The role of functional and molecular imaging in cancer drug discovery and development

B M SEDDON, MRCP, FRCR, PhD and P WORKMAN, BSc, PhD, FMedSci

Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, UK

Abstract. Studies of pharmacokinetics (which is what the body does to the drug) and pharmacodynamics (which is what the drug does to the body) are essential components of the modern process of cancer drug discovery and development. Defining the precise relationship between pharmacokinetics and pharmacodynamics is critical. It is especially important to establish a well understood pharmacological “audit trail” that links together all of the essential parameters of drug action, from the molecular target to the clinical effects. The pharmacological audit trail allows us to answer two absolutely crucial questions: (1) how much gets there; and (2) what does it do? During the pre-clinical drug discovery phase, it is essential that pharmacokinetic/pharmacodynamic (PK/PD) properties are optimized, so that the best candidate can be selected for clinical development. As part of contemporary mechanistic, hypothesis-testing clinical trials, construction of the pharmacological PK/PD audit trail facilitates rational decision-making. However, PK/PD endpoints frequently require invasive sampling of body fluids and tissues. Non-invasive molecular measurements, e.g. using MRI or spectroscopy, or positron emission tomography, are therefore very attractive. This review highlights the need for PK/PD endpoints in modern drug design and development, illustrates the value of PK/PD endpoints, and emphasises the importance of non-invasive molecular imaging in drug development. Examples cited include the use of PK/PD endpoints in the development of molecular therapeutic drugs such as the Hsp90 molecular chaperone inhibitor 17AAG, as well as the development of SR-4554 as a non-invasive probe for the detection of tumour hypoxia.

In this review, we describe the demands that modern cancer drug development is currently making on the biomedical community to produce improved pharmacological endpoints. We show how technical advances are leading to an increase in the number of innovative molecular therapeutic agents entering clinical trials, and we illustrate how these trials require greater mechanistic and hypothesis testing power. Many of the pharmacological endpoints and biomarkers that are currently being used are invasive in nature, requiring multiple blood and tissue samples, and we emphasise the importance and potential value of non-invasive techniques that are commonly grouped under the headings of functional or molecular imaging, together with related spectroscopic methods. We show how the use of these methods allow the construction of a pharmacological audit trail that links together all of the events from the administration of the drug, through its activity on the molecular target, to the downstream consequences of target modulation, including most importantly the clinical outcome. Judicious use of the appropriate endpoints improves decision-making and provides the basis for an informed and rational drug development process.

Modern drug discovery

Why do we need more cancer drugs? The simple answer is because of the therapeutic challenge that cancer continues to represent in the 21st century. One in three people will suffer from cancer in their lifetime, and one in four will die from it. The recently published World Cancer Report [1] states that in the year 2000 there were 10 million new cases of cancer world-wide. This figure is predicted to rise to 15 million new cases by the year 2020, due to steadily ageing populations, current trends in smoking prevalence, and unhealthy lifestyles. Cancer accounts for 12% of 56 million annual deaths globally from all causes. Worryingly, cancer has emerged as a major health problem in developing countries, as lifestyles of the developed world are adopted. Currently 61 drugs are approved by the Food and Drug Administration (FDA) of the USA [2], although of these only a relatively small proportion are in regular use [3]. The majority are cytotoxic agents with a low therapeutic index (the ratio of anticancer dose to dose that produces toxic effects), acting in a non-selective manner against proliferating cells of both cancerous and normal tissues. Because of this, the price of effective anticancer activity is frequently significant normal tissue toxicity. This, together with the ever-present problem of drug resistance, suggests that current cytotoxic agents have reached a plateau of effectiveness.

Clearly there is a need to produce new, more effective anticancer agents with novel modes of action [4, 5]. In the 1990s it took approximately 15 years to take a drug from laboratory discovery to FDA approval and widespread availability [6]. This can be broken down into pre-clinical development (6.5 years), Phase I testing (1.5 years), Phase II testing (2 years), Phase III testing (3.5 years), and FDA approval (1.5 years). Out of every 5–10 000 compounds evaluated pre-clinically, only five enter clinical trials, and of these only one gains regulatory approval. The cost of advancing a drug to the point of applying for FDA approval has been estimated to be $802 million [7]. Thus, the modern process of drug discovery and development
needs to be faster than before while minimizing costs, rapidly identifying the more promising candidates and discarding non-starters before too much money has been invested. Early anticancer agents were discovered largely serendipitously [8]. By necessity, there has now emerged a need for rational drug discovery of mechanism-based drugs acting on novel molecular targets. Such agents may produce cytostatic rather than cytotoxic effects, which is desirable in terms of minimizing normal tissue toxicity.

