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14. ABSTRACT

We have found that the monoclonal antibody specific for a specific epitope of CD151 that is hypothesized to be expressed on prostate cancer microparticles from metastatic prostate cancer patients is also expressed on a subpopulation of prostate cancer microparticles from localized prostate cancer patients undergoing radical treatment. Therefore, a new direction is to analyze prostate cancer microparticles from patients on active surveillance (low risk PCa patients under watchful waiting) and we hypothesize that these patients will have the lowest percentage of 1A5-positive prostate microparticles. Those patients that do have high levels of 1A5-positive prostate microparticles will be followed to determine if their disease progresses and requires radical therapy (surgery, radiation).

15. SUBJECT TERMS
Indolent cancer, active surveillance, biomarkers, prostatectomy

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Introduction:

While PSA testing has resulted in most prostate cancer cases being detected earlier and more curable, a subpopulation of patients initially diagnosed with localized disease will eventually progress to metastatic disease. An emerging area of clinical need is the ability for clinicians to determine at the time of radical treatment (prostatectomy or radiotherapy) which patients will eventually progress to metastasis and which ones will not. The difference between these two outcomes is most likely due to whether or not the tumor initially treated was of an indolent (non-aggressive) phenotype or of an aggressive phenotype. In this DoD fellowship proposal, I proposed research to evaluate the clinical utility of a metastasis-predicting monoclonal antibody called 1A5 (Zijlstra, Lewis, Degryse, Stuhlmann, & Quigley, 2008) that is specific for a unique epitope of CD151 to distinguish patients that have indolent vs. aggressive tumors at the time of radical therapy. The main objective was to determine if prostate cancer microparticles that bind this 1A5 mAb would indicate the presence of an aggressive tumor and increased propensity to metastatic progression and biochemical recurrence. The reason why we are analyzing 1A5-positive prostate cancer microparticles (PCMPs) is because individual circulating tumor cells are very rare in localized patients before and after prostatectomy. By developing a "fluid biopsy" based on enumeration of 1A5-positive prostate cancer microparticles to assess certain biological characteristics of the tumor or residual tumor cells, we will harness the translational potential of prostate cancer microparticles and the biomarkers on their surface to determine if patients have indolent PCa or aggressive PCa in a serial and non-invasive manner.

Body:

Important information regarding abbreviated term of funding by DoD fellowship:

Funds for this project were finally transferred to my financial institution in late November, funds which covered the term of September 1, 2012 to November 31, 2012. While this DoD fellowship is for a 2 year term, I will only be accepting funds for this first three month term because I will be accepting a new operating and salary grant for my new position as an independent scientist at the same institution effective Jan 1, 2013. This means that the DoD funding will only cover the first three months of the award term (September 1, 2012 to November 31, 2012). The purpose of this report is to detail what progress I had made during this 3 month period and my financial institution will be cancelling all further invoicing from the DoD and this will be expressed to you in the form of a letter written by our financial officer that is already submitted to Kimberly Carter. I am extremely grateful for the funds that DoD has provided for this first 3 month term and will describe to you the results of this important research albeit from this abbreviated time of the proposed 2 year term of the fellowship. I will be providing a complete record of the research findings in reference to the Statement of Work (SOW) that I provided for my fellowship application. Each item in the SOW will be italicized while the progress made towards completing the item within the abbreviated period will not be italicized.

Statement of Work (SOW)

Task 1. – Enumeration of CD151-positive prostate cancer microparticles in patient plasma

(timeframe = months 1-6). The major goal will be to compile data to determine if CD151+ve PCMPs are primarily present within prostate cancer patients that have metastasis or precedes the development of metastasis. All ethics regarding the retrospective analysis of human specimens and clinical data have been approved by the University of Western Ontario, and the London Health Sciences Centre at LRCP.

