and then to infants.

Where justified, extrapolation of efficacy data from adult to paediatric patients may be based on PK and/or PD data (for example, when a similar effect can be expected with similar mAb exposure). However, safety data for children cannot always be extrapolated from adult studies and additional studies may be required. The adverse event profile may differ in paediatric populations compared to adults. Data on the safety of the mAb in the paediatric population should therefore be generated unless its use is clearly inappropriate.

During clinical development, the timing of paediatric studies will depend on the product, the type of disease being prevented or treated, safety considerations (including the need for a juvenile toxicity study in animals) and

the efficacy and safety of alternative treatments (*58*). The justification for the timing and approach of a clinical programme which may include the paediatric population should be discussed in advance with the NRA.

6.6.2 Elderly population

The safety of mAb products should be investigated in elderly patients during clinical development unless there is no intention of using them in this age group. Adverse effects in the elderly population can be more severe, or less well tolerated, and may have more serious consequences than in younger populations. Therefore, it is important to determine whether the PK profile, efficacy, potency and safety of a mAb are different in the elderly compared to younger adults. If so, the elderly sub-population should be sufficiently represented in the main Phase III or Phase II/III clinical trials to permit the comparison of treatment effects, dose response and safety between older and younger patients – or investigated in separate studies. Population PK modelling and simulation PK data may also be used to support dosing in the elderly population.

6.6.3 Evaluation during pregnancy

The conducting of clinical trials in pregnant subjects may not be permitted in some countries and should be discussed with the NRA in advance. Where clinical trials during pregnancy are permitted, the inclusion of pregnant subjects should be based on an assessment of the potential benefits and risks for the mother, fetus and newborn, as well as on safety data gathered from nonclinical studies (including tissue cross-reactivity studies that include embryo-fetal and pregnancy protein targets) and from clinical trials in adults.

Understanding the process and likelihood of placental transfer of the mAb can also help in evaluating the risk of their administration during pregnancy. For mAbs that contain a constant region (Fc) of immunoglobulin G1 (IgG1) there is likely to be minimal active placental transfer during the first 20–22 weeks of pregnancy, due to the absence of the neonatal Fc receptor. However, the transport of mAbs across the placenta increases significantly towards the third trimester of pregnancy.

Longer-term observational studies are recommended to confirm the efficacy and safety of any mAb administered during pregnancy. Such studies would help assess whether gestational exposure to the mAb product poses a risk to the newborn, and whether such risk depends on the trimester of exposure. In all cases, the inclusion of pregnant women in clinical trials should be discussed with the NRA. Should the investigational mAb be inadvertently administered during pregnancy or pregnancy is confirmed soon after mAb administration, follow-up of the mothers and infants should be continued following birth and the findings supplied as part of the product submission package. Additional

notes on testing during pregnancy are provided in the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*).

6.7 Manufacturing and formulation changes

While manufacturing and formulation changes may be expected during product development, the Phase III studies should be conducted using mAbs manufactured according to the final manufacturing (commercial) process. If the product intended for commercial use is not available or has changed, a comparability exercise between the clinical and commercial product may be necessary to ensure that the change has not impacted the clinical performance of the product. Such a comparability exercise should normally follow a stepwise approach, starting with a comparison of the quality attributes of the active substance and relevant intermediates. However, this should not be limited to the routine release testing of the product but should also include more-extensive characterization parameters using a range of suitable analytical methods appropriate to the product and process changes in question. If differences are detected that might influence the clinical properties of the product, then nonclinical and/or clinical bridging studies (such as PK/PD studies and possibly immunogenicity studies) may be required. Further information can be found in the WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use (*7*) and in the WHO Guidelines on procedures and data requirements for changes to approved biotherapeutic products (*28*).

6.8 Phase IV and post-marketing studies

Phase IV studies may be required to further evaluate a mAb in order to obtain additional information on its safety or effectiveness, or both – especially if the product has been authorized for emergency use or was evaluated through a non-traditional regulatory pathway in which post-approval commitments were made. Such studies also provide an opportunity to evaluate the mAb in more diverse populations (for example, with regard to ethnicity or geographical location) and/or in groups with prior exposure to the infecting agent. Real-world evidence, such as that provided through the literature or derived from studies in other countries, may also provide supporting information. Post-marketing surveillance should also be conducted when it is anticipated that escape variants will emerge in order to test the activity of the mAb against newly recognized variant strains of the pathogen, or to monitor ADE. The requirements and plans for Phase IV studies and the use of real-world evidence and real-world data should be discussed with the NRA.

6.9 Statistical considerations

A number of general and specific statistical considerations, including the need for a statistical analysis plan, are outlined in section C.4 of the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*). Additional statistical considerations can be found in the ICH E9 Statistical principles for clinical trials guideline (*59*).

6.10 Pharmacovigilance systems and risk-management planning

Pharmacovigilance systems and risk-management plans should be developed by sponsors to include activities which reflect the risks associated with a specific mAb product and its intended use. Such risks may include potential reactogenicity, toxicity, ADE or reduced efficacy against circulating virus variants or antibiotic-resistant bacteria. A risk-management plan should be submitted and agreed with the NRA. This plan should note whether specific surveillance will need to be done and where relevant information may minimize risk.

Sponsors and prescribers are encouraged to facilitate the utilization of mAb products among those patients most likely to benefit from them. In addition, the genomic identification and characterization of mAb targets in locally circulating pathogens can augment antimicrobial stewardship and pharmacovigilance.

Further discussion of the key components of a risk-management plan can be found in the WHO Guidelines on the quality, safety and efficacy of biotherapeutic protein products prepared by recombinant DNA technology (*42*).

7. Authors and acknowledgements

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Appendix 1

Considerations regarding abbreviated submissions for mAbs against an infectious disease during a public health emergency

During a public health emergency, some NRAs may consider reviewing mAb products against the infectious agent supported by abbreviated submissions, and/or providing conditional marketing authorization, in order to expedite product availability. This process requires that an appropriate regulatory framework is in place that allows for the review of abbreviated submissions and outlines the conditions under which they may be considered.

Although it is not possible to outline a common regulatory pathway detailing the minimum nonclinical and clinical study requirements applicable to all situations and all regulators, one strategy that has evolved to reduce product development time during a public health emergency is the conducting of parallel nonclinical and clinical studies, as well as overlapping or combined Phase I/II and/or Phase II/III clinical trials. Such a condensed strategy may be acceptable to some NRAs under appropriate circumstances for supporting the issuing of a limited or temporary form of marketing authorization. However, as expectations and regulatory capacities for reviewing abbreviated submissions vary greatly between countries, and may differ for each outbreak, regular communication between the sponsor and NRA is strongly advised and should begin as early as possible. Ongoing discussions should be held to clarify commitments and their timelines, as well as post-authorization expectations, on the understanding that the full nonclinical and clinical programmes are continued until the requirements for full licensure are met. Additional points to consider during discussions will be situation dependent, but may include requirements to: (a) monitor the affinity of the mAb for the circulating pathogen strain; (b) review mAb product development plans during an evolving pandemic (for example, due to the difficulties of completing confirmatory trials as variant strains emerge); and/or (c) use real-world evidence and real-world data in supporting clinical data packages.

The use of platform technology in the manufacturing of mAbs may reduce the development time required for establishing and validating production processes and quality control methods (*1*). However, although mAbs produced within established platform technologies may provide some level of confidence with regard to product safety, most NRAs would still regulate such mAbs as any other new biological product. Therefore, platform technology might not

reduce the nonclinical and clinical regulatory expectations or requirements for marketing authorization.

During a public health emergency, it is important to determine the minimum nonclinical studies which can reasonably support the start of Phase I clinical trials of mAbs against the infectious disease. The characteristics and novelty of the candidate mAb product should be taken into consideration, along with the biology of the infection and target antigen. For a candidate product for which there is little or no clinical experience, NRAs may require a greater amount of toxicity data. In such cases, the nonclinical studies should focus on any unexpected direct and indirect consequences that might result from administration of the product. It is important to note that any limited nonclinical toxicity dataset must be of good quality, and be generated from relevant animal species following the principles of good laboratory practices to the fullest possible extent.

Interim data from ongoing toxicity studies and the submission of draft unaudited toxicity study reports may be sufficient to support proceeding to Phase I clinical trials. NRAs may require that the toxicity studies include the immediate effect on survival, vital physiological functions, histopathology data, safety pharmacology, local tolerability and/or TK assessments. In cases where clinical trials were initiated on a minimum safety data package, the nonclinical programme should continue in parallel with clinical development. An abbreviated nonclinical package should contain tissue cross-reactivity studies, PD proof-of-concept studies and a pivotal toxicity study. It is emphasized that the pivotal nonclinical toxicity study should be conducted in a pharmacologically relevant animal species at an age that reflects the proposed clinical target population for emergency treatment (for example, adult animals for pandemic pathogens primarily affecting the elderly, or juvenile animals for pandemic pathogens that primarily affect young children).

