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Guideline on predictive biomarker assays in the context of medicinal product lifecycle

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Executive summary

Predictive biomarker-guided medicines represent one of the most widely implemented regulatory concepts of personalised medicine in Europe, by linking the clinical use of a medicinal product to a corresponding predictive biomarker, with the intent to tailor treatment decisions to patients most likely to benefit in terms of efficacy and/or safety. The efficacy and/or safety of such a predictive biomarker-guided medicinal product relies on a corresponding analytically robust, reliable and clinically useful biomarker assay. For its use with a medicinal product, it may be a companion diagnostic (CDx) as defined in the In Vitro Diagnostics Regulation (IVDR).

This guideline describes the information needed for the submission of a Marketing Authorisation Application (MAA) for a biomarker-guided medicinal product. Guidance is provided on the generation of a robust data package for the biomarker and corresponding biomarker assay for the purpose of the marketing authorisation and post-authorisation applications. The guideline outlines best practices and the key evidence expected to be generated throughout the clinical development programme to support medicinal product use. Additionally, it provides general recommendations on how to reflect relevant information related to such biomarkers and their associated assays in the Summary of Product Characteristics (SmPC).

This guideline is primarily intended for medicinal product developers, but may also be useful for *in vitro* diagnostic (IVD)/CDx manufacturers working with medicinal products developers to prepare the marketing authorisation application, as it covers the interface with the IVDR. To facilitate understanding, where possible, terminologies from both the medicines and the IVDR frameworks are used.

1. Introduction

Technological advancements, among other factors, have resulted in an increase in prevention and treatment strategies that consider the variability in characteristics of (groups of) individuals. This has led to the development and definition of several closely related concepts such as precision medicine or personalised medicine. While there are no universally accepted definitions of these concepts, European Union (EU) Health Ministers described personalised medicine in the Council conclusions on personalised medicine for patients as "a medical model using characterisation of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention" [1].

Predictive biomarker-guided medicines development is among the most widespread applications of the personalised medicine concept, resulting in the clinical use of a medicinal product together with its corresponding predictive biomarker assay (refer to section 12 for definitions). Accordingly, patients are identified by biomarkers predictive for efficacy and/or safety for a treatment decision with the corresponding medicinal product. The efficacy and/or safety of such a medicinal product strictly relies on having a corresponding accurate, reproducible and clinically useful assay for predictive biomarker measurement. Therefore, the development of a predictive biomarker-guided medicinal product requires the identification and clinical validation of a biomarker, and the (co-)development of a corresponding biomarker assay.

If the use of predictive biomarker assays to identify patients who are most likely to benefit or be at increased risk of adverse reactions as a result of treatment with the corresponding medicinal product is mandatory, this has to be adequately reflected in the product information, in order to ensure the effective and safe use of the associated medicinal product [2].

The regulatory frameworks for medicinal products and IVDs are separate. Whereas the focus of the medicines sector is on the justification of the biomarker in the context of the use of the medicinal product, this justification cannot be provided unless there is a specific assay supporting reliable conclusions on the measurement of the biomarker.

Where a predictive biomarker assay in the context of the therapeutic use of a medicinal product fulfils the definition of a CDx, this creates an interface with the medical device legislation, notably Regulation (EU) 2017/746 on IVDR, particularly Article 2 (7) [3], which defines a CDx as:

"*a device which is essential for the safe and effective use of a corresponding medicinal product to*

(a) identify, before and/or during treatment, patients who are most likely to benefit from the corresponding medicinal product; or

(b) identify, before and/or during treatment, patients likely to be at increased risk of serious adverse reactions as a result of treatment with the corresponding medicinal product."

A CDx is essential for defining patients' eligibility for a specific treatment with a medicinal product through the quantitative or qualitative determination of (a) specific biomarker(s). An IVD is not a CDx if it is used to ensure that the concentration of a medicinal product in the human body stays within the therapeutic window during treatment (recital 12 of the IVDR [3]) or when it is used to establish the dosage of a medicinal product for patients that are already eligible to receive that medicinal product. Further considerations on when an IVD is considered to be a CDx are available in the Medical Device Coordination Group (MDCG) guidance on classification rules for *in vitro* diagnostic medical devices under Regulation (EU) 2017/746 [4].

Under the IVDR risk classification rules, CDx are assigned to risk class C (or higher if relevant rules apply), which is the second highest risk class. To place a CDx on the market, the manufacturer must receive certificates from the notified body (NB) for the quality management system and for the technical documentation of the device. The NB assessment of the technical documentation involves, among other elements, assessment of the data provided by the manufacturer for demonstration of the scientific validity, analytical performance and clinical performance of the device. The interface is addressed in the legislation through the CDx consultation procedure with Medicinal Product Authorities as part of CDx certification by NB, although the approval of a biomarker-guided medicinal product is independent from the CDx certification.

This guideline describes the interface and highlights important common ground between the pharmaceutical legislation and the IVDR, from a medicinal product regulatory framework perspective. Therefore, this guideline provides recommendations for the lifecycle management of predictive biomarker-guided medicinal products, i.e., when a corresponding predictive biomarker assay is required, which likely will be a candidate for a CDx as defined by the IVDR. It may be possible to use data from the (confirmatory) clinical trial(s) as evidence to support the clinical performance of the *candidate CDx*.

Throughout this guideline, the medicines framework terminology is preferentially used. Such is the case when using the terminology "assay" in a broad sense encompassing assays used in clinical trials and MAA irrespective of its status according to the IVDR. When an equivalent term is found in the IVDR, this term is referred to in brackets and italics. Because the classification as CDx is not in the remit of the Medicinal Product Authority, the terms "*candidate CDx*" or IVD are used when referring to predictive biomarker assays (refer to section 4 and 12 of the guideline).

2. Scope

This guidance provides the scientific requirements for the development and validation of predictive biomarkers and their corresponding assays for the purpose of MAA and post-authorisation applications (e.g., indication extensions) for biomarker-guided medicinal products, outlining the essential evidence to be generated throughout the clinical development programme.

Additionally, general guidance is provided on how to reflect information related to such predictive biomarkers and biomarker assays in the medicinal product information (the SmPC), as well as certain regulatory aspects developers should be aware of in the European regulatory framework for medicines.

The document also covers guidance for follow-on CDx candidates developed after the authorisation of the associated medicinal product. The document specifically addresses the interface between the medicines' framework and IVDR and uses both terminologies where they are related (IVDR terminology provided in *italics* and in brackets).

This guideline is primarily intended for medicinal product developers but may also be useful for IVD/CDx manufacturers working with medicinal product developers to prepare a marketing authorisation application, as it covers the interface with the IVDR.

The following aspects are out of scope for this guideline:

- *In vivo* diagnostics, as defined and covered in the Guideline on clinical evaluation of diagnostic agents [6]
- Classification & categorisation of IVDs
- Requirements for IVDs in the context of clinical trial approval (which is regulated under Regulation (EU) No 536/2014 on clinical trials on medicinal products for human use, also referred to as Clinical Trials Regulation (CTR) [7]) by Medicinal Product National Competent Authority (NCA) or requirements for CDx performance study approval by Device NCAs (under IVDR [3])
- Recommendations for conformity assessment under the IVDR [3]

3. Legal basis and procedural background

3.1. Procedural context for the assessment of predictive biomarker assays (candidate CDx)

In the medicinal product regulatory setting, two scenarios may occur in which an assessment of a predictive biomarker assay (*candidate CDx* (refer to section 4 and 12 of the guideline)) by a Medicinal Product Authority (European Medicines Agency (EMA) or NCA) is conducted.

1. During a **MAA** or **post-authorisation application procedure** (e.g., an extension of indication) for a biomarker-guided medicinal product

2. During a **CDx consultation procedure** (legally mandated by the IVDR) submitted by a NB to a Medicinal Product Authority.

