Template for Reporting Results of Biomarker Testing of Specimens From Patients With Tumors of the Head and Neck

Version: HeadNeckBiomarkers 1.0.0.0

Template posting date: February 2017

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Version Code
The definition of version control and an explanation of version codes can be found at www.cap.org (search: cancer protocol terms).

Summary of Changes
This is a new template.
Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (e.g., a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient’s medical record and thus readily available to the treating clinical team.

HEAD AND NECK

Select a single response unless otherwise indicated.

Note: Use of this template is optional.

+ RESULTS

+ Head and Neck Squamous Cell Carcinoma (HNSCC)

+ Human Papillomavirus (HPV) Testing

+ p16 Expression (by immunohistochemistry) as a Surrogate for Transcriptionally Active High-Risk HPV
  + ___ Negative (<50% diffuse and strong nuclear and cytoplasmic staining)
  + ___ Equivocal (70% but >60% diffuse and strong nuclear and cytoplasmic staining)
  + ___ Positive (>70% diffuse and strong nuclear and cytoplasmic staining)
  + ___ Indeterminate (explain): _________________________

+ HPV-DNA (by in situ hybridization [ISH])
  + ___ Negative (no signal)
  + ___ Positive (check all that apply)
    + ___ Punctate
    + ___ Diffuse
    + Subtype(s) (if available): _________________________
  + ___ Indeterminate (explain): _________________________

+ HPV E6/E7 mRNA (by ISH)
  + ___ Negative (no signal)
  + ___ Positive (cytoplasmic and/or nuclear signals)
    + Subtype(s) (if available): _________________________
  + ___ Indeterminate (explain): _________________________

+ HPV-DNA (by polymerase chain reaction [PCR])
  + ___ Negative (no signal)
  + ___ Positive
    + Subtype(s) (if available): _________________________
  + ___ Indeterminate (explain): _________________________

+ HPV E6/E7 mRNA (by reverse transcriptase polymerase chain reaction [RT-PCR])
  + ___ Negative (no signal)
  + ___ Positive
    + Subtype(s) (if available): _________________________
  + ___ Indeterminate (explain): _________________________

+ Data elements preceded by this symbol are not required.
+ Epstein-Barr Virus (EBV) Testing

+ *EBV Early mRNA (EBER) (by ISH)*
  + ___ Negative (no signal)
  + ___ Positive (nuclear signal)
  + ___ Indeterminate (explain): _________________________

+ NUT Midline Carcinoma

+ *NUT Expression (by immunohistochemistry [IHC])*
  + ___ Negative
  + ___ Positive
  + ___ Indeterminate (explain): _________________________

+ NUT Rearrangements (by Fluorescence ISH [FISH])
  + ___ No NUT rearrangement detected
  + ___ NUT rearrangement detected
    + Percentage of positive cells: _____%
    + Percentage of positive cells with classic rearrangement (if applicable): _____%
    + Percentage of positive cells with complex rearrangement (if applicable): _____%

+ *BRD4-NUT Fusion (by RT-PCR)*
  + ___ No BRD4-NUT fusions detected
  + ___ BRD4-NUT fusions detected

+ Other NUT Fusion (by RT-PCR)
  + ___ No NUT translocation detected
  + ___ NUT translocation detected
    + Fusion partner: ______

+ Salivary Gland Carcinoma

+ *(Hyalinizing) Clear Cell Carcinoma*

+ *EWSR1 Rearrangements (by FISH)*
  + ___ No EWSR1 rearrangement detected
  + ___ EWSR1 rearrangement detected
    + Percentage of positive cells: _____%
    + Percentage of positive cells with classic rearrangement (if applicable): _____%
    + Percentage of positive cells with complex rearrangement (if applicable): _____%
  + ___ Indeterminate (explain): _________________________

+ *EWSR1-ATF1 Fusion (by RT-PCR)*
  + ___ No EWSR1-ATF1 fusions detected
  + ___ EWSR1-ATF1 fusions detected

+ Other EWSR1 Fusion (by RT-PCR)
  + ___ No EWSR1 translocation detected
  + ___ EWSR1 translocation detected
    + Fusion partner: ______

+ Data elements preceded by this symbol are not required.
+ Mammary Analogue Secretory Carcinoma

+ **ETV6 Rearrangements (by FISH)**
  + ___ No ETV6 rearrangement detected
  + ___ ETV6 rearrangement detected
    + Percentage of positive cells: _____%
      + Percentage of positive cells with classic rearrangement (if applicable): _____%
      + Percentage of positive cells with complex rearrangement (if applicable): _____%
    + ___ Indeterminate (explain): _________________________

