Challenges in Cancer Pathology

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Clinical pathology forms the basis for diagnosis of all diseases where a disruption of tissue architecture is seen.

The bulk of current diagnostics is performed using traditional formalin fixation followed by paraffin embedding and microscopic analysis.

Molecular pathology is on the rise however. Today tissue is analyzed with gene panels where sets of genes are sequenced or analyzed with PCR/sequencing. In the future whole genome sequencing will be performed with an added step of in silico analysis of genes of importance for disease management.

This of great importance for genetic disease or in treatment decisions where the results are clear cut and dicotomous, present/not present.

Table 1 Examples of actionable genes for solid tumours

Tumour type	Genes	Abnormality	Drugs/indication
Non-small cell lung cancer	*EGFR*	Activating mutation	Response to EGFR TKI* treatment
	ALK	Translocations	Response to crizotinib treatment
Melanoma	*BRAF*	Activating mutation	Response to vemurafenib treatment
	KIT	Activating mutation	Response to imatinib treatment
GIST	*KIT*	Activating mutation	Response to imatinib treatment
Colorectal cancer	*KRAS*	Activating mutation	Resistance to anti-EGFR treatment
	NRAS	Activating mutation	Resistance to anti-EGFR treatment
	BRAF	Activating mutation	Poor prognosis and possible resistance to anti-EGFR treatment
Breast cancer	*ERBB2 (HER2)*	Amplification	Response to trastuzumab or lapatinib treatment
	BRCA1/2	Mutation	Response to PARP inhibitors
Ovarian cancer	BRCA1/2	Mutation	Response to PARP inhibitors

*EGFR TKI—tyrosine kinase inhibitors active against EGFR, such as gefitinib and erlotinib.

The current surge of targeted therapies increase the demand for the development of predictive markers in order to determine who might benefit from a particular form of treatment. Examples are: check-point inhibitors, anti-angiogenesis regimens and RTK inhibition.

This is part of the buzzwords: personalized medicine.

Novel targeted treatments are expensive and potentially harmful for the patient.

Many current studies depend on interpretation of RNA levels based on RNA sequencing histological material from patients included in the study. RNA levels do not always correspond to actual changes In protein expression.

Proteins are the units of biological function, RNA is a unit of potential function.

A major challenge is to integrate the findings of predictive relevance derived from sequencing into a histological context. Here pathology must serve an active function.

An example from ESMO 2020:

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700O - Kidney ccRCC Immune Classification (KIC) enhances the predictive value of T effector and angiogenesis signatures in response to Nivolumab

Maxime Meylan[1,2], Benoit Beuselinck, Cécile Dalban, Yann-Alexandre Vano, Nathalie Rioux-Leclercq, Catherine Sautes-Fridman, Eduard Roussel, Annelies Verbiest, Nathalie Chaput, Stephane Terry, Christine Chevreau, Marine Gross-Goupil, Aude Flechon, Brigitte Laguerre, Sylvie Chabaud, Florence Tantot, Diether Lambrechts, Bernard Escudier, Wolf-Hervé Fridman, Laurence Albiges

1. Centre de Recherche des Cordeliers, Sorbonne Université, Inserm, Université de Paris, F-75006, Paris, France.

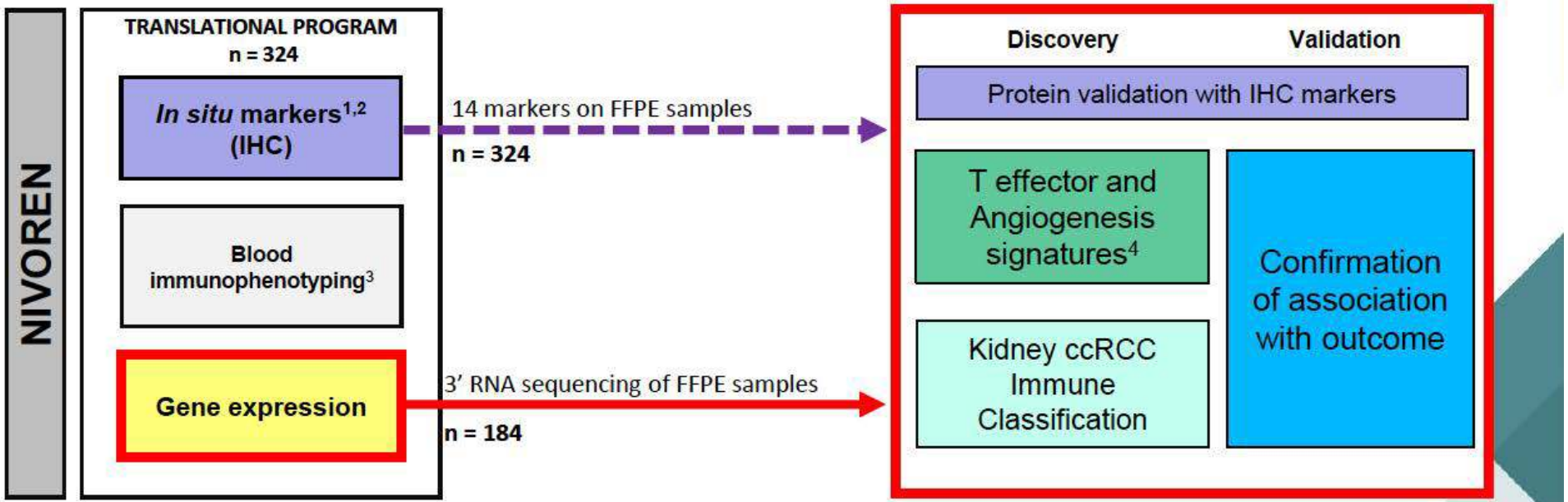
2. Programme Cartes d'Identité des Tumeurs, Ligue Nationale contre le Cancer, F-75013, Paris, France.

GETUG unicancer

2020

Material and Methods

Nivoren Translational Program



In situ markers and blood immunophenotyping preliminary results previously reported[1,2,3]

[1] *Vano YA. et al. ESMO 2019*
[2] *Vano YA. et al. ASCO-GU 2020*
[3] *Desnoyers A. et al. ESMO 2019*
[4] *McDermott, D.F., Huseni, M.A., Atkins, M.B. et al. Nat Med (2018).*

