

# Fluorescence In Situ Hybridization (FISH) or Other In Situ Hybridization (ISH) Testing of Uterine Cervical Cells to Predict Precancer and Cancer:

## Can FISH replace the Pap test?

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# My credentials

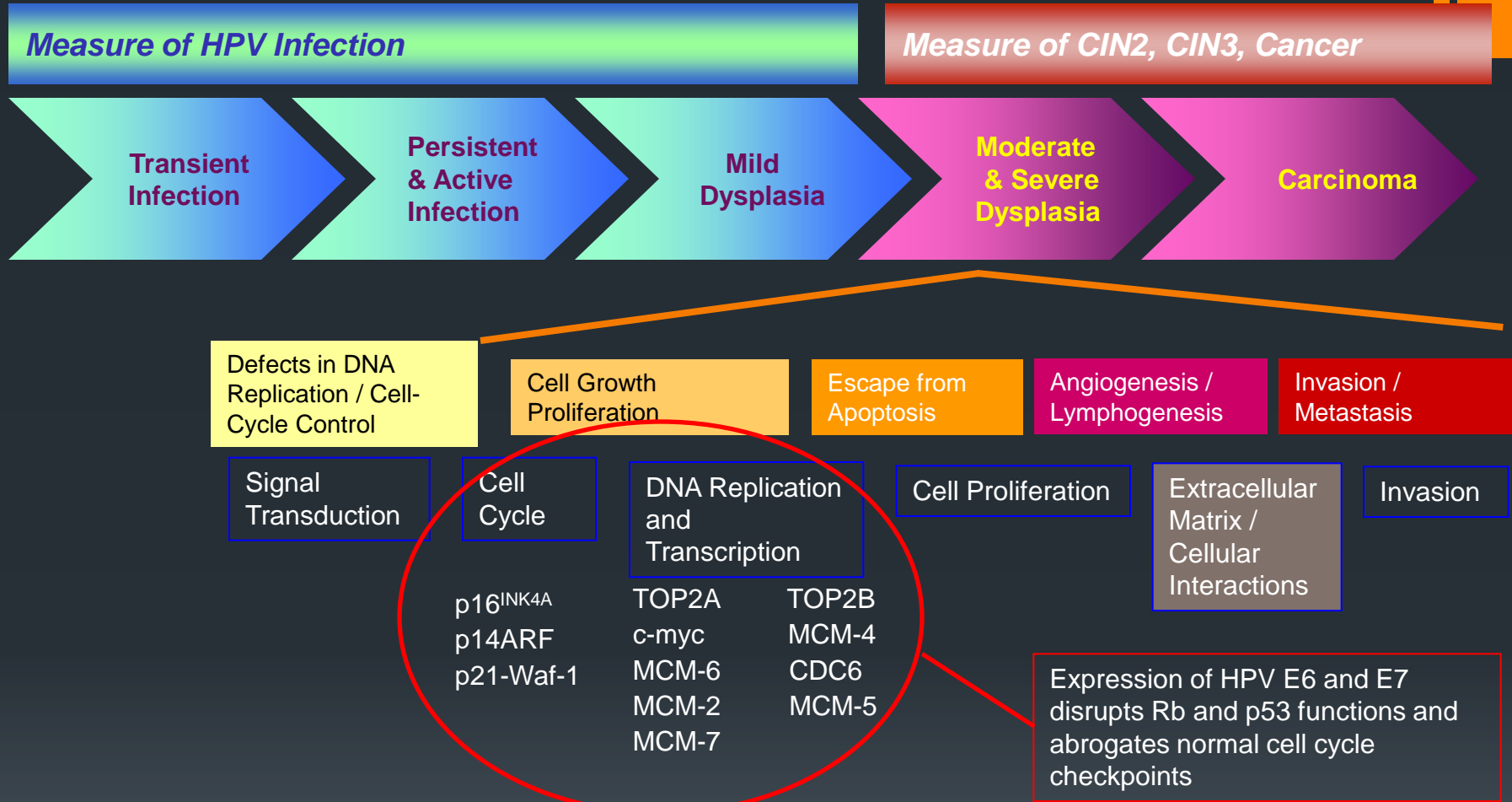
- Emeritus Professor of Pathology, UCLA
- Professor of Pathology, Oncology, and Gynecology/Obstetrics, The Johns Hopkins University (JHU)
- Cytopathologist at Johns Hopkins Hospital (JHH)



