Title: Ganetespib in combination with pemetrexed-platinum chemotherapy in patients with pleural Mesothelioma (MESO-02): A phase Ib trial

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Conflicts of Interest Statement

DAF has research grants with Astex Therapeutics, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Tesaro and has received Honoraria from Aldeyra, Astra Zeneca, Bayer, Boehringer Ingelheim, Inventiva, MSD, and Roche. MF has research grants with AstraZeneca, Boehringer Ingelheim, MSD and Merck and has received Honoraria from Achilles, AstraZeneca, Bayer, BMS, Celgene, Guardant Health, Merck, MSD, Nanobiotix, Novartis, Pfizer, Roche and Takeda. AH received a research grant from Synta Pharmaceuticals to conduct the study and provide ganetespib (the study drug) for free. GMW has received honoraria from AstraZeneca and Novametrics Consulting. The remaining authors declare no potential conflicts of interest.

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TRANSLATIONAL RELEVANCE

There remains an unmet need for effective therapies for front-line treatment of Malignant Pleural Mesothelioma (MPM). Heatshock protein 90 (Hsp90) inhibition reportedly induce apoptosis in MPM and mediate synergistic cisplatin-related toxicity in pre-clinical studies. Ganetespib, a potent small molecule Hsp90 inhibitor, demonstrates significant activity for down-regulating Hsp90 client protein levels with acceptable toxicity in single-agent phase II solid tumor studies. Furthermore, Hsp90 inhibition with ganetespib can enhance T-cell-mediated anti-tumor immune response. We present the MESO-02 trial of ganetespib plus pemetrexed and cisplatin/carboplatin in chemotherapy-naïve MPM patients. This novel combination was well-tolerated. We observed promising anti-tumor activity including partial responses, particularly in patients with epithelioid histology, and Loss of Heterozygosity was associated with shorter time to progression. Response rates of ganetespib are comparable or better than those observed in other novel-agent MPM trials. This study supports further investigation of ganetespib combination therapy to treat MPM in a large randomized controlled trial.

ABSTRACT

Purpose

Ganetespib, a highly potent, small molecule Heatshock protein 90 inhibitor, has potential efficacy in malignant pleural mesothelioma (MPM) via activity on critical survival pathways and known synergies with antifolates and platinum chemotherapy. We conducted a dose-escalation study to identify the Maximum Tolerated Dose (MTD) of ganetespib in chemotherapy-naïve MPM patients.

Experimental Design

MESO-02 (ClinicalTrials.gov: NCT01590160) was a non-randomized, multicentre, phase Ib trial of 3-weekly ganetespib (100 mg/m^2, 150 mg/m^2, 200 mg/m^2; days 1 and 15) with pemetrexed (500 mg/m^2; day 1) and cisplatin (75 mg/m^2; day 1) or carboplatin (area under concentration-time curve 5; day 1) in MPM patients. Dose-escalation was performed using the 3+3 design (cisplatin) and accelerated titration design (carboplatin). Secondary endpoints included best response, progression-free survival (PFS) and pharmacogenomic analyses.

Results

Of 27 patients enroled (cisplatin, *n*=16; carboplatin, *n*=11), 3 experienced dose-limiting toxicities: grade 3 nausea (cisplatin, *n*=1; carboplatin, *n*=1); grade 2 infusion-related reaction (carboplatin, *n*=1). Ganetespib's MTD was 200 mg/m^2. Partial response was observed in 14/27 patients (52%; 61% in 23 response-evaluable patients) and 13/21 (62%) with epithelioid histology. At the MTD, 10/18 patients (56%) had partial response, 15/18 (83%) had disease control, and median PFS was 6.3 months (95% CI 5.0-10.0). One responder exhibited disease control beyond 50 months. Global Loss of Heterozygosity was associated with shorter time to progression (Hazard Ratio 1.12, 95% CI 1.02-1.24; *p*=0.018).

Conclusions

Ganetespib can be combined safely with pemetrexed and platinum chemotherapy to treat patients with MPM. This class of agent should be investigated in larger randomized studies.

Key words: phase Ib, mesothelioma, heatshock protein, ganetespib, platinum therapy

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an incurable, rapidly lethal cancer arising most commonly from the parietal pleural mesothelium, and is associated with exposure to asbestos. Although the number of deaths due to MPM has increased globally[1] there has been no new licenced therapy since 2004. The combination of an anti-folate and platinum agent is an effective front-line treatment for MPM. Pemetrexed and cisplatin, the approved standard, has a response rate of 41.3%, with a median progression free survival (PFS) of 5.7 months and median overall survival (OS) of 12.1 months[2]. Using carboplatin instead of cisplatin has comparable activity[3], and a platinum combination with another anti-folate raltitrexed is also effective[4].

There remains an unmet clinical need for new, effective therapies that can improve outcomes in the front-line treatment setting. Addition of the vascular endothelial growth factor inhibitor bevacizumab to pemetrexed-cisplatin therapy can improve overall survival (18.8 months with bevacizumab vs. 16.1 months without; hazard ratio (HR) = 0.77, p=0.0167) and progression-free survival (9.2 months with bevacizumab vs. 7.3 months without; HR = 0.61, p<0.0001), but is not licenced[5], and is only recommended by National Comprehensive Cancer Network guidelines to be used in unresectable patients who are able to receive bevacizumab[6]. No positive randomized controlled studies have shown improved survival in either the maintenance[7] nor relapsed treatment settings[8-10]. A recent open label phase II trial did however show improvement in progression free survival with switch maintenance gemcitabine[11].

Heatshock protein 90 (Hsp90) is a molecular chaperone that mediates post-translational stabilisation of critical oncogenic signalling molecules, via a repertoire of client proteins that include oncogenic kinases relevant to MPM such as AXL and MET[12]. Hsp90 inhibition has been reported to induce apoptosis in MPM via an MCL1 dependent mechanism[13] and facilitates the evolution of drug resistance[14]. Acquisition of aneuploidy has been reported as a mechanism of resistance to Hsp90 inhibition[15].

