Plasmacytoid/diffuse urothelial carcinoma: a single institution immunohistochemical and molecular study of 69 patients

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This is the author's manuscript of the article published in final edited form as:
Perrino, C. M., Eble, J., Kao, C.-S., Whaley, R. D., Cheng, L., Idrees, M., … Grignon, D. (2019). Plasmacytoid/diffuse urothelial carcinoma: a single institution immunohistochemical and molecular study of 69 patients. Human Pathology. https://doi.org/10.1016/j.humpath.2019.04.012

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Key words: urothelial carcinoma; plasmacytoid; signet ring; diffuse; bladder.

Running title: Plasmacytoid urothelial carcinoma.

Conflicts of interest: The authors have no conflicts of interest to disclose.

Funding: All funding was provided by the Indiana University Department of Pathology and Laboratory Medicine.

Previous presentation: Portions of this data were presented at the United States and Canadian Academy of Pathology (USCAP) 2017 Annual Meeting as an abstract and a poster presentation.

Abstract (word count = 250)

Accurate diagnosis of plasmacytoid urothelial carcinoma (PUC) is important given its poor prognosis and frequent presentation at high stage. We aim to assess the clinicopathologic features, molecular aberrations, and follow-up data in a series of PUC cases from a single tertiary cancer center. Seventy-two urinary bladder, ureteral, and renal pelvic specimens with urothelial carcinoma with plasmacytoid differentiation were identified. Immunohistochemical (IHC) stains were performed on 48 cases. Among urinary bladder origin markers, GATA3 was most sensitive (96%). Breast carcinoma markers (ER, mammaglobin) were usually negative, but PR stained 1 case (4%). Neuroendocrine markers CD56 and TTF-1 were each positive in 1 case (4% and 4%, respectively). Gastrointestinal adenocarcinoma marker CDX2 was positive in 4 cases (15%), but nuclear β-catenin was negative in all cases. CD138 was positive in 83% and e-cadherin expression was lost in 57% of cases. Fluorescence in situ hybridization (FISH) using the UroVysion Bladder Cancer Kit and *FGFR3* mutational analysis using polymerase chain reaction (PCR) were performed on 15 cases; deletion of chromosome 9p21 was common (60%) and *FGFR3* mutations were detected in 60% of cases (5 cases had both deletion 9p21 and *FGFR3* mutations). Cases were divided into 3 morphologic groups: classic (29%), desmoplastic (35%), and pleomorphic (36%). The three morphologic subtypes had distinct survival outcomes (p=0.083), with median survival for all patients 18 being months versus 10 months for the desmoplastic group.

1. Introduction

Urothelial carcinoma accounts for approximately 90% of primary urinary bladder carcinomas and has a tremendous propensity for morphologic variations [1]. Accurate identification of variants is important for a variety of reasons, as some variants have prognostic significance and others may be mistaken for a benign entity or another tumor [1]. In the most recent edition of the *World Health Organization (WHO) Classification of Tumours of the Urinary System and Male Genital Organs*, the plasmacytoid variant is combined with signet ring (lacking extracellular mucin) and diffuse variants [1]. The plasmacytoid variant in particular has been associated with presentation at a higher pathologic T stage, a higher rate of positive surgical margins, and lower overall survival [2]. Awareness of this variant is crucial when interpreting ureteral and urethral margins on frozen section, given its propensity to infiltrate the soft tissue surrounding the urothelium rather than involving the overlying urothelium itself [3]. In addition, being alert to the unique pattern of discohesive, single cells that may infiltrate the sinusoids of lymph nodes is important in order to identify metastases [4].

One of the earliest references to primary signet ring cell carcinoma of the bladder in the literature is by Saphir in 1955, which describes cells with intracytoplasmic mucin that compressed the nuclei and gave them a crescent shape, cells with basophilic cytoplasm and eccentric nuclei, and cells resembling monocytes. He references another report of tumors composed almost exclusively of similar signet ring cells which were diffusely infiltrative and were associated with distant metastases [5]. In a subsequent study of signet ring cell carcinoma of the urinary bladder, Grignon et al. included linitis plastica-like growth in the definition and described similar cells to

those reported by Saphir [6]. Since that time, several case series have described the morphologic features of plasmacytoid urothelial carcinoma, the largest and most recent of which included 49 cases from The University of Texas MD Anderson Cancer Center [7,8,9,10,11,12,13,14,15,16,17,18]. While these studies describe plasmacytoid differentiation as having a central or eccentric nucleus and infiltrative architecture consisting of discohesive, single cells and linear arrangements of cells, they rarely offer much additional detail [1]. Some authors mention a few other morphologic observations such as the presence of a stromal response in a minority of cases, however these additional descriptions are minimal and not a focal point of any particular study [12,13,19,20,21]. Some cases of urothelial carcinoma have been described as having rhabdoid differentiation, however the morphologic features and images in these studies fall within the spectrum of plasmacytoid differentiation outlined in most other papers [22,23,24,25]. Lastly, reports of conventional urothelial carcinoma with an infiltrative growth pattern as single cells or cords of cells often with associated desmoplasia have shown a particularly poor prognosis in those cases [26].

We aim to describe the detailed morphologic features, immunohistochemical expression pattern, molecular aberrations, and clinical follow-up of a large series of urothelial carcinoma with plasmacytoid/diffuse differentiation from a single institution. Previously reported immunohistochemical stain results have been reported by variety of authors. However, we provide a description of the staining pattern for pertinent stains when applied to a large series of tumors which were all processed by one laboratory, thus eliminating inter-laboratory technical issues as a possible explanation for aberrant staining. Likewise, we hope to expand upon

previously reported information regarding the link between molecular aberrations detected in conventional urothelial carcinoma and the plasmacytoid variant.

