HSP90 Inhibitors in Lung Cancer: Promise Still Unfulfilled

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Abstract: Despite recent advances in the treatment of lung cancer, non–small cell lung cancer (NSCLC) remains the leading cause of cancer-related deaths in the United States and worldwide, with a 5-year survival rate of less than 17%. Analysis of the molecular drivers of NSCLC led to the recognition that NSCLC is a collection of distinct, molecularly driven neoplasms. Several subsets of NSCLC with clinical relevance to targeted therapies are defined based on alterations in *EGFR*, *ALK*, and other key oncogenic drivers. However, for many oncogenic drivers—such as mutant *KRAS*—targeted therapies are lacking. Heat shock protein 90 (HSP90) is an adenosine triphosphate (ATP)–dependent molecular chaperone that is critically required for the stability of its clientele, many of which are driver oncoproteins. Therefore, HSP90 inhibitors could prove to be an effective and alternate approach to treat patients with NSCLC that has a specific molecular background or that has acquired resistance to other drugs. Over the last 2 decades, several HSP90 inhibitors have been developed that produced promising preclinical and clinical results. The quest is far from over, however. In this review, we discuss the development and the preclinical and clinical profiles of some of the HSP90 inhibitors that may help to improve the targeted treatment of NSCLC.

Keywords
ALK, EGFR, HSP90 inhibitors, lung cancer, oncogenic drivers

Background

Non–small cell lung cancer (NSCLC) is the leading cause of cancer-related death in the United States and worldwide, causing approximately 160,340 deaths annually in the United States alone.[1] Recent advances in the treatment of NSCLC have come from the recognition that NSCLC is not a single disease entity, but rather a collection of distinct, molecularly driven neoplasms. A personalized approach based on the molecular alterations present in each patient's disease has led to the US Food Administration (FDA) approval of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors

(TKIs) in *EGFR*-mutant lung adenocarcinomas, and of crizotinib (Xalkori, Pfizer) and ceritinib (Zykadia, Novartis) in *ALK*-rearranged lung adenocarcinomas.[2] Despite the impressive response rates and improved survival achieved with these targeted agents, acquired resistance is inevitable, and targeted therapies for the majority of oncogenic drivers in NSCLC, including mutant *KRAS*, are lacking. Clearly, there is a need for novel therapeutic strategies for these patients. As described below, the heat shock protein 90 (HSP90) inhibitors may be an effective therapy for them.

Most HSPs are adenosine triphosphate (ATP)–dependent chaperones that protect a variety of "client" proteins from degradation, oxidative stress, hypoxia, and increases in temperature. HSP90, which is evolutionarily conserved and ubiquitously expressed, has been studied extensively because of its critical role in the folding, stabilization, activation, function, aggregation, and proteolytic degradation of several client oncoproteins in multiple tumor types.[3] Amplified human epidermal growth factor receptor 2 (HER2), B-Raf proto-oncogene serine/threonine kinase (BRAF), c-RAF, protein kinase B (AKT), met proto-oncogene product (MET), mitogen-activated protein kinase kinase (MEK), mutant *EGFR*, B-cell lymphoma 2 (BCL2), the *EML4-ALK* translocation product, androgen and estrogen receptors, and several cell cycle proteins are some of the HSP90 clients that are validated oncogenic drivers (Figure 1).[3,4] Hence, HSP90 represents an appealing molecular target for novel and exciting anticancer therapies under development. Comparatively, tumor cells are more HSP90-dependent than normal cells for proliferation and survival because the oncoproteins in cancer cells are often misfolded and require augmented HSP90 activity for correction.[5] Therefore, several HSP90 inhibitors have been developed for the treatment of cancer and have shown promise both preclinically and clinically in the treatment of NSCLC.

Structural Development of HSP90 Inhibitors

The development of HSP90 inhibitors began with 2 natural products: geldanamycin, a benzoquinone ansamycin (Figure 2I) and radicicol, a resorcyclic acid lactone (Figure 2II). These are commonly referred to as first-generation HSP90 inhibitors. Geldanamycin, derived from *Streptomyces hygroscopicus*, was shown to bind to the N-terminal nucleotide pocket of HSP90, leading to inhibition of the ATPase activity of HSP90.[4] Despite promising in vitro and in vivo efficacy, geldanamycin failed to reach any clinical trial owing to its poor solubility, chemical and metabolic instability, and hepatotoxicity.[4] On the other hand, radicicol, derived from *Monosporium bonorden*, was demonstrated to target the core of the ATP-binding pocket of HSP90, resulting in potent inhibition in vitro.[4] Owing to highly reactive structural difficulties, however, it failed to produce the same potency in vivo.[6]

To overcome these problems, geldanamycin derivatives were developed. Tanespimycin (17-N-allylamino-17-demethoxygeldanamycin [17-AAG]; Figure 2Ia) was the first geldanamycin derivative to be evaluated in the clinic.[7] This was followed by alvespimycin (17-dimethylaminoethylamino-17-demethoxygeldanamycin [17-DMAG]; Figure 2Ib). Although clinical activity was observed with these compounds, development was discontinued because of their unfavorable toxicity profiles.[3]

The next geldanamycin derivative to be developed was retaspimycin hydrochloride, or IPI-504 (17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride; Figure 2Ic). A reduced form of 17-AAG, it showed greater promise than the other first-generation HSP90 inhibitors owing to its water solubility and decreased hepatotoxicity.[3,4] The hydroquinone ring in IPI-504 was a more potent inhibitor of HSP90. Another geldanamycin analogue, a nonquinone, was WK88-1 (Figure 2Id); it also has fewer side effects and improved HSP90 binding properties.[8]

