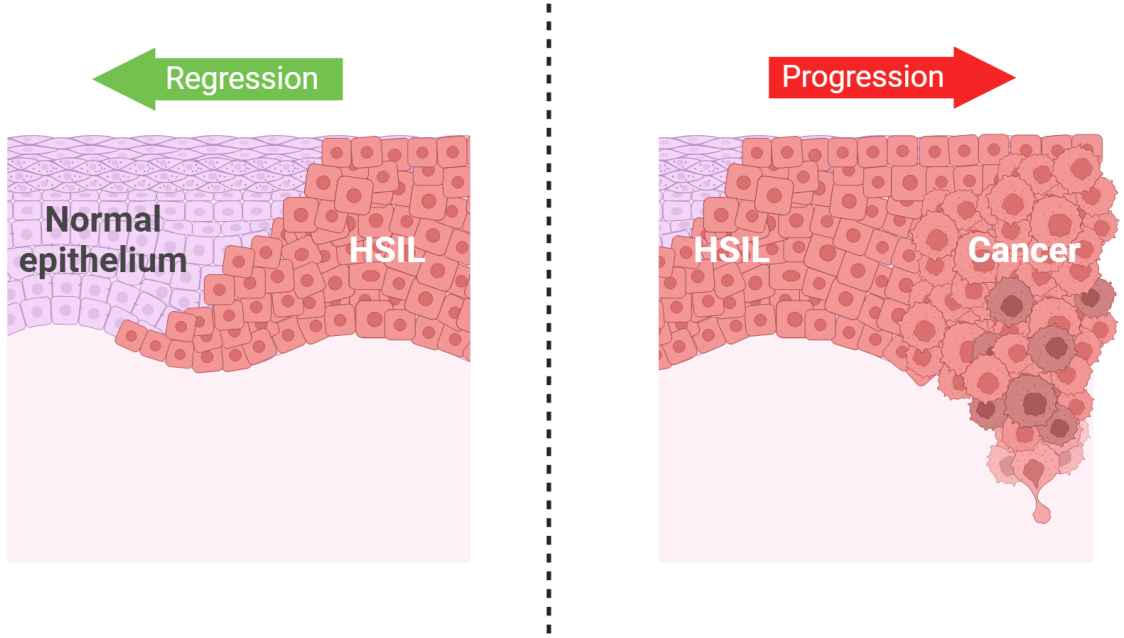
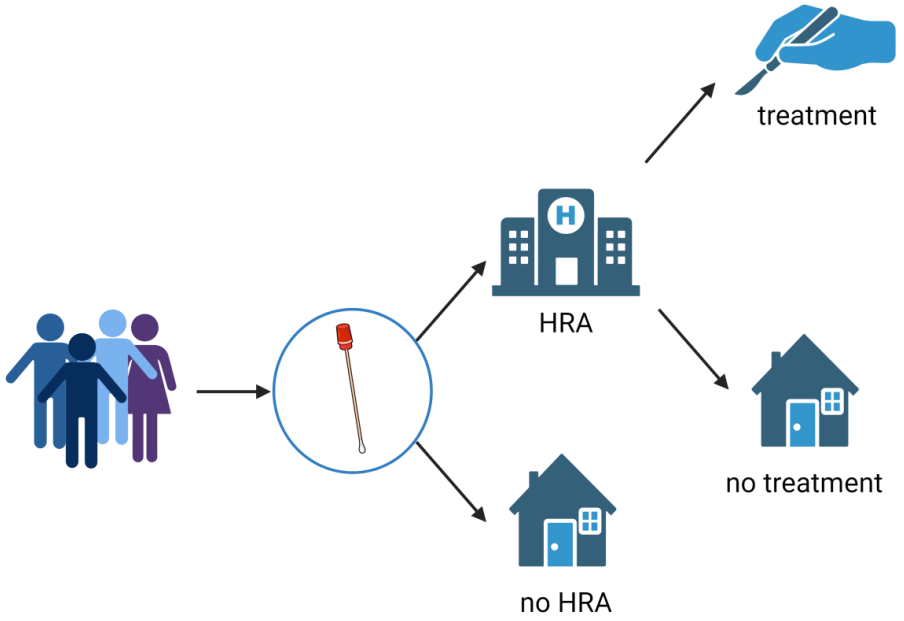
Biomarkers and anal dysplasia: How can we use them?

F. Dias Goncalves Lima, MD
Amsterdam University Medical Centers, The Netherlands





DISCLOSURES

- Nothing to disclose

INTRODUCTION

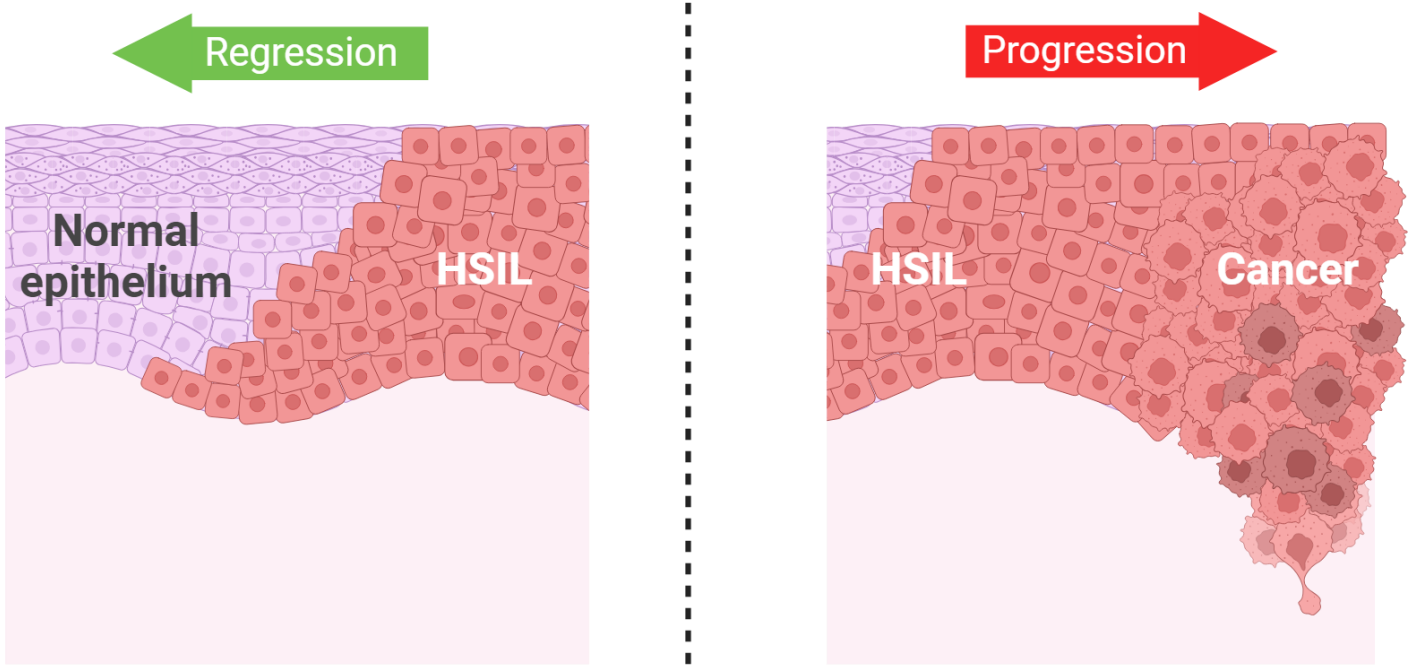
- ANCHOR: groundbreaking proof that treating HSIL can prevent anal cancer

- IANS guidelines on screening

- Challenges in clinical practice:
  - Limited capacity
  - HRA & treatments burden

How can we use biomarkers?

Palefsky *et al*. N Engl J Med. *2022*, Stier *et al*. Int J Cancer. 2024



1. Cancer-Risk Stratification

CASE: PATIENT A

- Man, 41yo

History

- HIV
- MSM
- Asymptomatic

DARE: normal

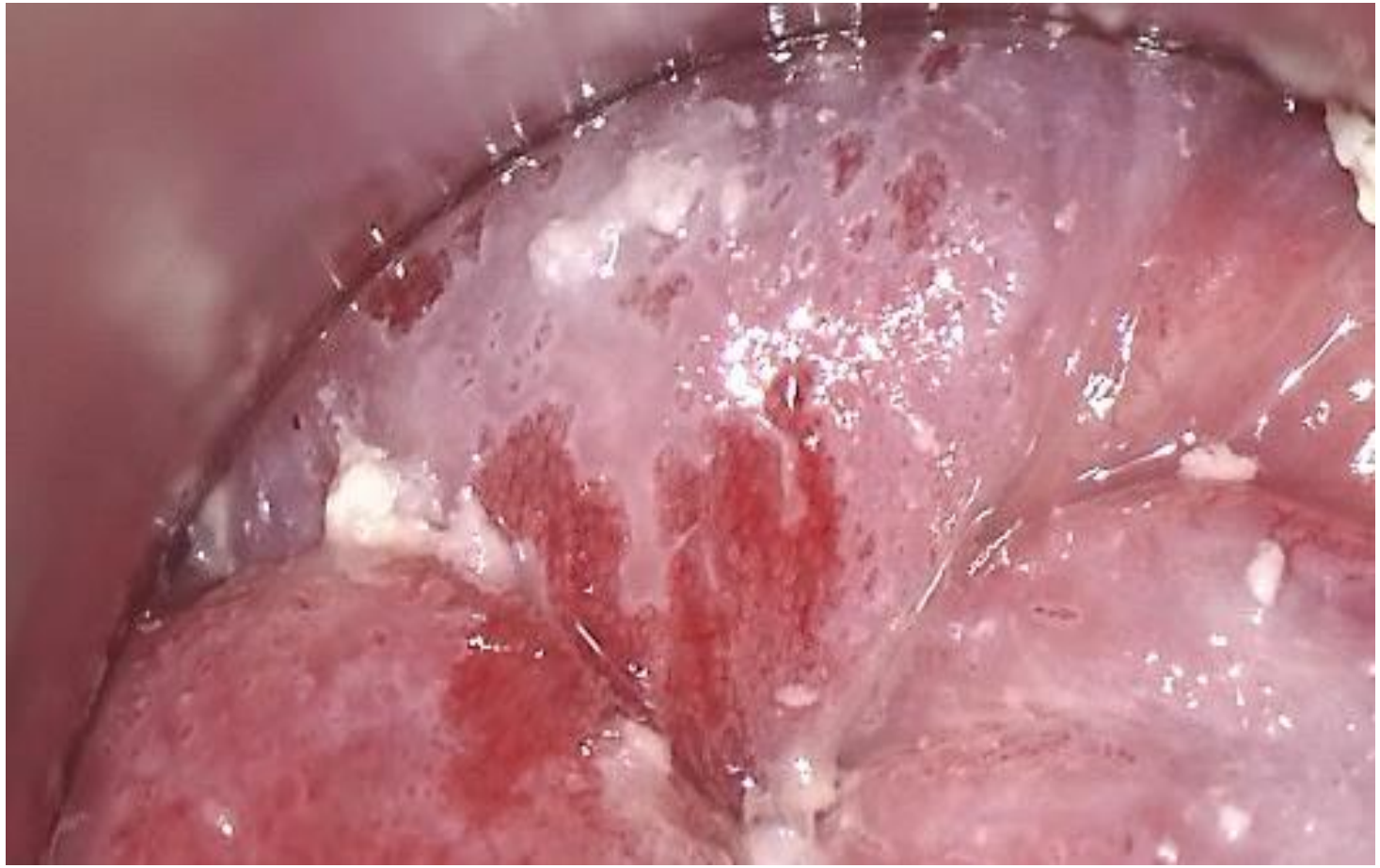
HRA: HSIL → start electrocautery (EC)