Thymidylate synthase (TS) is a Hsp90 client, implicated in anti-folate resistance which is downregulated following inhibition of Hsp90[16]. Furthermore, pre-clinical studies show that inhibition of Hsp90 mediates synergistic toxicity due to cisplatin[17]. Ganetespib (ADX-1612), an Hsp90 inhibitor, is a synthetic quadricyclic triazolone with a small molecular weight that binds to the adenosine triphosphate pocket in the N-terminus of Hsp90[17,18]. Single agent ganetespib demonstrates significant activity for down-regulating Hsp90 client protein levels with acceptable toxicity at a recommended dose of 200 mg/m^2 from phase II studies[18,19]. Furthermore, inhibition of Hsp90 with ganetespib has been shown to enhance T-cell-mediated anti-tumor immune response[20]. We hypothesized that the addition of ganetespib to pemetrexed and either cisplatin or carboplatin, can be safely delivered, that there might be a synergistic interaction clinically, and that patients harbouring genomic instability (reflected in somatic copy number alterations, loss of heterozygosity and homozygous deletions) might exhibit resistance to ganetespib.

MATERIALS AND METHODS

MESO-02 (ClinicalTrials.gov identifier: NCT01590160) was a multicentre phase I/II study of first line ganetespib with pemetrexed/platinum, in patients with malignant pleural mesothelioma. Here, we report the results of the phase Ib stage, in which the primary objective was to find a safe dose of ganetespib when combined with standard platinum and pemetrexed. A major secondary objective was to examine clinical efficacy. The trial was conducted in accordance with the World Medical Association Declaration of Helsinki and approved by the UK Medicines and Healthcare Products Regulatory Agency (clinical trial authorization number: 20363/0317/001-0001), the Research Ethics Service Committee East Midlands, Derby (REC reference no. 12/EM/0448), and the research and development

department of each participating National Health Service trust. All patients provided written informed consent.

Eligibility

Key inclusion criteria included: age 18 years or older; histopathological confirmation of MPM, with measurable disease using meso-modified Response Evaluation Criteria in Solid Tumors (RECIST) v1.0[21]; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1; adequate haematological status and organ function; and chemotherapy naïve. Key exclusion criteria included: evidence of CNS metastases that require local treatment prior to systemic cytotoxic chemotherapy; receipt of extensive radiation therapy (except drain site radiotherapy), systemic chemotherapy, or other anti-neoplastic therapy within 4 weeks before enrolment; uncontrolled intercurrent illnesses; known serious cardiac illness; and history of prior gastrointestinal illness. Detailed eligibility criteria are given in the study protocol (see Supplementary Appendix).

Treatment

Patients were given a one-hour intravenous ganetespib infusion on days 1 and 15 of each 21-day cycle, at one of three dose levels: 100 mg/m^2, 150 mg/m^2, or 200 mg/m^2. Patients also received a 10-minute intravenous pemetrexed infusion of 500 mg/m^2 (with vitamin B_{12} and folate supplementation) immediately after ganetespib infusion on day 1 only. All patients received either cisplatin (75 mg/m^2 intravenously over 2 hours), or carboplatin (AUC5 intravenously over 30 minutes), 30 minutes after the completion of pemetrexed infusion. Patients in the trial were initially only allowed cisplatin, and once the safety profile was shown to be acceptable carboplatin was allowed subsequently (at the clinician's discretion and influenced by expected patient tolerability). Having either platinum agent was incorporated in the trial to reflect routine practice.

Study design

Within the MESO-02 study there were separate cohorts for cisplatin or carboplatin, to ensure acceptable safety of the combination of ganetespib and platinum therapy specifically in MPM patients.

For patients receiving the ganetespib-pemetrexed-cisplatin triplet (i.e. the 'cisplatin cohort'), dose-escalation of ganetespib was conducted using the 3+3 design with a starting dose of 100 mg/m^2. In each cohort, if no dose-limiting toxicities (DLTs) were observed, recruitment proceeded to the next cohort of 3 patients. If there was one DLT, the cohort involved was expanded to 6. If there were no further DLTs in the cohort of 6, the next cohort was administered ganetespib with chemotherapy at the next highest dose. If 2 or more DLTs were observed in three or six patients at a given dose, dose-escalation would discontinue and no higher dose considered. The cohort at the estimate of the MTD was then expanded to 9 patients overall. The maximum planned sample size for the cisplatin cohort was 27 patients.

For patients receiving carboplatin with ganetespib and pemetrexed (i.e. the 'carboplatin cohort'), dose-escalation of ganetespib was conducted using an accelerated titration design with a starting dose of 100 mg/m^2. At dose levels below 200 mg/m^2, one patient would receive treatment; if no DLT was observed, the next patient would receive the next highest dose; otherwise, a 3+3 design would begin (i.e. the same as for the cisplatin-treated cohort). If ganetespib reached the estimate of the MTD, the cohort was expanded to 9 patients overall. An accelerated titration design was used here as the carboplatin cohort was introduced following a protocol amendment after 9 patients had been treated with cisplatin, none of whom experienced any DLTs. An accelerated escalation design towards 200 mg/m^2 ganetespib struck a balance between quickly moving towards a likely safe dose, whilst still

allowing for re-introduction of a 3+3 procedure if any DLTs were observed. The maximum planned sample size for the carboplatin cohort was 18 patients.

Patients who completed 6 cycles of chemotherapy without signs of disease progression in either cohort could go on to receive ganetespib as maintenance monotherapy, using the same dose they had already been given. Ganetespib would be given on days 1 and 15 of each 21-day cycle, and continued until toxicity, progression, or patients decided to stop.