# My disclosures

- Member, Scientific Advisory Board, BD-TriPath Oncology
- Consultant, Women's Health and Cancer, BD-Diagnostics


# Biomarkers and the Molecular Biology of Cervical Neoplasia





# Cervical Cancer in Developing Countries

- First or second most common cancer among women in many developing countries
- 370,000 out of 466,000 cases in the world (year 2000)
- 231,000 cervical cancer deaths worldwide, 80% in developing countries.
- Sankaranarayanan R, Budukh AM, Rajkumar R. Effective screening programmes for cervical cancer in low- and middle-income developing countries. Bull World Health Organ. 2001;79(10):954-62.



# Success and limitations of the Pap Smear

- Reduction of death rate by 70%  
in screened populations
- Annual new cases in US = ~12,000
- Annual deaths = < 5,000
- Inexpensive, non-invasive
- High false negative rate
- Need for frequent repetition
- Need for trained cytotechnologists and cytopathologists



# Improvements to the Pap smear

- Standardized reporting terminology, The Bethesda System (USA)
- Computerized scanners for Pap smears
- Liquid-based Pap tests
- HPV testing on residual of mildly abnormal Pap tests



# Pap smear terminology

- Negative = 90% population in USA
- ASC-US = 6% (<50% are HPV positive)
- SIL = 3%
- Cancer = <1%

