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APPLICATION OF BIOMARKERS IN DRUGS DEVELOPMENT

Innocent Apameio Jesse[1,2]*, Ndako Angulu Caleb[1], Godiya Mammam Peter[1], Eshimutu Yusuf, Bashiru Sani[3] and Mathias Mele[2]

[1]Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria
[2]Department of Microbiology, Federal University Wukari, Taraba State, Nigeria
[3]Department of Microbiology, Federal University Lafia, Nasarawa State, Nigeria
*Corresponding author: jesseinnocent75@gmail.com

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Abstract: Biomarkers are characteristics that are objectively measured and evaluated as an indicator of normal biologic processes or a pharmacologic response to a therapeutic intervention. Biomarkers fast track the development and discovery of new drugs and make the process most cost effective. There uses have been on the increase in guiding decisions in every phase of drug development. Safety and efficacy biomarkers are the two types of biomarkers. Safety biomarkers are used to ascertain the safety of a particular therapeutic intervention while efficacy biomarkers are used to demonstrate the effectiveness of a particular therapeutic intervention example include surrogate biomarkers, predictive biomarkers, diagnostic biomarkers, pharmacodynamic biomarkers, prognostic biomarkers. The development of a new drug involves discovery and development in the preclinical research and four phases in the clinical trials. Application of biomarkers in drugs development include the evaluation of dose-response and optimal regimen for desired pharmacologic effect, safety markers to determine dose-response for toxicity, and determination of the role of differences in metabolism. Biomarker cut across various aspects of life sciences but this review will focus on application of biomarkers in drugs development.

Keywords: Biomarkers, surrogate, pharmacodynamics, drug-development, metabolism, pharmacologic

Introduction

The high costs incurred when drugs fail during clinical trials has prompted interest in biomarkers as biological indicators for progress of disease, effect of therapeutic interventions, and/or drug-induced toxicity. One of the goals is to reduce rate at which drugs fail during the clinical and probably preclinical phases of drug development (Cummings *et al.*, 2010).

The fundamental question for drug discovery and development is no longer "How does this proven remedy work and how can it be made better?" but "Will a compound directed against this target work?" Some of the risks associated with clinical trials can be mitigated with biomarkers (Mayeux, 2004). Biomarker can fast track the development and discovery of new drugs especially antibiotics, cancer drugs and neurodegenerative disorder.

Biomarker definition

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or a pharmacologic response to a therapeutic intervention (Cummings *et al*., 2010; Halim, 2011; Mayeux, 2004). According to the FDA's definition, a biomarker is a measurable endpoint that can be used as an indicator of a particular disease or some other physiological state of an organism (Amur *et al.*, 2008). In practice, biomarkers include tools and technologies that can aid in understanding the prediction, cause, diagnosis, progression, regression, or outcome of treatment of disease (Mayeux, 2004). With these definitions, biomarkers include microbial culture, sensitivity testing, imaging (CT, MRI, PET, x-ray) or clinical laboratory testing which can span a whole range of laboratory testing including simple serum chemistries (blood glucose), immunochemistry, cell surface protein expression, drug metabolizing isoenzyme phenotype, blood pressure, psychometric testing, pain scales, pulmonary function tests, electrocardiogram, bone density, single gene mutations or global mutation scanning.

In genetics, a biomarker (identified as genetic marker) is a DNA sequence that causes disease or is associated with susceptibility to disease. They can be used to create genetic maps of whatever organism is being studied (Seyhan, 2010).

Historical Background

Medical practice in ancient times was performed mainly by physical examination and observation of the patient. However, testing of biological fluids for diagnostic and predictive purposes started around 6000 years ago with the analysis of human urine. Prior to Hippocrates (460 – 370 BC), Babylonian, Egyptian and Far Eastern cultures were familiar with the diagnostic utility of urine. Urine assessments by Sumerian and Babylonian physicians were documented in as far back as 4000 BC, when they first discovered that something other than physical evidence of disease could be utilized to make a clinical decision. In those days, whenever a patient was diagnosed with a serious disease, they would ask him/her to breathe into a sheep's nose. The animal would then be slaughtered and the liver removed and carefully inspected for evidence of disease. The resulting observation was to be used to predict the outcome of the patient's case and its treatment. The Babylonians based this diagnostic art on their theory that the liver was the centre of the human body's organs and that the whole of human physiology occurred there, which aligns with our modern perception of the metabolic importance of hepatic cells (Halim, 2011).

