The Role of Podoplanin in Cancer-associated Thrombosis

Cihan Ay

Clinical Division of Haematology and Haemostaseology
Department of Medicine I, Comprehensive Cancer Center Vienna
Medical University of Vienna, Vienna, Austria

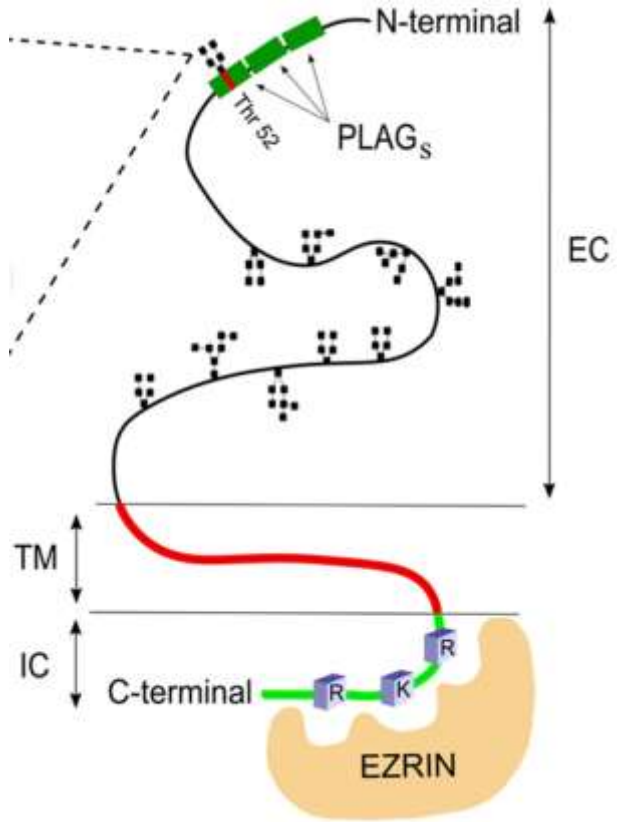
@Cihan_Ay_MD cihan.ay@meduniwien.ac.at

Podoplanin – a transmenbrane, mucin-type glycoprotein

- Expressed by a variety of different cells and normal tissues

Podoplanin in normal tissues

Organ	Cell type
Vasculature	Lymphatic endothelial cells
Lymphoid organs	Stromal reticular cells; Follicular dendritic cells
Skin	Focal expression in basal keratinocytes
Lung	Type I alveolar cells; Pleura
Kidney	Glomerular podocytes; Parietal epithelial cells of Bowman's capsule
Liver	Bile ducts; Peritoneum
Esophagus	Basal keratinocytes
intestine	Peritoneum
Central nervous system	Choroid plexus; Ependyma; Meninges
Peripheral nervous system	perineural fibroblasts
Ovary	Follicular granulosa cells; Germinal epithelium
Cervix	Basal keratinocytes
Breast	Myoepithelial cells
Prostate	Myofibloblasts
Testis	Fibromyocytes
Salivary gland	Myoepithelial cells
Bone	Osteocytes; Periosteum
Joint	Synovial cells



EC – extracellular domain, TM – transmembrane domain, IC - intracellular domain, PLAG - platelet aggregation-stimulating domain

Suzuki-Inoue et al. J Thromb Haemost 2017, Navarro-Núñez et al. Thromb Haemost 2013, Ugorski et al. Am J Cancer Res 2016