(1) During a MAA and post-authorisation application procedures

Biomarker assays that are used for patient selection (for efficacy and/or safety) define the patient population investigated in clinical trials and considered for the benefit/risk (B/R) assessment of a predictive biomarker-guided medicinal product. Evaluating the biomarker assay in terms of analytical data robustness and reliability to measure biomarker(s) is therefore key to understanding the B/R profile of the medicinal product in the intended population. It is important to note that during the MAA procedure, the Medicinal Product Authority does not assess the IVDR conformity of the biomarker assay (*IVD*) used in the clinical (pivotal) studies, even if it is a *candidate CDx.* This aspect falls under the legal remit of the NB performing a conformity assessment in a separate procedure.

The MAA assessment for a predictive biomarker-guided medicinal product evaluates the appropriateness of the biomarker assay for patient selection, including whether the choice of biomarker-identified populations in the pivotal study(ies) was justified (refer to section 5 of the

guideline). It also seeks to determine whether this supports the proposed target population described in the SmPC, and to assess the study outcome(s) in relation to the claimed conditions of use.

The analytical validation of the biomarker assay used in the pivotal clinical study(ies) is an important consideration; therefore, the analytical method is evaluated as part of the assessment report (refer to section 6 of the guideline). Based on this evaluation, a conclusion is drawn regarding the suitability, robustness and reliability of the data generated by the biomarker assay for defining the target/intended patient population and supporting the B/R assessment.

Clinical validation is described either by substantiating that biomarker status, as determined by the biomarker assay, is predictive of a clinical endpoint (for novel assays), or – where available - by a concordance study with a clinically valid reference assay. Moreover, cut-off point selection is described and discussed in detail since this is of particular importance for the B/R assessment (refer to section 8 of the guideline). This information is gathered from the MAA dossier or extension of indication variation dossier submitted by the applicant, where information on the development and validation of the predictive biomarker assay is expected to be included.

The description and assessment of the predictive biomarker assay are summarised in the dedicated sections on *"in vitro biomarker test for patient selection for efficacy / safety"* in the clinical assessment report and overview template [8], or, where applicable, in the corresponding assessment report templates for new indication and extensions of indication. The assessment of the predictive biomarker assay also supports the inclusion of information in the SmPC on the biomarker assay(s) used during the clinical development of the associated medicinal product (refer to section 10 of the guideline).

Further recommendations on the assessment of a predictive biomarker assay during a MAA or extension of indication are provided in the "*Frequently asked questions on medicinal products development and assessment involving companion diagnostic (CDx)*" [9].

(2) CDx consultation procedure

When a CDx consultation procedure is submitted by a NB, the Medicinal Product Authority is asked to provide a scientific opinion on the suitability of a CDx candidate intended for certification in relation to the concerned medicinal product. The opinion is provided based on the draft Summary of Safety and Performance (SSP) and the draft Instructions for Use (IFU) submitted by the NB. The opinion provided by the Medicinal Product Authority is subsequently considered by the NB in their final decision on issuing EU certification for the CDx candidate. The consultation procedure assessment by the Medicinal Product Authority is formally restricted to the information provided in the draft SSP and draft IFU but is also based on the MAA assessment for the corresponding medicinal product.

Detailed guidance on procedural aspects of the CDx consultation procedure is provided in the *"Guidance on the procedural aspects for the consultation to the European Medicines Agency by a notified body on companion diagnostics*" [5], and additional practical information can be found in the *"Questions & Answers - Practical arrangements on the companion diagnostics consultation procedure to the European Medicines Agency by notified bodies"* [10].

3.2. Legal basis and relevant guidelines

This guideline should be read in conjunction with the introduction and general principles of Annex I to Directive 2001/83/EC, as amended, all other relevant EU legislation, and EU and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. These include, but are not limited to:

EMA guidelines and relevant documents:

- Guidance on the procedural aspects for the consultation to the European Medicines Agency by a notified body on companion diagnostics (EMA/198592/2022)

- Guideline on clinical evaluation of diagnostic agents (CPMP/EWP/1119/98/Rev. 1)
- Guideline on good pharmacogenomic practice (EMA/CHMP/718998/2016)
- NOTICES FROM EUROPEAN UNION INSTITUTIONS, BODIES, OFFICES AND AGENCIES 2015/C 421/03 Council conclusions on personalised medicine for patients Guideline on the clinical evaluation of anticancer medicinal products Rev.6. (EMA/CHMP/205/95)
- Guideline on the investigation of subgroups in confirmatory clinical trials (EMA/CHMP/539146/2013)
- Qualification of novel methodologies for drug development: guidance to applicants (EMA/CHMP/SAWP/72894/2008)
- Scientific guidelines with SmPC recommendations EMA/813125/2012 rev. 8 (EMA/CHMP/483022/2019)
- Wording of therapeutic indication - A Guide for Assessors of Centralised Applications (EMA/CHMP/483022/2019)
- Reflection Paper on methodological issues in confirmatory clinical trials planned with an adaptive design (CHMP/EWP/2459/02)
- Frequently asked questions on medicinal products development and assessment involving companion diagnostic (CDx) (EMA/CHMP/821321/2022)
- Questions & Answers - Practical arrangements on the companion diagnostics consultation procedure to the European Medicines Agency by notified bodies (EMA/619893/2022)ICH M10 guideline on bioanalytical method validation and study sample analysis, Step5 (EMA/CHMP/ICH/172948/2019)

ICH guidelines:

- ICH guideline M10 on bioanalytical method validation and study sample analysis - Questions and Answers (EMA/CHMP/ICH/660315/2022)
- ICH guideline E18 on genomic sampling and management of genomic data, Step 5 (EMA/CHMP/ICH/11623/2016)
- ICH E6 (R3) Guideline for good clinical practice (GCP) - Step 5 (EMA/CHMP/ICH/162879/2026)

European Commission guideline and legislation:

- REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU (https://eur-lex.europa.eu/eli/reg/2017/746)
- EUROPEAN COMMISSION, NOTICE TO APPLICANTS, Revision 2 - A GUIDELINE ON SUMMARY OF PRODUCT CHARACTERISTICS (SmPC)

Medical Device Coordination Group (MDCG) guidance and relevant documentation:

- Guidance on general principles of clinical evidence for In Vitro Diagnostic medical devices (IVDs) (MDCG 2022-2)
- Guidance on Classification Rules for in vitro Diagnostic Medical Devices under Regulation (EU) 2017/746 (MDCG 2020-16)
- Questions & Answers regarding performance studies of in vitro diagnostic medical devices under regulation (EU) 2017/746 (MDCG 2025-5)
- Q&A on the interface between Regulation (EU) 536/2014 on clinical trials for medicinal products for human use (CTR) and Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) (MDCG 2022-10)

4. Context of use of biomarker assays during medicinal product development

The context of use (CoU) of a medicinal product with a biomarker refers to the specific circumstances, patient population, and purpose for which the biomarker is used, including its role in medicinal product development. It includes the (proposed) indication of the medicinal product, the intended purpose of the IVD, the target population, and conditions for use.

Data supporting the context of use should be submitted at MAA including support for the reliability of the biomarker assay(s), which is also an obligation from the Good Clinical Practice (GCP) and IVDR perspectives:

- ICH E6 (R3) Guideline for GCP [11] mandates that clinical trials are designed to protect the rights, safety and well-being of participants and to ensure the reliability of results. In particular, it is stated in ANNEX 1 (3.16.1) that *"The [medicine] sponsor should ensure that data acquisition tools are fit for purpose and designed to capture the information required by the protocol. They should be validated and ready for use prior to their required use in the trial".*
- The IVDR also requires that prior to a clinical performance study of an IVD, the analytical performance is specified in the clinical performance study plan (Annex XIII 2.3.2 (k) [3]). The analytical performance also needs to be described in the investigator's brochure to be prepared for a clinical performance study (Annex XIV section 2.3 [3]).

