# Hemostatic biomarkers in cancer progression

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# Disclosures

- Research Grant from the Italian Association for Cancer Research (AIRC)
- Speakers' bureau: Rovi, Pfizer, Sanofi
- Advisory Board: Bayer, Daichii-Sankyo

# Aim of my presentation

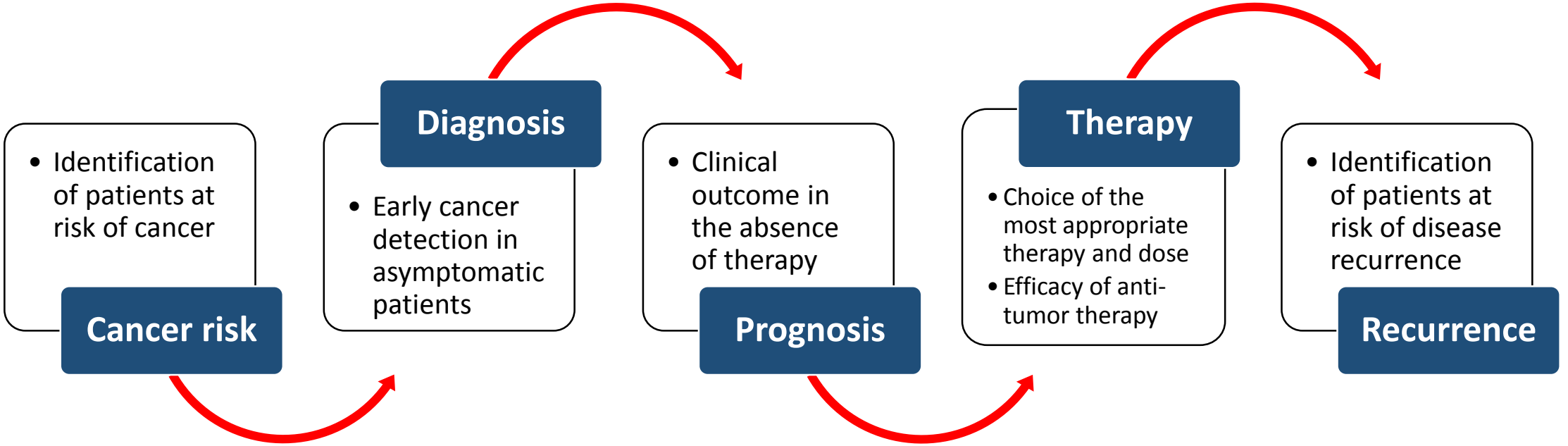
- To summarize the results of published studies on
  - ➢ blood clotting proteins and activation byproducts as biomarkers of cancer disease and progression
- and focus on ongoing research and future directions

# *What is a biomarker?*

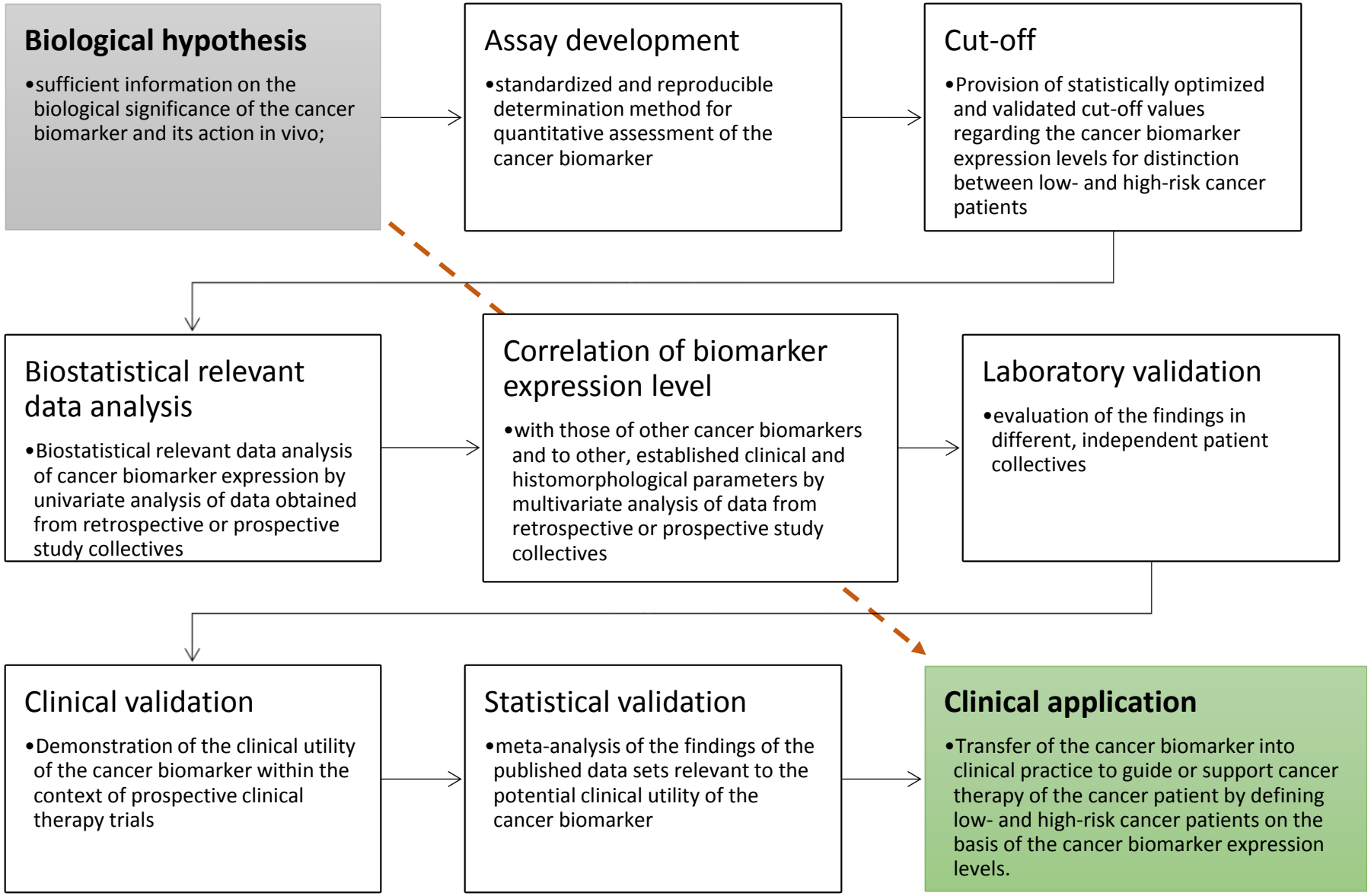
The National Institute of Health defines a biomarker as a cellular, biochemical, and/or molecular entity that can be **objectively** measured and can serve as an indicator of ongoing normal or pathogenic biological processes, or pharmacological responses to therapeutic interventions

