Human Papillomavirus DNA Methylation as a Biomarker for Cervical Precancer: Consistency across 12 Genotypes and Potential Impact on Management of HPV-Positive Women

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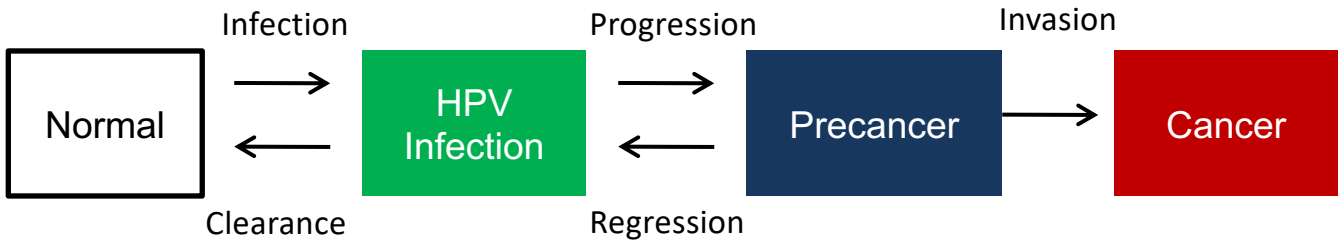
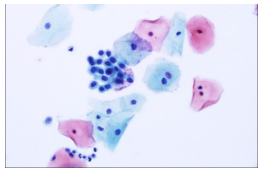
Biomarkers for HPV-Related Carcinogenesis

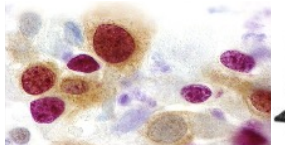


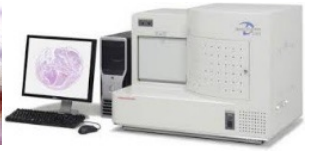
Cytology-based

Automated Cytology



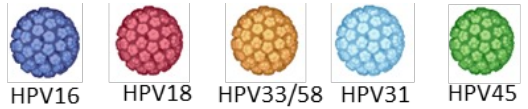
p16/Ki-67 dual stain (with automation)





Molecular

HPV testing with extended genotyping (DNA or mRNA)