One of the earliest recorded diagnostic tests for hormones in body fluids was documented in the time of Ikhnaton and Cleopatra, when Egyptian pharaohs tested for pregnancy by adding a patient's urine to a bag containing wheat and barley seeds. If the seeds germinated the woman was pregnant. If the barley seeds germinated first, it was an indication that the foetus was male, but if the wheat seeds germinated first then it indicated that the woman was carrying a female foetus. Testing of this pregnancy theory in 1963 showed 70% predictive value. Over the centuries, pregnancy testing became more sophisticated. In the early twentieth century scientists in several laboratories across Europe independently described the presence of a substance that promotes ovary development and growth in rabbits and mice, and they recognized that the substance was a specific hormone, now known as human chorionic gonadotropin (hCG). In 1928, German scientists Aschheim and Zondek developed the first bioassay for hCG in urine by injecting a woman's urine into an immature rat and looking for an estrous reaction;

hyperemia of the ovaries and growth of the follicles (Bush, 2004).

Another ancient diagnostic test was documented in Hindu cultures, utilizing the sweetness of urine and its ability to attract black ants to diagnose diabetes mellitus (Kraus *et al.*, 2011). Urine was once, and still is to a degree, regarded as a powerful fluid in many cultures. Towards the end of the 18th century, doctors with an interest in chemistry turned their attention to the scientific basis of urine analysis and to its use in practical medicine. To serve this interest, Boehringer Manheim launched the first urine dipstick in the mid-20th century (Halim, 2011).

The term "biological marker" was introduced in 1950s. The widespread use of the term "biomarker" dates back to as early as 1980. In 1998, a definition working group was set up to define biomarkers by the National Institutes of Health (Kraus *et al.*, 2011; Kraus *et al.*, 2013).

Technologies used for biomarker discovery

The information on biomarkers comes in the form of human genes, genetic variation, measurements of RNA, proteins and metabolites. Therefore, many approaches, including genomics, proteomics and metabolomics as well as imaging techniques hold promise for generating new biomarkers that can reflect the state of health or disease at the molecular level (Wellness, 2006). Different biomarkers can be quantitatively measured in biological samples (examples include; plasma, serum, cerebrospinal fluid, bronchoalveolar lavage, tissue biopsies, whole blood, urine, saliva) and include genomic biomarkers such as gene mutations or polymorphisms, transcriptomic biomarkers such as gene or microRNA (miRNA) expression profiling, allele/haplotype mapping, epigenomics, pharmacogenomics, and non-genomics, proteomics, metabolomics, glycomics, and other small molecules in samples. Researchers can now perform full genome, deep and transcriptome-sequencing of mRNA or miRNAs and determine gene copy-numbers and mass spectrometry-based analysis, which allows detection and measurement of selected compounds, proteins and other biomolecules.

New biomarker types and methodologies for their discovery are constantly emerging and existing technologies are evolving. Therefore, more critical to this process is to identify robust and clinically relevant multiple end point biomarkers using a combination of multi-omics approaches and validation of these biomarkers in clinically relevant human populations to see whether the distribution of biomarkers is Gaussian, and whether significant differences in values exist among different age, sex or race (Seyhan, 2010).

A robust biomarker discovery, development and validation effort must bring together multiple 'omics' technologies, data types, databases and bioinformatics and biostatistics to identify the most predictive biomarkers across DNA, RNA, protein, phenotype and metabolite domains (Nolan, 2006).

Application of biomarkers

These include;

i) Monitor the safety of a therapy
ii) Determine if a treatment is having the desired effect on the patient
iii) Predict patients who might respond better to a drug from a safety or efficacy perspective
iv) Potentially enable time and cost savings in clinical trials
v) Identification of mechanisms by which exposure and disease are related (Mayeux, 2004).

