Phase II trial of albumin-bound paclitaxel and granulocyte macrophage colony-stimulating factor as an immune modulator in recurrent platinum resistant ovarian cancer

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26 Running title: Albumin-bound paclitaxel & GMCSF in recurrent ovarian cancer

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Abstract

Background: Granulocyte macrophage colony-stimulating factor (GM-CSF) stimulates immunity via recruitment of antigen presenting cells and tumor specific T-cell stimulation. Albumin-bound paclitaxel (nab-paclitaxel) followed by GM-CSF may enhance antitumor responses and prolong remissions in ovarian cancer. Immune phenotypes present before treatment may identify responders to chemo-immunotherapy.

Methods: Recurrent platinum-resistant ovarian, peritoneal, or fallopian tube cancer patients received nab-paclitaxel, 100mg/m² days 1,8,15 followed by GM-CSF 250mcg days 16-26 every 28 days for 6 planned cycles. The primary endpoint was remission duration compared to immediate prior remission. Peripheral blood was evaluated by flow cytometry and interferon- γ ELISPOT.

Results: Twenty-one patients were enrolled. Six patients (29%) achieved a biochemical complete response and 9 (43%) a partial response for an overall response rate of 72%. Median time to progression was 4 months and 10% of patients achieved longer remissions than the immediate prior regimen. Median overall survival (OS) was 16.8 months. Fewer myeloid derived suppressor cells (MDSC) at enrollment significantly associated with complete response ($p=0.05$). T-cell responses to IGF1R-p1332-1346 ($r=0.827$, $p=0.0003$) and IGF1R-p1242-1256 ($r=0.850$, $p=0.0001$) during treatment correlated with time to progression.

Conclusions: Nab-paclitaxel combined with GM-CSF demonstrated biochemical responses in a majority of patients, although responses were not sustained. This combination did not demonstrate an advantage in OS over prior studies of nab-paclitaxel monotherapy. Agents that modulate MDSC should be studied as potential adjuvants to therapy. Strategies to expand T cells recognizing tumor-associated antigens biologically significant in ovarian cancer should also continue to be investigated.

56 **Keywords**

57 ovarian cancer

58 GM-CSF

59 nab-paclitaxel

60 myeloid derived suppressor cells

61 chemo-immunotherapy

62

63 **Introduction**

64 The emergence of resistance to platinum based chemotherapy heralds the majority of deaths
65 due to recurrent ovarian cancer. Response rates to commonly used cytotoxic agents in a
66 platinum resistant recurrence are modest, ranging from 10% to 15% with overall survival (OS) of
67 about 12 months based on randomized phase 3 trials.(1) Combination chemotherapy has not
68 shown consistent benefits and current guidelines recommend use of single agents and do not
69 support any combination in this setting except with bevacizumab.

70 The immune response to ovarian cancers has been recognized as an important prognostic
71 factor.(2, 3) CD8+ T cell infiltration of tumors improves prognosis, while regulatory T cells and
72 myeloid derived suppressor cells negatively impact survival.(2, 4) Although cytotoxic
73 chemotherapies have been traditionally thought to have immune suppressive effects, a greater
74 appreciation has evolved for their immunostimulatory properties and the potential for
75 combinations with immune-based therapies to exploit these synergies.

76 Paclitaxel administered as a single agent has shown significant activity when used in the
77 platinum resistant setting for recurrent ovarian cancer.(5) Albumin-bound paclitaxel (nab-
78 paclitaxel) avoids the need for steroid premedication, which may allow it to synergize with an
79 immunostimulatory agent since taxanes have been found to suppress myeloid derived
80 suppressor cells (MDSC) and stimulate the production of interferon- γ by cytotoxic T
81 lymphocytes.(6, 7) GM-CSF is a multifunctional cytokine originally developed to treat
82 chemotherapy-induced neutropenia that also possesses diverse immune properties. GM-CSF
83 has been studied as a therapeutic immunomodulating agent in several malignancies.(8) In
84 melanoma, GM-CSF has been evaluated alone and in combination with chemotherapy and has
85 been shown to increase dendritic cell number and activation in lymph nodes draining early stage

disease and demonstrates evidence of clinical benefit in select patients with advanced stage disease.(9-11)

