Heat shock protein 90 inhibitors in the treatment of cancer: current status and future directions

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Abstract

Introduction—Heat shock protein 90 (HSP90) serves as a critical facilitator for oncogene addiction. There has been augmenting enthusiasm in pursuing HSP90 as an anticancer strategy. In fact, since the initial serendipitous discovery that geldanamycin (GM) inhibits HSP90, the field has rapidly moved from proof-of-concept clinical studies with GM derivatives to novel second-generation inhibitors.

Areas covered—The authors highlight the current status of the second-generation HSP90 inhibitors in clinical development. Herein, the authors note the lessons learned from the completed clinical trials of first- and second-generation inhibitors and describe various assays attempting to serve for a more rational implementation of these agents to cancer treatment. Finally, the authors discuss the future perspectives for this promising class of agents.

Expert opinion—The knowledge gained thus far provides perhaps only a glimpse at the potential of HSP90 for which there is still much work to be done. Lessons from the clinical trials suggest that HSP90 therapy would advance at a faster pace if patient selection and tumor

pharmacokinetics of these drugs were better understood and applied to their clinical development. It is also evident that combining HSP90 inhibitors with other potent anticancer therapies holds great promise not only due to synergistic antitumor activity but also due to the potential of prolonging or preventing the development of drug resistance.

Keywords

cancer; clinical development; heat shock protein 90 inhibitors; patient selection

1. Introduction

Heat shock protein 90 (HSP90) is an ATP-dependent molecular chaperone that aids its 'client proteins' to fold properly. To achieve its role, HSP90 uses a complex cycle regulated by binding and hydrolysis of ATP and its various co-chaperones (HSP70, Aha1, p23, HSP-organizing protein (HOP), Cdc37). Inhibition of the HSP90 chaperone cycle leads to destabilization of these client proteins, their ubiquitination and ensuing degradation by the proteasome [1-4]. The acquired knowledge over the course of years that many of HSP90's clients are *bona fide* oncoproteins [e.g., human epidermal growth factor receptor (HER2), EGFR, CDK4, serine/threonine-protein kinase C-Raf (CRAF), serine/threonine-protein kinase B-Raf (BRAF), also known as Protein Kinase B (PKB) (AKT), mesenchymal epithelial transition factor (MET), BCR-ABL] has propelled this chaperone protein as a promising target for the treatment of cancers [1-4]. Importantly, HSP90 inhibition leads to concurrent effects on many oncogenic proteins and pathways, counteracting the numerous pathological traits displayed by cancer cells [2]. Numerous preclinical studies attest to the potential of HSP90 inhibition to result in tumor growth inhibition, reduction in metastatic potential and in sensitization of tumors to the effect of other therapies. There are several reviews that speak to these effects and we direct the readers to them for more information (Figure 1) [1-4].

The path to HSP90 inhibition was initially paved by two natural products, geldanamycin (GM) (Figure 2) [5] and radicicol (RD) (Figure 3) [6]. Both GM and RD were found to inhibit HSP90 by competing with ATP for binding to its N-terminal regulatory pocket. Unfortunately, these two compounds were precluded from reaching the clinic given their poor *in vivo* stability and toxicities stemming from their reactive chemical structures. Nonetheless, these pathfinder molecules served as tools for better understanding the biology of HSP90 in tumors, and ultimately sustained the process of bridging the gap between the HSP90 biology and subsequent call for HSP90 drugs. They also provided valuable pharmacophores for next-generation inhibitors, and as we shall see, several HSP90 clinical agents have incorporated in their structures the benzoquinone found in GM (Figure 2) or the resorcinol found in RD (Figure 3).

2. First-generation HSP90 inhibitors in clinic – lessons learned

Medicinal chemistry provided the path to first-generation HSP90 inhibitors with clinical potential. Replacement of the non-essential C-17 methoxy group of GM via substitution with various amines provided many semisynthetic derivatives, and among these was 17-AAG (Figure 2; 17-allyl-17-desmethoxygeldanamycin – tanespimycin), the first HSP90

inhibitor to have entered clinical trials (Table 1). 17-AAG retained the important biological features of GM but had an improved toxicity profile [7]. It has been explored in various dosing schedules and formulations as a single agent therapy [8-11]. The most promising clinical activity observed in these diverse Phase I trials was stable disease (SD). In Phase II trials, there were no objective responses noted with single agent tanespimycin in prostate cancer, melanoma or renal cell cancer, despite the presence of known target client proteins in these malignancies [12-15]. The lack of efficacy was attributed to multiple factors, including suboptimal doses and scheduling of tanespimycin in an attempt to avoid treatment-related toxicities, formulation issues and poor patient enrichment for those who might best benefit from therapy based on preclinical experience (e.g., HER2-amplified breast cancer, because HER2 is a sensitive HSP90 onco-client, Figure 1) [1-4].

On the contrary, activity has been reported when tanespimycin was combined with other cytotoxic or biologic agents (Table 1). Most prominently, a Phase II trial of tanespimycin in combination with trastuzumab in patients with HER2-positive metastatic breast cancer (MBC) progressing on prior trastuzumab, was the first to report Response Evaluation Criteria in Solid Tumors (RECIST)-defined efficacy for tanespimycin in solid tumors [16]. The reported overall response rate (ORR) and clinical benefit rate (CBR, defined as complete response [CR] + partial response [PR] + SD) were 22 and 59%, respectively [16], and median progression free survival (PFS) and overall survival (OS) were 6 and 17 months, respectively. Tanespimycin has also been evaluated in combination with bortezomib in patients with multiple myeloma (MM). In a Phase I/II trial, response rates (RRs) of 41, 20 and 14% were reported in bortezomib-naïve, -pre-treated and -refractory patients, respectively [17]. This led to a Phase III trial of this combination which was later suspended due to nonclinical reasons [18].

Nevertheless, 17-AAG failed to advance further given its poor pharmaceutical and toxicity profile. Besides the limiting solubility and formulation challenges, 17-AAG contains a benzoquinone moiety that undergoes reductive metabolism and detoxification by nicotinamide adenine dinucleotide phosphate (NADPH): quinone oxidoreductase (NQ01) (also called DT-diaphorase) before it acts against HSP90 [1-4,11]. It is this benzoquinone that causes increased hepatotoxicity and perhaps also constitutes a mechanism of drug resistance in patients with a mutation in NQ01 [19]. Naturally, all this information combined with the delayed hepatotoxicity of 17-AAG in the clinic with the twice-a-week continuous dosing schedule provided a further impetus for the development of non-ansamycin HSP90 inhibitors with an improved toxicity profile.

The solubility issue observed for the front-runner 17-AAG was surmounted when Kosan Biosciences discovered 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin (17-DMAG) (Figure 2; 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin-alvespimycin) [20]. This compound contains the ionizable N,N-dimethylethylamine group instead of the methoxy group at C-17. Importantly, the introduction of the ionizable amino group provided the much-needed improvement in water solubility, oral bio-availability and equal, if not greater, antitumor activity than 17-AAG [21]. These combined benefits allowed 17-DMAG to advance to clinical trials in 2004 where it was investigated both as an oral and as an intravenous (i.v.) agent (Table 1) [22-24]. Objective responses, including some CRs,

have been reported in patients with castrate-resistant prostate cancer (CRPC), acute myeloid leukemia and metastatic melanoma [23,24]. SD > 6 months has also been reported in three patients with chondrosarcoma, CRPC and clear cell renal cancer, respectively [24]. Toxicities reported in these trials included liver, lung, ocular and cardiac toxicities in addition to common side effects such as diarrhea, fatigue and nausea. Similar to tanespimycin, alvespimycin may be most beneficial in combination with trastuzumab. A Phase I trial of this combination reported one PR in a patient with HER2-positive MBC and 6 of the 28 other patients had SD lasting > 6 months [25]. Despite these objective responses, the clinical development of alvespimycin was halted in 2008 by Kosan due to strategic reasons [11]. However, this drug is still currently under evaluation by the National Cancer Institute for the treatment of patients with relapsed chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma, or B-cell prolymphocytic leukemia (NCT01126502).

