Published in final edited form as:

Am Soc Clin Oncol Educ Book. 2013 ; : 195–199. doi:10.1200/EdBook_AM.2013.33.195.

Targeting Molecular Aberrations in Urothelial Carcinoma: Are We Almost There?

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OVERVIEW

Advances in tumor biology and cancer genetics have led to the development of effective targeted therapies in oncology over the past decade. However, targeted drug development for urothelial carcinoma has been slower than for some other malignancies. The path forward in drug development is through a better understanding of the aberrant pathways driving urothelial tumor development. Steady progress has been made in the characterization of genomic alterations in urothelial carcinoma. The Cancer Genome Atlas (TCGA) project is well underway in the analysis of a large set of urothelial cancer specimens using multiple approaches and technologies. In addition, there are already many well-established mutations and genetic alterations in urothelial carcinoma that likely contribute in an important way to tumor development. In addition, urothelial cancer genome-wide association studies have identified common variants associated with urothelial cancer risk and protein expression that can potentially be therapeutically targeted. Furthermore, the MET pathway has emerged as an exciting target in multiple tumors, including urothelial carcinoma. Our knowledge of how to clinically target many emerging molecular aberrations in urothelial cancer is still in the early stages of development. However, there is much promise in the ongoing research being conducted in urothelial cancer molecular pathogenesis.

Urothelial carcinoma of the bladder is a common malignancy, causing approximately 15,000 deaths per year in the United States and 150,000 deaths per year worldwide. However, targeted drug development for urothelial carcinoma has been slower than that seen in some other malignancies such as melanoma, chronic myeloid leukemia (CML), gastrointestinal stromal tumors (GIST), and lung adenocarcinoma. The discovery of vemurafenib for melanoma tumors that harbor the B-Raf V600E mutations and the inhibition of ABL and c-Kit by imatinib for CML and GIST, respectively, have significantly changed clinical practice.

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Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked "L" indicate leadership positions. Relationships marked "I" are those held by an immediate family member; those marked "B" are held by the author and an immediate family member. Relationships marked "U" are uncompensated.

Employment or Leadership Position: None. **Consultant or Advisory Role:** David J. Kwiatkowski, Millennium; Novartis. **Stock Ownership:** None. **Honoraria:** David J. Kwiatkowski, Millennium. **Research Funding:** None. **Expert Testimony:** None. **Other Remuneration:** None.

Unfortunately, no drug has been approved by the United States Food and Drug Administration (FDA) for urothelial cancer in more than two decades. Other genitourinary malignancies such as renal cell carcinoma and prostate cancer have had more than a dozen new agents approved by the FDA in the past 7 years (Table 1). It is through our better understanding of the aberrant pathways driving tumor progression in renal cell carcinoma through the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways and in prostate cancer through the androgen receptor that we have made these therapeutic advances.

Urothelial cancer is a heterogeneous disease driven by a diverse array of molecular alterations; however, we still have not discovered a drug or combinations of drugs that interfere with the critical driver events involved in the development of urothelial cancers. Clinical trials using targeted agents known to be effective in other tumors have been disappointing to date in urothelial carcinoma with uniform low response rates (Table 2). Efforts are ongoing to identify important molecular targets and test novel therapeutics in clinical trials.

The National Cancer Institute (NCI) and the National Human Genome Research Institute have developed the TCGA project in 2006 with the goal of creating a genomic atlas of human cancer. The goal of the TCGA effort is to define the genetic and molecular characteristics of all common, and a number of less common, cancers. Arguably, the most important secondary goal of the TCGA project is to identify novel driver mutations of cancer development that can be targeted therapeutically. The TCGA project on urothelial carcinoma is well underway in the analysis of a large (> 100) set of specimens using multiple approaches and technologies with many new findings. There is an expected major publication on the TCGA findings in urothelial carcinoma later in 2013, and there is no doubt that many new potential targets for directed therapy will be unveiled at that time.