2. Materials and methods

A search of the pathology database at Indiana University (January 1996-September 2016) for in-house specimens from the urinary bladder, renal pelvis, or ureter with a final diagnosis of urothelial carcinoma with plasmacytoid or signet ring cell differentiation was performed. Pathology reports were reviewed and data including patient demographics (age, sex), types of specimens, estimated quantity of plasmacytoid differentiation specified, pathologic AJCC TNM stage, and clinical follow-up data was collected. In addition, all available slides from each case were re-reviewed and the cases were divided into three different morphologic groups (please refer to the "Results" section to see criteria used for distinction).

2.1 Immunohistochemistry

A representative block from 48 cases of urothelial carcinoma with plasmacytoid differentiation was selected. Unstained slides were cut at 5 μm in thickness. The antibodies used are listed in Table 1. Immunohistochemical stains were performed as previously described [14]. Twenty-six cases were initially stained with all 16 antibodies. An additional 22 cases were selected because of unique morphologic features (classic morphology, pleomorphic cells, desmoplastic stromal reaction, etc.) and were stained with e-cadherin and CD138. Positivity was interpreted as nuclear for β-catenin, CDX2, estrogen receptor (ER), GATA3, p53, p63, progesterone receptor (PR), and

TTF-1. Positivity was cytoplasmic for 34βE12, keratin 7, keratin 20, and mammaglobin. Positivity was membranous for CD56, CD138, e-cadherin, thrombomodulin, and uroplakin III. Staining was scored for intensity (0: no staining; 1: weak; 2: moderate; 3: strong) and proportion of cells stained (0: 0%; 1: >0 to ≤25%; 2: >25 to ≤50%; 3: >50 to ≤75%; 4: >75 to 100%).

2.2 UroVysion fluorescence in situ hybridization

Unstained sections were prepared from available tissue blocks from 15 cases and were deparaffinized with xylene for fluorescence in situ hybridization using the UroVysion probe set. Slides were treated with absolute ethanol, then air-dried and boiled in a glass staining-jar with 1× citrate buffer (pH 6.0) (Zymed, CA) within a beaker filled with distilled water on a hot block for 10 minutes. Slides were washed with distilled water and transferred to 2× sodium citrate buffer (SSC) for 5 minutes. The slides were air dried and digested with 0.75-mL pepsin (5 mg/mL in 0.01 N HCl with 0.9% NaCl; Sigma, St Louis, MO) at 37°C for 40 minutes. The slides were then washed with distilled water and 2×SSC, followed by air-drying. Chromosome enumeration probes (CEP) for chromosomes 3, 7, and 17, and the locus specific indicator (LSI) probe for 9p21 were labeled with fluorophores. CEP3, CEP7, and CEP17 probes were labeled with Spectrum Red, Spectrum Green, and Spectrum Aqua, respectively. LSI p16 (9p21) was labeled with Spectrum Gold (Vysis, Downers Grove, IL). The probes were diluted 1:10 with tDenHyb2 (Insitus, Albuquerque, NM). Five microliters of diluted probes were added to each slide in reduced light conditions. The slides were then covered with a 22×22 cover slip, sealed with rubber cement and put into an opaque plastic box wrapped with aluminum foil. The slides were denatured at 83°C for 12 minutes and hybridized at 37°C overnight. After hybridization, the

slides were washed and counter-stained with 10 µL DAPI (Insitus, Albuquerque, NM) and sealed with a 50×22 cover slip.

The stained slides were observed and documented using MetaSystem software (Belmont, MA) under 100× oil objective, using filters: SP-100 for DAPI, FITC MF-101 for Spectrum Green, Gold 31003 for Spectrum Gold, Aqua 31036V2 for Spectrum Aqua, and Texas Red Sp103 for Spectrum Red signals. Five sequential focus stacks with 0.4 µm intervals were acquired and integrated into a single image to reduce thickness-related artifacts.

For each case, 200 nuclei were counted. Each cell was simultaneously analyzed for the signals of chromosomes 3, 7, and 17, and 9p21. Chromosomal gain or loss was defined based on the Gaussian model and relative to normal controls. The cut-off values were set at the mean+3 SD of the number of disomic cells in control individuals. The mean+3 SD represents a specificity of 99.9%. Any tumor cases with a score beyond the cut-off value were considered to have either a gain or a loss of the designated chromosome(s).

2.3 *FGFR3* mutation analysis

FGFR3 mutational analysis was performed on 15 cases. For the *FGFR3* gene, exons 7, 10, and 15 were amplified by polymerase chain reaction (PCR) using previously established primers. PCR was performed with 3ml of isolated genomic DNA in a final volume of 50 ml containing 2.3 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM deoxynucleotide triphosphates, 2 mM each primer, and 2U Taq DNA polymerase (Bio-Rad, Hercules, CA, USA). Each PCR

protocol had an initial denaturing step of 95°C for 5 minutes, followed by 40 cycles of: 95°C for 30 seconds, 55°C (for exons 7 and 15) or 58°C (for exon 10) for 30 seconds, and 72°C for 30 seconds. There was a single final extension step at 72°C for 7 min. The PCR products were purified using the QIAquick PCR Purification kit (Qiagen Sciences, Germantown, MD, USA). DNA concentration of PCR products was measured and adjusted to 20 ng per microliter. Sequencing of the purified PCR product was then performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

3. Results

3.1 Epidemiology

A total of 72 specimens from 69 patients were identified. Among the 69 patients, 56 were male and 13 female. The median patient age was 68 years (range: 36 to 91 years). Among the 72 specimens, 5 were biopsies, 13 transurethral resections (TUR), 50 radical cystectomies (RC), 2 nephroureterectomies (NU), and 2 were ureterectomies. Among the 50 radical cystectomy cases, there were 41 radical cystoprostatectomies in men, 8 radical cystectomies with pelvic exenteration in women, and 1 radical cystectomy with vaginal margins in a woman who was already status-post hysterectomy. Among the biopsy/TUR specimens, 7 patients went on to have a RC. Of those 7 RC cases, 3 had plasmacytoid morphology and were included in the series. Four cases were excluded from analysis because 2 had no residual disease, 1 had urothelial carcinoma in situ only (no plasmacytoid morphology), and slides from 1 case (from 1996) could

not be located for re-review and plasmacytoid/signet ring morphology was not mentioned in the pathology report.

