Structure and Function of the Human Breast L2

Robert D. Cardiff, M.D., Ph.D
Welcome Trust
Δ TRUST
Dr. V. Seewaldt
December 8, 2021

Due Diligence

1. **Size of TDLU**
   a) **RDC and ADB**
   b) **Jindal et al**
   c) **Kinsler et al**
2. **How many Epithelial Cell types?**

TDLU:
SIZE AND NUMBER

Kensler KH, Liu EZF, Wetstein SC, Onken AM, Luffman CI, Baker GM, Collins LC, Schnitt SJ, Bret-Mounet VC, Veta M, Pluim JPW, Liu Y, Colditz GA, Eliassen AH, Hankinson SE, Tamimi RM, Heng YJ. **Automated Quantitative Measures of Terminal Duct Lobular Unit Involution and Breast Cancer Risk**. **Cancer Epidemiol Biomarkers Prev**. 2020 Nov;29(11):2358-2368. doi: 10.1158/1055-9965.EPI-20-0723. Epub 2020 Sep 11. PMID: 32917665; PMCID: PMC7642012.

Table 2. Quantitative TDLU measures and breast cancer risk factors among 1,083 controls.

	n	Median TDLU span (μm)	TDLU counts/mm^2	Median acini counts/TDLU	Median TDLU area (mm^2)
Age at BBD biopsy					
<40 years	244	0.56 (0.55–0.58)	0.48 (0.44–0.52)	7.56 (7.11–8.04)	0.11 (0.10–0.11)
40–49 years	431	0.52 (0.51–0.53)	0.49 (0.46–0.51)	7.52 (7.18–7.87)	0.09 (0.09–0.10)
50–59 years	272	0.47 (0.45–0.48)	0.43 (0.40–0.46)	5.32 (5.02–5.64)	0.07 (0.07–0.07)
≥60 years	136	0.46 (0.44–0.47)	0.38 (0.34–0.42)	4.33 (3.99–4.71)	0.07 (0.06–0.07)
P		<**0.001**	<**0.001**	<**0.001**	<**0.001**
BBD histologic subtype					
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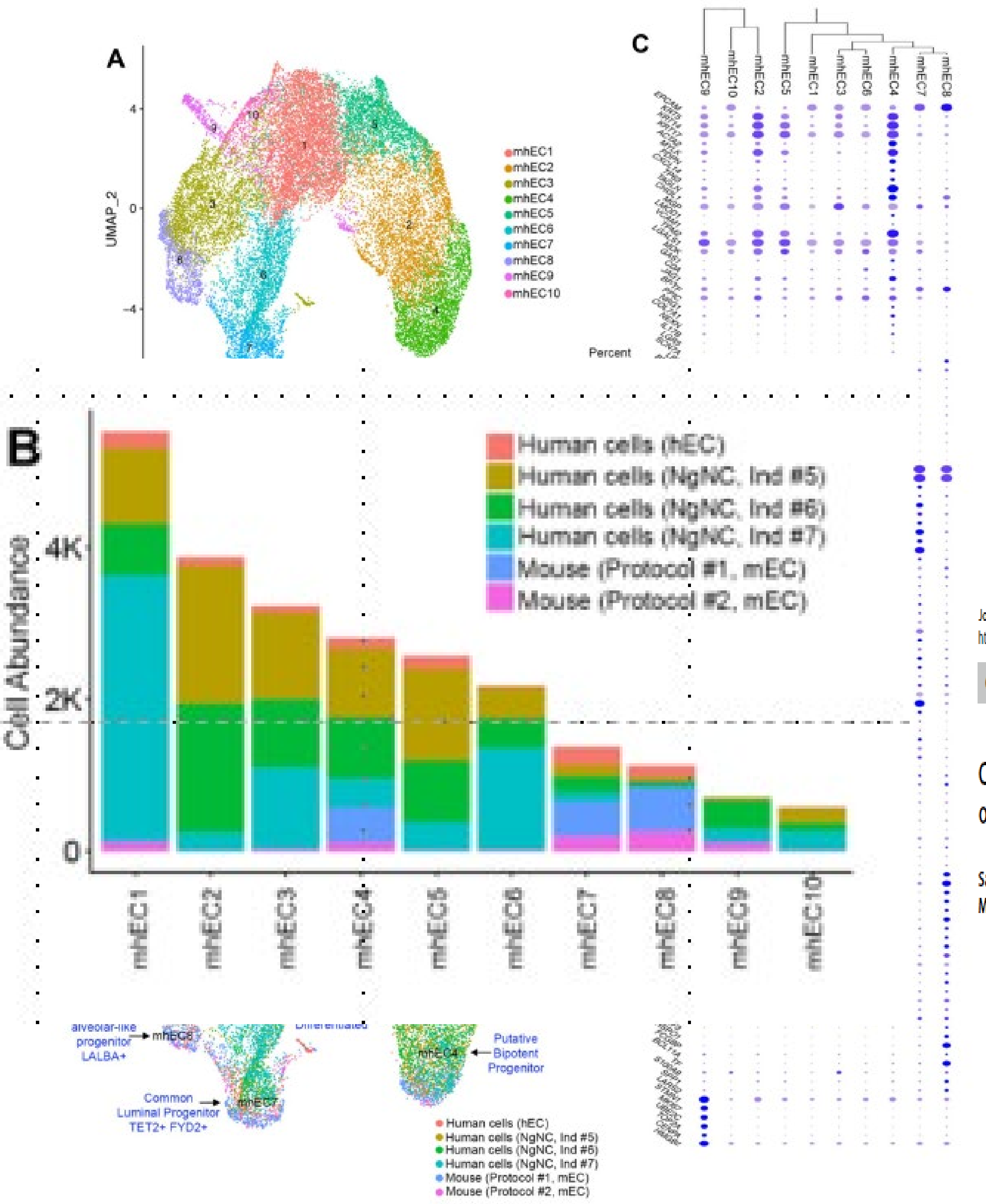


MOUSE-HUMAN MAMMARY EPITHELIUM sc-RNA-Seq

Journal of Mammary Gland Biology and Neoplasia (2021) 26:43–66
https://doi.org/10.1007/s10911-021-09486-3

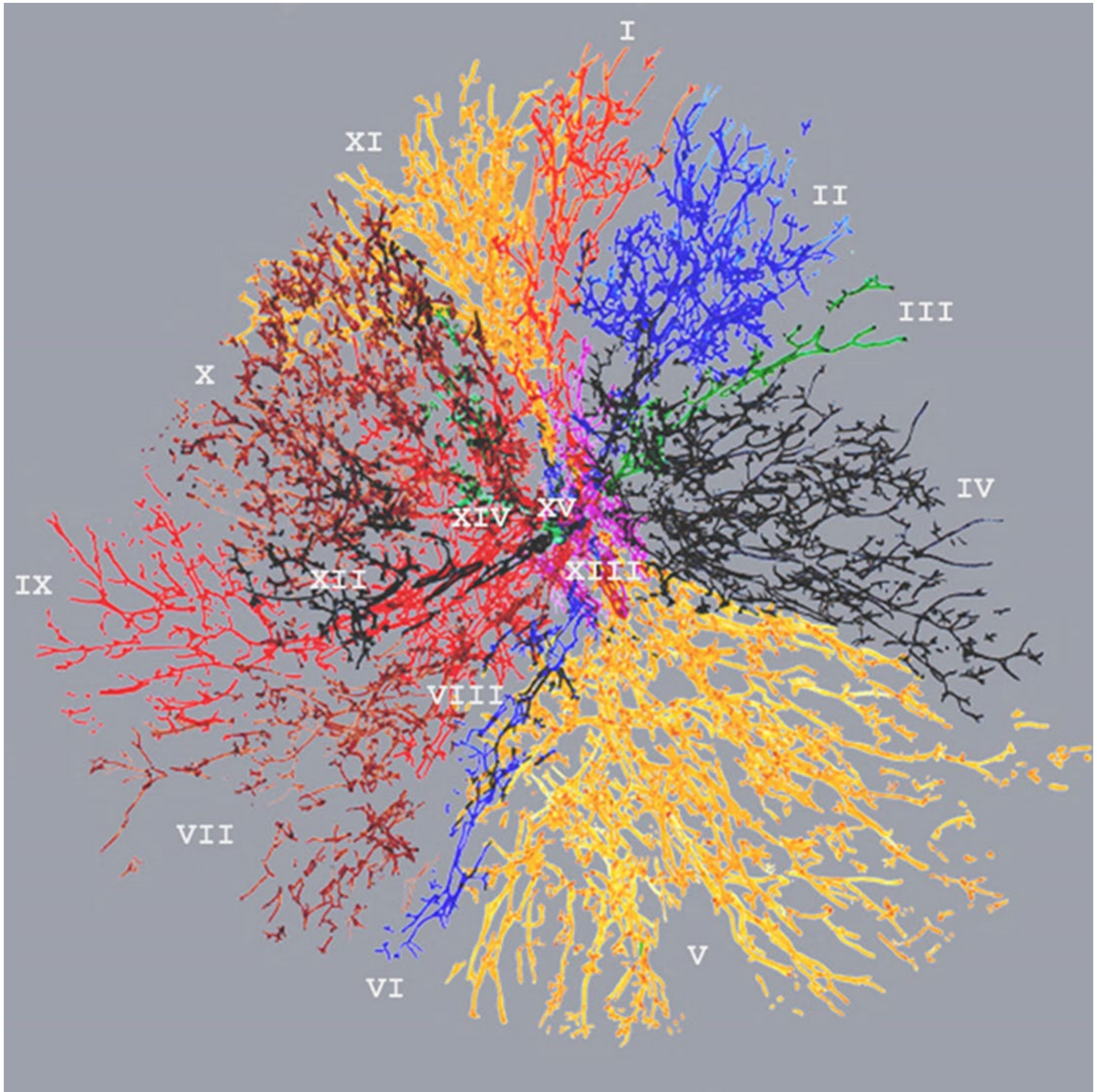
ORIGINAL PAPER

Check for updates

Characterization of Gene Expression Signatures for the Identification of Cellular Heterogeneity in the Developing Mammary Gland

Samantha Henry[1,2] · Marygrace C. Trousdell[1] · Samantha L. Cyrill[1] · Yixin Zhao[1] · Mary. J. Feigman[1] · Julia M. Bouhuis[3] · Dominik A. Aylard[4] · Adam Siepel[1] · Camila O. dos Santos[1]

COMPUTER RECONSTRUCTION
by Going



Subgross and Functional Histology

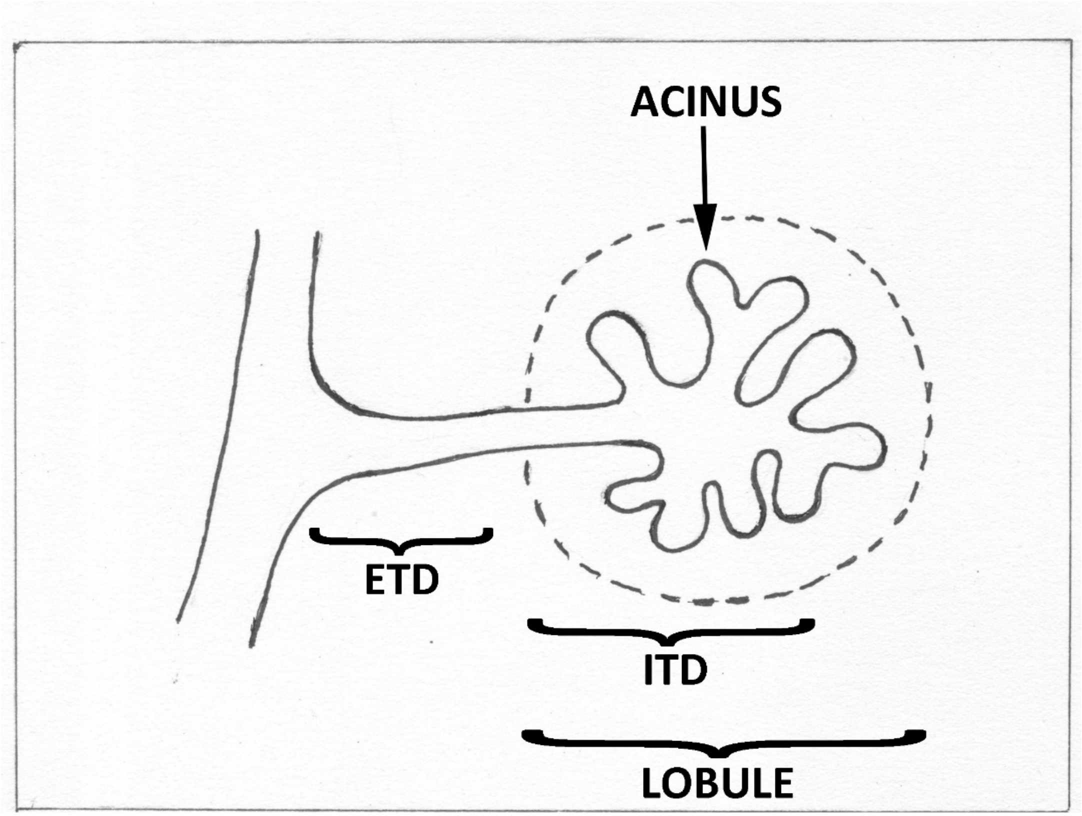
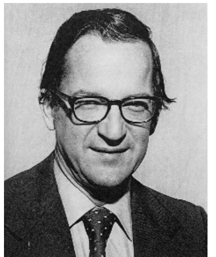
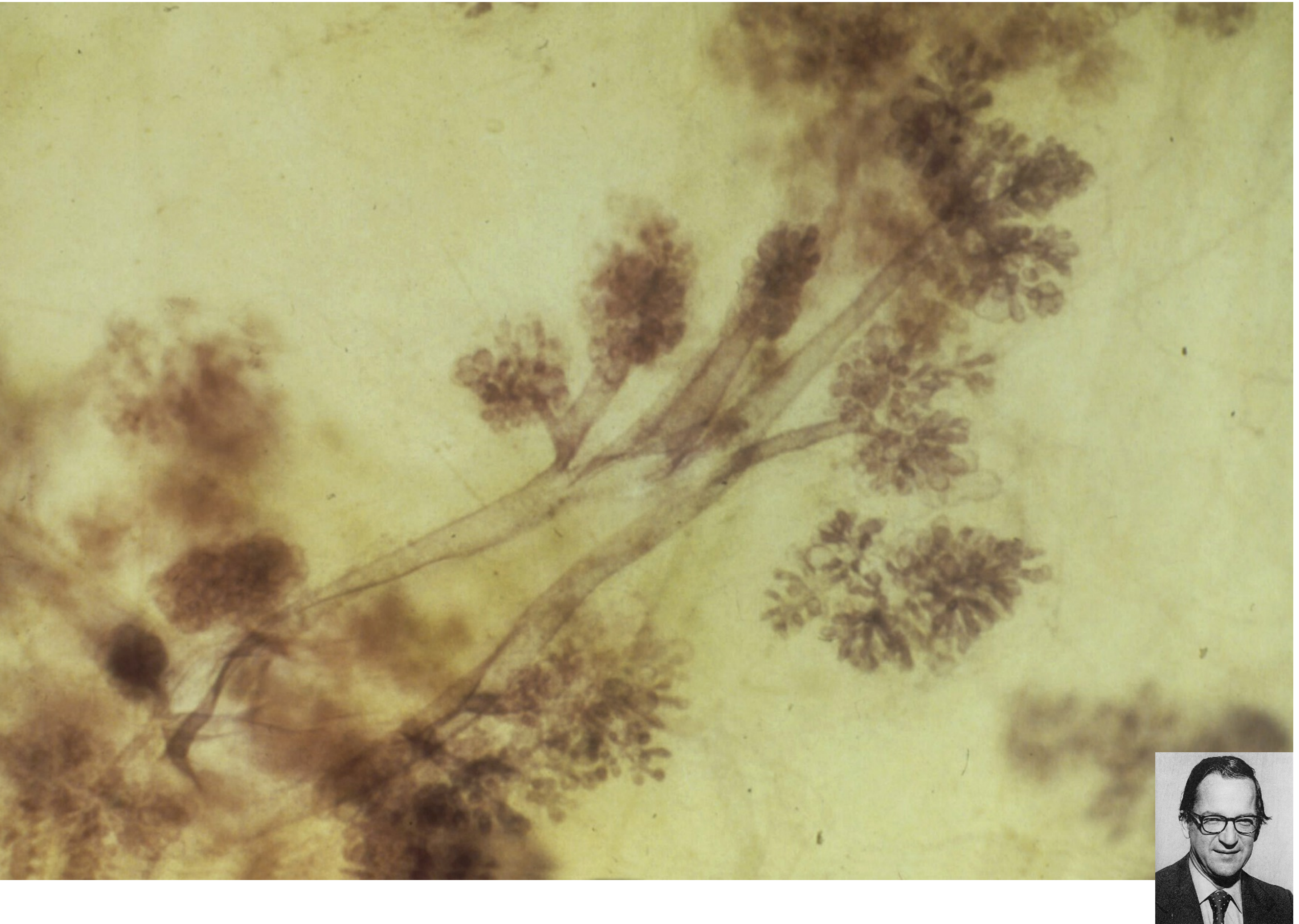


FIGURE 8

Diagram of Terminal Ductal Lobular Unit (TDLU). ETD: Extralobular Terminal Duct. ITD: Intralobular Terminal Duct, which is the axial core of the lobule. A: Acinus (or alveolus or ductule).