To develop a safer and more selective approach to target HSP90, a series of significant modifications of the parent compounds were made. The second-generation inhibitors either carry the resorcinol moiety of radicicol (Figure 2II) or are purine scaffold–based (Figure 2III). They also lack hepatotoxicity because of elimination of the benzoquinone moiety. NVP-AUY922 (also called luminespib or VER-52269; Figure 2IIa), ganetespib (STA-9090; Figure 2IIb), and AT13387 (onalespib; Figure 2IIc) are important resorcinol derivatives. NVP-AUY922 is a resorcinol derivative with a structure based on the 4,5-diarylisoxazole scaffold.[9] Unlike the geldanamycin analogues, it offers highest binding affinity for the N-terminal nucleotide-binding site of HSP90.[10] Ganetespib ([5-[2,4-dihydroxy-5-(1-methyl) phenyl]-4-(1-methyl-1H-indol-5-yl)-2,4-dihydro-[1,2,4] triazole-3-one]) is a small-molecule HSP90 inhibitor that contains a triazole, which contributes to its favorable pharmacologic and safety characteristics in comparison with all the first-generation inhibitors. Ganetespib is significantly smaller than the prototypical first-generation compound and most of the second-generation compounds (Figure 2).[11] Finally, AT13387 is a high-affinity and long-acting HSP90 inhibitor that binds within the N-terminal ATPase catalytic site of HSP90 overlapping the ATP binding site.[12]

Several additional HSP90 inhibitors are currently in development or in phase 1 studies, including the purine scaffold agents PU-H71 (Figure 2IIIa) and Debio 0932 (CUDC-305; Figure 2IIIb), the unique dihydroindazolone scaffold–based small molecule SNX-5422 (Figure 2IV), and DS-2248, which has an undisclosed structure (Figure 2V).

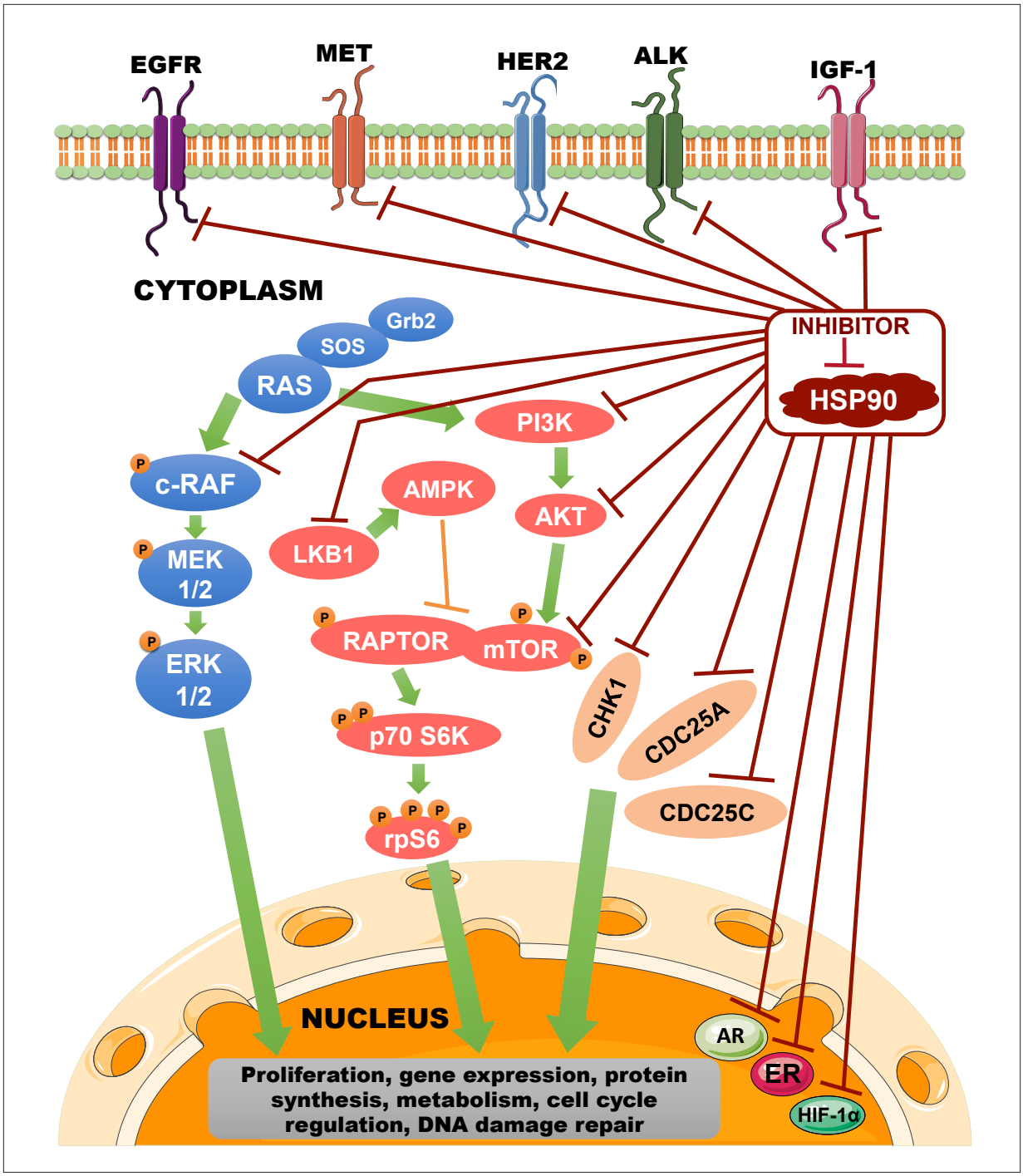

Figure 1. Targeted inhibition of HSP90 leading to the suppression or abrogation of several essential cell survival mechanisms. The use of HSP90 inhibitors leads to the degradation of HSP90 chaperone, which is involved in the processing and maturation of several client oncoproteins. This figure demonstrates some of the key direct and indirect target proteins critical for tumorigenesis that depend on HSP90 activity.

