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Extracellular vesicles in CAT pathogenesis

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Declaration of Conflict Of Interest

- I have no potential conflict of interest to report
X
- I have the following potential conflict(s) of interest to report

INTRODUCTION

- ✓ Cancer patients have a 5- to 7-fold increased risk of developing VTE
- ✓ It is estimated that approximately 4–20% of cancer patients will experience VTE at some stage, the rate being the highest in the initial period following diagnosis
- ✓ A diagnosis of VTE is a serious complication of cancer that adversely affects patient's quality of life and reduces overall survival rates
- ✓ Cancer-associated thrombosis is a multi-factorial process that has been associated with several mechanisms

*Khorana AA et al, Nat Rev 2022
Falanga A et al, Ann Oncol 2023*

THROMBOSIS IN CANCER IS PECULIAR

Patient-related

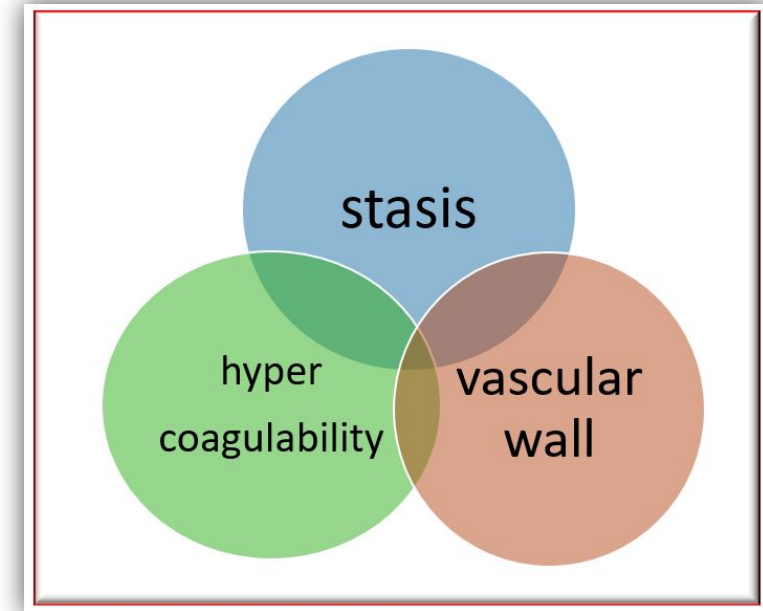
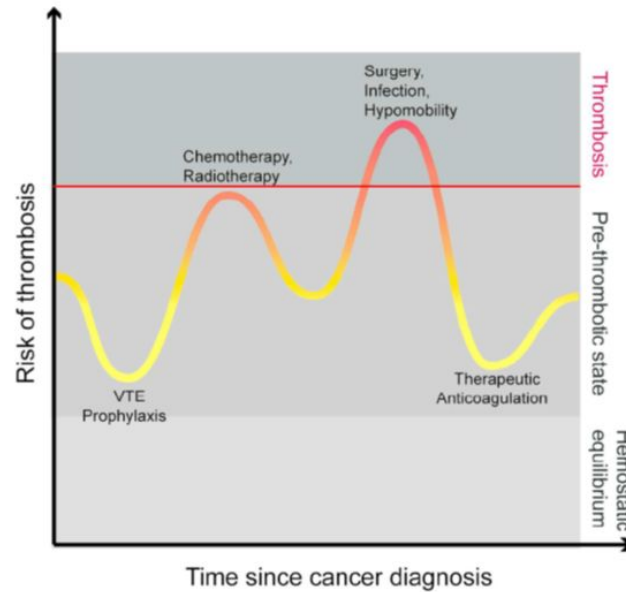
- Medical comorbidities
- Previous VTE
- Varicose veins
- Hereditary risk factors (i.e. FV Leiden)

Treatment-related

- Surgery
- Platinum-based and other chemotherapy
- Hormonal therapy
- Anti-angiogenesis agents
- Erythropoiesis stimulating agents
- Central venous catheters
- Blood transfusions
- Hospitalization - Immobility

