Review Article

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Cancer Biomarkers

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Abstract

In the field of oncology, the understanding of the complex molecular mechanisms that transform a normal cell into an aberrant one with the dysregulation of alternative complementary pathways have led to a significant progress in the biomarker technology backed by clinical diagnostic tests.We have been now able to achieve extremely targeted treatment approach for each individual. In this article we have projected certain specific predictive biomarkers, their invaluable role in cancer Chemotherapy and companion diagnostic tests that are gaining increasing acceptance in the cancer clinics.

Keywords: Cancer Biomarkers; Targeted Therapy and Personalized Medicine.

Introduction

With the rapid evolution of genetic and genomic technologies revolutionizing our approach to prognosis, screening and targetingof therepies, the age of personalized and predictive medicine has not only defined how clinical practice is evolving today, but also predicts how it will be practiced in the future. There are different types of cancer biomarkers; prognostic, pharmacodynamic and predictive[1]. Prognostic biomarkers anticipates the likely outcome of disease or dictate whether further therepy is required or not. More recent versions of prognostic biomarkers include the OncotypeDx test which forecasts the probability of breast cancer recurring after surgical intervention [2]. Then, there are pharmacodynamic biomarkers which measure the effect of a drug on the disease [3]. By contrast predictive biomarkers asses the likelihood that the tumor will respond to the drug. Thereby, allow a level of personalization to be introduced into the treatment regimen. The importance and necessity of these biomarkers are highlighted by the enormous healthcare expenditure on cancer drugs, and the estimated savings from patient selection and stratification based on the results of this biomarker diagnostic tests on predictive biomarkers with demonstrated clinical utility. The ideal approach to codevelopment of a targeted drug and companion diagnostic involves (a) identifying the mechanism of action of the drug and role of the drug target in the pathophysiology of the disease. This should further be verified by early phase clinical trials on the particular drug. The predictive biomarkers generally target a single gene or protein rather than multivariate classifier. Multivariate classifier reflects an incomplete understanding of action of drug or the role of its molecular target. (b) Development of an analytically validated test for measurement of that biomarker. The test should be reproducible and robust. (c) Use of that test to design and analyze clinical trial to evaluate the effectiveness of that drug and how the effectiveness relates to the biomarker value. We discuss the current commonly used predictive biomarkers in clinical practice and their accompanying diagnostic tests.

The Role of Cancer Biomarkers in Leukemia

CML and IMATINIB

Chronic myeloid leukemia are associated with a specific chromosomal translocation between ch 9 and

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22 resulting in the characteristic Ph chromosome, creating a fusion protein BCR-ABL,which acts as a constitutively active tyrosine kinase [4]. A selective inhibitor of BCR-ABL, Imatinib is now established as first line therapy [4]. In 30-50% of patients, secondary resistance to imatinib has developed. Newer drugs dasatinib and nilotinib act on imatinib resistant mutants. Mutation in the catalytic domain of BCR-ABL that confers resistance to imatinib is used as predictive biomarkers is becoming the gold standard for identifying patients that should be treated with dasatinib and nilotinib [5]. The most important prognostic indicator is the response to treatment at the hematologic, cytogenetic and molecular level. Measurement of minimal residual disease (MRD) using molecular tests is becoming the gold standard of measuring response to Therapy.

More than 100 different point mutations located between acid residues 244 and 500 have been reported in the literature. They are found in CML patients developing resistance to imatinib [7]. The various mutations cause different strengths of resistance, affect the specific therapeutic response and dictate the selection of the TKI for optimal response. The domain of BCR-ABL1 kinase consists of four major regions a) P loop b) ATP binding site c) catalytic domain d) A activation loop [8]. Mutations namely T3151, 5359V, mutations in P loop and mutations in the A loop. These mutations can be reliably detected by nested PCR amplification of the translocated ABL kinase domain, followed by direct sequencing of the entire amplified kinase domain. The sensitivity of the assay enables detection of mutations in samples containing at least 15-20% of mutated clones. In practice, screening for mutations is justified when an increase in the BCR-ABL1 transcripts is measured by RQ-PCR (especially when passing MMR level) or in any advanced phase disease such as in chronic phase patients who do not achieve cytogenetic response [9].

PML/RAR for ATRA and Arsenic Trioxide Treatment in Acute Promyelocytic Leukemia

APL constitutes 5-8% of AML cases, with an abnormal accumulation of promyelocytes in the blood and bone marrow [10,11]. The t (15:17)(q22; q12) results in fusion of the promyelocytic gene (PML) on ch15 with the retinoic acid receptor (RAR) gene on ch17.The PMLRAR fusion protein mediates a block in myeloid differentiation. The blasts are highly sensitive to anthracycline based Chemotherapy and differentiate in response to ATRA and Arsenic Trioxide treatment [10]. ATRA targets RAR component of the fusion protein whereas arsenic trioxide targets PML causing mutation and apoptosis. Cytogenetics, FISH and monoclonal anti PML Aband RT-PCR are necessary for genetic confirmation of aberrant PML-RAR [12]. RT-PCR is the only technique useful for the monitoring of MRD [10]. Sequential RT-PCR monitoring provides strongest predictor of relapse free survival in APL and is a very valid strategy to reduce rates of clinical relapse when coupled with preemptive Therapy [10]. Overall the PML-RAR translocation correlates with response to ATRA and arsenic trioxide.