An improved GM derivative, IPI-504/retaspimycin (Figure 2), was introduced by Infinity. This reduced quinone form of tanespimycin is more water soluble than tanespimycin or alvespimycin and also, by lacking the benzoquinone, less prone to hepatotoxicity [26]. Phase I clinical trials have been conducted in patients with non-small cell lung cancer (NSCLC), MM and metastatic gastrointestinal stromal tumor (GIST) (Table 1) [11,27]. Modest antitumor activity, including metabolic responses with FDG-PET, was reported in 2 out of 4 patients with NSCLC and 4 out of 18 patients with GIST. Single agent Phase II trials were also conducted in patients with CRPC, NSCLC and GIST [28-30]. Results were disappointing in CRPC with no radiological or prostate-specific antigen responses and two deaths related to liver failure and ketoacidosis [28].

Based on preclinical studies that the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) oncogenic fusion protein was highly sensitive to HSP90 inhibition by IPI-504 [31], a Phase II study of IPI-504 in refractory NSCLC stratified by ALK rearrangement status was initiated. The ORR was 67% in patients with ALK rearrangement compared to 8.3% in those without the ALK rearrangement [32]. In another Phase I/II trial of IPI-504 in patients with NSCLC who had progressed on prior EGFR tyrosine kinase inhibitors (TKIs), two out of three patients with ALK rearrangement had a PR and a third patient had SD > 7.2 months with 24% reduction in tumor size [29]. The RR in EGFR-mutated patients in this trial was poor (RR 4%). Most common side effects were diarrhea, nausea and fatigue, and grade 3 liver abnormalities were reported in nine patients [29]. Promising activity was also noted in patients with GIST which led to the Phase III retaspimycin in GIST trial. While the dose and schedule of IPI-504 was the same as the NSCLC trial, hepatotoxicity was very prominent in this trial with four treatment-related deaths leading to early study closure after 47 of the 195 planned patients were enrolled [33].

When combined with trastuzumab in patients with HER2-positive MBC, results from a Phase II trial revealed positive data, with 16 patients (62%) achieving SD with median on-study duration of 2.4 months (range 1.1 – 8.2 months). Contrary to expectations, there were no objective responses which was attributed to the lower dose of IPI-504 in this study (300 mg/m^2 weekly compared to 400 mg/m^2 twice weekly in the NSCLC trial) [34]. A Phase Ib combination trial of IPI-504 with docetaxel was conducted in 23 patients with NSCLC who had progressed on one or two prior lines of chemotherapy [34]. The ORR was 26% (6 PR, 7

SD) with higher responses in patients with squamous histology (43%) and those with a smoking history (33%) [34]. This has led to the ongoing randomized Phase II trial of this combination compared to docetaxel plus placebo in current and former smokers with pretreated NSCLC (NCT0 1262400). Another Phase Ib/II trial is evaluating the safety and combination of IPI-504 plus everolimus in patients with KRAS mutant NSCLC (NCT01427946).

3. HSP90 clinical agents: second generation

Taken as a whole, the GM derivatives have certainly fueled research efforts by both industry and academia to develop newer small-molecule synthetic HSP90 inhibitors with better bioavailability and toxicity profiles to permit administration of sufficiently high levels of inhibitor required to derive a therapeutic benefit. The quest for these much-improved small synthetic molecules resulted in the development of better HSP90 inhibitors coined as second generation. Studies with the first-generation HSP90 inhibitors also provided knowledge on tumor subtypes more likely to benefit from HSP90 inhibitors facilitating the clinical path of upcoming agents. From a pharmacophore perspective, most second-generation HSP90 inhibitors in clinical development either incorporate the resorcinol moiety of RD (Section 3.1, Figure 3) or mimic the purine-scaffold implemented in the design of the first synthetic HSP90 inhibitor, purine-scaffold (PU3) (Section 3.2, Figure 4), while just a few have a distinct scaffold (Section 3.3, Figure 5). We will next glimpse over the clinical development status of such inhibitors currently in clinical evaluation.

3.1 Resorcinol derivatives

The resorcinol core of RD, a critical element for binding to HSP90, is found in a number of clinical agents, namely NVP-AUY922, AT-13387, STA-9090 and KW-2478 (Figure 3, the resorcinol moiety is shown in blue), which are described below.

3.1.1 NVP-AUY922—NVP-AUY922 traces its origin to a high-throughput screen that employed inhibition of yeast HSP90 ATPase activity using the malachite green detection of inorganic phosphate [35]. The most potent hit emerging from this screen was the resorcinol-containing pyrazole CCT018159 – a molecule shown later by X-ray crystallography to bind to the ATP pocket in the N-terminal domain of yeast HSP90, similar to RD [36]. Scientists at Vernalis and the Cancer Research UK Center for Cancer Therapeutics used a structure-based approach to optimize this initial hit, and from the fruits of their labor emerged the isoxazole NVP-AUY922 (Figure 3), a potent HSP90 inhibitor [37].

Subsequently, this lead candidate developed by Novartis made it to clinical trials where it is being evaluated as a single agent as well as in combination therapy for various malignancies in multiple Phase I and Phase II trials (Table 2). The Phase I trial in advanced solid tumors established the maximum tolerated dose (MTD) of weekly i.v. infusions at 70 mg/m^2 [38]. Dose-limiting toxicities (DLTs) included atrial flutter, darkening of vision, diarrhea and fatigue. Interestingly, 20% of the patients developed night blindness and 7% had grade 3 or higher eye disorders at the MTD. Although there were no objective responses, 16 patients developed SD and 9 also had a partial metabolic response on flurodeoxyglucose positron emission tomography (FDG-PET) scans [38]. A Phase II expansion trial in HER2-positive

and estrogen receptor-positive breast cancer also reported two partial metabolic responses on FDG-PET and one of these was also a confirmed PR by RECIST [39]. A Phase II trial of monotherapy NVP-AUY922, conducted in patients with NSCLC progressing on two or more chemotherapy regimens (including patients with EGFR mutations who had progressed on EGFR TKI therapy), reported responses in patients with EGFR mutations (20% RR) and those with ALK rearrangements (32% RR) [40]. This led to the Phase II study of NVP-AUY922 plus erlotinib in patients with EGFR mutant lung cancer with acquired resistance to EGFR TKIs. Twenty-five patients were treated on this Phase II study. The study met its primary end point (defined as CR + PR at 8 weeks) with 5 of 22 patients (23%) demonstrating PR. Notably, three of these five patients had the T790M acquired mutation after EGFR TKI therapy, thus suggesting that the activity of this combination was not limited to patients with EGFRT790M mutation alone [41]. In a study of the combination of trastuzumab plus NVP-AUY922 in patients with HER2-positive MBC previously progressing on two prior anti-HER2 regimens, the ORR was 23% (5/22 patients) [42]. NVP-AUY922 is also being studied in Phase I/II studies in combination with other agents such as bortezomib in MM (NCT00708292), a phosphatidylinositol 3-kinase (PI3K) inhibitor BYL719 in advanced or metastatic gastric cancer (NCT01613950), an ALK inhibitor LDK378 in ALK-rearranged NSCLC (NCT01772797), with pemetrexed or docetaxel in NSCLC with EGFR mutations (NCT01646125) and cetuximab in KRAS-mutated colorectal cancer (NCT01294826).

3.1.2 AT-13387—Astex Pharmaceuticals applied a fragment-based drug discovery approach to find fragments with HSP90 binding affinity. This fragment screening consisted of a combination of nuclear magnetic resonance and high-throughput X-ray crystallography. One of the fragments identified was a phenolic chemotype [43] which was picked up and optimized via structure-guided design to ultimately led to the discovery of AT-13387 (Figure 3) [44].

