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Investigational New Drugs

Safety, PK/PD and preliminary anti-tumor activities of pegylated recombinant human arginase 1 (BCT-100) in patients with advanced arginine auxotrophic tumors

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Abstract

**Background:** The study determined the safety, pharmacokinetics/pharmacodynamics (PK/PD), and recommended Phase II dose of BCT-100 for arginine auxotrophic tumours in a non-Chinese population. **Methods:** This is a Phase I, 3 + 3 dose-escalation, open-label, multi-centre study in two arginine auxotrophic cancers - Malignant Melanoma (MM) and Castration Resistant Prostate Cancer (CRPC). Patients were enrolled to receive weekly intravenous BCT-100. The dose cohorts were respectively 0.5 mg/kg, 1.0 mg/kg, 1.7 mg/kg and 2.7 mg/kg. **Results:** There were 14 MM and 9 CRPC patients, 16 males and 7 females with median age of 71. No dose-limiting toxicities were reported. Among all the AEs, 18 were drug-related (mostly were Grade 1). Although there were individual variations in PKs amongst the patients in each cohort, the median arginine level was maintained at 2.5 uM (lower limit of quantification) in all 4 cohorts of patients after the second BCT-100 injection. Therapeutic Arginine Depletion was found in the 1.7 and 2.7 mg/kg/week cohorts when anti-tumor activities were observed. The two cohorts had a similar AUC (20947 and 19614 hr*ug/ml respectively). Since the 2.7mg/kg/week cohort had a more sustained arginine depletion for 2 weeks, the 2.7mg/kg/week dose is chosen as the future phase II dose. There were two complete remissions (1 MM & 1 CRPC), 1PR (MM) and 2 stable diseases with a disease control rate (CR+PR+SD) of 5/23 (22%). **Conclusions:** BCT-100 is safe in a non-Chinese population and has anti-tumor activities in both MM and CRPC. Weekly BCT-100 at 2.7 mg/kg is defined as the optimal biological dose for future clinical phase II studies.

**Keywords:** Arginine, Arginase, Advanced Solid Tumors, PEG-BCT-100
Introduction

Arginine is a semi-essential amino acid involved in a myriad of metabolic and signalling pathways and the synthesis of peptides and proteins. Arginine, and its derivative nitric oxide, are signalling molecules in cellular pathways, and Arginine and nitric oxide their concentrations regulate T cell proliferation and function [1]. In humans, the main source of arginine is from diet. In times of deficiency, such as pregnancy, cancer or significant inflammation, arginine homeostasis is maintained by the inter-organ resynthesis of arginine from citrulline. At a cellular level, the expression of the mitochondrial enzyme argininosuccinate synthetase (ASS1) in many somatic cells allows the intracellular production of citrulline as a precursor to arginine ensuring cell survival and maintenance of function under limiting conditions [2].

Arginine auxotrophic cancers are defined by low to absent ASS1 expression and hence cannot resynthesize arginine from citrulline, making them totally dependent on exogenous arginine for cellular metabolism and growth. We and others have shown that human hepatocellular carcinoma (HCC), melanoma, prostate cancer, acute leukaemias, and some paediatric malignancies are arginine auxotrophic and undergo cell death in vitro and in vivo, in the absence of exogenous arginine [3, 4].

Pegylated recombinant human arginase 1, BCT-100, is an established long-acting arginine depleting drug in clinical phase development. A phase I clinical study established an excellent drug safety profile, with sustained arginine depletion in patients with advanced hepatocellular carcinoma (HCC) in a Chinese population [5]. Improved survival was seen in a subsequent phase II study of single agent BCT-100 for relapsed HCC patients. It is increasingly understood that amino acid concentrations in the blood are subject to variation not only due to the composition of dietary intake, but also from differences in the genetic and mitochondrial composition between populations [6, 7]. In some cases drug pharmacology may also display population based changes secondary to genetic polymorphisms [8].

