

**CANNIESBURN
PLASTIC SURGERY UNIT**



**MULTICENTRE INTERVENTIONAL
TRIAL OF SENTINEL NODE BIOPSY IN
ORAL AND OROPHARYNGEAL
CANCER**

TRIAL PROTOCOL

INTRODUCTION

Head and neck squamous cell carcinoma (SCC) spreads via lymphatics to the regional draining lymph nodes in the neck, and this spread is thought to be embolic in nature¹. Since the presence of lymph node metastases is an important prognostic factor in Head and Neck cancer, decreasing survival by 50%², reliable staging of the neck in this disease is imperative to determine further management. Physical examination, magnetic resonance imaging, ultrasound scanning and computed tomography of the neck, have not proved reliable in assessing nodal involvement, with approximately 30% of patients with clinically clear necks (N0) containing occult metastases in neck dissection specimens³.

An accurate method for determining the presence of lymph node metastases in the neck is only available in the form of major surgery, by performing a neck dissection⁴.

Surgeons are reluctant to perform neck dissections for early clinical disease because of the associated morbidity. However, a "wait-and-see" approach results in a high proportion of patients developing metastatic disease after completing initial treatment. Conversely, performing a cervical lymph node dissection on all patients with the disease would lead to a high proportion of unnecessary surgery.

Since the treatment of early neck disease carries a better prognosis than late neck disease⁵, the management of the clinically N0 neck remains one of the continuing debates in oral and oropharyngeal cancer⁶.

In cutaneous malignant melanoma and breast cancer, a similar debate has centred around the investigation and treatment of regional lymph nodes and to this effect, the technique of sentinel node biopsy (SNB) is gaining popularity⁷. In this, a vital blue dye (such as Patent Blue V) and radiocolloid (such as Albures or Nanocolloid) are injected around a tumour, or the site of its excision. Lymphatic channels stain blue and these are mapped to the first echelon lymph node in the regional draining basin. The use of pre-operative lymphoscintigraphy and an intra-operative gamma probe aid localisation of radioactive sentinel nodes, some of which do not stain blue, yet contain microscopic deposits of tumour. In malignant melanoma, it has been shown that patients without palpable nodal disease and a sentinel node clear of metastatic disease are highly unlikely to have metastases elsewhere within the nodal group⁸. Sentinel node biopsy has also been used to successfully stage other cancers, such as penile⁹ cancer, carcinoma of the vulva¹⁰ and Merkel cell carcinoma¹¹.

Sentinel node biopsy is emerging as a means of determining the presence of nodal metastases in squamous cell carcinoma of the upper aerodigestive tract¹². Recent reports in the literature and on-going studies suggest the procedure will find a role in the management of the clinically N0 neck^{13,14}, although the procedure is not without technical difficulties^{15,16}.

Radiation doses to surgeons and pathologists participating in sentinel node procedures in breast cancer have been established to be low¹⁷.

AIMS

PRIMARY AIMS

To determine whether sentinel node biopsy can accurately determine the presence or absence of lymph node metastases in patients with T1/T2N0 oral and oropharyngeal cancer

SECONDARY AIMS

To map the anatomical site of the sentinel node in head and neck carcinoma for various sites of primary lesion in the oral cavity and oropharynx.

To determine the role of immunohistochemistry and multiple step sectioning in identifying micrometastases in the sentinel node in the absence of visible metastases by conventional staining methods

PATIENTS, MATERIALS AND METHODOLOGY

The methods described here for pre-operative lymphoscintigraphy and surgery are those used at Canniesburn Hospital. Other centres that join the study will have previously performed the technique using slightly different methods. The trial protocol accepts that different centres will use different techniques to harvest the sentinel node. For example, some centres will use blue dye and others will use radiocolloid only; some units will perform pre-operative lymphoscintigraphy and others will not. The centres participating in the study should have proven their experience in the technique of harvesting the sentinel node in the context of a neck dissection prior to embarking on sentinel node biopsy as an interventional procedure.

Although the actual method for harvesting the sentinel node will vary between units, it is imperative that all units use the same method for pathological examination of the sentinel node and that all units perform a modified radical neck dissection if the sentinel node is found to contain viable tumour cells.

