

ANÁLISIS DE IMAGEN EN INVESTIGACIÓN

Estudio de la microvascularización del neuroblastoma

Irene Tadeo y Rosa Noguera

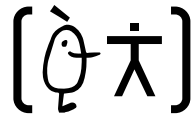
Dpto. Patología, Facultad de Medicina y

Odontología/INCLIVA

Valencia

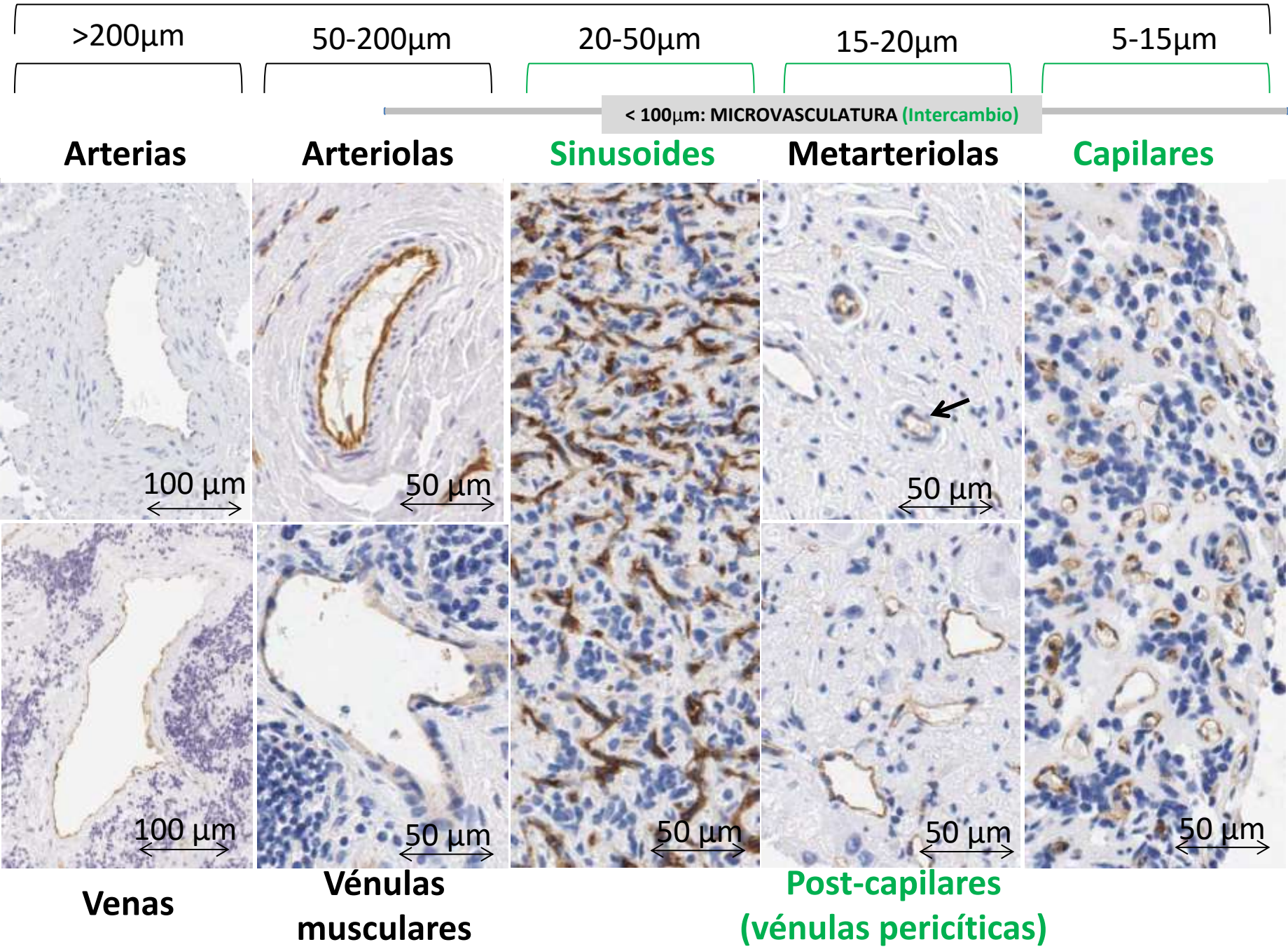


VNIVERSITAT
D VALÈNCIA



Facultat de Medicina i Odontologia
Departament de Patologia

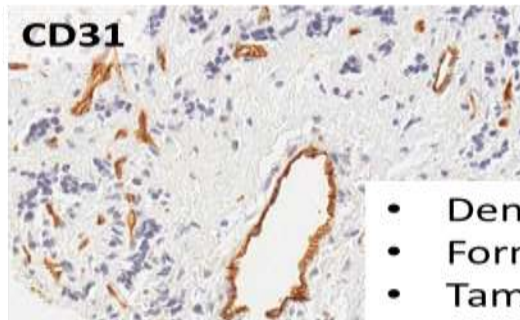
➤ ORGANIZACIÓN DEL SISTEMA VASCULAR: GLOBAL Y SEGMENTOS



➤ ORGANIZACIÓN DEL SISTEMA VASCULAR: GLOBAL Y SEGMENTOS

SISTEMA VASCULAR

- Vasos sanguíneos totales
- Diferenciación por segmentos:
 - 5-15 μ m, capilares
 - 15-20 μ m, post-capilares / metarteriolas
 - 20-50 μ m, sinusoides
 - 50-200 μ m, vénulas / arteriolas
 - >200 μ m, venas / arterias

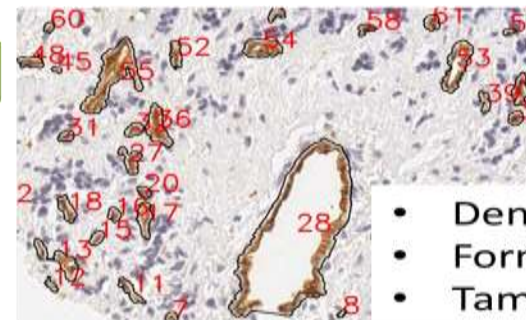


Angiopath

- Densidad \pm
- Forma?
- Tamaño?

PARÁMETROS

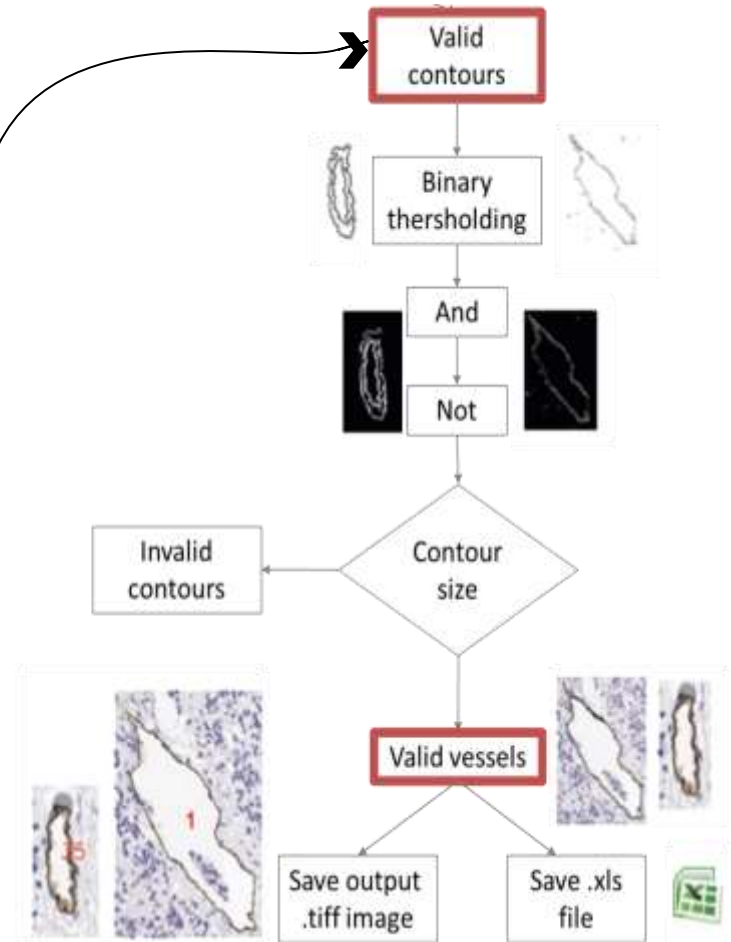
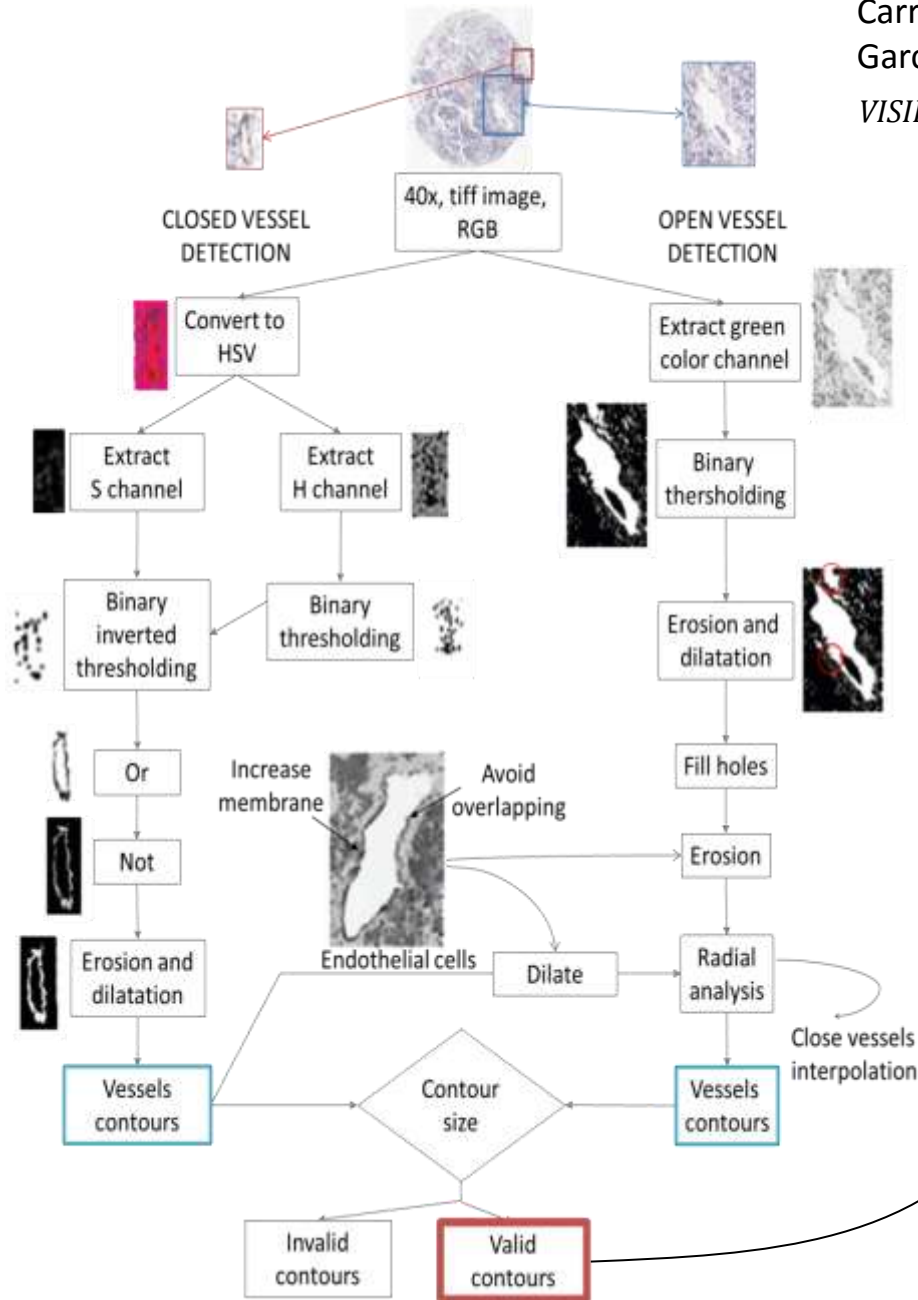
- Densidad:
 - Número de vasos /mm²
 - Área tisular que ocupan
 - Relativa por segmentos
- Tamaño:
 - Área
 - Longitud
 - Anchura
 - Perímetro
- Forma:
 - Aspecto
 - Redondez
 - Perímetro ratio
 - Factor de forma
 - Deformidad



- Densidad
- Forma <
- Tamaño

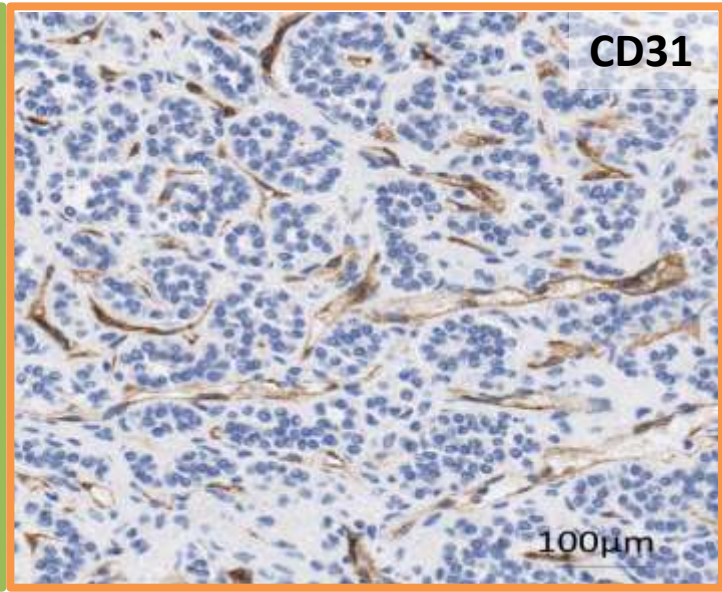
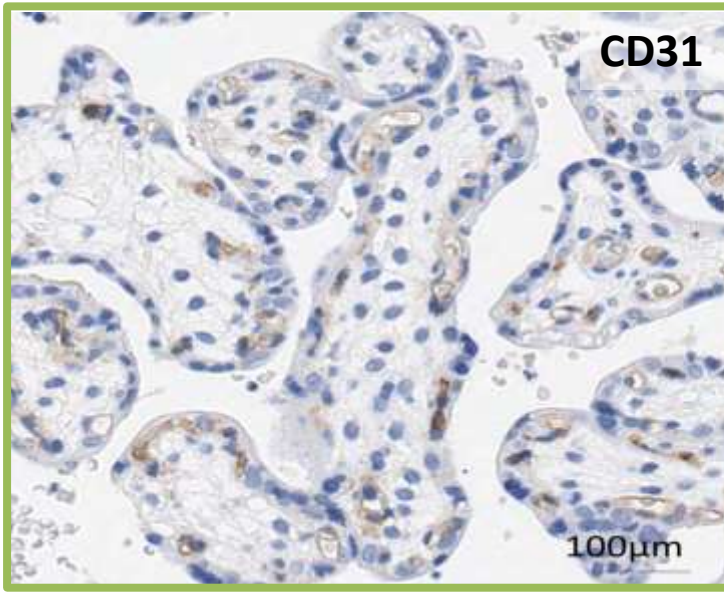
➤ DISEÑO DE LA HERRAMIENTA

TMA vessel segmentation based on color and morphological features: application to angiogenesis research. Fernández-Carrobles MM, Tadeo I, Bueno G, Noguera R, Déniz O, Salido J, García-Rojo M. *Scientific World Journal*. 2013 Dec 5;2013:263190
 VISILAB, E.T.S.I. Industriales, Universidad de Castilla-La Mancha

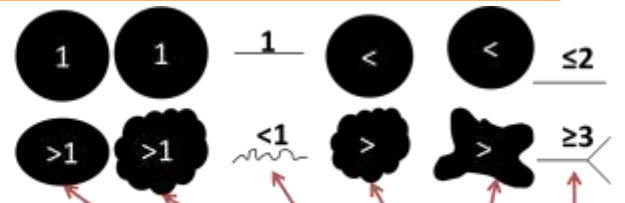


APLICACIÓN DE ANGIOPATH A LAS TMA

CONTROLES



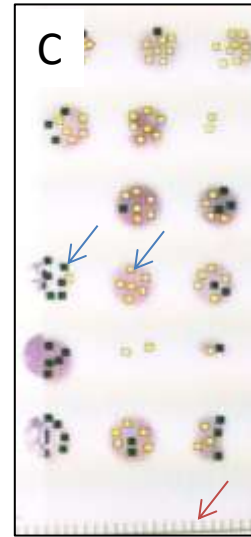
% density of vessels /
% area occupied by the vessels corresponding to a given segment out of the global density/stained area



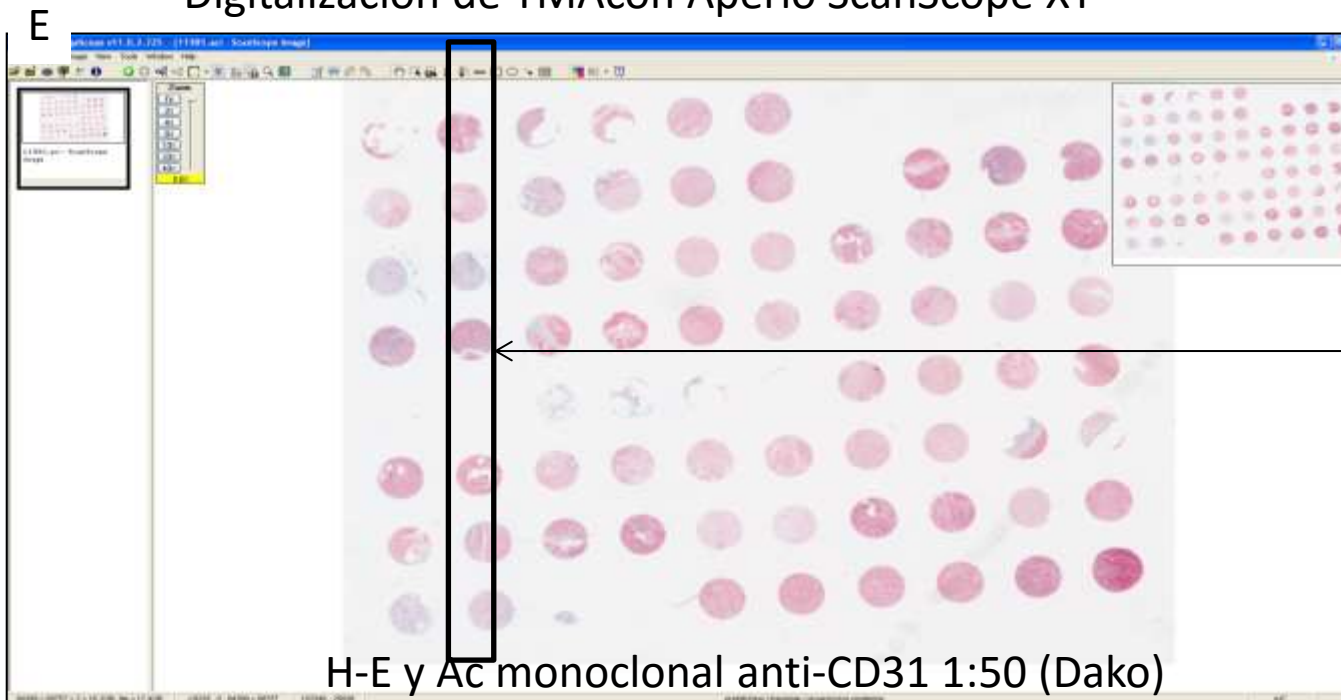
	Blood vessel segment	Density	Stained area (µm²)	% density of vessels / % area occupied by the vessels		Area (µm²)	Length (µm)	Width (µm)	Perimeter (µm)	Aspect	Roundness	Perimeter ratio	Deformity	Shape factor	Branching
				Relative density (%)	Relative stained area (%)										
Placenta	5_15 Capillaries	349	10875	78	41	31	9	5	27	2	2	0,867	107	0,3	2,4
	15_20 Post-cap/metarterioles	46	3753	10	14	82	17	9	52	2,2	2,8	0,821	473	0,45	3,3
	20_50 Sinusoid-like vessels	46	9160	= 10	34	199	28	14	98	2,4	4,1	0,732	1607	0,66	4
	50_200 Venules/arterioles	7	2771	2	10	396	66	15	190	5,3	7,5	0,751	4887	8,94	4,1
	TOTAL Global vascularization	448	26559	100	100	59	13	7	39	2,1	2,4	0,847	374	0,49	2,7
Adrenal gland	5_15 Capillaries	370	14013	50	11	37	9	5	28	1,9	1,8	0,872	106	0,23	2,5
	15_20 Post-cap/metarterioles	109	9683	+ 15	8	88	17	8	51	2,3	2,5	0,825	380	0,34	3,2
	20_50 Sinusoid-like vessels	214	47385	29	40	221	30	13	100	2,6	3,8	0,763	1711	1,28	3,9
	50_200 Venules/arterioles	51	45336	6	38	888	80	25	283	3,7	8,4	0,666	11995	15,92	5,3
	TOTAL Global vascularization	745	118563	100	100	159	21	9	70	2,3	2,9	0,819	1500	1,63	3,2



