

THE BCN HPV
COURSE

The transformation zone: fall of a myth? Paradigm shift?

ONGOING WORK

Silvia de Sanjosé, MD PhD on behalf of the HIV-HPV study group

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The Zoom meeting interface displays a slide with two histology images. The left image is a standard H&E stain of a tissue section. The right image is a fluorescence micrograph labeled "HLA DAPI", showing blue DAPI nuclear staining and green HLA staining. A white mouse cursor is positioned over the fluorescence image. On the right side of the meeting, a vertical list of participants is visible, including John Doorbar, Silvia de Sanjosé, Mark Schiffman, Tanvier.Omar, and Helen Kelly. The Zoom toolbar at the bottom shows icons for chat, mute, video, and other meeting controls.

Participants (7)

- Silvia de Sanjosé (Host, me)
- JD John Doorbar
- MS Mark Schiffman
- T Tanvier.Omar
- Chemtai Mungo
- HK Helen Kelly

Buttons: Invite, Mute All

Meeting Chat

can we delay 30 minutes our call for tomorrow?

Helen Kelly to You (Direct Message) 3:12 PM

HK Yes no problem !

You to Helen Kelly (Direct Message) 3:15 PM

Thanks

1 New Message ↓

Who can see your messages?

zoom CGR-ScreenFire

in 5 minutes

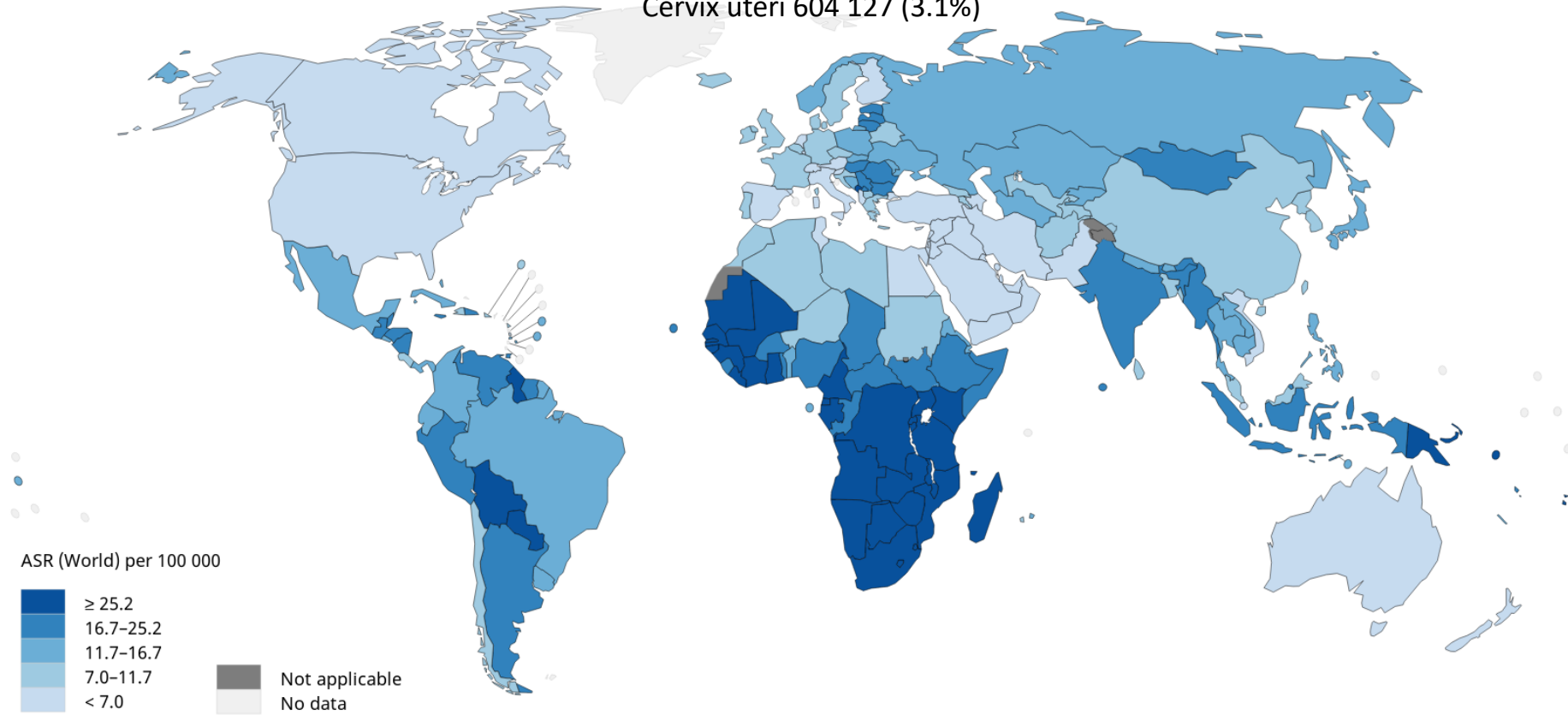
Meeting ID: 870 8449 3952

Buttons: Start, Snooze

CERVICAL CANCER REMAINS THE CANCER OF THE GLOBAL INEQUALITY

Estimated age-standardized incidence rates (World) in 2020, cervix uteri, all ages

Cervix uteri 604 127 (3.1%)

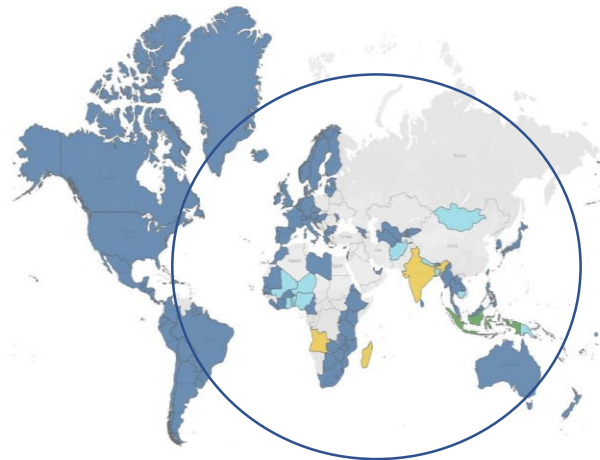


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Data source: GLOBOCAN 2020
Graph production: IARC
(<http://gco.iarc.fr/today>)
World Health Organization

WHO in 2030 to reach in 2100 Incidence 4x100,000 Vaccinate 90%, Screen 70%, Treat 90%

Vaccination



Introduction status

- National
- Subnational
- Demo complete*
- Projected - national

* Decision pending on national introduction

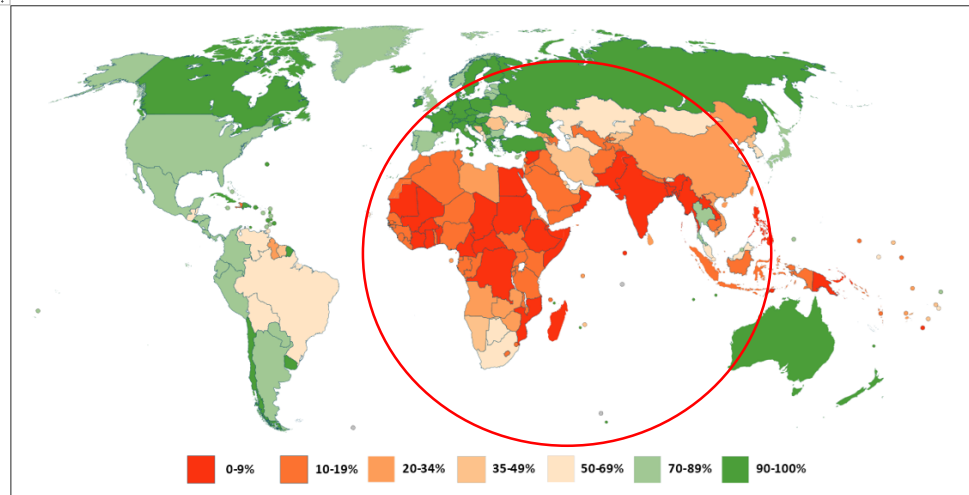
As of 17 Mar 2022

PATH
10404120

Still 245M of girls 9-14 are not receiving HPV vaccination

Screening

Figure 2. Ever in lifetime cervical cancer screening coverage in women aged 30-49 years in 2019 by country.



