

Understanding mechanisms of tumour irrigation to identify novel biomarkers



Gareth Owen

2020

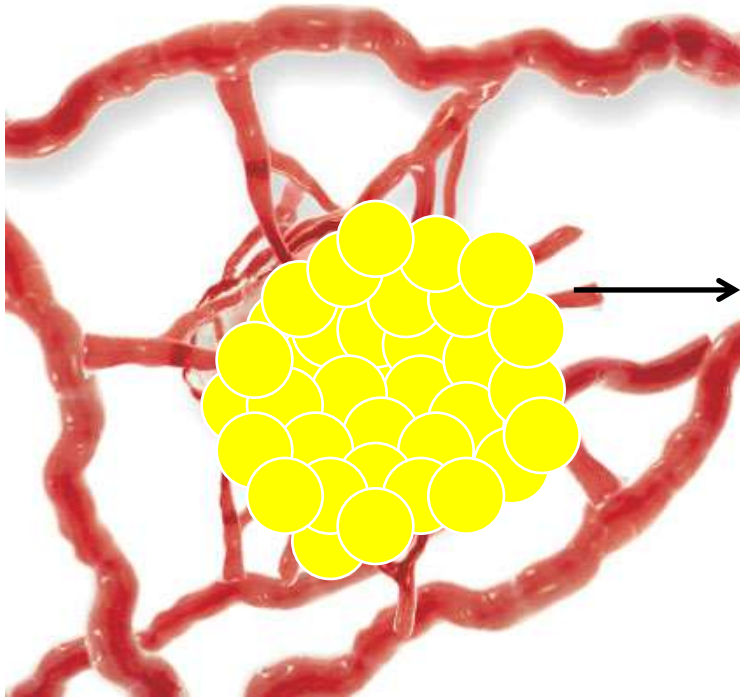


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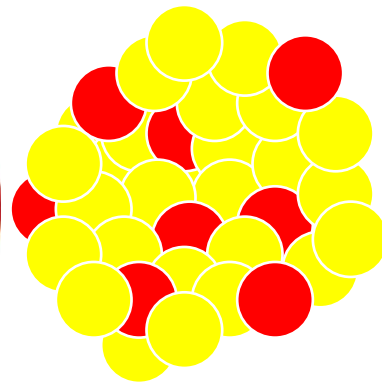


Instituto Milenio
**INMUNOLOGÍA E
INMUNOTERAPIA**

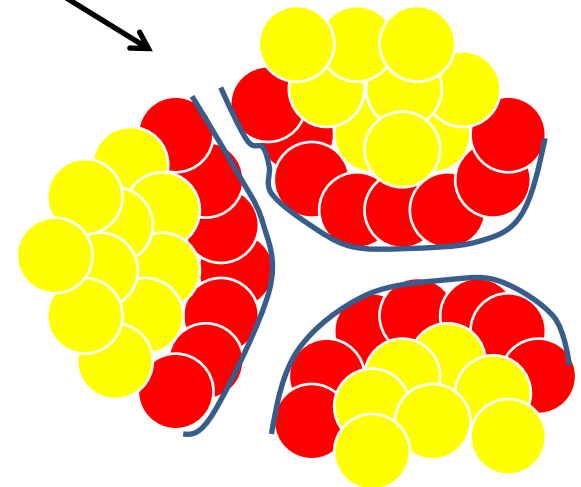
Tumor irrigation: What is Vasculogenic mimicry?



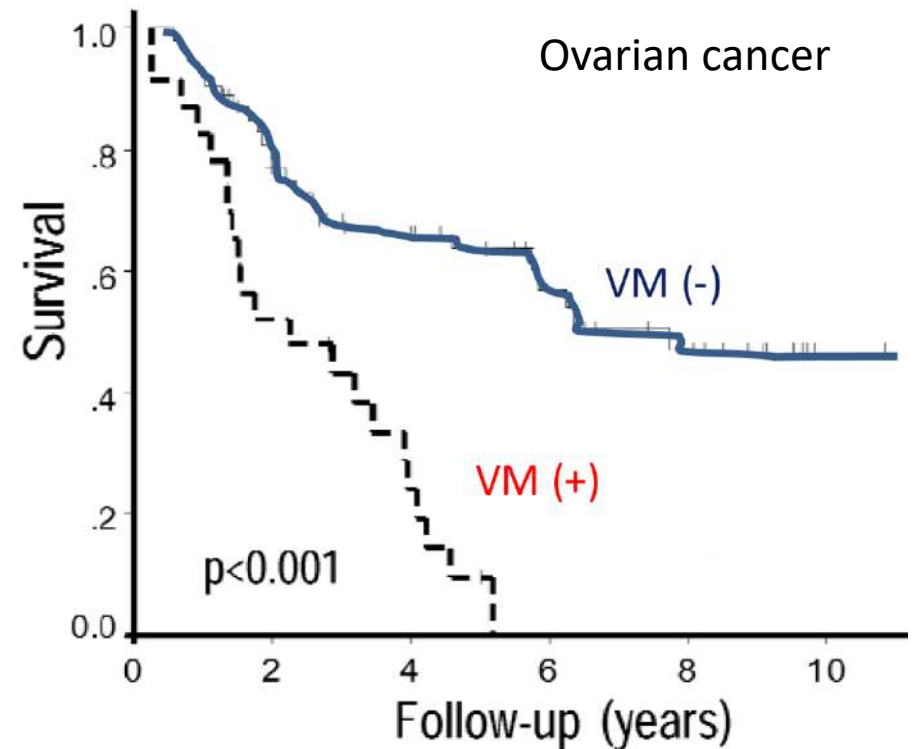
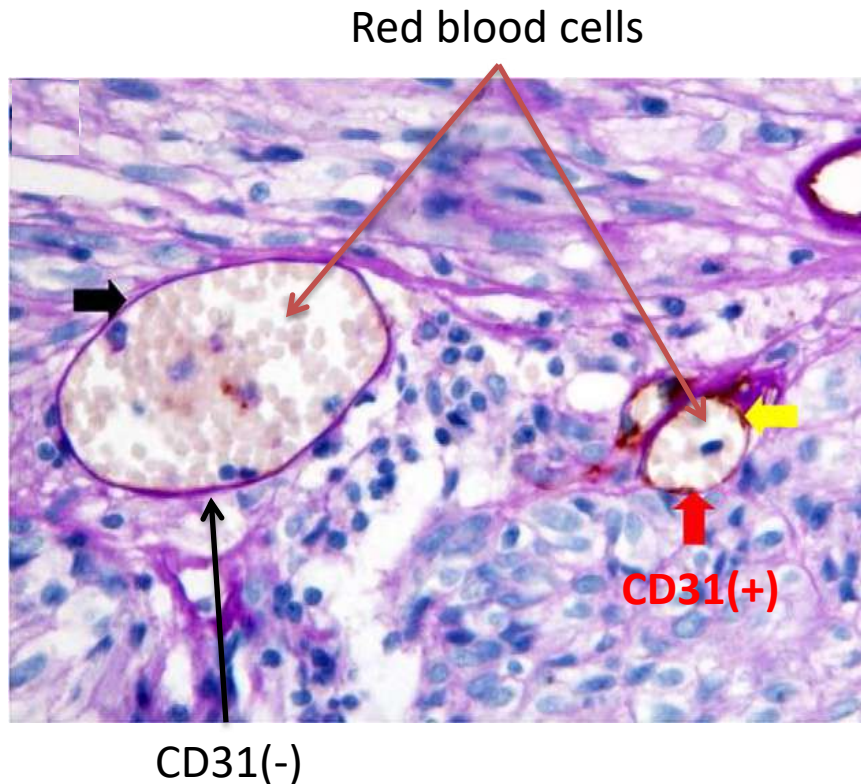
Bourgeoning tumour
or metastasis



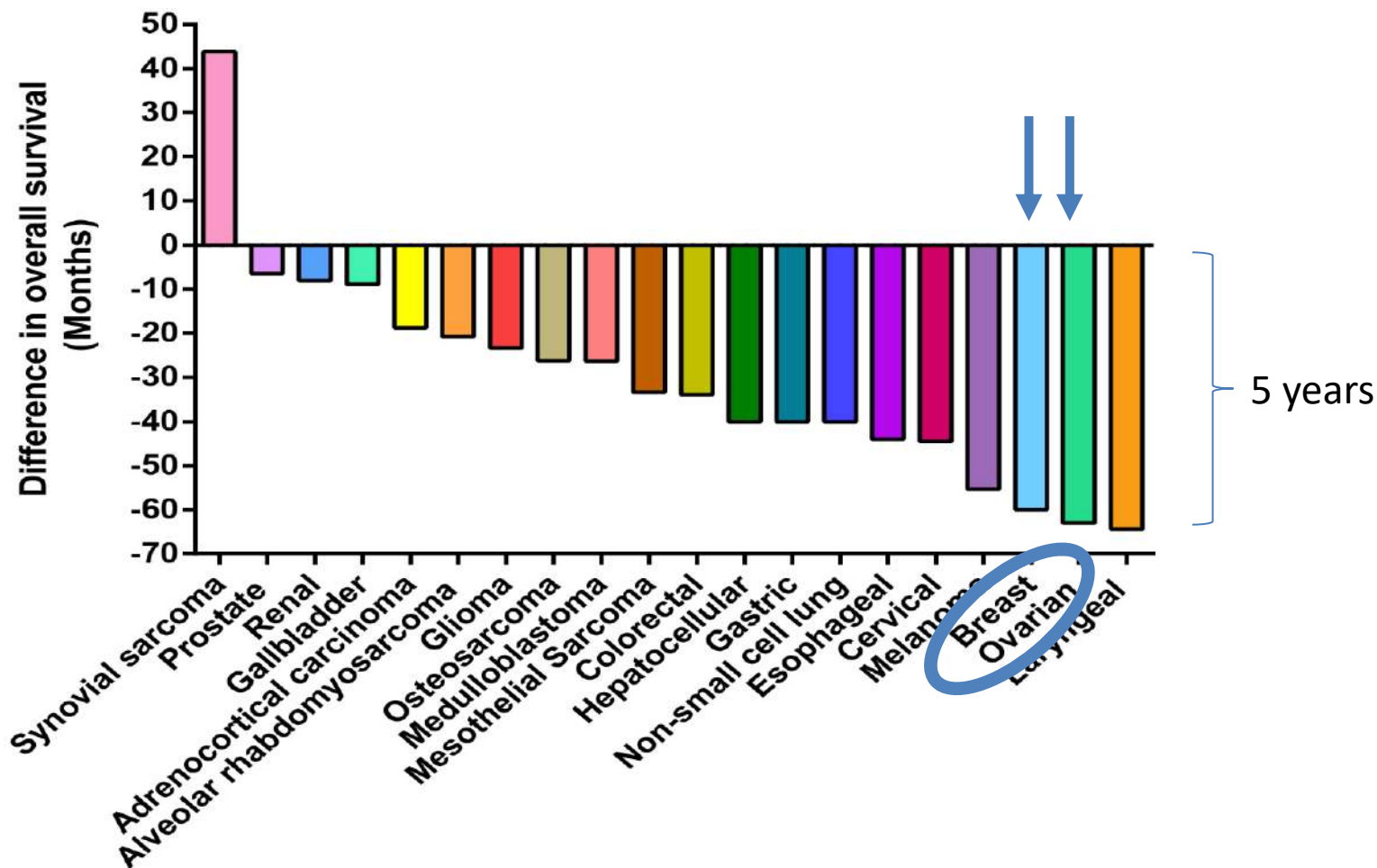
Tumour cell-lined
tubular structures



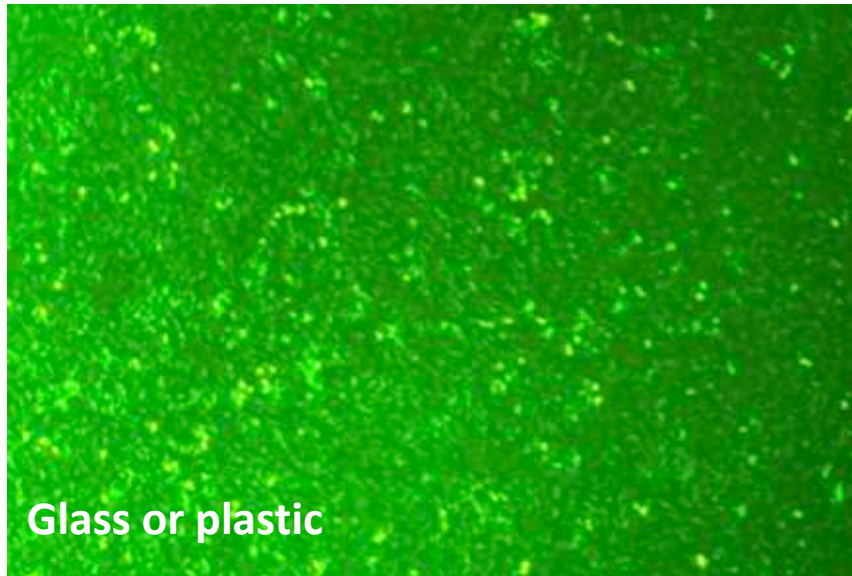
Vasculogenic mimicry positive tumours are associated with poor overall survival