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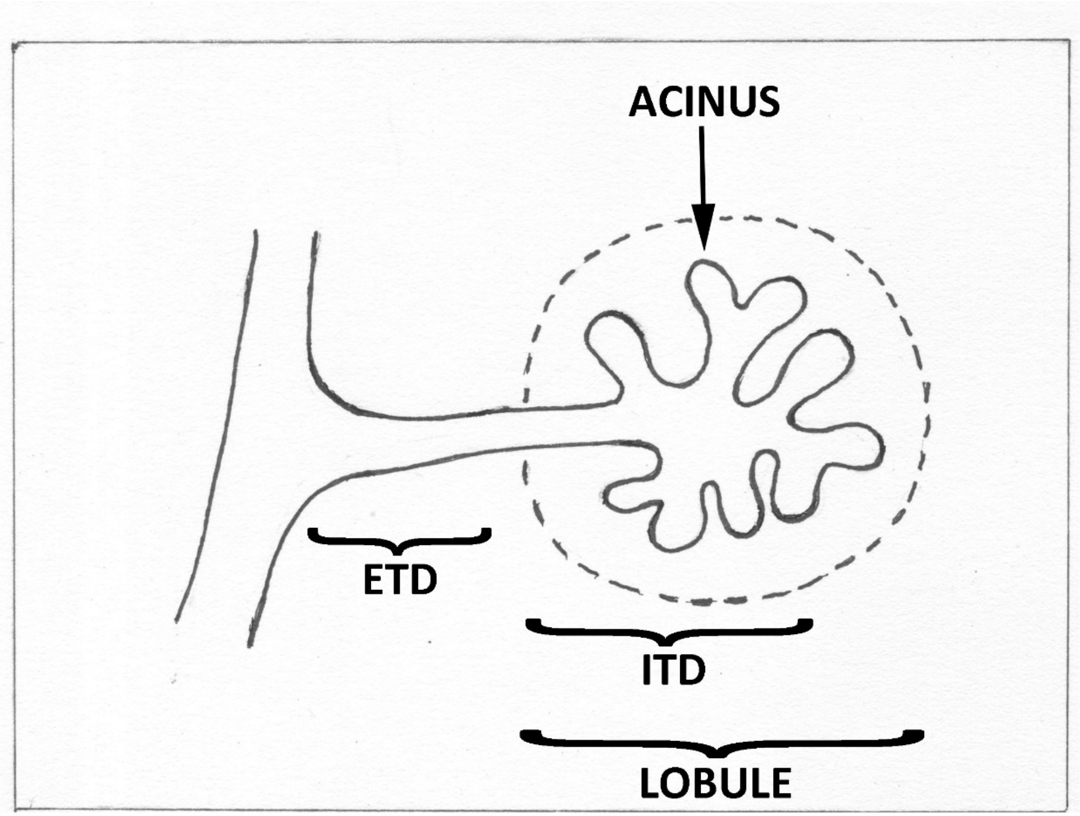

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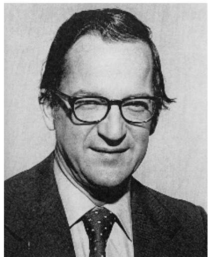
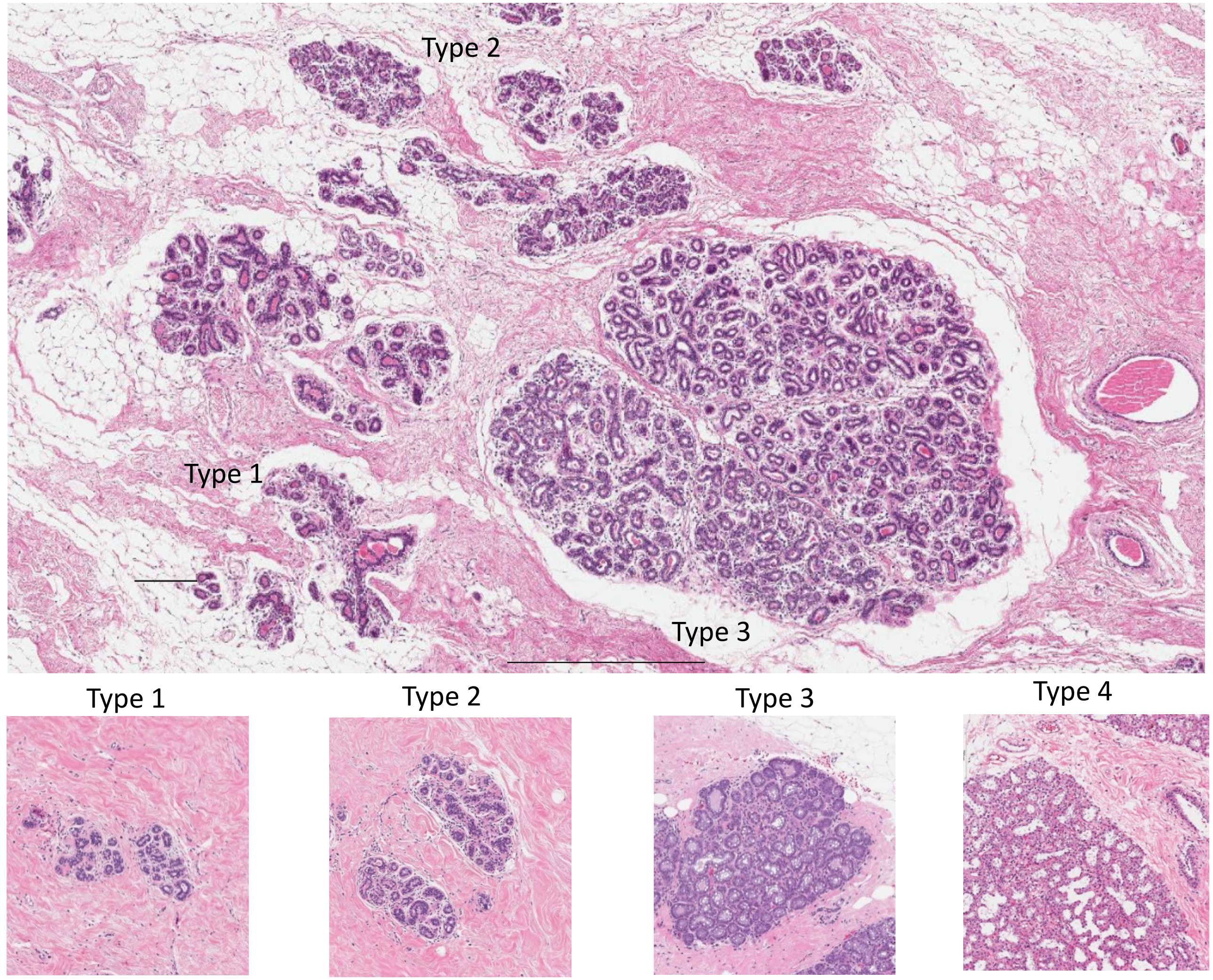
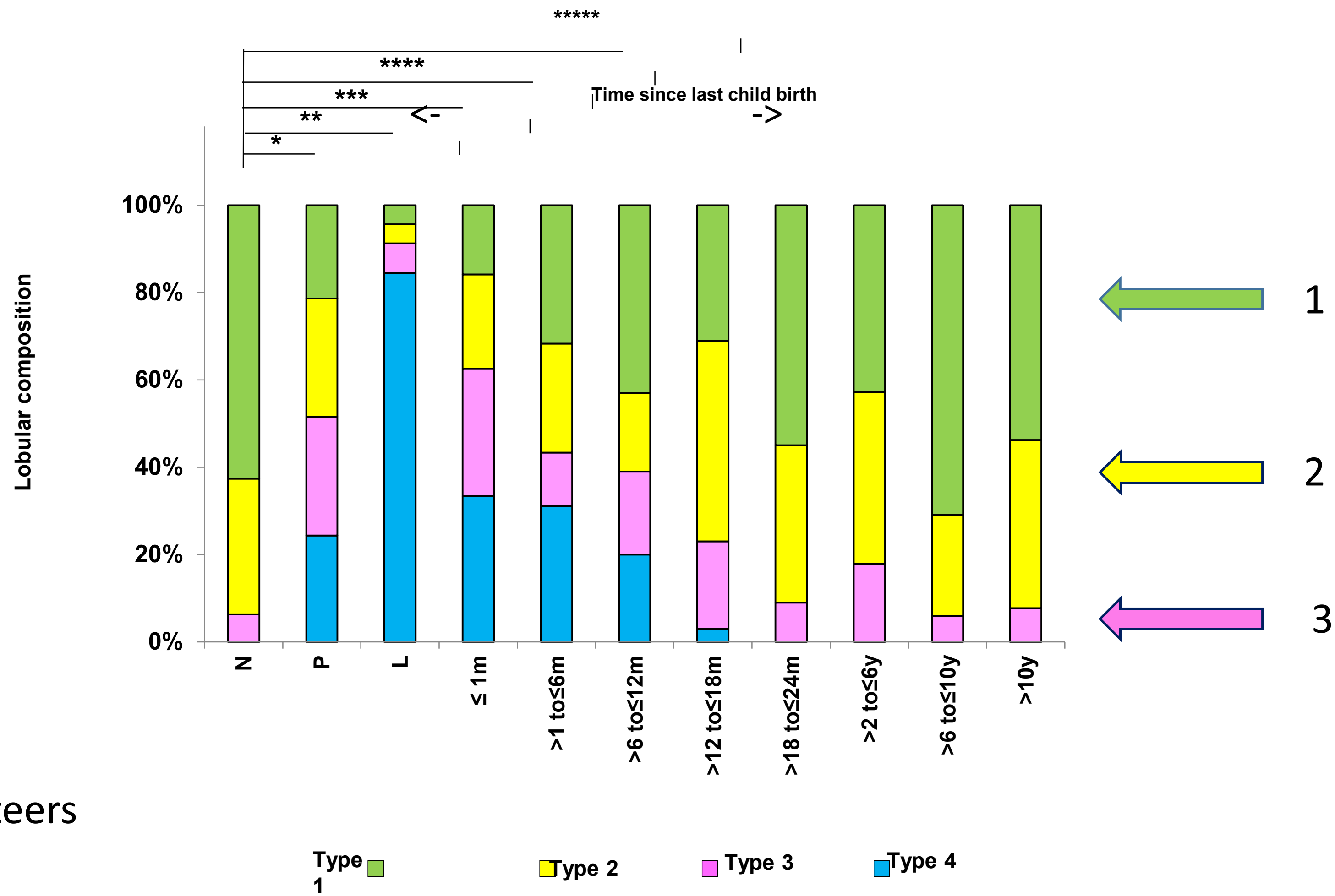




THE FOUR “TYPES” OF TDLU

JINDAL and SCHEDIN



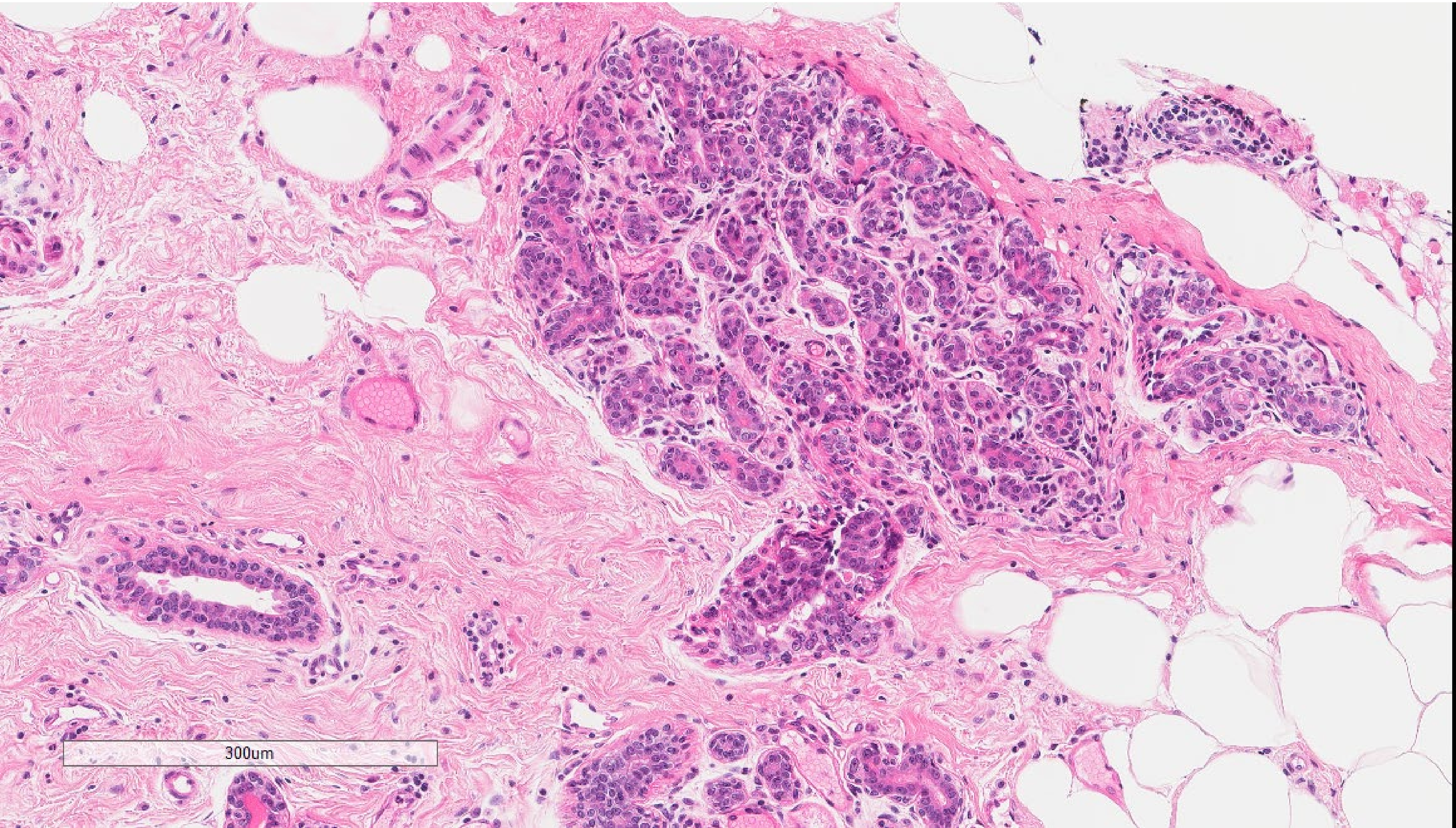
N=112 volunteers

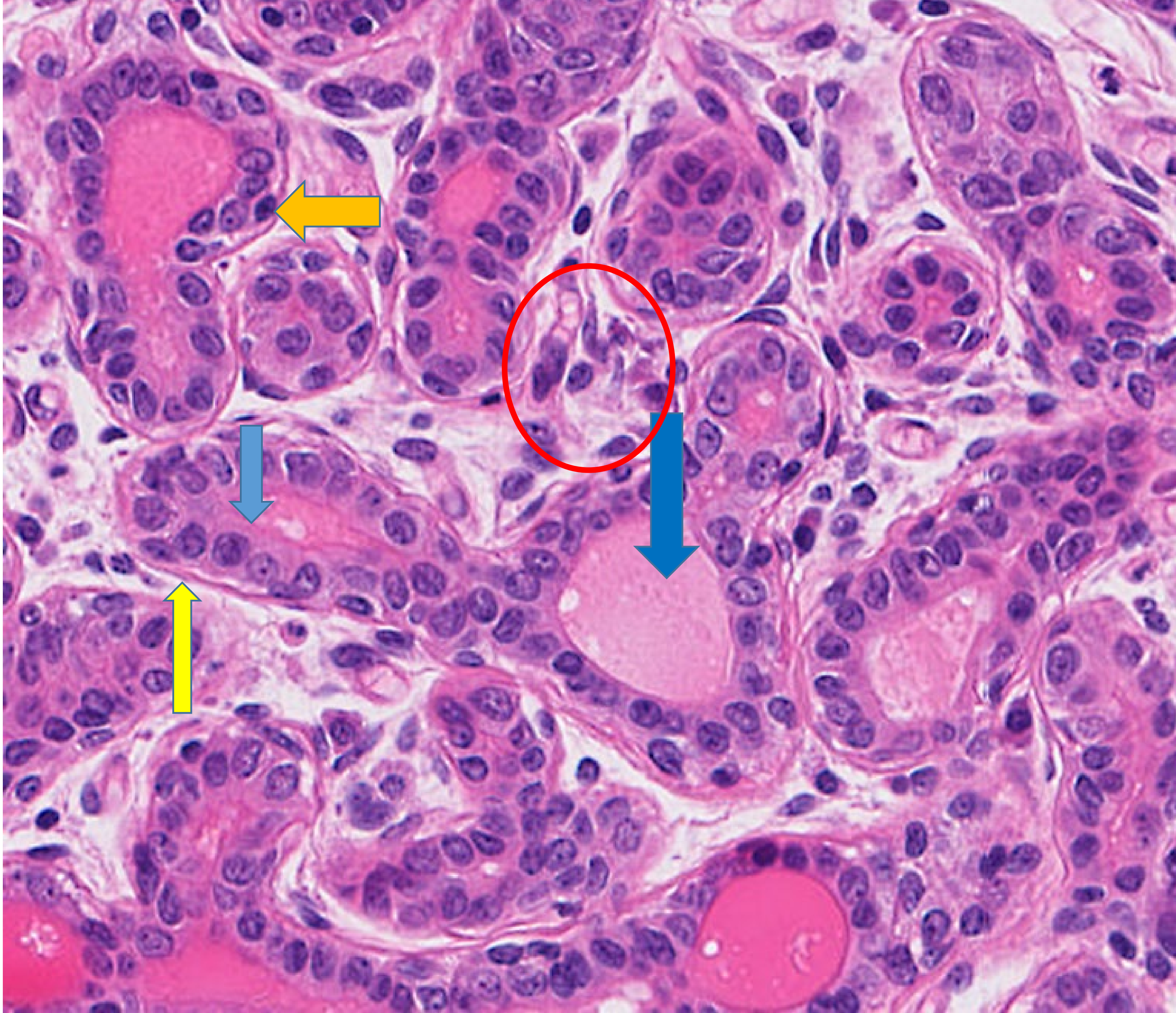
> NPJ Breast Cancer. 2020 Oct 16;6:55. doi: 10.1038/s41523-020-00196-3. eCollection 2020.

