



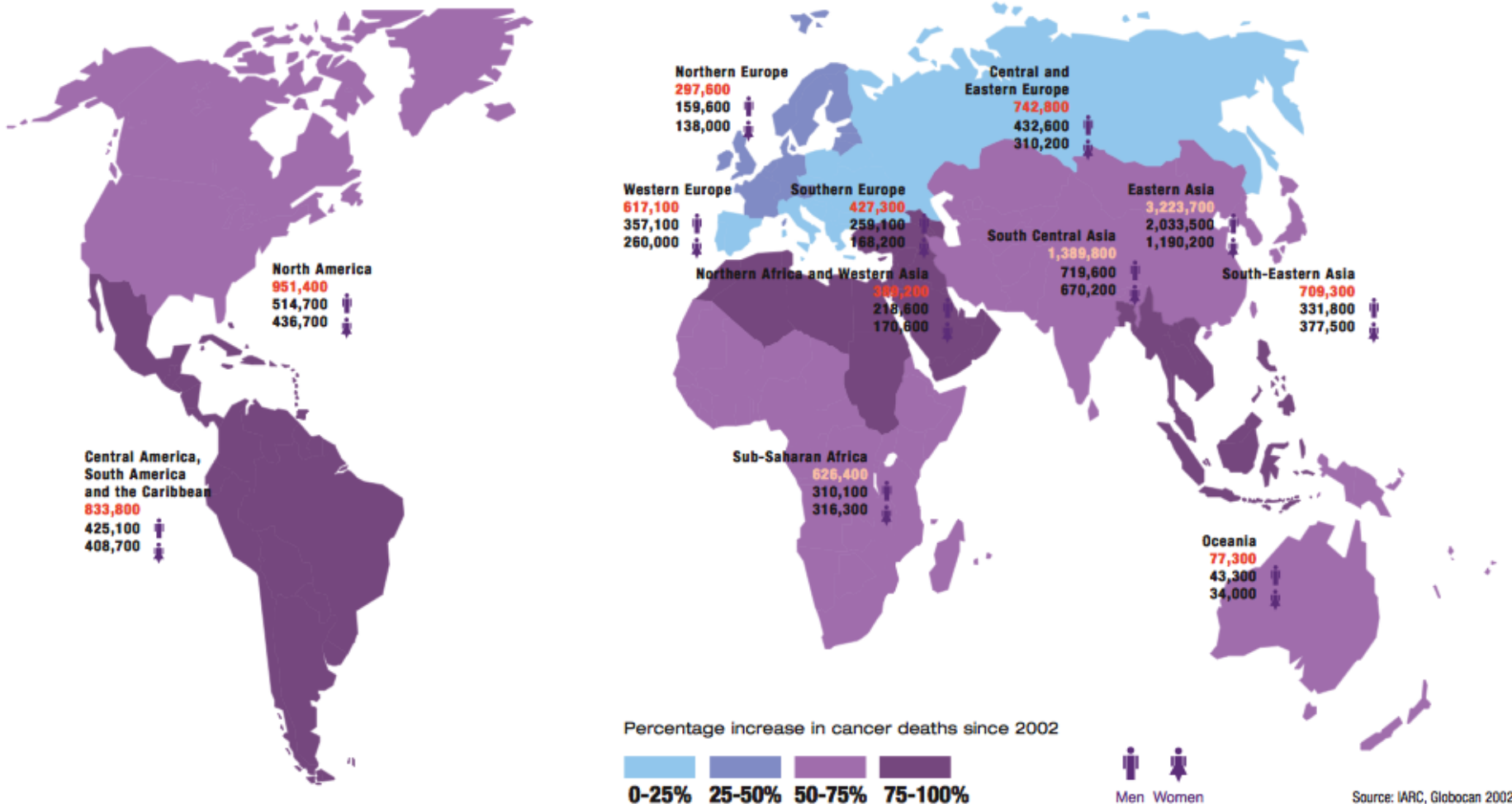
Reversing Cancer

Is Nasopharyngeal Cancer Treatable?

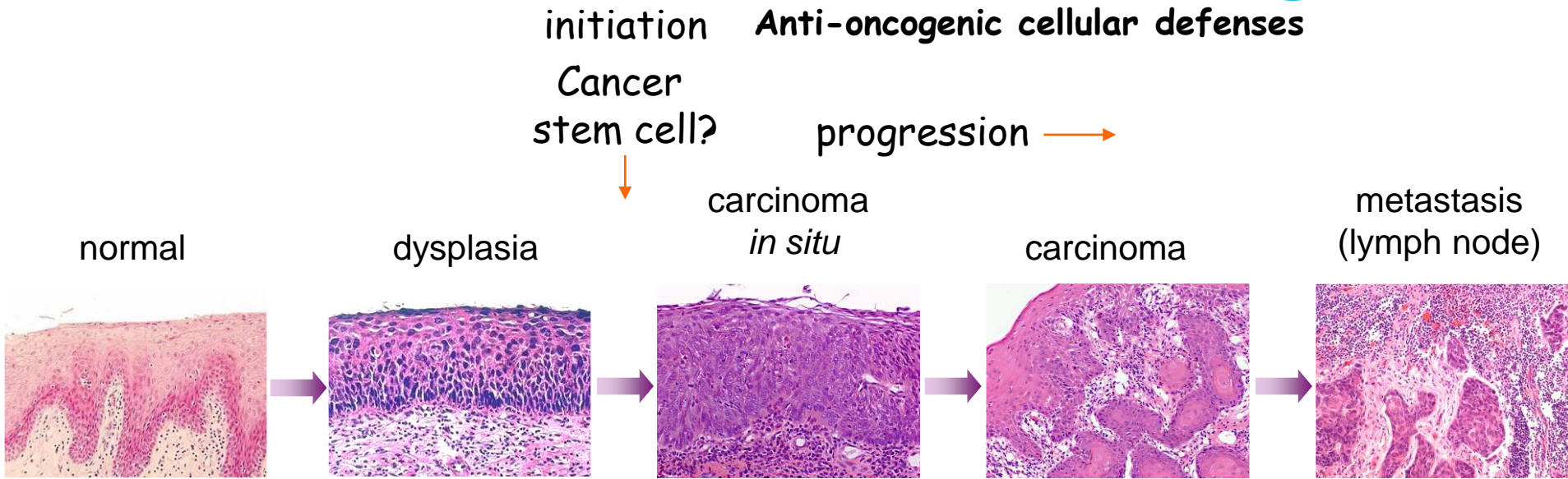
Huimei Lee and Vyomesh Patel
Cancer Research Malaysia

GLOBAL CANCER BURDEN

By **2020**, cancer **could kill 10.3 million**
people per year unless we act



Head and Neck (Oral) Cancer Progression



- Mobilize our community:**
- OC web-based genomics/proteomics data mining tools
 - Unlock the potential of tissue banks
 - Engage/train dental students
 - Funding mechanisms

