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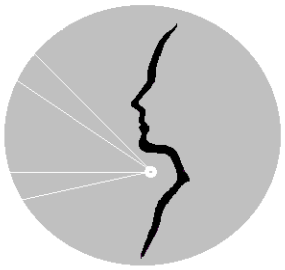
Danish Head and Neck Cancer Group



DAHANCA/EORTC guidelines for scoring and classification of p16-immunohistochemistry in HPV-related oropharyngeal cancer

Expression of p16 is highly correlated to Human Papillomavirus (HPV) in oropharyngeal cancer[1-4] and the strong prognostic impact of p16-immunohistochemistry (IHC) in radiotherapy of HNSCC has been demonstrated in several clinical trials [1,4,5].
The following recommendations on the scoring and classification p16-IHC are devised on the basis of a revision of DAHANCA-material [6] and recommendations set forward by El-Naggar et al[7] taking into account both the percentage of p16-positive tumour cells and the characteristic morphological profile of HPV-related HNSCC [7,8].

Interpretation of p16-IHC:

1. Strong and uniform p16-staining (*both* cytoplasmatic *and* nuclear) in > 70% of cancer cells of basaloid nonkeratinised/partially keratinised oropharyngeal carcinoma is classified as p16-positive and can be interpreted as HPV-positive
2. Absent or weak p16-staining of basaloid non-keratinised/partially keratinised oropharyngeal carcinoma is classified as p16-negative
3. Non-uniform/patchy p16-staining in conventional keratinising squamous cell carcinoma of the oropharynx is classified as p16-negative

Whether additional HPV-testing (in situ hybridisation/PCR-detection) would further optimise the correlation with HPV in the two last-mentioned situations is presently unresolved, and as such not routinely recommended by the DAHANCA.

Technical performance of p16-IHC on formalin fixed paraffin embedded (FFPE) tissue:

The following protocol represents an example of p16-IHC performed on a BenchMark® XT autostainer (Ventana Medical Systems, Illkirch, France):

FFPE sections are cut at 5 µm on Superfrost® plus charged glass slides (Menzel-Glaser), heated at 60°C for 1 hour and deparaffinised in the instrument with EZ prep solution (Ventana Medical Systems). Antigen retrieval is carried out using Cell Conditioning 1 solution (CC1, Ventana Medical Systems). Sections are incubated with murine anti-p16 antibody clone JC8 (Santa Cruz Biotechnology Inc) diluted 1:25 for 32 minutes. Specific reactions are detected using *ultra*View™

Universal DAB Detection Kit (Ventana Medical Systems) and the slides counterstained with haematoxylin.

Other commercially available antibodies can be used for incubation, for instance the E6H4 clone (MTM Laboratories) which has been used in several clinical trials and also recommended by NordiQC (Nordic immunohistochemical Quality Control)[9].

DAHANCA/Pernille Lassen

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