CLINICAL COURSE PATIENT A

Evaluation na 2x EC:

Persistent HSIL

→ Active monitoring



CLINICAL COURSE PATIENT A

Evaluation after 2y active monitoring:

- HSIL in complete regression

CASE: PATIENT B

- Man, 53yo

History:

- HIV
- MSM
- Asymptomatic

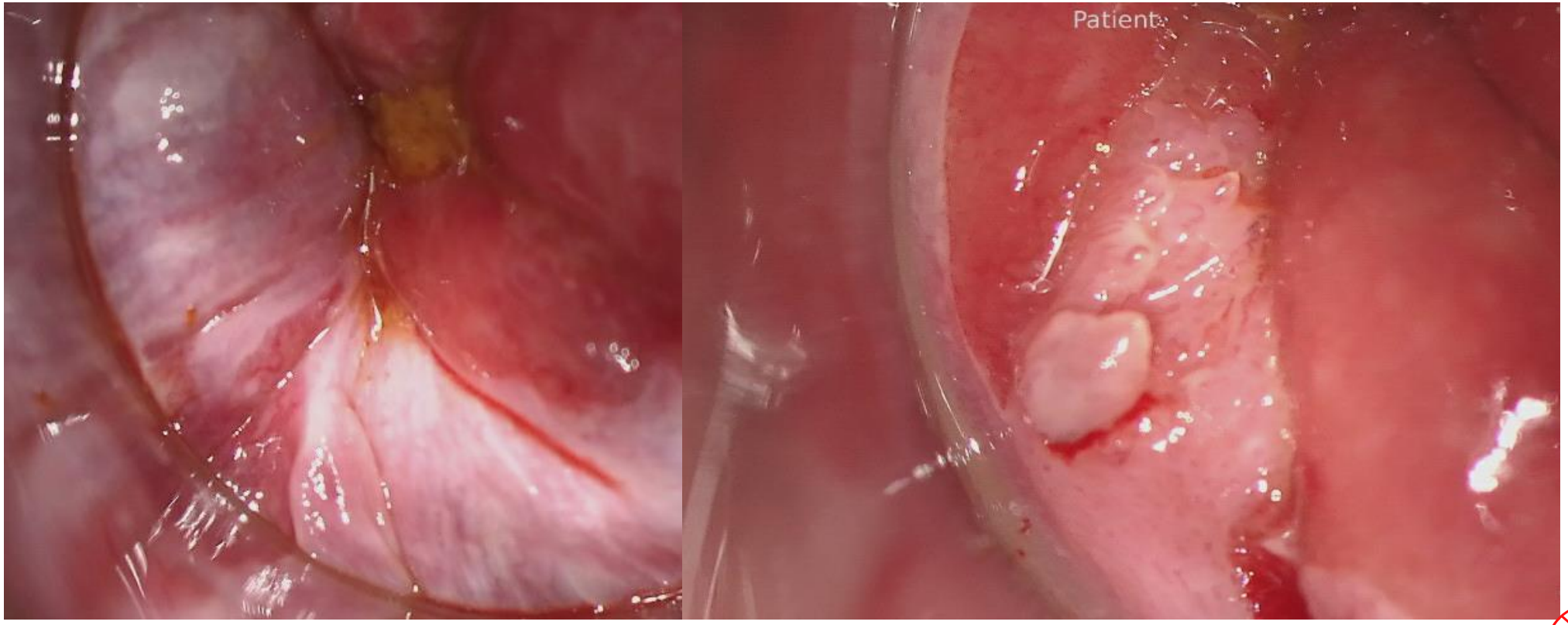
DARE: normal

HRA: HSIL

CLINICAL COURSE PATIENT B

Evaluation after 2x EC: clinical improvement HSIL

→ Once more 2x EC



CLINICAL COURSE PATIENT B

Emergency visit 5 months after 4th EC :

- Pain, bright red blood loss
- DARE:
  - Right posterior intra-anal: 3cm hard papule
  - Painful to touch
  - Visible blood

HRA

CLINICAL COURSE PATIENT B

PA: High suspicion for invasive squamous cell carcinoma

MRI + PET/CT: cT2N1M0 anal carcinoma

Therapy: Chemoradiation

Challenges in Treatment

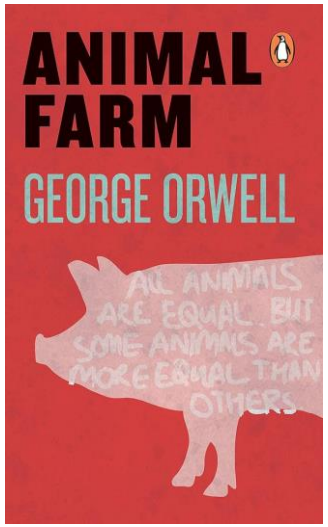
- Large proportion of anal cancers not prevented by treatment (43% in ANCHOR)

→ **Treat more (intensively)**

- Number needed to treat to prevent one cancer is high (438 in ANCHOR)
- 30% of HSIL regresses spontaneously in 1 year

→ **Treat less (often)**

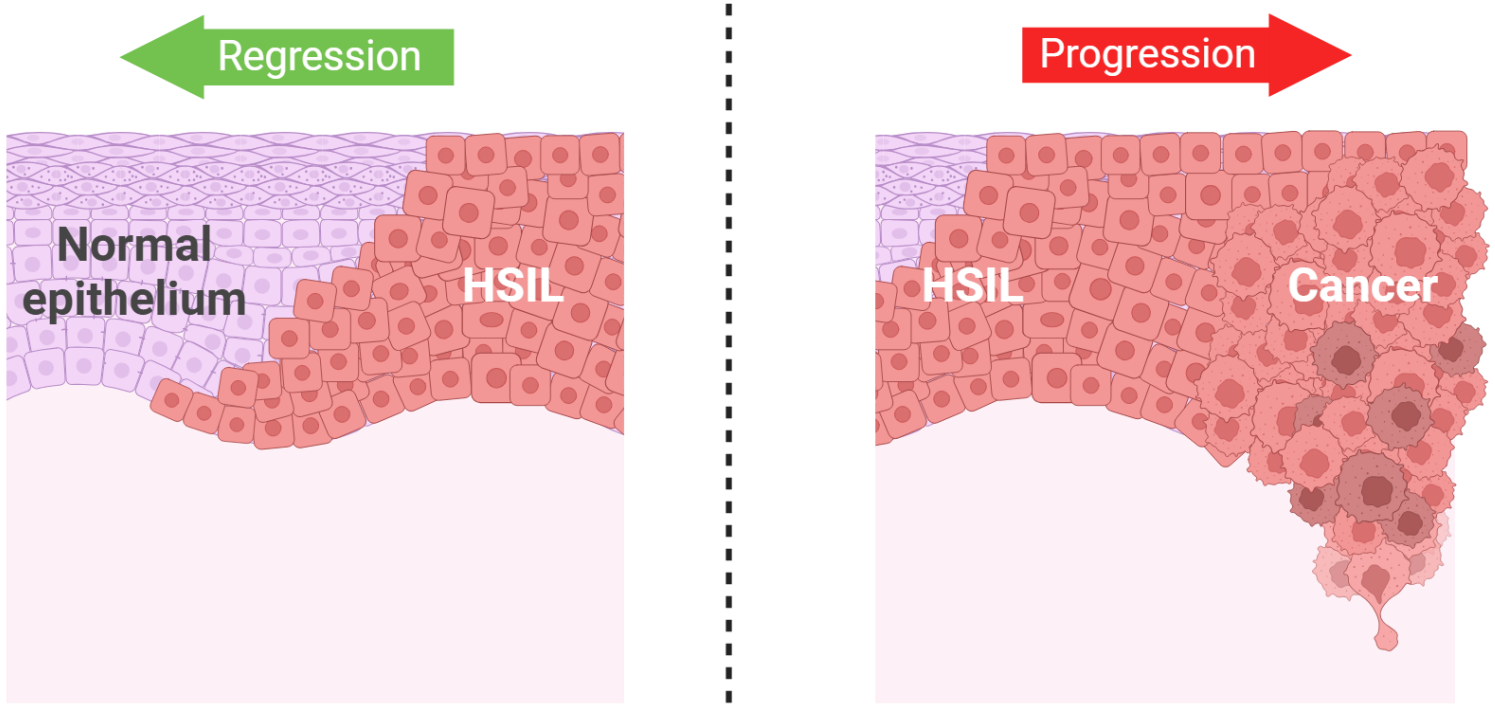
NOT ALL HSIL ARE EQUAL



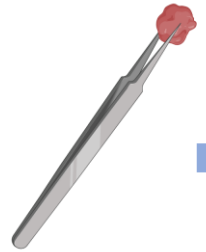
Palefsky *et al*. N Engl J Med. 2022

ROLE OF BIOMARKERS: CANCER RISK STRATIFICATION

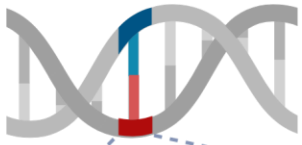
Differentiating between HSIL likely to **regress** and HSIL likely to **progress to cancer**