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

Many parts of the world have less than 10% of the population screened with limited efficacious tests

Treatment



No global compliance parameters

WHO elimination campaign

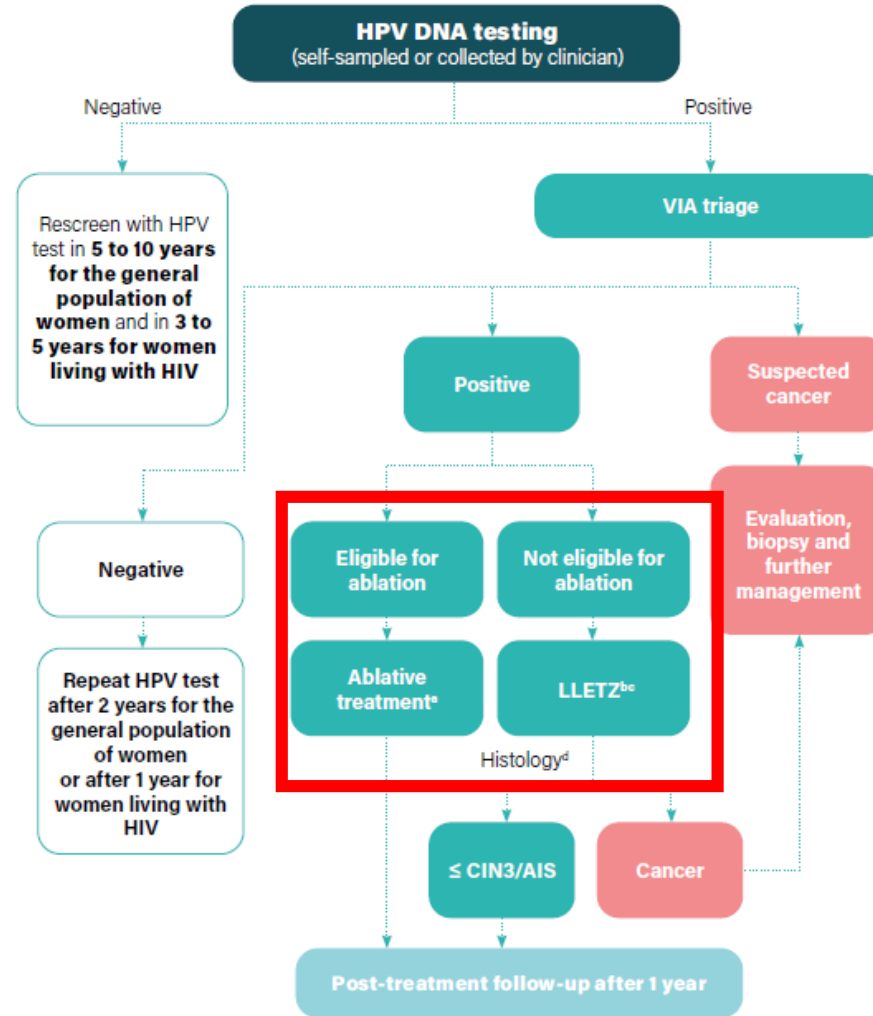
in 2030 to reach in 2100 Incidence $4 \times 100,000$

Vaccinate 90%, Screen 70%, Treat 90%

Thermal ablation may be the only feasible approach to reach high coverage

ALGORITHM 5. PRIMARY HPV DNA SCREENING AND VIA TRIAGE (SCREEN, TRIAGE AND TREAT APPROACH)

For both the general population of women and women living with HIV



Loose indications based on the transformation zone concept

^a Ablative treatment includes cryotherapy and thermal ablation.

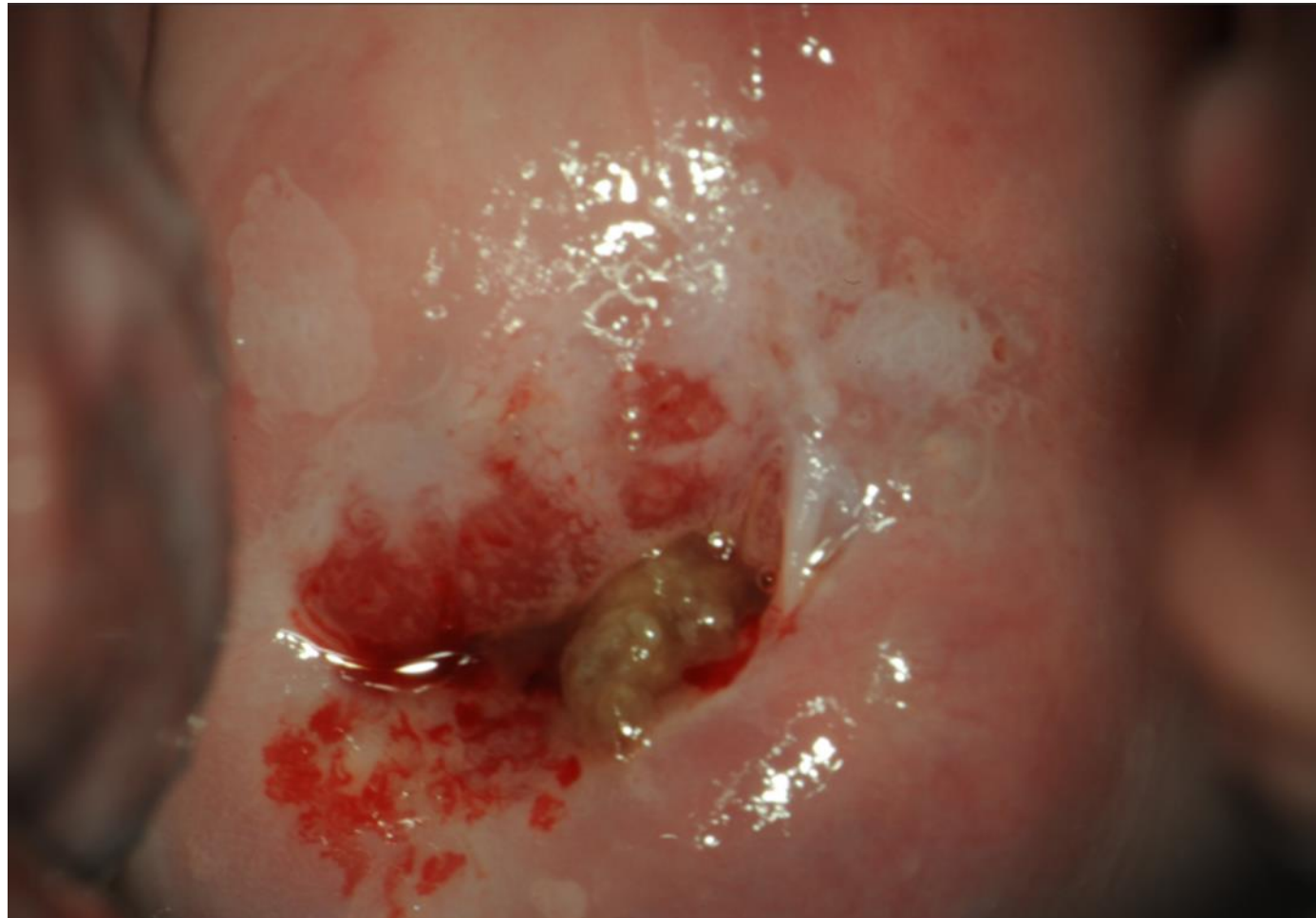
^b Cold knife conization (CKC) if LLETZ not available.

^c LLETZ and LEEP (loop electrosurgical excision procedure) indicate the same procedure.

^d Histology may not be available in certain settings; women should be advised to attend follow-up after 1 year or to report earlier, if they have any of the symptoms of cervical cancer.

