Tumour Genetics and Genomics to Personalise Cancer Treatment

Pei Jye Voon,1 MD, MMed, MRCP, Hwai Loong Kong,2,3 MBBS, MMed, FRCP (Edin)

Abstract

Personalising cancer treatment to optimise therapeutic efficacy while minimising exposure to the toxicities of ineffective drugs is the holy grail of medical oncology. Clinical parameters and conventional histopathological characterisations of cancers are no longer adequate to guide the practising oncologists in treatment planning. The explosion of knowledge in cancer molecular biology has led to the availability of tumour-specific molecules that serve as predictive and prognostic markers. In breast cancer, HER-2 positivity is a good predictor for success of anti-HER-2 trastuzumab monoclonal antibody therapy. K-ras mutational status predicts the likelihood of response to anti-EGFR monoclonal antibodies in advanced colorectal cancers. Similarly, EGFR mutational status in pulmonary adenocarcinoma is highly predictive for responses or otherwise to tyrosine kinase inhibitors. Notwithstanding our deeper understanding of tumour biology and the availability of predictive and prognostic laboratory tools, we are still far from achieving our dream of the perfect personalised cancer treatment, as each tumour in a particular patient is unique to itself. A much coveted, real-time, anti-tumour drug sensitivity testing in the future may one day pave the way for truly treating the right tumour with the right drug in the right patient.


Key words: Personalised cancer treatment, Predictive markers, Prognostic markers

Introduction

The goal of any medical therapy is to accurately deliver the right drug against the right disease in the right patient. We have taken this for granted in antimicrobial therapy. Conventional cancer treatment has not been able to achieve such specificity. However, the situation has improved dramatically over the past 2 decades, partly because of the exponential growth of our knowledge in tumour molecular biology. With this better understanding of what makes cancer tick, scientists and doctors are now able to design and utilise drugs that are more specifically tailored to treat cancer subtypes that are narrowly-defined by their molecular traits. The ultimate goal of personalised cancer therapies is to optimise therapeutic efficacy while minimising the exposure of patients to the toxicities of ineffective drugs.

Historical Perspectives

Tumour-specific cancer therapy is not a novel concept. The notion of drug-targeting can be traced back to historical examples of radioactive iodine therapy in differentiated thyroid carcinoma and androgen deprivation therapy in metastatic prostate carcinoma. One of the prototypical examples of tumour-specific therapy is endocrine therapy against hormone-receptor positive breast cancer. Back in the early 1970s, it was shown that approximately 50% of estrogen receptor (ER)-positive breast cancers achieved objective responses when treated with endocrine therapy. In contrast, patients with ER-negative tumours rarely did. This molecular specificity was further validated in the landmark meta-analysis of randomised adjuvant breast cancer treatment trials. Adjuvant use of tamoxifen, as
Tumour Heterogeneity: A Hurdle to Treatment Predictability

Anatomical classifications of cancer are rapidly becoming inadequate in cancer treatment today. The enormous biological heterogeneity within each tumour type (such as ‘breast cancer’ or ‘lung cancer’) makes such general labelling uninformative in contemporary cancer treatment. Indeed, treatment response rates in patients with a particular malignancy can vary from about 10% to more than 90%. The only sensible solution to this therapeutic chaos is to subdivide cancers into more homogeneous subgroups, so as to enhance the predictability of treatment responses. In the past, such subdivision was achieved through clinical and/or conventional histopathological criteria. In the era of tumour molecular biology, we now have the additional availability of tumour-specific (or near tumour-specific) molecular markers that allow the segregation of cancers into more distinctive subgroups.

Before a medical oncologist plans the treatment for a cancer patient, he or she would like to know the answers to 2 questions:

(i) What is the natural history of the cancer in this particular patient?
(ii) What drug or drugs are most appropriate against this cancer in this particular patient?