The development of new molecular therapeutics based on such a rational approach requires systematic progression through a number of pre-defined steps (Figure 1) [9]. This process has been streamlined in recent years by the introduction of a range of new technologies (Table 1). The first, crucial step is the identification of relevant molecular targets [5]. This has been advanced considerably by recent progress in the molecular biology, genetics and pathology of cancer together with the output from the Human Genome Project [10, 11] and systematic cancer genome sequencing and expression profiling [12, 13]. These have significantly increased our understanding of the genetic abnormalities and cognate deregulated molecular pathways that drive the development and progression of cancer. The genes and pathways that are most commonly hijacked in cancer are most likely to provide potential therapeutic targets. Examples include the receptor tyrosine kinase → Ras → Raf → MAP kinase pathway which regulates cell proliferation [14], the control of the cell cycle by the cyclin-dependent kinase–retinoblastoma gene product axis [15], and the PI3 kinase pathway that governs cell survival and many other cellular processes [16]. The frequency of a particular genetic abnormality in a specific tumour type, and the linkage of the abnormality to clinical outcome, e.g. survival, can be very important in target selection. Once a potential target has been identified, it is important that it is validated to confirm its role in tumourigenesis. This can be achieved by demonstration in model systems that mutation or altered expression of the gene produces the malignant phenotype [5]. Knockout of a dominant oncogenic function, e.g. by RNA interference technology [17], is also extremely valuable. There are, however, no hard and fast rules about target selection and validation. Guidelines such as those summarized above can be useful, but at the end of the day this is a judgement call in which the tractability or likely “drugability” of the target also carries significant weight. Thus, kinases are now known to be “druggable” following the development of the tyrosine kinase inhibitors Glivec (used in chronic myeloid leukaemia and gastrointestinal stromal tumours) and Iressa (used in epithelial tumours). In contrast, large surface protein–protein interactions are difficult to block with small molecule inhibitors.

Two recent examples serve to illustrate how systematic cancer genomic sequencing [13] and expression profiling [18] can identify new drug targets. The kinase BRAF was shown by high throughput DNA sequencing to be mutated in over 70% of melanomas and a lower proportion of other cancers [13]. The histone methyl transferase EZH2 was demonstrated by cDNA microarray expression profiling to be the over-expressed gene in prostate cancer as compared with normal prostate [18]. Both targets are enzymes for which inhibitors could be identified by various techniques, as discussed below.

A critical stage in the small molecule drug discovery process is “lead identification”. Robotic high throughput screening enables rapid screening of chemical libraries of tens to hundreds of thousands of small molecules, to identify a “hit” compound that is active against the selected target [19]. The likelihood of identifying hits is increased by screening against large numbers of structurally diverse compounds from a variety of sources. Typical current annual objectives of large pharmaceutical companies are to identify 100 targets, and to screen 500,000 compounds per target [20]. An alternative to the high throughput screening approach is to rationally design a chemical structure to suit
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Table 1. Examples of new technologies in modern drug discovery

<table>
<thead>
<tr>
<th>Phase of drug discovery</th>
<th>Technological advances</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>Target identification and validation</td>
<td>Genomics</td>
<td>Profiling of the sequence and expression of many thousands of genes or entire genomes simultaneously at the level of DNA and mRNA.</td>
</tr>
<tr>
<td></td>
<td>Proteomics</td>
<td>Profiling of the expression of thousands of genes at the level of the functional proteins.</td>
</tr>
<tr>
<td></td>
<td>Bioinformatics</td>
<td>The process of electronically representing and integrating biomedical information to make it accessible and usable – particularly important to process the enormous quantities of data produced by genomic and proteomic analyses.</td>
</tr>
<tr>
<td>Lead identification</td>
<td>Libraries of chemical compounds</td>
<td>Collections of chemical compounds, with widely diverse or more focused structures, that can be screened for activity against the selected molecular target.</td>
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<tr>
<td></td>
<td>Structure-based drug design</td>
<td>Design of a drug according to knowledge of the structural biology of the selected target.</td>
</tr>
<tr>
<td></td>
<td>Robotic high throughput screening</td>
<td>Automated assays for rapid screening of chemical libraries to identify compounds (&quot;hits&quot;) that act on the selected molecular target.</td>
</tr>
<tr>
<td>Lead optimization</td>
<td>Combinatorial chemistry</td>
<td>Generation of a wide diversity of chemical structures based on the selected &quot;hit&quot; compound to produce a lead compound.</td>
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<tr>
<td></td>
<td>Medicinal chemistry</td>
<td>Chemical optimization of lead compound to create a candidate drug molecule.</td>
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<tr>
<td></td>
<td>High throughput pharmacokinetics</td>
<td>Use of pharmacokinetic prioritization screen, e.g. cassette dosing of several compounds simultaneously in a single animal.</td>
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</table>