In principle, there are three potential stages of development for (predictive) biomarker assays used in clinical medicinal product development, but not all stages are sequential or mandatory:

Stage A) Exploratory/research setting

A biomarker assay is used for early exploration of a biomarker signal in association with a medicinal product or a medicinal product combination (e.g., by applying retrospective analysis). In a clinical trial setting, patients could be stratified on the basis of the biomarker but there would be no change in the treatment that they would receive on the basis of the assay results. In the context of the clinical trial, the assay would not fulfil the IVD definition according to the IVDR [9]. This setting is usually linked to early-phase clinical trials to potentially guide future patient selection, dose selection, or understanding the mechanism of action. This type of assay may later become an IVD – either an "*early candidate CDx*" (stage B) or a "*mature candidate CDx"* (stage C) if it proves to be robust, reliable, and clinically meaningful.

Stage B) IVD/"early candidate CDx" setting

A biomarker assay with an established analytical validity (*analytical performance*) and with a hypothesis that the biomarker is important in relation to the medicinal product (for example as a result of a study under the previous stage), can be used to support a clinical decision, for instance to include or exclude patients into a clinical trial, or to allocate them to treatment arms. This setting likely fulfils the definition of an IVD according to the IVDR [3, 4]. However, at this stage of development it is still uncertain if a biomarker-guided medicinal product development is justified. In cases where the clinical trial data confirm the biomarker hypothesis, the IVD can be termed an "*early candidate CDx*". The clinical trial data (considering patients receiving and not receiving the treatment) and the analytical (*analytical performance*) and clinical validation (*clinical performance)* of the IVD, may guide whether the IVD/*early candidate CDx* may be developed towards a *"mature candidate CDx"* (stage C).

Stage C) IVD/"mature candidate CDx" setting

At this stage, a biomarker assay with an established analytical validity (*analytical performance*) is used in the pivotal clinical trial(s) of a medicinal product in order to support a biomarker-guided use of the

medicinal product itself. This assay can therefore be considered as a "*mature candidate CDx"*. In the *mature candidate CDx* setting, the clinical validity in the context of the medicinal product development and the predictive biomarker hypothesis are to be confirmed. Co-development of the biomarker assay with the concerned medicinal product in a clinical development programme may occur for an initial MAA, but may also be required for extensions of indications post-approval MAA as a predictive biomarker assay is treatment and disease dependent. In the recommended co-development setting, the biomarker assay used in the pivotal clinical trial(s) (i.e., the *mature candidate CDx*), may be the subject of the conformity assessment according to the IVDR for its certification as a CDx device.

The three different stages illustrate that decisions on predictive biomarker assay/*candidate CD*x development may evolve during medicinal product development. Additionally, the suitability of the predictive biomarker assay/*candidate CDx* as a CDx device will be assessed at a later stage during the conformity assessment under the IVDR provisions.

In cases, where a *candidate CDx* has not been co-developed with the medicinal product, it is essential that a CDx candidate proposed for conformity assessment has a similar performance as the biomarker assay used in the pivotal clinical trial(s). This requires the bridging of analytical data to assure that the patient population identified by the proposed CDx candidate is comparable to the patient population originally selected by the pivotal biomarker assay. This ensures that the safe and effective use of the predictive biomarker-guided medicinal product as described in the SmPC is not impacted. For further information regarding bridging studies and requirements, please refer to section 9 "Bridging studies" of this guideline.

5. Scientific rationale for predictive biomarkers and their corresponding assays

In the context of medicinal product regulation, the concept of scientific rationale is similar to *scientific validity* in the IVDR. The successful development of a predictive biomarker assay depends on multiple factors. One that should be critically considered is the scientific rationale for the predictive role of the biomarker measured by the corresponding assay to determine the likelihood of benefit (or risk) from a treatment with a specific medicinal product in a specific target population. In this respect, the characteristics of the biomarker, such as its prevalence in the target patient population, relationship to disease pathogenesis and treatment effect, as well as the mechanistic rationale for its use, should be thoroughly explored in the context of the associated medicinal product development.

Generally, a biomarker may have a simultaneous diagnostic, prognostic, and, in some cases predictive role (refer to section 12 for terminology). Therefore, biomarker measurement can potentially serve different purposes at different timepoints of patient management. In the context of treatment with a specific biomarker-guided medicinal product, the predictive role of a biomarker is of particular relevance.

It is important to distinguish between the different roles that the same biomarker may have. For example, a biomarker may initially be used to detect or confirm the presence of a disease or condition, or to identify individuals with a specific disease subtype. At a later stage, the same biomarker may also have a predictive role by identifying patients who are more likely to respond to, or benefit from, a specific medicinal product for that disease or disease subtype.

For the development of predictive biomarker assays, the scientific rationale needs to distinguish between the prognostic and predictive roles for the same biomarker, and the rationale for medicinal product development in a specific patient population.

The link between the assumed therapeutic action of the medicinal product and the role of the biomarker(s) in relation to the targeted therapeutic indication should be clearly outlined (refer to section 4 and 12 of the guideline). For any single biomarker or multi-marker panel, mechanistic and

biological plausibility should be justified. In most cases, clinical data are necessary to substantiate the clinical impact of biomarker measurement.

In the case of novel biomarkers not previously used for patient selection, or where a scientific rationale exists but the current level of knowledge is insufficient to consider the biomarker as established, the patient selection strategy used in confirmatory trials should be adequately justified in the MAA dossier. This should be based on the available non-clinical/translational data, the mechanistic/biologic rationale, and, where available, clinical data. The scientific rationale (*scientific validity*) always need to be well justified when a biomarker is used to include or exclude patients from confirmatory clinical trial(s). In such cases, no data are collected for the subpopulation(s) excluded from the clinical trial(s), and this may hamper the MAA assessment of the biomarker's predictive value.

Choice of assay

As a chosen biomarker can be measured by different assay types (e.g. genomic/genetic versus proteomic formats), the rationale for the selected assay needs to be justified.

Cut-offs

Biomarker assays often provide a qualitative result classifying subjects into two or more groups. However, qualitative results are based on an underlying quantitative variable, which plays a crucial role in establishing the cut-offs between qualitative classifications (e.g. the gene variant allele frequency, the number of ligands (soluble proteins), or the number of cells expressing a certain protein on a tissue slide). The underlying rationale for the selected cut-off that defines a patient as having a positive biomarker status needs to be provided. Particularly, it should be clarified whether the cut-off is determined by the analytical sensitivity of the assay (e.g., the limit of detection) or by the quantitative relationship between the biomarker and clinical outcomes. The correlation of the chosen cut-off with clinical outcome(s) needs to be shown (for details refer to section 8 of the guideline).

The cut-off may also be based on approaches that include multiple parameters. For example, complex multi-parameter scoring algorithms are typically applied for semi-quantitative tissue-cut-slide assays like immunohistochemistry (IHC)-based technologies.

Considerations for multi-marker panels

In clinical studies, biomarker status may be determined by a single marker or by multiple markers (e.g. multiple markers that encompass a panel of genetic or genomic aberrations within one or several genes). Multiple markers can be measured using distinct assays or a single assay. The chosen multi-marker approach used to determine the biomarker status of the patient should be defined and scientifically justified.

Changes of the cut-off point and/or multi-marker panel

During clinical development, changes may be made to the assay or IVD. For example, the selected cut-off may need to be changed, or a multi-marker panel may be modified (e.g., by the addition or deletion of specific genetic or genomic aberrations). Changes require a sound scientific justification, together with an analysis and discussion of the reliability of the conclusions that can be drawn from the modified approach. Of note, from the IVDR perspective, such changes would also constitute changes to the IVD/*candidate CDx*.

6. Analytical validation of biomarker assays during medicinal product development

In section 6, only the term "samples", which is equivalent to *specimens* in the IVDR, is used throughout [3].

6.1. General aspects

Analytically validated assays should be used in clinical trials to fulfil the requirements by GCP ICH E6 (R3) Annex 1 (3.16.1) [11]. Beyond GCP requirements, more stringency on analytical assay validation is expected in situations where the reliability of the biomarker assay may impact patient safety, e.g., if the biomarker assay is used to select patients to be enrolled, guides the assignment of treatments (stage B in section 4 of the guideline), or where a clinical trial is designed to validate the predictivity of a biomarker (stage C).

