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# Luminespib plus pemetrexed in patients with non-squamous non-small cell lung cancer

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ABSTRACT

*Background:* Luminespib (AUY922) is a second-generation heat shock protein 90 (HSP90) inhibitor with demonstrated activity in non-small cell lung cancer (NSCLC). Since luminespib reduces levels of dihydrofolate reductase (DHFR), a key enzymatic target of pemetrexed, we assessed the safety and tolerability of luminespib in combination with pemetrexed in patients with previously treated metastatic non-squamous non-small cell lung cancer (NSCLC). We also sought to study the pharmacokinetics and correlate tumor dihydrofolate reductase (DHFR) expression with clinical response.

*Methods:* Patients received weekly luminespib at either 40 mg/m$^2$, 55 mg/m$^2$, or 70 mg/m$^2$ according to a standard 3 + 3 dose-escalation design along with pemetrexed at 500 mg/m$^2$ followed by an expansion at the maximum tolerated dose (MTD).

*Results:* Two-dose limiting toxicities (DLTs) were experienced in the 70 mg/m2 cohort, therefore the MTD was determined to be 55 mg/m$^2$. 69% (N = 9) of patients experienced ophthalmologic toxicity related to luminespib. Maximum serum concentration (*Cmax*) of luminespib was associated with increased grade 2 drug related adverse events (DRAEs) ($r_s = 0.74$, $P < 0.01$), with volume of distribution ($V_D$) inversely associated with the number of DRAEs ($r_s = -0.81$, $P = 0.004$) and ophthalmologic related DRAEs ($r_s = -0.65$, $P = 0.04$). The best response was partial response in one patient for 20 months, prior to expiration of all luminespib. Amongst patients treated at the MTD, the objective response rate was 14%.

*Conclusion:* In patients with previously treated metastatic NSCLC, the MTD of luminespib in combination with pemetrexed was 55 mg/m$^2$ per week. The combination of luminespib and pemetrexed demonstrated clinical activity. Tolerability of luminespib with pemetrexed is limited by ocular toxicity.

## 1. Introduction

Despite recent advances in therapy for non-small cell lung cancer (NSCLC), the prognosis for patients with metastatic disease who progress after prior therapy remains poor. Heat shock protein 90 (HSP90) is a molecular chaperone which facilitates protein trafficking, stabilization, and folding. HSP90 is induced in stress states including cancer [1]. HSP90 mediates numerous oncogenic driver proteins important in NSCLC pathogenesis including epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) [2].

Luminespib (AUY922) is an isoxazolyl resorcinol-based HSP90 inhibitor that is distinct from first-generation geldanamycin HSP90 inhibitors [3]. “Second-generation” inhibitors have drawn interest because of their increased potency, potentially preferable side effect profile, and more favorable pharmacokinetics [4]. We previously demonstrated preclinical activity of luminespib in a wide range of cancer cell lines [5]. A global phase II study of luminespib demonstrated clinical activity as a single-agent in patients with NSCLC, particularly

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those with *EGFR* mutations or *ALK* gene rearrangements [6]. It is plausible that a rationally selected combination of luminespib with a proven anticancer agent may increase its efficacy.

Pemetrexed is commonly used to treat NSCLC. Use is restricted to non-squamous disease and is effective in combination or as maintenance [7–9]. Pemetrexed is a folate antimetabolite which inhibits key enzymes in purine and pyrimidine synthesis, namely, thymidylate synthase (TS) and dihydrofolate reductase (DHFR) [10]. *In vitro* studies in cancer cell lines and xenograft models have demonstrated that HSP90 inhibition leads to cell cycle arrest through S-phase inhibition and downregulation of TS [11]. This is a mechanism similar to that of pemetrexed, which suggests that the combination of the two may be synergistic. Studies in murine cell lines show high binding affinity of HSP90 to chemically denatured DHFR and suggest that its chaperone activity likely involves maintaining DHFR in a folding-competent structure [12]. In addition, we found that DHFR mRNA is reliably decreased in human NSCLC cell lines exposed to luminespib [5]. Reduced DHFR expression has also been associated with pemetrexed responsiveness [13].

Therefore, we conducted a multicenter phase Ib trial, Translational Research in Oncology- United States (TRIO-US) L-05 (NCT01784640), of luminespib in combination with pemetrexed in patients with advanced NSCLC to determine the safety and tolerability of this combination. We also assessed the pharmacokinetics of luminespib in combination with pemetrexed and evaluated DHFR expression in enrolled patients.