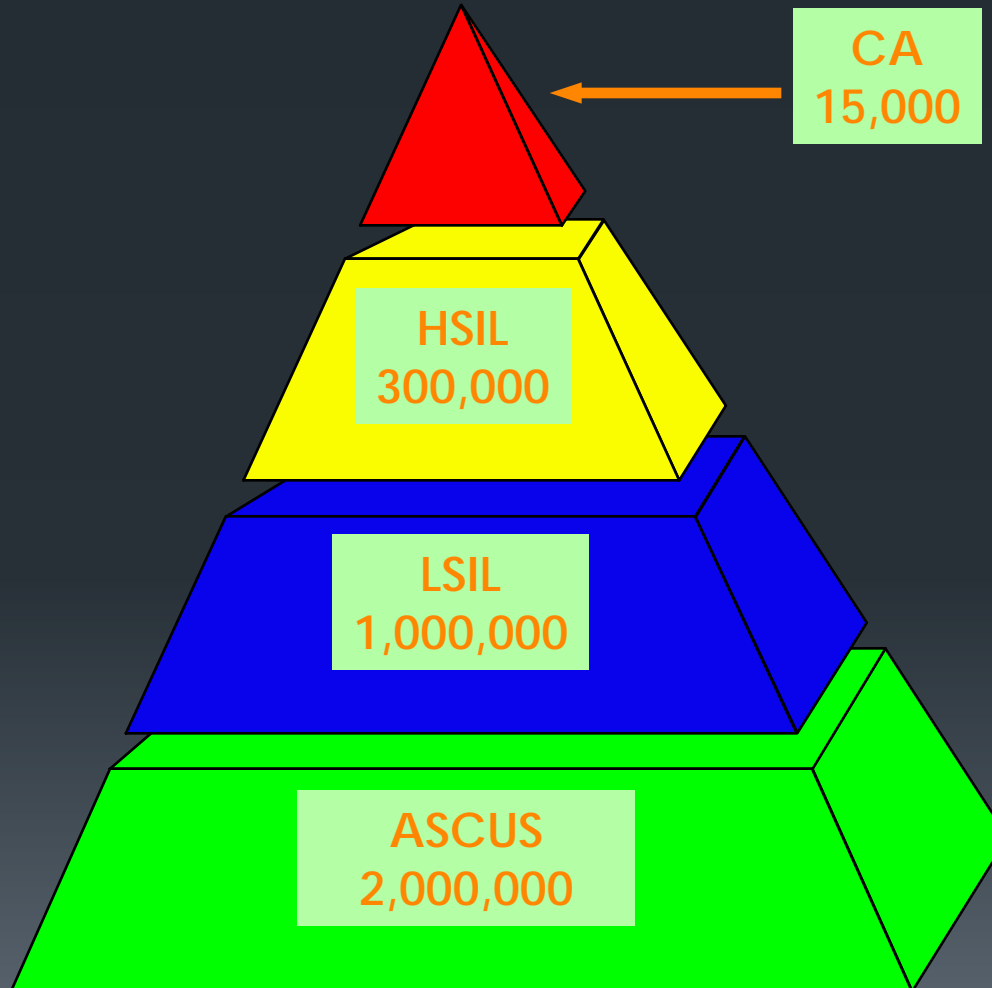




# Definitions:

- ASC-US = Atypical Squamous Cells of Undetermined Significance
- SIL = Squamous intraepithelial lesion
- CIN = Cervical Intraepithelial neoplasia
- False negative = Disease, but negative test
- False positive = NO disease, positive test

# Cervical Lesions: Pyramid of Pap Diagnoses (55 M/yr)





# Burden of ASCUS/LSIL

- 50 million annual Paps in USA
- 5-10% ASCUS/LSIL
- Economic impact: \$3-4 billion/year to evaluate, incl. colpo and biopsy
- Psychological burden substantial



# Human Papillomavirus (HPV)

- The cause of precursor lesions
- Usually clears spontaneously
- High prevalence in young women
- Prevalence declines sharply after age 35
- All cancers of cervix caused by HPV, but most HPV infections do not progress to cervical cancer - WHY?



# HPV tests

- Multivalent HPV-DNA Hybrid capture probe
- ImmunoHisto/Cyto Chemistry
- Specific genotyping for most common: 16, 18 & 45
- Consider expense vs. disease detected
- False positive and negative rates
- Negative predictive value most important



# Base test frequency upon

- HPV clearance: 8-24 months
- Frequency of screening
- Population risk factors
- Patient compliance
- Cultural factors
- Availability of treatment options



## My Charge by CMS:

- Determine if FISH will improve the test's value for those **Medicare beneficiaries** in whom it may be indicated

# Age distribution of Cervical Ca

## SEER Cancer Statistics Review 1975-2008

AGE	% NEW
<20	0.2
20-34	14.3
35-44	25.8
45-54	23.9
55-64	16.4
65-74	10.6
75-84	6.4
>85	2.5

AGE	IA1-IIA	IIB-IVB
<20	90%	10%
20-24	78.6	21.4
25-29	78.6	21.4
30-34	74.5	25.5
35-39	68.3	31.7
40-44	54.5	45.5
45-49	54.5	45.5
50-54	49.6	50.4
55-59	45.1	54.9
60-64	48.6	51.4
65-69	45.7	54.3
>70	41.2	58.8



