



**HKU, University of Toronto and  
Queen Elizabeth Hospital  
joint study on  
a trans-oral NP brush biopsy  
for early detection of nasopharyngeal cancer**

**Press Conference  
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# Speakers

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# Background on NPC

- Nasopharyngeal carcinoma (NPC, also known as “Guangdong” cancer) is common in southern China, Southeast Asia, the Arctic, North Africa and the Middle East
- Millions of individuals from southern China, Hong Kong as well as those of Chinese ancestry whose families have immigrated to other cities around the globe are at higher risk e.g. Toronto, Vancouver, Los Angeles, San Francisco, New York, Sydney



# Background on NPC

- NPC is more common in male, the male to female ratio is 3:2
- Patient age range: 15 to 90
- Peak age: 35 to 55
- Other risk factors include: increased age, certain ethnicities (e.g. southern Chinese)
- NPC is the 7<sup>th</sup> most prevalent cancer in Hong Kong, There are 862 new cases in 2011<sup>1</sup>

<sup>1</sup> Hong Kong Cancer Registry 2011



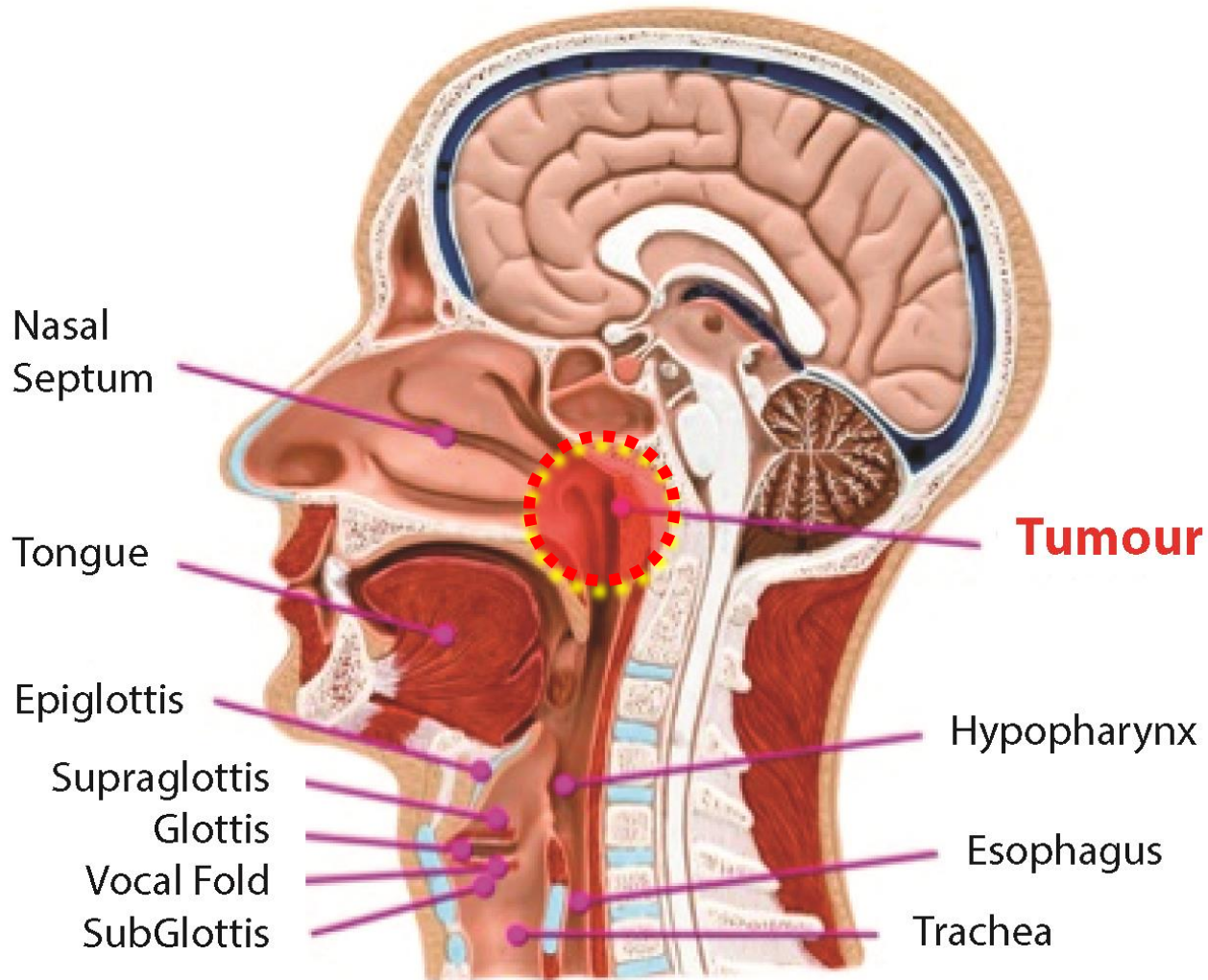
# Background on NPC: Causes

- **Genetic**
  - Strong familial predisposition
- **Environmental**
