AWARD NUMBER: W81XWH-17-1-0471

TITLE: Detection and Characterization of Treatment Emergent Neuroendocrine Prostate Cancer Using Hyperpolarized 13C Magnetic Resonance

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REPORT DATE: September 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| September 2018 | ANNUAL REPORT | 15 Aug 2017 - 14 Aug 2018 |

**4. TITLE AND SUBTITLE**

Detection and Characterization of Treatment Emergent Neuroendocrine Prostate Cancer Using Hyperpolarized 13C Magnetic Resonance

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-17-1-0471

**5c. PROGRAM ELEMENT NUMBER**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

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San Francisco, CA, 94158-2330

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The goal of this proposal is to discriminate biologically divergent treatment-emergent neuroendocrine prostate cancer (NEPC) from advanced adenocarcinoma based on the metabolic profile of NEPC tumors and to use real-time changes in metabolism to monitor therapeutic efficacy by using novel hyperpolarized (HP) 13C metabolic imaging techniques. In the 1st year of funding, our major activities focused on collating preliminary studies for Aims 1 and 2 to establish feasibility. To this end for Aim 1, the probes (glutamine and acetate) were optimized for hyperpolarization technique and tested in cells and baseline TRMAP studies. Correlative measures of NEPC presence were developed using ELISA and serum enzyme activities in addition to IHC quantification. Furthermore preliminary metabolic profiling of TRMAP tumors showed altered substrate utilization with the onset of CRPC further strengthening our hypothesis of metabolic adaptations with treatment resistance. For Aim2, we did exhaustive feasibility of labeling studies in biopsy samples, and for the first time showed viability and detected ex vivo measures of glycolytic activity.

**15. SUBJECT TERMS**
Hyperpolarized 13C MRI, pyruvate, treatment emergent neuroendocrine prostate cancer, metabolism

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | | 19b. TELEPHONE NUMBER *(include area code)* |

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## 1. INTRODUCTION

The goal of this proposal is to discriminate biologically divergent treatment-emergent neuroendocrine prostate cancer (NEPC) from advanced adenocarcinoma based on the metabolic profile of NEPC tumors and to use real-time changes in metabolism to monitor therapeutic efficacy by using novel hyperpolarized (HP) $^{13}C$ metabolic imaging techniques in order to develop a clinically translatable non-invasive imaging tool for early detection and appropriate therapeutic intervention. There is no current imaging or other diagnostic tools available to non-invasively detect NEPC. The successful completion of this project will confirm the utility of HP lactate production as a metabolic biomarker of NEPC and investigate the utility of acetyl CoA and glutamate as novel more specific biomarkers of NEPC, allowing for these biomarkers to be synergistically utilized to more accurately measure the occurrence of neuroendocrine dedifferentiation and the response of NEPC to therapy.

## 2. KEYWORDS

Hyperpolarized $^{13}C$ magnetic resonance imaging, pyruvate, lactate, metabolism, treatment emergent neuroendocrine prostate cancer, biomarkers, therapeutic resistance, castrate-resistant prostate cancer.

## 3. ACCOMPLISHMENTS

The major goal of Specific Aim 1: is to establish the molecular and metabolic signature of t-NEPC tumors and develop new HP $^{13}C$ labeled probes to identify neuroendocrine differentiation and its response to therapy. As a first steps towards this goal the following was accomplished

*3.1.a Optimization of HP [5-$^{13}C$]glutamine and [1-$^{13}C$]acetate*

[1-$^{13}C$]Pyruvate is prepared routinely and is available through our core imaging facility after being thoroughly quality controlled for each batch preparation to yield a $T_1$ of 48s (at 11.7T) and 22% polarization.

The acetate probe was prepared for polarization as detailed by Flori et al (PMID: 25201079). We achieved similar polarization and a $T_1$ of 56s on the 11.4T system. We then infused 50mM of the hyperpolarized [1-$^{13}C$]acetate in the two TRAMP mice at baseline (1 week after castration) and (n=1) of one of the mice after 1week on ARN509 (group II NEPC phenotype) and did not observe any metabolic byproducts. We will repeat these experiments with more group II (NEPC) cohort to further test its usefulness in that disease phenotype.