AIS: adenocarcinoma in situ; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; LLETZ: large-loop excision of the transformation zone; VIA: visual inspection with acetic acid.

Management of precancerous lesions relies on how we define what we see

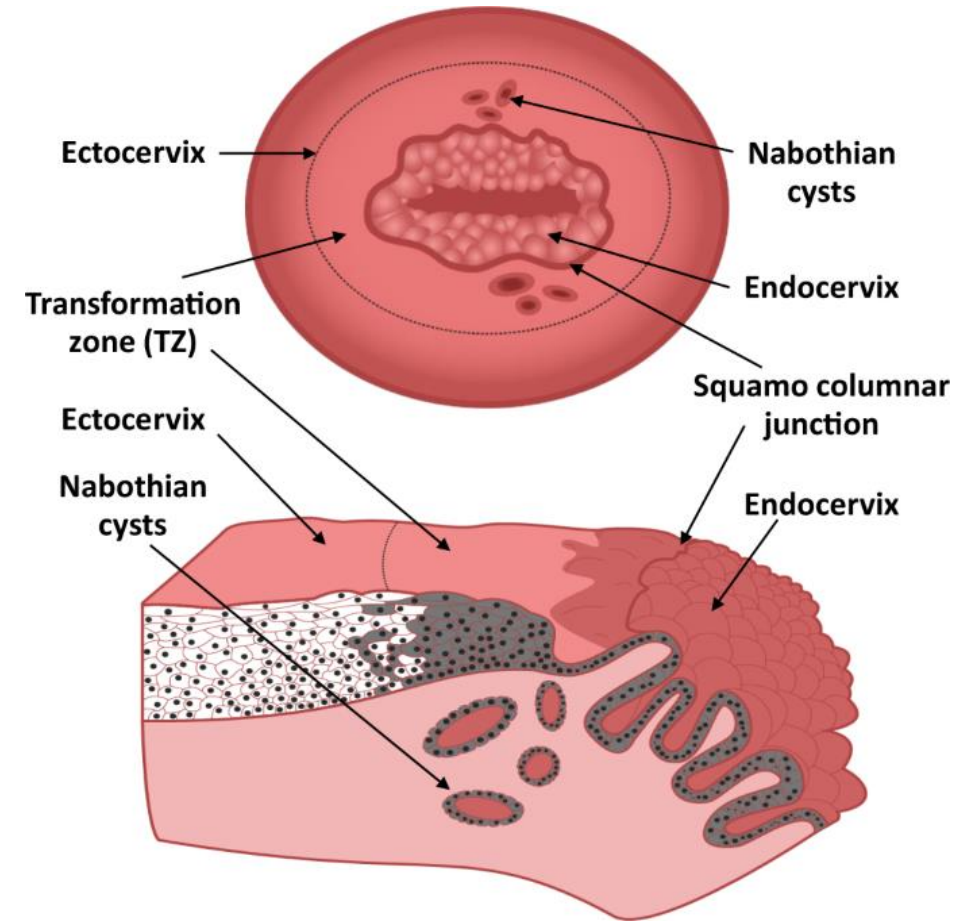


Is the cervix evaluable for treatability?

- FULLY EVALUABLE – Current SCJ (and lesions) fully visible
- EVALUABLE WITH CAVEAT – Current SCJ (and/or lesions) extends into canal and partly visible
- NOT EVALUABLE – Current SCJ is endocervical and/or completely not visible

Cervical precancerous lesions

- The invasive potential of cervical precancer involve an accumulation of somatic mutations in cells at the junction of metaplastic squamous and glandular epithelium (SCJ).
- **Cervical transformation zone (TZ), where metaplasia occurs***, is where we detect >90% of precancer
- Loose definition of what is TZ, Squamous columnar junction (SCJ) is more clearly defined

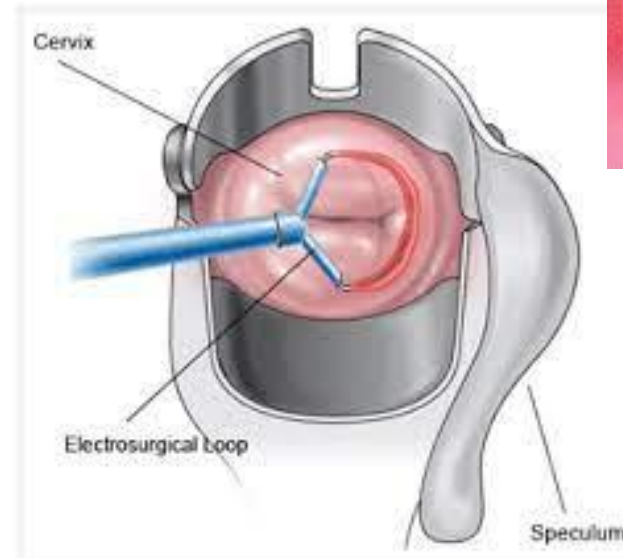
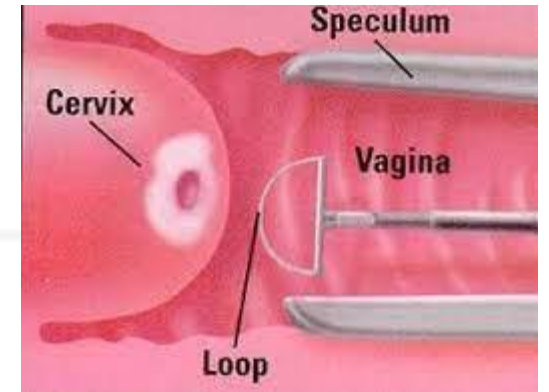
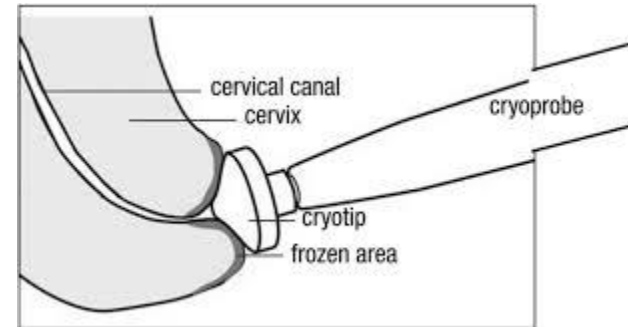


* Classical definition

Management of cervical precancer lesions. The aim is minimal tissue removal to minimize adverse events.

- Cryotherapy (cold)
- Thermal ablation (heat)

- Loop electrosurgical excision procedure (LEEP) or Large loop excision of the transformation zone (LLETZ)
- Laser conization



High recurrences after treatment in WLWH

In the general population <5% at 5 years follow up

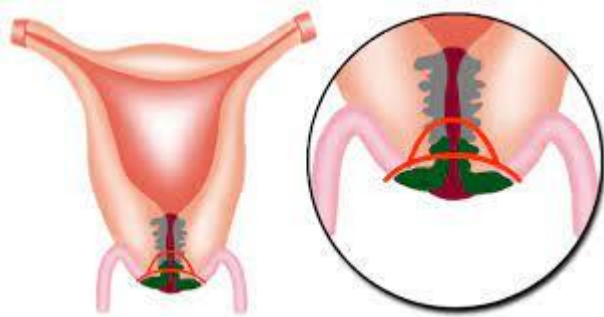
In women living with HIV

Type-specific persistence at 6 months associated with CIN2+ recurrence		
Overall	Cryotherapy	LEEP
73/196 (37%)	45/107 (42%)	28/89 (31%)

- Recurrent precancer
 - True recurrence of a clonal expansion from the original cell that generated the lesion -> initial failure of treatment, continued growth of the original clonal lesion, increased risk of secondary mutational events, and risk of invasion
 - Development of a new lesion -> implies much lower risk

TopHat conization

- WLWH, over 2-year follow-up,
- Recurrences of CIN2+ after TopHat conization
 - inadequate colposcope exam (endocervical lesions) 35 (29%)
 - ectocervical lesions 19 (24%)
- Hazard ratio 1.32; 95% confidence interval 0.75–2.31; $P = 0.338$



Lower recurrences

But, why recurrences?

