# *HPV-Positive HNSCC:*

## *Emerging Biomarkers for Predicting HPV+ HNSCCs and Potential Use of FDA-Approved MEK Inhibitors for Treating HPV Precancers and De-Intensifying HPV-Cancer Therapeutics*


Michelle A. Ozbun, Ph.D.

**The Maralyn S. Budke Endowed Professor of Viral Oncology**

Departments of Molecular Genetics & Microbiology, Obstetrics & Gynecology

The University of New Mexico School of Medicine

**The UNM Comprehensive Cancer Center**

The UNM Center for Infectious Disease and Inflammation



American Cancer Society

UNM COMPREHENSIVE CANCER CENTER

NIH National Institute of Dental and Craniofacial Research

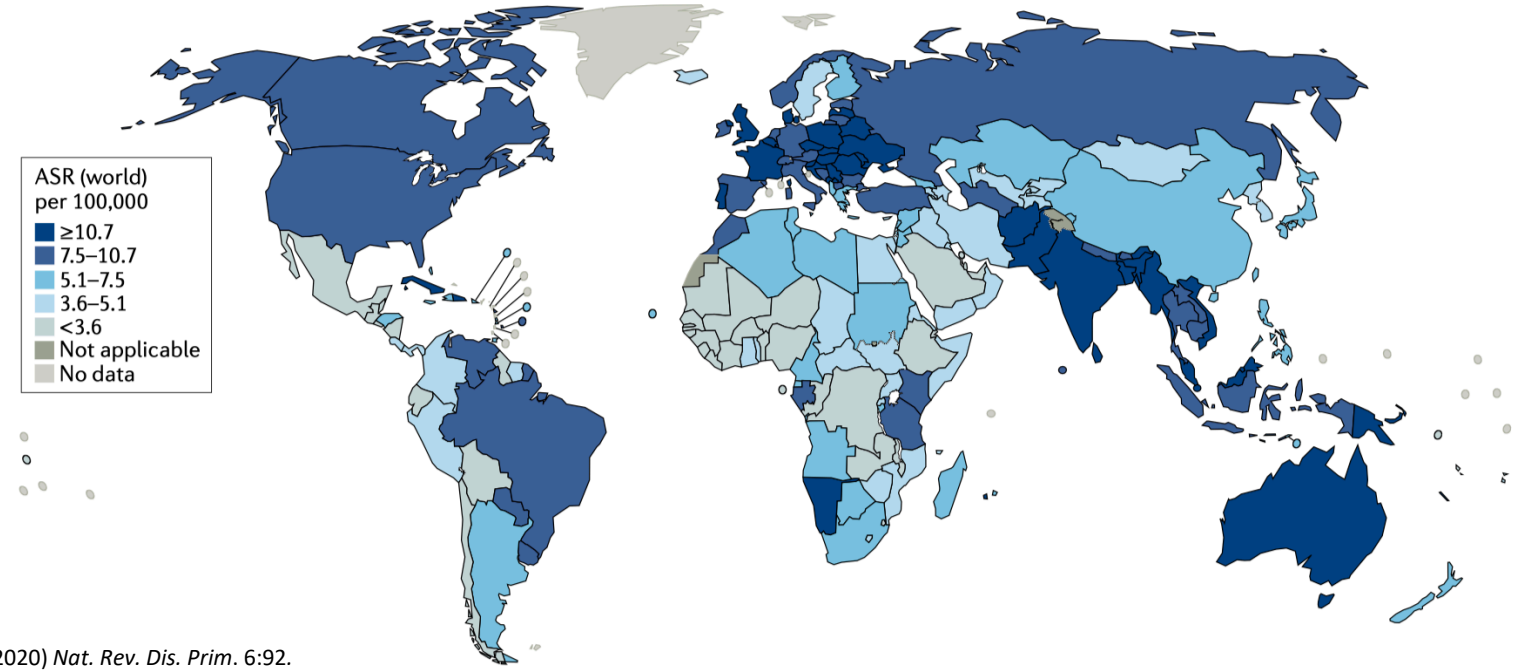
THE V FOUNDATION for Cancer Research

NIH National Institute of Allergy and Infectious Diseases

NIH NATIONAL CANCER INSTITUTE

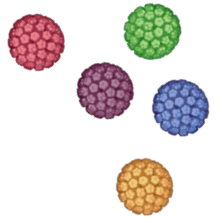
# HPV-Induced HNSCC: Challenges & Opportunities

- Worldwide, HPV-positive OPSCC constitutes ≈1/3 of all OPSCC cases.
- HPV-positive OPSCC are more prevalent in developed countries; **North America and Europe have the highest number of cases.**




Johnson *et al.* (2020) *Nat. Rev. Dis. Prim.* 6:92.

