

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

3 Tamas Hickish<sup>1</sup>, Thierry Andre<sup>2</sup>, Lucjan Wyrwicz<sup>3</sup>, Mark Saunders<sup>4</sup>, Tomasz Sarosiek<sup>5</sup>, Judit Kocsis<sup>6</sup>,  
4 Radim Nemecek<sup>7</sup>, Wojciech Rogowski<sup>8</sup>, Krzysztof Lesniewski-Kmak<sup>9</sup>, Lubos Petruzelka<sup>10</sup>, Ron N.  
5 Apte<sup>12</sup>, Prasant Mohanty<sup>11</sup>, Michael Stecher<sup>11</sup>, John Simard<sup>11</sup>, Aimery de Gramont<sup>13</sup>.

- 6 1. Poole Hospital NHS Foundation Trust, Longfleet Road, Poole, Dorset, BH15 2JB & Bournemouth  
7 University, Bournemouth, UK  
8 2. Oncology Department, Saint Antoine Hospital, and Pierre and Marie Curie University (Paris 6),  
9 Paris, France  
10 3. The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw,  
11 Poland  
12 4. Christie Hospital, Manchester, UK  
13 5. Oncology Department, Magodent, Warsaw, Poland  
14 6. University of Debrecen, Debrecen, Hungary  
15 7. Masaryk Memorial Cancer Institute, Brno, Czech Republic  
16 8. Clinical Department of Chemotherapy, Hospital Ministry of the Interior & Administration &  
17 Warmia & Mazury Oncology Centre, Olsztyn, Poland  
18 9. Gdynia Cancer Center Hospital. Gdynia, Poland  
19 10. General University Hospital in Prague, Prague, Czech Republic  
20 11. XBiotech USA, Inc, Austin, Texas  
21 12. Head, The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of  
22 Health Sciences, Ben-Gurion University of the Negev, 84105 Beer-Sheva, Israel.  
23 13. Oncology Department, Institut Hospitalier Franco-Britannique, Levallois-Perret, France

24

25 **Corresponding Author:**

26 Tamas Hickish

27

28 Castle Lane East

29 Bournemouth, Dorset BH7 7DW UK

30 Work Phone: 01202 303626

31 Email: [tamas.hickish@rbh.nhs.uk](mailto:tamas.hickish@rbh.nhs.uk)

32 Institution: Oncology Department, Royal Bournemouth Hospital NHS Foundation Trust

33

34

35 Total number of:

36 1) Abstract word count: 438

37 2) Text word count: 6794

38 3) Tables: 7

39 4) Figures: 2

40 5) Supplementary Tables: 6

41 6) Supplementary Figures: 3

42

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

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

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

20 **Findings:** Patients were randomized between May 20<sup>th</sup> 2014 and September 2<sup>nd</sup> 2015. The study was  
21 completed on November 3<sup>rd</sup> 2015. The observed CRR for MABp1 treated patients was 68 (33%) of 207  
22 and 19 (19%) of 102 for placebo (relative risk 1.76, 95% CI 1.12-2.76, 1-tailed p=0.0045). The most  
23 common grade 3-4 events were anemia (8 of 207 [4%] vs 5 of 102 [5%]), alkaline phosphatase  
24 increase (9 of 207 [4%] vs 2 of 102 [2%]), fatigue (6 of 207 [3%] vs 7 of 102 [7%]), and AST increase (6  
25 of 207 [3%] vs 2 of 102 [2%]) in MABp1 vs placebo, respectively. During the 8 week study period 18  
26 patients died in the MABp1 arm (9%) vs 11 (11%) in placebo. There were no deaths or serious  
27 adverse events related to therapy. There seemed to be a reduction in SAEs in the treatment arm (48  
28 (23%) of 207) versus placebo (32 (31%) of 102) although the difference did not reach statistical  
29 significance (1-tailed p=0.06).