PK evaluation in animal models may be omitted if sufficient human PK data is anticipated or becomes available. The abbreviated submission may also omit reproductive toxicity studies and carcinogenicity risk assessments – however, the provision of a scientific rationale for their omission is encouraged. Juvenile toxicity studies can be omitted when the target population for emergency treatment is not children, and on the understanding that the data gap would need to be addressed with a nonclinical juvenile toxicity study and/or clinical data/experience at a later time and prior to approval of the mAb for use in children (*2*). Similarly, large-scale Phase III efficacy trials may be approved in endemic regions without enrolling pregnant women – however, NRAs may require that developmental toxicity studies be conducted in parallel in order to support their eventual inclusion, either prior to the conclusion of the Phase III study or through their enrolment in a separate clinical study.

Since the use of a reduced toxicity dataset during a public health emergency provides less certainty about the safety of the mAb product, additional nonclinical data should be submitted as they become available, including data on any delayed effect observed at later time points in repeat-dose toxicity studies, histopathological data and the final signed audited reports. At the time of the full licensing application, the completed nonclinical data appropriate for the mAb should be submitted, or the application should be otherwise adequately justified.

Phase I and II studies of investigational mAbs against infectious diseases are, in general, expected to provide initial safety information and determine optimal dose(s). During a public health emergency, NRAs may consider recommending larger Phase I clinical studies to increase the early safety database, as well as the use of study populations similar to the eventual target population, thus facilitating timely initiation of Phase II clinical studies. This might be done by enrolling more trial sites than usual.

The epidemiology of the disease is likely to have a major impact on the timing and design of Phase III studies. In the face of an outbreak, and without any available preventive vaccines or other medications, mAb evaluation should still adhere to the principles of the phased approach but the intervals between clinical trial phases may be compressed to the point of overlap. For example, compressed timelines for clinical development may be achieved by initiating Phase III studies based on interim safety data from earlier-phase studies rather than on data from final study reports.

As the mAb product is intended for a foreign (non-endogenous) antigen, the early benefit–risk considerations may favour its safety profile in humans with underlying medical conditions. Therefore, under the circumstances of an emerging outbreak, epidemic or pandemic, consideration should be given to adjusting the trial-inclusion criteria to include those populations at higher risk from the emerging pathogen (for example, the immunocompromised, or those with cardiac, respiratory or renal diseases).

Phase II and Phase III clinical trials may be designed with prospectively planned adaptive features that allow for changes in design or analyses based on examination of the accumulated data at pre-specified interim points in the trial. Such adaptive features may make trials more efficient but also risk introducing complexities that would require advanced statistical plans and additional consultations with NRAs.

If the nature of a public health emergency affects the benefit–risk balance of a mAb product in such a way as to justify its accelerated development and conditional approval, the product sponsor would still be required to complete the full development work to the same standard required for a new mAb under non-emergency conditions should it be decided to subsequently submit the product for full licensure. The required supplementary data and expected timelines for their submission should be agreed between the sponsor and the NRA.

Regulatory processes and national requirements for the emergency assessment of products to be used during public health emergencies vary greatly between NRAs. For some NRAs, experience in considering products within an abbreviated development pathway may be limited and their capacities stretched due to the greater burden placed on resources. As part of good reliance practices (*3*), NRAs are strongly encouraged to implement evidence-based reliance on the assessments and decisions of trusted partner NRAs, WHO and regional regulatory bodies. This may be particularly valuable for NRAs with limited experience in reviewing applications for mAbs or with limited resources that may be further stressed during an epidemic or pandemic.

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Annex 3

Considerations in developing a regulatory framework for human cells and tissues and for advanced therapy medicinal products

Guidance documents published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes regulatory considerations for national regulatory authorities (NRAs).

Abbreviations

ATMP	advanced therapy medicinal product
CQA	critical quality attribute
DNA	deoxyribonucleic acid
GCP	good clinical practices
GLP	good laboratory practices
GMP	good manufacturing practices
HCTs	human cells and tissues for medicinal use
ICDRA	International Conference of Drug Regulatory Authorities
mRNA	messenger RNA
NRA	national regulatory authority
RNA	ribonucleic acid

1. Introduction

The use of cell, tissue and gene therapy products for the treatment of human diseases or physical conditions has generated wide interest due to their potential in addressing unmet medical needs. This very broad and diverse class of medicinal products (*1–3*) exhibits levels of complexity ranging from products that have been minimally manipulated prior to administration (such as unprocessed autologous cells and tissue grafts) to those that have undergone significant processing, culturing and/or other manipulation (such as substantially manipulated and/or genetically modified cells). Many countries have now established effective legal frameworks and regulations to protect donors and ensure outcomes in treated patients. Such frameworks and regulations reflect the diversity and complexity of this class of therapeutic product in terms of both product safety and efficacy.

Human cells and tissues which have undergone minimal manipulation are often used to provide the same essential functions in the recipient as they do in the donor, and are defined in this document as human cells and tissues for medicinal use (HCTs). Examples of HCTs include haematopoietic stem cells for the treatment of haematological malignancies, corneas to restore sight and skin grafts to treat burns. Such HCTs may be derived from living donors (for example, haematopoietic stem cells) or from the deceased (for example, heart valves, corneas and skin grafts). With advances in medicine, the number and variety of HCTs are steadily increasing. HCTs are most often regulated within transfusion or transplantation frameworks in which ethical principles and regulatory oversight are established to protect living donors, ensure the quality and safety of the donated material and improve outcomes for transplant recipients through best clinical practices and traceability (*4–6*).

Advanced therapy medicinal products (ATMPs) for human use are defined as cell and gene therapy products and tissue engineered products that are substantially manipulated and/or perform different functions in the recipient than in the donor. Although typically produced from substantially manipulated or genetically modified somatic cells or tissues, ATMPs can also include nucleic acids, and viral and non-viral vectors, as well as recombinant bacterial cells and recombinant oncolytic viruses (*7–9*). ATMPs are also very diverse and can include expanded autologous or allogeneic cells, engineered organs, viral products, genetically modified cells, and novel gene editing and/or edited products (*8–11*) (see Appendix 1). ATMPs may also be combined with medical devices, scaffolds or matrices as an integral part of the product ("combined ATMPs"). This wide variety of product types means that ATMPs have the potential to address a broad range of clinical indications, and may have inherent advantages over some existing treatments and current standards of care.

Some of these products are rapidly emerging as treatments that provide long-term benefits, potentially transforming the management of diseases such as thalassaemia, sickle cell disease, haemophilia, spinal muscular atrophy, Leber congenital amaurosis, certain cancers, monogenic inherited disorders and many other diseases (*2*, *12*, *13*).

ATMPs present unique challenges during their development and production that distinguish them from pharmaceuticals and from other biotherapeutic products. They are also distinguished from HCTs due to their substantial manipulation and/or non-homologous use. As a result, ATMPs can differ from other medicinal products in terms of their manufacturing and quality control requirements, nonclinical assessment, clinical development and post-market monitoring (*8*, *9*, *14*). An understanding of these challenges is therefore crucial in the development and establishment of a tenable regulatory framework for the oversight, authorization for marketing and clinical use of such products.

ATMP manufacturing often requires quality-by-design approaches, with additional considerations in their production stemming from the origin, sourcing and limitations of the starting materials (which may include HCTs) and from the manipulation processes that the starting material undergoes to generate the therapeutic product. For cells and tissues, as well as for nucleic acids and viral vectors, these methods can be complex and require specialized facilities and techniques for product manufacturing and formulation (*9*, *15*). This is particularly the case for genetically modified cells and directly administered vectors or nucleic acids. Therefore, manufacturing facilities for ATMPs are usually separate from the facilities where the starting materials are obtained and processed, and require specific manufacturing authorization by competent medicines authorities for their operation. In addition, any medical device used as part of a combined ATMP or in the administration of an ATMP also requires compliance with manufacturing and marketing regulations.

The nonclinical assessment of the safety and efficacy of ATMPs is challenging for many indications and especially for rare diseases. Such challenges include establishing relevant in vitro systems and/or animal models in which to study product safety and proof-of-concept of its functionality (*16*). Many associated limitations arise due to inherent differences in the immune systems and physiology of animal species and humans, and the lack of an established animal model of the disease. For cell-based therapies where multiple receptor-ligand interactions occur between the administered cell product and the surrounding host tissue, the physiological outcome of these interactions may also differ between species. Thus, when products are tested across species there are likely to be differences in the responses observed. Similarly, viral vectors may also present their own difficulties when studied using in vitro and/or animal models as they can differ in their tropism and will not necessarily infect

all species. Cell-based immunotherapies can also present challenges in their nonclinical assessment due to their exquisite specificity, and to complications such as host-versus-graft responses should human cells be administered to immunocompetent animals. This may be further complicated if the cell-based immunotherapy includes a species-specific genetic modification. Furthermore, the nonclinical testing of therapies which utilize genome editing technologies requires the use of human cells, humanized systems or testing of an animal-adapted version of the product to evaluate potential off-target effects.

