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The opportunities and challenges of personalized genome-based molecular therapies for cancer: targets, technologies, and molecular chaperones

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Abstract There are now unprecedented opportunities for the development of improved drugs for cancer treatment. Following on from the Human Genome Project, the Cancer Genome Project and related activities will define most of the genes in the majority of common human cancers over the next 5 years. This will provide the opportunity to develop a range of drugs targeted to the precise molecular abnormalities that drive various human cancers and opens up the possibility of personalized therapies targeted to the molecular pathology and genomics of individual patients and their malignancies. The new molecular therapies should be more effective and have less-severe side effects than cytotoxic agents. To develop the new generation of molecular cancer therapeutics as rapidly as possible, it is essential to harness the power of a range of new technologies. These include: genomic and proteomic methodologies (particularly gene expression microarrays); robotic high-throughput screening of diverse compound collections, together with *in silico* and fragment-based screening techniques; new structural biology methods for rational drug design (especially high-throughput X-ray crystallography and nuclear magnetic resonance); and advanced chemical technologies, including combinatorial and parallel synthesis. Two major challenges to cancer drug discovery are: (1) the ability to convert potent and selective lead compounds with activity by the desired mechanism on tumor cells in culture into agents with robust, drug-like properties, particularly in terms of pharmacokinetic and metabolic properties; and (2) the development of validated pharmacodynamic endpoints and molecular markers of drug response, ideally using noninvasive imaging technologies. The use of various new technologies will be exemplified. A major conceptual and practical issue facing the development and use of the new molecular cancer therapeutics is whether a single drug that targets one of a series of key molecular abnormalities in a particular cancer (e.g. BRAF) will be sufficient on its own to deliver clinical benefit (''house of cards'' and tumor addiction models). The alternative scenario is that it will require either a combination of agents or a class of drug that has downstream effects on a range of oncogenic targets. Inhibitors of the heat-shock protein (HSP) 90 molecular chaperone are of particular interest in the latter regard, because they offer the potential of inhibiting multiple oncogenic pathways and simultaneous blockade of all six ''hallmark traits'' of cancer through direct interaction with a single molecular drug target. The first-in-class HSP90 inhibitor 17AAG exhibited good activity in animal models and is now showing evidence of molecular and clinical activity in ongoing clinical trials. Novel HSP90 inhibitors are also being sought. The development of HSP90 inhibitors is used to exemplify the application of new technologies in drug discovery against a novel molecular target, and in particular the need for innovative pharmacodynamic endpoints is emphasized as an essential component of hypothesis-testing clinical trials.

Keywords Molecular pathology and genomics of cancer · New molecular targets · Technologies for drug discovery and development · HSP90 molecular chaperone inhibitors · Gefitinib · Imatinib · Trastuzumab

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Introduction

In many ways cancer drug discovery is unrecognizable from what it was even as little as 10 years ago. The

progressive elucidation of the molecular control pathways that are hijacked by cancers has provided us with a large number of potential targets for therapeutic intervention. At the same time, the putting together of a powerful tool kit of innovative technologies has allowed us to accelerate the pace and improve the efficiency of drug discovery [44].

Hence the focus of the first part of this commentary is on new targets and technologies. To illustrate how new drug discovery and development is now done, the second part of the article comprises a summary and update on the development of inhibitors of the heat-shock protein (HSP) 90 molecular chaperone. These are of particular interest because they provide a potential approach to block combinatorial oncogenesis within a single drug molecule. In addition, the first-in-class HSP90 inhibitor 17AAG is just completing phase I trials with promising early results.

From cancer genes to individualized therapies

Given that we now understand in increasing detail the molecular abnormalities that drive the process of malignant progression, the major strategy for drug discovery in cancer is to identify the genes and cognate biochemical pathways that are hijacked in cancer cells, to discover molecular reagents and biomarkers to identify pathways with these defects, and to develop drugs that counteract or exploit the deregulated control mechanisms. The vision is that we can exploit our growing knowledge of cancer genes and pathways by developing personalized therapies targeted to the molecular pathology of individual patients and their malignancies (see references 44 and 49, and Fig. 1).

Fig. 1 Strategy for exploiting knowledge of cancer genes and pathways in the development of personalized therapies targeted to molecular pathology of individual patients

A range of drugs that target the molecular pathology of cancer are now undergoing clinical trial (e.g. see reference 49, and Table 1). Proof of concept for the approach is provided by the regulatory approval of imatinib (Gleevec), trastuzumab (Herceptin), and gefitinib (Iressa). Various small-molecule cyclin-dependent kinase inhibitors, e.g. flavopiridol and CYC202 (*R*-roscovitine), are undergoing clinical evaluation. Furthermore, a wide range of innovative agents are in preclinical and clinical development. These include drugs that block the farnesylation of RAS and other protein targets; inhibitors of signal transduction kinases such as RAF-1, MEK, mTOR, and PI3 kinase; and drugs that block chromatin remodeling enzymes such as histone deacetylases [49].

The success with the first initial wave of molecular therapeutics that specifically attack the oncogenic pathways that are hijacked by cancer genome defects has provided encouragement for the view that this represents a major opportunity to develop innovative cancer drugs. Furthermore, the mechanism of action of these agents offers potential not only for improved therapeutic efficacy, but also for less-severe side effects compared with the previous generation of cytotoxic agents. The new agents may in fact be much more like tamoxifen—used chronically for long-term disease control and potentially for chemoprevention.