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

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

3 **Funding:** XBiotech

4

5

6

## 1 **Introduction**

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

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

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

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

9 Findings previously reported with the monoclonal antibody therapy in advanced cancer patients  
10 supported this hypothesis<sup>13</sup>. In the previous study, monotherapy with the antibody was associated with  
11 a 34% disease control rate. Key pharmacodynamic responses were also seen, including normalization  
12 of paraneoplastic thrombocytosis, reduction in metabolic rate and a lowering of systemic inflammation  
13 (serum IL-6 levels)—all measures known to correlate with overall survival in advanced cancer<sup>14,15,16</sup>.  
14 There was also recovery from key disease related morbidities, including reduction in fatigue, pain, and  
15 anorexia. Novel observations also included marked gains in LBM. In colorectal cancer, good outcomes  
16 in patients with symptomatic improvement suggested that symptom measures might represent a novel  
17 method to assess treatment benefit in advanced cancer. A phase III study was designed in order to  
18 confirm these earlier findings.  
19

1 **Methods:**

2 *Study Design and Participants*

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

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

15 Patients were thus required to have either any degree of unintentional weight loss (up to 20%) in the  
16 previous 6 months or serum Interleukin 6 levels  $\geq 10$  pg/ml<sup>17</sup>. They were also required to have one or  
17 more patient reported symptoms: anorexia, with a score of  $>10$ ; presence of fatigue, with a score of  
18  $>10$ ; presence of pain, with a score of  $>10$ ; decreased role, emotional and social function, with a score  
19 of  $< 90$ . Symptoms were captured using the European Organization for Research and Treatment of  
20 Cancer (EORTC) quality of life questionnaire (QLQ-C30).

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

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

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

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

### 9 *Randomisation and Masking*

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

22

### 23 *Procedures*

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

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

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

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

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



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

## 7 *Outcomes*

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

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

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

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

3 Other secondary measures involved assessment of pharmacodynamic responses to IL-1 antagonism,  
4 particularly those related to disease pathophysiology. Parameters measured were thus change in  
5 systemic inflammation as reported by serum IL-6 levels as well as assessment of paraneoplastic  
6 thrombocytosis. Univariable analysis was performed to evaluate the assumption of normality. Tukey's  
7 3-inter-quartile range (IQR) method of determining outliers was used as a guides<sup>21</sup>. To make the range  
8 more expansive (that detects more extreme values), we replaced IQR range with the range between  
9 5th and 95th quantile. Values higher than 3 times this range were considered extreme outliers.

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

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

### 23 *Statistical Analysis*

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

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

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

4 The primary endpoint was compared between the MABp1 and placebo arms using Pearson chi-square  
5 test. Relative risk and unadjusted odds ratio estimates are presented with 95% confidence intervals  
6 (95% CI). Accounting for the two components of the composite endpoint, 1-tailed type I error of 0.0125  
7 was used for determining statistical significance of the primary outcome.

8 Paraneoplastic thrombocytosis and systemic inflammation were assessed using analysis of covariance  
9 model (SAS GLM procedure), with classification groups (treatment arm and overall response status) as  
10 factor and baseline value as covariate. The difference in least-square means (LS Means) and 2-sided P  
11 values derived from analysis of covariance model were presented for comparison.

12 A sensitivity analysis was performed on the primary endpoint, stratified by ECOG status, gender,  
13 geographically and KRAS mutation status.

14

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

26 [NCT02138422]

27

#### 28 **Role of the funding source:**

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

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

3

## 4 **Results**

### 5 *Patients*

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

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

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

### 27 *Efficacy*

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

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

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

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

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

## 21 Secondary

22 Change in platelet count and IL-6 level were significantly different between treatment and placebo arms  
23 (Table 4). There was a worsening of paraneoplastic thrombocytosis after 8 weeks, with placebo  
24 patients exhibiting increased platelet counts compared to those receiving antibody therapy ( $40 \pm 8$  vs  
25  $14 \pm 5$ , 1,000 per  $\text{mm}^3$ ,  $p = 0.0052$ ). Placebo patients were found to have elevated systemic  
26 inflammation compared to the active treatment group as measured by serum IL-6 (LS means  $9.9 \pm 2.7$   
27 vs  $1.6 \pm 1.9$  pg/ml,  $p=0.012$ ). Baseline EORTC response was available for 309 patients, and week 8  
28 EORTC response was available for 241 patients (79 (77%) of 102 Placebo, MABp1 162 (78%) of 207  
29 MABp1,  $p=0.87$ . In the majority of cases, the reasons for the absence of completed questionnaires  
30 were for patients coming off study early due to disease progression, and hence they did not complete

1 the questionnaire.

