

**i. Title Page****Unilateral radiotherapy treatment for p16/HPV positive squamous cell carcinoma of unknown primary in the head and neck****Running title**

Unilateral radiotherapy for p16+ unknown primary

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None to declare.

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## **ii. Abstract, Keywords, and Level of Evidence**

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There are no conflicts of interest in the conduct of this study.

**Abstract**

**Objective/Hypothesis:** The outcomes of unilateral radiotherapy treatment for patients with p16/HPV positive squamous cell carcinomas affecting cervical lymph nodes with an unknown primary (SCCUP) are under-reported. Compared to radiating large volumes of the pharyngeal axis (the more common approach), this is potentially a much less toxic treatment for a good prognosis group.

**Study Design:** Retrospective cohort study.

**Methods:** We identified patients with SCCUP who were treated radically at our centre and did not have parotid, or isolated level IV or V nodal involvement. Failure free and overall survivals were calculated using Kaplan-Meier methods.

**Results:** From 2004 to 2012 there were 49 radically treated patients with SCCUP. Fourteen patients had bilateral neck treatment (they had bilateral nodal disease or suspected lesions in the base of tongue, though not proven with biopsy), 2 had surgery alone while 33 had unilateral radiotherapy (after neck dissection, excisional biopsy or definitively with concurrent chemotherapy). Of the 33 patients, 21 tested positive to p16/HPV and had median followed up of 57 months. In this group no isolated contralateral neck failures or putative primaries emerged. There was 1/21 (4.3%) ipsilateral neck failure, 1/21 (4.3%) concurrent contralateral neck and distant failure, and 1/21 (4.3%) patient with distant failure. The 5 year freedom from failure was 78% (CI: 56-100%) and overall survival was 90% (CI: 79-100%).

**Conclusion:** With no emergence of putative primaries and no isolated contralateral neck failures, this single institution experience in p16/HPV positive SCCUP patients suggests that unilateral radiotherapy may be an underutilised management strategy.

**Indexing words**

Squamous cell carcinoma

Neoplasms, unknown primary

Neck cancer

Cyclin-Dependent Kinase Inhibitor p16

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Radiotherapy

Levels of evidence

4

### **iii. Main text**

#### **Introduction**

Management of squamous cell carcinoma of unknown primary (SCCUP) in the head and neck remains a controversial issue and there is no general agreement regarding the best overall management. In most instances, the recommended management includes attempts to locate the primary with a thorough clinical examination of the mucosa and skin in the head and neck region, and imaging with computer tomography (CT), magnetic resonance imaging (MRI) and/or positron emission tomography (PET) (1-3). Further investigation should also include examination under-anaesthesia and biopsies of sites thought likely to harbour an occult primary (1). Tonsillectomy and more recently trans-oral resection of the base of tongue lymphoid tissue, has been recommended as many of these occult primaries are harboured in the lymphatic crypts of these sites (4-9).

Treatment involves performing either a surgical neck dissection followed by adjuvant (chemo)radiotherapy for those with adverse pathological risk factors for neck recurrence or definitive (chemo)radiotherapy (3). There is no agreement as to which management provides the best outcome. Furthermore, there is wide variation regarding the radiotherapy volumes required. Particularly controversial has been the issue of unilateral versus bilateral neck treatment and the volume of mucosa harbouring potential putative primary sites that should be encompassed prophylactically with radiotherapy. It is now known that a number of unknown primary cancers are associated with the human papilloma virus (HPV) which have a high probability of arising from the oropharynx (10). HPV oropharyngeal cancers are often difficult to see and/or palpate as they are small and often lie deep in the crypts of the tonsil or base of tongue.

Literature reporting on unilateral radiotherapy for unknown primaries has mostly been done in the era prior to p16 testing (a surrogate marker for HPV) (11-16). More contemporary data have few patients with p16 positive tumours that were treated with unilateral technique (17-19). While some studies report similar survival with unilateral radiotherapy compared with bilateral treatment (17, 18, 20), other studies do not agree (11). Of note, the National Comprehensive Cancer Network guidelines have classified the issue of unilateral versus bilateral treatment as category 3 where "no general agreement can be made" (3). Prior attempts at a randomised controlled trial have failed due to poor accrual (20). One review has advocated that bilateral radiotherapy be used where there is a higher chance of midline tumours (21).

In our institution we have treated patients with unilateral radiotherapy for a number of decades in cases where there is ipsilateral neck involvement and no suspicious primary after standard investigation (14). Unilateral treatment has the potential benefit of decreased dose to the contralateral submandibular and parotid glands, and constrictor muscles and carotid artery. However, with the increasing prevalence of HPV oropharyngeal cancers (22), we wanted to investigate whether our practice of unilateral radiotherapy remained appropriate for those patients who had p16/HPV positive cervical neck squamous cell carcinomas.

Therefore, we sought to examine the outcomes of these patients to see if our rate of isolated contralateral neck relapse and emergence of a putative primary is comparable with series reporting the outcomes of bilateral neck radiotherapy where the rate of contralateral neck and primary relapse is generally below an incidence of 10% (1). We also examined the progression free and overall survival of our cohort.

## **Methods**

Patient data were reviewed retrospectively on a protocol approved by a human research ethics committee. All patients were staged using AJCC 7<sup>th</sup> Edition (22).

We included patients who were radically treated between 1st January 2004 to 31st December 2012, aged 18 years and above and had histologically proven SCC involving a cervical lymph node where a primary was not evident after imaging (CT, MRI and/or PET) and an examination under anaesthesia. Excluded were patients with parotid, isolated level IV or level V nodal involvement. These patients are likely to have primaries that are involving skin or other primaries such as lung or oesophagus, and thus different from our population with occult oropharyngeal primary. In the identified group of patients, those who had surgery alone or had bilateral neck radiotherapy were excluded. Patients with who had unilateral radiotherapy and were p16/HPV positive were included for analysis.