nature medicine

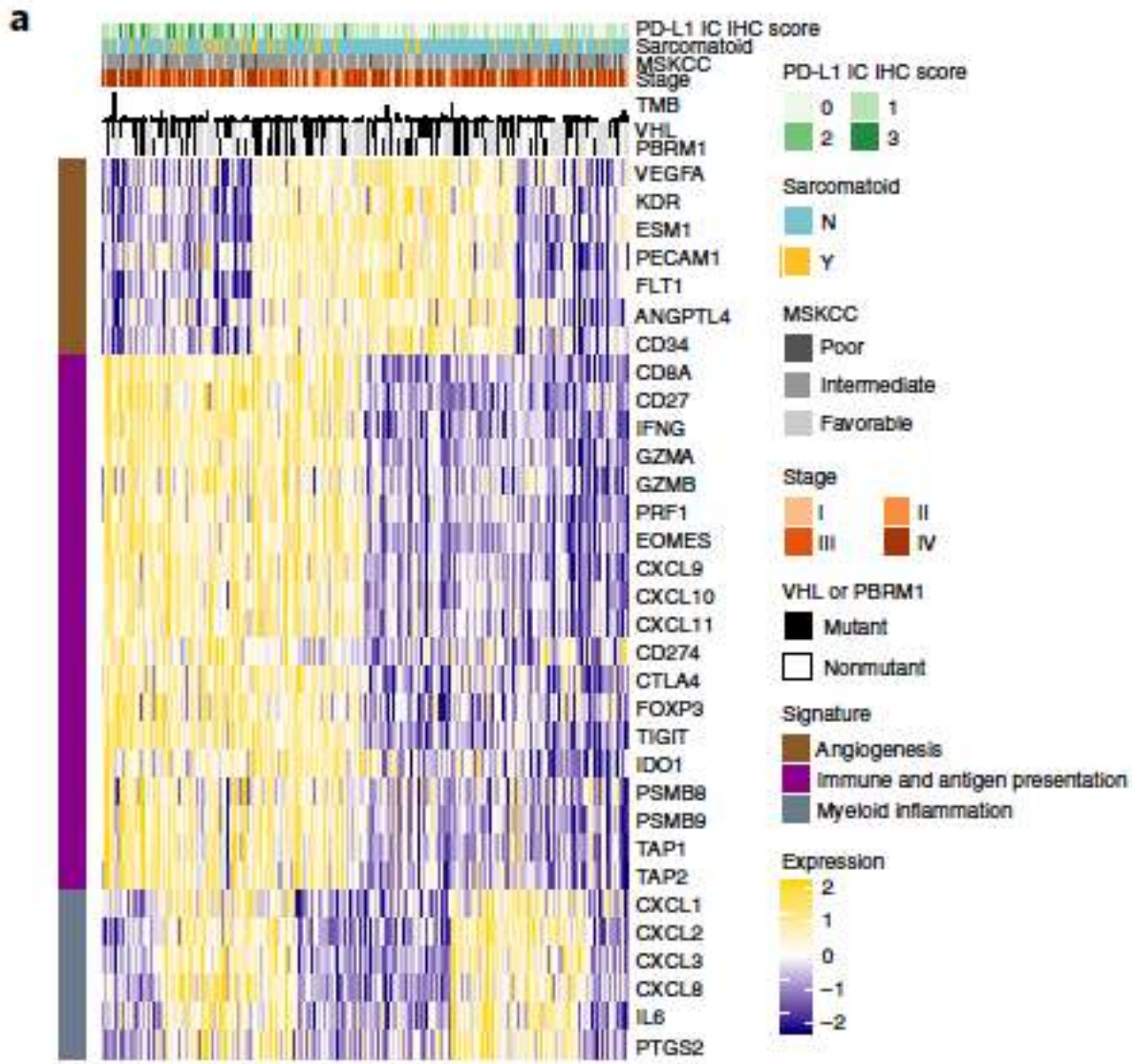
ARTICLES

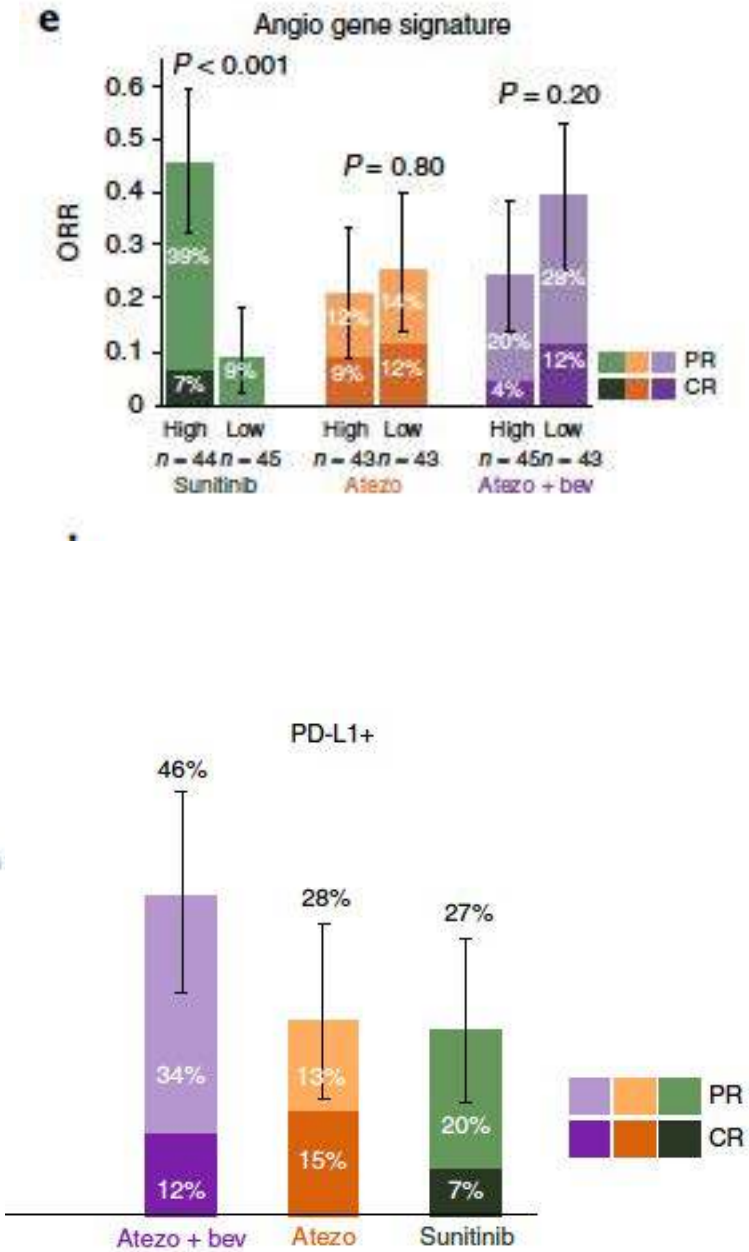
https://doi.org/10.1038/s41591-018-0053-3

Corrected: Publisher Correction

Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma

David F. McDermott[1*], Mahrukh A. Huseni[2], Michael B. Atkins[3], Robert J. Motzer[4], Brian I. Rini[5], Bernard Escudier[6], Lawrence Fong[7], Richard W. Joseph[8], Sumanta K. Pal[9], James A. Reeves[10], Mario Sznol[11], John Hainsworth[12], W. Kimryn Rathmell[13], Walter M. Stadler[14], Thomas Hutson[15], Martin E. Gore[16], Alain Ravaud[17], Sergio Bracarda[18], Cristina Suárez[19], Riccardo Danielli[20], Viktor Gruenwald[21], Toni K. Choueiri[22], Dorothee Nickles[2], Suchit Jhunjhunwala[2], Elisabeth Piault-Louis[2], Alpa Thobhani[23], Jiaheng Qiu[2], Daniel S. Chen[2], Priti S. Hegde[2], Christina Schiff[2], Gregg D. Fine[2] and Thomas Powles[24]


a
PD-L1 IC IHC score
Sarcomatoid
MSKCC
Stage
TMB
VHL
PBRM1
VEGFA
KDR
ESM1
PECAM1
FLT1
ANGPTL4
CD34
CD8A
CD27
IFNG
GZMA
GZMB
PRF1
EOMES
CXCL9
CXCL10
CXCL11
CD274
CTLA4
FOXP3
TIGIT
IDO1
PSMB8
PSMB9
TAP1
TAP2
CXCL1
CXCL2
CXCL3
CXCL8
IL6
PTGS2
PD-L1 IC IHC score
0
1
2
3
Sarcomatoid
N
Y
MSKCC
Poor
Intermediate
Favorable
Stage
I
II
III
IV
VHL or PBRM1
Mutant
Normutant
Signature
Angiogenesis
Immune and antigen presentation
Myeloid inflammation
Expression
2
1
0
−1
−2


e
Angio gene signature
P < 0.001
P = 0.80
P = 0.20
ORR
0.6
0.5
0.4
0.3
0.2
0.1
0
39%
7%
9%
12%
9%
14%
20%
4%
28%
12%
PR
CR
High Low
n = 44 n = 45
Sunitinib
High Low
n = 43 n = 43
Atezo
High Low
n = 45 n = 43
Atezo + bev
PD-L1+
46%
34%
12%
28%
13%
15%
27%
20%
7%
PR
CR
Atezo + bev
Atezo
Sunitinib

Becht *et al. Genome Biology* (2016) 17:218
DOI 10.1186/s13059-016-1070-5

Genome Biology

METHOD Open Access

Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression

Etienne Becht[1,2,3,4], Nicolas A. Giraldo[1,2,3], Laetitia Lacroix[1,2,3], Bénédicte Buttard[1,2,3], Nabila Elarouci[4], Florent Petitprez[1,2,3,4], Janick Selves[5,6], Pierre Laurent-Puig[7], Catherine Sautès-Fridman[1,2,3], Wolf H. Fridman[1,2,3] and Aurélien de Reyniès[4*]

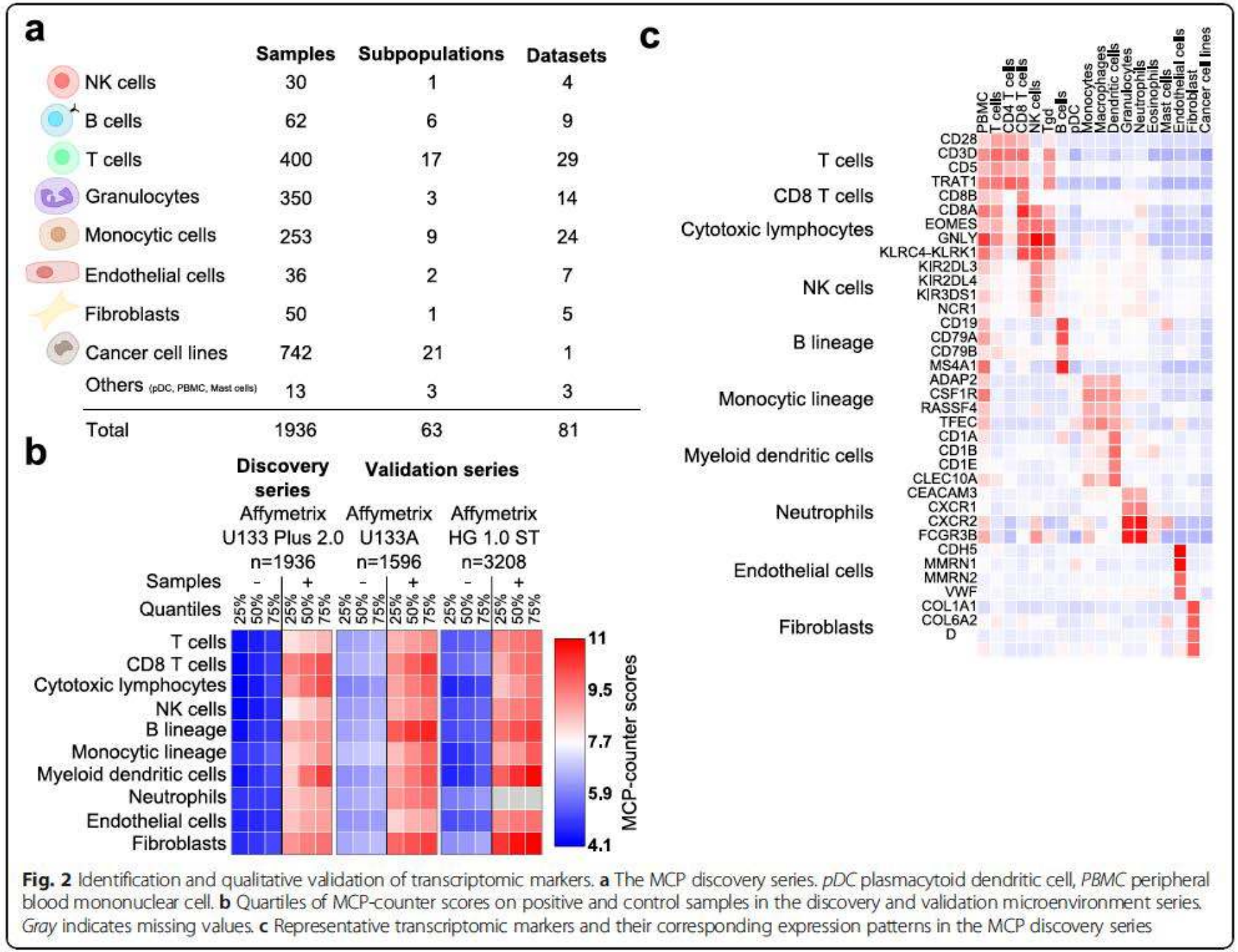
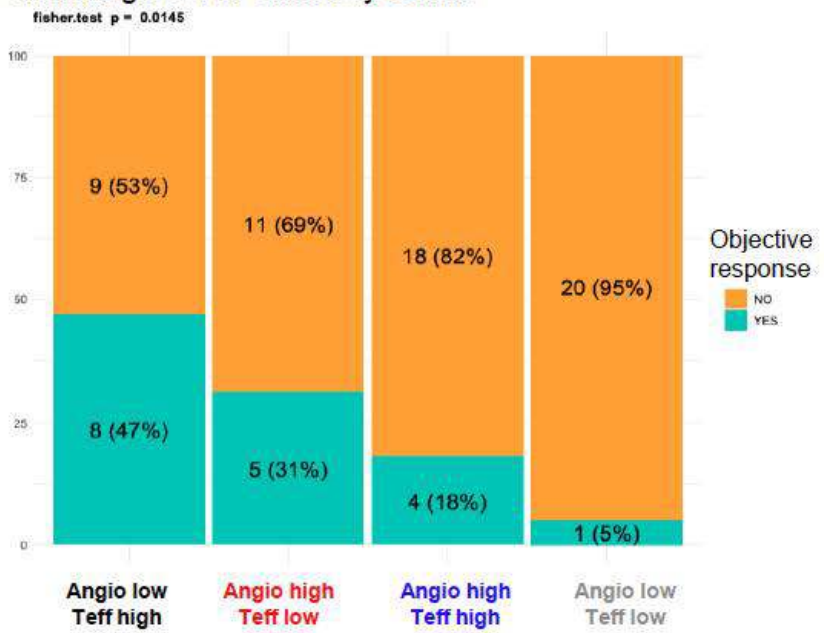