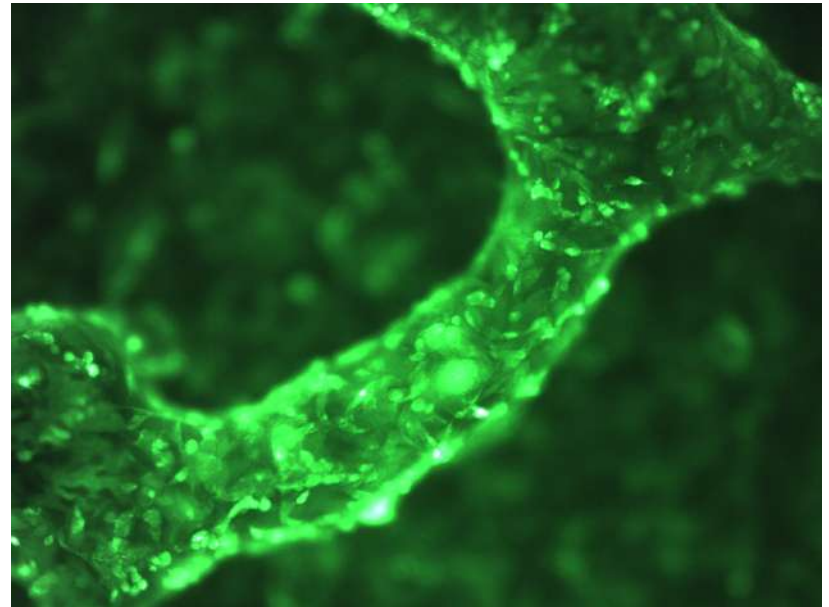
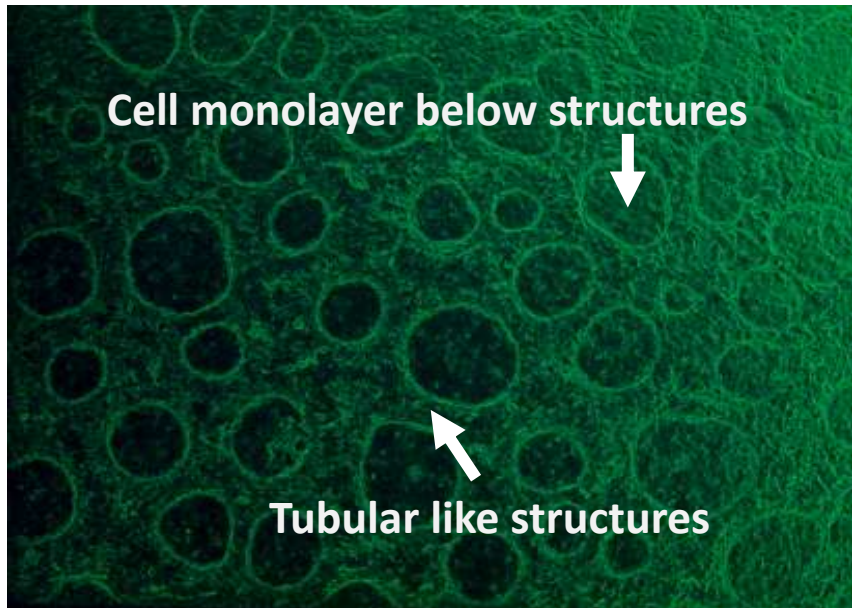
Vasculogenic Mimicry is associated with reduced survival time in virtually all cancers analysed



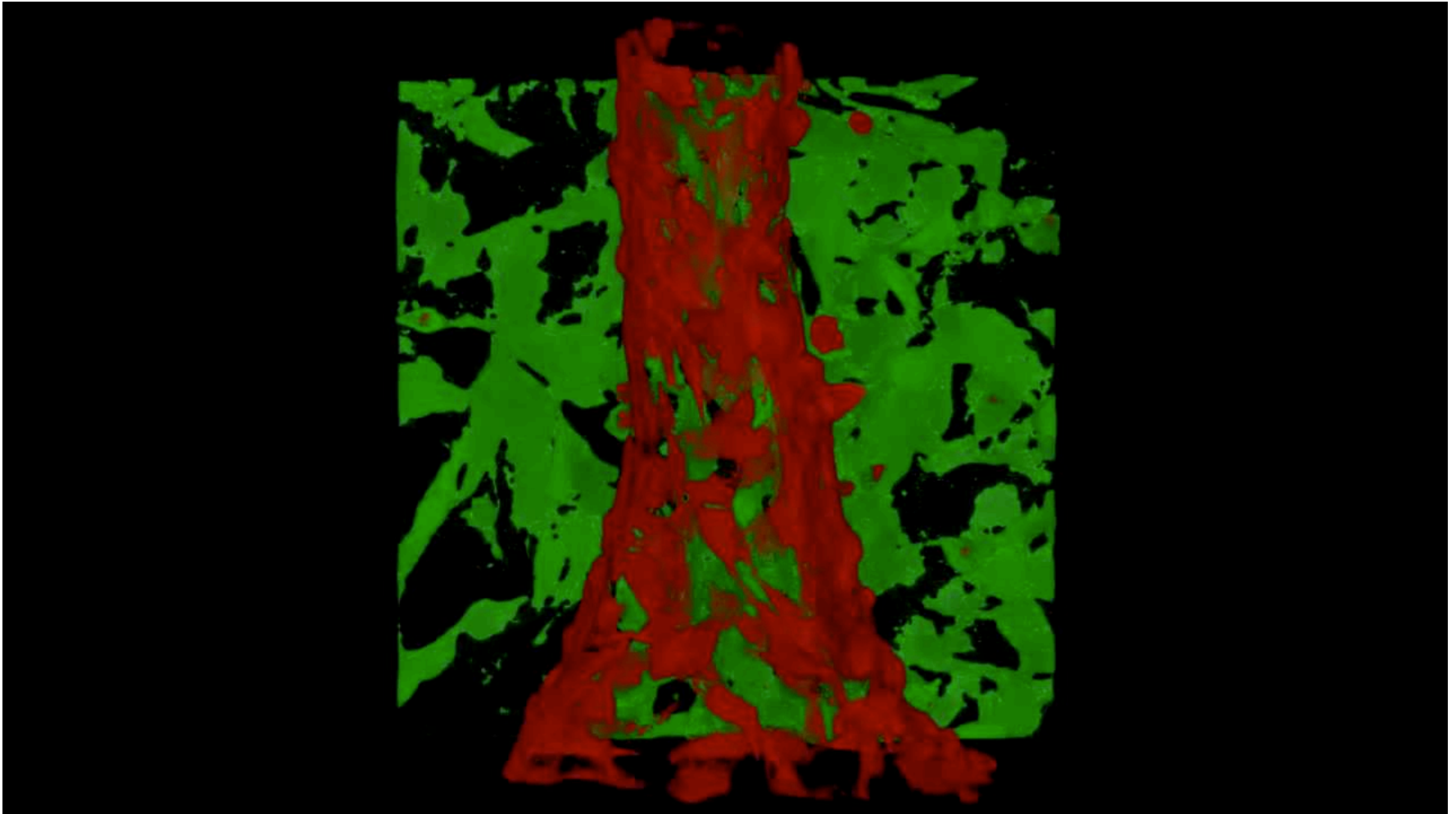
Vasculogenic Mimicry *in vitro* model



Creation of an ovarian cancer cell line expressing **GFP** that is capable of undergoing Vasculogenic Mimicry