# Role of biomarkers in malignant disease

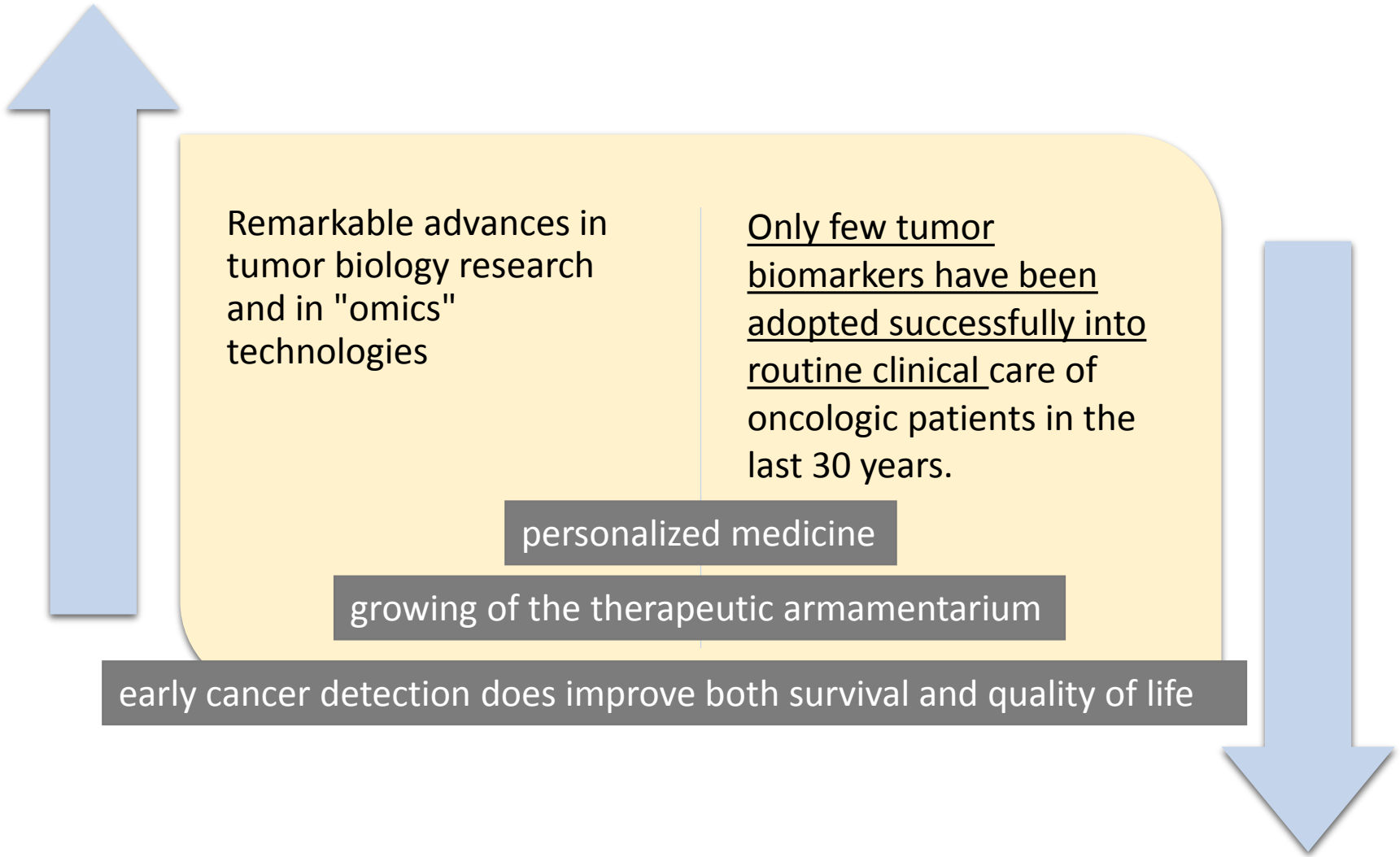
Biomarkers can play a crucial role in helping the diagnosis of early stage cancers (diagnostic biomarker), estimate the tumor aggressiveness, predict the likelihood of patient survival in the absence of treatment (prognostic biomarker), and predict the patient response to antitumor therapy (predictive biomarker).



# Requirements to be met before consideration of a cancer biomarker for clinical application



# Why we need new tumor biomarkers?

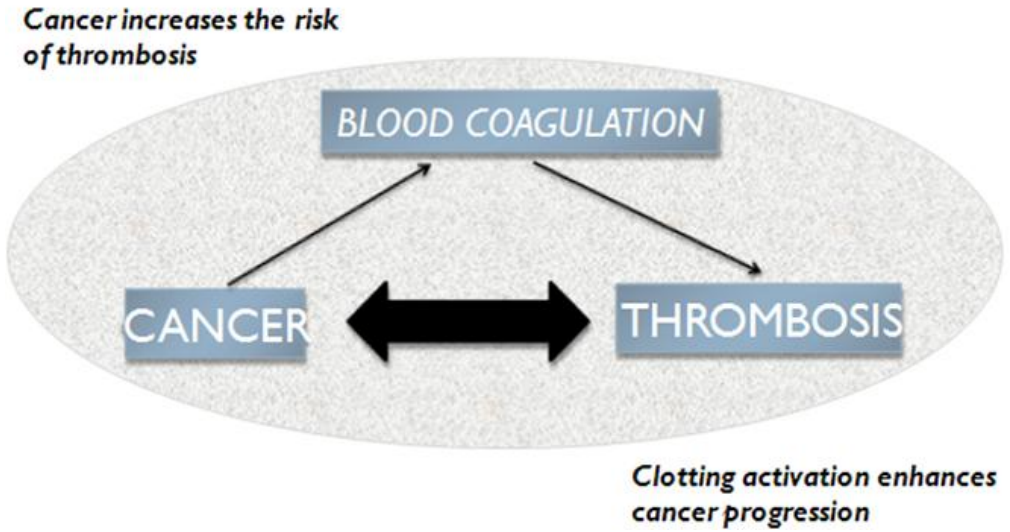



Mordente A, et al. Cancer Biomarkers Discovery and Validation: State of the Art, Problems and Future Perspectives, Advances in experimental medicine and biology 867 (2015) 9-26.

Diamandis E.P. . The failure of protein cancer biomarkers to reach the clinic: why, and what can be done to address the problem?, BMC medicine 10 (2012) 87.