Fig. 2 Identification and qualitative validation of transcriptomic markers. **a** The MCP discovery series. *pDC* plasmacytoid dendritic cell, *PBMC* peripheral blood mononuclear cell. **b** Quartiles of MCP-counter scores on positive and control samples in the discovery and validation microenvironment series. *Gray* indicates missing values. **c** Representative transcriptomic markers and their corresponding expression patterns in the MCP discovery series

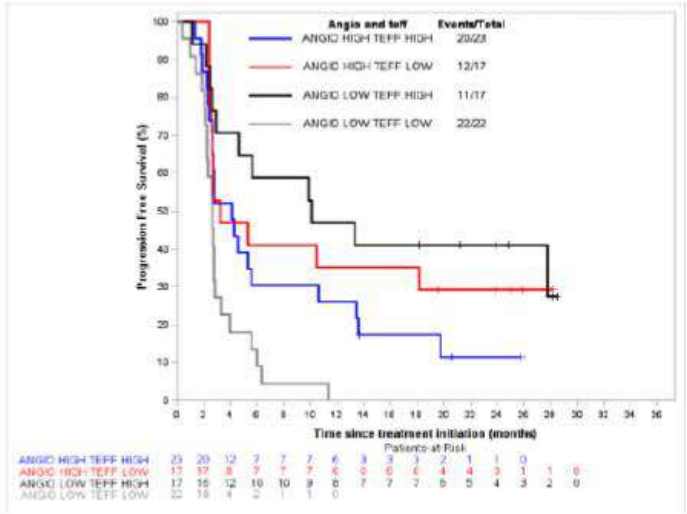
Angiogenesis and T effector signatures* associated with Nivolumab outcome in Discovery cohort (n=79)

ORR Angio & Teff Discovery cohort



PFS Angio & Teff Discovery cohort

*Without IFNG

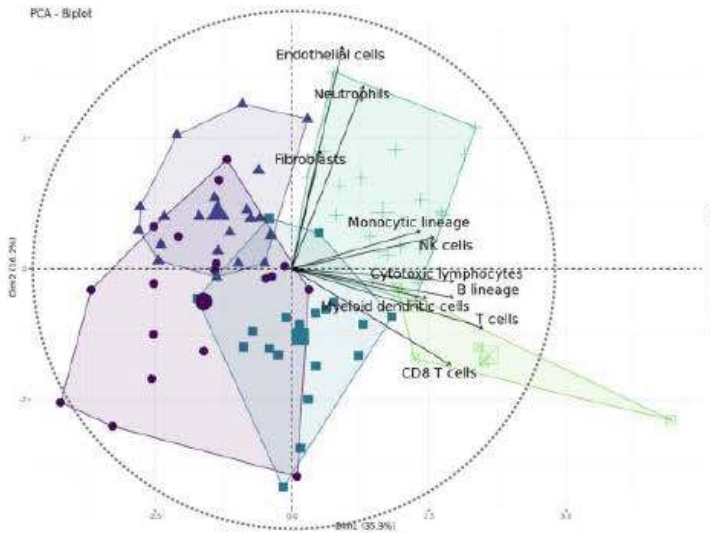


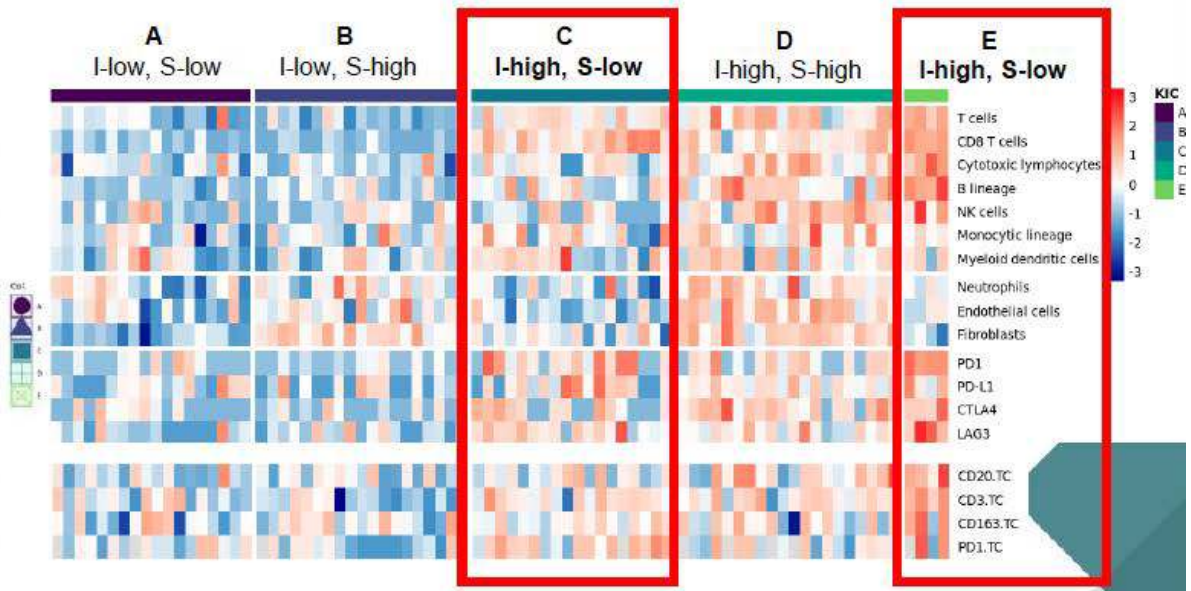
	Angio low Teff high	Angio high Teff high	Angio high Teff low	Angio low Teff low
Median PFS (months)	**10.1**	4.1	3.2	2.6

- Angio **low** Teff **high** associated with good outcomes (ORR + PFS)

Unsupervised clustering identified 5 KIC* subtypes in Discovery cohort (n=79)

KIC*: Kidney ccRCC Immune Classification





- PCA identified two main components:
  - **-Stromal** component (fibroblast, endothelial cells, neutrophils)
  - **-Immune** component (CD8 T cells, B cells)
- KIC* was confirmed on the KIRC TCGA cohort (n=530, data not shown)
- KIC* subtypes TME composition estimated by gene expression matches cell densities measured by IHC
- KIC* C & E displayed similar Immune-high, Stromal-low phenotype

