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REVIEW

Heat shock protein antagonists in early stage clinical trials for NSCLC

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ABSTRACT

Introduction: Cancer cells have a higher need of chaperones than normal cells to prevent the toxic effects of intracellular protein misfolding and aggregation. Heat shock proteins (Hsps) belong to these chaperones; they are classified into families according to molecular size. Hsps are upregulated in many cancers and inhibition can inhibit tumor growth by destabilizing proteins necessary for tumor survival. In non-small cell lung cancer (NSCLC), there are three different Hsp antagonist classes that are in (early) clinical trials: Hsp90, Hsp70 and Hsp27 inhibitors.

Areas covered: The rationale to use Hsp inhibitors in NSCLC will be summarized and phase I-III trials will be reviewed.

Expert opinion: Several Hsp90 inhibitors have been tested in phase I-III trials, until now none was positive in unselected NSCLC; therefore development of AUY922, ganetespib and retaspimycin was halted. Results seem more promising in molecularly selected patients, especially in *ALK*-rearranged NSCLC. Hsp27 is overexpressed in squamous NSCLC and is a mechanism of chemotherapy resistance. The Hsp27 inhibitor apatorsen is now tested in squamous NSCLC. No phase II/III data are known for Hsp70 inhibitors. Combination of Hsp inhibitors with heat shock transcription factor 1 inhibitors or focal adhesion kinase inhibitors might be of interest for future trials.

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1. Introduction

Non-small cell lung cancer (NSCLC) is increasingly subclassified as molecularly defined oncogene addicted tumors. For some of these tumors targeted agents are available. Examples are epidermal growth factor (*EGFR*)-mutated or anaplastic lymphoma kinase (*ALK*) translocated tumors [1,2]. However, resistance to targeted agents inevitably develops. Moreover, for other oncogenic drivers (e.g. Kirsten rat sarcoma viral oncogene (*KRAS*)), no targeted agents are available and still for most tumors no driver mutation has been identified. In these patients, there is an unmet need for new therapies.

Targeting the heat-shock protein (Hsp) family is an interesting area for development of new therapies. Hsps are chaperones which protect a variety of client proteins including driver mutation oncoproteins during their folding process and they are classified into families according to molecular size (e.g. Hsp90, Hsp70, small Hsp) [3]. The chaperoning is necessary to prevent the toxic effects of misfolding and aggregation of normal proteins. Cancer cells have an increased expression of chaperones such as Hsps compared to normal cells in response to the often hypoxic, acidotic, and nutrient-deprived tumor environment as was reviewed in Bagatell et al. [4]. Moreover, increased Hsp levels can cause impaired apoptotic signaling in tumor cells as was reviewed by Takayama et al. [5]. As such, Hsp can also promote tumor growth by stabilizing proteins necessary for tumor survival. In NSCLC, there are three different Hsp antagonist classes that are in (early) clinical trials (phase I-III): Hsp90, Hsp70, and Hsp27 inhibitors of which the first has been studied most extensively. Hsp90 is ubiquitously expressed but is overexpressed in many cancers [4]. Client proteins for Hsp90 are for example human epidermal growth factor receptor 2 (*HER2), EGFR, ALK, KRAS*, v-Raf murine sarcoma viral oncogene homologue 1 (*BRAF*), protein kinase B (*AKT*), mitogen-activated protein kinase (*MEK*), vascular endothelial growth factor receptor 1 (*VEGFR*), and cell cycle proteins [6,7]. In contrast, Hsp70 is not expressed or expressed at low levels in normal cells, but is highly induced by intracellular stress (e.g. in cancerous conditions) [8]. Hsp70 can inhibit apoptosis at different levels as was extensively reviewed by Wang et al. [9]. For example, Hsp70 can bind to death receptors which results in decreased activity of the death inducing signaling complex (DISC). At the premitochondrial level, Hsp70 for example inhibits stress-activated kinases, negatively interferes with the MAPK family kinase activity and stabilizes stress-activated kinases (e.g. Akt). At the mitochondrial level, heat-induced apoptosis is blocked by binding of Hsp70 to Bax, and at the postmitochondrial level Hsp70 can bind to apoptosis protease-activating factor-1 (Apaf-1) thereby inhibiting apoptosis [9]. Hsp27 is expressed in all human tissues but is often overexpressed in cancer. It is able to block apoptosis through inactivation of the caspase cascade [10].

There is a preclinical rationale to use Hsp antagonists in the treatment of cancer [11,12]. Moreover, there are also several preclinical studies evaluating Hsp inhibition in NSCLC cell lines specifically. For example, Hsp90 inhibition with 17-AAG down-

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Article highlights

- Hsps are upregulated in many cancers and inhibition can inhibit tumor growth by destabilizing proteins necessary for tumor survival
- In molecularly unselected NSCLC patients, results of Hsp90 inhibitors are disappointing
- Hsp90 inhibitors are more promising in *ALK*-rearranged patients
- To improve efficacy of Hsp90 inhibitors in molecularly selected NSCLC patients, combination therapy with TKIs seems the way forward
- Not many data exist for Hsp27 and Hsp70 inhibitors and till now, results are disappointing

This box summarizes key points contained in the article.

regulated repair protein Rad51 and DNA repair capacity which resulted in cytotoxicity [13]. Another Hsp90 inhibitor, retaspimycin (IPI-504) enhanced the antimitotic effects of docetaxel [14]. Comparable results were found for the Hsp90 inhibitor ganetespib: synergistic activity of ganetespib combined with docetaxel, paclitaxel, or vincristine was demonstrated [15]. Hsp27 overexpression promoted lung cancer growth in a NSCLC cell line [16]. Apatorsen (OGX-427, a Hsp27 inhibitor) enhanced erlotinib sensitivity as well as chemosensitivity to several chemotherapies commonly used in NSCLC treatment (pemetrexed, cisplatin, paclitaxel, gemcitabine) [17]. Despite this preclinical evidence, results of completed clinical trials have mostly been disappointing with a possible exception for *ALK* rearrangements. Results of these trials (phase I–III, divided into molecularly unselected and selected) and strategies to improve efficacy will be discussed below. A summary of completed trials and ongoing trials/trials without results is provided in Tables 1 and 2, respectively.

