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1. Introduction

In this era of personalised medicine, the focus of oncology drug development is shifting from classic chemotherapeutic drugs to rationally designed molecularly targeted agents (MTAs). This development has been accelerated by improved understanding of the key features of human tumour biology which have emerged over the last decade. A seminal paper by Hanahan and Weinberg (2000) proposed six vital elements for tumour formation, survival and progression. The six ‘Hallmarks of Cancer’ were sustained proliferative signalling, evasion of growth suppressors, resistance to cell death, replicative immortality, angiogenesis and activation of invasion and metastasis. Hanahan and Weinberg updated their findings in 2011 with further evidence describing the complexity of these hallmarks and the addition of further hallmarks, including modification of energy metabolism to fuel cell growth and evasion of immunosurveillance. The tumour micro-environment is also a critical factor in the regulation of tumour growth and progression, with multiple stromal cell types creating a succession of supportive tumour micro-environments enabling invasion of normal tissue and subsequent metastasis.

Recent successes have utilised these advances in understanding to create a strong biologic rationale for drug development, primarily focusing on targets of a single ‘Hallmark’. However, a number of challenges remain, not only in understanding the complex molecular pathways and networks, their interaction and mechanisms of resistance, but also in the drug development process through early incorporation of biomarkers to create rational drug development strategies. Challenges also lie in defining robust criteria to appropriately select patients for novel therapies. Effective trial design with integration of patient enrichment strategies is paramount to streamline drug development and deliver timely information to guide progress of drugs along the pipeline. The application of new technologies and novel strategies that address these problems will be discussed in detail in this chapter.

2. From hypothesis to proof of concept

Historically, the emphasis for drug development has focused on evidence-based medicine in large trials of unselected patient populations, with the benchmark endpoint for new drugs being overall survival or other intermediate endpoints. This ‘one size fits all’ paradigm did
not always take into account intra- and interpatient tumour heterogeneity commonly leading to large scale failure rates of multinational phase-III trials. Incorporating measures of pathway activity and tumour efficacy into early phase trials may help avoid failure in later phases of drug development. Early validation of pharmacodynamic assays to measure target blockade and assess optimal dose range and dosing schedule is essential. Establishing ‘proof-of-concept’ can then correlate anti-tumour activity in a selected patient population with validated predictive and intermediate endpoint biomarkers (De Bono & Ashworth., 2010).

For example, in patients with non-small cell lung cancer (NSCLC), correlation of epidermal growth factor receptor (EGFR) mutations with response to the EGFR inhibitors, gefitinib or erlotinib, occurred only after a number of negative trials. Although phase-II data in the second-line setting in patients with NSCLC was encouraging, when taken to a phase-III trial in an unselected group of patients with refractory disease, gefitinib failed to show a benefit in either overall survival or time-to-treatment failure when compared to placebo (Thatcher et al., 2005). In this context, it was only that retrospective analyses could help identify a subpopulation benefiting from treatment including being a female, never-smoker and of Asian origin. Similarly, erlotinib demonstrated progression-free and overall survival benefits both in the second-line setting and as maintenance therapy in patients with stable disease after first-line chemotherapy (Cappuzzo et al., 2010; Shepherd et al., 2005). However, the incremental benefits in these unselected patient populations were small, measured in weeks for progression-free survival and 1-2 months for overall survival. Ultimately it was the selection of patients based on EGFR mutation status that demonstrated a marked improvement in response rates and survival in phase-III trials comparing chemotherapy and gefitinib (Fukuoka et al., 2011), as well as chemotherapy and erlotinib in the first-line setting (Rosell et al., 2011).

We have witnessed similar studies in patients with advanced colorectal cancer (ACRC) treated with the monoclonal antibody cetuximab. Initially, treatment with cetuximab was conducted in patients with EGFR over-expression, assessed by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded (FFPE) tumour specimens (Cunningham et al., 2004). It was only later that the importance of Kirsten rat sarcoma-2 virus oncogene (KRAS) mutation was demonstrated; and this, in combination with an increased understanding of the complex EGFR downstream signalling cascade were the first steps in identifying a predictive biomarker for EGFR directed therapies in patients with ACRC. Several studies identified that patients with KRAS mutation did not respond to EGFR directed therapies, whereas patients who had wildtype (wt) KRAS tumours had response rates of over 50% (Lievre et al., 2006; Karapetis et al., 2008). More recently, it has been demonstrated that in fact, not all KRAS mutations are created equal. Although the presence of the majority of KRAS mutations precludes response to the EGFR inhibitors in ACRC, other KRAS mutations, particularly in codon 13, may predict a response similar to that demonstrated in wt KRAS tumours (De Roock et al., 2010).

These are just a few examples that demonstrate how the improved understanding of tumour biology supports a hypothesis-driven approach to the discovery of compounds to potentially generate more selective inhibition of key signalling proteins, pathways and networks. In this context, one of the most challenging tasks is the identification of the right target and more importantly whether this target is ‘druggable’. For example, although we know that RAS mutations are an early component of tumorigenesis and are identified in approximately 30% of human cancers, attempts to target RAS have been unsuccessful to
date as complex molecular structures constrain binding to the active site or pocket (Gysin et al., 2011). In contrast, selective inhibition of the v-raf murine sarcoma viral oncogene homologue B1 (BRAF) in patients with BRAF V600 mutant melanoma is associated with a dramatic improvement in response rates and survival. The strong biologic rationale of this approach was established through identification of the importance of the mitogen-activated protein kinase (MAPK) pathway in this disease and will be discussed at a later point in this chapter.

3. Biomarker development

Predictive and prognostic biomarkers are increasingly important in tailoring treatment decisions for individual patients. These markers are objectively measured to evaluate pathological processes or pharmacological responses to a therapeutic intervention, and can be any kind of molecule, substance, or genetic marker which is traceable (Atkinson et al., 2001). Predictive biomarkers provide information on response to a treatment, whereas prognostic biomarkers give information about outcome independent of the treatment effect. Historically, biomarkers have often been developed in retrospective analyses and were only in some cases prospectively applied. The retrospective approach was often criticised for being slow and difficult in practice, as well as raising concerns regarding heterogenous sample collection and validity. There are increasing efforts to incorporate new biomarker strategies into the earliest stages of clinical trial design, whether these are mutational analyses, clinical, or imaging measures, so that information can be gathered early and continually revisited during and after trial completion to inform the clinical development process.

As witnessed with a number of targeted agents, such as trastuzumab in human epidermal growth factor receptor-2 (HER2) positive breast cancer, the prospective analysis of HER2 as a predictive biomarker in clinical trials resulted in higher response rates and increased survival in this selected patient population, both in the metastatic and adjuvant setting (Slamon et al., 2001; Romond et al., 2005). This selective approach not only led to better outcomes for this subgroup, but ultimately to shorter and streamlined regulatory approval timelines. The use of trastuzumab in an unselected breast cancer population would undoubtedly have masked its true efficacy and potentially curtailed its development. Importantly this selective biomarker approach became a good example of what challenges researchers are facing when developing accurate, functional and standardised biomarker assays.