+ **ETV6-NTRK3 Fusion (by RT-PCR)**
  + ___ No ETV6-NTRK3 fusions detected
  + ___ ETV6-NTRK3 fusions detected

+ Mucoepidermoid Carcinoma

+ **MAML2 Rearrangements (by FISH)**
  + ___ No MAML2 rearrangement detected
  + ___ MAML2 rearrangement detected
    + Percentage of positive cells: _____%
      + Percentage of positive cells with classic rearrangement (if applicable): _____%
      + Percentage of positive cells with complex rearrangement (if applicable): _____%
    + ___ Indeterminate (explain): _________________________

+ **CRTC1-MAML2 Fusion (by RT-PCR)**
  + ___ No CRTC1-MAML2 fusions detected
  + ___ CRTC1-MAML2 fusions detected

+ **CRTC3-MAML2 Fusion (by RT-PCR)**
  + ___ No CRTC3-MAML2 fusions detected
  + ___ CRTC3-MAML2 fusions detected

+ Adenoid Cystic Carcinoma

+ **MYB Expression (by IHC)**
  + ___ Negative
  + ___ Positive
  + ___ Indeterminate (explain): _________________________

+ **MYB Rearrangements (by FISH)**
  + ___ No MYB rearrangement detected
  + ___ MYB rearrangement detected
    + Percentage of positive cells: _____%
      + Percentage of positive cells with classic rearrangement (if applicable): _____%
      + Percentage of positive cells with complex rearrangement (if applicable): _____%
    + ___ Indeterminate (explain): _________________________

+ **MYB-NFIB Fusion (by FISH)**
  + ___ No MYB-NFIB fusions detected
  + ___ MYB-NFIB fusions detected
    + Percentage of positive cells: _____%
      + Percentage of positive cells with classic fusion (if applicable): _____%
      + Percentage of positive cells with complex fusion (if applicable): _____%
    + ___ Indeterminate (explain): _________________________
+ **MYB-NFIB** Fusion (by RT-PCR)
+ ___ No **MYB-NFIB** fusions detected
+ ___ **MYB-NFIB** fusions detected

+ **Carcinoma ex Pleomorphic Adenoma/Pleomorphic Adenoma**

  + **HMGA2** (by FISH)
    + ___ No **HMGA2** rearrangement detected
    + ___ **HMGA2** rearrangement detected
      + Percentage of positive cells: _____%
      + Percentage of positive cells with classic rearrangement (if applicable): _____%
      + Percentage of positive cells with complex rearrangement (if applicable): _____%
    + ___ Indeterminate (explain): _________________________

  + **PLAG1** Expression (by IHC)
    + ___ Negative
    + ___ Positive
    + ___ Indeterminate (explain): _________________________

  + **PLAG1** (by FISH)
    + ___ No **PLAG1** rearrangement detected
    + ___ **PLAG1** rearrangement detected
      + Percentage of positive cells: _____%
      + Percentage of positive cells with classic rearrangement (if applicable): _____%
      + Percentage of positive cells with complex rearrangement (if applicable): _____%
    + ___ Indeterminate (explain): _________________________

+ **Salivary Duct Carcinoma**

  + Human Epidermal Growth Factor-2 (HER2 [ERBB2]) Expression (by IHC)
    + ___ Negative (score 0)
    + ___ Negative (score 1+)
    + ___ Equivocal (score 2+)
    + ___ Positive (score 3+)
    + ___ Indeterminate (explain): _________________________

  + **HER2** (ERBB2) Expression (by FISH)
    + ___ Negative (not amplified)
    + ___ Positive (amplified)
    + ___ Indeterminate (explain): _________________________

+ Number of observers: ______
+ Number of invasive cancer cells counted: ______

+ Using dual-probe assay
  + Average number of **HER2** (ERBB2) signals per cancer cell: ______
  + Average number of CEP17 signals per cancer cell: ______
    + **HER2** (ERBB2):CEP17 ratio: ______
+ Using single-probe assay
  + Average number of **HER2** (ERBB2) signals per cancer cell: ______

+ **Androgen Receptor** (by IHC)
+ ___ Negative
+ ___ Positive
+ ___ Indeterminate (explain): _________________________

+ Data elements preceded by this symbol are not required.
+ **Sinonasal Malignancies**

+ **INI-Deficient Sinonasal Carcinoma/Rhabdoid Tumor**
  + INI-1 (by IHC)
    + Intact (staining retained, negative for INI1 deletion)
    + Lost (staining lost, positive for INI1 alteration)
    + Indeterminate (explain): _________________________