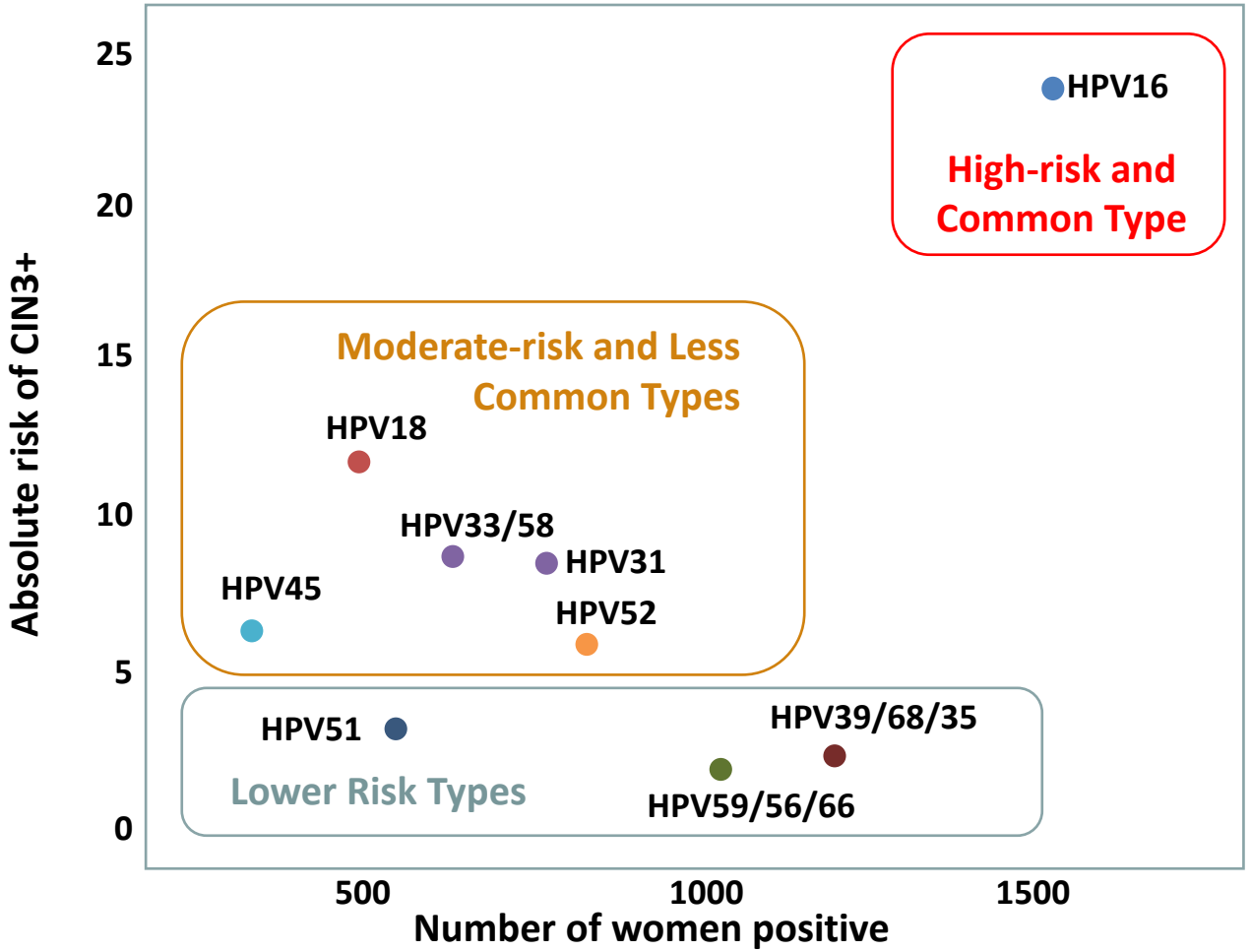


HPV methylation

Host gene methylation

HPV Genotyping: Implications for Screening and Management

- Extended HPV genotyping gives information about:
  - Individual risk
  - Insight into how common each type of virus is
- HPV16 is both high-risk and common
- Other types with lower risk
  - Consider different management?
- Challenge: Genotyping alone cannot distinguish between transient infection and precancer



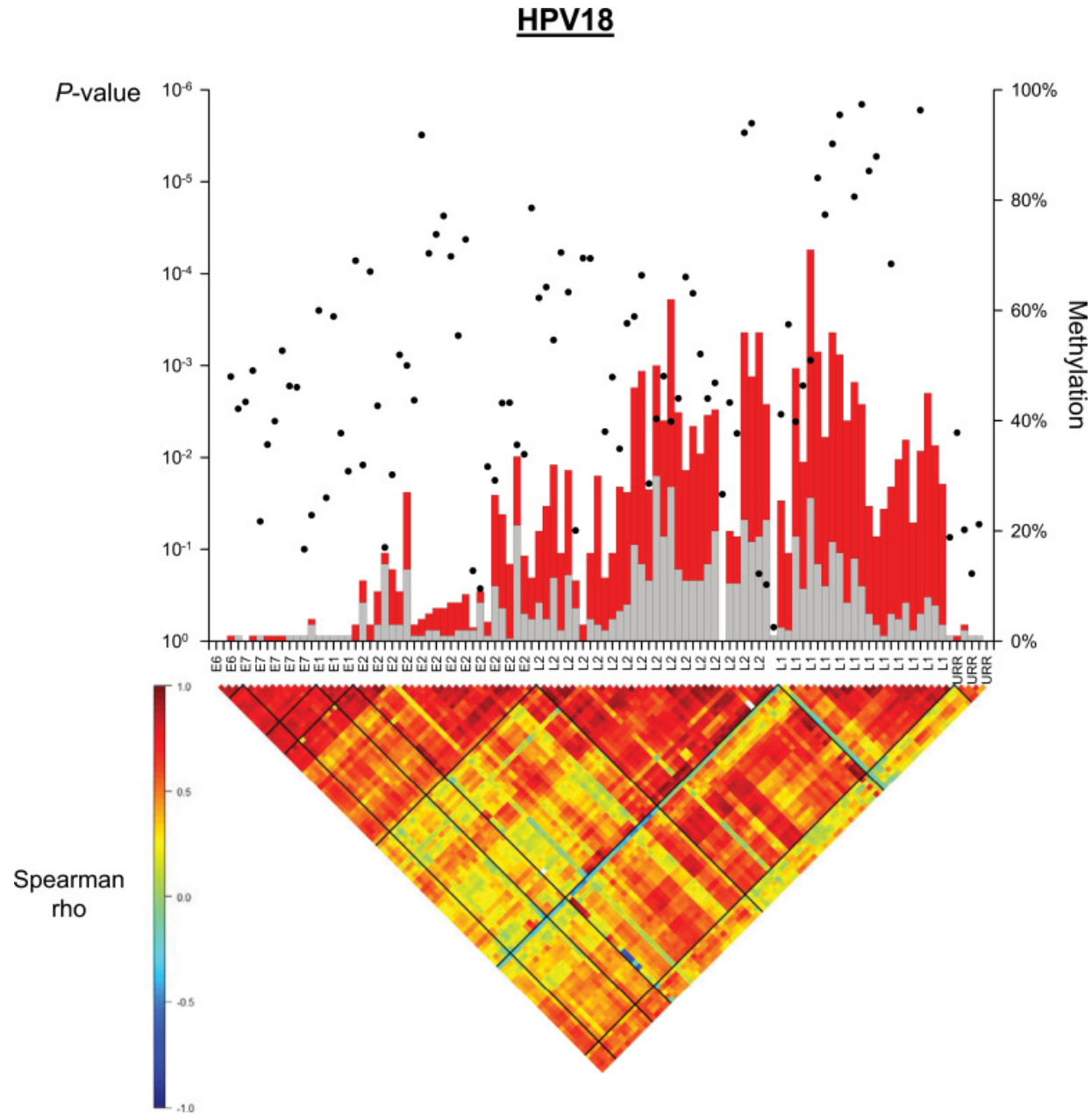
Adapted from Schiffman M, Hyun N, Raine-Bennett TR, et al. *Int J Cancer* 2016