Patient assessments

Collection of archival formalin-fixed paraffin-embedded (FFPE) diagnostic tissue was mandatory and undertaken at each patient's screening visit prior to registration. Haematological profiling comprised assessment of the haematocrit, haemoglobin, red cell count (RCC), white cell count with differential, and platelets, Biochemical profiling comprised sodium, potassium, urea, creatinine, chloride, bicarbonate, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST) or alanine aminotransferase (ALT), lactate dehydrogenase (LDH), albumin, total protein, calcium, phosphate, magnesium) and urinalysis (pH, protein, blood, ketones, glucose). These were conducted before each treatment cycle (full blood count and biochemistry, also before day 15 of cycles 1 and 2; urinalysis, also before day 15 of cycle 1). Patients underwent a CT scan of the chest and abdomen for disease response assessment within 28 days of registration, after chemotherapy cycles 2, 4 and 6, then every 6 weeks for 12 months. Scans were assessed according to meso-modified RECIST v1.0[21].

Outcome measures

Toxicities were graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE v4.0). A DLT was defined as any of the following adverse

events deemed definitely, probably, or possibly related to ganetespib therapy: grade 3 or 4 non-hematologic events except diarrhoea, nausea and vomiting lasting more than 48 hours despite maximum medical therapy; grade 4 thrombocytopenia or neutropenia lasting longer than 7 days; febrile neutropenia, any drug-related adverse event leading to an interruption of ganetespib for longer than 14 days; or any clinically significant toxicity leading to dose reduction for ganetespib. DLT assessment applied to cycles 1 and 2 only for patients in the cisplatin cohort, and cycle 1 only for the carboplatin cohort. Dose-escalation decisions and DLT reviews were made by members of the Trial Management Group.

Efficacy outcomes were progression-free survival (PFS), the time from study registration to confirmed disease progression or death by any cause and overall survival (OS), death by any cause, and best response rate (defined as the percentage of patients with a best overall response of complete response (CR) or partial response (PR) as per meso-modified RECIST v1.0). For DNA copy number analyses, genomic variables of interest were somatic copy number alterations (SNCA; number of somatic changes to chromosome structure that lead to gain or loss in copies of sections of DNA), global loss of heterozygosity (LOH; the number of somatic cells containing only one copy of an allele), and total homozygous deletions (total number of biallelic copy number losses). These were assessed in the baseline (diagnostic) tumor sample; 80 ng of DNA were extracted from archival diagnostic formalin fixed paraffin embedded (FFPE) tissue blocks with the QIAmp DNA Mini Kit (Qiagen) and analysed using the OncoScan FFPE Assay Kit (Thermo Fisher), which utilizes molecular inversion probe (MIPs) technology. MIP probes were added to the FFPE DNA for annealing performed for 16-18 hours. Gap fill reaction was then performed. Uncircularized MIP probes and genomic DNA were digested and circular MIP probes were linearized and amplified by PCR. Following a second round of PCR amplification amplicons were cleaved into smaller DNA fragments with the HaeIII enzyme to improve sample hybridization onto the OncoScan arrays. Samples were allowed to hybridize for 16-18 hours. After hybridization arrays were stained and washed and loaded

into the GeneChip Scanner[22]. BioDiscovery Nexus Express for OncoScan software was then used to define copy number alterations and loss of heterozygosity. The software uses the TuScan algorithm to generate an estimate of tumor ploidy and aberrant cell fraction at each copy number change. Samples were analysed retrospectively following completion of study enrolment, though blinded to knowledge of both safety and efficacy outcomes from the patients themselves.

Statistical Analysis

The MTD of ganetespib plus pemetrexed and platinum therapy was determined by the dose-escalation design and expansion phase in each platinum therapy cohort. Kaplan Meier methods were used to analyse PFS and OS. For exploratory genomic analyses, time to progression and associations with genomic variables (SCNA, LOH and total homozygous deletions at baseline) were assessed using separate Cox regressions, with each genomic variable included as a covariate and time to progression as an outcome (patients with no confirmed progression date were censored at last date known to be alive). Spearman's correlation was used to assess the association between best percentage change in total tumor burden (TTB, defined as the sum of six pleural measurements in millimetres determined by CT scan as per modified RECIST) from baseline and genomic variables. All analyses were conducted on data frozen on 4th February 2019 using Stata version 15.1[23].

RESULTS

Patient characteristics

Between 4th September 2013 and 10th November 2015, 27 chemo-naïve patients with a confirmed diagnosis of MPM (25 male, 2 female) were recruited. Patient characteristics are shown in Table 1. Median age was 66 years (range 37-76). Non-epithelioid MPM accounted for 22% of patients. Twenty-one patients (78%) had an ECOG PS 1. Only two patients were deemed to have a good prognostic score based on the EORTC prognostic scoring system[24]. There were 16 patients in the cisplatin cohort, and 11 in the carboplatin cohort.

Treatment delivered, toxicity and dose modifications

Patients in the cisplatin cohort were administered a median of 4 (range 1-6) treatment cycles (ganetespib and chemotherapy). Patients in the carboplatin cohort were administered a median of 3 (range 1-5) treatment cycles (see Supplementary Table 1). Five patients in the carboplatin cohort (45%) chose to withdraw from the study, with two doing so after experiencing adverse events; full reasons for treatment discontinuation/withdrawal from the study are provided in Supplementary Table 2. Maintenance ganetespib therapy was received by a total of 14 patients with a median of 2 cycles (range 1-60), and 2 patients had ≥10 cycles.

Supplementary Table 3 shows the DLTs observed in each ganetespib dose cohort. At the 200 mg/m^2 ganetespib dose level when the cohort was expanded from three to nine patients, one patient in the cisplatin cohort had a DLT (grade 3 toxicity comprising nausea lasting > 48 hours). In the carboplatin cohort at 200 mg/m^2 ganetespib, one patient in the first three treated experienced DLT (grade 2 infusion related reaction). An additional three patients were recruited to this dose level, and no more DLTs were observed. The cohort was expanded to nine patients, with one patient experiencing DLT (grade 3 nausea). Given the observed DLTs, 200 mg/m^2 was considered to be the MTD.

