Invited Article

The Changing Face of Cancer Therapeutics

Improved Outcome and Decreased Toxicity with Molecular Targeted Drugs

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Introduction

The treatment of patients with cancer has largely involved the administration of cytotoxic drugs with narrow therapeutic indices, with little selectivity for cancer cells over normal proliferating cells. The primary exception to this has been the successful administration of hormonal manipulation to treat breast and prostate malignancies. The development of hormonal manipulation arose from the observation by Sir George Beatson that breast carcinomas improved after bilateral oophorectomy. This led to the use of Tamoxifen and more recently aromatase inhibitors and oestrogen receptor antagonists. These targeted therapeutics are characterised by their ability to induce selective tumour cell death and achieve patient benefit with low toxicity, and have had a significant impact on the outcome of patients with early and advanced oestrogen receptor positive breast cancer.

Further advances in the understanding of tumour cell biology, the sequencing of the human genome, and the characterisation of the molecular differences between malignant and normal cells have, over the past two decades, resulted in the identification of a large number of critically important molecular targets. As with the identification of the importance of oestrogens and the oestrogen receptor, this has accelerated the development of molecularly targeted therapeutics and is rapidly revolutionising cancer medicine (Table 1). This brief review will describe some of the most important advances achieved and will attempt to predict what future cancer therapeutics will entail.

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Important Targets

Cancer, which comprises several hundred different diseases that differ in their genetic origin, manifests itself as a proliferating group of cells that are outside the normal regulatory framework of growth control. The cancer cell usually arises by the alteration or mutation of key cellular house-keeping genes, usually in a series of step-wise genetic changes, resulting in the increased activity of genes that promote cellular proliferation and inhibit cell death (called oncogenes) and the decreased or loss of activity of genes that negatively regulate cell proliferation (called tumour suppressor genes). This process, known as carcinogenesis, has been well characterised for several tumours. The identification of these genetic changes has allowed the development of anticancer drugs that can target the function of the cellular proteins involved in driving tumour growth. The list of relevant targets, which comprises hundreds of proteins, is rapidly increasing. These include proteins involved at different points of cell signaling, programmed cell death (apoptosis), and proteins involved in angiogenesis (the process by which tumours induce the growth of blood vessels to provide their blood supply). Drugs specifically targeting these proteins are now available. These are generally small molecule inhibitors or antibodies, although antisense and vaccine technologies are also being evaluated.

The Small Molecule Imatinib (Glivec™): An Oral Drug Curing Chronic Myeloid Leukaemia?

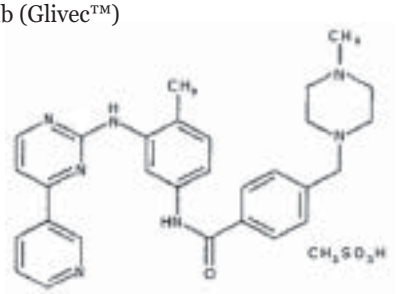
Imatinib, marketed by Novartis under the trade name Glivec™, exemplifies a rationally designed, molecularly targeted small molecule inhibitor (Figure 1). In 1960, Nowell and Hungerford described a causal relationship between chronic myeloid leukaemia (CML) and a consistent chromosomal abnormality.[1] This was identified as a translocation between chromosomes 22 and 9 that results in abnormally high levels of activated intracellular signaling by the tyrosine kinase c-Abl.[2] Preclinical studies have established this Bcr-Abl translocation to be the main genetic abnormality driving cellular proliferation in most CML cells.

Tyrosine kinases such as c-Abl are enzymes that catalyse the transfer of phosphate from adenosine triphosphate (ATP)

Table 1: *Examples of Recognised Important Targets 'Hijacked' by Tumour Cells*

Target	Molecular alteration	Tumour type	Therapeutic
HER2	DNA Amplification	Breast	Trastuzumab (Herceptin)
Bcr-Abl	Chromosomal Translocation	Chronic Myeloid Leukaemia	Imatinib (Glivec)
Bcl-2	Chromosomal Translocation	Follicular non-Hodgkin's Lymphoma	Bcl-2 antisense (Genasense)
c-Kit	Activating kinase mutation	GI stromal tumours (GIST)	Imatinib (Glivec)
B-Raf	Activating kinase mutation	Melanoma	BAY 43-9006
VEGFR	Loss of VHL Tumour suppressor	Renal	BAY 43-9006; Bevacuzimab (Avastin)
Ras	Activating mutation	Colorectal, pancreas	
EGFR	Activating mutation	Glioblastoma multiforme	Erlotinib (Tarceva)