HPV L1 methylation in four different carcinogenic HPV types

HPV type	Position	Odds Ratio	95%CI
16	5611	37.5	9.0 – 157.0
31	5524	12.2	4.3 - 34.4
18	7041	13.7	4.6 - 41.1
45	7042	40.0	3.1 - 525.0

- Increased methylation in precancer is observed in four common carcinogenic types
- In women with multiple infections, methylation shows causal type

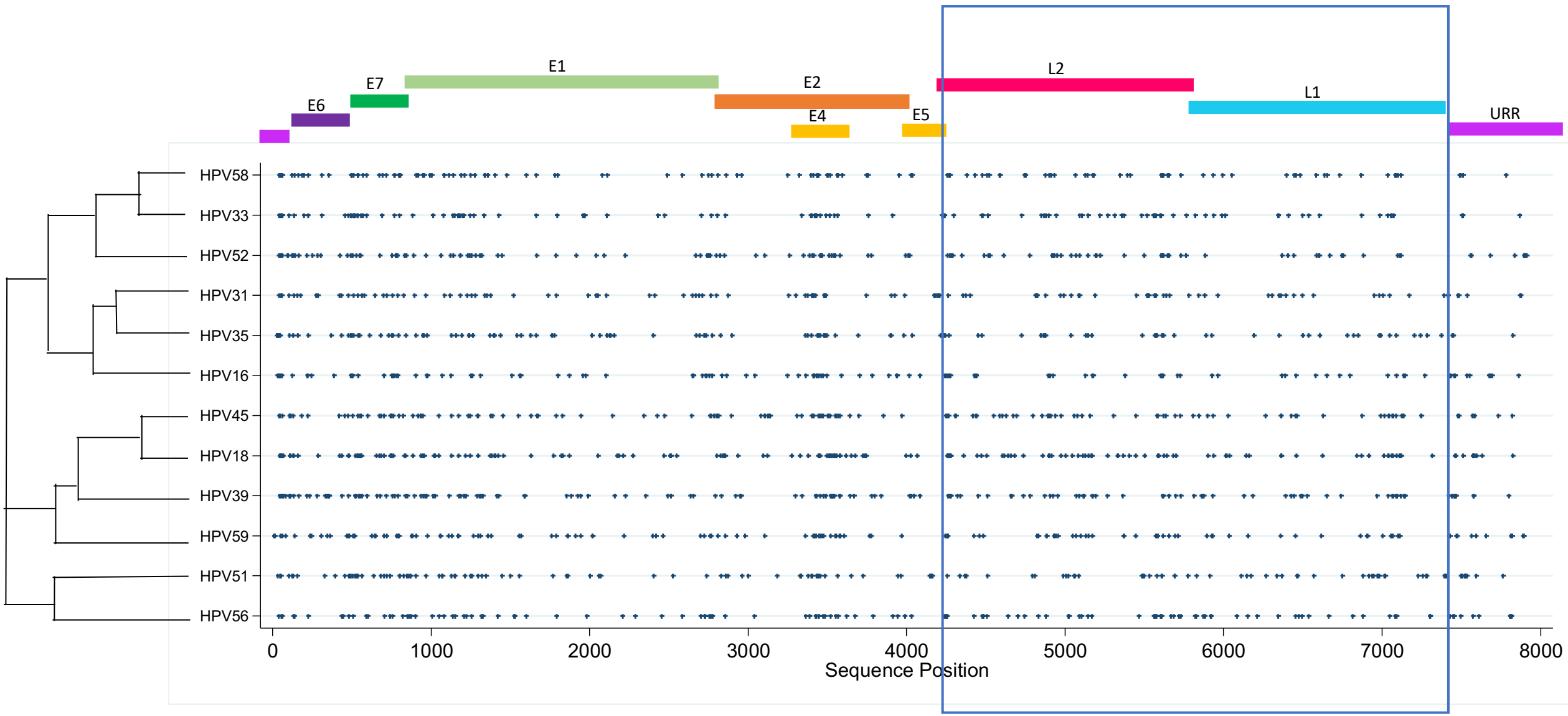
Mirabello et al. JNCI 2012; Wentzensen et al. JNCI 2012; Clarke et al., CEBP 2012



