MABp1, A Novel Antibody Therapy for Treating Advanced Colorectal Cancer: A 2:1 Randomized, Double Blind, Placebo-controlled, Phase 3 Study

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Total number of:
1) Abstract word count: 438
2) Text word count: 6794
3) Tables: 7
4) Figures: 2
5) Supplementary Tables: 6
6) Supplementary Figures: 3

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Background: An antibody (MABp1) targeting interleukin-1α previously demonstrated a 34% disease control rate and notable recovery from debilitating symptoms such as loss of lean body mass (LBM), fatigue, pain and anorexia in end-stage patients. Symptomatic improvement from treatment suggested that these symptom measures may represent a novel way to assess efficacy. A Phase III study was thus designed using these criteria to evaluate health status as a means to determine efficacy of the anti-tumor therapy in patients with advanced disease.

Methods: In this double-blind placebo-controlled randomized phase 3 trial, a central randomisation scheme with Interactive Web Response System was employed to assign patients (2:1) to receive either MABp1 or placebo. Patients enrolled had metastatic or unresectable disease, failed oxaliplatin and irinotecan, ECOG status 1-2, systemic inflammation or weight loss, and other disease-related morbidities that are poor prognosticators. Patients received 4 bi-weekly i.v. infusions of MABp1 or placebo at 7.5 mg/kg and were assessed for response. The primary endpoint, Clinical Response Rate (CRR), was determined for a modified intent to treat population, which included all patients that received at least one dose of MABp1 or placebo. CRR was prospectively defined as stable or increased LBM (measured by Dual Energy X-ray Absorptiometry) and stable or improved health status in two or three of the categories pain, fatigue and anorexia (reported using EORTC-QLQ-C30) from baseline to week 8. [NCT02138422]

Findings: Patients were randomized between May 20th 2014 and September 2nd 2015. The study was completed on November 3rd 2015. The observed CRR for MABp1 treated patients was 68 (33%) of 207 and 19 (19%) of 102 for placebo (relative risk 1.76, 95% CI 1.12-2.76, 1-tailed p=0.0045). The most common grade 3-4 events were anemia (8 of 207 [4%] vs 5 of 102 [5%]), alkaline phosphatase increase (9 of 207 [4%] vs 2 of 102 [2%]), fatigue (6 of 207 [3%] vs 7 of 102 [7%]), and AST increase (6 of 207 [3%] vs 2 of 102 [2%]) in MABp1 vs placebo, respectively. During the 8 week study period 18 patients died in the MABp1 arm (9%) vs 11 (11%) in placebo. There were no deaths or serious adverse events related to therapy. There seemed to be a reduction in SAEs in the treatment arm (48 (23%) of 207) versus placebo (32 (31%) of 102) although the difference did not reach statistical significance (1-tailed p=0.06).

Interpretation: A new symptom-based endpoint was found to be useful in evaluating responses to a therapy that targets tumor-related inflammation. Using this approach, an antibody derived from human

immunity against an endogenous mediator of inflammation was shown to provide clinical benefit in advanced colorectal cancer.

Funding: XBiotech

Introduction

Colorectal cancer is the second leading cause of malignancy in the industrialized world and the incidence is increasing with economic development and aging worldwide[1]. Half of all patients diagnosed currently will progress and succumb to the disease[2]. Disease progression is typically associated with significant morbidities related to the underlying disease process as well as to treatment-related toxicities. In this population, the benefit of further therapy must be weighed against increasing morbidities and loss of life quality related to the therapy itself. A substantial and growing need therefore exists for a way to evaluate new anti-cancer agents with respect to their ability to offer unequivocal clinical benefit during therapy to patients suffering from advanced colorectal and other forms of cancers.

The European Medicines Agency (EMA) has provided a regulatory path to encourage and expedite the development of anti-cancer agents that improve patient health status while prolonging life. These guidelines enable development of anti-cancer agents based on an effect that improves debilitating symptoms in patients, particularly where the effect is the result of an anti-tumor mechanism and the clinical measures are considered prognosticators for overall survival[3]. The current study design was developed based on this concept in collaboration with the EMA's Scientific Advice Working Party (SAWP).

The treatment agent used in the study was a human monoclonal antibody derived from a human with natural neutralizing antibodies against interleukin-1α (IL-1α). The IL-1 pathway, and specifically IL-1α, is a highly desirable target for anti-cancer therapy because of its pathological role in both local and systemic effects of cancer[4]. IL-1 is a key source of inflammatory signaling in the tumor microenvironment, where it occurs as a result of malignant cells or infiltrating leukocytes or stromal cells[5]. IL-1 can by itself drive varied inflammatory processes, such as COX-2 upregulation, but it also induces several inflammation-inducing mediators (cytokines/chemokines, matrix-metalloproteinases, angiogenic factors, etc.), which result in amplification of the inflammatory response and the creation of a pro-tumor environment. IL-1 activity in the tumor microenvironment is thus implicated in the promotion of tumor invasiveness and metastasis[6,7,8]. In addition, IL-1 activity induces expression of adhesion molecules on endothelial cells, tumor cells and leukocytes and thus increases cell infiltration at sites of tumors and promotes metastatic spread of the malignant cells. In experimental tumor systems and in patients, effects of IL-1 activity in the tumor microenvironment with respect to tumorigenesis and tumor invasiveness (growth, angiogenesis, local spread and metastasis) have been described[9,10]. The IL-1 pathway also contributes to suppression of anti-tumor immune mechanisms such as immune recruiting

and activating myeloid-derived suppressor cells (MDSCs) and T regulatory cells[11,12]. IL-1α and cytokines it induces, like interleukin-6 (IL-6), cause fever, fatigue, anorexia, and acute phase protein secretion. IL-1 signaling via the hypothalamus-pituitary-adrenal axis may mediate metabolic pathology, involving heightened gluconeogenesis and loss of lean body mass (LBM). IL-1-signaling at the site of muscle can also affect a direct breakdown of muscle tissue. As IL-1 signaling mediates these myriad local and systemic responses in the context of malignant tumors, neutralization with a monoclonal antibody was believed to have the potential to antagonize tumor growth and to reverse debilitating morbidities associated with the disease.

Findings previously reported with the monoclonal antibody therapy in advanced cancer patients supported this hypothesis[13]. In the previous study, monotherapy with the antibody was associated with a 34% disease control rate. Key pharmacodynamic responses were also seen, including normalization of paraneoplastic thrombocytosis, reduction in metabolic rate and a lowering of systemic inflammation (serum IL-6 levels)—all measures known to correlate with overall survival in advanced cancer[14,15,16]. There was also recovery from key disease related morbidities, including reduction in fatigue, pain, and anorexia. Novel observations also included marked gains in LBM. In colorectal cancer, good outcomes in patients with symptomatic improvement suggested that symptom measures might represent a novel method to assess treatment benefit in advanced cancer. A phase III study was designed in order to confirm these earlier findings.

Methods:

Study Design and Participants

The study was conducted at forty-two outpatient oncology clinics in the European Union and Russia, and was an 8 week, randomized (2:1), double blind, placebo-controlled design. [NCT02138422]

All patients included in the study were expected to have both metastatic and symptomatic disease. Furthermore, inclusion criteria focused on symptomatic elements of the disease that correlate with prognosis. That is, patients were systematically selected with multiple symptoms that portend *poor* outcomes. Patients were required to have failed both oxaliplatin and irinotecan in prior regimens for metastatic disease. Patients were a minimum of 18 years but also included those beyond 70 years of age. Eastern Cooperative Oncology Group (ECOG) status 0 patients were excluded, with only ECOG 1 or 2 eligible. Disease related morbidities were required and were separated into two domains, to ensure that patients had evidence of key pathophysiological symptoms that could be measured respectively through DEXA and self-reported outcomes. Patients were required to have at least one abnormality in each domain.

Patients were thus required to have either any degree of unintentional weight loss (up to 20%) in the previous 6 months or serum Interleukin 6 levels ≥10 pg/ml[17]. They were also required to have one or more patient reported symptoms: anorexia, with a score of >10; presence of fatigue, with a score of >10; presence of pain, with a score of >10; decreased role, emotional and social function, with a score of < 90. Symptoms were captured using the European Organization for Research and Treatment of Cancer (EORTC) quality of life questionnaire (QLQ-C30).

Serum chemistries, blood counts and IL-6 levels were required to assess eligibility. There were no restrictions regarding histologies permitted nor for molecular aberrations, such as KRAS mutation. Progressive disease was established based upon failure of both oxaliplatin and irinotecan based regimens, as well as the presence of metastatic or inoperable disease. The estimated life expectancy in this population is approximately 4.6 months, although this could be less due to exclusion of ECOG 0 patients[18].

