 doi:10.20944/preprints202107.0281.v1

Article

Clinical Application of Next Generation Sequencing in Recurrent Glioblastoma

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Summary: Glioblastoma (GBM) remains a disease with poor survival and with limited options. The purpose of this retrospective study is to determine if routine genomic profiling could guide treatment selection and impact survival outcomes. Although our study is limited by its sample size, we were able to demonstrate that there is a significant population of patients who may benefit from genomically-informed target therapy. There are some limitations to our analysis and its applicability. Access to next generation sequencing technology, availability of evidence to suggest off-label use of targeted drugs, and the timely implemention of therapeutic strategies makes our results difficult to generalize in a broader context. However, we argue that with advances in genomic sequencing and its expanded use, treatment options for patients with recurrent GBM may broaden. Furthermore, our results may inform future basket studies in patients with recurrent GBM, as well as larger studies to validate targeted strategies.

Abstract:

BACKGROUND: Glioblastoma (GBM) is driven by various genomic alterations. Next generation sequencing (NGS) could yield targetable alterations that may impact outcomes. The goal of this study was to describe how NGS can inform targeted therapy (TT) in this patient population.

METHODS: The medical records of patients (pts) with a diagnosis of GBM from 2017-2019 were reviewed. Records of patients with recurrent GBM and genomic alterations were evaluated. Objective response rates and disease control rates were determined.

RESULTS: A total of 87 pts with GBM underwent NGS. Forty percent (n = 35) were considered to have actionable alterations. Of the 35, 40% (n=14) pts had their treatment changed due to an alteration. The objective response rate (ORR) of this population was 43%. The disease control rate (DCR) was 100%. The absolute mean decrease in contrast enhancing disease was 50.7% (95% CI 34.8 – 66.6).

CONCLUSION: NGS for GBM, particularly in the recurrent setting, yields a high rate of actionable alterations. We observed a high ORR and DCR, reflecting the value of NGS in deciding on TT to match alterations that are likely to respond. In conclusion, patient selection and availability of NGS may impact outcomes in select pts with recurrent GBM.

Keywords: Glioblastoma; Precision Medicine; Targeted Therapy; Genomics; Neuro-Oncology

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1. Introduction

Recurrent primary glioblastoma (GBM) is associated with a high mortality rate, and effective treatments remain limited. Despite recognizing their initial biological heterogeneity, newly diagnosed GBM has been largely treated uniformly since Stupp and colleagues demonstrated improved survival with concurrent temozolomide (TMZ) and radiotherapy, followed by maintenance temozolomide; however, almost 50% of patients diagnosed with GBM show progression within the first two years of diagnosis.[1] A better understanding of genomic alterations that drive cancer progression as well as increasing availability of targeted therapeutics has created a paradigm shift in the treatment of other cancers. For example, routine genomic profiling for melanoma and lung cancer can identify targetable alterations that change management, but this practice has not yet translated to patients with intrinsic brain tumors. [2,3] This has largely been due to the lack of uniform effectiveness of single agents, however, targeted individual patient-level sequencing may open the door for inclusion of GBM patients in larger clinical trials based on mutational rather than tumor histology. It may also reveal targetable alterations for which approved drugs already exist, and, thus, providing additional therapeutic options that may impact individual patient outcomes.

Next generation sequencing (NGS) is an umbrella term describing genomic analysis that identifies unique sequences of DNA and RNA. These sequences may be copy number variants (CNV), as well as alterations within the DNA (e.g. mutations) and RNA transcriptome (e.g. fusions). In the setting of solid tumors, this technique has been routinely employed as a means to stratify patients with advanced lung, melanoma, ovarian, and breast cancers.[4] Alterations involving the epidermal growth factor receptor (EGFR) tyrosine kinase or anaplastic lymphoma kinase (ALK) receptor can lead to constitutively active and unchecked cellular proliferation in lung adenocarcinoma.[5,6] In the setting of advanced lung cancer, testing that supports certain targetable alterations in EGFR or ALK, it is routine for practitioners to prescribe osimertinib or crizotinib, respectively. [7-9] This type of precision medicine is appealing, however, it has not so easily translated to patients with recurrent GBM (rGBM) due to lack of robust biomarker enriched clinical studies showing benefit beyond the standard of care. It is notable that routine sequencing of patients with rGBM has not been widely adopted and data utilization for clinical actionability can vary. [10] Additionally, the cost of NGS can be prohibitive, further making widespread adoption difficult. [11] However, more centers are beginning to publish their own experiences with NGS and its implications for therapeutic applicability.[12]

