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Progress Report	☐ 6 Month Report ☒ Year 1 Report ☐ Year 2 Report ☐ Year 3 Report	☒ Final Report ☐ No-cost Extension

Award Name: 2019 #cureMEdullo powered by Carson Leslie Foundation Young Investigator Award in Medulloblastoma Research

Supported By: Carson Leslie Foundation/CureMedullo

Project Title: Cerebrospinal fluid derived circulating tumor DNA as an actionable biomarker in medulloblastoma

Grant Award Term:	7/1/2019-6/30/2020
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Mentor:	**Additional Mentor or Sponsor:**
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Human Use? No **Assurance Status:** **Assurance Number:** **IRB Expiration Date:**	**Animal Use?** No **Assurance Status:** **Assurance Number:** **IACUC Expiration Date:**
ClinicalTrials.gov Identifier:	
Budget:	$50,000
Notes:	

This report has been reviewed and approved by: Kavita Bhalla, PhD, Scientific Reviewer	Date: 9/30/2020

Title: Cerebrospinal fluid derived circulating tumor DNA as an actionable biomarker in medulloblastoma

Description of Reporting Period Activities

1. Study Background and Aims

Medulloblastoma is among the most common malignant brain tumors in young children. Despite advances in management and research, 30-40% of medulloblastoma patients fail conventional treatment and ultimately relapse. Efforts to improve patient outcome are hindered by the lack of sensitive biomarkers to complement radiographic monitoring, to detect early disease relapse, and to track clonal evolution.

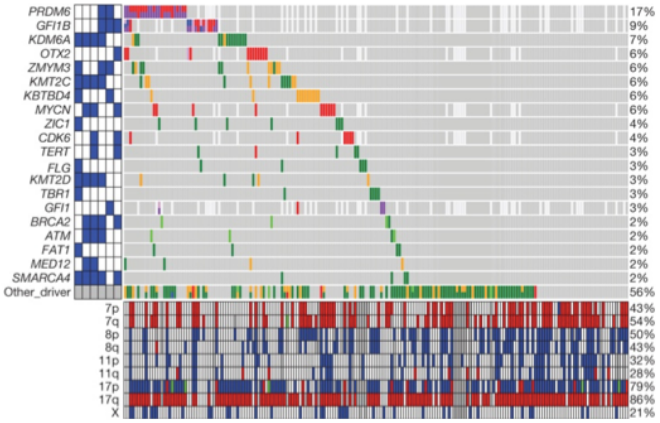

Fig. 1. Oncoprint showing recurrent genetic alterations and frequent chromosomal copy number gains (red) and losses (blue) in Group 4 medulloblastoma (Northcott et al., Nature 2017).

In recent years, there has been significant progress in the use of tumor-derived cell-free DNA (cfDNA) as a biomarker for patients with brain tumors. While studies have demonstrated feasibility in detecting and molecularly profiling cfDNA, published reports have been largely limited to patients with high-grade glioma and application of targeted sequencing approaches. In medulloblastoma, there is a lack of highly recurrent driver gene mutations. In contrast, medulloblastoma genomes are typified by frequent chromosomal alterations and focal copy number variation (CNVs) (Fig. 1). We hypothesize that cell-free DNA can be detected in cerebrospinal fluid (CSF) collected from children with medulloblastoma and genomic profiling of such, primarily through low-coverage whole-genome sequencing (lcWGS) for inferring tumor-specific CNVs, will be informative as a personalized biomarker to reflect treatment response and predict relapse. In addition, we hypothesize that evolution of somatic genomic alterations during disease course will be captured by serial profiling of cfDNA derived from CSF. To test the above hypotheses, we plan to analyze the longitudinally banked CSF samples from patients with medulloblastoma enrolled on two completed prospective trials (SJMB03/SJYC07) and correlate such findings with genomic profiles of primary tumors, clinical features and outcome.