➤ APLICACIÓN DE ANGIOPATH A LAS TMA_s DE NB

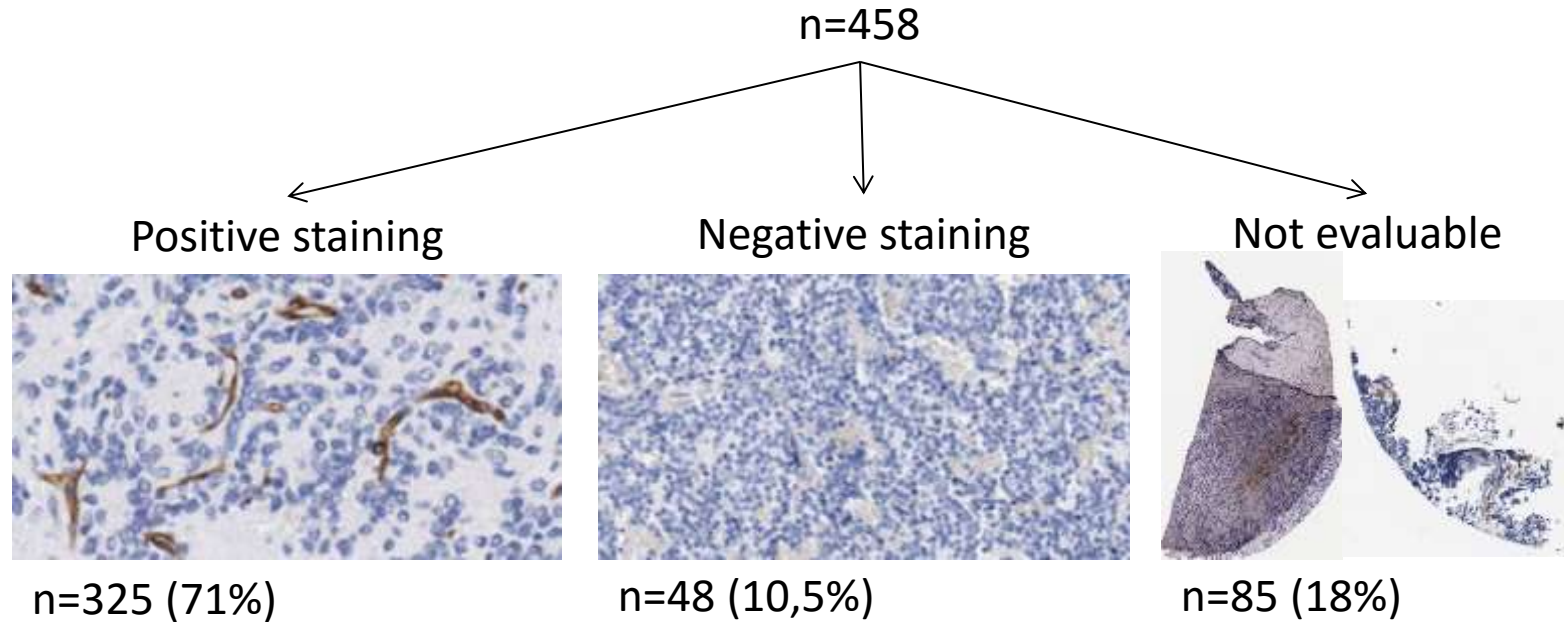


Digitalización de TMA con Aperio ScanScope XT



H-E y AC monoclonal anti-CD31 1:50 (Dako)

➤ APLICACIÓN DE ANGIOPATH A LAS TMA_s DE NB



→ n= 325 (100%) have capillaries (5-15 μ m), 67.7% of the total vessels.

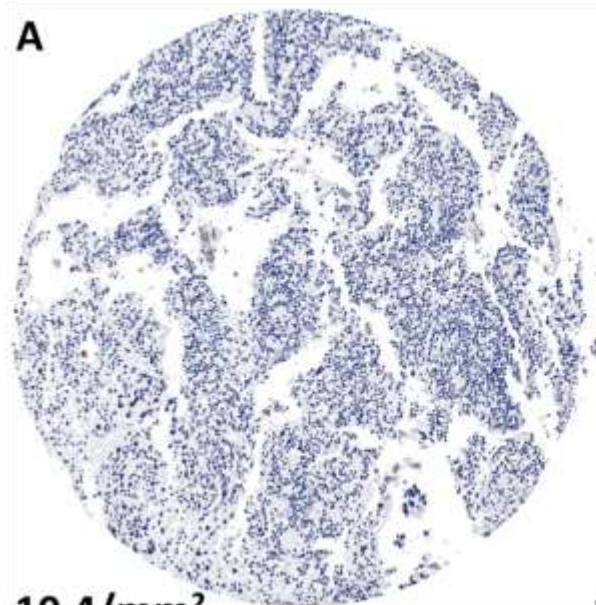
→ n= 319 (98%) have post-capillary venules /metarterioles (15-20 μ m), 8%, “ “.

→ n= 319 (98%) have sinusoids(20-50 μ m), 10% “ “.

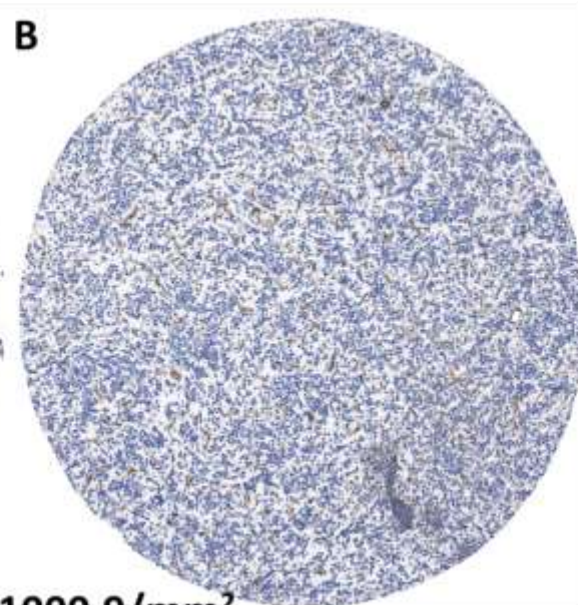
→ n= 268 (83%) have venules and arterioles (50-200 μ m), 1.7% “ “.

→ n= 55 (18%) have veins and arteries (>200 μ m), 0,1% “ “.

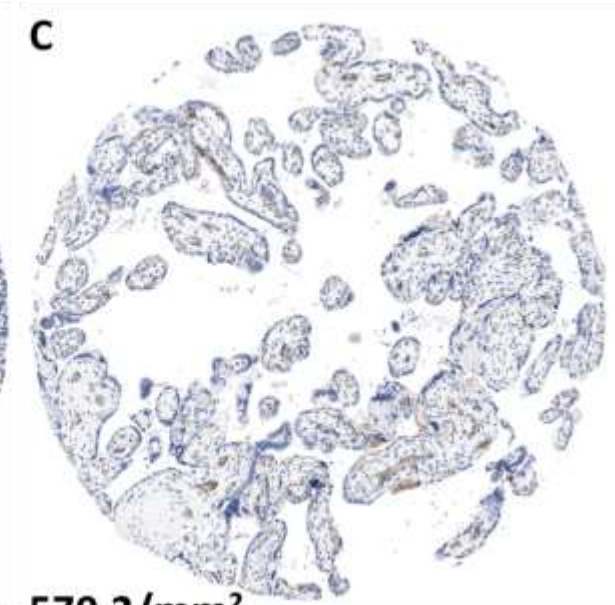
➤ APLICACIÓN DE ANGIOPATH A LAS TMA_s DE NB



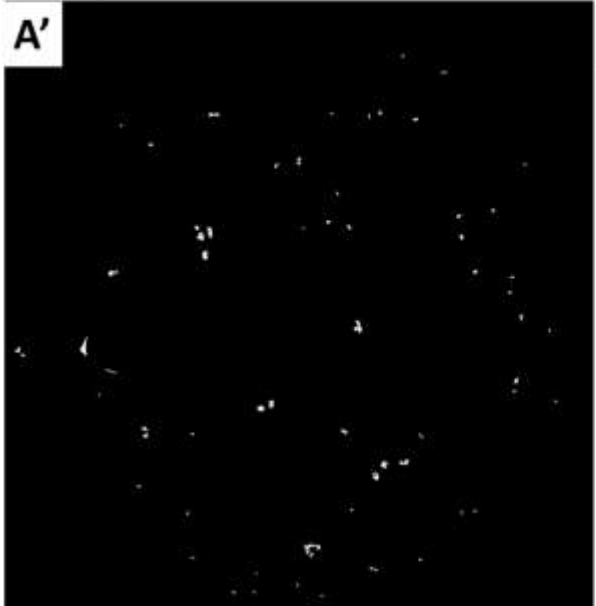
10.4/mm²



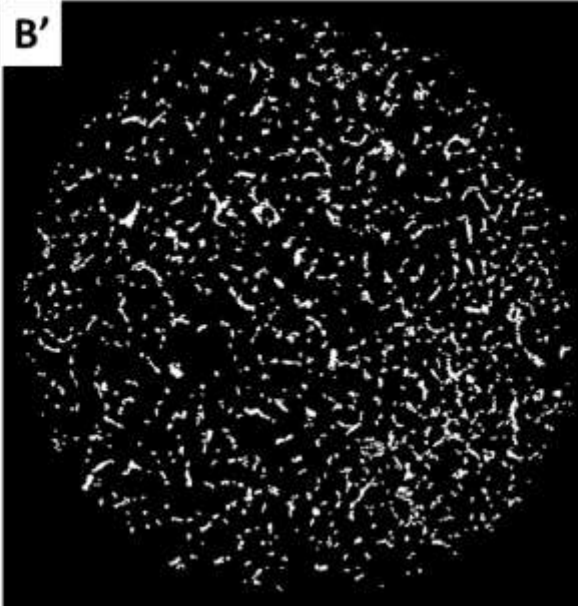
1000.9/mm²



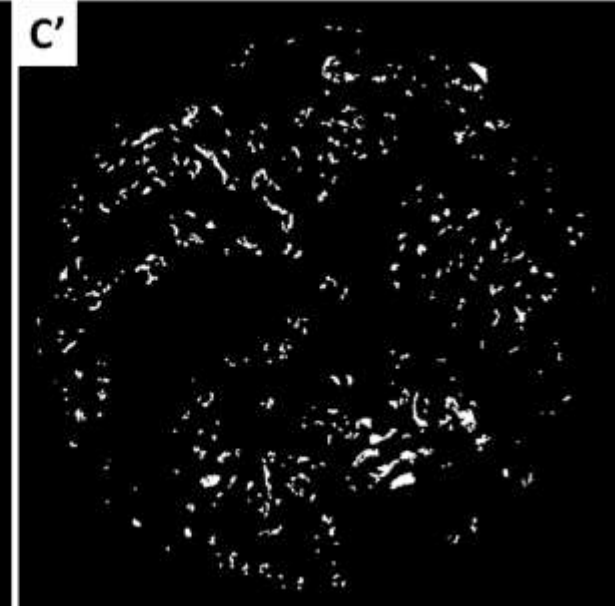
570.2/mm²



A'



B'



C'

INTEGRACIÓN CON LOS DATOS CLINICOBIOLOGICOS

The international Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report, Cohn et al, J Clin Oncol 2009

INRG Stage	Age (months)	Histologic Category	Grade of Tumor Differentiation	MYCN	11q Aberration	Ploidy	Pretreatment Risk Group
L1/L2		GN maturing; GNB intermixed					A Very low
L1		Any, except GN maturing or GNB intermixed		NA			B Very low
				Amp			K High
L2	< 18	Any, except GN maturing or GNB intermixed		NA	No		D Low
					Yes		G Intermediate
	≥ 18	GNB nodular; neuroblastoma	Differentiating Poorly differentiated or undifferentiated	NA	No		E Low
					Yes		H Intermediate
					Amp		N High
M	< 18			NA		Hyperdiploid	F Low
	< 12			NA		Diploid	I Intermediate
	12 to < 18			NA		Diploid	J Intermediate
	< 18			Amp			O High
	≥ 18						P High
MS	< 18			NA	No		C Very low
					Yes		Q High
					Amp		R High

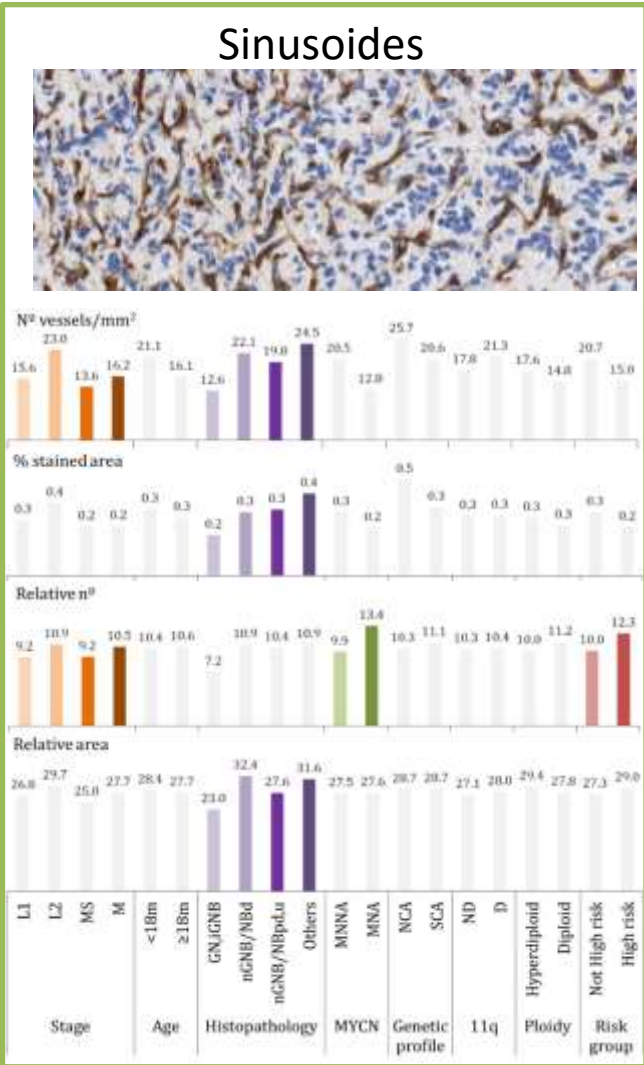
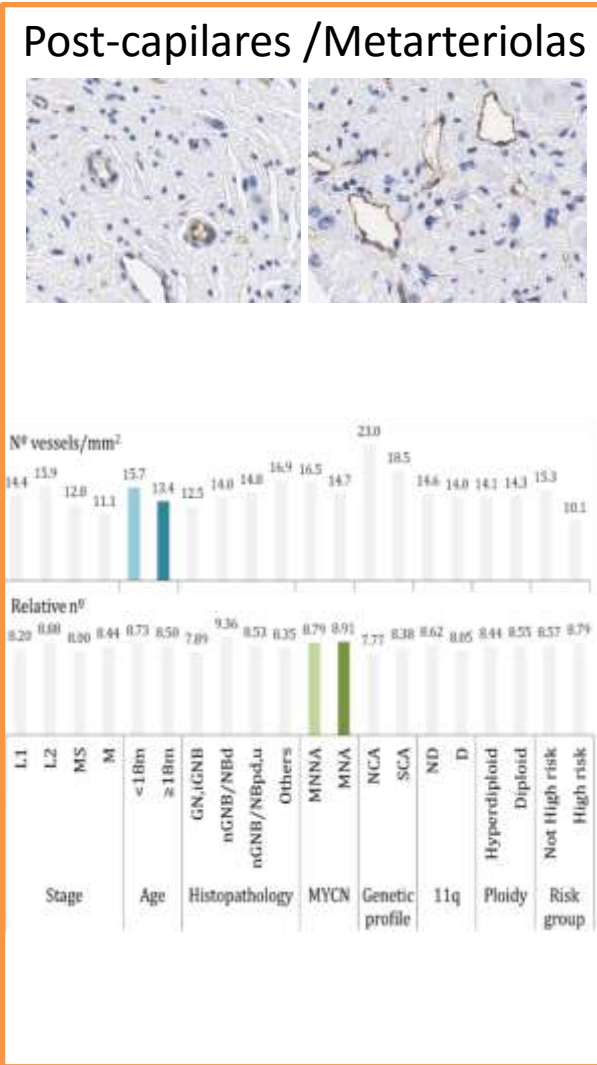
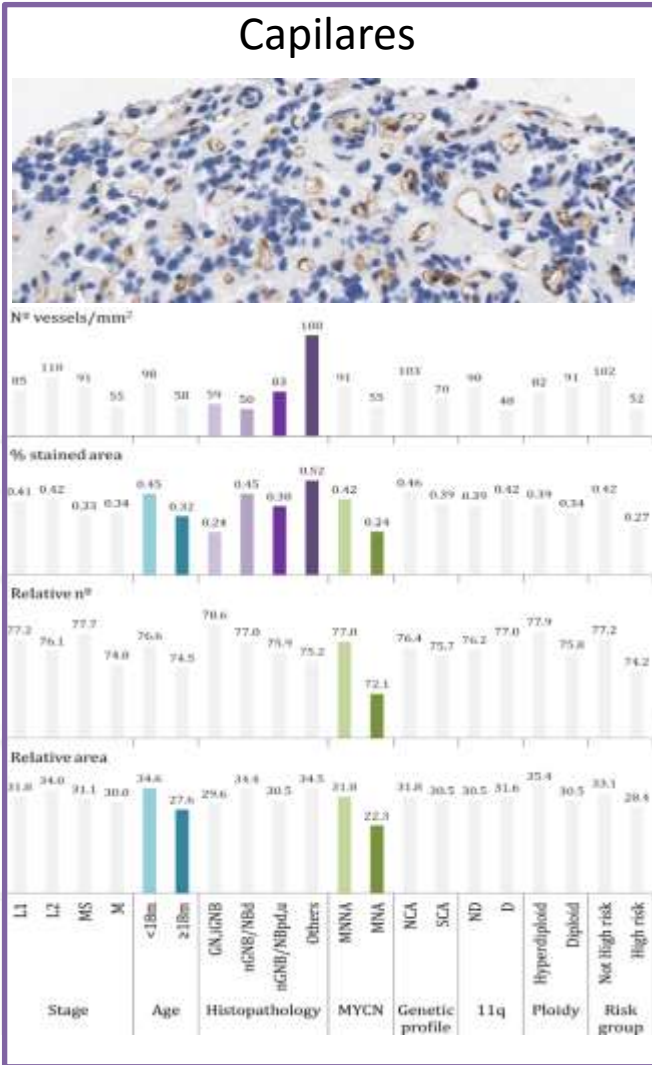
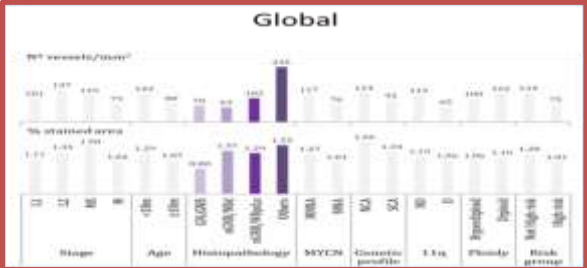


[NeuPAT: an intranet database supporting translational research in neuroblastic tumors.](#)

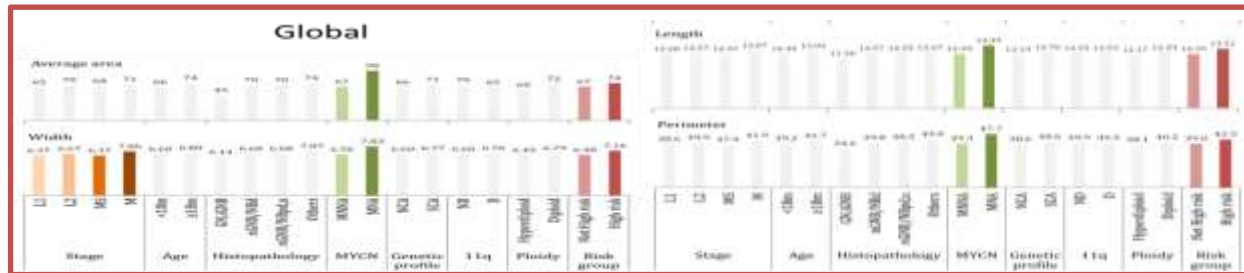
Villamón E, Piqueras M, Meseguer J, Blanquer I, Berbegall AP, Tadeo I, Hernández V, Navarro S, Noguera R.