3.2 Gross findings

Among resection cases, 83% (45/54) had a mass and 17% (9/54) had no mass present. Three cases (5%, 3/54) had a scar, all of which did not have a grossly identifiable mass. The presence or absence of a scar was not stated in the remaining 94% (51/54) of cases. Among cystectomies, 34% (17/50) had a thickened bladder wall. Of note, the two ureterectomy specimens were described as having no gross abnormality. The two nephroureterectomies each had a mass described but no scar or thickening of the renal pelvis wall/proximal ureter was noted.

3.3 Histopathology and morphologic groups

After reviewing all of the cases three main morphologic groups were recognized. The *first group* was dubbed the "*classic*" group and were composed of cells somewhat resembling invasive lobular carcinoma of the breast. The cells had small to medium nuclei (generally two to three times larger than a lymphocyte), some of which were eccentrically located (plasmacytoid) and others were located in the center of the cell (monocytoid) (Fig. 1). Cells with signet ring-like morphology were also a feature of this group. Overall, the cells were singly scattered and discohesive or in loose aggregates forming linear cords. The *second group* was the "*pleomorphic*" group, in which cases were composed of cells with pleomorphic nuclei which were larger and had more atypia than the cells in the classic category (nuclei were generally at

least four times the size of a lymphocyte). A subset of these cases had markedly bizarre nuclear atypia which at times was rhabdoid in appearance (prominent nucleolus, eccentric eosinophilic cytoplasmic inclusion). This group was usually seen in association with more typical plasmacytoid cells. The *third group* of cases was were the "*desmoplastic*" group. Cells in this category were surrounded by an exuberant, desmoplastic stromal response. While a minority of the cells in the desmoplastic cases closely resembled the cells in the classic group, most of the cells were pleomorphic with more abundant cytoplasm. At times the cells in this third group more closely resembled conventional high grade urothelial carcinoma.

Overall, 29% (21/72) of cases were in the classic category, 35% (25/72) in the exuberant desmoplasia category, and 36% (26/72) of cases were in the pleomorphic category. Among cases with an exuberant, desmoplastic stromal response, 84% (21/25) were composed of pleomorphic cells and 16% (4/25) were composed of cells with classic morphology. A total of 6 cases were noted to have marked/bizarre nuclear atypia, 3 of which were included in the category with exuberant desmoplasia and 3 of which were included in the pleomorphic category. Interestingly, a few of the cases in the classic category consisted of closely packed cells resembling a discohesive sheet, often located beneath the luminal surface of the urinary bladder.

All cases were initially diagnosed as urothelial carcinoma with plasmacytoid (93%, 67/72) or signet ring (7%, 5/72) differentiation. The amount of plasmacytoid differentiation ranged from 5% to 100% (median 25%). Additional variant differentiation was identified in 32% (23/72) of cases; additional variants included clear cell (4%, 1/23), giant cell (4%, 1/23), glandular (13%, 3/23), micropapillary (13%, 3/23), nested (13%, 3/23), sarcomatoid (31%, 7/23), small cell (4%,

1/23), and squamous differentiation (18%, 4/23). Concomitant urothelial carcinoma in situ (UCIS) was present in 43% (31/72) of specimens.

Overall, among resections the pathologic T category was most commonly pT4 (48%, 26/54), followed by pT3 (37%, 20/54), pT2 (13%, 7/54), and pT1 (2%, 1/54) (Table 2). In the classic category, 14% (3/21) of cases were pT1, 33% (7/21) pT2, 29% (6/31) pT3, and 24% (5/21) pT4. In the desmoplastic category, 0% (0/25) were pT1, 12% (3/25) pT2, 36% (9/25) pT3, and 52% (13/25) pT4. In the pleomorphic category, 11% (3/26) were pT1, 35% (9/26) pT2, 23% (6/26) pT3, and 31% (8/26) pT4.

A positive ureteral margin was present in 9% (5/54) of all resection cases (3 invasive in soft tissue, 2 urothelial dysplasia). When analyzed by morphologic group, positive ureteral margins were present in 25% (4/16) of classic resection cases, 32% (7/22) of desmoplastic resection cases, and 19% (3/16) of pleomorphic resection cases; of these cases, 2 classic cases, 1 desmoplastic case, and no pleomorphic cases were positive based on soft tissue invasion surrounding the ureteral lumen. The ureter was involved in 39% (21/54) of all resection cases (12 invasive in soft tissue only, 1 invasive arising from the urothelium only, 5 UCIS only, 1 urothelial dysplasia only, 1 invasive arising from the urothelium and in the soft tissue, and 1 invasive in soft tissue and UCIS). A positive urethral margin was present in 4% (2/50) of radical cystectomies (1 invasive carcinoma, 1 UCIS). The urethra was involved in 10% (5/50) of radical cystectomies (2 invasive carcinoma, 1 invasive in soft tissue, 2 UCIS).

