Published in final edited form as:

*Clin Genitourin Cancer*. 2016 February ; 14(1): e81–e90. doi:10.1016/j.clgc.2015.07.017.

# Molecular Signature of Response to Pazopanib Salvage Therapy for Urothelial Carcinoma

**Patrizia Pinciroli**[1,2], **Helen Won**[3], **Gopa Iyer**[4,5], **Silvana Canevari**[1], **Maurizio Colecchia**[6], **Patrizia Giannatempo**[7], **Daniele Raggi**[7], **Marco A. Pierotti**[8], **Filippo G. De Braud**[7], **David B. Solit**[2,4,5], **Jonathan E. Rosenberg**[4,5], **Michael F. Berger**[3], and **Andrea Necchi**[7]

[1]Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

[2]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY

[3]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

[4]Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY

[5]Weill Cornell Medical College, Cornell University, New York, NY

[6]Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

[7]Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

[8]Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

### Keywords

Advanced disease; Angiogenesis; Clinical benefit; Next-generation sequencing; Pazopanib

## Introduction

Progress in developing new treatments for urothelial carcinoma (UC) has been stagnant for more than 20 years. A paradigm shift is needed to advance treatment for UC. For patients with advanced disease and in whom chemotherapy regimens have failed, a variety of single-agent or combination therapies, including molecularly targeted agents, have yielded modest response rates and poor survival durations.[1,2] Although immunotherapy portends new promise, vinflunine is the only approved drug in Europe (European Medicines Agency) for progressive UC after platinum-based therapy, and the US Food and Drug Administration has not approved any agent.[3] Advancements in genomic profiling of UC, mainly through The

Address for correspondence: Andrea Necchi, MD, Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, ViaG.Venezian 1, 20133 Milano, Italy Fax: +39-02-2390-3150; contact: andrea.necchi@istitutotumori.mi.it.

**Disclosure**: The authors have stated that they have no conflicts of interest.

Supplemental Data: Supplemental tables and figure accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clgc.2015.07.017.

Cancer Genome Atlas (TCGA) project, recently made exceptional steps forward to uncover pertinent information on the underlying UC biology and potentially druggable pathways.[4] The strategy to molecularly characterize patients who had achieved an extreme response to targeted drugs recently helped investigators to put known relevant alterations into the context of a clinical benefit from those compounds.[5-7] This paradigm might best apply to anti-angiogenics, for which a proportion of approximately 5% to 10% complete or sustained responders has been reported for therapies including pazopanib.[8,9]

## Case Report

### Patients and Methods

Forty-one patients with UC in whom 1 or multiple chemotherapy regimens had failed were enrolled in an open label, phase II trial of pazopanib,[9] a multikinase inhibitor with distinct antiangiogenic activity targeting various transmembrane receptors, including vascular epidermal growth factor receptor 1-3, platelet-derived growth factor receptor (PDGFR*)* $\alpha$ and $\beta$, *c-stem cell growth factor receptor (KIT)*, and fibroblast growth factor receptor (*FGFR)*. Despite a promising 17% response rate, progression-free and overall survival were poor. Nevertheless, 2 sustained partial responses (PRs) were reported at long term follow-up.[10] On the basis of the availability of tumor samples and the quality of response, we selected 3 patients for molecular interrogation. Patient 1 had achieved a 32-month PR (Figure 1 and Supplemental Table 1 in the online version) in the third-line meta-static setting, patient 2 had stable disease of 2.7 months in the fourth-line setting, and patient 3 experienced progressive disease during second-line treatment.

DNA was extracted from formalin-fixed paraffin-embedded tumor and matched normal blood samples of each patient, and then profiled using the custom hybridization capture panel Memorial Sloan Kettering (MSK)-Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) assay for the targeted sequencing of all exons of 341 cancer-relevant genes. We analyzed the presence of somatic variants, small insertions and/or deletions, and copy number alterations. The MSK-IMPACT was analytically validated for sensitivity, specificity, reproducibility, and was able to detect variants with 10% frequency at 98% power ($\alpha = 0.05$) when coverage was at least 100×.[11] The study was approved by the internal review board and ethics committee of the Fondazione IRCCS Istituto Nazionale dei Tumori, Milano.