Figure 1: Two-dimensional chemical structure of Imatinib (Glivec™)



to tyrosine residues on specific signaling proteins. These tyrosine amino acid residues function as cellular switches, with phosphorylation usually resulting in 'switching on' of cell signaling. Chemical compounds that can inhibit the function of these signaling kinase switches have been developed. Imatinib (Glivec™) is a small molecule inhibitor of c-Abl that binds to the ATP-binding site of this kinase, blocking its signaling function and inducing the cell death of Bcr-Abl positive CML cells. Clinical trials have confirmed that imatinib induces complete haematological and cytogenetic responses in patients with CML. Randomised clinical trials have reported that imatinib is superior to interferon and cytarabine. Complete haematological responses were observed in 97% of imatinib patients compared to 56% in the cytarabine and interferon arm, with complete cytogenetic responses being reported in 74% of the Imatinib arm compared to 8% in the interferon and cytarabine arm.[3] Responses to imatinib were rapid with most patients achieving a complete haematologic response within the first 4 to 6 weeks of therapy. Imatinib is administered orally, twice daily and is well tolerated with minimal toxicity.[3] It is now the standard of care for the first line treatment of patients with CML. It is hoped that many of the patients achieving cytogenetic complete responses from imatinib therapy will be cured of the disease. Imatinib is also active in patients with transformed CML. However, it is less active in this setting, presumably because the tumour cells have by this stage developed other genetic changes and the reversal of Abl signaling alone is no longer able to block tumour cell proliferation.

Imatinib is also active in the treatment of gastrointestinal stromal tumours (GIST) - a tumour type that is being diagnosed with increasing frequency. This neoplasm can arise from any organ in the gut, or from the omentum or mesentery and is highly refractory to chemotherapy and radiotherapy. It is rapidly fatal unless curative surgery is feasible. Abnormal activation of the transmembrane receptor c-KIT by constitutive mutations occurs in the majority of GISTs, leading to uncontrolled tumour growth. c-KIT is another tyrosine kinase enzyme switch that can also be inhibited by the chemical imatinib. A study of 147 patients with unresectable GIST treated with imatinib reported a partial response rate in 66% of patients and an additional 17% of patients had minor responses or stable disease.[4] KIT and PDGFRα mutational status were highly significant in predicting response. The patients have been followed up for a median of 21 months so the durability of response is not yet known. However, while secondary resistance and late progression is seen in GIST patients treated with imatinib, most remain on the drug for a prolonged period.

Many other small molecule chemical inhibitors of various key cellular proteins involved in tumour growth are now being evaluated in the clinic. Gefitinib (Iressa™, AstraZeneca) blocks epidermal growth factor signaling (erbB1 or EGFR) and has been approved for the treatment of patients with non-small cell lung carcinoma. There are high expectations that these novel agents will have a major impact on the care of cancer patients.

The Antibodies Trastuzumab (Herceptin™) and Cetuximab (Erbitux™)

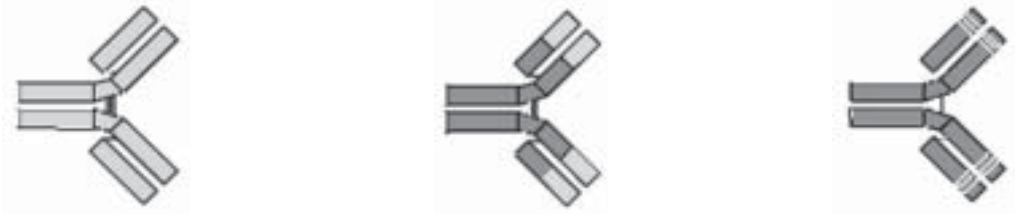
An alternative means of targeting key cellular proteins is through the development of monoclonal antibodies against the protein of interest. These antibodies were initially generated by immunising mice to raise monoclonal antibodies to the target human receptor. To overcome the development of a human anti-mouse antibody (HAMA) immune response against murine monoclonal antibodies in treated patients, human or mouse chimeric (or partially 'humanised') antibodies have been produced. These primarily retain the small portion of the murine protein sequences responsible for antigen binding, with the remainder of the molecule being composed of human immunoglobulin (Figure 2). Fully humanised monoclonal antibodies have also been produced, for example ABX-EGF (Abgenix, Inc), using the Xenomouse™ transgenic strain. These antibodies do not contain mouse protein sequences and should not be immunogenic. Therefore they have a slower clearance rate than mouse or mouse-derived monoclonal antibodies, facilitating repeat administration.

In 1962, Stanley Cohen isolated a novel protein, now known as epidermal growth factor (EGF), from the submaxillary gland of mice. It promoted accelerated eruption of incisors and eyelid opening in newborn animals.[5] After four decades of research, it is now known that EGF and its family of related receptors (EGFR, HER2, erbB3 and erbB4) play an important role in the modulation of tumour cell proliferation and apoptosis. They are normally found on epithelial cells but are often over-expressed in tumour cells. Drugs that target these receptors, and their downstream signalling pathways, are widely viewed as potential anticancer therapeutic agents.