Grade 3 and 4 toxicities are summarized in Table 2. There were no grade 4 toxicities at the 100 mg/m^2 ganetespib dose level in either the cisplatin or carboplatin cohorts. At the 150

mg/m^2 dose in the cisplatin cohort, one patient experienced grade 4 hearing impairment. The most common grade 3 and 4 adverse effects were all related to anaemia (6 patients; 22%), decreased neutrophil counts (4 patients, 15%), and nausea/vomiting (4 patients, 15%).

Four patients in the cisplatin cohort had reduced cisplatin (median 56 mg/m^2; range 37-56) for a median of 1 cycle (range 1-2). No dose reductions in ganetespib were required in the cisplatin cohort. In the carboplatin cohort, four patients had reduced carboplatin (median 405 mg/m^2; range 250-480), all for 1 cycle. One patient had their ganetespib dose reduced from 150 mg/m^2 to 112mg/m^2 (1 of 2 treatment cycles), and another patient assigned to receive 200 mg/m^2 ganetespib did not receive ganetespib on day 15 of cycles 1 and 2 due to haematological toxicities. Dose reductions for pemetrexed occurred in four patients in the carboplatin cohort (reduction from 500 mg/m^2 to 375 mg/m^2) and occurred for a median of 1 cycle (range 1-2).

Efficacy outcomes

Median observed follow-up time for all patients was 10.7 months (range 2.3-49.4). Median follow-up time was 12.3 months (range 3.6-49.4) in the cisplatin cohort, and was 8 months (2.3-20.8) in the carboplatin cohort. Overall, 23 patients were evaluable for response (i.e. had at least one evaluable response assessment conducted following treatment). Partial response (≥30% reduction in total tumor burden from baseline) was observed in 14 out of 27 patients (Objective Response Rate (ORR) 52%; 95% CI 32%-71%), with 10 out of 18 patients (56%; 95% CI 31%-78%) receiving 200 mg/m^2 of ganetespib achieving a partial response (Figure 1). When only based on patients with evaluable disease the ORR was 61% (14/23). Twenty-two out of 27 patients (81%) had disease control (partial response or stable disease), and one patient had progressive disease. For the 15 evaluable patients treated at the MTD, all (100%) had disease control. Patients with non-epithelioid histology had a lower ORR (1/6; 17%) compared to patients with epithelioid histology (13/21; ORR 62%; 95% CI

38%-82%). Five out of 6 patients with non-epithelioid histology received lower ganetespib doses (100 mg/m^2 or 150 mg/m^2). However, all but one of the 6 patients with non-epithelioid MPM had disease control, stable disease or better (<20% increase in tumor burden). One patient receiving the MTD of ganetespib exhibited a prolonged response, reaching its nadir after 12 months, and was sustained for over 40 months.

Nineteen patients had disease progression, and 21 out of 27 patients died. Sixteen patients progressed and died later, and 5 patients died without prior reported progression, the causes of which were MPM. All 5 of these patients had withdrawn from the trial due to patient choice of treatment delay > 28 days and were followed up only for assessing overall survival. In all patients, the median PFS was 5.8 months (95% CI 5.0-8.0) (Supplementary Figure 1), though was not significantly different between the different platinum therapies (median PFS of 5.8 months in both cohorts; HR for cisplatin vs. carboplatin 0.81 (95% CI 0.34-1.91, *p*=0.63)) (Supplementary Figure 2). As with tumor volume, PFS was shown to improve with increasing doses of ganetespib, though not statistically significant (log-rank test for trend, *p*=0.10) (Supplementary Figure 3). In patients who had 200 mg/m^2, the median PFS was 6.3 months (95% CI 5.0-10.0). Median overall survival was 11.5 months (95% CI 8.0-19.5) (Supplementary Figure 4), but differed across platinum therapies (cisplatin = 14.4 months, 95% CI 6.3-28.7; carboplatin = 10.6 months, 95% CI 6.3-19.5) (Supplementary Figure 5). Overall survival at the MTD was 16.3 months (95% CI 8.0-21.7) (Supplementary Figure 6).

Somatic copy Number Alterations and clinical outcomes

Aneuploidy has been shown to be associated with acquired resistance to Hsp90. In cell lines selected for resistance to ganetespib, we have previously reported large chromosomal alterations[13]. It was hypothesized that response rate to ganetespib-triplet therapy might therefore be higher for patients harbouring MPMs with a lower somatic copy number alterations. Genome-wide data was acquired for 11 of 27 patients (3 on 100 mg/m^2

ganetespib (2 cisplatin, 1 carboplatin), 8 on 200 mg/m^2 ganetespib (3 cisplatin, 5 carboplatin); remaining 16 patients provided non-viable samples); Supplementary Table 4 shows the distribution of these variables over key baseline characteristics. As exploratory analyses, correlations and hazard ratios between genomic variables and the change in total tumor burden and time to progression respectively are shown in Table 3, with mean and standard errors of each genomic variable by best response category presented in Supplementary Table 5. Four patients did not have evaluable responses post-baseline. One of 7 response-evaluable patients with genomic data recorded (male, cisplatin cohort, 100 mg/m^2 ganetespib) had non-epithelioid histology and stable disease as best response. All other response evaluable patients in this exploratory analysis achieved partial response and had epithelioid histology. Total LOH was associated with tumor shrinkage (i.e. higher baseline LOH associated with smaller reduction in total tumor burden; Spearman's correlation = -0.703, *p*=0.078; Supplementary Figure 7) and a 12% increase in the risk of disease progression (HR = 1.12, 95% CI 1.02-1.24, *p*=0.018).

DISCUSSION

In MESO-02, we investigated the safety, tolerability and efficacy of intravenous ganetespib combined with standard pemetrexed and platinum therapy in patients with MPM. The MTD of ganetespib was found to be 200 mg/m^2 in both the cisplatin and carboplatin cohorts. The trial successfully passed the phase Ib stage. However, the manufacturer of ganetespib decided not to proceed to the randomized phase II study following a strategic review and mixed findings from other solid tumor trials of ganetespib.