AKT, protein kinase B; ALK, anaplastic lymphoma kinase; AMPK, adenosine monophosphate–activated protein kinase; AR, androgen receptor; CDC25A, cell division cycle 25A; CDC25C, cell division cycle 25C; CHK1, checkpoint kinase 1; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERK, extracellular signal–regulated kinase; Grb2, growth factor receptor–bound protein 2; HER2, human epidermal growth factor receptor 2; HSP90, heat shock protein 90; HIF-1α, hypoxia-inducible factor 1-alpha; IGF-1, insulin-like growth factor 1; LKB1, serine/threonine liver kinase B1; MET, met proto-oncogene product; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; p70 S6K, p70 S6 kinase; PI3K, phosphoinositide 3-kinase; RAF, rapidly accelerated fibrosarcoma; RAPTOR, regulatory-associated protein of mTOR; RAS, rat sarcoma; rpS6, ribosomal protein S6; SOS, salt overly sensitive.

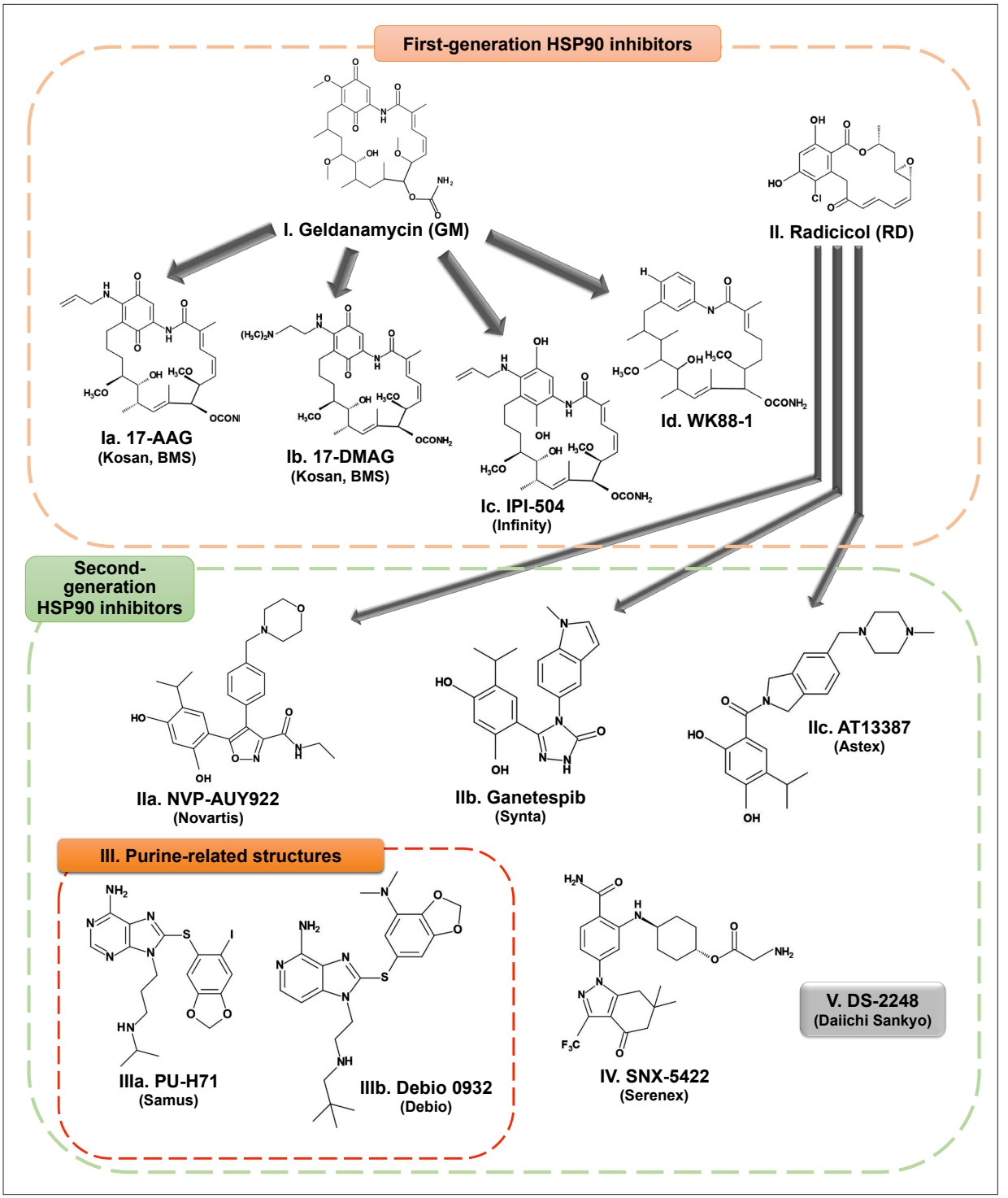

Figure 2. Structural development of HSP90 inhibitors. Geldanamycin (I) and radicicol (II) are the 2 natural compounds considered to be the first HSP90 inhibitors. 17-AAG (Ia), 17-DMAG (Ib), IPI-504 (Ic), and WK88-1 (Id) are the 4 important geldanamycin derivatives with potential antitumor activity in non–small cell lung cancer cells in vivo and in vitro. The first group of second-generation HSP90 inhibitors were generated by chemically modifying radicicol and include agents such as NVP-AUY922 (IIa), ganetespib (IIb), and AT13387 (IIc). PU-H71 (IIIa) and Debio 0932 (IIIb) are 2 of the purine scaffold–based second-generation HSP90 inhibitors with promise. SNX-5422 (IV) is a unique pyrazole-containing scaffold that is water-soluble and orally available. DS-2248 (V) is in clinical trials, but the structure of the drug is yet to be disclosed. BMS, Bristol-Myers Squibb.

Preclinical Studies on Therapeutic Targets of HSP90 Inhibitors in Lung Cancer

Preclinical studies have demonstrated that HSP90 activity significantly increases in cancer cells[5] and that the continued activity of HSP90 is required for oncogene-driven tumorigenesis because inhibition of HSP90 leads to growth inhibition and apoptosis. In this section, we discuss the important oncogenic targets (Figure 1) of HSP90 inhibitors.

EGFR-Mutant Non–Small Cell Lung Cancer

EGFR mutations are found in almost 20% of advanced lung adenocarcinomas[13] and can be targeted by EGFR TKIs. Unfortunately, within 9 to 12 months of treatment initiation, resistance to these drugs develops.[14] Because mutant *EGFR* is a client protein of HSP90, one strategy to overcome such acquired resistance is to target the mutant-*EGFR* protein with HSP90 inhibitors.[15] HSP90 inhibition in lung cancer harboring mutant *EGFR* was first demonstrated with geldanamycin and its derivatives 17-AAG, 17-DMAG, and IPI-504 in both cell line and animal models.[16-18] Efficacy was observed in *EGFR*-mutant cell lines and animal models with acquired resistance to EGFR TKIs mediated via a secondary *EGFR* mutation, T790M.[18-21] In addition, the resorcinol derivatives NVP-AUY922 and ganetespib were effective against *EGFR*-mutant NSCLC.[22,23] AT13387 was able to suppress EGFR signaling for a sustained period both in vitro and in vivo.[24] This is in contrast to ganetespib, where inhibition of EGFR was not sustained in vivo.[23] The prolonged pharmacodynamic action of AT13387 makes it a deserving candidate for further investigation in several ongoing phase 1 and 2 clinical trials.