The first question is answered through the use of prognostic markers, which provide information on the cancer outcome without consideration of the treatment that the patient will be receiving. In contrast, the selection of the most appropriate cancer treatment against a specific cancer in a particular patient is best made through an appreciation of its predictive marker profile. Predictive markers play an important role by providing probabilistic information on the likelihood of benefiting from a specific therapy. Predictive markers thus guide the choice of treatment. Potential candidate predictive markers in a particular tumour tend to be the direct targets of drugs, molecules that signal downstream of the primary target, molecules that involved in DNA repair, or polymorphisms in genes involved in drug metabolism.

Some of the molecular markers can have both predictive and prognostic values. This review will discuss how currently available predictive and prognostic molecular markers are used to personalise treatment of the three common tumour types, namely breast, colorectal and lung cancers.

Breast Cancer

Predictive and prognostic markers of breast cancer have been widely studied for the past few decades. The earlier markers encompass routine clinical and conventional pathological parameters such as patient age, menopausal status, tumour size, histological grade, axillary nodal status, and hormonal receptors status. Adjuvant endocrine therapy may be appropriate for a small, low-grade, ER-positive breast cancer in an elderly postmenopausal lady, whereas adjuvant chemotherapy is often required in a young premenopausal lady with a large, high-grade, hormone-receptor negative, node-positive breast cancer. These traditional markers have been widely used in guidelines such as the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology and the St Gallen’s Consensus. In addition, a validated tool such as Adjuvant! Online utilises these clinical-pathological factors in assessing recurrence risk in breast cancer, and the positive impact of adjuvant endocrine therapy and chemotherapy.

The advent of molecular assays examining various gene expressions in the tumour cells has enabled us to identify new prognostic and predictive molecular signatures. Specifically, gene expression profiling can reveal different molecular signatures for tumour with high and low risk of progression according to the differential expression of finite number of genes. These signatures have been validated in independent cohorts of patients. One of the commercially-available assays, Oncotype Dx™ has been recommended by ASCO panelists in 2007 and NCCN Clinical Practice Guidelines in Oncology to be useful to predict the risk of recurrence in patients with node-negative and ER-positive disease treated with tamoxifen, without chemotherapy. Predictive value of Oncotype Dx™ for magnitude of chemotherapy benefits in such a cohort of patients was further validated by subsequent publication. Other commercially available assays include Mammaprint™, which provides a 70-gene signature of fresh frozen breast tumour.

Further treatment specificity is informed through the tumour’s molecular profile. One of the successful stories is the use of trastuzumab as targeted therapy in HER-2 positive breast cancer. HER-2 is a member of the HER (ErbB) transmembrane receptor tyrosine kinase family which includes HER-1 (c-ERB-1), HER-2 (c-ERB-2), HER-3 (c-ERB-3) and HER-4 (c-ERB-4). Overexpression of HER-2 protein is found in 15% to 25% of newly
diagnosed invasive breast cancer. Enhanced cell signalling secondary to the overexpression of HER-2 protein will culminate in increased cell proliferation, cell motility, cell survival, angiogenesis, invasion and metastasis. HER-2 has been widely studied as a therapeutic target and as a predictive marker for cancer therapy using specific HER-2 antagonists. Trastuzumab is a humanised monoclonal antibody which binds to the extracellular domain of the HER-2 receptor and thus inhibiting signalling from this receptor. The predictive value of HER-2 for response to trastuzumab in breast cancer has been shown in both adjuvant and advanced disease settings. One of the pivotal trials using chemotherapy with additional trastuzumab in the advanced breast cancer setting has reported a higher response rate (50% vs 32%, P < 0.001) and statistically significant benefit in progression-free survival (median, 7.4 vs 4.6 months; P < 0.001) and overall survival (median survival, 25.1 vs 20.3 months; P = 0.01). In the adjuvant breast cancer treatment setting, efficacy of trastuzumab has been evaluated in several prospective randomised trials. A meta-analysis of these trials showed that the addition of 1 year of trastuzumab to standard adjuvant chemotherapy significantly improved disease-free survival (RR 0.62; 95% CI, 0.56 to 0.68). Superiority was also observed for patients receiving trastuzumab with respect to mortality (RR, 0.66; 95% CI, 0.43 to 0.77). Besides trastuzumab, small molecule lapatinib antagonizes both EGFR and HER-2 by competing with the ATP for binding to the intracellular domain of these receptors. A randomised phase III trial had shown promising results of combination of lapatinib with capecitabine in patients with previously treated advanced breast cancer.