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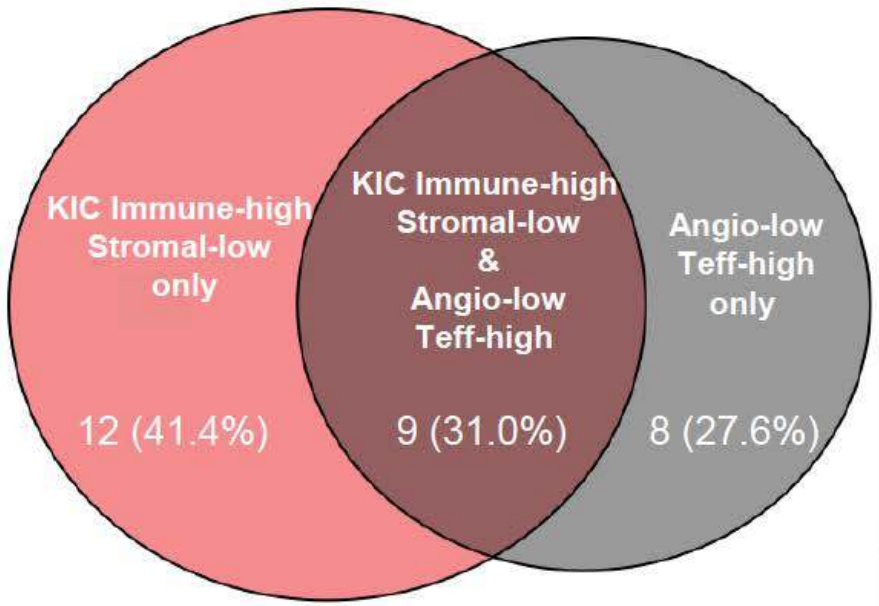
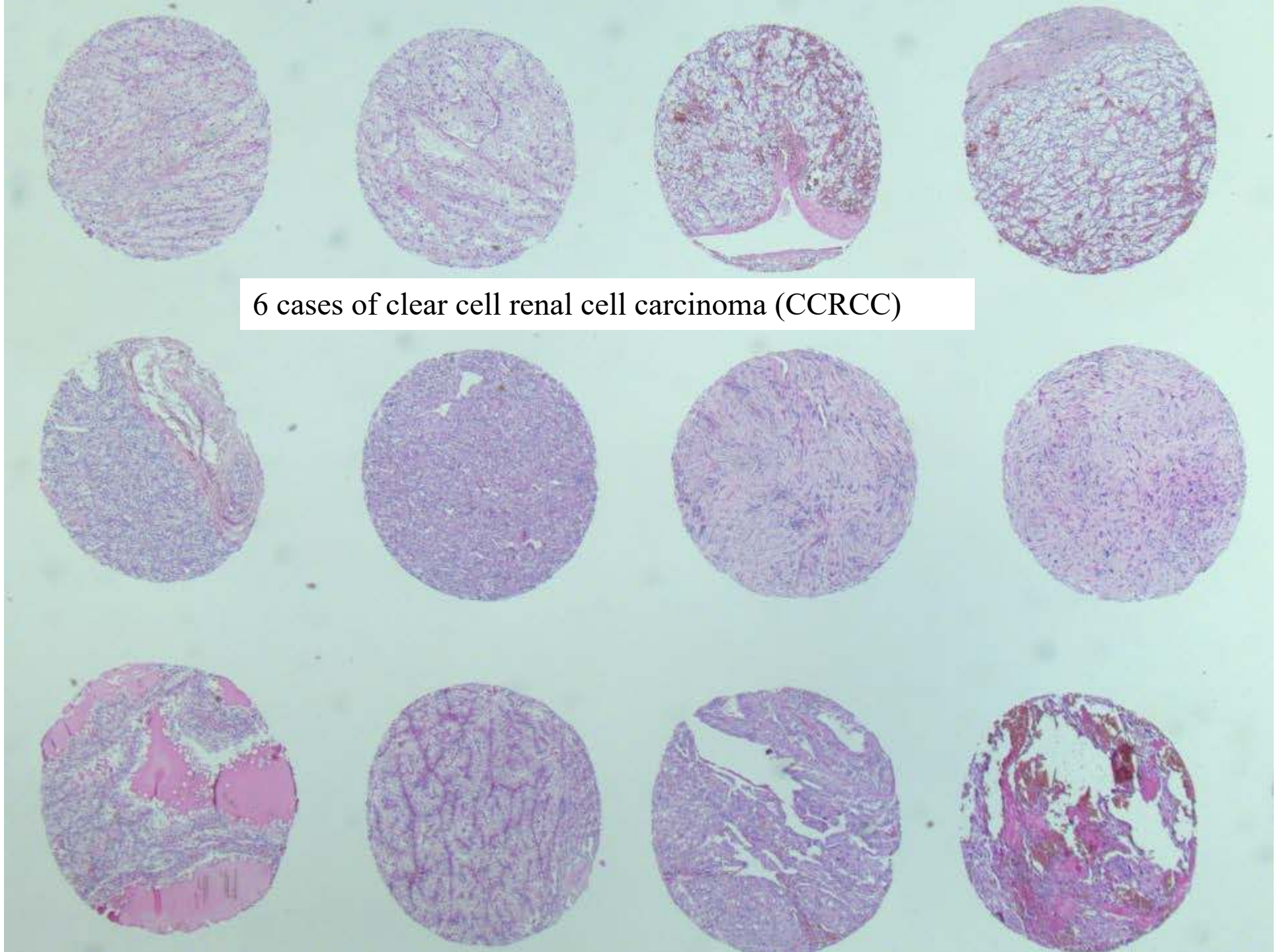
Only partial overlap is observed between KIC* Immune-high, Stromal-low and Angio-low, Teff-high tumors

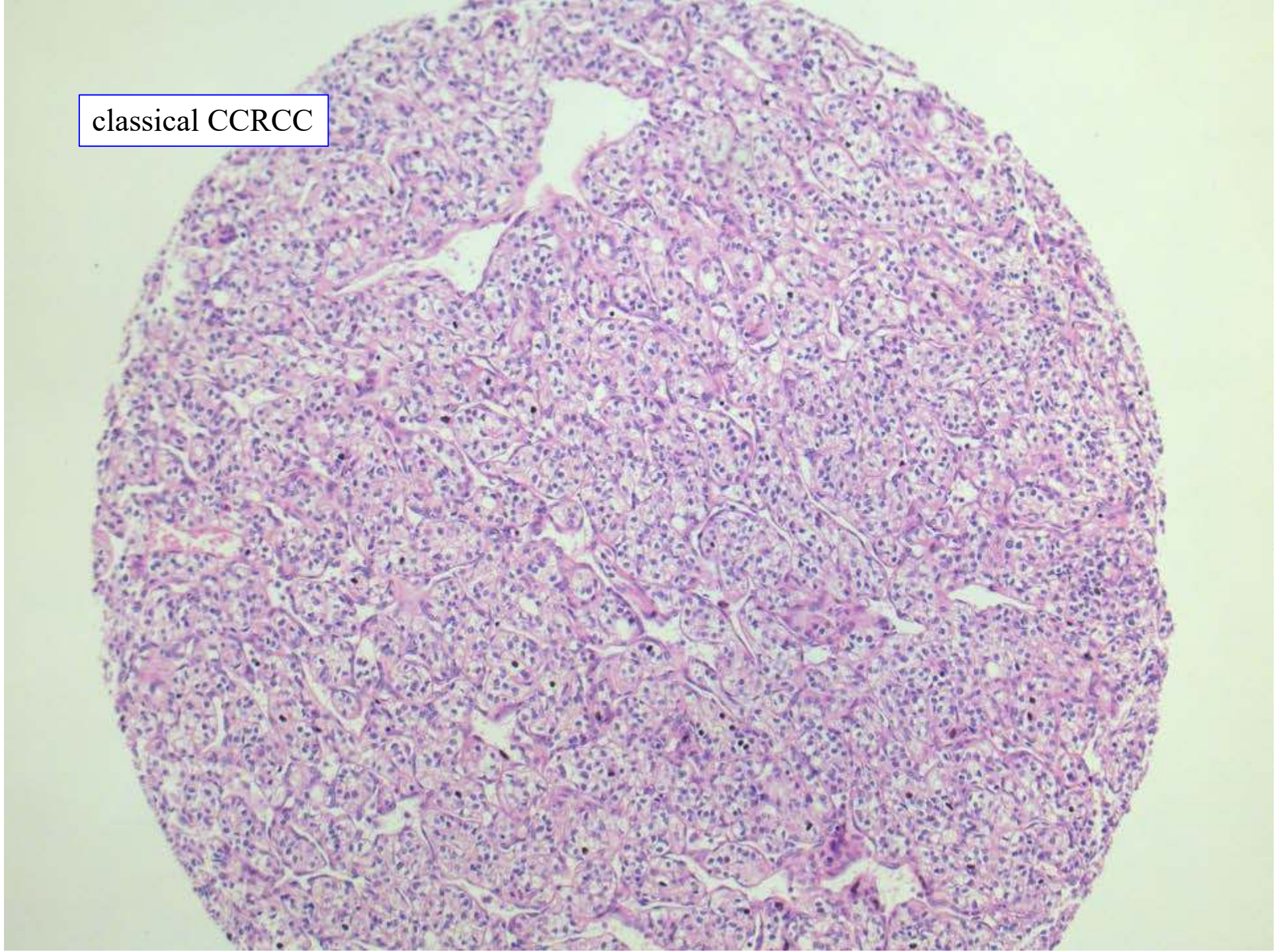
- 31% overlap between KIC Immune-high, Stromal-low and Angio-low, Teff-high
- Angio-low, Teff-high and KIC KIC Immune-high, Stromal-low mostly identify different tumors