- Highest methylation found in late gene regions encoding the viral capsid, not in the promoter
- High correlation between sites within HPV genes

Wentzensen et al. JNCI 2012

CpG Sites in HPV Genomes, ~80-120 per HPV type



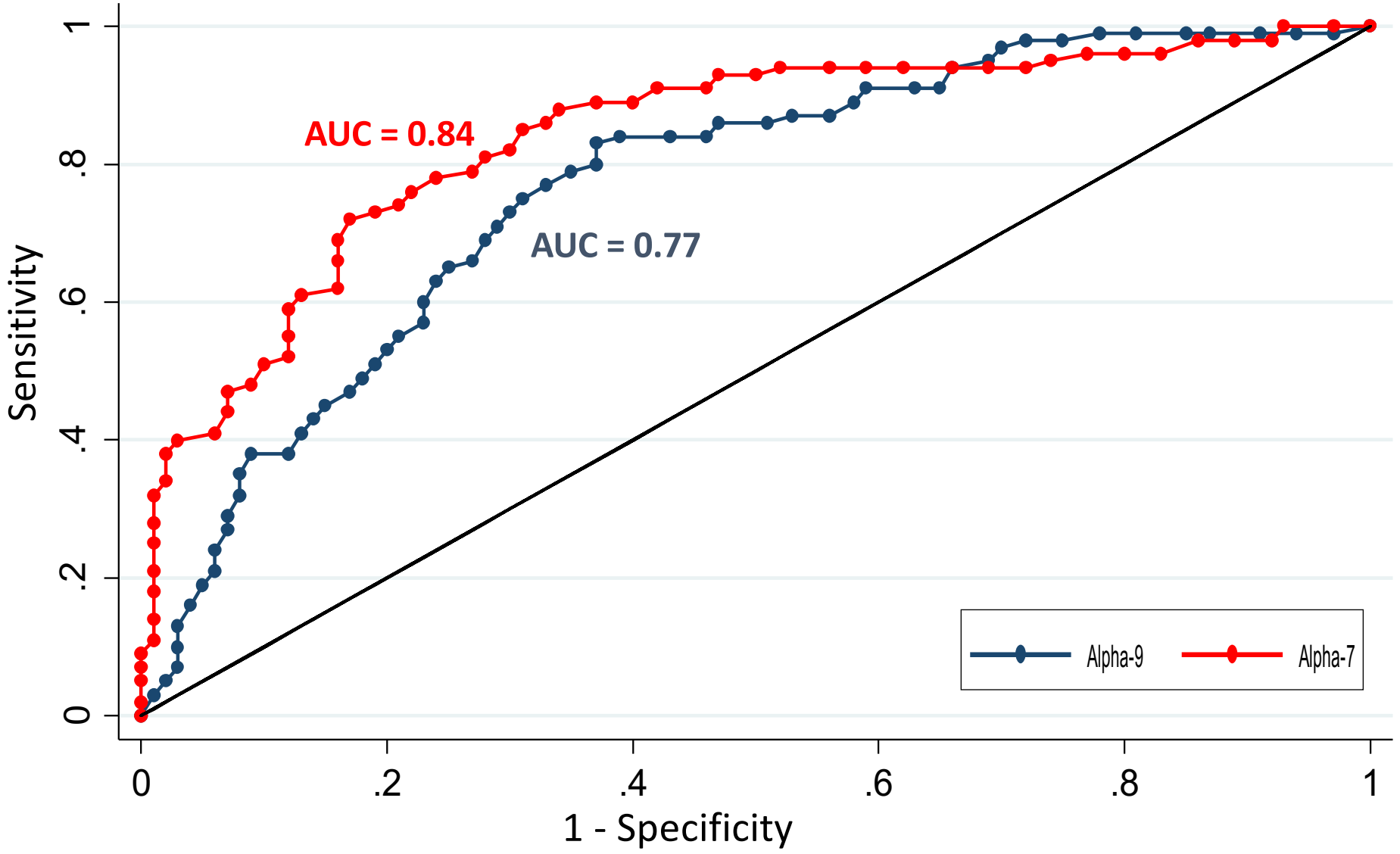
HPV Methylation in 12 High-Risk Types – PaP Cohort

