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Biomarkers improving decisions making in clinical trials: explanations and examples

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ABSTRACT

Biomarkers are originate from physiological processes, medical imaging, tissues, or chemicals. They are potentially useful indicators for every type of disease and have important roles on drug discovery and development, disease progression tracking, prognosis, diagnosis, and therapy response. Biomarkers provide precision measurement results, lower bias result, also faster early warning signal. Therefore, they can be used as a basis for clinical decisions. Biomarkers found in tissues, blood, and other bodily fluids. Depend on their purpose of usage, biomarkers can be categorized of diagnostic, prognostic to predicts disease progression, pharmacodynamic to measures the effect of a drug on a biological system, and predictive to predicts response to a specific treatment. In clinical trials, biomarker can be used as basic of decisions making. Therefore, several steps required to incorporate biomarkers in clinical trials are determine roles and functions of biomarkers, choose specific test and laboratory according to purpose of trial, describe test and protocol, carry out and report analysis validation appropriate for trial, implement test in trial, and plan sustainability of biomarker uses in future research. Biomarker have been used as basis of decision making in clinical trials in phase I-IV to recruit participants or making a decision whether the trial will be terminated or continued. In this review, we outlined general explanations about molecular biomarkers, step of biomarkers incorporation in clinical trial, and examples of several studies using molecular biomarkers in clinical trial as basis of decision-making.

ABSTRAK

Biomarker berasal dari proses fisiologis, pencitraan medis, jaringan, atau senyawa kimiawi dan dapat digunakan sebagai indikator berbagai macam jenis penyakit. Biomarker berperan penting pada penemuan dan pengembangan obat, memahami progresi penyakit, prognosis, diagnosis, dan memahami respon terhadap terapi. Biomarker digunakan karena dapat memberikan hasil pengukuran yang presis, rendah bias, dan memberi tanda awal yang lebih cepat. Oleh karena itu, biomarker dapat digunakan sebagai dasar pengambilan keputusan klinik. Biomarker dapat ditemukan di jaringam darah, dan cairan tubuh lain. Berdasarkan tujuan penggunaannya, biomarker dibedakan menjadi biomarker prognostik untuk memprediksi progresi penyakit, farmakodinamik untuk mengukur efek obat di sistem biologis, dan prediktif untuk memprediksi respon terhadap perlakuan tertentu. Pada uji klinik, biomarker dapat digunakan sebagai dasar pengambilan keputusan. Oleh karena itu, terdapat langkah untuk memasukkan biomarker ke uji klinik. Langkah-langkah tersebut antara lain menentukan peran dan fungsi biomarker, memilih metode pengujian dan laboratorium sesuai tujuan pengujian, mendeskripsikan jenis uji beserta protokol uji klinik, melakukan dan melaporkan analisis validasi biomarker yang sesuai untuk pengujian, dan merencanakan keberlanjutan penggunaan biomarker bagi penelitian lain di masa depan. Biomarker telah digunakan sebagai bahan pengambilan keputusan pada uji klinis tahap I-IV untuk menentukan peserta atau membuat keputusan jika uji klinis dilanjutkan atau dihentikan. Pada kajian pustaka ini, kami menjelaskan secara ringkas mengenai biomarker molekuler, tahapan penggunaan biomarker pada uji klinik beserta contoh penggunaan biomarker molekuler pada uji klinik sebagai dasar pengambilan keputusan.

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INTRODUCTION

The identification of medicinal chemicals useful for treating and managing disease conditions is part of a complex process known as drug discovery. Researchers usually discover new drugs by gaining a new understanding of how a disease develops, which allows them to create drugs with the aim of stopping or fighting the effects of the disease. Drug candidate identification, synthesis, characterization, screening, and clinical trials of therapeutic efficacy are all part of the drug discovery process.[1] Drug discovery is very costly for its research and development to clinical trials. From the time a new drug molecule is discovered until it can be used commercially for patient treatment, it takes about twelve to fifteen years to develop.[2]

Developing and designing new drugs through several stages of target validation, compound screening, lead optimization, preclinical, phase I - IV, and approval to launch. At the clinical trial stage, starting from phase I to phase III, it is necessary to determine the selecting respondents with the aim of improving early decision making in drug discovery.[3] Biomarker technology can help determine selecting respondents as early detection of disease. This is due to the effectiveness of research with the same diversity of respondents. Biomarker technology can also achieve the goal of precision medicine as one of the ways in drug development.