Additional new targets from cancer genomics

A further tranche of new targets and drugs can be expected to emerge over the next 5–10 years as the genes involved in all stages of the malignant progression of every tumor type are elucidated. Historically, cancer genes have been discovered and cloned by a variety of means, including the dissection of major chromosomal abnormalities, i.e. translocations, amplifications, and deletions; transfection of dominant oncogenes into

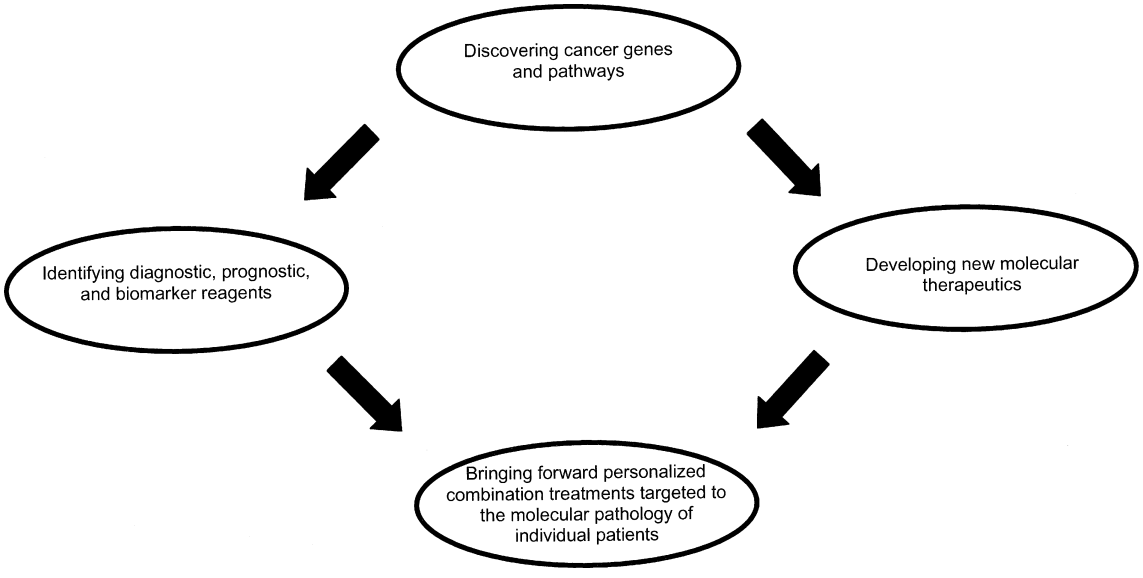

Table 1 Examples of novel drugs acting on cancer genome targets (for further details see reference 49)

Imatinib	A small molecule that shows activity in chronic myeloid leukemia and gastrointestinal stromal tumors via inhibition of the BCR-ABL and c-KIT receptor tyrosine kinases, respectively
Trastuzumab	A monoclonal antibody active in ERBB2-positive breast cancers
Gefitinib	A small-molecule inhibitor of the epidermal growth factor receptor tyrosine kinase active in non-small-cell lung, hormone-refractory prostate, and head and neck cancer
Various small-molecule cyclin-dependent kinase inhibitors, e.g. flavopiridol and CYC202 (*R*-roscovitine)	Undergoing clinical evaluation
Inhibitors of RAS farnesylation, RAF-1, MEK, PI3 kinase, mTOR, and histone deacetylases	In preclinical and clinical development
Wide range of other innovative agents	In preclinical and clinical development, e.g. potential for BRAF inhibitors
17AAG	A small-molecule inhibitor of the HSP90 molecular chaperone that is completing phase I clinical with promising early results

NIH3T3 cells; various genetic and molecular studies in model organisms such as yeast, fly, and worm; and also from studies of inherited predisposition [31].

The discovery of new cancer genes should be accelerated by the impact of the Cancer Genome Project [42]. The aim here is to use the information and technologies obtained via the Human Genome Project [18, 38] to carry out a systematic, high-throughput, genome-wide screen for somatic mutations in human cancer cell lines and tissues.

The likely success of this approach is exemplified by the recent unexpected discovery that *BRAF* is an oncogene that is activated in about 70% of melanomas, 10% or more of colorectal cancers, and a smaller subset of other tumors [11]. This exciting finding, made under the auspices of the Cancer Genome Project (Sanger Centre, Hinxton, UK), indicates that the kinase encoded by the *BRAF* oncogene is an excellent target for drug discovery. One possibility is that drugs could be developed that would be selective for the mutationally activated BRAF. Such a drug would be effective in the genomically defined subset of tumors that express and are driven by the mutant kinase gene. This approach would be of particular benefit in metastatic melanoma for which therapeutic options are restricted, especially because the mutation rate is particularly high in this cancer. This discovery illustrates a number of points: (1) the power of a high-throughput genome-based approach in the discovery of new cancer genes and drug targets; (2) the potential for discovering new drugs targeted to a particular molecular pathology; (3) the value of understanding the biological function of the cancer gene and the biochemical pathway in which it operates; and (4) the downstream commercial challenges posed by the development of ''niche'' drug products that may have high therapeutic value but in a genomically restricted subset of cancer patients [43].

New technologies for drug discovery

Although drugs such as imatinib, trastuzumab, and gefitinib represent major technical achievements, as well as genuine medical advances, in each case there was a considerable delay between the discovery of the target and the regulatory approval of the drug. In the case of imatinib, more than 40 years elapsed between the discovery of the Philadelphia chromosome translocation and the marketing of imatinib. To accelerate drug discovery and patient benefit, the power of a range of effective, often high-throughput technologies is now being harnessed (see Figs 2 and 3).

As already discussed, high-throughput DNA sequencing and associated genomic and bioinformatic techniques are being used to speed up gene discovery and hence the identification of new molecular targets. RNAi technology is proving to be a powerful and simple means of knocking out gene function as part of target validation. Genomic and proteomic technologies are now having an impact across all areas of basic research and drug development. A particular advantage is the large number of genes, mRNAs, and proteins that can be interrogated in a single experiment. For a more extensive recent commentary on this area see Weinstein [41] and Workman [45].