# HPV-Induced HNSCC: Challenges & Opportunities

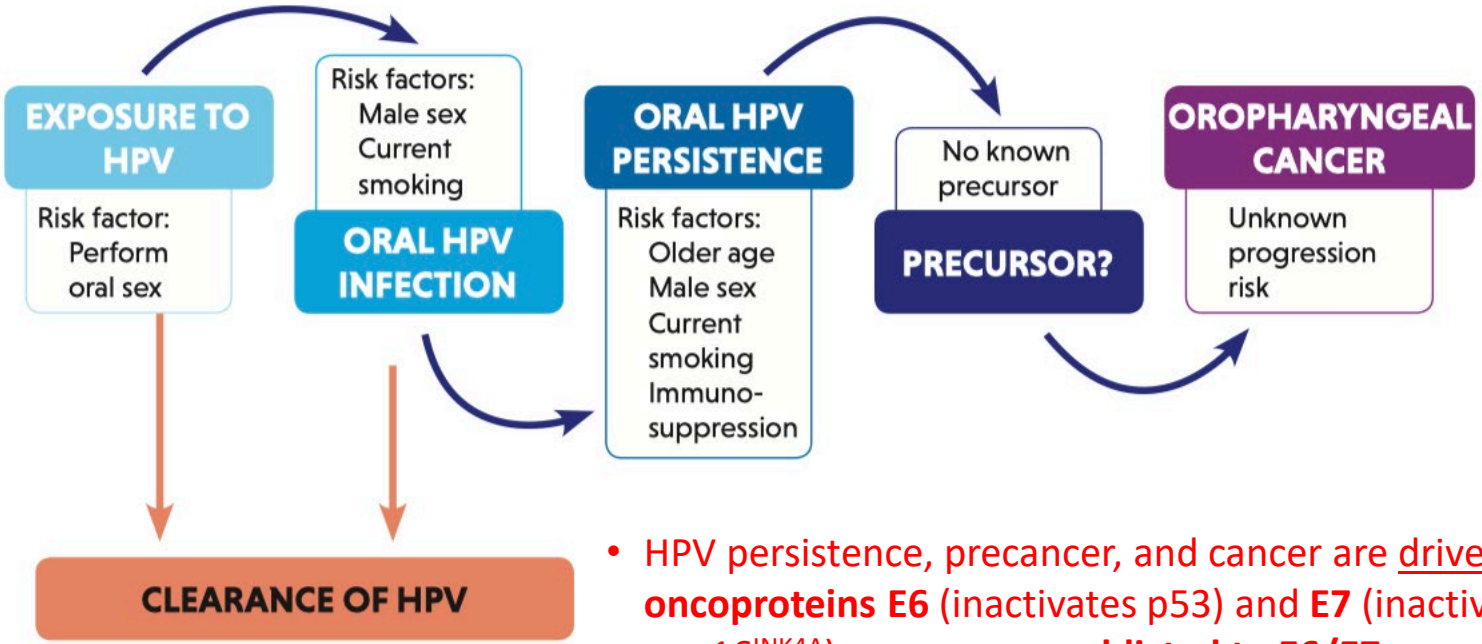


- In the US, HPV vaccine coverage averages 54.2% (13- to 17-year-olds)
- Unvaccinated individuals and those currently infected are at risk for HPV-precancers and cancers
- HPV-related cancer incidence is expected to increase through the year 2040 and beyond, with HPV+ OPSCC outnumbering CxCa cases.

Beachler et al. *J. Natl. Cancer Inst.* 2016;108:djv302.

Canfell et al. *Lancet* 2020;395:591-603.

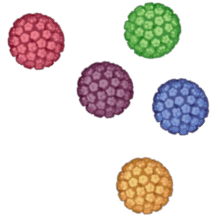
# HPV-Induced HNSCC: Challenges & Opportunities




- HPV persistence, precancer, and cancer are <u>driven</u> by **oncoproteins E6** (inactivates p53) and **E7** (inactivates Rb -> $p16^{INK4A}$); **cancers are addicted to E6/E7 expression**.

D'Souza *et al.* (2020) *www.HPVWorld.com*. 131.

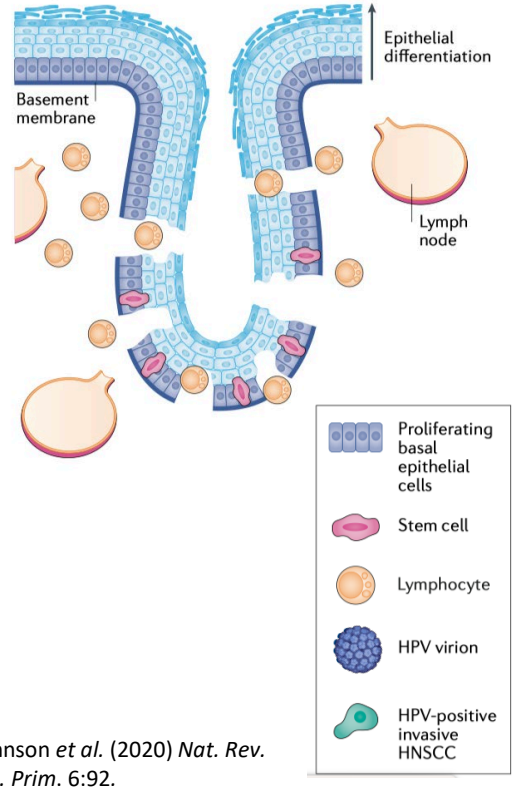
# HPV-Induced HNSCC: Challenges & Opportunities