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

11 Change in other markers of inflammatory response, such as platelet-lymphocyte ratio (PLR), neutrophil-  
12 to-lymphocyte ratio (NLR) and CRP were evaluated. The baseline NLR and PLR were well balanced  
13 between MABp1 and placebo arms (NLR  $4.6\pm 2.7$  and  $4.5\pm 3.2$  ( $p=0.87$ ), PLR  $196\pm 90$  and  $207\pm 139$  ( $p=$   
14  $0.42$ ) respectively). Average change at 8 weeks in NLR was 0.78 (95% CI 0.36 to 1.19) in MABp1 and  
15 1.1 (95% CI 0.55 to 1.65) in placebo,  $p= 0.35$ . Similarly no significant change in PLR was observed;  
16 average change 41 (95% CI 17 to 66) in MABp1 and 26 (95% CI 10 to 43) in placebo,  $p= 0.32$ . With the  
17 high variability in the CRP level, detecting statistical significance was not possible.

18 Computed tomography analysis for tumor response based on RECIST criteria showed that after 4  
19 cycles of therapy, 35 (17%) patients in the treatment arm had stable disease (SD) compared to 12  
20 (12%) patients in the placebo arm. These findings suggested an increased risk of disease progression  
21 in the placebo arm compared to the treatment arm (HR 1.26 (95 CI 0.93-1.70,  $p=0.14$ ). There were no  
22 significant differences between arms with respect to patient reported outcomes.

### 23 Post hoc Analysis

24 The primary endpoint was a composite measure of performance with respect to lean body mass and  
25 pain, fatigue and anorexia. The break-out of the performance for each of the components measured for  
26 this clinical response endpoint showed that the responders indeed exhibited substantial and significant  
27 *improvement* in key individual measures for health status. The 87 patients who met the prospective  
28 definition for clinical response criteria showed robust improvement for lean body mass ( $1.4\pm 1.3$ , median  
29 1.1, kg), as well as reduction in fatigue ( $-10.85\pm 22.9$  [median -11.0]) and pain ( $-12.66\pm 23.3$  [median -  
30 16.0]) (Table 6). Appetite improved significantly on average but there was no median change (-

1 13.80±27.7 [median 0.0]). The changes presented above are the absolute change from baseline. We  
2 also calculated the LS mean change after adjusting for baseline values and presented the findings by  
3 clinical response status in Table 6. Post hoc analysis further demonstrated that clinical response was  
4 prognostic for overall survival as well as for improvement with respect to all other endpoints, including  
5 clinical, laboratory, radiologic and patient reported outcomes. Survival data was available for 175  
6 patients (126 of 222 (57%) non-responders and 49 of 87 (56%) responders). At the last follow-up 110  
7 (87%, 95% CI 81 to 92%) non-responders and 25 (51%, 95% CI 38 to 66%) responders had died (log-  
8 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)  
9 months for non-responders and responders respectively (see web appendix p5). Overall survival was  
10 also compared between the study arms. Survival data was available for 59 placebo patients and 116  
11 MABp1 patients. At the follow-up, 42 (71%, 95% CI 59 to 82%) of placebo patients compared to 93  
12 (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)).  
13 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  
14 placebo and MABp1 arms respectively (see web appendix p6).

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

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

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

1 38,000 to 39,000) (ANCOVA analysis showed change was statistically significant (p=0.00017)).

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

## 6 *Safety*

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

13 The most common grade 3-4 events were anemia (8 of 207 [4%] in the MABp1 arm vs 5 of 102 [5%] in  
14 placebo), alkaline phosphatase increase (9 of 207 [4%] in MABp1 vs 2 of 102 [2%] in placebo), fatigue  
15 (6 of 207 [3%] in MABp1 vs 7 of 102 [7%] in placebo), and AST increase (6 of 207 [3%] in MABp1 vs 2  
16 of 102 [2%] in placebo). There were no deaths related to therapy. One patient discontinued therapy  
17 due to an upper extremity DVT, which occurred one week after study drug administration. This event  
18 was assessed as probably related by the investigator, but not related by the sponsor based on analysis  
19 of similar events.

