Published in final edited form as:
Clin Cancer Res. 2014 December 1; 20(23): 5927–5936. doi:10.1158/1078-0432.CCR-14-0804.

Phase II clinical trial of amatuximab, a chimeric anti-mesothelin antibody with pemetrexed and cisplatin in advanced unresectable pleural mesothelioma

Raffit Hassan[1], **Hedy L. Kindler**[2], **Thierry Jahan**[3], **Lyudmila Bazhenova**[4], **Martin Reck**[5], **Anish Thomas**[1], **Ira Pastan**[6], **Jeff Parno**[7], **Daniel J. O'Shannessy**[8], **Penny Fatato**[8], **Julia D. Maltzman**[8], and **Bruce A. Wallin**[8]

[1]Thoracic and GI Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

[2]University of Chicago, Chicago, IL

[3]University of California, San Francisco, CA

[4]University of California, San Diego ,CA

[5]Department of Thoracic Oncology, Lung Clinic Grosshansdorf, Member of the German Center for Lung Research (DZL), Grosshansdorf, GER

[6]Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

[7]United BioSource Corp., Blue Bell, PA

[8]Morphotek, Inc., Exton, PA

Abstract

Purpose—Amatuximab is a chimeric monoclonal antibody to mesothelin, a cell surface glycoprotein highly expressed in malignant pleural mesothelioma (MPM). Based on its synergy with chemotherapy in pre-clinical studies, we evaluated the antitumor activity of amatuximab plus pemetrexed and cisplatin in patients with unresectable MPM.

Experimental Design—In a single-arm phase II study, amatuximab 5 mg/kg was administered on days 1 and 8 with pemetrexed, 500 mg/m^2 and cisplatin, 75 mg/m^2 on day 1 of 21-day cycles for up to 6 cycles. Patients with response or stable disease received amatuximab maintenance until disease progression. Primary endpoint was progression-free survival (PFS) at 6 months. Secondary endpoints were overall survival (OS), response rate and safety.

Results—Eighty nine patients were enrolled at 26 centers. Median of five cycles (range 1–6) of combination treatment was administered and 56 (63%) patients received amatuximab maintenance. Combination therapy resulted in no overlapping toxicities. Eleven patients (12.4%) had amatuximab-related hypersensitivity reactions. Responses included partial responses in 33

Corresponding author: Raffit Hassan, Thoracic and GI Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4264. Tel: (301) 451-8742; Fax: (301) 402-1344; hassanr@mail.nih.gov.

Conflicts of interest: DJO, PF, JDM, BAW are employees of Morphotek, Inc. The other authors have no conflicts of interest.

(40%) and stable disease in 42 (51%). Six month-PFS rate was 51% (95% CI: 39.1, 62.3), median PFS 6.1 months (95% CI: 5.8, 6.4) and median OS 14.8 months (95% CI: 12.4, 18.5) with 29 patients alive at data cut-off.

Conclusions—Amatuximab with pemetrexed and cisplatin was well-tolerated with objective tumor response or stable disease rate of 90% by independent radiological review. Although PFS was not significantly different from historical controls, the median OS was 14.8 months with a third of patients alive and 5 continuing to receive amatuximab at the time of analysis.

Keywords

malignant pleural mesothelioma; CA125; mesothelin; megakaryocyte potentiating factor; monoclonal antibody

Introduction

Malignant pleural mesothelioma (MPM) is an aggressive disease with poor prognosis. Although patients with a limited tumor burden may benefit from surgical resection, most patients have advanced disease at diagnosis and are not candidates for surgery (1). For patients who are not eligible for curative surgery, the median survival with supportive care alone is approximately 6 months whereas with the current standard treatment, a combination of cisplatin and pemetrexed, the median survival is 12 months (2–3).

Mesothelin is a glycosylphosphatidyl inositol (GPI)-anchored membrane glycoprotein, which is present in a restricted set of normal adult tissues such as the mesothelium (4). In contrast, mesothelin is highly expressed in many epithelial cancers. More than half of all the ovarian cancers and lung adenocarcinomas and nearly all epithelial mesotheliomas and pancreatic ductal adenocarcinomas express mesothelin (5–9). Although the normal biological function of mesothelin is unknown, growing evidence suggests that it may play a role in tumorigenesis and metastasis in mesothelioma (10). Its limited expression in normal human tissue and high expression in tumor makes mesothelin an excellent target antigen for antibody-based immunotherapy (11).

The mesothelin gene encodes a 71-kDa precursor protein that is cleaved into a soluble 31-kDa fraction, megakaryocyte potentiating factor (MPF) and the 40-kDa mesothelin (12). Mesothelin binds to CA125, a specific epitope expressed on MUC16, a transmembrane mucin. The interaction between CA125, which is present on a majority of mesothelioma cells, and mesothelin, has been suggested to facilitate implantation and metastasis of mesothelioma (13–15). Serum mesothelin, MPF and CA125 could be potentially useful as biomarkers for mesothelioma (16–20).

Amatuximab (MORAb-009) is a chimeric high-affinity monoclonal IgG1/k antibody targeting mesothelin (21). *In vitro*, amatuximab elicits antibody-dependent cellular cytotoxicity (ADCC) against mesothelin expressing tumor cell lines and inhibits heterotypic cell adhesion of mesothelin-positive tumor cells to CA125-expressing tumor cells. In tumor xenograft studies, combination treatment with amatuximab plus chemotherapy led to a greater reduction in the growth of mesothelin-expressing tumors than either amatuximab or

chemotherapy alone. In a phase I study of patients with mesothelin-expressing cancers, weekly infusions of amatuximab were well tolerated and the maximum tolerated dose was identified as 200 mg/m^2 (22). Dose limiting toxicities were grade 4 transaminitis and grade 3 serum sickness. Other adverse events at least possibly related to amatuximab included grade 1 or 2 drug hypersensitivity. In the phase I study, amatuximab treatment resulted in an increase in serum CA125, possibly due to inhibition of binding of tumor shed CA125 to mesothelin present on the serosal lining of pleural and peritoneal cavities (23). Based on its safety in the phase I study and pre-clinical studies showing synergy with chemotherapy, amatuximab was combined with pemetrexed and cisplatin in a single-arm phase II study in patients with unresectable MPM.