Comput Biol Med. 2013 Mar;43(3):219-28

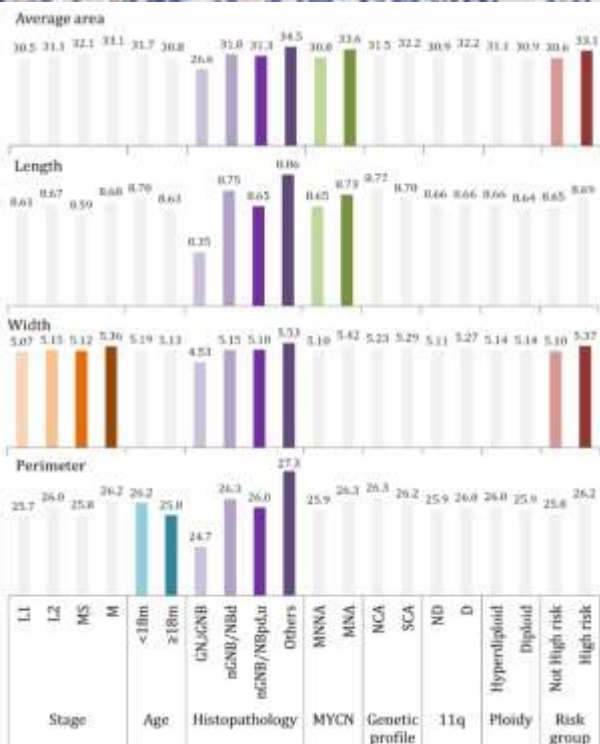
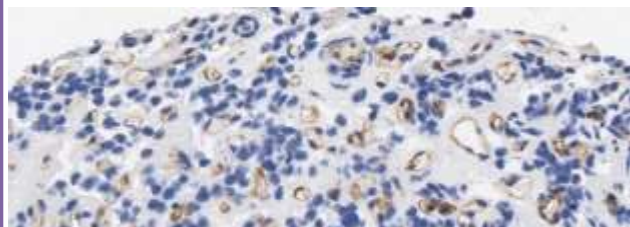
Parámetros de CANTIDAD



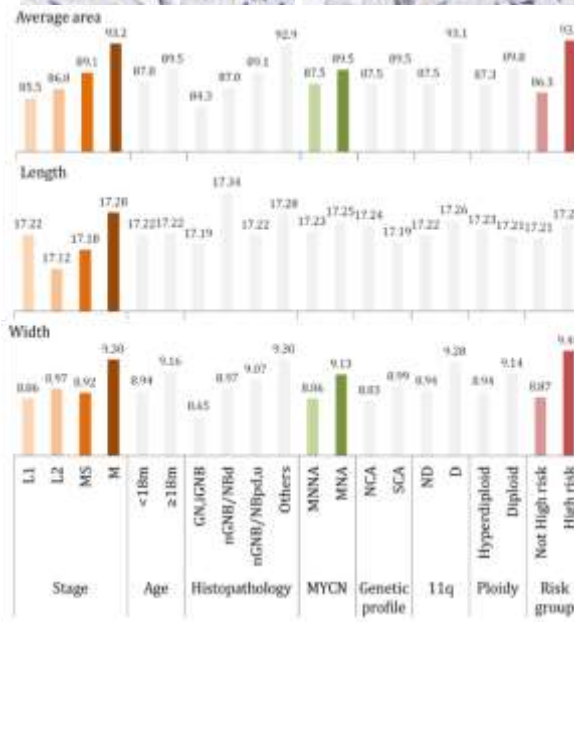
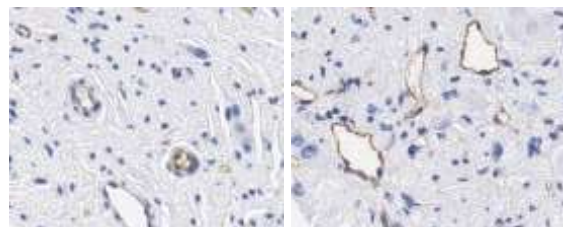
Parámetros de TAMAÑO



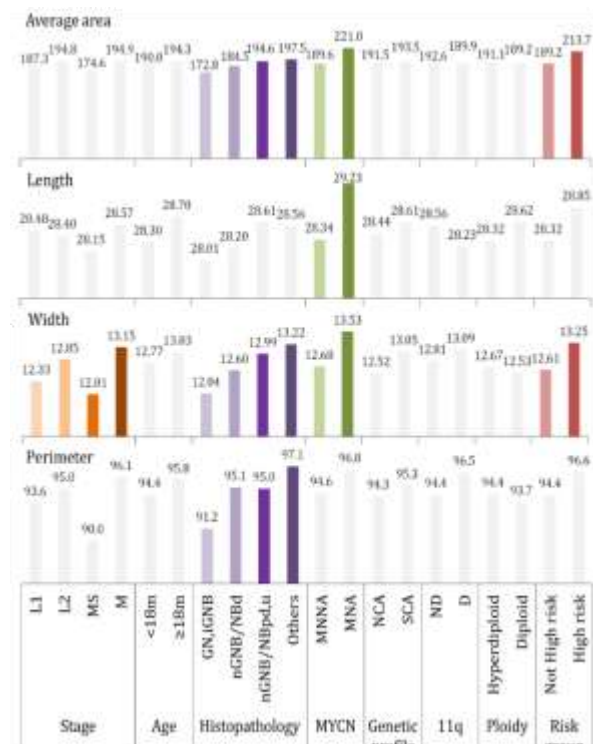
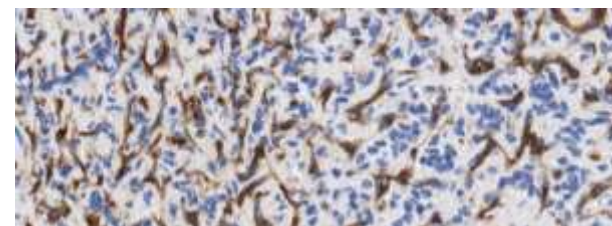
Capilares



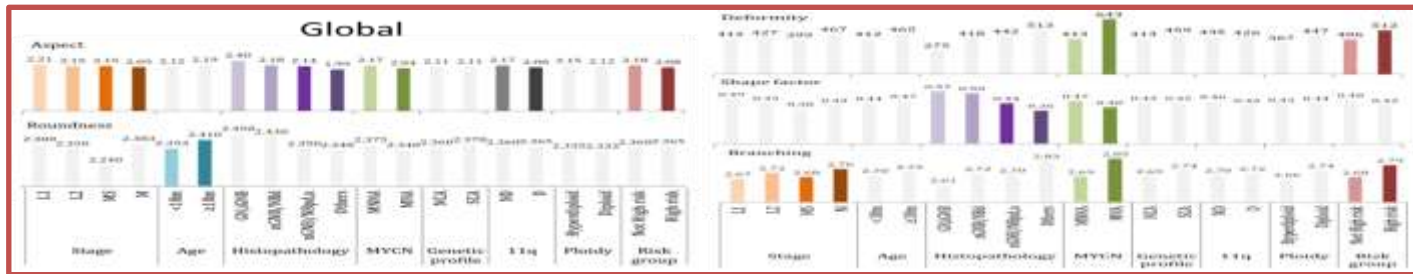
Post-capilares /Metarteriolas



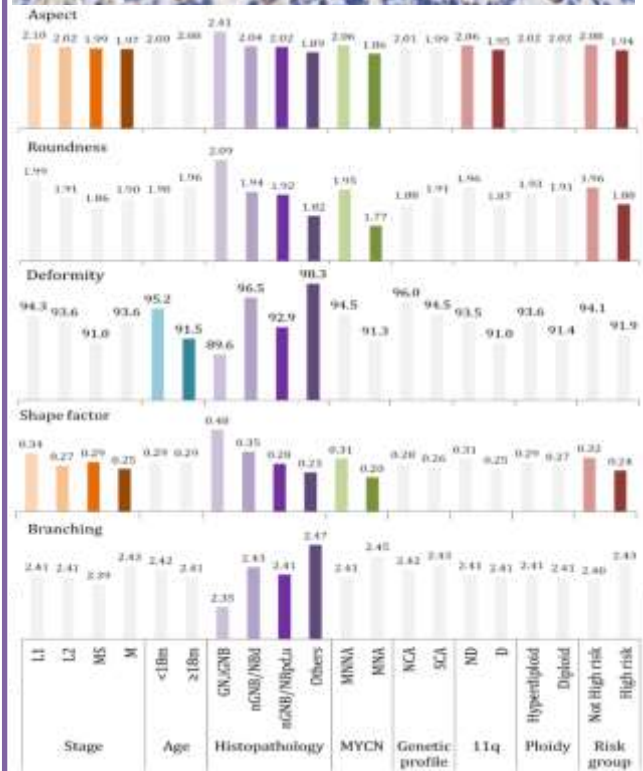
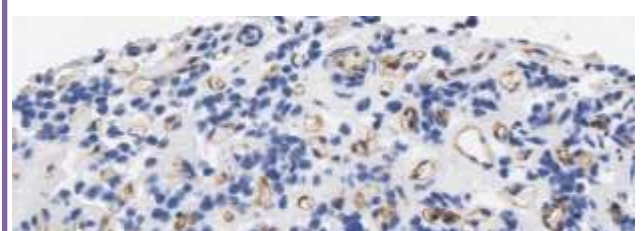
Sinusoides



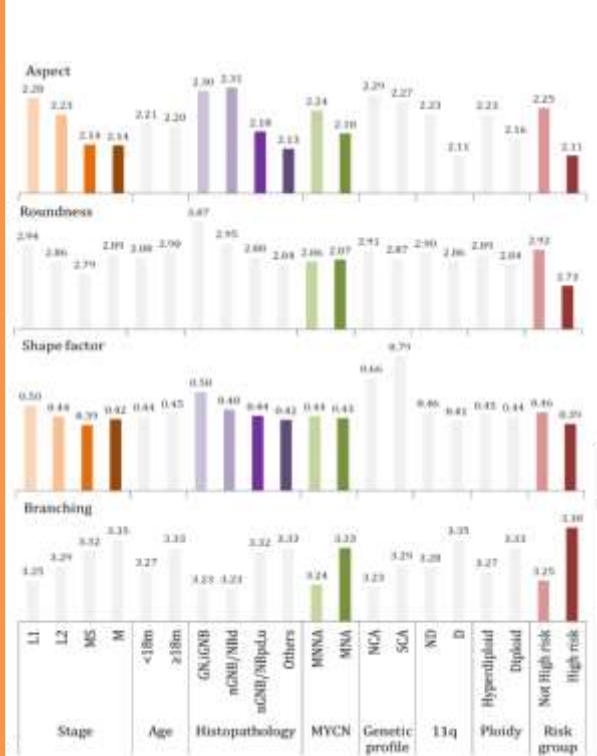
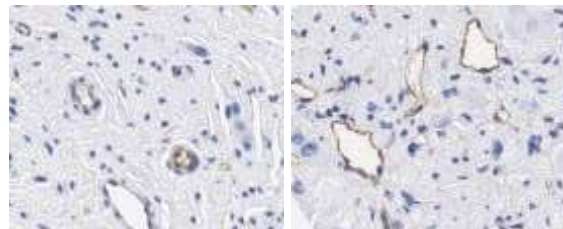
Parámetros de FORMA



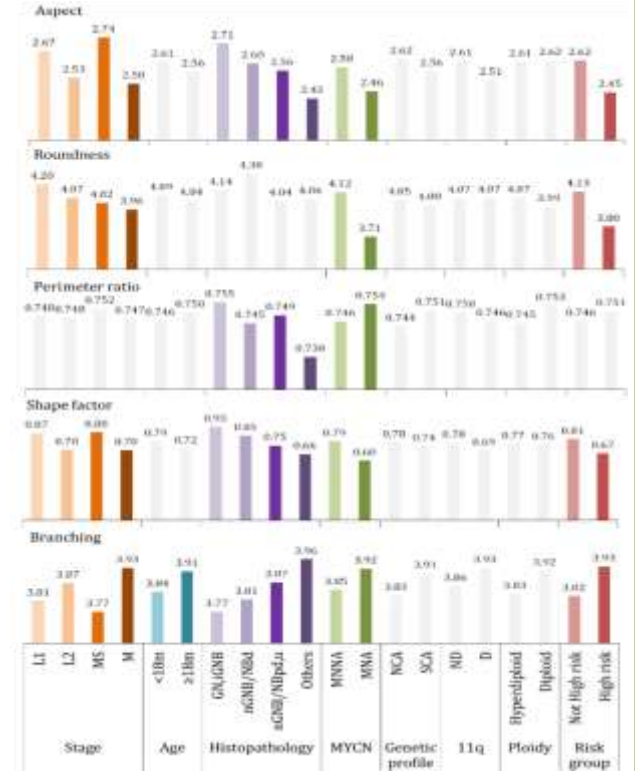
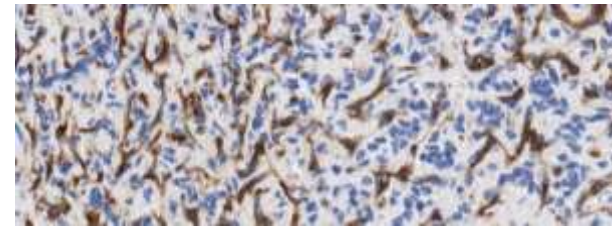
Capilares



Post-capilares /Metarteriolas



Sinusoides





RESULTADOS

Supervivencia libre de evento (EFS)

Vascularización global

	Variable	HR (95% CI)	p-value
a, b	NON-INFORMATIVE		

Capilares

	Variable	HR (95% CI)	p-value
a,b	NON-INFORMATIVE		

Vasos tipo sinusoide

	Variable	Wald	HR (95% CI)	p-value
a	Stage	20.1		0.000
	MYCN	16.4	3.2 (1.8-5.8)	0.000
	length	8.6	0.8 (0.7-0.9)^	0.003
	11q	8.1	2.2 (1.2-3.9)	0.004
b	MYCN	24.1	4.3 (2.4-7.7)	0.000
	11q	17.1	3.0 (1.8-5.1)	0.000
	length	8.0	0.8 (0.7-0.9)^	0.004
	Age	5.3	1.9 (1.1-3.2)	0.021

Venas/arterias

	Variable	Wald	HR (95% CI)	p-value
a	-			
b	relative area	8.3	1.03 (1.01-1.05)	0.004
	MYCN	6.8	6.4 (1.5-25.7)	0.009
	aspect	4.0	1.3 (1.0-1.7)	0.044

Post-capilares/metarteriolas

	Variable	Wald	HR (95% CI)	p-value
a	Stage	22.04		0.000
	Histopathology	6.9		0.072
	width	7.7	1.4 (1.1-1.7)	0.005
	Genomic profile	4.9	2.5 (1.1-5.7)	0.027
	length	4.4	0.7 (0.5-0.9)^	0.035
b	MYCN	13.3	2.8 (1.6-5.0)	0.000
	perimeter ratio	10.6	0.0 (0.0-0.03)^	0.001
	11q	9.1	2.2 (1.3-3.8)	0.003
	Age	5.8	2.0 (1.1-3.5)	0.016
	area	5.5	1.02 (1.0-1.03)	0.018

Venulas/arteriolas

	Variable	Wald	HR (95% CI)	p-value
a	Stage	20		0.000
	MYCN	17.4	3.9 (2.0-7.5)	0.000
	11q	6.36	2.1 (1.1-3.8)	0.012
	relative area	6.35	0.9 (0.95-0.99)^	0.012
b	MYCN	24.0	4.9 (2.6-9.4)	0.009
	11q	16.0	3.2 (1.8-5.8)	0.000
	relative area	8.9	0.9 (0.94-0.98)^	0.037
	Age	6.8	2.2 (1.2-4.1)	0.009
	length	4.3	1.0 (1.00-1.04)	0.037



RESULTADOS

Supervivencia global (OS)

Vascularización global

	Variable	HR (95% CI)	p-value
a, b		-	

Capilares

	Variable	HR (95% CI)	p-value
a, b		-	

Vasos tipo sinusoide

	Variable	Wald	HR (95% CI)	p-value
a	MYCN	24.1	5.0 (2.6-9.5)	0.000
	Stage	19.9		0.000
	length	9.8	0.8 (0.7-0.9)^	0.002
	relative density	6.6	1.0 (1.0-1.1)	0.002
	11q	5.6	2.1 (1.1-4.0)	0.017
b	MYCN	27.3	5.9 (3.0-11.5)	0.001
	length	10.2	0.8 (0.7-0.9)^	0.001
	11q	10.1	2.6 (1.4-4.9)	0.001
	Age	8.8	2.8 (1.4-5.5)	0.003
	relative density	4.5	1.0 (1.0-1.1)	0.033

Venas/arterias

	Variable	HR (95% CI)	p-value
a, b		-	

Post-capilares/metarteriolas

	Variable	Wald	HR (95% CI)	p-value
a		-		
b	MYCN	18.4	4.0 (2.1-7.7)	0.000
	Age	7.3	2.6 (1.3-5.4)	0.007
	width	7.2	1.4 (1.1-1.8)	0.007
	11q	6.4	2.2 (1.2-4.0)	0.011
	length	4.3	0.7 (0.5-0.9)^	0.037