Among resection cases, 93% (50/54) included lymph nodes, 72% (36/50) of which had lymph node metastases. The majority of cases with positive lymph nodes were N2 (83%, 30/36), and a smaller number were N1 (14%, 5/36) and N3 (3%, 1/36). The median number of positive lymph nodes was 4 (range: 1-32), the median size of metastatic deposits was 1.3 cm (range: 0.1-3.9 cm), and extranodal extension was present in 64% (23/36) of cases.

Among radical cystoprostatectomies/cystectomies, 52% (26/50) had involvement of adjacent organs (Table 3). Among radical cystoprostatectomies, 37% (15/41) had involvement of the prostatic stroma and 37% (15/41) of the seminal vesicles (10 cases with both prostate and seminal vesicles; 5 cases prostate only; 5 cases seminal vesicles only). In women who underwent radical cystectomy and pelvic exenteration, 25% (2/8) had involvement of the cervix, 25% (2/8) of the uterus, 25% (2/8) of the fallopian tube, 13% (1/8) of the ovary, and 13% (1/8) of the vagina. Among radical cystectomies, 6% (3/50) had involvement of the rectum and 2% (1/50) of the skeletal muscle of the abdominal wall. One patient who underwent ureterectomy had involvement of the spermatic cord (spermatic cord was biopsied during surgery).

3.4 Immunohistochemical stains

For this study, 14 immunohistochemical markers were performed on 26 cases (Table 3). Two additional stains, CD138 and e-cadherin, were performed on 48 cases. Among urothelial origin markers, GATA3 was the most sensitive (96%, 25/26), followed by p63 (62%, 16/26), uroplakin III (46%), and thrombomodulin (19%, 5/19). Keratin 7 (85%, 22/26) and keratin 20 (77%, 20/26) were positive in most cases. Breast carcinoma markers were usually negative; PR stained 1 case

(intensity 1, proportion 1), while mammaglobin and ER were negative in all cases. Neuroendocrine markers CD56 and TTF-1 were each positive in 1 case (intensity 1, proportions 1 and 2 respectively). Gastrointestinal adenocarcinoma marker CDX2 showed nuclear positivity in 4 cases, while nuclear β-catenin staining was absent in all cases. Nuclear p53 staining was present in 96% (25/26) of cases. CD138 was positive in 83% (40/48) and e-cadherin was positive in 44% (21/48) of cases.

Notably, cases with associated exuberant desmoplasia were more likely to have retained expression of e-cadherin (68%, 13/19) (Fig. 2). In contrast, e-cadherin expression was retained only in a minority of classic (36%, 5/14) and pleomorphic (20%, 3/15) cases. Otherwise, differential staining between the morphologic categories was not noted. CD138 was positive in most cases (classic 10/14, desmoplastic 18/19, pleomorphic 12/15). Similarly, p63 was positive in most cases (classic 3/9, desmoplastic 7/8, pleomorphic 6/9).

3.5 Molecular

UroVysion FISH detected polysomy of chromosomes 3 (13%, 2/15), 7 (20%, 3/15), and 17 (20%, 3/15) and deletion of chromosome 9p21 (60%, 9/15). *FGFR3* mutations were detected by PCR in 60% (9/15) of cases (exon 7 in 6 cases, exon 10 in 6 cases, and exon 15 in 8 cases).

3.6 Clinicopathologic results

A total of 69 patients were diagnosed with plasmacytoid urothelial cancer. Five patients presented with metastatic disease, whereas 64 patients were diagnosed with presumed clinically localized plasmacytoid urothelial bladder cancer. Among patients with locoregional disease, 58 underwent radical cystectomy and the remainder elected for bladder preservation approaches. At the time of cystectomy, 6 patients were found to have pathologically organ-confined disease (≤pT2 N0), and 51 were diagnosed with non-organ-confined disease (pT3,T4 or N+), 2 of which were noted to have diffuse peritoneal carcinomatosis. Twenty-eight (40.5%) patients had positive surgical margins, most of which had extensive infiltrative disease not amenable to complete excision.

Neoadjuvant chemotherapy appeared to have little effect on this cohort. Of the 14 patients who received neoadjuvant cisplatin-based chemotherapy, 10 patients (71.4%) had lymph node involvement and 12 (85.7%) had pT3/4 disease at cystectomy. Only one patient was found to have a pathologic complete response with pT0N0. Thirteen patients received adjuvant chemotherapy, 2 of which received neoadjuvant chemotherapy as well.

Median survival for all patients with plasmacytoid urothelial cancer from the time of initial diagnosis was 18 months (IQR 8-78 months) (Fig. 3A), and 14 (IQR 5-74) months from the time of cystectomy for patients undergoing radical cystectomy. Of note, there appeared to be three morphologic subtypes of plasmacytoid disease; classic, desmoplastic and pleomorphic, with distinct survival outcomes (p=0.083). Patients with desmoplastic disease tended to have the worst clinical outcomes (median survival 10 months), whereas pleomorphic and classic subtypes had somewhat better survival outcomes (Fig. 3B).

4. Discussion

The plasmacytoid/diffuse variant is included with a number of variants with established prognostic or potential clinical significance outlined in the latest edition of the *WHO Classification of Tumours of the Urinary System and Male Genital Organs* [1]. The WHO, International Consultation on Urologic Disease (ICUD), European Association of Urology (EAU), American Joint Committee on Cancer (AJCC), and the College of American Pathologists (CAP) have all recommended documenting the presence of divergent differentiation/variant morphology in pathology reports [1,27,28,29]. However, the difficulty of detecting the plasmacytoid variant both in community practices and large academic medical centers is well described in the literature [30,31]. With this in mind, we report the clinicopathologic features of a large, single-institution case series of urothelial carcinoma with plasmacytoid/diffuse differentiation.