Materials and methods

Patients

Patients with histologically confirmed, chemotherapy-naive MPM who were not candidates for curative surgery were assessed for eligibility. The study was approved by the Institutional Review Boards of participating institutions and informed consent was obtained prior to enrolment. The trial was registered at clinicaltrials.gov (identifier NCT00738582).

Eligibility criteria included age ≥ 18 years, epithelial type or biphasic (mixed) MPM with low sarcomatous content, radiographically measurable disease, Karnofsky performance status (KPS) score of ≥70, adequate bone marrow reserve [absolute neutrophil count (ANC) ≥1.5 × 10^9/L; platelet count ≥100 × 10^9/L; hemoglobin ≥ 9 g/dL], hepatic function [bilirubin ≤1.5 times the upper limit of normal (ULN); alanine transaminase (ALT) and aspartate transaminase (AST) ≤2.5 × ULN; alkaline phosphatase ≤3.0 × ULN], and normal renal function (serum creatinine ≤2.0 mg/dL and a calculated creatinine clearance ≥60 ml/min) based on the standard Cockcroft and Gault formula. Pregnant women were not eligible, and all men and women of reproductive potential were required to use an approved method of birth control. Patients were excluded if they had predominantly mesothelioma of the sarcomatous type, disease located primarily in the peritoneum, prior systemic therapy or radiotherapy, known central nervous system tumor involvement, treatment within three months of the start of the trial with other immunomodulatory therapy and known hypersensitivity to any of the following: monoclonal antibodies or biologic therapy, pemetrexed, cisplatin or other platinum containing compounds.

Treatment

Amatuximab 5 mg/kg by intravenous infusion over 1 hour was administered on days 1 and 8 with pemetrexed (500 mg/m^2 by intravenous infusion over 10 minutes) and cisplatin (75 mg/m^2 by intravenous infusion over 2 hours) administered on day 1 of 21-day cycles for up to six cycles. Patients with objective response or stable disease continued to receive amatuximab maintenance (5 mg/kg on days 1 and 8 of a 21-day cycle) until disease progression. Supportive treatment included premedication with acetaminophen 650 mg and diphenhydramine 25 to 50 mg 30 minutes prior to amatuximab infusion; folic acid (350 μg to 1 mg PO daily) starting at least five days prior to the first dose of pemetrexed; vitamin B12 (1 mg approximately every 9 weeks); dexamethasone 4 mg PO twice daily on the day

before, the day of and the day following pemetrexed. Dose adjustments for adverse events and management of infusion-related adverse events are described in Supplemental Appendix A.

Assessments

Tumor measurements were performed at baseline and thereafter on day 8 of every third cycle starting with cycle 3 until disease progression or treatment discontinuation. Response was assessed using the modified RECIST for the assessment of response in malignant pleural mesothelioma (24). A complete blood count and a comprehensive metabolic panel were performed on days 1 and 8 of each cycle during the combination therapy phase and on day 1 of each cycle during the amatuximab maintenance phase. Safety was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) scale, version 3.0.

Pharmacokinetic assessments were performed and will be reported as a separate manuscript. Serum mesothelin and MPF were measured using Morphotek proprietary assays. CA125 levels (U/mL) were measured using an automated commercial assay. All assays were run according to the manufacturer's instructions, blinded to patient data. Relative changes in MPF and CA125 levels were compared with the patients best overall radiological response during treatment. Changes in serum mesothelin levels were not assessed since amatuximab is known to interfere with mesothelin assays.

Study design and statistics

This was a multicenter, single-arm phase II study. The primary objective was to determine the 6-month progression-free survival (PFS) of amatuximab plus pemetrexed and cisplatin. In the phase III trial of cisplatin and pemetrexed in MPM, the median Time to Progression was 5.7 months which corresponded to a 6-month PFS response rate of 48.2% (2). By adding amatuximab to the combination we sought to demonstrate an improvement in median PFS to 8.7 months corresponding to 6-month PFS response rate of 62%. The study utilized Simon's optimal 2-stage design (25) and set the probability for accepting a poor drug to 10% (alpha=0.10) and the probability of rejecting a good drug at 15% (beta=0.15). Kaplan-Meier (K-M) methodology was used to estimate the median of time-to event endpoints (26). A two-sided 95% confidence interval (CI) was constructed using the methodology of Brookmeyer and Crowley (27). K-M estimates at selected time points (e.g. 3, 6, 9, and 12 months) were determined and corresponding 95% CIs were constructed using the log-log transformation methodology.

The secondary endpoints were objective response rate (ORR), overall survival (OS), overall progression-free survival, and safety and tolerability of the combination.

The objective response rate (ORR) was based on an independent assessment of total tumor measurement. An exact two-sided 95% CI for the ORR was constructed. Simple descriptive statistics (i.e. number and percentage of patients) were used to summarize safety data. All patients who received at least one dose of amatuximab were included in the safety analysis and OS efficacy assessment. The population for the PFS analysis was defined as all patients who received treatment and underwent at least one post baseline imaging assessment, or who had died.

