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Gene Fusion Characterization of Rare Aggressive Prostate Cancer Variants - Adenosquamous Carcinoma, Pleomorphic Giant Cell Carcinoma, and Sarcomatoid Carcinoma: An Analysis of 19 Cases

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Abstract

Aims: We evaluated the molecular underpinnings of rare aggressive prostate cancer variants adenosquamous, pleomorphic giant cell, and sarcomatoid carcinomas.

Methods and Results: We retrieved 19 tumors with one or more variant(s) and performed ERG immunohistochemistry, a next-generation sequencing assay targeting recurrent gene fusions, and fluorescence in situ hybridization (FISH) for *ERG* and *BRAF*. Divergent differentiation included: sarcomatoid (n=10), adenosquamous (n=7), and pleomorphic giant cell carcinoma (n=7). Five patients had more than one variant. Four had variants only in metastases. *ERG* rearrangement was detected in 9 (47%, 7 via sequencing, showing *TMPRSS2-ERG* and one *GRHL2-ERG* fusion, and 2 via FISH, showing rearrangement via deletion). Of these, ERG immunohistochemistry was positive in the adenocarcinoma for 8/9 (89%) but only 5/9 (56%, typically decreased) in the variant. One patient had false-positive ERG immunohistochemistry in the sarcomatoid component despite negative FISH. Two (11%) harbored *BRAF* fusions (*FAM131A-BRAF* and *SND1-BRAF*).

Conclusions: *ERG* gene fusions are present in these rare prostate cancer variants with a close frequency to conventional prostate cancer (9/19, 47%). ERG immunohistochemistry usually detects rearrangement in the adenocarcinoma but is less sensitive for the variant histology with weak to negative staining. Adenosquamous and sarcomatoid variants particularly can occur together. Molecular assessment may be an additional tool in select cases to confirm prostatic origin of unusual tumors. The presence of 2 *BRAF* gene rearrangements suggests that this gene fusion may be enriched in this setting, as RAF kinase fusions have been previously reported in 1-2% of prostate cancers.

Introduction:

The overwhelming majority of prostate cancer is conventional acinar adenocarcinoma. Although architectural and cytological variations are common, such as atrophic, pseudohyperplastic, or foamy gland variants, unusual histological variants such as adenosquamous carcinoma, sarcomatoid carcinoma, pleomorphic giant cell carcinoma are exceedingly rare and are known to behave aggressively. (1, 2) Approximately half of adenosquamous and sarcomatoid carcinomas have been reported in the setting of previous treatment for conventional prostatic adenocarcinoma, suggesting evolution from the conventional carcinoma component. (3) However, understanding of the molecular characteristics of these rare variants is currently scant. To our knowledge, only a few publications assessing a handful of cases have examined the molecular characteristics of these rare variants. (4-7) Better understanding of the molecular features of these tumor variants could shed light on the clinical and biologic diversity of prostate cancer and potentially have therapeutic significance. We studied a cohort of rare prostatic carcinoma variants including adenosquamous carcinoma, sarcomatoid carcinoma, pleomorphic giant cell carcinoma for their molecular profiles, using fluorescence in situ hybridization and next generation sequencing.

Materials and methods:

Following institutional review board approval from the Henry Ford Health System, we retrieved tumors from 19 patients with rare prostatic carcinoma variants including adenosquamous carcinoma, sarcomatoid carcinoma, pleomorphic giant cell carcinoma or a combination of more than one of these, from the pathology archives of the participating institutions, from 2013 to 2019. Patients in whom a diagnosis of urothelial carcinoma could not be disproven were excluded from the cohort, using selective immunohistochemistry for prostate-specific antigen, prostate specific acid phosphatase, prostein, and NKX3.1. Antibodies against p63 and high molecular weight cytokeratin were used to support squamous differentiation in selected cases and as applicable to argue against urothelial carcinoma. Hematoxylin and eosin stained slides and immunohistochemistry slides were reviewed by two of the authors (MA & SRW). The final cohort included a total of 19 patients with prostate cancer showing one or more of these variants found either within the prostate (15 patients) or in a metastatic lesion (4 patients). ERG immunohistochemistry was performed on 17 tumors with sufficient material using anti-human ERG antibody clone EP111 (Dako, Carpinteria, CA) in a Dako automated instrument.

Fluorescence in situ hybridization:

Fluorescence in situ hybridization analysis for *ERG* and *BRAF* genes were performed using bacterial artificial chromosome derived break-apart probes, using methods described previously. (8, 9) *ERG* fluorescence in situ hybridization was performed on 12 specimens and (after finding unexpected *BRAF* fusions in 2 index cases using the sequencing assay) *BRAF* fluorescence in situ hybridization was tested in 15 tumors (Table 1). Fluorescence in situ hybridization results were reviewed by four of the authors (MA, NP, SC, & SRW).

Next generation sequencing:

A multiplex RNA fusion panel (Archer® FusionPlex® Solid Tumor Kit) was performed on RNA extracted from representative formalin-fixed, paraffin-embedded tissue tumor blocks. Tissue sections were marked for the areas of variant histology and macro-dissected from the slide, if not already pure by histologic review. Briefly, this assay is a targeted sequencing assay that uses anchored multiplex polymerase chain reaction to prepare target-enriched cDNA libraries from RNA to detect fusions and other mutations in over 50 genes linked to known solid tumors, using methods previously described. (10) Genes targeted in this assay include: *AKT3*, *ALK*, *ARHGAP26*, *AXL*, *BRAF* (fusion and V600E mutation), *BRD3*, *BRD4*, *EGFR* (fusion and mutation), *ERG*, *ESR1*, *ETV1*, *ETV4*, *ETV5*, *ETV6*, *EWSR1*, *FGFR1*, *FGFR2*, *FGFR3*, *FGR*, *INSR*, *MAML2*, *MAST1*, *MAST2*, *MET* (fusion and mutation), *MSMB*, *MUSK*, *MYB*, *NOTCH1*, *NOTCH2*, *NRG1*, *NTRK1*, *NTRK2*, *NTRK3*, *NUMBL*, *NUTM1*, *PDGFRA* (fusion and mutation), *PDGFRB*, *PIK3CA*, *PKN1*, *PPARG*, *PRKCA*, *PRKCB*, *RAF1*, *RELA*, *RET*, *ROS1*, *RSPO2*, *RSPO3*, *TERT*, *TFE3*, *TFE3*, *TFEB*, *THADA*, and *TMPRSS2*. Seventeen formalin-fixed, paraffin-embedded tissue blocks of different tumors were tested via this method, four of which failed quality metrics, whereas the remaining 13 tumors were informative and yielded positive or negative results. Sequencing results were reviewed by three molecular pathologists (JCGG, LAF, & DAC) and two of the authors (MA & SRW).