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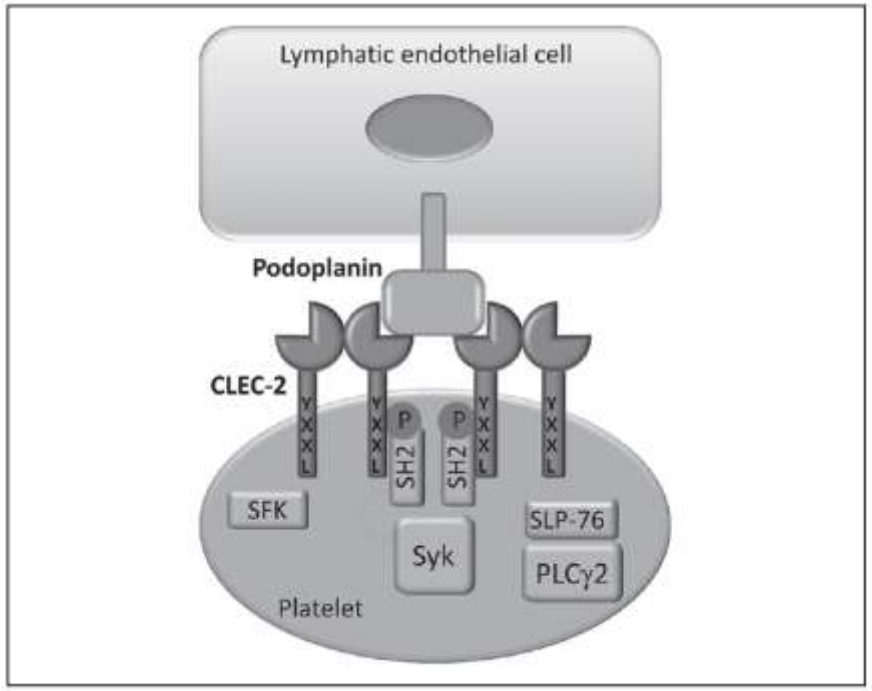
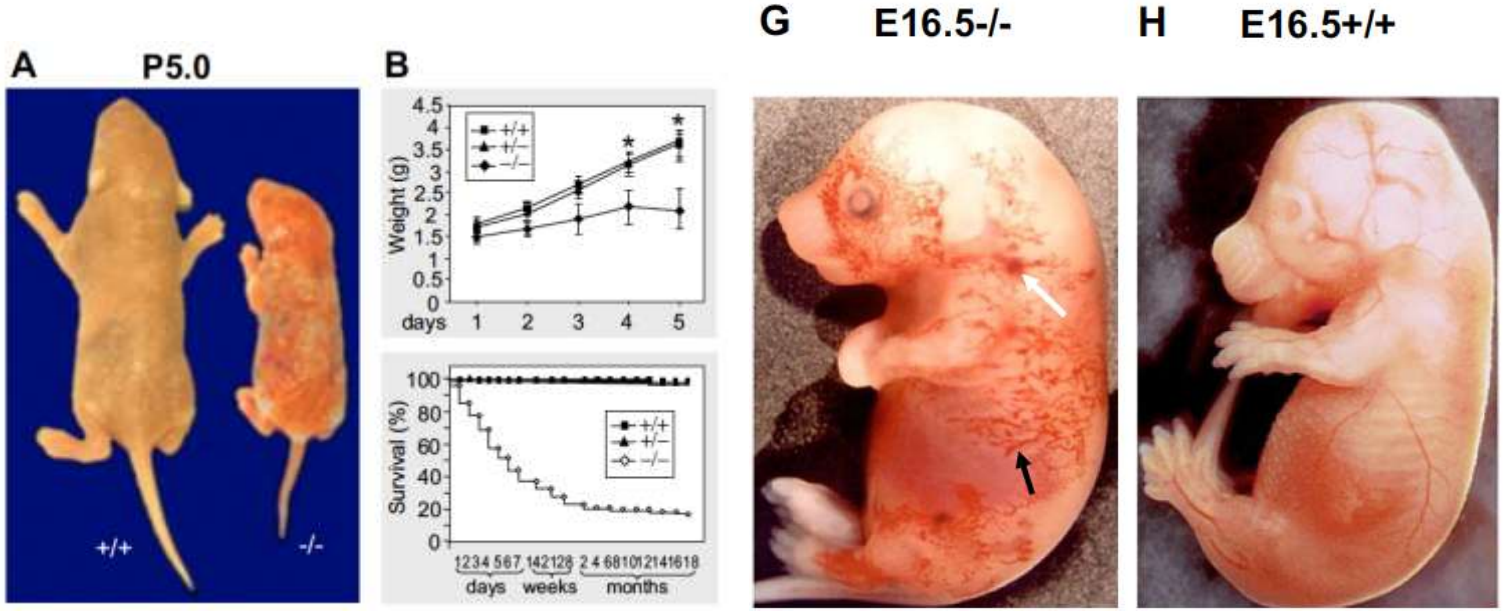
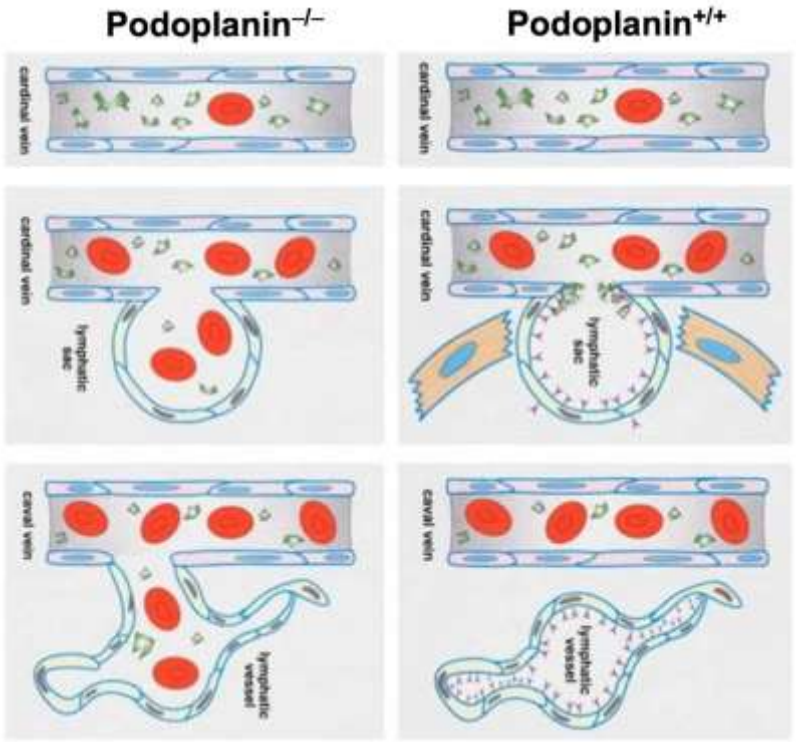
- Activates platelets via C-type lectin receptor type 2 (CLEC-2)



Suzuki-Inoue K et al. J Thromb Haemost 2017, Navarro-Núñez et al. Thromb Haemost 2013

Podoplanin-mediated platelet activation is critically involved in seperation of blood and lymphatic vessels



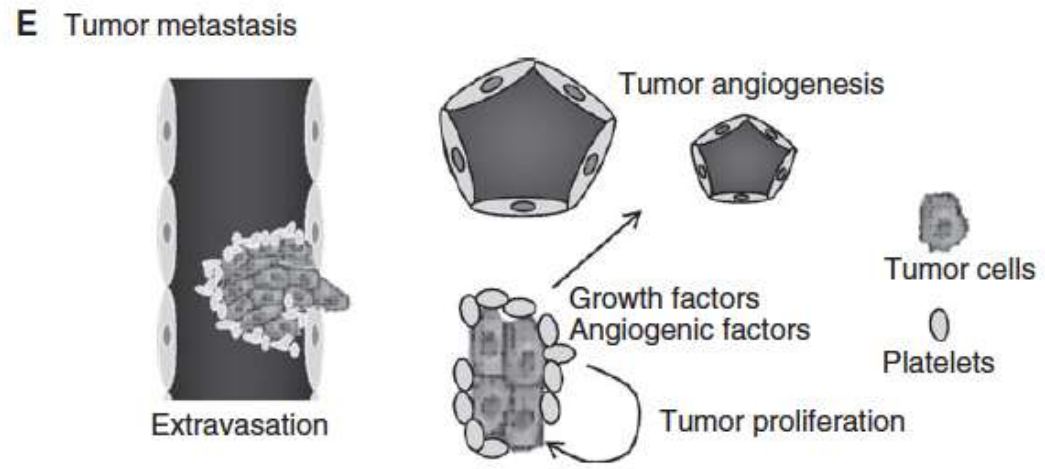


- Podoplanin□-/-□ mice
  - Significantly smaller, ectatic vessels and skin bleeding
  - Approximately 20% reach fertility age
- Blood-filled capillary network (black arrows) in podoplanin-/-□, but not in podoplanin□+/+□ embryos

