Companion and complementary diagnostics as tools of precision medicine

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Abstract

Companion diagnostics (CDx) is an important element in the realization of precision medicine. The FDA defines a CDx assay as an in vitro diagnostic device that provides information that is essential for the safe and effective use of a corresponding therapeutic product. Most CDx assays have been developed prospectively using the drug-diagnostic codevelopment model, in which the assay is developed in parallel to the drug. However, a CDx assay is not only important during clinical development, but just as important as a treatment decision tool when the drug is regulatory approved and routinely used in the clinic. Owing to the central role of the CDx assay in the treatment of individual patients, regulators have imposed strict requirements on assay quality. Before a CDx assay can be used in the clinic, careful analytical and clinical validation must be performed to document the accuracy, reproducibility, and clinical performance.

Key points

- A companion diagnostic device is an in vitro diagnostic device that provides information essential for the safe and effective use of a corresponding therapeutic product.
- The first drug that had a companion diagnostic linked to its use was trastuzumab (Herceptin), which in 1998 obtained FDA approval together with the immunohistochemical assay HercepTest for the treatment of HER2-positive metastatic breast cancer.
- Most companion diagnostics are developed in parallel with the drug they are meant to guide using the drug-diagnostic codevelopment model.
- Companion diagnostics are classified as high-risk medical devices that require regulatory approval based on the comprehensive documentation of their analytical and clinical validity.
- The analytical platforms for companion diagnostics are diverse and include imaging, immunohistochemistry, in situ hybridization, polymerase chain reaction, and next-generation sequencing.
- For more than 20 years, companion diagnostics have played a significant role in individualizing pharmacotherapy and implementing precision medicine.

Introduction

The goal of individualized therapy has been on the agenda of healthcare providers for centuries, most likely millennia. Interpretations of texts from the ancient Greek era by Hippocrates seem to indicate that even in the 5th century BC, therapy should be

adapted to the individual patient's needs and characteristics. It was further described that each human body is different and responds differently to therapy; therefore, the same treatment is not suitable for everybody (Konstantinidou et al., 2017). Taking a big jump to the 19th century and one of the fathers of modern medicine, Sir William Osler expressed the issue of patient heterogeneity in this way: "If it were not for the great variability among individuals, medicine might as well be a science and not an art" (Woodcock and Lesko, 2009). This statement underlines the need to individualize therapy, which was outlined half a century ago in the principles of rational use of drugs or rational pharmacotherapy (Klett and Moseley, 1965; Galbrecht and Klett, 1968). Here, the goal was that the individual patients should receive medications appropriate to their clinical needs in order to optimize the benefits and minimize harm. Already then, these principles were translated into "the right drug for the right patient in the right dose at the right time," which is an expression we still use when discussing precision medicine today (Jørgensen and Hersom, 2019).

One might argue that precision medicine was already introduced 50–60 years ago. However, there is one major difference when we compare then and now, and that is our increased knowledge of the molecular understanding of the pathophysiology and mechanisms of action of drugs, which has been instrumental in the implementation of a more individualized therapy and how we understand precision medicine today (Jørgensen, 2019a). During the past few decades, we have experienced significant advances in molecular medicine, which have enabled us to understand and describe part of the heterogeneity observed between patients with apparently the same disease. A development that has been most visible within hematology and oncology, where a number of targeted drugs guided by predictive biomarkers have led to improved treatment outcomes for many patients. These predictive biomarker assays are most often developed in parallel with the drug using the prospective drug-diagnostic codevelopment model, which leads to contemporaneous regulatory approval of both drugs and diagnostics (Jørgensen and Hersom, 2018). It is important, as the diagnostic assay needs to be available at the same time as the drug to support the treatment decision process for the individual patient. The name that has been adopted by the regulators for these predictive biomarker assays are companion diagnostics (CDx) (Food and Drug Administration, 2014). This chapter will discuss different subjects related to the background and development of companion and complementary diagnostics, as well as clinical and regulatory aspects related to their use.

Early application of predicative biomarker in oncology drug development

The first time we see predictive biomarker testing integrated in an anti-cancer drug development project was in the 1970s when the selective estrogen receptor modulator tamoxifen (Nolvadex, AstraZeneca) was developed for the treatment of metastatic breast cancer (Morgan et al., 1976). In a single-arm clinical trial published by Morgan et al. in 1976, 72 women with advanced breast cancer were treated with tamoxifen. All patients enrolled in the trial were tested for estrogen receptor (ER) status and arylsulfatase B and glucose-6-phosphate dehydrogenase enzyme activity. The objective response rate (ORR) following tamoxifen treatment for all 72 enrolled patients was 38%. However, for the subgroup of patients with positive ER status and no prior treatment with chemotherapy, the ORR was 74%. None of the patients who tested negative for ER achieved remission. Based on the trial results, the investigators concluded that tamoxifen was effective in the treatment of patients with advanced breast cancer, and that ER status and specific enzymes might be useful in selecting patients for hormone manipulation (Morgan et al., 1976). Today, testing for hormone receptor status is routine before initiating treatment with selective estrogen receptor modulators or aromatase inhibitors (Thürlimann et al., 2005). Although the trial by Morgan et al. was conducted more than 45 years ago, the principles described here still apply to the current drug-diagnostic codevelopment model. The purpose of this model is to identify a predictive biomarker as early as possible during the discovery and research phases, which can subsequently be used to select patients who are likely to respond to the drug during clinical development. However, in the described clinical trial with tamoxifen, testing for estrogen receptor status was not used as a patient selection criterion, as we know from today's enrichment trial design (Thürlimann et al., 2005). Although it was the first example of combining drugs and diagnostics in oncology, it was not until a decade or two later that the prospective enrichment trial design proved its real value (Jørgensen and Hersom, 2019).

In the early 1980s, the gene encoding the human epidermal growth factor receptor 2 (*HER2*) was discovered and cloned. A few years after this discovery, Dennis Slamon and colleagues found the link between amplification of the *HER2* gene and a poor prognosis in patients with breast cancer (Slamon et al., 1987). In a paper published in *Science* in 1987, they suggested that the *HER2* gene product serves as a growth factor receptor that plays an important role in the pathogenesis of this type of breast cancer. Furthermore, they proposed that a specific antagonist should be developed, which could block signaling from the receptor and hopefully inhibit the growth of HER2 positive tumors. This antagonist later became the monoclonal antibody trastuzumab (Herceptin, Roche/Genentech), which specifically binds to subdomain IV of the extracellular region of the HER2 transmembrane receptor (Jørgensen and Hersom, 2019). When Genentech subsequently entered clinical development with trastuzumab, an immunohistochemistry (IHC) assay that could detect HER2 protein expression levels in tumor tissue, called the clinical trial assay (CTA), was developed. In early clinical trials with trastuzumab, the CTA was used for patient selection, and different studies demonstrated a clear link between HER2 overexpression and the efficacy of trastuzumab. By using the CTA for patient stratification, Genentech formed the basis for the prospective enrichment clinical trial design, which is well established in today's cancer drug development. In September 1998, the Food and Drug Administration (FDA) contemporaneously granted approval to trastuzumab and a new optimized HER2 IHC assay, named HercepTest (Dako/Agilent), through a new coordinated procedure (Jørgensen, 2019a).

In 2019, Dennis Slamon, Axel Ullrich, and Michael Shepard received the Lasker-DeBakey Clinical Medical Research Award for their research, which led to the development of trastuzumab. In relation to this event, the *New England Journal of Medicine* published an article by the former American Society of Clinical Oncology (ASCO) President Daniel Hayes entitled 'HER2 and Breast Cancer - A Phenomenal Success Story' (Hayes, 2019). The development of trastuzumab was definitely a phenomenal success story. Trastuzumab was the first monoclonal antibody targeted toward an oncoprotein that was shown to be effective and it significantly changed the treatment of women with HER2 positive breast cancer and the approach to cancer drug development in general. Not only was this a scientific and medical achievement, but it also paved the way for the prospective drug-diagnostic codevelopment model where a predictive biomarker assay, a CDx, is developed in parallel to the drug and used to select the patients most likely to respond (Jørgensen, 2019a; Jørgensen et al., 2021). Since the approval of trastuzumab and the IHC assay HercepTest in 1998, the number of FDA-approved drug-CDx combinations in hematology and oncology has steadily increased. Especially within the last decade, the development has taken off and the number of target drugs approved by the FDA with a CDx assay will soon reach 50 (Food and Drug Administration, 2021a).

