RESEARCH ARTICLE

Circulating Tumor DNA Analysis Defining the Genomic Landscape of Paragangliomas and Pheochromocytomas

Lana Khalil[1], Ali F. Al Sbihi[3], Jill Tsai[2], Leylah M Drusbosky[2], Hiba I Dada[2], Bassel El-Rayes[4], Walid Shaib[5*]

[1]Department of Hematology and Oncology, Winship Cancer Institute, Emory University, Atlanta, USA

[2]Guardant Health, Redwood City, USA

[3]Department of Internal Medicine, Wayne State University/Detroit Medical Center/Sinai Grace Hospital, Detroit, USA

[4]Department of Hematology and Oncology, O'Neal Cancer Center, University of Alabama, Tuscaloosa, USA

[5]Northwest Georgia Oncology Centers Wellstar, Marietta, USA

ABSTRACT

Background: Paragangliomas (PGLs) and Pheochromocytomas (PCCs) are rare cancers. There is not standard of care treatments for these cancers. The genomic landscape of PGLs and PCCs is not reported. The aim of this study is to report the mutational difference and assess the feasibility of Next Generation Sequencing (NGS) testing by Circulating Tumor DNA (ctDNA) from patients with PGLs and PCCs.

Methods: Molecular alterations in 46 plasma samples were tested using Guardant360® or Guardant360® CDx ctDNA assays from multiple institutions 2016-2021. Single nucleotide variants and indels in 54-83 genes with copy number amplifications and fusions in selected genes were detected.

Results: A total of 46 patients (24 PGLs and 22 PCCs) were included. For the 24 PGLs patients, the median age was 55 (range: 28-78); 14 (58%) were male. Of the 22 PCCs patients, the median age was 56 (range: 28-86); 12 (54.5%) were male. The identified genetic alterations were present in 16 (67%) PGLs and 17 (77%) PCCs patients. The 16 PGLs mutations include: *TP53* (44%), followed by *ATM* (25%), *FGFR2* (19%), *APC* (13%), *BRAF* (13%), *BRCA1* (13%), *CCND2* (13%), *FGFR3* (13%), *IDH2* (13%), *KRAS* (13%), *PDGFRA* (13%), *RB1* (13%), *TERT* (13%), *ALK* (6%), *ARID1A* (6%), *BRCA2* (6%), *CCND1* (6%), *CDK6* (6%), *CDK12* (6%), *EGFR* (6%), *FGFR1* (6%), *KIT* (6%), *MET* (6%), *NF1* (6%), *NRAS* (6%), *PIK3CA* (6%), *PTEN* (6%) and *ROS1* (6%). The 17 PCCs alterations include: *TP53* (41%), followed by *ATM* (35%), *NF1* (24%), *FGFR1* (18%), *APC* (13%), *EGFR* (12%), *MET* (12%), *MYC* (12%), *NOTCH* (12%), *PDGFRA* (12%), *TSC1* (12%), *AR* (6%), *ARID1A* (13%), *BRAF* (6%), *BRCA1* (6%), *BRCA2* (6%), *CCND1*(6%), *CDK6* (6%), *CHEK2* (6%), *ERBB2* (6%), *EZH2* (6%), *FGFR2* (6%), *IDH2* (6%), *KIT* (6%), *KRAS* (6%), *NRAS* (6%), *NTRK1*(6%), *NTRK2* (6%) and *VHL* (6%).

Conclusion: Liquid biopsy was feasible to detect alterations in PGLs and PCCs patients. ctDNA is a non-invasive method with the ability to detect alterations that could help personalize the treatment options for patients. We report a high rate of Homologous Recombinant Deficiencies (HRD) among the PGLs/PCCs patients highlighting the need for prospective evaluation on clinical trials.