Uhrin P et al. Blood. 2010;115(19):3997-4005

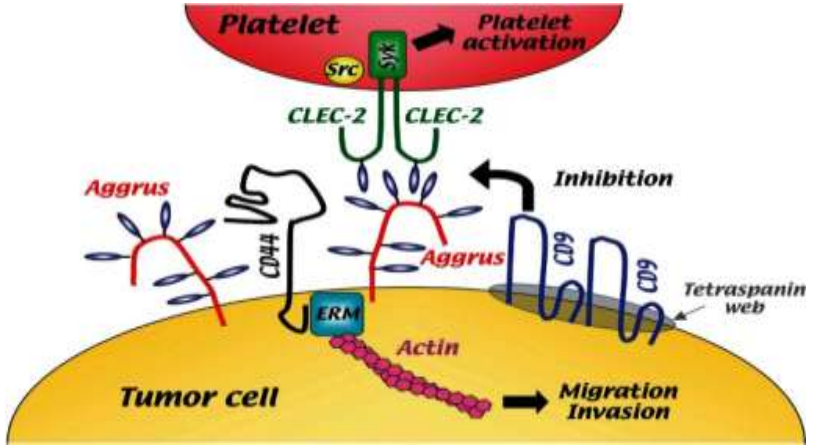
Podoplanin expression in tumors

Tumor type	Frequency
Squamous cell carcinoma	80% of squamous cell carcinoma (lung, larynx, cervix, skin, and esophagus), 25% of oral carcinoma
Germinal tumors	Seminoma (98%), Embryonal carcinoma (69%), Teratoma (29%), York sac tumor (25%)
Brain tumors	Anaplastic astrocytoma (25%), Glioblastoma (47%), Germinoma (98%), Immature teratoma (71%)
Others	Mesothelioma (93%), Kaposi's sarcoma (90-100%), Lymphangioma (100%)



Podoplanin expression in primary brain tumors associated with

- Grade of malignancy
- Poor survival

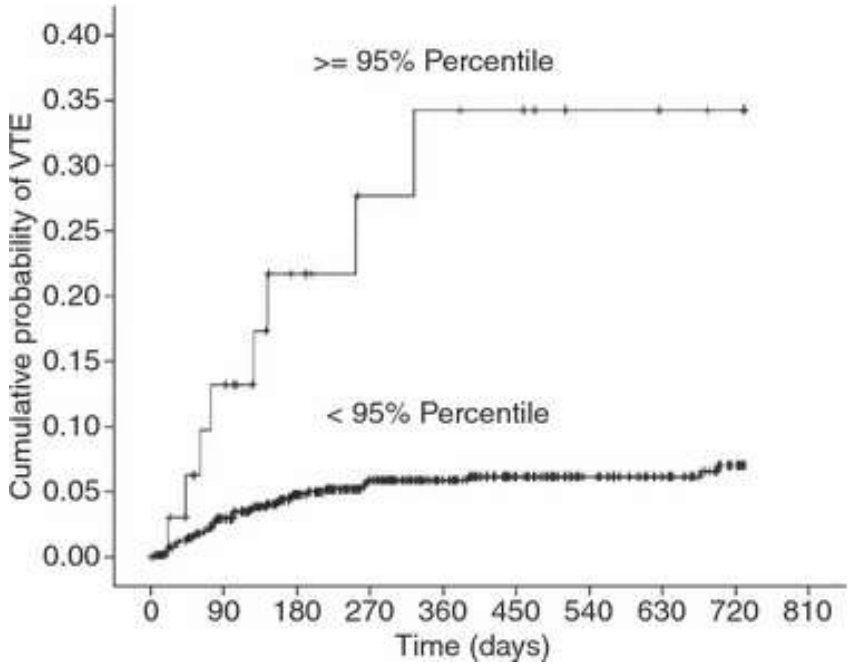


Tsuruo T et al. Proc Jpn Acad Ser B Phys Biol Sci 2008

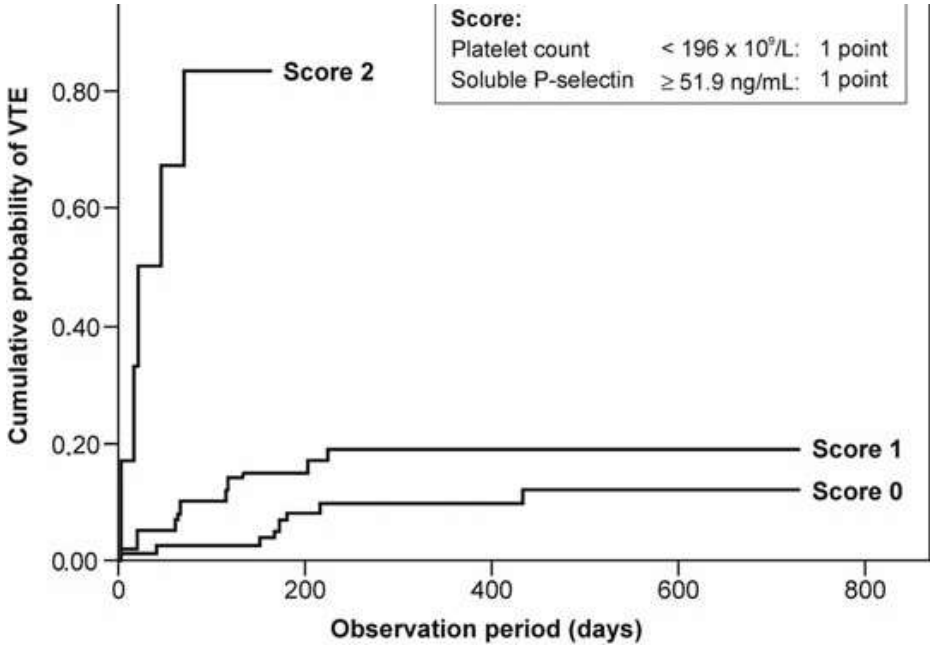
Suzuki-Inoue K et al. J Thromb Haemost 2017

