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Frankly Invasive Carcinoma Ex-intraductal Carcinoma: Expanding on an Emerging and Perplexing Concept in Salivary Gland Tumor Pathology

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Abstract

Intraductal carcinoma (IDC) of the salivary glands is an uncommon and enigmatic tumor, our understanding of which is rapidly evolving. Recent studies have demonstrated multiple IDC subtypes and consistent gene fusions, most frequently involving *RET*. Because IDC is a ductal proliferation surrounded by flattened myoepithelial cells, it was previously presumed to be analogous to breast ductal carcinoma in situ, but recent evidence has shown that the myoepithelial cells of fusion-positive IDC harbor the same genetic alterations of the ductal cells and are therefore neoplastic. In addition, there are rare reports of fusion-positive IDC with overt areas of irregular invasion lacking myoepithelial cells, but this phenomenon is not well documented or understood. This study aims to better characterize these frankly invasive carcinoma ex-IDC. All cases of frankly invasive carcinoma ex-IDC were obtained from the authors' files. Inclusion criteria included a component of concurrent or antecedent IDC and/or a fusion known to be associated with IDC. Immunohistochemistry (S100, SOX10, mammaglobin, androgen receptor, p63, p40) and molecular analysis (targeted RNA sequencing or large panel DNA next generation sequencing) was performed. Clinical follow-up was obtained from medical records. Ten cases of frankly invasive carcinoma ex-IDC were identified. The tumors occurred in 8 men and 2 women ranging from 33 to 82 years (mean, 66.3). All but one case arose in the parotid gland. In 4 cases, the IDC component was intercalated duct type. It was mixed apocrine/intercalated duct in two, and in the remaining 4 cases, no residual IDC was identified. The frankly invasive carcinomas were remarkably heterogeneous, ranging from minimally to widely invasive beyond the confines of the IDC, low-grade to high-grade, with morphologies that varied from duct-forming to those having clear cell or sarcomatoid features, to frankly apocrine. The original diagnoses for these cases were (adeno) carcinoma, not otherwise specified (n = 6), salivary duct carcinoma (n = 3), and secretory carcinoma (n = 1). All cases harbored fusions: *NCOA4*::*RET* (n = 6), *TRIM33*::*RET* (n = 2), *TRIM27*::*RET* (n = 1), and *STRN*::*ALK* (n = 1). Clinically, one tumor recurred locally, cervical lymph node metastases occurred in five patients, and distant metastasis later developed in four of these patients. Our findings highlight striking diversity in frankly invasive carcinomas that arise from fusion-positive IDC, a tumor which may serve as a precursor neoplasm like pleomorphic adenoma. These carcinomas vary in their extent of invasion, grade, histologic appearances, and clinical behavior. Importantly, in contrast to pure IDC, which is believed to be indolent, many frankly invasive cases were aggressive. Because *RET* and *ALK* fusions are targetable, it is important to recognize the broad spectrum of frankly invasive carcinomas that can arise from IDC, particularly because some cases are completely overrun or recur without any recognizable IDC component. These results suggest fusion analysis may be of clinical benefit on any salivary gland (adeno) carcinoma, not otherwise specified or salivary duct carcinoma.

Keywords Intraductal carcinoma · Salivary · *NCOA4:RET* · *TRIM33:RET* · *TRIM27:RET* · *STRN:ALK*

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Introduction

Intraductal carcinoma (IDC) is an uncommon salivary gland neoplasm previously known as "low-grade salivary duct carcinoma" or "low-grade cribriform cystadenocarcinoma" [1, 2]. In the past few years, our understanding of IDC has dramatically improved. Far from a monolithic entity, it is now clear that there are at least four subtypes of IDC which are morphologically and genetically unique. Intercalated duct type IDC is most common; it is diffusely S100 and SOX10 positive and most often harbors *NCOA4*::*RET* (in about half), [3–5] although *STRN*::*ALK, TUT1*::*ETV5*, *KIAA1217*::*RET* and fusion-negative cases have also been reported [3, 6]. Purely apocrine IDC is negative for S100 protein and SOX10, and strongly positive for androgen receptor. This subtype does not harbor fusions but rather has a complex mutational profile (e.g., *PIK3CA* and *HRAS* mutations, *TP53* loss) reminiscent of salivary duct carcinoma [4, 5, 7, 8]. Oncocytic IDC has an intercalated duct-like phenotype with oncocytic cytomorphology, and may have *TRIM33*::*RET*, *NCOA4*::*RET*, or *BRAF* mutations [9]. Lastly, there are mixed IDCs with intercalated duct-like areas juxtaposed with oncocytic or apocrine areas. Mixed IDCs may harbor *TRIM27*::*RET* or *TRIM33*::*RET* [5, 9, 10].

Because IDC consists of a ductal proliferation surrounded by attenuated myoepithelial cells, it was previously presumed that IDC was conceptually analogous to breast ductal carcinoma in situ. A recent study, however, demonstrated that the myoepithelial cells of fusion-positive IDC harbor the same alterations as the ductal cells, strongly pointing to the fact that IDC is actually a biphasic (i.e., containing neoplastic ducts and myoepithelial cells) salivary gland neoplasm similar to pleomorphic adenoma, epithelial-myoepithelial carcinoma, and others [11].

When IDC is entirely intraductal, i.e., has a complete intact layer of myoepithelial cells, it usually behaves indolently, regardless of subtype. When IDC loses this myoepithelial cell layer and exhibits frank tissue invasion, however, it appears to have the capacity to be much more aggressive. This phenomenon is well documented for purely apocrine IDC, which is known to give rise to salivary duct carcinoma. This association is intuitive, as apocrine IDC and salivary duct carcinoma are essentially histologically and genetically identical [4, 5, 7, 8]. The occurrence of frankly invasive carcinomas arising from the other types of IDC, however, is much less well understood, with only a few reported cases [1, 3–5, 12–14]. We sought to more completely characterize the histology, immunoprofile, and behavior of frankly invasive carcinomas that arose from fusion-positive IDC.

