

# Vaccines and Vaccination During and Post Covid Pandemics



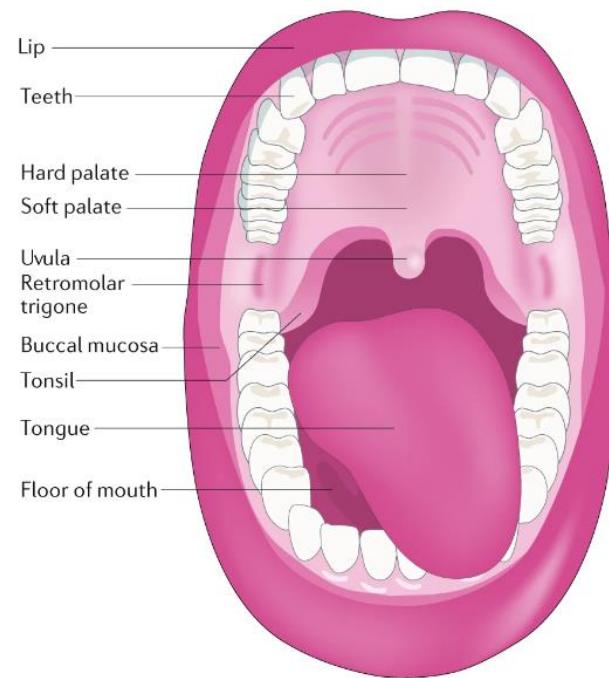
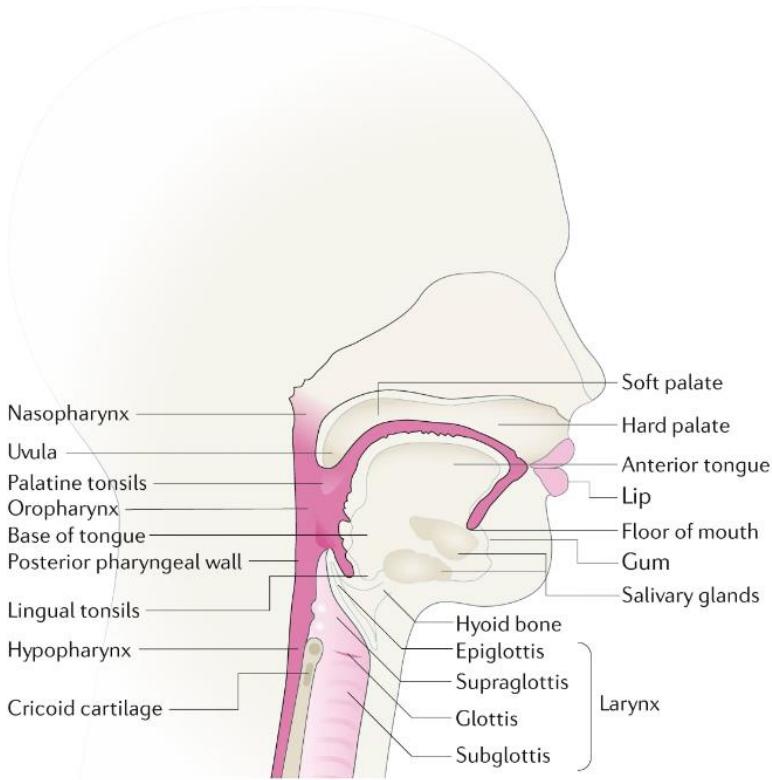
**Role of E6\* isoform expression as a marker of active  
HPV infection in head and neck cancers**

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**INT - IRCCS “Fondazione G. Pascale”, Naples, Italy**  
**Molecular Biology and Viral Oncology Unit**

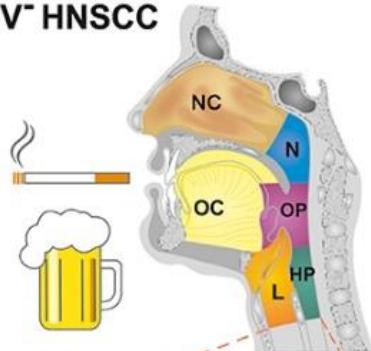
# HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)



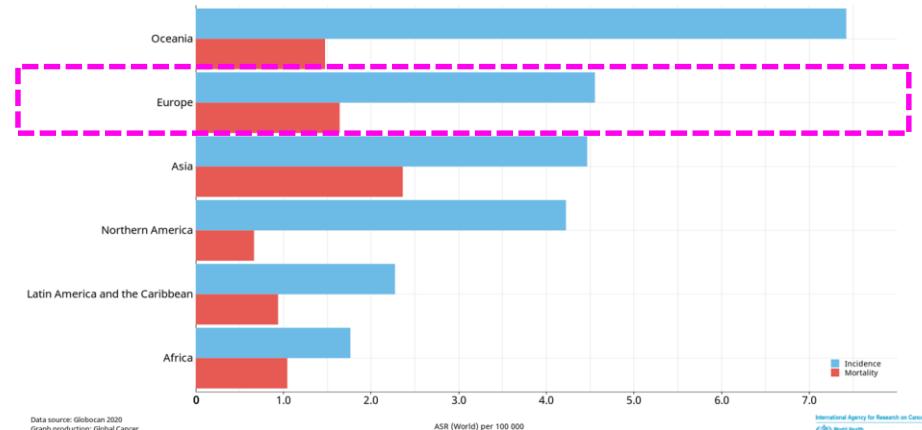
(Johnson et al., 2020)

# HNSCC RISK FACTORS AND EPIDEMIOLOGY

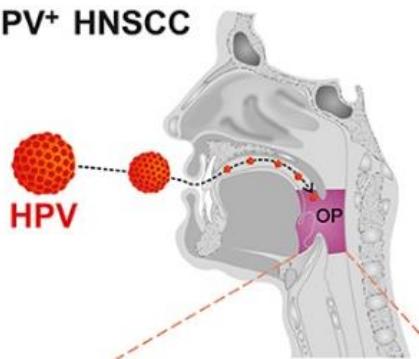
## HPV<sup>-</sup> HNSCC



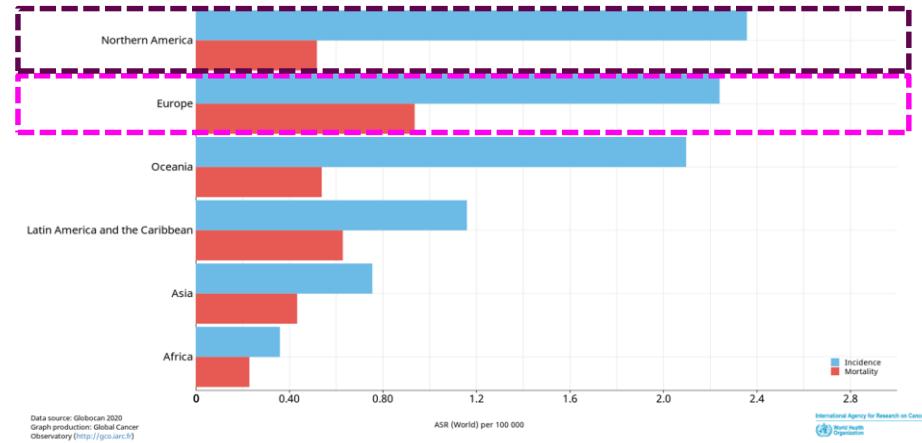
Estimated age-standardized incidence and mortality rates (World) in 2020, both sexes, all ages



## HPV<sup>+</sup> HNSCC



Estimated age-standardized incidence and mortality rates (World) in 2020, oropharynx, both sexes, all ages

