Amsterdam UMC
University Medical Centers

DNA methylation testing on clinician-collected cervical and self-collected vaginal samples for the detection of CIN3 in high-risk HPV positive women

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RISCC DECISION MODELING CENTER Amsterdam Public Health

Disclosure

No potential COI

Main questions

1. Can methylation testing on cervical scrapes replace cytology as triage test in HPV-positive women?

2. Can methylation testing be recommended for vaginal self-samples?

3. Can methylation testing be used together with cytology in HPV-positive women?

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Accuracy of cytology for detecting CIN3+ in HPV-positive women

Study name	Country	Primary test	Sensitivity	Specificity	Ref
POBASCAM	Netherlands	Co-testing	0.75	0.78	Dijkstra, CEPB 2014
VUSA-SCREEN	Netherlands	Co-testing	0.71	0.86	Rijkaart, IJC 2012
HPV program	Netherlands	HPV alone	0.82	0.73	Aitken, BMC Med 2019
Meta-analysis	28 studies	HPV alone/ co-testing	0.78	0.73	IARC Handbook 2022

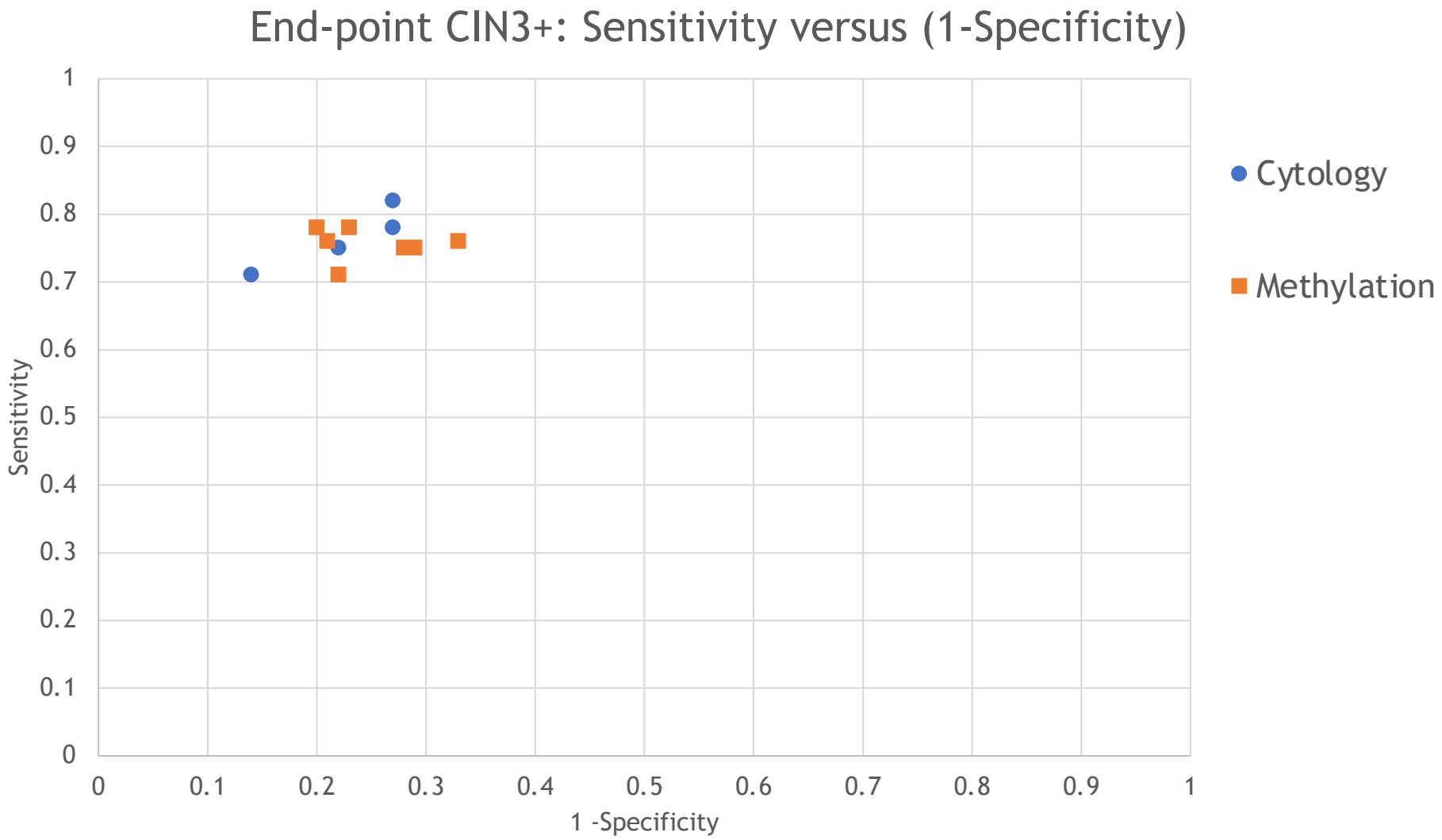
QIAsure test

- Methylation of two disease-related genes: FAM19A4 and miR124-2
- PCR test
- Regulatory approval: CE-IVD
- Qiagen

FAM19A4/miR124-2 methylation analysis of cervical scrapes for detecting CIN3+ in HPV-positive women

Study name	Country	Setting	Size	Age	Ref
POBASCAM (FAM19A4 only)	Netherlands	Co-testing	218	19-62	DeStrooper, CPR 2014
COMETH (FAM19A4 only)	Netherlands	Referral	532	18-70	Luttmer, BJC 2016
PaVDAg	Scotland	Co-testing	161	30-61	Bonde, IJC 2021
Valgent4	Denmark	Cytology	424	30-65	Bonde, IJC 2021
Slovenian HPV prev.	Slovenia	Co-testing	928	30-76	Bonde, IJC 2021
VUSA-SCREEN	Netherlands	Co-testing	871	29-61	Bonde, IJC 2021
VUSA-SCREEN	Netherlands	Co-testing	979	29-61	Vink, CMI 2021

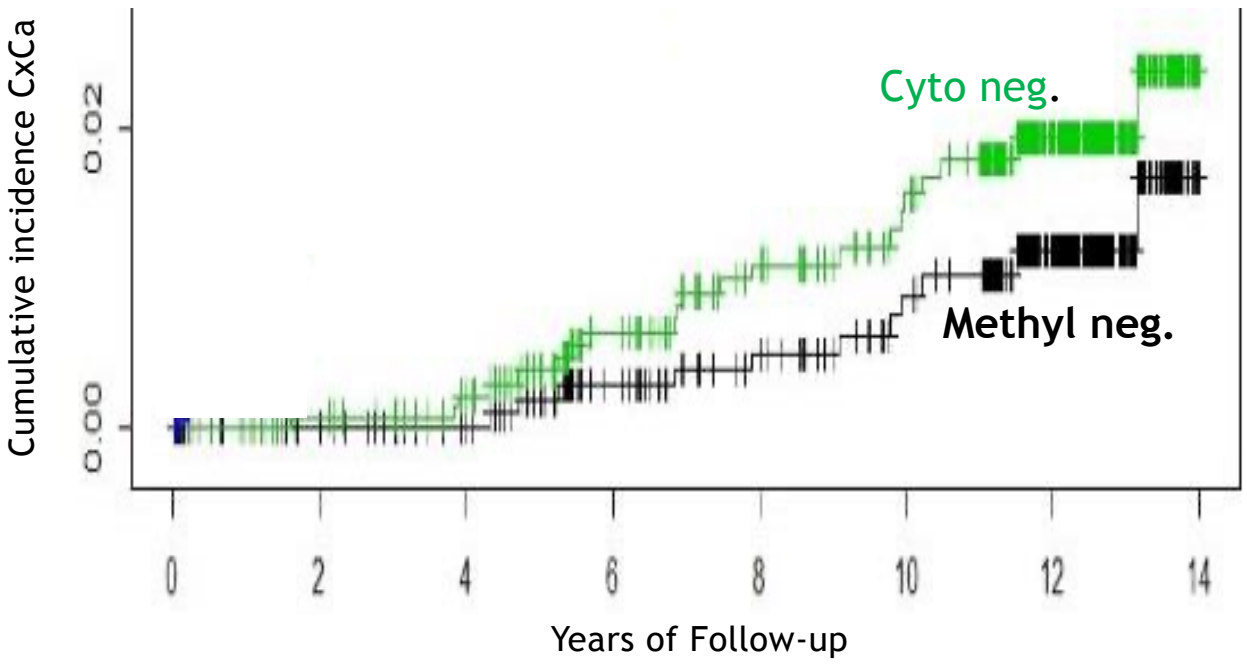
FAM19A4/miR124-2 methylation versus cytology as triage test in HPV-positive women



