Phase III Randomized Study of Second Line ADI-PEG 20 Plus Best Supportive Care versus Placebo Plus Best Supportive Care in Patients with Advanced Hepatocellular Carcinoma

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Abstract:	Purpose: Arginine depletion is a putative target in hepatocellular carcinoma (HCC). HCC often lacks argininosuccinate synthetase, a citrulline to arginine-repleting enzyme. ADI-PEG 20 is a cloned arginine degrading enzyme – arginine deiminase – conjugated with polyethylene glycol. The goal of this study was to evaluate this agent as a potential novel therapeutic for HCC after first line systemic therapy. Patients and methods: Patients with histologically proven advanced HCC and Child-Pugh up to B7 with prior systemic therapy, were randomized 2:1 to ADI-PEG 20 18 mg/m2 vs. placebo intramuscular (IM) injection weekly. The primary endpoint was overall survival (OS), with 93% power to detect a 4 to 5.6 months increase in median OS (1-sided α = 0.025). Secondary endpoints included progression-free survival (PFS), safety, and arginine correlatives. Results: 635 patients were enrolled: median age 61, 82% male, 60% Asian, 52% hepatitis B, 26% hepatitis C, 76% stage IV, 91% Child-Pugh A, 70% progressed on sorafenib and 16% were intolerant. Median OS was 7.8 months for ADI-PEG 20 vs 7.4 for placebo (p = 0.88, HR=1.02) and median PFS 2.6 months vs. 2.6 (p = 0.07, HR=1.17). Grade 3 fatigue and decreased appetite occurred in less than 5% of patients. Two patients on ADI-PEG 20 had ≥ grade 3 anaphylactic reaction. Death rate within 30 days of end of treatment was 15.2% on ADI-PEG 20 vs. 10.4% on placebo, none related to therapy. Post-hoc analyses of arginine assessment at 4, 8, 12 and 16 weeks, demonstrated a trend of improved OS for those with more prolonged arginine depletion. Conclusions: ADI-PEG 20 monotherapy did not demonstrate an OS benefit in second line setting for HCC. It was well tolerated. Strategies to enhance prolonged arginine depletion and synergize the effect of ADI-PEG 20 are underway.

Phase III Randomized Study of Second Line ADI-PEG 20 Plus Best Supportive Care versus Placebo Plus Best Supportive Care in Patients with Advanced Hepatocellular Carcinoma

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ABSTRACT (289 words)

Purpose: Arginine depletion is a putative target in hepatocellular carcinoma (HCC). HCC often lacks argininosuccinate synthetase, a citrulline to arginine-repleting enzyme. ADI-PEG 20 is a cloned arginine degrading enzyme – arginine deiminase – conjugated with polyethylene glycol. The goal of this study was to evaluate this agent as a potential novel therapeutic for HCC after first line systemic therapy**.**

Patients and methods: Patients with histologically proven advanced HCC and Child-Pugh up to B7 with prior systemic therapy, were randomized 2:1 to ADI-PEG 20 18 mg/m^2 vs. placebo intramuscular (IM) injection weekly. The primary endpoint was overall survival (OS), with 93% power to detect a 4 to 5.6 months increase in median OS (1-sided $\alpha = 0.025$). Secondary endpoints included progression-free survival (PFS), safety, and arginine correlatives.

Results: 635 patients were enrolled: median age 61, 82% male, 60% Asian, 52% hepatitis B, 26% hepatitis C, 76% stage IV, 91% Child-Pugh A, 70% progressed on sorafenib and 16% were intolerant. Median OS was 7.8 months for ADI-PEG 20 vs 7.4 for placebo ($p = 0.88$, HR=1.02) and median PFS 2.6 months vs. 2.6 ($p = 0.07$, HR=1.17). Grade 3 fatigue and decreased appetite occurred in less than 5% of patients. Two patients on ADI-PEG 20 had ≥ grade 3 anaphylactic reaction. Death rate within 30 days of end of treatment was 15.2% on ADI-PEG 20 vs. 10.4% on placebo, none related to therapy. Post-hoc analyses of arginine assessment at 4, 8, 12 and 16 weeks, demonstrated a trend of improved OS for those with more prolonged arginine depletion.

Conclusions: ADI-PEG 20 monotherapy did not demonstrate an OS benefit in second line setting for HCC. It was well tolerated. Strategies to enhance prolonged arginine depletion and synergize the effect of ADI-PEG 20 are underway.

INTRODUCTION

Arginine, a nonessential amino acid in humans, is acquired through external arginine rich foods, and is also synthetized in two steps from citrulline via argininosuccinate synthetase (ASS1) and argininosuccinate lyase (ASL) enzymes (1). HCC cells frequently lack ASS1, and thus cannot metabolize citrulline into arginine (2-3). ADI-PEG 20 is an arginine degrading enzyme, arginine deiminase, cloned from *M. hominis* and produced in *E. coli* and conjugated with polyethylene glycol. ADI-PEG 20 turns external supplies of arginine into citrulline (4). Restricting arginine sources through the degradation of external sources via ADI-PEG 20, combined with the lack of ASS1, renders arginine depletion a putative target for HCC.

Three phase II clinical trials have evaluated ADI-PEG 20 in advanced HCC, and have collectively suggested an improvement in survival (2, 5-6), across different etiologies of HCC (2).

Thus we embarked on the reported herein randomized phase III trial of ADI-PEG 20 plus best supportive care versus placebo plus best supportive care in patients with advanced HCC in the second line setting.

PATIENTS AND METHODS

This was a multi-institutional, randomized, placebo-controlled phase III clinical trial. The Institutional Review Board (IRB) of each institution reviewed and approved the protocol. Written informed consent was obtained from each patient. The study was registered with www.clinicaltrials identifier NCT 01287585.

Patients' Eligibility

Men and women ≥18 years of age, with unresectable locally advanced, or metastatic histologically confirmed HCC, with at least one measurable lesion by RECIST 1.1 criteria (7), and who had received at least one prior systemic therapy with documented progression of disease or adverse events that resulted in discontinuance of that therapy were eligible. Previous local therapy, e.g. hepatic artery embolization, was allowed, as long as there was an untreated target lesion and/or evidence of progression of disease by RECIST 1.1 prior to enrollment. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and a Child-Pugh score of A6 that was later amended to B7 in view of the excellent safety profile of ADI-PEG 20. Adequate hematologic function (absolute neutrophil count ≥ 1,500/mcL, platelets ≥ 50,000/mcL) and PT/INR ≤ 1.7 times upper limit of normal, adequate hepatic function (total bilirubin ≤ 3 mg/dL, albumin ≥ 2.8 g/dL, AST/ALT ≤ 5 times the upper limit of normal) plus an adequate creatinine of ≤ 1.5 mg/dL or a creatinine clearance of ≥ 60 mL/min were required. Serum uric acid ≤ 8 mg/dL, with anti-hyperuricemic treatment allowed, was required in view of prior hyperuricemia with ADI-PEG 20 treatment in this population (2, 5-6).