  - Consumption of salted fish and other preserved foods containing elevated levels of nitrate/nitrosamines
  - Reduced fruit and vegetable consumption
- Cigarette smoking, betel nut chewing, alcohol consumption, and occupational exposures<sup>1</sup>
- **Infection with Epstein Barr Virus (EBV, a human herpesvirus)**
  - Infection is believed to be necessary, but not sufficient for developing NPC

<sup>1</sup> Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2006;15:1765–1777.



# Background on NPC





# The Challenges of Diagnosis

- Early NPC is inconspicuous with minimal symptoms
- Nasopharynx is not easily accessible and therefore, routine detection of NPC is difficult without special equipment such as endoscope
- Endoscopic examination is subjective and some NPC are not visible even with endoscope
- Most patients present late stage with advanced disease
- Early stage is highly curable by radiation therapy, but it is rather difficult to treat at the late stage



# Background on NPC: Epstein Barr Virus

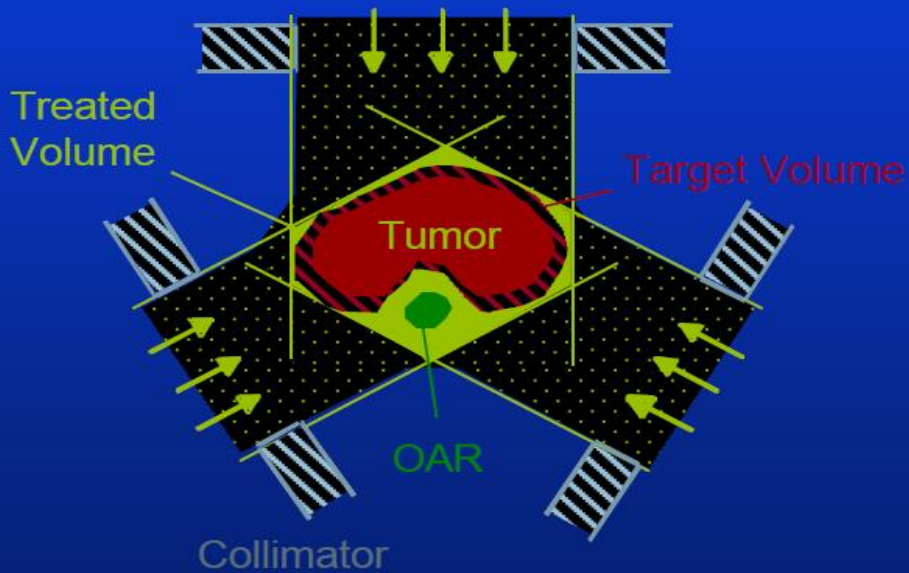
- EBV found in the earliest stage of the development of NPC (pre-neoplastic or stage 0 carcinoma-in-situ)
- Patients with NPC have EBV DNA detectable in their tumor cells
- Presence of EBV DNA in NP epithelial cells strongly supports NPC diagnosis