## 2. Patients and methods

### 2.1. Patient population

Patients were 18 years or older with histologically confirmed stage IV non-squamous, NSCLC who had progressed on one or more prior lines of therapy. Eligible patients had at least one measurable lesion defined by RECIST (1.1) and had an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2. Adequate organ function (AST and ALT ≤ 1.5 × Upper Limit of Normal (ULN), or ≤ 2.5 of ULN if liver metastases are present, serum creatinine ≤ 1.5), expected survival time of ≥ 3 months, and at least a four-week washout from prior systemic therapy were required. Patients with unresolved ≥ grade 1 diarrhea, baseline QTc ≥ 450 ms, history of prior cancer within 3 years of enrollment, untreated central nervous system metastasis, prior treatment with pemetrexed, prior anti-neoplastic treatment with any HSP90 or HDAC inhibitors, or ongoing infection were excluded from the study. All patients provided written informed consent. The study was approved by the Institutional Review Board at all participating institutions and overseen by the Jonsson Comprehensive Cancer Center Data Safety Monitoring Board (DSMB).

### 2.2. Study design

Patients were assigned to one of three cohorts according to a standard 3 + 3 dose-escalation design. The expansion cohort at maximum tolerated dose (MTD) was planned to enroll twenty patients: ten patients with *EGFR* mutations, five with *ALK* gene rearrangements, and five with wild-type *KRAS*, *EGFR* and *ALK*.

Patients in each cohort received weekly intravenous luminespib at one of three doses, 40 mg/m$^2$ (cohort one), 55 mg/m$^2$(cohort two), or 70 mg/m$^2$(cohort three), in combination with pemetrexed 500 mg/m$^2$ intravenous every 21 days. Luminespib was administered on day 1 (over 60 min) at the specified dose for that cohort, followed by pemetrexed 500 mg/m$^2$ infusion (over 15 min). Participants continued to receive weekly luminespib and every 3 week pemetrexed until end of study, defined as progression of disease, death, toxicity precluding further therapy, or withdrawal of consent. Our primary objective was to assess safety and tolerability. Secondary endpoints were pharmacokinetic parameters, objective response rate (ORR) in evaluable patients, and correlation of tumor DHFR expression with response.

### 2.3. Assessments

Response was assessed using Computed Tomography (CT) scans with IV contrast of the chest and abdomen by RECIST (1.1). Additional studies were performed at the discretion of the treating physician.

Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0). DLTs were defined as drug related adverse events (DRAEs) which were either grade 4 non-hematologic toxicity, grade 4 neutropenia, thrombocytopenia requiring platelet transfusion, or grade 3 non-hematological toxicity lasting greater than seven days. Patients were evaluated weekly for treatment-related toxicities throughout cycles one and two and at the beginning of each subsequent 21-day cycle. ECGs and blood samples were obtained at predefined timepoints pre- and post- luminespib infusion. Ophthalmologic examinations before treatment and after cycles one and three included assessments of visual acuity, intraocular pressure, slit-lamp test, dilated fundus, color-vision test, and if indicated, electroretinography.

#### 2.3.1. Pharmacokinetics

Whole-blood samples for pharmacokinetic (PK) analysis were drawn at prescheduled timepoints (cycle 1 day 1: 0, 1, 2, 4, and 24 h; cycle 1 day 8: 0 and 1 h). Luminespib levels in plasma were measured using a validated liquid chromatography and tandem mass-spectrometry method by Covance Inc. (Princeton, NJ). PK parameters were defined using non-compartmental analysis by the linear trapezoidal method. PK calculations were confirmed using the *PKSolver* package [14].

### 2.4. Immunohistochemistry

DHFR expression was estimated using immunohistochemical (IHC) analysis of pretreatment formalin-fixed paraffin-embedded (FFPE) tumor tissue using the PA5-14267 DHFR antibody (ThermoFischer Scientific, Waltham, MA). Slides were optimized and compared to IHC for DHFR in the Human Protein Atlas. Slides were analyzed by two pathologists blinded to patient outcomes.

### 2.5. Statistical analysis

All statistical analyses were performed using the *R* programming language [15] and plotted using the ggplot2 package [16]. Nonparametric analysis of adverse events and pharmacokinetic parameters was performed using the Spearman's rank-order correlation. Descriptive statistics were used for the survival data given the limited sample size.

## 3. Results

### 3.1. Patients

18 patients were screened, and a total of 13 patients were enrolled in this phase 1b study (Table 1). Nine were enrolled in the dose-escalation phase and four in the expansion phase. At that time, the sponsor (Novartis; Basel, Switzerland) transferred luminespib to Vernalis (Winnersh, UK), so trial accrual and enrollment terminated early.