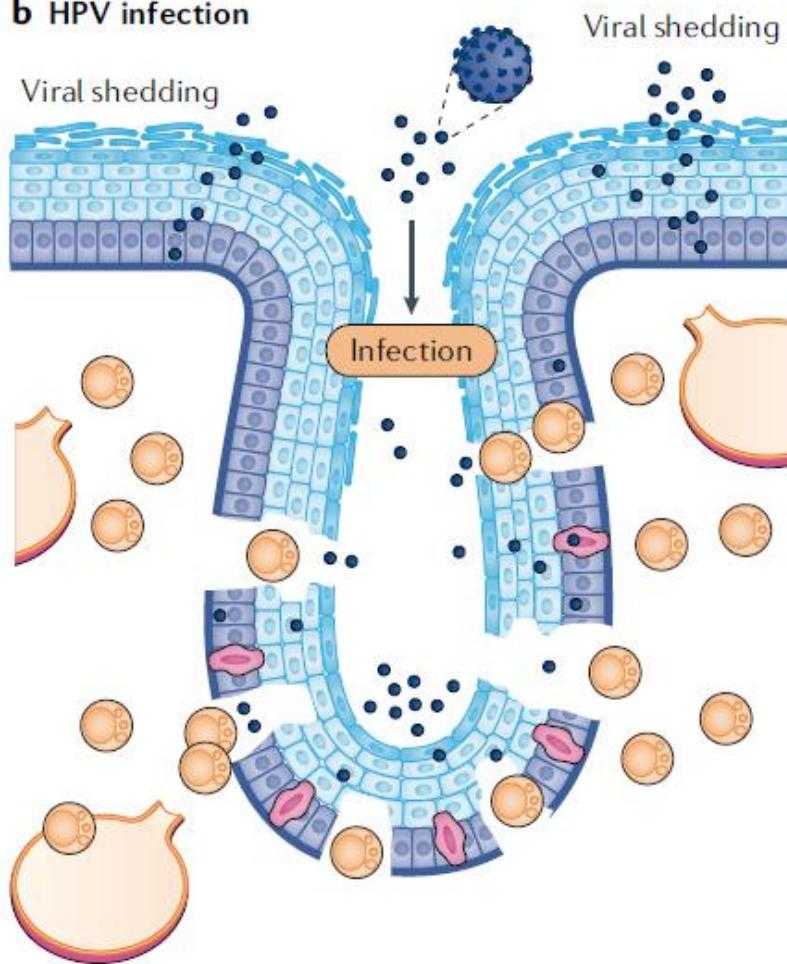


(Fialovà et al., 2020)

(GLOBOCAN, 2018)

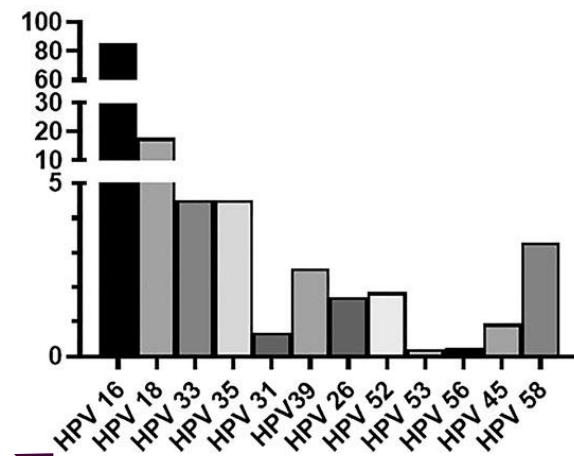
# HNSCC AND HPV

b HPV infection



(Johnson et al., 2020)

Prevalent HPV genotypes within the Head and Neck region

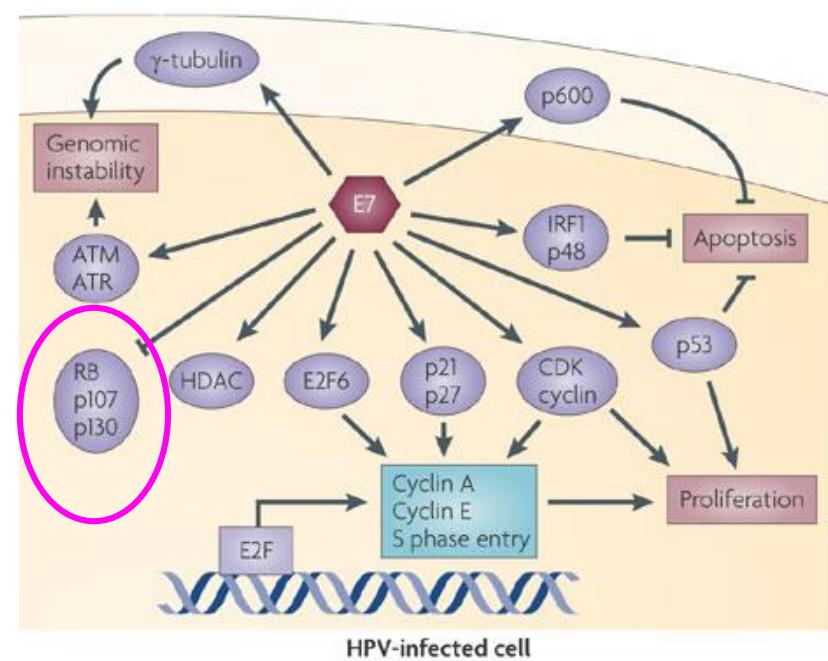
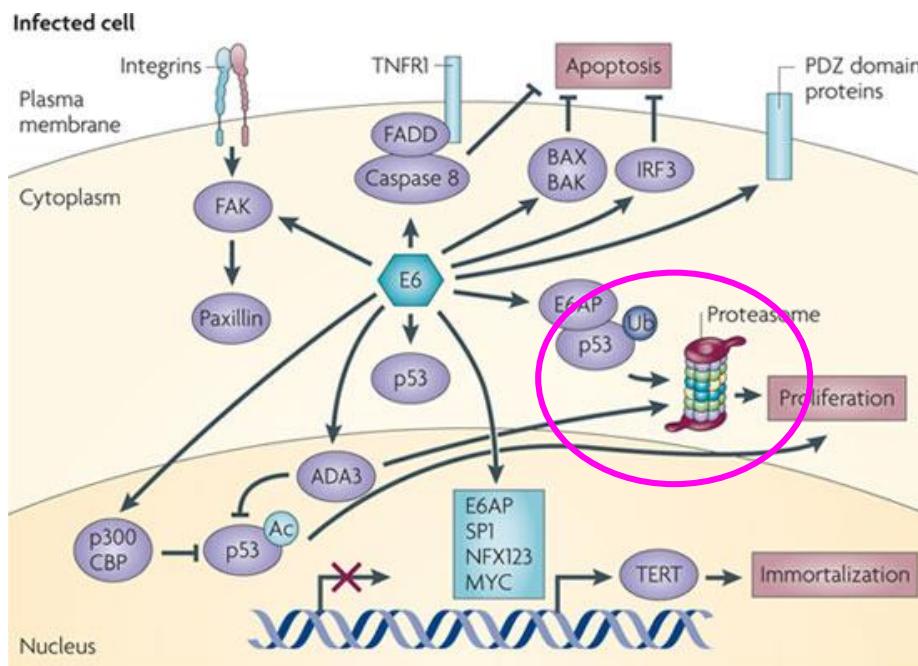


(Brennan et al., 2021)

Group	α-HPV types	Comments
I	16	Most potent HPV type, known to cause cancer at several sites
I	18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	Sufficient evidence for cervical cancer

(IARC, Lancet Oncology, 2009)