Ganetespib was well tolerated. At the MTD found in MESO-02, three patients out of 18 (17%) experienced a DLT. Overall, five patients (18.5%) withdrew from the study due to unacceptable/serious adverse events, all of which were grade 2-3. This phase Ib study was

not powered to detect improvements in efficacy measures compared to previous work. However, our results were highly encouraging; of fifteen patients evaluable for response at the MTD, 10 (67%) had a best response of PR and 5 (33%) had stable disease. Median PFS at the MTD was 6.3 months and Median OS at the MTD was 16.7 months. One patient (male, 63 years old at registration, epithelioid histology, baseline ECOG PS of 0) treated at the MTD received maintenance treatment with no observed progression after 60 cycles. Whilst these results were obtained from a small sample of MPM patients compared to other studies, they indicate that this ganetespib-pemetrexed-platinum therapy combination may be worth investigating in a larger randomized double-blind placebo-controlled trial, with histology, performance status, previous lines of therapy and baseline LOH as key stratification factors.

Prior to the setup of MESO-02 most trials used 200 mg/m^2 ganetespib as monotherapy, given weekly for 3 weeks over a 4-week cycle. The regimen used in our trial was considered appropriate given the addition of pemetrexed and platinum agents, and that patients could continue to have ganetespib as maintenance. The aim was to ensure the majority of patients would complete and tolerate at least one cycle of treatment (24 of 27 patients completed at least one full cycle). Whilst our study did not explore the tolerability of higher doses of ganetespib when combined with pemetrexed and platinum therapy, higher doses of ganetespib may still be tolerable.

MESO-02 recruited a higher percentage of male patients and patients with performance status of at least 1 compared to previous MPM studies[5,7-10], which led to 93% of patients in our trial with a poor baseline EORTC prognosis score, Despite the relatively high rate of non-epithelioid MPM in the treated cohort (22%) and the trend towards lower response in this histological subtype, there was a significant overall response rate (52%) with 62% of patients with epithelioid disease responding to treatment. These response rates are among the highest reported for any combination treatment in advanced MPM, though the confidence intervals are expectedly wide due to the small number of patients. However, this suggests

potential activity over and above that of standard chemotherapy, making Hsp90 inhibition a real possibility for treating MPM[2]. Six patients had non-epithelioid MPM; one patient received the MTD of ganetespib (200 mg/m^2), and another achieved a PR as best response. The poorer response of patients with non-epithelioid MPM may be due to the majority of patients (5 out of 6) receiving doses lower than the MTD.

These data are consistent with preclinical evidence supporting a role for Hsp90 in mediating DNA repair including homologous recombination, which may underpin synergy of Hsp90 inhibitors with platinum drugs[25,26]. Furthermore, thymidylate synthetase, which has been shown to correlate with pemetrexed activity is a putative mediator of anti-folate resistance, and is downregulated following by Hsp90 inhibition[27-29].

Acquisition of aneuploidy has been reported to be associated with resistance to Hsp90 inhibition[15]. We therefore hypothesized that patients harbouring genomic instability (reflected in LOH) might exhibit resistance to ganetespib. In our exploratory analyses, we observed a statistically significant effect on time to progression, with shorter time to progression for more genomically unstable MPMs. However, the results of this need to be interpreted with caution as increasing genomic instability *per se* may be negatively prognostic, and that we only had a relatively small number of cases for these analyses[30]. Nevertheless, our results indicate that patients harbouring MPM with high levels of LOH may fail to benefit from addition of ganetespib. LOH was associated with a worse clinical outcome, when considering both total tumor burden and time to progression. Our study was underpowered to detect any interaction between specific copy number alterations and sensitivity to Hsp90 inhibition.

Chemo-immunotherapy has transformed the front-line treatment of non-small cell lung cancer and is currently being developed in studies such as DREAM (NCT04334759), PrE505 (NCT02899195), IND227 (NCT02784171) and BEAT-meso (NCT03762018). Of note, the single arm PrE505 phase II study had an overall survival of 21 months, significantly greater than expected with the standard of care[31] and a phase III trial, PrE0506/DREAM3R, is planned. However, combined immune-checkpoint inhibition (ICI) with ipilimumab and

nivolumab was recently announced as being superior to pemetrexed-platinum in the pivotal Checkmate 743 phase III trial[32]. This could herald an imminent change of practice in the first-line setting, creating new second-line development opportunities for novel, non-ICI or ICI-chemotherapy combinations. Recent evidence suggests that Hsp90 inhibition, through its upregulation of interferon response genes, can enhance ICI therapy and pave the way for possible future ICI or chemo-immunotherapy combination studies[33]. In conclusion, ganetespib can be safely administered to patients with MPM at 200 mg/m^2 when combined with pemetrexed and platinum-based chemotherapy. This novel triplet also shows a potential signal of activity, for which further evaluation of ganetespib or other Hsp90 inhibitors should be done in a larger randomized trial.

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Author contributions:

DAF originated the study concept. DAF and AH initially designed the trial, along with DT, SD and MF. Patients were recruited by site principal investigators DAF, SD, MF, and DT. Statistical analyses were performed by GMW. Genomic analyses were conducted by AS and SB. Study management conducted by YN, JC and LF. All authors were involved in writing the paper and approved the final version.