Precision medicine is also known as personalized medicine, by changing the patient's overall treatment strategy for the purpose of overcoming the problem of ineffective patient treatment to avoid increasing the cost of care.[4] Biomarkers are defined as characteristics that are measured as indicators of biological processes, pathogenic processes or biological responses due to exposure or therapeutic interventions.[5] Biomarkers can be used as early disease diagnosis to optimize decision making in clinical practice, on the other hand biomarkers are used as a "tool" to identify subgroups of patients with cancer genetic mutations and optimize drug dose adjustments.[6]

The development of more adaptive biomarkers helps understand the mechanism of action of drugs, select appropriate patients for clinical trials, monitor and predict toxicity issues, and aid in decision-making on drug regulation and development.[7] Biomarkers also help contribute to the development of more adaptive drugs with a rapid regulatory process, so the design of phase I, phase II, and phase III clinical trials that are typically used in drug development stages tend to become less important.[8] This results in regulatory strategies being readjusted with the aim of achieving improved drug safety and quality, significantly reducing development costs during research, and the approval process for new drug development.

Biomarkers are used for the purpose of stratification and enrichment of trials. Biomarker stratification is used to measure the cancer status of all patients and determine the treatment that matches their biomarker status or level. Biomarker enrichment of trials is used to select patients who are suitable for a given treatment and provide positive benefits based on specific biomarker criteria.[9] Both biomarkers can certainly be utilized in early decision making in drug development. This review article will discuss the types of biomarkers, examples of the application of biomarkers as the role of molecular biology in improving early decision making in drug discovery and the stages of drug development starting from preclinical to commercialized drugs.

DISCUSSION

History and Definition of Biomarkers

History

Although the word "biomarkers"

was first introduced in 1973, the phrases "chemical markers" (1949) and "biological markers" (1957) have been used previously.[10] When the word "surrogate" first appeared in the early 1980s, it meant "biomarker replacement.[11] Biomarkers linked to certain disease improvements are recommended as surrogate endpoints or surrogate indicators.[12] The Food and Drug Administration (FDA) states that biomarkers are quantifiable and potentially useful indicators for every type of disease and having important roles on drug research, disease progression tracking, prognosis, diagnosis, and therapy response.[10] Biomarkers may originate from physiological processes, medical imaging, tissues, or chemicals.

Clinical outcome assessment (COA) and biomarkers are different. The patient's physical state, emotional state, and chance of survival are the main focuses of COA. While there are various uses for biomarkers, one of them is the ability to forecast clinical outcomes; yet, gaining regulatory clearance for new medications depends on COA. Drug approval for marketing may be predicated on the validation of biomarkers. In situations where therapy is unavailable, the U.S. Food and medication Administration may utilize biomarkers to support accelerated medication approval.[13]

The development of biomarkers necessitates collaboration from other scientific disciplines for scientific validation. Biomarkers are evolving quickly due to data analysis and technological advancements. Pre-analytical and analytical validation are two of the stages in the biomarker development process.[10] Biomarker detection is currently guaranteed in accordance with sample quality analysis and standardization, which covers sample collection, storage, and measurement protocols.

The biomarker's performance, including the test's repeatability, reliability, and suitable degrees of specificity and sensitivity, is assessed during the clinical validation stage. The biomarker must receive regulatory permission and pass relevant agency inspection and evaluation before being used widely. Clinical advantages are shown at the last stage of development to indicate the utility of the biomarker in clinical practice.

The advantages of biomarkers

In the early phases of the drug development process, biomarkers are utilized in clinical practice to uncover signals/pathways of toxicity and efficacy as well as to customize therapy or healthcare.[14]

Biomarkers can help in prognosis, treatment, and diagnosis. Once the appropriate biomarkers are identified, there is a huge potential for improved clinical outcomes and therapy. Additionally, biomarkers will assist patients in receiving medications that are suitable for their previously thought-out treatment plan. As a result, less medication will be administered needlessly and the toxicity that results.[15]

The efficacy of biomarkers in illness diagnosis, disease staging, and therapy selection determines their usage in clinical settings. The primary attributes of biomarker usage, including sensitivity, specificity, accuracy, positive predictive value, and negative predictive value, must all be met. However, the benefits and drawbacks of the biomarker must be taken into account prior to application in a clinical context.[16]

Biomarkers that have been approved and those that are currently being tested may be classified based on their clinical application. The clinical applications are counterbalanced by the increasing in-corporation of biomarkers in the process of therapeutic development. Biomarkers may be classified into many categories, such as diagnostic, prognostic, pharma-codynamic, and predictive.[17]

TABLE 1. Advantages and disadvantages of biomarkers.