All patients had ECOG performance status of 0 or 1. Patient and tumor characteristics are shown (Table 1). Median age was 65 years (range 46–94). 61.5% (N = 8) were women. The population was split nearly equally between white (N = 7) or other races (N = 8). The majority of patients (N = 10) were never smokers. 69% of patients (N = 9) harbored an *EGFR* mutation, and 44% (N = 4) had exon 20 insertion in *EGFR*. This disproportionate enrollment of *EGFR* mutations, and specifically exon 20 insertions, was based on data showing particular benefits in this group and limited alternate treatment options. All

**Table 1**
Patient Demographics, Tumor Characteristics, and Response.

| Age (y) | Sex | Race | Ever Smoker | Prior Lines | Last Regimen | Tumor EGFR mut | Tumor DHFR | luminespib (mg/m2) | Best Response | Time on Trial (m) | Reason Off Trial |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 67 | F | White | Yes | 2 | targeted | none | Neg | 40 | PD | 1.2 | PD |
| 74 | M | Asian | No | 2 | targeted | L858R | Neg | 40 | SD | 1.7 | WDC |
| 85 | M | Asian | No | 3 | targeted | L858R | Neg | 40 | NA | 1.0 | SAE |
| 94 | F | Black | No | 1 | targeted | Exon 20 ins | Neg | 40 | SD | 11.2 | PD |
| 58 | F | White | Yes | 1 | chemo | none | Neg | 55 | PD | 1.4 | PD |
| 71 | M | White | No | 1 | chemo | L858R | Neg | 55 | PD | 1.4 | PD |
| 46 | F | White | No | 1 | chemo | Exon 20 ins | Neg | 55 | PD | 1.4 | PD |
| 65 | M | White | No | 2 | targeted | none | NA | 55 | SD | 1.6 | AE, WDC |
| 65 | F | White | Yes | 1 | targeted | L858R | Pos | 55 | PR | 19.6 | Supply Ended |
| 52 | M | White | No | 3 | chemo | Exon 20 ins | Neg | 55 | SD | 5.5 | PD |
| 64 | F | Other | No | 2 | chemo | Exon 19 del | Neg | 55 | PD | 1.7 | Rapid Decline |
| 75 | F | Asian | No | 3 | chemo | none | NA | 70 | NA | 0.2 | DLT |
| 50 | F | Hispanic | No | 3 | IO | Exon 20 ins | Neg | 70 | NA | 0.4 | DLT |

Abbreviations: AE = adverse event, del = deletion, DHFR = dihydrofolate reductase, EGFR = epidermal growth factor receptor, ins = insertion, IO = immuno-oncology drug, m = months, mut = mutation, NA = not available, Neg = negative by immunohistochemistry, PD = progressive disease, Pos = positive by immunohistochemistry, PR = partial response, SAE = serious adverse event, SD = stable disease, DLT = dose limiting toxicity, WDC = withdrew consent, y = years.

patients harboring a sensitizing *EGFR* mutation (N = 5) (defined as an exon 19 deletion or L858R mutation) were treated with either a first or second generation EGFR-TKI. Four patients harbored an exon 20 mutation (associated with de novo resistance to approved EGFR-TKIs), and two of them were previously treated with an EGFR-TKI [17]. An equivalent number of patients received chemotherapy (N = 6) or targeted therapy (N = 6) as their last regimen prior to the trial.

### 3.2. DLTs and MTD

Cohort one enrolled four patients after the third patient experienced atrial fibrillation which was concerning for a possible DLT. However, after review by the DSMB, the event was determined to not meet DLT criteria (grade 2), so cohort two was opened. Three patients were enrolled in cohort two without a DLT. A total of two patients enrolled in cohort three, and both experienced a DLT, grade 3 thrombocytopenia and grade 3 supraventricular tachycardia (SVT). The MTD was therefore determined to be luminespib 55 mg/m2 with pemetrexed 500 mg/m2.

