

Novel strategies of coagulation inhibition for reducing tumor growth and angiogenesis

Yona Nadir, MD, PhD

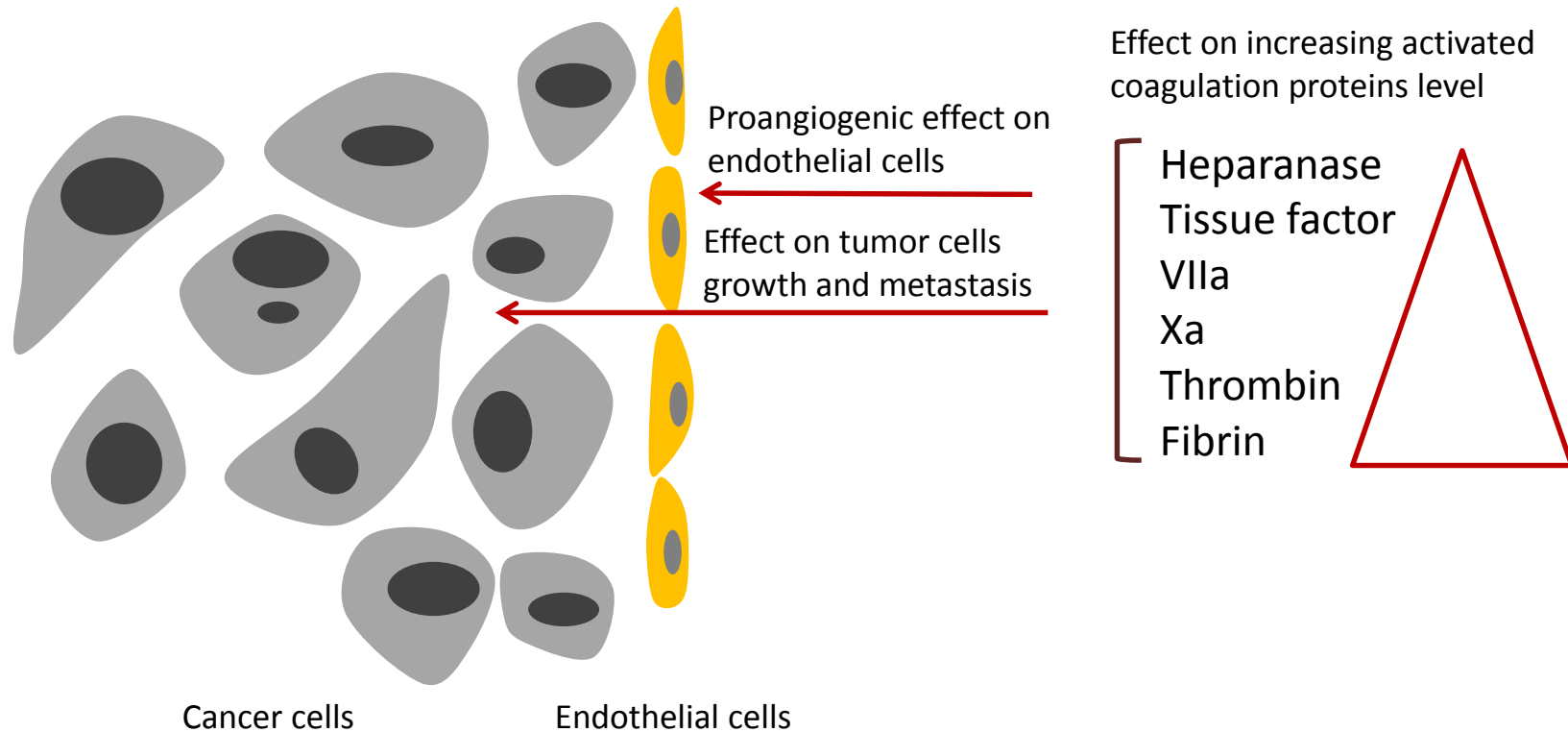
Thrombosis and Hemostasis Unit

Rambam Health Care Campus, Haifa, Israel

Disclosures for Yona Nadir

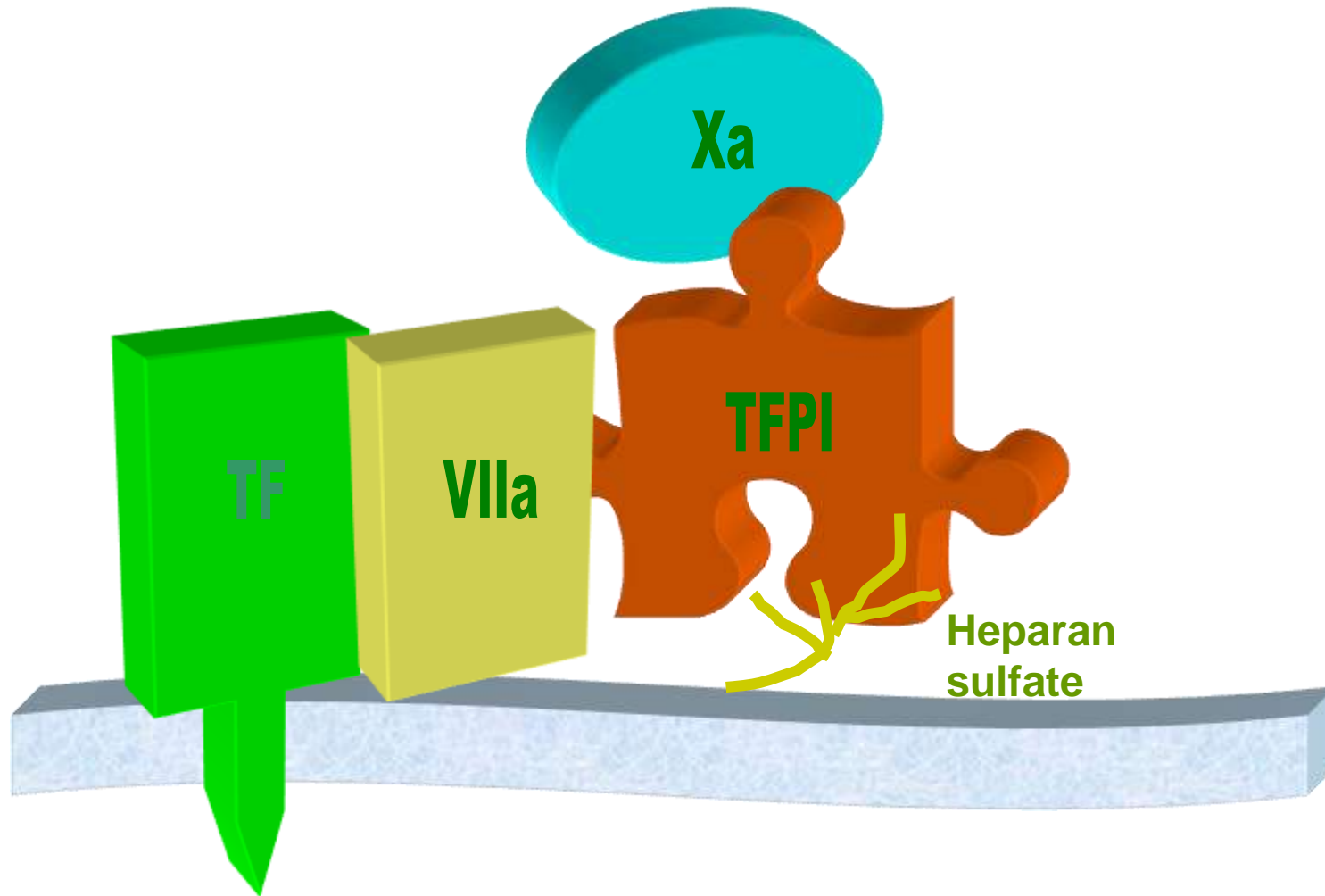
Research Support/P.I.	<i>[No relevant conflicts of interest to declare or Company Name(s)]</i>
Employee	<i>[No relevant conflicts of interest to declare or Company Name(s)]</i>
Consultant	<i>[No relevant conflicts of interest to declare or Company Name(s)]</i>
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Speakers Bureau	<i>[No relevant conflicts of interest to declare or Company Name(s)]</i>
Other (Specify)	<i>[No relevant conflicts of interest to declare or Company Name(s)]</i>

Coagulation proteins are involved in enhancement of tumor growth



There is paucity of information about involvement of the intrinsic coagulation system in tumor growth.

Inhibition of tissue factor



Inhibition of tissue factor

Phase I study of an **anti-TF antibody ALT-836** in subjects with acute respiratory distress syndrome (ARDS)

	Placebo	0.06 mg/kg ALT-836	0.08 mg/kg ALT-836	0.10 mg/kg ALT-836	Total
	(n = 3)	(n = 5)	(n = 5)	(n = 5)	(n = 18)
Mortality by study day 28 (treatment related)	0	0	1	0	1
Patients with non-fatal SAEs (treatment related)	2	1	0	1	5
Total number of AEs (treatment related)	20	18	29	20	87
Patients with hematuria AEs (treatment related)	0	2	2	5	9
Patients with anemia AEs (treatment related)	2	1	2	3	8

*Currently larger phase I/II study is being conducted

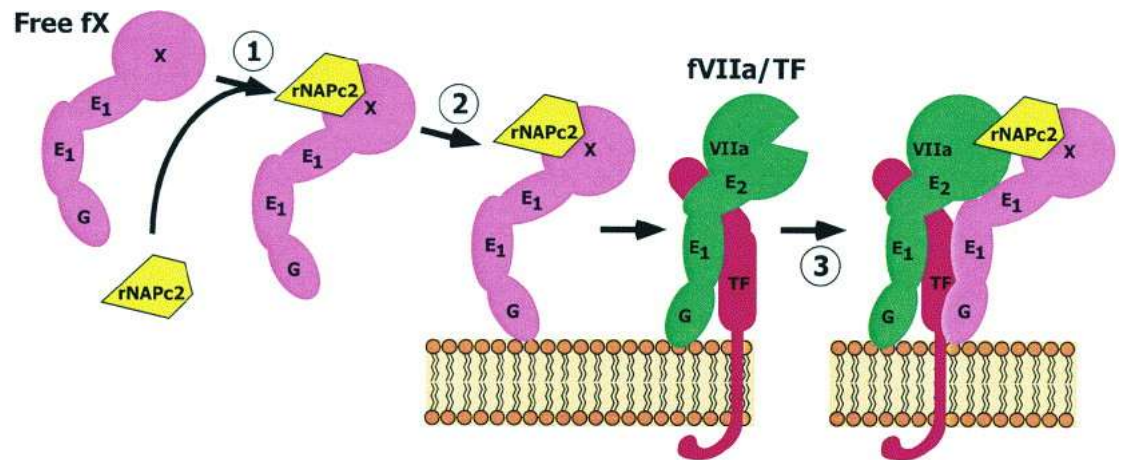
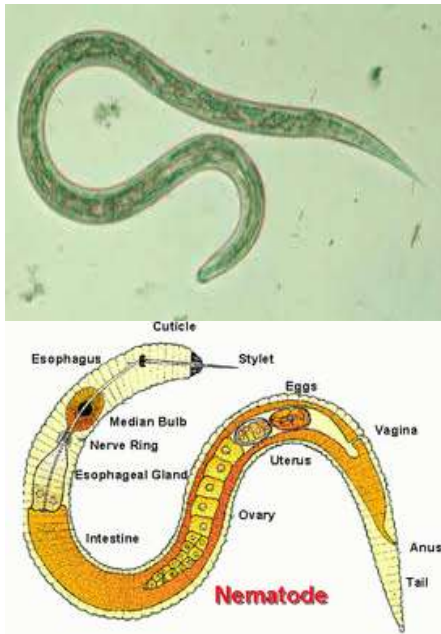
Morris *et al*, BMC pulmonary medicine, 2012

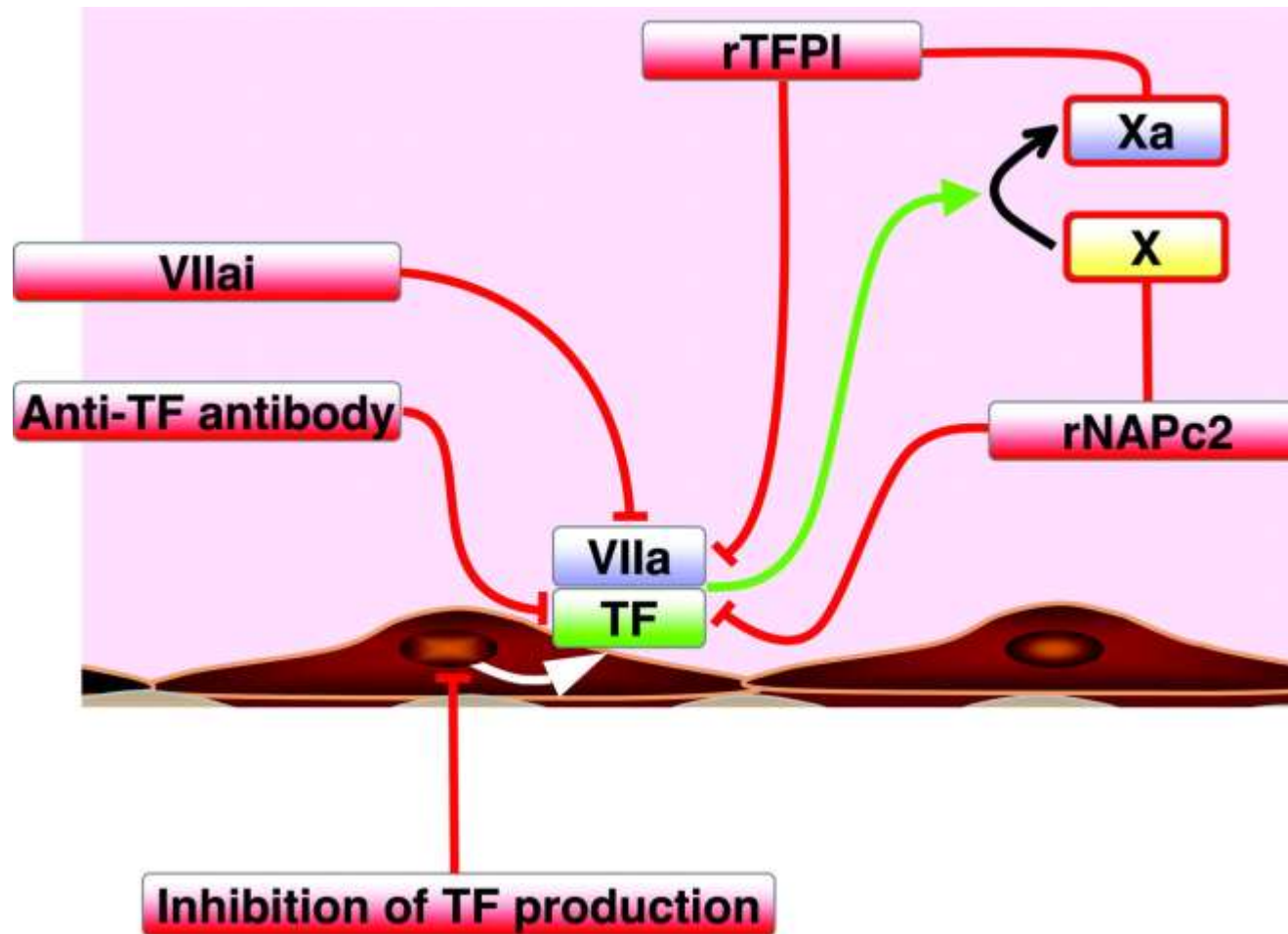
Inhibition of tissue factor and factor VIIa