INCLUSION CRITERIA

patients with histologically proven T1/T2N0 oral or oropharyngeal SCC accessible to injection.

EXCLUSION CRITERIA

pregnancy, lactation, patients undergoing elective treatment to the neck (either surgery or radiotherapy).

METHOD OF SENTINEL NODE BIOPSY

SNB consists of three main areas: Lymphoscintigraphy, Surgery and Pathology.

(A): LYMPHOSCINTIGRAPHY

Patients undergo lymphoscintigraphy up to one day prior to surgery. A maximum of 40MBq ^{99m}Tc-labelled Human Serum Albumin (Albures or Nanocoll) is injected throughout the normal mucosa surrounding the tumour edge and submucosa on the deep aspect of the tumour in a volume of approximately 0.5-1.0ml. A syringe with a permanently secured needle is used for injection, to prevent inadvertent spillage of colloid into the mouth. Colloid is injected at as many points as necessary in an attempt to completely surround the tumour. A mouthwash is used immediately following injection to prevent pooling or swallowing of residual radioactivity by the patient.

Static lymphoscintigraphy is performed at 15 minutes, 30 minutes and one hour post injection in two planes or until the appearance of radioactive nodes. It is usual to see hot spots 15 minutes post injection. If nodes are still absent one hour after injection, the lymph nodes are either too close to the injection site or radiocolloid has leaked out of the injection site.

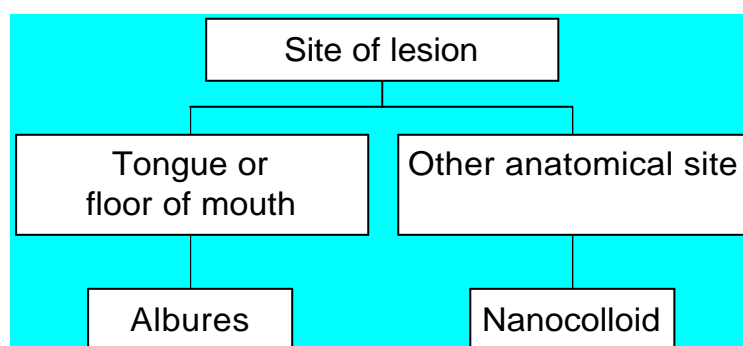
Either a ^{57}Co marker is employed to trace the patient outline or a flood source of a ^{57}Co or $^{99\text{m}}\text{Tc}$ is placed behind the patient to produce a silhouette of the patient outline. From the point of view of radiation dose the marker pen is preferable. A gamma camera fitted with a low energy, general purpose (LEGP) collimator is used to image the patient. A 20% window centred on the 140keV photopeak is selected and the camera interfaced to a suitable computing system. The locations of radioactive lymph nodes are marked on the patients' skin: the position of a ^{57}Co solid source pen is observed on the cameras' persistence display and the pen moved until its position overlies that of a radioactive node. This position is then marked on the skin using indelible ink. During the skin marking, a lead plate of an appropriate thickness (eg 3mm) is used to shield the injection site.

Following image acquisition a software mask is applied to all images to eliminate radioactivity from the injection site. A region of interest, drawn around the image of the site of injection, is used as the basis for the mask applied.

The technique for lymphoscintigraphy may vary slightly between centres.

CHOICE OF COLLOID

Two colloids are commonly used for lymphoscintigraphy in Europe: Albures and Nanocoll. Albures has a mean particle size of 500nm and is a slower moving particle that remains in first echelon (sentinel) nodes but requires a high density of terminal lymphatic vessels at the injection site. For these reason, Albures is the colloid of choice in the tongue and floor of mouth. Nanocolloid has a mean particle size of 50nm and is a faster moving colloid which finds lymphatic vessels despite injection into tissues with low densities of terminal lymphatics. However, it moves readily from sentinel nodes to subsequent echelon nodes and for these reasons nanocolloid is the colloid of choice in non-floor of mouth/non-tongue primaries. The choice of colloid should be recorded.



The choice of colloid may vary slightly between centres.