This compound is being evaluated in Phase I trials in patients with advanced solid tumors (Table 2). The various dosing schedules being studied include weekly or twice-weekly infusions [45], every 3 weeks of a 28-day cycle and a twice a week (2 days in a row) for the first 3 weeks of a 28-day cycle [46]. A dose of 260 mg/m^2 has been identified as the once-weekly MTD [45]. One durable RECIST PR (8 months) was reported in an imatinib-relapsed metastatic GIST patient with c-kit mutations in exons 11 and 17. Three SD 6 months (follicular cell thyroid carcinoma, metastatic uveal melanoma, GIST) were also observed. Phase II studies are currently ongoing. HSP70 induction, a surrogate readout of HSP90 inhibition, was noted in peripheral blood mononuclear cells (PBMCs) at all doses and exhibited dose dependence. In addition to diarrhea and fatigue that has been reported with this class of agents, this compound also showed interesting toxicity of reversible visual changes of blurred vision, diplopia, flashes and light–dark accommodation [45]. It is currently under evaluation in three Phase I/II trials either alone or in combination with: i) abiraterone in the treatment of CRPC which is no longer responding to abiraterone (NCT01685268); ii) crizotinib in the treatment of NSCLC (NCT01712217); and iii) imatinib in patients with unresectable and/or metastatic GIST whose tumor has progressed following treatment with a maximum of three TKIs (NCT01294202).

3.1.3 STA-9090 (ganetespib)—STA-9090 (Figure 3) is a novel resorcinolic triazolone inhibitor which was discovered and developed by Synta Pharmaceuticals [47]. It has been or is currently being tested in over 25 clinical trials in both hematologic and solid tumors, including a Phase III trial in NSCLC as summarized below [11,48].

Two Phase I clinical trials in advanced solid malignancies evaluated ganetespib in different dosing schedules: intravenously weekly for 3 weeks in a 28-day cycle or twice-weekly dosing for 3 weeks in a 28-day cycle, respectively. The recommended Phase II dose for the weekly dosing was established at 200 mg/m^2 [49]. Ganetespib was well tolerated with predominantly grade 1 or 2 toxicities that were easily manageable. DLT included grade 3 amylase elevation, grade 3 diarrhea and grade 3 and 4 asthenia. One patient with metastatic colorectal cancer achieved a PR and 23 patients achieved SD. Disease control rate defined as PR + SD > 16 weeks was noted in 24.5% of the patients (Table 2) [49]. DLT of elevated liver enzymes was reported in the twice-weekly dosing trial. In this trial, one patient with metastatic melanoma achieved PR and two others with NSCLC achieved SD [50]. Two additional Phase I studies evaluated single agent ganetespib in hematologic malignancies, concluding that the doses for further study in this population are 200 mg/m^2 once weekly [51] and 90 mg/m^2 twice weekly [52]. The most common adverse events in both these trials were mild-to-moderate diarrhea and fatigue that were reversible and easily manageable. DLT in the once-weekly dosing trial was documented as elevated liver enzymes in one patient. Although there were no formal responses, three patients had a hematologic response [51]. In the twice-weekly dosing trial, DLTs included hyperbilirubinemia/hyponatremia, QTc prolongation and transaminitis [52].

Single agent ganetespib has also been evaluated in Phase II trials in patients with NSCLC [53], MBC [54] and GIST [55]. The drug was well tolerated, with the most frequent toxicities being grade 1/2 diarrhea, nausea and fatigue. In advanced NSCLC, ganetespib activity was evaluated in three cohorts based on prospective genotypic characterization of tumors for the presence of EGFR or KRAS mutation or the absence of these mutations. Ganetespib showed encouraging single agent activity in the non-mutant EGFR or KRAS cohort, specifically in the crizotinib-naïve patients whose tumors harbored the EML4-ALK rearrangement. Among eight such patients, there were four PRs and a median PFS of 8.1 months. This formed the basis for a Phase II study of ganetespib monotherapy in patients with crizotinib-naïve ALK-positive disease which has been initiated (NCT015 62015).

There is a strong preclinical rationale for combining HSP90 inhibitors with taxanes, due to synergistic antitumor activity [56,57]. A randomized Phase II GALAXY-I trial recently reported extended OS with ganetespib and docetaxel compared to docetaxel alone in the second-line setting in patients with advanced NSCLC who are at least 6 months from initial diagnosis of advanced disease [58]. Encouraged by these results the GALAXY-2 trial, a Phase III trial of this combination has been initiated and is currently accruing patients (NCT01798485).

It is well established that HER2 is a very sensitive client protein of HSP90 inhibition [1-4] and proof of concept for HSP90 inhibition was provided by tanespimycin in combination with trastuzumab in HER2-positive trastuzumab refractory MBC [16]. A Phase II trial of

single agent ganetespib was conducted in an unselected cohort of MBC patients [54]. Although the study did not meet its prespecified criteria for ORR in the first-stage of the Simon's 2-stage model in this heavily pretreated unselected population, activity was noted in trastuzumab refractory HER2-positive and triple-negative breast cancer (TNBC) [54]. Clinically, anecdotal evidence of single agent activity has been reported in TNBC patients treated on Phase I and Phase II trials including an ongoing Phase II monotherapy trial (ENCHANT, NCT01677455) [59]. Ganetespib also showed enhanced cytotoxic effects when combined with chemotherapeutic agents such as doxorubicin and taxanes [59] and the ENCHANT-1 trial has been expanded to evaluate the combination of ganetespib and weekly paclitaxel, and Synta Pharmaceuticals is planning another trial of ganetespib and docetaxel in TNBC. A Phase I trial of ganetespib plus paclitaxel plus trastuzumab is ongoing in HER2-positive trastuzumab-refractory MBC (NCT020 60253).

Patients with GIST who had failed prior therapy with imatinib and sunitinib were treated with single agent ganetespib weekly for 3 weeks in a 28-day cycle [55]. Correlatives included positron emission tomography (PET) imaging, and HSP90 client proteins were evaluated using pre- and post-treatment biopsies. The primary end point was CBR (CR + PR + SD > 16 weeks). In this study, 12 of 23 patients in this Simons 2-stage model achieved SD, and 7 of the 123 patients reported a > 20% decrease in the standardized uptake value (SUV) as measured by PET imaging. However, paired tumor biopsies in four patients did not show prolonged inhibition of activated KIT or its downstream pathways. These data, therefore, suggested that once-weekly treatment might not be optimal in patients with GIST and therefore accrual is limited to patients with platelet-derived growth factor receptor alpha (PDGFRA) mutations to allow evaluation of other schedules and combinations.

3.1.4 KW-2478—In their pursuit for small-molecule HSP90 leads, Kyowa Hakko Kirin Pharma used a binding assay where HSP90 was fixed onto plates and the compounds to be screened were then added to the wells to identify competitive inhibitors of a labeled RD. Their endeavors resulted in the identification of several resorcinol-based leads which were subsequently optimized via X-ray crystallography, cell-based assays and *in vivo* models to eventually provide the clinical candidate KW-2478 (Figure 3) [60].

A Phase I study of this compound was reported in patients with relapsed/refractory MM, CLL or B-cell non-Hodgkin's lymphoma [60]. KW-2478 (14 – 99 mg/m^2) was administered intravenously over 60 min once-daily on days 1 – 5 of a 14-day cycle. No DLTs were reported up to 99 mg/m^2. Drug-related toxicities included grade 1/2 hypertension in one patient and grade 3 QTc prolongation in another. Another Phase I/II trial of KW-2478 in combination with bortezomib, when administered on days 1, 4, 8 and 11 of a 21-day cycle in patients with relapsed and refractory MM, was recently reported at the Annual Society of Hematology meeting in 2013 (Table 2) [61]. A total of 95 patients were treated: 15 on the Phase I trial and 80 on the Phase II trial. The most common adverse events included diarrhea, nausea, vomiting, fatigue and peripheral neuropathy. Five patients had grade 4 thrombocytopenia and three patients had grade 4 neutropenia. In the Phase I trial, HSP70 induction was observed in the PBMC of all patients. Of the 80 patients on the Phase II trial, 79 patients were evaluable for response using the International Myeloma Working Group

response criteria. ORR was 39% and PFS was 26.4 weeks. In bortezomib-naïve patients (n = 50), the ORR was 48% [61].