#### *HPV analysis*

As p16 was not routinely performed on tumour samples, all pathological specimens were recalled for re-testing. A repeat p16 status was tested using immunohistochemistry where there was an adequate sample. Testing for HPV was also performed on both p16 positive and negative cases using HPV RNA ISH testing. Our method for testing for p16 and HPV are outlined in a prior publication (23).

#### *Diagnostic work-up*

In our institution all patients with a SCCUP have examination under anaesthesia, diagnostic CT and PET scan. In general a palatine tonsillectomy or biopsy was recommended if there was tonsillar tissue still present. Directed biopsies of potential mucosal sites were performed according to the discretion of the surgeon and the clinical and radiological examination findings. In this series, tonsil biopsies or tonsillectomies were done in 71% (15/21) of cases (7 tonsillectomies and 8 tonsil biopsies) and 62% (13/21) had a biopsy of the base of tongue. Of the six cases who did not have a tonsil biopsy or tonsillectomy, one case had a record of prior tonsillectomy.

#### *Treatment*



All patients were discussed at our weekly multi-disciplinary meeting where the decision of surgical versus nonsurgical management was made. Patients had either a modified radical levels I/II to IV neck dissection (or excisional biopsy) with adjuvant (chemo)radiotherapy or upfront definitive chemo-radiotherapy. Adjuvant radiotherapy was recommended in patients who had stage N2a and above neck disease or nodal extracapsular extension. Chemotherapy was given concurrently in cases where extra-capsular extension was present. A salvage neck dissection was performed after definitive chemo-radiotherapy if there was an incomplete nodal metabolic response without distant metastasis on PET imaging three months after completion of treatment (there were no cases in our series).

Patients treated with radiotherapy were simulated with a thermoplastic mask in the supine position. Scars were wired if an operation was performed and contrast was given if patients were treated definitively in order to better delineate the gross nodal disease. In general, volumes encompassed the ipsilateral retro-pharyngeal lymph nodes and levels II to V. Where there was a large node in level IIa or if level Ib was involved, volumes would encompass level Ib as well. In one patient where there was an ipsilateral retropharyngeal lymph node involved, bilateral retropharyngeal lymph nodes were encompassed but the lower neck (levels III and IV) received unilateral radiotherapy. Candidate mucosal sites were not purposefully covered although typically the ipsilateral tonsil and lateral portion of the base of tongue received 50 to 60Gy, depending on the dosimetry achieved. Radiotherapy was delivered with either 3-dimensional conformal radiotherapy (3D-CRT) or intensity modulated radiotherapy (IMRT). Figure 1 shows indicative 3D-CRT and IMRT plans for patients who were treated with unilateral technique.

The standard regimen for patients treated definitively with chemo-radiation was cisplatin weeks 1, 4 and 7. If patients were unfit for cisplatin they received either carboplatin-5 FU combination, or carboplatin or cetuximab. Adjuvant chemotherapy was usually weekly cisplatin concurrently with radiation.

Patients were followed-up post completion of treatment with either a clinical examination if the gross nodal disease was resected or a PET-CT if definitive treatment was given. In cases where there was incomplete response to treatment, then a therapeutic neck dissection was performed. In general, follow up entailed 3-monthly reviews by an Ear, Nose and Throat surgeon or Radiation Oncologist for the first 2 years, 4-monthly for the 3<sup>rd</sup> year and 6 monthly until 5 years.

The primary endpoint of this study was the incidence of isolated contralateral nodal recurrence and emergence of a putative primary. Failure free and overall survival estimates were also calculated using the Kaplan-Meier method. Failure free survival was defined as the time from diagnosis until the first of local relapse, nodal relapse, contra-nodal relapse, distant metastasis or death. Overall survival was taken as the time of diagnosis until death from any cause.

## Results

Figure 2 summarises the selection of SCCUP patients for our study. During our study period we identified 49 patients with unknown primaries who were treated radically. Fourteen patients had bilateral neck radiotherapy (either definitively or as adjuvant treatment after neck dissection) for the following reasons: 8 patients had bilateral neck disease, 6 patients referred with unknown primaries and examination under anaesthesia (EUA) and biopsies did not reveal a primary but PET was suspicious for a primary in base of tongue and hence physician elected to treat bilaterally. Of the remaining 35 patients, two had surgery alone. One patient had N1 disease without extra-capsular extension while another patient had N2a disease but declined to have adjuvant radiotherapy. The remaining 33 patients who had unilateral radiotherapy had their pathology specimens recalled. The two patients who only had surgery without radiation also had their pathology samples recalled.

We were able to obtain a p16 status on 27/33 (81.8%) of patients (either on initial or subsequent retesting of pathology samples). The remainder of the patients had fine needle

aspirates which were inadequate for p16/HPV testing or had laboratory samples which could not be obtained. Of the 27 patients, 21/27 (77.8%) were p16 positive while 6/27 (22.2%) were p16 negative. These 27 cases were also tested for HPV RNA ISH. Among the p16 positive group, 16 tested positive to HPV RNA ISH. In the remaining 5 patients with p16 positive results, HPV testing failed due to poor quality RNA in the formalin fixed paraffin embedded samples. In the p16 negative group, all tested negative to HPV RNA ISH except one sample which failed testing. The two surgery alone cases were p16 and HPV RNA ISH positive.