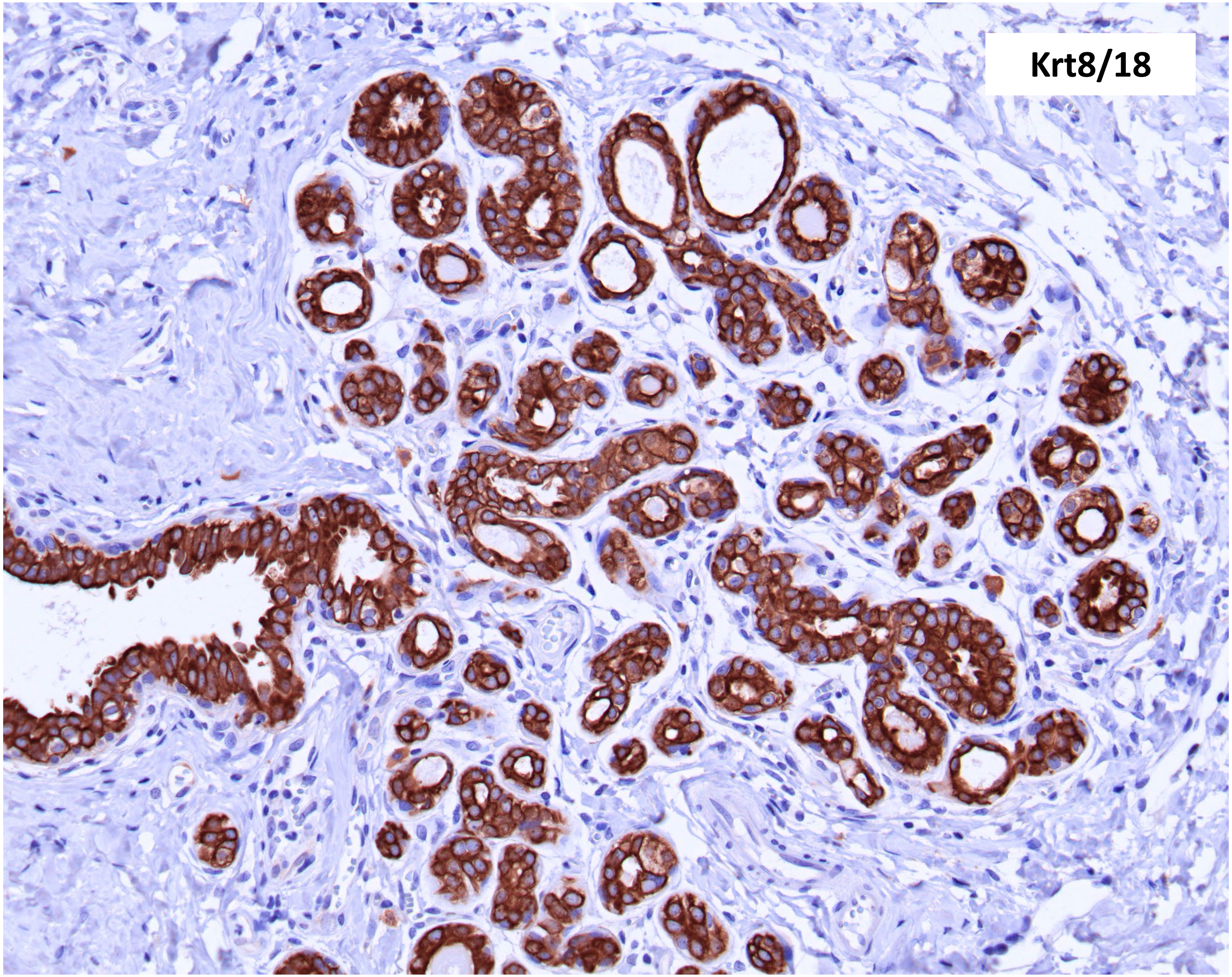
Characterization of weaning-induced breast involution in women: implications for young women's breast cancer

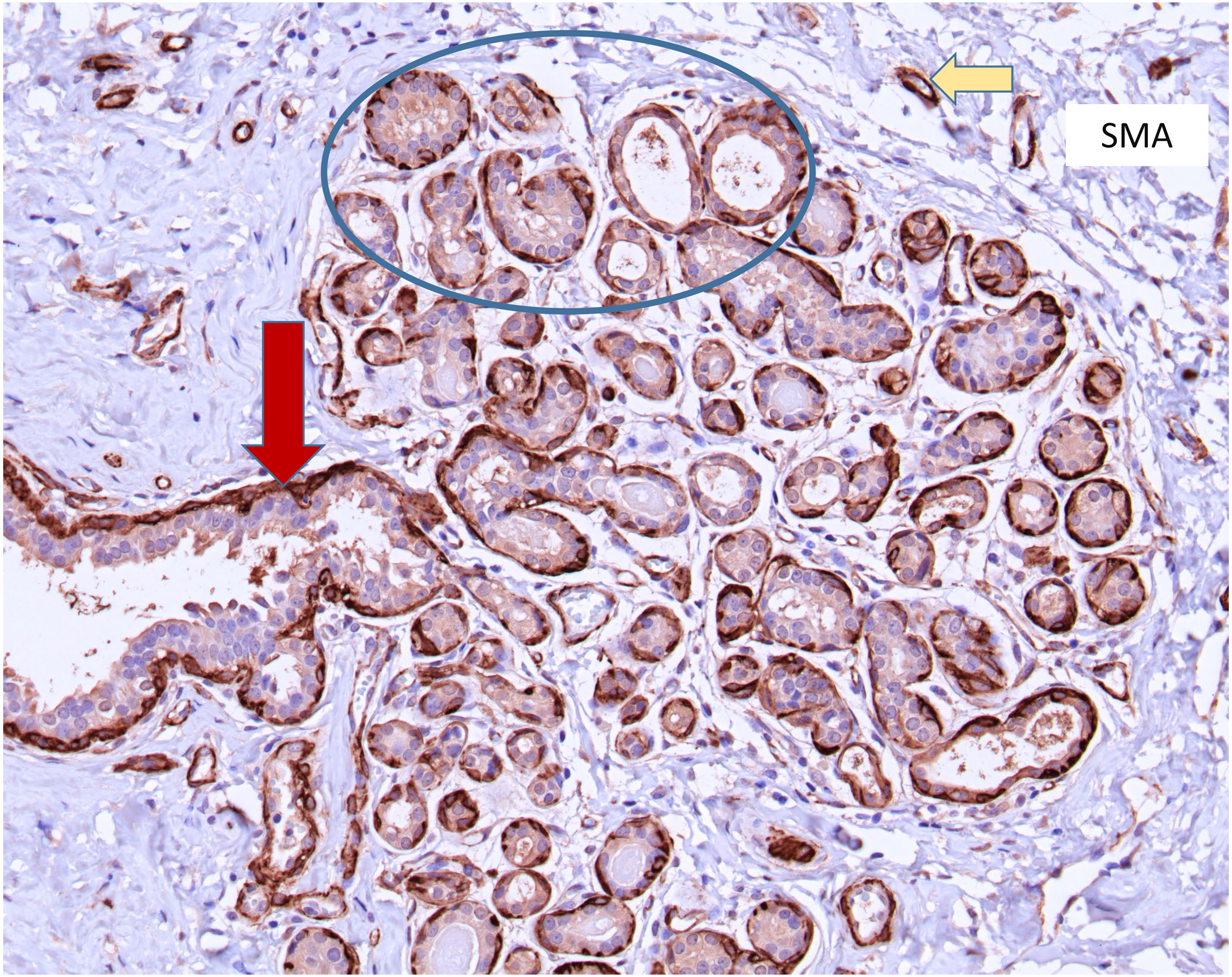
Sonali Jindal [1 2], Jayasri Narasimhan [1], Virginia F Borges [# 3 4], Pepper Schedin [# 1 2 3 4]