High-throughput screening (HTS) is an extremely effective way of identifying small-molecule ''hits'' that act on a novel drug target [1]. Large compound collections from tens of thousands up to millions are required for screening campaigns involving biochemical or cell-based assays. Where the structure of the target is known or can be modeled, HTS is complemented by methods such as *in silico* screening of virtual libraries containing millions of ''drug-like'' compounds against the target of interest, using sophisticated computer algorithms [21]. Fragment-based screening, which involves using X-ray crystallography or nuclear magnetic resonance methods to search for very low molecular weight compounds that show weak interactions with the target, can also be profitable [5]. The use of a combination of these hit-finding methods can be highly synergistic. Following the identification of a screening hit, or more likely a series of hits against a given molecular target, the quality and potential of the hit is evaluated. Practical factors such as physicochemical properties [22], feasibility of synthesis, and overall

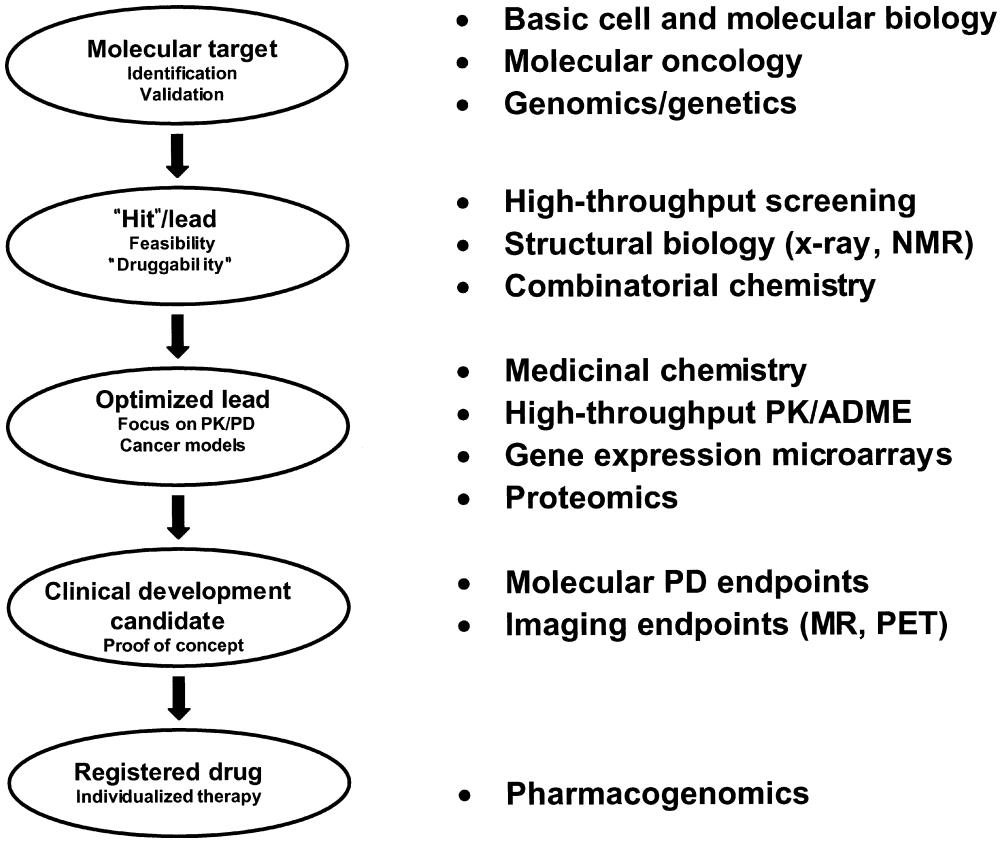

Fig. 2 The impact of new technologies at various stages of the drug discovery process (*PK* pharmacokinetics, *PD* pharmacodynamics, *NMR* nuclear magnetic resonance, *ADME* absorption, distribution, metabolism, and excretion, *MR* magnetic resonance, *PET* positron emission tomography)

"druggability" are important. Combinatorial chemistry and other new chemical methods can be used not only to create chemical diversity for HTS, but also to make more targeted libraries and for "lead explosion" to establish initial structure-activity relationships [15, 36]. Parallel synthesis methodology is valuable at this stage.

Optimization of a selected lead series towards the profile of desired properties is often focused on two main areas: (1) potency and selectivity; and (2) pharmacokinetics and absorption, distribution, metabolism, and excretion (ADME) properties. Robust assays, preferably high-throughput, need to be put in place for all these properties. These assays are formulated into a hierarchical test cascade [1]. Structure-based optimization, for example exploiting the X-ray cocrystal structure of the target–inhibitor complex, can be highly complementary to classical medicinal chemistry-based optimization. An important area for chemical innovation at the interface with bioscience is that of chemical biology [2, 37].

The ability to convert potent and selective lead compounds with activity on cancer cells in culture into agents with robust drug-like properties, particularly in terms of pharmacokinetic and metabolic properties, remains a particular challenge. It is difficult to predict such properties *ab initio*. In vitro ADME methods and higher throughput pharmacokinetic techniques, such as cassette or cocktail dosing, can be extremely valuable when used carefully with suitable lead series [33].

Mechanism of action and pharmacodynamic endpoints

It is absolutely essential during both preclinical and clinical development that particular key milestones are met. Such milestones can often constitute *go*/*no-go* decision points. As shown in Fig. 4, it is critical to know that active plasma and tissue concentrations of drug can be achieved in animals and patients. Next it is important to demonstrate the desired activity on the intended molecular target (e.g. kinase inhibition), followed by modulation of the corresponding biochemical pathway (e.g. RAS $\rightarrow$ ERK signaling) and also the achievement of the desired downstream biological effect (e.g. inhibition of proliferation, blockade of angiogenesis, or induction of apoptosis). Finally, these molecular and cellular events need to be linked to the therapeutic response, e.g. tumor cytostasis or regression. It is important that pharmacokinetic/pharmacodynamic relationships are established and that a pharmacological "audit trail" is constructed, consisting of measured parameters for each of the levels of analysis mentioned above (see Fig. 4, and references 46 and 48 for more details).