Unlike standard chemotherapeutics which are frequently dosed according to a maximum tolerated dose (MTD), anti-metabolite drugs may seek an optimal biological dose – the lowest dose to safely achieve target metabolite depletion [9, 10]. Here we present an open-label, phase I dose-escalation study in two arginine auxotrophic cancers - malignant melanoma (MM) and castration resistant adenocarcinoma of prostate (CRPC) which are not prevalent in the
Chinese populations. Primary objectives were to determine the safety, pharmacokinetics/pharmacodynamics (PK/PD), and recommended Phase II in a non-Chinese setting.

**Patients and Methods**

*Study Design and Treatment*

The study was conducted at 3 centers (Loma Linda University; California Cancer Associates For Research and Excellence; John Wayne Cancer Institute) in the United States. The protocol was approved by the local institutional review board at each study site. Written consents were collected from all patients before enrollment. The trial was registered in clinicaltrials.gov (identifier NCT02285101) and was conducted in compliance with ICH guidance of Good Clinical Practice.

Eligible patients were enrolled sequentially into cohorts in a standard 3+3 dose-escalation design. The four dose levels of weekly, intravenous administration of BCT-100 were 0.5 mg/kg, 1.0 mg/kg, 1.7 mg/kg, and 2.7 mg/kg respectively. At each dose level, safety parameters were monitored for 3 weeks after the first dose. Patients with no dose limiting toxicity following the first dose received two additional doses at the same dose level on Day 22 (Week 4) and Day 29 (Week 5). If there was no evidence of progressive disease or dose limiting toxicity (DLT) patients continued to receive BCT-100 treatment until disease progression.

**Eligibility**

The study population included adult patients (> 18 years-old) with histologically confirmed diagnosis of stage IIIb/IV malignant melanoma or CRPC. Patients must have been deemed unsuitable for standard therapies or whose cancers have progressed on current standard of care. Other inclusion criteria included Karnofsky performance status of 80% or above; a negative urine pregnancy test for female patients; and willingness to use an effective contraceptive method during the study period for all patients. The main exclusion criteria included: chemotherapy within 4 weeks of BCT-100 treatment or other cancer treatments (e.g., targeted biologic) within 2 weeks; significant hepatic, renal or bone marrow dysfunction (total bilirubin >3.0 mg/dL, evidence of bile duct obstruction, serum albumin <2.5 g/dL, serum ALT or AST >5 x upper limit of normal, serum creatinine ≥1.5 mg/dL, ANC <1.5 x 10⁹/L, platelets <50 x 10⁹/L, or INR >2.0); significant cardiac or
pulmonary disease defined by New York Heart Association Class III or IV; significant active infection; use of an investigational drug within 4 weeks of enrolment; and prior treatment with arginine depleting agent.

Safety evaluations
Patients who received at least one dose of BCT-100 were included in the intent-to-treat (ITT) population analysis and considered evaluable for safety analysis. Safety assessments involved monitoring and recording all adverse events (AEs) and serious adverse events (SAEs) between the administration of study drug and 30 days after the last dose. AEs and SAEs were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03. A DLT was defined as treatment-related Grade 2 cardiac AEs, Grade 3 or 4 non-hematologic clinical or laboratory toxicity, Grade 4 hematologic or liver function AEs, or any life-threatening SAE leading to permanent discontinuation of study drug.

Pharmacokinetics (PK) and pharmacodynamics (PD) evaluations
Blood samples were collected at baseline, 1, 2, 4, and 24 hours after the first dose administration to establish Cmax and initial clearance. Additional blood samples were collected on Day 4, 8, 15, and 22 to establish the terminal T1/2 and duration of arginine depletion after the single dose administration. For Weeks 4 through 13, pre-dose and 1hour post dose samples were obtained. Blood was processed to obtain serum and aliquots were frozen at -80C until assayed. Plasma BCT-100 level was measured by ELISA and corresponding arginine level was measured by LC-MS/MS (WuXi AppTec, China). PK parameters were analyzed using non-compartmental method and linear trapezoidal method.