Types of biomarkers in clinical trials

There are two types of biomarkers use in clinical trials, this include safety biomarkers and efficacy biomarkers (Fig. 1).

Safety biomarkers

Application of the most sensitive procedures to identify toxicity as early as possible in clinical development before engagement into expensive phase III trials is essential (Boulton & Dally, 2010). Thus, at phases I and II, careful selection of the correct tests should be mandatory, and the selection of those tests should be based on the compound profile and pre-clinical toxicology data. In addition to physical examination, vital signs, and electrocardiogram (ECG), constantly monitored safety lab biomarkers can act as common vital organ function tests applied across different therapeutic areas or as specialized testing applied to detect unique toxicities (Halim, 2011). Safety testing can be classified as follows: liver safety tests, renal safety tests, hematology safety biomarkers, bone safety biomarkers and basic metabolic safety biomarkers.

Efficacy biomarkers

The purpose of efficacy testing differs fundamentally from safety monitoring in that biomarkers are being used to demonstrate a change in all, or at least a good proportion of treated subjects; in other words, the more positive the biomarker, the higher the efficacy of a drug. Efficacy biomarkers can be classified into the following groups: surrogate, predictive, diagnostic, pharmacodynamic (PD), and prognostic biomarkers (Hurko, 2009).

From the illustration in figure 2 different classes of biomarkers; drug metabolizing enzyme, drug receptor, and intermediary pathway substrate polymorphisms as predictive of a drug response, an intermediary signal produced from the interaction of a drug with its receptor as a PD biomarker, and a surrogate biomarker to demonstrate the final drug action. The diagram shows that panels 1 and 4 have similar pharmacological pathway components, in terms of quality and quantity, but the magnitude of the endpoints' action can be significantly affected by the rate of converting the inactive drug to an active one. Panels 2 and 3, compared to Panel 1, show that two subjects may have the same efficiency of drug metabolizing enzymes but, due to mutations in the drug receptor or downstream intermediary protein substrate, the drug does not perform its intended final action (Kraus *et al.*, 2011).