Advantage	Disadvantages
Precision measurement results	Biomarker sampling is have to done at the right time
Lower bias result	Cost of biomarker analysis is expensive
Faster early warning signal	Storage of biomarker samples to keep them valid
Biomarker validity can be used as a basis for clinical decisions	Use of biomarkers requires ethical considerations

TABLE 2. Categories of biomarkers based on their clinical uses

Type of biomarker	Function
Diagnostic biomarker	Detect the presence of a specific disease and act as an indicator of medical condition
Prognostic biomarker	Predicts the likelihood of disease progression
Pharmacodynamic biomarker	Measures the effect of a drug on a biological system
Predictive biomarker	Predicts individual's response to a specific treatment.

Types of Biomarkers

Biomarkers are biological molecules found in tissues, blood, and other bodily fluids that provides information about a biological state or condition for various purposes, such as detecting diseases, monitoring disease progression, predicting treatment response, evaluating the efficacy of drugs or interventions[18] that act as indicators of a biological state or process and are invaluable in various stages of healthcare to enable earlier diagnosis.[19] Biomarkers divided to four categories based on their clinical uses including diagnostic, prognostic, pharmacodynamic, and predictive (TABLE 2).[20] Some current clinical uses of biomarkers and their effect on development and application of cancer therapeutics briefly summarizes in this review.

Diagnostic biomarker is essential for early detection of cancer. This biomarker used to detect the presence of a specific disease and act as an indicator of medical condition in elevated levels of certain molecules, such as prostate-specific antigen (PSA) for prostate cancer, fecal occult blood test (FOBT) for colorectal cancer, or CA-125 for ovarian cancer,[19] and helps in detecting or confirming the presence of a disease or condition of interest. For example, PSA as a long used cancer diagnostic biomarker for prostate cancer and mammogram to detect abnormalities in breast tissue, potentially indicating breast cancer.[21] Diagnostic biomarker can classify patients into subtypes based on molecular characteristics,[20] and elevated levels of this diagnostic biomarker may suggest the presence of cancer, and thus can be used as a screening tool in healthy individuals or can support other diagnostic measures such as imaging and biopsy.[19]

Prognostic biomarker can predict the likelihood of disease progression, inform individualized treatment plans independent of therapy to anticipate and manage negative medication reactions.[22] For instance, CA-125 is often used as a monitoring biomarker for ovarian cancer, indicating how well the disease is responding to treatment and elevated

PSA levels in the blood might indicate a higher risk of developing prostate cancer. Certain biomarkers can forecast a patient's overall lifespan, regardless of the treatment. Some examples include CA 19-9 and CA 125 to predict overall survival rates in pancreatic ductal adenocarcinoma (PDAC) and ovarian cancer,[23,24] and carcinoembryonic antigen (CEA) in various cancers, including colorectal, lung, and pancreatic cancer.[19]

Pharmacodynamic biomarker measures the effect of a drug on a biological system, such as changes in gene expression or protein levels. Often used to evaluate the safety and efficacy of new drugs during clinical trials and measures the effect of a drug on a particular biological process that suggests whether a drug has reached its target and provides a cellular response.[20] For example, measures mitogen-activated protein kinase (MAPK) pathway inhibition (via pERK levels) in non-small-cell lung cancer (NSCLC) can indicate if the drug is directly interacting with its target.[25] With additional monitoring tumor cell proliferation markers (cyclin D1, Ki67) or tumor growth (via PET/CT scans) can be measured alongside pERK.[20] This combined monitoring helps assess drug effectiveness in trials and guides treatment decisions for individual patients. Ultimately, these advancements could allow for personalized drug dosing, minimizing side effects and ensuring optimal treatment for each patient.