The Role of Cancer Biomarkers in Solid Malignancies

BRAF V600E for Vemurafenib in Melanoma

Melanoma accounts for about 80% of deaths from skin cancer, with a 5 year survival rate of 15%. Approximately 60% of melanoma harbor activating mutations in BRAF. The specific BRAF inhibitor.

Vemurafinib demonstrates a very good antitumor response rate in patients having BRAF V600E mutation. Again RT-PCR has been successful in detecting this mutation. Allele specific PCR qualitative assays also confirm V600E mutation in hairy cell leukemia [13]. Another specific BRAF inhibitor, GSK2118436, has also shown significant activity in patients with metastatic melanoma and is undergoing phase 111 study [14].

HER 2 and Breast Cancer

Breast cancer accounts for 14% of cancer deaths in women. The HER2 gene is amplified in 25% of tumors and portends a poor prognosis. Trastuzumab is a recombinant monoclonal antibody that targets HER 2 protein overexpression of HER2 occurs in 80% of patients .In several large clinical studies , trastuzumab had a major impact on HER2 positive metastatic breast cancer , and in combination with chemotherapy increases both survival and response rate [15]. About 70% of HER 2 positive patients do not respond to the drug and resistance to treatment develops rapidly in all patients [16]. The ability to reliably identify patients who might benefit from trastuzumab is a vital issue owing to a high cost of therepy and also due to the potential cardiotoxicity associated with treatment.

EML4-ALK for Crizotinib in Non Small Cell Lung Cancer

EML4-ALK fusion oncogene defines a distinct clinicopathologic subset of non small cell lung cancer with an overall incidence of approximately 5%. Non small cell lung cancer is a leading cause of cancer mortality. Crizotinib is a targeted therepy against ALK

that has shown encouraging response rates in patients with EML4-ALK fusion gene and has proved to be the latest champion in cancer wars. Crizotinib may also benefit ALK positive non Hodgkins Lymphoma or inflammatory myofibroblastictumor [17].Crizotinib has a potential role in treating neuroblastoma, a devastating childhood cancer, in which ALK gain of function mutation has been reported in 10% of patients [18]. To identify ALK rearrangement, FISH and RT-PCR play a major role. The efficacy of Crizotinib is quite impressive with an overall response rate of 57% and rate of stable disease of 33%.

EGFR for Erlotinib and Gefitinib in Non Small cell Carcinoma

The EGF receptor is a transmembrane protein with cytoplasmic kinase activity. The currently available EGFR tyrosinase kinase inhibitors,Erlotinib and Gefitinib , is standard FDA approved monotherepy for NSCLC as a second line treatment as well for maintenance irrespective of the EGFR status.

Mutation analysis for EGFR status in NSCLC is the preferred method. There are various techniques among which peptide nucleic acid – locked nucleic acid PCR clamp technique has a sensitivity of 97% and specificity of 100% [19]. Mutation detection kits for EGFR mutation such as Genzyme and QIAGEN are a further advancement in the field of companion diagnostics for these biomarkers.

KRAS against Cetuximab or Panitumumab in Colorectal Cancer

Mutations in KRAS oncogene are overexpressed in colorectal, pancreatic, lung and even ovarian cancer [20]. KRAS mutation in colorectal cancer selects patients who do not benefit from anti EGFR receptor therepy, cetuximab or panitumumab. It is an important predictive biomarker for poor response to this anti EGFR receptor therapy [21]. Therefore, KRAS is a drug response specific biomarker. KRAS mutations detectedby allelespecific PCR, single strand conformational polymorphism, nucleic acid sequencing are all helpful in the detection of KRAS mutation.

Monoclonal Antibodies as Therepeutic Agents

An antigen CD33 in acute myeloid leukemia is now being used to measure minimal residual disease in acute leukemia. CD33 is a 67 kd surface glycoprotein that is expressed in the leukemic blasts in over 80% of cases. Monoclonal antibodies against CD33 are routinely used in the characterization of leukemia. A humanized IgG4 K anti CD33 mAb has been developed. This antibody has been conjugated to the chemotherapy agent calicheamicin through a hydrozone linker to form the therapeutic agent as gemtuzumab ozogamicin (Mylotarg) [22].

More recent studies have suggested that gemtuzumabozogamicin may yield a higher complete remission rate when administered with intensive Chemotherapy.

Conclusion

These predictive biomarkers along with their companion diagnostic test are effective weapons used in this battle of cancer with mankind. It is important if the pathologist and oncologist come on the same platform and agree upon mutation testing and reporting procedures to ensure optimum patient care. Pathologist will be central because of their crucial role in appropriate tumor specimens for testing, choosing the molecular diagnostic lab to be used, assisting in the selection of a suitable test and interpreting the result of mutation analysis. Such specialized laboratories have to be developed as they would be immensely useful in bringing down economic burden in cancers due to Chemotherapy.

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