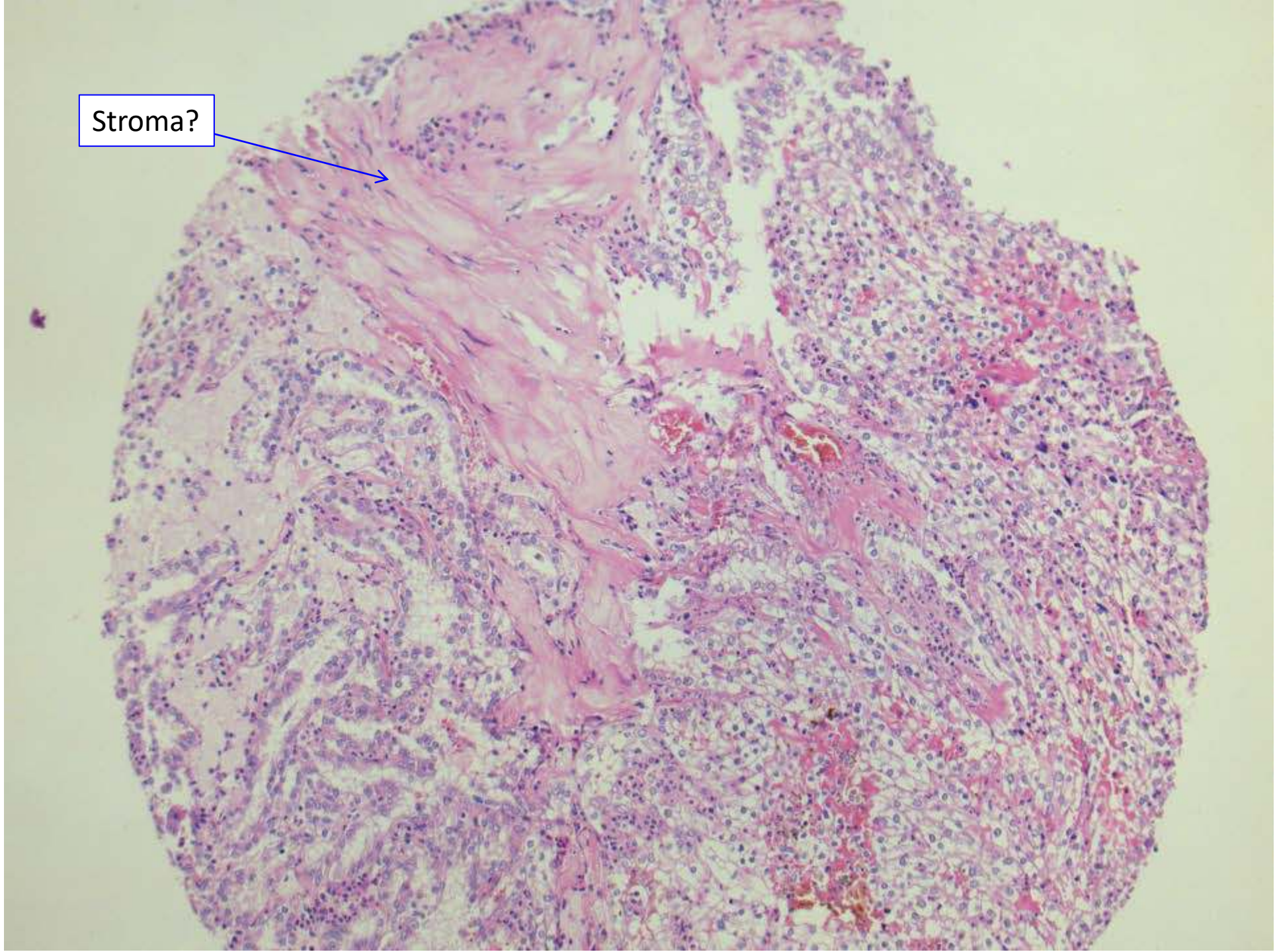
KIC*: Kidney ccRCC Immune Classification