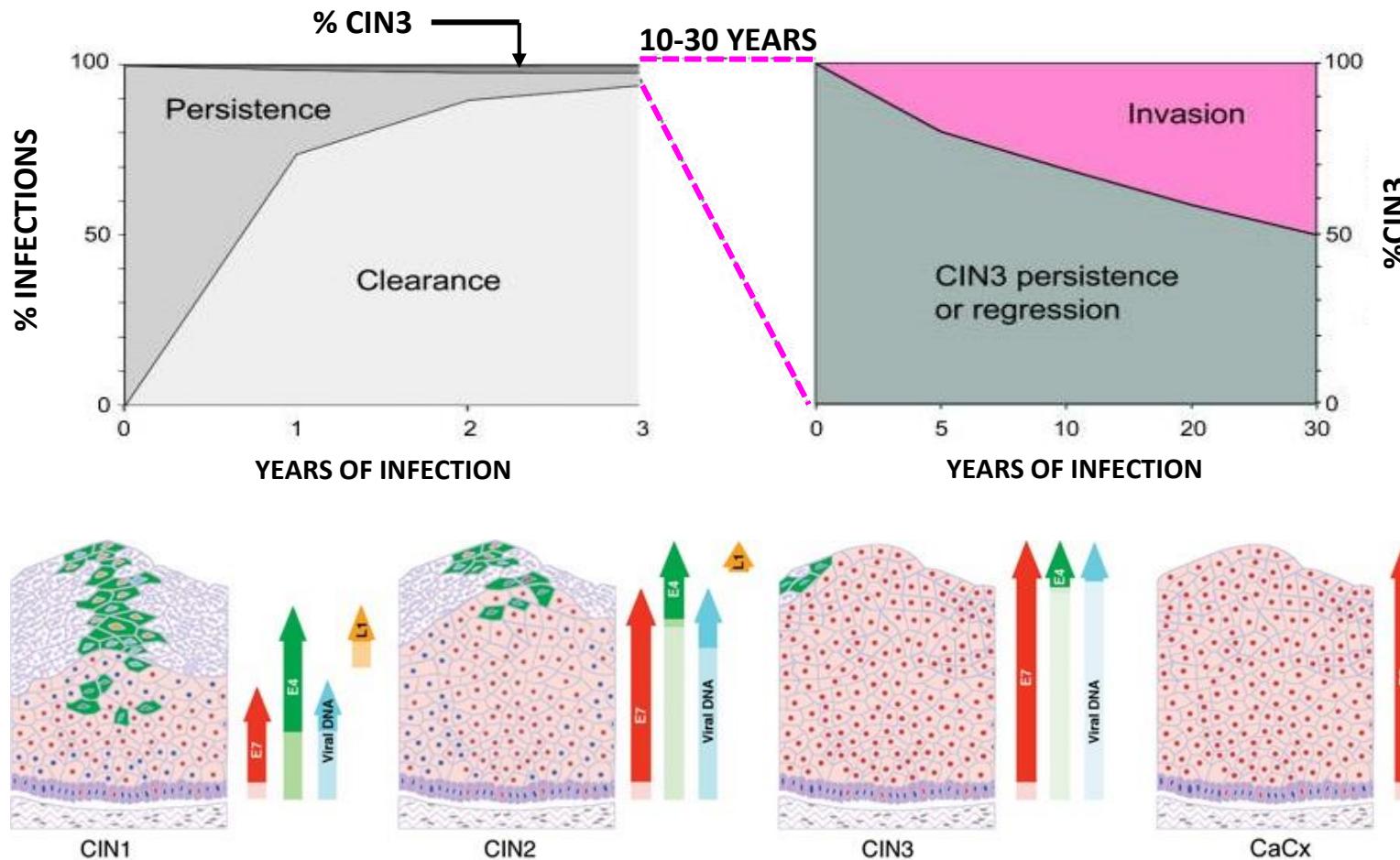
# ONCOGENIC ACTIVITY OF HPV16 E6 AND E7



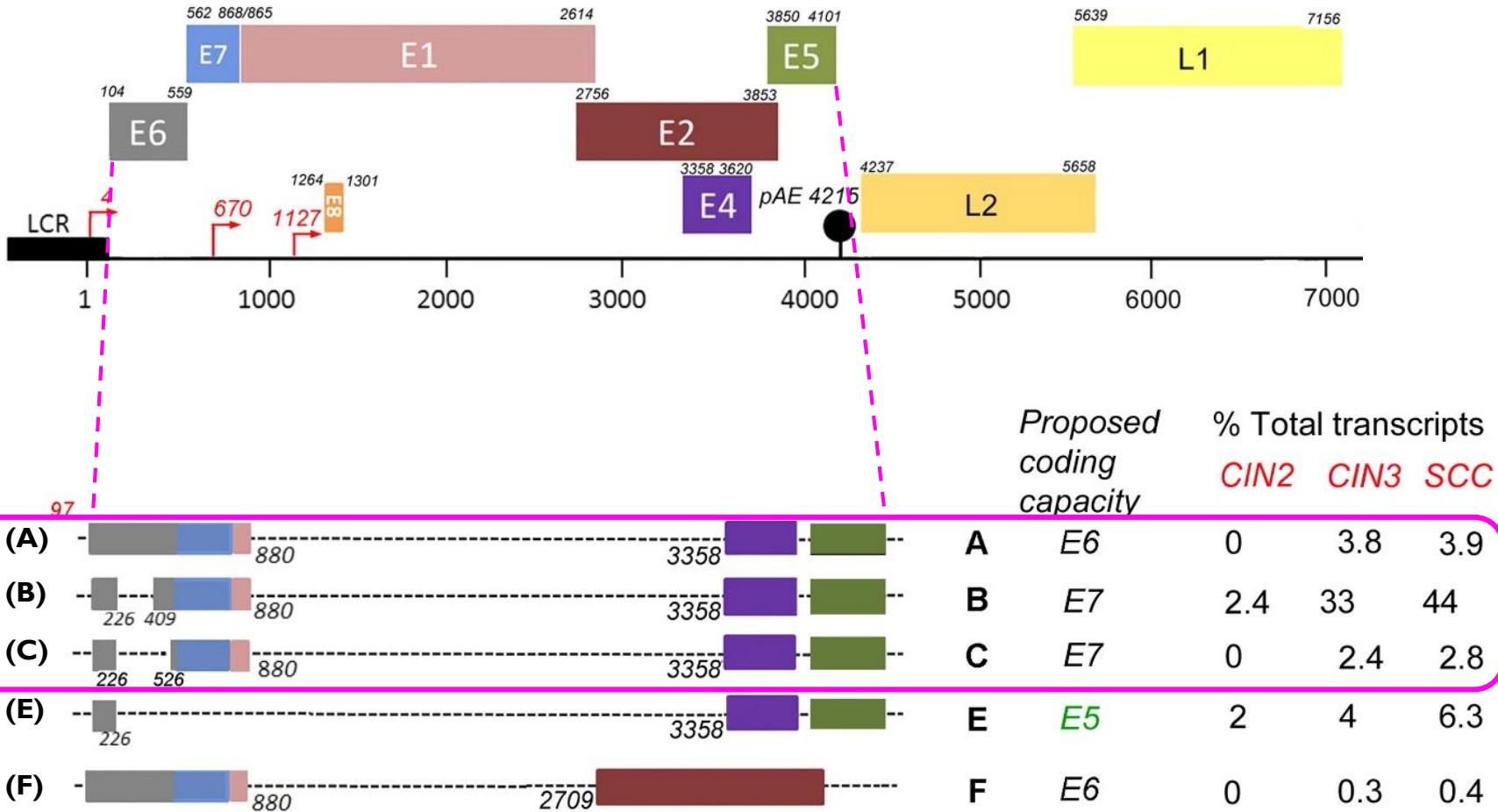
(Moody and Laimins, 2010)

# HPV-RELATED CANCER DEVELOPMENT

## Cervical cancer model

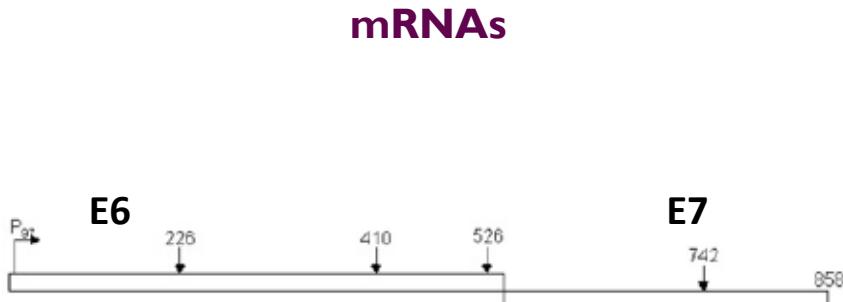


# HPV GENOME AND GENE EXPRESSION

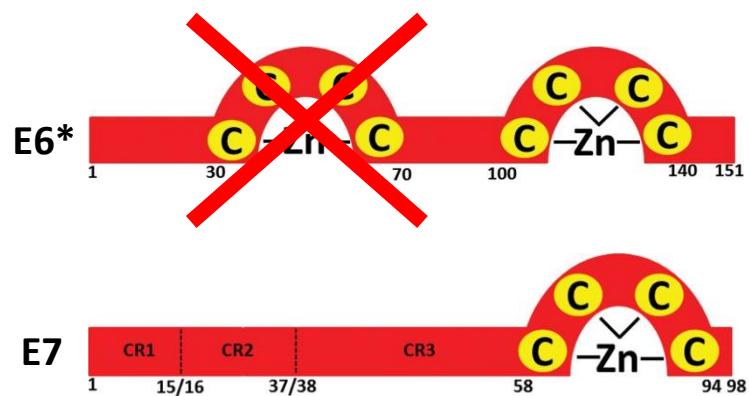
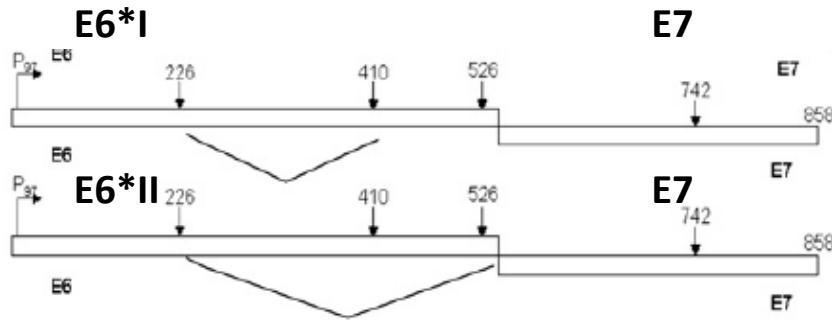
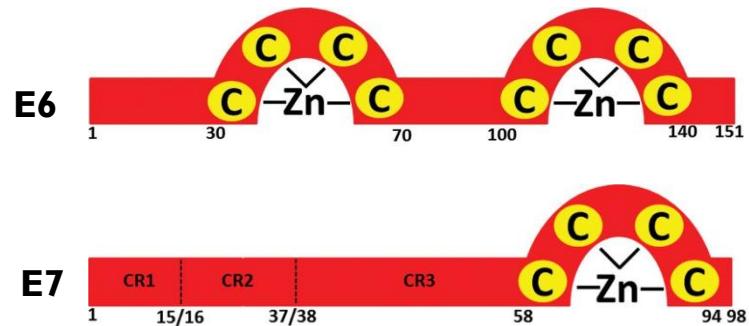


