STUDY PROTOCOL

HPV PREVALENCE IN THE MOUTH AND OROPHARYNX OF THE TONSILLECTOMY POPULATION

ACRONYM: OROMOUTH

Version:	5.0
Date:	15-Jun-2015
Chief Investigator:	Hisham Mehanna
Study Coordinating Centre:	Cancer Research UK Clinical Trials Unit (CRCTU) and Institute of Head and Neck Studies and Education (InHANSE)
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InHANSE Institute of Head and Neck Studies and Education

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UNIVERSITY OF BIRMINGHAM

This study will be conducted in facilities funded through Advantage West Midlands (AWM) which forms part of the Science City Research Alliance of the University of Warwick and University of Birmingham.

SIGNATURE PAGE

Oromouth Study Protocol version 5.0, 15-Jun-2015.

THIS PROTOCOL HAS BEEN APPROVED BY:

Name:	Hisham Mehanna	**Study Role:**	Chief Investigator
Signature:	_____	**Date:**	DD / MON / YYYY

This protocol describes the Oromouth study and provides information about procedures for patients taking part in the Oromouth study.

AMENDMENTS

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
1	03rd Oct 2011	1.1	Substantial amendment	Ethical recommended amendment
N/A	04th April 2012	1.2	Non-substantial amendment	Change in study contact details
N/A	26th Jul 2012	1.3	Non-substantial amendment	Change in document formatting
2	06th Sep 2012	2.0	Substantial amendment	Change of sponsor and rewording
3	10th Dec 2012	3.0	Substantial amendment	Change in pilot study data use
4	20th May 2014	4.0	Substantial amendment	Adapting to CRCTU template and clarification on study duration. Addition of SAE reporting section.
5	15th Jun 2015	5.0	Substantial amendment	Change in recruitment target and extension of study.

STUDY SUMMARY

Title	HPV prevalence in the mouth and oropharynx of the tonsillectomy population
Design	Prospective cross-sectional study with a longitudinal sub-study
Aims	To determine the age and gender distribution of HPV detection in tonsils, oropharyngeal scrapes, nail brushings, oral fluid and urine and also the HPV antibodies in blood
Primary Objectives	• To estimate the overall, age and gender specific prevalence of HPV in the oropharynx in patients undergoing tonsillectomy for non-malignant conditions
Secondary Objectives	• To compare the HPV oropharynx prevalence between different sites– in order to identify the different reservoirs of HPV infection • To describe the distribution of HPV seropositivity in the study population by age and gender • To describe HPV-type distribution overall by age, gender, and sampling site • To describe distribution by age and gender of patients who are both HPV PCR positive and anti-HPV L1 sero-positive – predicting persistent HPV infection • To provide preliminary estimates of the clearance and persistence of HPV infection and antibodies in the oropharynx, as detected in follow-up oral fluid, finger tips/nail brushings and urine samples • To assess the presence of anti-L1 HPV antibodies in saliva as a marker of vaccination or previous infection in the oral cavity • To assess the presence of HPV in the urine • To assess the presence of HPV in finger tips/nail brushings • To provide preliminary evaluation of the performance of potential non-invasive tests for the detection of HPV infection in the mouth against HPV detection rates in tonsillectomy and oropharyngeal scrapes • To identify the change in overall prevalence and distribution of HPV in tonsils over time, and to describe preliminary data on age and gender specific changes over time • To provide preliminary data on the potential effect of HPV vaccination in the prevention of oral infection
Duration	6 months setup and pilot, recruitment over 36 months and 12 months for analysis
Population	Prospective cohort of up to 1000 eligible patients (minimum set of samples) undergoing tonsillectomy and a historical comparison group of 1250 patients who underwent tonsillectomy in early 2000's
Eligibility	**Inclusion criteria for prospective cohort:** The following patients are eligible: • Patients undergoing tonsillectomy for non-cancer reasons including operations for: recurrent tonsillitis, snoring surgery or obstructive sleep apnoea • Age 0 to 65 years • Patient or parent(s)/legal guardian(s) has given written informed consent/assent

Eligibility	**Exclusion criteria:** The following patients are not eligible to join the study: • Patients undergoing adenoidectomy alone with no tonsillectomy • Patients with previous oropharyngeal cancer or oral cancer, or any other head and neck cancer such as nasopharyngeal or laryngeal cancer that is currently undergoing or previously has been diagnosed or treated • Learning disability preventing an adult over 16 years old from giving their own consent
Primary Outcome Measure	• Occurrence of HPV in tonsil samples
Secondary Outcome Measure	• Occurrence of HPV in oral fluid, fingertip/nail brushings, oropharyngeal scrapes and urine samples • Occurrence of HPV infection clearance in follow-up oral fluid, fingertip/nail brushings and urine samples collected at approximately 3 - 6 months after the first HPV positive sample • Occurrence of persistent HPV infection in follow-up oral fluid, fingertip/nail brushings and urine samples collected at approximately 3 - 6 months after the first HPV positive sample • Occurrence of HPV infection detected in follow-up oral fluid, fingertip/nail brushings and urine samples collected at approximately 3 - 6 months after the first HPV negative sample • Occurrence of anti-HPV L1 antibodies in serum • Occurrence of anti-HPV L1 antibodies in oral fluid sample
Study procedures and sample collection	• Study questionnaire – if 16 years old or over • Oral fluid samples 20ml blood serum sample in adults >16years, 10ml blood in child <16 years old; • 20- 30ml urine sample • Finger tips/nail brushings Oropharyngeal scrape samples: posterior pharyngeal wall and base of tongue • Tonsillectomy specimen • Longitudinal subset: second oral fluid, finger tips/nail brushings and urine samples 3-6 months after operation

CONTACT NAMES AND NUMBERS

Key Contacts	Laboratories
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ABBREVIATIONS

a/b	Alpha/beta
ABPI	Association of the British Pharmaceutical Industry
AIC	Akaike Information Criterion
AO	Abstract Only
CRCTU	Cancer Research Clinical Trials Unit
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CV	Curriculum Vitae
DCF	Data Clarification Form
DNA	Deoxyribonucleic Acid
EU	European Union
GCP	General Practitioner
GP	Good Clinical Practice
HPA	Health Protection Agency
HPV	Human Papillomavirus
ICF	Informed Consent Form
GCP	Good Clinical Practice
InHANSE	Institute of Head and Neck Studies and Education
ISF	Investigator Site File
ISH	In-Situ Hybridisation
mls	millilitres
NHS	National Health Service
NS	Not Significant
OPSCC	Oropharyngeal Squamous Cell Carcinoma
PCR	Polymerase Chain Reaction
PIS	Patient Information Sheet
QA	Quality Assurance
R&D	Research and Development
REC	Research Ethics Committee
RNA	Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
RNO	Registration Number
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAP	Statistical Analysis Plan
SSDL	Site Signature and Delegation Log
TMG	Trial Management Group
TSC	Trial Steering Committee
UK	United Kingdom
UKHAN	UK Head and Neck
USA	United States of America
VENICE	Vaccine European New Integrated Collaboration Effort
WBC	White Blood Cell
WHO	World Health Organisation
WMA	World Medical Association

