Worldwide Clinical Trials

# Biomarkers in Oncology Studies

## The Science, the Medicine, and the Impact on Development

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Advances in discovery research with an emphasis on precision therapeutics, coupled with a need for patient stratification to inform appropriate pharmacotherapy in oncology, necessitate an integration of scientific, medical, and operational considerations for biomarker development. Both pharmacodynamic and disease-related analytes have gained ascendancy in both discovery and development, with molecular, histologic, radiographic, and physiologic biomarkers identified across multiple tumor types, particularly as advances in the biology of oncology have provided additional insights for specific targets. Evolving alongside the advances in science supporting biomarker development are implications for trial operations. They span the spectrum of domains from the place of a given biomarker in a treatment decision algorithm to the acquisition and processing of that biomarker and on to the eventual clinical and commercial implication that may derive from a program of research that has entwined product use with access and clinical decision-making. This publication provides an overview of biomarker development within oncology and points to additional strategic considerations.

Within the world of clinical research in oncology, biomarkers act as guides. They do not tell drug developers how a patient feels or how well a patient functions, but they can tell investigators and practicing healthcare providers alike whether an intervention has a rationale for application in a given cancer type or has caused a change in a disease state or process, predicated on an assumption that a given analyte or mosaic of assessments can accurately serve as a pharmacodynamic marker supporting target engagement or as disease-related proxy for an eventual clinical outcome.

Biomarkers can be used across the continuum of cancer care, including risk assessment, screening, and differential diagnosis. Prognostic biomarkers can provide insight into the expected course of a disease, while predictive biomarkers can provide insight into a patient's anticipated response to a drug or intervention. Biomarkers can also play a role in therapeutic monitoring, providing insights into disease status, safety, and the efficacy of an ongoing treatment. Pharmacodynamic biomarkers in particular provide insights into the pharmacologic effects of a drug on its target, including proof of mechanism (that the treatment hits its intended target), proof of concept (that the drug induces the intended biologic outcome in its target), optimal dosing levels (and toxicity avoidance), as well as insights into drug response and resistance mechanisms. And biomarkers can frequently

## Biomarkers and Trial Design

The scientific rationale for platform trial designs, such as umbrella or basket studies, are facilitated by access to a suite of viable biomarker assessments.[2] Accessibility to appropriate analytes can enable innovative trial designs based on several different stratum:

A) Patient enrichment (only patients with specific histology/biomarker profiling are included).

B) An all-comers patient design (stratification at baseline based on biomarkers status).

C) Specification of subgroups focusing on patients with a specific histology/biomarker profile within the overall umbrella of all patients included.

do this in close temporal proximity to treatment and with a small sample size.

Thousands of molecular, histologic, radiographic, and physiologic biomarkers are known; thousands more may yet be identified.[1] These facts raise a number of scientific, clinical, and operational questions where biomarkers are concerned.

The scientific rationale prompting pharmacodynamic biomarker development specific to a given compound begins during discovery and enabling investigational new drug (IND) activity — long before the basic design elements of a clinical study are taken into consideration.[3] Classically, these explorations are initially approached through an examination of primary pharmacology given a mechanism of action that has been generated in the course of drug discovery. Retrospective studies that use specimens and data collected during prospective trials may be undertaken, with a mandate that the results of one study need be reproduced in others.[4]

The metrics used to evaluate biomarker performance include sensitivity, specificity, positive predictive value, negative predictive value, receiver operating characteristic curves, as well as discrimination and calibration. Practically speaking, biomarkers used for clinical assessments are expected to have additional characteristics: They should be routine, simple, rapid, robust, cost-effective, reproducible, specific, quantitative, and standardized. Taken together, these characteristics (shown in Figure 1) both determine the viability of a biomarker for clinical use and define the limits and barriers that govern their utilization.

Because prompt determination of treatment efficacy is a necessity in oncology drug development, a developer benefits from those biomarkers that can be collected and assayed quickly and in a standardized manner. As an example, given that the treatment response of solid malignancies has historically been defined by the absence of clinical symptoms, as well as the stability/regression of lesions on radiological imaging over time, the ability to rely on biomarkers to detect changes that correlate with changes to the disease progression — and that can serve as proxies for that outcome, which might otherwise require weeks or months to detect — is of interest to developers and patients.

