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Review

How to develop a successful cancer drug – molecules to medicines or targets to treatments?

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Abstract

Cancer chemotherapy remains the only treatment modality with curative activity against multiple forms of metastatic malignancy. Over the past decade, cytotoxic and anti-endocrine drugs have been supplemented by targeted therapies that seek to exploit the molecular lesions that underlie the carcinogenic process or maintain the cancer phenotype. Success with, for example, Imatinib and Trastuzumab has suggested that identification and validation of the drug target is the starting point for the optimal route to the development of active drugs. However, in reality, our understanding of the biology of cancer is still too rudimentary to allow drug developers to rely on the simplistic linear pathway of target identification and validation, lead identification and optimisation, followed by Phase I, II and III trials. As pre-clinical and clinical drug developers investigate the second wave of targeted agents, it is worthwhile reflecting on experience gained during the initial development of cytotoxic drugs. For example, the clinical activity of alkylating agents and antimetabolites was demonstrated before the targets for these drugs were defined in any detail. Recent experience with signal transduction modifiers has again shown that agents initially developed to exploit one target may actually hit other targets, and that interaction with these other targets may be responsible for the clinical activity of the compound. Using lung cancer, the world's single biggest cancer problem, as an example the development of recently evaluated drugs, both cytotoxic and targeted, is reviewed. On the basis of this Review, it is concluded that drug developers should design pre-clinical studies and early clinical trials in a manner that allows both the pharmacology of the drug as well as the biology of the target to inform the development process.

Keywords: Cancer drug; Targets; Treatment; Trials; Lung

1. Introduction

The drug development process can be defined as comprising two generic approaches to the identification of new therapies. On the one hand, chemicals are identified by screening against tumour models of some type, and if there is evidence of a therapeutic index in pre-clinical *in vivo* studies they are advanced to clinical trials. In this empirical approach, the target for the drug does not have to be defined and is termed here as the "molecules to medicines" route to drug discovery. On the other hand, the drug development process may start by the identification of a target (i.e. gene or gene product) that is believed to be linked to the molecular pathology of cancer. Increasingly sophisticated clinical molecular pathology (e.g., genomics and proteomics) and pre-clinical target validation studies (e.g., genetically modified tumour cell lines and animals, antisense and siRNA approaches) are then used to validate the target as one worthy of exploitation. Subsequently, contemporary drug development techniques (e.g., high throughput screening and/or structure-based design) are used to develop a clinical trial candidate whose activity it designed to operate *via* the intended target. In this more rational approach, knowledge of the drug target and its biology

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are central to the development process, and is termed here as the "targets to treatments" route to drug discovery.

Michel Clavel (1946–1993) suffered from a non-Hodgkin's lymphoma and was treated with cyclophosphamide, vincristine, doxorubicin, cytosine arabinoside and methotrexate (J.-Y. Blay, personal communication). These five cytotoxic drugs all exemplify the molecules to medicines approach to cancer drug discovery, i.e.:

- Cyclophosphamide was designed as a nitrogen mustard pro-drug that would be activated by tumour phosphoramidases, whereas in fact it is hepatic cytochrome P450 enzymes that are largely responsible for cyclophosphamide metabolism.
- The vinca alkaloids were identified by screening extracts of the periwinkle *Vinca rosea*.
- Doxorubicin was discovered as an antitumour product of the soil microrganism *Streptomyces peucetius* (var. *caesius*).
- Cytosine arabinoside was one of a very large number of nucleoside analogues synthesised and screened for potential and antimetabolite activity.
- Methotrexate was an analogue of the antifolate aminopterin, itself developed after Farber and colleagues had shown that folate supplements appeared to promote leukaemia progression in children.

Thus, for all of the above widely used cytotoxic drugs, the target and mechanism of action was largely or completely unknown at the time the agent was first shown to have clinical activity.