Keywords: Paragangliomas; Pheochromocytomas; Liquid biopsy; Pheochromocytoma; Cholangiocarcinomas; Pancreas

INTRODUCTION

Pheochromocytomas (PCCs) and Paragangliomas (PGLs) are rare cancers originating from the autonomic nervous system PGLs and adrenal medulla PCCs chromaffin cells. PCCs and PGLs can be either sympathetic or parasympathetic secretory cancers, with the sympathetic lesions being more active, symptomatic and common in the abdomen and pelvis [1]. PGLs are less common than PCCs, and secrete norepinephrine as opposed to the PCCs which secrete epinephrine [2]. Most PCCs and PGLs tumors are benign with a 15%-20% risk of metastasis [3,4]. Both cancers are heterogenous diseases with no standard of care treatment guidelines established [5].

PGLs cancers are increasing at a rate of 3-5 fold over the past 40 years [6]. This could be related to better means of detection due to the improvement in imaging and biochemical tests. PGLs can be sporadic or hereditary syndromes. Germline variants in PGLs genes are found in 40% of patients with PGLs, making the tumor form the most heritable of all human malignancies. Somatic

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Correspondence Walid Shaib

Northwest Georgia Oncology Centers Wellstar, Marietta, Georgia, USA

E-mail wshaib@ngoc.com

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mutations are common in PGLs [7] and detected in around 30% of cases [8,9].

PGLs have consistent histological features that they share with PCCs with high heritability rates [10,11]; 25%-30% of these tumors develop under conditions of a hereditary tumor syndrome [12] a third of which affect the Von Hippel Lindau (*VHL*) gene [12] with 25%-30% of the tumors having somatic RET, *VHL*, Neurofibromin 1 (*NF1*) and MYC-associated factor X (*MAX*) mutations [13-16]. All PGLs exhibit malignant potential [7], and a few harbors malignant potential with (<5%), specifically in patients with the hereditary forms, like *VHL*, Multiple Endocrine Neoplasia type 2 (MEN2 with RET), and an increased risk of disseminated disease in patients with *SDHB* mutations [17]. Circulating Tumor DNA (ctDNA) testing is now performed for all cancer types. As opposed to traditional tissue biopsies, liquid biopsies are faster to result, less invasive, have the potential to reflect all metastatic sites (i.e. tumor heterogeneity), detect current (real time) and non-archived mutations, can monitor responses of therapy through serial sampling, and carry lower cost to perform [18-21]. ctDNA testing is now recommended to guide the treatment in many cancer types [22]. There is no standard of care for the treatment of PGLs and PCCs. Current management is based on case series and reports. Understanding the genomic landscape is of utmost importance in this disease as it may define the treatment through targeted therapies.

MATERIALS AND METHODS

This is a retrospective analysis of molecular alterations in 46 ctDNA samples from PGLs or PCCs patients who underwent Guardant360® or Guardant360® CDx from different institutions. This test detects single nucleotide variants in 54-83 genes, copy number amplifications, fusions, and indels in selected genes. Samples from patients between the years 2016 and 2021 were analyzed. Patient-specific covariates included gender and age. Ethical approval was not required given the de-identified nature of the data collected in a retrospective fashion, no consents were required, through a data transfer agreement between guardant health and Emory University.

Next Generation Sequencing (NGS)

ctDNA (liquid biopsy) testing was done by Guardant Health (Guardant360®). Guardant Health ctDNA testing is a College of American Pathologists (CAP)-accredited and Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory and detects Single-Nucleotide Variants (SNV), indels, fusions and copy number alterations in 83 genes, including the most prevalent tumor suppressor genes in human cancers, with a reportable range of ≥ 0.04%, ≥ 0.02%, ≥ 0.04% and ≥ 2.12 copies, respectively, and these include the detection of microsatellite instability. The test specificity is at the rate of >99.99% [23]. ctDNA gets extracted from the plasma using the QIAmp Circulating Nucleic Acid Kit (Qiagen, Inc.,). Hybrid-capture sequencing libraries are captured from up to 30 ng ctDNA and labeled with non-random oligonucleotide barcodes (IDT, Inc.,), followed by library preparation, hybrid capture enrichment (Agilent Technologies, Inc.,), and sequencing at 15,000 × read depth of the critical exons in the targeted panel by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.,), these are then reported through bioinformatics analysis [24]. NGS data were interpreted by N-of-One, Inc., (Lexington, MA, USA).

