Review Article

Molecular Pathology of New Anti-cancer Agents And Role of Histopathologist in Selecting These Therapies "Targeted Therapy"

Shahzad Shafqat Qureshi

Since the Start of chemotherapy there has been a search for more specific anti-cancer agents which should selectively act against the tumor cells and spare the normal cells. We know that conventional anti-cancer drugs act against all dividing cells in our body and because of the fact that most of the tumor cells have high proliferative index they are at the selective disadvantage. There are two important considerations in this regard, firstly some tumor grow slowly rendering them less sensitive to these agents and paradoxically some normal cells divide very rapidly such as bone marrow, gut lining epithelium and hair follicle resulting in some of the well known side effects of chemotherapy such as myelosuppression, gastrointestinal disturbances and alopecia. With our better understanding of the molecular biology of cancer, new exploitable differences between normal and tumor cells have been discovered against which we can apply more specific agents. Majority of these molecular targets are protein products of oncogenes and oncosuppressor genes including growth factor receptors and their enzymatic intracytoplasmic domains. Various monoclonal antibodies directed against these proteins or small molecule inhibitors of their enzymatic intracytoplasmic domains are increasingly being used in cancer therapy such as imatinib mesylate, trastuzumab, cetuximab and gefitinib. This article reviews the molecular biology of these agents and the new and promising role of histopathologist in selecting these therapies for individual patients, known by various names such as targeted/customized/individualized or personalized therapy.

Key Words

anticancer-agent, molecular targets, oncogenes, oncosuppressor genes, growth factors, growth factor receptors, monoclonal antibodies, small molecule inhibitors, targeted therapy.

Abbreviations

IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; CML, chronic myeloid leukemia; GIST's, gastrointestinal stromal tumors; NSCLC, Non small cell lung cancer.

Introduction

The conventional anticancer drugs act mainly by killing the dividing cells irrespectively whether they belong to the tumor or normal body tissues. This results in several well known side effects particularly relating to tissue with high proliferative rate thus causing problems such as alopecia, G.I disturbances and myelosuppression etc. Therefore, there has always been a desire to find more specific agents which should act against tumor cells only and spare the normal cells. This was only possible if we could find some exploitable differences between the normal and tumor cells. With our better understanding of the molecular biology of cancer particularly with discovery of genes and their protein products involved in oncogenesis such as oncogenes, onco-suppressor genes, DNA repair genes and apoptosis controlling genes, these was a whole new world of potential molecular targets which could be exploited for the development of new anticancer drugs.

This also poses new challenges to the histopathologist who eventually will have to provide the extremely critical information regarding selection of anticancer therapy for those patients who would respond to that particular drug. As these drugs are very expensive and may have to be taken for long time, this makes the histopathologist job all the more important and critical in clinical decision making.

Cancer Related Genes

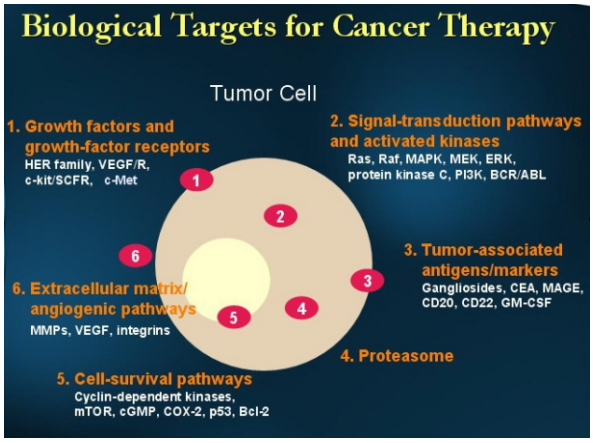
It was known for a very long time that various carcinogenic agents including chemicals, radiation, viruses etc. cause cancer but the vital question that how come these variable factors bring about the some change in the cell, that is malignant transformation,

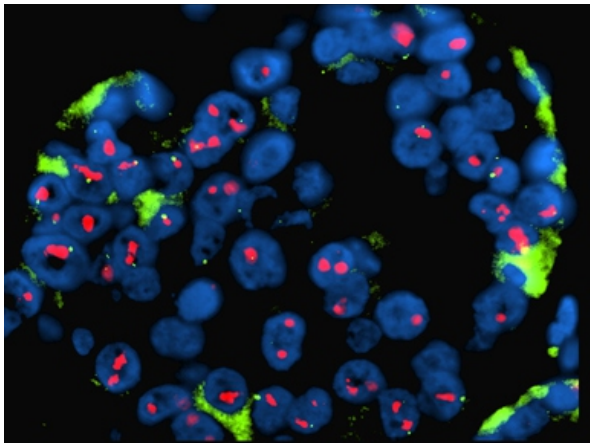
remained unanswered.