We did a heads-up comparison of the different glutamine preparations published thus far (see table below) and found that the use of water and NaOH yielded the highest polarization and concentration. Although we were able to maintain the impurity profile (pyroglutamate, pGlu, and glutamate, glu) of the preparation low and steady over many days, we were not able to eliminate the metabolite of interest (glu) that is already present along with the precursor (glutamine, gln) during the preparation for hyperpolarization. At 3T where the imaging of the TRAMP mice is conducted, the homogeneity does not allow resolution of the pGlu and Glu peaks as they are only 0.5ppm apart, making it difficult to discern any “new” formation of Glu in the tumor. However, we will try in a few preliminary TAMP tumors to assess this thoroughly.

| Prep | [Gln] (M) | % Gln | % Glu | % pGlu | Gln T1 (s) | % polarization | n |
|---|---|---|---|---|---|---|---|
| Gln in glycerol-$H_2O$[1] | 0.245 | 98.0 | 0.0 | 2.0 | 15.4 | 5.7 | 1 |
| Gln in DMSO-CsOH[2] | 2.7 | 98.7 ± 0.0 | 0.1 ± 0.0 | 1.2 ± 0.0 | 16.5 ± 0.5 | 19.8 ± 8.2 | 2 |
| Gln in $H_2O$-NaOH | 4 | 91.1 ± 4.4 | 2.1 ± 1.1 | 6.8 ± 3.5 | 18.0 ± 1.3 | 18.3 ± 3.0 | 7 |
| Same as above with radical addition before HP experiment[3] | 4 | 50 | 24 | 26 | 13.19 | 15.5 | 1 |
| Dt-Gln in H2O-NaOH[4] | 4 | 82.7 ± 4.1 | 10.7 ± 2.5 | 6.5 ± 1.8 | 21.3 ± 1.2 | 10.5 ± 1.3 | 4 |

*3.1.b Preliminary TRAMP neuroendocrine tumor phenotype establishment and its assessment using biochemical assays and immunohistochemical staining quantification.*

We found that the protracted treatment of 2 weeks of ARN-509 was rather devastating on the mice and significantly impacted the mice survival. The 2-week timeframe was based on our initial data to achieve maximal neuroendocrine tumor burden. We are conducting preliminary work to assess NEPC burden with only one week of ARN-509 treatment. While clinical serum neuroendocrine markers are used for selectivity and (not specificity) of the presence of neuroendocrine disease, there is no pre-clinical equivalents other than pathologic staining. We investigated the use of neuron specific enolase (NSE) enzyme activity of the mouse serum and compared it to the immunohistochemical staining of NSE and synaptophysinA in the TRAMP

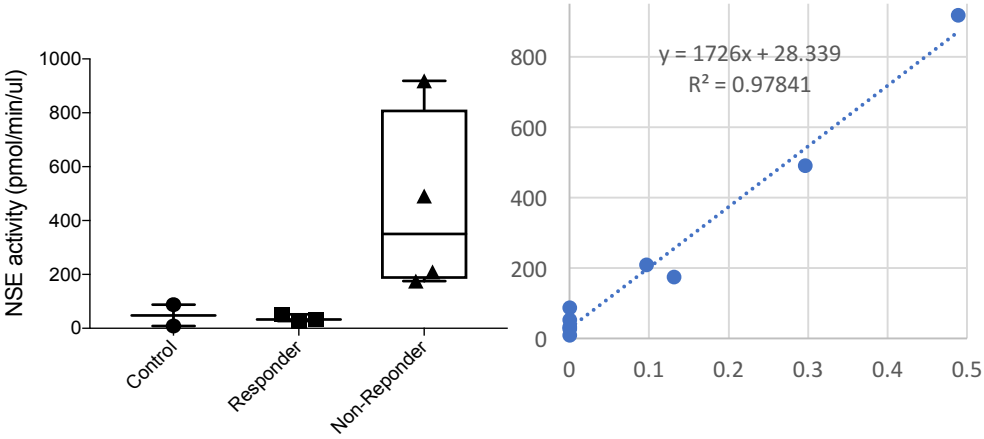



**Figure. 1:** The bar graph denotes increase in serum NSE activity with development of CRPC and the line graph shows the strong correlation between the serum NSE activity and mean NEPC staining.

