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Clinicopathologic, Immunohistochemical, and Molecular Characteristics of Ovarian Serous Carcinoma With Mixed Morphologic Features of High-grade and Low-grade Serous Carcinoma

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汇报人：李擒龙

WHO Classification of tumours of the ovary[a,b]

Epithelial tumours

Serous Tumours

Mucinous tumours

Endometrioid tumours

Brenner tumours

Seromucinous tumours

Undifferentiated carcinoma

Serous Tumours	
Benign	
Serous cystadenoma	8441/0
Serous adenofibroma	9014/0
Serous surface papilloma	8461/0
Borderline	
Serous borderline tumour / Atypical proliferative serous tumour	8442/1
Serous borderline tumour - micropapillary variant / Non-invasive low-grade serous carcinoma	8460/2*
Malignant	
Low-grade serous carcinoma	8460/3
High-grade serous carcinoma	8461/3

高、低级别浆液性癌的比较

	高级别	低级别
比例	90-95%	5-10%
年龄	平均63岁	平均比高级别小10岁
大体	双侧，囊实性，出血坏死易见	双侧，囊性多见，细乳头，罕见坏死，常见钙化
光镜	实片+裂隙，常有乳头、腺管、筛状区域， 核大、多形、核仁显著、嗜酸性、多核 MI>12/10HPF，常伴坏死	散在细胞浸润性生长，微乳头，少见粗大乳头 细胞小，核形态相对单一，常缺乏核仁，轻-中度异型 MI通常2-3/10HPF，无坏死，常伴SBT
分子改变	＞95% *TP53*突变 40-50% *BRCA*1/2失活	*KRAS*，*BRAF*突变（二者互斥） *ERBB2, CTNNB1, PIK3CA* 突变
IHC	P53突变型表达	P53野生型表达
处理	手术、化疗	IA 、IB：观察 IC/II：观察、手术、化疗、激素治疗 III、Ⅳ：手术、化疗
预后	75-80%确诊时II期以上 5年生存率25% 术后残存瘤＞2cm预后差	I期：5年生存率＞90% II期以上：5年85%，10年50% 完全减瘤术预后好

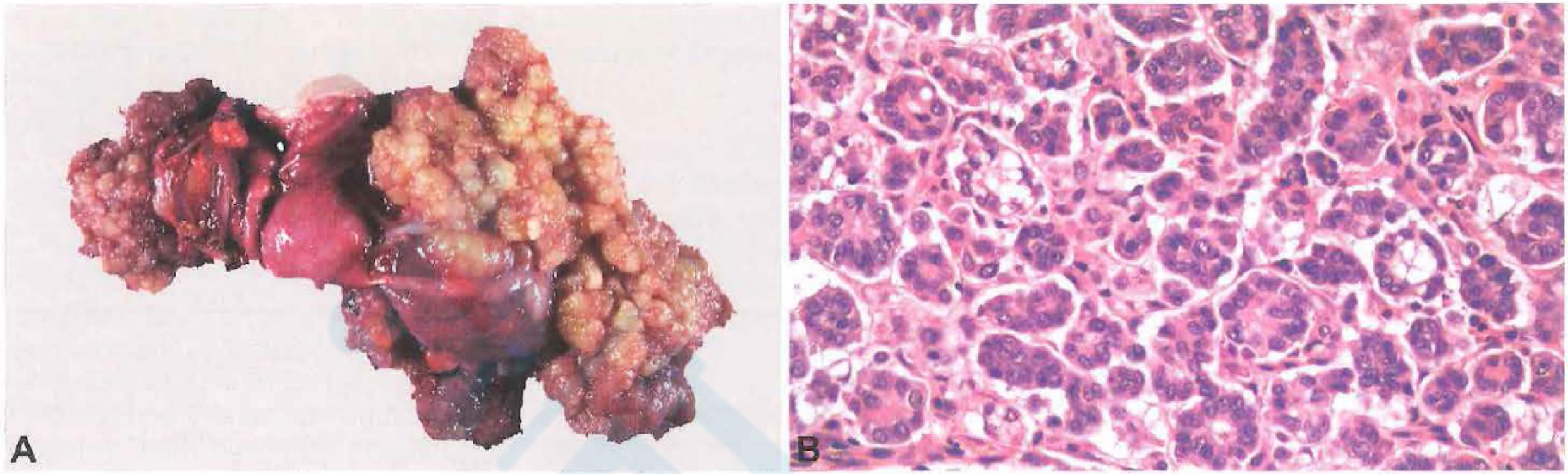

Fig. 1.11 **A** Low-grade serous carcinoma. Bilateral tumours with extensive exophytic papillary growth. **B** Stromal invasion is characterized by micropapillae infiltrating stroma in a haphazard pattern. Nuclei are relatively small, rounded and uniform in size and often contain a single, small but prominent nucleolus. Note the absence of mitotic figures in this field.