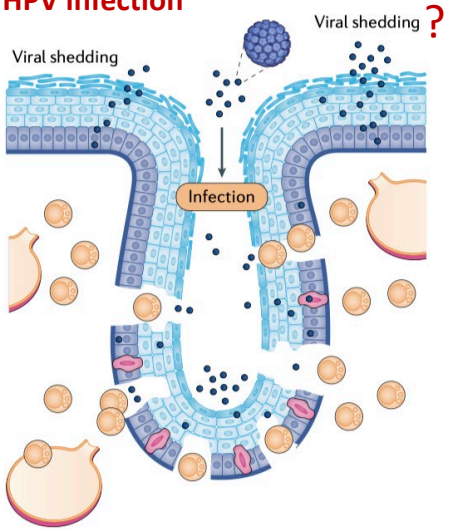
- Challenge 1: In contrast to the HPV disease at the cervix, no screening programs or identifiable pre-malignant lesions have been characterized for HPV+ OPSCC.
- HPV+ OPSCC have increased overall survival (OS) compared to those with HPV-negative disease
  - This group of younger patients could benefit from therapeutic deintensification to reduce the long-term toxicities in anticipation of longer survival
  - Challenge 2: Need to increase our understanding of HPV infections/ disease to provide rationale for de-intensifying therapies.

# Challenge 1: HPV+ OPSCC Arises in Oral Crypts

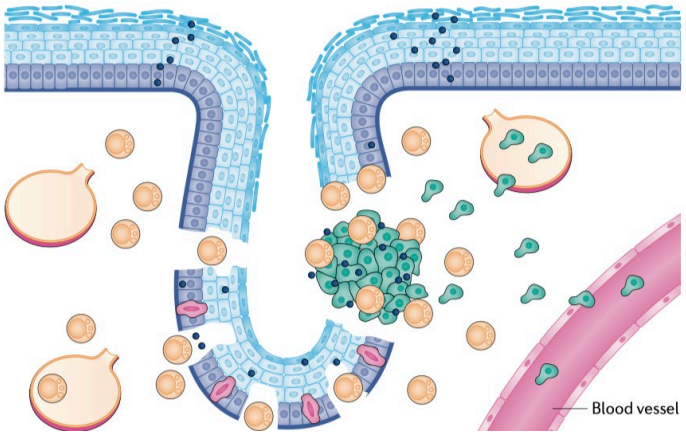
**Normal tonsil crypt**




**HPV infection**




**HPV-mediated malignant transformation**

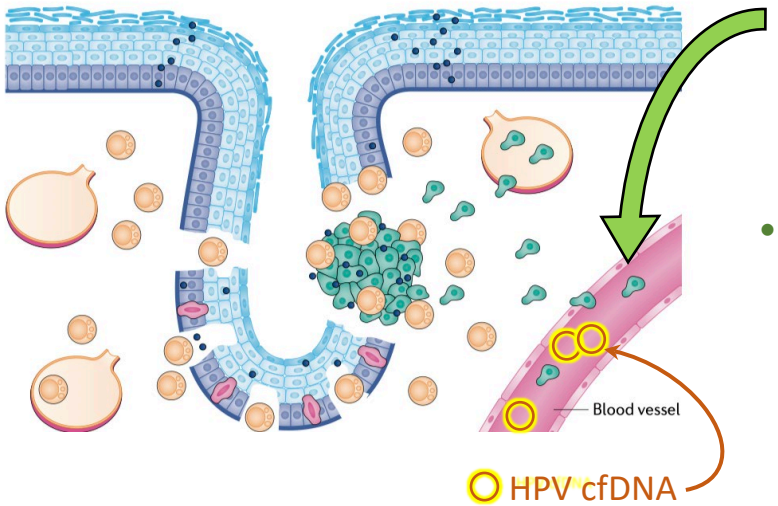



- Precancers thought to arise in the crypts
- Difficult to sample premalignant cells

Johnson *et al.* (2020) *Nat. Rev. Dis. Prim*. 6:92.

# Potential Screening Biomarker: HPV cfDNA

**HPV-mediated malignant transformation**




- HPV circulating-tumor DNA (ctDNA): released by cancer cells under necrosis, apoptosis, or *via* active secretion mechanisms in the bloodstream compartment
- HPV circulating-free DNA (cfDNA): A prospective study reported that cfHPV DNA detection in plasma is specific biomarkers with high sensitivity, representing a useful non-invasive diagnostic approach to identify HPV-related HNSCCs

➢ A case-control study showed that in a subset of patients, **cfHPV16 DNA was detected 3 years before the clinical diagnosis of HPV16-related HNSCC** (none of the controls were +)

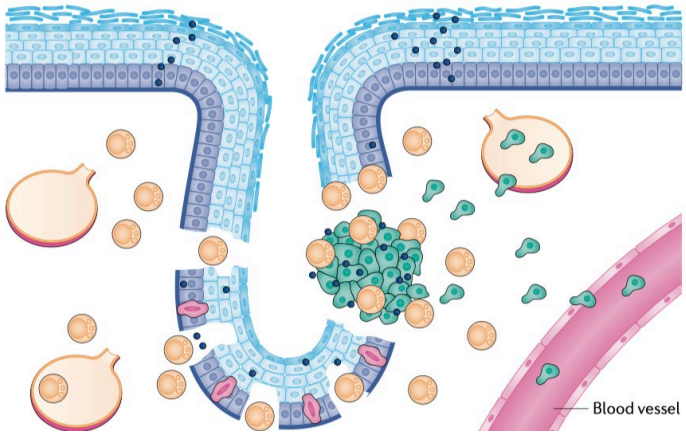
Modified from: Johnson *et al.* (2020) *Nat. Rev. Dis. Prim*. 6:92.