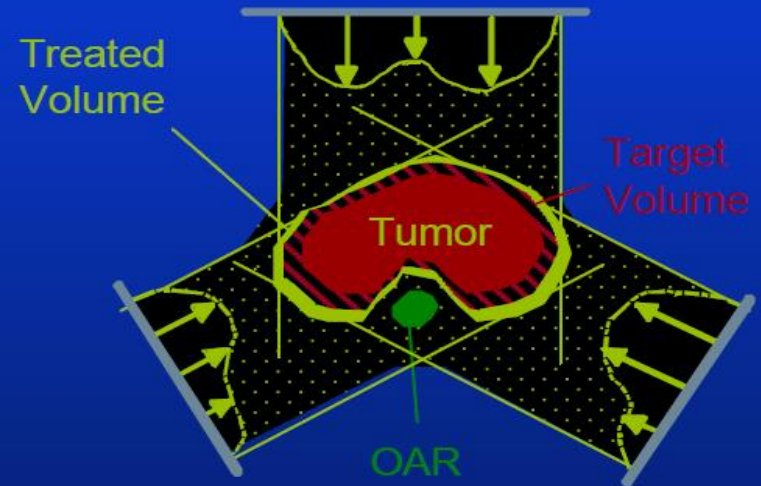


# IMRT Principles

## "Classical" Conformation



## Intensity Modulation





# Current Treatment

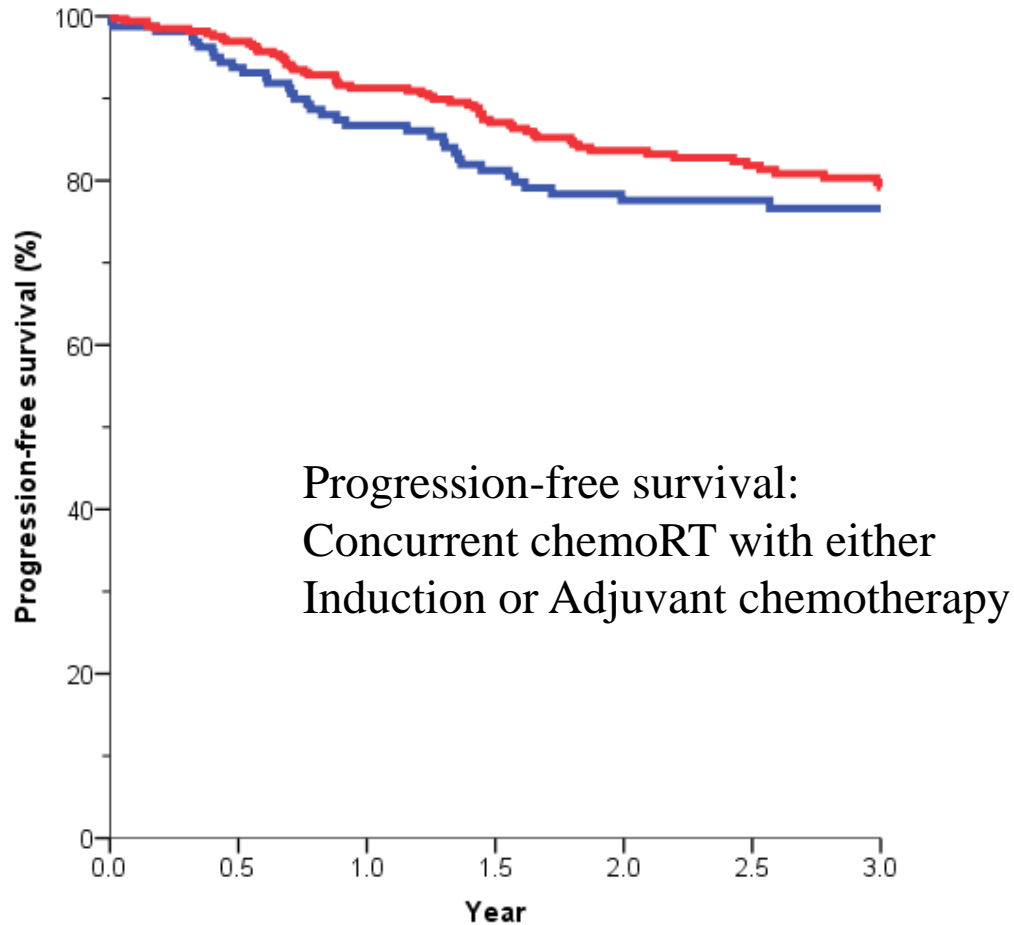
- Early stages (stage I & II)
  - radiotherapy (IMRT – intensity modulated radiotherapy) alone
- Late stages (stages III & IV)
  - radiotherapy plus concurrent chemotherapy  $\pm$  additional chemotherapy
- Results: 3 year progression-free survival
  - > 95% in early stages<sup>1</sup>
  - ~ 80% in late stages<sup>2</sup>