## Results

The mean coverage of the targeted regions in each patient's tumor ranged from 455 to 722× (Supplemental Table 2 in the online version). In the 3 patients, we found a total of 95 significant non-synonymous single nucleotide variants (SNVs), splicing events (SEs), frameshift deletions (FDs) and insertions (FI), stop gain SNVs (sg.SNVs), and upstream mutations (Supplemental Table 3 in the online version). Among these alterations, 31 were truncating mutations (FD, FI, sg.SNV, and SE) and 24 were registered in the Catalogue Of Somatic Mutations in Cancer database and were thus considered “likely deleterious” (LD) and “recurrently altered” (RA), respectively; 7 alterations were LD and RA (detailed in Supplemental Figure 1 and Supplemental Table 4 in the online version). Further-more,

patient 1 harbored the most alterations with 74 somatic events (55 genes), 34 of which were RA and/or LD (involving 25 genes). More in detail, patient 1 harbored an *ERBB3* RA mutation (R475W), an neurofibromin 1 truncation and alterations in chromatin-remodeling genes reported at high frequency in TCGA, such as multiple *lysine (K)-specific methyltransferase 2D* and *CREB binding protein* mutations, an *E1A binding protein p300* frameshift alteration, and a *lysine (K)-specific methyltransferase 2C* point mutation. Finally, patient 1 had 2 LD mutations in *mouse DNA polymerase epsilon*, which is known to be involved in DNA repair and chromosomal DNA replication.

Patient 2 also had an *AT rich interactive domain 1A frameshift and a lysine (K)-specific methyltransferase 2A* point mutation.

Mutations of mammalian target of rapamycin (*mTOR*) and erb-b2 receptor tyrosine kinase 2 (*ERBB2)* occurred at different sites in both responders; in particular, in patient 2 the *mTOR* mutation (D928N) was RA (COSM3471344) and the *ERBB2* mutation (S310F) is a hotspot driver mutation in UC that is known to be activating.[12] Importantly, we found a significant copy number amplification (CNA) of epidermal growth factor receptor (*EGFR*) with a fold-change of 17 in patient 1 (Figure 2 and Supplemental Table 5 in the online version).

The refractory case (patient 3) presented a deletion in cyclin-dependent kinase inhibitor-2A (Figure 2 and Supplemental Table 5 in the online version) and showed mutations in lysine (K)-specific demethylase 6A (*KDM6A*), stromal antigen 2 (*STAG2*), *FGFR3* (S249C) and an H1047R phosphatidylinositol-4, 5-Bisphosphate 3-Kinase, catalytic Subunit alpha (*PIK3CA*), which is an activating mutation, occurs in a hotspot in the catalytic domain, and induces the growth factor-independent proliferation.[12] Interestingly, in patient 3 we found the *FGFR3* mutation S249C, which is a known activating mutation and the most frequently observed mutation in the gene. Interestingly, the same activating mutation, associated with gene amplification, has been reported in a patient who had achieved a significant response to pazopanib in another study.[13]

Many of the mutations found in the 3 patients were validated (www.cbioportal.org; see Supplemental Table 4 in the online version)[4] or were predicted to have a deleterious effect on protein function using the PolyPhen-2 algorithm (http://www.broadinstitute.org/oncotator).