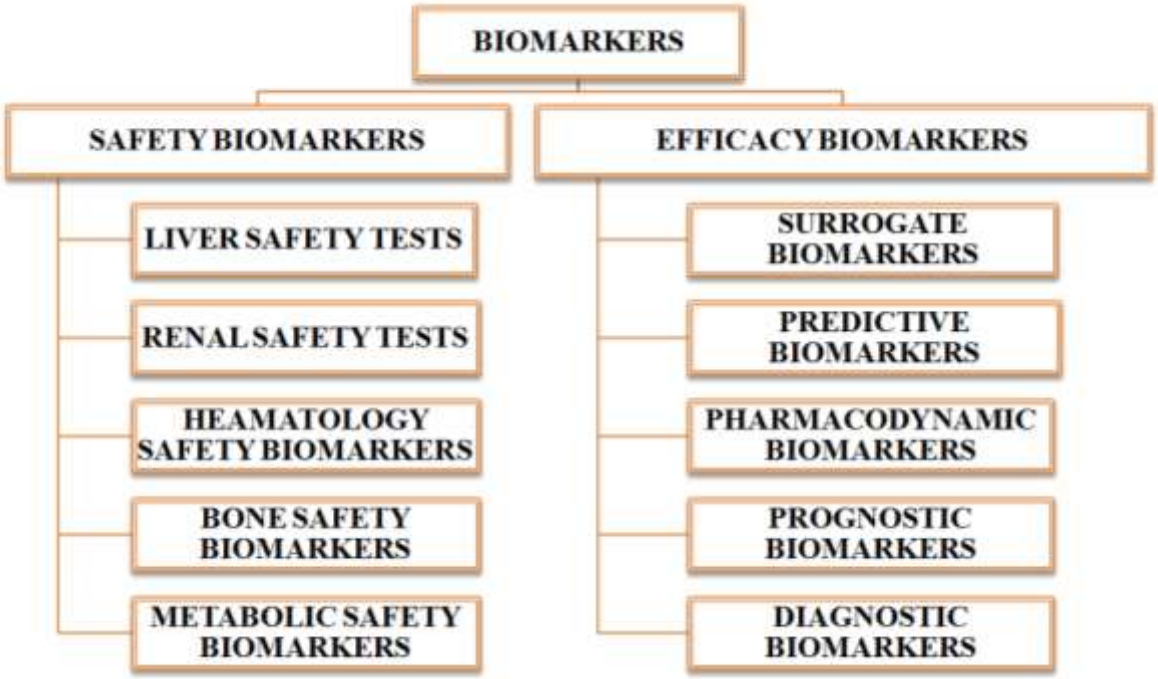
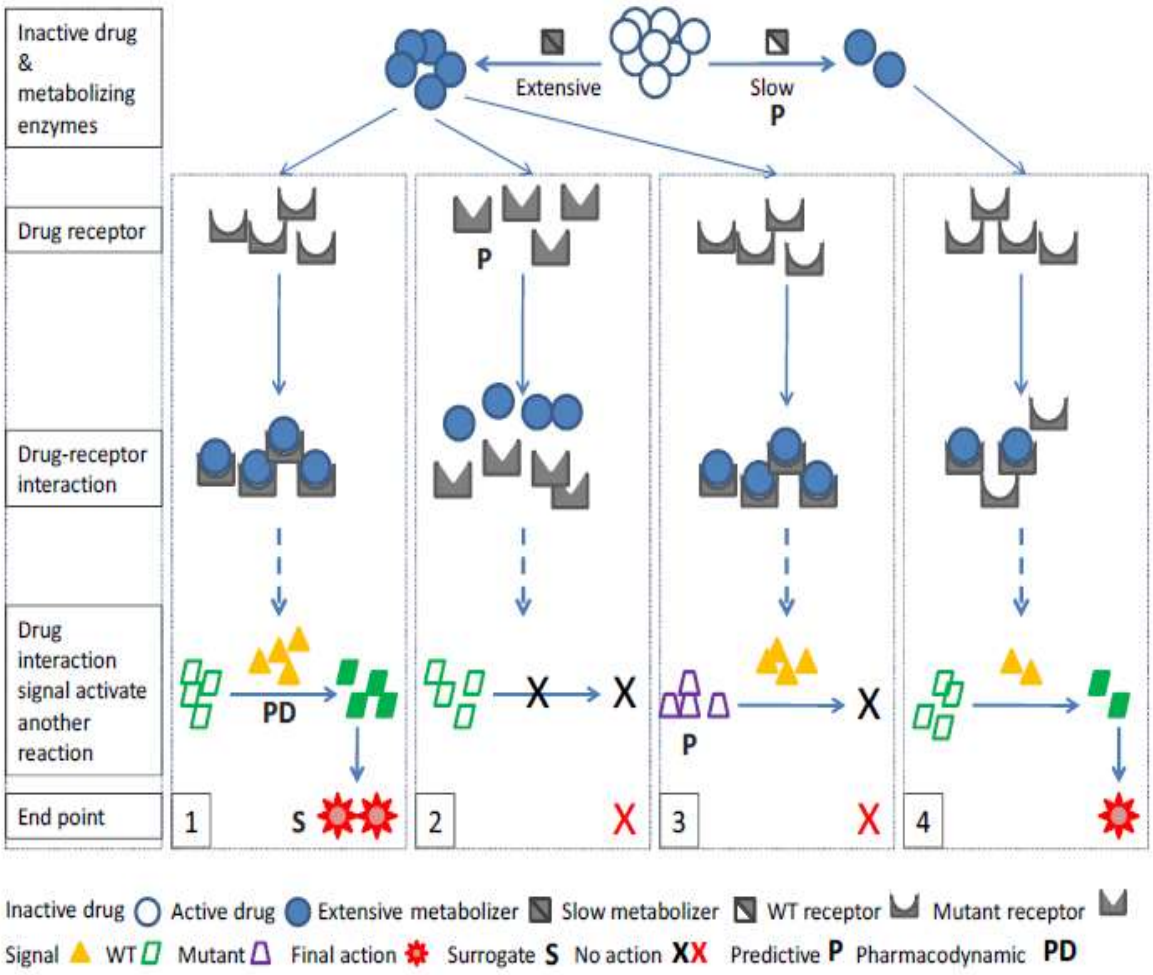


