Methylation in predicting progression of untreated high grade cervical intraepithelial neoplasia

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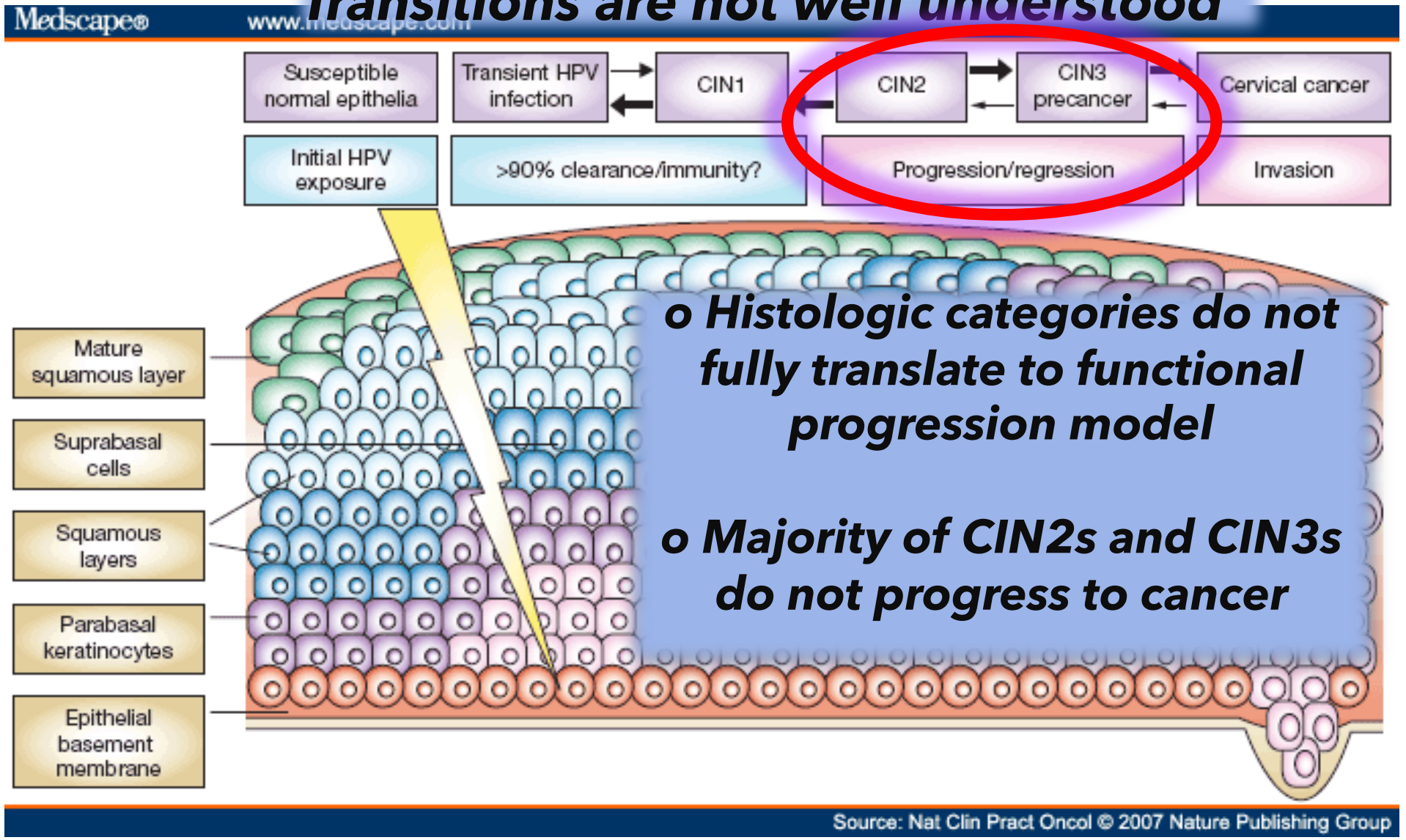
Cervical cancer progression model