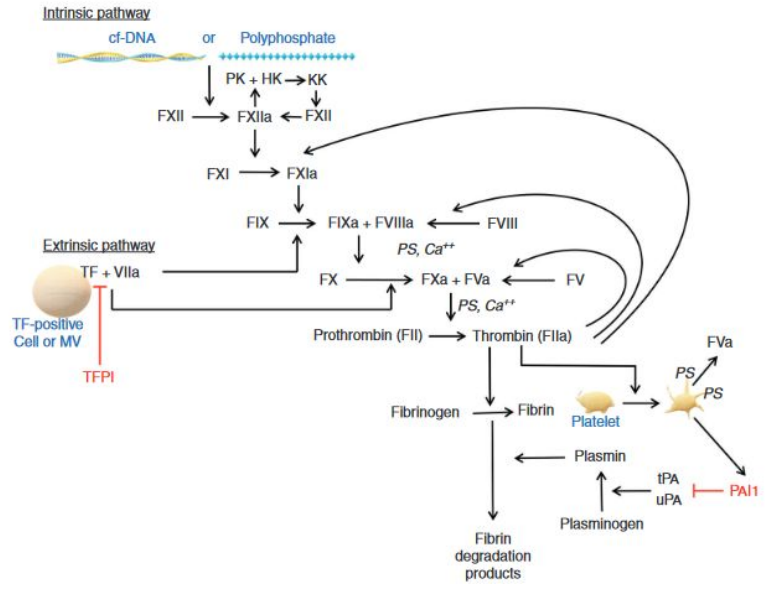
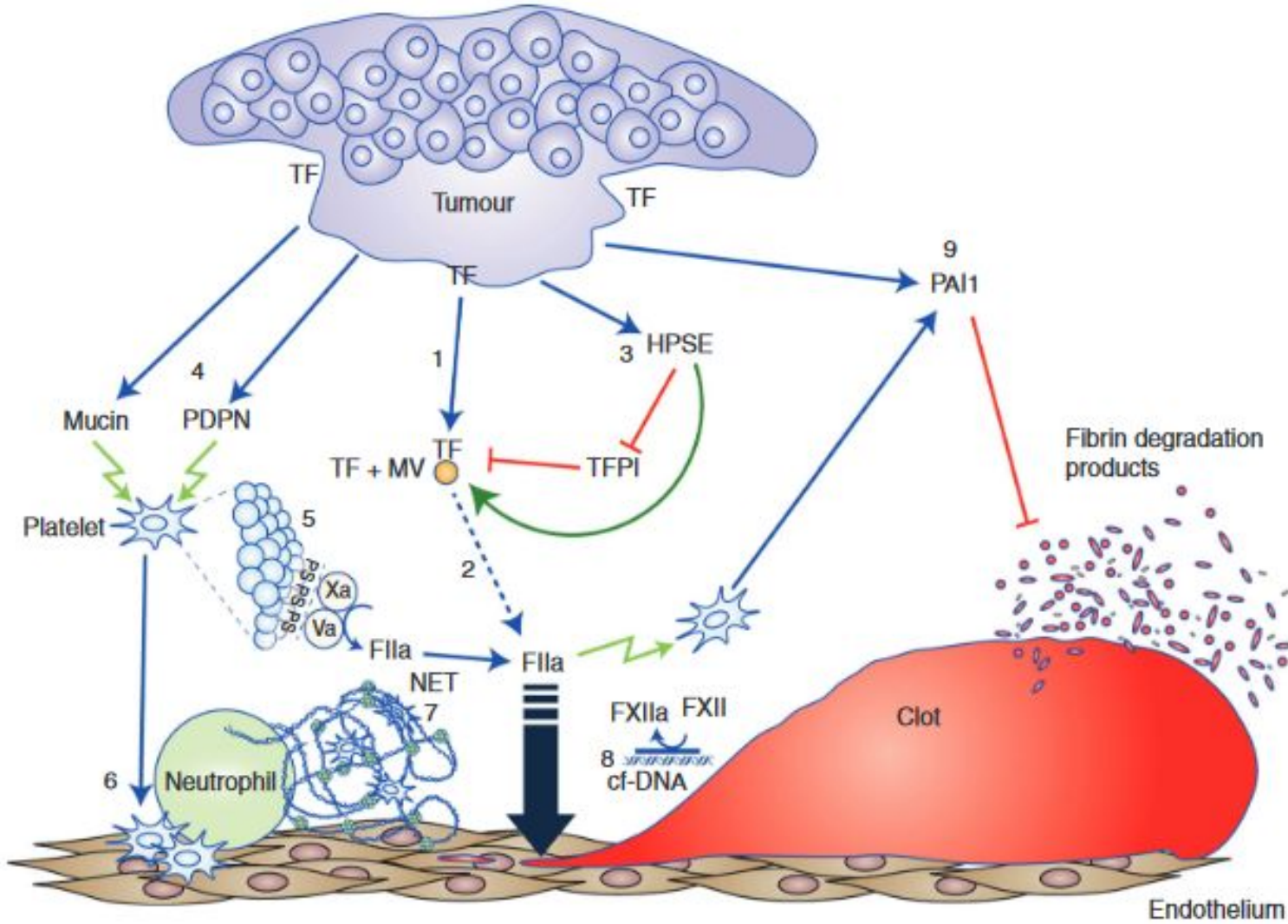
Tumor-related

- Site of cancer
- Stage of cancer
- Histological grade
- Time since cancer diagnosis