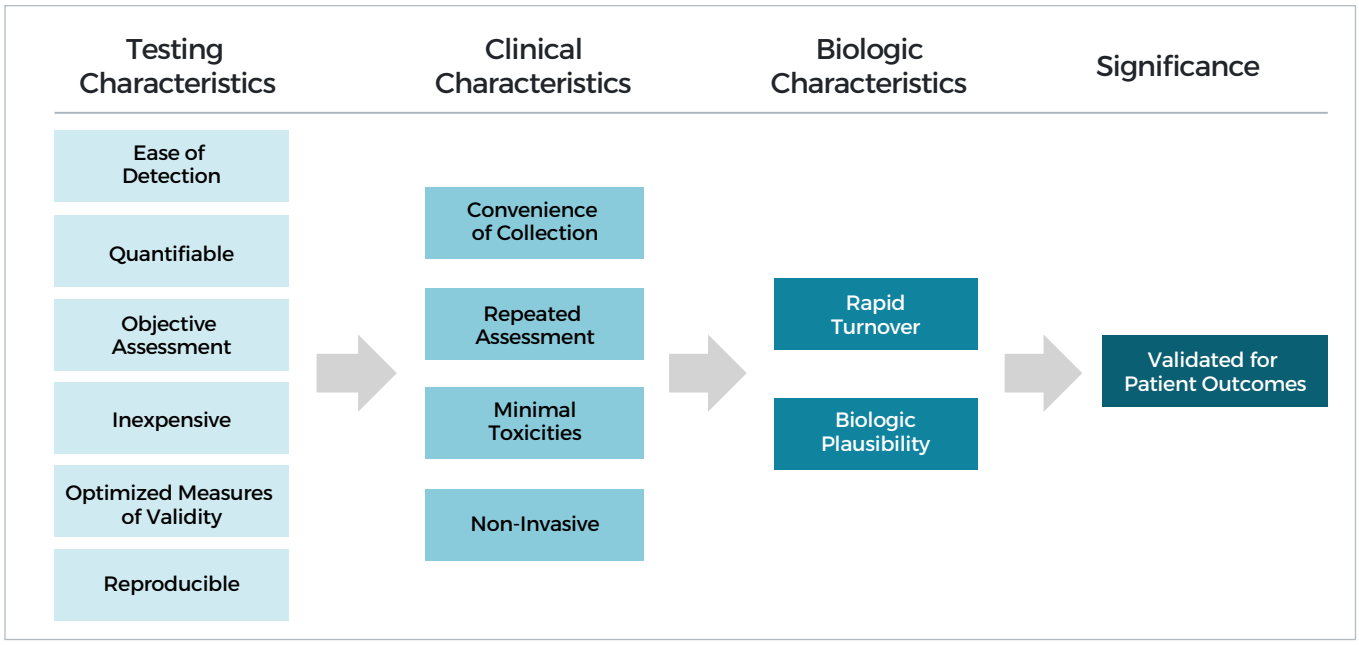


*Figure 1: Features of an ideal circulating biomarker.*

## Circulating Biomarkers in Oncology Studies

Biomarkers that can be drawn from blood, plasma, and serum have been increasingly studied, validated, and incorporated into oncology trials over the last few decades.[5] They lend themselves to studies that demand rapid, standardized, and reproducible assessments. The blood draws required to capture the biomarkers can often be performed by individuals with no specialized training. In many cases the assays involved with isolating and measuring the biomarkers may be available as a kit that can be used on site; in other cases, the samples may be sent off to any of dozens of labs that can perform the assays and provide data within hours. While there are emerging biomarker technologies involving the analysis of exosomes, metabolomics, and microbiomes, many of these have yet to be validated, lack uniformity in analysis and interpretation, and may be more complicated to process.[5]

Several types of circulating biomarkers may lend themselves to oncology studies:

- Non-specific markers of disease burden
- Tumor markers
- Circulating tumor cells

Each possesses characteristics that may be of use in specific situations, as outlined below. There are other types of circulating biomarkers, such as circulating nucleic acids, but these often rely on industry-created assays that, at this time, may introduce both processing delays and costs that compromise their utility in the fast-paced environment of oncology studies, particularly if dosing algorithms depend upon timely access to biomarker data. The concept of a "fit-for-purpose" biomarker stratagem will be developed in a companion publication.