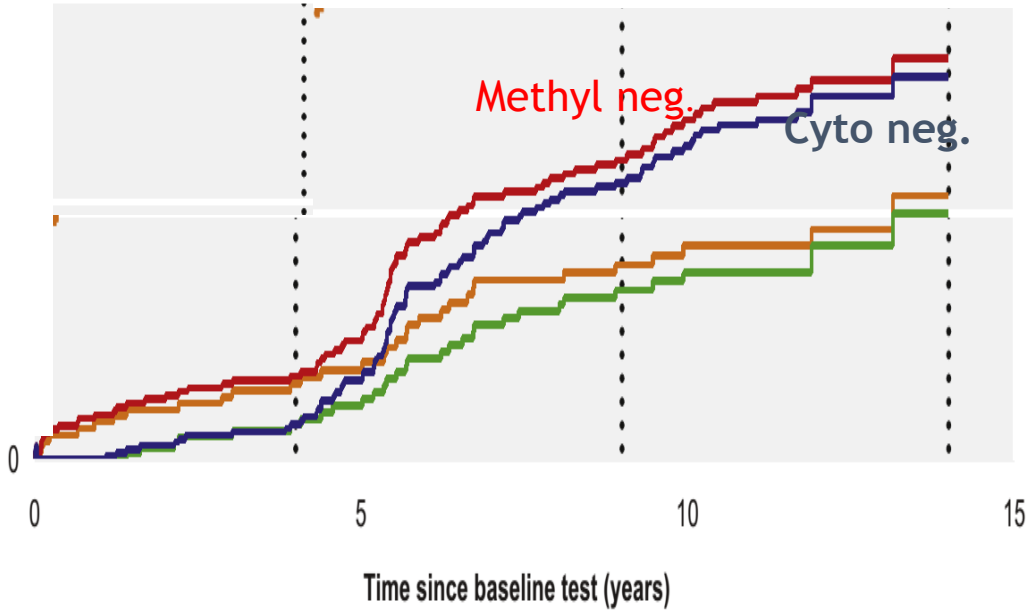
Cytology and Methylation have similar accuracy for detection of CIN3+

Long-term risk of CIN3+ and cancer in HPV-positive women after negative cytology versus negative FAM19A4/miR124-2: POBASCAM

Cancer risk difference:
0.71% (95% CI: 0.16 to 1.4%)



CIN3+ risk difference:
−0.73% (95% CI: −3.0 to 1.5%)



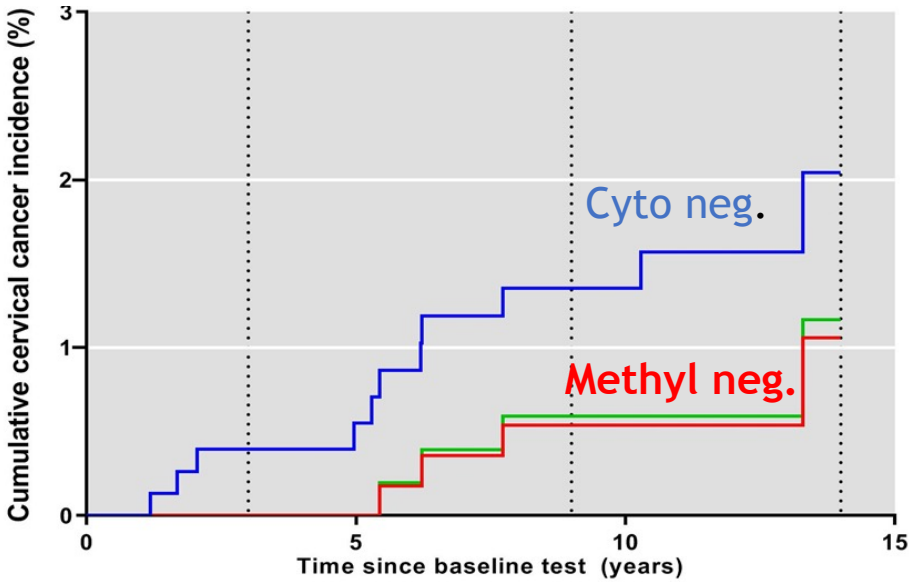
Long-term cancer risk lower after negative methylation, CIN3+ risks similar

