

DNA methylation assays used in cervical cancer screening

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(est. 1988, Cancer Research Society)

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Disclosure

- **No conflicts of interest**
- **Mariam El-Zein**, David Cheishvili, Moshe Szyf, and Eduardo Franco hold a patent related to the discovery “DNA methylation markers for early detection of cervical cancer”, registered at the Office of Innovation and Partnerships, McGill University, Montreal, Quebec, Canada (October 2018).

Commercial or research assays

| | Company | Assay | Target gene regions |
|--|---|-------|--|
| QIASure Methylation Test® | Qiagen Hilden, Germany | qMSP | <i>EAM19A4</i> : Family with sequence similarity 19 (chemokine (C-C motif)-like) member A4 <i>Hsa-miR-124</i> : Human microRNA 124 |
| GynTect® assay | Oncnostics Jena, Germany | qMSP | <i>ASTN1</i> : Astrotactin1 <i>DLX1</i> : Distal-less homeobox 1 <i>ITGA4</i> : Integrin subunit α 4 <i>RXFP3</i> : Relaxin family peptide receptor 3 <i>SOX17</i> : SOX17 <i>ZNF671</i> : Zinc finger protein 671 |
| CONFIDNECE Marker™ | Neumann Diagnostics Budapest, Hungary | qMSP | <i>POU4F3</i> : POU Class 4 Homeobox 3 |
| Cervi-M | Ingenuity Healthcare Mumbai, India | qMSP | <i>PAX1</i> : Paired Box Gene 1 |
| Precursor-M Test® | Self-screen B.V. Amsterdam, Netherlands | qMSP | <i>CADM1</i> : Cell adhesion molecule 1 <i>MAL</i> : Myelin and lymphocyte protein <i>miR124-2</i> : MicroRNA 124-2 |
| PAX1 DNA Detection kit ZNF582 DNA Detection kit | iStat Biomedical Co. Ltd New Taipei City, Taiwan | qPCR | <i>PAX1</i> : Paired Box Gene 1 <i>ZNF582</i> : Zinc finger protein 582 |
| S5 classifier | Research assay (Attila Lorincz Team) S5®CareLYFE (China) | PSQ | <i>EPB41L3</i> : Erythrocyte membrane protein band 4.1 like 3 <i>HPV16L1</i> , <i>HPV16L2</i> <i>HPV18L2</i> <i>HPV31L1</i> <i>HPV33L2</i> |

QIASure, performance

| CIN3+ Endpoint | Sensitivity, % | Specificity, % | Reference |
|--------------------------------|----------------|----------------|---|
| 68/254 HPV+ brush self-samples | 69.4 | 76.4 | De Strooper et al., Gynecol Oncol, 2016 |
| 72/538 HPV+/- cervical samples | 77.8 | 69.3 | Leeman et al., IJC, 2018 |
| 248/2384 HPV+ cervical samples | 77.2 | 78.3 | Bonde et al., IJC, 2020 |
| 169/979 HPV+ cervical samples | 71.3 | 78.3 | Vink et al., CMI, 2021 |

GynTect, performance

| CIN3+ Endpoint | Sensitivity, % | Specificity, % | Reference |
|---------------------------------|----------------|----------------|--|
| 93/306 HPV+/- cervical samples | 67.7 | 82.6 | Schmitz et al., Clin Epigenetics, 2017 |
| 55/280 HPV+/- cervical samples | 64.8 | 94.6 | Schmitz et al., BMC Cancer, 2018 |
| 19/87 HPV+/- brush self-samples | 31.6 | 95.9 | Klischke et al., J Virol Methods, 2021 |



Comparison

Physician-collected,
hrHPV+
cervical samples, n=149



| | Endpoint | Sensitivity, % | Specificity, % |
|----------------|-------------|----------------|----------------|
| QIAure | CIN2+, n=60 | 63.3 | 67.4 |
| | CIN3+, n=42 | 78.6 | 68.2 |
| | Cancer, n=7 | 100 | - |
| GynTect | CIN2+, n=60 | 56.7 | 87.6 |
| | CIN3+, n=42 | 66.7 | 84.1 |
| | Cancer, n=7 | 100 | - |