2. Heat-shock protein inhibitors

2.1. Hsp90 inhibitors

Completed and ongoing Hsp90 antagonist clinical trials in NSCLC were recently reviewed by Chatterjee et al. [37] and will be summarized below. Two other Hsp90 inhibitors, 17-AAG and 17-DMAG, were the first in their class, but these were never examined in NSCLC clinical trials and will not be discussed. Development of these two drugs has been abandoned because of toxicities and lack of response.

2.1.1. Retaspimycin (IPI-504)

Molecularly unselected patients: in a phase Ib trial (N = 16, including 6 NSCLC patients), retaspimycin was tested in combination with docetaxel. Dose limiting toxicities (DLTs) were febrile neutropenia, fatigue, sinus bradycardia, elevated aspartate aminotransferase (AST) and acute respiratory distress syndrome. Maximum tolerated dose (MTD) MTD and recommended phase II dose (RP2D) was 450 mg/m^2 intravenous (iv) for retaspimycin in combination with docetaxel 75 mg/m^2 iv once every three weeks. Median number of cycles was three (range 1–11) [18]. In the subsequent expansion cohort with previously treated metastatic NSCLC patients objective response rate (ORR) was 26%, but was highest in those with wild-type *KRAS* (36%), former smokers (33%) and squamous histology (43%) [18]. This combination was subsequently tested in a double-blind, randomized, placebo controlled phase II trial comparing docetaxel with retaspimycin or placebo in previously treated advanced NSCLC patients with a smoking history (N = 226 (NCT01362400)). Co-primary end points were overall survival (OS) in the entire patient population and OS in the squamous histology population. For both endpoints, the addition of retaspimycin did not show an improvement. Subgroup analyses based on biomarkers (*KRAS*-mutated, p53, plasma levels of Hsp90-alpha) could not identify a patient population with more benefit (press release, results have not been published, no further details in this press release) [19]. As a result, retaspimycin will not be developed further.

Molecularly selected patients: monotherapy retaspimycin was evaluated in single-arm phase II study including 76 advanced NSCLC patients who had prior treatment with EGFR-tyrosine kinase inhibitors (TKI) and tissue available for molecular analysis. 28 patients had an *EGFR*-mutation, 12 a *KRAS*-mutation and three had an *ALK* rearrangement. Median number of prior treatments was four. Starting dose was 400 mg/m^2 retaspimycin iv on days 1, 4, 8, and 11 of a 21-day cycle for 75 patients, but because of hepatotoxicities observed in another trial with gastrointestinal stromal tumors, the dose was lowered to 225 mg/m^2 for those still on treatment and for the last enrolled patient. Objective response rate (ORR) was 7% for the overall study population, 4% in the *EGFR*-mutation subgroup, and 67% in the *ALK*-rearrangement subgroup (2 out of 3 patients). The third *ALK*-rearranged patient had a prolonged stable disease (SD, 7.2 months) [27]. Based on these results, a 2-arm phase II trial in *ALK*-rearranged patients was launched: one arm including ALK-TKI naïve patients, one arm including patients with prior exposure to an ALK-TKI (NCT01228435). Retaspimycin was given at 225 mg/m^2 twice a week for two weeks followed by 10 days off therapy, one cycle was 21 days. Primary end point was ORR. The trial was closed because of poor accrual: three patients were enrolled (one ALK-TKI naïve, two ALK-TKI pretreated). None of these patients responded [28]. Another phase Ib/II study studied retaspimycin in combination with everolimus in previously treated advanced *KRAS*-mutated NSCLC (NCT01427946). Primary end point of the phase Ib part is defining the MTD and RP2D, primary end point of the phase II part is ORR. The study has been completed but results are awaited.

2.1.2. AUY922

Molecularly unselected patients: In a phase I study in patients with solid-organ malignancies (primarily breast, ovarian and colon, N = 101), RP2D was AUY922 70 mg/m^2 once weekly. DLTs were diarrhea, asthenia/fatigue, anorexia, atrial flutter, and visual disturbances (night blindness, photopsia, blurred vision, and visual impairment) [20]. As far as we know, there are no phase II trials with AUY922 in molecularly unselected NSCLC patients. An ongoing phase Ib study (NCT01784640) with escalating doses of AUY922 iv weekly and pemetrexed iv three-weekly in previously treated stage IV NSCLC patients has completed recruitment and results are awaited. In the expansion phase, patients will be allocated to treatment groups according to their molecular status (*EGFR, KRAS, ALK*, triple negative).

Table 1. Heat-shock protein inhibitor trials with results in molecularly unselected and selected non-small cell lung cancer patients.