Transitions are not well understood



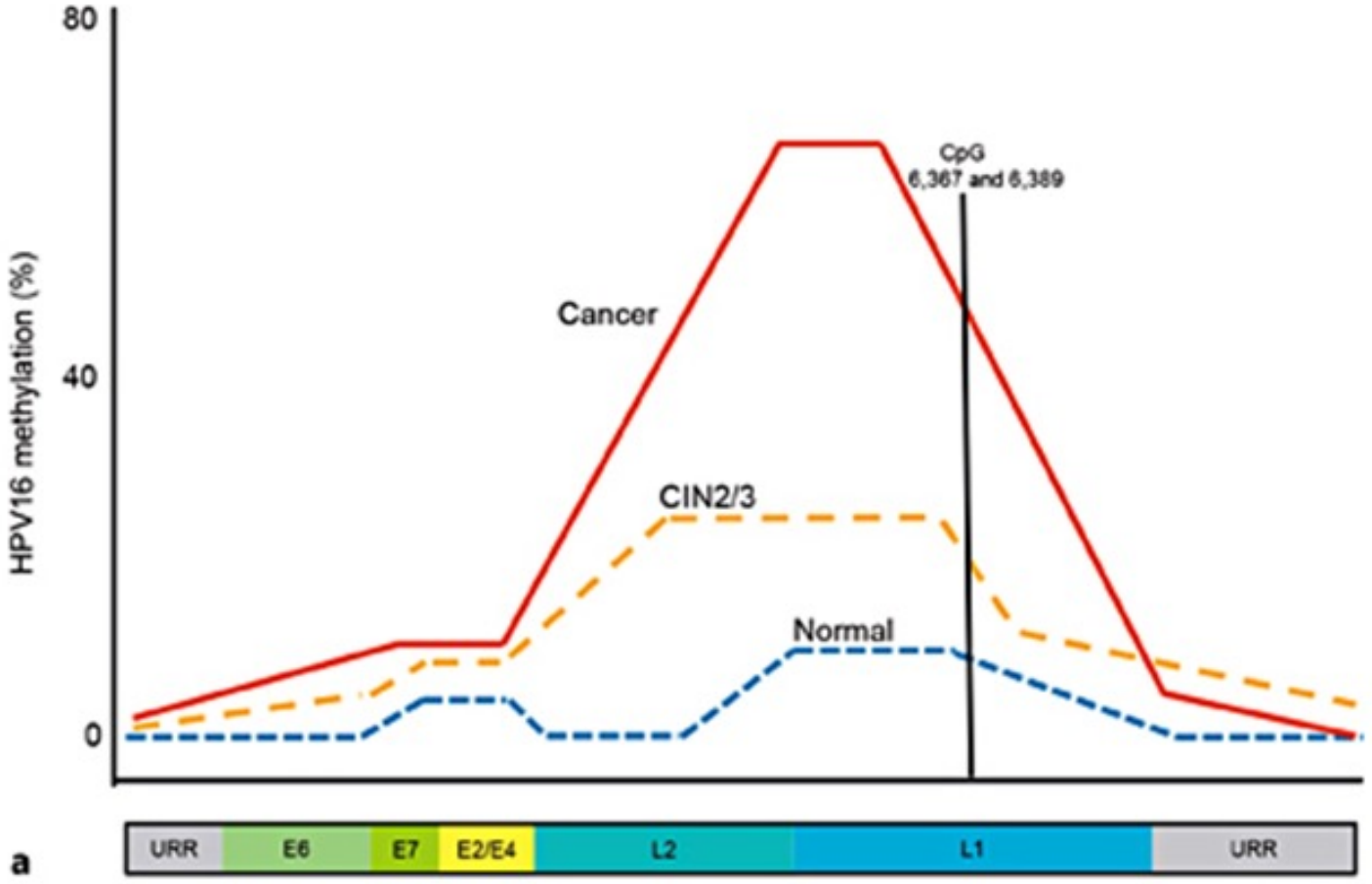
- Histologic categories do not fully translate to functional progression model
- Majority of CIN2s and CIN3s do not progress to cancer

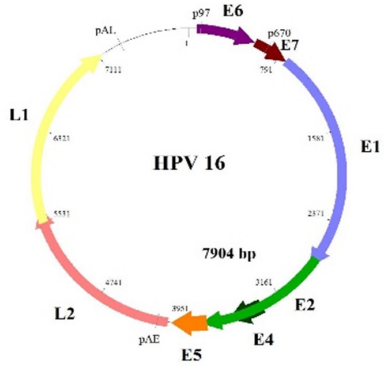
Background

- **An accurate prognostic biomarker** to distinguish HPV-infected women at **risk for progression** to CIN3 or cancer from those who will regress spontaneously without treatment would change the outline of future cervical cancer screening programs.