3.2 Purine derivatives

The development of HSP90 inhibitors based on the purine scaffold was achieved via structure-based design, using the X-ray crystal structures of GM, RD and ADP bound to the N-terminal nucleotide-binding domain of HSP90 [62].

These molecules illustrate the ATP-binding pocket as having a distinguishing Bergerat fold, which is seen only in the GHKL ATPase protein family [63]. This knowledge played an important role and provided the blueprint for the design of synthetic HSP90 inhibitors. The first purine-based inhibitor, which was also the first reported synthetic HSP90 inhibitor, was PU3 (Figure 4). It was rationally designed by Chiosis *et al.* using the co-crystal structures of HSP90 with its ligands (i.e., GM, RD and adenine nucleotides) [64]. Importantly, PU3 was the first synthetic molecule to demonstrate phenotypic properties similar to GM [64]. The essential motif found in PU3, purine-linker-aryl (Figure 4), has been incorporated by numerous research groups in the design and development of potent drug candidates with favorable pharmaceutical properties. These efforts have culminated in the advancement to clinical trials of CNF2024/BIIB021, PU-H71, MPC-3100 and Debio 0932 (CUDC-305) (Figure 4).

3.2.1 CNF2024/BIIB021—Conforma Therapeutics discovered BIIB021 (Figure 4), a compound which distinguishes from the other purine-based compounds by that the aryl moiety that is attached to the N9 position of the purine and not the typical C8 position [65]. This was possible without a loss in activity because the NH_2 group found on the 6-position of the purine scaffold was moved to the 2-position of the purine, and hence this change maintained the optimal six bond distance between the NH_2 and the aryl group needed for potent binding of the pharmacophore (purine-linker-aryl, Figure 4). This HSP90 inhibitor was initially developed by Conforma Therapeutics and later by Biogen Idec, and it was the first synthetic inhibitor to enter clinical evaluation (Table 2).

BIIB021 has been evaluated in Phase I trials in various dosing regimens in patients with CLL and advanced solid tumors [11]. The monotherapy MTD was 800 mg twice weekly. DLTs included syncope and dizziness, and grade 3 or 4 toxicities included fatigue, hyponatremia and hypoglycemia. In patients with CLL, there was one incidence of grade 3 liver toxicity [11]. Response evaluation revealed SD in 11 of 16 patients with solid tumors and 1 patient with CLL had a 39% reduction in the lymph node size.

A Phase II trial was conducted in patients with GIST who had progressed on prior imatinib and sunitinib [66]. Two dosing schedules: 600 mg twice weekly and 400 mg three times a week were evaluated in 12 and 11 patients, respectively. Although the treatment was well tolerated with no evidence of liver toxicity, there were no radiological responses noted by RECIST criteria, and the duration of response was very brief (25 – 138 days). Interestingly, metabolic responses were noted by FDG-PET in 3 of 12 patients on the 600 mg twice weekly cohort and 2 of 9 patients on the 400 mg three times a week cohort [66]. The combination of BIIB021 with trastuzumab was tested in a Phase I trial in patients with

HER2-positive MBC [67]. The MTD was established at 600 mg twice weekly. DLTs included diarrhea and partial seizure that presented as aphasia, and other adverse events included fatigue, nausea, dizziness, headache and rash. Of the 30 patients enrolled, 2 patients had confirmed PR by RECIST and an additional 10 patients had SD. Three patients had metabolic PR (> 25% decline in the SUV_{max}) with FDG-PET and an additional 16 patients had SD as well. Although other Phase II combination studies were planned, the clinical development of this drug has been halted by Biogen Idec due to the company's exit from the oncology field [68].

3.2.2 PU-H71—PU-H71 (Figure 4) is a purine-based compound discovered at Memorial Sloan-Kettering Cancer Center and developed by Samus Therapeutics and NCI [62,69]. A unique feature of this molecule is that it has an endogenous iodine atom (^{127}I), which has been conveniently replaced with the PET radionuclide ^{124}I to result in the imaging agent ^{124}I-PU-H71 [70]. In fact, the PET agent is identical to PU-H71, and its physical half-life of 4.02 days allows serial imaging for monitoring tumor PU-H71 concentrations for multiple days. ^{124}I-PU-H71 has been tested clinically in a Phase 0, first-in-human trial in patients with advanced solid tumors to determine the microdose biodistribution of PU-H71 in patients, including tumor uptake and retention, as well as gather tracer radiation dosimetry data important for clinical development of this promising tumor-imaging agent. Preliminary results have confirmed the feasibility of this approach in detecting PU-H71 in tumors using HSP90-targeted PET [70]. Tracer uptake at the metastatic tumor sites in this study correlated well with baseline CT and/or FDG-PET scans. Accrual to this trial is ongoing (Table 2).

PU-H71 is also being evaluated in twice weekly, 2 weeks on, 1 week off in a 21-day cycle in a Phase I trial of patients with advanced solid tumors and lymphoma (NCT01 393509). This trial also incorporates the ^{124}I-PU-H71 PET as a noninvasive means to determine tumor pharmacokinetics (PK) and intratumoral drug concentration. A mix of ^{124}I-PU-H71 and unlabeled PU-H71 is given during cycle 2 followed by serial PET imaging. Thus far, data from this ongoing study show that ^{124}I-PU-H71 is useful in visualizing PU-H71 uptake, and tumor concentrations as measured by biopsies correlates well with tumor concentrations calculated by ^{124}I-PU-H71 PET imaging [71]. This agent is also being studied at a different dosing schedule at the NCI in patients with advanced solid tumors and low-grade non-Hodgkin's lymphoma patients who have not responded to standard treatment (NCT01581541).

3.2.3 MPC-3100—Myrexis Inc. discovered MPC-3100 (Figure 4) after initiating a drug discovery program aimed at finding HSP90 inhibitors using the purine-scaffold [72]. Despite the large number of analogs synthesized, they retained the 1,3-benzodioxole moiety found in PU-H71 because no other functionalities had more favorable PK profiles. In the end, the extensive SAR carried out on the pendant N-9 piperidine moiety and of the 1,3-benzodioxole ring, complemented by in vitro and PK data, provided MPC-3100 as their best drug candidate [72]. Nonetheless, the presence of bromine instead of iodine rendered MPC-3100 of reduced activity when compared to PU-H71.

MPC-3100 was evaluated in a Phase I trial in 26 patients with recurrent or refractory cancer (Table 2) [73]. Patients received MPC-3100 either daily for 21 days with a week off in a 28-

day cycle (doses: 50,100, 165, 245 or 340 mg/m^2) or daily continuously for 28 days (at 240 or 320 mg every 12 h). The most common adverse events were grade 1 or 2 diarrhea, nausea, vomiting and fatigue. The DLT was supraventricular tachycardia at a dose of 245 mg/m^2. Grade 1 – 3 gastrointestinal and grade 1/2 visual adverse events were observed at total daily doses > 600mg/day; however, these were reversible and resolved following discontinuation of study drug [73].

3.2.4 Debio 0932 (CUDC-305)—Curis developed CUDC-305 (Figure 4) after studying the structure requirements and compound design of the purine scaffold established by others [62,69,74]. This compound is similar to PU-H71; however, the nitrogen at 3-position is replaced by a carbon and the iodine by dimethylamine, and it also appears that replacement of N with C diminishes activity [75].