Methods

Case Selection

Cases of frankly invasive carcinoma ex-IDC were retrieved from the authors' surgical pathology archives and consultation files. Two frankly invasive cases had been previously published [4, 12]. Another frankly invasive case arose following an IDC that was previously published in its pure form [3, 5]. All cases were reviewed, and various histologic features were tabulated. Inclusion criteria for "frankly invasive" carcinoma ex-IDC were: (1) tumor was overtly invasive at the histologic level; (2) there was a lack of myoepithelial cells in the invasive area(s) as demonstrated by routine microscopy and/or immunohistochemistry; and (3) the overtly invasive carcinoma was associated with a concurrent or prior IDC in the same location and/or it harbored a fusion known to occur in IDC. "Frank invasion" is a qualitative term that does not refer to extent of invasion. Any available clinical and follow-up information was collected for each case from the electronic medical record.

Immunohistochemistry

Immunohistochemistry for S100 (Ventana Medical Systems, Tucson, USA), SOX10 (Ventana), androgen receptor (AR) (Ventana) mammaglobin (Dako, Glostrup, Denmark); and either p40 (BioCare Medical, Concord, USA) or p63 (BioCare) was performed, with appropriate controls, on 4-µm whole-slide sections using standardized automated protocols on Ventana BenchMark Ultra autostainers (Ventana). All immunohistochemical signals were visualized using the Ultra view polymer detection kit (Ventana).

Molecular Analysis

Five cases were subjected to targeted RNA sequencing for fusions as previously described [15]. Briefly, whole-slide tissue sections were cut at 10 µm, and Qiagen AllPrep kits (Qiagen, Germantown, MD) were used for RNA isolation. A sequencing library was made using a modified TruSight RNA Pan-Cancer kit (Illumina, San Diego, CA) with 1425 genes. Sequencing was performed on the NextSeq 550 (Illumina, San Diego, CA) with a minimum of 6,000,000 mapped reads. Fusions were called using the Star-Fusion algorithm [16]. All fusions were manually reviewed via the Integrated Genomics Viewer (Broad Institute, Cambridge, MA). Four cases underwent large panel DNA next generation sequencing by Foundation Medicine using methods previously described [17]. The remaining case was subjected to

NextGen Sequencing as part of the MGH Pathology targeted Solid Fusion Assay [12].

Results

Ten cases of frankly invasive carcinoma ex-IDC were identified. They are summarized in Table 1. The tumors occurred in 8 men and 2 women ranging from 33 to 82 years (mean, 66.3). Nine cases arose in the parotid gland; the remaining case was from the sublingual gland. Patients presented nonspecifically, with facial swellings of several months' duration, with or without pain.

In 4 cases, the IDC component was documented as intercalated duct type, demonstrating classic histologic and immunophenotypic features. Two IDCs were mixed apocrine/intercalated duct type, with approximately half of each morphology showing each phenotype. In the remaining 4 invasive carcinomas, no residual IDC was identified, and no prior IDC history was documented. These cases were nevertheless included because they harbored fusions known to occur in IDC (as detailed below). For the 6 cases where IDC was found, it was adjacent to the invasive tumor in 5 of 6. For the remaining tumor, the invasive carcinoma occurred 42 months following complete excision of a mixed apocrine/intercalated duct IDC that lacked any frank invasion.

Six of 10 frankly invasive carcinomas were originally diagnosed as some variation of carcinoma, not otherwise specified (NOS): cystadenocarcinoma, NOS (n = 2); adenocarcinoma, NOS (n = 2); microinvasive carcinoma (n = 1); and carcinoma with clear cell features (n = 1). Three cases were diagnosed originally as salivary duct carcinoma. Two of them were seen only as recurrences and metastases, and another arose from a preceding mixed apocrine/intercalated duct IDC. One case arising from an intercalated duct IDC was originally diagnosed as secretory carcinoma.

Histologically, the frankly invasive carcinomas ex-IDC were remarkably heterogeneous and therefore difficult to summarize. In three cases, the invasion was minimal (≤ 1 mm) in an otherwise typical IDC (Fig. 1). In these cases, the invasive carcinoma closely resembled IDC at the cellular level, with epithelioid cells with amphophilic cytoplasm and bland, oval nuclei, arranged as small nests (Fig. 1). Although small, these foci were recognizable as invasive based on irregularity of the nests with a stromal reaction, and confirmed by a total loss of myoepithelial cells on immunohistochemistry (Fig. 1). The remaining cases were widely invasive from the IDC, or had no concurrent IDC component.

Two of the widely invasive carcinomas were low-grade and partially cystic, while 5 were high-grade and solid. One of the low-grade cystic carcinomas was strikingly papillary and mucin-producing, somewhat resembling mucinous adenocarcinoma of salivary glands (Fig. 2). The other low-grade cystic tumor had solid areas composed of back-to-back and fused tubules containing amphophilic cytoplasm and monotonous round nuclei (Fig. 3). This appearance resembled low-grade sinonasal adenocarcinoma, so much so that the possibility of metastasis from the sinonasal tract was originally considered, but there was no clinical or pathologic evidence of a primary sinonasal tumor.

The 5 high-grade, widely invasive carcinomas shared in common an elevated mitotic rate and necrosis but were otherwise strikingly different in their appearances. One case presented with multiple nodules, made up of nests and sheets of clear cells with focal duct formation (Fig. 4). For this case, a residual IDC component was only found in retrospect, with extensive additional sampling following the discovery of its genetic alteration (Fig. 4D). Another case consisted of a tubulopapillary ductal proliferation in addition to overtly sarcomatoid tumor cells with spindled cells growing as loose fascicles, with focal deposition of osteoid matrix with osteoclast-type giant cells (Fig. 5). The remaining three high-grade widely invasive carcinomas were indistinguishable from salivary duct carcinoma, with clear-cut apocrine differentiation apparent on routine microscopy (Fig. 6).

The immunohistochemical findings are summarized in Table 2. Seven of 9 cases were positive for S100, and 4 of 5 were positive for SOX10. The cases negative for S100 and SOX10 were overtly apocrine carcinomas, which were strongly positive for AR in contrast to the other cases which were negative and lacked apocrine morphology. Mammaglobin was positive in 5 of 7. All cases tested (7 of 7) were negative for p63 and/or p40.

