Nasopharyngeal Carcinoma – Molecular Biology & Biomarkers

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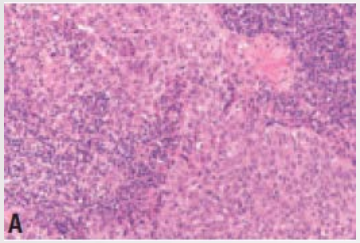
DISCLOSURES

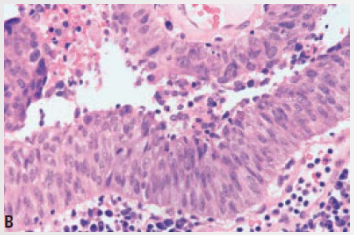
For Brigette Ma

- Novartis – Research grant, advisory board
- MSD, BMS – speaker tour, advisory board
- Merck Serono – Research grant, advisory board
- Boerhinger Ingelheim – advisory board
- Roche – speaker tour

Histological classification of NPC

Non-keratininzing, differentiated subtype.



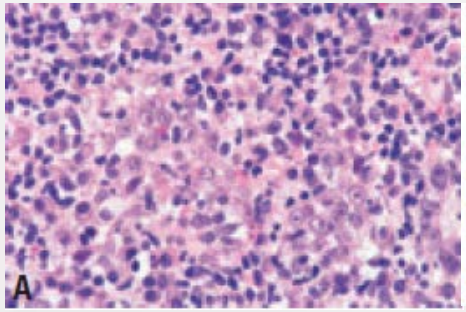


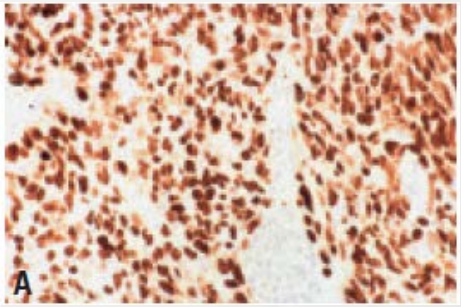
Papillary features

Table 2.04 Frequency of histological subtypes of nasopharyngeal carcinoma.

Current WHO classification	High incidence population		Intermediate incidence	Low incidence population	
	Hong Kong*	Singapore {2318}	Tunisia {323}	Japan {2497}	U.S.A. {2735}
Keratinizing squamous cell carcinoma	1%	17%	8%	13%	25%
Nonkeratinizing carcinoma	99%	83%	92%	87%	75%
- Undifferentiated	92%	42%	76%		
- Differentiated	7%	41%	16%		
Basaloid squamous cell carcinoma	<0.2%	NA	NA	NA	NA

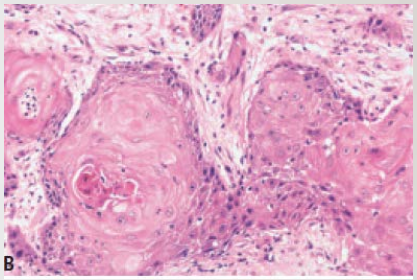
NA = Not available; *Queen Elizabeth Hospital, 2001-2003





Non-keratinizing undifferentiated subtype, EBER+ve

Keratinizing squamous subtype



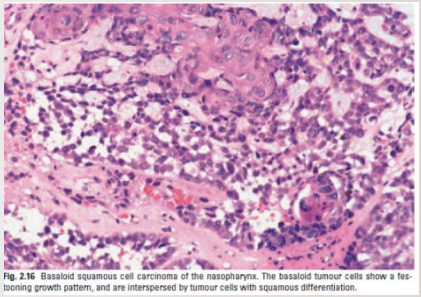


Fig. 2.16 Basaloid squamous cell carcinoma of the nasopharynx. The basaloid tumour cells show a festooning growth pattern, and are interspersed by tumour cells with squamous differentiation.

Basaloid squamous subtype

Tumours of the nasopharynx, JKC Chan, Chapter 2

https://www.iarc.fr/en/publications/pdfs-online/pat-gen/bb9/bb9-chap2.pdf

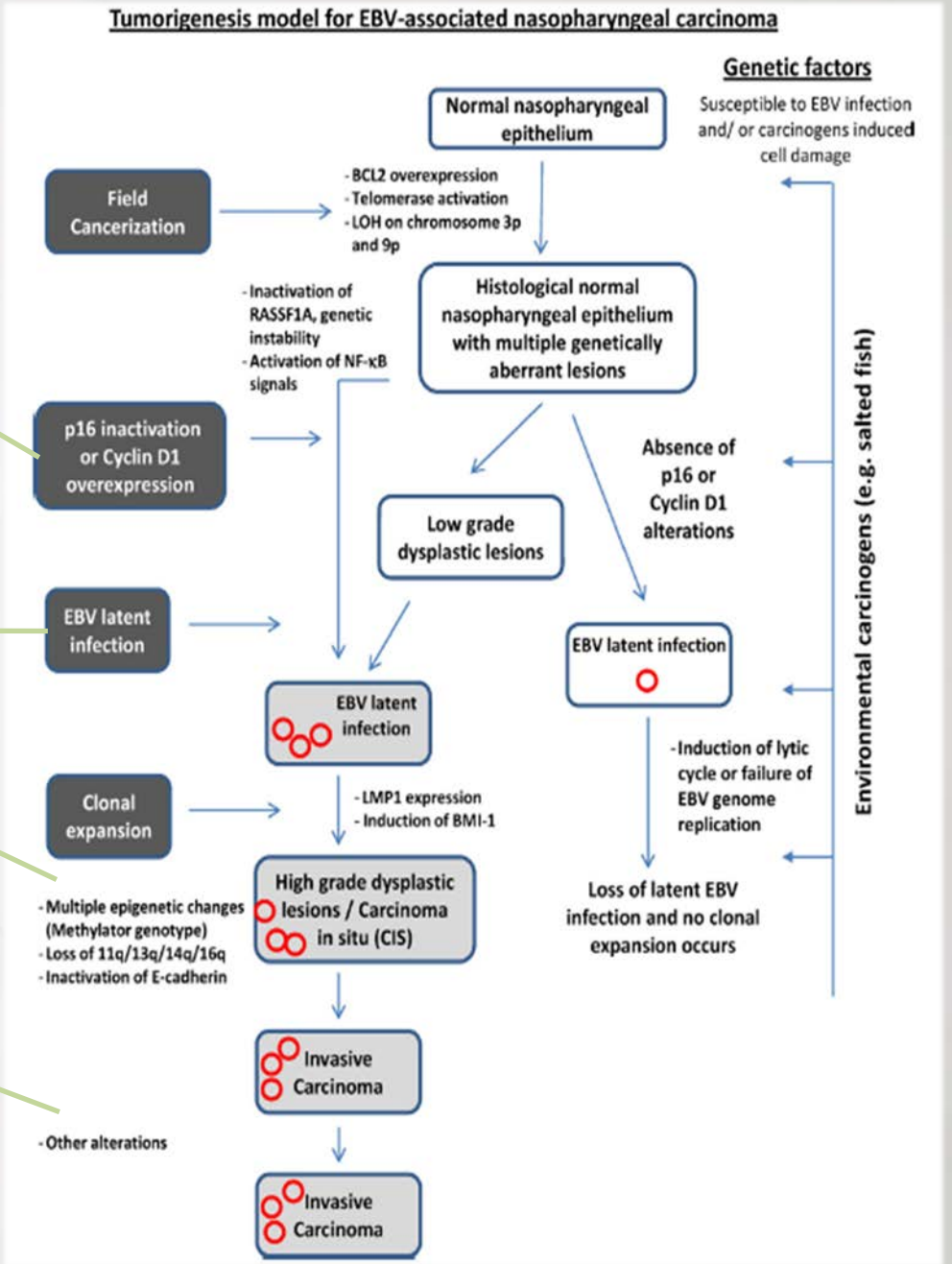
Tumorigenesis model of NPC

Oncogenes activation:
- Most frequent: CCDN1 (16% amp), PIK3CA (20% amp), LTBR (7-10% amp)

Role of Epstein Barr Viral infection

Inactivation of tumor suppressor genes
- Cell cycle regulators: p16, RASSF1A
- Cadherins and matrix matelloproteinases
- Wnt/β-catenin signaling pathway
- RAS signaling pathway

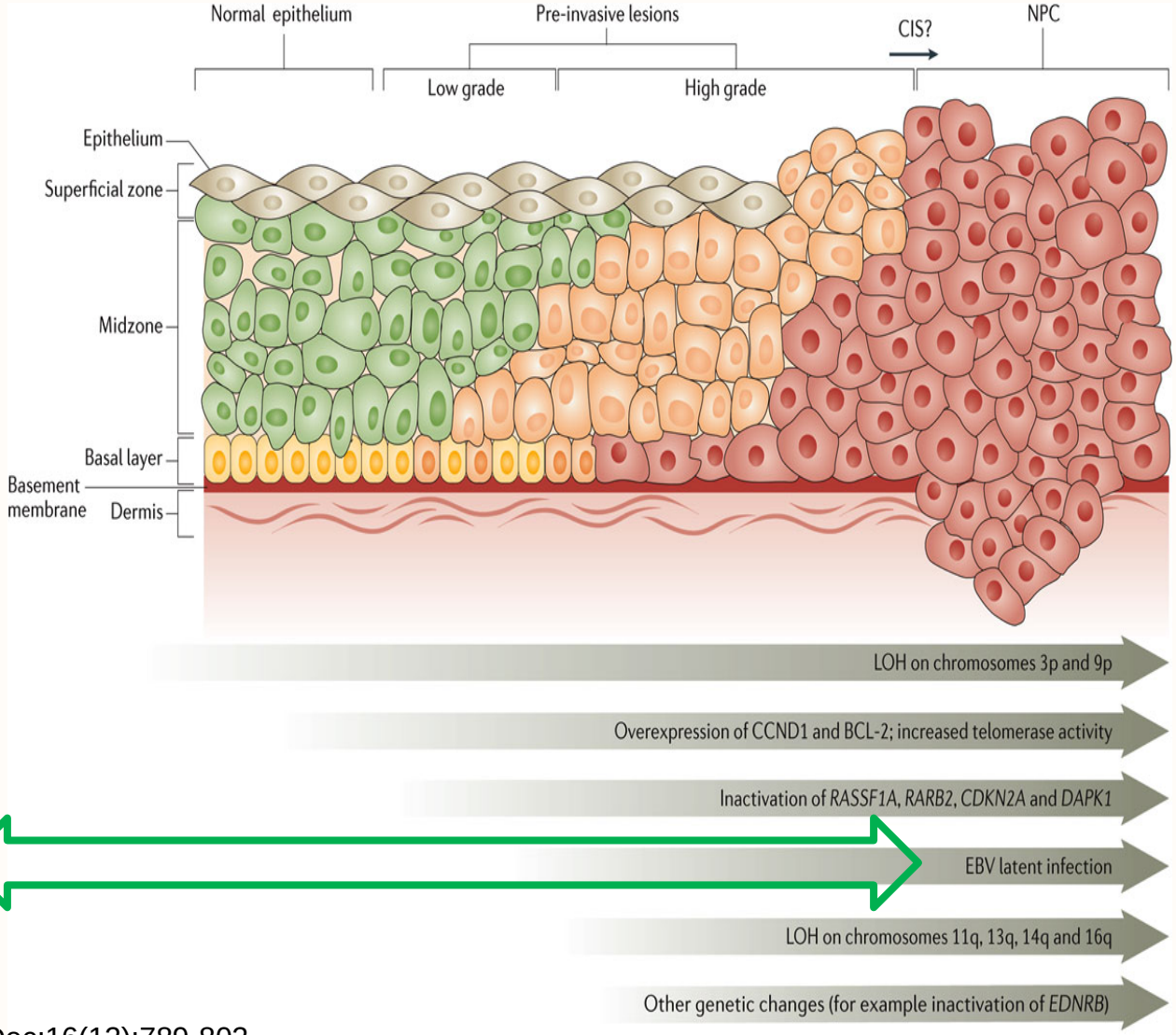
Tumor stromal microenvironment: immune, hypoxia and angiogenesis



KW Lo, G Chung & KF To. Seminars in Cancer Biology 22 (2012) 79– 86

Pathogenic role of EBV - Evidence

1) Patients show elevated anti-EBV ABs & circulating EBV DNA.
2) Consistent presence of EBV genome +ve monoclonal episomes.
3) Presence of EBV in high grade pre-invasive lesions.
4) EBV latent gene expression



LS Young et al. Nat Rev Cancer. 2016 Dec;16(12):789-802

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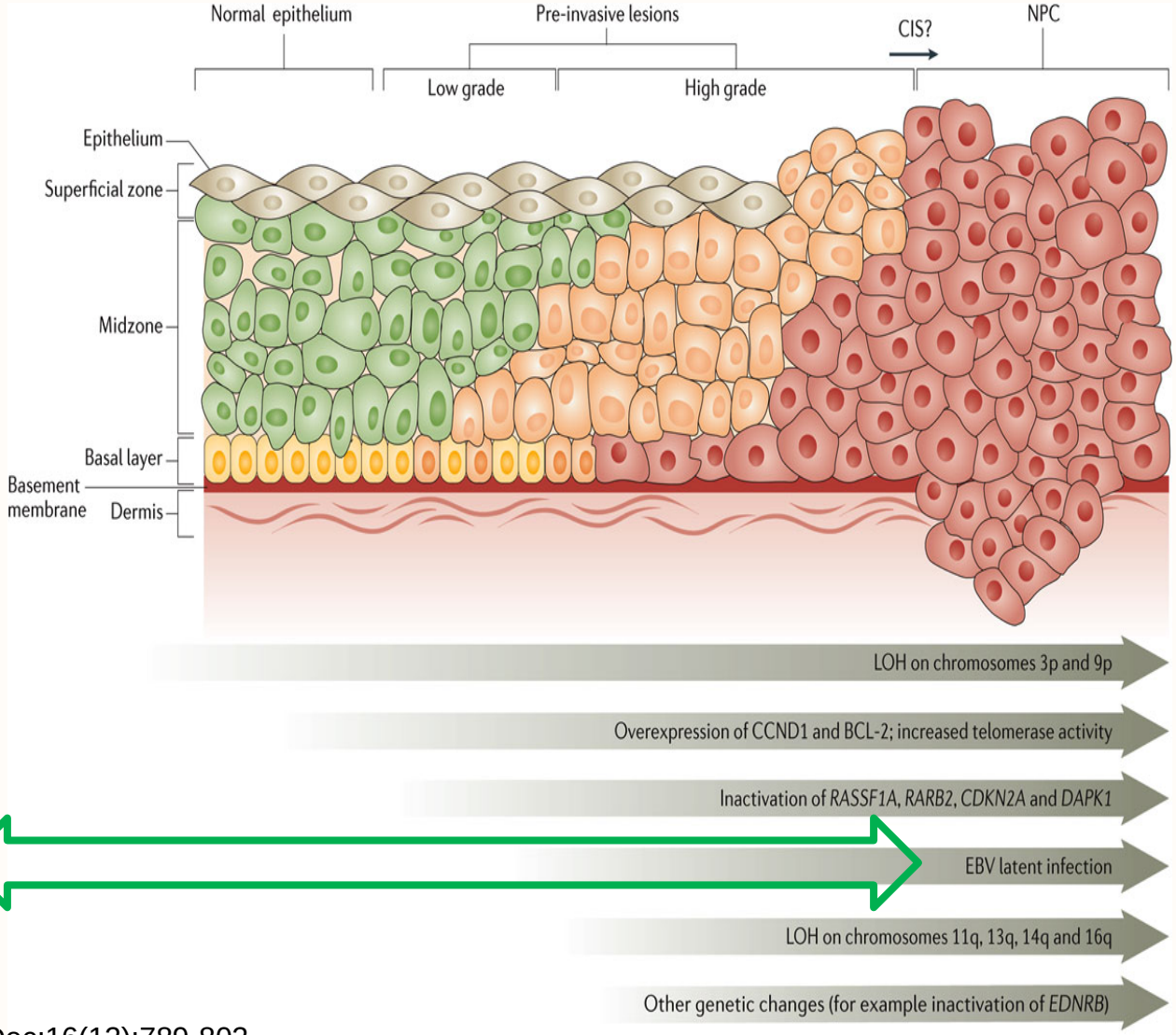
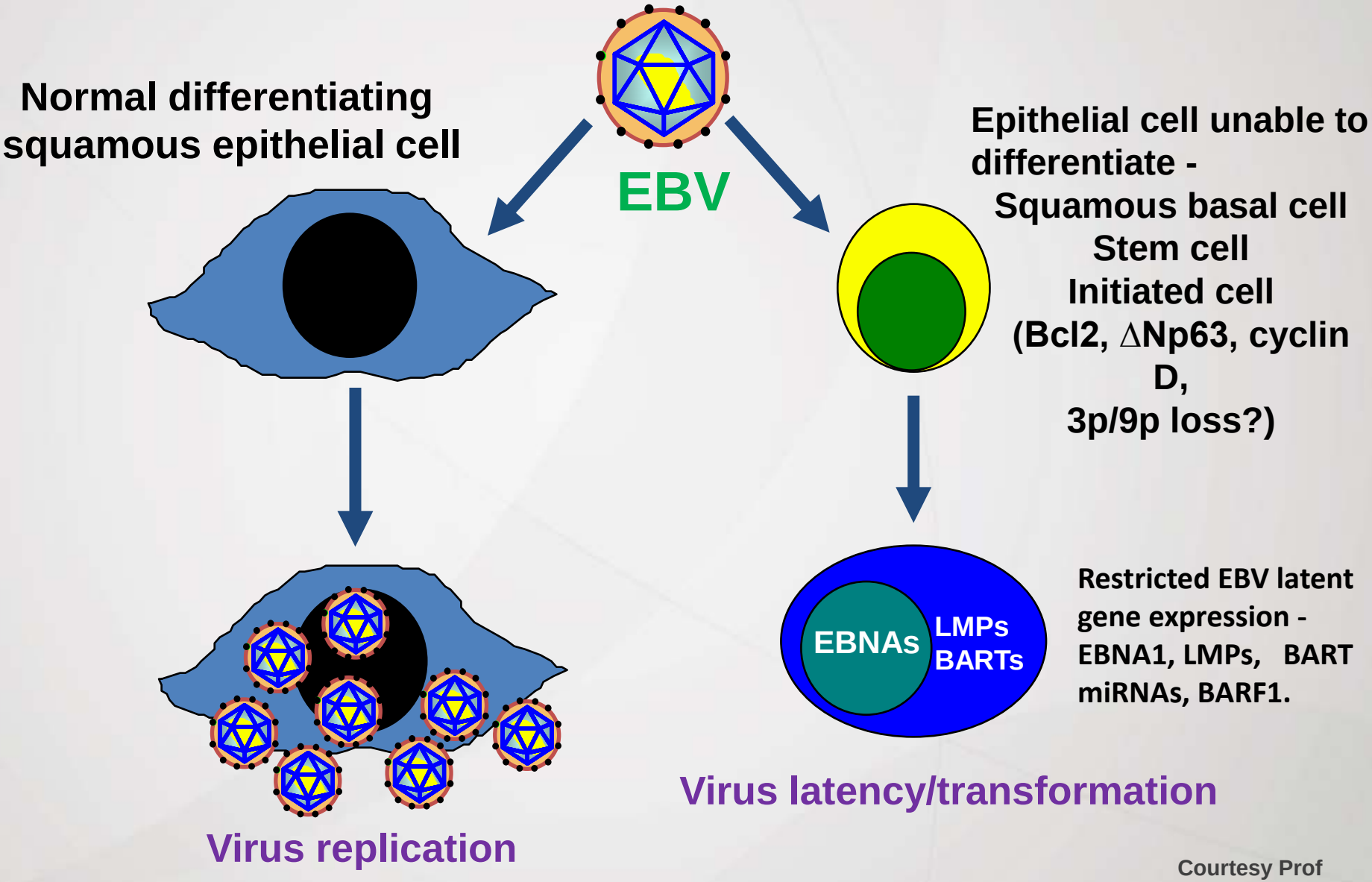