Type Hsp inhibitor	Trial type	*N*	Treatment	Primary study objective	Outcome	AE grade ≥3 in ≥ 5% of patients	Further development	Reference
HSP90								
Molecularly unselected								
Retaspimycin	Phase Ib	16 (6 NSCLC)	Retaspimycin + docetaxel	Safety	DLTs: febrile neutropenia, fatigue, sinus bradycardia (hospitalization), elevated AST, ARDS RP2D: retaspimycin 450 mg/m^2 iv + docetaxel 75 mg/m^2 Q3 W	See DLTs, except sinus bradycardia (grade 1)	No	[18]
Retaspimycin	Randomized, double-blind, placebo controlled phase II	226 NSCLC	A: Retaspimycin + docetaxel B: placebo + docetaxel	OS OS in squamous histology	No improvement (no further details in press release)	Not mentioned in press release	No	[19]
AUY922	Phase I	101, *N* NSCLC unknown	AUY922 monotherapy	Safety	DLTs: diarrhea, asthenia/fatigue, anorexia, atrial flutter/fibrillation, visual disturbances RP2D: 70 mg/m^2 iv once weekly	See DLTs	Novartis: no rights were returned to Vernalis	[20]
Ganetespib	Phase I	53 (10 NSCLC)	Ganetespib monotherapy	Safety	DLTs: amylase elevation, diarrhea, asthenia RP2D 200 mg/m^2 iv day 1,8,15 Q4 W	Fatigue 11.3% Asthenia 7.5% Diarrhea 7.5% Hypophosphatemia 7.5% Increased ALT 7.5% Dehydration 5.7% Hyperbilirubinemia 5.7% Hyponatriemia 5.7%	No	[21]
Ganetespib	Randomized phase II	381	A: Ganetespib + docetaxel B: docetaxel	PFS in two AdC subgroups: elevated LD and *KRAS*+	No benefit in median PFS Elevated LD: A: 2.9 months, B: 2.7 months, HR 0.77 (p = 0.11) *KRAS*+: A: 3.9 months, B: 3.0 months, HR 1.11 (p = 0.34)	Neutropenia A 41%, B 42% Fatigue A 6% B 4% Anemia A 8% B 2% Dyspnea A 7% B 3% Asthenia A 5% B 3% Leukopenia A 10% B 6% Febrile neutropenia A 9% B 5%	No	[22]
Ganetespib	Randomized phase III	672 AdC NSCLC with diagnosis > 6 months before study entry	A: Ganetespib + docetaxel B: docetaxel	OS	No benefit in median OS A: 10.9 months B: 10.5 months, HR 1.11 (p = 0.33)	A: 65% grade ≥ 3 B: 54% grade ≥ 3 Not further specified in WCLC 2016 presentation	No	[23]
Onalespib	Phase I	62 (10 NSCLC)	Onalespib monotherapy	Safety	DLT with twice weekly regimen: visual disturbances RP2D 120 mg/m2 twice weekly for 3 weeks in a 4-week schedule DLT with once weekly regimen: no formal DLT RP2D 260 mg/m^2 iv once weekly for 3 weeks in a 4-week schedule	See DLTs	Yes	[24]
Onalespib	Phase I	31 (1 NSCLC)	Onalespib	Safety	DLTs: liver enzyme abnormalities, gastrointestinal hemorrhage RP2D: 160 mg/m^2 iv day 1,2,8,9,15,16 in a 4-week schedule)	See DLTs	Yes	[25]
Debio0932	Phase I	50 (7 NSCLC) Extension cohort + 30 (15 NSCLC)	Debio0932	Safety	DLTs: febrile neutropenia, diarrhea, asthenia RP2D: 1000 mg once daily orally	See DLTs	Yes	[26]
Molecularly selected								
Retaspimycin	Single-arm phase II	76 EGFR-TKI pretreated	Retaspimycin monotherapy	ORR	All patients: 7% *EGFR*+: 4% *ALK*+: 67%	Fatigue 7.9% Nausea 7.9% Diarrhea 10.5% Vomiting 7.9% Anorexia 5.3% Dyspnea 7.9% Liver function test abnormality: 11.8%	No	[27]

(*Continued*)

Table1. (Continued).