Confocal Microscopy: Cancer Cells

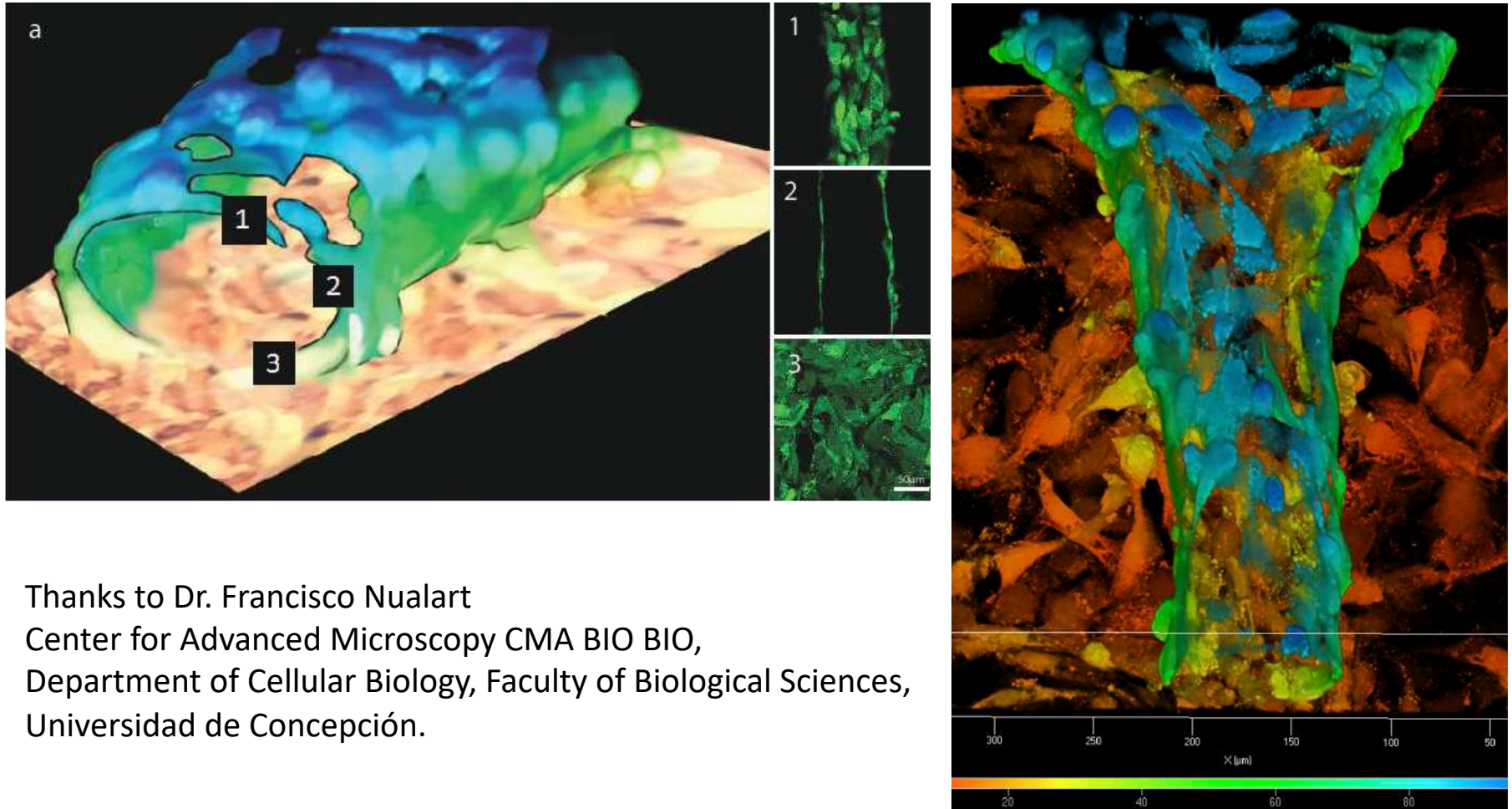


Elevated Cancer Cells

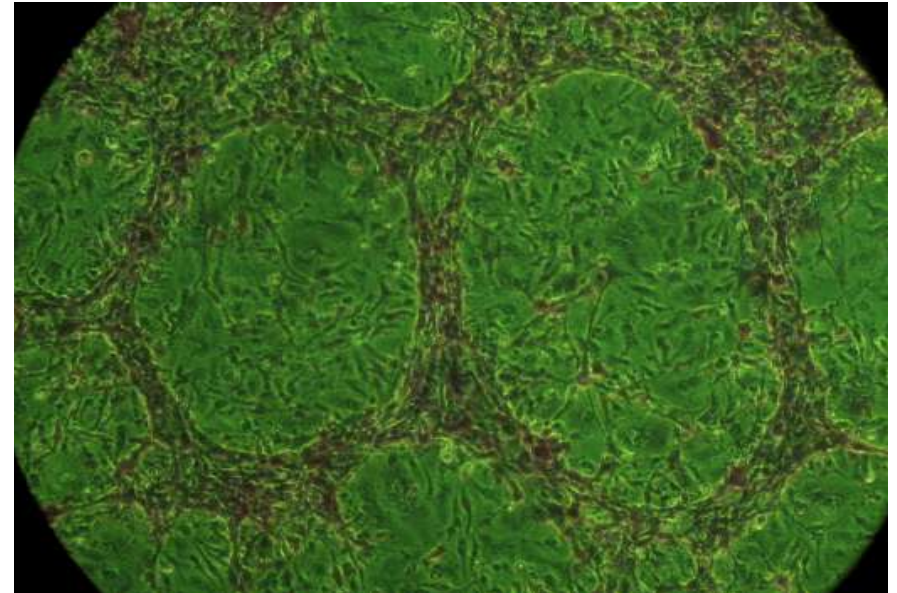
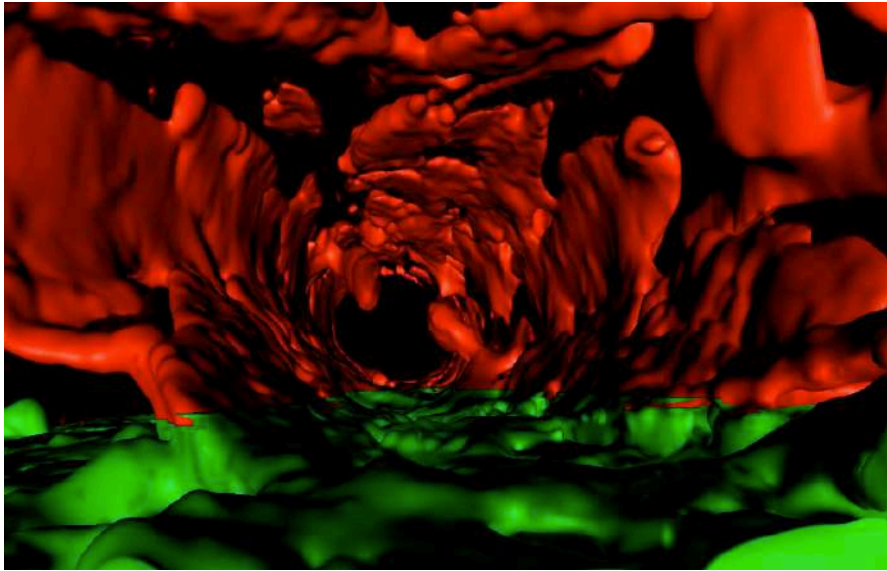
Monolayer of cancer cells

VIDEO

Z Stack Confocal Microscopy demonstrates cancer cells (fluorescence) at different heights within the matrigel



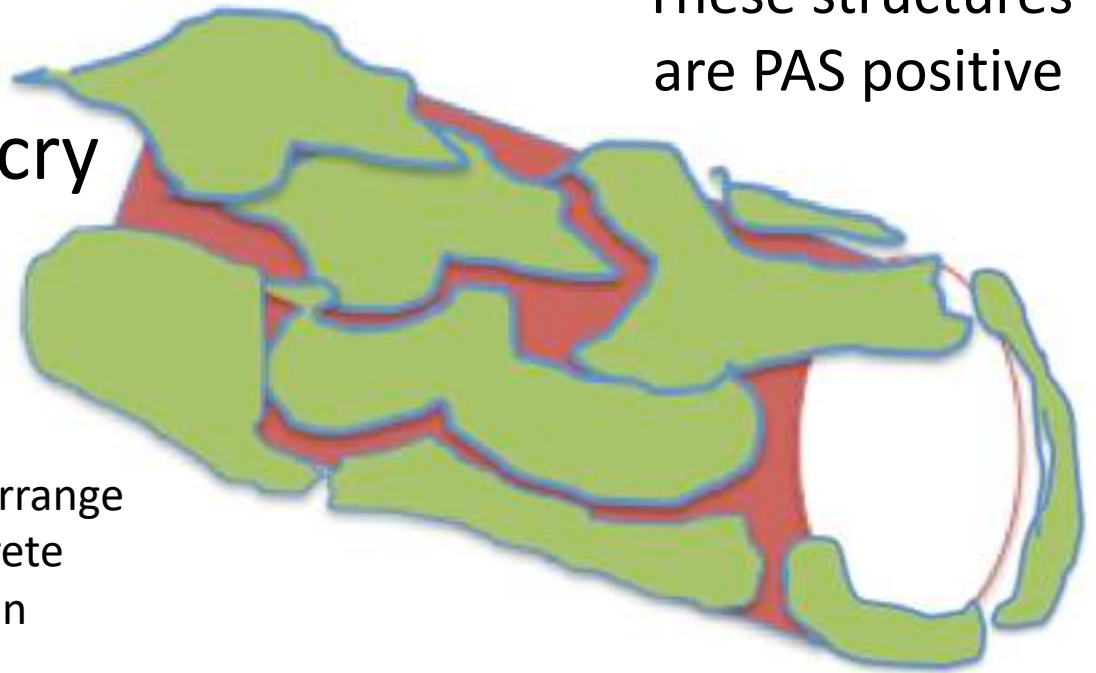
Thanks to Dr. Francisco Nualart
Center for Advanced Microscopy CMA BIO BIO,
Department of Cellular Biology, Faculty of Biological Sciences,
Universidad de Concepción.



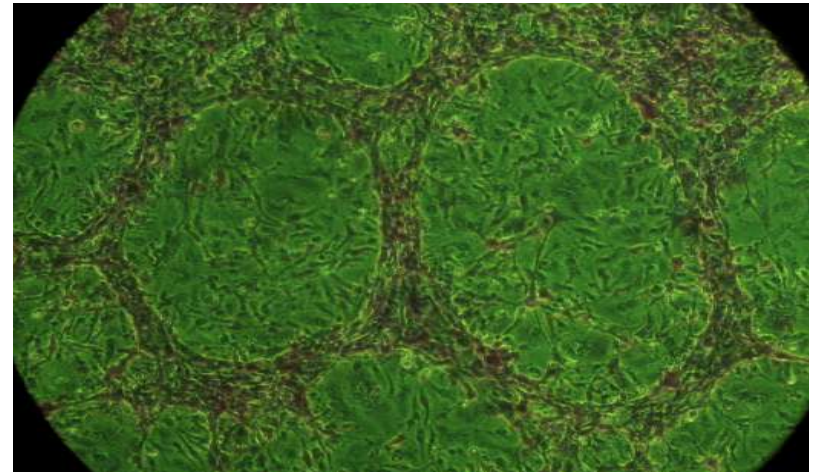
Vasculogenic Mimicry in vitro.

Cancer cells grown on matrigel rearrange
to form a hollow tube then secrete
glycoproteins to line the lumen

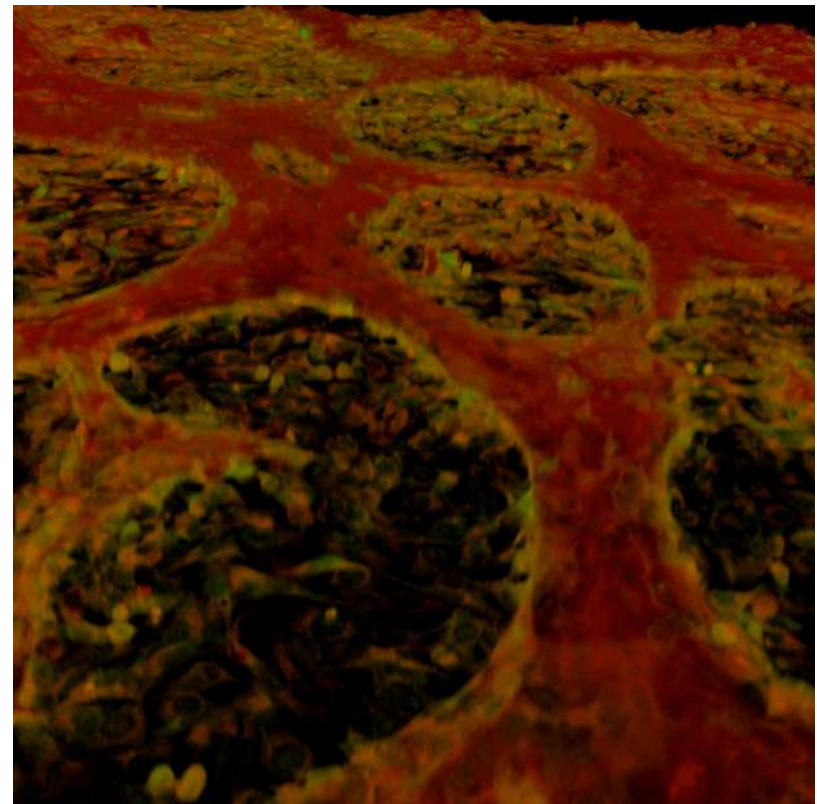
These structures
are PAS positive



Tubular structures are lined with a glycoprotein-rich layer

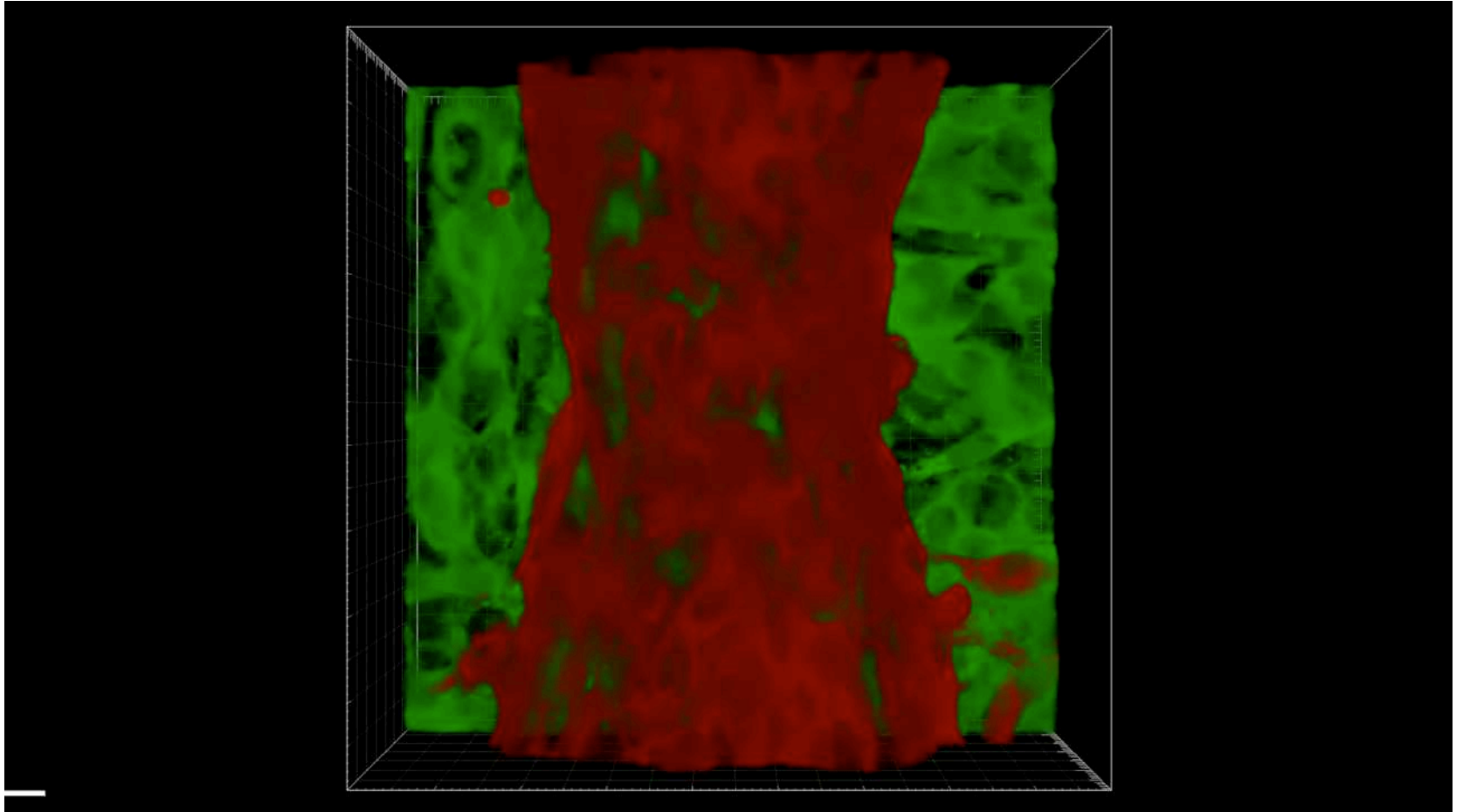


PAS stained structures give off red fluorescence under confocal microscopy when excited using an argon laser exposition of 488nm