tumors. In this process we also established a quantitative image analysis tool to quantify %

[1] Gallagher, F. A., Kettunen, M. I., Day, S. E., Lerche, M., & Brindle, K. M. (2008). 13C MR spectroscopy measurements of glutaminase activity in human hepatocellular carcinoma cells using hyperpolarized 13C-labeled glutamine. *Magnetic Resonance in Medicine : Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *60*(2), 253–257. http://doi.org/10.1002/mrm.21650

[2] Cabella, C., Karlsson, M., Canapè, C., Catanzaro, G., Colombo Serra, S., Miragoli, L., et al. (2013). In vivo and in vitro liver cancer metabolism observed with hyperpolarized [5-13C]glutamine. *Journal of Magnetic Resonance*, *232*, 45–52. http://doi.org/10.1016/j.jmr.2013.04.010

[3] Salamanca-Cardona, L., Shah, H., Poot, A. J., Correa, F. M., Di Gialleonardo, V., Lui, H., et al. (2017). In Vivo Imaging of Glutamine Metabolism to the Oncometabolite 2-Hydroxyglutarate in IDH1/2 Mutant Tumors. *Cell Metabolism*. http://doi.org/10.1016/j.cmet.2017.10.001

[4] Qu, W., Zha, Z., Lieberman, B. P., Mancuso, A., Stetz, M., Rizzi, R., et al. (2011). Facile Synthesis [5-13C-4-2H2]-L-Glutamine for Hyperpolarized MRS Imaging of Cancer Cell Metabolism. *Academic Radiology*, *18*(8), 932–939. http://doi.org/10.1016/j.acra.2011.05.002

neuroendocrine staining. Preliminary results suggest a very strong correlation between the serum NSE activity and the estimated % NEPC. We are targeting a 60% neuroendocrine staining prevalence for the groupIII NEPC mice.

*3.1.c Metabolic profiling of adenocarcinoma and CRPC TRAMP tumors*

We have performed [U-$^{13}$C]glucose labeling studies on a cohort of TRAMP mice (n=3 each) as well as infusion of [U-$^{13}$C]glutamine (n=1 each) , who did (androgen sensitive) and did not respond (androgen insensitive or CRPC) to primary AR inhibition (**Figure 1**). The representative spectrum from the tumors labeled with [U-$^{13}$C] glucose clearly shows an increase in flux to lactate, alanine and glutamate pools with CRPC. The graphs show the fractional enrichment (ratio of the concentrations of the $^{13}$C enriched metabolite to the total pool). And with glucose labeling, the flux to lactate and alanine are increased in CRPC alongside the TCA intermediates aspartate and glutamate. While the glutamine infusion produced similar trends (n=1) each, the magnitude of the change between adenocarcinoma and CRPC is smaller in comparison to glucose infusion. This seems to suggest that hyperpolarized C2- labeled pyruvate might yield a large change in glutamate signal levels rather than hyperpolarized glutamine.