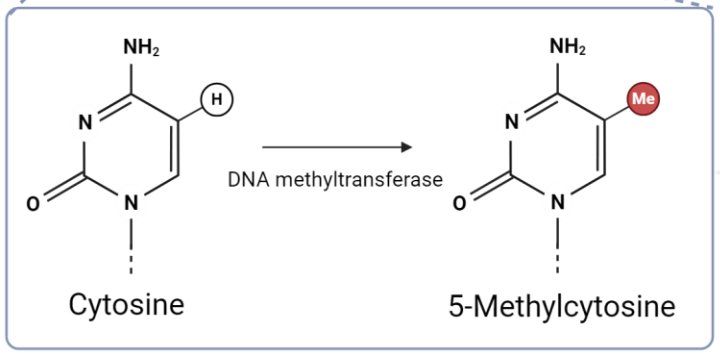


DNA METHYLATION MARKERS

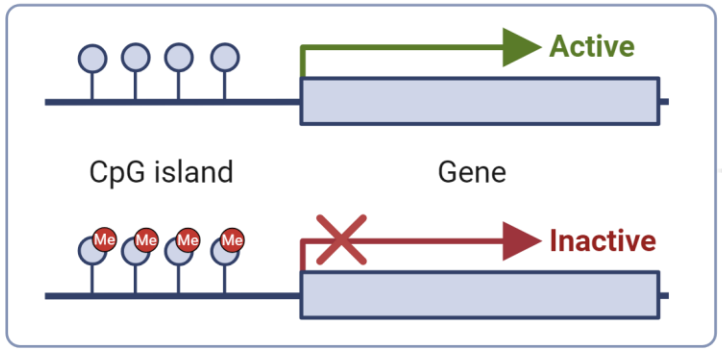


qPCR





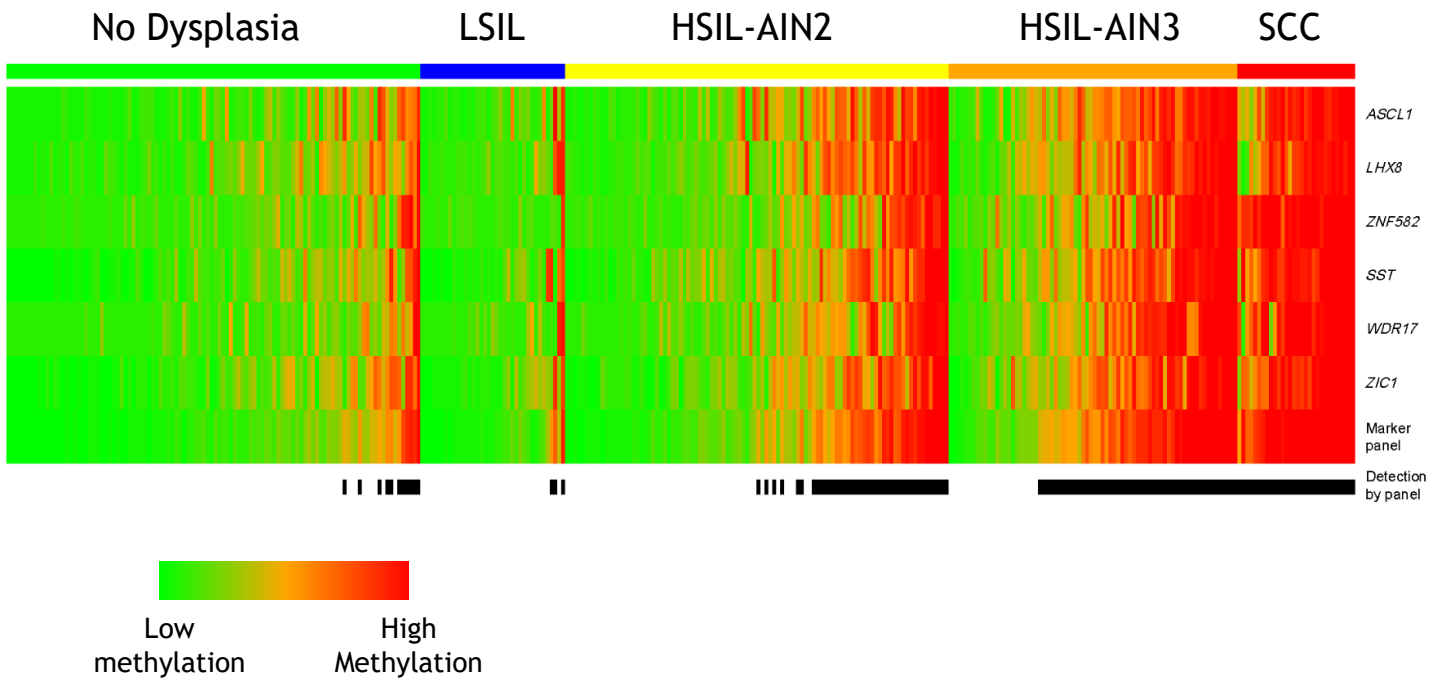
Inactivation of
Tumor Suppressor Genes

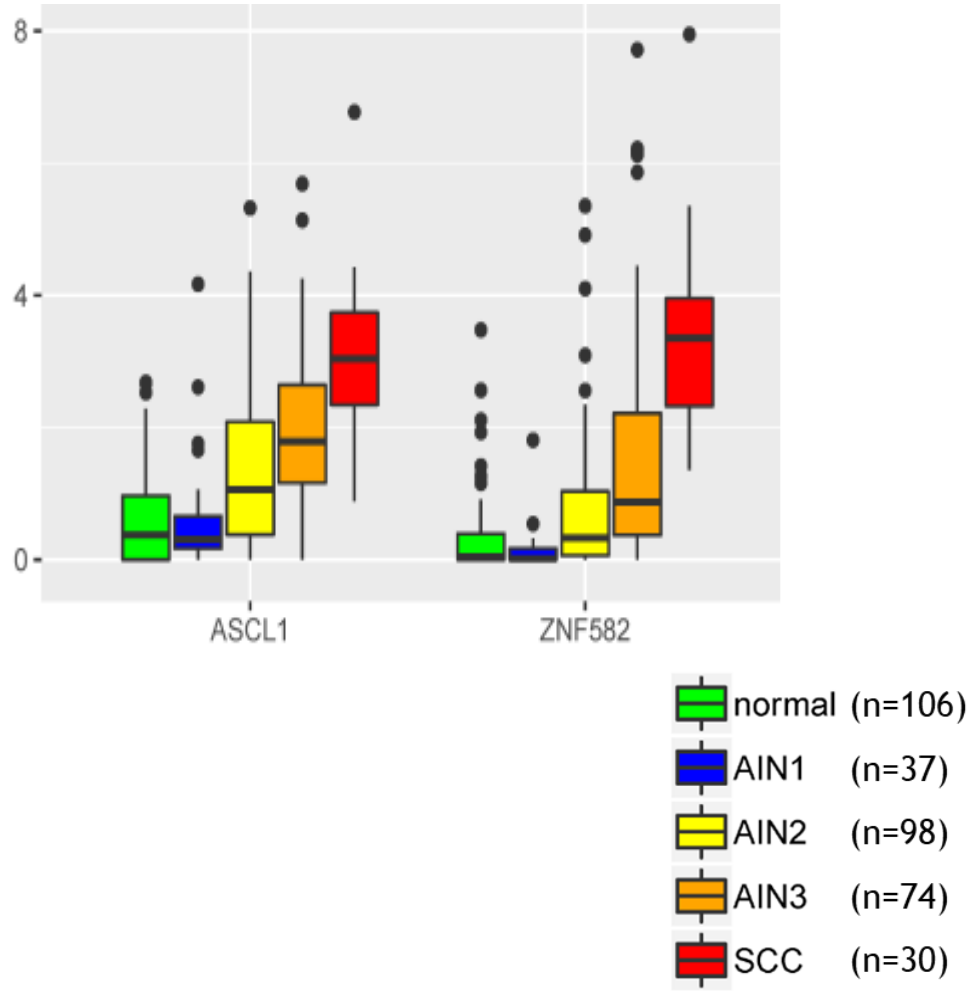


Carcinogenesis

Unmethylated Methylated

ANAL CARCINOGENESIS: INCREASE IN DNA METHYLATION





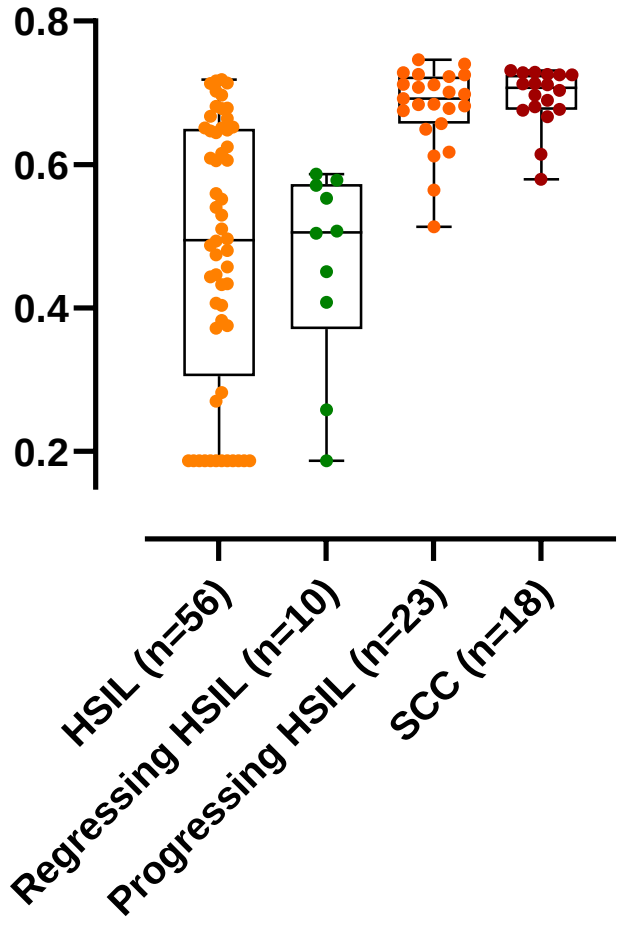
Van Der Zee *et al.* CID 2021

METHYLATION IS HIGH IN HSIL PROGRESSING TO CANCER

Case	Sex	HIV status	Age at final diagnosis (year)		Preceding HGAIN						Final diagnosis		
1	M	pos	62	Methylation result:					1	2	3		
				Diagnosis:					AIN3	AIN3	≈ SCC		
				t=					-12M	-7M	0		
2	M	pos	59	Methylation result:					1	2	3a	3b	
				Diagnosis:					AIN3	AIN2	≈ SCC	AIN2	
				t=					-5.5M	-2M	0	0	
3	F	neg	49	Methylation result:						1	2		
				Diagnosis:						AIN3	SCC		
				t=						-5M	0		
4	M	neg	60	Methylation result:					1	2	3		
				Diagnosis:					AIN3	≈ SCC	SCC		
				t=					-5M	-2.5M	0		
5	M	pos	51	Methylation result:						1	2		
				Diagnosis:						AIN3	≈ SCC		
				t=						-3M	0		
6	M	pos	47	Methylation result:						1	2		
				Diagnosis:						AIN2	SCC		
				t=						-5M	0		
7	M	pos	51	Methylation result:	1	2	3	4	5	6	7a	7b	7c
				Diagnosis:	AIN3	AIN2	AIN3	AIN2	AIN2	AIN3	≈ SCC	SCC	SCC
				t=	-28M	-20M	-18M	-16.5	-9.5M	-6M	0	0	0
8	M	pos	58	Methylation result:			1a	1b	2	3	4a	4b	4c
				Diagnosis:			AIN3	AIN2	AIN3	AIN2	≈ SCC	≈ SCC	≈ SCC
				t=			-9M	-9M	-2.5M	-2M	0	0	0
9	M	pos	62	Methylation result:				1	2	3	4		
				Diagnosis:				AIN2	AIN2	≈ SCC	SCC		
				t=				-42M	-30M	-0.5M	0		
10	M	pos	62	Methylation result:				1	2	3	4		
				Diagnosis:				AIN2	AIN2	AIN2	≈ SCC		
				t=				-24M	-13M	-3M	0		