EX10-0218DV-12-TDLU 3-HE-10X-RDC

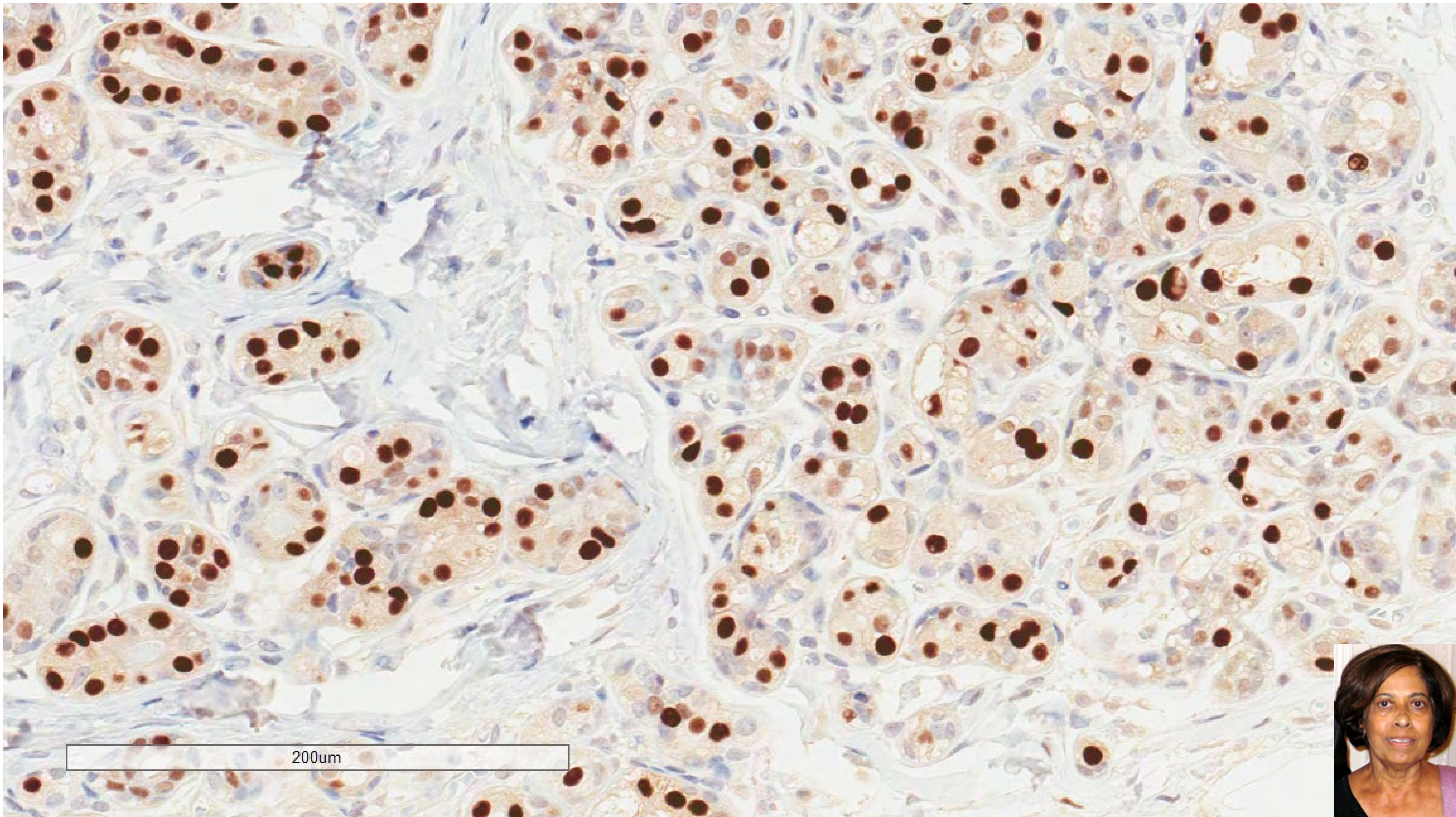




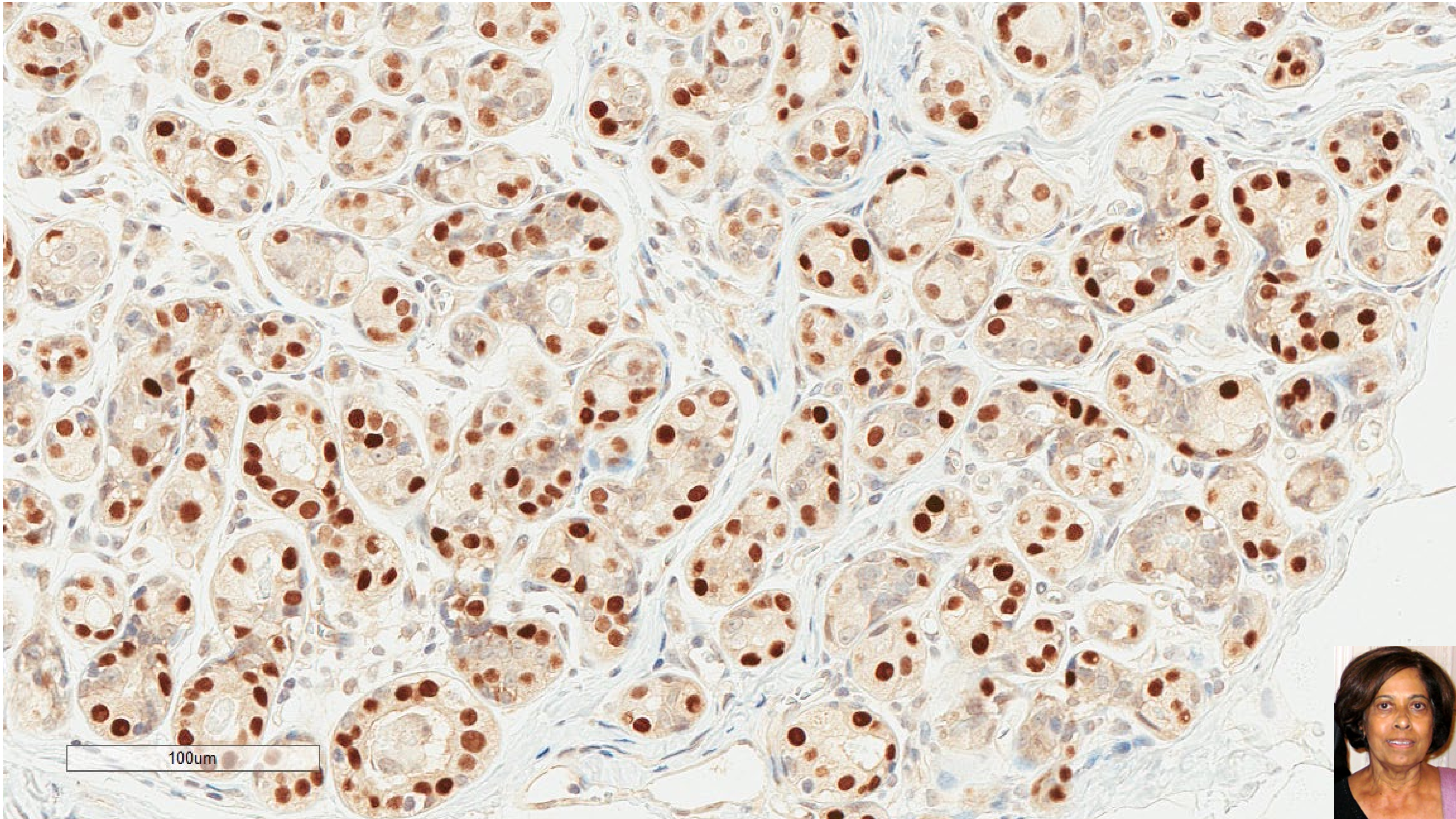

Krt8/18


SMA

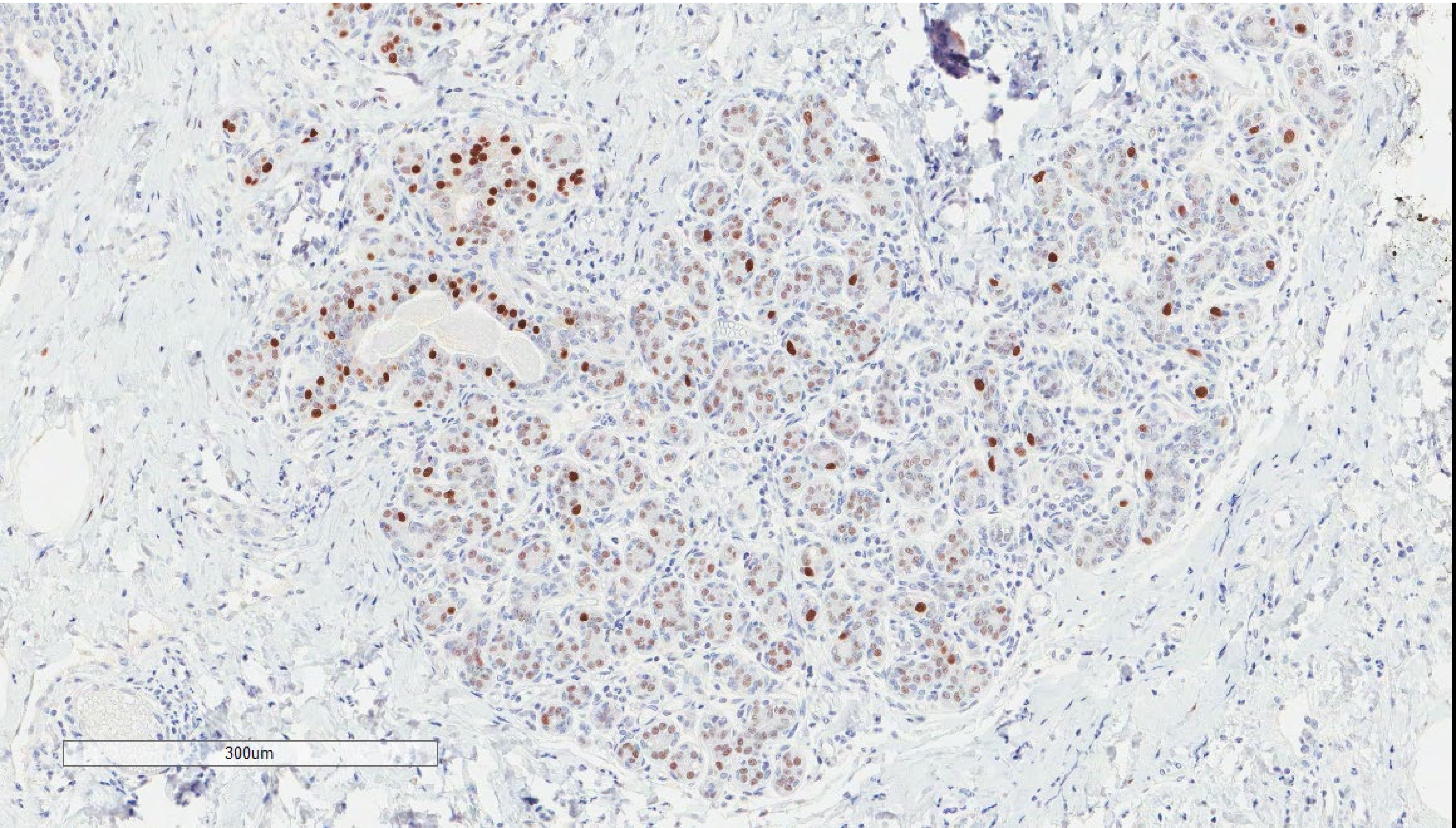
EX10-0218DV-3-TDLU1-ER-20X-RDC



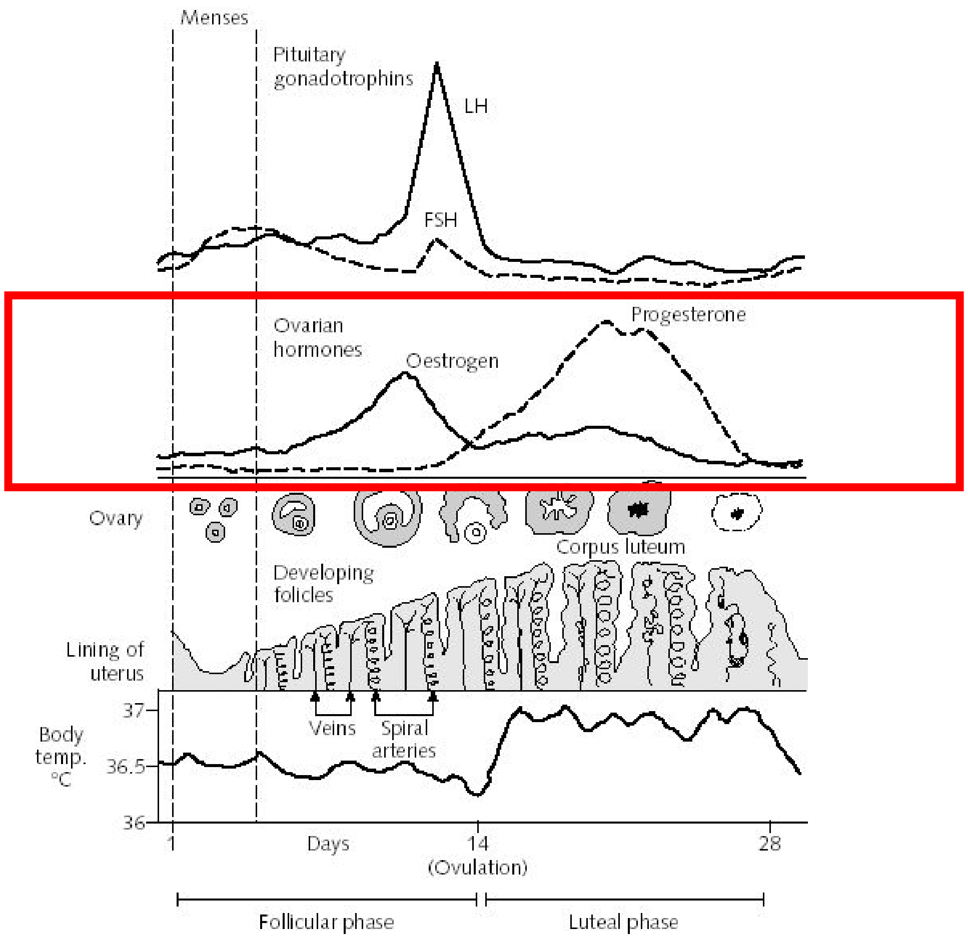
EX10-0218DV-3-TDLU1-PR-20X-RDC



EX10-0218DV-12-TDLU1-ER-10X-RDC

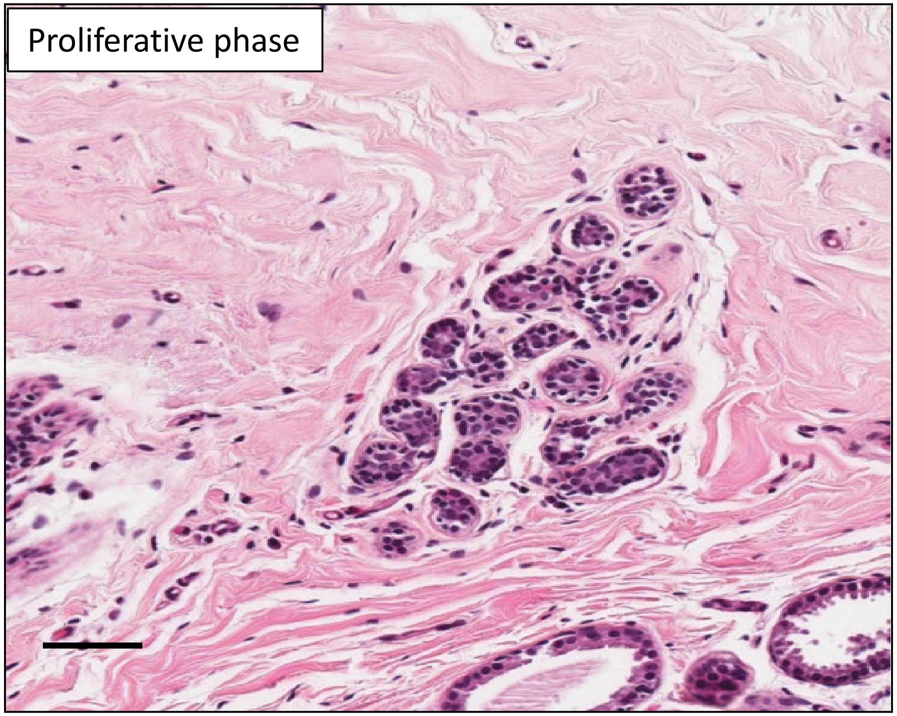


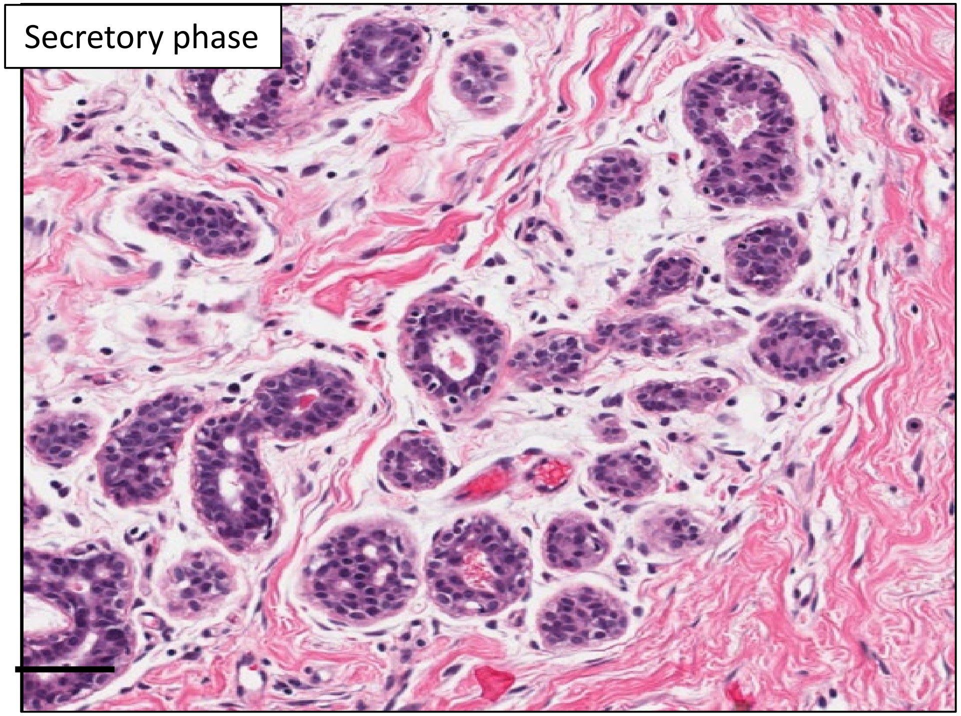
The "Menstrual Cycle"