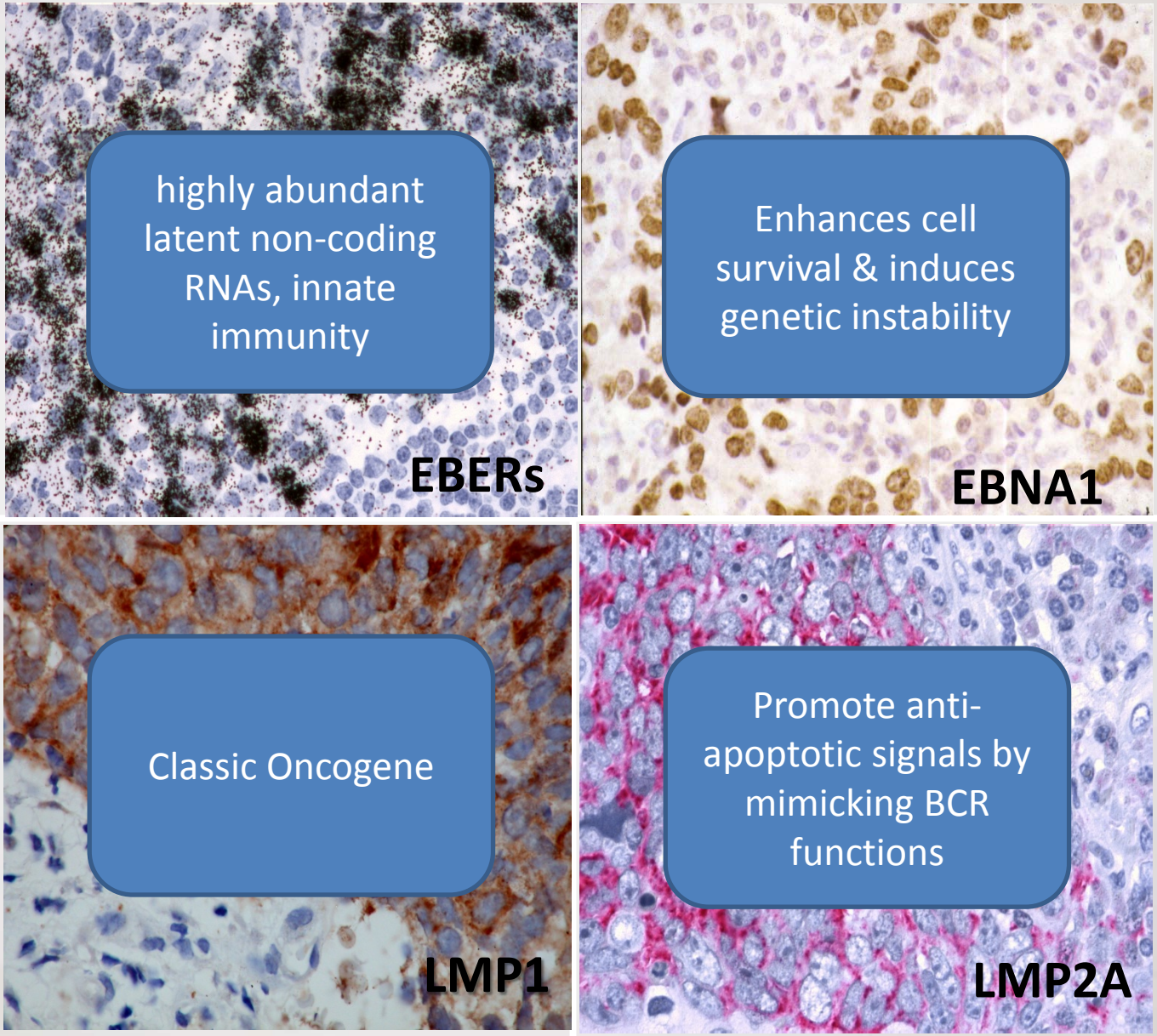
LS Young et al. Nat Rev Cancer. 2016 Dec;16(12):789-802

EBV infection of epithelial cells



Courtesy Prof Lawrence Young

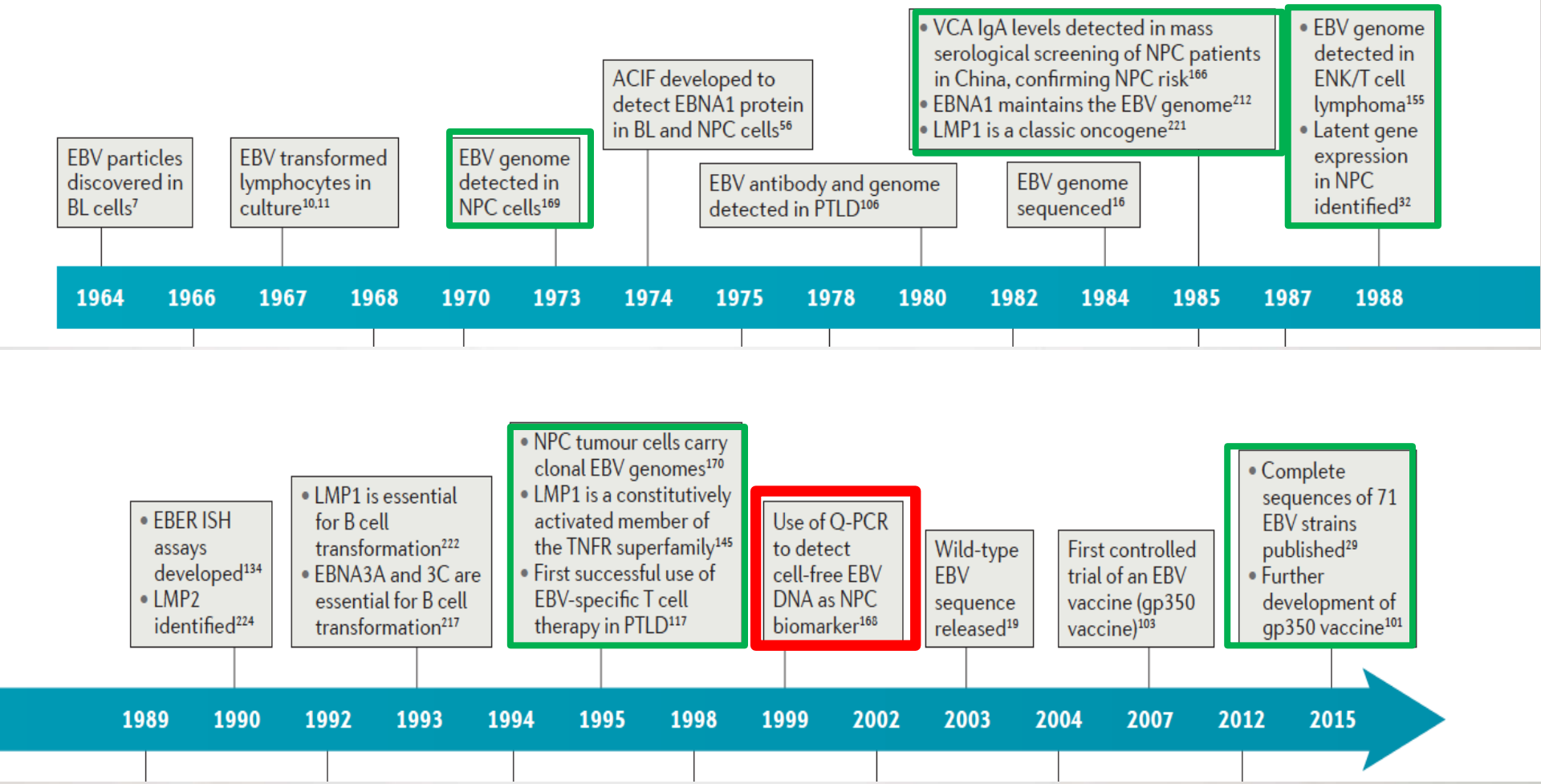
Type II EBV latent gene expression in NPC



Courtesy Prof Lawrence Young

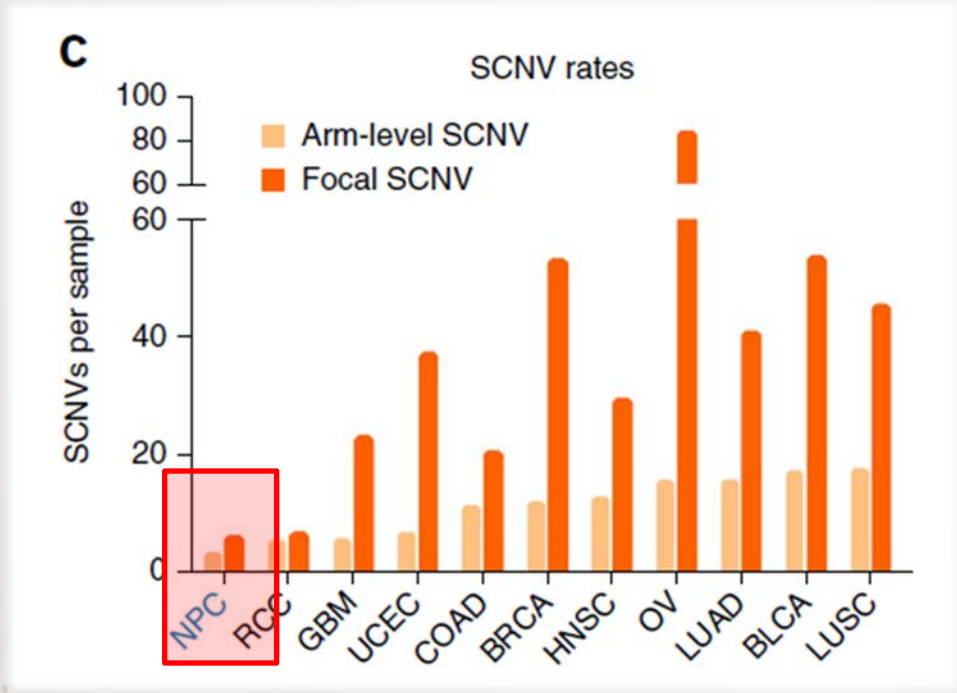
LS Young et al. Nat Rev Cancer. 2016 Dec;16(12):789-802

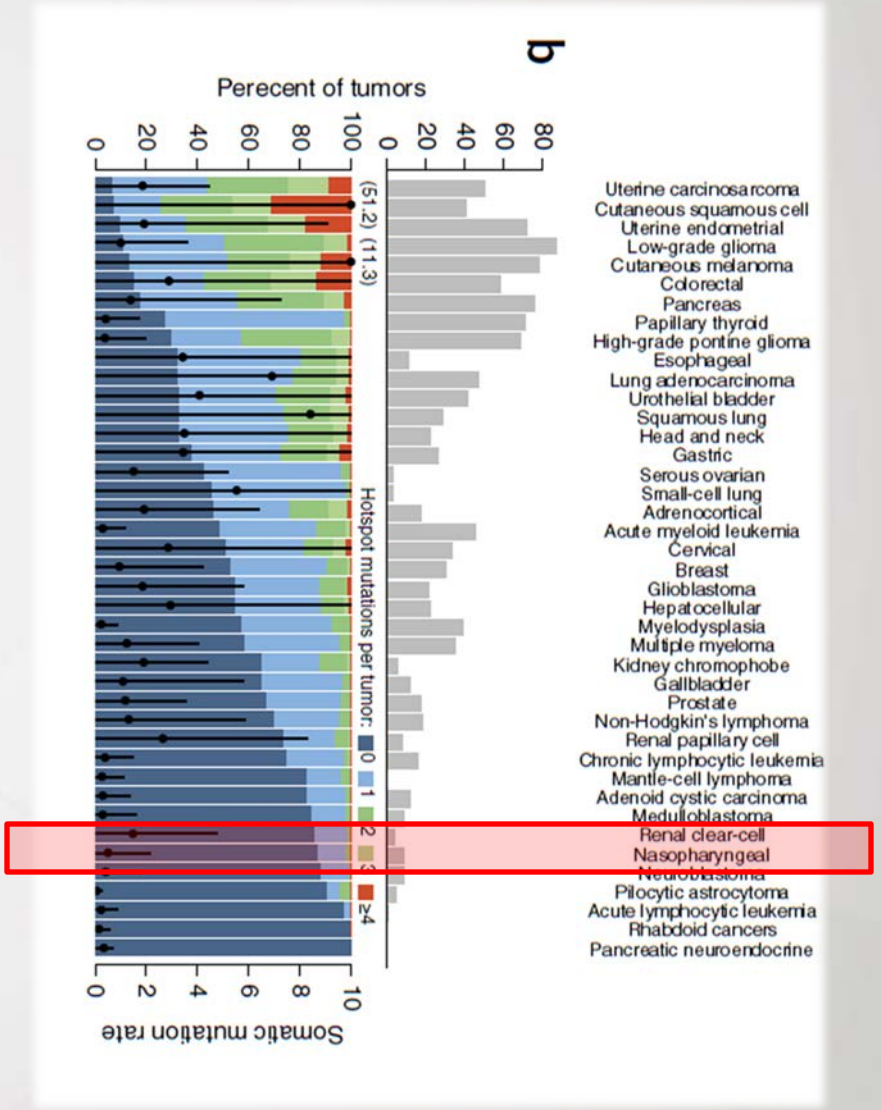
Translational research of EBV in NPC – clinical applications



First Report on the Whole Exome Sequencing WES) of NPC from TCGA (2014)

- National University of Singapore
- Whole-exome, targeted deep sequencing, SNP array analysis of ~ 60 primary NPC tumors



