Why consider therapeutic approaches for HPV-associated lesions and cancers?

500,000 new cases/288,000 cervical cancer deaths per year (worldwide)  
(2nd most common cause of cancer death in women)

Prophylactic vaccines will only protect from new infections by vaccine HPVs

Vaccine implementation has been challenging.  
(US 2007: 25% of girls ages 13-17, 10% of all females ages 18-26  
1.1% of Hispanic women)

Cervical cancer develops decades after the initial HPV infection

It will take decades before such vaccines will result in a measurable decline of HPV-associated cancers
HPV genome integration is a hallmark of malignant progression

HPV-associated cancers only express two viral proteins, E6 and E7
Mechanisms of HPV-associated carcinogenesis

HPV oncoproteins target cellular signaling pathways that are frequently mutated in human solid tumors:

- pRB pathway >80%
- p53 >60%
- Telomere maintenance ~100%
Evidence that HPV E6/E7 expression is necessary and sufficient for cervical carcinogenesis

HPV16 E6/E7 expression in primary epithelial cells causes histopathological abnormalities reminiscent of CIN3.

HPV16 E6/E7 expression causes cervical cancer in transgenic mice.

Extinguishing HPV E6/E7 expression in cervical cancer cell lines causes cellular senescence/G1 arrest/apoptosis.

HPV-associated cancers are the only human solid tumors where the carcinogenic agent is known at a molecular level.
HPV-associated cancers are driven by expression of the E6 and E7 oncoproteins

HPV E6 and E7 oncoproteins should be excellent drug targets
**HPV E6/E7 Oncoproteins**

<table>
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<tr>
<th>E6</th>
<th>CXXC - X&lt;sub&gt;29&lt;/sub&gt; - CXXC</th>
<th>CXXC - X&lt;sub&gt;29&lt;/sub&gt; - CXXC</th>
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<th>E7</th>
<th>LXXC&lt;sub&gt;X&lt;/sub&gt; CKII CXXC - X&lt;sub&gt;29&lt;/sub&gt; - CXXC</th>
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- No cellular homologues
- No intrinsic enzymatic activities
- No specific DNA binding activities
- Associate with and functionally modify host cellular protein complexes
HPV E6 and E7 function through protein/protein interactions
(E6/p53; E7/pRB)

Protein/protein interactions are difficult to target by small molecule approaches
Identification of therapeutic targets

Are HPV oncoproteins associated with cellular enzymatic activities that are necessary for their activities?

Does expression of HPV oncoproteins induce perturbations of cellular signal transduction networks that may be harnessed for therapy?
Are HPV oncoproteins associated with cellular enzymatic activities that are necessary for their activities?
Proteomic analysis of HPV16 E7 associated host cellular protein complexes

- Construction of stable cell lines expressing double tagged E7
- Sequential immunoaffinity purification with anti-FLAG and anti-HA resin
- SDS-PAGE and stain
- Identification of protein components by mass spectrometry
HPV E6 and E7 oncoproteins associate with and reprogram cellular enzymes

HPV16 E6 retargets the cellular cullin 2 ubiquitin ligase complex to the retinoblastoma tumor suppressor protein, pRB

HPV16 E6 retargets the cellular E6AP ubiquitin ligase to the p53 tumor suppressor protein
Are HPV-positive cancer lines sensitive to the proteasome inhibitor, Bortezomib?

EC50s:

- CaSki (HPV16): ~10-15 nM
- SiHa (HPV16): ~30-35 nM
- HeLa (HPV18): ~25 nM

Will test on HPV16 oncogene expressing primary human epithelial cells

Karin Hellner

collaboration with Jochen Lorch/ Marshall Posner
Identification of therapeutic targets

Does expression of HPV oncoproteins induce perturbations of cellular signal transduction networks that may be harnessed for therapy?

“Under the Streetlight” Approach

“You study what you can see”

“Unbiased” approach

“You see what you can study”
Identification of therapeutic targets

“Under the Streetlight” Approach

“You study what you can see”
HPV E6 expression activates mTOR signaling

Jennifer Spangle based on
Lu et al, JBC 279: 35664-70, 2004
Are HPV-positive cancer lines susceptible to mTOR inhibitors?

**EC50s for RAD001:**

- CaSki (HPV16): ~15 µM
- SiHa (HPV16): ~25 µM
- HeLa (HPV18): ~27 µM

Will test on HPV16 oncogene expressing primary human epithelial cells
Will test combination with 2-deoxyglucose

Karin Hellner
collaboration with Jochen Lorch/Marshall Posner
Identification of therapeutic targets

“Unbiased” approach

Genetic Screens

Systems Biology

“You see what you can study”
Identification of therapeutic targets

“Unbiased” approach

Genetic Screens

“You see what you can study”
Essential kinases for cervical cancers

Precedence: Gleevec® inhibits the bcr/abl kinase that is critical for the growth of certain leukemias (CML) that have the Philadelphia translocation

Can we identify a kinase target for a “cervical cancer-specific Gleevec”? 

Ed Harlow
Dorre Grueneberg
Karl Munger
Amy Baldwin
Karin Hellner
Miranda Grace
Haoxuan Tong
Essential kinases for cervical cancers: Experimental Strategy

“100 Hits” Kinase shRNA library
Targets a subset of 80 kinases that potently kill a variety of cancer cell lines

Identify essential kinases for each cell population

- Normal Keratinocytes
- Cervical Carcinoma
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PAK3 and SGK2 become essential upon HPV E6 expression
PAK3 Inhibitor Screen
Collaboration with Greg Cuny/Jun Xian (PCDD)

123,751 Compounds

Primary screen (8.4 µM)

578 Hits (0.47%)

Secondary screen (10, 1, 0.1 µM)

137 “Confirmed” Hits (23.7%)

Decoding Scaffold analysis

16 “Drug-like” compounds

IC50

4 Compounds chosen for SAR

IC50

Biological Assays Specificity

Karin Hellner
Identification of therapeutic targets

“Unbiased” approach

Systems Biology

“You see what you can study”
An integrative approach to identify cellular network perturbations induced by viral oncoprotein expression

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Dorre Grueneberg (HMS)
The RNAi Consortium (Broad)

Marshall Posner (DFCI)
Jochen Lorch (DFCI)

Greg Cuny (PCDD)
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(LSUHSC, New Orleans, LA)
Lessons and Take Home Messages

- Virus associates cancers offer unique opportunities for prevention, diagnosis and therapy

- Some mechanistic insights obtained with HPV-associated cancers should be generally applicable to non-virus associated human cancers

- Unbiased genetic screens and integrative, system biology based approaches will define novel therapeutic targets