Type Hsp inhibitor	Trial type	*N*	Treatment	Primary study objective	Outcome	AE grade ≥3 in ≥ 5% of patients	Further development	Reference
Retaspimycin	2-arm phase II	Unknown planned, enrolled 3	A: retaspimycin monotherapy in ALK-TKI naive patients B: retaspimycin monotherapy in ALK-TKI pretreated patients	ORR	A: no responses B: no responses	No SAEs, otherwise grading AE not mentioned	No	[28]
AUY922	Single-arm phase II	112	AUY922 monotherapy	ORR, DCR	Preliminary PR: *EGFR*+: 18% *ALK*+: 25% (crizotinib naive: 50%) *KRAS*+: 0% Triple neg.: 13%	Abstract: <10% grade ≥ 3, not specified	Novartis: no rights were returned to Vernalis	[29]
AUY922	Phase I/II	37 *EGFR*+ with acquired resistance to EGFR-TKI	AUY922 + erlotinib	Phase I: safety Phase 2: ORR	DLT: junctional cardiac rhythm RP2D AUY922 70 mg/m^2 iv once a week ORR at RP2D 16% independent of T790M	Diarrhea 16% Elevated AST 8% Elevated ALT 8% Decreased lymphocytes 32%	Novartis: no rights were returned to Vernalis	[30]
AUY922	Single-arm phase II	10 (first part) *EGFR* exon 20 insertion	AUY922 monotherapy	ORR	1 PR (10%) Three SD (30%) for >3 months	Hypertension 20% Hypophosphatemia 7% Transaminitis 7%	Novartis: no Rights were returned to Vernalis	[31]
Ganetespib	Phase II, 3 cohorts based on molecular status	15 *EGFR*+ 17 *KRAS*+ 66 *EGFR/KRAS* wt	Ganetespib monotherapy	PFS rate at 16 weeks	*EGFR*+ 13.3% *KRAS*+ 5.9% *EGFR/KRAS* wt 19.7% 8 patients with PR, retrospectively 4 were ALK+	Diarrhea 8.1% Fatigue 14.1% Dyspnea 12.1% Hyponatremia 10.1% Elevated AST 5.1% Elevated lipase 5.1%	No	[32]
Ganetespib	Phase I	12 ALK+, ALK-TKI naive	Ganetespib + crizotinib	Safety	DLTs: ALT, AST, lipase and amylase elevation PR 67%, DCR 83% RP2D: ganetespib 200 mg/m^2 iv days 1 and 8 Q3W + crizotinib 250 mg twice daily	See DLTs	No	[33]
Hsp27								
Molecularly unselected								
Apatorsen	Phase I	42 (3 NSCLC)	Apatorsen monotherapy	Safety	DLTs: intracranial hemorrhage in previously undiagnosed brain metastases, no MTD defined 1/3 of patients SD	See DLTs	Yes	[34]
Apatorsen	Phase II	155	A: pem/carbo + placebo B: pem/carbo + apatorsen	PFS	Median PFS: A: 4.9 months B: 6.0 months (HR 0.92, p = 0.67)	Anemia A 22% B 20% Trombocytopenia A 27% B 28% Neutropenia A 33% B 22% Leukopenia A 22% B 13% Fatigue A 13% B 5% Dyspnea A 3% B 5%	Yes	[35]
Molecularly selected: no completed trials								
Hsp70								
Molecularly unselected								
Hsp70 NK-based adoptive immunotx	Phase I	12 (1 NSCLC)	Ex vivo Hsp70 peptide activated, autologous NK cells	Safety	No grade ≥3	No grade ≥3	Yes	[36]
Molecularly selected: no completed trials								

Hsp: heat-shock protein; *N*: number; AE: adverse event; NSCLC: non-small cell lung cancer; DLTs: dose limiting toxicities; AST: aspartate aminotransferase; ARDS: acute respiratory distress syndrome; RP2D: recommended phase II dose; mg: milligram; iv: intravenous; Q: every; W: weeks; OS: overall survival; ALT: alanine aminotransferase; PFS: progression free survival; AdC: adenocarcinoma; LD: lactate dehydrogenase; KRAS+: Kirsten rat sarcoma viral antigen mutated; HR: hazard ratio; EGFR-TKI: epidermal growth factor receptor-tyrosine kinase inhibitor; ORR: objective response rate; EGFR+: epidermal growth factor receptor mutated; ALK+: anaplastic lymphoma kinase translocated; SAE: serious adverse event; DCR: disease control rate; PR: partial response; neg: negative; SD: stable disease; NK: natural killer cell; immunotx: immunotherapy

Table 2. Ongoing trials or completed trials without results with heat shock protein inhibitors in molecularly unselected and selected non-small cell lung cancer patients.

Hsp inhibitor (clinicaltrials.gov number)	Phase Patient group	Treatment	Primary outcome	Clinicaltrials.gov status
Hsp90				
Molecularly unselected				
Retaspimycin: no ongoing trials or completed trials without results	N/A	N/A	N/A	N/A
AUY922 (NCT01784640)	1b + expansion cohort (expansion cohort allocation to treatment groups based on molecular status: *EGFR*+, *KRAS*+, *ALK*+, triple neg NSCLC)	AUY922 iv + pemetrexed iv	Safety	Active, not recruiting
Ganetespib: no ongoing trials or completed trials without results	N/A	N/A	N/A	N/A
Onalespib (NCT02503709)	Phase I Solid tumors	Onalespib + CDK inhibitor AT7519M	Safety	Recruiting
Debio0932: no ongoing trials or completed trials without results	N/A	N/A	N/A	N/A
Molecularly selected				
Retaspimycin (NCT01427946)	Phase Ib/II *KRAS*+ NSCLC	Retaspimycin + everolimus	Part 1b: MTD, RP2D Part 2: ORR	Completed No results posted
AUY922 (NCT01646125)	Open-label randomized phase II Previously treated (EGFR-TKI platinum-based chemotherapy) *EGFR*+ NSCLC	A: AUY922 monotherapy B: pemetrexed or docetaxel monotherapy	PFS	Completed No results posted
AUY922 (NCT01752400)	Single arm phase II *ALK*+ NSCLC with acquired resistance to ALK-TKI	AUY922 monotherapy	ORR	Active, not recruiting
AUY922 (NCT01772797)	Phase Ib *ALK*+ NSCLC with acquired resistance to ALK-TKI	AUY922 + ceritinib	Safety	Completed No results posted
AUY922 (NCT01922583)	Single arm phase II NSCLC with driver mutation except classical sensitive *EGFR*+	AUY922 monotherapy	ORR	Recruiting
Ganetespib (NCT01562015)	Single arm phase II *ALK*+ NSCLC, ALK-TKI naive	Ganetespib monotherapy	ORR	Completed No results posted
Onalespib (NCT01712217)	Phase I/II *ALK*+ NSCLC	Onalespib ± crizotinib	Part I: safety Part II: ORR	Active, not recruiting
Onalespib (NCT02535338)	Phase I/II *EGFR*+ NSCLC, acquired resistance to EGFR-TKI	Onalespib + erlotinib	Part I: safety Part II: ORR	Recruiting
Debio0932: no ongoing trials or completed trials without results	N/A	N/A	N/A	N/A
Hsp27				
Molecularly unselected				
Apatorsen (NCT02423590)	Open-label randomized phase II Squamous histology NSCLC	A: gemcitabin/carboplatin B: gemcitabin/carboplatin + apatorsen	PFS	Recruiting
Molecularly selected				
No ongoing trials or completed trials without results	N/A	N/A	N/A	N/A
Hsp70				
Molecularly unselected				
Hsp70 NK cell-based adoptive immunotx (NCT02118415)	Open label, randomized phase II Stage IIIA/B NSCLC, completed chemoradiotherapy	A: Hsp70 NK cell-based adoptive immunotx B: observation	PFS	Recruiting
Molecularly selected				
No ongoing trials or completed trials without results	N/A	N/A	N/A	N/A