ALK-Positive Non–Small Cell Lung Cancer

A total of 2% to 5% of patients with NSCLC carry rearrangements in anaplastic lymphoma kinase (ALK), frequently with echinoderm microtubule-associated protein-like 4 (EML4).[25,26] Soda and colleagues first identified the *EML4-ALK* rearrangement as an oncogene and showed that the EML4-ALK fusion protein possesses strong transforming activity.[26] Several ALK inhibitors (eg, crizotinib, ceritinib, and alectinib [Alecensa, Genentech]) have shown significant efficacy in both preclinical and clinical studies, but acquired resistance is unavoidable.[27] Because ALK fusion proteins are clients of HSP90,[3] HSP90 inhibition seemed a rational therapeutic approach to pursue, especially in patients with acquired resistance to ALK TKIs. In addition to strong growth-inhibitory effects in NSCLC with EML4-ALK expression, 17-AAG was able to deplete expression of EML4-ALK and downstream effectors, including phospho-AKT, phospho-ERK1/2, and phospho-S6,[28] and was able to inhibit cell growth in crizotinib-resistant NSCLC.[27] In vivo treatment of *EML4-ALK*–driven xenografts with 17-DMAG[28] or IPI-504[29] resulted in significant tumor regression. Moreover, IPI-504 induced EML4-ALK degradation, triggering rapid depletion of phospho-ERK, phospho-STAT3, and phospho-AKT, followed by growth arrest and apoptosis.[29] Second-generation HSP90 inhibitors also have shown activity against *ALK*-positive cancers. Ganetespib alone or in combination with an ALK inhibitor other than crizotinib was active in treating *ALK*-positive NSCLC cells that were crizotinib-naive or that had acquired resistance to crizotinib, including cells with secondary *ALK* mutations.[30] NVP-AUY922 also showed potential against *ALK*-driven NSCLC.[9] Finally, AT13387 showed strong efficacy against *ALK*-positive disease in vitro and in vivo[31] and is being evaluated in the clinic as a treatment for *ALK*-positive disease.

KRAS-Mutant Non–Small Cell Lung Cancer

KRAS is mutated in 25% of adenocarcinomas of the lung, and no effective targeted therapies exist for these patients.[32] Many of the KRAS downstream mediators require HSP90 chaperonage. Hence, HSP90 inhibition could be a promising therapeutic intervention for *KRAS*-mutant NSCLC. Although KRAS is not a client protein, *KRAS*-mutant NSCLC cell lines were sensitive to 17-AAG.[33] 17-AAG decreased the expression of several KRAS downstream mediators, including c-RAF and AKT.[33] In addition, in vivo activity against *KRAS*-mutant NSCLC was observed with 17-DMAG, and the same study concluded that *KRAS* mutations could predict HSP90 inhibition in vivo.[33] IPI-504 was also effective against *KRAS*-driven tumors.[34] Furthermore, IPI-504 in combination with the mammalian target of rapamycin (mTOR) inhibitor rapamycin induced significant regression of tumors in a mouse model of *KRAS*/*TP53*-mutant NSCLC.[34] Strong antitumor activities were observed for both AUY922 and ganetespib in *KRAS*-mutant NSCLC cell lines as well.[9,22,35] Strong in vivo efficacy, either as a single agent or in combination, also was demonstrated in several solid tumors and xenograft models bearing *KRAS*-mutant NSCLCs.[34-37]