Siravegna *et al.* (2022) *Clin Cancer Res* 28: 719–727.

Galati *et al.* (2022) *Tumor Virus Res.* 30:1335-1343.
Rettig *et al*. (2022) *Int. J. Cancer* 151(7):1081-1085.

# Potential Screening Biomarkers: E6 Antibodies

**HPV-mediated malignant transformation**




- HPV16 E6 serum Abs have high sensitivity (>90%) and specificity (>99%) for the diagnosis of concurrent HPV16-positive OPSCC
- HPV16 E6 Abs are strongly associated with HPV-induced tumors at the time of or prior to cancer diagnosis (>100-fold risk), **preceding cancer diagnosis by ≈ 5-15 years**
- E6 Abs are rare in cancer-free individuals (<1% prevalence)

➢ Positive predictive value for HPV+ OPSCC risk by E6 Ab is low; the number needed to screen to identify one HPV+ OPSCC likely exceeds several thousands.

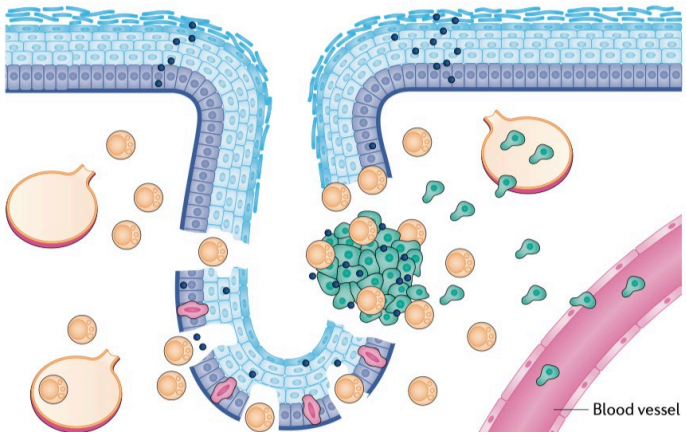
Modified from: Johnson *et al.* (2020) *Nat. Rev. Dis. Prim*. 6:92.

Galati *et al.* (2023) *Tumor Virus Res.* 14:200245.
Kreimer *et al.* (2019) *Ann Oncol* 30:1335-1343.

Busch *et al.* (2022) *EClinicalMedicine* 53:101659.
D'Souza *et al.* (2023) *Cancer* 129:2373-2384.

# Potential Screening Biomarkers: E6 Ab + ctDNA

**HPV-mediated malignant transformation**




- These biomarkers are both detectable in 85–90% of HPV-positive OPSCC patients at diagnosis with 95–100% specificity
- Rettig *et al.* compared both in the pre-diagnostic setting to understand their temporal relationship with respect to clinical disease manifestation:
  - 9 cases, 45 matched controls

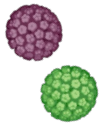
➢ In this exploratory cohort, HPV16 E6 antibodies were more commonly detected than cf/ctHPV16 DNA in blood collected prior to diagnosis of HPV16-positive HNC.

➢ E6 seroconversion occurs **before tumor DNA is shed into the circulation at detectable levels among individuals later diagnosed with HPV-positive OPC**

Modified from: Johnson *et al.* (2020) *Nat. Rev. Dis. Prim.* 6:92.

Rettig *et al*. (2023) *Oral Oncology* 141:106417.

# Challenge 2: Increase understanding of HPV disease to provide rationale for de-intensifying therapies



- HPV persistence, precancer, and cancer are **driven by expression of E6 and E7 oncoproteins** (inactivates p53, inactivates Rb -> $p16^{INK4A}$, respectively).
- **HPV+ cancers are addicted to E6/E7 expression**.
- *Can we suppress HPV E6/E7 expression to restore tumor suppressor functions and render cancer cells susceptible to lower doses of cancer treatments?*

# FDA-Approved MEK Inhibitors Suppress Papillomavirus E6 and E7 Expression

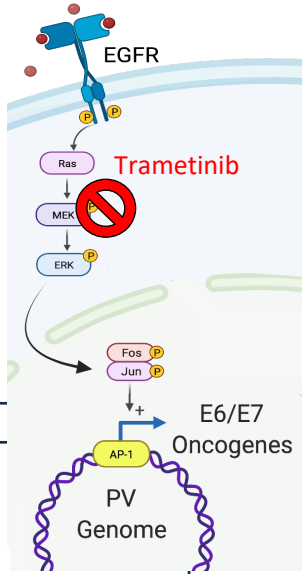
**PLOS PATHOGENS** (2021) 17(1): e1009216

RESEARCH ARTICLE

## MEK/ERK signaling is a critical regulator of high-risk human papillomavirus oncogene expression revealing therapeutic targets for HPV-induced tumors