De Strooper IJC 2018, Dick Gyn Oncol 2019

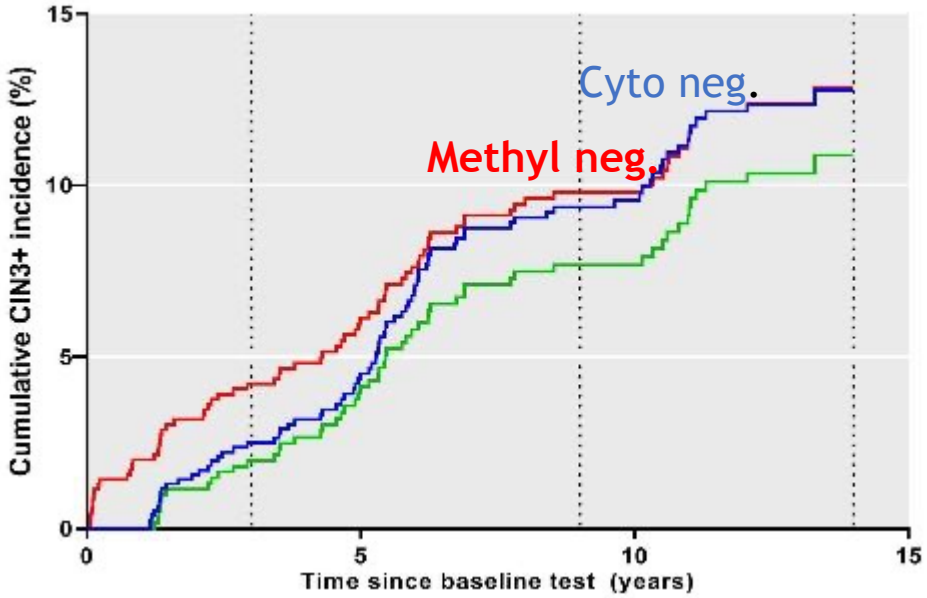
Long-term risk of CIN3+ and cancer in HPV-positive women after negative cytology versus negative FAM19A4/miR124-2: VUSASCREEN

Cytology negative
FAM19A4/miR124-2 methylation negative
FAM19A4/miR124-2 and/or cytology negative

Cancer risk difference cytology- vs methylation-
0.98% (95%CI 0.26 to 2.0 %) at 14 years



CIN3+ risk difference cytology- vs methylation-
-0.07% (95%CI: -1.9 to 1.9%) at 14 years



Vink et al. CMI 2021

FAM19A4 methylation analysis for detecting CIN3+ and association with age in COMETH study

Age	Sensitivity	Specificity	1-NPV	PPV
< 30 years	46%	82%	8.3%	26%
≥ 30 years	88%	63%	4.5%	38%

In women <30 years, clinical sensitivity of methylation testing is much lower than in older women.

Are CIN3 lesions detected in young women early, small lesions with a high chance of regression?