- Would allow more focused interventions on lesions with true progressive potential
- Reduce repetitive examinations
- Eliminate treatments for women with regressive CIN

Levels of DNA methylation across the genome of HPV16

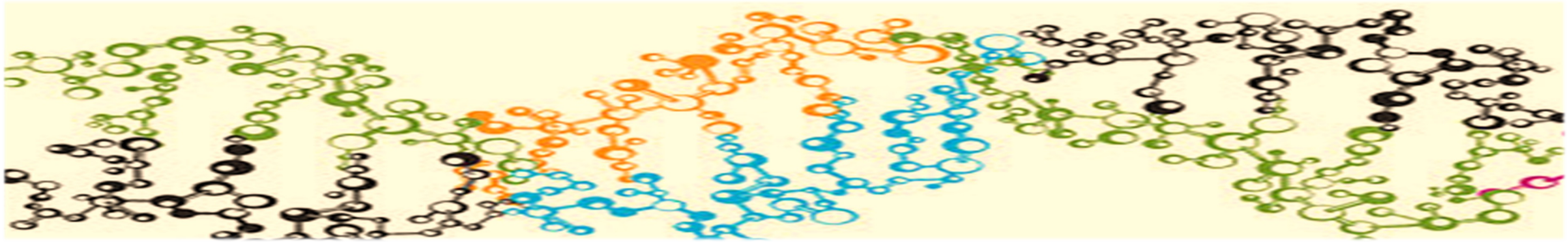




Mirabello, IJC, 2013 /Lorincz, acta cytologica, 2016

Could a DNA methylation biomarker panel to discriminate between progression and regression among women with CIN2?

Materials and Methods



Untreated Cervical Intraepithelial Neoplasia, grade 2 (UCIN2) -STUDY

- **Recruitment:** young patients 18-30 years with biopsy-confirmed CIN2
  - Informed consent
  - Exclusion criteria:
    - CA/CIN3/VIN3/VAIN3
    - Previous CIN3 LEEP
    - Pregnancy or lactation
    - HIV or immunosuppressive medicine
    - Language barrier
    - Large lesion in all four quadrants
    - Type III transformation zone (Lesion not completely seen)

Started in **September 2013** and is ongoing

Biopsies always overviewed in the Gyno-Pathological meetings

During the follow-up visit ("LEEP-visit") if recruited to the study: HPV-DNA sample is collected

FOLLOW UP PROTOCOL

6 MO
- **Pap-smear**
- If needed, biopsies

12 MO
- **Pap-smear**
- **HPV-DNA**
- If needed, biopsies
- **IF COLPOSCOPY AND BIOPSIES NORMAL -- > CONTROL AT 24MO**

18 MO
- **Pap-smear**
- If needed, biopsies

24 MO
- **Pap-smear**
- **HPV-DNA**
- **biopsies 6 and 12 o'clock** and if nessesary treatment and FU according to the normal guidelines

If biopsies had CIN3, or patient doesn't want to continue the study or moves to another city → LEEP

ISRCTN91953024, . University of Helsinki Institutional Review Board approved the protocol; 24/04/2013; ref: 131/13/03/03/2013

Methods

- Pyrosequencing methylation assays were run on exfoliated cervical cells

- **HOST gene**: EPB41L3 (CpG 438, 427, 425)
- **VIRAL HPV genes**:
  - **HPV16**: L1 (CpG 6367, 6389) and L2 regions (CpG 4256, 4261, 4265, 4269, 4275, 4281)
  - **HPV18**: L2 regions (CpG 4256, 4261, 4265, 4269, 4275, 4281)
  - **HPV31**: L2 regions (CpG 6352, 6364)
  - **HPV33**: L2 regions (CpG 5557,5560, 5566)