Any history of untreated variceal bleed within 3 months rendered patients ineligible, as did any serious inter-current illnesses, known brain metastases, clinically significant cardiac history, uncontrolled hypertension, ongoing infections, and/or known HIV infection. The study also excluded pregnant women and patients with other malignancies that might have affected patients' outcome.

Treatment Plan

Eligible patients were randomized (2:1) to receive either weekly ADI-PEG 20 18 mg/m^2 or placebo by IM injection in a double-blinded fashion. Four weekly treatments were defined as one cycle of therapy. Patients in both groups continued to receive best supportive care. Patients were assessed for adverse events weekly, and had a doctor visit with physical examination and safety blood work every other week. Computed tomography (CT) or magnetic resonance imaging (MRI) scans were performed at baseline and at the end of every 12 weeks (3 cycles). Patients could continue to receive treatments unless one of the following occurred at any time during the course of therapy: unacceptable adverse events, death, or progression of disease.

As diet was a potential source of arginine, dietary restrictions for arginine-rich foods were also strongly recommended.

Study Objectives

The primary outcome was OS. Secondary objectives included the assessment of safety and tolerability using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.02, tumor response rate, PFS and time to progression (TTP), the latter two defined as the time from randomization to the date of radiologic disease progression per the RECIST criteria 1.1 or death, and the time from randomization to the date of radiologic disease progression respectively. Disease control rate was defined as the percentage of patients with confirmed CR, PR or stable disease (SD).

Immunogenicity Assay

Peripheral blood anti-ADI-PEG 20 antibody titers were assessed using a validated single-tier semi-quantitative ELISA-based assay. The assay was specifically developed

for this study based on the recommendations of Mire-Sluis et al. (8). In brief, microtiter plates were coated with ADI-PEG 20. Diluted human plasma samples were added to the plate, incubated for 60 minutes, and then treated with a goat anti-human IgA+IgG+IgM/HRP conjugate. Tetramethylbenzidine was added to the plate allowed to react and then the absorbance at 450 nm was recorded. Samples with absorbance greater than the cut-point were considered to be positive, and vice versa. The titer of asample was based on the highest dilution that yielded a positive signal.

Pharmacodynamic Assay

Peripheral blood arginine levels were measured in samples at baseline and at the start of each cycle using liquid chromatography (HPLC) with tandem mass spectrometric detection (MS/MS). The assay was specifically developed and validated for this study. Briefly, arginine and an isotopically labelled internal control were extracted from human plasma samples by protein precipitation. The supernatant was loaded on to a Venusil ASB C18 column. The mobile phase was 0.1% formic acid in water:acetonitrile (95:5, v:v). Detection was performed with positive ion electrospray using a Sciex API 5000. The ratio of the peak areas arising from the arginine and isotopically labelled internal standard was used to quantify the arginine level.

Pharmacokinetic Assay

Peripheral blood ADI-PEG 20 levels were measured in samples at baseline and at the start of each cycle using a fluorometric enzyme activity-based assay designed to detect the production of ammonia. The assay, based on the method of Banerjee et. al. (9), was specifically developed and validated for this study. It was conducted in two parts: the

conversion of arginine to citrulline and ammonia; and the reaction of ammonia with o-phthaldialdehyde (OPA) to produce a fluorescent isoindole-derivative. Plasma samples were added to assay buffer. Arginine was then added, allowed to react, and then development reagent (OPA and reducing agent) was added. The plate was read using a fluorometric plate reader with excitation set at 405 nm and emission set at 460 nm. Human plasma samples spiked with known amounts of ADI-PEG 20, and treated in the same manner as the unknown samples, were used to generate a calibration curve.

Statistical Analyses

All statistical analyses were based on intent-to-treat populations. The safety population comprised all patients who were randomly assigned into the study and who received at least 1 dose of study medication.

Based on historical data (10-11), it was estimated that patients would have a median OS of 4 months after progressing on sorafenib. An improvement of 40% resulting in a median OS of 5.6 months was deemed to be clinically significant. It was estimated that 633 patients needed to be accrued (original assumption was a 12-month enrollment period plus 6-month follow up) with 487 deaths to be reached to demonstrate such difference with an overall 1-sided type I error rate (α) of 0.025, and an overall type II error rate (β) of 0.07. Treatments were compared using a log-rank test stratified by the 3 levels of region Asia vs. North America and Europe, and by prior sorafenib exposure (non-sorafenib failure vs. sorafenib failure). OS, PFS, and TTP were summarized using the Kaplan-Meier method, with 95% confidence intervals analyzed by treatment group. Analysis of OS was at the time of the 487th death.

Safety, tolerability, and adverse events were summarized using descriptive

statistics.

All pharmacokinetic and pharmacodynamic analyses were summarized descriptively. The relationship between anti-ADI-PEG 20 antibodies and changes in arginine was assessed using Pearson's Rho.

In addition, a post-hoc analysis was performed on the ADI-PEG 20 group. The relationship between arginine depletion (based on blood samples taken at weeks 4, 8, 12, and 16) and OS was studied.

ASS1 Expression Induced by Sorafenib in HCC Cell Lines

ASS1 protein expression in both untreated and drug treated human HCC cell lines was assessed by western blot as described (12).

RESULTS

Patients Disposition

Between July 2011 and February 2015, a total of 854 patients were assessed for eligibility and 635 unique patients were randomly assigned to receive ADI-PEG 20 (n=424) or placebo (n=211). Details are further depicted in the consort diagram (figure 1).

Demographics

Demographics were as detailed in table 1. Worth noting is that a total of 332 (52.3%) had hepatitis B as etiology of HCC, commensurate with 338 (53.2%) patients

accrued in Asia. The group of prior sorafenib failure encompassed 549 (86.5%), versus 86 (13.5%) non-sorafenib failure patients.

Treatment

The median number of doses administered of ADI-PEG 20 was 11 (range 0-145) vs. 11 (0-98) for placebo, with a duration of exposure of 10 (range 0-146) and 11 (range 0-109) weeks respectively.

Safety and Tolerability

The incidence of adverse events was similar between the two treatment groups including grade 3 or more events (table 2). There was a statistical difference in the between the two groups. Fatigue was the most frequent adverse event. Grade 3 skin puritis or rash was limited to 1 (0.2%) and 2 (1%) patients who received ADI-PEG 20 and placebo, respectively.