Clinical evaluations
Computed tomography scans were performed at baseline, Week 5, Week 13 and thereafter as clinically indicated. MRI, ultrasonography and bone scans were additionally used to confirm treatment response when deemed clinically necessary. After the enrollment of 15 patients, a protocol amendment was made to have efficacy evaluations on Week 9 instead of Week 5 because of operational challenges. Tumor measurements were assessed and recorded according to RECIST 1.1 guidelines. Plasma PSA levels were monitored for CRPC patients throughout the study.
Statistical analysis
Statistical analyses were performed on an Intent-to-treat basis. All statistical analysis was performed using SAS® Software version 9.4 (SAS Institute, Cary, NC, USA). Survival analysis was computed by the Kaplan-Meier method. Progression-free survival (PFS) was calculated from the date of first-dose administration to the date of documented progression or death.

Results

Demographics
A total of 23 patients received BCT-100: in the study at dose levels of 0.5 mg/kg (3 patients), 1.0 mg/kg (3 patients), 1.7 mg/kg (3 patients), and 2.7 mg/kg (14 patients). There were 9 CRPC patients and 14 melanoma patients. Table 1 shows the demographics of the enrolled patients. The median age was 71 years, and 16 (69.6%) patients were male. The number of patients with baseline Karnofsky performance of ‘80’, ‘90’, ‘100’ were 4 (17.4%), 13 (56.5%), and 5 (21.7%) respectively.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n, %)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (30.4)</td>
</tr>
<tr>
<td>Male</td>
<td>16 (69.6)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Median</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>White</td>
<td>22 (95.7)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
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</tr>
<tr>
<td>Non-Hispanic</td>
<td>22 (95.7)</td>
</tr>
<tr>
<td>Karnofsky Performance Scale</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td>90</td>
<td>13 (56.5)</td>
</tr>
<tr>
<td>80</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>Cancer type (n, %)</td>
<td></td>
</tr>
<tr>
<td>Castration Resistant Adenocarcinoma of Prostate</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Malignant Melanoma</td>
<td>14 (60.9)</td>
</tr>
</tbody>
</table>

Table 1 Demographics and baseline characteristics of the enrolled patients
Safety and tolerability

All 23 patients were evaluable for DLTs. The median number of weeks that the patients received treatment was 12 (range, 9 to 12) at 0.5 mg/kg cohort, 8 (range, 5 to 10) at 1.0 mg/kg cohort, 12 (range, 7 to 13), and 8.5 (range 1 to 13) at 2.7 mg/kg cohort. The majority of the patients (82.6%) had ≥ 6 weeks study visits, including the two-week safety monitoring period (three patients had > 13 weeks of planned visits). No patients experienced a dose-limiting-toxicity (DLT) event. Therefore, the optimal biological dose is defined as 2.7 mg/kg. The number of subjects with a SAE reported at different dose levels (level 1 to 4) were 1, 1, 2, and 4, respectively. No statistically significant relationship was found between dose levels and number of subjects reporting SAE (p = 0.393). No treatment-related SAEs or deaths were reported. Therefore, the optimal biological dose is defined as 2.7 mg/kg.

A total of 261 AEs was reported during the study period. Among system organ classes, 42 AEs were listed under “Investigations”, 32 were listed under “gastrointestinal disorders” and 30 were listed under “general disorders and administrations site conditions”. The most common AEs were nausea (8 subjects, 34.8%), back pain (8 subjects, 34.8%), fatigue (7 subjects, 30.4%), and anemia (7 subjects, 30.4%). Among all AEs, only 18 AEs were deemed drug-related; most of these adverse events were grade 1 (88.9%). The most common drug-related toxicities were fatigue, nausea, and thrombocytopenia (Supplementary Table 1).