Any clinical development programme for HCTs and ATMPs also requires special regulatory consideration as these medicinal products are often being developed for the treatment of rare diseases. Such considerations may include the need to account for the lack of adequately documented natural history data for the disease, as well as the need to evaluate clinical safety and efficacy in very small patient populations. Furthermore, interpretation of efficacy from controlled clinical trials for some ATMPs may be difficult if there is no suitable comparator or if there is a limited effect in the overall population and the subgroup of individuals having benefit is not known. Some ATMPs, such as cells harbouring integrated nucleic acids or systemically administered integrating vectors, may have effects that last for years or decades. Under these circumstances, it is important to assess the need for adequate long-term patient follow-up (*17*).

Countries in all regions of the world are receiving – or have received – clinical trial applications and/or regulatory authorization submissions from companies or non-profit organizations interested in providing access to HCTs and ATMPs. With the growing number of such products and submissions, it is important for regulatory authorities to be aware of the regulatory considerations, challenges and need for adequate supporting data to assure product quality, safety and efficacy, and to avoid unnecessary delays in patient access.

Given the highly varied nature of HCTs and ATMPs, it is not surprising that different national or regional regulatory frameworks have evolved for the oversight of these medicinal products around the world. However, despite their differences, all such frameworks are intended to ensure the highest standards in protecting donors, and ensuring the quality, safety and efficacy of the administered products. Any such regulatory framework should also be based on sound scientific and ethical principles, and include the requirement for comprehensive evaluation of the benefit–risk ratio applicable to each of the different categories of HCTs and ATMPs.

Effective regulatory decision-making will depend on establishing strong, risk-based regulatory frameworks for the oversight of ATMPs – and of HCTs where these are not sufficiently regulated under an existing transfusion or transplantation framework within a regulatory jurisdiction. Achieving the right balance is crucial – while under-regulation may expose recipients to risk,

excessive regulations may deter innovation and hinder access to novel therapies. The key elements of an effective regulatory framework for these types of medicinal product include:

- a clear definition of the categories that constitute HCTs and ATMPs; and
- a risk stratification of the HCTs and ATMPs and a level of regulatory oversight that is appropriate for each category.

In most regulatory jurisdictions with existing legislation and regulations applicable to ATMPs, such products are regulated as medicinal products to ensure their quality, safety and efficacy before authorization for use in the patient population. The regulatory requirements for ATMPs will differ based on stage of product development. As more knowledge is acquired about the product and its safety and efficacy, and as the product moves from investigational to post-authorization use, the requirements will need to be adapted to an appropriate level of stringency, and cover an increasing number of parameters. For HCTs, the regulations will concentrate on the control of possible transmission of communicable diseases and contaminants, as well as on ensuring product quality and safety for its intended use, underpinned by ethical considerations for both the donor and recipient (*4*, *5*). Additionally, the regulatory expectations for ATMPs will also include requirements to address the added risks inherent in such complex, highly manipulated medicinal products (*18*–*25*). Furthermore, it will be important to ensure that appropriate long-term post-market surveillance systems are in place, particularly where any adverse reaction to an ATMP may not become evident for many years. In all cases, regulatory decisions should be based on the totality of the available information and on a comprehensive benefit–risk assessment covering the development phase through to the post-authorization phase. Any possible risks which may be introduced as a result of subsequent changes to the production process must also be considered.

As an integral part of their regulatory framework(s), national regulatory authorities (NRAs) with only limited experience of reviewing applications for HCTs and ATMPs, or with limited resources, are encouraged to have mechanisms in place for evidence-based reliance on the assessments and decisions of trusted partners and NRAs with more longstanding experience and expertise in this area. NRAs with limited experience are encouraged to consider the entirety of the product life-cycle (development, licensure and post-market), vigilance, patient access and sustainability when setting up their regulatory framework. This will include any decision-making processes with regard to reliance on the assessments and decisions of more experienced NRAs. The utilization of regulatory reliance for both initial marketing applications and post-approval amendments will help to ensure increased global access to safe and effective

HCTs and ATMPs. As NRAs gain experience and expertise, they can consider additional activities (for example, the reviewing of more complex applications in line with their increased capacity and resources) and/or further implement reliance approaches (for example, by participating in work-sharing procedures and/or establishing recognition pathways).

2. Background

In its 2014 resolution WHA67.20, the Sixty-seventh World Health Assembly called for increased WHO support and guidance in strengthening the capacity of countries to regulate increasingly complex biological products, including new medicines based on gene therapy, somatic cell therapy and tissue engineering (*26*). In addition, the WHO Expert Committee on Biological Standardization has on several occasions highlighted the importance of global-level standardization in the technical and regulatory approaches to these advanced therapies, and the key role of WHO in promoting such standardization (*27–29*). A consensus was reached by the Committee that global regulatory convergence for advanced medicinal products was needed and that WHO should collaborate with international groups active in this area. As part of this process, the harmonization of definitions and terminology would be particularly helpful for countries now in the process of setting out their own national requirements. Although a variety of relevant guidelines and regulations currently exist or are in development in different regions of the world, the Committee noted during its meeting in October 2021 that there was a high degree of commonality among different NRAs in the ways in which minimally manipulated cells are regulated (30). However, there remained a need to identify common principles for the regulatory evaluation of more complex medicinal products. In addition, during discussion at the 2018 International Conference of Drug Regulatory Authorities (ICDRA), participants noted the potential impact of HCTs and ATMPs on global public health and the need, especially in low- and middle-income countries, to build scientific knowledge and strengthen regulatory capacity to provide oversight of these novel medicinal products. Identified priorities in support of strengthening such regulatory capacity included:

- defining HCTs and ATMPs;
- developing regulatory requirements for HCTs and ATMPs based on sound scientific and risk-based principles; and
- promoting convergence in establishing minimum global standards for ATMPs.

The ICDRA recommendations to WHO were to:

> *... develop with Member States a "current state of the art" document capturing areas where agreement among experienced regulatory authorities exists, noting where harmonization has yet to be achieved, and documenting existing areas of uncertainty; areas covered could include definitions, quality attributes, standards, and clinical development pathways. (31).*

In response, WHO established an international working group on the standardization of cell and gene therapy products in 2019 to provide expert advice on the development of a WHO considerations document on developing a regulatory framework to assure the quality, safety and effectiveness of these medicinal products. As a first steps in developing the current document the following priorities were identified:

- Provide guidance on the categorization of HCTs and ATMPs, along with definitions of the key terms relevant to this area.
- Summarize the history of ATMPs under development or that have been approved, including examples of the challenges faced in their development, identified solutions and currently unresolved issues.
- Describe the key elements of a regulatory framework that would help to assure the quality, safety and effectiveness of HCTs and ATMPs including:
  - regulatory requirements for different risk categories of products; and
  - the need for adequate oversight of these products through their entire life-cycle, including the investigational phase to post-market surveillance, where relevant.
- Develop a proposal on how such a regulatory framework for the various risk categories could be implemented in countries with different levels of regulatory maturity.
- Provide useful information and references to key resources relevant to the development, manufacture and regulation of ATMPs.

3. Purpose and scope

The current document represents a further step in responding to resolution WHA67.20 (*26*), and to the above recommendations of the WHO Expert Committee on Biological Standardization (*27–29*) and the 2018 ICDRA (*31*). By outlining a number of fundamental principles and concepts in the regulatory oversight of different types of HCTs and ATMPs, the document is intended

to advance and promote both regulatory convergence and the practice of regulatory reliance across all jurisdictions – whether or not adequate regulations are currently in place. It is intended that this will in turn facilitate both the development of and access to advanced medicinal products. The document also outlines a number of priorities in harmonizing regulatory frameworks in order to improve product safety, ensure efficacy, and prevent the exploitation of donors and patients. In this regard, the document also serves to highlight the crucial importance of strengthening national regulatory systems for the oversight of these vitally needed medicinal products.

However, it is also acknowledged that in some countries, many or all HCTs may be regulated within existing regulatory frameworks on transplantation and transfusion. Thus, rather than providing comprehensive guidance on this topic, the current document is instead intended to serve as a foundation for the development of future WHO guidance on assuring the quality, safety and efficacy of HCTs and ATMPs.