20 During the 8 week study period, 18 patients died in the MABp1 arm (9%) vs 11 (11%) in placebo.

21 There were no deaths related to therapy, and all appeared to be related to the patient's underlying  
22 disease. The event terms reported for the deaths by arm are as follows:

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

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

31



## 1 **Discussion**

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

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

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

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

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

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

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

10 In the post hoc analysis we undertook a complete deconstruction of the clinical response criteria,  
11 separately evaluating individual components of the prospectively defined combined endpoint, as well as  
12 all other measures, with respect to responders and across study arms. Since the response criteria  
13 required only stabilization or improvement in symptoms, importantly this additional analysis  
14 demonstrated the positive magnitude of change with respect to component measures: responders had  
15 significant gains in lean body mass ( $1.41 \pm 1.3$  kg); and clinically significant reductions in fatigue ( $-$   
16  $10.85 \pm 22.9$ ), pain ( $-12.66 \pm 23.3$ ) and anorexia ( $-13.80 \pm 27.7$ ) were associated with the endpoint. The  
17 changes were highly significant in the ANCOVA analysis (Table 6). Moreover, patients meeting clinical  
18 response criteria improved with respect to virtually every other measure of anti-tumor activity evaluated  
19 in the study, including: 5-fold reduced incidence of SAEs ( $p < 0.0001$ ); two-fold increase in likelihood of  
20 stable disease at 8 weeks ( $p = 0.0062$ ); and a median overall survival of 11.5 versus 4.2 months (HR  
21 0.31, 95% CI 0.20 to 0.48,  $p < 0.0001$ ). Results from ANCOVA model comparing least squares mean  
22 change, adjusted for baseline values, are presented in Table 6.

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

29  
30 The clinical response endpoint has thus offered new perspective on the natural history of colorectal  
31 cancer. With nineteen percent of placebo patients achieving the response criteria, this finding suggests  
32 that even in advanced disease, compensatory responses to tumors, likely in part involving

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

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

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

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

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

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

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

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

29

1 **Author Contributions:**

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

6

7 **Conflicts of Interest:**

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

12 **Acknowledgements:**

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

16

17

18

19

20

21

22

23

1

2

### 3 **Research in context**

#### 4 **Evidence before this study:**

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

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

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

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

#### 28 **Added value of this study:**

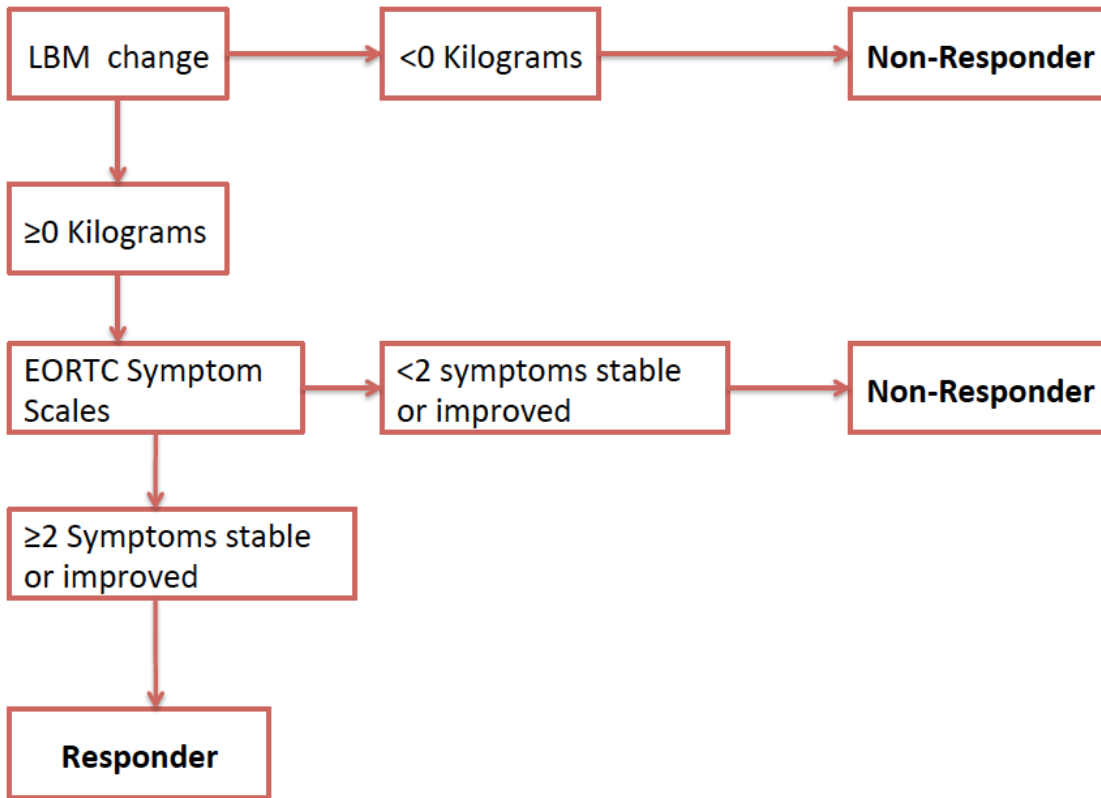
1 Extensive prior work has been performed examining the relationship of key functional and metabolic  
2 parameters and their significance in predicting survival outcomes for patients with refractory  
3 malignancies. The majority of this work has focused on the prognostic value of baseline results of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