One case of anaphylactic reaction (grade 3) and one of anaphylactic shock (grade 4) occurred in patients who received ADI-PEG 20. The two cases of anaphylaxis occurred after the third and eighth injections, respectively. In the first case, there was no anti-ADI-PEG 20 antibody detected at the time of the anaphylaxis. In the second case, the antibody titer was 10^3.

There were 64 (15.2%) deaths in the ADI-PEG 20 group that occurred within 30 days of the last dose of study drug, vs. 22 (10.5%) in placebo group. The deaths due to causes other than disease progression were 22/64 (34.4%) in the ADI-PEG 20 group and 12/22 (54.5%) in the placebo group. These included ten gastrointestinal bleeds (6 in the ADI-PEG 20 group and 4 in the placebo group); eight liver failures (6 and 2,

respectively); and one intracranial hemorrhage, one brain stem infarction, and one tumor embolus, all in the ADI-PEG 20 group. One cardiac arrest on ADI-PEG 20 and four respiratory failures (2 in each group) were reported. Sepsis/infection occurred in three (2 in the ADI-PEG 20 group and 1 in the placebo group). Four patients sustained general health deterioration (1 in the ADI-PEG 20 group and 3 in the placebo group). One patient in the ADI-PEG 20 group died of unexplained abdominal pain.

Outcome

The median OS as depicted in figure 2A, was 7.8 months (95% confidence interval [CI], 6.77-8.57) for the ADI-PEG 20 group vs. 7.4 months (95% CI, 6.37-9.03) for the placebo group, with a p value of 0.884 (hazard ratio = 1.022 [95% CI, 0.847-1.233]). At the time of analysis there were 322 deaths (75.9%) in the ADI-PEG 20 group and 165 (76.7%) in the placebo group. Forest plot and sensitivity anlayses are shown in supplemental figure 1.

Planned subgroup analyses assessed geographical region and prior sorafenib treatment. In Asia, the median OS was 6.2 months (n=226; 95% CI, 4.90- 7.13) for the ADI-PEG 20 group vs. 6.5 months (n=112; 95% CI, 5.40- 8.30) for the placebo group. In North America or Europe, the median OS was 8.6 months (n=198; 95% CI, 7.30- 10.03) for the ADI-PEG 20 group vs. 7.8 months (n=99; 95% CI, 6.37- 10.17) for the placebo group. There was no significant difference in OS for the treatment and placebo groups when the geographical regions were analyzed.

For those patients who had failed prior sorafenib, the median OS was 7.3 months (n=367; 95% CI, 6.33- 8.17) for the ADI-PEG 20 group vs. 7.7 months (n=182; 95% CI, 6.53-9.47) for the placebo group. For those who had failed a chemotherapy that did not

include sorafenib (non-sorafenib), the median OS was 6.5 months (n=57; 95% CI, 4.93-9.3) for the ADI-PEG 20 group vs. 5.7 months (n=29; 95% CI, 3.43-6.53) for the placebo group. There was no significant difference in OS for the treatment and placebo groups when prior sorafenib failure or non-sorafenib subgroups were analyzed. However, for the non-sorafenib group, the Chi-squared value for the comparison was 2.84, p=0.092.

The median PFS as depicted in figure 2B, was 2.6 months for both groups with ADI-PEG 20 95% CI, 2.6-2.63 vs. 2.6-2.7 for the placebo group, and a p value of 0.075 (hazard ratio = 1.175 [95% CI, 0.964-1.432]).

There were no complete responses, while 2 and 6 partial responses were reported for the ADI-PEG 20 and placebo groups respectively.

There was no difference in AFP (supplemental table 1) and AFP decrease did not correlate with arginine levels (supplemental table 2).

Immunogenicity

The median baseline value for anti-ADI-PEG 20 antibodies was 0 in both the ADI-PEG 20 and placebo groups. The median post baseline change was an increase to a titer of 2 around 8 weeks with a plateau at a titer of 3 at 12 weeks in the ADI-PEG 20 group. . The titer remained 0 in the placebo group. Changes from baseline in anti-ADI-PEG 20 antibodies and blood arginine levels were correlated at all time points tested (weeks 2, 4, 8, 12, 16; $p<0.0001$). Levels of anti-ADI-PEG 20 antibodies did not correlate with adverse events. No attempt was made to discern neutralizing antibodies from non neutralizing antibodies.

Pharmacokinetics

In the ADI-PEG 20 group, the mean blood ADI-PEG 20 levels were highest at weeks 2 and 4, and then decreased to a plateau level at week 12, to ~45% of the highest levels. This was commensurate with the development of anti-ADI-PEG 20 antibodies.

Pharmacodynamics

Circulating arginine level markedly decreased after the first dose of ADI-PEG 20. The median arginine level remained depleted for 8 weeks (data not shown). A patient was defined as having arginine depletion if their arginine level reached and remained below 10 μM post ADI-PEG 20 dosing.

A post-hoc analysis was performed to determine if there was a relationship between arginine depletion and OS. For this analysis, at each of the specified timepoints (4, 8, 12 and 16 weeks), ADI-PEG 20 treated patients were divided into two groups. One group consisted of patients with arginine depletion, as defined above, and one group consisted of those whose blood arginine level no longer met the arginine depletion as defined at the specified timepoint. Patients that had died, progressed, withdrawn consent or were otherwise uanable to have a blood draw at the specified timepoint were excluded from these analyses. At each timepoint, the number of patients who had their arginine level assayed along with their median OS are presented in supplemental table 3. At all four timepoints tested, the patients with arginine depletion trended to have improved OS.