potentially malignant disorders

molecular events
diagnostic markers

(chemo)prevention

- roadblocks:
- tissues
 - enabling techniques
 - molecular candidates

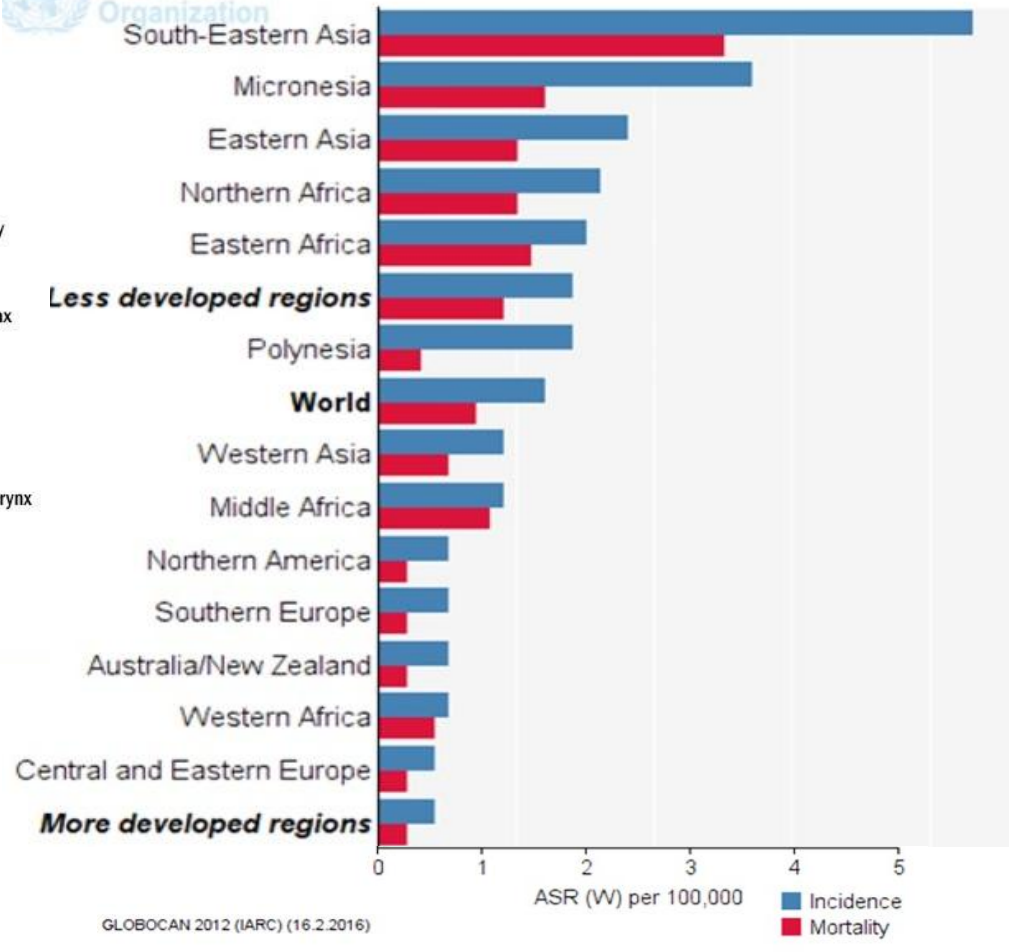
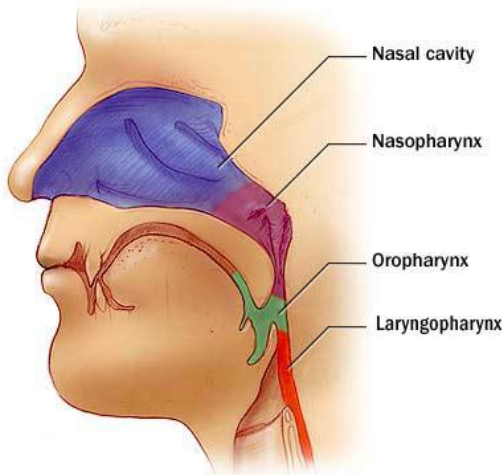
epithelial stem cells, predictive cellular systems, animal models

molecular events
diagnostic markers

molecular targeted therapies

- NGS (many): LCM
tumor/tumor-metastasis
OC genome, epigenome, miRNAs
proteomics, integration!

International Agency for Cancer Research **Nasopharynx: both sexes, all ages**



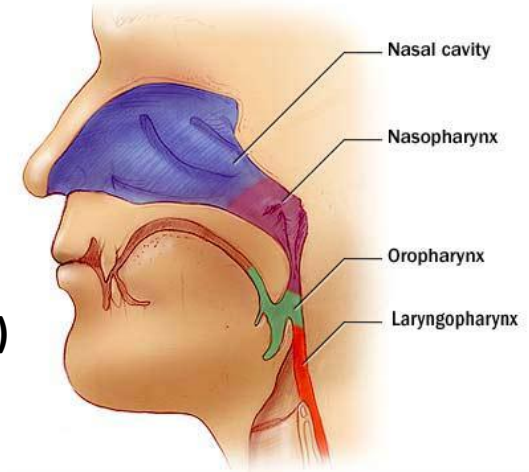
- NPC is more prevalent in Asian region
- Majority of the patients diagnosed at late stage

Signs and symptoms

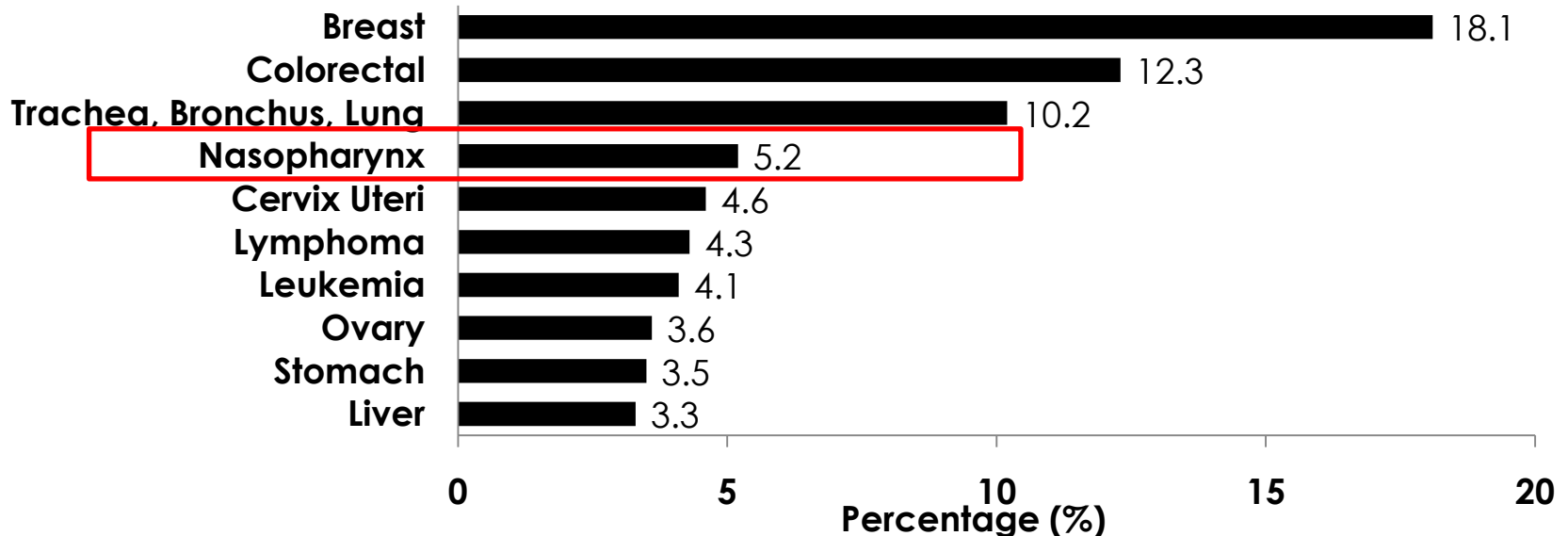
- Hearing loss, ringing in the ear, or feeling of fullness in the ear (especially on one side only)
- Ear infections that keep coming back
- Nasal blockage or stuffiness
- Nosebleeds
- Headache
- Facial pain or numbness
- Trouble opening the mouth
- Blurred or double vision

Nasopharyngeal Carcinoma

- Cancer occur at the back of the nose.
- 4th most common cancer among Malaysians;
3rd most common in **Malaysian men**.
- Risk factors:
 - **Genetic**
 - **Viral (Epstein-Barr Virus)**
 - **Diet (preserved food)/ environmental (occupational hazards)**



10 Most Common Cancers in Malaysia 2007



Nasopharyngeal Carcinoma: Challenges

- **Current treatment modality**

- Radiotherapy/ IMRT
- Chemotherapy
- Surgery

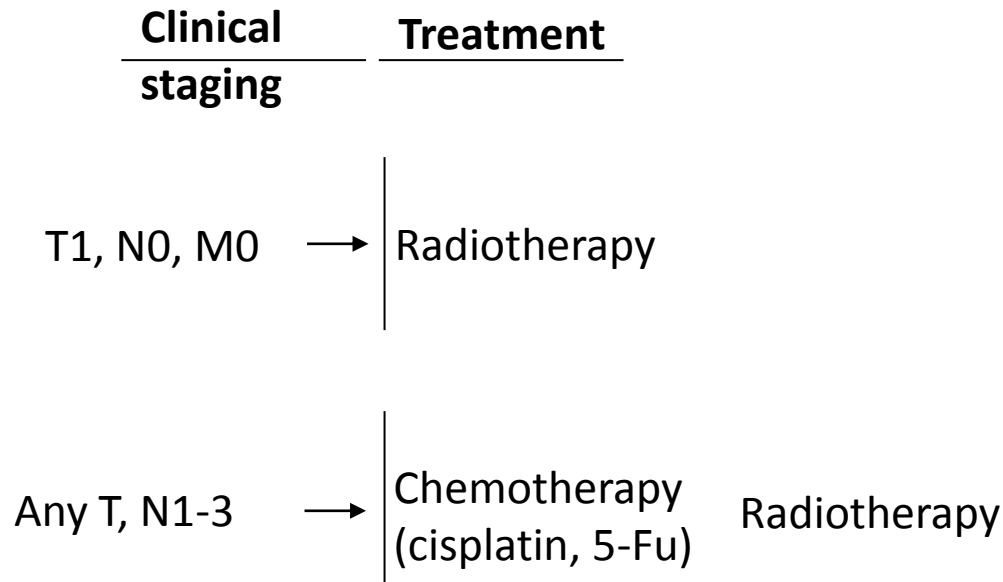


- **Challenges:**

- > 80% survival and good local control (concurrent CRT/ IMRT) – for early stage patients
 - Poor prognosis, 5-year survival → 41 - 63%
 - Poor quality of life – dry mouth, unable to swallow, loss of taste
- Relapse/ metastasis → leading cause of death in NPC
- No specific biomarkers/ therapeutic target