Debio-0932 is an oral HSP90 inhibitor that has been evaluated in a Phase I trial in patients with advanced solid tumors and lymphoma (Table 2) [76]. It was evaluated either as daily dosing or every other day dosing. The drug was well tolerated at doses up to 1600 mg every 2 days and 1000 mg/day. The most common adverse events were diarrhea, asthenia and decreased appetite with no ocular or cardiotoxicity being reported. One DLT of febrile neutropenia was observed with the every-other-day dosing and two DLTs of diarrhea and asthenia were reported with the daily dosing. Overall, this inhibitor showed promising activity with PR in two patients, one with KRAS-mutant lung cancer and the other with breast cancer. Specifically, of the eight patients with lung cancer, one had PR and four others had SD [76]. The daily dosing cohort is being expanded to enroll an additional 30 patients and a Phase I/II trial of Debio-0932 in combination with standard-of-care in the first- and second-line treatment of NSCLC has been initiated (NCT01714037).

3.3 Other chemotypes

3.3.1 SNX-5422/PF-04929113—Researchers at Serenex discovered SNX-5422 (Figure 5), which is a compound based on a novel dihydroindazolone scaffold. To identify their initial hits, a chemoproteomics-based strategy was used which employed the screening of their chemical library versus a diverse array of possible targets [77]. This screen found compound–protein interactions which involved HSP90. The subsequent synthetic design was complemented by structural information and ultimately resulted in the lead candidate SNX-2112. To improve the oral bioavailability of SNX-2112, a glycine ester moiety was introduced resulting in the prodrug SNX-5422 (Figure 5). SNX-5422 was advanced by Serenex in 2007 to clinical trials and was further developed with Pfizer. The development was discontinued initially due to concerns of ocular toxicity, including irreversible retinal damage seen in animal models and reported in a Phase I study (Table 2) [78]. However, more recently, this drug has now been acquired by Esanex Inc., a privately owned Lilly-Ventures biotech company and the program for SNX-5422 has been restarted [79]. It is currently being evaluated as a Phase I/II trial in patients with advanced HER2-positive cancers, including metastatic NSCLC, urothelial cancers, esophagogastric and breast cancers (NCT01848756). Patients will receive 100 mg/m^2 every other day orally for 3 weeks in a 28-day cycle. Ophthalmological examinations, including visual acuity, visual field, ophthalmoscopy, dark adaptation are planned at screening, at end of cycle 1, at end of cycle

3 and then every 3 months thereafter. Another Phase I trial is evaluating the combination of SNX-5422 with erlotinib in patients with NSCLC that are resistant to EGFR TKIs (NCT01851096).

3.3.2 HSP990 (NVP-HSP990)—Novartis discovered HSP990 (Figure 5) via high-throughput screening (HTS) and subsequent follow-up structure-based lead optimization [80]. It was consequently advanced to clinical trials as an oral agent. HSP990 was evaluated in a Phase I trial in advanced solid tumors in weekly (n = 53; 2.5 – 60 mg) and twice weekly (n = 11; 25 mg) dosing schedules (Table 2). The most common side effects reported were diarrhea, increased liver enzymes, anemia and cholestasis. DLTs included grade 3 diarrhea, grade 3 QTc prolongation, grade 4 alanine aminotransferase/aspartate aminotransferase, grade 3 tremors and grade 2 neurologic toxicity, including ataxia, confusion and visual hallucination. Dose escalation was limited by neurologic toxicity and the MTD was established at 50 mg weekly [81]. Nonetheless, the further advancement of NVP-HSP990 has been stopped due to its failure to achieve clinically meaningful responses at the MTD [82].

3.3.3 XL888—Exelixis discovered XL888 (Figure 5), a novel tropane-derived inhibitor of HSP90. First, the initial hits were found via an HTS campaign of 4.1 million compounds using an in-house chemical library [83]. The challenges encountered during the structure-based approach used for their optimization efforts pertained to reducing the high molecular weights (> 650 amu) and polar surface areas (> 135 Å^2), which were outside the ideal range for orally bioavailable drugs [83].

The first Phase I safety study of XL888 was terminated by the sponsor Exelixis [11] with no publicly disclosed specific reason (Table 2). However, a Phase I study of XL888 in combination with vemurafenib in patients with unresectable BRAF-mutated stage III/IV melanoma is underway (NCT01 657591). This study is supported by preclinical data which demonstrated that signaling proteins involved in intrinsic and acquired resistance to BRAF inhibitors are clients of HSP90 and inhibiting HSP90 restores sensitivity to vemurafenib [84].

4. Biomarkers and diagnostics – unmet need in HSP90 therapy

As with the development of any novel targeted therapy, identification of biomarkers and companion diagnostic assays are crucial not only to ascertain target inhibition but also to better identify patients who are most likely to respond or be resistant to therapy.

With HSP90 inhibitors, target inhibition in clinic has been evaluated by either measuring the levels of client oncoproteins in surrogate tissues pre- and post-therapy (RAF-1, AKT, C-KIT, CDK4) [11] or by evaluating the upregulation of other co-chaperones such as HSP70 or HSP27 or other factors involved in the heat shock response using PBMCs [85]. Effective target inhibition certainly helped optimize drug dosing and scheduling in many of these trials. However, although HSP70 upregulation in the serum or PBMCs has served as a pharmacodynamic (PD) readout of HSP90 inhibition, it has not predicted tumor response [11,86-88]. This comes perhaps as no surprise since HSP90 inhibitors are known to be

preferentially retained in tumor cells and not normal cells [89-91], and also there seems to be a fundamental difference between the HSP90 species in normal and tumor cells [90,91]. Perhaps for the same reasons, the clinical utility of other serum biomarkers such as soluble insulin growth factor binding protein and HER2 extracellular domain also remains to be validated [92].

Few trials have undertaken pre- and post-treatment tumor biopsies from patients on HSP90 inhibitor therapy for PD analysis. Hence data are limited and inconsistent due to small sample sizes, low sensitivity of traditional immunohistochemical staining methods employed in these trials and tumor heterogeneity [11]. One potential way to overcome these barriers is to utilize noninvasive molecular imaging that not only allows visualization of the target but also permits assessment of whole body tumor burden and heterogeneity, evaluates tumor response and characterizes the PK and PD changes in the tumors. ^{18}F-FDG PET has been utilized in highly glycolytic tumors such as GIST as a PD correlate of antitumor activity [11,93]. Metabolic PR and SD have also been reported using FDG-PET imaging [38,66]. PET with either radiolabeled antibodies against other specific targets such as HER2, VEGF receptor, androgen receptor, and the like [94-96] or with radiolabeling the therapeutic drug itself have also been explored [70,71].

Last, although HSP90 tumor expression might seem to be an appropriate strategy to best select patients who might respond to therapy, available data do not suggest a predictive role for this marker. To date, the only reliable way to predict response to HSP90 inhibition has been to identify those patients whose cancer is driven by a particular oncogene that is a very sensitive HSP90 client (e.g., HER2 in breast cancer or chimeric ALK in NSCLC) [97]. Nonetheless, clinical success in other tumor types that harbor HSP90 onco-clients has been limited so far and the failure of HSP90 inhibitors in these tumors remains poorly understood.

5. Conclusion

The past few years have shown intense activity in the discovery and optimization of synthetic HSP90 inhibitors. Most of these second-generation HSP90 inhibitors that have progressed to clinical development either incorporate the resorcinol moiety of the natural product RD or mimic the purine-scaffold implemented in the design of the first synthetic HSP90 inhibitor, PU3. Studies with these new agents have brought evidence that the liver toxicity associated with the first-generation GM derivatives was pharmacophore-(i.e., benzoquinone) and not target (i.e., HSP90)-mediated. In fact a number of the new agents show manageable and reversible toxicity profiles (predominantly diarrhea and fatigue) and hold promise for further advancement in the clinic. Diarrhea seems to be an on-target effect [54] and lasts for 24 – 48 h and can be easily controlled with anti-diarrheal medications prophylactically. Ocular toxicity has also emerged as a concerning side effect with a few but not all second-generation inhibitors [45,78], but whether it is a class effect/on or off target effect, remains to be further elucidated.