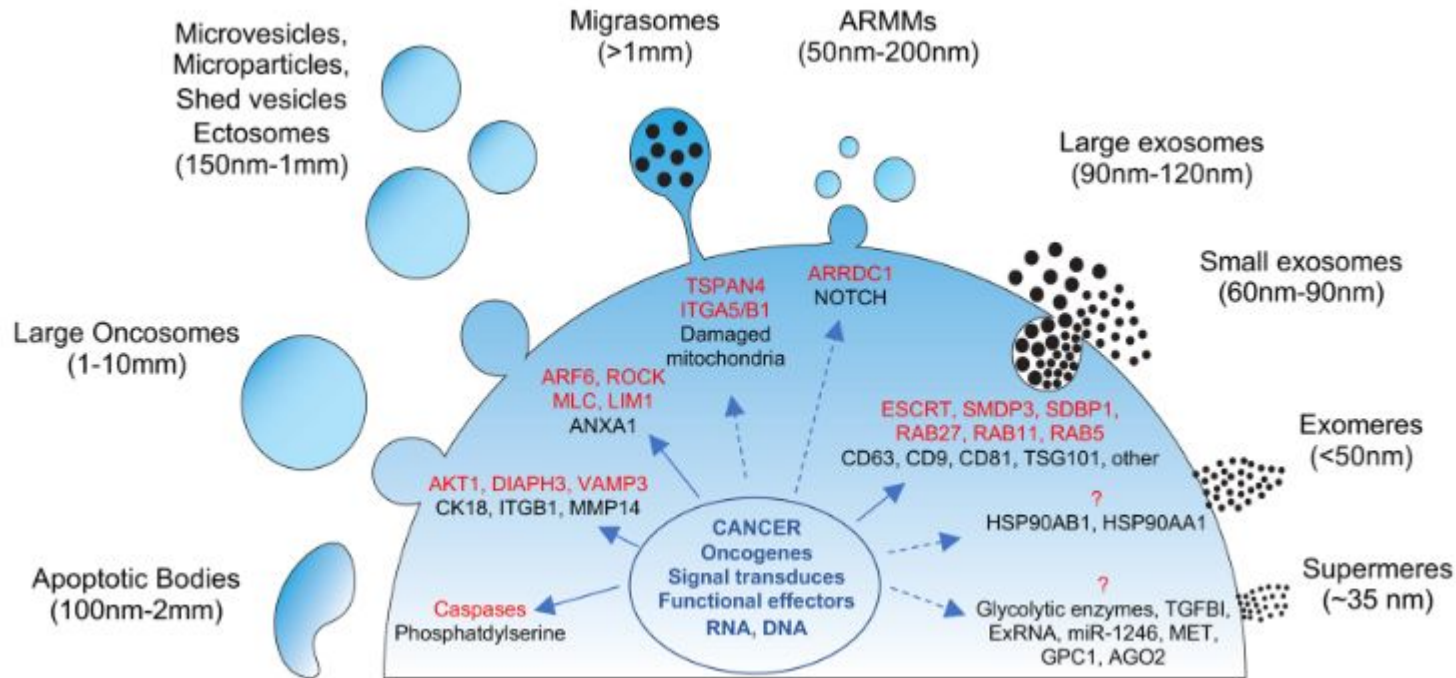


*Adapted from Ay C et al Thromb Haemost 2017
Campello E. et al. BJC 2019*

CANCER-ASSOCIATED COAGULOPATHY



EXTRACELLULAR VESICLES



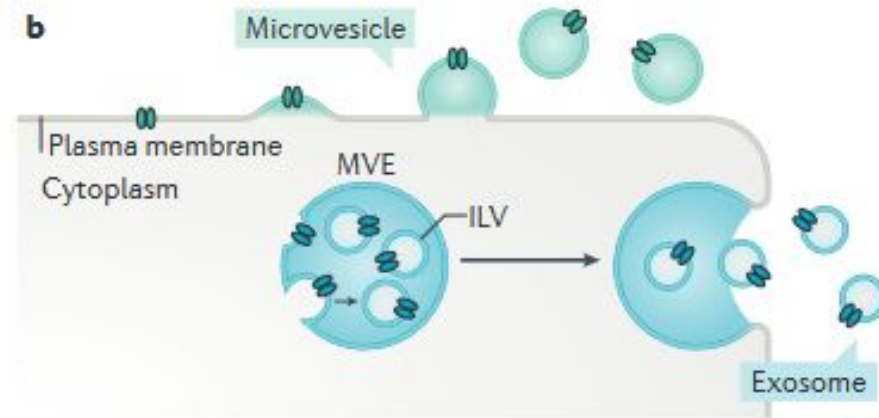
ISEV definition: “the generic term for naturally released particles from the cell that are delimited by a lipid bilayer and cannot replicate”

Extracellular vesicles are a heterogeneous group of cell-derived membranous structures extensively associated with intercellular communication in both physiological and pathological situations, including thrombosis and cancer.

Under physiological conditions, several cell types are able to release EV

EXTRACELLULAR VESICLES types

a	Exosomes	Microvesicles
Origin	Endosome	Plasma membrane
Size	50–150 nm	50–500 nm (up to 1 μ m)
Other names (according to their origin, size and morphology)	<ul style="list-style-type: none">• Protasomes• Tolerosomes• Dexosomes• Nanovesicles• Exosome-like vesicles and others	<ul style="list-style-type: none">• Microparticles• Blebbing vesicles• Shedding vesicles• Oncosomes• ARRM• Migrasomes• Neurospheres• Apoptotic bodies



The two major populations of EVs released by tumor cells are microvesicles and exosomes.

- ✓ Microvesicles (ectosomes/ microparticles) are lipid bilayer-enclosed sacs (100 to 1000 nm of diameter) released from the plasma membrane to the extracellular milieu.
- ✓ Exosomes differ from microvesicles by their size (30 to 100 nm), formation process (endosomal origin) and protein content.

EXTRACELLULAR VESICLES in cancer

Under physiological conditions, several cell types are able to release microvesicles, however, cells malignant transformation stimulates the release of EVs

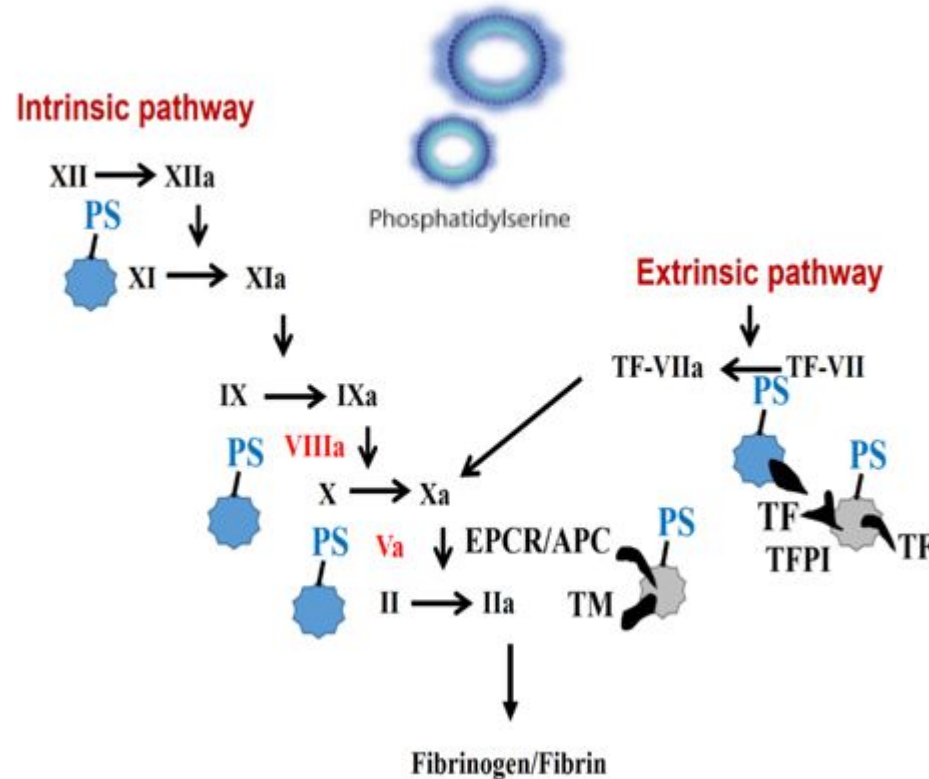
Table 1 Oncogenic Induction of Cellular Vesiculation

Oncogenic Pathway	Impact on Vesiculation	Reference
K-ras	Increased emission of TF-containing procoagulant microvesicles in colorectal cancer cells expressing mutant K-ras	Yu et al ⁷⁴
EGFRvIII	Increase in vesiculation and incorporation of EGFRvIII into microvesicles (oncosomes) in glioma cells transformed with this oncogene	Al-Nedawi et al ²³
p53	Increase in production of TF-containing microvesicles in colorectal cancer cells upon deletion of p53 gene	Yu et al ⁷⁴
p53	Increase in exosome production in cells, in which irradiation triggered p53 expression	Yu et al ⁷⁵
EGFR/AKT	Activation of the EGFR and AKT pathways stimulated vesiculation of prostate cancer cells	Di Vizio et al ²⁸