HER2 gene amplification was first observed to be a potential biomarker in breast cancer when its presence in 25% of axillary lymph-node positive breast cancers was correlated with worse prognosis (Slamon et al., 1987). Additional studies confirmed that HER2 protein over-expression was also a poor prognostic marker in breast cancer, correlating with decreased relapse-free and overall survival (Ravdin et al., 1995). The trastuzumab clinical trials were initially designed using HER2 over-expression measured by IHC with a centralised sponsor developed assay, which was particularly important as there was no standardised assay at that time. As the testing of HER2 was expanded from central to local laboratories, with incorporation of fluorescence in-situ hybridisation (FISH) in addition to IHC, there were concerns about the correlation and regulation of such assays. Although the results of the five adjuvant trastuzumab trials in HER2 positive early stage breast cancer clearly showed a significant clinical benefit in both progression-free and
overall survival, the testing algorithms for HER2 were not consistent across these trials. HER2 testing included either IHC supported by FISH testing for intermediate IHC result (IHC2+) or reliance on FISH testing alone to assess gene amplification ratios. Concern was generated at the lack of accuracy and validation of HER2 testing in some instances as several assays were in use, including both validated assays, but also so called “home brew” assays developed in local pathology laboratories. Sub-studies from two of the adjuvant trials demonstrated that approximately 20% of HER2 assays performed at the primary treatment site were incorrect compared to re-evaluation in a high volume, central laboratory (Paik et al., 2002; Roche et al., 2002). Furthermore, the sensitivity of IHC itself was of concern. For example, one study demonstrated that commercially available US Food and Drug Administration (FDA) approved IHC methods were significantly less accurate than FISH at correctly characterising tumours with known HER2 status. Depending on the IHC method and use of HER2 antibody, correlation with FISH positivity ranged between 67-83%, with greater susceptibility to inter-observer variation (Bartlett et al., 2001).

Clearly in the case of IHC testing, several contributing factors may further impact on sensitivity and specificity including initial sample processing, time to and type of fixation, analytic variables of assay validation, equipment calibration, use of standardised laboratory procedures, training of staff, test reagents, use of standardised control materials and use of automated laboratory methods.

Slamon et al. (1989) demonstrated that a proportion of breast cancers known to have gene amplification and over-expression of HER2, in fact lose membrane staining after paraffin embedding and are negative on IHC assessment. Loss of antigenicity resulting in a potential false negative IHC can be affected by poor standardisation of fixative methods.

To overcome this lack of concordance in HER2 testing, which can so markedly impact on patients’ prognosis and survival, an American Society of Clinical Oncology (ASCO) panel developed guidelines to improve the accuracy of HER2 testing (Wolff et al., 2007). These recommendations covered over 30 aspects of testing and requirements including the HER2 testing algorithm, optimal FISH and IHC testing and interpretation, tissue handling, internal validation and quality assurance procedures, optimal external proficiency, laboratory accreditation and regulatory requirements, statistical requirements for assay validation and international external quality assessment initiatives. Despite these guidelines, there were concerns that IHC assessment still lacked sufficient sensitivity to be used alone to decide on HER2 status (Carlson., 2008) though this remains the standard initial assessment in most laboratories.

In 2010, the addition of trastuzumab to first-line chemotherapy in HER2 positive advanced gastric cancer demonstrated a survival benefit (Bang et al., 2010). Similar to breast cancer, approximately 20-30% of gastric and gastro-oesophageal junction (GOJ) cancers show HER2 over-expression, but the testing criteria in gastric specimens differs significantly (Albarello et al., 2011). This is related to the increased frequency of heterogeneity of HER2 positivity in gastric cancer compared with breast cancer, as well as variations in membrane staining and the number of stained cells necessary to diagnose a positive case. In addition there is also less stringent correlation between HER2 amplification and protein over-expression with more than 20% of cases carrying HER2 amplification, often of low level, without HER2 expression. Clinically in this group of patients, there is no apparent benefit from adding trastuzumab to chemotherapy (Bang et al., 2010). Similarly, Hofmann et al. (2008a) demonstrated concordance between FISH and IHC of 93%, with 7% of specimens demonstrating FISH positivity with negative or equivocal IHC staining.
Discordant findings have also been demonstrated with HER2 testing on surgical specimens compared to biopsy alone, with more than 10% of cases showing discrepant results (Yano et al., 2006). As a result, if only gastric or GOJ cancer biopsy samples are available for HER2 testing, current guidelines recommend sampling of at least 6 different areas of the tumour for HER2 analysis. New IHC scoring criteria have also been developed for gastric and GOJ cancers and were validated by Hofmann et al. (2008b), further demonstrating that the analysis of HER2 based on the breast cancer guidelines may lead to false negative reporting in gastric cancer specimens.

This example demonstrates that although an assay may have progressed through thorough validation and review processes in one cancer sub-type, its use cannot be assumed for other malignancies and re-validation needs to be incorporated into early phase trials, particularly when the drug is readily available and may otherwise rapidly proceed to clinical practice. Furthermore, when several IHC assays exist, it is of the utmost importance that laboratories validate their internal IHC and FISH procedures according to international guidelines.

In this context it is paramount that biomarker development is orchestrated collaboratively in large multi-institutional networks. The integration of biomarkers early in drug development and correlation with clinical observations can generate early signals of unexpected efficacy or resistance that can then be used to change the direction of development of a particular drug and enhance outcomes.

Furthermore new health information technologies (HIT) are a pivotal part of biomarker development and need to be linked into routine practice to support the large-scale information of tumour biology and clinical data. The use of HIT will also support the integration of a variety of data sets including gene expression profiles, metabolic, immunohistochemical profiles and clinical outcome data. The development of next generation sequencing, functional genomic screening and transcriptional analysis offers detailed insight not only into DNA sequence, but also into mRNA profiles, protein structure and metabolic pathways. The enormity of the information that is available needs parallel information technologies to interpret and link these findings to their regulated networks.

The ultimate application of these technologies involves the modelling of interacting pathways to make phenotypic predictions and develop complete system models to advance personalised drug development. The incorporation of molecular biology and information technology can thus maximise the interpretation, application and targeting of these complex oncological systems. In this context, bioinformatics has evolved to combine sequence matching and pattern discovery with modelling of dynamic biological systems to enhance the drug discovery process.