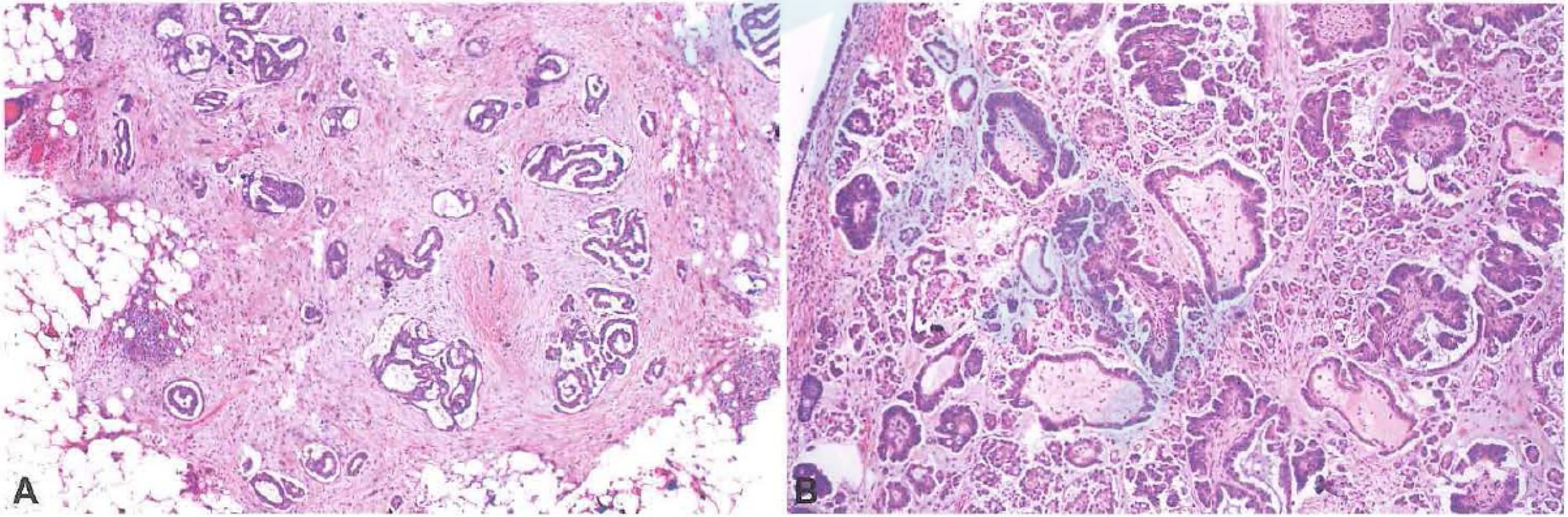

Fig. 1.12 Invasive low-grade serous carcinoma. **A** Micropapillae and gland-like structures haphazardly infiltrating adipose tissue. **B** Macropapillae admixed with micropapillae, displaying a haphazard pattern of infiltration {2084}.

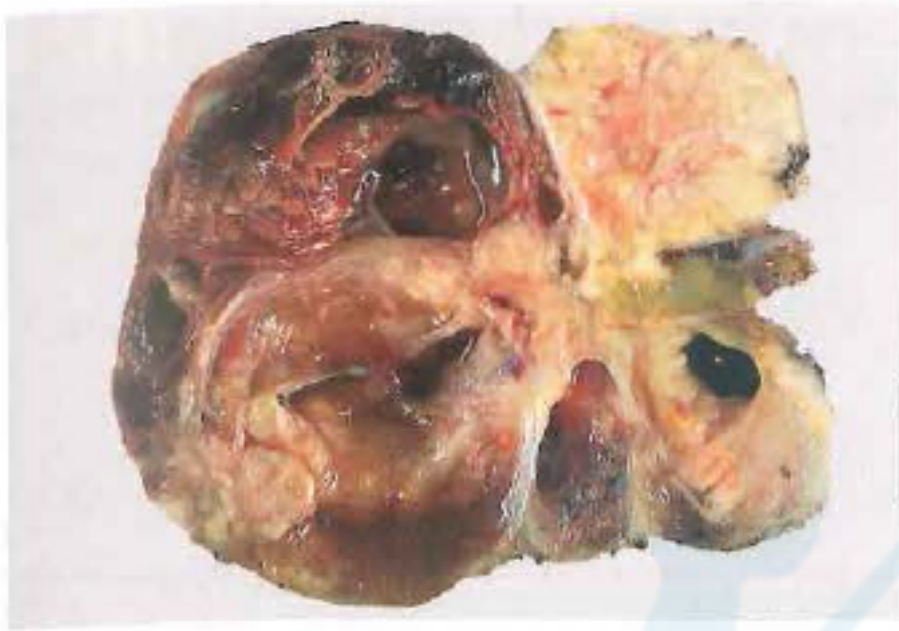

Fig. 1.13 High-grade serous carcinoma. Note the solid growth and large fluid filled cysts.

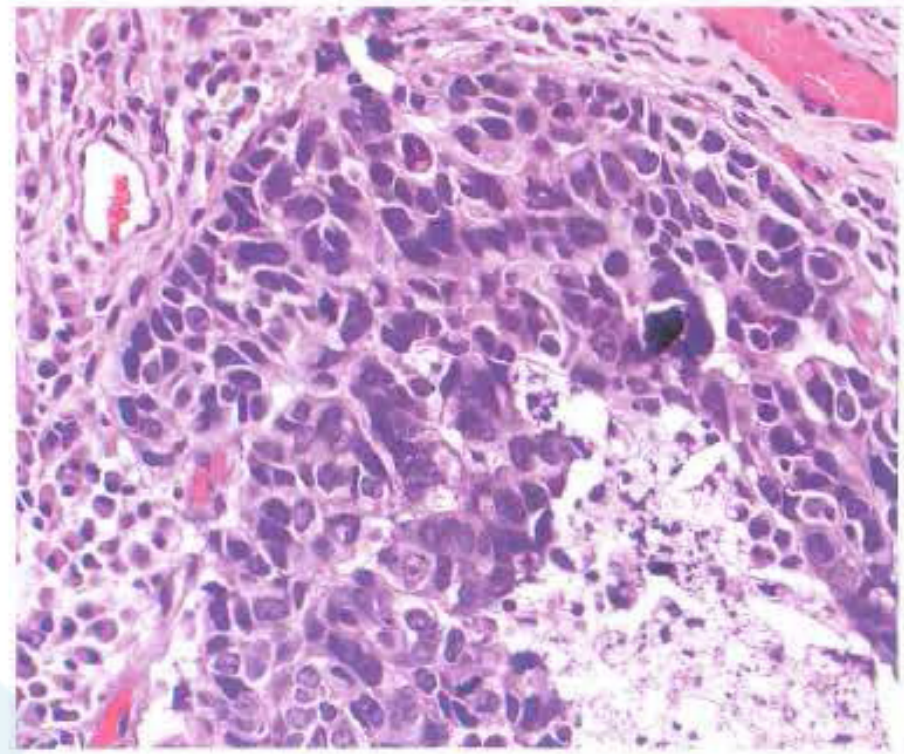

Fig. 1.16 High-grade serous carcinoma. Marked cytological atypia, mitotic figures and necrosis are seen.