Recent improvements in our understanding of the molecular pathology of cancer have provided, for the first time, a mechanistic framework for understanding carcinogenesis and tumour progression. This understanding has also provided multiple targets against which therapies may be directed, and the notable success stories of Trastuzumab and Imatinib illustrate the potential of the targets to treatments route to drug discovery. However, targeted therapies have yet to have a major impact in many forms of cancer, or on the overall global burden of cancer deaths.

2. Lung cancer as a global problem and the need for new treatments

The World Health Organisation estimate that there are currently 10 million new cases of cancer *per* year, and that this will rise to 15 million by 2020 [1]. There are some 6 million cancer deaths each year, 12% of all deaths worldwide, and lung cancer is the single most common cause of cancer death (17% overall, 23% in males and 11% in females). Tobacco is the major carcinogen in lung cancer and an important carcinogen in multiple other tumour types, and it is estimated that tobacco-related mortality will rise from 100 million in the 20th century to 1000 million by the end of the current century [2]. In some developed countries, for example the UK, smoking prevention has resulted in a substantial decrease in the number of deaths from lung cancer, and epidemiological studies suggest that the decline will continue for the next 20 years at least [3]; however, in other developed countries, such as France and Japan, epidemiological studies indicate that lung cancer deaths are continuing to rise and are set to do so for the near future. Statistics for lung cancer in developing countries are less robust, but a cause for major concern, and lead to the conclusion that, in addition to redoubling efforts to prevent lung cancer, new treatments are also needed. The need for new treatments is clear from results with currently available drugs which are of very limited benefit. For example, in England and Wales, 5-year survival rates in lung cancer patients are <10% [4].

3. Recently developed treatments for the lung cancer

Give the magnitude of the lung cancer problem, and the need for new treatments, the disease has understandably been the focus of intensive pre-clinical and clinical research which has resulted in the identification of a number of promising new drug treatments, both cytotoxic and targeted agents. Selected examples of these new treatments are reviewed below specifically from the perspective of whether these agents were developed by turning molecules into medicines, or targets into treatments.

4. Novel cytotoxic therapies for the treatment of lung cancer

Standard first-line therapy for the treatment of non-small cell lung cancer (NSCLC) involves cisplatin or carboplatin in combination with a second generation cytotoxic drug, e.g., a taxane, gemcitabine or vinorelbine. Intensive clinical research is ongoing; however, there is no clear consensus that any one platinum/cytotoxic doublet, or indeed triplet of drugs, is superior [5]. Year 2004 has seen the identification of a further second generation cytotoxic drug with unequivocal activity on NSCLC, the antifolate pemetrexed, and as the most recent cytotoxic drug to be identified for the treatment of lung cancer, the development of pemetrexed will be reviewed to determine whether the molecule resulted from the systematic identification and exploitation of a target, or the optimisation of a molecule.

Antifolates were first used for the treatment of leukaemia by Faber and colleagues in the late 1940s; however, it was not until the late 1950s that the primary

target of methotrexate (MTX), by then the most important antifolate, was identified, namely dihydrofolate reductase (DHFR). As illustrated in Fig. 1, a large volume of biochemical pharmacology research over the intervening 50 years has identified thymidylate synthase (TS) and glycinamide ribonucleotide formyl transferase (GARFT) as key direct and indirect targets for antifolate drugs. As a result of the inhibition of these enzymes, tumour cells are unable to sustain the *de novo* synthesis of purine nucleotides (ATP/GTP and dATP/dGTP) and thymidylate (TTP) required for RNA and DNA synthesis, and hence cell division [6,7]. In particular, the identification of TS as an indirect target for MTX, inhibition being due primarily to the depletion of reduced folate co-factor pools resulting in insufficient 5,10-methylene tetrahydrofolate for *de novo* thymidylate synthesis, was a major stimulus for the development of direct selective TS, as opposed to DHFR, inhibitors. Clinical data with the first selective antifolate TS inhibitor (CB3717) were reported in the early 1980s; however, despite showing signs of clinical activity, dose-limiting nephrotoxicity, often complicated by unpredictable myelo-suppression, led to the cessation of clinical trials. The nephrotoxicity of CB3717 was shown to be due to the poor solubility of the drug under acidic conditions, and raltitrexed was developed as a more soluble derivative with improved antitumour potency, which has been registered for clinical use in certain countries [7]. At the same time as developing pure TS inhibitors, a number of selective GARFT inhibitors, i.e. anti-purine antifolates, have also