In the later half of the last century, it was realized that all of these carcinogenic agents must be acting on the same targets in the cells, probably at the genetic level. These targets were found to be important sets of genes which control some of the most critical phenomenons such as cell growth/proliferation, differentiation, DNA repair and programmed cell death (apoptosis). These normal genes are called protoncogenes and when they become abnormal, called oncogenes. The mechanisms causing these genetic abnormalities include structural and numerical chromosomal aberrations such as mutations, translocations, inversions and amplification, deletions respectively. The detailed account of these genes and their protein products (oncoproteins) are beyond the scope of this review article, suffice is to say that these genes encode proteins that are mainly involved in the mitogenic signal transduction pathways including growth factors, growth factor receptors with their enzymatic intracytoplasmic domains, signal transuding proteins, nuclear transcription factors and cyclins.

Exploitable Differences Between Normal And Cancer Cells

With the completion of human genome project in 2000, in which the entire mapping of genes with functional grouping was achieved, coupled with advancement in biotechnology, resulted in much more efficient detection of exploitable differences between normal and cancer cells. [1,2] This has helped in development of new anticancer drugs. Previously gene analysis used to be very labor intensive but now genes can he analyzed by gene arrays or FISH technique and their expression (protein products) by IHC.

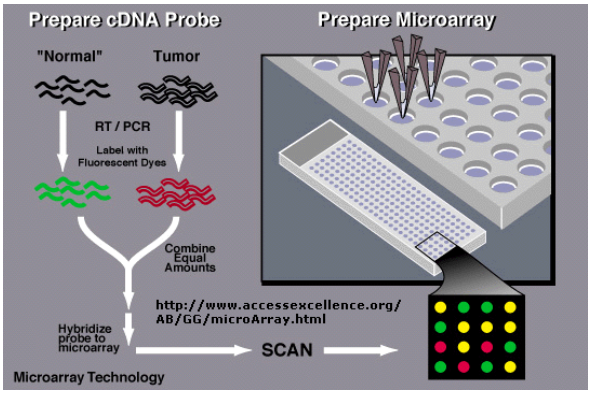




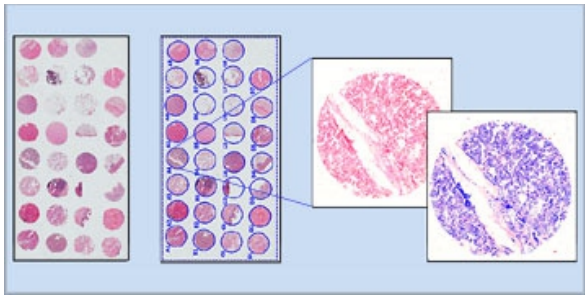
Fish Technique

Gene Arrays

Gene arrays can detect the transcriptional activity of thousands of genes in a small number of samples and differences in the transcriptional activity of normal tissue and cancers can be highlighted. [3] These screens often produce a few hundred genes that have a significant difference in activity (low or high) from normal tissues. [4]



Gene Micro Array



Tissue Micro Array

Tissue Micro arrays

This technology allows hundreds of tissue samples to be arrayed in a single paraffin-embedded block from which many sections can be cut for immunohistochemistry for protein localization & expression. [5,6]

Bioinformatics

With advent of array technologies, there has been a vast increase in bioinformatics resulted in many sophisticated systems of data analysis such as treeview and Michael Eisen's cluster, widely used and freely downloadable from the internet. [7,8]

2 Classes Of New Anticancer Drugs

I. Monoclonal antibodies

These relatively large molecules are designed to bind and selectively inhibit the extracellular domain of growth factor receptor proteins, so native growth factor cannot bind to receptors. [9, 10] They show high affinity for the receptors particularly when there is amplification of the growth factor receptor proteins. They are chimeras proteins produced by protein engineering with both murine and human subunits, therefore not eliciting immune reaction. The major disadvantage is that these are large molecules which cannot be absorbed through the gut, therefore they are given parentally and under hospital supervision. Because of their large size they also cannot enter intracellular environment so used only against protein targets with extracellular domain.