In 2017, our group began to routinely send fresh frozen paraffin embedded (FFPE) newly diagnosed high grade glioma samples to Strata Oncology® (Strata) for sequencing. As part of a non-therapeutic clinical protocol, patients were consented to submit tumor tissue at no cost. As we looked back at institutional experience, we sought to understand the impact of upfront and routine sequencing of patients diagnosed GBM and if these data informed therapeutic changes in the setting of disease recurrence.

2. Materials and Methods

Data collection

For this study, we retrospectively reviewed all patients with a diagnosis of wildtype isocitrate dehydrongenase (*IDH*) gene glioblastoma, who had their tumor sequenced using Strata from 2017-2019. Research Electronic Data Capture (REDCap) was used to filter these patients, retrieve demographic data, and identify responses to treatment. Collected variables included age, sex, Ki67 immunohistochemistry, telomerase reverse transcriptase (*TERT*) mutation status, O[6]-methylguanine-DNA methyltransferase (*MGMT*) promotor methylation status, alterations on the Strata profiling report, and various clinical time points defining treatment response. Only patients with actionable alterations listed on their Strata profile were included in this study. There were three types of alterations collected: hotspot mutations, gene fusions, and copy number variants (CNV). An alteration was defined as "actionable" if it met criteria set

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forth by Li and colleagues and described in "Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer" (Table 2b).[13] We retrospectively reviewed patient medical records to determine important characteristics (Table 1). The study was approved by the institutional Office of Human Research Ethics.

Table 1. Table 1. Patient demographics.

Variable		
Age	Mean (sd)	56 (14.9)
Age	<55	6 (43%)
	≥55	8 (57%)
Gender	Female	3 (21%)
	Male	11 (79%)
Surgical Status	biopsy	4 (29%)
	STR	4 (29%)
	GTR[1]	6 (42%)
Ki67[2]	<30	2 (14%)
	≥30	10 (72%)
	Unknown	2 (14%)
TERT[3]	Mutant	11 (79%)
	Wildtype	3 (21%)
MGMT[4]	Methylated	8 (57%)
	Unmethylated	6 (43%)

[1]GTR: Gross total resection, as defined as ≥90% resection of enhancing tissue

[2]Ki67: monoclonal antibody for immunohistochemical staining to define proliferation index

[3]TERT: Telomerase reverse transcriptase

[4]MGMT: O-6-Methylguanine-DNA Methyltransferase

All patients had their initial tumor tissue from diagnosis profiled via Strata. Patients included in this analysis had undergone standard of care (SOC) with radiation therapy and temozolomide (TMZ) followed by maintenance temozolomide with or without the addition of tumor treating fields (Optune®), followed by their first progression.[1] However, patients were not limited to the number of progressions in order to be included in the analysis. Those included in the analysis needed to demonstrate disease progression as documented by gadolinium-enhanced magnetic resonance imaging (MRI) or contrast-enhanced computer tomography (CT); the latter only being inclusionary if a patient was unable to tolerate an MRI.

Treatment response was graded using Response Assessment in Neuro-Oncology (RANO) criteria.[14] Objective response rate (ORR) was determined as the percentage of those patients who achieved a partial response or a complete

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response as their best response. Disease control rate (DCR) was determined as the percentage of complete, partial, or stable disease response by RANO criteria at a subsequent follow up imaging analysis following targeted treatment initiation. Baseline imaging (at initial progression) was compared to subsequent imaging (after starting targeted treatment) to determine the absolute mean change in lesion size by RANO criteria.

