



# HSP90 inhibition: two-pronged exploitation of cancer dependencies

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The early clinical hypothesis for inhibiting HSP90 in cancers was based on the dependence of certain key client proteins in malignant cells – including a host of well-characterized oncoproteins – on the activity of HSP90 for their function and stability. The additional concept has been established that cancer cells have heightened dependence on the efficient maintenance of intracellular proteomic homeostasis, central components of which are HSP90 and other heat shock proteins. We evaluate the evidence that inhibiting HSP90 in cancer exploits both of these biological vulnerabilities very effectively, we review the current status of the discovery and development of HSP90 inhibitors and we identify routes to improve their clinical efficacy, based on emerging knowledge.

## Oncogene addiction and proteotoxic stress in cancer cells

The classical driver-mutation view of oncogenesis and cancer progression supports, in concept and now in medical practice, the rational design of novel therapeutics that target such driver oncogenes as susceptible nodes in the complex signaling pathways and networks that regulate the hallmark traits of malignancy [1,2]. The addition of individual cancers to specific oncogenes is, however, only a single component – albeit a major one – of this molecularly multifaceted disease. To maintain local growth conditions that sustain tumor development, cancer cells must regulate their microenvironment and also profoundly alter intracellular homeostasis to cope with the biosynthetic demands of rapid clonal expansion in the low nutrient conditions imposed by deregulated cellular proliferation [1,3]. These processes can occur by upregulation of pre-programmed stress responses that are present latently in all cells; although the activation of these stress management systems is commonly independent of specific oncogene mutation, cancer cells are thought to be particularly dependent on the upregulation of such non-oncogene pathways for survival [3–5]. Moreover, it has become increasingly evident that, owing to the proteotoxic pressures of aneuploidy, gene copy number variation, chromosomal translocation and missense mutations – which all

increase the cellular load of proteins with suboptimal stability – alteration of proteostasis is an additional hallmark feature of cancer [3]. Standing at the center of oncogenic proteostasis, which also crucially entails the functional and structural stabilization of a host of known oncoproteins, is the molecular chaperone heat shock protein 90 (HSP90) [5].

The central role of HSP90 in chaperoning cancer was first identified in 1994 by a landmark study linking a natural product – the benzoquinone geldanamycin – that had known antitumor activity with the HSP90-dependent degradation of the viral oncoprotein v-SRC [6]. This first example of oncogene-mediated targeting of cancer cells by an HSP90 inhibitor, and the subsequent exploration of the ATPase-dependent biochemistry of chaperone-client interactions with this inhibitor [7,8], has been followed in recent years by the cataloging of over 200 identified HSP90 client proteins – many of which, such as ERBB2/HER2, anaplastic lymphoma receptor tyrosine kinase (ALK) and BRAF, have oncogenic activity (<http://www.picard.ch>).

The fast pace of HSP90 client protein discovery has been matched by the explosion of academic publications and patents on chemical inhibitors of HSP90, a drug discovery and development effort that has now culminated in the entry of several different HSP90 inhibitor chemotypes into Phase I and Phase II clinical trials in cancer [9–12]. This present review charts the journey of HSP90 inhibitors from the soil of Kalamazoo County – the region of Michigan State where

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geldanamycin-producing bacteria were first discovered – to cancer clinics around the world (see timeline in Fig. 1). We also offer a perspective on the current and future application of these agents in treating cancer by exploitation of the unique dual activity of targeting oncogene addiction and, at the same time, the non-oncogene dependence of cancer cells on altered proteostasis [13,14]. Importantly, we identify potential routes to improve the clinical efficacy of HSP90 inhibitors – based on our expanding knowledge of the basic science of the molecular chaperone, together with our accumulating preclinical and clinical experience with the first-generation drugs and their second-generation, synthetic small-molecule successors.

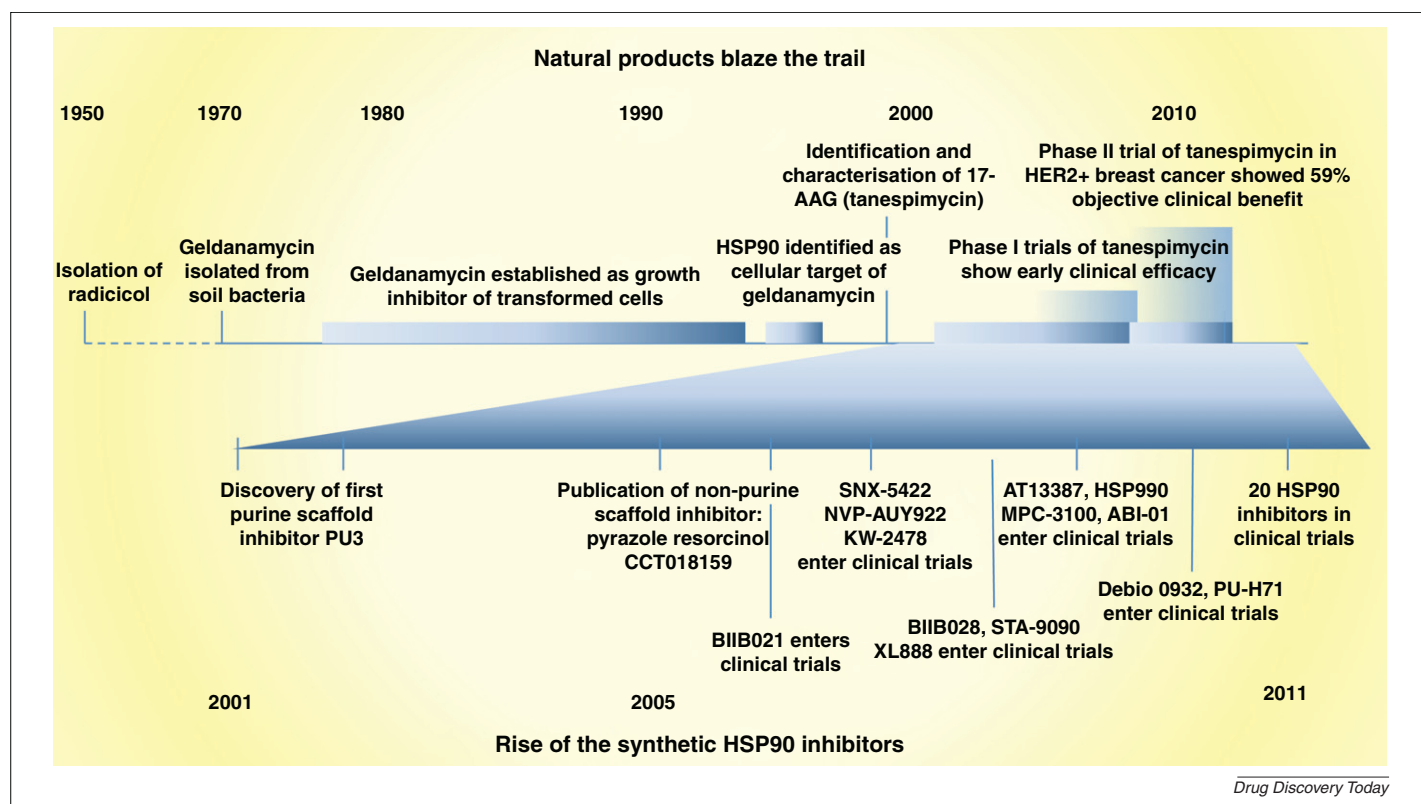
### From geldanamycin to the discovery of drug-like synthetic small-molecule HSP90 inhibitors

