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European Journal of Cancer 38 (2002) 2189–2193

European Journal of Cancer

www.ejconline.com

Challenges of PK/PD measurements in modern drug development

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Received 31 May 2002; accepted 1 August 2002

1. The need for PK and PD endpoints

Modern cancer drug development can be characterised by a number of important principles [1–4]. These include: (1) A focus on the genes and pathways that are driving the molecular pathology and progression of human cancers, providing an intellectual framework and a source of new molecular targets for improving drug efficacy and selectivity; (2) Application of new technologies, such as high throughput screening, combinatorial chemistry and gene expression microarrays, to accelerate the discovery and development process; (3) Use of pharmacokinetic (PK) and pharmacodynamic (PD) endpoints to enhance the rationality and hypothesis-testing power of early clinical trials; and (4) Development of diagnostic, prognostic and pharmacogenomic biomarkers to allow the targeting of individualised treatments to patients most likely to benefit from a particular therapy. The exploitation of the cancer genome to discover new markers of molecular pathology, on the one hand, and novel therapeutic agents, on the other, is illustrated in Fig. 1. Against this exciting background, molecular imaging technologies have tremendous potential to impact on postgenomic drug discovery and development.

Due to advances in the molecular biology, genetics and pathology of cancer, underpinned by the Human Genome [5,6] and Cancer Genome Projects [7], the discovery of new drug targets is no longer the rate-limiting step in the development of innovative cancer drugs. Similarly, advances in high throughput screening, structure-based design and combinatorial chemistry have markedly advanced our ability to identify small molecule drug leads and to optimise them for clinical trial. Probably the major challenge for postgenomic drug development is to develop methodologies to enhance the quality and quantity of information obtained from early clinical trials of new agents, to use this information to improve the decision-making during clinical development, and in particular to develop endpoints and biomarkers that allow us to move towards the individualisation of treatment regimens.

Particular questions that need to be answered during early clinical development are:

1. Is the drug reaching the concentrations required for biological activity in the blood and tumour tissue?
2. Is the drug hitting the desired molecular target, e.g. inhibiting a particular kinase?
3. Is the drug modulating the biochemical pathway in which the molecular target functions, e.g. the Ras→Erk pathway?
4. Is the drug achieving the desired biological effect, e.g. inhibition of proliferation, cell cycle arrest, inhibition of invasion, angiogenesis and metastasis, or induction of apoptosis?

The availability of appropriate PK and PD assays allows each of these questions to be answered, sequentially or in parallel, so that drug development can be carried out in a rational, hypothesis-testing fashion (Fig. 2). Eventual linkage of PD effects to clinical response is important.

Increasingly, early clinical studies no longer simply involve dose escalations to define the maximum tolerated dose and to catalogue the toxic side-effects. Instead, they can be designed to determine whether a particular gene or pathway is an important factor in malignant progression. Another advantage is that decisions can be taken relatively early as to whether the

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0959-8049/02/$ - see front matter
PII: S0959-8049(02)00395-7

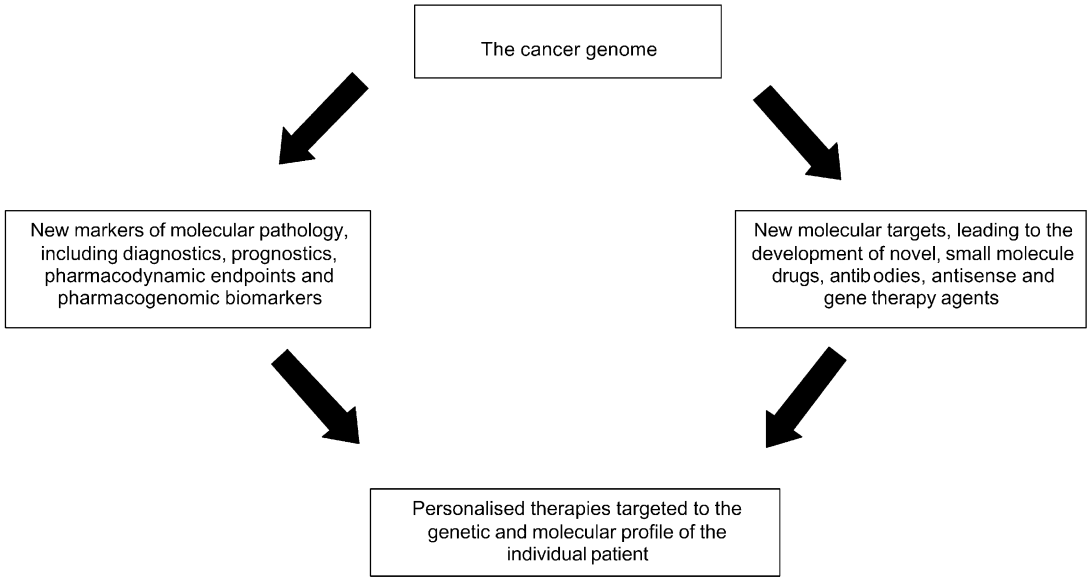

Fig. 1. Exploitation of the cancer genome for molecular pathology and novel therapeutics. Modern, postgenomic therapies will increasingly be targeted to the genetic and molecular profile of individual patients. The use of appropriate prognostic markers to identify patients likely to benefit from a particular therapy is extremely important, as is the application of pharmacodynamic endpoints to demonstrate that the target has been modulated in the intended way.