Tumor-associated antigens overexpressed in ovarian cancer may play biologic roles in maintaining the malignant phenotype and promoting resistance to chemotherapy. A number of tumor-associated antigens have been reported to mediate resistance to taxane chemotherapy. IGFBP-2 is overexpressed in high grade serous ovarian cancers and has been shown to reduce the efficacy of taxanes.(12, 13) Mutations in p53, seen in almost all high grade serous ovarian cancers, have been shown to be related to resistance to microtubule stabilizers such as taxanes.(14, 15) Absent or low expression of IGF1R is associated with improved responses to taxane-based neoadjuvant chemotherapy in breast cancer patients.(16) IGF1R expression is also associated with resistance to taxane-based chemotherapy in ovarian cancer cells.(17) PRAME, a cancer-testis antigen, is overexpressed in epithelial ovarian cancer and has been proposed as a potential target for immunotherapy.(18)

We hypothesized that pairing weekly nab-paclitaxel with GM-CSF may enhance and prolong antitumor immunity through increased antigen presentation allowing proliferation of tumor-associated antigen-specific T cells. We further hypothesized that a favorable immune environment, as has been seen in ovarian cancers with improved prognosis, may predict successful application of chemo-immunotherapy.

Methods

Patient population

After informed consent was obtained, patients were enrolled in this trial approved by the University of Washington Human Subjects Division (NCT00466960). Major enrollment criteria included: recurrent platinum resistant or refractory ovarian, fallopian tube, or primary peritoneal cancer, with platinum resistance defined as a treatment free interval following platinum therapy

of less than 6 months, persistent disease at the completion of a platinum based therapy, or progression of disease on a platinum based regimen; an elevated CA-125 greater than the institutional norm, 35, on two occasions greater than 7 days apart; and at least 3 weeks elapsed time from prior chemotherapy or radiation therapy. Patient characteristics are shown in Table 1. Other inclusion criteria included age ≥ 18 years, an absolute neutrophil count $\geq 1500/\mu\text{L}$; platelets $\geq 100,000/\mu\text{L}$; creatinine $\leq 2.0\text{mg/dL}$; total bilirubin $\leq 1.5\text{ mg/dL}$; SGOT ≤ 2.5 times the upper limit of normal or <5 times the upper limit of normal with documented report of hepatic metastases. Patients with a history of other invasive malignancies within the previous 5 years were excluded, with the exception of non-melanoma skin cancer.

Study Design

This was a Phase II study of GM-CSF in combination with nab-paclitaxel. Nab-paclitaxel was delivered by IV infusion over 30 minutes at a dose of 100mg/m^2 on days 1, 8, and 15. GM-CSF was self-injected subcutaneously at 250 mcg on days 16-26 of each 28 day cycle. Patients were treated to maximum response or for 6 months, and then started maintenance therapy of GM-CSF as a single agent, 250 mcg on days 1-15 of the 28 day cycle for a maximum of six cycles. Patients, who had to discontinue nab-paclitaxel due to toxicity prior to the completion of all cycles of therapy, could still receive maintenance GM-CSF in the absence of disease progression or excessive toxicity. Patients were pretreated with ondansetron 8mg intravenously, 30 minutes prior to the administration of nab-paclitaxel for anti-emetic prophylaxis. Subjects could also be premedicated with 650 mg of acetaminophen orally, 30 minutes prior to nab-paclitaxel for prophylaxis against acute hypersensitivity reaction. Toxicities were evaluated by CTEP CTCAE v 3.0 criteria.

The primary objective was to determine whether GM-CSF administration during and after cytotoxic chemotherapy could induce a longer remission than experienced with the most recent

platinum-containing regimen.(19, 20) Biochemical responses were based on Rustin criteria for CA-125 tumor kinetics.(21) Progression was defined as a CA-125 greater than the institutional norm and double the patient's nadir value at enrollment. Partial response (PR) was defined as a 50% decrease in serum CA-125 from initially elevated samples. Complete response (CR) was defined as either a 75% reduction serially in CA-125 or when CA-125 fell below the institutional norm of 35 U/mL. The study's secondary endpoints were to determine the extent to which GM-CSF can increase the numbers of monocytes and peripheral circulating antigen presenting cells. Additional secondary endpoints were to determine the extent that GM-CSF can increase the number and functional status of T-cells that recognize tumor associated antigens and foreign pathogens.