Other emerging breast cancer therapy predictive markers are TOPO2a gene amplification and Tau expression which predict sensitivity to anthracyclines and paclitaxel, respectively. Besides that, BRCA1/2 mutation is another promising predictive marker for the use of a PARP inhibitor in metastatic breast cancer.

**Colorectal Cancer**

The cornerstones of colorectal chemotherapy are fluoropyrimidines, oxaliplatin and irinotecan. The selection of these agents is largely based on data derived from phase III randomised clinical studies. Adjuvant chemotherapy using fluoropyrimidines, with or without oxaliplatin, is the standard of care for stage III colon cancer patients.

Stage II colon cancer represents a particularly difficult treatment conundrum for oncologists. Traditionally, clinico-pathological features including intestinal obstruction and perforation, poorly differentiated tumours, and lymphovascular invasion are used to prognosticate and influence the adjuvant therapy decision in this group of patients. However, the use of these clinical predictors is more empirical than quantitative. We now have a multi-gene RT-PCR assay for colon cancer, analogous to the breast cancer Oncotype DX test. This assay utilises differential expression of 12 genes to predict the risk of recurrence in patients with stage II colon cancer. However, the current assay is not sufficiently discriminatory to accurately predict the therapeutic benefit of adjuvant chemotherapy.

Tumour microsatellite instability (MSI) status may provide information on prognosis and the usefulness of fluoropyrimidines. Patients whose tumours demonstrate high levels of MSI appear to have a better prognosis than those with microsatellite-stable (MSS) tumours. Paradoxically, these cancers may also be resistant to certain cytotoxic drugs, including fluoropyrimidines. However, there is still considerable debate about the predictive value of MSI status. In addition, it is not known whether the predictive value of MSI for fluoropyrimidines is translatable to oxaliplatin-based regimens such as FOLFOX, which has become the standard regimen for stage III disease. Thus, MSI status is still not widely used as a decision tool in community oncological practice.

EGFR, a transmembrane tyrosine kinase receptor that stimulates colorectal cancer cell growth and survival by signalling through the MAPK, PI3K and JAK/STAT pathways, is a target for treatment. Cetuximab and panitumumab are monoclonal antibodies that target EGFR at its extracellular domain. They have significant antitumour activity against advanced colorectal cancers, especially when used in conjunction with systemic chemotherapy. These antibodies were initially used empirically, without regard for the molecular profile of the colorectal tumours. Several studies also showed no significant correlation between EGFR expression and response to cetuximab therapy. Acne-like skin rash was found to be an indirect crude clinical predictor of antitumour response to cetuximab.

K-ras is a downstream molecular effector of the EGFR signalling pathway. In recent years, K-ras mutation status has emerged as a useful predictive marker of the likelihood of positive response to either monoclonal antibody. Data from the CRYSTAL trial, a randomised phase III-trial in metastatic colorectal cancer patients comparing FOLFIRI chemotherapy with or without cetuximab as first line systemic treatment, showed significantly better overall response rate (59.3% vs 43.2%, P = 0.0025) and progression-free survival (P = 0.0167; HR 0.68; 95% CI, 0.05 to 0.934) in favour of the cetuximab arm if the tumours harboured wildtype K-ras. In contrast, subgroup analysis showed K-ras mutation as a negative predictor of response to cetuximab. These findings were consistently reproduced.
in other clinical trials utilising panitumumab or cetuximab with better response rates and progression-free survivals in patients with wildtype K-ras colorectal cancers.\textsuperscript{38,42}

Other downstream signalling mediators have also been extensively studied in predicting treatment response to anti-EGFR monoclonal antibodies.\textsuperscript{43,44} These downstream intracellular molecules include BRAF, PI3KCA and PTEN. Mutations of these genes have been found to confer resistance to anti-EGFR antibodies.