### *Non-Specific Markers of Disease Burden*

Biomarkers categorized as non-specific markers of disease burden often occur as macromolecules that either are released into the bloodstream as a consequence of cell membrane integrity loss or are aberrantly upregulated by rapidly dividing cancer cells. Lactate dehydrogenase (LDH) is an example of one type of non-specific marker, associated as it is with cellular damage arising from disease conditions ranging from myopathies to hemolytic anemias, to cancer. Similarly, cell death products (CDPs) such as caspase-cleave cytokeratin 18, HMGB1, RAGE, and DNase may also be readily discernable, as they are released into the bloodstream by apoptotic or necrosing malignancies. However, researchers note that while CDPs may reflect the on-target killing of neoplastic cells, they may also include off-target effects on healthy tissues and may not be useful biomarkers in clinical practice.[5]

Despite their lack of specificity and the degree to which context may determine the interpretability of a given data point (e.g., which type of cancer is being studied?), many circulating non-specific biomarkers of disease burden can provide useful insights. Because they have been studied for years, many of these markers have established normal values against which the values collected from individual trial participants can be readily compared. LDH, for example, is often used to facilitate stratification in clinical trials and has been incorporated into the Royal Marsden Hospital[6] and the Gustave Roussy[7] prognostic scoring systems. Indeed, because the level of LDH in melanoma patients has proven to be a clinically significant factor associated with treatment response, progression-free survival, melanoma-specific survival, and overall survival, it has been incorporated into the American Joint Committee on Cancer's Tumor, Nodes, and Metastasis (TNM) classification and stage grouping criteria to distinguish the M1a/b/c/d (0) from M1a/b/c/d (1) forms of distant metastasis in melanoma.[8] Finally, the processes for collecting and detecting biomarker value are often robust, well-established, and inexpensive. This is of particular importance when a developer must collect data quickly in a study scenario.

## *Tumor Markers*

Tumor markers are biomarkers captured in blood, plasma, or serum that can provide more specific insights about tumor burden and response to an investigational product (IP).[9] Some well-known tumor markers include cancer antigen 125 (CA 125), prostate-specific antigen (PSA), and carcinoembryonic antigen (CEA). CA 125 has been used to monitor response assessment in relapsed ovarian cancer, though concerns have been raised about a decline in the sensitivity of the biomarker in certain ovarian cancer scenarios.[5] PSA finds its way into the bloodstream in the presence of prostate malignancies. It provides a non-invasive means of monitoring disease response in studies exploring the use of prostate-cancer targeting chemotherapies or androgen receptor axis-targeted therapies.[5] Elevated serum CEA levels are associated with breast, colorectal, gastric, lung, pancreatic, and ovarian cancers. As with other biomarkers, it has its limitations: It has been validated as a prognostic surrogate for patients undergoing CRC resection and it is considered a predictor of disease recurrence during follow-up after a CRC resection. However, it is considered a weak screening tool for colorectal cancer (CRC), and it has also been detected in a variety of non-CRC scenarios, including inflammatory bowel disease, cigarette smoking, diverticulitis, pancreatitis, liver disease, and alcohol consumption.[5] The latter observation reflects upon the importance of