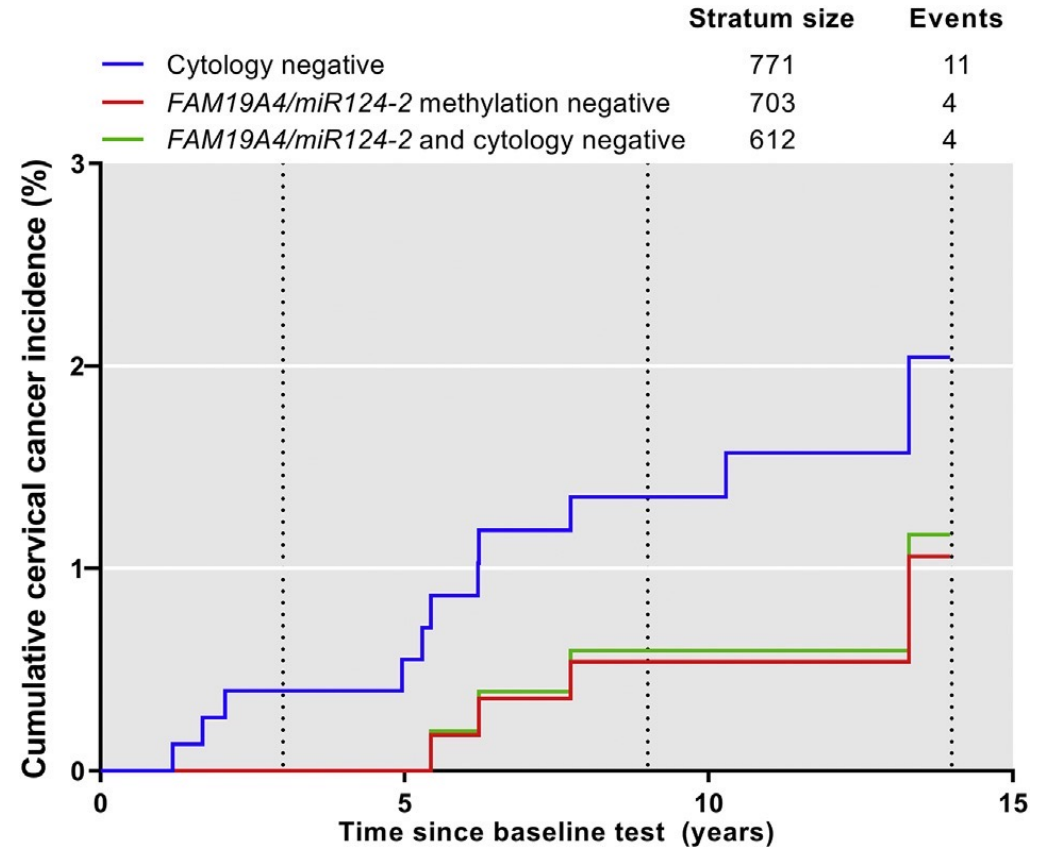
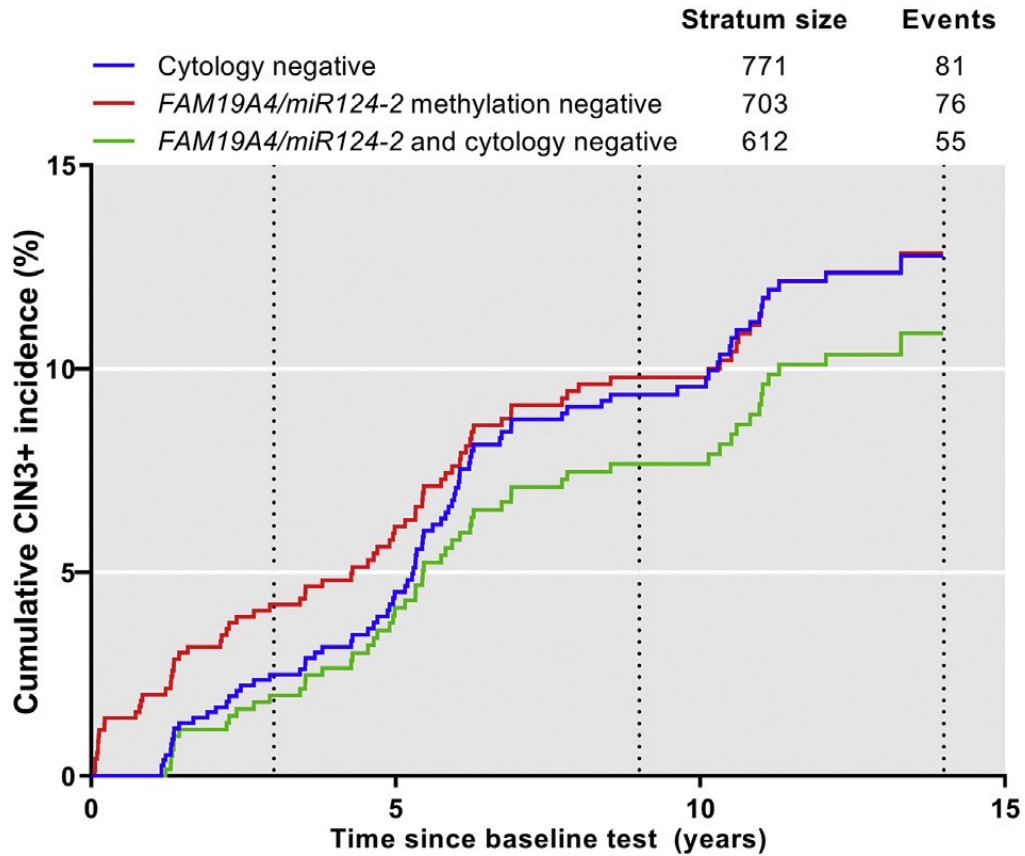
Other methylation markers...