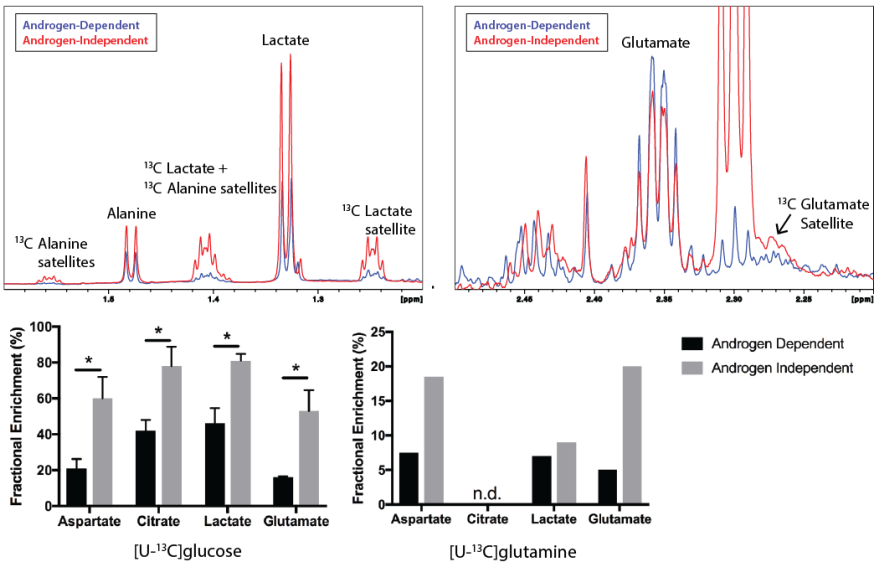
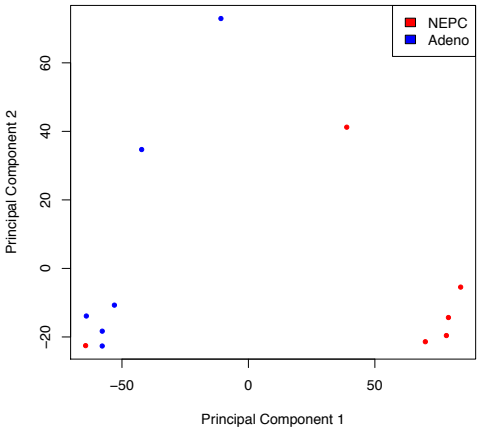



**Figure 2.** Top row shows the spectra overlaid from androgen dependent and independent TRAMP tumors and the bottom bar graphs shows the fractional enrichment of the different metabolites in the two TRMAP tumors.




*3.1.e Preliminary genetic profiling of adenocarcinoma and NEPC TRAMP tumors*

6 TRAMP tumors for NEPC and CRPC from the preliminary imaging data were processed in a whole mouse genome array and found significant upregulation of ChromograninB in the NEPC cohort (along with other neuroendocrine components along with glutamate and GABA receptors). And principal component analysis (image on left) shows an initial stratification in the genes between the two groups (NEPC vs CRPC) (with one NEPC tumor overlapping with the adenocarcinoma group).

*3.2 Estimating metabolic fluxes in patient derived prostate biopsy samples*

In Aim 2, we proposed to define the molecular and metabolic signature of metastatic NEPC tumors from patient biopsy samples. Therein we also proposed to quantitatively capture the spectrum of **metabolic fluxes** associated with NEPC. Such an endeavor has never been published thus far and in order to optimize the procedures, this was undertaken in primary

prostate biopsies (rather than the metastatic biopsies from the WCDT cohort, which is of limited availability and shared over a large number of researchers). The initial results are very encouraging and shows not only increased intracellular labeling, but also efflux of lactate in malignant compared to benign prostate biopsy tissue, while reaffirming our earlier observation of higher total lactate pool in the same.

