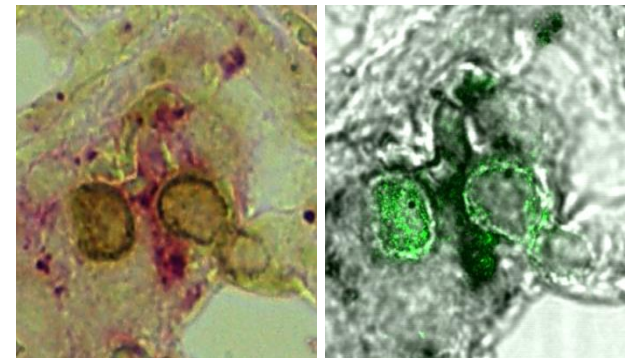


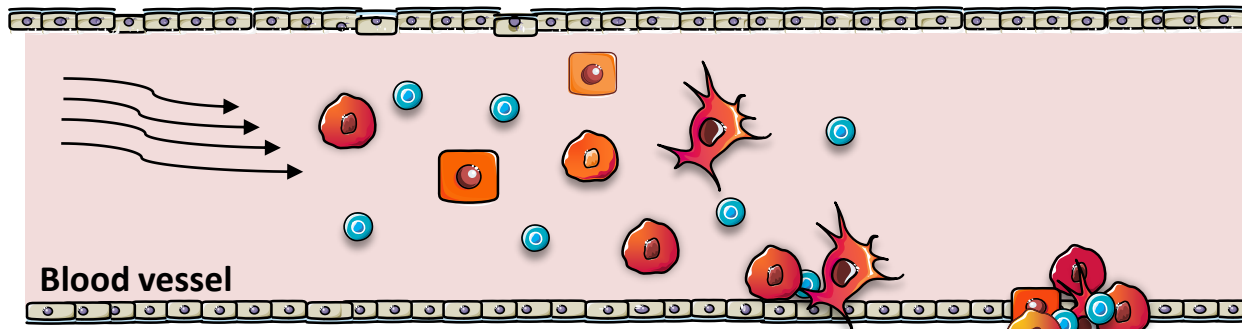
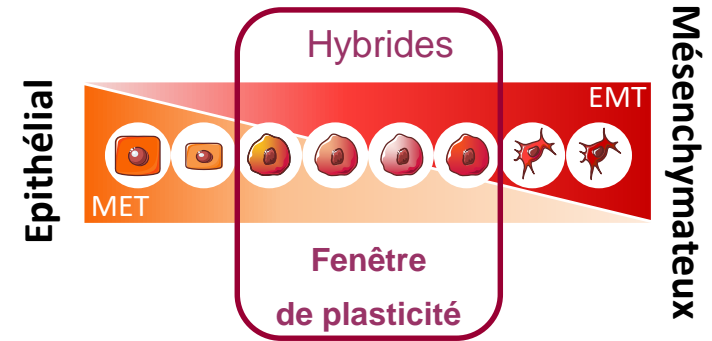
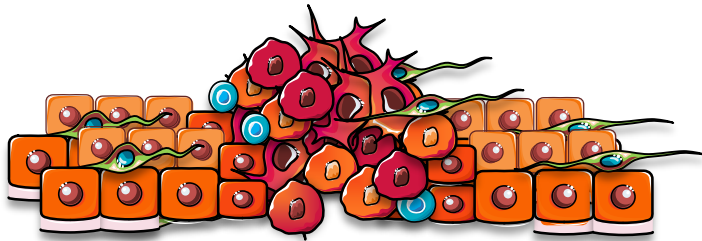


# Transitions Epithélio-Mésenchymateuses Coagulation Cellules TumORAles Circulantes



## PEM: Plasticité Epithélio-Mésenchymateuse

Tumeur Primaire



Blood vessel

Métastases

# TEM: Transitions Epithélio-Mésenchymateuses

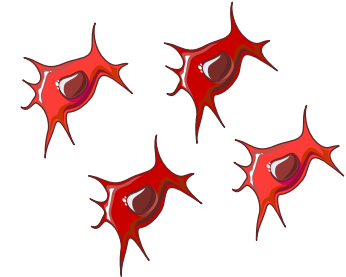
**Cellules  
Epithéliales**



**Phénotypes  
intermédiaires**



**Dérivés mésenchymateux**



**Perte de caractéristiques  
épithéliales**



**Gain de caractéristiques  
mésenchymateuses**

**Facteurs de  
transcription  
(Snail, ZEB)**

**Contacts cellules-cellules:**

- Jonctions serrées (Occludin/ZOs)
- Jonctions adhérentes (E-Cadherin/ $\beta$ -catenin)
- desmosomes (Desmoplakin)

**Cytosquelette:**

- kératines

**Cytosquelette:**

- **Vimentine**

**Protéases:**

- MMPs

**Autres:**

- FSP1,  $\alpha$ -SMA, FN, PDL-1

**Invasivité augmentée**

**Potentiel de survie augmentée**

**Souchitude**

**Création d'un stroma permissif**

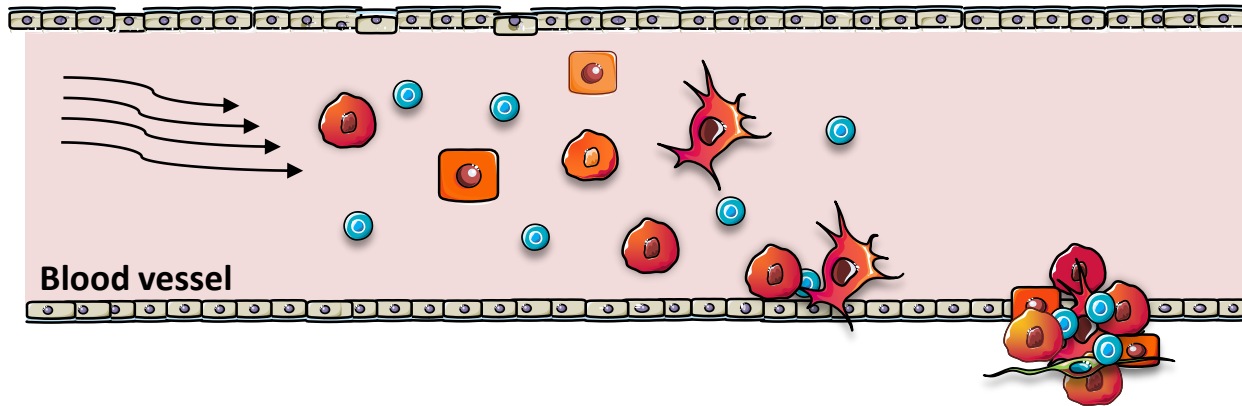
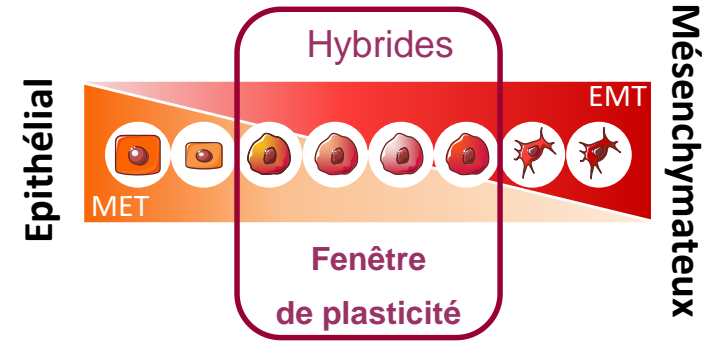
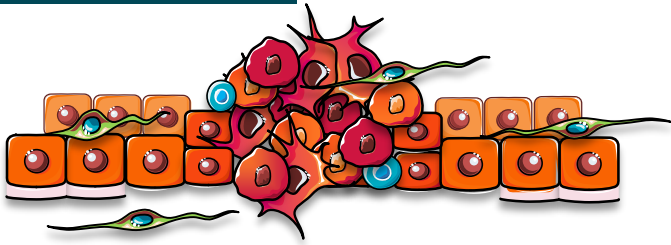
**Echappement au système**

**immunitaire**

**Métabolisme altéré**

# PEM: Plasticité Epithélio- Mésenchymateuse

Tumeur Primaire



- Les CTCs constituent une population très hétérogène: nombreux phénotypes (« single » ou « clusters »)
- Les CTCs Hybrides TEM+ auraient une compétence métastatique plus élevée:

Quels sont les mécanismes impliqués? Coagulation?