therapy shows the properties required to progress to the next stage. If not, the development can be terminated and resources redirected to overcome the problem or allocated to a more promising area.

PK and metabolism properties are, in general, relatively straightforward to determine, particularly with the impact of tandem mass spectrometry harnessed to liquid chromatography [8]. However, PK studies are usually carried out on samples of plasma or serum. Non-invasive assays that allow the measurement of PK behaviour in tumour and normal tissues can provide valuable additional data.

Even more important though is the development of assays to determine the PD effects of drugs. A range of relatively invasive methodologies are available to measure PD effects in normal and tumour samples. The use of molecular assays such as northern and western blotting, enzyme-linked immunosorbent assay (ELISA), RNase protection, and real time polymerase chain reaction methods can be extremely informative. The use of gene expression microarrays in particular is enhancing our ability to identify biomarkers and molecular PD endpoints [9,10]. However, surgically invasive assays have a number of limitations and therefore the development of less invasive imaging technologies is extremely important. The measurement of vascular parameters such as blood flow is a good example of how valuable information can be generated using non-invasive methods such as magnetic resonance spectroscopy/imaging (MRS/I) and position emission tomography (PET). For example, such methods have proved useful in the assessment of drugs that exert vascular damaging effects (e.g. combrestatin A4 phosphate) or anti-angiogenic effects (e.g. inhibitors of vascular endothelial growth factor receptor signalling).

Two projects from the work of the Centre will be described briefly in order to illustrate and exemplify progress and potential in the development of non-invasive endpoints.

2. Development of SR4554 as a hypoxia detection agent

Hypoxia is an extremely important factor in the malignant progression of human cancers [11,12]. Low oxygen levels are a major driving force for tumour angiogenesis and hypoxia is involved directly in resistance to radiation therapy and chemotherapy, as well in the selection for a more aggressive phenotype, including loss of p53. Furthermore, tumour hypoxia has been shown to be an independent prognostic factor in determining metastasis and survival in various types of cancer [13].

The fluorine-containing nitroimidazole agent SR4554 was developed as a non-invasive marker of tumour hypoxia [14–18]. Reduction of the nitro group by reductase enzymes leads to retention of the agent in the hypoxic regions of tumours. The fluorine-containing metabolites and adducts generated and retained in this way can be detected by MRS/I (in the case of the naturally abundant ^{19}F) or PET (in the case of the ^{18}F isotope). The approach has been validated in relatively anoxic animal tumours and human tumour xenografts

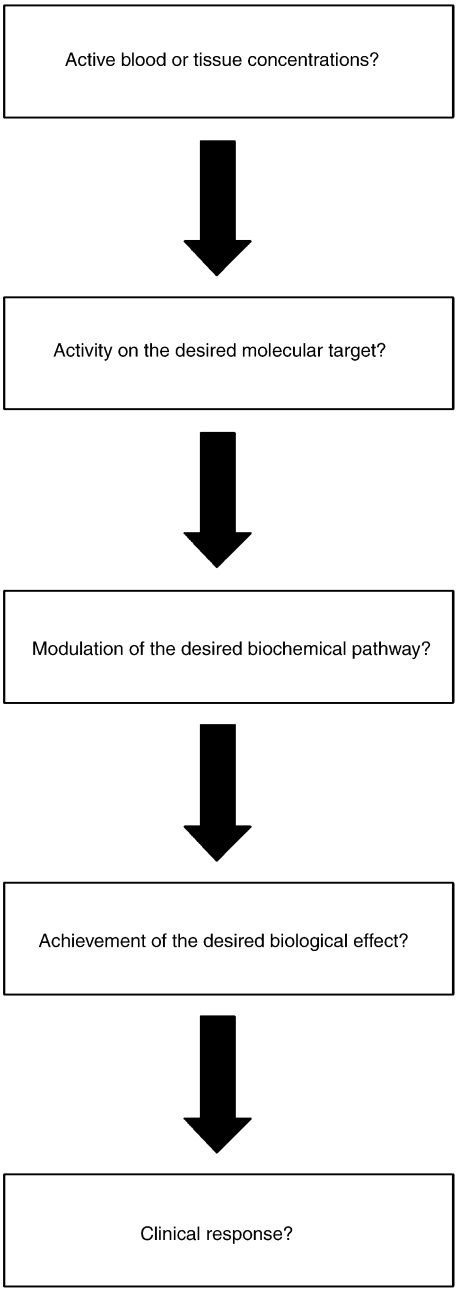

Fig. 2. Key questions that need to be answered in contemporary drug development. PK and PD endpoints to answer these key questions are essential.