Subtask 1a*. Enumeration of CD151+ve PCMPs will be done by flow cytometric analysis of patient plasmas and this procedure has already been optimized into a standard operating protocol at the Gerald C. Baines Translational Cancer Research Centre. The first paper that describes the work using these models will be submitted by month 18 to a high-profile cell biology journal. There are a total of 206 plasma samples (four different cohorts) and these will be analyzed in a double-blind manner in triplicate (618 samples for flow cytometric analysis). Milestones will be the completion of 400 samples by month 3 and completion of all samples by month 5.*

In terms of milestones we have been able to collect 145 plasma samples for Subtask 1a, thus falling short 66 plasmas of our projected goal for month 3 (206). We have met our N=20 target in the age matched volunteer cohort, and met our N=78 target in the cohort of men who have undergone RRP and exhibited no biochemical recurrence over the next 4 years (Group A). However, we have only been able to acquire N=11 plasmas for the cohort of men who have undergone RPP and have exhibited biochemical recurrence within 4 years (Group B). This is

because when I had made the initial inquiry for these patients, I neglected to exclude radiation treatment post-RRP (projected was N=64 as proposed), and so with the proper exclusion criteria, only N=11 was available. We intend to acquire the remaining 53 plasmas from other biobanks (Princess Margaret Hospital GU Biobank) with REB ethics already in hand. However, we do have N=36 in the cohort of patients with prostate cancer who have developed bone metastasis as confirmed by radiology, which is close to the target of N=48 initially projected.

Results for Subtask 1a - The %age of 1A5+ve prostate microparticles was the lowest in the age-matched healthy volunteer group out of all four groups (11.1±7.6%). However, when we analyzed Group A plasmas, we found a bimodal distribution of 1A5+ve prostate microparticles according to plasmas collected pre-RRP (Figure 1). While the majority of plasmas in Group A had low %age of 1A5+ve prostate microparticles (5%-21%), there were many plasmas that had moderate to high %ages of 1A5+ve prostate microparticles (35%-60%). All plasmas analyzed in Group B and C had high levels of 1A5+ve prostate microparticles (47.9±30.7% and 50.0±27.2% respectively, mean±SD), which was expected based on our preliminary data for this proposal (Figure 1). Figure 2 is a representative plasma analyzed by nanoscale flow cytometry for the 1A5-FITC and PSMA-RPE markers. The right panel reveals the co-expression of the two markers in contrast to the single population that only binds the PSMA mAb (below red gate). The left panel represents all the events that are dual positive and gated into a sizing histoplot, revealing the sizes of the 1A5+ve microparticles in the sample.

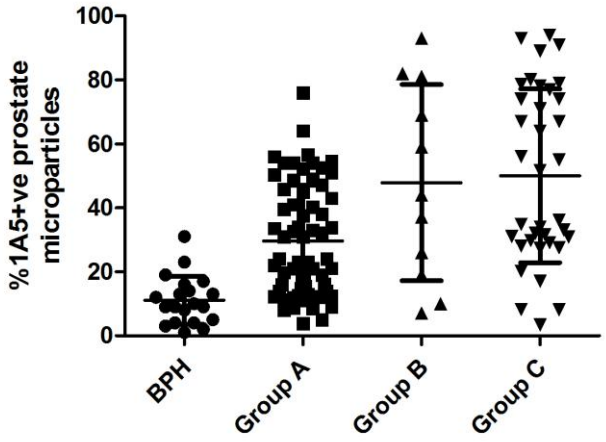

Figure 1. Proportion of prostate microparticles that bind 1A5 mAb. BPH represents age matched patients with enlarged prostates but no prostate cancer. Group A represents baseline plasmas from patients prior to RRP and have not progressed to metastatic disease within a 4 year period. Group B represents plasmas from patients who have undergone RRP and have progressed to metastatic disease within a 4 year period. Group C represents plasmas from patients with bone metastases.