<sup>1</sup>Su SF et al. Int J Radiat Oncol Biol Phys. 2012;82(1):327-33

<sup>2</sup>Lee A et al. ECCO 2013



# HKNPCSG 0501 Study Stages III & IV





# Introduction: Ten Year Collaborative Project

- From year 2001 to 2010, HKU, University of Toronto and the Queen Elizabeth Hospital jointly developed a trans-oral brush and method to access and biopsy of the Nasopharynx (NP), for subsequent retrieval of the epithelial cells for EBV DNA quantitation



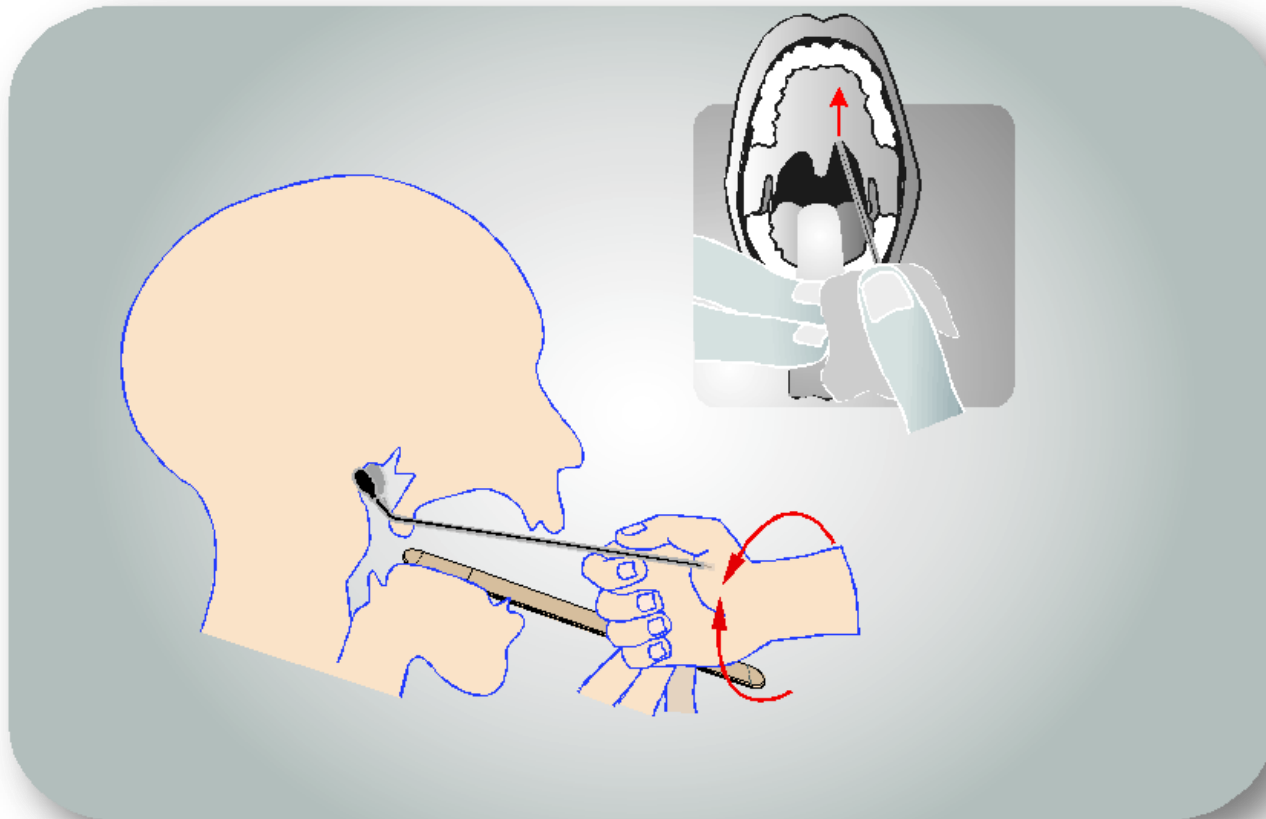
# Introduction: Objectives of This Study