[11–16] and also more recently in the P22 tumour which has an oxygen profile that more closely mimics many human tumours [17].

The relatively hydrophilic side-chain was designed to discourage penetration into the nervous system and thereby to avoid the neurotoxicity seen with some nitroimidazoles. The side-chain also encourages renal rather than metabolic clearance.

On the basis of these promising preclinical results, SR4554 has now entered a phase I clinical trial at our Institute, in association with the Royal Marsden Hospital and under the auspices of Cancer Research UK [19]. The drug has been given safely up to doses of 1400 mg/m^2 and the pharmacokinetic properties are reproducible and suitable for a hypoxia imaging agent. Furthermore, the fluorine signal has been shown to be detectable in the tumour tissue of treated patients. SR4554 therefore shows promise, and clinical studies are continuing.

Potential applications include the selection of patients for therapies for which hypoxia is a direct determinant of response (e.g. radiation and bioreductive drugs), the monitoring of the PD effects of antivascular and anti-angiogenic agents, and the prediction of outcome (e.g. metastasis and survival) in those tumours for which hypoxia is an independent prognostic indicator.

3. Development of PD endpoints for the Hsp90 molecular chaperone inhibitor 17AAG

The Hsp90 molecular chaperone is an exciting new molecular target for cancer treatment [20,21]. Of particular relevance is the fact that Hsp90 is responsible for the folding, stability and function of a range of oncogenic 'client' proteins, including Raf-1, erbB2, CDK4, oestrogen and androgen receptors and mutant p53. Inhibition of Hsp90 leads to degradation of the oncogenic client proteins by the ubiquitin proteasome pathway. Hence Hsp90 inhibitors may provide a one-step combinatorial attack on multiple oncogenic pathways in the cancer cell [20].

The first-in-class Hsp90 inhibitor 17AAG has entered a clinical trial at our institution [22] and elsewhere under the auspices of the US National Cancer Institute and Cancer Research UK. A combination of protein analysis, e.g. by western blotting [23–25] and also gene expression profiling by microarray analysis [10] has defined a specific molecular signature for Hsp90 inhibition. This involves the depletion of client proteins, such as Raf-1 and CDK4, and the increased expression of members of the Hsp70 gene family.

Several of these changes have now been validated in cell culture and human tumour xenografts and these are being used as PD markers in the phase I clinical trials of 17AAG. A decrease in client proteins and an increase in Hsp70 has been demonstrated in the peripheral blood lymphocytes and tumour tissue of patients treated with 17AAG [22].

A non-invasive PD endpoint would clearly be desirable. Recent studies have shown changes in phosphoethenolamine and phosphocholine in tumour cells and xenografts treated with 17AAG, as detected by MRS [26,27]. These unusual changes may be indicative of drug-induced alterations in membrane turnover or lipid signalling. Further studies are underway to elucidate the mechanism of these effects. We are monitoring

patients treated with 17AAG to determine whether similar changes can be seen in the clinic.

4. Concluding remarks

PK and PD endpoints are an essential component of the rational development and evaluation of new cancer drugs. They provide enormously helpful information and inform the decision-making process. Particular emphasis should be focused on the development of PD endpoints. Biomarkers that indicate directly whether the molecular target has been modulated are extremely valuable. Endpoints that provide evidence for intervention in a biochemical pathway are also very useful, as are methods to confirm whether a downstream biological effect has been achieved. Minimally invasive assays, e.g. involving MRS/I, PET and so on, have major advantages, not least for the patient. Significant resources should be put into the development of such assays. This will be very challenging technically, especially where the need is to measure a specific molecular event, such as the inhibition of phosphorylation of a signalling protein or the blockade of a protein-protein interaction.

This timely development of PK and PD endpoints requires the close collaboration of many disciplines, including chemistry, physics and pharmacology. In the case of new drug development that is carried out under the auspices of Cancer Research UK, the development, validation and use of PK and PD endpoints is fostered and monitored by the establishment of a Pharmacokinetic and Pharmacodynamic Technology Advisory Committee (PTAC).

Acknowledgements

Work carried out in the Cancer Research UK Centre for Cancer Therapeutics (http://www.icr.ac.uk/cctherap/index.html) is funded primarily by Cancer Research UK. Paul Workman is a Cancer Research UK Life Fellow. I thank my Centre colleagues and members of the Cancer Research UK Pharmacokinetic and Pharmacodynamic Advisory Committee for valuable discussions.

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