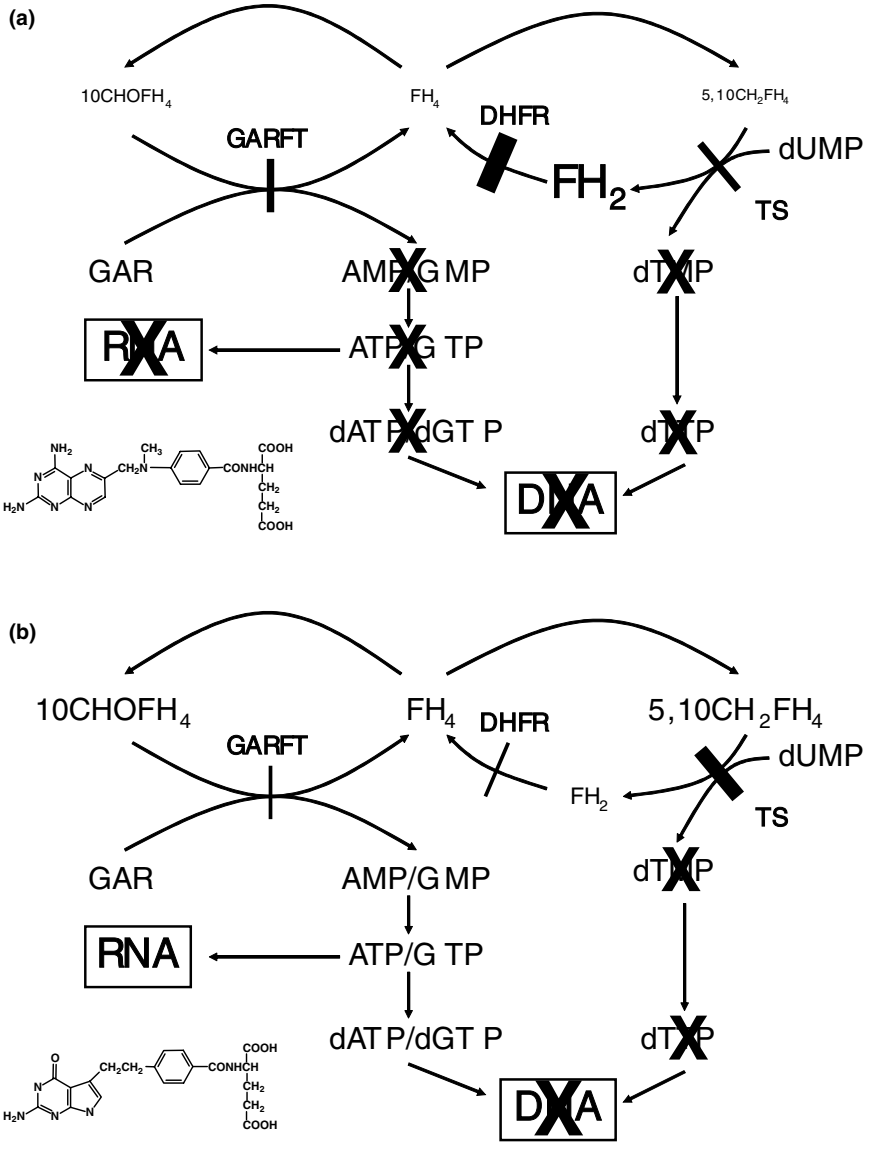

Fig. 1. Mechanism of action of methotrexate and pemetrexed: (a) methotrexate inhibits dihydrofolate reductase (DHFR) thereby decreasing reduced folate co-factor pools, indirectly inhibiting thymidylate synthase (TS) and glycinamide ribonucleotide transformylase (GARFT), and depleting nucleotide precursors for RNA and DNA synthesis; (b) pemetrexed primarily inhibits TS, with DHFR and GARFT as additional *loci*.

been brought to clinical trials. These compounds are still under investigation; however, pronounced antitumour activity has yet to be reported [7].