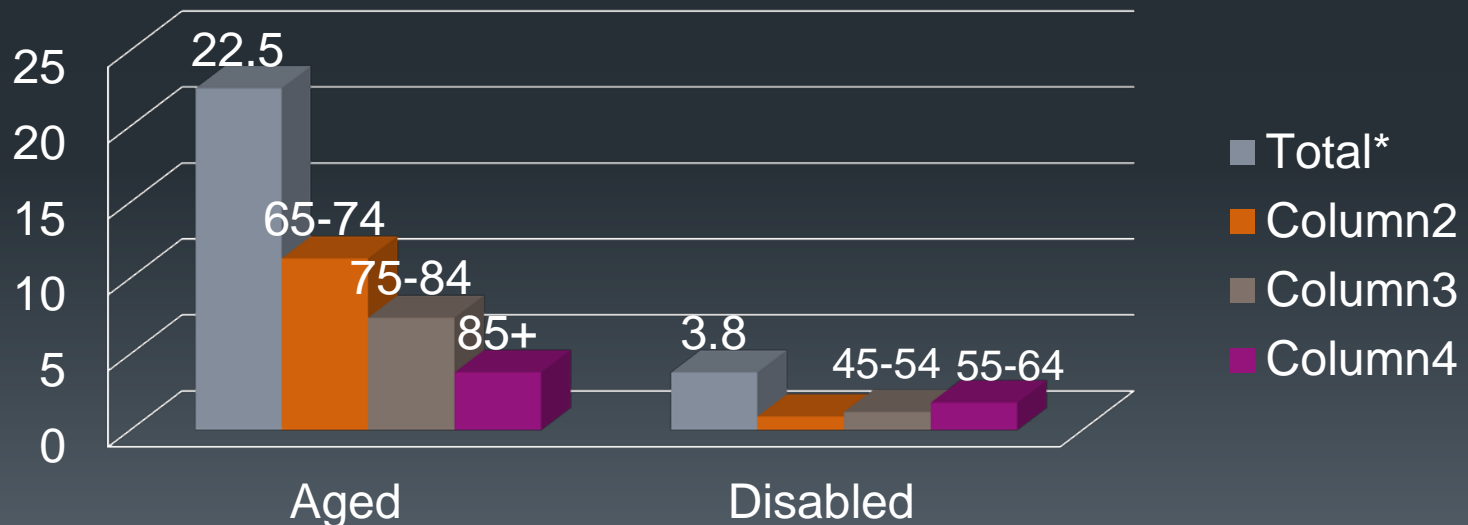


## Medicare Beneficiaries – for 2010, as of December 2011, in millions

	Total - Mil	Male	Female	
Aged - yrs	39.6	17.2	22.5	
65 – 74	21.2	10.0	11.3	
75 – 84	12.8	5.4	7.4	
85 plus	5.6	1.8	3.8	
Disabled	8.0	4.2	3.8	
44 & under	1.9	1.0	0.9	
45 – 54	2.5	1.3	1.2	
55 – 64	3.7	1.9	1.8	

# Who are Female Medicare Beneficiaries?

**\*In millions of patients  
By age in years**





# 2013 Screening Guidelines

- <20 years: No testing
- 20-29 years: Pap only; if ASC-US, reflex HPV
- 30-65 years: Pap plus HPV
- >65: No testing if negative Paps and/or HPV in prior decade
- HPV for primary screening not recommended in USA



# 2013 Intervals for Screening

- Three annual negative Paps = 3 year interval
- Pap and HPV negative = 5 year interval



# AHRQ Technology Assessment

- Role of in situ hybridization (ISH) tests, to detect chromosomal abnormalities or HPV DNA on cervical cytologic specimens (Pap tests), and
- Their clinical validity for identification of precancerous lesions or cervical cancer.



# The Four Questions

- 1. What ISH tests have been used in cervical cytology or histology specimens based on literature search?
- 2. For ISH tests for TERC or MYC, HPV 16 or 18, determine ***analytic validity*** and conditions that impact validity.
- 3. For ISH tests for TERC or MYC, HPV 16 or 18, what is the ***clinical validity*** to detect CIN 3+, factors that impact validity, and applicability to Medicare Beneficiaries?
- 4. For ISH tests for TERC or MYC, HPV 16 or 18, what is published evidence for ***clinical utility*** and harms?



# Literature Search

- Q 1. Identify ISH tests  
Total: 1462 abstracts, 227 full texts
- Q 1. 135 appropriate full texts
- Q 2. Analytic Validity, 16 texts: TERC probe = 2;  
HPV 18 or 18 = 14
- Q 3. Clinical Validity, 10 texts: TERC probe +/- MYC = 8  
HPV 16 or 18 = 3 (one study had both probe types)
- Q 4. Clinical utility and harms: 0 texts



## Q 1: Scientific evidence

- Literature search revealed ISH tests for:
  - TERC (telomerase RNA component gene, 3q26)
  - MYC (myelocytomatosis oncogene, 8q24)
  - HPV 16 and HPV 18
- Gain of TERC linked to development of High-Grade SIL and cervical cancer
- HPV 16 and 18 account for > 70% of all cervical cancers