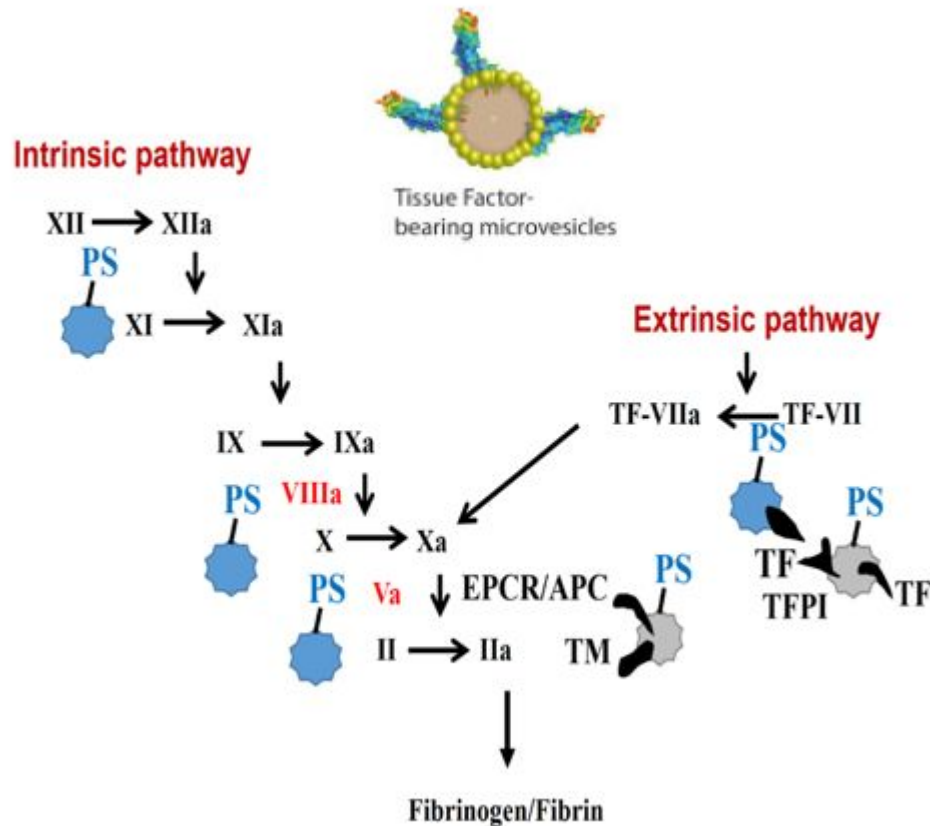
TF, tissue factor; EGFRvIII, epidermal growth factor receptor variant III; EGFR, epidermal growth factor receptor.

EXTRACELLULAR VESICLES in cancer - composition

As seen with tumor cells, cancer cell-derived EVs may expose procoagulant phospholipids such as *phosphatidylserine*, which constitute a suitable platform for the assembly of the blood coagulation complexes.



EXTRACELLULAR VESICLES in cancer - composition



Several studies demonstrated that EVs isolated from cultured cells expose TF to similar extents as observed in the producing cells.

Indeed, cancer driver mutations and the overactivation of signaling pathways increase the expression of TF in tumor cells, as well as trigger the emission of TF-bearing EVs.

Other cancer-related phenomena, such as the epithelial-mesenchymal transition, may also promote the release of EVs containing TF

TF+EVs in patients with cancer

Table 1. Clinical studies associating tissue factor (TF)-containing extracellular vesicles and venous thromboembolism.

Cancer Type	TF Measurement	VTE
Pancreatic, non–small cell lung, ovarian, colorectal and breast [15,41,42]	TF antigen (IFC)	Yes
Colon, lung, bladder, pancreatic, prostate, rectal, bile duct, brain, cholangio, liver, lymphoma, renal cell, testis and other types of cancer [15,41,42]	TF activity (FXa generation assay)	Yes
Gastrointestinal, lung, pancreatic, prostatic, breast, liver, uterine and brain [15,41,42]	TF antigen (FACS)	Yes
Pancreatic [43,44,45]	TF activity (FXa generation assay), TF antigen (FACS or ELISA)	Yes
Breast [43,46]	TF activity (FXa generation assay), TF antigen (FACS)	Yes/ No
Soft tissue sarcoma [47]	TF antigen (FACS)	No
Non-Hodgkin lymphoma, colorectal, breast, stomach, lung and pancreatic [48]	TF antigen (ELISA), TF activity (FXa generation assay)	No
Multiple myelomas [49]	TF activity (FXa generation assay)	No
Ovarian [50,51]	TF antigen (ELISA), TF activity (FXa generation assay or FGT)	No
Small cell lung cancer [52]	TF activity (FXa generation assay)	No
Gastric, colorectal and brain [53]	TF activity (FXa generation assay)	No

TF, tissue factor; IFC, Impedance-based flow cytometry; FACS, fluorescence-activated cell sorting; ELISA, enzyme-linked immunosorbent assay; FGT, fibrin generation test.

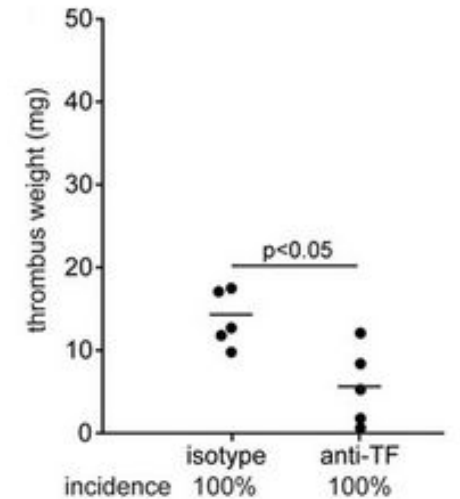
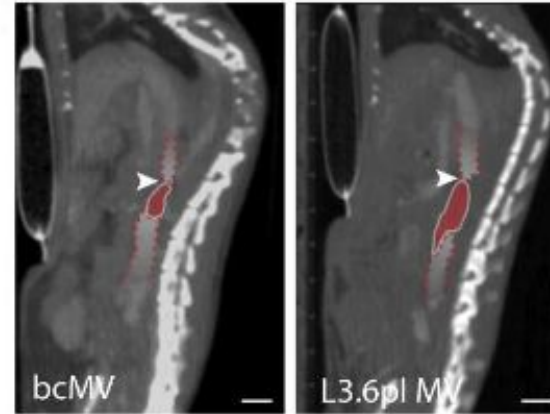
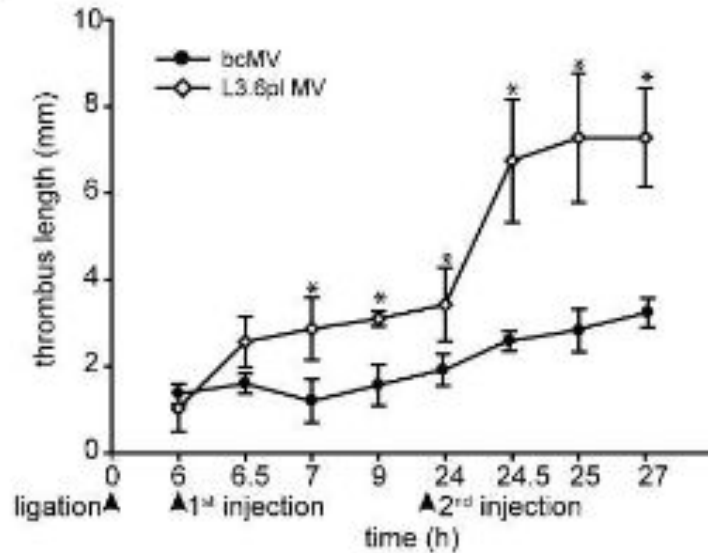
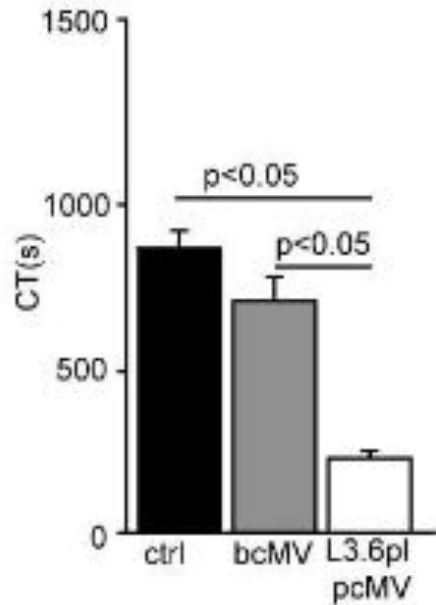
TF+EVs in patients with cancer