The team of the 'Recurrence' project

- Epidemiology: Mark Schiffman, Kanan Desai, Helen Kelly, Silvia de Sanjosé at NCI, US
- Molecular Evaluation: John Doorbar, Ademola Aiyenuro, Heather Griffin, Konstanze Schichl, at Cambridge, UK
- Case selection/Pathology:
 - Tanvier Omar, South Africa
 - Jaume Ordi & Marta del Pino, Spain
 - Michael Chung, Kenya
 - Chemtai Mungo, Malawi

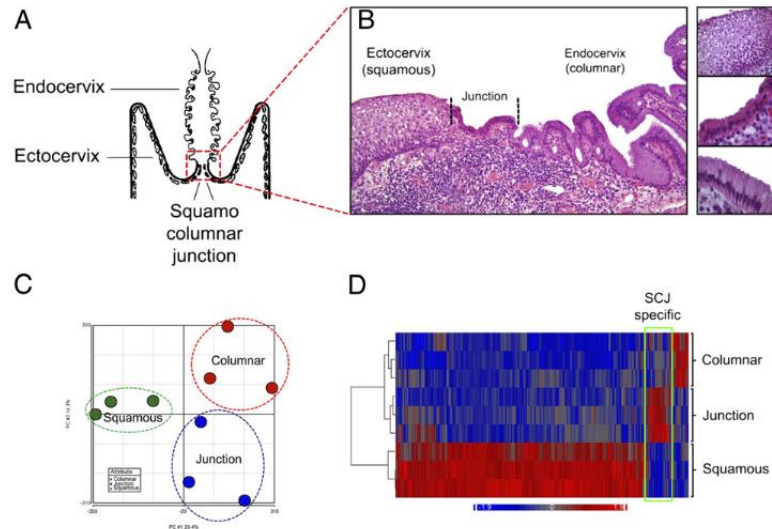
Overall objectives of the 'Recurrence' project

CLARIFYING THE MALIGNANT POTENTIAL OF NEW AND "RECURRENT" ANOGENITAL PRECANCERS IN HIV-INFECTED INDIVIDUALS TO GUIDE OPTIMAL MANAGEMENT OF HPV-HIV DUAL INFECTION

1. Describe the cellular composition of the transformation zone and confirm distribution and function of reserve cells (RC)
2. Evaluate impact of SCJ removal in the cervix after 1 year in patients with recurrences
3. Confirm patterns of recurrences in a long follow up cohort in Kenya
4. Provide implications for treatment of precancerous lesions

- What is the impact of removing TZ/SCJ?

The junction cells hypothesis



...our findings suggest that carcinogenic HPV-related CINs and cervical cancers are linked to a small, discrete cell population that localizes to the SC junction of the cervix, expresses a unique gene expression signature, and is not regenerated after excision. Herfs et al. 2012 PNAS, Herfs & Crum Nature 2015

Krt7 are identified as the key cells in the junction

The Herfs theory

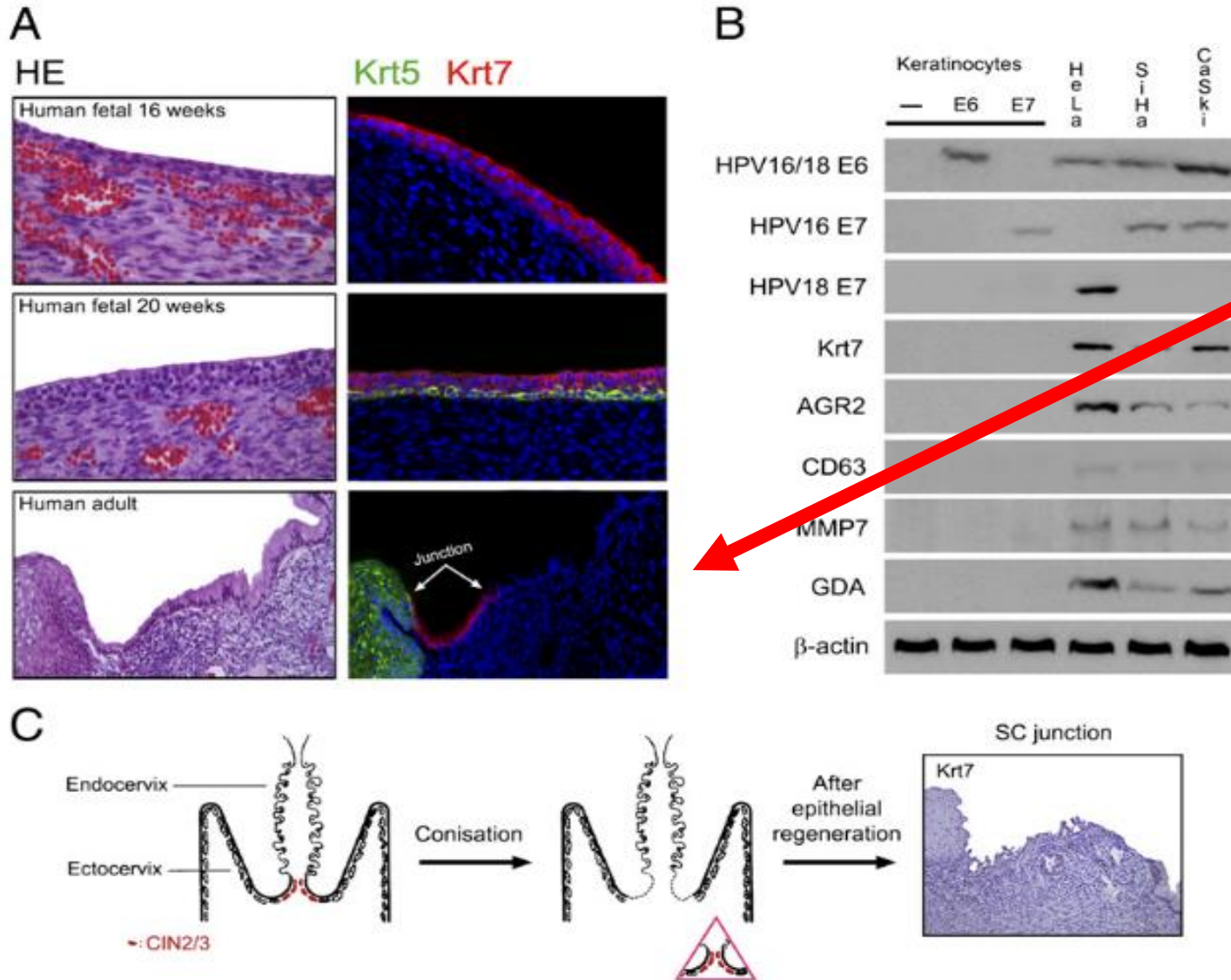
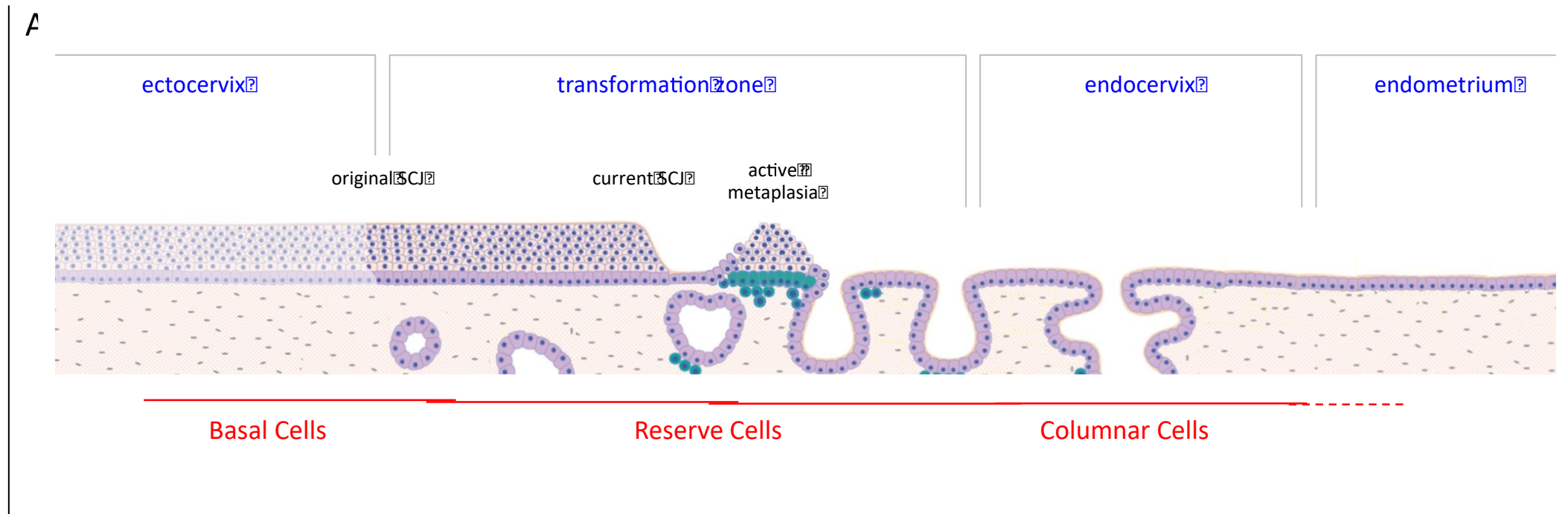