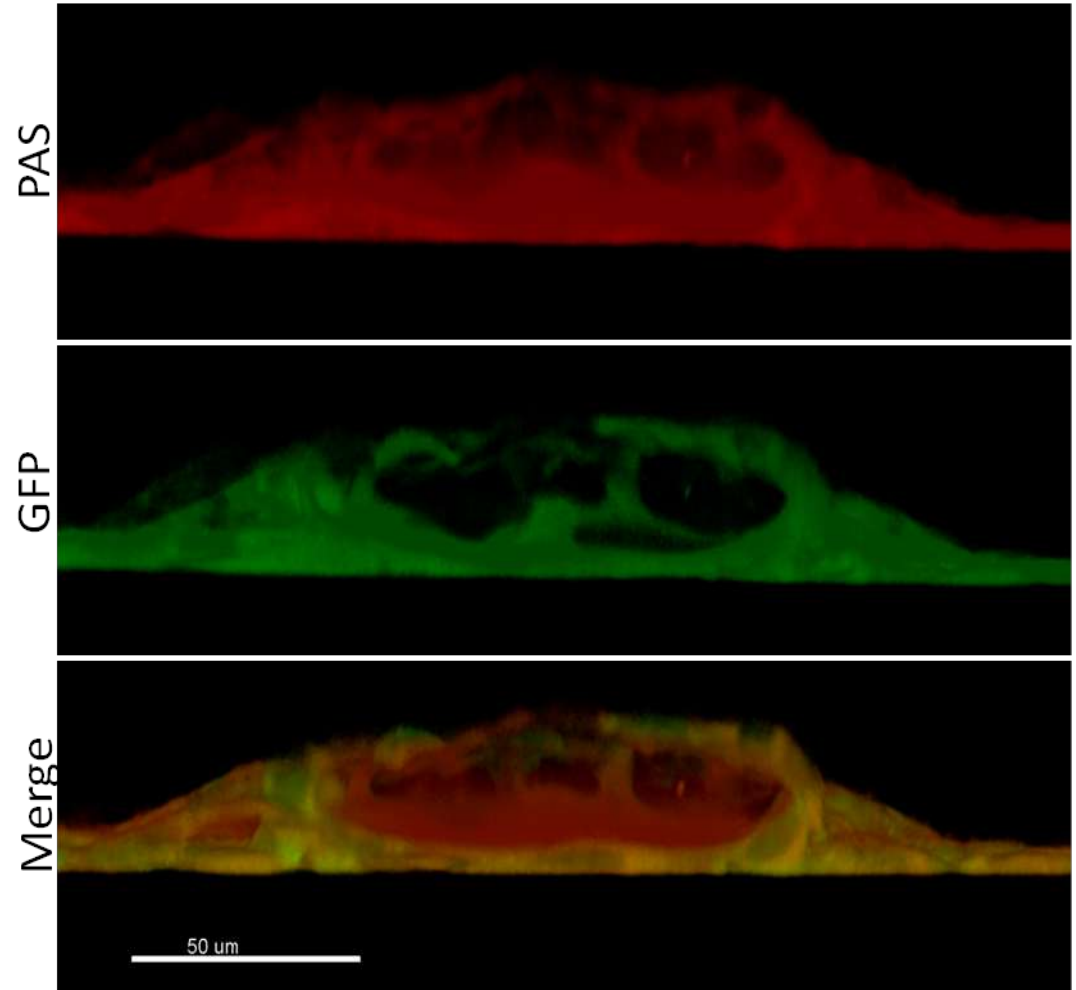
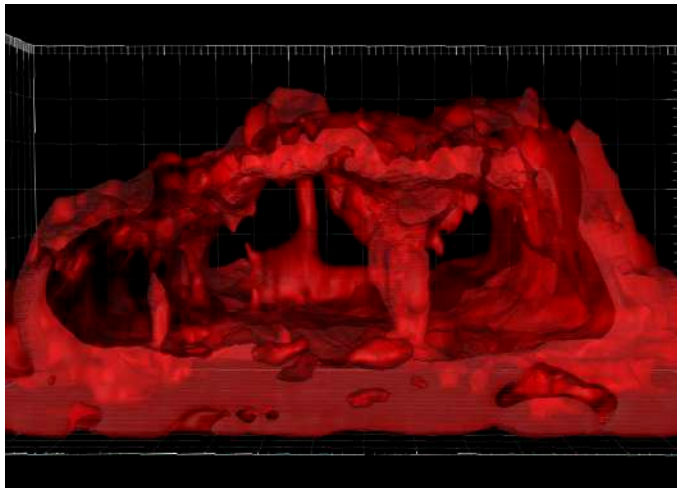
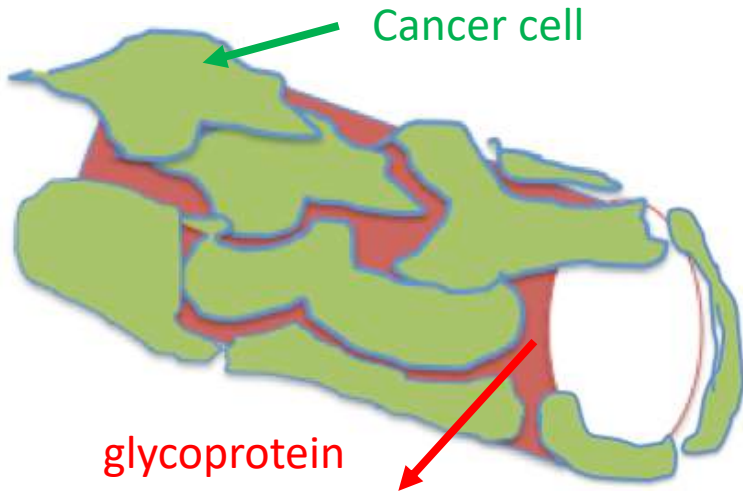
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Tubular structures are lined with a glycoprotein-rich layer



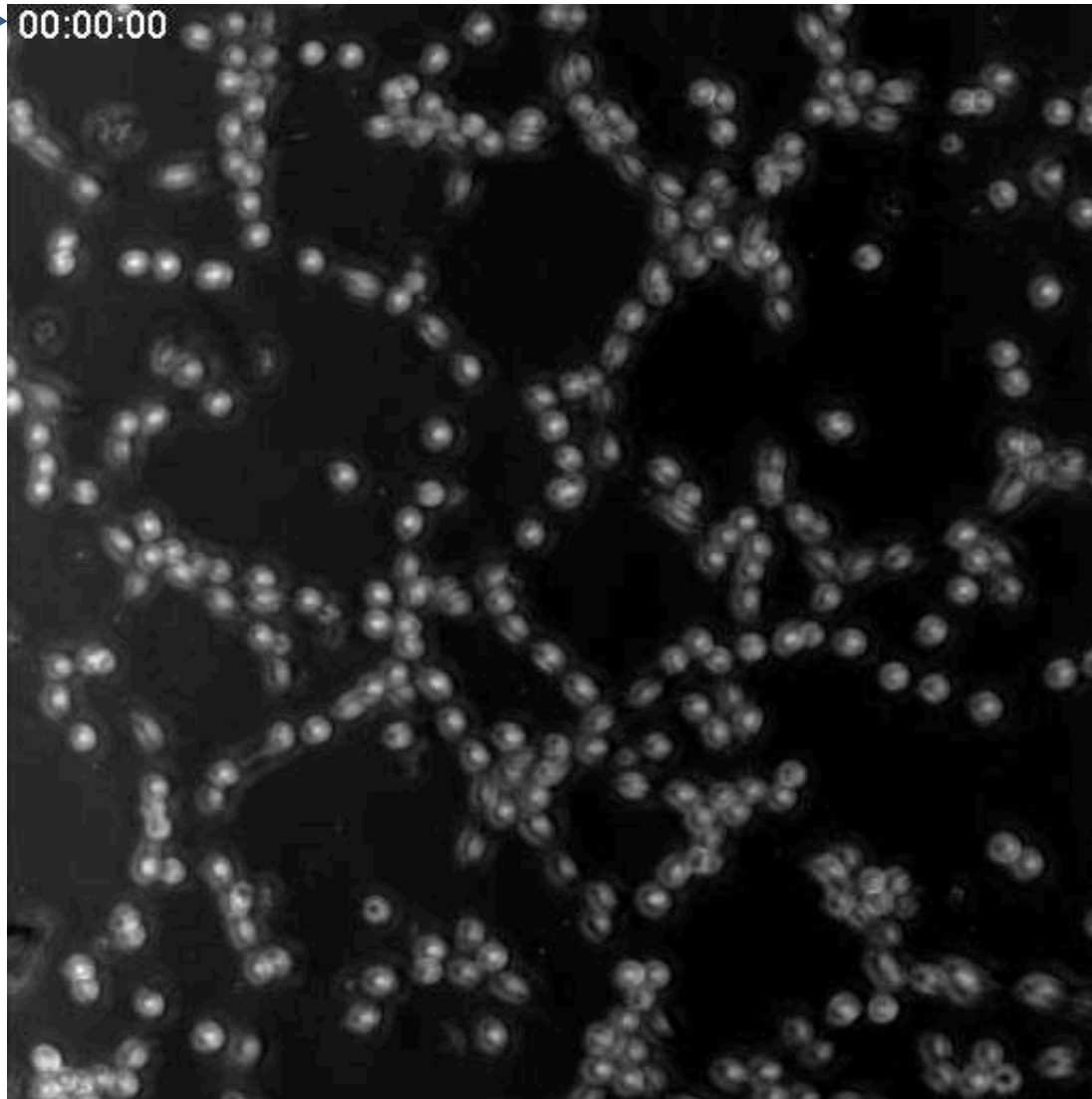
The tubular structure corresponds exclusively to PAS staining (not cancer cells)

Vasculogenic Mimicry vessels have an internal glycoprotein rich membrane surrounded by cancer cells



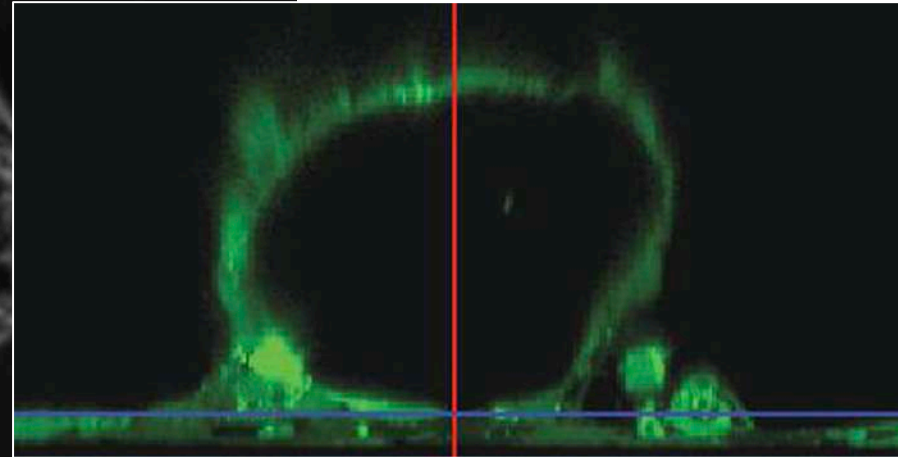
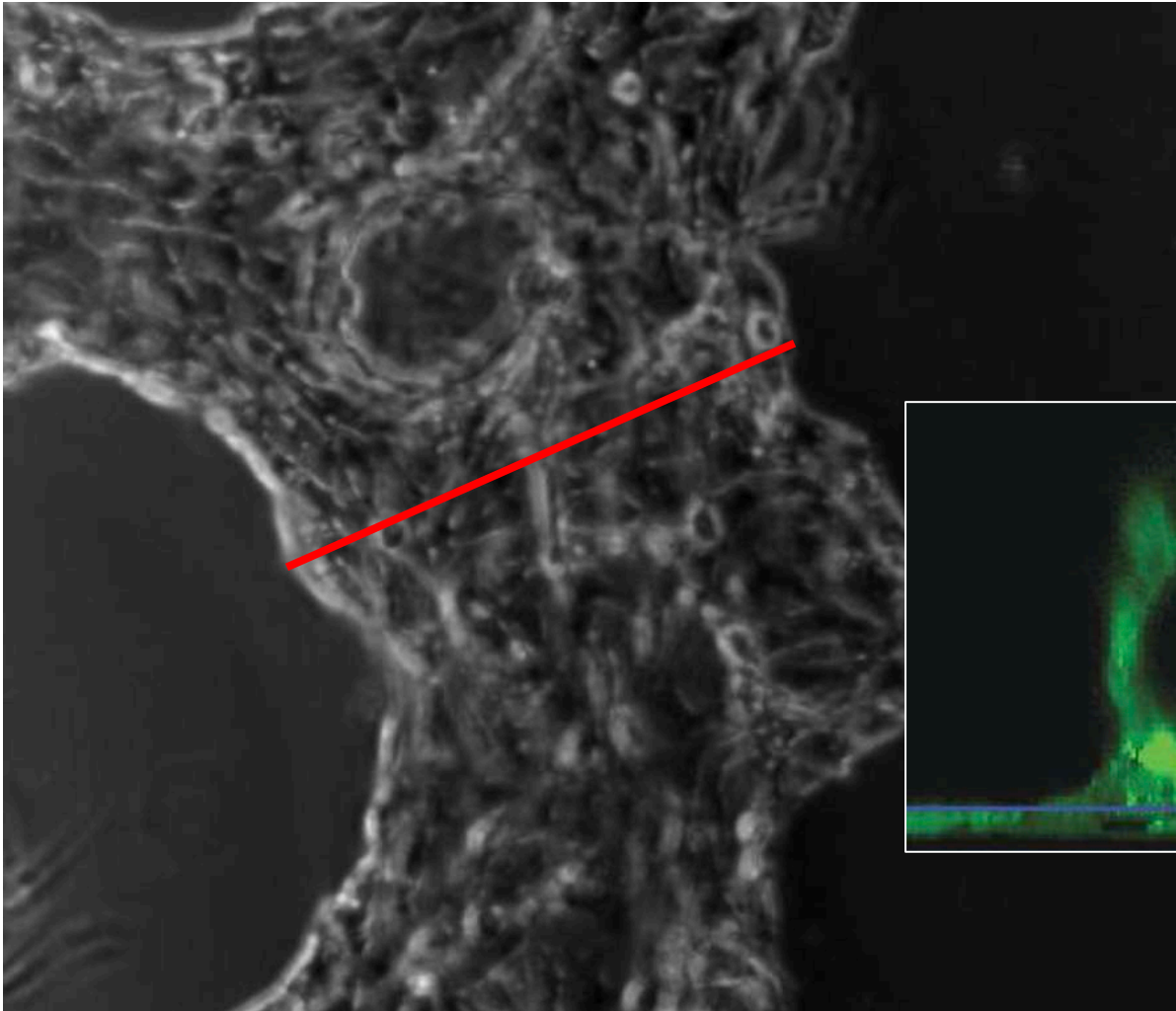
Time lapse of the formation of tubular structures