- Nested case-control study of individuals in the Pap cohort
  - 360 <CIN2 (controls), 299 CIN3 (cases)
- Next-generation bisulfite sequencing (Illumina HiSeq; Robert Burk)
  - L1 and L2 genes, approximately 9 CpGs per type
  - Each type = separate PCR reaction
- Absolute two-year risks of cervical precancer for type-specific DNA methylation, weighted back to full-cohort (~30,000 HPV+ individuals)

Clarke et al., Clin Cancer Res 2018

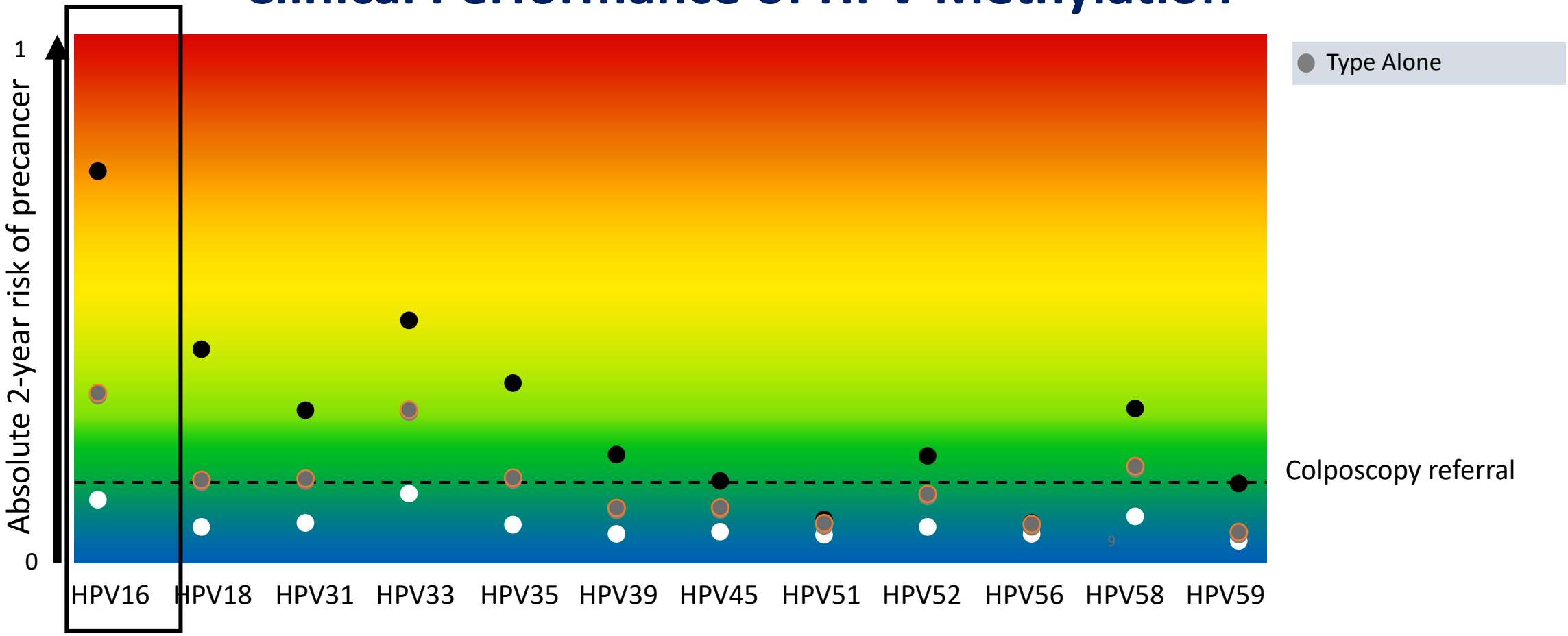
Performance of Top CpG Sites by HPV Type