- Develop an ambulatory mass screening tool for early detection of NPC, using an EBV–DNA based testing system
- An non-invasive test that can be performed easily by medical professionals or trained medical personnel without need for special tools
- Well tolerated in outpatient settings



# Introduction: Trans-oral Approach

- For the first time to utilise trans-oral approach to brush NP



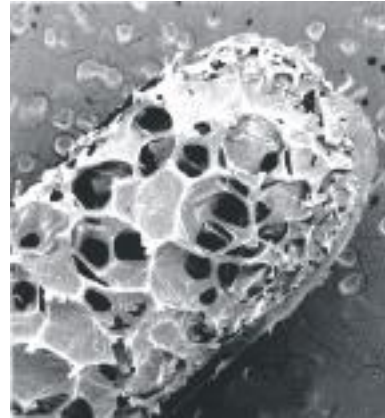


# Introduction: Trans-oral Approach

## ■ Innovative Angled Brush and Tip Design



- Electron micrographs of brush surface
- Intact epithelial cell samples



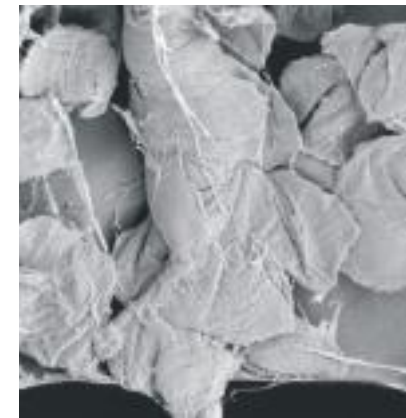
Before



After



150X

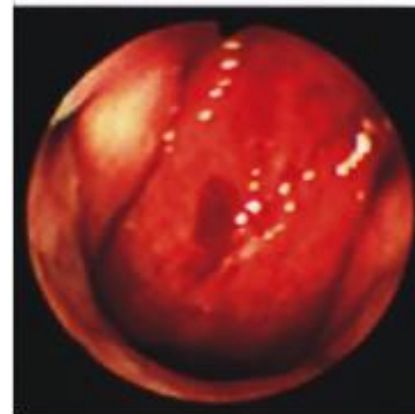
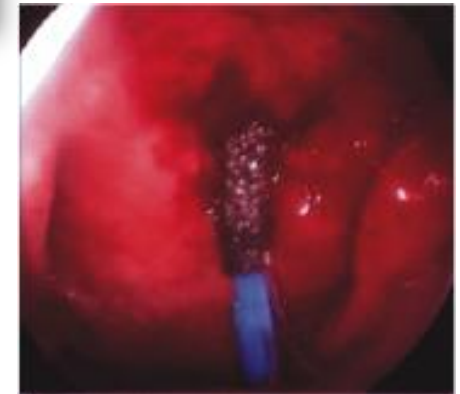
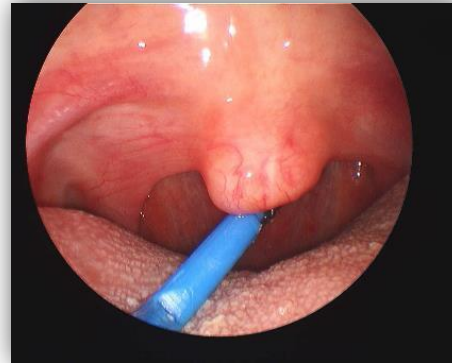


600X



# Introduction: Access Route to Nasopharynx

- Oral access to Nasopharynx
- Brushing location in the Nasopharynx
- Post-brushing mucosal surface of Nasopharynx







# Introduction: Preservation of Samples

- With the solution developed by our research team, DNA is stable up to 30 days without refrigeration
- Allows sample collection and screening in remote locations, e.g. Guangxi, Yunnan of China, and Nunavut of Canada





# Clinical Trial in Toronto and Hong Kong



# Clinical Trial

- Clinical trial period: 2001 to 2010
- Total 600 subjects are recruited from Toronto, Canada and Hong Kong, China
- Inclusion: patients who have confirmed NPC before radiation; high risk individuals from Hong Kong or immigrants in Canada, or those with family history of NPC
- Exclusion: immunosuppressed, or treated NPC patients



# Study Method

- Using a specially designed angled brush to enter the NP, to collect fresh epithelial/pre-neoplastic/tumor cells for testing