Pharmacodynamic endpoints may be measured on tumor biopsies or surrogate normal tissue such as peripheral blood lymphocytes, skin or buccal mucosa. Alternatively, and preferably, minimally invasive assays employing techniques such as positron emission

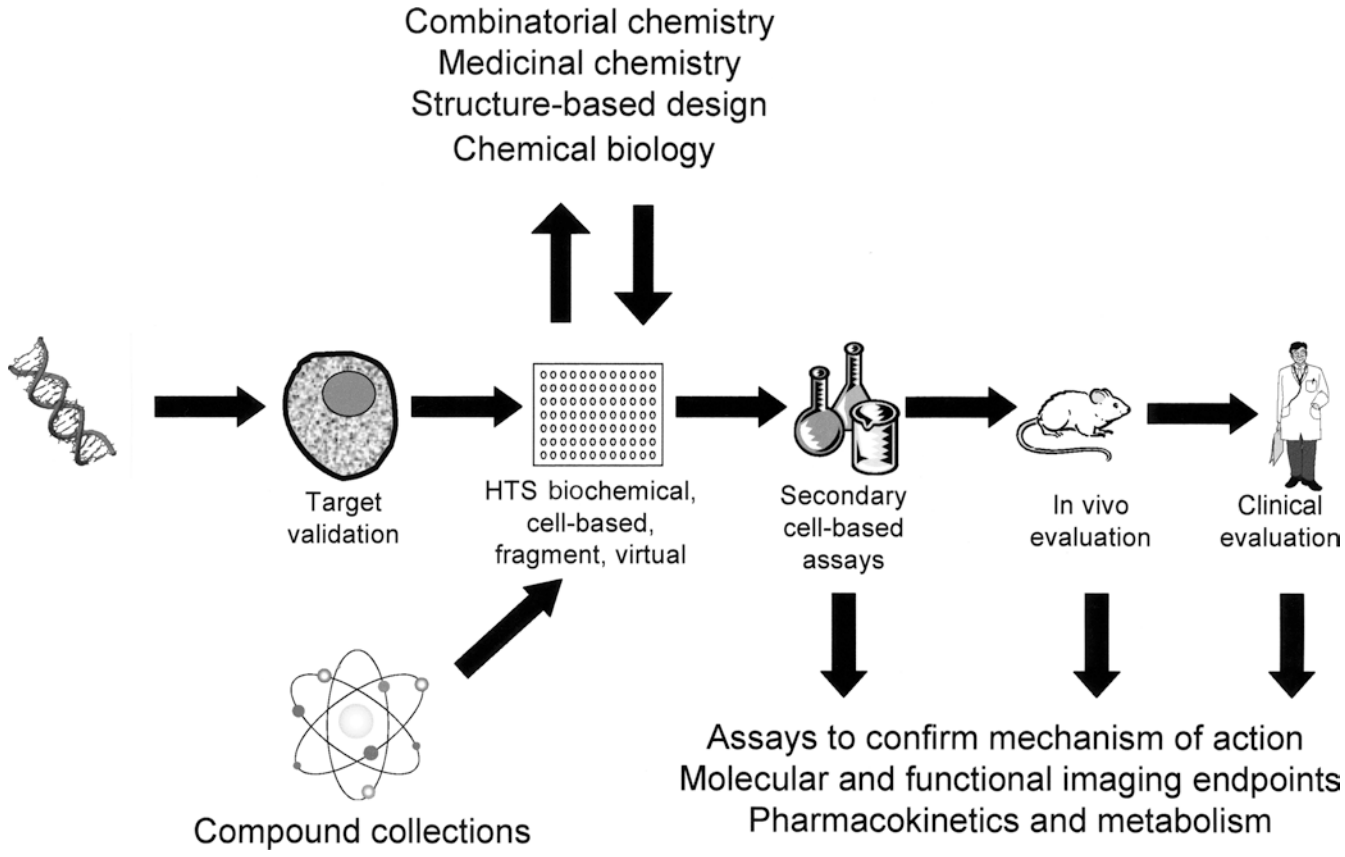

Fig. 3 Process of contemporary drug discovery (*HTS* high-throughput screening)

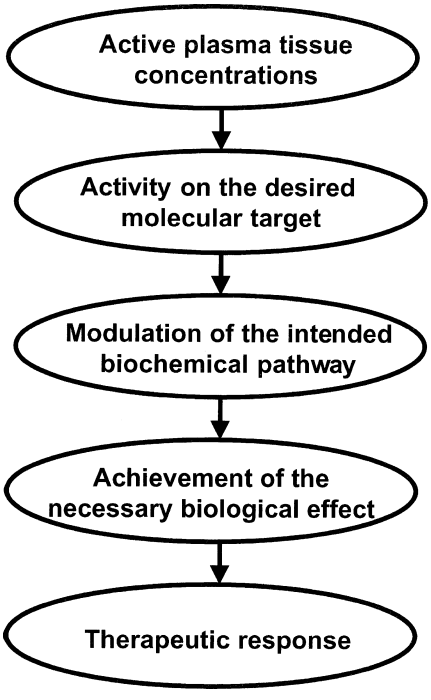

Fig. 4 Key milestones in preclinical and clinical drug development. Measurements made at each milestone allow construction of a pharmacological "audit trail" (see references 45 and 46)

tomography (PET) and magnetic resonance spectroscopy/imaging (MRS/MRI) can be extremely valuable [46, 48].

Invasive molecular endpoints can for example involve changes in protein phosphorylation, as measured by Western blotting, enzyme-linked immunosorbent assay (ELISA), or immunohistochemistry. Genome-wide expression profiling by microarray and also global proteomic analysis can provide a rich source of potential pharmacodynamic endpoints, as well as helping to understand the cellular mode of action of a drug, which may not always be as intended [8, 9, 45].

Current issues in the development of new molecular cancer therapeutics

Although rich in potential and showing signs of considerable promise, the new genome-based approach is not without its challenges (e.g. see references 3, 10, and 43). This is exemplified by the recent clinical trial results with gefitinib [14, 19]. The trials concerned were randomized, double-blind, phase III studies in which gefitinib when used in combination with chemotherapy (gemcitabine and cisplatin or paclitaxel and carboplatin) failed to improve survival in patients with chemotherapy-naive advanced non-small-cell lung cancer (NSCLC). This was perhaps surprising given that gefitinib has activity as a single agent in NSCLC, as well as in head and neck malignancy, and in hormone-refrac-

tory prostate cancer [12]. In addition, studies in preclinical models showed a benefit for the combination of gefitinib with chemotherapy. There are a number of possible explanations for the inability of gefitinib to improve clinical outcome for the particular tumor type and chemotherapy regimens concerned. One is that gefitinib and cytotoxic therapy are each maximally effective against the same tumor cell population; hence there is no additive, let alone synergistic, interaction. Another possibility is that gefitinib may block cell-cycle progression in tumor cells, thereby antagonizing the effects of cytotoxic therapy. These factors presumably outweigh potentially advantageous interactions such as blockade by gefitinib of survival pathways that might be used by cancer cells to protect themselves against cytotoxic damage. It could also be speculated that for some reason, possibly relating to changes in signaling pathways, gefitinib may be more effective in the biological context of previous exposure to chemotherapy.