# Coagulation activation in cancer

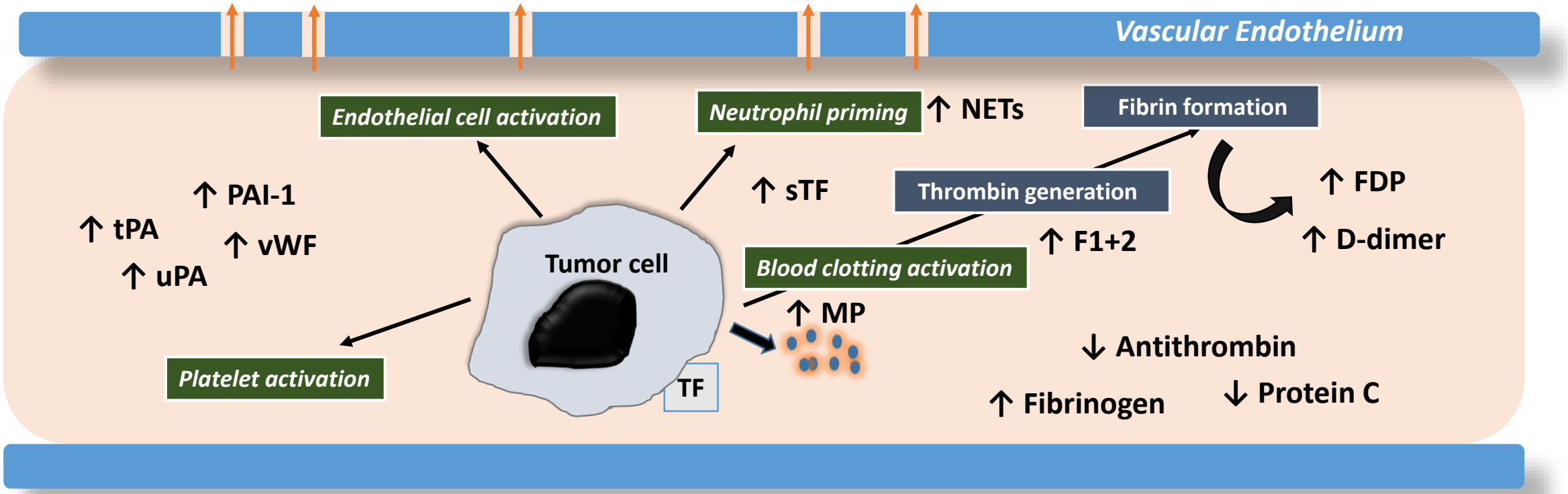
- Malignant disease is characterized by a hemostatic imbalance, usually shifted towards a procoagulant direction, and a high incidence of thrombotic complications.
- The mechanisms of hemostasis that are critically involved in thrombosis are also implicated in tumor progression, angiogenesis, and metastatic spread.




*Increased synthesis of hemostatic factors*
*Blood clotting activation*
*Fibrinolytic system activation*

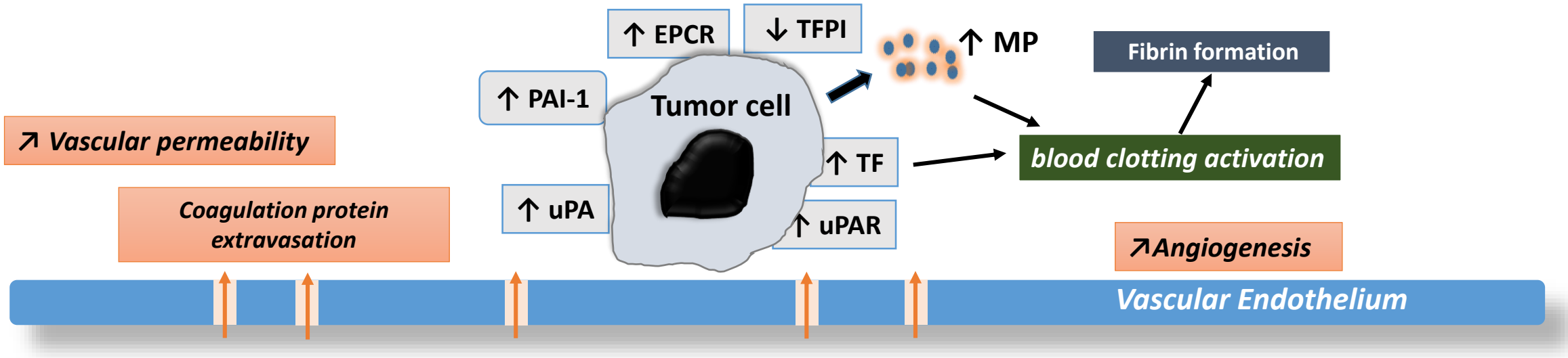
*Falanga A., Marchetti M., Vignoli A. Coagulation and cancer: biological and clinical aspects. J. Thromb. Haemost. 2013*