+ **Biphenotypic Sinonasal Sarcoma**

  + **PAX3 Rearrangements (by FISH)**
    + No PAX3 rearrangement detected
    + PAX3 rearrangement detected
      + Percentage of positive cells: ______%  
        + Percentage of positive cells with classic rearrangement (if applicable): ______%  
        + Percentage of positive cells with complex rearrangement (if applicable): ______%

  + **PAX3-MAML3 Fusion (by RT-PCR)**
    + No PAX3-MAML3 fusions detected
    + PAX3-MAML3 fusions detected

+ **Paraganglioma**

  + SDHB (by IHC)
    + Intact (staining retained, negative for SDH alteration)
    + Lost (staining lost, positive for SDH alteration)
    + Indeterminate (explain): _________________________

+ **METHODS**

+ **Head And Neck Squamous Cell Carcinoma (HNSCC)**

+ **HPV Testing**
  + P16 (by IHC)
  + Primary Antibody
    + E6H4
    + Other (specify clone): ______
  + HPV DNA (by ISH)
  + Subtypes Separately Tested
    + High-Risk (specify test/vendor): _________________________
    + Low-Risk (specify test/vendor): _________________________
    + Specific Subtype: _____ (specify test/vendor): _________________________

  + **HR-HPV E6/E7 mRNA** (by ISH)
    + High-Risk (specify test/vendor): _________________________
    + Low-Risk (specify test/vendor): _________________________
    + Specific Subtype: _____ (specify test/vendor): _________________________

+ **HPV DNA (by PCR)**
  + Subtypes Separately Tested
    + High-Risk (specify test/vendor): _________________________
    + Low-Risk (specify test/vendor): _________________________
    + Specific Subtype: _____ (specify test/vendor): _________________________

+ **HR-HPV E6/E7 mRNA by RT-PCR**

+ Data elements preceded by this symbol are not required.
+ EBV Testing
  + ___ EBER by ISH
  + ___ EBV DNA by PCR

+ **NUT Midline Carcinoma**

  + **NUT Rearrangements**
    + ___ Breakapart FISH
    + ___ BRD4-NUT fusion transcript RT-PCR
    + ___ NUT immunohistochemistry

+ **Salivary Gland Carcinoma**

  + (Hyalinizing) Clear Cell Carcinoma

  + **EWSR1 Rearrangements**
    + ___ Breakapart FISH
    + ___ EWSR1-ATF1 fusion transcript RT-PCR

+ **Mammary Analogue Secretory Carcinoma**

  + **ETV6 Rearrangements**
    + ___ Breakapart FISH
    + ___ ETV6-NTRK3 fusion transcript RT-PCR

+ **Mucoepidermoid Carcinoma**

  + **MAML2 Rearrangements**
    + ___ Breakapart FISH
    + ___ CRTC1-MAML2 fusion transcript RT-PCR
    + ___ CRTC3-MAML2 fusion transcript RT-PCR

+ **Adenoid Cystic Carcinoma**

  + **MYB Rearrangements**
    + ___ Breakapart FISH
    + ___ MYB-NFIB fusion FISH
    + ___ MYB-NFIB fusion transcript RT-PCR
    + ___ MYB immunohistochemistry

+ **Carcinoma ex Pleomorphic Adenoma/Pleomorphic Adenoma**

  + **HMGA2**
    + ___ Breakapart FISH

  + **PLAG1**
    + ___ Breakapart FISH
    + ___ PLAG1 Immunohistochemistry

+ **Salivary Duct Carcinoma**

  + **Her2 Expression (by IHC)**
    + ___ US Food and Drug Administration (FDA) cleared (specify test/vendor): ________________
    + ___ Laboratory-developed test
+ **Primary Antibody**
  + ___ 4B5
  + ___ HercepTest
  + ___ A0485
  + ___ SP3
  + ___ CB11
  + ___ Other (specify): __________________________

+ **HER2 (ERBB2) Expression (by FISH)**
  + ___ FDA cleared (specify test/vendor): ____________________
  + ___ Laboratory-developed test (specify probe): _______________________

+ ___ Androgen receptor immunohistochemistry

**+ Sinonasal Malignancies**
  + ___ INI-1
  + ___ PAX-3

**+ Paraganglioma**
  + ___ SDHB immunohistochemistry

**+ COMMENT(S)**

____________________________________________________________________
____________________________________________________________________

*Note: Fixative type, time to fixation (cold ischemia time), and time of fixation should be reported if applicable in this template or in the original pathology report.*

*Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (www.genenames.org; accessed May 9, 2016).*

