Rational Use of Biomarkers in Oncology Clinical Trials: A Paradigm Shift towards Precision Medicine

Biomarkers discovered and used in clinical trials have been approved as companion diagnostics and used routinely in making treatment decisions. This article will give an overview of cancer biomarkers, their discovery using traditional approaches and more recently through genomics and proteomics technologies and their validation through clinical trials.

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Biomarkers are biological indicators of early disease detection (diagnostic), disease progression and outcome (prognostic), and response to therapy (predictive). The inclusion of biomarkers in patient selection has led to superior drug response rates and increased overall survival in pivotal clinical trials. Also, use of biomarkers to select drug sensitive patients have greatly improved the quality of life by improving therapeutic efficacy and reducing toxicity. Biomarkers discovered and used in clinical trials have been approved as companion diagnostics and used routinely in making treatment decisions.

Definition of Biomarkers

Diagnostic biomarkers: Diagnostic biomarkers allow disease detection and/or disease staging. Traditionally, diagnostic biomarkers in cancer came from histopathology. The WHO classification of solid and hematological tumors are based on histopathological examination of the tissues and available as monographs, or blue books for consultation (whobluebooks.iarc.fr/). For example, WHO recognizes 30 subtypes of lymphoma based on their histopathology, which has improved the accuracy of patient diagnosis significantly, without impacting drug development, or treatment decisions, because of molecular heterogeneity within the subtypes[1]. For example, gene expression profiling of diffuse large B-cell lymphoma (DLBCL) has identified three distinct molecular subtypes that are treated differently.

Other molecular rearrangements have aided in the diagnosis of solid tumors such as ALK-fusion for the diagnosis and therapy of ALK-positive non-small cell lung cancer. Diagnostic markers in many instances have become both predictive and prognostic. For example, estrogen receptor positive (ER+) breast cancer is a diagnostic marker, as well as a predictive marker for hormone inhibition therapy, and a prognostic marker of good clinical outcome, when compared with hormone receptor negative tumors[2].

Predictive vs. prognostic biomarkers: There is considerable confusion in our understanding of what distinguishes a predictive biomarker from a prognostic biomarker. Predictive biomarkers are associated with response to treatment. Tumors positive for the marker will show differential treatment effects compared with tumors negative for the marker. As an example, in non-small cell lung cancer (NSCLC), tumors harboring activating mutations in epidermal growth factor receptor (EGFR) benefited more from erlotinib (Tarceva) treatment (hazard ratio, HR 0.10) compared to tumors harboring wild-type EGFR treated with erlotinib (HR 0.78)[3]. In this example, both groups benefited from treatment HR <1, however, there was a quantitative difference in benefit between EGFR mutant vs. EGFR wild-type group (quantitative interaction) [2, 4].

The benefit can also be qualitative, in which case the biomarker positive group benefits from the therapy, whereas there is a lack of benefit to the negative biomarker group including harmful effects from the treatment. For example, use of anti-EGFR monoclonal antibody cetuximab provides benefit to metastatic colorectal cancer patients harboring wild-type KRAS, but patients harboring mutant KRAS fare poorly in the presence of the drug[5]. This makes KRAS a predictive marker of response to anti-EGFR therapy

in metastatic colon cancer. Surprisingly, the status of KRAS is not a predictive biomarker of anti-EGFR tyrosine kinase inhibitor (erlotinib or gefitinib) in non-small cell lung cancer[6] indicating deeper biological differences between the two cancer types.

A prognostic biomarker provides information on disease outcome, such as disease progression, disease recurrence or death, independent of drug treatment[2]. For example, activating mutations in phosphatidyl-inositol-3-kinase catalytic subunit alpha (PIK3CA) show worse prognosis in women with HER2-positive metastatic breast cancer, regardless of treatment[7,8]. A prognostic biomarker may reveal the underlying mechanism of disease progression and can guide the development of novel therapies.

Biomarker Detection in Clinical Settings

Platform Technologies: Biomarkers are derived from tumor tissues or other body fluids and detected by histopathological, immunohistochemical (IHC), fluorescence, ELISA, and PCR based techniques. Tumor tissue-derived biomarkers, such as overexpression of genes are detected by IHC, such HER2 overexpression in HER2+ breast cancer. Chromosomal translocation such as BCR-Abl fusion in Philadelphia chromosome is detected by fluorescence in situ hybridization (FISH). ELISA methods are used to detect proteins in blood or other body fluids such as Carbohydrate antigen 19-9 (CA19-9) from the serum of pancreatic cancer patients. More recently DNA and RNA sequencing have expanded the scope of biomarker detection from limited tissue material. Mutations in EGFR, BRAF, KRAS and other oncogenes are detected by sequencing and is used routinely in clinical settings as predictive and prognostic markers. Similarly, mass-spectrometric approaches have identified biomarkers in complex body fluids such as serum and saliva. Biomarkers discovered using high throughput proteomics methods are validated in the clinic using more robust multiplex ELISA methods.