3. Results

Patients

There were at total of 87 patients with GBM at our institution that had Strata profiling performed. Thirty-five (40%) of those patients had a tumor that revealed alterations considered actionable (Table 2a). Of these 35 patients, 14 (40%) patients were placed on a TT due to an alteration found on their report (Table 1). The mean age at diagnosis was 56 years. Patients with *MGMT* promotor methylation made up 57% (n = 8) of the population.

Table 2a. Actionable alterations.

Alterations	Type	Tier	Grade	Alterations	Type	Tier	Grade
ALK[15]	Fusion	II	D	MET[16]	CNV	II	C
ATM[17,18]	Hotspot	II	C	MET[19,20]	Hotspot	II	C
BRAF V600E[21]	Hotspot	II	D	MET[16,22]	Fusion	II	C
BRCA1[23]	Hotspot	II	D	NF1[24,25]	Fusion	II	C
BRCA2[23]	Hotspot	II	D	NTRK1[26-28]	Fusion	I	A
EGFR[29,30]	Hotspot	II	C	NTRK2[26-28]	Fusion	I	A
EGFR-SEPT14[31]	Fusion	II	C	NTRK3[26-28]	Fusion	I	A
FGFR1[32]	Hotspot	II	C	PTPRZ-MET[16,22]	Fusion	II	C
FGFR2[32]	Hotspot	II	C	RET[33]	Hotspot	II	D
FGFR3[32]	Hotspot	II	C	RET[34]	Fusion	II	D
IDH1[35]	Hotspot	II	C	ROS1[36,37]	Fusion	II	D
IDH2[38]	Hotspot	II	C	SMO[39]	Hotspot	II	D
KIT[40]	Hotspot	II	C				

Table 2b. Criteria per Li et al used to determine the level of evidence of each treatment used to target alterations found on STRATA sequencing reports.[13]

Tier	Grade	
I	A	FDA approved therapy for disease in question
	B	Large studies, not yet approved
II	C	Approved in other diseases, some studies in disease in question
	D	Pre-clinical data, case reports
III		VUS, not clear association with cancer
IV		Benign variant

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Sequencing results and outcomes

The most common alterations were seen in *EGFR* (63%), *CDKN2A* (60%), and the *TERT* promotor (51%). The most common actionable alterations were amplifications in *EGFR* (63%), *KIT* (17%), and *PDGFRα* (17%), as well as various *EGFR* mutations (14%). Of the 14 patients placed on targeted treatment, 12 (86%) eventually had progression of disease following treatment and either went on to a subsequent line of therapy or were referred to hospice.

We calculated an ORR of 43% (6 of 14 patients). Additionally, the DCR at first imaging timepoint following progression and the intiation of targeted treatment was 100% (14 of 14 patients) response per RANO criteria, with those patients meeting the criteria for complete response (CR), partial response (PR), or stable disease (SD).[41]

The absolute mean decrease in contrast enhancing disease was 50.7% (95% CI 34.8 – 66.6) when considering the best response to targeted therapy initiation. Table 3 illustrates the best response obtained per patient while on targeted treatment when compared to the MRI at disease progression, prior to the start of targeted therapy. Three agents (afatinib, selpercatinib, and cabozantinib) resulted in a complete response by RANO criteria. The most frequently used treatments in our cohort were afatinib, osimertinib, and a combination of dabrafinib and trametinib.

Table 3. Individual patient response by RANO criteria.

Alteration	**Treatment**	**Response (%)**
EGFR-SEPT14 fusion EGFR amp EGFR vIII deletion	Afatinib	100
MET exon 14 deletion MET amp	Cabozantinib	100
RET amp	Selpercatinib	100
BRAFV600E	Dabrafenib/trametinib	72
EGFR amp	Osimertinib	53
NF1 exon 23 splice donor site mutation	Trametinib	52
EGFR p.A289T	Afatinib[1]	46
MET amp	Crizotinib	45
PDGFR amp, KIT amp	Imatinib	41
EGFR-SEPT14 fusion EGFR amp	Osimertinib	39
SQSTM1-NTRK2 Fusion	Larotrectinib	26
TPM1-ALK fusion	Alectinib	25
EGFR vIII deletion EGFR amp	Osimertinib	23
BRAFV600E	Dabrafenib/trametinib	4