Although there was a clear clinical hypothesis underpinning inhibition of HSP90 as an anticancer strategy – the combinatorial depletion of the oncoprotein clientele that could be more therapeutically powerful than inhibiting a single driver oncoprotein – major pharmaceutical companies were initially slow to move into this field, probably owing to a perceived risk of the adverse effects of inhibiting such an abundant cellular protein with a key role in normal homeostasis. This meant that, as well as enabling the characterization of HSP90 structure and function, the discovery and development of early drug-like inhibitors of HSP90 was carried out largely in academic research institutes and small biotechnology companies.

Initial discovery and development of HSP90 inhibitors was stimulated by the risk-taking and trail-blazing work with the

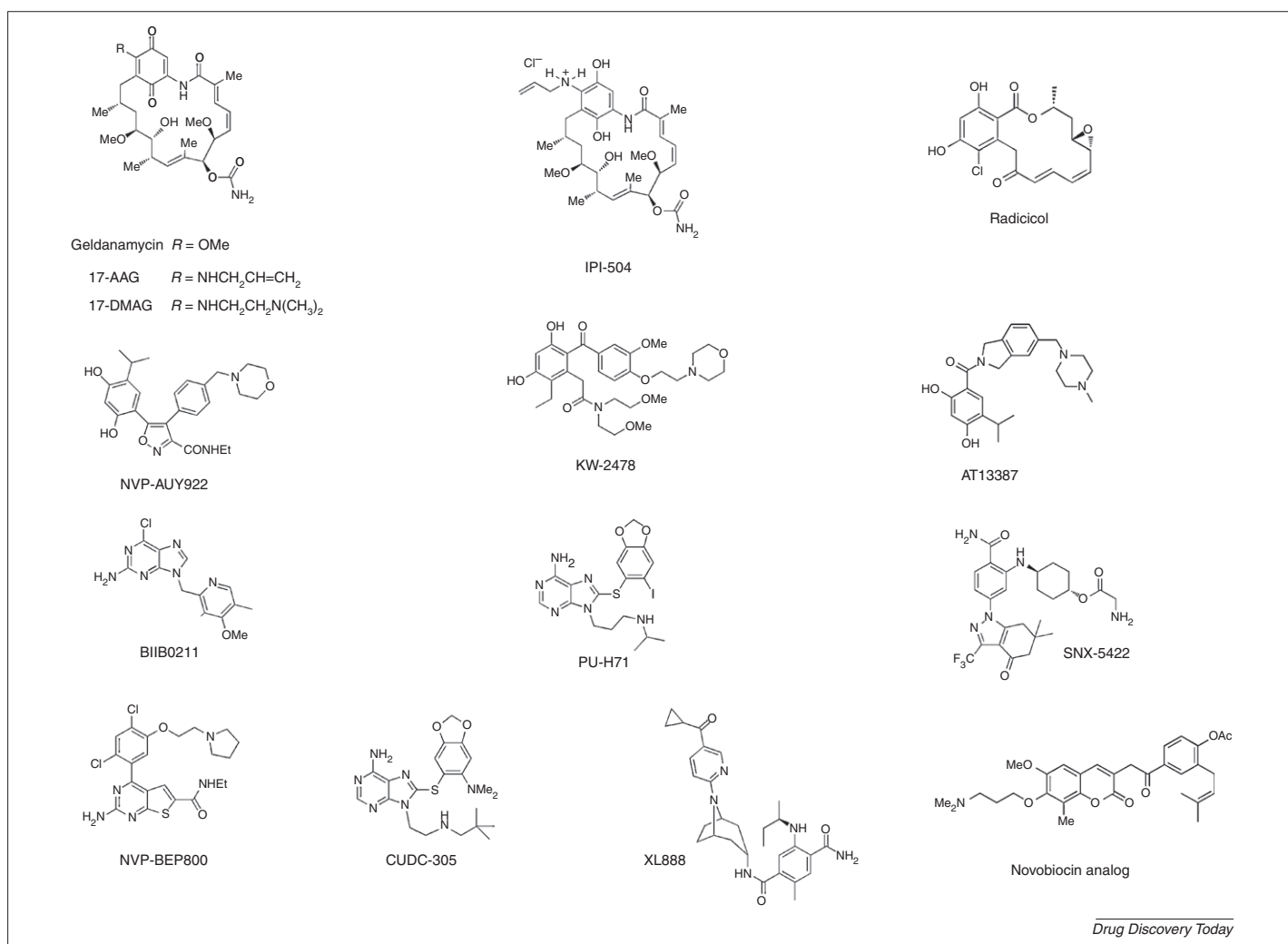
benzoquinoid ansamycins. The isolation of geldanamycin from the geldanus variant of the filamentous soil bacterium *Streptomyces hygroscopicus* and the initial characterization of its biocidal properties was first published in 1970 [15] (see Fig. 2 for the chemical structure of geldanamycin and other key agents discussed here). That early study demonstrated not only potent antibiotic activity but also the growth-inhibitory effects of geldanamycin on HeLa-derived KB cancer cells [15]. Subsequent studies in the 1980s established the antitumor activity of geldanamycin in virally transformed cells, but it was not until the mid-1990s – with the identification of HSP90 as the molecular target of geldanamycin [6,16,17] – that the development of natural-product-based HSP90 inhibitors began in earnest. Although geldanamycin itself proved too unstable and toxic, particularly to the liver, to justify clinical development, the close analog 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) was found to exhibit markedly improved pharmacokinetic properties combined with greater tolerability and therapeutic index in human tumor xenograft models in mice, thus stimulating its entry into preclinical development [18].