Recombinant nematode anticoagulant protein c2 (rNAPc2)

Phase I/II studies were conducted in cancer patients, acute coronary syndrome

No further progress to phase III study

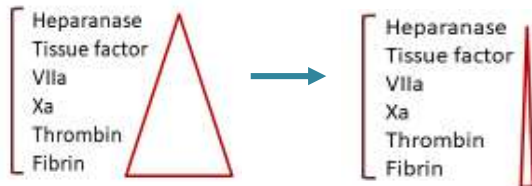




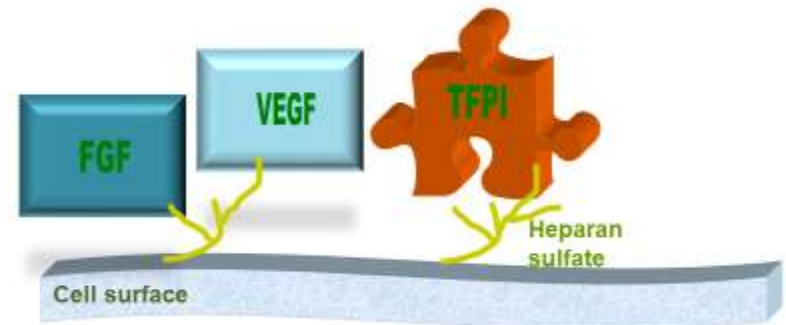
Heparins



Inhibition of coagulation

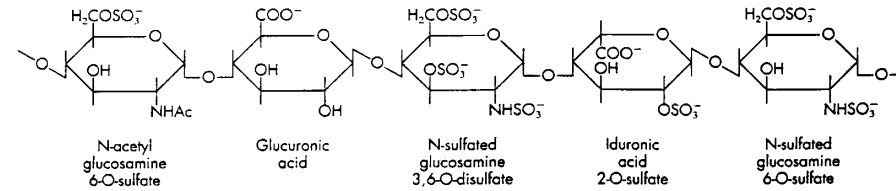


Competitive inhibitors to HS



Anti-angiogenic, anti-metastatic effect

Heparins as anti-tumor drugs



The antithrombin-binding structure of heparin.

A recently published Cochrane analysis assessed the efficacy and safety of heparins in ambulatory cancer patients.

The 18 randomized controlled trials (RCTs) included in the analysis, enrolled 9575 participants. Either UFH or LMWH was used.

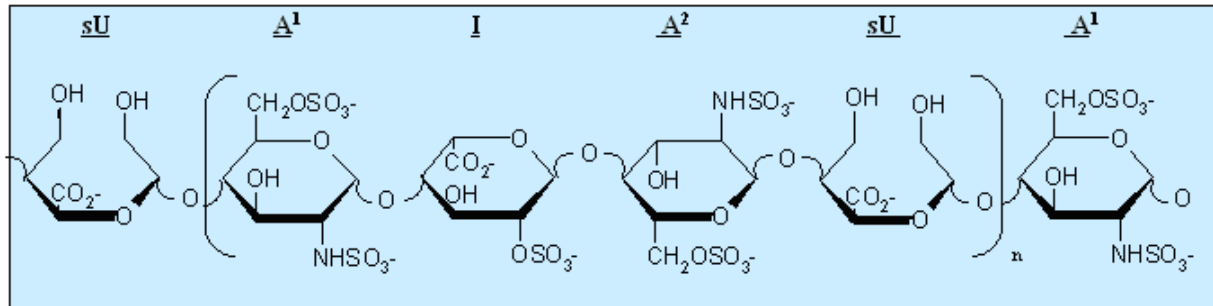
Findings:

No effect on 12-month and 24-month mortality, with a decrease in VTE along with an increase in minor and major bleedings.

Effect on survival in subgroup of non-advanced patients.

Akl et al, 2017

Glicol-split heparin derivatives



Weak anticoagulant activity.

Strong inhibitor to heparanase and releases VEGF and FGF from ECM.

Inhibits metastasis in animal models.

SST0001 - phase I study in advanced multiple myeloma

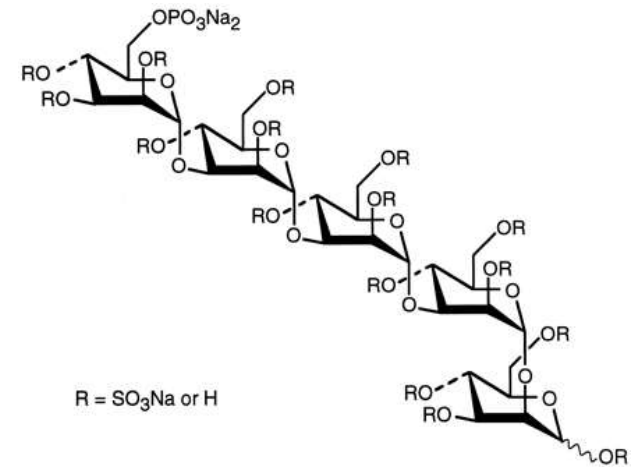
Heparin mimetic PI-88

Phase III study in HCC completed.

Phase II study in advanced melanoma completed.

No effect on survival

Side effects: bleeding, HIT, hepatotoxicity



PI-88 Phosphomannopentaose SO₄

Heparanase under physiological conditions

Heparanase levels are highest in the:

Placenta

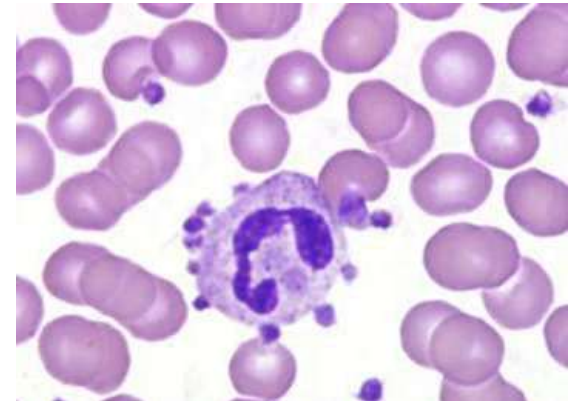
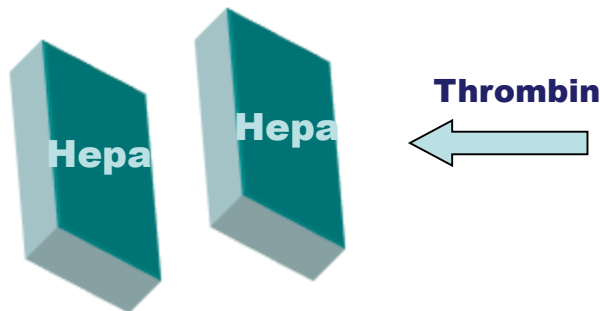
Platelets

Neutrophils

Monocytes

Thrombin is a selective inducer of heparanase release from platelets and granulocytes *via* protease-activated receptor-1

Tatour, Shapira *et al*, Thrombo Haemost, 2017

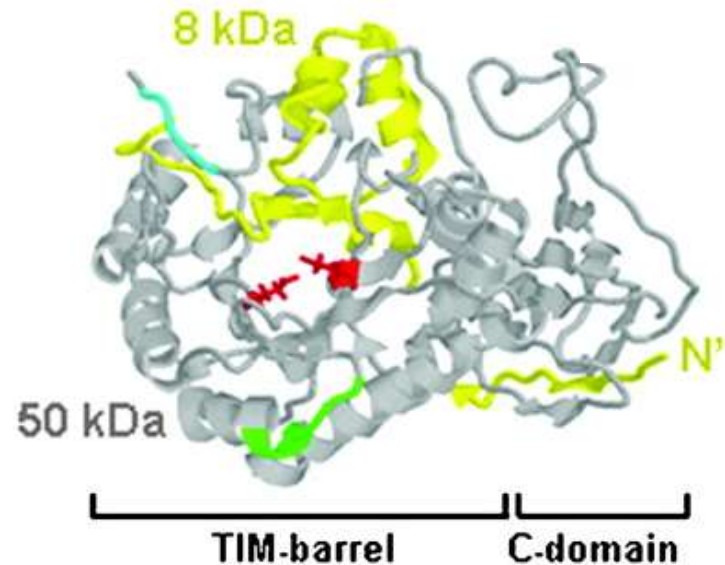


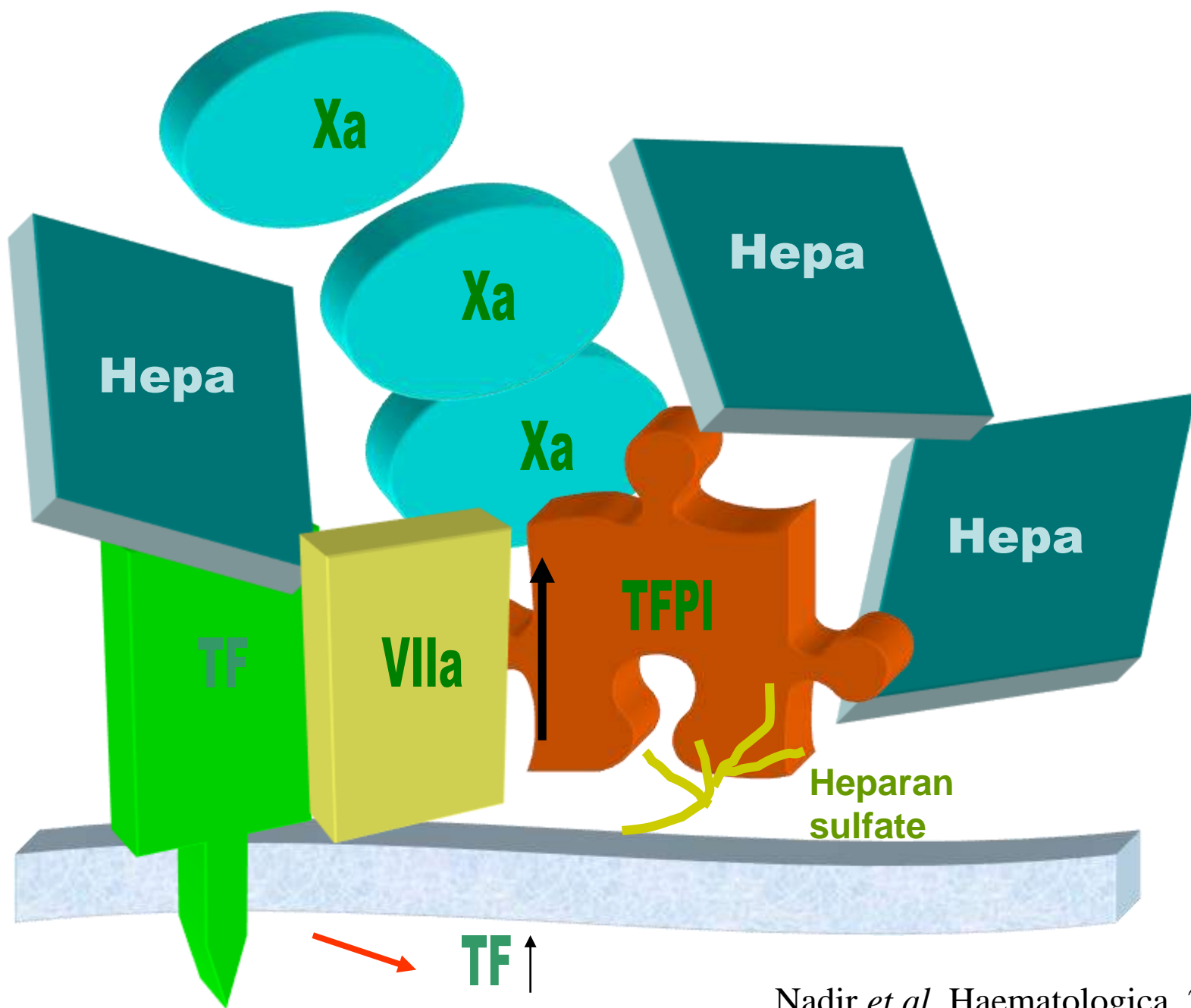
Heparanase enhances:

Angiogenesis

Inflammation

Cancer





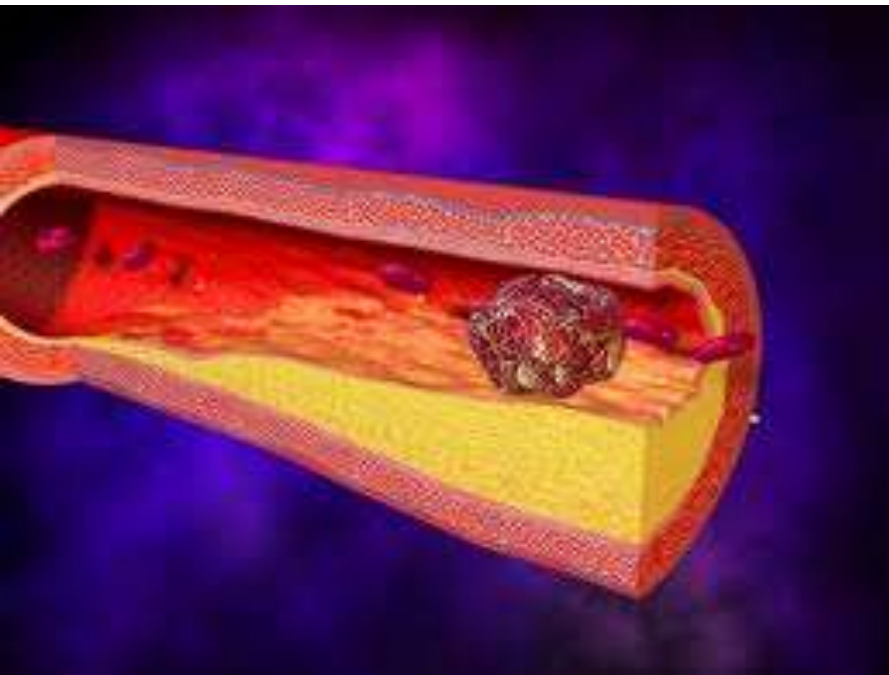
Nadir *et al*, Haematologica, 2010

Heparanase regulates thrombosis in vascular injury and stent-induced flow disturbance

[Baker AB](#), [Gibson WJ](#), [Kolachalama VB](#), [Golomb M](#), [Indolfi L](#), [Spruell C](#), [Zcharia E](#), [Vlodavsky I](#), [Edelman ER](#).