A spectrum of morphologic features exists in these cases, a finding which has been suggested but not extensively explored in the literature. After reviewing all of the cases for this study, three main morphologic groups emerged: (1) classic (29% of cases); (2) desmoplastic (35% of cases); and (3) pleomorphic (36% of cases). Some authors have previously applied the term “rhabdoid” to cases with marked pleomorphism [22,23,24,25,32]. Notably, a total of 6 cases in our series were noted to have marked/bizarre nuclear atypia, but we considered these changes to be within the spectrum of plasmacytoid differentiation (3 placed in the desmoplastic category, 3 in the pleomorphic category).

We applied an extensive immunohistochemical panel to a large number of cases processed in the same laboratory. Caution should be exercised when utilizing immunohistochemistry to determine whether or not the tumor is urothelial in origin, since some stains such as PR for breast carcinoma (1 case; intensity 1 and proportion 1) and CDX2 for gastrointestinal carcinoma (4 cases; intensity 1-2 and proportion 1-2) can occasionally be positive in the plasmacytoid variant. Staining for ER, mammaglobin, and nuclear β-catenin were notably absent in all cases. Finally, all tested urothelial origin markers were positive, with GATA3 (96%) being the most frequent. A recent study showed similar findings to ours (occasional positive CDX2 and PR), and in addition they report staining for GCDFP-15 (24%, 11/45 cases positive) and p-CEA (49%, 22/45 cases) [33].

Loss of e-cadherin and positive staining for CD138 have been touted as useful findings suggestive of plasmacytoid urothelial carcinoma. However, a recent study calls into question the utility of CD138 staining given that positivity in benign urothelium and a variety of benign and malignant urothelial tumors was found [34]. We observed a loss of e-cadherin in the majority of cases (57%) and CD138 positivity in 83% of cases. Notably, cases with associated exuberant desmoplasia were more likely to have retained expression of e-cadherin (68%, 13/19). In contrast, e-cadherin expression was retained only in a minority of classic (36%, 5/14) and pleomorphic (20%, 3/15) cases. Otherwise, differential staining between the morphologic categories was not noted.

The median overall survival for the entire cohort was 21 months. Subsequent analysis of overall survival for each morphologic category showed that there was a statistically significant

difference between the groups. Cases within the desmoplastic category had the worst overall survival (median 12 months). Notably, 88% (22/25) of cases within the desmoplastic category were high stage (pT3 or pT4) in comparison to 48% (10/21) of cases in the classic category and 54% (14/26) of cases in the pleomorphic category. While conclusions are difficult to draw given that the number of cases in each category is small, this raises the question of whether cases with desmoplasia inherently have a worse outcome or if they have a similar prognosis when matched stage for stage with the other groups. The association of worse outcome with certain morphologic features has been alluded to in a prior study by Jimenez et al. The authors identified 3 architectural patterns (nodular, trabecular, infiltrative) within the invasive component of urothelial carcinoma and found that cases with any amount of an infiltrative pattern (narrow cords or single cells, desmoplasia, necrosis) had a worse outcome (median survival 29 months versus 85 months for cases without the infiltrative pattern) [26]. While their findings did not reach statistical significance, they do offer further support that the presence of desmoplasia in urothelial carcinoma could suggest a worse prognosis.

The molecular aberrations that give rise to urothelial carcinoma with plasmacytoid differentiation are still largely unknown. One study that used UroVysion FISH found a homozygous deletion of chromosome 9p21 in 15% of cases and a relative deletion in 70% of cases, therefore the authors suggested that chromosome 9 may play an important role in the pathogenesis of this variant [8]. We performed UroVysion FISH on 15 cases and found chromosome 9 to be most commonly aberrant, with a deletion of chromosome 9p21 in 60% of cases. A prior study using mutation analysis via capillary electrophoresis did not show any

mutations of *FGFR3*, however we detected *FGFR3* mutations in 60% of cases [8]. The significance of these findings needs to be further elucidated in future studies.

In conclusion, it is important for the practicing pathologist to be aware of the range of morphologic features of the plasmacytoid variant and to be aware of the pitfalls related to immunohistochemical staining. Finally, further studies regarding the association between desmoplasia and outcome as well as the molecular aberrations leading to the plasmacytoid variant are warranted.

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Figure Legends

Fig. 1. Plasmacytoid urothelial carcinoma: 3 morphologic groups.

A, Three cells types are present, including plasmacytoid (circle), monocytoid (arrow), and signet ring (arrowhead) (H&E, X400). B, A solid expansile nest of tumor cells is present beneath the luminal surface of the urinary bladder (H&E X100). C, Classic category, small cells with discohesive cells and cells arranged in a linear pattern are present (H&E X200). D, Pleomorphic category, pleomorphic cells with bizarre atypia bordering on rhabdoid differentiation are present (H&E X400). E, Desmoplastic category, an exuberant desmoplastic response is present surrounding tumor cells (H&E, X100). F, Pleomorphic cells with marked nuclear atypia with eccentric, eosinophilic cytoplasm, bordering on rhabdoid differentiation (H&E X400).

Fig. 2. Immunohistochemical findings.

A-C, Classic category (A, H&E X400), with positive expression of CD138 (B, CD138 X400) and loss of e-cadherin (C, e-cadherin X400). D, Desmoplastic category (D, H&E X200), with retention of e-cadherin (D inset, e-cadherin X200). E-F, Desmoplastic category (H&E X200), with an absence of staining for CD138 (E inset, CD138 X200) and retention of staining for e-cadherin (F, e-cadherin, X400).