The natural product radicicol, which exemplifies a distinct resorcylic macrolactone chemotype, had been isolated considerably earlier (Fig. 1) and subsequently proved to be a valuable additional tool in the investigation of HSP90 function [19,20]. This early work with the natural-product-based inhibitors was essential for the biological validation of HSP90 as a drug target, as well as establishing the technical druggability of the N-terminal domain HSP90 ATP site at which the inhibitors bind, thereby blocking the ATPase cycle that drives the functionality of the



**FIGURE 1**

Timeline of HSP90 inhibitor development from the first discovery of geldanamycin to the present period in which 20 next-generation HSP90 inhibitors are in clinical trials for the treatment of a variety of cancer types.

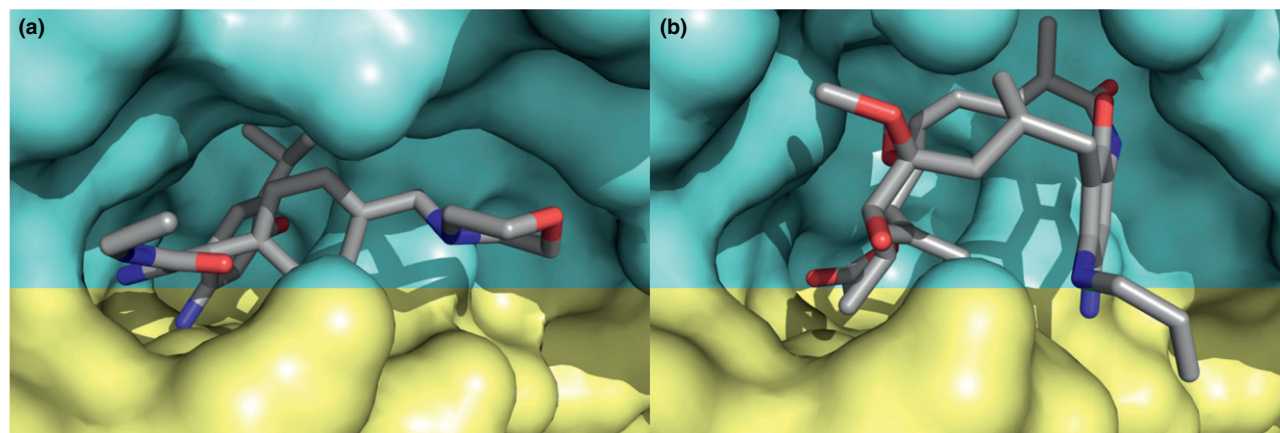
**FIGURE 2**

Chemical structures of HSP90 inhibitors. N-terminal binding radicicol and geldanamycin analogs (geldanamycin, 17-AAG, 17-DMAG and IPI-504); next-generation synthetic resorcinol-containing chemotype agents (NVP-AUY922, KW2478 and AT13387); purine-scaffold chemotype agents (BIIB021, PUH71); other chemotypes (SNX5422, NVP-BEP800, CUDC-305 and XL888); and the C-terminal-binding HSP90 inhibitor novobiocin.

molecular chaperone. In addition, these natural product inhibitors have enabled us to define a suite of pharmacodynamic biomarkers of HSP90 inhibition. The now widely used molecular biomarker signature comprises specific on-target depletion of client proteins combined with mechanism-based induction of heat shock proteins as part of a pharmacologic audit trail [21]. This on-target signature has been of paramount importance in the subsequent effort to design and develop more-potent, drug-like, well-tolerated and thus clinically efficacious HSP90 inhibitors.

The discovery of inhibitors that mimicked the natural products in competing with normal nucleotide binding at the N-terminal domain HSP90 ATP site – but which were more potent than 17-AAG while retaining selectivity for HSP90 over most other ATPase enzymes – was facilitated by the structure of the highly divergent N-terminal domain ATP-binding site, placing HSP90 in the small GHKL (Gyrase, HSP90, Histidine Kinase, MutL) subgroup of ATPases that contain the unique Bergerat fold geometry (Fig. 3) [22]. The unusual shape of this deep cavity when compared with most other ATP-binding proteins explains the high molecular selectivity of most HSP90 inhibitors.

Using the full gamut of discovery techniques, including rational ATP substrate-based mimicry, structure-based design using X-ray crystallography, biophysical approaches, virtual screening and high-throughput biochemical screening of compound libraries, two leading small-molecule classes of HSP90 inhibitor were discovered that went on to be developed clinically. One of these classes is the Institute of Cancer Research (ICR)/Vernalis/Novartis resorcylic pyrazole/isoxazole series, members of which share the anchoring resorcinol warhead used by radicicol. These are exemplified by the clinical investigational isoxazole drug NVP-AUY922 (VER52296) and are based on the original high-throughput screening discovery of the prototype CCT018159 at the ICR [23–27]. The other class was led by the Memorial Sloan-Kettering Cancer Center (MSKK)/Conforma/Biogen Idec purine scaffold series, exemplified by the clinical candidates BIIB028 and PUH71, which originated from the modeling approach taken at MSKK that yielded the first prototype synthetic small-molecule HSP90 inhibitor PU3 [28,29]. These two initial classes of small-molecule agents share with the geldanamycins and radicicol binding to HSP90 N-terminal ATP site via a complex, canonical series of hydrogen-bonding interac-



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**FIGURE 3**

Targeting the human N-terminal HSP90 ATP-binding site. Surface-filling representations of the N-terminal domain of HSP90 with HSP90 inhibitors **(a)** NVP-AUY922; PDB ID: 2VCI and **(b)** 17-AAG modeled from 17-DMAG; PDB ID: 10SF.

tions involving Asp93 (in human HSP90) and other residues, often mediated by essential water molecules, together with additional hydrophobic interactions (Fig. 3). Although there are differences in detail and in entropic versus enthalpic contributions, this common core binding mechanism provides the basis for impressive nucleotide mimicry in hydrogen-bonding interactions and shape complementarity, and has been emulated by most of the other HSP90 inhibitory chemotypes that have since emerged [8–10]. These agents have rapidly followed down the path to the clinic that was previously marked by the geldanamycin derivatives. The field has since seen a rapid expansion of the HSP90 inhibitor portfolio (Fig. 2) with most major pharmaceutical companies now possessing pipeline agents, obtained either by internal discovery and development efforts or through acquisition.