# *Tumor cell-induced blood clotting activation in the intravascular compartment*

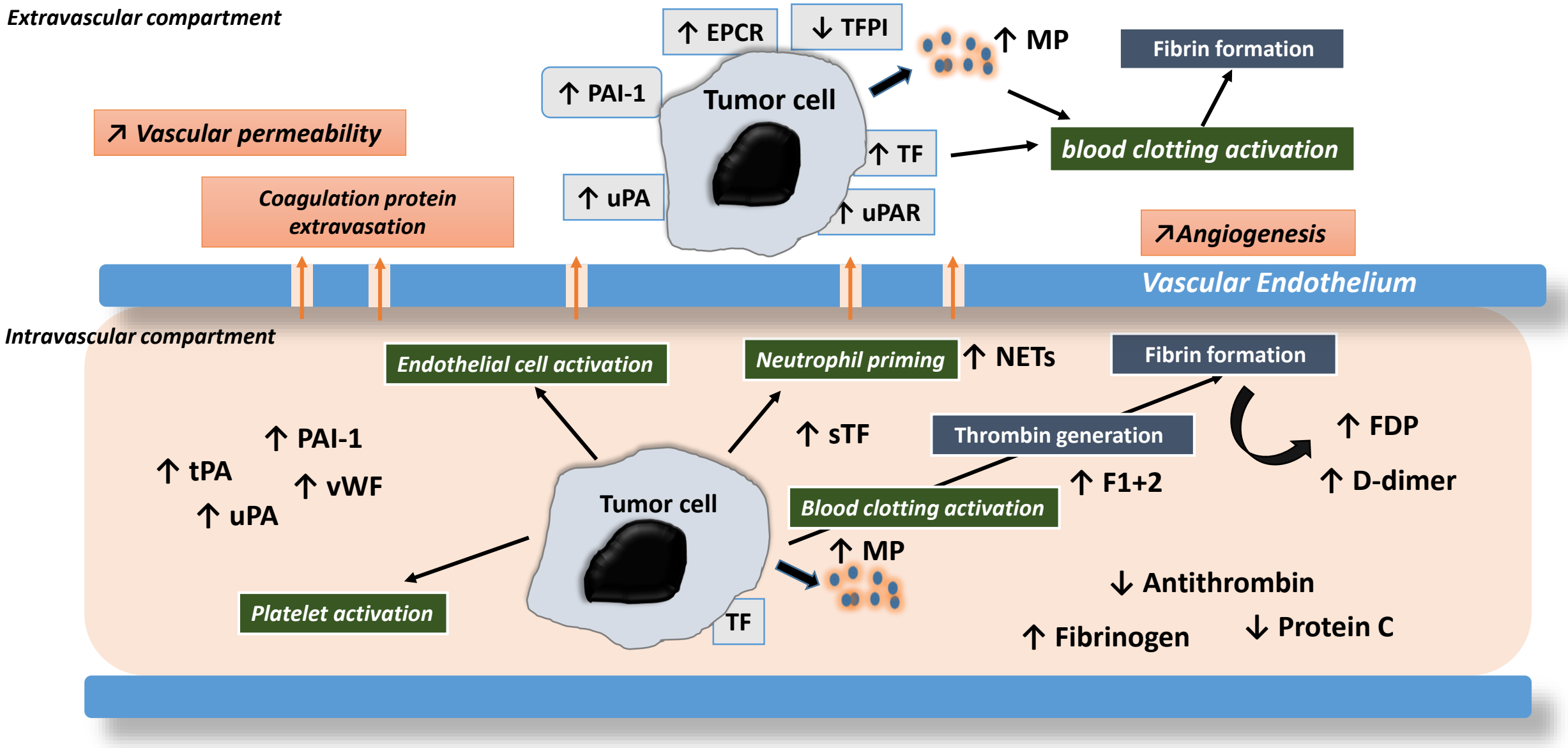



*Falanga A & Marchetti M. Thromb Res, 2018*

# *Tumor cell-induced blood clotting activation in the extravascular compartment*




*Falanga A & Marchetti M. Thromb Res, 2018*




*Falanga A & Marchetti M. Thromb Res, 2018*

# May thrombotic biomarkers be a relevant tool in predicting cancer outcomes?

Hemostatic markers (cellular and humoral) have been evaluated in cancer patients in relation to:

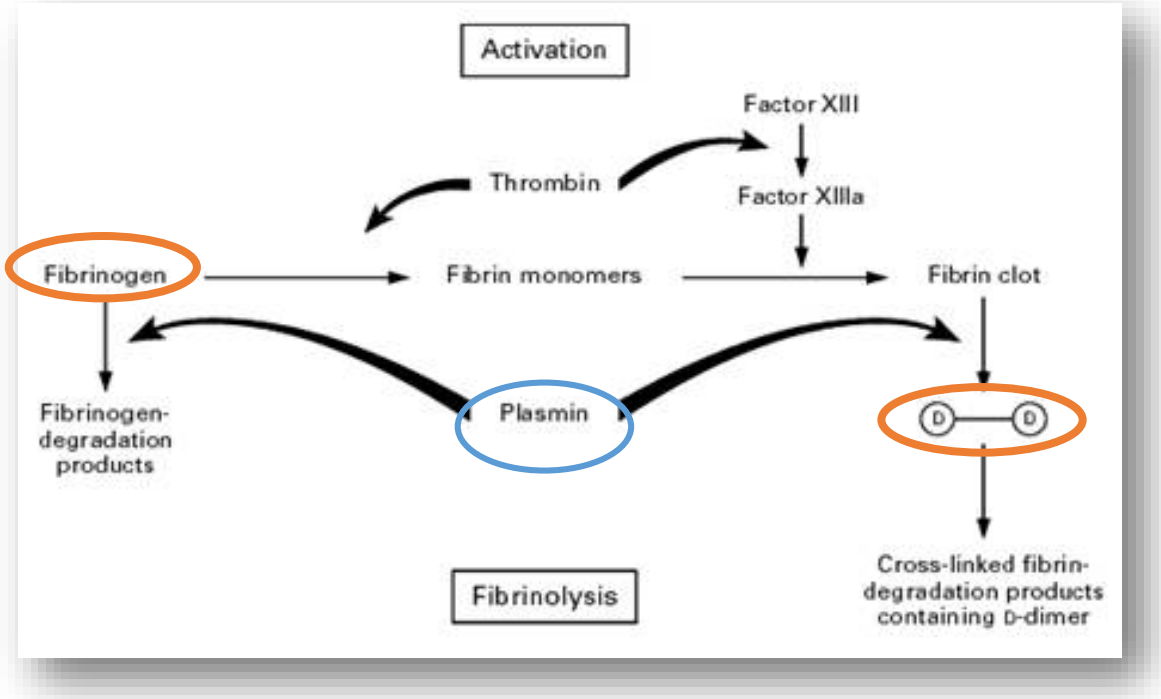
- **overall survival (OS)**
- **disease specific survival (DSS)**
- **disease free survival (DFS)**
- **progression free survival (PFS)**
- **tumor response to therapies**

# D-dimer and fibrinogen

Are the most studied hemostatic biomarkers

*well standardized, large availability in most hospitals, low cost, use in pre-operative routine screening, ….*

*lung*
*colorectal*
*gastric*
esophageal
ovarian
renal
pancreatic
breast cancer
…….