**Lung Cancer**

Despite remarkable advances in our understanding of non-small cell lung cancer (NSCLC) over the past decades, the survival for patients with advanced disease treated with chemotherapy remains poor, with a median survival of 8 to 10 months.\textsuperscript{45} It is now clear that NSCLC is a highly heterogeneous cancer. Not only are there different histological subtypes of NSCLC, even within the subgroup of adenocarcinoma, one can further subdivide it into various molecular subgroups that have distinctively different responses to targeted therapy. Efforts to improve the outcome of this dismal disease focus on the identification of predictive molecular markers that optimise treatment selection.

Histology is emerging as an important factor when choosing the appropriate chemotherapy in NSCLC. In the largest phase III trial to compare two chemotherapy regimens, previously untreated patients with advanced stage IIIB or IV NSCLC were randomly assigned to cisplatin plus pemetrexed or cisplatin plus gemcitabine.\textsuperscript{46} Overall, there was no difference in median survival between the two arms (10.3 months with both regimens). However, in a preplanned subset analysis, survival in the 847 patients with adenocarcinoma was significantly prolonged with cisplatin plus pemetrexed compared to cisplatin plus gemcitabine (median survival of 12.6 vs 10.9 months, respectively). Conversely, cisplatin plus gemcitabine was superior to cisplatin plus pemetrexed in the 473 patients with squamous cell carcinoma with median survival of 10.8 versus 9.4 months, respectively (HR 1.23; 95% CI, 1.00 to 1.51; \(P = 0.05\)).

Besides that, there is a growing body of information to suggest that chemo-sensitivity may be predicted by the tumour’s molecular profile. The level of expression of the ERCC-1 gene, the RRM-1 gene, and the thymidylate synthase gene appear to influence the treatment responses to platinum, gemcitabine and pemetrexed chemotherapy, respectively.\textsuperscript{47-49}

In the era of oral EGFR tyrosine kinase inhibitors (TKIs), retrospective analysis had suggested that certain clinical characteristics such as female gender, adenocarcinoma histology, Asian ethnicity and a history of never/light smoking were associated with increased response to these oral drugs.\textsuperscript{50,51} Over the past 2 years, it has become clear that specific molecular aberrations offer much greater predictive value to TKIs than the conventional clinico-pathological predictors. The efficacy of erlotinib in pre-treated advanced NSCLC was established in a phase III study, BR21.\textsuperscript{52} A significant improvement in median survival was seen in patients receiving erlotinib compared with placebo (6.7 months vs 4.7 months, HR 0.70; 95% CI, 0.58 to 0.85). Additional biomarker studies were also reported in this study.\textsuperscript{52} Erlotinib treatment was associated with prolonged survival in patients whose tumours exhibited EGFR overexpression by immunohistochemistry (HR 0.68; 95% CI, 0.49 to 0.95) or EGFR amplification by gene copy number (HR 0.44; 95% CI, 0.23 to 0.82).

Discovery of molecular predictors of EGFR TKIs sensitivity such as the somatic activating mutations in exon 18-21 of EGFR (commonly exon 19 deletions and L858R point mutation in exon 21) is a major breakthrough in the management of NSCLC.\textsuperscript{53}

The Iressa Pan-Asia Study (IPASS), a randomised phase III trial conducted in Asia where patients were selected by clinical characteristics for EGFR mutation (never/light smoker, adenocarcinoma subtype), were randomised to gefitinib or carboplatin and paclitaxel.\textsuperscript{54} A superior PFS favoring gefitinib (HR 0.74; 95% CI, 0.65 to 0.85) was seen. Furthermore, in patients receiving gefitinib, higher response rates (43% vs 32.2%) and superior quality of life were reported. In the IPASS study, in preplanned subset analysis, patients with EGFR mutations had a prolonged PFS with gefitinib compared with chemotherapy (HR 0.48; 95% CI, 0.36 to 0.64) whilst patients without EGFR mutation had a poorer PFS with gefitinib (HR 2.85; 95% CI, 2.05 to 3.98). Response rate for EGFR mutation positive and negative patients was 71.7% and 1.1%, respectively.