A two-week washout from previous cancer therapies or from agents used to treat symptoms, such as corticosteroids or stimulants was mandatory. Subjects with mechanical obstructions, uncontrolled medical disorders or dementia were excluded.

The study was performed in accordance with the declaration of Helsinki and in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP). The study protocol and all its amendments were reviewed and approved by the appropriate independent ethics committees and all patients provided written informed consent prior to participation.

An Independent Data Monitoring Committee (IDMC) was established to assess safety of the intervention at a pre-specified interim analysis, which occurred after 50% enrollment. The IDMC was also responsible for recommending adjustments to the sample size based upon the number of subjects that were not evaluable for the primary endpoint at the interim safety analysis.

Randomisation and Masking

The study employed a non-stratified randomisation plan. A central randomisation scheme with Interactive Web Response System (IWRS) was employed to facilitate effective randomisation and allocation concealment. The scheme used a block randomisation technique, randomly assigning participants within blocks (block size 6) based on a 2:1 allocation ratio to MABp1 or placebo. The randomization sequence was generated using Oracle Clinical (OC) Remote Data Capture (RDC) application (Oracle Corporation, Redwood City, CA USA). When a patient was randomized by the site investigator, the RDC generated a unique randomization sequence number (randomization code). The randomization code and the study arm assignment were safely retained in the backend of the OC database. The contract drug distribution organization had one-time access to download the un-blinded list for the purpose of labeling and shipping of the study drugs. The patients, investigators, the Clinical Research Organization (CRO), and the sponsor were blinded to treatment allocation until after completion of the study and database lock.

Procedures

Patients were randomized to treatment with antibody (7.5 mg/kg) plus best supportive care (BSC) versus placebo plus BSC, with intravenous administration every two weeks for a total of 8 weeks (4 doses). BSC did not include any agents with proven anti-cancer effect or other agents that might conceivably confound measurement of the primary endpoint, such as corticosteroids, megestrol acetate or stimulants. No information was collected on subsequent anti-neoplastic therapy that patients received after coming off study either during the 8 week study period or in the extension period, as this would not affect the primary endpoint assessment which occurred at 8 weeks.

Patient welfare was paramount to study design. The study was thus conceptualized in a manner that would provide the potential for all patients in the study to receive antibody therapy and further, to allow patients to continue on therapy as long as they were deemed to be benefiting. After completion of the scheduled 8 week treatment regimen, all patients from either arm were eligible to receive MABp1 in an *open label extension* phase of the study. Treatment allocation from the 8 week study period was not revealed after completion of the study, therefore neither patients nor caregivers were aware whether patients entering the open label extension were transferring from placebo or merely staying on active drug. Assessment of primary and secondary endpoints were based on data collected during the 8 week study period. There were no further prospective assessments of efficacy in the open label extension, where study visits included only safety assessment. The open label extension is still ongoing.

During the 8 week study period, tumor assessments, DEXA scans, and administration of the EORTC questionnaire were performed at baseline, prior to dosing, and again at 8 weeks of therapy. Patients were assessed for adverse events and had routine laboratory assessments (chemistries and hematology) every 2 weeks. Patients were required to discontinue therapy for any adverse event of grade 3 or greater with a relationship assessed by the investigator of probably or definitely related to study therapy, or any clinical adverse event, laboratory abnormality or concurrent illness, which in the opinion of the investigator, indicated that continued participation in the study was not in the patient's best interest. There were no requirements for dose modifications, however doses could be delayed for up to 7 days in the event of adverse events that were not related to study drug administration.

DEXA is an imaging modality used to determine the mass (in grams) of bone, fat and lean body compartments[19]. DEXA is an accurate and precise method for measuring body composition, with a coefficient of variation for serial measurements of LBM between 0.4% and 1.3%[20]. Analysis of DEXA images was performed by a central imaging vendor. A board certified radiologist, blinded to treatment allocation, was responsible for reviewing DEXA images for artifacts and confirming correct placement of cut-lines, and the plausibility of the calculated numbers. Patients were scanned with the same DEXA machine using the same software version, at both screening and week 8. The use of IV or oral contrast was restricted within 14 days of receiving the baseline or follow up scan.

Tumor measures were performed using CT or MRI imaging within four weeks prior to dosing and after 8 weeks from first treatment. No additional radiologic assessments were performed after week 8. Tumor assessment was performed by a board certified radiologist at a central vendor, blinded to treatment allocation, utilizing RECIST guidelines (v1.1).

The EORTC QLQ-C30 questionnaire (version 3) is a validated quality of life instrument for assessment of cancer related symptoms. It consists of 30 items that encompass 3 symptom scales (pain, fatigue, and nausea/vomiting), 6 single-item symptom items, 5 functional scales (physical, cognitive, role, emotional, and social), and one scale assessing global health status/quality of life. Each scale consists of 2-5 items. All items have four response categories (not at all, a little, quite a bit, and very much), except for 2 items assessing overall health status/quality of life, which use a seven-point scale.

Outcomes

Clinical response rate (CRR), as defined in the protocol, was a composite endpoint that involved measuring body compartments (to determine lean body mass (LBM)) using dual energy X-ray absorptiometry (DEXA), and the use of the EORTC-QLQ-C30 instrument to assess patient reported outcomes with respect to fatigue, pain and anorexia from baseline to week 8. Patients had to maintain or improve LBM and maintain or improve in regards to two-of-three of the categories of pain, fatigue and anorexia (Figure 1). The clinical response endpoint was prospectively designed as part of the Scientific Guidance procedure with the EMA. The combination of the novel but objective DEXA measurement together with the established but self-reported measures of health status were deemed to be a compelling assessment of clinical performance. These measures were thus combined to create a composite endpoint that was a direct measure of clinical benefit and one that was expected to correlate with overall survival. CRR was not re-assessed during the open label extension.

Evaluation of secondary endpoints was planned to compare treatment versus placebo groups as a whole largely as a measure of drug safety during the 8 week study period. To further elucidate the relevance of the prospective clinical response criteria on an exploratory basis, secondary measures were also examined to further fully characterize the nature of the prospective clinical response. Secondary measures were as follows: EORTC QLQ-C30 for functional performance (role, work and social functions); global quality of life (QoL); adverse and serious adverse events; tumor response (RECIST); paraneoplastic thrombocytosis; and systemic inflammation.

The study design does not enable a comparison of overall survival between treatment arms. The fundamental concept of the study was to establish a clinical response endpoint that could evaluate treatment efficacy rapidly enough to reasonably allow for all patients to have access to active treatment. On recommendation of the study chair, the protocol was amended as of November 20th, 2014 to follow up patients for survival as a measure of safety. Approximately one third of patients had discontinued treatment by the time the amendment came into effect. The availability of overall survival data provided

the opportunity for post hoc survival analysis of outcomes for clinical responders as defined by the primary endpoint.

Other secondary measures involved assessment of pharmacodynamic responses to IL-1 antagonism, particularly those related to disease pathophysiology. Parameters measured were thus change in systemic inflammation as reported by serum IL-6 levels as well as assessment of paraneoplastic thrombocytosis. Univariable analysis was performed to evaluate the assumption of normality. Tukey's 3-inter-quartile range (IQR) method of determining outliers was used as a guides[21]. To make the range more expansive (that detects more extreme values), we replaced IQR range with the range between 5th and 95th quantile. Values higher than 3 times this range were considered extreme outliers.

Testing for hematologic parameters, including platelets and serum IL-6 levels, was performed at screening, and subsequently every two weeks at each dosing visit. Platelet counts were determined by a central laboratory using automated cell counters. Serum IL-6 levels were measured at XBiotech with a commercially available ELISA kit from eBioscience (catalog # 88-7066). An analysis of covariance (ANCOVA) statistical model was used to assess both change in IL-6 levels and platelet counts. The response in terms of IL-6 decrease was determined at last visit compared to baseline. A relative change of <25%, computed as [(post-pre)/pre], was considered a decrease in IL-6.

Safety was assessed by comparing the incidence of serious adverse events (SAEs) and adverse events (AEs) between groups during the 8 week study period and classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events (v4.03) (CTCAE). Assessment of SAEs included all adverse events, which included events *related to underlying, cancer related progression*. Assessment of AEs and laboratory examinations occurred every two weeks while on study.

Statistical Analysis

The trial was designed to have 80% power to detect 20% effect size, with one-sided alpha of 0.0125 and 2:1 allocation ratio. The alpha level was set to 0.0125 in order to account for the two-component composite endpoint.