Pharmacokinetics and Pharmacodynamics

A total of 22 patients were included in the PK/PD analysis; one patient received only one dose and was therefore excluded for analysis. Overall, plasma BCT-100 concentrations reached a maximum at 2 hours post-dose (Figure 1a). The half-life was 263.63 hour (CV, 5.58%). Following the first dose of BCT-100, the mean C_max at 0.5 mg/kg, 1.0 mg/kg, 1.7 mg/kg, and 2.7 mg/kg were 26.54 (CV, 3.11%), 45.35 (CV, 4.37%), 66.44 (CV, 6.74%), and 59.01 (CV, 6.49%) ug/ml, respectively. The mean AUC_∞ was 6474.69 (CV, 0.84%) hr*ug/ml for 0.5 mg/kg dose level. Similar No significant differences in the mean AUC_∞ were observed from 1.0 mg/kg to 2.7 mg/kg dose levels, which were 14935.15 (CV, 3.85%) hr*ug/ml, 20947.64 (CV, 3.57%) hr*ug/ml, and 19614.86 (CV, 5.41%) hr*ug/ml, respectively. In essence, the PK parameters are similar in cohort 2 to 4.
**Figure 1. Pharmacokinetics and Pharmacodynamics of BCT-100.** (a) Plasma BCT-100 level after the first dose. (b) Changes of plasma arginine level at 0 – 6 hours after the first dose administration of BCT-100. (c) Changes of plasma arginine level in different cohorts from Day 1 to 14 (336 hours) after the first dose administration; cohort 4 had the lowest mean plasma arginine level.
The time taken to achieve the lower limit of quantification (LLOQ; 2.5 uM) of arginine in plasma was 1 hour following a single dose for all cohorts (Figure 1b). The median arginine level was maintained at LLOQ after the second dose on week 4. Patients who received 2.7 mg/kg of BCT-100 had the lowest arginine levels at day 14 (Figure 1c). A significant positive relationship was found between arginine depletion duration and BCT-100 dose level ($p = 0.001$) (Supplementary Table 2).

Serial blood samples collected on Week 1, 5, 7, 9, 11, and 13 were analyzed for the presence of anti-PEG-BCT-100 antibodies (Supplementary Figure 1). The antibodies were detected and remained at low mean titer level, and there was no impact on arginine depletion with BCT-100.

**Clinical Outcomes**

![Clinical Outcomes Graph](image)
Figure 2. (a) Waterfall plot of the percentage change in best tumor response. The tumor response was defined as the minimum percentage change of tumor size after 13-week treatment. For a subject without a 13-week treatment, the tumor assessment records in his last visit was used. For patient 014, 007, 008, 009, and 001, only non-target lesion assessments were performed. (b) Swimmer plot of the all 23 patients.

 Patients who received with three doses or more were included in disease response evaluation. Overall two subjects attained complete response (CR), one patient achieved a partial response (PR), and two patients had stable disease (SD) during treatment (Figures 2a and 2b). Three further patients had unconfirmed stable disease (only non-target lesions were assessed). The first patient was a male 63-year of age with history of multiple skin melanoma resections [11]. He presented with brain metastases, which were successfully resected. He then relapsed with extensive pleural and mediastinal disease that progressed through two immunotherapy strategies. The patient was recruited into the 1.7 mg/kg BCT-100 cohort. CT documented a PR at weeks 5 and 13. Subsequent CT scans in weeks 22 to 78 confirmed an ongoing CR (Figure 3). His last BCT-100 dose was on week 80 when he developed acute ITP from which he made a full recovery on steroids and was deemed not treatment related. He was taken off treatment at the discretion of by his physicianPI.
The second CR (case #014) was a 77-year old male with progressive osseous-only CRPC. Before enrollment to the 2.7mg/kg/week cohort, he was previously treated with Sipuleucel-T for 5 months, achieving a best response of stable disease. He then received a combination therapy of JNJ-56021927, Abiraterone Acetate and Prednisone for a year, but ended due to progressive disease in his bones. The patient was enrolled to the 2.7mg/kg/week cohort. He attained stable disease on serial CT scans on Week 5, 13, 16 and 19. Serial imaging followed by a final bone scan and a PET scan on week 61 revealed no evidence of disease and the patient was taken off treatment as of week 61. He was deemed to have attained complete response on week 61.

