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Fibroblast Growth Factor Receptor 3 Alterations and Response to PD-1/PD-L1 Blockade in Patients with Metastatic Urothelial Cancer

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Abstract

Prior studies have demonstrated that fibroblast receptor 3 (FGFR3)-mutant urothelial cancers (UCs) are associated with decreased T-cell infiltration. As *FGFR3* mutations are enriched in luminal-like UC and luminal-like UC has been shown to be relatively less responsive to PD-1/PD-L1 inhibition (checkpoint inhibition [CPI]), these data have led to the speculation that *FGFR3* mutations may be causally related to poor T-cell infiltration and that UC patients harboring *FGFR3* mutations may be suboptimal candidates for CPI. Using data derived from two clinical trials exploring CPI in metastatic UC, we demonstrate no statistically significant difference in response rates in patients with FGFR3-mutant versus wild-type UC. We present hypothesis-generating data, suggesting that similar response rates may be explained by a "balancing out" of previously identified independent positive and negative predictors of CPI sensitivity; that is, compared with *FGFR3* wild-type UC, FGFR3-mutant UC is associated with a similar tumor mutational burden, lower T-cell infiltration, but also lower stromal/transforming growth factor beta (TGF-β) signals. Based on our findings, *FGFR3* mutation status is not a biomarker of resistance to CPI. Indeed, the single-agent activity of both FGFR3 inhibitors and CPI in FGFR3-mutant UC, and potential non–cross resistance provide a strong pragmatic rationale for combination approaches.

Patient summary

In this report, we examined the impact of a mutated gene found in a subset of urothelial cancers on response to treatment with immunotherapy. We found that patients with tumors harboring mutations in the gene *FGFR3* respond to immunotherapy similarly to patients without such mutations.

Keywords

Bladder cancer; Immune checkpoint blockade; FGFR3; PD-1 blockade; PD-L1 blockade; Urothelial cancer

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Immune checkpoint inhibition (CPI) has changed the landscape of treatment for metastatic urothelial cancer (mUC). However, a minority of patients respond to treatment, prompting the pursuit of biomarkers and mechanisms underlying resistance to guide combination approaches. Prior studies have shown that luminal I, or luminal papillary, urothelial cancer (UC) harbors lower T-cell infiltration compared with other subtypes, and is also associated with lower response rates with CPI [1,2]. Further, an in silico analysis previously reported a correlation between fibroblast receptor 3 (*FGFR3*) alterations and decreased T-cell infiltration [3]. As *FGFR3* mutations (mFGFR3) are enriched in luminal UC, these data have led to the speculation that *mFGFR3* may causally be related to poor T-cell infiltration. These observations have further fueled the conjecture that UC patients harboring *mFGFR3* may be suboptimal candidates for CPI and have stimulated interest in combining CPI with *FGFR3* inhibition as a means of overcoming CPI resistance.

We used the IMVigor 210 cohorts 1 and 2, a phase 2 trial exploring the PD-L1 inhibitor atezolizumab in patients with mUC, to assess the association between *mFGFR3* and response to CPI [4]. The characteristics of IMVigor 210 have been described [4] and cohort

characteristics are summarized in Supplementary Table 1. Hybrid capture-based next-generation sequencing data were available for 274 patients, among whom 49 had tumors harboring mFGFR3. There was no statistically significant difference in objective response rate with CPI or overall survival (OS) in patients with and without *mFGFR3* in an analysis combining IMVigor 210 cohorts 1 and 2 (Fig. 1, Supplementary Table 2, and Supplementary Fig. 1 and 2), or when cohorts 1 and 2 were analyzed separately (Supplementary Fig. 3). We confirmed these findings in a second cohort utilizing whole exome sequencing data from CheckMate 275 [2], a single-arm phase 2 trial exploring the PD-1 inhibitor nivolumab in patients with mUC (Fig. 1, Supplementary material, Supplementary Table 1, and Supplementary Fig. 1 and 2).

We sought to reconcile our observations from these two clinical trial cohorts with prior observations correlating *FGFR3* alterations with decreased T-cell infiltration. We first focused on *FGFR3* gene expression because (1) prior studies correlated increased *FGFR3* gene expression, along with mutations, with decreased T-cell infiltration [3] and (2) *FGFR3* gene expression may encompass other mechanisms of increased FGFR3 signaling (eg, amplifications, gene fusions) [5]. We confirmed that *mFGFR3* was associated with increased *FGFR3* gene expression compared with wild-type (WT) UC in the IMVigor 210 cohort (Supplementary Fig. 4). Consistent with prior studies, *FGFR3* expression, or mFGFR3, was also negatively correlated with a T-cell gene signature (Supplementary Fig. 5 and Fig. 2). However, there was no statistically significant difference in response rates or OS with CPI among groups separated based on *FGFR3* gene expression (Supplementary Fig. 6 and 7).