Companion diagnostics

The term CDx was not used when the HercepTest assay was contemporaneously approved with trastuzumab in 1998. At that time, this type of predictive biomarker assay was most often termed pharmacodiagnostics or theranostics (Jørgensen and Hersom, 2019). In an article by Nickolas Papadopoulos and colleagues published in *Nature Biotechnology* in 2006, the term CDx appeared for the first time (Papadopoulos et al., 2006). Here, the authors discussed the development and use of targeted anticancer drugs and concluded that the therapeutic potential of these types of drugs would only be realized if CDx assays were developed concomitantly. Furthermore, they stated that this type of assay could simplify the drug discovery process, make clinical trials more efficient and informative and be used to individualize therapy. Another aspect discussed was the quality standards applied in drug development compared with CDx development. Here, the authors emphasized that similar strict standards used in drug development should be applied to CDx assay development. Finally, it was concluded that choosing which patients to treat is as important as choosing which drugs to use, and errors could have serious consequences. The view on the strict requirements and controlled development of CDx assays have subsequently been expressed by other researchers (Lyman and Moses, 2016; Jørgensen, 2016; Hayes et al., 2013). A few years ago, Daniel Hayes was interviewed by *Expert Review of Molecular Diagnostics* for a special issue on Companion Diagnostics and here he said that 'A bad tumor biomarker test is as bad as a bad drug,' and continued by stating that people need to value biomarker tests as much as they value drugs and that researchers should do a biomarker study with the same amount of rigor as therapeutic trials (Hayes and Raison, 2015).

The first regulatory guidance document on CDx was issued by the FDA in 2014 and here, they defined this type of assay for the first time (Food and Drug Administration, 2014). According to this guidance document, a CDx assay is an in vitro diagnostic device that provides information that is essential for the safe and effective use of a corresponding therapeutic product. Furthermore, the FDA specifies four areas where a CDx assay could be essential: I) to identify patients who are most likely to benefit from the therapeutic product; II) to identify patients likely to be at increased risk of serious adverse reactions as a result of treatment with the therapeutic product; III) to monitor response to treatment with the therapeutic product for the purpose of adjusting treatment (e.g., schedule, dose, discontinuation) to achieve improved safety or effectiveness; and finally, IV) to identify patients in the population for whom the therapeutic product has been adequately studied, and found safe and effective, i.e. there is insufficient information about the safety and effectiveness of the therapeutic product in any other population. The fourth item mentioned in the definition is related to the type of study design most often used for clinical validation of CDx assays: the enrichment trial design (Jørgensen and Hersom, 2018). In their definition, the FDA emphasizes that clinical outcome data cannot be extrapolated to any population other than the one the CDx assay defines and if a drug is used for other types of patients, it will be regarded as an off-label prescription. A similar opinion on the use of CDx assays and off-label prescriptions has recently been expressed by the European Medicines Agency (EMA) also (Enzmann et al., 2019).

In the guidance document on CDx from 2014, the FDA emphasized various important safety aspects of assay performance in relation to the care of individual patients (Food and Drug Administration, 2014). Here, it is said that if a CDx assay is essential in treating patients, health care professionals must be able to rely on the test results, as an inadequate performance of such an assay could have severe therapeutic consequences. False-negative or false-positive test results can lead to withholding appropriate therapy or to administering inappropriate therapy. The key role of the CDx assay in patient care is further underlined, as it is stated that the use of a CDx must be included in both the labeling for the drug and the diagnostic assay, including the labeling of any subsequent generic equivalents. For drugs with a CDx assay linked to their use, this requirement underlines that testing is essential and must be performed before the drug can be prescribed to the patient and treatment initiated.

Complementary diagnostics

Besides the nearly 50 drugs that have been approved by the FDA with a CDx assay, a small number of drugs have a complementary diagnostic linked to their use. The term complementary diagnostics (CoDx) was introduced by the FDA in relation to the approval of nivolumab (Opdivo, Bristol-Myers Squibb) for second-line treatment of non-squamous non-small cell lung cancer (NSCLC) in

2015 (Jørgensen and Hersom, 2018). This relatively new regulatory class of predictive biomarkers has mainly been used for different indications related to immune checkpoints inhibitors and PARP inhibitors. To date, no guidance document has been issued on the subject, and no official definition is currently available. However, from presentations and publications by FDA officials, it can be deduced that a CoDx assay is a test that is not essential for the safe and effective use of the corresponding therapeutic product, but it can identify a biomarker-defined subset of patients that respond particularly well and aid the risk/benefit assessments for individual patients (Beaver et al., 2017; Food and Drug Administration, 2018a). Based on this preliminary definition, a CoDx, in contrast to a CDx, is not essential for the use of a corresponding therapeutic product, which means that testing with this type of assay is not mandatory before the drug is prescribed. This difference is also reflected in the prescribing information for drugs that have a CoDx linked to their use, as no information about testing is included in the labeling text (Jørgensen and Hersom, 2018).

A CoDx is not something that can be specified in the submission files for a drug-diagnostics combination. During the review of the New Drug Application (NDA) or Biologics License Application (BLA) for the drug and the Premarket Approval Application (PMA) for the CDx assay, the FDA may determine that the test is not essential for a safe and effective use of the drug under evaluation (Beaver et al., 2017). For most oncological and hematological drugs, this decision will be based on the clinical documentation with focus on the ability of the CDx assay to predict responding patients.

Drug-diagnostic codevelopment

Today, CDx assays play a significant role in the treatment of an increasing number of hematological and oncological patients. Most drugs that have a CDx linked to their use would lose their value without these assays. The CDx assays detect patients who are most likely to benefit from the different targeted drugs, who are often found at a low prevalence. For instance, a drug such as crizotinib (Xalkori, Pfizer), which is indicated for treatment of patients with metastatic NSCLC whose tumors are *ROS1*-positive, which is found in 1–2% of the patients only (Socinski et al., 2021). However, it is not only in relation to patient care that the CDx assays play a significant role but just as much in relation to clinical drug development. As described earlier in this chapter, the event that formed the foundation for the current drug-diagnostic codevelopment model was the development of trastuzumab. Based on a solid molecular understanding of the pathogenesis of HER2 positive breast cancer, Dennis Slamon and colleagues suggested the development of an antagonist that might improve the therapeutic outcome for this group of patients (Slamon et al., 1987). For any drug development project, a molecular understanding of the pathophysiology and the mechanism of the drug is of paramount importance and the essence of the drug-diagnostic codevelopment model. Fig. 1 illustrates the traditional prospective drug-diagnostic codevelopment model where the three main events related to CDx assay development are highlighted: (1) generation of a biomarker hypothesis, (2) assay development and analytical validation, and (3) clinical validation. For the model described in Fig. 1, it is important that the different CDx development stages are aligned with the drug development path and the clinical trials.

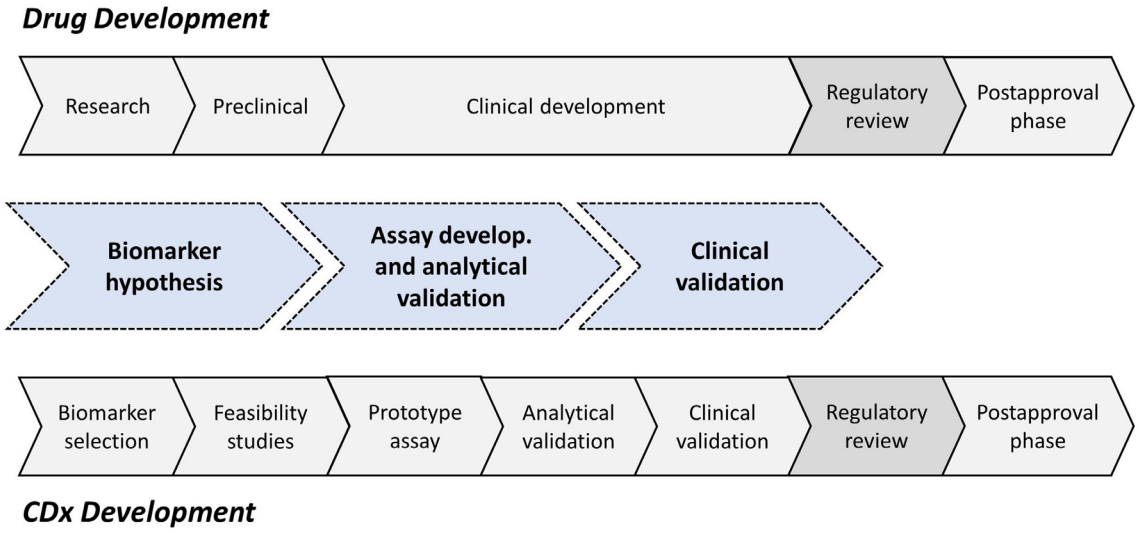

Fig. 1 The drug-diagnostic codevelopment model with an aligned development of drug and diagnostic followed by a contemporaneously regulatory approval. The three main events for the CDx assay development are: (1) generation of the biomarker hypothesis; (2) assay development and analytical validation; and (3) clinical validation.