Of particular potential importance is the possibility that there may be a subset of NSCLC patients who have molecular characteristics that predispose them to be responsive. This may not relate simply to the level of expression of the epidermal growth factor receptor molecular target, but could feasibly correlate with the flux through the receptor tyrosine kinase → RAS → RAF → MEK → ERK1/2 signal transduction pathway (potentially measurable using antibodies to phospho-ERK1/2) or with the expression of any number of genes that could be detected by microarray profiling. Pharmacogenomic analysis is required to identify such genes, and studies of this type will need to be an important part of the future clinical evaluation of gefitinib and other molecular therapeutics. We discuss later in this section the possibility that the optimal use of gefitinib may require a combination involving other molecular therapeutics to take out additional oncogenic pathways in NSCLC and other tumor types.

In the case of trastuzumab, although this agent clearly improves the response of ERBB2-positive breast cancer patients to cytotoxic chemotherapy, when used with anthracyclines it does have significant toxicity [12]. In addition, whereas imatinib is extremely active in the early phase of chronic myeloid leukemia (CML), it produces only short-lived responses in the accelerated and blast crisis stages of the disease; furthermore, acquired resistance to the drug is seen in chronic-phase patients, often due to mutation of the BCR-ABL kinase to a form that is no longer susceptible to imatinib [49].

One of the most important characteristics that may limit the effectiveness of signal transduction inhibitors and other molecular cancer therapeutics is the fact that the malignant progression of most cancers is probably driven by multiple oncogenic defects. Extensive epidemiological data would support the view that 5–7 rate-limiting genes are involved, although there may be as many as 10–12 oncogenic abnormalities in tumors such as pancreatic cancer. The concept of a stepwise accumulation of genetic and epigenetic abnormalities driving malignant progression is probably best exemplified in colorectal cancer [39]. Here, combinatorial oncogenesis involves a conspiracy between mutations in genes such as *RAS*, *APC*, and *P53*, which combine together to accelerate the conversion of normal cells into full-blown invasive and metastatic cancer. Although the precise source and role of genetic instability and its involvement in driving early- versus late-stage malignancy remains a highly controversial issue, there is no doubt that a high level of genetic chaos is a common feature of the major epithelial cancers such as those of the lung, breast, and bowel, as well as in the leukemias, as evidenced by the presence of large-scale amplifications, deletions, and translocations [26]. Genes involved in checkpoint control, mismatch repair, and telomere maintenance may all contribute to genomic instability and the progressive accumulation of cancer-causing defects.

The concept and reality of multistep combinatorial oncogenesis has a number of implications for the development and use of molecular cancer therapeutics. Principle among these is the issue as to whether therapeutic "correction" of a single oncogenic defect will be sufficient to achieve a significant or optimal therapeutic effect—or whether it will in fact be necessary to attend to all or at least several of the key molecular abnormalities to put the brake on combinatorial oncogenesis.

The potential problem is illustrated in Fig. 5. In the particular model example shown (Fig. 5A), the normal cell is transformed into a fully malignant cancer cell by the deregulation of three "mission-critical" pathways, most likely involving the hijacking of normal controls on proliferation signaling, cell-cycle regulation, and survival/apoptosis [13]. Pharmacological modulation of the first pathway, involving genes A–D, is without significant therapeutic effect (Fig. 5B). Similarly, intervention in the second oncogenic pathway, involving genes E–H, also confers little or no therapeutic benefit, either alone or in combination with modulation of the first pathway (Fig. 5C). However, simultaneous intervention in all three oncogenic pathways does have a major therapeutic effect (Fig. 5D). So, the model presented in Fig. 5 would predict that combinatorial oncogenesis would require combinatorial therapy. How do the data stack up against this prediction?

Surprisingly, perhaps, there are a number of published examples in which molecular correction of a single oncogenic abnormality can bring about a therapeutic effect, even in the context of multiple genetic abnormalities [40]. Examples include knockout of oncogenes such as *RAS* or *MYC*, or reintroduction of a lost tumor suppressor gene such as *P53*, *APC*, or *PTEN*. To explain such results, one can invoke the "house of cards" model and the oncogene addiction/tumor suppressor gene hypersensitivity concept [40]. In the house of cards model, the tumor requires each of the molecular abnormalities to power up malignancy; remove any one of the molecular batteries and the cancer cell collapses like a house of cards. In the related oncogene addiction/tumor suppressor gene