Low methylation — High Methylation

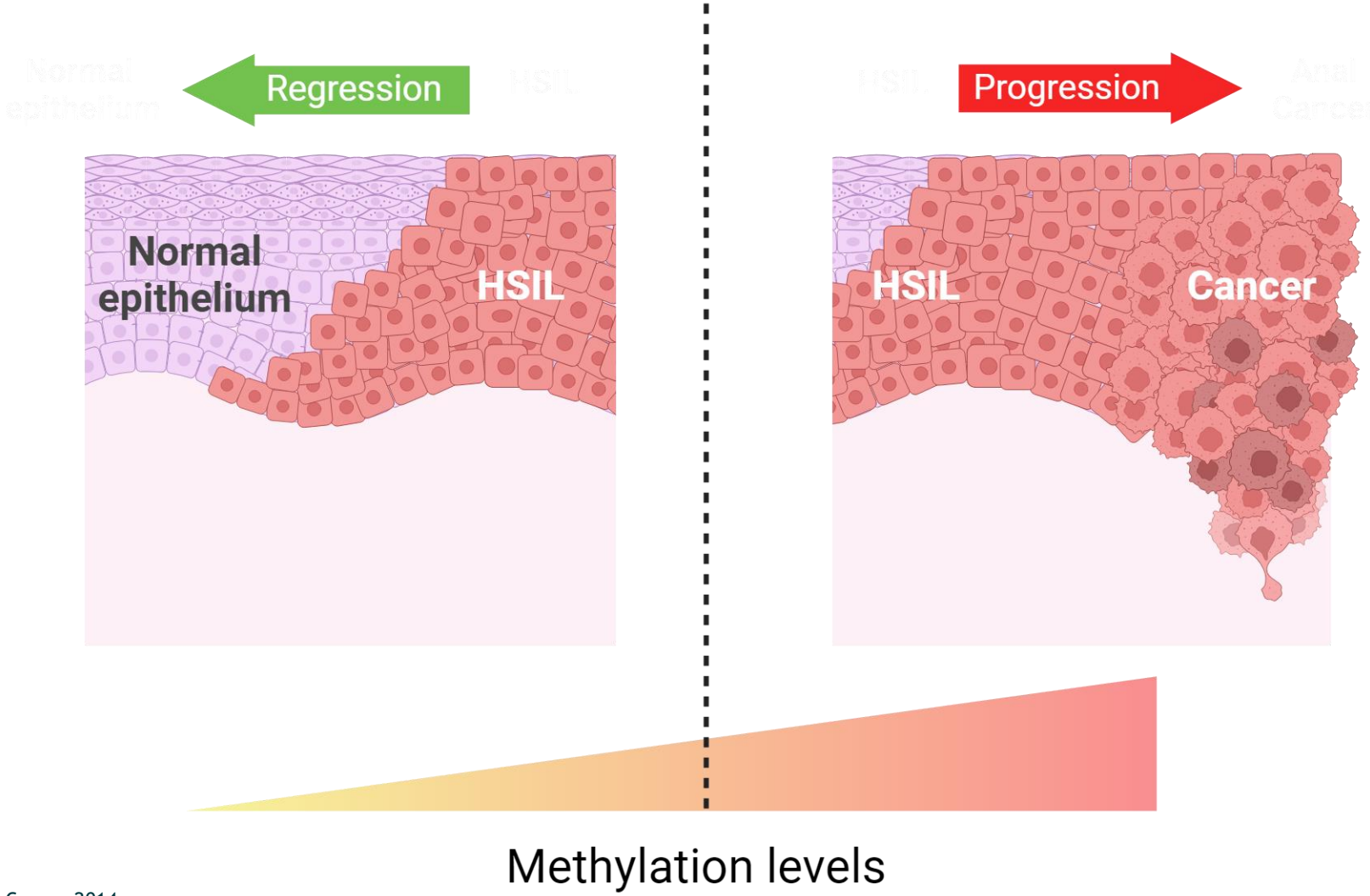
ASCL1/ZNF582 methylation predicted probability



Van Der Zee *et al.* CID 2021; Rozemeijer et al. Tumor Virus Res, 2023

*PreCursor-M AnoGYN

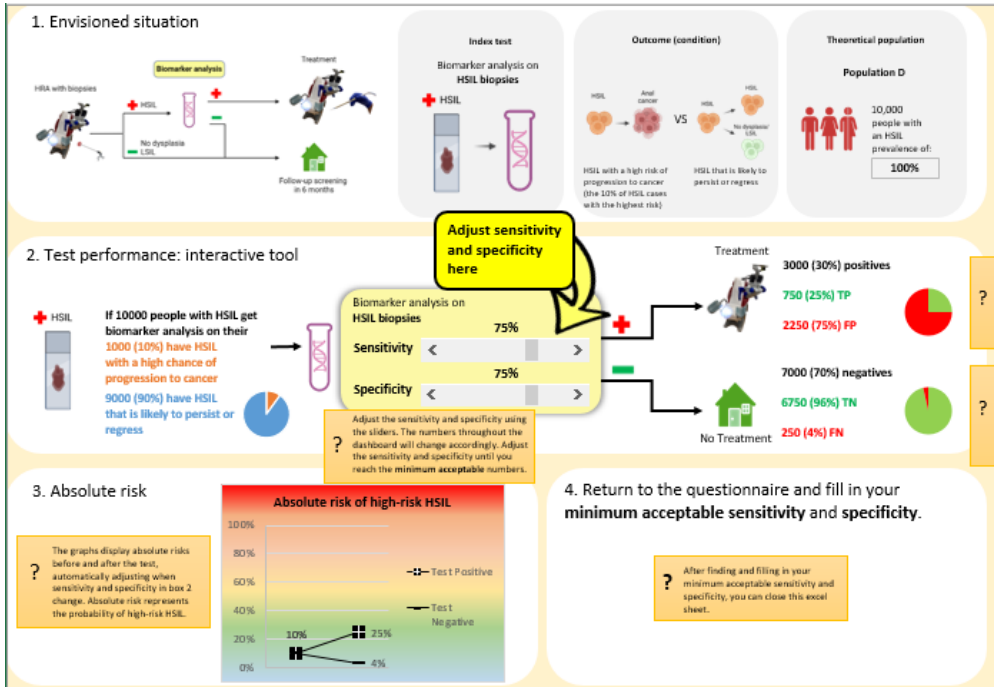
CONCEPT



Steenbergen et al. Nat Rev Cancer 2014

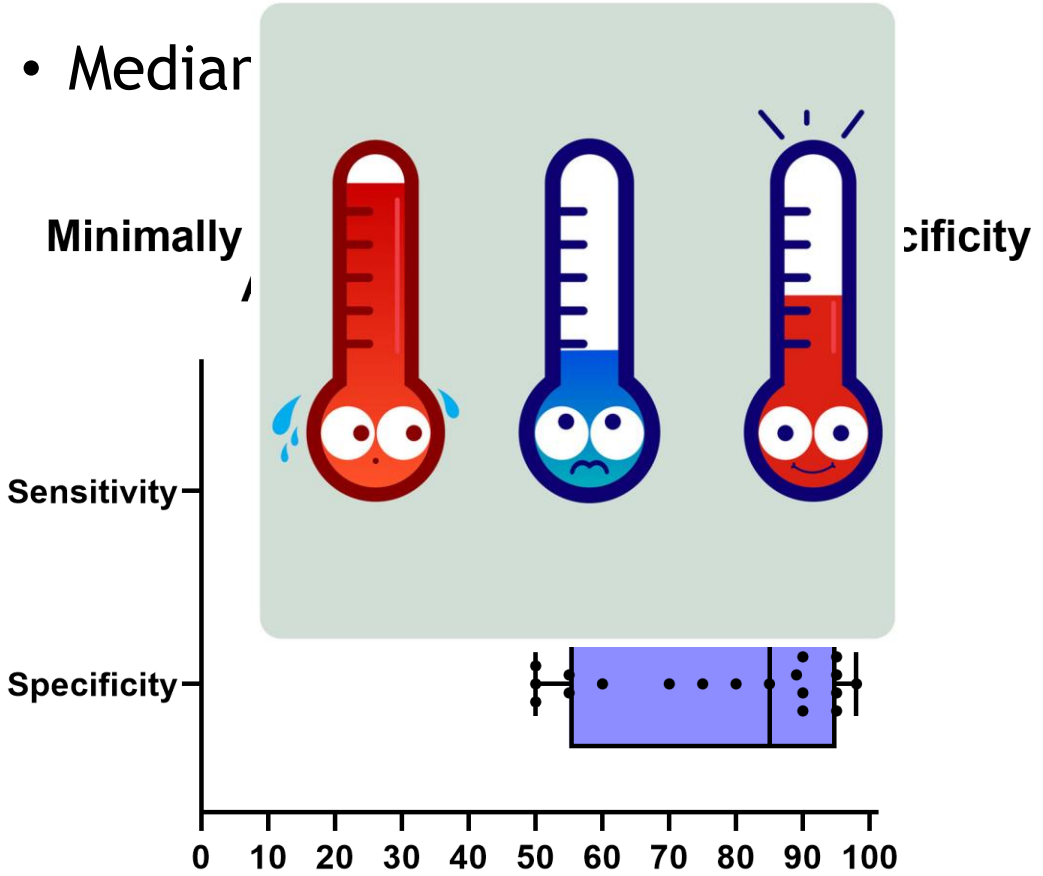
THRESHOLD SETTING: eDELPHI SURVEY

- Experts from around the world
- Multiple survey rounds
- Tool for calculations



Results Round 1:

- Median Sensitivity = 89%
- Median



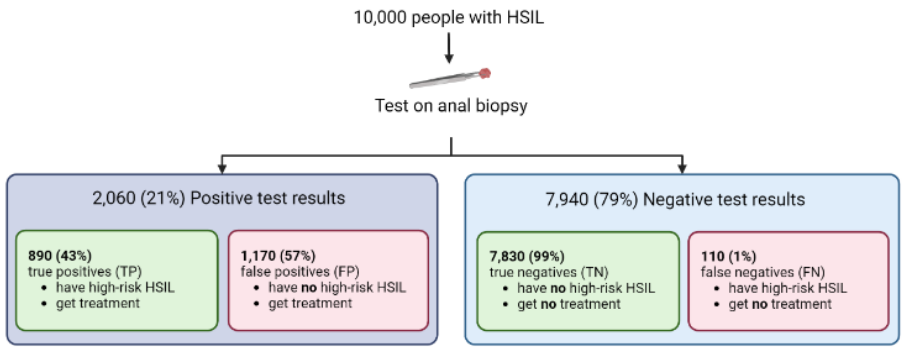
eDELPHI ROUND 2 IS STILL ONGOING

*

Question 3

For the detection of HSIL with a high chance of progression to cancer in biopsies, the median of participants answered that:
- the minimally acceptable sensitivity was 89%, and
- the minimally acceptable specificity was 87%.

This translates in the following theoretical scenario:



Statement: The above mentioned sensitivity and specificity and corresponding numbers of false and true positives and negatives are acceptable. A lower sensitivity or specificity would be insufficient.