Table 1 summarizes the demographic, tumour and treatment characteristics of the unilateral radiotherapy patient population. The population of interest of p16 positive patients treated with unilateral radiotherapy were followed for a median of 57 months (20 to 102 months). The neck nodal levels that were involved with disease were level II (20 patients), III (10 patients), level IV (2 patients) and RP nodes (1 patient). For the 12 patients with p16 positive N2b disease, there were 9 patients with  $\leq 4$  nodes involved, while 3 patients had  $>4$  nodes involved. This includes 2 patients with one nodal level, 7 with 2 nodal levels and 3 patients with 3 nodal levels involved with tumour.

During our follow up period, there were no isolated contralateral neck relapses. One case had an ipsilateral neck failure. This patient originally had N2b disease and was treated with surgery followed by chemo-radiotherapy. Unfortunately he relapsed in a retropharyngeal lymph node which was on the superior edge of the radiotherapy field 4 years after his original radiotherapy. Attempted salvage was made with re-irradiation but he subsequently developed distant metastasis. Other cases of relapse included one patient who had a concurrent relapse in the contralateral neck with distant metastasis and another who had distant metastasis alone. Table 2 summarizes the patterns of failure of this population and as comparison we also included patients where the p16 status was negative or unknown. The two surgery alone patients had occult primaries emerging at 2.8 and 3.3 years from the date of neck dissection (one in the ipsilateral base of tongue and one in the ipsilateral tonsil). The

only case of an occult primary emerging after radiotherapy was in a p16 negative patient who had an oral cavity (retromolar trigone) lesion that was not seen on initial clinical examination or imaging.

The 5 year failure free survival for the p16 positive unilateral radiotherapy population was 78% (CI: 56-100%). Overall survival was 90% (CI: 79-100%) at 5 years. Figure 3 and 4 demonstrates these survival curves.

Ten out of 21 patients (47.6%) needed a temporary feeding tube during treatment. The median duration of the feeding tube was 36 days. One patient had dysphagia after neck dissection which was further exacerbated by radiotherapy and he required long-term percutaneous enteral feeding. None of the remaining patients (20/21, 95%) were feeding tube dependent long term.

## Discussion

Our study suggests that selected patients who have a p16/HPV positive cervical lymph node where a primary cannot be found after thorough evaluation can be safely treated with unilateral radiotherapy. In our series where the median follow up was 57 months (range: 20-102), there were no isolated relapses in the contralateral neck or primary. One case had an ipsilateral neck failure where bilateral radiotherapy would not have altered the outcome. Another case had a concurrent contralateral neck failure and distant disease, where again bilateral radiotherapy would not have likely altered the outcomes. Our patients were evaluated by a multi-disciplinary team which included expert Head and Neck surgeons, radiation oncologists, radiologists, nuclear medicine physicians, and medical oncologists. All patients had PET imaging along with examination under anaesthesia. Patients who had a suspicious lesion in the base of tongue on examination or imaging were treated with bilateral neck radiotherapy despite negative biopsies. This treatment paradigm is in keeping with studies that have safely treated early oropharyngeal primaries with unilateral radiotherapy, which excluded known base of tongue involvement (24-26). Our series also excluded the

outcomes of patients with isolated level IV, V and parotid nodal disease as they are likely to represent a different disease entity.

The outcomes of patients with p16/HPV positive SCCUP who have unilateral radiotherapy are not well described in the literature. The overall survival of our population at 5 years (90%) compares favourably with recent publications on p16/HPV positive SCCUP patients where the overall survival is reported to be between 75 to 90 per cent at 2 to 5 years (17, 19, 27-30).

This figure also resembles the overall survival (85%) of T1N1-N2b (ICON-S stage I) p16 positive oropharyngeal carcinomas in a large validation cohort for the new staging system of oropharyngeal cancers (31). The overall survival of p16/HPV positive SCCUP patients are mostly better compared with p16 negative tumours, although this has not been universally found (27). As expected, the number of patients (around 20) with p16 positive SCCUP in these studies is small. In most instances radiotherapy treatment when given includes coverage of bilateral neck and comprehensive mucosal radiation of the pharyngeal axis. One study did include some patients who had unilateral radiotherapy and were p16/HPV positive, however their numbers were small (9 patients) and their outcomes were not reported separately from the general group (19).

There are a wide range of treatment philosophies for treating occult head and neck primaries. A number of studies advocate a large volume of irradiation to encompass possible occult primary disease in the pharyngeal axis and bilateral neck (32-36). Our results compare well with these studies despite having a smaller volume irradiated and likely resultant reduced toxicity. The results of our study are comparable with the outcomes of our previously published series with unilateral radiotherapy (14). They also compare well with series where comprehensive mucosal radiation was performed where the emergence rate of an occult primary is between 0 to 10% (1, 21, 37). The rate of contralateral neck failures reported in the literature is between 0 and 5% in most instances for bilateral neck radiotherapy (1, 21, 37).

Radiating a large volume of the pharyngeal axis to prevent a small putative primary being missed is fraught. In one study where the whole pharyngeal axis was radiated to 60Gy, 46% of patients required dilation for oesophageal stricture (36). Other series have reported a stricture rate of between 4% and 14% (33, 35). In our series we did not purposely radiate the pharyngeal axis and despite this, no occult primaries emerged in the p16 positive population. Furthermore, nearly all (95%) of our cases who were cured of their disease did not require long-term enteral feeding (the one case who did probably also had complications from his surgery). Unfortunately, we do not have good long term toxicity data as it was a retrospective review. However, it is likely that sparing the contralateral parotid gland, submandibular gland and pharyngeal axis with unilateral radiotherapy is likely to result in better outcomes for xerostomia and also long-term dysphagia.