High tumor mutational burden (TMB) and T-cell gene signatures have been shown to be independently associated with response to CPI [4]. Further, our group and others have demonstrated that gene signatures derived from stromal elements are independently associated with CPI resistance [4,6]. We hypothesized that the lack of association between *mFGFR3* and response to CPI, despite a negative correlation with T-cell infiltration, might be due to an imbalance in these other parameters. In the IMVigor 210 cohort, we found no statistically significant difference in TMB between *mFGFR3* versus WT tumors; however, *mFGFR3* tumors demonstrated significantly lower expression of a fibroblast TGF-β response signature or our epithelial to mesenchymal transition (EMT)/stromal signature versus WT tumors (Fig. 2). These findings support the concept that the lower T-cell infiltration in *mFGFR3* tumors may be counterbalanced by a lower level of stromal-mediated immune suppression (Fig. 2) culminating in similar sensitivity to CPI in *mFGFR3* and WT UC.

Given that *FGFR3* inhibition is being pursued in combination with CPI in clinical trials, we sought to explore the spectrum of gene expression perturbed by *mFGFR3* and FGFR3 inhibition in vitro. We obtained gene expression data for a panel of *FGFR3* WT and *mFGFR3* cell lines (Supplementary material) and also performed RNA sequencing of *mFGFR3* cell lines (RT4, SW780, and MGHU3) after knocking down FGFR3 with two independent siRNAs (Supplementary Fig. 8) [7]. Compared with *FGFR3* WT cells, *mFGFR3* cells were associated with decreased expression of several immune-related genes particularly related to interferon response; conversely, expression of these genes increased with *FGFR3* siRNA (Supplementary Fig. 9). FGFR3-mutant cells also demonstrated

decreased expression of EMT- and TGF-β–related genes compared with WT cells; again, these gene signatures were increased in cells treated with *FGFR3* siRNA (Supplementary Fig. 9). Similar to our data derived from human samples, these findings highlight the possibility of a complex relationship between the downstream impacts of *mFGFR3* and antitumor immunity, and underscore the importance of considering the totality of the immunomodulatory effects of FGFR3 inhibition.

At least two retrospective analyses of patients with *mFGFR3* UC enrolled in phase I/II trials of FGFR3 inhibitors have indicated infrequent responses to prior CPI in such patients [8,9]; however, given that the majority of patients do not respond to CPI, analyses centered on patients seeking enrollment on trials are at a risk of selection bias for CPI-progressing patients. An analysis of the IMVigor 211 phase 3 study previously revealed a relatively low response rate with atezolizumab in patients with *mFGFR3* tumors, although with overlapping 95% confidence intervals for response rate compared with FGFR3 WT tumors [10].

There are potential limitations to our study. Despite confirming that *FGFR3* alterations were associated with decreased inferred T-cell infiltration in the IMVigor dataset and decreased interferon response gene signatures in cell culture, these findings do not confirm a causal relationship. We lacked information on *FGFR3* gene fusions, but probed the relationship between *FGFR3* expression and response to CPI to try and encompass mechanisms of increased *FGFR3* signaling beyond mutations. Our cell culture data are hypothesis generating, and the lack of a tumor microenvironment in such systems precludes a comprehensive understanding of the immunomodulatory effects of FGFR3 inhibition and contextualization of changes in gene signatures largely ascribed to stromal cells in studies utilizing bulk human tumor transcriptome data (ie, EMT, TGF-β). However, we hypothesize that cross-talk likely exists between the epithelial and stromal compartments with regard to such signatures. The source of specimens from which sequencing data (eg, primary tumors vs metastases, bladder vs upper urinary tract, etc.) and intratumoral heterogeneity were derived could potentially impact our results. Further, we observed a different prevalence of *mFGFR3* in the two clinical trials, which may be at least in part related to technical differences in the next-generation sequencing approaches employed.

There are several implications of our findings: (1) Patients with mUC harboring *mFGFR3* should not be denied treatment with CPI. Despite prior analyses demonstrating an association between *mFGFR3* and decreased T-cell infiltration, and hypothesis-generating data presented in our analysis highlighting at least one potential causative mechanism underlying such observations, there is currently no direct experimental or clinical evidence for FGFR3 signaling driving CPI resistance. (2) Clinical trials evaluating combinations with CPI should probe the balance of immunomodulatory effects of FGFR3 inhibition.