*All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (http://varnomen.hgvs.org; accessed June 21, 2016).*
A. Head and Neck Squamous Cell Carcinoma (HNSCC)

Human Papillomavirus Testing

Human papillomavirus (HPV)-related head and neck lesions have garnered much attention in recent years, mainly due to the rising incidence of HPV-related oropharyngeal squamous cell carcinoma (OPSCC). Transcriptionally active, high-risk HPV is present in the majority of OPSCC in the United States and at least a significant minority of OPSCC patients worldwide, with rates rapidly increasing over the past several decades, despite the decrease in smoking rates. This change has been linked to changes in sexual practices, and the disease is overwhelmingly one of white men in their 50s and 60s. Patients typically have smaller primary tumors (often clinically occult), present with neck metastases (80% to 85%) that are often cystic, and have tumors that are nonkeratinizing in appearance. High-risk HPV types cause the cancers, and more than 90% of the time it is HPV type 16. While there are many carcinogenic/transforming HPV proteins, the early gene products E6 and E7 appear to play the most significant roles. Although both E6 and E7 can interact with many host proteins, their main contribution to tumorigenesis is through negative regulation of the tumor suppressor proteins p53 and Rb, respectively. Of particular importance is the degradation of Rb by E7, because this leads to a paradoxical overexpression of the tumor suppressor protein p16, which is consistently overexpressed in the nuclei and cytoplasm of tumors with transcriptionally active, high-risk HPV. P16 immunohistochemistry thus serves as a very good surrogate marker of active HPV in these tumors.

Despite the tendency for HPV-related tonsillar squamous cell carcinoma (SCC) to metastasize to neck lymph nodes early in the course of disease (a feature that is usually associated with aggressive disease in cancers), the prognosis is better than for HPV-negative carcinomas. HPV-related oropharyngeal SCC is associated with a 30% to 50% reduction in the risk of death compared to HPV-negative SCC. However, the improved prognosis may be offset in patients who have a strong history of tobacco use and/or are current (heavy) smokers. HPV-related oropharyngeal SCC responds better to both primary chemoradiation and surgical treatment. This may be because these tumors have lower mutation rates and are less genetically complex than HPV-negative cancers. There are now definitive prospective studies showing that the prognosis of HPV-related OPSCC patients has improved such that the head and neck oncology community is essentially united in the concept that all new patients should be tested for high-risk HPV.

How to test for high-risk HPV is not clearly defined, however, and different groups vary in recommendations about use of HPV-specific testing, surrogate marker testing such as p16, or a combination of these. Further, many of the recommendations are site and clinical scenario specific. For OPSCC, for instance, there is broad acceptance of p16 as a good surrogate marker for HPV. The cutoffs listed above refer mainly to these tumors. For other sites this is not well vetted.

Currently, for OPSCC, prognosis and counseling critically depends on these test results. Small changes in treatment within the standard of care are made for HPV-positive OPSCC patients currently, and there are many clinical trials underway to de-intensify and tailor treatments specifically for these patients. There are many HPV-specific detection methods, including those that detect HPV DNA (PCR, ISH, fluid-based tests such as those used in gynecologic cytology) and those that detect HPV mRNA (RT-PCR, ISH, fluid-based tests). These have quite variable sensitivity, specificity, prognostic power, and availability. Tests can be performed on formalin-fixed paraffin-embedded small biopsies and resection specimens; on cytology cell blocks, fluid aspirates, and smears; or on saliva specimens. Given the predominance of bulky cervical nodal disease in patients with OPSCC, both surgical and cytology specimens from the neck are common. Cytology specimen-based diagnosis, confirming metastatic carcinoma and then providing HPV-specific and/or surrogate marker testing, is increasingly common.

High-risk HPV has been detected in most of the specific SCC variants in the oropharynx. When associated with active HPV, these SCC variants appear to have the same favorable prognosis. Although its significance is not established, active high-risk HPV has also been detected in most oropharyngeal small cell carcinomas, although many of these patients have developed progressive and metastatic carcinoma much more akin to the clinical behavior of a high-grade neuroendocrine carcinoma at any site. Although a minority of non-oropharyngeal SCC have transcriptionally active high-risk HPV (particularly those of the sinonasal tract and nasopharynx, and less
often oral cavity, larynx, and hypopharynx), current data have not clearly demonstrated prognostic benefit or altered treatment responses for these tumors.