4. Developments of new rationally designed targeted therapies

Several recent phase-I trials of molecularly targeted agents have demonstrated remarkable progress when patients were selected based on their molecular profile and subsequently treated with an agent directed against this specific target. The shift from 'one size fits all' to molecularly defined subpopulations has been particularly successful in the treatment of patients with advanced BRAF mutant cutaneous melanoma. Two pivotal phase-I trials, showed encouraging response rates and improved survival rates with the selective BRAF inhibitors, vemurafenib (PLX4032) and GSK 2118436, in a disease notoriously resistant to standard chemotherapies. Another trial in patients with NSCLC who were carriers of the EML4-ALK fusion protein showed remarkable response rates with
the new ALK inhibitor, crizotinib. The successful development of such agents is of course complex but can be simplistically considered as having three key components: the right target (strong biologic rationale, druggable), the right drug (selective, right formulation, tolerable side-effect profile) and the right biomarker (reproducible, validated) (Figure 1). This paradigm can be further evidenced by the success of imatinib and CAL-101 in haematological malignancies and reflects the limitations that have impacted on the use of other agents, such as sorafenib in melanoma or bevacizumab in breast and other malignancies.

Sorafenib is an oral multikinase inhibitor of vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR)-β and Raf-1 (Wilhelm et al., 2006). Although it was initially developed as a RAF inhibitor, sorafenib showed only moderate IC50s for all three RAF isoforms and also had inhibitory effects on several other receptor tyrosine kinases including VEGFR2, VEGFR3, PDGFRβ, cKIT and FLT3. Sorafenib has demonstrated significant improvements both in clinical benefit rate and survival in renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC) (Escudier et al., 2005; Llovet et al., 2008). Correlative markers were incorporated into these trials including phosphorylated ERK (pERK) immunostaining and soluble c-KIT, VEGFR2, VEGFR3 and VEGF levels. As yet however, there is no validated biomarker to predict the target patient population. Despite a good biologic rationale to support its use in melanoma and promising early phase trials, sorafenib failed to show a clinical benefit in phase II-III trials (Eisen et al., 2006; Hauschild et al., 2009). Unlike the early phase trials for the selective BRAF inhibitors, patients were not selected for BRAF mutations, one of the key drivers in cutaneous...
melanoma, nor were the pharmacodynamic markers from the early phase trials translated into the design of the phase-III trials. The failure of this drug development programme in melanoma could have been mitigated if phase-II data had been critically reviewed and early ‘go or no-go’ decisions had been incorporated in the decision making process for the phase-III trials.

Similarly, the development of bevacizumab as a drug targeting the ‘angiogenic switch’ and tumour-associated neo-vasculature met with much anticipation (Hanahan & Folkman, 1996). Bevacizumab is a humanised monoclonal antibody targeting VEGF-A and its binding to VEGFR2. There was early pre-clinical evidence that it not only inhibited the formation of new blood vessels, but also caused regression of existing micro-vessels and stabilised the mature vasculature to improve drug delivery. Significant clinical benefit Has been demonstrated with bevacizumab in combination with chemotherapy in advanced colorectal cancer but despite promising data regarding potential clinical, biochemical and radiological parameters, a predictive biomarker remains elusive (Hurwitz et al., 2004; Jubb & Harris, 2010). Although bevacizumab is now approved in several disease entities, the broad use in many tumour types remains controversial, bearing in mind its associated cost and toxicity. In this context, the lack of proven and validated biomarkers to predict the patient population most likely to benefit is often criticised and in part may have contributed to the withdrawal by the FDA of its approval in metastatic breast cancer.

On the contrary, the development of selective BRAF inhibitors for BRAF V600 mutation positive advanced cutaneous melanoma commenced with a strong biologic rationale and its success was facilitated by the validation of an associated predictive biomarker (Figure 2). Aberrant activation of the MAPK pathway has been demonstrated in over 80% of primary melanomas, due to abnormalities at various levels’ along the RAS-RAF-MEK-ERK pathway with subsequent acceleration of cell growth, proliferation and differentiation (Platz et al., 2008). BRAF mutations are among the most studied, occurring in 36-59% of primary melanomas (Houben et al., 2004; Jakob et al., 2011; Long et al., 2011) and 42-66% of metastatic melanomas and have been characterised as oncogenic mutations (Davies et al., 2002; Karasarides et al., 2004). Early phase trials with the selective BRAF inhibitors, vemurafenib (PLX4032) and GSK 2118436, have demonstrated response rates far higher than standard chemotherapy with impressive improvements in survival (Chapman et al., 2011; Flaherty et al., 2010; Kefford et al., 2010; Ribas et al., 2011.). Thus, the identification of ‘the right target’, the BRAF mutation, lent itself to the development of ‘the right drug’, the selective BRAF inhibitors, whose efficacy could be predicted by ‘the right biomarker’, presence of a BRAF mutation. Activating mutations or translocations of the anaplastic lymphoma kinase gene (ALK) have been identified in several types of cancer, with the EML4-ALK fusion gene evident in 2-7% of all NSCLC. EML4-ALK is an aberrant fusion gene that encodes a cytoplasmic chimeric protein with constitutive kinase activity. It is more prevalent in patients who are never or light smokers and in patients with adenocarcinoma histology. Crizotinib is a selective inhibitor of the ALK and MET tyrosine kinases and has shown unprecedented response rates and clinical benefit in a phase-I trial of heavily pretreated patients with advanced NSCLC harbouring ALK rearrangement (Kwak et al., 2010). The study incorporated molecular analysis of tumour samples with prospective tumour genotyping, including analysis via FISH, IHC and reverse-transcriptase-polymerase-chain-reaction (RT-PCR). FISH positivity for ALK rearrangement strongly correlated with aberrant expression of the ALK protein on IHC and many patients, though not all, also had positive results for EML4-ALK.
on the RT-PCR assay. The use of prospective tumour genotyping not only potentiated the development of diagnostic approaches for these patients but has also streamlined rapid drug development for crizotinib. Remarkably, there were only three years between target identification, initiation of the phase-I trial and enrolment on the phase-III registration trial and stands in contrast to more than ten years from the initial unsuccessful trials of EGFR inhibitors in non-genotyped unselected patients to the phase-III trials that demonstrated benefit of EGFR inhibitors in EGFR-mutant tumours (Kwak et al., 2010). Again, there is strong supporting evidence for ‘the right target’ and ‘the right drug’ in this setting, whilst development of ‘the right biomarker’ has been incorporated into the phase-I trials to assist in overcoming the many complexities inherent with new assay validation.