Statistical power was based on the biology of successive ovarian cancer recurrences exhibiting progressively shorter times to progression.(19, 22) We assumed that the expected ratio of time to progression is 0.7 times for patients with recurrent disease treated with the combination compared to initial line of platinum chemotherapy. If the true time to progression of nab-paclitaxel/GM-CSF were equal to the time to progression following initial therapy, a study containing 30 subjects would provide 80% power to demonstrate the predicted effect of nab-paclitaxel/GM-CSF. If nab-paclitaxel/GM-CSF had a 20% longer time to progression than platinum chemotherapy, only 20 subjects are necessary for an 80% power of detection.(20)

Flow Cytometry

PBMC were evaluated by flow cytometry for circulating monocytes, dendritic cells, and myeloid derived suppressor cells (MDSC). The PBMC profiling panel antibody cocktail contains: CD3-PE CF594 (#UCHT1; BD Biosciences), CD4-FITC (#RPA-T4; Biolegend), CD8-PerCP/Cy5.5 (#SK1; BD Biosciences), CD11C-AF700 (#BLY6; BD Biosciences), CD14-V450 (#MOP9; BD Biosciences), CD16-APC H7 (#3G8; BD Biosciences), CD19-PE-Cy5 (#HIB19; Biolegend),

CD45-AmCyan (#2D1; BD Biosciences), CD56-PE-Cy7 (#NCAM16.2; BD Biosciences), CD122-APC (#TU27; Biolegend), CD123-PE (#9F5; BD Biosciences), HLA-DR BV605 (#L243; Biolegend). Myeloid dendritic cells were defined as CD45+, HLA-DR+, and CD11C+. Plasmacytoid dendritic cells were defined as CD45+, HLA-DR+ and CD123+. For staining MDSCs the following antibodies were used: CD11b-PE (#ICRF44; Biolegend), CD33-PerCP-Cy5.5 (#WM53; Biolegend), HLA DR-PE-Cy7 (#L243; Biolegend), and CD45-AmCyan (#2D1; BD Biosciences). MDSC were defined as HLADR-CD33+CD11b+CD45+. PBMCs were incubated with the appropriate antibody cocktail for 30 minutes at 4°C in FACS buffer (PBS/1%FBS). The cells were washed twice with FACS buffer then fixed with 2% paraformaldehyde before data acquisition. Samples were acquired using either a BD LSR II (for PBMC panel samples) or a FACS CANTO2 (MDSC panel samples) flow cytometer (BD Bioscience), and analyzed using FlowJo software v9.

Enzyme-linked immunosorbent spot assay (ELISPOT)

Interferon gamma (g) ELISPOT was performed as previously described.(23) Each antigen was assessed in six replicates of 2×10^5 /well. Recombinant human p53 (JPT), PRAME (JPT), insulin-like growth factor binding protein 2 (Leinco) and 1 µg/mL of individual peptides IGF1R p1196-1210, IGF1R 1242-1256, IGF1R 1332-1346 (CPC Scientific) were tested as common ovarian cancer antigens in addition to tetanus toxoid (0.5 U/mL), CEF peptide pool, and phytohemagglutinin (PHA) (2.5 µg/mL) plate controls. These MHC class II epitopes of IGF1-R have been shown to be broadly immunogenic in cancer patients.(24) All samples for each patient were cryopreserved then thawed and analyzed simultaneously to ensure comparability. Data are presented as precursor frequencies calculated at 1/frequency of interferon gamma secreting cells in 10^6 PBMC derived from measured spots per well, corrected for background. A representative data set is shown in Supplemental Figure 2.

Statistical Analysis

Statistical analysis to describe patient and response characteristics was performed using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA). Response rates were compared to disease related characteristics and tested for significance using chi-square tests. Wilcoxon matched-pairs signed ranks tests were used to compare baseline and follow up cell population changes within patients. The Mann-Whitney test was used to compare differences in cell populations between patients with and without a complete response. Kaplan-Meier curves estimated median survivals for overall survival (OS) and progression free survival (PFS), and also compared treatment and disease-related characteristics using log-rank tests.

Results

Feasibility and safety of combination chemo-immunotherapy.