(Chen et al, Virol., 2014)

# HPV16 E6/E7 mRNA AND ONCOPROTEINS



## CODING POTENTIAL

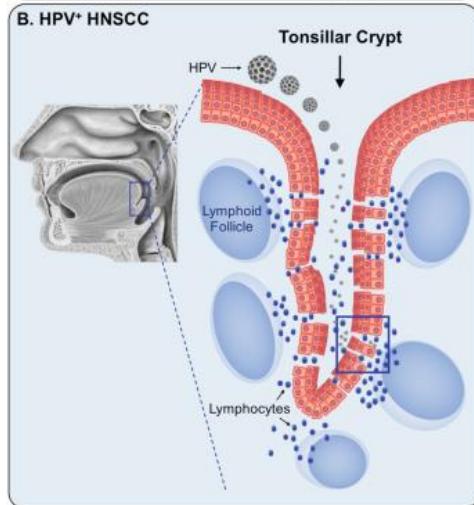
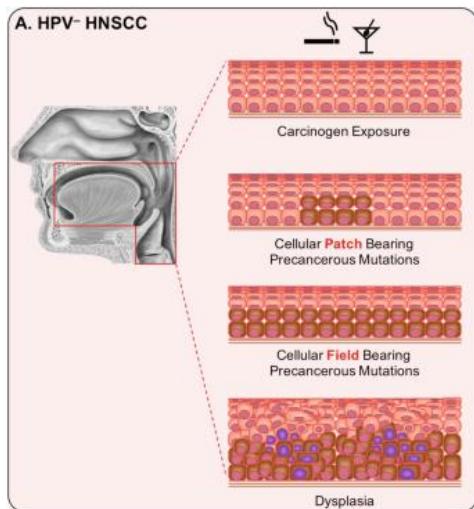


(Mc Farlane and Graham, Biochem Soc Trans., 2010)

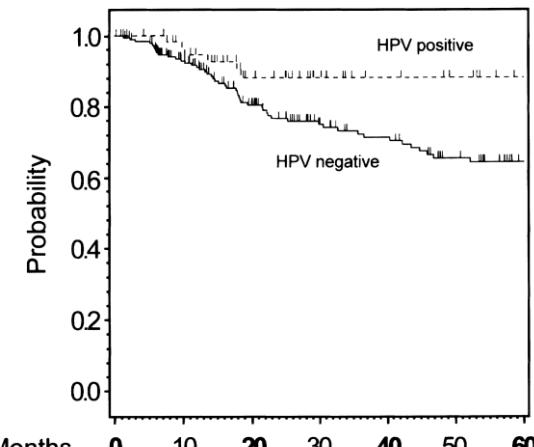
Andrea Cerasuolo

(Miller et al, Biochem J., 2012)

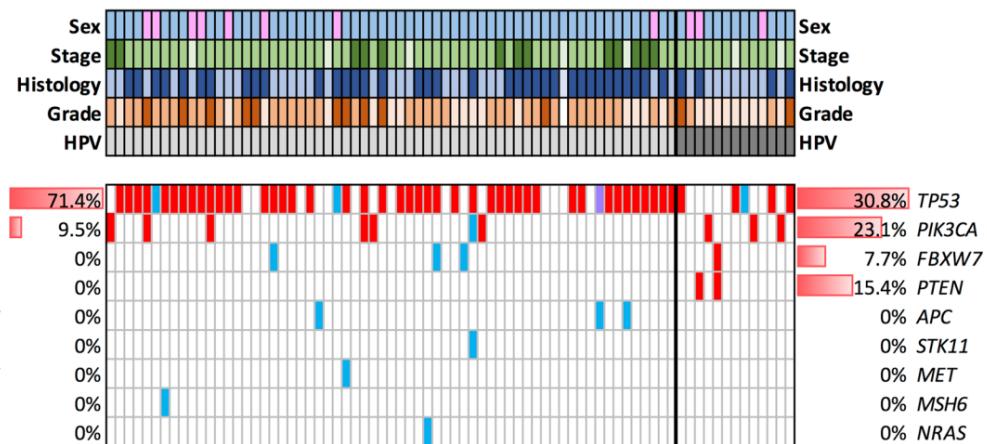
# HPV- HNSCC vs HPV+ HNSCC



(Faraji et al., 2018)

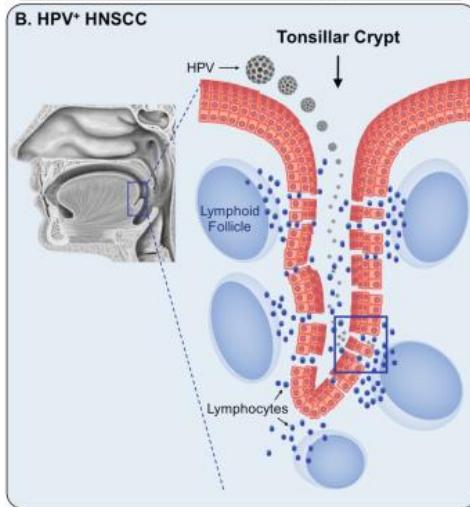
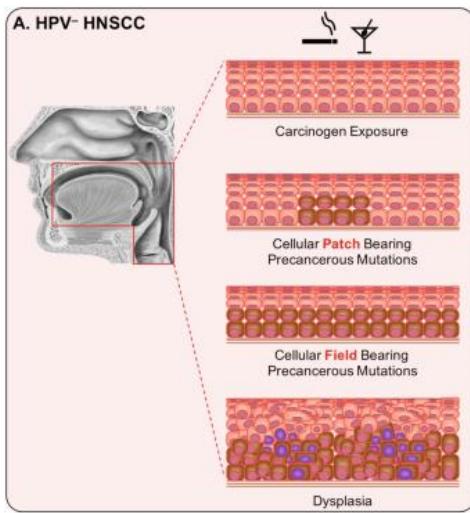


(Gillison et al., 2000)

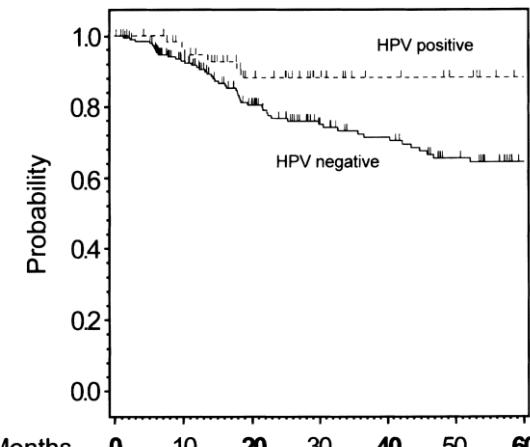


(Fernández-Mateos et al., 2020)