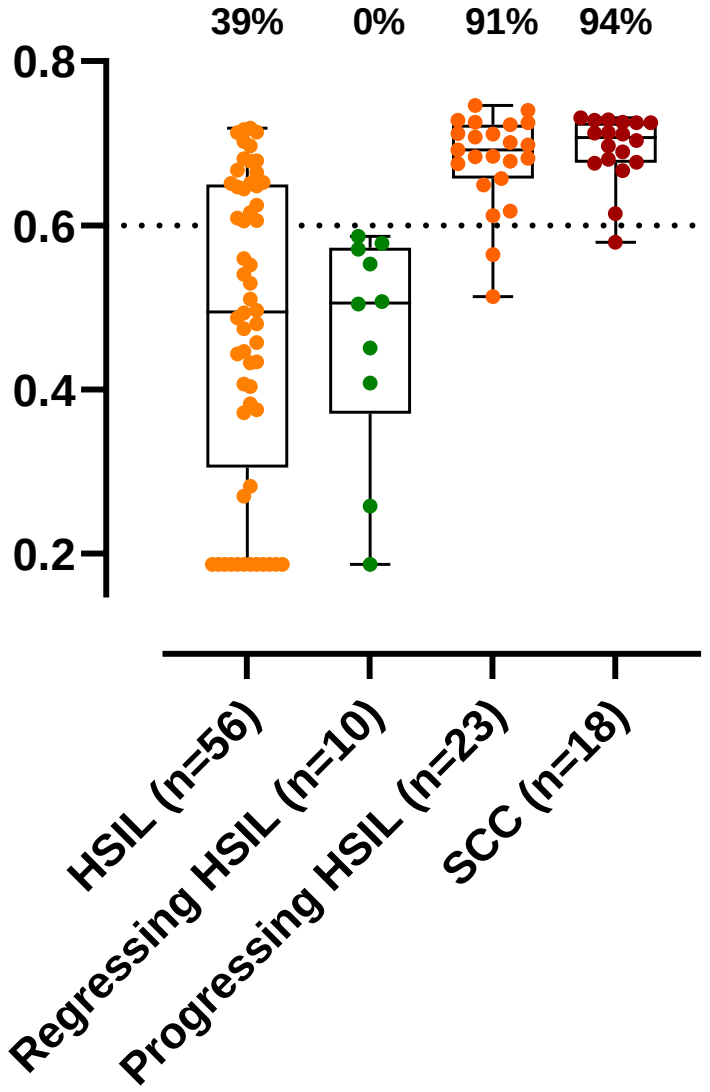
Do you agree with this statement?

Strongly Disagree	Moderately Disagree	Neutral	Moderately Agree	Strongly Agree	Do not know
○	○	○	○	○	○

Scan to participate

THRESHOLD SETTING

- At least 89% sensitivity for high-risk HSIL
- As high as possible to increase specificity



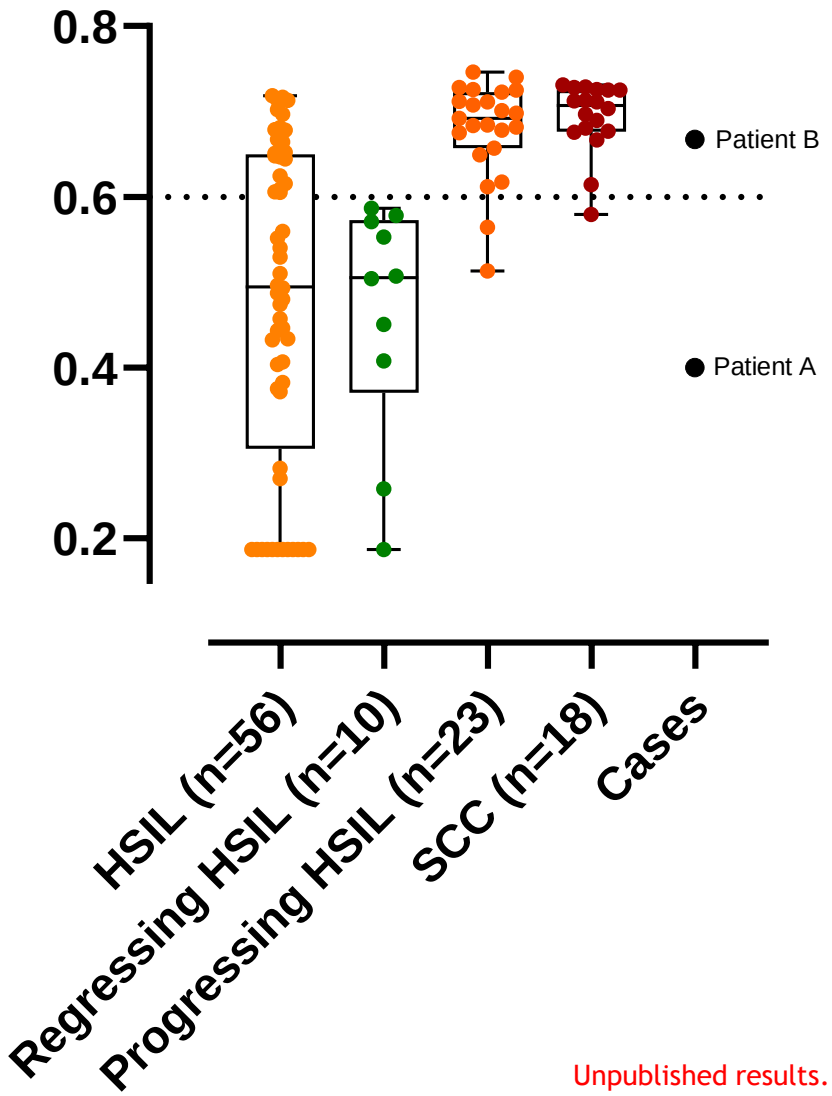
Dias Goncalves Lima et al, manuscript in preparation;
Rozemeijer et al. Tumor Virus Res, 2023

*PreCursor-M AnoGYN

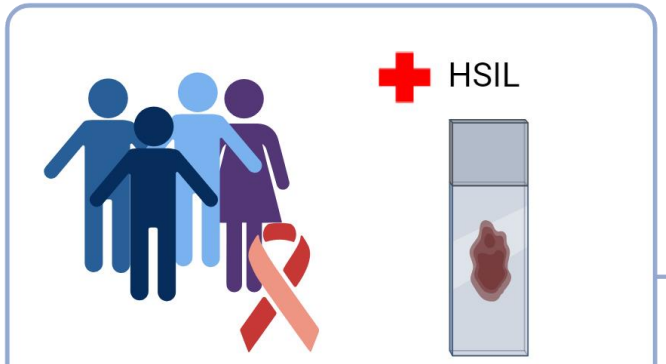
BACK TO THE CASES

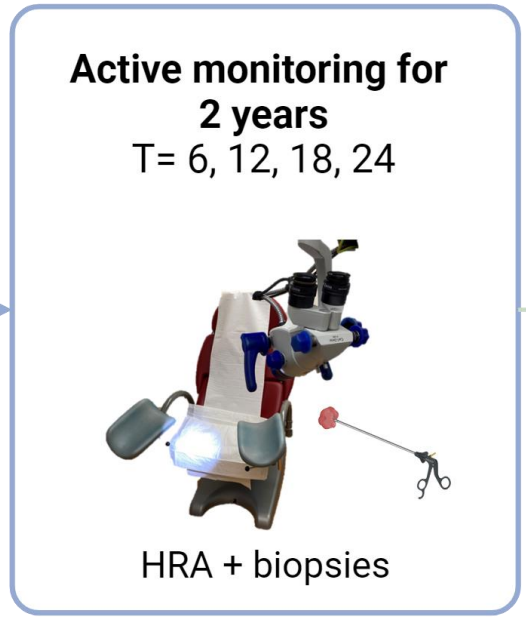
Biomarkers could stratify HSIL by its risk of progression to cancer and determine propper treatment indication

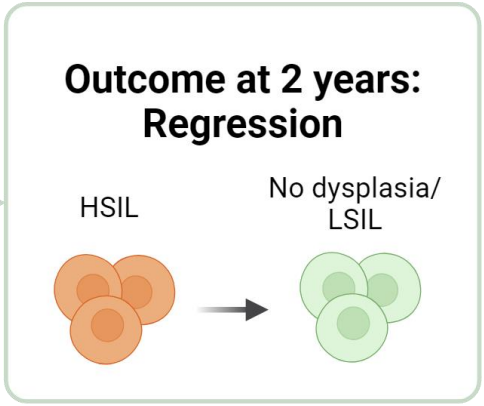
***ASCL1/ZNF582* methylation predicted probability**

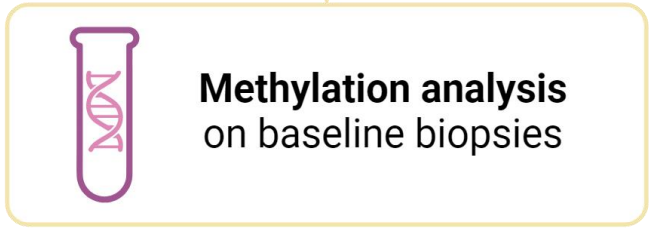


CLINICAL VALIDATION: MARINE STUDY

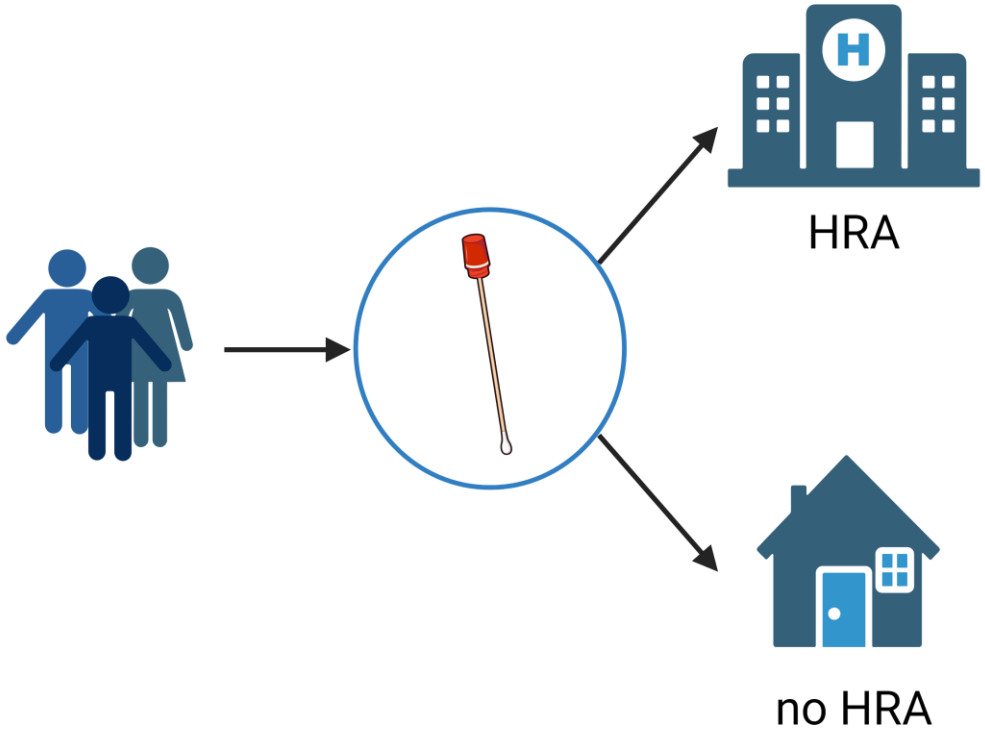








Dias Goncalves Lima et al. BMJ Open, 2022



2. Screening

CASE: SCREENING IN THE NETHERLANDS

- 18 million people
- All MSMLWH>35yo → HRA indication
- Now: screening with swabs
- Next step: Screening of other risk groups