HPV Type	Gene	CpG Site	AUC
16	L1	5602	0.84
31	L1	6363	0.75
33	L1	7034	0.73
35	L2	4244	0.81
52	L2	4258	0.77
58	L1	6446	0.74
18	L1	7041	0.86
39	L1	5731	0.85
45	L1	7088	0.82
59	L1	5584	0.91
51	L2	5533	0.71
56	L1	5570	0.71



Clarke et al., Clin Cancer Res 2018

Clinical Performance of HPV Methylation



Potential to detect HPV positivity, genotype, and methylation status all from the same sample

Clarke et al., Clin Cancer Res 2018

Performance of Methylation vs. Current Triage Strategies

	Cytology	HPV16/18	Cytology and HPV16/18	Methylation
Threshold	ASC-US+	Either 16 or 18 positive	ASC-US+ or 16 or 18 positive	Sensitivity fixed at 80%
Positivity	**48.7%**	30.8%	**63.7%**	**38.5%**
Sensitivity	76.6%	56.7%	89.9%	80.0%
Specificity	**54.1%**	71.8%	**38.8%**	**65.6%**
PPV	14.3%	16.8%	12.8%	18.9%
1-NPV	4.2%	5.7%	2.5%	3.0%

Methylation has better performance compared with cytology and HPV16/18 genotyping

Clarke et al., Clin Cancer Res 2018

Methylation Assay Development and Clinical Validation

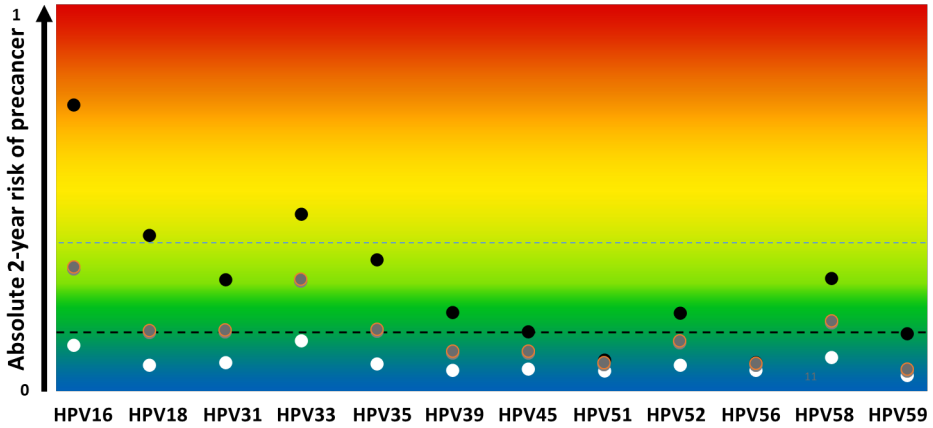
- Next-generation bisulfite sequencing assay (Sarah Wagner)
  - Ion Torrent S5 System
  - 11 HPV types (~10-15 sites per type), 10 host genes
  - Four PCR reactions, agnostic to HPV type
- Validation Studies:
  - PaP Study (KPNC) – validate site-specific performance in samples that were previously tested
  - SUCCEED Study – validate assay in samples with multiple HPV infections

Clinical Validation in NCI Studies

- Large head-to-head comparisons with different triage markers (IRIS, KPNC)
- Diverse populations (e.g., STRIDES, Mississippi)
- Self-collected samples, cis-gender and transgender individuals (Selfie)
- International populations, people living with HIV (AVE Network Studies)
- Anal precancers and cancers (ACES, Mt. Sinai)

Summary

- Methylation is an ideal test for cervical cancer screening and management in both high- and low-resource settings
  - Objective, amenable to automation
  - Quantitative – thresholds may be tailored to different settings
  - Self-collected sampling
- Unprecedented risk stratification from a single test
  - HPV positivity, genotype, methylation status
- Several validation studies
  - Different populations
  - Different sample types (clinician vs. self-collect)
  - Different anatomic sites (cervix, anal)



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