Expectations regarding the extent and stringency of analytical validation for the biomarker assay increase from the research stage (stage A) to the IVD stages (stages B and C). Analytical validation acceptance criteria should be predefined and appropriately justified, proportionate to the stage of development and to the risks and benefits of the assay. Analytical validation at the research stage (stage A*)* focuses on establishing the suitability of the biomarker assay's use based on preliminary acceptance criteria. In contrast, when the biomarker assay has a medical purpose (i.e., fulfils the definition of an IVD), typically used in the later or pivotal stages of medicinal product development (stages B and C), analytical validation involves confirmation of the assay's suitability for the predefined validation attributes and acceptance criteria.

6.2. Assay considerations

Specific recommendations for analytical validation of different biomarker assay formats are beyond the scope of this guideline, given their diversity (e.g., genomic, genetic, proteomic, other omic platforms) as well as the different grades of complexity of assays (e.g., more simple ligand binding assays *versus* highly complex IHC assays) currently used in biomarker-guided medicinal product development. Moreover, these technologies are constantly being improved (e.g., genomic variant and mutation analysis evolved rapidly from Sanger sequencing and polymerase chain reaction (PCR)-based approaches to next-generation sequencing (NGS) technologies). Therefore, it is recommended to consider general guidelines for common scenarios instead of specific recommendations that might become outdated. In particular, the principles stated in the ICH M10 guideline on bioanalytical method validation and on study sample analysis [12], together with the corresponding Questions and Answers document [13], should be considered where applicable. For NGS-based assay platforms used for the detection of germline mutations, recommendations in the guideline on good pharmacogenomic practice should be followed [14]. For IVD/*candidate CDx,* the IVDR requirements of Annex I section 9.1(a) shall be considered [3].

With respect to the use of an analytically validated biomarker assay (*IVD/candidate CDx* stages B and C) in the context of a clinical trial, several general analytical rules apply. These requirements are comparable in both the medicinal product framework and the IVDR, and include, for example, the known analytical validation attributes of sensitivity, specificity, precision (repeatability and reproducibility, e.g. inter-/intra-assay, operator-to-operator, lot-to-lot) and cut-offs. For quantitative assays, the derivation of the linear range, including controlling for known relevant interference, and limits of detection, are applicable [3, 12, 13]. When available, internationally recognised standard materials, should be included in the characterisation of the assay. For analytical assay validation purposes, typically biobank samples are used, and these samples should be sufficiently representative of the future target population and should target the cut-off, if applicable. It is considered essential that the development of the assay (e.g., cut-off selection or scoring rule for combined biomarkers) is based on a different dataset from that used for final analytical validation. Otherwise, analytical performance may be overestimated. A cut-off which is not based only on analytical assay sensitivity (e.g., limit of detection (LOD)) may also be selected. In such cases, the clinical cut-off which optimises the predictive clinical performance should be derived from a provisional cut-off from early clinical trials (e.g., stages A or B) and subsequently be confirmed in the confirmatory clinical trial(s) (stage C).

6.3. Considerations for specific assays

If tissue-based assays (cut-slide assays, e.g., IHC assays, or fluorescence *in situ* hybridisation (FISH) assays) are used, separate analytical validation studies should be performed to confirm the suitability of each intended tissue type as specified in the (proposed) clinical indication (e.g., to evaluate tissue histology-dependent matrix effects).

The use of innovative biomarker assay technologies or formats in medicinal product development may require confirmation of the suitability of the method by a recognised and analytically validated state-of-the art method. In such cases it is recommended to seek scientific advice from an NCA or EMA, or to consider an EMA Qualification Opinion on the suitability of novel methodologies for medicinal product development [15].

6.4. Characterisation studies

Additional characterisation studies may be needed to confirm the CoU of the biomarker assay. These are illustrated via the oncology setting and may be applicable to other contexts.

(1) Tissue heterogeneity is often a critical feature of most solid cancers. Samples may consist of a mixture of tumour tissue and accompanying benign or normal tissue which impacts the reliable detection of the biomarker (panel) and may lead to false negative testing results. Therefore, especially for cut-slide tissue assays like IHC or fluorescence *in situ* hybridisation (ISH)/FISH, tissue heterogeneity should be analysed to confirm the biomarker status. Equally, for the detection of genetic aberrations such as somatic mutations, e.g. by NGS or PCR assays, high levels of tumour tissue heterogeneity may undermine the validity of pooled results, and the combined estimate may not accurately represent individual outcomes. Characterisation studies to evaluate the biomarker mutation status in heterogeneous tumour tissue samples may follow different approaches. Characterising the full range of molecular heterogeneity within a tumour tissue sample is essential to ensure that the genetic biomarker profile of a patient is accurately reflected. Depending on the outcome of such studies, it may be required to define a minimum threshold of tumour content in the sample or to include initial tumour cell isolation (e.g., by microdissection technology) in the assay platform strategy.

(2) Another scenario is present in the metastatic tumour setting which may require the assessment of the biomarker status between the primary tumour *versus* metastatic tumour samples.

(3) In case of liquid biopsy (e.g., measuring the biomarker(s) in circulating tumour cells, circulating free deoxyribonucleic acid (DNA), or cell free micro ribonucleic acid (microRNA)), the concordance of the biomarker status between the primary tumour tissue versus liquid biopsy samples should be evaluated.

(4) For some very rare rapidly growing tumours, such as glioblastomas, a stringent characterisation of the suitable window of measurement is required to counteract the high risk of false testing results. Generally, the acceptable window for patient testing should be justified based on the persistence of the biomarker tested (e.g., human leukocyte antigen (HLA) on normal tissue *versus* markers of infection or tumour markers).

(5) Where archival samples are used (e.g., for evaluation of the relationship of the biomarker status in archival tumour tissue samples *versus* fresh liquid biopsy samples (see (2)) or compared to fresh metastatic tumour samples (see (3))), justification that these samples match the biomarker samples of patients in the intended clinical indication should be provided.

6.5. Assay adaptations

A biomarker assay might be further adapted during clinical medicinal product development. In case of substantial changes to the biomarker assay, the maintenance of analytical (and clinical) performance in the context of the use of the medicinal product must be ensured, and respective data need to be established to confirm this. Analytical performance differences, also in cases of assay improvement, need to be justified in terms of maintained performance with regards to the use of the medicinal product.

Specifically, a *candidate CDx* might be further adapted during clinical development to optimise patient selection (e.g., changing the exact composition of a biomarker panel by adding and/or deleting genomic aberrations, or changing the cut-off). It is also essential to ensure that datasets used to derive an adapted assay are distinct from the dataset(s) used for its analytical validation. If a single dataset is split into a training set for the derivation of the optimised assay and a test set for the analytical validation, this approach must be pre-specified and transparently documented.

For the MAA of a biomarker-guided medicinal product, it is essential to connect all clinical data collected. Therefore, if the methodological approach or the *candidate CDx* assay platform has undergone changes, adequate justification on the continuity of earlier data relevance needs to be provided in the dossier. This exercise may also require supportive bridging data for the different assay versions used throughout clinical development. In cases where clinical samples are limited or not available, the alternative use of biobank samples and their suitability must be justified.

For further details, refer also to section 9 "Bridging studies" of the guideline.

6.6. Considerations for centralised versus decentralised testing

In the clinical development setting, two options are generally available, i.e., sample testing with a single assay performed in a single testing laboratory (centralised testing) and sample testing using the same or different assays in different laboratories (decentralised or local testing). Each approach has specific benefits and limitations with regards to practicality, regulatory implications and potential impact on data quality and reliability. The decision for one or the other approach will be largely determined by the CoU and the available analytical options for measuring the biomarker. On one end of the spectrum is a setting where a Conformité Européenne (CE)-marked IVD/CDx for local patient testing is available, which would facilitate the decentralised approach. On the other end of the spectrum is a highly complex or novel analytical approach which requires specialised expertise and thus fosters the preference for central testing. And in between, there is a wide range of possible scenarios.