By molecular analysis (Table 1), 4 of 5 invasive carcinomas that had a demonstrable intercalated duct subtype IDC component were found to harbor *NCOA4*::*RET*. Two additional carcinomas, both clinically recurrent tumors diagnosed as salivary duct carcinoma, also were found to have *NCOA4*::*RET*. One carcinoma arising from a mixed intercalated duct/apocrine IDC harbored *TRIM27*::*RET* while the other had *TRIM33*::*RET*. The low-grade cystic sublingual gland carcinoma without an identifiable IDC component harbored *TRIM33*::*RET* by RNA sequencing, and the high-grade carcinoma with sarcomatoid features was found to have *STRN*::*ALK*.

Most of the frankly invasive carcinomas ex-IDC were treated with surgery. Five of the cases were also treated with radiation, three received chemotherapy, and two of the apocrine cases received androgen deprivation therapy. Two patients with *NCOA4*::*RET* positive carcinoma received cabozatanib, an agent that targets tyrosine kinases like *RET*, with some clinical response. Follow up ranged from 0 to 104 months (mean, 28.3). Of the 9 cases with follow up, 6 recurred and/or metastasized, all of which showed high-grade histology. Four of those cases were metastatic to regional cervical lymph nodes on presentation, with four

Table 1 Clinical and molecular features of cases of frankly invasive carcinoma ex-intraductal carcinoma

Case	Age	Sex	Site	Original diagnosis	Primary invasive carcinoma size (cm)	IDC component subtype	Genetic alteration (testing modality)	Treatment	Clinical course	Outcome	Follow-up duration
1	40	M	Parotid gland	Carcinoma with clear cell features	Multifocal, largest 4.5	Intercalated duct	*NCOA4::RET* (RNA seq)	Surgery, CT, RT	Metastases to multiple bilateral cervical lymph nodes on presentation; metastases to lungs at 6 months	DOD	7 months
2	82	F	Parotid gland	Microinvasive carcinoma	0.1	Intercalated duct	*NCOA4::RET* (RNA seq)	Surgery	No residual disease	NED	1 month
3	33	M	Sublingual gland	Cystadeno-carcinoma, NOS	8.6	None identified	*TRIM33::RET* (RNA seq)	Surgery	No residual disease	NED	13 months
4	70	M	Parotid gland	Salivary duct carcinoma	1.7	Mixed apocrine/intercalated duct	*TRIM27::RET* (RNA seq)	Surgery	Local recurrence at 42 months with cervical lymph node metastases	NED	42 months
5	82	M	Parotid gland	Cystadeno-carcinoma, NOS	2.0–3.0	Intercalated duct	*NCOA4::RET* (RNA seq)	Surgery	Unknown	Unknown	None
6	61	M	Parotid gland	Secretory carcinoma	0.1	Intercalated duct	*NCOA4::RET* (DNA NGS)	Surgery	No residual disease	NED	24 months
7	77	M	Parotid gland	Adenocarcinoma NOS with spindle cell features	Unknown (primary tumor not available)	None identified	*STRN::ALK* (DNA NGS)	Surgery, CT, RT	Metastases to multiple bilateral cervical lymph nodes on presentation, metastases to cervical nodes at 34 and 40 months, lung metastases at 45 months	AWD	48 months
8	79	M	Parotid gland	Salivary duct carcinoma	Unknown (primary tumor not available)	None identified	*NCOA4::RET* (DNA NGS)	Surgery, RT, ADT, cabozantinib	Metastases to multiple ipsilateral cervical lymph nodes on presentation; dermal, pulmonary, and pleural metastases at 12 months	DOD	31 months

Table 1 (continued)

Case	Age	Sex	Site	Original diagnosis	Primary invasive carcinoma size (cm)	IDC component subtype	Genetic alteration (testing modality)	Treatment	Clinical course	Outcome	Follow-up duration
9	63	M	Parotid gland	Salivary duct carcinoma	Unknown (primary tumor not available)	None identified	*NCOA4*::*RET* (DNA NGS)	CT, RT, ADT; cabozantinib	Facial skin involvement and metastases to cervical and axillary lymph nodes at presentation; dermal metastases in neck and chest wall at 12 months; spine metastases at 43 months; esophageal cancer at 98 months	DWDOC	104 months
10	76	F	Parotid gland	Adenocarcinoma NOS	0.1	Mixed apocrine/intercalated duct	*TRIM33*::*RET* (DNA NGS)	Surgery, RT	No residual disease	NED	13 months

IDC intraductal carcinoma, *M* male, *F* female, *CT* chemotherapy, *RT* radiotherapy, *ADT* androgen deprivation therapy, *NED* no evidence of disease, *FISH* fluorescence in situ hybridization, *RNA seq* RNA sequencing, *DNA NGS* large panel DNA next generation sequencing, *NOS* not otherwise specified, *AWD* alive with disease, *DOD* died of disease, *DWDOC* died with disease of other cancer