The most recent antifolate, pemetrexed, combines properties of a number of antifolate drugs in a manner that results in a favourable therapeutic index when patients are supplemented with folic acid and vitamin B12 to overcome sporadic and unpredictable anti-proliferative toxicity [8]. The biochemical pharmacology of pemetrexed has been carefully investigated and the drug acts primarily as a direct TS inhibitor, whilst also having the ability to inhibit GARFT and DHFR, albeit with less potency than TS [9]. This spectrum of enzyme inhibition produces effects on folate and nucleotide pools that are distinct from those of MTX, and also may limit cross-resistance with other antifolates [10]. In a Phase III clinical trial of pemetrexed in relapsed NSCLC, published in 2004, Hana and colleagues [11] clearly demonstrated that pemetrexed has equivalent antitumour activity to docetaxel, a standard treatment for relapsed NSCLC, but at the cost of considerably less haematological toxicity – making pemetrexed a valuable addition to lung cancer therapy. Looking at the overall development of pemetrexed, the evolution of the compound can be traced back to MTX, a drug which showed activity well before its target was identified, to the selective antifolate TS inhibitors, which in turn led to a multi-targeted compound – pemetrexed. Hence, the drug is an example of a molecule to a medicine (MTX) to a target (TS) and then to a treatment (pemetrexed).

5. The development of targeted therapies for lung cancer

The development of targeted therapies starts with the identification of a gene or gene product that is linked to the molecular or cellular pathology of the tumour. The key molecular pathological lesions in cancer are oncogene activation, tumour suppressor gene loss of function and the activation of immortality genes. Lung cancer has been extensively investigated and key molecular lesions are summarised in Table 1. Therapeutic interventions to exploit this molecular pathological information have now been extensively investigated, and promising clinical results are beginning to emerge.

Table 1
The molecular pathology of lung cancer

Tumour suppressor gene loss of function	
P53	50% NSCLC and 75-100% SCLC
Rb	15-30% NSCLC and 90% SCLC
p16	70% NSCLC
Oncogene activation	
RAS	*KRAS* mutation in NSCLC
EGFR	EGFR overexpression in NSCLC
MYC	Overexpression of *MYC* family members

Data extracted from [14,15].
NSCLC, non-small cell lung cancer; SCLC, small lung cancer; EGFR, epidermal growth factor receptor.

6. Therapies designed to target tumour suppressor gene loss in lung cancer

As summarised in Table 1, loss of tumour suppressor gene function is a very common event in lung cancer. Mechanistically, three key tumour suppressor genes (p53, p16 and Rb) involved in the regulation of progression through the G1/S boundary of the cell cycle malfunction in lung cancer. As illustrated in Fig. 2, transition from G1 into S phase requires the activity of a family of transcription factors collectively termed E2F, which are components of transcriptional complexes required for the activation of key S-phase genes [12]. In non-dividing cells, E2F is held in an inactive form by Rb binding and the release of Rb that is required for G1/S transition involves Rb phosphorylation. The phosphorylation of Rb can be catalysed by the cyclin-dependent kinases (CDKs) 4/6 and 2, sequentially, following their activation by cyclin partners. In addition to the cyclical fluctuation of cyclin levels during the cell cycle, and post-translational modification by phosphorylation, CDK activity is negatively regulated by the binding of endogenous peptide inhibitors. Two key CDK-inhibitory peptides are p16 and p21, that latter being the product of an important p53-regulated gene [13]. Thus, in lung cancer, unrestrained CDK activity (due to epigenetic silencing of p16, deletion of p16, or reduced p21 expression due to loss of p53 function) or loss of Rb (due to mutation or deletion) results in deregulated cell growth and unrestrained cell division [14,15].