The major aspects addressed in this document include:

- provision of definitions for key terms;
- the categorization of HCTs and ATMPs;
- use of a risk-based approach to the regulatory oversight of HCTs and ATMPs;
- the key elements of an effective regulatory framework; and
- provision of useful information on references and resources relevant to the manufacture, development and regulation of HCTs and ATMPs.

It should be noted that definitions of HCTs and ATMPs can vary between countries and regions. For the purposes of this and future WHO regulatory guidance documents in this area, the definitions and terms provided in the **Terminology** section below apply. It should also be noted that germ cells and/or potentially heritable genetic modifications are outside the scope of the current document and of the definitions contained herein. The document also does not apply to xenogeneic products or to organs for transplantation. Similarly, vaccines intended to elicit an immune response against infectious diseases are also outside the scope of this document, and are excluded from the definition of a gene therapy product. A large body of guidance on such prophylactic vaccines already exists and should be consulted instead. However, therapeutic vaccines – such as those under development for the treatment of cancer – fall within the scope of this document. Finally, although ethical principles are a key aspect requiring consideration in any product development process, particularly when donated human materials are involved, this issue is not addressed in the current text.

4. Terminology

The definitions given below apply to the terms as used in this WHO document. These terms may have different meanings in other contexts, or in other international or regional regulatory documents. It should also be noted that in this document, unless otherwise indicated, the term "cells" refers to human cells, excluding anucleated cells such as red blood cells and platelets.

Allogeneic: referring to cells and tissues donated by one person and used to treat a medical condition in another person.

Autologous: referring to cells and tissues taken from, and used to treat a medical condition in, the same person.

Advanced therapy medicinal product (ATMP): any cell or gene therapy product or tissue engineered product that has been substantially manipulated and/or performs a different function in the recipient than in the donor. ATMPs are usually produced from genetically modified and/or substantially manipulated somatic cells or tissues. ATMPs also include nucleic acids, viral and non-viral vectors, recombinant bacterial cells and recombinant oncolytic viruses. **Xenogeneic** cells and tissues are included in the definition of ATMPs but are not within the scope of this document due to the complexity of their application.

Cell therapy product: a product composed of human nucleated cells intended for replacement or reconstitution, and/or for the treatment or prevention of human diseases or physiological conditions, through the pharmacological, immunological or metabolic action of its cells or tissues.

Combined ATMPs: ATMPs that include a medical device(s), scaffold or matrix as an integral part of the product and where the device or additional supporting structure has a role/function in the product's overall effect and is not intended to be removed or used solely for administration purposes.

Critical quality attribute (CQA): any physical, chemical, biological and/or microbiological property and/or characteristic of a medicinal product that should be within an appropriate limit, range or distribution to ensure the desired product quality.

Gene editing: a method which allows for genetic material to be added, removed or altered in a sequence-specific manner in the genome. Currently, the most commonly used approaches are based on zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) or clustered regularly interspersed short palindromic repeats (CRISPR) together with Cas9-endonuclease (CRISPR Cas9) (*32*).

Gene therapy product: a medicinal product containing nucleic acids (for example, plasmids, messenger RNA (mRNA) or DNA) that are intended to regulate, repair, replace, add or delete a genetic sequence. The intended therapeutic effect is dependent upon the encoded gene used. Gene therapy products include those containing non-viral vectors (for example, lipid

nanoparticles) or viral vectors that are used in vivo, as well as cells that have been modified by these types of vectors ex vivo. They may contain plasmids, mRNA or DNA, and may also include oncolytic viruses that are not genetically modified to express a transgene.

> Within this definition, gene edited products are considered to be gene therapy products. However, vaccines intended to elicit an immune response to prevent infectious diseases (for example, mRNA, plasmid DNA or viral-vectored vaccines) are excluded from this definition and are not considered to be gene therapy products within the definition of an ATMP. It should be noted that the scope of what constitutes a gene therapy product may vary between regulatory authorities and, in some jurisdictions, might include prophylactic vaccines against infectious diseases.

Homologous use (same essential function/s): the concept that the essential functions of the cells or tissues in the recipient are the same, or highly similar, to their functions in the donor. For example, infusion of bone marrow cells for haematopoietic reconstitution would be considered homologous use, whereas the use of bone-marrow-derived mononucleated cells for the treatment of spinal cord injury, heart failure or osteoarthritis would be considered non-homologous use.

Human cells and tissues for medicinal use (HCTs): human cells and tissues that have undergone minimal manipulation, and which may be used to provide the same essential functions in the recipient as they do in the donor.

In vivo gene therapy product: a gene therapy product administered directly into the recipient.

Minimal manipulation: the concept that cells or tissues do not undergo processing steps that could substantially alter their risk profile (which could include characteristics such as structural properties and functionality), or that could induce their differentiation, activation, proliferation potential or metabolic activity. Minimally manipulated cells and tissues must not have a systemic effect and must depend on their own metabolic activity for their primary function.

> Cell or tissue processing steps that are considered minimal include sizing, rinsing and washing with solutions such as saline. Depending on local legal frameworks, the definition of minimal manipulation may also include other processing steps such as cutting, grinding, centrifugation, freeze-drying, antibiotic treatment, washing, sterilization/irradiation, cell separation or removal, cell suspension, concentration, filtering and/or cryopreservation.

Regulatory convergence: a voluntary alignment of regulatory approaches and requirements across countries and regions that may include the gradual adoption of international technical guidance documents and standards, and internationally recognized scientific principles, practices and procedures.

Regulatory framework: the collection of laws, regulations, guidelines and other regulatory instruments through which a government regulates HCT and ATMP research and development, manufacturing, clinical evaluation, marketing, promotion and post-market safety monitoring, as well as human cell and tissue donation, procurement, testing, processing, preservation, storage, distribution, clinical use, traceability and biovigilance.

Regulatory harmonization: a process by which technical guidance documents are developed to achieve uniform regulatory requirements among participating jurisdictions.

Regulatory reliance: the act whereby a regulatory authority in one jurisdiction may take into account and give significant weight to – that is, totally or partially rely upon – evaluations performed by another regulatory authority or trusted institution in reaching its own decision. The relying authority remains responsible and accountable for the decisions taken, even when it relies upon the decisions and information of others (*33*).

Tissue engineered product: a medicinal product composed of nucleated human cells that are substantially manipulated and/or used in a non-homologous way, and intended for the repair, replacement, reconstitution or regeneration of tissues. Some tissue engineered products may incorporate medical devices and/or natural or artificial scaffolds such as extracellular matrix proteins.

Xenogeneic: denoting cells, tissues or organs originating from one species and administered to an individual of another species.

5. Classification of HCTs and ATMPs

Minimal manipulation and homologous use are the concepts that have been embraced by numerous regulatory authorities when distinguishing between HCTs and ATMPs (*4*, *7*, *8*, *24*, *34*). Definitions of these concepts are provided in the Terminology section above, and their application illustrated in Appendices 1 and 2 below. For the purposes of the current document, cells and tissues that are recovered and which undergo only minimal manipulation (simple processing such as washing or sizing) and which are used to achieve the same essential function(s) in the recipient as they do in the donor (homologous use) are considered to be HCTs. Most minimally manipulated cells and tissues have fewer uncertainties in their risk profile to consider in a risk assessment compared to substantially manipulated cells or tissues. As a result, their regulatory requirements mainly focus on ensuring the quality and safety of the cells and tissues, and on the protection of donors and recipients through compliance

with the relevant ethical principles of transplantation frameworks. The quality and safety elements of HCT regulation primarily aim to prevent possible disease transmission and to mitigate risks associated with their origin, or that may arise during cell or tissue procurement and/or processing. When homologous use of human cells and tissues is intended, evidence of their clinical performance must be provided while product-specific clinical studies are usually not required.

Human cells or tissues may also provide the starting material for cell- or tissue-based ATMPs, and thus need to comply with the regulatory requirements applied to the donation, procurement and testing of such cells and/or tissues. The greater complexity of ATMPs compared to HCTs arises because ATMPs usually require controlled manufacturing processes with significant manipulation of the cellular or genetic starting material, and this can include expansion and/or purification steps (Appendix 1). In addition, their safety and efficacy cannot be predicted without well-controlled clinical studies due to the biological complexity of cells and tissues, and because their structure and/or function may be changed by the manipulation and production processes. Depending on the product and disease, clinical studies may require an innovative "fit for purpose" design which considers the complexity of both the ATMP and the treatment. Therefore, ATMPs require comprehensive regulation and demonstration of safety and efficacy, with robust data required to show high product quality, biological activity and manufacturing consistency, both prior to marketing authorization and following any manufacturing process changes (*1*, *4*, *5*, *9*, *35*). Further information on cell and gene therapy product regulation is provided in Appendix 3 below. In addition, regulations for ATMPs based on replicating and non-replicating viral vectors, viable viruses (for example, oncolytic viruses) or other potentially infectious agents which could be shed from the recipient should include separate considerations to address the possibility of their release into the environment, and the resulting induction of disease in (or transmission to) third parties. As strategies need to be in place to mitigate the risk of such an occurrence, this type of product should be subjected to an environmental risk assessment to evaluate the potential adverse effects of their transmission and/or release into the environment.