mRNA, messenger RNA.

the molecular target, based on its three-dimensional structure determined by X-ray crystallography or nuclear magnetic resonance spectroscopy [21]. These techniques can also be used in high throughput to identify hits.

Having identified a "hit", the next priority is to convert it into a genuine lead in a process termed "lead optimization". Combinatorial chemistry, the automated chemical synthesis of libraries of large numbers of structural analogues, has been an important advance in both lead identification and lead optimization [22]. Lead optimization is the process by which an analogue of related chemical structure but exhibiting superior pharmacological properties of selectivity and potency against the selected target is identified. Medicinal chemistry allows further optimization by specific structural modification of the selected analogue [23]. A key component of this process involves optimization of the lead compound with respect to suitable "drug-like" properties in experimental animals, measuring absorption, distribution, metabolism and excretion in pharmacokinetic (PK) studies. This is critically important because a frequent point of failure in drug discovery programmes is suboptimal PK properties when the new agent is first tested in the intact animal [5]. PK describes "what the body does to the drug". Equally important at this stage of drug development are pharmacodynamics or "what the drug does to the body". Specifically, it is essential to confirm that the drug is hitting the selected molecular target in vivo. Furthermore, it is crucial to develop and validate robust PK and pharmacodynamic (PD) endpoints in pre-clinical models, which can then be used in clinical studies to enhance their mechanistic and hypothesis-testing power [24].

Pharmacokinetics, pharmacodynamics and the pharmacological audit trail

In the early clinical development of a new drug, a number of key issues need to be addressed. Is the drug reaching concentrations in the blood and tumour necessary to achieve biological activity? Is the drug hitting the selected molecular target, e.g. BRAF? Is the drug modulating the biochemical pathway in which the molecular target functions, e.g. the Ras-Raf-ERK1/2-MAP kinase pathway? Is the drug achieving the desired biological effect, e.g. inhibition of cell proliferation? These issues can be formulated into a hierarchy of PK and PD endpoints, forming an informative test cascade that provides a pharmacological audit trail [24, 25]. This audit trail allows us to ask two crucial questions about the new drug: how much gets there, and what does it do? (Figure 2). Furthermore, the pharmacological audit trail provides a rigorous and logical evaluation process enabling systematic testing of the new drug, during which rational decisions can be made in the face of the progressive accumulation of information and knowledge. Each defined PK or PD endpoint can be systematically checked, thus avoiding a costly failure at a later stage of drug development. In the current climate of very high costs of drug development, early identification of unsuitable drugs is essential.

The initial clinical context in which a new drug is tested is a Phase I study. Ideally, the defined PK and PD endpoints of the audit trail, already evaluated and validated pre-clinically, should be addressed within this study. Conventionally, it should include dose escalation to a maximum tolerated dose, monitoring of toxicity, and recommendation of the dose for a Phase II trial. In addition, PK studies are performed, measuring drug concentrations in the plasma by high performance liquid chromatography and an appropriate detection method such as ultraviolet or mass spectrometry [26]. Increasingly, plasma and tumour samples are also collected for evaluation of PD endpoints, which can be assessed by a range of assays, including northern and western blotting, ELISA, immunohistochemistry, real-time polymerase chain reaction and gene expression microarrays. In addition to
Peripheral blood lymphocytes, surrogate normal tissues such as skin and buccal mucosa may also be sampled. However, while collection of serial blood samples from patients is relatively straightforward, acquisition of single or serial tumour and normal tissue samples presents technical, logistical, or indeed ethical challenges.