Department of Biomedical Engineering, University of Texas at Austin, Austin, Texas

J Am Coll Cardiol, 4/2012



An assay to evaluate heparanase procoagulant activity

Heparanase procoagulant activity is significantly elevated in hypercoagulable clinical set-ups:

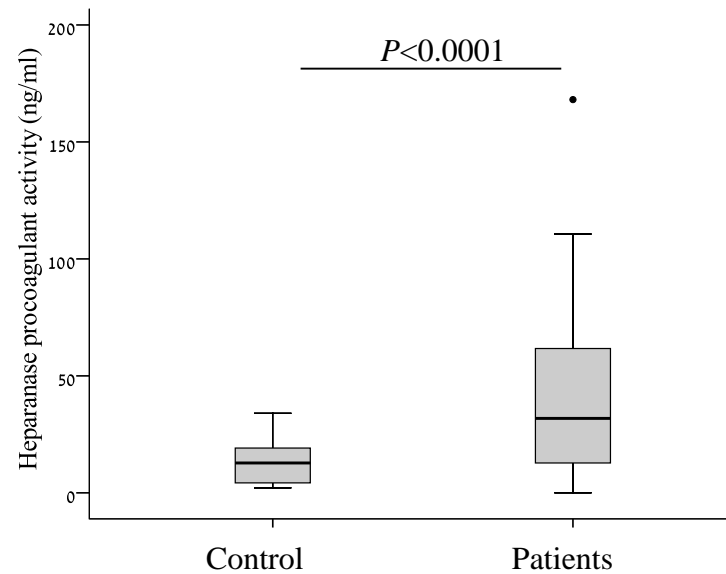
1. End of pregnancy (Thromb Res, 2011)
2. Following orthopedic surgery (Thromb Res, 2012)
3. Oral contraceptive use (Hum Reproduction, 2013)
4. Patients with lung cancer (Thromb Res, 2014)
5. Shift work *vs.* day work female nurses (Ann Hematol, 2015)
6. Patients with diabetic foot (Thromb Res, 2016)



Heparanase procoagulant activity is elevated in lung cancer patients at presentation

	Patients (n=65)	Controls (n=20)	p
Age	65 ± 10	63 ± 12	NS
Males (%)	77%	75%	NS
Anti-platelet therapy	23%	20%	NS
Current smoking	82%	65%	p=0.05
Thrombotic event in the past	3/65	1/20	NS

Heparanase procoagulant activity is elevated in lung cancer patients at presentation

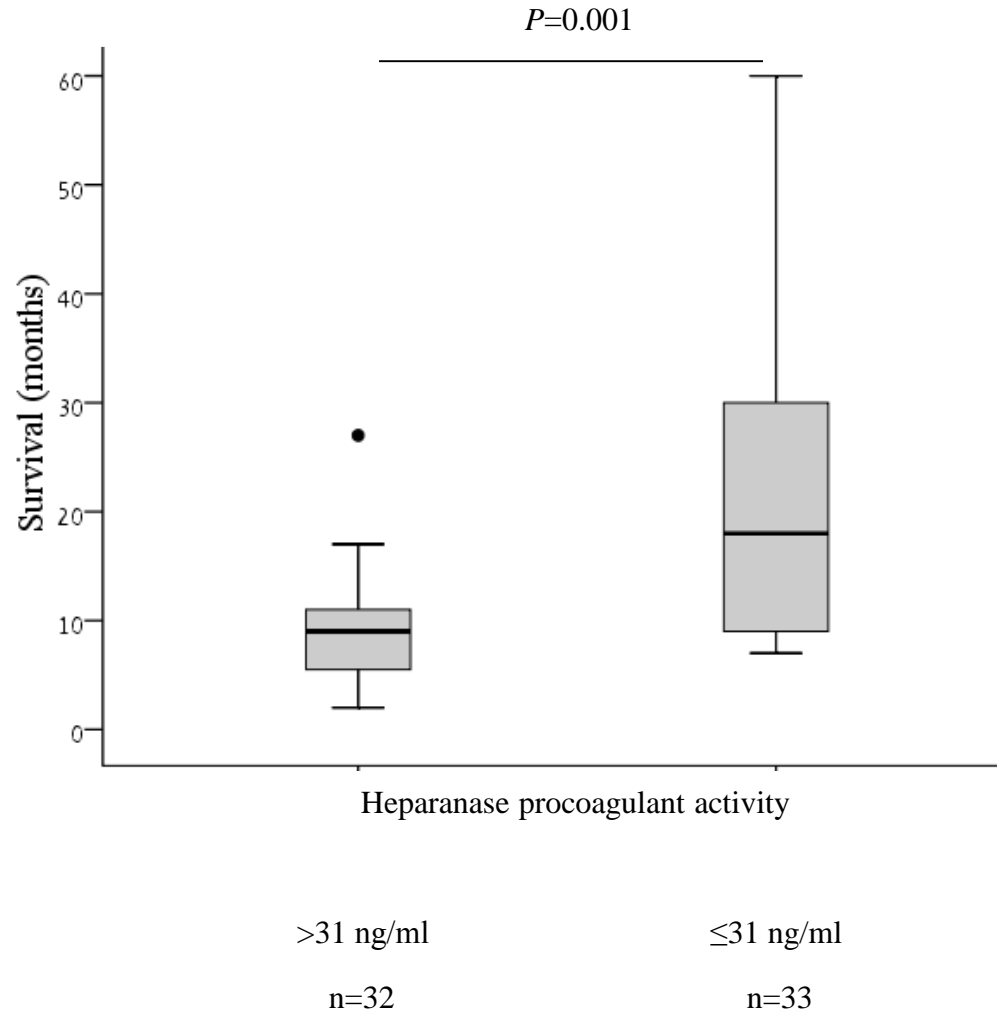


Heparanase procoagulant activity is elevated in lung cancer patients at presentation

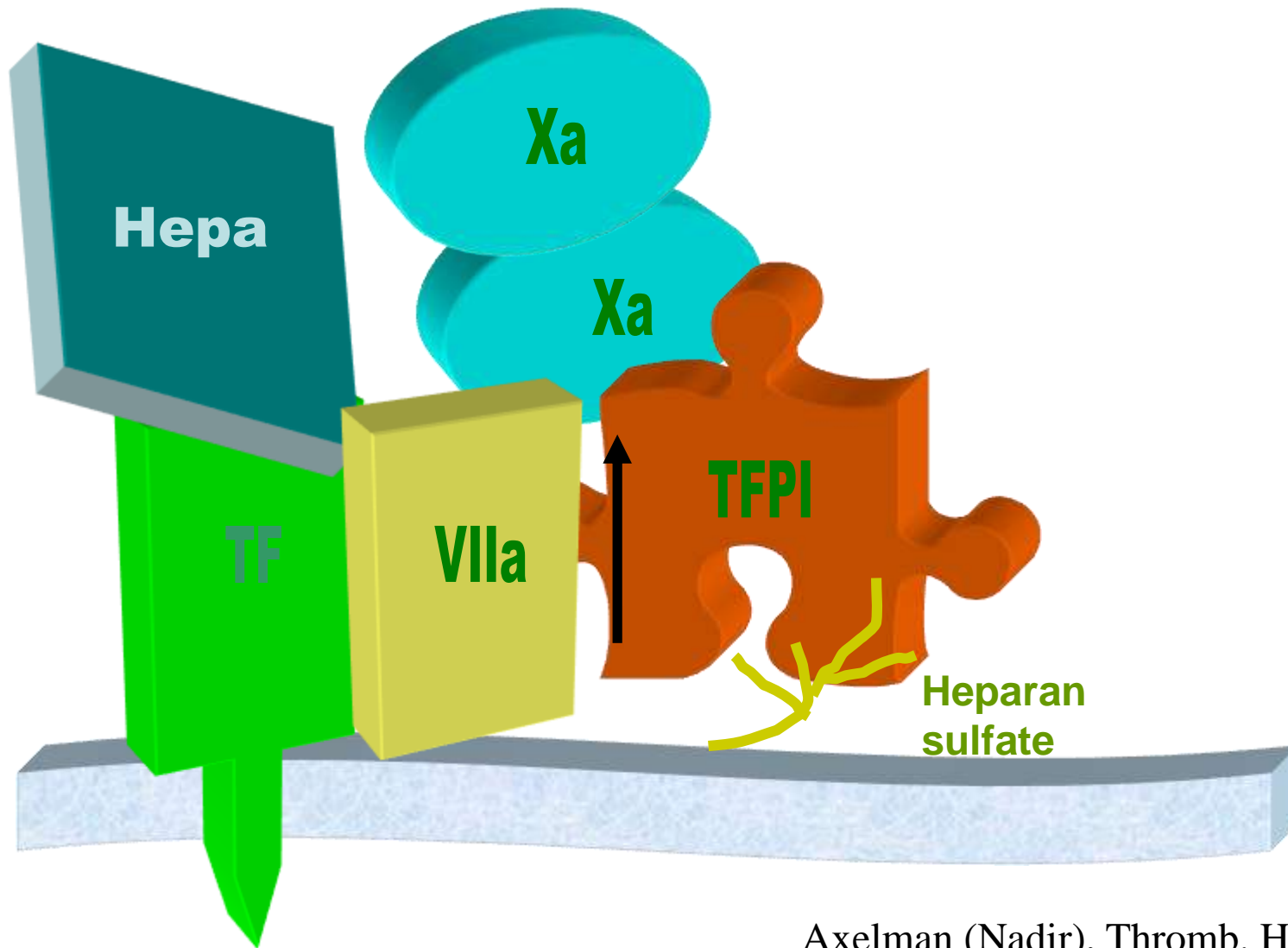
n=65

Type of lung cancer	NSCLC (Adeno - 32%, squamouse - 25%)
Thrombotic events	5 (2 subclavian, 1 PE, 1 CVA, 1 leg ischemia)
Survival (months)	15.5 \pm 13.5, 2 patients survived beyond 48 months
Stage 3A-4 at presentation	82%

Low heparanase procoagulant activity at presentation predicts longer survival

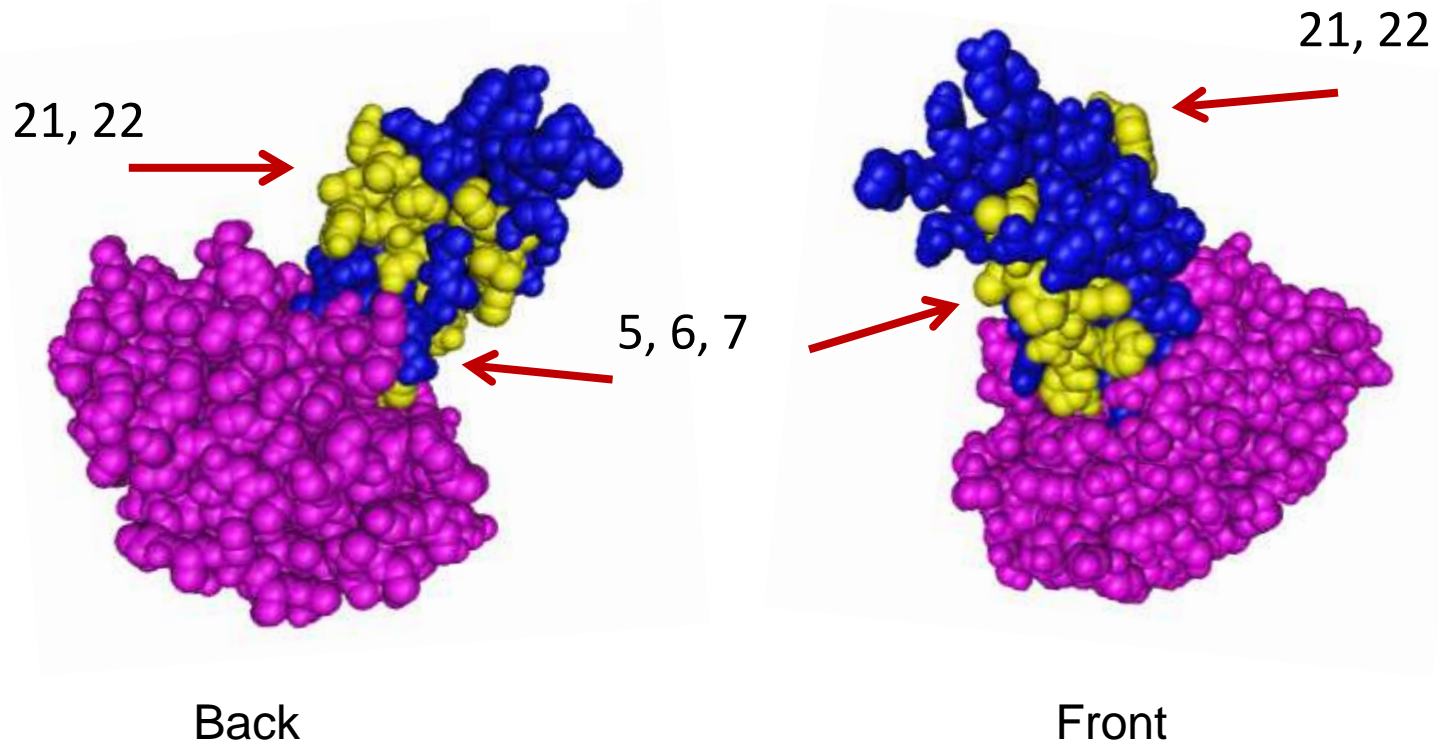


Novel inhibitors of heparanase procoagulant effect



Novel inhibitors of heparanase procoagulant effect

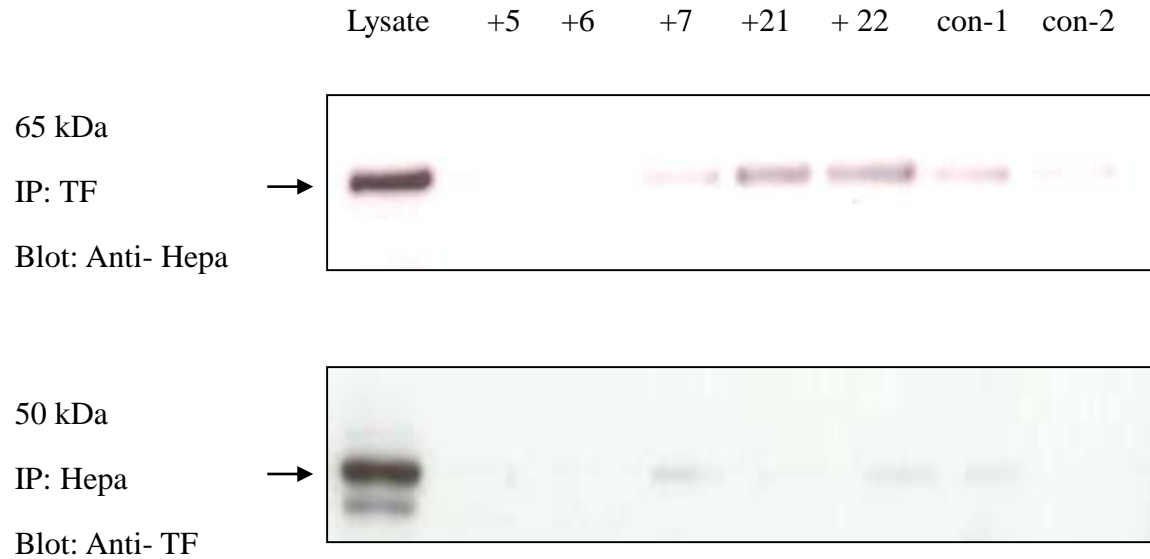
TFPI-2



Peptide 5	amino acids	35-48	ICLLPLDYGPCRAL
Peptide 6	amino acids	33-46	AEICLLPLDYGPCR
Peptide 7	amino acids	31-44	NNAEICLLPLDYGP
Peptide 21	amino acids	72-82	NANNFYTWEAC
Peptide 22	amino acids	74-87	NNFYTWEACDDACW