A post-hoc exploratory objective was to determine optimal thresholds for biomarker levels to predict survival. These optimal thresholds were determined using Maximally Selected Chi Square Statistics (28). Other post-hoc exploratory investigations included an analysis of OS by EORTC prognostic score category (low risk versus high risk) (29) using Kaplan-Meier methodology, and a stepwise multivariate Cox regression analysis of OS utilizing the categorized biomarker data (above or below the optimal threshold for baseline CA125, mesothelin, and MPF) and three categorized elements of the EORTC prognostic score (baseline WBC above or below 8.3×10^9/L, baseline ECOG score (0 versus 1 or 2), and gender (male versus female). A 0.10 level of significance was used for selection and retention of factors in the Cox model. EORTC prognostic variables of histology and probability of diagnosis were not included in the model since all patients had confirmed diagnosis of epithelioid mesothelioma.

An Independent Data Monitoring Committee (IDMC) was established to review safety data after 8 patients had completed one cycle, and after 17 and 33 patients had completed 6 cycles. Because of the rapid enrollment of this study, this initial safety review occurred after 17 patients had completed one cycle. In addition, the IDMC reviewed all safety data on a quarterly basis.

Results

Patient characteristics

Between February 2009 and October 2010, 89 patients from 26 sites in the North America and Europe were enrolled. Twelve patients were enrolled over the design-specified target of 77, under the a priori assumption of an approximate 10% loss-to-followup and non-evaluability for tumor assessment. All patients received at least one dose of amatuximab. Eighty three (93%) patients had at least one post-baseline imaging assessment and were evaluable for efficacy. One patient withdrew consent after their third cycle.

Patient demographics and baseline disease characteristics are shown in Table 1. The median age was 67 years (range, 46 –80 years), 78% were male, 89% were Caucasian, and 93% had a KPS score ≥80. Eighty-eight percent had stage III/IV disease and 89% had epithelial histology. Sixty-three percent reported prior exposure to asbestos.

Treatment

Forty patients (45%) completed 6 cycles of the combination therapy phase. Reasons for discontinuation of combination therapy included adverse events (35%), progressive disease (12%), investigator discretion (3%) and other (5%). Fifty-six patients (63%) entered the amatuximab maintenance phase. Reasons for discontinuation of maintenance therapy were progressive disease (73%), adverse events (5%), and other (4%). A median of five cycles of combination therapy (range, 1–6) and six cycles of maintenance therapy (range, 1–30) were administered. Sixteen patients received ≥10 cycles and eight patients received ≥20 cycles of amatuximab maintenance, the longest being 52 cycles.

Efficacy

Efficacy data are shown in Table 2. The study did not meet the pre-specified design criterion of 43 responders in the first 77 patients and the study did not meet its primary endpoint. The Kaplan-Meier estimate of 6 month PFS was 51.3% (95% CI: 39.1, 62.3) (Figure 1A), median PFS was 6.1 months (95% CI: 5.8, 6.4) and median overall survival was 14.8 months (95% CI: 12.4, 19.2) (Figure 1B). There were no complete responses; 33 out of 83 patients had partial responses [ORR: 39.8% (95% CI: 29.2, 51.1)]. Forty-two patients (51%) had stable disease as the best response. The disease control rate was 90%. As of April 23, 2012, 29 patients are alive and 5 remain on amatuximab maintenance. A sub-group of patients who received at least 4 cycles of combination chemotherapy (n=60) had a median OS of 19.2 months (95% CI: 13.5, 20.8). The post-hoc exploratory analysis of the median OS by EORTC prognostic score category showed that those subjects with a score indicating a low risk (n=25) had a median OS of 20.7 months (95% CI: 16.0, 28.6) as compared to those with a score indicating a high risk (n=64) having a median OS of 12.6 months (95% CI, 11.3, 17.1).

Safety

Treatment-emergent adverse events are shown in Table 3. Among the most common adverse events seen in ≥ 15% of patients during the combination therapy phase were nausea (71%), fatigue (61%), anorexia (43%), vomiting (32%), constipation (30%), anemia (29%), neutropenia (29%), diarrhea (28%), and weight decrease (20%). Hypersensitivity reactions (12%) as well as infusion related reactions (9%) were also seen. The Serious Adverse Events (SAE) during the combination therapy phase were hypersensitivity reactions (4 patients), neutropenia (4 patients), atrial fibrillation (3 patients), hyponatremia (3 patients), anemia (2 patients), dehydration (2 patients), and pneumothorax (2 patients). Adverse events which led to discontinuation of treatment were: hypersensitivity reactions to amatuximab (8 patients), increasing serum creatinine (6 patients), fatigue (3 patients), neutropenia (2 patients), worsening in the patients' general condition (2 patients), nausea and vomiting (2 patients), development of a pneumothorax (2 patients), and one patient each for anemia, thrombocytopenia, dyspnea, pericarditis and pericardial effusion. One additional patient was discontinued in order to undergo a thoracotomy.

During the amatuximab maintenance phase, the most common adverse events (>15%) seen were dyspnea (23%), nausea (20%), peripheral neuropathy (18%), fatigue (18%), and non-cardiac chest pain (16%). Grade 3 and 4 adverse events were dyspnea (3 patients), fatigue (3 patients), abdominal pain (2 patients), and flank pain (2 patients). SAEs seen during the maintenance phase were dyspnea (in 4 patients) and fatigue (2 patients). Adverse events which led to discontinuation of treatment were hyperbilirubinemia (1 patient), peritonitis (1 patient), and overdose/cardiopulmonary arrest (1 patient). The latter was reported in a 58-year old man who suffered a cardiac arrest related to cocaine use, one week after the first dose of maintenance amatuximab, from which he was successfully resuscitated.

