Comprehensive Genomic Analysis of Metastatic Mucinous Urethral Adenocarcinoma Guides Precision Oncology Treatment: Targetable EGFR Amplification Leading to Successful Treatment With Erlotinib

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Introduction

Malignancies of the male urethra are relatively rare, with an approximate rate of 1.3 per million people. They are most often of squamous-cell or transitional-cell histology, with adenocarcinoma composing only about 5% of male urethral cancers. Mucinous adenocarcinoma of the male urethra (MAU) is extremely rare, with only a handful of cases reported in the literature since 1961. A recent study of primary urethral cancers in men and women found no association between histology and overall survival, but other work focusing only on men reported better overall survival among men with urethral adenocarcinoma than those with other histology types. These MAU most commonly occur in the prostatic urethra and must be distinguished from mucinous adenocarcinoma of the prostate, bladder, or colon.

A result of the rarity of this diagnosis is that experience in systemic therapy is lacking. Clear treatment options exist for primary malignancies of the bladder, prostate, and colon, as well as for urothelial-type or squamous-cell cancers of the urethra. At best, systemic therapy for MAU must be an extrapolation from one of these other malignancies.

Advances in genomics offer some hope for rare cancers because the genetic underpinnings of the malignancy can provide a rationale for treatment selection. To date, there is no published data on genome sequencing of MAU. Herein we describe a case of a man with MAU that was analyzed by comprehensive genomic profiling, along with subsequent treatment outcomes. Furthermore, we identified 6 additional cases of MAU treated at Mayo Clinic and confirmed in archival tissue that the targetable epidermal growth factor receptor (EGFR) overexpression observed in our patient was also present in these 6 cases.

Case Report

Standard of Care

The patient initially presented at 67 years old with a 10-year history of prostate cancer that was treated with brachytherapy. He then presented with urinary hesitancy and a weak stream, and urinalysis revealed atypical cells. A computed tomographic scan of the abdomen and pelvis revealed nothing abnormal, but cystoscopy demonstrated a stricture of the membranous urethra. Biopsy was performed, and samples were obtained from the stricture, from the prostate, and throughout the bladder.

Biopsy results of the bladder and prostate were benign, but the urethral biopsy revealed a high-grade glandular dysplasia/adenocarcinoma-in-situ in perurethral glands. Cytokeratin 20 was expressed in most of the glands, and cytokeratin 7 was negative. There was no expression of p63, but p53 was expressed in most of the glands. Prostate-specific antigen and Papanicolaou staining was negative. A screening colonoscopy to rule out a colon primary malignancy was normal.

The patient underwent a segmental resection of the urethra with subsequent surveillance. After 8 months, surveillance imaging demonstrated new retroperitoneal lymphadenopathy, with a needle biopsy confirming metastatic disease. The patient was then treated with 6
cycles of gemcitabine and cisplatin on a 21-day cycle with complete response, at which point chemotherapy was discontinued. Therapy was complicated by peripheral neuropathy and progressive neutropenia. Unfortunately, recurrent disease was noted on the first posttreatment computed tomographic scan at 2 months. Single-agent gemcitabine was then resumed, with no response.

The patient was then enrolled onto a clinical study providing integrated genomic and transcriptomic analysis of advanced malignancies. Materials and Methods for this study are located in the Supplemental Data in the online version. To that end, we chose a bilateral pelvic lymphadenectomy and extended left retroperitoneal lymphadenectomy for tissue acquisition and debulking of measurable disease. Pathology again demonstrated a poorly differentiated mucinous adenocarcinoma. Unfortunately, imaging 1 month later again demonstrated further progressive disease, and we began treatment with dose-reduced capecitabine 1000 mg by mouth twice daily, and intravenous oxaliplatin 130 mg/m² every 3 weeks (CapeOx) while awaiting results. Carcinoembryonic antigen (CEA) at the time of progression was 6.4 ng/mL (Figure 1A). Positive CEA immunohistochemistry staining has been previously reported in MAU case studies,⁴ ⁷–⁹ and while CEA is not established as a biomarker for MAU, it was nonetheless checked, given its utility in mucinous adenoma of the colon. After 2 cycles of CapeOx, the lymphadenopathy had resolved, and the CEA fell to 1.5 ng/mL. However, the patient had a decline in performance status as well as progressive neuropathy and neutropenia, so therapy was again discontinued.