**Adrian J. Luna[1], Rosa T. Sterk[1], Anastacia M. Griego-Fisher[1], Joon-Yong Chung[2], Kiersten L. Berggren[1], Virginie Bondu[1], Pamela Barraza-Flores[1¤], Andrew T. Cowan[3,4], Gregory N. Gan[5], Emrullah Yilmaz[4,6], Hanbyoul Cho[7], Jae-Hoon Kim[7], Stephen M. Hewitt[2], Julie E. Bauman[8], Michelle A. Ozbun[1,4]***

https://pubmed.ncbi.nlm.nih.gov/33481911/



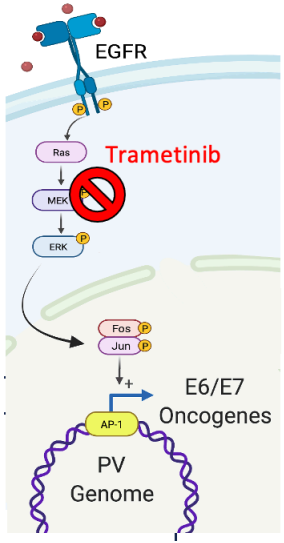

Antiviral Research 216 (2023) 105667

## The antiviral effects of a MEK1/2 inhibitor promote tumor regression in a preclinical model of human papillomavirus infection-induced tumorigenesis

Adrian J. Luna [a,1], Jesse M. Young [a,2], Rosa T. Sterk [a], Virginie Bondu [a], Fred A. Schultz [b], Donna F. Kusewitt [b,d], Huining Kang [c,d], Michelle A. Ozbun [a,d,*]

https://pubmed.ncbi.nlm.nih.gov/37429527/

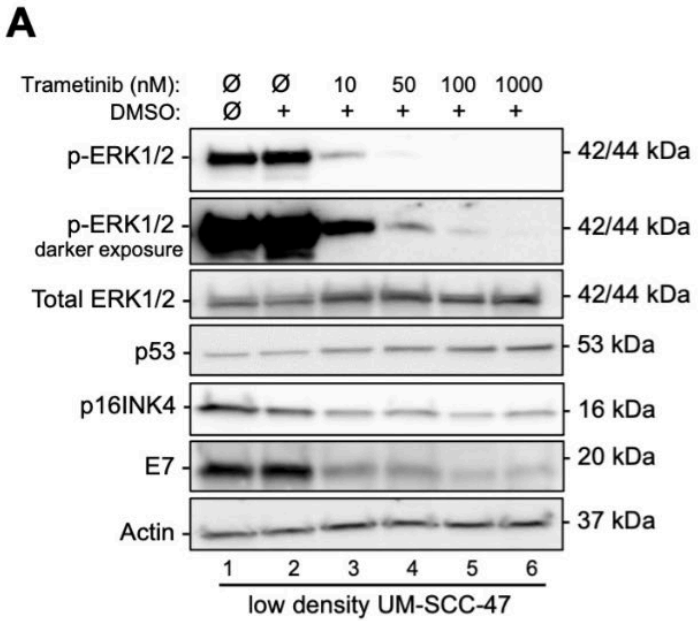
# HPV16 E6/E7 Transcription in HNSCC cells is MEK/ERK Signaling-Responsive

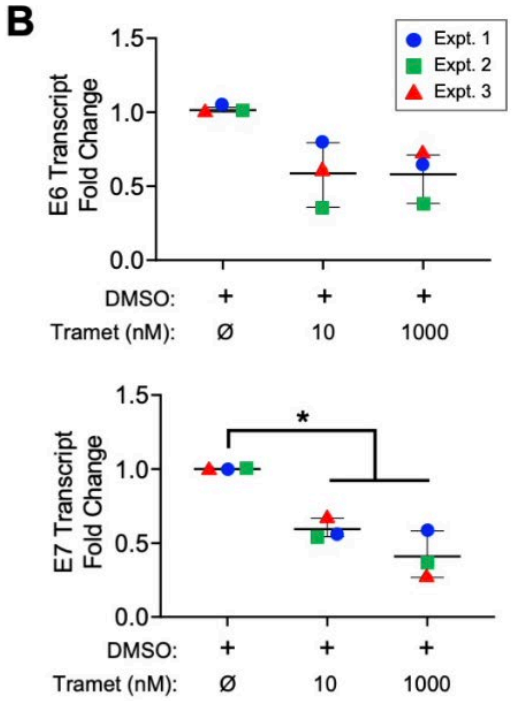




HPV genome integration invariably includes the LCR upstream of E6/E7

UM-SCC-47 HNSCC cells with integrated HPV16 genomes treated with **trametinib** for 24 h



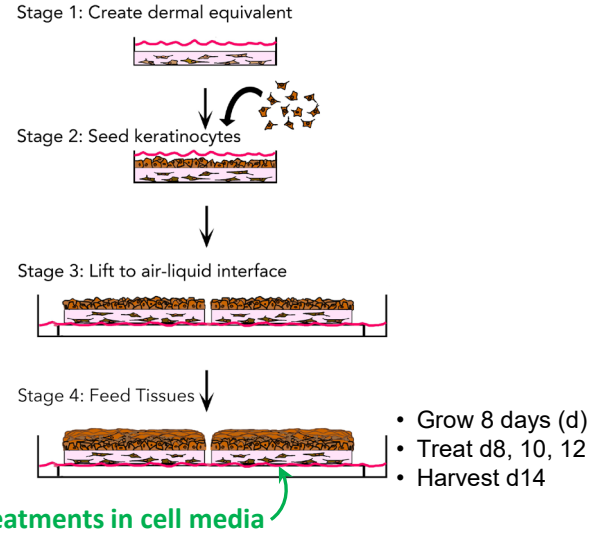

Trametinib, an allosteric **MEK1/2 inhibitor**, has anti-viral effects in cell culture