Biomarker assessment

As depicted in Figure 2A a strong correlation was observed between pre-treatment serum mesothelin levels and serum MPF levels [r = 0.77 ($p<0.0001$)]. Based on the current data set

we estimated optimal thresholds of baseline serum mesothelin, MPF and CA125 to predict OS as 33.14 ng/mL, 4.7 ng/mL, and 6 U/mL, respectively. Patients with low pre-treatment mesothelin levels had a significantly longer OS compared to patients with high baseline mesothelin levels [18.5 months (95% CI: 13.2, not reached) vs. 12.5 months (95% CI: 10.5, 16.7)] (Figure 2B). A similar association was observed with pre-treatment MPF wherein patients with low levels at baseline had superior OS compared to those with high levels [18.5 months (95% CI: 13.2, not reached) vs. 12.8 months (95% CI: 11.3, 16.0)] (Figure 2C) and CA125 [20.7 months (95% CI: 10.7, not reached) vs. 13.3 months (95% CI: 11.6, 18.2)] (Figure 2D). The stepwise multivariate Cox regression analysis utilizing these three categorized biomarkers and three categorized elements of the EORTC prognostic score resulted in a final model that retained the following factors: baseline mesothelin ($p = 0.004$), baseline ECOG status ($p = 0.046$), and baseline CA125 ($p = 0.070$). The final model was based on the 77 subjects for whom the values of all six candidate factors were known. Baseline MPF was not selected because, although associated with OS in a univariate setting, it strongly correlated with baseline mesothelin.

Serum MPF data from before and after treatment were available for 59 patients who were also evaluable for response. For each patient, the relative change in serum MPF levels at the time of best overall radiological response (stable disease, partial response or progressive disease) was displayed in a waterfall plot (Figure 3). The two patients with progressive disease experienced an increase in serum MPF, whereas 17 of 21 (81%) patients with a confirmed partial response had a decrease in MPF levels from baseline. Among 32 patients with stable disease as the best response, 10 (31%) had an increase whereas 22 (69%) had a decrease in serum MPF with treatment.

Discussion

This multi-center phase II study demonstrated that amatuximab in combination with pemetrexed and cisplatin was well-tolerated and resulted in a disease control rate of 90% and median PFS of 6.1 months by independent radiological review in the primary efficacy population.

Response rate of 40% by independent radiologic review is comparable to the 45.5% observed with cisplatin and pemetrexed alone (2). Reproducibly measuring tumor response is challenging in MPM and to avoid investigator bias in response assessment, we used independent radiologic review. The study did not meet the primary endpoint of three month improvement in PFS over historical controls. Nevertheless, with all the caveats associated with a cross trial comparison, the median OS of 14.8 months compares favorably with 13.3 months in the fully supplemented subset of patients in the phase III study of cisplatin and pemetrexed (2). However, direct comparison of results of this trial with the phase III study of cisplatin and pemetrexed is difficult due to differences in patient populations studied and frequency of response assessment. For example, the phase III trial of cisplatin and pemetrexed had more patients with poor performance status (48% patients with KPS 70/80 vs. 30% in our trial) and unfavorable histology (22% with mixed or sarcomatoid histology vs. 11% mixed histology in our trial). However our trial had more patients with advanced stages of disease (87% patients with stage III/IV vs. 77% in the phase III trial of cisplatin

and pemetrexed). The improved median OS with 33% of patients alive at the time of analysis also exceeds the literature based expectations of outcomes for MPM (30–31). Post-progression second-line treatment which may influence the duration of OS is not known for our patients.

The extended final plateau of the Kaplan-Meier curve of overall survival of patients suggests that the combination may be particularly effective in a subgroup of patients. Hypothetically, the prolongation of OS with no improvement in PFS may be a function of the mechanism of action of amatuximab whereby the immune system may be modulated to control tumor growth. Although the full extent of the mechanism of action of amatuximab and its synergy with chemotherapy is not known, amatuximab elicits antibody-dependent cellular cytotoxicity (ADCC) against mesothelin expressing tumor cell lines and inhibits heterotypic cell adhesion of mesothelin positive tumor cells to CA125 expressing tumor cells (21).

The combination chemotherapy was well tolerated with no overlapping toxicities. Hypersensitivity reactions as well as infusion-related reactions were the most common amatuximab-related AEs. These are expected given the fact that amatuximab is a chimeric monoclonal antibody, with human constant regions and murine variable regions which contain non-self epitopes than can stimulate immune responses. The hypersensitivity and infusion reactions were not life-threatening and responded to supportive care.

The development of targeted agents to which only a subset of patients responds depends on the identification of robust predictive biomarkers. In this study, we investigated the effect of pre-treatment levels of serum CA125, mesothelin and MPF levels on survival. Previous reports which identified a significant correlation between serum mesothelin and MPF levels in MPM have involved a relatively small number of patients (32–33).Our finding of a strong correlation between pre-treatment serum mesothelin levels and serum MPF levels [$r = 0.77$ ($p<0.0001$)] confirm these findings in a large cohort. This correlation may be attributable to the release of MPF by physiological cleavage at the furin cleavage site of the mesothelin precursor protein (34). Univariate analyses using optimal biomarker thresholds identified from this dataset showed that patients with low baseline mesothelin, MPF and CA125 levels had a significantly longer OS compared to patients with high levels. A multivariate analysis which included three categorized biomarkers (baseline mesothelin, MPF and CA125 levels) and three elements of the EORTC prognostic score (baseline WBC above or below 8.3 × 109/L, baseline ECOG 0 versus >=1, and gender) showed that baseline mesothelin, baseline ECOG performance status, and baseline CA125 were prognostic of overall survival. It is to be noted that the number of patients included in this analysis was limited. Future studies should explore the changes in these circulating biomarkers with treatment and their potential to provide an early assessment of treatment efficacy.