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1. BACKGROUND AND RATIONALE

1.1. RAPIDLY INCREASING PUBLIC HEALTH PROBLEM

In the EU, oropharyngeal squamous cell carcinoma (OPSCC) currently accounts for 93,342 new cases (ASR-w of 6.9) and 42,962 deaths a year (ASR-w of 3.1) (Globocan 2008). It is estimated that in Eastern Europe, the burden of OPSCC is higher, with incidence rates in males of 9.1 per 100,000 (WHO HPV centre).

Worryingly, the incidence of OPSCC has been increasing dramatically in the EU and USA over the past decade. For example, the incidence of oropharyngeal cancer in England has more than doubled in the last decade (National Cancer Intelligence Network, 2010); whilst in Sweden it is increasing at the rate of 2% per year in men.

1.2. CAUSE OF THE PROBLEM

The rising incidence in Europe and the USA has recently been attributed to a new distinct disease entity called Human Papillomavirus-related oropharyngeal squamous cell carcinoma (HPV-OPSCC), caused by the Human Papillomavirus (HPV), which also causes most cervical and anal cancers (Mehanna, 2010). HPV infection is sexually-transmitted, and in the mouth may be linked to oral sex. Other bodily fluids e.g. saliva may potentially also be routes of transmission, especially before the onset of sexual activity. A meta-analysis of the world literature performed by our group shows that the proportion of HPV-OPSCC has more than doubled in Europe and the USA over the past decade (rising from 35% to 73% in Europe for example). Incidence in Europe and the USA is expected to continue to rise significantly due to the lag time between HPV infection and disease presentation (~25 yrs.), prompting concerns by some experts of an epidemic of viral-induced malignancy (Sturgis 2010).

1.3. HUMAN PAPILLOMAVIRUS VACCINATION AND EU ACTION

On 10 April 2008, the European Parliament adopted a resolution on combating cancer in the enlarged EU, and Council conclusions on reducing the European burden of cancer were adopted on 10 June 2008. The resolution recommended the 'cost-effective integration of appropriate HPV testing for cervical cancer screening and HPV vaccination to protect young women from cervical cancer'. The Vaccine European New Integrated Collaboration Effort (VENICE) and the European Cervical Cancer Association reports show that currently over half of the EU countries do not have vaccination programmes. The remaining member states vaccinate girls only.

1.4. GAPS IN THE LITERATURE

The vaccination of girls only may not protect males, especially if herd immunity is not achieved due to incomplete uptake of the vaccine in girls. Therefore, in view of the recent rise in HPV-oropharyngeal carcinoma, some experts are raising the possibility of the vaccination of boys as well as girls. Yet, to date insufficient data is available on oropharyngeal HPV infection to allow the accurate assessment of the need for and cost effectiveness of including boys in the HPV vaccination programmes in the EU.

Furthermore, there is little information on the prevalence of HPV infection in the mouth in the general population or on the age and gender distribution of this infection. The data that is available consists of small studies. These are usually retrospective and most of the study cohorts are over ten years old – therefore most of the prevalence data available is at least ten years old. Furthermore, the studies used a variety of sample types and different HPV detection techniques. Therefore the results are widely varying with reported HPV prevalence rates in the mouth between 0% and 31%.

Table 1: Frequency with which high-risk HPV types were detected in oral exfoliated cell samples using PCR methods (> 10 cases).

Study	City	Period of patient accrual	Age range	HPV detection	Type of oral sample	No of samples	HPV+ rate
D'souza 2007	Baltimore, USA	2000-5	<50yrs-34%, >50yrs-66%	PCR	Oral rinse and brush	200	6%
Smith 2007	Iowa , USA	n/a	2-20	PCR	Oral exfoliated cells	1235	1.9%
Rintala 2006	Finland	N/A	28	PCR	Scrapings	462	15-27%
Smith 2004	Iowa, USA	1994-97	18-70, 34% <55	PCR	Oral exfoliated cells	333	10.8%
Summersgill 2001	Iowa	<2001	<20	PCR	Swabs or oral rinses	268	8.7% <7yrs, 0% 7-12, 5.2% 13-20
Koch 1997	Denmark	<1997	0-17	PCR	Oral rinses	392	0.26%
Puranen 1996	Finland	1981-96	0.3-11.6	PCR	Oral scrapings	98	31.6%
Rice 1996	London	<1996	3-11	PCR	Buccal swabs	267	17%
Jennison 1990		<1990	preschool	PCR	Oral scrapings	21	24%

Table 2: Frequency with which high-risk HPV types were detected in tonsillectomy samples using PCR methods (> 10 cases).

Study	Year	City	Period of patient accrual	Age range	HPV detecti on	Number of samples	Number positive for HPV
Klinkenberg	2010	Netherlands	1997-98	29.2	PCR	195	2 (1%)
Ernster	2009	USA	1979-82; 1997-2001	> 21 years	PCR	226	0 (0%)
Kim	2007	Korea	1995-2005	NS	RT-PCR	69	3 (4%)
Sisk	2006	USA	AO	paediatric patients	PCR	50	1 (2%)
Ribeiro	2006	Brazil	NS	2-13 years	PCR	64	5 (7.8%)
Mammas	2006	Greece	1995-2000	2-14 years	PCR	106	6 (5.7%)
Chen	2005	Sweden	2001-02	23.2 years	PCR	206	13 (6.3%)
Strome	2002	USA	1987-95	52.5 years	PCR	48	0 (0%)
Klussman	2001	Germany	"new cases"	52 years	PCR	14	0 (0%)

AO (abstract only): includes unspecified number of low-risk types; prevalence fell with increasing age from 11.5% in preschool children to 0% in older adults.
NS: Not Significant.

This data is essential for the assessment of the need for and cost effectiveness of including boys in the HPV vaccination programmes, and for the determination of the age of viral acquisition in the general population and hence the age of start of vaccination programmes (Kim,Vaccine 2008; Smith Pediatr Infect Dis J. 2007), as it may be that young people acquire earlier in the mouth than in the cervix, due to oral sex experimentation starting at a younger age than sexual intercourse. This data is also essential for the evaluation of the potential effectiveness of screening programmes, and preventative educational programmes (Pediatr Infect Dis J. 2007).