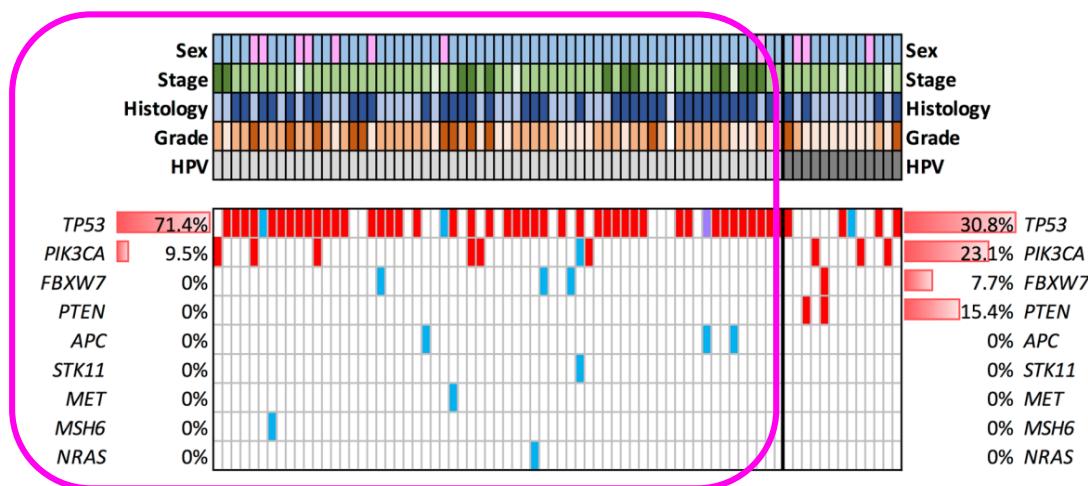
# HPV- HNSCC vs HPV+ HNSCC



(Faraji et al., 2018)

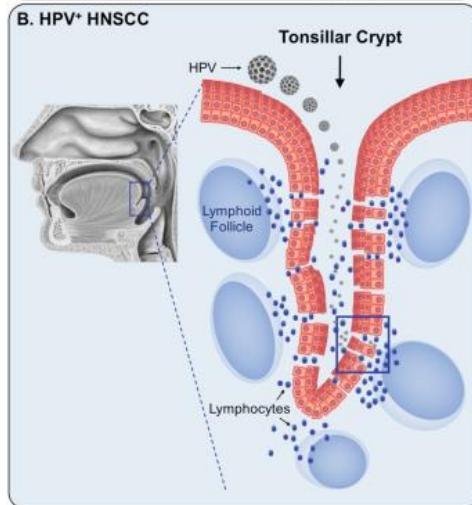
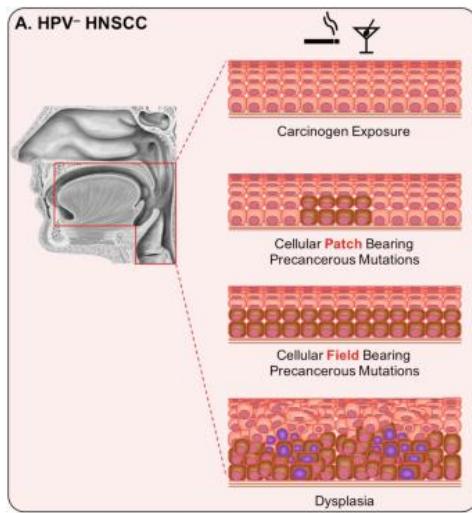


(Gillison et al., 2000)

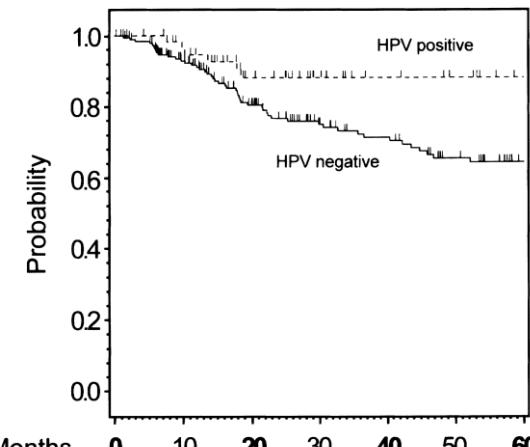


(Fernández-Mateos et al., 2020)

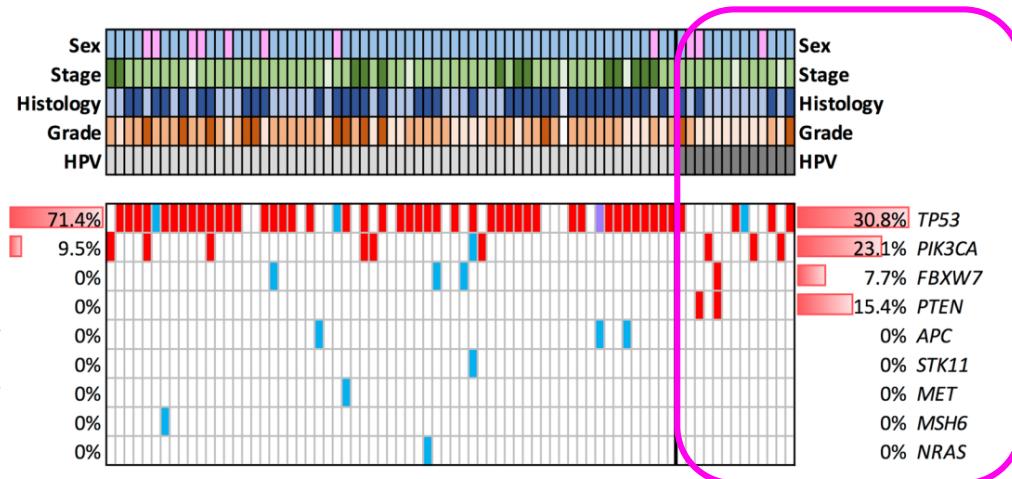
# HPV- HNSCC vs HPV+ HNSCC



(Faraji et al., 2018)



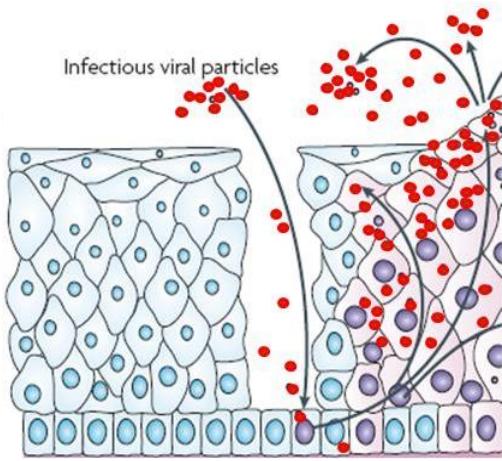
(Gillison et al., 2000)



(Fernández-Mateos et al., 2020)

# HPV DETECTION IN HNSCC

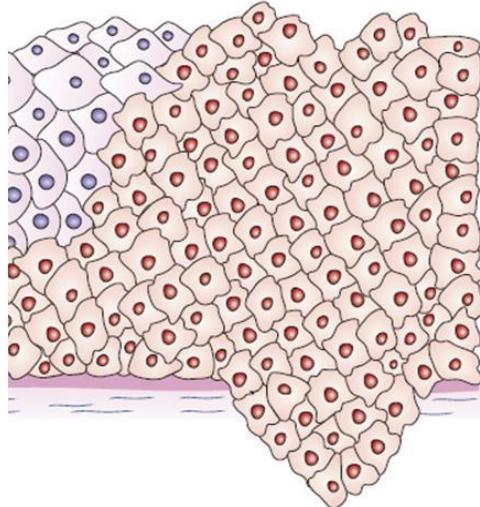
A.



HPV DNA positivity does not distinguish between transient (A) and transforming (B) infections.



B.



Markers of active HPV infection (i.e. p16, mRNA E6/E7 etc.) are needed for accurate diagnosis of HPV-related HNSCCs and patients treatment.

# AIM OF THE STUDY

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The aim of the study was to identify HPV-related HNSCCs through:

- 1) Analysis of HPV DNA prevalence in HNSCCs and head and neck dysplasia;
- 2) Analysis of the viral load in HPV16-positive samples;
- 3) Analysis of HPV16 E6 and E6\*I transcripts expression;
- 4) Analysis of the correlation between viral load and E6\*I expression levels.