Cancer, platelets and venous thromboembolism

- In solid tumors a high platelet count is associated with risk of VTE[1]



- Primary brain tumours lower platelet count is associated with risk of VTE[2]



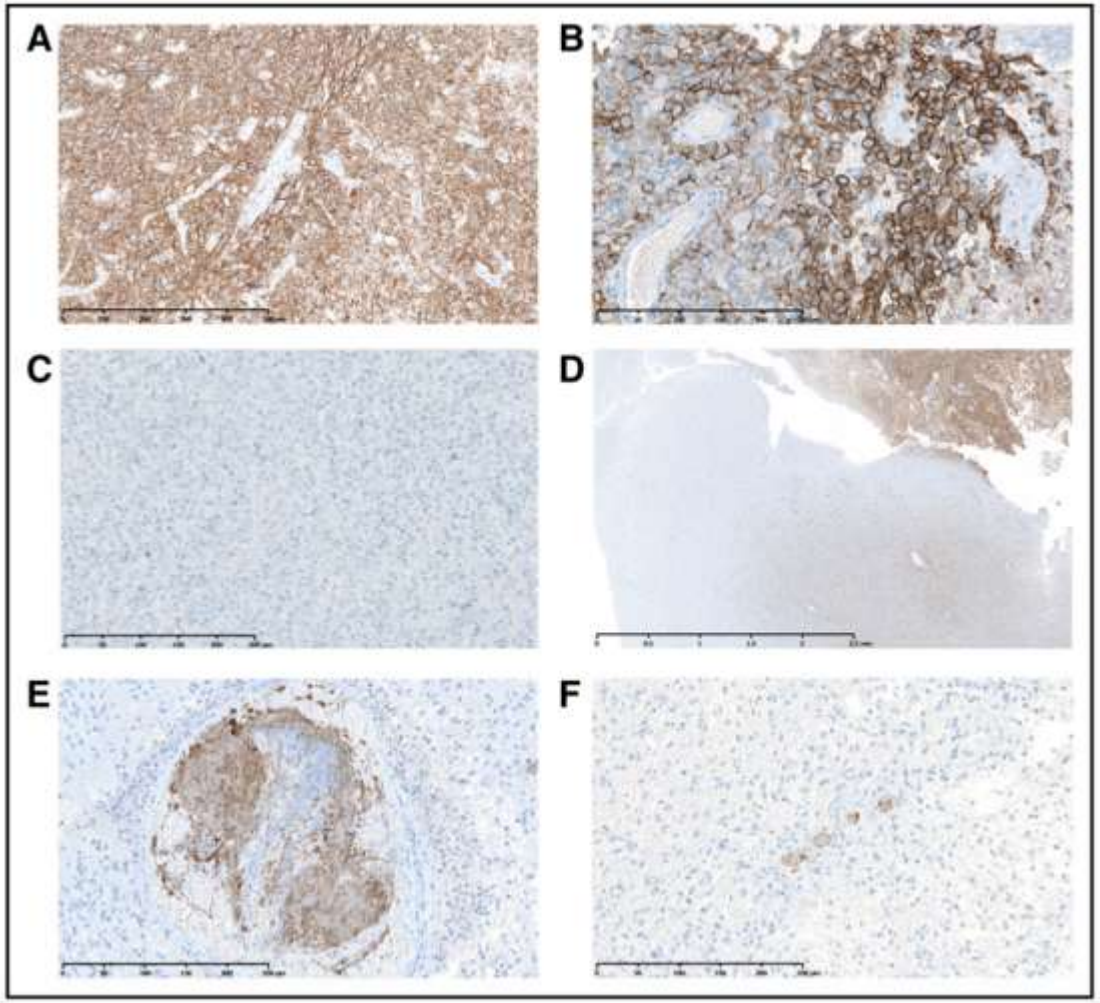
[1]Simanek et al, JTH 2009, 8:114; [2] Thaler et al, Neuro Oncol 2014, 16:1645

Role of podoplanin in brain cancer-associated thrombosis

- Hypothesis
  - Podoplanin, due to its ability in activating blood platelets, is involved in the procoagulatory state and the development of VTE in patients with primary malignant brain tumors

Riedl J et al. Blood 2017;129: 1831-9

Immunohistochemical staining against podoplanin and intratumoral platelet aggregates in brain tumor specimens



A, B: Representative examples of high podoplanin (+++)-expressing tumors

C: Representative example of a podoplanin-negative (–) tumor.

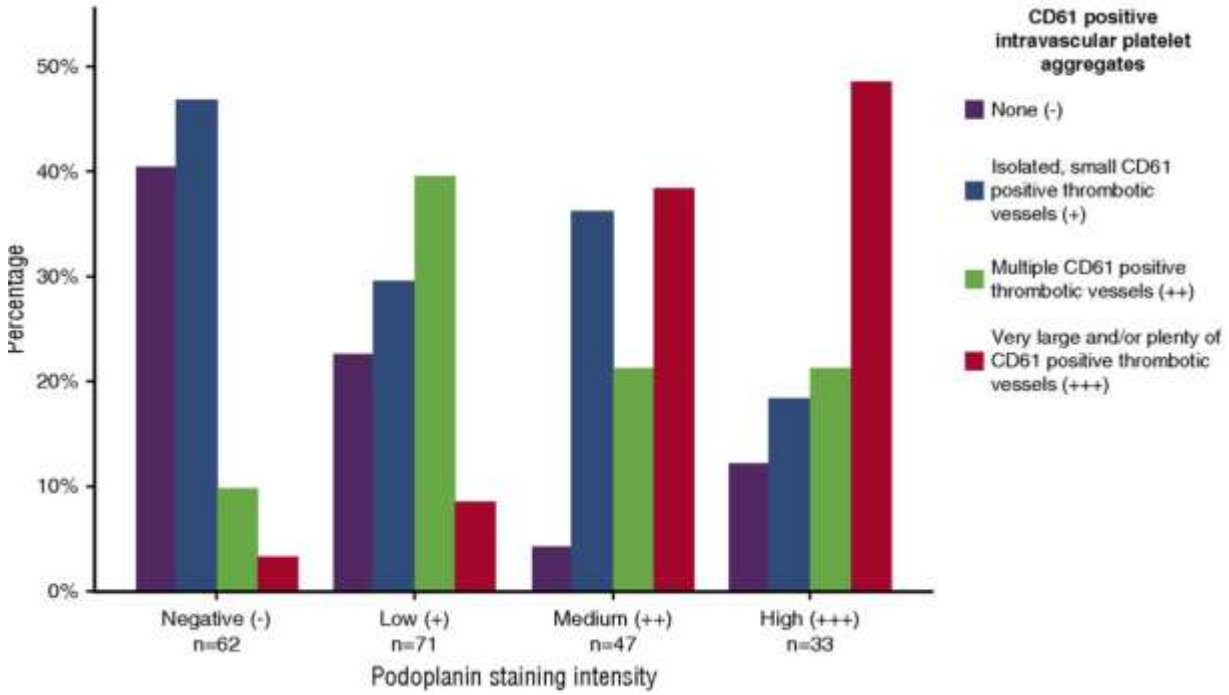
D: Invasion zone of a podoplanin-expressing tumor