**Genomic Analysis and Targeted Therapy**

The genome sequencing results were returned during cycle 2 of CapeOx therapy and analyzed by the study genomic tumor board. Sequencing statistics are shown in Supplemental Table 1 in the online version. A total of 219 somatic point mutations and insertion/deletions were identified, with nonsynonymous coding mutations constituting the vast majority (77%) (Supplemental Figure 1 in the online version). The remaining mutations were composed of frameshift (10%), STOP gained (5%), codon deletion (5%), codon change plus codon deletion (2%), and splice site acceptor/donor changes (< 1%). Point mutations deemed functionally significant and clinically relevant were observed in, TP53, and LRP1B, and relevant frameshift mutations were observed in DYRK1A and WHSC1. Copy number alterations were observed across much of the genome (Figure 2), with those of potential functional and clinical relevance reported in EGFR, CCND1, FGF3, FGF4, and FGF19 (Supplemental Table 2 in the online version; Figure 2). CCND1, FGF3, FGF4, and FGF19 are located in a chromosomal region demonstrating an amplification, with a log₂ of 2.64.

The most striking copy number alteration was a focal amplification of EGFR, with a log₂ of 5.38, and a corresponding RNA overexpression, with a log₂ of 7.69. Notably, this focal amplification was restricted to the EGFR gene and was not a part of the broader chromosomal gain observed within chromosome 7 (Figure 2). Immunohistochemical staining for EGFR in a Clinical Laboratory Improvement Amendments (CLIA) setting demonstrated 3+ positive staining with cytoplasmic and membranous EGFR expression.
(Figure 1). On the basis of these findings, it was the consensus of the genomic tumor board to recommend anti-EGFR therapy as a potential therapeutic approach for this tumor.

The patient did well for 14 months after CapeOx therapy until again developing recurrent adenopathy, with a CEA of 4.6 µg/L and increasing retroperitoneal lymphadenopathy. At that point, he was enrolled onto a clinical trial testing targeted therapies for cancers with genomic alterations and initiated therapy with erlotinib (ClinicalTrials.gov, NCT02091141).

The patient has now been on the trial for 1 year. He has experienced a reduction in his lymphadenopathy, with index lesions decreasing from 2.3 × 1.3 cm to 2.1 × 1.1 cm, and from 1.8 × 1.3 cm to 0.6 × 0.4 cm (Response Evaluation Criteria in Solid Tumors = —34%, partial response). CEA declined to 0.9 ng/mL (Figure 1A). The patient developed a grade 2 acneiform rash consistent with erlotinib use that responded to topical steroids and minocycline. His quality of life was well maintained, and he was physically active, with no limitations to his normal activities.

### Historical Cases

An additional 6 cases of MAU were identified in the Mayo Clinic archives and tissue stained for EGFR expression. In all 6 cases, immunohistochemistry for EGFR was positive, with staining varying from focal membranous positivity to 3+ positive staining with cytoplasmic and membranous EGFR expression (Figure 1B; Supplemental Figure 2 in the online version).

### Discussion

Primary mucinous adenocarcinoma of the male urethra is a rare disease with no standard treatment approach. Only 13 cases have been reported in the literature since 1961, so when selecting systemic therapy for advanced disease, the clinician is most likely to be biased by the anatomic location of the disease and select a regimen suited for either bladder cancer or colon cancer. While established evidence has contributed to the successful treatment of common tumors, treatment of rare tumors is largely based on extrapolation from other diagnoses that may be only weakly related.