Apart from TCGA effort, there has been steady progress in the characterization of genomic alterations in urothelial carcinoma, dating back to the discovery of the first oncogene, HRAS, in a urothelial cancer cell line more than 3 decades ago.¹ Several recent discoveries are notable. Gui et al described whole-exome sequencing of nine urothelial carcinoma specimens, with confirmatory studies of novel genes with mutations in a larger set of 88 urothelial carcinoma samples.² Mutations were frequently (13% to 21%) identified in multiple genes involved in chromatin remodeling, including histone demethylases (*UTX*), histone acetyltransferases (*CREBBP*, *EP300*), a SWI/SNF-related gene (*ARID1A*); and less commonly in histone methyltransferases (*MLL*, *MLL3*) and others. For all of these genes, most of the mutations identified were clearly inactivating, suggesting that it was the genes' loss of function that was important in urothelial carcinoma development. Chromatin remodeling genes play critical roles in the regulation of the epigenetic structure of chromatin. They have various activities including "writing" of covalent histone modifications (acetylation, methylation), "erasing" of such modifications (deacetylases, demethylases), insulating chromatin regions from modification or expression, and remodeling the three dimensional structure of chromatin to either enhance or repress transcription. The report by Gui et al and the TCGA findings indicate that mutations in chromatin regulator genes are very common, perhaps nearly universal in urothelial

carcinoma, and seen at a higher rate than perhaps any other common adult malignancy.² Hence, these alterations are very likely to contribute in an important way to urothelial carcinoma development by altering gene expression in a global manner. Unfortunately, our knowledge of how to clinically target mutations in these chromatin regulator genes is at a very early stage, and it is likely that each specific alteration will require a different approach.

PIK3CA, TSC1, and most recently TSC2 (the latter two genes are the cause of the human tumor suppressor gene syndrome tuberous sclerosis complex [TSC]) are recognized to be mutated in a collective 20% to 30% of urothelial carcinoma (COSMIC [www.sanger.ac.uk/genetics/CGP/cosmic/] and TCGA data).³ These mutations have common effects in activating mTOR, a master kinase that regulates cell growth through enhancement of protein synthesis and other effects. Hence, patients with urothelial carcinoma with mutations in these genes are potential candidates for therapy targeting mTOR (for TSC1, TSC2 mutations) or more directly PIK3CA for mutations in that gene. Multiple drugs that target PI3K are in current clinical development for diverse cancers (e.g., ZSTK474, GDC-0941), and it is hoped that these drugs will show effectiveness for PIK3CA-mutant urothelial carcinoma. Notably, PIK3CA mutations in urothelial carcinoma are primarily in the central PIK domain, not H1047R, and effectiveness of these agents for that category of mutation will require further evaluation. Recently Iyer et al reported that a single patient on a clinical trial of everolimus (mTOR inhibitor) for urothelial carcinoma had a sustained complete response lasting more than 2 years.⁴ This patient's cancer was found to have mutations in both TSC1 and NF2, as well as other genes. At least four other patients on this clinical trial also had TSC1 mutations and transient minor responses to everolimus. It is unknown what caused the dramatic response of the single complete responder. Everolimus has been shown to be effective for the treatment of several tumors that develop in TSC and for PEComas—a type of sarcoma in which TSC1/TSC2 gene mutations are common. Complete loss of either TSC1 or TSC2 is known to occur in TSC tumors. Hence, collectively these observations suggest that the TSC1 mutation was an important factor in the complete response to everolimus by the patient with urothelial carcinoma.

Urothelial carcinoma has been known for many years to have a wide variety of genomic copy number changes, including loss of chromosome 9p containing the CDKN2A gene and amplification of multiple genomic regions. We have recently used multiplex inversion probe analysis to perform a genome-wide screen for copy number alterations in urothelial cancer. We then selected nine genomic regions of amplification and developed a multiplex ligation-dependent probe analysis (MLPA) assay, which was then used on a set of both early-stage and more advanced-stage urothelial carcinomas. One or more amplification events for one of the nine genomic regions were seen in only two of 23 (9%) Ta grade 1 or grade 1 to 2 cancers. In contrast, one or more amplifications were seen in 11 of 20 (55%) Ta grade 3 cancers and in 20 of 41 (49%) T2 grade 2 cancers ($p = 0.0020$ and 0.0011 , respectively). Furthermore, amplification of the E2F3/SOX4 region alone was also seen more commonly in the Ta grade 3 and T2 grade 2 cancers (11 of 61) than in Ta grade 1 or 1 to 2 cancers (0 of 23, $p = 0.03$). Several of the genomic regions analyzed for amplification by MLPA included genes that are potential therapeutic targets: CCND1 (CDK4 inhibitors), ERBB2 (lapatinib, trastuzumab), and MDM2. These preliminary results suggest that analysis of these nine

genomic regions might have use as a prognostic and therapeutic marker in urothelial carcinoma, although further study and validation is required.