An interesting recent development has been the discovery of various inhibitors that are proposed to target alternative sites on the HSP90 molecule, such as a non-ATP-binding region of the N-terminal domain, a region between the N-terminal and middle domains or a putative ATP-binding site in the C-terminal domain [30–34]. Of particular note, compounds based on the aminocoumarin antibiotic novobiocin, a bacterial DNA gyrase inhibitor, appear to bind to a putative ATP site at the C-terminal domain of HSP90—a mechanism of action that has the potential advantage over N-terminal binding in that at least some of these compounds do not appear to activate the cytoprotective heat shock response while retaining client depletion activity [35]. The natural product macrocycle Sansalvamide A has been reported to bind between the N-terminal and middle domains and thereby allosterically block the interaction of HSP90 with a set of important regulatory co-chaperones that contain a tetratricopeptide-repeat (TPR) region [32].

Currently, there are 20 drugs that have entered trials in cancer patients, and many more compounds in preclinical development (see next section and Refs [9–12]). This represents a huge increase in research output compared with just ten years ago, when the first geldanamycin derivatives entered preclinical development. As a result, the major challenges that remain no longer seem to focus on the identification of novel drug-like chemical entities that inhibit the chaperone selectively, but rather are centered on the

selection of clinical disease settings and stratified patient groups that will be needed to demonstrate the requisite efficacy for the regulatory approval of current clinical investigational drugs. As we will describe, devising the appropriate approval strategies relies heavily on what we have learnt from the pioneering clinical studies on tanespimycin and its more soluble analog 17-DMAG (alvespimycin, 17-dimethylaminoethylamino-17-demethoxygeldanamycin), together with emerging data on the newer synthetic small-molecule inhibitors.

### Lessons from pathfinder clinical trials

The Phase I trials of the first-in-class geldanamycin-based pathfinder drugs tanespimycin and alvespimycin in advanced solid tumors were the first to demonstrate the achievement of the pharmacodynamic and proof-of-mechanism endpoints of HSP90 client depletion together with HSP70 induction [via activation of heat shock factor (HSF)1] that indicate substantial target engagement at doses that were relatively well tolerated [36–38]. Although toxicity-associated adverse events and formulation issues were dose-limiting, the maximum tolerated doses for both drugs did reveal evidence of therapeutic efficacy. Dose-limiting toxicities included constitutional, gastric and hepatic (transaminitis) effects; however, the hepatic toxicity was not considered to be an on-target toxicity, but was probably caused by metabolism of the quinone moiety, as has been borne out by experience with other chemotypes.

Some encouraging instances of single-agent clinical benefit were observed in patients with advanced disease in Phase I trials; for example, in a study with alvespimycin one patient with castration-resistant prostate cancer showed a complete response and a further four patients with prostate cancer, melanoma, chondrosarcoma and renal carcinoma, respectively, showed clinical benefit (partial response or stable disease) [37]. Furthermore, it was suggested that in malignant melanoma patients treated with tanespimycin there was a possible association of prolonged disease stabilization in patients with the mutant BRAF or NRAS status of the cancer [38,39], consistent with the role of HSP90 client proteins BRAF and CRAF in this disease [40,41].



The early clinical experience led to the progression of tanespimycin into single-agent Phase II clinical trials. In a selected cohort of patients with metastatic melanoma, limited objective responses were observed in two concurrent trials, regardless of BRAF mutation status [42,43]. It was suggested that the period of target inhibition achieved was insufficient to obtain the necessary level of BRAF and/or CRAF client protein depletion, and this was probably limited by the weekly dosing schedules imposed by the drug formulation and side-effects [42]. Similarly, a Phase II trial of tanespimycin in metastatic hormone-refractory prostate cancer was curtailed on account of the lack of response as measured by circulating prostate-specific antigen (PSA) biomarker levels [44] and a Phase II trial in renal cancer also showed no objective responses [45].

Promising clinical responses have been obtained in Phase I and Phase II studies in which tanespimycin has been administered in combination with a molecularly targeted agent. When tanespimycin was combined with the multikinase inhibitor sorafenib in a range of advanced malignancies clinical effect was demonstrated in 75% (9/12) and 67% (4/6) of renal cancer and melanoma patients, respectively; these results were considered to compare favorably with the single-agent activity for either drug [46]. Of particular interest is that tanespimycin has also been tested clinically in combination with the HER2-targeting monoclonal antibody trastuzumab, specifically in a patient cohort that displayed trastuzumab-refractory disease. Phase I combination tolerance and Phase II combination efficacy studies clearly demonstrated the benefit of this treatment strategy for patients with HER2-positive breast cancer [47,48]. In the recently published Phase II trial, a 22% response rate [by objective response evaluation criteria in solid tumors (RECIST)] was observed with an overall clinical benefit rate of 59% in the same patient cohort [48]. These responses are a striking improvement on those noted in previous Phase II trials with HSP90 inhibitors and provide validation of the clinical impact that HSP90 inhibitors can have in specific tumor types with particular driver oncogenes and where the key oncoprotein is an HSP90 client. Moreover, it is important to note that the clear therapeutic benefit was shown in a molecularly defined tumor type where the defining oncoprotein – HER2 in this case – is not only a client of HSP90 but one that shows extremely high sensitivity to depletion upon HSP90 inhibition [25,49].