Multi-omics Approaches: In recent years, technological breakthroughs in genomics and proteomics have resulted in a shift from the use of a single biomarker to multiple biomarkers for disease classification, diagnostics, and prognosis. This is specifically true for oncology indications, where genetic and biochemical heterogeneity of tumor cells and the need to use combination therapies to derive maximum efficacy require a deeper understanding of the molecular features of the tumor and its microenvironment. These molecular features can be accurately assessed by the use of carefully selected biomarkers.

This multi-omics biomarker discovery approach has found extensive application in the area of cancer immunotherapy - a rapidly developing field of cancer treatment, where the host immune response is boosted to elicit an anti-tumor response. The efficacy of immune-boosting checkpoint inhibitors is closely associated with molecular features

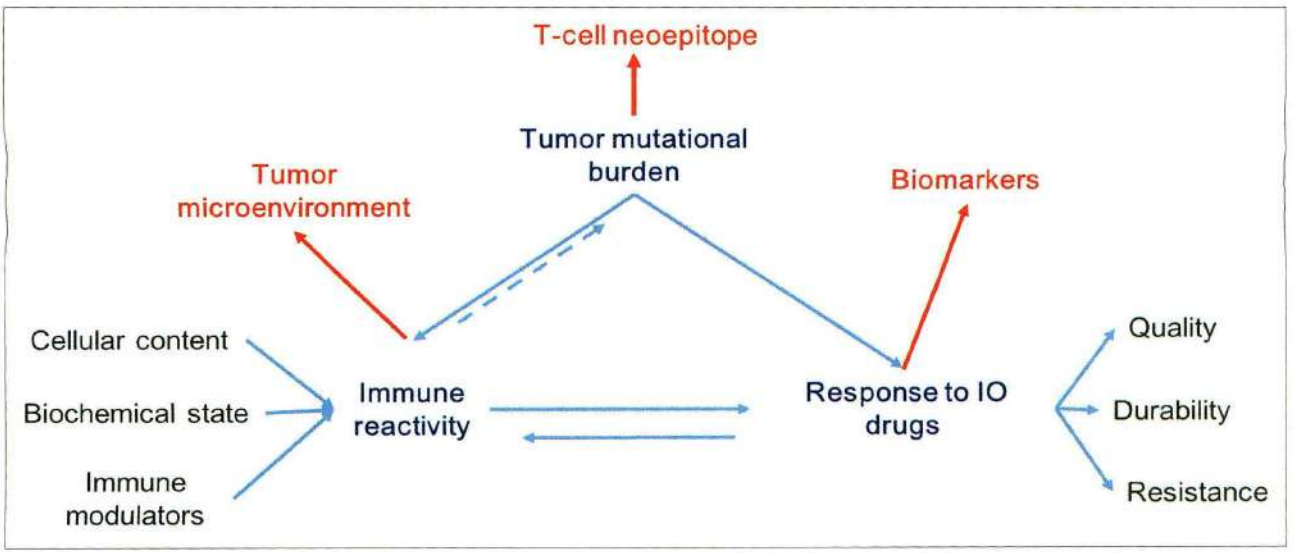

Figure 1. Biomarkers of response to immuno-oncology drugs combine analysis of tumor cell intrinsic and extrinsic factors. Exome sequencing identifies protein-altering genetic changes in tumor cells that contribute to the generation of immunogenic peptides (T-cell neoepitopes) mediating recognition between tumor cells and cytolytic killer T-cells (CD8 T-cells). Whole transcriptome sequencing provides information on the tumor microenvironment defining the immune reactivity of the tumor. Together, the tumor cell and tumor microenvironment analysis determines response to cancer immunotherapy drugs [9-11]

present in tumor cells and the tumor microenvironment. Both exome and RNA-sequencing analyses reveal critical determinants of drug response. The scope of such an analysis is schematically represented in Figure 1.

Biomarkers Make Meaningful Differences in Clinical Trials

A review of clinical trials conducted between 2006-2015 (9985 trials) reveal a low Phase-I to approval success rate for oncology drugs compared to other non-oncology disease areas (5.1% vs. 11.8% respectively)[12]. Further, the success of a biomarker-driven clinical trial was 3-times higher than a trial without biomarkers (25.9% vs. 8.4% respectively) [12]. Therefore, biomarker discovery has become mandatory for the clinical development of therapeutic molecules in all disease areas, particularly in oncology.