Urothelial cancer genome-wide association studies have identified common variants associated with urothelial cancer risk.^{5,6} A missense variant rs2294008 in the prostate stem cell antigen (PSCA) gene showed consistent association with urothelial cancer. Resequencing of the PSCA genomic region showed that rs2294008 is the only common missense single nucleotide polymorphism in PSCA, identifying rs2294008 as a new urothelial cancer susceptibility locus. We have found that the genetic variant rs2294008 is a strong predictor of PSCA protein expression in both non-muscle-invasive and muscle-invasive urothelial tumors.⁷ A monoclonal antibody against PSCA has emerged as a novel cancer therapy currently being tested in clinical trials for prostate and pancreatic cancers. Clinical studies of urothelial carcinoma are needed to validate PSCA as a therapeutic target. This presents an exciting opportunity to target a genomic variant to assess therapeutic efficacy.

The c-MET proto-oncogene encodes the tyrosine kinase receptor of the hepatocyte growth factor (HGF), which modulates expression of cytoskeletal proteins, cell-cycle regulators, matrix metalloproteinases, and antiapoptotic effectors. HGF dysregulation is associated with tumor growth, metastasis, and invasion.^{8,9} Oncogenesis, tumor angiogenesis, and metastasis are driven by dysfunctional pathway activation through MET gene amplification, overexpression, mutation, or autocrine loop formation in many tumors, including urothelial cancer.^{9–13} MET expression has been associated with a worse prognosis in many tumors and may be associated with more aggressive urothelial tumors. In one preclinical study, expression of MET correlated with differentiation in five urothelial cancer cell lines in the absence of point mutations. In addition, expression of MET in primary urothelial cancer of the bladder (142 patients) was positively associated with histologic grade, stage classification, tumor size, and nodular tumor growth ($p = 0.002$, 0.004 , 0.01 , and 0.02 , respectively).¹⁰ Indicators for poor long-term survival were invasive cancer, multiple tumors, and MET overexpression ($p = 0.0006$, 0.01 , and 0.04 , respectively). In these studies, the MET protooncogene demonstrated that it plays an important role in the progression of bladder carcinogenesis. Cordon-Cardo et al demonstrated the prognostic value of MET expression in urothelial bladder cancer using antibody arrays.¹⁴ The serum protein, MET, was top ranked in identifying urothelial carcinoma and was associated with pathologic stage and grade ($p < 0.001$) and survival ($p = 0.044$) when validated by immunohistochemistry of tissue microarrays containing urothelial tumors (173 patients). Hypoxia has been shown to result in the upregulation of both MET and VEGF, and activated MET can indirectly promote angiogenesis and tumor growth by upregulating VEGF expression and IL-8.^{15–17} Evidence also supports cooperative roles for MET and VEGFR2 in the proliferation and survival of the tumor endothelium.¹⁷ Inhibition of MET can amplify the effects of VEGFR blockade and leads to rapid, robust, and progressive regression of tumor vasculature, increased intratumoral hypoxia and apoptosis, and reduced tumor invasiveness and metastasis in vivo.^{18,19} Thus, simultaneous inhibition of both MET and VEGFR2 should result in near-complete inhibition of major survival pathways induced by hypoxia. Cabozantinib (XL184) is an oral agent that inhibits multiple receptor tyrosine kinases, primarily targeting MET and VEGFR2. Cabozantinib has shown activity in multiple solid

tumors in phase I, II, and III clinical trials. Preclinical data supports the use of cabozantinib in urothelial cancer.²⁰ Currently, there is an ongoing phase II trial of cabozantinib for patients with advanced urothelial cancer.