**Strong association of these biomarkers with different cancer outcomes, even independently from VTE**

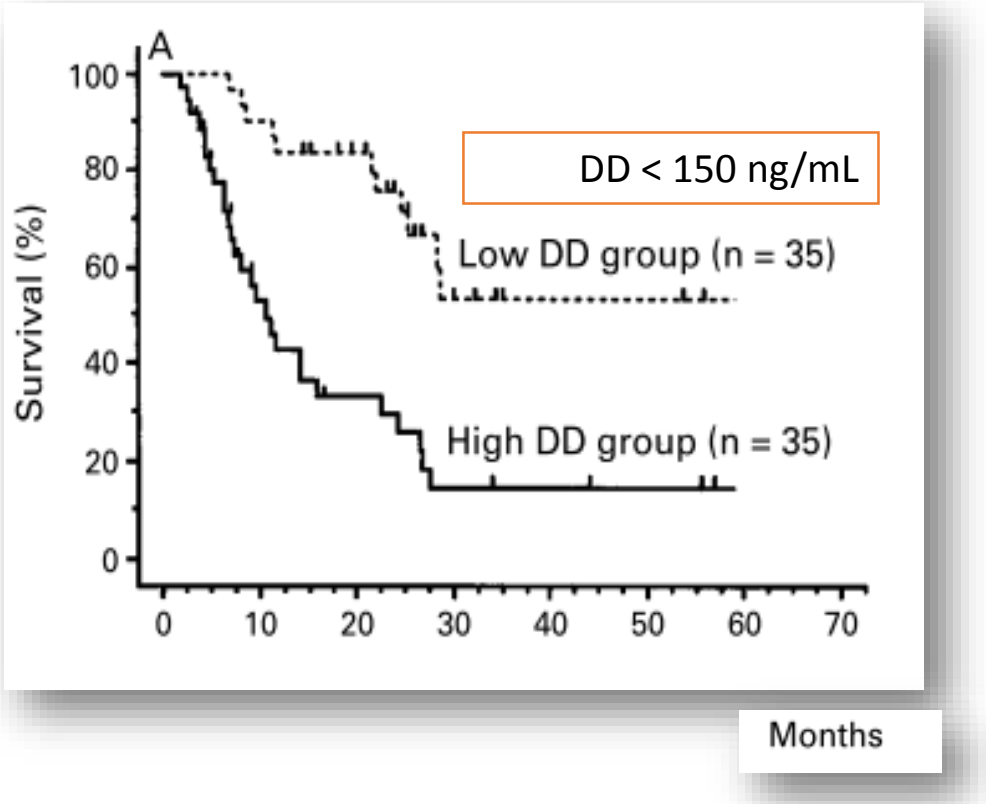
# The case of lung cancer

- NSCLC is the main type of lung cancer, account approximatively 85% of all lung cancer cases
- The majority of NSCLC patients are diagnosed at more advanced stages of the disease (IIIB and IV NSCLC).
- Patients with advanced disease are usually offered chemotherapy with the option of surgery. Radiation is an option for patients not candidates for surgery. Molecular-targeted therapy plays an increasingly important role
- NSCLC show only limited sensitivity of chemotherapy, with an overall response rate of 30-40%
- Existing biomarkers and predictors for NSCLC are not satisfactory due to the lack of adequate sensitivity and specificity

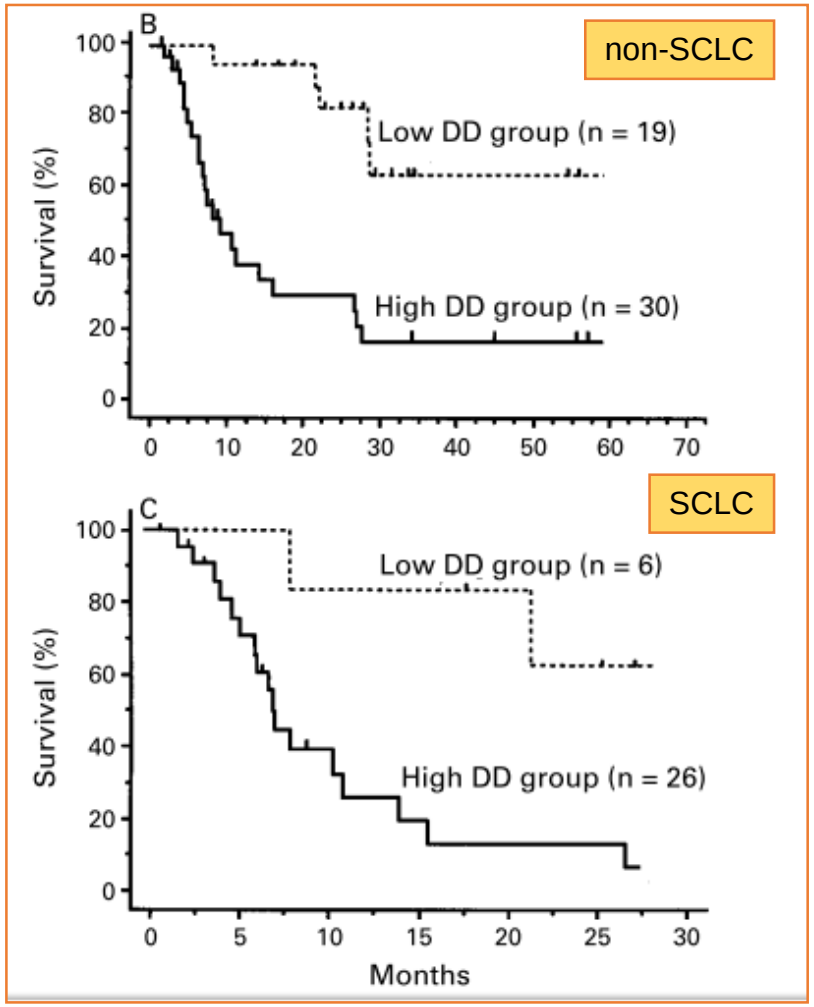
# Elevated D-dimer plasma levels have been frequently found in association with large tumor burden, clinical progression and poor prognosis

| Author | N patients | Study | Cancer type | Outcome | HR (95% CI) by multivariate analysis | Dimer cut-off | VARIABLES |
|---|---|---|---|---|---|---|---|
| Buccheri G 2003 | 826 newly diagnosed | **Retr** | 733 NSCLC 93 SCLC | OS | 1.3 (1.0-1-6) p<0.05 | 1.0 ug/mL (vs <0.4ug/mL) Pretreatment levels | Tumor stage, Karnofsky PS, therapy, age, serum proteins, TPA, CNS metastases, lymph node status |
| Zhang PP 2013 | 232 Stage I, II, IIIA | **Retr** | NSCLC | OS | 1.54 (1.11-2.78) p=0.03 | 0.3 ug/mL Preoperative levels | Age, Gender, Histology, Tumor size, TNM stage, postoperative VTE, surgery |
| Chen Y 2016 | 393 newly diagnosed | **Retr** | SCLC | PFS OS | 1.42 (1.09-1.85) p=0.09 1.58 (1.13-2.12) p=0.007 | 0.5 ug/mL Pretreatment levels | Age, Karnofsky PS, tumor stage, n of metastatic sites, LDH, CEA |
| Taguchi O 1997 | 70 newly diagnosed | **Pros 6 Y FU** | 49 NSCLC 21 SCLC | OS | 3.9 (1.6- 9.2) p<0.001 | 0.15 ug/mL Preteatment levels | Tumor stage, tumor size, performance status, tumor histology |
| Altiay G 2007 | 78 Stage III and IV | **Pros** | 60 NSCLC 18 SCLC | OS | 4.32 (2.18-8.55) p<0.001 | 0.65 ug/mL Preteatment levels | Tumor stage, Karnofsky PS |
| Komurcuoglu B 2011 | 100 newly diagnosed | **Pros 2 Y FU** | 87 NSCLC 13 SCLC | OS | 5.1; (1.01-1.19) p=0.013 | 500 ng/dl pretreatment levels | Gender, tumor histology, ECOG, PS |
| Ge LP 2015 | 82 stage IIIB and IV | **Pros** | NSCLC | PFS | 1.58 (1.04-2.24) p= 0.011, after 1 cycle 1.82 (1.28-2.24) p=0.006, after 2 cycle | 0.55 ug/L before and during CT | Tumor stage, treatment response, serum CEA and Cyfra pos, number of metastatic sites |
| Zhu YX 2016 | 74 newly diagnosed | **Pros** | SCLC | PFS OS | 4.08 (1.40-14.72) p=0.013 5.12 (1.71-15.42) P=0.009 | 0.65 ug/ml After 2 cycle of CT | Age, gender, ECOG PS, tumor stage, CT response, fibrinogen, NSE, CEA, LDH |

Pre-treatment levels of D-dimer predicted OS independent of stage, tumor size, performance status or histology in 70 lung cancer patients (non-SCLC and SCLC).