**Non-invasive molecular imaging in drug discovery**

Because of the issues inherent in tumour sample collection and because of the need to develop new methodologies for evaluation of PK and PD endpoints, there has been growing interest in the use of non-invasive functional and molecular imaging and spectroscopic techniques in the process of drug discovery. The National Cancer Institute in the USA has established five In Vivo Cellular and Molecular Imaging Centres, and has committed $78.7 million of its 2004 budget to "stimulate and accelerate discovery and development of imaging methods and biosensors to identify the biological and molecular properties of pre-cancerous or cancerous cells" [27]. In the UK, Cancer Research UK’s Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee has been established under the auspices of the Cancer Research UK Phase I/II Clinical Trials and New Agents Committees, and particularly recognises the importance of minimally invasive PK and PD technologies in hypothesis-testing clinical trials of innovative therapies [28]. This is especially important given the recent shift in drug discovery from conventional cytotoxic agents to novel agents acting on specific molecular targets. The recognition that such drugs may be more likely to be cytostatic than cytotoxic means that the traditional methods of evaluating antitumour activity by reduction in tumour size [29, 30] may no longer be appropriate or adequate [4]. Furthermore, there is a need to confirm the desired mechanism of action on the intended molecular target and biochemical pathway.

At present, there is a range of imaging techniques that can be used as part of a drug discovery programme (Table 2). These technologies may be used in different ways (Figure 3). The drug itself can be directly monitored in blood, normal tissue and tumour (PK), and the effects of the drug (PD) can be monitored in the context of the tumour. This can be at a physiological level, evaluating drug effects on structural and functional endpoints, for example tumour size or tumour vasculature. It can also be at a molecular level, assessing generic biological endpoints such as bioenergetic status, by monitoring a molecule within a particular biochemical or molecular pathway, or assessing specific biological endpoints such as expression of cellular receptors. All of these approaches have in common the use of a reporter probe, either endogenous or externally administered, that is detectable by the selected imaging technique.

Considering first techniques assessing the tumour at a physiological level using externally administered reporter probes, CT, MRI and ultrasound (US) scanning can all assess tumour size and structure. CT, when used with iodinated contrast agents, provides information on such endpoints as tumour perfusion [31] and the integrity of the blood–brain barrier. Dynamic contrast-enhanced MRI is based on the temporal and spatial changes in signal intensity following the rapid injection of a paramagnetic

**Figure 2.** The pharmacological audit trail used in the development of novel anticancer agents.
contrast agent such as gadolinium-DTPA (a low molecular weight contrast agent in routine clinical use), thus providing information on tumour perfusion, vessel density and permeability, and blood volume [32, 33]. Larger molecular weight “blood pool” contrast agents such as ferric oxide particles coated in dextran may also be used to evaluate blood volume, in addition to assessing the permeability of tumour vessels to large particles [34, 35]. Doppler US techniques can detect blood flow, velocity and flow resistance [36]. More recently, intravenously administered microbubble contrast agents have been used with US as reporter probes to enhance signal from tumour vessels [37].

Techniques that employ endogenous reporter probes to assess the tumour microenvironment at a physiological level include diffusion weighted MRI, which utilizes measurement of changes in the rate and diffusion of water molecules [38, 39]. This has been used as an early indicator of response to anticancer agents, used either alone [40] or in combination with radiotherapy [41]. Furthermore, early indications suggest that diffusion weighted MRI performed prior to treatment can predict response to anticancer therapy [42]. Blood oxygenation level dependent (BOLD) 1H MRI depends upon the paramagnetic properties of deoxyhaemoglobin in producing magnetic field inhomogeneities, which result in shortening of the T2* relaxation time [43–45]. In T2* weighted MR images, deoxyhaemoglobin acts as an endogenous reporter probe such that tissues with vessels containing deoxygenated blood appear dark. The signal thus depends on vessel density, blood oxygenation, and blood volume and flow.

Techniques currently in clinical use for evaluation and imaging at a molecular level are magnetic resonance spectroscopy (MRS) and positron emission tomography (PET). Both detection methods can be used to directly monitor blood, tissue and tumour PK of drugs containing appropriate nuclei with magnetic properties (MRS), e.g. 19F detected by 19F MRS [46, 47], or those with radionuclide labels (PET), e.g. [11C]temozolamide [48]. Importantly, such PK monitoring can be performed in real-time, in contrast with traditional PK studies of blood and tumour samples, which are analysed retrospectively.