Fig. 4. Topographic specificity of the SC junction immunophenotype. (A) Fluorescence micrograph of human cervix at 16 wk (*Top*) showing diffuse Krt7 immunopositivity. At 20 wk of gestation, basal Krt5 expression emerges (*Middle*). In the adult cervix (*Bottom*), the Krt7 staining is limited to the SC junction. For each case, a corresponding histology image [hematoxylin–eosin staining (HE)] is shown. (B) Western blots of lysates of control (–), HPV16 E6- or E7-expressing primary human keratinocyte cultures, cervical adenocarcinoma (HeLa), and squamous carcinoma (SiHa, CaSki) reacted with antibodies specific for the SC junctional cells. Only cervix-derived tumor cells (HeLa, SiHa, and CaSki) score positive. (C) Schematic illustration of the squamocolumnar junction before and after LEEP (*Left* and *Center*). Absence of Krt7 staining in a “new” SC junction following LEEP (*Right*).

Current Model of HPV Disease



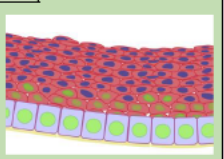
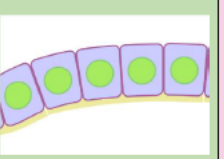
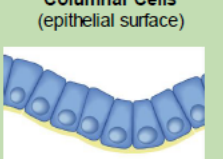
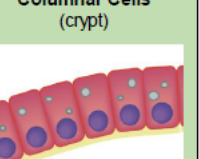

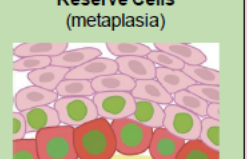
Site of Infection affects Disease Outcome

Five distinctive cell-types in the cervix architecture

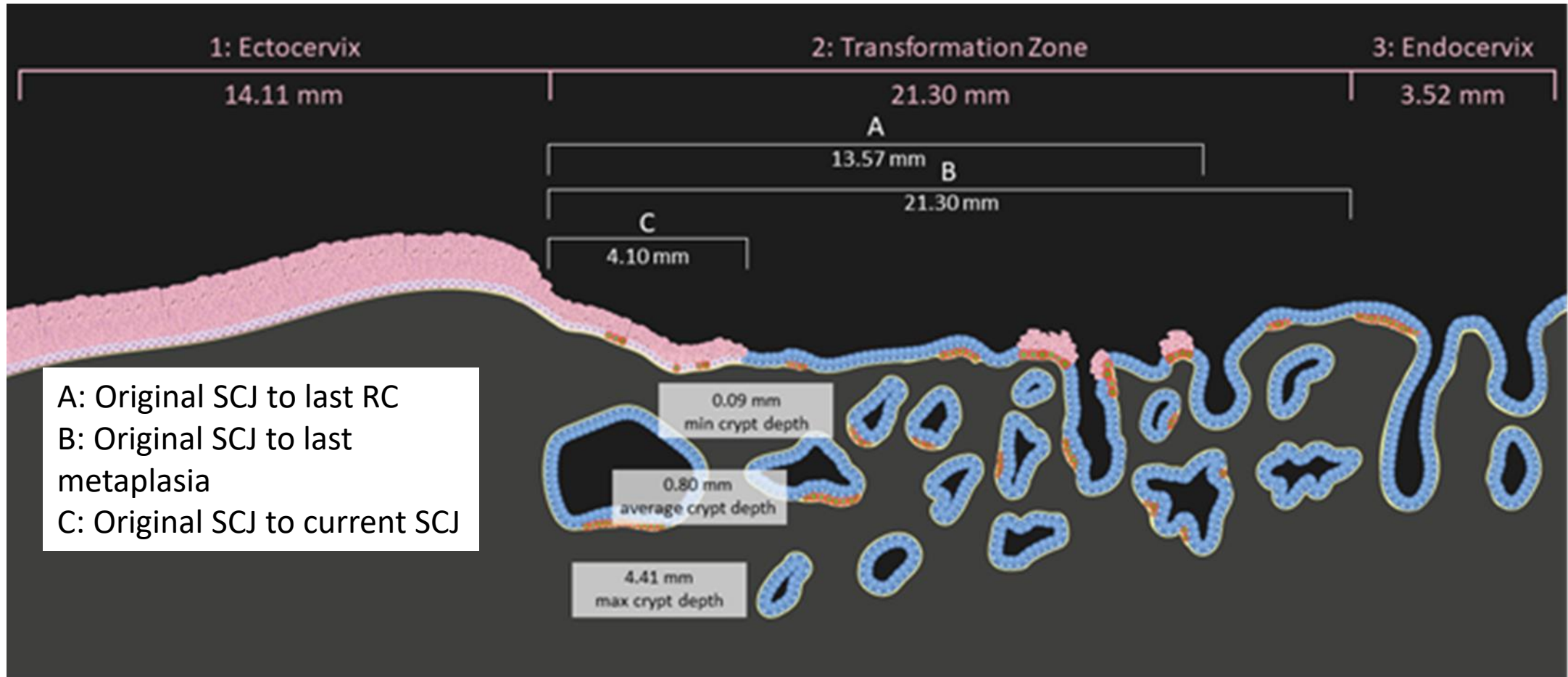
B

<p>Ectocervix Stratified Cell Layers</p> <p>Cytokeratin 13 (K13 +)</p> <p>Structural component of the cell present in stratified epithelial layers of mucosal epithelial tissue. Type I keratin (ref)</p> <p>P63 +ve</p> <p>The TA isoform of P63, which is detected by P63 antibodies, is a nuclear transcription factor required during epithelial differentiation (ref).</p>	<p>Ectocervix Basal Cell Layers</p> <p>Cytokeratin 5 (K5 +)</p> <p>Structural component of the cell present in the basal epithelial layers of all stratified epithelial tissue. Type II keratin (ref)</p> <p>P63 +ve</p> <p>The Delta isoform of P63, which is detected by P63 antibodies, is a nuclear transcription factor that controls 'stratification potential' (ref).</p>	<p>Transformation Zone Columnar Cells (Epithelial Surface and Crypt)</p> <p>Cytokeratin 17 (K17 +) P63 -ve</p> <p>Simple type II epithelial keratin found in many columnar cell types. Structural component of the cell (ref)</p> <p>Transcription factor that is consistently absent from cells that lack stratification potential (ref)</p> <p>Cytokeratin 8 (K8 +)</p> <p>Simple type II epithelial keratin found in all columnar cell types. Structural component of the cell (ref)</p>		<p>Reserve Cells Small Clusters</p> <p>Cytokeratin 17 (K17 +)</p> <p>Structural component of the cell. K17 is a type I keratin that is sometimes described as a stress keratin (ref)</p> <p>P63 +ve</p> <p>The Delta isoform of P63, which is detected by P63 antibodies, is a nuclear transcription factor that controls 'stratification potential' (ref). The P63/K17 combination is a reserve cell characteristic.</p>	<p>Reserve Cells Metaplasia</p> <p>Cytokeratin 5 (K5 +)</p> <p>Structural component of the cell present in the basal epithelial layers of all stratified epithelial tissue including the stratifying cells of the cervical transformation zone. Type II keratin (ref)</p> <p>P63 +ve</p> <p>The Delta and TA isoforms of P63 are detected by P63 antibodies, and are present in metaplastic epithelial tissue undergoing stratification (ref).</p>
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C