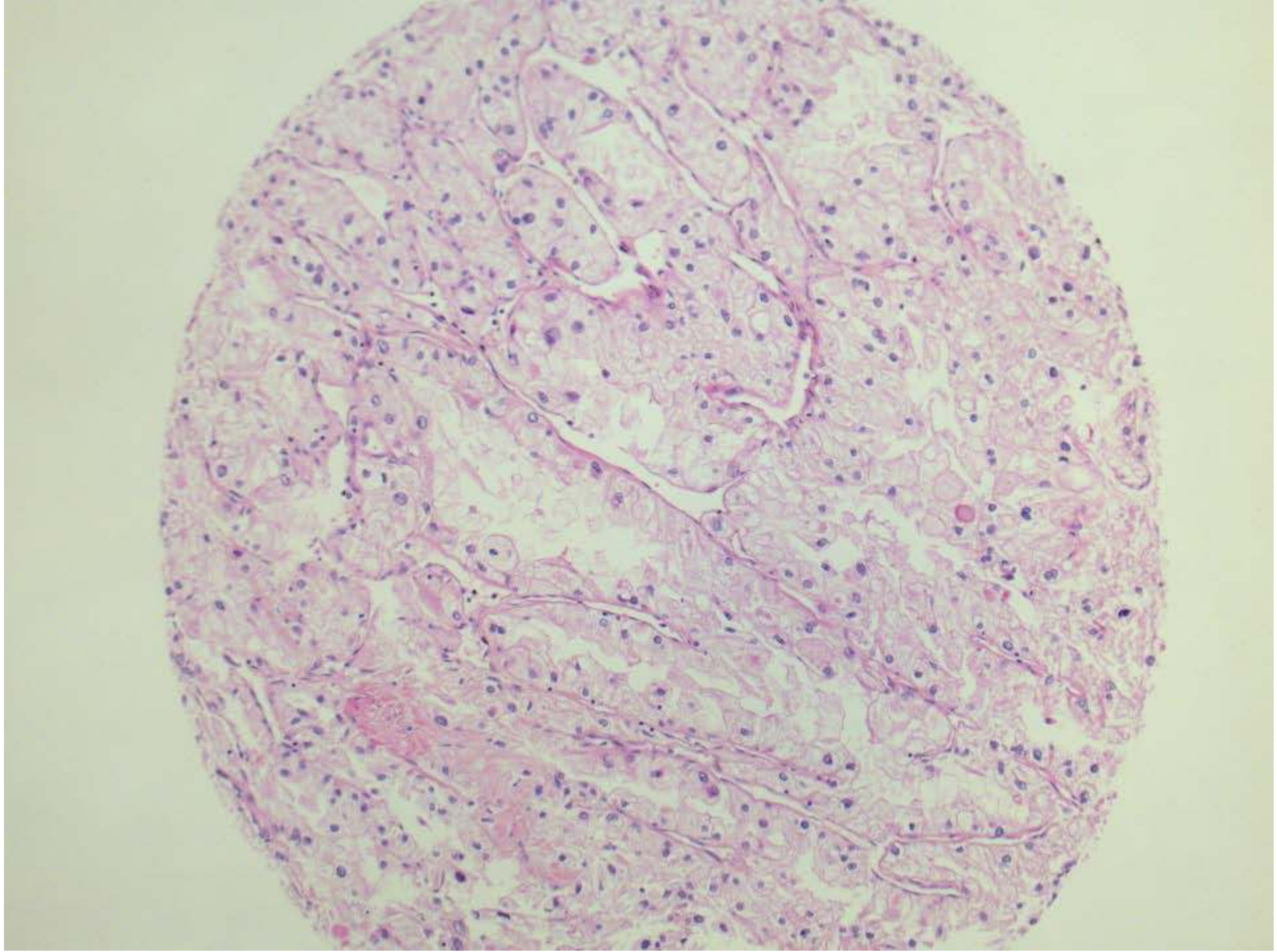


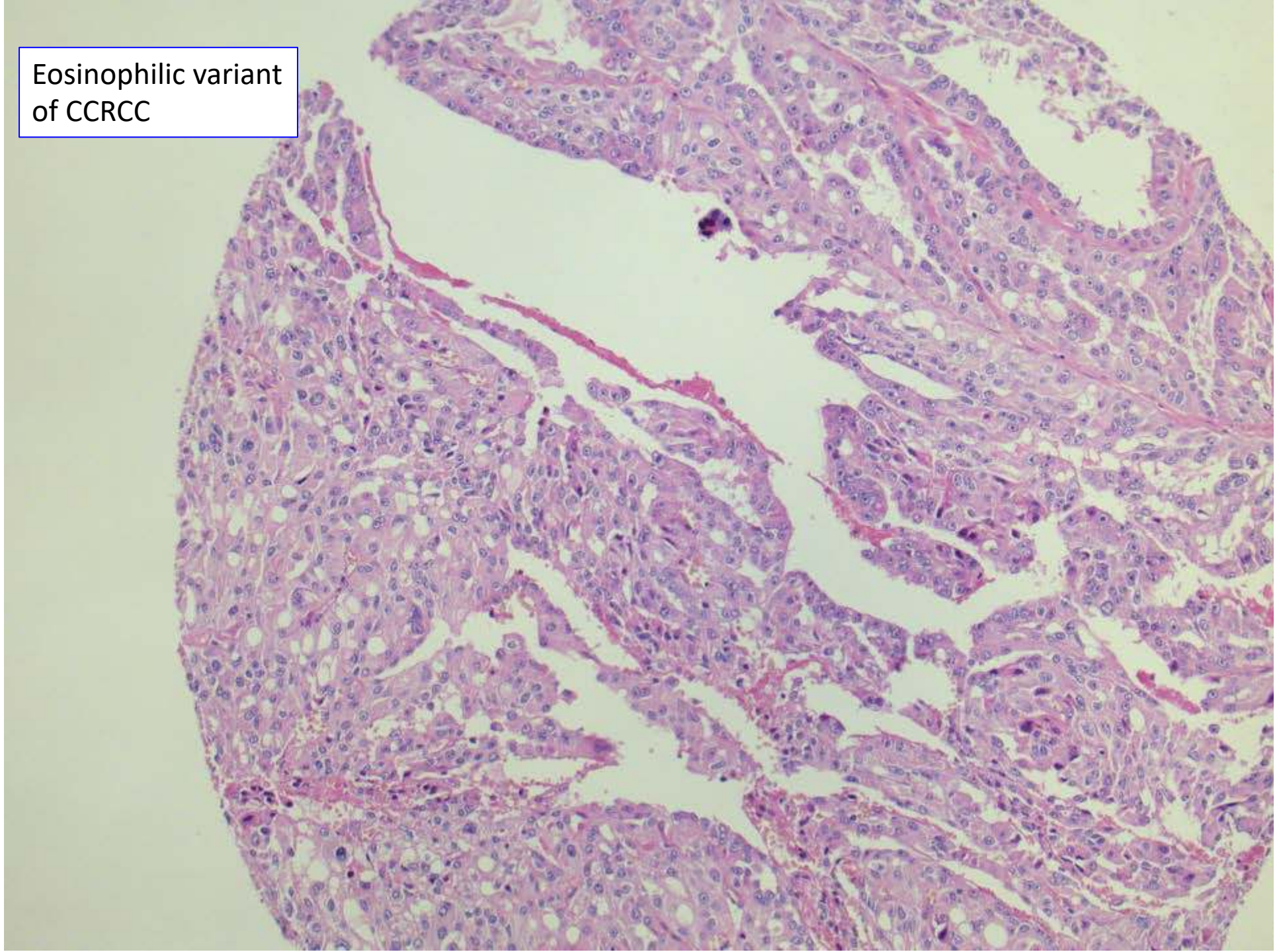


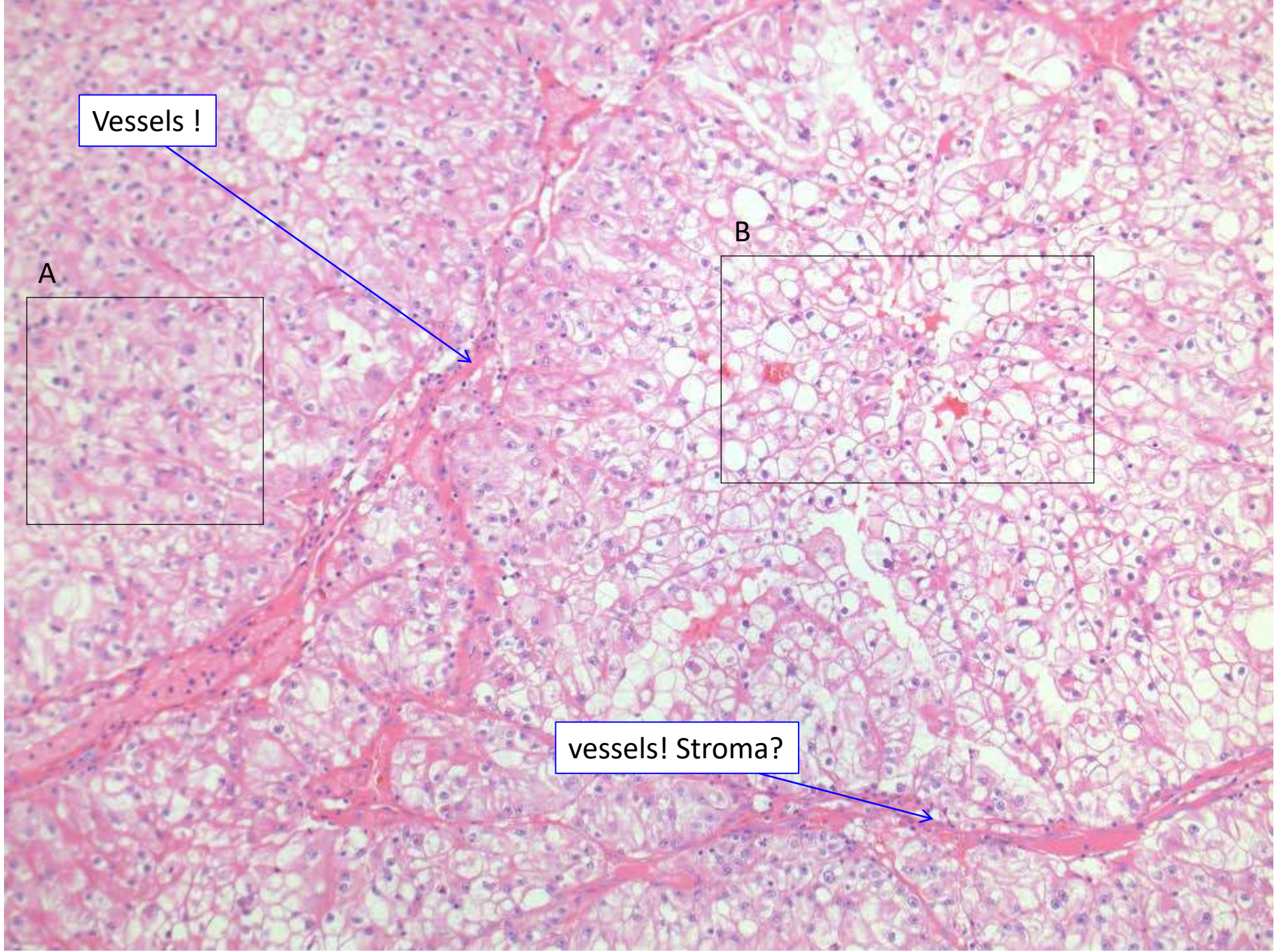
6 cases of clear cell renal cell carcinoma (CCRCC)