The centralised approach has the benefit of data generation within a consistent testing environment; however, it requires the transfer of samples from local sites to the central testing facility. The transfer may impact sample stability and storage conditions requiring additional sample transport validation. The timeframe of the results availability is influenced by both the sample shipping time and processing time at the central facility.

The most important advantage for decentralised testing is the potentially faster availability of results for both the treating physicians and the patients, and it may be crucial in cases of unstable samples. However, this approach also presents risks related to the consistency of assay performance across different laboratories. For instance, where different assays are used locally, comparable assay performance needs to be ensured for the consistent determination of biomarker status. This may include bridging studies and/or consideration of round-robin tests. Therefore, in decentralised settings, the use of the same assay across all sites is strongly recommended, or if not operationally feasible, similar assay technology with comparable performance characteristics must be considered.

If a panel where multiple markers are used to determine the biomarker status of a patient, then a uniform definition of the set of markers across all sites is required.

A complex analytical assay platform (e.g. cut-slide assays, like IHC) is associated with specific challenges in ensuring adequate technical expertise at local sites and hence achieving reproducible results across the local laboratories. Therefore, in such cases, centralised testing is generally recommended to ensure consistency and reliability of study data.

Candidate CDx aspects

If it is decided to develop a *candidate CDx* (stages B and C), it might be more challenging to (co-)develop a suitable *candidate CDx* when testing is performed in a decentralised rather than a centralised setting, as the assays used across the different testing laboratories would need to be compared to ensure equal performance. Therefore, in a decentralised scenario the use of the same assay as *candidate CDx* across the different testing laboratories is the recommended approach. In cases where different local assays are used, a *candidate CDx* status may be assigned to one of them, or, alternatively, to an additional (novel) assay platform. For both scenarios, a prospectively defined strategy for assuring comparable performance characteristics between the *candidate CDx* and different local assay platforms should be in place. For example, testing results of different local assays may be confirmed by a (central) *candidate CDx*.

At the time of the marketing authorisation, it is essential to ensure that reliable and robust data are available to support the B/R assessment of the corresponding medicinal product, especially when decentralised testing was applied. Due to the complexity and variety of decentralised testing approaches, developers may also consider seeking scientific advice from EMA.

Additional considerations for analytical suitability of biomarker assays during medicinal product development beyond the EU

During a global development programme of a biomarker-guided medicinal product and corresponding biomarker assay(s), clinical studies may be performed within the EU, partially within and outside the EU, or entirely outside the EU. While EU legislation applies to clinical studies conducted in the EU, GCP compliance and assays being fit for purpose are generally required. Challenges in consistency of safety and efficacy data may arise from variability in (patient) populations, analytical assay performance, and/or testing laboratory accreditation standards.

Therefore, in order to ensure consistency of the testing results during clinical development, it is strongly recommended that patient samples are centrally tested, regardless of their origin (EU or non-EU). Where testing is conducted outside the EU, analytically validated assays should be used in a laboratory compliant with accepted standards, such as ISO15189 or CAP/CLIA.

In situations where centralised testing may not be feasible, it is recommended that the same assay, or at least a similar assay technology with comparable performance characteristics, is used across all concerned local accredited testing laboratories.

Generally, the recommendations provided in section 6.6 "Considerations for centralised versus decentralised testing" of the guideline also apply in a global testing setting, particularly with respect to aspects relevant to a *candidate CDx*. Also, in a global setting, the assurance of reliable and robust testing data generated in clinical studies within and outside the EU must be confirmed at the time of MAA to support the B/R assessment of the associated biomarker-guided medicinal product.

7. Samples for biomarker analysis during medicinal product development

In section 7, the term "samples", which is equivalent to *specimens* in the IVDR, is used throughout [3].

Samples for biomarker analyses are collected at all stages of medicinal product development. Different sample types may be collected for analysis: blood and blood fractions (plasma, serum, buffy coat, red blood cells), tissue biopsies (from surgery, autopsy, transplant), urine, saliva/buccal cells, and any other types of biospecimens (e.g. breast milk, cell lines, semen, ascites, hair). Sample types relevant to the context of use should be collected, processed, and stored under conditions that preserve their stability and reliability with respect to the intended downstream analyses. To ensure sample quality that supports decision-making, the general principles for sample collection, processing, transport, storage, and disposition, as outlined in the ICH E18 guideline, should be followed [16]. According to ICH E6(R3) on GCP [11], biological samples may not be collected or stored from any subject without first obtaining informed consent based on adequate information regarding the purpose, potential risks, and intended use of the samples (including information on potential biobanking).

The source and quality of the sample for biomarker analyses performed in confirmatory studies should be appropriately justified in relation to type and purpose, because they may be obtained under variable conditions (leading to uncertain sample quality) and the time between sample collection and study treatment initiation can vary widely. Freshly obtained samples collected using standardised procedures for collection and processing are generally preferred.

7.1. Pre-screening

Screening procedures (i.e., relevant procedures actively evaluating potential participants for enrolment in clinical trials) are an integral part of clinical development and are described in the clinical trial protocols. Generally, *pre-screening* refers to the pre-selection of potential trial participants based on archival data that were collected independently of the clinical trial data and are already available to the investigator, sometimes prior to obtaining informed consent for participation in the trial.

In the context of biomarker assay development, pre-screening can lead to so-called pre-screening bias. Pre-screening bias can occur, for example, when a local test result is used to include or exclude patients from the planned screening procedures. Patients whose samples are considered biomarker-positive by a local test are usually retested using a central test (which might be the *candidate CDx*) and will be included in the trial only if biomarker positivity is confirmed by this central test. However, patients with biomarker-negative results based on the local test will be excluded from the trial. This may lead to the underrepresentation of patients whose samples are negative by the local test but positive when assessed using the central test. Therefore, biomarker-based pre-screening (selection) should generally be avoided.

Furthermore, it is encouraged to thoroughly analyse the prevalence of the biomarker based on epidemiological data, to document pre-screening procedures, and to test (to the extent possible) a proportion of local test-negative samples using the *candidate CDx* to estimate the magnitude of the potential bias. Pre-screening bias may be estimated by comparing the treatment effect in patients with and without available pre-screening tests. While pre-screening may be inevitable in instances of very low prevalence of biomarker-positive populations in the context of large-scale genome profiling (e.g. tumour samples), estimating the proportion of potentially underrepresented populations is highly recommended. In any scenario, the pre-screening processes and the number of patients screened in a study need to be accurately and thoroughly documented to understand the potential for bias.

7.2. Baseline samples

A baseline sample refers to the initial sample taken for measurement from a participant before the start of the intervention or treatment in a clinical trial. Baseline sample collection and analysis is crucial for the investigation of the impact and value of the biomarker(s) of interest. The timing of baseline sample collection (e.g., tissue biopsies) should generally occur as close as possible to the initiation of study treatment, preferably during the screening period, following an appropriate wash-out period after any prior therapy, in order to minimise residual treatment effects and avoid confounding the interpretation of baseline data. Additional aspects to consider regarding source and timing of sampling include intra-patient variability in biomarker expression, which can impact on the performance of the biomarker, as well as the temporal variation in biomarker levels over time.

Consideration should be given to the need to retain all samples collected which may be required for future use during the course of product development. In addition to samples from patients enrolled in the trial, samples of patients who were excluded from the trial due to their biomarker status may be valuable for future bridging studies. For further information regarding bridging, refer to section 9 "Bridging studies" of the guideline.

7.3. Missing data

During clinical development, collection of biomarker samples (*specimens*) should be as complete as possible, according to the planned sampling schedule. Missing samples are more commonly encountered when biomarker testing is performed at a timepoint distant from baseline sample collection, as is often the case in retrospective analyses. Reasons for missing samples should be adequately documented, and any potential bias should be investigated [17]. There can be causes of missing data other than missing samples.