Fig. 3. Overall survival for patients with plasmacytoid urothelial carcinoma.

A, Overall survival (OS) for all patients with plasmacytoid urothelial carcinoma (median [IQR] OS: 20.99 [8.87-82.79], 69 total patients). B, OS of patients with plasmacytoid urothelial carcinoma by morphologic category (p=0.0336; median [IQR] OS classic: 20.63 [10.41-NR], 21 patients; median [IQR] OS desmoplastic: 11.56 [7.69-22.93], 25 patients; median [IQR] OS pleomorphic: 82.79 [17.74-85.55], 23 patients

Table 1. Antibodies used in this study

Antibody	Clone	Dilution	Company
β-catenin	14	Prediluted	Cell Marque, Rocklin, CA
CD56	123C3	Prediluted	Dako, Santa Clara, CA
CD138	M115	Prediluted	Dako, Santa Clara, CA
CDX2	DAK-CDX2	Prediluted	Dako, Santa Clara, CA
Keratin 7	OV-TL	Prediluted	Dako, Santa Clara, CA
Keratin 20	K_S20.8	Prediluted	Dako, Santa Clara, CA
E-cadherin	NCH-38	Prediluted	Dako, Santa Clara, CA
Estrogen receptor	EP1	1:50	Dako, Santa Clara, CA
GATA3	L50-823	Prediluted	Biocare Medical, Concord, CA
Mammaglobin	31A5	Prediluted	Cell Marque, Rocklin, CA
P53	DO-7	Prediluted	Dako, Santa Clara, CA
P63	BC4A4	1:100	Dako, Santa Clara, CA
Progesterone receptor	PgR 636	Prediluted	Dako, Santa Clara, CA
Thrombomodulin	1009	1:100	Dako, Santa Clara, CA
TTF-1	86763/1	Prediluted	Dako, Santa Clara, CA
Uroplakin	AU-1	1:30	Cell Marque, Rocklin, CA

Table 2. Clinicopathologic features of 72 cases of urothelial carcinoma with plasmacytoid differentiation

Total patients	69
Male	56 (81)
Female	13 (19)
Age median (Range)	68 years (36-91 years)
Total cases (n)	72
Biopsy	5 (7)
TUR	13 (18)
Radical cystectomy	50 (69)
Nephroureterectomy	2 (3)
Ureteretectomy	2 (3)
pT category[a]	
pT1	1 (2)
pT2[b]	1 (2)
pT2a	2 (4)
pT2b	4 (7)
pT3a	7 (13)
pT3b	13 (24)
pT4[c]	1 (2)
pT4a	23 (42)
pT4b	2 (4)
pN category[a]	
pN0	14 (26)
pN1	5 (9)
pN2	31 (58)
pN - not applicable	4 (7)
pM category[a]	
pM1	2 (4)
pM – not applicable	52 (96)
Positive ureteral margin (n=54 resections)	5 (9)
Dysplasia	2 (40)
Soft tissue invasion	3 (60)
Positive urethral margin (n=50 cystectomies)	2 (4)
Urothelial carcinoma in situ	1 (50)
Soft tissue invasion	1 (50)
Involvement of adjacent organs (n=50 cystectomies)	26 (52)
Prostate	15 (58)
Seminal vesicle	15 (58)
Both prostate and seminal vesicle	10 (38)
Prostate only	5 (19)
Seminal vesicle only	5 (19)
Vagina	1 (4)
Cervix	2 (8)
Uterus	2 (8)

Ovary	1 (4)
Fallopian tube	2 (8)
Rectum	3 (12)
Skeletal muscle	1 (4)
Spermatic Cord	1
Median % plasmacytoid differentiation (range)	30% (5-100%)
Other variant morphology present	23 (32)
Clear cell	1 (4)
Giant cell	1 (4)
Glandular	3 (13)
Micropapillary	3 (13)
Nested	3 (13)
Sarcomatoid	7 (31)
Small cell	1 (4)
Squamous	4 (18)
Tumor size median (range)	4.7 cm (0.5 – 14.4 cm)
Concomitant urothelial carcinoma in situ present	31 (43)
Lymphvascular space invasion present	40

Abbreviations: TUR, transurethral resection of the urinary bladder.
[a]Pathologic pTNM categories assigned to resection specimens (n=54).
[b]Tumor involved muscularis propria only on initial transurethral resection (not on subsequent radical cystectomy), therefore not possible to reliably designated as T4a versus T4b.
[c]Nephroureterectomy, therefore no subcategories of T4 staging in the AJCC 7th edition.
[d]Spermatic cord was biopsied during a ureterectomy and was involved by tumor.

Table 3. Immunohistochemical stains performed in this study

Stain	Intensity				Proportion					Negative	Positive
	0	1	2	3	0	1	2	3	4	n (%)	n (%)
β-catenin (nuclear)	26	0	0	0	26	0	0	0	0	26 (100)	0 (0)
CD56	25	1	0	0	25	1	0	0	0	25 (96)	1 (4)
CD138	8	15	14	11	8	14	7	9	10	8 (17)	40 (83)
CDX2 (nuclear)	22	3	1	0	22	3	1	0	0	22 (85)	4 (15)
Keratin 7	4	3	8	11	4	7	1	2	12	4 (15)	22 (85)
Keratin 20	6	4	11	5	6	8	4	4	4	6 (23)	20 (77)
E-cadherin	27	9	6	6	27	10	3	3	5	27 (56)	21 (44)
Estrogen receptor	26	0	0	0	26	0	0	0	0	26 (100)	0 (0)
GATA3	1	4	4	17	1	4	3	1	17	1 (4)	25 (96)
Mammaglobin	26	0	0	0	26	0	0	0	0	26 (100)	0 (0)
P53	1	5	6	14	1	4	5	4	12	1 (4)	25 (96)
P63	10	1	10	5	10	8	2	2	4	10 (38)	16 (62)
Progesterone receptor	25	1	0	0	25	1	0	0	0	25 (96)	1 (4)
Thrombomodulin	21	2	3	0	21	5	0	0	0	21 (81)	5 (19)
TTF-1	25	0	1	0	25	0	1	0	0	25 (96)	1 (4)
Uroplakin	14	4	7	1	14	7	1	1	3	14 (54)	12 (46)