Results:

A total of 19 patients were analyzed (**Table 1**) and revealed the following; prostate carcinoma with sarcomatoid (n=10), adenosquamous (n=7, **Figure 1**), and pleomorphic giant cell carcinoma (n=7, **Figure 2**) divergent differentiation. Five patients had more than one variant (adenosquamous and sarcomatoid in 4 patients; and sarcomatoid and pleomorphic giant cell in 1 patient). Divergent differentiation was present only

in metastases for 4 patients. These metastatic sites included pelvic lymph node dissections, a perirectal mass (likely representing a replaced lymph node), a retroperitoneal mass (also likely representing a lymph node), and a bone (femoral head) metastasis.

Molecular characterization:

ERG rearrangement was detected in 9 tumors (47%, 7 via sequencing, showing *TMPRSS2-ERG* & *GRHL2-ERG* fusions, and 2 via fluorescence in situ hybridization, showing rearrangement via deletion). Of note a *GRHL2-ERG* fusion has been previously reported in prostate cancer in an annotation database from the Cancer Genome Atlas as TCGA-V1-A9OT, (11) which was noted to be Gleason score 3+3=6 (Grade Group 1) in the original pathology report (without mention of any variant histology) and the available whole slide image demonstrated a small focus of Gleason score 3+3=6 (Grade Group 1) cancer (https://cancer.digitalslidearchive.org/). Two tumors (11%) were detected to harbor *BRAF* gene fusion by sequencing and confirmed by fluorescence in situ hybridization (**Figure 3,** cases 2 and 4), with fusions being *FAM131A-BRAF* and *SND1-BRAF* (**Table 2**). Breakpoints involved exons 10 and 9 of *BRAF*, respectively, consistent with prior reports of retaining the C-terminus tyrosine kinase domain in the fusion. (9) *BRAF* V600E mutation was, however, not detected.

Some of the remaining tumors were incompletely characterized. In 5 specimens, fluorescence in situ hybridization was negative for *ERG* and *BRAF* rearrangement. The sequencing assay detected no definite fusions; however, it did not meet quality control metrics and could not be interpreted as reliable. This includes 1 autopsy specimen, in which sequencing could not be performed due to the suboptimal material (patient 5). For tumors with sequencing showing *TMPRSS2-ERG*, confirmatory fluorescence in situ hybridization was deemed not necessary, as this is the prototypical fusion described in prostate cancer. Confirmatory fluorescence in situ hybridization was attempted in the tumor with *GRHL2-ERG* fusion, but was unsuccessful, possibly related to preanalytical factors, being a metastatic bone specimen. One tumor had *ERG*-positive fluorescence in situ hybridization in the low-grade adenocarcinoma component, but a negative fluorescence in situ hybridization was found in the sarcomatoid component, suggesting co-existence of two different neoplastic clones.

Three patients had histologically disparate tumors (**Figure 4**) at different sites (1- adenosquamous carcinoma only in the lymph nodes but not the primary tumor, 2- separate adenosquamous and sarcomatoid

metastases, and 3- conventional and pleomorphic carcinoma in different metastatic sites). Of these, two were confirmed to have the same fusion in both components: case 2 demonstrated the *FAM131A-BRAF* fusion in sequencing of two separate specimens (sarcomatous perirectal mass and adenosquamous carcinoma involving lymph nodes). The prostatectomy from this patient, which did not exhibit variant histology, was not available for testing. The tumor from patient 1 was confirmed to have *ERG* rearrangement in both glandular and squamous components of lymph node metastases using fluorescence in situ hybridization. The third (case 4) had conventional adenocarcinoma (single cells and solid growth) with *SND-BRAF* fusion in a testicular metastasis at initial presentation. A retroperitoneal tumor biopsy from the same patient demonstrated pleomorphic giant cell carcinoma; however, there was insufficient tissue for molecular testing to verify the same fusion in the second site.

ERG immunohistochemistry

ERG immunohistochemistry was performed in 17 tumors (**Table 3, Figure 5**). One patient (patient 1) had *ERG* rearrangement detected via fluorescence in situ hybridization (deletion) but had negative immunohistochemistry in both the adenocarcinoma and the variant (false negative). In 5 patients with *ERG* rearrangement, staining was clearly positive in the adenocarcinoma but markedly decreased or negative in the variant. In 1 patient, ERG staining was weak in both components, and in another (patient 4), ERG staining was negative in the variant tumor but positive in a separate, unrelated low-grade prostatic adenocarcinoma. Finally, one tumor which showed negative *ERG* fluorescence in situ hybridization showed negative immunohistochemistry in the adenocarcinoma but variable negative to moderate staining of the sarcomatoid component (patient 16). Overall, 8/9 (89%) tumors with confirmed *ERG* rearrangement had positive staining in the associated adenocarcinoma, whereas only 5/9 (56%) had definite positive staining in the variant component.

Discussion:

Adenosquamous carcinoma, sarcomatoid carcinoma, pleomorphic giant cell carcinoma are extremely rare variants of prostate adenocarcinoma that are typically associated with a dismal prognosis. (1, 12-14) A substantial fraction of these unusual morphologies are encountered in the post-treatment setting, suggesting that they often evolve from usual carcinoma (**Table 3**). Although the optimal treatment for these variants is not yet clarified, likely due to their rarity, it is important to discriminate them from the mimics, especially

urothelial carcinoma, due to the markedly different treatment implications of these diagnoses. Additionally, the possibility of coexistence of prostatic adenocarcinoma and urothelial carcinoma, or both metastatic to the same lymph node, is conceivable and usually requires a robust morphological assessment and a judicious immunohistochemistry.