(B): SURGERY

At operation, 1-2 ml of Patent Blue V dye is injected throughout the normal mucosa and submucosa surrounding the tumour. Patent Blue V dye is injected prior to the skin incision to minimise the risk of disrupting lymphatic channels draining the primary tumour. In order to approximate the same injection sites as for radiocolloid, all

injections should be made by one person. A suitable incision is made in the neck in such a position as to facilitate excision of the incision scar should a subsequent neck dissection be necessary. The hand held gamma probe is used to identify radioactive sentinel nodes, including those marked pre-operatively during lymphoscintigraphy. To reduce detection of radiation from the injection site, a series of malleable sterilised lead plates may be used to mask the injection site, thus aiding in-vivo identification of radioactive nodes. Radioactive nodes are excised and radioactivity within the node is confirmed *ex-vivo*. Blue stained lymphatics, if seen, will be followed to the first draining lymph node, which is harvested. Sentinel nodes are labelled according to their colour and radioactivity. The anatomical neck level of sentinel nodes are noted. Although sentinel nodes should be harvested prior to treatment of the primary, the proximity of the sentinel node to the injection site may require a further search for sentinel nodes following excision of the primary. If sentinel nodes are sought after excision of the injection site, the nodes are unlikely to be blue stained.

Because of the relatively high radioactivity still present in the injection sites and the proximity to the sentinel node, detection of scattered radiation must be avoided as far as possible. As well as the use of lead plates as above, the gamma probe must have a well collimated detector which excludes gamma radiation except over a small angle in front of it. The pulse height analysis window should be set just to include the ^{99m}Tc photopeak with a cut-off on the low energy side at about 130 keV. The calibration should be checked at regular intervals of not more than one month (depending on make and model of instrument) and a quick check of calibration should be devised to be carried out before each use. It may be necessary to call on appropriate scientific/technical assistance to ensure that the gamma probe is at its optimum settings and to make an estimate of its sensitivity at these settings.

The details of the exact surgical methodology to retrieve the sentinel node may vary slightly between centres.

(C): PATHOLOGY

Sentinel nodes are fixed in 10% neutral buffered formalin and after fixation are bisected through the hilum, if this is identifiable, or through the long axis of the node. If the thickness of the halves is more than 2mm the slices are further trimmed to provide additional 2mm thick blocks. If sentinel nodes are found to be free from tumour on initial histological examination step-serial sections will be prepared at an additional six levels in the block at approximately 150 micron intervals. One H&E stained section will be prepared at each level. If the nodes still appear histologically negative, an immediately adjacent section from each level will be examined by immunocytochemistry using the multi-cytokeratin antibody AE1/AE3. (It is advisable to mount two or more short sequences of serial sections at each level to allow for possible technical problems with section preparation).

If a neck dissection is subsequently performed during the period of the study, all non-sentinel nodes over approximately 2.5mm in maximum diameter are identified in their anatomical groups. Each node is bisected through the hilum (or long axis, if the hilum is not identifiable) and both halves are processed for histological examination. Larger nodes are trimmed in the manner detailed above for sentinel nodes. One H&E stained section is prepared from each block and is examined for the presence of nodal involvement by tumour.

The interpretation of the histopathology and immunocytochemistry of sentinel lymph nodes will be categorised as follows:-