A total of 21 patients enrolled in this single institution clinical trial. Patients were heavily pretreated and had received a median of 3 prior regimens with over half of patients (52%) also having received bevacizumab as part of a regimen prior to enrollment (Table 1). Seven (33%) patients received all six planned cycles of nab-paclitaxel and GM-CSF and of these, five received an additional 1 to 4 maintenance cycles. Fourteen patients (67%) of patients received at least 4 cycles. 19 of 21 patients ultimately left the study due to recurrence. Of 393 adverse events (AE), 359 (92%) were grade 1 or 2. The most common were hematologic, with anemia, leukopenia, and lymphopenia making up 21% of all AE related of any grade. One patient died during the study at home, unattended, cause unknown, and one patient left the study due to a grade 3 toxicity, an allergic reaction leading to persistent urticaria. Severe adverse events, grades 3 or 4, that were possibly, probably, or definitely related were uncommon, comprising 8% of all AE. The majority of these, 10 of the 15 total adverse events $\geq$ grade 3, were bone

marrow related toxicities (Table 2). The remaining were fatigue, hypokalemia, neuropathy, and urticaria.

Biochemical responses were observed in the majority of patients but were of short duration.

Of the 21 enrolled patients, 6 (29%) achieved a complete response (CR) and 9 (43%) a partial response (PR) for an overall response rate of 72%. Three (14%) had stable disease for a disease control rate of 86%. Three patients (14%) did not have a measurable response to the treatment. Ratio of time to progression on nab-paclitaxel/GM-CSF over the time to progression on the most recent platinum based regimen was greater than 1.0 for only 2 out of 21 (10%) patients indicating transient responses (Fig. 1A) and the primary endpoint was not achieved. When time to progression is studied at a first recurrence, the percentage of ovarian patients that will have a time to progression greater than their previous remission has been reported to be 3% to 8%.(19, 22) The median time to progression was 4 months (Fig. 1B). The median OS was 16.8 months, which is comparable to what has been reported in other phase 2 studies of nab-paclitaxel both as a single agent (17.4 months) and in combination with bevacizumab (17.2 months) in persistent or platinum resistant ovarian cancer.(25, 26) (Fig. 1C).

Patients with a complete biochemical response to chemo-immunotherapy have significantly lower levels of circulating MDSC in peripheral blood prior to the start of treatment than partial and non-responders

We considered whether there were any immunologic differences in patients classified as partial responders from non-responders at baseline. We found that there were no significant differences between PR and non-responders with respect to MDSC, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), and monocytes. (Supplemental Table 1). We then examined the baseline MDSC, mDC, pDC and monocytes of complete responders compared to

partial and non-responders. Median percentages of CD45+ cells made up of mDC (CR: Median 1.54% range 0.86-5.73%, PR and non-responders: median 2.00% range 0.45-9.06%, $p=0.77$), pDC (CR: median 0.25% range 0.18%-0.46%, PR and non-responders: median 0.37% range 0.14%-1.19%, $p=0.23$) and monocytes (CR: median 20.75% range 7.31%-26.2%, PR and non-responders: median 12.75% range 4.87-43%, $p=0.74$) were also not significantly different in those patients who achieved a complete response compared to non-responders. Only baseline percentage MDSC (CD33+CD11b+CD45+) was associated with achieving a complete response to nab-paclitaxel and GM-CSF (CR: median 0.59% range 0.17%-0.88%, PR and non-responders: median 1.09% range 0.29%-25.4%, $p=0.05$) (Fig. 2). The single patient with the highest percentage of MDSC was a partial responder.

An increase in circulating IGF1R specific T-cells is associated with response to combination chemo-immunotherapy.

Interferon- γ ELISPOT responses against measurements of overall cellular immune competency such as PHA, CEF, and tetanus toxoid did not significantly change from baseline to follow-up levels after starting treatment. When we looked at whether immunity against tumor-associated antigens correlated with response, however, the fold change achieved above baseline ELISPOT responses after treatment with nab-paclitaxel and GMCSF significantly correlated with time to progression for both IGF1R class II restricted epitopes p1332-1346 ($r=0.8269$, $p=0.0003$) and p1242-1256 ($r=0.8496$, $p=0.0001$) (Table 3).(27) Measures of interferon- γ ELISPOT responses to p53, IGFBP2, PRAME and PHA, CEF, and tetanus toxoid did not reveal significant correlations to time to progression (Table 3).