Venulas/arteriolas

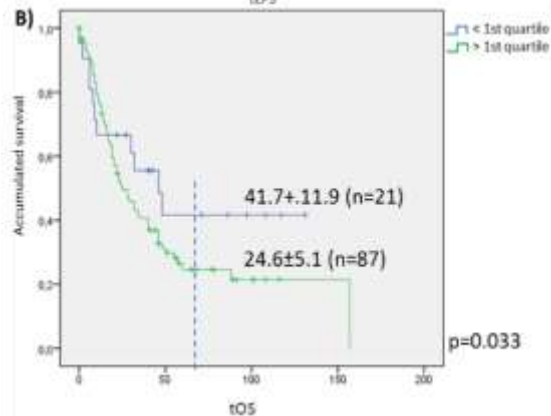
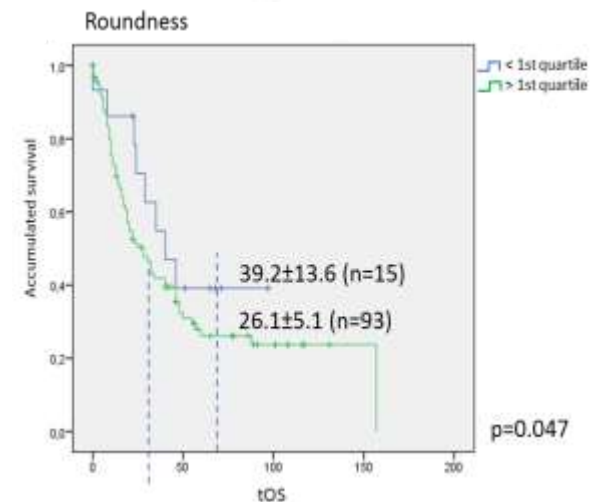
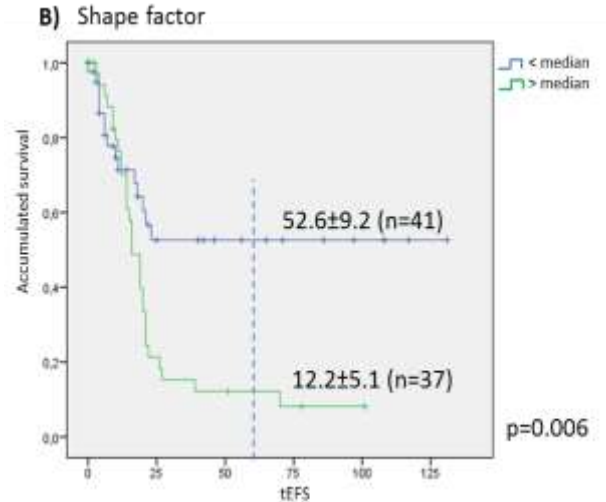
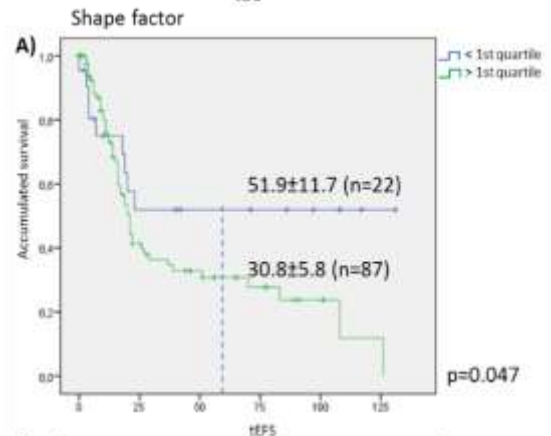
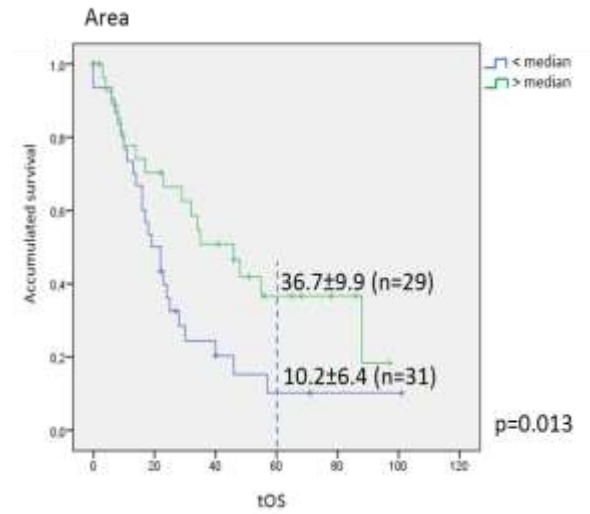
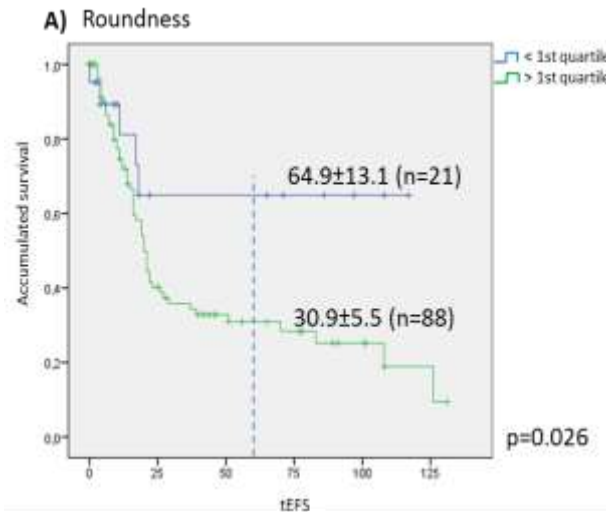
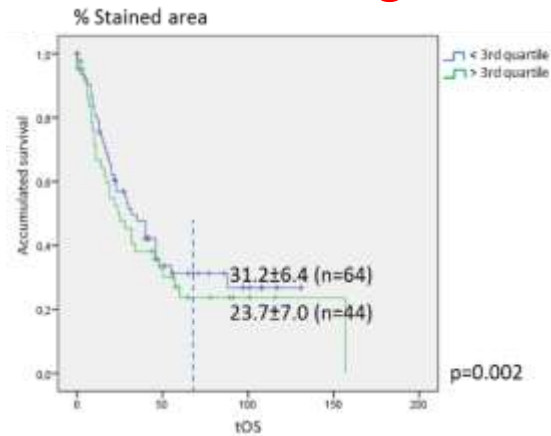
	Variable	Wald	HR (95% CI)	p-value
a	MYCN	28.1	7.9 (3.6-17.1)	0.000
	Stage	9.9		
	11q	7.1	2.6 (1.3-5.3)	0.007
	relative stained area	6.7	0.96 (0.94-0.99)^	0.010
	shape factor	3.9	1.0 (1.00-1.02)	0.048
	% stained area	3.7	1.4 (0.9-2.1)	0.053*
b	MYCN	32.6	8.5 (4.0-17.8)	0.000
	11q	9.7	3.0 (1.5-5.9)	0.002
	Relative stained area	7.1	0.96 (0.93-0.99)^	0.007
	shape factor	6.3	1.0 (1.00-1.02)	0.012
	Age	5.2	2.3 (1.1-4.9)	0.022
	% stained area	4.5	1.5 (1.0-2.3)	0.032

HR/UHR

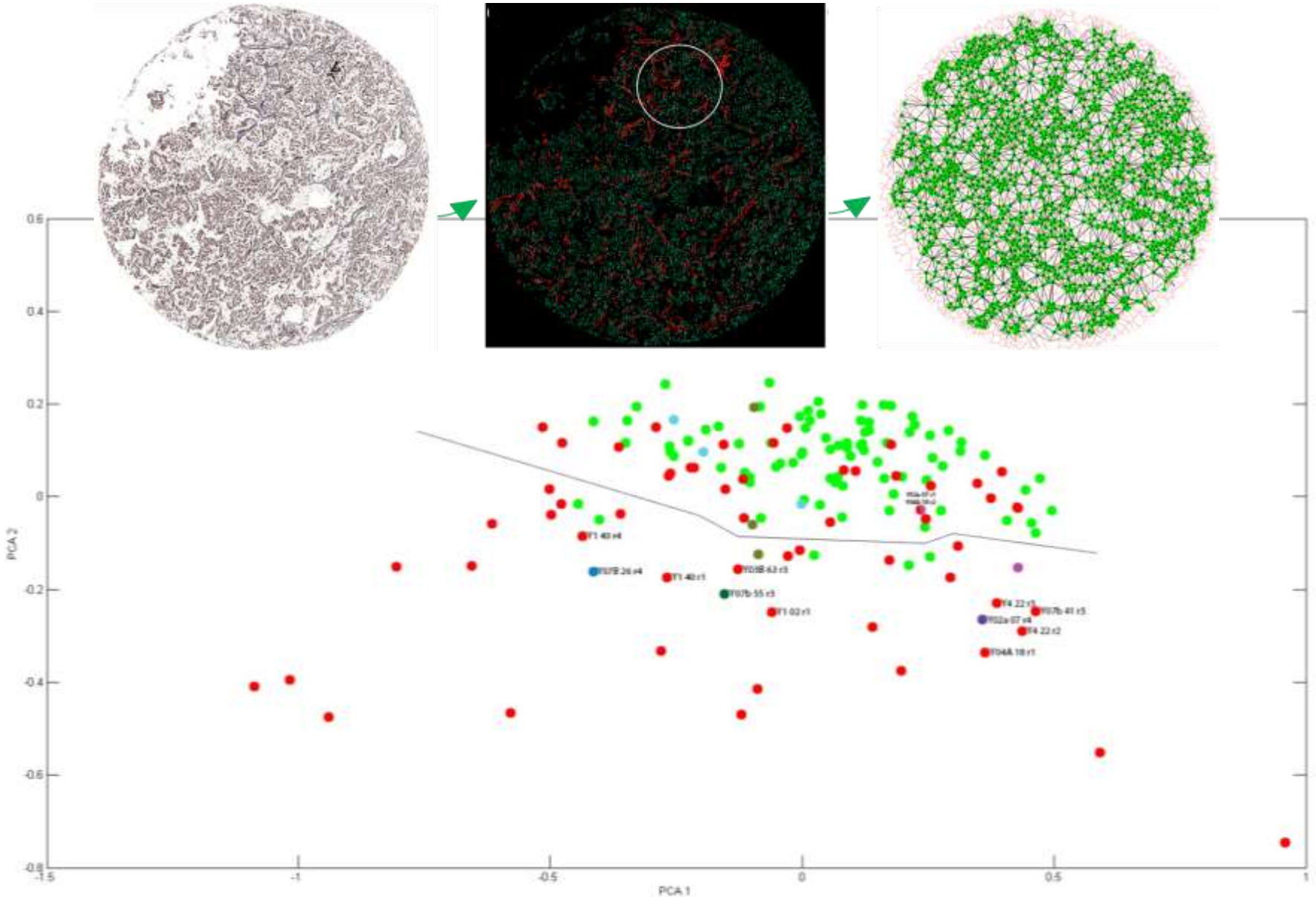
Vascularización global

Capilares

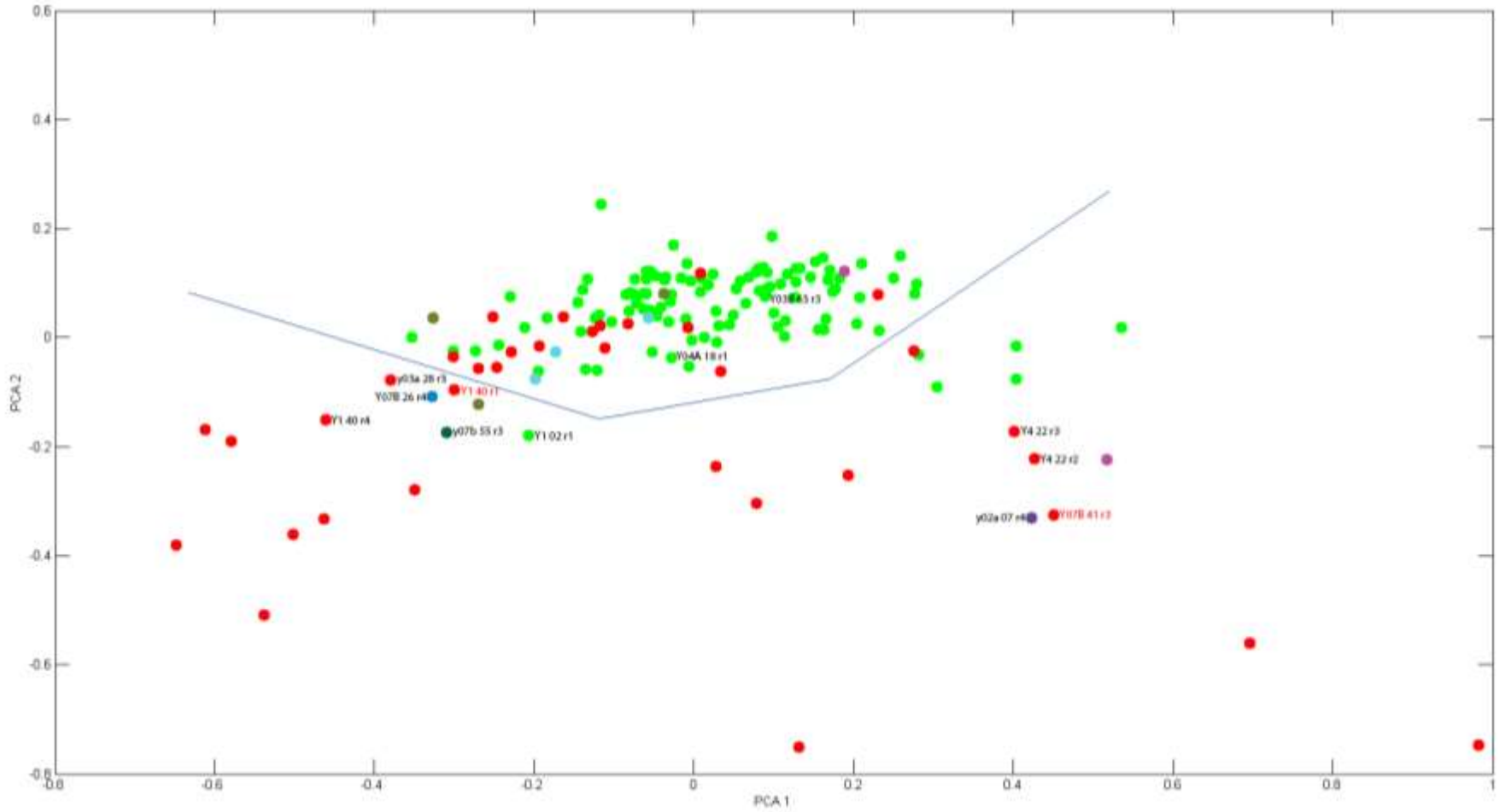
Venulas/arteriolas



Integración topológica

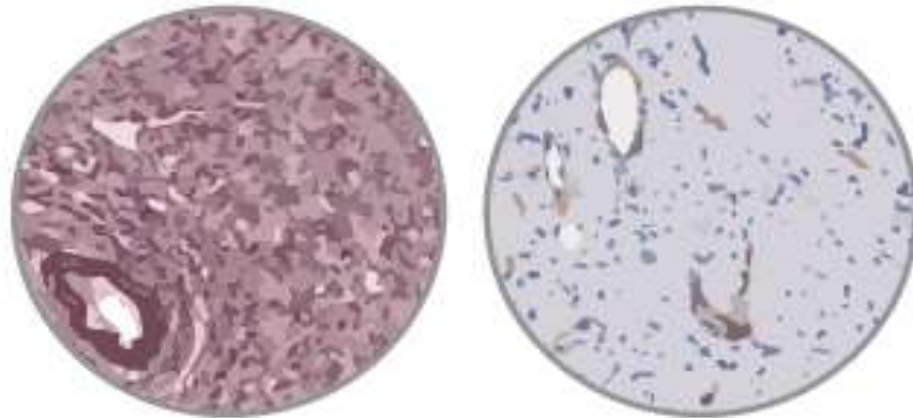


Integración topológica

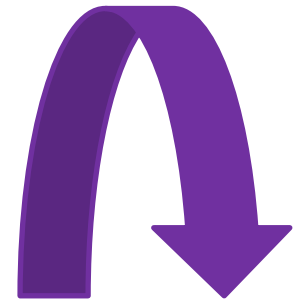




Estudio del armazón arquitectónico y del sistema vascular de los tumores neuroblásticos
Tesis doctoral D^a IRENE TADEO CERVERA (depositada el 11/09/2014. Dpto. Patología, UV para su defensa)

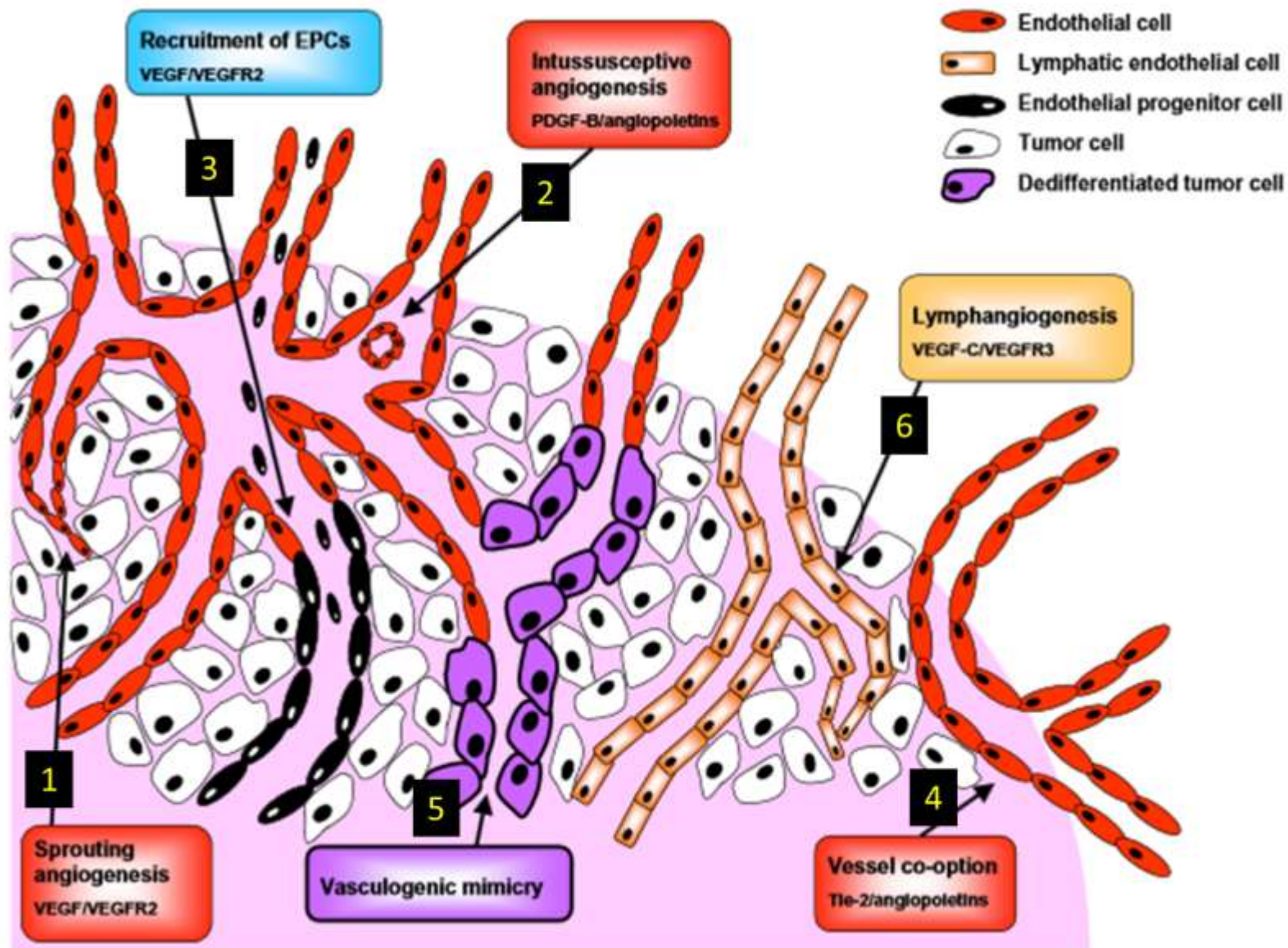


- Organización global y diferenciación por segmentos del sistema vascular sanguíneo
- Diseño de la herramienta Angiopath
- Aplicación a las TMAs
- Interacción con los datos de Neupat
- Mecanismos de vascularización tumoral y factores implicados
- Relaciones del compartimento vascular con la MEC



Multipotencialidad terapéutica

MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

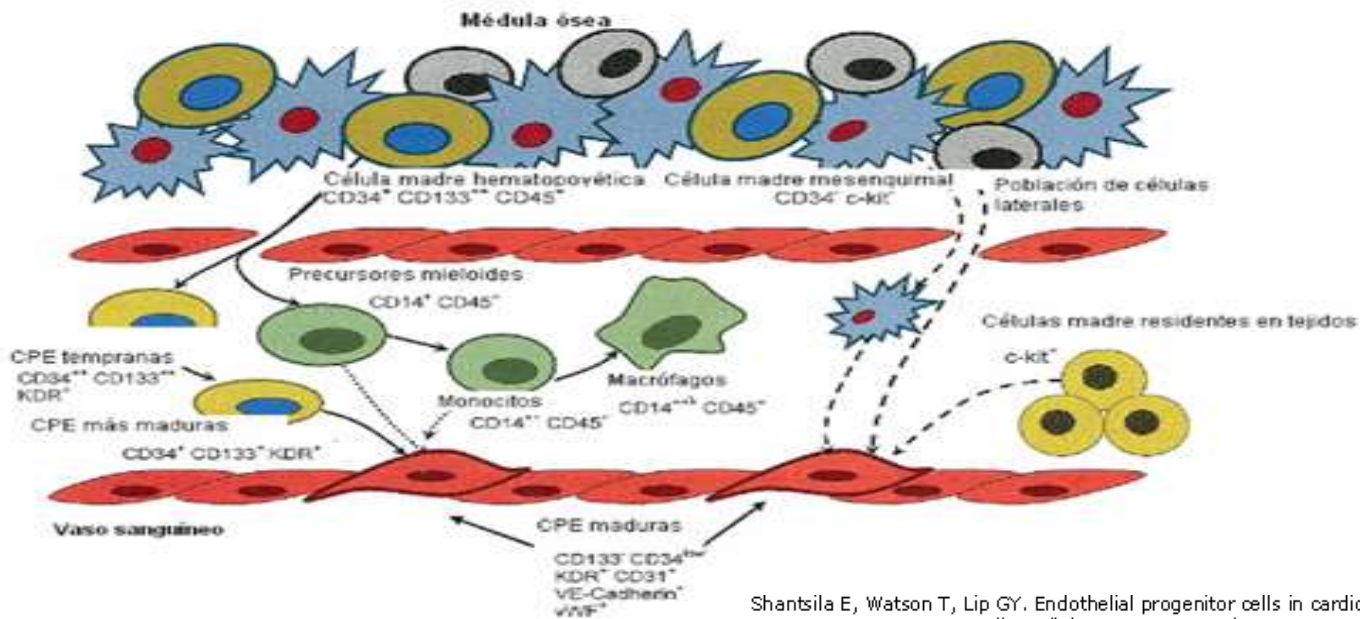


[Tumour vascularization: sprouting angiogenesis and beyond.](#)

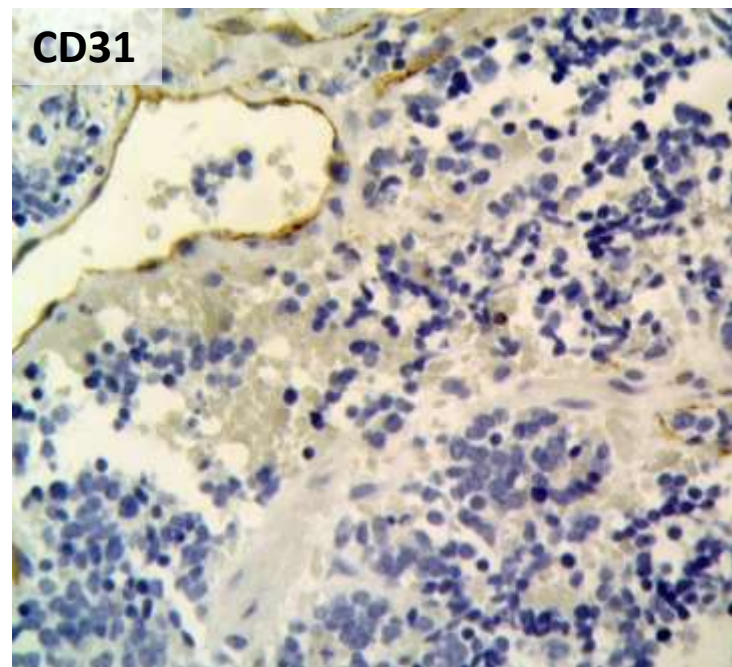
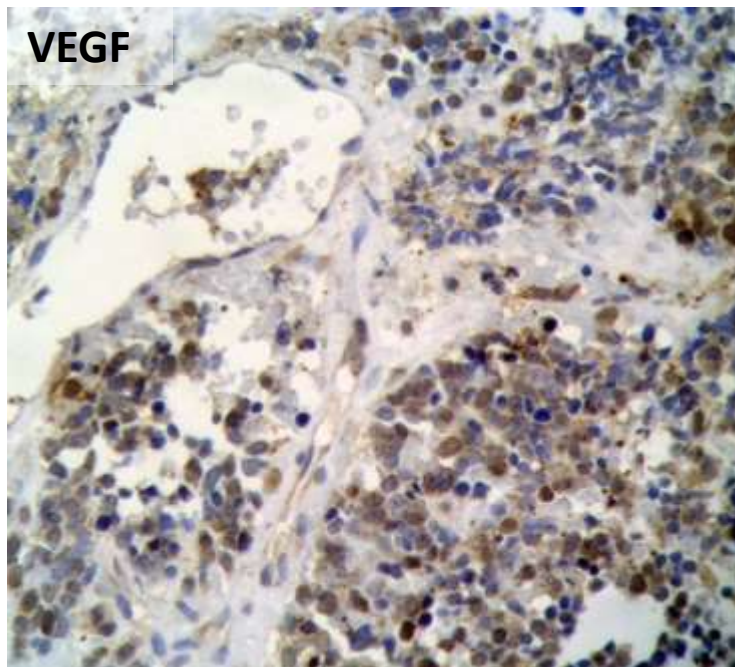
Hillen F, Griffioen AW.