Examples are Trastzumab, Cetuximab and Bevacizumab.

Trastuzumab (Herceptin)

This monoclonal antibody reduces the level of protein by binding to it, inducing endocytosis and causing facilitative antibody-dependant cell mediated cytotoxicity. It binds to the extracellular domain of the HER2 receptors which is an ERBB tyrosine kinase family along with EGFR (HER1/ERBBI). [11, 12] The HER2 gene is known to be amplified in 20 30% of breast cancers resulting in over expression of HER 2 protein on tumor cell surface. [13] The HER2 amplification is found to be an independent adverse prognostic factor. [14]

There are now a large number of reported trials of Herceptin. [15,16,17,18] One of the early phase II studies used Trastuzumab as a monotherapy in patients with metastatic breast cancer who had failed conventional chemotherapy. The overall response rate was 15% and median duration of response 9 months by IHC and 19% when HER2 gene amplification was evaluated by more sensitive FISH method. [19]

In a large phase III study with 469 patients, response rate increased from 31% to 54% with addition of Herceptin with first line chemotherapy in metastatic breast cancer and median survival increased from 20 to 26 months. [20]

Cetuximab (Erbitux)

This monoclonal antibody is directed against the extracellular domain of the EGFR protein (ERBBI/HER I). EGFR has been shown to be over expressed in a number of human cancers including colorectal and head & neck cancer which result in increased transmembrane signal, greater nuclear transcription of genes related to cell proliferation and increased apoptosis. [21, 22, 23] Cetuximab is being used in many clinical trials as mono or combination therapy in metastatic colorectal cancer. [24, 25, 26, 27] In a phase III trial of 329 patients, results showed a response rate of 18% in combination groups and 10% in monotherapy group. In 2004 FDA approved Cetuximab for its use in this group of patients. Screening and detection of KRAS mutation in colorectal cancer is also shown to be good predictor of response to EGFR inhibitors. [28] In the large FLEX trial, the Cetuximab plus Cisptatin / Vinorelbine demonstrated a significant survival advantage in advanced non small cell lung cancer (NSCLC), whereas in the smaller BMS-099 trial a similar but not significant trend was found in the Cetuximab plus Carboplatin + Taxane arm. [29,30]

Bevacizumab (Avastin)

Acts against vascular endothelial growth factor (VEGF) which is a potent promoter of angiogenesis and survival of endothelial cells in tumors. [31, 32, 33] VEGF is expressed in the majority of solid cancers including colorectal & breast cancer and is found to be an independent poor prognostic factor. [34] The Bevacizumab binds to VEGF thus blocking the signal for angiogenesis.

A phase III study of 925 randomized patients with metastatic colorectal cancer who received Irinotecan /5 Fluorouracil/Leucovorin with or without Bevacizumab showed increase in median survival from 16 to 20 months with addition of Bevacizumab. [35] FDA approved this drug in 2004 for its use as first line treatment with 5 Fluorouracil in metastatic colorectal cancer. Bevacizumab alone or in combination therapy has also shown promising results in metastatic breast cancer. [36] An open-label,

Phase 3 trial showed initial therapy of metastatic breast cancer with Paclitaxel plus Bevacizumab prolongs progression free survival, but not overall survival, as compared with Paclitaxel alone. [37] Targeting vascular endothelial factor receptor has also shown promising results in Thyroid and CNS tumors. [38,39,40]

Rituximab (MabThera)

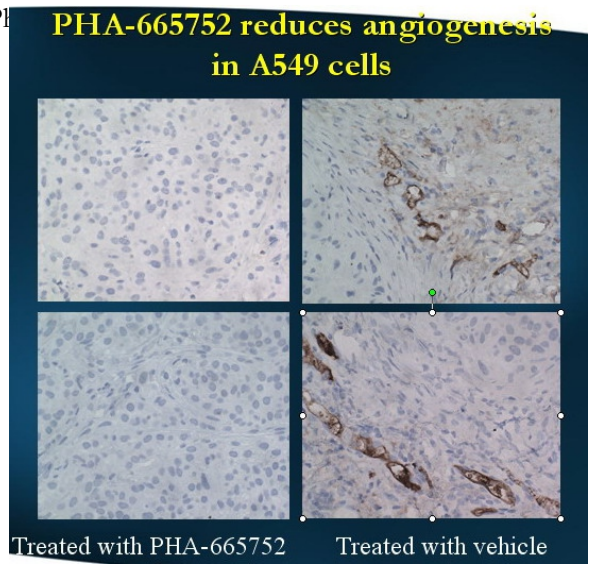
Rituximab is a monoclonal anti-CD20 antibody which in combination with chemotherapy improves overall survival compared to chemotherapy alone when used for induction therapy for patients with newly diagnosed or relapsed indolent lymphoma. [41,42] Randomized controlled trials have demonstrated that maintenance treatment with Rituximab prolongs progression free survival but evidence of effect on overall survival is lacking. Conclusion was Rituximab maintenance therapy should be added to standard therapy of patients with relapsed or refractory follicular lymphoma following successful induction treatment. [43]