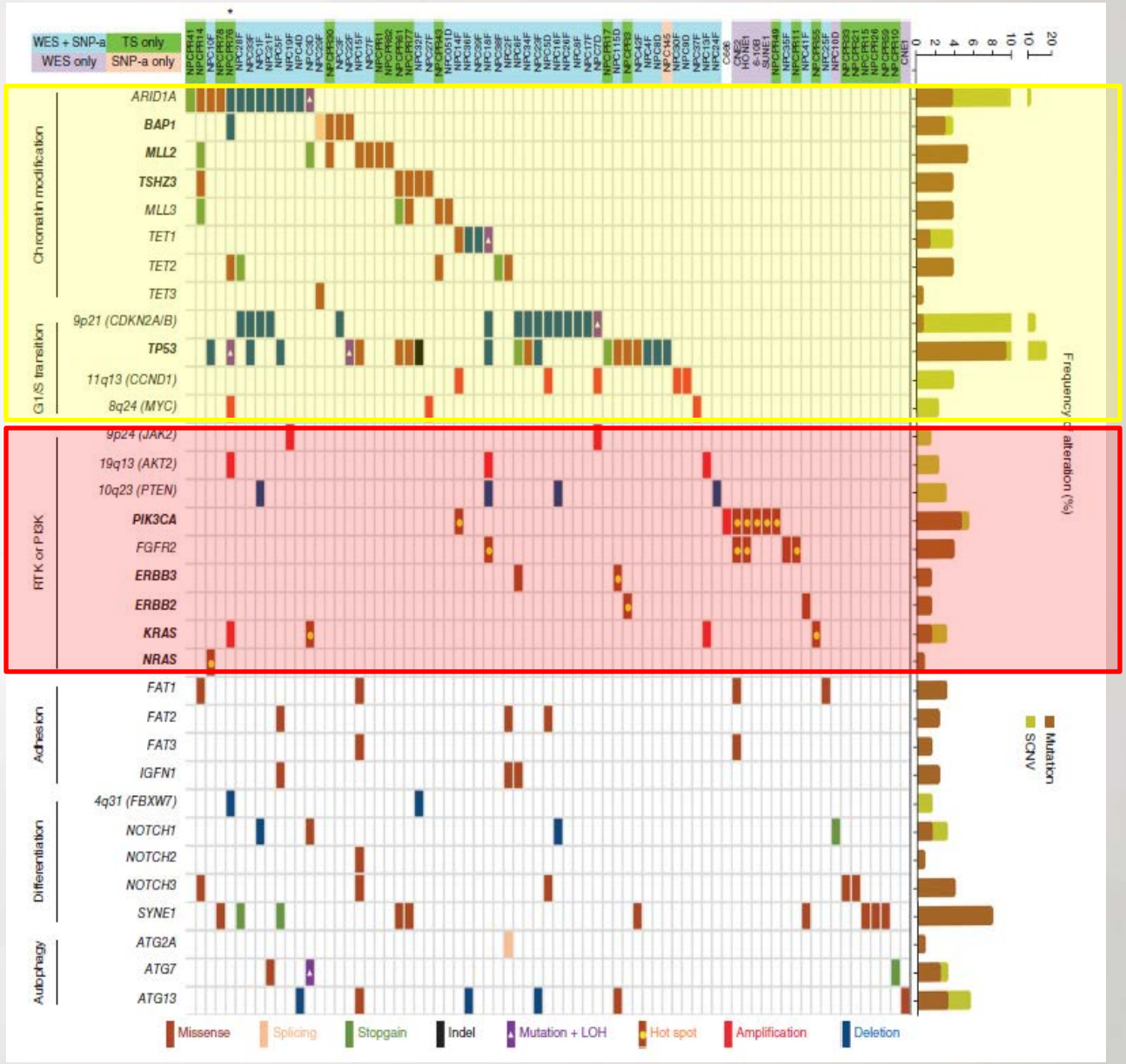


Lin et al, 46 | 8 | AUG 2014 Nature Genetics
Chang 2015, Nature biotech, November

WES Genomic landscape of NPC

- Genetic alterations in chromatin modification and cell cycle regulation are most frequent

- Actionable RTK gene mutations/amp occur in low frequencies:
- PIK3CA mutations 6%
- Prevalence: < 1%
  - ERBB2/ERBB3 mutation
  - KRAS mutation
  - AKT2 amplification
  - PTEN loss



Whole exome and genome sequencing of nasopharyngeal carcinoma

nature COMMUNICATIONS

ARTICLE

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Exome and genome sequencing of nasopharynx cancer identifies NF-κB pathway activating mutations

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Principal Investigator:
Kwok W. Lo, PhD, CUHK

Collaborators:
Broad Institute of Harvard and MIT
University of Pittsburgh
UCSF, San Francisco
The University of Hong Kong
Seoul National University, Sth Korea
St. Luke's Medical Center, Philippines

- **WES** of 111 NPC microdissected primary and recurrent NPC. **WGS** of 15 primary tumors
- **Clonal analysis** (n = 4 paired primary & recurrent tumors).
- **Clinical correlation**: Overall survival, survival after recurrence. Stratified by LMP-1 status.
- Mutational spectrum, somatic copy number alteration, structural variants. Functional analysis on candidate drivers/ TSG

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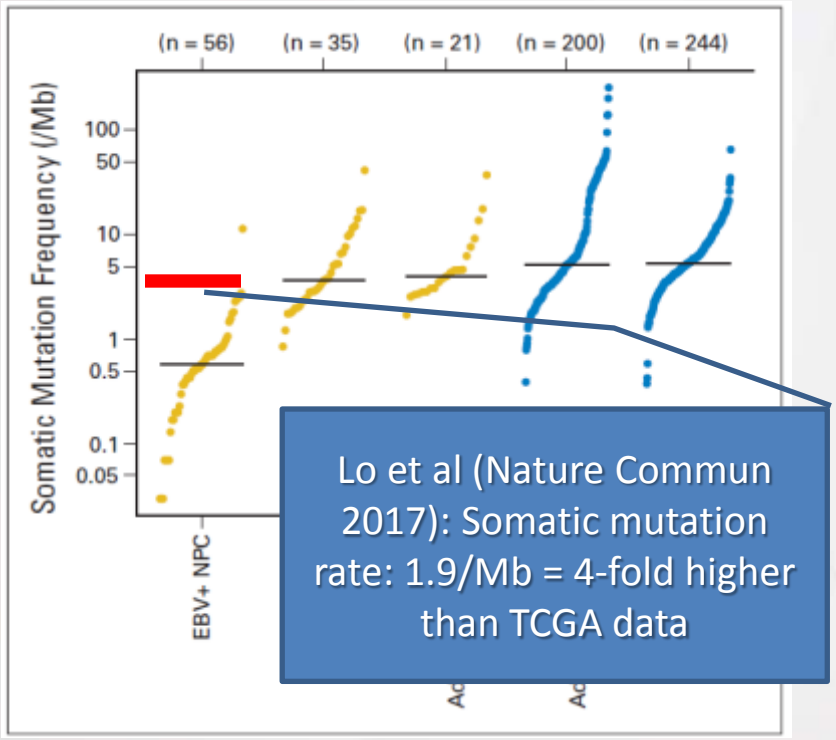
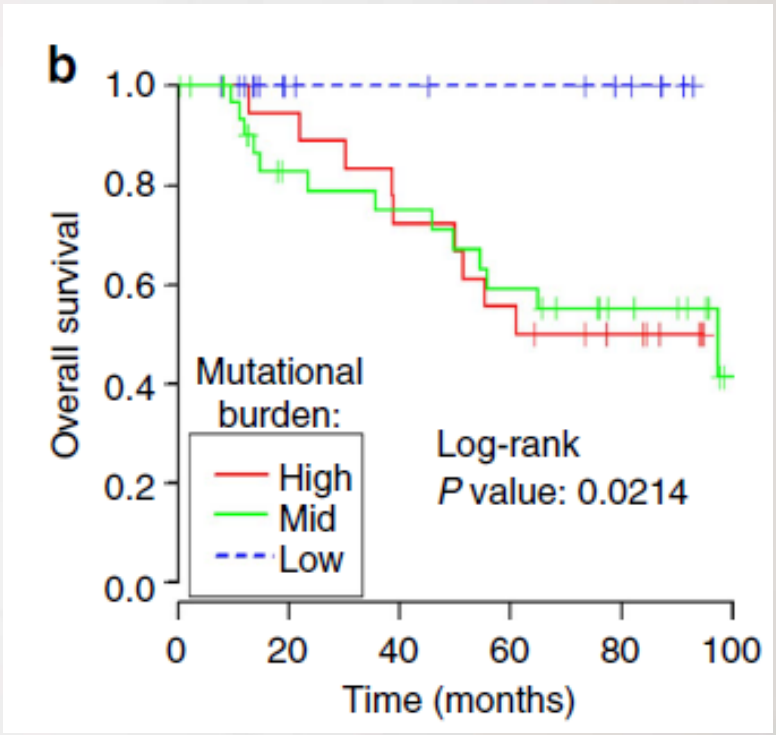
WGS/WES of NPC

Mutational burden



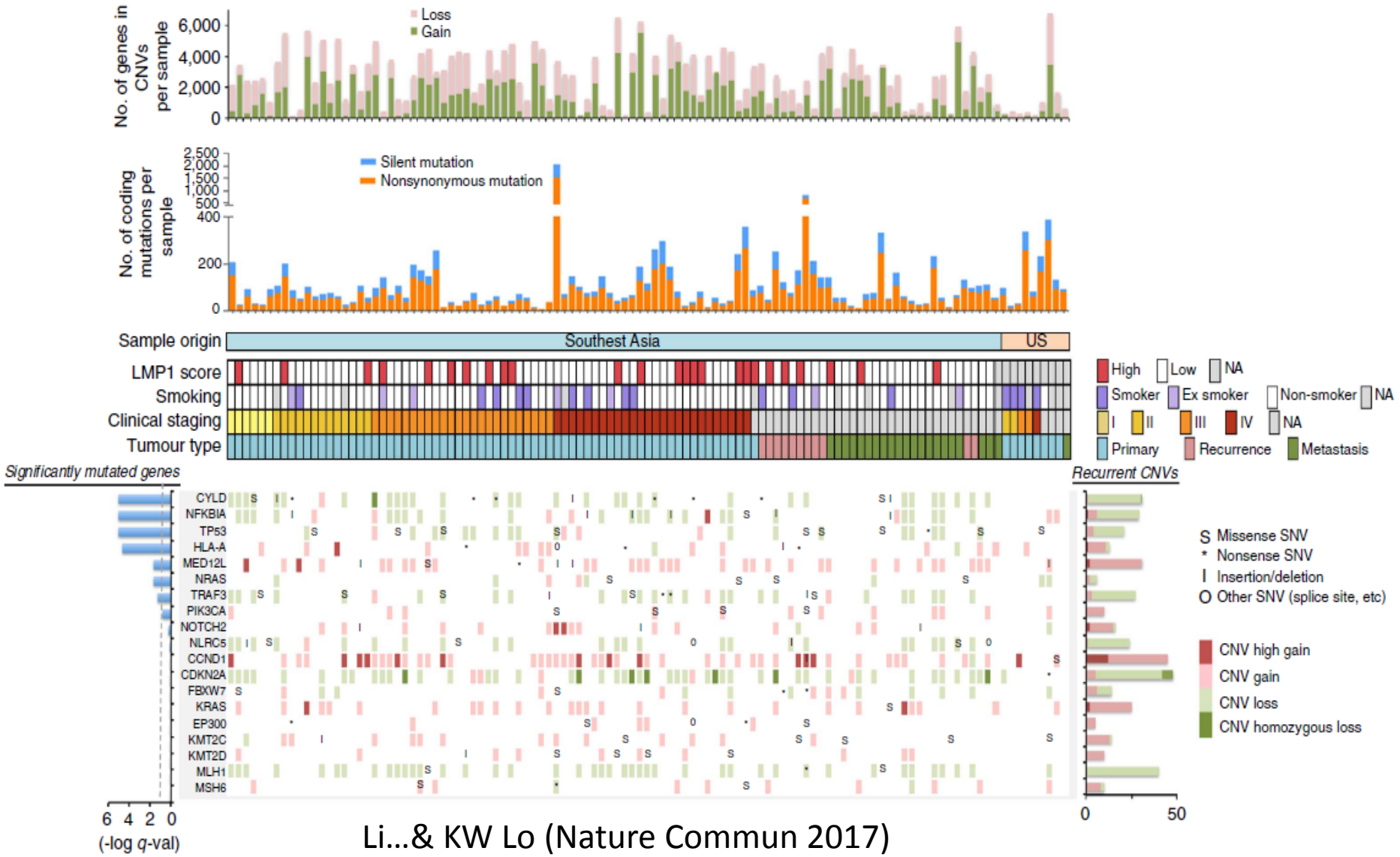
Bruce et al, J Clin Oncol 33. 2015

Prognostic significance



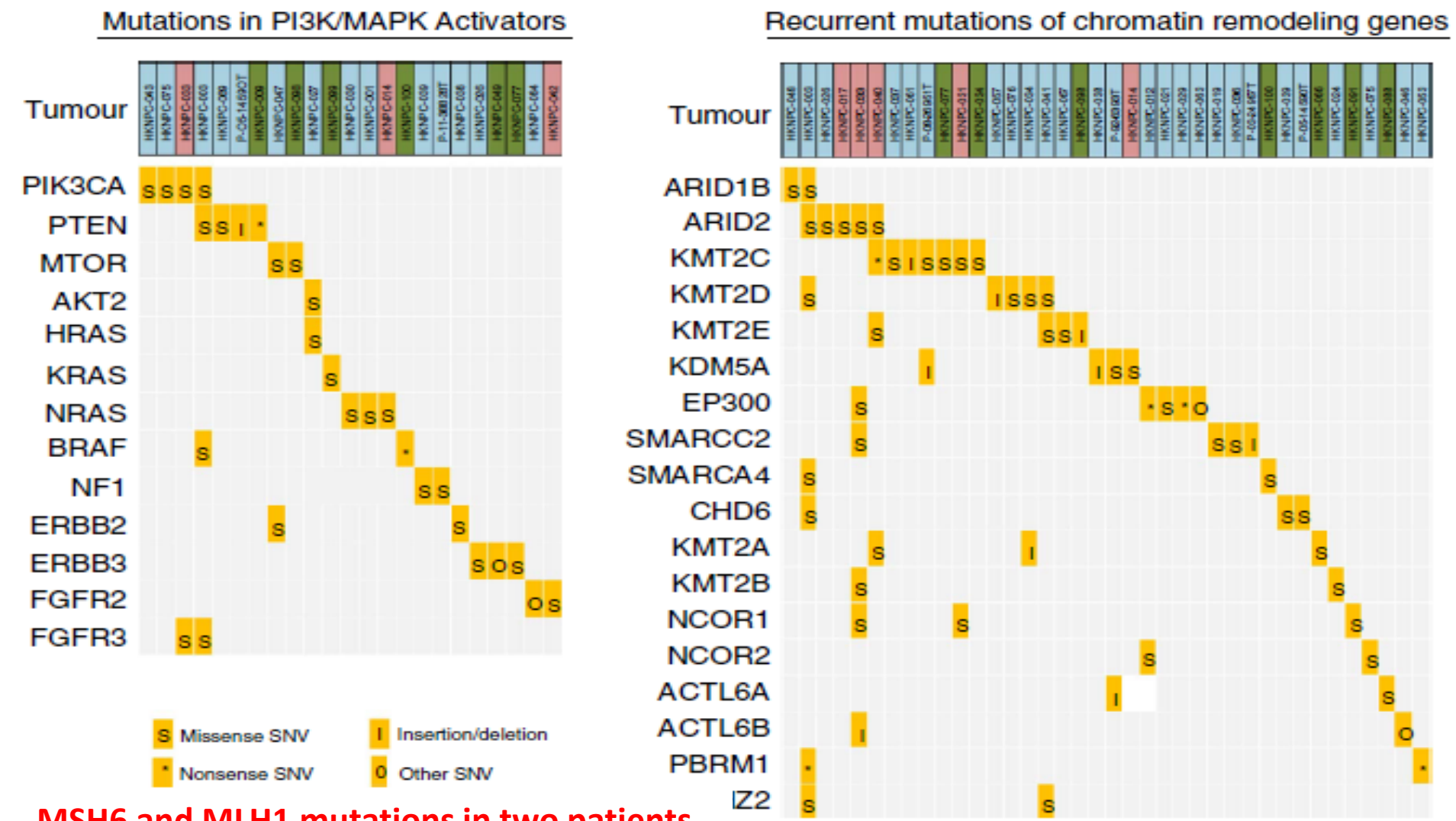
Li…& KW Lo (Nature Commun 2017)

Genomic landscape



Li…& KW Lo (Nature Commun 2017)