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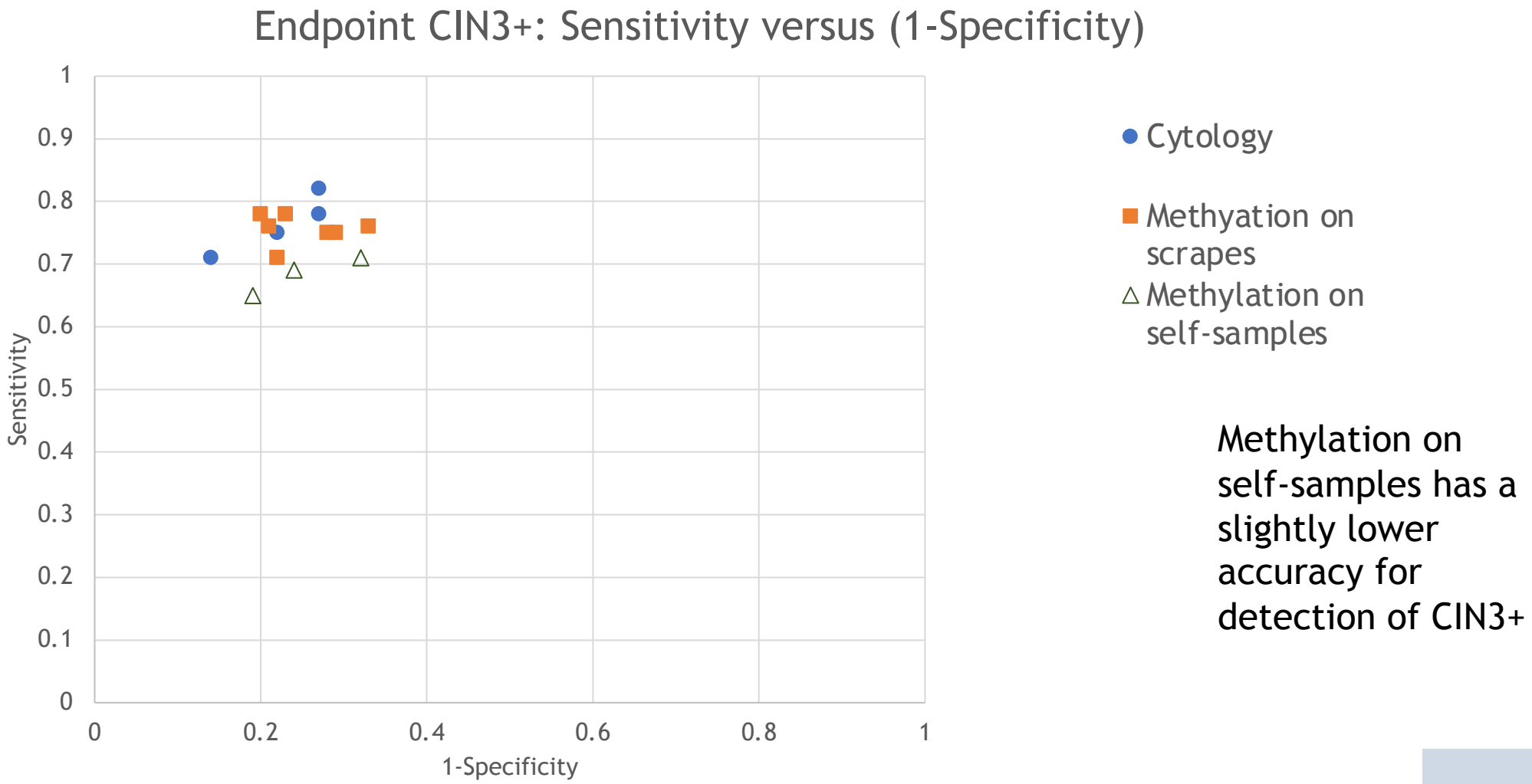
3. Can methylation testing be used together with cytology in HPV-positive women?

FAM19A4/miR124-2 methylation analysis on self-collected samples in COMETH and PROHTECT3 study

Study name	Population	Self-sampling method	Size	Reference
COMETH	Referral	scrape	532	Luttmer, BJC 2014
COMETH	Referral	lavage	532	Luttmer, BJC 2014
PROHTECT3	Non-responder	lavage/brush	643	DeStrooper GynOnc 2016

FAM19A4/miR124-2 methylation versus cytology as triage test in HPV-positive women



Methylation on self-samples has a slightly lower accuracy for detection of CIN3+

Methylation marker discovery & validation on self-samples: LHX8/ASCL1/ST6GALNAC5

1 *1. Discovery screen (450K markers) 2. Hierarchical clustering (28 markers)*

Infinium 450K Beadchip Array
PROHTECT-1
68 lavage self-samples:
29 controls
39 CIN3

Extra: 4 SCC

2 *3. Building a DNA methylation classifier (12 markers)*

Multiplex qMSP

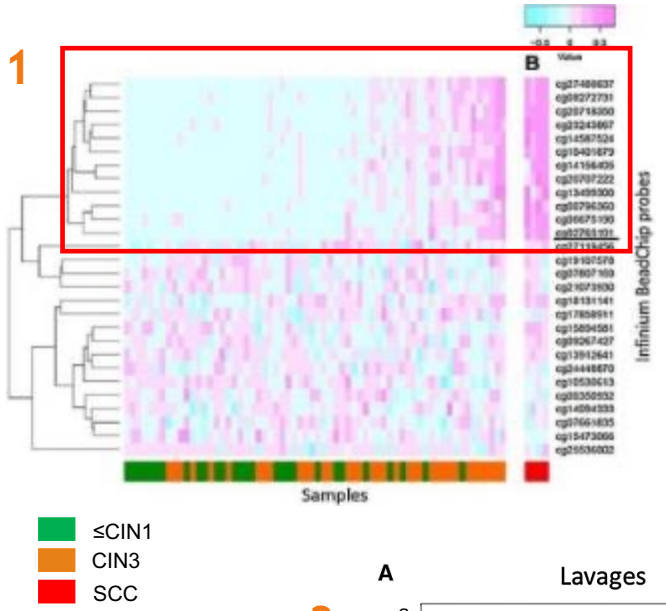
PROHTECT-1	*PROHTECT-2*
245 lavage self-samples:	246 brush self-samples:
214 controls	174 controls
31 CIN3	72 CIN3