| | Endpoint | Sensitivity, % | Specificity, % | Reference |
|---|--------------------------------------|----------------|----------------|---|
| <i>POU4F3</i> | 77 CIN3+/1287 HPV+ cervical samples | 89.6 | 60.9 | Kocsis et al., IJC, 2017 |
| <i>CADM1</i> , <i>MAL</i> , <i>miR124-2</i> | 95 CIN3+/247 HPV+ cervical samples | 94.7 | 78.9 | De Strooper et al., J Clin Pathol, 2014 |
| <i>PAX1</i> <i>ZNF582</i> | 37 HSIL/150 HPV+/- cervical samples | 91.9 97.3 | 72.8 76.7 | Liang et al, clin transl Oncol, 2020 |
| <i>ZNF582</i> | 93 CIN2+/154 HPV+/- cervical samples | 69.9 | 84.3 | Zhang et al., Onco Target Ther, 2021 |

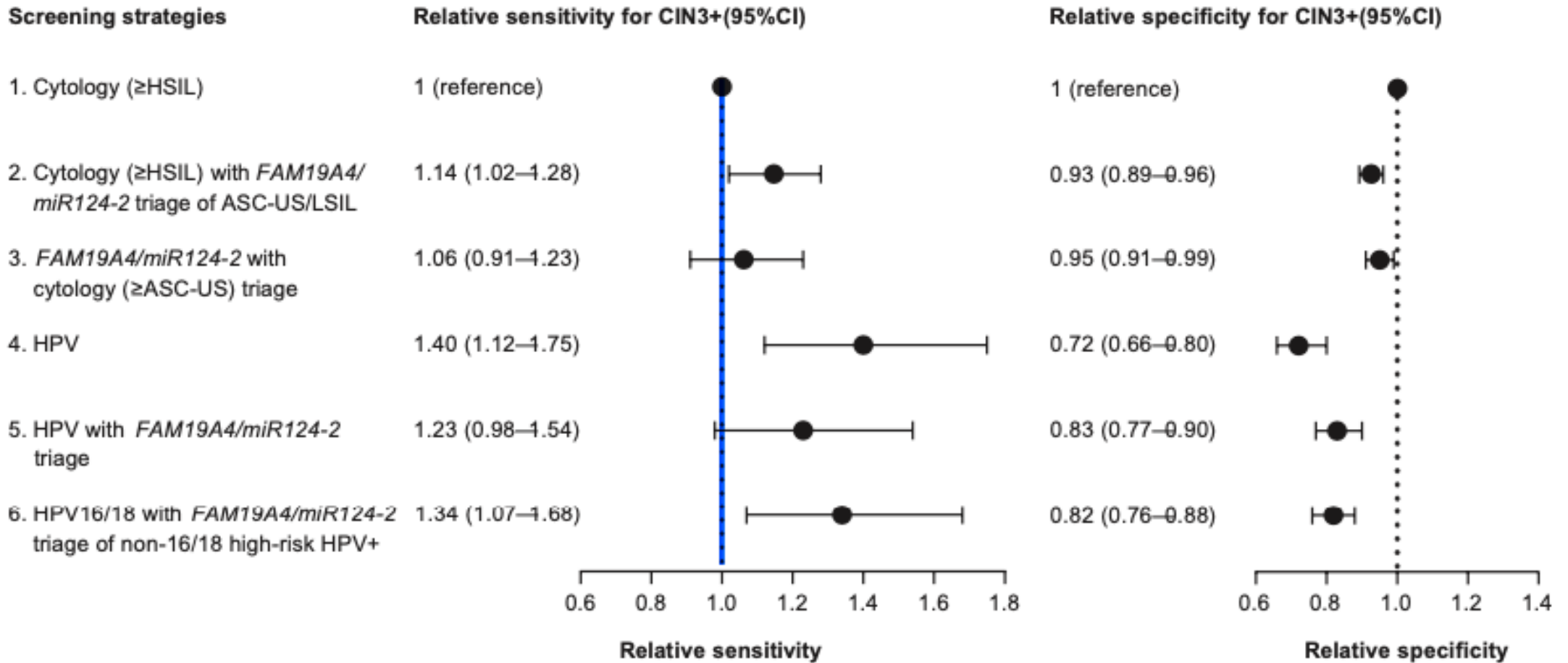
S5 classifier

| CIN3+ Endpoint | Sensitivity, % | Specificity, % | Reference |
|-------------------------------------|----------------|----------------|--|
| 52/257 HPV+ cervical samples | 93.2 | 41.8 | Cook et al., IJC, 2018 |
| 19/341 HPV+ cervical samples | 84.0 | 63.0 | van Leeuwen et al., IJC, 2019 |
| 58/316 HPV+ cervical samples | 70.3 | 76.6 | Hernández-López et al., Clin Epigenetics, 2019 |
| 421 HSIL+/588 HPV+ cervical samples | 89.1 | 76.6 | Gu et al., Clin Epigenetics, 2020 |
| 48/366 HPV+ cervical samples | 77.1 | 35.0 | Ramirez et al., IJC, 2021 |