Actionable alterations are uncommon in NPC



MSH6 and MLH1 mutations in two patients

NF-kB pathway aberrations are common in NPC

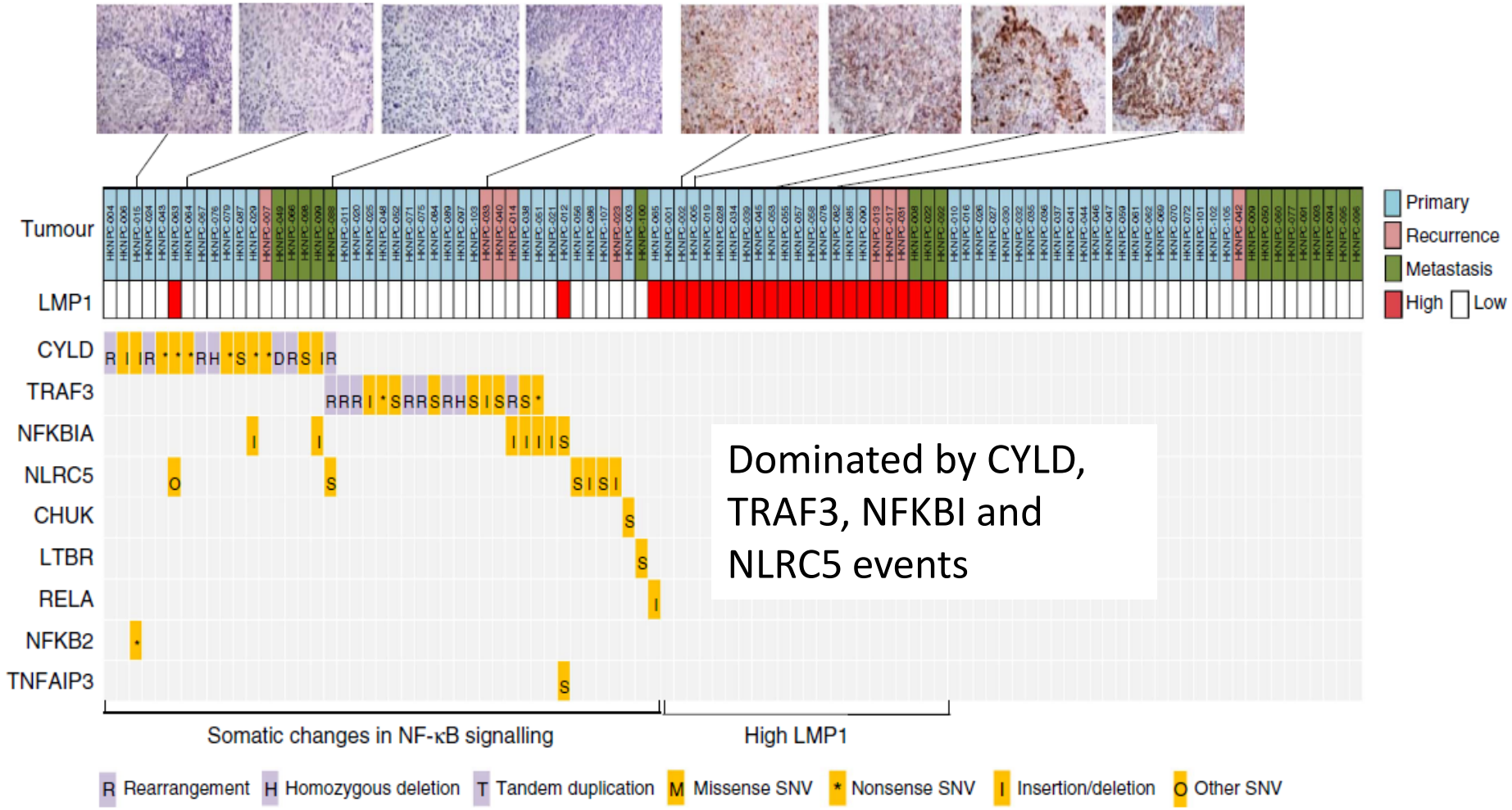
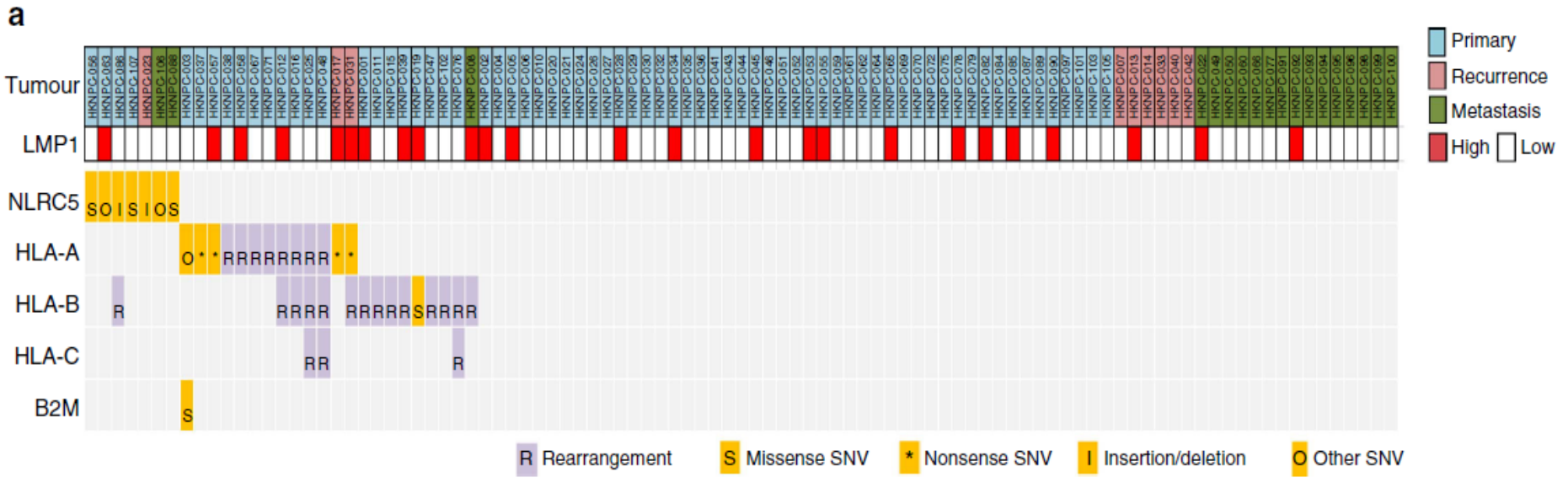
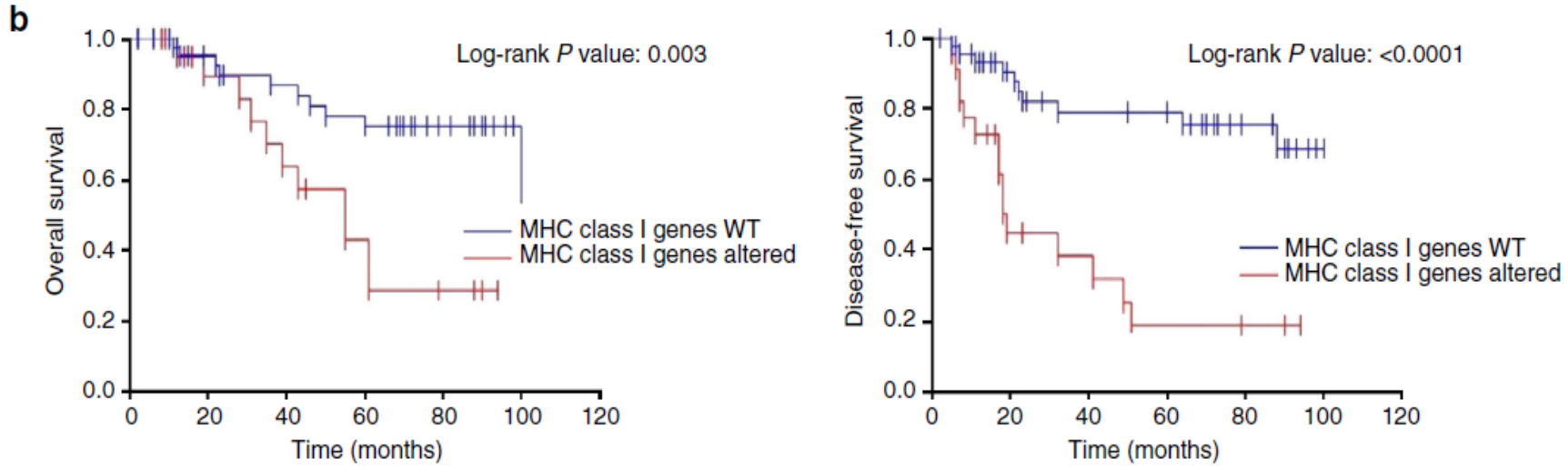


Figure 6 | Genomic aberrations in NF-κB pathways in NPC. Mutual exclusivity between LMP1 overexpression and NF-κB somatic alterations in NPC tumours ($P = 0.00014$). LMP1 staining of representative tumours are also shown.

MHC class 1 gene alterations are found in 30% of NPC

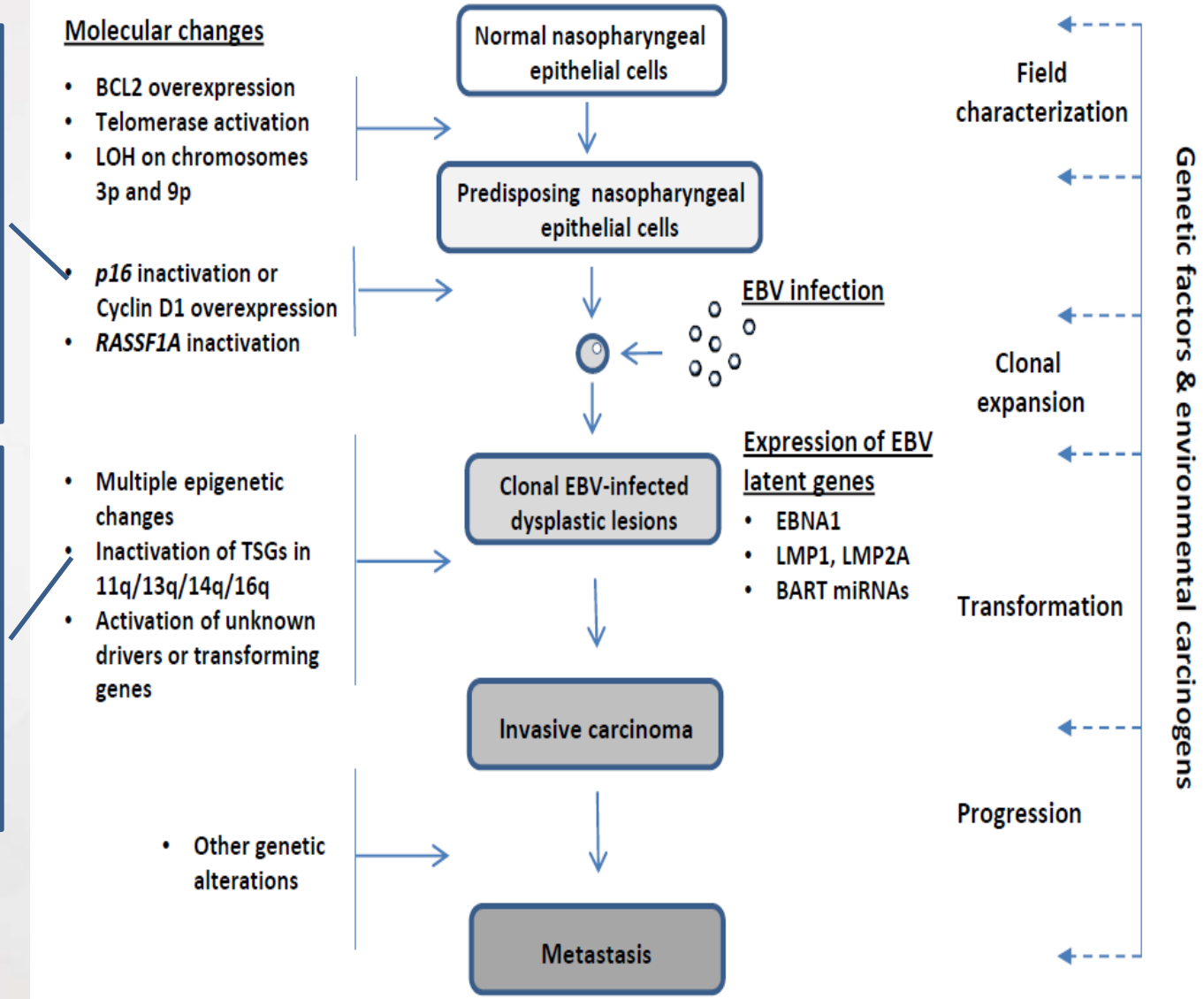




Epigenetic dysregulation plays important role in early pathogenesis of NPC

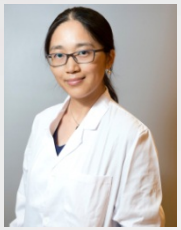
Chr 3p & 9p:
- Promoter hypermethylation and homozygous deletion of p16 in >85% NPC
- Hypermethylation of RASSF1A, DLEC1, BLU > 60% NPC

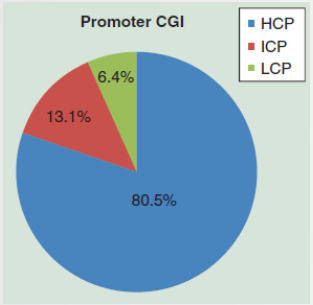
Chr 11, 13, 14, 16:
- TSG Regulators of NKkappaB and other signaling pathways, Cadherin superfamily



Semin Cancer Biology 22 (2012) 79– 86

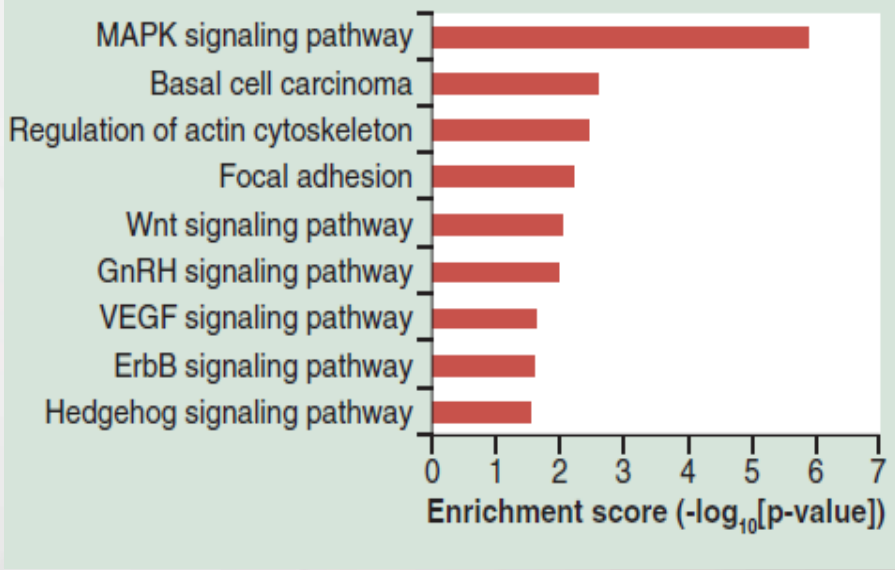
Mapping out the epigenetic alterations affecting signaling pathways in NPC



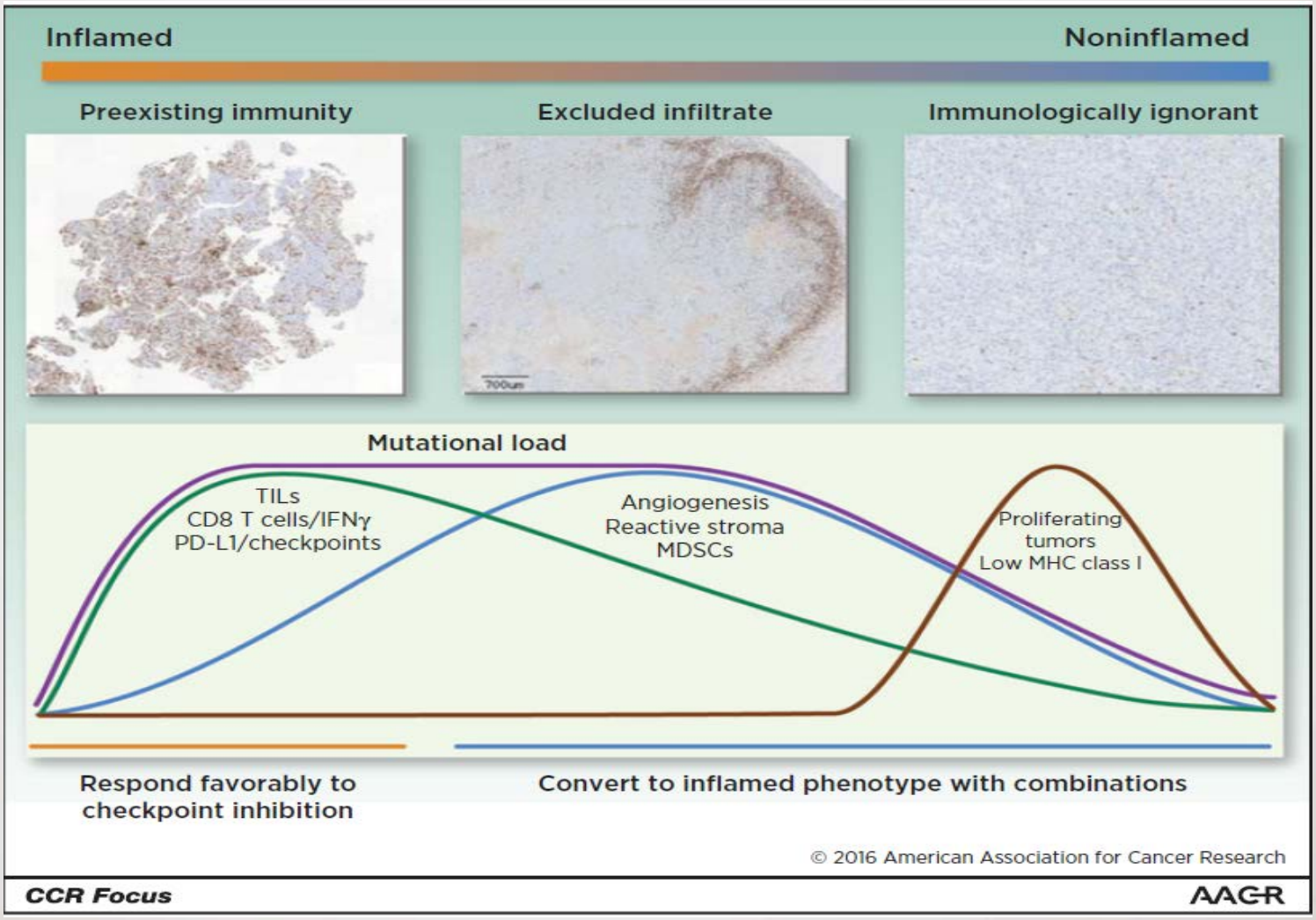


- Prof Qian Tao, L Li and CUHK team
- Methylnomic analysis of NPC cell lines & primary tumors
- Identified over 2000 hypermethylated genes.
- Functional analysis of tumor suppressor genes modulating key signaling pathways: Wnt, MAPK, TGF-β, Hedgehog and ErbB