- Amplify the DNA using Quantitative Polymerase Chain Reaction (Q-PCR) technology , and then determine the presence and quantity of EBV DNA from the genome of the epithelial cells
- Find the correlation between the EBV DNA detection levels with the presence or absence of NPC
- Confirmed NPC by biopsy and pathologic diagnosis
- Two years follow-up period post testing





# Clinical Trial: Result

- Brushing biopsy retrieve samples from the NP identical to the more invasive forcep biopsy method
- Without the need of general anesthesia, surgery time and recovery period of patient
- Brush biopsy result is comparable to forcep biopsy



# Clinical Trial: Result

- 578 subjects completed the clinical trial
  - 263 females and 315 males
- Brushing may identify **submucosal disease**
  - 5 patients who had negative endoscopy, but brushed positive had confirmed NPC
- Brushing may identify **small primary tumors**
  - 1 patient with positive brushing, negative endoscopy and negative biopsy, was found to have advanced disease with lymph node metastases



# Clinical Trial: Result

- Brushing may identify **very early disease**
  - 1 patient with negative endoscopy and negative biopsy developed the disease one year after the positive brushing
- Detection of EBV DNA in patients in pre-neoplastic or Stage 0 disease (Carcinoma-in-situ)



# Early Stage Detection and Comparison with Endoscopy

## Comparison of Endoscopy and Trans –oral NP Brush Biopsy<sup>1</sup>

	Naso-Endoscopy		Brush Biopsy	
	TP	FN	TP	FN
T1	12	<b>3</b>	15	0
T2	32	<b>0</b>	31	1
T3	9	<b>1</b>	10	0
T4	10	<b>0</b>	10	0

Abbreviations: TP, true positive; FN, false negative.