Lastly, predictive biomarker predicts individual's response to a specific treatment. For instance, HER_2 testing can identify breast cancer patients who are more likely to benefit from a particular type of therapy.[26] Thus biomarkers suggest individuals who will respond best to a specific treatment. The development of predictive biomarkers alongside novel therapeutics to identify patients likely to benefit from a particular treatment or experience adverse effects referred to companion diagnostics.[20] An early example of this approach was using estrogen receptor assays to guide prescribing the drug tamoxifen. Since then, various companion diagnostics have emerged, such as measuring HER2 levels before prescribing pertuzumab[27] and assessing PD-L1 levels prior to pembrolizumab treatment as immunotherapy for various cancers.[28] These diagnostics categorized patients based on molecular biomarkers to enable more precise targeting of therapies, improved selection of patients for clinical trials, faster identification and development of effective drugs for personalized medicine. This interdependence between diagnostics and therapeutics is exemplified by the FDA's concurrent approval of vemurafenib and a companion assay detecting the V600E mutation targeted by the drug.[20] By better identifying eligible patients for clinical trials and rapidly pinpointing effective personalized treatments, companion diagnostics facilitate optimized drug development and precision medicine approaches.

Phases of Clinical Trials

The utilisation of clinical trials is imperative in the process of drug development as it enables the testing of novel therapeutic approaches and/or new medications on human subjects, thereby ensuring their efficacy and safety in authentic patient environments. A clinical trial is a study that is conducted in a methodical manner with the purpose of determining whether or not a medicine or technology is safe and effective in the treatment, prevention, or diagnosis of a disease or medical condition. Furthermore, clinical trials are undertaken with the aim of enhancing comprehension of the unfamiliar, testing a theory, and doing research relevant to public health. This process is mostly conducted by gathering the data and analysing it in order to draw conclusions.[29]

Pharmaceutical firms and other drug developers must do through preclinical assessments, outline the structure of

clinical trials, and officially submit this information together with a clinical strategy to regulatory authorities. Upon receiving approval from regulatory authorities, the suggested technique may proceed to the phase I clinical study, which involves testing on people for the first time. Each research has certain predetermined criteria for patient eligibility, determining who can or cannot participate.[30]

Phase 0 (micro-dosing studies) is the first phase of a clinical trial, followed by phases I, II, III. The first phase of the trial is referred to as the non-therapeutic phase, the third phase is known as the therapeutic confirmatory phase, and the fourth phase is referred to as the post-approval or post-marketing monitoring phase. Phase 0 and phase II are both considered exploratory trial phases.

Phase 0

In certain instances, such as cancer, a preliminary stage known as phase 0 may be initiated. This stage entails administering minuscule quantities of the novel medication to a restricted number of individuals, including patients. This study is conducted with the aim of expeditiously examining the potential mechanisms and effects of the medicine.[30] A micro-dose is defined as a dosage that is less than one hundredth of the NOAEL. Additionally, it is less than one hundredth of the pharmacologically active dose, which is measured in milligrams per kilogram for intravenous administration and milligrams per square meter for oral administration.[31]

Phase 0 techniques use *in vivo* human data to select preclinical candidates, leading to higher quality pharmaceuticals entering clinical development. This might potentially lead to lower attrition rates in subsequent stages of development. And, since this might be accomplished with less resources than conventional phase I procedures (for example, by allowing for the early termination of non-viable compounds), it may increase the number of medications that could reach clinical development.[32, 31]

Phase I

A small number of healthy volunteers or patients who are afflicted by a well-defined ailment (for example, infections or cancer) are often the subjects of phase I clinical trials, which are the initial stage in the process of developing a new therapy. In these kinds of studies, the major objective is to determine the maximum tolerated dose (MTD) of the new therapeutic agent, in addition to determining whether or not the candidate treatment is safe and whether or not it presents any adverse effects.[33] The safety and tolerability of the therapeutic agent are evaluated at this phase. Often, a single dosage is administered first, followed by short-term multi-dose trials. The evaluations are conducted on a limited number of healthy persons, often between twenty and eighty people. A number of other factors, including the dosage, are being explored.[30]

In addition, the phase I trial will provide information about the pharmacokinetics of the drug, which includes its absorption, distribution, metabolism, and elimination, as well as its pharmacodynamic features, which include its effects on the body's biochemical and physiological processes. All participants are aware of the medications and dosages that are being administered in these trials, which are carried out in a manner that is referred to as a "open label" model.[34] The high success rate of phase I trials is a testament to the efficacy of clinical transition testing in verifying the safety of a medicine, which is then verified in human testing in most circumstances. Despite the absence of established safety, the early human testing are designed to be gradual and careful, minimising the likelihood of injury.[29,34]