E: A very large $CD61^+$ intravascular thrombosis

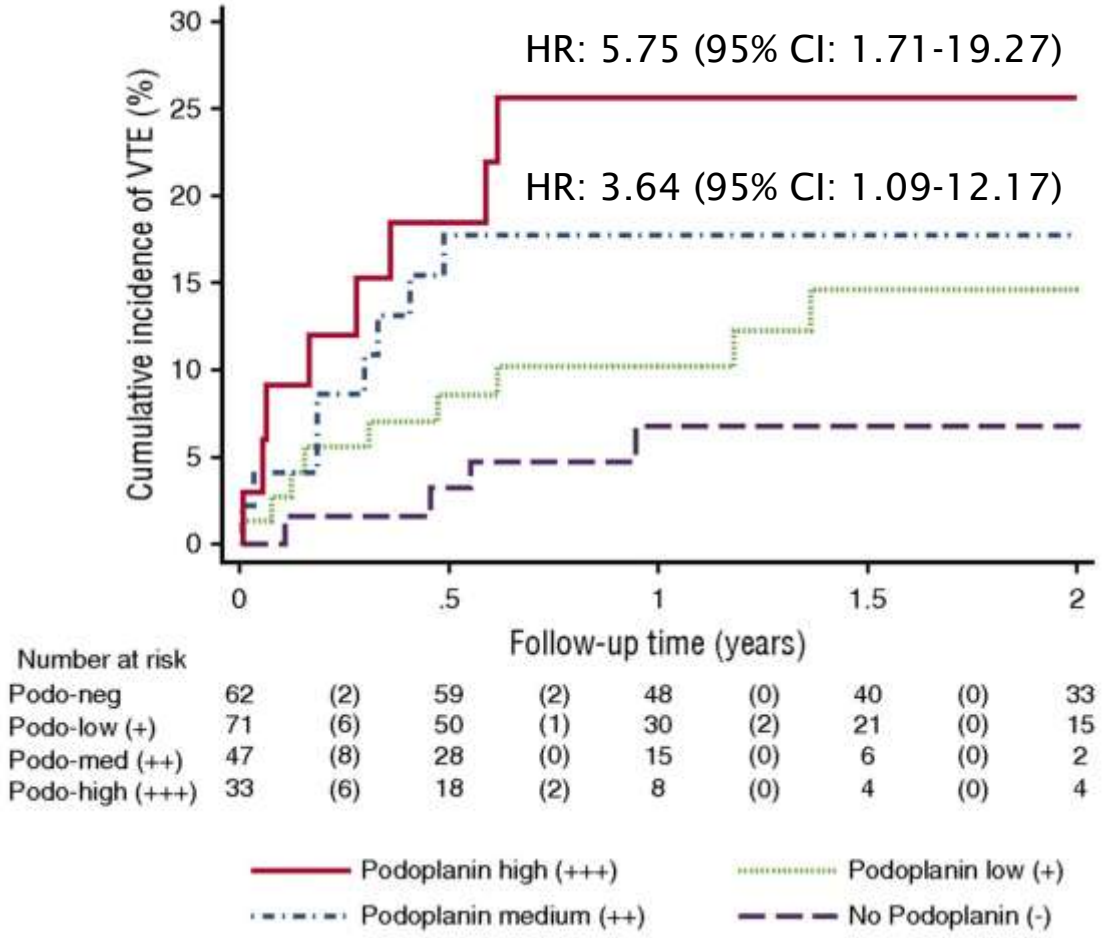
F: Tumor sample with multiple $CD61^+$ thrombotic vessels

Riedl J et al. Blood 2017;129: 1831-9

Correlation between podoplanin expression levels and intratumoral intravascular platelet aggregates (p<0.001)



Cumulative incidence of VTE according to expression levels of podoplanin (p=0.019)



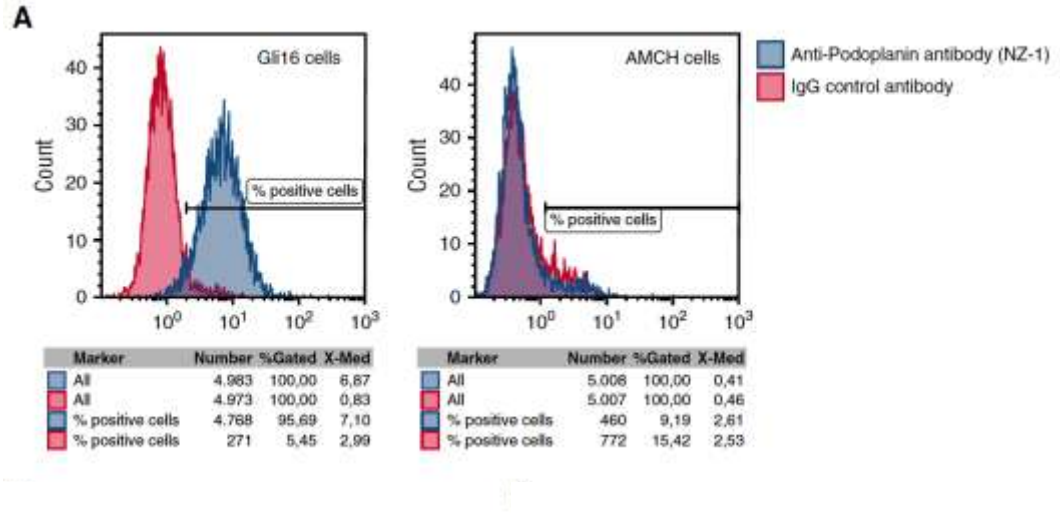
Riedl J et al. Blood 2017;129: 1831-9

Podoplanin expression, peripheral blood count parameters, and biomarkers of blood coagulation