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REFERENCES

1. Odgerel CO, Takahashi K, Sorahan T, et al. Estimation of the global burden of mesothelioma deaths from incomplete national mortality data. *Occup Environ Med* 2017; **74**(12): 851-8.
2. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol* 2003; **21**(14): 2636-44.
3. Santoro A, O'Brien ME, Stahel RA, et al. Pemetrexed plus cisplatin or pemetrexed plus carboplatin for chemonaive patients with malignant pleural mesothelioma: results of the International Expanded Access Program. *J Thorac Oncol* 2008; **3**(7): 756-63.
4. van Meerbeeck JP, Gaafar R, Manegold C, et al. Randomized phase III study of cisplatin with or without raltitrexed in patients with malignant pleural mesothelioma: an intergroup study of the European Organisation for Research and Treatment of Cancer Lung Cancer Group and the National Cancer Institute of Canada. *J Clin Oncol* 2005; **23**(28): 6881-9.
5. Zalcman G, Mazieres J, Margery J, et al. Bevacizumab for newly diagnosed pleural mesothelioma in the Mesothelioma Avastin Cisplatin Pemetrexed Study (MAPS): a randomised, controlled, open-label, phase 3 trial. *Lancet* 2016; **387**(10026): 1405-14.
6. Ettinger DS WD, Akerley W, Bazhenova LA, Borghaei H, Camidge DR, Cheney RT, Chirieac LR, D'Amico TA, Dilling T, Dobelbower M, Govindan R, Hennon M, Horn L, Jahan TM, Komako R, Lackner RP, Lanuti M, Lilenbaum R, Lin J, Loo Jr BW, Martins R, Otterson GA, Patel JD, Pisters KM, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Sharma N, Swanson SJ, Stevenson J, Tauer K, Yang SC, Gregory K, Hughes M. NCCN Guidelines Insights: Malignant Pleural Mesothelioma, Version 3.2016. *Journal of the National Comprehensive Cancer Network* 2016; **14**(7): 825-36.
7. Buikhuisen WA, Burgers JA, Vincent AD, et al. Thalidomide versus active supportive care for maintenance in patients with malignant mesothelioma after first-line chemotherapy (NVALT 5): an open-label, multicentre, randomised phase 3 study. *Lancet Oncol* 2013; **14**(6): 543-51.
8. Krug LM, Kindler HL, Calvert H, et al. Vorinostat in patients with advanced malignant pleural mesothelioma who have progressed on previous chemotherapy (VANTAGE-014): a phase 3, double-blind, randomised, placebo-controlled trial. *Lancet Oncol* 2015; **16**(4): 447-56.
9. Maio M, Scherpereel A, Calabro L, et al. Tremelimumab as second-line or third-line treatment in relapsed malignant mesothelioma (DETERMINE): a multicentre, international, randomised, double-blind, placebo-controlled phase 2b trial. *Lancet Oncol* 2017; **18**(9): 1261-73.
10. Gregorc V, Gaafar RM, Favaretto A, et al. NGR-hTNF in combination with best investigator choice in previously treated malignant pleural mesothelioma (NGR015): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol* 2018; **19**(6): 799-811.
11. S A Burgers CdG, R Cornelissen, J G Aerts, B Biesma, R V Heemst, M Youssef-El Soud, H J M Groen, A J Staal-van den Brekel, G Bootsma, J H E M Schijen, P Baas, E Giovannetti, J F de Vries, F A Hogenboom, D C M de Wit, M C W Mahn- Schaefers, F Lalezari, V van de Noort, J Stigt. Switch maintenance gemcitabine after first-line chemotherapy in patients with malignant mesothelioma: A multicenter open label phase II trial (NVALT19). October 2019: Annals of Oncology.
12. Ou WB, Hubert C, Corson JM, et al. Targeted inhibition of multiple receptor tyrosine kinases in mesothelioma. *Neoplasia* 2011; **13**(1): 12-22.
13. Busacca S, Law EW, Powley IR, et al. Resistance to HSP90 inhibition involving loss of MCL1 addiction. *Oncogene* 2016; **35**(12): 1483-92.
14. Fitzgerald DM, Hastings PJ, Rosenberg SM. Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance. *Annu Rev Cancer Biol* 2017; **1**: 119-40.
15. Chen G, Bradford WD, Seidel CW, Li R. Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy. *Nature* 2012; **482**(7384): 246-50.
16. Lee JH, Park JH, Jung Y, et al. Histone deacetylase inhibitor enhances 5-fluorouracil cytotoxicity by down-regulating thymidylate synthase in human cancer cells. *Mol Cancer Ther* 2006; **5**(12): 3085-95.