<sup>1</sup> Subset of all NPC confirmed patients with tumor staging available

- Brush biopsy method identified four cases of false negative from endoscopy
- Brush biopsy method has 1 false negative from early part of the study





# Comparison with MRI and Endoscopy

# Tumor Development

Pre-Neoplastic

**TRANS-ORAL NP BRUSH BIOPSY**

**MRI**

**ENDOSCOPY**

**Brushing Sample Required  
= 5000 cells**

**0.25 cm<sup>3</sup> =  
25 Millions cells**

**1 cm<sup>3</sup> =  
100 Millions cells**





# Clinical Trial: Result

<b>Sensitivity<sup>1</sup></b>	<b>98.9%</b>
Specificity <sup>2</sup>	99.3%

<sup>1</sup>True Positive Rate = positive case/(true positive + false negative)

<sup>2</sup>True Negative Rate = negative case/(true negative + false positive)



# Summary

- This study is the first to describe a trans-oral NP brush biopsy method
- It is safe, well tolerated by patients, easy to perform and non invasive
- EBV DNA collection in real-time from live and growing tumor cells
- The detection of EBV DNA from NP accurately correlates with the presence or absence of NPC, accuracy better than all existing tests available



# Summary

- Q-PCR method provides an objective measurement that indicates the presence or absence of early cancer, as well as submucosal tumors, that may not be visible on routine endoscopy
- Substantial improvement in accuracy by elimination of human subjectivity
- The sample preservation system and associated protocol is simple, routine, large-scale screening method for individuals at risk of developing NPC globally



# Conclusion

- Trans-oral NP brushing in combination with Q-PCR EBV DNA testing, is a simple, accurate, and non-invasive method for large scale mass screening of NPC, which is applicable globally in both developed and less-developed medical settings



# Further Clinical Studies

- Further clinical studies ongoing
  - Testing post-radiation patients for detection of early local recurrence
  - Testing high risk patients with persistent positive serology



# To Monitor for Local Recurrence

- NP may harbor persistent disease after radiotherapy or chemotherapy
- There may be local recurrence after an initial remission of disease
- The gold standard investigations for confirming local persistent or recurrent disease is with NP endoscopy and biopsy. However, these are invasive procedures and uncomfortable. They also need expertise for performing these procedures
- Serum EBV DNA is useful in detection of distant metastases but is not very sensitive in detecting small volume local disease
- Trans-oral NP brush biopsy may be a useful non-invasive tool in post-treatment monitoring of local recurrence





# To Monitor for Local Recurrence

- We started a prospective pilot study on testing out the sensitivity and specificity of trans-oral NP brush biopsy in detecting early local recurrence in NPC patients after treatment
- Follow-up scheme:

Time	Tests performed
Before treatment	Trans-oral NP brush biopsy, NP biopsies, serum EBV DNA
Post-radiotherapy 10 weeks	Trans-oral NP brush biopsy, NP biopsies, serum EBV DNA
Every 3 months post-radiotherapy for 2 years	Trans-oral NP brush biopsy, serum EBV DNA, clinical follow up
At suspicion of NP recurrence	Trans-oral NP brush biopsy, NP biopsies, serum EBV DNA

- The results of trans-oral NP brush biopsy will be correlated with NP biopsies and clinical findings and compared with serum EBV DNA
- Recruitment is in progress



# Testing High Risk Individuals

- EBV IgA antibody is a tumour marker for screening of NPC
- Raised EBV IgA antibodies may indicate presence of NPC
- Even if no NPC, there is a quadruple risk of developing NPC subsequently

Chien, Y.-C. et. al. (2001). Serologic markers of Epstein–Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *New England Journal of Medicine*, 345(26), 1877–1882.



# Testing High Risk Individuals

- Further clinical studies planned
  - Collaborative study with Singapore Hospitals, and The University of Toronto
  - Use trans-oral NP brush biopsy for follow up of high risk individuals with persistently positive EBV antibody serology
  - Compare with conventional routine endoscopy and biopsy



# Q & A



# Brushing Demonstration

**Brushing doctor: Dr Raymond Ng**

**Narrator: Prof William I Wei**