### 3.3. Toxicity

The most common adverse events were fatigue, hyperglycemia, anemia, dyspnea, nausea, and diarrhea. There was only one grade 4 event, respiratory failure, unrelated to either pemetrexed or luminespib. The most common DRAE was visual complaints/ ophthalmologic toxicity (*N* = 9, 69%) followed by fatigue (*N* = 6, 46.2%) (Table 2). Four patients experienced blurred vision, and this occurred after the first or second cycle in all patients. Two of those patients had a dose interruption or dose reduction, and this led to resolution of the blurred vision. Other frequent DRAEs (experienced in > 30% of patients) included anemia (*N* = 5, 38.5%), diarrhea (*N* = 5, 38.5%) and anorexia (*N* = 4, 31%). By the end of trial, over half of patients had QTc prolongation (defined as QTcF > 440 msec) (N = 7, 53.8%), with one patient experiencing QTc prolongation as early as after cycle 1. At the MTD, 71% of 7 patients (*N* = 5) required a dose reduction, with a median relative dose intensity (RDI) of 88%. RDI was not correlated to number of grade 1, grade 2, or grade 3 AEs experienced.

### 3.4. Pharmacokinetics

The pharmacokinetic parameters of luminespib are shown in Fig. 1. Mean maximum concentration ($C_{max}$) of luminespib with pemetrexed at the MTD was 122 ng/m L. The area under the curve from zero to infinity ($AUC_{0\text{-}inf}$) for luminespib was 166.4, 679.6, and 2083.2 ng x h/m L for each cohort, respectively (Fig. 1). Elimination half-life ($t_{1/2}$) reliably increased with luminespib dose, and at the MTD was 5.64 h with an observed clearance of 153.5 L/m2/h (Fig. 1). We explored the correlation of pharmacokinetic parameters with the onset of toxicities (not shown). $C_{max}$ of luminespib was associated with increased grade 2 DRAEs ($r_s = 0.74$, $p < 0.01$), while the volume of distribution ($V_D$) was inversely associated with number of DRAEs ($r_s = -0.81$, $P = 0.004$) and ophthalmologic related DRAEs ($r_s = -0.65$, $P = 0.04$).

### 3.5. Antitumor activity

Two patients (15%) withdrew consent and two others died from disease progression. Both events occurred within two months of enrollment. Patients treated at the MTD were evaluable for response (*N* = 7). The ORR was 14% in patients treated at the MTD. The median PFS was 1.4 months. There was one partial response. This patient received luminespib 55 mg/m$^2$ for 20 months, ending when the drug supply ended. The other six patients treated at the MTD all progressed within 6 months.

### 3.6. DHFR expression was seen in the one responder

85% (11) of the pre-treatment tumor specimens were successfully analyzed for DHFR expression using IHC. All patients had absent expression or cytoplasmic expression only. The only patient with the characteristic cytoplasmic and membranous DHFR IHC staining of the tumor was the only durable responder (Supplementary Figure S1).

## 4. Discussion

Based upon promising preclinical data, this trial examined the safety and tolerability of luminespib with pemetrexed in patients with advanced non-squamous NSCLC. The MTD of luminespib in combination with pemetrexed was 55 mg/m$^2$, which was lower than the MTD when given as a single-agent or with erlotinib [18–20]. This was associated with synergistic toxicity, which may be due to combined DHFR inhibition by luminespib and pemetrexed. We observed ophthalmologic toxicity related to luminespib in 69% of patients, primarily manifesting as blurred vision. This ophthalmologic toxicity was in the range observed in a single-agent phase II trial (80%) or in combination with erlotinib (60%) [6,20]. However, low grade ocular toxicity was reversible with dose reductions and interruptions. These rates of ocular toxicity are higher than those seen with first-generation HSP90 inhibitors, which are associated with dose-limiting hepatotoxicity because of a benzoquinione moeity [21]. The increased rates of ocular

**Table 2**
Drug Related Adverse Events by Luminespib Dose*.

| | 40 mg/m2 (N = 4) | | | 55 mg/m2 (N = 7) | | | 70 mg/m2 (N = 2) | | | All (N = 13) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grade (N) | | | Grade (N) | | | Grade (N) | | | All Grades (N) | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | |
| **opthalmologic toxicity** | 2 | 0 | 0 | 3 | 3 | 0 | 0 | 1 | 0 | **9** | **(69%)** |
| blurred vision | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 4 | (31%) |
| night blindness | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | (15%) |
| flashing lights | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | (15%) |
| floaters | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | (8%) |
| dry eyes | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | (8%) |
| eye pain | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | (8%) |
| photophobia | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | (8%) |
| diplopia | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | (8%) |
| retinopathy | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | (8%) |
| color vision disturbance | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | (8%) |
| **fatigue** | 1 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | **6** | **(46%)** |
| **diarrhea** | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 0 | **5** | **(38%)** |
| **anemia** | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | **5** | **(38%)** |
| **anorexia** | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | **4** | **(31%)** |
| **nausea** | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | **3** | **(23%)** |
| **leukopenia** | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | **3** | **(23%)** |
| **muscle weakness** | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | **2** | **(15%)** |
| **neutropenia** | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | **2** | **(15%)** |
| **thrombocytopenia** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | **2** | **(15%)** |
| **dehydration** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **2** | **(15%)** |
| **supraventricular tachycardia** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | **1** | **(8%)** |

*Including most commonly experienced adverse events (occurring in > 10% of all patients) and all grade 3 or greater adverse events. There were no grade 4 drug related adverse events.