**HR = 4.7 (95% CI 1.8- 11.7), p<0.0005**



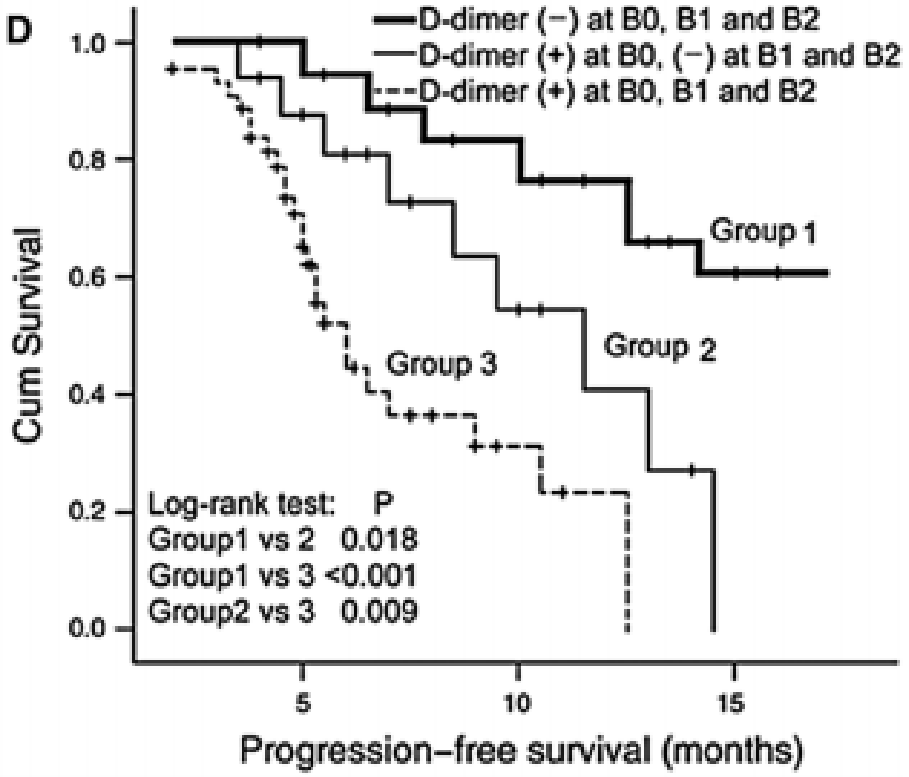

*Taguchi O. et al, Thorax 1997*

# Patients with positivity of D-dimer before and during chemotherapy had significantly shorter PFS compared with those with negativity.

82 patients with advanced **NSCLC** (stage III B and IV), entering first-line chemotherapy with cisplatin-based regimen

Blood specimens collected at:
B0: 1–3 days before the first cycle of CT
B1: 1–3 days before the second cycle of CT
B2: before the third cycle of CT.

Assays:
prothrombin time (PT)
D-dimer
serum carcinoembryonic antigen (CEA)
Cyfra 21-1




. The normal upper limits of plasma D-dimer, and PT, and serum CEA and Cyfra 21-1 were 0.55 μg/L, 9–12 s, 5 μg/L and 7 ng/mL, respectively, according to the recommendations of the corresponding manufactures.

*Ge LP et al, Clin Transl Oncol 2015*

# Two meta-analyses suggested that high preoperative D-dimer level is associated with poor prognosis of lung cancer

| Author | n. studies | n. patients | Study outcome | HR |
|---|---|---|---|---|
| Zhou YX et al, 2013 | 7 | 1,377 | OS | 1.12 (1.02-1.23) |
| Ma X et al, 2014 | 11 | 1,625 | 10 studies OS<br><br>1 study DFS | 2.06 (1.64-2.58)<br><br>3.38 (1.17-9.75) |