Phase II

Phase II studies are conducted to evaluate whether the new therapy has enough promising effectiveness to justify continued exploration in a large-scale randomized phase III trial, and to also analyse its safety. In this phase of studies commonly encompass a sample size of 100–500 patients, and they may be conducted across multiple institutions situated in diverse nations.[30,35] In the initial stage of phase II, which is referred to as phase IIa, the objective is to further refine the dosage that is necessary to deliver the therapeutic effect or monitored endpoints that are intended for the clinical candidate. Phase IIb studies may be started if the appropriate dosage levels have been established. The purpose of the phase IIb is to have a better understanding of the overall effectiveness of the candidate medications by testing them on a smaller group of participants.[30]

One of the most prevalent components of a phase II design is the inclusion of a control group that is not administered the medicine but rather gets a placebo. One of the usual practices that is connected to this is known as blinding the study, which means that patients are not informed about whether they are taking the treatment or a placebo.[34,30] It is also possible that the physician is not told about whether the test agent or the placebo is being delivered in many particular instances. The method in question is referred to be a double-blind study. In phase III, the data from the phase II study are utilized to choose a dosing regimen (amount, duration, and frequency) for extended trials in a wider sample of patients, with the goal that these studies will be definitive.[34]

Phase III

During phase III, the medication candidate's effectiveness is assessed in a more extensive group of patients. These studies are usually conducted with randomization and involve a large number of patients, ranging from 1,000 to 5,000, across multiple clinical trial centers. Their purpose is to determine the effectiveness of the candidate compound compared to the current standard of care or a placebo. Additionally, they investigate potential interactions with other medications and re-evaluate various doses to identify the optimal dose for maximum medication effectiveness.[30] Similar to phase II, the majority of phase III trials are conducted using randomization and blinding. These trials are said to be crucial in the industry since they will determine the success or failure of the medicine. The phase III trial design integrates both scientific and economic factors. When a medicine is very successful, a statistically significant beneficial impact may be shown with a relatively small number of patients.[34]

A new medicine application (NDA) is filed to the regulatory bodies after the phase III trial has been completed in order to establish that the medicine is both safe and effective. In order to better prove either the safety or the effectiveness of a product, regulatory evaluations may result in requests for further information or even demands for new clinical studies. In an ideal scenario, these assessments will result in regulatory clearance, which will include labelling requirements, as well as permission to market (review and approval).[30] In order for the medicine to be approved, it must possess sufficient pharmaceutical quality, therapeutic efficacy, and safety considerations. A good "risk-benefit ratio" is required for it to be considered. Priority is given to medications that provide significant clinical advancements in the treatment of an illness. On the other hand, the approval of regulatory agencies does not always mean that clinical studies are finished.[34,30]

Step of Biomarkers Incorporation in Clinical Trials

Basically, biomarkers can be used as decision-making material in early phase (phase 0-II) and late phase (phase III-IV) clinical trials. The use of biomarkers in late-stage clinical trials is recommended because they have gone through analytical validation studies, so the biomarkers used in late-stage clinical trials have been well characterized and have been used for a long time. For example, the use of kidney biomarkers (e.g., serum creatinine, creatinine clearance, cystatin C). In late-stage clinical trials, biomarkers are used to measure drug safety, as proof of concept to understand disease activity and disease burden, and to determine surrogate end points. Surrogate endpoints are laboratory measures or physical signs used in clinical trials as a substitute for clinically meaningful endpoints such as pain scales, function, or survival rates as an effect of therapy.[36]

Biomarkers can be used for decision making in early stage clinical trials. However, the use of biomarkers in the early stages has problems, namely the limited initial information regarding therapy obtained from clinical trials.[37] Therefore, the following steps (FIGURE 1) were taken to maintain the validity of the use of biomarkers in early-stage clinical trials.