When considering the mutations specific for each patient or common among them (Supplemental Table 6 in the online version), we observed that in the responding patients 3 genes (*mTOR, FAT atypical cadherin 1*, and *ERBB2*, and only the latter 2 considering LD mutations) were mutated, an 8 genes (*nuclear factor erythroid 2-like 2, PIK3CA, FGFR3, polo-like kinase 2, folliculin, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4, KDM6A*, and *STAG2*; 7 LD) were specifically mutated in the nonresponding patient. The most altered pathways of the analyzed patients are summarized in Supplemental Table 7 in the online version. In particular, the chromatin-remodeling genes were the most frequently altered, as with previous reports.[4]

## Discussion

*EGFR, ERBB2*, and pazopanib targets (*PDGFR, KIT*, and *kinase insert domain receptor*) share the same signaling pathways, involving the *RAS-RAF* and the phosphoinositide 3-kinase (*PI3K)-AKT* pathways. These pathways were found to be heavily altered, especially at the receptor level, in all 3 patients, although for many identified alterations there were no conclusive indications about their biological effect. The most relevant alterations (LD and/or RA) included the *EGFR* CNA in patient 1, mutations in *ERBB2* in both responders, and in *FGFR3* and *phosphoinositide-3-kinase, catalytic, alpha polypeptide* in the non-responding patient. These alterations might be putative biomarkers of sensitivity to pazopanib.

To summarize, many issues affect the efforts to clinically contextualize the molecular alterations we have detected, and of course a number of biases should be acknowledged. First, and most important, it is unknown whether these molecular alterations are linked to drug response or whether they represent prognostic biomarkers independent of the treatment delivered. Second, the increasing molecular characterization of extreme re-sponders to targeted agents is also raising concerns about the clinical relevance of specific alterations (*FGFR3* mutation and response to pazopanib is paradigmatic). Third, the mechanisms underlying the activity of multitargeted tyrosine kinase inhibitors like pazopanib and the role of specific mutations have not been definitively elucidated. An important finding in our study is the association of the burden of molecular alterations with the sustained response to pazopanib. In this huge amount of complexity, the role of mutations in multiple epigenetic regulatory genes deserves special attention, because inactivating alterations in some of these genes might lead to sensitivity to histone deacetylase inhibitors, and a clinical trial with one of these compounds (ie, mocetinostat) is currently under way (NCT02236195).

## Conclusion

The functional interpretation of the altered network dynamics during pazopanib treatment and the possible *EGFR*-, *ERBB2*-, *FGFR3*-, or *PI3K*-targeting with sequences or combinations of antiangiogenic drugs and in patient-enriched study designs might deserve further investigation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported in part by funds obtained through an Italian law that allows taxpayers to allocate 0.5 percent share of their income tax contribution to a research institution of their choice.

## References

1. Sonpavde G, Sternberg CN, Rosenberg JE, Hahn NM, Galsky MD, Vogelzang NJ. Second-line systemic therapy and emerging drugs for meta-static transitional-cell carcinoma of the urothelium. Lancet Oncol. 2010; 11:861–70. [PubMed: 20537950]
2. Sonpavde G, Jones BS, Bellmunt J, Choueiri TK, Sternberg CN. Future directions and targeted therapies in bladder cancer. Hematol Oncol Clin North Am. 2015; 29:361–76. [PubMed: 25836940]

