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University Hospitals Bristol NHS
NHS Foundation Trust

headandneck 5000

To clarify the role of human papillomavirus in head and neck cancer by identifying HPV driven cancers

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Scientific Outline

Summary

Most cancers of the head and neck are squamous cell cancer. Tobacco and alcohol use are risk factors. Oral infection with human papillomavirus (HPV), especially HPV type 16, is a risk factor for oropharyngeal cancer (OPC). The proportion of OPCs caused by HPV infection varies and is estimated to be around 30% in Europe (though around 70% of OPC in head and neck 5000 are HPV seropositive). The role of HPV as a cause of cancers outside the oropharynx requires further investigation. In addition, the ability to detect tumours driven by other serotypes using serology (both for OPC and outside the oropharynx) is unclear.

We have already measured HPV serology measurements at the German Cancer Research Center (DKFZ) in Heidelberg. Assays included the E6 and E7 proteins of all high-risk HPV available at the DKFZ laboratory, i.e. HPV 16, 18, 31, 33, 35, 45, 52 and 58. For HPV 16 and 18 this also included E1 and E2.

We will measure molecular markers including Human Papilloma Virus (HPV) DNA and RNA and cellular protein p16ink4a on up to 1000 formalin fixed tissue blocks selected on the basis of their HPV serology and location.

Keywords: Human papillomavirus, lifestyle behaviour, survival

Proposed work

We will transfer up to 1000 formalin fixed tissue blocks selected on the basis of their HPV serology and location from the Head & Neck 5000 study to DKFZ for processing and to be tested for molecular markers, such as Human Papilloma Virus (HPV) DNA and RNA and cellular protein p16ink4a

Blocks will be sectioned according to a standard protocol used previously by DKFZ (summarized below). Head and Neck 5000 will identify appropriate samples to be used with an assessment of tissue availability in each block. Blocks of paraffin embedded tissue will be sectioned to provide material for H+E examination, determination of p16 status (immunohistochemistry) and to extract DNA and RNA for HPV testing and subtyping

DNA and RNA will be extracted from formalin-fixed paraffin embedded (FFPE) tissue sample sections (1); appropriate negative controls will be included to monitor cross-contamination. HPV DNA analysis will be conducted using Multiplex Papillomavirus Genotyping (2,3). Samples positive for HPV and/or beta-globin will be considered DNA valid. HPV RNA analysis, that is, detection of viral transcripts, will be performed by HPV type-specific reverse transcription polymerase chain reaction (RT-PCR) and hybridization assays (1), which amplify HPV E6*I and ubiquitin C (ubC) cDNA as a cellular mRNA QC. Specimens that will be HPV E6*I and/or ubC mRNA-positive will be considered RNA valid.

(1) Halec G, Schmitt M, Dondog B, et al. Biological activity of probable/possible high-risk human papillomavirus types in cervical cancer. Int J Cancer. 2013; 132(1):63–71.
(2) Schmitt M, Bravo IG, Snijders PJ, et al. Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol. 2006; 44(2):504–512.
(3) Schmitt M, Dondog B, Waterboer T, et al. Homogeneous amplification of genital human alpha papillomaviruses by PCR using novel broad-spectrum GP5þ and GP6þ primers. J Clin Microbiol. 2008; 46(3):1050–1059.

P16 IHC will be undertaken on a single tissue section taken from the relevant blocks with the utilization of appropriate positive and negative batch controls.