Discussion

Efforts to stimulate immune responses against tumors in platinum resistant ovarian cancer have recently yielded promising results.(28) Integrating existing cytotoxic agents with immune

therapies may exploit underlying synergies. In our study, the addition of GM-CSF did not prolong remission compared to prior remissions achieved with platinum-based chemotherapy, however, lower baseline levels of the subset of peripheral blood MDSC may identify patients who could achieve a CR. Immunity against tumor-associated antigens with chemotherapy is also significantly associated with improvement in time to progression.

Several factors may contribute the absence of longer remissions with this chemotherapy. GM-CSF has been shown to increase peripheral MDSC and monocytes, but we did not see this elevation in our patients when it was given in combination with nab-paclitaxel (Supplemental Fig. 1).(29) This may suggest that the dose selected, based on a study that included women with ovarian cancer treated with GM-CSF, could be insufficient to generate a durable response.(29) It could also reflect antagonistic effects of taxane chemotherapy, which has been reported to decrease circulating myeloid cells when given with carboplatin.(30) Our patients were also very heavily pretreated, having received a median of 3 prior lines of chemotherapy (range of 1-13), which may make them less able to respond to chemotherapy based on poor bone marrow reserve and may have also contributed to the percentage of patients able to complete all planned cycles.

Some features of the study limit the extent to which we may attribute immunomodulatory effects of the combination to GM-CSF alone. All patients in the study received GM-CSF. There may be overlapping effects on T cell function from GM-CSF and nab-paclitaxel. GM-CSF may also have direct effects on tumor growth. GM-CSF production by colon cancer cells is an independent favorable prognostic factor.(31) GM-CSF has also been reported to inhibit breast cancer growth and metastasis.(32) Finally, while biochemical response criteria in ovarian cancer clinical trials using CA-125 are well defined, immune therapies could act through mechanisms that may demand re-evaluation these definitions.(33-35) The use of CA-125 response criteria may also have been a factor in the observed completion rates since these levels were checked

278 monthly, allowing earlier and more frequent opportunities to establish progression, since CA-
279 125 changes have been shown to precede RECIST responses.(36) Inflammatory conditions
280 unrelated to malignancy are known to elevate CA-125 levels. Generating an effective
281 inflammatory immune response could falsely elevate CA-125, beyond levels seen with well-
282 characterized cytotoxic chemotherapies.(37) Thus, biochemical assessment of clinical response
283 may not be precise when used in the contexts of immune therapy or chemo-immunotherapy.

284 MDSC infiltrating ovarian cancers are associated with poorer prognosis and promote and
285 maintain the ovarian stem cell pool.(2) Our data demonstrate that patients who responded to
286 chemo-immunotherapy had significantly lower subset of peripheral MDSC at baseline Lower
287 baseline levels of this subset of MDSC in the peripheral blood may suggest a more favorable
288 immune milieu, allowing cytotoxic chemotherapies to exploit the immune environment.

289 The ability to increase antigen specific T cells secreting interferon- γ with chemo-immunotherapy
290 correlated with the best outcomes. This supports the wealth of data identifying the importance
291 of T cells in ovarian cancer prognosis not only when infiltrating tumor, but also when circulating
292 in the periphery.(3, 4, 38) High neutrophil to lymphocyte ratios correlate with poor survival and
293 absolute lymphocyte count has been associated with survival independent of tumor infiltrating T
294 cells in ovarian cancer.(38, 39) T cells exhibiting a Th1 phenotype by secreting interferon- γ in
295 response to IGF1R showed the most significance in our study. IGF1R is biologically significant
296 in the early stages of chemoresistance in ovarian cancer cells(17) and can potentiate paclitaxel
297 resistance in lung, and breast cancer.(16, 40, 41) Presumably, most advanced stage patients
298 would have high expression of IGF1R in their therapy-refractory cancers.

299 As immune-based therapies become incorporated into the treatment of ovarian cancers,
300 identification of accessible immune biomarkers of clinical response will be crucial. Peripheral
301 blood biomarkers such as MDSC subsets should be investigated for their ability to identify

patients most likely to respond to chemo-immunotherapy. Our findings also support rationales for targeting both immunomodulatory cells such as MDSC and tumor-associated antigens through induction and expansion of Th1 T cell responses in ovarian cancer. Antigens that promote tumor growth in the face of cytotoxic chemotherapy represent important targets that could be exploited to overcome chemoresistance in ovarian cancer. Systemic immune markers may chart real time changes in the tumor immune environment and reflect tumor immune suppression. Furthermore, agents that modulate immune suppressive cell populations and strategies to augment tumor-associated antigen-specific T cell responses should be evaluated as adjuvants to existing therapies.