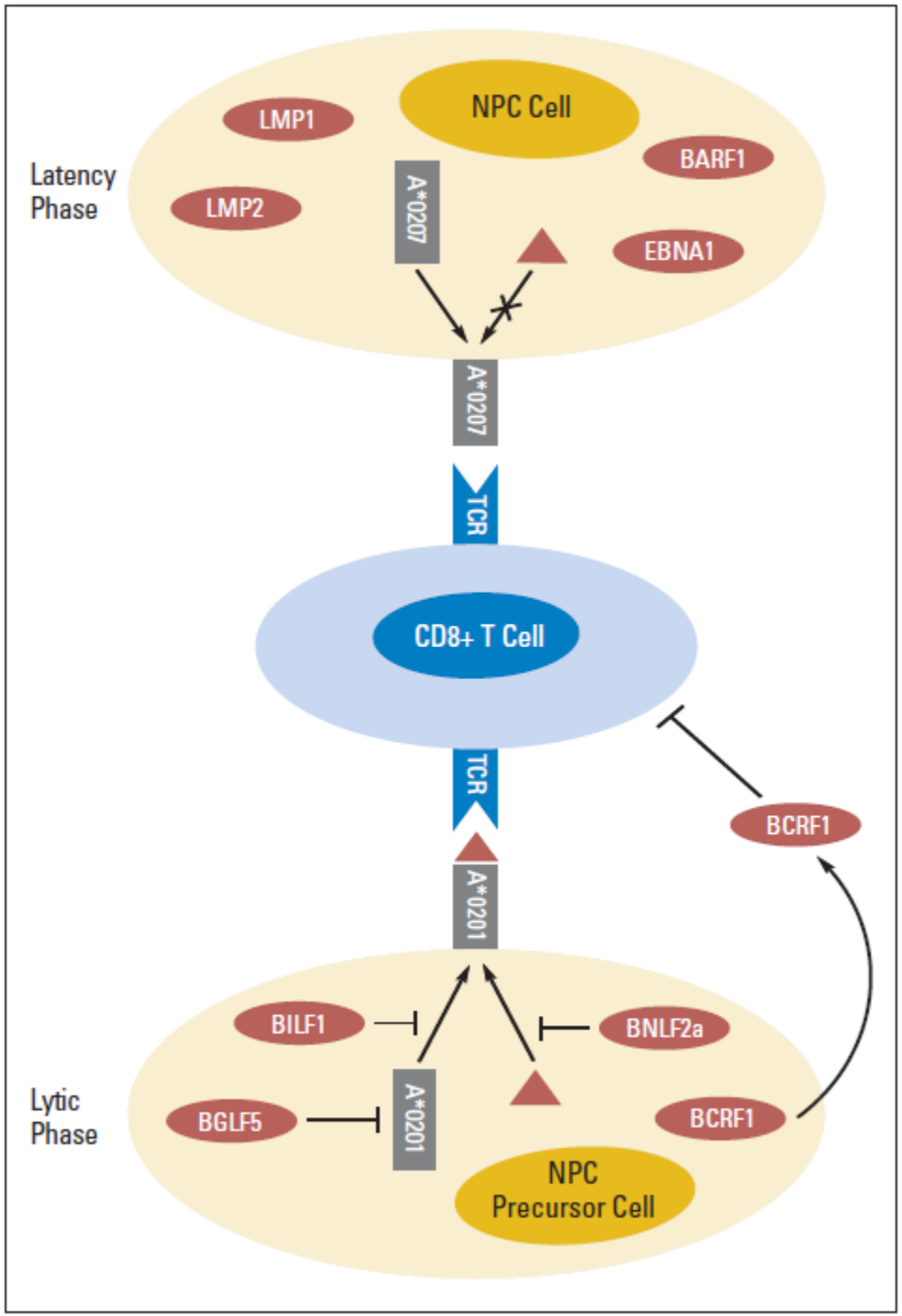
Epigenomics (2015) 7(2), 155–173



NPC is the archetypal ‘immune-hot’ tumor



Hegde Clin Cancer Res; 22(8); 1865–74. 2016 AACR



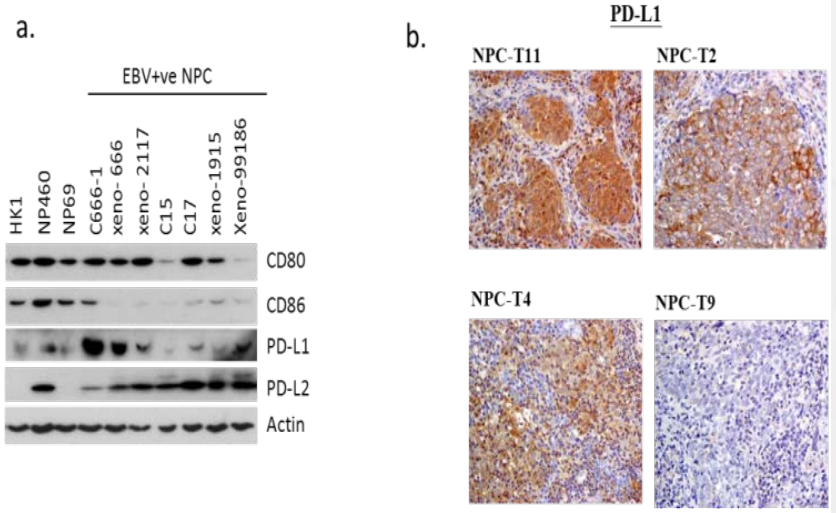
Immune evasion in NPC

Journal of Clinical Oncology 33, no. 29 (Octobe
2015) 3346-335

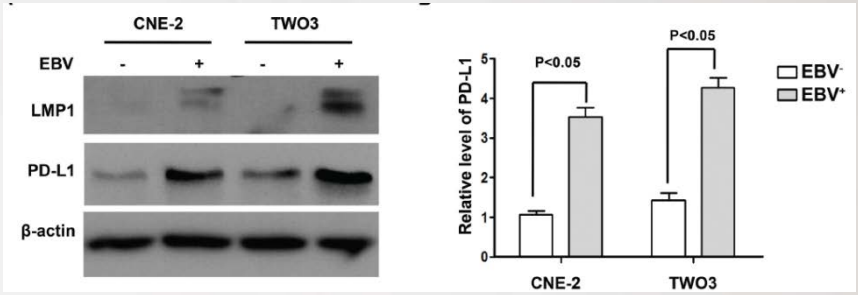
Programmed cell death-1 receptor and ligand (PD1/PD-L1) expression in NPC

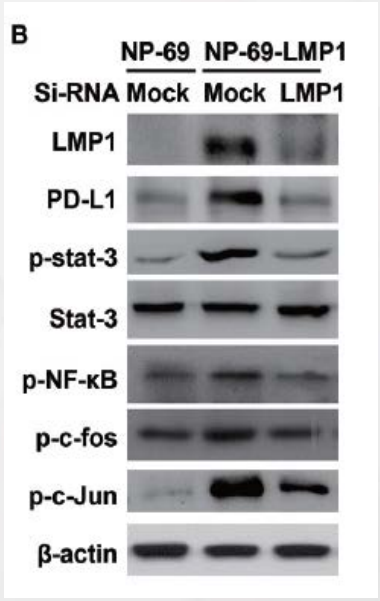
- PD-L1 is upregulated in EBV+ve NPC: Prognostic significance of PD-L1 expression in advanced NPC = conflicting.
- Variable expression rate in literature

Figure 1 (unpublished confidential data, Prof KW Lo, CUHK)



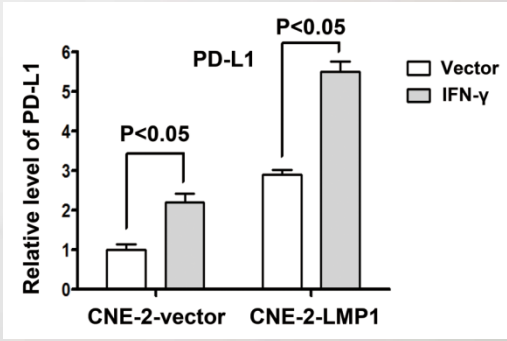
- **EBV infection upregulates PD-L1 in vitro**





- **← LMP-1 upregulates PD-L1 via STAT3, MAPKs/AP-1, NF-κB in vitro**

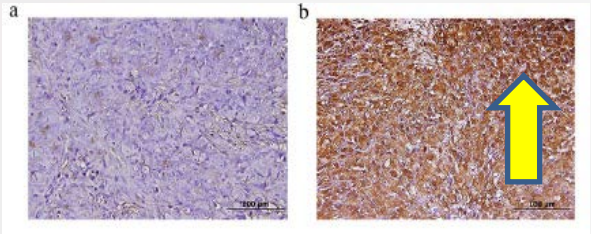
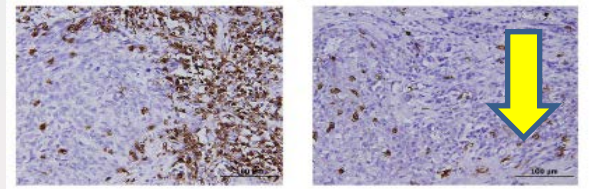
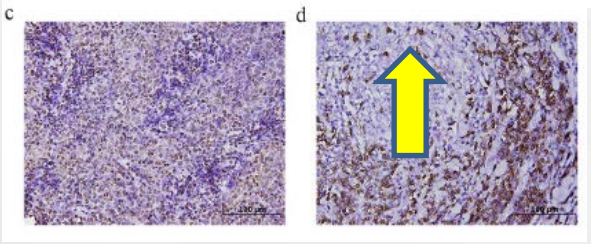
- **PD-L1 up-regulation after IFN-γ exposure ↓**



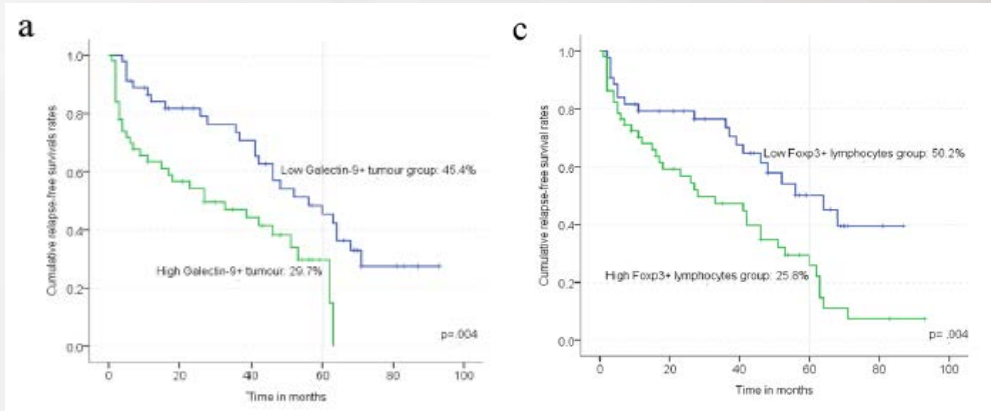
Fang 2014 Oncotarget;5(23):12189-202

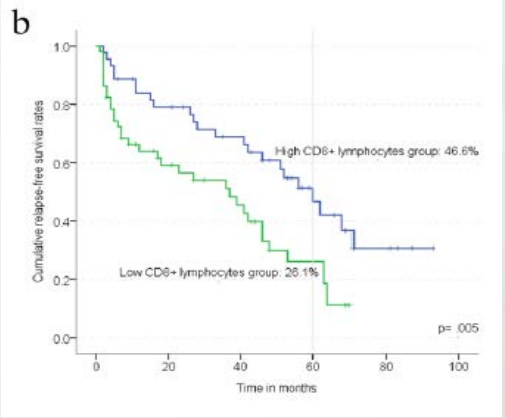
Changes in the tumor microenvironment of primary NPC vs recurrent tumors following cytotoxic therapy

- Paired primary and recurrent (local or distant) of mainly non-keratinizing NPC tumors in 95 patients, who received cytotoxic therapy

	Pre-Rx	Post-Rx
Galectin-9		
CD8+		
Foxp3		

Relapse-free survival curves





Summary:

1. The checkpoint protein Galectin-9/Tim-3 pathway could be an important immune escape pathway in NPC
2. TIL density is prognostic

Chen et al 2017 Scientific Report | 7: 10349 |

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Antitumor Activity of Nivolumab in Recurrent and Metastatic Nasopharyngeal Carcinoma: An International, Multicenter Study of the Mayo Clinic Phase 2 Consortium (NCI-9742)

Brigette B.Y. Ma, Wan-Teck Lim, Boon-Cher Goh, Edwin P. Hui, Kwok-Wai Lo, Adam Pettinger, Nathan R. Foster, Jonathan W. Riess, Mark Agulnik, Alex Y.C. Chang, Akhil Chopra, Julie A. Kish, Christine H. Chung, Douglas R. Adkins, Kevin J. Cullen, Barbara J. Gitlitz, Dean W. Lim, Ka-Fai To, K.C. Allen Chan, Y.M. Dennis Lo, Ann D. King, Charles Erlichman, Jun Yin, Brian A. Costello, and Anthony T.C. Chan

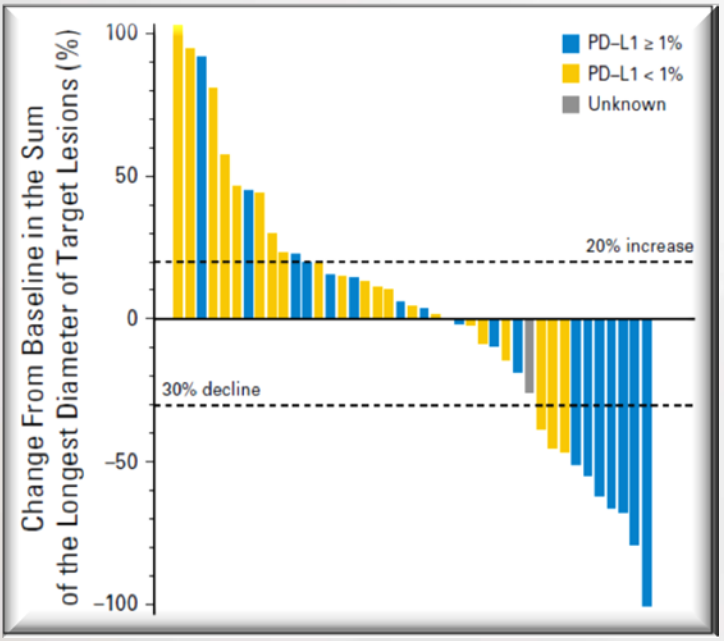


Table A2. Biomarker Characteristics in Responders

Patient No.	PD-L1 Expression, Tumor Cells, %	PD-L1 Expression, Immune Cells, %	HLA-A	HLA-B	RECIST Response	Site of Metastases
015	10	35	Expressed	Expressed	PR	Lung
022	70	< 1	Expressed	Expressed	PR	Lung
001	< 1	< 1	Loss	Loss	PR	Liver, nodal
005	40	< 1	Expressed	Loss	PR	Liver, lung
043	< 1	< 1	Loss	Loss	PR	Liver, nodal
016	< 1	< 1	Expressed	Expressed	PR	Liver, lung, bone
044	5	< 1	Expressed	Expressed	PR	Distant nodes
040	90	< 5	Expressed	Loss	PR	Bone, locoregional
027	5	50	Loss	Loss	CR	Soft tissue/bone

Abbreviations: CR, complete response; HLA, human leukocyte antigen; PD-L1, programmed death-ligand 1, PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors.