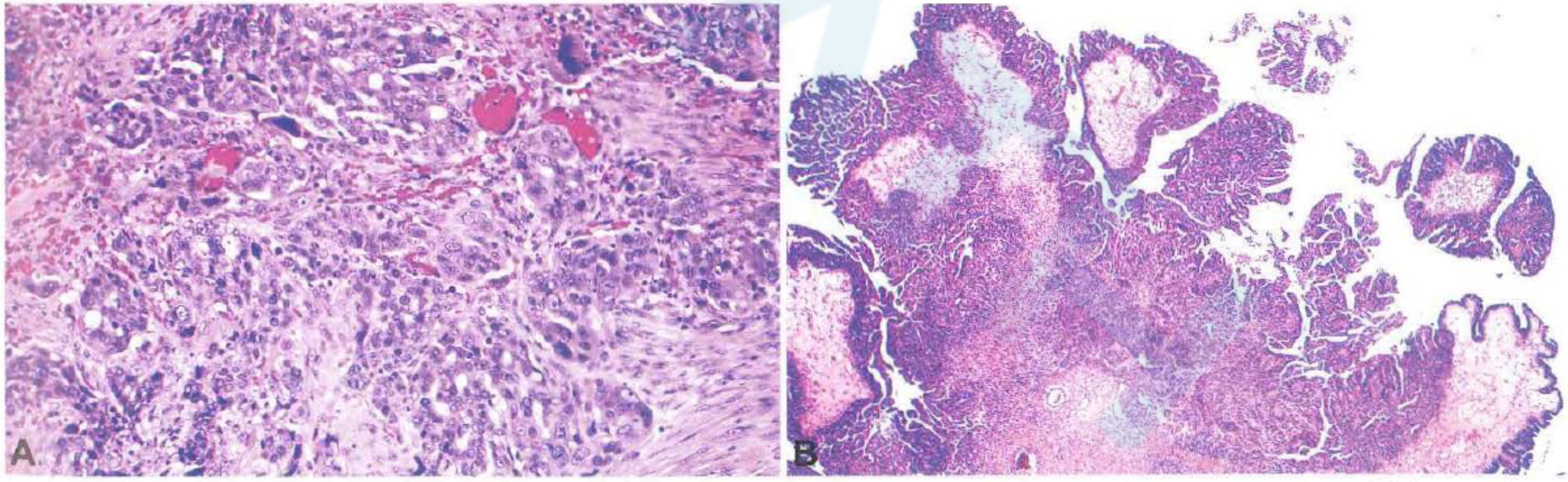

Fig. 1.14 A High-grade serous carcinoma, invasive. Solid masses of cells with slit-like spaces, high-grade nuclear atypia. B High-grade serous carcinoma. This low-magnification view displays a complex papillary serous proliferation which architecturally does not appear to be infiltrating the underlying stroma. The cytological features at higher magnification (not shown) are identical to typical high-grade serous carcinoma and warrant such a diagnosis.

Table 1.1 Immunohistochemical staining of ovarian carcinoma types {38,506,951A,953,1169A,1772A,2085}

Carcinoma Type	PAX8, positive	WT1, positive	TP53 aberrant*	CDKN2A, diffuse#	ER pos.	PR pos.
LGSC	100%	100%	0	0	96%	50%
HGSC	98%	92%	93%	60%	80%	30%
MC	50-60%	0%	50%	14%	6%	0
EC	84%	4%	11%	6%	86%	72%
CCC	99%	0%	12%	9%	13%	6%

* Aberrant expression (associated with *TP53* mutation) refers to either overexpression (strong nuclear expression > 60% of tumour cell nuclei) or complete absence (< 5% of tumour cell nuclei), which is different from the TP53 wild type pattern (not associated with *TP53* mutation); # diffuse bloc staining in > 90% staining; LGSC, low-grade serous carcinoma; HGSC, high-grade serous carcinoma; MC, mucinous carcinoma; EC, endometrioid carcinoma; CCC, clear cell carcinoma

Despite the clear distinction between HGSCA and LGSCA based on their morphologic and molecular features in most cases, in our daily consult and in-house practice, we not infrequently encounter cases that show morphologic features of both HGSCA and LGSCA, making the diagnosis and classification of these tumors very difficult.

Very little is known about the characteristics of this morphologically challenging group. A few case reports and small series of cases have described instances of HGSCA coexisting with SBT or LGSCA, or both, at presentation, or as metachronous recurrences after SBT and/or LGSCA, and molecular genetics and clonality studies on these cases are limited.

The aim of this study is to describe the morphologic features of this relatively uncommon group of ovarian serous carcinomas with mixed morphologic features of HGSCA and LGSCA, that we term **indeterminate grade serous carcinomas (IGSCAs)**, and to investigate the immunohistochemical (IHC), molecular, and clinical characteristics of this diagnostically challenging group of ovarian epithelial neoplasms.

MATERIALS AND METHODS

Case Selection

over 650 ovarian carcinoma cases (1995-2012)

476 classic HGSCA（13 for IHC and molecular analysis）

31 classic LGSCA （19 for IHC and molecular analysis,
27 for histologic evaluation）

22 IGSCA （19 for IHC and molecular analysis，
19 for histologic evaluation）

Histologic Analysis

The following histologic features were recorded for the ovarian neoplasms:

①the histologic appearance of the ovarian tumor,
②the presence of associated lesions (serous cystadenoma, serous adenofibroma, usual SBT, micropapillary, or cribriform SBT),
③the LGSCA was focal (<50% of the tumor) or predominant (>50%),
④the pattern of invasion (micropapillary, small solid nests, cribriform nests, macropapillae, solid sheets, irregular glands, or combinations of patterns),
⑤the presence or absence of lymph-vascular space invasion,
⑥nuclear features,
⑦mitotic index.

Histologic Analysis

The fallopian tube slides, which for the most part were sampled with only 1 representative section per tube, were reviewed for the presence of papillary hyperplasia, SBT, STIC, or serous carcinoma.

As immunostains were not performed in the majority of cases, **STIC** was defined as marked nuclear atypia with loss of polarity in areas showing loss of cilia of the tubal epithelium without invasion.

Histologic Analysis

Peritoneal involvement was evaluated for the following features:
①site,
②associated lesions (endosalpingiosis, noninvasive implants of SBT),
③focal versus predominant LGSCA (< or >50%),
④the pattern of invasion (same as for the ovarian tumors),
⑤lymphvascular space invasion,
⑥nuclear features,
⑦mitotic index.

Immunohistochemistry

Diffuse cytoplasmic staining was considered as an abnormal BRAF V600E (VE1) staining pattern, consistent with mutant BRAF protein expression.

Diffuse strong nuclear staining or complete absence of nuclear staining with p53 antibody were considered abnormal p53 staining patterns consistent with mutant p53 protein expression.

Molecular Testing

next-generation sequencing [NGS]

the hotspot mutation sites of the 50 most common solid tumor genes

(including ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, and VHL.)

Clinical Data

The overall survival, mutation status and the frequency of mutations were compared between three patient groups (HGSCA, LGSCA, and IGSCA) .