## Q 2: Analytic validity

- 14 studies compared ISH tests including HPV 16 or 18 with another HPV test, total of 852 patients.
- Comparative results varied in HPV genotypes captured, both within and across studies.
- Study design quite variable: all described performance of index tests to permit replication.
- Half of studies reported use of positive and negative controls
- Some criteria for scoring in majority of studies
- No study reported reproducibility on same sample, across operators, instruments, reagent lots, different laboratories.
- No study addressed yield of useable results, proficiency testing or interlaboratory exchange programs.



Q 3. Clinical Validity:  
LSIL or ASC-US;  
NILM or ASC-US with +HPV



## Q 3. Clinical Validity in LSIL to detect CIN 2+

- 7 studies for TERC; 2 studies for TERC or MYC; 1 all HPV+
- Sensitivity ranged from 0.24 to 1.00
- Specificity ranged from 0.38 to 1.00
- Meta analysis of 7 studies of TERC in LSIL for **CIN 2+**:
  - summary sensitivity 0.76 (95% CI 0.60, 0.86)
  - summary specificity 0.79 (95% CI 0.50, 0.93)
- Meta analysis of 5 studies of TERC in LSIL for **CIN 3+**:
  - summary sensitivity 0.78 (95% CI 0.65, 0.87)
  - summary specificity 0.79 (95% CI 0.51, 0.93)



## Q 3. Clinical Validity in LSIL to detect CIN 2+

- 2 studies compared TERC or MYC vs other tests:
- FISH for TERC or MYC; TERC or MYC or onc-HPV; and HC-2 for onc-HPV:
  - HC-2 for HPV most sensitive
  - FISH for TERC or MYC most specific
- FISH for TERC, HC-2 for onc-HPV, and combo of both: consistent pattern of higher sensitivity and lower specificity from combined test compared to solo tests



## Q 3. Clinical Validity in LSIL to detect CIN 2+

- 3 studies of FISH for HPV 16 or 18
- CIN 2+: sensitivities from 0.75 to 0.81  
specificities from 0.00 to 0.88
- 2 studies of FISH for HPV 16 or 18
- CIN 3+: sensitivities were 0.83 and 0.80  
specificities were 0.42 and 0.17



## Q 3. Clinical validity in ASC-US Paps

- FISH for TERC and/or MYC (3 studies)
  - outcome for CIN 2+: sensitivity 0.75 to 0.82  
specificity 0.87 to 0.93
  - outcome for CIN 3+: sensitivity 0.25 to 0.87  
specificity 0.67 to 0.89
- FISH for TERC versus other tests (one study)
  - outcome similar to LSIL results



## Q 3. Clinical validity in ASC-US Paps

- FISH for HPV 16 or 18:
  - 1 study for CIN 2+: sensitivity 1.00, specificity 0.57
  - 2 studies for CIN 3+: sensitivities of 0.25 and 1.00  
specificities of 0.44 and 0.67
- HPV+ with Normal Paps  
no relevant data found




## Q 4. Clinical utility and harms

- No studies: ISH testing not currently used in practice.



# Research Gaps

- Analytic validity: need to establish common thresholds, probe sets, controls and procedures; place for automation
- Bigger studies needed to yield more precise estimates and patients with CIN 3+
- Studies comparing ISH tests as add-ons to Pap/HPV tests
- Compare to HPV genotyping
- Study combined testing as a panel including TERC, HPV, etc.
- Role of ISH in adenocarcinoma of the cervix will likely need a panel of ISH probes to cover the variability of chromosomal changes between squamous cell carcinoma and endocervical adenocarcinoma.
- Effects of vaccines on natural history of HPV infections



# ISH for cervical cancer screening: Are we ready yet? “NO!”

- Lack of standardization of probes and procedures.
- Inadequate clinical testing to evaluate ISH as an add-on or replacement for HPV and/or Pap testing .
- Evidence as yet is too immature.
- Medicare Beneficiaries are generally beyond the age of screening (>65).