17. Roh JL, Kim EH, Park HB, Park JY. The Hsp90 inhibitor 17-(allylamino)-17-demethoxygeldanamycin increases cisplatin antitumor activity by inducing p53-mediated apoptosis in head and neck cancer. *Cell Death Dis* 2013; **4**: e956.
18. Proia DA, Bates RC. Ganetespib and HSP90: translating preclinical hypotheses into clinical promise. *Cancer Res* 2014; **74**(5): 1294-300.
19. Goldman JW, Raju RN, Gordon GA, et al. A first in human, safety, pharmacokinetics, and clinical activity phase I study of once weekly administration of the Hsp90 inhibitor ganetespib (STA-9090) in patients with solid malignancies. *BMC Cancer* 2013; **13**: 152.
20. Mbofung RM, McKenzie JA, Malu S, et al. HSP90 inhibition enhances cancer immunotherapy by upregulating interferon response genes. *Nat Commun* 2017; **8**(451): 1-8.
21. Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. *Ann Oncol* 2004; **15**(2): 257-60.
22. Foster JM OA, Togneri FS, Vasques FR, Hau D, Taylor M, Tinkler-Hundal E, Southward K, Medlow P, McGreeghan-Crosby K, Halfpenny I, McMullan DJ, Quirke P, Keating KE, Griffiths M, Spink KG, Brew F. Cross-laboratory Validation of the OncoScan® FFPE Assay, a Multiplex Tool for Whole Genome Tumour Profiling. *BMC Medical Genomics* 2015; **8**(5): 1-13.
23. StataCorp. Stata Statistical Software: Release 15.: College Station, TX: StataCorp LLC; 2017.
24. Fennell DA, Parmar A, Shamash J, et al. Statistical validation of the EORTC prognostic model for malignant pleural mesothelioma based on three consecutive phase II trials. *J Clin Oncol* 2005; **23**(1): 184-9.
25. Fang Q, Inanc B, Schamus S, et al. HSP90 regulates DNA repair via the interaction between XRCC1 and DNA polymerase beta. *Nat Commun* 2014; **5**: 5513.
26. Stecklein SR, Kumaraswamy E, Behbod F, et al. BRCA1 and HSP90 cooperate in homologous and non-homologous DNA double-strand-break repair and G2/M checkpoint activation. *Proc Natl Acad Sci U S A* 2012; **109**(34): 13650-5.
27. Abu Lila AS, Kato C, Fukushima M, Huang CL, Wada H, Ishida T. Downregulation of thymidylate synthase by RNAi molecules enhances the antitumor effect of pemetrexed in an orthotopic malignant mesothelioma xenograft mouse model. *Int J Oncol* 2016; **48**(4): 1399-407.
28. Righi L, Papotti MG, Ceppi P, et al. Thymidylate synthase but not excision repair cross-complementation group 1 tumor expression predicts outcome in patients with malignant pleural mesothelioma treated with pemetrexed-based chemotherapy. *J Clin Oncol* 2010; **28**(9): 1534-9.
29. Zucali PA, Giovannetti E, Destro A, et al. Thymidylate synthase and excision repair cross-complementing group-1 as predictors of responsiveness in mesothelioma patients treated with pemetrexed/carboplatin. *Clin Cancer Res* 2011; **17**(8): 2581-90.
30. Ivanov SV, Miller J, Lucito R, et al. Genomic events associated with progression of pleural malignant mesothelioma. *Int J Cancer* 2009; **124**(3): 589-99.
31. Patrick M. Forde ZS, Valsamo Anagnostou, Hedy L. Kindler, William T. Purcell, Bernardo H. L. Goulart, Arkadiusz Z. Dudek, Hossein Borghaei, Julie R. Brahmer, Suresh S. Ramalingam. PrE0505: Phase II multicenter study of anti-PD-L1, durvalumab, in combination with cisplatin and pemetrexed for the first-line treatment of unresectable malignant pleural mesothelioma (MPM)—A PrECOG LLC study. *J Clin Oncol* 2020; **38**(suppl; abstr 9003).
32. Squibb BM. Bristol Myers Squibb Announces Positive Topline Result from Pivotal Phase 3 Trial Evaluating Opdivo® (nivolumab) plus Yervoy® (ipilimumab) vs. Chemotherapy in Previously Untreated Malignant Pleural Mesothelioma. Opdivo plus Yervoy prolonged survival compared to chemotherapy at interim analysis in CheckMate -743; 2020.
33. Mbofung RM, McKenzie JA, Malu S, et al. HSP90 inhibition enhances cancer immunotherapy by upregulating interferon response genes. *Nat Commun* 2017; **8**(1): 451.

TABLES

Table 1. Patient characteristics at baseline.

Characteristic	No. (N = 27)	%
Age (years)		
Median (range)	66 (37-76)	
Sex		
Female	2	7
Male	25	93
Histology		
Epithelioid	21	78
Non-epithelioid	6	22
ECOG Performance Status		
0	6	22
1	21	78
EORTC Prognostic Score		
Good	2	7
Poor	25	93
Platinum Treatment		
Cisplatin	16	59
Carboplatin	11	41
Ganetespib Dose (mg/m^2)		
100	5 (1 Carboplatin)	
150	4 (1 Carboplatin)	
200	18 (9 Carboplatin)	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EORTC, European Organisation for Research and Treatment of Cancer.

Table 2. Number of patients with maximum grade 3 or 4 adverse events, per platinum-therapy cohort and ganetespib dose level (number of patients experiencing grade 4 shown in parentheses).

Ganetespib Dose (mg/m^2)	Cisplatin			Carboplatin		
	100	150	200	100	150	200
	(n = 4)	(n = 3)	(n = 9)	(n = 1)	(n = 1)	(n = 9)
Haematological/ Biochemical AEs						
Anemia	1	.	3	.	.	2
Hyperglycemia	.	.	1	.	.	.
Hyperkalemia	.	.	.	.	.	1
Hypokalemia	.	.	.	.	.	1
Hyponatremia	.	.	.	.	.	1
Neutrophil count decreased	.	.	2(1)	1(1)	.	1
Platelet count decreased	.	.	.	.	.	3(1)
Symptomatic AEs						
Acute kidney injury	.	.	1	.	.	.
Anxiety	.	.	1	.	.	.
Apnea	.	.	1	.	.	.
Ascites	.	.	.	.	.	1
Chest wall pain	.	.	1	.	.	.
Diarrhea	.	.	1	.	.	1
Dyspnea	1	.	.	.	.	.
Hearing impaired	.	1(1)	.	.	.	.
Infections (chest)	.	1	.	.	.	.
Lung infection	.	.	.	.	.	1
Nausea	.	.	3	.	.	1
Pleural effusion	.	.	.	.	.	1
Sepsis	.	.	1(1)	.	.	.
Sleep apnea	.	.	1	.	.	.
Syncope	.	.	.	1	.	.
Upper respiratory infection	1	.	.	1	.	1
Vasculitis	.	.	1	.	.	.
Vomiting	.	.	2	.	.	.
Wound infection	.	.	.	.	.	2
Total Number of Patients	**3**	**2**	**7**	**1**	**.**	**6**

Table 3. Genomic variables and associations with best reduction in total tumor burden (TTB) and time to progression. TTB is the unidimensional measure corresponding to the sum of six pleural measurements determined by CT scan as per modified RECIST.