Disappointingly – and apparently for non-clinical reasons [47] – the strong clinical findings, especially in breast cancer, were not considered sufficient for the pharmaceutical sponsor of the trial, Bristol-Myers Squibb, to pursue the development of the KOS953 formulation of tanespimycin. It has been suggested that this decision could have been related to the formulation issues that have plagued the early geldanamycin derivatives [50]. In addition, preclinical studies by ourselves and others have shown that intrinsic resistance to 17-AAG can be caused by low expression or an inactivating polymorphism of the *NQO1* (NAD(P)H/quinone oxidoreductase I) gene, encoding an enzyme that catalyses the reduction of tanespimycin to the more potent hydroquinone [51,52]. We have also previously shown that the mechanisms underlying the acquired *in vitro* resistance to tanespimycin in glioblastoma and melanoma cells involve reduced *NQO1* mRNA and protein levels and expression of the inactive *NQO1* polymorphism [53]. It seems that the probable quinone-dependent hepatotoxicity along

with the uncertain commercial viability of the drug contributed to the termination of tanespimycin development [50]. Despite this setback, however, the clear demonstration of the clinical efficacy of tanespimycin not only highlights HER2-positive breast cancer as a promising route to approval for the next generation of HSP90 inhibitors but in addition unequivocally validates HSP90 as a legitimate clinical drug target in cancer.

In a single-agent Phase II trial in patients with molecularly defined advanced non-small-cell lung cancer (NSCLC), the pharmaceutically more ‘well-behaved’ 17-AAG hydroquinone analog IPI504 (retaspimycin hydrochloride) from Infinity Pharmaceuticals showed an overall objective response rate of 7% (5/76) irrespective of epidermal growth factor (EGFR) mutation; although this was below the threshold success rate of 20%, clinical benefit was observed in all patients with ALK rearrangements (3/3). Despite the low prevalence of ALK translocation in NSCLC, the striking efficacy of an HSP90 inhibitor in this genetic background is a strong indication that the ALK oncogenic driver HSP90 client protein can be targeted by clinically achievable doses [54].

Novartis has taken the resorcylic isoxazole NVP-AUY922 discovered by ICR/Vernalis (as VER52296) into clinical testing and it is currently in Phase I/II clinical trials in patients with NSCLC, HER2-positive advanced breast cancer, gastric cancer and refractory gastrointestinal stromal tumor (GIST) (<http://clinicaltrials.gov>). Novartis is also testing NVP-AUY922, alone and in combination with the proteasome inhibitor bortezomib plus dexamethasone, in a Phase I/II trial in multiple myeloma. Dose-limiting toxicity with NVP-AUY922 has included diarrhea, fatigue and the ocular effects of darkening of vision and night blindness that are also seen with several other HSP90 inhibitors. The purine scaffold drug BIIB021, previously with Biogen Idec, is currently being developed by Premiere Oncology and is in several Phase I clinical trials and two Phase II trials in patients with GIST and estrogen receptor positive (ER+) metastatic breast cancer. Dose-limiting toxicity included syncope and dizziness in patients with advanced solid tumors [11]. PUH71 (licensed to Samus Therapeutics) is in a Phase I clinical trials in patients with advanced solid tumors, lymphoma and myeloproliferative disorders [11].

Using a chemical proteomics approach, Serenex has identified the orally active 2-aminobenzamide compound SNX-5422 [55]. Phase I results in patients with refractory solid tumors and lymphomas have shown no objective responses but long-lasting disease stabilization was observed [56]. However, the development of SNX-5422, subsequently acquired by Pfizer (now PF04929113), has been discontinued owing to ocular toxicity seen in a separate Phase I study [56]. Debiopharm initiated a Phase I clinical trial with the orally bioavailable, dimethylamino-bearing imidazopyridine, Debio 0932 (formerly Curis CUDC-305). Of interest, this agent demonstrated an ability to cross the blood–brain barrier effectively and reach therapeutic levels in an intracranial glioblastoma model [57]. XL888 from Exelixis is being evaluated in a Phase I trial in patients with solid tumors (<http://clinicaltrials.gov/ct2/show/NCT00796484>).

Another resorcinol-containing drug, KW2478 developed by Kyowa Hakko Kirin, is currently in Phase I/II clinical trials in combination with bortezomib in patients with relapsed and/or refractory multiple myeloma (<http://clinicaltrials.gov/ct2/show/NCT01063907>). The resorcylic dihydroxybenzamide AT13387

developed by Astex Therapeutics was discovered using a fragment-based drug discovery approach using NMR screening and X-ray crystallography [58]. In preclinical studies, this agent is characterized by a long duration of client protein depletion. AT13887 has entered clinical development with an intravenous (i.v.) formulation and a twice a week dosing schedule. The oral 2-aminothieno [2,3-*d*]pyrimidine NVP-BEP800 was discovered in collaborative work carried out by ICR, Vernalis and Novartis based on a combination of fragment and *in silico* screening technologies [59]. Ganetespib (STA9090), developed by Synta Pharmaceuticals Corporation, is a novel resorcinol-containing triazole agent (precise structure undisclosed) that is currently undergoing clinical testing for the i.v. treatment of hematological and solid malignancies [60]. Sanofi-aventis has discovered a class of potent tricyclic imidazo[4,5-*c*]pyridine HSP90 inhibitors by building on compounds that bound in an induced hydrophobic pocket, 10–15 Å away from the ATP/resorcinol binding site that is also used by other inhibitors, so as to incorporate the canonical binding to Asp93 [61]. Other HSP90 inhibitors include those from Myrex/Myriad Pharmaceuticals, Chroma Therapeutics, Daiichi Sankyo, Exelixis, Wyeth, Merck and AstraZeneca [11].

The activity of HSP90 inhibitors seen in the clinic to date illustrates their potential, especially in HER2-positive breast cancer and ALK-rearranged NSCLC. Apart from these, however, the HSP90 inhibitors have, to date, arguably not shown as much clinical activity as the dual mechanism of action on multiple oncogene addiction and proteotoxic stress might have predicted [13,14]. In fact, close inspection of the data from preclinical mouse models shows that the dominant response is a cytostatic growth arrest rather than tumor regression [13]. This is consistent with the observation in *in vitro* cell culture models of a more predominant cell cycle blockade with less-extensive apoptosis [62]. To maximize the therapeutic benefit of HSP90 inhibitors in the clinic, it is important to attempt to draw out the lessons from these pioneering clinical studies that instruct the way to achieve future effectiveness of next-generation inhibitors and suggest strategies to exploit tumor dependence on particular oncoproteins that are sensitive HSP90 clients or the more general non-oncogene dependence on upregulated proteostatic pathways.