3. Bellmunt J, Theodore C, Demkov T, et al. Phase III trial of vinflunine plus best supportive care compared with best supportive care alone after platinum-containing regimen in patients with advanced transitional cell carcinoma of the urothelial tract. J Clin Oncol. 2009; 27:4454–61. [PubMed: 19687335]
4. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014; 507:315–22. [PubMed: 24476821]
5. Iyer G, Hanrahan AJ, Milowsky MI, et al. Genome sequencing identifies a basis for everolimus sensitivity. Science. 2012; 338:221. [PubMed: 22923433]
6. Van Allen EM, Mouw KW, Kim P, et al. Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. Cancer Discov. 2014; 4:1140–53. [PubMed: 25096233]
7. Wagle N, Grabiner BC, Van Allen EM, et al. Activating mTOR mutations in a patient with an extraordinary response on a phase I trial of everolimus and pazo-panib. Cancer Discov. 2014; 4:546–53. [PubMed: 24625776]
8. Sonpavde G, Pond GR, Rosenberg JE, et al. Complete response as an intermediate endpoint in patients receiving salvage systemic therapy for urothelial carcinoma. Clin Genitourin Cancer. 2015; 13:185–92. [PubMed: 25458370]
9. Necchi A, Mariani L, Zaffaroni N, et al. Pazopanib in advanced and platinum-resistant urothelial cancer: an open-label, single group, phase 2 trial. Lancet Oncol. 2012; 13:810–6. [PubMed: 22819172]
10. Necchi A, Pennati M, Zaffaroni N, et al. Analysis of plasma cytokines and angiogenic factors in patients with pretreated urothelial cancer receiving pazopanib: the role of circulating interleukin-8 to enhance the prognostic accuracy. Br J Cancer. 2014; 110:26–33. [PubMed: 24231947]
11. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn. 2015; 17:251–64. [PubMed: 25801821]
12. Young CD, Zimmerman LJ, Hoshino D, et al. Activating PIK3CA mutations induce an EGFR/ERK paracrine signaling axis in basal-like breast cancer. Mol Cell Proteomics. 2015; 14:1959–76. [PubMed: 25953087]
13. Palma N, Morris JC, Ali SM, Ross JS, Pal SK. Exceptional response to pazopanib in a patient with urothelial carcinoma harboring FGFR3 activating mutation and amplification. Eur Urol. 2015; 68:168–70. [PubMed: 25766722]

### Clinical Practice Points

- The mechanisms that underlie the therapeutic response of urothelial carcinoma (UC) to targeted drugs remain largely unclear.
- We sequenced archival tissue from 3 outlier patients who had been treated with pazopanib after chemotherapy treatment had failed: a 32-month partial response (patient 1) in the third-line setting, a 3-month stable disease (patient 2) in the fourth-line setting, and a primary refractory case (patient 3). Tumor and matching germline DNA were sequenced using a targeted next-generation deep sequencing assay to identify somatic variants, small insertions or deletions, and copy number alterations.
- In responding patients we found a significant focal epidermal growth factor receptor copy number amplification and an erb-b2 receptor tyrosine kinase 2 *(ERBB2)* S310F activating mutation. *ERBB2* mutations occurred at different sites in patients 1 and 2, and multiple alterations in chromatin-remodeling genes.
- Patient 3 showed mutations in fibroblast growth factor receptor 3 *(FGFR3)* and *phosphoinositide-3-kinase, catalytic, alpha polypeptide*, which could attenuate response to pazopanib.
- In summary, we reported peculiar genomic alterations that might be putative variants of differential sensitivity to pazopanib in advanced UC.