MRS and PET can also be used in a number of ways to monitor PD endpoints at a molecular level. In MRS, a radiofrequency pulse excites a selected peak in the MRS spectrum, and the resulting signal is measured as a function of time. This signal is then processed to generate a map of the concentration of the metabolite of interest, which can be used to monitor changes in tumour metabolism.

Table 2. Techniques for functional and molecular imaging potentially useful in drug discovery programmes

<table>
<thead>
<tr>
<th>Imaging technique</th>
<th>Use</th>
<th>Reporter molecule/contrast agent</th>
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<tbody>
<tr>
<td>Magnetic resonance imaging</td>
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<tr>
<td>• Dynamic contrast-enhanced MRI</td>
<td>• Measurement of kinetic parameters of tumour vasculature</td>
<td>Gadolinium DTPA</td>
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<td></td>
<td>• Measurement of blood volume</td>
<td>Iron oxide particles</td>
</tr>
<tr>
<td>• Diffusion weighted MRI</td>
<td>• Measurement of parameters associated with rates and diffusion of water molecules</td>
<td>Diffusion weighted MRI</td>
</tr>
<tr>
<td>• BOLD MRI</td>
<td>• Measurement of parameters of tissue/tumour permeability and perfusion</td>
<td>Deoxyhaemoglobin</td>
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<tr>
<td>Ultrasound scanning</td>
<td>• Measurement of changes in tissue/tumour perfusion</td>
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</tr>
<tr>
<td>CT</td>
<td>• Measurement of tumour size and functional properties (tumour perfusion, blood–brain barrier breakdown)</td>
<td>Iodine-based contrast agents</td>
</tr>
<tr>
<td>Magnetic resonance spectroscopy</td>
<td>• Measurement of endogenous reporter molecules (metabolites) to evaluate tumour microenvironment</td>
<td>31P (adenosine triphosphate, phosphomonoester, inorganic phosphate, intracellular pH)</td>
</tr>
<tr>
<td></td>
<td>• Measurement of exogenous reporter molecules to evaluate tumour microenvironment:</td>
<td>19F (perfluorocarbons)</td>
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<tr>
<td></td>
<td>• Oxygenation</td>
<td>19F (SR-4554)</td>
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<tr>
<td></td>
<td>• Hypoxia</td>
<td>19F (fluorodeoxyglucose)</td>
</tr>
<tr>
<td></td>
<td>• Glucose utilization</td>
<td>19F (SFU, gemcitabine)</td>
</tr>
<tr>
<td></td>
<td>• Measurement of drug pharmacokinetics in tumour and normal tissues</td>
<td>31P (cyclophosphamide, ifosfamide)</td>
</tr>
<tr>
<td></td>
<td>• Measurement of generic biological endpoints:</td>
<td>13C]thymidine, [19F]fluorothymidine</td>
</tr>
<tr>
<td></td>
<td>• Cellular proliferation</td>
<td>19F]fluorodeoxyglucose</td>
</tr>
<tr>
<td></td>
<td>• Glucose utilization</td>
<td>[13N]H20</td>
</tr>
<tr>
<td></td>
<td>• Tissue perfusion</td>
<td>[15O]CO</td>
</tr>
<tr>
<td></td>
<td>• Blood volume</td>
<td>[13C]methionine</td>
</tr>
<tr>
<td></td>
<td>• Amino acid metabolism</td>
<td>13C]thymidine</td>
</tr>
<tr>
<td></td>
<td>• Measurement of specific biological endpoints:</td>
<td>[124I]labelled antibodies/peptides</td>
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<tr>
<td></td>
<td>• Detection of thymidylate synthase inhibition</td>
<td>[124I]anti-erbB2 antibody</td>
</tr>
<tr>
<td></td>
<td>• VEGF/VEGF receptor inhibition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Over-expression of erbB2</td>
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MRI, magnetic resonance imaging; BOLD, blood oxygenation level dependent; PET, positron emission tomography; VEGF, vascular endothelial growth factor.