[1]Combined with temozolomide

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Case Example

A 72-year-old female presented with seizures, with imaging revealing a left temporal lesion. She underwent a subtotal resection and was found to have a GBM with methylguanine methyltransferese (*MGMT*) promotor hypermethylation and *IDH* wildtype. She went on to complete standard chemoradiotherapy, which was complicated by pancytopenia. Strata profiling revealed potentially actionable alterations involving the mesenchymal-to-endothelial transition (*MET*) gene. Given treatment-limiting pancytopenia during chemoradiation, she was started on crizotinib, in conjunction with alternating electric tumor treating fields. A subsequent MRI revealed a partial response. Unfortunately, disease progression was observed two months later. Crizotinib was discontinued. She was started on low dose daily temozolomide. However, subsequent MRI revealed progression, mirroring a precipitous clinical decline. Given the partial response that she had with crizotinib, we reasoned that a more potent MET inibitor with better brain penetration, could be considered.[42] Therefore, she was started on cabozantinib (Figure 1A). [43] She remained on cabozantinib for 22 days but was forced to stop treatment due to thrombocytopenia. A subsequent MRI revealed a complete response (Figure 1B). Platelets recovered after one month off therapy; followed by an MRI revealing disease progression (Figure 1C). She was restarted on dose-reduced cabozantinib. MRI four weeks later revealed a partial response (Figure 1D). Unfortuntately, the patient continued to clinically decline and was transitioned to hospice.

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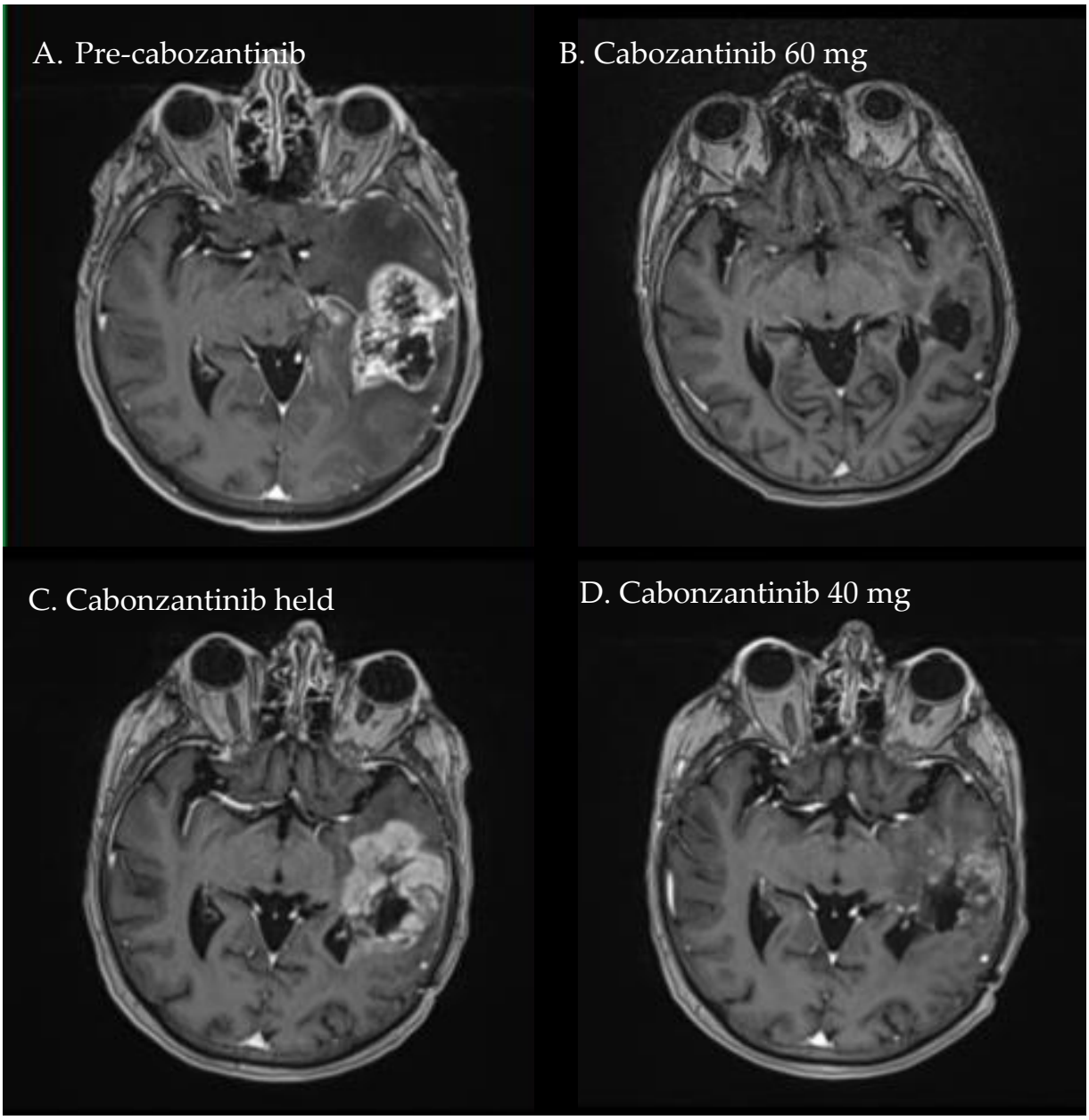