2. Small molecule inhibitors

These are relatively small molecules which can be absorbed through oral route and penetrate intracellular environment. [44] They inhibit intracellular enzymatic tyrosine kinase domain of receptor proteins. [45] Examples are Imatinib mesylate, Gefitinib & Erlotinib.

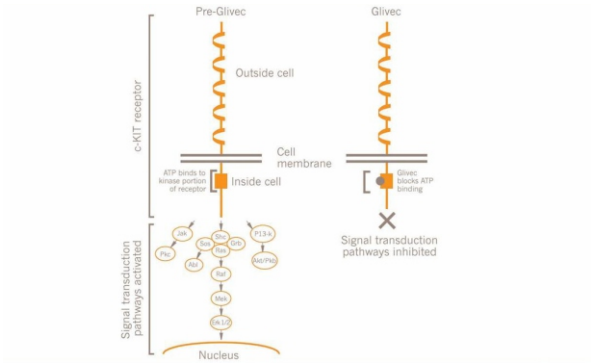
Imatinib mesylate (Gleevec) & Nilotinib

Imatinib is a small molecule inhibitor of tyrosine kinase domain of two proteins - activated ABL kinase protein (caused by fusion of BCR-ABL in

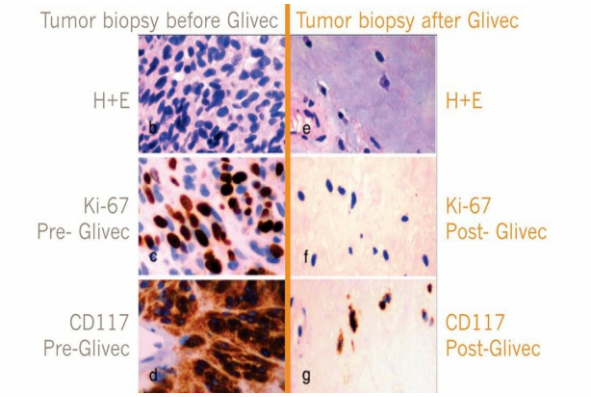


KIT protein in GIST's. [46,47] Imatinib is highly effective in CML with a 91% 2 year survival rate. [48,49] Pre clinical in vitro studies have shown that Nilotinib, a new BCR-ABL tyrosine kinase inhibitor, is more potent than Imatinib against CML cells by a factor of 20 to 50. Out of 33 Patients with blastic phase of disease 13 had a hematologic response, of 46 patients with accelerated phase 33 had a hematologic response and 22 had a cytogenetic response; 11 out of 12 patients with the chronic phase showed a complete hematologic remission. Conclusion was that Nilotinib has a relatively good safety profile and is active in Imatinib resistant CML. [50, 51] The response rate of GIST's to Gleevec is 54% among 147 patients with inoperable or metasatic GIST's (Whereas GIST'S are very resistant to conventional chemotherapy). [52,53] A phase III randomized intergroup trial assessing Imatinab mesylate at two dose levels in patients with unresectable or metastatic GIST expressing the KIT receptor tyrosine kinase showed statistically significant response. [54] A study on Japanese patients showed good efficacy and safety profile of Imatinib mesylate in advanced GIST. [55] A prospective multicentre phase 2 study of imatinib mesylate in Korean patients with metastatic or unresectable GIST is showing encouraging results. [56] Imatinib is taken as once daily oral tablet with very few side effects.

