Spectrum of FGFR2/3 Alterations in Cell-Free DNA of Patients with Advanced Urothelial Carcinoma

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Abstract. Detecting genomic alterations (GAs) in advanced urothelial carcinoma (aUC) can expand treatment options by identifying candidates for targeted therapies. Erdafitinib is FDA-approved for patients with platinum-refractory aUC with activating mutation or fusion in FGFR2/3. We explored the prevalence and spectrum of FGFR2/3 GAs identified with plasma cfDNA NGS testing (Guardant360) in 997 patients with aUC. FGFR2/3 GAs were detected in 201 patients (20%) with characterized activating GAs in 141 (14%). Our results indicate the Guardant360-based FGFR2/3 GA detection rate is similar to those described from previous studies employing tumor tissue testing, suggesting that plasma-based cfDNA NGS may non-invasively identify candidates for anti-FGFR targeted therapies.

A recent wave of new agents has been introduced to the arsenal of treatments for patients with aUC, including immune checkpoint inhibitors, targeted therapies and antibody-drug conjugates. In April 2019, erdafitinib, a potent tyrosine kinase inhibitor (TKI) of FGFR1–4, received FDA accelerated approval for adult patients with locally advanced or metastatic urothelial carcinoma exhibiting susceptible activating mutations or fusions in the FGFR2 or FGFR3 (FGFR2/3) genes who had progression on platinum-containing chemotherapy. Other FGFR
inhibitors, including infgratinib, pemigatinib, rogaratinib and Debio-1347, are under investigation in urothelial cancer.

Platinum-based chemotherapy remains the first-line standard of care for patients with aUC, with median overall survival noted between 9 and 15 months in older trials [1, 2]. However, for patients who have progression following this initial treatment, the median overall survival may only be estimated at 5–7 months [3]. Erdafitinib demonstrated the median overall survival may only be estimated at approximately seven days. All panel versions included in the study cohort included assessment for FGFR2/3 fusions and sequencing of all critical exons of FGFR2/3 harboring sensitizing SNVs. Guardant360 testing is validated and intended for the detection of somatic alterations.

During the specified study time frame, a total of 1,349 samples from 1,096 unique patients with aUC (a number of patients had testing performed at multiple time points) had associated G360 results for analysis. Median patient age was 69 (range 28–93); 28% were female.

Presumed somatic GAs were identified in 1,192 (88%) cfDNA tests from 997 unique patients. Of these, 201 (20%) patients with aUC had ≥1 fusion and/or nonsynonymous SNV in FGFR2/3; 141 patients (14%) had at least one characterized activating FGFR2/3 GA while the remainder of GAs were functionally uncharacterized variants of uncertain significance (VUS); VUS are generally not used to recommend treatment with targeted agents based on lack of clear functional significance, but could possibly be reclassified in the future with additional investigation and generated data. Of patients with FGFR2/3 GAs, the median age was 70 (range 39–92) and 27% were female.

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GAs were observed more often in FGFR3 (n = 125) than FGFR2 (n = 30). The majority (82%) of the observed GAs in FGFR3 were predicted to be activating, while most FGFR2 GAs (86%) were VUS (Figs. 1a, b). However, there was a greater diversity of unique variants in FGFR2 (n = 59) vs. FGFR3 (n = 30) (Figs 1c, d); for the latter, this was primarily driven by the S249C variant, which comprised 46% of nonsynonymous FGFR3 SNV observations (Fig. 2a).
Fig. 1. Occurrences and unique variants in FGFR2/3. The number and proportion of both characterized and VUS GAs observed in cfDNA in FGFR3 (a) and FGFR2 (b) across the aUC cohort. Excluding occurrences of the same variant seen in multiple patients, the number and proportion of both characterized and VUS unique variants present in FGFR3 (c) and FGFR2 (d) across the cfDNA aUC cohort. VUS, variant of uncertain significance.

The second most common FGFR3 non-synonymous SNV, Y373C, accounted for 16% of cases. There were no unique significantly recurrent FGFR2 variants. In both genes, VUS were individually uncommon. FGFR3 fusions were identified in 34 (3.1%) patients. The vast majority (n = 32, 94%) of these involved TACC3 as the fusion partner, but JAKMIP1 and TNIP2 were detected as fusion partners in one patient each.

Copy number-adjusted clonality was determined by calculating the relative variant allele fraction (VAF) and correcting for copy number, as previously described [12]. The median copy number-adjusted clonality of nonsynonymous SNVs was higher in FGFR3 (0.80) than FGFR2 (0.20); this remained true when limiting to characterized activating mutations (0.84 vs. 0.17) (Fig. 2b).

When examining the co-occurrence of characterized activating GAs, there was very little overlap between activation of FGFR2 and FGFR3. However, two patients were identified in the cohort whose samples contained activating point mutations in both genes. One patient’s sample had an overall maximum VAF of 8.2% and a total of 17 GAs identified including FGFR2 N549K and FGFR3 S249C at VAF of 1.5% and 1.4%, respectively. The other patient’s sample had an overall maximum VAF of 2.5% and a total of 9 GAs identified including FGFR2 S252W and FGFR3 G370C at VAF of 2.0% and 0.7%, respectively. A recent study utilizing tissue NGS across multiple cancer types identified a degree of co-occurrence in FGF/FGFR GAs, but observed mutual exclusivity between FGFR2 and FGFR3 in particular [13]. While no clinical information is available regarding these patients to better understand this intriguing phenomenon, the ability of cfDNA sampling to capture potential genomic heterogeneity may provide unique further insights into co-occurrence vs mutual exclusivity of biologically relevant pathways in future work.