3 *4. Validation of DNA methylation classifier (3 markers)*

Multiplex qMSP

PROHTECT-3	*PROHTECT-3B*
199 lavage self-samples:	287 brush self-samples:
134 controls	178 controls
22 CIN2	28 CIN2
35 CIN3	56 CIN3
7 SCC	16 SCC
1 AdCA	1 ACIS
	8 AdCA

1



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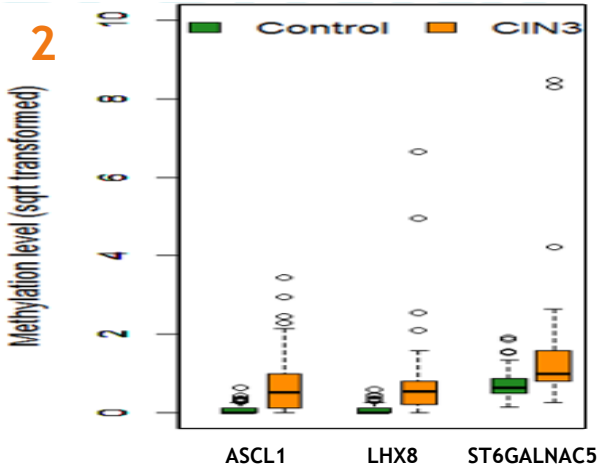

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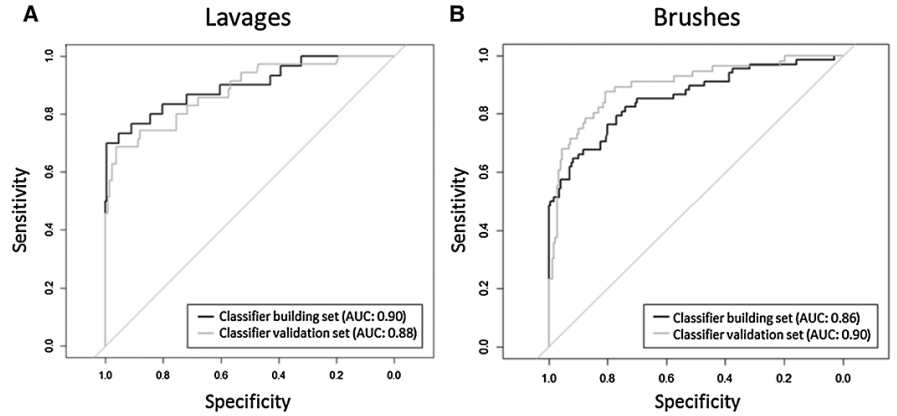

Verlaat et al. (2018) CCR

LHX8/ASCL1/ST6GALNAC5 methylation analysis of cervical scrapes for detecting CIN3+ in HPV-positive women

Study name	Population	Collection	Size	Sens	Spec	Ref
PROHTECT3 validation	Non-responder	Self-collected Lavage	153	74%	79%	Verlaat, CCR2018
PROHTECT3b	Non-responder	Self-collected Brush	169	88%	81%	Verlaat, CCR2018
IMPROVE (LHX8/ASCL1)	Primary HPV	Cervical scrape	715	77%	75%	Verhoef, IJC2021