Hours after plating
(+30mins)

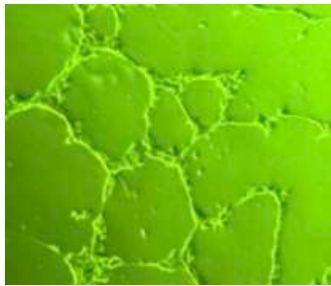


HEY-A8 ovarian cancer cell line on Matrigel

Time lapse of the formation of tubular structures

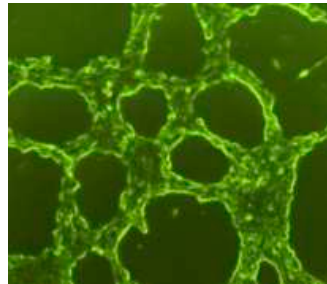


Vasculogenic Mimicry formation Time lapse



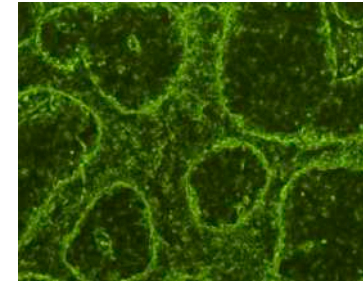
2 HRS

Organisation /
connections



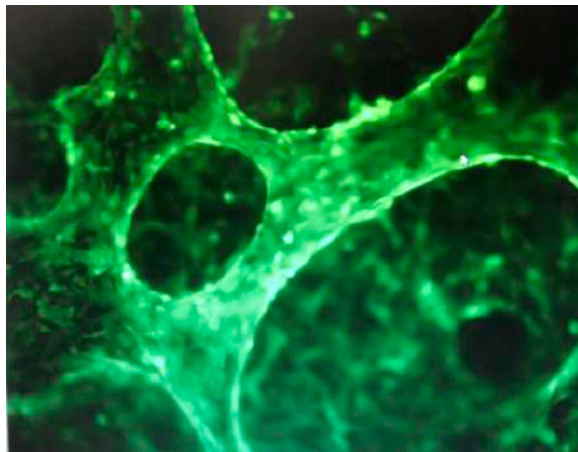
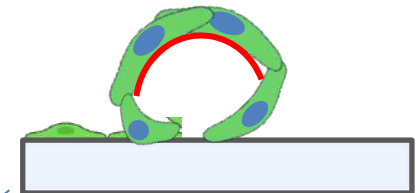
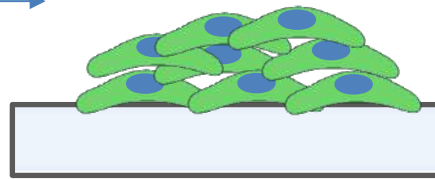
8 HRS

Proliferation



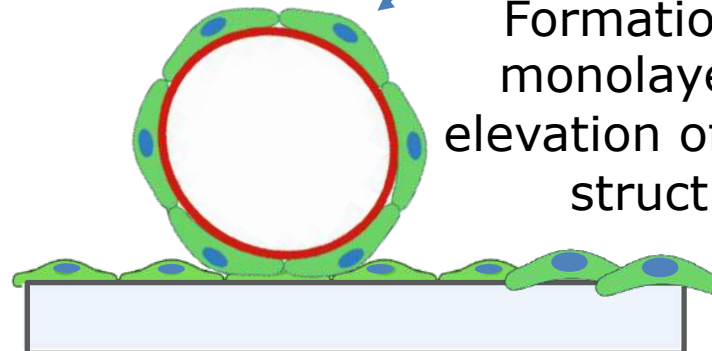
24-48 HRS

Stabilization and
formation of lumen

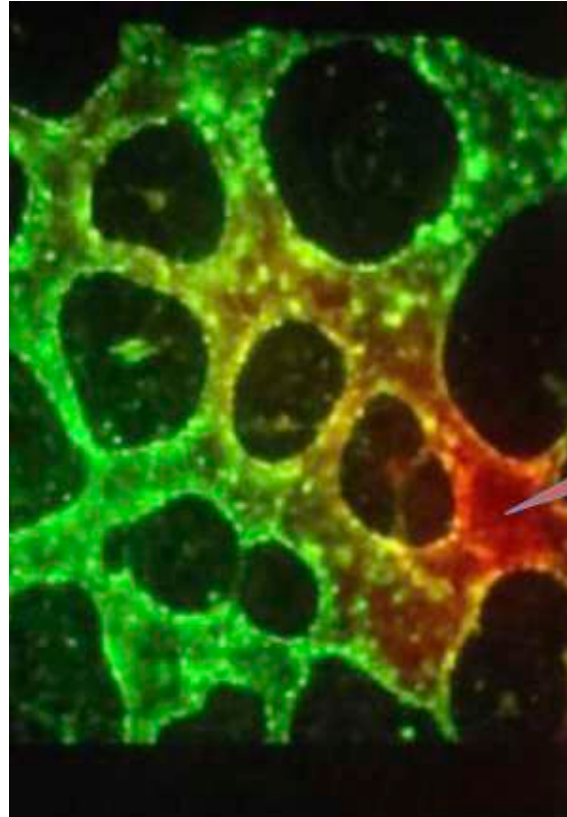
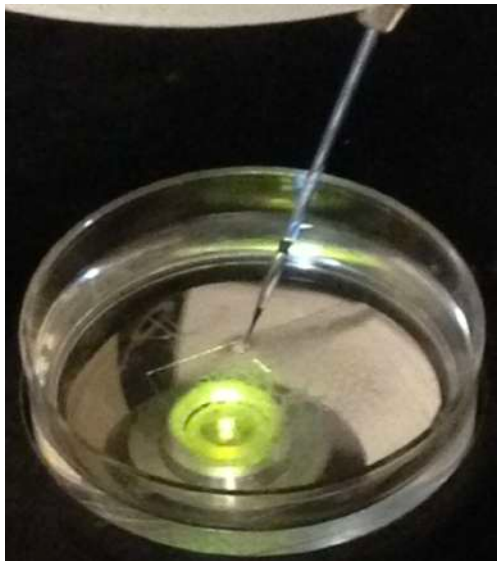
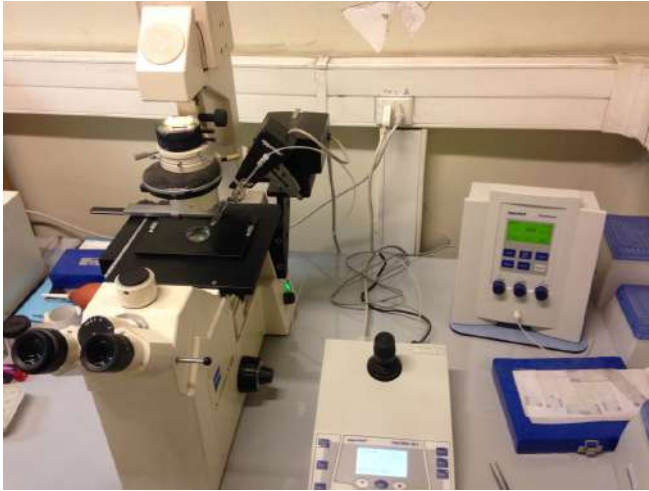


72-96 HRS

Formation of a
monolayer and
elevation of tubular
structure



Microinjection on Trypan Blue with Pressure Pump

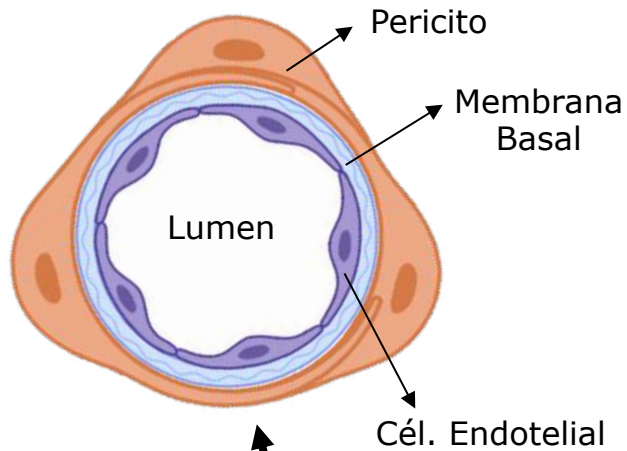


Dye Injection

Thanks to Dr. Alfonso Gonzalez,
Universidad San Sebastian, Chile

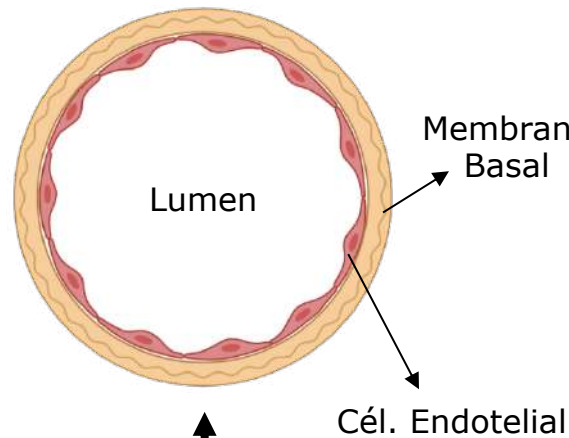
¿Cuál es la diferencia entre Mímica Vasculogénica y Angiogénesis *in vivo*?