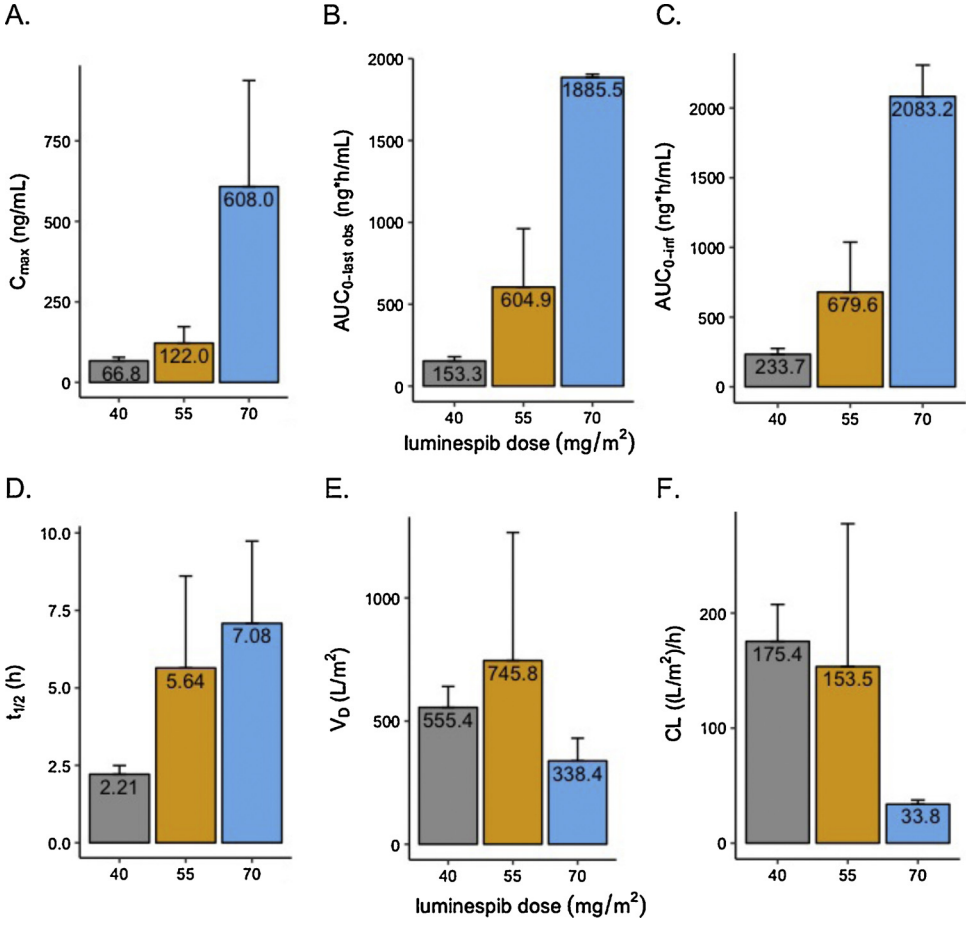


**Fig. 1.** Noncompartmental pharmacokinetic analysis of luminespib, depicting mean of the maximum concentration ($C_{max}$) (A), area under the curve (AUC) of luminespib from time zero to last observation (B), an improved estimation of AUC – extrapolation of AUC from zero to infinity (C), half-life ($t_{1/2}$) (D), volume of distribution ($V_D$) (E), and clearance (CL) (F). Means are stated on each plot with standard error bars depicted above.

toxicity may be explained by prolonged inhibition of HSP90 by luminespib in photoreceptors and a higher retina to plasma exposure ratio [22].

HSP90 is potentially appealing target in cancer because the list of oncogenes which it stabilizes is long. This phase I study demonstrates that the combination of luminespib and pemetrexed has clinical activity, but the trial was not designed nor powered to look at efficacy. However, experience from other trials looking at the combination of a second-generation HSP90 inhibitor with chemotherapy for NSCLC patients have been disappointing. The randomized phase II trial of ganetespib and docetaxel in advanced NSCLC patients (GALAXY-1) failed to demonstrate progression-free survival (PFS) benefit [23]. The only phase III trial of an HSP90 inhibitor in NSCLC (GALAXY-2) randomized 677 patients with advanced NSCLC to docetaxel or docetaxel plus ganetespib, but failed to improve PFS and was terminated early [24].