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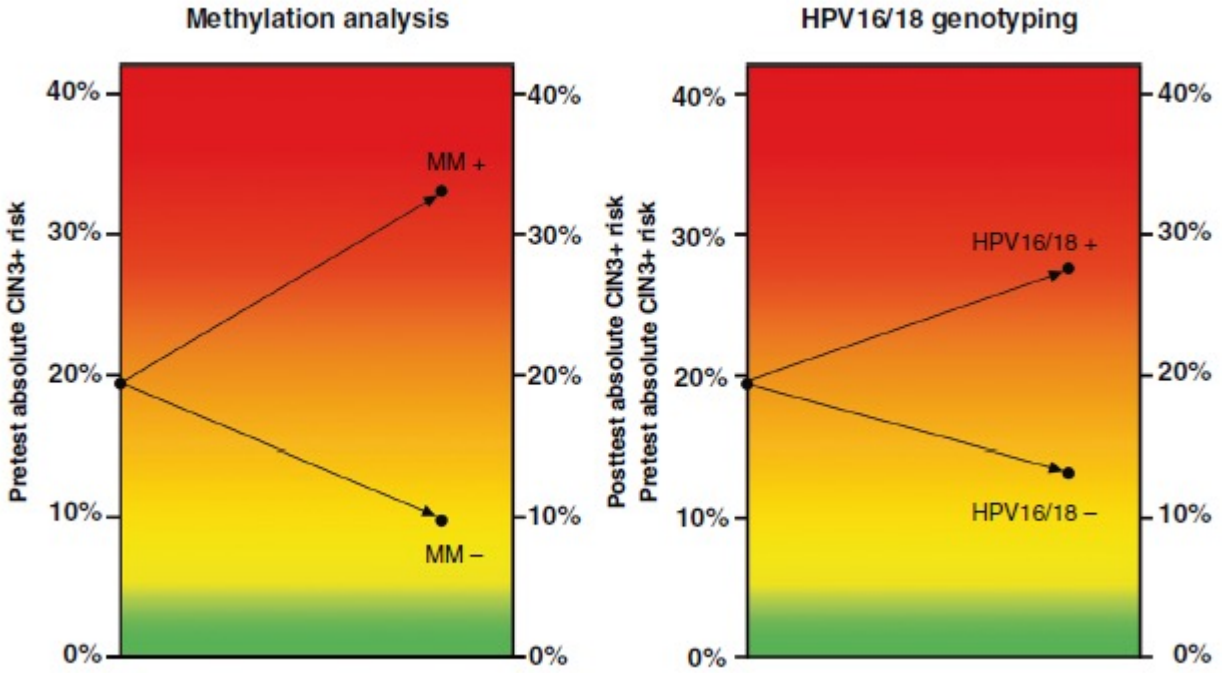


VUSA SCREEN AND POBASCAM DATA:

Methylation predicts CIN3+ risk in women with ASCUS/LSIL

Risk-stratification of HPV-positive women with low-grade cytology by *FAM19A4/miR124-2* methylation and HPV genotyping

Stèfanie Dick[1,3], Frederique J. Vink[1,3], Daniëlle A. M. Heideman[1], Birgit I. Lissenberg-Witte[2], Chris J. L. M. Meijer[1]✉ and Johannes Berkhof[2]

British Journal of Cancer (2022) 126:259 – 264

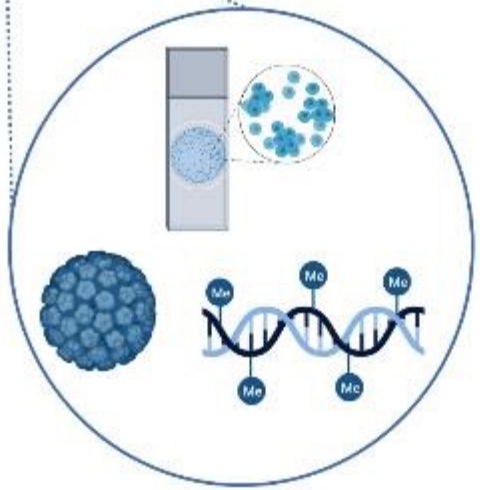
CONCERVE study (Kremer, Dick et al. JCO 2022)

Inclusion criteria
- CIN2/3
- Age 18-55 years
- Small lesion (<50%)

Baseline & follow-up
6-12-18-24 months
- Self-sample
- Cervical scrape
- Colposcopy

Treatment indication
- Volume >50%
- CIN2 > CIN3 > cancer
- AIS
- Transformation zone not visible