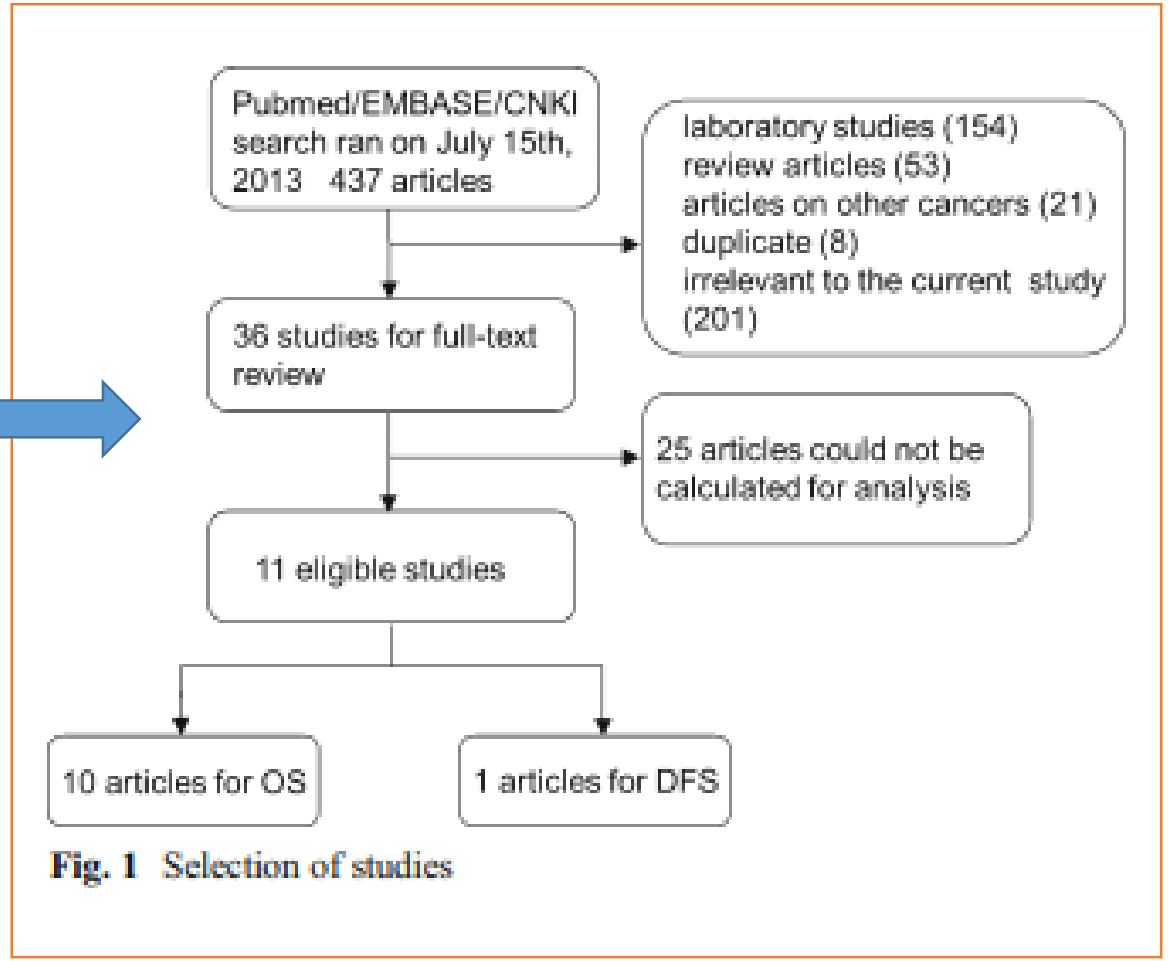
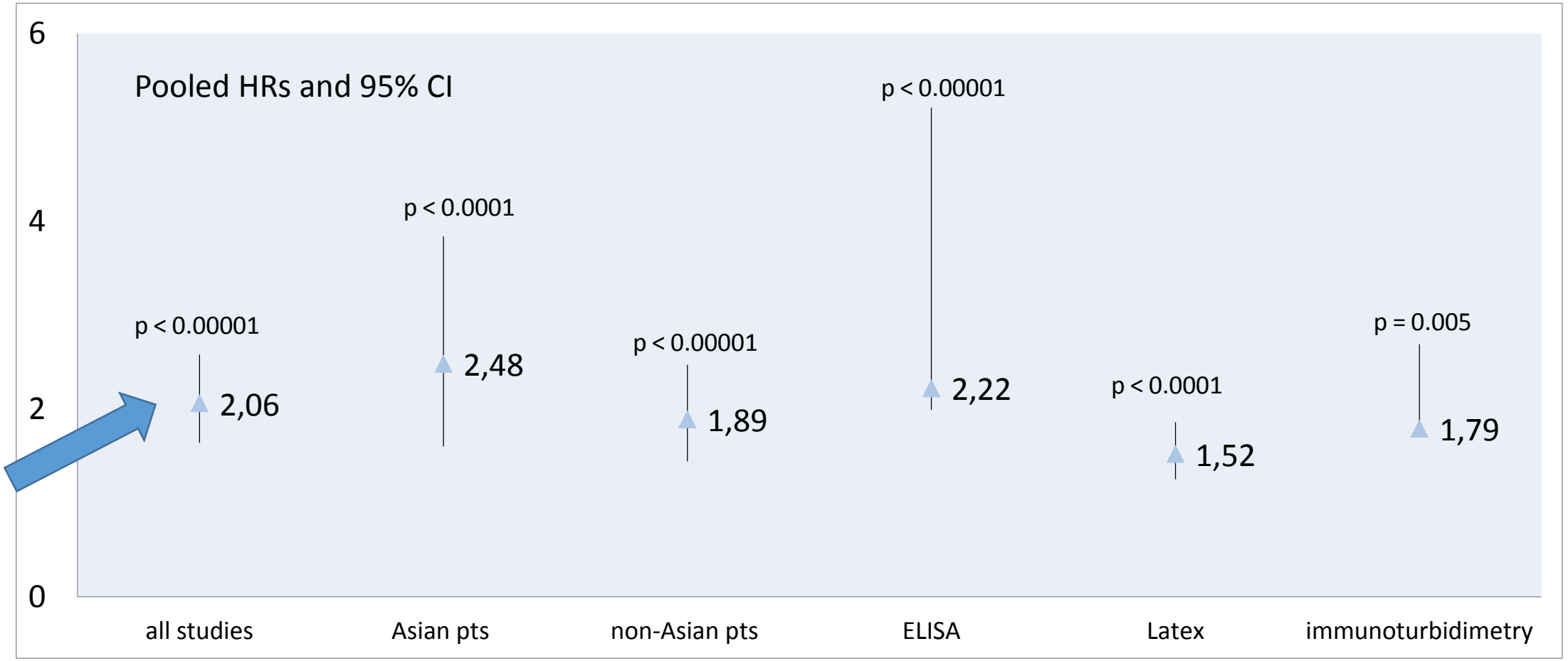


Fig. 1 Selection of studies

# Prognostic role of D-dimer in patients with lung cancer: a meta-analysis

11 eligible studies published between 1996 and 2013.
The estimated pooled HR and 95 % CI for OS of all studies was 2.06 (95 % CI 1.64–2.58, $p < 0.00001$) and for DFS in one study was 3.38 (95 % CI 1.17–9.75, $p = 0.002$).




*Modified from Ma X et al. Tumor Biology, 2014*

# D-Dimer in *colorectal cancer*

- Circulating D-dimer levels are better predictors of overall survival and disease progression than CEA levels in patients with ***metastatic colorectal carcinoma.*** (Blackwell K, et al. Cancer 2004)

- Is circulating D-dimer level a better prognostic indicator than CEA ***in resectable colorectal cancer***? Our experience on 199 cases. (Pedrazzani et al. Int J Biol Markers. 2010)

- High levels of D-dimer correlated with disease status and poor prognosis of **inoperable metastatic colorectal cancer** patients treated with **bevacizumab.** (Zhu L et al. J Can Res Ther 2014)

- High pretreatment plasma D-dimer predicts poor survival of colorectal cancer: insight from a **meta-analysis of observational studies.** (Lu SL et al. Oncotarget 2017)

# High levels of D-dimer correlated with disease status and poor prognosis of inoperable metastatic colorectal cancer patients treated with bevacizumab