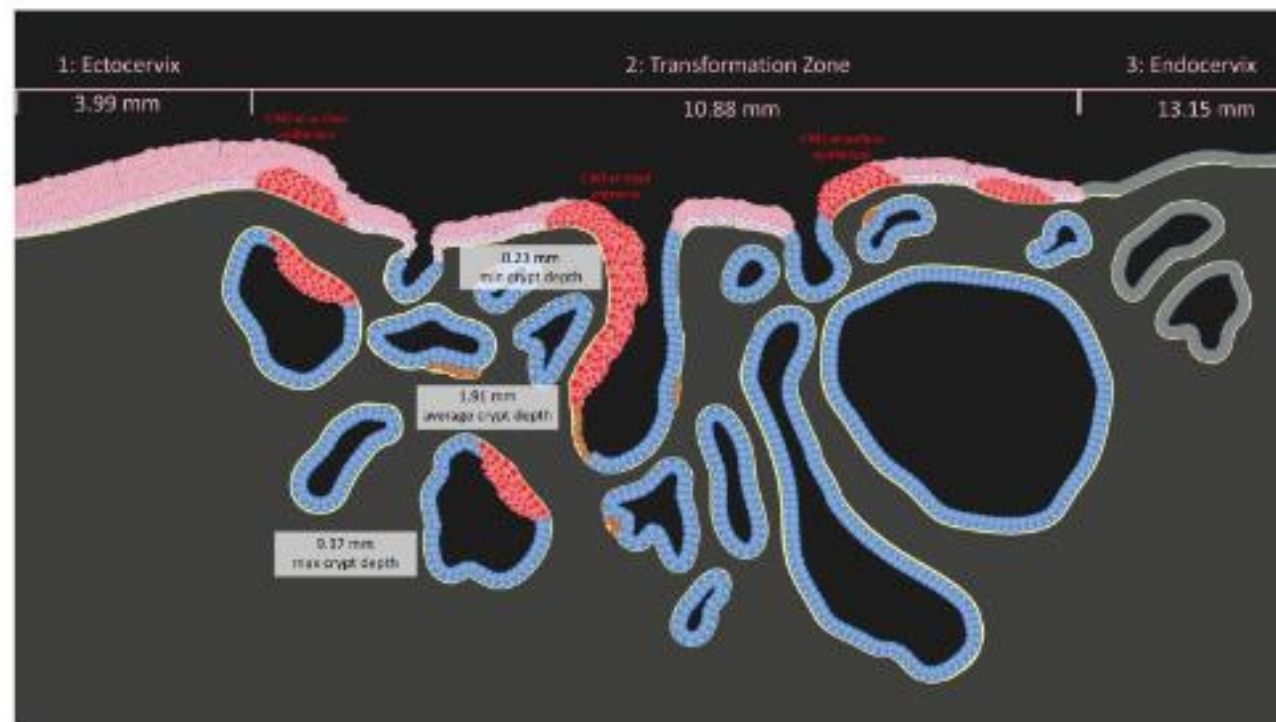
<p>Stratified Cells</p>  <p>Morphology varies according to distance from the epithelial basal layer, but becoming flatter and more elongated towards the epithelial surface (ref)</p>	<p>Basal Cells</p>  <p>Closely packed polarised rectangular cells with high nuclear to cytoplasm ratio (ref)</p>	<p>Columnar Cells (epithelial surface)</p>  <p>Closely packed polarised columnar cells with nucleus close to the basolateral surface (ref)</p>	<p>Columnar Cells (crypt)</p>  <p>Closely packed polarised columnar cells with mucin secretory vesicles and basolateral nuclei (ref)</p>	<p>Reserve Cells (small clusters)</p>  <p>Irregularly shaped spherical or elongated cells occurring individually or in small between the columnar cell layer and the basal lamina (ref)</p>	<p>Reserve Cells (metaplasia)</p>  <p>Irregularly shaped cuboidal-like cells which are more closely packed with the emergence of stratified progeny in the cell layers above (ref)</p>
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What are we calling the transformation zone ?



Measurements based on 15 hysterectomies and 15 conizations

Table 2: **Disease types and location of crypts within the TZ.** Analysis of 15 cases treated for HPV Neoplasia to determine the average lengths of ectocervix, TZ including disease types, and endocervix, as well as the approximate location of original SCJ and last neoplasia. The average depth of crypts was also determined.



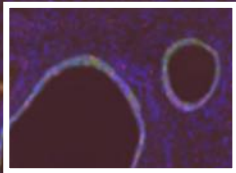
Preliminary findings

- Crypts are identified far distant from SCJ, including RC
- In conizations, crypts with RC are deeper than in the hysterectomy samples