S5 – Classifier:

$$S5 = 30.9\times EPB41L3 + 13.7\times HPV16\text{-}L1 + 4.3\times HPV16\text{-}L2 + 8.4\times HPV18\text{-}L2 + 22.4\times HPV31\text{-}L1 + 20.3\times HPV33\text{-}L1$$

- The mean methylation of the CpGs within a gene or HPV types at nucleotides and the proportion of CpGs methylated in HPV16-L2 sites.


Results

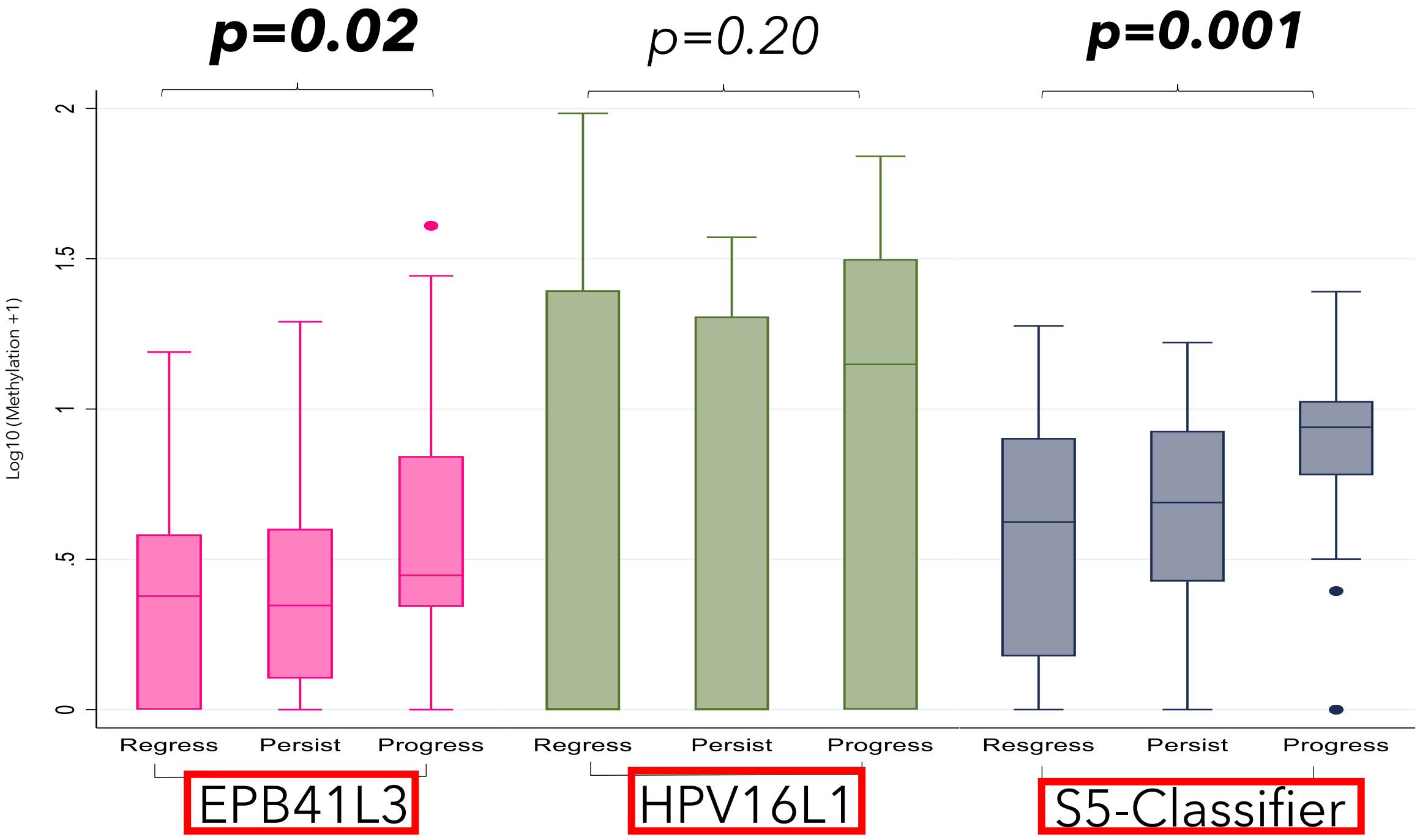
Clinical outcome groups

- ***149 women*** were included to the analyses
  - Follow-up: 6 – 24 months
  - Mean age 26 years (range: 25.9 to 27.0 years)
  - Overall **77.8%** (116/149) of the women were **positive for hrHPV**
- Three clinical outcome groups where defined:

Progression to CIN3 or cancer (≥CIN3) *(n=25)*

Regression to less than CIN1 (<CIN1) *(n=88)*

Persistence (CIN2 or conversion to persistent CIN1) *(n=36)*

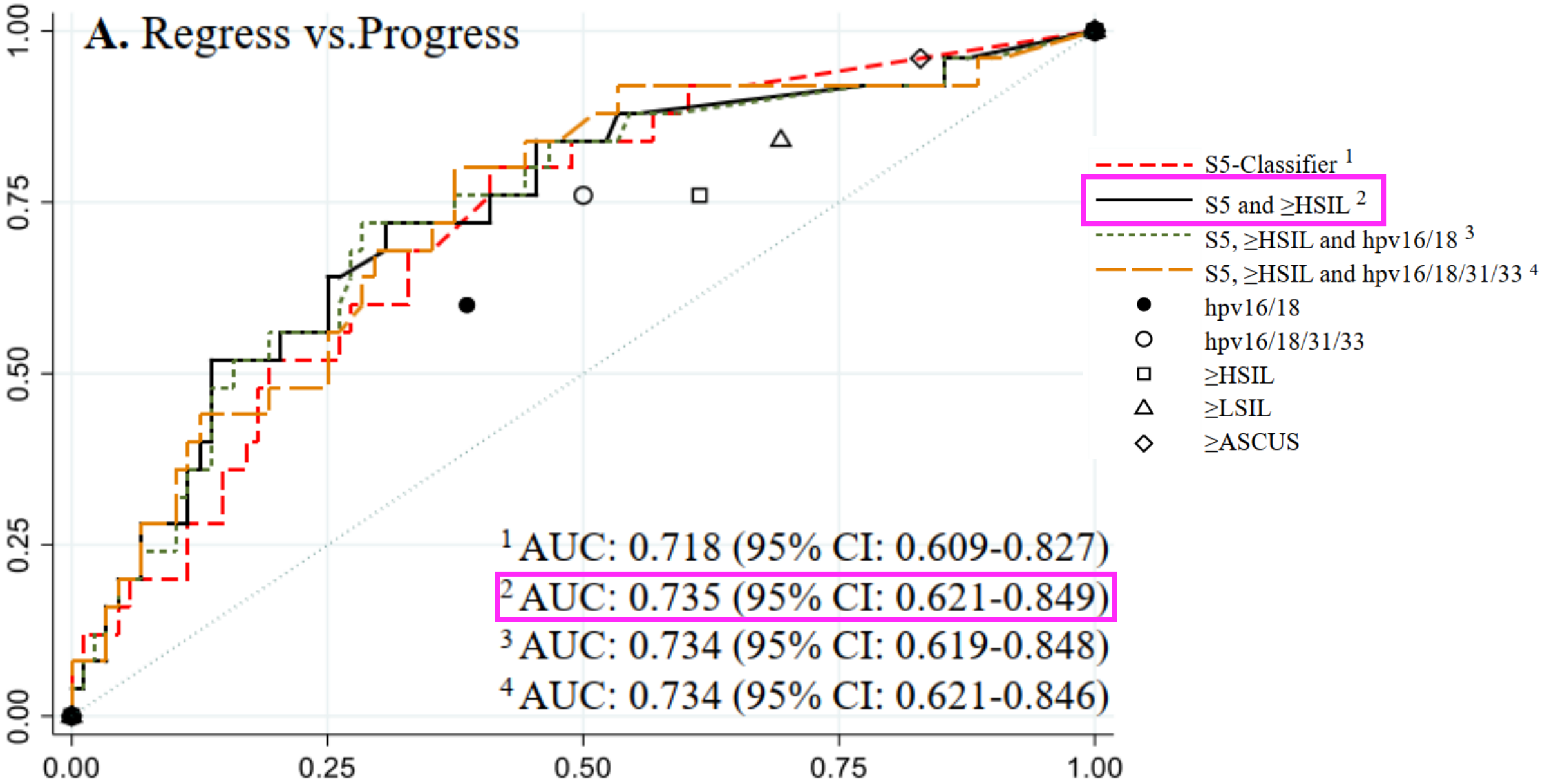

p=0.02
p=0.20
p=0.001
Log10 (Methylation +1)
0
.5
1
1.5
2
Regress
Persist
Progress
Regress
Persist
Progress
Resgress
Persist
Progress
EPB41L3
HPV16L1
S5-Classifier

Compared markers and cutoffs	Specificity[1] % (95% CI)	Sensitivity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	McNemar test for sensitivity p-value
