Phase II Study of Ensituximab, a Novel Chimeric Monoclonal Antibody, in Adults with Unresectable, Metastatic Colorectal Cancer

Richard Kim[1], Nilofer Azad[2], Michael A. Morse[3], Elizabeth Poplin[4], Amit Mahipal[5], Benjamin Tan Jr. [6], Sharon A Mavroukakis[7], Massimo Fantini[7], Kwong Y. Tsang[7], Anjum Zaki[7], Jose Torrealba[8], Philip M. Arlen[7], Muhammad Shaalan Beg[9]

[1]Department of Gastrointestinal Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL

[2]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

[3]Department of oncology, Duke University Medical Center, Durham, NC

[4]Department of Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

[5]Department of Oncology, Mayo Clinic, Rochester, MN

[6]Department of Oncology, Washington University School of Medicine Siteman Cancer Center, St. Louis, MO

[7]Precision Biologics, Inc., Bethesda, MD

[8]Department of Pathology, University of Texas Southwestern Medical Center of Dallas, Dallas, TX

[9]Department of Oncology, University of Texas Southwestern Medical Center of Dallas, Dallas, TX

Corresponding Author: Corresponding author: Richard D. Kim MD, H. Lee Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612; Phone: 813-745-1277; Fax: 813-449-8553; E-mail: richard.kim@moffitt.org

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Abstract

Purpose: Patients with metastatic colorectal cancer (CRC) refractory to chemotherapy have limited treatment options. Ensituximab (NEO-102) is a novel chimeric monoclonal antibody targeting a variant of MUC5AC with specificity to CRC.

Patients and Methods: Single arm, phase II trial assessed the efficacy and safety of ensituximab in patients with advanced, refractory cancer who expressed MUC5AC antigen in tumor tissue. Ensituximab was administered intravenously every 2 weeks with 3 mg/kg as recommended phase II dose (RP2D). A minimum sample size of 43 patients was required based on the assumption that ensituximab would improve median overall survival by 7 months using a one-sided significance level of 10% and 80% power. Written informed consent was obtained from all patients.

Results: Sixty-three patients with advanced, refractory CRC were enrolled and 53 subjects were treated in phase 2 arm. Median age was 58 years and 46% of the patients were female. Among 57 evaluable patients, median OS was 6.8 months. No responses were observed, and stable disease was achieved in 21% of the patients. The most common treatment related adverse events at RP2D included fatigue (38%), anemia (30%), nausea (15%), vomiting (11%), increased bilirubin (9%), constipation (8%), decreased appetite (6%) and diarrhea (6%). Serious adverse events at least possibly related to ensituximab occurred in 4 patients and included anemia, nausea, increased bilirubin and hypoxia. No patients discontinued treatment due to drug related adverse events.

Conclusions: Ensituximab was well tolerated and demonstrated modest antitumor activity in patients with heavily pretreated refractory CRC.

Translational Relevance

Patients with chemotherapy refractory, advanced colorectal cancer have poor prognosis with limited systemic therapeutic options. MUC5AC is overexpressed in malignant colorectal cancer tissue compared to normal colon mucosa. Ensituximab is a novel chimeric monoclonal antibody that targets MUC5AC. The antitumor activity is primarily through antibody dependent cellular cytotoxicity. In this trial, ensituximab demonstrated promising antitumor activity in patients with chemotherapy refractory colorectal cancer with median overall survival comparable to available therapeutic agents including regorafenib and TAS-102. The study drug was well tolerated with manageable adverse events. No correlation between MUC5AC expression level in tumor tissue and overall survival was observed. The role of ensituximab could be further explored in future trials utilizing combination therapies.

INTRODUCTION

Despite significant improvement in the survival of patients with colorectal cancer (CRC) over the past decade, almost all patients with metastatic disease will succumb to the disease, resulting in a significant number of deaths every year(1). Modest progress has been made recently with approval of regorafenib and TAS-102 for advanced, refractory CRC based on the results of large phase III studies(2,3). However, median overall survival (OS) for patients with chemotherapy refractory CRC remains less than a year(4). Therefore, there is an urgent need for development of additional novel therapeutic options for patients with CRC who have disease progression through standard therapies.

Ensituximab (NEO-102) is a novel chimeric monoclonal antibody (mAb) targeting a glycosylated variant of MUC5AC with specificity to colorectal and pancreatic cancer. This mAb was uniquely developed from a colon cancer tumor associated antigen (TAA) vaccine preparation tested in Hollinshead's laboratory and an early phase clinical trial(5). Its mechanism of action is primarily through antibody dependent cellular cytotoxicity (ADCC)(6). The development of the drug ensituximab has been previously published(6).

MUC5AC is a mucin which contains cysteine regions and participates in the formation of extracellular gels(7). It is typically expressed in stomach and respiratory tract(8). However MUC5AC can potentially be an important biomarker as the protein is expressed in the fetal and precancerous colon mucosa but to a lesser extent in normal colon(9). Overexpression of MUC5AC antigen has been associated with pancreatic cancer and CRC(10). Interestingly MUC5AC is expressed in colon cancer in an aberrantly glycosylated form and ensituximab is able to discriminate the aberrantly glycosylated form from native MUC5AC(6). Pre-clinical data

demonstrates ensituximab targets only MUC5AC-positive tumors and spares MUC5AC antigen in non-malignant tissue(6).