RESULTS

Demographic Data

	HGSCA	IGSCA	LGSCA	
I and II	7.7%	10.5%	26.3%	***P = 0.42***
III and IV	92.3%	89.5%	73.7%	
Median age	63.2 range: 40.5 to 73.9	53.6 range: 23.1 to 86.7	57.4 range:28.3 to 89.6	***P = 0.52***

RESULTS

Morphologic Features of LGSCA versus IGSCA

Parameters	n (%) LGSCA (N = 27)	n (%) IGSCA (N = 19)
Comparison of gross morphologic features between LGSCA and IGSCA		
Ovarian tumor		
Not resected	1 (4)	0
No tumor	1 (4)	0
BLT only	2 (8)	1 (5)
Focal CA < 50%	13 (48)	5 (26)
CA > 50%	10 (37)	13 (68)
Associated ovarian findings		
Serous AF	1 (4)	0
TSBT	8 (30)	3 (16)
MPSBT	8 (30)	8 (42)
T/MPSBT	5 (19)	0
AF/T/MPSBT	1 (4)	1 (5)
No associated lesion	0	6 (30)

AF indicates adenofibroma; CA, carcinoma; MPSBT, micropapillary serous borderline tumor; TSBT, typical serous borderline tumor.

TABLE 1. Morphologic Features of LGSCA versus IGSCA

Parameters	n (%) LGSCA (N = 27)	n (%) IGSCA (N = 19)
Comparison of microscopic features between LGSCA and IGSCA		
Ovarian tumor		
Nuclei	Small, uniform	Enlarged, overlapping, irregular
Mitotic rate (mean) (MF/10 HPF)	3	11
> 12 MF/10 HPF (%)	0	43
Pattern of stromal invasion		
Micropapillae only	1 (4)	5 (28)
Solid sheets, alone or with other patterns	2 (9)	4 (22)
Micropapillae and solid nests	8 (35)	3 (17)
Cribriform nests	4 (17)	2 (11)
Solid nests only	2 (9)	2 (11)
Micropapillae and glands	2 (9)	0
Macropapillae	1 (4)	1 (5)
Other combinations	3 (13)	1 (5)
Lymph-vascular invasion	0	0
Fallopian tube lesions (STIC)	0	0

AF indicates adenofibroma; CA, carcinoma; MPSBT, micropapillary serous borderline tumor; TSBT, typical serous borderline tumor.

HGSCA

pleomorphism, overlapping

31

88

(*P* <0.0001)

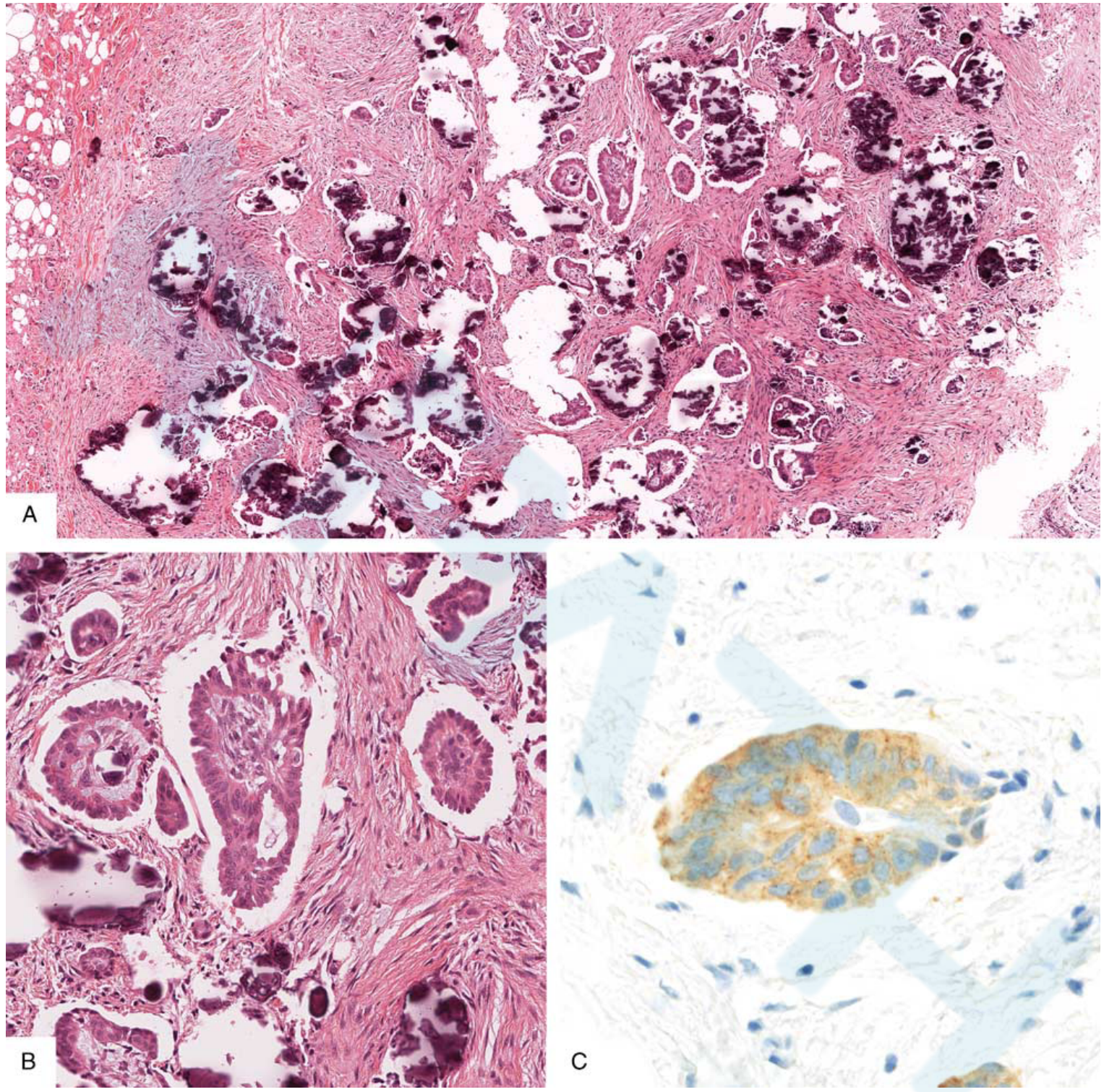

FIGURE 1. Classic LGSCA with a BRAF V600E mutation (1/19), infiltrating papillae (A), LG nuclear features (B), and a corresponding mutant BRAF V600E immunostain (C).