One third of breast cancers overexpress human epidermal growth factor receptor 2 (HER2) due to HER2 gene amplification (high copy number of HER2 DNA).[6] This is associated with an inferior prognosis, with shorter disease-free and overall survival. A recombinant monoclonal antibody, trastuzumab, targets this receptor. Patients randomised to receive chemotherapy with trastuzumab as first line therapy in metastatic disease, when compared to patients receiving chemotherapy alone, have improved response rates and survival.[7] Also, when used as a single agent, trastuzumab produces durable objective responses in women who had not previously received chemotherapy for their metastatic disease.[6] However, only breast cancer patients with HER-2 gene amplification significantly benefit from trastuzumab. Trastuzumab is largely non-toxic although it has been uncommonly associated, in patients previously exposed to the anthracycline doxorubicin (adriamycin), with cardiac toxicity usually manifested as asymptomatic decreases in the left ventricular ejection fraction.[8] These decreases appear to be reversible and are probably related to the role of HER2 signaling in cardiac repair and remodelling following anthracycline induced injury. The clinical role of trastuzumab in other tumour types overexpressing HER2 is being explored, and may important in a small subgroup of patients with non-small cell lung carcinoma.

Cetuximab is a monoclonal antibody directed against the extracellular domain of the EGF receptor (EGFR). It arrests cells in the G1 phase of the cell cycle, inhibiting the growth of EGFR expressing tumour cells.[9] A recent trial randomised 329 heavily pre-treated patients, with histologically proven metastatic colorectal carcinoma, that progressed whilst on or within 3 months of an irinotecan-based regimen, to receive either cetuximab alone or cetuximab with irinotecan. All the patients had EGFR expressing tumours. The researchers found that 22.9% of patients who received combination treatment of cetuximab and irinotecan had a partial response and tumour growth was delayed by approximately 4.1 months. For patients

Figure 2: Schematic representation of humanised mouse monoclonal antibodies. In the case of the chimeric antibody, the intact rodent variable regions (grey) are attached to human constant (FC) regions (black). In fully humanised antibodies, the antigen binding regions from the rodent antibody variable region, called complimentarity determining regions (CDR) (3 in the heavy chain and 3 in the light chain [grey]), are combined with a human antibody that does not contain any mouse protein sequences and should, therefore, not be immunogenic



Mouse monoclonal antibody

Chimeric (partially humanised) monoclonal antibody

Fully humanised antibody

who received cetuximab alone, the tumour response rate was 10.8% and tumour growth was delayed by 1.5 months.[10] Cetuximab is well tolerated: allergic reactions and a reversible acne-like skin rash are the most relevant side effects.[10] In February 2004, the US Food and Drug Administration (FDA) approved cetuximab for use in patients with colorectal carcinoma. The FDA has also approved a diagnostics kit for the assessment of the EGFR status of patients' tumours.

Anti-angiogenesis and the Antibody Bevacizumab (Avastin™)

Anti-angiogenic drugs are another exciting development in the armamentaria to treat cancer. Bevacizumab is a recombinant humanised monoclonal antibody against vascular endothelial growth factor (VEGF), a protein that promotes growth of blood vessels, which tumours need to survive. In a study of 815 patients with colorectal cancer treated with a standard chemotherapy regimen of irinotecan, fluorouracil and folinic acid (IFL), the addition of bevacizumab was shown to confer a survival advantage.[11] Patients treated with the bevacizumab combination had a median overall survival of 20.3 months, while patients treated with standard chemotherapy alone had a median survival of 15.6 months. Tumours were reduced in size by at least half in 45% of patients who received bevacizumab and in 35% of patients who received chemotherapy alone. Overall, more patients receiving the bevacizumab combination responded to treatment (44.9% versus 34.7%) and their response was maintained for longer (10.4 months versus 7.1 months). All differences were statistically significant. The incidence of gastrointestinal perforation, though rare, may be increased by the addition of bevacizumab to IFL. High blood pressure was higher in patients treated with the bevicizumab combination (22.4% and 8.3%; $p < 0.01$), but was easily managed with oral anti-hypertensive medication.

Conclusion

Over two hundred new anticancer molecular targeted drugs are at present being evaluated in clinical trials. An even larger number of compounds are undergoing preclinical tests. Their greatest value will probably lie in their use in strategically selected combinations based on tumour biology. Improved diagnostics and therapeutics are rapidly changing the treatment of patients with cancer, and it is envisioned that cancer survival will increase in the years ahead.

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