Vaso Sanguíneo



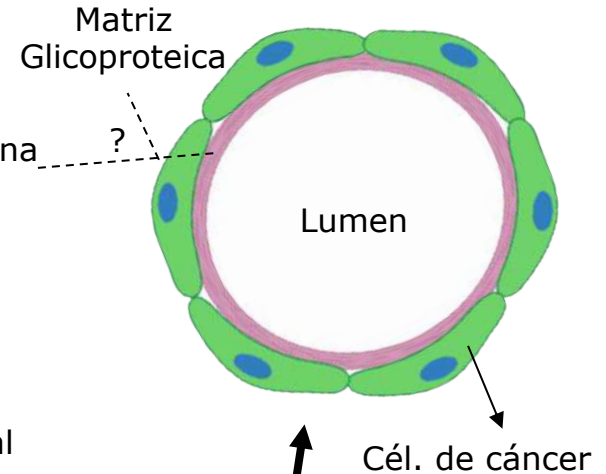
CD31(+) / **PAS (+)**

Vaso Linfático



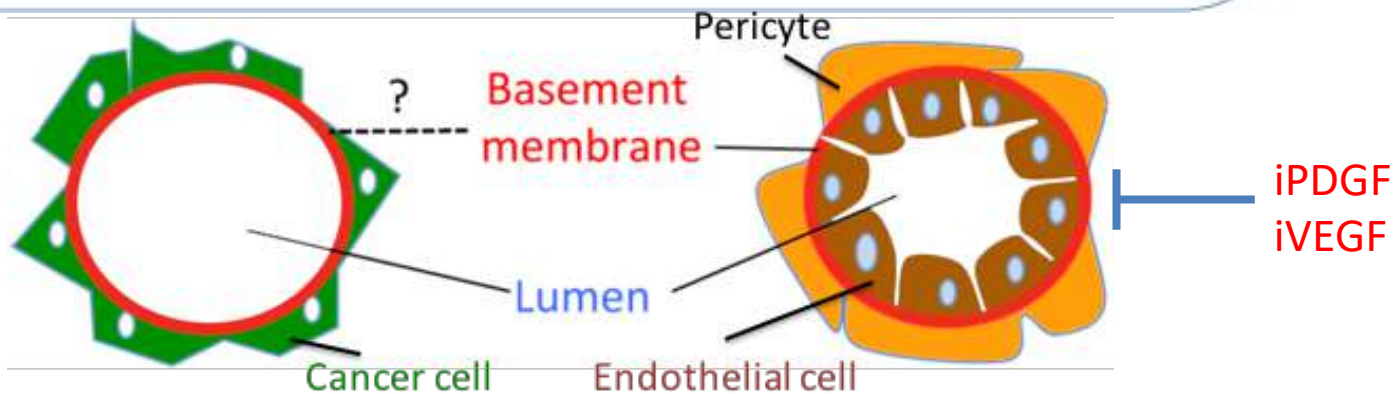
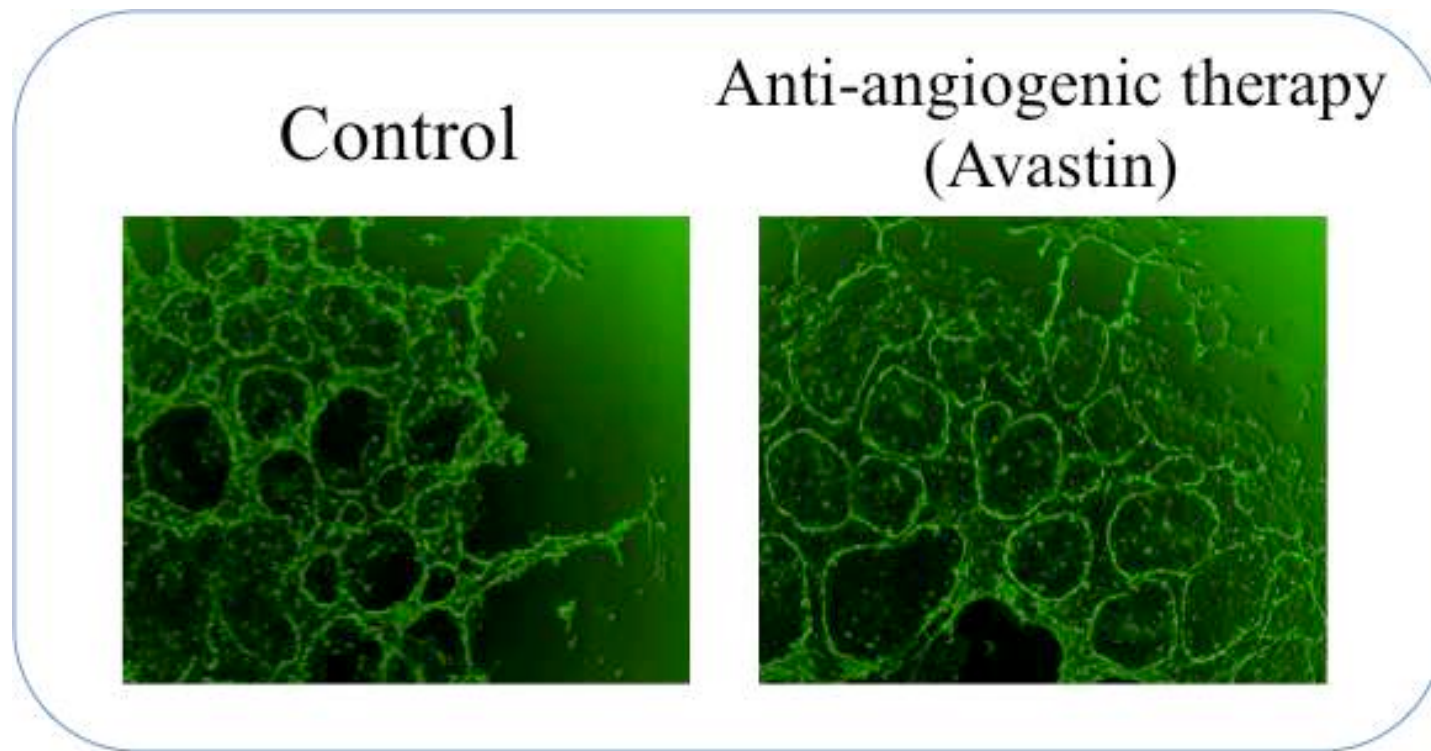
CD31(+) / **PAS (+)**

Mímica Vasculogénica

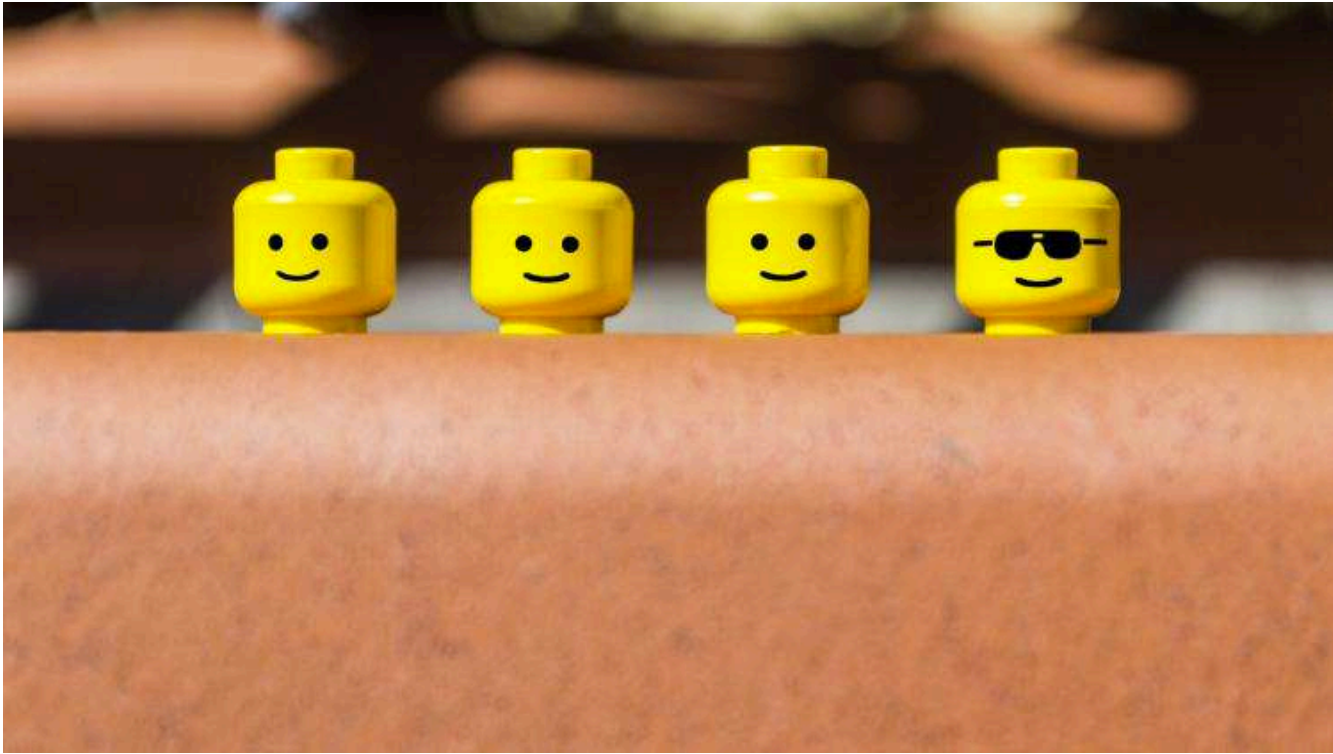


CD31(-) / **PAS (+)**

Vasculogenic Mimicry and Angiogenesis are separate processes



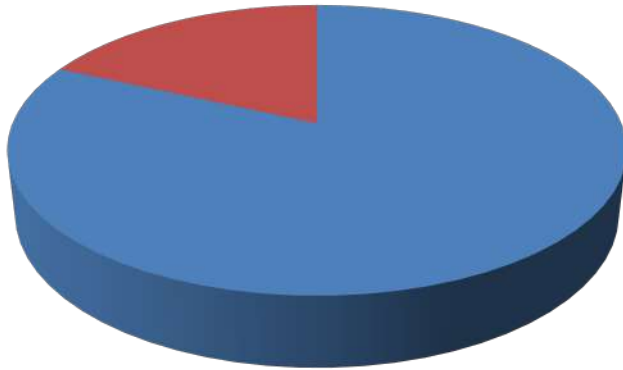
Can all cancers undergo
Vasculogenic Mimicry ?



Cultivos primarios de cáncer gástrico y ovario también son capaces de generar VM

Cáncer de Ovario

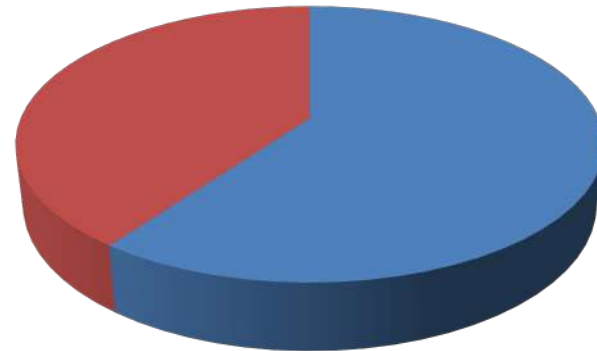
VM+
(n=6)
18%



Total de
muestras
(n=27)
82%

Cáncer Gastrointestinales

VM+
(n=4)
40%



Total de
muestras
(n=6)
60%



The Next Step

Mechanism

The Bioinformatic Approach

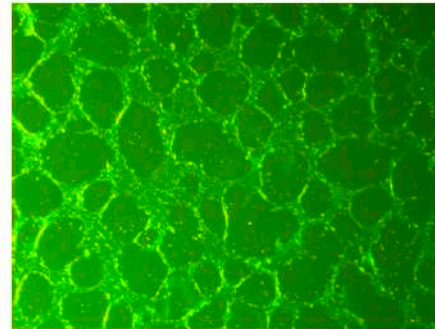


SickKids
THE HOSPITAL FOR
SICK CHILDREN

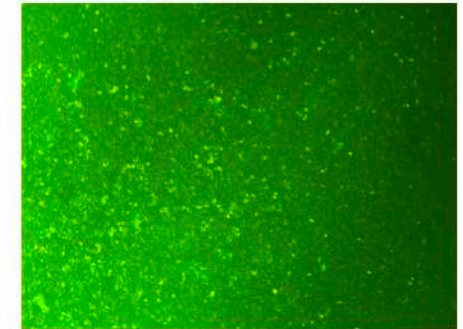
Microarray

Upregulated Genes	Fold Change	P Value
ZNF253	1,578	0,031
ZNF267	1,576	0,038
SCEL	1,571	0,043
NBPF12	1,567	0,023
ZNF615	1,529	0,023
IFNA14	0,5656	0,023
TRBV6-5	0,5940	0,029
KRTAP5-7	0,6039	0,024
KRTAP10-12	0,6069	0,023
IGLL5	0,6352	0,029

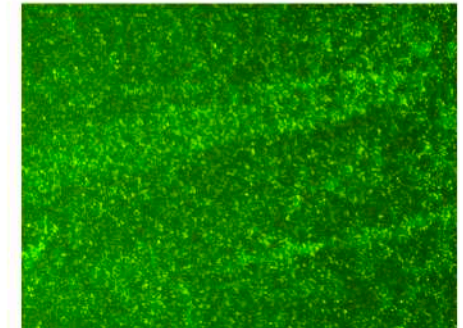
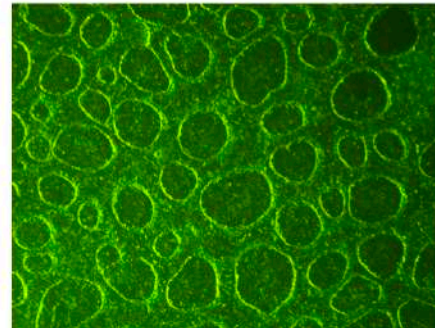
Day 1