DISCUSSION

This phase III trial of ADI-PEG 20 monotherapy at a dose of 18 mg/m^2 did not meet its primary object of improving OS vs. placebo in patients with advanced HCC who had failed prior systemic therapy. However, patients with arginine depletion trended to

have improved OS. A similar trend of improved OS with arginine depletion has been shown with ADI-PEG 20 in a prior phase II HCC trial with ADI-PEG 20 monotherapy (2). In this study we did not formally statistically assess this relationship in the post-hoc analysis as there may be a selection bias as some patients came off study early due to progression and thus could no longer contribute to the pharmacodynamic analysis. Furthermore, such an analysis might also be affected by other aspects, incuding the next line of therapy. Nonethelsss, if there is a therapeutic utility of ADI-PEG 20 monotherapy, it would appear to be limited primarily to those patients in whom prolonged arginine suppression is obtained. Also, although antidrug antibodies were determined, neutralizing antibodes were not determined. Prior studies seem to indicate both a general lack of correlation between neutralizing antibodies and arginine levels, and the possibility of still present efficacy despite the presence of neutralizing antibodies for ADI-PEG 20 (2, 13), and unpublished data, Polaris Pharmaceuticals-data on file) . The same has been shown for other therapeutic agents (e.g., cetuximab, rituximab, and panitumumab) where the presence of antidrug antibodies often did not have a clinical effect (14). Strategies to prolong ADI-PEG 20 induced arginine suppression include: (a) an increased dose of ADI-PEG 20 (36 mg/m^2) which has shown modest indication of benefit as monotherapy in a malignant pleural mesothelioma population (15), (b) combination with cytotoxic agents which may blunt the immune response to ADI-PEG 20 and has been shown to both delay and decrease the generation of antibodies to ADI-PEG 20 in several ongoing clinical trials, and (c) developing a new ADI that would not be so quickly neutralized by antibodies. This latter approach is currently under investigation, and would mirror the success observed with asparaginase from *Erwinia chrysanthemi* in patients with acute

lymphocytic leukemia (ALL) who have developed antibodies to *E. coli* asparaginase (16), as well as by developing a new formulation (17).

ADI-PEG 20 was well tolerated, as observed in other studies in HCC (3,5 and 6). Local injection site reactions, rash, pruritus and anaphylaxis were expected (18, 19), and at occurred at a rate of 0.4%. This compares favorably with other pegylated non-human enzymes used in the treatment of patients (21, 22). Local injection site reactions, rash and pruritus in the placebo control group were consistent with the intramuscular injection and placebo solution.

One case of brain stem infarction and one of intracerebral hemorrhage occurred in the ADI-PEG 20 treated group, thus with an occurrance rate of 0.4%. In cirrhotic patients intracerebral hemorrhage are observed (230 and reported at 1.3% (24).

Respiratory failure, consistent with hepatopulmonary syndrome, is a well known complication of cirrhosis, same for infection (25, 26).

We noted in previous studies that archived HCC tissues samples were 70-75% of samples ASS1 deficient (2,3). While this study was ongoing an experiment was conducted across multiple HCC cell lines which demonstrated an increase in ASS1 expression in some cell lines treated with sorafenib (Supplemental figure 2). For those cell lines with little to no expression of ASS1 (SK-HEP-1, SNU398, SNU449, and Tong) sorafenib treatment had little impact on ASS1 level. Sorafenib treatment also had little impact on the ASS1 level of a cell line with a relatively high ASS1 expression level (PLC5). However, of the six cell lines with intermediate ASS1 expression, four (HepG2, Huh7, SNU182, and Malhavu) demonstrated an increase in ASS1 expression upon treatment with sorafenib, one (Hep3B) demonstrated a small decrease in ASS1

expression, and one (HCC36) demonstrated little change on ASS1 expression after sorafenib treatment. Considering that 86% of patients on the ADI-PEG 20 received prior sorafenib, up-regulation of ASS1 expression may have contributed to the lack of efficacy in the patient population in this study.

At the time of the design of the study, the 4 months median OS anticipated for the placebo group seemed reasonable. But in retrospect, it would be hard to justify given our present understanding of OS in the good performance status, favorable Child-Pugh population that is selected for clinical trials. The reported herein 7.4 months median OS for the placebo group is commensurate with current data (27-30) and reflective of this selection bias.

Going forward, capitalizing on the attributes that may help potentiate the efficacy of ADI-PEG 20 would be critical. Another arginine deprivation approach in HCC has been investigated with pegylated recombinant human arginase (31).

In summary, ADI-PEG 20 at the dose of 18 mg/m^2 proved to be ineffective in prolonging OS in patients with advanced HCC who failed prior therapy. However, those with arginine depletion from ADI-PEG 20 were observed to have a superior OS to those who did not achieve prolonged depletion. New studies of ADI-PEG 20 are currently focused on maximizing arginine depletion through elucidating and testing potential synergistic effects and on modulating its antigenic structure as well as formulation.

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Table 1. Demographics of ADI-PEG 20 and placebo groups

Parameter	ADI-PEG 20 (n=424)	Placebo (n=211)
Median Age (years)	61	62
Male Gender	83% (352)	80% (168)
Asia Accrual	53% (226)	53% (112)
Etiology		
HBV	53% (226)	50% (106)
HCV	26% (112)	26% (55)
Alcohol	12% (51)	15% (32)
NASH	6% (24)	6% (12)
Other	13% (57)	14% (29)
ECOG 0/1	98% (414)	98% (207)
Child Pugh		
A	91% (387)	89% (188)
B7	9% (37)	10% (22)
BCLC		
B	18% (77)	19% (40)
C	82%(347)	81% (170)
Baseline AFP>=400 ug/L	210 (49.5%)	107 (50.7%)
Prior therapy		
Sorafenib failure	70% (299)	69% (146)
Sorafenib intolerance	16% (68)	17% (36)

Other	13% (57)	14% (29)
1 prior chemotherapy	73% (311)	79% (167)
≥ 2 prior chemotherapies	27% (113)	21% (44)

Table 2. Treatment-emergent adverse events by treatment group and CTCAE grade

	ADI-PEG 20 (N=421) CTCAE Grade (%)					**Placebo (N=209) CTCAE Grade (%)**				
Grade 1-5 AEs in Patients with ≥ 7.5% Grade 1-2 Events	**1-2**	**3**	**4**	**5**	**Total**	**1-2**	**3**	**4**	**5**	**Total**
Fatigue	21.4	1.9	0	0	23.3	23.5	3.3	0	0	26.8
Decreased appetite	21.0	1.9	0	0	22.8	18.2	1.4	0	0	19.6
Nausea	18.6	0.5	0	0	19.0	17.2	0.5	0	0	17.7
Abdominal Pain	14.2	4.3	0	0.2	18.8	14.8	2.4	0	0	17.2
Edema peripheral	16.2	2.4	0	0	18.5	17.3	1.4	0	0	18.7
Pyrexia	18.3	0	0	0	18.3	18.7	0.5	0	0	19.1
Cough	14.9	0.2	0	0	15.2	17.3	0.5	0	0	17.7
Abdominal distension	13.3	1.2	0	0	14.5	16.3	0.5	0	0	16.7
Diarrhea	12.8	1.0	0	0	13.8	15.6	1.0	0.5	0	17.2

Pruritus	13.1	0.2	0	0	13.3	12.5	0.5	0	0	12.9
Ascites	10.0	2.6	0	0	12.6	9.5	3.3	0	0	12.9
Vomiting	11.9	0.7	0	0	12.6	12.4	0	0	0	12.4
Constipation	11.9	0.2	0	0	12.1	13.9	1.0	0	0	14.8
Adbominal pain upper	11.7	0.2	0	0	11.9	10.5	1.0	0	0	11.5
Dyspnea	9.5	1.7	0.5	0.2	11.9	8.6	2.9	0	0	11.5
Back pain	10.3	0.5	0	0	10.7	9.1	2.4	0	0	12.0
Insomnia	10.3	0.2	0	0	10.5	8.2	0.5	0	0	8.6
Rash	10.2	0	0	0	10.2	7.6	0.5	0	0	8.1

Figure 1. Consort diagram

Figure 2.