RESULTS

Patient demographics

Between 2016 and 2021, a total of 46 locally advanced unresectable or metastatic disease PCCs and PGLs patients underwent Guardant360® or Guardant360® CDx testing; 24 PGL and 22 PCC patients. The median age of PGLs patients was 55 (range: 28-78); 14 (58%) patients were male. The median age of PCC patients was 56 (range: 28-86); 12 (54.5%) patients were male.

Molecular alterations

Genetic alterations were identified in 16 (67%) PGLs and 17 (77%) PCCs patients. In PGLs patients, *TP53* mutation was the most common detected alteration (44%), followed by *ATM* (25%), then *FGFR2* (19%), *APC* (13%), *BRAF* (13%), *BRCA1* (13%), *CCND2* (13%), *FGFR3* (13%), *IDH2* (13%), KRAS (13%), *PDGFRA* (13%), *RB1* (13%), *TERT* (13%), *ALK* (6%), *ARID1A* (6%), *BRCA2* (6%), *CCND1* (6%), *CDK6* (6%), *CDK12* (6%), *EGFR* (6%), *FGFR1* (6%), *KIT* (6%), *MET* (6%), *NF1* (6%), *NRAS* (6%), *PIK3CA* (6%), *PTEN* (6%) and *ROS1* (6%). The alteration frequencies by gene and types are shown in Table 1.

Table 1. Ongoing clinical trials which assess different therapies for PPGL.

Trial number and Trial name	Study title	Estimate/actual number of participants	Status
NCT05133349 EASOAIPPGL	A prospective phase II efficacy and safety study of anlotinib in metastatic or locally advanced pheochromocytoma/paraganglioma: Open-label single-arm, exploratory trial	20	Recruiting
NCT03946527 LAMPARA	Lanreotide in metastatic pheochromocytoma/paraganglioma	40	Recruiting
NCT03839498	Study of axitinib (AG-013736) with evaluation of the VEGF-pathway in pheochromocytoma/paraganglioma	25	Recruiting

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NCT00843037 SNIPP	Study of sunitinib in patients with recurrent paraganglioma/pheochromocytoma	25	Active, not recruiting
NCT04924075 MK-6482-015	Belzutifan/MK-6482 for the treatment of advanced Pheochromocytoma/Paraganglioma (PPGL) or Pancreatic Neuroendocrine Tumor (pNET)	140	Recruiting
NCT03008369	Lenvatinib in treating patients with metastatic or advanced pheochromocytoma or paraganglioma that cannot be removed by surgery	3	Active, not recruiting, has results
NCT04860700	The efficacy and safety of anlotinib in patients with metastatic pheochromocytoma or paraganglioma	31	Recruiting
NCT03206060	Lu-177-DOTATATE (Lutathera) in therapy of inoperable pheochromocytoma/paraganglioma	90	Recruiting
NCT02302833	Cabozantinib S-malate in treating patients with metastatic pheochromocytomas or paragangliomas that cannot be removed by surgery	22	Recruiting
NCT04711135	Study to evaluate safety and dosimetry of lutathera in adolescent patients with GEP-NETs and PPGL	8	Recruiting
NCT04394858	Testing the addition of an anticancer drug, olaparib, to the usual chemotherapy (Temozolomide) for advanced neuroendocrine cancer	76	Recruiting
NCT05142241 RARE2	Testing the combination of anti-cancer drugs talazoparib and temozolomide in patients ≥ 18 years old with advanced stage rare cancers	34	Recruiting
NCT04276597 PUTNET	Phase-II study of Lu177DOTATOC in adults with STTR(+)pulmonary, pheochromocytoma, paraganglioma, unknown primary, thymus NETs (PUTNET), or any other Non-GEP-NET	50	Recruiting
NCT00107289	Phase-II study of Lu177DOTATOC in adults with STTR(+)pulmonary, pheochromocytoma, paraganglioma, unknown primary, thymus NETs (PUTNET), or any other non-GEP-NET	200	Recruiting
NCT02721732	Pembrolizumab in treating patients with rare tumors that cannot be removed by surgery or are metastatic	202	Active, not recruiting
NCT04400474 The CABATEN	Trial of cabozantinib plus atezolizumab in advanced and progressive neoplasms of the endocrine system. The CABATEN study	144	Recruiting
NCT02834013	Nivolumab and ipilimumab in treating patients with rare tumors	818	Recruiting
NCT03034200	Phase 2 study of ONC201 in neuroendocrine tumors	28	Active, Not recutting
NCT03541720	18F-fluorodopamine PET studies of neuroblastoma and pheochromocytoma	20	Recruiting
NCT01850888	MIBG for refractory neuroblastoma and pheochromocytoma	100	Recruiting
NCT04284774	Tipifarnib for the treatment of advanced solid tumors, lymphoma, or histiocytic disorders with HRAS gene alterations, a pediatric match treatment trial	49	Recruiting