1.5. STUDY RATIONALE

There is little information on the distribution of HPV within the oropharynx and the reservoirs of infection within the mouth. Different studies show different rates for different sites within the oropharynx. However, since these studies use different types of samples and different HPV detection techniques, no reliable data can be surmised from them. Furthermore there is no data on the

effectiveness of vaccination for the prevention of HPV infection in the mouth, or on persistence of HPV infection in the mouth or the effect of HPV vaccination on HPV infection in the mouth. The study rationale is to gather data on the occurrence of HPV in the mouth and determine if it is common enough to implement a vaccination programme for boys as well.

2. AIMS, OBJECTIVES AND OUTCOME MEASURES

To provide current data on the prevalence, distribution and natural history of HPV infection in the mouth, which are essential requirements for the assessment, development and evaluation of cost-effectiveness of prophylactic vaccination and screening programmes.

2.1. OBJECTIVES

2.1.1. PRIMARY OBJECTIVE:

- To estimate the overall, age and gender specific prevalence of HPV in the oropharynx in patients undergoing tonsillectomy for non-malignant conditions

2.1.2. SECONDARY OBJECTIVES:

- To compare the HPV oropharynx prevalence between different sites–in order to identify the different reservoirs of HPV infection
- To describe the distribution of HPV seropositivity in the study population by age and gender
- To describe HPV-type distribution overall by age, gender, and sampling site
- To describe distribution by age and gender of patients who are both HPV PCR positive and anti-HPV L1 sero-positive – predicting persistent HPV infection
- To provide preliminary estimates of the clearance and persistence of HPV infection and antibodies in the oropharynx, as detected in follow-up oral fluid, finger tips/nail brushings and urine samples
- To assess the presence of anti-L1 HPV antibodies in saliva as a marker of vaccination or previous infection in the oral cavity
- To assess the presence of HPV in the urine
- To assess the presence of HPV in finger tips/nail brushings
- To provide preliminary evaluation of the performance of potential non-invasive tests for the detection of HPV infection in the mouth against HPV detection rates in tonsillectomy and oropharyngeal scrapes
- To identify the change in overall prevalence and distribution of HPV in tonsils over time, and to describe preliminary data on age and gender specific changes over time
- To provide preliminary data on the potential effect of HPV vaccination in the prevention of oral infection

2.2. OUTCOME MEASURES

2.2.1. PRIMARY OUTCOME

- Occurrence of HPV in tonsil samples

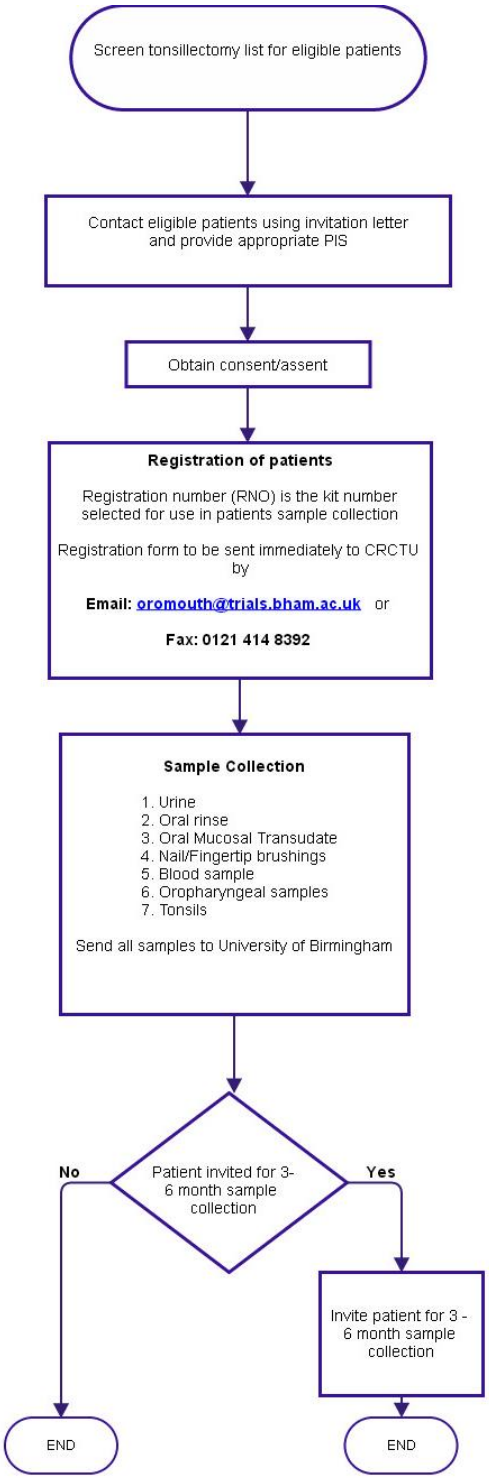
2.2.2. SECONDARY OUTCOME

- Occurrence of HPV in oral fluid, fingertip/nail brushings, oropharyngeal scrapes and urine samples
- Occurrence of HPV infection clearance in follow-up oral fluid, fingertip/nail brushings and urine samples collected at approximately 3 - 6 months after the first HPV positive sample
- Occurrence of persistent HPV infection in follow-up oral fluid, fingertip/nail brushings and urine samples collected at approximately 3 - 6 months after the first HPV positive sample
- Occurrence of HPV infection detected in follow-up oral fluid, fingertip/nail brushings and urine samples collected at approximately 3 - 6 months after the first HPV negative sample
- Occurrence of anti-HPV L1 antibodies in serum

- Occurrence of anti-HPV L1 antibodies in oral fluid sample

3. STUDY DESIGN

OROMOUTH is a prospective, multi-centre, cross sectional study.



4. ELIGIBILITY CRITERIA

4.1. INCLUSION CRITERIA

The following patients are eligible:

1. Patients undergoing tonsillectomy for non-cancer reasons including operations for: recurrent tonsillitis, snoring surgery or obstructive sleep apnoea
2. Age 0 to 65 years
3. Patient, parent(s) or legal guardian(s) has given informed written consent

4.2. EXCLUSION CRITERIA

The following patients are not eligible to join the study:

1. Patients undergoing adenoidectomy alone with no tonsillectomy
2. Patients with previous oropharyngeal cancer or oral cancer, or any other head and neck cancer such as nasopharyngeal or laryngeal cancer that is currently undergoing or has previously been diagnosed or treated
3. Learning disability preventing an adult over 16 years old from giving their own consent

5. SCREENING AND INFORMED CONSENT/ASSENT

Potential patients will be identified from the list of patients having tonsillectomy at the participating hospital by the research nurse. All eligible patients or their parent(s)/ legal guardian(s) (if they under 16 years old), will be contacted by invitation letter before their operation date to inform them of the study. On or before the date of the operation, the patients will have the study explained to them by the research nurse. Children and young people under the age of 16 will also have the study explained to them and their parent(s) or legal guardian(s). If they are willing to participate in the study, they will be asked to give written consent. Parents will give consent and assents will be obtained from young people (aged between 11 and 15 years) in addition to their parent(s) or legal guardian(s) consent.