	Podoplanin-positive tumors (+, ++, and +++), n = 151	Podoplanin-negative tumors (–), n = 62	*P*
Platelet count, × 10^9/L	227 (186-285)	286 (241-355)	<.001
Hemoglobin, g/dL	13.6 (13.0-14.5)	13.9 (13.0-14.6)	.232
Leukocyte count, × 10^9/L	8.68 (6.30-12.15)	7.11 (5.36-10.55)	.029
sP-selectin, ng/mL	36.8 (28.7-47.9)	38.8 (28.8-51.2)	.513
D-dimer, μg/mL	0.85 (0.46-1.92)	0.42 (0.23-0.79)	<.001
F1+F2, pmol/L	210 (154-325)	194.5 (123-260)	.047
FVIII activity, %	224 (156-295)	179.5 (147-239)	.006

Riedl J et al. Blood 2017;129: 1831-9

Podoplanin expression and tumor cell–induced platelet aggregation and platelet activation *in-vitro*



Riedl J et al. Blood 2017;129: 1831-9

Regular Article

blood

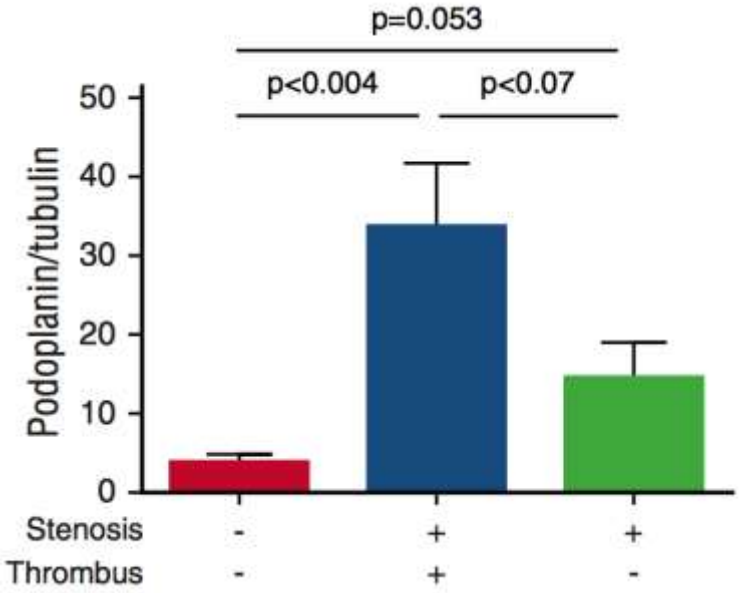
THROMBOSIS AND HEMOSTASIS

Mice with a deficiency in CLEC-2 are protected against deep vein thrombosis

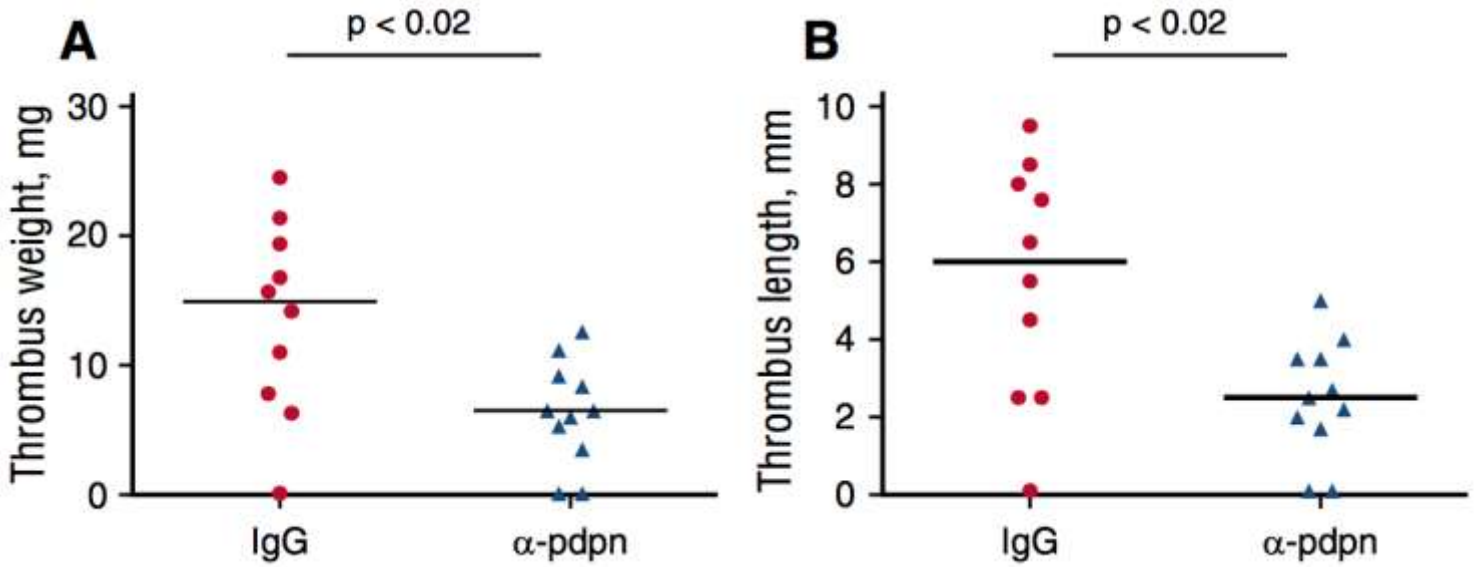
Holly Payne,* Tatyana Ponomaryov,* Steve P. Watson, and Alexander Brill

Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom

Podoplanin expression in the IVC vessel wall increases with thrombosis



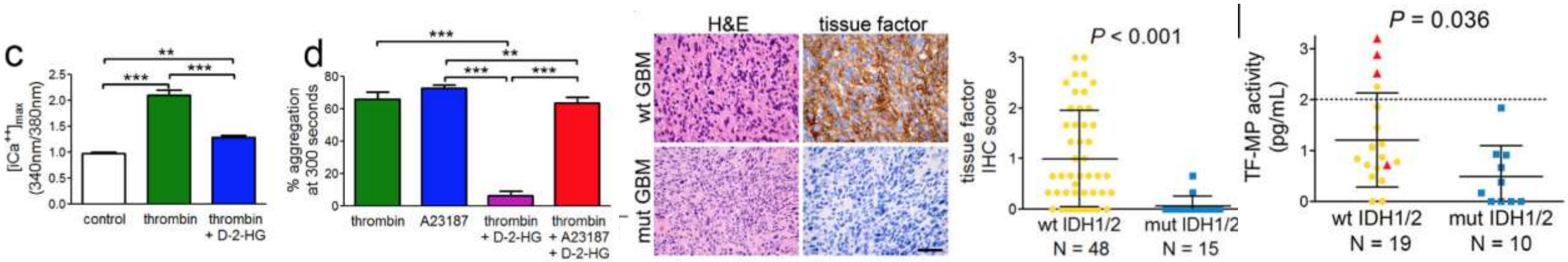
Anti-podoplanin antibody decreases size of thrombi in murine DVT



Payne H et al. Blood 2017

Mutant IDH1 and thrombosis in gliomas

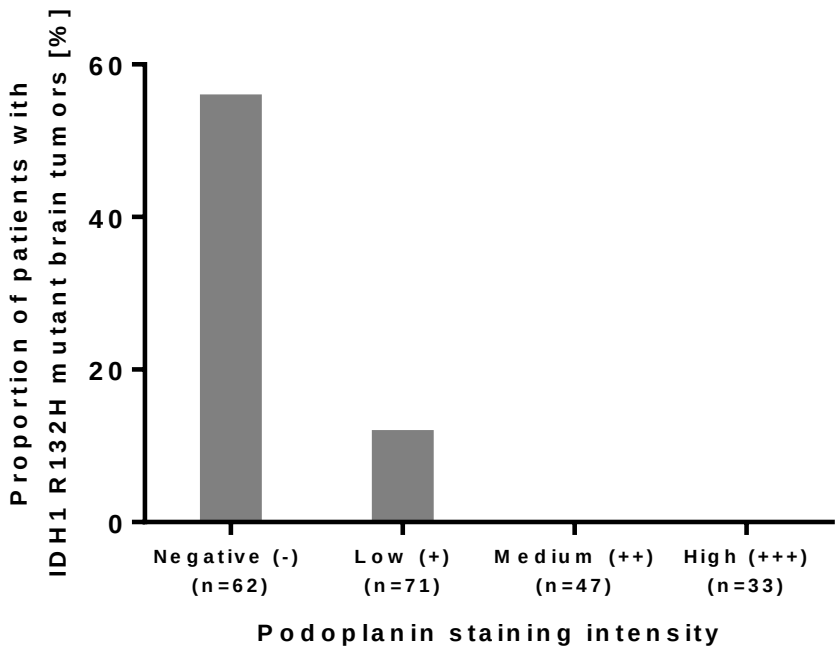
- Mutant isocitrate dehydrogenase 1 (IDH1) is common in gliomas, and produces D-2-hydroxyglutarate (D-2-HG), an oncometabolite associated with DNA hypermethylation
- Full effects of IDH1 mutations on glioma biology and tumor microenvironment are unknown, but mutant IDH1 tumors have are less aggressive behavior
- VTE rate in patients with wild-type IDH1: 26-30%
- VTE rate in mutant IDH1 gliomas: 0%