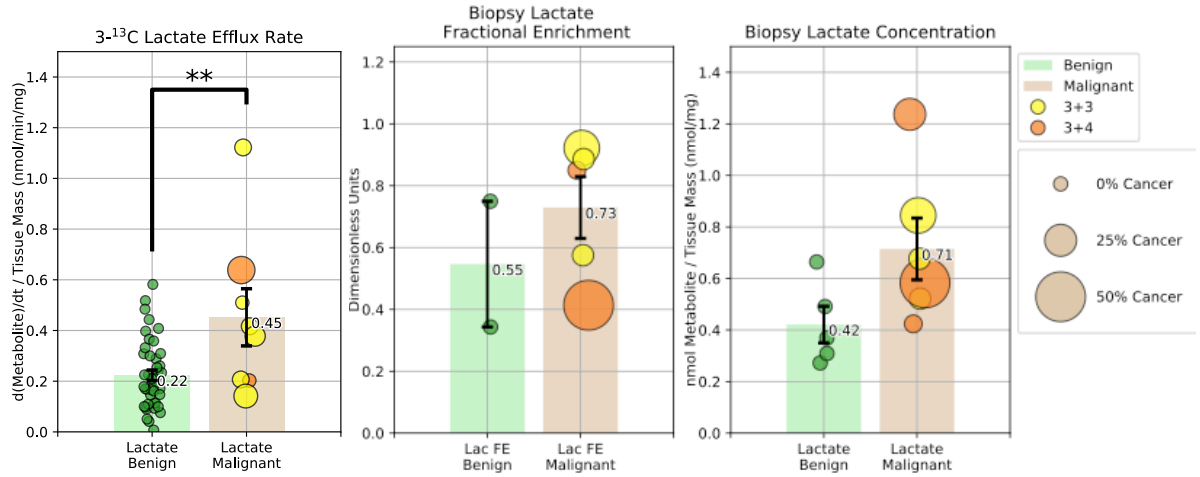



**Figure 3. Labeling of biopsy tissues.** The left graph demonstrates that the lactate efflux rate is higher in malignant prostate tissue as measured by lactate concentration in the media in which the biopsy tissue was cultured. The middle graph shows that the intracellular lactate flux is also higher in cancer tissues and reaffirms our previous observations of increased lactate concentration.

The preliminary work of metabolic profiling of TRAMP tumors as well as determining fluxes in biopsy samples were presented in Experimental NMR Conference held in Orlando, Florida in May 2018 by graduate students Jinny Sun and Jeremy Bancroft Brown respectively (see appendix)

In the next reporting period, we hope to start generating data using the HP $^{13}$C probes as well as the metabolic genetic profiling of the TRMAP tumors with the NEPC phenotype. And for Aim 2 under the auspices of Dr. Agarwal (the co-Pi) on this grant, access the WCDT biopsy samples for metabolic profiling using HR-MAS NMR technique.

## 4. IMPACT

The biggest impact from the preliminary studies thus far, has been

i. A rational approach to developing hyperpolarized biomarkers. For example, using the metabolomics data form the TRAMP labeling studies, its seems that using HP pyruvate may show a larger % change in glutamate signal than when using HP glutamine and maybe a better alternative, considering the poor performance in terms of polarization and T1 characteristics of the latter probe.

ii. We have shown for the first time, that it is feasible to keep biopsy tissue viable and measure the glycolytic process *in situ*. We have developed methodology for the same that can be used to optimize such measures for other tissues. To facilitate this, detailed procedures will be published in a manuscript shortly.

There is nothing significant to report with regards to technology transfer and impact to society beyond science and technology.

## 5. CHANGES/PROBLEMS

It seems from our preliminary data, that hyperpolarized [2-$^{13}$C] pyruvate might be a better marker of NEPC disease than hyperpolarized glutamine. Initial studies for imaging will also be conducted using this probe in addition to the ones already prescribed.

## 6. PRODUCTS

Nothing to report

## 7. PARTICIPANTS

| | |
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| Funding Support: | |

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| Funding Support: | |

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| Nearest person month worked: | *12* |
| Contribution to Project: | *Metabolomics and biopsy labelling studies respectively* |
| Funding Support: | Graduate program |

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| Funding Support: | |

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| Funding Support: | |

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| Researcher Identifier (e.g. ORCID ID): | |
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| Contribution to Project: | *Neuroendocrine assays and IHC quantification* |
| Funding Support: | |

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  *"Nothing to Report."*

- **What other organizations were involved as partners?**

  *"Nothing to Report."*