MET-Amplified Non–Small Cell Lung Cancer

MET amplification is detected in 5% of adenocarcinomas of the lung[13] and in an additional 5% of tumors with acquired resistance to EGFR TKIs.[38] Furthermore, *MET* mutation, which occurs in 3% to 8% of NSCLCs,[39,40] may be a targetable oncogenic driver in NSCLC, given that dramatic responses were seen in patients with *MET*-amplified and *MET*-mutant tumors treated with a MET inhibitor.[41-43] To date, targeting the MET pathway with HSP90 inhibition has been limited to the setting of acquired resistance to EGFR inhibitors in which *MET* amplifications are found. AUY922 has been shown to induce growth inhibition of *MET*-amplified NSCLC cell lines resistant to gefitinib (Iressa, AstraZeneca) and erlotinib (Tarceva, Genentech/

Astellas), as well as xenograft models generated from parental HCC827.[44] SNX-2112 was shown to attenuate EGFR cross-talk and activation of the MET receptor by MET degradation in *EGFR*-mutant NSCLC.[45] The efficacy of HSP90 inhibition in overcoming MET-mediated resistance was first demonstrated in hepatocyte growth factor (HGF)–transfected Ma-1 cells with the L858R *EGFR* mutation, which were not sensitive to erlotinib but underwent growth inhibition followed by apoptosis after 17-DMAG treatment.[46] WK88-1 also was effective against gefitinib-resistant NSCLC cells and xenograft models with amplified *MET*.[8]

HER2/ROS1/RET/BRAF

HSP90 inhibitors have shown potential against the oncogenic driver mutant *HER2,* as well as novel targets such as *ROS1* and *RET*. HER2 is a member of the ERBB family, and the *HER2* gene is a well-known proto-oncogene. Between 13% and 20% of NSCLCs overexpress HER2. The use of the humanized monoclonal antibody trastuzumab (Herceptin, Genentech) in patients with NSCLC did not produce promising results in preclinical or clinical studies.[47] HSP90 inhibition seemed suitable as an alternate approach to targeting HER2, given that HER2 is an HSP90 client protein. Geldanamycin and its derivatives 17-AAG[17,48,49] and AUY922,[22,50] along with ganetespib,[11,23] have shown potential antitumor activities in *HER2*-mutant NSCLC cells in murine lung adenocarcinomas both in vitro and in vivo.

Aberrant *ROS1* gene fusions/rearrangements occur in 2% of lung adenocarcinomas,[51] and oncogenic ROS1 kinase activity leads to the onset of multiple proliferative pathways,[52] resulting in a distinct subset of molecularly defined NSCLCs.[53] Chromosomal rearrangements in the *RET* gene also have recently been found in 1% to 2% of cases of NSCLC.[53-56] Ganetespib is so far the only HSP90 inhibitor that has exhibited strong antitumor activities in NSCLC cell lines, with oncogenic rearrangements in *ROS1* and *RET* genes in addition to *ALK* rearrangements.[30]

BRAF mutations, especially *BRAF* V600E, are oncogenic drivers and have been found most frequently in melanoma and colorectal cancer, but activating mutations are present in 2% to 3% of NSCLC cases.[57,58] Interestingly, BRAF is an HSP90 client, and studies using AT13387[59] or a novel molecule XL888[60] in *BRAF* V600E melanoma models have produced promising preclinical results. These studies suggest that targeting BRAF in lung cancer with HSP90 inhibitors may be an effective therapeutic strategy.

DNA Repair and Cell Cycle Checkpoint Mechanisms

Cancer is characterized by aberrant cell cycle regulation, persistent DNA damage, and other epigenetic changes. Several HSP90 clients control the levels of genomic instability in cancer cells. Therefore, HSP90 inhibition is a reasonable approach for sensitizing cancer cells to agents that induce cell cycle arrest or DNA damage. Interestingly, HSP90 inhibitors, alone or in combination with taxanes, often induce G_2/M arrest. 17-AAG alone or in combination with paclitaxel enhanced the sensitivity of NSCLC overexpressing EGFR/HER2 to paclitaxel both in vitro and in vivo.[61,62] 17-AAG also induced G_2/M arrest by degrading the expression of CHK1 and CDC25A, 2 important cell cycle regulators.[63] IPI-504 showed synergy with docetaxel, inhibiting NSCLC cells in vitro and in xenograft tumor.[64] This combination also significantly depleted expression of anaphase-promoting complex components.[64] Ganetespib has demonstrated synergy with docetaxel both in vitro and in vivo.[37] Moreover, the 17-AAG–induced disruption of CHK1 expression sensitized the cells to gemcitabine, a nucleoside analogue and antimetabolite that specifically blocks cell cycle progression.[63] HSP90 inhibition also can be synergistic with radiotherapy. 17-AAG in combination with irradiation depleted the expression of CDC25C and CDC2 in lung cancer cell lines, inducing G_2/M arrest.[65] In contrast, 17-DMAG was able to sensitize *KRAS*-mutant NSCLC cells to radiotherapy by hindering their DNA repair mechanisms.[66] Ganetespib also inhibited the DNA repair mechanism in response to irradiation-induced DNA damage[36] and showed additive or synergistic activities with drugs used for advanced malignancies, such as etoposide.[67] Moreover, the combination of ganetespib and irradiation generated persistent DNA damage, leading to senescence.[36]

Clinical Development of HSP90 Inhibitors

The initial clinical application of HSP90 inhibitors was primarily focused on targeting molecularly defined subtypes of lung cancer. However, combining these inhibitors with other cytotoxic therapeutics may be more efficacious. Several past and ongoing clinical studies have involved many such HSP90 inhibitors, given either as single agents or as part of combination therapeutics. In this section, we focus on the antitumor activities of clinically significant HSP90 inhibitors.