1000 μ m

HPV infection is manifest as thin HSIL in deeper crypts

P16/MCM/DAPI



1ST LLETZ

Treated for CIN3

B12-39660-3

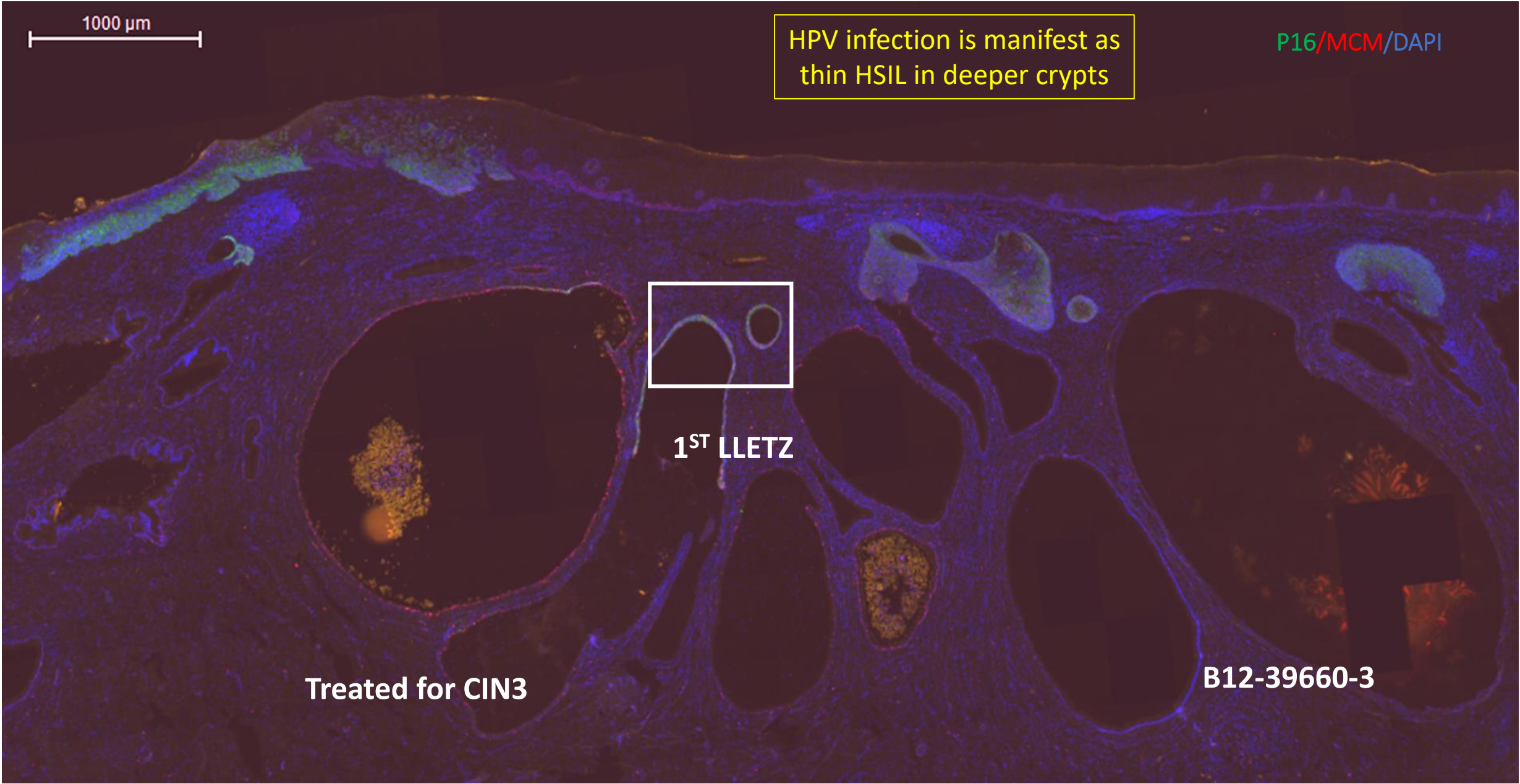
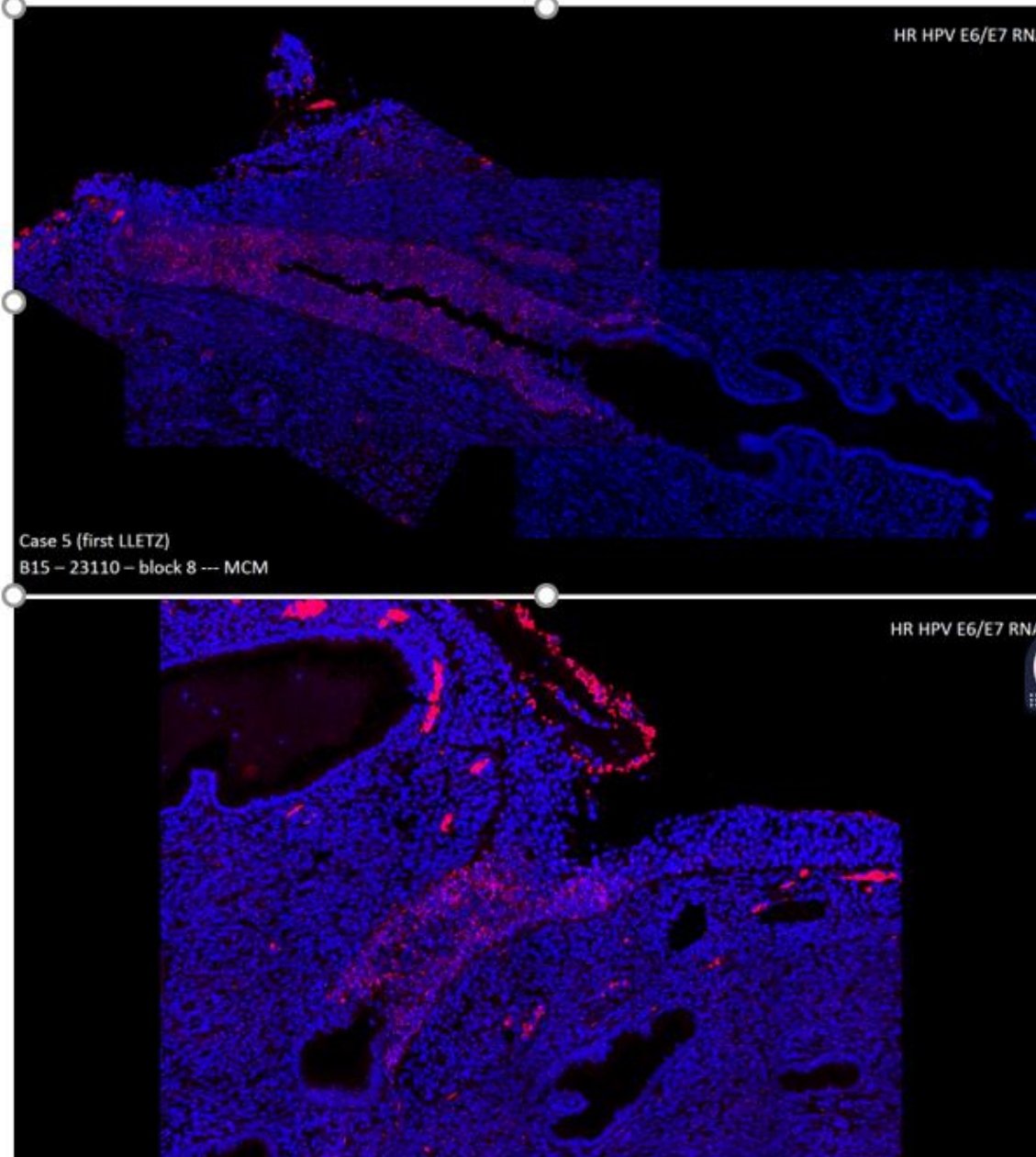