- **Search for biomarker for diagnosis and treatment options (molecular targets)**

Urgent need of new targeted therapy



NCCN, 2010

- Development of novel therapies for NPC has been slow
- Little advances beyond standard cytotoxic approaches
- Exploratory phase II studies

Challenges in NPC research



APS | Acta
Pharmacologica
Sinica

Altmetric: 0 Citations: 16

[More detail >>](#)

Letter to the Editor | Published: 01 July 2008

Cell line cross-contamination in biomedical research: a call to prevent unawareness

Armando Rojas , Ileana Gonzalez & Héctor Figueroa

Acta Pharmacologica Sinica **29**, 877–880 (2008) | [Download Citation ↓](#)

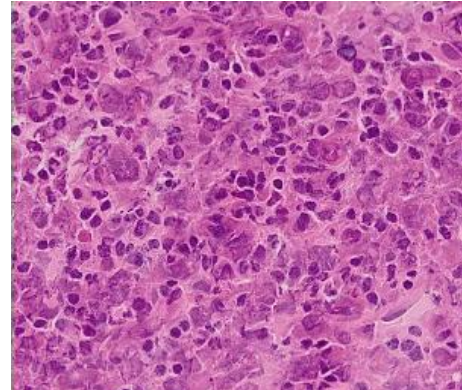
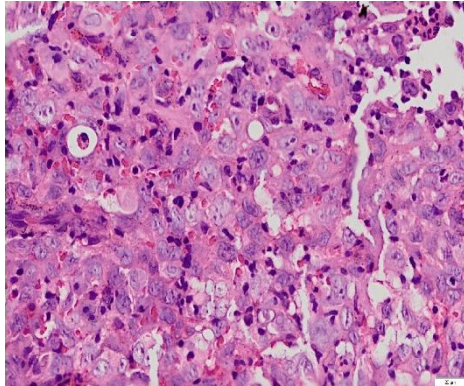
- Many NPC cell lines (CNE-1, CNE-2, AdAH, NPC-KT and HONE) are contaminated by HeLa
(Chan et al, 2008; Strong et al, 2014)
- Solution – make new NPC cell lines

Characterization – type of NPC

FFPE

Fresh frozen

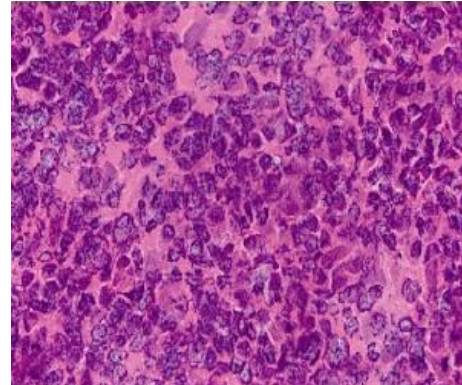
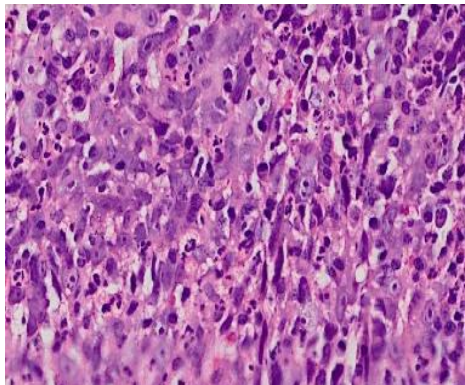
NPC 248



Type 3 (non-keratinizing, undifferentiated)

- High nuclei to cytoplasmic ratio
- Indistinct cytoplasmic margins

NPC 253

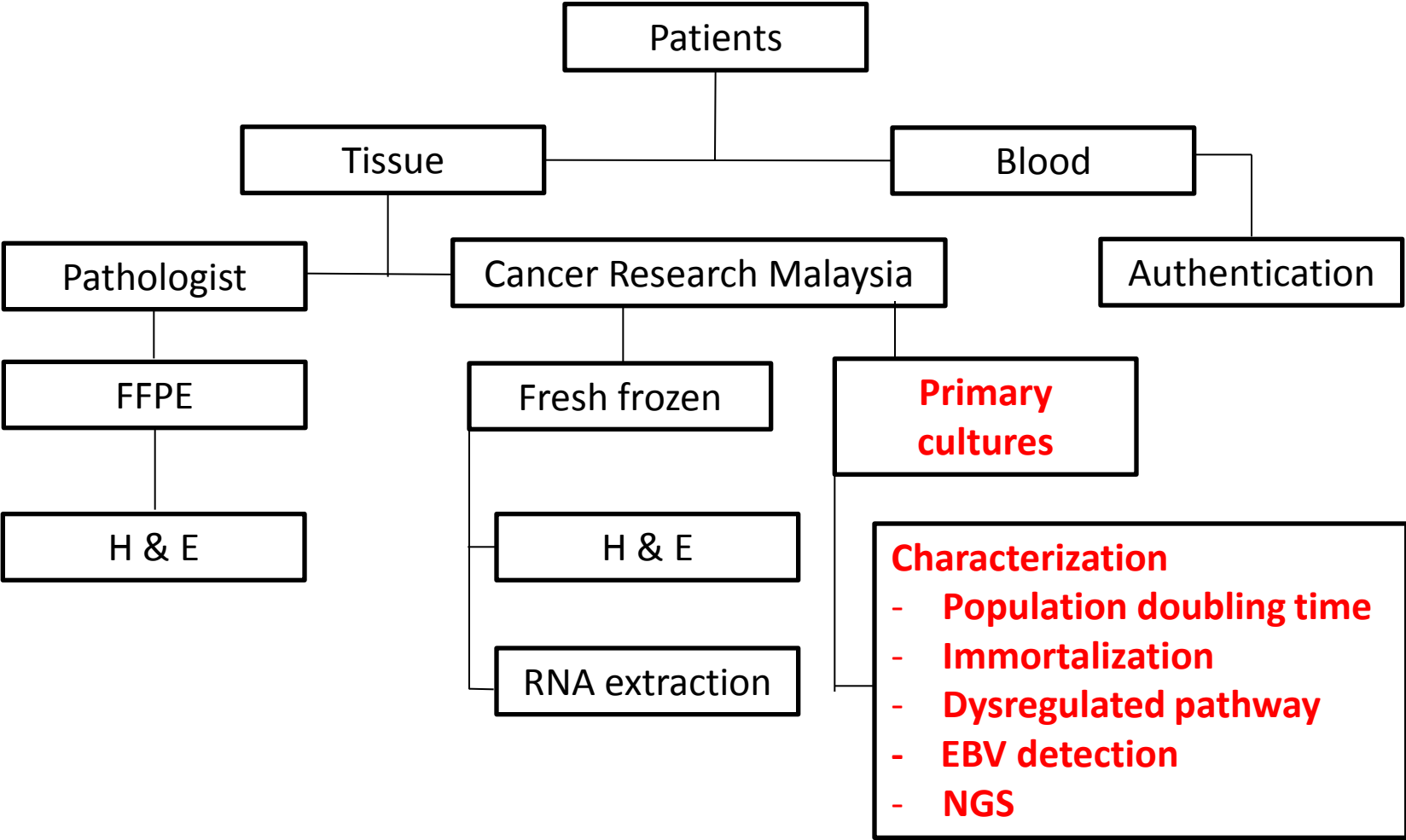


Type 2 (non-keratinizing, differentiated)