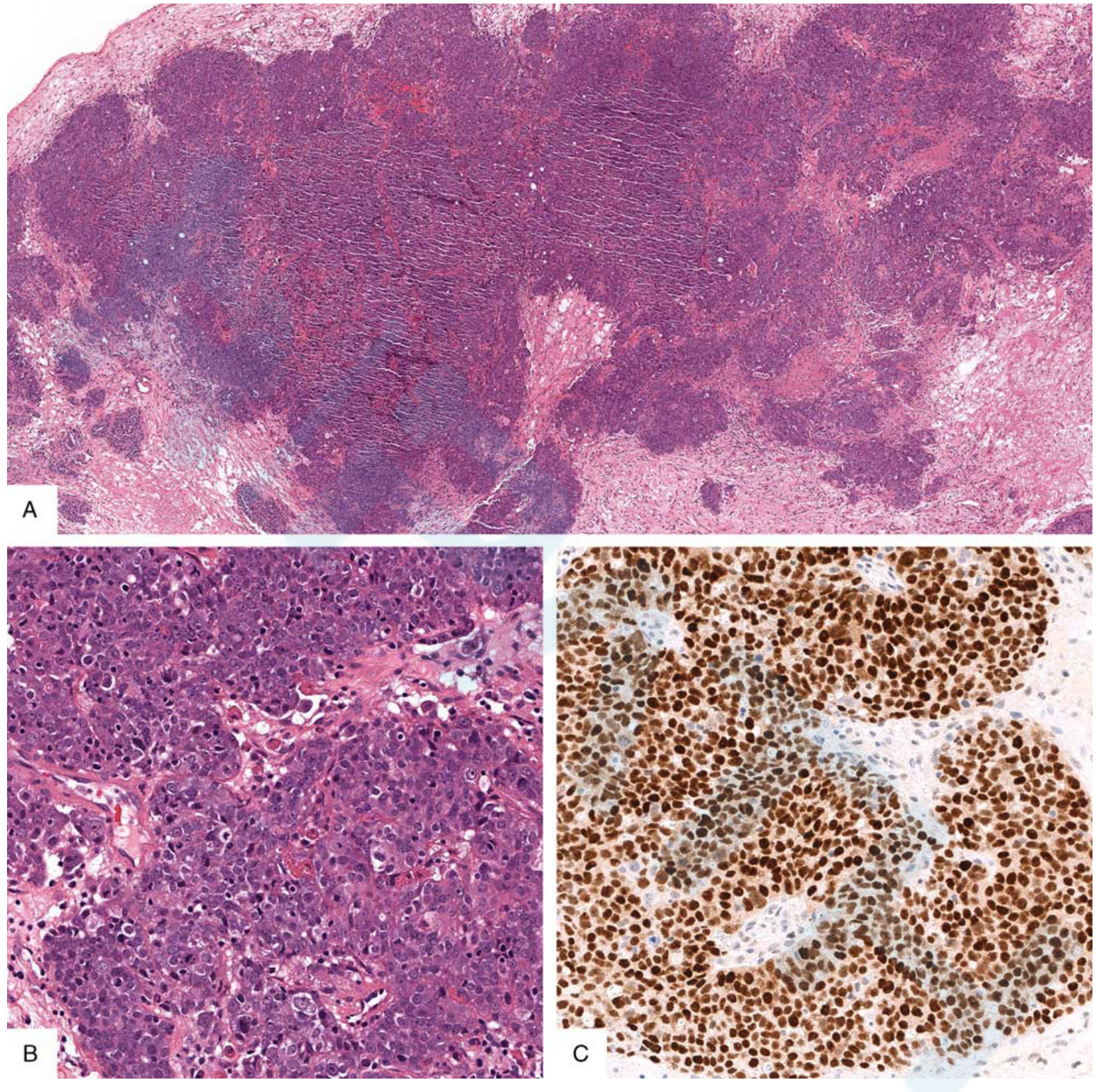

FIGURE 2. Classic HGSCA with a TP53 mutation,
solid, and focal slit-like space architecture (A), high nuclear grade (B),
and diffuse p53 (mutational) immunostaining (C).

The majority of cases in this study had only 1 representative section of each fallopian tube. Given this caveat, none of the patients with LGSCA or IGSCA with evaluable fallopian tube tissue had STIC of the fallopian tube.

RESULTS

IHC Data

TABLE 2. IHC Results

Groups	p53 IHC Type	p53 IHC # of Patients	BRAF V600E IHC Type	BRAF V600E IHC # of Patients
Classic LGSC	Mutant	0	Mutant	1
	Wild	19	Wild	18
Classic HGSC	Mutant	12	Mutant	0
	Wild	1	Wild	13

	LG area	HG area	# of Patients	LG area	HG area	# of Patients
IGSCA	NA	Mutant	1	NA	Wild	2
	NA	Wild	1	Wild	Wild	17
	Equivocal	Mutant	1			
	Mutant	Mutant	1			
	Wild	Equivocal	1			
	Wild	Mutant	1			
	Wild	Wild	13			

NA indicates not available.