17-AAG and 17-DMAG

Although 17-AAG was the first HSP90 inhibitor introduced into the clinic in a phase 1 trial, it was never examined in lung cancer. In the early phase 1 trials in patients with solid-organ malignancies, hepatotoxicity and diarrhea were the dose-limiting toxicities. Similar toxicities and ocular toxicity were seen with the second HSP90 inhibitor, 17-DMAG. Owing to the lack of objective responses and the presence of severe side effects, these drugs were abandoned, and new, second-generation HSP90 inhibitors were developed.[68]

IPI-504

After a small phase 1 trial in patients with gastrointestinal stromal tumor or soft-tissue sarcoma,[69] IPI-504 was tested in patients with molecularly defined (*EGFR* mutation and *ALK*

Table 1. Previous Trials of Heat Shock Protein 90 Inhibitors in Non–Small Cell Lung Cancer

Agent(s)	Phase	Number of Patients	Setting	Study Results	Reference
IPI-504	2	76	Advanced NSCLC with prior therapy	- Median PFS, 2.86 mo - ORR, 7% - 2 PRs among patients with *ALK* rearrangement	70
IPI-504 and docetaxel	1	23	Advanced NSCLC with prior systemic therapy	- Higher response among patients with squamous histology (43%) and among former smokers (33%)	71
AUY922	2	112	Advanced NSCLC with prior systemic therapy	- ORR of 25% in patients with *ALK* gene rearrangement - ORR of 50% in patients with crizotinib-naive *ALK*-positive disease - 18% ORR in patients with *EGFR* mutation	74
AUY922 and erlotinib	1/2	37	Advanced NSCLC that had progressed while patient on TKIs	- *EGFR* T790M identified by second tumor biopsy in 10 of 25 patients (40%) - ORR in 4 of 25 patients (16%; 95% CI, 6%-35%) - *EGFR* T790M in 3 of 4 patients with PR	75
AUY922 and erlotinib	1	10	Advanced NSCLC and *EGFR* exon 20 insertion	- 1 PR - 3 patients with disease stabilization lasting more than 3 mo and PFS lasting more than 6.1 mo	76
Ganetespib	2	99	Advanced NSCLC with prior systemic therapy	- PFS at 16 wk: 13.3% among patients with *EGFR* mutation, 5.9% among patients with *KRAS* mutation, 19.7% among patients with wild-type *KRAS/EGFR* - *ALK* gene rearrangement in 4 patients with PR	79
Ganetespib and docetaxel	2	381	Advanced disease with prior systemic therapy	- Increased hemoptysis and decreased efficacy in non-adenocarcinoma after first 71 patients enrolled, so study limited to adenocarcinoma - Median survival of 3.9 mo in combination group vs 3.0 mo in control group - OS of 7.6 mo in combination group vs 6.4 mo in control group; HR, 1.23	80

ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; HR, hazard ratio; mo, months; NSCLC, non–small cell lung cancer; ORR; objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; TKI, tyrosine kinase inhibitor; wk, weeks.

rearrangement status known) advanced NSCLC that had progressed on previous TKI therapy.[70] The median progression-free survival (PFS) was found to be 2.86 months, and the objective response rate was 7% (Table 1). Disappointingly, the activity of IPI-504 in patients with *EGFR*-mutant disease was found to be only 4%. Interestingly, 2 partial responses were seen in 3 patients with *ALK* rearrangements.[70] This study suggests that IPI-504 monotherapy may be effective in patients with *ALK*-rearranged lung cancer.

The combination of IPI-504 with docetaxel was tested in a phase 1b trial that included patients with lung cancer[71] and a subsequent expansion cohort of patients with pretreated metastatic NSCLC. Interestingly, the highest response rates were observed in patients with squamous histology (43%), former smokers (33%), and patients with wild-type *KRAS* status (36%), compared with an overall response rate of 26%. Of note, this response rate is considerably higher than the historical 8% response rate with docetaxel alone. The most common toxicities encountered included fatigue, nausea, and diarrhea. Based on this study, a phase 2 study evaluating IPI-504 and docetaxel (NCT01362400) in NSCLC was completed; however, results have not yet been published (Table 2). Unfortunately, because of treatment-related deaths due to liver toxicity and lack of efficacy, this compound is not being further developed.[72]

AUY922

AUY922 was first evaluated in a phase 1 study conducted in patients with solid-organ malignancies (primarily breast, ovarian, and colon cancers). Major dose-limiting toxicities included visual symptoms (43% of patients), diarrhea, asthenia, anorexia, and atrial flutter.[73] Subsequently, AUY922 was examined in a phase 2 trial in advanced NSCLC, and patients were grouped on the basis of molecular targets, including mutations in *EGFR* and *KRAS, ALK* rearrangement, and wild-type NSCLC.[74] The study revealed that the objective response rate was highest among patients with the *ALK* rearrangement