- High nuclei to cytoplasmic ratio
- Spindled-like, dark and elongated nuclei

Cross comparison of FFPE tissues and fresh frozen biopsy from ENT surgeon

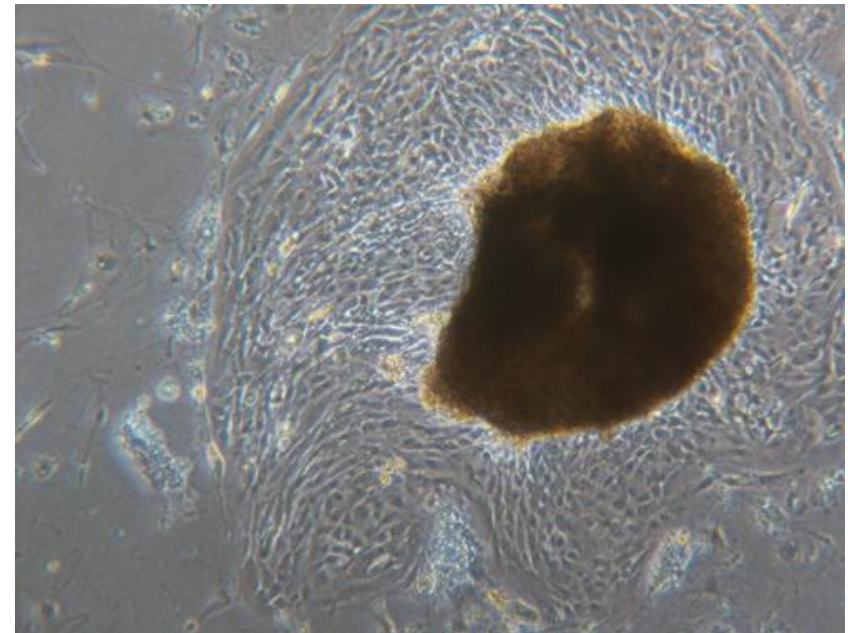
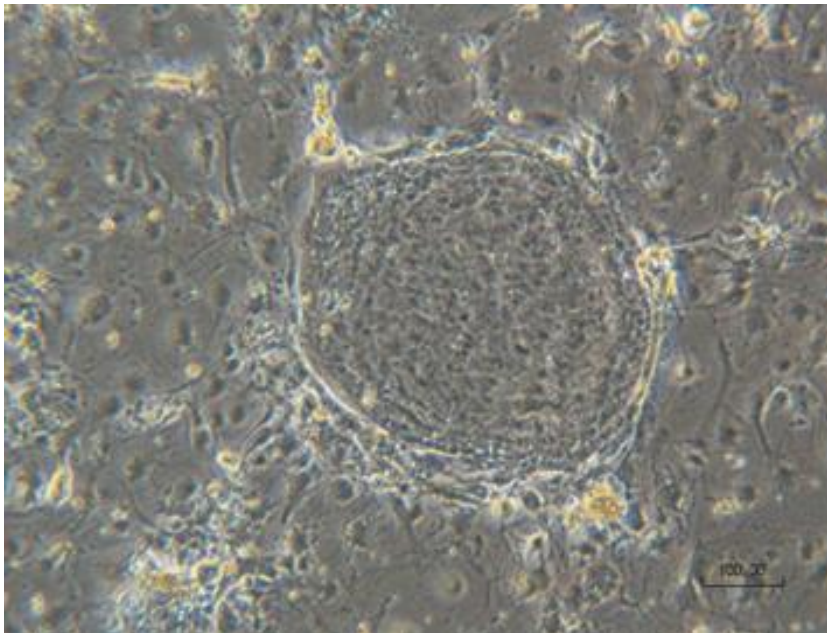
Research Approach



Conditional reprogramming and long-term expansion of normal and tumor cells from human biospecimens

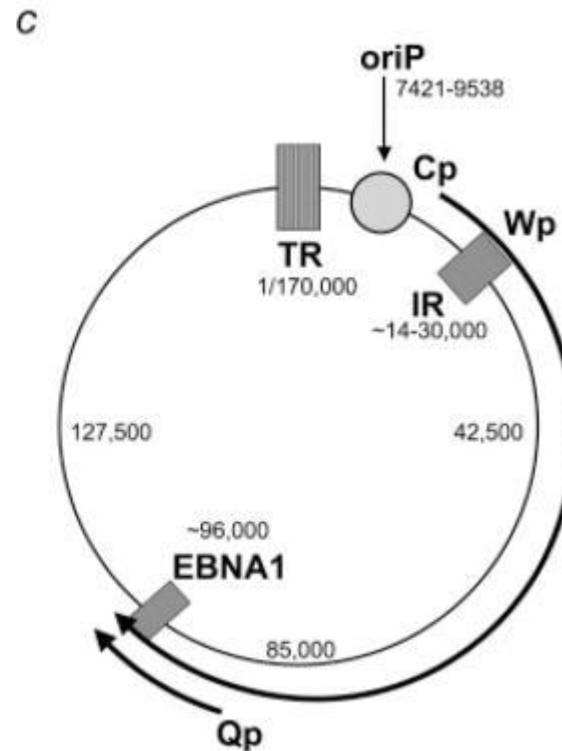
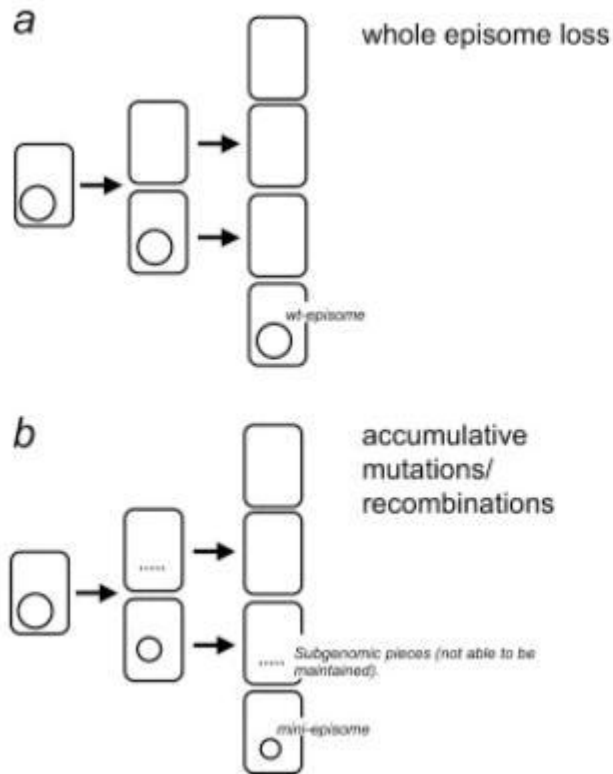
Xuefeng Liu^{1,2,8}, Ewa Krawczyk^{1,2,8}, Frank A Supryniewicz^{1,2}, Nancy Palechor-Ceron^{1,2}, Hang Yuan^{1,2}, Aleksandra Dakic^{1,2}, Vera Simic^{1,2}, Yun-Ling Zheng³, Praathibha Sripadhan^{1,2}, Chen Chen^{1,2}, Jie Lu^{1,2}, Tung-Wei Hou^{1,2}, Sujata Choudhury^{1,2}, Bhaskar Kallakury^{1,2}, Dean Tang⁴, Thomas Darling⁵, Rajesh Thangapazham⁵, Olga Timofeeva^{3,6}, Anatoly Dritschilo⁶, Scott H Randell⁷, Christopher Albanese¹⁻³, Seema Agarwal^{1,2} & Richard Schlegel^{1,2}