5.1. INFORMED CONSENT / ASSENT

It is the responsibility of the investigator or research nurse (delegated by the Principal Investigator as captured on the Site Signature and Delegation Log (SSDL)) to obtain written informed consent/assent for each patient prior to performing any study related procedure. Age specific Patient Information Sheets (PIS) are provided to facilitate this process. Investigators must ensure that they adequately explain the aim, anticipated benefits and potential hazards of taking part in the study to the patient, parent(s) or legal guardian(s). The investigator should also stress that the patient, parent(s) or legal guardian(s) are completely free to refuse to take part or withdraw from the study at any time. The patient, parent(s) or legal guardian(s) should be given ample time (e.g. 24 hours) to read the PIS and to discuss their participation with others outside of the site research team. The patient, parent(s) or legal guardian(s) must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient, parent(s) or legal guardian(s) to refuse to participate in the study without giving a reason must be respected.

If the patient, parent(s) or legal guardian(s) expresses an interest in participating in the study they should be asked to sign and date the latest version of the Informed Consent Form/Assent Form. The investigator or research nurse must then sign and date the form. A copy of the Informed Consent Form and Assent Form should be given to the patient, their parent(s) or legal guardian(s), a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF). Once the patient is entered into the study the patient's Registration Number (RNO) should be entered on the Informed Consent Form/Assent Form maintained in the ISF. In addition, if the patient, their parent(s) or legal guardian(s) has given explicit consent/assent a copy of the signed Informed Consent Form/Assent Form must be sent in the post to the OROMOUTH Study Office for review.

Details of the informed consent/assent discussions should be recorded in the patient's medical notes, this should include date of, and information regarding, the initial discussion, the date consent/assent was given, with the name of the study and the version number of the PIS and Informed Consent Form/Assent Form. Throughout the study the patient, their parent(s) or legal guardian(s) should have the opportunity to ask questions about the study and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent/re-assent the patient in which case the process above should be followed and the patient's right to withdraw from the study respected.

Electronic copies of the PIS and Informed Consent Form/Assent Form are available from the OROMOUTH Study Office and should be printed or photocopied onto the headed paper of the local institution.

Details of all patients approached about the study should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent/assent their General Practitioner (GP) should also be informed that they are taking part in the study. A GP Letter is provided electronically for this purpose.

6. STUDY ENTRY

Patients should be registered into the study once eligibility is confirmed and informed consent/assent has been obtained. The patients unique RNO will correspond to the ID number of the kit used in the sample collection for that patient. The Registration Form confirming patients' eligibility should be completed and sent to the OROMOUTH Study Office by email or fax using the details below:

Email: oromouth@trials.bham.ac.uk

Fax: **0121 414 8392**

The following details are captured on the Registration Form:

1. Name of registering hospital
2. Name of investigator
3. Patient's initials
4. Date of written informed consent
5. Confirmation of eligibility criteria

Registration confirmation will be sent from the OROMOUTH Study Office by email to notify the site of the patient's registration on the OROMOUTH database.

7. STUDY PROCEDURES AND SAMPLE COLLECTION

All samples should be collected in accordance to the study procedure manual developed by GSK with details of sample collection kits, volume of samples to be collected and storage temperature requirements.

For a patient to be eligible for inclusion in the data analysis, the following samples are a minimum requirement:

- Oral samples
- Blood
- Oropharyngeal brushings
- Tonsils

1. After written consent is obtained, the patients (above 16 years old only) will complete a short study questionnaire including age, gender, history of tobacco/alcohol use, sexual history including age of start of sexual intercourse, lifetime numbers of sexual partners, engagement in oral sex and lifetime number of oral sex partners etc.

2. An HPV vaccination history will also be taken. Consent/assent will also be taken from the patient, parent(s) or their guardian(s), if applicable, to obtain the patient's HPV vaccination status from vaccine and medical health records from their GP and/or other relevant health and educational authorities. The data requested will include confirmation of HPV vaccination status, including dates, number of doses and the type of vaccine given.

3. Oral fluid samples will be collected for HPV PCR and HPV antibody tests, using an oral rinse and an exudate collection device respectively.

4. A 20 - 30 ml urine sample will be collected for HPV PCR test.

5. Blood serum will be taken for HPV and biomarker serology tests. This can be taken after the patient has been anaesthetised if necessary. 20 mls of blood will be taken from patients aged 16 years and above, 10 mls from patients below 16 years old.

6. Once the patient is anaesthetised, two brush cytology scrapings using cytobrushes (provided) will be taken: one from the posterior pharynx including above and below the soft palate so that the adenoidal area is included, and a second separate brush scrape will be taken from the back of the tongue (base of tongue).

7. Tonsillectomy will then be performed and the tonsillar tissue will be placed in 10% buffered formalin and sent to the pathology department for paraffin embedding according to a specific protocol.

8. Stratified sampling will be employed to ensure that sufficient numbers of patients are recruited from all age groups, especially the age groups between 10 and 20 years old.

7.1. PROSPECTIVE LONGITUDINAL SUBSET STUDY

A random selection of around 150 patients will be asked to give oral fluid and urine samples and brushings from their finger tips and under the nails approximately 3 - 6 months after their operation to provide pilot data on persistence and clearance of HPV. These patients may be contacted by phone or letter to arrange an appointment in a clinic or a visit by the researcher at home to obtain the specimens.

7.2. HISTORICAL COMPARISON GROUP

95,000 tonsillectomy samples collected for the National Prospective Tonsillectomy Audit between 2003 -2004 have been archived by the Health Protection Agency (HPA) and now constitute an ethics approved research tissue bank called the National Anonymous Tonsil Archive (REC ref. 08/H0404/54). We will match the patients in our study with a sex and age matched cohort of approximately 1250 anonymised tonsillectomy samples from the above research tissue bank. Tissues from the anonymised tonsillectomy samples will be tested for HPV DNA by PCR and other techniques to provide a historical comparison group of HPV prevalence and distribution. The HPA have confirmed their approval to provide the tonsil samples, provided ethical approval is granted for our study.