Populations to screen

Risk Category A
Cancer incidence >17/100,000

Persons with HIV
- Men who have sex with men (MSM) *age 35+*
- Transgender women (TW) *age 35+*
- Men (not MSM) *age 45+*
- Women *age 45+*

Vulva Dysplasia or Vulva Cancer

MSM without HIV *age 45+*
TW without HIV *age 45+*

Solid organ transplant recipients
10 years post transplant

Risk Category B
Cancer incidence <10/100,000

Shared Decision-Making Age 45+
with history of:

- Cervical/Vaginal HSIL or Cervical/Vaginal Cancer
- Perianal Warts
- Persistent Cervical HPV 16+
- Other immunosuppression or on chronic systemic steroid therapy

Challenges in Screening: Capacity

=1,000 persons

Now: 13,000 MSMLWH >35y

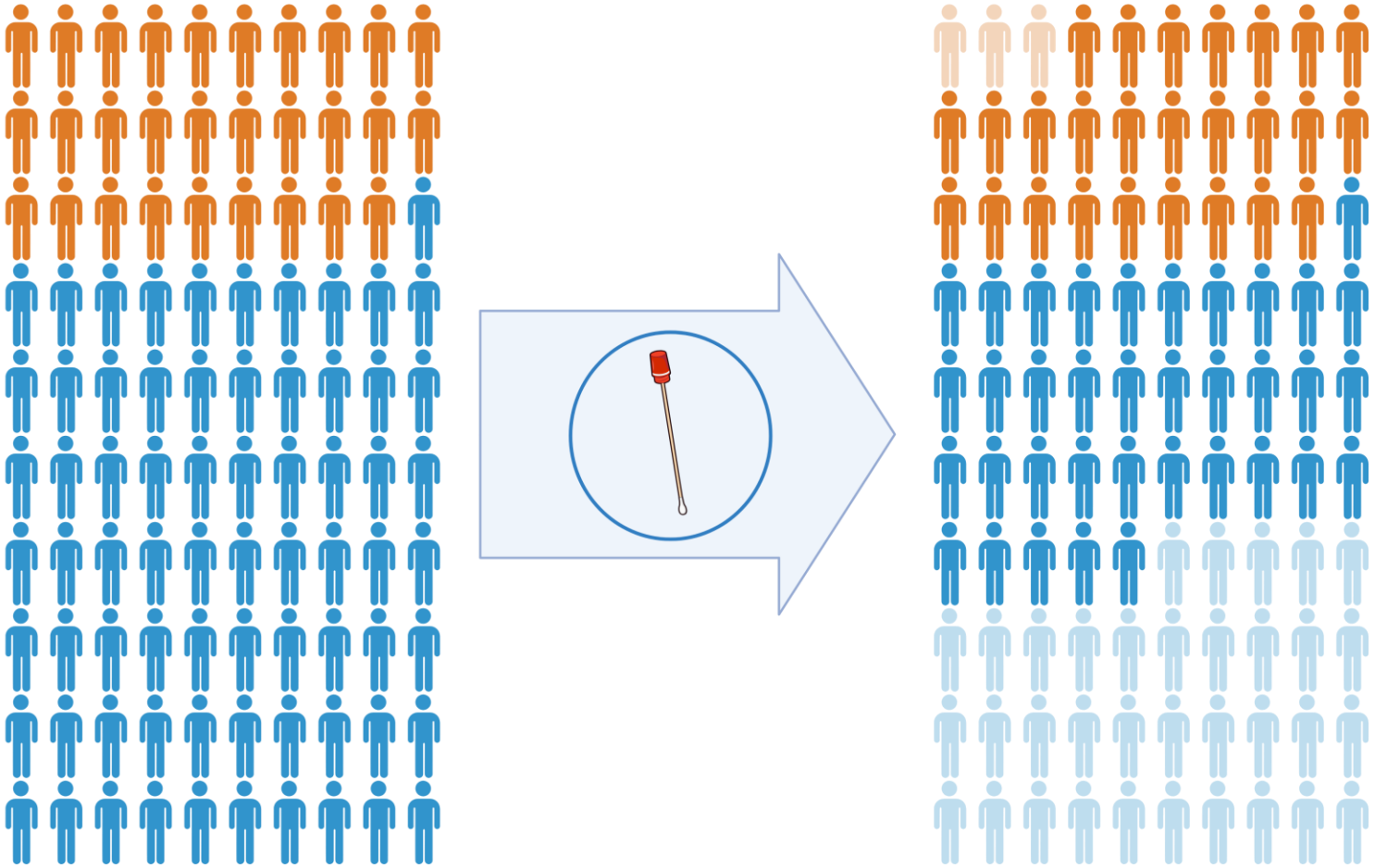
Future:

13,000 MSMLWH >35y
10,000 vulva (pre-)cancer
6,000 PLWH >45y
120,000 MSM without HIV >45y
5,000 organ transplant recipients
= **154,000**

NKR register; Marcus et al. BMC Public Health 2013; Stichting HIV Monitoring

hrHPV testing

HSIL detection: **89%**
HRA referral: **62%**



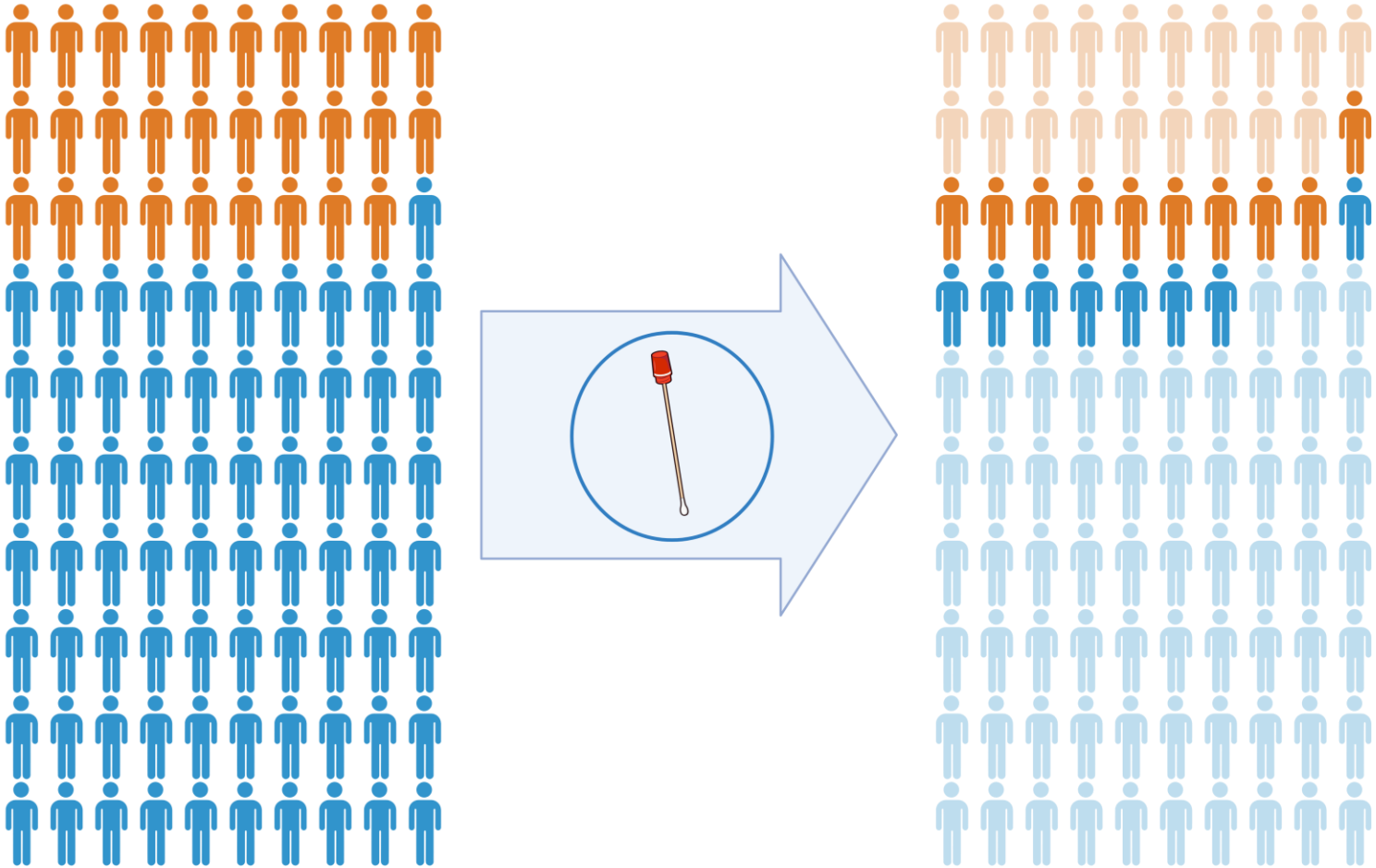
HSIL

no HSIL

Rozemeijer et al, manuscript in preparation

Cytology (HSIL)