# IDENTIFICATION OF $\alpha$ -HPV SEQUENCES

HNSCC	N°HPV+ (%)	N°HPV16+ (%)
OSCC	6/9 (67%)	5/6 (83%)
OPSCC	5/14 (36%)	4/5 (80%)
NPC	1/1 (100%)	1/1 (100%)
LSCC	1/5 (20%)	1/1 (100%)
HPC	0/1 (0%)	0/1 (0%)

OSCC = oral squamous cell carcinoma;

OPSCC = oropharyngeal squamous cell carcinoma;

NPC = nasopharyngeal carcinoma;

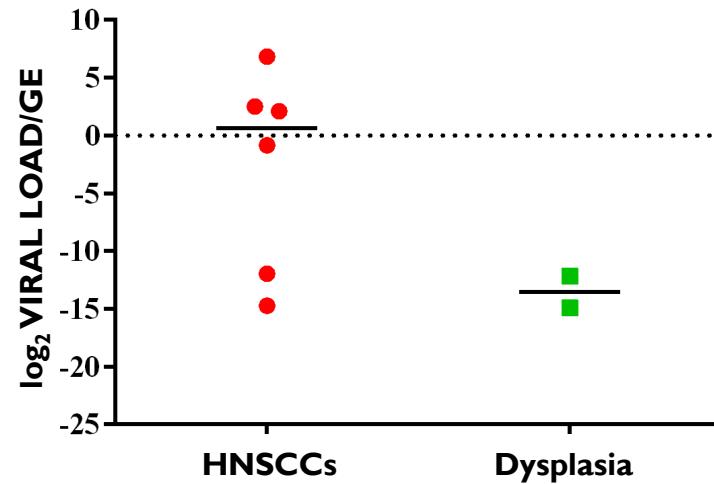
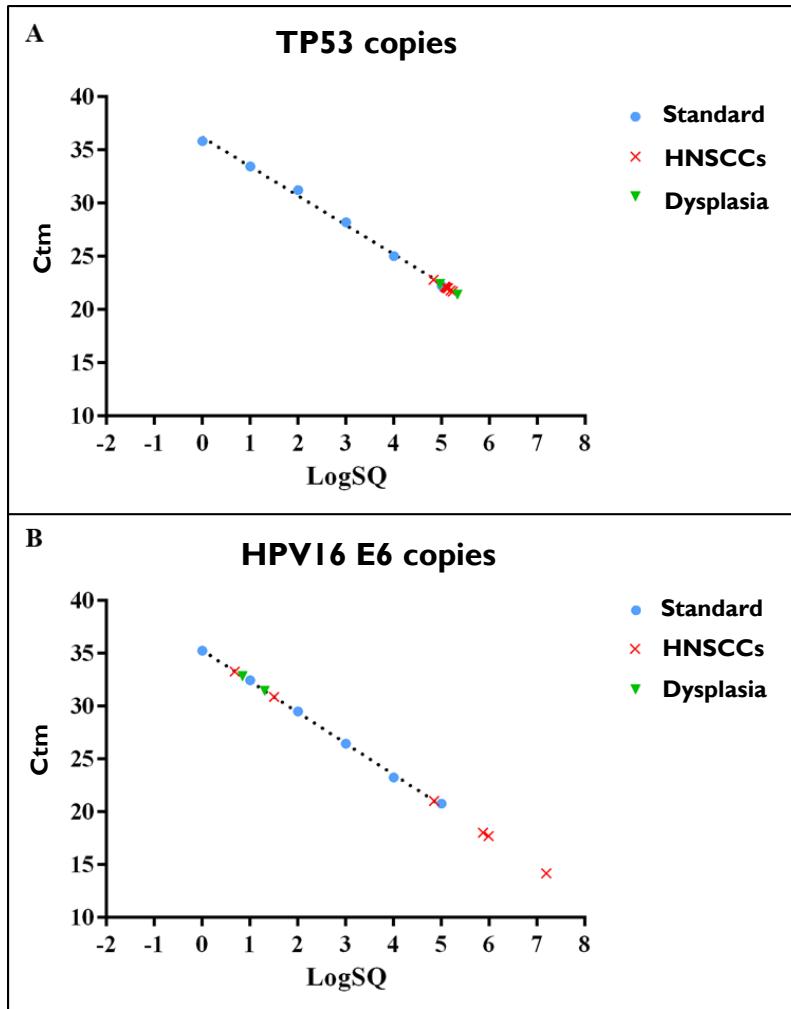
LSCC = laryngeal carcinoma;

HPC = hypopharyngeal carcinoma

The 45% (14/31) of HNSCCs and the 33% (3/9) of dysplasia was positive for HPV DNA.

The most frequently detected genotype in all samples was HPV16, followed by HPV33, regardless of the anatomic site.

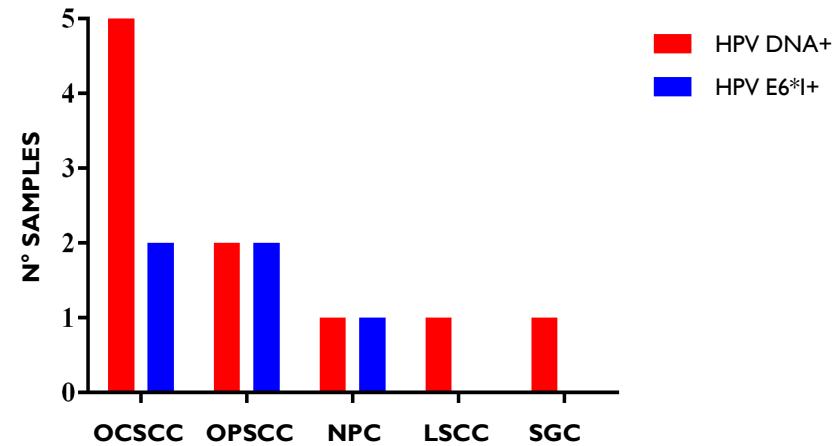
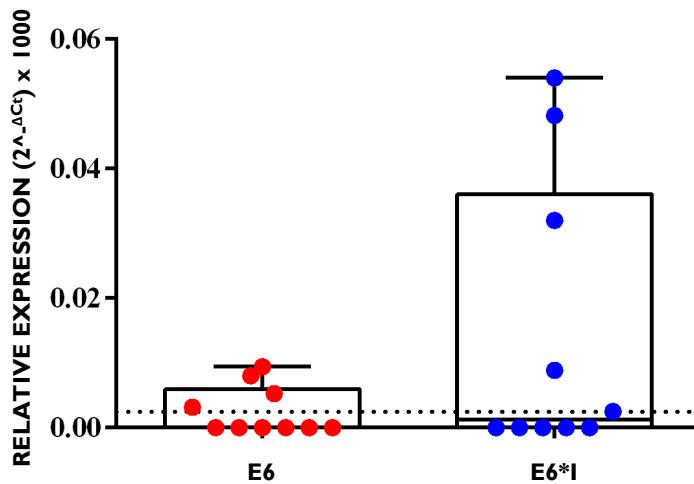
# VIRAL LOAD IN HNSCC AND DYSPLASIA



The 33.3% of HNSCCs showed medium-high viral load, the 33.3% low viral load, the remnant cases were negative.

All dysplasia showed low viral load.