If the above is not possible for any logistical reasons, then as an alternative, we may analyse an anonymised sample of up to 650 tonsil samples previously harvested in the PROTECT study in 2000-01, which are currently available to the Chief Investigator. The samples are totally anonymised, and so the only data available is the gender data and whether the age is below or above 9 years of age. These samples have equal numbers of males and females, and the age distribution between older than and younger than 9 years old is equally distributed. Ethics approval has been obtained by the PROTECT study to transfer the samples to our group for use in further ethically-approved studies.

7.3. SAMPLE SHIPMENT

Samples collected from the registered patients, should be sent to the InHANSE laboratory at the University of Birmingham (address below) using the approved courier indicated in the OROMOUTH Sample Collection Instructions.

InHANSE Laboratory
CRC Building
School of Cancer Sciences,
3rd Floor
University of Birmingham
Birmingham.B15 2TT, UK

InHANSE will dispatch samples to other laboratories (as appropriate) for testing.

7.4. SAMPLE ANALYSIS

All samples will be analysed using laboratory manuals and standard operating procedures applicable to that organisation.

Table 3: List of laboratories analysing samples

Testing Laboratory	Sample
DDL Diagnostic Laboratory Diagnostic Service Unit Visseringlaan, 25 2288 ER Rijswijk The Netherlands	• Fresh Tonsils • Archive Tonsils • Oral rinse
Universiteit van Antwerpen Vaxinfectio – CEV Campus Drie Eiken, gebouw R, 2e verd. Room R 2.10 Universiteitsplein 1 2610 Wilrijk Belgium	• Urine
CEVAC Center For Vaccinology UZ GENT - 1BA De Pintelaan 185 BE-9000 Gent Belgique	• Serum
InHANSE Laboratory CRC Building School of Cancer Sciences, 3rd Floor, University of Birmingham Birmingham.B15 2TT, UK	• Nail Brushings • White Blood Cells • Serum

Testing Laboratory	Sample
GlaxoSmithKline Vaccines Clinical Immunology R & D Department/Building 7 Rue de l'Institut, 89 B-1330 Rixensart – Belgium	• Oral Mucosal Transudate • Urine • Fresh Tonsils • Archive Tonsils • Oropharyngeal brushings

1. The oral fluid, nail brushings, oropharyngeal scrapes and urine samples will be tested for HPV DNA by PCR. We may also test them for HPV viral load, viral RNA and p16 RNA. They may also undergo other detection methods, such as in-situ hybridisation (ISH). The oral fluid may also be tested for anti HPV L1 antibodies. Other antibodies e.g. anti E6/7, nucleic acids, other HPV related proteins and total antibodies may also be assayed.

2. The tonsil samples will be tested for HPV DNA by PCR. They may also undergo other forms of detection of HPV such as in-situ hybridisation and other tests that may be developed in future for HPV detection. The tonsil samples will also be examined histologically for pre-malignancy to examine the molecular profile and genetic changes within the pre-malignant sample and to compare it to those of the normal tonsil, using immunohistochemical, DNA, mRNA and proteomics techniques.

3. The blood samples will be tested for antibodies to the HPV L1, and other HPV antibodies. Also other protein testing may be performed. Samples may undergo other forms of detection for HPV within the blood, such as HPV nucleic acids and HPV virus particles.

8. ADVERSE EVENT REPORTING

The collection and reporting of Adverse Events (AEs) will be in accordance with the Research Governance Framework for Health and Social Care and the requirements of the National Research Ethics Service (NRES). Definitions of different types of AE are listed in Appendix 1. The investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient with reference to the protocol.

8.1. REPORTING REQUIREMENTS

Investigators should only report AEs that meet the definition of an SAE (see Appendix 1 for definition) and which are thought to be related to the patients' participation in the OROMOUTH study.

8.1.1.1. EXPECTED SERIOUS ADVERSE EVENTS

We are not expecting any SAEs to occur as a result of participation in this non-interventional study thus all SAEs reported will be categorised as unexpected.

8.1.2. REPORTING PERIOD

Details of all SAEs will be documented and reported on the day of sample collection.

8.2. REPORTING PROCEDURE

AEs defined as serious and related to the OROMOUTH protocol should be reported on an SAE Form. When completing the form, the investigator will be asked to define the causality and the severity of the AE which should be documented using the CTCAE version 4.0.

On becoming aware that a patient has experienced an SAE, the investigator (or delegate) must complete, date and sign an SAE Form. The form should be faxed together with a SAE Fax Cover

Sheet to the OROMOUTH Study Office using one of the numbers listed below as soon as possible and no later than 24 hours after first becoming aware of the event:

To report an SAE, fax the SAE Form with an SAE Fax Cover Sheet to:

0121 414 8392 or 0121 414 7989

On receipt the OROMOUTH Study Office will allocate each SAE a unique reference number. This number will be transcribed onto the SAE Fax Cover Sheet which will then be faxed back to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the OROMOUTH Study Office. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE. The SAE Fax Cover Sheet completed by the OROMOUTH Study Office should be filed with the SAE Form in the ISF.

For SAE Forms completed by someone other than the investigator the investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the OROMOUTH Study Office in the post and a copy kept in the ISF. Investigators should also report SAEs to their own Trust in accordance with local practice.

8.2.1. STUDY OFFICE

On receipt of an SAE Form seriousness and causality will be determined independently by the Clinical Coordinator. An SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the sample collection will be regarded as a related SAE.

8.3. REPORTING TO THE RESEARCH ETHICS COMMITTEE

8.3.1. UNEXPECTED AND RELATED SERIOUS ADVERSE EVENTS

The OROMOUTH Study Office will report all events categorised as Unexpected and Related SAEs to the Research Ethics Committee (REC) within 15 days.

8.3.2. OTHER SAFETY ISSUES IDENTIFIED DURING THE COURSE OF THE STUDY

The REC will be notified immediately if a significant safety issue is identified during the course of the study.

8.4. REPORTING TO INVESTIGATORS

Details of all Unexpected and Related SAEs and any other safety issue which arises during the course of the study will be reported to Principal Investigators. A copy of any such correspondence should be filed in the ISF.

9. DATA HANDLING AND RECORD KEEPING

9.1. DATA COLLECTION

The Case Report Form (CRF) will comprise of the forms listed in Table 4.