The use of HSP90 inhibitors for molecularly defined subsets of NSCLC has shown somewhat better results [25,20,6,26]. However, the greatest benefit appears to be in patients with *ALK* gene rearrangements [27,28], with a 32% response rate with single-agent luminespib in a phase II trial [6]. EGFR and the EML4-ALK translocation product are both client proteins of HSP90 which are degraded by its inhibition, and this may explain why luminespib may be particularly effective in these patients [29,5,30]. Luminespib has demonstrated activity in patients harboring *EGFR* mutations, even T790M + disease, suggesting a role in overcoming acquired resistance, but the utility seems limited in the era of third-generation EGFR-TKIs [20,31,6]. None of the patients with a sensitizing *EGFR* mutation in this trial were previously treated with a third-generation EGFR-TKI because none were approved at the time. By today's standard of care, most of these patients would have either received front-line osimertinib or osimertinib after progression on a prior EGFR-TKI. In our trial, the longest responder harbored an L858R mutation, but mutation testing for T790 M was unable to be performed. Only two of four patients with an exon 20 insertion were previously treated with an EGFR-TKI because of low response rates of 3–8% to first and second generation TKIs [17]. Studies have suggested a role for luminespib in patients with exon 20 insertions, for whom treatment options are limited [32,6]. One of two patients with an *EGFR* exon 20 insertion evaluable for response had stable disease for 5.5 months.

Concerns have been raised that once weekly treatment may not achieve sustained oncoprotein suppression, and perhaps newer orally administered HSP90 inhibitors such as TAS-116 or SNX-5422 may be able to overcome this issue [4]. Multiple redundant mechanisms overcome the effects of HSP90 inhibition through activation of the heat shock response (HSR) which induces alternative heat shock proteins (HSP70, HSP40, HSP27) and has also been considered a potential impediment [33]. The first-in-class C-terminal HSP90 inhibitor (aminoxyrone), unlike other N-terminal HSP90 inhibitors, does not elicit the HSR, and may be a promising avenue given its novel mechanism of action [34,35].

In our trial, one patient was still responding to treatment at 20 months prior to the supply of drug ending, suggesting that further studies should be undertaken to understand which subset of patients respond best to HSP90 inhibition, although it is possible that the result was driven entirely by pemetrexed. None of the trials using HSP90 inhibitors have identified a lead candidate as a predictive biomarker. Our observation of tumor DHFR expression in the one durable responder is interesting, but requires prospective validation.

In summary, luminespib with pemetrexed had a manageable safety profile similar to that of luminespib in other trials, and was limited by ophthalmologic toxicity, and to a lesser extent, cardiac toxicity. The trial ended early due to a change in its sponsor. There was clinical activity in previously treated patients with metastatic non-squamous NSCLC, but were luminespib to be evaluated further for clinical use, biomarkers to select the subset of patients likely to benefit luminespib would be key, particularly given the associated toxicities.

## Disclosures

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.05.022.

## References

[1] F.H. Schopf, M.M. Biebl, J. Buchner, The HSP90 chaperone machinery, Nat. Rev. Mol. Cell Biol. 18 (6) (2017) 345–360, https://doi.org/10.1038/nrm.2017.20.

[2] R. Garcia-Carbonero, A. Carnero, L. Paz-Ares, Inhibition of HSP90 molecular chaperones: moving into the clinic, Lancet Oncol. 14 (9) (2013) e358–69, https://doi.