The clinical development of the second-generation inhibitors continued in tumor subtypes where first-generation inhibitors demonstrated response. As such, the clinical activity observed for all HSP90 chemotypes in HER2-positive breast tumors and NSCLC with ALK

translocations provides convincing data for the addiction of these tumors to HSP90. Also validated is the ability of these HSP90 agents to provide efficacy enhancement in well-chosen combination strategies. Noteworthy is resensitization of tumors to the effect of other targeted therapies, such as the case for trastuzumab in HER2-positive MBC progressing on trastuzumab, bortezomib in MM refractory to bortezomib and erlotinib in mEGFR NSCLC progressing on EGFR inhibitors. Of value is also their ability to provide enhanced cytotoxic effects with chemotherapy such as taxanes in refractory NSCLC patients. In fact, combination strategies might enhance antitumor activity even in those tumors where despite strong preclinical rationale, single agent HSP90 inhibitor has failed to demonstrate efficacy, perhaps due to the lack of a sensitive client protein or lack of sustained inhibition due to ineffective dose and scheduling or due to tumor evolution and drug resistance [57].

6. Expert opinion

But more importantly, the clinical studies so far also highlight the limitations of the current HSP90 field. They cement the notion that in unselected patient populations, HSP90 studies have limited probability of success, underscoring the need to augment efforts toward a better understanding of HSP90-addicted tumor types. However, finding out the particularities of a tumor that renders it addicted to HSP90 remains elusive. So far, identification of sensitive tumors in clinic appears to be trial-and-error-based. The activity seen in HER2-positive breast cancer and chimeric ALK NSCLC has a strong foundation provided by preclinical studies. Nonetheless, these preclinical studies predicted many other tumor types to harbor HSP90-addicted onco-proteins, and their clinical benefit has been limited thus far.

Further, not all tumors that express the 'highly sensitive client' are responsive to HSP90 therapy (e.g., not all HER2-positive tumors respond equally well to an HSP90 inhibitor). This is likely a consequence of the complex molecular networks regulated by HSP90 in a tumor-by-tumor manner; these may interconnect distinctly and as determined by the tumor's specific genetic background. As such, inhibition of HSP90 in tumors expressing a 'sensitive HSP90 client' may result in distinct outcomes.

Studies also indicate that the sensitivity of a tumor to pharmacologic inhibition may not be driven by the expression of total HSP90 in the tumor cell, but rather by the presence of actively chaperoning HSP90 species [91,98,99]. These species, whose presence are regulated by co-chaperone recruitment and likely also by post-translational modifications, maintain multiple oncogenic pathways dependent on HSP90 activity. In fact, recent proteomic and biochemical studies support the notion that tumor selection in the HSP90 field should be based on more sophisticated functional proteomic insights that measure not only the network of tumor-driving HSP90 clientele but also the abundance and the biochemical nature of the HSP90 fraction available for inhibitor binding [91,98,99].

Ideally, clinical trials of targeted cancer therapy also require knowledge of whether effective tumor concentrations are achieved and whether the target is appropriately modulated at the site of action, the tumor cell. Knowledge on both such topics is, however, sorely lacking in the HSP90 field. Although preclinical studies indicate the antitumor activity of HSP90 inhibitors to be largely mediated by their ability to engage the target over the time of

treatment, as described above, no validated assays are available to answer this question for HSP90 inhibitors in clinic. Therefore it is difficult to conclude that HSP90 inhibition fails to provide benefit in a particular disease setting, when we cannot answer the obvious: whether the HSP90 inhibitor in question given at a dose and on a schedule actually optimally engaged the target in that setting.

Selection of a proper dose and schedule that are needed to achieve antitumor efficacy is also poorly understood in HSP90 therapy. Plasma PK generally provides data relevant to the design of therapeutic dosing, with the plasma area under the curve often used as a metric of systemic drug exposure. However, for HSP90 inhibitors, the concentration and duration of retention of drug in tumor tissues, and not in blood, determine their antitumor effect. Animal and clinical data have shown that blood concentrations do not predict nor mimic tumor concentrations of HSP90 inhibitors [11,69,70]. Nevertheless, only one agent, PU-H71, currently incorporates in its clinical development an assay that provides real-time measurement of the inhibitor concentrations in individual tumors [70].

To conclude, the HSP90 multifaceted chaperone, although not fully understood, remains an enticing target for cancer therapy. In retrospect, the rush for better HSP90 drugs has left unanswered much on patient selection and proper target engagement and this is perhaps slowing down the advancement of these inhibitors in the clinic. Thus, the field should focus more attention on these topics if the fulfillment of the target's promise is to be achieved soon. As such, we believe the successful transition of the current HSP90 inhibitors to approval will be limited to those that not only have a favorable therapeutic index but also incorporate companion diagnostics to inform on a more judicious implementation and clinical use.

Acknowledgments

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the fact that they bind to the same pocket. It shows the importance of HSP90's post-translational modifications on the anticancer activity of small-molecule inhibitors. [PubMed: 23867252]

Article highlights

- Several second-generation heat shock protein 90 (HSP90) inhibitors have entered the clinical oncology arena. These are based on a restricted number of favorable pharmacophores.

- The dose-dependent hepatotoxicity noted with the first-generation geldanamycin is not observed in the second-generation HSP90 inhibitors, indicating it to be chemical- rather than target-related in nature. The second-generation HSP90 inhibitors, except a select few, are in general well tolerated with mostly grade 1/2 toxicities.

- The clinical development path of second-generation inhibitors follows in the footsteps of path-finding first-generation agents and has focused on a small select subset of tumors.

- Combining HSP90 inhibitors with other potent anticancer therapies holds promise not only due to synergistic antitumor activity but also due to the potential of prolonging or preventing the development of drug resistance.

- A clear understanding on HSP90 target engagement in the several ongoing studies is still sorely lacking. Dose and schedule appears to be mostly toxicity-rather than target saturation-driven.

- Understanding of tumors that are more likely to draw a benefit from HSP90 therapy remains limited. The field should move from the 'HSP90 client protein selection criteria' toward a more sophisticated biomarker based on a functional proteomic approach.

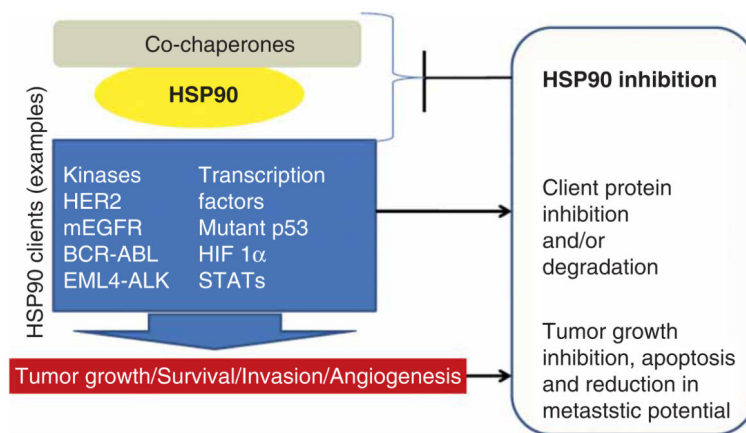This box summarizes key points contained in the article.