Therapeutic approaches to overcoming the loss of tumour suppressor gene function during G1/S transition have included p53 gene therapy, the use of peptides based on p16 and direct inhibitors of CDKs. Gene therapy has yet to produce convincing clinical benefit [16]; however, small molecule CDK inhibitors have been extensively studied in clinical trials and investigations are ongoing [17]. The first generation compound flavo-

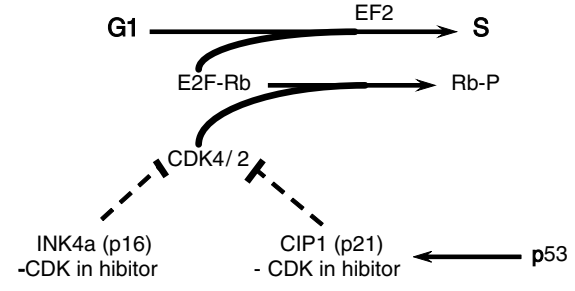

Fig. 2. The G1/S phase transition and the role of the p53, Rb and p16 tumour suppressor genes.

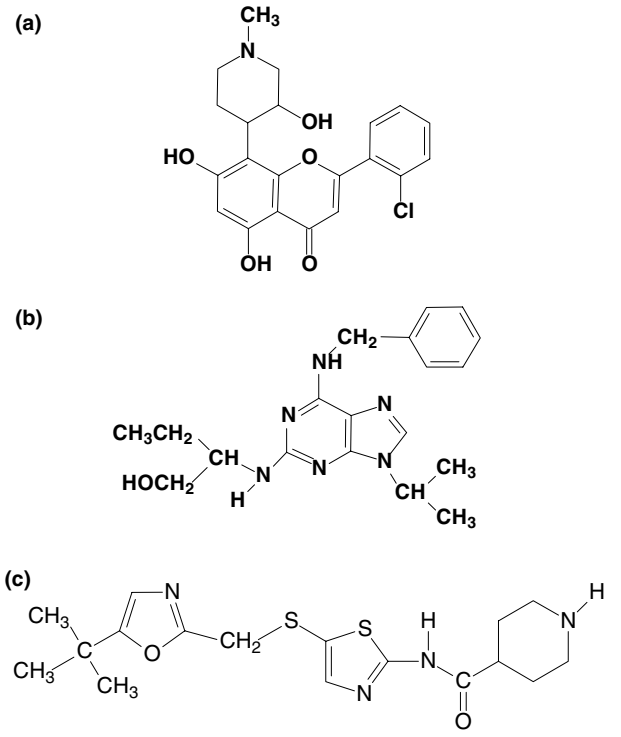

Fig. 3. CDK inhibitor chemical structures: (a) Flavopiridol; (b) R-roscovitine (CYC-202); (c) BMS-387032.

piridol (Fig. 3) did not demonstrate significant single agent activity in lung cancer, and Phase III trials have been suspended. CYC-202 (Fig. 3), also known as R-roscovitine, is currently in clinical trials for NSCLC in combination with cytotoxic drugs and the more selective CDK2 inhibitor BMS-387032 (Fig. 3) is currently completing single agent Phase I trials. Only BMS-387032 has been reported to have a dose-limiting anti-proliferative toxicity, namely neutropenia, an effect that might be predicted for a compound designed to prevent cell cycle progression. Interestingly, recent pre-clinical data using both molecular genetic approaches in human tumour cell lines and knock-out mouse models have questioned the validity of CDK2 as a drug target [18,19], and if the CDK inhibitors currently in clinical trials – in particular BMS-387032 – do demonstrate significant activity it may not be mediated by the intended CDK target. Thus, if CDK inhibitors originally designed to act *via* CDK2 are useful anticancer drugs this could represent a further example of the success of the molecule to medicine approach to drug development.