Table 4: OROMOUTH Case Report Form

Form	Summary of data recorded	Schedule for submission

Registration Form	RNO, Initials, Date of Birth, eligibility checklist: confirmation of eligibility	Emailed or faxed as soon as possible after registration
Baseline Form	Details of HPV vaccination history and demographic information	By post/courier within 2 weeks
Patient Details Form	Patient address and personal information	By post/courier within 2 weeks
Sample Data Form	Information on samples collected	By post/courier within 2 weeks
Second Sample Data Form	Sample collected 3 – 6 months after surgery	By post/courier within 2 weeks
Discontinuation Form	Information regarding discontinued patients	Immediately upon patients' discontinuation
Serious Adverse Event Form	See Section 8	Immediately (no later than 24 hours) after first discovering that the patient has experienced an SAE

The CRF must be completed, signed/dated and returned to the OROMOUTH Study Office by the investigator or an authorised member of the site research team (as delegated on the SSDL) within the timeframe listed above.

Entries on the CRF should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change. Refer to the General Paper CRF Completion Guidelines for more information.

Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning.

In all cases it remains the responsibility of the investigator to ensure that the CRF has been completed correctly and that the data are accurate.

On completion, the top copy of each form must be submitted to the OROMOUTH Study Office and the bottom copy filed in the ISF.

Study forms may be amended by the OROMOUTH Study Office, as appropriate, throughout the duration of the Study. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

9.2. ARCHIVING

It is the responsibility of the Principal Investigator to ensure all essential study documentation and source records (e.g. signed Informed Consent Forms, ISFs, patients' hospital notes, copies of CRFs etc.) at their site are securely retained for at least 10 years after the end of the study. Do not destroy any documents without prior approval from the CRCTU Document Storage Manager.

10. QUALITY MANAGEMENT

OROMOUTH study will be coordinated by the CRCTU and InHANSE at the University of Birmingham according to the current guidelines for Good Clinical Practice (GCP).

10.1. SITE SET-UP AND INITIATION

All sites will be required to sign a Clinical Study Site Agreement prior to participation. In addition all participating Investigators will be asked to sign the necessary agreements and supply a current CV to the OROMOUTH Study Office. All members of the site research team will also be required to sign the SSDL, which should be returned to the OROMOUTH Study Office. Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting covering aspects of the study design, protocol procedures, Sample collection and reporting of data and record keeping. Sites will be provided with an ISF containing essential documentation, instructions, and other documentation required for the conduct of the study. The OROMOUTH Study Office must be informed immediately of any change in the site research team.

10.2. CENTRAL MONITORING

Where a patient has given explicit consent/assent, sites are requested to send in copies of signed Informed Consent Forms/Assent Forms for in-house review.

Research staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Research staff will check incoming CRFs for compliance with the protocol, data consistency, missing data and timing. Sites will be sent Data Clarification Forms (DCFs) requesting missing data or clarification of inconsistencies or discrepancies.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to Trial Steering Committee (TSC) and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the study protocol to the Research Ethics Committee (REC).

10.3. AUDIT AND INSPECTION

The Investigator will permit study-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

10.4. NOTIFICATION OF SERIOUS BREACHES

Sites are requested to notify the OROMOUTH Study Office of a suspected study-related serious breach of GCP and/or the study protocol. Where the OROMOUTH Study Office is investigating whether or not a serious breach has occurred, sites are also requested to cooperate with the OROMOUTH Study Office in providing sufficient information to report the breach where required and in undertaking any corrective and/or preventive action.

- The conditions and principles of GCP in connection with that study or;
- The protocol relating to that study, within 7 days of becoming aware of that breach
- For the purposes of this regulation, a “serious breach” is a breach which is likely to effect to a significant degree:
    - The safety or physical or mental integrity of the patients of the study; or
    - The scientific value of the study

11. END OF STUDY DEFINITION

The end of study will be the date of last visit of the last patient recruited. The OROMOUTH Study Office will notify the REC that the study has ended and a summary of the clinical study report will be provided within 12 months of the end of the study.

12. STATISTICAL CONSIDERATIONS

12.1. DEFINITION OF OUTCOME MEASURE

See section 2.2.1 and 2.2.2.

12.2. ANALYSIS OF OUTCOME MEASURE

The statistical analyses will be described in detail in a statistical analysis plan (SAP). All of the tests will be two-sided with an alpha level of 0.05.

12.2.1. PRIMARY OUTCOME:

The overall prevalence will be estimated as the number of patients with HPV detected in tonsils divided by the total number of patients with available samples. An exact 95% confidence interval will be computed. The prevalence per age and gender group will be calculated similarly.

The relationship between prevalence and age will be explored using logistic regression with the age as a continuous covariate and gender and centre as categorical covariate. The number of degrees (one, two, three) of the model and possible variable transformation (e.g. log-transformation of age) could be determine based on graphical analysis of prevalence versus age relationship and on model fit criteria (e.g. AIC).

12.2.2. SECONDARY OUTCOME:

The overall prevalence will be estimated as the number of patients with HPV detected in oral fluid, oropharyngeal scrapes and urine samples divided by the total number of patients with available samples. An exact 95% confidence interval will be computed.

The prevalence per age and gender group will be calculated similarly. Prevalence rate will be compared among centres using a Cochran-Mantel-Haenszel test with stratification by age group.

Relationship between the prevalence of HPV in tonsils and the prevalence of HPV in oropharyngeal scrapes, oral fluid, and urine samples will be analysed by computing kappa coefficient. Specificity, sensitivity, positive predictive value and negative predictive value of HPV detection in the different sample types will be derived using the oral sample results as the reference.

Relationship between presence of anti-HPV L1 antibody in oral fluid samples and occurrence of HPV in tonsils and/or in oropharyngeal scrape samples will be analysed by computing kappa coefficiency. Specificity, sensitivity, positive predictive value and negative predictive value of anti-HPV L1 antibody assay will be derived using the oral sample results as the reference.

The rate of HPV persistence will be calculated as the proportion of baseline HPV-positive patients who again test HPV-positive in oral fluid, fingertip/nail brushings and urine samples collected after approximately 3 - 6 months. The rate of oral HPV infection clearance will be calculated as the proportion of baseline HPV-positive patients who test HPV-negative in oral fluid, fingertip/nail brushings and urine samples collected after approximately 3 - 6 months.

HPV incidence will be calculated as the proportion of baseline HPV-negative patients who test HPV positive in oral fluid, fingertip/nail brushings and urine samples collected after approximately 3 - 6 months. Exact 95% confidence intervals will be computed.

Multi variable regression models will be used to calculate the odds ratios of different risk factors for having HPV DNA in the tonsil and in the oropharynx, Variables to be included in the model (e.g. serological markers and clinical risk factors such as HPV vaccination status, tobacco and alcohol use) will be selected on the basis of descriptive statistics/possible causal relationship and/or a stepwise approach. Analysis of results may be performed to identify a possible marker or combination of markers for screening for persistent HPV infections in the oropharynx.