Biomarker hypothesis

A strong biomarker hypothesis is key to a successful drug-diagnostic codevelopment project. Such a hypothesis is often deduced during the research/preclinical or early clinical development activities. This hypothesis should point in the direction of a link between the biomarker status/level and the efficacy outcome of the investigational drug. The basic research on trastuzumab is an excellent example to illustrate how such a hypothesis can be generated (Jørgensen et al., 2021). Based on the observation of *HER2* gene amplification in women with breast cancer, Dennis Slamon and colleagues suggested the development of an antagonist that could block the HER2 receptor. A mouse monoclonal antibody directed toward HER2 was developed, and pharmacological experiments suggested a link between HER2 expression and the efficacy of trastuzumab. When Genentech entered clinical development with a humanized version of this antibody, they had also developed an IHC assay that could detect HER2 expression in the tumor tissue from women with breast cancer. This assay was used to enrich the study population with HER2 positive patients in the different clinical trials conducted with trastuzumab. This hypothesis turned out to be correct, and a number of years later, when trastuzumab was approved by the FDA, they approved the HER2 assay simultaneously (Jørgensen et al., 2021). The biomarker hypothesis generated during the early part of a drug development project will normally also make up the foundation for the intended use of the CDx assay when it is regulatory approved. In this context the intended use means, what is stated on the regulatory label that the assay can be used for.

Assay development and analytical validation

When a biomarker has been identified based on the proposed hypothesis, the next step is to choose the analytical platform and start the development of a prototype assay (Ellison and Stanforth, 2019). The choice of platform depends on the type of biomarker and the specimen to be analyzed. Looking at the analytical platforms for the currently FDA-approved CDx assays, they are mainly dominated by methods for detection of different types of genomic biomarkers (Food and Drug Administration, 2021a). These platforms include next-generation sequencing (NGS), polymerase chain reaction (PCR), and different types of in situ hybridization (ISH) assays. Depending on the platform, they are suitable for detecting gene copy number changes, mutations, rearrangers, and fusions. Another platform is IHC, which is used to detect the presence of specific proteins in various types of tissue specimens, including receptor proteins. Approximately 25% of the FDA-approved CDx assays are based on IHC (Jørgensen, 2021b). When the analytical platform has been selected, a prototype assay must be developed, which will most often form the technical foundation for the final assay. Depending on the platform chosen, the key reagents should be selected and tested. For assays such as ISH, PCR, and NGS, the primer and probe design should be examined carefully, and often, a comparison of their sequences to those of the human genome is made. For IHC assays, the primary antibody formulation should be tested for specificity, sensitivity, and stability (Ellison and Stanforth, 2019). In general, the prototype assay is also exposed to a number of different tests to ensure robustness and reliability, which is often referred to as assay verification. This testing is important because the prototype assay is used in early clinical trials to confirm or disprove the biomarker hypothesis as early as possible. If the hypothesis can be confirmed, the next step for some assays is to have a cut-off value for the assay determined, as it will be for IHC, testing for gene copy numbers, and tumor mutation burden etc. The cutoff value is normally based on results from the prototype assay used in an early clinical trial with the investigational drug.

An important step in the development of the CDx assay is analytical validation. According to the FDA Code of Federal Regulations, validation of a medical device means confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use can be consistently fulfilled (Food and Drug Administration, 2022a). Based on prespecified performance specifications, the analytical validation should establish evidence that the CDx assay meets the specifications according to the intended use. An important aspect of the validation is that the assay can classify samples as positive or negative in an accurate and reliable manner based on the proposed cutoff value (Ellison and Stanforth, 2019). Here, a "cutoff value" could also mean the dichotomous answer on the presence or absence of a given mutation. The studies conducted in relation to the validation will vary depending on the type of assay, for example, genomic (PCR or NGS) or slide-based (IHC or ISH), but for both groups, testing will comprise the same principles. Typically, for slide-based assays, the studies will include sensitivity, specificity, precision, reproducibility, and robustness. Furthermore, aspects such as stability of assay components, samples, stained slides, and assessment of preanalytical variables, including cold ischemic time and fixation will be performed. For assays based on other platforms, studies may also include the assessment of accuracy, limit of detection, limit of blank, interfering substances, and other testing that is specific to the platform in question. Finally, an external analytical validation study should be performed to document the reproducibility of the CDx assay across different laboratories. Such a study, which is conducted at multiple clinical laboratories representing the user sites, includes at least an assessment of inter- and intra-laboratory reproducibility (Ellison and Stanforth, 2019).

An example of an external analytical validation study was when the *HER2* IQFISH pharmDx assay, developed to run on the automated Dako Omnis staining platform, was tested across three different clinical laboratories in the USA and Europe (Viale et al., 2016). The *HER2* IQFISH pharmDx is a fluorescence in situ hybridization (FISH) assay designed to quantitatively determine *HER2* gene amplification in formalin-fixed paraffin-embedded breast cancer tissue specimens. In addition to an evaluation of inter- and intra-laboratory reproducibility, the external analytical validation study also included different method comparison studies. Here, the *HER2* IQFISH pharmDx assay for Dako Omnis was compared to the manual version of the assays and the PathVysion *HER-2* DNA Probe Kit (Abbott Molecular). Finally, a comparative assessment of the staining quality of the three assays were performed.

Clinical validation

The aim of the clinical validation is to demonstrate that the CDx assay is able to detect the biomarker of interest in the intended use population and to identify patients who are expected to benefit from the investigational drug (Jørgensen and Nielsen, 2017). In the prospective drug-diagnostic codevelopment model, the clinical validation of the CDx assay is performed in a late phase pivotal clinical trial at the same time as safety and efficacy are documented for the investigational drug, as outlined in Fig. 1. It is important to conduct clinical validation of the CDx assay with the "final" analytical validated vision in order to ensure optimal performance in relation to the selection of the correct patient population. With the recent appearance of tumor-agnostic drugs, where a biomarker solely defines the indication, the analytical validation of the CDx assay becomes even more important before the start of a pivotal clinical trial. Examples of these types of drugs are the tropomyosin receptor kinase (TRK) inhibitors larotrectinib (Vitrakvi, Loxo Oncology/Bayer) and entrectinib (Rozlytrek, Roche/Genentech), which have recently been approved for patients with solid tumors harboring a neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion (Drilon et al., 2018; Paz-Ares et al., 2021; Jørgensen, 2020).

However, due to challenges with the alignment and timing of the development of the drug and the CDx assay, it is sometimes tempting to start a clinical trial with the prototype assay and then replace it with the analytically validated version during the conduct of the trial. Unfortunately, such a strategy is not recommendable, as it makes it difficult to interpret the results from the clinical trial, since this approach divides the patient population into two: one population selected with the prototype assay and another selected with the final analytically validated version of the assay. If different versions of the assay are used in a pivotal clinical trial, a subsequent bridging study needs to be performed, which is both resource- and time-intensive. For the clinical validation of a CDx assay, it is advisable to follow the "one rule," which is only to use one version of the assay, which must be the final analytically validated version, and furthermore, only to use one testing site in order to reduce possible interlaboratory variability (Jørgensen and Nielsen, 2017; Paz-Ares et al., 2021).

Clinical enrichment trial designs

Looking at the list of CDx assays approved through the FDA PMA process, there is one common denominator when it comes to clinical validation, namely the enrichment trial design (Food and Drug Administration, 2021a). However, it is important to remember that this design should only be used if there is a clear indication of a relationship between the CDx assay test result and the outcome following treatment with the investigational drug, for example, from previously conducted preclinical pharmacological studies or early clinical trials. If such a relationship is questionable, it would be unethical to use this design; instead, an all-comers trial design should be used, where the enrolled patients are tested for the biomarker in question but the assay result is not used as a selection criterion for enrollment in the trial. Originally, the enrichment design was built on the traditional randomized controlled clinical trial, but with an extra step included in relation to the patient selection process. Here, all patients are tested with the CDx assay, and only the CDx positive patients are enrolled in the trial and subsequently randomized to the investigational drug or the 'standard' treatment as outlined in Fig. 2 (Jørgensen and Nielsen, 2017).

The randomized enrichment trial design has been used several times for the development of different drug-CDx combinations. It was also the design of the phase III trial that documented the safety and efficacy of trastuzumab in HER2 positive patients with metastatic breast cancer, which lead to the approval of the drug and the HercepTest assay (Slamon et al., 2001). A few years after this approval, Richard Simon of the US National Cancer Institute published an article where he presented an alternative sample calculation for this phase III trial (Simon and Maitournam, 2004; Simon, 2006). In the phase III trial, an enrichment design was used and here, 469 HER2 positive patients were randomized to receive either trastuzumab plus chemotherapy or chemotherapy alone. However, if an all-comers trial design had been used instead, without testing for HER2 positivity, the calculations showed that the number of patients needed to be enrolled would have been 8050 in order to demonstrate the same statistically significant difference between the two treatment arms as in the original phase III trial. This would have been 17.2 times more patients and shows how important the clinical enrichment trial design was for the development of trastuzumab, as it has been for a number of other target cancer drugs developed over the past more than 20 years (Skoulidis et al., 2021).