Previously, only a few studies have evaluated molecular characteristics of these rare variants. Rodrigues et al found 3 sarcomatoid prostate cancers to have *ERG* rearrangement via deletion using fluorescence in situ hybridization. (4) Another study reported 2 cases of prostatic adenocarcinoma with squamous transformation, which harbored *SPOP* mutation and *PTEN* deletion in one and *TMPRSS2-ERG* fusion and *PTEN* mutation in another. (5) Recently, Lotan et al studied 8 prostatic adenocarcinomas with pleomorphic giant cell features and found DNA damage repair mutations but not *ERG* fusions. (7)

Recurrent gene rearrangements involving *ERG* or other members of the ETS family of genes occur in approximately 50% of prostate adenocarcinoma. (15) From our study, *ERG* gene fusions appear to be also present in these rare prostate cancer variants with a similar frequency to the conventional prostate cancer (9/19, 47%). Of note, ERG immunohistochemistry does not demonstrate an ideal correlation with rearrangement in these variants, with 8 of 9 rearranged tumors showing positive staining in the prostatic adenocarcinoma but only 5 of 9 showing positive staining in the variant, typically with a decreased distribution and intensity. We interpret one tumor as showing a false-positive immunohistochemical result in the sarcomatoid component, since fluorescence in situ hybridization was negative for rearrangement in this case and the admixed adenocarcinoma showed negative immunohistochemistry. Anecdotally, we have occasionally encountered weak to moderate ERG immunohistochemical staining in non-vascular spindle cell lesions of soft tissue (unpublished observations), which may account for this finding. This is also in keeping with the findings of others including occasional ERG immunohistochemical positivity in other mesenchymal and spindle cell tumors. (16)

Interestingly, the presence of *BRAF* gene rearrangements in two tumors (10% of our cohort) suggests that this gene fusion may be enriched in this setting, as RAF kinase fusions have been previously reported in only 1-2% of prostate cancers, including from multiple large scale genetic profiling studies as assessed via cbioportal.org. (9, 17-21) Additional clinical and molecular studies regarding such fusions will be necessary to better understand targeting the RAF kinase pathways as a potential therapy option in these patients. *SND1-BRAF* fusion has been reported in pancreatic acinar cell carcinoma and lung cancer, (22-26) and a fusion similar to

FAM131A-BRAF has been described in pilocytic astrocytoma (*FAM131B-BRAF*). (27, 28). A new 5' fusion partner of *ERG*, *GRHL2,* was identified in one of the tumors (case 18). Although not well characterized at present, this fusion is also noted in an additional case in the Cancer Genome Atlas fusion data as case TCGA-V1-A9OT-01A. (11) *GRHL2* is reported to be a coregulator of the androgen receptor, similar to *TMPRSS2* and other fusion partner genes of *ERG.* (29) The original pathology report and scanned slide from this case demonstrated a Gleason score 3+3=6 (Grade Group 1) prostatic adenocarcinoma, but without variant histology.

Besides the relatively small number of patients, one limitation of our study is that fluorescence in situ hybridization and sequencing were performed with assays against known gene fusions. Because a substantial fraction of the evaluated tumors (8/19, 42%) yielded negative results, we cannot exclude the presence of novel gene fusions. Additional studies using other techniques (e.g. RNA-seq) may be helpful in shedding more light on these rare variants of prostate cancer. And as noted recently by Lotan et al, other non-fusion molecular mechanisms may be responsible for pathogenesis of some of these tumors. (7) Gene fusions may be difficult to target therapeutically, making these findings of limited value for treatment; however, this may be of relevance to diagnostic pathology practice, if fluorescence in situ hybridization or molecular studies are employed to attempt to confirm the prostatic origin of histologically unusual tumors occurring in the prostate or at distant sites after treatment.

Conclusions:

ERG gene fusions are present in rare prostate cancer variants adenosquamous, sarcomatoid, and pleomorphic giant cell carcinomas with a close frequency to conventional prostate cancer (47%). Adenosquamous and sarcomatoid variants in particular can sometimes occur together. Molecular assessment may be an additional tool in confirming prostatic origin for tumors with unusual morphology, either occurring in the prostate or at distant, metastatic sites in the setting of known prostate cancer. ERG immunohistochemistry is less robust than molecular techniques when assessed in the variant components of these tumors. The presence of 2 *BRAF* gene rearrangements suggests that this gene fusion may be enriched in this setting, as RAF kinase fusions have been previously reported in 1-2% of prostate cancers. Further study will be helpful to determine whether therapy targeting BRAF may be of value in patients with rearranged tumors.

Author contributions:

Drafting the manuscript: Alhamar

Critical revision and final approval of the manuscript: all authors

Data collection, analysis, and interpretation: all authors

Conception / design: Williamson

Figure Legends:

Figure 1: This adenosquamous prostate cancer shows a transition from adenocarcinoma (top) to squamous cell carcinoma (bottom).

Figure 2: This carcinoma with pleomorphic giant cell features shows a transition from solid adenocarcinoma (top) to bizarre giant tumor cells (center and bottom).

Figure 3: Fluorescence in situ hybridization for *BRAF* in case 4 confirms the sequencing results, showing multiple copies of the *BRAF* probes, with several demonstrating widely separated signals.

Figure 4: Tumor 2 with *BRAF* rearrangement demonstrated a lymph node metastasis composed partly of glandular structures (A). There is partial p63 reactivity in the glandular component (B) and partial positivity for NKX3.1 in the adjacent cancer (C), which also showed partial positivity for prostate-specific antigen (D). Squamous differentiation was also present in the same lymph node, (E) and sarcomatoid carcinoma was present in a separate mass (F). A *FAM131A-BRAF* gene fusion was demonstrated in both morphologies by sequencing.