An earlier phase I study established the maximum tolerated dose (MTD) or recommended phase 2 dose (RP2D) of ensituximab at 3.0 mg/kg intravenously (IV) every 2 weeks with encouraging early signs of clinical activity(11). Although there were no partial responses, median OS of patients heavily pretreated with advanced CRC was 12 months (n=15). Furthermore, the drug was very well tolerated with few grade 3 or 4 adverse events. In the study, an immunohistochemistry (IHC)-based companion diagnostic assay was developed to ensure that patients' tumors expressed the NEO-102 target, which correlated preclinically to anti-tumor responses. The study screened 75 patients and NEO-102 antigen expression was seen in 47% of the colon cancer specimens. Based on encouraging preliminary results of the phase I trial which included 15 patients with colon cancer, an expansion cohort in the original Phase 1 clinical trial was added in an amendment to evaluate efficacy (OS) in patients with refractory colorectal cancer (CRC) who had progressive disease during or relapse after at least 2 lines of standard regimens. Thus, we now report the results of the phase II study of ensituximab in refractory CRC patients whose tumors tested at least ≥20% positive for NEO-102 target antigen by IHC.

PATIENTS AND METHODS

Patient Population

Patients at least 18 years of age, diagnosed with advanced refractory CRC for whom standard treatment was no longer effective or did not offer curative or life-prolonging potential, were eligible to participate in this study. Patients were required to have recurrent or progressive disease after at least 2 standard chemotherapy regimens, and tumor sections were required to stain ≥20% positive for NEO-102 antigen target as determined by the Department of

Pathology at a central laboratory or in a CLIA laboratory of a participating site. Patients were required to have measurable disease per Response Evaluation Criteria in Solid Tumors (RECISTv1.1), adequate organ function, Karnofsky performance score of ≥70%,and to have completed chemotherapy ≥2 weeks and immunotherapy ≥4 weeks prior to enrollment. Patient with uncontrolled brain metastases or uncontrolled concomitant illness were excluded. Medications associated with prolongation of QT/QTc interval were not allowed. Written informed consent was obtained from all patients.

Study Design and Objectives

The primary objective of this multi-center phase II study was to assess safety and efficacy of ensituximab in patients with refractory CRC. All patients provided written informed consent prior to participation. Specifically, all patients signed IHC pre-screening consent and only signed protocol treatment informed consent if their tumor tissue stained ≥20% positive for NEO-102 antigen target. The study was a single arm study with a primary endpoint of OS which was compared to historical control(3). The secondary endpoints included overall response rate (ORR) as per RECIST criteria 1.1, safety and tolerability, and exploration of select immunologic correlates associated with administration of ensituximab.

Patients who received at least 2 (two) doses of ensituximab were considered evaluable for response in the study. Patients continued the study drug until disease progression or development of unacceptable drug related adverse events. The study protocol was approved by the Institutional Review Board at participating sites and was conducted in accordance with the Declaration of Helsinki and other Good Practice Guidelines.

Investigational Regimen

In the phase II part, all patients with CRC received ensituximab at 3.0 mg/kg intravenously every two weeks. Six weeks of dosing (4 doses of ensituximab) plus 2 weeks for evaluation was considered one course (57 days). At the conclusion of the first course if restaging scans showed stable disease (SD) or clinical response per RECIST1.1, patients without unacceptable toxicity were allowed to proceed with additional courses of ensituximab.

Dose reductions were mandated for grade 3 or higher toxicities related to the study drug. The study drug could be resumed at a lower dose once the toxicity resolved to grade 1 or baseline prior to the next scheduled dose of ensituximab. If toxicity did not resolve to grade 1 or baseline parameters within 14 days, ensituximab was discontinued. Dose re-escalation was not allowed.

Assessments

Determination of NEO-102 Target

An immunohistochemical assay was designed and validated to assess the expression of NEO-102 on tumor tissue as previously described(11). Laboratories were trained to perform the immunohistochemistry (IHC) testing for NEO-102 antigen on prospective subject's archived unstained tumor slides or tumor block. Clinical sites with CAP/CLIA laboratories capable of conducting the IHC included Johns Hopkins University Hospital (JHUH) and Duke University. Clinical sites who did not have the capability or chose to use the Central Laboratory sent slides or tumor block to University of Texas Southwestern (UTSW) Medical Center.

Assessment of Response

Patients were evaluated for response serologically with CEA levels and radiographically with computed tomography (CT) of the chest, abdomen and pelvis and/or magnetic resonance

imaging (MRI) at the completion of every course (57 days). Response was determined locally by RECIST1.1 and patients continued courses of ensituximab until disease progression, unacceptable toxicity, withdrawal of consent, or physician's decision to discontinue. Patients were monitored for safety during and after dosing. Safety evaluations included vital signs, physical exam, performance status evaluation, complete blood count, blood chemistries, coagulation studies, and urinalyses. Adverse events (AE) were assessed according to the NCI Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 4.03.

Correlative Assays

HACA (Human Antibody to Chimeric mAb)/ HAMA (Human Anti-mouse Antibody)

To ensure that human anti-mouse antibodies were not developed against ensituximab, serum of patients receiving the investigational regimen was tested at BioReliance (Rockville, MD). During cycle 1 only, blood samples (5 ml) were drawn into red-top tubes for HACA/HAMA in the first 10 subjects in this study. HACA/HAMA was measured by ELISA using a commercially available assay kit (BioReliance) qualified for use in this application. Samples were drawn on days 1, 4, 15 and 57.