This study demonstrates that cfDNA NGS analysis identifies fusions and a broad spectrum of SNVs in FGFR2/3 at a similar rate to historically reported tumor tissue testing, with previous studies demonstrating that tissue analysis detects FGFR3 mutations in up to 15%–20% of advanced/metastatic urothelial cancers [4, 14–19]. FGFR2/3 fusions are less common at a frequency of 2.2%–2.4% in patients with aUC who had tissue testing, [18, 20] with TACC3 being the most common fusion partner, consistent with the current study [4, 21]. FGFR3-TACC3 fusions are reported to be enriched in younger (≤50) patients (12%), Asians (13%), and never smokers (5.6%) [20].

Concordance of plasma cfDNA results with tumor tissue across solid tumors has been previously researched; overall concordance can vary depending on a variety of factors, including timing of sample collection and overlap of assay panels utilized, but in studies controlling for these variables, analytical concordance is quite high [9, 10, 22, 23]. Such comparisons are limited specifically for FGFR2/3 GAs in aUC; a cohort comparison study found a similar overall frequency of FGFR3 GAs between plasma and tissue-based datasets in lower tract aUC [24] and a pilot concordance study for patients with metastatic urinary tract cancer found that plasma genomic profiling detected FGFR2/3 GAs in 4 of 5 patients in whom they were detected by tissue testing [25].

This study explored both fusions and point mutations across both FGFR2/3 with an analysis of functionally activating and uncharacterized (VUS) GAs in both. It should be noted that the BLC2001 study of erdafitinib included only activating mutations and fusions in both FGFR2/3; thus, this study cohort did not include patients with activating FGFR2 point mutations [4]. While the present analysis suggests that FGFR2 point mutations known to have func-
tionally activating properties appear rare in advanced urothelial cancer, they may be potential drug targets. However, it is not clear whether these specific GAs would be ideal targets for anti-FGFR therapeutics and further exploration of this question is warranted.

The difference in copy number-adjusted clonality between FGFR2 (0.20) and FGFR3 (0.80) suggests that FGFR3 GAs are frequently early truncal mutations, whereas FGFR2 GAs may arise later in a heterogeneous fashion [12]. While further study is needed to understand the therapeutic impact of subclonal alterations, especially those with low relative VAF, there may be potential clinical benefit for matching targeted therapies and there is much more to learn regarding response to immunotherapy. Therefore, capturing the components of dynamic tumor heterogeneity over time with serial samples may prove very important.

Especially given that tumors can increase in genomic complexity over time and with previous exposure to systemic treatment, cfDNA NGS offers the opportunity to assess the current genomic profile of the evolving disease versus “snapshot” testing of archival tissue. Peripheral blood samples for cfDNA analysis can be obtained in a non-invasive manner at the time of consideration of post-progression therapy compared to tissue acquisition, which can be invasive, costly, not feasible, and logistically challenging. Moreover, cfDNA NGS testing using plasma also provides the opportunity to track the evolution of GAs across multiple time points in the treatment course, exploring response to treatment as well as the emergence and types of resistance mechanisms.

The present study has several inherent limitations, including the potential for selection and confounding biases, retrospective nature, and absence of matched tumor tissue testing and clinical outcomes data, highlighting the need for prospective validation of our generated hypotheses. In the absence of available matched tissue specimens or tissue testing information from this particular patient cohort, we compared observed alteration frequencies to previ-
ously published analyses of these GAs in cohorts with tissue testing performed, but we cannot rule out potential cohort differences, which could affect this comparison. These are common limitations when studying such cohorts derived through standard of care clinical testing genomic databases; however, outcomes-focused studies are ongoing [26].

To conclude, genomic profiling via cfDNA is a fast, minimally invasive tool that may be used to help inform decision-making for patients with advanced solid tumors. The results from our large retrospective study indicate a detection rate of FGFR2/3 GAs identified in plasma cfDNA using the G360 assay that is similar to historical rates based on conventional tumor tissue testing. Our hypothesis-generating results can contribute to ongoing discussions about identification of patients who can benefit from erdafitinib and emerging therapies in clinical trials.

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AUTHOR CONTRIBUTIONS

All authors contributed to careful interpretation of data and writing the article. Additionally, PG/LAK/RAT contributed to conception, LAK/AIH contributed to performance of work, and PG/LAK/AIH had access to data.

CONFLICTS OF INTEREST

Petros Grivas MD PhD reports grants and consulting/advisory board involvement from AstraZeneca, Bayer, Bristol-Myers Squibb, Clovis Oncology, GlaxoSmithKline, Genentech, Immunomedics, Merck, Mirati Therapeutics, Pfizer, and QED Therapeutics; grants from Bavarian Nordic, Debiopharm, Kure It Cancer Research, and Oncogenex; and consulting/advisory board involvement from Driver, Dynaia Health, EMD Serono, Exelixis, Foundation Medicine, Genzyme, Heron Therapeutics, Janssen, Roche, and Seattle Genetics, outside the submitted work.

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