Pap cytology: ≤LSIL vs ≥HSIL	38.6 (28.4-49.6)	62.3 (49.0-74.4)	41.3 (31.1-52.1)	59.6 (45.8-72.4)	Ref
EPB41L3	38.6 (28.4-49.6)	70.5 (57.4-81.5)	44.3 (34.2-54.8)	65.4 (50.9-78.0)	0.28
S5-Classifier	38.6 (28.4-49.6)	83.6 (71.9-91.8)	48.6 (38.7-58.5)	77.3 (62.2-88.5)	0.005

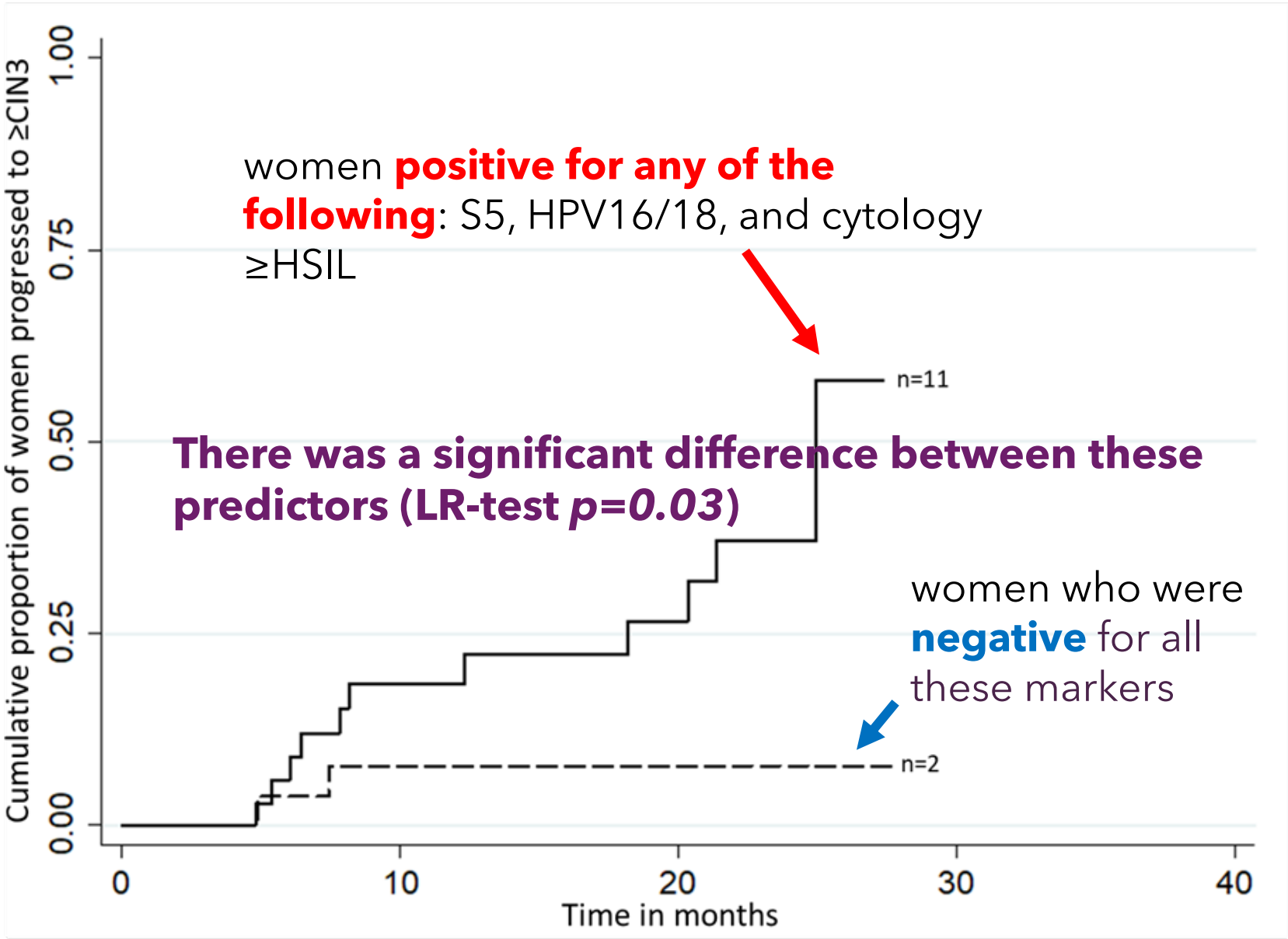
[1] Empirically assessed at the threshold that yielded the closest specificity to the cytology comparison.