In PCCs patients, *TP53* mutation was the most common alteration detected (41%), followed by *ATM* (35%), *NF1* (24%), *FGFR1* (18%), *APC* (13%), *EGFR* (12%), *MET* (12%), *MYC* (12%), NOTCH1 (12%), *PDGFRA* (12%), *TSC1* (12%), *AR* (6%), *ARID1A* (13%), *BRAF* (6%), *BRCA1*(6%), *BRCA2* (6%), *CCND1* (6%), *CDK6* (6%), *CHEK2* (6%) *ERBB2* (6%), *EZH2* (6%), *FGFR2* (6%), *IDH2* (6%), *KIT* (6%), *KRAS* (6%), *NRAS* (6%), *NTRK1* (6%), *NTRK2* (6%) and *VHL* (6%)). These alterations are summarized by frequencies and types. In PGLs (21%) and PCCs (41%) alterations were associated with targeted therapies that are approved in other indications.

DISCUSSION

The genomic landscape and the treatment paradigms of PGLs and PCCs cancers are not well established and are extrapolated from either case series or from other disease states like neuroendocrine tumors. Current therapeutic approaches for PPGL include radioactive Iodine-131-Metaiodobenzylguanidine (MIBG-Azedra) [25,26] selected by the 23I-MIBG scintigraphy scan positive in metastatic lesions which constitute about 50% of all PPGL [27,28]. No other treatments have been approved specifically for these tumors. Other radionuclide therapy that has been reported is the Peptide Receptor Radionuclide Therapy (PRRT) using the radiolabeled somatostatin analogue (177LutetiumDOTA0-Tyr3) octreotate (177Lu-DOTATATE) extrapolated from neuroendocrine tumor treatment. Benign and malignant PPGL overexpress somatostatin receptors which is more prominent in *SDHB* gene mutation [29]. Its efficacy and safety were assessed by a study of 30 inoperable PPGL cases (27 PGLs, 3 PCCs) [30,31].

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Case reports and series have reported good outcomes utilizing targeted therapies in some unselected patients. Targeted therapies are possible treatment options in unselected patients with metastatic PGLs and PCCs [32]. These include small molecule tyrosine kinase inhibitors and monoclonal antibodies. Sunitinib malate (Sutent) is reported through case series as a possible treatment of PGLs and PCCs [33-37]. A phase II trial of sunitinib in patients with progressive PGLs or PCCs (the SNIPP trail) in 2019 showed a survival advantage of progression free survival of 13.4 months and a reported disease control rate of 83%, only 3 patients (13%) achieved partial response. The trial emphasized that patients with germline variants in RET or SDH subunits alterations may have the greatest benefit despite poor overall response rate [38]. Temozolomide is reported to have some activity through case report and series in unselected patients [39-41]. The importance of evaluation for genetic mutations is also reflected by a study that detected a 67% clinical benefit of temozolomide for progressive PGLs and PCCs with a correlation between *SDHB*-mutation and hypermethylation of the Methylguanine-Deoxyribonucleic Acid Methyltransferase (MGMT) promoter region [42-44].