Cytokines

Blood samples (5 ml) were drawn for cytokine analysis during Cycle 1 in 10 ml red-top tubes (combined with HACA blood draw in same tube) in the first 10 subjects enrolled in the Phase 2 component of this study to evaluate the toxicity risk of cytokine release syndrome (CRS). Samples were drawn on days 1, 4, 15 and 57. Serum was processed via centrifugation within 2 hours of blood draw and samples were frozen and stored at -20˚C until assays were performed. Th1 and Th2 cytokines (IL-1b, IL-2, IL-6, IL-8, IL-10, IL-12p70, GM-CSF, TNF-a, IFN-g)(12) were evaluated using the Human ProInflammatory 9-plex Ultra-

Sensitive Kit, measuring nine specific cytokines in a 96-well MULTI-ARRAY or MULTI-SPOT plate, using a sandwich immunoassay format at BioReliance in accordance with U.S. FDA Good Laboratory Practice regulations (21 CFR Part 58).

Other Research Studies

Blood samples were drawn during cycle 1 on Days 1, 15 and 57 on all patients enrolled in this study. Blood was collected into two 8 ml blue-top (sodium citrate) and one 10 ml red-top tube and stored at room temperature until processed to recover serum, plasma and peripheral blood mononuclear cells (PBMCs). The recovered specimens were aliquoted and cryopreserved under appropriate conditions according to standard operating procedures (SOPs). A selection of the research studies planned to be conducted utilizing both the subjects' serum as well as PBMCs included anti-idiotype assay, natural killer (NK) cell assay, ADCC and Regulatory T-cell assay. The following materials and methods were used to complete these assays.

- **Cell lines and culture**

Human pancreatic carcinoma cell lines ASPC-1 and CFPAC-1 were obtained from the American Type Culture Collection (Manassas, VA). Cancer cells were maintained in RPMI 1640 (Corning Life Science, Manassas, VA, USA). Culture medium was supplemented with 10% USA-sourced and heat-inactivated HyClone fetal bovine serum defined (GE Healthcare Life Sciences, Issaquah, WA, USA), 100 U/mL penicillin, 100 μg/mL streptomycin (Corning Life Science, Manassas, VA, USA).

- **FcγRIIIa (CD16) genotyping**

DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini kit (Qiagen, CA), and stored at −80°C until use. The polymorphism of CD16 was determined by performing allele-specific droplet digital polymerase chain reaction (ddPCR) using the TaqMan array for CD16 (rs396991) (Life Technologies, Grand Island, NY), as previously described(13).

- **Radioactive *in vitro* ADCC assay**

Patient PBMC effectors were thawed the evening prior to conducting the assay and allowed to rest overnight in RPMI 1640 medium containing L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin (Corning Life Science, Manassas, VA, USA) and 10% human AB serum (Gemini Bio-Products, West Sacramento, CA). On the day of the assay, human pancreatic cancer cell line ASPC-1 was used as target in a 4-hour ^{111}In-release assay. Cancer cell lines were labeled with 20 μCi ^{111}In-oxyquinoline (GE Healthcare, Silver Spring, MD) at 37°C for 20 minutes and then seeded at 3000 cells/well in 96-well round-bottom culture plates. Cancer cells were then treated with 10 μg/mL of human IgG1 isotype control antibody (Thermo Fisher Scientific, Waltham, MA, USA) or ensituximab and then PBMCs were added as effectors at effector-to-target (E:T) ratios of 50:1 and 25:1.

Assays listed above were performed for 4 hours following a previously described procedure(13) and specific ADCC lysis was determined using the following equation:

Percent lysis = (experimental − spontaneous)/(complete − spontaneous) × 100.

- **Non-radioactive *in vitro* ADCC assay**

Purified NK cells were obtained from human donor PBMCs using the EasySep Human NK Cell Isolation Kit (StemCell Technologies, Vancouver, BC, Canada) according to the manufacturer's

protocol. Purified NK cells were exposed to vehicle control (RPMI-1640 medium supplemented with L-glutamine, 10% FBS, and antibiotics) or IL-15 superagonist (25 ng/mL) for 48 hours prior to use as effectors. On the day of the assay, CFPAC-1 cells were used as target cells following a non-radioactive ADCC assay procedure previously described(14)

Target cells were treated with human IgG1 isotype control antibody (Thermo Fisher Scientific, Waltham, MA, USA) or ensituximab (10 µg/mL), and then purified NK cells were added at effector-to-target (E:T) ratios of 6.25:1 and 12.5:1.

After 4-hour incubation at 37°C, ADCC activity was analyzed using the Celigo Imaging Cytometer (Nexcelom Bioscence LLC, Lawrence, MA, USA). Specific ADCC lysis was calculated as previously described(15)

Statistical Analysis

Grothey et al.(3) in the CORRECT study demonstrated that the median OS was 5.0 months in subjects with previously treated metastatic colorectal cancer randomized to placebo. In this Phase 2 design, subjects were assumed to be recruited for a period of 24 months and followed for survival for 12 months. To determine an improvement over 5.0 month survival, estimated sample size was 43 subjects, assuming that treatment with ensituximab would improve the median OS by 40% (7.0 months) using a one-sided significance level of 10% and 80% power for this single-arm phase II trial. The sample size was estimated using the software: http://www.swogstat.org/stat/public/one_survival.htm.

Data from the ADCC assay were analyzed using GraphPad Prism (GraphPad Software, La Jolla, CA). Comparisons between two groups were conducted by T-test, and $p < 0.05$ was considered statistically significant.