Ma B, Lim WT, Goh BC et al. J Clin Oncol 36. © 2018

HLA class 1

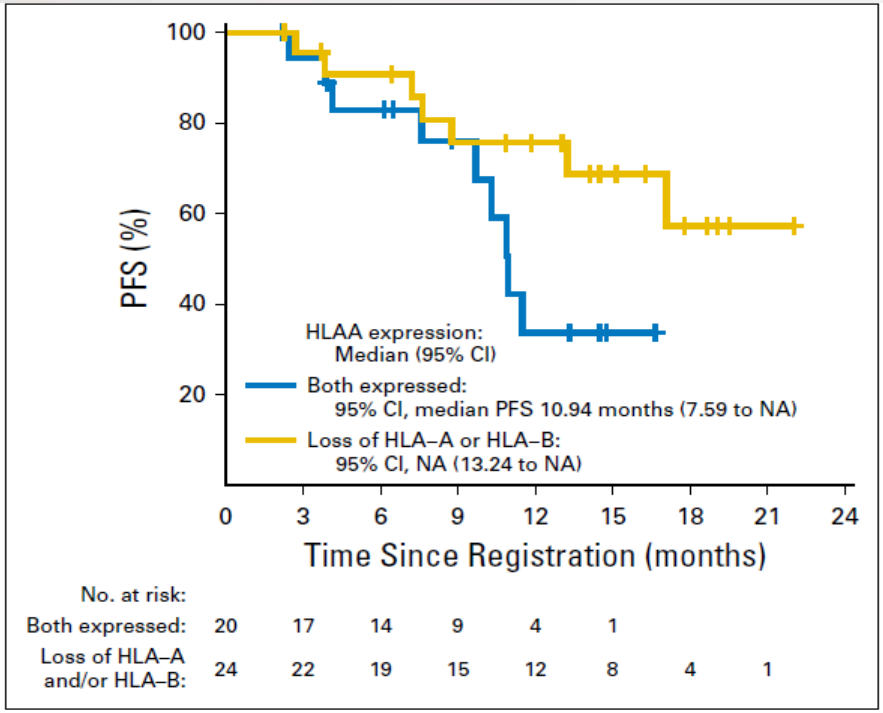


Fig 3. Progression-free survival curves of patients with tumors expressing both HLA-A and HLA-B (blue line), versus loss of HLA-A and/or HLA-B expression (gold line). HLA, human leukocyte antigen; NA, not achieved; PFS, progression-free survival.

PD-L1

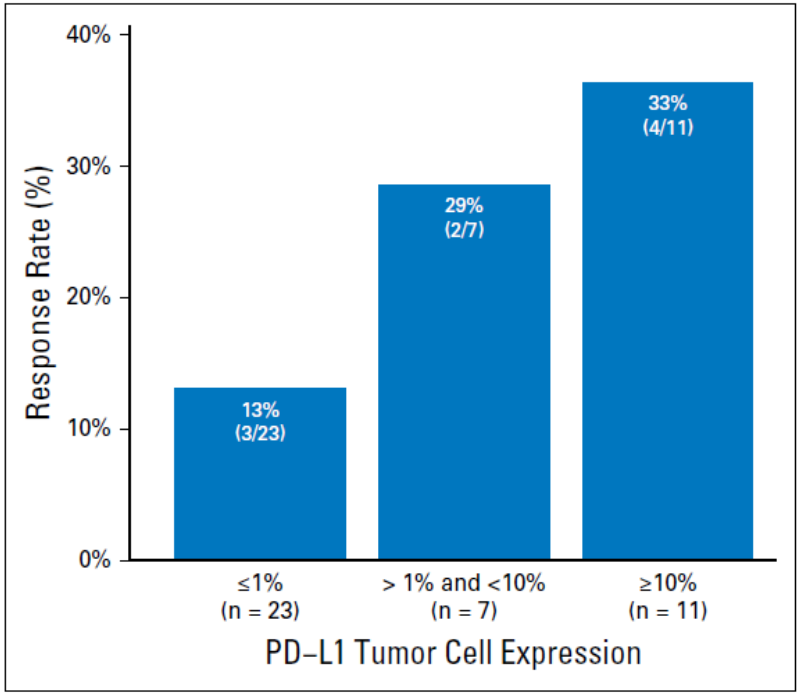


Fig A3. Descriptive summary on the proportion of patient with response (defined by Response Evaluation Criteria in Solid Tumors) to nivolumab according to the level of programmed death-ligand 1 (PD-L1) expression in tumor cells.

Ma B, Lim WT, Goh BC et al. J Clin Oncol 36. © 2018

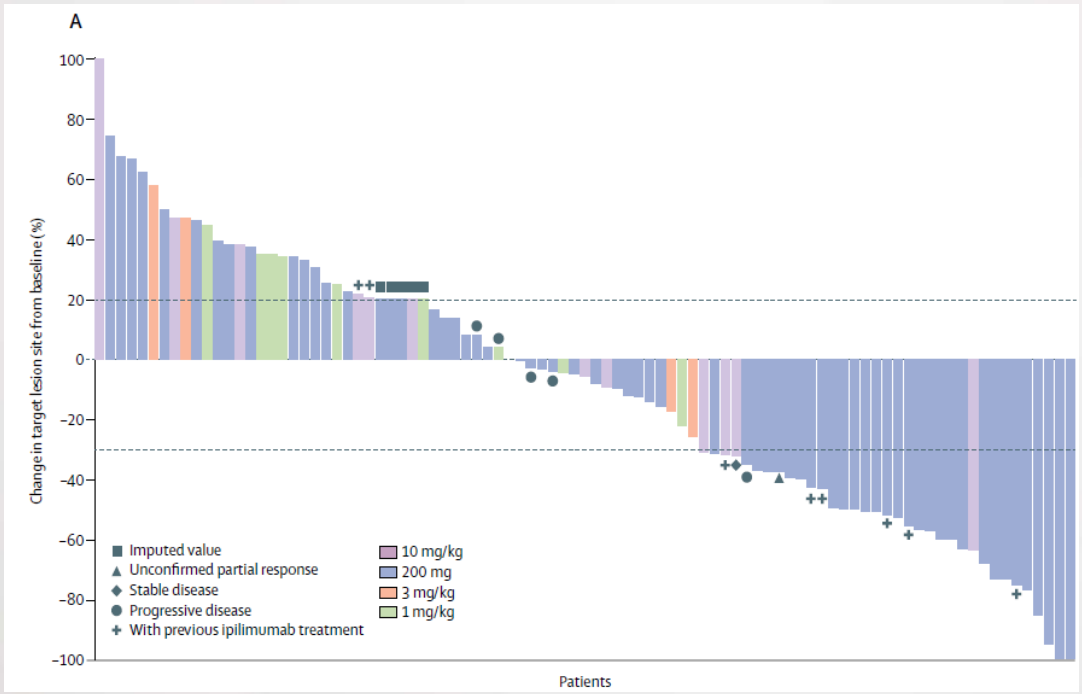
Camrelizumab (SHR-1210) alone or in combination with gemcitabine plus cisplatin for nasopharyngeal carcinoma: results from two single-arm, phase 1 trials

Wenfeng Fang, Yunpeng Yang*, Yuxiang Ma*, Shaodong Hong*, Lizhu Lin*, Xiaohui He, Jianping Xiong, Ping Li, Hongyun Zhao, Yan Huang, Yang Zhang, Likun Chen, Ningning Zhou, Yuanyuan Zhao, Xue Hou, Qing Yang, Li Zhang*

Summary

Background Platinum-based doublet chemotherapy regimens, preferentially gemcitabine plus cisplatin, are generally

Lancet Oncol 2018



- IgG4 PD-1 inhibitor
- Monotherapy: 34%
- Safe to combine with cisplatin-gemcitabine
- TMB via WES
- No relationship with outcome. Sample size could be underpowered

Today's talk

- Molecular biology

- Biomarkers (*diagnostic, prognostic, predictive*):
  - Biomarkers in clinical use: EBV-based
  - Biomarkers in research: immunological, gene signatures, micro-RNA, circulating tumor cells.

Biomarkers in NPC

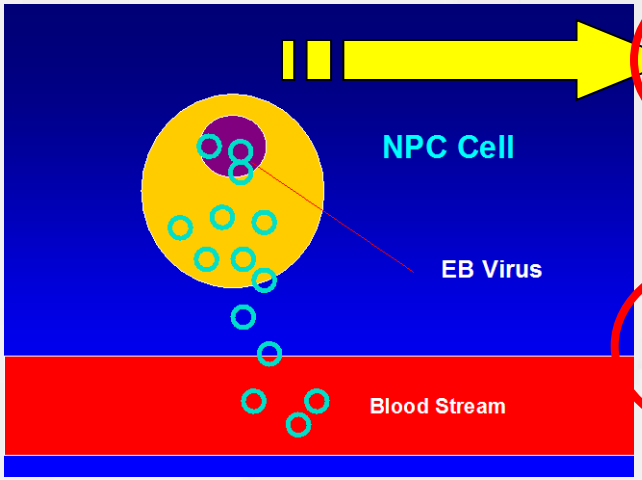
EBV-related

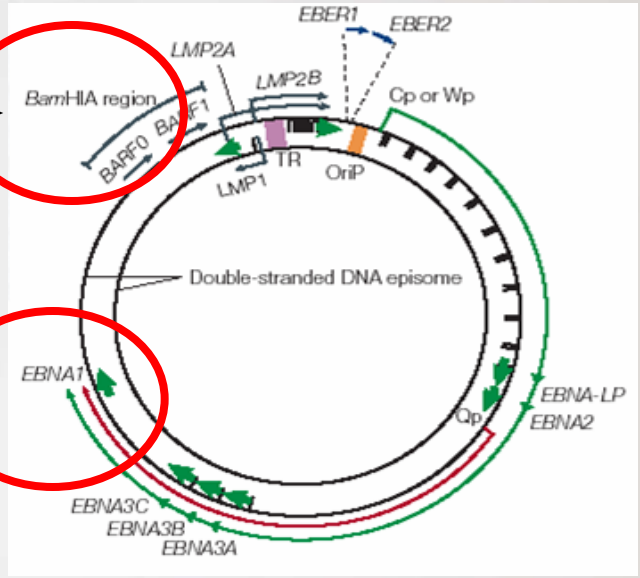
- EBER
- Anti-EBV antibodies
- Cell free circulating EBV DNA

Non-EBV related

- Immune
- Gene signatures

Quantitative Real-Time PCR for measuring tumor-derived plasma EBV DNA





- Lo YMD et al: using the DNA fragment that corresponds to the *Bam*HI-W & EBNA-1 regions of EBV genome. Quantitative PCR using β-globin gene as control
- Concordance between plasma & tumor derived EBV DNA. Monoclonal in origin, reflects tumor burden
- Sensitivity 98%, specificity 93% c/w control
- Half-life after RT or surgery: 4.4 days.

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Lo YMD, et al Cancer Res., 59: 1188-1191, 1999.

Plasma EBV DNA as a prognostic marker of survival after CRT

Plasma Epstein-Barr Virus DNA and Residual Disease After Radiotherapy for Undifferentiated Nasopharyngeal Carcinoma

Anthony T. C. Chan, Y. M. Dennis Lo, Benny Zee, Lisa Y. S. Chan, Brigette B. Y. Ma, Sing-Fai Leung, Frankie Mo, Maria Lai, Stephen Ho, Dolly P. Huang, Philip J. Johnson

Background: **Epstein-Barr virus (EBV) DNA can be detected and quantified in the plasma of patients with EBV-related tumors, such as nasopharyngeal carcinoma (NPC). Although NPC at early stages can be cured by radical radiotherapy, there is a high recurrence rate in patients with advanced NPC. The pretreatment level of circulating EBV DNA is a prognostic factor for NPC, but the prognostic value of post-treatment EBV DNA has not been studied. We designed a prospective study in Hong Kong, China, to investigate the value of plasma EBV DNA as a prognostic factor for NPC.** ***Methods:*** **One hundred seventy NPC patients, without metastatic disease at presentation, were treated with a uniform radiotherapy protocol. Circulating EBV DNA was measured by real-time quantitative polymerase chain reaction before treatment and 6–8 weeks after radiotherapy was completed. Risk ratios (RRs) were determined with a Cox regression model, and associations of various factors with progression-free and overall survival and recurrence rates were determined with a stepwise Cox proportional hazards model. All statistical tests were two-sided.** ***Results:*** **Ninety-nine percent of patients achieved complete clinical remission. Levels of post-treatment EBV DNA dominated the effect of levels of pretreatment EBV DNA for progression-free survival. The RR for NPC recurrence was 11.9 (95% confidence interval [CI] = 5.53 to 25.43) for patients with higher post-treatment EBV DNA and 2.5 (95% CI = 1.14 to 5.70) for patients with higher pretreatment EBV DNA. Higher levels of post-treatment EBV DNA were statistically significantly associated with overall survival (*$P<.001$*; RR for NPC recurrence = 8.6, 95% CI = 3.69 to 19.97). The positive and negative predictive values for NPC recurrence for a higher level of post-treatment EBV DNA were 87% (95% CI = 58% to 98%) and 83% (95% CI = 76% to 89%), respectively.** ***Conclusion:*** **Levels of post-treatment plasma EBV DNA in patients with NPC appear to strongly predict progression-free and overall survival and to accurately reflect the post-treatment residual tumor load. [J Natl Cancer Inst 2002;94: 1614–9]**

Nasopharyngeal carcinoma (NPC) is endemic in southern China, and nearly all patients harbor Epstein-Barr virus (EBV) in their tumor tissues (*1,2*). Most patients with early-stage NPC will achieve a complete clinical remission after radiotherapy, with or without concurrent chemotherapy, with no clinical evidence of residual disease (*3–7*). Despite this high rate of initial local control, disease will subsequently recur in 30%–40% of patients with advanced NPC at the local site or with distant metastases (*3*). Such treatment failures, presumably, represent residual microscopic disease that is not detected by current imaging procedures. If such patients with a high risk of recurrence could be identified, they might benefit from more intensive primary treatment or adjuvant therapy, and those at low risk of recurrence could be spared such potentially toxic treatment.

Prompted by reports that tumor-derived DNA can be detected in the plasma and serum of cancer patients (*8,9*), we developed a real-time quantitative polymerase chain reaction (PCR) assay for measuring circulating tumor-derived EBV DNA in patients with NPC (*10–12*). The level of pretreatment EBV DNA is strongly associated with overall survival and is a more powerful prognostic factor than stage (*13*). In a small case–control study (*11*), we have previously observed that patients who relapsed after radiotherapy often had residual high levels of EBV DNA, whereas patients with continuous clinical remission had continuous low or undetectable levels of EBV DNA.

We now report a large prospective study involving 170 patients with a median follow-up of more than 2 years after radiotherapy. We sought to more rigorously test our original hypothesis that the levels of EBV DNA after completion of conventional treatment, perhaps in combination with pretreatment levels, might be associated with the presence or absence of residual disease. We assumed that residual disease would eventually be detected as disease recurrence and that because such disease is usually incurable, it would ultimately be reflected by a statistically significant decrease in survival.

Patients and Methods

Patients

One hundred seventy patients with newly diagnosed NPC were recruited to the study between September 25, 1997, and October 5, 1999. The study was approved by the Ethics Committee of the Chinese University of Hong Kong. Patients were investigated uniformly with endoscopic examination of the nasopharynx and computed tomography of the nasopharynx and neck. In patients with advanced disease that had metastasized to the supraclavicular lymph nodes [stage N3b, according to the

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Correspondence author: Philip J. Johnson, M.D., F.R.C.P., Department of Clinical Oncology, The Chinese University of Hong Kong, Prince of Wales Hospital, 30–32 Ngan Shing St., Shatin, New Territories, Hong Kong Special Administrative Region (e-mail: pjohnson@clo.cuhk.edu.hk).

See "Notes" following "References."

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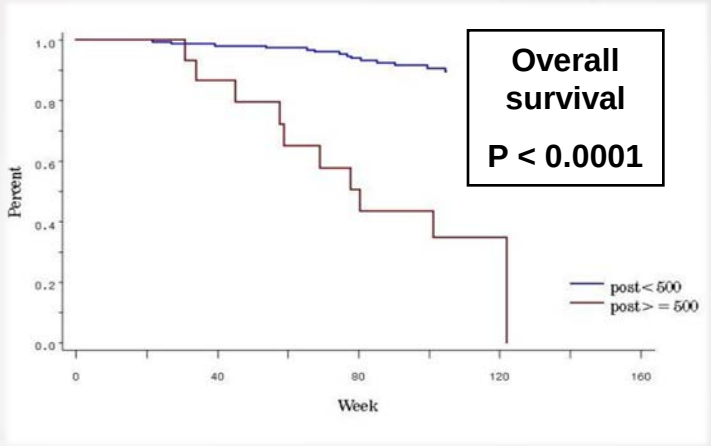

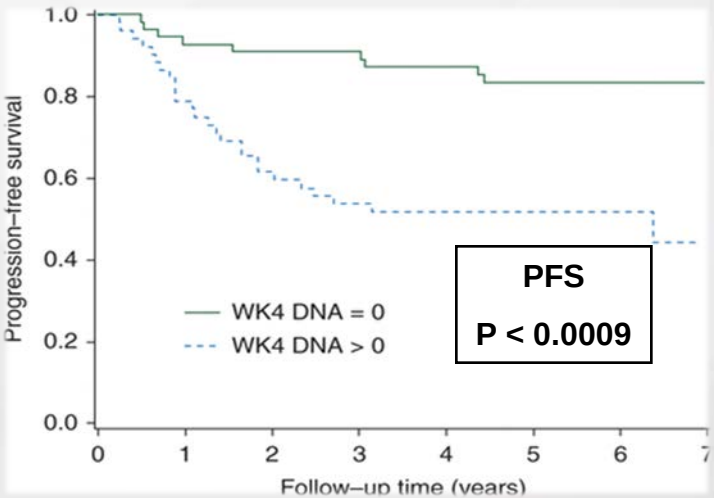

- Plasma EBV DNA level taken 6-8 weeks post RT: OS analysis using cutoff 500 copies/ml. Relative risk for recurrence: 11.9 (5.5-25.4)

- Plasma EBV DNA level taken week 4 during RT: HR 12.02; 95% CI 2.78–51.93, P = 0.0009), PFS (HR 4.05, 95% CI 1.89–8.67, P = 0.0003).