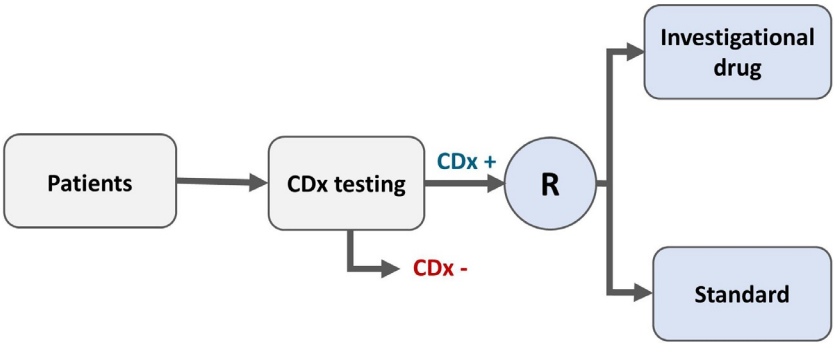

Fig. 2 The randomized enrichment trial design. All test positive patients are randomized to either of the two treatment arms. CDx + = test-positive; CDx − = test-negative; R = randomization.

Until around 2010, the randomized enrichment trial design was the dominating design in documenting the clinical efficacy of drug-CDx combinations and clinical validation of the assay. However, in the past decade, an even simpler trial design has been introduced; in fact, it is as simple as it possibly can be. This is the nonrandomized enrichment trial design with no comparator or randomization, as shown in Fig. 3. Within the past 10 years, 15–20 targeted drugs and corresponding CDx assays have obtained regulatory approval based on efficacy data from this simple trial design.

An example of a drug recently approved based on clinical efficacy documentation from a nonrandomized enrichment trial is sotorasib (Lumakras, Amgen), which is an inhibitor of the RAS GTPase family targeting the specific the KRAS *G12C*-mutated protein (Skoulidis et al., 2021). To demonstrate the efficacy of sotorasib in *KRAS G12C*-mutated metastatic NSCLC, data from 124 patients enrolled in a single-arm, open-label, multicenter trial were analyzed. Before enrollment in the trial, tumors from the patients were tested locally for *KRAS G12C* mutation, which was subsequently confirmed in a central laboratory using the therascreen *KRAS* RGQ PCR Kit (Qiagen). In May 2021, sotorasib was approved, under the provisions of the accelerated approval regulations, for patients with *KRAS G12C*-mutated locally advanced or metastatic NSCLC, as determined by an FDA-approved test. Along with the approval of sotorasib, the therascreen *KRAS* RGQ PCR Kit was approved as a CDx assay (Food and Drug Administration, 2021a).

For decades, cancers have been classified according to the organ from where the tumor arises. The rationale behind this classification was that the origin of the tumor was closely linked to its biological behavior, and hence, this characteristic could be used to guide the selection of an optimal therapy (Jørgensen, 2020; Raze and Santos, 2018). However, in most cases, this has not been the case, and the past decades have taught us that cancer is a group of heterogeneous diseases where complex molecular mechanisms play a key role. The development within molecular medicine has enabled us to study these mechanisms, which has fostered a greater understanding of the pathogenesis that drives the disease. The increased molecular understanding of the pathophysiology has slowly changed the way cancers are classified and therapies are developed. Many cancers can now be divided into subgroups based on their molecular characteristics, which is also reflected in the way drugs are developed using the drug-diagnostic codevelopment model. With the approval of larotrectinib, the use of molecular diagnostics for the diagnosis and treatment of cancer has entered a new era. In 2018, larotrectinib, a selective tropomyosin receptor kinase (TRK) inhibitor, was approved for the treatment of patients with solid tumors harboring neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion. Clinical trials with larotrectinib have shown that its efficacy is independent of tumor type and site, and the drug has demonstrated efficacy across a number of different traditionally defined cancer diseases (Drilon et al., 2018; Hong et al., 2020a). A drug like larotrectinib has been termed tumor- or site-agnostic, and for documenting the clinical efficacy of such a drug, a specific type of enrichment design is used, which is the basket trial design shown in Fig. 4.

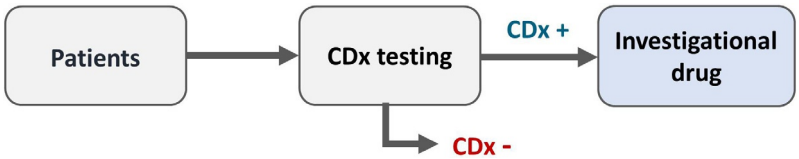

Fig. 3 The nonrandomized enrichment trial design. Alle test positive patients are treated with the investigational drug. CDx + = test-positive; CDx - = test-negative.

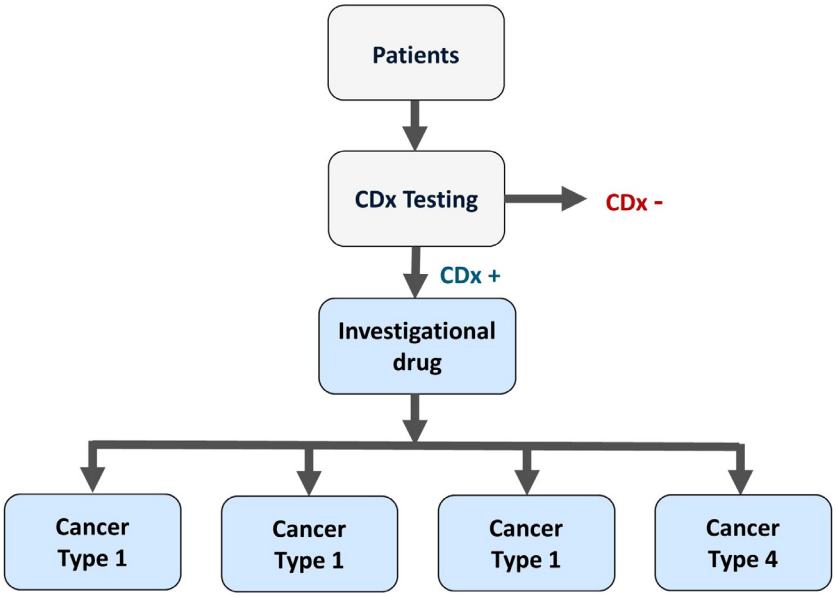

Fig. 4 The basket enrichment trial design. All test positive patients are treated with the same investigational drug independently of tumor type. CDx + = test-positive; CDx – = test-negative.

Unlike the traditional enrichment trial, which focuses on a single tumor type, the basket trial included patients with different tumors. However, a common denominator for these tumors is that they all harbor the same molecular aberration, and all patients will need to be tested positive before they can be enrolled in the trial, as shown in Fig. 4. In essence, a basket trial can be seen as a set of parallel sub-trials that share the same overall concept and design, and where the outcome data can be analyzed as a whole as well as stratified according to tumor type (Jørgensen, 2020). A typical question asked in a basket trial is whether the efficacy of the targeted drug differs by tumor type, and if so, in which tumor type the drug works (Cunanan et al., 2017). Initially, larotrectinib was approved by the FDA based on 55 patients with *NTRK* gene fusion-positive tumors with an overall ORR (95% CI) of 75% (61%, 85%) across 12 different classically defined tumor types, and recently, a larger cohort of patients based on data from 153 patients has been published in *Lancet Oncology* (Hong et al., 2020a). This analysis showed that 121 of 153 patients responded to treatment with larotrectinib, which corresponds to an overall ORR (95% CI) of 79% (72%, 85%) across the different tumor types. In particular, patients with different types of soft tissue sarcomas seem to respond particularly well to the treatment, which was also the largest group included in the analysis. The recent published data confirmed the efficacy data submitted to the FDA in 2018 in relation to the approval of the drug. Fig. 5 shows the different *NTRK* gene-positive tumor types included in the analyses. The FoundationOne CDx (Foundation Medicine) assay is a CDx assay approved by the FDA for detecting *NTRK* gene fusions in patients with solid tumors who may benefit from treatment with larotrectinib (Food and Drug Administration, 2021a).

Bridging studies

As previously briefly discussed, a bridging study will be required if a prototype assay or a CTA has been used to select patients in a pivotal clinical trial inserted of the final analytical validated version of the CDx assay (Food and Drug Administration, 2016). The aim of a bridging study is to demonstrate that the analytically validated assay has performance characteristics that are very similar to those of the prototype assay or CTA. In such a study, preferably the original pivotal clinical trial samples should be used for the comparison between the two assays, both with respect to analytical concordance as well as clinical outcome data. A reanalysis of the primary outcome must be performed to ensure that the use of the new assay does not alter the overall conclusion regarding the safety and efficacy of the drug in the selected patient population. If not all samples are available from the original pivotal clinical trial, it is important to document that the subset is representative of the overall trial with respect to sample characteristics and patient demographic data (Ellison and Stanforth, 2019).