# CTC, coagulation, métastases

## □ Données de la littérature reliant la coagulation au cancer:

- Armand Trousseau a décrit dès 1860 un lien entre les thromboembolismes et les cancers
- Plus récemment, un lien entre les CTCs et les thromboses associées au cancer a également été décrit

### Short Communication

Circulating tumour cells are associated with increased risk of venous thromboembolism in metastatic breast cancer patients

M Mego<sup>1,2,3</sup>, U De Giorgi<sup>1,2</sup>, K Broglio<sup>4</sup>, S Dawood<sup>2</sup>, V Valero<sup>2</sup>, E Andreopoulou<sup>2</sup>, B Handy<sup>2</sup>, JM Reuben<sup>1</sup> and M Cristofanilli<sup>\*,2</sup>

British Journal of Cancer (2009) 101, 1813–1816

frontiers in  
**ONCOLOGY**

MINI REVIEW ARTICLE

published: 10 September 2012  
doi: 10.3389/fonc.2012.00115



Do circulating tumor cells play a role in coagulation and thrombosis?

Garth W. Tormoen<sup>1\*</sup>, Kristina M. Haley<sup>2</sup>, Ross L. Levine<sup>3</sup> and Owen J. T. McCarty<sup>1,4</sup>

**jth** journal of  
thrombosis and haemostasis™

**isth**  
International Society of  
Thrombosis and Haemostasis

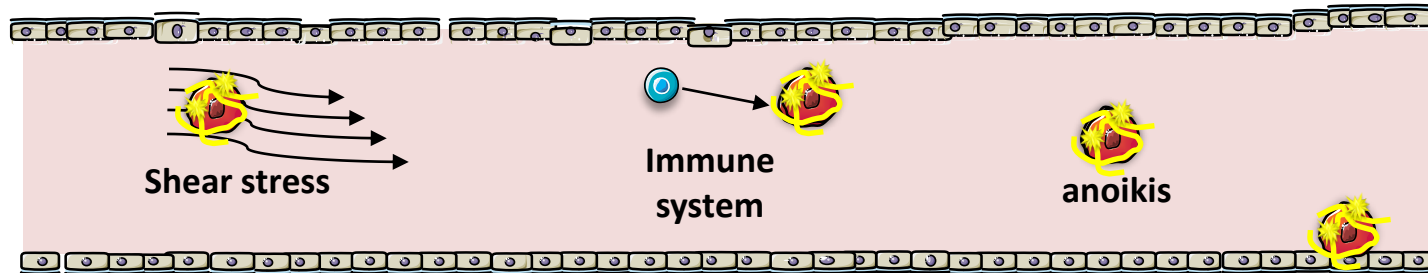
Brief Report

Circulating tumor cell count and thrombosis in metastatic breast cancer

G. Beinse, F. Berger, P. Cottu, M.-E. Dujaric, I. Kriegel, M.-N. Guilhaume, V. Diéras, L. Cabel, J.-Y. Pierga, F.-C. Bidard ✉

First published: 05 August 2017 | <https://doi.org/10.1111/jth.13792>

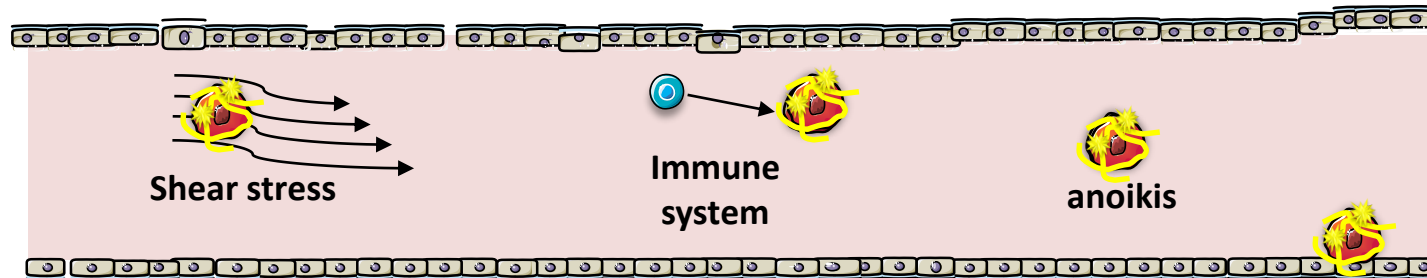
# CTC, coagulation, métastases



- Littérature importante qui relie la coagulation à la formation de métastases:

*Biggerstaff JP, Degen JL, Gil-Barnabé AM/Muschel RJ, Im JH/Muschel RJ, Labelle M/Hynes RO, Palumbo JS, Rak JW*

# CTC, coagulation, métastases



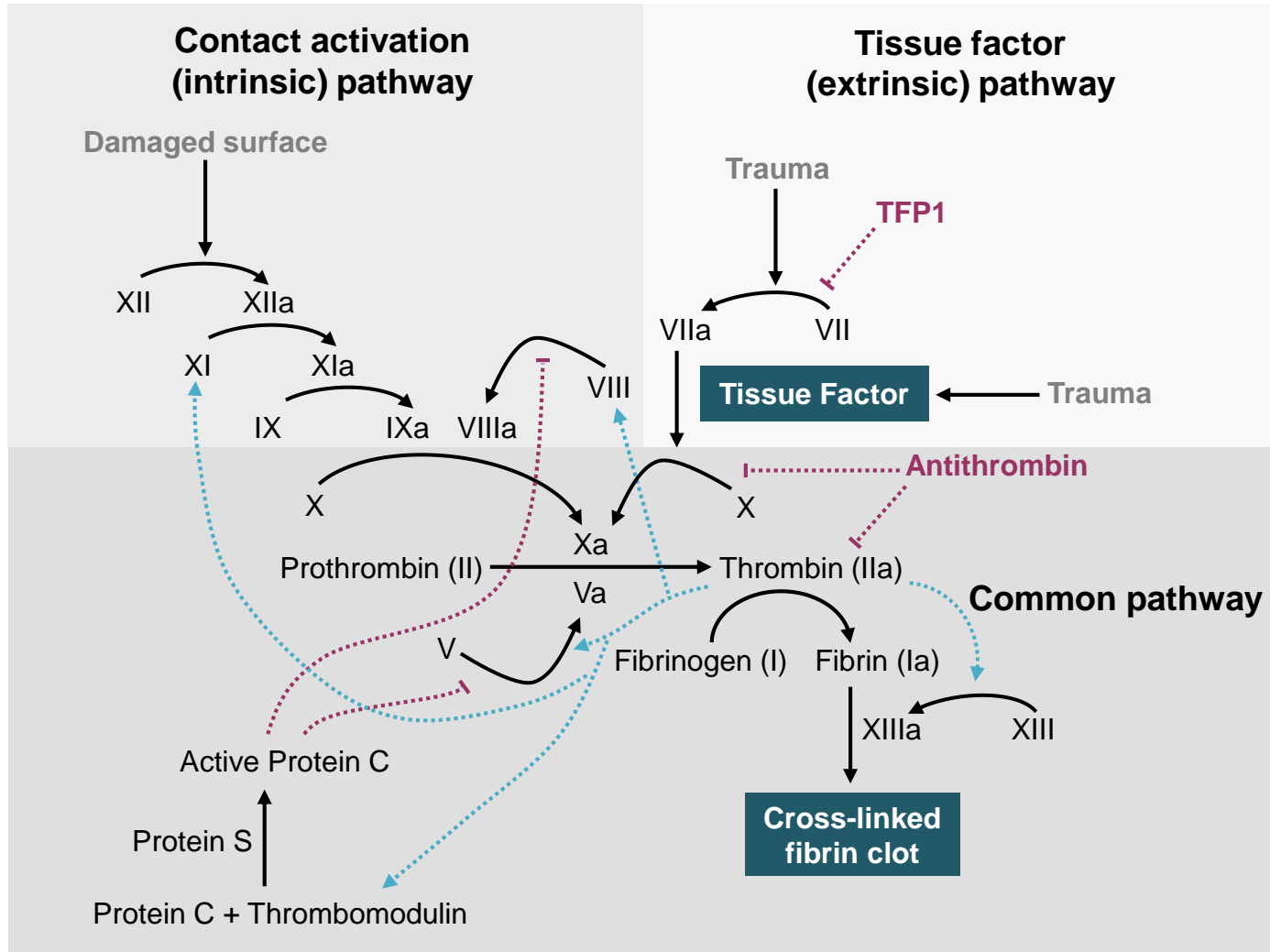
- Données expérimentales du labo suggérant un lien entre TEM/coagulation/CTC  
Des **μarrays** ont identifiés le **Facteur Tissulaire (FT)** comme un gène induit par la TEM dans divers modèles cellulaires de TEM