Reports of worse outcomes as a result of unilateral radiotherapy need to be taken with caution (11, 12). Some older studies had inclusion criteria which were broad and included, for instance, patients with isolated supraclavicular nodes (11). These patients are much more likely to have a putative primary outside the head and neck, and hence likely to have uncontrolled disease in the oesophagus or thorax. Another study treated patients with an enface electron technique, which is unlikely to give adequate coverage to the deep tissues of the ipsilateral neck (12). Recent studies report no difference in the overall and recurrence free survival between unilateral and bilateral radiotherapy (17, 19).

The discovery of an occult primary for patients who initially present with a head and neck SCCUP has improved with diagnostic approaches such as PET imaging (38-40) and tonsillectomies (5, 41, 42). More recently, studies have shown that trans-oral resection of the lingual tonsil can increase the yield of investigations for unknown primaries (6-9, 43). It is known that p16 or HPV positive oropharyngeal cases often present with nodal disease, while the primary disease is asymptomatic and not easily detected on conventional imaging (44). It is thought that this is due to the primary emerging from deep in the basal cell layer of the crypts of the palatine or lingual tonsil with minimal mucosal changes. A recent review

also showed that the reported prevalence of p16 unknown primaries decreased in populations where random biopsies of the upper aero-digestive tract and tonsillectomies were performed (45). Presumably this is because many p16 positive occult primaries were discovered after these investigations.

The number of cases who had tonsillectomies (7/21, 33.3%) in our group was not high and no patients had a trans-oral resection of the base of tongue. Despite this, our unilateral treatment did not seem to have a detrimental impact on outcomes. We would speculate that PET imaging helped detect a number of cases who had possible base of tongue lesions that had FDG avidity (although not equivocally positive) and were excluded from unilateral treatment. Our other cases probably had small lesions harboured in the tonsillar fossa, the glosso-tonsillar sulcus or lateral base of tongue area which were sterilized by unilateral conformal radiotherapy. It is likely, however, that this area in most cases would have received at least somewhere between 50 and 60Gy in our conformal plans with the wash dose. The only case where a primary emerged after radiotherapy was a p16 negative case where there was an undetected oral cavity (retromolar trigone) primary. It is likely a combination of a low dose of radiotherapy and a more radio-resistant tumour in this area resulted in the primary being uncontrolled.

The majority (90%) of our cases were treated with conformal radiotherapy. In the era of IMRT with its inherent rapid falloff in dose, we consider that a PTV should be constructed to include the ipsilateral tonsil and ipsilateral base of tongue in a prophylactic dose for p16 positive CUPs. There is currently active research into treatment de-intensification for HPV/p16 positive oropharyngeal cancers; however the role of de-intensification by reduction in radiotherapy volumes tends to be over looked. Our results for unilateral treatment of lateralised tonsil cancers (24) and the results of this study suggest that unilateral RT would be safe, and clinically it is an effective way to reduce acute and late treatment toxicities.

A comparable experience of published unilateral treatment can be found in reports of unilateral radiotherapy for small (T1 and T2) known tonsillar SCCs as many, if not all, of our cases were likely to have small occult oropharyngeal primaries. A recent large series, which also contained a comprehensive review of published unilateral tonsil experience, showed that very few contralateral neck failures occurred in selected patients treated with unilateral radiotherapy (46). In their series primaries centred on the palatine tonsil which did not have greater than 1cm of soft palate or base of tongue involvement and up to N2b nodal staging were permitted to have unilateral treatment. The currently open NRG HN-002 (NCT02254278) de-escalation trial for p16 positive oropharyngeal SCC allowed for unilateral radiotherapy if only one nodal level was involved and lateralised tonsillar primaries. While it is not possible to know how lateralised the primaries were in our series, the treatment that we delivered for multiple nodal levels of involvement seemed safe. Unlike the published series or the NRG HN-002, our cohort allowed for much more extensive N2b disease yet isolated contralateral neck relapses did not occur.

We do advise caution is using p16 positivity alone to direct patient management. Our institution has published a series of patients with parotid SCC which are highly likely to have originated from a skin primary (23). We found that 31% of these cases were p16 positive but HPV negative. Another study had similar findings (47). In our study we only included patients where the pattern of nodal involvement was more likely from a pharyngeal axis primary (i.e. level II or III cervical neck involvement), all cases who were p16 positive were also HPV positive (although in a minority of cases (17.1%) the HPV RNA ISH testing failed on the samples provided). Furthermore, the two p16 and HPV positive cases who had surgery alone had relapses in the oropharynx. We would postulate from this that taking into account both the pattern of cervical nodal involvement and p16 status, and HPV status if possible, better determines the putative primary site.

We acknowledge that our study is a retrospective review with a small number of patients.

However, most published studies of unknown primary are small, with considerable



heterogeneity in the management paradigm. Prior attempts at randomised trials looking at the issues of unilateral versus bilateral radiotherapy have failed and it is unlikely that one will be successful in the near future. We therefore remain dependent upon institutional series to guide management. Our current series does suggest that unilateral treatment is safe and should be considered in a prospective study. While at present comprehensive mucosal and bilateral radiotherapy is a common treatment recommendation (1), we propose that unilateral radiotherapy may be considered for patients who have p16 positive SCCUP with N2a/b disease after a comprehensive diagnostic work up..

