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## Urachal Carcinoma Shares Genomic Alterations with Colorectal Carcinoma and May Respond to Epidermal Growth Factor Inhibition

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## Abstract

Metastatic urachal carcinoma is a rare, understudied, and aggressive malignancy with limited treatment options. Histologically, urachal carcinomas resemble enteric adenocarcinomas and anecdotally respond to systemic therapies utilized in colorectal cancer. Targeted exome sequencing of archival primary tumor tissue from a patient with metastatic urachal cancer revealed *EGFR* amplification and wild-type *KRAS*. The patient was treated with cetuximab, a monoclonal antibody directed against EGFR, as a single agent, and achieved a response lasting more than 8 mo. Subsequent whole-exome sequencing revealed no additional alterations likely to be associated with cetuximab sensitivity. Formalin-fixed, paraffin-embedded tumor specimens from nine additional urachal cancers were subjected to targeted exome sequencing. Mitogen-activated protein kinase (MAPK) pathway mutations were found in four of the nine samples, but no *EGFR* amplification was detected. Importantly, *APC* mutations were detected in two of the nine patients. To our knowledge, this is the first report of a response to single-agent cetuximab in a patient with metastatic urachal cancer and of molecular analysis to probe the basis for sensitivity. On the basis of these findings and the histologic, and now genomic, similarities with colorectal cancer, monoclonal antibodies directed at EGFR could be used in the treatment of metastatic urachal cancer.

**Patient summary**—Urachal cancers are morphologically and genomically similar to colon adenocarcinomas and may respond to drugs targeting the epidermal growth factor receptor.

## Keywords

Urachal carcinoma; Genomic alterations; Epidermal growth-factor; Inhibition

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Urachal cancer is an aggressive bladder malignancy arising from a vestigial remnant. Patients with metastatic urachal cancer have poor prognosis, with median survival of ~1.3 yr [1]. Given the rarity of urachal cancer, prospective trials to guide the treatment of patients with metastatic disease are lacking, there are no standard chemotherapeutic regimens, and management strategies are empiric and highly institution-dependent. Histologically, urachal cancer resembles enteric adenocarcinoma and anecdotally may respond to chemotherapy used to treat colorectal cancer [1]. Comprehensive molecular analyses of urachal cancer are lacking; however, an analysis of *KRAS* and *BRAF* mutations in urachal cancer previously identified *KRAS* mutations in four out of seven specimens [2].

A 35-yr-old male presented with metastatic urachal cancer to the lungs. A partial cystectomy was performed as palliative treatment for severe hematuria. Pathological analysis confirmed the diagnosis of mucinous urachal carcinoma, and the possibility of colorectal adenocarcinoma invading the bladder was excluded. He was subsequently treated with two cycles of gemcitabine-FLP (5-fluorouracil, leucovorin, cisplatin) that resulted in transient disease stabilization. However, treatment was discontinued because of severe treatment-related toxicities including fatigue, nausea, vomiting, and diarrhea. Paclitaxel plus carboplatin was then administered but was discontinued owing to disease progression (Fig. 1A). Given the lack of treatment options, targeted exome sequencing was performed on archival tissue from the primary tumor (Foundation One; Foundation Medicine, Cambridge, MA, USA) and revealed *EGFR* amplification and wild-type *KRAS* (Table 1). On the basis of

these findings, the patient was started on treatment with the anti-EGFR monoclonal antibody cetuximab, and experienced a 25% decrease in tumor burden that lasted for more than 8 mo (Fig. 1B). Treatment was generally well tolerated with the exception of an acneiform rash that was treated with doxycycline and topical steroids.

Anti-EGFR antibodies have been approved by regulatory agencies for use in metastatic colorectal cancer since 2004 [3]. However, in 2009, on the basis of emerging evidence demonstrating that activating *KRAS* mutations (resulting in constitutively activated MAPK signaling downstream of *EGFR*) conferred resistance to treatment, the US Food and Drug Administration restricted use of anti-EGFR antibodies to patients with wild-type *KRAS* tumors [4,5]. The National Comprehensive Cancer Network guidelines extended this restriction to patients with wild-type *KRAS* and *NRAS* tumors. Activating mutations in several other genes downstream of *EGFR*, such as *BRAF* and *PIK3CA*, have also been associated with EGFR inhibitor resistance in some, but not all, studies and therefore have not yet been fully integrated into standard clinical decision-making [6]. Predictors of sensitivity have proven even more elusive; analyses using model systems and human specimens have identified putative treatment response biomarkers such as *EGFR* amplification, alterations in the tyrosine kinase adaptor gene *IRS2*, and increased EGFR ligand expression [7–9].