Despite the probable level of depletion of HER2 in trastuzumab-refractory breast cancer likely being sufficiently sustained to achieve an impressive clinical effect – although this was not directly measured in the clinical study [47] – the same dose and schedule (weekly 450 mg/m<sup>2</sup>) was reported to cause only moderate depletion of mutant V600E BRAF and other RAF isoforms in malignant melanoma [42], in contrast to more-impressive effects seen in initial Phase I observations [38]. The even greater sensitivity to HSP90 inhibition of HER2 compared with mutant BRAF has been noted *in vitro* [24]. Data on these and other clients have led to the suggestion that the sensitivity of HSP90 clients can be viewed as a functional hierarchy that translates to differential cellular sensitivity to HSP90 inhibition. Thus, it could be argued that the limited activity of tanespimycin in RAF-dependent melanoma and therapeutic success in HER2-positive breast cancer is a function of the sensitivity of the relevant HSP90 clients – HER2 and RAF – to depletion after HSP90 inhibition. Therefore, the failure to achieve sufficient RAF depletion levels in melanoma could be attributable to the limited tolerability and formulation of tanespimycin, which

precluded higher frequency scheduling in the clinical trials, rather than the dispensability of HSP90 activity for clinical cancers driven by RAF and other less sensitive client proteins. Therefore, revisiting melanoma and other potential disease indications for HSP90 inhibitors with second-generation clinical investigational agents exhibiting improved tolerability and pharmaceutical properties can be seen as essential for determining the broader profile of clinical effectiveness under conditions of appropriately sustained target inhibition and oncoprotein depletion.

Interestingly, whereas oncoprotein depletion was not always seen, by contrast all clinical reports have noted the induction of the canonical heat shock protein HSP72, regardless of the eventual efficacy of the HSP90 inhibitor in the trial. This suggests that, in cancer types where the clinical hypothesis is dependent on oncogene addiction to an oncoprotein that is highly HSP90-dependent, the most appropriate pharmacodynamic biomarker to test for target inhibition will be the depletion of the client itself, rather than the increased expression of HSP72, which is a useful biomarker of target engagement but is an overly sensitive molecular response. Focusing more on the key clients in a refined pharmacological audit trail approach [21] should give a much clearer picture of the relationship between target inhibition and clinical benefit in every particular therapeutic context.

As highlighted earlier, based on the experience to date, breast cancer and NSCLC appear to be promising disease indications in which HSP90 inhibitors could potentially gain approval (See Box 1 for summary of approval strategies). Given the limited spectrum of clinical activity to date, an additional approach to improving the use of HSP90 inhibitors in the clinic is to adopt more-rigorous preclinical evaluation strategies that inform the clinical hypotheses going forward [63]. Profiling in large molecularly defined cancer cell line panels might be useful. However, the challenges of this approach are shown by the findings from an integrated genomic and pharmacologic study in a panel of 84 NSCLC lines that KRAS mutation confers sensitivity to HSP90 inhibitors [64]. Although *in vitro* observation translated to *in vivo* sensitivity of a KRAS-driven genetically engineered mouse lung adenocarcinoma model [64], it is not clear that this translates into clinical activity in such tumors.

Nevertheless, it is reasonable to anticipate an improvement in clinical benefit – and also in our understanding of the factors that

#### BOX 1

##### Routes to regulatory approval for HSP90 inhibitors

- Use of pharmaceutically optimized, second-generation HSP90 inhibitors with sustained target inhibition and improved tolerability properties.
- Treatment of cancers addicted to oncoproteins that are highly dependent on HSP90.
- Selection of individual patients with appropriate oncoprotein client status (amplified, mutated, translocated).
- Treatment of cancers in which survival is dependent on buffering of proteotoxic stress, especially multiple myeloma.
- Use in mechanistically relevant combinations, including molecularly targeted drugs acting directly on the relevant client proteins.
- In multiple myeloma, combination with other proteotoxic drugs, for example bortezomib.

influence therapeutic responses – through the combination of more-detailed preclinical modeling and subsequent patient stratification, coupled with the use of more-potent and fully optimized second-generation HSP90 inhibitors that exhibit improved tolerability and pharmaceutical properties and that can therefore deliver profound and sustained depletion of the relevant pathogenic driver oncoprotein.

### Targeting non-oncogene dependency by HSP90 inhibition

As introduced earlier, in addition to causing the degradation of a number of key driver oncoproteins, HSP90 inhibitors have profound effects on cellular proteostasis. This is illustrated by the clinical effectiveness of HSP90 inhibitors in the myeloid malignancy, multiple myeloma, a cancer that is characterized by extreme, potentially toxic overload of unfolded protein [65,66]. Multiple myeloma is a special case owing to the excess production of immunoglobulin from bone marrow plasma cells, but the proteotoxic burden of many cancer types, particularly those with a severely abnormal karyotype [67], could potentially be exploited therapeutically by HSP90 inhibitors as a differential dependence of tumor versus normal cells. This proteostasis vulnerability is supported by several clinical examples of increased expression of HSP90 and its canonical partner HSP72 correlating with prognosis in NSCLC [68] and with progression in melanoma [69] and breast cancer [70]. Recent results show that the expression of nuclear (activated) HSF1 is an independent prognostic factor in breast cancer, particularly the ER+ subgroup [71]. It is intriguing that, as we have discussed above, breast cancer and NSCLC are disease settings in which more-promising clinical responses with HSP90 inhibitors have been seen.

In addition to the clinical outcome relationships with the overall levels of chaperones, the intriguing discovery that HSP90 exists in a more-active ‘superchaperone’ complex (containing regulatory co-chaperones) in cancerous but not healthy cells [72] has recently been supported by a comprehensive proteomic study in chronic myelogenous leukemia cells [73]. Furthermore, the latter study also identified cancer-cell-specific signaling networks of HSP90 client proteins [73]. These studies further support and inform the concept that expression of HSP90 and other heat shock proteins might indicate non-oncogene dependence. By contrast, the HSF1-dependent increase in the expression of HSP72, HSP27 and other heat shock proteins following treatment with HSP90 inhibitors probably represents an intrinsic resistance mechanism to HSP90 inhibitors [74,75]. Activation of HSF1 following release from HSP90 after inhibitor treatment – as with heat shock – initiates the rapid transcriptional upregulation of a host of pro-survival proteins [76] including HSP72, which itself has a direct antiapoptotic function [77,78]. Not only can HSF1 activation ameliorate the therapeutic interference with tumor proteostasis that HSP90 inhibition induces but the upregulation of pro-survival proteins could also protect tumor cells from HSP90-inhibitor-mediated oncoprotein targeting.