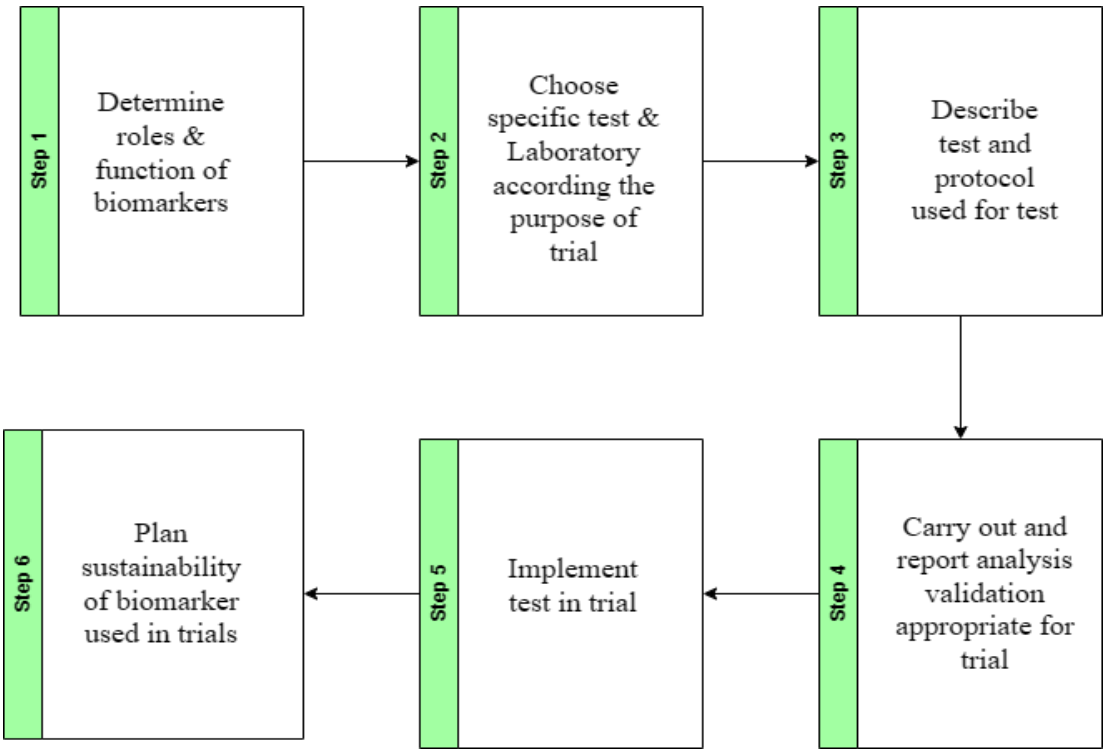

FIGURE 1. Steps of incorporating biomarkers in early phase clinical trial

The first step of incorporating biomarkers in clinical trial is determining roles & functions of biomarker in clinical trial. First, researchers have to decide whether the biomarker will be integral or integrated. Integral biomarker is essential for the research and have to be performed real time. Integrated biomarker is to answer urgent questions in research which there is hypothesis that have to tested statistically, but the trial could be hold without usage of biomarker. Exploratory biomarkers are not required in the research. It is neither integrated nor integral biomarker. Integrated biomarker would require most data when explorative requiring the least.[37]

Step two choosing biomarker test that fit for research purposes and a laboratory that supports to perform those tests. A fit for one test is not permitted to use. The next step is to define the biomarker test, laboratory, and protocol of the test run in laboratory chosen. Biomarker test used for pharmacodynamics and pharmacokinetics test have to be performed in laboratory that well-equipped and experienced with the determined assay. To determine patient eligibility in clinical trial, it is permitted to used less mature assay since biomarker for investigational drug is not yet known widely. Researchers also must give attention to the important factors that affect how to perform test. So, they must describe target analyte, specimen type, platform, pre-analytic processing, test components, controls, where the test would be performed, and scoring procedures of test.[37]

Step four is to performed validation test for biomarker appropriate for trial. Analytical validation test performed to ensure the test can give sufficiently accurate and reliable result. Once the test had been justified adequate for the purposes of trial, biomarker can be implemented in trial according to protocols in step 2. Lastly, researcher need to think how biomarker used in the future to use in future research, clinical trial, or clinical setting. After the end of trial, researchers should determine whether the trials confirm the hypothesis related to biomarker and whether the next step trial using the biomarker would be continued or not.[37]

Example of Biomarkers Incorporation in Clinical Trials

Examples of the use of biomarkers for decision making in clinical trials are presented in the table below.