### iii. References

1. Galloway TJ, Ridge JA. Management of squamous cancer metastatic to cervical nodes with an unknown primary site. *Journal of Clinical Oncology*. 2015;JCO. 2015.61. 0063.
2. Miller FR, Karnad AB, Eng T, Hussey DH, Stan McGuff H, Otto RA. Management of the unknown primary carcinoma: long-term follow-up on a negative PET scan and negative panendoscopy. *Head & neck*. 2008;30(1):28-34.
3. Pfister DG, Ang K-K, Brizel DM, Burtress BA, Busse PM, Caudell JJ, et al. Head and neck cancers, version 2.2013. *Journal of the National Comprehensive Cancer Network*. 2013;11(8):917-23.
4. Kim SH, Koo BS, Kang S, Park K, Kim H, Lee KR, et al. HPV integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR and c - myc during tumor formation. *International Journal of Cancer*. 2007;120(7):1418-25.
5. Mcquone SJ, Eisele DW, Lee DJ, Westra WH, Koch WM. Occult tonsillar carcinoma in the unknown primary. *The Laryngoscope*. 1998;108(11):1605-10.
6. Mehta V, Johnson P, Tassler A, Kim S, Ferris RL, Nance M, et al. A new paradigm for the diagnosis and management of unknown primary tumors of the head and neck: a role for transoral robotic surgery. *The Laryngoscope*. 2013;123(1):146-51.
7. Abuzeid WM, Bradford CR, Divi V. Transoral robotic biopsy of the tongue base: a novel paradigm in the evaluation of unknown primary tumors of the head and neck. *Head & neck*. 2013;35(4):E126-E30.
8. Nagel TH, Hinni ML, Hayden RE, Lott DG. Transoral laser microsurgery for the unknown primary: role for lingual tonsillectomy. *Head & neck*. 2014;36(7):942-6.
9. Durmus K, Rangarajan SV, Old MO, Agrawal A, Teknos TN, Ozer E. Transoral robotic approach to carcinoma of unknown primary. *Head & neck*. 2014;36(6):848-52.
10. SIVARS L, TANI E, NÄSMAN A, RAMQVIST T, MUNCK-WIKLAND E, DALIANIS T. Human Papillomavirus as a Diagnostic and Prognostic Tool in Cancer of Unknown Primary in the Head and Neck Region. *Anticancer research*. 2016;36(2):487-93.
11. Grau C, Johansen LV, Jakobsen J, Geertsen P, Andersen E, Jensen BB. Cervical lymph node metastases from unknown primary tumours. Results from a national survey by the Danish Society for Head and Neck Oncology. *Radiother Oncol*. 2000;55(2):121-9.
12. Reddy SP, Marks JE. Metastatic carcinoma in the cervical lymph nodes from an unknown primary site: results of bilateral neck plus mucosal irradiation vs. ipsilateral neck irradiation. *International journal of radiation oncology, biology, physics*. 1997;37(4):797-802.
13. Marcial-Vega VA, Cardenes H, Perez CA, Devineni VR, Simpson JR, Fredrickson JM, et al. Cervical metastases from unknown primaries: radiotherapeutic management and appearance of subsequent primaries. *International journal of radiation oncology, biology, physics*. 1990;19(4):919-28.
14. Sinnathamby K, Peters L, Laidlaw C, Hughes P. The occult head and neck primary: to treat or not to treat? *Clinical Oncology*. 1997;9(5):322-9.
15. Glynne-Jones RG, Anand AK, Young TE, Berry RJ. Metastatic carcinoma in the cervical lymph nodes from an occult primary: a conservative approach to the role

- of radiotherapy. *International Journal of Radiation Oncology\* Biology\* Physics*. 1990;18(2):289-94.
16. Weir L, Keane T, Cummings B, Goodman P, O'Sullivan B, Payne D, et al. Radiation treatment of cervical lymph node metastases from an unknown primary: an analysis of outcome by treatment volume and other prognostic factors. *Radiother Oncol*. 1995;35(3):206-11.
  17. Straetmans J, Vent J, Lacko M, Speel E-J, Huebbers C, Semrau R, et al. Management of neck metastases of unknown primary origin united in two European centers. *European Archives of Oto-Rhino-Laryngology*. 2015;272(1):195-205.
  18. Fakhrian K, Thamm R, Knapp S, Molls M, Pigorsch S, Haller B, et al. Radio (chemo) therapy in the management of squamous cell carcinoma of cervical lymph nodes from an unknown primary site. *Strahlentherapie und Onkologie*. 2012;188(1):56-61.
  19. Perkins SM, Spencer CR, Chernock RD, Haughey BH, Nussenbaum B, Adkins DR, et al. Radiotherapeutic management of cervical lymph node metastases from an unknown primary site. *Archives of Otolaryngology–Head & Neck Surgery*. 2012;138(7):656-61.
  20. Lige A, Gentil J, Crehange G, Montbarbon X, Pommier P, Peignaux K, et al. Impact of target volumes and radiation technique on loco-regional control and survival for patients with unilateral cervical lymph node metastases from an unknown primary. *Radiother Oncol*. 2009;93(3):483-7.
  21. Strojan P, Ferlito A, Medina JE, Woolgar JA, Rinaldo A, Robbins KT, et al. Contemporary management of lymph node metastases from an unknown primary to the neck: I. A review of diagnostic approaches. *Head & neck*. 2013;35(1):123-32.
  22. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Annals of surgical oncology*. 2010;17(6):1471-4.
  23. McDowell LJ, Young RJ, Johnston ML, Tan TJ, Kleid S, Liu CS, et al. p16 - positive lymph node metastases from cutaneous head and neck squamous cell carcinoma: No association with high - risk human papillomavirus or prognosis and implications for the workup of the unknown primary. *Cancer*. 2016.
  24. Liu C, Dutu G, Peters LJ, Rischin D, Corry J. Tonsillar cancer: the Peter MacCallum experience with unilateral and bilateral irradiation. *Head & neck*. 2014;36(3):317-22.
  25. Chronowski GM, Garden AS, Morrison WH, Frank SJ, Schwartz DL, Shah SJ, et al. Unilateral radiotherapy for the treatment of tonsil cancer. *International Journal of Radiation Oncology\* Biology\* Physics*. 2012;83(1):204-9.
  26. O'sullivan B, Warde P, Grice B, Goh C, Payne D, Liu F-F, et al. The benefits and pitfalls of ipsilateral radiotherapy in carcinoma of the tonsillar region. *International Journal of Radiation Oncology\* Biology\* Physics*. 2001;51(2):332-43.
  27. Tribius S, Hoffmann AS, Bastrop S, Görögh T, Haag J, Röcken C, et al. HPV status in patients with head and neck of carcinoma of unknown primary site: HPV, tobacco smoking, and outcome. *Oral oncology*. 2012;48(11):1178-84.
  28. Keller LM, Galloway TJ, Holdbrook T, Ruth K, Yang D, Dubyk C, et al. p16 status, pathologic and clinical characteristics, biomolecular signature, and long - term outcomes in head and neck squamous cell carcinomas of unknown primary. *Head & neck*. 2014;36(12):1677-84.
  29. Demiroz C, Vainshtein JM, Koukourakis GV, Gutfeld O, Prince ME, Bradford CR, et al. Head and neck squamous cell carcinoma of unknown primary: neck