A. **Kaplan-Meier curves depicting OS for the ADI-PEG 20 and placebo cohorts**
B. **. Kaplan-Meier curves depicting PFS for the ADI-PEG 20 and placebo cohorts**

Supplemental Figure 1. Forest plot and sensitivity analyses

Supplemental Figure 2. Depiction of ASS1 level expression pre- and post-treatment with sorafenib

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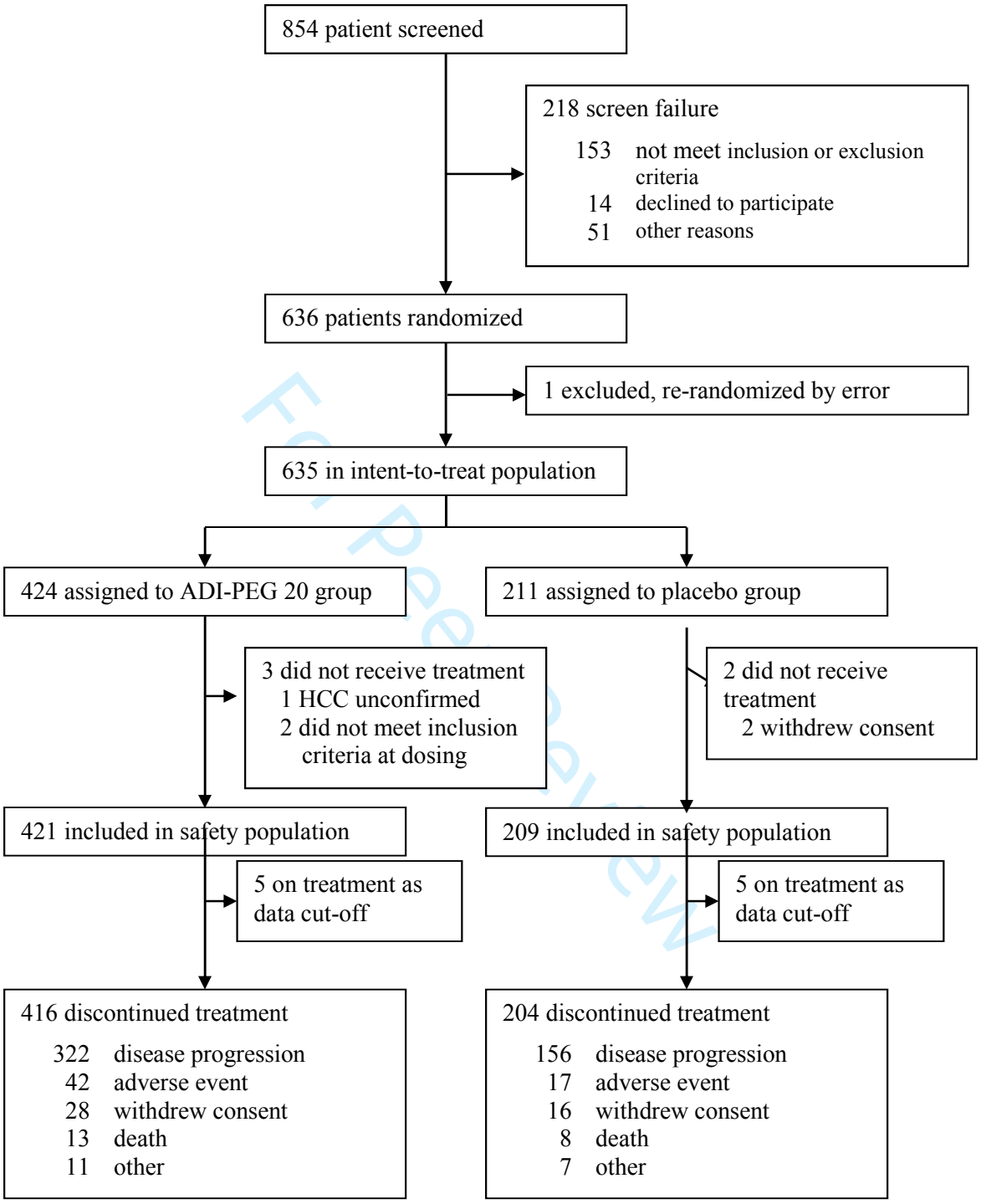
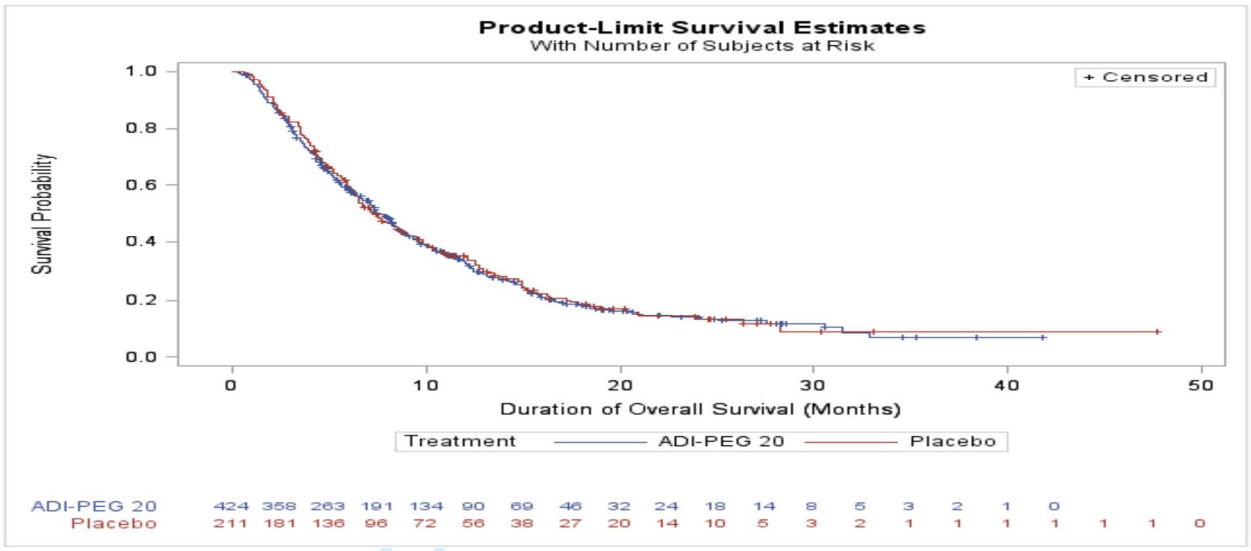
The statistical comments of Marinela Capanu, PhD, Memorial Sloan Kettering Cancer are appreciated.