TABLE 3. Examples of the use of predictive biomarkers for clinical trials

Target biomarkers	Agents	Conditions	Clinical trial phase	References
LSD-1 and HK34me2/1	Tranyl-cypromine (TCP)	LSD1+, HK34me2+ HK34me1+, Relapsed/refractory Acute Myeloid Leukemia (AML)	Phase I/IIa	38
Claudin 18.2	Zolbetu-ximab	CLDN18.2+ (confirmed by IHC) Advanced/metastatic gastric cancer	Phase II (completed)	39
Human Estrogen Receptor -2 (HER2)	Pembroli-zumab	HER2+ metastatic gastric/ gastro-oesophageal junction adenocarcinoma	Phase III	40
PD-L1, & Microsatellite instability (MSI)	Pembroli-zumab	HER2+, high status MSI and PD-L1 expression, metastatic gastric/gastro-oesophageal junction adenocarcinoma	Phase III	40
Hormone Receptor (HR) & HER2	Palbociclib & fulvestrant	HR-, HER2-, metastatic breast cancer	Phase IV	41

Example of the use of biomarkers for phase I and IIa clinical trials is in the clinical trial with National Clinical Trial (NCT) number NCT02261779, which tested tranylcypromine (TCP) using the biomarkers LSD1 and HK34me2/1. LSD1 is a biomarker in various tumors. LSD1 attaches to chromatin, precisely in the fusion zone with protein complexes. This protein complex has transcription factors that bind to DNA. Upregulation of LSD1 affects the cancer cell cycle through inhibition of the tumor suppressor p53 and also enhances the growth, invasion ability, and metastasis of cancer cells through the methylation/demethylation process. Therefore, LSD1 is an epigenetic target that has the potential to be used as a biomarker. LSD1 can be considered to select appropriate therapy for patients across a wide range of tumors.[38]

Several clinical trials have been conducted on LSD1 inhibitors. Clinical trials on TCP, one of the LSD1 inhibitors, were carried out using two biomarkers, namely LSD1 and HK34me2/1, as epigenetic markers that were demethylated by LSD1. Phase I/II clinical trials evaluated double-agent TCP/all-trans-retinoic-acid (ATRA) in relapsed/refractory (R/R) acute myeloid leukemia (AML). The TCP/ATRA phase I/II clinical trial aims to study the safety and efficacy of therapy in R/R AML. The clinical trial was carried out on 18 patients who were not eligible for intensive treatment. It was found that 20% of patients experienced remission, with two patients experiencing complete remission and one patient experiencing partial remission. Simultaneous TCP/ATRA treatment showed myeloid differentiation in patients who did not experience remission. Median overall survival (OS) was 3.3 mo, and OS at 1 yr was 22%. Some patients given TCP/ATRA had upregulated H3K4me1/2 in AML blast cells and white blood cells. AML blast cell differentiation can be induced by a combination of TCP/ATRA drugs. Administration of this drug combination was responded to by patients with R/R AML with tolerable toxicity and manageable side effects. Further clinical trials are needed to explore the potential of TCP/ATRA in treating AML and improving patient outcomes.[38]

Another example of the use of biomarkers for phase II clinical trials is the clinical trial with NCT number NCT01630083 using Claudin 18.2 (CLDN 18.2) as a biomarker and zolbetuximab, a monoclonal antibody that specifically binds to CLDN 18.2. CLDN 18.2 belongs to the tight junction protein family and is a very selective biomarker because it is little expressed in normal cells and has abnormal expression in primary malignant cancers, one of which is gastric/gastroesophageal junction cancer (GE/GEJ cancer). Phase II clinical trials in gastroesophageal adenocarcinoma patients were carried out with the criteria that volunteers were not eligible to receive standard trastuzumab therapy and expressed CLDN 18.2 in ≥40% of tumor cells with an expression value ≥ 2. Patients were divided into two groups randomly by comparing the number of respondents in each group 1:1 and given first-line therapy in the form of EOX (epirubicin 50 mg, oxaliplatin 130 mg, and capecitabine 625 mg) with or without zolbetuximab (initial dose 800 mg, subsequent dose 600 mg). The results show that the combination of first-line EOS therapy with zolbetuximab improves progression free survival (PFS) and OS and has benefits that outweigh the risks. The efficacy of zolbetuximab was higher in the subpopulation with very high CLDN 18.2 expression (expression value ≥2 in ≥70% of tumor cells). In addition, the combination of first-line chemotherapy with zolbetuximab can increase T cell infiltration and increase the release of inflammatory cytokines. This shows that zolbetuximab has a higher curative effect in GE/GEJ cancer patients with CLDN 18.2 expression ≥2, so that the zolbetuximab trial can be continued to phase III.[39]