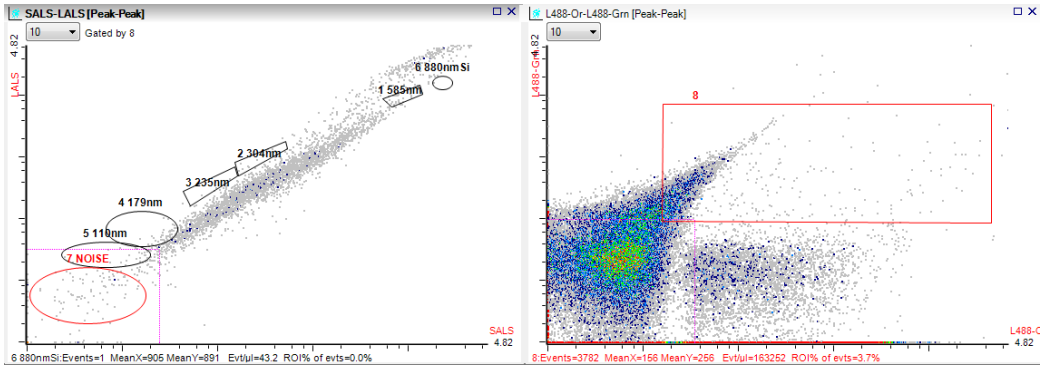

Figure 2. Nanoscale flow cytometry of prostate cancer microparticles in plasma from a metastatic prostate cancer patient. The panel on the left represents the size range of dual positive microparticles (Y axis:1A5-FITC, X axis: PSMA-RPE) as gated in red in the right panel. At least 250,000 events were analyzed. The gates in the left panel represent the location of different sizing beads.

Subtask 1b. *Immunohistochemistry will be performed on prostate tissue biopsy sections with the corresponding plasma samples analyzed in Subtask 1a. There are a total of 186 tissue sections (there are 20 volunteer plasmas that will not have accompanying tissue biopsies) and these will be stained with the 1A5 mAb to assess CD151+ve status. These sections will be stained and imaged in a double-blinded manner. Milestones will be the completion of 100 tissue sections by month 2 and the completion of all tissue sections by month 4.*
We have acquired 97 sets of sections that are paired to the plasmas we have already analyzed in subtask 1a although we do not currently have sufficient N's for group B.
Results for Subtask 1b - Due to the results observed in Group A, we have elected not to perform immunohistochemistry staining with the 1A5 mAb because of the large %age of patients in this group that exhibit high %age of 1A5+ve prostate microparticles (bimodal distribution). This finding was not anticipated because we had hypothesized that these patients that did not progress over a 4 year followup would exhibit a low %age of1A5+ve prostate cancer microparticles. Since this was not observed, performing the immunostains was not a priority because the 1A5 mAb is a valuable and limited resource. This step was initially proposed to confirm the possibility of low 1A5 status in prostate cancer lesions as predicted in their plasmas. We address future directions in the final section of this Body section.

Subtask 1c. *Statistical analysis of data accumulated during Task 1. The last month (month 6) will be spent with Mr. Larry Stitt, our statistician, and all statistical analyses will be completed by month 6. If additional sample analysis is required, we have set aside one additional month for analysis of plasma samples and tissue sections.*
These analyses have not been performed by Mr. Stitt because this final report only summarizes progress and results for the first 3 months of the fellowship whereas this subtask 1c was due for completion in month 6.

Task 2. Correlation of CD151-positive prostate cancer microparticles to circulating tumour cells in patient whole blood.
(timeframe, months 1-24). This objective will compare the ability of CD151+ve microparticles versus CTCs to predict the onset of metastasis in prostate cancer patients when first diagnosed at the LRCP. All ethics regarding the recruitment and analysis of human specimens and clinical data have been approved by the University of Western Ontario, and the London Health Sciences Centre at LRCP. Patients are already being recruited into the two patient cohorts and the analyses being described in Subtask 2a and 2b have been performed.
Subtask 2a. *Longitudinal collection of whole blood samples from patient cohorts will occur continuously over the entire 2 year period and beyond if necessary.*
Collection of whole blood in a prospective manner has not been an issue thus far for the first three months of this study (Sept-Nov).