In mesothelioma, objective assessment of radiologic response to treatment is difficult and markers of response could complement radiologic assessment to discriminate between effective and ineffective treatments. Serum mesothelin has previously been reported as a potential biomarker of response in mesothelioma (35). However amatuximab binds to the same epitope on mesothelin as one of the antibodies used in the mesothelin assay (MESOMARK® Assay, Fujirebio Diagnostics, Inc., Malvern, PA USA) and interferes with

the measurement of serum mesothelin. This precludes the use of serum mesothelin as a biomarker of response in patients treated with amatuximab. In this study, we investigated the relative change in serum MPF levels with treatment as a biomarker of response. Although data were limited, we observed that patients with progressive disease had increases in serum MPF with treatment whereas a majority of patients with partial responses had decreases in MPF levels.

We conclude that amatuximab plus pemetrexed and cisplatin has activity in pleural mesothelioma. The single-arm design and the limited patient numbers preclude a definitive conclusion regarding its survival advantage over cisplatin plus pemetrexed. However, a median OS of 14.8 months with a third of patients alive at the time of analysis is suggestive of antitumor activity of the combination of amatuximab plus pemetrexed/cisplatin. A randomized, placebo controlled study is planned to investigate the survival benefit of this combination. Discovery of tissue or serum biomarkers predictive of response to therapy will be a priority in any further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, and in part by Morphotek, Inc., under a Cooperative Research and Development Agreement with National Cancer Institute.

References

1. Sugarbaker DJ, Garcia JP, Richards WG, Harpole DH, HealyBaldini E, DeCamp MM, et al. Extrapleural pneumonectomy in the multimodality therapy of malignant pleural mesothelioma - Results in 120 consecutive patients. Annals of Surgery. 1996; 224:288–294. [PubMed: 8813257]
2. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J Clin Oncol. 2003; 21:2636–2644. [PubMed: 12860938]
3. Manzini VD, Brollo A, Franceschi S, Dematthaeis M, Talamini R, Bianchi C. Prognostic Factors of Malignant Mesothelioma of the Pleura. Cancer. 1993; 72:410–417. [PubMed: 8319172]
4. Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int J Cancer. 1992; 50:373–381. [PubMed: 1735605]
5. Ordonez NG. Value of mesothelin immunostaining in the diagnosis of mesothelioma. Mod Pathol. 2003; 16:192–197. [PubMed: 12640097]
6. Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R, Brackett D. Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. Am J Clin Pathol. 2005; 124:838–845. [PubMed: 16416732]
7. Argani P, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: Identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res. 2001; 7:3862–3868. [PubMed: 11751476]
8. Hassan R, Kreitman RJ, Pastan I, Willingham MC. Localization of mesothelin in epithelial ovarian cancer. Appl Immunohistochem Mol Morphol. 2005; 13:243–247. [PubMed: 16082249]

9. Miettinen M, Sarlomo-Rikala M. Expression of calretinin, thrombomodulin, keratin 5, and mesothelin in lung carcinomas of different types: an immunohistochemical analysis of 596 tumors in comparison with epithelioid mesotheliomas of the pleura. Am J Surg Pathol. 2003; 27:150–158. [PubMed: 12548160]
10. Hassan R, Schweizer C, Lu KF, Schuler B, Remaley AT, Weil SC, et al. Inhibition of mesothelin-CA-125 interaction in patients with mesothelioma by the anti-mesothelin monoclonal antibody MORAb-009: Implications for cancer therapy. Lung Cancer. 2010; 68:455–459. [PubMed: 19744744]
11. Kelly RJ, Sharon E, Pastan I, Hassan R. Mesothelin-targeted agents in clinical trials and in preclinical development. Mol Cancer Ther. 2012; 11:517–525. [PubMed: 22351743]
12. Hassan R, Bera T, Pastan I. Mesothelin: A new target for immunotherapy. Clin Cancer Res. 2004; 10:3937–3942. [PubMed: 15217923]
13. Rump A, Morikawa Y, Tanaka M, Minami S, Umesaki N, Takeuchi M, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. J Biol Chem. 2004; 279:9190–9198. [PubMed: 14676194]
14. Gubbels JAA, Belisle J, Onda M, Rancourt C, Migneault M, Ho M, et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. Molecular Cancer. 2006; 5
15. Bateman AC, AlTalib RK, Newman T, Williams JH, Herbert A. Immunohistochemical phenotype of malignant mesothelioma: Predictive value of CA125 and HBME-1 expression. Histopathology. 1997; 30:49–56. [PubMed: 9023557]
16. Hassan R, Remaley AT, Sampson ML, Zhang J, Cox DD, Pingpank J, et al. Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and ovarian cancer. Clin Cancer Res. 2006; 12:447–453. [PubMed: 16428485]
17. Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, et al. Mesothelin-family proteins and diagnosis of mesothelioma. Lancet. 2003; 362:1612–1616. [PubMed: 14630441]
18. Hollevoet K, Nackaerts K, Thimpont J, Germonpre P, Bosquee L, De Vuyst P, et al. Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am J Respir Crit Care Med. 2010; 181:620–625. [PubMed: 20075387]
19. Onda M, Nagata S, Ho M, Bera TK, Hassan R, Alexander RH, et al. Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. Clin Cancer Res. 2006; 12:4225–4231. [PubMed: 16857795]
20. Hellstrom I, Raycraft J, Kanan S, Sardesai NY, Verch T, Yang Y, et al. Mesothelin variant 1 is released from tumor cells as a diagnostic marker. Cancer Epidemiol Biomarkers Prev. 2006; 15:1014–1020. [PubMed: 16702385]
21. Hassan R, Ebel W, Routhier EL, Patel R, Kline JB, Zhang J, et al. Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. Cancer Immun. 2007; 7:20. [PubMed: 18088084]
22. Hassan R, Cohen SJ, Phillips M, Pastan I, Sharon E, Kelly RJ, et al. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. Clin Cancer Res. 2010; 16:6132–6138. [PubMed: 21037025]
23. Hassan R, Schweizer C, Lu KF, et al. Inhibition of mesothelin–CA-125 interaction in patients with mesothelioma by the anti-mesothelin monoclonal antibody MORAb-009: Implications for cancer therapy. Lung Cancer. 2010; 68:455–459. [PubMed: 19744744]
24. Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. Ann Oncol. 2004; 15:257–260. [PubMed: 14760119]
25. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989; 10:1–10. [PubMed: 2702835]
26. Kaplan EL, Meier P. Nonparametric-Estimation from Incomplete Observations. J Am Stat Assoc. 1958; 53:457–481.
27. Brookmeyer R, Crowley J. A Confidence-Interval for the Median Survival-Time. Biometrics. 1982; 38:29–41.
28. R. Miller and D. Siegmund 1982 MSCSS. Biometrics. 38:1011–1016.