Establishing new NPC cultures



Challenges in NPC research

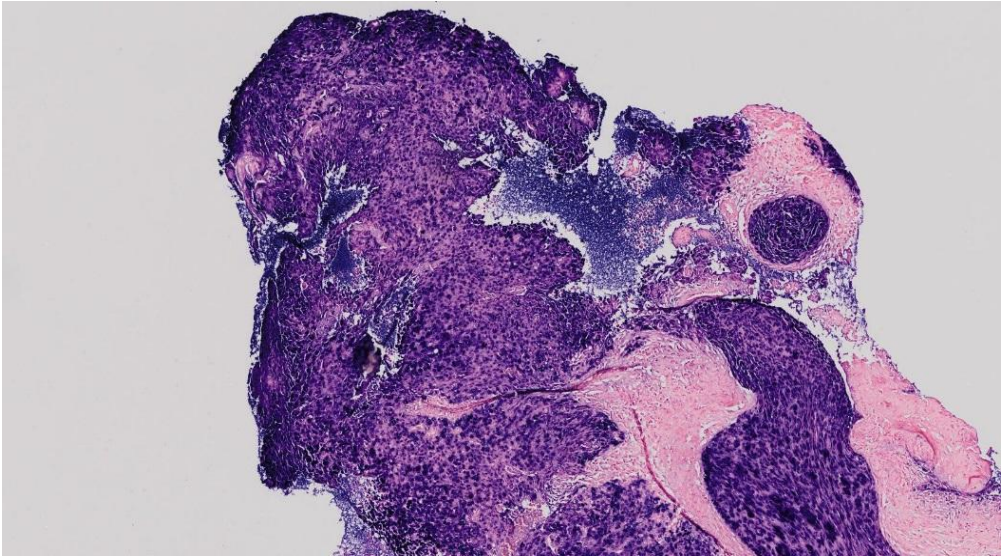
Loss of EBV in available cell lines



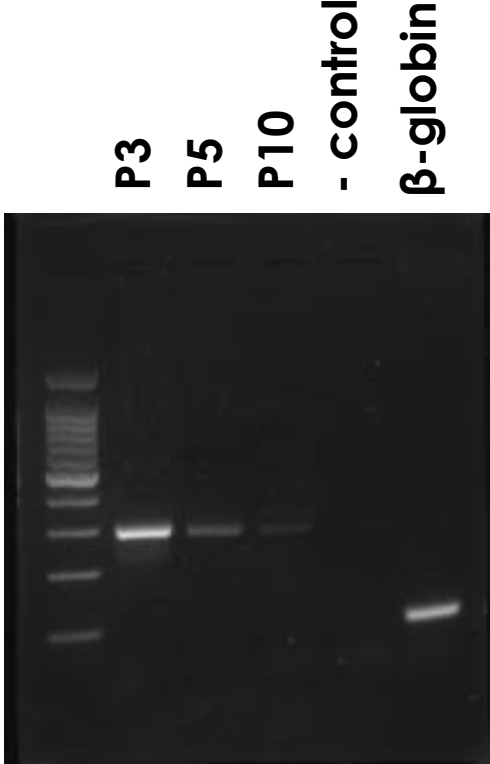
Strong et al. 2014;
Dittmer et al. 2008

Characterization - EBV

NPC associated with Epstein Barr virus infection



EBER ISH – to detect EBV in FFPE tissue



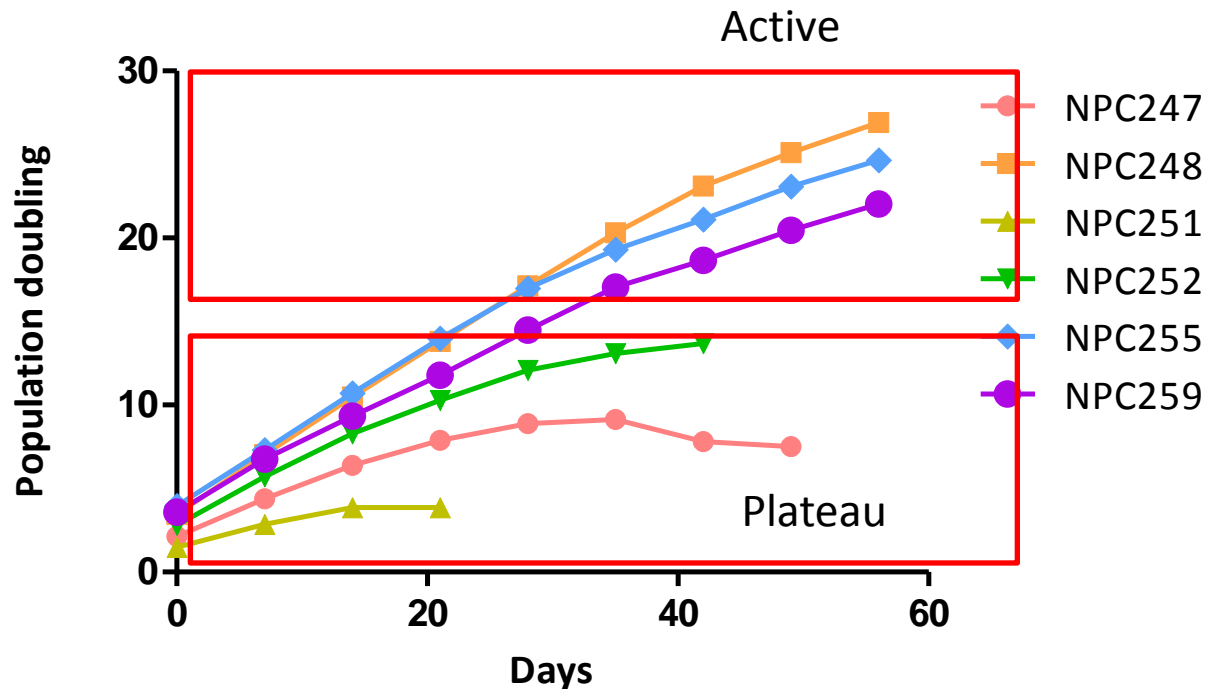
NPC 248

Loss of EBV detected at late passage

How do we maintain EBV in NPC cultures?