Chan ATC…YMD Lo JNCI 02;94:1614-9.

Leung SF et al Ann Oncol. 2014 Jun;25(6):1204-8

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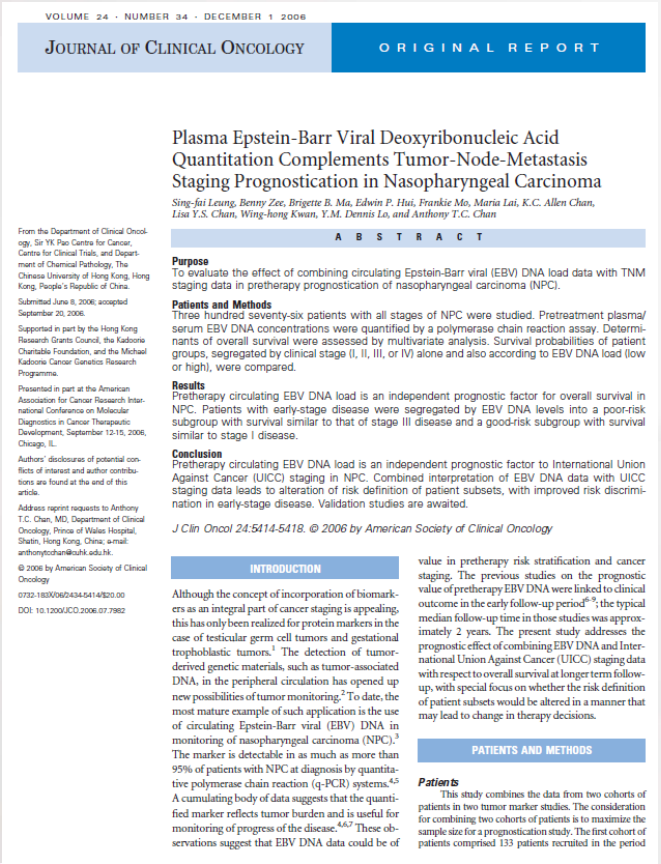
Plasma EBV DNA is a better discriminator of survival than TNM stage for stage I-II NPC

- 2 cohorts: N = 133 ('93-4), N = 243 ('97-00). T1-T2a received brachytherapy
- Most = RT alone. 38 had cisplatin-RT



VOLUME 24 · NUMBER 34 · DECEMBER 1 2006

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Plasma Epstein-Barr Viral Deoxyribonucleic Acid Quantitation Complements Tumor-Node-Metastasis Staging Prognostication in Nasopharyngeal Carcinoma

Sing-fai Leung, Benny Zee, Brigette B. Ma, Edwin P. Hui, Frankie Mo, Maria Lai, K.C. Allen Chan, Lisa Y.S. Chan, Wing-hong Kwan, Y.M. Dennis Lo, and Anthony T.C. Chan

From the Department of Clinical Oncology, Sir YK Pao Centre for Cancer, Centre for Clinical Trials, and Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong, People's Republic of China.

Submitted June 8, 2006; accepted September 20, 2006.

Supported in part by the Hong Kong Research Grants Council, the Kadoorie Charitable Foundation, and the Michael Kadoorie Cancer Genetics Research Programme.

Presented in part at the American Association for Cancer Research International Conference on Molecular Diagnostics in Cancer Therapeutic Development, September 12-15, 2006, Chicago, IL.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/06/2434-5414/$20.00

DOI: 10.1200/JCO.2006.07.7982

A B S T R A C T

Purpose
To evaluate the effect of combining circulating Epstein-Barr viral (EBV) DNA load data with TNM staging data in pretherapy prognostication of nasopharyngeal carcinoma (NPC).

Patients and Methods
Three hundred seventy-six patients with all stages of NPC were studied. Pretreatment plasma/serum EBV DNA concentrations were quantified by a polymerase chain reaction assay. Determinants of overall survival were assessed by multivariate analysis. Survival probabilities of patient groups, segregated by clinical stage (I, II, III, or IV) alone and also according to EBV DNA load (low or high), were compared.

Results
Pretherapy circulating EBV DNA load is an independent prognostic factor for overall survival in NPC. Patients with early-stage disease were segregated by EBV DNA levels into a poor-risk subgroup with survival similar to that of stage III disease and a good-risk subgroup with survival similar to stage I disease.

Conclusion
Pretherapy circulating EBV DNA load is an independent prognostic factor to International Union Against Cancer (UICC) staging in NPC. Combined interpretation of EBV DNA data with UICC staging data leads to alteration of risk definition of patient subsets, with improved risk discrimination in early-stage disease. Validation studies are awaited.

J Clin Oncol 24:5414-5418. © 2006 by American Society of Clinical Oncology

INTRODUCTION

Although the concept of incorporation of biomarkers as an integral part of cancer staging is appealing, this has only been realized for protein markers in the case of testicular germ cell tumors and gestational trophoblastic tumors.[1] The detection of tumor-derived genetic materials, such as tumor-associated DNA, in the peripheral circulation has opened up new possibilities of tumor monitoring.[2] To date, the most mature example of such application is the use of circulating Epstein-Barr viral (EBV) DNA in monitoring of nasopharyngeal carcinoma (NPC).[3] The marker is detectable in as much as more than 95% of patients with NPC at diagnosis by quantitative polymerase chain reaction (q-PCR) systems.[4,5] A cumulating body of data suggests that the quantified marker reflects tumor burden and is useful for monitoring of progress of the disease.[4,6,7] These observations suggest that EBV DNA data could be of value in pretherapy risk stratification and cancer staging. The previous studies on the prognostic value of pretherapy EBV DNA were linked to clinical outcome in the early follow-up period[6-9]; the typical median follow-up time in those studies was approximately 2 years. The present study addresses the prognostic effect of combining EBV DNA and International Union Against Cancer (UICC) staging data with respect to overall survival at longer term follow-up, with special focus on whether the risk definition of patient subsets would be altered in a manner that may lead to change in therapy decisions.

PATIENTS AND METHODS

Patients

This study combines the data from two cohorts of patients in two tumor marker studies. The consideration for combining two cohorts of patients is to maximize the sample size for a prognostication study. The first cohort of patients comprised 133 patients recruited in the period

Table 2. Actuarial Survival of Patient Groups With Different UICC Stages and With Different EBV DNA Levels Within UICC Stages

Stage	No. of Patients	5-Year Survival (%)	95% CI (%)
I	36	92	83 to 100
II	119	80	73 to 88
III	95	73	64 to 82
IV	126	47	38 to 56
I + II, low DNA*	108	91	85 to 97
I + II, high DNA*	47	64	53 to 75
III + IV, low DNA	73	66	50 to 81
III + IV, high DNA	148	54	44 to 65

Abbreviations: UICC, International Union Against Cancer; EBV, Epstein-Barr virus.

*Low DNA denotes low EBV DNA levels of < 4,000 copies/mL; high DNA denotes EBV DNA levels of ≥ 4,000 copies/mL.

Leung SF, et al. J Clin Oncol 24:5414-5418.

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VOLUME 36 · NUMBER 31 · NOVEMBER 1, 2018

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Analysis of Plasma Epstein-Barr Virus DNA in Nasopharyngeal Cancer After Chemoradiation to Identify High-Risk Patients for Adjuvant Chemotherapy: A Randomized Controlled Trial

Anthony T.C. Chan, Edwin P. Hui, Roger K.C. Ngan, Stewart Y. Tung, Ashley C.K. Cheng, Wai T. Ng, Victor H.F. Lee, Brigette B.Y. Ma, Hoi C. Cheng, Frank C.S. Wong, Herbert H.F. Loong, Macy Tong, Darren M.C. Poon, Anil T. Ahuja, Ann D. King, Ki Wang, Frankie Mo, Benny C.Y. Zee, K.C. Allen Chan, and Y.M. Dennis Lo

CU Medicine HONG KONG

- UICC (6th Ed) stage IIB, III, IVA or IVB NPC
- No clinical and radiological evidence of distant metastasis (M0)
- No clinical evidence of persistent loco-regional disease after RT or CRT
- ECOG 0 or 1
- Adequate organ function
- Detectable plasma EBV-DNA (>0 copy/ml) at 6-8 weeks after completion of RT or CRT

RANDOMISE

Stratification:
- RT vs CRT
- Stage II/III vs IV

EBV-DNA PET-CT (0 month)	Treatment Arm	EBV-DNA PET-CT (6 months)
Arm 1		
√ √	Adjuvant Chemotherapy (Cisplatin-gemcitabine x 6)	√ √
Arm 2		
√ √	Clinical Observation and Surveillance	√ √

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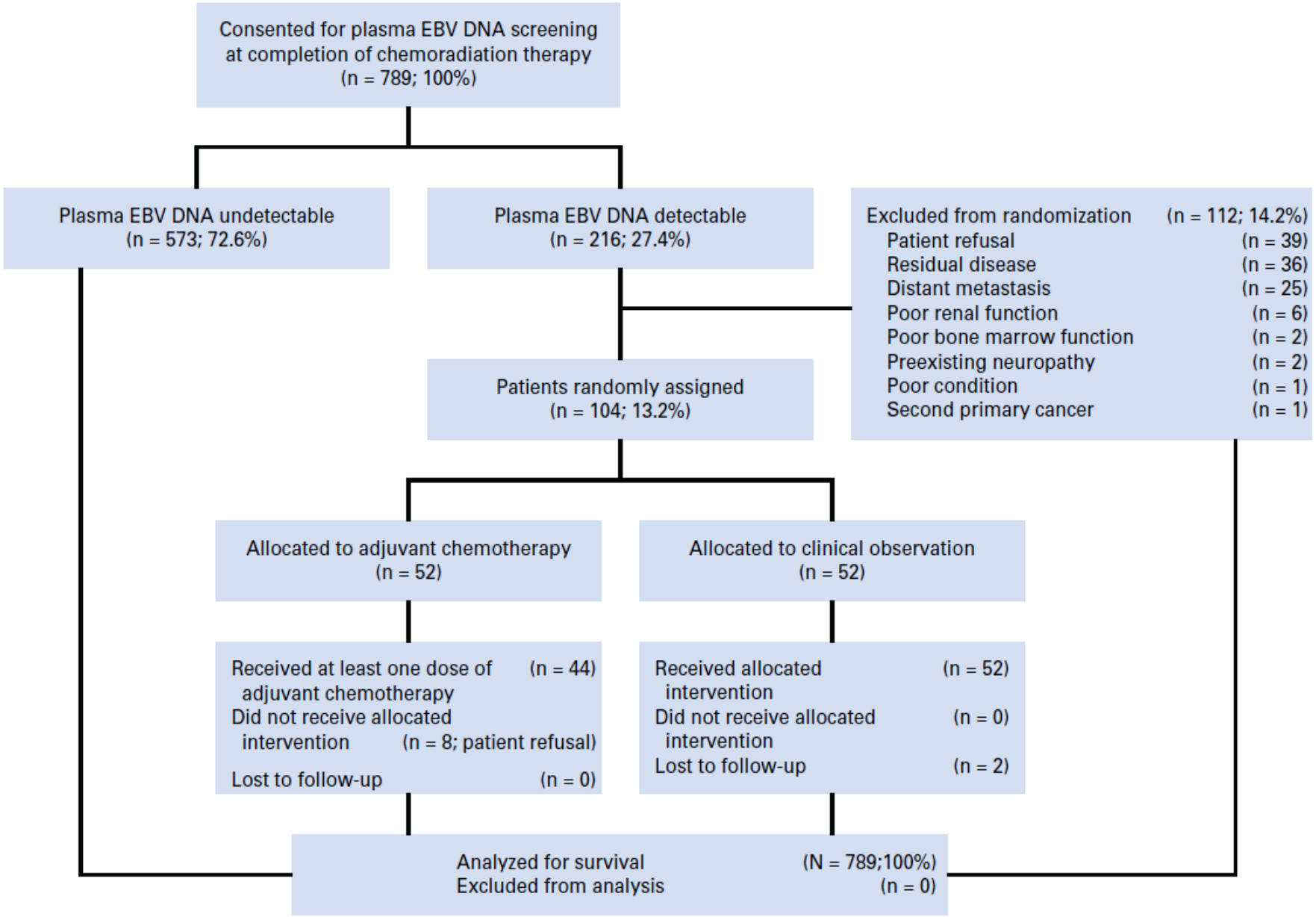

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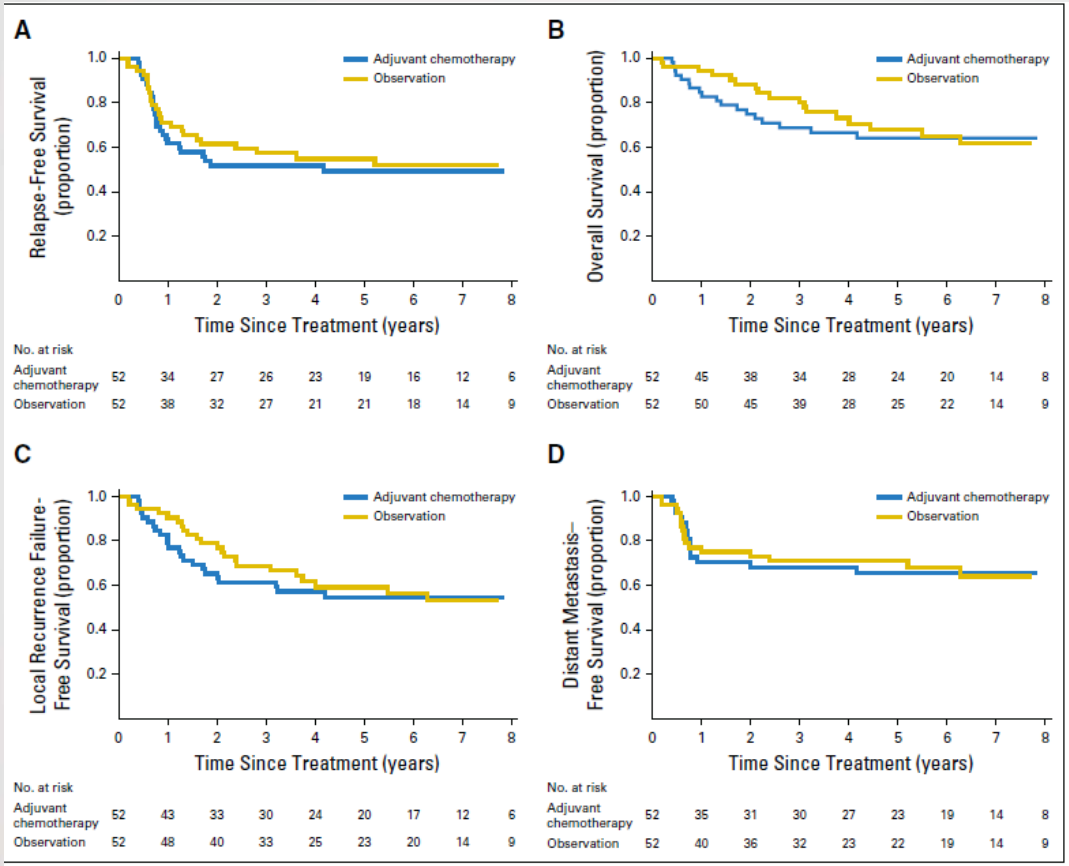
EBV DNA is prognostic but not predictive

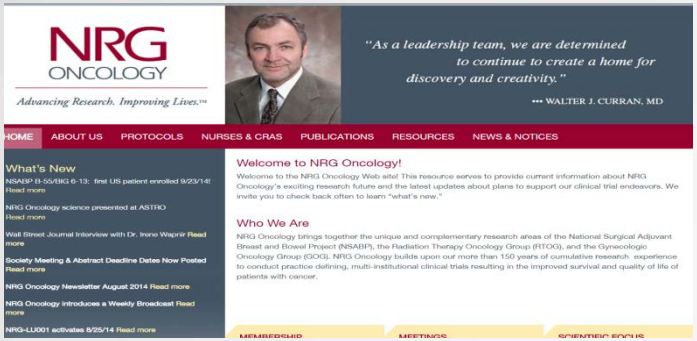


Recursive-partitioning Analysis (RPA) presented by Dr Hui (ESMO Asia 2017, oral, oncology Pro):

- Low risk group: EBV DNA <50 and stage II/III (n=518), 5yr OS 89.2%
- Intermediate risk group: EBV DNA <50 and stage IV (n=155). 5yr OS 78.3%
- High risk group: EBV DNA ≥50 (n=116). 5-year OS 42.2% ($p < 0.001$).

https://oncologypro.esmo.org/Meeting-Resources/ESMO-Asia-2017-Congress/Biomarker-analysis-of-randomized-controlled-trial-RCT-of-adjuvant-chemotherapy-CT-using-plasma-EBV-DNA-to-identify-patients-pts-at-higher-risk-of-relapse-after-radiotherapy-RT-or-chemoradiation-CRT-in-nasopharyngeal-cancer-NPC-336O

Randomized Phase II and Phase III Studies of Individualized Treatment for NPC Based on plasma EBV DNA



NRG ONCOLOGY

NRG-HN001
ClinicalTrials.gov NCT02135042

RANDOMIZED PHASE II AND PHASE III STUDIES OF INDIVIDUALIZED TREATMENT FOR NASOPHARYNGEAL CARCINOMA BASED ON BIOMARKER EPSTEIN BARR VIRUS (EBV) DEOXYRIBONUCLEIC ACID (DNA)

This trial is part of the National Clinical Trials Network (NCTN) program, which is sponsored by the National Cancer Institute (NCI). The trial will be led by NRG Oncology with the participation of the network of NCTN organizations: the Alliance for Clinical Trials in Oncology, ECOG-ACRIN Medical Research Foundation, Inc., and SWOG.