Hsp: heat-shock protein; N/A: not applicable; EGFR+ epidermal growth factor receptor mutated; KRAS+: Kirsten rat sarcoma viral antigen mutated; ALK+: anaplastic lymphoma kinase translocated; neg: negative; NSCLC: non-small cell lung cancer; iv: intravenous; CDK: cyclin-dependent kinase; MTD; maximum tolerated dose; RP2D: recommended phase 2 dose; ORR: objective response rate; TKI: tyrosine kinase inhibitor; PFS: progression-free survival; NK: natural killer; immunotx: immunotherapy

Molecularly selected patients: in a single-arm phase II trial (N = 112) including advanced NSCLC with progression after at least two prior systemic therapies, patients were stratified according to molecular status (*EGFR*-mutated (31%), *KRAS*-mutated (25%), *ALK*-translocated (12%), triple negative (28%)). AUY922 was given at 70 mg/m^2 once weekly as a 1-hour infusion. Primary end points were ORR and disease control rate (DCR). Preliminary data showed different percentages of partial responses (PR) across the different molecular cohorts: 25% (*ALK*-translocated), 18% (*EGFR*-mutated), 0% (*KRAS*-mutated), and 13% (triple negative). In the *ALK*-rearrangement subgroup, RR was 50% in the crizotinib-naïve patients. One of the included patients with a durable response had an *EGFR* exon 20 insertion [29]. Based on these results, AUY922 was further evaluated in *EGFR*-mutated and *ALK*-rearranged NSCLC patients. In a phase I/II trial including *EGFR*-mutated patients with acquired resistance to EGFR-TKIs (N = 37), increasing doses of AUY922 were combined with erlotinib 150 mg once daily (except for cohort 1: 75 mg once daily). MTD and RP2D for AUY922 was 70 mg/m^2 iv once a week. DLT was a junctional cardiac rhythm because of QTc prolongation. Primary end points for the phase II part were ORR and adverse events. For the 25 patients treated at the MTD, ORR was 16% and was independent from T790M status [30]. This is interesting, as T790M is a resistance mechanism to first-generation EGFR-TKIs such as erlotinib [38], and it is possible that AUY922 combined with erlotinib can overcome this resistance. Another open-label randomized phase II trial compared AUY922 (70 mg/m^2 iv once a week) to pemetrexed (500 mg/m^2 every three weeks) or docetaxel (75 mg/m^2 every three weeks) in previously treated (EGFR-TKI as well as platinum-based chemotherapy) advanced *EGFR*-mutated NSCLC patients (NCT01646125). The trial has been completed but results are not yet available. AUY922 (70 mg/m^2 iv once a week) has also been tested in NSCLC patients with an *EGFR* exon 20 insertion in a single arm phase 2 study. Primary end point was ORR and a Simon two-stage design was used. In the first part of the trial (N = 10), one patient had a PR and three had a stable disease (SD) for >3 months. Results from the second stage of the trial are awaited [31]. For *ALK*-rearranged NSCLC patients, several trials are ongoing or have been completed, but for now without results available. NCT01752400 is a single-arm phase II study evaluating AUY922 in *ALK*-rearranged NSCLC patients with acquired resistance to ALK-TKIs. Primary end point is ORR. NCT01772797 is a phase Ib trial evaluating ceritinib combined with AUY922 in the same patient population as NCT01752400. Another single-arm phase II trial (NCT01922583) is including NSCLC patients with a driver mutation (e.g. *ALK, ROS1, HER2, BRAF, EGFR* exon 20 insertion or T790M, *RET*, except the classical sensitive *EGFR*-mutation). Treatment is with monotherapy AUY922, primary end point is ORR.

Unfortunately, approximately 2 years ago development of AUY922 has been terminated by Novartis and the rights were returned to Vernalis [39,40].