- Urothelial carcinoma with plasmacytoid differentiation can be divided into 3 morphologic categories: (1) classic; (2) pleomorphic; and (3) desmoplastic
- Cases with plasmacytoid differentiation often present at a high stage, and within our dataset cases assigned to the desmoplastic group had a worse outcome
- Urinary bladder origin immunohistochemical stains are sensitive for detecting the plasmacytoid variant
- Molecular aberrations present in conventional urothelial carcinoma are also present in the plasmacytoid variant

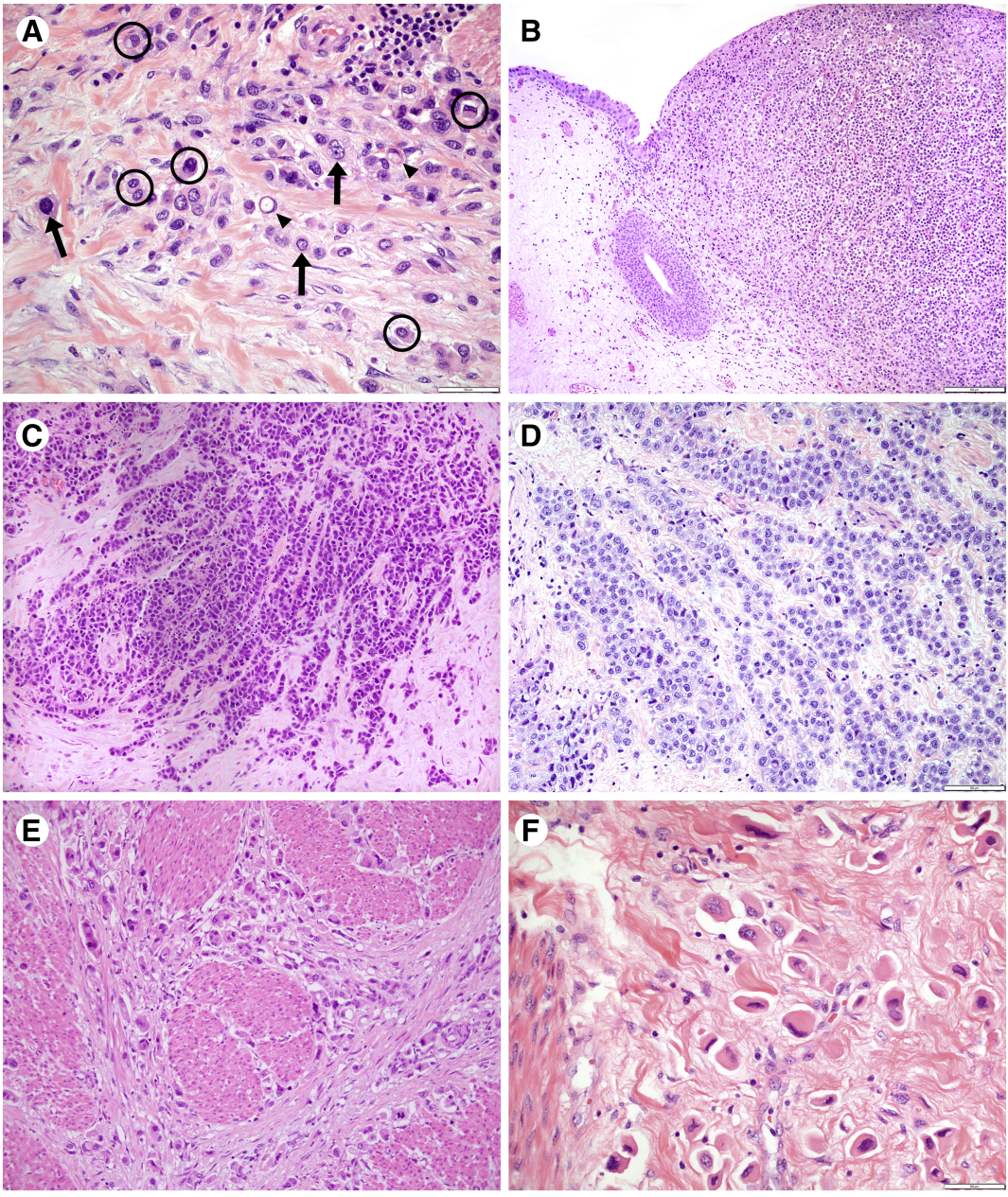


Figure 1

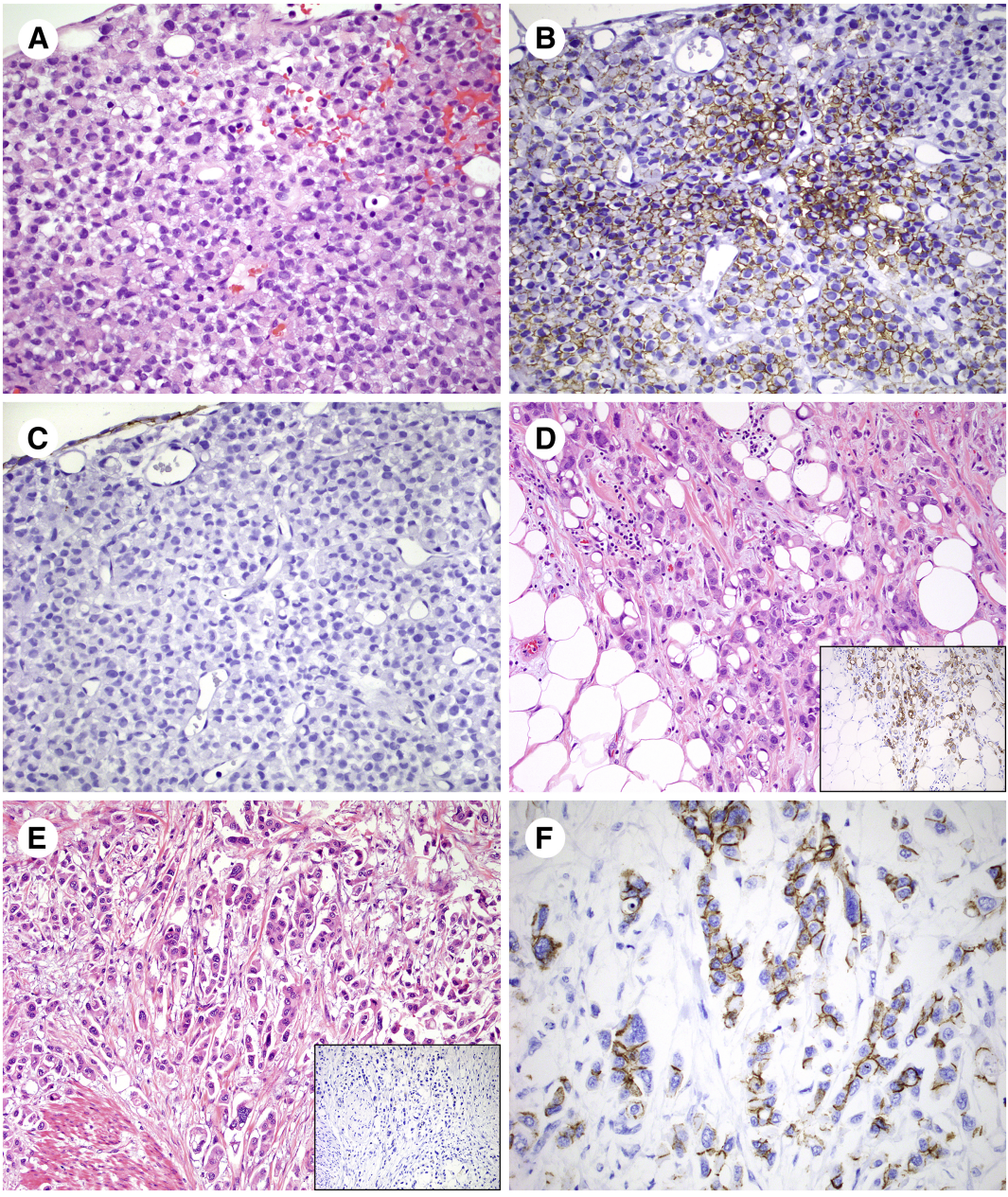
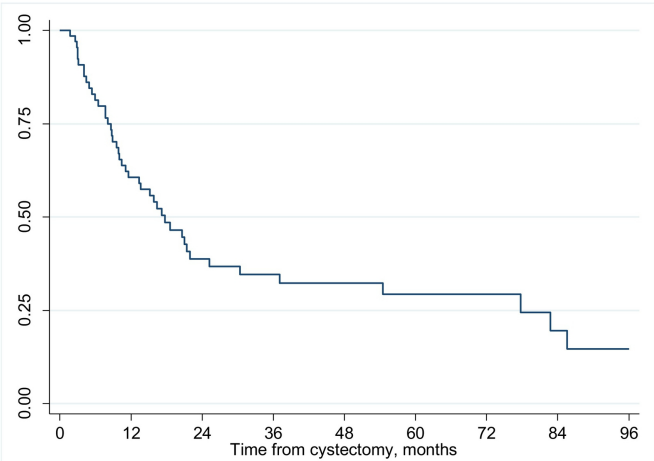


Figure 2

A


1.00
0.75
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12
24
36
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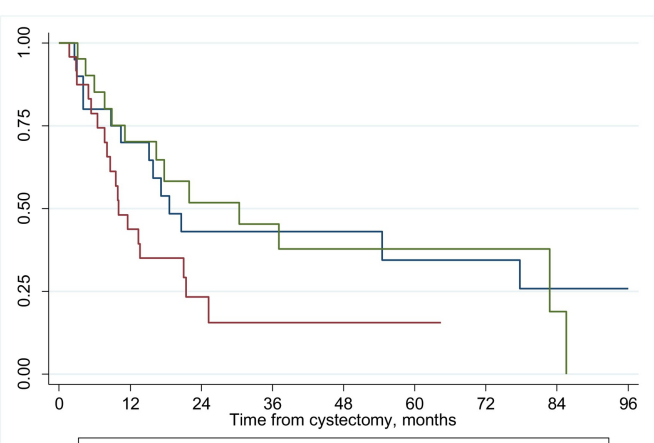
72
84
96
Time from cystectomy, months

B


1.00
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96
Time from cystectomy, months
Classic
Desmoplastic
Pleomorphic

Figure 3