To realize fully the benefits of dual exploitation of oncogene and non-oncogene vulnerability by HSP90 inhibitors – and potentially to shift the predominant effects of HSP90 inhibition from stasis to regression in a wider spectrum of tumors – it is becoming increasingly evident that co-targeting of the heat-shock response and

HSP90 could be a powerful therapeutic approach [4,74,75,79]. Of note, dual knockdown of the core HSP70 isoforms – HSC70 and HSP72 – elicited marked tumor-specific sensitization to HSP90 inhibition *in vitro* [80]; furthermore, use of a chemical tool HSP70 inhibitor in combination with 17-AAG has demonstrated the pharmacologic proof-of-concept for simultaneously targeting HSP70 and HSP90 [81].

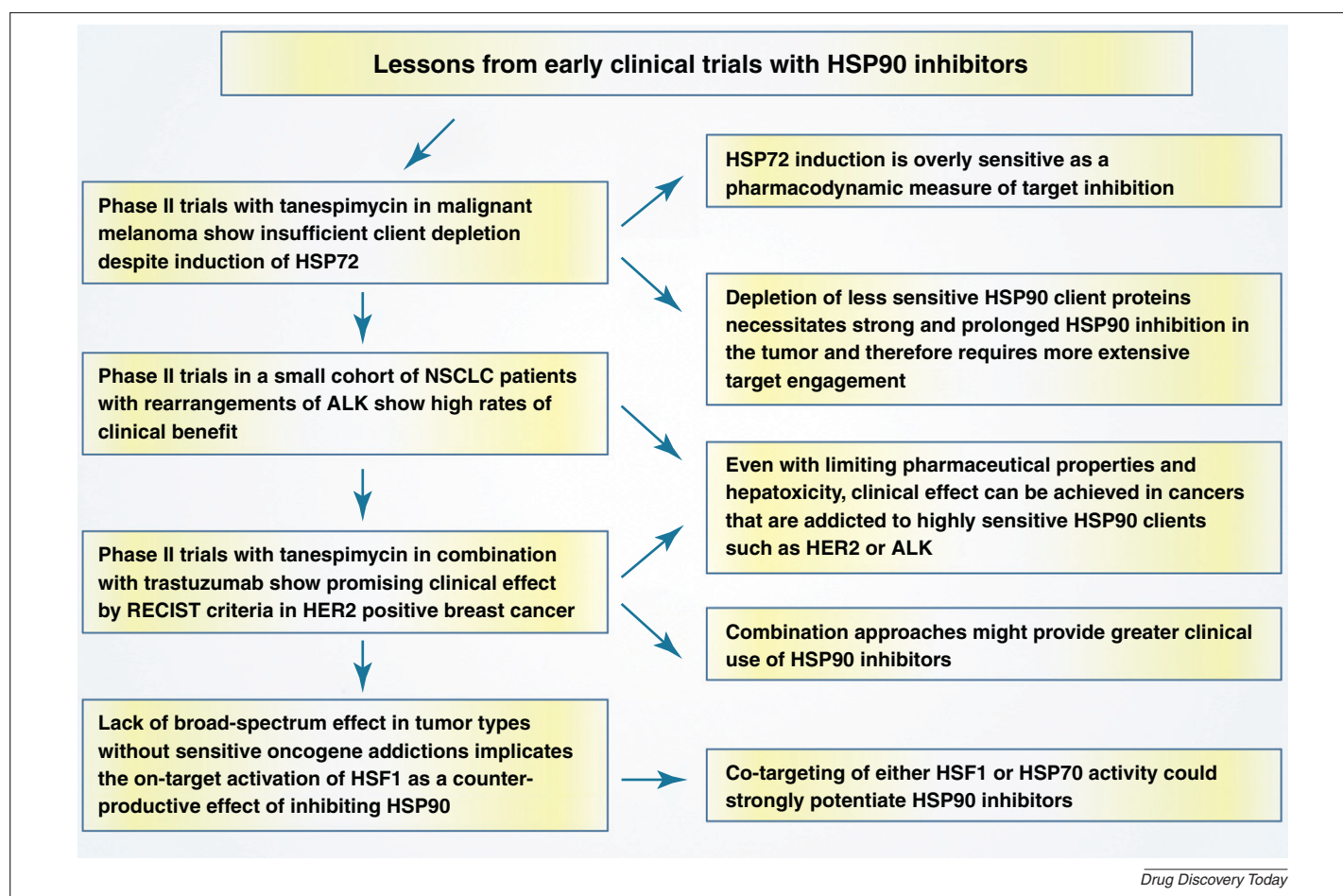
An alternative strategy to direct co-targeting of key chaperones, especially HSP70 and HSP90 isoforms, is to block completely the transcriptional induction of heat shock proteins by activated HSF1. In addition to use in combination, inhibition of HSF1 activity could prove to be an effective anticancer approach in itself, particularly in instances where increased expression of HSF1 and molecular chaperones is evident [71,74,79]. Although as a non-liganded transcription factor HSF1 itself is not conventionally druggable, chemical tools that block HSF1 activation, such as KNK437, have already been employed in multiple myeloma models to achieve therapeutic sensitization to HSP90 inhibition [82]. As mentioned earlier, there is also potential for the use of certain C-terminal HSP90 inhibitors that deplete HSP90 clients without apparently inducing the heat shock response [35].

### New biological perspectives on HSP90 inhibitors

We have outlined herein several possible approaches to optimize the clinical use of HSP90 inhibitors, based on experience gained from the laboratory and early clinical trials (Fig. 4). Although clinical studies continue apace, fundamental research questions remain to be answered concerning the cellular context-specific nature of HSP90 function and the precise structural determinants of the interactions of HSP90 with client proteins and co-chaperones alike.