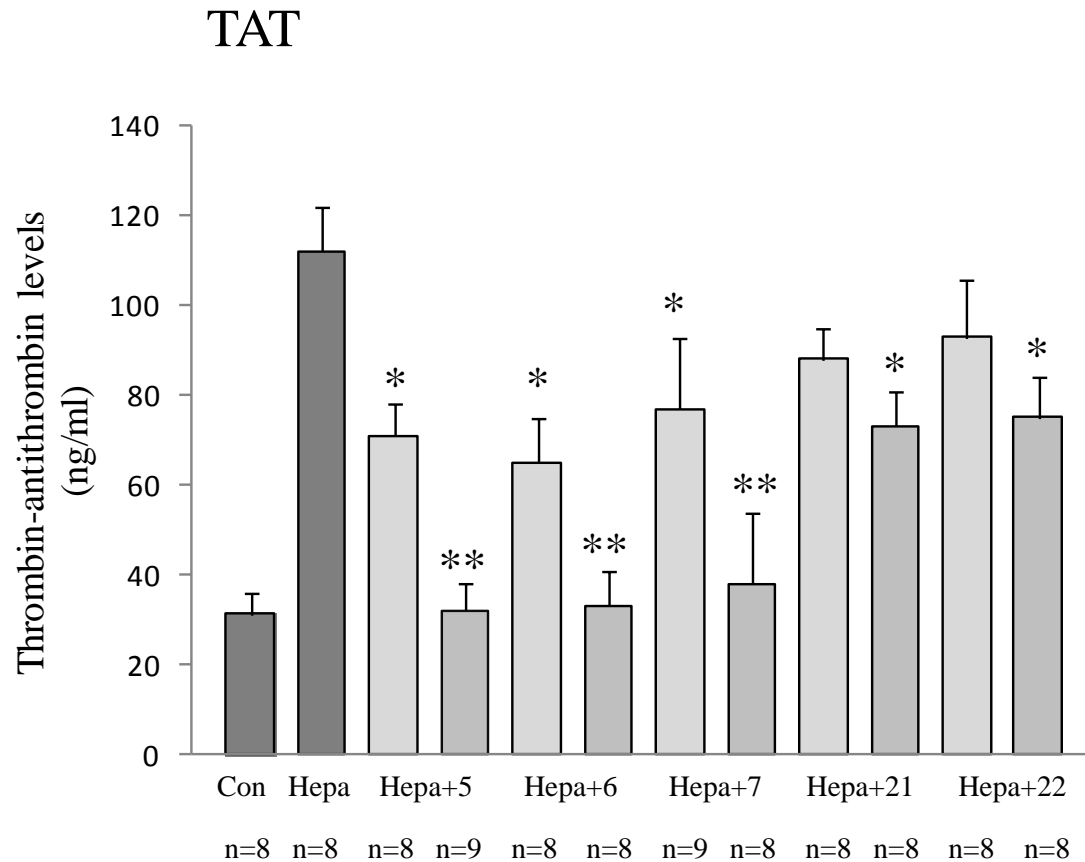
Axelmann (Nadir), Thromb. Haemost., 2014

Novel inhibitory peptides in CO-IP



Peptides inhibit TF – heparanase complex

Inhibitory peptides in a mouse model

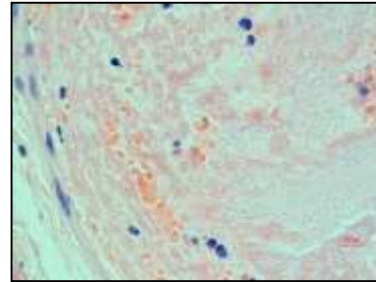
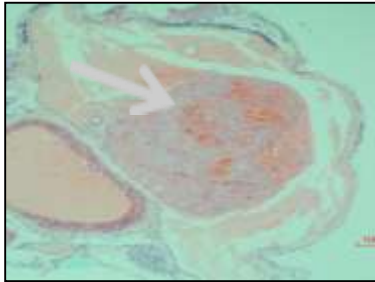


* $p < 0.05$, ** $p < 0.001$

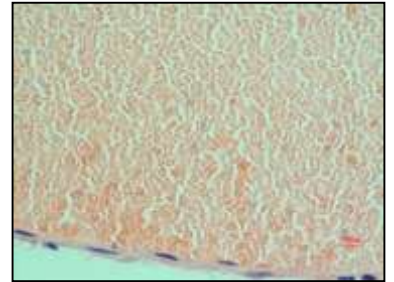
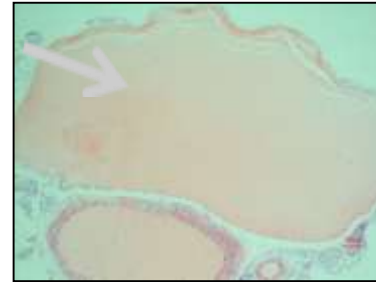
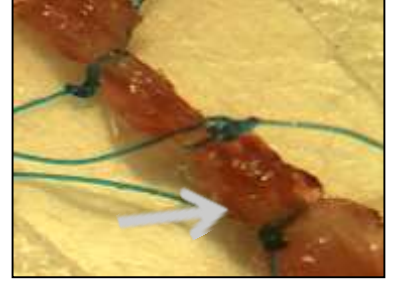
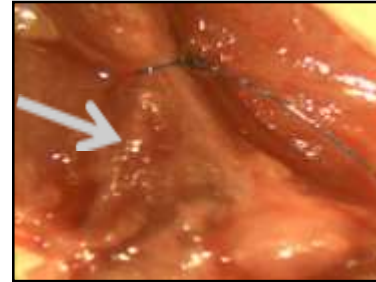
ICR mice



IVC thrombosis model

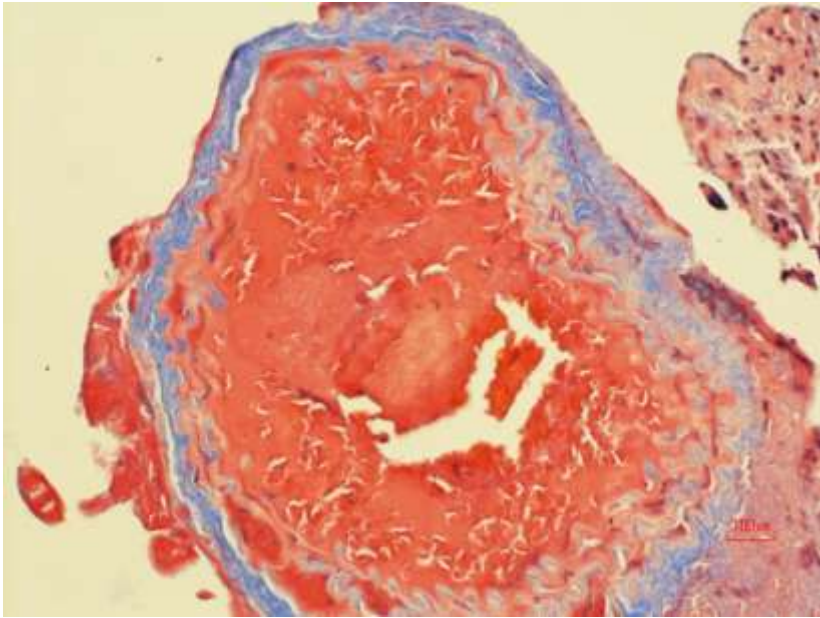


Control

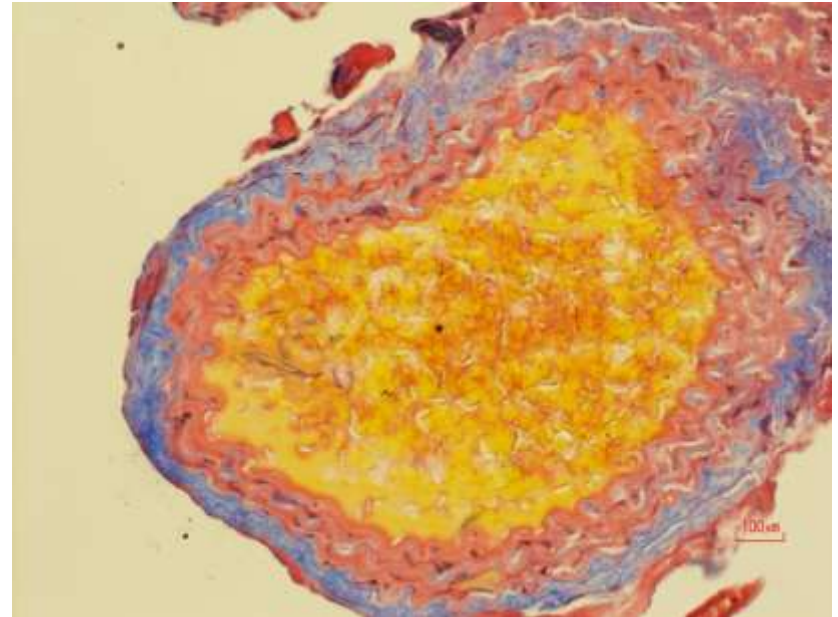


With inhibitory peptide

Carotid thrombosis model



Control



With inhibitory peptide

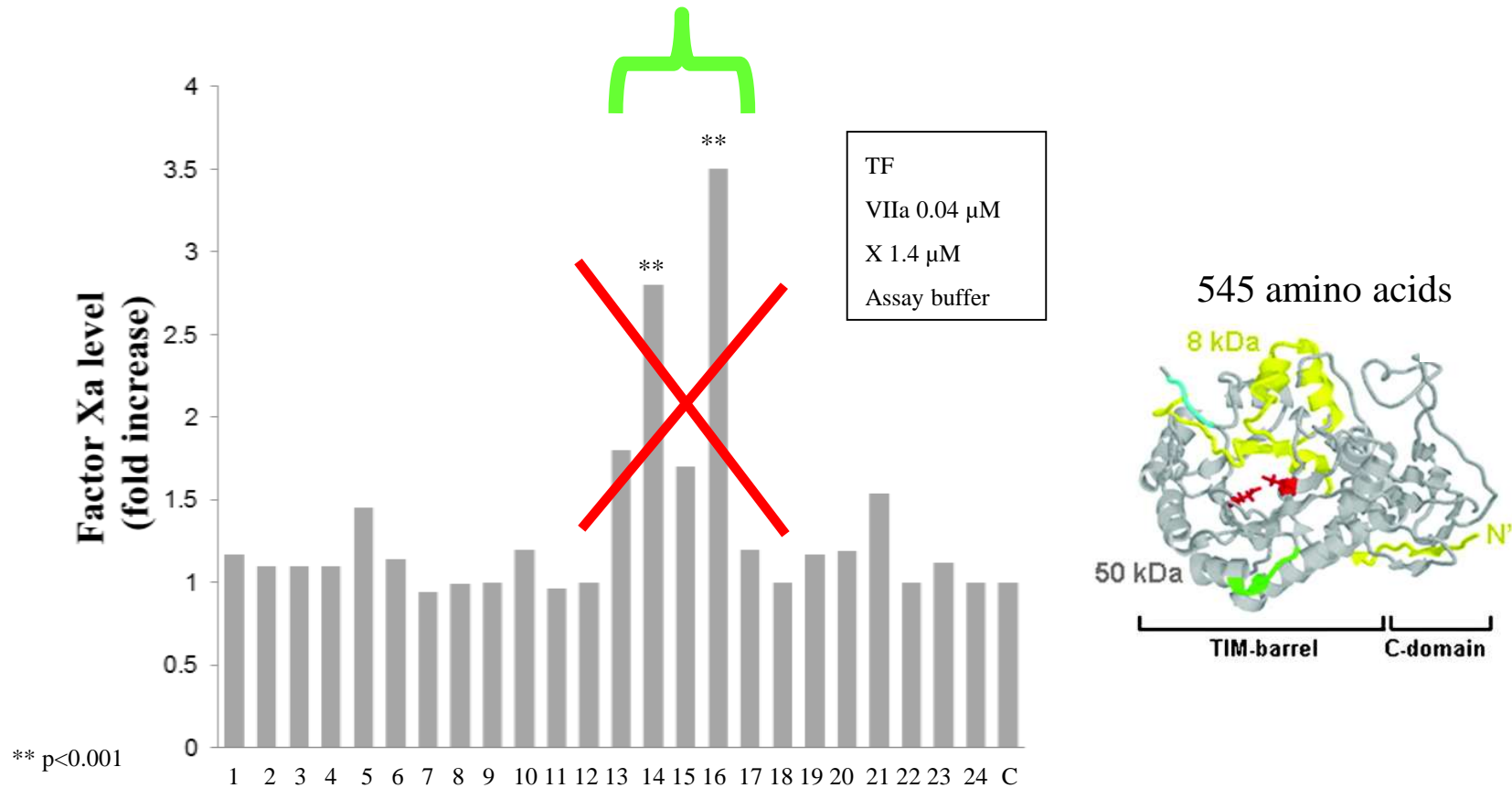
MSB staining

Red – early thrombus

Yellow – red blood cells

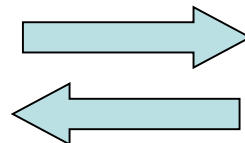
Involvement of heparanase procoagulant domain in bleeding and wound healing