Cancer Metastasis Rev. 2007 Dec;26(3-4):489-502. Review.

MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

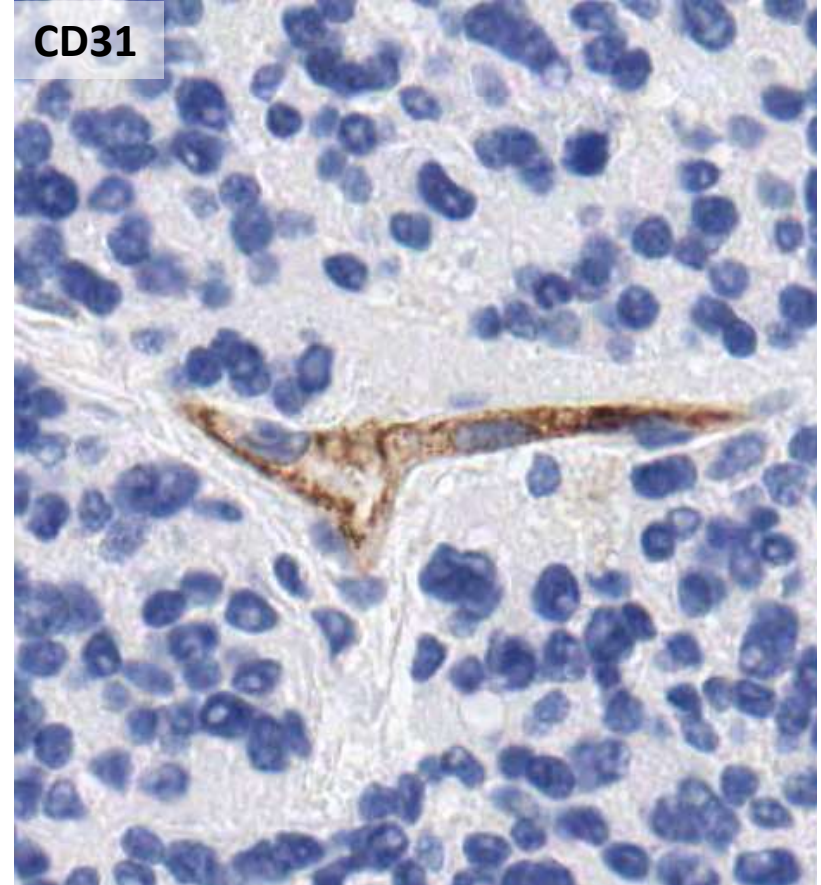
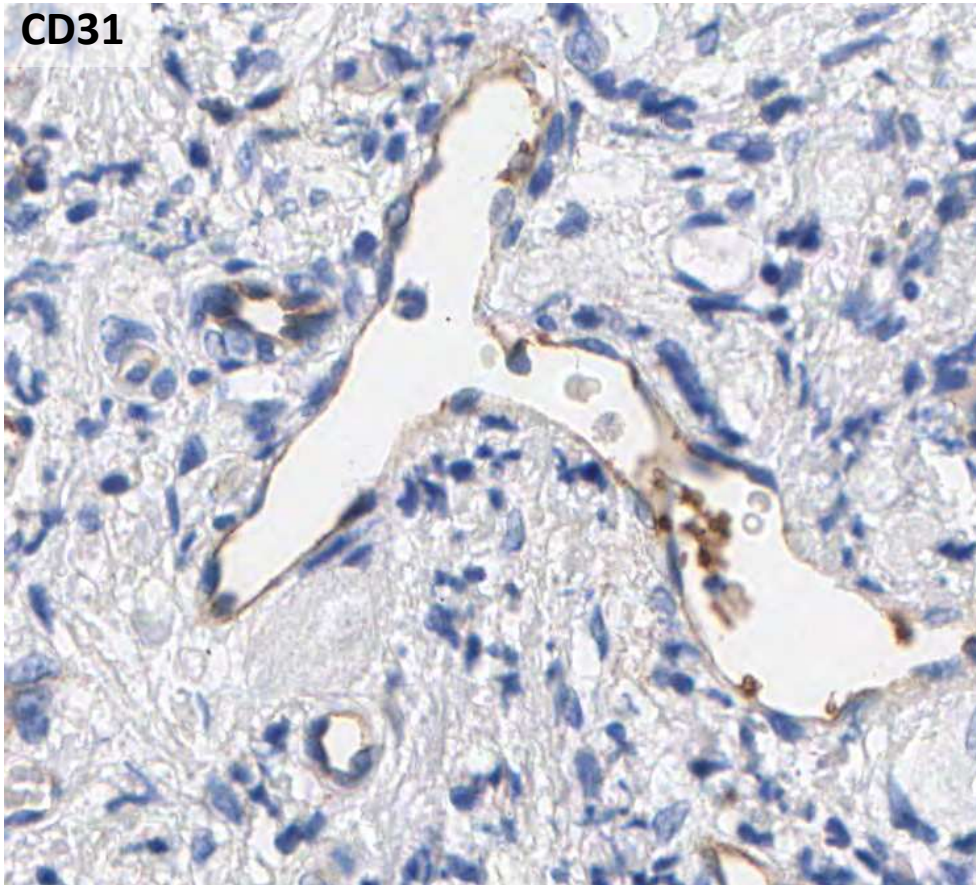


Shantsila E, Watson T, Lip GY. Endothelial progenitor cells in cardiovascular disorders. *J Am Coll Cardiol* 2007;49:741-52).



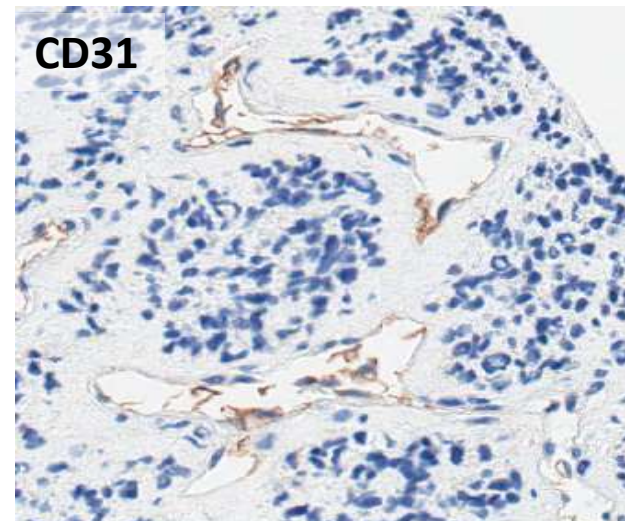
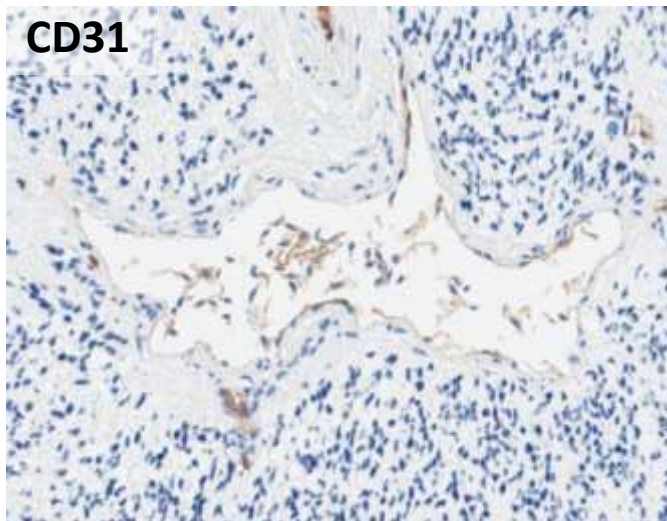
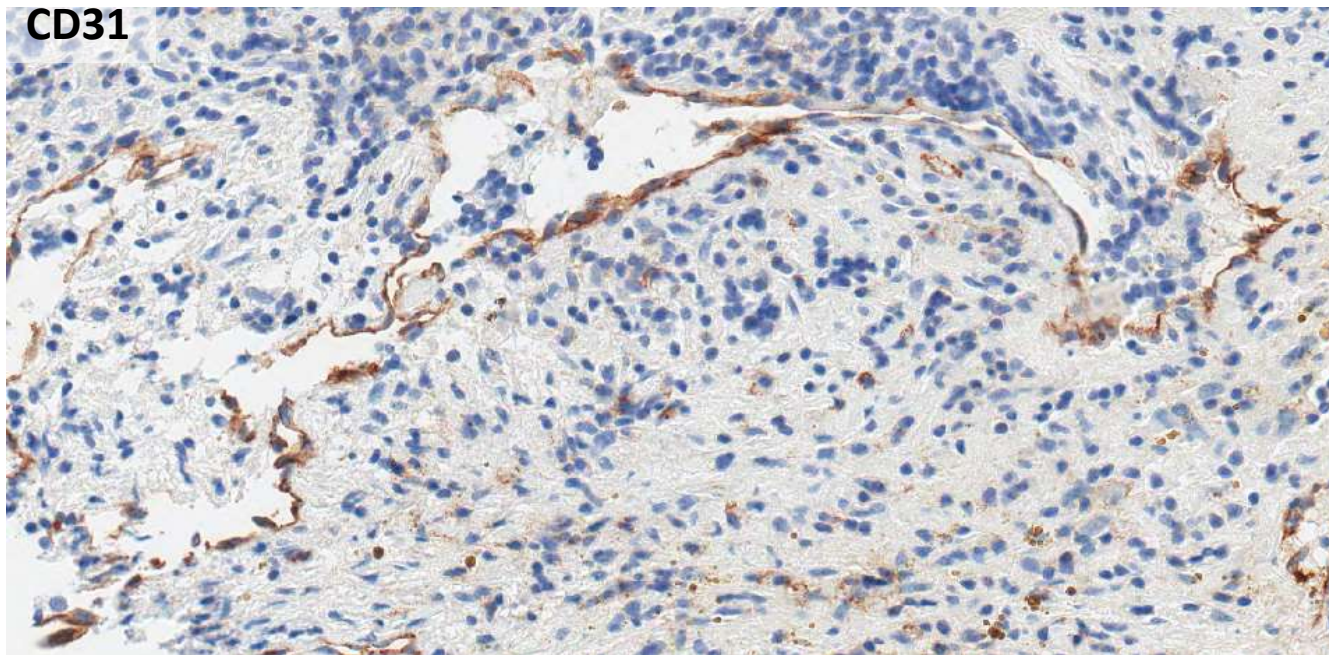
MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

1. Angiogénesis por gemación



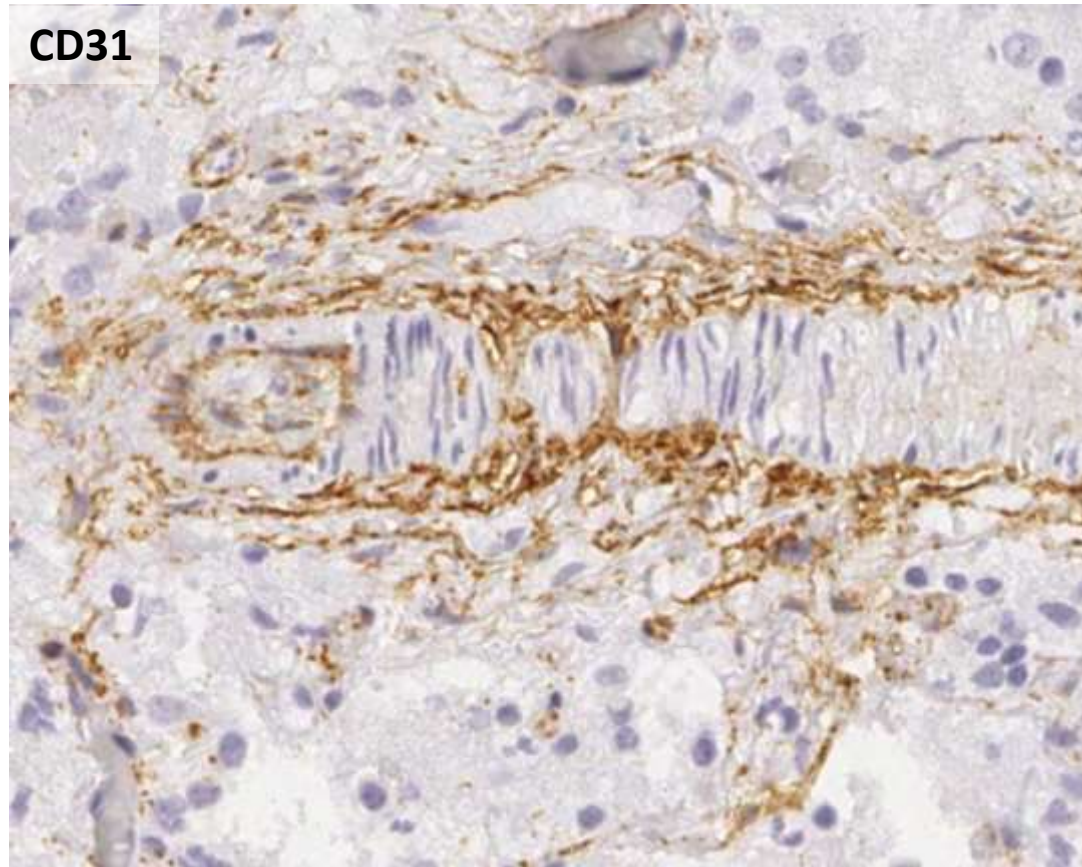
MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

Vasos irregulares

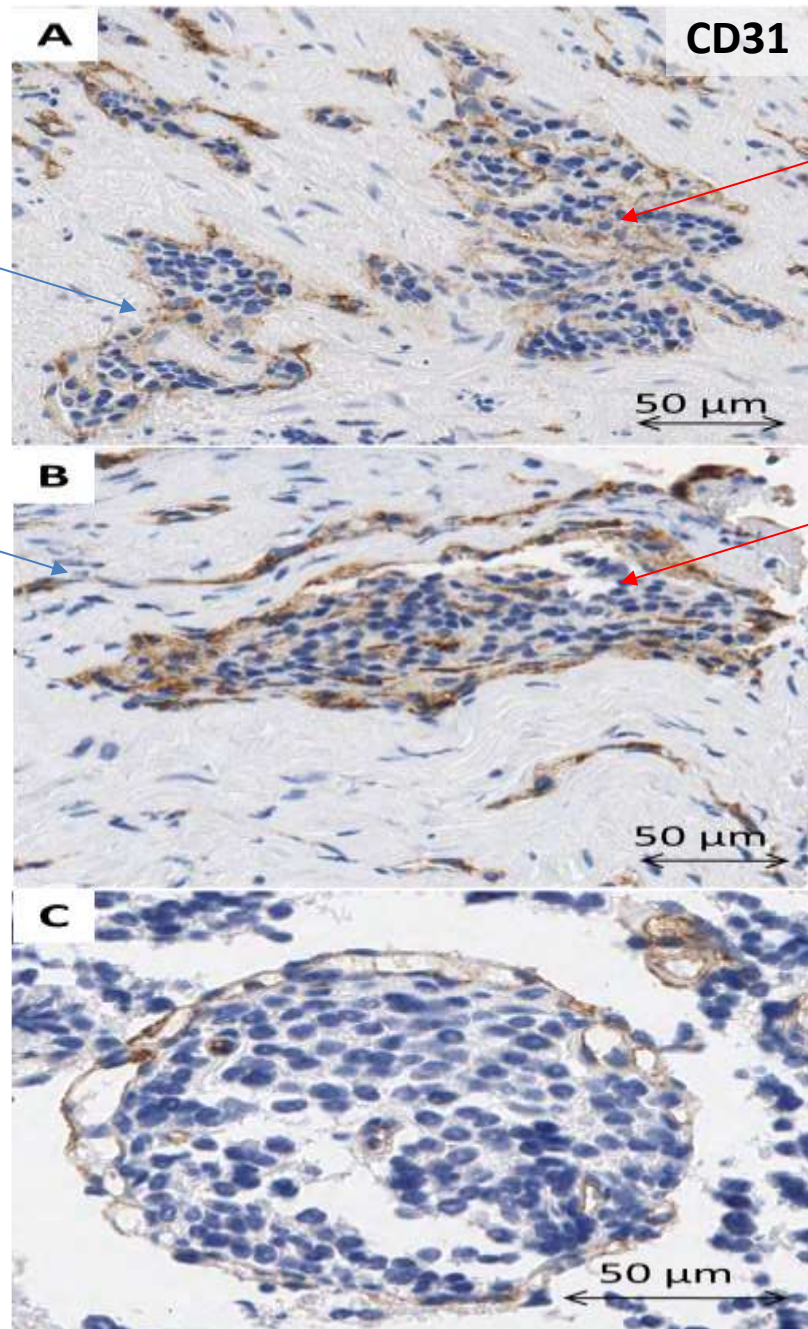


MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

2. Angiogénesis por intususcepción



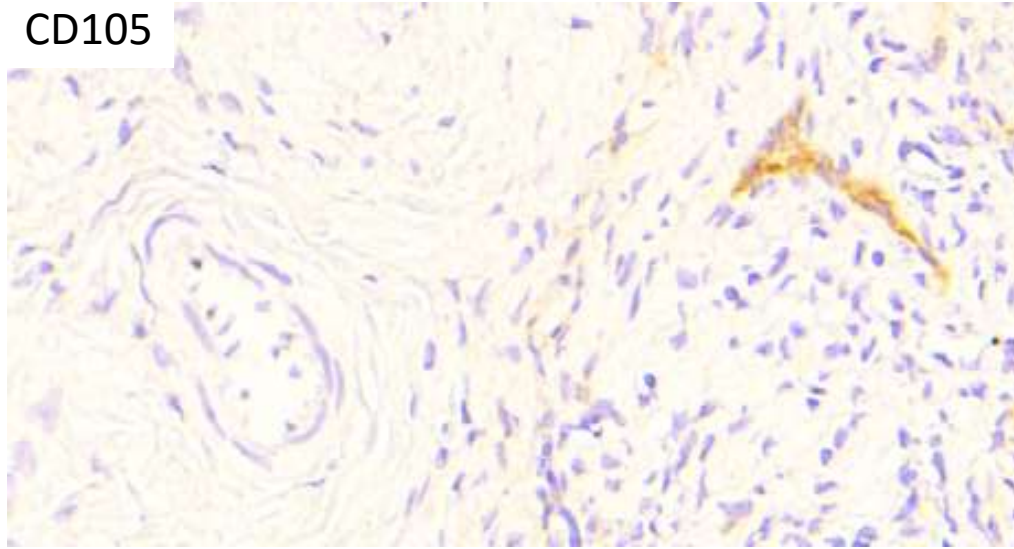
MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS



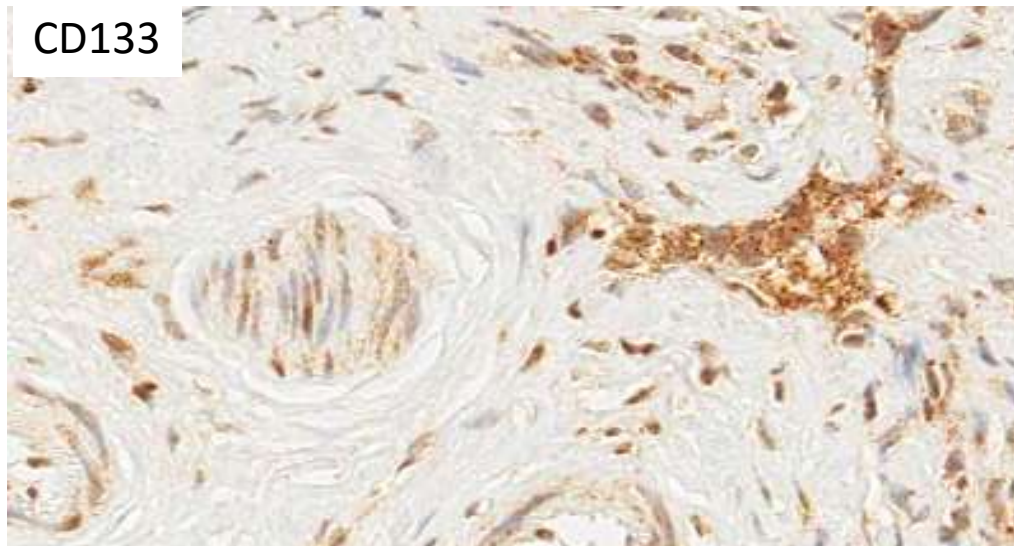
➤ MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

3. Angiogénesis por reclutamiento de células progenitoras endoteliales

CD105

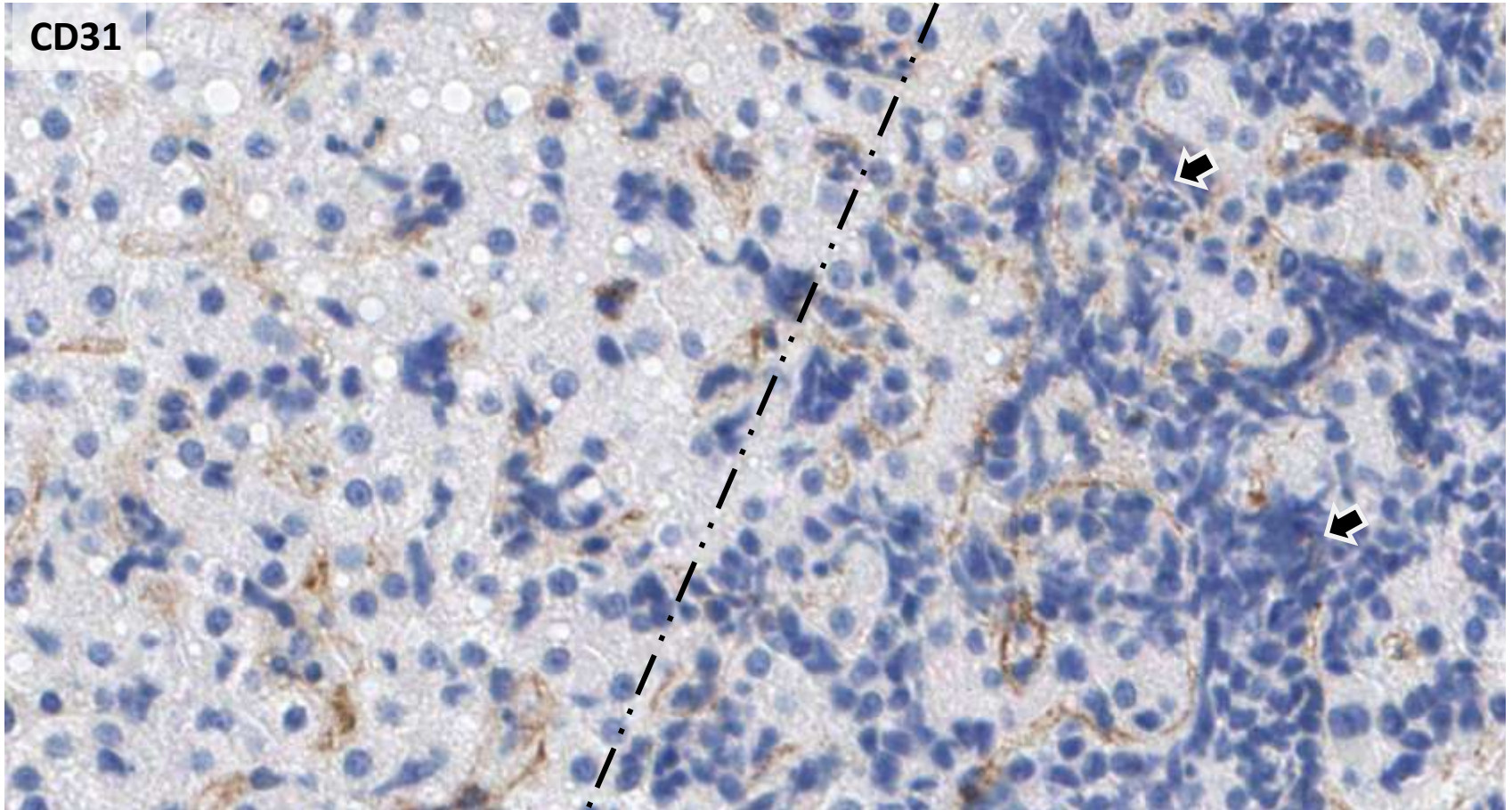


CD133



MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS

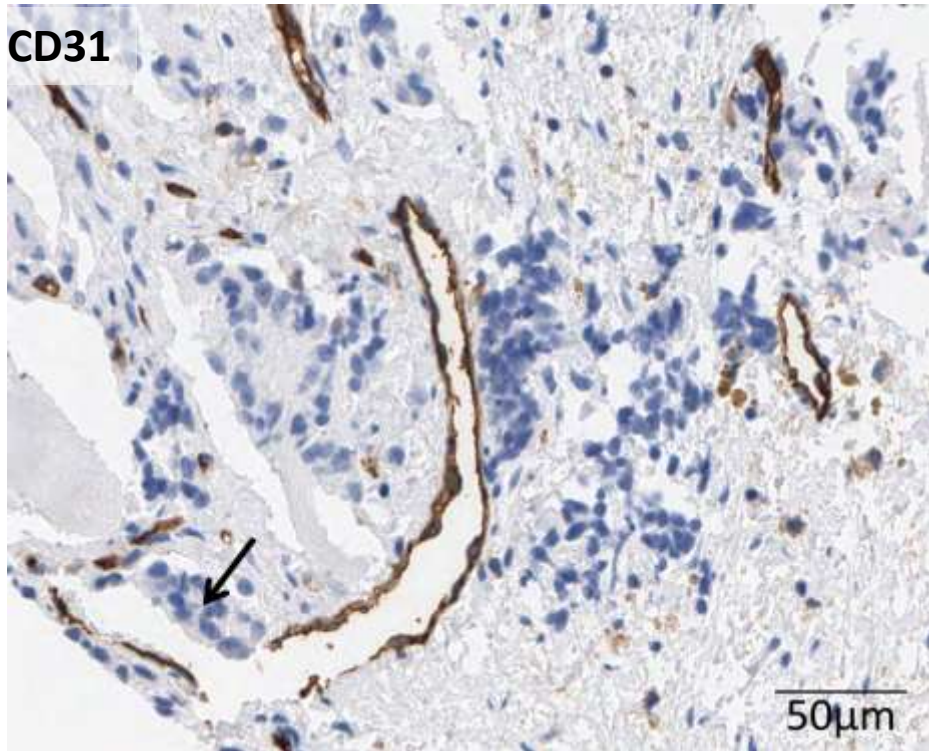
4. Angiogénesis por cooptación



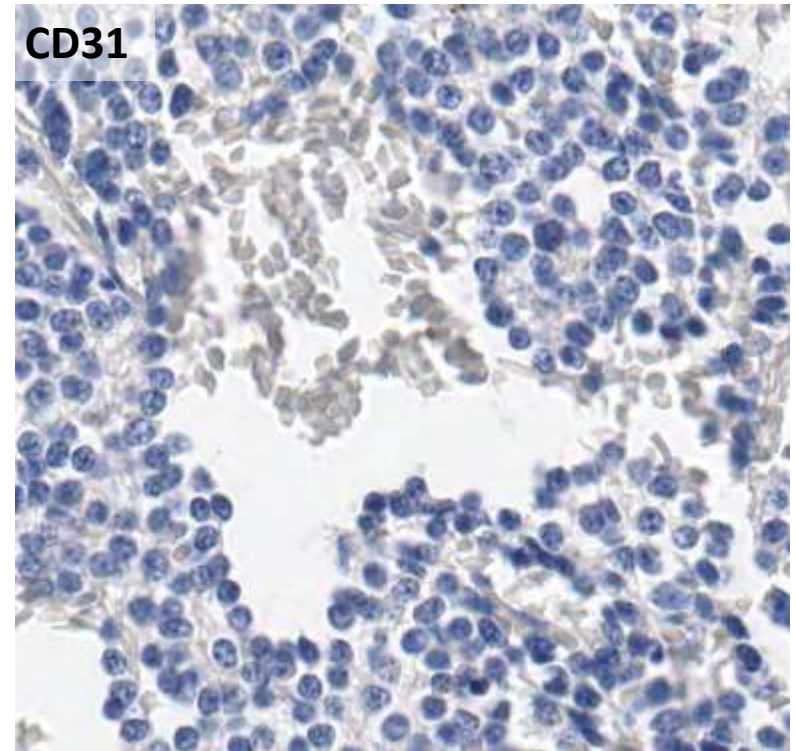
Hígado

Neuroblastoma

5. Mimetismo vasculogénico

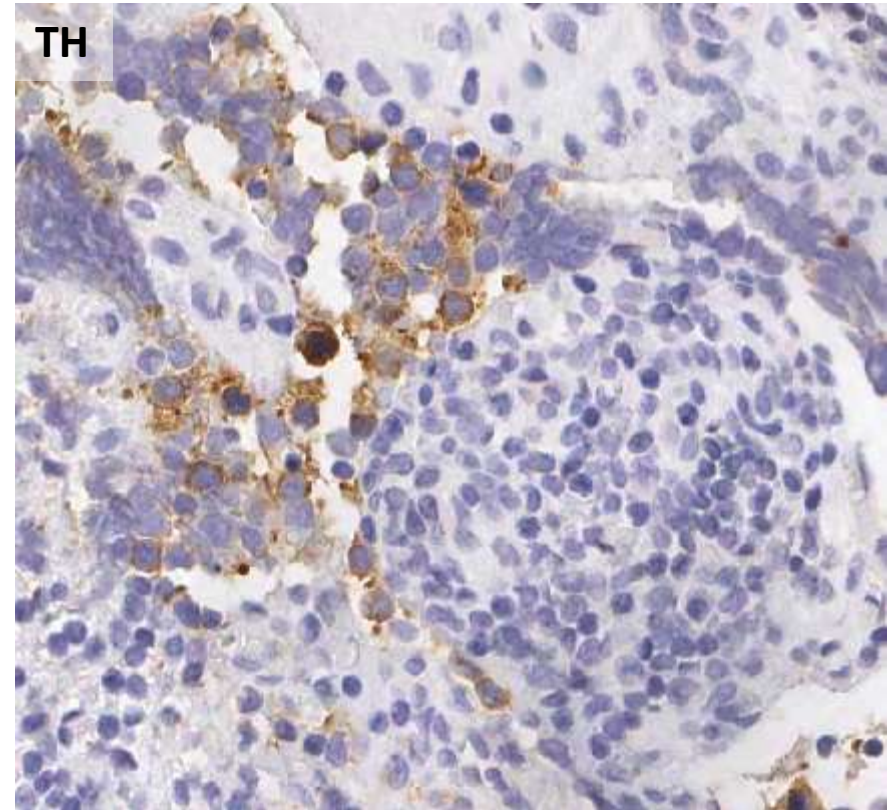
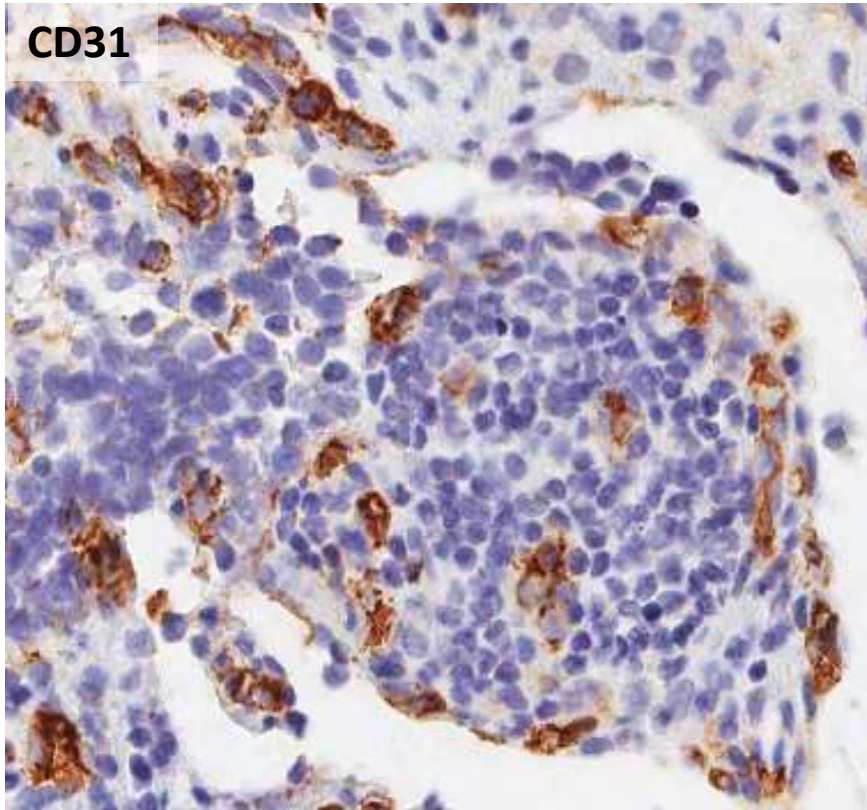


Túnica íntima con mosaicismo

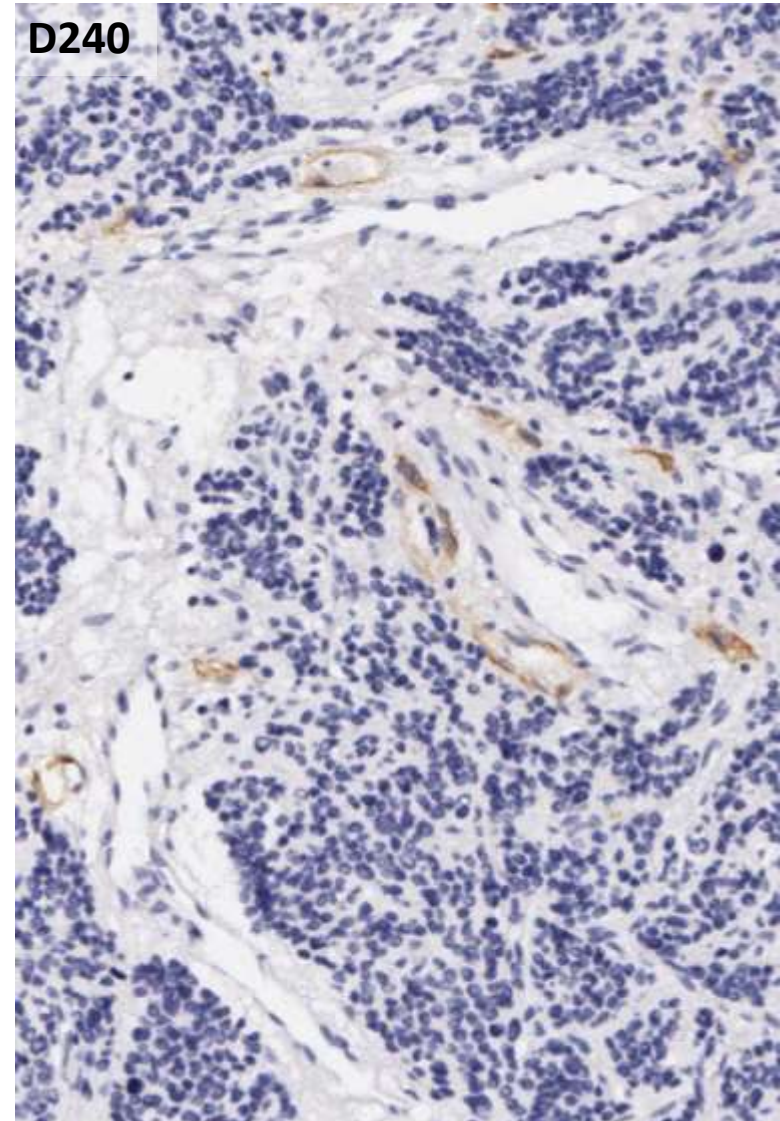
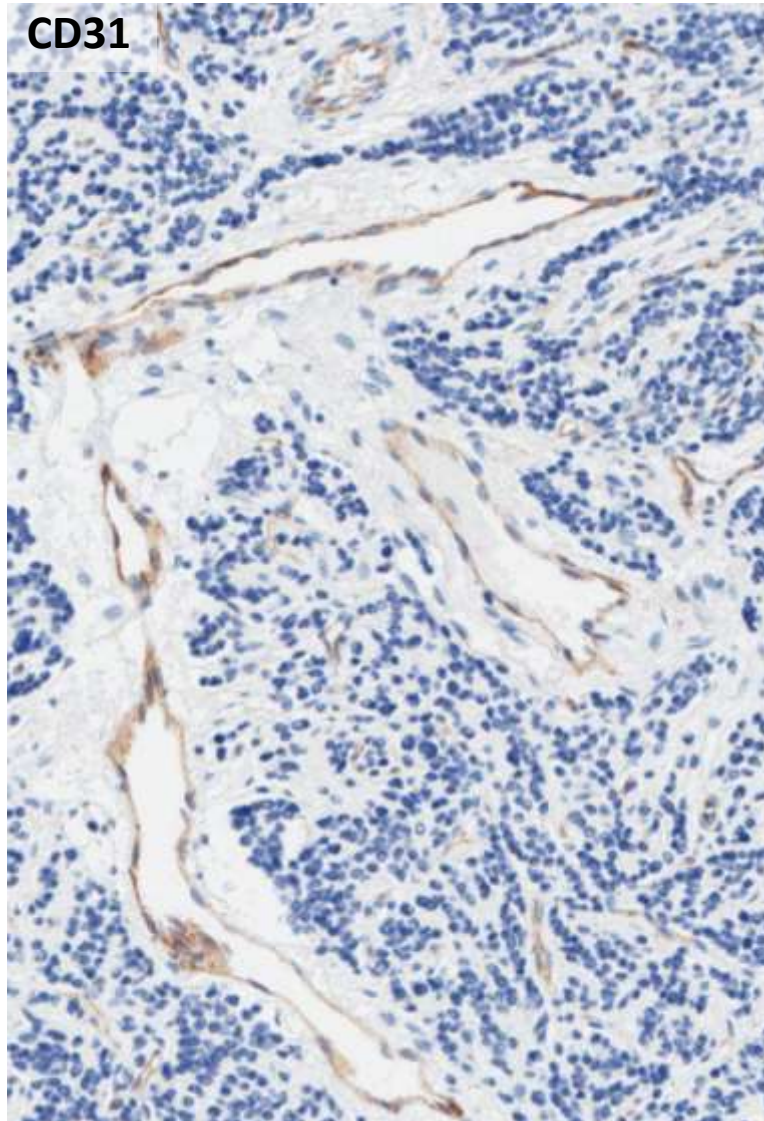