Biomarker	Study design	Cancer type	Number of patients	Biomarker cutoff or numeric variable	Statistic	95% CI
Extracellular vesicles	Prospective	Mixed	728	≥ 4.62 nM PS	HR 0.95	0.55–1.64
	Prospective	Pancreas	60	Per 2-fold increase	HR 1.5	1.0–2.4
Tissue factor-positive extracellular vesicles		Brain	119		HR 0.9	0.7–1.3
		Stomach	43		n/a	n/a
		Colorectal	126		HR 0.9	0.6–1.6
	Prospective	Multiple myeloma	122	> 11.8 fM Xa/min	OR 1.4	0.4–4.7
	Prospective	Mixed	648	$\geq 13\%$	sHR 1.0	0.99–3.8
	Retrospective	Pancreaticobiliary	117	≥ 2.5 pg/ mL	OR 4.78	1.64–13.98
	Prospective	Pancreatic	140	≥ 2.37 pg/ mL	HR 10.5	1.5–72.4
	Meta-analysis	Solid tumor	n/a	n/a	OR 1.76	1.21–2.56

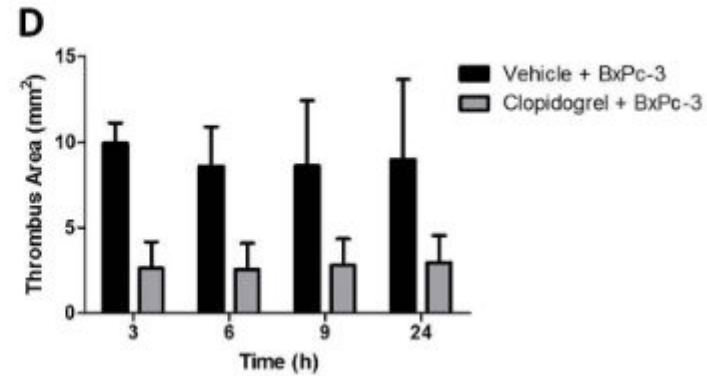
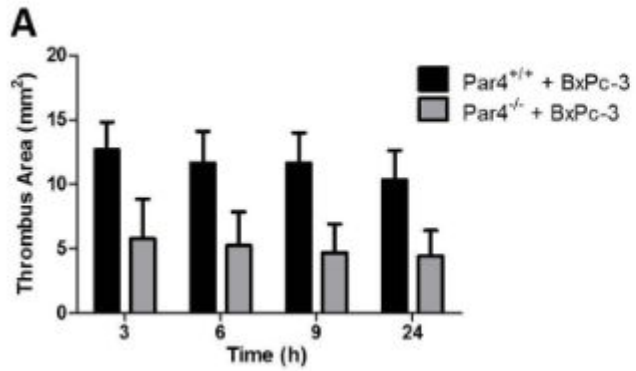
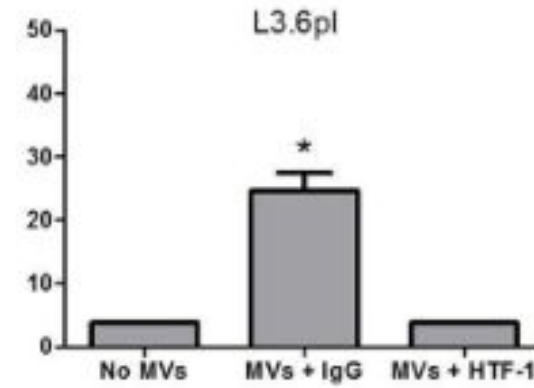
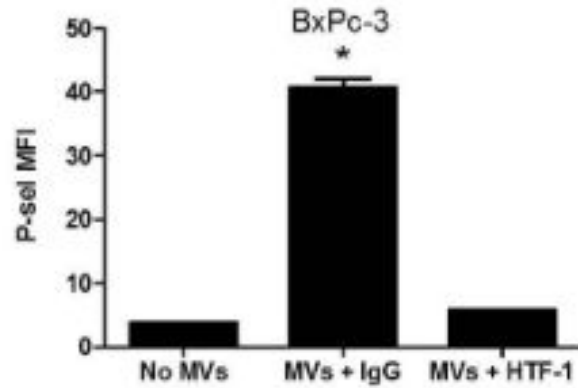
TF+EVs flow-cytometry OR 2.97 (95% CI 1.81 4.86)
 TF+EV activity assays OR 1.25 (95% CI 0.95 1.65)