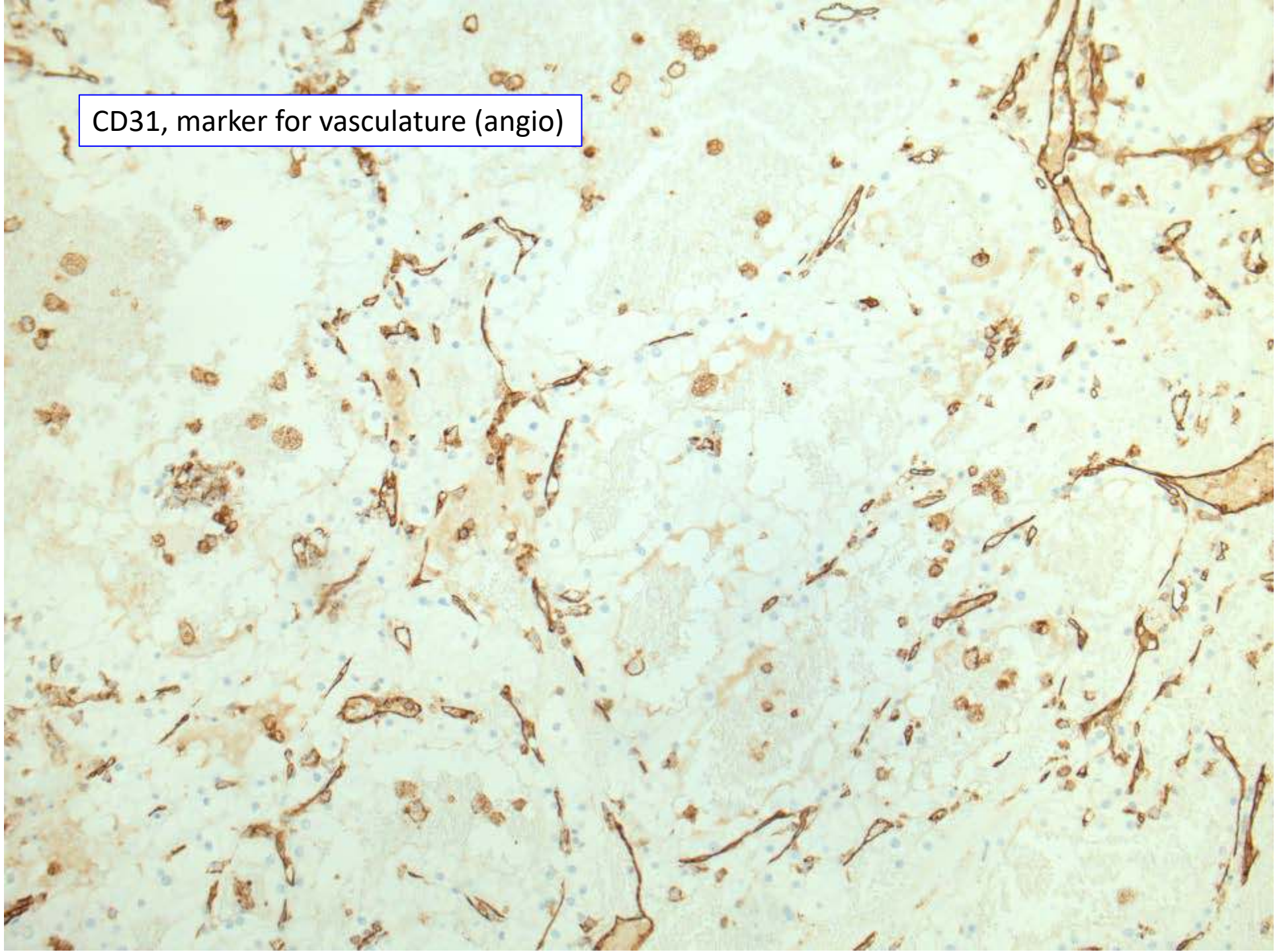

classical CCRCC

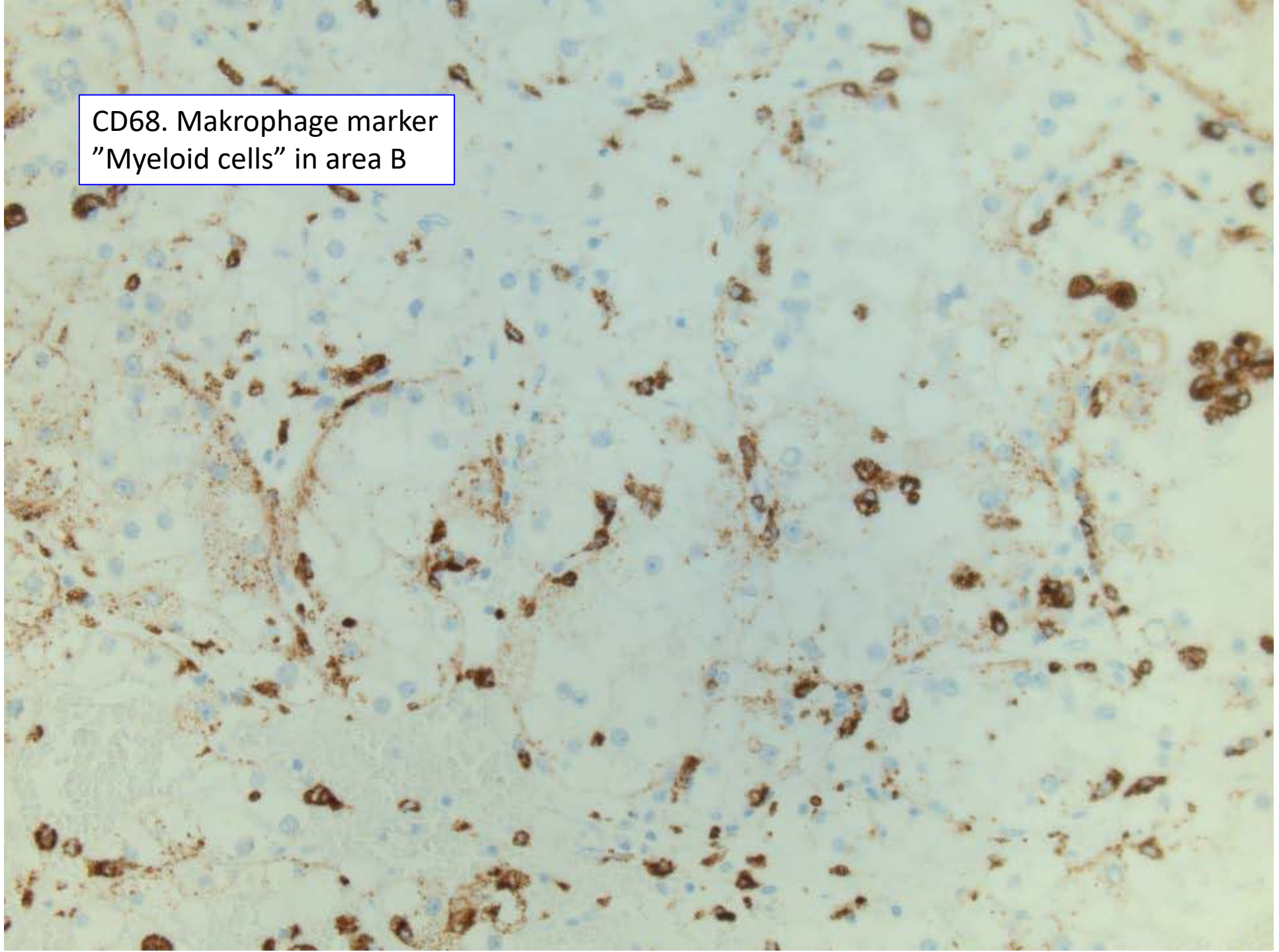

Stroma?