dissection and radiotherapy or definitive radiotherapy. *Head & neck*.

2014;36(11):1589-95.

30. Sivars L, Näsman A, Tertipis N, Vlastos A, Ramqvist T, Dalianis T, et al. Human papillomavirus and p53 expression in cancer of unknown primary in the head and neck region in relation to clinical outcome. *Cancer medicine*. 2014;3(2):376-84.

31. O'Sullivan B, Huang SH, Su J, Garden AS, Sturgis EM, Dahlstrom K, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. *Lancet Oncol*. 2016;17(4):440-51.

32. Chen AM, Li BQ, Farwell DG, Marsano J, Vijayakumar S, Purdy JA. Improved dosimetric and clinical outcomes with intensity-modulated radiotherapy for head-and-neck cancer of unknown primary origin. *International journal of radiation oncology, biology, physics*. 2011;79(3):756-62.

33. Frank SJ, Rosenthal DI, Petsuksiri J, Ang KK, Morrison WH, Weber RS, et al. Intensity-modulated radiotherapy for cervical node squamous cell carcinoma metastases from unknown head-and-neck primary site: M. D. Anderson Cancer Center outcomes and patterns of failure. *International journal of radiation oncology, biology, physics*. 2010;78(4):1005-10.

34. Colletier PJ, Garden AS, Morrison WH, Goepfert H, Geara F, Ang KK. Postoperative radiation for squamous cell carcinoma metastatic to cervical lymph nodes from an unknown primary site: outcomes and patterns of failure. *Head & neck*. 1998;20(8):674-81.

35. Klem ML, Mechalakos JG, Wolden SL, Zelefsky MJ, Singh B, Kraus D, et al. Intensity-modulated radiotherapy for head and neck cancer of unknown primary: toxicity and preliminary efficacy. *International journal of radiation oncology, biology, physics*. 2008;70(4):1100-7.

36. Sher DJ, Balboni TA, Haddad RI, Norris CM, Jr., Posner MR, Wirth LJ, et al. Efficacy and toxicity of chemoradiotherapy using intensity-modulated radiotherapy for unknown primary of head and neck. *International journal of radiation oncology, biology, physics*. 2011;80(5):1405-11.

37. Jereczek-Fossa BA, Jassem J, Orecchia R. Cervical lymph node metastases of squamous cell carcinoma from an unknown primary. *Cancer treatment reviews*. 2004;30(2):153-64.

38. Johansen J, Buus S, Loft A, Keiding S, Overgaard M, Hansen HS, et al. Prospective study of 18FDG - PET in the detection and management of patients with lymph node metastases to the neck from an unknown primary tumor. Results from the DAHANCA - 13 study. *Head & neck*. 2008;30(4):471-8.

39. Rudmik L, Lau HY, Matthews TW, Bosch JD, Kloiber R, Molnar CP, et al. Clinical utility of PET/CT in the evaluation of head and neck squamous cell carcinoma with an unknown primary: a prospective clinical trial. *Head & neck*. 2011;33(7):935-40.

40. Fogarty GB, Peters LJ, Stewart J, Scott C, Rischin D, Hicks RJ. The usefulness of fluorine 18-labelled deoxyglucose positron emission tomography in the investigation of patients with cervical lymphadenopathy from an unknown primary tumor. *Head & neck*. 2003;25(2):138-45.

41. Waltonen JD, Ozer E, Schuller DE, Agrawal A. Tonsillectomy vs. deep tonsil biopsies in detecting occult tonsil tumors. *The Laryngoscope*. 2009;119(1):102-6.

42. Randall DA, Johnstone PA, Foss RD, Martin PJ. Tonsillectomy in diagnosis of the unknown primary tumor of the head and neck. *Otolaryngology--Head and Neck Surgery*. 2000;122(1):52-5.