The FoundationOne CDx assay is an NGS laboratory-developed test designed to detect variations in 324 genes. This assay has been approved by the FDA as a CDx assay for the detection of genetic aberrations in patients who may benefit from one of twenty-five different FDA-approved targeted drugs, including the tumor-agnostic drug larotrectinib, as mentioned earlier (Jenkins et al., 2019; Food and Drug Administration, 2020a). The approval of the FoundationOne CDx as CDx assay for these drugs were often based on both analytical concordance studies as well as a reanalysis of clinical outcome data.

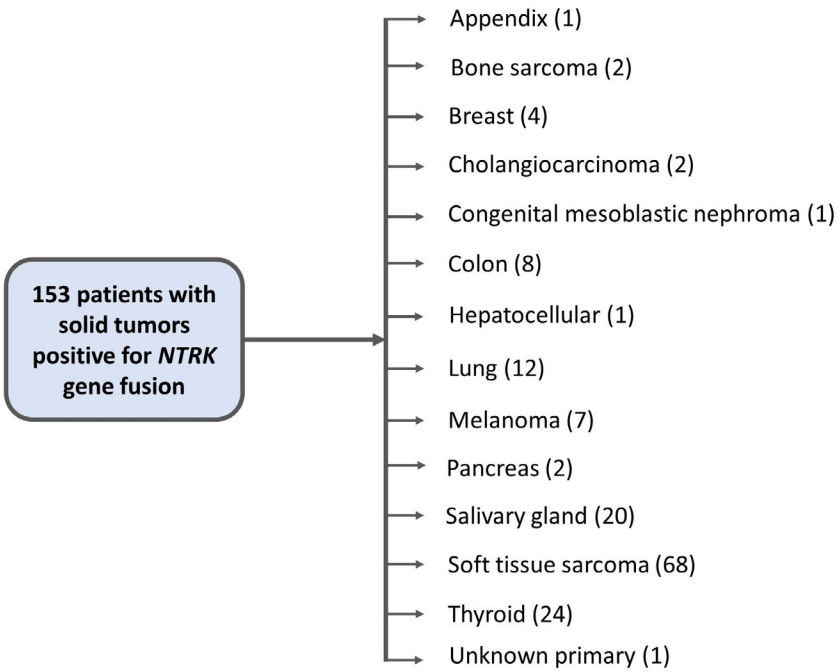

Fig. 5 The analysis published in *Lancet Oncology* demonstrated efficacy of the tumor-agnostic drug larotrectinib across a range of different tumor types with an ORR of 79% (Hong et al., 2020a). The figures in brackets after the tumor type is the number of patients included in the analysis with the specific tumor type.

Osimertinib

The small-molecule inhibitor osimertinib (Tagrisso, AstraZeneca) is an example of a drug that would probably not have been developed had it not been for the simultaneous development of a CDx assay. Osimertinib is a third-generation EGFR tyrosine kinase inhibitor (TKI), which was originally developed for the treatment of NSCLC patients who had developed resistance to first- and second-generation inhibitors, whose tumors harbor an *EGFR* T790M mutation (Jørgensen, 2019c). A flowchart showing the timelines for the codevelopment of osimertinib and the PCR-based CDx assay for selection of patients with T790M mutations is shown in Fig. 6.

The approval of osimertinib was based on efficacy data from two single-arm open-label phase II enrichment trials, which included a total of 411 metastatic NSCLC patients with *EGFR* T790M mutation (Food and Drug Administration, 2015). In Study 1 (N=201) the ORR (95% CI) was 57% (50%, 64%) and in Study 2 (N=210) it was 61% (54%, 68%). In both trials, the patients were prospectively selected based on the presence of a positive *EGFR* T790M mutation status using a PCR assay (cobas *EGFR* mutation Test (Roche Molecular Systems)). In November 2015, osimertinib and the cobas *EGFR* mutation Test were contemporaneously approved by the FDA based on accelerated approval (Jenkins et al., 2019). The development of osimertinib demonstrated the effectiveness of the drug-diagnostic codevelopment model. The first patient dosed in the phase I trial was in March 2013, and only 27 months later was AstraZeneca ready to submit an NDA for the first osimertinib indication, as shown in Fig. 6. Osimertinib has subsequently been approved for both adjuvant therapy and first-line treatment of adult patients with metastatic NSCLC whose tumors have *EGFR* exon 19 deletions or exon 21 L858R mutations using the cobas *EGFR* mutation Test as a CDx assay (Jenkins et al., 2019).

Regulatory requirements

The past decades' changes in drug development with the use of predictive biomarkers have also been reflected in the regulatory requirements for both drugs and diagnostics. Over the past 20 years, the drug-diagnostic codevelopment has played an increasing role, mainly in hematology and oncology, where an increasing number of drugs have been developed together with a CDx assay. This has resulted in a growing number of discussion papers and guidance documents issued by the regulators in the USA, the European Union (EU), Japan, and other countries. In particular, the FDA have been at the forefront of discussions and implementation of regulatory strategies for CDx assays and drug-diagnostic codevelopment (Jørgensen, 2019c).

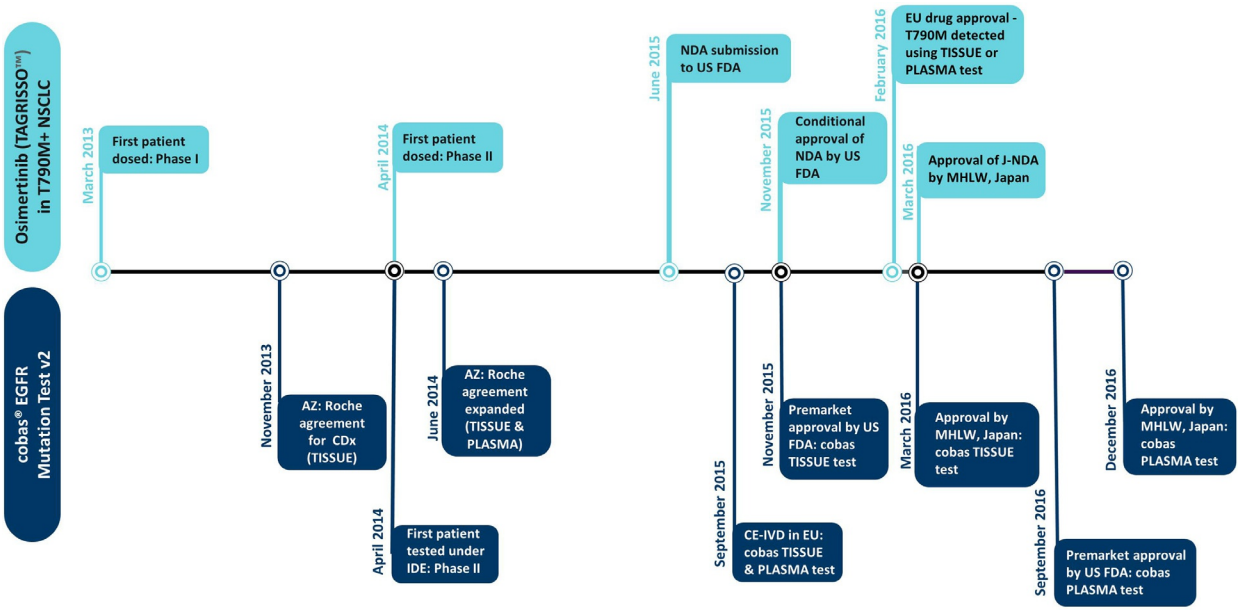

Fig. 6 Timelines for the codevelopment of osimertinib and its CDx assay for the treatment of NSCLC patients with *EGFR* T790M mutation positive tumors (Jenkins et al., 2019). Reprinted from Jenkins S et al. (2019) Osimertinib (Tagrisso) and the cobas EGFR Mutation Test v2. In Jørgensen JT (eds.), Companion and Complementary Diagnostics. London: Academic Press/Elsevier, with permission from Elsevier.

The Food and Drug Administration

In 1998, trastuzumab together with the HercepTest, obtained regulatory approval for treatment of patients with HER2 positive metastatic breast cancer in the USA. This approval was granted through a new coordinated process between the FDA's Center for Devices and Radiological Health and the Center for Biologics Evaluation and Research. The HercepTest assay became the first ever regulatory approved CDx (Jørgensen et al., 2021). Since then, the number of FDA-approved CDx assays have increased considerably and at the end of 2021, the number has reached 48, representing different assays technologies and platforms (Food and Drug Administration, 2021a).