Fig. 1: Classification of biomarker



Source: Halim (2011)

Fig. 2: Illustration of surrogate, predictive, pharmacodynamic biomarkers

Surrogate biomarkers

According to the Food and Drug Administration, a surrogate biomarker is "a laboratory or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a measure of how a patient feels, functions, or survives and that is expected to predict the effect of therapy" (Hurko, 2009). A clinical endpoint is a characteristic or variable that reflects a patient's health status, usually related to efficacy, and is usually acceptable as evidence of efficacy for regulatory purposes (Fig. 2). A surrogate biomarker can be used to assess the benefit of or harm from a therapeutic agent, based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence that links the biomarker to the clinical outcome (Amur *et al.*, 2008).

Despite this new drug development work path, which has increased time and cost, it is interesting to examine the development of new therapeutics for the treatment of AIDS as a case study for the development of surrogate end point biomarkers for other diseases. The average drug for AIDS was developed in three years, because the FDA has allowed the use of 'viral load' as a surrogate biomarker as a measure of probable clinical benefit with later confirmation of a mortality benefit in phase IV studies following registration. The experience with AIDS drug development demonstrates that innovations can accelerate the development of drugs without compromising safety. Therefore, validated and clinically relevant surrogate end point biomarkers can improve diagnosis, precision medicine, targeted therapy and monitor activity and therapeutic response (Seyhan, 2010).

Surrogate biomarkers are hugely beneficial when substituted for clinically significant endpoints, also known as patient-oriented outcomes (Amur *et al*., 2008; Halim, 2011).

Predictive biomarkers

Predictive biomarkers can stratify patient populations into responders and non-responders, predict whether or not a drug will have the intended effect, or forecast the extent to which a drug can be effective or toxic in different patient populations (Fig. 2). The discovery of Cytochrome P450-2D6 (CYP2D6) polymorphism in 1977 opened the door for research on the impact of such metabolizing enzyme's genetic variability on the efficacy and toxicity of drugs. However, 34 years after this discovery, only 76 genetic and genomic biomarkers, mainly CYP2D6 followed by CYP2C19, are on FDA labels of 70 approved drugs, for antiviral, antifungal, antibacterial, oncology, psychiatry and cardiovascular drugs (Cummings *et al.*, 2010).

Predictive biomarkers in personalized medicine

Completion of the human genome project getting to about two decades now enormously facilitated our understanding of human genetics and the associated biology, and it has become increasingly clear that patients with different genetic makeup manifest diseases differently and respond to medication differently in terms of both efficacy and safety. Also, there is a rapidly spreading notion that uncertainty about which patients might respond positively or negatively to a particular treatment regimen has significant consequences on patient health and attrition rate in drug discovery, that empirical drug development is unsustainable, and that biomarkers can provide guidance and help with these issues (Colbum, 2003). In this respect, the personalization of medicine, via targeting the right population, offers the potential for mitigating the problem of universalizing therapy into a single, all-encompassing solution. If two populations with genetic and biological makeup similar to Panels 1 and 2 depicted in Fig. 2 use the same drug, Panel 1's population would observe the desired effect while the population in Panel 2 would only be exposed to the side effects of the drug. The population depicted by Panel 4 will need to double the dose used for Panel 1 to get same value (Halim, 2011).