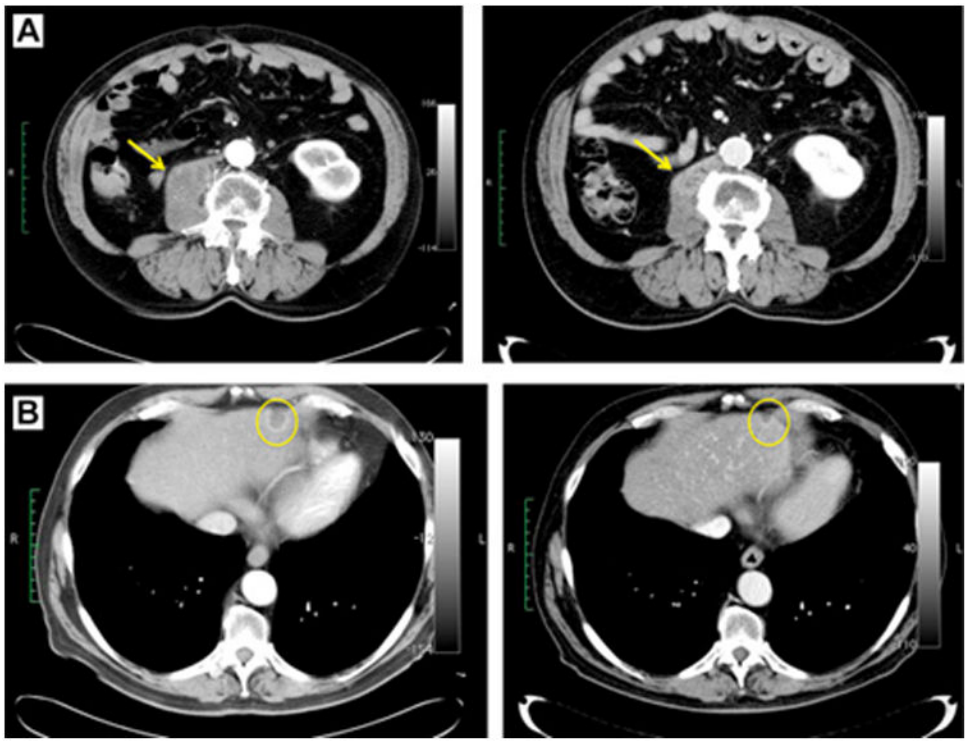


**Figure 1.**

Computed Tomography Scans Showing the Best Response in (A) Retroperitoneal and (B) Hepatic Disease of Patient 1. Left Panels: Baseline Examination; Right Panels: Best Response (Achieved After 4 Weeks of Pazopanib Treatment)

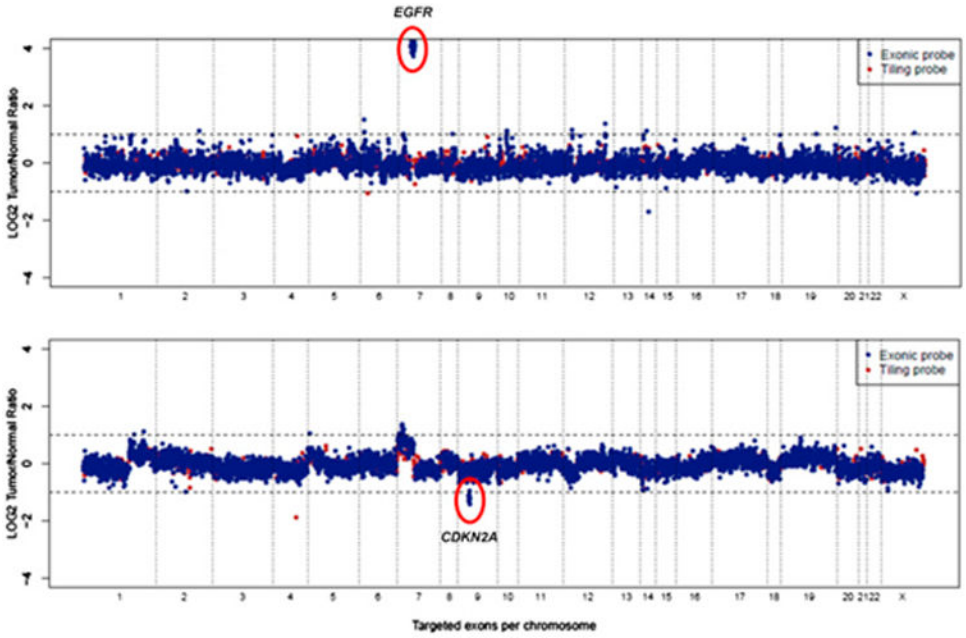


**Figure 2.**

Copy Number Alterations in Patients 1 and 3 (Top and Bottom Panel, Respectively). Each Dot Represents a Target Exon. The Genomic Regions Affected by Significant Copy Number Amplification (*EGFR* in Patient 1) and Deletion (*CDKN2A* in Patient 3) Are Highlighted in Red