Acknowledgements

This study was supported by a grant from Celgene.

JBL is supported by the Department of Defense, Ovarian Cancer Research Program, Ovarian Cancer Academy: Early-Career Investigator Award, W81XWH-14-1-0161.

MLD was supported by the Athena Distinguished Professor of Breast Cancer Research, P50 CA083636, U01 CA154967, and is a Komen Scholar.

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Figure Legends

Table 1. Baseline patient characteristics

Table 2. Adverse events by CTEP CTCAE v 3.0 criteria

Figure 1. A. Waterfall plot of time to progression ratios with annotated biochemical response. SD= stable disease, CR= complete response, PR= partial response, NR= no response. B. Progression free survival. Median = 4 months. C. Overall survival. Median = 16.8 months.

Figure 2. Flow cytometry showing percentage of CD45+ peripheral blood mononuclear cells made up by (A) myeloid derived suppressor cells, (B) myeloid dendritic cells, (C) plasmacytoid dendritic cells, and (D) monocytes prior to starting chemo-immunotherapy in patients who ultimately achieved a complete response (CR) versus those who did not. * p=0.05

Table 3. Time to progression ratio correlated with fold change from baseline to post-therapy in interferon- γ ELISPOT responses to peptides from tumor-associated and foreign antigens seen in PBMC.

Table 1. Baseline Patient Characteristics (n=21)			
Characteristic	Median (range)	No.	%
Disease Site			
Fallopian tube	-	1	5%
Ovarian	-	19	90%
Peritoneal	-	1	5%
Grade			
High	-	14	67%
Low	-	3	14%
Histology			
Serous	-	19	90%
Non-serous	-	2	10%
Ascites present at baseline	-	7	33%
Age, years	61 (30 - 92)	-	-
Number of prior regimens	3 (1 - 13)	-	-
Prior bevacizumab therapy	-	11	52%
Received neoadjuvant chemotherapy	-	4	19%
Time from last platinum chemotherapy, months	18 (0.3 - 29.4)	-	-
CA-125 (U/mL) at enrollment	416 (30 - 8725)	-	-

Table. 1

Table 2. Adverse Events (n=393)				
Most Common	Possibly, Probably, or Definitely Related		All AEs	
	No.	% of Related AEs	No.	% of All AEs
Anemia	39	12%	41	10%
Leukopenia	23	7%	23	6%
Lymphopenia	20	6%	20	5%
Fatigue	13	4%	14	4%
Neutropenia	11	3%	11	3%
Nausea	5	2%	11	3%
Hair loss/alopecia	9	3%	11	3%
Hypoalbuminemia	9	3%	10	3%
Vomiting	4	1%	10	3%
Diarrhea	7	2%	9	2%
AE Gradings				
1	202	62%	235	60%
2	108	33%	124	32%
3	12	4%	27	7%
4	2	1%	3	1%
5	1	<1%	4	1%

Table 2

Time to Progression Ratio vs Fold Change				
Antigen	Pearson r	95% confidence interval	R square	P value (two-tailed)
IGF1R-p1196-1210	0.5056	-0.03429 to 0.8171	0.2556	0.0651
IGF1R-p1242-1256	0.8496	0.5807 to 0.9513	0.7218	0.0001
IGF1R-p1332-1346	0.8269	0.5279 to 0.9435	0.6838	0.0003
IGFBP2.Leinco	0.3676	-0.2982 to 0.7927	0.1351	0.2661
p53.JPT-CST	-0.02268	-0.5667 to 0.5351	0.0005143	0.9414
PRAME.JPT-CST	-0.0817	-0.5869 to 0.4693	0.006675	0.7813
PHA	-0.08759	-0.5908 to 0.4647	0.007673	0.7659
CEF	-0.1818	-0.6498 to 0.3862	0.03304	0.534
tt	-0.1071	-0.6035 to 0.4491	0.01148	0.7155

Table 3

5. Figure

Figure 1