org/10.1016/s1470-2045(13)70169-4.
[3] T. Taldone, A. Gozman, R. Maharaj, G. Chiosis, Targeting Hsp90: small-molecule inhibitors and their clinical development, Curr. Opin. Pharmacol. 8 (4) (2008) 370–374, https://doi.org/10.1016/j.coph.2008.06.015.
[4] R.N. Pillai, S.S. Ramalingam, Throwing more cold water on heat shock protein 90 inhibitors in NSCLC, Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer 13 (4) (2018) 473–474, https://doi.org/10.1016/j.jtho.2018.02.010.
[5] E.B. Garon, R.S. Finn, H. Hamidi, J. Dering, S. Pitts, N. Kamranpour, et al., The HSP90 inhibitor NVP-AUY922 potently inhibits non-small cell lung cancer growth, Mol. Cancer Ther. 12 (6) (2013) 890–900, https://doi.org/10.1158/1535-7163.Mct-12-0998.
[6] E. Felip, F. Barlesi, B. Besse, Q. Chu, L. Gandhi, S.W. Kim, et al., Phase 2 study of the HSP-90 inhibitor AUY922 in previously treated and molecularly defined patients with advanced non-small cell lung Cancer, J. Thorac. Oncol 13 (4) (2018) 576–584, https://doi.org/10.1016/j.jtho.2017.11.131.
[7] T. Ciuleanu, T. Brodowicz, C. Zielinski, J.H. Kim, M. Krzakowski, E. Laack, et al., Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study, Lancet 374 (9699) (2009) 1432–1440, https://doi.org/10.1016/s0140-6736(09)61497-5.
[8] L. Gandhi, D. Rodriguez-Abreu, S. Gadgeel, E. Esteban, E. Felip, F. De Angelis, et al., Pembrolizumab plus chemotherapy in metastatic non-small-Cell lung Cancer, N. Engl. J. Med. 378 (22) (2018) 2078–2092, https://doi.org/10.1056/NEJMoa1801005.
[9] J.H. Schiller, A new standard of care for advanced lung Cancer, N. Engl. J. Med. 378 (22) (2018) 2135–2137, https://doi.org/10.1056/NEJMe1804364.
[10] C. Shih, V.J. Chen, L.S. Gossett, S.B. Gates, W.C. MacKellar, L.L. Habeck, et al., LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes, Cancer Res. 57 (6) (1997) 1116–1123.
[11] G.P. Nagaraju, O.B. Alese, J. Landry, R. Diaz, B.F. El-Rayes, HSP90 inhibition downregulates thymidylate synthase and sensitizes colorectal cancer cell lines to the effect of 5FU-based chemotherapy, Oncotarget 5 (20) (2014) 9980–9991.
[12] M. Yonehara, Y. Minami, Y. Kawata, J. Nagai, I. Yahara, Heat-induced chaperone activity of HSP90, J. Biol. Chem 271 (5) (1996) 2641–2645.
[13] D. Zhang, N. Ochi, N. Takigawa, Y. Tanimoto, Y. Chen, E. Ichihara, et al., Establishment of pemetrexed-resistant non-small cell lung cancer cell lines, Cancer Lett. 309 (2) (2011) 228–235, https://doi.org/10.1016/j.canlet.2011.06.006.
[14] Y. Zhang, M. Huo, J. Zhou, S. Xie, PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel, Comput. Methods Programs Biomed 99 (3) (2010) 306–314, https://doi.org/10.1016/j.cmpb.2010.01.007.
[15] R Development Team. R: a Language and Environment for Statistiacl Computing, R Foundation for Statistical Computing, Vienna, Austria, 2010.
[16] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer, New York, NY, 2009.
[17] S. Vyse, P.H. Huang, Targeting EGFR exon 20 insertion mutations in non-small cell lung cancer, Signal Transduct. Target. Ther. 4 (1) (2019) 5, https://doi.org/10.1038/s41392-019-0038-9.
[18] T. Doi, Y. Onozawa, N. Fuse, T. Yoshino, K. Yamazaki, J. Watanabe, et al., Phase I dose-escalation study of the HSP90 inhibitor AUY922 in Japanese patients with advanced solid tumors, Cancer Chemother. Pharmacol 74 (3) (2014) 629–636, https://doi.org/10.1007/s00280-014-2521-x.
[19] C. Sessa, G.I. Shapiro, K.N. Bhalla, C. Britten, K.S. Jacks, M. Mita, et al., First-in-human phase I dose-escalation study of the HSP90 inhibitor AUY922 in patients with advanced solid tumors, Clin. Cancer Res. 19 (13) (2013) 3671–3680, https://doi.org/10.1158/1078-0432.Ccr-12-3404.
[20] M.L. Johnson, Hart E.M. Yu HA, B.B. Weitner, A.W. Rademaker, J.D. Patel, et al., Phase I/II study of HSP90 inhibitor AUY922 and erlotinib for EGFR-Mutant lung Cancer With acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors, J. Clin. Oncol.: Offi. J. Am. Soc. Clin. Oncol. 33 (15) (2015) 1666–1673, https://doi.org/10.1200/jco.2014.59.7328.