The primary efficacy analysis was conducted on a modified intent-to-treat population. As defined in the protocol, this population included only those subjects who had been randomised and received at least a single dose of therapy. Patients missing primary endpoint data, and patients that received restricted therapies were considered non-responders.

The initial sample size was 276, which factored in a 5% drop-off rate. During the planned interim review of safety data, the IDMC recommended increasing the oversampling to 20% to account for patients with missing endpoint data.

The primary endpoint was compared between the MABp1 and placebo arms using Pearson chi-square test. Relative risk and unadjusted odds ratio estimates are presented with 95% confidence intervals (95% CI). Accounting for the two components of the composite endpoint, 1-tailed type I error of 0.0125 was used for determining statistical significance of the primary outcome.

Paraneoplastic thrombocytosis and systemic inflammation were assessed using analysis of covariance model (SAS GLM procedure), with classification groups (treatment arm and overall response status) as factor and baseline value as covariate. The difference in least-square means (LS Means) and 2-sided P values derived from analysis of covariance model were presented for comparison.
A sensitivity analysis was performed on the primary endpoint, stratified by ECOG status, gender, geographically and KRAS mutation status.

Clinical sites followed-up patients after study completion or discontinuation and assessed their survival status. An analysis was performed to assess overall survival and a log-rank test was used for comparing between groups. Survival was assessed for all patients that entered the study after the November 2014 protocol amendment, which enabled collection of survival data. Survival duration was defined as time from first study drug administration to date of death. Patients event-free at the last follow-up time point were censored. Patients that went off study prior to the protocol amendment, were lost to follow up or who withdrew consent were not included in survival analysis. Univariate Cox model was used for evaluating the association of the grouping variable and computing hazard ratio (HR). Hazard ratio along with 95% confidence intervals (CI) from the Cox model is reported in the result. Significance was tested at 2-sided p of 0.05. SAS 9.3 (SAS Institute Inc., Cary, NC) was used for statistical analysis.
[NCT02138422]

Role of the funding source:

The sponsor provided the study drug. The study chair (TH), in collaboration with the sponsor, developed the protocol and this report and were responsible for conduct of the study. The sponsor performed data collection. Data review and analysis was performed by the sponsor and TH, who had

access to the study data. An independent data monitoring committee was responsible for unblinded assessment of safety data.

Results

Patients

Findings were based on a total of 458 patients screened, 333 randomized, and 309 receiving at least one dose of therapy between May 20th, 2014 to November 3rd, 2015 (See Figure 2, flow chart showing the patient disposition). The median follow-up duration in the 8 week blinded study period for MABp1 and placebo patients was 49 (IQR 48-50) days and 49 (IQR 48-51) days respectively. The 8 week study period ended after completion of the last patient, last visit, however an open label extension is still ongoing. A total of 202 patients continued in the open label phase of the study and received the active therapy. This included 140 (68%) of 207 patients from treatment and 62 (61%) of 102 patients from placebo arms.

The demographic and baseline characteristics were well balanced between the arms. Comparison of important variables did not show any statistical difference (age p=0.53, sex p=0.54, ECOG p= 0.45, prior antineoplastic medications p= 0.26, body weight p=0.37) (Table 1). The KRAS status of tumors was analyzed and showed no imbalance in distribution between arms. BRAF mutation status was not however captured and therefore the distribution between study arms is not known, although this could have been informative.

There were no differences in corticosteroid (placebo, 1 of 102; MABp1, 1 of 207) or megestrol acetate (placebo 0 of 102; MABp1, 1 of 207) use between patients in either the MABp1 or placebo arms, and no patients that received these agents were responders. In the open label extension 24% (49 of 202) of patients received corticosteroids. Usage of corticosteroids in the open label did not change the AE profile, which suggests that the combination is safe. It is not possible to assess the effect of steroids on the efficacy of MABp1 as no endpoint data was collected during the extension. No patients received anti-neoplastic therapies in the 8 week study or the open label extension.

Efficacy

A clinical response was prospectively defined as a co-primary measure, which included (1) lean body mass as measured by dual energy X-ray absorptiometry; and (2), the EORTC categories pain, fatigue or anorexia. Demonstration of a statistically significant enhancement in rate of clinical responses in the treatment arm versus the placebo was considered a successful primary outcome. The primary efficacy analysis was performed in the 309 patients (207 MABp1 and 102 placebo) who received at least one dose of therapy. The per-protocol population, excluding patients who discontinued therapy prior to week 8 assessment, consisted of 169 MABp1 and 83 placebo patients.

The MABp1 therapy arm had a significant improvement in CRR compared to placebo. As shown in Table 2, patients demonstrated significantly higher CRR with respect to placebo (33% and 19%, relative risk 1.76 (95% CI 1.12 to 2.76, one-tailed p=0.0045)).

Efficacy analysis in the per-protocol population also demonstrated significant improvement in the composite primary endpoint in MABp1 patients: 68 (40%) of 169 MABp1 and 19 (23%) of 83 placebo subjects were responders (relative risk 1.76, (95% CI 1.14 to 2.72, one-tailed p= 0.0033)).

There were 5 (4.9%) of 102 patients in the placebo group who received restricted therapy and were per protocol non-responders. In MABp1 and placebo arms 52 (25%) of 207 and 29 (28%) of 102 were per protocol non-responders for disease progression or missing data (p=0.53).

A sensitivity analysis was performed on the primary endpoint, stratified by ECOG status, gender, geographically and KRAS mutation status. The results were consistent with the benefit observed in the overall MABp1 group (Table 3). Response on individual components of the primary endpoint, i.e. LBM, pain, fatigue, and appetite, did not show any difference between groups (Table 5)

Secondary

Change in platelet count and IL-6 level were significantly different between treatment and placebo arms (Table 4). There was a worsening of paraneoplastic thrombocytosis after 8 weeks, with placebo patients exhibiting increased platelet counts compared to those receiving antibody therapy (40±8 vs 14±5, 1,000 per mm^3, p = 0.0052). Placebo patients were found to have elevated systemic inflammation compared to the active treatment group as measured by serum IL-6 (LS means 9.9±2.7 vs 1.6±1.9 pg/ml, p=0.012). Baseline EORTC response was available for 309 patients, and week 8 EORTC response was available for 241 patients (79 (77%) of 102 Placebo, MABp1 162 (78%) of 207 MABp1, p=0.87. In the majority of cases, the reasons for the absence of completed questionnaires were for patients coming off study early due to disease progression, and hence they did not complete

the questionnaire.

Results from covariance analysis for change in EORTC scores showed no difference between arms. To examine if possible asymmetric distribution of EORTC scores affects the covariance analysis, we re-analyzed the EORTC measures using a mixed model with restricted maximum likelihood (REML) variance component and the results were not different. Assessment of univariate normality of change in IL-6 identified four extreme observations. Three times of 5th and 95th quantile range was 222 mg/ml; the outlying observations reported a change of 275, 746, 1216, and 10176 mg/ml. These four observations were removed from analysis. The univariate analysis of the platelet count did not demonstrate any significant asymmetry; the skewness and kurtosis were within acceptable range of normal univariate distribution (see web appendix p7).

Change in other markers of inflammatory response, such as platelet-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR) and CRP were evaluated. The baseline NLR and PLR were well balanced between MABp1 and placebo arms (NLR 4.6±2.7 and 4.5±3.2 (p=0.87), PLR 196±90 and 207±139 (p= 0.42) respectively). Average change at 8 weeks in NLR was 0.78 (95% CI 0.36 to 1.19) in MABp1 and 1.1 (95% CI 0.55 to 1.65) in placebo, p= 0.35. Similarly no significant change in PLR was observed; average change 41 (95% CI 17 to 66) in MABp1 and 26 (95% CI 10 to 43) in placebo, p= 0.32. With the high variability in the CRP level, detecting statistical significance was not possible.

Computed tomography analysis for tumor response based on RECIST criteria showed that after 4 cycles of therapy, 35 (17%) patients in the treatment arm had stable disease (SD) compared to 12 (12%) patients in the placebo arm. These findings suggested an increased risk of disease progression in the placebo arm compared to the treatment arm (HR 1.26 (95 CI 0.93-1.70, p=0.14). There were no significant differences between arms with respect to patient reported outcomes.