Specific aims:

1. We will investigate the feasibility detecting tumor-specific alterations in CSF derived cfDNA from patients with medulloblastoma with next generation sequencing (NGS)
2. We will evaluate the correlation between detectability of tumor-specific alterations in CSF derived cfDNA and disease burden (metastasis vs. no metastasis)/course (relapse vs. no relapse)
3. We will evaluate the correlation between quantity of CSF derived cfDNA and disease burden/course (metastasis vs. no metastasis)/course (relapse vs. no relapse)
4. We will compare somatic alterations in cfDNA derived from serial CSF samples in patients with relapse for possible clonal evolution

With support of the Conquer Cancer – #cureMEdullo powered by Carson Leslie Foundation Young Investigator Award, I was able to execute planned experiments to address the above specific aims over the past 12 months, detailed as follows.

2. Studies and Results

I launched and spearheaded this transdisciplinary project under the supervision of Dr Paul Northcott from Department of Developmental Neurobiology, and Dr Giles Robinson from Division of Neuro-Oncology. I received support from members of the Northcott Lab, Rahul Kumar (PhD Student) and Kyle Smith (Bioinformatics Research Specialist), as well as collaborators from within St. Jude Children's Research Hospital and outside of our institution. Funding was allocated for purchase of relevant reagents and NGS. Following initial proof-of-concept results generated during the first six-month of the project, I have expanded the study to include 144 patients with medulloblastoma enrolled on SJMB03 (n=109) and SJYC07 (n=35). Progress and findings according to the predefined specific aims are described below.

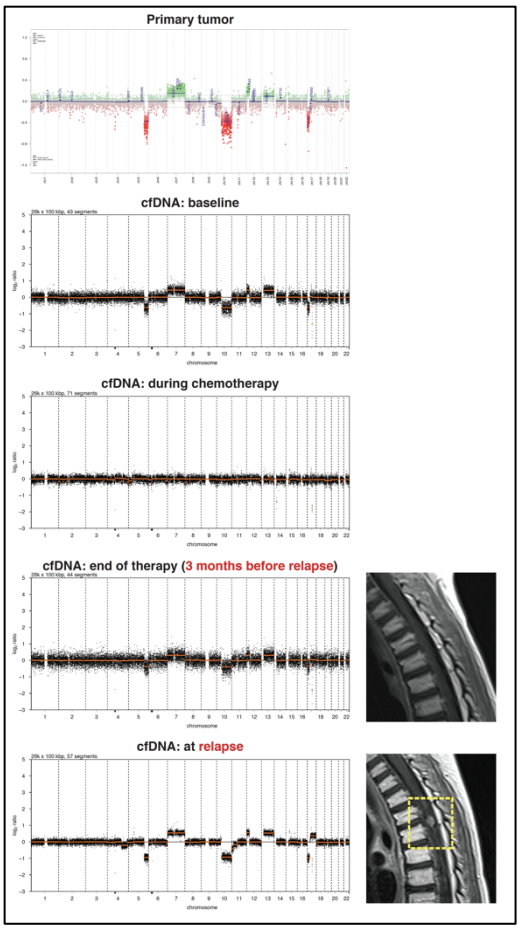

Fig. 2. Comparison of genome-wide copy number profiles from primary tumor (upper panel), and serial cfDNA samples in a patient with Group 3 medulloblastoma. Somatic CNVs resolve with treatment but re-emerge 3 months before radiographic relapse (yellow bracket).