Figure 1. (A) Baseline MRI at progression. (B) MRI at four weeks following cabozantinib, revealing complete response. (C) After four weeks of holding cabozanitnib, MRI revealing progression. (D) After four weeks of dose-reduced cabozantinib, MRI revealing partial response.

4. Discussion

Current management of GBM involves maximal safe resection followed by adjuvant chemoradiation and maintenance chemotherapy with or without the incorporation of Optune®. At present, overall survival continues to stand at approximately 14 months.[1] The utility of a limited cadre of validated biomarkers has been recognized as a complemenary measure in the practice of neuro-oncology. Prime examples of validated and clinically impactful biomarkers are mutations in *IDH*, co-deletion of short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q), as well as *MGMT* promoter methylation status to guide responsiveness to conventional chemoradiotherapy.[44] These alterations play a diagnostic, prognostic, and predictive role in the management of high grade glioma.[45]

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However, despite multiple validated and commercially available assays, a broader and deeper analysis of tumor tissue is not routinely performed at diagnosis, nor is it used at the time of disease recurrence.

In our study, we demonstrate that 40% of profiled patients had targetable alterations. This rate of alterations appeared similar to the 46% among patients with primary brain tumors described by Siegel and colleagues.[46] Although we present a small number of patients, our study demonstrates that routine sequencing of high grade glioma can detect a clinically significant number of patients with potentially actionable alterations which can influence treatment decisions. In the absence of standardized second line agents, and even though efficacy is still unproven, we've demonstrated that there is the potential to impact management in select GBM populations and have shown repeated clinically significant treatment responses in individual patients.

Despite showing that almost half of our patients had actionable alterations, the therapeutic potential of these biomarkers is not fully defined or validated in biomarker-enriched clinical trials. We demonstrated that in the cohort of patients that had actionable alterations and who went on to receive targerted therapy, the ORR was 43% and the DCR was 100%. The absolute mean decrease in lesion size was estimated to be 50.7% (95% CI 34.8 – 66.6), suggesting a robust initial response to NGS-informed targeted therapy.

Our data suggest that matching a patient with a potentially susceptible alteration combined with a rationally developed therapeutic strategy can provide a meaningful response and clinical benefit. The highlighted case above demonstrated that even in the setting of progression after one targeted therapy, re-challanging with a more potent kinase inhibitor with better brain penetrance can lead to disease control. Additionally, this case also highlights that particularly sensitive patient populations can respond to lower concentrations of drugs. However, when one considers the clinical evidence for cabozantinib in rGBM, it is clear that the majority of study subjects did not benefit from it per Wen and colleagues.[19] A closer look at the aforementioned study suggests that subjects were not selected by *MET* status; however, one may argue that it would not be feasible given that *MET* alterations occur in less than 2% of newly diagnosed GBM.[19] Our findings suggest that for those 2% of patients, the treatment may provide a clinical benefit.