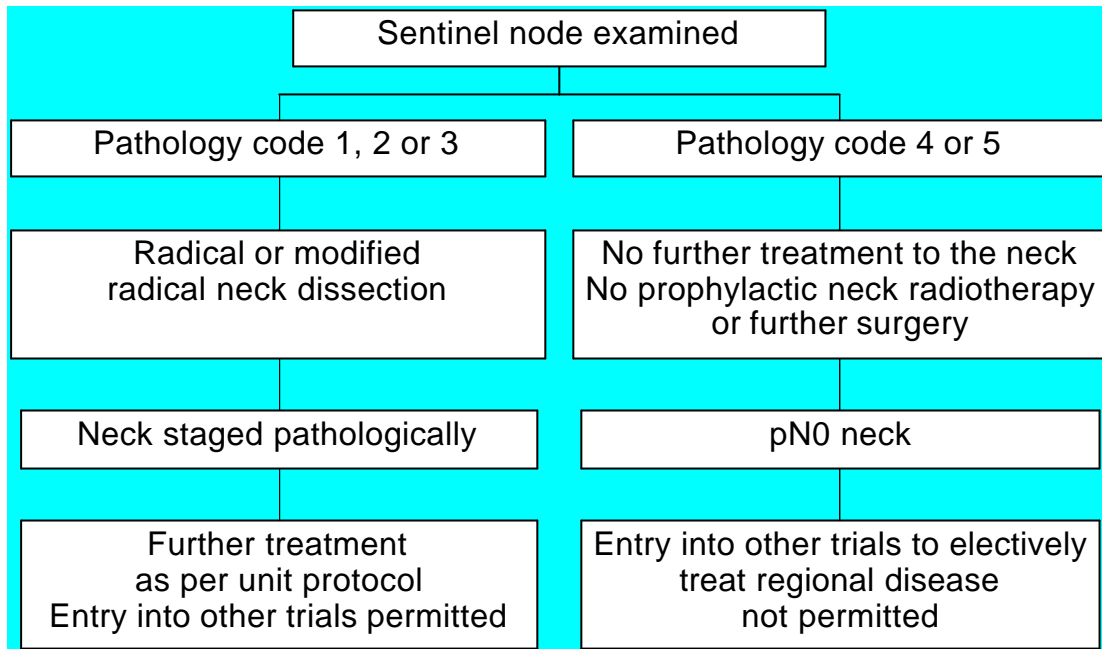
PATHOLOGY CODE	DESCRIPTION
1	Tumour positive on first H&E examination
2	Initially tumour negative, but tumour positive on examination of H&E of step serial sections
3	Negative at stages 1 and 2 but positive by immunohistochemistry. To be categorised as tumour positive there must be cells which are both positive by immunocytochemistry and are cytologically seen to be nucleated cells with the characteristics of viable epithelial cells in both the immunocytochemical preparation and the serial H&E section. Cytokeratin positivity lacking the cytological features of viable tumour cells is categorised as 4.
4	Cytokeratin positivity not showing the features of viable tumour cells. This positivity is likely to represent either dying tumour cells, possibly apoptotic cells, characterised by being eosinophilic bodies lacking normal nuclei, or macrophages with phagocytosed tumour products. Usually these cells will be single and not small cohesive groups. The decision to allocate nodes to this category requires careful comparison of the serial H&E and immunocytochemical preparations.
5	Negative at all stages.
6	Negative on first H&E examination. Further examination not performed since other sentinel nodes contained viable tumour either on H&E or immunohistochemistry

It is important that all centres examine the sentinel node using the same methodology and report the sentinel node pathology in the same manner.

FURTHER TREATMENT FOR PATIENTS WITH SENTINEL LYMPH NODES CONTAINING TUMOUR

In the event that any lymph node contains viable tumour either by routine histology or through immunohistochemistry and multiple sectioning, the patient will undergo a radical or modified radical neck dissection. For tumours that drain to lymph nodes on both sides of the neck, a neck dissection will only be performed on the side of the neck in which a sentinel node containing tumour was found. The neck dissection should take place within four weeks of the sentinel node biopsy, and any adjuvant radiotherapy should start within six weeks of the neck dissection. Radiotherapy should not be administered prior to neck dissections.

Patients will undergo further treatment to the neck following sentinel node biopsy according to the following flow chart:



FOLLOW-UP

Follow-up will take place for all patients entered into the trial. Patients will be seen three monthly for the first year, four monthly for the following two years and six monthly until 5 years post sentinel node biopsy.

At any stage, if nodal disease is detected, patients will be offered treatment to the neck in the form of surgery. All other treatments for metastatic disease will be given according to local protocols, however, patients with regional failure must undergo a neck dissection, if fit for surgery.

END POINTS

PRIMARY END POINTS:

- Date of regional recurrence (in the absence of: primary disease recurrence or a second primary in a site likely to spread to the site of regional failure)