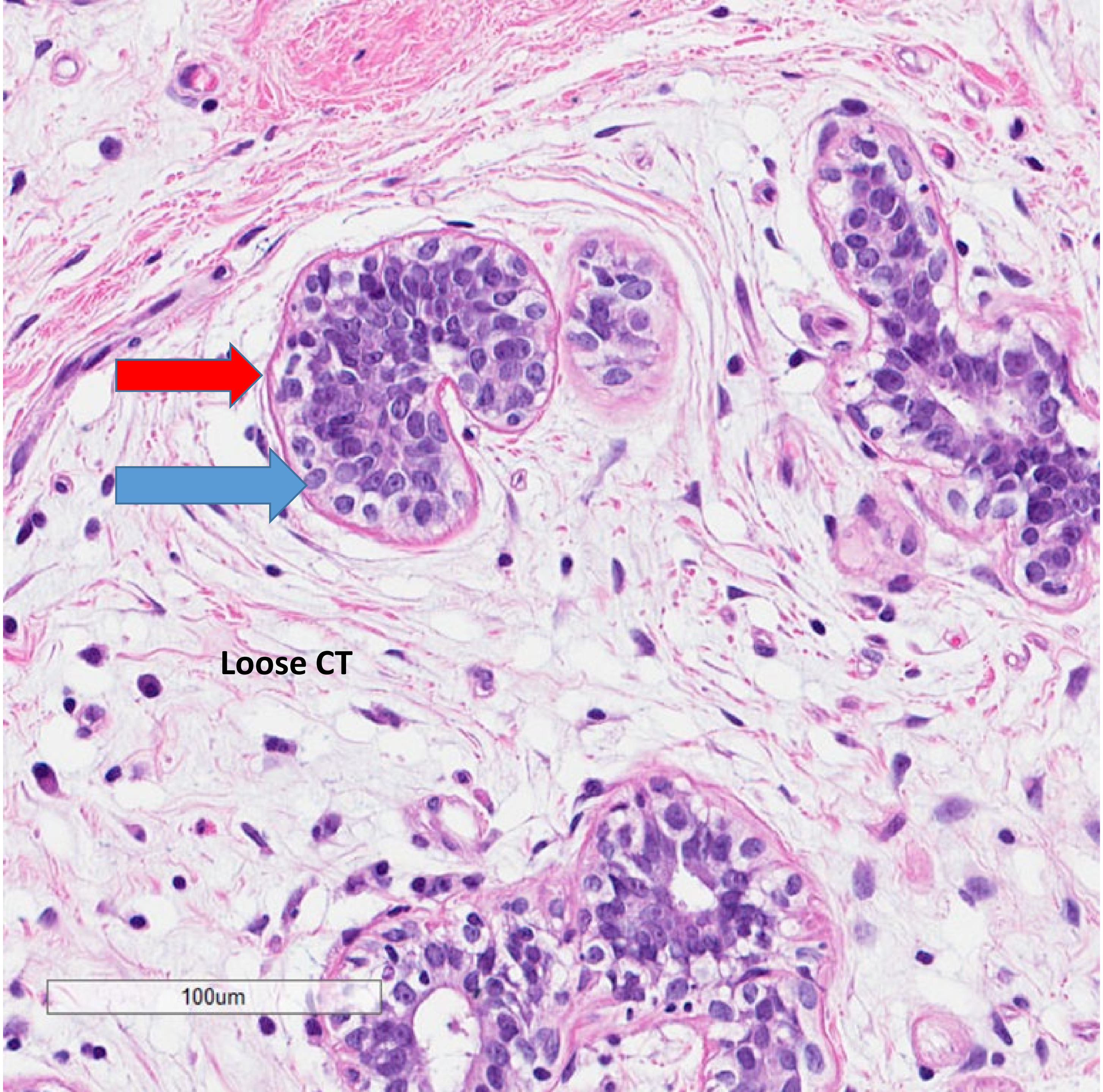


Human Menstrual Cycle

Human Menstrual Cycle





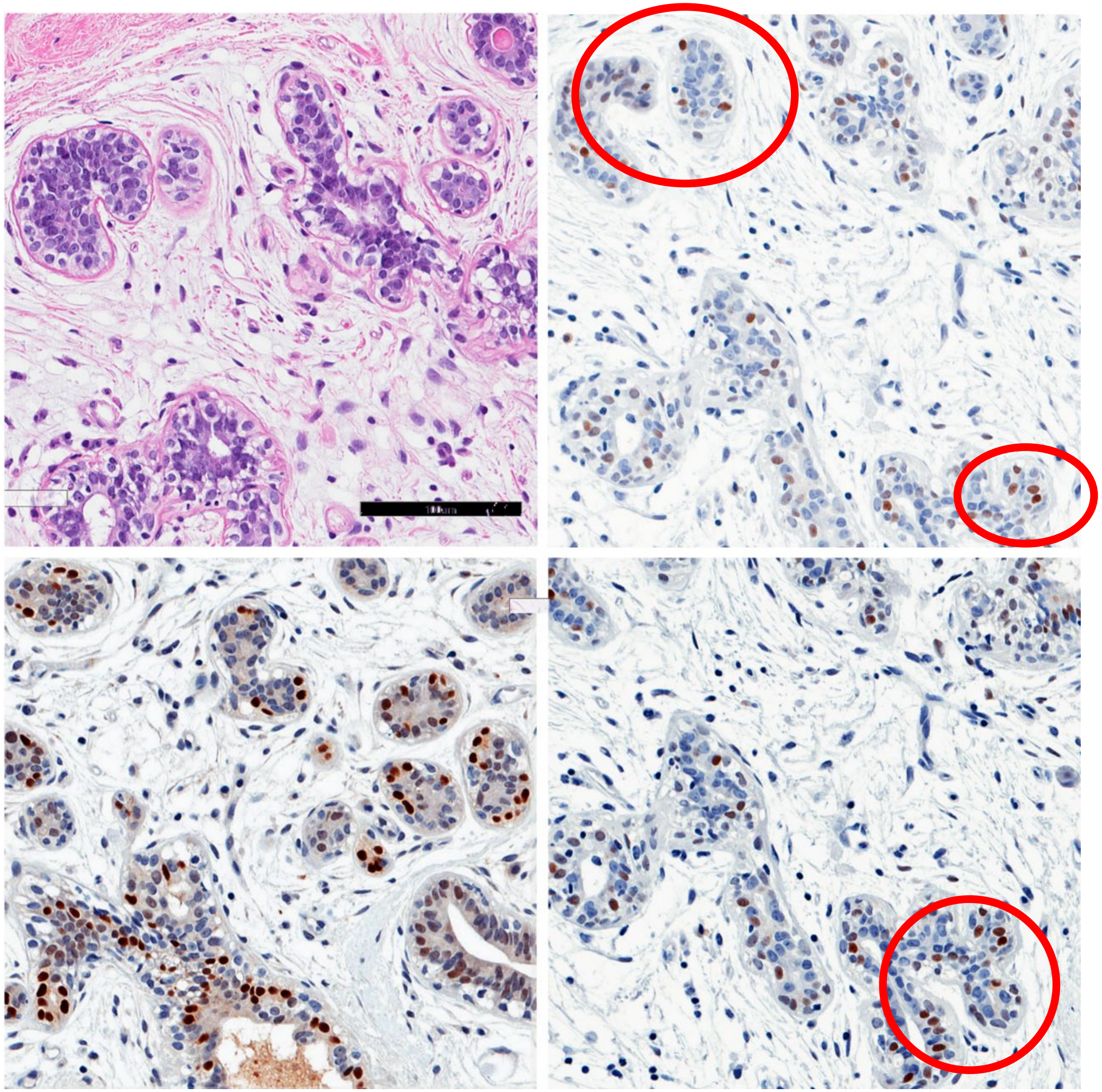


Loose CT
100um

ST12-0010-PANEL-HE-KI-ER-PR-20X-RDC

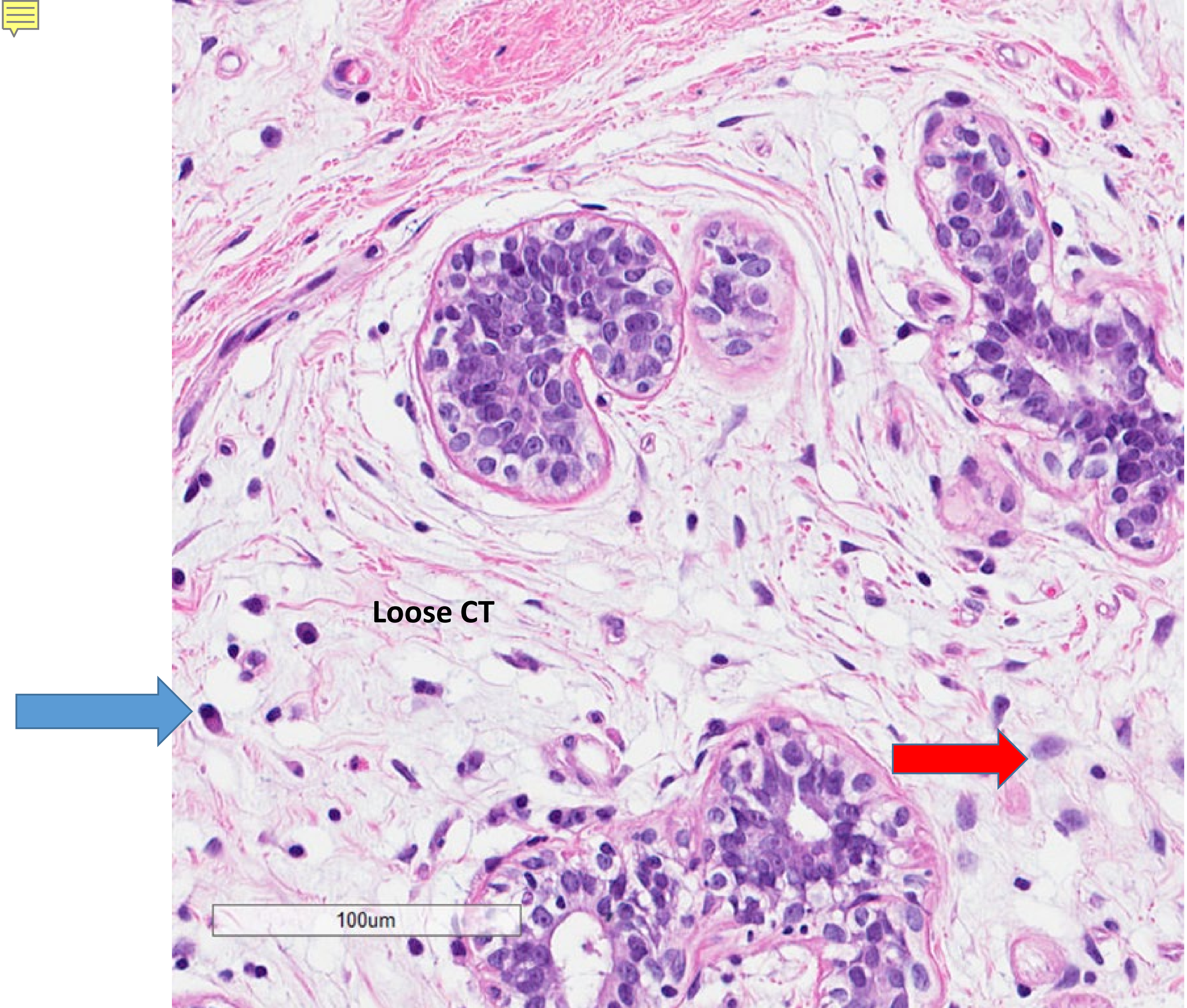
H&E

KI67

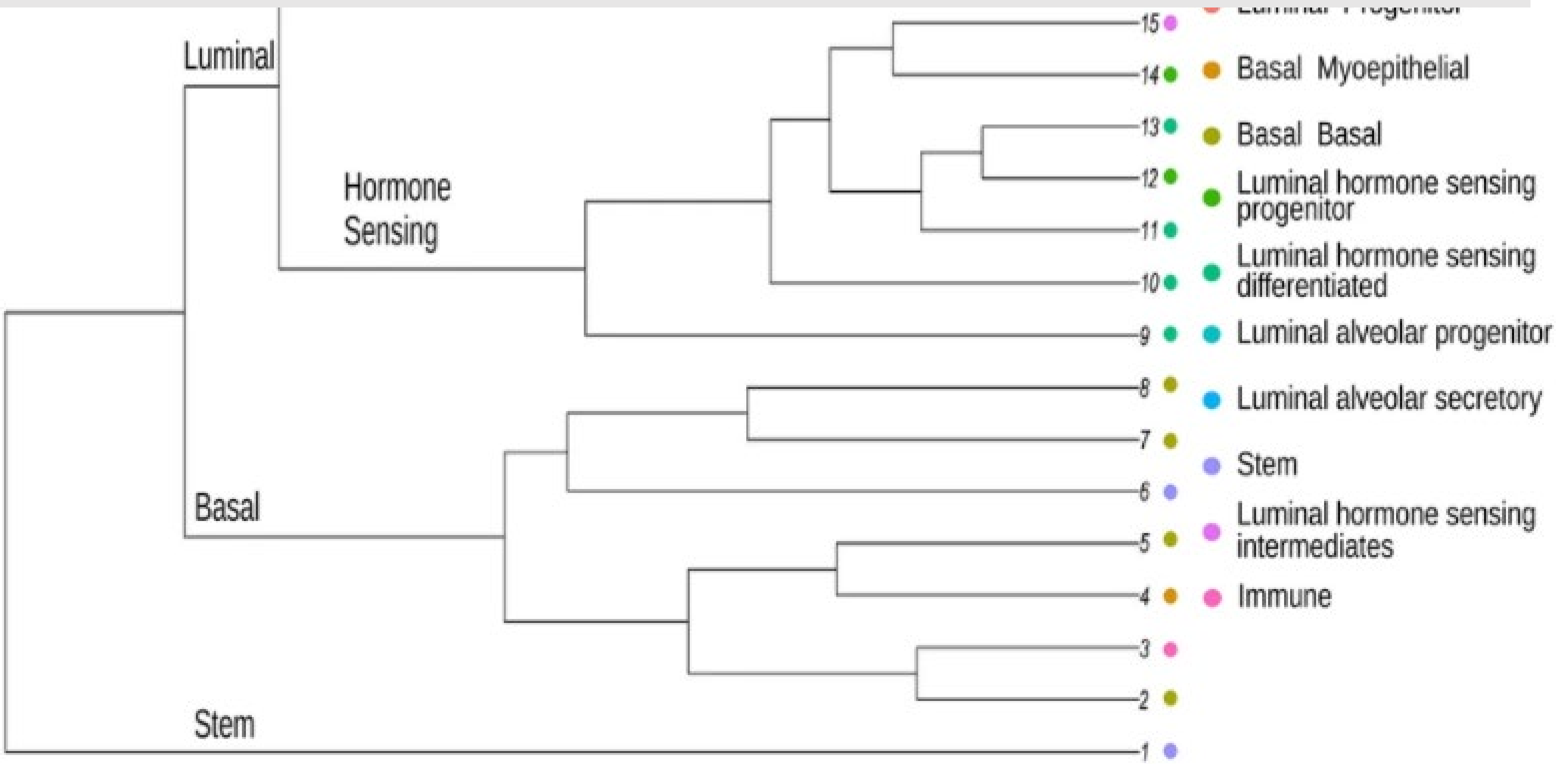


ER

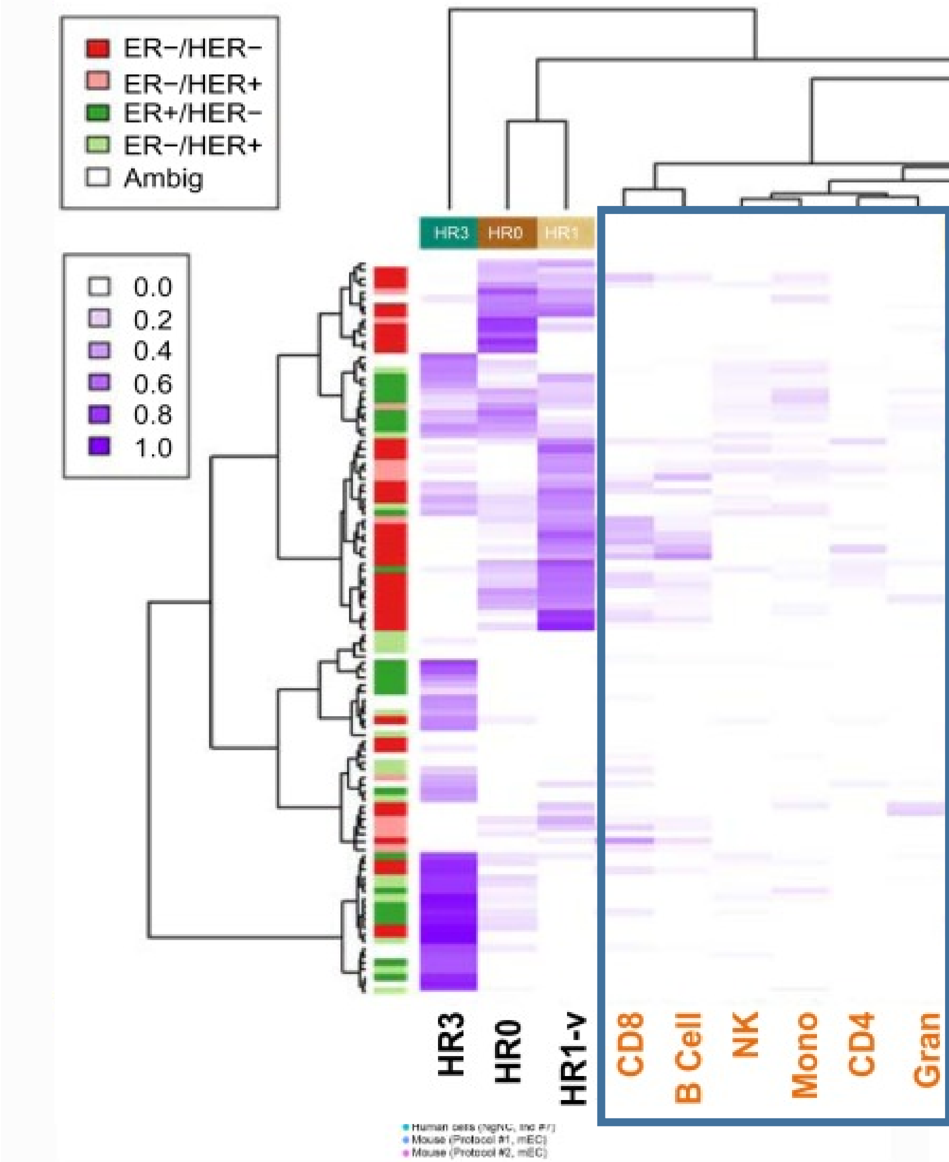
PR


Loose CT
100um

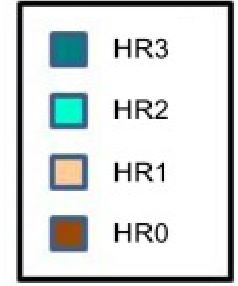
How many types of ME Cells exist?



Martín E. García Solá, Micaela Stedile, Inés Beckerman & Edith C. Kordon
Journal of Mammary Gland Biology and Neoplasia **volume 26**, pages29–42 (2021)



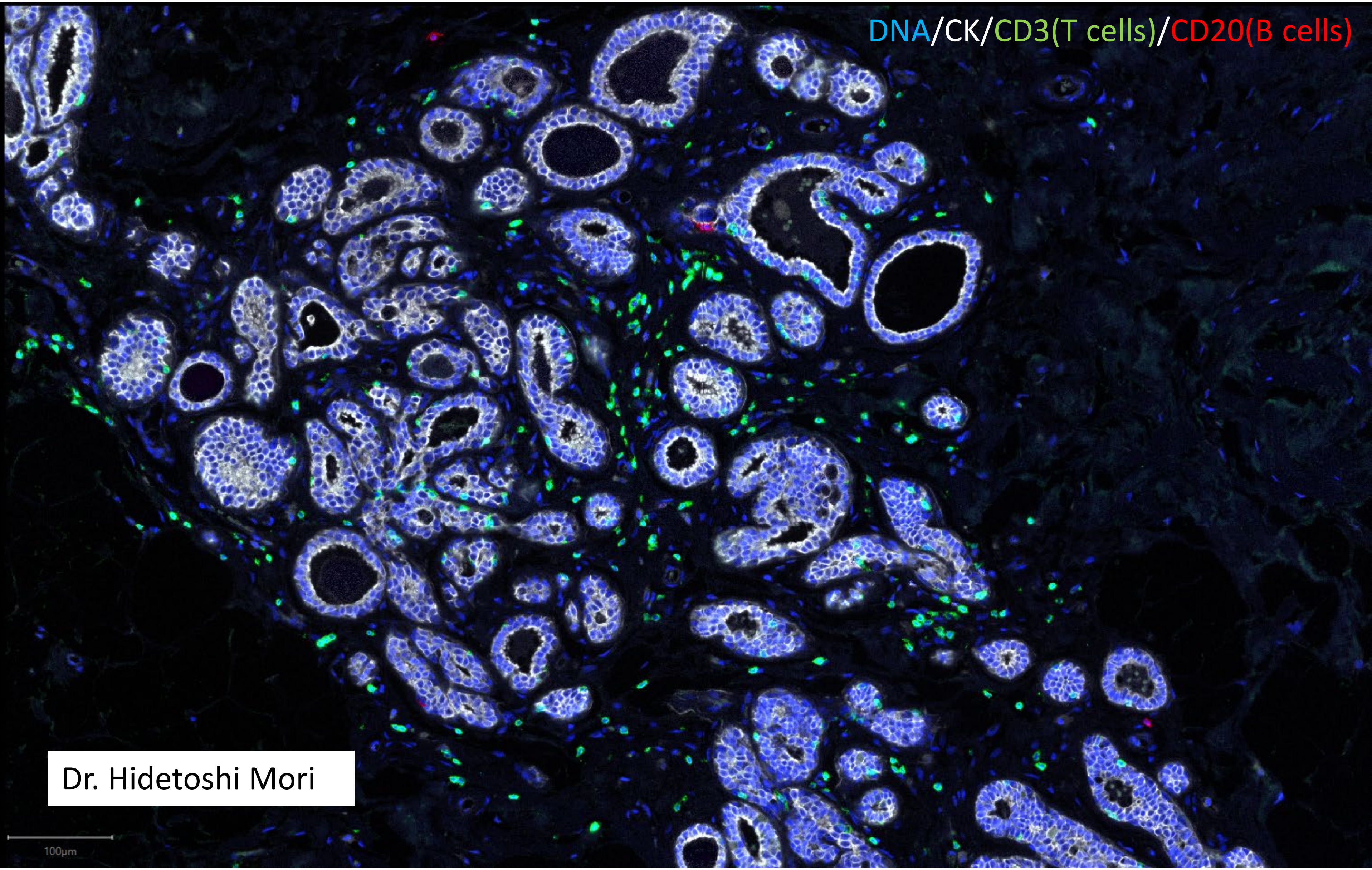
The evolutionary conserved basis of murine and human breast epithelial identity. (A) UMAP plot showing the distribution of epithelial cells identified to be present in murine and human breast tissue datasets (mhEC). (B) Cell abundance distribution of murine (Protocol #1 and Protocol #2, mEC) and human (hEC, NgNC Ind #5, NgNC Ind #6, NgNC Ind #7) breast tissue datasets. (C) Dot plot and dendrogram branching showing the average and percentage of expressed genes that distinguished and classified clusters of epithelial cell lineages present in murine and human breast tissue datasets. (D) UMAP plot illustrating the lineage identity of epithelial cell lineages present in murine and human breast tissue datasets

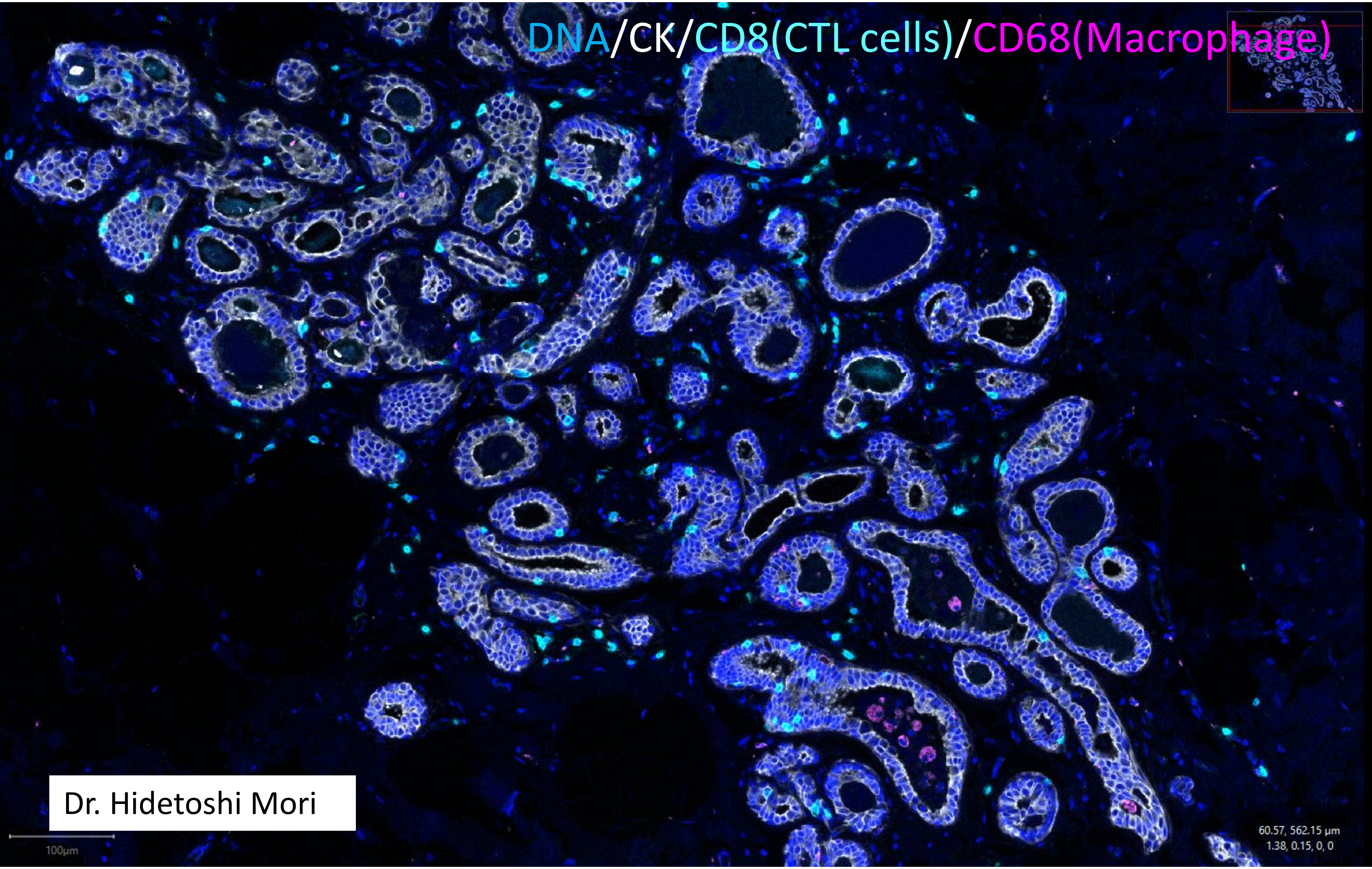


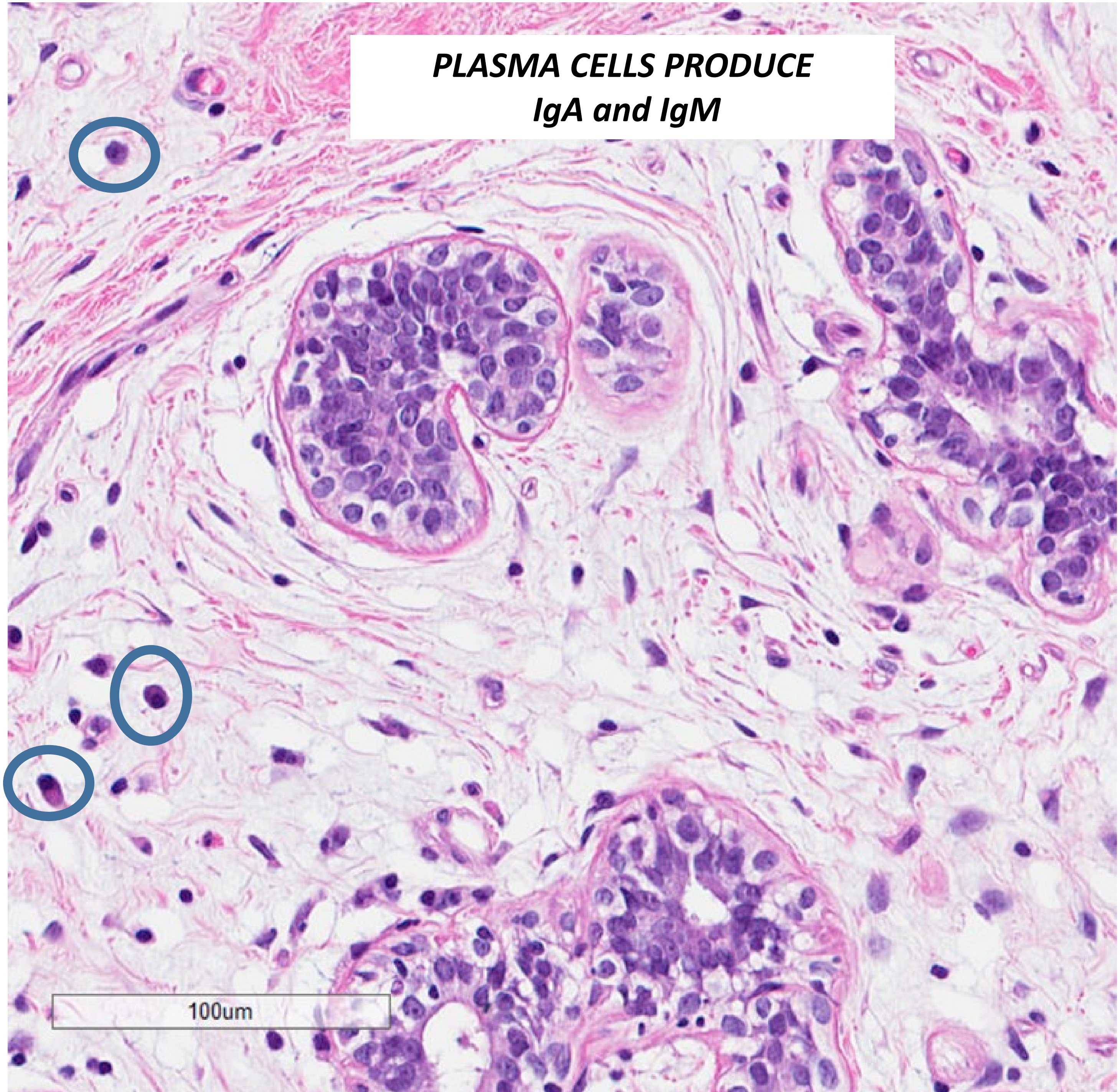
NON- ME
Cells Stroma

Normal cell-type epigenetics and breast cancer classification: a case study of cell mixture-adjusted analysis of DNA methylation data from tumors
Eugene Andrés Houseman[1], Tan A Ince[2]

Henry, S., Trousdell, M.C., Cyrill, S.L. *et al.* Characterization of Gene Expression Signatures for the Identification of Cellular Heterogeneity in the Developing Mammary Gland. *J Mammary Gland Biol Neoplasia* **26,** 43–66 (2021). https://doi.org/10.1007/s10911-021-09486-3


DNA/CK/CD3(T cells)/CD20(B cells)
Dr. Hidetoshi Mori
100µm


DNA/CK/CD8(CTL cells)/CD68(Macrophage)
Dr. Hidetoshi Mori
100μm
60.57, 562.15 μm
1.38, 0.15, 0, 0