7. Therapies designed to overcome oncogene activation in lung cancer

As indicated in Table 1, overexpression of the epidermal growth factor receptor (EGFR) and activating mutations of *RAS* genes, in particular *KRAS* at codons 12, 13 and 61, are frequently described events in lung cancer [14,15]. However, in both cases, the impact of these events on disease prognosis is not well established. Thus the situation in lung cancer is clearly distinct for that of *BCR-ABL* translocation in chronic myeloid leukaemia and *C-ERBB2/HER2* amplification in breast cancer, events which are clearly diagnostic or prognostic and targets that have been successfully exploited with novel therapeutics; Imatinib and Trastuzumab, respectively.

Notwithstanding the lack of a clear prognostic effect of either EGFR overexpression or *RAS* mutation, these targets have been extensively studied, the former leading to effective new treatments for NSCLC. Therapeutic approaches to treat EGFR-expressing tumours include both blocking antibodies and small molecule EGFR tyrosine kinase inhibitors. The latter have been studied in Phase III trials in NSCLC and the two lead compounds are gefitinib and erlotinib (Fig. 4). Gefitinib is already registered in certain countries for the treatment of relapsed NSCLC and 2004 has seen reports of the first Phase III clinical trial to unequivocally demonstrate that an EGFR tyrosine kinase inhibitor, erlotonib, can improve survival in patients with relapsed NSCLC, when compared with placebo [20]. Recent molecular pathology studies have suggested that patients who benefit most from treatment with gefitinib and erlotinib may be those with activating mutations in the *EGFR* gene [21–23]. However, the magnitude of the beneficial effect of erlotonib in the recently reported Phase III trial may exceed the frequency of *EGFR* mutations described to date, suggesting additional determinants of sensitivity, which may be related to the pharmacology of the particular EGFR tyrosine kinase inhibitor used. Regardless of the final outcome of the current intensive investigations into the molecular and clinical pharmacology of EGFR tyrosine kinase inhibitors, 2004 has established beyond doubt that these compounds do have significant activity

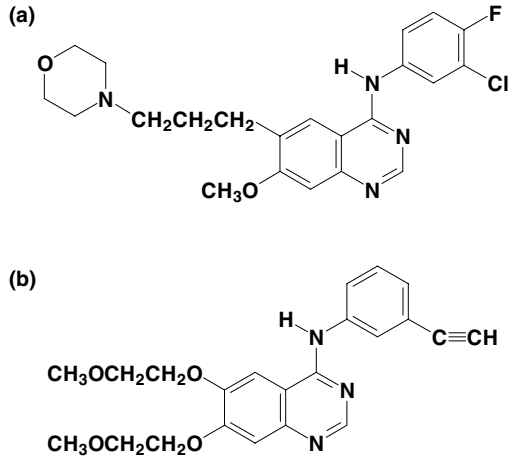

Fig. 4. Gefitinib and erlotinib chemical structures: (a) Gefitinib; (b) Erlotinib.

in NSCLC and hence their development is clearly an example of a target being exploited to develop a drug.

The second oncogenic lesion to be targeted in lung cancer is mutant *RAS*. Mutant *RAS* itself has proven intractable, to date, as a drug target, i.e. molecules that block the activity of the mutant protein, which inappropriately maintains the GTP-bound active confirmation, have not been identified and hence two alternative approaches have been investigated: blocking the post-translational modification of the ras protein that is required for membrane localisation and signal transduction, or blocking the mitogen-activated protein (MAP) kinase pathway downstream of ras, notably at C-raf kinase [24]. The post-translational modification of ras proteins involves prenylation in a reaction that transfers farnesyl residues to ras in order to facilitate appropriate membrane localisation and interaction with upstream and downstream signalling complexes. The transfer of the farnesyl residue to ras is catalysed by a farnesyl transferase, and a number of farnesyl transferase inhibitors (the FTIs) have been developed and tested, some in lung cancer patients [25]. A major limitation of FTIs is that they lack any selectivity for mutant as opposed to wild-type ras. Furthermore, post-translational modification by farnesylation involves many proteins in addition to ras, and hence where activity is seen it is not possible to unequivocally attribute the effects to an interruption of ras-signalling. In pre-clinical models, which included lung tumours, the FTIs demonstrated significant activity, albeit in a manner that was independent of mutant ras status, and, as a result, the compounds have progressed to clinical trials. These have not as yet revealed significant activity, either as single agents or in combination. Indeed, a Phase III trial in lung cancer of one FTI (lonafarnib) in combination with carboplatin and paclitaxel has recently been suspended due to a lack of efficacy. Should FTIs subsequently be shown to be active in lung cancer, there is currently no definitive evidence that any effect would be due specifically to the interruption of mutant ras-dependent signalling, and as such it would be hard to champion these drugs as examples of successful target exploitation.