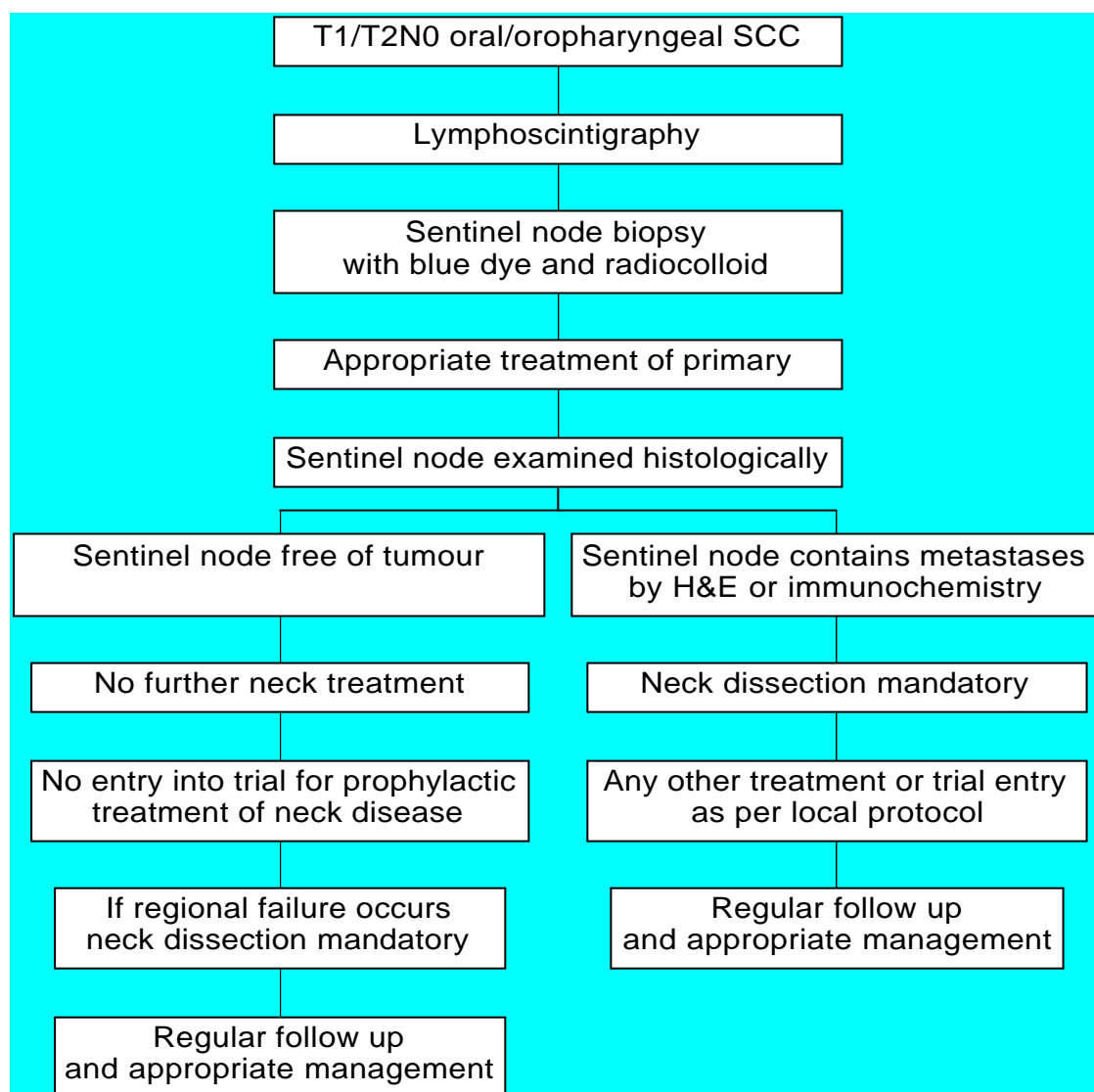
SECONDARY END POINTS:

- Date of death
- survival to 60 months post entry into trial
- Development of a second primary tumour in the upper aerodigestive tract
- Development of a local recurrence

ROLE OF PARTICIPATING CENTRES

A lead clinician will be nominated for each centre and will enter patients. Clinicians should have demonstrated their experience with sentinel node biopsy on at least 10 patients with prior to entering patients in the multicentre study.