TF+EVs-mediated clotting activation

The procoagulant capacity of TF+Evs has been well demonstrated in mouse models

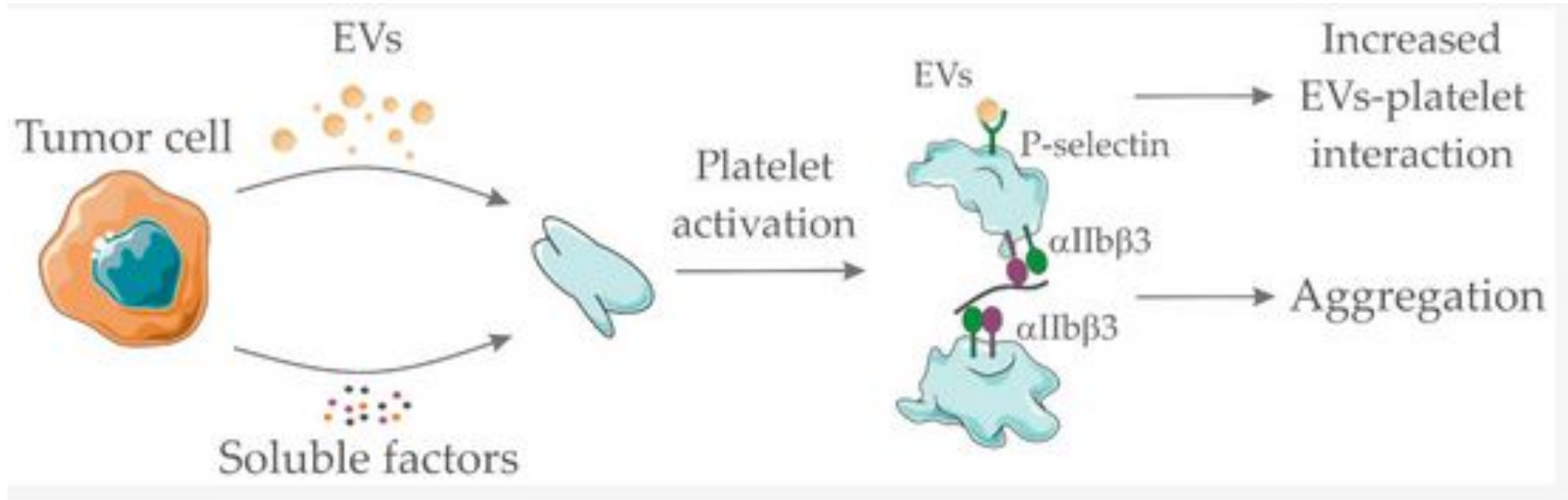


TF+EVs-mediated platelets activation



TF+EVs-mediated platelets activation

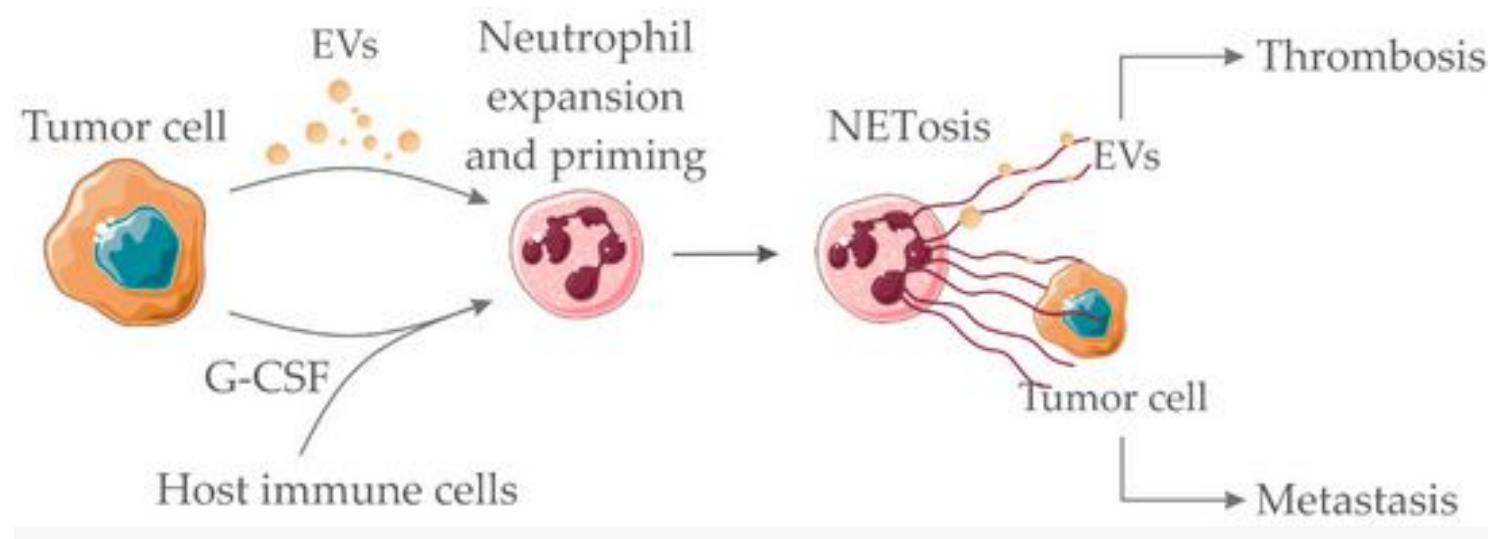
Pro-hemostatic interactions between tumor cell-derived soluble factors/EVs and platelets.



- ✓ Tumor-derived EVs and/or tumor-derived soluble factors interact with platelets promoting their activation.
- ✓ Platelet activation accounts for integrin exposure and further interaction with fibrinogen, thus enabling platelet aggregation.
- ✓ In addition, platelet activation promotes P-selectin exposure which serves as a ligand for PSGL1-containing EVs.
- ✓ EVs interaction with platelets favor their accumulation at the site of thrombotic injury.

EVs-mediated leukocytes activation

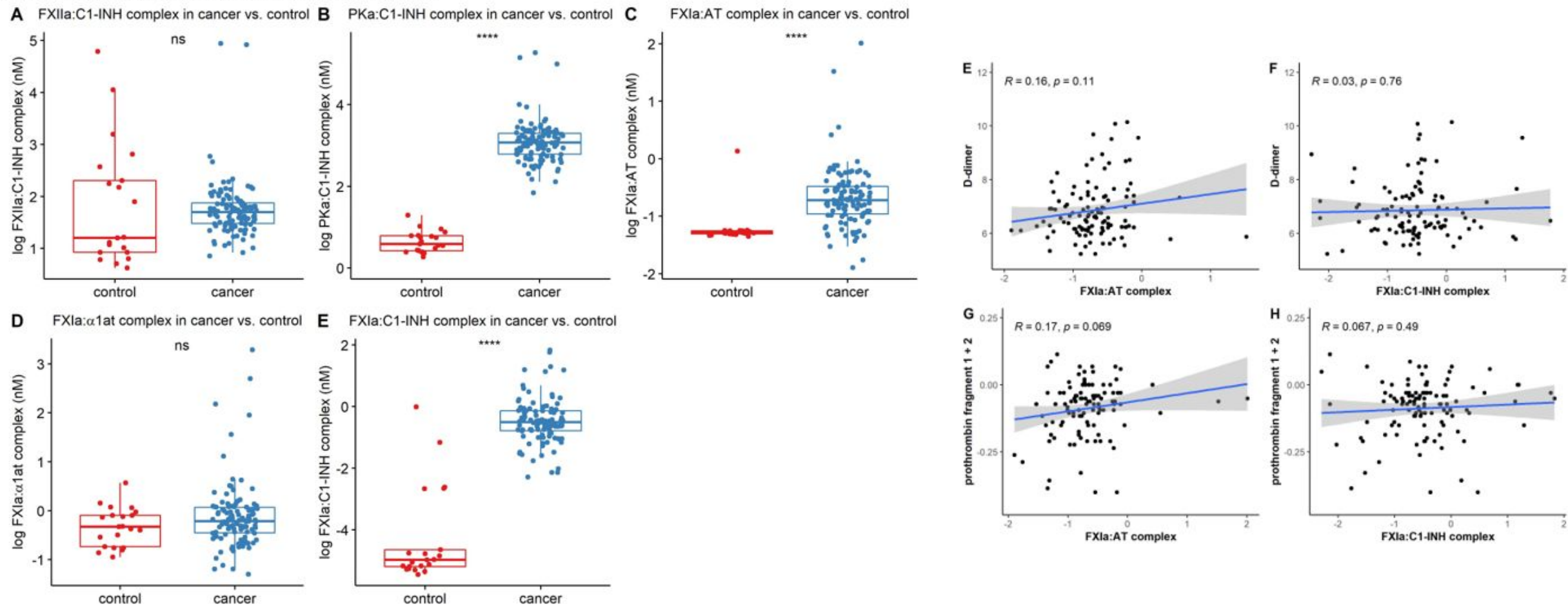
Pro-hemostatic interactions between tumor cell-derived soluble factors/EVs and neutrophils