**Epstein-Barr Virus Testing**

Epstein-Barr virus is an established etiologic agent for cancer development, specifically for nasopharyngeal carcinomas, lymphoepithelial carcinomas at other sites, and also for several types of hematopoietic malignancies. Exposure to EBV is widespread in humans, and the virus establishes persistent asymptomatic infection in lymphocytes. It also infects epithelial cells, specifically in the oropharynx and nasopharynx, establishing replicative status and shedding virus into the saliva throughout a host's life. EBV also establishes several different forms of latency in cells, resulting in complex and varying expression profiles in infected cells. EBV is strongly associated with nasopharyngeal carcinoma worldwide. Tumors are particularly concentrated in distinctive geographical regions such as southern China and Southeast Asia, among the Inuit in Alaska and Greenland, and in the Middle East and north Africa.6,7

Nasopharyngeal carcinoma is highly associated with viral infection, predominantly EBV, but also rarely HPV. The World Health Organization classifies nasopharyngeal carcinoma into 3 major types: keratinizing, same morphology as conventional SCC at other anatomic subsites; nonkeratinizing, further subdivided into differentiated and undifferentiated; and basaloid.

EBV is universally seen in undifferentiated tumors regardless of geographic location. In endemic areas, keratinizing tumors are often positive as well, but in Western countries, these are consistently negative. Basaloid nasopharyngeal carcinoma is very rare and is frequently, but not consistently, EBV related.6,7

Plasma and serum EBV DNA, assessed by RT-PCR, is highly sensitive and specific for nasopharyngeal carcinoma. EBV early RNA (EBER) is strongly and diffusely expressed by EBV-related tumors, and in situ hybridization for EBER in is very helpful diagnostically, as well, particularly in specimens from nodal metastases where nasopharyngeal carcinoma is suspected. EBER is strongly and diffusely expressed by EBV-related tumors, so the assay is highly sensitive and specific.9 There are a number of commercially available assays, and individual laboratory-developed ISH assays are common as well. Routine ISH testing of new nasopharyngeal carcinoma cases in clinical practice is common since studies suggest that EBV-related tumors have somewhat better prognosis and treatment response and, further, to differentiate from the subset of nonkeratinizing (and perhaps basaloid) nasopharyngeal carcinomas that are related to HPV, rather than EBV.

**NUT Midline Carcinoma**

NUT midline carcinoma is a rare, aggressive, squamous cell carcinoma variant uniquely defined by NUT gene translocations.9 It has a predilection, as its name implies, for midline sites and can occur in both children and adults. About one-third occur in the head and neck, frequently, the sinonasal tract.10 The majority (approximately two-thirds) show a NUT-BRD4 fusion [t(15;19)(q14, p13.1)], while the remainder show a NUT-BRD3 fusion [t(9;15)(q34.2;q14)], the newly described NSD3-NUT fusion, or another uncharacterized NUT fusion.11 The presence of a NUT fusion is specific for this entity and can be used to diagnostically define it.

Both breakapart fluorescence in situ hybridization (FISH) or RT-PCR for the fusion transcript are commonly used testing methodologies. But perhaps the most common method of testing is by immunohistochemistry for NUT protein since the fusions result in overexpression. As with many other immunohistochemical surrogates for fusion, NUT overexpression performs well (sensitivity 87%, specificity almost 100%),12 but NUT protein-positive, fusion-negative cases can occur.13-15

Clinical response to BRD inhibitor OTX015/MK-8628 has recently been documented.16
B. Salivary Gland Carcinoma

(Hyalinizing) Clear Cell Carcinoma – \textit{EWSR1} Translocation Analysis

Hyalinizing clear cell carcinoma is a distinct, typically low-grade salivary tumor with clear cell features, characteristic fibrohyaline stroma, and a largely squamous phenotype. It is essentially defined by the \textit{EWSR1-ATF1} translocation \([t(12;22)(q13;q12)]\) in over 80% of cases.\textsuperscript{17} This translocation is seen in nonsalivary tumors as well, notably clear cell sarcoma (soft parts melanoma) and angiomatoid fibrous histiocytoma, among others.\textsuperscript{18} Within tumors of the head and neck region, this specific translocation is largely restricted to clear cell carcinoma and its odontogenic counterpart, clear cell odontogenic carcinoma.\textsuperscript{19} The major value of testing for the translocation is to aid in distinction from mimics like clear cell mucoepidermoid carcinoma and squamous cell carcinoma with clear cell change, as these entities have a different biologic behavior.