The wide range of medicinal products of varying risk profiles that constitute ATMPs requires consideration of their regulation as an overall class of product. Due to the substantial manipulation required to produce most ATMPs, controlled manufacturing processes are required to ensure consistency of production and acceptable levels of batch-to-batch variation. This includes assurance of product identity, purity, biological activity and freedom from adventitious agents (for example, viruses and prions) (*36*). Therefore, an important aspect in the development of ATMPs is the identification of critical quality attributes (CQAs) for each product. Due to the biological complexity of ATMPs, their production control requires multiple CQAs. Ideally, these CQAs

would correlate with clinical outcome (such as potency correlating with product efficacy) – though this may not always be possible or feasible. Examples of potential CQAs include:

- minimum percentage of a certain cell type as determined by specific cell surface markers;
- percent viability of cells;
- in vitro or in vivo potency;
- ratio of full to empty viral capsids; and
- correct genomic sequence.

Long-term safety and efficacy follow-up of individuals treated with ATMPs can also present challenges as these products may exert long-term effects following even a single administration. For example, lentivirus-vector-transduced CD34+ cells that are systemically administered to correct a genetic defect could exert their effect for years through the integrated presence of the vector in cells. Thus, the risk of insertional mutagenesis should be addressed in nonclinical and clinical studies, and safety surveillance monitoring systems that allow for longer-term follow-up of all treated patients should be in place to identify any emerging serious adverse reactions, including the development of malignancy (*37*). A risk-based approach should be used to determine the duration of any long-term safety surveillance requirements. Careful consideration is needed to ensure the optimal collection of necessary data without this being unduly burdensome for patients receiving the gene therapy products.

It should also be noted that the risk profiles of HCTs and ATMPs are not always clear, or easy to address, and that HCTs do not necessarily have a lower risk profile than ATMPs. For example, the use of fresh versus frozen cells/tissues may have a significant impact on treatment outcome, while the risks of using a vector can differ depending on whether it is used in vivo or for ex vivo transduction. The risk identification should also take into account the level of scientific knowledge supporting the use of a medicinal product (for example, on the biology of cells and tissues and their normal functionality) as well as prior manufacturing experience for similar products. Special attention should be paid to medicinal products used for the first time, and/or where there is limited or no knowledge of their safety or efficacy in humans, or experience in their production.

6. Regulatory expectations for HCTs and ATMPs

Working towards the global convergence of regulatory expectations for HCTs and ATMPs, and ultimately regulatory harmonization, will facilitate global access to these potentially transformative medicinal products. The harmonization of

regulations and regulatory expectations will be crucial in supporting timely product development and access – in part, because it will allow product developers to submit regulatory applications more efficiently and cost-effectively across different jurisdictions.

As an initial step towards convergence, it is useful to consider cell, tissue and gene therapy products as belonging to one of two broad categories based on the risks arising from their processing and/or manufacturing:

1. HCTs – where the minimal processing of the cells or tissues and their intended homologous use introduces fewer uncertainties in their risk profile; or
2. ATMPs – which require complex manufacturing steps or are composed of cells not being used for the same essential functions, thus introducing greater uncertainty in their risk profile.

Such category determinations can be made by answering the following fundamental questions:

- Is the product a gene therapy product and/or does it include genetically modified cells?
- Is the product intended for blood transfusion or organ transplantation?
- Is the product minimally manipulated?
- Is the product intended for homologous use?

A schematic illustration of the application of these questions in classifying HCTs and ATMPs is provided in Appendix 3 below.

Although HCTs do not usually require marketing authorization, their donation, processing and transplantation must generally be authorized by competent authorities to ensure their quality and safety, and to protect donors and recipients. In addition, the facilities and establishments dedicated to the procurement and processing of HCTs may also require approval/licensing by competent authorities. The use of HCTs for the treatment of diseases or physiological conditions may also require approval from a local or institutional ethics committee, while information on the effectiveness of the treatment is typically collected through clinical studies and/or registries. Furthermore, any post-approval changes in the processing of HCTs may also require an assessment of associated risks, along with an evaluation of the impact of the change(s) on product specifications and release criteria.

For ATMPs across a spectrum of complexities and risks (see Appendix 1 below), regulations based on stringent requirements for product quality, safety and efficacy, and on assuring manufacturing consistency, have been established

in many jurisdictions. For countries developing regulatory frameworks for HCTs and ATMPs, it is strongly recommended that such regulations are aligned with other relevant regulations that may already be established in the jurisdiction, with any additional requirements adapted to reflect the specificities of HCTs and ATMPs.

7. A risk-based approach to the regulatory oversight of HCTs and ATMPs

Although HCTs and ATMPs have the potential to bring considerable benefits to individuals for a wide range of diseases, they can also cause serious harm if they are not prepared and used properly, or not supported by adequate nonclinical and clinical evidence. Therefore, it is important for regulators to have a good understanding of a product before they approve its use in order to minimize the risk of introducing unproven therapies for which there is an insufficient body of evidence to support clinical use (*38*). For ATMPs in particular, developers may benefit from early discussions with, and regulatory guidance from, the regulatory authority before initiating clinical studies to ensure that risks are identified and appropriately mitigated. There will need to be careful consideration of product development and deployment under appropriate regulatory oversight. The conducting of investigational studies or deployment of these medicinal products, especially ATMPs, without appropriate regulatory oversight and adequate safety monitoring can result in severe adverse outcomes for product recipients. Similarly, a failure to ensure the containment of ATMPs manufactured using replicating microbial vectors could pose a risk to third parties and/or to the environment. Thus, it is vital that all regulatory authorities are familiar with the potential risks and regulatory considerations for HCTs and ATMPs, and with the appropriate level of regulation required in each case. ATMPs should also be authorized by a competent regulatory authority that has evaluated the product's quality, safety and efficacy. This will be essential in preventing patients from receiving treatments and therapies that have no proven benefit.

A scientifically sound, risk-based approach is a practical way to regulate HCTs and ATMPs and has been adopted in most current national and international guidelines. A risk-based approach involves identifying and taking into consideration all of the various risks and risk factors that may impact product quality, safety and efficacy, including risk factors that may be inherent to the HCTs or ATMP, and ensuring that those risks are mitigated. Since HCTs and many ATMPs are derived or prepared from living organisms or are themselves living organisms, the risk of infectious disease transmission is a fundamental concern and must be mitigated. Additional potential risks can vary and are largely dependent on the type of cells or tissues, or ATMP. The mitigation of

such risks may include the need for appropriate human leukocyte antigen (HLA) matching in certain transplants and applications, and consideration of the potential immunogenicity, tumorigenicity, genotoxicity, implant failure and insertional mutagenesis of the product.

The manipulation of cells and tissues can increase the risk of their transformation and tumorigenicity, and also of their unwanted immunogenicity and other severe toxicities (*39*, *40*). Many gene therapy products are manufactured using recombinant forms of common viruses, the wild types of which can be human pathogens. Therefore, gene therapy vectors are usually constructed to not contain those parts of their native genomes that make them pathogenic or allow them to replicate. However, other risks associated with gene therapy products remain, including replication-competent virus contaminants, undesired immunogenicity and insertional mutagenesis leading to tumorigenicity. Good manufacturing practices (GMP), good laboratory practices (GLP), and adequate nonclinical and clinical studies conducted under good clinical practices (GCP) are required to identify and mitigate as many risks as possible to ensure patient safety.

For cells and tissues destined for allogeneic transplantation, it is crucial that proper measures are in place to screen the donors (either living or deceased) for relevant communicable disease risks that might be associated with disease transmission from the donor to the recipient. Tests to perform generally include those for certain viruses (such as hepatitis B virus, hepatitis C virus and human immunodeficiency virus) as well as other infectious agents that may be locally or globally relevant. In addition, appropriate testing of the cells or tissues to detect contamination (such as microbiological cultures) should be performed to protect recipients.

The entities that perform donor screening or testing, or that recover, process, store and/or distribute HCTs are generally registered by the regulatory authority overseeing them and should comply with current good tissue practices, where adopted (*41*, *42*). Registration involves, at a minimum, recording the name and physical location of the establishment providing the HCTs, as well as a detailed list of the different cells or tissues being offered by the establishment. This will facilitate the implementation of traceability systems between the donor and recipient, which will be vitally important if an infectious agent is identified or suspected in either the donor or recipient of the HCTs. It will also facilitate the ability to recall entire lots or classes of products in a timely manner should issues such as bacterial or viral contamination be identified. In addition, it should be verified that donor screening and testing, as well as the recovery, processing, storage, distribution and use of the HCTs, do not introduce other risks to the recipients. It should also be verified that the HCTs do not meet the criteria of being ATMPs – in which case they would require specific marketing authorization.