Figure 5: Tumor 15 with *ERG* rearrangement shows positive ERG immunohistochemical staining in a large gland of the prostatic adenocarcinoma (A, right), but weak to negative staining in the sarcomatoid component (A, left). At higher magnification, the sarcomatoid tumor cells vary from negative to weakly positive (B). Tumor 16 was interpreted as having false-positive ERG immunohistochemistry. The associated prostatic

adenocarcinoma (C) is negative for ERG immunohistochemistry; however, the spindle cell component showed a variable reaction, ranging from negative to moderate positivity (D).

References:

1.	Mazzucchelli R, Lopez-Beltran A, Cheng L, et al. Rare and unusual histological variants of prostatic carcinoma: clinical significance. *BJU Int.* 2008;102:1369-1374.

2.	Grignon DJ. Unusual subtypes of prostate cancer. *Mod Pathol.* 2004;17:316-327.

3.	Epstein JI, Algaba F. Squamous neoplasms. In: Moch H, Humphrey PA, Ulbright T.M., et al., eds. *WHO Classification of Tumours of the Urinary System and Male Genital Organs*. Lyon: International Agency for Research on Cancer; 2016:170.

4.	Rodrigues DN, Hazell S, Miranda S, et al. Sarcomatoid carcinoma of the prostate: ERG fluorescence in-situ hybridization confirms epithelial origin. *Histopathology.* 2015;66:898-901.

5.	Dizman N, Salgia M, Ali SM, et al. Squamous Transformation of Prostate Adenocarcinoma: A Report of Two Cases With Genomic Profiling. *Clin Genitourin Cancer.* 2019.

6.	Ray ME, Wojno KJ, Goldstein NS, et al. Clonality of sarcomatous and carcinomatous elements in sarcomatoid carcinoma of the prostate. *Urology.* 2006;67:423 e425-423 e428.

7.	Lotan TL, Kaur HB, Alharbi AM, et al. DNA Damage Repair Alterations are Frequent in Prostatic Adenocarcinomas with Focal Pleomorphic Giant Cell Features. *Histopathology.* 2019.

8.	Bhalla R, Kunju LP, Tomlins SA, et al. Novel dual-color immunohistochemical methods for detecting ERG-PTEN and ERG-SPINK1 status in prostate carcinoma. *Mod Pathol.* 2013;26:835-848.

9.	Palanisamy N, Ateeq B, Kalyana-Sundaram S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nat Med.* 2010;16:793-798.

10.	Jebastin JAS, Smith SC, Perry KD, et al. Pseudosarcomatous myofibroblastic proliferations of the genitourinary tract are genetically different from nodular fasciitis and lack USP6, ROS1 and ETV6 gene rearrangements. *Histopathology.* 2018;73:321-326.

11.	Kim P, Zhou X. FusionGDB: Fusion Gene annotation DataBase. Available at: https://ccsm.uth.edu/FusionGDB/gene_search_result.cgi?page=page&type=quick_search&quick_search=15390. Accessed 12/30/2019.

12.   Alharbi AM, De Marzo AM, Hicks JL, et al. Prostatic Adenocarcinoma With Focal Pleomorphic Giant Cell Features: A Series of 30 Cases. *Am J Surg Pathol.* 2018;42:1286-1296.

13.   Parwani AV, Herawi M, Epstein JI. Pleomorphic giant cell adenocarcinoma of the prostate: report of 6 cases. *Am J Surg Pathol.* 2006;30:1254-1259.

14.   Parwani AV, Kronz JD, Genega EM, et al. Prostate carcinoma with squamous differentiation: an analysis of 33 cases. *Am J Surg Pathol.* 2004;28:651-657.

15.   Kumar-Sinha C, Kalyana-Sundaram S, Chinnaiyan AM. Landscape of gene fusions in epithelial cancers: seq and ye shall find. *Genome Med.* 2015;7:129.

16.   Yaskiv O, Rubin BP, He H, et al. ERG protein expression in human tumors detected with a rabbit monoclonal antibody. *Am J Clin Pathol.* 2012;138:803-810.

17.   Abida W, Cyrta J, Heller G, et al. Genomic correlates of clinical outcome in advanced prostate cancer. *Proc Natl Acad Sci U S A.* 2019;116:11428-11436.

18.   Baca SC, Prandi D, Lawrence MS, et al. Punctuated evolution of prostate cancer genomes. *Cell.* 2013;153:666-677.

19.   Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell.* 2015;161:1215-1228.

20.   Abida W, Armenia J, Gopalan A, et al. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. *JCO Precis Oncol.* 2017;2017.

21.   Gao Q, Liang WW, Foltz SM, et al. Driver Fusions and Their Implications in the Development and Treatment of Human Cancers. *Cell Rep.* 2018;23:227-238 e223.

22.   Chmielecki J, Hutchinson KE, Frampton GM, et al. Comprehensive genomic profiling of pancreatic acinar cell carcinomas identifies recurrent RAF fusions and frequent inactivation of DNA repair genes. *Cancer Discov.* 2014;4:1398-1405.

23.   Jang JS, Lee A, Li J, et al. Common Oncogene Mutations and Novel SND1-BRAF Transcript Fusion in Lung Adenocarcinoma from Never Smokers. *Sci Rep.* 2015;5:9755.

24.   Ross JS, Wang K, Chmielecki J, et al. The distribution of BRAF gene fusions in solid tumors and response to targeted therapy. *Int J Cancer.* 2016;138:881-890.

25.   Wood LD, Klimstra DS. Pathology and genetics of pancreatic neoplasms with acinar differentiation. *Semin Diagn Pathol.* 2014;31:491-497.

26.   Chou A, Kim Y, Samra JS, et al. BRAF gene rearrangements can be identified by FISH studies in pancreatic acinar cell carcinoma. *Pathology.* 2018;50:345-348.

27.   Roth JJ, Santi M, Pollock AN, et al. Chromosome band 7q34 deletions resulting in KIAA1549-BRAF and FAM131B-BRAF fusions in pediatric low-grade Gliomas. *Brain Pathol.* 2015;25:182-192.