2.1.3. Ganetespib

Molecularly unselected patients: In a phase I trial including 53 patients with solid malignancies (10 NSCLC), DLTs were amylase elevation, diarrhea, and asthenia. MTD was 216 mg/m^2 and RP2D was 200 mg/m^2 iv day 1, 8 and 15 in a 4-weekly schedule. DCR was 24.4% [21]. This trial was followed by a phase I trial combining ganetespib with docetaxel (NCT01183364) because of preclinical evidence that these drugs act synergistically [15]. As far as we know, results have not been published. The randomized phase II GALAXY-1 trial evaluated docetaxel 75 mg/m^2 day 1 with/without ganetespib 150 mg/m^2 day 1 and 15 in a 21 days schedule in advanced NSCLC patients with progression after first-line chemotherapy (N = 381). In the original study design the primary end point was PFS for all patients. However, based on an interim analysis, patients with non-adenocarcinoma histology were excluded because of increased risk of hemoptysis and lack of efficacy. Primary end point was changed to PFS in two adenocarcinoma subgroups: those with elevated lactate dehydrogenase (eLDH, N = 114) and *KRAS*-mutated patients (N = 89) [22]. Rationale for changing to PFS in eLDH patients as a primary end point was the preclinical finding that hypoxia inducible factor-1α (HIF-1α) is a client protein of Hsp90, that it is overexpressed in aggressive and treatment-resistant cancers (preclinical data from colon and hepatocellular cancer) and that HIF-1α is sensitive to ganetespib in preclinical rectal cancer models [41–43]. eLDH was used as a surrogate marker for HIF-1α since LDH subunit A expression is regulated by HIF-1α [44,45]. For KRAS, this change was based on the modest clinical evidence that *KRAS*-mutated patients benefited from ganetespib monotherapy [32],and preclinical evidence that several RAS effectors in the MAPK and PI3K/AKT pathway are Hsp90 clients (evidence from a vemurafenib-resistant melanoma cell line treated with XL888 (HSp90 inhibitor) and evidence from a breast cancer xenograft treated with another Hsp90 inhibitor (Pu-H71)) [46,47]. PFS was not superior in the combination arm (hazard ratio (HR) 0.77, p = 0.11 and HR 1.11, p = 0.34, respectively). One of the exploratory analyses showed a benefit regarding PFS and OS with the combination arm in the subgroup of adenocarcinoma patients with a diagnosis of advanced NSCLC more than six months before study entry (N = 177). HR for PFS was 0.74 (p = 0.04), and HR for OS was 0.69 (p = 0.02) [22]. Based on the results of these exploratory analyses, the randomized phase III GALAXY-2 trial was launched including only pretreated advanced adenocarcinoma patients with a diagnosis of advanced NSCLC more than 6 months before study entry (NCT01798485). Primary end point was OS [48]. This trial was terminated early (672 patients randomized) based on the results of a preplanned interim analysis: there was no statistically significant improvement in the combination arm compared to docetaxel monotherapy [23,49]. Median OS was 10.9 months in the experimental arm and 10.5 months in the comparator arm (HR 1.11, 95% CI 0.90–1.37, p = 0.33).

Molecularly selected patients: in a phase II study, 99 previously treated advanced NSCLC were enrolled in three cohorts based on their molecular status (*EGFR*-mutated (N = 15), *KRAS*-mutated (N = 17), *EGFR/KRAS* wild type (N = 66)). Ganetespib was administered at the RP2D. Primary end point was PFS rate at 16 weeks and was, respectively, 13.3%, 5.9%, and 19.7%. Four patients had a PR. Patients were retrospectively tested for *ALK*-rearrangements and 8 were tested positive. All patients with a PR were *ALK* rearranged [32]. Ganetespib is still being evaluated in this subgroup of patients. NCT01579994 was a phase I trial of increasing doses

of ganetespib combined with crizotinib 250 mg twice daily in ALK-TKI naïve ALK-rearranged patients. Twelve patients were included, MTD for ganetespib was 200 mg/m2 on days 1 and 8 of a 21-day cycle. DLTs were ALT, AST, lipase and amylase elevation. 67% had a PR, DCR was 83% [33]. NCT01562015 is a single-arm phase II trial of ganetespib monotherapy in ALK-TKI naïve ALK-rearranged patients with as a primary end point ORR. Results are awaited.

2.1.4. Onalespib (AT13387)

Molecularly unselected patients: in a phase I trial including 62 patients with advanced pretreated solid malignancies (10 NSCLC), DLT with a twice weekly regimen was visual disturbances, and MTD and R2PD for this regimen was onalespib 120 mg/m^2 twice weekly for 3 weeks in a 4-week regimen. With a once weekly regimen, no formal DLTs were encountered but several moderately severe adverse events were seen: diarrhea, nausea, vomiting, fatigue, and systemic infusion reactions. RP2D was 260 mg/m^2 for 3 weeks in a 4-week regimen [24]. In another phase I study (N = 31, 1 NSCLC), a different schedule was tested with six doses in a four-week schedule (day 1, 2, 8, 9, 15, 16). DLTs were liver enzyme abnormalities and gastrointestinal hemorrhage. RP2D was 160 m/m^2/dose [25]. As far as we know, there are no phase II trials with monotherapy onalespib in molecularly unselected NSCLC patients. One phase I trial is evaluating onalespib in combination with the cyclin-dependent kinase (CDK) inhibitor AT7519M in patients with solid tumors (NCT02503709).

Molecularly selected patients: Onalespib can delay resistance to crizotinib or erlotinib in *ALK*- or *EGFR*-activated xenograft models, respectively [50]. For molecularly selected NSCLC patients, no clinical trials have been completed. However, two trials are ongoing: in patients with an *ALK*-rearrangement (phase I/II NCT01712217, onalespib with/without crizotinib) and in patients with an *EGFR* mutation who have been treated with an EGFR-TKI (phase I/II NCT02535338, onalespib with erlotinib).