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nucleus that possesses nuclear spin when placed in a magnetic field. When the pulse is terminated the nucleus relaxes, emitting energy, hence allowing detection of molecules containing the nucleus. MRS data are usually expressed in the form of a spectrum, in which different peaks correspond to different chemical compounds containing the nucleus. However, spatial images of chemical concentrations can also be produced [49]. MRS-detected endogenous reporter probes can be used to evaluate factors including tissue metabolism, bioenergetic status, pH and phospholipid membrane turnover. This can be achieved by measuring a range of metabolites, by $^{31}$P MRS (e.g. adenosine triphosphate, inorganic phosphate), and also by $^1$H MRS (N-acetylaspartate, choline, lactate) which has been used particularly in the brain [49, 50]. Spectra of these metabolites may change during a course of anticancer treatment, thus giving an indication of response to therapy and thereby acting as a PD endpoint [51–53]. MRS-detected externally administered fluorinated reporter probes can be used to measure cellular hypoxia (SR-4554 [54]), cellular oxygenation (fluorocarbon relaxometry [55, 56]), and glucose utilization (fluorodeoxyglucose [57]), although the last two approaches have not yet entered the clinic.

PET is the non-invasive dynamic measurement of the three-dimensional distribution within the body of compounds labelled with positron-emitting isotopes. Externally administered PET-labelled reporter probes can be used to evaluate generic biological endpoints that can act as PD endpoints relevant to a particular molecular target. For example, a biological effect of a new drug may be to inhibit cellular proliferation, which can be monitored by $^{11}$C-thymidine [58]. Other generic biological endpoints that can be explored by PET include glucose utilization ($^{18}$F-fluorodeoxyglucose [59, 60]), tumour perfusion ($^{15}$O$\text{H}_2\text{O}$ [61]), and blood volume ($^{15}$O$\text{CO}$ [62, 63]).$^{18}$F-fluorodeoxyglucose was used recently to demonstrate the rapid response of gastrointestinal tumours to imatinib mesylate (Glivec) [64]. PET can also be used to measure specific biological endpoints that are directly relevant to a particular molecular target. For example, use of $^{124}$I anti-erbB2 antibodies to detect over-expression of the ErbB2 gene [65] can identify patients suitable for therapy with the anti-erbB2 antibody Herceptin, which is used in the treatment of breast cancer. In an interesting recent application, PET was used to show that the thymidylate synthase inhibitor AG337 was able to increase the tumour uptake of $^{14}$C-thymidine [66]. This increased uptake measures the salvage pathway for thymidine and provides a PD endpoint that demonstrates thymidylate synthase inhibition by the drug. Furthermore, considerable effort is going into the development of reporter gene readouts used with PET to study the efficiency of gene expression following gene therapy [67]. For example, the herpes simplex virus enzyme thymidine kinase, when expressed in transfected mammalian cells, converts PET substrates into a detectable form that can be imaged as an indicator of gene transfer efficiency [68].

To illustrate further the role of imaging technologies in drug discovery and development, two specific examples from our own work will be discussed in more detail: the Hsp90 inhibitor 17AAG, and the hypoxia probe SR-4554.
Hsp90 inhibitor 17AAG

Hsp90 is a molecular chaperone that has recently emerged as a new target for anticancer therapies [69, 70]. It is responsible for folding, stability and function of a range of oncogenic client proteins, e.g. RAF-1, ErbB2, and its inhibition results in degradation of the client proteins by the ubiquitin-proteasome pathway. Thus, the inhibition of a single target, Hsp90, has the potential to affect multiple oncogenic pathways [69].

The first Hsp90 inhibitor, 17AAG, recently entered Phase I study at our institution [71] and at four centres in the USA. In the pre-clinical phase of development, a specific molecular signature of Hsp90 inhibition was characterized, using protein analysis by western blotting [72–74] and gene expression profiling by microarray analysis [75]. The features are of depletion of client proteins and a simultaneously increased expression of the Hsp70 gene family. This molecular signature has been validated in cell culture studies and in vivo animal models [69, 73, 76]. As a direct result of this work, it has been possible to incorporate PD assays into Phase I studies by evaluating client protein and Hsp70 expression profiles in peripheral lymphocytes and tumour tissue of treated patients. This has provided evidence of achievement of the desired molecular effect of Hsp90 inhibition [71]. We are now extending this work to study the relationship between the plasma PK of 17AAG, alterations in the protein and gene expression profiles in peripheral blood lymphocytes and tumour biopsies, and the clinical response of the patients, particularly in melanoma where prolonged stable disease has been observed [77]. This represents a good example of the construction of a detailed audit trail during pre-clinical and clinical drug development [25]. In addition to its use with 17AAG, the PD endpoints can be used in the development of second-generation Hsp90 inhibitors.