A


Product-Limit Survival Estimates
With Number of Subjects at Risk
+ Censored
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Duration of Overall Survival (Months)
Treatment ADI-PEG 20 Placebo
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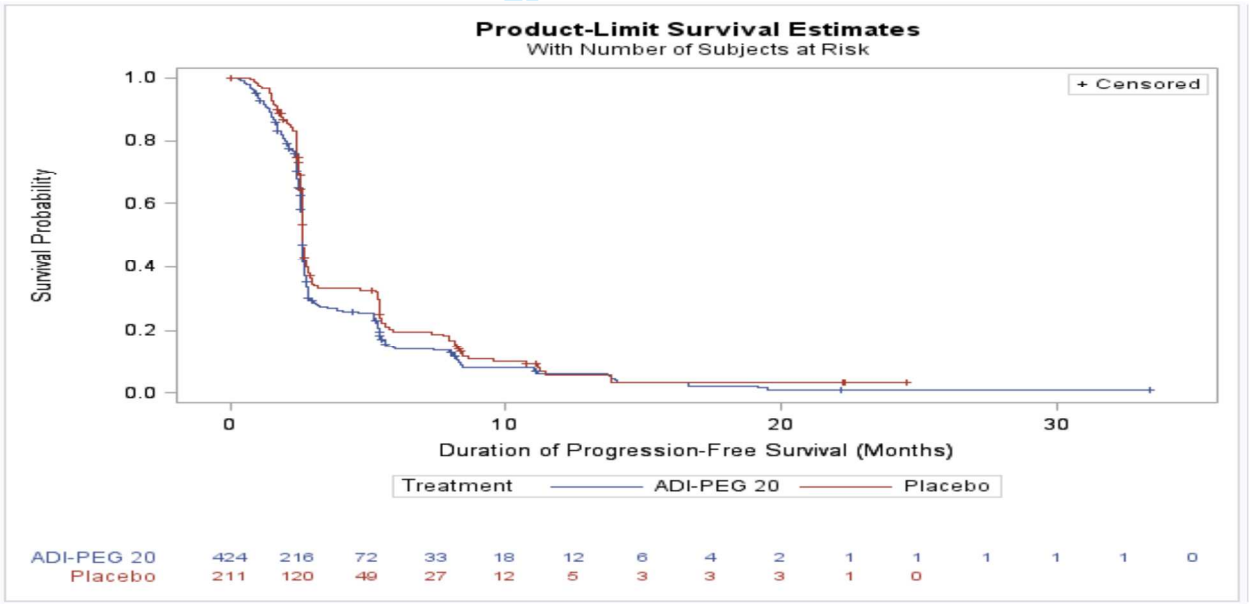
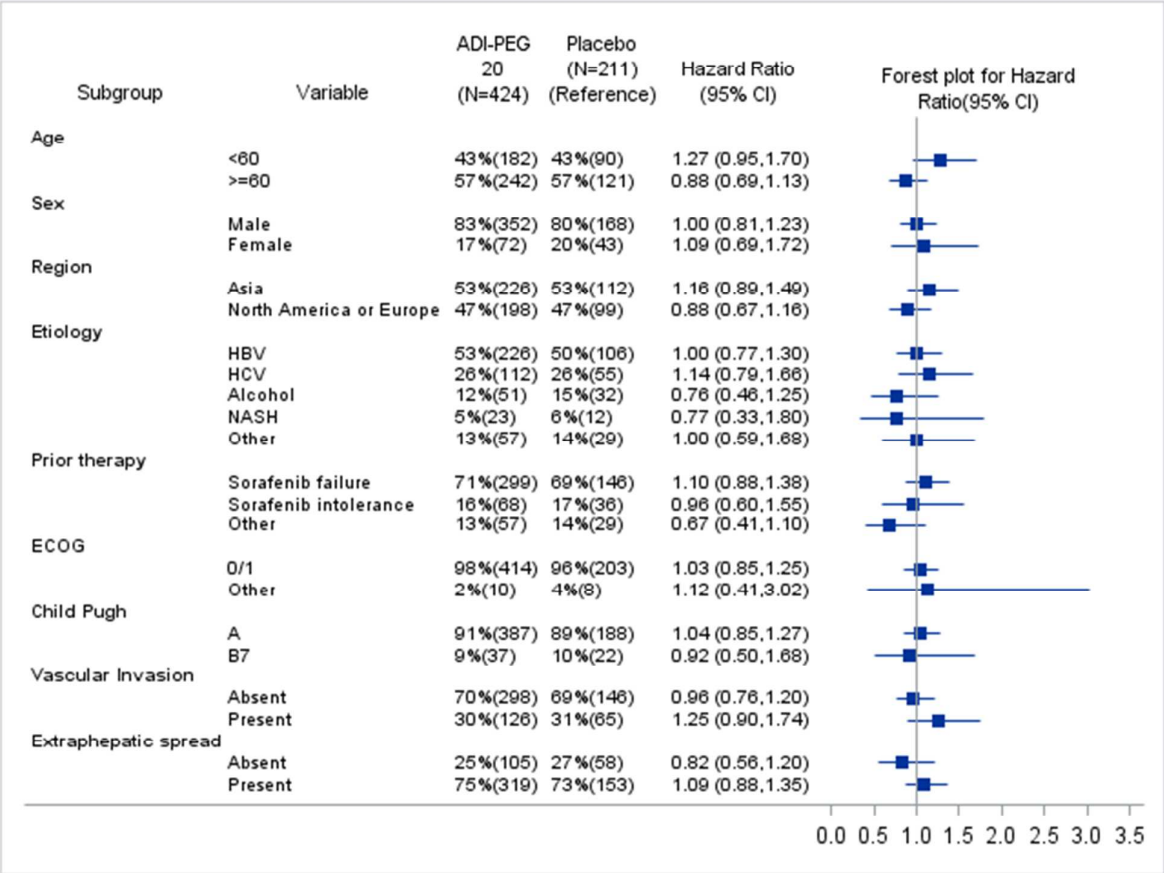
Placebo 211 181 136 96 72 56 38 27 20 14 10 5 3 2 1 1 1 1 1 0