Table 2. Current Heat Shock Protein 90 Inhibitor Trials in the Setting of NSCLC

Agent(s)	Phase	Setting	Primary Endpoint	Clinical Trial Number
IPI-504	1/2	Advanced NSCLC, second line and beyond	ORR, MTD	NCT00431015
IPI-504 and docetaxel	2	Advanced NSCLC, second line and beyond	ORR, PFS	NCT01362400
IPI-504 and everolimus (Afinitor, Novartis)	1b/2	Advanced NSCLC conferring *KRAS* mutation, second line and beyond	ORR	NCT01427946
Ganetespib and crizotinib	2	Advanced NSCLC conferring *ALK* rearrangement	MTD, PFS	NCT01579994
Ganetespib	2	Stage IIIB or IV NSCLC	PFS	NCT01031225
Ganetespib	2	*ALK*-rearranged NSCLC	ORR	NCT01562015
Ganetespib and docetaxel (GALAXY-2)	3	Advanced NSCLC, second line and beyond	OS	NCT01798485
AUY922 and pemetrexed (Alimta, Lilly)	1B	Recurrent or metastatic NSCLC (nonsquamous)	DLT	NCT01784640
AUY922 vs docetaxel vs pemetrexed	2	NSCLC with *EGFR* mutations	PFS	NCT01646125
AUY922	2	*ALK*-positive NSCLC	OR	NCT01752400
AUY922	1	*EGFR* exon 20 insertion NSCLC	PFS	NCT01854034
AUY922 and trastuzumab	2	*HER2*-positive NSCLC	OR	NCT01798485
AT13387 and erlotinib	1/2	Recurrent or metastatic NSCLC	ORR, DLT	NCT02535338
AT13387 alone or in combination with crizotinib	1/2	*ALK*-rearranged NSCLC	ORR, DLT	NCT01712217

DLT, dose-limiting toxicity; GALAXY-2, A Phase 3 Study of Ganetespib in Combination With Docetaxel Versus Docetaxel Alone in Patients With Advanced NSCLC; MTD, maximum tolerated dose; NSCLC, non–small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

(25%) and was as high as 50% in crizotinib-naive, *ALK*-positive patients. In contrast to results with other HSP90 inhibitors, a higher response rate was seen in patients with *EGFR*-mutant disease (18%) than in patients with wild-type *EGFR* (13%). No responses were seen in patients with *KRAS* mutations.

Several follow-up studies have looked at AUY922 in *EGFR*-mutant NSCLC. The ability of the combination of AUY922 and erlotinib to overcome acquired erlotinib resistance was examined in patients with *EGFR*-mutant NSCLC.[75] In a phase 1/2 trial, a total of 37 patients were treated. The most common side effects were diarrhea, skin rash, hyperglycemia, and night blindness. A partial response was seen in 4 of 25 patients treated at the maximum tolerated dose, for a partial response rate of 16% (95% CI, 5%-36%). No complete responses were observed. In addition, 10 patients achieved stable disease, 4 of them for longer than 6 months. Additionally, the *EGFR* T790M mutation was identified by a second tumor biopsy in 10 of 25 patients (40%) and the *EGFR* T790M mutation was present in 3 of 4 patients with a partial response. Unfortunately, the phase 2 segment did not meet its endpoint of complete and partial response.[75] Finally, AUY922 is being examined in patients with *EGFR* exon 20 insertions, whose tumors inherently lack sensitivity to EGFR TKIs (NCT01124864). Early reports suggest that this may be an effective strategy, given that partial responses and prolonged stable disease have been observed.[76] Unfortunately, Novartis stopped further development of AUY922 in December of 2014.[77]

Ganetespib

Ganetespib is the HSP90 inhibitor that has been developed furthest in patients with NSCLC. Ganetespib was first examined in a phase 1 trial of patients with pretreated metastatic solid-organ malignancies. Of the 53 patients, 10 had NSCLC, and 6 of these had stable disease for at least 8 weeks. The most common toxicities were diarrhea, fatigue, and nausea or vomiting.[78] This phase 1 trial was followed by a phase 2 study[79] in which ganetespib was given to 99 patients divided into 3 cohorts based on mutational status (mutant *EGFR*, mutant *KRAS*, or wild-type *EGFR* and *KRAS*). At 16 weeks, the PFS rate was 13.3% for the patients with mutant *EGFR*, 5.9% for those with mutant *KRAS*, and 19.7% for those with wild-type *KRAS/EGFR*. Interestingly, all 4 of the patients with wild-type *KRAS/EGFR* who had a confirmed partial response harbored the *ALK* gene rearrangement, and there appeared to be significant single-agent activity in this patient population. Serious treatment-related adverse events occurred in 8 patients (8.1%), of which 2 resulted in death (cardiac arrest and

renal failure). The most common adverse events were diarrhea, fatigue, nausea, and anorexia.[79] In summary, single-agent HSP90 inhibition failed to demonstrate significant activity in *EGFR*-mutant or *KRAS*-mutant NSCLC, but it appeared to improve survival in patients with the *ALK* gene rearrangement. Currently, the activity of ganetespib in *ALK*-driven diseases is being followed further in 2 clinical trials. In the first study (NCT01562015), investigators are looking at ganetespib alone in patients with *ALK*-rearranged NSCLC. The other study (NCT01579994) is specifically looking at crizotinib in combination with ganetespib (Table 2).