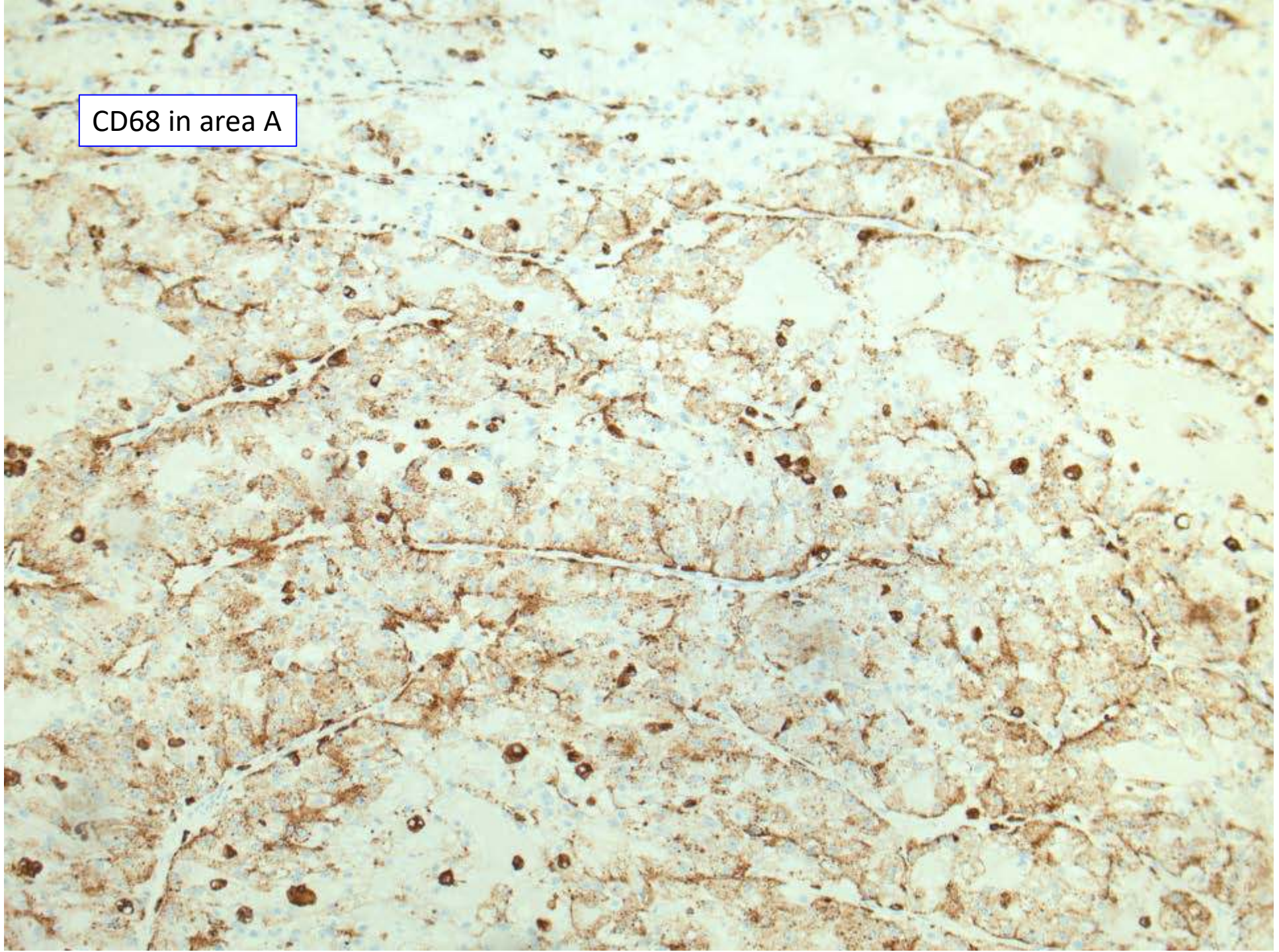


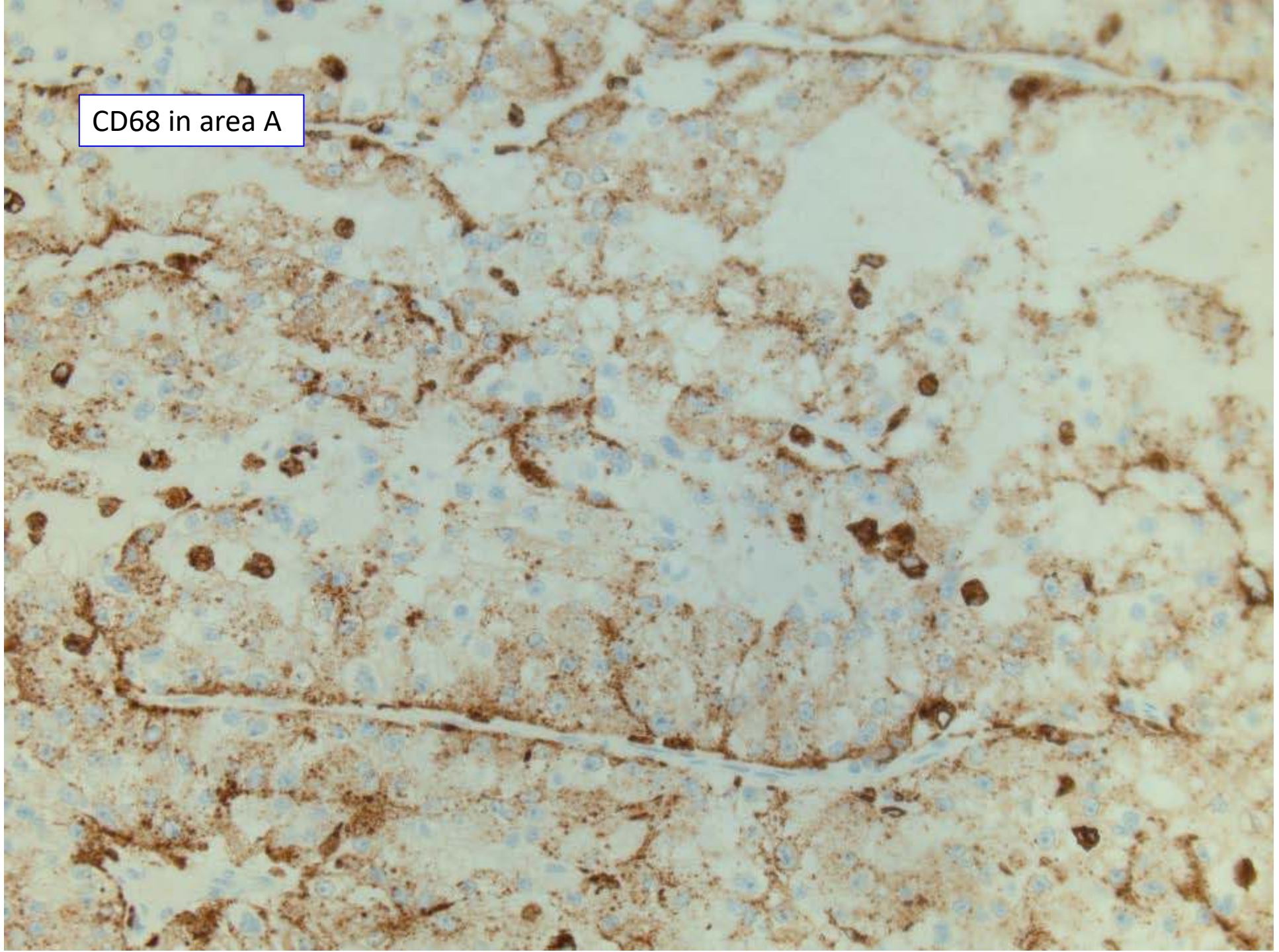

Eosinophilic variant
of CCRCC

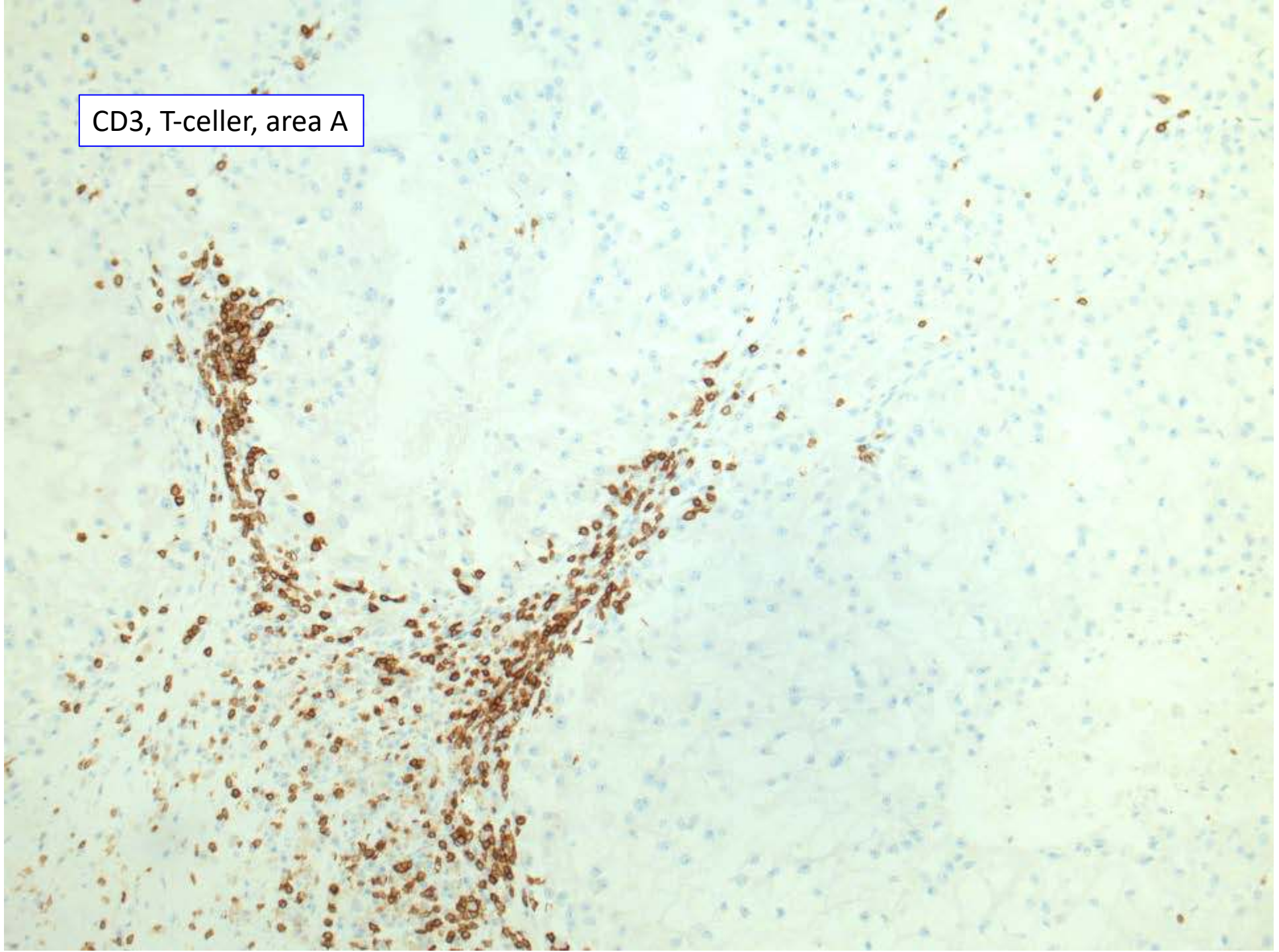

Vessels !
A
B
vessels! Stroma?

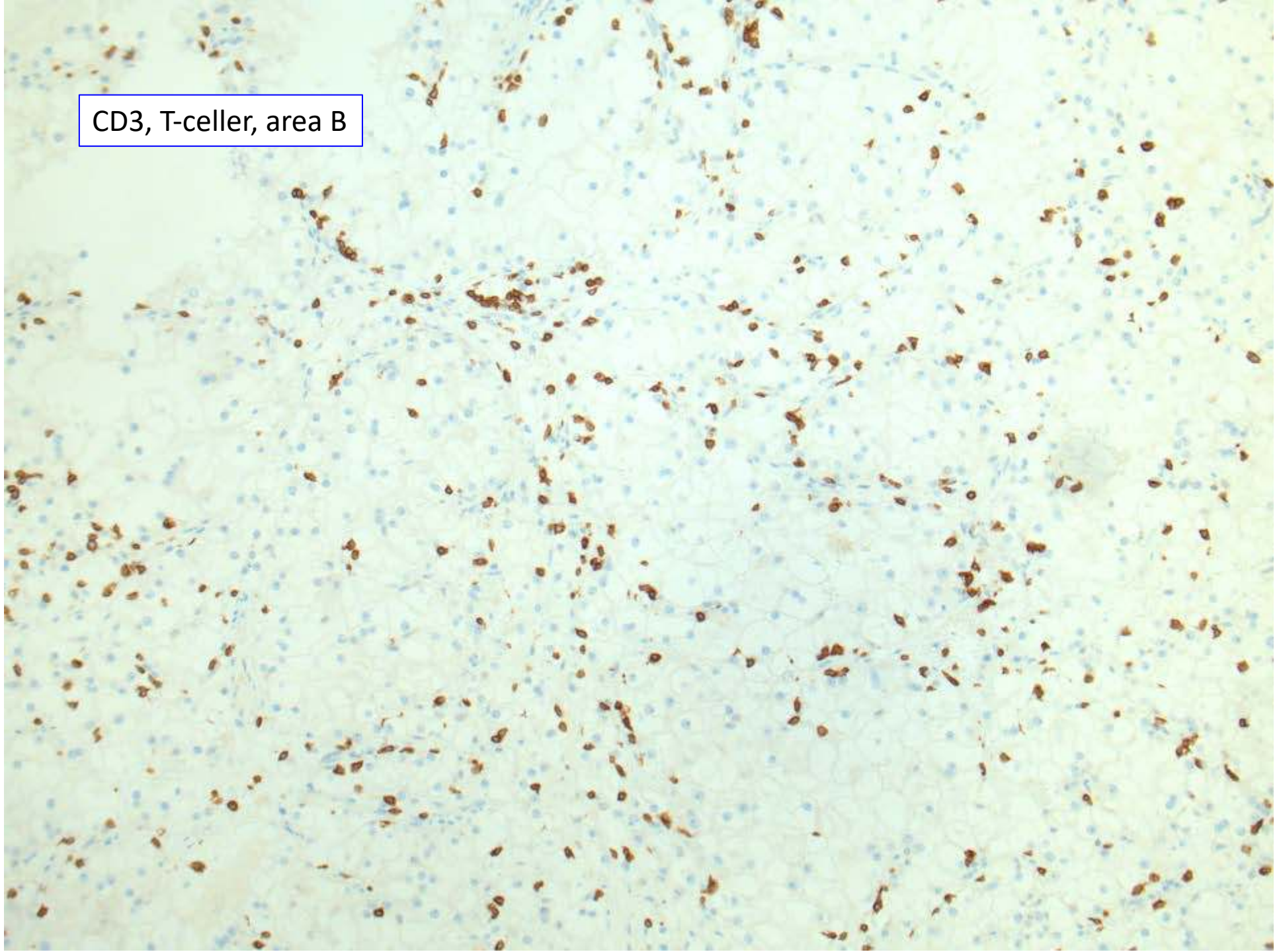

CD31, marker for vasculature (angio)


CD68. Makrophage marker
”Myeloid cells” in area B


CD68 in area A


CD68 in area A


CD3, T-celler, area A


CD3, T-celler, area B

Summing up:

Pathology is still golden standard for diagnosis and grading of malignancies.

Molecular pathology offers important services in diagnosis of mutations in genes of therapeutic importance.

Much of the research into treatment prediction addresses mRNA levels in tumour tissue and here histopathological correlation is an important challenge.

The histological site for actual ***protein expression*** in tumour tissue is probably of highest importance for interpretation and of high impact for prediction.