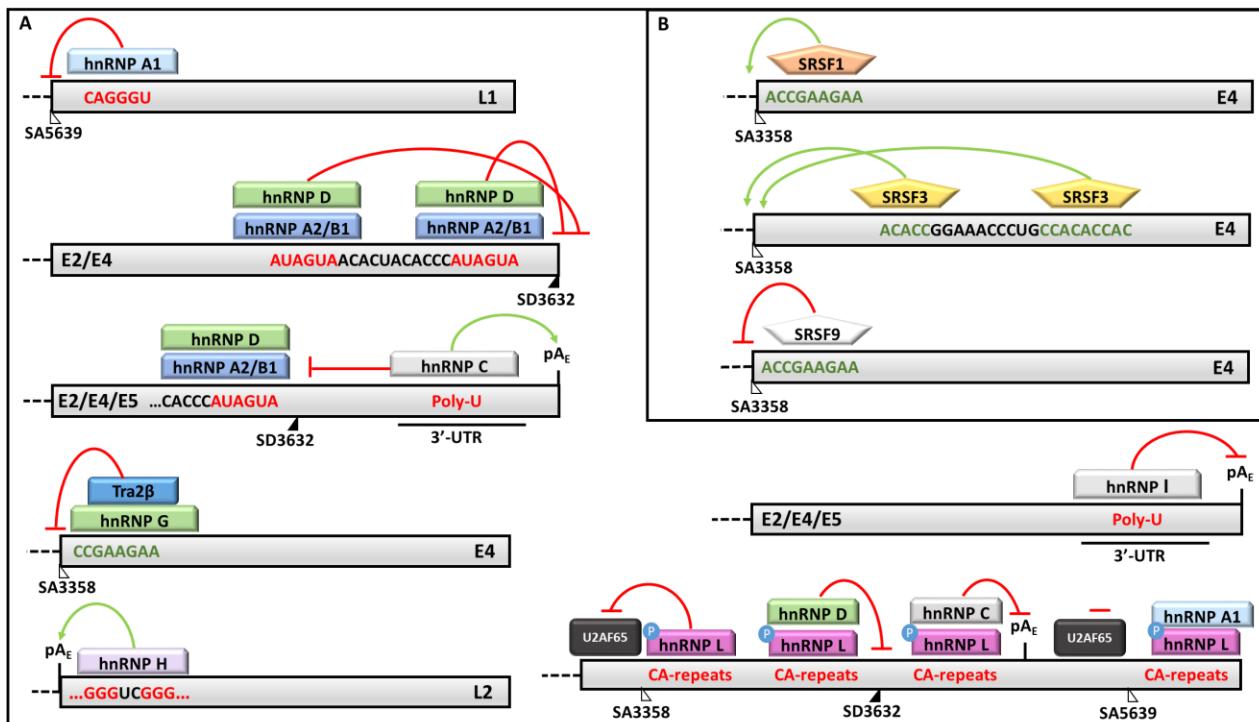
# HPV16 GENE EXPRESSION STUDY



OSCC = oral squamous cell carcinoma; OPSCC = oropharyngeal squamous cell carcinoma; NPC = nasopharyngeal carcinoma; LSCC = laryngeal squamous cell carcinoma; SGC = salivary gland carcinoma

VL (viral load)	E6*I+	E6*I-	P-value
medium-high VL > median	3	0	0,047
low VL < median/ND	1	5	

# HPV16 E6/E7 mRNA SPLICING



Splicing factors	Binding site
hnRNPA1	? <sub>(E)</sub> /SA5639 <sub>(L)</sub>
hnRNPA2/B1	? <sub>(E)</sub> /SA5639 <sub>(L)</sub>
Sam68	? <sub>(E)</sub>
BRM	? <sub>(E)</sub>
SRSF1	SA3358 <sub>(E)</sub>
SRSF2	? <sub>(E)</sub>
SRSF3	SA3358 <sub>(E)</sub>

(Cerasuolo et al., 2020)

*E=early genes splicing;  
L=late genes splicing*

# HPV16 E6/E6\* EXPRESSION IN CERVICAL AND OROPHARYNGEAL CANCER

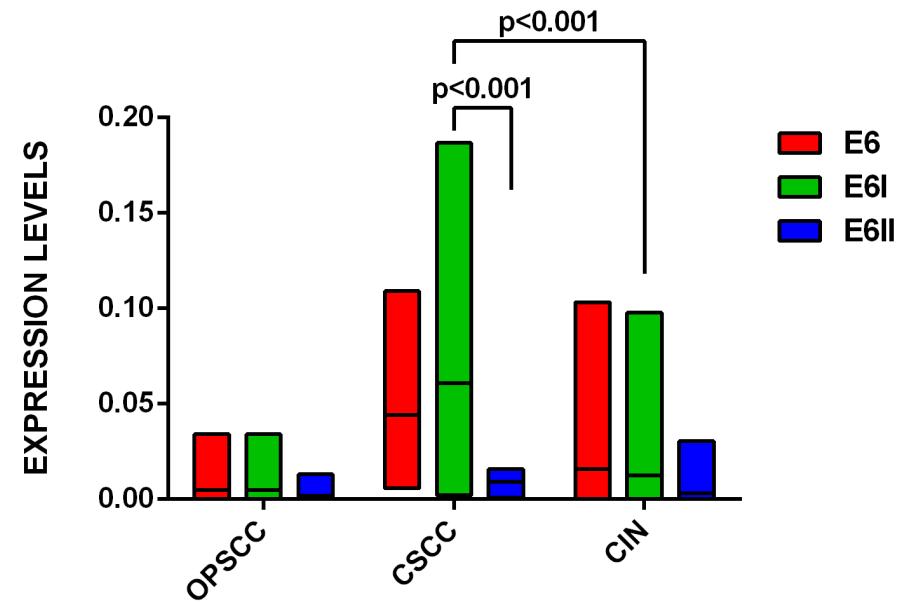
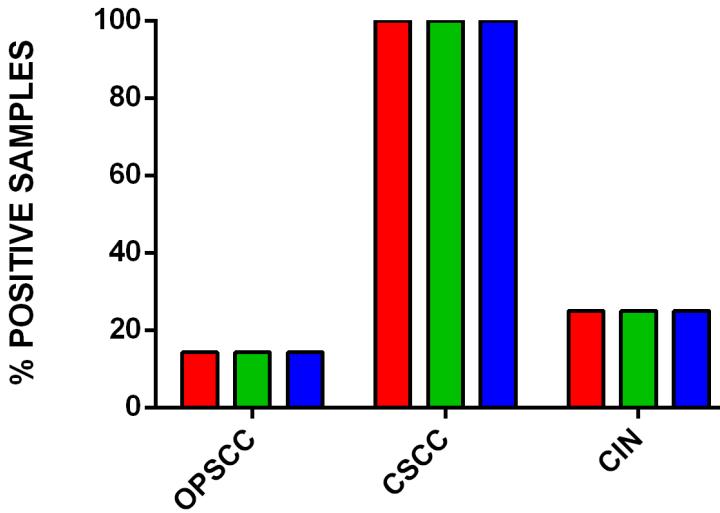
www.impactjournals.com/oncotarget/