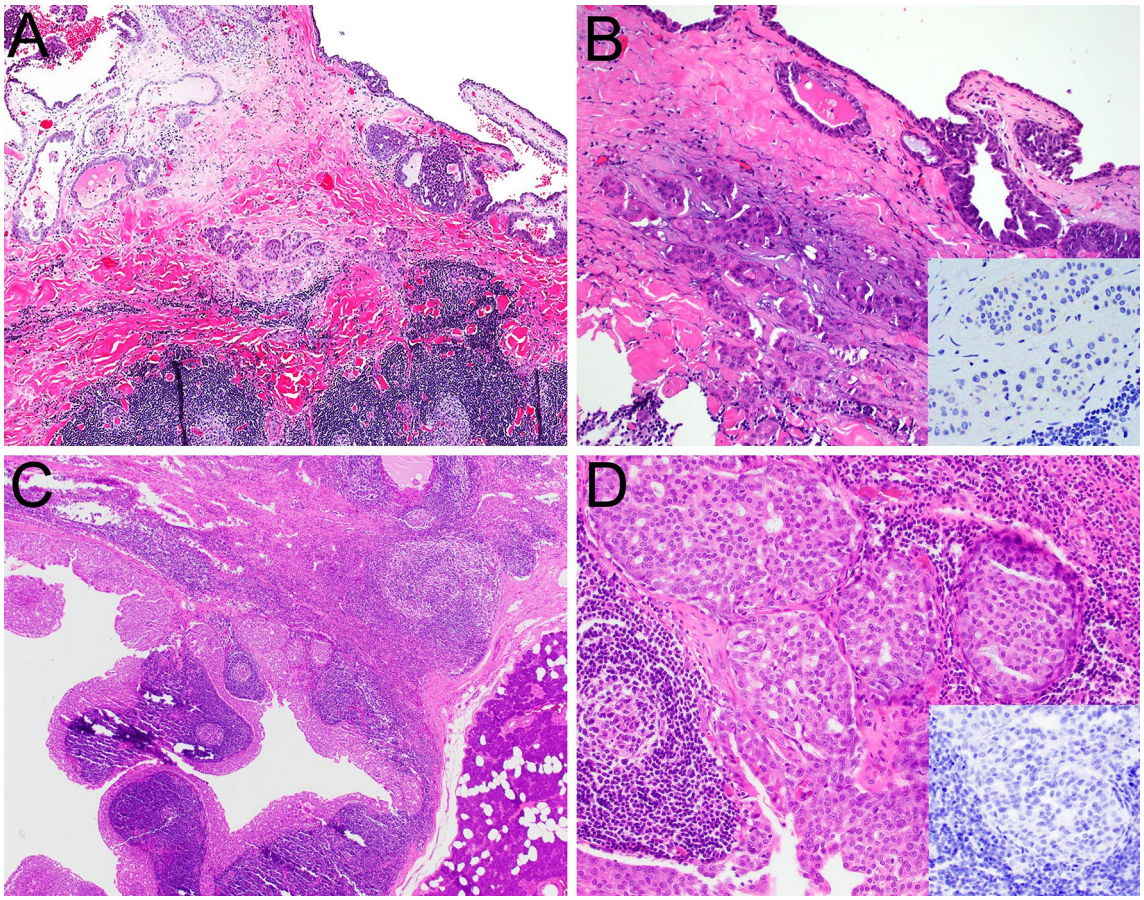

Fig. 1 In Case 2 (**A**, **B**) and Case 6 (**C**, **D**), the frankly invasive carcinoma was microscopic, in the setting of an otherwise classic intercalated duct-type IDC. At low power (**A** and **C**), the invasive focus is difficult to see, though slightly more apparent on high power (**B** and **D**). In both cases, a complete loss of myoepithelial cells by immunostaining (in this figure, p63) confirmed frank microinvasion (insets). Case 2 was originally called microinvasive carcinoma ex-IDC, and Case 6 was originally called secretory carcinoma

subsequently metastasizing to lung (4 of 4), skin (2 of 4), and bone (1 of 4). Two patients succumbed to their disease, at 7 and 31 months.

Discussion

In the emerging molecular evolution of salivary gland tumors, considering ongoing reclassification schema with frequent nomenclature changes/refinements along with uncovering of the molecular foundations of its various subtypes, perhaps no tumor has undergone more upheaval than IDC. Although much has been learned about this tumor, including the grouping of these tumors of variable genetic drivers under one umbrella term, unanswered questions remain. Most center around its terminology and biologic potential. Although it is attractive to draw comparisons between IDC and breast ductal carcinoma in situ, the analogy falls apart on closer scrutiny. At least one IDC with intact myoepithelial cells has recurred in bone, inconsistent with an in situ process [18]. Moreover, a recent study demonstrated that the myoepithelial cells of fusion-positive IDC harbor the same rearrangement as the ductal cells, indicating that they are also neoplastic [11]. Although this provides clear evidence that IDC is not "carcinoma in situ" but rather a biphasic neoplasm, it remains unclear whether it is benign or low-grade malignant with a pushing invasive border. These questions have led some investigators to endorse yet another name change for IDC to "intercalated duct carcinoma" [3].

Another confounding finding has been IDCs that show obvious areas of conventional invasion with concomitant loss of myoepithelial cells [1, 3–5, 12–14]. While previously thought to be analogous to invasive mammary carcinoma arising from ductal carcinoma in situ, this is a difficult phenomenon to understand or even describe in light of what is now known about IDC. "Carcinoma ex-IDC" is awkward because IDC already is considered a carcinoma. Even "invasive carcinoma ex-IDC" may not be appropriate since it has not been firmly established that conventional