In general, the eligibility criteria for patient enrolment, should outline an inclusion requirement of baseline biomarker data. Exceptions (where this might not be possible) must be well justified. In any case, the population with a biomarker result needs to be representative of the overall study population and this should be demonstrated considering that missing data may not occur at random but may be related to other factors such as disease state (e.g., tumour size) or operational reasons (e.g., non-compliant study site) with a large potential for study results bias. Since biomarker data is used to select/exclude patients from a treatment (sub)group, statistical methods to handle missing biomarker data and addressing potential bias need to be pre-specified in the analysis plan.

8. Clinical validation of biomarkers and validation of biomarker assays

In the guidance below, the term B/R is related to the B/R of medicinal products and is used as applied in the medicines' framework.

A biomarker may be predictive of the efficacy or safety outcomes of a medicinal product. This implies that the indication of the medicinal product should cover the biomarker-defined target population with a positive B/R balance. The predictive ability of the biomarker(s) for patient clinical outcome may initially be hypothesised prior to clinical development based on the mechanism of action and may progressively evolve, becoming more evident as clinical development advances.

The aim of predictive biomarker-guided medicine development is to (co-)develop a medicinal product along with (an) appropriate IVD(s) able to identify the patient population that would benefit from the medicinal product, or that has a lower risk of serious adverse reactions. Consequently, a predictive biomarker IVD can be considered clinically valid if it separates patients with a favourable B/R profile from patients with an unfavourable or clinically meaningfully worse B/R profile.

Consequently, in order to clinically validate a predictive biomarker IVD, it needs to be shown that the biomarker status, as measured by the IVD, is predictive of a clinical outcome relevant for the B/R assessment. This is demonstrated by evidence of a differential treatment effect according to biomarker status, i.e. by showing that a biomarker-by-treatment interaction exists. Generally, such an investigation requires data from the overall patient population, rather than from a population pre-selected based on biomarker status, as this allows comparison of the treatment effect between biomarker-positive and biomarker-negative patients. Without inclusion of biomarker-negative patients, the predictive effect of the biomarker may not be elucidated. Therefore, biomarker-negative patients should be included in the clinical development programme whenever possible.

The treatment-by-biomarker interaction may be investigated during different development steps of the medicinal product, serving different purposes (exploratory or confirmatory). During early clinical development, the interaction can be investigated in an exploratory way to identify potential signals, or to propose cut-off value(s) for the biomarker assay that serve to generate hypotheses for the next development steps. (Potential) surrogate endpoints (e.g. pharmacodynamic endpoints or endpoints

indicating clinical activity) that may allow prediction of the interaction for later clinically relevant outcomes might be investigated at this stage. The findings regarding the predictive value of the biomarker and the proposed cut-off would be usually considered exploratory and need to be validated (or confirmed) in the subsequent development step(s). In late clinical development, the investigation of the treatment-by-biomarker interaction should serve to clinically validate the biomarker assay. When designing the pivotal trial(s) for the medicinal product, which also serves to generate the pivotal evidence for the IVD, the following scenarios are of relevance:

(1) ***Biomarker-stratified***

There is a prior rationale that the B/R ratio may be improved in the biomarker-defined target population, but it cannot be excluded that the B/R may also be positive in a broader patient population, or there is uncertainty whether the selected cut-off is appropriate. Therefore, both biomarker-positive and biomarker-negative patients are selected for the study. This allows for the investigation of the clinical validity of the biomarker assay by analysing the treatment-by-biomarker interaction according to a pre-specified plan. Randomisation of patients should generally be stratified by (appropriately defined) biomarker status aiming to ensure adequate balance in the experimental and control arms, which is particularly important for small studies and/or rare biomarkers. The results may (or may not) support that biomarker status is predictive of a favourable B/R balance, i.e. that the biomarker assay is (or is not) clinically valid for patient selection. Of note, the absence of an interaction cannot be concluded based on a non-significant interaction. And vice versa, even if there is clear evidence of an interaction, this does not necessarily imply a negative B/R in patients with a negative biomarker status (who may simply have a smaller overall benefit). Indeed, as a treatment effect in the broader patient population may be driven by the effect in the patients with positive biomarker status, subgroup analyses can help clarify the treatment effect in the disjoint subgroups of patients with positive and negative biomarker status. For continuous biomarker assays, this means that subgroup analyses based on the selected cut-off - or disjunct biomarker subgroups in case of multiple pre-specified cut-offs (e.g., biomarker below cut-off A, biomarker between cut-off A and cut-off B, and biomarker above cut-off B) - should be provided.

(2) ***Biomarker-targeted***

There is a strong prior rationale that the proposed treatment has a positive B/R ratio *only* in the biomarker-defined target population. Therefore, only patients with positive biomarker status are included in the study that serves as the basis to support clinical validity. For continuous biomarkers, these would be patients above (or below) a specific cut-off. While it is possible to confirm a positive B/R balance in patients with positive biomarker status, the biomarker-by-treatment interaction cannot be investigated but assumes no (sufficient) treatment effect in the patients with negative biomarker status. This approach requires a strong scientific rationale, usually based on previous clinical or non-clinical data. As only patients above or below a specific cut-off are included, a clinical justification of the cut-off is particularly important.

The assumption at the planning phase of the study to investigate the B/R profile of the medicinal product can also be that the B/R in the target population is independent of the biomarker status. However, exploratory subgroup analysis to investigate the consistency of the treatment effect across the overall population could show a strong treatment-by-biomarker interaction which suggests that B/R may be positive only in a biomarker-defined target population. The claim of a biomarker assay being predictive on this basis may be acceptable only after careful assessment of the credibility of such a finding, particularly if the B/R balance in the overall population is unconvincing and the intention is to identify a subpopulation with a convincing B/R (refer to Guideline on the investigation of subgroups in confirmatory clinical trials [18]). In many cases, replication of such findings will be required for confirmation of clinical validation. However, if there is a concern that the B/R may be negative in patients with a specific biomarker status, the possibility that the biomarker is predictive should not be disregarded even if uncertainties remain (precautionary principle).

In case there are sub-entities of the biomarker assay (e.g., different mutations in multi-marker panels), explorative subgroup analyses should be provided to support that the biomarker assay is predictive across the entire population of patients with positive biomarker status. Adaptive two-stage designs of a clinical trial that include an interim analysis can allow for timely restriction of the study to only biomarker-positive patients while generating data in biomarker-negative patients, thereby limiting exposure of patients to a potentially ineffective or unsafe treatment in case of scenario (1) above. A decision on the cut-off could be made in the first stage and confirmed in the second stage. However, specific considerations that apply for design and analysis of adaptive studies need to be taken into account, including appropriate pre-specification and multiplicity control, which can be read in the Reflection Paper on methodological issues in confirmatory clinical trials planned with an adaptive design [19].

Single arm trials are usually not suitable to establish that a biomarker is predictive because, in such a setting, predictive and prognostic roles cannot be disentangled without additional assumptions. When a randomised controlled trial is not feasible, seeking scientific advice is recommended.

Validation of cut-offs

The cut-off of the biomarker assay that differentiates between patients with a positive biomarker status and negative biomarker status needs to be provided. Even if the biomarker assay provides a qualitative result (usually binary, i.e. biomarker present or absent), there is usually an underlying quantitative variable (refer to section 5 of the guideline). The clinical validity of the chosen cut-off needs to be justified.

The choice of the cut-off for a biomarker assay should be guided by its predictive effect, i.e. the aim should be to select it in a way that it differentiates patients with a positive B/R from those with a negative B/R. This could be based on an exploration of the quantitative relationship between the biomarker and clinical outcomes, which may be confirmed in subsequent steps. Note, when the biomarker is also prognostic, the cut-off for a prognostic effect will usually not be identical to the cut-off for a predictive effect. If the cut-off is exclusively based on analytical sensitivity of the assay (e.g. the LOD), but is not associated with clinical validity, it remains uncertain whether all patients with a positive biomarker status can be expected to benefit from treatment.