Biomarkers have become particularly important for targeted therapies and patient selection during clinical trials. In the early days of cancer treatment, non-targeted therapies, such as chemotherapy, or radiation therapy did not require specific biomarkers for patient selection. Histopathological examination of tumor tissue helped in tumor staging, which guided treatment decisions. With the advent of targeted therapies, biomarkers for selecting patients who will benefit from treatment became pivotal in designing Phase-II and III clinical trials. In 2005, AstraZeneca's EGFR inhibitor gefitinib was tested in a Phase-III multicenter clinical trial involving 1692 patients.

The trial failed to show improvement in benefit between the placebo and the treated groups, although indications of benefit to certain patient subgroups, such as never smokers or Asian origin were noted[13]. However, follow up molecular studies, investigating the mechanism for the lack of benefit, discovered that only patients harboring activating mutations in EGFR were super responsive to the EGFR tyrosine kinase inhibitors erlotinib and gefitinib[14-16]. These findings resulted in the rescue of the drugs, which have become the standard of care treatment for NSCLC patients harboring activating mutations in EGFR. Similarly, approval of crizotinib against NSCLC tumors harboring anaplastic lymphoma kinase fusion (ALK-fusion) has become the standard of care treatment within four years after the discovery that 3-5 per cent of NSCLC tumors harbor ALK-fusion genes[17] and ROS fusion genes[18]. Such accelerated clinical development was only possible because biomarkers for selecting tumors that will benefit from therapy were well established and FISH assays to detect such fusions were in place.

Biomarkers for Drug Repurposing

Drug repurposing or drug repositioning is finding new uses for existing drugs against new disease indications. Repurposed drugs may be approved for one disease indication, or may have failed clinical development due to inadequate efficacy or unacceptable toxicity. An example of an approved drug repurposed for a totally different indication is the cyclogenase-2 inhibitor (COX2) Celebrex (celecoxib). Celebrex and its generic counterpart celecoxib reduce inflammation and is approved for osteoarthritis, rheumatoid arthritis and acute pain and other indications.

However, the drug has been repurposed for use against colon polyps based on the finding that COX2 overexpression increases the risk of colorectal cancer and a clinical trial to that effect demonstrated a decrease in the risk of additional polyp formation in individuals with colorectal cancer[19]. Drug repurposing requires identification of diagnostic biomarkers associated with disease mechanisms. In the example above, the discovery that COX2 is highly overexpressed in colon cancer and inflammation is a key mediator of colon polyp formation led to the repurposing of COX2 inhibitor in this disease indication, which is considered a milestone discovery in colon cancer research. Another example is the use of the Type-2 diabetic drug metformin in preventing cancer. Metformin inhibits mitochondrial complex-I, reducing the generation of ATP, thereby increasing AMP levels that trigger AMPK kinase activation resulting in an increase in glucose metabolism[20]. New discoveries made in the last few years have identified pleiotropic effects of metformin on cellular pathways, such as inhibition of reactive oxygen species (ROS) generation, inhibition of p53-mediated cyclin-D1 expression, inhibition of autophagy and insulin-like growth factor signaling triggering a flurry of over 200 clinical trials in cancer (www.clinicaltrials.gov). Drug repurposing will rely heavily on the discovery of biomarkers for patient stratification, and for measuring positive effect of drugs in the repurposed disease indications.

Future of Biomarkers in Precision Medicine and Personalized Therapies

Biomarker discovery is a critical bottleneck to ensure the success of drugs in clinical trials. The cost of new drug development has skyrocketed in the last decade reaching over 1 billion dollars in discovery/development cost and running clinical trials. The burden of failure in late stage clinical trials results in a significant erosion in company's market value, winding down of future research activities and blunting innovation that small companies bring to the table.

A recent example is the failure of BMS's drug Opdivo (nivolumab) in the first line treatment of advanced non-small cell lung cancer. The results of the failed clinical trial demonstrated that PD-L1, which is

used routinely as a biomarker for selecting patients might not be robust enough to ensure approval of BMS's drug. The lack of positive clinical trial data erased 20 per cent of BMS's market cap in a day and prevented the market adoption of its drug to a competing product Keytruda (pembrolizumab) from Merck, which got approved for the same indication. The Opdivo CheckMate trial and other unsuccessful clinical trials emphasize the need to identify robust biomarkers very early during drug development, and design efficacy and toxicity studies around these biomarkers to evaluate their utility, before transitioning the drug into pivotal clinical trials.

A large number of technological platforms including next generation sequencing and mass-spectrometry are available for the rapid discovery of biomarkers in complex tissues and body fluids[21]. This robustness of these technologies is well suited for clinical adoption and is rapidly gaining momentum with the regulatory authorities. Equipped with multi-omics-based biomarkers the era of precision medicine will enter into the next phase of delivering personalized medicine, where each patient will receive a tailored therapy at the right time and at the right dose to maximize efficacy and avoid adverse toxicity - fighting cancer and still experiencing a better quality of life. ■

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