43. Patel SA, Magnuson JS, Holsinger FC, Karni RJ, Richmon JD, Gross ND, et al. Robotic surgery for primary head and neck squamous cell carcinoma of unknown site. *JAMA Otolaryngol Head Neck Surg.* 2013;139(11):1203-11.
44. Lam M, O'Sullivan B, Gullane P, Huang SH. Challenges in establishing the diagnosis of human papillomavirus - related oropharyngeal carcinoma. *The Laryngoscope.* 2016;126(10):2270-5.
45. Boscolo-Rizzo P, Schroeder L, Romeo S, Pawlita M. The prevalence of human papillomavirus in squamous cell carcinoma of unknown primary site metastatic to neck lymph nodes: a systematic review. *Clinical & Experimental Metastasis.* 2015;32(8):835-45.
46. Huang SH, Waldron J, Bratman SV, Su J, Kim J, Bayley A, et al. Re-evaluation of Ipsilateral Radiation for T1-T2N0-N2b Tonsil Carcinoma at the Princess Margaret Hospital in the Human Papillomavirus Era, 25 Years Later. *International Journal of Radiation Oncology\* Biology\* Physics.* 2017;98(1):159-69.
47. Beadle BM, William WN, Jr., McLemore MS, Sturgis EM, Williams MD. p16 Expression in cutaneous squamous carcinomas with neck metastases: A potential pitfall in identifying unknown primaries of the head and neck. *Head & neck.* 2013;35(11):1527-33.

**Figure Legends**

Figure 1. Example 3D-conformal (A) and IMRT (B) plans of patients who were treated with unilateral radiotherapy for SCCUP (shaded areas show PTVs).

Figure 2. Population of patients treated for squamous cell carcinoma of unknown primary (SSCUP). \*Excludes cases with parotid, isolated level IV or level V nodal involvement.

Figure 3. Kaplan-Meier curve for Failure Free Survival for patients treated with unilateral radiotherapy for p16 positive SCCUP

Figure 4. Kaplan-Meier curve for Overall Survival for patients treated with unilateral radiotherapy for p16 positive SCCUP

**Tables**

Table 1. Characteristics of patients with SCCUP treated with unilateral radiotherapy.

| Variable     |                    | p16 positive<br>(N=21) | p16 negative,<br>(N=6) | p16 unknown,<br>(N=6) |
|--------------|--------------------|------------------------|------------------------|-----------------------|
| Age          | Median (range)     | 53 years (42-75)       | 65 (52-80)             | 56 (54-74)            |
| Gender       | Female             | 3 (14%)                | 0 (0%)                 | 0 (0%)                |
|              | Male               | 18 (86%)               | 6 (100%)               | 6 (100%)              |
| N stage      | N1                 | 1 (5%)                 | 1 (17%)                | 1 (17%)               |
|              | N2a                | 8 (38%)                | 0                      | 1 (17%)               |
|              | N2b                | 12 (57%)               | 2 (33%)                | 2 (33%)               |
|              | N3                 | 0 (0%)                 | 3 (50%)                | 2 (33%)               |
| ECE          | No                 | 11 (69%)               | 3 (50%)                | 1 (17%)               |
|              | Yes                | 5 (31%)                | 2 (33%)                | 2 (33%)               |
|              | No neck dissection | 5 (31%)                | 1 (17%)                | 3 (50%)               |
| Smoking Pack | 10 or less         | 15 (71%)               | 1 (17%)                | 3 (50%)               |
|              | More than 10       | 4 (19%)                | 5 (83%)                | 2 (33%)               |
|              | Missing            | 2 (10%)                | 0 (0%)                 | 1 (17%)               |
| Overall      | Definitive CRT     | 3 (14%)                | 1 (17%)                | 3 (50%)               |
|              | ND and RT          | 4 (19%)                | 3 (50%)                | 1 (17%)               |
|              | ND and CRT         | 13 (62%)               | 2 (33%)                | 2 (33%)               |
|              | EB and CRT         | 1 (5%)                 | 0 (0%)                 | 0 (0%)                |
| Radiotherapy | Conformal          | 19 (90%)               | 6 (100%)               | 6 (100%)              |
|              | IMRT               | 2 (10%)                | 0 (0%)                 | 0 (0%)                |

CRT=chemo-radiotherapy, ND=modified radical neck dissection, RT=radiotherapy,  
EB=excisional biopsy, IMRT=intensity modulated radiotherapy

Table 2. Patterns of failure of first failure for patients treated with unilateral radiotherapy

| Pattern of failure                | p16 positive<br>unilateral<br>radiotherapy<br>(N=21) | p16 negative<br>(N=6) | p16 unknown<br>(N=6) |
|-----------------------------------|------------------------------------------------------|-----------------------|----------------------|
| Primary                           | 0                                                    | 1 (16.7%)             | 0                    |
| Ipsilateral neck                  | 1 (4.3%)                                             | 0                     | 0                    |
| Contralateral neck                | 0                                                    | 1 (16.7%)             | 0                    |
| Contralateral neck<br>and distant | 1 (4.3%)                                             | 1 (16.7%)             | 1 (16.7%)            |
| Distant metastasis<br>only        | 1 (4.3%)                                             | 0                     | 1 (16.7%)            |



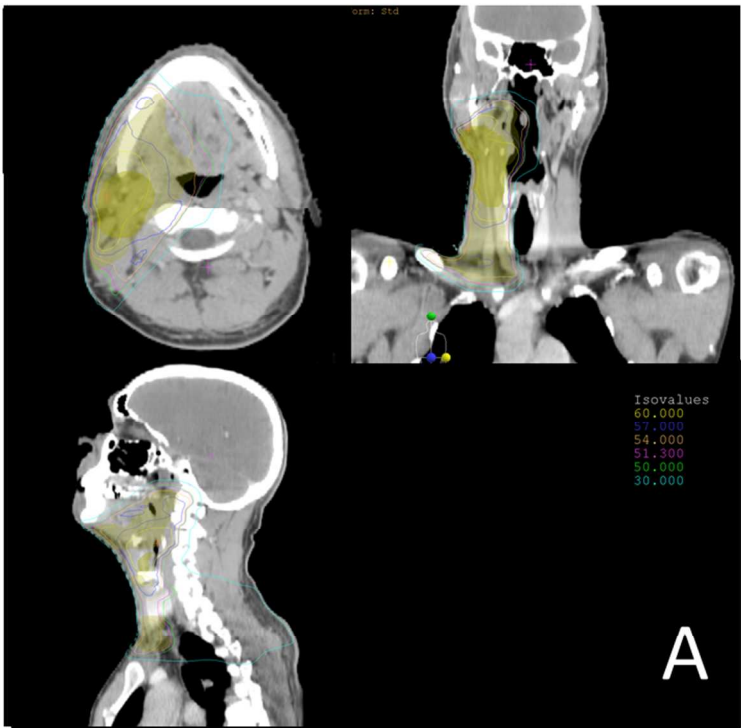


Figure 1. Example 3D-conformal (A) and IMRT (B) plans of patients who were treated with unilateral radiotherapy for SCCUP (shaded areas show PTVs).