The PR case was an ocular melanoma (case #015) who attained 38% tumor regression by RECIST on week 20 of BCT-100. The two patients had documented Stable Disease. One was a one bladder MM (case #012) who had tumor size decrease (12.9% reduction) after 3 injections of treatment on week 5. He was taken off treatment at week 5 due to respiratory distress of unspecified cause. The second was a patient with case of CRPC (case #002), his tumor remained within a had tumor progression of 6.5% of baseline size on week 12 but and he withdrew from treatment because of an increased PSA increase (from 14 to 36 ng/ml) at the PI’s discretion.

The plasma PSA levels increased in all the other CRPC patients consistent with progressive disease were found to have increased throughout the study. All, except patient 014 mentioned...
above, had progressive disease. Patients 007, 008 & 009 had a non-target lesion classified as unconfirmed stable disease or non-CR/non-PD with no new lesions as per RECIST assessment criteria.

Discussion

The current study evaluated BCT-100 PEG-Arginase in patients with one of two common arginine auxotrophic cancers, malignant melanoma and/or castration resistant prostate cancer, which are prevalent in a non-Chinese population [5, 12].

In terms of patient demographics, all but one of the patients were Caucasian hence these patients were not only ethnically different but likely to have different dietary habits than Chinese subjects. Minimal inter-subject variations in PK were seen, and BCT-100 was effective in depleting plasma arginine at doses of 1.7 mg/kg/week and 2.7 mg/kg/week (3rd and 4th cohort) which is similar to the PK/PD profile reported in the our previous Phase I trial conducted in Hong Kong [12]. A single dose of BCT-100 at 2.7mg/kg/week led to a sustained arginine depletion up to 14 days. Thus BCT-100 has the same arginine depleting activity across different ethnic groups whose dietary arginine intakes, and underlying genomic and metabolic composition, may vary greatly. No DLT toxicities were observed, and the incidence of drug-related AEs in this study was low with only 2 patients experiencing a higher than grade 2 toxicity—one case of grade 4 thrombocytopenia diagnosed to be acute idiopathic thrombocytopenia (ITP) which responded well to steroid therapy; the other being grade 4 itching of unknown cause. This low incidence of drug-related AEs is also in keeping with our previous reports. With the similar PK/PD parameters and safety profiles in these two ethnic groups, BCT-100 at 2.7mg/kg/kg/week can now safely be chosen as our optimal biological dose for our future studies in arginine auxotrophic cancers.

It is well established by the study of human samples that metastatic melanoma and prostate carcinoma tumours are rationale targets for the use of the arginine depleting therapeutics, due to the low to absent expression of ASS1 [4, 13, 14]. The encouraging safety-findings highlight the biological differences between normal somatic cells which tolerate the absence of arginine due to resynthesis from citrulline, via ASS1 expression, compared to malignant cells which undergo cell death [15, 16].
Study of patient samples from larger Phase II/III studies will allow for the correlation of tumour responses with biological pathways that lie outside of canonical arginine metabolism. Currently, there are three clinically relevant arginine depleting drug candidates in clinical development: ADI-peg, Pegzilarginase, and BCT-100. The emergence of anti-drug antibodies, particularly on prolonged administration is the main concern in for non-human arginase drug candidates, such as ADI-peg - a pegylated Mycoplasma protein derivative. In the reported ADI-peg clinical studies, including the randomized phase III global study in HCC [17], more than half of all subjects eventually tested positive for rising titers of anti-ADI-peg antibodies rendering the drug ineffectual as an arginine depleting agent in a majority of patients. [13]. In this study we found that the incidence of anti-drug antibody to BCT-100 is low. All patients in the four study cohorts had arginine depletion to an unquantifiable level (LLOQ) throughout the entire treatment period, thus anti-drug antibodies, even if present, had no impact on arginine depletion with BCT-100.

As arginine depletion must be maintained for a period of many months in order to sustain remissions, or achieve cure, the method of drug administration and toxicity profile is critical, ADI-peg is administered by deep intramuscular injection with incidences of skin/injection site reactions such as local pain and anaphylactic reactions [17, 18]. Apart from minor grade 1 skin reactions at injection sites, none of these side effects have been reported in our studies of BCT-100. Pegzilarginase (pegylated human recombinant arginase with cobalt as co-factor instead of the naturally occurring Manganese) has a seemingly different toxicity profile to BCT-100, and to date its use has focused on developing the drug as an enzyme replacement therapy [19].