15 **Implications of all the available evidence:**

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

23

1 **Figure 1. Response Flow Chart**

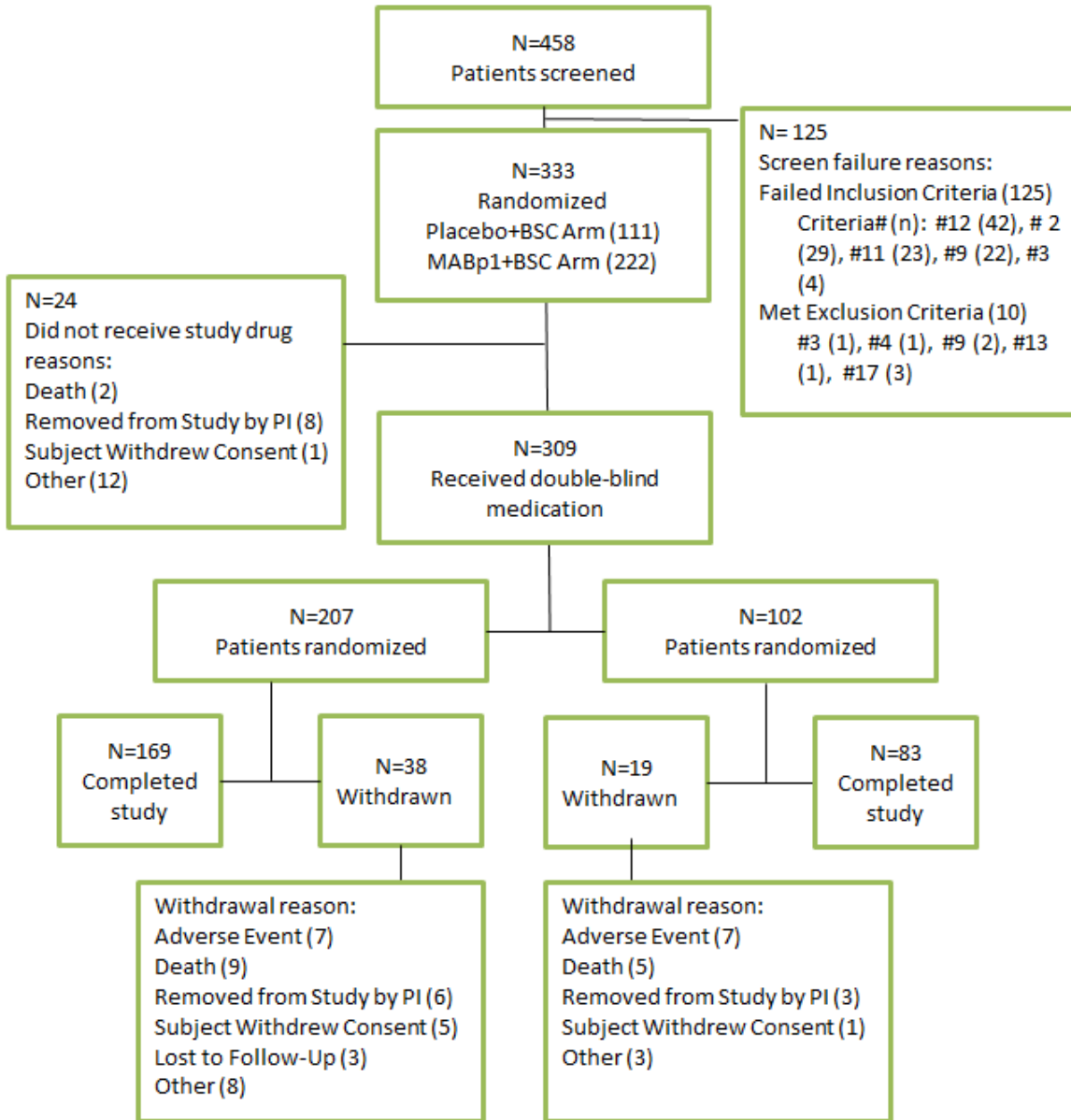


2

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



1 **Figure 2. Disposition of Patients**



2

3 A total of 458 patients were screened and 333 randomized. Three-hundred and nine patients received

4 at least one dose of therapy between May 20<sup>th</sup>, 2014 to November 3<sup>rd</sup>, 2015. A total of 202 patients