Subtask 2b. *CellSearch™ instrument training will occur for the first 1 month. During this time, personnel from the Dr. Allan laboratory (Dr. Ben Hedley and Mr. David Goodale) will perform CTC analysis from patient whole blood until the PI (Dr. Hon Leong) attains instrument competency. Meanwhile, 0.5 mL of whole blood will be processed and the plasma will be analyzed by flow cytometry by the PI. After training, CTC enumeration by CellSearch analysis will occur for the next 24 months until blood collection is halted.*

For the first 3 months, David Goodale has been performing the CTC analysis for all baseline whole bloods collected (N=6 for Cohort A, patients post-RRP and N=4 for Cohort B, patients with no history of RRP and have progressed to metastatic prostate cancer).

Results for Subtask 2b - As seen in Figure 3, we only have baseline measurements for plasmas from recruited patients, but we already observe that most of the whole bloods analyzed from patients post-RRP have zero CTC counts, whereas the whole bloods from patients with metastatic disease have CTC counts >2.

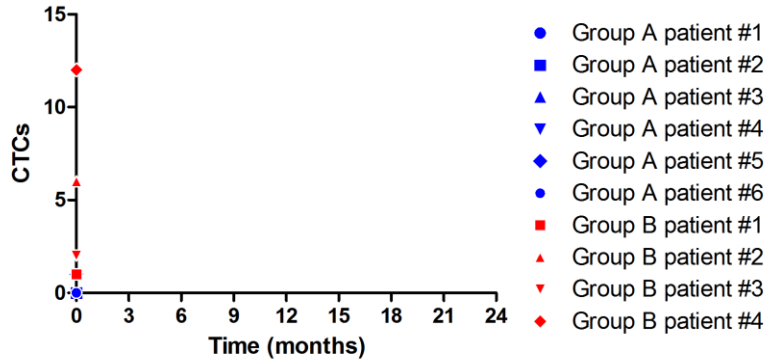

Figure 3. Enumeration of circulating tumor cells (CTCs) by CellSearch Instrument. Group A represents whole blood analyzed from post-RRP patients at baseline. Group B represents whole blood analyzed from metastatic prostate cancer patients.

Subtask 2c. *Enumeration of CD151+ve PCMPs by flow cytometry will occur for the next 24 months until blood collection is halted. Patient endpoints are: biochemical failure, confirmed metastasis, death.*

This subtask is unique because we are performing serial analyses of whole blood from patients collected every three months. However, due to the 3 month period of progress in this final report, we have only collected baseline samples of 6 patients that have recently had an RRP procedure and 4 patients that have castration resistant metastatic disease and are currently on chemotherapy over this 3 month period. We will continue to follow these patients and collect blood for every visit they make (typically every 3 or 6 months).

Results for Subtask 2c - As seen in Figure 4, we only have baseline measurements for plasmas from recruited patients. With a single timepoint, this data is not interpretable without more time points. We will continue this blood collection and analysis.

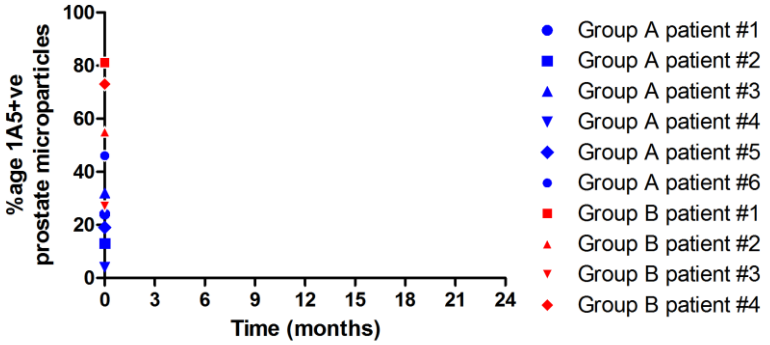

Figure 4. Proportion of prostate cancer microparticles that bind 1A5 mAb. Group A represents whole blood analyzed from post-RRP patients at baseline. Group B represents whole blood analyzed from metastatic prostate cancer patients.