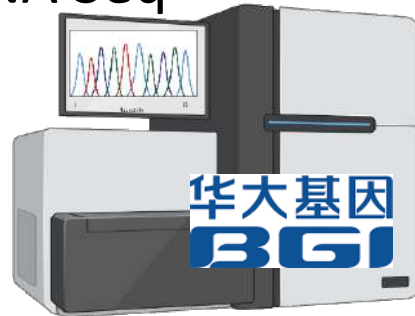
Denatured



Day 4



RNA Seq



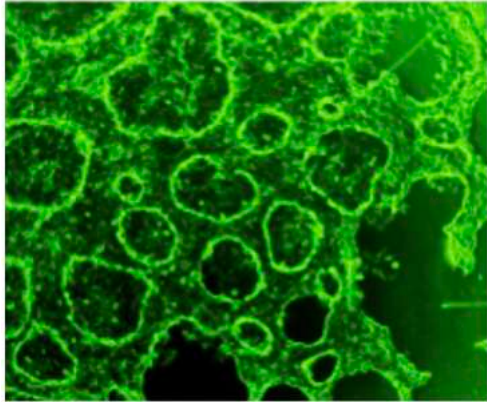
华大基因
BGI

N=4

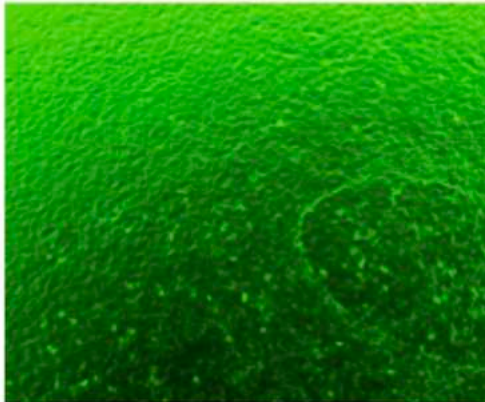


The Matrix Approach

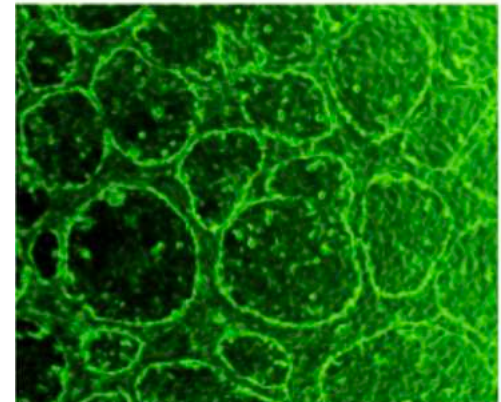
Matrigel



Colagen 1

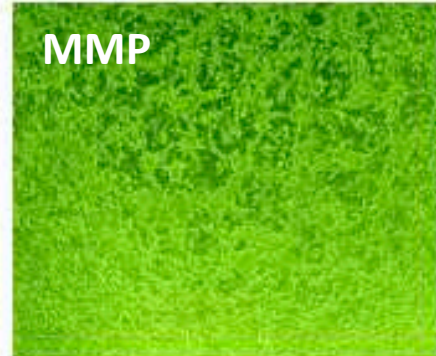
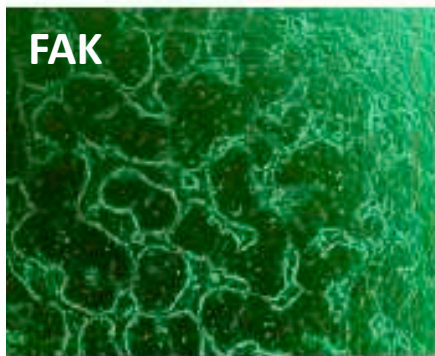
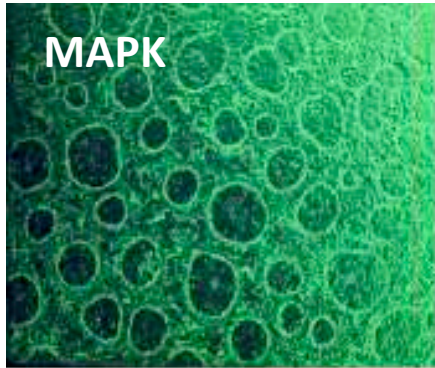


Laminin 111



Vasculogenic Mimicry can occur upon a matrix containing exclusively Laminin 1

The Inhibition Approach (Chemical and siRNA)



**Laminin is required to trigger
Vasculogenic Mimicry**

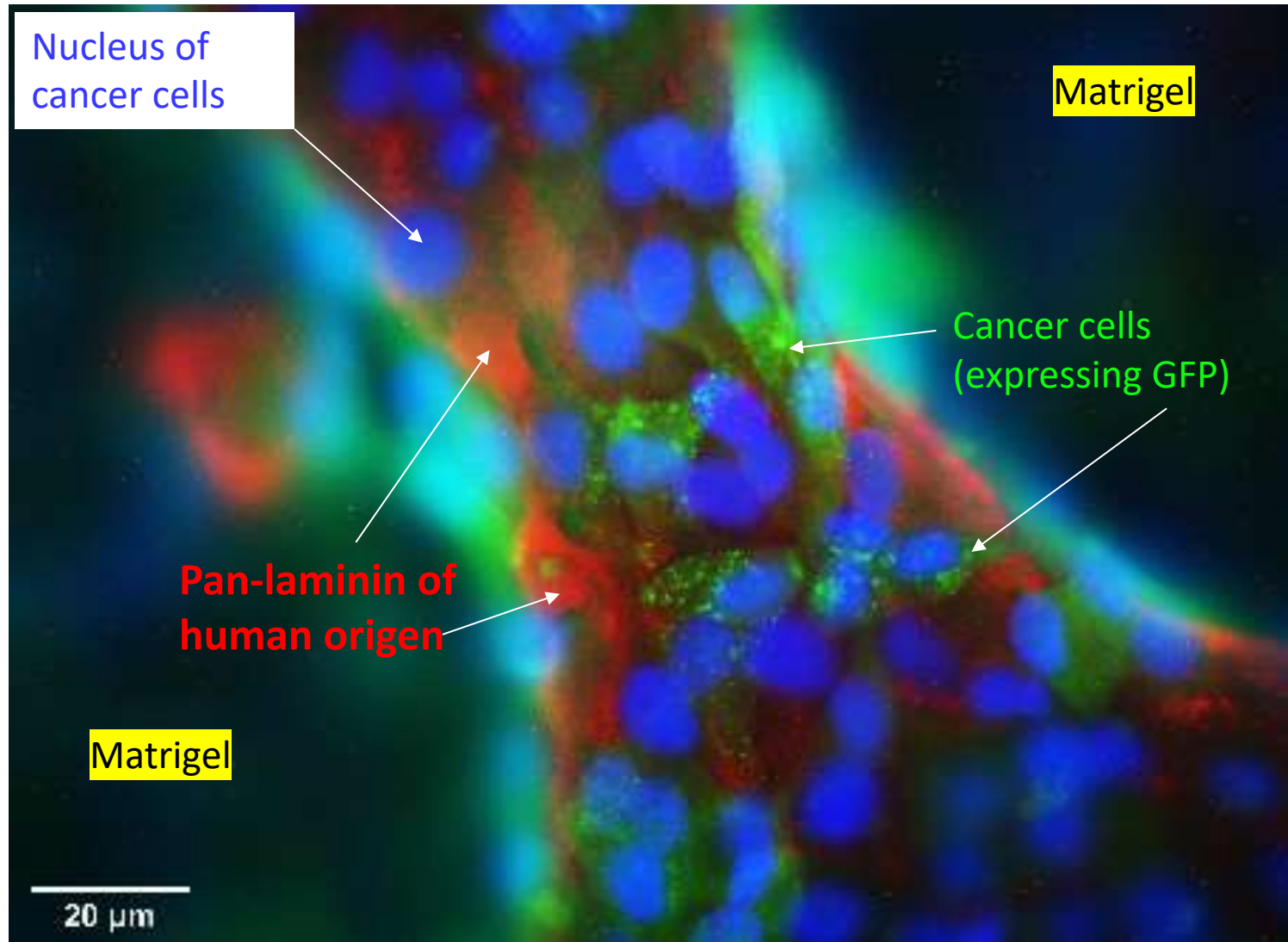


However

**Does laminin make up part of the matrix
within the tubular structure?**

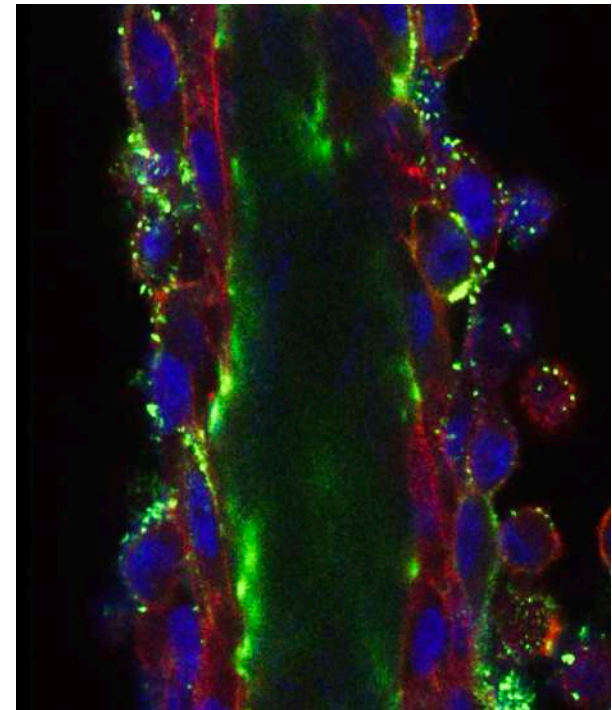
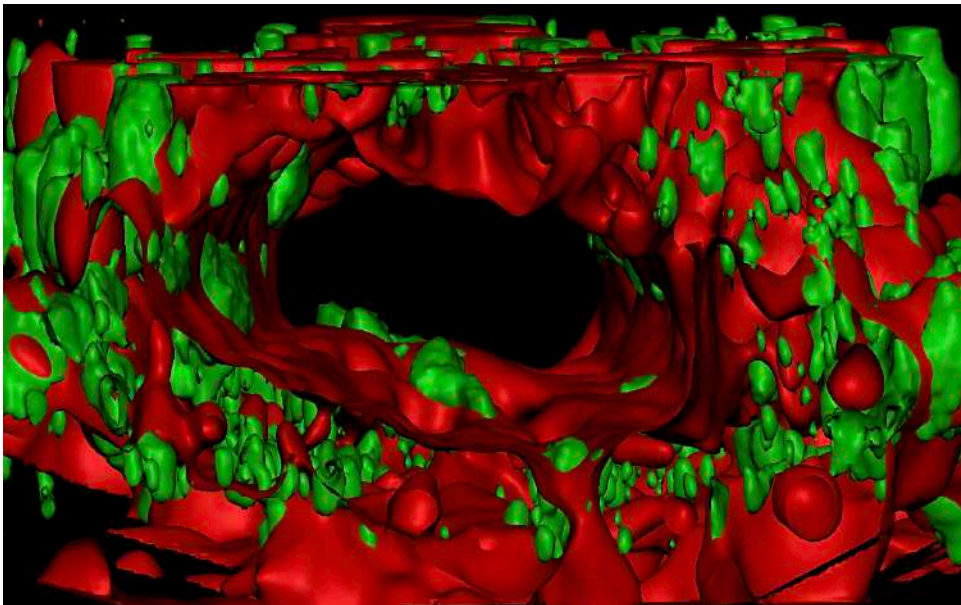
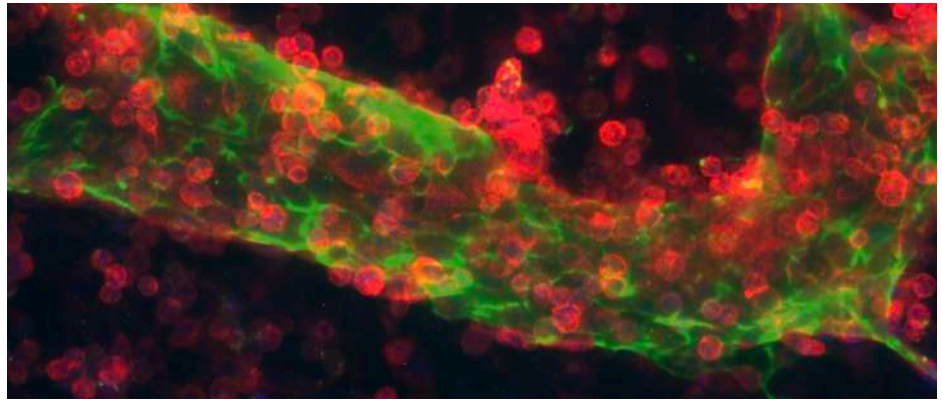
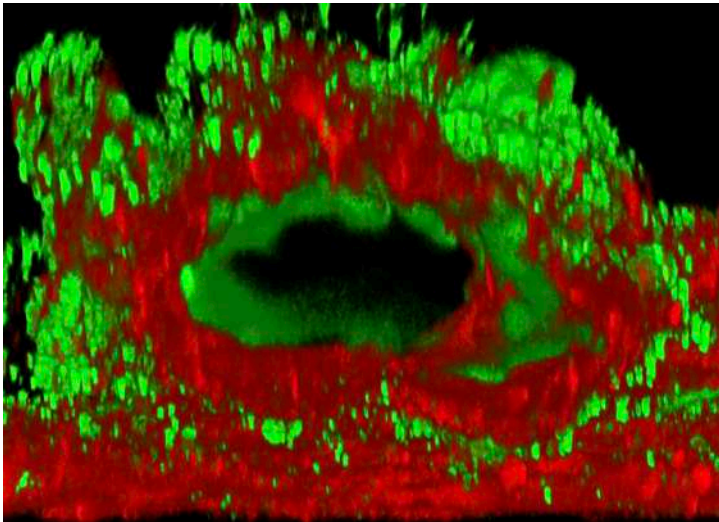