Both breakapart FISH and RT-PCR for the fusion transcript are viable testing methodologies in the appropriate morphologic and immunophenotypic context. Without this context, however, \textit{EWSR1} FISH is not specific. Aside from other \textit{EWSR1} translocated tumors (Ewing sarcoma, extraskeletal myxoid chondrosarcoma, desmoplastic small round cell tumor, soft tissue type myoepithelioma, among others) that may occur in this region, a subset of salivary, high-grade, clear cell myoepithelial carcinomas have been recently reported to show \textit{EWSR1} rearrangements as well, though the translocation partner here is unknown.\textsuperscript{20}

Mammary Analogue Secretory Carcinoma – \textit{ETV6} Translocation Analysis

Mammary analogue secretory carcinoma is a recently described, distinct, low-grade salivary gland tumor, historically categorized under acinic cell carcinoma and adenocarcinoma not otherwise specified, but now recognized to show a striking resemblance to secretory carcinoma of the breast.\textsuperscript{21,22} In addition to this shared morphologic appearance, this tumor also shares an \textit{ETV6-NTRK3} translocation \([t(12;15)(15)(p13;q25)]\).\textsuperscript{22-28} Of note, this translocation is well described in other nonsalivary tumors: infantile fibrosarcoma, cellular mesoblastic nephromas, acute myeloid leukemias, and a subset of radiation-associated papillary thyroid carcinomas.\textsuperscript{21,24,28,30}

In the context of primary salivary gland tumors, application of testing for the translocation is diagnostic, mainly to distinguish this tumor from zymogen-poor acinic cell carcinoma, low-grade cribriform cystadenocarcinoma, and mucoepidermoid carcinoma.\textsuperscript{22} Similarly, translocation studies may aid in establishing the diagnosis of mammary analogue secretory carcinoma with high-grade transformation.\textsuperscript{25} In these scenarios, both breakapart \textit{ETV6} FISH and RT-PCR for the fusion transcript are viable testing methods, though traditionally RT-PCR only detects about 65% of cases detected by paired breakapart FISH in paraffin tissue.\textsuperscript{21,25-27} Aside from technical limitations of RNA-based testing on paraffin tissue, a small subset of mammary analogue secretory carcinomas may have an alternate translocation with a currently unknown partner.\textsuperscript{20}

A phase 1a/1b clinical trial of the oral TRK Inhibitor LOXO-101 is available and may thus be relevant in the rare aggressive cases of mammary analogue secretory carcinoma.

Mucoepidermoid Carcinoma – \textit{MAML2} Translocation Analysis

Mucoepidermoid carcinoma is still the most common salivary gland carcinoma and serves as prototypical translocation-associated salivary gland carcinoma, known to frequently harbor a \textit{CRTC1-MAML2} translocation \([t(11;19)(q21;p13)]\) in 40% to 80% of cases,\textsuperscript{31} and a \textit{CRTC3-MAML2} translocation \([t(11;15)(q21;q26)]\) in roughly 5% of cases.\textsuperscript{32,33} This translocation may be of potential prognostic and diagnostic significance.

Both translocations historically favored low- to intermediate-grade mucoepidermoid carcinomas and were even purported as an independent prognosticator.\textsuperscript{33-35} However, in others, the \textit{MAML2} translocations have been documented in sizeable subsets of high-grade tumors, and the prognostic value is actually muted to absent, suggesting that the perceived prognostic value was an artifact of misclassification of high-grade mucoepidermoid carcinomas in earlier series. Conversely, other genetic events (ie, CDKN2A/p16 alterations)\textsuperscript{39} adversely modulate the favorable prognosis of a translocation-positive tumor.
Ultimately, rather than prognostic value, \textit{MAML2} translocation testing has evolved into a diagnostic capacity serving as adjuncts in separating variant morphologies of mucoepidermoid carcinoma (ie, oncocytic) from their mimics\textsuperscript{40} or delineating high-grade mucoepidermoid carcinoma from more aggressive entities such as adenosquamous carcinoma and salivary duct carcinoma.\textsuperscript{36}

While highly prevalent in mucoepidermoid carcinoma, \textit{MAML2} rearrangements are not considered diagnosis defining; translocation negativity does not necessarily supersede the histomorphologic diagnosis, let alone conventional staging for prognosis. Both \textit{MAML2} FISH using breakapart methodology and RT-PCR for \textit{CRTC1} or \textit{CRTC3-MAML2} fusion transcripts are feasible techniques for detection in paraffin tissue, though the former is more widely utilized and provides coverage of both translocations.\textsuperscript{33,36} While there is some controversy regarding the presence \textit{CRTC1-MAML2} translocations in some Warthin tumors, the diagnostic verification of these cases is suspect, and in large series focusing on Warthin tumors with high-quality morphologic verification, the translocation is nonexistent.