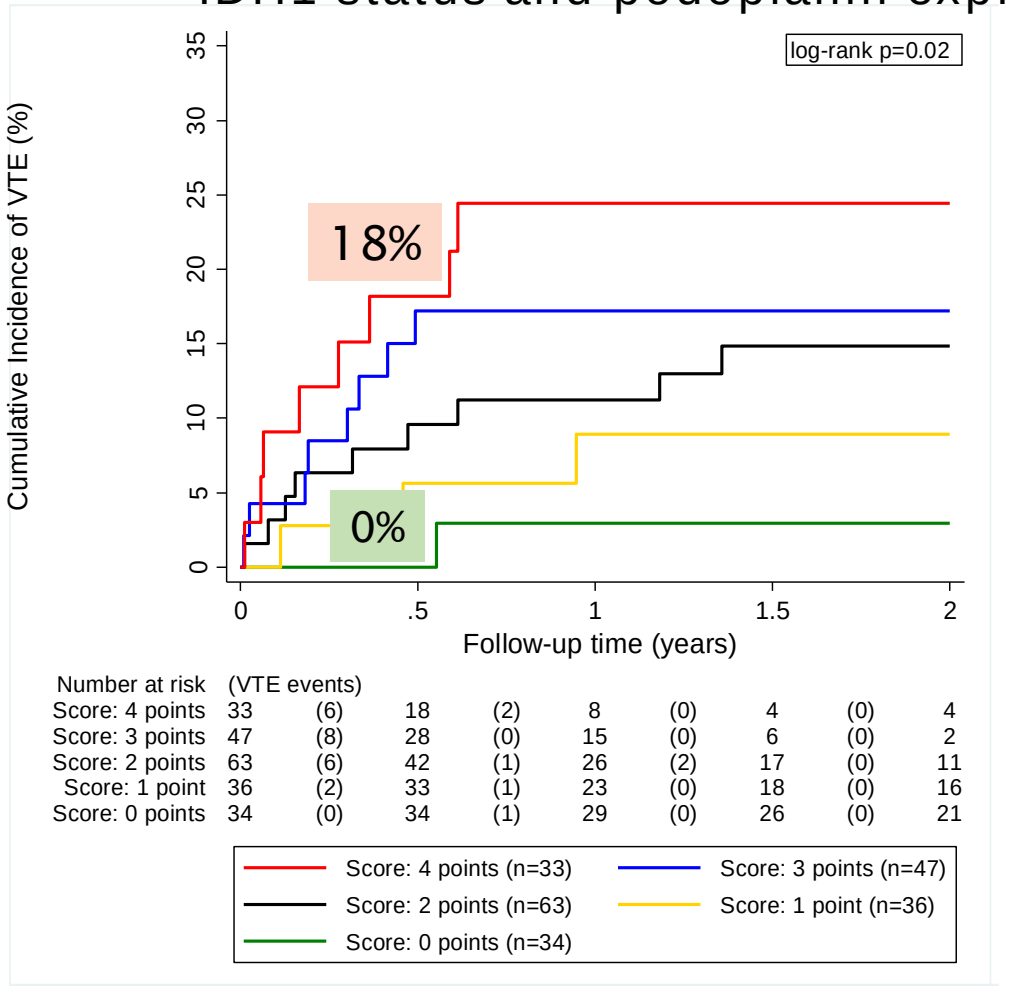
Unruh D et. al. Acta Neuropathol. 2016

Combined effect of IDH1 and podoplanin in glioma

Proportion tumors with IDH1 mutation and different levels of podoplanin expression



Cumulative incidence of VTE according to IDH1 status and podoplanin expression



Score

IDH1 status (mut=0, wt=1)

Podoplanin expression levels (no=0, low=1, medium=2, high=3)

Mir Seyed Nazari P. et al. J Thromb Haemost 2018 (in press)

Podoplanin methylation and mRNA expression levels in IDH1 mutant and wildtype tumors

- Data extracted from The Cancer Genome Atlas (TCGA)
  - Podoplanin methylation was strongly increased in IDH1 mutant compared to wildtype tumors
  - Mean podoplanin mRNA levels were significantly lower in both low-grade gliome (LGG) and glioblastoma (GBM) patients with an IDH1 mutation than those with an IDH1 wildtype tumor ($p<0.0001$)
  - Higher podoplanin methylation was associated with lower podoplanin mRNA expression only in LGG patients (Spearman's $rho=-0.64$, $p<0.0001$) but not GBM patients ($rho=-0.15$, $p=0.35$)

Mir Seyed Nazari P. et al. J Thromb Haemost 2018 (in press)

Summary & Take Home Messages

- In primary brain tumors, podoplanin expression is associated with intratumoral platelet aggregates, procoagulant state and high risk of VTE
- IDH1 mutation and podoplanin overexpression in primary brain tumors appear to be exclusive
  - Patients with IDH1 wildtype and high podoplanin expression have an increased VTE risk (6-month VTE risk of 18.2%)
  - Patients with IDH1 mutation and no podoplanin expression have a low VTE risk (6-month VTE risk of 0%)
- Podoplanin is able to induce platelet aggregation via the receptor CLEC-2 on platelets
- Podoplanin and CLEC-2 might represent attractive drug targets for prevention of (brain) cancer-associated VTE.

The „Pabinger-Lab“

<u>Podoplanin Project</u>

Julia Riedl

Pegah Mir Seyed Naza

Podoplanin expression in acute promyelocytic leukemia contributes to coagulopathy

- RNA-sequencing-based characterization of APL (n = 30) compared to other acute myeloid leukemia (n = 400) samples and normal promyelocytes
  - Podoplanin (PDPN) gene was aberrantly expressed in APL promyelocytes and was the most distinctive transcript for APL
  - PDPN was the most specific surface marker for APL in flow cytometry
  - All-trans retinoic acid therapy rapidly decreases podoplanin expression
  - Functional studies
    - PDPN-expressing primary APL cells, but not PDPN-negative primary leukemias induced platelet binding, activation and aggregation.
  - PDPN expression on leukemia cells in a xenograft model was associated with thrombocytopenia (platelet consumption) and prolonged bleeding time in vivo

Lavallée VP et al. Leukemia. 2018

Mutant IDH1 and thrombosis in gliomas

- Mutant isocitrate dehydrogenase 1 (IDH1) is comp… produces D-2-hydroxyglutarate (D-2-HG), an oncometab… DNA hyper…ylation
- Full effects of IDH1 mutations o… tumor mic…re unknown, but mutant ID… ess aggres…
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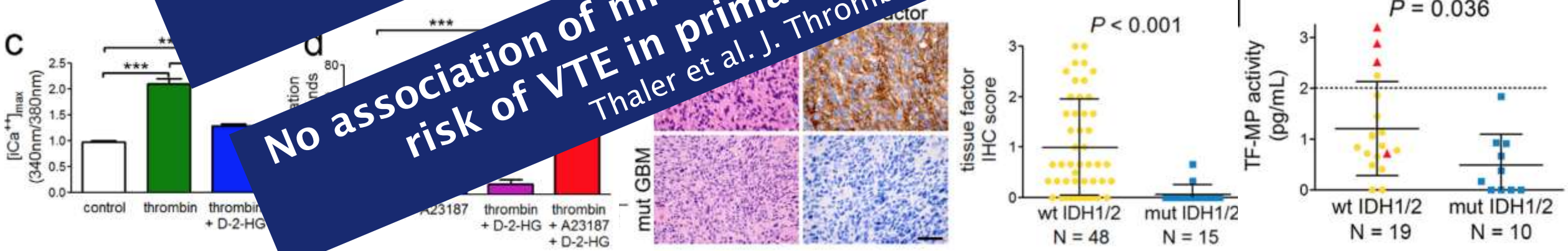
- VTE … gliomas: 0%

No correlation of tissue factor (TF) expression and VTE risk in primary brain tumors (gliomas)
Thaler et al. Thromb. Res. 2013

No association of microvesicle-associated TF activity with risk of VTE in primary brain tumors (gliomas)
Thaler et al. J. Thromb. Haemost. 2012



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