Genomic tumor analysis offers a tool to provide precise and unbiased data to guide the treatment of rare cancers by identifying novel targets. Furthermore, single tumor profiling may reveal recurrent abnormalities that are previously unrecognized. The findings from this case led to the retrospective analysis of a handful of MAU cases seen at Mayo Clinic and revealed a previously unrecognized biomarker that can easily and inexpensively be tested for in future cases. Without genomic testing that included copy number alterations, this novel target would have remained overlooked and untreated.

However, identification of novel targets is only the first step. Access to treatment is critical for the physician’s ability to then act on the genomic testing results. “Basket” studies, such as the My Pathways study (NCT02091141), on which this patient is enrolled, provides a pathway for obtaining targeted therapies by basing eligibility on the presence of molecular genomic markers and not pathologic diagnosis.
In our patient’s case, his disease responded to a traditional bladder cancer regimen (gemcitabine–cisplatin), a traditional colon cancer regimen (CapeOx), and a novel targeted therapy (erlotinib). The bladder cancer regimen was the most toxic and led to only a brief response. The colon cancer regimen led to a more durable response even with dose reduction, but was similarly toxic. Targeted therapy has unequivocally provided the highest quality of life for the patient while achieving disease control for a full year.

**Conclusion**

Although primary mucinous adenocarcinoma of the male urethra is rare, genomic testing has led to a novel treatment approach that would not have otherwise been considered. Furthermore, the availability of therapies through basket trials will provide unprecedented access to novel treatments for patients with previously untreatable disease.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Clinical Practice Points

- Male mucinous adenocarcinoma of the urethra (MAU) is rare and lacks any standard options for systemic therapy.
- Genomic analysis of this rare tumor has demonstrated a targetable amplification of epidermal growth factor receptor (EGFR).
- Erlotinib is effective in EGFR-amplified MAU.
- Follow-up immunohistochemistry in this case and 6 other archival cases demonstrate that EGFR overexpression is a recurrent abnormality.
- These findings have identified a new biomarker, EGFR, which can be tested for in future MAU cases to inform treatment decisions.
Figure 1.
Treatment and Response Course With Representative IHC Staining of MAU Tissues. (A) CEA Levels Over Time Relative to Treatments Received. CEA Levels Were Not Obtained Before March 2014. Computed Tomographic Scans Before Enrollment Onto Clinical Trial NCT02091141 in August 2015 and Demonstrated Continuing Response to EGFR Inhibitor Erlotinib in August 2016. Red Ring Indicates Area of Tumor Shrinkage. (B) Representative IHC Staining of Archival MAU Tissues. Intensity and Proportion of Positive Cells for EGFR IHC Were Recorded. Staining Intensity was Scored From 0 to 3+ and Was Defined as Follows: 0, No Staining or Weak Staining in < 10%; 1+, Weak Staining in > 10%; 2+, Moderate Staining; 3+, Strong Staining Based on Staining Score. Tumors With 1+, 2+, or 3+ Expression Were Interpreted as Positive, and Tumors With No Expression (0 Score) Were Interpreted as Negative. Cytoplasmic or Membranous Distribution of Positive Staining Was Also Recorded and Evaluated at High Magnification. Hematoxylin and Eosin and EGFR IHC Staining at 20x Magnification of Case Study MAU With 3+ Positive Staining With Cytoplasmic and Membranous EGFR Expression, Archival Patient 1 MAU Sample With Focal Membranous Positivity, and Archival Patient 2 MAU Sample With 3+ Positive Staining With Cytoplasmic and Membranous EGFR Expression
Abbreviations: CEA = carcinoembryonic antigen; EGFR = epidermal growth factor receptor; IHC = immunohistochemistry; MAU = mucinous adenocarcinoma of urethra.
Figure 2.
Genomewide Copy Number Alterations. Red Indicates Copy Number Gain; Green, Copy Number Loss. Note Focal Amplification of *EGFR* on Chromosome 7