[21] K. Jhaveri, T. Taldone, S. Modi, G. Chiosis, Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers, Biochim. Biophys. Acta 1823 (3) (2012) 742–755, https://doi.org/10.1016/j.bbamcr.2011.10.008.
[22] D. Zhou, Y. Liu, J. Ye, W. Ying, L.S. Ogawa, T. Inoue, et al., A rat retinal damage model predicts for potential clinical visual disturbances induced by Hsp90 inhibitors, Toxicol. Appl. Pharmacol. 273 (2) (2013) 401–409, https://doi.org/10.1016/j.taap.2013.09.018.
[23] S. Ramalingam, G. Goss, R. Rosell, G. Schmid-Bindert, B. Zaric, Z. Andric, et al., A randomized phase II study of ganetespib, a heat shock protein 90 inhibitor, in combination with docetaxel in second-line therapy of advanced non-small cell lung cancer (GALAXY-1), Ann. Oncol. 26 (8) (2015) 1741–1748, https://doi.org/10.1093/annonc/mdv220.
[24] R. Pillai, D. Fennell, V. Kovcin, T. Ciuleanu, R. Ramlau, D. Kowalski, et al., PL03.09: phase 3 study of Ganetespib, a heat shock protein 90 inhibitor, with docetaxel versus docetaxel in advanced non-small cell lung cancer (GALAXY-2), J. Thorac. Oncol. 12 (1) (2017) S7–S8, https://doi.org/10.1016/j.jtho.2016.11.009.
[25] M.A. Socinski, J. Goldman, I. El-Hariry, M. Koczywas, V. Vukovic, L. Horn, et al., A multicenter phase II study of ganetespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer, Clin. Cancer Res. 19 (11) (2013) 3068–3077, https://doi.org/10.1158/1078-0432.Ccr-12-3381.
[26] L.V. Sequist, S. Gettinger, N.N. Senzer, R.G. Martins, P.A. Jänne, R. Lilenbaum, et al., Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non–small-cell lung cancer, J. Clin. Oncol 28 (33) (2010) 4953–4960, https://doi.org/10.1200/JCO.2010.30.8338.
[27] J. Sang, J. Acquaviva, J.C. Friedland, D.L. Smith, M. Sequeira, C. Zhang, et al., Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer, Cancer Discov. 3 (4) (2013) 430–443, https://doi.org/10.1158/2159-8290.Cd-12-0440.
[28] A. Tanimoto, T. Yamada, S. Nanjo, S. Takeuchi, H. Ebi, K. Kita, et al., Receptor ligand-triggered resistance to alectinib and its circumvention by Hsp90 inhibition in EML4-ALK lung cancer cells, Oncotarget 5 (13) (2014) 4920–4928.
[29] T. Shimamura, A.M. Lowell, J.A. Engelman, G.I. Shapiro, Epidermal growth factor receptors harboring kinase domain mutations associate with the heat shock protein 90 chaperone and are destabilized following exposure to geldanamycins, Cancer Res. 65 (14) (2005) 6401–6408, https://doi.org/10.1158/0008-5472.Can-05-0933.
[30] E. Normant, G. Paez, K.A. West, A.R. Lim, K.L. Slocum, C. Tunkey, et al., The Hsp90 inhibitor IPI-504 rapidly lowers EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models, Oncogene 30 (22) (2011) 2581–2586, https://doi.org/10.1038/onc.2010.625.
[31] T.S. Mok, Y.-L. Wu, M.-J. Ahn, M.C. Garassino, H.R. Kim, S.S. Ramalingam, et al., Osimertinib or platinum–pemetrexed in EGFR T790M–positive lung cancer, N. Engl. J. Med 376 (7) (2017) 629–640, https://doi.org/10.1056/NEJMoa1612674.
[32] Z. Piotrowska, D.B. Costa, M. Huberman, G.R. Oxnard, J.F. Gainor, R.S. Heist, et al., Activity of AUY922 in NSCLC patients with EGFR exon 20 insertions, J. Clin. Oncol. 33 (Suppl.15) (2015) 8015, https://doi.org/10.1200/jco.2015.33.15_suppl.8015.
[33] X. Wang, M. Chen, J. Zhou, X. Zhang, HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy (Review), Int. J. Oncol. 45 (1) (2014) 18–30, https://doi.org/10.3892/ijo.2014.2399.
[34] S. Bhatia, D. Diedrich, B. Frieg, H. Ahlert, S. Stein, B. Bopp, et al., Targeting HSP90 dimerization via the C terminus is effective in imatinib-resistant CML and lacks the heat shock response, Blood 132 (3) (2018) 307.
[35] J.C. Byrd, HSP90 inhibition without heat shock response, Blood 132 (3) (2018) 241.