Post hoc Analysis

The primary endpoint was a composite measure of performance with respect to lean body mass and pain, fatigue and anorexia. The break-out of the performance for each of the components measured for this clinical response endpoint showed that the responders indeed exhibited substantial and significant *improvement* in key individual measures for health status. The 87 patients who met the prospective definition for clinical response criteria showed robust improvement for lean body mass (1.4±1.3, median 1.1, kg), as well as reduction in fatigue (-10.85±22.9 [median -11.0]) and pain (-12.66±23.3 [median -16.0]) (Table 6). Appetite improved significantly on average but there was no median change (-

13.80±27.7 [median 0.0]). The changes presented above are the absolute change from baseline. We also calculated the LS mean change after adjusting for baseline values and presented the findings by clinical response status in Table 6. Post hoc analysis further demonstrated that clinical response was prognostic for overall survival as well as for improvement with respect to all other endpoints, including clinical, laboratory, radiologic and patient reported outcomes. Survival data was available for 175 patients (126 of 222 (57%) non-responders and 49 of 87 (56%) responders). At the last follow-up 110 (87%, 95% CI 81 to 92%) non-responders and 25 (51%, 95% CI 38 to 66%) responders had died (log-rank p <0.001). The median survival was 4.2 (95% CI 3.2 to 5.3) months and 11.5 (95% CI 8.3 to 13.2) months for non-responders and responders respectively (see web appendix p5). Overall survival was also compared between the study arms. Survival data was available for 59 placebo patients and 116 MABp1 patients. At the follow-up, 42 (71%, 95% CI 59 to 82%) of placebo patients compared to 93 (80%, 95%CI 73 to 87%) of MABp1 patients had died (log-rank p=0.25, HR 0.81 (95% CI 0.56 to 1.16). The median survival was 6.3 (95% CI 4.1 to 8.9) months and 6.1 (95% CI 4.4 to 7.2) months for placebo and MABp1 arms respectively (see web appendix p6).

Clinical response was significantly associated with lower death (hazard ratio 0.31, 95% CI 0.20 to 0.48, p<0.0001). Moreover, subjects achieving response criteria had a significant reduction in the incidence of SAEs due to any cause (29.3% [65 of 222] vs 5.7% [5 of 87], p<0.0001) compared to non-responders. Patients that experienced a clinical response were also more likely to achieve stable disease (RECIST V1.1) (24.1% [21 of 87] vs 11.7% [26 of 222]; p=0.0062) at the week 8 endpoint.

A similar effect was observed when stratified based on EORTC self-reported symptoms and global QoL, and pharmacodynamic endpoints (see web appendix p4). Response to these measures was prognostically associated with overall survival. However, the survival benefit associated with the clinical response endpoint appeared to be stronger than the individual measures. This prompted an analysis to assess if any additive interaction existed between the components of the primary endpoint, i.e. DEXA and EORTC measures. We used a multivariate Cox model to assess the additive interaction and also calculate the relative excess risk due to interaction (RERI). Interaction term for DEXA and EORTC symptoms was observed on the additive model (p=0.043). This indicated that the joint effect captured in the primary endpoint was stronger than that of the individual component measures.

Secondary measures improved among the prospectively defined clinical responders (see web appendix p2). A reduction was seen in serum IL-6 levels (-3.38±6.31 pg/ml vs 10.3±2.2 pg/ml) and there was an increase in paraneoplastic thrombocytosis in the non-responder group (median change 23,000/mm^3, IQR -11,000 to 60,000), while platelet counts decreased in responders (median -11,000/mm^3, IQR -

38,000 to 39,000) (ANCOVA analysis showed change was statistically significant (p=0.00017)).

Patient functional performance and global quality of life (QOL) also showed marked improvements in responders versus non-responders: QOL (4.32 vs -6.98, p=<0.0001); role function (3.87 vs -13.43, p=<0.0001); emotional function (10.03 vs -2.33, p<0.0001); and social function (10.16 vs -6.71, p<0.0001).

Safety

The most common AEs reported (>10%) were abdominal pain, peripheral edema, fatigue, anemia, constipation, decrease in weight, asthenia, decreased appetite, and nausea. A total of 159 patients receiving experimental therapy and 79 patients receiving placebo had at least one adverse event. The majority of these events were grade 1 or 2, and appeared to be related to the underlying CRC. The prevalence of AEs was similar in treatment and placebo groups (Table 7). The incidence of SAEs in the placebo arm compared to treatment arm was 33 (32%) and 47 (23%) respectively (p=0.07).

The most common grade 3-4 events were anemia (8 of 207 [4%] in the MABp1 arm vs 5 of 102 [5%] in placebo), alkaline phosphatase increase (9 of 207 [4%] in MABp1 vs 2 of 102 [2%] in placebo), fatigue (6 of 207 [3%] in MABp1 vs 7 of 102 [7%] in placebo), and AST increase (6 of 207 [3%] in MABp1 vs 2 of 102 [2%] in placebo). There were no deaths related to therapy. One patient discontinued therapy due to an upper extremity DVT, which occurred one week after study drug administration. This event was assessed as probably related by the investigator, but not related by the sponsor based on analysis of similar events.

During the 8 week study period, 18 patients died in the MABp1 arm (9%) vs 11 (11%) in placebo. There were no deaths related to therapy, and all appeared to be related to the patient's underlying disease. The event terms reported for the deaths by arm are as follows:

- Placebo causes of death: Anemia (1); disease progression (2); dyspnea (1); renal failure(1); liver failure (1); respiratory failure (1); death* (2); general health deterioration (1); and thromboembolic event (1).
- MABp1 causes of death: disease progression (5); CNS metastasis (1); obstruction (1); hepatic failure (1); condition aggravated (2); renal impairment (1); ileus (1); peritonitis (secondary to surgical complication) (1); dehydration (1); respiratory failure (1); hip fracture (1); death* (1); and cardiopulmonary failure (1).

*Died at home after coming off study, presumed to be disease progression

Discussion

A monoclonal antibody targeting the potent inflammatory cytokine IL-1a was derived from a natural human immune response and used to block tumor-related inflammation in advanced colorectal cancer patients. Earlier findings in advanced cancer patients suggested anti-neoplastic effects of antibody monotherapy, including unique observations of resolution of disease-related morbidities. Clinical responses seen were expected to have strong prognostic value and to be useful as novel endpoints to evaluate therapy.

In the present study, a composite primary endpoint consisting of radiological and patient self-reported outcomes was thus used to assess morbidities associated with disease progression in patients with advanced symptomatic colorectal cancer. Subjects that were stable or improved over an 8 week study period with respect to the composite endpoint were considered to have a favorable disease course and prognosis. Patients from either treatment or placebo arms meeting the endpoint criteria would be considered responders while those with progression would be considered non-responders. The study was powered to show a significant enhancement in responder rate for subjects receiving MABp1 monotherapy versus placebo.

The primary finding of the study was a significant increase in the number of responders for subjects receiving antibody monotherapy versus placebo (relative risk 1.76, p=0.0045). Pharmacodynamic measures of MABp1 activity—systemic inflammation and thrombocytosis—were secondary endpoints of the study. A significant reduction in systemic inflammation (Serum IL-6, LS means 1.6±1.9 vs 9.9±2.7 pg/ml, p=0.012) and thrombocytosis (platelet count, 14±5 vs 40±8 (x 1,000/mm^3), p = 0.0052) was seen in the treatment group compared to placebo. The findings confirmed that MABp1 monotherapy rendered significant clinical benefit to patients with advanced colorectal cancer.

Inflammation has long been recognized as a central feature in malignancy, both in the transformation process but also in creating a pro-tumor microenvironment rich in essential remodeling and angiogenic factors[22,23,24]. Efficacy and conversely treatment failure with cytotoxic chemotherapy may also be explained in part by the impact these agents have of inflammatory mechanisms that affect the tumor microenvironment[25]. While the role of inflammation is well established, a targeted anti-inflammatory approach to the treatment of cancer has yet to yield an approved therapy.

A novel endpoint developed in collaboration with the SAWP was established based on the earlier findings where systemic improvements in patients were seen from therapy. Patient self-reporting and objective radiological imaging used in a combined endpoint was expected to provide as a crucial

measure of health status, and to serve as an important metric of underlying disease progression. Even though the novel endpoint was not a validated surrogate for overall survival, since the endpoint provided an unequivocal measure of clinical benefit, successful outcome of the double-blind placebo controlled study was considered to be suitable for registration.

Symptom-based measures have been used in the development of an anti-cancer agent[26]. Nevertheless, a misconception is that an outcome based on symptoms, even if those include objective radiological measures, would be more suited for assessing palliative therapy. To clarify the importance and relevance of the primary endpoint with respect to patient outcomes, we performed detailed post hoc evaluation of subjects that achieved the prospectively defined response criteria.