Characterization – immortalization

Reach 100 population doubling to be immortal



Transfect with TERT to reach immortalization

On going work

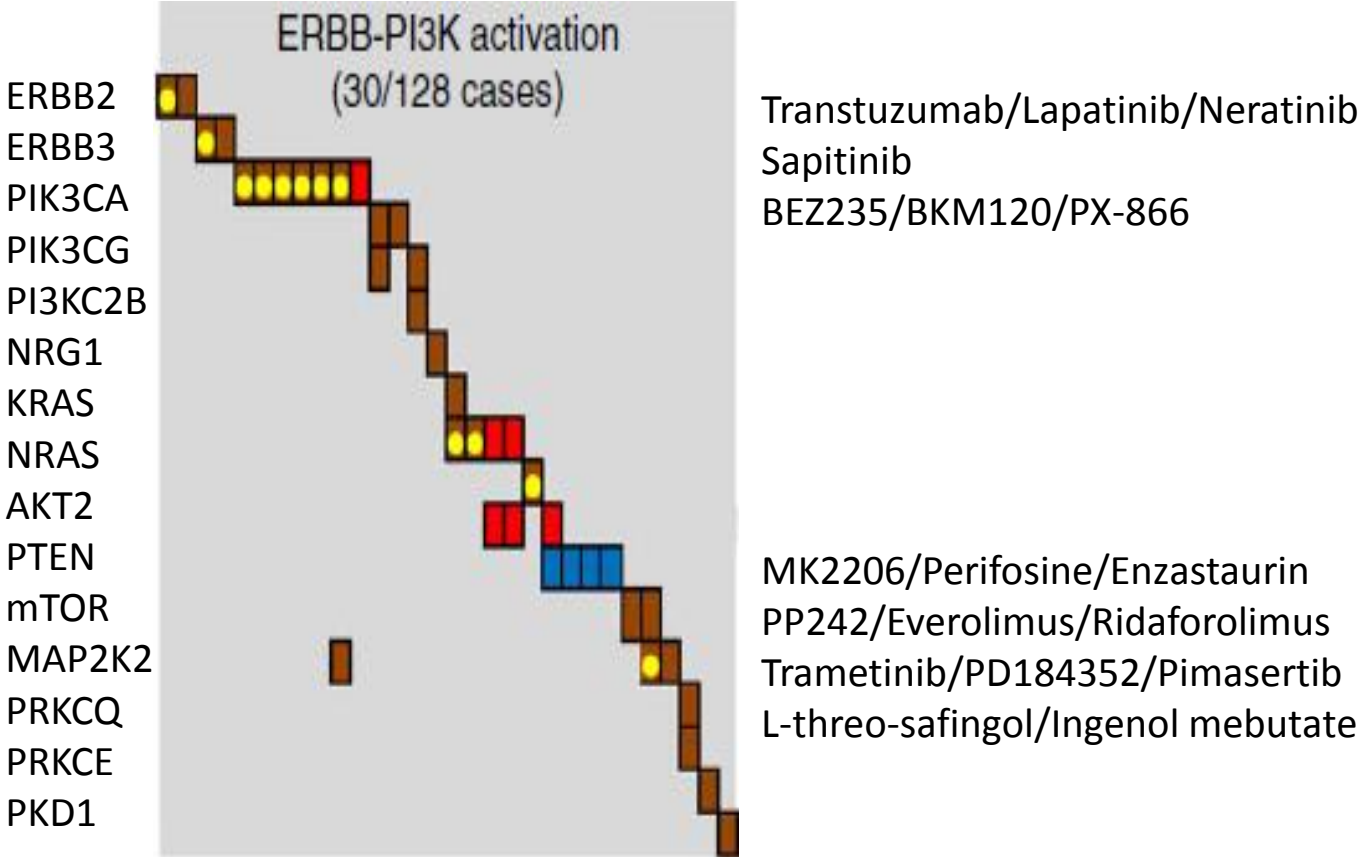


RNA-seq data is being analyzed

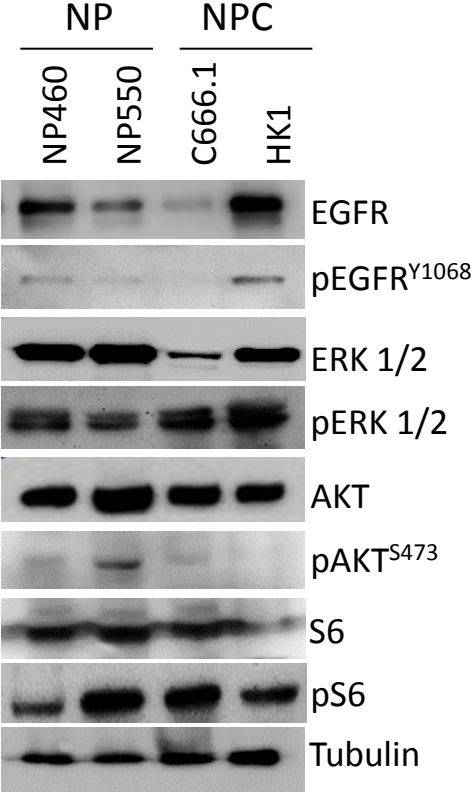
Data still being collected to characterize new primary cultures

Meanwhile, work is on going using existing cell lines to test new therapy

Druggable mutations in NPC

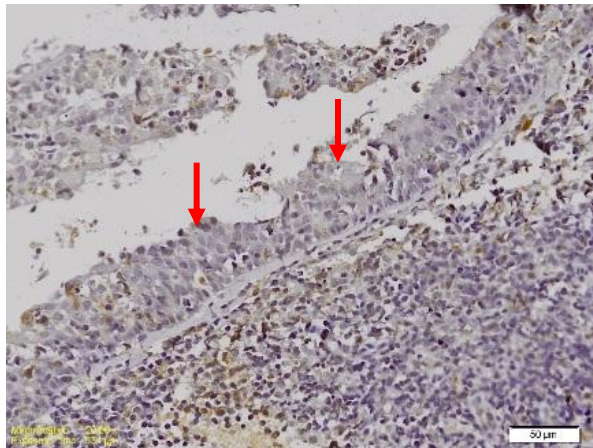


Overexpression of pERK1/2 in NPC cell lines

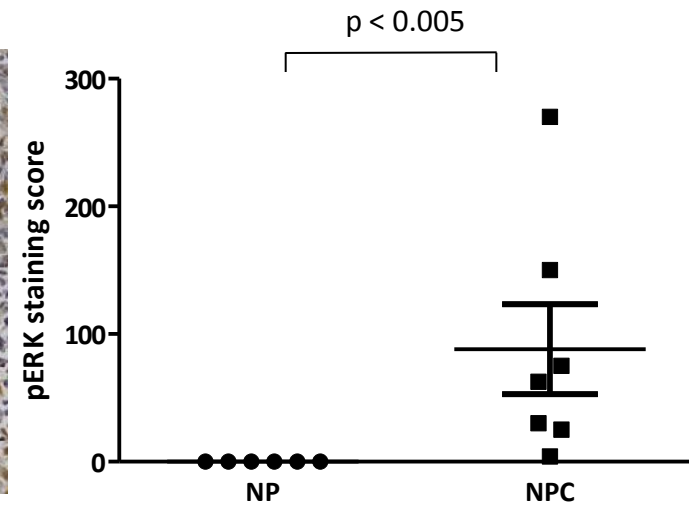
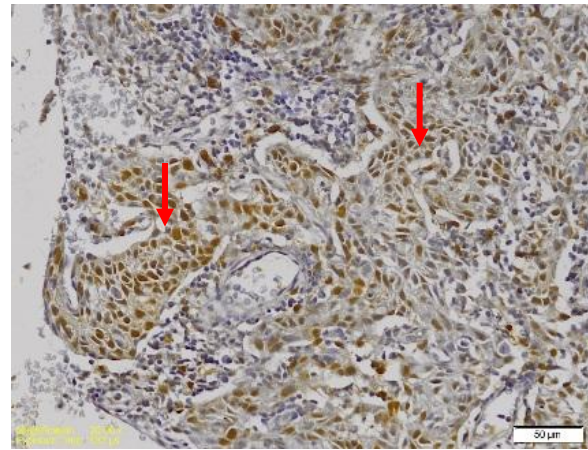


Overexpression of pERK in NPC clinical samples

Non-malignant
nasopharynx tissue (NP)



NPC tissue



Expression profiling of 21 biomolecules in locally advanced nasopharyngeal carcinomas of Caucasian patients

[Dimitrios Krikelis](#)[†] ✉, [Mattheos Bobos](#)[†], [Georgia Karayannopoulou](#), [Liliana Resiga](#), [Sofia Chrysafi](#), [Epaminontas Samantas](#), [Dimitrios Andreopoulos](#), [Vassilios Vassiliou](#), [Elisabeta Ciuleanu](#) and [George Fountzilias](#)

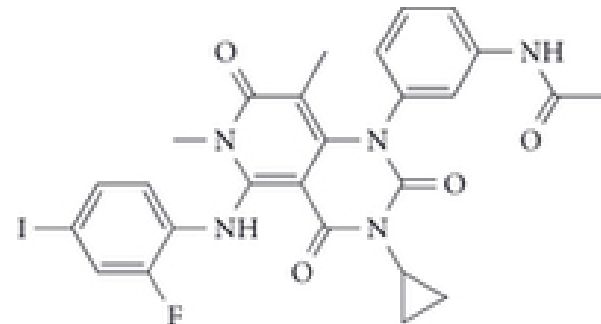
[†] Contributed equally

Frequency of immunohistochemical expression of the examined biomarkers in nasopharyngeal carcinoma (sorted from the most frequently expressed to the least frequently ones)

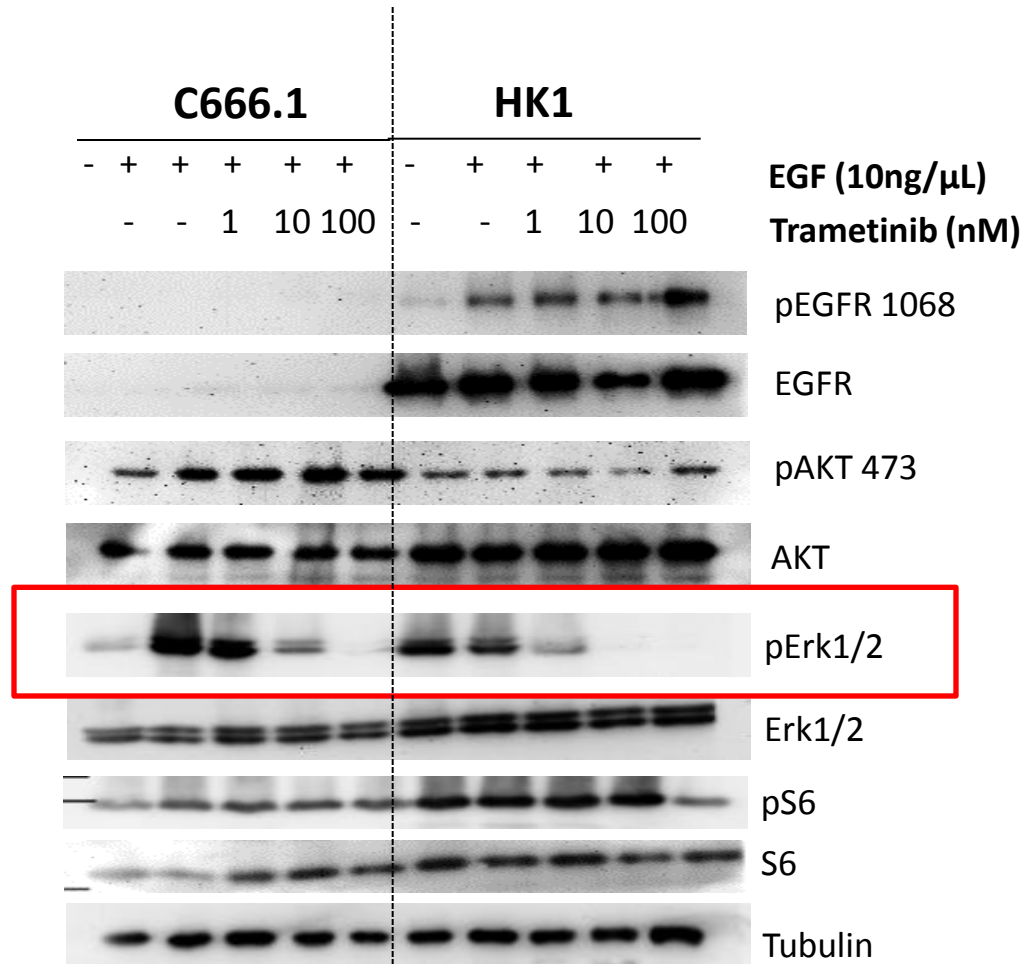
Biomarker	No of Cases	Positive (%)	Negative (%)
Phosho-p44/42 MAPK ^{Thr202/Tyr204}	95	60 (63.2)	35 (36.8)