Among females in the target age class for vaccination (12 to 24 years), exploratory analyses will compare the HPV prevalence rate in HPV vaccinated and non HPV vaccinated patients using a Fisher's exact test. Association between HPV vaccine and oral HPV infection will be assessed using a case-control approach. Cases will be patients with HPV positive oral sample and controls will be patients with HPV negative oral sample. The exposure to vaccine will be compared between the case and the control groups using logistic regression, OR and its 95% confidence interval will be derived.

Prevalence of HPV in tonsils will be described and compared between the current cohort and the comparison historical cohort using a Cochran-Mantel-Haenszel test with stratification by age group and gender.

12.3. SAMPLE SIZE CONSIDERATIONS

An analysis of current literature suggests that in a population recruited between 1990 and 2000, the prevalence of oral HPV was about 6.5%, varying from 0% - 10%. However, in an analysis of oropharyngeal scrapes and oral washings, the prevalence of HPV detection was much higher at 31%.

Pilot data of patients having tonsillectomy at University Hospital Coventry over two 3-month time periods are shown below in table 3. The number of tonsillectomy operations performed at the Heart of England NHS Foundation Trust - is approximately 50% of that for University Hospital Coventry quoted below.

Table 5: The number of tonsillectomy operations performed at University Hospital Coventry.

Age Group*	Number of Monthly recruitment (%)
0-4	7 (12%)
5-9	12 (20%)
10-14	7 (12%)
15-19	10 (17%)
20-24	6 (10%)
25-29	5 (8%
30-34	4 (7%)
35-39	5 (8%)
40-44	1 (2%)
45-49	1 (2%)
50-69	1 (2%)
Total	**59**

*There was an equal distribution of males and females in the group.

We plan to enrol 925 patients. The precision of the overall prevalence estimate that would be achieved with such a number is summarised in Figure 1 for prevalence ranging from 1 to 31 %. For example, if the observed prevalence is 10 %, the 95 % confidence interval for the overall population will be 8.4 to 11.8 % corresponding to a relative precision of 17 %.

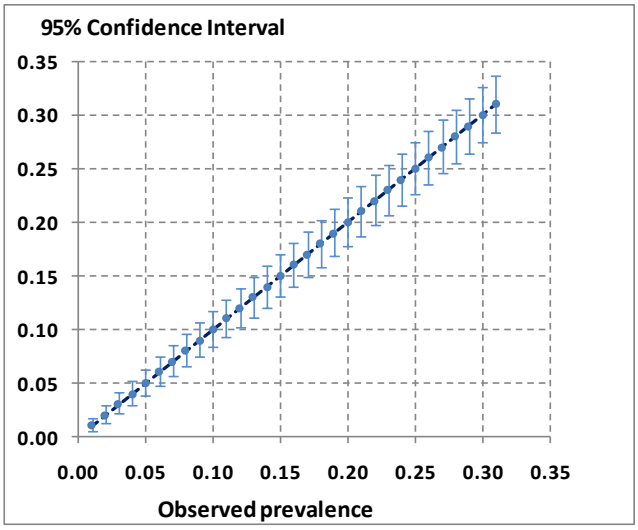


Figure 1: Precision (95% confidence interval) of the prevalence estimate versus the prevalence observed in a random sample of 1250 patients.

We will also undertake a stratified sampling strategy to allow comparisons between those aged 14 and under (non-exposed, approximately one third of the cohort), and patients over 14 years of age (exposed) to allow the determination of the age of acquisition of infection and the optimal age of start of vaccination. The stratified sampling strategy will recruit patient subgroups as follows:

Table 6: The stratified sampling strategy for patient recruitment.

Age group	Sample size (%)
0-4	150 (16.5%)
5-9	150 (16.5%)
10-14	150 (16.5%)
15-19	150 (16.5%)
20-24	150 (16.5%)
25-34	100 (11%)
35-44	60 (5.5%)
45-65	15 (1.0%)
Total	**925**

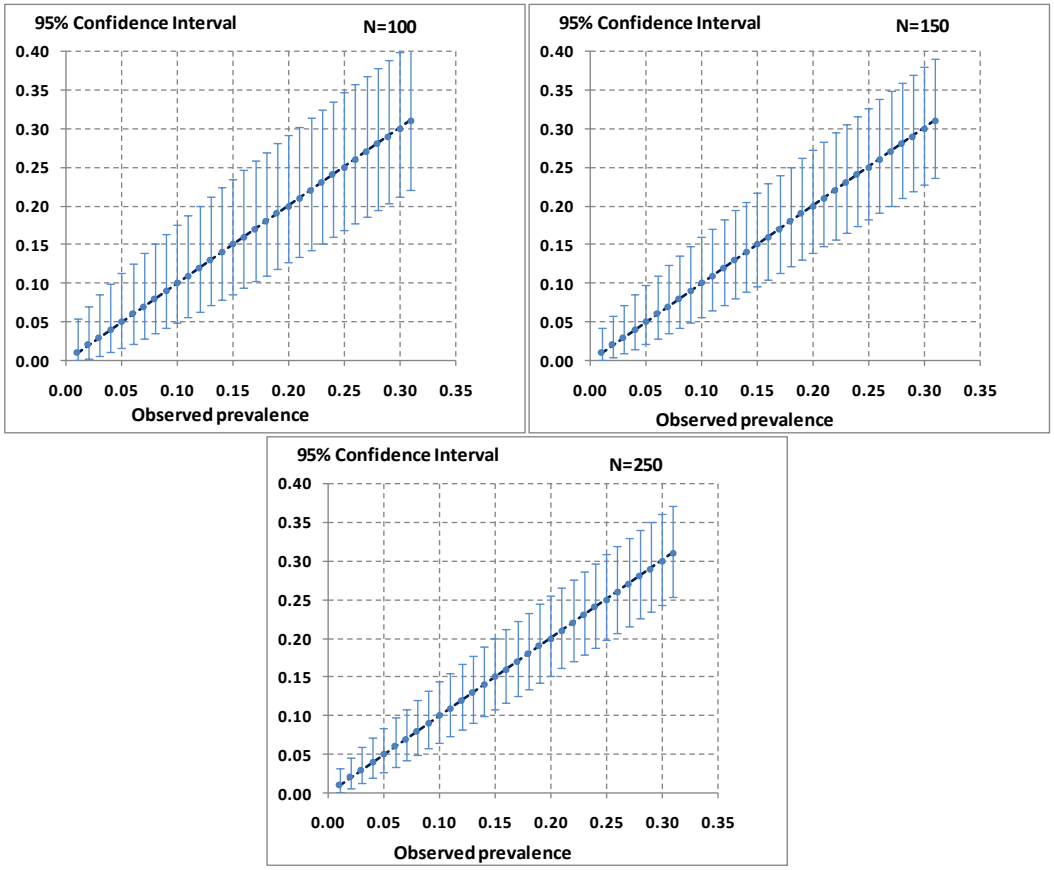


Figure 2: Precision (95% confidence interval) of the prevalence estimate versus the prevalence observed in a random sample of 100, 150, and 250 patients.