FAM19A4 and *Hsa-miR-124*, longitudinal analysis



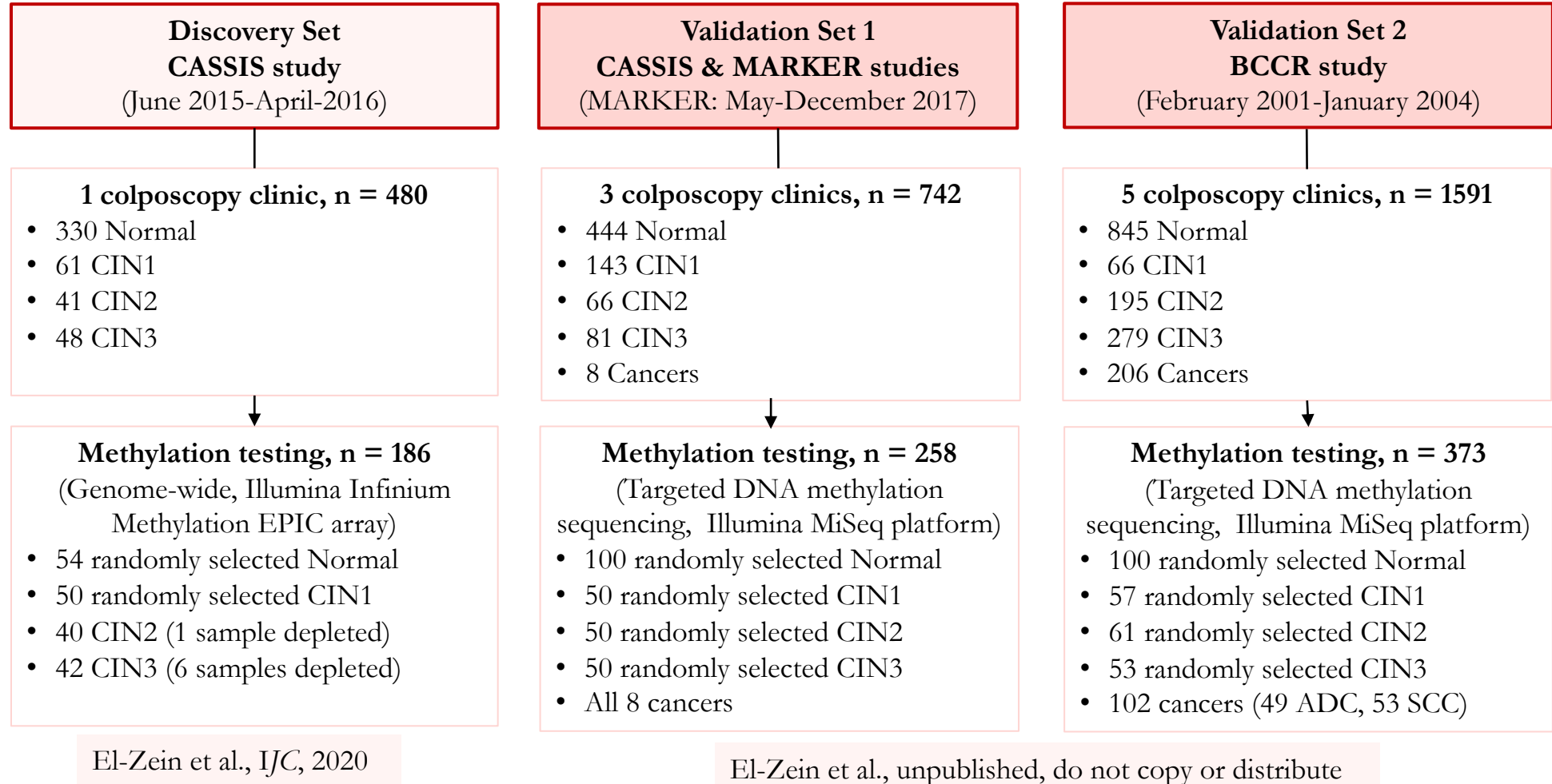
FAM19A4 and *Hsa-miR-124*, 308 women living with HIV



Use of Methylation analysis

- Primary triage, hrHPV-positive women
- Secondary triage for CIN3+, women with minor cytological abnormalities
- Exit test, women leaving the screening programme
- Management, hr-HPV-positive women of childbearing age

Discovery/validation of methylation markers



CASSIS: Cervical And Self-Sample In Screening

MARKER: Methylation Analysis Revealing Key Epigenetic Regulation

BCCR: Biomarkers of Cervical Cancer Risk

Discovery Set

Genome-wide DNA methylation profiles using Infinium Methylation EPIC array