Subtask 2d. *Statistical analysis of data accumulated during Task 2. The last 2 months (month 23-24) will be spent with Mr. Larry Stitt, our statistician, and all statistical analyses will be completed by month 24. In terms of milestones for this objective, the uro-oncology fellows have already recruited patients into the two patient cohorts: patients that have undergone RPP, and*

patients that have not undergone RPP and have confirmed metastasis (N=12 and N=5 respectively). CTC enumeration has already been performed on these 17 patients in a longitudinal manner since March 2011. We expect to achieve full recruitment of patients into each cohort by the end of the year. This research proposal is expected to yield at least one publication that describes the relationship of cancer microparticle counts in prostate cancer patients with cancer progression to metastasis. This paper will be submitted by month 26 or later to a high-profile clinical oncology publication.
There is no analysis provided in this report because this final reporting period only covers the first 3 months of this fellowship while this subtask will require data compiled from a 2 year period.

Key Research Accomplishments:

Due to the short timeframe of research performed (September 1, 2012 to November 31, 2012), there are no major key research accomplishments to report. However, we are committed to completing the study because of the patients recruited in Subtasks 2b-c and will be receiving another 40 patient plasmas for group C in Subtask 1a-b.

Reportable Outcomes:

Manuscripts, abstracts, presentations - none

Degrees obtained that are supported by this award - Not applicable

Development of cell lines, tissue or serum repositories - We have accumulated 155 plasma samples and 97 tissue sections within the first 3 months of this project. These plasmas and sections are currently stored in a -80°C freezer.

Informatics (Databases) - As part of the plasma and sections provided by OICR tumor bank, we have also received an excel spreadsheet of the anonymized samples which contains all clinical information within a 7 year followup period.

Funding applied for based on work supported by this award - Prostate Cancer Canada Movember Pilot Grant awarded in July 2012. Title: Non-invasive staging of prostate cancer: detection of circulating prostate microparticles using unique metastasis-specific antibody 1A5.

Employment opportunities recieved based on training by this award - I have been awarded a salary and operating grant for unrelated work but this training award did provide invaluable experience in terms of performing clinical/translational research.
http://www.lhsc.on.ca/About_Us/LHSC/Publications/Homepage/Prostate-Cancer-Research.htm

Conclusion:

While it is too early to make definitive conclusions on the effectiveness of the 1A5 mAb to distinguish patients at risk of progressing to metastatic disease, we are able to demonstrate that 1A5+ve prostate microparticles are abundant in a subpopulation of localized PCa patients that

did not progress within 4 years of RRP. This was not expected and has prompted us to realize that this cohort of patients (Group A) are actually candidates for medium to high risk prostate cancer and thus eligible for radical treatment. In retrospect, such patients eligible for radical treatment are the wrong patient cohort to be evaluating the 1A5 mAb. Hence, this localized PCa cohort was not the group that would have resulted in a low %1A5+ve prostate cancer microparticle result and that the cohort we should have assembled would be patients that are considered low-risk and on active surveillance. We are now recruiting patients on active surveillance to determine the %1A5+ve prostate cancer microparticle profile in this more homogenous patient population. This patient cohort is of the highest priority for our laboratory.

"So what" - Although the 1A5 mAb was immunoreactive for prostate microparticles in pre-RRP patient plasmas, we may have selected the wrong cohort for "low-risk" indolent cancer. Therefore, we will set our sights on analyzing plasmas from patients on active surveillance, which is now the ideal cohort for determining which patients will progress and be submitted to RRP.

References: none since there have been no publications arising from this work as of yet.

Appendices: none