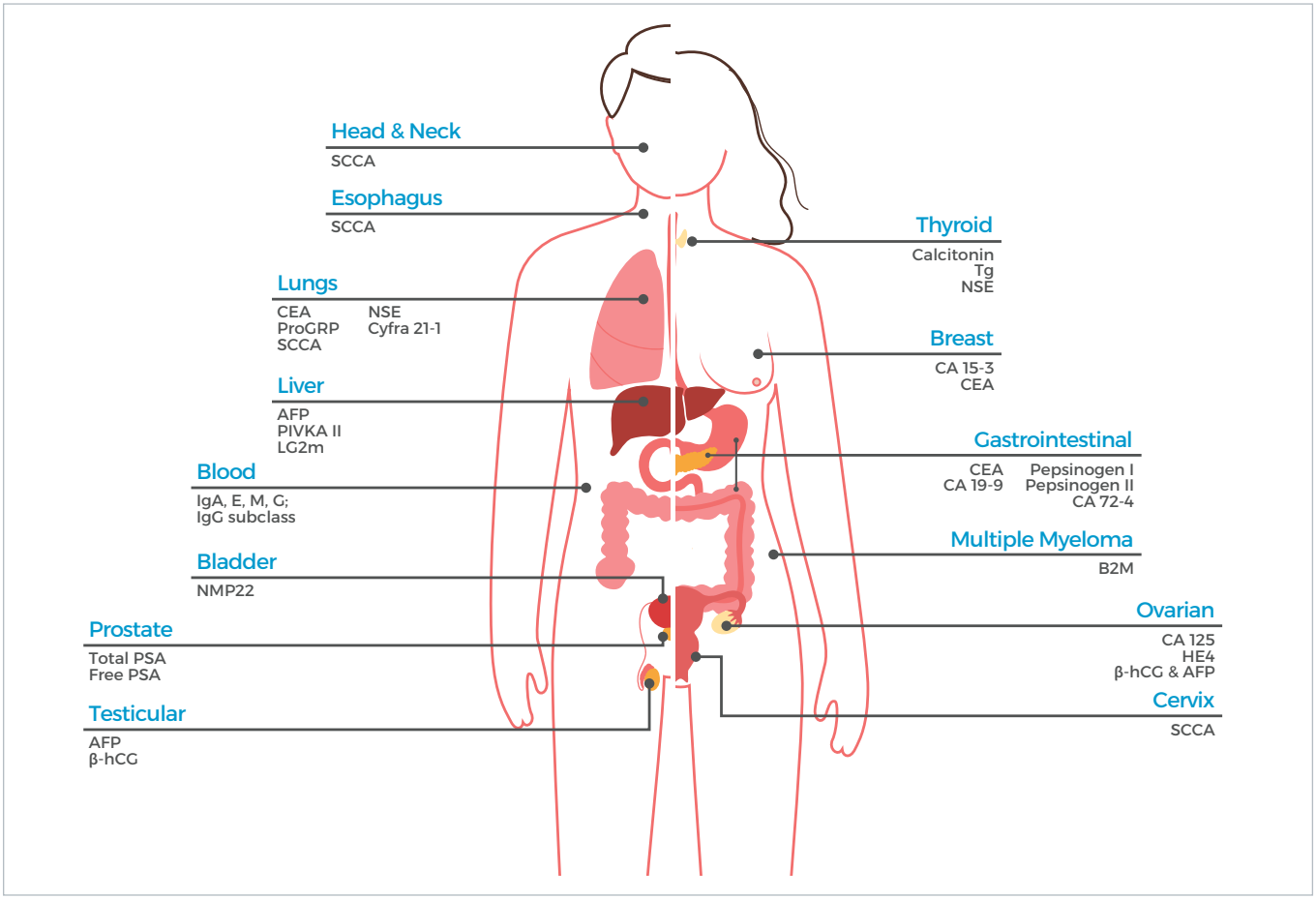


*Figure 2: Tumor markers associated with different cancer types. Source: Gawel et al.[9]*

specificity determinations as well as sensitivity within a biomarker development program. It becomes critical for developers to know the strengths and weaknesses of the biomarkers they might consider using in a study and to take steps to ensure that the weaknesses of the agents do not compromise the integrity of the data collected.

## *Circulating Tumor Cells*

Circulating tumor cells (CTS) are cells from a tumor that have sloughed off and are loose in the bloodstream. Their presence in the bloodstream is positively indicative of the presence of a specific tumor, but their presence in the bloodstream can be very difficult to detect as they are greatly outnumbered by normal cells. According to some researchers, detection is complicated by a signal-to-noise ratio of a billion to one.[10] At the same time, their presence in the bloodstream reflects an increased threat to the individual because CTS can give rise to a metastasis of the original tumor in a new location. Once a tumor has metastasized and established itself in a new location, the CTS may become more heterogeneous and less indicative of the state of the original tumor.[10]

Prior to metastasis, however, CTS have proven useful as candidate surrogate biomarkers for a variety of solid cancers (primarily breast, prostate, lung, liver, pancreatic, and gastric cancers, as well as melanoma). In clinical trials, CTS have been used to monitor treatment responses, as their use helps lessen exposure to radiation from repeated imaging assessments that would otherwise be used to monitor treatment response. In most cases, the decrease or clearance of CTS is associated with a positive response to treatment.[10]

## *Immuno-Oncology Biomarkers*

Immuno-oncology is evolving rapidly and there are already many immuno-oncology (IO) products that have multiple indications — including tumor-agnostic indications. IO biomarkers may provide valuable information on the patient's immune status, response to therapy, and overall determination of what patient population will benefit most from this type of therapy — which is fundamentally unique among anticancer therapies.