ATMPs require the same risk-based approach as HCTs to prevent the transmission of infectious diseases and the mitigation of any other potential risks which may be inherent in the product. In addition, ATMPs require compliance with other key regulatory practices including:

- GMP to ensure that the ATMPs used for clinical trials and clinical use are manufactured under a quality management system with investigational phase-appropriate quality controls, and procedures in place for the management of process changes.
- GLP applied, where feasible, in required nonclinical studies used to gather safety data for HCTs and ATMPs to ensure that the risks are understood and mitigated before use in humans. Pharmacodynamic (PD), pharmacokinetic (PK) and biodistribution analysis included in a toxicology study is not necessarily required to be conducted under GLP.
- GCP applied to all clinical studies on ATMPs with proper design and control to ensure the collection of robust and reliable safety and efficacy data for the product and appropriate long-term follow-up of patients.

These aspects require that the regulatory authority must have the capacity and expertise to evaluate and authorize both clinical trial and marketing authorization applications, and to oversee post-market surveillance to monitor the long-term safety and efficacy of authorized ATMPs. In addition, ensuring compliance with GMP, GLP and GCP requires that the regulatory authority and/or its inspectorate have the capacities and expertise needed to perform the necessary inspections.

It is essential that the safety of all authorized ATMPs be continually monitored while they are being used in medical practice. This will include the implementation of a pharmacovigilance system for such products in which all authorization holders should participate. To this end, the product authorization holder should have a system for compiling, processing and evaluating information on suspected adverse reactions, and for communicating this information to the regulatory authority. This will enable the early detection of risks and effective mitigation of their consequences for patients, and will inform the design of appropriate post-authorization studies to monitor product safety and efficacy.

8. Considerations in the development of a regulatory framework

The diversity of HCTs and ATMPs may require tailoring of the regulatory framework to adapt to the range of medicinal products that a country may

authorize for use within its jurisdiction. Use of HCTs that do not require marketing authorization can potentially be administered in settings with less experienced regulatory systems. However, appropriate regulations and oversight must be in place to ensure donor rights and safety, and respect for ethical standards to minimize the risk of communicable disease transmission, to ensure that the HCTs are of appropriate quality, and that safety standards (including with regard to coding, traceability and biovigilance) have been appropriately applied for the intended use. It is also important to ensure that mechanisms are put in place for both ethical and inspectional oversight, and that medicinal products can be traced and recalled if necessary.

Due to their risks, manufacturing complexity and intended use, the regulatory oversight of ATMPs requires robust quality assurance mechanisms and the demonstration of safety and efficacy in clinical trials before their authorization for use. Where the necessary experience for such regulatory oversight is not yet in place, several options exist based on the principles of good reliance practices (*33*). For jurisdictions with minimal experience in the regulation of ATMPs and with less well developed safety surveillance systems, it may be possible to have cell therapy or tissue engineered products marketed following an external regulatory review process by a more experienced regulatory authority. Such an approach would be based both on the external review of the nonclinical and clinical evidence and on the external implementation of appropriate surveillance measures. Jurisdictions with limited resources and experience of ATMPs could also rely on the review and approval of clinical trials and/or marketing applications by jurisdictions with greater experience in regulating ATMPs. For jurisdictions that already have some experience with cell therapy and tissue engineered products and that have an adequate safety surveillance system in place, it may be feasible to review and approve less complex ATMPs that have had fewer potential risks introduced during their manipulation. For jurisdictions with more extensive experience in the approval of simple ATMPs and which have established safety surveillance systems, it may be reasonable to approve more complex ATMPs and allow their local investigational use in controlled clinical trials under an appropriate regulatory framework and with ethics committee oversight. Such regulatory authorities may also review marketing applications and post-approval changes for these ATMPs and make decisions regarding their approval. There are also intermediate states between these various options that an individual jurisdiction could consider. In general, good reliance practices would also help to minimize the risk of authorizing unproven therapies that have not undergone controlled clinical trials and/or that have an insufficient body of evidence to support their marketing authorization.

9. Collaboration and strengthening global regulatory capacity for the oversight of HCTs and ATMPs

WHO encourages regulatory cooperation and reliance between authorities and other entities involved in the oversight of HCTs and ATMPs. Existing opportunities for joint reviews and inspections, agency visits, collaboration in the reviewing of medicinal products for rare and ultra-rare diseases, regulatory activities based on reliance, and so on could all be further expanded and would positively impact upon the global accessibility of these products. Sharing knowledge, expertise and experience is crucial for strengthening global regulatory capacity for the oversight of HCTs and ATMPs in all regions of the world. For those regulatory authorities now in the process of investing resources in strengthening their regulatory capacity and building up their expertise there would be significant benefits in collaborating with a more experienced regulatory authority. In addition, strengthening regulatory capacity and advancing global convergence in the regulation of HCTs and ATMPs will provide further opportunities for clinical research. This is particularly the case in the field of HCTs and ATMPs intended for the treatment of rare diseases.

To increase access to high-quality, safe and effective ATMPs, collaboration between regulators regionally and globally, including through regulatory networks (*43*–*46*), is encouraged to share knowledge and experience and to leverage resources more efficiently. The convergence of regulatory requirements in different jurisdictions will increase efficiencies and promote opportunities for reliance. Such regulatory reliance is even more crucial in promoting access to ATMPs since regulators in many countries currently have limited or no experience in the authorization of these products.

Authors and acknowledgements

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Based on the outcome of the working group meeting, a preliminary first draft was prepared by: P. Marks and G. Raychaudhuri, US FDA, USA; and

I. Knezevic and S.H. Yoo, World Health Organization, Switzerland. The draft was then subjected to review by the working group and by: C.A. Bravery, consultant, United Kingdom; I.U. Oh, Ministry of Food and Drug Safety, Republic of Korea; P. Salmikangas, consultant, Finland; and J. Wang, National Institutes for Food and Drug Control, China. Following incorporation of the working group feedback, the resulting document was posted on the WHO Biologicals website from 20 December 2021 to 24 January 2022 for a first round of public consultation.

An informal consultation on the document was then held virtually on 7–9 February 2022 and attended by: *Working group members*; C.A. Bravery, consultant, United Kingdom; G. Jotwani, Indian Council of Medical Research, India; P. Marks and G. Raychaudhuri, US FDA, USA; Y. Maruyama, Pharmaceuticals and Medical Devices Agency, Japan; F.C. Melo and R.M. Parca, National Health Surveillance Agency, Brazil; I.U. Oh, Ministry of Food and Drug Safety, Republic of Korea; I.G. Reischl, Federal Office for Safety in Health Care, Austria; P. Salmikangas, consultant, Finland; J. Wang, National Institutes for Food and Drug Control, China; and K. Warre-Cornish, NIBSC, United Kingdom; *State actors*: J. Arcidiacono, US FDA, USA; C. Buchholz, Paul-Ehrlich-Institut, Germany; B. Domínguez-Gil, National Transplant Organization, Spain; M.L. Fraga and C. Milne, European Directorate for the Quality of Medicines & Healthcare, France; S. Kellathur and L.L. Ong, Health Sciences Authority, Singapore; M.B.C. Koh, National Blood Bank, Singapore; Z. Park, Ministry of Food and Drug Safety, Republic of Korea; M. Rosu-Myles, Health Canada, Canada; and S. Van Der Spiegel, European Commission; *Observers from non-state actors in official relations*: G. Stacey and J-H. Trouvin, International Alliance of Biological Standardization, Switzerland; *Representation from intergovernmental and other entities*: F. Atouf and J. Jacques, United States Pharmacopeia, USA; K. Francissen, Roche, USA; K. Ho, Roche, Switzerland; I. Irony, Janssen, USA; C. Koerner, Novartis, USA; K. Nichols, International Society for Cell and Gene Therapy, USA; G. O'Sullivan, International Society for Cell and Gene Therapy, Australia; K. Quillen, Boston University, USA; and by C. Ondari, E. Chatzixiros, R.G. Balocco, U. Loizidesi, Y. Maryuningsih, I. Knezevic and S.H. Yoo, World Health Organization, Switzerland.