Oncotarget, 2017, Vol. 8, (No. 21), pp: 34070-34081

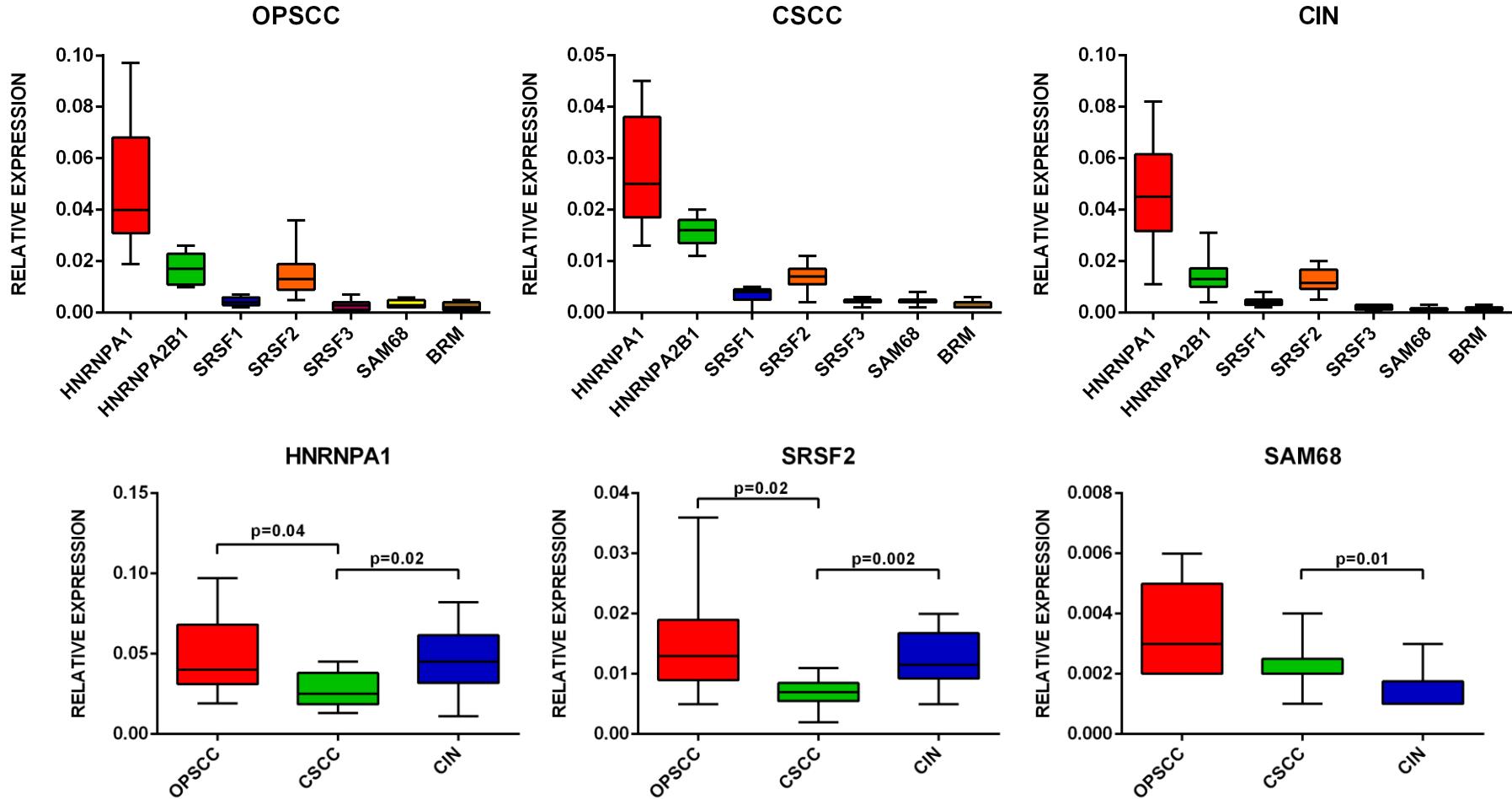
Research Paper: Pathology

## Comparative analysis of HPV16 gene expression profiles in cervical and in oropharyngeal squamous cell carcinoma

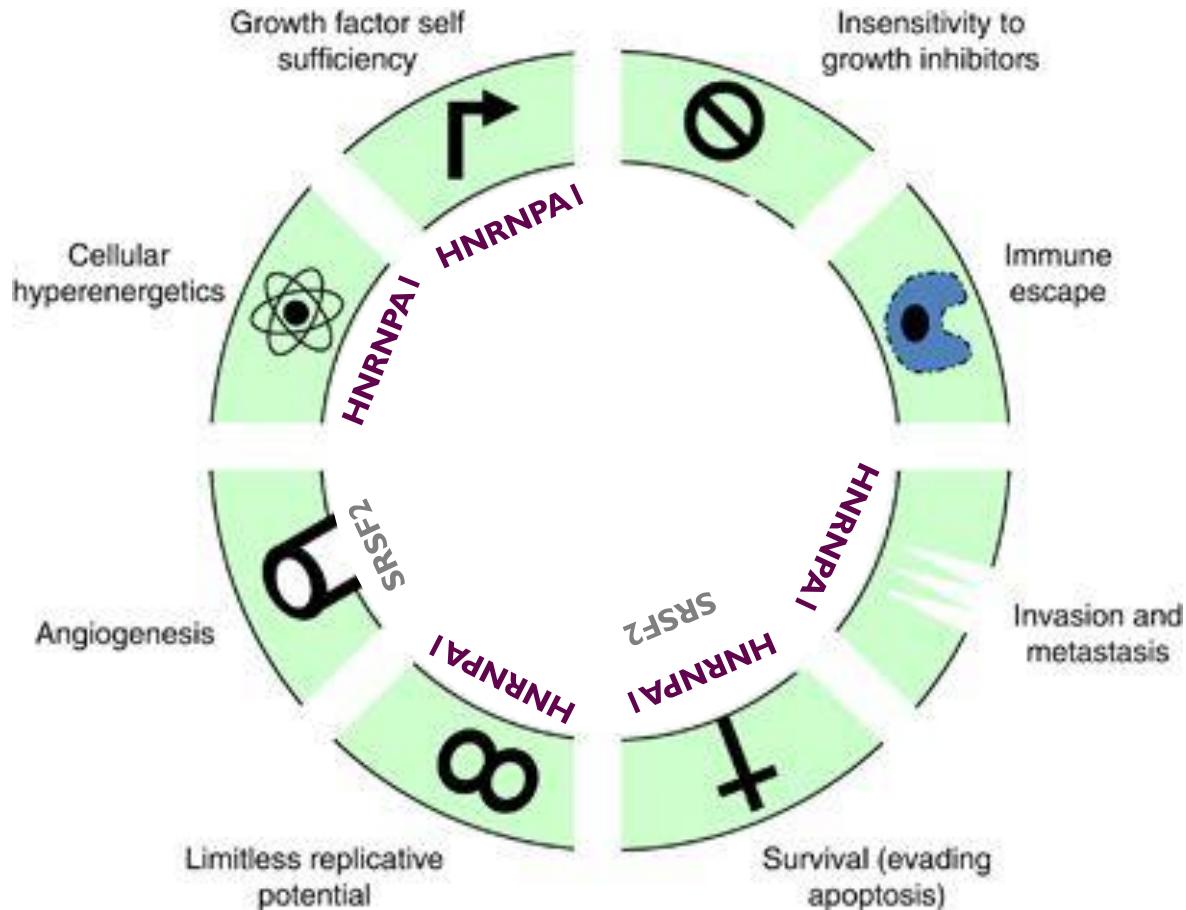
Andrea Cerasuolo<sup>1</sup>, Clorinda Annunziata<sup>1</sup>, Marianna Tortora<sup>1</sup>, Noemy Starita<sup>1</sup>, Giovanni Stellato<sup>2</sup>, Stefano Greggi<sup>2</sup>, Maria Grazia Maglione<sup>3</sup>, Franco Ionna<sup>3</sup>, Simona Losito<sup>4</sup>, Gerardo Botti<sup>4</sup>, Luigi Buonaguro<sup>1</sup>, Franco M. Buonaguro<sup>1</sup> and Maria Lina Tornesello<sup>1</sup>



# EXPRESSION PATTERN OF HPV16 SPlicing REGULATION FACTORS



# HNRNPA1 AND SRSF2 IN CANCER



## HNRNPA1

- Lung cancer
- Burkitt lymphoma
- Multiple Myeloma
- Leukemia
- Cervical cancer

## SRSF2

- Leukemia
- Cervical cancer



HNSCC???

# CONCLUSIONS

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- HPV16 is the most frequently detected genotype in HNSCCs;
- The HPV viral load in HNSCCs is highly variable;
- HPV16 E6\*I expression levels correlate with HPV DNA positivity in HNSCCs with medium-high viral load ( $P=0,047$ );
- Human splicing factors HNRNPA1 and SRSF2 are higher expressed in OPSCC than CSCC and may play a major role in the production of high levels of E6\*I in this tumor
- The integration of different markers of viral infection (HPV DNA, viral load, E6\*I mRNA expression) is the optimal strategy to identify HPV-related HNSCCs.

# FUTURE PERSPECTIVES

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› [mBio](#). 2021 Jan 19;12(1):e03224-20. doi: 10.1128/mBio.03224-20.

## Development of DNA Vaccine Targeting E6 and E7 Proteins of Human Papillomavirus 16 (HPV16) and HPV18 for Immunotherapy in Combination with Recombinant Vaccinia Boost and PD-1 Antibody

Shiwen Peng <sup>1</sup>, Louise Ferrall <sup>1</sup>, Stephanie Gaillard <sup>2 3</sup>, Chenguang Wang <sup>4</sup>, Wei-Yu Chi <sup>1</sup>,  
Chuan-Hsiang Huang <sup>1</sup>, Richard B S Roden <sup>1 2</sup>, T-C Wu <sup>1 2 3</sup>, Yung-Nien Chang <sup>5</sup>,  
Chien-Fu Hung <sup>6 2 3</sup>

Affiliations + expand

PMID: 33468698 PMCID: [PMC7845631](#) DOI: [10.1128/mBio.03224-20](#)

› [Nanomedicine](#). 2020 Oct;29:102254. doi: 10.1016/j.nano.2020.102254. Epub 2020 Jun 30.

## A therapeutic HPV16 E7 vaccine in combination with active anti-FGF-2 immunization synergistically elicits robust antitumor immunity in mice

Hanghang Xie <sup>1</sup>, Congyan Shu <sup>2</sup>, Hongmei Bai <sup>1</sup>, Pengyan Sun <sup>3</sup>, Hongxian Liu <sup>1</sup>, Jialong Qi <sup>1</sup>,  
Sijin Li <sup>1</sup>, Chao Ye <sup>1</sup>, Fulan Gao <sup>1</sup>, Mingcui Yuan <sup>1</sup>, Yongjun Chen <sup>1</sup>, Manchang Pan <sup>4</sup>, Xu Yang <sup>1</sup>,  
Yanbing Ma <sup>5</sup>

Affiliations + expand

PMID: 32615335 DOI: [10.1016/j.nano.2020.102254](#)

# ACKNOWLEDGEMENTS



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**Dr Maria Lina Tornesello**

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**Dr Rosalia Anna Rega**

**Dr Rossella De Cecio**

**Dr Gerardo Ferrara**

**THANK YOU FOR YOUR ATTENTION!**