The phase 2 GALAXY trial examined the effect of ganetespib in combination with docetaxel in pretreated patients with advanced NSCLC. Patients were given docetaxel alone (75 mg/m^2) on day 1 or in combination with ganetespib (150 mg/m^2) on days 1 and 15 of a 3-week cycle. It was found early on during this trial that the combination therapy resulted in hemoptysis and had no efficacy in the patients without adenocarcinoma, following which only patients with adenocarcinoma were enrolled. The combination therapy was favored in the group with adenocarcinoma (n=253) with respect to PFS (hazard ratio [HR], 0.82; *P*=.0784) and overall survival (OS; HR, 0.84; *P*=.1139). For patients with adenocarcinoma, the combination of ganetespib and docetaxel improved PFS (4.5 months in the combination arm vs 3.2 months in docetaxel arm; HR, 0.82). Additionally, OS in the patients with adenocarcinoma who received combination therapy was improved (7.6 vs 6.4 months; HR, 1.23). It was also observed that those with advanced disease diagnosed more than 6 months before study entry (n=177) responded better to the combination with regard to PFS (5.3 vs 3.4 months; adjusted HR, 0.74; *P*=.0417) and OS (11.0 vs 7.4 months; adjusted HR, 0.69; *P*=.0191).[80] Hence, this subpopulation was further explored in a phase 3 trial, GALAXY-2.

GALAXY-2 accrued patients who had pretreated advanced NSCLC and who had been diagnosed with advanced NSCLC more than 6 months before study entry. Patients were randomly assigned in a 1:1 fashion to docetaxel or the combination of ganetespib and docetaxel (NCT01798485).[81] Unfortunately, the preplanned interim analysis concluded that docetaxel in combination with ganetespib was unlikely to demonstrate a statistically significant improvement in OS compared with docetaxel alone. Synta Pharmaceuticals has announced[82] that it will be terminating the phase 3 portion of GALAXY-2.

AT13387

Promising results in preclinical studies led to a phase 1 study with AT13387 that determined the maximum tolerated dose: 260 mg/m^2 per week for 3 weeks of a 4-week cycle, given intravenously. Major side effects included infusion-related symptoms, gastrointestinal effects, and fatigue.[83] Currently, 2 more phase 1/2 trials of AT13387 are ongoing. In one of these trials (NCT01712217), patients with *ALK*-rearranged NSCLC receive AT13387 with or without crizotinib (Table 2). In the other trial (NCT02535338), patients with recurrent or metastatic NSCLC who have the *EGFR*-activating mutation receive erlotinib and AT13387 (Table 2).

Discussion and Future Directions

As discussed earlier, the first-generation inhibitors (Figure 2) were abandoned in large part because of significant toxicity. The second-generation HSP90 inhibitors, including AUY922, ganetespib, and AT13387, appear not to have the toxic effects of the first-generation HSP90 inhibitors. Ganetespib did proceed to a phase 3 trial looking at it in combination with docetaxel, but this trial recently was closed prematurely owing to futility. Although this class of drugs was first tested in phase 1 trials in 1999 and show considerable in vivo activity, no FDA-approved HSP90 inhibitors are available. A number of phase 1 and phase 2 trials are still open that are looking at specific molecular alterations in NSCLC. The way forward for this class of agents likely is through rationally designed combinations in distinct molecular subtypes, such as *ALK*, *KRAS*, *EGFR,* and *HER2* (Table 2).

In addition to the current drugs, alternate methods to target HSP90 are now available. Based on preclinical data, it seems plausible that targeting co-chaperones, such as CDC37, AHA1, and p23, would increase the efficacy of HSP90 inhibitors.[84] Although most of the HSP90 inhibitors target the N-terminal ATP-binding pocket of HSP90, the C-terminal also can be targeted by drugs such as the aminocoumarin antibiotics novobiocin, clorobiocin, and coumermycin A1.[85,86] The natural product celastrol was reported to disrupt the protein–protein interaction between HSP90 and its co-chaperone CDC37, leading to the destabilization of several HSP90 client kinases.[87] The use of such compounds might provide a new paradigm for targeting HSP90. In addition, previous studies have demonstrated that treatment with HSP90 inhibitors leads to the activation of heat shock transcription factor 1 (HSF1), which in turn leads to the upregulation of its transcriptional targets[88] HSP27, HSP70, and HSP90. Therefore, targeting HSF1 or its downstream targets (HSP27 and HSP70) in addition to HSP90 might be an effective strategy to overcome resistance to HSP90 inhibitors.[3] Furthermore, in the past 5 years, global analyses have recognized several proteins as partners in HSP90 interactions. Careful genetic and biochemical analysis of such proteins might provide effective avenues in targeted cancer therapeutics. Finally, a novel class of HSP90 drug conjugates that take advantage of the abundance of HSP90 in tumor cells to deliver a toxic payload are currently being developed by Synta.[89]

Conclusion

HSP90 plays an essential role in the assembly of multiple-protein chaperone complexes, which in turn help stabilize several client oncoproteins. The advantage of using HSP90 inhibitors is that they are able to inhibit multiple targets simultaneously, potentially improving patient outcomes. Preclinical data have shown potential for these drugs in NSCLC, either alone or in combination. HSP90 inhibitors have shown promise in overcoming TKI resistance in *EGFR*-positive and *ALK*-rearranged NSCLC in early clinical trials. Unfortunately, the combination of ganetespib and taxanes in unselected patients was not beneficial in the phase 3 GALAXY-2 trial. The path forward for this class of agents likely is through rationally designed combinations in molecularly defined patient populations. To this end, a number of phase 1 and phase 2 trials intended to elucidate the role of HSP90 inhibitors in the treatment of NSCLC are currently recruiting patients. The hope is that the results of these trials will translate into strategies for treating our patients more effectively.

Disclosures

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