FLOW CHART OF SIMPLIFIED TRIAL PROTOCOL



FURTHER INFORMATION

For further information, please contact:

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REFERENCES (COLLATED DECEMBER 1999)

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MINIMUM DATA SET

PATIENT DETAILS

Patient Identification Number

Centre name

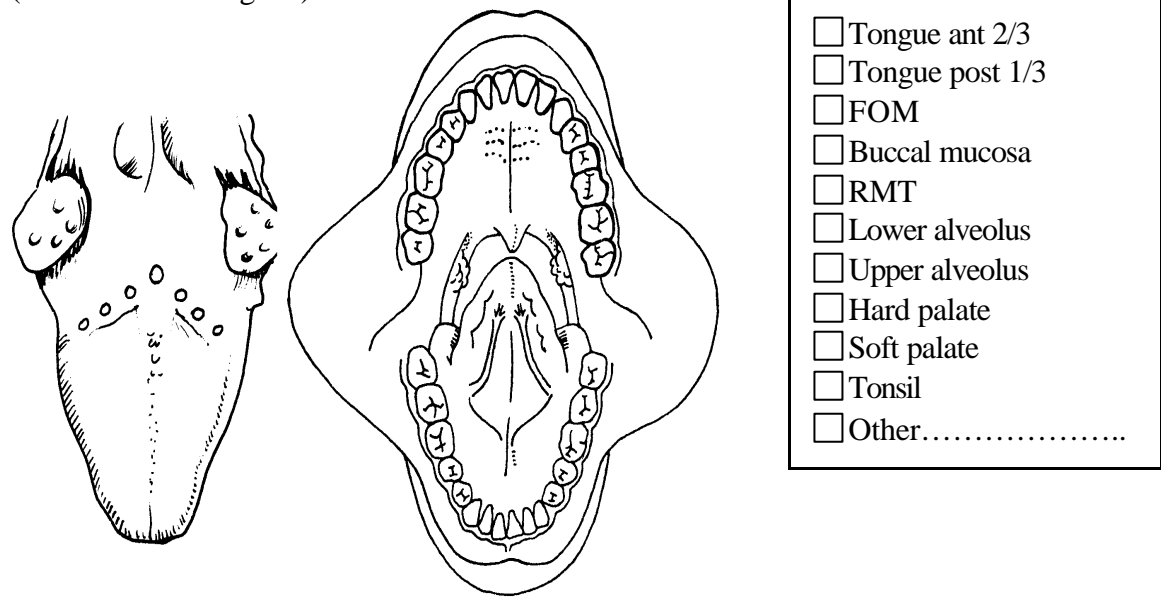
Lead clinician in charge

Date of birth
(day/month/year)

Sex Male
 Female

Date of sentinel node biopsy
(day/month/year)

Tumour site:
(indicate site on diagram)



- Tongue ant 2/3
- Tongue post 1/3
- FOM
- Buccal mucosa
- RMT
- Lower alveolus
- Upper alveolus
- Hard palate
- Soft palate
- Tonsil
- Other.....

Clinical T stage

Treatment to primary: Excision
 External Beam Radiotherapy
 Interstitial radiotherapy
 Other (state).....

MINIMUM DATA SET

Patient ID number: Centre name:

LYMPHOSCINTIGRAPHY

Type of colloid injected	<input type="checkbox"/> Nanocoll <input type="checkbox"/> Albures <input type="checkbox"/> Other (state).....
No of scintigraphy nodes	<input type="text"/>

SURGERY

No of radioactive only nodes	<input type="text"/>
No of blue only nodes	<input type="text"/>
No of hot blue nodes	<input type="text"/>
Lymph node basins explored	<input type="checkbox"/> Lt neck (levels I-V) <input type="checkbox"/> Rt neck (levels I-V) <input type="checkbox"/> Other (state).....
Any non-sentinel nodes excised	How many..... Which nodal levels.....

Length of time for SNB: (tick appropriate box)

- <15 mins
- 15-30mins
- 30-45mins
- 45-60mins
- >1 hour

Technical difficulties:

- none and all sentinel nodes removed
- some difficulties but all sentinel nodes removed
- severe difficulties and all sentinel nodes not removed
- abandoned with no neck surgery
- abandoned with neck dissection

Whole procedure of sentinel node biopsy

- satisfactory
- unsatisfactory

Reconstruction of defect

- None/not applicable
- Graft
- Local flap (eg nasolabial, buccal mucosa, etc)
- Distant Flap (e.g. pec. major, deltopectoral, etc)
- Free tissue transfer

Complications of node biopsy	<input type="text"/>
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MINIMUM DATA SET

Patient ID number:
Centre name:

PATHOLOGY

Pathological T stage:

Tumour thickness in mm:

Summary of sentinel node information:

	Node 1	Node 2	Node 3	Node 4	Node 5	Node 6
Anatomical level						
Dimensions in mm						
Blue stained?						
Radioactive?						
Pathology code						

Pathology codes

1. Tumour positive on first H&E examination
2. Initially tumour negative, but tumour positive on examination of H&E of step serial sections
3. Negative at stages 1 and 2 but positive by immunohistochemistry. To be categorised as tumour positive there must be cells which are both positive by immunocytochemistry and are cytologically seen to be nucleated cells with the characteristics of viable epithelial cells in both the immunocytochemical preparation and the serial H&E section. Cytokeratin positivity lacking the cytological features of viable tumour cells is categorised as 4.
4. Cytokeratin positivity not showing the features of viable tumour cells. This positivity is likely to represent either dying tumour cells, possibly apoptotic cells, characterised by being eosinophilic bodies lacking normal nuclei, or macrophages with phagocytosed tumour products. Usually these cells will be single and not small cohesive groups. The decision to allocate nodes to this category requires careful comparison of the serial H&E and immunocytochemical preparations.
5. Negative at all stages.

THIS FORM SHOULD NOW BE SENT TO TAIMUR SHOAB OR GARY ROSS BY E-MAIL FOR ENTRY INTO THE MULTICENTRE DATABASE. ALL CENTRES WILL HAVE ACCESS TO THE DATA WITHIN THE MULTICENTRE DATABASE UPON COMPLETION OF TEN CASES ON THE CONDITION THAT THEY DO NOT USE THE RESULTS OF OTHER CENTRES FOR PUBLICATION. E-MAIL ADDRESS: sentinel@canniesburn.org

MINIMUM DATA SET

Patient ID number: Centre name:

NECK DISSECTION DETAILS

REASON FOR NECK DISSECTION

<input type="checkbox"/> Sentinel node positive (pathology codes 1,2 or 3) <input type="checkbox"/> Non sentinel node (excised during sentinel node biopsy) positive for tumour <input type="checkbox"/> Development of lymph node disease at follow up <input type="checkbox"/> Other (state).....
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FOR EACH NECK SIDE DISSECTED, STATE:

Side of neck dissection

<input type="checkbox"/> Left <input type="checkbox"/> Right

Date of lymph node dissection:
(day/month/year)

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Nodal levels of neck cleared:

<input type="checkbox"/> Level I <input type="checkbox"/> Level II <input type="checkbox"/> Level III <input type="checkbox"/> Level IV <input type="checkbox"/> Level V <input type="checkbox"/> Other (state).....

Non-nodal structures preserved:

<input type="checkbox"/> Spinal Accessory <input type="checkbox"/> Sternomastoid <input type="checkbox"/> Internal Jugular
--

COMPLETE THE FOLLOWING TABLE FOR EACH NECK DISSECTION:

	Number of nodes examined	Number of nodes with tumour	Number of nodes with extra capsular spread
Level 1			
Level 2			
Level 3			
Level 4			
Level 5			
Other (state)			

MINIMUM DATA SET

Patient ID number: Centre name:

OTHER END POINTS

Date of development of local recurrence

(day/month/year)

Date of development of 2nd primary

(day/month/year)

Site of development of 2nd primary

Date of death

(day/month/year)

ADJUVANT THERAPY**RADIOTHERAPY**

	To primary	To neck
Start date: (day/month/year)		
Finish date: (day/month/year)		
Total dose received:		
Number of fractions:		

CHEMOTHERAPY

Chemotherapy details (include date given, drugs used and doses administered):

MINIMUM DATA SET

Patient ID number:
Centre number:

FOLLOW UP

Tick the appropriate boxes

	Alive and disease free	Alive but with disease	Dead of disease	Dead from another cause
3 months				
6 months				
9 months				
12 months				
16 months				
20 months				
24 months				
28 months				
32 months				
36 months				
42 months				
48 months				
54 months				
60 months				

Data entry forms are available from Taimur Shoaib or from the multicentre web site:

gary.ross@canniesburn.org or tshoaib@canniesburn.org

www.canniesburn.org/sentinel

Please return forms at each stage of their completion to Taimur Shoaib or Gary Ross for entry into the multicentre database.