## Tissue Factor Induced by Epithelial-Mesenchymal Transition Triggers a Procoagulant State That Drives Metastasis of Circulating Tumor Cells

Cancer  
Research  
76: 4270–82, 2016.

Morgane Bourcy<sup>1</sup>, Meggy Suarez-Carmona<sup>1</sup>, Justine Lambert<sup>1</sup>, Marie-Emilie Francart<sup>1</sup>,  
Hélène Schroeder<sup>2</sup>, Céline Delierneux<sup>3</sup>, Nicolas Skrypek<sup>4,5</sup>, Erik W. Thompson<sup>6</sup>,  
Guy Jérusalem<sup>2</sup>, Geert Berx<sup>4,5</sup>, Marc Thiry<sup>7</sup>, Silvia Blacher<sup>1</sup>, Brett G. Hollier<sup>8</sup>, Agnès Noël<sup>1</sup>,  
Cécile Oury<sup>3</sup>, Myriam Polette<sup>9</sup>, and Christine Gilles<sup>1</sup>

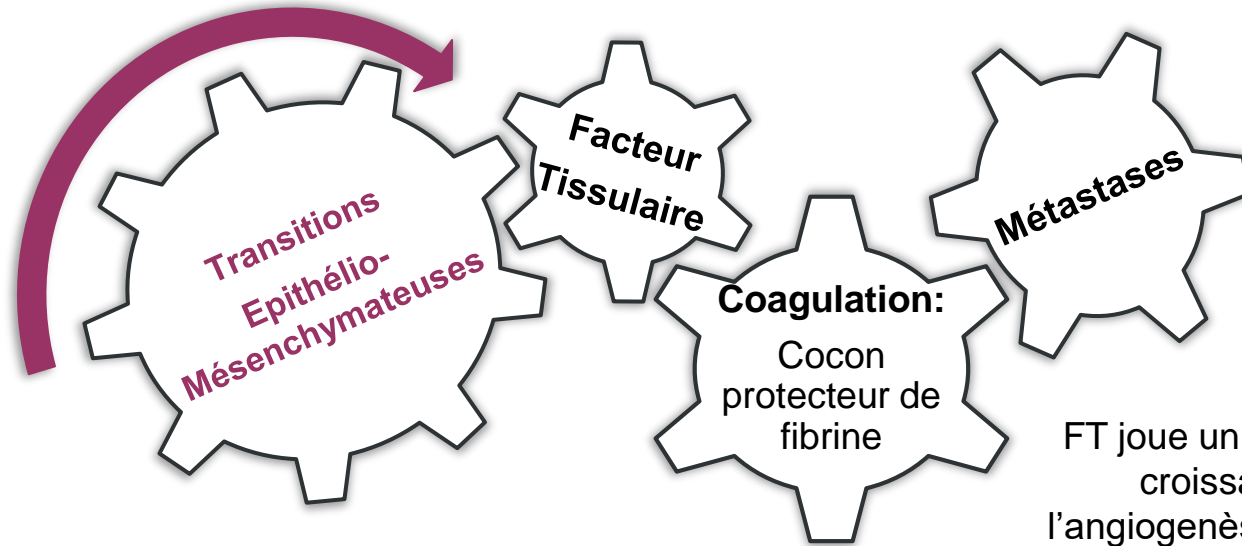
# Cascade de Coagulation





# TEM/TF/coagulation et CTC

Surexpression de FT dans divers cancers a été associée avec des paramètres cliniques défavorables

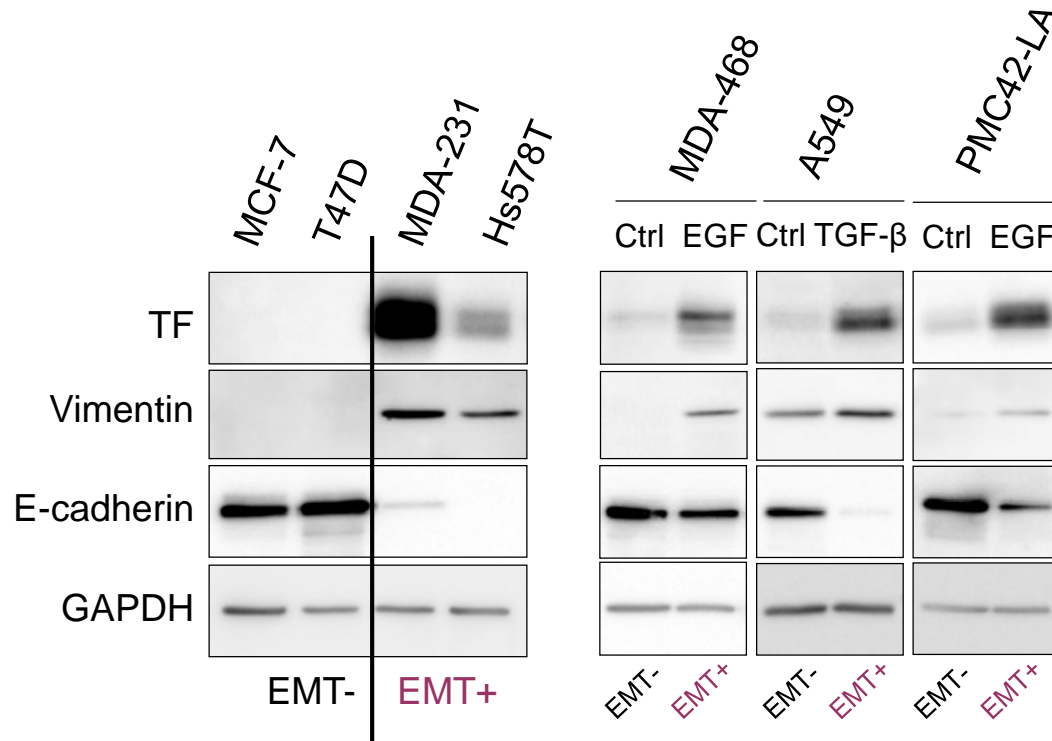


**Impact pour les CTCs?**

FT joue un rôle crucial dans la croissance tumorale, l'angiogenèse et la formation de métastases

# EMT and TF expression

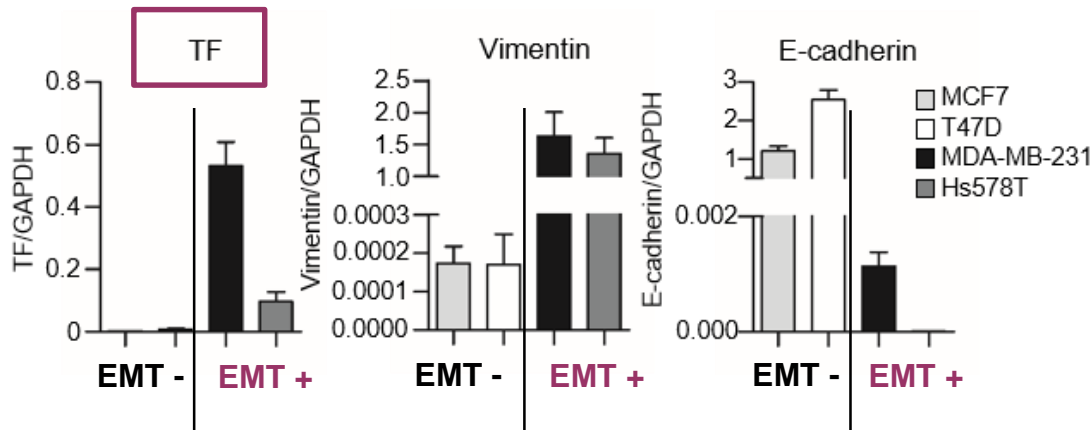
- Confirmation of the microarray on different cellular models of EMT