Crispel *et al*, JTH, 2017



Heparanase procoagulat peptides

Heparanase derived



Heparanase procoagulant
inhibitory peptides

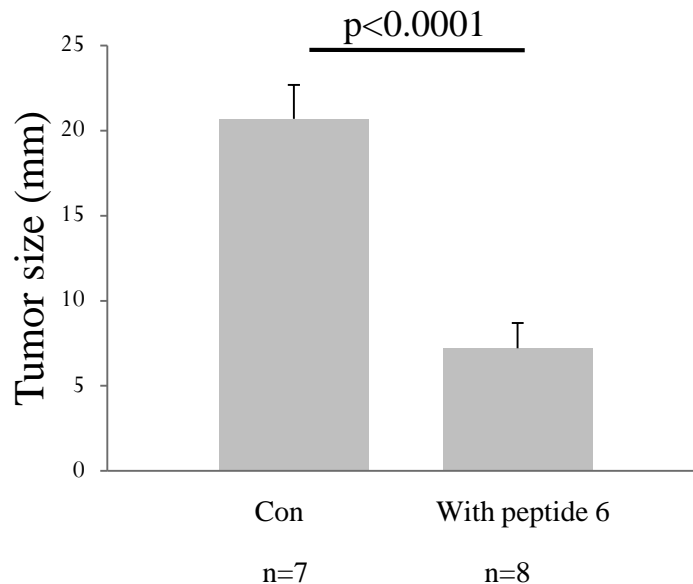
TFPI-2 derived

Inhibitory peptides in a mouse tumor model



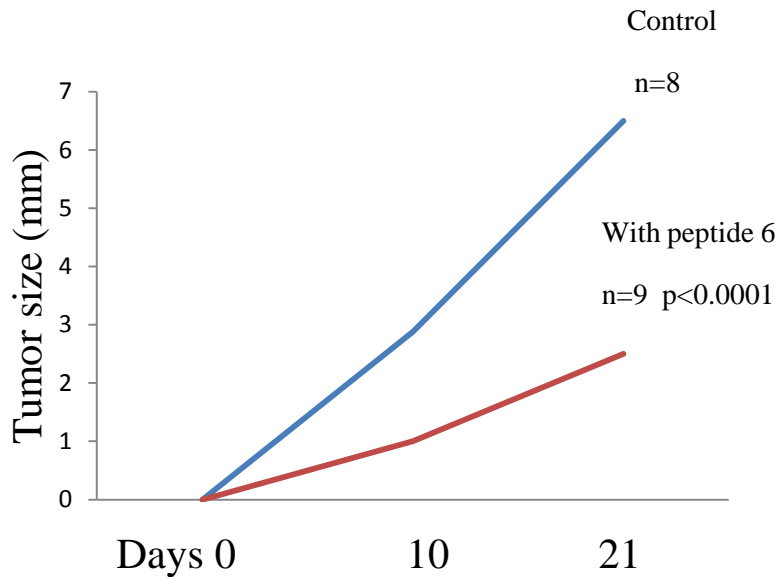
B16 melanoma
Peptides 5,6,7
3 mg/kg

C57BL/6 mice



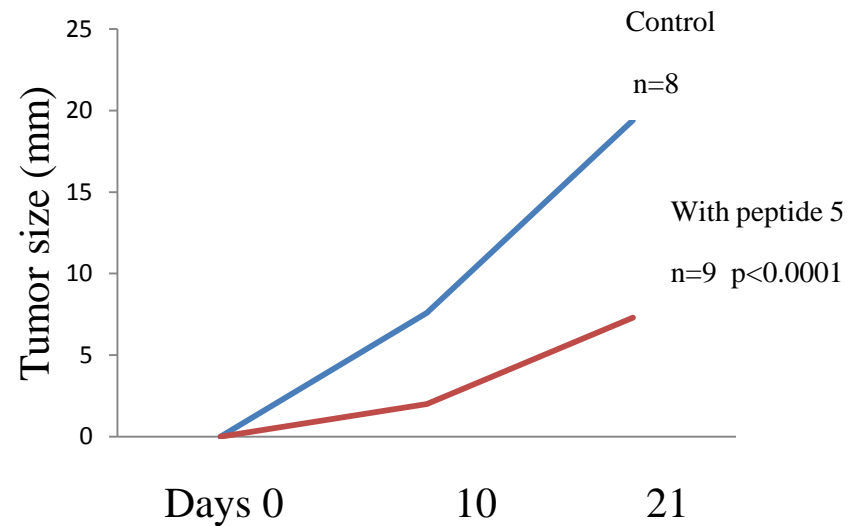
Crispel (Nadir) Thromb. Haemost., 2016

Inhibitory peptides in a mouse tumor model



231-MDA human breast Ca
Peptides 5,6,7
3 mg/kg

SCID mice

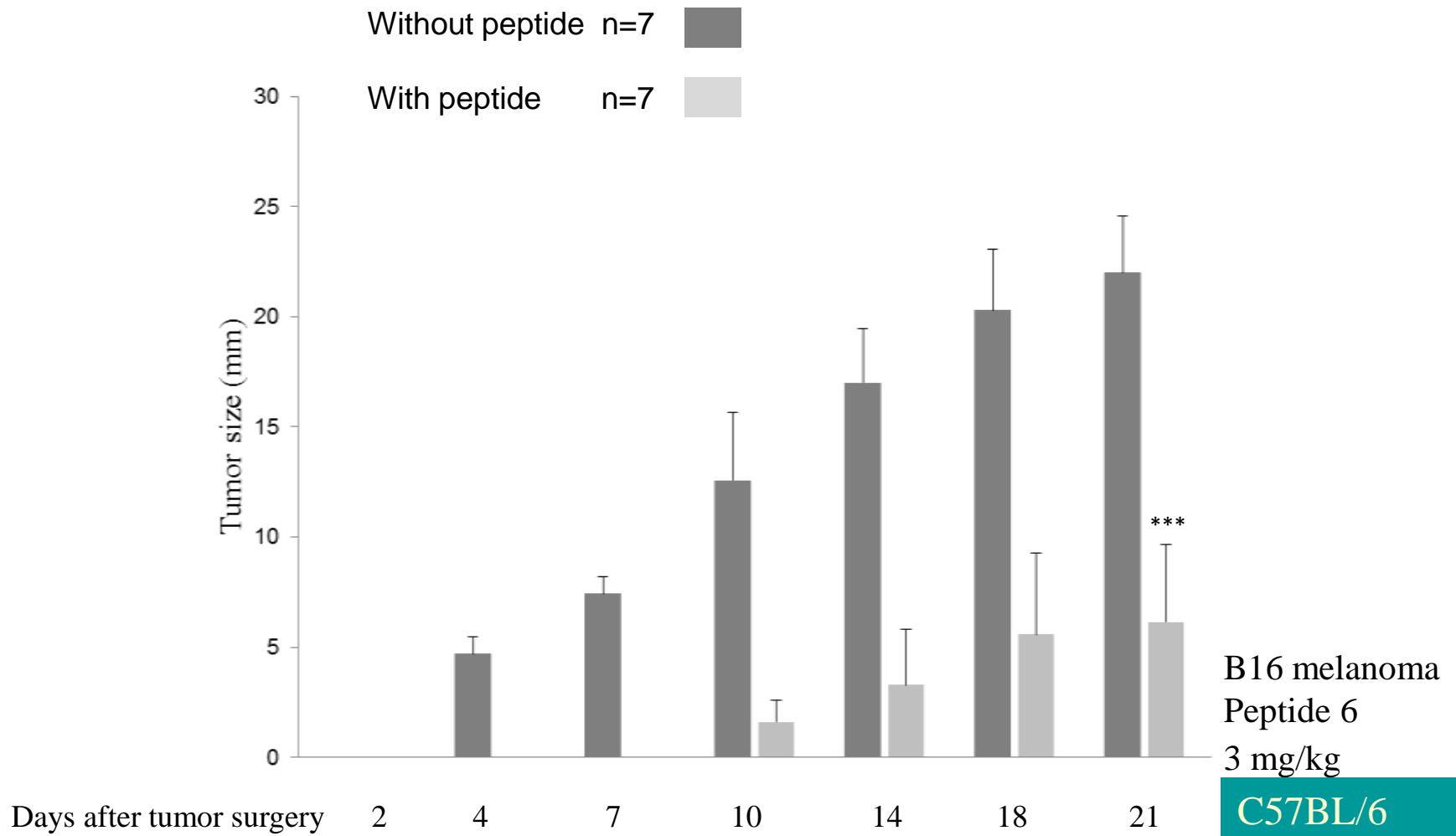


EMT6 mouse breast
Peptides 5,6,7
3 mg/kg

BALB/c mice



Effect of peptides on cancer relapse

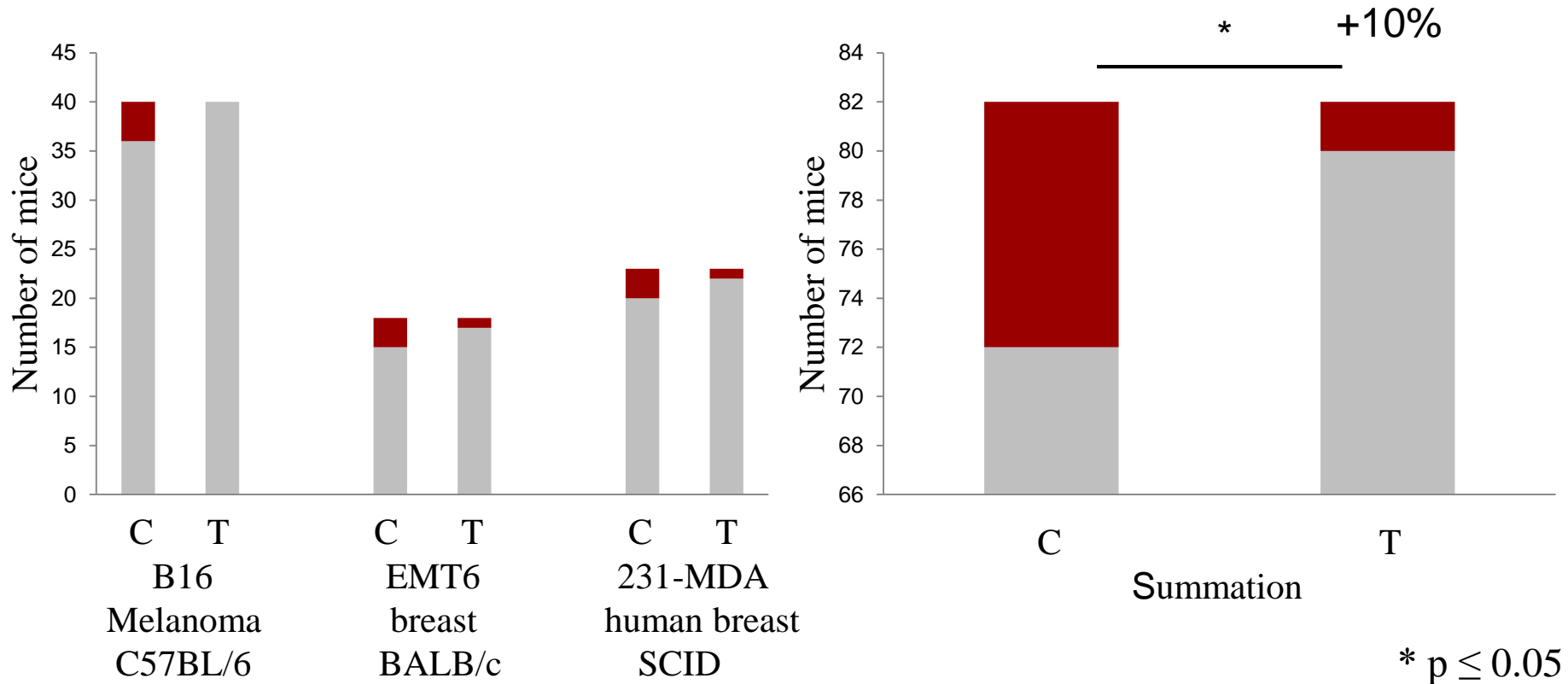


At day 21
7/7 non-treated group developed tumors
2/7 in treated group - no relapse
***p<0.0005

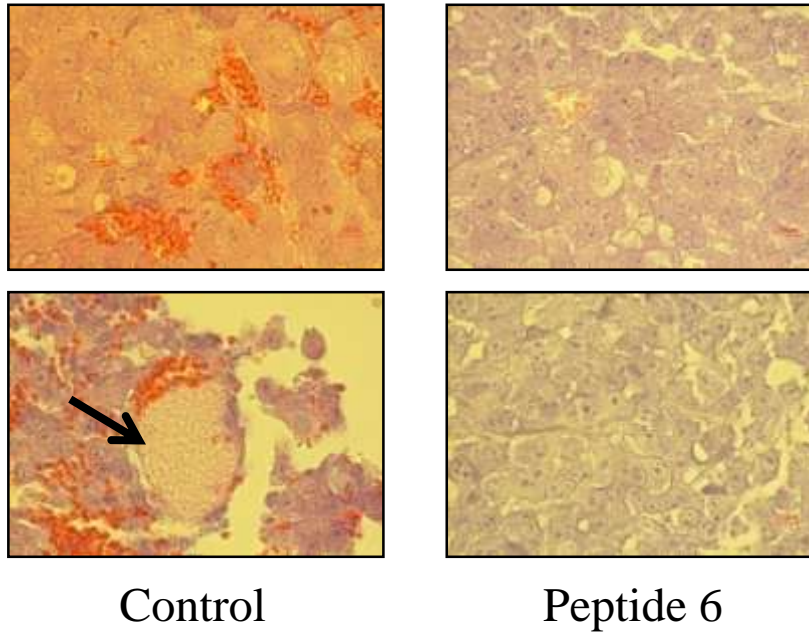


Effect of peptides on survival

Number of mice at the end of the 3 weeks experiments



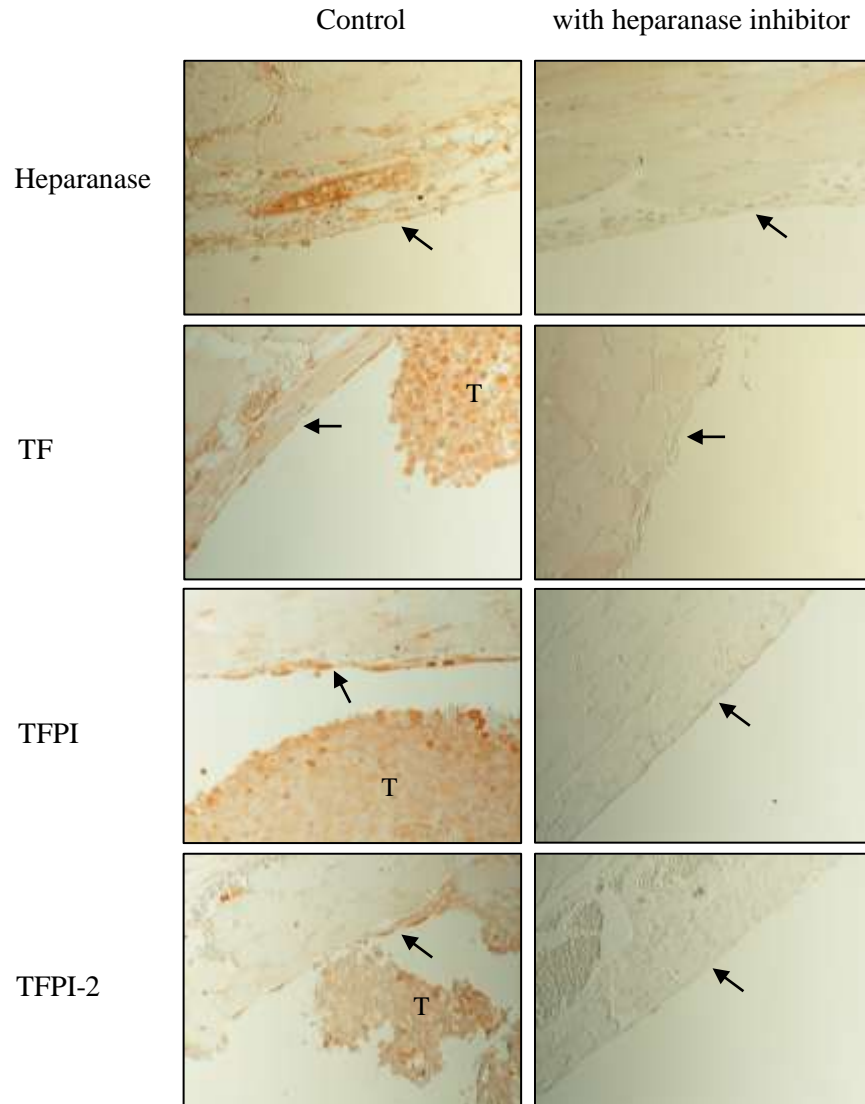
Tumors with the peptide are less vascular and with no thrombi