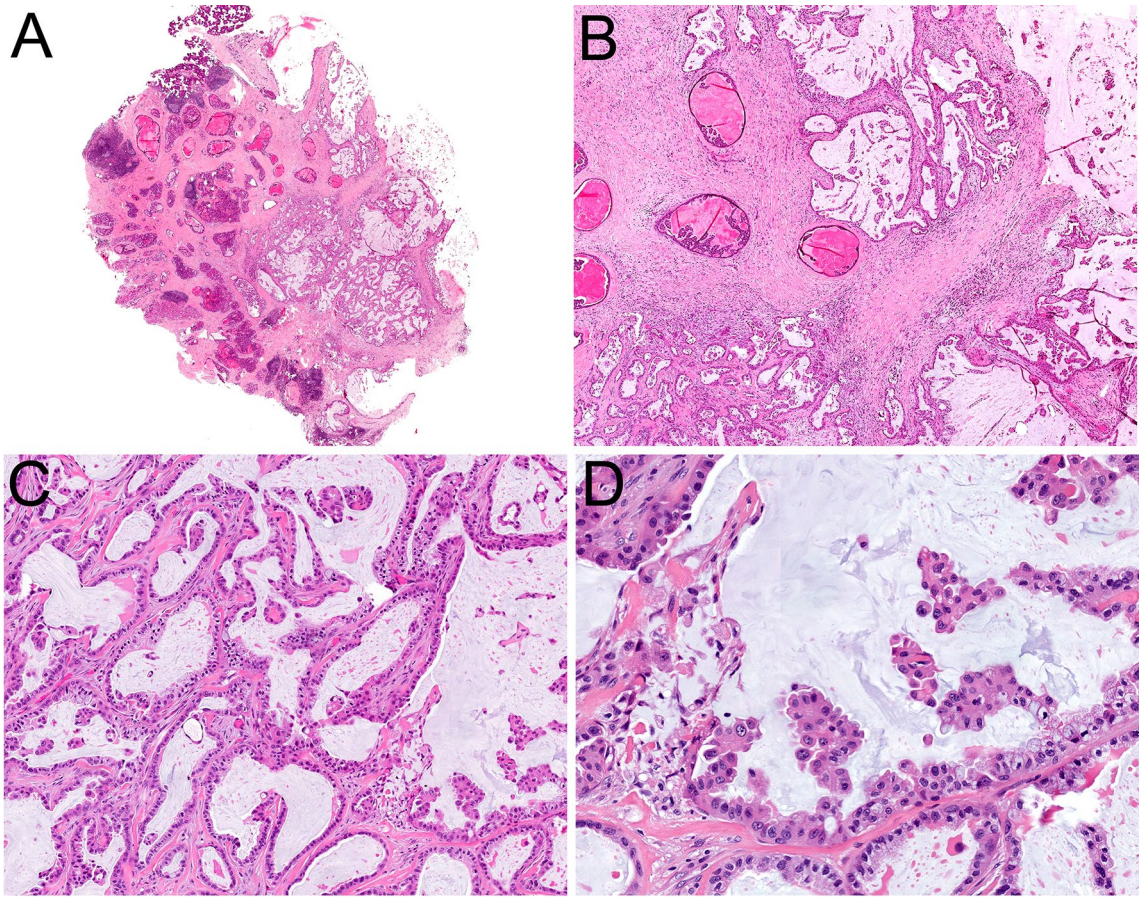

Fig. 2 Case 5 was originally called low-grade cystadenocarcinoma, not otherwise specified. At low power, it had approximately 50% conventional intercalated duct like IDC (left) and 50% frankly invasive carcinoma (right) (**A**, **B**). The frankly invasive areas were cystic and tubulopapillary, with extensive mucin deposition (**C**). The tumor cells were cuboidal with bland nuclei and eosinophilic to clear cytoplasm. The mitotic rate was low (**D**)

IDC is noninvasive. For the purposes of this study, we used the term "frankly invasive carcinoma ex-IDC," where "frankly" is qualitative, referring to a conventionally invasive growth pattern and concomitant loss of myoepithelial cells. It remains to be clarified what consensus terminology will be for this phenomenon in the future.

We believe that is it is appropriate to regard invasive carcinomas as having arisen from IDC when a fusion known to occur in IDC is found, regardless of whether an IDC component is seen. In one case in this series, the tumor met criteria for salivary duct carcinoma with no IDC component, and only the knowledge of the patient's prior history of IDC allowed that link to be connected. In another case, conventional IDC component represented a very small percentage of the tumor volume, and it was only found in retrospect following extensive additional sampling. With these cases in mind, it is not difficult to imagine a scenario where an IDC is obliterated, missed on sampling, or not assessable because the frankly invasive carcinoma was a recurrence or metastasis. Further, it is well accepted to regard carcinomas as carcinoma ex-pleomorphic adenoma when they harbor *PLAG1* or *HMGA2* fusions seen in pleomorphic adenoma, regardless of whether there is histologic or clinical evidence of a precursor tumor [19, 20].

Previously reported cases of frankly invasive carcinoma ex-IDC are few and not consistently well-detailed. In this study dedicated to the phenomenon, we demonstrated that frankly invasive carcinoma ex-IDC is protean. Cases can be minimally or widely invasive beyond the IDC, small or large, low-grade or high-grade, cystic or solid, duct-forming, clear cell, sarcomatoid, or apocrine. With this variability in mind, frankly invasive carcinoma ex-IDC is probably not a single entity. Indeed, IDC may serve as a precursor neoplasm like pleomorphic adenoma, which can give rise to a variety of frankly invasive carcinoma types. It is also interesting to note that the tumors that behaved aggressively in this series were all high-grade and widely invasive beyond the IDC, suggesting a probable prognostic role for grading and describing extent of conventional invasion beyond the IDC, similar to what is done when reporting a carcinoma ex-pleomorphic