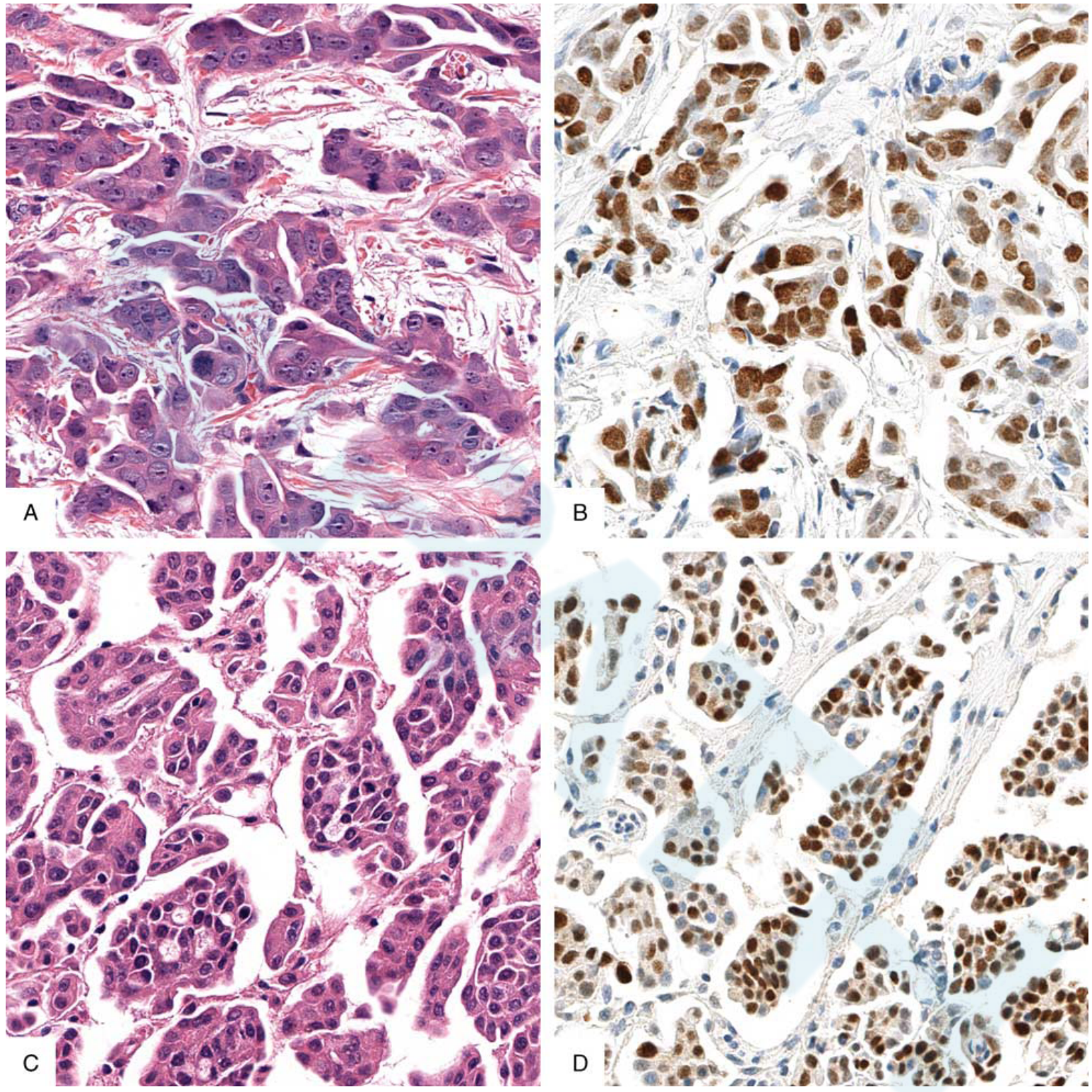

FIGURE 3. IGSCA case with identical missense TP53 mutations in the HG component (A) and LG component (C). Immunostains for p53 were interpreted as equivocal in the HG component (B) and wild-type in the LG component (D).

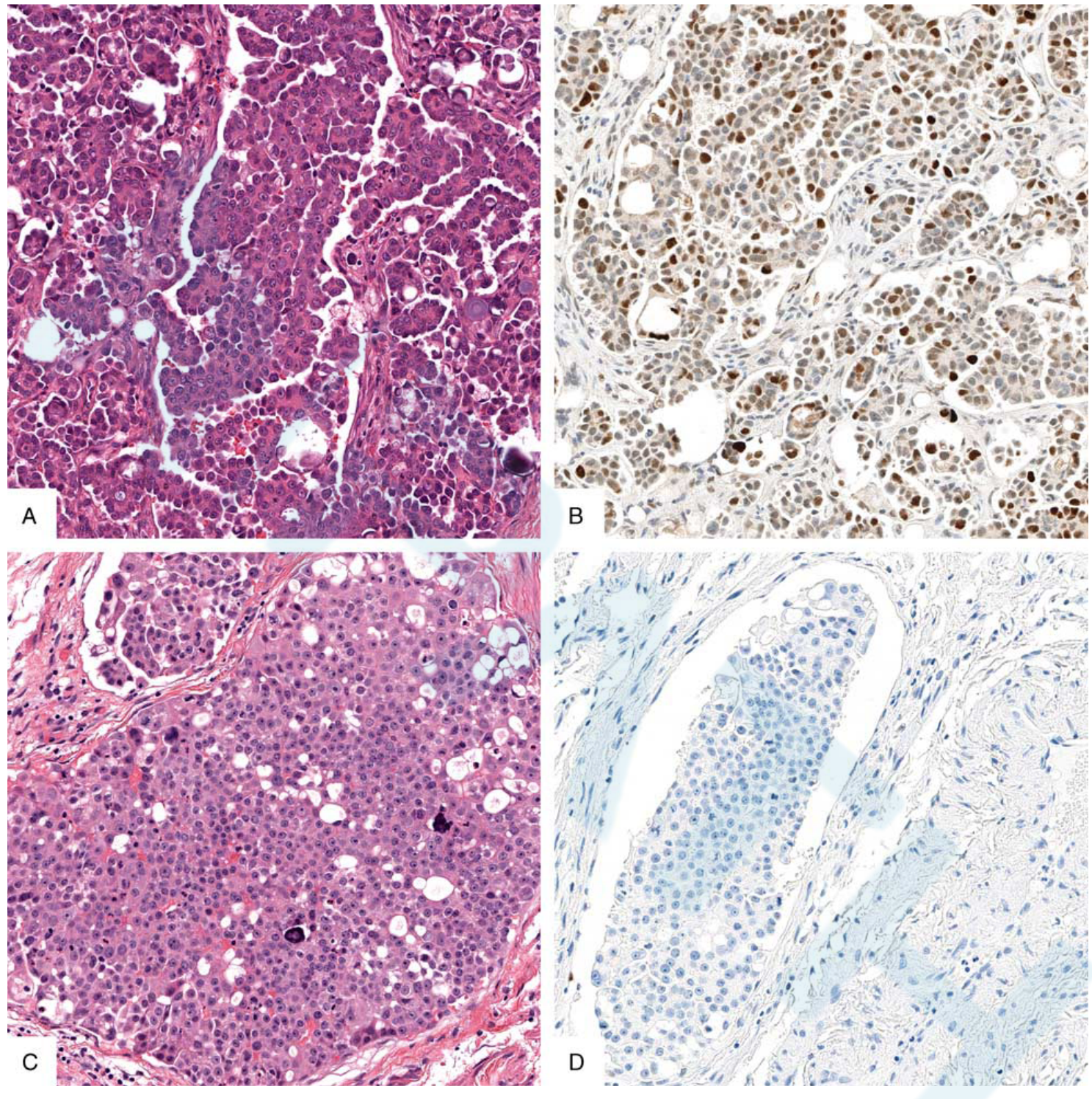

FIGURE 4. IGSCA cases with only unusual ambiguous HG cytologic features. One had a BRAF non-V600E mutation, diffusely infiltrating micropapillae (A), and wild-type p53 immunostaining pattern (B). The other had a truncating TP53 mutation, focal solid areas (C), and a null-type mutational p53 immunostaining pattern (D).