B

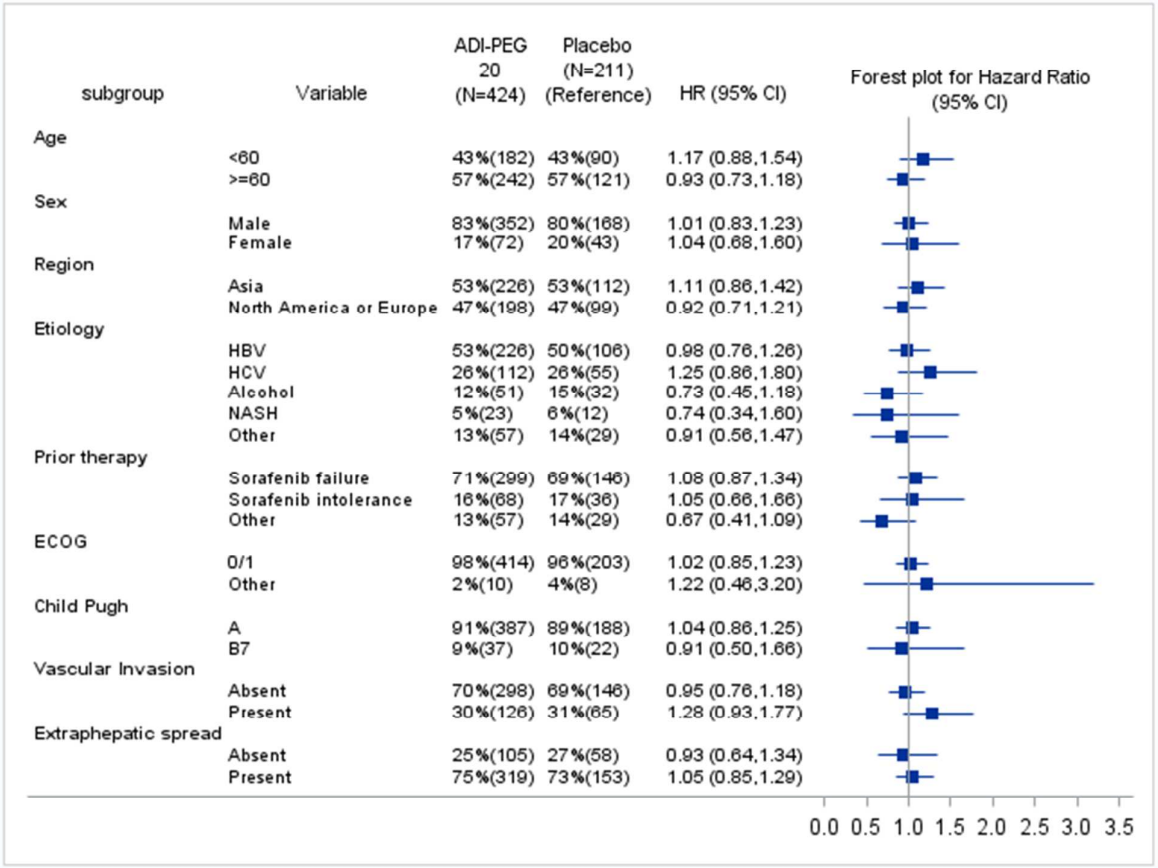

Product-Limit Survival Estimates
With Number of Subjects at Risk
+ Censored
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
Duration of Progression-Free Survival (Months)
Treatment ADI-PEG 20 Placebo
ADI-PEG 20 424 216 72 33 18 12 6 4 2 1 1 1 1 1 0
Placebo 211 120 49 27 12 5 3 3 3 1 0

Forest plots of demographics of ADI-PEG 20 and placebo groups

- Primary Analysis: The primary analysis includes deaths that occurred before the date of the 487th events.

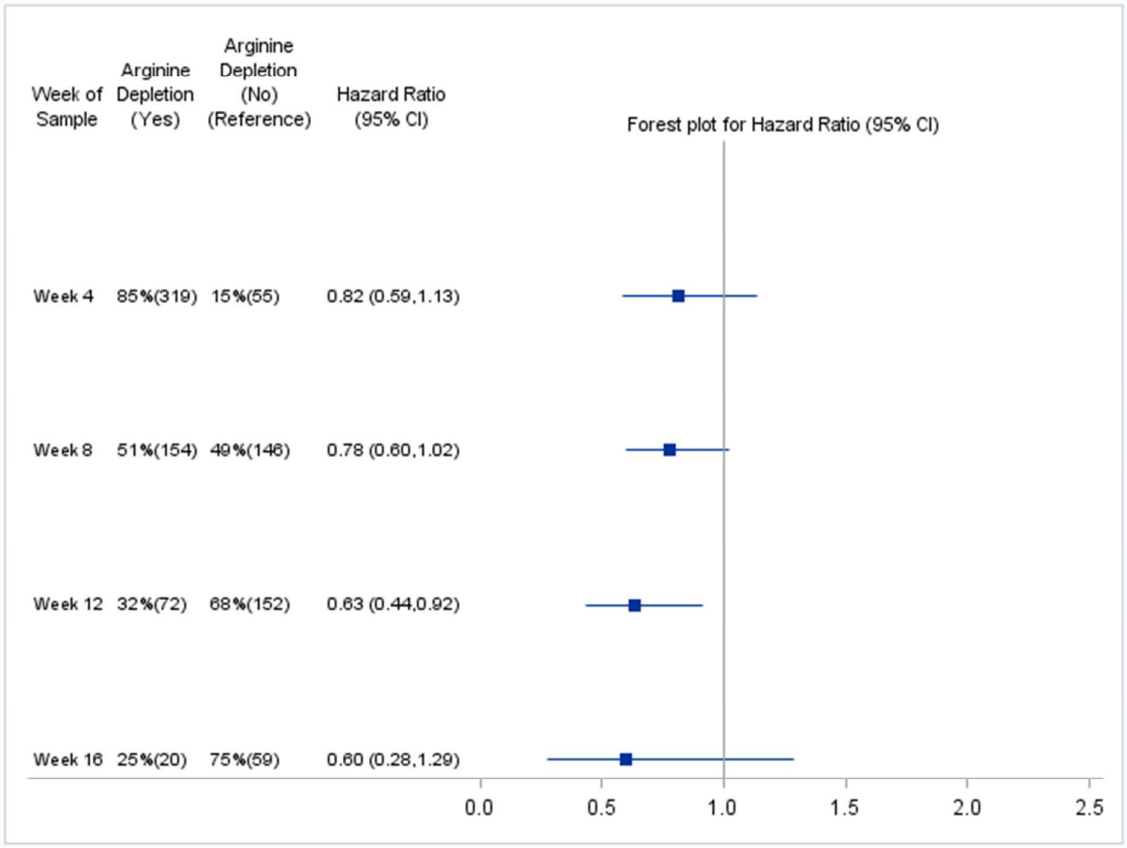


- Sensitiveity Analsyis: The sensitivity analysis includes all deaths.