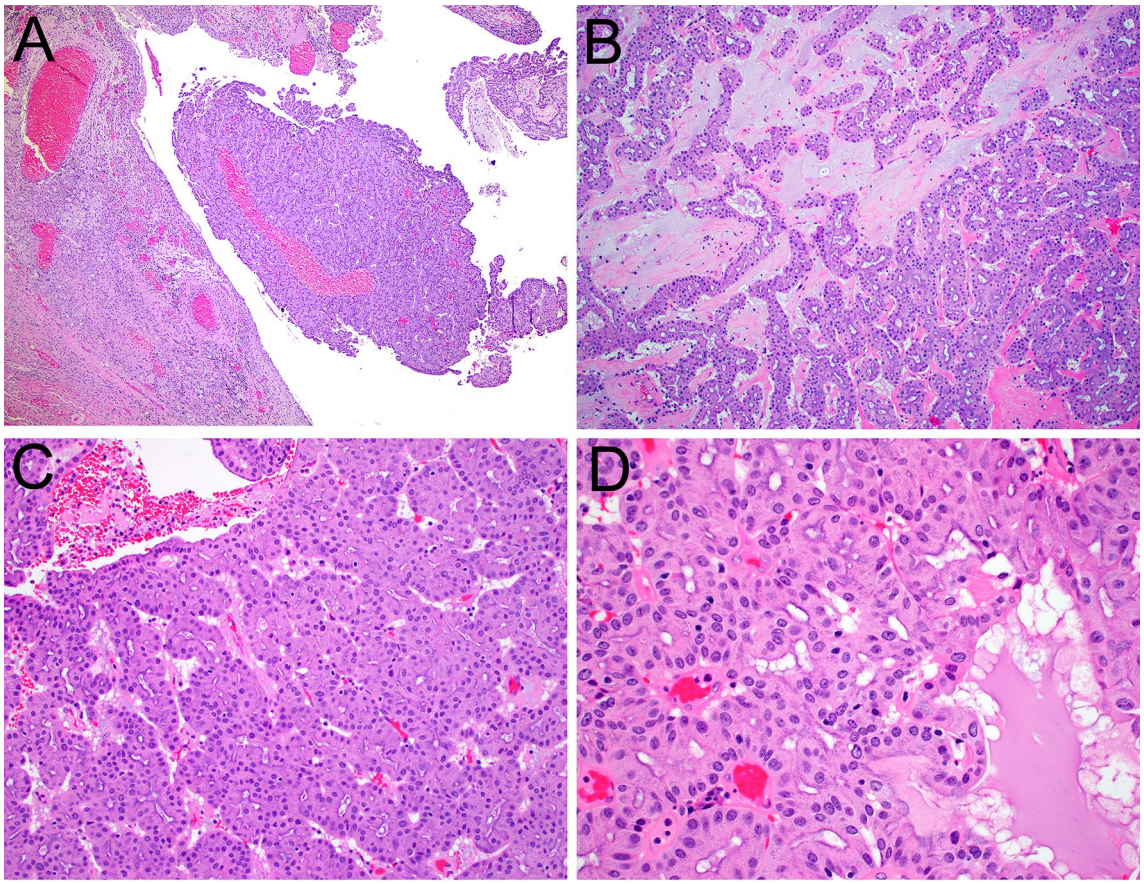

Fig. 3 Case 3 was originally diagnosed as low-grade cystadenocarcinoma, not otherwise specified. It was quite cystic, with solid mural nodules (**A**). At higher power, the carcinoma consisted of interconnecting tubules, trabeculae, and acini with amphophilic cytoplasm (**B**, **C**). The tumor cell nuclei were uniform and round, with minimal mitotic activity (**D**). Although the tumor was completely submitted, conventional IDC was not identified in this case

adenoma [21]. Frankly invasive carcinomas ex-IDC appear to be much less common than carcinoma ex-pleomorphic adenoma, so additional studies will be needed to confirm the importance of grading and extent of overt invasion. It is also possible that these frankly invasive carcinomas represent a form of high-grade transformation, a well-described phenomenon where a low-grade carcinoma transforms into a higher-grade carcinoma that can demonstrate remarkably variable appearances [22]. After all, adenoid cystic carcinomas that have undergone high-grade transformation lose their myoepithelial cell component, similar to frankly invasive carcinoma ex-IDC [23]. This concept, however, is difficult to reconcile with these cases when considering the low-grade histologic features seen in a subset. In salivary gland tumors, high-grade transformation is, by definition, associated with high-grade histology.

Despite their differences, the frankly invasive carcinomas shared some characteristics. First, many of the cases that arose from known intercalated duct IDC or had fusions associated with this subtype had evidence of an intercalated duct-like phenotype with at least focal duct formation and staining with S100, SOX10, and/or mammaglobin. Nevertheless, two frankly invasive cases with *NCOA4*::*RET* that presumably arose from a prior intercalated duct IDC were purely apocrine so while some frankly invasive carcinomas ex-IDC retain the characteristics of their precursor IDC, this is not always the case. Although no purely apocrine IDC were included in this study, finding that some frankly invasive carcinomas arising from non-apocrine IDC can be entirely apocrine was unexpected. Interestingly, although to this point *TRIM33*::*RET* had only been described in oncocytic IDC, this fusion may not be very specific because the frankly invasive carcinomas in this series lacked any oncocytic features, and the IDC precursor found in one of them was mixed intercalated duct/apocrine. Second, many of the frankly invasive carcinomas ex-IDC were difficult to classify. The majority were given an NOS designation, and even with hindsight there is no better WHO category to assign these cases. Only the cases of salivary duct carcinoma ex-IDC met diagnostic criteria for any specific entity. Assigning

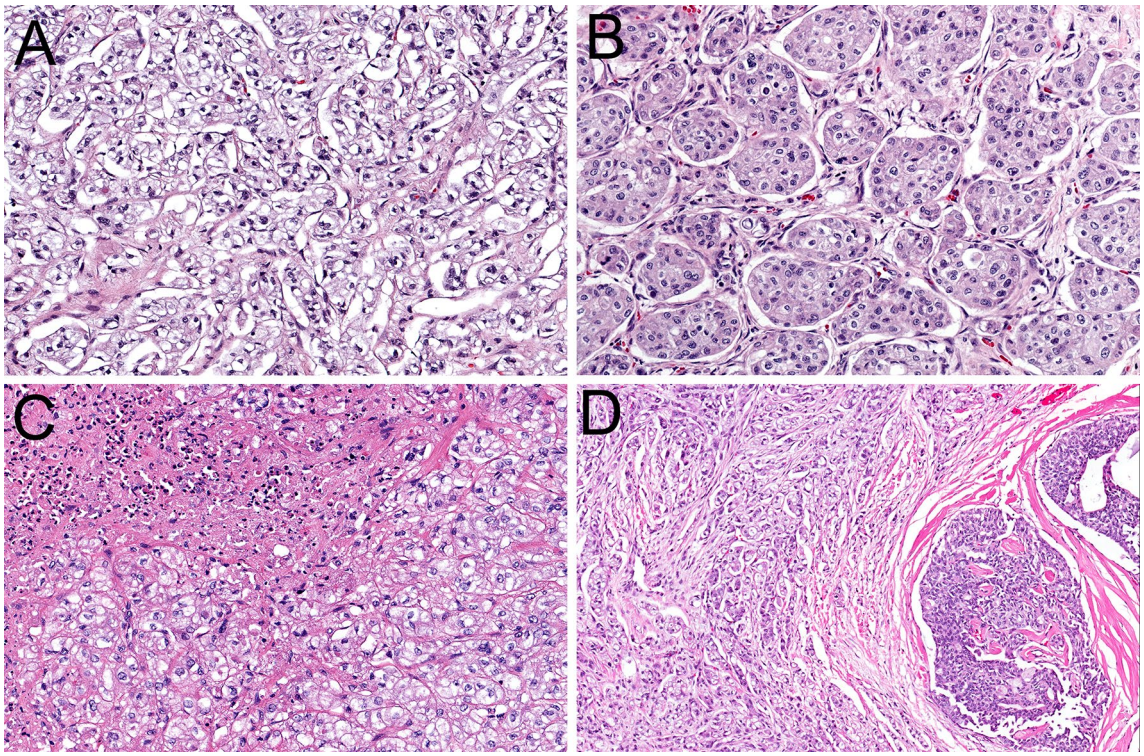

Fig. 4 Case 1 was originally diagnosed as high-grade carcinoma with clear cell features. The extensively invasive carcinoma was multifocal, and made up of clear cells arranged as solid sheets and nests (**A**–**C**), with zones of necrosis and pleomorphic nuclei (**C**). A very small residual intercalated duct type IDC component (right) was observed only in retrospect, following the finding of *NCOA4*::*RET*, with additional tissue sampling (**D**)