12.4. POWER CALCULATIONS

For the historical comparison analysis, assuming a two-sided test, 0.05 alpha level, baseline prevalence of 2 to 8%, and exclusion of 150 HPV vaccinated women from the analysis, we would have approximately 80% power to detect an absolute increase in overall HPV prevalence of at least 2 to 4% (see table below). Exploratory analyses of changes in HPV prevalence by gender and age and by vaccination status will be descriptive.

12.5. PLANNED INTERIM ANALYSIS

We will undertake an interim analysis when approximately 50% of the target recruitment has been achieved.

Table 7: Comparison Analysis.

Power	Alpha	Sample size historical cohort	Sample size Oromouth cohort	Prevalence historical cohort	Prevalence Oromouth cohort	Minimal detectable difference
0.80	0.05	1100	1100	2.0%	4.1%	2.1%
0.80	0.05	1100	1100	5.0%	8.1%	3.1%
0.80	0.05	1100	1100	8.0%	11.6%	3.6%

13. STUDY ORGANISATIONAL STRUCTURE

13.1. SPONSOR

This study will be sponsored by the University of Birmingham.

13.2. COORDINATING CENTRE

The study is being conducted under the auspices of the CRCTU and InHANSE, University of Birmingham according to their local standard operating procedures.

13.3. TRIAL MANAGEMENT GROUP

The Chief Investigator, Principal Investigators, Study Statistician, Senior Trial Manager and Study Coordinator will form the TMG. The TMG will be responsible for the day-to-day conduct of the study. They will be responsible for study set-up, promotion of on-going management of the study, monitoring patient accrual, the interpretation of the results and preparation and presentation of relevant publications.

13.4. TRIAL STEERING COMMITTEE

The TSC is made up of three representatives from GSK Biologicals (Funder), three Sponsor representatives and one independent member. The TSC will meet by mutual agreement on an as needed basis. The TSC will:

- Facilitate the research collaboration
- Address key study issues, including but not limited to:
    - Data analysis (including interim study results)
    - Interpretation
    - Publications

14. FINANCE

This is an investigator-initiated and investigator-led study funded by an educational grant from GSK Biologicals.

No individual per patient payment will be made to NHS Trusts, Investigators or patients.

15. ETHICAL CONSIDERATIONS

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, 1964, amended by the 48th WMA General Assembly, Somerset West, Republic of South Africa, 1996 (website:http://www.wma.net/en/30publications/10policies/b3/index.html) (Appendix 2).

The study will be conducted in accordance with the Research Governance Framework for Health and Social Care, the applicable USTUDYK Statutory Instruments, (which include the Data Protection Act 1998 and Human Tissue Act 2008) and the Guidelines for Good Clinical Practice (GCP). The protocol will be submitted to and approved by the Research Ethics Committee (REC) prior to circulation.

Before any patients are enrolled into the study, the Principal Investigator at each site is required to obtain local Research and Development (R&D) approval. Sites will not be permitted to enrol patients until written confirmation of R&D approval is received by the OROMOUTH Study Office.

It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

16. CONFIDENTIALITY AND DATA PROTECTION

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. With the patient's consent/assent, their specific patient identifiers e.g. full name, date of birth, National Health Service (NHS) number, address, post code, hospital number, and GP details will be collected on the Patient Details Form.

Patients will be identified using only their unique RNO, initials, and date of birth on the CRF and correspondence between the OROMOUTH Study Office and the participating site. However patients are asked to give permission for the OROMOUTH Study Office to be sent a copy of their signed Informed Consent Form/Assent Form which will not be anonymised. This will be used to perform in-house monitoring of the consent/assent process. The investigator must maintain documents not for submission to the OROMOUTH Study Office (e.g. Patient Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete study records, provided that patient confidentiality is protected.

The OROMOUTH Study Office will maintain the confidentiality of all patient's data and will not disclose information by which patients may be identified to any third party other than those directly involved in the treatment of the patient and organisations for which the patient has given explicit consent/assent for data transfer (e.g. health and educational authorities to obtain HPV vaccination information). Representatives of the OROMOUTH study team may be required to have access to patient's notes for Quality Assurance (QA) purposes but patients should be reassured that their confidentiality will be respected at all times. The laboratories where sample analyses will be carried out will only identify samples by RNO. The research nurses will not have access to the result of the sample analyses.

17. INSURANCE AND INDEMNITY

University of Birmingham employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical study they undertake whilst in the University's employment.

In terms of liability at a site, NHS Trust and non-Trust hospitals have a duty to care for patients treated, whether or not the patient is taking part in a clinical study. Compensation is therefore available via NHS indemnity in the event of clinical negligence having been proven.

The University of Birmingham cannot offer indemnity for non-negligent harm. The University of Birmingham is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation.

18. PUBLICATION POLICY

Results of this study will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the TMG and authorship will be determined by mutual agreement.

Any secondary publications and presentations prepared by Investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the study was performed with the support of University of Birmingham. Intellectual property rights will be addressed in the Clinical Study Site Agreement between Sponsor and site.

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APPENDIX 1 - DEFINITION OF ADVERSE EVENTS

Adverse Event
Any untoward medical occurrence in a patient or clinical trial subject participating in the trial which does not necessarily have a causal relationship with the treatment received.
Comment:
An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Related Event
An event which resulted from the administration of any of the research procedures.

Serious Adverse Event
Any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening*
- Requires hospitalisation** or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly/birth defect
- Or is otherwise considered medically significant by the Investigator***

Comments:
The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on patients/event outcome or action criteria.
* Life threatening in the definition of an SAE refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
**Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment (e.g. line insertion), elective procedures (unless brought forward because of worsening symptoms) or for social reasons (e.g. respite care) are not regarded as an SAE.
*** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.

Unexpected and Related Event
An event which meets the definition of both an Unexpected Event and a Related Event.

Unexpected Event
The type of event that is not listed in the protocol as an expected occurrence.

APPENDIX 2 - WMA DECLARATION OF HELSINKI

Recommendations guiding physicians
in biomedical research involving human subjects
Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964
and amended by the
29th World Medical Assembly, Tokyo, Japan, October 1975
35th World Medical Assembly, Venice, Italy, October 1983
41st World Medical Assembly, Hong Kong, September 1989
and the
48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The Health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the

minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.

NOTES

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