1. Development of a strong biologic rationale
   Mutations along the MAPK pathway present in up to 80% of metastatic melanomas
   In vitro evidence with vemurafenib (PLX4032) and GSK 2118436 of selective inhibition of BRAF V600E and impaired tumour growth in mouse models

2. Biomarkers for early phase trials
   Prognostic Biomarker: BRAF aberrations
   Predictive Biomarker: BRAF V600E aberrations (and V600K with GSK118436)
   Pharmacodynamic Biomarkers: pMEK and pERK

3. Confirmation of a clinical response
   Phase 1/2 trials (vemurafenib): RR 50-80%; PFS >7m
   Phase 1/2 trials (GSK 2118436): RR 60%; PFS 8.3m
   Phase 3 trial (vemurafenib vs dacarbazine): RR 48% v 5%; PFS 5.3m v 1.6m
   OS at 6m 84% v 64%

4. Dissecting the Mechanisms of Resistance
   Longitudinal biopsies pre-treatment, on treatment and on progression
   On-target effect demonstrated by suppression of pMEK and pERK, and decreased staining of proliferative markers on IHC (cyclin D1 and Ki67)
   Resistance possible through alternate signalling in MAPK pathway or via bypass pathway signalling
   Vemurafenib
   Some tumours demonstrate increased pMEK/pERK on progression with reactivation of MAPK pathway
   Evidence of NRAS and MEK mutations which mediate signalling via the MAPK pathway
   Evidence of PTEN loss and increase pAkt demonstrates activation of PI3K-AKT-mTOR pathway
   GSK 2118436
   Abnormal PTEN associated with shorter PFS and loss of inhibition of the PI3K-AKT pathway
   CDKN2A and KIT deletion associated with shorter PFS

MAPK: mitogen-activated protein kinase; IHC: immunohistochemistry;
BRAF: v-raf murine sarcoma viral oncogene homologue B1;
pMEK: phosphorylated MEK; pERK: phosphorylated ERK; pAKT: phosphorylated AKT; RR: response rate; PFS: progression-free survival; OS: overall survival;
PI3K: phosphatidylinositol 3-kinase; mTOR: mammalian target of rapamycin

Fig. 2. Selective BRAF Inhibitors for BRAF mutant Metastatic Melanoma
In haematological malignancies, the development of the phosphatidylinositol 3-kinase (PI3K) inhibitor, CAL-101, has shown encouraging results in advanced non-hodgkins lymphoma (NHL), mantle cell lymphoma and chronic lymphocytic leukaemia (CLL) (Herman et al., 2010). CAL-101 is a selective inhibitor of the PI3K p110 δ isoform that is primarily expressed on cells of haematopoietic origin and has a key role in B cell maturation and function. Through inhibition of PI3K signalling, CAL-101 can induce apoptosis of primary CLL and acute myelogenous leukaemia (AML) cells and a range of other leukaemia and lymphoma cell lines (Lannutti et al., 2010). In phase–I studies, CAL-101 has demonstrated durable clinical responses in a number of haematological malignancies, including NHL (Flinn et al., 2009). Reduction in phosphorylated AKT (pAKT) as a marker of PI3K activation provides ‘proof-of-mechanism’ for this agent and later phase trials are underway in B cell malignancies with markers along the PI3Kδ pathway acting as predictive biomarkers. These recent ‘proof-of-concept’ studies were the first of their kind where molecular profiles were used for selection of ‘new in class’ compounds and demonstrate that when patients are appropriately selected, convincing benefit can be realised in the earliest of trials, setting the stage for rapid drug approval. This phase-I experience has convinced investigators that tumour profiling and patient selection will become a routine part of cancer drug development.

5. Challenges in drug development

5.1 Mechanisms of resistance

Despite the advances in parallel drug and biomarker development in early clinical trials, one of the major challenges remaining is the understanding of mechanisms that cause primary and acquired or secondary resistance. Primary resistance is characterised by lack of efficacy of an agent from treatment initiation, whereas acquired resistance develops after an initial response of some degree over a period of time. As evidenced by all currently approved molecularly targeted agents, initial treatment may yield response rates far higher than standard chemotherapy with impressive disease control, but inevitably resistance and tumour progression develops. Importantly, understanding the mechanisms of resistance can lead to rationally designed drug combinations incorporating targeted agents, antibodies, or cytotoxics. This approach should include continuous analysis of tumour material via biopsies on disease progression or surrogate markers such as circulating tumour cells (CTCs) or circulating free DNA (cfDNA). In this context, cancer treatment could follow strategies as witnessed by the treatment of tuberculosis with quadruple combination regimens or human immunodeficiency virus (HIV) with highly active antiretroviral therapy (HAART). In a similar way, cancer drugs will be used in parallel or sequentially to block different driver pathways and networks simultaneously. Although there are a number of mechanisms of resistance that are particular to molecularly targeted agents and are intrinsic to the pathway they inhibit, there are other mechanisms that are common to both cytotoxic chemotherapy and molecularly targeted agents falling into three main categories: decreased uptake, such as occurs with water-soluble drugs like the folate antagonists; impaired capacity of cytotoxic drugs to induce cell kill via a combination of altered cell cycle checkpoints, increased or altered drug targets, repair of DNA damage and inhibition of apoptosis; or increased drug efflux (Gottesman et al., 2002; Szakacs et al., 2006).
The presence of efflux pumps is one of the best described mechanisms of resistance and is thought to be common to both cytotoxic chemotherapy and the molecularly targeted agents. P-glycoprotein (P-gp), otherwise known as the multidrug transporter, is an energy dependent efflux pump that has been identified as a major mechanism of multidrug resistance (MDR) in cultured cancer cells. It is the product of the MDRI gene in humans and is one member of a large family of ATP-dependent transporters known as the ATP-binding cassette (ABC family). P-gp is widely expressed in many human cancers including cancers of the gastrointestinal tract, hematopoietic system, genitourinary system and childhood cancers. P-gp can detect and bind a large variety of hydrophobic natural-product drugs as they enter the plasma membrane including chemotherapeutic agents such as doxorubicin, vinblastine and paclitaxel, as well as anti-arrhythmics, antihistamines and the HIV protease inhibitors (Robert., 1999). Increased drug efflux was initially thought to be a significant mechanism of resistance for the tyrosine kinase inhibitor imatinib in patients with CML (Mahon et al., 2003). However, it is not fully understood how much impact this resistance mechanism has on molecularly targeted drugs as a prime source of resistance.

Another relevant mechanism of resistance that has been illustrated in a number of cancers involves the disruption of interacting proteins and receptors on the plasma membrane level impacting on receptor binding and subsequent drug efficacy. For example, EGFR is a membrane-bound receptor whose signalling involves a complex pathway of ligand binding, receptor homo- and heterodimerisation with ERBB2 and other family members, followed by internalisation and recycling of the ligand-bound receptor. Significant EGF-dependent signalling may occur during the process of internalisation and alterations in EGFR trafficking have been linked to cellular responses (Wiley et al., 2003). Analysis of EGFR trafficking in resistant lung cancer cell lines demonstrated increased internalisation of EGFR compared to parental drug-sensitive cells, which interestingly could be overcome by the action of irreversible EGFR inhibitors (Kwak et al., 2005). Similarly in breast cancer, one of the proposed mechanisms of resistance to trastuzumab involves membrane-associated glycoprotein mucin-4 (MUC4) which may block the inhibitory actions of trastuzumab by directly binding with HER2 and preventing interaction between the drug and the molecular target (Nagy et al., 2005).