5 continued in the open label phase of the study and received the active therapy. This included 140

6 (68%) of 207 patients from treatment and 62 (61%) of 102 patients from placebo arms.

7

8

1 **Table 1 Demographic and Other Baseline Characteristics (mITT)**

	Treatment Group		Total (N= 309)
	MABp1+BSC (N= 207)	Placebo+BSC (N=102)	
<b>Age, year</b>			
Mean	63±10	63±9	63±10
Median	64	63	63
Min-Max	31-83	38-84	31-84
<b>Age distribution, n(%)</b>			
<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%)
<b>Sex, n(%)</b>			
Female	79 (38)	43 (42)	122 (39)
<b>*Race, n(%)</b>			
White	202 (98)	101 (99)	303 (98)
Asian	2 (1)	0	2 (1)
<b>Geographic Region, n(%)</b>			
EU	176 (85)	91 (89)	267 (86)
Georgia	15 (7)	4 (4)	19 (6)
Russia	16 (8)	7 (7)	23 (7)
<b>*KRAS Mutation Status, n(%)</b>			
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)
<b>ECOG Performance Status</b>			
1	170 (82%)	80 (78)	250 (81)
2	37 (18%)	22 (22)	59 (19)
Days on Study	48±9	49±10	49±9
<b>Baseline Weight, kg</b>			
Mean	74±20	76±16	75±18
Median	72	75	74
Min-Max	36-172	43-154	36-172
<b>Baseline Serum IL-6</b>			
Median (pcg/ml)	9.9 (4.6-28)	9.8 (4.3-25)	
<b>Histology, n(%)</b>			
Adenocarcinoma	204 (99%)	100 (98)	304 (98)
Adenocarcinoma in situ	1 (0%)	1 (1)	2 (1)
Other	2 (1%)	1 (1)	3 (1)
<b>Number of prior chemotherapy regimens for metastatic disease, n(%)</b>			

	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)

1 Abbreviation: ECOG= Eastern Cooperative Oncology Group

2 • Race was missing for 4 patients and KRAS Mutation Status was missing for one patient

3

4 **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)	

5

6

7

8

9

10

11

12

1 **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)

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

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

7

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

Change in Self-Reported Outcomes and Pharmacodynamic 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 <sup>3</sup> )	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

10 \*Four observations with extreme value were removed.

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

6

7 **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 (%)			
<b>LBM Response</b>	105 (51%)	46 (45%)	6%	0.18	1.11 (0.89, 1.39)
<b>Pain</b>	93 (45%)	45 (44%)	1%	0.45	1.01 (0.82, 1.25)
<b>Fatigue</b>	94 (45%)	46 (45%)	0%	0.48	1.0 (0.81, 1.25)
<b>Appetite</b>	114 (55%)	49 (48%)	7%	0.12	1.16 (0.91, 1.47)

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

13

14

15

16

17

18

19

20

1 **Table 6. Post Hoc Analysis to Assess Change for Individual Measures of Self-Reported and Pharmacodynamic**  
 2 **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 <sup>3</sup> )	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

3  
 4 Individual components of the primary endpoint, as well as all other outcomes, were assessed with  
 5 respect to the primary endpoint. Each of these measures were positively correlated with the  
 6 prospectively defined response. Individual measures of the primary endpoint showed not just  
 7 stabilization but significant improvement, including gain in lean body mass (1.41±0.30kg (p0.00044);  
 8 and reductions in fatigue (-8.35±2.42; p<0.0001), pain (-10.01±2.75; p<0.0001) and anorexia (-  
 9 9.83±3.11p<0.0001). An increase in EORTC scores indicates improvement, except that a reduction in  
 10 scores for pain, appetite, and fatigue represent improvement. Least-square mean (LSM), computed by  
 11 fitting analysis of covariance (ANCOVA) model with overall response status as factor and baseline  
 12 value as covariate.