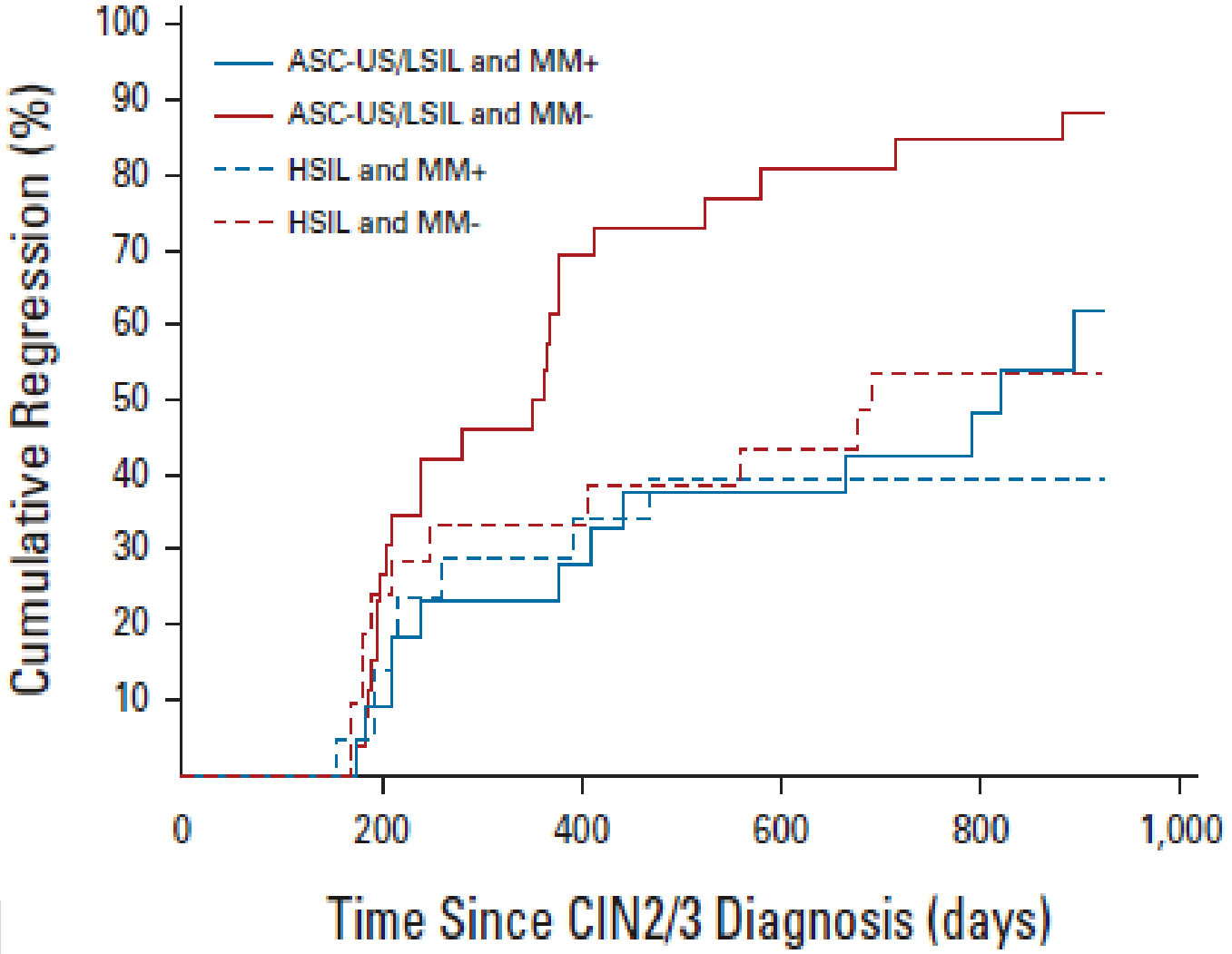
Exclusion criteria
- Pregnancy
- History of CIN
- AIS
- Transformation zone not visible



Study endpoint
- Absence of CIN2+
  or
- HPV negative NILM

Study design and methods:
Kremer (2019) *BMJ Open*

CONCERVE:
Cytology and FAM19A4/miR124-2 on cervical scrapes



Methylation predicts CIN2/3 regression in women with ASCUS-LSIL, but not in women with HSIL

Conclusions

- Strong evidence that DNA methylation on cervical scrapes has similar accuracy as cytology for detecting CIN3+
- DNA methylation testing on vaginal self-samples is promising. Evidence on clinical performance is emerging.
- DNA methylation can be used as a triage test in HPV-positive women with ASCUS/LSIL

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  Birgit Lissenberg-Witte

Amsterdam UMC
University Medical Centers

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RISCC

Thank you

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