Mimetismo endotelial completo

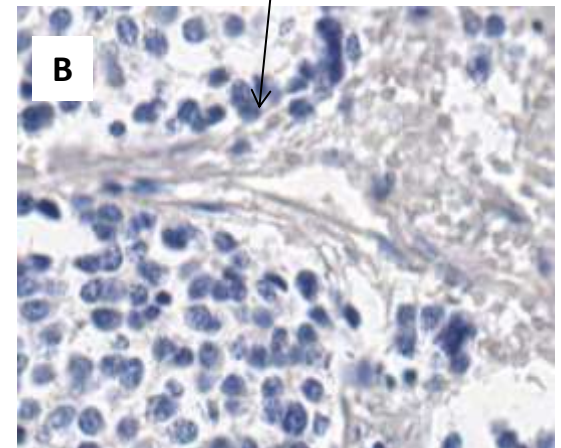
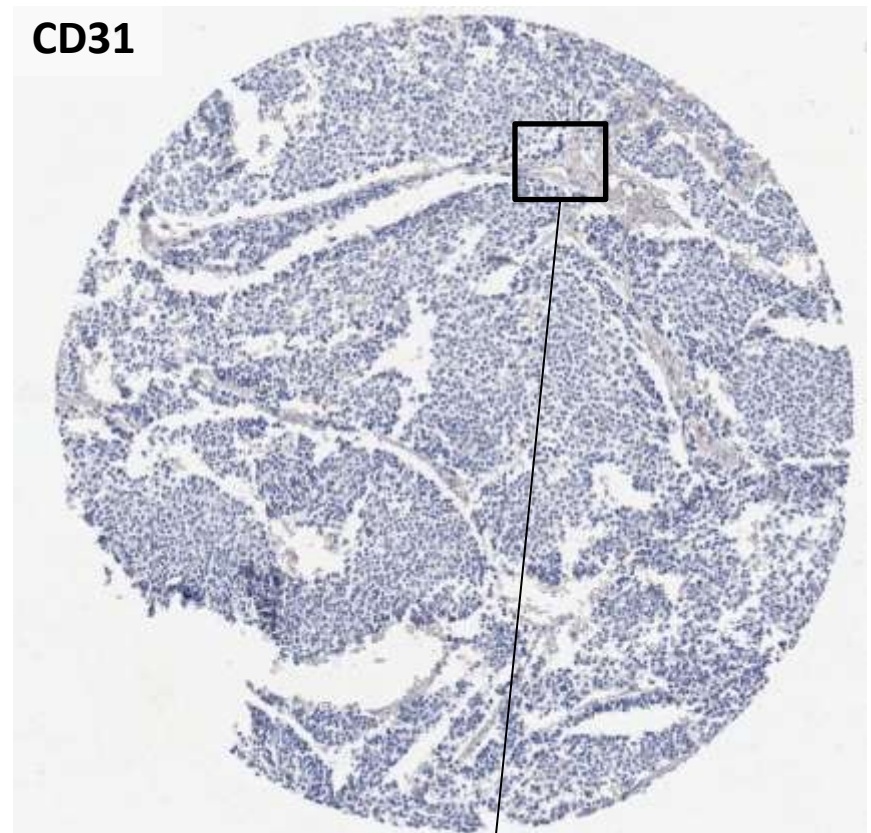
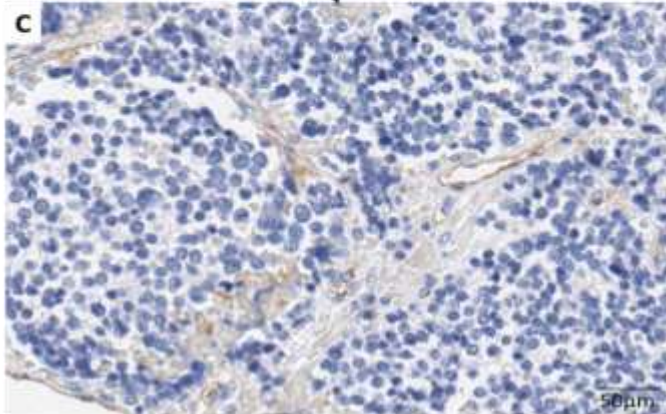
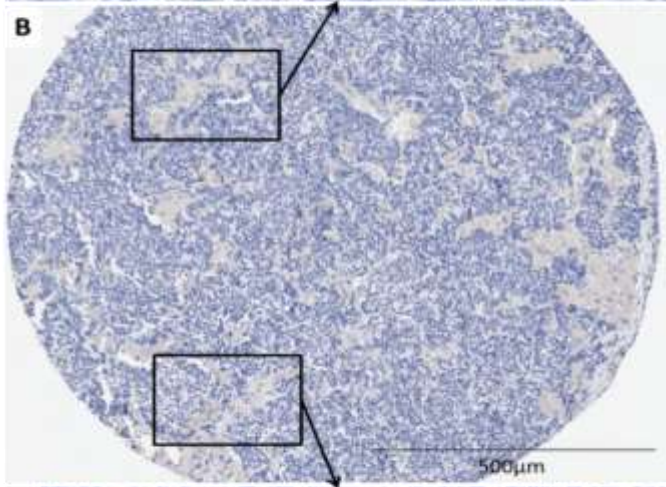
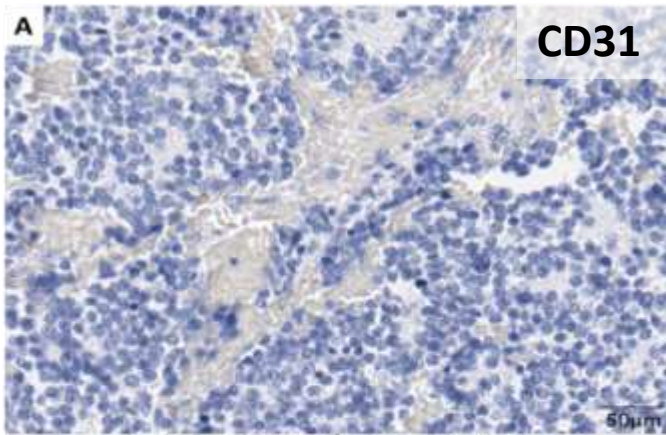
➤ MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS



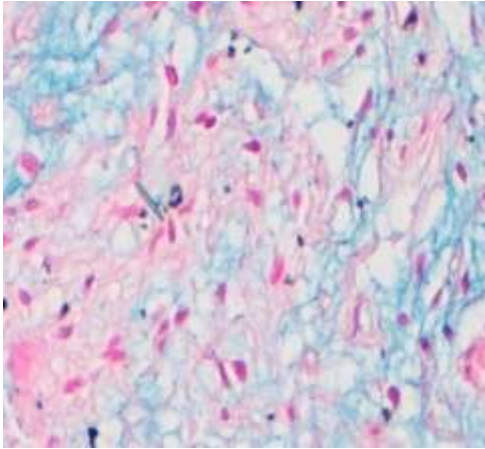
6. Linfoangiogénesis



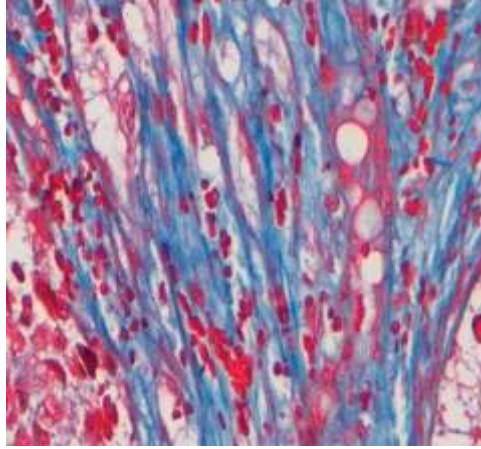
MECANISMOS DE VASCULARIZACIÓN TUMORAL Y FACTORES IMPLICADOS



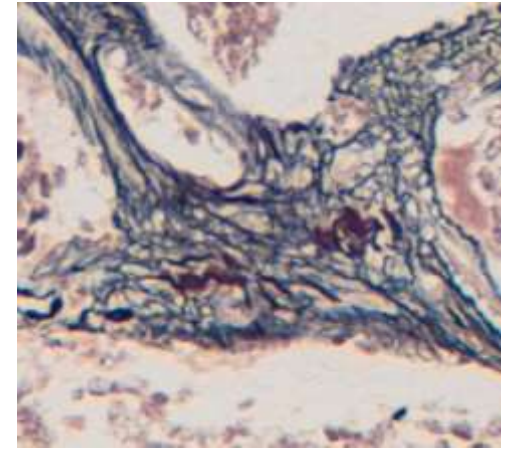
➤ RELACIÓN DE LA MORFOLOGÍA VASCULAR CON MEC TUMORAL



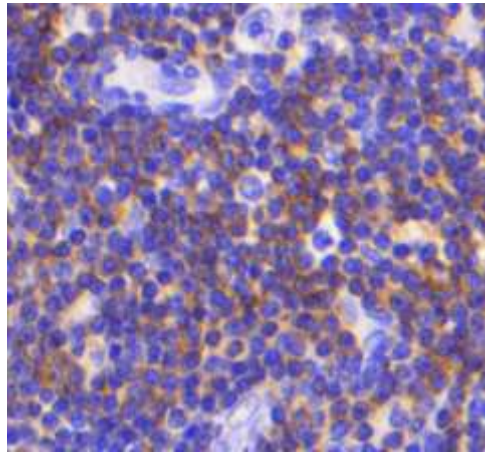
Azul alcian
(Mucopolisacaridos ácidos)



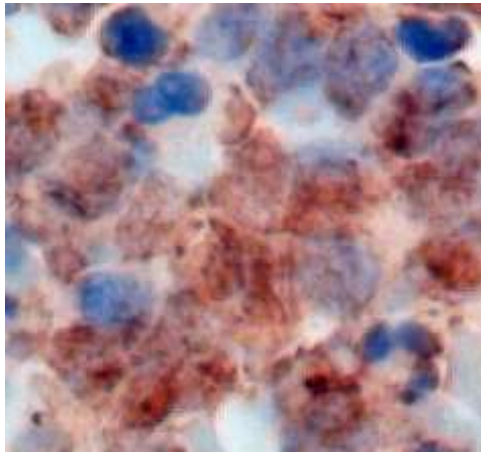
Tricrómico Masson
Fibras de colágeno



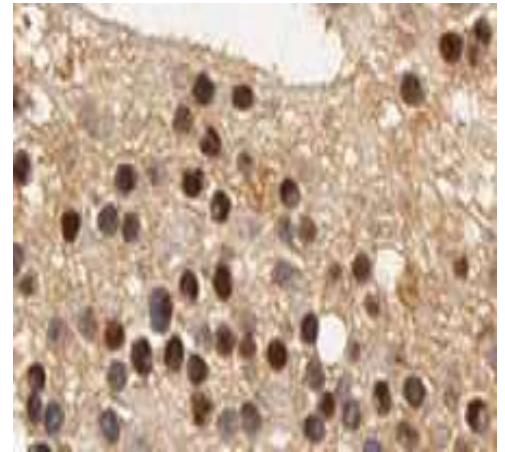
Tinción Gomori
Fibras de reticulina



Ac antiCD7
Células linfoides

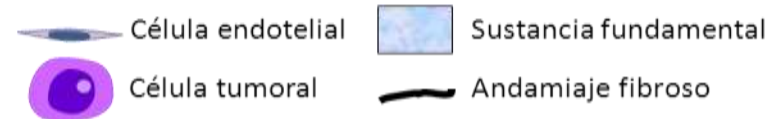
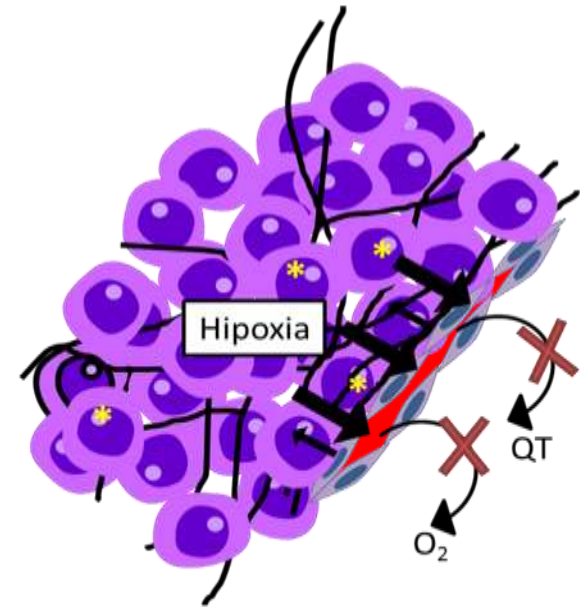
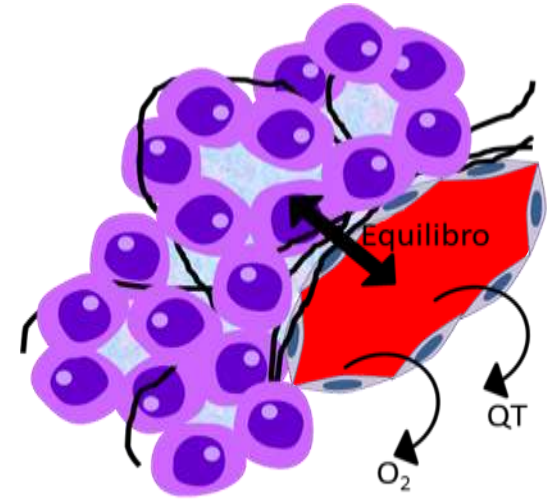
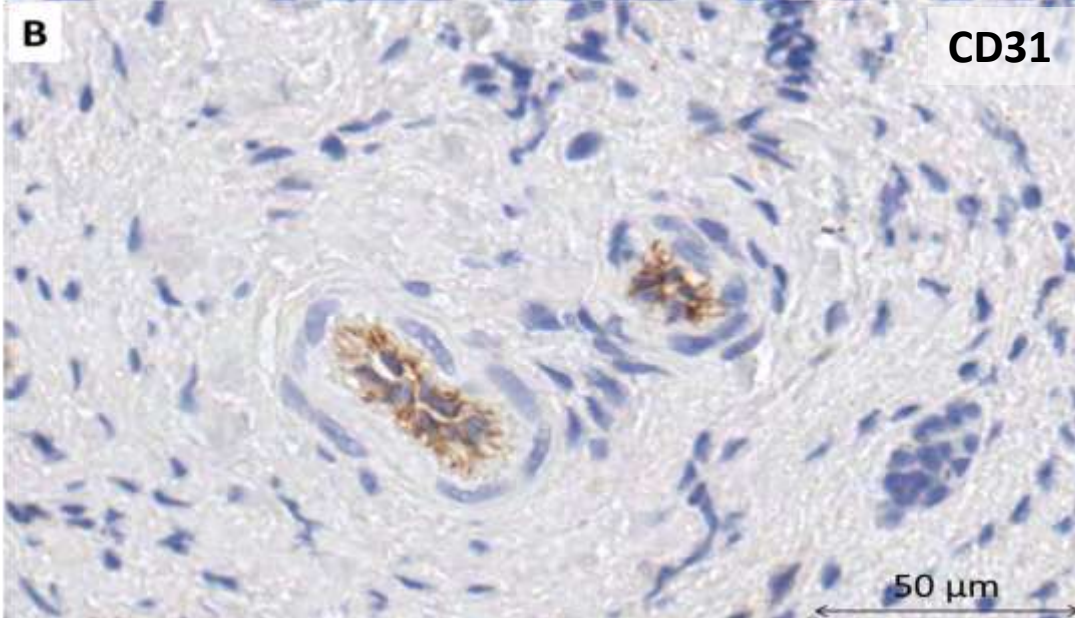
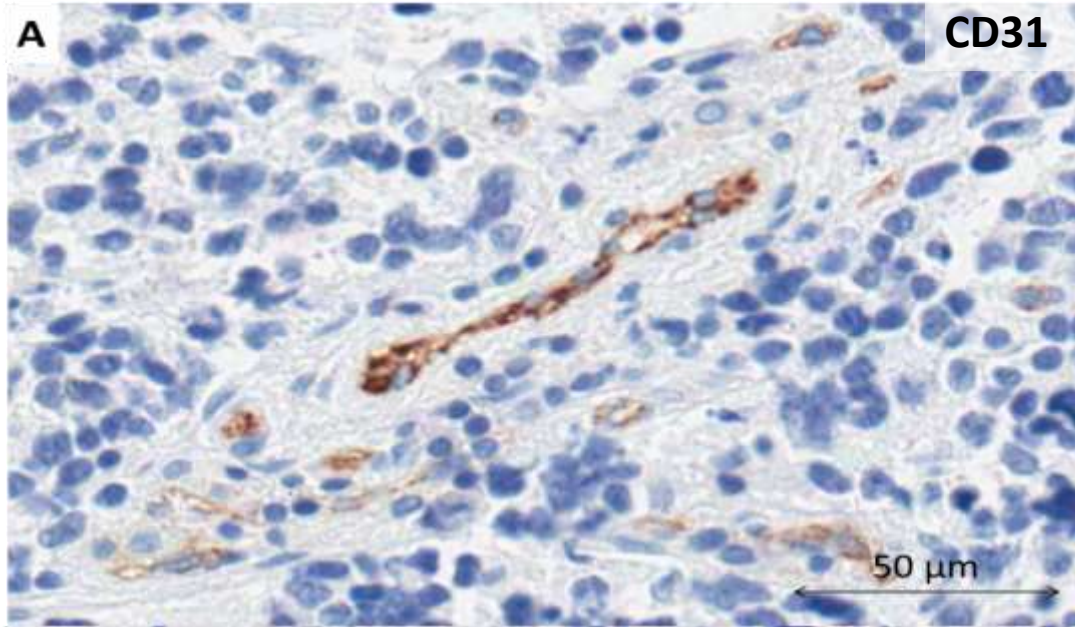