Primary or secondary mutations and aberrations at the level, up- or downstream of the target are also frequently studied mechanisms of resistance to the molecularly targeted agents. For example, primary resistance to the EGFR targeted agents, gefitinib and erlotinib, has been associated with the presence of a KRAS mutation in 20-30% of NSCLC patients, or via an insertion mutation in exon 20 of EGFR, which represents fewer than 5% of all known mutations in the EGFR gene (Hammerman et al., 2009). Secondary resistance to the EGFR inhibitors after an initial response is mediated by the T790M mutation in 50-59% of patients, characterised by the substitution of methionine for threonine at position 790 (T790M) in EGFR (Pao et al., 2005). In this case, biological understanding of primary and secondary resistance allows for development of rationally designed drugs. Pre-clinical evidence demonstrated that an irreversible inhibitor of EGFR, such as neratinib (HKI-272), could overcome resistance induced by T790M-mutant EGFR and such agents are currently in clinical development (Kobayashi et al., 2005; Kwak et al., 2005).

Recent advances in the treatment of melanoma have further assisted in the understanding of the complexity of resistance mechanisms. For example although secondary BRAF mutations have not been identified as a cause of BRAF inhibitor resistance, mutations elsewhere along the MAPK pathway have been implicated, including secondary NRAS and MEK mutations.
MEK mutations have been demonstrated to cause reactivation of ERK signalling despite BRAF or MEK inhibition both in vitro and in vivo (Corcoran et al., 2011; Emery et al., 2009; Wagle et al., 2011). Similarly NRAS mutations, such as the NRAS Q61K mutation, have been demonstrated in BRAF mutant melanoma cell lines resistant to vemurafenib, and in a nodal biopsy from a patient who progressed after an initial response on treatment (Nazarian et al., 2010). The presence of an NRAS mutation can result in persistently elevated pMEK and pERK levels despite BRAF inhibition and is thought to signal through RAS and subsequently through RAF isoforms other than BRAF (Nazarian et al., 2010).

Signalling via the CRAF isoform is also a significant mechanism of resistance, with increased CRAF activity and a switch from BRAF to CRAF dependency demonstrated in BRAF mutant melanoma cell lines that are resistant to RAF inhibition (Montagut et al., 2008). Importantly, sensitivity to MEK inhibition was maintained in these cell lines, supporting further novel drug combinations, such as a non-selective RAF inhibitor or selective CRAF inhibitor with a MEK or BRAF inhibitor to overcome this mechanism of resistance.

Amplification of the mutant BRAF allele has also been implicated in resistance via increased pMEK and subsequently pERK signalling, though the evidence for this lies in studies of BRAF mutant colorectal cancer cell lines. In three such cell lines, BRAF amplification was demonstrated as a mechanism of acquired resistance to MEK inhibitors with cross-resistance to BRAF inhibitors, although to a lesser degree (Corcoran et al., 2010; Little et al., 2011). Preclinical studies showed that increased concentrations of RAF or MEK inhibitors, as well as the combination of the two agents, could suppress ERK phosphorylation and downstream signalling (Corcoran et al., 2010).

Changes in signalling upstream of a target pathway as well as bypass signalling along alternate pathways have also been demonstrated as mechanisms of resistance (Figure 3). In this context the insulin-like growth factor 1 receptor (IGF1R) which signals upstream of the PI3K-AKT-mTOR and MAPK pathways has been found to contribute to resistance in a number of malignancies. For example, activity of trastuzumab was impaired in breast cancer cells that over-expressed both HER2 and IGF1R, but its activity could be restored when IGF1R activation was blocked (Lu et al., 2001). Moreover, in vitro models have demonstrated that IGF1R physically interacts with and induces phosphorylation of HER2 in trastuzumab-resistant cells, but not in trastuzumab-sensitive cells, with subsequent increased signalling through the PI3K-AKT-mTOR and MAPK pathways. Again, inhibition of IGF1R signalling either by antibody blockade or tyrosine kinase inhibition restored trastuzumab sensitivity, demonstrating another potential therapeutic mechanism to overcome secondary resistance to trastuzumab. Similar findings were also evident in BRAF V600E melanoma cell lines resistant to BRAF inhibition, providing early evidence for the combination of IGF1R and MEK inhibition in this setting. (Villanueva et al., 2010).

A number of other preclinical studies have also demonstrated aberrant activation of the PI3K-AKT pathway at other levels that contributes to both primary and secondary resistance in BRAF mutant cell lines (Jiang et al., 2011; Shao et al., 2010). Just as the combination of IGF1R inhibition with MEK inhibition is being investigated to overcome resistance mediated along the IGF1R and MAPK pathways, there may be a biologic rationale for the combination of PI3K and MEK inhibitors (Jiang et al., 2010). In such cases, phosphorylated AKT may act as a marker of activity of the PI3K-AKT-mTOR pathway and thus, may be used as a biomarker to select when the combination of PI3K inhibitors and BRAF/MEK inhibitors is appropriate to block both the PI3K and MAPK pathways respectively.
PTEN loss (PTEN-) and subsequent lack of inhibition on the PI3K-AKT-mTOR pathway has also been demonstrated to confer resistance to BRAF inhibition. Paraiso et al. (2011) showed that in cell lines with PTEN loss compared to cell lines with normal PTEN, BRAF inhibition with vemurafenib was associated with increased AKT signalling and decreased apoptosis. Dual treatment of PTEN- cell lines with both vemurafenib and a PI3K inhibitor could then restore increased levels of apoptosis (Paraiso et al., 2011).

Exemplified by preclinical and clinical examples in melanoma, signalling via the PI3K-AKT-mTOR pathway mediates an important MAPK-pathway independent mechanism of resistance in a variety of cancers and demonstrates a complex crosstalk between these pathways (Corcoran et al., 2011). Measurement of phosphorylated ERK and phosphorylated AKT to determine pathway activity may therefore help to guide therapeutic choices and combinations of selective BRAF, MEK or PI3K/AKT inhibitors. Thus, knowledge of secondary resistance mechanisms will increasingly influence decision making processes for further drug development and rational drug combinations.