Figure 1. The chaperone HSP90 has received significant attention in cancer because the many client proteins it regulates are involved in numerous processes that are dysregulated in cancer
Inhibition of HSP90 function by small molecules results in client protein inactivation leading to tumor growth inhibition, apoptosis and reduction of its metastatic potential. HSP90: Heat shock protein 90.
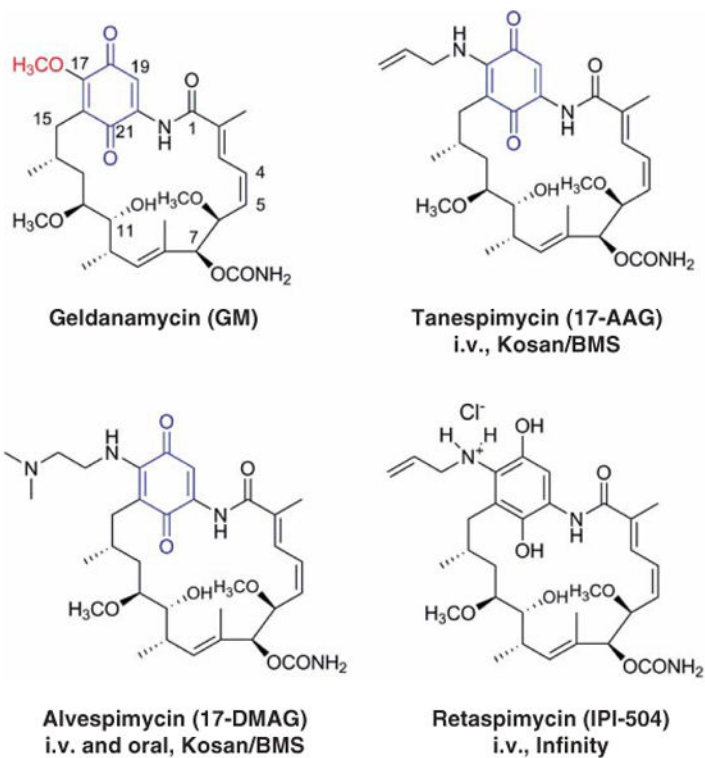
Figure 2. Chemical structures of ansamycin-based HSP90 inhibitors: GM and its derivatives 17-AAG, 17-DMAG and IPI-504 (benzoquinone moiety shown in blue and the methoxy group at C17 is shown in red)

17-DMAG: 17-desmethoxy-17-N,N-dimethylaminoethylaminogeldanamycin; GM:
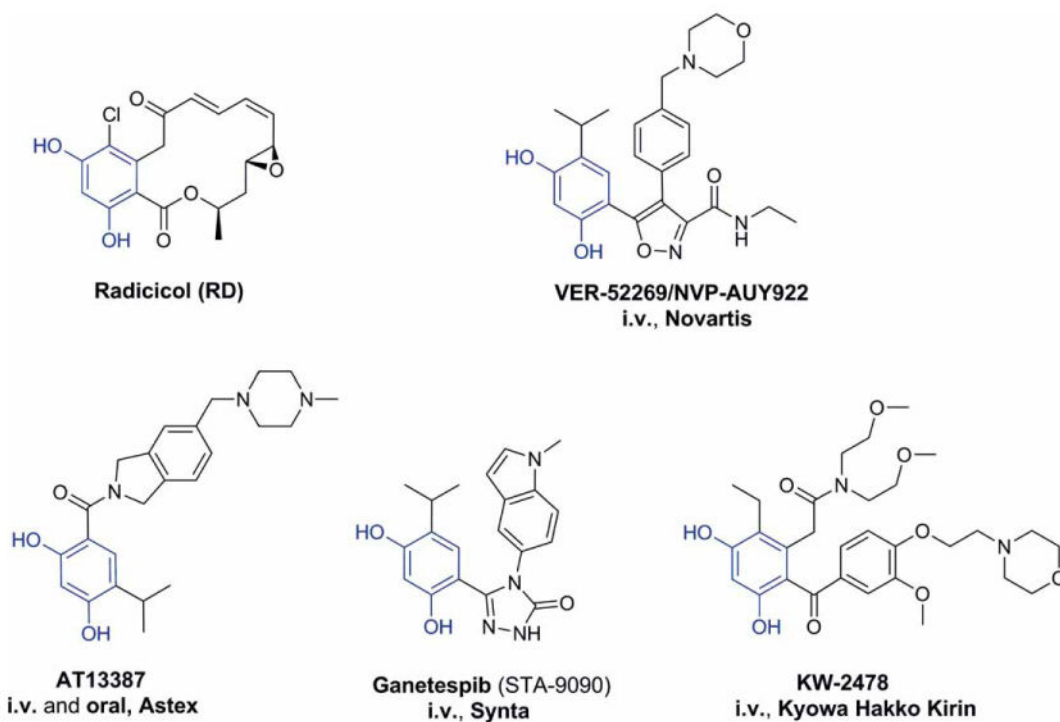
Geldanamycin; i.v.: Intravenous.

Figure 3. Chemical structures of resorcinol-based HSP90 inhibitors: RD and the resorcinol incorporating NVP-AUY922, AT13387, Ganetespib and KW2478 (in blue is shown the resorcinol moiety)
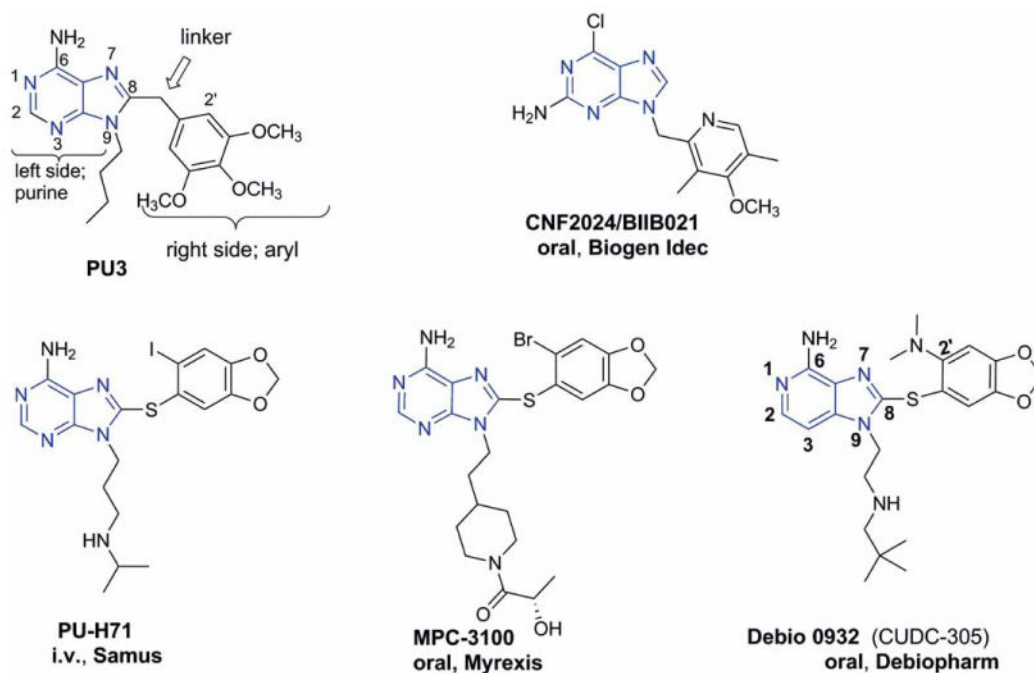HSP90: Heat shock protein 90; i.v.: Intravenous; RD: Radicicol.

Figure 4. Purine and purine-like inhibitors of HSP90: PU3, PU-H71, MPC-3100 and BIIB021 (in blue is shown the purine and purine-like core) are presented

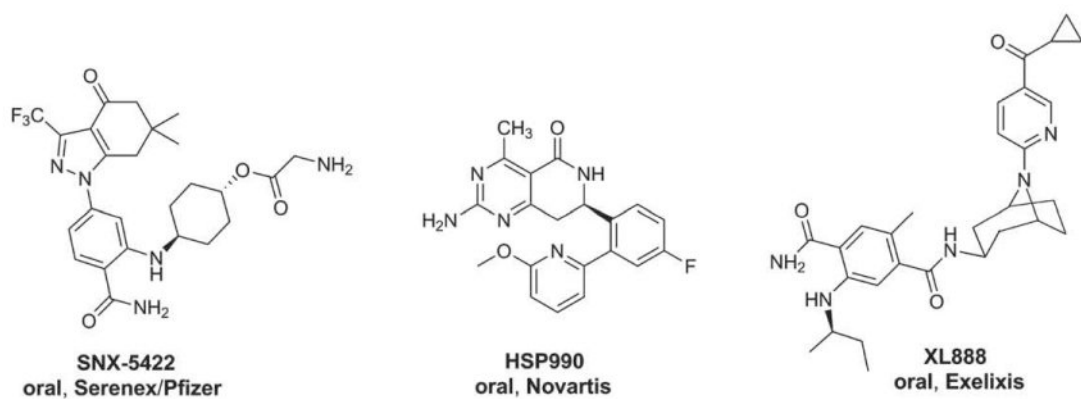HSP90: Heat shock protein 90; i.v.: Intravenous.