28.   Cin H, Meyer C, Herr R, et al. Oncogenic FAM131B-BRAF fusion resulting from 7q34 deletion comprises an alternative mechanism of MAPK pathway activation in pilocytic astrocytoma. *Acta Neuropathol.* 2011;121:763-774.

29.   Paltoglou S, Das R, Townley SL, et al. Novel Androgen Receptor Coregulator GRHL2 Exerts Both Oncogenic and Antimetastatic Functions in Prostate Cancer. *Cancer Res.* 2017;77:3417-3430.

Patient	Location of Divergent Differentiation	Type of Divergent Differentiation	Overall molecular status	Fluorescence in situ hybridization BRAF	Fluorescence in situ hybridization ERG	Next Generation Sequencing
1	Lymph node metastasis	Adenosquamous	*ERG*	Negative	5'deletion	Not done
2	Lymph node metastasis and perirectal mass	Adenosquamous & sarcomatoid	*BRAF*	Positive	Negative	*FAM131A-BRAF* fusion
3	Prostate	Sarcomatoid	**Negative**	Negative	Negative	Quality control fail x2
4	Retroperitoneal metastasis	Pleomorphic giant cell	*BRAF*	Positive + aneuploid	Negative	*SND1-BRAF* fusion
5	Prostate/bladder	Pleomorphic giant cell	**Negative**	Negative	Negative	Not done
6	Prostate	Sarcomatoid	*ERG*	Negative	Not done	*TMPRSS2-ERG* fusion
7	Prostate	Adenosquamous	*ERG*	Negative	Not done	*TMPRSS2-ERG* fusion
8	Prostate	Adenosquamous & sarcomatoid	*ERG*	Negative	Not done	*TMPRSS2-ERG* fusion
9	Prostate/bladder neck	Sarcomatoid	**Negative**	Negative	Negative	Quality control fail
10	Prostate	Adenosquamous & sarcomatoid	**Negative**	Negative	Negative	Quality control fail
11	Prostate	Pleomorphic giant cell	**Negative**	Negative	Negative	Negative
12	Prostate	Adenosquamous & sarcomatoid	*ERG*	Not done	Not done	*TMPRSS2-ERG* fusion
13	Prostate	Sarcomatoid	**Negative**	Negative	Negative	Negative

14	Prostate	Pleomorphic giant cell	**Negative**	Negative	Negative	Negative
15	Prostate	Pleomorphic giant cell	***ERG***	Not done	Not done	*TMPRSS2-ERG* fusion
16	Prostate	Sarcomatoid	**Negative**	Negative	Negative	Quality control fail
17	Prostate/bladder neck	Sarcomatoid & pleomorphic giant cell	***ERG***	Negative	5' deletion	Negative
18	Femoral head metastasis	Adenosquamous	***ERG***	Not done	Failed	*GRHL2-ERG* fusion
19	Prostate	Pleomorphic giant cell	***ERG***	Not done	Not done	*TMPRSS2-ERG* fusion

Table 1: Types of divergent differentiation and overall molecular status.

| Fusions	Reads (# / %)	Start Sites	Segments
GRHL2 (exon 4) → ERG (exon 2)	111 / 75	58	GRHL2(+) chr8:102570812→102571040 ERG(-) chr21:39817544→39817327
FAMA1A (exon 1) → BRAF (exon 10)	41 / 42	17	FAM131A(+) chr3:184053800→184053911 BRAF(-) chr7:140482957→140482930
SND1 (exon 10) → BRAF (exon 9)	308 / 83	63	SND1(+) chr7:127361341→127361454 BRAF(-) chr7:140487384→140487348

**Table 2**: Molecular characteristics of the unique fusions in the cohort.

| Patient	Age	Type of Divergent Differentiation	Prior treatment	Overall molecular status	ERG IHC-adenocarcinoma	ERG IHC - variant
1	63	Adenosquamous	No	*ERG*	Negative	Negative
2	68	Adenosquamous & sarcomatoid	ADT	*BRAF*	Negative	Negative
3	77	Sarcomatoid	No	Negative	Negative*	Negative
4	55	Pleomorphic giant cell	No	*BRAF*	Negative	Negative
5	91	Pleomorphic giant cell	ADT and radiation	Negative	Negative	Negative
6	77	Sarcomatoid	Radiation	*ERG*	Positive	Negative
7	77	Adenosquamous	ADT	*ERG*	Weak	Weak
8	58	Adenosquamous & sarcomatoid	ADT (short interval from diagnosis)	*ERG*	Positive	Focal
9	86	Sarcomatoid	Radiation	Negative	Negative	Negative
10	71	Adenosquamous & sarcomatoid	Radiation	Negative		
11	76	Pleomorphic giant cell	ADT	Negative		
12	67	Adenosquamous & sarcomatoid	ADT	*ERG*	Positive	Negative / equivocal
13	86	Sarcomatoid	No	Negative	Negative	Negative
14	67	Pleomorphic giant cell	ADT	Negative	Negative	Negative
15	78	Pleomorphic giant cell	Likely**	*ERG*	Positive	Focal weak
16	67	Sarcomatoid	Brachytherapy	Negative	Negative	Variable negative to moderate
17	90	Sarcomatoid & pleomorphic giant cell	Radiation	*ERG*	Variable weak to strong	Negative
18	68	Adenosquamous	ADT (short interval from diagnosis)	*ERG*	Positive	Positive, slightly decreased intensity
19	68	Pleomorphic	No	*ERG*	Focal weak	Focal