2.1.5. Debio0932

Molecularly unselected patients: Debio0932 has been evaluated in a phase I trial including 50 patients with advanced previously treated cancer (7 NSCLC). DLTs were febrile neutropenia, diarrhea and asthenia and were reached at 1600 mg orally once daily. The extension cohort consisted of another 30 patients (15 NSCLC), these patients were treated with 1000 mg once daily. Approximately half of the NSCLC patients showed SD (one metabolic PR) [26]. The subsequent phase I/II trial in molecularly unselected NSCLC patients that combined debio0932 with standard of care chemotherapy has been terminated (NCT 01714037) for unknown reasons.

Molecularly selected patients: as far as we know, no trials exist.

2.2. Hsp27 inhibitors

2.2.1. Apatorsen (OGX-427)

Molecularly unselected patients: A phase I study of apatorsen (OGX-427, Hsp27 inhibitor) in patients with solid tumors (N = 42, 3 lung cancer) showed that apatorsen was well tolerated and a MTD was not defined. Approximately 1/3 of the patients had stable disease, no confirmed responses were reported [34]. Apatorsen has also been evaluated in previously untreated stage IV non-squamous NSCLC in the randomized double-blind phase II Spruce trial (N = 155). In this trial, the chemotherapy backbone was pemetrexed 500 mg/m^2 day 1 with carboplatin AUC5 day 1 and apatorsen 600 mg iv in a 3-weekly schedule. Primary end point was PFS. Median PFS was 6.0 months in the experimental arm versus 4.9 months in the comparator arm (HR 0.92, p = 0.67) [35]. Hsp27 is overexpressed in 70–98% of squamous NSCLC patients and Hsp27 upregulation by chemotherapy can be a mechanism of chemotherapy resistance. Apatorsen enhanced sensitivity to gemcitabin in a pancreatic cancer cell line [51]. Based on this background is the open-label phase II Cedar™ trial currently recruiting advanced squamous cell histology NSCLC patients: randomization is between first-line gemcitabine 1250 mg/m^2 day 1, 8/carboplatin AUC5 day 1 with or without apatorsen 600 mg iv weekly in a 3-weekly schedule (NCT02423590) [52].

Molecularly selected patients: apatorsen combined with erlotinib significantly enhanced antitumor effects when compared to erlotinib alone when tested in an *EGFR*-mutated, erlotinib-resistant cell line [17]. As far as we know, there are no currently ongoing clinical trials in molecularly selected NSCLC patients.

2.3. Hsp70 inhibitors

2.3.1. Hsp70-peptide targeted natural killer cell based adoptive immunotherapy

Molecularly unselected patients: a specific amino acid sequence (TKD) of Hsp70 has been identified as a tumor-selective recognition structure for natural killer (NK) cells. Preclinically, cytolytic NK cell activity against Hsp70-positive tumors can be enhanced when peripheral blood lymphocytes are incubated with TKD plus low-dose interleukin-2 [53,54]. One phase I trial tested the treatment of NSCLC (and colon cancer) patients with *ex vivo* Hsp70 peptide activated, autologous natural killer (NK) cells. No severe adverse events were observed, but also no responses were observed (1 patient SD) [36]. This cell based adoptive immunotherapy is currently tested in a randomized phase II trial including stage IIIA/B NSCLC patients after chemoradiotherapy (NCT 02118415).

Molecularly selected patients: as far as we know, no trials are ongoing in this specific patient population.

3. Conclusion

Hsps are upregulated in many cancers and inhibition can inhibit tumor growth by destabilizing proteins necessary for tumor survival [4]. From the Hsp inhibitors in clinical trials, Hsp90 inhibitors are the most studied in NSCLC. In molecularly unselected NSCLC patients, results of Hsp90 inhibitors are disappointing. Despite preclinical evidence that Hsp90 inhibition enhances the cytotoxicity of especially taxanes, this was not demonstrated in clinical trials. Most trials did not report whether there was a translational research part in the trial aiming to identify a subset of patients with possible higher benefit of Hsp90 inhibition. Retaspimycin, AUY922, and ganetespib will not be developed further and for onalespib only phase I trials exist. In molecularly selected NSCLC patients,

KRAS seems to be a negative predictive marker for Hsp90 inhibitor effect. Results are more promising especially in *ALK*-rearranged patients however number of included *ALK*-rearranged patients is still limited. Although monotherapy retaspimycin was not effective in those patients, AUY922 monotherapy as well as ALK-TKI combination regimens trials are ongoing (AUY922 rights were returned to Vernalis) and the same is true for ganetespib and onalespib. Retaspimycin had also disappointing results in *EGFR*-mutated patients and despite a modest 16-week PFS rate for ganetespib; no trials are currently ongoing with one of these drugs in this patient population. Monotherapy AUY922 for *EGFR* exon 20 insertions or T790M is still being evaluated as is onalespib combined with erlotinib.

For Hsp27 and Hsp70 inhibitors, not many data exist. Apatorsen combined with pemetrexed/carboplatin was not superior to chemotherapy alone in untreated stage IV non-squamous NSCLC. Results for squamous cell NSCLC are awaited and no molecularly selected trials are ongoing. Hsp70 inhibitors are still in phase I development.