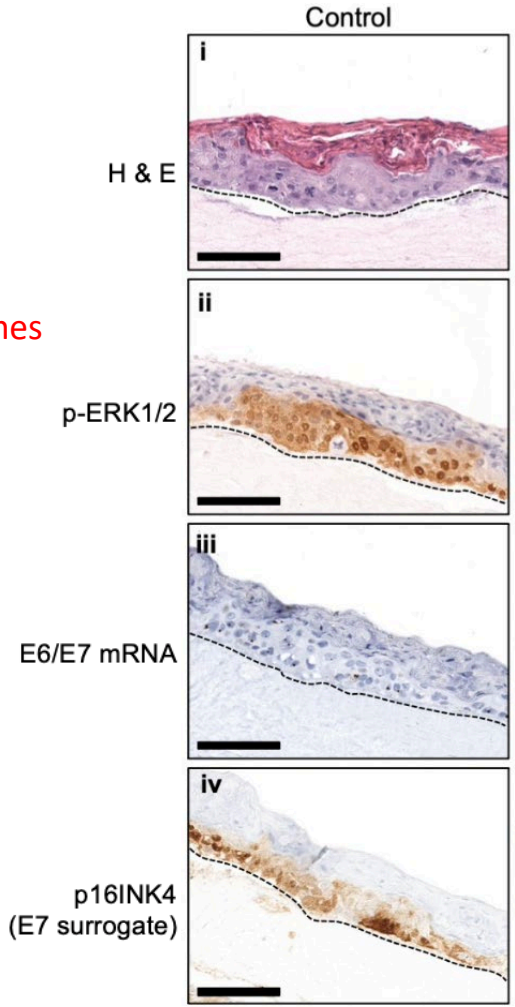
Luna *et al.* (2021) PLoS Pathog. **17**(1):e1009216

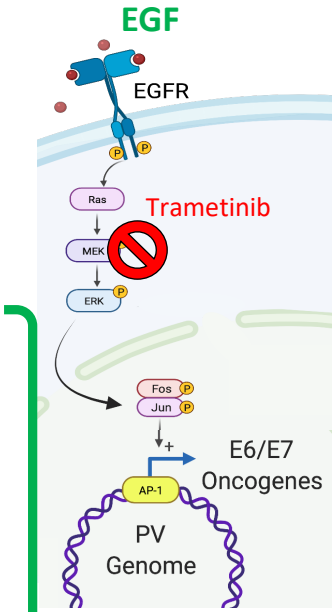
# HPV16 E6/E7 Transcription in Raft Tissues is MEK/ERK Signaling-Responsive