**Adenoid Cystic Carcinoma – MYB Expression and Translocation Analysis**

Adenoid cystic carcinoma is among the earliest described salivary gland carcinomas and is defined by a highly infiltrative biphasic salivary gland neoplasm composed of epithelial (luminal) and myoepithelial (basal) cells arranged in cribriform, tubular, and solid growth patterns. Overexpression of MYB and activation of its downstream targets are now implicated in the pathogenesis of this tumor.\textsuperscript{44} Specifically, an \textit{MYB-NFIB} translocation [t(6;9)(q22-23;p23-24)] is the main mechanism for this.

While not diagnosis defining, MYB status can thus be used diagnostically, especially on small biopsies. Translocation of \textit{MYB} protooncogene (nearly always with transcription factor \textit{NFIB}) has been demonstrated by breakapart or fusion FISH or fusion transcript RT-PCR in 30\% to 80\% of cases.\textsuperscript{45-47} Furthermore, MYB protein overexpression, which can be detected by IHC, is seen in 60\% to 80\% of adenoid cystic carcinomas, including a large proportion of fusion-negative cases.\textsuperscript{45-47}

\textit{MYB-NFIB} is highly specific for adenoid cystic carcinoma, regardless of tissue of origin (eg, salivary gland, breast, bronchus, sinus); however, as implied above, sensitivity is variable and may be quite low. While MYB protein expression is more sensitive, showing strong and diffuse nuclear immunohistochemical staining for MYB within the abluminal component, it is not entirely specific since a subset of other salivary gland tumor types can also show immunoreactivity for MYB.\textsuperscript{45} It is now known that a subset (approximately one-third) of MYB negative tumors have an alternate translocation, \textit{MYBL1-NFIB}.\textsuperscript{48}

**Carcinoma ex Pleomorphic Adenoma/Pleomorphic Adenoma – PLAG1 and HMGA2 Translocation Analysis**

Pleomorphic adenoma is the most common salivary gland tumor type overall, and can undergo malignant transformation, designated generically as \textit{carcinoma ex pleomorphic adenoma}. By conventional karyotyping, translocations involving 12q13-15\textsuperscript{49} or 8q12\textsuperscript{50} have been reported in 40\% to 70\% of pleomorphic adenomas. The genes involved are now known to be \textit{HMGA2} [12q13-15] and \textit{PLAG1} [8q12].

Translocation-positive pleomorphic adenomas tend to have more of a classic morphology and occur in younger individuals.\textsuperscript{50} Translocation assessment may provide value on fine-needle aspirates or small biopsies of pleomorphic adenomas. However, these translocations are also useful in confirming origin from pleomorphic adenoma in histologically malignant tumors (carcinoma ex pleomorphic adenoma). It must be noted that over half of carcinoma ex pleomorphic adenomas show imbalanced translocations, with amplification of \textit{PLAG1} or \textit{HMGA2}.\textsuperscript{51,52}

As the translocations may be complex and may involve a variety of partners, FISH is among the most viable clinical methods for testing. Additionally, for PLAG1, IHC for overexpression has also been utilized. However, like other immunohistochemical markers used as surrogate markers, PLAG is sensitive, present in over 90\% of pleomorphic adenomas and carcinoma ex pleomorphic adenomas, but not specific, as it can be expressed in other tumor types and can be difficult to interpret.\textsuperscript{53,54}
Salivary Duct Carcinoma – Her2 and Androgen Receptor Analysis

Salivary duct carcinoma is generally a high-grade malignant neoplasm, most commonly arising in the parotid gland, with poor outcomes. While morphologically akin to a high-grade ductal carcinoma of breast, it is now understood that, when properly classified, the vast majority of salivary duct carcinomas recapitulate the “luminal AR-positive/molecular apocrine” type of breast carcinoma. Less common histologic variants, papillary, micropapillary, mucin-rich, basal like, and sarcomatoid, are still under the umbrella of this phenotype. In situ disease has also been reported.

Given the similarities to ductal carcinoma of breast, assessment for ERBB2 (HER2) is of potential therapeutic interest, though it has no diagnostic utility. There are no established meaningful thresholds for HER2 IHC and HER2 FISH testing in salivary duct carcinoma, but by extrapolation and for standardization, breast criteria are generally adopted. Roughly 20% to 40% of salivary duct carcinomas show 3+ positivity for HER2 by IHC, and FISH amplification of HER2 is noted in 20% to 30% of cases. Objective tumor responses in patients treated with HER2-directed therapy in combination with bevacizumab and chemotherapy have been reported, though complete responses are rare. A subset of salivary duct carcinomas may harbor mutations in TP53, HRAS, or PTEN loss, which may decrease efficacy of anti-ERBB2 therapy.