PLASMA CELLS PRODUCE
IgA and IgM
100um

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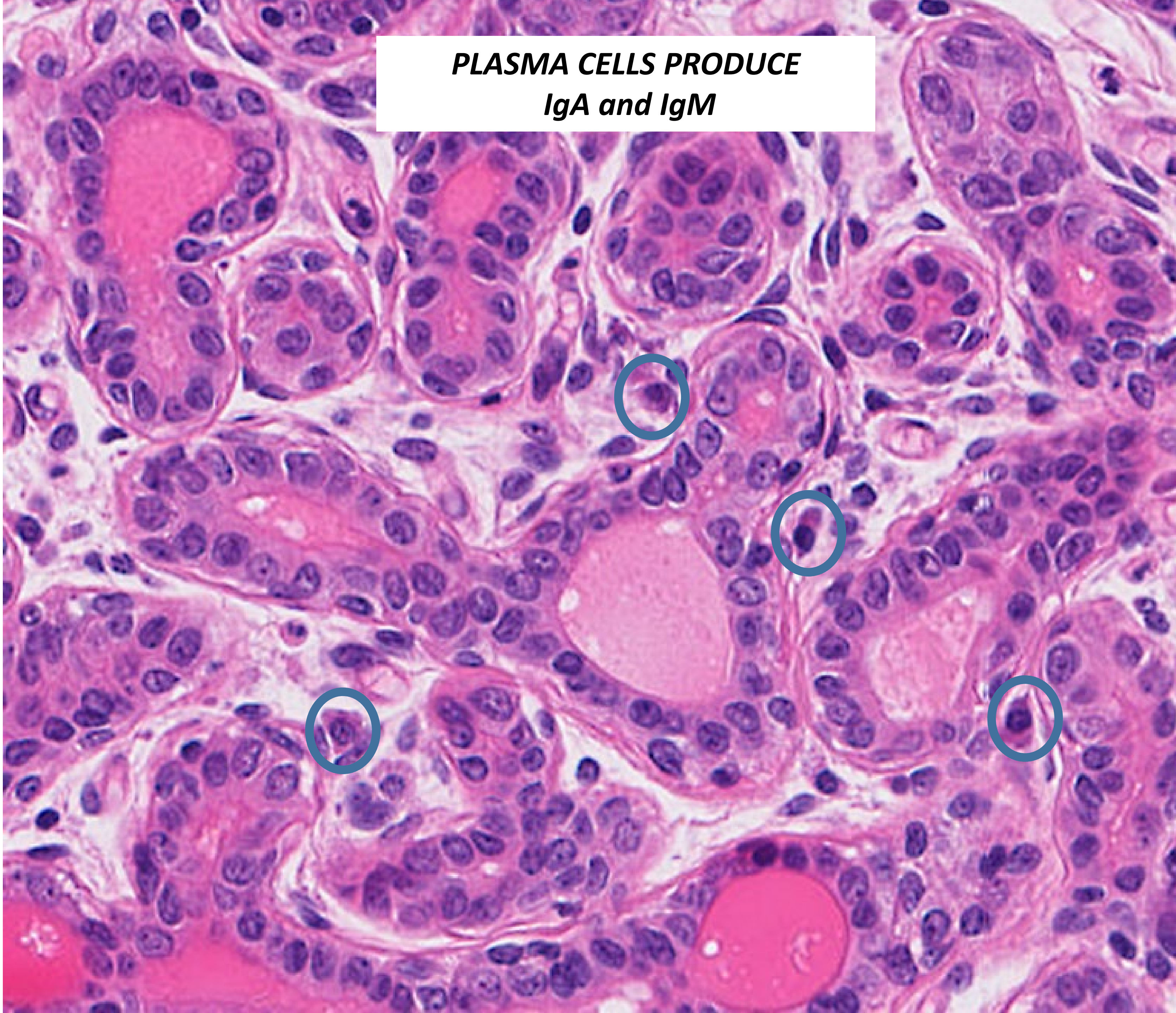
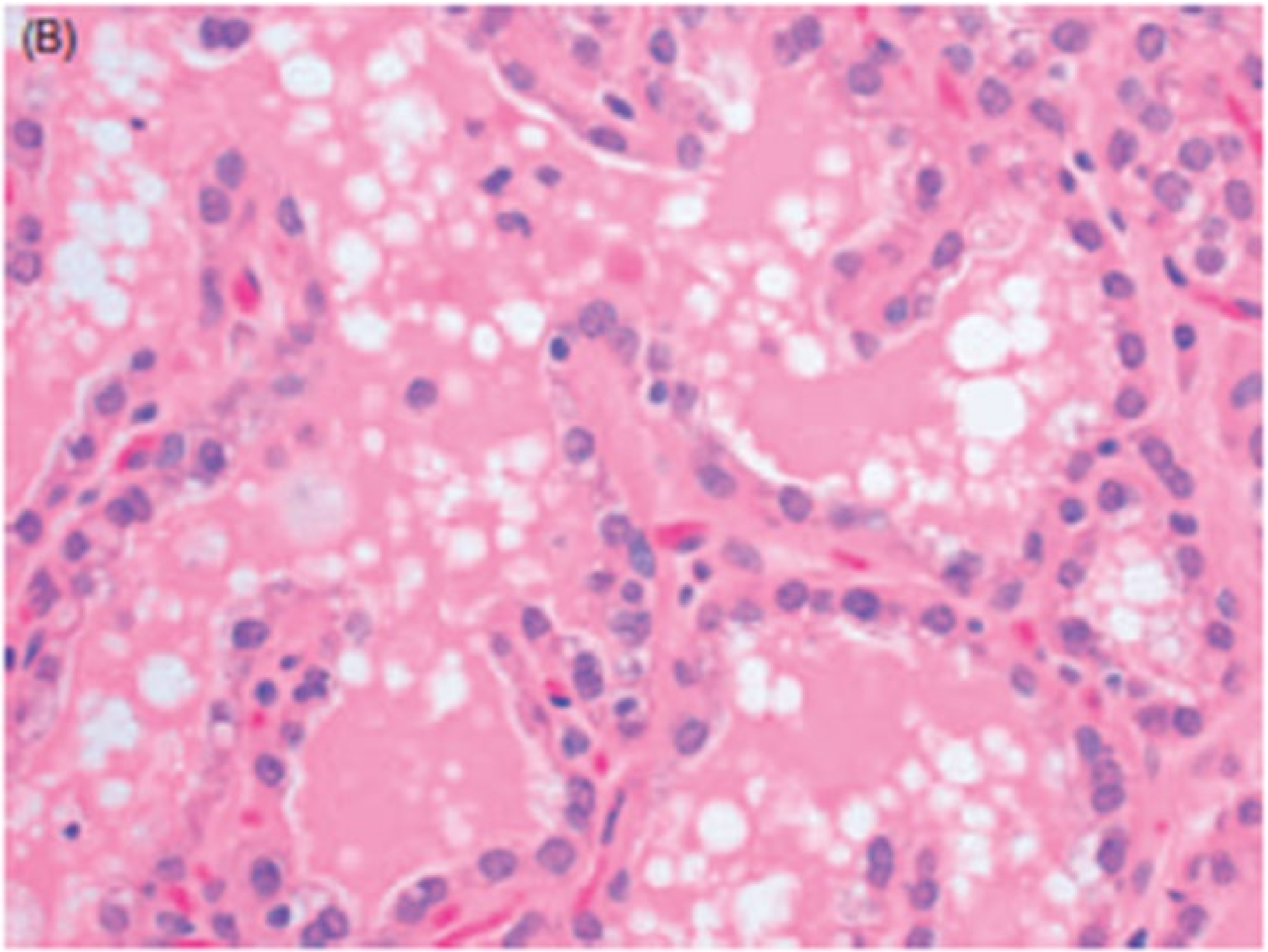


LACTATION subgross



MAMMARY NATURAL HISTORY: Ageing

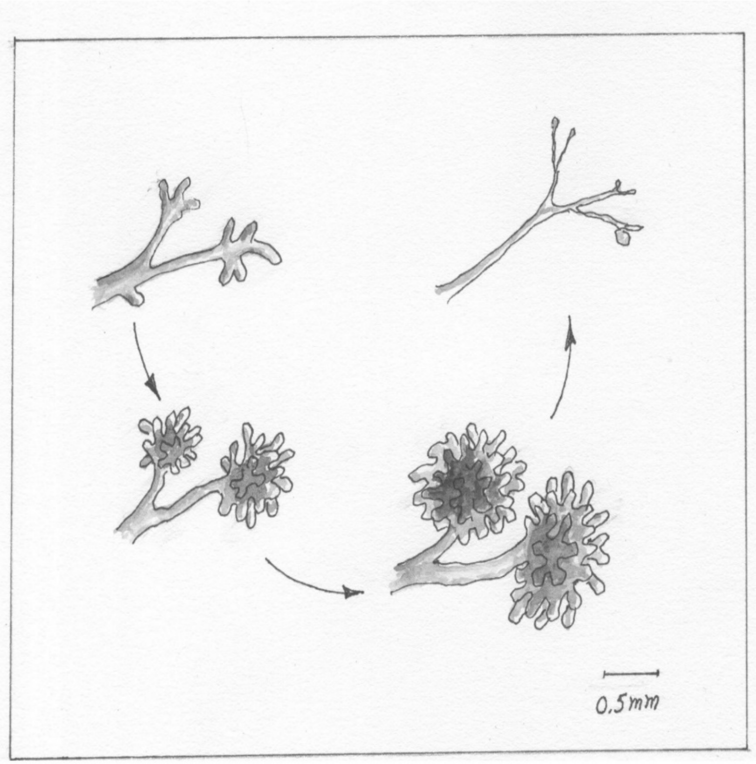
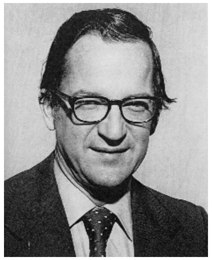
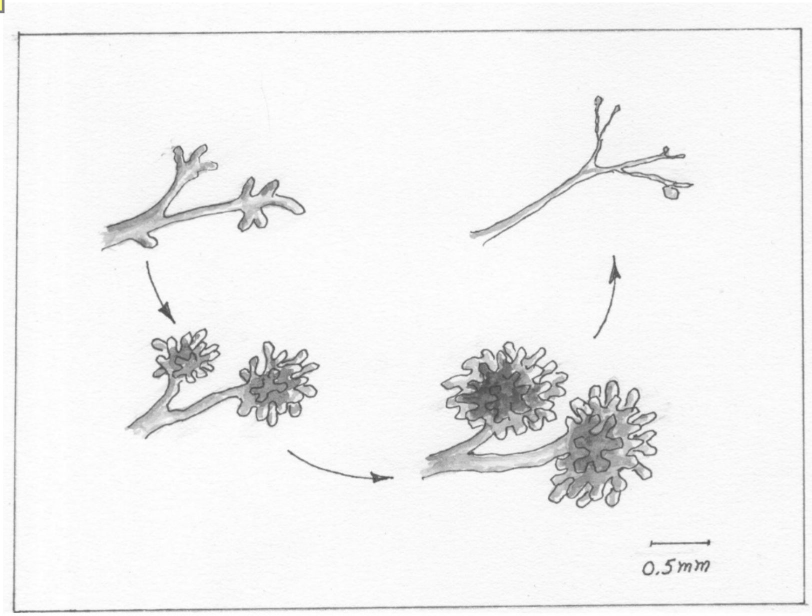


FIGURE 11

Diagram illustrating the natural history of a normal human mammary lobule which progresses from premenarche (upper left) through the reproductive years (bottom left and bottom right), to post-menopausal (senescent) atrophy (upper right).





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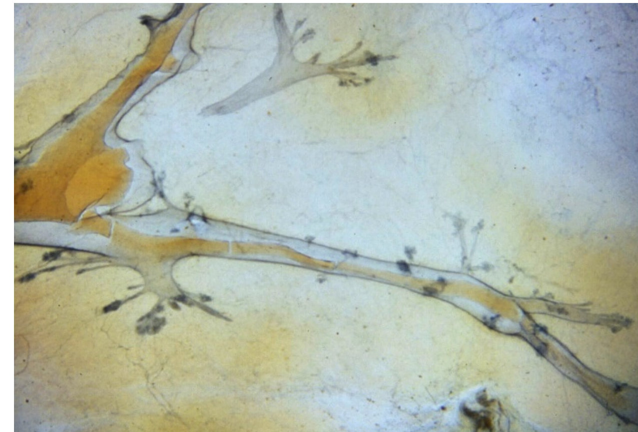
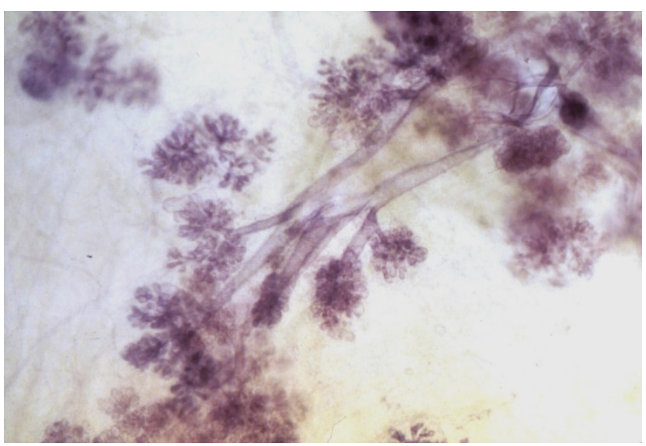


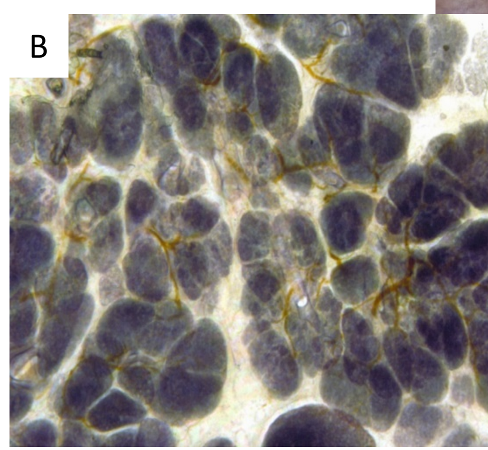
Post-menopausal

Young Adult



Reproductive

B



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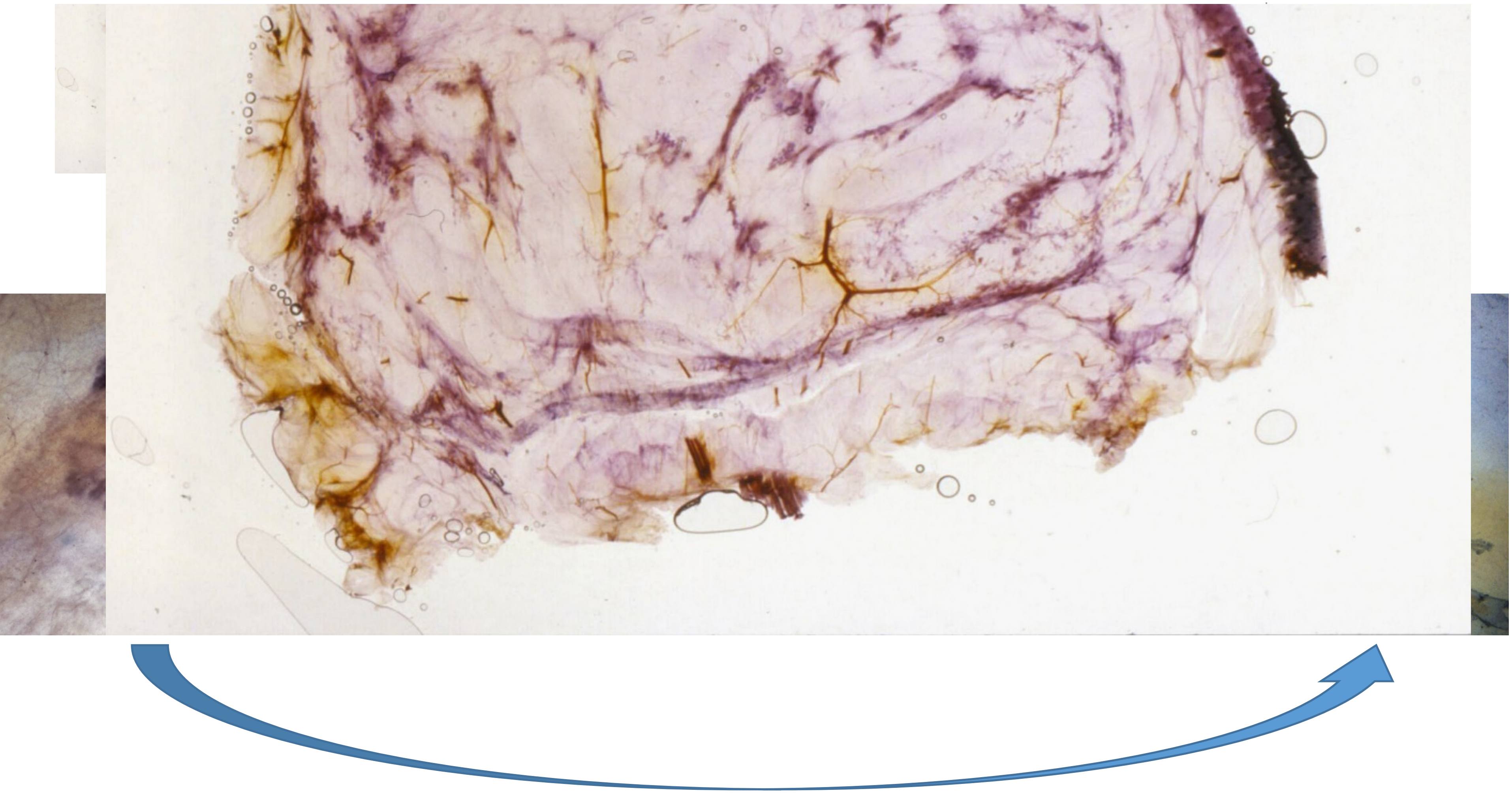
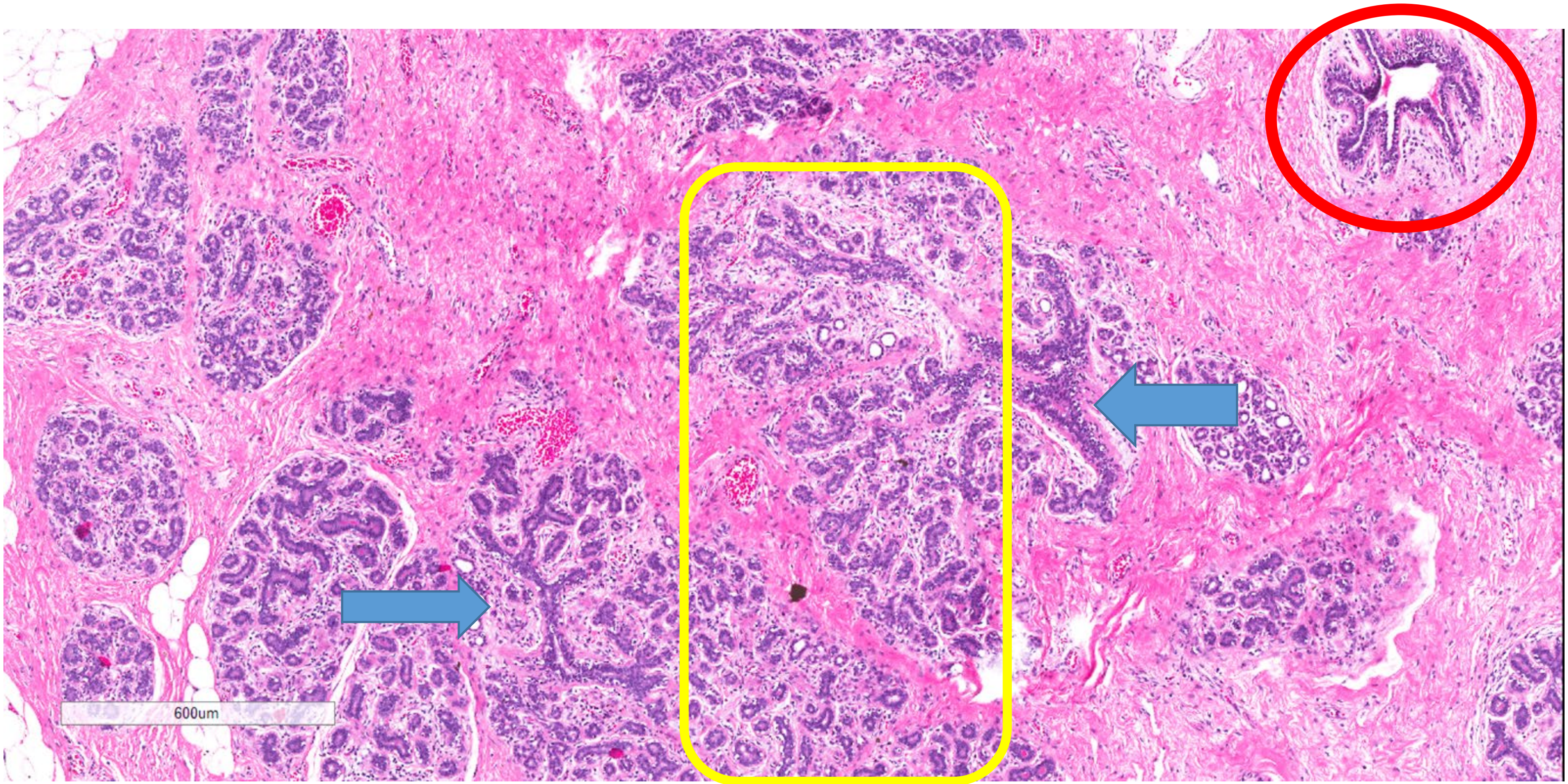


TCGA-01 TDLU 30 YO HI 3-HE-4X-psd-RDC1



BY POPULAR DEMAND:

WHAT ARE THE CHARACTERISTICS OF THE MAMMARY EPITHELIUM?