HER-2, PD-1, PD-L1, and MSI were used as biomarkers in the phase

III pembrolizumab clinical trial (NCT03615326) KEYNOTE-811 protocol. Pembrolizumab is a humanized IgG4 monoclonal antibody that inhibits the programmed death-1 (PD-1) receptor. PD-1 is a checkpoint component of immune regulation in the tumor microenvironment.[42] The clinical trial was conducted in a randomized, double blind system with placebo and pembrolizumab combined with standard gastric/gastro-esophageal junction adenocarcinoma therapy: trastuzumab and chemotherapy 5-fluorouracil and cisplatin or capecitabine and oxaliplatin in patients with untreated/unresectable metastatic gastric/gastro-esophageal junctional adenocarcinoma. Clinical trials were conducted in two interims. The first interim was carried out on 264 volunteers (efficacy population), and the second interim aimed to test safety in all volunteers who received at least one dose of treatment. Of the 433 volunteers involved, 217 were given pembrolizumab, and 216 were given placebo. The results of the clinical trial showed that treatment with pembrolizumab and standard therapy resulted in a significant 22.7% improvement in clinical outcomes compared to treatment with standard therapy. This shows that there is synergy between inhibiting HER2 and PD-1. Pembrolizumab treatment plus standard chemotherapy has guaranteed safety due to adverse events, in accordance with previous studies on the administration of pembrolizumab and standard therapy. In addition to HER-2, PD-1, and MSI, PD-L1 expression and MSI are also utilized as biomarkers to support the clinical trial. From supporting data, it was found that 0.7% of participants with high PD-L1 expression and high MSI status responded better to PD-1 inhibitors.[40] Until March 2024, the clinical trial will continue by recruiting more volunteers from outside the United States.[43]

Biomarkers play a crucial role in determining the eligibility criteria for volunteers and assessing the disease status of patients participating in phase IV clinical trials. Like previous clinical trials, phase IV clinical trials use biomarkers to determine the criteria for eligible volunteers and the disease status of patients who are eligible to take part in clinical trials. In the PRECYCLE protocol clinical trial, biomarkers were employed to assess the quality of life (QoL) and time to deterioration (TTD) in patients, supported by the eHealth system for documenting patient-reported QoL. Metastatic breast cancer patients (n = 960) with positive criteria for HR+ and HER2-hormone receptors joined this study. A total of 62.5% of participants received the Cyclin Dependent Kinase (CDK) 4/6 inhibitor palbociclib in the first line of treatment, and 37.5% of participants received palbociclib in the second line. First- and second line therapy is accompanied by endocrine therapy (AI, fulvestrant). Clinical trials were carried out, with follow-up for 48 months after volunteer recruitment. The eHealth system can apparently improve QoL and reduce TTD. The eHealth system allows patients to receive oral therapy at any time if needed without having to wait long and improves communication between patients and doctors. The study also found that the eHealth system helped in monitoring side effects and adherence to treatment. Overall, the use of eHealth technology in cancer care has shown promising results in improving patient outcomes and quality of life.[41]

CONCLUSION

Molecular biology has an essential role in early decision-making that have been used in clinical practice to personalize medication or healthcare, monitoring prognostic conditions, select the treatments and evaluate drug efficacy. Selection of the most suitable patients to receive targeted treatment can detected by testing specific biomarkers including protein overexpression, mutations in driver genes, or other specific molecular profiles. By selecting patients based on biomarkers, doctors

can avoid administering ineffective treatments. Therefore, the likelihood of patients experiencing side effects from prescription medication will be reduced.

In clinical trials of new drugs, biomarkers are often used as ultimate measures clinical outcomes, to ensure that the stratify patient populations under study has a molecular profile relevant to the mechanism of the drug being tested. Biomarkers enable a more personalized and targeted approach to avoid potentially harmful treatments. Although many treatment decisions are still made without the aid of biomarkers, the future may invent more tools to guide the development and utilization of clinical cancer therapies.

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