Specific Aim 1: *To investigate the feasibility of detecting tumor-specific alterations in CSF derived cfDNA from patients with medulloblastoma*, cfDNA was extracted from supernatants of pre-centrifuged CSF samples obtained at baseline (NucleoSnap DNA Plasma kit, Macherey-Nagel). The extracted cfDNA was quantified by qPCR and up to 2ng of cfDNA was used for preparation of NGS libraries, using the Accel-NGS 2S Hyb library kit (Swift Bioscience). NGS libraries were successfully generated from all baseline cfDNA samples. lcWGS targeting 3x mean coverage was then performed for each sample. A computational framework for generation of CNV plots and automated calling of large-scale CNVs was devised (cfdna v0.1.0) (Fig. 2). Broad and focal CNVs were called by the above pipeline and verified by manual inspection. CNV profiles of cfDNA based on lcWGS are compared with those from the corresponding primary tumors derived from methylation arrays. The detectability of tumor-specific CNVs, implicating the presence of tumor-derived cfDNA, was annotated accordingly. Despite the low cfDNA yield (median=1ng/ml of CSF, 50pg-2ng used for library preparation), we were able to detect tumor-specific CNVs from 71% of cfDNA samples collected at baseline. This confirms feasibility of profiling CSF-derived cfDNA for children with medulloblastoma using our study pipeline.

Specific Aim 2: *To evaluate the correlation between detectability of tumor-specific alterations in CSF derived cfDNA and disease burden*, we compared the detectability of tumor-specific CNV in baseline CSF samples and the metastatic status in patients. We observed a significantly higher detection rate in patients with metastatic disease (91%, high disease burden), than in those with localized disease (50%, low disease burden) (Fisher's exact, $p<0.001$). Such correlation confirmed the observation from our pilot data and supports the role of cfDNA profiling as a surrogate for tumor burden. Longitudinal CSF samples throughout treatment and during follow-up were then profiled for a subset of patients who were treated on a risk-stratified manner on SJMB03 (n=81). Tumor-specific CNVs were observed to resolve with treatment in patients who remained in remission, and either persisted or re-emerged in those who had subsequent

progression (Fig. 3). In ~50% of patients who achieved disease remission before relapse, we were able to detect CNVs in cfDNA samples collected > 3 months before radiographic progression. Furthermore, the detectability of CNV in cfDNA samples collected either after the radiation-phase of treatment, or at end of therapy readily stratified the progression-free and overall-survival of patients, either as univariate analysis or in a multivariable model when disease risk was taken into account (Fig. 4). These results provide evidence for the correlation between findings from cfDNA profiling and disease burden in patients beyond the resolution of imaging.