Personalized medicine and companion diagnostics (CDx)

Recent advances in cancer research have focused on drug candidates with specific molecular targets including mutated genes in cancer cells. To achieve the greatest benefit from such types of therapeutic agents, populations that are positive for the target should be identified and exclusively treated, and in order to do that, an *in vitro* diagnostic test (IVD) should be readily available. This IVD can be an existing test for a biomarker that is classified by the FDA as "known valid;" in other words, the biomarker is accepted by the scientific community at-large as a predictor of clinical outcomes, such as LDL-c, HbA1c, and CYP2C19. When a biomarker appears to have predictive value but is not yet replicated or widely accepted, it is classified by the FDA as "probable valid," as in the cases of EGFR and KRAS mutations. These types of biomarkers can be used in targeted therapies to demonstrate the efficacy or toxicity of an agent during a drug's clinical development, and then become "known valid" when treatment is approved. This approach mandates co-development of an IVD with a drug- a companion diagnostic (Walton, 2010).

Co-development can occur during any stage of drug development but, ideally, a biomarker should be integrated early in the drug's development program so that trial data will support both drug and test approval. Clinical qualification of a biomarker should be prospective, but the retrospective path remains a possibility. Under any circumstances, the biomarker assay should be analytically validated before testing clinical samples. Only a few oncology drugs and IVD have been approved thus far. Despite of the biological, analytical, clinical, regulatory, and project management hurdles, co-development of drugs and IVD appears to be the future in facilitating the personalized medicine approach. After the end of phase II and prior to initiation of pivotal phase III trial, in which the predictive biomarker will be used for patient randomization, both CDER (the Center for Drug Evaluation and Research; the branch of FDA responsible for drug approval) and CDRH (the Center for Devices and Radiological Health; the branch of FDA responsible for approval of medical devices), should approve the approach of co-development (David *et al.*, 2017).

Diagnostic biomarkers

These are the biomarkers used for diagnosis of an existing disease and staging of disease biomarkers. Diagnostic biomarkers can also be used to stratify patients by disease type and response to treatment. Biomarkers that can reveal the status of the target (example, at the level of receptor expression, genetic polymorphism, gene expression, somatic mutation) in individual patients may provide significant predictive value for treatment response (Seyhan, 2010).

Pharmacodynamic (PD) biomarkers

These are the biomarkers which demonstrate that a drug hits its target and impacts its biochemical pathway. Such types of biomarkers are necessary to demonstrate proof of the drug's mechanism of action (POM), i.e. markers of pharmacological response (Fig. 2). This class constitutes the majority of biomarkers in early phases of drug discovery (preclinical, phase I, and, probably phase II). In correlation with pharmacokinetic (PK) measurements, this class of biomarkers can help to determine effective dose and dose schedule. The biomarker illustration in Fig. 2 shows that detection of an intermediary signal can indicate that the drug hit its target and the magnitude of the signal can reflect the efficacy of the interaction (Davis *et al.*, 2013).

The contribution of biomarkers to the goals of phase I oncology trials was analyzed to reveal that biomarkers supported the proposed mechanism of action in 39% of the trials, contributed to dose selection for subsequent phase II studies in 13%, contributed to the selection of dosing schedule for phase II studies in 8%, and biomarkers were considered by

the authors to be potentially useful for selecting a patient population in subsequent studies in 19% of the trials. These biomarkers were determined in serum (36.8% of total), tumor tissue (25.6%), peripheral blood mononuclear cells (22.7%), normal solid tissue (3.7%), and cerebrospinal fluid (0.2%), in addition to 10.9% by special *in vivo* imaging. The non-imaging biomarkers included proteins, cytokines, and enzyme activity in serum, cerebrospinal fluid (CSF), or tissue lysates, proteins by immunohistochemistry (IHC), and DNA and RNA gene expression (Halim, 2011).

Prognostic biomarkers

Prognostic biomarkers can predict the risk or outcome of a disease in patient population without the involvement of therapy. For example, a population that tested positive for a given prognostic biomarker can survive longer or live better than another that tested negative. In addition to its predictive power, prognostic biomarkers may help enrich a clinical trial by choosing people more likely to respond to treatment (Hodge, 2009). Examples of prognostic biomarkers include prostatic specific antigen to predict survival in prostatic cancer patients.

Drugs development process

They development of a new drug is broadly divided into two stages the preclinical research and the clinical trials (Fig**.** 3).