Association between the different clinical outcome comparisons and different markers

Clinical outcome	OR (95% CI)			
	S5- Classifier	**Pap cytology ≤ASC-US vs ≥LSIL**	**HPV16/18 genotyping**	**HPV 16/18/31/33 genotyping**
Regression	1·00	1·00	1·00	1·00
Persistence	1·33 (0·58-3·07)	1·00 (0·43-2·33)	1·99 (0·91-4·35)	**3·50 (1·44-8·52)**
Progression	**3·39 (1·35-8·50)**	2·32 (0·73-7·42)	2·38 (0·96-5·91)	**3·17 (1·15-8·68)**


A. Regress vs.Progress
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
S5-Classifier [1]
S5 and ≥HSIL [2]
S5, ≥HSIL and hpv16/18 [3]
S5, ≥HSIL and hpv16/18/31/33 [4]
hpv16/18
hpv16/18/31/33
≥HSIL
≥LSIL
≥ASCUS
[1] AUC: 0.718 (95% CI: 0.609-0.827)
[2] AUC: 0.735 (95% CI: 0.621-0.849)
[3] AUC: 0.734 (95% CI: 0.619-0.848)
[4] AUC: 0.734 (95% CI: 0.621-0.846)

Cumulative proportions of women who progressed to CIN3+ by time since the diagnosis of CIN2



S5-classifier

Prognostic biomarker

- *The first biomarker* that can distinguish whether the HPV infection will become a CIN3+ or disappear without treatment

CONCERVE study – HOST FAM19A4/miR124-2 methylation

- 114 Women with CIN2/3 were prospectively followed for 24 months – FU every 6mo.
  - median age, 30 years; range, 20-53 years
  - 80 CIN2
  - 34 CIN3
- 65.8% of women (75/114) did not receive surgical treatment surgical treatment.
- **Baseline negative FAM19A4/miR124-2 result**
  - more regression (74.7%) than women with a positive methylation result (51.4%,).
  - highest regression when cytology was ASCUS/LSIL (88.4%) or HPV16 neg (85.1%).

Kremer et al, JCO May 2022

Conclusions

- S5 DNA methylation-classifier and FAM19A4/miR124-2 methylation shows high potential to be a **prognostic biomarker** to identify women with progressive cervical disease

- Methylation marker in combination with cytology could be a useful triage test for women with CIN2 at risk of progression

Thank you!

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