TDLU: SIZE AND NUMBER

Kensler KH, Liu EZF, Wetstein SC, Onken AM, Luffman CI, Baker GM, Collins LC, Schnitt SJ, Bret-Mounet VC, Veta M, Pluim JPW, Liu Y, Colditz GA, Eliassen AH, Hankinson SE, Tamimi RM, Heng YJ. **Automated Quantitative Measures of Terminal Duct Lobular Unit Involution and Breast Cancer Risk**. **Cancer Epidemiol Biomarkers Prev**. 2020 Nov;29(11):2358-2368. doi: 10.1158/1055-9965.EPI-20-0723. Epub 2020 Sep 11. PMID: 32917665; PMCID: PMC7642012.

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P		0.88	0.44	**0.04**	0.34

Normal TDLU with Multiplex (INCE)

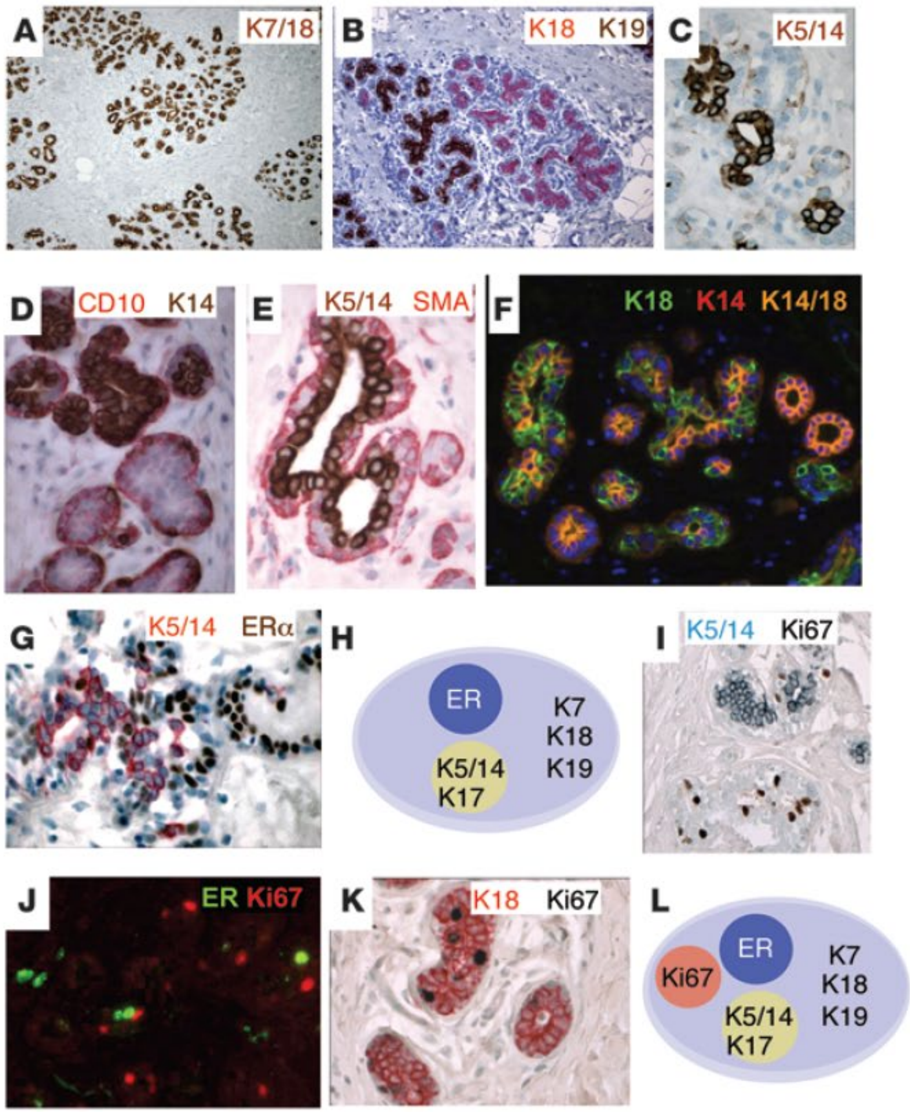


Figure 1

Expression of intermediate filaments and ER in normal human breast. Single and double IHC with immunoperoxidase (**A**–**E**, **G**, **I**, and **K**) and merged IHC images (**F** and **J**) of normal human FFPE sections are shown. (**A**) K7/18 (brown). (**B**) K18 (red) and K19 (brown). (**C**) K5/14 (brown). (**D**) CD10 (red) and K14 (brown). (**E**) K5/14 (brown) and SMA (red). (**F**) K18 (green) and K14 (red). Merged $K14^+K18^+$ appears yellow. (**G**) K5/14 (red) and ER (brown). We designated this population of cells $K5/14/17^+$ because the tissue sections were not stained simultaneously with these markers. (**H**) Differentiation states of normal luminal epithelial cells, based on expression of ER and keratins. (**I**) Ki67 (brown) and K5/14 (blue). (**J**) ER (green) and Ki67 (red). (**K**) K18 (red) and Ki67 (brown). (**L**) Differentiation states of normal luminal epithelial cells, based on ER, keratins, and Ki67. Representative images were selected from multiple patient samples ($n = 36$). Original magnification, ×20 (**A**); ×40 (**B**); ×200 (**F**); ×400 (**C**, **G**, and **I**–**K**); ×600 (**D** and **E**). See http://sylvester.org/ince for additional high-resolution images.

J Clin Invest. 2014 Feb 3; 124(2): 859–870.

Taxonomy of breast cancer based on normal cell phenotype predicts outcome

Sandro Santagata,[1] Ankita Thakkar,[2] Ayse Ergonul,[2] Bin Wang,[2] Terri Woo,[1] Rong Hu,[3,4] J. Chuck Harrell,[5] George McNamara,[2] Matthew Schwede,[6] Aedin C. Culhane,[6] David Kindelberger,[1] Scott Rodig,[1] Andrea Richardson,[1] Stuart J. Schnitt,[7] Rulla M. Tamimi,[3,4] and Tan A. Ince[2]

Normal TDLU with Multiplex (INCE)

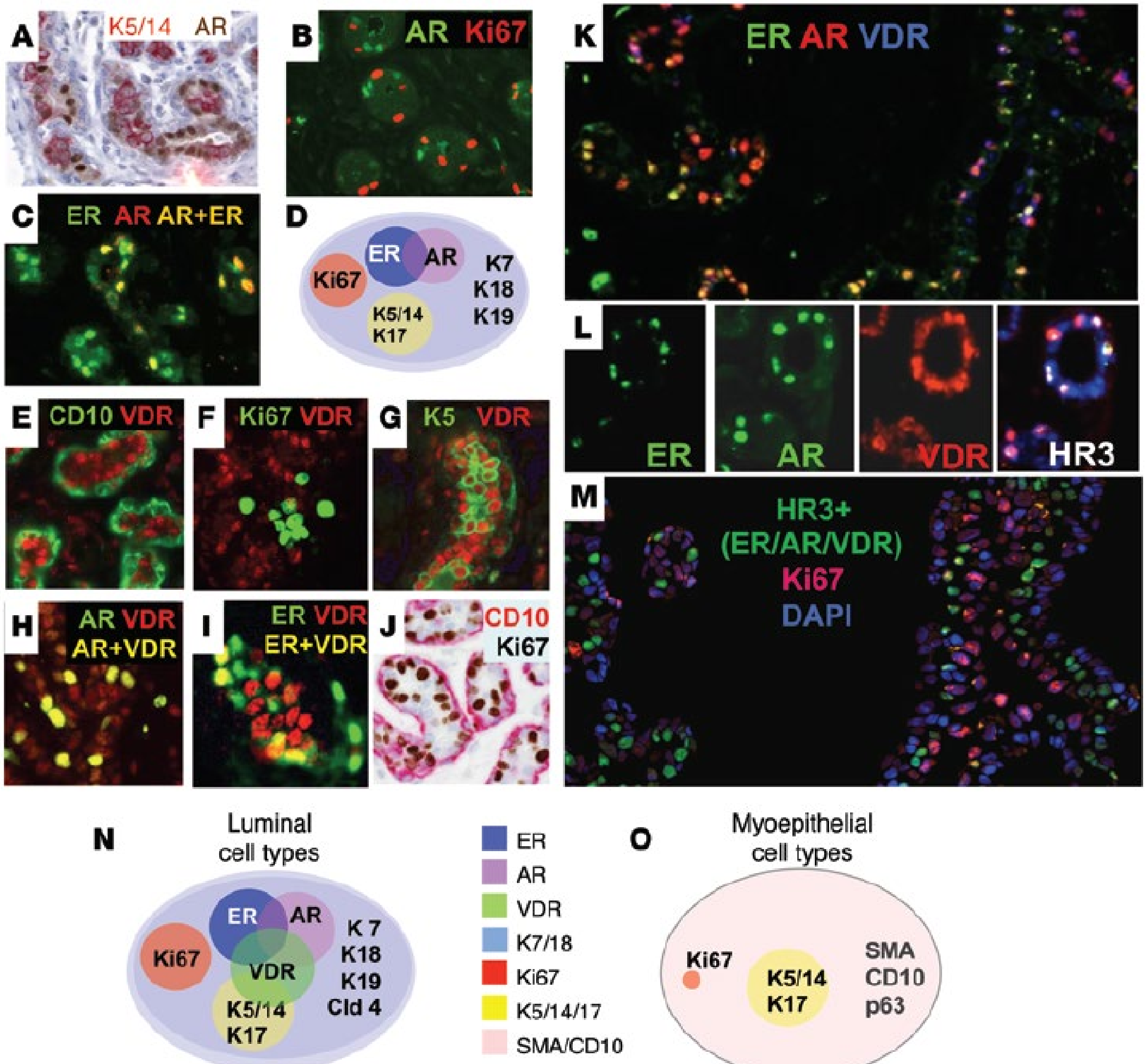


Figure 2
Expression of intermediate filaments, ER, AR, and VDR in normal human breast. Double IHC (**A** and **J**) and merged images (**B**, **C**, **E**–**I**, and **K**–**M**) of normal human breast FFPE sections…

Human Mammary Epithelium (TAN INCE)

Table 1
Cellular differentiation states in normal human breast lobules

Cell type		ER	AR	VDR	K5/14/17	Ki67	Cld-4	K7/8/18	CD10/SMA/p63
Luminal									
L1 (HR0)	$Ki67^+$	–	–	–	–	+	+	+	–
L2 (HR0)	$K18^+$	–	–	–	–	–	+	+	–
L3 (HR0)	$K5^+$	–	–	–	+	–	+	+	–
L4 (HR1)	ER^+	+	–	–	–	–	+	+	–
L5 (HR1)	AR^+	–	+	–	–	–	+	+	–
L6 (HR1)	VDR^+	–	–	+	–	–	+	+	–
L7 (HR1)	$K5^+VDR^+$	–	–	+	+	–	+	+	–
L8 (HR2)	ER^+AR^+	+	+	–	–	–	+	+	–
L9 (HR2)	ER^+VDR^+	+	–	+	–	–	+	+	–
L10 (HR2)	AR^+VDR^+	–	+	+	–	–	+	+	–
L11 (HR3)	$ER^+AR^+VDR^+$	+	+	+	–	–	+	+	–
Myoepithelial									
My1	$CD10^+$	–	–	–	–	–	–	–	+
My2	$K5^+$	–	–	–	+	–	–	–	+

IHC of normal breast sections from multiple donors (*n* = 36) with 14 different markers identified multiple normal breast cell subtypes. We grouped the 11 differentiation states in the luminal layer of human breast lobules (L1–L11) into HR0–HR3. All luminal cells expressed K7/8/18 and Cld-4. In the myoepithelial layer, all cells expressed CD10/SMA/p63, with 2 subtypes that were either $K5/14/17^-$ (My1) or $K5/14/17^+$ (My2).

scRNA Seq

García Solá, M., Stedile, M., Beckerman, I. *et al.* An Integrative Single-cell Transcriptomic Atlas of the Post-natal Mouse Mammary Gland Allows Discovery of New Developmental Trajectories in the Luminal Compartment. ***J Mammary Gland Biol Neoplasia*** **26,** 29–42 (2021). https://doi.org/10.1007/s10911-021-09488-1

Henry, S., Trousdell, M.C., Cyrill, S.L. *et al.* Characterization of Gene Expression Signatures for the Identification of Cellular Heterogeneity in the Developing Mammary Gland. ***J Mammary Gland Biol Neoplasia*** **26,** 43–66 (2021). https://doi.org/10.1007/s10911-021-09486-3

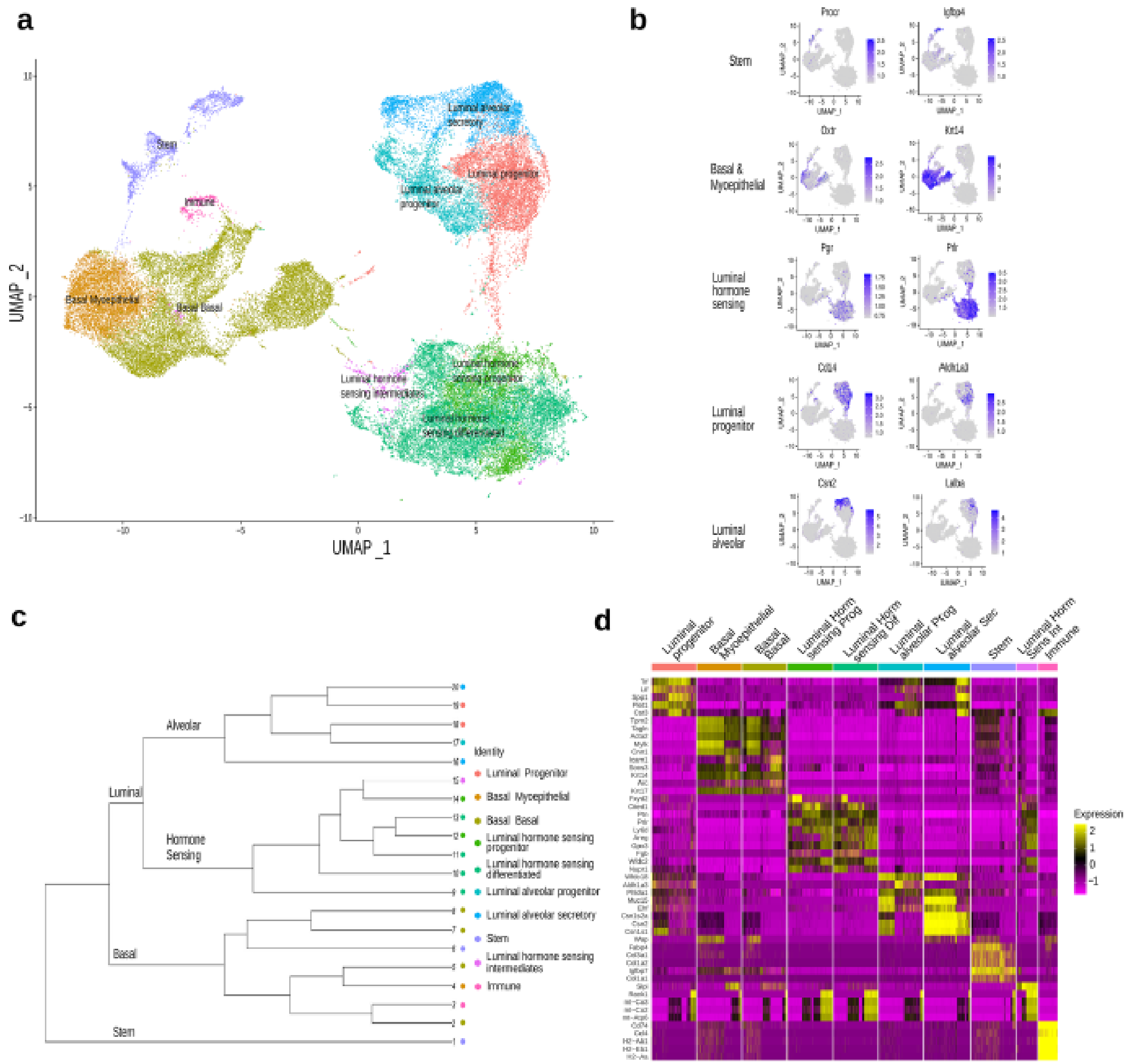


Fig. 2 Identification of mammary gland clusters based on their transcriptomic features. **(a)** UMAP plot showing the identification of the obtained cell clusters with putative mammary epithelial subpopulation by color code. **(b)** UMAP displaying relative expression levels of mammary subpopulation markers. **(c)** Dendrogram representing cell clusters in hierarchical structure. Subpopulation distribution is indicated in each main branch. **(d)** Heatmap highlighting top 5 key marker genes used to infer the identities of clusters. The bars located above the figure indicate the associated cell subpopulations

García Solá, M., Stedile, M., Beckerman, I. *et al.* An Integrative Single-cell Transcriptomic Atlas of the Post-natal Mouse Mammary Gland Allows Discovery of New Developmental Trajectories in the Luminal Compartment. *J Mammary Gland Biol Neoplasia* **26,** 29–42 (2021). https://doi.org/10.1007/s10911-021-09488-1