Androgen receptor (AR) positivity essentially defines salivary duct carcinoma; most high-grade ductal carcinomas that are AR negative represent high-grade transformation of another tumor type. Aside from its diagnostic utility, a subset of cases has been shown to benefit from antiandrogen therapy either alone or concurrently with radiation. There are currently no thresholds for defining positivity for AR from a diagnostic or therapeutic standpoint.

C. Sinonasal Malignancies

INI-Deficient Sinonasal Carcinoma/Rhabdoid Tumor

Recently, a subset (up to 6% of primary sinonasal carcinomas) of poorly differentiated/undifferentiated carcinomas of the sinonasal tract have now been characterized by loss of SMARCB1 (INI-1) on chromosome 22q11.2 akin to rhabdoid tumors. Tumors are usually phenotypically undifferentiated but may show squamous or glandular differentiation and do show some rhabdoid morphology. The current diagnosis rests on establishing INI-1 deficiency in the context of the aforementioned morphology. Currently this is most frequently documented by IHC, though deletions can be evaluated by FISH. Limited data suggest that the latter is only 75% sensitive in detecting the INI-1–deficient phenotype.

Biphenotypic Sinonasal Sarcoma – PAX3 Translocation Analysis

Biphenotypic sinonasal sarcoma, or low-grade sinonasal sarcoma with neural and myogenic features, is an increasingly recognized, locally aggressive sarcoma of the sinonasal tract, seen most commonly in middle-aged women. Key morphologic features include a cellular, spindled, herringbone architecture; “hemangiopericytomatous” vasculature; and secondary proliferation of respiratory glandular elements. While, initially, confirmation of diagnosis was established by expression of neural (typically at least focal S-100) and myogenic (typically smooth muscle actin [SMA] and/or muscle specific actin [MSA] staining), recent work has demonstrated a recurring t(2;4)(q35;q31.1) translocation, most commonly resulting in PAX3-MAML3 gene fusion.

The utility of testing for this translocation is currently restricted to diagnostic application: to distinguish this entity from both more indolent (ie, cellular schwannoma) and more aggressive (ie, synovial sarcoma, desmoplastic melanoma) spindle cell neoplasms of the sinonasal tract. Both breakapart FISH for PAX3 and RT-PCR for the PAX3-MAML3 fusion transcript are viable testing methodologies. As with other translocations, breakapart FISH appears more sensitive than RT-PCR for the PAX3-MAML3 fusion transcript. PAX3 rearrangements are detectable by FISH in 96% of biphenotypic sinonasal sarcomas, while MAML3 is confirmed as the fusion partner in only 79% of cases. However, while this translocation is, to date, specific for biphenotypic sinonasal sarcoma; PAX3 rearrangements are also seen in alveolar rhabdomyosarcomas (typically partnered with FOXO1), which are not uncommon in the sinonasal tract. Thus, as with other diagnostic translocations, morphologic and immunophenotypic context is critical for appropriate use of breakapart FISH.
D. Paraganglioma

Familial Paraganglioma - SDHB Immunohistochemistry

Head and neck paragangliomas typically consist of carotid body, vagal, and jugulotympanic tumors; primary laryngeal and thyroid paragangliomas are uncommon. Unlike pheochromocytoma, most head and neck paragangliomas are phenotypically “parasympathetic” and rarely present with adrenergic symptomatology. While classically approximately 10% of paragangliomas are familial, the prevalence of germline mutations in several “sporadic” cohorts suggest that this is actually closer to 30% or higher. Most mutations in head and neck paragangliomas involve the succinate dehydrogenase complex subunits B, C, and D (SDHB, SDHC, SDHD); SDHA and SDHAF2 mutations are rare. The majority of these in head and neck paragangliomas are SDHD mutations, which are more strongly associated with multifocal disease. However, SDHB mutations have a higher risk of malignancy, including nonparaganglioma tumors.

While genetic testing and counseling is the reference standard for evaluating for hereditary disease, SDHB loss by immunohistochemistry has been variably employed as a screening method on paragangliomas and pheochromocytomas to help triage patients that should be evaluated by a geneticist and/or tested for germline mutations. Destabilization of the succinate dehydrogenase complex from any mutation will result in a loss of SDHB protein, which is part of the catalytic core. Normal “intact” staining is represented as cytoplasmic granular immunopositivity. SDHB loss in SDH-deficient tumors is denoted by absence of staining, with an important caveat that the internal controls (ie, stroma, vessels) still retain granular positivity. Aside from technical failure, embolization and trauma may decrease SDHB staining, leading to a false-positive result.

References