Based on comments received from the first round of public consultation and the outcomes of the above informal consultation, a second draft of the document was prepared by the working group and by S.H. Yoo and R.A. Isbrucker, World Health Organization, Switzerland. The revised document was posted on the WHO Biologicals website for a second round of public consultation from 5 July to 9 September 2022. The document was also discussed during the October 2022 meeting of the WHO Expert Committee on Biological Standardization and additional revisions proposed for consideration. Based on the comments received during the second round of public consultation and

from the Committee, a revised document (WHO/BS/2023.2441) was prepared by R. Isbrucker, World Health Organization, Switzerland, with inputs from: C.A. Bravery, consultant, United Kingdom; G. Jotwani, Indian Council of Medical Research, India; P. Marks and G. Raychaudhuri, US FDA, USA; Y. Maruyama, Pharmaceuticals and Medical Devices Agency, Japan; F.C. Melo, National Health Surveillance Agency, Brazil; I.G. Reischl, Federal Office for Safety in Health Care, Austria; and E. Chatzixiros, World Health Organization, Switzerland.

Further changes were made to document WHO/BS/2023.2441 by the WHO Expert Committee on Biological Standardization.

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Appendix 1

Examples of HCTs and ATMPs demonstrating the broad range of product complexity and primary potential risks of concern

Product class	Product type	Processing	Indication	Potential clinical risks
HCT	Allogeneic bone marrow cells	Collection of the bone marrow	Haematopoietic reconstitution	Infection; graft failure
HCT	Allogeneic amniotic membrane	Collection and freeze drying, sizing	Treatment of ocular wounds	Infection; immunogenicity
HCT	Allogeneic virus-specific T cells, non-engineered	Collection, selection, washing and freezing of selected T cells (no culture and/or expansion)	Treatment of severe infections	Infection; immunogenicity
ATMP/ CTP	Autologous PBMCs	Collection, isolation and expansion of the cells, washing, formulation	Treatment of cardiac infarction	Infection; altered reactogenicity
ATMP/ TEP	Autologous cultured chondrocytes	Collection, expansion, formulation	Cartilage repair	Poor, non-hyaline cartilage
ATMP/ GTP in vivo	Adeno-associated virus + SMN1 gene	Most viral genes replaced by the SMN1 cassette, virus expansion, purification, formulation	Treatment of spinal muscular atrophy	Viral infection; immunogenicity; immune-related acute liver failure
ATMP/ CTP	Allogeneic pluripotent stem cells (iPSC/hESC)	Collection, purification, expansion, differentiation, formulation	Treatment of retinitis pigmentosa	Immunogenicity; tumorigenicity

Table *continued*

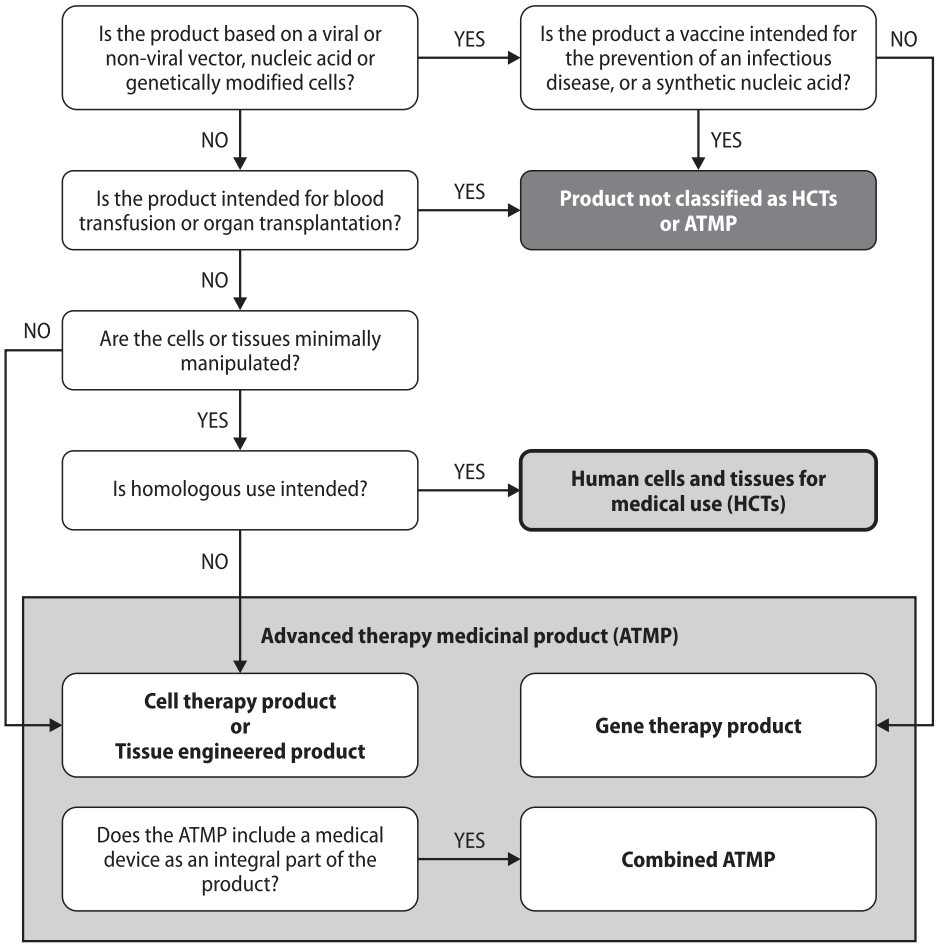
Product class	Product type	Processing	Indication	Potential clinical risks
ATMP/ GTP ex vivo	Lentivirus + globin gene in autologous CD34+ cells	Lentivirus vector production using plasmids, purification and transduction into patient CD34+ cells, cell expansion, formulation	Treatment of beta-thalassaemia	Insertional mutagenesis; oncogenesis; viral infection
ATMP/ GTP ex vivo	Allogeneic CD19 CAR T cells	Construction of the CAR into lentivirus vector, removal of HLA genes from the T cells by gene editing, expansion, formulation	Haematopoietic malignancies	Genotoxicity; immunotoxicity; off-target editing; insertional mutagenesis; neurotoxicity

CAR = chimeric antigen receptor; CTP = cell therapy product; GTP = gene therapy product; hESC = human embryonic stem cell; HLA = human leukocyte antigen; iPSC = induced pluripotent stem cell; PBMCs = peripheral blood mononuclear cells; TEP = tissue engineered product.

Appendix 2

Proposed general schema for the classification of HCTs and ATMPs

Note: ATMPs can be subcategorized according to their degree of processing and their mode of application – factors that directly impact upon the risks associated with their use.



Appendix 3

Useful information for cell and gene therapy products regulation

Currently, a number of international initiatives are actively working on promoting information sharing and international convergence with regard to the regulation of cell and gene therapy products. Examples of such information for manufacturers and regulators include, but are not limited to:

- International regulatory frameworks for cell and gene therapies. International Pharmaceutical Regulators Programme (IPRP); 11 August 2021 (https://admin.iprp.global/sites/default/files/2021-09/IPRP_CTWG-GTWG_Frameworks_2021_0811_0.pdf, accessed 28 April 2023).

  The IPRP Cell Therapy and Gene Therapy Working Groups share regulatory frameworks and guidelines on ATMPs provided by participating regulatory authorities to help manufacturers access global regulatory requirements. Links to further information on the laws and regulations in specific jurisdictions are provided in the above document.

- Manufacture of advanced therapy medicinal products for human use. In: Guide to good manufacturing practices for medicinal products; Annex 2A. Geneva: Pharmaceutical Inspection Co-operation Scheme (PIC/S); 2022 (https://picscheme.org/docview/4590, accessed 28 April 2023).

  PIC/S provides specific GMP requirements for ATMPs as Annex 2A in their GMP guideline. The annex is divided into two parts: Part A, covering specific considerations in ATMP manufacturing (from process of control over seed lots and cell banks to finishing activities and testing); and Part B, encompassing considerations of particular product types (such as gene therapy products).

- Nonclinical biodistribution considerations for gene therapy products. S12. ICH Harmonised Guideline. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH); 2023 (Document S12; https://database.ich.org/sites/default/files/ICH_S12_Step4_Guideline_2023_0314.pdf, accessed 28 April 2023).

  ICH Guideline S12 provides guidance on nonclinical biodistribution studies during the development of gene therapy products. The document covers the design of nonclinical biodistribution studies and considerations in the interpretation and application of the resulting data to support the design of clinical trials.

- INN nomenclature scheme for cell therapy products (CTP). Geneva: World Health Organization; 2015 (INN Working Doc. 13.323 revision 4; https://www.who.int/publications/i/item/inn-13-323-4, accessed 28 April 2023); and Mandatory information for INN selection and publication for cell-based therapies including cell-based gene therapy substances. Geneva: World Health Organization; 2020 (INN Working Doc. 20478; https://www.who.int/publications/i/item/inn-20-478, accessed 28 April 2023).