The FDA have a long tradition of regulating in vitro diagnostic tests, which dates back to the passage of the Medical Device Regulation Act in 1976 (Roscoe et al., 2015; Scherf et al., 2010). This has influenced the regulation of CDx assays, despite the fact that the HercepTest was approved only a couple of decades later. Another important event that influenced the regulations for CDx assays was the publication of the *Drug-Diagnostic Co-Development Concept Paper* by the FDA in 2005 (Hinman et al., 2006). This paper describes the preliminary thoughts of FDA on how to co-develop drugs and diagnostic tests. The intention of this document was not to serve as a guideline, but more as a discussion paper reflecting perspectives under consideration by the FDA at that time. This concept paper addressed four key areas related to CDx development: analytical assay validation, clinical assay validation, clinical assay utility, and labeling. This concept paper has served as an important source of inspiration for discussions related to drug-diagnostic codevelopment and the development of regulatory requirements for CDx assays. The intention of the FDA was that this concept paper should be followed by guidance documents on drug-diagnostic codevelopment. However, it took nearly 10 years before the first guidance document on In Vitro *Companion Diagnostic Devices* was issued and a couple of years later, the FDA issued a draft guidance on the *Principles for Codevelopment of an* In Vitro *Companion Diagnostic Device with a Therapeutic Product*, which could be regarded as a kind of equivalent to the concept paper from 2005 (Food and Drug Administration, 2014, 2016).

As discussed previously, CDx assays play a central role as important treatment decision tools for a number of hematological and oncological drugs, which is also reflected in the FDA classification of these assays. Most CDx assays are high-risk devices and are classified as Class III, which requires submission of a PMA. Compared to other types of in vitro diagnostic (IVD) submissions, such as a 510(k), the PMA requires the most comprehensive documentation level, which further underlines the critical role of CDx assays (Jørgensen, 2021b). Submission of a PMA has also been the situation for nearly all FDA-approved CDx assays, except for two. These are the MRDx BCR-ABL Test (MolecularMD Corporation) and FerriScan (Resonance Health Analysis Services Pty), which are both Class II devices. Furthermore, two CDx assays, KIT D816V Mutation Detection by PCR for Gleevec Eligibility (ARUP Laboratories) and PDGFRB FISH for Gleevec Eligibility (ARUP Laboratories), have both been approved through a humanitarian device exemption (HDE) application (Food and Drug Administration, 2021a). Approval through the HDE program follows a similar thinking as known from the Orphan Drug Act and is reserved for rare diseases or conditions with a low patient prevalence (Food and Drug Administration, 2019).

Over the years, the US FDA have issued several important guidance documents describing the requirements for the development of CDx assays, a couple of these have already been mentioned in this chapter. In 2014, the guidance document on In Vitro *Companion Diagnostic Devices* was issued, in which a CDx assay was regulatory defined for the first time (Food and Drug Administration, 2014). Furthermore, this guidance document outlined a number of administrative procedures for the development of a therapeutic product for which a CDx assay is evaluated to be essential. A couple of years later, in 2016, the draft guidance on the *Principles for Codevelopment of an* In Vitro *Companion Diagnostic Device with a Therapeutic Product* was issued (Food and Drug Administration, 2016). This guidance document aims in a practical manner to provide information on the design and implementation of a successful drug-diagnostic codevelopment program. Over the last decade, cancer drugs have been developed for smaller and smaller molecular-defined subsets of patients. This is often the case in tumor-agnostic drug development, as for larotrectinib, where a drug is effective across multiple tumor types that harbor a specific molecular aberration; however, often at a very low frequency. The *NTRK* gene fusion, which is required for the initiation of treatment with larotrectinib, is found only in approximately 1% of all patients with solid tumors (Jørgensen, 2020). By the end of 2018, a guidance document on *Developing Targeted Therapies in Low-Frequency Molecular Subsets of a Disease* was issued by the FDA, describing the approach for the evaluation of targeted therapies in small groups of patients (Food and Drug Administration, 2018b). In 2020, the FDA issued a guidance document on *Developing and Labeling* In vitro *Companion Diagnostic Devices for a Specific Group of Oncology Therapeutic Products*, which changed the previous policy regarding the use of CDx assays (Food and Drug Administration, 2020b). With this guidance document, the FDA made it possible to broaden the labeling claims for a CDx assay to cover not one drug but a specific group of drugs if certain requirements were fulfilled. An example could be a CDx assay that identifies NSCLC patients with tumors harboring the most common *EGFR* mutations, such as exon 19 deletions or exon 21 (L858R) substitution mutations. When the FDA recently approved the ONCO/Reveal Dx Lung & Colon Cancer Assay (Pillar Biosciences), it was stated that the assay is intended as a CDx to identify patients with NSCLC or Colorectal Cancer (CRC) who may benefit from treatment with a number of targeted therapies in accordance with these drugs approved therapeutic product labeling. This list for CRC includes the drugs cetuximab (Erbitux, Eli Lilly and Co) and panitumumab (Vectibix, Amgen), and for NSCLC it covers all EGFR TKI approved by the FDA. With regard to the approval of the ONCO/Reveal Dx Lung & Colon Cancer Assay, no prospective clinical data were presented but only data from concordance studies with relevant FDA-approved CDx assays (Food and Drug Administration, 2021b). As previously mentioned, no official FDA definition exists for CoDx, and consequently, no guidance documents are available so far.

Drug-diagnostic codevelopment is an integrated process involving different scientific and medical disciplines, why the above-mentioned guidance documents have all been issued based on a cooperation between the different centers within the FDA. Most of the guidance documents mentioned in this chapter were developed based on inputs from the Center for Biologics Evaluation and Research, the Center for Drug Evaluation and Research, the Center for Devices and Radiological Health, and the Oncology Center of Excellence.

The European Union and other countries

So far, the EU has adopted a very different approach to regulating CDx assays. Until May 2022, the regulatory framework for CDx assays in the EU was the IVD Directive 98/79/EC issued in 1998 (European Parliament, 1998). However, this directive did not mention CDx assays in the definition of an IVD, and likewise the risk classification system did not consider this type of assay. Until May 2022, any CDx assay developed for the EU were classified as general IVD, which means low-risk devices. Through the so-called self-certification procedure, the manufacturer performed a conformity assessment where they stated that the essential requirements of the IVD Directive were fulfilled, after which they could CE-marked their CDx assay. This conformity assessment was without any direct involvement and control of the national competent authorities in the EU counties. Compared with the PMA approval process in the US, the EU self-certification procedure must be regarded as a very different regulatory path, which does not consider the critical role of these types of assays in the treatment of individual patients (Jørgensen and Hersom, 2018).

However, the situation will soon change. In April 2017, the European Commission and the European Parliament adopted new regulations on IVD medical devices (EU/2017/746), which is more up-to-date with the current understanding of the role of CDx assays (European Parliament, 2017; Ritzhaupt et al., 2020). Originally, the new IVD Regulation was planned to enter into force in May 2022, but because of the extraordinary circumstances mainly caused by the COVID-19 pandemic, the European Commission has partly postponed the effective date for the CDx part to May 2026 (European Parliament, 2021). When the regulation is fully implemented, all CDx assays used in the EU must comply with the new requirements, which means a number of substantial changes, including a new risk-based classification system that considers patient impact and safety more seriously. CDx assays are no longer considered to be low-risk devices. According to the new IVD regulation, these assays are now classified as class C, which means a high individual risk or moderate public health risk, where an erroneous result would put the patient in an imminent life-threatening situation or would have a major negative impact on the outcome (Pignatti et al., 2014). Another major change is that self-certification is no longer possible. According to the new IVD Regulation, it is required that the conformity assessment must be performed by an independent notified body and that this notified body also has to consult the EMA or one of the national competent authorities. Furthermore, the general requirements for both the technical and clinical performance documentation, as well as post-market surveillance activities have been strengthened (Jørgensen and Hersom, 2018; European Parliament, 2017). CoDx assays are not mentioned in the new IVD regulations and no guidance documents have been issued for this type of assay. Table 1 provides a brief overview of the regulations for CDx and CoDx in the EU and the USA.

The EU is not the only region that has strengthened and updated its requirements for CDx. Several countries have recently issued regulatory guidance documents. In 2013, the Pharmaceutical and Medical Devices Agency of Japan issued a guidance document on drug-diagnostic codevelopment (Pharmaceuticals and Medical Devices Agency, 2013). Likewise, in recent years, other countries, such as Canada and Australia, have also issued different guidance documents to support the development of CDx and the concept of drug-diagnostic codevelopment (Craig, 2017).

FDA-approved CDx-drug combinations

Since the turn of the century, the number of FDA-approved drug-CDx combinations in hematology and oncology has steadily increased (Fig. 7). Especially within the last decade, the development has taken off, and by the end of 2021, the number of targeted drugs approved with a CDx assay by the FDA has reached 48. These drugs and their CDx biomarkers are listed in Table 2 and can be roughly divided into two classes: small-molecule inhibitors and antibody-based compounds (Food and Drug Administration, 2021a, 2022b). The small-molecule inhibitors are by far the largest group and make up more than 75% of all targeted cancer drugs that have a CDx linked to their use. These drugs have diverse mechanisms of action; however, the majority can be classified as tyrosine kinase inhibitors, but here, we also find PARP inhibitors, IDH inhibitors, methyltransferase inhibitors etc. The second group is the antibody-based drugs and here, the major part is the monoclonal antibodies, but among this group, we also find the antibody drug conjugate ado-trastuzumab emtansine (Kadcyla, Roche/Genentech) and the recently approved bispecific antibody amivantamab (Rybrevant, Janssen Biotech) (Jørgensen, 2021c).