		giant cell				

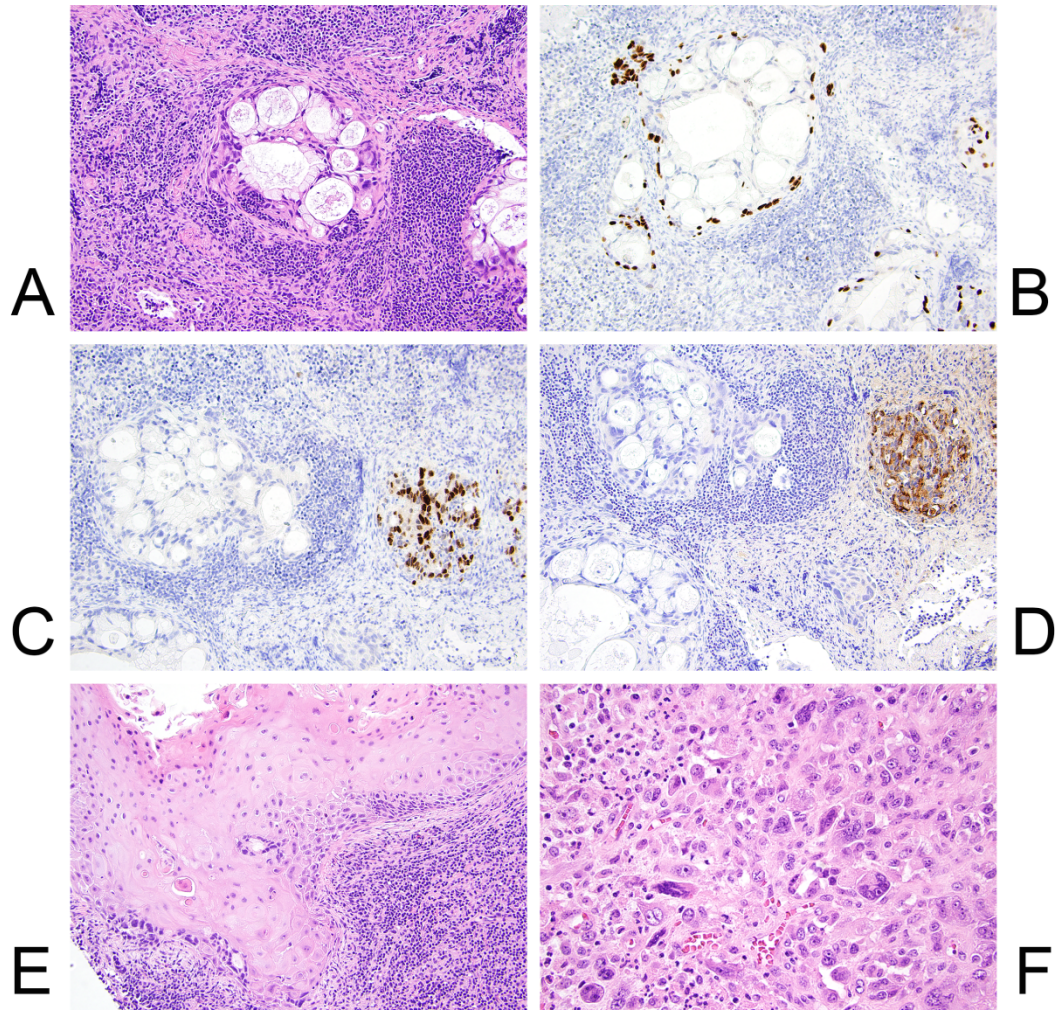**Table 3:** Age, treatment history, and correlation of *ERG* molecular status with immunohistochemistry. *A low-grade prostatic adenocarcinoma was positive for *ERG* fluorescence in situ hybridization, but the sarcomatoid component was negative, interpreted as two unrelated tumor clones. **Specific