To further explore the mechanistic underpinnings of cetuximab sensitivity in our patient with wild-type *KRAS* urachal cancer, we first confirmed the *EGFR* amplification by fluorescent in situ hybridization (FISH; Fig. 2A). Wholeexome sequencing (WES) subsequently performed on the primary tumor to define the pattern of co-altered genes revealed 54 nonsynonymous single-nucleotide variants (SNVs), 106 segmental amplifications (28 segments with estimated copy number 6) and 36 segmental deletions (12 segments with homozygous deletions) (Fig. 2B). No additional alterations known to be associated with sensitivity or resistance to anti-EGFR antibodies were identified. Notably, a homozygous *APC* deletion was identified (Supplementary methods).

We next sought to explore the prevalence of mutations downstream of *EGFR*, determine if *EGFR* amplification is a common genomic event, and identify additional novel putative therapeutic targets in urachal cancer. We identified archival formalin-fixed, paraffin-embedded tumor specimens from nine additional patients treated at our institution during 2000–2014. Slides were reviewed by an expert genitourinary pathologist (M.C-M.) and targeted exome sequencing was performed using the MSK-IMPACT platform [10] (Table 1 and Supplementary Fig. 1). In addition, FISH was performed to evaluate *EGFR* amplification. There were no *EGFR* amplifications detected by next-generation sequencing analysis or FISH. Mutations in the MAPK pathway were present in four of the nine tumors: two *KRAS* mutation (G13D, G12 V), one *NRAS* mutation (Q61K), and one *MAP2K1* (C121S) kinase domain alteration (Table 1). Importantly, two of the nine tumors had *APC* truncating mutations (R1450\*, R554\*) that are frequently found in colorectal adenocarcinoma. Seven of nine tumors had *TP53* mutations (2 truncating mutations, 1 in-frame mutation, and 4 missense mutations). Inactivation of tumor suppressor genes such as *TP53* is a prototypic event in the cancer genome heretofore thought to be undruggable,

although multiple efforts to target *TP53* mutant tumors are in preclinical and early-phase clinical development [11].