# EMT and TF expression

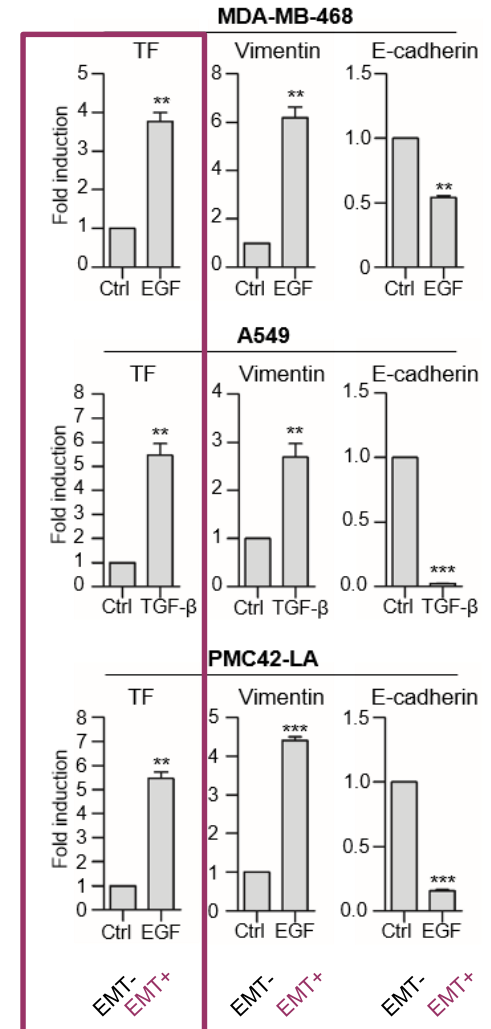
« EMT-inducible » cell models

« Stable » cell models



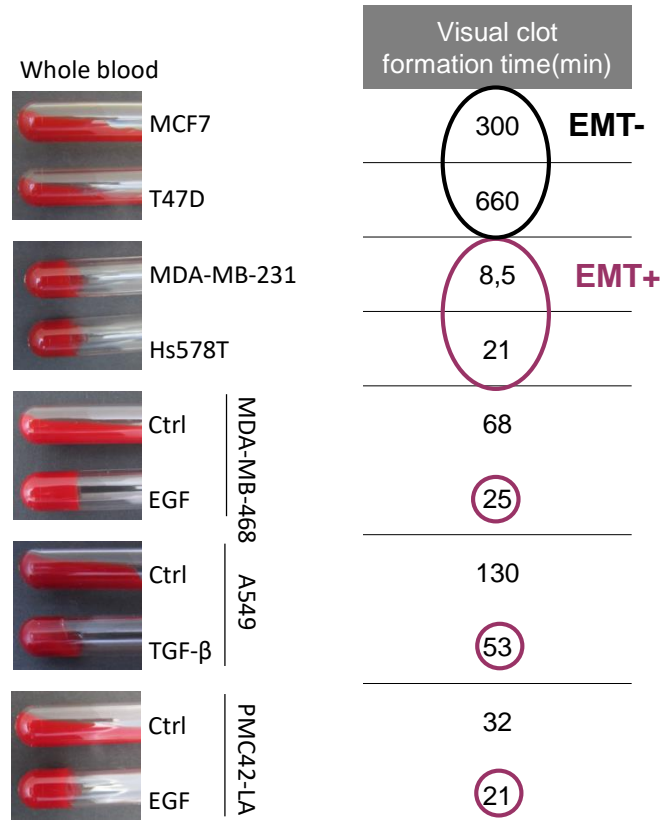
RT-qPCR

→ EMT associates with enhanced TF expression

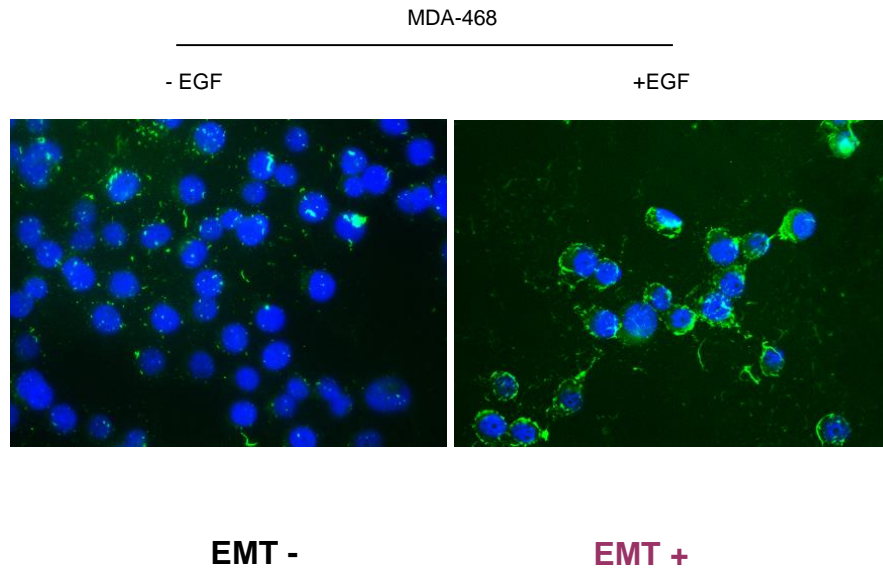


# EMT /TF /coagulant properties

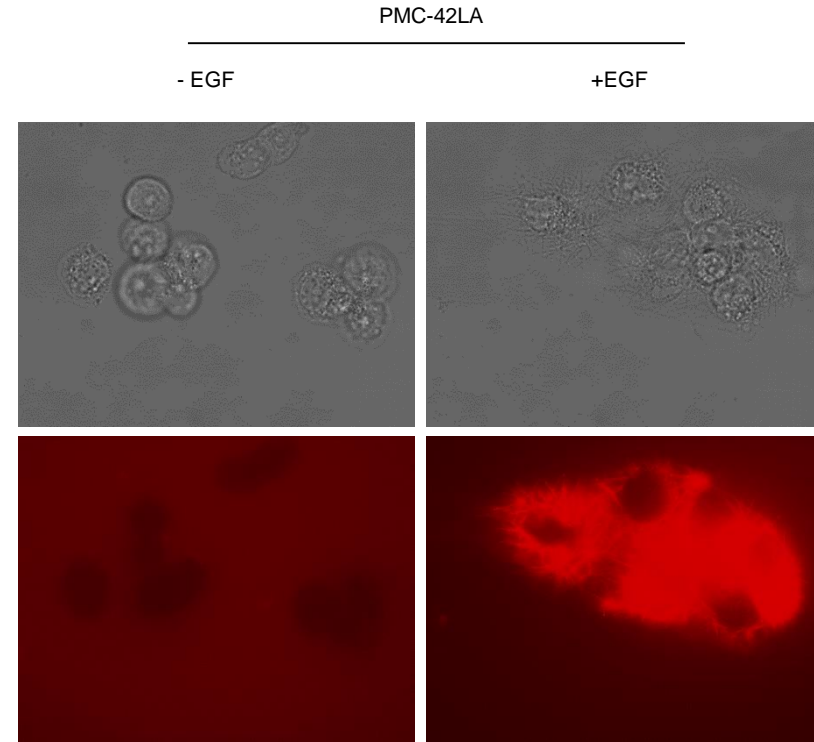
## In vitro coagulation assays



# EMT /TF /coagulant properties



*In vitro* sfibrin formation assays with FITC-labeled fibrinogen: Static conditions