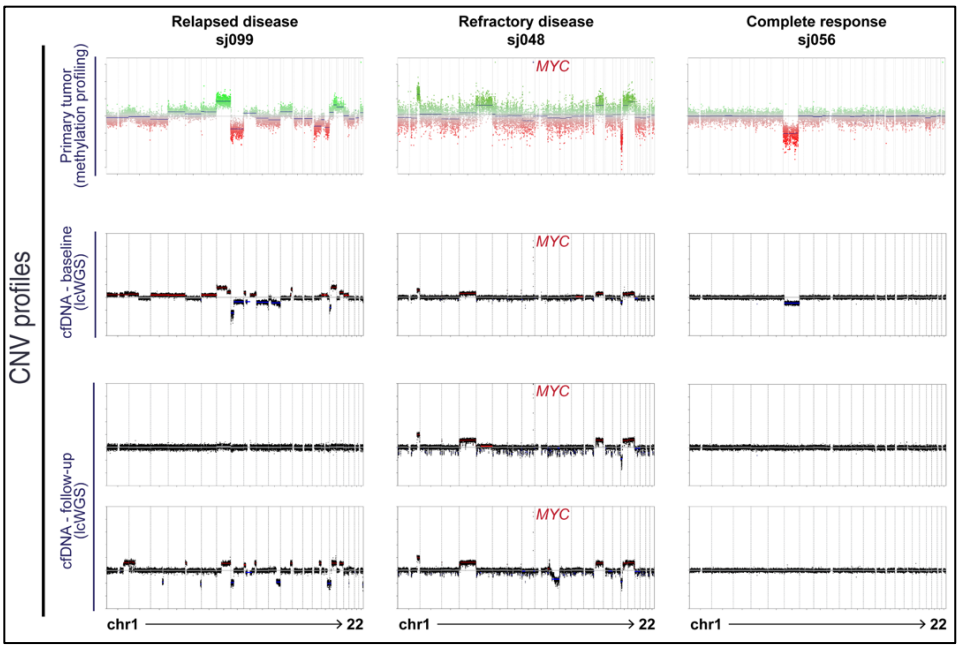

Fig. 3. Tumor-specific CNV detectability reflects disease course. Exemplary data comparing copy-number profile of primary tumor, and longitudinal cfDNA samples in three patients with disease relapse after initial response, persistent refractory disease, and complete response.

Specific Aim 3. *To evaluate the correlation between quantity of CSF derived cfDNA and disease burden/course*, cfDNA was quantified using qPCR based on primers against *ALU* sequence. Although significantly higher quantities of cfDNA was detected in CSF samples (baseline and follow-up samples) with CNV detected (median cfDNA concentration 0.8 vs 0.37ng/ml of CSF, Wilcoxon p=0.003); such difference was subtle and did not correlate significantly with disease burden or clinical course. Such lack of correlation was likely due to the inadvertent inclusion of genomic DNA that could not be discerned from the cell-free fragments during quantification.

Specific Aim 4. *To demonstrate clonal evolution in patients with medulloblastoma who relapsed or progressed*, we compared the CNV profiles obtained from cfDNA extracted from CSF samples at baseline and relapse/progression. In addition to CNVs that were shared between the sample pairs, we also observed CNVs that were divergent and specific to either the diagnostic, or relapse samples. Loss of 10q, for example, appeared to be a recurrent relapse-specific event. Emergence of focal amplicons at relapse were also anecdotally observed (Fig. 5). Such observations provide evidence for clonal selection with treatment in patients with recurrent medulloblastoma based on a non-invasive, liquid biopsy approach.

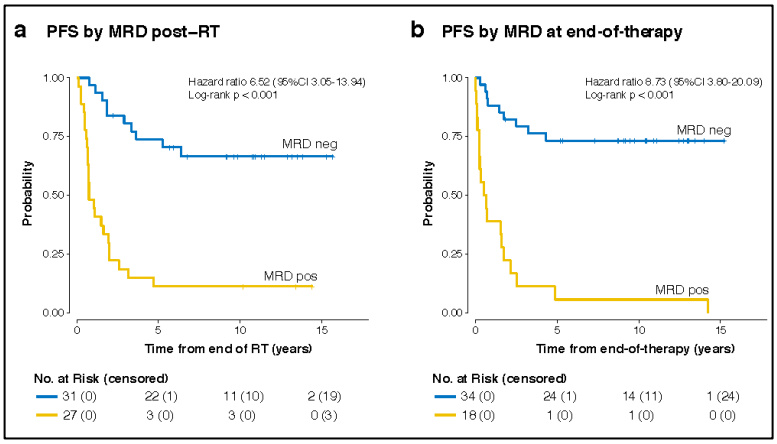

Fig. 4. cfDNA CNV detectability and patient outcome. Detectability of tumor-specific CNV in cfDNA samples after (a) radiation (post-RT) and (b) at completion of therapy was significantly associated with patient's progression-free survival (PFS)