One of the greatest fundamental research challenges in the HSP90 field is to identify which features make a protein become a client of HSP90. This cannot currently be predicted by the DNA sequence, protein sequence or 3D structure of the client [83,84]. The research challenge is exemplified by the example of BRAF; for reasons that remain unclear, the common, activated and oncogenic V600E mutant and many other mutants are much more highly dependent on HSP90 for their activation and stability, as compared with the wild-type protein [40,41]. With a better understanding of specific HSP90-client-protein interactions, it could be possible to induce selectively the functional inactivation, misfolding and degradation of specific, individual oncogenic client proteins, or groups of these, thus avoiding potential adverse effects of multiple client depletion through targeting HSP90. By contrast, such greater molecular selectivity could lose the initially conceived advantages of combinatorial depletion of multiple oncoprotein clients, with its attendant benefits for preventing the induction of resistance through alternative HSP90 clients.

Although the underlying mechanism is emerging, the clear hierarchy of clients in terms of dependence on HSP90 and sensitivity to HSP90 inhibition seems likely to have major implications for the clinical efficacy of HSP90 inhibitors. One possible contributory determinant of client sensitivity is the function of HSP90 co-chaperones such as CDC37 or AHA1, which have been shown to affect cancer cell sensitivity to HSP90 inhibition [85,86]. These and other co-chaperones could potentially represent new targets for therapeutic intervention. Drugs acting on these

**FIGURE 4**

Scheme showing the evaluation of clinical data from trials of geldanamycin analogs and lessons learned for the future clinical application of next-generation HSP90 inhibitors.

proteins could feasibly be differentiated clinically from HSP90 inhibitors in terms of therapeutic and adverse effect profiles.

### Horizon scanning

As emphasized in this review, HSP90 inhibitors clearly possess mechanistic potential to exploit cancer vulnerabilities involving addiction to oncogenic client proteins and dependence on the proteotoxic stress response. Considerable progress has been made in basic biology research on HSP90 and in the exploitation of this knowledge for drug discovery and development. Initial experience with first-generation natural product inhibitors has demonstrated potential therapeutic use and has also revealed hurdles to overcome. A large number of pharmaceutically optimized HSP90 inhibitors are now in preclinical and clinical development.

Progress to regulatory approval of the first HSP90 inhibitor is likely to depend on two key factors. First, the evaluation of second-generation inhibitors with improved pharmaceutical and tolerability properties will be important to give the best chance of therapeutic activity being revealed. Many such agents are now in clinical development and the availability of fit-for-purpose, pharmaceutically optimized HSP90 inhibitors is no longer likely to be a limiting factor. Second, the careful selection of disease type with molecular stratification to reveal responsive patients is also likely to be crucial. This will probably focus on cancers such as

HER2-positive breast tumors and NSCLC with ALK translocations, for which there is strong evidence for addiction to a sensitive HSP90 client and emerging clinical response data. Other molecularly stratified disease indications need to be identified.

Additional exploitation of proteotoxic stress dependence – as best exemplified in multiple myeloma – will also be important, potentially in combination with the approved proteasome inhibitor bortezomib. Furthermore, from the oncogenic client addiction standpoint, the most optimal clinical use for HSP90 inhibitors might be in combination with other targeted agents, including in the above-mentioned molecularly defined indications where activity has already been observed, as with tanespimycin plus trastuzumab in HER2-positive breast cancer. Modulating a driver oncoprotein using a combination of a drug that inhibits its biochemical function (e.g. kinase activity) together with its overall depletion at the protein level via HSP90 inhibition could be especially damaging for the cancer cell, particularly if proteotoxic stress is also induced. Examples include the combinations of HSP90 inhibitors with the BRAF inhibitor vemurafenib in mutant BRAF melanoma or with androgen-receptor-targeted therapies in prostate carcinoma (because this pathogenic receptor is also a client protein). Such an approach can be viewed as tackling cancer through a network biology strategy, resulting in breakdown of even a robustly evolved oncogenic system [87].



However, although the combinatorial action of HSP90 inhibitors will probably reduce the risk of drug resistance arising, resistance could still be possible under the conditions of Darwinian selection imposed during clinical treatment. In addition to the cytoprotective heat shock response, the major resistance mechanisms seen with tanespimycin is reduced NQO1 expression/activity, which can be circumvented by using the non-quinone drugs [51–53]. However, although it has been considered difficult to mutate essential drug-binding residues without also losing ATP binding and, hence, cell viability, there is now precedent for selection of inhibitor-resistant forms of HSP90 in organisms that synthesize and secrete geldanamycin and radicicol [88,89].

Although, understandably, prioritizing stratified tumor types where therapeutic activity has already been revealed is crucial, it is important that trials should be carried out in cancers with sensitive driver oncoprotein clients in which little effect has been observed to date in Phase II trials of tanespimycin. Use of improved next-generation HSP90 inhibitors, especially in combination with targeting of the driver mutation, could enable therapeutic effects to be seen in additional settings.

Looking further ahead, the potential for combinatorial targeting of HSP90 together with HSF1 or HSP70 is an exciting prospect, but one that remains to be validated fully in preclinical animal models. Certainly, this might be one way to maximize the therapeutic window of HSP90 inhibitors, particularly in cases where dependence on HSF1 activation is high. Indeed, such cancers that are driven by pathogenic HSP90 clients and also display evidence of non-oncogenic addiction to HSF1 and HSP90 could be especially vulnerable. Also important will be the judicious combination of HSP90 inhibitors with cytotoxic drugs or radiation therapy.

Finally, it should also be noted that other interesting therapeutic applications for HSP90 inhibitors are now being considered, particularly in protein folding disorders such as Alzheimer's disease, Parkinson's disease and prion disease, and also in the treatment of viral and protozoan infections, where there are exciting prospects for these drugs in addition to their emerging potential in cancer therapy [90].

### Conflict of interest statement

All the authors are or were employees of the Institute of Cancer Research, which has a commercial interest in HSP90 inhibitors and operates a reward to inventors scheme. Paul Workman and Swee Sharp received research funding from Vernalis for the discovery of HSP90 inhibitors and intellectual property from this programme was licensed to Vernalis Ltd and Novartis. Paul Workman has been a consultant to Novartis, is a founder and scientific advisory board member of Chroma Therapeutics, and is scientific advisory board member of Astex Pharmaceuticals and Nextech Invest.

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