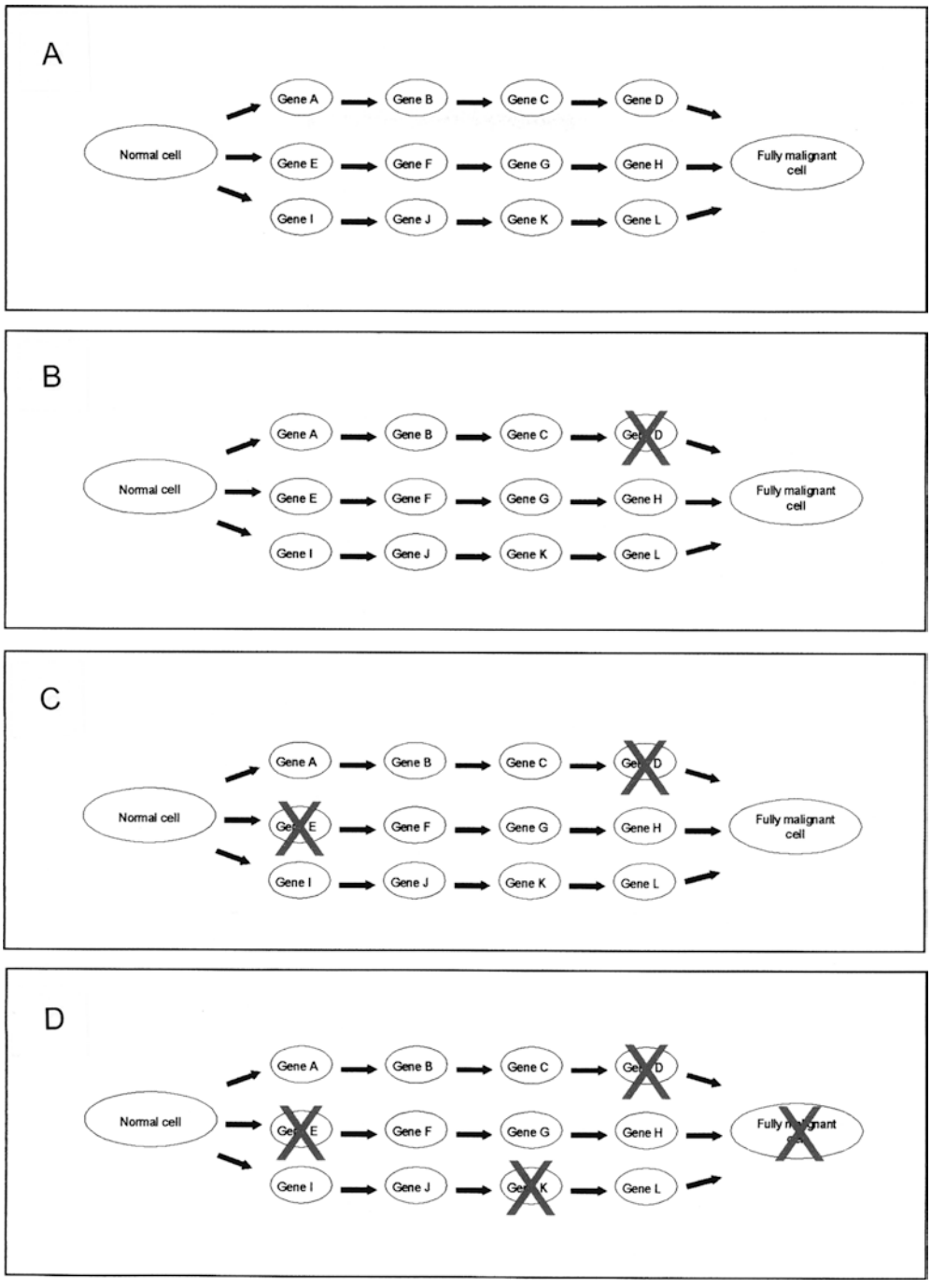

Fig. 5A–D Combinatorial oncogenesis may require combinatorial therapy. In this model, the malignancy is driven by three "mission-critical" pathways. The first pathway comprises the products of genes A–D, the second pathway the products of genes E–H, and the third pathway the products of genes I–L. As shown, the inhibition of one or two of the pathways may be insufficient for a significant therapeutic effect—combinatorial therapeutic blockade of all pathways is required for optimal treatment

hypersensitivity concept, genome instability and selection for malignancy leads to the "hard-wiring" of mission-critical oncogenic pathways and the loss of alternative or redundant signal transduction pathways. As a result, the cancer cell develops a dependence on, or addiction to, the hard-wired oncogenic pathways, together with enhanced sensitivity to reactivation of tumor suppressor functions. Because of this, treatment with a molecular therapeutic that inhibits an activated, hard-wired oncogenic pathway or reactivates a lost tumor suppressor function results in a preferential response in the cancer cell compared with its normal counterpart. It is clearly possible to invoke the oncogene addiction model to explain why a selective anticancer effect can be obtained with molecular cancer therapeutics that hit signal transduction pathways that are activated in cancer cells but that are also important for normal cell function. Probably the best example of this is the selective activity of mTOR inhibitors [e.g. rapamycin (sirolimus) derivatives] and PI3 kinase inhibitors (e.g. LY2940022) against cancer cells that have lost PTEN tumor suppressor gene function, thereby activating the PI3 kinase–AKT–mTOR pathway [27].

How does the clinical experience fit with the oncogene addiction model and the need for the correction of single versus multiple molecular abnormalities? The activity of imatinib in chronic-phase CML and gastrointestinal stromal tumors can be cited as supporting the oncogene addiction model. It is likely, however, that these are cancers in which only a single genetic defect is driving malignancy, i.e. *BCR-ABL* and mutant c-*KIT* respectively. Indeed, the lower activity in imatinib in acute and blast-phase CML and also in acute lymphocytic leukemia, where additional mutations are present, supports the view that combinations of agents may be needed to block these multiple defects. A similar argument can be made to account for the partial, although usually incomplete, responses that are seen with other molecular cancer therapeutics such as trastuzumab and gefitinib. It appears possible, then, that oncogene addiction to a single hard-wired, mission-critical pathway is partial rather than absolute. Oncogene addiction may well be present but in most cases there may be overlapping dependence on several genes and pathways. If this is correct, it would follow that treatment with a targeted drug cocktail would be advantageous. In addition, this would be likely to decrease the likelihood of resistance arising to a single agent, as seen in the clinic with imatinib in CML. This is entirely analogous to the use of multiple drug cocktails in HIV/AIDS. On the other hand, as we target several oncogenic pathways that are also used by normal cells, the key question then becomes: can we retain a therapeutic window between malignant and normal cells?

The development of HSP90 inhibitors

Given the above discussion on the likely advantage of a combinatorial blockade of multistep oncogenesis, the development of HSP90 inhibitors is brought into particularly sharp focus. The factors contributing to the "credentialing" or validation of HSP90 as a therapeutic target, together with the likely advantages of this therapeutic approach, are summarized in Table 2. HSP90 is not a product of a cancer gene per se but rather it is a protein that is required for the malignancy-driving properties of a number of bona fide oncogenes [24, 29].