Best percentage reduction in TTB (mm) from baseline (n = 7)			
Genomic Variable (measured at baseline)	**Spearman Correlation**		**p-value**
Total SCNA	0.0714		0.879
Total LOH	-0.703		0.0782
Total Homozygous Deletions	-0.0541		0.908
Time to Progression from treatment start (n = 11)			
Genomic Variable (measured at baseline)	**Hazard Ratio**	**95% CI**	**p-value**
Total SCNA	1.01	0.99 - 1.02	0.295
Total LOH	1.12	1.02 - 1.24	0.018
Total Homozygous Deletions	1.24	0.89 - 1.73	0.201

Abbreviations: TTB, Total tumor burden; SCNA, Somatic Copy Number Alterations; LOH, Loss of Heterozygosity.

FIGURES LEGENDS AND CAPTIONS

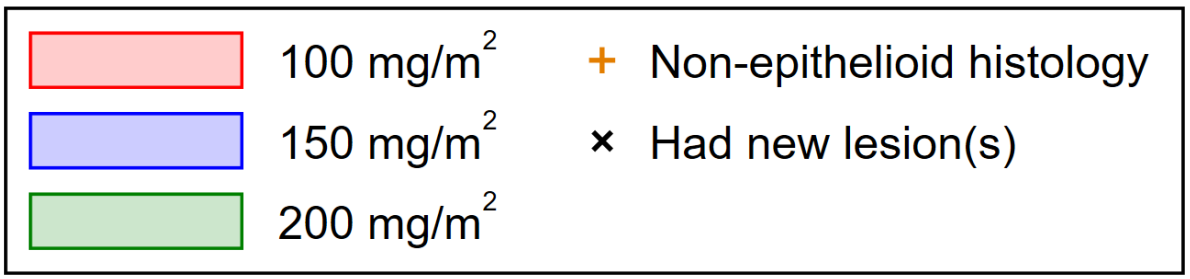
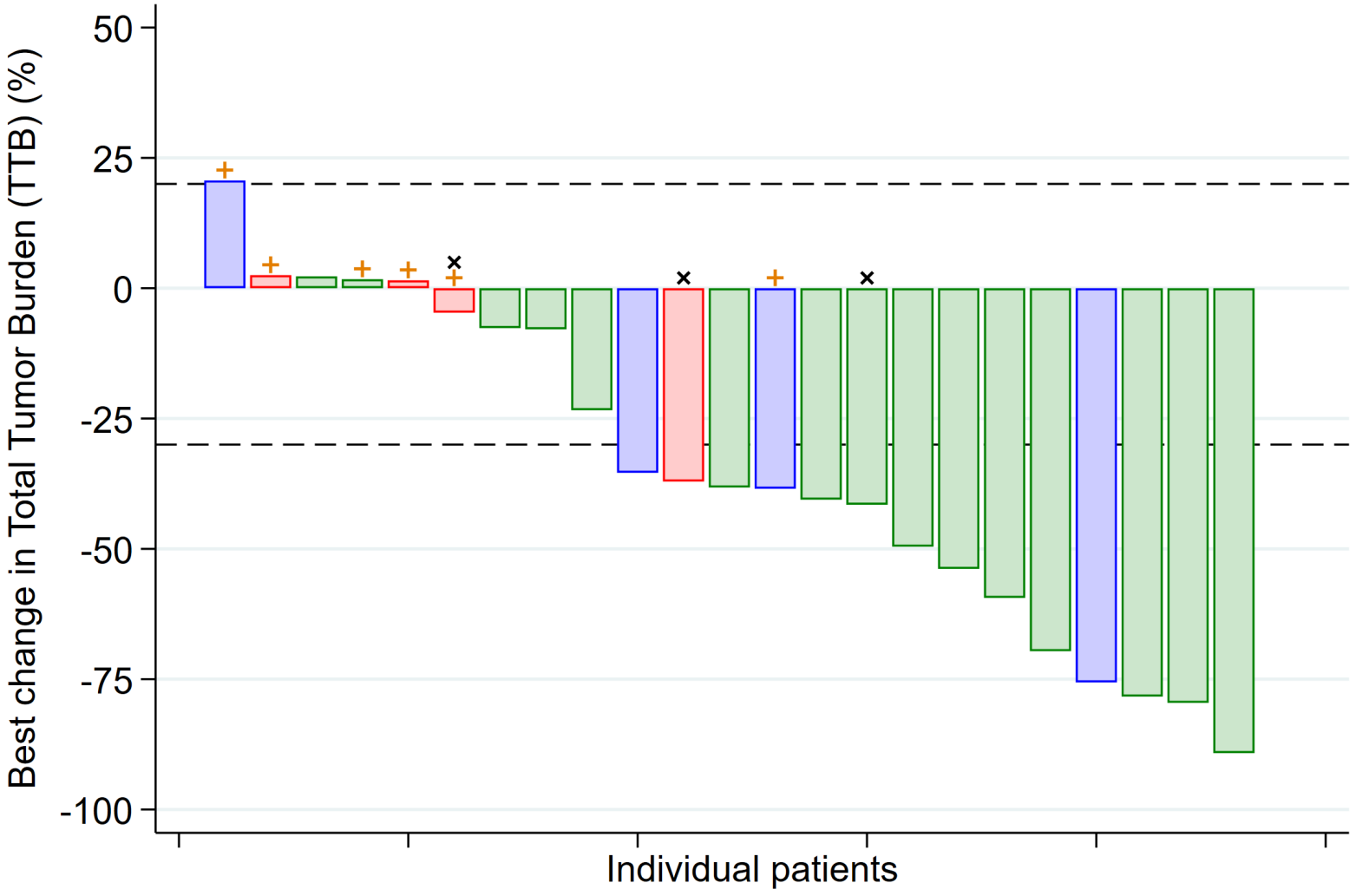


Figure 1. Waterfall plot of best response by ganetespib dose in response-evaluable patients. Total Tumor Burden (TTB) is the unidimensional measure corresponding to the sum of six pleural measurements determined by CT as per modified RECIST.

Figure 1


50
25
0
-25
-50
-75
-100
Best change in Total Tumor Burden (TTB) (%)
Individual patients