RESULTS

Patient disposition, demographics, and baseline characteristics

Out of 238 patients with CRC that were screened by IHC, 142 (60%) patients had tumor that was positive for the NEO-102 target antigen. Sixty-three patients with advanced, metastatic CRC were enrolled in the phase I and phase II portions of this study.

The demographics are depicted in **Table 1**. Of 63 patients (all dose levels) with metastatic colorectal cancer, the median age was 58 years (range 32-79) with 46 (73%) patients younger than 65 years of age and 34 patients (54%) male. The ethnic background of study population was predominantly White (76%) followed by African American (19%), Hispanic or Latino (6%), Asian (3%) and unknown (2%). Eleven (17%) patients had Karnofsky Performance score (PS) of 100, 13 (21%) of 90, 28 (44%) of 80, and 11 (17%) of 70, which was the minimum PS for eligibility. The mean time from initial diagnosis to study entry was 3.79 years (±2.22 years) with a range of 1.0-9.8 years. All patients had undergone prior cancer-directed therapy, from 2 to 9 prior therapies (median: 3).

Three (5%) patients withdrew from the study therapy, 3 (5%) patients were removed by the investigator, 2 (3%) did not meet criteria for further therapy, 2 (3%) developed dose limiting toxicity (DLT), 1 (2%) patient died (unrelated to study therapy), and 3 (5%) came off study for other reasons (intercurrent illness). The remaining patients were removed from the study therapy due to progression of disease. Fifty-seven (90%) were evaluable for the primary endpoint (OS) .

Efficacy

The primary efficacy evaluation of OS was conducted on all patients who received 2 or more infusions of NEO-102. Fifty-seven patients with metastatic colorectal cancer were evaluable for

OS (all dose levels) (**Table 2**). The median OS was 6.8 months (95% CI: 5.4-8.0 months) (**Figure 1**) which is significantly larger than the OS of 5.0 months of the historical control (p=0.007). Univariate Cox regression analyses show that none of the risk factors (CEA, the number of prior therapies, and the number of doses) were significantly associated with OS. The proportion of patients who had SD was 21.5% (12/57) with 95% confidence interval of 11.4% to 33.9%. Evaluating only patients who received the recommended phase 2 dose (RP2D) of 3 mg/kg (n=48), the median OS was 6.4 months (95% CI: 5.2-8.0 months). Thirty-one evaluable patients (65%) were alive at 6 months, and 16 patients (28%) were alive at 1 year, while 3 patients lived beyond 2 years.

Of the 57 patients with metastatic colorectal cancer at all dose levels who were evaluable for response, 12 (21%) achieved stable disease (SD) at the end of the first course (C1D57). There were no clinical responses (complete or partial responses). Out of these 57 patients, 49 patients were evaluable for progression-free survival (data available on date of progressive disease), and 8 patients were removed from the study prior to development of progressive disease (taken off study for toxicity, patient withdrawal of consent, and investigator discretion). The median progression-free survival of these 49 patients is 56 days, a mean progression-free survival of 70.4 days with a 95% confidence interval of 60.1 to 80.6 days.

There was no statistically significant correlation between OS and the level of NEO-102 IHC antigen expression.

Safety data

The most common treatment-related AEs in patients with CRC who received ensituximab at the MTD/RP2D (3 mg/kg) included fatigue (38%), anemia (30%), nausea (15%), vomiting (11%), increased bilirubin (9%), constipation (8%), and chills, decreased appetite, headache and

diarrhea (6%). AEs in patients with CRC at the RP2D and for all subjects regardless of dose level (1.5 mg/kg, n=3; 2 mg/kg, n=1; 3 mg/kg, n=53; 4 mg/kg, n=6) are contained in **Table 3**. Treatment-emergent serious AEs regardless of attribution are listed in **Table 4Error! Reference source not found.**. Treatment-emergent serious AEs at least possibly related to ensituximab occurred in 2 patients at the RP2D (4%) and included nausea (2%), and hypoxia (2%). Study drug administration at the RP2D was interrupted in 11 patients (17%) for treatment related AEs regardless of attribution and included anemia (2%), hemolysis (2%), abdominal pain (6%), ascites (2%), small intestinal obstruction (2%), bile duct obstruction (2%), sepsis (2%), ALT/AST increase (2%), bilirubin increase (2%), headache (2%) and hypoxia (2%). Ensituximab drug therapy was discontinued in 3 patients (8%) at the RP2D due to following AEs: anemia (2%), hemolysis (2%) and hypoxia (2%). Overall, all AEs were self-limiting, reversible, and most events were mild in severity.

Correlative studies

Human Antibody to Chimeric mAb (HACA)

HACA assay used a validated, enzyme-linked immuno-sorbent assay (ELISA) technique. Results are available on 12 patients. The % analytical recovery (%AR) fell within 80 to 120% of the nominal concentration for all standards between or equal to 250 and 7.8 ng/mL. The % coefficient of variation (% CV) of the calculated concentrations for all standards between or equal to 250 and 7.8 ng/mL was $\leq$ 20% and the % CV of the calculated concentrations for standards at 3.9 ng/mL was $\leq$ 25%. The r^2 of the standard curves was $\geq$ 0.980. All patient samples had concentrations of HACA less than the assays lower limit of detection (LOD) of 3.9 ng/mL.