Rationale of using trametinib

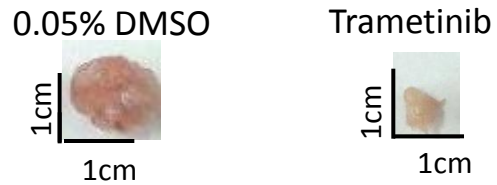
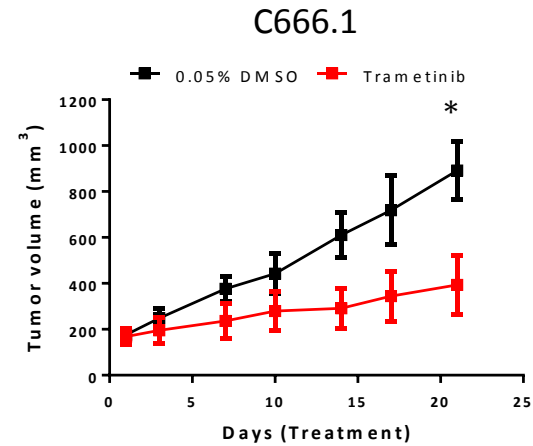
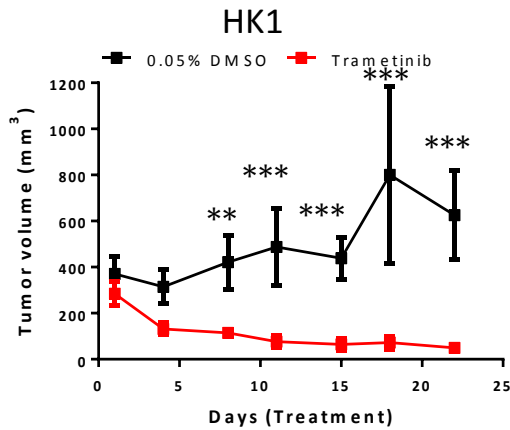
- Reversible, selective, allosteric inhibitor of MEK1/2 activation
- Low C_{\max} (peak concentration) to C_{trough} ratio and long circulating half-life, sustaining effective drug levels
- Approved for BRAF^{V600E} or ^{V600K} metastatic melanoma and other indications



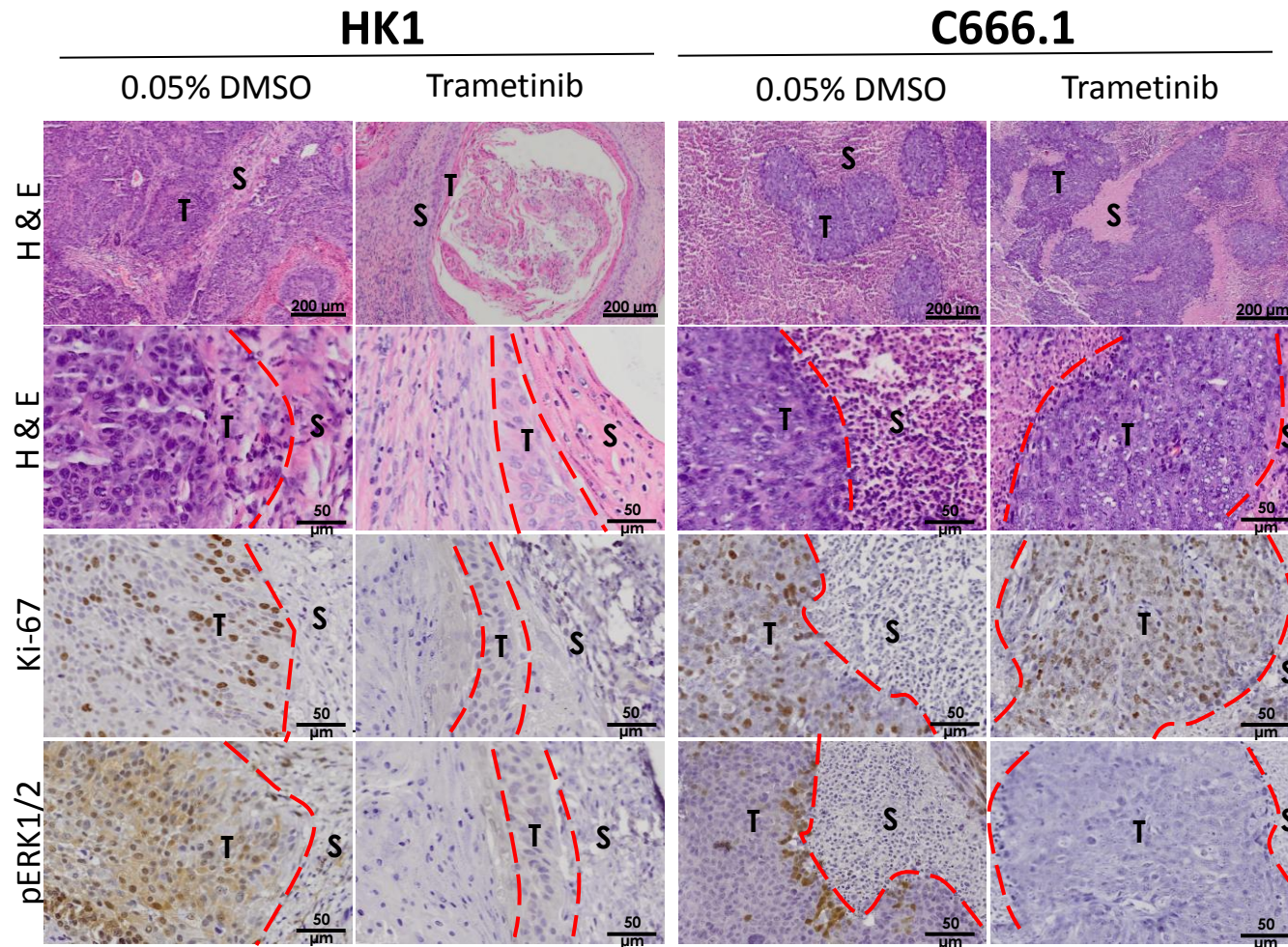
Trametinib reduced pERK expression



Effect of trametinib in NPC xenograft models



Immunohistochemical staining on mouse models



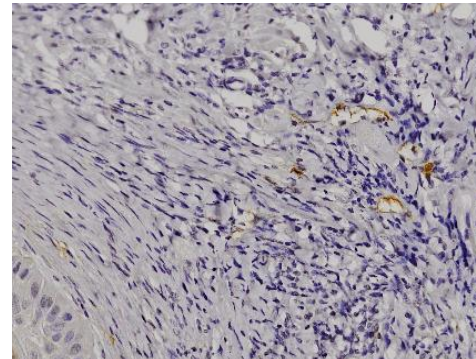
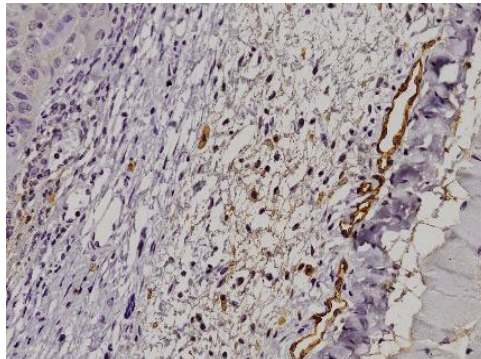
Trametinib reduced lymphatic vessels in NPC xenograft models