Homologous Recombination Deficiency (HRD) is a genomic alteration resulting from mutations in genes involved in double-stranded DNA breaks' repair with Homologous Recombination DNA Damage Repair (HR-DDR) deficiencies prevalent among many tumor types [45] including breast, ovarian, pancreatic and prostate cancers [46,47] with approved treatment targets [48-50]. In this study, a high prevalence of these HRD alterations are reported. Poly-ADP Ribose Polymerase (PARP) inhibition has emerged as a potential therapeutic strategy in the treatment of cancers. These agents inhibit DNA repair mechanisms and could have an important implication in the treatment of PGLs/PCCs patients given the high frequency of the HRD alterations reported here. Combining PARP inhibitors to an alkylating agent, such as temozolomide, have shown response efficacy in small cell lung cancer proving potential future hope in this aggressive disease [51]. This same combination is now under study. An ongoing trial (NCT04394858) is awaited to define the efficacy of targeting this pathway in patients with PPGL [52].

Other targeted alterations are reported in this study with high prevalence that are worth testing in prospective studies. These include the Fibroblast Growth Factor (FGF) [53,54], (Erdafitinib, Pemigatinib, Infigratinib) are currently approved for the treatment of selected patients with advanced urothelial and bile duct cancers [55]. In our study, we report a high prevalence of these alterations at a rate of 19% in PGLs (*FGFR3* (13%), *FGFR1* (6%)) and 24% in PCCs (*FGFR1* (18%), *FGFR2* (6%)). Isocitrate Dehydrogenase 1 and 2 (*IDH1*/*IDH2*) which are enzymes that physiologically convert isocitrate to α-ketoglutarate. This target (Ivosidenib and Enasidenib) is druggable and approved for selected patients with Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS) [56] and cholangiocarcinomas [57,58]. *IDH2* alterations are present in 13% of the PGLs and 6% of the PCCs as reported in this analysis. Other important alterations that could be targetable are: *BRAF* with V600 E mutation previously identified in a patient with PCCs [59], (Vemurafenib, Encorafenib) [60], PDGFRA/*KIT* (Imatinib) [61-63], *MET* with exon 14 mutation (Tepotinib) [63-65], the latter study showed Exon 14 mutations identified on three samples, *PIK3CA* (Idelalisib, Copanlisib, Duvelisib) [66,67], *ROS1* (Crisotinib) [68], *ERBB2* (Trastuzumab, Pertuzumab, Lapatinib, Fam-trastuzumab Derutecan) [69-73], *EZH2* (Tazemetostat) [74], *NTRK* (Larotrectinib, Entrectinib) [75,76].

CONCLUSION

PPGL are rare tumors, and the effectiveness of current treatment modalities remains limited throughout the literature. There are many limitations to this report. This is retrospective analysis of the prevalence of mutations in rare cancers. These genomic data were obtained from a de-identified database with very limited clinical information available. There are no treatments detailed and no clinical outcomes. No data available on when these samples were obtained (prior or after treatments). The gene panel is limited to 74 genes failing only. Another limitation is the lack of comparing tissue to liquid testing. This study proves that ctDNA genomic testing is feasible for PGLs and PCCs diseases and could have important implications on patients choosing to participate in trials and for physicians to design trials for these drug gable targets reported here, specifically the HRD alterations. The data lacks the specificities of the anatomic location and the burden of disease. Our findings in this paper help implement a personalized treatments approach that might improve PGLs/PCCs patients' outcomes. Furthermore, the sample size reported here-given the rarity of the disease- does not allow clinically meaningful survival outcomes even if these survival data were available. The need for further studies in the era of NGS and its continuous improvement to further identify PPGL mutations and thus guiding therapy, is important. We prove that liquid genomic testing modality is feasible in this rare cancer as these tumors shed circulating DNAs.

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