Sequencing (NGS) Results

Genes	Pathogenic Alterations Found by NGS Panel and Their Frequency
Classic HGSCA (n = 13)	
TP53, oncomorphic	c.583A > T, p.Ile195Phe (2/13)
TP53, oncomorphic	c.524G > A, p.Arg175His (2/13)
TP53, oncomorphic	c.695T > G, p.Ile232Ser (1/13)
TP53, truncating	c.1013_1014del, p.Phe338* (1/13)
TP53, truncating	c.493C > T, p.Gln165* (1/13)
TP53, oncomorphic	c.404G > A, p.Cys135Tyr (1/13)
TP53, oncomorphic	c.731G > A, p.Gly244Asp (1/13)
TP53, truncating	c.1024C > T, p.Arg342* (1/13)
TP53, truncating	c.328del, p.Arg110Valfs*13 (1/13)
TP53, truncating	c.788del, p.Asn263Ilefs*82 (1/13)
TP53, oncomorphic	c.329G > T, p.Arg110Leu (1/13)
Classic LGSCA (n = 18)	
KRAS	c.35G > A, p.Gly12Asp (5/18)
NRAS	c.182A > G, p.Gln61Arg (3/18)
BRAF	c.1406G > C, p.Gly469Ala (1/18)
BRAF	c.1799T > A, p.Val600Glu (1/18)
ERBB2	c.2313_2324dup, p.Tyr772_Ala775dup (1/18)
ERBB2	c.2325_2328delins16, p.Tyr772_Ala775dup (1/18)

	IGSCA with mixed HG and LG areas (n = 18, including 2 cases lacking LG component)	
	Pathogenic alterations found by NGS panel in each tumor area	
	HG	LG
TP53 oncomorphic	c.832C > T, p.Pro278Ser	c.832C > T, p.Pro278Ser
TP53 oncomorphic	c.747_748delinsTT, p.Arg249_Pro250delinsSerSer	c.747_748delinsTT, p.Arg249_Pro250delinsSerSer,
TP53 oncomorphic	c.856G > A, p.Glu286Lys	c.856G > A, p.Glu286Lys
NRAS	c.182A > G, p.Gln61Arg	c.182A > G, p.Gln61Arg
BRAF	c. 1790T > G, p.Leu597Arg	NA
TP53, truncating	c.394A > T, p.Lys132*	NA

RESULTS

Association of IHC and NGS Results

TABLE 4. IHC and NGS Concordance in Classic HGSCA, Classic LGSCA, and IGSCA Cases With Mixed Features

		IHC Result/Sequencing Result (# of Patients)					
	Concordance (%)	+/+	+/−‡	−/+	−/−	Equivocal/+	Equivocal/−
p53/TP53							
Classic HG	92.3	12/13		1/13			
Classic LG	100				18/18		
IGSCA, HG*	77.8	2/18	1/18		12/18	2/18	1/18
IGSCA, LG*	81.3	1/16		1/16	12/16	1/16	2/16
BRAF V600E							
Classic HG	100				13/13		
Classic LG	100	1/18		1/18§	16/18		
IGSCA, HG†	100			1/18§	17/18		
IGSCA, LG†	100				16/16		

*Among the 18 IGSCA patients overall, considering any mutation found in either the LG or HG block, 2 patients had mutant p53 via IHC only, 2 had *TP53* mutation via NGS only, and 2 had mutation found via both methods.

†Among the 18 IGSCA patients overall, considering any found mutation for *BRAF* V600E in either the LG or HG block, only 1 patient had mutation found via NGS only.

‡Plus sign (+) is a p53 mutant protein staining pattern either nuclear overexpression or complete loss of nuclear expression. Minus sign (−) is a p53 normal staining pattern.

§These 2 patients had *BRAF* non-V600E mutations by NGS and the IHC was negative for mutant BRAF V600E protein in both.

Survival and Prognostic Features

The only statistically significant parameters affecting patients' survival in all 3 groups were WHO 2-tier classification system with the addition of IGSCA category and TP53 mutation status.

The IGSCA group's overall survival was more similar to classic HGSCA with a 5-year survival rate.

Group	5-year survival
IGSCA	46.5% (95% CI: 19.7%-73.4%)
HGSCA	72.7% (95% CI: 45.9%-99.5%)
LGSCA	87.7% (95% CI: 71.6%-100%)