3. Significance and Clinical Impact of the research

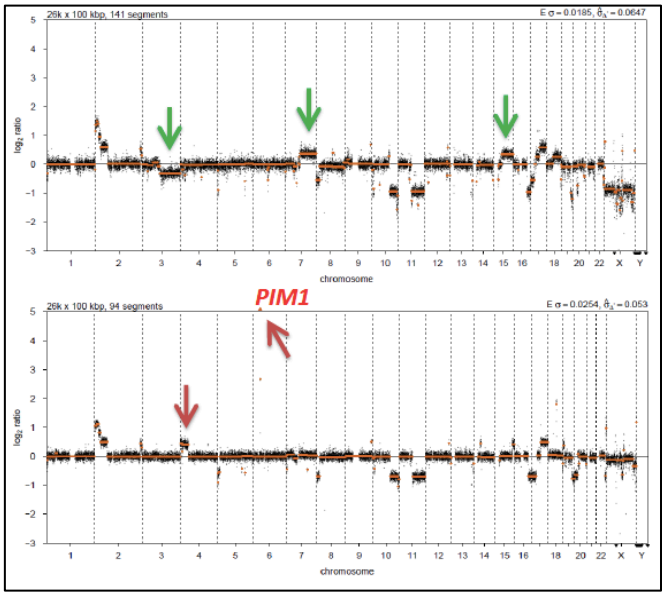

Fig. 5. Copy-number profiles derived from cfDNA at baseline (upper panel) and relapse (lower panel) from a patient with Group 3 medulloblastoma. Divergent CNVs are indicated by green (baseline-specific) and red arrows (relapse-specific), respectively. The relapse sample acquired focal amplification of *PIM1,* a serine/threonine kinase implicated in progression of prostate and breast cancer.

The approach to managing patients with medulloblastoma has remained largely unchanged over the past 20 years. Risk and treatment stratification rely primarily on specific clinico-pathological features (i.e. extent of resection, metastatic status, histopathology). Biologically and clinically relevant medulloblastoma subgroups have emerged (WNT, SHH, Group 3, and Group 4), and further heterogeneity in the form of subtypes within subgroups has recently been described. Nevertheless, there is no consensus as to how the numerous subgroups/subtypes should be integrated with other clinico-pathological risk factors to formulate a personalized yet practical treatment stratification schema. In contrast, despite the enhanced resolution of modern MRI techniques, inconclusive findings are often encountered, and recurrence might not be unambiguously diagnosed until significant tumor load is present. The evaluation of CSF-derived cfDNA as a sensitive biomarker of minimal residual disease (MRD) might play a pivotal role in treatment stratification as well as in prediction or early detection of relapse. Similar to the use of MRD guided therapy in pediatric acute lymphoblastic leukemia, we envisage that CSF-derived cfDNA may represent a sensitive biomarker of treatment response in medulloblastoma, where treatment may be personalized through intensification in those patients with persistently detectable tumor-derived cfDNA (such as inclusion of novel agents and/or autologous stem cell transplantation) and de-escalation in patients with favorable clinico-pathological findings and early molecular remission (such as dose reduction or elimination of craniospinal irradiation). Interpretation of inconclusive MRI features might be aided by results from CSF-derived cfDNA analysis and less invasive determination of tumor-specific alterations can be achieved to inform the use of targeted agents.

Current literature on the use of liquid biopsy for brain tumors remains anecdotal. Published reports have been largely restricted to universally fatal high-grade gliomas, thus preventing in-depth analysis of the prognostic value provided by liquid biopsies. Our study thus demonstrated the feasibility and clinical utility of cfDNA profiling in children with medulloblastoma. To circumvent the absence of highly recurrent trackable mutations in medulloblastoma, we made use of pervasive broad and focal CNVs as an indicator of tumor-derived cfDNA. Our experimental pipeline allowed genomic profiles to be generated from cfDNA quantities in the subnanogram range. Collectively, the outcomes of these studies are of immediate clinical relevance and MRD detection and prediction of treatment response in children with medulloblastoma. This study support prospective integration of cfDNA evaluation into medulloblastoma clinical trials such that, akin to leukemia, future medulloblastoma management will personalize therapy according to MRD response. Broadly, our findings encourage the investigation of MRD-guided therapy using CSF-derived liquid biopsies for other malignant CNS tumors in children and adults.

4. Future Plans

Validation study with an independent cohort: Our study provided robust initial data on the clinical utility of cfDNA profiling in prospectively curated cohort of patients with medulloblastoma. To validate our findings, we plan to replicate these experimental analysis based on medulloblastoma patients treated on the ongoing, risk-stratified SJMB12 study. Verification with an independent clinical cohort is often considered as a necessarily step before biomarkers could be introduced into study protocols for upfront usage where treatment decisions are based upon.