There are currently over 100 oncology therapeutic clinical trials using MET inhibitors. The Center for Cancer Research (CCR) at the NCI maintains a webpage of these trials: <https://ccrod.cancer.gov/confluence/display/CCRHGF/Home>. Targeting the HGF/MET signaling pathway is an exciting approach to inhibiting tumor growth and metastases.

CONCLUSION

The current emphasis in oncology drug development is on targeted therapies that exploit the vast amount of knowledge we are acquiring from tumor pathogenesis. Current available therapies targeting molecular aberrations in other tumors have not demonstrated significant efficacy as single agents in urothelial cancers. The development of effective therapies for this devastating disease remains dependent on the progress we make in scientific discovery. Furthermore, it appears clear from the existing genomic datasets that the diversity of mutations seen in urothelial carcinoma will require personalized targeted therapy—similar to lung adenocarcinoma, in which multiple different agents are used for different mutation subsets. Hence, going forward, we can anticipate that targeted clinical trials for urothelial carcinoma will be designed in a mutation-specific manner.

So are we almost there? No, but we are well on our way.

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KEY POINTS

- Targeting molecular aberrations has revolutionized clinical management of multiple cancers, but progress in urothelial cancer has been relatively slow.
- Clinical trial results of targeted therapies in urothelial cancer have been negative when applied to the urothelial cancer population in a nonspecific manner.
- Urothelial cancer is a heterogeneous disease that is driven by a diverse array of molecular alterations.
- Personalized therapy for specific molecular targets is likely to be the way forward in urothelial carcinoma and will require cooperative multi-institutional studies to have enough patients for each molecular subset of disease.

TABLE 1.

FDA-Approved Drugs for Genitourinary Tumors in the Past 7 Years

Year of FDA Approval	Renal Cell Carcinoma	Prostate Cancer	Urothelial Cancer
2006	Sunitinib		
2007	Temsirolimus		
	Sorafenib		
2008		Degarelix	
2009	Everolimus		
	Bevacizumab		
	Pazopanib		
2010		Sipuleucel-T	
		Cabazitaxel	
2011		Abiraterone	
		Denosumab	
2012	Axitinib	Enzalutamide	

Abbreviation: FDA, U.S. Food and Drug Administration.

TABLE 2.
Selected Clinical Trials of Single-Agent Targeted Therapy with Metastatic Urothelial Cancer

Author	Line	Agent	Target	No.	RR %	OS Months
Bellmunt ²¹	First	Sunitinib	EGFR, VEGFR-1/2, c-KIT, PDGFR a/b, FLT3, and RET	38	8	8.1
Quinn ²²	First	Eribulin	tubulin	37	38	9.4
Sridhar ²³	First	Sorafenib	B-Raf, c-Raf, VEGFR-2/3, PDGFR-b	17	0	5.9
Gomez-Abuin	Second	Bortezomib	Proteasome inhibitor	20	0	NR
Wulfing ²⁴	Second	Lapatinib	HER1 and HER2	59	3	4.5
Petrylak ²⁵	Second	Gefitinib	EGFR	31	3	3
Dreicer ²⁶	Second	Sorafenib	B-Raf, c-Raf, VEGFR-2/3, PDGFR-b	27	0	6.8
Gallagher ²⁷	Second	Sunitinib	EGFR, VEGFR-1/2, c-KIT, PDGFR a/b, FLT3 and RET	45	7	6.9
Twardowski ²⁸	Second	Aflibercept	VEGF, PDGF	22	4.5	NR
Milowsky ²⁹	Second	Everolimus	PI3K/Akt/mTOR	45	5	10.5
Necchi ³⁰	Second	Pazopanib	VEGFR-1/2/3, PDGFR a/b, and c-KIT	41	17.1	4.7
Milowsky ³¹	Second	Dovitinib	FGFR3	44	3	NR

Abbreviations: EGFR, epidermal growth factor receptor; FGFR3, fibroblast growth factor receptor 3; FLT3, Fms-like tyrosine kinase 3; mTOR, mammalian target of rapamycin; NR, not reported; OS, overall survival; PDGFR, platelet-derived growth factor receptor; RR, response rate; VEGFR, vascular endothelial growth factor receptor.