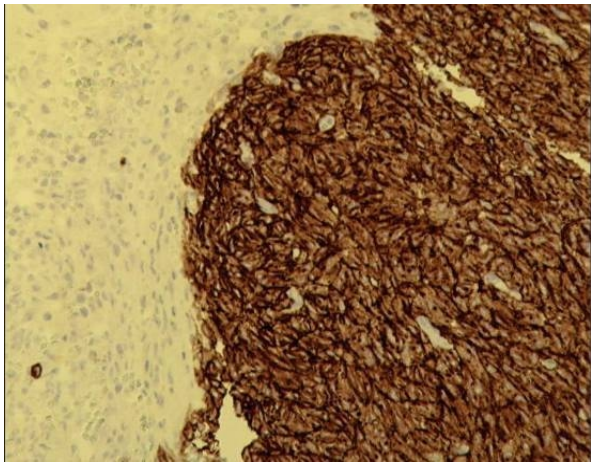
C kit gene mutation causes receptor protein which is constitutively active even when no ligand is bound to it. As GIST's grow more due to reduction in apoptosis than cell proliferation, conventional chemotherapy is less effective (7% response rate to



Rationale for use of Glivec in GIST



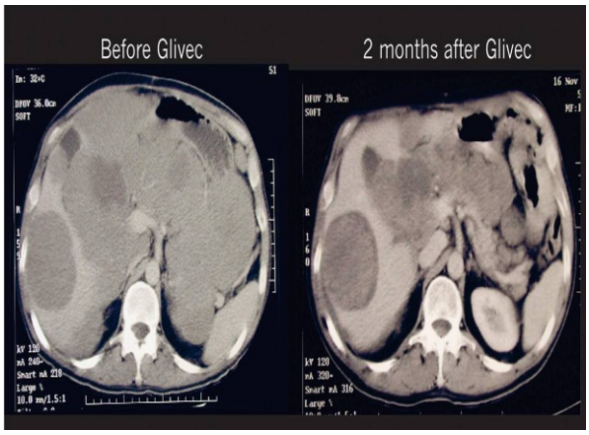
Treatment of unrescetable and/or metastatic GISTs with Glivec



GIST histopathology CD 117 staining

Erlotinib (Tarceva) & Gefitinib

These are the small molecule inhibitor of intracellular tyrosine kinase domain of EGFR. [58] This causes multiple effects like decrease cell proliferation, increase in apoptosis, inhibition of angiogenesis & invasiveness. EGFR is expressed in 40-80% as patients with non small cell lung cancer (NSCLC) and has been proposed a target for NSCLC therapy. [59,60,61]



Tumour liquefaction after treatment with Glivec

Several EGFR inhibitors are being evaluated in treatment of advanced NSCLC. [62, 63] The EGFR TK inhibitors (TKI s) Erlotinib and Gefitinib are in the clinical development for NSCLC treatment. The study of Cappuzzo et al was the first to show that high EGFR copy number correlated significantly with improved survival in patients treated with Gefitinib. [64] EGFR protein expression is assessed by IHC and EGFR gene copy number of FISH. The mutations so detected are potential biomarkers that may predict sensitivity to anti-EGFR therapy. Erlotinib is currently the only approved EGFRTKI for advanced NSCLC therapy in the USA and European Union. The 2 months survival advantage observed with Erlotinib compared with placebo in the pivotal phase III BR. 21 trial led to its approval for the second-line / third-line therapy of patients with advanced disease while Gefitinib is approved for use in Japan. [65]

Conclusion

There has been a wave of new anticancer drugs which are increasingly being used in cancers as targeted therapies. Majority are still in development or clinical trial phase whilst some have been approved by FDA for treatment of certain type of cancers. The role of histopathology laboratory in testing of tumors for the appropriate molecular pathology / protein targets is well established. Immunohistochemistry might be used as an initial screening test to identify negative or strongly positive cases and some other tests such as FISH could be performed on the intermediate cases.

controls. As the number of targeted cancer therapies increases, there will be a concomitant increase in the range of tissue based tests needed to select therapy in individual patients so called 'customized' therapy.

It is important to realize that unlike conventional anticancer drugs, these agents inhibit /prevent growth of cancer cells (cytostatic) rather than kill cancer cells (tumorcidal). Therefore use of these drugs alone of in combination with conventional anti cancer drugs will help in stabilizing the disease with long remission rather than to cure cancer. In the future, there agents may allow long term control of cancer to produce a chronic disease with a morbidity rather than an immediate mortality comparable with rheumatoid arthritis, chronic bronchitis or atherosclerosis.

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