Although mechanisms of secondary resistance are well described for several new targeted agents, challenges remain, particularly with anti-angiogenic or multitargeted agents such as bevacizumab, sunitinib and sorafenib. The complexity of resistance mechanisms to anti-angiogenic therapy reflects the difficulty in developing anti-angiogenic agents in parallel with corresponding biomarkers. So far, two main resistance mechanisms for anti-angiogenic agents have been proposed: firstly, evasive resistance with adaptation to circumvent specific angiogenic blockade, and secondly, intrinsic or pre-existing indifference (Bergers & Hanahan, 2008). Evasion of anti-angiogenic therapy may occur via up-regulation of alternative pro-angiogenic signalling circuits or via a number of alterations in the micro-environment, including recruitment of vascular progenitor cells and pro-angiogenic monocytes from the bone marrow, increased and tight pericyte coverage protecting tumour blood vessels and increased capacity for invasion without angiogenesis.

Alternate pro-angiogenic signals that have been implicated in preclinical studies include fibroblast growth factor (FGF)-1 and -2, ephrin A1 and A2 and angiopoietin-1. To establish the significance of these up-regulated genes, preclinical studies used the combination of FGF signalling suppression with VEGFR inhibitors and demonstrated that the combination of these agents attenuated re-vascularisation and slowed tumour growth (Casanovas et al., 2005). These findings were also seen clinically in patients with glioblastoma treated with the VEGFR inhibitor cediranib (Batchelor et al., 2007). After initial response, peripheral blood levels of FGF2 increased when patients progressed, suggesting that signalling through FGF assists in restoring angiogenesis. Elevated levels of pro-angiogenic factors such as VEGF and placental growth factor (PGF) have been previously proposed as predictive biomarkers for tumour response (Bocci et al., 2004). However there is also evidence that the expression of pro-angiogenic growth factors such as FGF, PDGF and others increase in advanced stages of metastatic breast cancer, resulting in alternate pathway signalling (Relf et al., 1997). Thus, there is uncertainty regarding the significance of these factors; whether the presence of pro-angiogenic factors in peripheral blood are in fact markers of response or resistance, or neither. Understanding the complex regulatory networks, the interaction of pro- and anti-angiogenic factors and contributing components of the micro-environment, illustrates the difficulties to-date in target and biomarker development, as well as the potential mechanisms by which anti-angiogenic therapy can be optimised.
<table>
<thead>
<tr>
<th>Malignancy and Drug</th>
<th>Target</th>
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<tr>
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<td>Estrogen receptor</td>
<td>ER/PR status on IHC</td>
<td>Loss of ER expression Epigenetic changes in ER gene Increased drug metabolism ER/HER2 cross-talk PI3K-AKT pathway activation Alterations in co-regulatory proteins (Ring et al., 2004)</td>
</tr>
<tr>
<td>Trastuzumab</td>
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<td>MUC4 binding to HER2 (Nagy et al.; 2005) HER2 &amp; IGFIR crosstalk (Lu et al., 2001) PI3K-AKT pathway signalling and PTEN loss</td>
</tr>
<tr>
<td>Bevacizumab*</td>
<td>VEGF-A and VEGFR2</td>
<td>Nil currently validated</td>
<td>Alternate pro-angiogenic signalling circuits (e.g. FGF) Bone marrow derived vascular progenitor cells &amp; pro-angiogenic monocytes Increased pericyte coverage (Bergers &amp; Hanahan, 2008)</td>
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<td>Melanoma</td>
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<td>Nil currently validated</td>
<td>Alternate pro-angiogenic signalling, PDGFR mt Glucose-regulated protein 78 (Chiou et al., 2010)</td>
</tr>
<tr>
<td>Vemurafenib/ GSK 2118436</td>
<td>BRAF V600E/K mt</td>
<td>BRAF mt status</td>
<td>Upstream: IGFIR, PDGF upregulation, NRAS mt (Nazarian et al., 2010; Villanueva et al., 2010) Target level: BRAF amplification, CRAF activity (Corcoran et al., 2011; Montagut et al., 2008) Downstream: MEK mt (Corcoran et al., 2011) Alternate pathway signalling: PI3K-AKT-mTOR activation</td>
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<td>Lung Cancer</td>
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</tr>
<tr>
<td>Malignancy and Drug</td>
<td>Target</td>
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<td>Colorectal Cancer</td>
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<td>Cetuximab</td>
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<tr>
<td>CAL-101</td>
<td>PI3K p1108</td>
<td>pAkt and markers along the PI3Kδ pathway</td>
<td></td>
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</tbody>
</table>

*Evidence for bevacizumab also applies to colorectal cancer, NSCLC, renal cell carcinoma and other malignancies
ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; IHC: immunohistochemistry; mt: mutation
IGF1R: insulin growth factor-1 receptor; PDGF: platelet derived growth factor
EGFR: epidermal growth factor receptor; PI3K: phosphatidylinositol-3-kinase
PTEN: phosphatase and tensin homologue

Fig. 3. Selected Examples of Molecularly Targeted Therapies and Mechanisms of Drug Resistance

5.2 The breakthroughs and dilemmas of recurrent tumour biopsies
Although many mechanisms of resistance can be identified through studies of cell lines and xenograft models, it is often through correlation with patients’ tumour specimens that valid conclusions can be drawn about the significance of these resistance mechanisms in the clinical setting. To this end, access to longitudinal tumour biopsies and assessment of these in ‘real-time’ may change the treatment paradigm for patients.

The need for longitudinal tumour biopsies is evidenced at a number of levels. Firstly, it assists in understanding and mapping the complex molecular networks, communication with the micro-environment, angiogenesis and other ‘hallmarks’. As technologies in tumour analysis improve, for example with high throughput genetic sequencing and unravelling the cancer genome, findings on the pre-clinical level can be investigated and explored clinically and changes in tissue can be correlated with therapeutic response.

Secondly, there are multiple variables that can affect the accuracy of mutational analysis on tumour tissue, not least that the tumour itself can develop new mutations and aberrations.
that drive tumorigenesis. Studies of concordance or lack thereof, between archival primary tissue and biopsies of metastatic disease have demonstrated this in breast, colorectal and other malignancies. Analyses of HER2 over-expression in primary breast cancer and metastatic sites demonstrate that up to 12% of patients may have HER2 negative primary breast cancers with HER2 positivity at the metastatic sites, and subsequent potential therapeutic benefit from trastuzumab (Zidan et al., 2005). Conversely, up to 30% of tumours could switch from HER2 positive status on primary tissue to HER2 negative status on metastatic tissue, again significantly impacting on future treatment decisions (Locatelli et al., 2010).