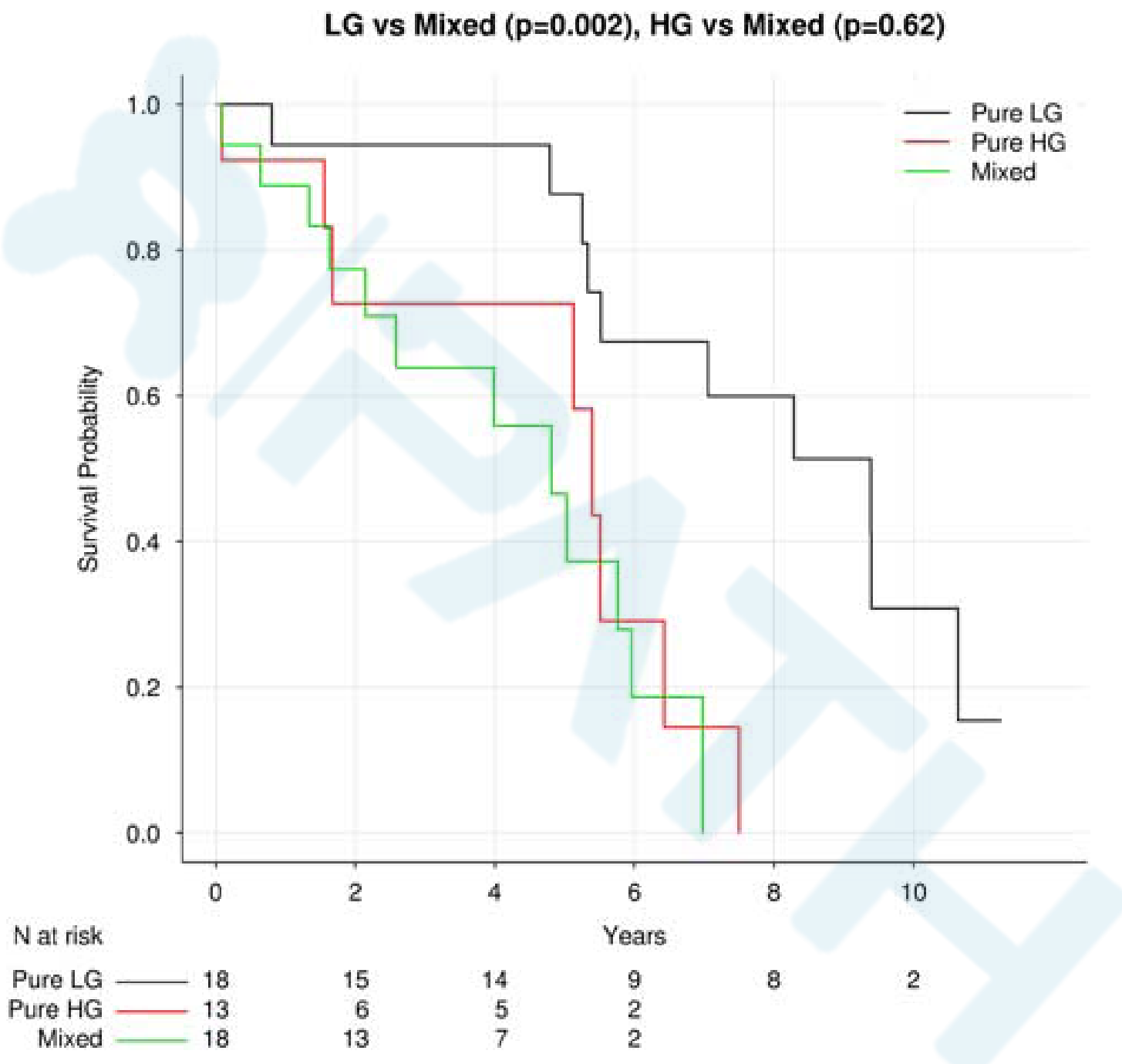

FIGURE 5. Survival comparison between 3 groups

The survival for those with the TP53 mutation was worse as compared with those without the mutation. (P= 0.03)

TP53 mutation	5-year survival
With	65.3% [95% CI: 40.2%-90.3%]
Without	73.3% [95% CI: 56.2%-90.5%]

DISCUSSION

IGSCA are generally identified by the architectural patterns of LGSCA with the presence of areas with HG nuclear features and mitotic index coexisting with areas with small uniform nuclei that resemble LGSCA.

IGSCA are uncommon, comprising 3.4%, or 22 of 650 ovarian carcinoma cases reviewed for this study. It is somewhat similar to that reported in 1 series by Malpica et al, in which the incidence of serous carcinoma of low malignant potential coexisting with HGSCA was estimated as 2% (1 case of 50 classic HGSCA).

The IGSCA group patients were slightly younger than patients with classic LGSCA at the time of diagnosis, however, this was not statistically significant.

Somewhat surprisingly, patients with IGSCA in this study had a poor prognosis, more similar to that of HGSCA than LGSCA.

IGSCA had patterns of stromal invasion similar to that of the LGSCA analyzed.

Overall, 96% of our LGSCA had coexisting lesions. In contrast, 1/3 of the IGSCA had no identifiable coexisting neoplasm, most of those with a coexisting tumor had an MPSBT alone or in combination with a conventional SBT, most of them comprised the majority of tumor in the ovaries they involved (68%).

The majority of IGSCA (12/18, 67%) lacked the most common genetic alterations seen in the classic HGSCA and classic LGSCA, including TP53, KRAS, and BRAF mutations respectively.

Of the 4 cases with mutations and an LG and HG components, 3 had TP53 mutations and 1 had an NRAS mutation.

It is important to note that in IGSCA with both LG areas and HG areas, the mutation results in both components were identical, suggesting at least baseline similarity in molecular driver mutations despite differences in the microscopic appearance.

Our case series included 2 tumors with the morphology of IGSCA without the LG component. One of our cases had a TP53 mutation and 1 a BRAF non-V600E mutation. This data indicates that at least some “moderately differentiated” serous carcinomas may have molecular features unlike typical HGSCA, but still have a poor prognosis, and deserve further study, such as more extensive NGS.

This study and those mentioned in the previous paragraphs document that HGSCA may coexist with, or arise after an SBT or LGSCA, that most such HGSCAs do not have TP53 mutations, and that they likely have a poor prognosis. The relationship between the HG and LG components remains ill-defined.

Our findings suggest that IGSCAs are a rare, potentially clinically aggressive variant of serous carcinoma. They have an overall survival similar to classic HGSCA, rather than LGSCA.

This morphologic heterogeneity has important implications for the interpretation of small biopsy and fluid specimens for the initial diagnosis of serous carcinoma, as the aggressive behavior of an IGSCA could be initially unrecognized in a small specimen. As seen by the molecular data, since most of these tumors lack TP53 mutations, IHC stains for p53 would not be of value in this differential.

Conclusions

1. IGSCA is a rare, but morphologically distinct tumor that provides a diagnostic conundrum for pathologists within the existing 2-tier grading system. These tumors have morphologic characteristics that make them difficult to assign to either classic HGSCA or LGSCA.
2. Molecular analysis suggests that such defined IGSCAs infrequently show TP53, RAS/RAF, or ERBB2 mutations typically seen in classic serous carcinomas of HG or LG, but most are negative for these alterations.
3. Although small in number in our study, TP53 mutant IGSCA may actually have a more aggressive course than classic HGSCA, and p53 wild-type IGSCA may still portend a clinical course similar to HGSCA, providing for diagnostic pitfalls, especially in small biopsies.
4. Further genomic study of IGSCA may provide needed diagnostic, prognostic, and theranostic biomarkers in this rare, but difficult group of serous carcinomas.

Thanks for your attention!