- Discovery set: 186 cervical specimens (54 normal, 50 CIN1, 40 CIN2, 42 CIN3)

7715 differentially methylated CpGs among 756,737 probes

- methylation level significantly correlated ($p < 0.05$) with progression from normal to CIN grades 1 to 3

79 top CpGs

- 10% average increase or decrease in methylation between CIN3 and normal

16 CpGs

- hypermethylated between CIN3 and normal
- highest effect size (Cohen $D > 1.3$) between CIN3 and normal
- highest Spearman correlation ($r > 0.4$) with lesion progression

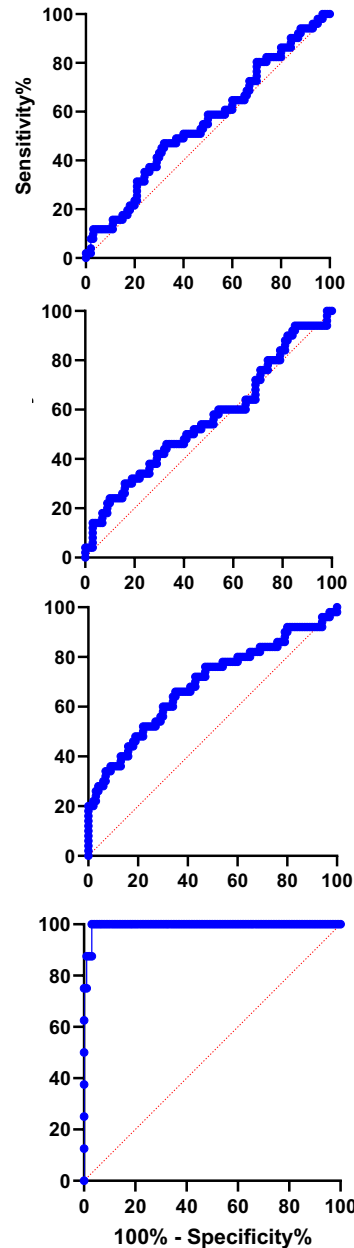
5 CpGs

- identified by penalized regression as the minimal number required for differentiating CIN3 from normal