Evidence for laminin expression within tubular structures



Laminin comes from the cancer cell, not the matrigel matrix

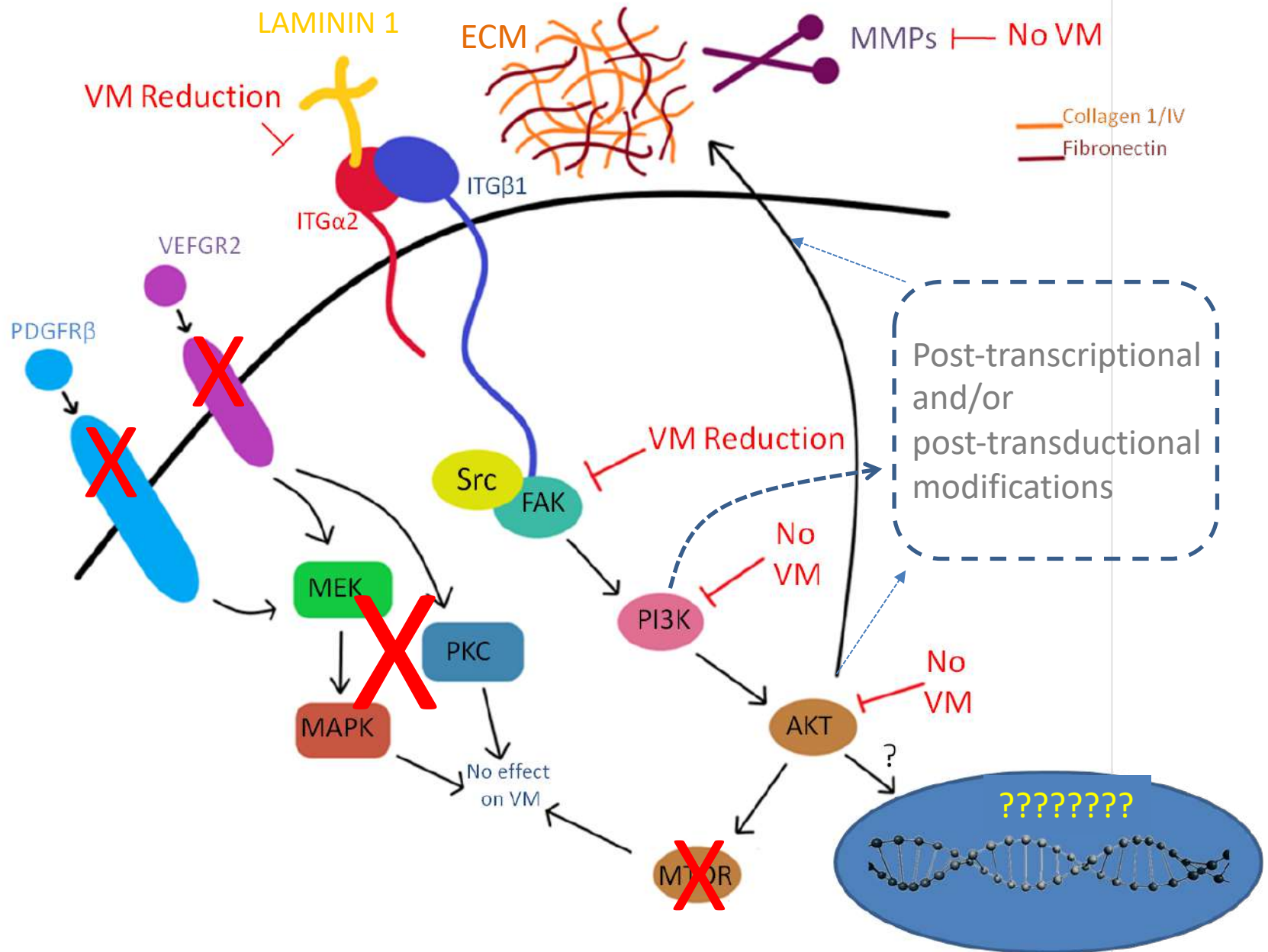
Evidence for laminin expression within tubular structures



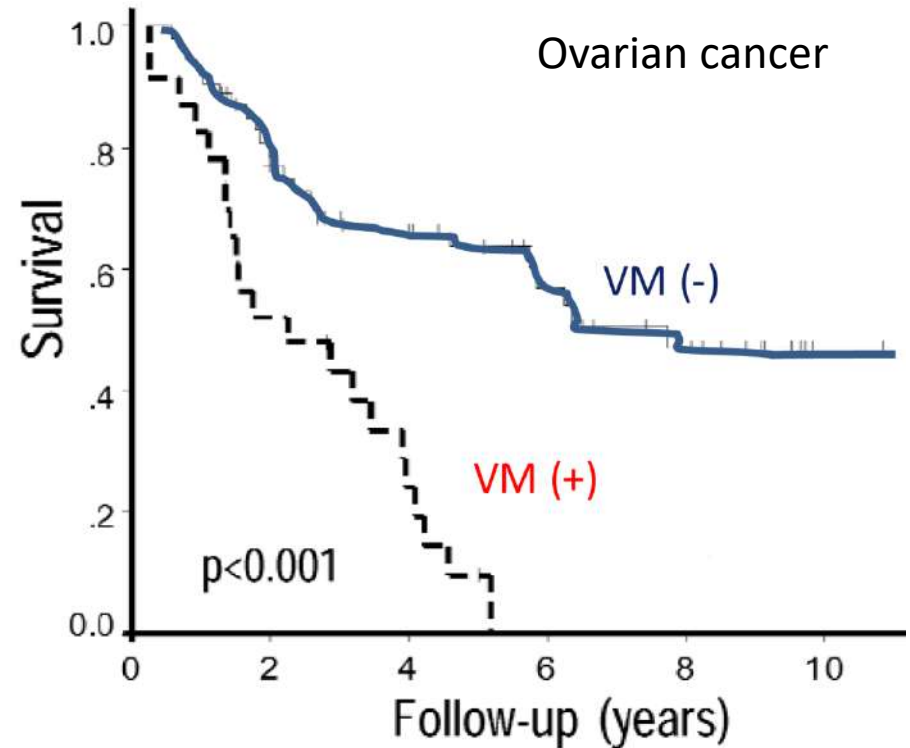
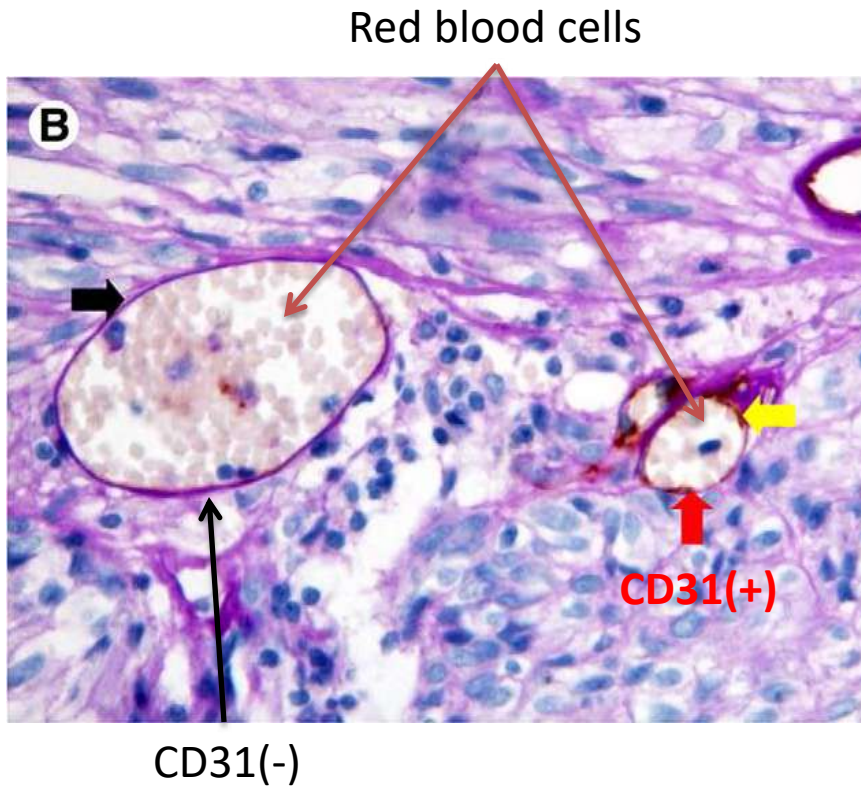
HEY-A8 con IF para pan-laminina

MDA-231 con IF para pan-laminina

Preliminary Model of Vasculogenic Mimicry Formation



Search for a new biomarker for both Vasculogenic mimicry and poor overall survival



Those who did the work:



Andres Valdivia
Dusan Racordon
Varina Aldana
Alejandra Sandoval
Gabriel Mingo
Javiera Pradenas



Medical Team
(Patient samples)



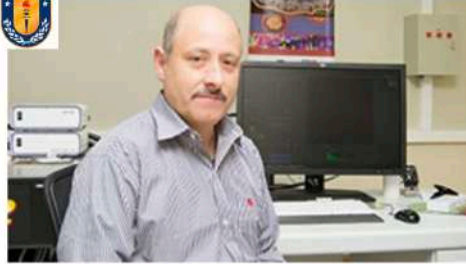
Dra Carolina Ibañez



Dr. Mauricio Cuello



Dr. Beatriz Sanchez. & Dr Alejandro Corvalan
Pontificia Universidad Católica de Chile



Dr. Francisco Nualart
Universidad de Concepción



Dr. Alfonso Gonzalez,
Universidad San Sebastian



Dr. Jorge Brañes

OPEN

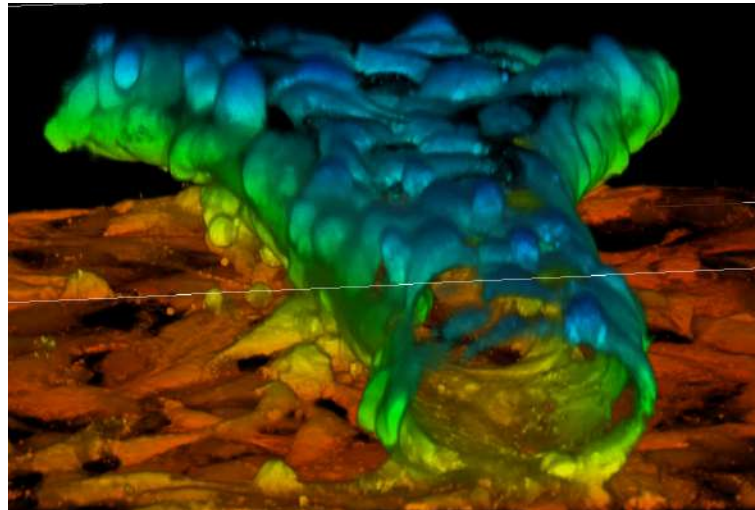
Structural and functional identification of vasculogenic mimicry *in vitro*

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Dusan Racordon¹, Andrés Valdivia¹, Gabriel Mingo¹, Rafaela Erices^{1,2}, Raúl Aravena^{1,3}, Felice Santoro⁴, María Loreto Bravo^{4,5}, Carolina Ramírez¹, Pamela González¹, Alejandra Sandova^{1,4,6}, Alfonso González^{2,13}, Claudio Retamal^{14,15}, Marcelo J. Kogan⁶, Sumie Kato⁷, Mauricio A. Cuello⁷, German Osorio¹², Francisco Nualart¹², Pedro Alvarez¹³, Araceli Gago-Arias¹³, Daniella Fabri¹³, Ignacio Espinoza^{2,13}, Beatriz Sanchez^{7,13}, Alejandro H. Corvalán^{1,4,7,8}, Mauricio P. Pinto¹ & Gareth I. Owen^{1,4,5,6,7,8}

Fact or Fiction, It Is Time for a Verdict on Vasculogenic Mimicry?

Andrés Valdivia¹, Gabriel Mingo¹, Varina Aldana¹, Mauricio P. Pinto², Marco Ramirez³, Claudio Retamal⁴, Alfonso Gonzalez⁴, Francisco Nualart⁵, Alejandro H. Corvalan^{2,6} and Gareth I. Owen^{1,2,6,7,8}



Mingo G, Valdivia , Aldana V, Pradenas J, González P, Leyton L, Andrea Ravasio, Bertocchi C, Nualart F, Owen GI.