Cytokines

The serum samples from 13 subjects were used for this study. Detection range for IL-1β, IL-2, IL-8, IL-10, IL- GMCSF was 2500-0.61pg/mL, TNFα 12p70 and IFNγ was 2500 -2.44pg/mL. The standards and serum samples were run in triplicate with 4 time points of each subject in the same plate for avoiding plate variation. There are no elevations of serum IL-1β, IL-2, IL-10, IL-12p70, GMCSF, IFNγ, and TNFα in these 13 subjects at D4, D15 and D57 after NEO-102 infusion comparing to BL before infusion. There were unrelated significant elevations in IL-8 concentrations in 3 subjects. Overall, the cytokine level after NEO-102 infusion indicates there is no indication of significant cytokine release after NEO-102 infusion.

Research Immune Studies

Of the 43 patients with samples available the following genotypes were identified: *FCGR3A 158* V/V (N=7), *FCGR3A 158* F/F (N=14) and *FCGR3A 158* V/F (N=22).

Comparison of the ability of V/V versus F/F PBMCs treated with Ensituximab to induce ADCC on human pancreatic cancer cell line, ASPC-1 that expresses the NEO-102 antigen, is shown in **Table 5A**.

Although PBMCs with CD16 V/V polymorphism showed a stronger ADCC activity *in vitro* compared to PBMCs with CD16 F/F polymorphism, there was no correlation between CD16 polymorphism as analyzed above and patient clinical outcomes. The lack of correlation between CD16 polymorphism and patient outcomes suggests that other immune-related factors (under investigation) may impact the efficacy of Ensituximab *in vitro*.

Ensituximab-mediated ADCC against human carcinoma cells is enhanced by an IL-15 superagonist

To evaluate the ability of an IL-15 superagonist to modulate the ADCC activity of ensituximab, NK cells isolated from PBMCs from two healthy donors were treated with an IL-15 superagonist prior to being added in the non-radioactive ADCC assay as effectors. As shown in Table 5B, IL-15 superagonist significantly enhanced NEO-102-mediated ADCC against CFPAC-1 cells (high expression of NEO-102 antigen), compared with the IgG1 isotype control, at the highest E:T ratio in both donors. These data demonstrated that treatment of NK cells with IL-15 effectively enhances NEO-102-mediated ADCC against NE0-102-positive carcinoma cells *in vitro*.

Discussion

We performed this clinical trial to assess the clinical efficacy of NEO-102 in patients with refractory metastatic colorectal cancer with expression of the NEO-102 target antigen. The results from our study clearly demonstrated that ensituximab was very well tolerated in heavily pretreated patients with advanced CRC. Anemia was the only grade 3 or 4 event which occurred in more than 5% of patients. The mechanism of anemia is unclear but could be related to hemolysis. The most frequent treatment related AEs included fatigue, anemia, nausea, vomiting, increased bilirubin and constipation. Treatment was interrupted due to adverse events in 21% of the patients treated at the RP2D, while only 3 of those patients' (6%) AEs were treatment related. Most of the adverse events were grade 1 or 2 and were easily managed with standard supportive measures.

In terms of efficacy, median survival was 6.8 months which was significantly higher than historical control of 5 months. However, OS is similar to those achieved in clinical trials with regorafenib or TAS-102 in refractory CRC patients(2,3). Patients were heavily pretreated with a median of 3 lines of prior therapies including regorafenib. Approximately, 60% of the patients

had ECOG performance status of 2, consistent with real world setting. No clinical responses were observed in this trial, although 21% of the patients experienced stable disease, some of which were durable. Three patients received treatment for more than 2 years. One-year OS was 28%. The benefit of ensituximab was observed even in patients previously treated with regorafenib. There was no correlation between degree of expression of NEO-102 on IHC with response to ensituximab or OS, suggesting that intensity of staining or percentage of tumor cells positive may not be a good predictive marker for clinical benefit. Better selection of patients with predictive biomarker may lead to improved outcomes with ensituximab.

In this study, patients with refractory CRC were only eligible based on ≥ 20% positive on IHC for NEO-102 target antigen on the tumor samples. Pre-clinical data demonstrated that antibody-staining results with NEO-102 demonstrated specific immunoreactivity with cancer tissues from colon cancer patients, whereas only weak binding, if at all, was observed in normal pancreas or colon tissues which will potentially lead to less toxicity. In our study, 60% of the patients with metastatic colorectal cancer were found to be eligible based upon biomarker requirements.

Extensive correlative analyses were performed as part of this study. Ensituximab was previously tested for antibody-dependent cell cytotoxicity (ADCC) activity against several colorectal and pancreatic tumor cell targets *in vitro*(6). In addition, we performed ADCC on the pancreatic cancer cell line ASPC-1 using PBMCs from cancer patients and evaluated the impact of the CD16 polymorphism on the ADCC activity mediated by ensituximab. We observed *in vitro* that patients harboring the FcγRIIIA 158 V/V genotype had a higher ensituximab-mediated ADCC activity compared to F/F genotype. No correlation was observed between CD16 polymorphism and response or survival of patients in this study, suggesting that other mechanisms may be involved in part in the clinical efficacy of ensituximab. In addition, we evaluated the effects of an IL-15 superagonist on the ability of human NK cells to perform ADCC mediated by ensituximab

and demonstrated that treatment with the IL-15 superagonist significantly enhanced the ADCC mediated by ensituximab against the high NEO-102 positive carcinoma cell line (CFPAC-1), compared with the IgG1 isotype control, in both donors tested. A recent study showed that IL-15 superagonist is able to modulate the phenotype of human NK cells towards a more active cytotoxic function, increasing expression of NK activating receptors, anti-apoptotic factors and factors involved in the NK cytotoxicity, as well as down-regulating gene expression of NK inhibiting receptors and factors involved in the NK cell exhaustion(14). Patients with metastatic malignant melanoma or metastatic renal cell cancer receiving a human recombinant IL-15 in a recent clinical trial showed that IL-15 can be administered safely and effectively in cancer patients. IL-15 administration modulated the function and proliferation of NK cells in these cancer patients, rendering the immune system more aggressive against cancer cells(16).