254x190mm (96 x 96 DPI)

Author

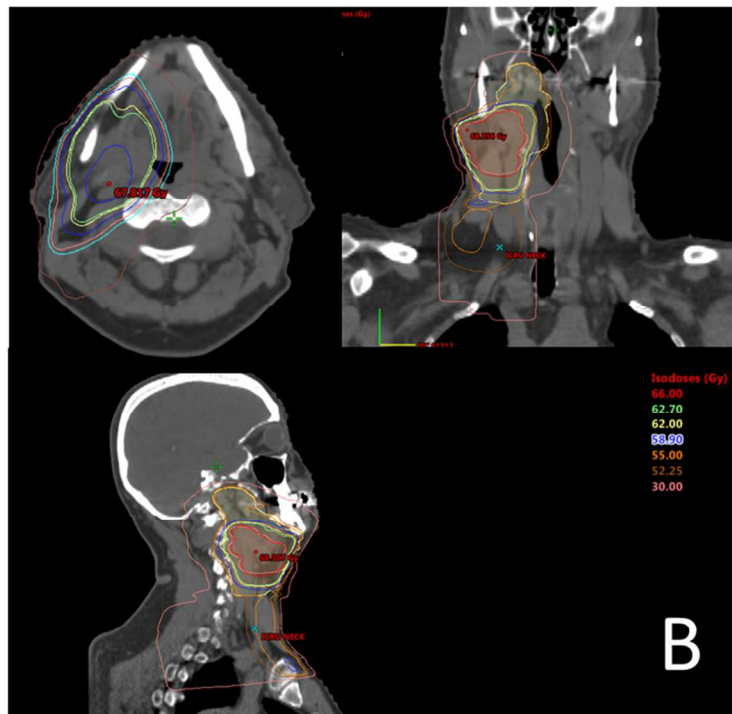


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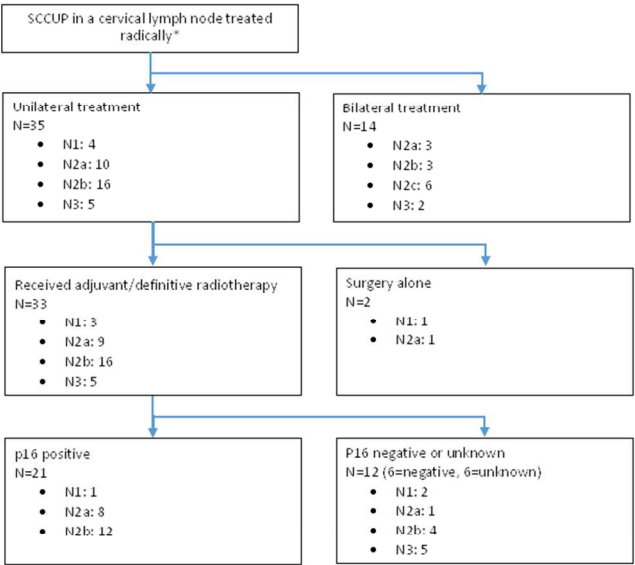


Figure 2. Population of patients treated for squamous cell carcinoma of unknown primary (SSCUP).  
\*Excludes cases with parotid, isolated level IV or level V nodal involvement.

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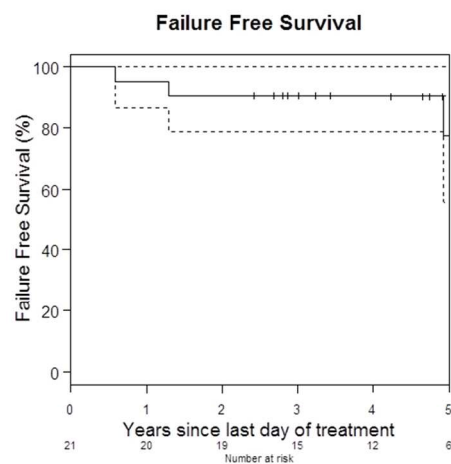


Figure 3. Kaplan-Meier curve for Failure Free Survival for patients treated with unilateral radiotherapy for p16 positive SCCUP

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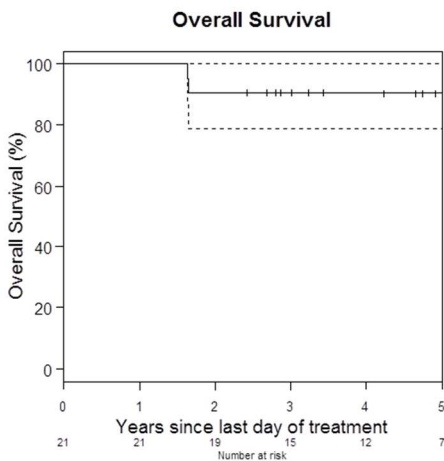


Figure 4. Kaplan-Meier curve for Overall Survival for patients treated with unilateral radiotherapy for p16 positive SCCUP

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