Some of the validated IO biomarkers are already being used on a large scale. However, the presence or absence of a single IO biomarker may not be enough to completely understand the complex interactions taking place within the complex tumor microenvironment. Therefore, the idea of a "composite biomarker" that is made up of several individual biomarkers combined in a specific algorithm is attractive for clinical development. Published results are proving that these panels of biomarkers can better predict response to therapy more precisely than one single biomarker.[11]

Some of the most utilized IO biomarkers include:

- PD-L1 expression on tumor cells — and, although not an absolute predictor, high expression of PD-L1 is associated with better treatment response rates in certain tumors.
- Microsatellite Instability (MSI) — defined by an accumulation of genetic errors in the microsatellite regions of the DNA and DNA Mismatch Repair Deficiency (MMRD), referring to errors in the DNA repair functions.
- Tumor-Infiltrating Lymphocytes (TILs) — the presence of immune cells in the tumor microenvironment.
- Tumor Mutational Burden (TMB) — the number of mutations present in a tumor. The higher the number, the higher the likelihood that the immune cells will recognize them as a result of increased immunogenicity.

The IO biomarkers research field is rapidly evolving and clinical trials are playing a major role in finding new biomarkers as well as composite algorithms to better utilize known biomarkers.

## Operational Considerations

There are several important factors to take into consideration when weighing the use of a biomarker in an early phase clinical trial.

- The role to be played by the biomarker must be defined. What is it measuring? What does that measurement represent? How valid is that measurement relative to the endpoint in question?
- A biomarker needs to be selected that is appropriate for the role, and a lab that is equipped to perform the assay on the samples must be found. If the lab needs to be offsite from the facility where the study is taking place, this can add to costs of the trial and increase the time to results.
- Testing and operating procedures must be determined and codified. This includes a scoring procedure that is either quantitative, semiquantitative, or qualitative. Instructions for collection, handling, and processing of the sample must be provided to the sites before the materials arrive at the site. It is also important to conduct a trial run of the biomarker test and performance reporting before the trial actually commences. This will streamline collection and testing of the samples when patients are involved.
- Finally, it is important to consider how the biomarker might be used in the future. Is there a role for the biomarker in a later phase trial? Will the biomarker raise flags for regulators at a later date? If so, what can be done to address regulators' concerns proactively?

### The Operational Implications of Biomarkers

- Validity of biomarker vs. protocol endpoints
- Accessibility to a laboratory facility
- Criticality for scoring algorithms
- Collection, handling, processing
- Turnaround time
- Cost
- Longer-term strategic impact

Researchers are required to provide protocols that describe the plans and quality assurance measures for a study. The FDA requires developers to submit a Context of Use Statement for Biomarker Qualification that details the manner of use, interpretation, and purpose of a biomarker in drug development.[12] The Context of Use statement contains five elements:

- The identity of the biomarker
- The aspect of the biomarker that is measured and the form in which it is used for biological interpretation
- The species and characteristics of the animal or subjects studied
- The purpose of the biomarker's use in drug development
- The drug development circumstances for applying the biomarker

In completing this form, the developer will have addressed many of the key considerations enumerated above.

### *On Collecting Biomarker Samples*

When collecting biomarker samples, the method of collection should utilize non- or minimally-invasive techniques whenever possible. Sample collection and preservation should also take into consideration the limitations and characteristics of the trial setting itself. What patient privacy provisions are in place? Is there sufficient refrigeration nearby (if necessary) for preservation of the sample? Are there any special handling requirements associated with sample collection and testing that must be accommodated? Finally, trial personnel should draw a sample sufficient for analysis (and the possible retesting of the sample), but not more than this minimum.