In the post hoc analysis we undertook a complete deconstruction of the clinical response criteria, separately evaluating individual components of the prospectively defined combined endpoint, as well as all other measures, with respect to responders and across study arms. Since the response criteria required only stabilization or improvement in symptoms, importantly this additional analysis demonstrated the positive magnitude of change with respect to component measures: responders had significant gains in lean body mass (1.41±1.3 kg); and clinically significant reductions in fatigue (-10.85±22.9), pain (-12.66±23.3) and anorexia (-13.80±27.7) were associated with the endpoint. The changes were highly significant in the ANCOVA analysis (Table 6). Moreover, patients meeting clinical response criteria improved with respect to virtually every other measure of anti-tumor activity evaluated in the study, including: 5-fold reduced incidence of SAEs ($p<0.0001$); two-fold increase in likelihood of stable disease at 8 weeks ($p=0.0062$); and a median overall survival of 11.5 versus 4.2 months (HR 0.31, 95% CI 0.20 to 0.48, $p<0.0001$). Results from ANCOVA model comparing least squares mean change, adjusted for baseline values, are presented in Table 6.

Another fundamental observation to come from this post hoc analysis was the finding that neither EORTC nor DEXA measures alone revealed significant differences between arms. These endpoints could not individually therefore serve as a measure of treatment response. These findings confirmed that the combination of radiological and self-reported measures used as the primary endpoint were in fact crucial in identifying patients that were experiencing clinically important recovery or treatment responses to therapy.

The clinical response endpoint has thus offered new perspective on the natural history of colorectal cancer. With nineteen percent of placebo patients achieving the response criteria, this finding suggests that even in advanced disease, compensatory responses to tumors, likely in part involving

immunoregulatory mechanisms, can and do still operate to facilitate recovery from debilitating symptoms and even control progression of the underlying disease process. With this in mind, it should be emphasized that the therapeutic agent used in the study was an antibody isolated from a natural immune response. While a great deal of attention has been given recently to the possible role for enhancing cell mediated immunity to treat cancer, less focus has been given to the potential for augmenting humoral immunity to fight the disease. The presence of natural anti-tumor and immunomodulatory antibodies in human plasma has been documented for some time[27,28,29]. This study represents the first evidence that these antibodies can be useful as therapeutic agents in cancer.

Findings presented here represent the first evidence that antibodies produced as a result of natural humoral immunity can play a role in regulating disease progression in human cancer. Investigating the nature of the clinical responses seen in the placebo subjects, specifically whether these responses were related to endogenous humoral immunity, was beyond the scope of the study. We did, however, confirm that responses in placebo patients were not the result of endogenous anti-IL-1a antibody responses (Data not shown). This raises the possibility that endogenous humoral responses may be regulating disease progression in placebo patients that showed positive clinical courses and that the antibody repertoire in such subjects, if investigated in other studies, might be future sources for additional candidate therapeutic antibodies.

The clinical response criteria used in the study has a number of advantages compared to traditional endpoints. Overall survival studies require large studies and typically long follow-up times. Moreover, there is considerable patient variability in OS outcomes in advanced stage treatments as a result the heterogeneity of patient populations with respect to prior and subsequent therapies[30]. Clinical response criteria evaluated here enable rapid assessment of treatment effect. Sample sizes and study durations using the clinical response criteria are relatively modest, reducing the time and cost of development for new agents. The clinical response endpoint also provides an assessment of patient trajectory after only 8 weeks of therapy, such that patients can be maintained on monotherapy versus placebo for the duration of the endpoint assessment, and further enabling a crossover of all patients to active therapy (which in our experience is a paramount consideration to patient welfare). Finally, the clinical response endpoint is itself a direct measure of crucial aspects of health status, making treatment response an unequivocal measure of patient benefit.

The study design is not without limitations. The responder analysis used is not a traditional endpoint in oncology studies, thus exploring and communicating the value of the endpoint with respect to patient outcomes will require further efforts. While the primary endpoint correlates with substantial overall

individual survival benefit, it is at present difficult to translate the treatment response rate into a customary overall survival expectation for the entire treatment population. The present study involved a relatively small sample population and due to the advanced nature of the subjects enrolled, all patients could not be factored into the endpoint analysis due to disease progression. Since the outcome of the study is binary—patients are either responders or non-responders—patients failing to reach the 8 week endpoint were necessarily considered non-responders. The addition of non-responders to each arm is dilutive of the potential treatment effect, making it more difficult to achieve significance of the primary endpoint. The effect of this difficulty was highlighted with the relatively strong performance with respect to analysis of the per protocol population, where 68 (40%) of 169 MABp1 and 19 (23%) of 83 placebo subjects achieved clinical responses (95% CI 1.14 to 2.72, one-tailed p= 0.0032).

The concept behind the primary endpoint was to establish means of evaluating a targeted cancer therapy using a direct and critical measure of clinical benefit. Patients achieving the primary endpoint in the study had markedly improved overall survival, relatively stable tumor burden and dramatically reduced incidence serious adverse events. Similar to tumor response measures, however, the ability to extrapolate response rates to the entire treated population with respect to overall survival benefit will vary depending on a number of factors, including the durability of the treatment effect, type and phenotype of the targeted tumor, stage of disease and the use of post progression therapy.

A large global Phase III study for MABp1 monotherapy is ongoing in colorectal cancer with overall survival as the primary endpoint. It is also considered that MABp1 may work to improve efficacy of cytotoxic chemotherapy, where disease progression may in part be related to the induction of inflammation and angiogenic factors in the tumor microenvironment[31,32]. These combination studies with MABp1 are currently being planned.

A first-of-a-kind therapeutic antibody derived from natural human immunity has been used to treat advanced colorectal cancer. Monotherapy with the antibody was intended to augment endogenous immunoregulatory mechanisms in patients to help antagonize the chronic inflammatory process involved in tumor growth and disease progression. A novel endpoint used recovery of debilitating symptoms to evaluate anti-tumor activity of the therapy. The finding of significant response to therapy offers a highly innovative new approach to treat advanced cancer.

Author Contributions:

TH, PM, MDS, and JS were involved in the study design, data analysis, and generation of the manuscript. TH, TA, LW, JK, RN, WR, KLK, LP, MPS, TS and ADG were study investigators and collected data. All authors reviewed, edited, and made the final decision to submit the manuscript for publication.

Conflicts of Interest:

MS and PM are employees of and hold stock options for XBiotech. JS is an employee of and holds stock options for XBiotech and holds patents related to anti-interleukin-1α therapy. TA is a consultant for Roche and Bayer. TH reports research funding paid to his institution by XBiotech. All other authors declare that they have no competing interests.

Acknowledgements:

We would like to thank Dr. Charles Dinarello for his advice and support in the development of MABp1, including participation in the EMA Scientific Advice meeting were he explained the history of IL-1 as a therapeutic target and the importance of IL-1 alpha blockade in cancer.

Research in context

Evidence before this study:

Prior to initiation of the pivotal phase 3 trial, an extensive literature review was performed to assess the validity of functional and metabolic parameters as measures of clinical outcomes and prognosis for overall survival in advanced cancer patients.

Results for lean body mass were obtained by searching PubMed for: [lean body mass] [prognosis] [advanced cancer] [survival]. With the exception of the previous trial utilizing MABp1 in advanced cancer, no trials were identified that showed an improvement in lean body mass or a correlation with changing lean body mass and survival.

The following terms were used to investigate the EORTC questionnaire: [EORTC QLQ C30] [Improvement] [survival] [prognosis]. After filtering results for the previous 5 years, 21 articles were found. Several studies were identified, for multiple tumor types, which showed that baseline results in global QoL, symptoms, and functional domains were prognosticators for survival. Further, these studies showed that worsening of these domains with treatment was predictive for worse survival. Finally, there were reports of studies evaluating changes in EORTC domains during treatment for tumor types, including prostate, NSCLC, ovarian, hepatocellular, and colorectal cancer. These trials revealed that improvement in global QoL, and domains such as cognitive function, physical function, emotional function, social function, were all associated with prolonged survival.

Finally, the contribution of IL-6 levels and platelet counts were searched. The following pubmed search terms were used to investigate IL-6: [interleukin-6 level] [prognosis] [advanced cancer] [survival]. The effects of platelets were assessed by searching pubmed for: [platelets] [prognosis] [advanced cancer] [survival]. Numerous reports were found for both searches which showed a correlation between elevated IL-6 levels and platelet counts and survival for several tumor types, including pancreatic, endometrial, ovarian, gallbladder, hepatocellular, non-small cell lung cancer, gastric, renal, and colorectal cancer.