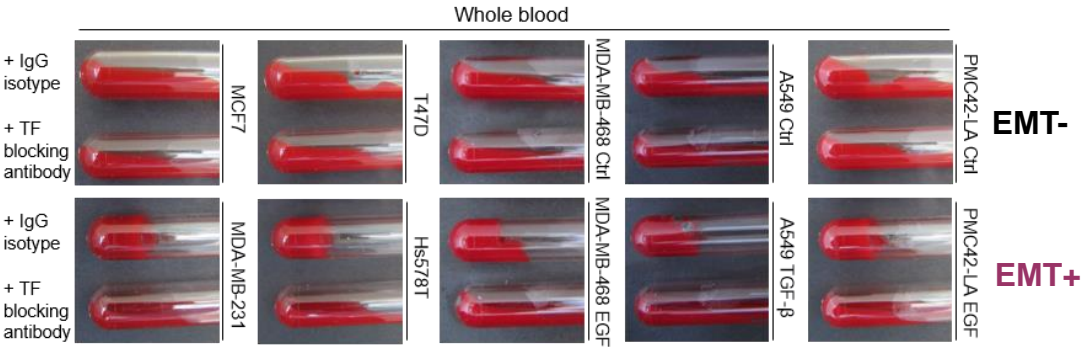


EMT -	EMT +
-------	-------

*In vitro* sfibrin formation assays with:  
Under flow conditions in  $\mu$ fluidics chambers

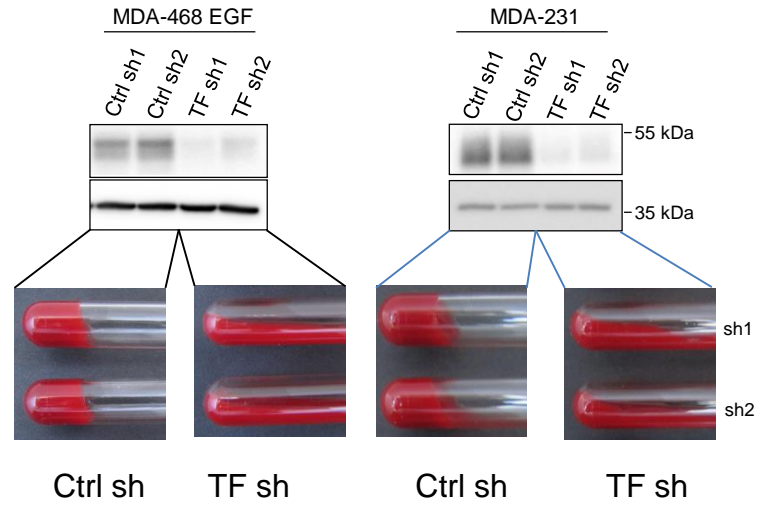
→ EMT associates with enhanced coagulant properties

# EMT /TF /coagulant properties



Visual clot formation time (min)		
	IgG isotype	TF blocking antibody
MCF-7	No clot*	No clot*
T47D	No clot*	No clot*
MDA-231	6	44
Hs578T	14	63
MDA-468 Ctrl	12	No clot*
MDA-468 EGF	6	No clot*
A549 Ctrl	No clot*	No clot*
A549 TGF-β	28	No clot*
PMC42-LA Ctrl	10	No clot*
PMC42-LA EGF	8	60

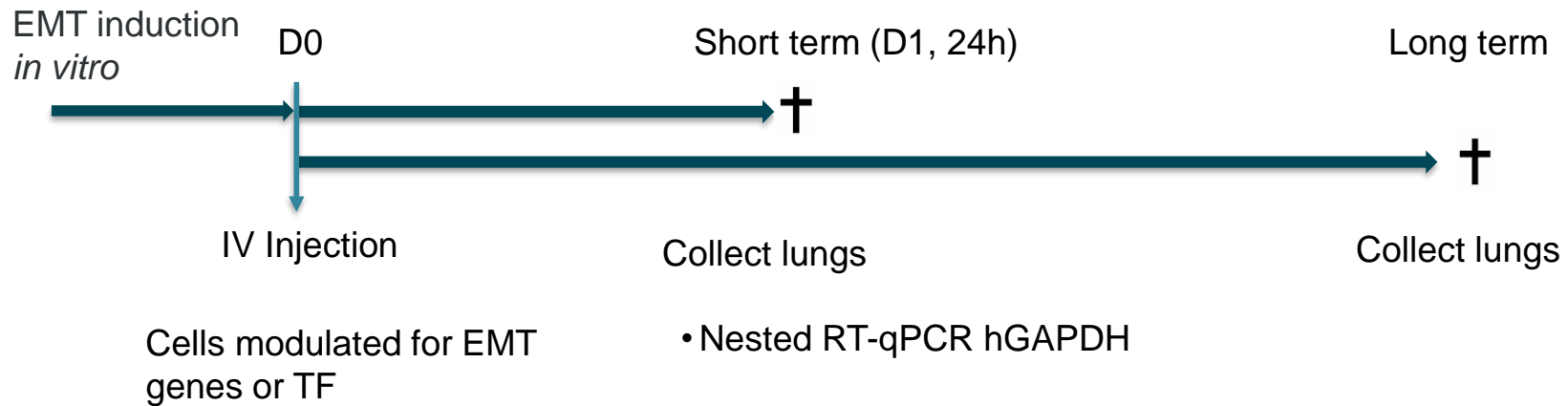
**In vitro coagulation assays**



**→ EMT-associated coagulant properties are TF-dependent**

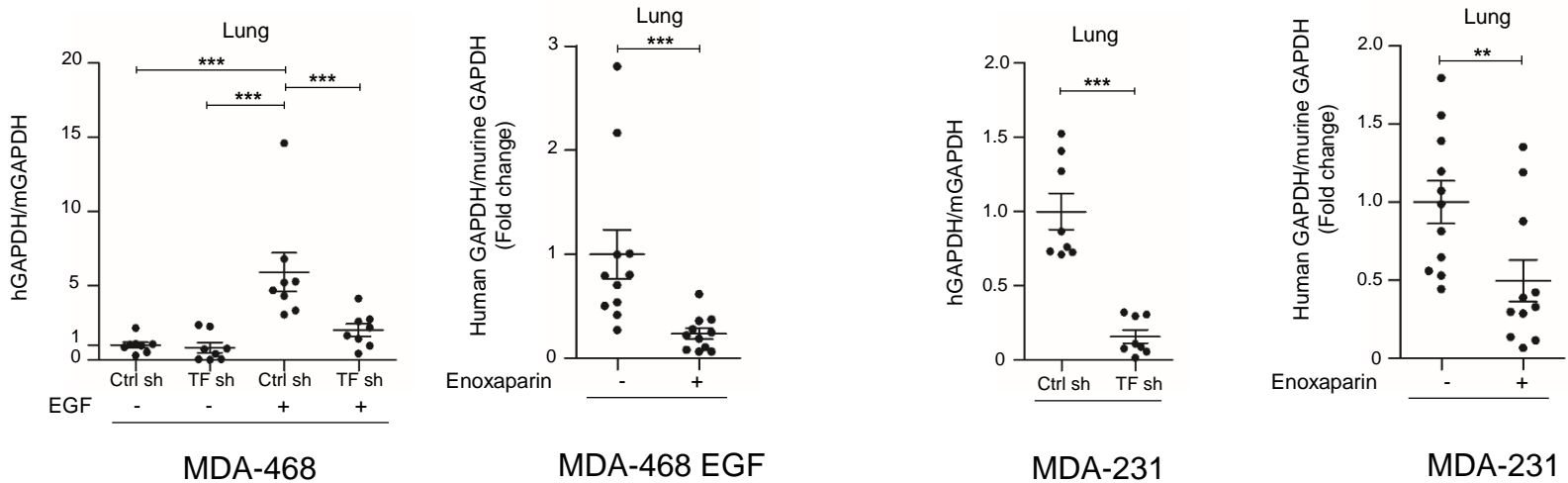
# EMT-TF-coagulation axis in early colonization