treatment information was not available but the variant was diagnosed 2 years after biopsy diagnosis, suggesting likely interval treatment. In 2 patients with treatment history, the variant was found only a short time after initiating androgen deprivation therapy, suggesting that progression was not a typical therapy-related event. Shaded fields = marked decrease in ERG staining in the variant. IHC = immunohistochemistry. ADT = androgen deprivation therapy.
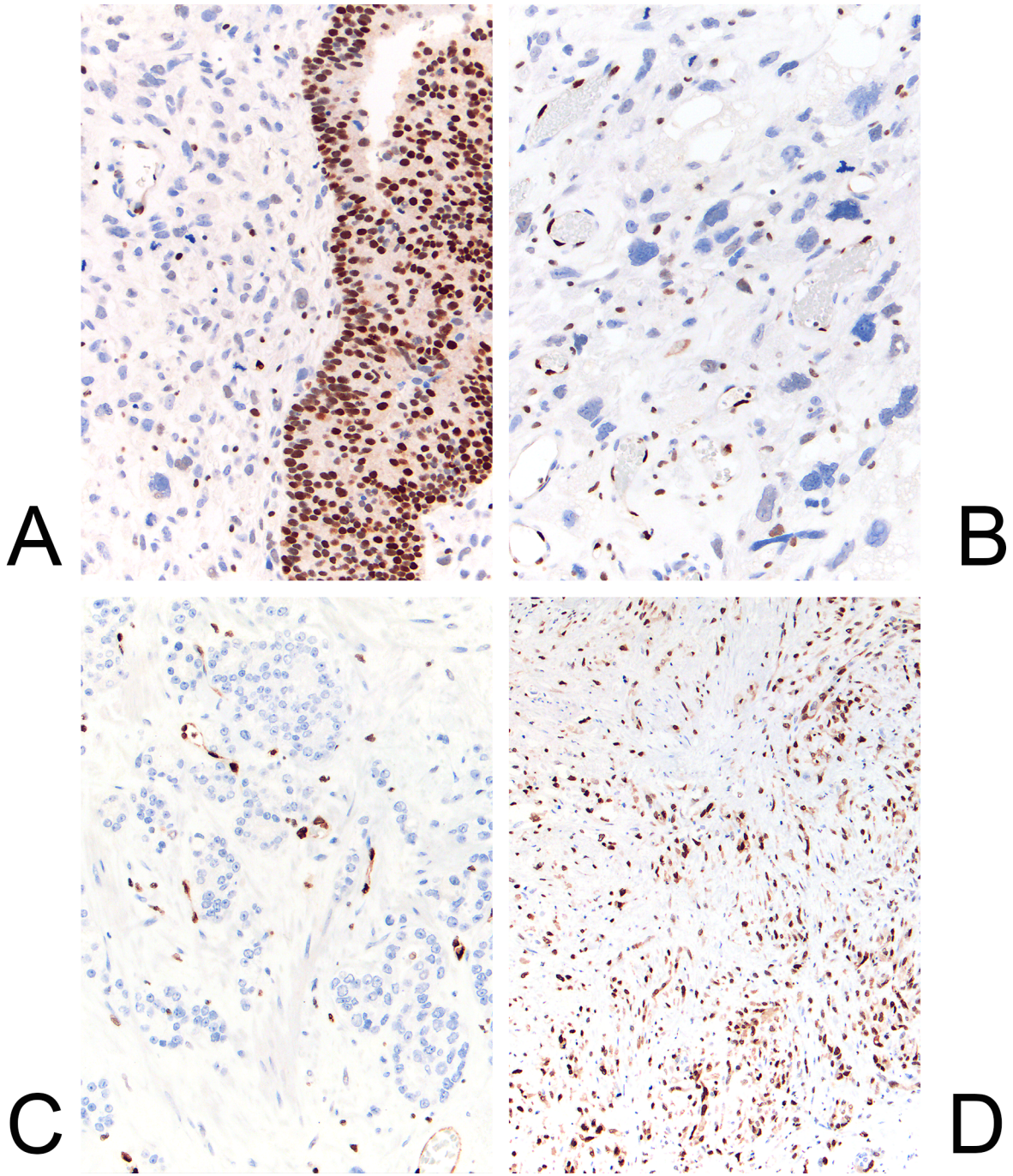
his_14205_f1.tif
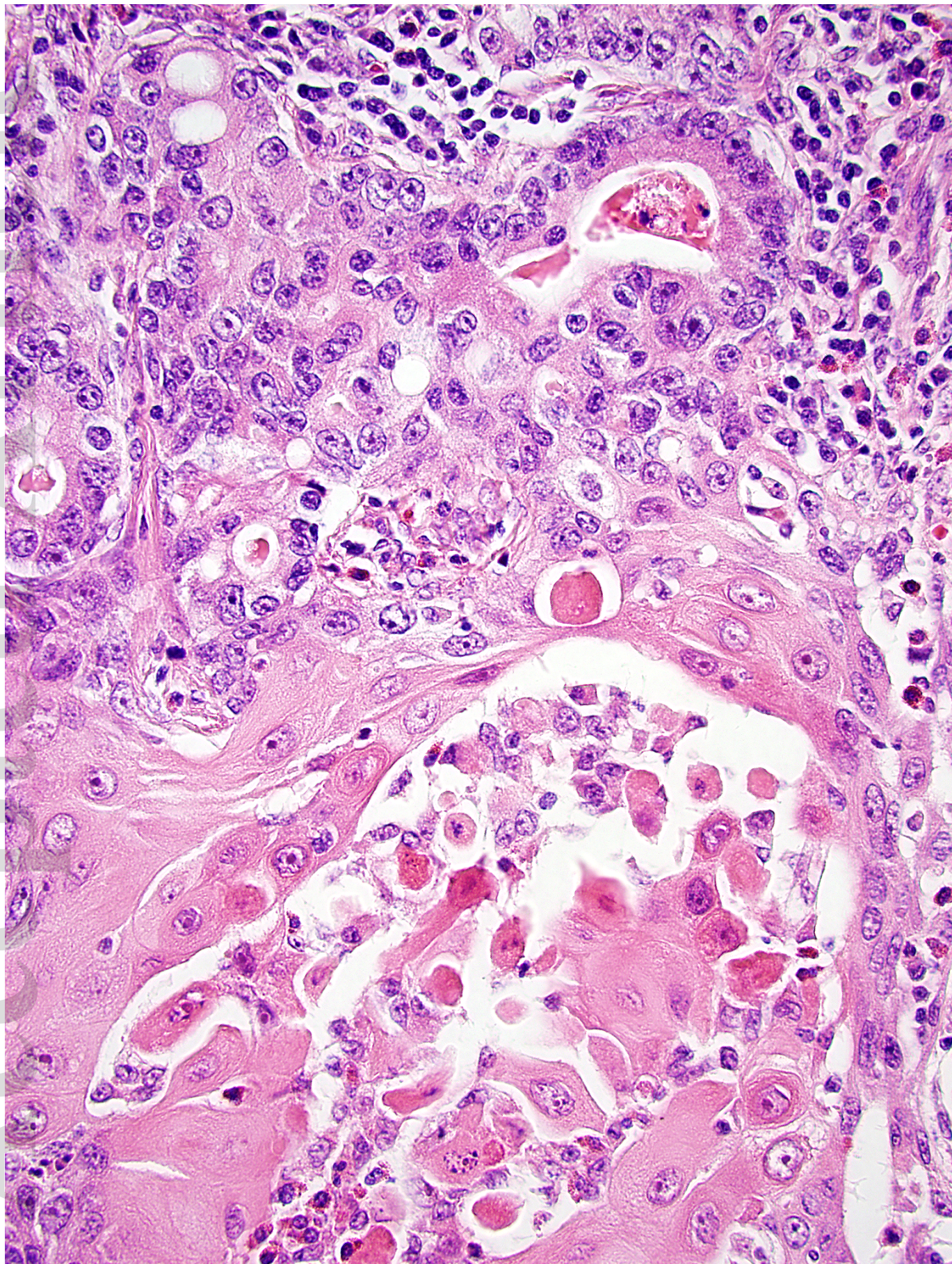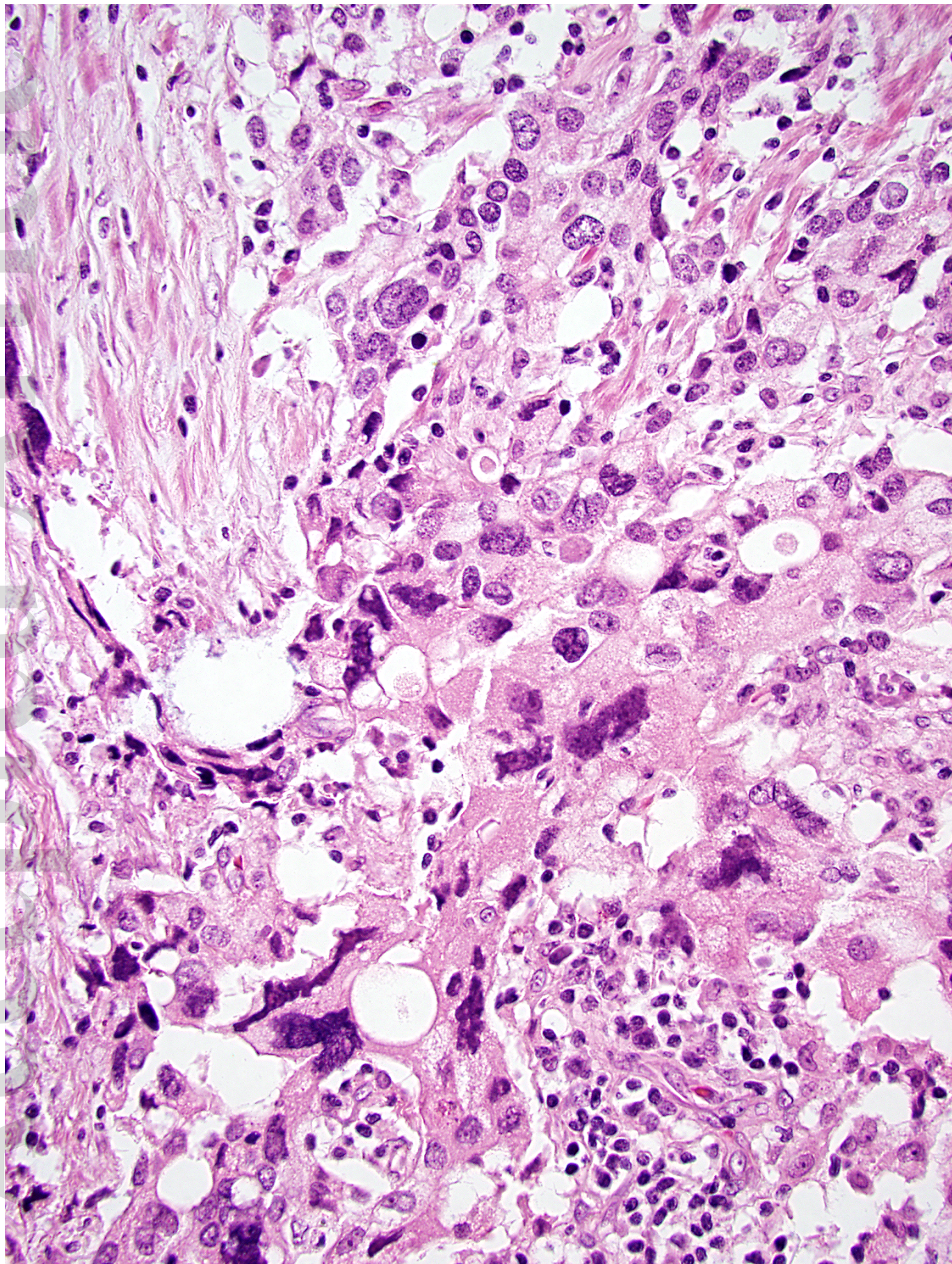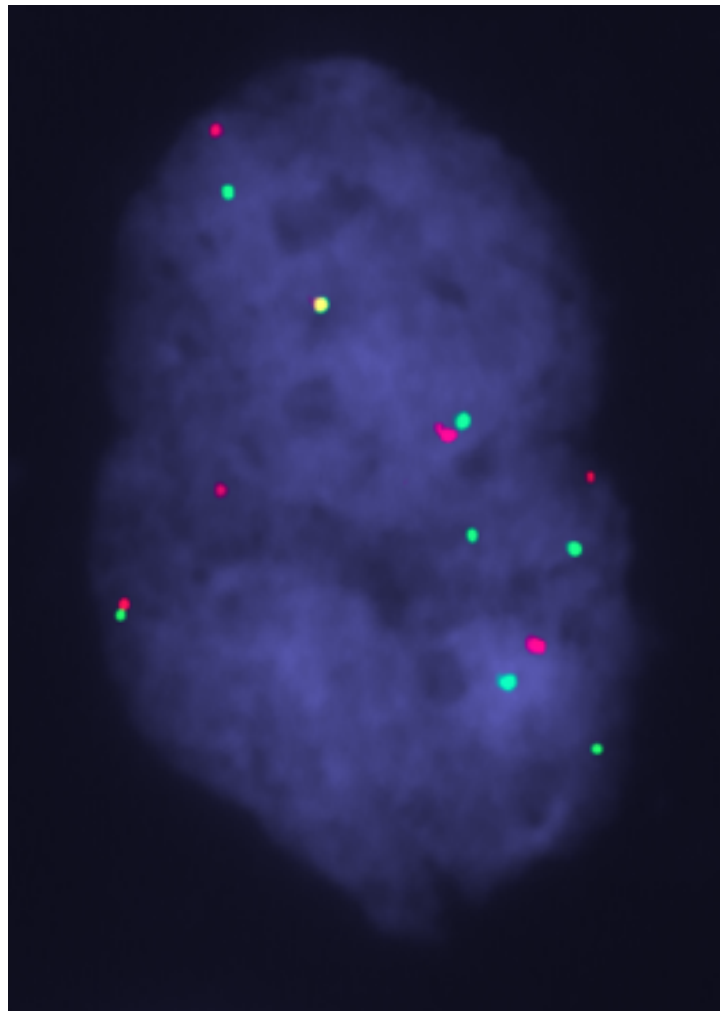
his_14205_f2.tif

his_14205_f3.tif

his_14205_f4.tif

A

B

C

D

his_14205_f5.tif