Future directions

With the Food and Drug Administration (FDA) permiting surrogate endpoints (i.e. ORR) to guide its approval pathway for cancers with significant unmet need, biomarker enriched studies have the potential to bring targeted therapy to rare and poorly responsive advanced malignancies.[47] Examples have emerged in various single-arm, biomarker-enriched studies leading to accelerated approval for a number of indications. Larorectinib and entrectinib standout as prime examples. Drilon et al. evaluated larotrecetinib in 55 patients with NTRK fusion alterations from 17 different histologies and demonstrated an ORR of 75%.[27] This gene fusion was also present in 1.4% of glioma patients.[48] The study ultimately lead to FDA approval of larotrectinib in NTRK fusion-positive solid tumors.[49] Similarly, entrectinib was approved with a similar indication after a pooled analysis of multiple studies showed an ORR of 57% in those subjects that had various NTRK fusion alterations to their advanced solid tumors. [28] With such a high DCR, our data sugget that pooled studies enriched for patients with molecular drivers could demonstrate a high ORR, which could ultimately lead to accelerated regulatory approval.

Limitations

There are inherent limitations to this study. This is a single institution retrospective analysis with a small cohort size and limited power. We cannot make strong statistical inferences to support the adopation of NGS in clinical practice based on the limited numbers that we report. Additionally, we had to rely on retrospectic review of patient records that may not fully capture disease assessment, complications related to disease and therapy, and compliance with targeted medications. Despite using RANO criteria for all radiographic disease assessment, imaging review was not centralized.

 doi:10.20944/preprints202107.0281.v1

There are also inherent limitations to using NGS platforms. In particular, NGS profiles can evolve over time. Depending on the assay, the number of genes being queried can expand and new data can be generated to support the use of targeted therapy. During the study period, the Strata panel expanded from 88 genes to 409 genes. Therefore, not every patient received the same extensive profiling, especially those who were initially profiled in 2017. Another important factor that can impact the generlizability of our outcomes is that our NGS data came from archival tissue samples from when patients were intially diagnosed. Therefore, at the time these data could be applied in the clinical setting, we may not be fully taking into account the role of temporal tumor evolution and the intratumoral genomic heterogeneity of these samples. When disease heterogeneity at time of recurrence is taken into account, it can certainly impact the development of biomarker-driven studies in high grade glioma; however, it is encouraging that our results suggest that matching targeted therapy with well validated genomic data can lead to robust reponses in certain patients.

5. Conclusions

The wide-spread availability of NGS and its gradual adoption may provide clinically impactful data that can guide clinical decision-making in the setting of recurrent GBM. Although there are inherent limitations to our retrospective single center analysis, it is clear that patients with rGBM and sensitizing alterations can have meaningfully robust responses to targeted therapy. With continuous optimization of NGS assays, these tests may provide practice-changing, hypothesis-driven, biomarker-enriched basket studies in GBM.

Author Contributions: Conceptualization, D.Z., M.C., S.K.; methodology, D.Z., S.M., K.P. N.R.S.K.; formal analysis, D.Z, J.W. S.M. N.R..; data curation, D.Z., J.W. ; writing—original draft preparation, D.Z., S.K..; writing—review and editing, D.Z., M.C., N.R. S.K.; supervision, S.K.; project administration, K.P. All authors have read and agreed to the published version of the manuscript.

Funding: None

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of University of North Carolina at Chapel Hill (protocol code 19-0988 on 7/1/2020)

Informed Consent Statement: Patient consent was waived due to this being a retrospective study.

Data Availability Statement: Deidentified patient data is stored at the University of North Carolina at Chapel Hill RedCap® database. Data analysis was done with Excel®. Review of data can be arranged through the corresponding author.

Acknowledgments: The authors would like to acknowledge the editorial contributions of Dr. William Kim (Division of Medical Oncology, UNC School of Medicine) and that of Dr. Jason Merker (Department of Pathology and Laboratory Medicine & Genetics, UNC School of Medicine).

Conflicts of Interest: The authors declare no conflict of interest

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