| CpG | Gene Name | r | Adjusted P-value |
|------------|-----------|--------|-----------------------|
| cg03419058 | ATP10A | 0.4599 | 1.78×10^{-6} |
| cg13944175 | HAS1 | 0.4008 | 5.72×10^{-4} |
| cg01650149 | FMN2 | 0.4507 | 2.93×10^{-6} |
| cg20405017 | CA10 | 0.4153 | 2.35×10^{-5} |
| cg21678377 | DPP10-AS1 | 0.4408 | 5.78×10^{-6} |
| cg17233763 | RALYL | 0.4513 | 2.84×10^{-6} |
| cg02547394 | SOX1 | 0.4286 | 1.12×10^{-5} |
| cg19717586 | NTM | 0.4315 | 9.88×10^{-6} |
| cg00027083 | EPB41L3 | 0.4428 | 5.07×10^{-6} |
| cg00688962 | KCNIP4 | 0.4480 | 3.64×10^{-6} |
| cg11358689 | NTM | 0.4625 | 1.78×10^{-6} |
| cg18384778 | USP29 | 0.4231 | 1.63×10^{-5} |
| cg10182317 | CLVS2 | 0.4603 | 1.78×10^{-6} |
| cg18897632 | SOX11 | 0.4350 | 8.17×10^{-6} |
| cg08305436 | LINC01785 | 0.4310 | 1.02×10^{-5} |
| cg18343957 | MDGA2 | 0.4051 | 4.44×10^{-5} |

El-Zein et al., *IJC*, 2020

Validation Set 1



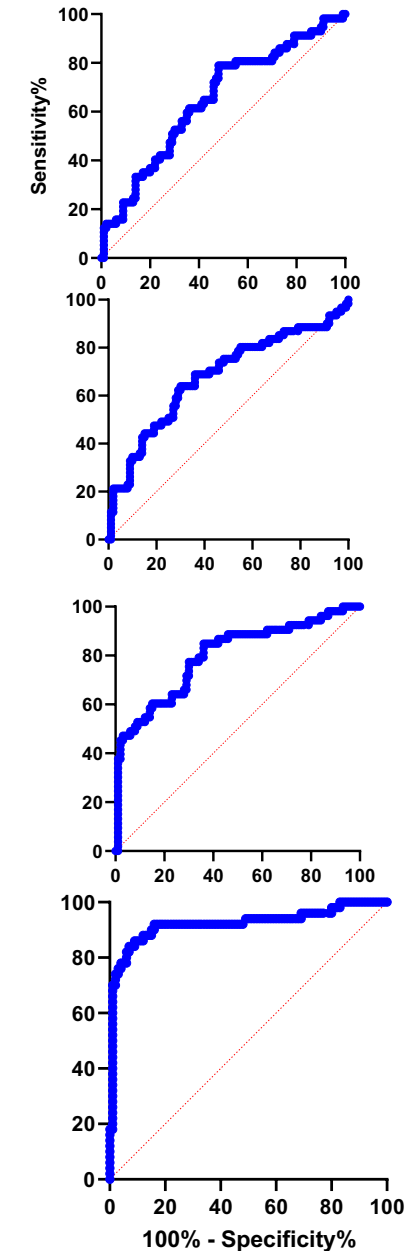
Normal vs CIN1

Normal vs CIN2

Normal vs CIN3

Normal vs Cancer

Validation Set 2



El-Zein et al., unpublished, do not copy or distribute

Final comments...

- Validity of our biomarkers demonstrated
- Translational research warranted
- Next steps:
 - evaluate and validate the prospective prognostic potential of these markers in a cohort study (new patients) and determine risk of progression of low- & high- grade CIN lesions
 - validation in self-collected samples