HSIL detection: **44%**
HRA referral: **18%**



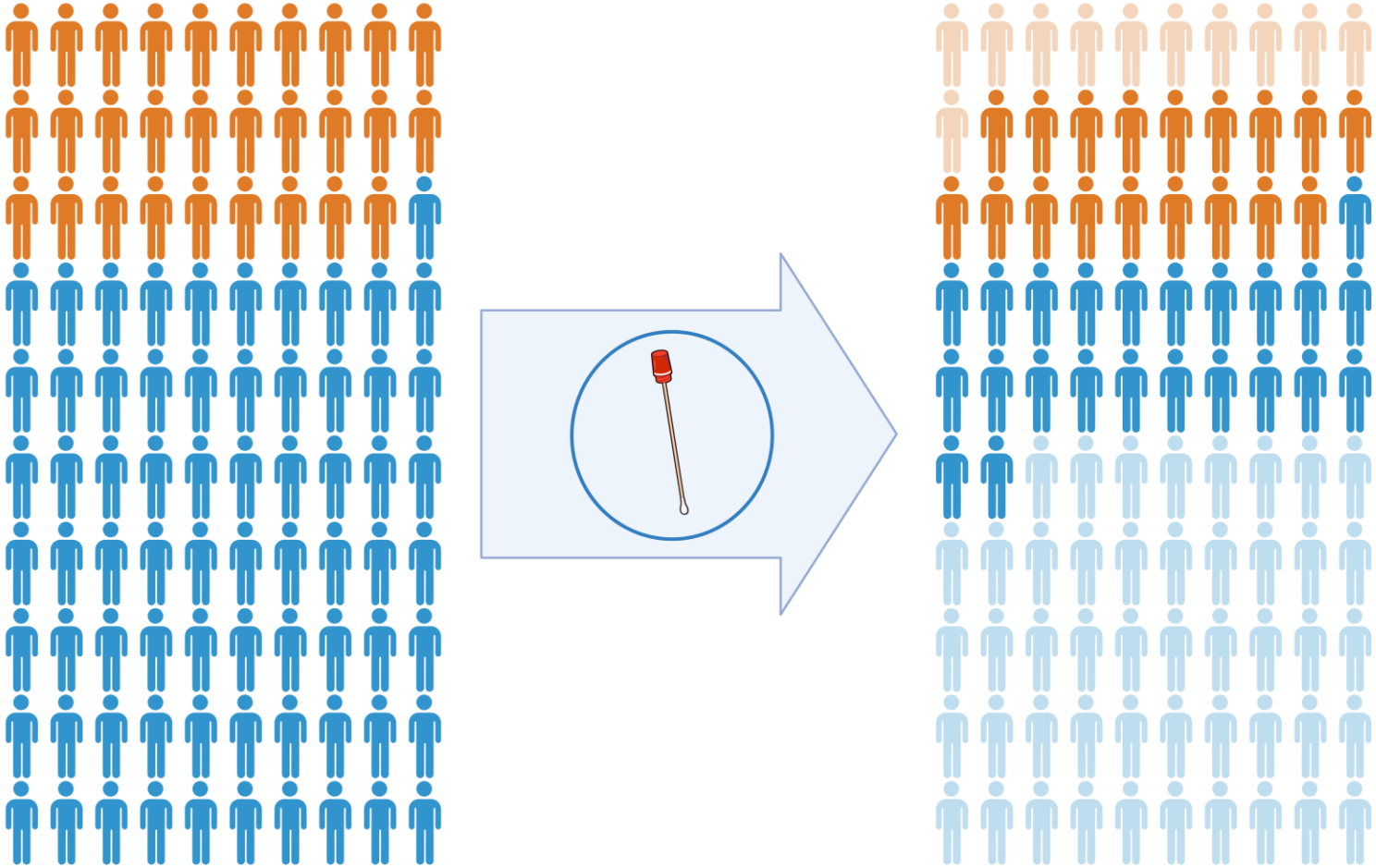
HSIL

no HSIL

Rozemeijer et al, manuscript in preparation

hrHPV + cytology (ASCUS) co-testing

HSIL detection: **64%**
HRA referral: **41%**

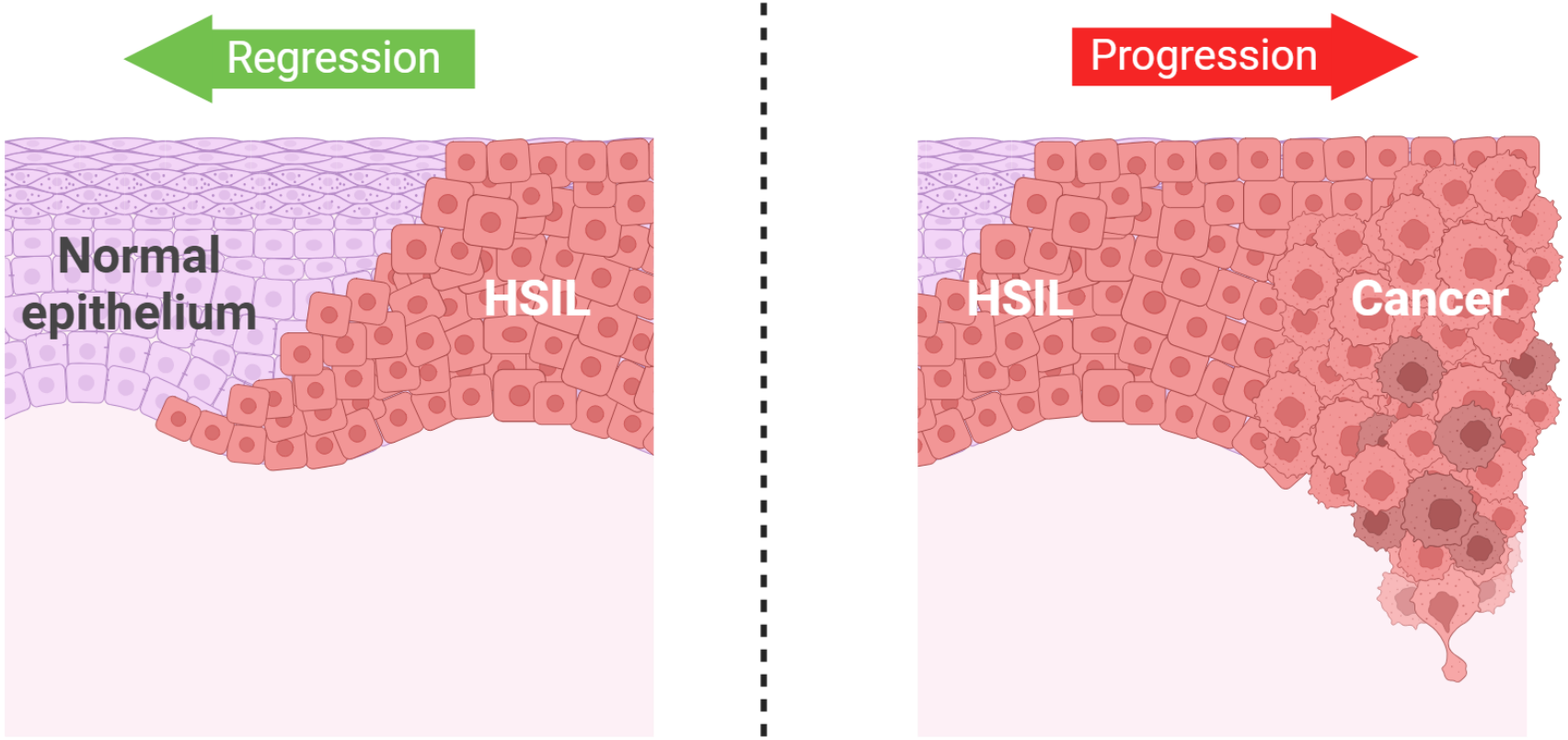


HSIL

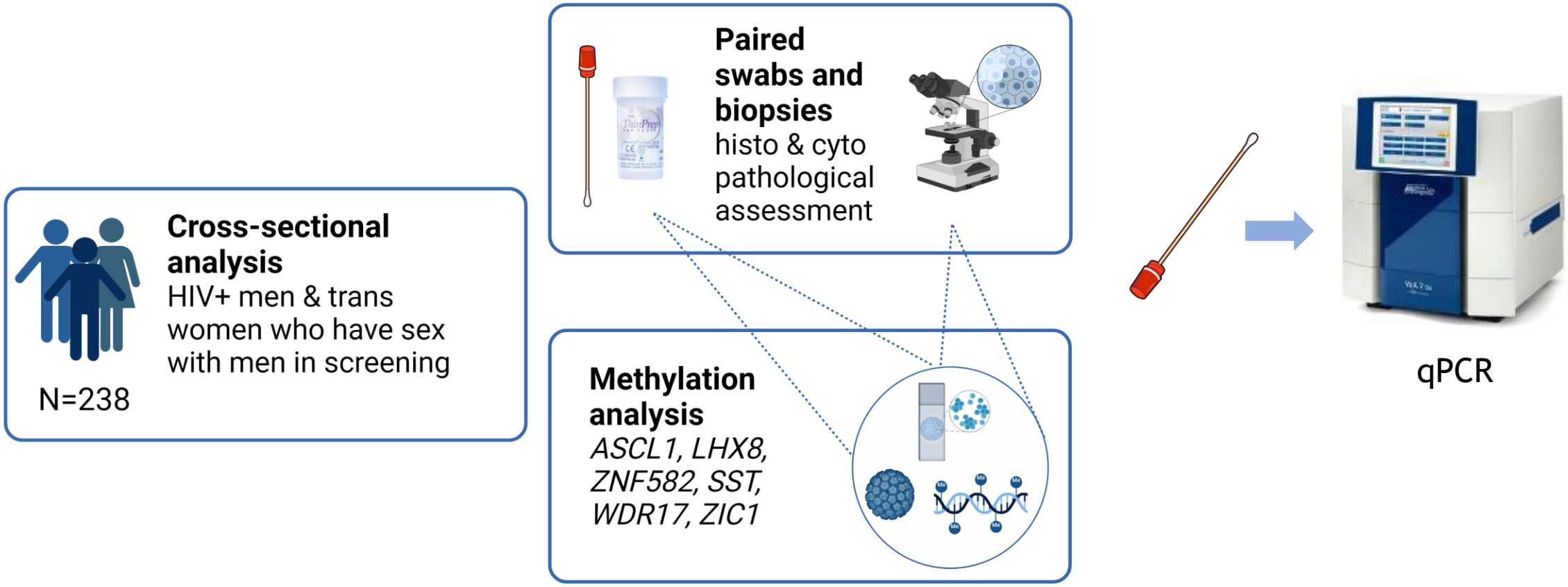
no HSIL

Rozemeijer et al, manuscript in preparation

ROLE OF BIOMARKERS: TARGETED SCREENING



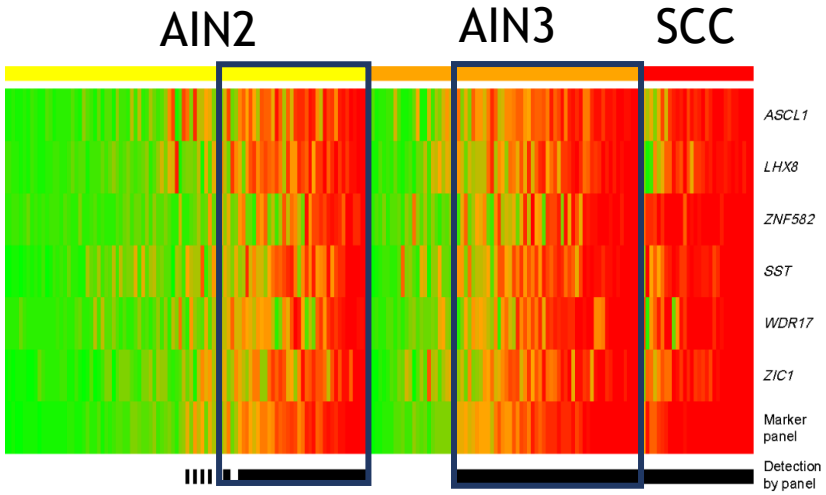
FEASIBILITY OF METHYLATION ANALYSIS IN ANAL SWABS