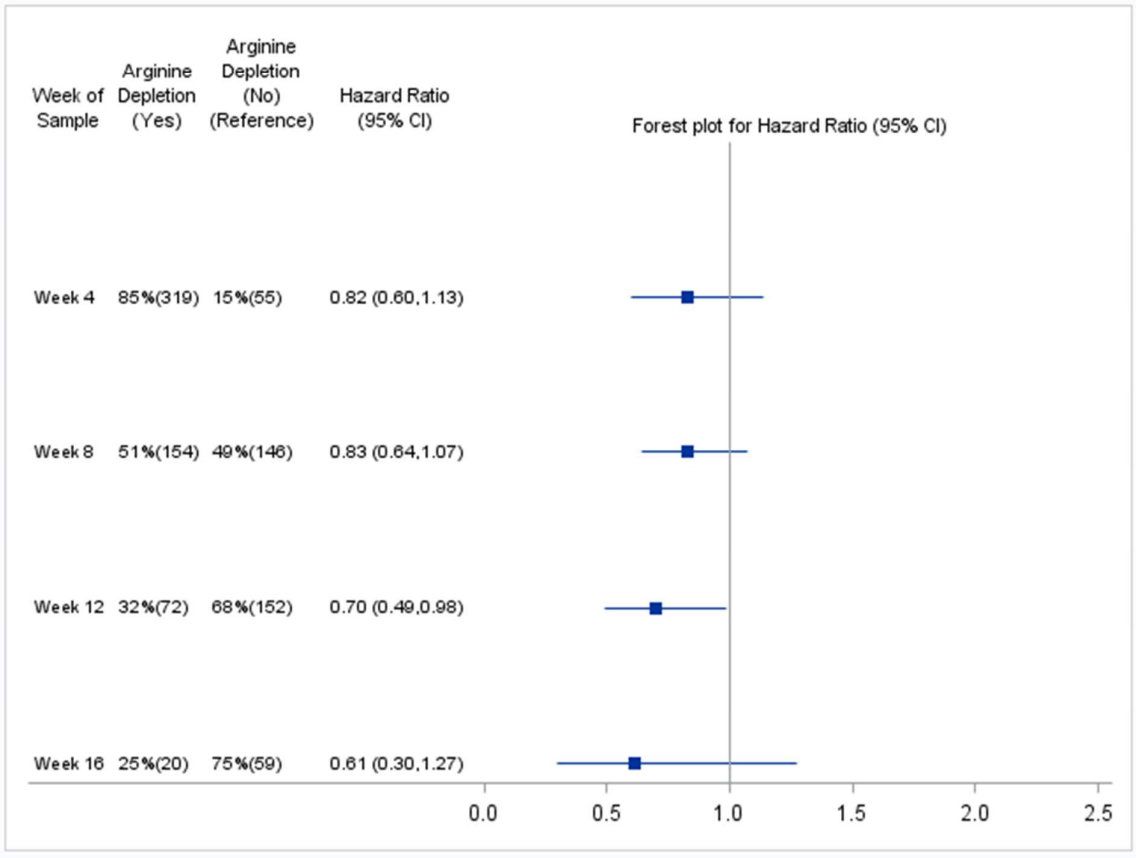


Forest plot of Arginine depletion

- Primary Analysis: The primary analysis includes deaths that occurred before the date of the 487th events.

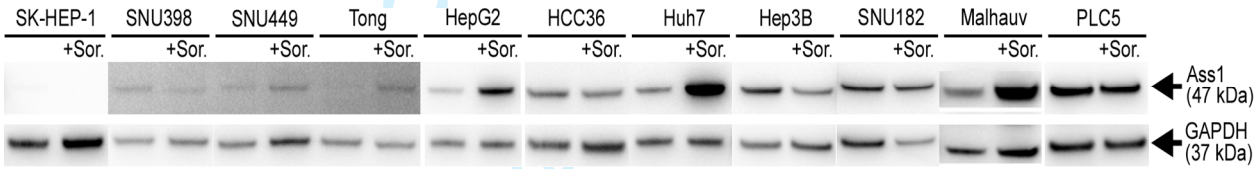


- Sensitiveity Analsyis: The sensitivity analysis includes all deaths.



Supplemental Figure 2. Depiction of ASS1 level expression pre- and post-treatment with sorafenib

After establishing an IC50 for sorafenib, each cell line was treated with sorafenib starting with a concentration below the IC50, and then over the course of 6 to 8 weeks subjected to increasing concentrations of sorafenib up to a maximum tolerated dose (1 to 4 μM depending upon the cell line). After a 24 hour recovery period the ASS1 level was measured by IHC and compared with the pre-treatment ASS1 level.



Supplemental table 1. AFP levels at baseline.

Parameter	ADI-PEG 20 (n=424)	Placebo (n=211)
Baseline AFP>=400 ug/L	210 (49.5%)	107 (50.7%)
Asia	126 (29.7%)	67 (31.8%)
Non-Asia	84 (19.8%)	40 (19.0%)
Baseline AFP>=200 ug/L	233 (55.0%)	120 (56.9%)
Asia	141 (33.3%)	71 (33.6%)
Non-Asia	92 (21.7%)	49 (23.2%)

Supplemental table 2. Correlation between arginine depletion and AFP decrease

		AFP decrease =50% or greater		
Total	Arginine depletion	No	Yes	P-value
	No	39	1	0.2320
	Yes	349	35	
Week 4				
	No	51	2	0.6679
	Yes	299	9	
Week 8				
	No	132	5	1.0000
	Yes	143	6	
Week 12				
	No	137	7	0.7211
	Yes	68	2	
Week 16				
	No	56	3	0.5673
	Yes	20	0	

Supplemental table 3. Patients confirmed with/without arginine depletion and median OS (months).

Week of Sample	**4**		**8**		**12**		**16**	
Arginine Depletion	**n**	**OS**	**n**	**OS**	**n**	**OS**	**n**	**OS**

No	55	5.9	146	8.3	152	10.5	59	15.1
Yes	319	8.2	154	11.5	72	15.8	20	27.5