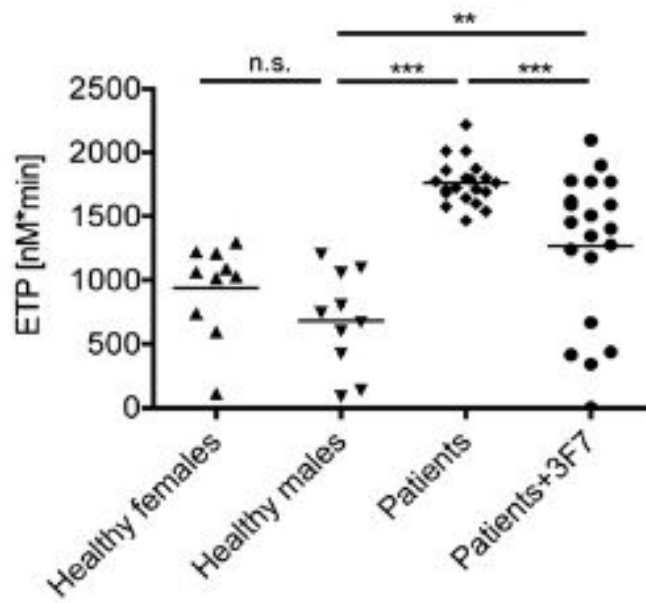
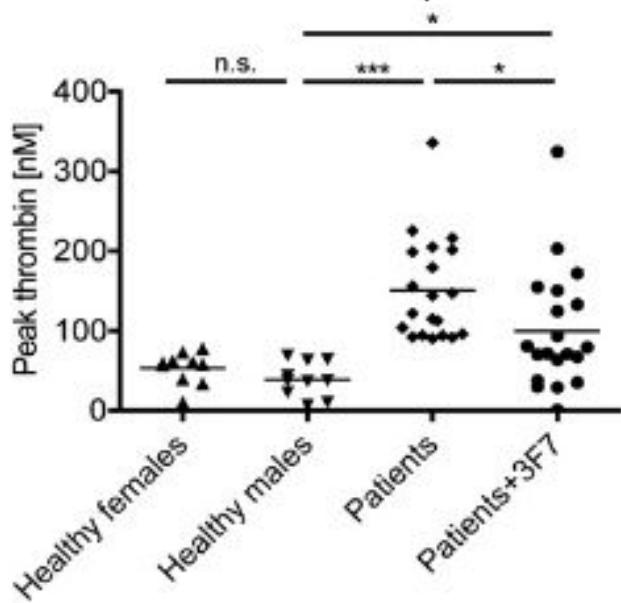
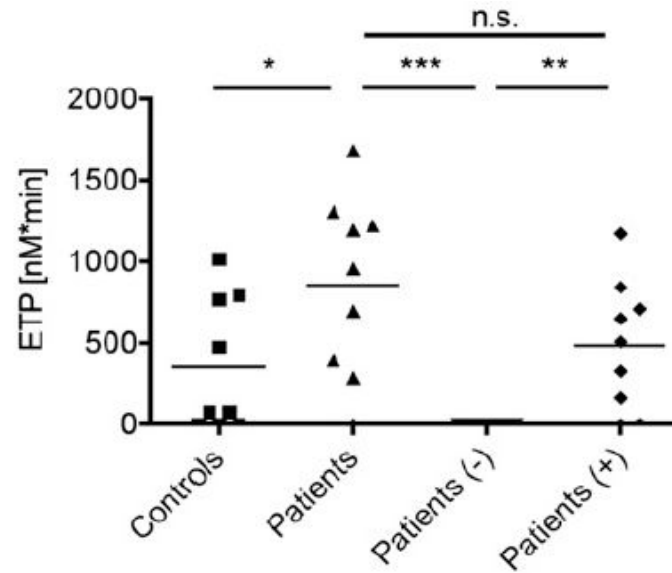
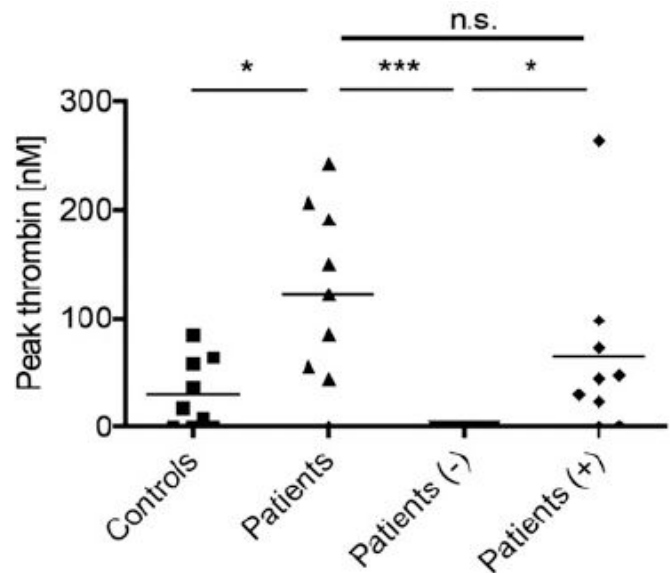
- ✓ Tumor-derived EVs and/or tumor/host-derived soluble factors (such as granulocyte colony-stimulating factor (G-CSF) and other cytokines) interact with neutrophils promoting NETosis.
- ✓ NETs act as scaffolds for the binding of procoagulant tumor-derived EVs, therefore, amplifying thrombus formation.