## Mice models: experimental metastasis assays



# EMT-TF-coagulation axis in early colonization

## Short term experimental metastasis assays

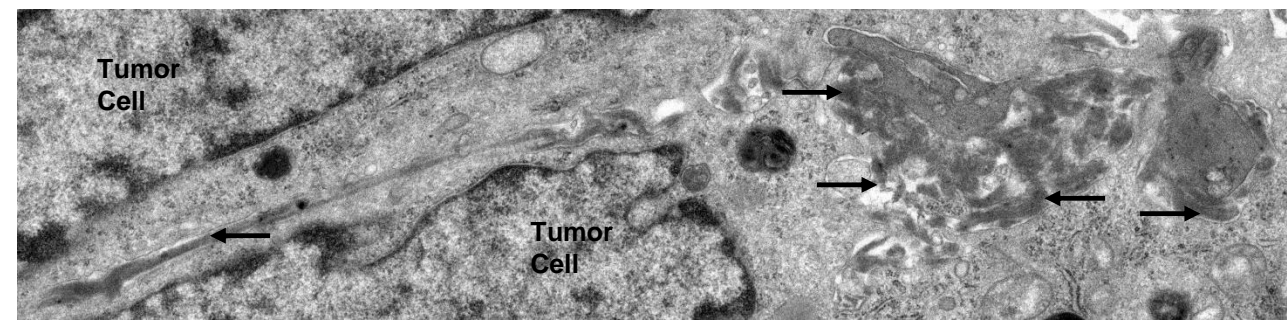
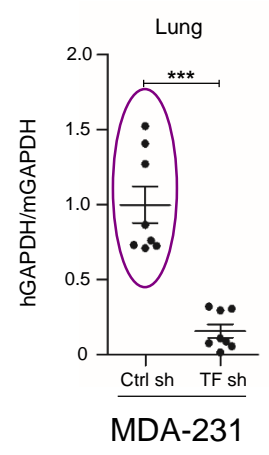
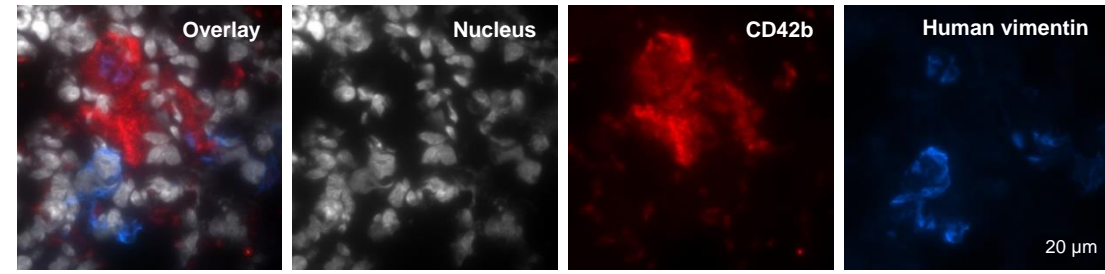
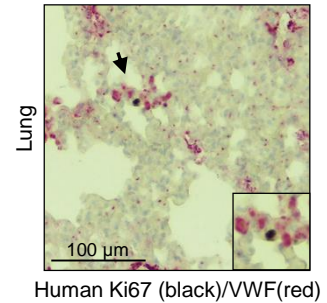
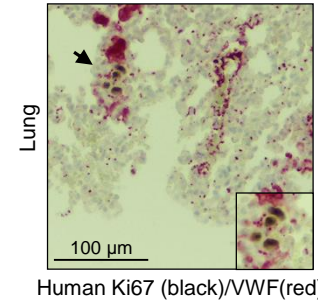
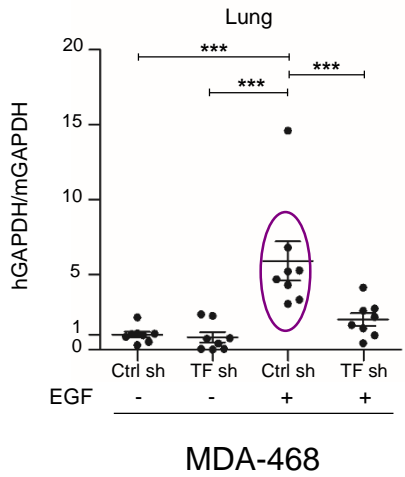


→ EMT-associated early metastatic colonization is TF/coagulation-dependent



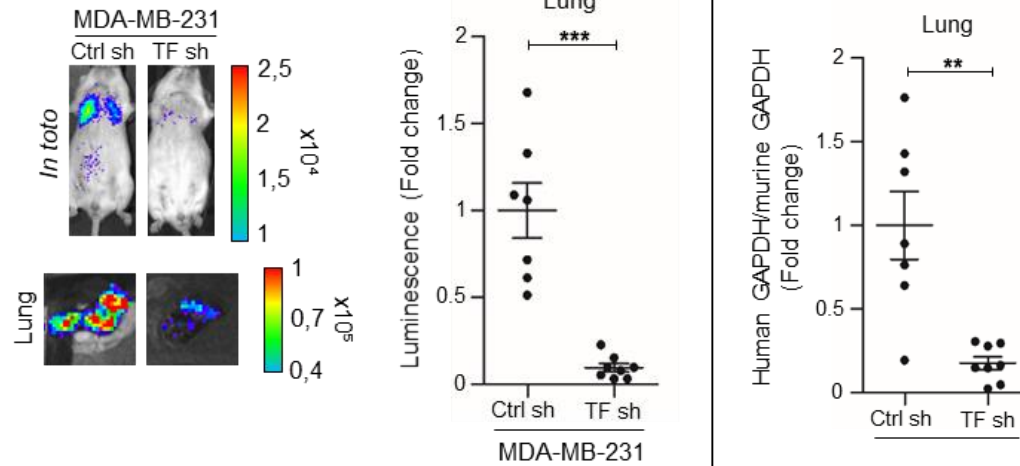
# EMT-TF-coagulation axis in early colonization