This study showed that ensituximab appeared to have modest activity with good safety profile in patients with refractory colorectal cancer. The study drug was relatively well tolerated with mild and manageable side effects. Previously conducted phase I trial demonstrated predictable pharmacokinetic profile and elevation of IL-6 and IL-8 post treatment. These findings provide supporting rationale for investigating combination therapies using ensituximab with IL-15 or other agents such as chemotherapy, to enhance efficacy in this patient population.

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Table 1: Demographics for Subjects with CRC receiving Ensituximab

Demographic	**Results**	
	3 mg/kg (N=53)	All Subjects (1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg) (N=63)
Age		
Median (min/max)	58 (32/79)	58 (32/79)
< 65	46 (73%)	46 (73%)
≥ 65	17 (27%)	17 (27%)
Gender		
Male	28 (53%)	34 (54%)
Female	25 (47%)	29 (46%)
Ethnicity		
Hispanic or Latino	4 (8%)	4 (6%)
Not Hispanic or Latino	49 (92%)	59 (94%)
Race		
Asian	2 (4%)	2 (3%)
Black or African American	11 (21%)	12 (19%)
White	39 (74%)	48 (76%)
Unknown	1 (2%)	1 (2%)
Weight: Mean (SD)	79 (21.5)	80 (21.2)
Median (min/max)	78 (43/144)	79 (43/144)
Karnofsky Performance Status		
100%	9 (17%)	11 (17%)
90%	12 (23%)	13 (21%)
80%	23 (43%)	28 (44%)
70%	9 (17%)	11 (17%)
≤ 60%	0	
Mean (SD)	84.0 (9.68)	83.8 (9.7)
Median (min/max)	80 (70-100)	80 (70-100)
Number of Prior Lines of Therapy		
Median (min/max)	3 (2/9)	3 (2/9)

Table 2: Evaluable Patients with Metastatic Colorectal Cancer

Variable	1.5 mg/kg (N=3)	2 mg/kg (N=1)	3 mg/kg (N=48)	4 mg/kg (N=5)	All Subjects (N=57)
Median Survival (months)	12.2	1.4	6.4	7.5	6.8
95% CI for the Median Survival Time	11.4 , 26.6	-, -	5.2 , 8.0	1.3 , 23.7	5.4 , 8.0

Table 3: Incidence of Treatment-Emergent, Treatment-Related Adverse Events

System Organ Class Preferred Term	3 mg/kg (N=53)	All Subjects (1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg) (N=63)
Subjects with at Least 1 Treatment-Related AE	43 (81%)	51 (81%)
Blood and lymphatic system disorders	16 (30%)	20 (32%)
Anemia	16 (30%)	20 (32%)
Hemolysis	3 (6%)	3 (5%)
Cardiac disorders	0	2 (3%)
Tachycardia	0	2 (3%)
Gastrointestinal disorders	15 (28%)	16 (25%)
Abdominal pain upper	0	1 (2%)
Anal incontinence	0	1 (2%)
Constipation	4 (8%)	5 (8%)
Diarrhea	3 (6%)	4 (6%)
Mouth ulceration	0	1 (2%)
Nausea	8 (15%)	8 (13%)
Vomiting	6 (11%)	6 (10%)
General disorders and administration site conditions	24 (45%)	30 (48%)
Asthenia	0	1 (2%)
Chills	3 (6%)	5 (8%)
Fatigue	20 (38%)	23 (37%)
Gait disturbance	1 (2%)	1 (2%)
Malaise	1 (2%)	1 (2%)
Mucosal inflammation	1 (2%)	1 (2%)
Pyrexia	2 (4%)	5 (8%)
Hepatobiliary disorders	1 (2%)	2 (3%)
Hyperbilirubinemia	1 (2%)	2 (3%)
Immune system disorders	1 (2%)	2 (3%)
Allergic transfusion reaction	1 (2%)	1 (2%)
Cytokine release syndrome	0	1 (2%)