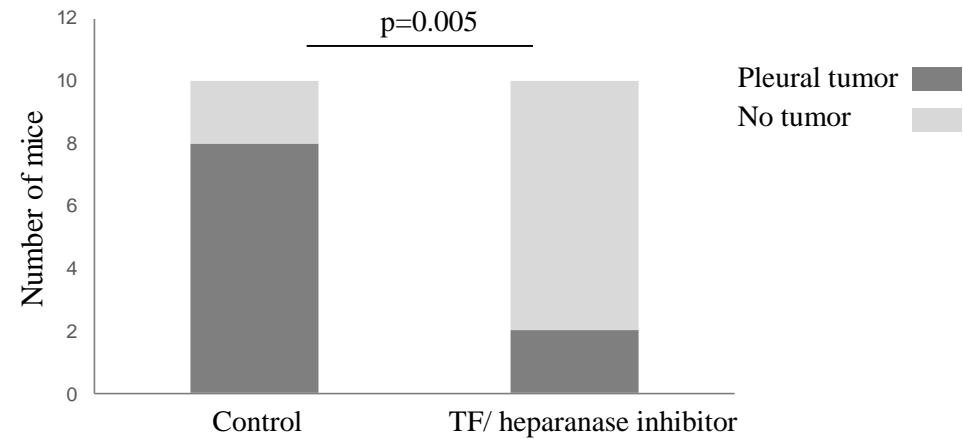
Staining by H&E x10 magnitude

No hemorrhages or metastasis were detected in the organs of both groups

Heparanase / TF inhibition reduces intra-pleural tumor growth

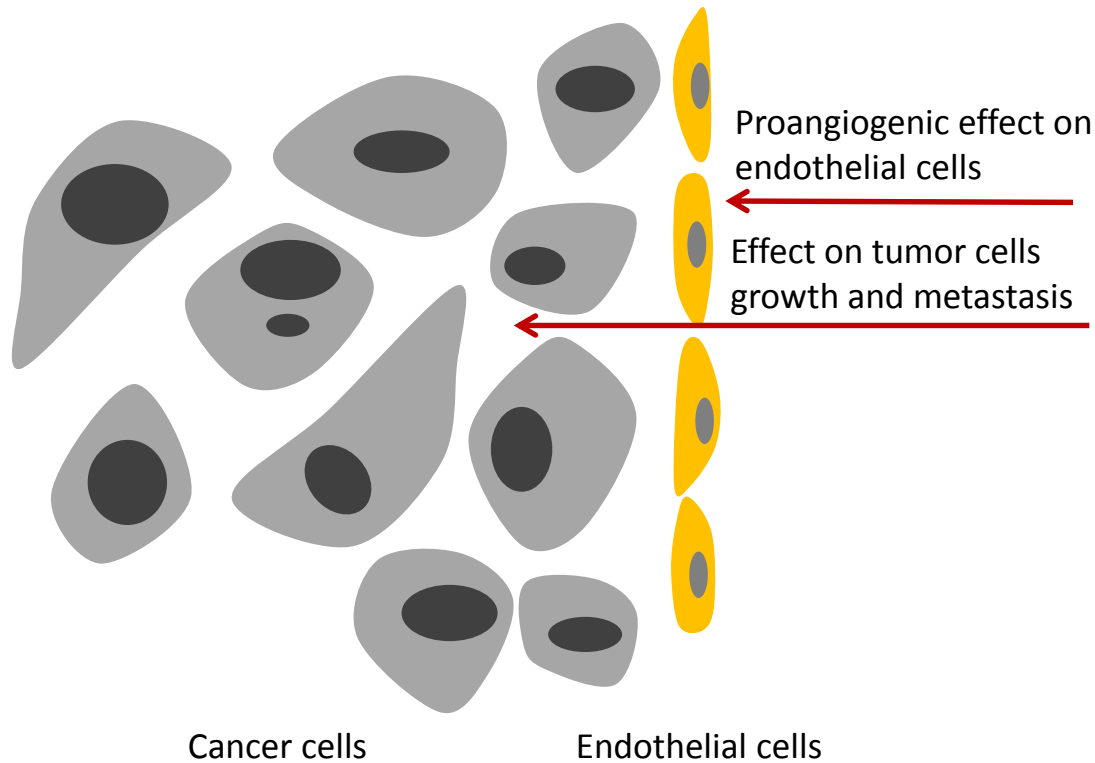


EMT6 breast carcinoma cells



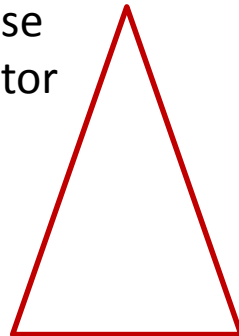
Hardak *et al*, Unpublished data

Heparanase inhibitory peptides in cancer

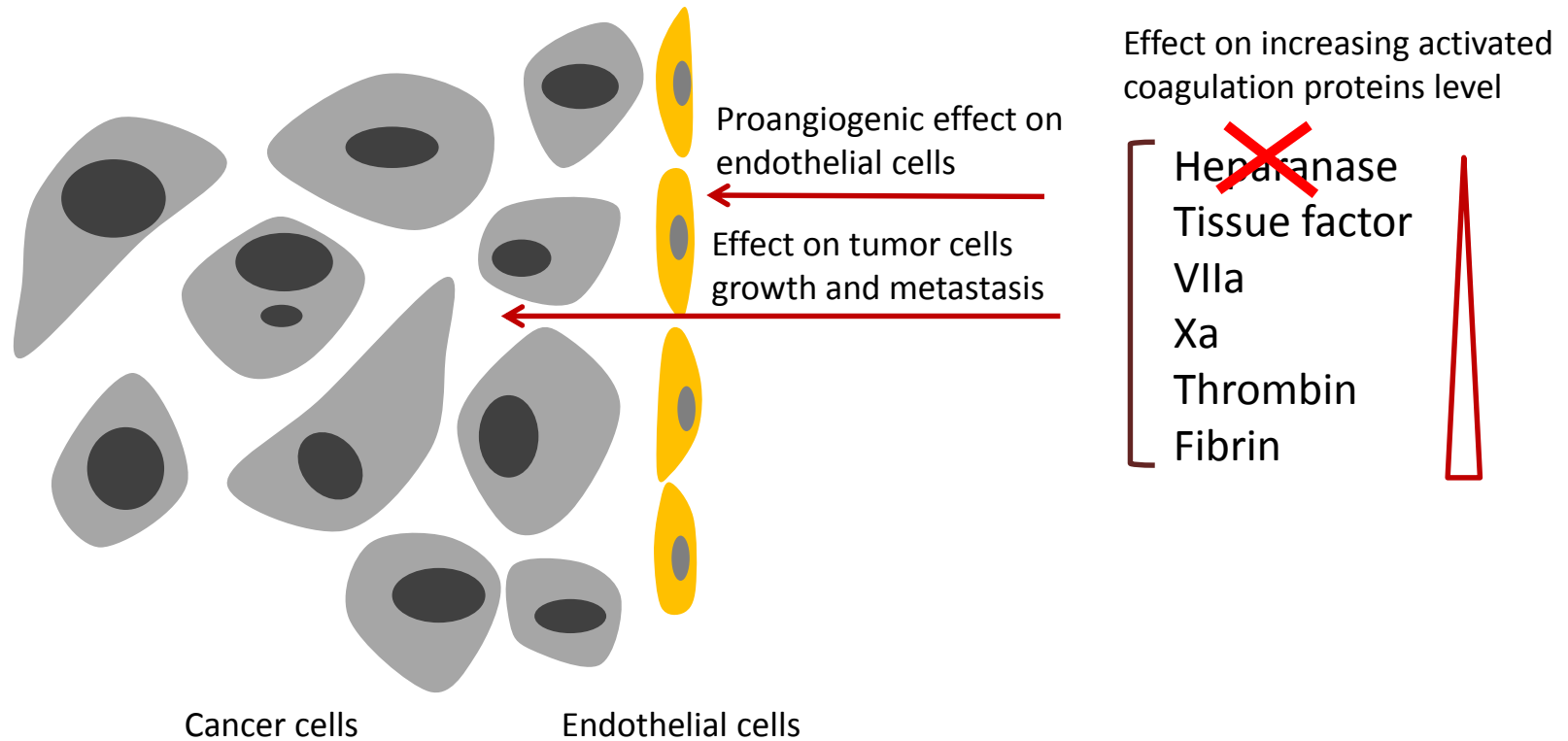


Effect on increasing activated coagulation proteins level

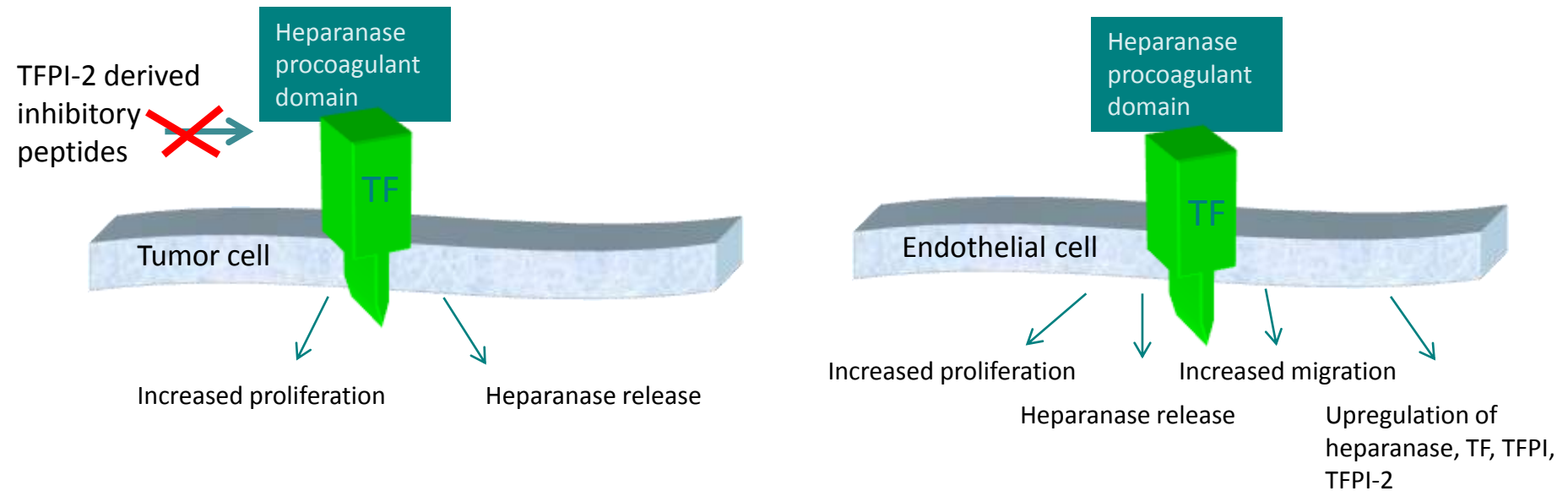
Heparanase
Tissue factor
VIIa
Xa
Thrombin
Fibrin



Heparanase inhibitory peptides in cancer



Non hemostatic effects of heparanase procoagulant domain



Conclusions

An ideal anticoagulant drug to inhibit cancer growth

- ✓ A weak anticoagulant effect
- ✓ Involved at the beginning of the coagulation cascade
- ✓ Has a dominant anti-tumor effect

Heparin mimetics?

Heparanase / TF inhibitory peptides?

THANK YOU



Cancer and Coagulation Lab

Shourouk Ghanem (MS student)

Mifleh Tatour (MS student)

Dr. Inna Kogan (Post doc)

Dr. Yonatan Crispel (Post doc)

Dr Emilia Hardak (Associate member in CRIR)

Prof. Eli Peled (Associate member in CRIR)

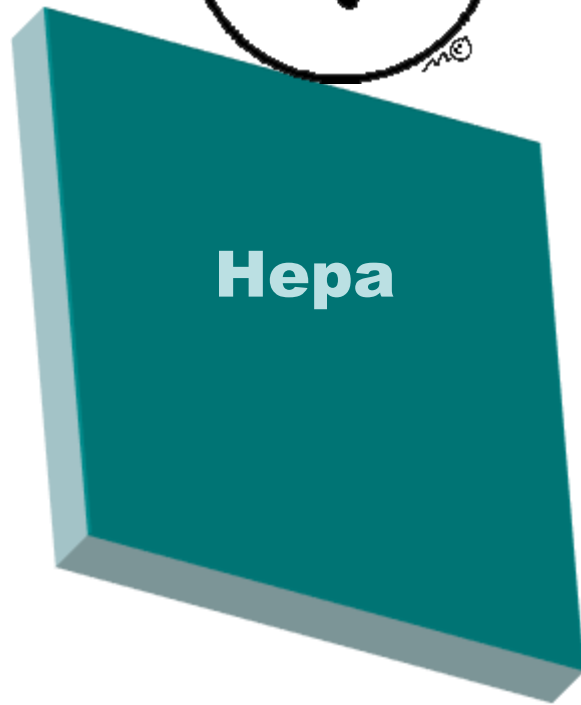
Dr. Neta Nevo (Fellowship)