In scenario (1) above, in case of uncertainty regarding the cut-off prior to the study, the study might be used to explore and define the cut-off. In that case, the study should allow for a subsequent evaluation and confirmation of the cut-off in a separate dataset to avoid overfitting. This could be achieved, for example, by using a smaller proportion of patients to derive the cut-off and the remaining patients to validate it. This should be clearly pre-specified and preferably be temporally separated (i.e., the first enrolled patients are used for the cut-off derivation, the later enrolled patients for the cut-off confirmation).

In scenario (2) above, the validity of the chosen cut-off for patient selection could be supported by investigating the interaction of the biomarker in subjects above the cut-off. It should be shown that the treatment effect is decreased for biomarker values close to the cut-off, but, at the same time, that subjects whose biomarker value is close to the cut-off still have a positive B/R.

In the setting where the biomarker-by-treatment interaction is evident only after the results are available, unless there is a strong rationale for a cut-off, further validation in independent data is usually required.

9. Bridging studies

In section 9, the term "samples", which is equivalent to *specimens* in the IVDR, is used throughout [3].

This guideline primarily addresses considerations related to the co-development of a single biomarker assay with a corresponding medicinal product. However, bridging studies may be required in the following scenarios where different biomarker assays are used.

"Internal" bridging within the same development:

- *Co-development but major assay changes*: the *early/mature candidate CDx* undergoes significant changes (i.e., with potential impact on assay performance) during the clinical development programme of the medicinal product (e.g., during the pivotal clinical trial).

- *Different assays used for a biomarker*: different biomarker assays (measuring the same biomarker) may be used during the clinical development programme of the medicinal product, either concurrently or sequentially (e.g., when decentralised testing is used, refer to section 6.6 of the guideline, specifically paragraph "*candidate CDx* aspects"). Several scenarios can apply. In the absence of a co-developed assay, a CDx candidate may differ from the assay used in the medicinal product development (i.e., was not co-developed) but will be the first CDx to be certified after medicinal product authorisation.

"External" bridging, e.g., across different IVD developments:

- *Follow-on CDx candidates*: The device seeks the same clinical indication and intended purpose as a Conformité Européenne (CE) certified predecessor CDx device, and targets the same biomarker, but it may use the same or a different technology [9].

In all these scenarios, bridging studies need to be conducted. The aim of a bridging study is to show that two assays (original and new) are equivalent, meaning that the biomarker-selected populations are highly similar for the two assays. In other words, it needs to be confirmed that the clinical benefit of the medicinal product is maintained if a new IVD is used instead of the one used in the original clinical trial.

In a bridging study, the primary focus is to assess the concordance between the original and the new assay, by using a set of samples analysed by both assays. The concordance should be evaluated using positive percentage agreement (PPA) and negative percentage agreement (NPA). Overall percentage agreement (OPA) is only considered relevant if the distribution of the samples used to establish OPA mirrors the actual frequency in the real-world population (e.g., if a mutation occurs in 30% of the patients in the indication, then approximately 30% of the samples used to calculate OPA should be true positives with respect to the mutation). In any case, acceptance criteria for analytical equivalence between the original and new assay should be pre-defined and appropriately justified in terms of PPA and NPA. Acceptance criteria should be expressed in terms of lower bounds of the 95% confidence intervals. They should be justified by clinical and analytical considerations and not by the available sample size. For example, the concordance range should consider the extent of variability of the assay as determined in the analytical validation studies. Ranges are expected to be narrower for genetic-based assay (e.g. NGS) versus targeted proteomic assays (e.g. IHC).

The choice of samples for a bridging study should allow for a robust evaluation of PPA and NPA (i.e. both positive and negative samples are needed) and be appropriately justified with respect to the target indication. The samples should be representative of the target population in which the assay will be used and should cover a wide range of different biomarker values, especially around and close to the threshold. For bridging studies performed within the clinical development programme of the medicinal product, a representative subset of the retained (*left-over*) samples (i.e. samples originating from the initial clinical study) should be used. If samples collected outside the clinical development of the drug are used (e.g. biobank samples), justification that these are representative for the context in which the assay will be used should be provided.

If the samples from pivotal clinical trial(s) are available for bridging, the concordance should be evaluated by analysing the primary and secondary endpoints as per the original clinical trial, but with samples categorised according to the new assay instead of the original one and then comparing the results from this re-analysis to the original clinical trial results. Generally, concordance should always be evaluated whenever possible, but it is acknowledged that this may not be practically feasible in many cases (e.g. for follow-on CDx candidate). Of note, modelling and simulation approaches to extrapolate concordance in the absence of access to the original samples are currently discouraged due to the high uncertainty and variability associated with these approaches.

When reporting the outcome of a concordance analysis, the following information should be presented in the MAA submission: the reference assay that was used, the original data/clinical trials that were used as a reference, and a description of the samples analysed (origin, comparability to the original population and a flowchart of sample inclusion/exclusion). Both the point estimate and the two-sided 95% confidence intervals of PPA and NPA should be provided, and the lower boundary of the confidence interval should exceed the threshold that was pre-specified as success criterion. Specifically, in the case of continuous markers, additional analyses such as the correlation of the assay outcomes or a more granular concordance analysis across a range of cut-offs may deliver relevant supportive information.

10. Summary of Product Characteristics (SmPC)

The SmPC is the basis of information for healthcare professionals on how to use medicinal products safely and effectively. The Public Assessment Report contains comprehensive information on the scientific assessment of the medicinal product. Information on the correct use of the IVD device is available in the IFU.

Information on specific aspects of treatment with an approved predictive biomarker-guided medicinal product related to its use with the validated assay for the purpose of patient selection should be described in the relevant sections of the SmPC to a sufficient extent to ensure the safe and effective use of the medicinal product. Information on the biomarker(s) and biomarker assay(s) should be included and/or updated in relevant sections of the SmPC in line with the SmPC guideline [8, 20, 21, 22] and considering the examples given by the SmPC advisory group on pharmacogenomic information in the SmPC [23].

In line with established guiding principles, inclusion of information about the biomarker and the biomarker assay (including CDx) is foreseen in sections 4.1, 4.2, 4.3, 4.4, 4.8 and 5.1 of the SmPC, as relevant [9].

SmPC sections 4.1, 4.2, 4.3

When the indication or contraindication is dependent on the predictive biomarker and its detection or measurement by a corresponding predictive biomarker assay, inclusion of the biomarker reference in section 4.1 (Indication) or in section 4.3 (Contraindication) is mandatory. For example, the indication in section 4.1 of the SmPC can refer to a particular genotype and/or phenotype (e.g. the expression of a gene or protein). Situations where the medicinal product should not be prescribed for safety reasons, i.e. contraindications, are in the scope of the section 4.3. Such circumstances could also include a particular genotype or phenotype.

Description of the predictive biomarker should be included with as much precision as possible (including cut-off values where applicable, e.g. for continuous biomarkers) in order to adequately define the intended patient population with a positive B/R profile. Sections 4.1 and 4.3 of the SmPC reflect the essential and mandatory use of predictive biomarker assays. More detailed information may be included in other SmPC sections, such as section 4.2. The extent of information provided in sections 4.1 and 4.2 may depend on the simultaneous diagnostic role of the predictive biomarker, with the objective of clearly specifying the patient population with a positive B/R profile and, consequently, excluding patients with a negative B/R profile from treatment. Where the patient population defined by a biomarker used for disease diagnosis overlaps with that defined by a predictive biomarker, the level of detail included in sections 4.1 and 4.2 should be determined on a case-by-case basis, provided that it is sufficient to ensure the safe and effective use of the medicinal product and a clear definition of the patient population with a positive B/R profile.

For indications with multiple or composite biomarkers where it is not reasonable to specify the cut-off or other detailed information in section 4.1, a reference to section 4.2, where the biomarker status will be further characterised, should be included. The following sentence will be included in section 4.1 "For

biomarker-defined patient selection, see section 4.2". Similarly, when the patient population is defined by other biomarkers not predictive for treatment with a specific medicinal product, the extent of information in sections 4.1 and 4.2 can be decided on a case-by-case basis.