  During the 61st INN Consultation in 2015, a USAN-INN-harmonized nomenclature scheme for cell therapy products was formally finalized and approved by the members of the INN Expert Group designated to deal with the selection of international nonproprietary names. The Mandatory information for INN selection and publication for cell-based therapies including cell-based gene therapy substances document is provided as an annex to the INN application form to be used for requesting a new INN.

- Human genome editing: recommendations. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/9789240030381, accessed 28 April 2023); Human genome editing: a framework for governance Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/9789240030060, accessed 28 April 2023); and Human genome editing: position paper. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/9789240030404, accessed 28 April 2023).

  WHO provides recommendations on the governance and oversight of human genome editing in nine areas, including human genome editing registries. WHO has also provided a new governance framework that highlights specific tools, institutions and scenarios to illustrate the practical challenges in implementing, regulating and overseeing research into the human genome.

- Principles on the donation and management of blood, blood components and other medical products of human origin. Report of the Secretariat. In: Seventieth World Health Assembly. Provisional agenda item 13.2. 3 April 2017 (Document A70/19: https://apps.who.int/iris/bitstream/handle/10665/274793/A70_19-en.pdf?sequence=1&isAllowed=y, accessed 28 April 2023).

  In this report to the Health Assembly, WHO sets out 10 principles for promoting ethical practices in the donation and management of medical products of human origin, including voluntary consent of the donor, and ensuring the safety, quality and efficacy of donation, while also providing key considerations in the implementation of these principles.

- WHO guiding principles on human cell, tissue and organ transplantation. Geneva: World Health Organization; 2010 (Document number: WHO/HTP/EHT/CPR/2010.01; https://apps.who.int/iris/handle/10665/341814, accessed 28 April 2023).

  WHO recommends 11 guiding principles which are intended to provide an orderly, ethical and acceptable framework for the acquisition and transplantation of human cells, tissues and organs used for therapeutic purposes.

- First WHO International Reference Reagent for lentiviral vector integration site analysis (NIBSC code: 18/144; https://www.nibsc.org/documents/ifu/18-144.pdf, accessed 28 April 2023); and First WHO International Reference Reagent for CD4 T-cells (human) (NIBSC code: 15/270; https://www.nibsc.org/documents/ifu/15-270.pdf, accessed 28 April 2023).

  The UK Medicines and Healthcare products Regulatory Agency (MHRA) distributes WHO international measurement standards for assuring the quality of biological products. The above two WHO international reference reagents are available for cell and gene therapy products. The First WHO International Reference Reagent for lentiviral vector integration site analysis is suitable for use as a qualitative reference material for the detection of the 10 defined lentiviral vector integration sites. The First WHO International Reference Reagent for CD4 T-cells (human) is intended for use as a cellular control for CD4 T-cell enumeration by flow cytometry.

Annex 4

New and replacement WHO international reference standards for biological products

The provision of global measurement standards is a core normative WHO activity. WHO international reference standards are widely used by manufacturers, regulatory authorities and academic researchers in the development and evaluation of biological products. The timely development of new reference standards is crucial in harnessing the benefits of scientific advances in new biologicals and in vitro diagnosis. At the same time, management of the existing inventory of WHO international reference standards requires an active and carefully planned programme of work to replace established materials before existing stocks are exhausted.

The considerations and guiding principles used to assign priorities and develop the programme of work in this area have previously been set out as WHO Recommendations.[8] In order to facilitate and improve transparency in the priority-setting process, a simple tool was developed as Appendix 1 of these WHO Recommendations. This tool describes the key considerations taken into account when assigning priorities, and allows stakeholders to review and comment on any new proposals being considered for endorsement by the WHO Expert Committee on Biological Standardization.

A list of current WHO international reference standards for biological products is available at: https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/catalogue.

At its meetings held via video conference on 20–24 March 2023, the WHO Expert Committee on Biological Standardization made the changes shown below to the previous list. In addition, the Committee recommended that two further antibody preparations be added to the First WHO International Reference Panel of antibodies to SARS-CoV-2 variants of concern established at its previous meeting. Each of the WHO international reference standards shown in the table below should be used in accordance with their instructions for use (IFU).

[8] Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO Expert Committee on Biological Standardization: fifty-fifth report. Geneva: World Health Organization; 2006: Annex 2 (WHO Technical Report Series, No. 932; https://iris.who.int/handle/10665/43278, accessed 20 September 2023).

Additions[9]

Material	Unitage	Status
Biotherapeutics other than blood products		
Vascular endothelial growth factor 165	9000 IU/ampoule	First WHO International Standard
Blood products and related substances		
Blood coagulation factor VIII concentrate	9.5 IU/ampoule	Ninth WHO International Standard
In vitro diagnostics		
Antibodies to human leukocyte antigen (negative plasma)	No unitage assigned	WHO International Reference Reagent
Antibodies to human leukocyte antigen (negative serum)	No unitage assigned	WHO International Reference Reagent
Antibodies to human leukocyte antigen (strong positive plasma)	No unitage assigned	WHO International Reference Reagent
Antibodies to human leukocyte antigen (weak positive plasma)	No unitage assigned	WHO International Reference Reagent
Antibodies to citrullinated peptide/ protein	260 IU/ampoule	First WHO International Standard
Hepatitis B virus DNA for NAT-based assays	5.69 $\log_{10}$ IU/vial	Fifth WHO International Standard
Vaccines and related substances		
Meningococcal serogroup C polysaccharide	0.965 ± 0.024 mg/ampoule	Second WHO International Standard

[9] Unless otherwise indicated, all materials are held and distributed by the Medicines and Healthcare products Regulatory Agency, Potters Bar, Herts, EN6 3QG, United Kingdom.

Material	Unitage	Status
Antibodies to Rift Valley fever virus for neutralization assays (human plasma)	250 IU/ampoule	First WHO International Standard
Antibodies to Rift Valley fever virus for binding assays (human plasma)	250 IU/ampoule (anti-glycoprotein immunoglobulin G)	First WHO International Standard

SELECTED WHO PUBLICATIONS OF RELATED INTEREST

WHO Expert Committee on Biological Standardization
Seventy-sixth report.
WHO Technical Report Series, 1045, 2023 (xvi + 330 pages)

WHO Expert Committee on Biological Standardization
Seventy-fifth report.
WHO Technical Report Series, 1043, 2022 (xii + 252 pages)

WHO Expert Committee on Biological Standardization
Seventy-fourth report.
WHO Technical Report Series, No. 1039, 2022 (xv + 157 pages)

WHO Expert Committee on Biological Standardization
Report of the seventy-second and seventy-third meetings.
WHO Technical Report Series, No. 1030, 2021 (xvii + 269 pages)

WHO Expert Committee on Biological Standardization
Seventy-first report.
WHO Technical Report Series, 1028, 2021 (xii + 102 pages)

WHO Expert Committee on Biological Standardization
Seventieth report.
WHO Technical Report Series, No. 1024, 2020 (xvi + 227 pages)

WHO Expert Committee on Biological Standardization
Sixty-ninth report.
WHO Technical Report Series, No. 1016, 2019 (xv + 251 pages)

WHO Expert Committee on Biological Standardization
Sixty-eighth report.
WHO Technical Report Series, No. 1011, 2018 (xvi + 380 pages)

WHO Expert Committee on Biological Standardization
Sixty-seventh report.
WHO Technical Report Series, No. 1004, 2017 (xviii + 591 pages)

Website: https://www.who.int/health-topics/Biologicals#tab=tab_1

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This report presents the recommendations of a WHO Expert Committee commissioned to coordinate activities leading to the adoption of international recommendations for the production and control of vaccines and other biological products used in medicine, and the establishment of international biological reference materials.

Following a brief introduction, the report summarizes a number of issues brought to the attention of the Committee at its meeting held virtually in March 2023. Of particular relevance to manufacturers and national regulatory authorities are the discussions held on the development and adoption of new and revised WHO Recommendations, Guidelines and guidance documents. Following these discussions, the following two documents were adopted on the recommendation of the Committee: (a) Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases; and (b) Considerations in developing a regulatory framework for human cells and tissues and for advanced therapy medicinal products.

Subsequent sections of the report provide information on the current status, proposed development and establishment of international reference materials in the areas of: biotherapeutics other than blood products; blood products and related substances; in vitro diagnostics; standards for use in high-throughput sequencing technologies; standards for use in public health emergencies; and vaccines and related substances.

A series of annexes is then presented which includes an updated list of all WHO Recommendations, Guidelines and other documents related to the manufacture, quality control and evaluation of biological products (Annex 1). The above two WHO documents adopted on the advice of the Committee are then presented as part of this report (Annexes 2 and 3). Finally, all new and replacement WHO international reference standards for biological products established during the March 2023 meeting are summarized in Annex 4. The updated full online catalogue of WHO international reference standards is available at: https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/catalogue.

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