In patients with advanced colorectal cancer, retrospective analyses have assessed the concordance of KRAS mutation status and other alterations along the MAPK and PI3K-AKT-mTOR pathways between primary tumours and metastatic sites. Loupakis et al. (2008) assessed PTEN status which regulates the PI3K-AKT-mTOR pathway, and demonstrated that PTEN loss occurred in 37% of tumours with associated lack of response to cetuximab and irinotecan. Interestingly the reported PTEN concordance between primary tumours and metastases was 60% compared to 95% for KRAS mutations. In those patients who were KRAS wild-type and PTEN positive on metastases, there was evidence for improved RR and PFS indicating the importance of pathway profiling to predict clinical response.

These examples underline the importance of tumour assessment not only for patients who develop metastatic disease after resection of a primary cancer, but also for patients with progressive disease on treatment. Understanding of the ‘driving’ pathway, receptor or network before treatment initiation, especially with new molecularly targeted agents, will become standard of care for several new treatments and guide us in the decision making algorithm even in advanced stages of disease.

This can be further evidenced by a recent study in a cohort of heavily pre-treated phase-I patients who were tested for aberrations in the MAPK and PI3K-AKT-mTOR pathways and then treated with drugs targeting these pathways (Tsimberidou et al., 2011). Impressively, those patients with molecular alterations treated with targeted therapy had a response rate of 29% (complete response or partial response) compared to 8% in the group without alterations. The proportion of patients with stable disease beyond 6 months and the median survival were also higher in this patient group.

Importantly the recent early phase-I melanoma studies with selective BRAF inhibitors have incorporated tumour biopsies at baseline, on-treatment and on-progression biopsies to analyse the changes in pathway signalling (McArthur et al., 2011; Nathanson et al., 2011). The tumour analyses included not only immunohistochemical staining, but also Sequenom MassARRAY of over 400 gene mutations, such as BRAF, RAS, PIK3CA, AKT1/2, CDK4 and others. Following this approach, patients were selected for the BRAF mutation at baseline and monitored during treatment with the measurement of phosphorylated MEK and ERK levels to confirm target inhibition. On progression, a number of potentially significant genetic alterations were identified, including NRAS and MEK1 mutations indicating continuing MAPK-pathway signalling. In addition PTEN loss and an increase in pAKT were observed, demonstrating activation of the PI3K-AKT-mTOR pathway as a possible alternate signalling pathway (Figure 2).

Clearly the risk-benefit of serial tumour biopsies needs to be well balanced and risks and disadvantages acknowledged. For example, in some cancers like NSCLC, access to tumour tissue is restricted by the site of disease with an increased potential risk of pneumothorax,
bleeding and other complications secondary to a lung biopsy. Tissue biopsies also run the risk of sampling error, in part from tumour heterogeneity. As discussed with HER2 testing in gastric cancer, multiple biopsies may be required to minimise the chance of missing the alteration of interest, in this case HER2 amplification and protein over-expression. In addition, sample handling, fixation, validation of assays, inter-observer variability and assessment, all contribute to the accuracy of the final result on which clinical decisions are made.

Finally, new technologies also need to be validated prior to routine introduction into clinical care. Although the ability to sequence the genome and perform genetic profiling on patients’ tumours dramatically escalates the information available on an individual patient, the significance of this information is still, as yet, often unknown. The presence of a mutation does not determine its significance in tumorigenesis, such that inhibition of a given mutation will not correlate with clinical benefit, if the mutation was an incidental finding rather than an oncogenic mutation.

5.3 The role of circulating tumour cells and circulating free DNA

Detection of circulating tumour cells (CTCs), and circulating free DNA (cfDNA) in peripheral blood specimens potentially presents an easily accessible ‘liquid biopsy’ without the risk of tumour biopsies and further, may not only provide a predictive biomarker for a given treatment, but also contain information on molecular aberrations and changes in pathway signalling while on treatment.

There is increasing evidence that CTCs can be used as a surrogate endpoint for progression-free and overall survival and thus, allow an earlier assessment of the clinical benefit of a particular agent to streamline drug development and regulatory approval. Such ‘surrogate endpoints’ may accelerate drug development as long as adequate and well controlled clinical trials establish that the new drug has an effect on this surrogate, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, and that this surrogate endpoint can predict clinical benefit and survival (Atkinson et al., 2001).

The enumeration of CTCs and their utility as a prognostic and predictive biomarker has been best characterised in breast, colorectal and prostate cancers with further evidence in other malignancies including melanoma and lung cancers. The most widely used and FDA approved method for CTC enumeration and molecular characterisation is the CellSearch system, which involves the immunomagnetic capture of CTCs using antibodies against the epithelial cell adhesion molecule (EpCAM), expressed on the cell surface of most epithelial malignancies. Additional cell identification includes the detection of pan-cytokeratin antibodies, DAPI nuclear staining (4,6-diamidino-2-phenylindole staining to detect nucleated cells) and CD45 negative selection to demonstrate the detected cell is not a leucocyte.

The presence of CTCs at baseline in metastatic breast cancer has not only been demonstrated to have prognostic significance but has also been shown to be the strongest predictor of overall survival when compared to age, hormone receptor status, HER2 status and metastatic site. It also maintains its prognostic value independent of line of treatment, site of recurrence and disease phenotype (Cristofanilli et al., 2005). Preliminary studies in breast cancer suggest that CTC enumeration may even be superior to radiological evaluation in predicting response to treatment and outcome. It may provide a more reproducible indication of disease status compared to current imaging methods, particularly in view of inter-reader
variability in confirming radiological response which can vary by up to 15% compared to 1% variability for CTC counts (Budd et al., 2006).

In castrate resistant prostate cancer, the presence of CTCs at baseline and lack of a decline during treatment is also indicative of poor response and survival. In multivariate analyses, CTC counts and PSA doubling time have been demonstrated as the only independent predictors for clinical outcome as compared to PSA level, Gleason Score, bone metastases and age (De Bono et al., 2008). Additionally, there is now evidence that CTCs may be a potential surrogate biomarker in metastatic prostate cancer trials. The randomised, double-blind phase III trial in metastatic prostate cancer, in which abiraterone was compared to placebo, was the first of its kind to demonstrate the utility of CTCs in this setting. CTCs were measured at baseline and repeated at 4, 8 and 12 weeks post treatment. Pre-treatment CTCs were strongly correlated with OS, as was a fall in CTC count on treatment (Reid et al., 2010; Scher et al., 2011). Particularly in the setting of castrate resistant prostate cancer where there may be inter-observer variation regarding radiological progression, CTCs may provide an accurate and reproducible alternative.

In patients with metastatic colorectal cancer, higher baseline CTC counts correlate with shorter PFS and OS. Again, conversion of an unfavourable baseline CTC count to a favourable count at 3-5 weeks after starting treatment is associated with longer PFS and OS compared with patients with unfavourable counts at both time points. Baseline and follow-up CTC levels also remain strong predictors of PFS and OS after adjustment for clinically significant factors (Cohen et al., 2008).