The HSP90 family comprises HSP90α, HSP90β, the endoplasmic reticulum homologue GRP94, and the mitochondrial counterpart TRAP1. HSP90 is a molecular chaperone involved in protein folding. It is not, however, a generic chaperone that is required for the folding of cell proteins. Nor is it only involved under stress conditions such as heat shock. Rather, it is responsible under normal cellular conditions for the later stage folding and maintenance of the correct conformation and functional activity of a relatively restricted selection of "client" proteins. Many of the clients on this "celebrity A list" have oncogenic activity. They include several oncogenic kinases such as ERBB2, RAF-1, CDK4, POLO-1, and MET. In addition, HSP90 clients also include mutant P53, HIF-1α, estrogen/androgen receptors, and the catalytic component of telomerase hTERT. Thus inhibition of HSP90 activity leads to incorrect folding and subsequent degradation by the ubiquitin-proteasome pathway of all the above-mentioned oncogenic clients. As a result, HSP90 inhibitors are likely to block all six of the so-called "hallmark traits" of malignancy [16] and therefore have potential for one-step combinatorial therapy against a broad range of cancers. Furthermore, based on the work of Lindquist and colleagues [35], it might be speculated that inhibition of HSP90 could uncover synthetic lethal mutations in cancer cells.

Encouragingly for the approach, certain natural products that were known to have anticancer activity were found to target HSP90 [24, 29]. In particular, these include radicicol and geldanamycin (see Fig. 6 for chemical structures). These agents work by competing

Table 2 HSP90 target validation (for further details see reference 24)

Molecular chaperone involved in protein folding
Overexpressed in human tumors (e.g. due to stress and oncoproteins)
Essential for stability and function of many oncogenic "client" proteins e.g. ERBB2, RAF-1, CDK4, POLO-1, MET, mutant P53, HIF1α, estrogen/androgen receptors, and telomerase hTERT
Inhibition likely to block all six "hallmark traits" of cancer
Potential for one-step combinatorial therapy against a broad range of malignancies
May uncover synthetic lethal mutations in cancers
Natural products that target HSP90 have anticancer activity
Proof of concept for therapeutic selectivity demonstrated in human tumor xenograft models
First-in-class inhibitor 17AAG now showing evidence of biological and clinical activity at well-tolerated doses

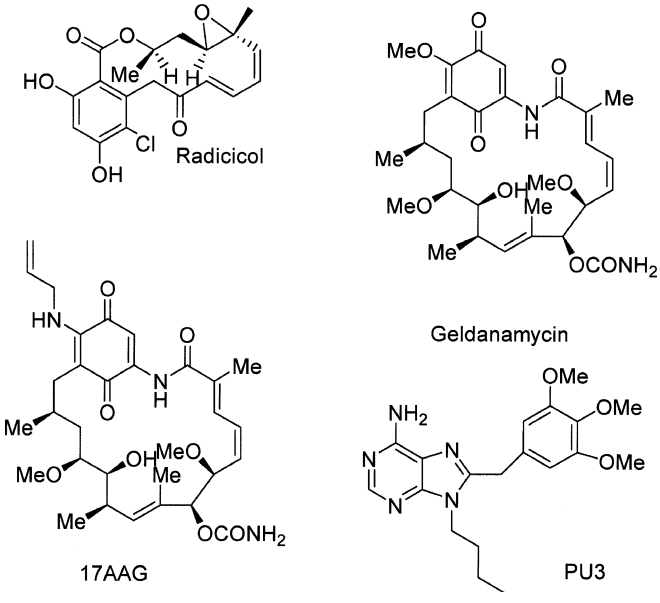

Fig. 6 Chemical structures of HSP90 inhibitors

with ATP for binding at the nucleotide-docking site located in the N-terminal domain of HSP90 [32, 34]. ATP binding and hydrolysis are essential for the functioning of the chaperone and drug binding prevents the correct assembly of mature HSP90/client protein/cochaperone complexes. This appears to result in recruitment of a ubiquitin ligase to the immature complex, leading to proteasomal degradation of client protein [24].

Proof of concept for therapeutic selectivity towards cancer cells was exemplified with the geldanamycin analog 17AAG (Fig. 6) in human tumor xenograft models grown in immunosuppressed mice [20]. Furthermore, 17AAG has entered clinical trials as the first-in-class inhibitor of HSP90 and is now showing consistent molecular evidence of the desired mechanism of action, together with early indications of therapeutic activity [4].

We have shown that treatment of human colon cancer cells with 17AAG leads to combinatorial depletion of key oncogenic client proteins such as RAF-1 and AKT, consistent with the demonstrated inhibition of the ERK1/2 and PI3 kinase signaling pathway and the downstream induction of cell-cycle arrest and apoptosis [8, 17].

We have used global gene expression microarray profiling to investigate genes that might be involved in sensitivity to 17AAG, as well as to identify potential pharmacodynamic markers of effective HSP90 inhibition [8]. In addition, we used proteomic analysis to identify global responses to HSP90 inhibition by 17AAG at the protein level (collaboration with Professor Mike Waterfield and colleagues, Ludwig Institute for Cancer Research, University College London, London, UK). A molecular signature of HSP90 inhibition has been defined, consisting of depletion of client proteins such as RAF-1, CDK4, and ERBB2 at the protein level (with no effect at the mRNA level) together with upregulation of HSP70 at both the mRNA and protein levels [24]. In some cancer cell lines, HSP90 itself is upregulated. We routinely determine the molecular signature of HSP90 inhibition by Western blotting. In addition, we are also developing ELISA assays for greater sensitivity and more straightforward quantification.

In terms of the expression of genes that may confer sensitivity or resistance, we have shown that high levels of the quinone reductase NQO1/DT-diaphorase cause considerable sensitization toward 17AAG, which has a 17-allylamino group, although not to the major metabolite of 17AAG, which has an amino moiety at the 17 position, or to geldanamycin, which has a methoxy group at the 17 position [20]. The results suggest a role for activation via quinone metabolism, although the HSP90 mechanism is retained. Further work is required to elucidate the details and full significance of the effect.

Interestingly, our studies have also suggested that tumor lines that respond to treatment by expressing increased levels of the HSP90 target itself may recover more rapidly from the effects of 17AAG and therefore be less sensitive to the drug [8].