SOLAR ET AL

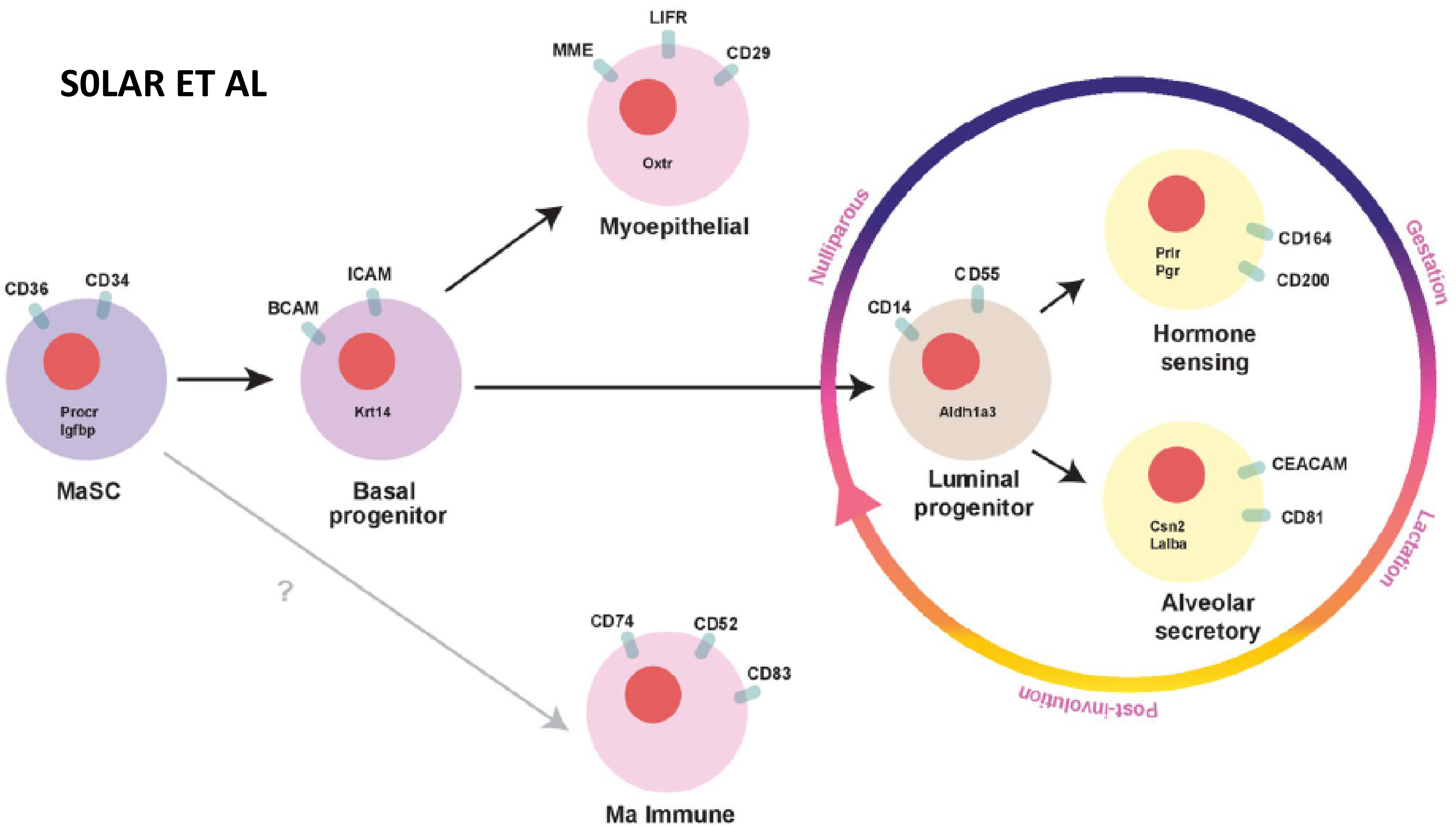
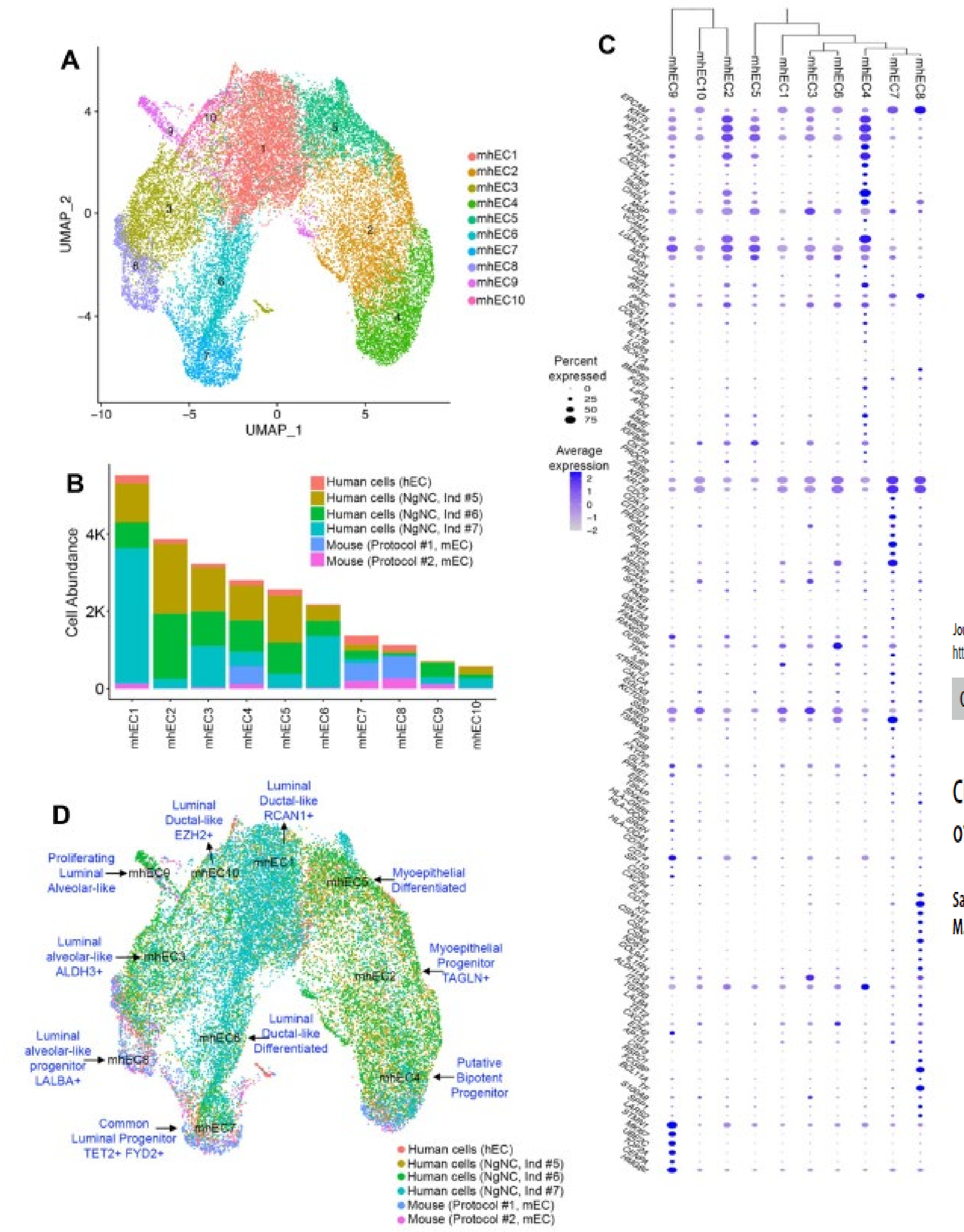


Fig. 7 Diagram of MEC hierarchy from multipotent MaSC to the cell lineages determined by our sc-RNAseq integrative analysis. In each oval a few featured genes/proteins for each mammary cell sub-type are displayed. Small green ovals on the borders represent membrane proteins recognizable by antibodies suitable for FACS. Genes encoding intracellular proteins are indicated inside the "cells". Arrows represent the general pseudotemporal trajectory of mammary cell differentiation. The circle compassing the luminal sub-populations indicates the cycling pattern of these cell types throughout post-natal life of female mice. Its colors depict the developmental process: progenitor cells of nulliparous females are at the beginning of our luminal pseudotime (purple), differentiated hormone-sensing and alveolar cells are intermediate stages (pale orange) and, at the end, luminal progenitors arise again in post-involution samples (yellow)



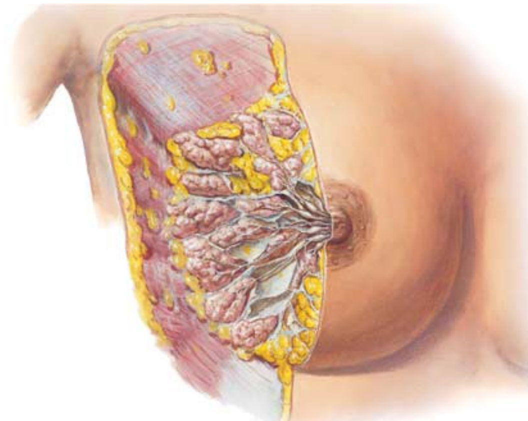
MOUSE-HUMAN MAMMARY EPITHELIUM sc-RNA-Seq

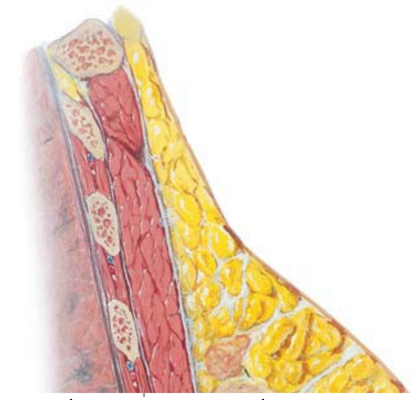
Journal of Mammary Gland Biology and Neoplasia (2021) 26:43–66
https://doi.org/10.1007/s10911-021-09486-3

ORIGINAL PAPER

Characterization of Gene Expression Signatures for the Identification of Cellular Heterogeneity in the Developing Mammary Gland

Samantha Henry[1,2] · Marygrace C. Trousdell[1] · Samantha L. Cyrill[1] · Yixin Zhao[1] · Mary. J. Feigman[1] · Julia M. Bouhuis[3] · Dominik A. Aylard[4] · Adam Siepel[1] · Camila O. dos Santos[1]



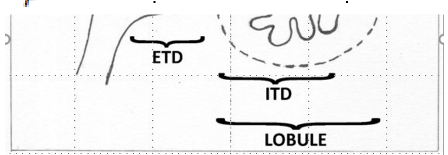


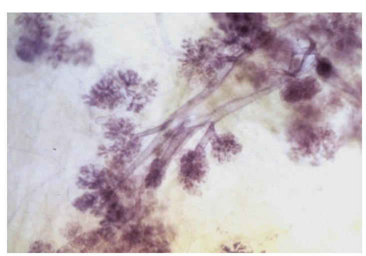
THE HUMAN BREAST

THE INTERMISSION

Table 2. Quantitative TDLU measures and breast cancer risk factors among 1,083 controls.

	n	Median TDLU span (μm)	TDLU counts/mm²	Median acini counts/TDLU	Median TDLU area (mm²)
Age at BBD biopsy					
<40 years	244	0.56 (0.55–0.58)	0.48 (0.44–0.52)	7.56 (7.11–8.04)	0.11 (0.10–0.11)
40–49 years	431	0.52 (0.51–0.53)	0.49 (0.46–0.51)	7.52 (7.18–7.87)	0.09 (0.09–0.10)
50–59 years	272	0.47 (0.45–0.48)	0.43 (0.40–0.46)	5.32 (5.02–5.64)	0.07 (0.07–0.07)
≥60 years	136	0.46 (0.44–0.47)	0.38 (0.34–0.42)	4.33 (3.99–4.71)	0.07 (0.06–0.07)
P		**<0.001**	**<0.001**	**<0.001**	**<0.001**





TO BE CONTINUED NEXT WEEK WITH PATHOLOGY

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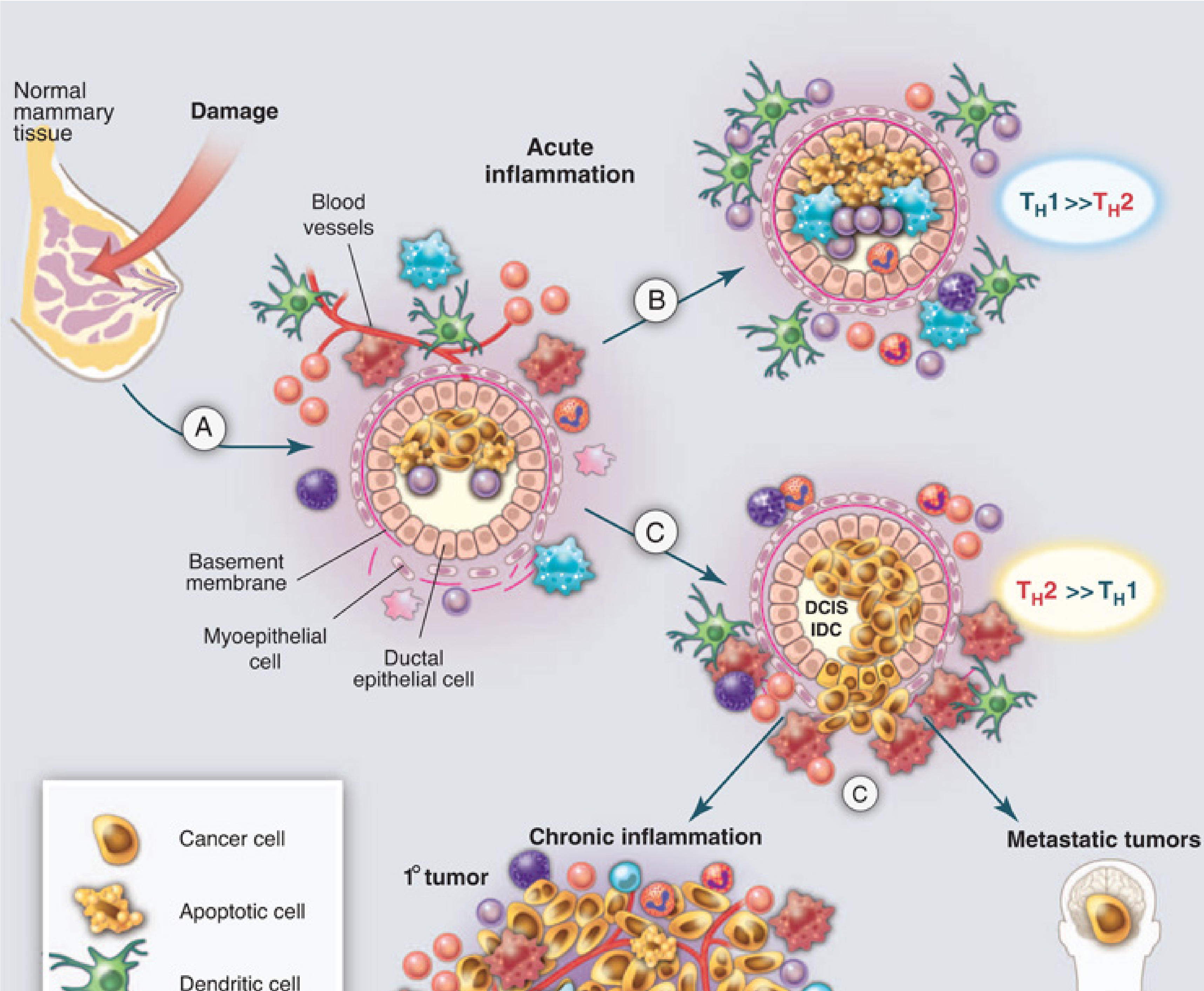
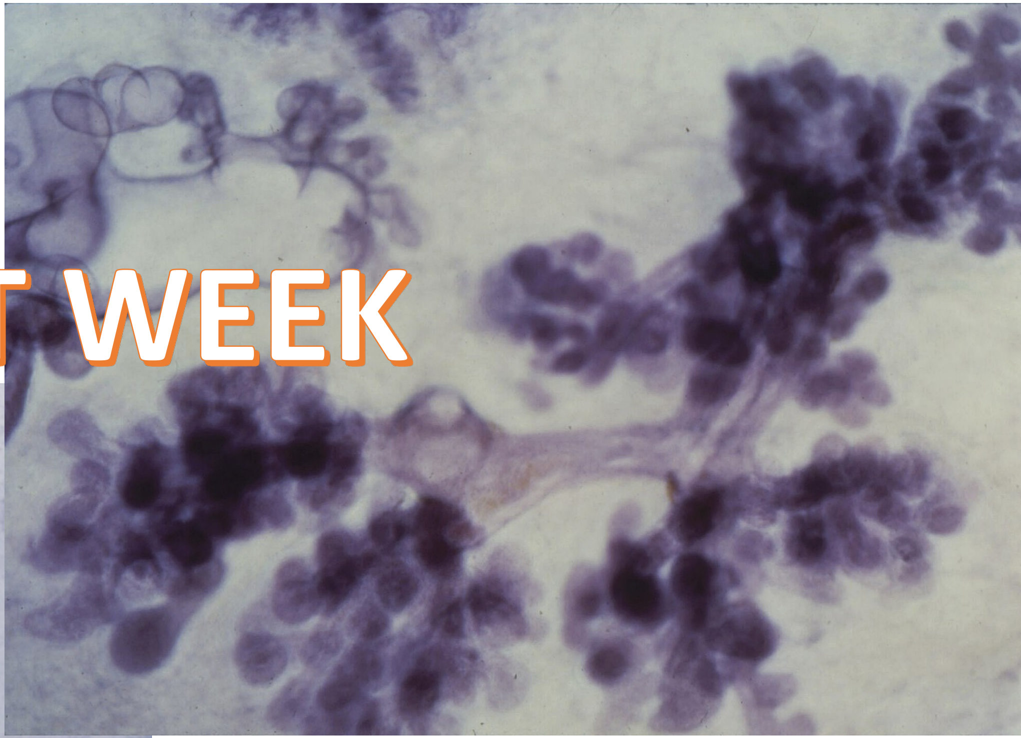
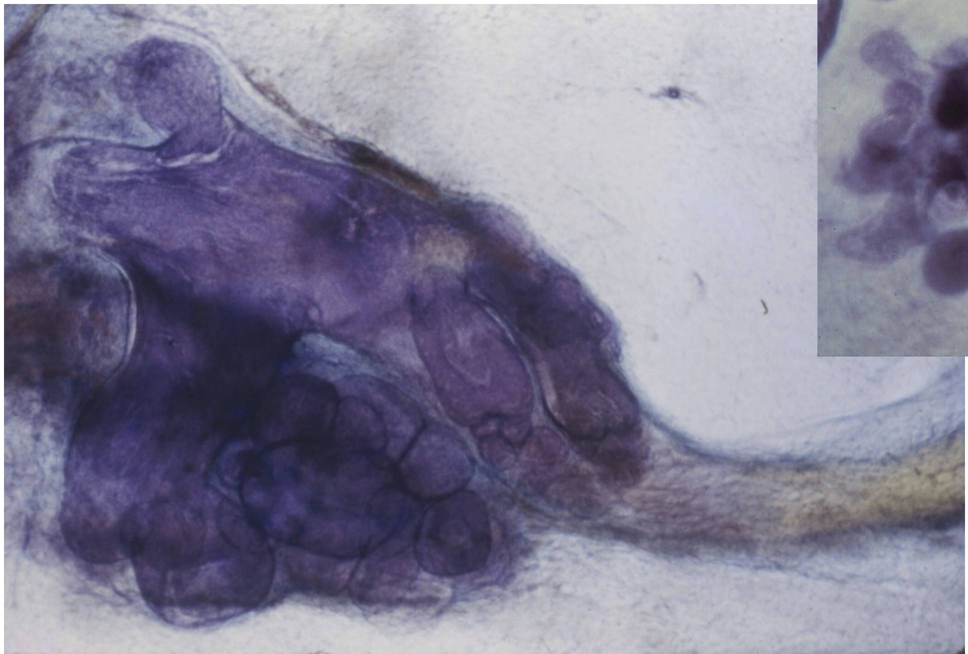


Normal
mammary
tissue
Damage
Acute
inflammation
Blood
vessels
$T_H1 >> T_H2$
B
A
C
Basement
membrane
Myoepithelial
cell
Ductal
epithelial cell
DCIS
IDC
$T_H2 >> T_H1$
C
Chronic inflammation
Metastatic tumors
1° tumor
Cancer cell
Apoptotic cell
Dendritic cell

What goes wrong when cells go wrong?





NEXT WEEK

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