29. Fennell DA, Parmar A, Shamash J, Evans MT, Sheaff MT, et al. Statistical validation of the EORTC prognostic model for malignant pleural mesothelioma based on three consecutive phase II trials. J Clin Oncol. 2005; 23:184–189. [PubMed: 15625372]

30. Ruffie PA. Pleural mesothelioma. Curr Opin Oncol. 1991; 3:328–334. [PubMed: 2069994]

31. Schildge J, Kaiser D, Henss H, Fiebig H, Ortlieb H. Prognostic factors in diffuse malignant mesothelioma of the pleura. Pneumologie. 1989; 43:660–664. [PubMed: 2608642]

32. Iwahori K, Osaki T, Serada S, Fujimoto M, Suzuki H, Kishi Y, et al. Megakaryocyte potentiating factor as a tumor marker of malignant pleural mesothelioma: Evaluation in comparison with mesothelin. Lung Cancer. 2008; 62:45–54. [PubMed: 18394747]

33. Creaney J, Yeoman D, Demelker Y, Segal A, Musk AW, Skates SJ, et al. Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. J Thorac Oncol. 2008; 3:851–857. [PubMed: 18670302]

34. Onda M, Nagata S, Ho M, Bera TK, Hassan R, Alexander RH, et al. Megakaryocyte potentiation factor cleaved from mesothelin precursor is a useful tumor marker in the serum of patients with mesothelioma. Clin Cancer Res. 2006; 12:4225–4231. [PubMed: 16857795]

35. Wheatley-Price P, Boming Yang B, Demetris Patsios D, Devalben Patel D, Clement Ma C, Wei Xu W, et al. Soluble Mesothelin-Related Peptide and Osteopontin As Markers of Response in Malignant Mesothelioma. J Clin Oncol. 2010; 8:3316–3322. [PubMed: 20498407]

Translational Relevance

Mesothelin, a cell surface differentiation antigen highly expressed in malignant mesothelioma may play a role in tumor metastasis because of its interaction with CA125. Amatuximab, a chimeric anti-mesothelin monoclonal antibody elicits antibody-dependent cellular cytotoxicity against mesothelin expressing tumor cells and inhibits heterotypic adhesion of mesothelin-positive tumor cells to CA125-expressing tumor cells. In tumor xenograft studies, combination of amatuximab with chemotherapy showed superior anti-tumor activity compared with chemotherapy alone. Results of this phase II clinical trial of amatuximab with pemetrexed and cisplatin in patients with unresectable pleural mesothelioma show that this treatment was safe and well tolerated. Although there was no improvement in progression-free survival, the median overall survival was superior to historical controls. In a multivariate analysis, baseline mesothelin, baseline ECOG performance status, and baseline CA125 were prognostic of overall survival. A phase III study is planned to validate these findings.