HNSCC tissues with integrated HPV16 genomes

**3D Organotypic Epithelial (Raft) Tissues**



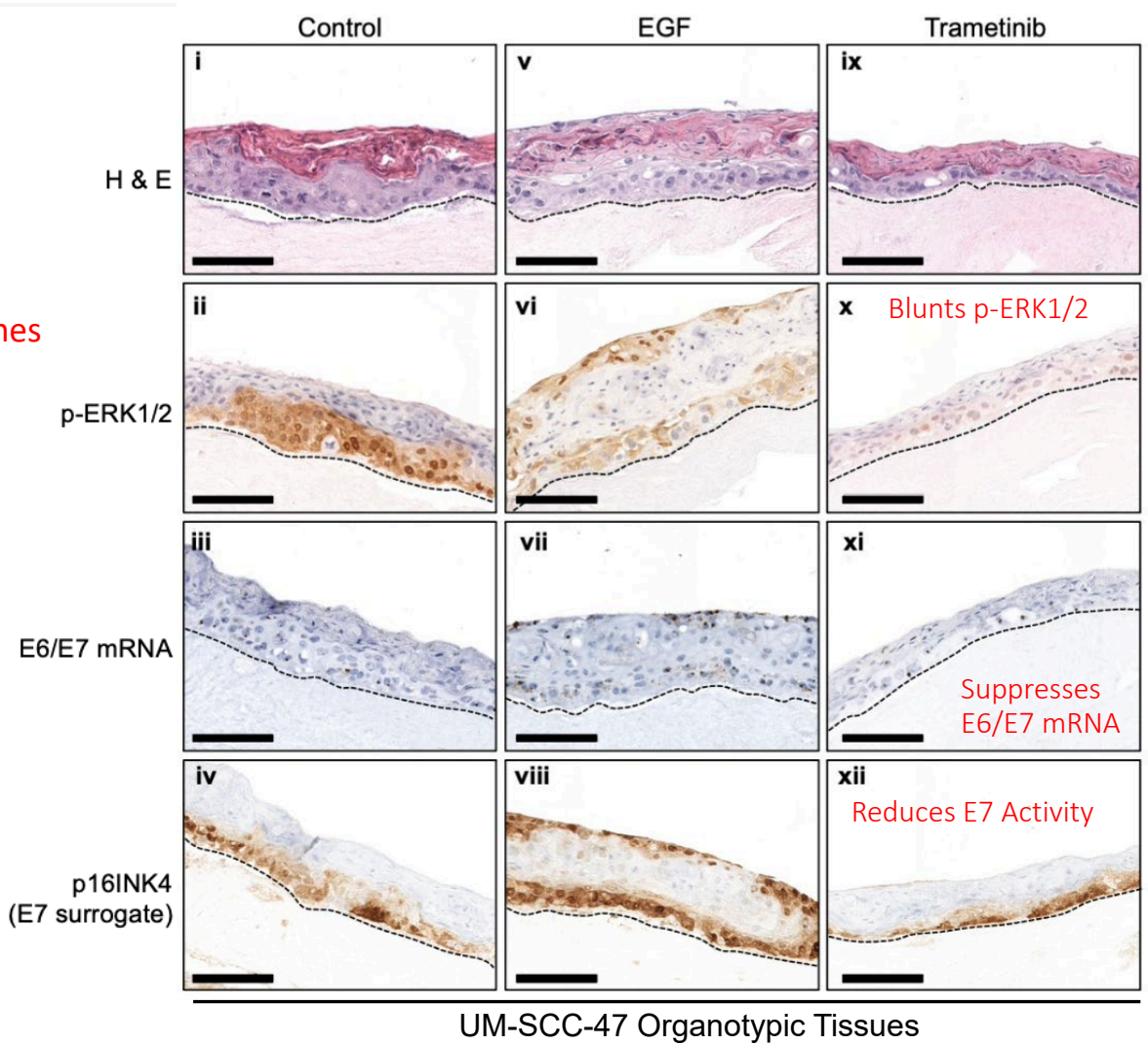

UM-SCC-47 Organotypic Tissues

Luna *et al.* (2021) PLoS Pathog. **17**(1):e1009216

# HPV16 E6/E7 Transcription in Raft Tissues is MEK/ERK Signaling-Responsive

HNSCC tissues with integrated HPV16 genomes

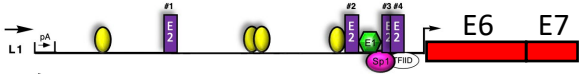
Trametinib, an allosteric **MEK1/2 inhibitor**, has anti-viral effects in the tissue context