Several case reports for patients with urachal cancer have described responses to chemotherapy regimens that are effective in patients with colorectal cancer [1,12]. However, to the best of our knowledge this is the first report describing a response to single-agent cetuximab and probing of the molecular basis for sensitivity. Level I evidence, developed in the context of prospective randomized controlled trials, is the gold standard to guide the care of patients with cancer given the often narrow therapeutic index, modest benefits, and high cost of many systemic anticancer therapies. However, there are a multitude of pragmatic challenges associated with the generation of high-level evidence for the care of patients with rare cancers, including inadequate understanding of disease pathogenesis, lack of model systems, insufficient interest/funding from key stakeholders in drug development, and the absence of an international infrastructure to ensure adequate trial accrual. Alternative approaches to evidence development, such as umbrella and *n*-of-1 trials, may ultimately prove beneficial [13]. In the meantime, use of all the tools currently available is critical for optimizing the care of patients in need of better therapies. Whether the presence of EGFR amplification or the lack of a *KRAS* mutation, or both, conferred sensitivity to cetuximab in our patient remains unknown. However, given the histologic, and now genomic, similarities between urachal and colonic adenocarcinoma (eg, presence of *APC* mutations) and the standard role of *KRAS* mutations in predicting lack of benefit for EGFR monoclonal antibodies in colorectal cancer, EGFR monoclonal antibodies with or without chemotherapy should be considered a potential treatment strategy for metastatic wild-type *KRAS* urachal cancers. Multicenter and multinational collaborations are needed to validate these findings and advance the care of patients with this rare and understudied malignancy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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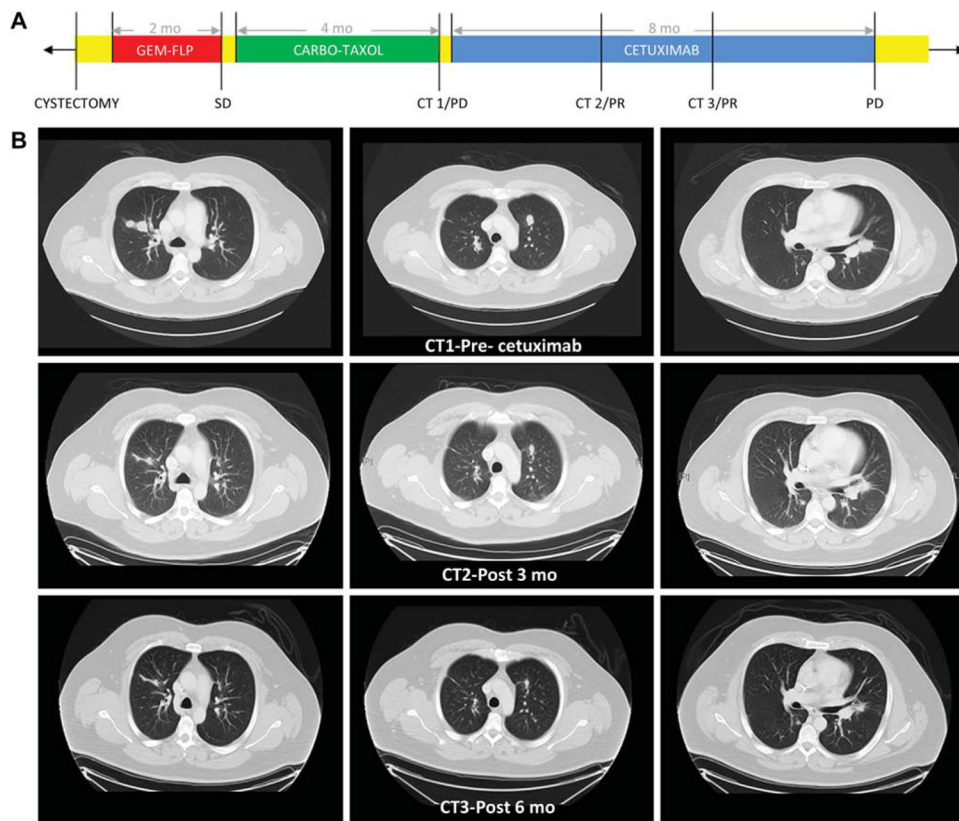
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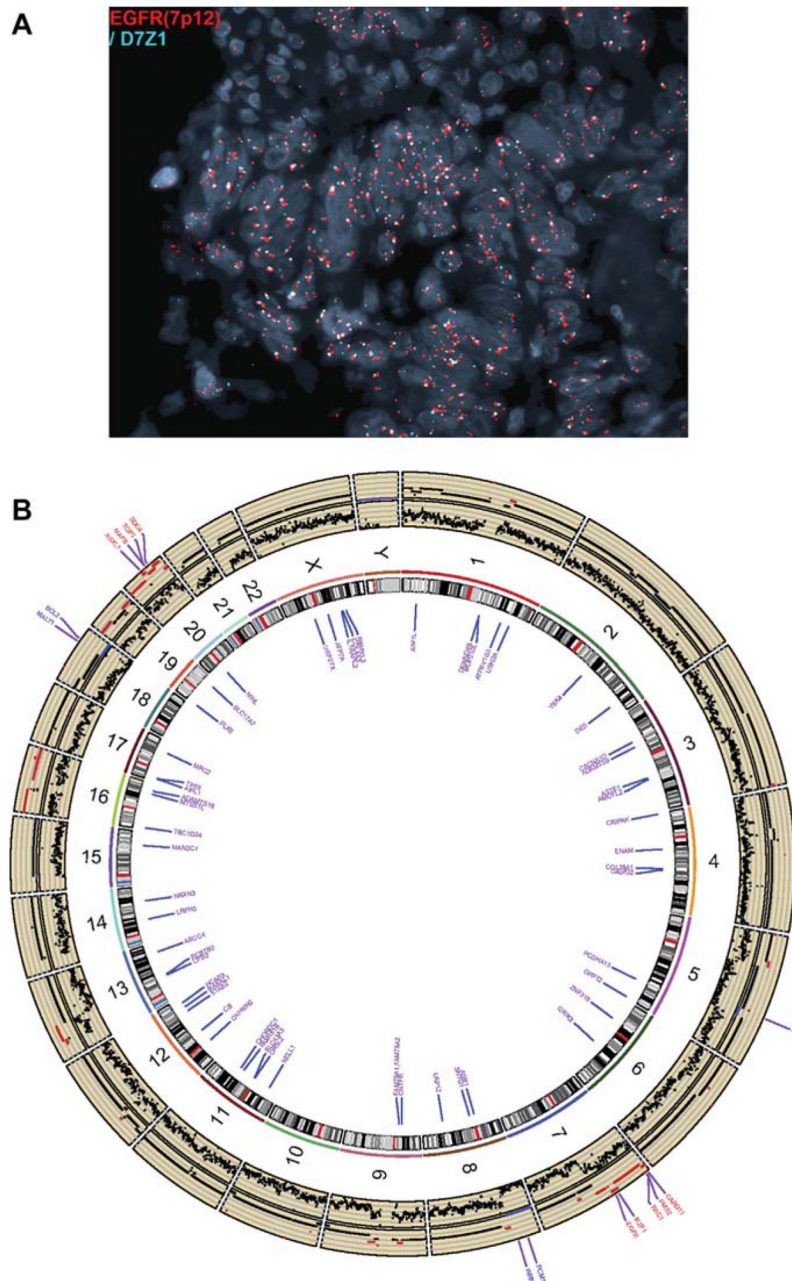
## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.eururo.2016.04.037>.