Figure 3. HR-HPV* E6/E7 positive staining (red) at crypt entrance



hrHPV is detected at the entry of the crypts

1000 μ m

P63/K17/DAPI

HSIL lesions are observed under columnar epithelium in the crypts

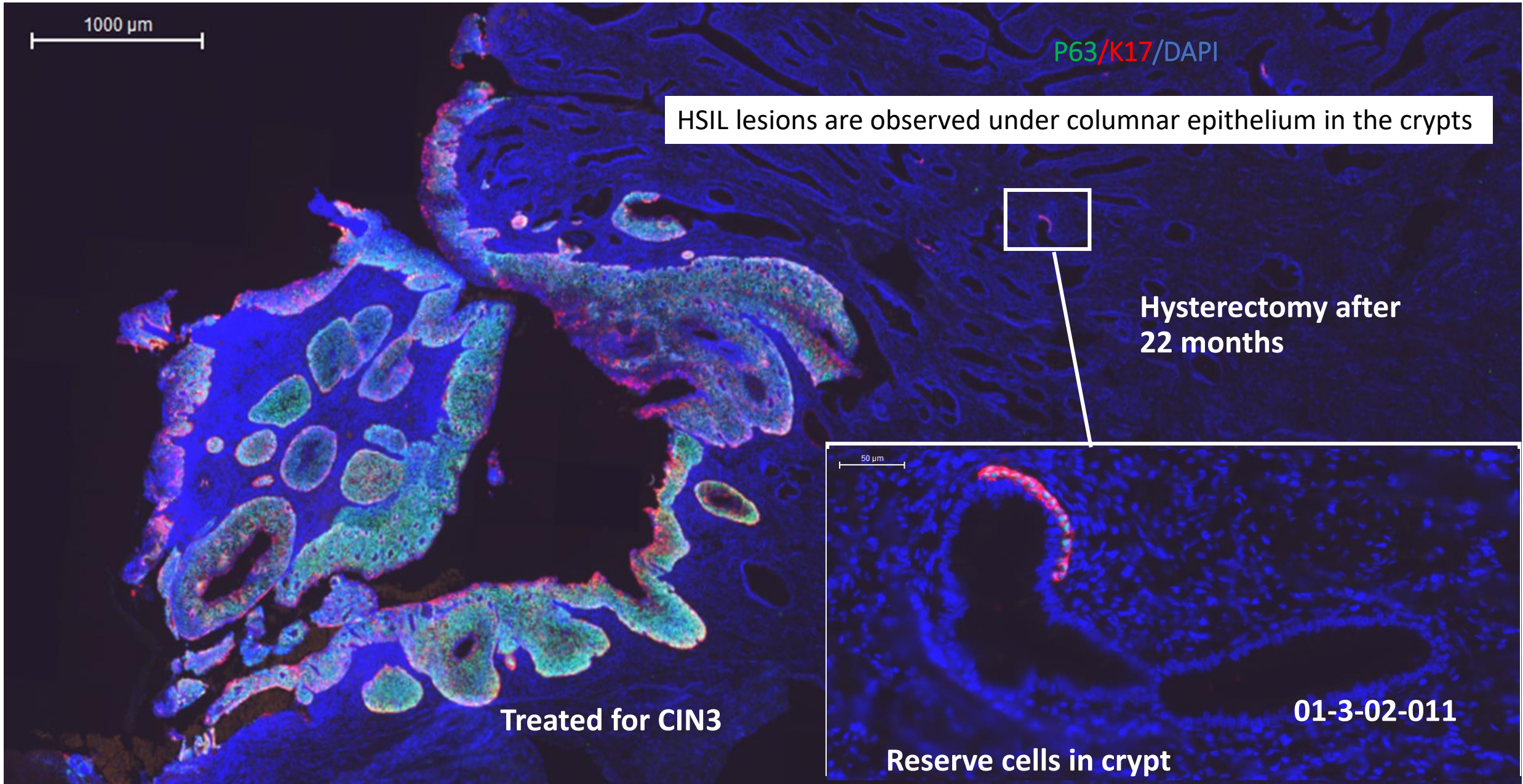
Hysterectomy after
22 months

Treated for CIN3

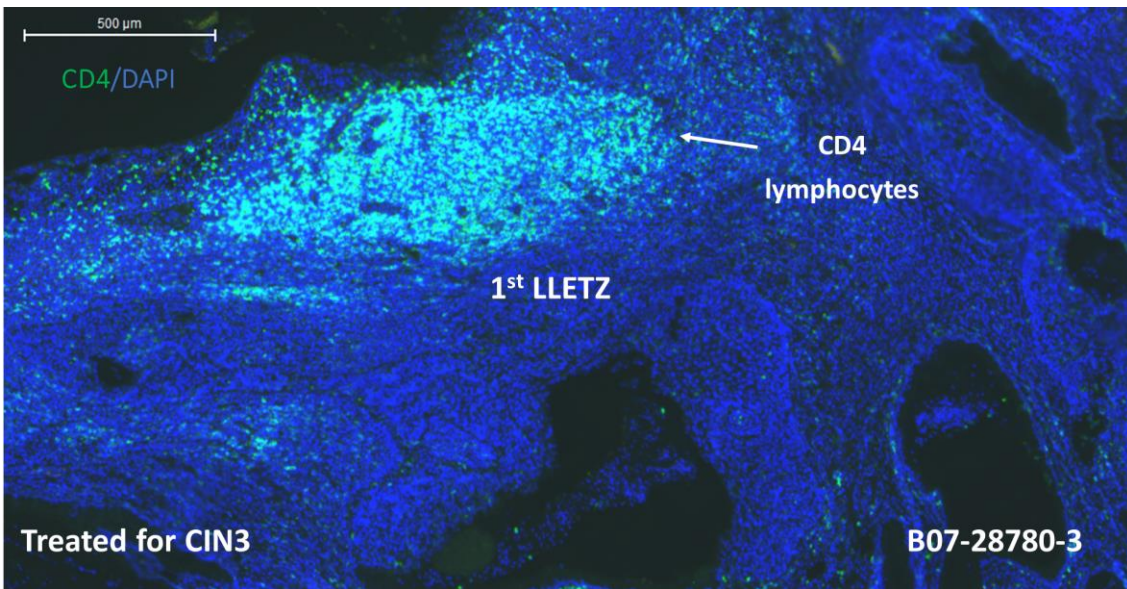
50 μ m

01-3-02-011

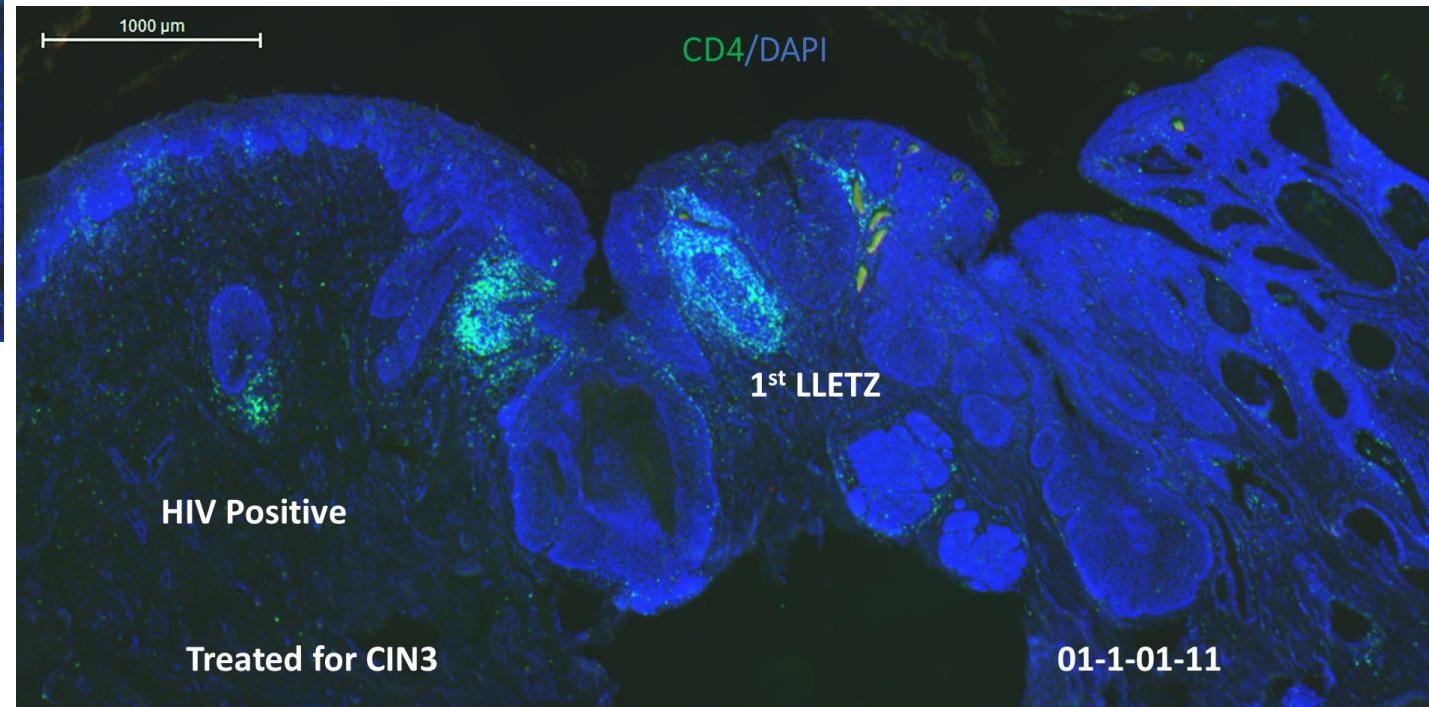
Reserve cells in crypt



Immune response show different patterns by HIV status



CD4 cells are more compact in non HIV
A more diffuse, and less dense accumulation of CD4 is more often identified in samples from WLWH



Discussion

- Our data suggest that RC are located under the columnar epithelium and far from SCJ
- The RC are identified in distant crypts, can be infected by HPV and can be identified as HSIL
- The impact of RC is not visible in regular colposcopy exams, but could be suspected if HPV persists after first treatment.
- To date, our data is not consistent with the existence of junctional cells.

Preliminary implications

- In settings where thermal ablation is being prompted to manage precancer, high level of recurrences, particularly in WLWH, may require a profound revision of the recommendations
- It remains unclear based on our data whether regular LEEP is sufficient in reducing recurrences in WLWH
- New treatment approaches are needed to reach deeper in the canal without affecting the reproductive outcomes.

Thank you for your attention