Ac antiALK
Células neuroblásticas



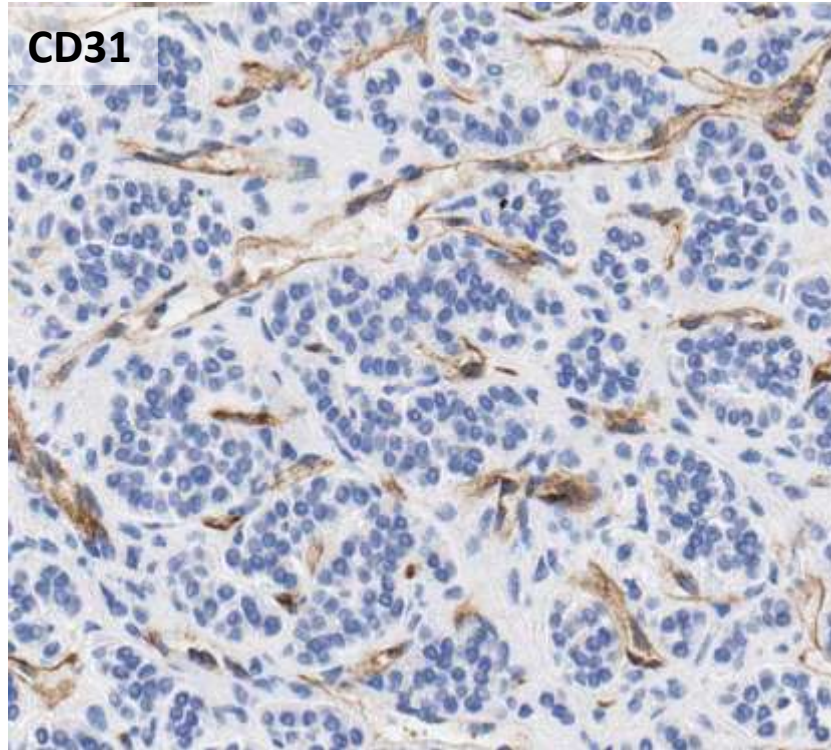
Ac antiSOX2
Células madre

➤ RELACIÓN DE LA MORFOLOGÍA VASCULAR CON MEC TUMORAL

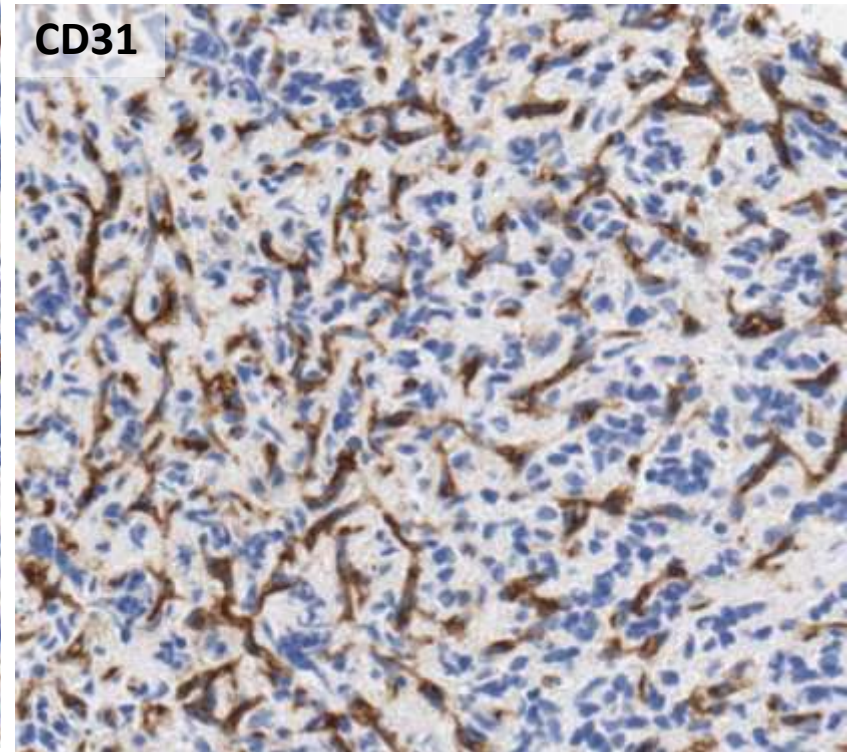


➤ RELACIÓN DE LA MORFOLOGÍA VASCULAR CON MEC TUMORAL

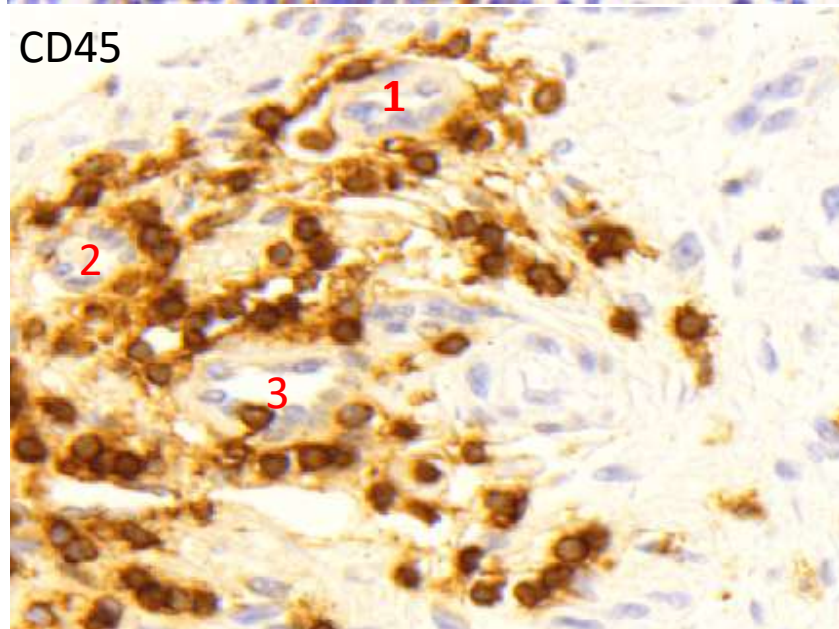
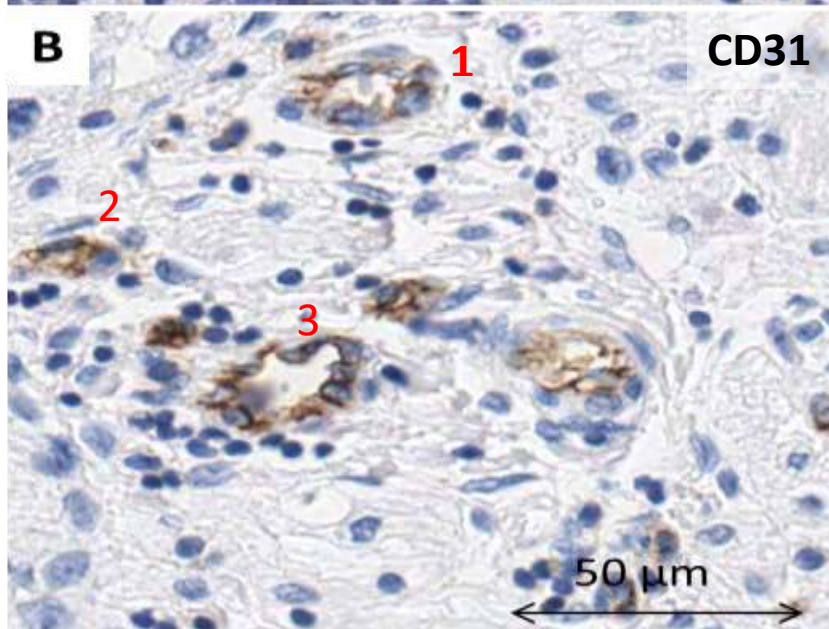
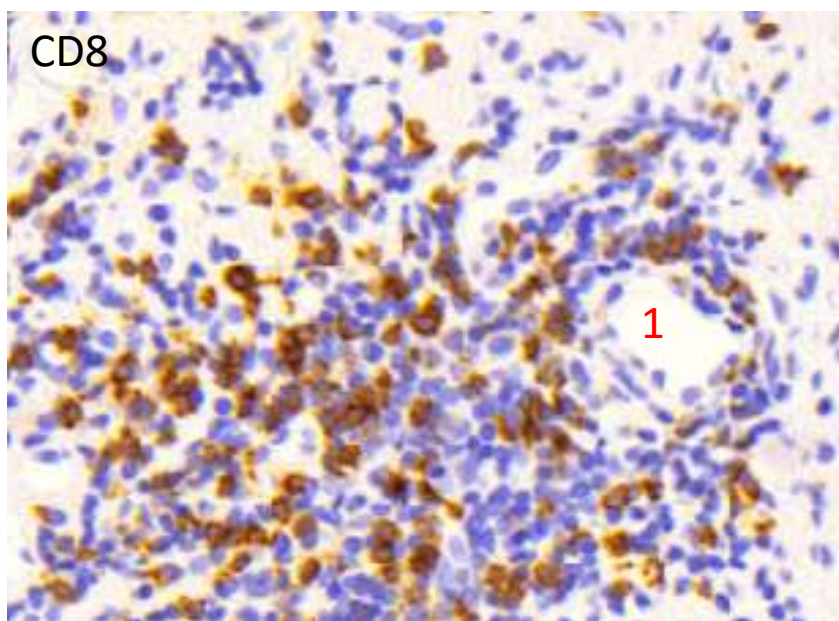
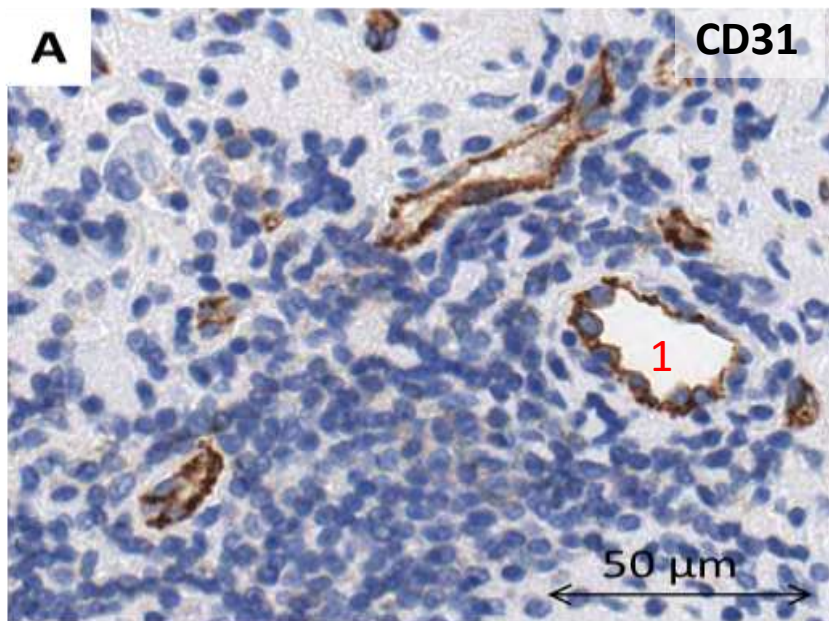
Sinusoides en glándula suprarrenal



Vasos tipo sinusoidal en NB



➤ RELACIÓN DE LA MORFOLOGÍA VASCULAR CON MEC TUMORAL



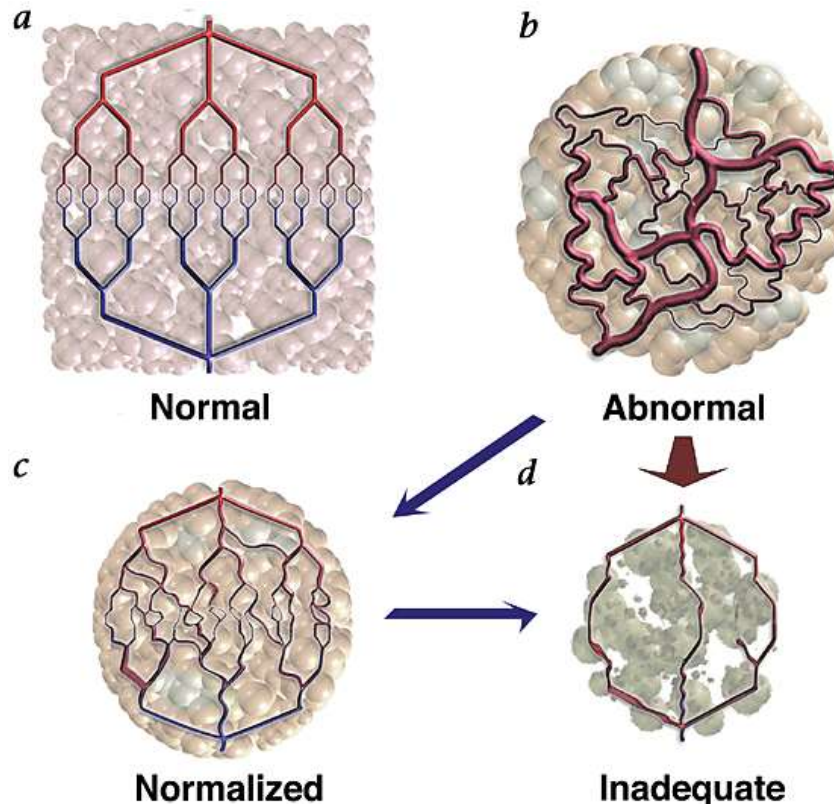
Multipotencialidad terapéutica

[Quantitative modeling of clinical, cellular, and extracellular matrix variables suggest prognostic indicators in cancer: a model in neuroblastoma.](#)

Tadeo I, Piqueras M, Montaner D, Villamón E, Berbegall AP, Cañete A, Navarro S, Noguera R.
Pediatr Res. 2014 Feb;75(2):302-14.

[Biotensegrity of the extracellular matrix: physiology, dynamic mechanical balance, and implications in oncology and mechanotherapy.](#)

Tadeo I, Berbegall AP, Escudero LM, Alvaro T, Noguera R.
Front Oncol. 2014 Mar 4;4:39.



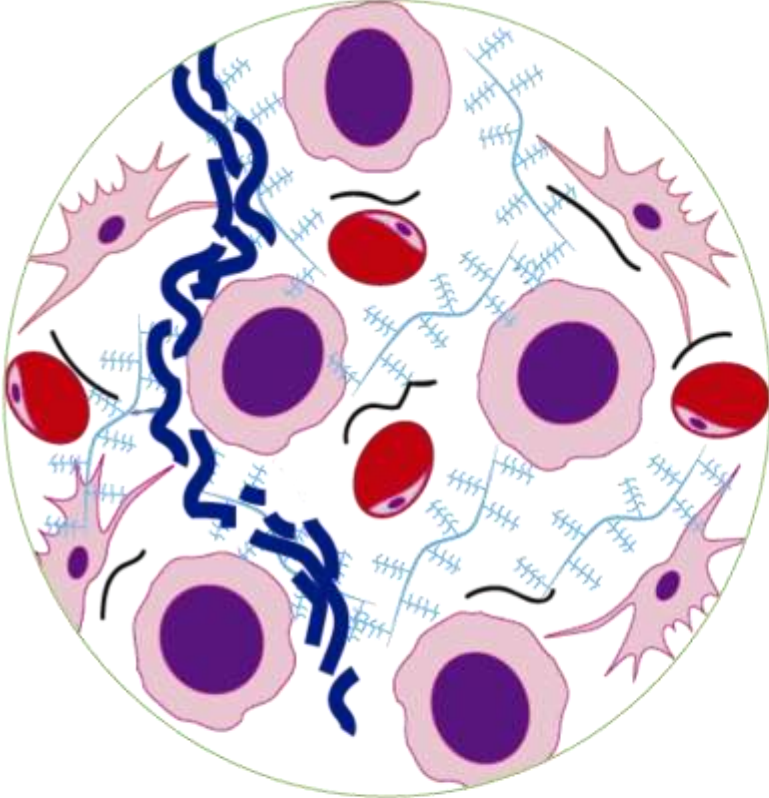
Normalizing tumor vasculature with anti-angiogenic therapy: A new paradigm for combination therapy

Rakesh K. Jain

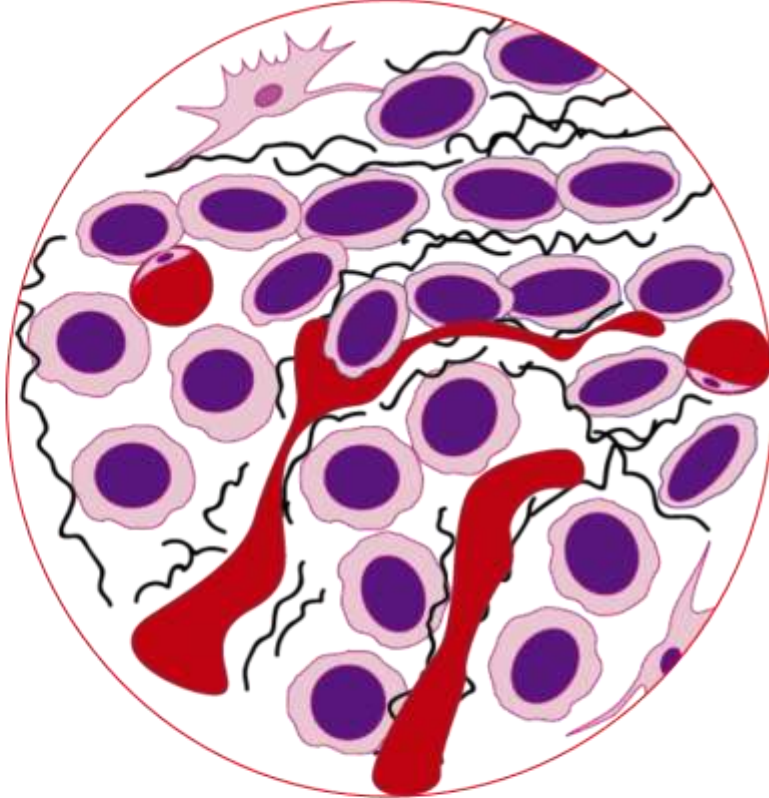
Nature Medicine 7, 987 - 989 (2001)





Multipotencialidad terapéutica





FAVORABLE



DESFAVORABLE



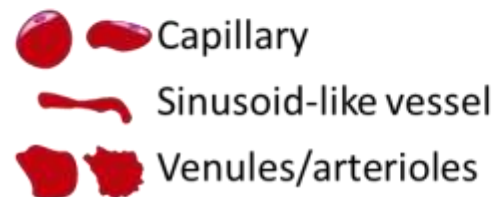
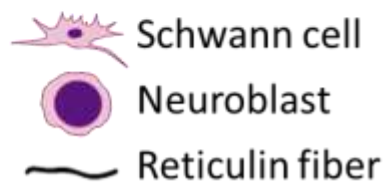
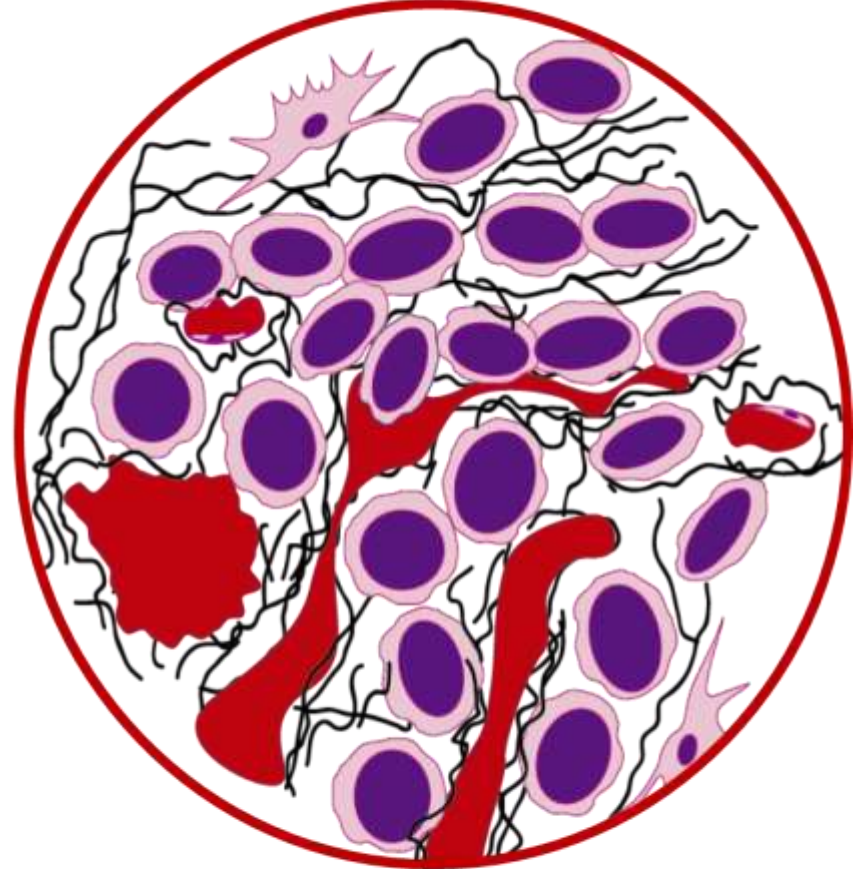
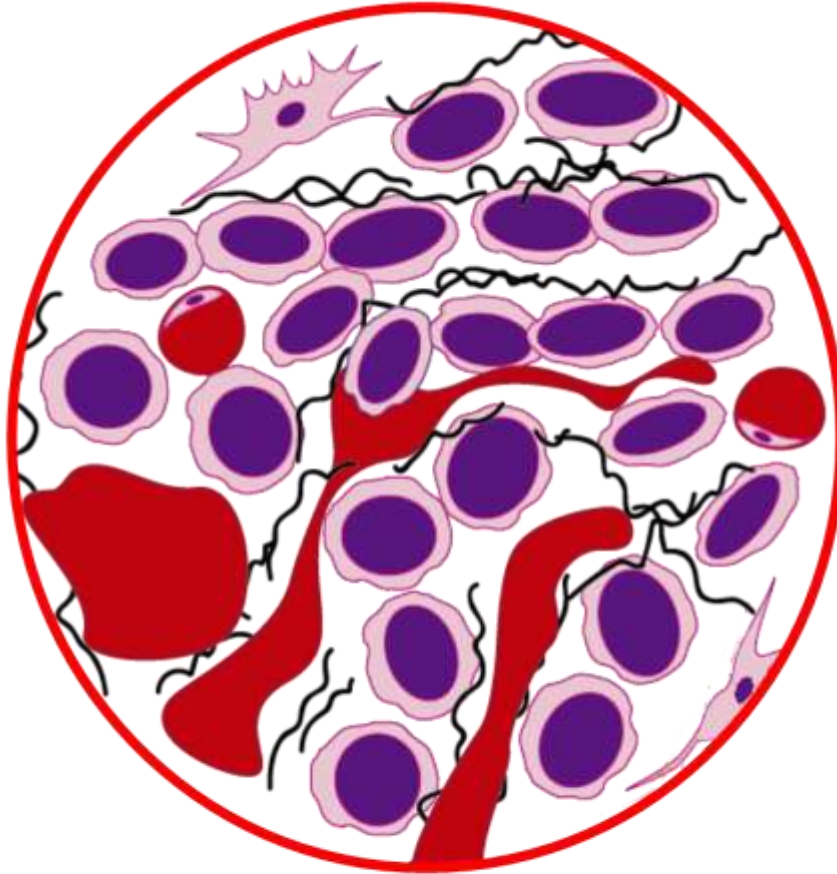
-  Schwann cell
-  Neuroblast
-  Migrating neuroblast
-  Capillary

-  Sinusoid-like vessel
-  Reticulin fiber
-  Proteoglycan
-  Collagen type I

Multipotencialidad terapéutica

ALTO RIESGO

RIESGO ULTRA ALTO



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- Elisa Alonso

- VISILAB, E.T.S.I. Industriales. U. Castilla La Mancha.

- Gloria Bueno
- Mila Fernandez

- Financiación:



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- Alejo Sempere
- Cristina Armengod

- Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío

- Luis M. Escudero
- Daniel Sánchez
- Mila Fernandez

- Los clínicos

- Los pacientes

UNIVERSITAT DE VALÈNCIA [Q*] Facultat de Medicina i Odontologia



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