**Fig. 1.** Clinical information for the index patient. (A) Timeline demonstrating treatment course. CT = computed tomography scan; SD = stable disease; PR = partial response; PD = progression of disease. Yellow represents periods without chemotherapy treatment. (B) CT images of lung metastases before and after 3 and 6 mo of cetuximab treatment.





**Fig. 2.** Molecular alterations observed for the index patient. (A) Fluorescent in situ hybridization confirming EGFR amplification (6 copies of EGFR [orange] in the absence of multiple copies of centromere 7 [green]). (B) Circos plot of copy number variant (CNV) and single nucleotide variant (SNV) data. The raw CNV data (log ratio of read number) are plotted in the first track outside the ideogram. The estimated absolute copy number (CN) is plotted in the second track (blue represents CN = 0 and red represents CN = 4). Known cancer genes [9] with CN = 0 (blue) or CN = 6 (red) are labeled in the next track. Genes with

nonsynonymous SNVs are labeled in the track inside the ideogram in purple color. Supplementary Tables 1–4 provide complete gene lists for CNV and SNV data.

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Histology and genomic alterations in *APC*, *TP53*, and *PI3K* pathway genes as determined by next-generation sequencing in a cohort of patients with urachal cancer and by whole-exome sequencing in the index patient with urachal cancer

Table 1

Gene	Frequency (%)	Patients										
		Index	1	2	3	4	5	6	7	8	9	
<i>TP53</i>	80	W47del <sup>a</sup>	R213* <sup>b</sup>	L257Rfs*6 <sup>b</sup>	H179del <sup>a</sup>	R248Q <sup>c</sup>	G266R <sup>c</sup>	R248W <sup>c</sup>	R175H <sup>c</sup>			
<i>APC</i>	20	DEL			R1450*					R554* <sup>b</sup>	Q1406Efs*2 <sup>b</sup>	
<i>KRAS</i>	20					G13D <sup>c</sup>	G12V <sup>c</sup>					
<i>NRAS</i>	10									Q61K <sup>c</sup>		
<i>RASAL1</i>	10	R342H <sup>c</sup>										
<i>EGFR</i>	10	AMP										
<i>MAP2K1</i>	10									C121S <sup>c</sup>		
<i>MAP3K19</i>	10	R139Q <sup>c</sup>										
<i>GNAS</i>	10									R201C <sup>c</sup>		
<i>PIK3CA</i>	10		E545K <sup>c</sup>									
<i>MET</i>	10								AMP			
<i>ERBB3</i>	10						P583S <sup>c</sup>					
<i>ERBB4</i>	10		X497_Splice <sup>b</sup>									
<i>ERBB2</i>	0											
<i>HRAS</i>	0											
<i>BRAF</i>	0											
<i>RAF1</i>	0											
<i>NFI</i>	0											
Histology		Mucinous	Mucinous	Mucinous	Mucinous	Mucinous	Mucinous	Enteric	SRC	ADC	Mucinous	Mucinous

DEL = deletion; AMP = amplification; SRC = signet ring cell; ADC = adenocarcinoma not otherwise specified.

<sup>a</sup>In-frame mutation.

<sup>b</sup>Truncating mutation.

Missense mutation.

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