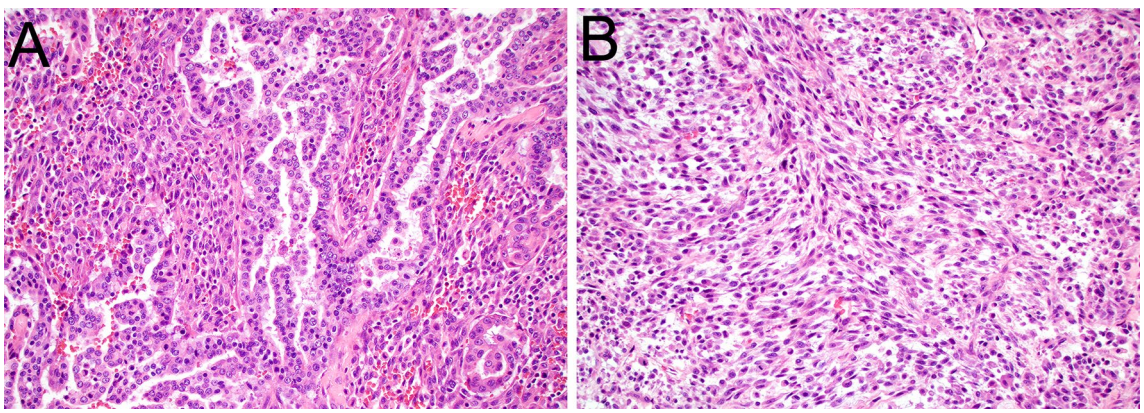

Fig. 5 Case 7 was originally diagnosed as high-grade adenocarcinoma, not otherwise specified, with spindle cell features. It was seen as a recurrence; the original tumor was not available for review or testing. The carcinoma consisted of a tubulopapillary epithelial proliferation (**A**) associated with overly malignant spindled cells arranged in loose fascicles (**B**). No residual IDC was seen despite extensive tissue sampling

all frankly invasive carcinomas ex-IDC with one diagnostic term makes little sense when considering their variable appearances and behavior. Similar to pleomorphic adenoma, it seems appropriate to name the carcinoma when possible (e.g., salivary duct carcinoma if high-grade and apocrine) and use (adeno)carcinoma, NOS ex-IDC for those that defy more precise classification. In fact, an S100/SOX10/mammaglobin-positive staining pattern in an invasive carcinoma, NOS that is otherwise difficult to type may be a clue to the possibility that the tumor arose from IDC. Additional tissue sampling and/or genetic analysis could be employed in such cases to address the possibility.