Preanalytical steps for the sample can be performed at a central laboratory or a local laboratory. A central lab may have more carefully controlled procedures, but the time it takes to ship the sample to the lab may have an adverse affect on the biomarker. A shipping delay may also compromise the timely delivery of analytical insights. It should be noted, though, that local labs come with their own risks, and these may outweigh the benefits afforded by proximity. Local labs may lack the expertise and equipment required by a more sophisticated assay. Extra training, on-site monitoring, and quality control measures may be required before the lab is fully qualified to perform a new assay.

### *Turnaround Times*

Ultimately, the turnaround time associated with the use and analysis of a biomarker includes the time it takes to acquire the sample, perform a preliminary pathological analysis at the clinical site (or to ship the sample to a central lab for analysis, if applicable), ship the sample to a certified CLIA lab, perform the requisite workflow at the CLIA lab, and then prepare and return the patient test report to the clinical site. Where the assays involve gene expression or the detection of genetic mutations, the testing lab may need up to 4 weeks following receipt of a sample to perform the assay and report the results. Developers may be able to expedite turnaround time by sending the specimen directly to the CLIA testing lab and asking the lab to work weekends, accept weekend shipments, or utilize longer workdays. If the testing lab assay time is three days or less, batching samples might also offer a way to reduce costs while still achieving acceptable turnaround times.

### *Variable Costs*

Cost remains a major consideration when it comes to the use of biomarkers in an oncology study. Simply put, the assays associated with biomarker testing can be very expensive, and the more a study relies on biomarker assays, the higher the costs become. Careful oversight and management of the number of sample shipments to a central lab (and strategizing when to batch shipments) provides one area of potential cost savings.

Random sampling and group testing have been proposed as ways to reduce the cost of biomarker testing. Random sampling is a design that measures biomarkers for a random sub-sample of subjects, while group testing is the practice of physically pooling specimens from multiple subjects and assaying the pooled samples for the presence of the molecular alteration. Both random sampling and group testing designs have been shown to be cost-efficient compared to the standard design, and group testing designs have been shown to achieve much higher cost-efficiency than random sampling designs. Group testing has shown to be useful for statistical efficiency; it also has the advantage of overcoming a limited availability of specimen while simultaneously alleviating patient confidentiality concerns. At the same time, group testing precludes the possibility of the biomarker assay delivering personalized insights. The group-tested sample may indicate that a change is occurring in study participants, but it cannot tell clinicians the degree to which that change is occurring in an individual patient.

### *Issues of Adoption and Access*

Although the incorporation of pharmacodynamic or disease-related biomarkers in the context of the clinical development of precision therapeutics may be heuristically and operationally appealing on its own, the impact on eventual commercialization may prompt additional strategic considerations. For example, the rate of biomarker testing traditionally has been lower in community-based oncology settings. The reasons for this are multivariate, relating to ethnicity, age, insurance status (covered versus not), and the nature of commercial versus Medicaid coverage. Key domains to be considered in the course of clinical development have been identified and include:

- Awareness about the need for biomarkers for a precision medicine for specific cancers
- Access and affordability
- Reimbursement policies
- Gap between testing and availability of results
- Strength of evidence supporting its use

These limitations are in part related to the historical evidence customarily available for a given biomarker. Unlike therapeutics, biomarker tests have not encountered the same demands for evidence (both clinical and economic) accorded to a given therapeutic agent. Evidence generation strategies — operating in parallel with traditional clinical development activities — are required to ensure that adequate levels of information are available for patients and healthcare providers at the time of product approval. Reimbursement commensurate with the quality of these data will facilitate utilization.[13]

## Summary

Innovation in oncology is not limited to drug development. The increasing prevalence of streamlined multi-phase clinical trials necessitates validation of biomarkers, or a "fit for purpose" early phase biomarker stratagem that can support dose optimization decisions in real-time. However, the availability, cost, and specificity/sensitivity of a given biomarker will guide its utility during clinical development.

Under the FDA's recent Project Optimus, a more thorough assessment of the dose-exposure-response relationship is required prior to initiation of a pivotal investigation.[14] While traditional safety and radiographic imaging remain important, the increasing amount of clinical data available through biomarkers is enabling disease- and drug-specific response assessments that are more rapid and quantifiable, which in turn can better support dose selection for accelerated clinical development.

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