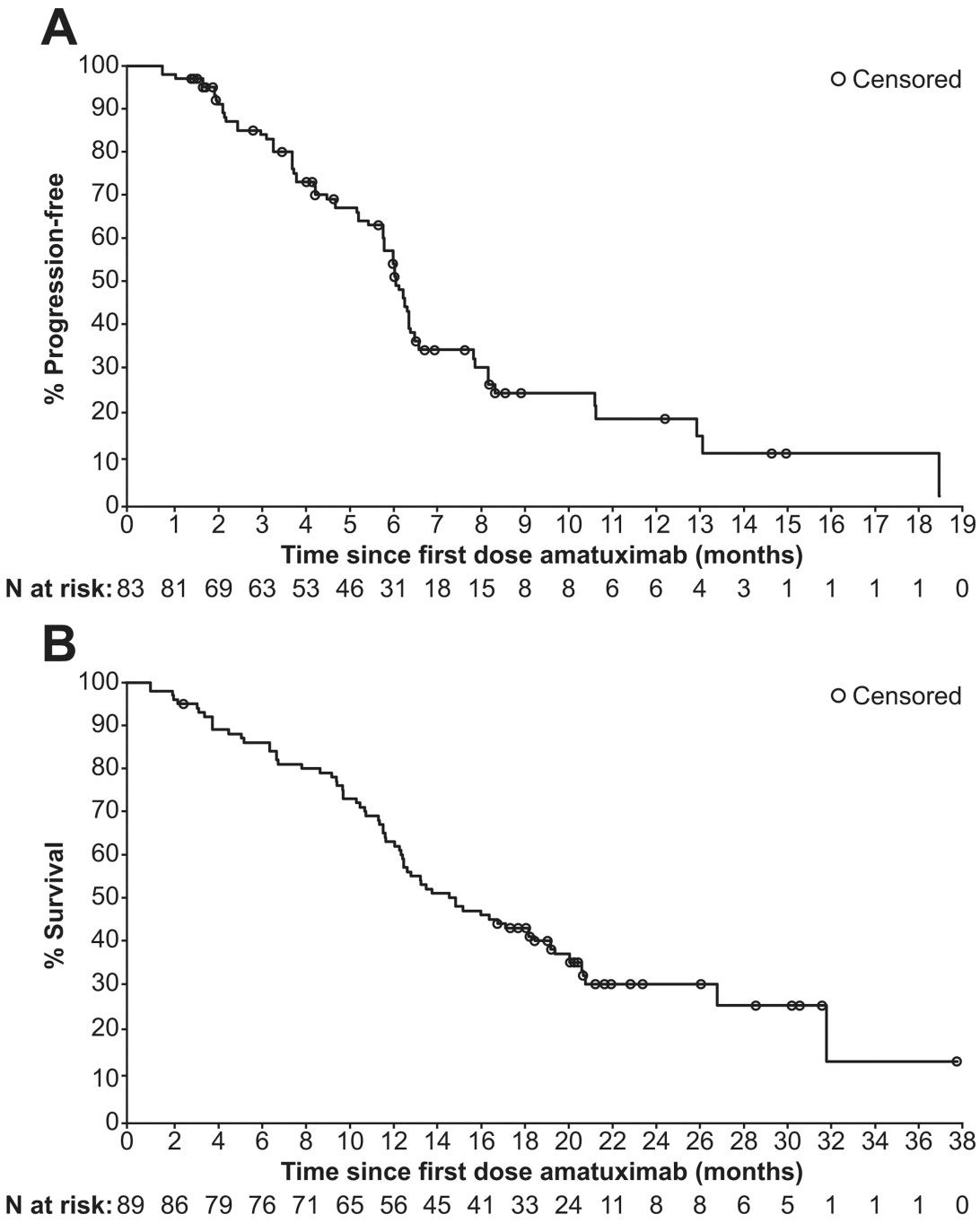

Figure 1.

(A) Progression free survival and (B) overall survival of patients treated with amatuximab and chemotherapy

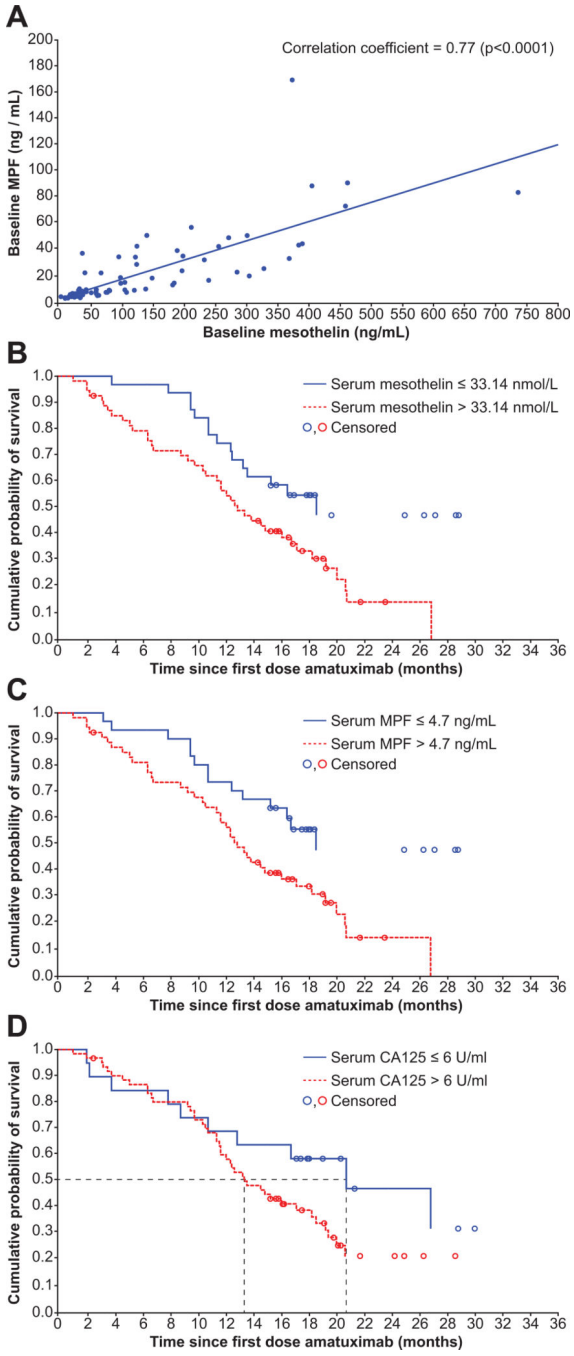

Figure 2.

Correlative markers. (A) Scatter plot showing correlation between baseline serum mesothelin and MPF. (B) Kaplan-Meier curve showing overall survival of patients with mesothelin levels above and below the optimal threshold of 33.14 nmol/L. (C) Kaplan-Meier curve showing overall survival of patients with MPF levels above and below the optimal threshold of 4.7 ng/mL. (D) Kaplan-Meier curve showing overall survival of patients with CA125 levels above and below the optimal threshold of 6 U/ml.