EVs-mediated intrinsic pathway activation

Figure 1. Complexes in pancreatic cancer versus healthy controls.



EVs-mediated intrinsic pathway activation



EVs-mediated procoagulant state

EVs-derived molecules and their possible prothrombotic roles.

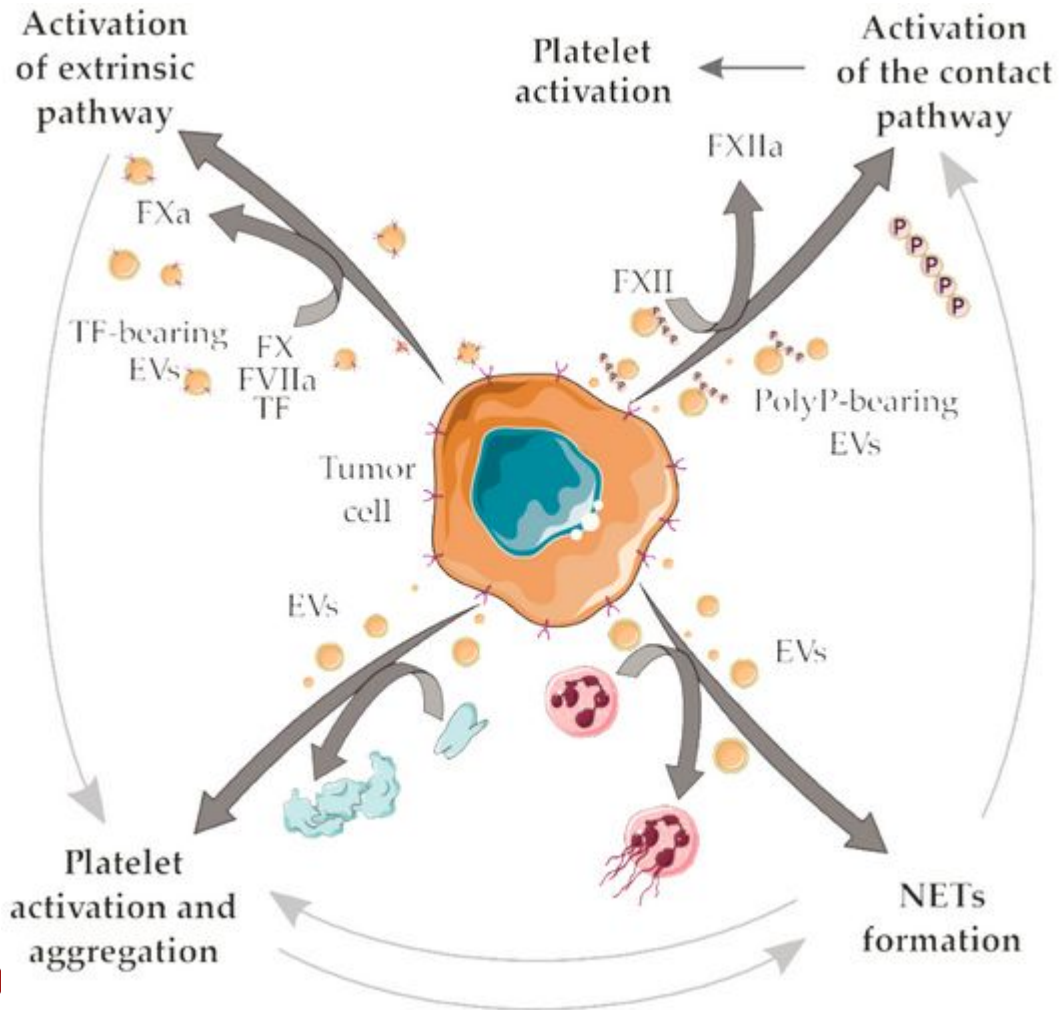
Table 2. EVs-derived molecules and their possible prothrombotic roles.

EVs-Linked Molecules	Suggested Effect
TF	Activation of the extrinsic pathway, fibrin generation, and platelet activation/aggregation in a thrombin-dependent manner
Podoplanin	Platelet aggregation
PSGL-1	Accumulation of the EVs at the site of thrombosis through binding to platelets via P-selectin
Integrins	Accumulation of the EVs at the site of thrombosis through binding to platelets
Unknown	Neutrophil activation and NETs release
Unknown	Binding to NETs
PolyP	Activation of the contact pathway, fibrin generation, and platelet activation/aggregation in a thrombin-dependent manner

EVs, extracellular vesicles; TF, tissue factor; PSGL-1, P-selectin glycoprotein ligand-1; PolyP, polyphosphate; NETs, neutrophil extracellular traps.

EVs-mediated procoagulant state

Possible crosstalk between the proposed mechanisms for tumor-derived EVs in cancer-associated thrombosis.



- ✓ Tumor-derived TF + EVs initiate the extrinsic pathway of coagulation, culminating in the activation of the FX and initial thrombin-generation.
- ✓ Further thrombin generation accounts for an indirect mechanism for platelet activation/aggregation. EVs may elicit direct platelet activation/aggregation.
- ✓ Tumor-derived polyP+ EVs initiate the contact pathway of coagulation, mediating FXII activation
- ✓ Interaction of neutrophils with tumor-derived EVs may support NETs release thus eliciting several NETs-dependent prothrombotic mechanisms. NETs provide additional surfaces that support the contact pathway activation.
- ✓ In addition, crosstalk between platelets and NETs may play an important role in the establishment of cancer-associated thrombosis.

CONCLUSIONS

A number of prothrombotic mechanisms in CAT rely on complex interactions between tumor-derived EVs and vascular cells or with components of the hemostatic system

Initiation of the extrinsic pathway by TF+ EVs or initiation of the contact pathway by polyP+ EVs have been described in different type of cancer

The interaction of tumor-derived EVs with platelets (promoting platelet activation and/or platelet aggregation) or neutrophils (facilitating NETs release) may provide additional positive feedback

One or more mechanisms may take place and predominate in a certain cancer type for the establishment of the prothrombotic state

EVs are promising players for the detection of novel therapeutic strategy that could attenuate cancer-associated hypercoagulability



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THANK YOU FOR YOUR TIME!