4. Expert opinion

Although there is preclinical evidence and mechanistic rationale for effectiveness of Hsp inhibitors in NSCLC, the results of clinical trials are disappointing. These disappointing clinical trials resulted in discontinuation of the development of some of these compounds. A possible explanation for the disappointing results of the Hsp90 inhibitor trials in molecularly unselected patients is the activation of a heat-shock transcription factor 1 (HSF1) stress response upon Hsp90 inhibition. This was demonstrated in fibroblasts from wild type and HSF1 knockout mice. Hsp90 inhibition with 17-AAD resulted in a HSF1 stress response in the wild-type fibroblasts, and Hsp90 inhibition was more cytotoxic in the HSF1 knock-out cells. However, no Hsp90 inhibitor was used that has been tested in NSCLC patients. Hsp90 inhibition in HSF1 wild type, but not in HSF1 knock-out fibroblasts resulted in upregulation of other Hsps such as Hsp27, Hsp72 but also Hsp90 [55]. In the same study, a mouse breast cancer xenograft was treated with 17-AAD and this resulted in Hsp27 upregulation [55]. One can speculate that upregulation of these Hsps can result in blocking of apoptosis in the cancer cells. Maybe the use of a HSF1 antagonist or the combination of different Hsp antagonists would result in improved outcomes. Preclinical effect was shown for the HSF1 inhibitor 2,4-bis(4-hydroxybenzyl)phenol and for the combination of VER-155008 and 17-AAD ((a Hsp70 and Hsp90 inhibitor, respectively) [56,57]. 2,4-bis(4-hydroxybenzyl)phenol induced growth arrest and apoptosis of lung cancer cells and cotreatment of this compound with chemotherapy (paclitaxel, cisplatin) or radiation potentiated the effects of both chemo- and radiotherapy [57]. VER-155008 also inhibited cell cycle progression in a lung cancer cell line and acted synergistically with 17-AAD. Moreover, VER-155008 could sensitize the lung cancer cells to radiation [56]. At the moment, there are no clinical data with a HSF1 antagonist or a combination of a Hsp90 and a Hsp27 or Hsp70 inhibitor. Another strategy is to combine an Hsp90 inhibitor with a focal adhesion kinase (FAK) inhibitor as there is preclinical evidence that these two inhibitors act synergistically. In a NSCLC cell line, 17-AAG enhanced the inhibitory effects of PF-573228 (FAK inhibitor) on the FAK/Pi3K/Akt survival pathway and the Ras/Raf/MAP kinase survival pathway. Cotreatment significantly suppressed tumor growth compared to single-agent treatment [58]. As most of the Hsp90 inhibitors only target the N-terminal ATP-binding pocket of HSP90, cotargeting the C-terminal could be an option to increase efficacy. This C-terminal can be targeted by drugs such as novobiocin, HDN-1, chaetocin, and coumermycin A1 [59,60].

It is also possible that targeting of the Hsp itself is not enough to obtain meaningful clinical activity. There are preclinical data that the efficacy of Hsp90 inhibitors increases when co-chaperones such as CDC37, AHA1, and p23 are also targeted [61]. Currently, no clinical trials are evaluating these combinations in NSCLC or other cancer types.

Another explanation is that the level of Hsp inhibition reached in a patient is insufficient to effectively inhibit tumor cell Hsps because of undesirable off-target and/or Hsp-related adverse events. Examples of commonly observed DLTs for Hsp90 inhibitors are diarrhea and visual disorders such as night blindness or blurred vision likely caused by retinal dysfunction as Hsp90 inhibition affects the photoreceptors in the retina (overview adverse events also in Table 1, retinal dysfunction is reversible) [20,62]. Maybe the new, highly selective Hsp90 inhibitor, TAS-116 can overcome these DLTs as preclinically in a rodent model, potent antitumor activity and minimal toxicity was found. Tumor/retina ratio was high for TAS-116 compared to other Hsp90 inhibitors (5.7 vs. 0.9–2.3). Furthermore, TAS-116 did not inhibit CYP450 within the effective concentration range which is a favorable characteristic to combine this compound with other drugs [63]. As far as we know, there are currently no clinical trials with this compound. A new (preclinical) option is the development of Hsp90 drug conjugates (HDCs) that take advantage of the abundance of Hsp90 in the tumor cells to deliver a toxic payload to these cells. STA-12–8666 is an example of a HDC: a Hsp90 inhibitor fused to SN-38, the active metabolite of irinotecan [64].

A different way to move forward is to combine Hsp90 inhibition with radiation, as this can sensitize cells to radiation. For example, ganetespib accentuated G2-M arrest that was caused by radiation in NSCLC cell lines and significantly reduced clonogenic survival. However, when carboplatin-paclitaxel were added ganetespib could only sensitize some, but not all cell lines [65].

In contrast to the disappointing results in molecularly unselected NSCLC patients, some efficacy was shown in molecularly selected patients, for example *ALK*-rearranged patients. *In vitro* and *in vivo*, ganetespib overcame multiple forms of crizotinib resistance, including *ALK*-translocations [66]. In an *ALK*-rearranged NSCLC cell line Hsp90 inhibition with 17-DMAG was also able to overcome ligand-triggered resistance to alectinib, a newer ALK-TKI [67]. As discussed earlier, early phase clinical trials showed the highest ORR in this patient subgroup. However, side effects of Hsp90 antagonist therapy are usually worse than those of second-generation ALK-inhibitors. Moreover, *ALK*-rearranged patients often relapse in the brain and the second generation ALK-TKI have a higher penetration of the blood–brain barrier (BBB) than most Hsp90 inhibitors. The only two Hsp90 inhibitors

that have been reported to have a good BBB penetration rate are Debio0932 and NMS-E973 [68]. Only Debio0932 has been evaluated in a phase I trial. Toxicity was manageable, but with limited clinical activity [26]. No data are available for this compound in *ALK*-rearranged patients. It would be interesting to evaluate this drug in this patient population in combination with an ALK-TKI, to evaluate whether resistance to the ALK-TKI can be delayed, without severe adverse events and with adequate control of central nervous system metastases.

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