However, the undesirability of repeated tumour biopsies has driven the search for non-invasive PD endpoints. Changes in the concentration of phosphoethanolamine, phosphocholine and phosphodiester metabolites have been detected by MRS in human tumour xenografts following treatment with 17AAG, suggesting possible changes in cell membrane turnover and alterations in lipid signalling [78]. Although the precise mechanistic relationship between the MRS changes is not yet understood, we are now looking for similar changes in the tumours of patients treated with 17AAG at our institution. In addition, radiolabelled choline has been used to study alterations in choline metabolism in treated tumour cells, establishing the potential for the use of PET-detected [11C]choline to study the PD effects of 17AAG in tumours in the clinic [79]. Future clinical studies will determine whether these findings are reproducible in humans.

Development of SR-4554 as a hypoxia reporter probe

SR-4554 is a novel agent that has been developed for use as a reporter probe to detect tissue and tumour hypoxia [80]. It is specifically targeted to hypoxic cells where it is detected by 19F MRS. It is of particular interest in the context of the present review, in that while its pre-clinical development illustrates the use of the pharmacological audit trail, it is in fact a non-therapeutic, diagnostic reporter probe that can be employed as a prognostic and PD assay in its own right.

Tumour hypoxia is recognized to be of considerable clinical importance [81]. It has been unequivocally shown to occur in human tumours [82] and to relate to a poor clinical outcome following anticancer treatment [83–85]. Hypoxia has long been known to be associated with radioresistance [86] and radiotherapy treatment failure [85], but it has more recently become apparent that hypoxia also results in treatment failure following surgery [84] and resistance to chemotherapy [87–89]. Furthermore, it acts as a selective pressure for the development of a more malignant tumour phenotype [90], leading to enhanced tumour growth and progression [91]. The molecular basis for the effects of hypoxia has emerged with the discovery of the hypoxia inducible factor (HIF), which plays a central role in oxygen homeostasis and adaptability of the tumour microenvironment under conditions of normal oxygenation and in response to hypoxia [92, 93].

Thus, detection of hypoxia within tumours by SR-4554 could provide prognostic information, enabling identification of patients likely to have a poor clinical outcome. Such patients might be suitable for more intensive conventional therapies or novel therapies targeted to the HIF pathway. An alternative approach is the use of therapeutic strategies aiming to reverse hypoxia (hyperbaric oxygen [94], ARCON [95]), to increase the effectiveness of conventional therapies (radiosensitizers [95]), or to exploit hypoxia (bioreductive cytotoxic agents [96, 97]). A further role for SR-4554 lies in its use as a PD assay to monitor response to hypoxia-modifying therapies, and also the effects of antiangiogenic and antivascular agents. In addition, SR-4554 might have a role in the detection of hypoxia in normal tissues in disease processes such as stroke and ischaemic heart disease.

SR-4554 is a fluorinated 2-nitromidazol (Figure 4), that was specifically designed to be used as a non-invasive hypoxia probe [80]. This function is achieved by selective bioreduction of the nitro group under hypoxic conditions by intracellular reductase enzymes. This process does not occur under oxic conditions. A number of reactive metabolites are formed, which are then covalently bound within the cells. The presence of the metabolites, detected by 19F MRS by virtue of the possession of three magnetically equivalent 19F atoms within the side chain, indicate cellular hypoxia. The use of SR-4554 as a hypoxia probe has been validated in pre-clinical studies [98–102]. Thus, selective retention of SR-4554 bioreduction products within tumour cells has been demonstrated, and has been

Figure 4. Chemical structure of the hypoxia probe SR-4554.
shown to correlate with tumour pO$_2$ measured by polarographic electrode (Figure 5), confirming that it is achieving the desired function of detecting hypoxia. SR-4554 is rapidly absorbed after oral and intraperitoneal administration in the mouse and undergoes extensive renal excretion [100, 103], demonstrating pharmacokinetic properties that are favourable for use as a hypoxia reporter probe. It was designed with a relatively hydrophilic side chain, to minimize crossing of the blood–brain barrier, thereby reducing the risk of neurotoxicity encountered with nitroimidazoles used as radiosensitizers. Low nervous system penetration without compromise of tumour uptake has been confirmed in pre-clinical studies [100].