Where the use of specific methods, samples (*specimens)* (e.g. blood vs tissue), or analytes (e.g. DNA vs RNA) is mandatory to ensure the selection of patients with a positive B/R profile, this can be outlined in section 4.2. of the SmPC.

It is recommended that the wording in section 4.2 (Posology) of the SmPC refers to a CE marked IVD or a validated test, i.e. "… should be assessed by a CE marked IVD with the corresponding intended purpose. If the CE marked IVD is not available, an alternative validated test should be used.".

SmPC sections 4.4 and 5.1

The biomarker and methods for its detection or measurement in clinical trials (e.g. clinical trial assay or CDx with its brand name if applicable) should be described in section 5.1. The use of brand names in this section can be required as an additional measure for safe and/or effective use of the medicinal product (e.g. when distinctive information on concurrently used tests/assays is needed). Section 5.1 needs to clearly state which types of samples (*specimens*) were used in the clinical trial as the basis of the presented data. Similarly, the type of analyte is to be specified (e.g. DNA, RNA, circulating tumour DNA (ctDNA)), unless this information is already reflected in section 4.2 as a result of the assessment on the mandatory scope of the information. When information on the predictive biomarkers tested in clinical trials is extensive (e.g. numerous genetic alterations or composite biomarkers) and could thus dilute the description of the clinical trials results in section 5.1, such information can be included under subsection 'Mechanism of action' describing predictive biomarker(s).

In cases where the indication/target population is restricted to a subpopulation of trial population(s) (e.g., defined by a predictive biomarker), it should be noted in the text whether the primary analysis was positive in all enrolled patients. Nevertheless, a description of treatment effect estimates in all enrolled patients is not necessary as it is not aligned with the restricted indication. All results should be subsequently included for the restricted indication only.

While the main descriptive information on the use of biomarkers and biomarker assays is included in section 5.1, additional description may need to be provided in section 4.4, particularly in case where there are uncertainties related to biomarker detection or measurement for the identification of patients who are likely to benefit, or where specific precautions are required. Section 4.4 may also include special warning and precautions for use related to biomarkers that are relevant to safety (with cross-reference to section 4.8), for example in cases of deficiency of certain enzymes.

11. Post-approval follow-up

If, at the time of the marketing authorisation, uncertainties remain on predictive biomarkers used to select patients and/or certain performance characteristics of the corresponding predictive biomarker assays, these aspects may need to be addressed post-approval depending on their potential impact on the B/R profile of the selected patient population.

12. Definitions and abbreviations

This section provides definitions of key terms and abbreviations which are used throughout the document for clarity and consistency. Where available, the definitions align with official definitions.

Analytical validation	Demonstration that a bioanalytical method is suitable for its intended purpose [12]

B/R	Benefit/Risk
Biobank samples	Samples (*specimens*) which have been collected, processed and stored in a biobank for use in current or future clinical studies; collected under clinical protocols.
Biomarker assay	The test or device used to detect/measure the biomarker.
Biomarker status	The positive/negative outcome of the biomarker assay, defined for all biomarkers to be included in a medicinal product indication. The status can simply refer to the detection of the biomarker in the sample for qualitative biomarkers (i.e., being above or below the detection limit), or it can be based on the dichotomisation of a continuous value for continuous biomarkers (i.e., being above or below an established cut-off).
Biomarker-defined target population	The population where the medicinal product is expected to exhibit a positive B/R based on the biomarker status. The co-development of the medicinal product and biomarker assay aims to identify such a population as close as possible.
Biomarker-selected population	The population selected based on the biomarker status (positive or negative), in which the medicinal product has shown a positive B/R and is hence reflected in the SmPC. Can be either the population with a positive biomarker status or the one with a negative biomarker status.
Candidate CDx	A biomarker assay used in pivotal clinical trials for the development of a predictive biomarker-guided medicinal product in order to establish the data for B/R assessment; and that is (co-)developed towards a CDx as defined in the IVDR. Depending on the stage of clinical development, this can either be an "early" or a "mature" *candidate CDx*.
CE	Conformité Européenne (European Conformity)
Clinical validation of a predictive biomarker assay	The process to establish the ability of an assay to differentiate the efficacy or safety of a medicinal product based on biomarker status.
Co-developed CDx	An IVD device that is developed in a clinical development programme together with the concerned medicinal product, either in view of an initial marketing authorisation or post-approval extension of the indication. A co-developed CDx is usually the device used in a pivotal clinical trial for the concerned medicinal product. Alternatively, the CDx may be developed in the context of a bridging study.
Companion diagnostic (CDx)	Defined in Article 2(7) of Regulation (EU) 2017/746 [3] as follows: a device which is essential for the safe and effective use of a corresponding medicinal product to: (a)

	identify before and/or during treatment patients who are most likely to benefit from the corresponding medicinal product; or (b) identify before and/or during treatment patients likely to be at increased risk of serious adverse reactions as a result of treatment with the corresponding medicinal product.
CoU (Context of Use)	A commonly used statement which clearly describes the way in which the predictive biomarker (assay) is to be used in relation to the medicinal product development. This terminology (although linked) is broader in meaning than the "intended purpose" of the IVD according to the IVDR. The context of use (CoU) of a medicinal product or tool is a formal, concise statement defining the specific circumstances, patient population, and purpose for which it is used, often focusing on its role in drug development, clinical trials, or regulatory approval. It includes the intended use, target population, and conditions for use. For biomarkers, the CoU specifically outlines the category (e.g., prognostic, predictive) and its role in drug development. It ensures that the product is "fit for purpose" and provides relevant data to support regulatory or scientific decisions. The context of use (CoU) of a medicinal product with a biomarker refers to the specific circumstances, patient population, and purpose for which the biomarker is used, often focusing on its role in drug development, clinical trials, or regulatory approval. It includes the intended use, target population, and conditions for use.
ctDNA	Circulating tumour DNA
CTR	Clinical Trials Regulation [7]
DNA	Deoxyribonucleic acid
EMA	European Medicines Agency
EU	European Union
FISH	Fluorescence *in situ* hybridisation
GCP	Good Clinical Practice
HLA	Human leukocyte antigen
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IHC	Immunohistochemistry

IFU (instructions for use)	According to the IVDR Art 2 (14) [3], "instructions for use" means the information provided by the manufacturer to inform the user of a device's intended purpose and proper use and of any precautions to be taken.
Intended purpose	According to the IVDR Art2 (12) [3], "intended purpose" means the use for which a device is intended according to the data supplied by the manufacturer on the label, in the instructions for use or in promotional or sales materials or statements or as specified by the manufacturer in the performance evaluation.
ISH	*In situ* hybridisation
IVD	*In vitro* diagnostic medical device
IVDR	Regulation (EU) 2017/746 on in vitro diagnostic medical devices [3]
LOD	Limit of Detection
MAA	Marketing Authorisation Application
MDCG	Medical Device Coordination Group
NB	Notified Body
NCA	National Competent Authority
NGS	Next-generation sequencing
NPA	Negative percentage agreement
Omic	Referred as multiple omic technologies (e.g., genomic, proteomic, transcriptomic)
OPA	Overall percentage agreement
PCR	Polymerase Chain Reaction
PPA	Positive percentage agreement
Predictive biomarker	A biomarker, which in association with a specific treatment, identifies patients that are more likely to experience a certain effect on the clinical outcome.
Predictive biomarker assay	An assay to detect or/and quantify a predictive biomarker.

Prognostic biomarker	A biomarker which identifies patients that are more likely to experience a certain clinical outcome, regardless of treatment.
RNA	Ribonucleic acid
Round-robin test	A procedure in which multiple laboratories analyse identical samples using the same method to evaluate the reproducibility and performance of that method across different settings [24].
SmPC	Summary of Product Characteristics
SSP	Summary of safety and performance (IVDR terminology)

13. References

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