System Organ Class Preferred Term	3 mg/kg (N=53)	All Subjects (N=63)
Injury, poisoning and procedural complications	2 (4%)	2 (3%)
Incision site rash	1 (2%)	1 (2%)
Infusion related reaction	1 (2%)	1 (2%)
Investigations	11 (21%)	12 (19%)
Aspartate aminotransferase increased	1 (2%)	1 (2%)
Blood bilirubin increased	5 (9%)	6 (10%)
Blood creatinine increased	1 (2%)	1 (2%)
Blood pressure increased	1 (2%)	1 (2%)
Haptoglobin decreased	1 (2%)	1 (2%)
Neutrophil count decreased	1 (2%)	1 (2%)
Platelet count decreased	1 (2%)	1 (2%)
Weight decreased	2 (4%)	2 (3%)
Metabolism and nutrition disorders	5 (9%)	6 (10%)
Decreased appetite	3 (6%)	3 (5%)
Hyperglycemia	1 (2%)	1 (2%)
Hypokalemia	1 (2%)	1 (2%)
Hypovolemia	0	1 (2%)
Vitamin B12 deficiency	1 (2%)	1 (2%)
Musculoskeletal and connective tissue disorders	5 (9%)	6 (10%)
Arthralgia	2 (4%)	2 (3%)
Back pain	1 (2%)	2 (3%)
Musculoskeletal pain	1 (2%)	1 (2%)
Myalgia	1 (2%)	1 (2%)
Nervous system disorders	5 (9%)	6 (10%)
Dizziness	2 (4%)	2 (3%)
Headache	3 (6%)	4 (6%)
Hypoesthesia	1 (2%)	1 (2%)
Psychiatric disorders	0	1 (2%)
Insomnia	0	1 (2%)
Renal and urinary disorders	1 (2%)	1 (2%)
Chromaturia	1 (2%)	1 (2%)

System Organ Class **Preferred Term**	**3 mg/kg** **(N=53)**	**All Subjects** **(N=63)**
Respiratory, thoracic and mediastinal disorders	6 (11%)	7 (11%)
Dyspnea	4 (8%)	4 (6%)
Hypoxia	1 (2%)	1 (2%)
Nasal congestion	0	1 (2%)
Pharyngeal edema	1 (2%)	1 (2%)
Upper-airway cough syndrome	1 (2%)	1 (2%)
Skin and subcutaneous tissue disorders	1 (2%)	2 (3%)
Night sweats	1 (2%)	1 (2%)
Pruritus	0	1 (2%)
Vascular disorders	7 (13%)	9 (14%)
Flushing	7 (13%)	8 (13%)
Hypertension	0	1 (2%)

Table 4: Incidence of Treatment-Emergent Serious Adverse Events Regardless of Attribution

System Organ Class Preferred Term	3 mg/kg (N=53)	All Subjects (1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg) (N=63)
Subjects with at Least 1 Serious AE	17 (32%)	23 (37%)
Blood and lymphatic system disorders	1 (2%)	5 (8%)
Anemia	1 (2%)	4 (6%)
Febrile neutropenia	0	1 (2%)
Cardiac disorders	0	1 (2%)
Left ventricular dysfunction	0	1 (2%)
Gastrointestinal disorders	7 (13%)	8 (13%)
Abdominal pain	3 (6%)	3 (5%)
Dysphagia	1 (2%)	1 (2%)
Gastrointestinal hemorrhage	1 (2%)	2 (3%)
Nausea	1 (2%)	1 (2%)
Small intestinal obstruction	2 (4%)	2 (3%)
General disorders and administration site conditions	1 (2%)	1 (2%)
Pyrexia	1 (2%)	1 (2%)
Hepatobiliary disorders	2 (4%)	2 (3%)
Bile duct obstruction	1 (2%)	1 (2%)
Hyperbilirubinemia	1 (2%)	1 (2%)
Jaundice	1 (2%)	1 (2%)
Infections and infestations	4 (8%)	5 (8%)
Catheter site infection	1 (2%)	1 (2%)
Influenza	1 (2%)	1 (2%)
Pneumonia	1 (2%)	1 (2%)
Sepsis	1 (2%)	2 (3%)
Investigations	0	1 (2%)
Blood bilirubin increased	0	1 (2%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	2 (4%)	2 (3%)
Neoplasm	2 (4%)	2 (3%)
Nervous system disorders	2 (4%)	2 (3%)
Headache	1 (2%)	1 (2%)

System Organ Class Preferred Term	3 mg/kg (N=53)	All Subjects (1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg) (N=63)
Syncope	1 (2%)	1 (2%)
Renal and urinary disorders	1 (2%)	2 (3%)
Acute kidney injury	0	1 (2%)
Urinary tract obstruction	1 (2%)	1 (2%)
Respiratory, thoracic and mediastinal disorders	5 (9%)	6 (10%)
Dyspnea	3 (6%)	4 (6%)
Hypoxia	1 (2%)	1 (2%)
Respiratory failure	2 (4%)	2 (3%)
Vascular disorders	1 (2%)	1 (2%)
Jugular vein thrombosis	1 (2%)	1 (2%)

Table 5A: CD16 Polymorphism and ADCC

DONOR (n=2)	% Specific Killing (± SEM)		
	Eff/Target Ratio	Control	Ensituximab
VV1	50:1	1.1 ±1.66	31.0 ± 1.098
	25:1	-.03 ± 1.54	19.1± 1.66
VV2	50:1	-1.7 ± 1.67	28.4 ± 1.40
	25:1	0.7 ± 0.84	18.1 ± 1.32
FF1	50:1	-1.3 ± 0.45	22 ± 1.94
	25:1	0.6 ± 1.11	11.9 ± 0.10
FF2	50:1	0.3 ± 1.67	19.3 ± 2.51
	25:1	1.6 ± 2.4	11.8 ± 0.14

Data represent the correlation between the polymorphism of the NK FcγRIIIA receptor and the NEO-102 ADCC activity. A single nucleotide polymorphism (SNP) of the FCGR3A gene results in two allotypes of the NK FcγRIIIA with valine (V) or phenylalanine (F) at amino acid 158. In this way, FcγRIIIA can have three different genotypes: V/V, V/F, F/F. Each genotype has a different avidity for the NEO-102 Fc region.