Recent evaluation of CTCs in patients with NSCLC has also suggested prognostic significance (Krebs et al., 2011). CTCs in patients with NSCLC were found more commonly with stage IV (32%) compared to stage IIIB disease (7%) and in those patients with five or more CTCs detected, both PFS and OS were inferior. Particularly with the complexities in obtaining longitudinal tissue biopsies, further investigation of a prognostic 'liquid biopsy' and incorporation into early phase trials is of importance.

In patients with advanced melanoma, recent studies have demonstrated good correlation between CTC status and tumor-node-metastasis stage, underlining the prognostic role of CTCs (Mocellin et al., 2006). The predictive value of CTCs was so far limited by the fact that treatment options consisted of bio-chemotherapies with no effects on clinical outcomes. However, the presence of circulating melanoma cells after adjuvant treatment for stage III melanoma has been shown to correlate with inferior relapse-free and overall survival and may be a useful indicator of systemic subclinical disease (Koyanagi et al., 2005). Isolation and molecular characterisation of these cells, combined with analysis of cfDNA, presents an opportunity to obtain further information about the pathways driving tumorigenesis, invasion and metastasis. In addition to evaluating the role of CTCs in melanoma, one study found good correlation between CTCs and cfDNA suggesting both markers may be a useful determinant of disease status and treatment effect. Patients with measurable CTC or cfDNA showed poorer disease outcome compared with patients without these markers, and patients with both markers showed the most inferior disease outcome, despite the fact that the treatment regimens were heterogenous and consisted of bio-chemotherapies of limited clinical benefit (Koyanagi et al., 2006).

5.4 Optimising trial design

Given the diversity of novel compounds discovered over the last decade, clinical trial design for the evaluation of these targeted agents has evolved with the agents being tested. Many
of these agents do not cause typical chemotherapy-induced side effects such as myelosuppression around which early phase trial design has been based. Therefore design of clinical trials of novel agents has had to develop in order to evaluate these agents appropriately and efficiently.

In a standard dose escalation phase I trial, cohorts of three to six patients are treated at pre-defined dose levels, dose-limiting toxicity (DLT) is observed and the maximum tolerated dose (MTD) is defined as the dose level where >33% of patients treated have experienced a DLT. Dose levels are commonly defined using modification of the original Fibonacci design (increasing dose by fixed increments of 100%, 67%, 50%, 40% followed by 33% for all subsequent levels) but slow attainment of the MTD and exposure of significant numbers of patients to low doses have been criticisms of this approach (Rogatko et al, 2007). An accelerated trial design (Simon et al, 1997) is now a widely accepted alternative to the Fibonacci dose-definition model and many trials now allow individual patients to be dose-escalated within a study if safe to do so, aiming to minimise those being exposed to ineffective doses. Therefore there are many combinations of model-based and rule-based designs that allow flexibility of the recruitment structure in a trial and can be appropriately adapted to the agent under consideration (Ivy et al, 2010; Parulekar et al, 2004; Rogatko et al, 2005; Korn et al, 2001; Cannistra et al, 2008; Sleijfer et al, 2008; Bria et al., 2009).

The appropriateness of the primary endpoint of maximum tolerated dose (MTD) has been challenged for some of these agents and consideration has been given instead to the concept of optimal biological dose (OBD) (O’Reilly et al., 2010; Le Tourneau et al., 2009). Targeted biological agents are more commonly cystostatic rather than cytotoxic, therefore other endpoints should be considered when evaluating treatment efficacy (Rixe et al., 2007; Gelmon et al., 1999) including novel radiographic assessment and immunotherapy assessment (Wolchok et al., 2009).

There has also been an increasing realization that patients need to be appropriately selected for certain agents based on tumour biology and molecular characteristics. The question is whether patient selection should take place at the outset of drug development, as a targeted approach which is then diversified; or whether a broader recruitment strategy should prevail initially, followed by testing within a targeted population. There is therefore a critical need to integrate and validate novel biomarkers into drug development from the earliest stages of evaluation, incorporating tumour and non-tumour tissue samples to apply these biomarkers appropriately and guide patient selection.

Overall, in the era of development of molecularly targeted agents, appropriately designed hypothesis-testing trials should be conducted. Patients should be selected rationally according to tumour biology and molecular characteristics and above all, an element of flexibility should be allowed within the trial design to enable response to unexpected findings, whether that be toxicity or efficacy.

6. Conclusion

The increased understanding of tumour biology and genetics along with improvements in laboratory methodologies and IT-systems will continue to make a tremendous impact on oncology drug development. Critical to future oncology drug development is the incorporation of biomarkers from the earliest stages and supported by applied bioinformatics. In addition, the use of new preclinical models and novel clinical trial designs incorporating intermediate surrogate biomarker endpoints will be essential not only for the
better understanding of mechanisms of action of new targeted drugs, but also in supporting confidant ‘go or no-go decisions’. The ‘personalised medicine’ approach involving molecular characterisation of the tumour and its context within the micro-environment and immune system, will help to define the right treatment, for the right patient at the right time. Increasing our understanding on how to combine established and novel therapeutics in an efficient timeframe is critical to improved outcomes for the treatment of solid malignancies.

7. Acknowledgments

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8. References


Jakob, J.A.; Bassett, R.L.; Ng, C.S.; et al. (2011) Clinical characteristics and outcomes associated with BRAF and NRAS mutations in metastatic melanoma. *J Clin Oncol* 29 (suppl; abstr 8500)


Novel Oncology Drug Development Strategies in the Era of Personalised Medicine


McArthur, G.; Ribas, A.; Chapman, P.B.; et al. (2011) Molecular analyses from a Phase 1 trial of vemurafenib to study mechanism of action and resistance in repeated biopsies from BRAF mutation positive metastatic melanoma patients. *J Clin Oncol* 29 (suppl, abstr 8502).


Nathanson, K.L.; Martin, A.; Leteoro, R.; et al. (2011) Tumor genetic analyses of patients with metastatic melanoma treated with the BRAF inhibitor GSK2118436 (GSK436). *J Clin Oncol* 29 (suppl; abstr 8501)


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Drug discovery and development process aims to make available medications that are safe and effective in improving the length and quality of life and relieving pain and suffering. However, the process is very complex, time consuming, resource intensive, requiring multi-disciplinary expertise and innovative approaches. There is a growing urgency to identify and develop more effective, efficient, and expedient ways to bring safe and effective products to the market. The drug discovery and development process relies on the utilization of relevant and robust tools, methods, models, and validated biomarkers that are predictive of clinical effects in terms of diagnosis, prevention, therapy, and prognosis. There is a growing emphasis on translational research, a bidirectional bench to the bedside approach, in an effort to improve the process efficiency and the need for further innovations. The authors in the book discuss the current and evolving state of drug discovery and development.

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