## Short term experimental metastasis assays

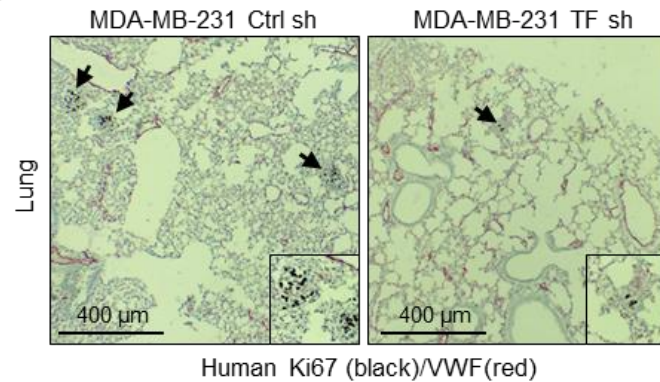


# EMT-TF-coagulation axis in metastases

## Long term experimental metastasis assays

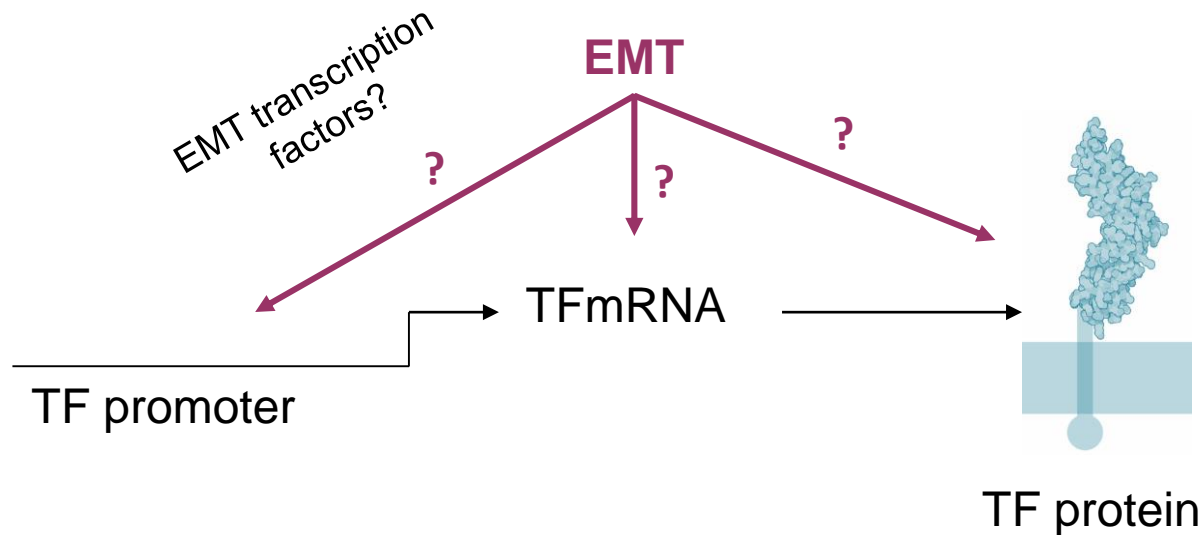


**C**

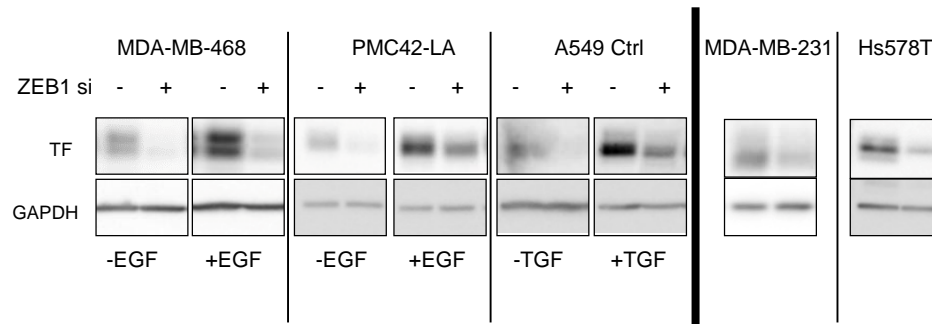


# Molecular mechanisms of TF regulation

- An EMT-coagulation axis contributes to CTCs' early colonization potential
- TF is a major player in this EMT-coagulation-early metastasis axis
- What are the molecular mechanisms linking EMT and TF expression?

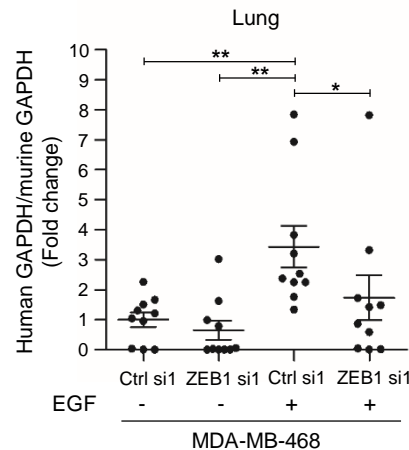


# ZEB1 regulates TF expression

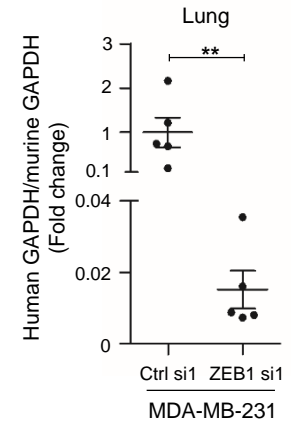
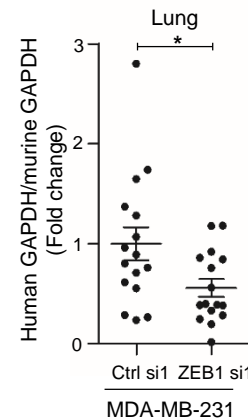


		Visual clot formation time (min)
Ctrl si1	MDA-MB-468 Ctrl	36
ZEB1 si1		157
Ctrl si1	MDA-MB-468 EGF	17
ZEB1 si1		50
Ctrl si1	MDA-MB-231	12
ZEB1 si1		16

**In vitro coagulation assays**

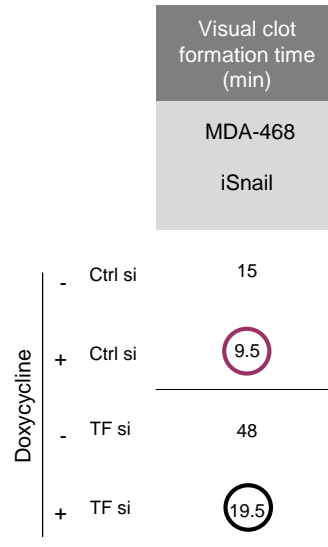
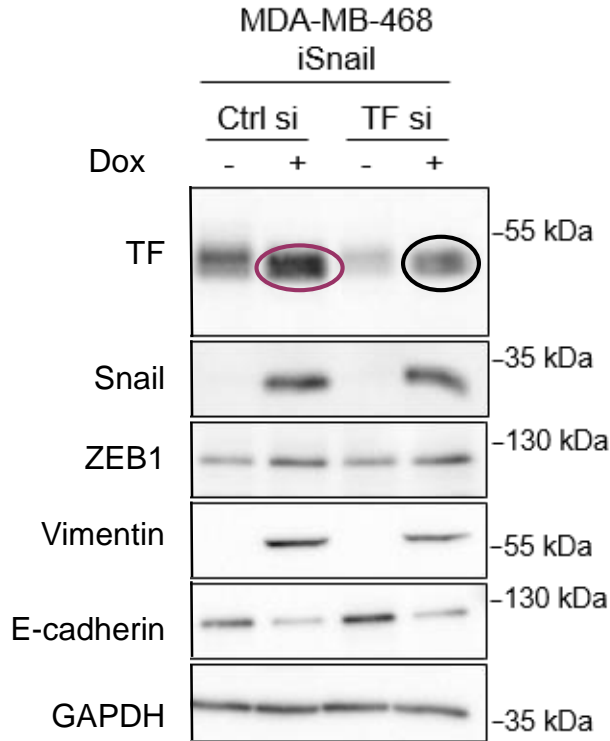


**Short term experimental metastasis assays**

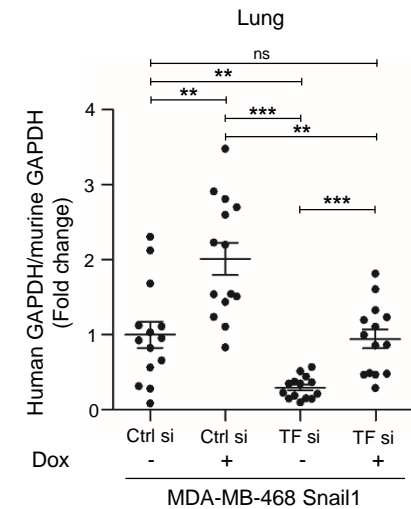


**Long term experimental metastasis assays**

# Snail regulates TF expression



**In vitro coagulation assays**



**Short term experimental metastasis assays**

→ Snail induces coagulant properties and early colonization through a TF-dependent mechanism

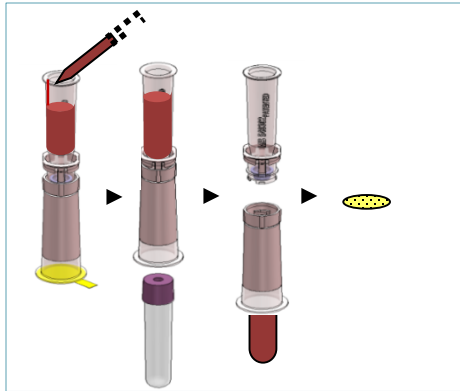
→ ChIP so far suggest that snail may directly bind TF promoter