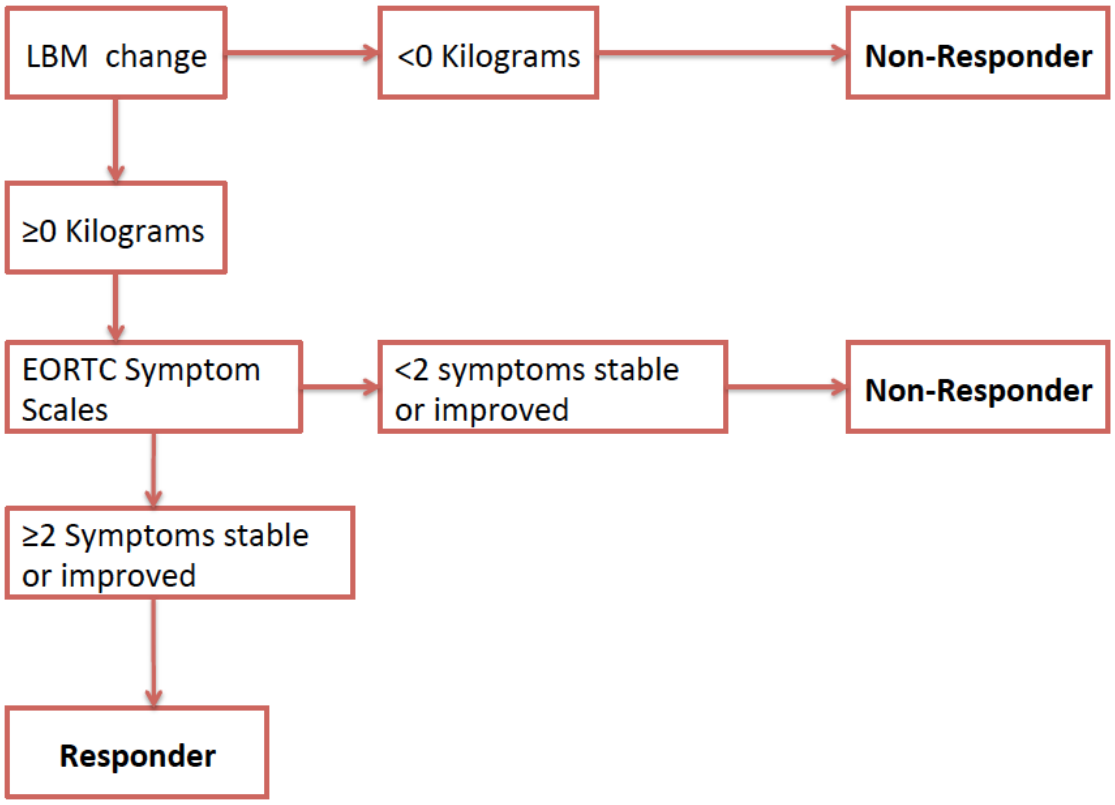
Added value of this study:

Extensive prior work has been performed examining the relationship of key functional and metabolic parameters and their significance in predicting survival outcomes for patients with refractory malignancies. The majority of this work has focused on the prognostic value of baseline results of the EORTC QLQ C30, IL-6 levels, and platelet levels. However, improvement in QoL and functional domains, as measured by the EORTC instrument, with treatment has also been shown to predict prolonged survival. Less evidence surrounding lean body mass change was found, presumably because there are no agents that have demonstrated the ability to increase lean body mass in cancer. The results from the current study show an improvement in a co-primary endpoint of lean body mass change and symptoms as assessed by the EORTC QLQ C30 questionnaire in patients with refractory colorectal cancer and disease associated symptoms. A response as assessed by this co-primary endpoint, was also associated with improvement in global QoL and functional domains, as well as reduction in IL-6 levels and stabilization of platelet counts. These results validate this novel endpoint as an important measure of clinical benefit in patients with refractory disease, and based on prior research, suggest that this endpoint is a surrogate for overall survival benefit.

Implications of all the available evidence:

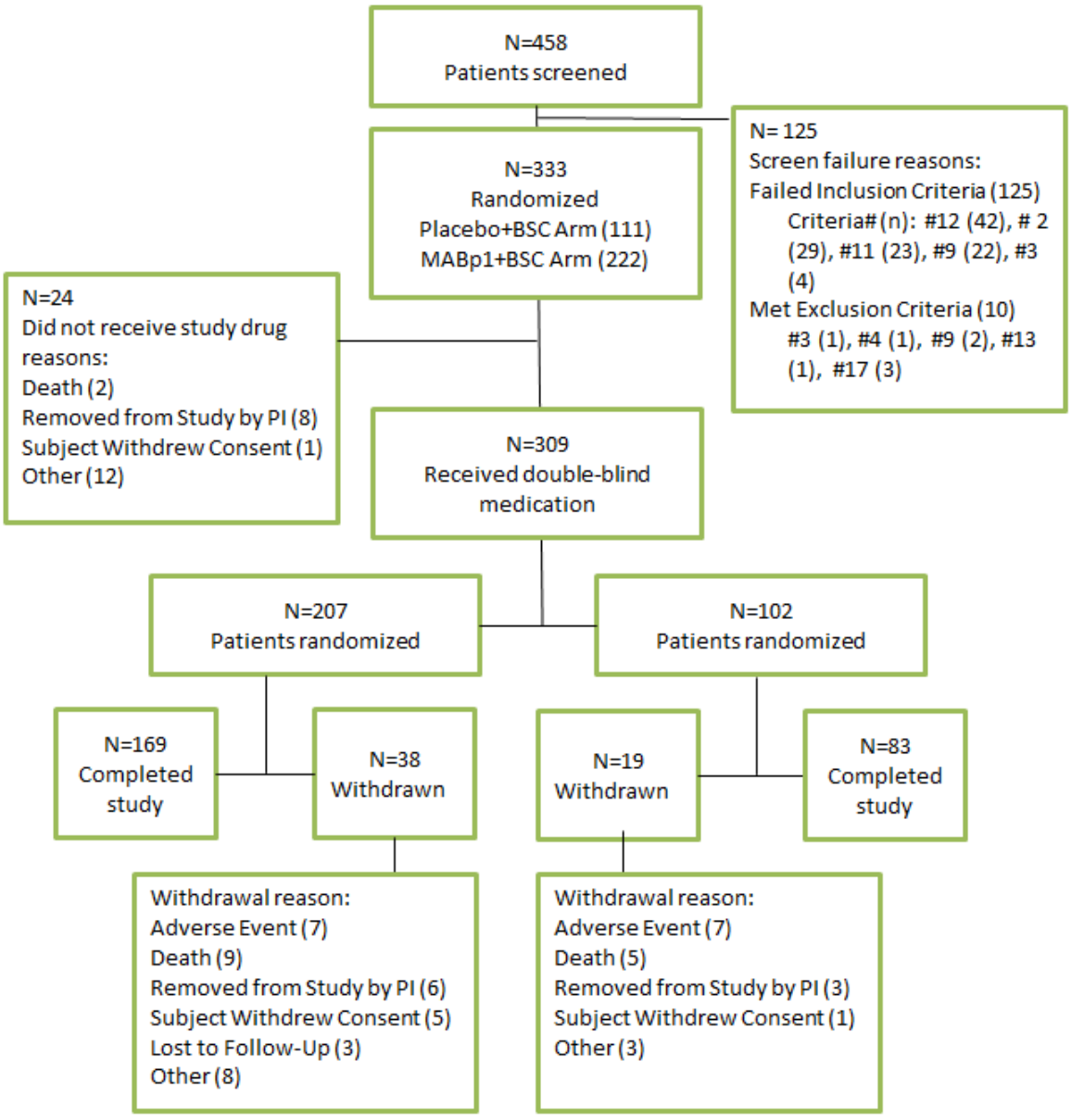
Patients with refractory cancer, who are suffering from disease related symptoms, have few available treatment options. In this setting, the available treatments are frequently associated with toxicities, which may result in these therapies having little to no overall clinical benefit for the individual patient. For this population, clinical benefit should be determined by assessing changes in symptoms that are known to predict morbidity and mortality. In this study, novel objective response criteria has been used to establish the efficacy of MABp1, thus providing a potential blueprint for development of a new class of agents that selectively target the disease pathophysiology.

Figure 1. Response Flow Chart



The primary endpoint of the study was a comparison of response rates between MABp1 monotherapy and placebo arms. Response criteria included DEXA measure of lean body mass (LBM) and patient self-reported assessment of health status using the EORTC-QLQ-C30 questionnaire. From baseline to the 8-week study endpoint, a subject was considered to have achieved a response if they were found to have stable or increased lean body mass, *and* stable or improved symptoms in two-out-of-three of the categories of pain, fatigue and anorexia. Patients in either the treatment or placebo arms could therefore qualify as responders. Responder analysis therefore was a measure of either clinical progression or improvement of individuals across study arms.