Thus, in terms of the pharmacological audit trail, SR-4554 has been shown to have appropriate “drug-like” properties, achieving necessary blood and tissue concentrations for activity (as a hypoxia probe), and to have achieved the desired function (detection of hypoxia). On the basis of these encouraging pre-clinical results, SR-4554 entered Phase I study at the Institute of Cancer Research in association with the Royal Marsden Hospital, under the auspices of Cancer Research UK [54]. PK properties were as predicted by the pre-clinical animal studies, with rapid plasma elimination and high renal clearance being demonstrated. PK parameters such as peak levels and area under the curve increases linearly with dose, and there is very good reproducibility between and within patients. In addition, SR-4554 is well tolerated and has been administered safely up to doses of 1400 mg m$^{-2}$. Crucially, it has been detected in tumours by MRS at these doses. It thus shows early promise as a non-invasive hypoxia probe, and clinical studies are continuing.

Conclusions

Drug development is now an extremely sophisticated and costly activity. New technologies are having a tremendous impact on the process, and new molecular targets are emerging from our increasingly detailed understanding of the molecular pathology and genomics of cancer. This approach is validated by the clinical activity and regulatory approval of Glivec, Herceptin and Iressa. The unprecedented supply of molecular targets coupled with the speed with which innovative new drugs can now be developed against these targets, using technologies such as high throughput screening, combinatorial chemistry and structural biology, presents us with many exciting opportunities but also a series of formidable challenges. To which targets should we give priority? How can we make sure that only the best and most promising projects are taken forward? On what basis can we terminate those that are not going to be successful?

In the present article we have emphasised the importance of the pharmacological audit trail [24, 25]. It supplies a conceptual framework for planning new drug development programmes, and also a set of performance indicators against which informed decisions on individual projects can be made. Priorities between projects in a portfolio can be decided in a rational way. The audit trail provides a hierarchy of questions and the corresponding experimental assays or endpoints to address these questions, linking all of the important aspects of drug action from the expression of the molecular target, through PK and PD endpoints, to the eventual biological and clinical outcome.

Clearly, the availability of relevant PK and PD endpoints is essential for rational and efficient decision-making in clinical drug development. They not only provide the basis for stop/go decisions, but also facilitate the selection of the optimal dose and schedule. Most of the current assays are, however, invasive in nature and thus present logistic and ethical challenges. Non-invasive PD endpoints are urgently required. Developments in molecular and functional imaging are now beginning to have a significant impact. Although there are instances where particular molecular events can be monitored, molecular and functional imaging methods are generally more applicable to monitoring events downstream of the drug target and the biochemical pathway in which it operates. Assays for biological processes such as proliferation, apoptosis and angiogenesis have generic utility across multiple target projects and hence are likely to be the most cost effective to develop in terms of return on investment. Developing a method specific to each molecular target is extremely costly and in most cases will be unrealistic. Moreover, if the drug development project is terminated, the method developed may have little or no subsequent application. Emphasis should therefore be placed on the development of robust PD endpoints that will be useful across a range of target projects. In addition to PD endpoints, biomarkers that predict which patients are more likely to benefit from a new therapeutic agent are also extremely important, particularly as we enter the era in which individualization of therapy is likely to become a reality. This approach is exemplified by the use of techniques that identify hypoxic tumours, such as the hypoxia probe SR-4554.

The development and use of PK and PD endpoints, whether by invasive or non-invasive means, requires the involvement of multidisciplinary teams and in particular a close integration of the drug development/clinical pharmacology and molecular/functional imaging communities. The Cancer Research UK Pharmacokinetic and Pharmacodynamic Technology Advisory Committee has been established to respond to this need, and in particular

Figure 5. Correlation between the retention of SR-4554 bio-reduction products in tumour cells detected by $^{19}$F magnetic resonance spectroscopy (MRS) indicating cellular hypoxia (3 h $^{19}$F fluorine retention index) and tumour pO$_2$ measured by polarographic electrode (pO$_2$ values <2.5 mmHg) in groups of P22 tumour-bearing female SCID mice. Tumour hypoxia was increased by hydralazine (HDZ) and combretastatin A-4 phosphate (CA-4-P), decreased by carbogen and nicotineamide (C/N), or unmodulated (air). Error bars indicate standard error of the mean. (Modified from reference [102]).
to ensure that the most appropriate PK/PD assays are developed in a timely way to support Cancer Research UK’s clinical trials portfolio.

The continued development of novel molecular and functional imaging technologies for non-invasive measurement of PK and PD endpoints will be extremely important for the development of cancer drugs over the next decade.

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