Figure 5. Other HSP90 inhibitors: SNX-5422, HSP990 and XL888 are presented
HSP90: Heat shock protein 90.

Table 1

Summary of clinical data – first-generation HSP90 inhibitors

HSP90 inhibitor	Clinical stage	Combination	Disease	Response	Ref.
Tanespimycin	Phase I	No	Advanced solid tumors	SD in metastatic melanoma	[8]
	Phase II	No	Prostate, papillary and clear cell RCC, melanoma V600E harboring BRAF mutation	SD in melanoma	[13,14]
	Phase I/II	Bortezomib	Naïve, pretreated, refractory MM	Objective responses	[17]
	Phase II	Trastuzumab	HER2 BC progressing on trastuzumab	Objective responses	[16]
Alvespimycin	Phase I	No	Solid tumors, AML	Objective responses in CRPC, AML and metastatic melanoma and SD in peritoneal mesothelioma, head and neck cancer, renal cancer, chondrosarcoma	[23,24]
	Phase I	Trastuzumab	HER2 BC progressing on trastuzumab	Objective responses and SD	[25]
Retaspimycin	Phase I	No	MM	SD	[27]
	Phase II	No	CRPC	No response	[28]
	Phase II	Trastuzumab	HER2 BC progressing on trastuzumab	SD	[34]
	Phase I/II	No	NSCLC progressing on EGFR inhibitor	Objective responses in patients with ALK rearrangement, EGFR and KRAS wild-type and SD in patients ALK rearrangement	[29,32]
	Phase Ib	Docetaxel	NSCLC progressing on chemotherapy	Objective responses in patients with squamous histology, KRAS wild type and smoking history	[34]
	Phase III	No	GIST progressing after TKI	No response	[33]

ALK: Anaplastic lymphoma kinase; AML: Acute myeloid leukemia; BC: Breast cancer; CRPC: Castrate-resistant prostate cancer; GIST: Gastrointestinal stromal tumor; KRAS: Kirsten rat sarcoma viral oncogene homolog; MM: Multiple myeloma; NSCLC: Non-small cell lung cancer; RCC: Renal cell carcinoma; SD: Stable disease; TKI: Tyrosine kinase inhibitor.

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Table 2

Summary of clinical data – second-generation HSP90 inhibitors

HSP90 inhibitor	Clinical stage	Combination	Disease	Response	Ref.
NVP-AUY922	Phase I	No	Advanced solid tumors	SD	[38]
	Phase II	No	NSCLC progressing on chemotherapy	Objective responses in patients with ALK rearrangement, EGFR/KRAS and ALK wild-type and mEGFR	[40]
	Phase II	Erlotinib	mEGFR NSCLC progressing on EGFR inhibitors	Objective responses in EGFRT790M and SD	[41]
	Phase Ib/II	Trastuzumab	HER2+ MBC refractory to trastuzumab	Objective responses	[42]
	Phase II	No	HER2+ or ER+ BC	Objective responses	[39]
	Phase I/II	Bortezomib	MM	Ongoing	NCT00708292
	Phase I/II	PI3K inhibitor	Advanced or metastatic gastric cancer	Ongoing	NCT01613950
	Phase I/II	ALK inhibitor	ALK-rearranged NSCLC	Ongoing	NCT01772797
	Phase I/II	Docetaxel	NSCLC with EGFR mutations	Ongoing	NCT01646125
	Phase I/II	Cetuximab	KRAS-mutated colorectal cancer	Ongoing	NCT01294826
AT-13387 Ganetespib	Phase I	No	Refractory solid tumors	Objective response in GIST and SD in follicular cell thyroid cancer, metastatic uveal melanoma and GIST	[45]
	Phase I/II	No	CRPC progressing on abiraterone	Ongoing	NCT01685268
		Abiraterone	CRPC progressing on abiraterone	Ongoing	NCT01685268
	Phase I/II	No	NSCLC	Ongoing	NCT01712217
		Crizotinib	NSCLC	Ongoing	NCT01712217
	Phase I/II	No	Unresectable and/or metastatic GIST progressing on TKI	Ongoing	NCT01294202
		Imatinib	Unresectable and/or metastatic GIST progressing on TKI	Ongoing	NCT01294202
	Phase I	No	Unselected solid tumors	SD and 1 PR in colorectal cancer, PR in metastatic melanoma and SD in NSCLC	[49,50]
	Phase I/II	No	Hematological malignancies	No formal responses	[51,52]
	Phase II	No	NSCLC genotypic characterization of tumors for the presence of EGFR or KRAS mutation or the absence of these mutations	Responses in crizotinib-naïve patients with the EML4-ALK rearrangement	[53]
	Phase II	No	NSCLC crizotinib-naïve patients with the EML4-ALK rearrangement	Ongoing	NCT01562015
	Phase II	Docetaxel	NSCLC progressing on chemotherapy; AML; RCC	Objective responses	[58]
	Phase III	Docetaxel	NSCLC progressing on chemotherapy	Ongoing	NCT01798485

HSP90 inhibitor	Clinical stage	Combination	Disease	Response	Ref.
	Phase II	No	Unselected MBC	Objective not met but activity noted in trastuzumab refractory HER2+ and TNBC	[54]
	Phase II	No	GIST progressing on TKI	SD	[55]
	Phase I	Paclitaxel and trastuzumab	HER2+ trastuzumab refractory MBC	Ongoing	NCT02060253
KW-2478	Phase I	No	Lymphomas and leukemias	Completed	NCT00457782
BIIB021	Phase I/II	Bortezomib	Relapsed and refractory MM	Objective responses	[61]
	Phase I	No	CLL and advanced solid tumors	SD	[11]
	Phase II	No	GIST progressing on TKI	Metabolic responses as noted on FDG-PET	[66]
	Phase I	Trastuzumab	HER2+ BC	Objective responses with metabolic responses on FDG-PET as well	[67]
PU-H71	Phase I	No	Advanced solid tumors and lymphoma	Ongoing	NCT01393509
MPC-3100	Phase I	No	Recurrent or refractory cancer	SD	[73]
CUDC-305	Phase I	No	Advanced solid tumors	SD and PR in 2 patients (mKRAS NSCLC and BC)	[76]
	Phase I/II	Chemotherapy	NSCLC progressing on chemotherapy	ongoing	NCT01714037
SNX-5422	Phase I	No	Advanced solid tumors	Terminated due to ocular toxicity	[78]
	Phase I/II	No	Several HER2+ tumor types (NSLC, esophagogastric, breast, urothelial)	Ongoing	NCT01848756
NVP-HSP990	Phase I	No	Advanced solid tumors	No responses (drug development halted)	[81,82]
XL888	Phase I	No	Advanced solid tumors	Terminated	[11]
	Phase I	Vemurafenib	Unresectable mBRAF melanoma	Ongoing	NCT01657591

ALK: Anaplastic lymphoma kinase; AML: Acute myeloid leukemia; BC: Breast cancer; CLL: Chronic lymphocytic leukemia; CRPC: Castrate-resistant prostate cancer; EML4-ALK: Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; FDG: Fluorodeoxyglucose; GIST: Gastrointestinal stromal tumors; NSCLC: non-small cell lung cancer; MBC: Metastatic breast cancer; MM: Multiple myeloma; PET: Positron emission tomography; PI3K: Phosphatidylinositol 3-kinase; PR: Partial response; SD: Stable disease; TKI: Tyrosine kinase inhibitor; TNBC: Triple-negative breast cancer.