VC

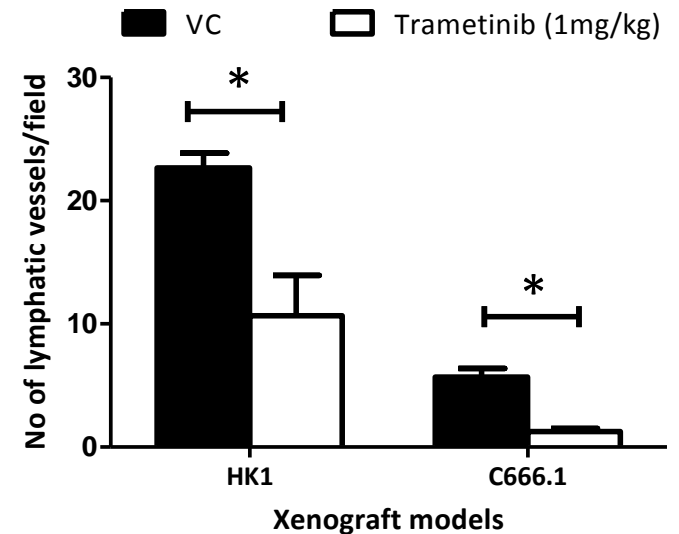
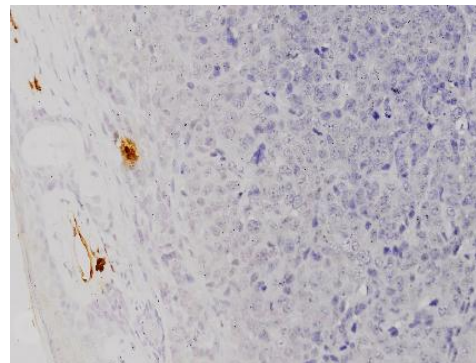
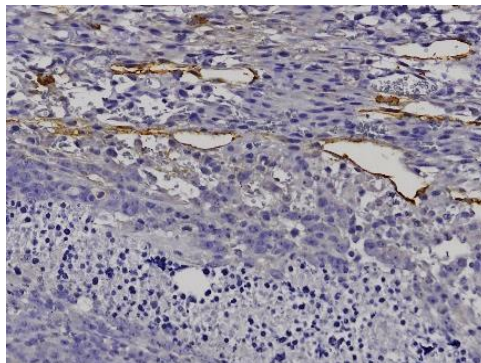
Trametinib (1mg/kg)

Lyve-1

HK1

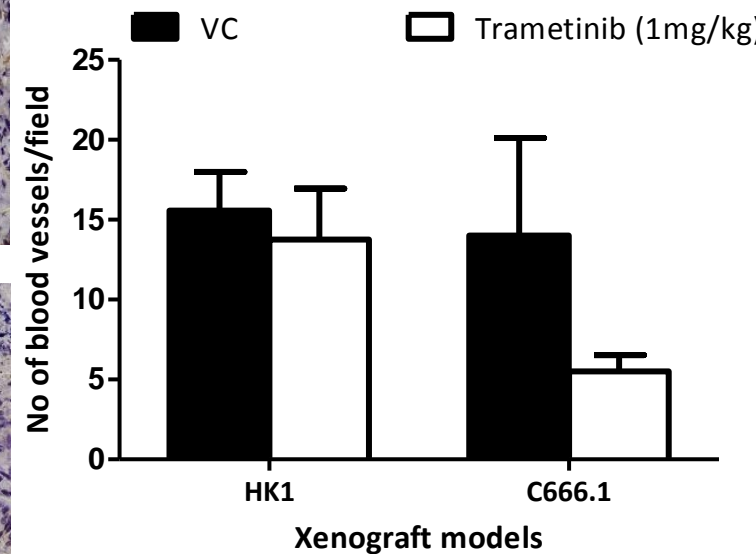
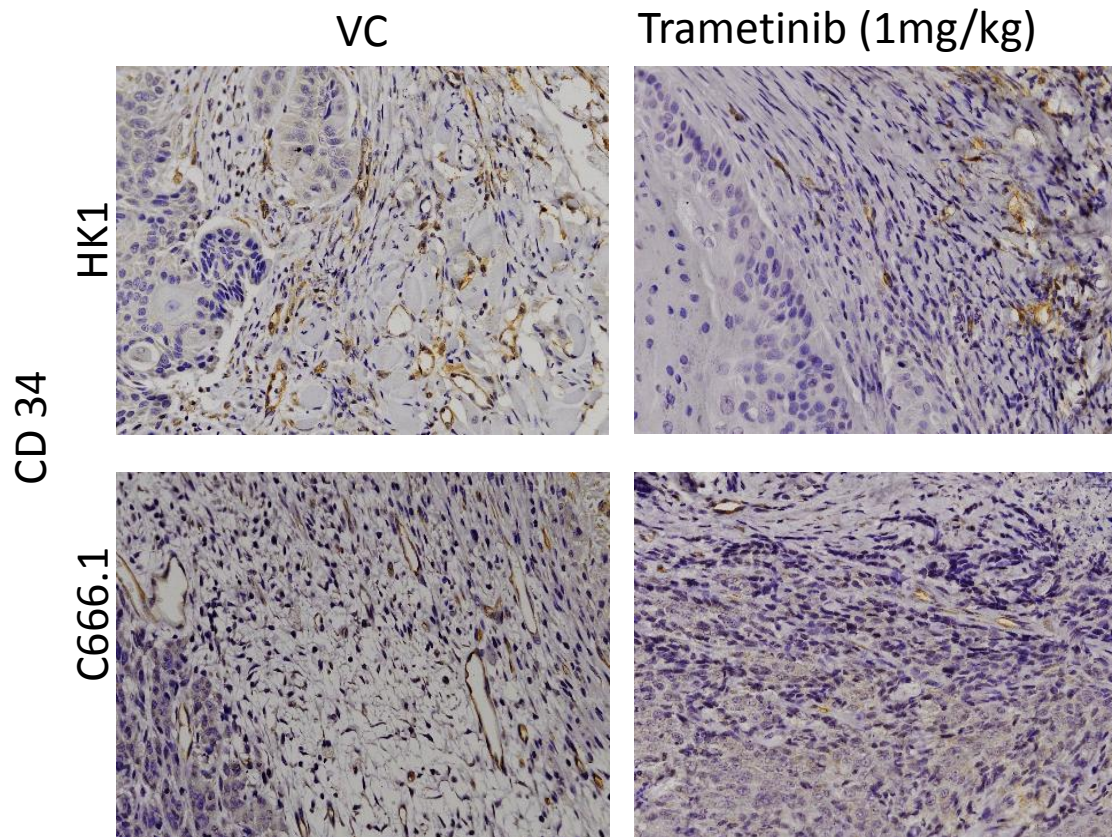


C666.1

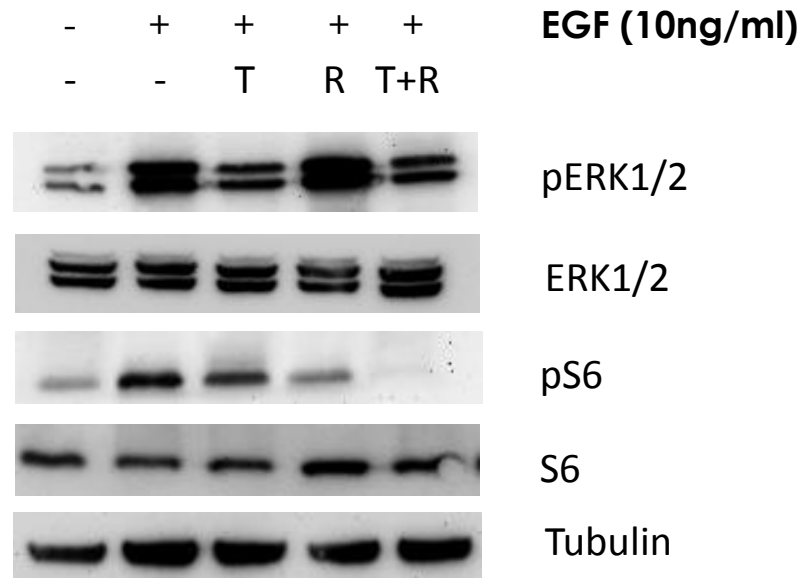


* 0.05

Trametinib did not affect blood vessels in NPC xenograft models



Combination treatment



Summary

- pERK1/2 is overexpressed in NPC
- Our data demonstrate that trametinib reduced cell proliferation in NPC *in vitro* and *in vivo* by reducing pERK1/2 expression
- Trametinib might be anti-lymphangiogenic in NPC xenograft model and zebrafish
- **Addressing challenges (how, why, what)**
- **Team work/collaboration/sharing (NPC biopsies for example)**
- **Key observations with potential for clinical evaluation**
 - **pERK activation in NPC for exploiting for clinical evaluation**

Acknowledgement



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