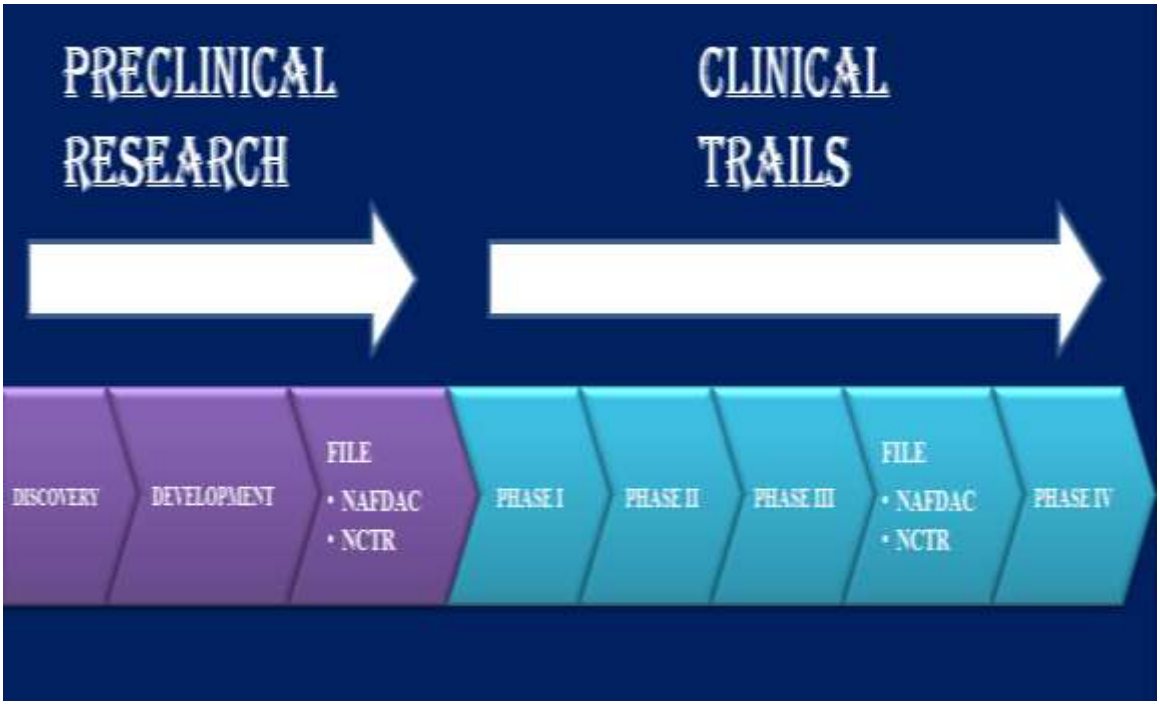


Fig. 3: Drug development process

Pre-clinical research

Before testing a drug in people, researchers must find out whether it has the potential to cause serious harm, also called toxicity. Preclinical research involve both *In Vitro* and *In Vivo* (NCTR, 2018).

National Agency for Food and Drug Administration and Control (NAFDAC) requires researchers to use good laboratory practices (GLP), defined in medical product development regulations, for preclinical laboratory studies in Nigeria. The GLP regulations are found in NAFDAC good clinical practice guidelines 2015. These regulations set the minimum basic requirements for; study conduct, personnel, facilities, equipment, written protocols, operating procedures, study reports and a system of quality assurance oversight for each study to help assure the safety of NAFDAC-regulated product (NAFDAC, 2015). Usually, preclinical studies are not very large. However, these studies must provide detailed information on dosing and toxicity levels. After preclinical testing, researchers review their findings and decide whether the drug should be tested in humans.

Clinical trials

Any investigation in participants intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of one or more investigational medicinal product(s), and/or to identify any adverse reactions to one or more investigational medicinal product(s) and/or to study absorption, distribution, metabolism and excretion of one or more investigational medicinal product(s) with the object of ascertaining its safety and/or efficacy. This includes clinical trials carried out in either one site or multiple sites (NAFDAC, 2015).