Several trials combining CPI with FGFR3 inhibitors have been initiated. Despite a potentially complex relationship between *mFGFR3* and antitumor immunity, given that non–cross resistance of drugs with single-agent activity may underlie the benefit of most combination regimens [11], the finding that patients with *mFGFR3* UC are responsive to

CPI similarly to those with WT UC may provide an even greater pragmatic rationale for moving such combinations forward.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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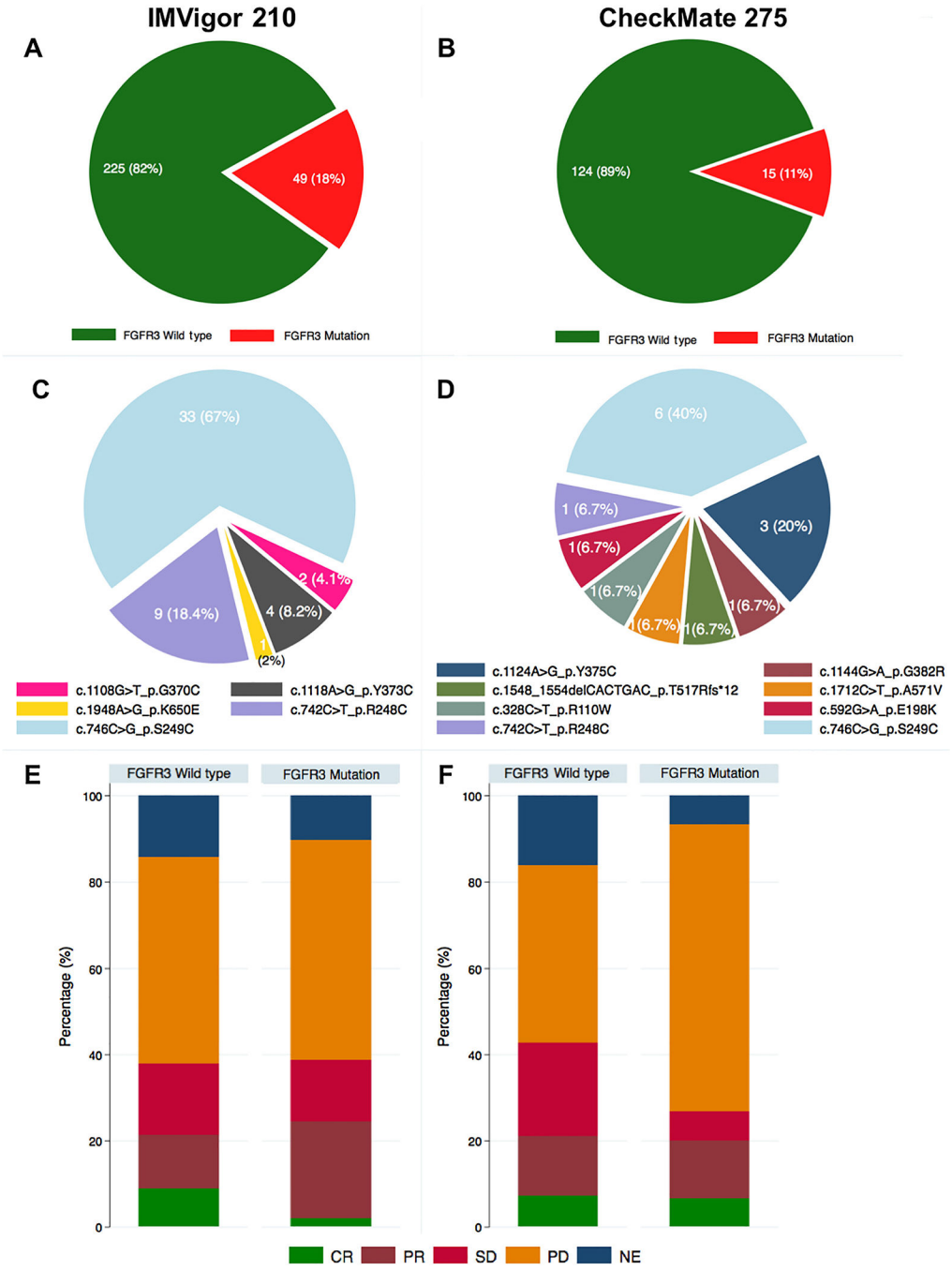

Fig. 1 –.

Association between *FGFR3* mutations and response to treatment with atezolizumab in IMVigor 210 or nivolumab in CheckMate 275 cohorts. (A) Targeted exome sequencing data were available for 274 patients (Foundation One; Foundation Medicine) from the IMVigor 210 cohort, among whom 49 had tumors harboring *FGFR3* mutations. (B) Whole exome sequencing data were available for 139 patients from the CheckMate 275 cohort, among whom 15 had tumors harboring *FGFR3* mutations. Distribution of specific *FGFR3* mutations among (C) 49 tumors in IMVigor 210 and (D) 15 tumors in CheckMate 275. (E)