Prof. Benjamin Brenner

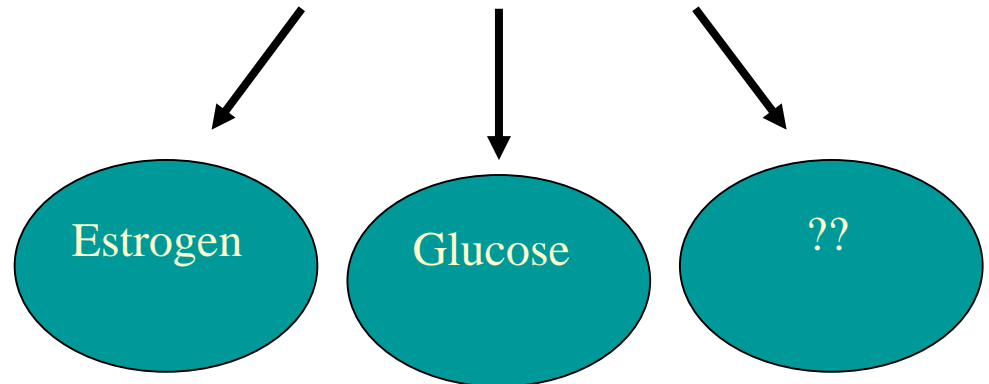
Prof. Israel Vlodavsky

Dr. Judith Attias

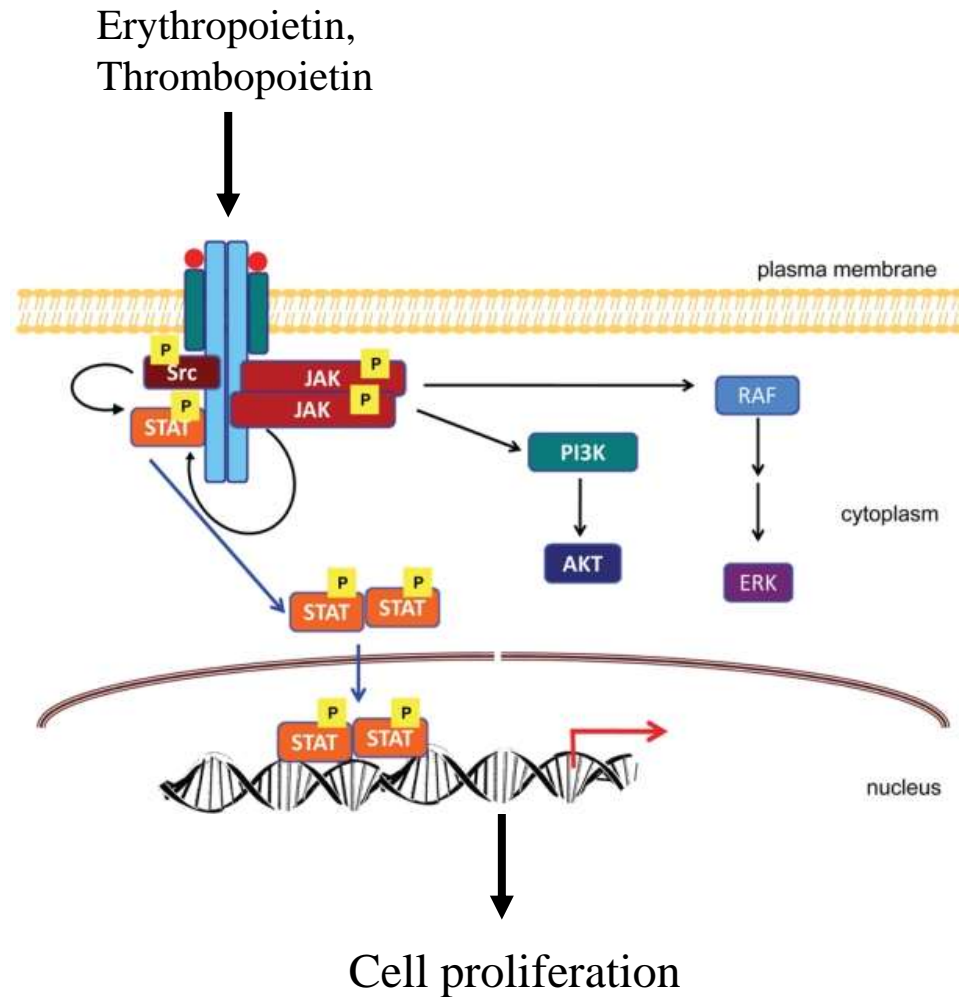
Dr. Neta Ilan



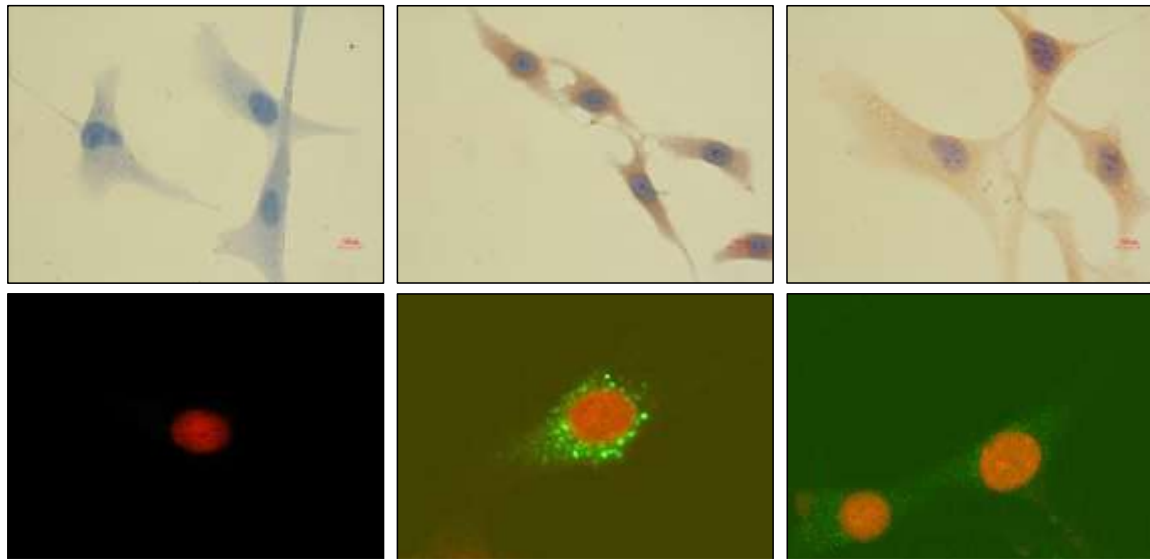
What increases heparanase?



JAK-2 activation and heparanase



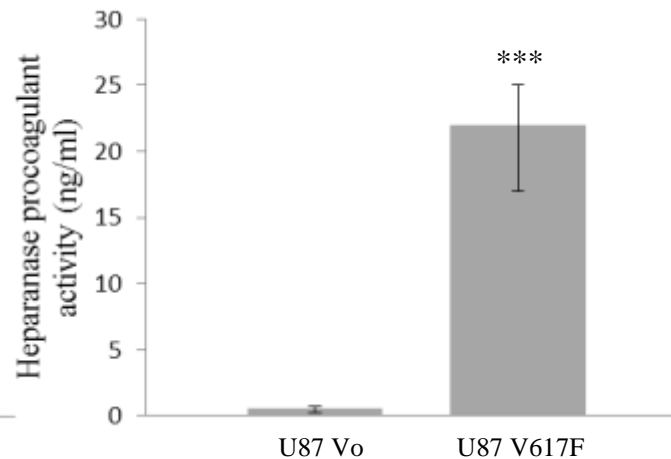
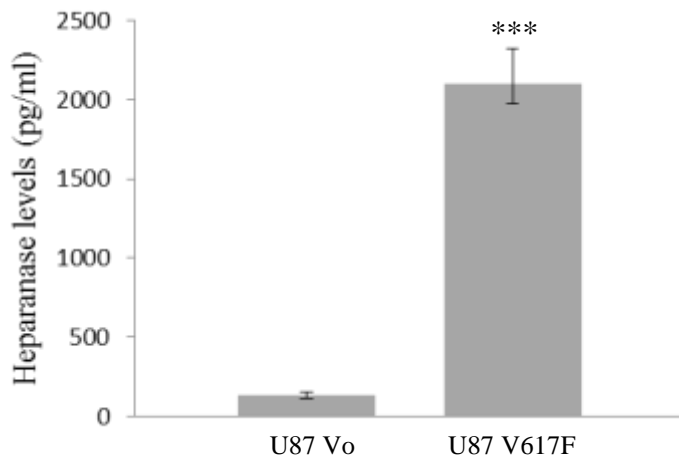
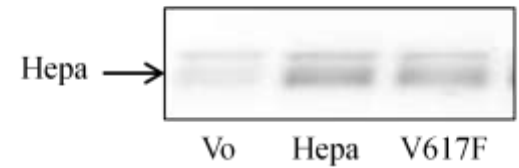
Heparanase protein level and procoagulant activity are elevated in JAK-2 V617F cells



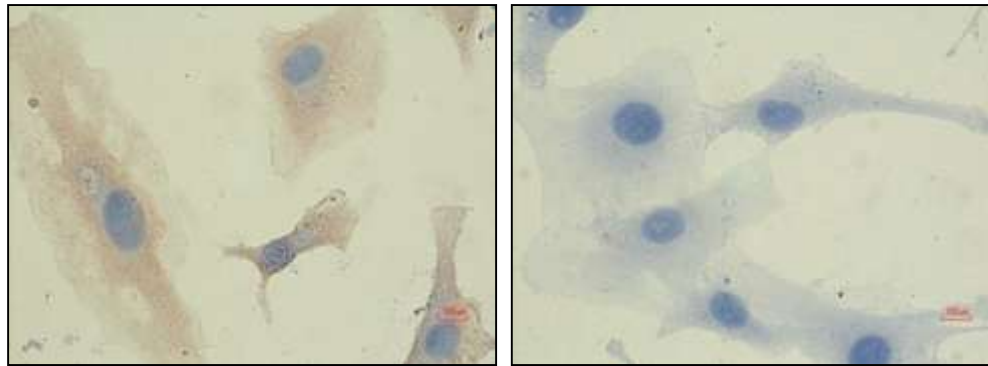
U87 Vo

U87 Hepa

U87 V617F

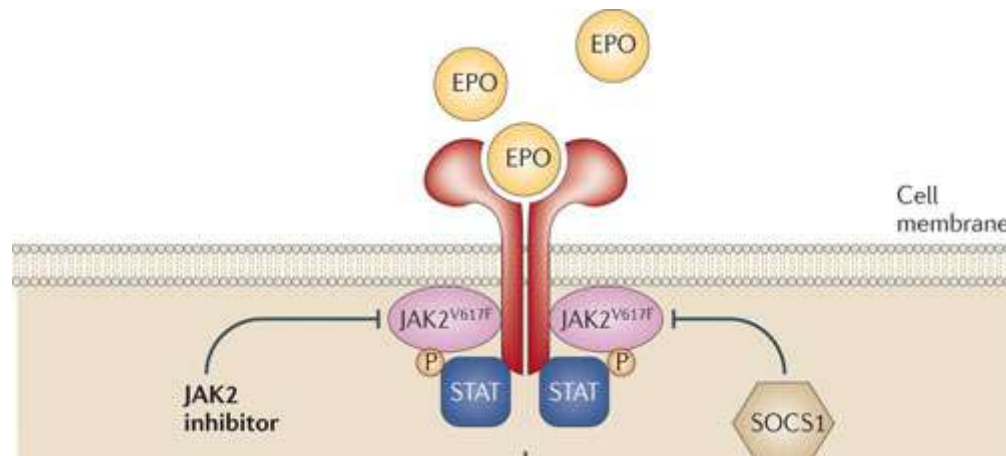


JAK-2 inhibitor reduces heparanase protein level and procoagulant activity

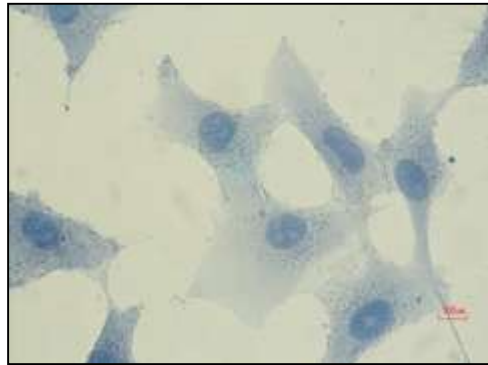


U87 V617F

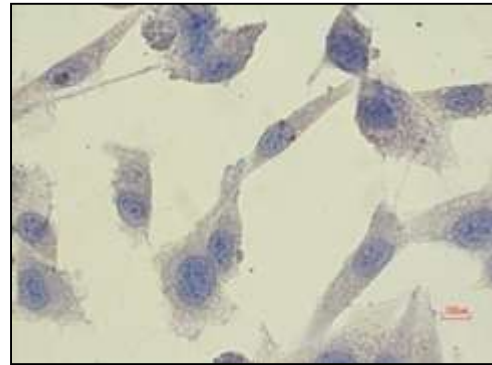
U87V617F + Ruxolitinib



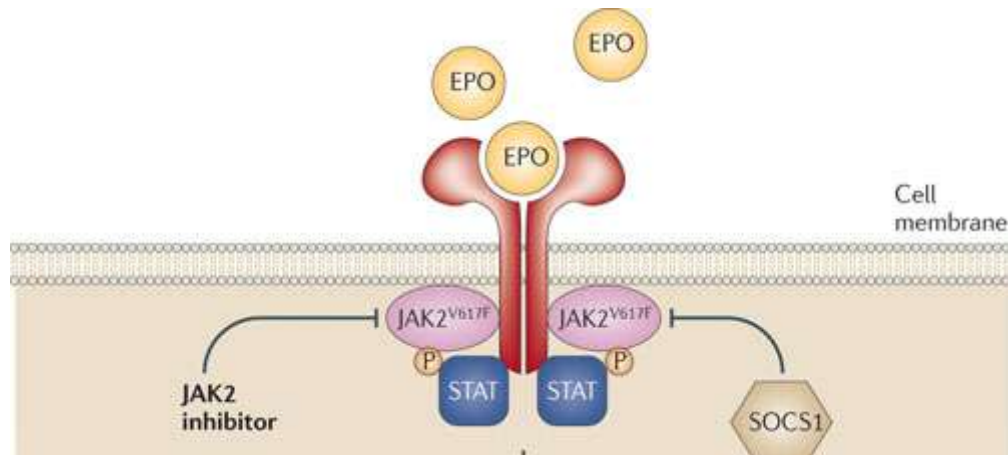
JAK-2 activator- erythropoietin increases heparanase level and procoagulant activity