Figure 2. Disposition of Patients



A total of 458 patients were screened and 333 randomized. Three-hundred and nine patients received at least one dose of therapy between May 20th, 2014 to November 3rd, 2015. A total of 202 patients continued in the open label phase of the study and received the active therapy. This included 140 (68%) of 207 patients from treatment and 62 (61%) of 102 patients from placebo arms.

Table 1 Demographic and Other Baseline Characteristics (mITT)

	Treatment Group		Total (N= 309)
	MABp1+BSC (N= 207)	Placebo+BSC (N=102)	
Age, year			
Mean	63±10	63±9	63±10
Median	64	63	63
Min-Max	31-83	38-84	31-84
Age distribution, n(%)			
<65 years	112 (54%)	60 (59%)	172 (56%)
≥65 to <75 years	72 (35%)	32 (31%)	104 (34%)
>75 years	23 (11%)	10 (10%)	33 (11%)
Sex, n(%)			
Female	79 (38)	43 (42)	122 (39)
*Race, n(%)			
White	202 (98)	101 (99)	303 (98)
Asian	2 (1)	0	2 (1)
Geographic Region, n(%)			
EU	176 (85)	91 (89)	267 (86)
Georgia	15 (7)	4 (4)	19 (6)
Russia	16 (8)	7 (7)	23 (7)
*KRAS Mutation Status, n(%)			
KRAS Mutation	85 (41%)	37 (36)	122 (39)
KRAS wild-type	91 (44%)	56 (55)	147 (48)
Test Not Done	30 (14%)	9 (9)	39 (13)
ECOG Performance Status			
1	170 (82%)	80 (78)	250 (81)
2	37 (18%)	22 (22)	59 (19)
Days on Study	48±9	49±10	49±9
Baseline Weight, kg			
Mean	74±20	76±16	75±18
Median	72	75	74
Min-Max	36-172	43-154	36-172
Baseline Serum IL-6			
Median (pcg/ml)	9.9 (4.6-28)	9.8 (4.3-25)	
Histology, n(%)			
Adenocarcinoma	204 (99%)	100 (98)	304 (98)
Adenocarcinoma in situ	1 (0%)	1 (1)	2 (1)
Other	2 (1%)	1 (1)	3 (1)
Number of prior chemotherapy regimens for metastatic disease, n(%)			

	Treatment Group		Total (N= 309)
	MABp1+BSC (N= 207)	Placebo+BSC (N=102)	
2	55 (27)	29 (28)	84 (27)
3	56 (27)	33 (32)	89 (29)
4	42 (20)	21 (21)	63 (20)
5	23 (11)	7 (7)	30 (10)
≥6	27 (13)	12 (12)	39 (13)

Abbreviation: ECOG= Eastern Cooperative Oncology Group

- Race was missing for 4 patients and KRAS Mutation Status was missing for one patent

Table 2: Results of CRR Primary Analysis

	MABp1+BSC	Placebo+BSC
N	207	102
Clinical Response, n (%)	68 (33%)	19 (19%)
Difference (effect size)	14%	
P value from Pearson Chi-Square test (one-tailed)	0.0045	
Relative Risk (95% CI)	1.76 (1.12, 2.77)	

Table 3: Sensitivity Analysis of the Response Rate

Sensitivity Analysis	Xilonix+BSC		Placebo+BSC		Difference (effect size)	*P value (Pearson Chi-Square test)	Relative Risk (95% CI)
	N	CRR, n (%)	N	CRR, n (%)			
ECOG 1	170	57 (34%)	80	16 (20%)	14%	0.014	1.68 (1.03, 2.73)
ECOG 2	37	11 (30%)	22	3 (14%)	16%	0.14	2.18 (0.68, 6.97)
Female	79	24 (30%)	43	3 (7%)	23%	0.002	4.35 (1.39, 13.63)
Male	128	44 (34%)	59	16 (27%)	7%	0.162	1.27 (0.78, 2.05)
KRAS Wild-Type	85	30 (35%)	37	6 (16%)	19%	0.02	2.18 (0.99, 4.78)
KRAS Mutation	91	26 (29%)	56	10 (18%)	11%	0.07	1.60 (0.84, 3.06)

CRR: clinical response rate, ECOG: Eastern Cooperative Oncology Group

The primary endpoint was subjected to sensitivity analysis. Responders were stratified by ECOG status, gender, geography and KRAS mutation status. Such stratification has limitations with the relatively small sample population of the study. The results were nevertheless considered consistent with the primary endpoint analysis.

Table 4: Comparison of Pharmacodynamic Outcomes between Treatment arm and Placebo

Change in Self-Reported Outcomes and Pharmcodynamic Measures from Baseline to 8 Weeks	LS Mean±Standard Error		P (LS mean difference)
	Placebo+BSC (n=102)	Xilonix+BSC (n=207)	
Serum IL-6 Levels* (pg/mL)	9.90±2.71	1.6±1.9	0.012
Platelet Count (1000/mm^3)	40±8	14±5	0.0052
Global QOL (Score)	-4.03±2.27	-2.36±1.58	0.547
Physical Function (Score)	-3.38±2.19	-5.11±1.53	0.5183
Role Function (Score)	-7.83±3.02	-6.83±2.12	0.786
Emotional Function (Score)	1.37±2.34	2.50±1.64	0.692
Social Function (Score)	0.00±3.06	-0.89±2.14	0.811

*****Four observations with extreme value were removed.

Individual analysis of EORTC and DEXA measures alone revealed significant differences between arms. Comparison between arms for pharmacodynamic measures shows a significant reduction in serum IL-6 levels and in thrombocytosis. There was no difference in self-reported measures between arms. These findings confirm that the combined primary endpoint were critical to measuring response to therapy.

Table 5: Post Hoc Analysis, Comparing Individual Elements of the Primary Endpoint by Arm

Outcome Measures	Xilonix+BSC (N=207)	Placebo+BSC (N=102)	Difference (effect size)	P value (1-sided Pearson Chi-Square test)	Relative Risk (95% CI)
	Objective Response, n (%)	Objective Response, n (%)			
LBM Response	105 (51%)	46 (45%)	6%	0.18	1.11 (0.89, 1.39)
Pain	93 (45%)	45 (44%)	1%	0.45	1.01 (0.82, 1.25)
Fatigue	94 (45%)	46 (45%)	0%	0.48	1.0 (0.81, 1.25)
Appetite	114 (55%)	49 (48%)	7%	0.12	1.16 (0.91, 1.47)

Post hoc analysis was performed for individual measures of the combined primary endpoint. Each of the composite measures were individually analyzed to assess possible differences between arms. No individual measures were found to be different between arms. These findings confirm that the combined primary endpoint was a more relevant readout than any of the component measures with respect to therapeutic activity of the antibody.

Table 6. Post Hoc Analysis to Assess Change for Individual Measures of Self-Reported and Pharmacodynamic Outcomes

	LS Mean±Standard Error		P value
	Non-responder	Responder	
Change in Lean Body Mass (kg)	0.072±0.22	1.41±0.30	0.00044
Change in Global QoL (Score)	-6.98±1.56	4.32±2.08	<0.0001
Change in Physical Function (Score)	-9.85±1.49	4.12±1.91	<0.0001
Change in Role functioning (Score)	-13.43±2.08	3.87±2.77	<0.0001
Change in Emotional functioning (Score)	-2.33±1.61	10.03±2.15	<0.0001
Change in Social functioning (Score)	-6.71±2.11	10.16±2.81	<0.0001
Change in Platelet Count (x1000/mm^3)	33.3±5.2	-2.0±0.79	<0.00017
Change in IL-6 (pg/mL)	10.3±2.2	-3.38±6.31	0.00071
Change in Fatigue (Score)	10.81±1.81	-8.35±2.42	<0.0001
Change in Pain (Score)	13.70±2.07	-10.01±2.75	<0.0001
Change in Appetite, Score	14.46±2.33	-9.83±3.11	<0.0001
Incidence of Serious Adverse Events	29.3% (65 of 222)	5.7% (5 of 87)	<0.0001
Incidence of Stable Disease	11.7% (26 of 222)	24.1% (21 of 87)	0.0062

Individual components of the primary endpoint, as well as all other outcomes, were assessed with respect to the primary endpoint. Each of these measures were positively correlated with the prospectively defined response. Individual measures of the primary endpoint showed not just stabilization but significant improvement, including gain in lean body mass (1.41±0.30kg (p0.00044); and reductions in fatigue (-8.35±2.42; p<0.0001), pain (-10.01±2.75; p<0.0001) and anorexia (-9.83±3.11p<0.0001). An increase in EORTC scores indicates improvement, except that a reduction in scores for pain, appetite, and fatigue represent improvement. Least-square mean (LSM), computed by fitting analysis of covariance (ANCOVA) model with overall response status as factor and baseline value as covariate.