Mutational profiling of cfDNA: Within a subset of study patients with primary tumors that harbor mutations in well-defined driver genes, we will perform exome- or gene-panel based capture of the NGS libraries already prepared for the purpose of lcWGS. The relevant loci will be interrogated for each patient's cfDNA samples and mutant allele frequencies (MAFs) determined accordingly. Longitudinal trends of the MAFs will be evaluated and correlated with each patient's clinical course, and mutational profiles at baseline and progression compared. Such analysis will also allow us to incorporate the small proportion (10-15%) of medulloblastoma patients with copy-number neutral primary tumors.

cfDNA profiling for other pediatric CNS tumors: We intend to expand the scope of our research to other high-grade, pediatric CNS tumors where biomarkers are lacking and the role of cfDNA profiling is undefined. These include atypical teratoid/rhabdoid tumors, pineoblastomas and other CNS embryonal tumors. The routine collection of CSF as disease evaluation in patients with these tumors likewise allow evaluation of feasibility and utility for profiling tumor-derived cfDNA in pediatric CNS tumors other than medulloblastoma.

In all, our study laid the groundwork for follow-up translational research in the use of cfDNA profiling for medulloblastoma and other malignant CNS tumors.

Key findings. The current study provided solid data on the feasibility and clinical utility of profiling CSF-derived cfDNA in children with medulloblastoma. We demonstrated that tumor-derived cfDNA could be used as a surrogate of minimal residual disease and a prognostic biomarker in medulloblastoma. This would allow for the first time, design of a treatment strategy where regimen intensities are adapted based on disease response at the molecular level. cfDNA profiling complements imaging and CSF cytologic assessment in the detection of early relapse, and serial liquid biopsies serve as a non-invasive alternative to repeated neurosurgical procedures in evaluating the genomic profiles of relapsed medulloblastomas.

Intellectual Property. N/A.

Further Funding. St. Jude Comprehensive Cancer Center Developmental Funds; PI: Paul Northcott, PhD, Giles Robinson, Cerebrospinal fluid derived circulating tumor DNA as a biomarker of medulloblastoma relapse, Jan-Dec 2020, USD 100,000. External funding (NIH R21 grant) for a validation study applied for (PI: Paul Northcott).

Career Progress. Interim results were presented as an oral platform presentation by the Grantee at the 2019 Society for Neuro-Oncology Annual Meeting, and at the St. Jude Comprehensive Cancer Center Postdoctoral Symposium 2020. The Grantee was awarded first runner up in oral presentation in the Postdoctoral Symposium.

Human Subjects Information. N/A

Laboratory Animals Information. N/A

Multimedia. Interim findings have been shared with the Carson-Leslie Foundation for publicity and fund-raising purposes. Our study has been presented at the American Association of Neurological Surgeons 2020 meeting (presenter: Rahul Kumar) and awarded the James T. Rutka Pediatric Brain Tumor Award. It has also been accepted for presentation at the International Society of Pediatric Neuro-Oncology meeting in November 2020. We are in the process of summarizing the findings as a manuscript for submission to a top-tiered peer-reviewed journal.

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SIGNATURE PAGE

I certify that the information contained in all components of this progress report is true, complete, and accurate to the best of my knowledge.

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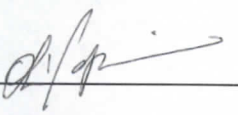
Recipient Signature: 

Date: August 31, 2020

Required for mentored grants (ie. YIA, CDA, LIFe, GO YIA)

I certify that I have reviewed and approved this progress report.

Mentor Printed Name: Paul A. Northcott

Mentor Signature: 

Date: August 31, 2020

Please attach the completed and signed signature page as a required component of your Progress Report.