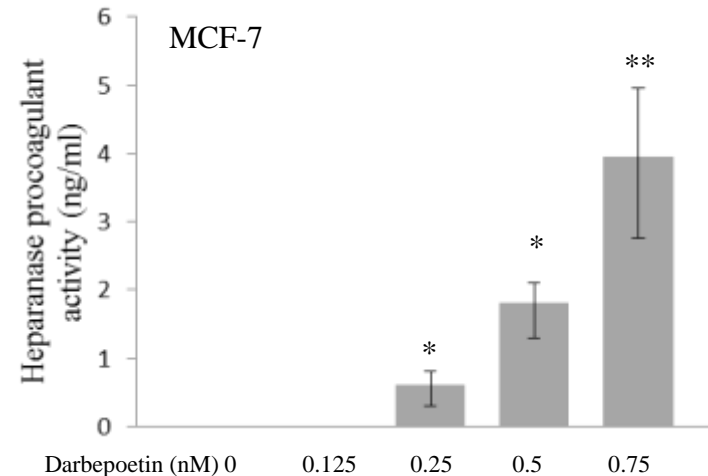
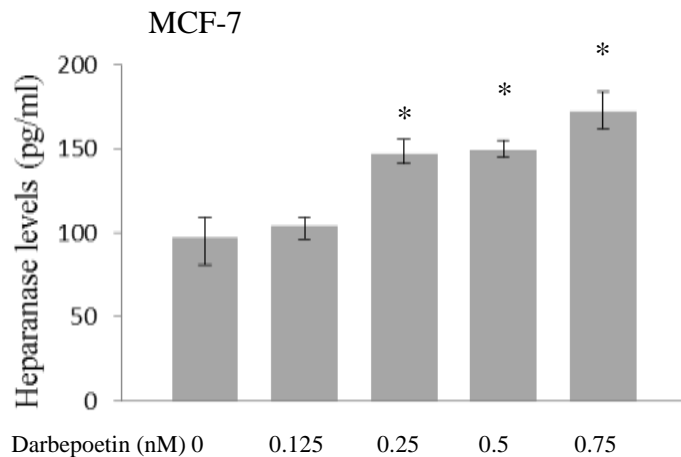
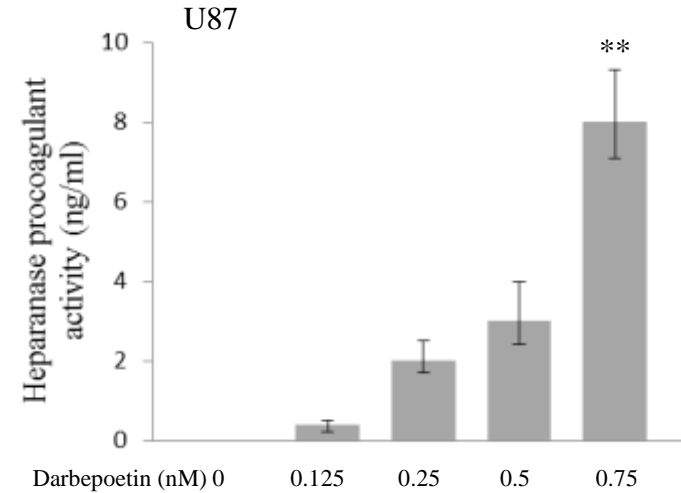
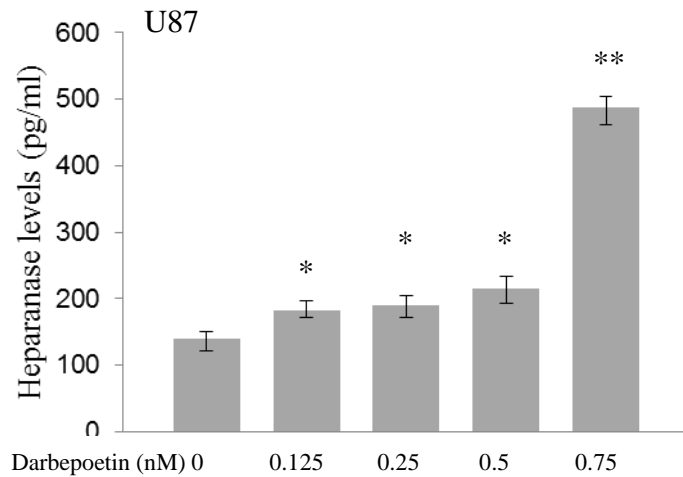
U87



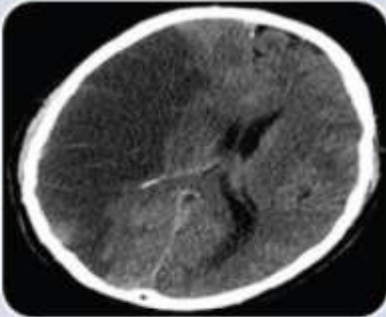


U87 + Darbepoietin



JAK-2 activator- erythropoietin increases heparanase level and procoagulant activity



Thrombosis is the major cause of morbidity and mortality in MPD patients

		
Arterial thrombosis <ul style="list-style-type: none">• Myocardial infarction• Unstable angina• Ischemic stroke• Transient ischemic attack• Acute peripheral and visceral thromboembolism	Venous thrombosis <ul style="list-style-type: none">• Deep venous thrombosis (legs and arms)• Pulmonary embolism• Unusual sites venous thrombosis (visceral vein thrombosis and cerebral sinus and venous thrombosis).• Superficial venous thrombosis	Microcirculatory disturbances <ul style="list-style-type: none">• Erythromelalgia• Seizures• Migraine• Vertigo• Tinnitus• Scintillating scotomas• Amaurosis fugax

JAK-2 V617F and thrombosis

2905 ET patients

JAK2 V617F mutation was associated with an increased risk of both

Venous thrombosis (OR 2.09; 95% CI, 1.44-3.05)

Arterial thrombosis (OR 1.96; 95% CI, 1.43-2.67)

Thrombosis at presentation (OR 1.88; 95% CI, 1.38-2.56).

Ziakas et al, Haematologica, 2008

In PV and PMF correlation is less clear

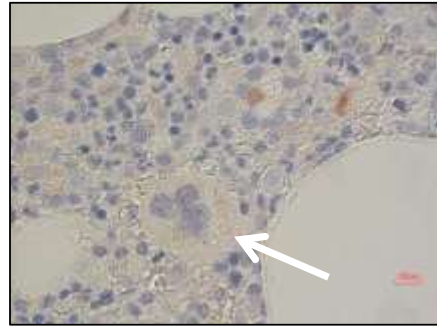
Heparanase and TFPI are increased in the bone marrow of MPD

10 BM biopsies of CML patients

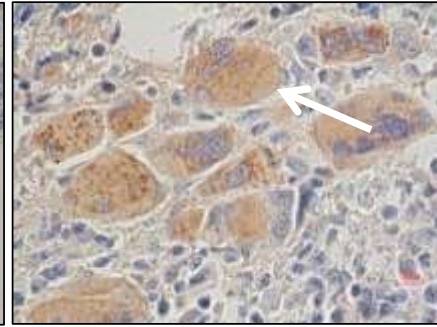
10 BM biopsies of ET patients
5 JAK-2 mutation +

10 BM biopsies of PV patients
10 JAK-2 mutation +

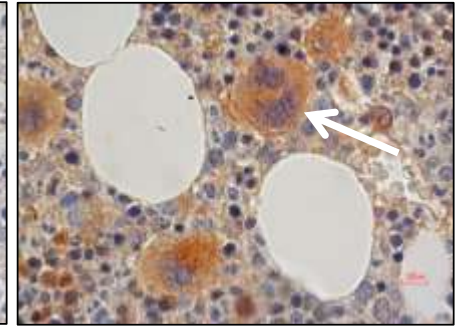
10 BM biopsies of MF patients
4 JAK-2 mutation +



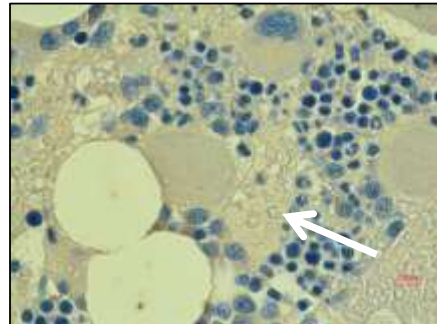
CML- Hepa



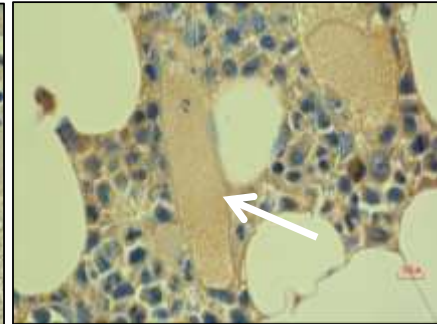
MPD- Hepa



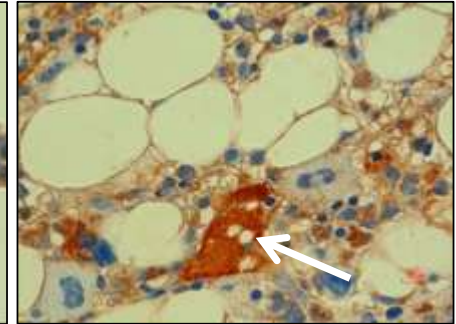
JAK-2 positive ET - Hepa



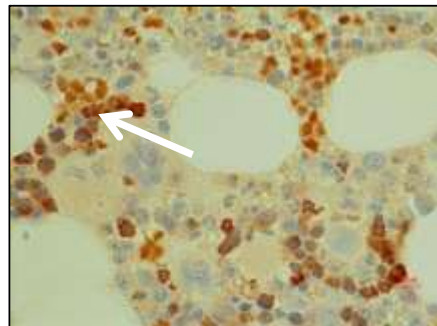
CML - TFPI



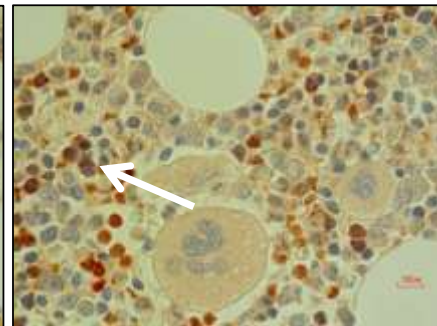
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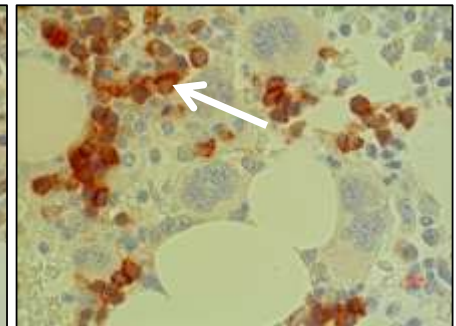
JAK-2 positive ET - TFPI



CML - TF

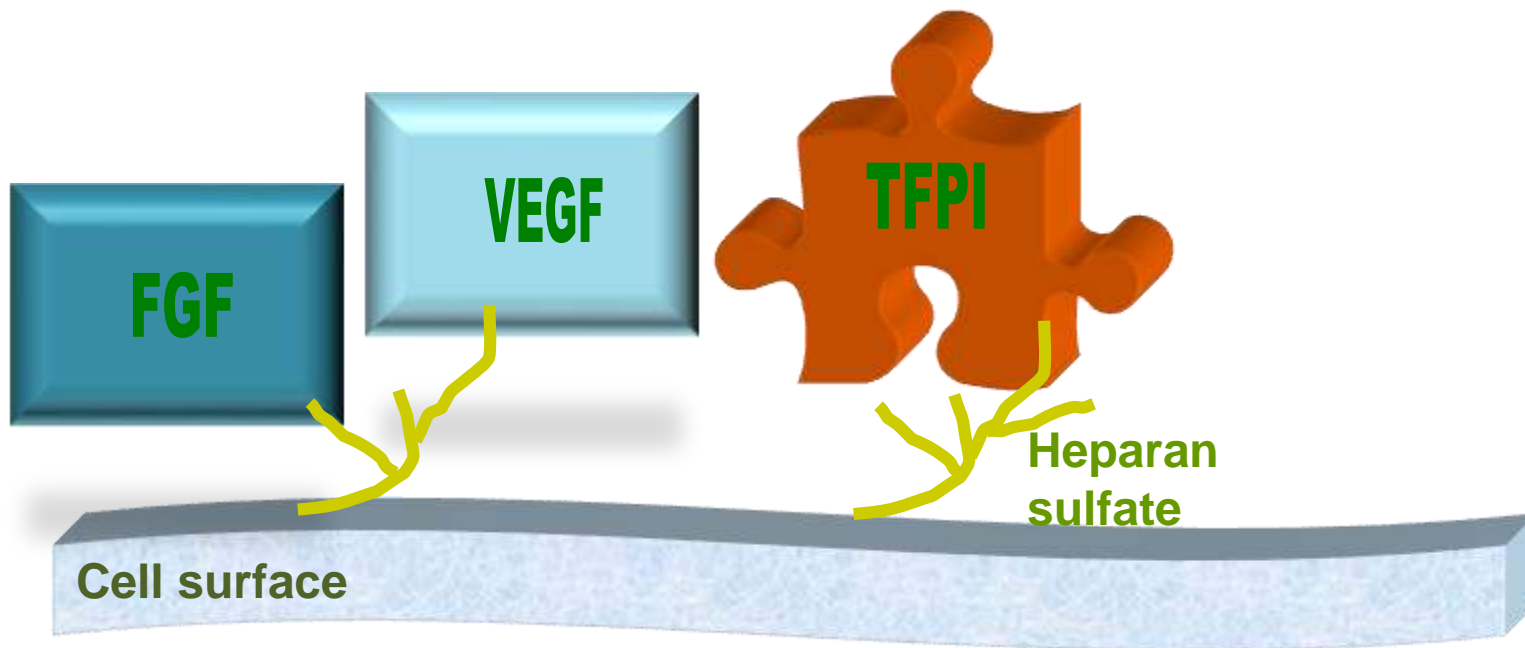


MPD - TF

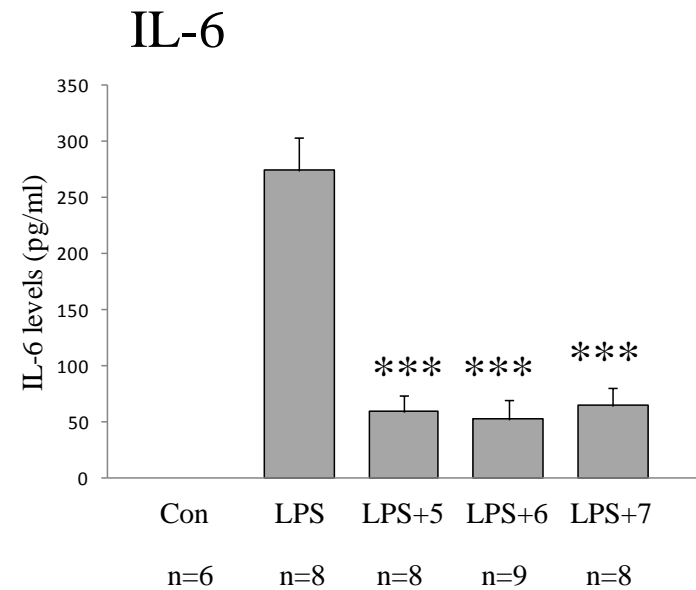
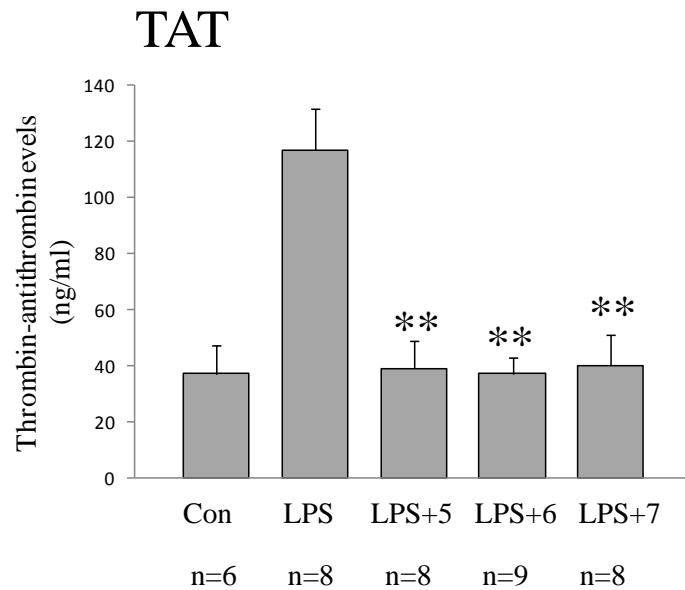


MPD - CD71

PAR-1 and PAR-2 are present on systemic vascular endothelial cells and on brain microvascular endothelium.



Novel inhibitory peptides in a mouse sepsis model



** $p < 0.001$, *** $p < 0.0001$

ICR mice



Effect of peptides on tube formation