Luna *et al.* (2021) PLoS Pathog. **17**(1):e1009216

# MEK Inhibitors Have Growth Suppressive Effects in HPV Tumor Xenografts

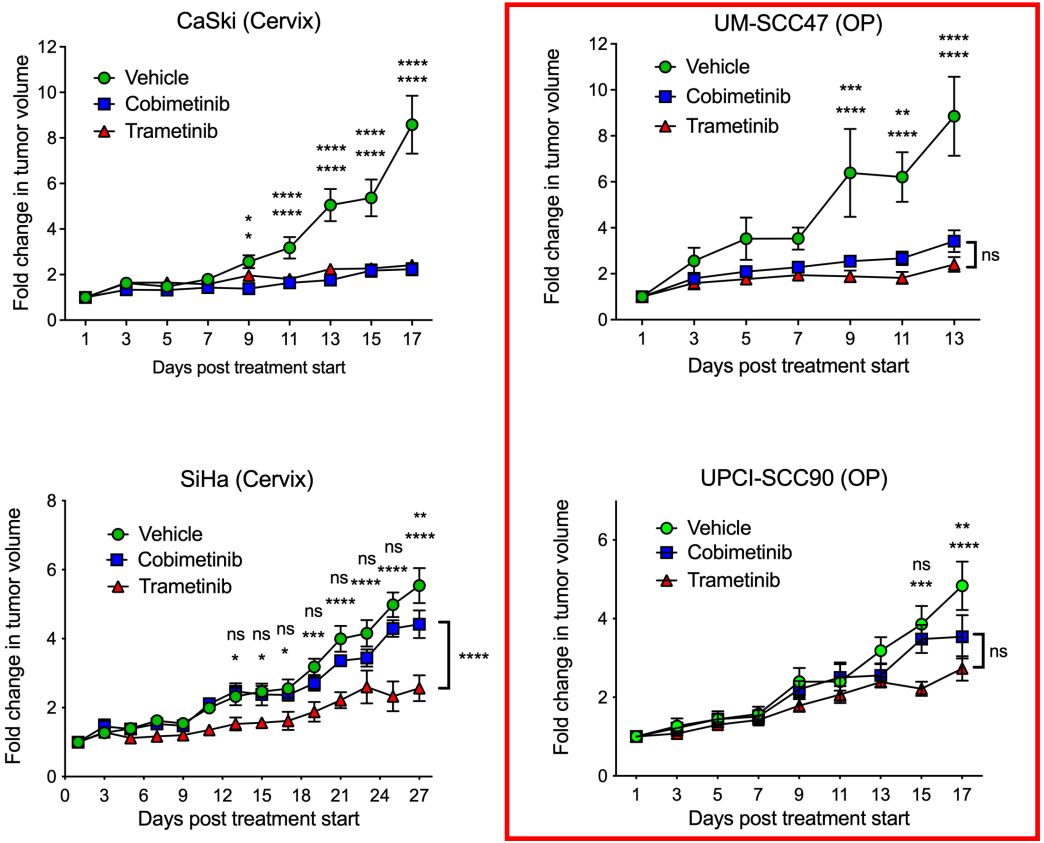
SCC cells with integrated HPV16 genomes




HPV genome integration invariably includes the LCR upstream of E6/E7

**FDA-Approved Small Molecules**

- Cobimetinib = MEK1 inhibitor
- Trametinib = MEK1/2 inhibitor

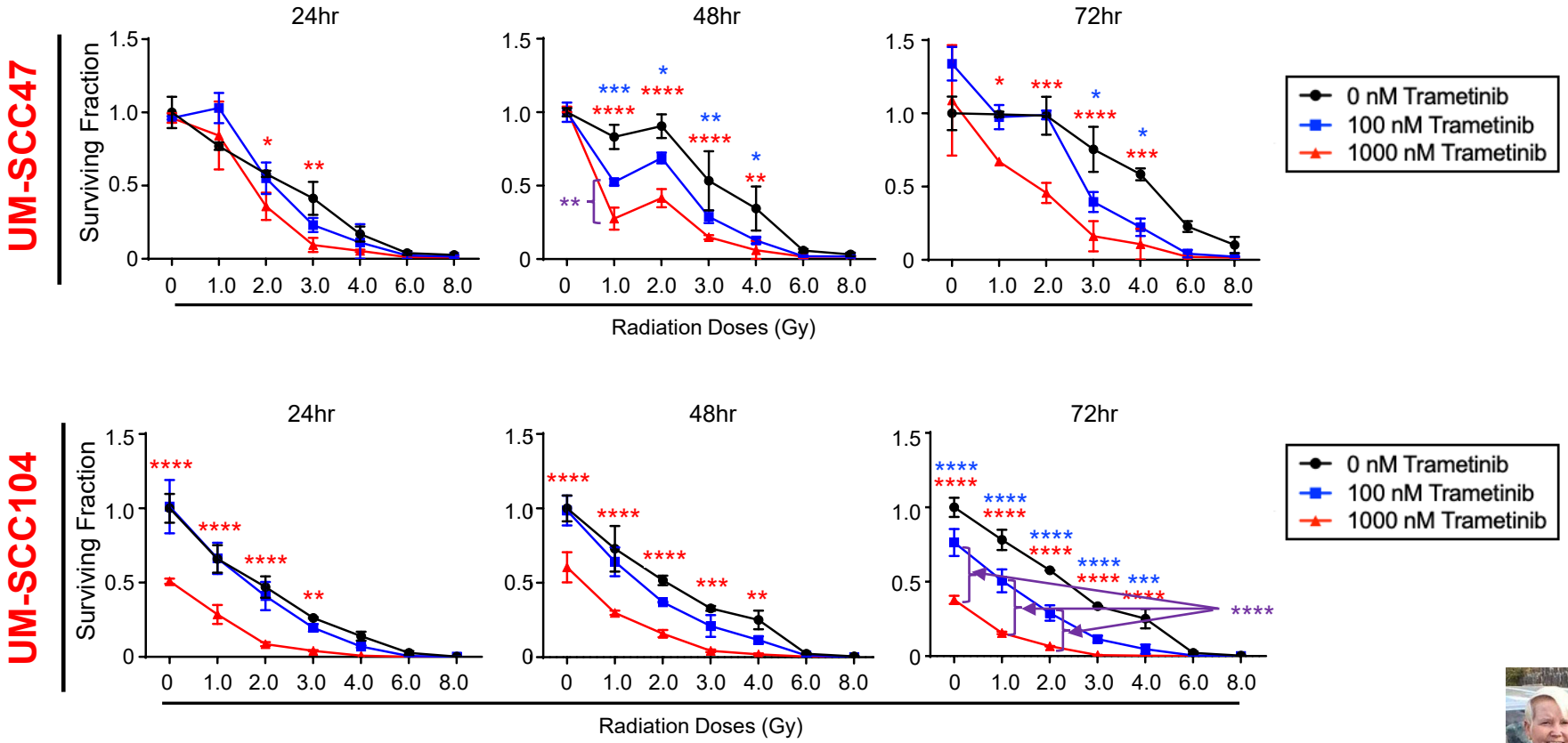



n=5-6 mice/group
2-way Anova fold changes, NSG mice

MEK1/2 inhibition is superior to MEK1 inhibition (tissue analyses are pending)

Berggren, Ozbun unpublished

# Trametinib Sensitizes HPV16+ OPSCC Cell Lines to Radiation *in vitro*




Berggren, Ozbun unpublished

# SUMMARY

- HPV cfDNA and/or E6 antibodies in blood are biomarkers for HPV+ OPSCC
  - Non-invasive screening
  - The presence of E6 Abs precedes cancer diagnosis by ≈ 5-15 years
- MEK1/2 inhibition effectively suppresses HPV E6 and E7 expression
  - Suppresses tumor growth in the absence of T cell involvement (may be more effective in immunocompetent hosts)
  - Sensitizes HPV+ OPSCC cells to lower radiation doses
  - ➢ Reduced E6/E7 expression leads to decreased PD-L1 expression and restored MHC Class I presentation – *could this relieve immune checkpoint blockade?*
- **MEK/ERK signaling represents a targetable HPV pathway for precancers and cancers**