Another promising predictive marker is oncogenic fusion gene of EML4-ALK which is present in 2% to 7% of NSCLC. Patients whose lung cancers harbour this fusion gene tend to be younger with little or no exposure to smoking and had adenocarcinoma. In a recently published open-labelled, multicentered, two-part phase 1 trial of crizotinib, an oral ALK and MET tyrosine kinases inhibitor had shown overall response rate of 57% and 6-months progression-free survival of 72% in previously treated patients.\textsuperscript{55}

Tumour heterogeneity may impact on sensitivity, as well as resistance, to specific cancer drugs. An example is the acquisition of EGFR TKI resistance in NSCLC through MET amplification.\textsuperscript{56} A rare population of MET-positive clone may already be pre-existing in the untreated lung tumour, only to be selected for during disease progression after exposure to EGFR TKIs.
Patient Stratification in Clinical Trials

Patient stratification is not only useful for clinical practice, it may potentially improve the way clinical trials are conducted as well. By limiting eligibility for clinical trials to specific subgroups of patients, based on pre-specified tumour molecular profiles, we may be able to enrich and differentiate the treatment groups such that meaningful responses to experimental drugs are more likely to be achieved. This may help reduce wastage and increase trial efficiency.

Limiting drug testing to smaller, more specific, subgroups of patients increases the chances of trial success. This should sufficiently offset the disincentive to the pharmaceutical industry of a potentially smaller market share for each new drug. One example is the development of cetuximab, which has become an important agent in the management of metastatic colorectal carcinoma. Cetuximab, as monotherapy or in combination with other chemotherapeutic agents, was initially investigated as a therapeutic agent in treatment of metastatic colorectal carcinoma, without regard to the tumour K-ras mutational status.37,57 The end results showed modest treatment responses. Confidence in using this drug waned. A decision was subsequently taken by the company that developed the drug to refine the treatment specificity by using K-ras mutational status as a discriminator. It then became clear, through a number of major clinical studies alluded to in the section on colorectal cancer, that the benefit of cetuximab was restricted to patients whose tumours lacked a K-ras mutation.41,42,58 Cetuximab is now a standard drug for K-ras wildtype colorectal cancer. The decision to restrict drug usage, based on tumour molecular profile, has paid off eventually.

Conclusion

In spite of major advances in our knowledge of tumour genetics and genomics and the subsequent discovery of a wide array of molecular tumour markers, we are still far from achieving the ‘perfect personalised cancer treatment’ goal. The fact that we are unable to achieve 100% response rate even in molecularly-defined subgroups clearly indicates the heterogeneity that must exist in such subgroups. Generally, molecularly-defined personalised treatment at this time still entails a fair dose of statistical probability and better molecular tools are still required.

Another unsolved problem in practical oncology is tumour heterogeneity over time. This is the hypothesis of evolution of genetic aberrations in tumour over time and after exposure to the initial therapies. Darwinian forces select for tumour clones with genetic aberrations that confer drug resistance. At the time of relapse, the metastases may harbour a molecular profile that may differ from that of the primary tumour. In light of this, we may need to consider whether multiple samplings are needed to fully characterise the tumours that we are treating, in order to ensure that optimal therapies are given.

Essentially, each tumour in a particular patient is unique, as defined by the complex repertoire of upregulated and down-regulated genes, whose status may vary dynamically over time. Hopefully, real-time sensitivity testing against a panel of anticancer drugs may eventually become available, in a way that we carry out antimicrobial testing for infected specimens from particular patients. Some early successes are seen in the use of patient-derived lung cancer and colon cancer xenografts.59,60 The use of such patient-derived tumour models in immunosuppressed animals may give us invaluable insights into the molecular complexity of primary human cancers, and may provide useful preclinical models to predict treatment sensitivities in real time.

Personalised cancer treatment holds many promises. It is also fraught with theoretical and practical challenges. Notwithstanding its limitations, however, personalised cancer treatment is a worthy goal to achieve. We have made a small but significant step towards it.

REFERENCES