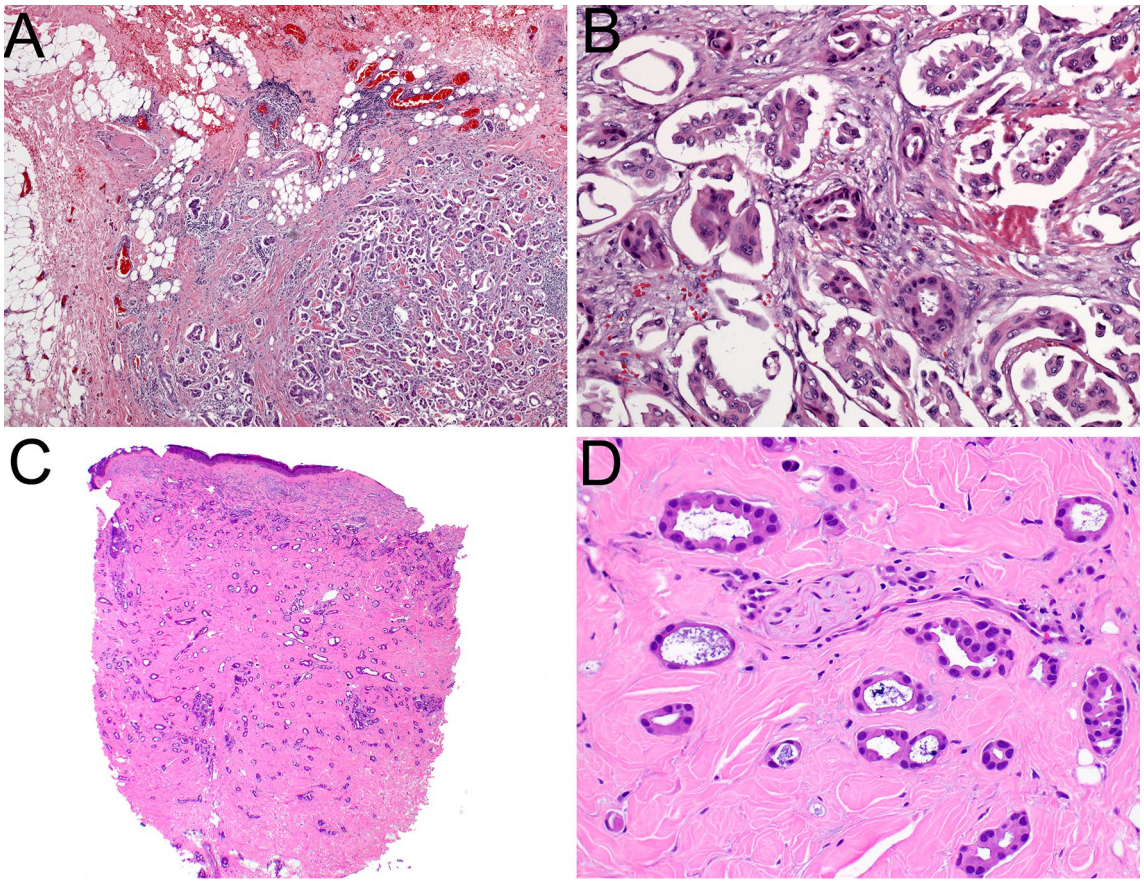

Fig. 6 Cases 4 (**A**, **B**) and 9 (**C**, **D**) were diagnosed as salivary duct carcinoma. Case 4 (**A**) recurred locally following excision of a mixed intercalated duct/apocrine IDC. The frankly invasive recurrence was indistinguishable from micropapillary variant of salivary duct carcinoma, with nests showing prominent retraction artifact, apocrine snouting, and nuclear pleomorphism (**B**). Case 9 was seen as a local recurrence in skin (**C**); the original primary tumor was not available for review or testing. It consisted of widely infiltrative tubules with overtly apocrine features. Although the frankly invasive tumors in these cases were entirely apocrine (i.e., salivary duct carcinoma), Case 4 had a known precursor IDC which was mixed apocrine-intercalated duct with *TRIM27*::*RET*, and although Case 9 had no documented IDC, it harbored *NCOA4*::*RET*

The widely invasive apocrine carcinoma with *TRIM27*::*RET* warrants particular attention. First, it occurred 42 months following the complete excision of a mixed intercalated duct/apocrine IDC that lacked frank invasion, and had an entirely intact myoepithelial cell layer. To our knowledge, this occurrence has not been previously reported, and suggests that even IDCs without frank invasion have some capacity to recur as aggressive, invasive carcinomas. Second, its recurrence as a salivary duct carcinoma raises interesting questions. Had the patient's prior history of an IDC not been known, there was nothing about the salivary duct carcinoma to suggest that it had arisen from an IDC. This finding, when combined with the two cases in this series of recurrent/metastatic salivary duct carcinoma with *NCOA4*::*RET* and reports of rare salivary duct carcinomas with IDC-associated fusions, essentially confirms that some salivary duct carcinomas arise from fusion-positive IDC [21–23]. This concept has significant therapeutic implications.

While unraveling the full spectrum of IDC and invasive carcinomas arising from it is interesting, it is also potentially quite impactful to patients because *RET* and *ALK* fusions are targetable [24, 25]. While conventional (not frankly invasive) IDC is usually indolent and is treated with excision only, it has been demonstrated that frankly invasive carcinomas ex-IDC can be very aggressive, and these patients could benefit from targeted kinase-inhibitor based therapies. Given that an IDC component is not always seen, a strong case is made for evaluating for fusions in all cases of (adeno)carcinoma NOS, particularly aggressive cases and/or tumors showing a suggestion of intercalated duct-like differentiation. Similarly, given the existence of salivary duct carcinomas with not only IDC-associated fusions (*RET*, *ALK*) but also targetable *NTRK3* fusions, [26, 27] a case can also be made for evaluating all salivary duct carcinomas for fusions as well. If available, RNA sequencing or large panel DNA next-generation

Table 2 Immunohistochemical features of the frankly invasive carcinomas ex intraductal carcinoma

Case	CK7	S100	SOX10	Androgen receptor	Mammaglobin	p40/p63	Others
1	+	+	+	−	+	–	DOG1 (+); estrogen receptor (−); progesterone receptor (−); GATA3 (−)
2	+	+	ND	−	+	–	GCDFP-15 (−); estrogen receptor (−); progesterone receptor (−)
3	+	+	+	−	−	–	DOG1 (+); CD117 (+ focal); GATA3 (+ weak); CK5/6 (−) Calponin (−)
4	+	−	−	+	+	–	GCDFP-15 (+); CK5/6 (+); EMA (+); CK20 (−); Her-2 (−); SMA (−); estrogen receptor (−)
5	ND	+	ND	−	ND	ND	None
6	+	+	+	−	+	–	GATA3 (+); DOG1 (−); CD117 (−); GCDFP-15 (−)
7	+	+	ND	−	+	ND	TTF1 (−); Napsin A −)
8	+	−	ND	+	−	–	GCDFP-15 (+); SMMS-1 (−); estrogen receptor (−); progesterone receptor −)
9	ND	ND	ND	+	ND	ND	Estrogen receptor (−); progesterone receptor (−); HER (2+) but not amplified by FISH
10	+	+	+	−	ND	–	None

ND not done

sequencing may be the best modalities for this purpose because it can detect many different fusions, and because *NCOA4*::*RET*, a common fusion in frankly invasive carcinomas ex-IDC, is often very difficult to detect by fluorescence in situ hybridization [4].

In summary, similar to pleomorphic adenoma, IDC can give rise to frankly invasive carcinomas that harbor the same fusions as IDC (e.g., involving *RET* or *ALK*) but with a spectrum of appearances and behavior. While some frankly carcinomas ex-IDC resemble salivary duct carcinoma, others are difficult to classify and have a wide range of features. Cases with high-grade histology and invasion far beyond the IDC may behave more aggressively. Given that *RET* and *ALK* fusions are targetable, it is desirable to identify carcinomas that arose from IDC and therefore may have these genetic alterations. Therefore, consideration should be given to fusion analysis for any frankly invasive salivary carcinoma, NOS or salivary duct carcinoma, particularly those behaving in an aggressive manner.

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Data Availability Availability of dataand material is possible upon reasonable request, deidentified for maintenance of anonymity and compliance with IRB approval.

Code Availability Not applicable.

Declarations

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical Approval All procedures performed in this retrospective data analysis involving human participants were in accordance with the ethical standards of the institutional review board (IRB 112017-073), which did not require informed consent.

Informed Consent The IRB-approved study was classified as exempt, which does not require informed consent.

Consent for Publication Consent forpublication was obtained from all individual participants for whom identifying information is uniquely included in this manuscript.

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