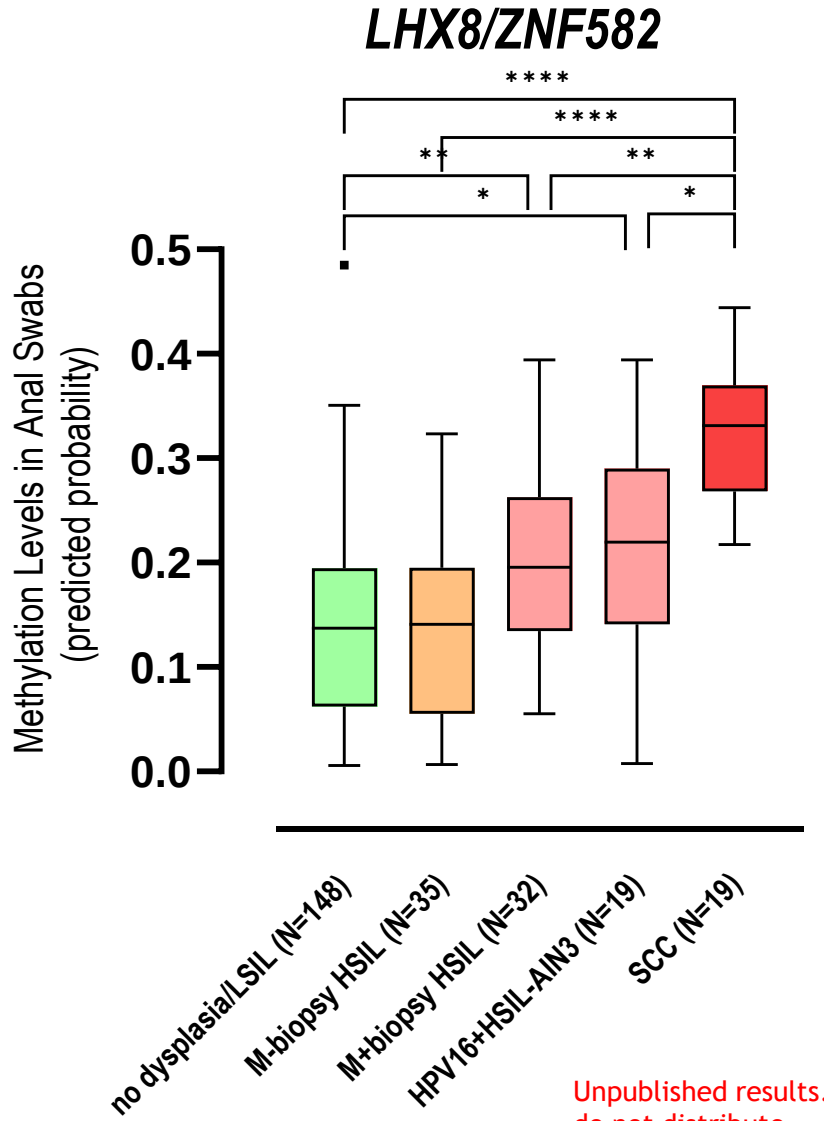


Dias Goncalves Lima et al. under review

METHYLATION OF ANAL BIOPSIES WAS REFLECTED IN ANAL SWABS

- Detect highest-risk lesions
- More specific screening
- Quality control after HRA





Dias Goncalves Lima et al. under review

ONGOING & FUTURE RESEARCH

Biopsies

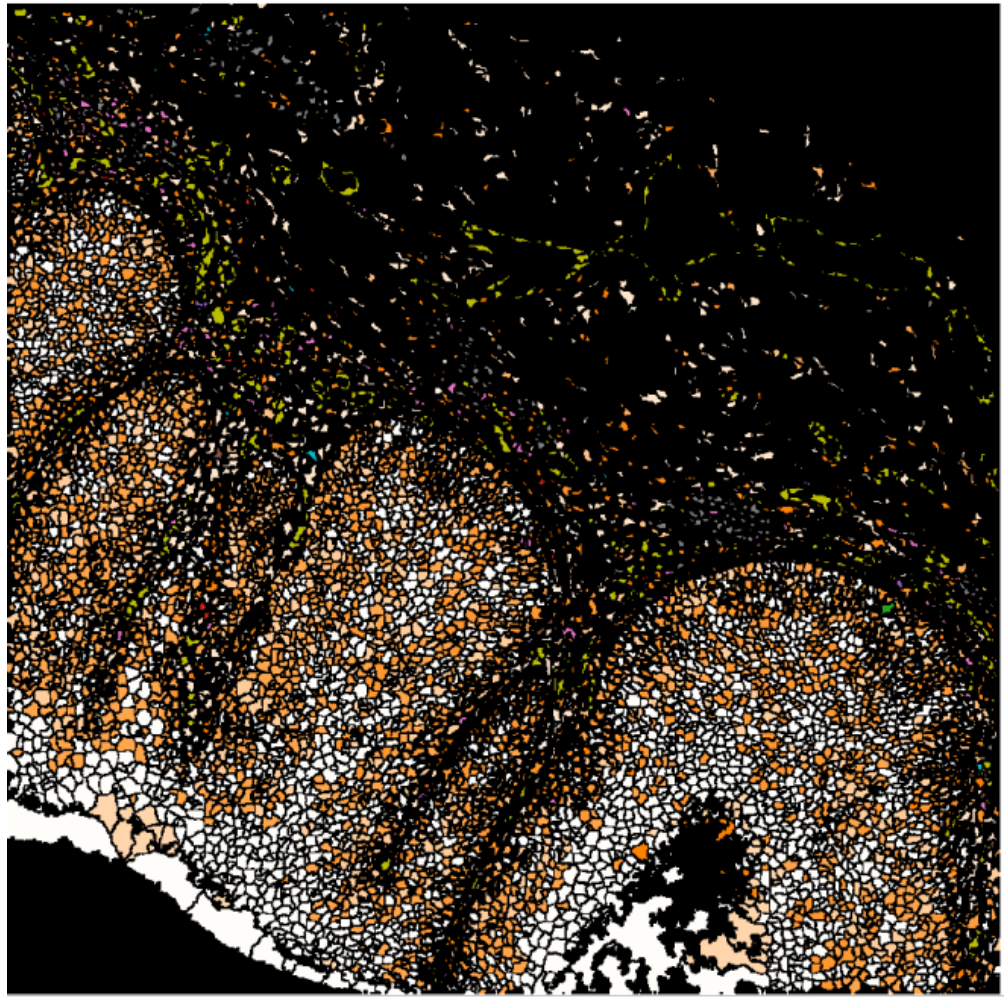
- Tumor immune microenvironment

Swabs

- New markers

General

- What does the patient think?
- Cost effectiveness



TAKE TO WORK

Screening for- and treating HSIL can prevent anal cancer

However,

- Most HSIL does not progress to cancer
- Some HSIL regress spontaneously

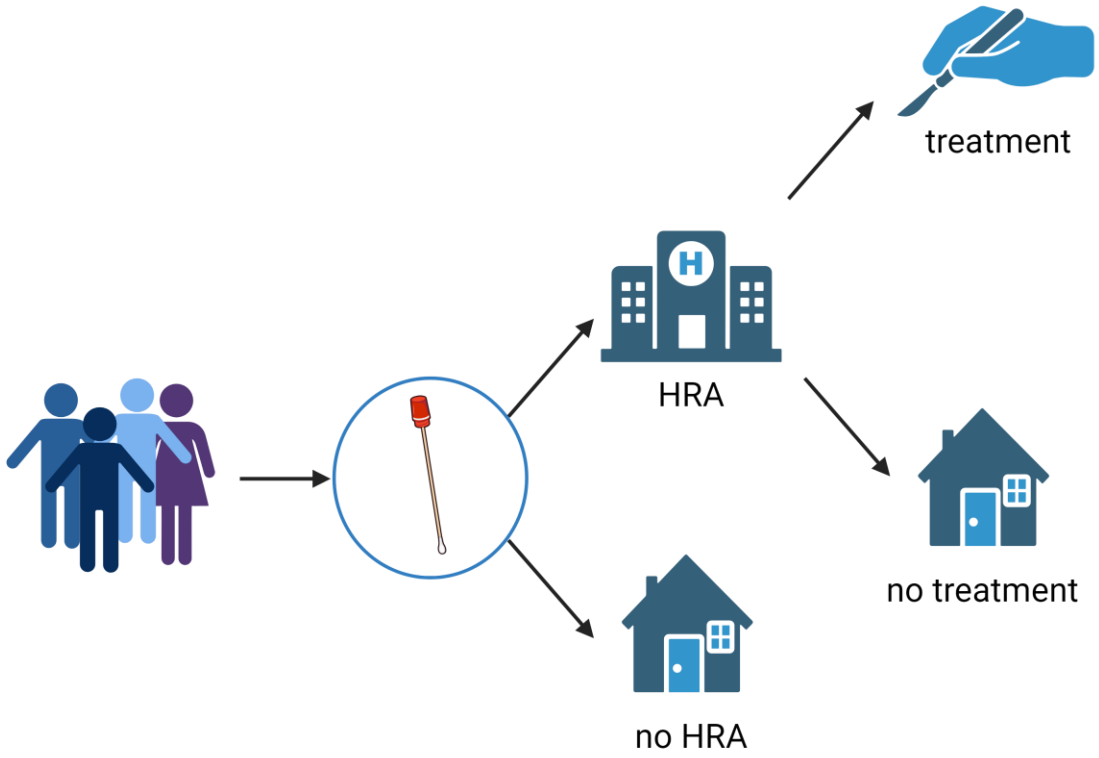
- Treating HSIL sometimes fails to prevent anal cancer

- Installing efficient large-scale screening is challenging

HOW CAN WE USE BIOMARKERS?

1. Cancer-risk stratification of HSIL for treatment indication

2. Improving screening efficiency by targeting high-risk lesions



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Karien Gosens
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eDELPHI ROUND 2 IS STILL ONGOING

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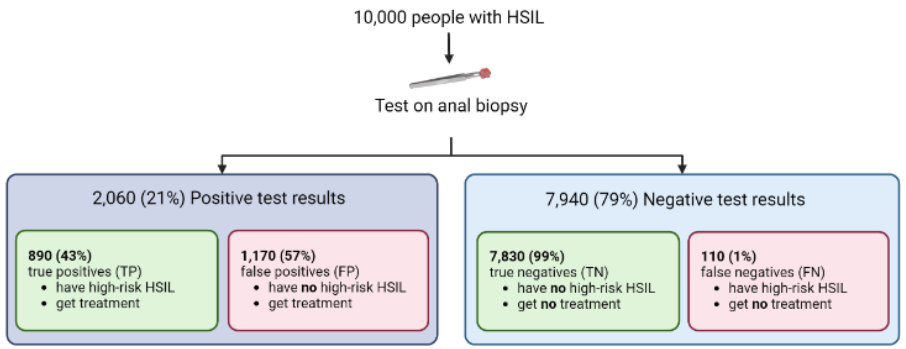

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Scan me to participate