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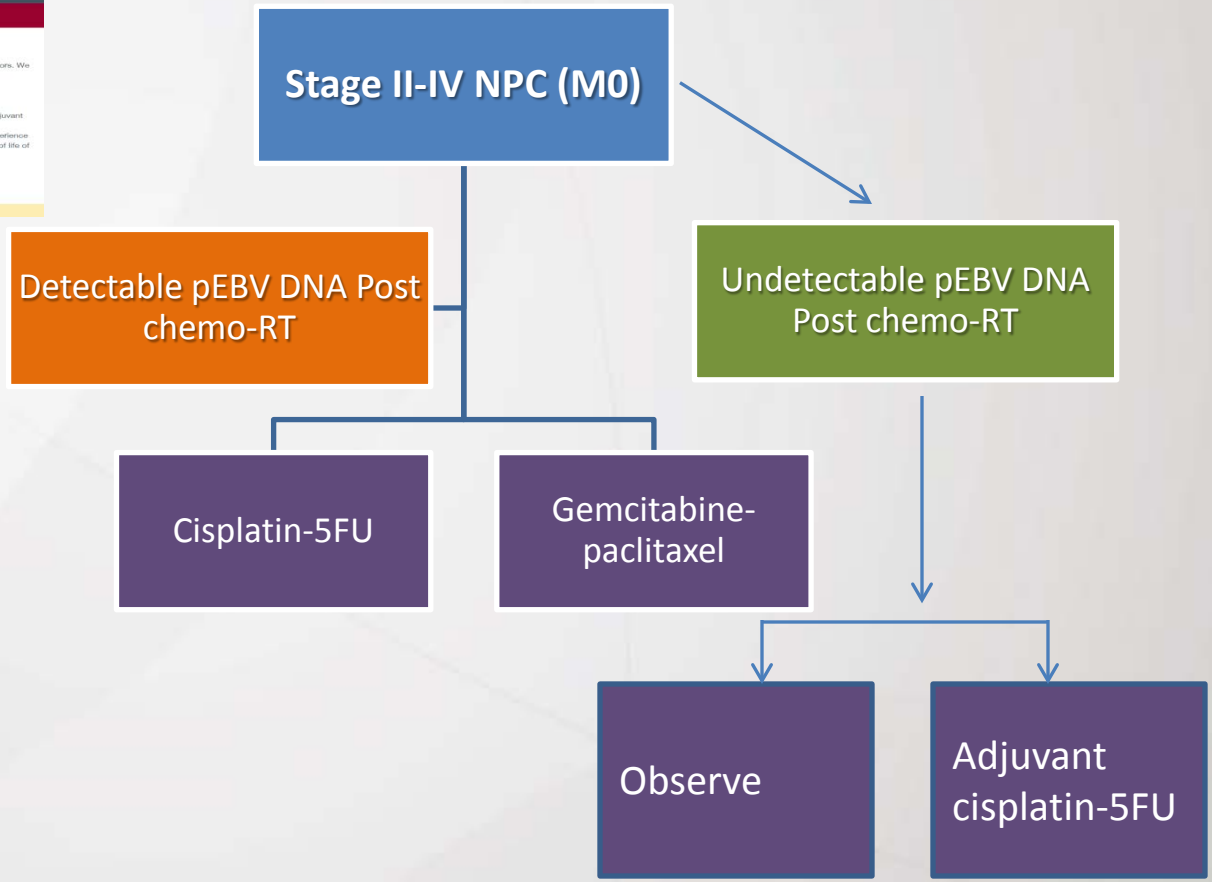

https://clinicaltrials.gov/ct2/show/NCT02135042

Screening Study for Early Detection of Nasopharynx Cancer

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 10, 2017 VOL. 377 NO. 6

Analysis of Plasma Epstein–Barr Virus DNA to Screen for Nasopharyngeal Cancer

K.C. Allen Chan, F.R.C.P.A., John K.S. Woo, F.R.C.S., Ann King, F.R.C.R., Benny C.Y. Zee, Ph.D., W.K. Jacky Lam, F.R.C.S., Stephen L. Chan, F.R.C.P., Sam W.I. Chu, B.Sc., Constance Mak, B.S.N., Irene O.L. Tse, B.N., Samantha Y.M. Leung, B.N., Gloria Chan, R.N., Edwin P. Hui, F.R.C.P., Brigette B.Y. Ma, M.D., Rossa W.K. Chiu, F.R.C.P.A., Sing-Fai Leung, F.R.C.R., * Andrew C. van Hasselt, F.R.C.S., Anthony T.C. Chan, F.R.C.P., and Y.M. Dennis Lo, F.R.S.

ABSTRACT

BACKGROUND

Circulating cell-free Epstein–Barr virus (EBV) DNA is a biomarker for nasopharyngeal carcinoma. We conducted a prospective study to investigate whether EBV DNA in plasma samples would be useful to screen for early nasopharyngeal carcinoma in asymptomatic persons.

METHODS

We analyzed EBV DNA in plasma specimens to screen participants who did not have symptoms of nasopharyngeal carcinoma. Participants with initially positive results were retested approximately 4 weeks later, and those with persistently positive EBV DNA in plasma underwent nasal endoscopic examination and magnetic resonance imaging (MRI).

RESULTS

A total of 20,174 participants underwent screening. EBV DNA was detectable in plasma samples obtained from 1112 participants (5.5%), and 309 (1.5% of all participants and 27.8% of those who initially tested positive) had persistently positive results on the repeated sample. Among these 309 participants, 300 underwent endoscopic examination, and 275 underwent both endoscopic examination and MRI; of these participants, 34 had nasopharyngeal carcinoma. A significantly higher proportion of participants with nasopharyngeal carcinoma that was identified by screening had stage I or II disease than in a historical cohort (71% vs. 20%, P<0.001 by the chi-square test) and had superior 3-year progression-free survival (97% vs. 70%; hazard ratio, 0.10; 95% confidence interval, 0.05 to 0.18). Nine participants declined to undergo further testing, and 1 of them presented with advanced nasopharyngeal carcinoma 32 months after enrollment. Nasopharyngeal carcinoma developed in only 1 participant with negative EBV DNA in plasma samples within 1 year after testing. The sensitivity and specificity of EBV DNA in plasma samples in screening for nasopharyngeal carcinoma were 97.1% and 98.6%, respectively.

CONCLUSIONS

Analysis of EBV DNA in plasma samples was useful in screening for early asymptomatic nasopharyngeal carcinoma. Nasopharyngeal carcinoma was detected significantly earlier and outcomes were better in participants who were identified by screening than in those in a historical cohort. (Funded by the Kadoorie Charitable Foundation and the Research Grants Council of the Hong Kong government; ClinicalTrials.gov number, NCT02063399.)

From the Li Ka Shing Institute of Health Sciences (K.C.A.C., W.K.J.L., S.W.I.C., C.M., I.O.L.T., S.Y.M.L., G.C., R.W.K.C., Y.M.D.L.), the Department of Chemical Pathology (K.C.A.C., W.K.J.L., S.W.I.C., C.M., I.O.L.T., S.Y.M.L., G.C., R.W.K.C., Y.M.D.L.), State Key Laboratory of Oncology in South China, Sir Y.K. Pao Centre for Cancer (K.C.A.C., W.K.J.L., S.L.C., S.W.I.C., C.M., I.O.L.T., S.Y.M.L., G.C., E.P.H., B.B.Y.M., R.W.K.C., S.-F.L., A.T.C.C., Y.M.D.L.), Department of Otorhinolaryngology, Head and Neck Surgery (J.K.S.W., W.K.J.L., A.C.H.), Department of Imaging and Interventional Radiology (A.K.), Jockey Club School of Public Health and Primary Care (B.C.Y.Z.), and Department of Clinical Oncology (S.L.C., E.P.H., B.B.Y.M., S.-F.L., A.T.C.C.), Chinese University of Hong Kong, Prince of Wales Hospital — all in Hong Kong. Address reprint requests to Dr. K.C. Allen Chan or Dr. Lo at the Department of Chemical Pathology, Chinese University of Hong Kong, Prince of Wales Hospital, 30–32 Ngan Shing St., Shatin, Hong Kong, or at allen@cuhk.edu.hk or loym@cuhk.edu.hk.

*Deceased.

N Engl J Med 2017;377:513-22.
DOI: 10.1056/NEJMoa1701717
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- Pilot study: N = 1318 40-60yrs male subjects, 69 had detectable pEBV DNA had NP scope. FU 2 yrs.
- 3 confirmed NPC
- Ongoing: 20,000 subjects

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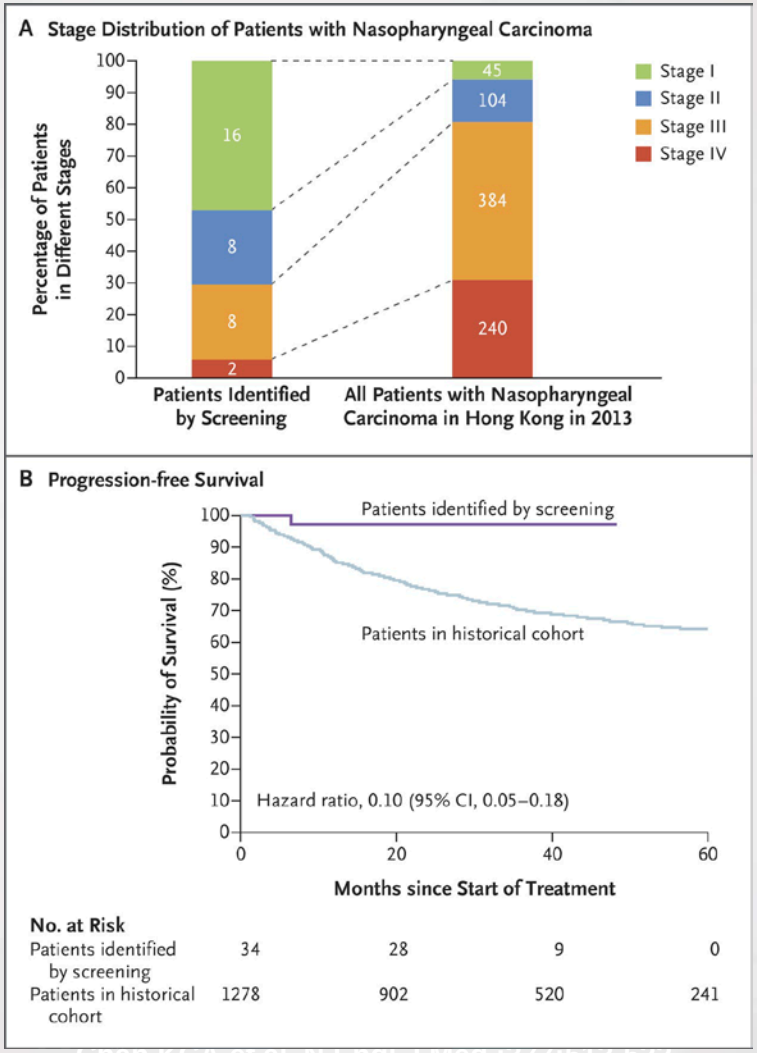
https://www.lihs.cuhk.edu.hk/en-us/newsandevents/news.aspx?udt_524_param_detail=159

KC Chan et al Cancer 2013

Stage Distribution and Progression-free Survival among the Participants with NPC Identified by Screening



Chan KCA et al. N Engl J Med ;377:513-522

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Sensitivity and Specificity of the Two-Stage Screening Protocol for the Detection of Nasopharyngeal Carcinoma.

Table 2. Sensitivity and Specificity of the Two-Stage Screening Protocol for the Detection of Nasopharyngeal Carcinoma.*

Finding	Screen-Positive (N = 308)†	Screen-Negative (N = 19,865)
Confirmed nasopharyngeal carcinoma by the screening protocol or nasopharyngeal carcinoma reported to have developed within 1 yr — no.	34	1
No nasopharyngeal carcinoma within 1 yr after screening — no.	274	19,864
Sensitivity — % (95% CI)	97.1 (95.5–98.7)	
Specificity — % (95% CI)	98.6 (98.6–98.7)	
Positive predictive value — % (95% CI)	11.0 (10.7–11.3)	
Negative predictive value — % (95% CI)	99.995 (99.99–100.00)	
Proportion of stage I/II disease in the 34 cases of nasopharyngeal carcinoma identified by screening — % (95% CI)	70.6 (69.6–72.5)	

* Screen-positive is defined as persistently positive for plasma EBV DNA at baseline and at follow-up. Screen-negative is defined as negative for plasma EBV DNA either at baseline or at follow-up.

† The participant who had declined further investigation but in whom advanced nasopharyngeal carcinoma developed 32 months after screening is not included in this number.

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Practice guidelines

clinical practice guidelines

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Nasopharyngeal cancer: EHNS–ESMO–ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

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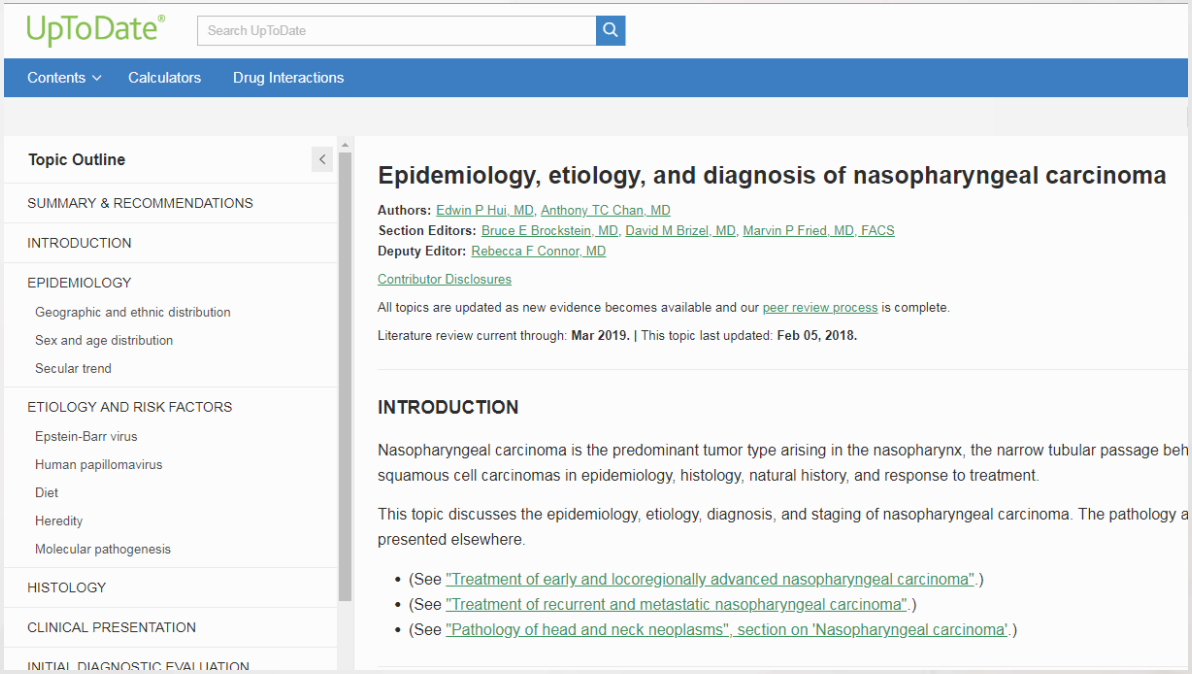

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