13  
 14  
 15  
 16  
 17  
 18

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

AE Preferred Term	Xilonix, n=207					Placebo, n=102				
	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%)

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

6  
7  
8  
9  
10  
11  
12  
13  
14  
15

# 1 References

- <sup>1</sup> 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.
- <sup>2</sup> Obrand DI, Gordon PH. Incidence and patterns of recurrence following curative resection for colorectal carcinoma. *Dis Colon Rectum*. 1997 Jan;40(1):15-24.
- <sup>3</sup> Guideline on the evaluation of anticancer medicinal products in man, 13 December 2012. EMA/CHMP/205/95/Rev.4. Oncology Working Party
- <sup>4</sup> Dinarello CA. Interleukin-1 $\alpha$  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.
- <sup>5</sup> Tjomsland V, et al. Interleukin 1 $\alpha$  sustains the expression of inflammatory factors in human pancreatic cancer microenvironment by targeting cancer-associated fibroblasts. *Neoplasia*. 2011 Aug;13(8):664-75.
- <sup>6</sup> Tomimatsu S, Ichikura T, Mochizuki H. Significant correlation between expression of interleukin-1 $\alpha$  and liver metastasis in gastric carcinoma. *Cancer*. 2001 Apr 1;91(7):1272-6.
- <sup>7</sup> Ricote et al. Interleukin-1 (IL-1 $\alpha$  and IL-1 $\beta$ ) and Its Receptors (IL-1R1, IL-1RII and IL-1RAcP) in Prostate Carcinoma. *Cancer* 2004;100:1388-96.
- <sup>8</sup> Miller LJ, et al. Interleukin-1 family expression in human breast cancer: interleukin-1 receptor antagonist. *Cancer Invest*. 2000;18:293–302.
- <sup>9</sup> 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
- <sup>10</sup> Salven et al. Interleukin-1 $\alpha$  promotes angiogenesis in vivo via VEGFR-2 pathway by inducing inflammatory cell VEGF synthesis and secretion. *FASEB J*. 2002 July 18; 14:71-73.
- <sup>11</sup> 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.
- <sup>12</sup> 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.
- <sup>13</sup> 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-1 $\alpha$  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.
- <sup>14</sup> 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.
- <sup>15</sup> 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.
- <sup>16</sup> 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.
- <sup>17</sup> 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.
- <sup>18</sup> 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.
- <sup>19</sup> 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>
- <sup>20</sup> 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.
- <sup>21</sup> Tukey JW, *Exploratory Data Analysis*. Addison-Wesley, 1977, 43-44.
- <sup>22</sup> Rajiput and Wilber. Roles of inflammation in cancer initiation, progression and metastasis. *Front Biosci*. 2010;2: 176-83.
- <sup>23</sup> Terzic et al. Inflammation and colon cancer. *Gastroenterology*. 2010 138(6): 2101-2114.
- <sup>24</sup> Gao et al. Role of inflammation-associated microenvironment in tumorigenesis and metastasis. *Curr Cancer Drug Targets*. 2014. 14(1): 30-45.



- 
- <sup>25</sup> Sophia Ran The role of TLR4 in chemotherapy-driven metastasis. *Cancer Res.* 2015. 75(12): 2405–2410.
- <sup>26</sup> Harrison, C et al. JAK inhibition with Ruxolitinib versus best available therapy for myelofibrosis. *New England Journal of Medicine.* 2012 366(9): 787-798.
- <sup>27</sup> Jager et al. Identification of tumor antigens as potential targets for immunotherapy by serological expression cloning. *Cancer Immunol Immunother.* 2004 53(3):144-7
- <sup>28</sup> Turano and Caruso. The role of human autoantibodies against gamma-interferon. *Journal of Antimicrobial Chemotherapy.* 1993. 32, Suppl. A, 99-105
- <sup>29</sup> Shapir and Shoenfeld. Facing the Enigma of Immunomodulatory Effects of Intravenous Immunoglobulin.
- <sup>30</sup> 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
- <sup>31</sup> Singel and Segal. Neutrophils in the tumor microenvironment: trying to heal the wound that cannot heal. 2016 *Immunol Rev.* 273(1): 329-43.
- <sup>32</sup> Daenen et al. Chemotherapy enhances metastasis formation via VEGFR-1-expressing endothelial cells. 2011 *Cancer Res*; 71(22) 6976-6985.