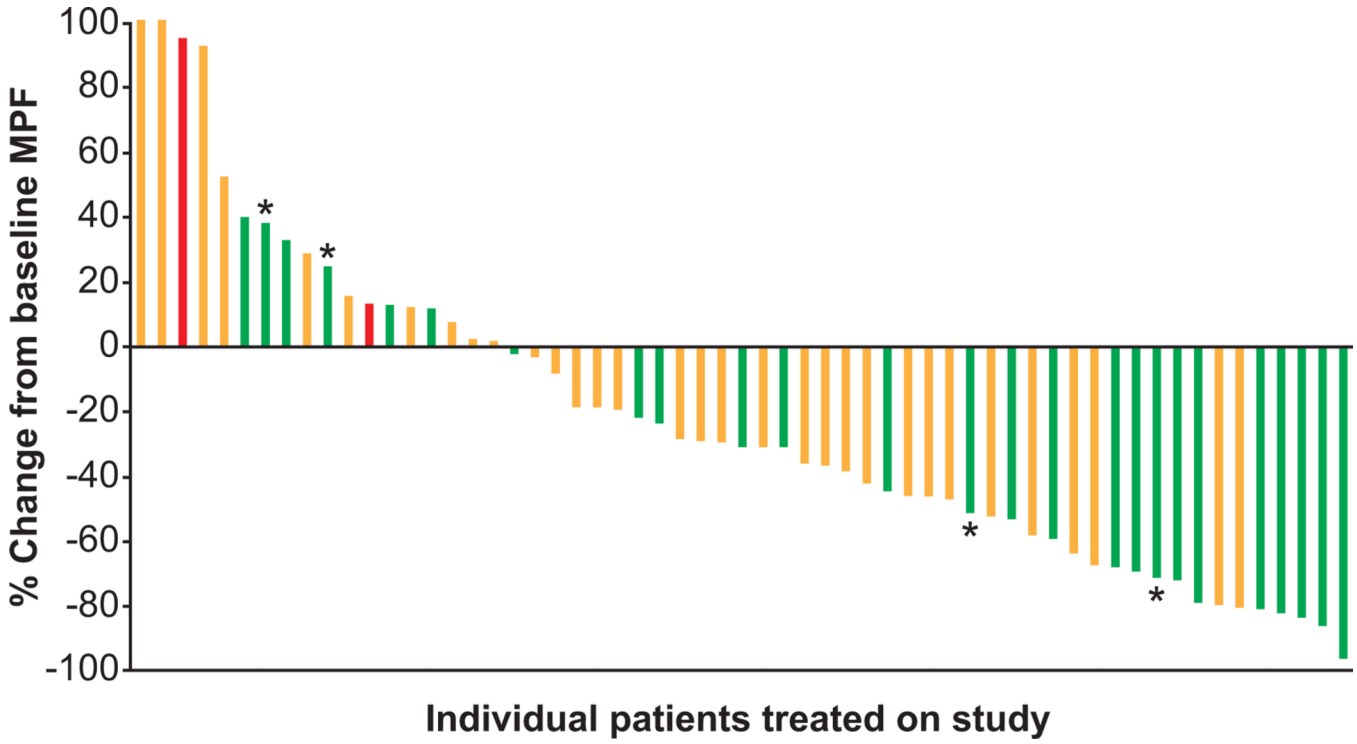

Figure 3.

Waterfall plot depicting the relative change in serum MPF between pre- and post-therapy samples and the best radiological response in each of the 59 patients with available data. *Indicates patients who had an unconfirmed partial response. Green, orange and red bars indicate patients with partial response, stable disease and progressive disease respectively. Abbreviations: MPF, megakaryocyte potentiating factor.

TABLE 1

Patient demographics and baseline disease characteristics

Patient characteristic	Number of patients (%)
Number of patients	89
Age (yr)	
Median (range)	67 (46–80)
Gender	
Male	69 (78)
Female	20 (22)
Race	
White	79 (89)
African American	2 (2)
Other	6 (X)
Asian	2 (2)
Karnofsky Performance Status	
100	22(24.7)
90	40 (44.9)
80	21 (23.6)
70	6 (6.7)
Stage of disease	
IV	43 (48)
III	35 (39)
II	5 (6)
IB	4 (5)
IA	2 (2)
Histology	
Epithelial	79 (88.8)
Mixed	10 (11.2)
Exposure to asbestos	
Yes	56 (62.9)
No	20 (22.5)
Unknown	13 (14.6)
EORTC[a] prognostic score	
Low-risk	25 (28.1)
High-risk	64 (71.9)
Smoking history	
Yes	58 (65.2)

Patient characteristic	Number of patients (%)
No	31 (34.8)

[a]EORTC, European Organization for Research and Treatment of Cancer

TABLE 2

Efficacy results

Outcome studied	Number of patients (%)
Number of patients	83
Responses, number of patients (%)	
PR	33
SD	42
PD	8
Objective Response Rate, %	33
95% CI	(29.2, 51.1)
6-month PFS, %	51.3%
95% CI	(39.1, 62.3)
PFS, months	
Median	6.1
95% CI	(5.8, 6.4)
OS, months	
Median	14.8
95% CI	(12.4, 19.2)

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; OS, overall survival

TABLE 3

Treatment emergent adverse events with a frequency of >15%

Adverse events	Any Grade	Grade 3 or 4
	Number of patients (%)	
Combination phase		
Nausea	63 (70.8)	4 (4.5)
Fatigue	54 (60.7)	9 (10.1)
Anorexia	38 (42.7)	5 (5.6)
Vomiting	28 (31.5)	2 (2.2)
Constipation	27 (30.3)	1 (1.1)
Anemia	26 (29.2)	10 (11.2)
Neutropenia	26 (29.2)	15 (16.9)
Diarrhea	25 (28.1)	1 (1.1)
Weight decrease	18 (20.2)	2 (2.2)
Dysgeusia	17 (19.1)	0
Asthenia	15 (16.9)	1 (1.1)
Peripheral edema	15 (16.9)	0
Dizziness	15 (16.9)	0
Non-cardiac chest pain	15 (16.9)	3 (3.4)
Dyspnea	14 (15.7)	3 (3.4)
Rash	14 (15.7)	0
Single-agent phase		
Dyspnea	13 (23.2)	3 (5.4)
Nausea	11 (19.6)	0
Peripheral neuropathy	10 (17.9)	0
Fatigue	10 (17.9)	3 (5.4)
Non-cardiac chest pain	9 (16.1)	0