Table 7. Adverse Events (>10%) Occurring During the 8 Week Period

	Xilonix, n=207					Placebo, n=102				
AE Preferred Term	Grade I/II	Grade III	Grade IV	Grade V	Total	Grade I/II	Grade III	Grade IV	Grade V	Total
Abdominal pain	31 (15.0%)	5 (2.4%)			36 (17.4%)	10 (9.8%)	2 (2.0%)			12 (11.8%)
Fatigue	21 (10.1%)	6 (2.9%)			27 (13.0%)	6 (5.9%)	7 (6.9%)			13 (12.7%)
Oedema peripheral*	24 (11.6%)	4 (1.9%)			28 (13.5%)	5 (4.9%)	2 (2.0%)			7 (6.9%)
Anaemia	13 (6.3%)	8 (3.9%)			21 (10.1%)	2 (2.0%)	5 (4.9%)		1 (1.0%)	8 (7.8%)
Weight decreased	21 (10.1%)				21 (10.1%)	8 (7.8%)				8 (7.8%)
Constipation	21 (10.1%)				21 (10.1%)	6 (5.9%)				6 (5.9%)
Asthenia	17 (8.2%)	2 (1.0%)			19 (9.2%)	7 (6.9%)	3 (2.9%)			10 (9.8%)
Nausea	18 (8.7%)				18 (8.7%)	11 (10.8%)	1 (1.0%)			12 (11.8%)

*Fluid overload in the form of peripheral edema or ascites could potentially confound the assessment of lean body mass as measured by DEXA. However, the composite endpoint was intended to correct for this potential confounder. Only 2.9% of responders in the MABp1 arm (2 of 68) and 5.3% (1 of 19) responders in the placebo had developed evidence of fluid overload (edema or ascites) at the week 8 assessment.

1 References

[1] Lozano R, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380: 2095e128.

[2] Obrand DI, Gordon PH. Incidence and patterns of recurrence following curative resection for colorectal carcinoma. Dis Colon Rectum. 1997 Jan;40(1):15-24.

[3] Guideline on the evaluation of anticancer medicinal products in man, 13 December 2012. EMA/CHMP/205/95/Rev.4. Oncology Working Party

[4] Dinarello CA. Interleukin-1α neutralisation in patients with cancer. Lancet Oncol. 2014 May;15(6):552-3. doi: 10.1016/S1470-2045(14)70164-0. Epub 2014 Apr 17.

[5] Tjomsland V, et al. Interleukin 1α sustains the expression of inflammatory factors in human pancreatic cancer microenvironment by targeting cancer-associated fibroblasts. Neoplasia. 2011 Aug;13(8):664-75.

[6] Tomimatsu S, Ichikura T, Mochizuki H. Significant correlation between expression of interleukin-1alpha and liver metastasis in gastric carcinoma. Cancer. 2001 Apr 1;91(7):1272-6.

[7] Ricote et al. Interleukin-1 (IL-1α and IL-1b) and Its Receptors (IL-R1, IL-RII and IL-1Ra) in Prostate Carcinoma. Cancer 2004;100:1388-96.

[8] Miller LJ, et al. Interleukin-1 family expression in human breast cancer: interleukin-1 receptor antagonist. Cancer Invest. 2000;18:293–302.
[9] Voronov et al. IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci U S A. 2003 March 4; 100 (5): 2645–2650

[10] Salven et al. Interleukin-1α promotes angiogenesis in vivo via VEGFR-2 pathway by inducing inflammatory cell VEGF synthesis and secretion. FASEB J. 2002 July 18; 1471-73.

[11] Apte et al. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. Cancer Metastasis Rev. 2006 Sep;25(3):387-408.

[12] Hong DS, Janku F, Naing A, Falchook GS, Piha-Paul S, Wheler JJ, Fu S, Tsimberidou AM, Stecher M, Mohanty P, Simard J, Kurzrock R. Xilonix, a novel true human antibody targeting the inflammatory cytokine interleukin-1 alpha, in non-small cell lung cancer. Invest New Drugs. 2015 Jun;33(3):621-31. doi: 10.1007/s10637-015-0226-6. Epub 2015 Mar 31.

[13] Hong DS, Hui D, Bruera E, Janku F, Naing A, Falchook GS, Piha-Paul S, Wheler JJ, Fu S, Tsimberidou AM, Stecher M, Mohanty P, Simard J, Kurzrock R. MABp1, a first-in-class true human antibody targeting interleukin-1α in refractory cancers: an open-label, phase 1 dose-escalation and expansion study. Lancet Oncol. 2014 May;15(6):656-66. doi: 10.1016/S1470-2045(14)70155-X. Epub 2014 Apr 17.

[14] Yeh KY, Li YY, Hsieh LL, et al. Analysis of the effect of serum interleukin-6 (IL-6) and soluble IL-6 receptor levels on survival of patients with colorectal cancer. Jpn J Clin Oncol. 2010 Jun;40(6):580-7. doi: 10.1093/jjco/hyq010. Epub 2010 Mar 1.

[15] Stone RL, Nick AM, McNeish IA, et al. Paraneoplastic thrombocytosis in ovarian cancer. N Engl J Med. 2012 Feb 16;366(7):610-8. doi: 10.1056/NEJMoa1110352.

[16] Quinten C, Coens C, Mauer M, et al. Baseline quality of life as a prognostic indicator of survival: a meta-analysis of individual patient data from EORTC clinical trials. Lancet Oncol. 2009 Sep;10(9):865-71. doi: 10.1016/S1470-2045(09)70200-1. Epub 2009 Aug 18.

[17] Wallengren O., Lundholm K., Bosaeus I. Diagnostic Criteria of Cancer Cachexia: Relation to Quality of Life, Exercise Capacity, and Survival in Unselected Palliative Care Patients. Support Care Cancer. 2013; 21:1569-1577.

[18] Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med. 2007 Nov 15;357(20):2040-8.

[19] International Atomic Energy Agency, Human Health Series No. 15. "Dual Energy X Ray Absorptiometry for Bone Mineral Density and Body Composition Assessment". http://www.iaea.org/Publications/index.html

[20] Toombs R.J., Ducher G., Shepherd J.A., and De Souza M. The Impact of Recent Technological Advances on the Trueness and Precision of DXA to Assess Body Composition. *Obesity* (2012) 20**,** 30–39.

[21] Tukey JW, Exploratory Data Analysis. Addison-Wesley, 1977, 43-44.
[22] Rajiput and Wilber. Roles of inflammation in cancer initiation, progression and metastasis. Front Biosci. 20102:176-83.
[23] Terzic et al. Inflammation and colon cancer. Gatroenterology. 2010 138(6):2101-2114.
[24] Gao et al. Role of inflammation-associated microenvironment in tumorigenesis and metastasis. Curr Cancer Drug Targets. 2014. 14(1):30-45.

[25]Sophia Ran The role of TLR4 in chemotherapy-driven metastasis. Cancer Res. 2015. 75(12): 2405–2410.
[26] Harrison, C et al. JAK inhibition with Ruxolitinib versus best available therapy for myelofibrosis. New England Journal of Medicine. 2012 366(9):787-798.
[27] Jager et al. Identification of tumor antigens as potential targets for immunotherapy by serological expression cloning. Cancer Immunol Immunother. 2004 53(3):144-7
[28] Turano and Caruso. The role of human autoantibodies against gamma-interferon. Journal of Antimicrobial Chemotherapy. 1993. 32, Suppl. A, 99-105
[29] Shapir and Shoenfeld. Facing the Enigma of Immunomodulatory Effects of Intravenous Immunoglobulin.
[30] Shi et al. Individual Patient Data Analysis of Progression-Free Survival Versus Overall Survival As a First-Line End Point for Metastatic Colorectal Cancer in Modern Randomized Trials: Findings From the Analysis and Research in Cancers of the Digestive System Database. *J Clin Oncol* 2015 *33:22-28*
[31] Singel and Segal. Neutrophils in the tumor microenvironment: trying to heal the wound that cannot heal. 2016 Immunol Rev. 273(1):329-43.
[32] Daenen et al. Chemotherapy enhances metastasis formation via VEGFR-1-expressing endothelial cells. 2011 Cancer Res; 71(22) 6976-6985.