Table 5B: IL-15 superagonist enhances NEO-102-mediated ADCC activity

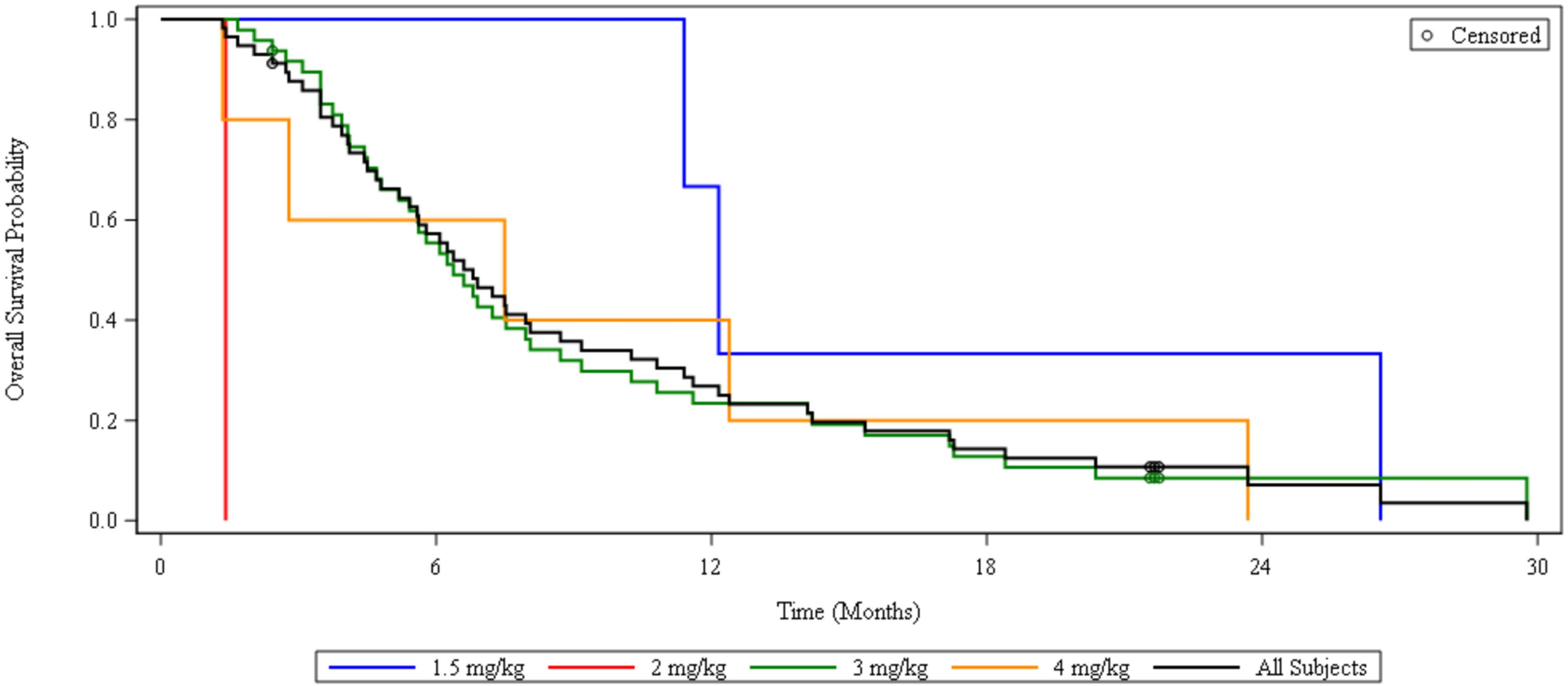
		% specific lysis (SD) (Donor 1)		% specific lysis (SD) (Donor 2)	
Antibody	**E:T ratio**	Untreated	IL-15 superagonist	Untreated	IL-15 superagonist
IgG1	12.5:1	7.7 (1.21)	9.5 (2.5)	1.9 (0.7)	10.9 (1.3)
	6.25.1	-1.1 (1.85)	5.8 (5.5)	-0.6 (3.8)	1.7 (4.6)
NEO-102	12.5:1	14.1 (3.9)	**22.1 (1.4)***	1.6 (4.2)	**19.7 (0.4)***
	6.25.1	7.2 (4.7)	4.3 (4.1)	2.1 (0.6)	**11.4 (1.7)***

NK cells isolated from three normal donors were treated with IL-15 superagonist (25ng/ml) or medium control (untreated) for 48 hours prior to be used as effector cells in a 4h non-radioactive ADCC assay using Celigo Imaging cytometer.
CFPAC-1 (human pancreatic cancer cell line) cells were stained with calcein AM and used as targets at 3,000 cells/well.
Results are expressed in % specific lysis (SD).

* Statistically significant ($p < 0.05$) by Student's t-test (NEO-102 + IL-15 superagonist vs NEO-102 untreated)

Figure 1: Overall survival of patients with metastatic colorectal cancer treated with ensituximab at different dose levels

Figure 1



At Risk						
1.5 mg/kg	3	3	2	1	1	0
2 mg/kg	1	0	0	0	0	0
3 mg/kg	48	26	11	6	1	0
4 mg/kg	5	3	2	1	0	0
All Subjects	57	32	15	8	2	0

AACR American Association for Cancer Research

Clinical Cancer Research

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Richard D Kim, Nilofer S Azad, Michael A. Morse, et al.

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