Objective response rate in patients with FGFR3-mutant versus wild-type tumors with atezolizumab in the IMVigor 210 cohort. The response rates (complete and partial responses) were 24% (95% CI: 14%, 39%) and 21% (95% CI: 16%, 27%) in *FGFR3*-mutant group and wild-type group, respectively ($p = 0.8$). (F) Objective response rate to nivolumab in the CheckMate 275 cohort. When considering only known *FGFR3* hotspot mutations in the CheckMate 275 cohort, 12/139 (8.6%) harbored mutations and the objective response rate (complete and partial responses) in patients with *FGFR3-mutant* tumors was 20% (95% CI: 6%, 51%) versus 21% (95% CI: 15%, 29%) in patients with *FGFR3* wild-type tumors ($p = 0.2$). All p values are based on the chi-square test. CI = confidence interval; CR = complete response; NE = not evaluable; PD = progression of disease; PR = partial response; SD = stable disease.

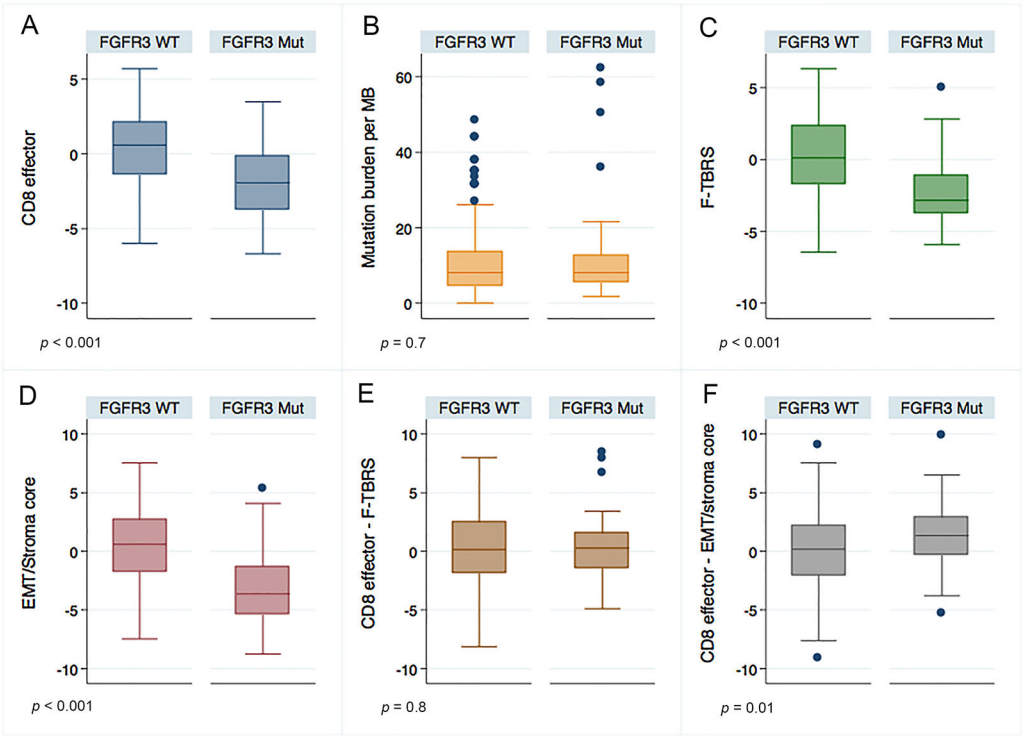

Fig. 2 –.

Relationship between *FGFR3* mutations and previously identified independent predictors of response to PD-1/PD-L1 blockade. Compared with wild-type tumors, neoplasms with *FGFR3* mutations in the IMVigor 210 cohort were associated with (A) lower CD8 T-cell gene signature expression (estimated difference of CD8 effector expression level −2.34 [95% CI: −3.14, −1.54; $p < 0.001$]), (B) no statistically significant difference in tumor mutational burden (estimated difference of mutation burden: 1.88 [95% CI: −2.09, 5.86; $p = 0.7$]), and (C) lower fibroblast TGF-β response signature (F-TBRS) (estimated difference of F-TBRS: −2.43 [95% CI: −3.21, −1.65; $p < 0.001$]) and (D) EMT/Stroma_core signature expression (estimated difference of EMT/Stroma_core: −3.63 [95% CI: −4.62, −2.64; $p < 0.001$]). The balance of CD8 T-cell gene signature expression and expression of the stromal signatures was either (E) not significantly different in the FGFR3-mutant versus wild-type tumors (F-TBRS; estimated difference 0.09 [95% CI: −0.79, 0.97, $p = 0.8$]) or (F) higher in FGFR3-mutant versus wild-type tumors (EMT/Stroma- core signature; estimated difference: 1.28 [95% CI: 0.42, 2.14; $p = 0.01$]). Gene expression is $\log_2$ transformed. All p values are based on the Wilcoxon test. CI = confidence interval; Mut = mutant; TGF-β = transforming growth factor beta; WT = wild type.