Several drugs listed in Table 2 are approved for more than one indication, which requires the use of different CDx assays. This is the case for crizotinib (Xalkori, Pfizer), imatinib (Gleevec, Novartis), pembrolizumab (Keytruda, Merck & Co.), and olaparib (Lynparza, AstraZeneca). As an example, crizotinib, an inhibitor of ALK, ROS1, and c-Met receptor tyrosine kinases, which is approved for treatment of patients with metastatic NSCLC whose tumors are ALK/*ALK* or *ROS1*-positive. Currently, three CDx assays are available and linked to the use of crizotinib for these two indications: the Vysis *ALK* Break Apart FISH Probe Kit (Abbott Molecular), the Ventana ALK (D5F3) CDx Assay (Ventana Medical Systems), and the Oncomine Dx Target Test (Life Technologies Corporation) (Food and Drug Administration, 2021a; Jørgensen, 2021c).

Table 1 Brief overview on companion (CDx) and complementary diagnostics (CoDx) regulations in the USA and the EU.

	United States of America	*European Union*
Definition CDx	An IVD device that provides information that is essential for the safe and effective use of a corresponding therapeutic product	A device which is essential for the safe and effective use of a corresponding medicinal product
Definition CoDx	An IVD device not essential for the safe and effective use of the drug, but the device identifies a biomarker-defined subset of patients that responds differentially to a drug and aids in the risk/benefit assessment for individual patients[a]	NA
Risk Classification CDx	Class III[b]	Class C
Risk Classification CoDx	Class III[b]	NA
CDx Information in Drug Labeling	Yes[c]	No[d]
CoDx Information in Drug Labeling	No	No

[a]Preliminary FDA definition (Beaver et al., 2017).
[b]Two FDA-approved CDx assays MRDx BCR-ABL Test and FerriScan) are Class II devices (Jørgensen, 2021b).
[c]Patients must be selected based on an FDA-approved companion diagnostic.
[d]For a few recent EMA-approved drugs, it is stated that biomarker status should be assessed by a CE-marked IVD medical device.

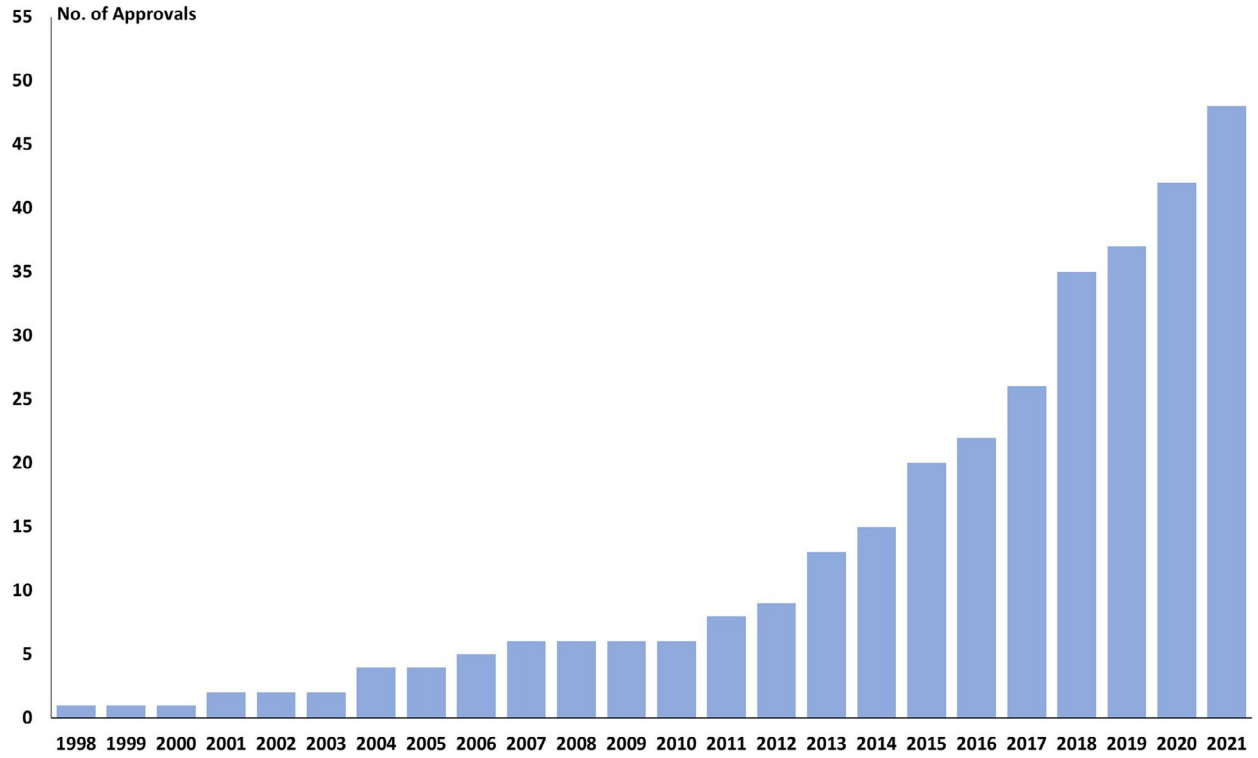

Fig. 7 The cumulative number of FDA-approved drug-CDx combinations by year. The total number of drugs that have a CDx assay linked to their use was 48 by the end of 2021.

In 2021, several interesting drug-CDx combinations were approved by the FDA, one of which was amivantamab along with the NGS-based Guardant360 CDx assay (Guardant Health), as listed in Table 2. Amivantamab is a bispecific antibody targeting both EGFR and MET and is indicated for treatment of metastatic NSCLC patients with *EGFR* exon 20 insertion mutations (Food and Drug Administration, 2021a; Park et al., 2021). Another drug approved in 2021 was sotorasib (Lumakras, Amgen), an RAS GTPase inhibitor indicated for treatment of patients with *KRAS G12C*-mutated NSCLC. *KRAS* mutations are often associated with resistance to targeted therapies, and sotorasib is the first in the class of drugs targeting KRAS (Food and Drug Administration, 2021a; Hong et al., 2020a). Along with sotorasib, the FDA approved two CDx assays, the Qiagen therascreen *KRAS* RGQ PCR kit and the Guardant360 CDx assay. The Qiagen CDx assay is for analysis of tissue specimens, whereas the assay from Guardant Health is for analysis of liquid biopsies (plasma samples). The use of liquid biopsies is more convenient to the patients and the health care professionals, but it also carries the risk of false-negative test results (Rolfo et al., 2018). This is why the FDA requires testing based on tumor tissue if the test result based on a liquid biopsy is negative. The reflex testing on the tumor specimen must then performed with the Qiagen therascreen *KRAS* RGQ PCR kit. (Food and Drug Administration, 2021a, Jørgensen, 2021c).

Table 2 List of FDA-approved drugs that have a CDx assay linked to their use (Food and Drug Administration, 2021a).

Antibody Based Drugs	*CDx Biomarker*
Amivantamab	*EGFR*
Trastuzumab, Pertuzumab, Ado-trastuzumab emtansine	HER2/*HER2*
Dostarlimab	MMR
Atezolizumab, Cemiplimab, Nivolumab, Pembrolizumab	PD-L1
Cetuximab, Panitumumab	*RAS (KRAS/NRAS)*/EGFR
Pembrolizumab	TMB-H
Small Molecule Inhibitors	*CDx Biomarker*
Alectinib, Brigatinib, Ceritinib, Crizotinib, Lorlatinib	ALK/*ALK*
Nilotinib	*BCR-ABL1*
Binimetinib, Cobimetinib, Dabrafenib, Encorafenib, Trametinib, Vemurafenib	*BRAF V600E or V600K*
Niraparib, Olaparib, Rucaparib, Talazoparib	*BRCA1/BRCA2*
Afatinib, Dacomitinib, Erlotinib, Gefitinib, Osimertinib	*EGFR*
Tazemetostat	*EZH2*
Pemigatinib, Infigratinib	*FGFR2*
Erdafitinib	*FGFR2 or FGFR3*
Midostaurin, Gilteritinib	*FLT3*
Olaparib	*HRR*
Ivosidenib	*IDH1*
Enasidenib	*IDH2*
Abemaciclib	Ki-67
Sotorasib	*KRAS G12C*
Imatinib	c-KIT/*KIT*, *PDGFRB*
Capmatinib	*MET*
Larotrectinib	*NTRK1/2/3*
Alpelisib	*PIK3CA*
Pralsetinib	*RET*
Crizotinib	*ROS1*
Venetoclax	*TP53*

Since 1998, the FDA have approved 48 CDx assays based on several different analytical platforms. Until 2011, the dominant technologies were IHC and in situ hybridization (ISH), when the first CDx assay based on a PCR platform was approved, namely the cobas 4800 *BRAF V600* Mutation Test (Roche Molecular Systems). This assay is used to detect the *BRAF V600E* mutation in patients with melanoma who might be candidates for treatment with vemurafenib (Zelboraf, Roche/Genentech) (Jørgensen, 2021b). To date, the PCR technology makes up the largest group of CDx assays, as shown in Fig. 8. A total of 16 assays based on this technology have obtained regulatory approval, corresponding to 33% of all FDA-approved CDx assays (Food and Drug Administration, 2021a).