In collaborative studies published recently, we have identified the new gene product AHA1 as a novel cochaperone that activates the essential ATPase activity of HSP90 and which is upregulated in human tumor cells by stress, heat shock, and pharmacological HSP90 inhibitors [30]. Using a combination of gene expression microarrays, proteomics (two-dimensional gel electrophoresis with MALDI mass spectrometry) and Western blotting, we showed that *AHA1* gene expression is upregulated at the level of both mRNA and protein in response to treatment of human tumor cells with the HSP90 inhibitors radicicol and 17AAG. The mechanistic, pharmacological, and therapeutic significance of these observations is now under investigation.

Having shown good activity in xenograft models and an acceptable therapeutic index in animal models, 17AAG has been taken into clinical trials in our own institution and at our four centers in the USA under the auspices of the US National Cancer Institute and Cancer Research UK (formerly the Cancer Research Campaign). In the UK trial at the Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, and the Royal Marsden Hospital [4], 17AAG has been given weekly by intravenous infusion at doses up to 450 mg/m^2/week. Pharmacokinetic studies show that plasma concentrations are above the IC_{50} for inhibition of tumor cell growth for prolonged periods. In addition, depletion of RAF-1, CDK4, and the SRC family kinase LCK has been clearly demonstrated in peripheral blood lymphocytes, together with upregulation of HSP70. Furthermore, depletion of RAF-1 and CDK4 alongside increased expression of HSP70 has also been observed in malignant tissue by comparing tumor biopsies taken before and after treatment. Consistent with these molecular changes, we have seen evidence of disease stabilization in some patients. RNA has been prepared from certain tumor biopsies to allow global expression profiling to be carried out. This should generate valuable results to compare with those from in vitro cell-culture exposures [8].

Although relatively invasive assays are providing valuable information by demonstrating that 17AAG is able to inhibit its molecular target both in peripheral blood lymphocytes and in tumor biopsy material, minimally invasive assays such as those involving PET and MRS/MRI would have major advantages [46, 48]. In collaboration with Professors Martin Leach, John Griffiths, and colleagues (Cancer Research UK Biomedical Magnetic Resonance Group, St George's Hospital Medical School, London, and Cancer Research UK Clinical Magnetic Resonance Research Group, Institute of Cancer Research and Royal Marsden Hospital, Sutton, UK), we have noted interesting changes in human xenograft tumors following treatment with 17AAG, in particular an unusual increase in the levels of phosphoethanolamine and phosphocholine [7]. These may be indicative of alterations in lipid signaling and/or membrane turnover. In addition, we are collaborating with Professor Pat Price and Dr. Eric Aboagye (Cancer Research UK PET Oncology Group, Molecular Imaging

Centre, Manchester, and Cancer Research UK PET Oncology Group, MRC Cyclotron Unit, Hammersmith Hospital, Imperial College School of Medicine, London, UK) to use labeled choline PET tracers to monitor the effects of 17AAG in tumors [23]. Overall, the potential to use molecular or functional imaging to monitor the pharmacodynamic effects of the new molecular cancer therapeutics is an exciting area.

17AAG shows significant promise and demonstrates proof of concept for HSP90 inhibition in humans. It does, however, have a number of potential limitations. These include:

- Limited stability and complex formulation
- Modest potency against the HSP90 target
- Substrate for P-glycoprotein
- Activated by polymorphic NQO1/DT-diaphorase
- Metabolism by polymorphic cytochrome P450
- Low oral bioavailability
- Limited therapeutic index

Because of these potential issues, several groups are seeking small-molecule, synthetic inhibitors of HSP90 as alternatives to the existing natural products. A range of approaches are likely to be taken, including those described earlier in this commentary and depicted in Fig. 3.

One interesting lead that has emerged is the synthetic purine-based compound PU3 (Fig. 6). This agent has been shown to inhibit HSP90 in cancer cells and to retard their growth [6]. PU3 appears to behave like the natural product agents, competing with ATP at the nucleotide-binding site of the N-terminal domain of HSP90 [6]. Another interesting compound is novobiocin. This appears to act in a different way by binding to the C-terminal domain of HSP90 [25]. Given the attractiveness of the target and the encouraging results with 17AAG, it appears likely that more synthetic chemical inhibitors of HSP90 will emerge.

There are many challenges ahead with HSP90 inhibitors. Some of the important outstanding questions include:

- What is the optimal treatment regimen?
- How should the drug be used as a single agent?
- How should the drug be used in combination with cytotoxics, e.g. paclitaxel [28]?
- Will any tumor types be particularly sensitive?
- Are any particular client proteins especially important for response in certain tumor settings?
- Will particular genomic abnormalities predispose to sensitivity or resistance?

Conclusions

The following overall conclusions can be drawn:

- Proof of principle is now established that targeting cancer genome abnormalities and the molecular pathology of cancer can be clinically beneficial.
- New molecular targets continue to emerge from cancer genomics.
- Blocking multistep oncogenesis will most likely require combinatorial therapies.
- This may be delivered in individualized cocktails of molecularly targeted agents.
- HSP90 inhibitors such as 17AAG may block multiple oncogenic pathways in a single drug.
- Deployment of multidisciplinary skills and new technologies is required to accelerate the pace and improve the efficiency of drug discovery against new molecular targets.
- Clinical development strategies must pay close attention to the proposed mechanism of action and a pharmacological audit trail must be constructed to allow rational decision-making, including *go/no-go*.
- Demonstration of proof of concept is invaluable in hypothesis testing phase I clinical trials.
- Pharmacodynamic and pharmacogenomic markers are essential for success.

The explosion of new molecular targets and the development and application of many powerful technologies should accelerate the discovery of innovative molecular therapeutics. There are many challenges ahead and the risks associated with each individual agent remain considerable, but the prospects for overall success with individualized therapies targeted to the molecular pathology of the individual patient are excellent [47, 49]. This exciting translational work requires many disciplines (e.g. chemistry, biology, and medicine) and organizations (e.g. academia, biotech, and large pharmaceutical companies) to work together internationally to accelerate patient benefit.

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