An alternative approach to interrupting mutant ras-mediated signalling is to inhibit enzymes downstream of ras, and raf in particular has been the subject of intensive investigations. The most advanced raf inhibitor is sorafenib (BAY 43-9006) [26], and preliminary results of Phase II trials with the compound were reported in 2004 [27]. Interestingly, although originally developed as a C-raf inhibitor, sorafenib is now known to inhibit multiple kinases, in particular B-raf, vascular endothelial growth factor receptor 2 (VEGFr2), Flt-3 and c-kit. Although studies in lung cancer have yet to be reported, sorafenib has significant activity in renal cell cancer, which begs the question of the target responsible for clinical activity in this disease. The activity of sorafenib against VEGFr2, coupled with the known clinical activity of the anti-VEGF antibody bevacizumab in renal cancer, at the least raises the possibly that VEGFr2, and not C-raf, is the target for sorafenib in this malignancy, and further studies to address this possibility are clearly warranted. If it does transpire that sorafenib is active by virtue of VEGFr2 inhibition, it will again be an example of the success of the molecule to medicine approach.

8. Conclusions

This short article has reviewed recent developments in the treatment of lung cancer, the most common cancer worldwide that, in the absence of effective new treatments, is set to account for many millions of deaths during the current century. Although the prognosis for lung cancer patients currently remains dismal, recent developments have identified a number of new drugs with clinical activity which, it is hoped, will offer patients both more therapeutic options and a greater chance of survival with a satisfactory quality of life. In reviewing the development of these new drugs, it is clear that for both targeted and cytotoxic agents the drug may ultimately be found to act on a target other than that originally intended. Hence all those involved in both pre-clinical and clinical drug development should maintain an ''open-mind'' at all times to all possibilities. The two extremes of the drug development spectrum have been described as ''molecules to medicines'' and ''targets to treatments'' for the purposes of this article, and it is of course recognised that this is a false dichotomy and that both approaches should be applied on every project. Conceptually, compounds should be viewed as both potential drugs and pharmacological probes that permit 'proof-of-principle' clinical studies. Once this concept is accepted, the drug development process has to be modified to promote, in particular, the early development and validation of the pharmacological assays (both pharmacokinetic and pharmacodynamic) that will be needed in clinical trials. Delaying the development of such assays until the time a clinical trial candidate is identified will result in either unacceptable delays to the initiation of clinical trials, or trials going ahead without the required tools to hand with the result that patients are not managed in an optimal and contemporary manner.

On the occasion of the 16th European Organisation for Research and Treatment of Cancer/National Cancer Institute/American Association for Cancer Research (EORTC/NCI/AACR) meeting, patients are still receiving, and oncologists are still heavily dependent on, drugs of the type used to treat Michel Clavel. Developments over the past decade have provided examples of a number of targeted therapies with significant clinical activity, and these are now beginning to emerge for the treatment

of lung cancer. Combining the molecules to medicine and targets to treatments approaches to drug development requires multidisciplinary teams that embrace the full spectrum of expertise needed to understand the disease, develop the drug, delivery the drug to the clinic and treat the patient, and only through consistent team-work can such an approach be brought to fruition.

Conflict of interest statement

None declared.

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