In vitro and in vivo, BCT-100 synergizes with a number of chemotherapeutic agents in different arginine auxotrophic cancer models [3]. In our previous Phase II HCC study, BCT-100 in combination with oxaliplatin-based chemotherapy had led to a significant improvement in OS for patients with ASS1 deficient HCC tumours (NCT02089633). We plan to test the efficacy of BCT-100 in combination with oxaliplatin-based chemotherapy in ASS deficient HCC patients. Separately, a UK national AML study sponsored by BloodWise (EUDRACT number: 2011-000749), of BCT-100 in combination with low dose cytarabine in AML patients >65 year, has recently been concluded (EUDRACT number: 2011-000749). Results of this study should be available soon. Currently, there is an ongoing phase II international study in Europe and Australia testing the single agent activity of BCT-
100 in a number of pediatrics cancers (EUDRACT number: 2017-002762-44)—such as AML, sarcoma, glioma and neuroblastoma. Study of these cohorts patient samples from larger Phase II/III studies will allow for the correlation of tumour responses with biological pathways that lie outside of canonical arginine metabolism.

In conclusion, BCT-100 at a dose of 2.7mg/kg/week is safe and effective in inducing prolonged and sustained arginine depletion in humans across ethnic groups, that induces remission and disease stability in a number of patients and the drug has anti-cancer activities in the arginine auxotrophic cancers with relapsed/refractory malignant melanoma [11], prostate cancer and HCC [5]. Although immunotherapy is increasingly used for some tumors such as melanoma and prostate cancer, significant numbers of these patients still relapse. BCT-100 could address this unmet medical need.

**Declarations**

**Funding:** Bio-Cancer Treatment International Limited

**Conflicts of interest:** Dr Paul N.M. Cheng and Dr Angela M. Liu are employees of Bio-Cancer Treatment International Ltd. There are no other conflicts of interest.

**Availability of data and material:** The data set that support the findings of this study are available to researchers who provide a methodologically sound proposal.

**Code availability:** Not applicable

**Authors’ contributions:** PC and AB designed the study and performed research. PC, AL and FM analyzed the data and wrote the manuscript. All authors critically reviewed and approved the manuscript.

**Ethics approval:** The study protocol was approved by the Institutional Review Boards at all participating centers.

**Consent to participate:** All patients provided written informed consent prior to enrollment.

**Consent for publication:** The patient and study members have provided consent for publication.
References


Supplementary Tables and Figures

Supplementary Table 1: Summary of possible drug-related AEs.

<table>
<thead>
<tr>
<th></th>
<th>Total no. of AEs</th>
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<tbody>
<tr>
<td></td>
<td>Grade 1</td>
</tr>
<tr>
<td>Fatigue</td>
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<tr>
<td>Dehydration</td>
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</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1</td>
</tr>
<tr>
<td>ALT elevated</td>
<td>1</td>
</tr>
<tr>
<td>AST elevated</td>
<td>1</td>
</tr>
<tr>
<td>Hot flashes</td>
<td>1</td>
</tr>
<tr>
<td>Night sweats</td>
<td>1</td>
</tr>
<tr>
<td>Intermittent diarrhea</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Itching</td>
<td></td>
</tr>
<tr>
<td>Maculopapular rash</td>
<td>1</td>
</tr>
<tr>
<td>Vision changes</td>
<td>1</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>1</td>
</tr>
</tbody>
</table>

ALT alanine Transaminase; AST Asparate Transaminase

Supplementary Table 2. Relationship between dose levels and effective arginine depletion (AD) duration. Effective AD is defined as the level of plasma arginine below 8 uM. P-value is obtained by linear regression model.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Dose Level (mg/kg)</th>
<th>Number of Patients</th>
<th>Effective AD Duration</th>
<th>p-value</th>
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**Supplementary Figure 1.** Changes of mean plasma PEG-BCT-100 antibody titer across study period (error bars: ± SEM).