In the last 6 years, NGS has also been used as a CDx platform. By the end of 2016, the first assay based on this technology was approved by the FDA, which was the FoundationFocus CDx*BRCA* Assay (Foundation Medicine). It is a laboratory-developed assay for the detection of *BRCA1* and *BRCA2* alterations in tumor tissue from patients with ovarian cancer, who might be candidates for treatment with rucaparib (Rubraca, Clovis Oncology) (Food and Drug Administration, 2021a), Jørgensen, 2021b). Currently, eight different NGS-based assays have been approved by the FDA as CDx assays, as shown in Fig. 8.

Future direction for CDx assays

Within the past 20 years, significant progress has been made in the treatment of hematological and oncological patients using different types of targeted drug therapies, most often guided by a CDx assay (Jørgensen, 2019a). When looking at the pipeline of the different pharmaceutical and biotech companies, it is a development that will most likely continue, and there are several good reasons why such a strategy is worth pursuing. By investigating data from different public databases, several studies have shown that using CDx assays in clinical drug development increases the probability of a successful outcome (Li et al., 2021; Lara Gongora et al., 2020; Parker et al., 2021). It has been shown both for hematological and oncological drugs that the biomarker-based enrichment trial design improves the success rate severalfold compared to a traditional all comers design (Lara Gongora et al., 2020; Rodriguez et al., 2021). The drug-diagnostic codevelopment strategy also seems to reduce both the cost and time spent on clinical development (Lara Gongora et al., 2020, Parker et al., 2021).

When it comes to CDx assays, our main focus has so far been on genomic biomarkers. As shown in Fig. 8, approximately 70% of all CDx assays are genomic-based; however, changes may be underway. Despite an initial high response rate and improved progression free survival in patients treated with targeted therapy, we see development of resistance. If we should try to understand

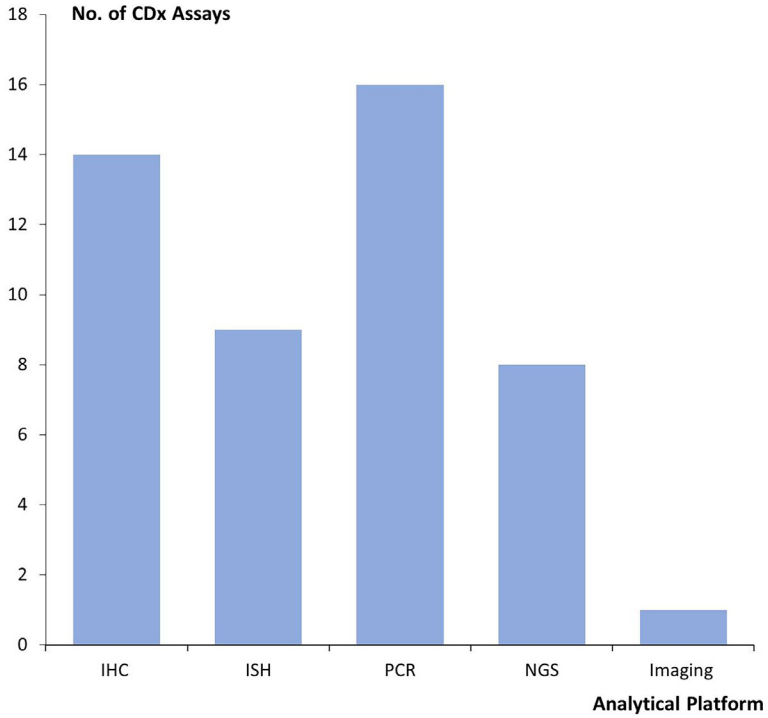

Fig. 8 FDA-approved CDx assays by analytical platform at the end of 2021 (*N*=48). IHC, immunohistochemistry; ISH, in situ hybridization; PCR, polymerase chain reaction; NGS, next generation sequencing.

these mechanisms and further improve the treatment for patients with cancer, we will likely need to integrate proteomics to a much larger extent than today when we are developing new CDx assays. In a paper recently published in *Cell* by a group of researchers from the National Cancer Institute in the US, the integration of proteomics with genomics was suggested, which they named 'proteogenomics' (Rodriguez et al., 2021). The reason for this integration is that proteins and their post-translational modifications add an extra layer of complexity that is hidden from genomics and furthermore, the proteins are targets for most of our pharmacological interventions. Hopefully, such an integration may be able to narrow down the gap between genotype and phenotype and enable us to obtain a more complete picture of the cancer biology, leading to improved treatment options.

The increased focus on proteomics will likely mean that we will see the first CDx assay based on mass spectrometry being introduced within the years to come (Blackler and Duncan, 2019). Furthermore, in relation to proteomics, IHC has been used for decades and is still widely used, as shown in Fig. 8, which is a technology that will continue to be developed and improved. We will see more multiplex assays being introduced and likely linked to different types of image analysis (IA) and artificial intelligence (AI), which will ease the interpretation of the stained tissue slides. IHC is often hampered by both relatively large intra- and inter-observer variation, and the use of IA and AI will make this type of assay more robust and reproducible (Li et al., 2020). Such a combination could turn IHC into a strong technology, which will also be important in relation to improving several of the current CDx assays used, such as the HER2 and PD-L1 expression assays, used in relation to the treatment of patients with different HER2 inhibitors and immune checkpoint inhibitors.

Despite the increased focus on proteomics, CDx assays based on NGS will continue to play a very central role. Eight different NGS assays have already been approved as CDx, and we will continue to see their panels be updated to include an increasing number of targeted drugs. The FoundationOne CDx and the FoundationOne Liquid CDx assays (Foundation Medicine), are laboratory-developed NGS assays approved by the FDA for use as a CDx for a number different targeted drugs, both in relation to tissue samples as well as liquid biopsies (Food and Drug Administration, 2021a). NGS assays developed for liquid biopsies and the detection of ctDNA have recently been approved as companion diagnostics. These types of assays have several advantages over tissue-based assays, but as previously described, there is an issue with sensitivity, which may result in false negative test results. The reason for this seems to be that some tumors do not shed sufficient amounts of DNA into the circulation to be detected by this technology (Rolfo et al., 2018). Even the most sensitive assays appear to achieve a maximum sensitivity of approximately 85% in advanced-stage disease, which must be expected to be somewhat lower in early-stage disease. For this reason, the FDA requires reflex testing on a tissue sample if a CDx assay based on a liquid biopsy result is a negative test result (Food and Drug Administration, 2021a). Despite the issue of false-negative test results, the use of this type of CDx assay will increase in the years to come owing to the convenience of using a blood sample over a tissue biopsy.

Cancer is a very complex and heterogeneous disease in which the detection of a single biomarker is not always sufficient to guide treatment. In the years to come, we will very likely see more complex CDx assays being developed that will be algorithm-based composite biomarkers with inputs from different sources, which will include both omics and non-omics data (Jørgensen and Hersom, 2018). Such an approach may help us further understand the complex disease mechanisms and hopefully enable us to optimize therapies for individual patient.

Conclusion

Predictive biomarkers and CDx assays are important elements in the realization of precision medicine, and the use of the drug-diagnostic codevelopment model has enabled us to development a number of targeted hematological and oncological drugs that match the needs of the individual patient. A CDx assay is not only important during clinical development, but just as important as a treatment decision tool when the drug is approved and used routinely in the clinic. In fact, many targeted drugs would lose their value without a CDx assay linked to their use. As the analyses in this chapter show, the number of drug-CDx combinations has increased substantially over the past decade, and this development is expected to continue in the years to come. We will see new types of CDx assays being developed that rely on data input from both omics and non-omics sources using artificial intelligence, which hopefully will lead to a further improvement in treatment of the individual patient.

Conflict of Interest Disclosure

Jan Trøst Jørgensen is an employee of the Dx-Rx Institute, and has worked as a consultant for Agilent Technologies, Alligator Biosciences, Argenx, Azanta, Biovica, Euro Diagnostica, Leo Pharma and Oncology Venture and has given lectures at meetings sponsored by AstraZeneca, Merck Sharp & Dohme, and Roche.

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