# EMT-TF-coagulation in human CTCs

---

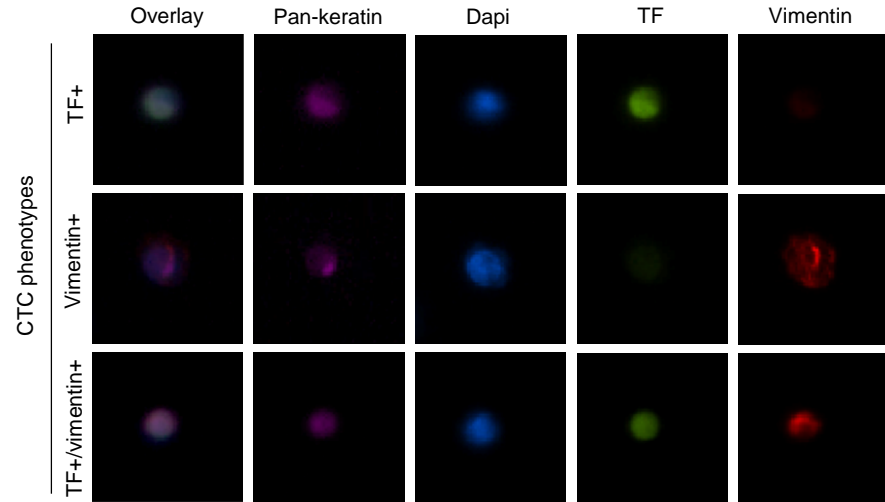
- ❑ Is this EMT-TF-coagulation axis important for CTCs in human?
- ❑ Does it have a clinical significance?
- ❑ Are coagulant CTCs nastier than others?

# Vim+/TF+ CTCs in breast cancer patients

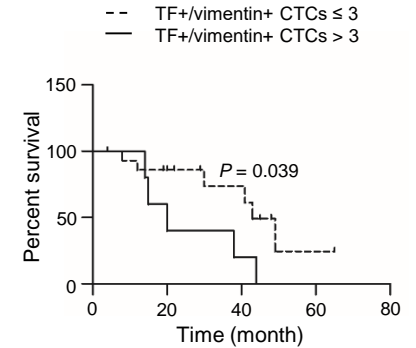
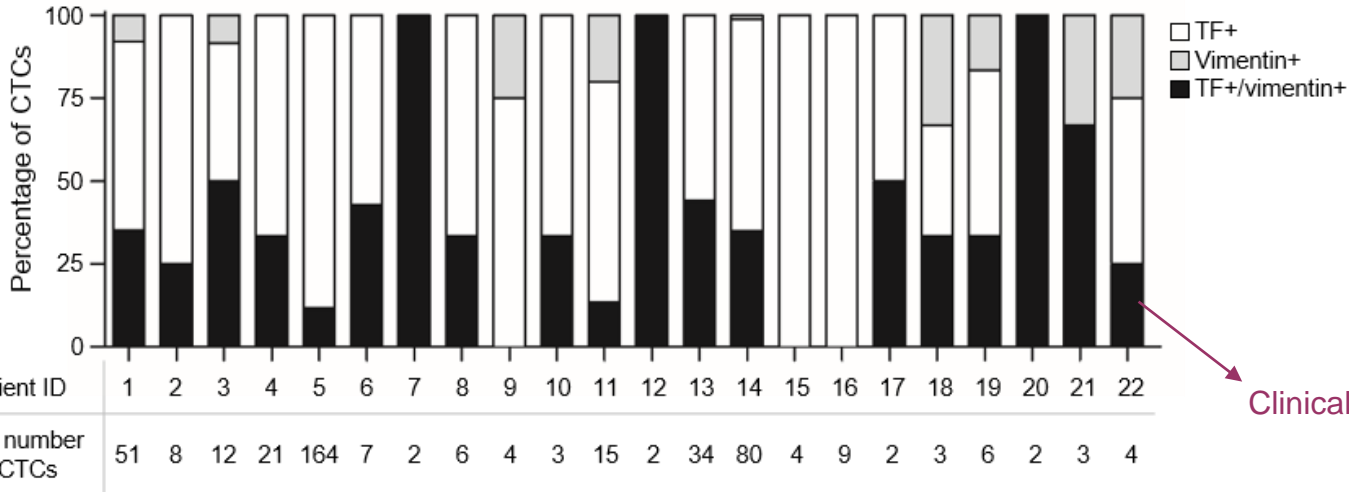


Filters: ScreenCell

CTCs isolated from metastatic breast cancer patients

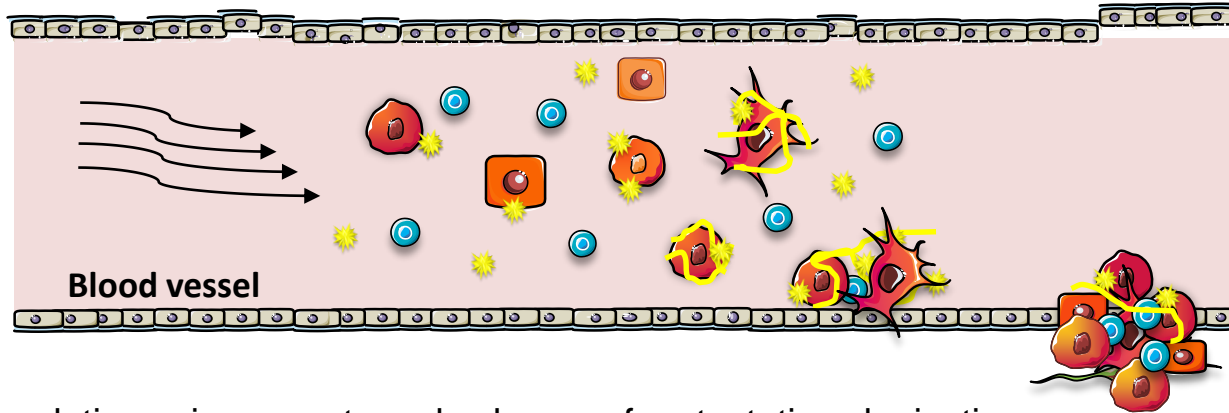


Breast cancer patient-derived CTCs



Clinical significance?

# Conclusions



- ❑ An EMT-coagulation axis supports early phases of metastatic colonization
  - ❑ TF is a major player in this axis
  - ❑ EMT actors regulate TF expression: a miR-dependent regulatory mechanism of TF mRNA by vimentin has been evidenced
  - ❑ These mechanisms could provide mesenchymally-shifted CTCs with enhanced metastatic competence
- ➔ Further characterize the molecular regulation of TF by EMT for targeting/inhibiting perspectives
  - ➔ Further examine EMT/TF expression on CTCs from breast and lung cancer patients: clinical significance of EMT+/TF+ CTCs?
  - ➔ Isolate and further characterize coagulant CTCs from breast and lung cancer patients (droplet  $\mu$ fluidic approach)



# THANK YOU



*Lab. Developmental Tumor Biology,  
LBTD/GIGA-Cancer, ULg, Belgium*

**Dr Morgane Bourcy**  
**Marie-Emilie Francart**

Justine Lambert  
Aline Vanwynsberghe  
Anthony Genna  
Pr Sylvia Blacher

*Molecular Angiogenesis Laboratory, ULg*

**Dr Ingrid Struman**

*Lab. Thrombosis and Hemostasis, ULg*

Dr Cécile Oury

*Department of Oncology, CHU, Belgium*

**Pr Guy Jerusalem**  
**Dr Hélène Schroeder**

*Inserm UMR-S 1250, Reims, France*

**Pr Myriam Polette**

Pr Philippe Birembaut

*Institute of Health and Biomedical Innovation,  
Queensland University of Technology, Australia*

**Pr Rik Thompson**

*Australian Prostate Cancer Research Center,  
Queensland University of Technology, Australia*

**Pr Brett Hollier**

*Maastricht University, CARIM, The Netherlands*

**Pr Johan Heemskerk**

*Department for Molecular, Biomedical Research, VIB,  
Gent, Belgique*

Pr Geert Berx

*Institut of Molecular and Cell Biology, Singapore*

Pr Walter Hunziker