Clinical trials are generally divided into Phases I to IV. It is not possible to draw distinct lines between the phases, and diverging opinions about details and methodology do exist. A brief description of the individual phases, based on their purposes as related to clinical development of medicinal products, is given below:

Application of biomarkers in preclinical and clinical trials

Uses of biomarkers from pre-discovery to late clinical drug development (Table 1) and decision making is critical to evaluate activity in animal models, link animal and human pharmacology via proof-of-mechanism or other observations, evaluate safety in animal models and assess human safety early in development. Additionally, every stage of drug development has its own specific set of biomarkers that may or may not be applicable to other stages (Boulton & Dally, 2010).

Table 1: Clinical trials, time requirement and number of volunteers in various stages of drugs development

	Preclinical testing		Phase I	Phase II	Phase III		NAFDAC		Phase IV
Years	3.5		1	2	3		2.5	12 Total	
Test population	Laboratory and animal studies		20-80 healthy volunteers	100 - 300 patient volunteers	1000-3000 patient volunteers		Review process / approval		
Purpose	Assess safety and biological activity	File •NAFDAC •NCTR	Determine safety and dosage	Evaluate effectiveness . Look for side effects	Verify effectiveness , monitor adverse reactions from long-term use	File •NAFDAC •NCTR			Additional post marketing testing required by NAFDAC
Success rate	5,000 compounds evaluated		5 enter trials				1 approved		

To have any value, biomarkers must be robust, and be reproducible and be accessible (that is present in body fluids and measurable). A biomarker must also be sensitive and specific to distinguish true positives from false negatives. They should indicate not only the disease presence but also the disease response to time and treatment. And most importantly, detection of a biomarker should be clinically relevant and provide clinical benefits to the patient (that improved survival and quality of life).

Examples of biomarkers in preclinical trials are serum chemistries, cell surface protein expression, drug PK/PD measurements, drug metabolising isoenzyme phenotype, serum transaminases, genomic expression profile, drug distribution or receptor occupancy via imaging (Colbum, 2003).

Uses of biomarkers in late drug development are the evaluation of dose-response and optimal regimen for desired pharmacologic effect, safety markers to determine dose-response for toxicity, and determination of the role of differences in metabolism. Once validated, for example, a biomarker can be used in dose selection for phase II/III clinical trials based on the biomarker's PK/PD relationship and projected therapeutic index as well as to differentiate candidate compounds from other compounds. The biomarkers that measure PK/PD relationship also provide valuable feedback to discover whether the mechanism does or does not translate to clinical trials. Target specific biomarkers can also be used to stratify patients by disease type or response to treatment. This strategy was effectively used with the drugs trastuzumab (Herceptin®) and imatinib (Gleevec®) to stratify patients based on their pharmacogenetic polymorphisms (Quenot *et al.*, 2013).

Biomarkers in clinical studies have been used for diagnosis, a tool for staging disease, as indicators of disease status, and to predict and/or monitor clinical response to a therapeutic intervention (examples include electrocardiogram, PET brain image, serum chemistries, auto-antigens in blood, bone densitometric measurement, pulmonary function test). Biomarkers used in late clinical development are psychometric testing, pain scales, imaging studies, culture status (antimicrobials), pulmonary function tests, serum chemistries and electrocardiogram. Moreover, biomarkers have the potential to reveal prognostic information about the future health status of a patient whereas diagnostics classify patients at one point in time.

Conclusion

The high costs incurred when drugs fail during clinical trials has prompted interest in biomarkers as biological indicators for progress of disease, effect of therapeutic interventions and drug-induced toxicity. The role of biomarkers has been exponentially increasing in guiding decisions in every phase of drug development, from drug discovery to preclinical evaluations through each phase of clinical trials and into post-marketing studies. The application of biomarkers in drugs development is helping the drug industry achieve the goal of quick and cost-effective research, especially in poorly served areas such as viral diseases, neurodegenerative disorders and cancer.

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Conflict of Interest

The authors declared no conflict of interest.

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