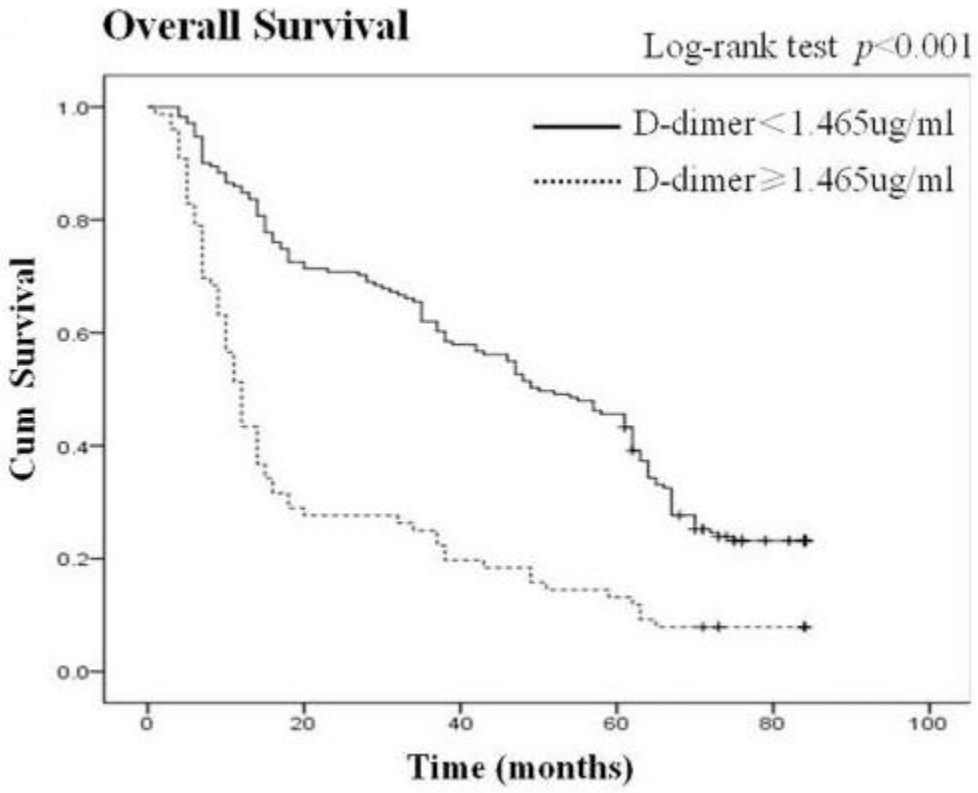
| Factors | Grouping | PFS | | OS | |
|---|---|---|---|---|---|
| | | HR (95% CI) | P | HR (95% CI) | P |
| ECOG-PS score | 0 | | | | |
| | 1/2 | 2.48 (1.46-4.00) | 0.001 | 6.55 (2.75-15.58) | <0.001 |
| Primary focal | Removal | | | | |
| | Unremoval | - | - | 1.65 (0.66-4.10) | 0.285 |
| Metastatic organ (number) | 1 | | | | |
| | ≥2 | 1.53 (0.81-32.88) | 0.192 | 0.39 (0.12-1.30) | 0.124 |
| Therapeutic quality | First line | | | | |
| | Second line | 2.94 (1.64-5.30) | <0.001 | 3.90 (1.79-8.52) | 0.001 |
| D-dimer grouping (μg/ml) | <0.56 | | | | |
| | 0.56-0.94 | 0.94 (0.44-1.98) | 0.861 | 1.58 (0.53-4.71) | 0.397 |
| | 0.94-1.90 | 0.70 (0.32-1.52) | 0.365 | 0.80 (0.27-2.38) | 0.971 |
| | ≥1.90 | 1.26 (0.58-2.72) | 0.556 | 3.52 (1.28-9.67) | 0.015 |

PFS=Progression-free survival, OS=Overall survival, HR=Hazard ratio, CI=Confidence interval, ECOG-PS=Eastern Cooperative Oncology Group Scale of Performance Status

*Zhu L. et al. J Can Res Ther 2014*

# Plasma D-dimer levels are increased in gastric cancer patients and may be a valuable biomarker for peritoneal dissemination, with high D-dimer levels predicting poor outcomes for gastric cancer patients

Kaplan-Meier curve for OS for patients stratified by plasma D-dimer levels



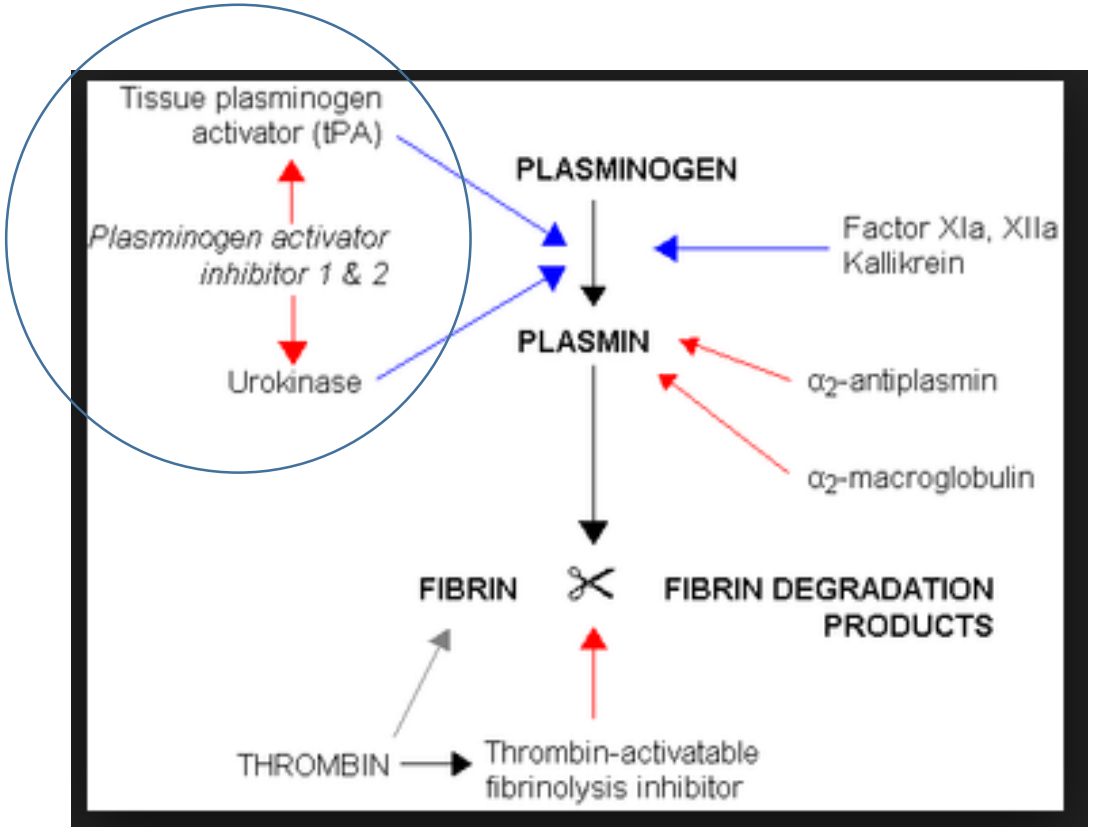

**Multivariate analysis of the prognostic factors for gastric cancer patients using the Cox regression model.**

| Parameters | Multivariate analysis | | |
|---|---|---|---|
| | Hazard ratio | 95%CI | P value |
| Invasion depth (T1/T2/T3/T4) | 1.50 | 1.23–1.82 | <0.001 |
| Lymph node metastasis (positive/negative) | 1.74 | 1.22–2.49 | 0.002 |
| Peritoneal dissemination (positive/negative) | 3.86 | 2.60–5.72 | <0.001 |
| Tumor size, cm (≥5/<5) | 1.44 | 1.07–1.94 | 0.015 |
| D-dimer, μg/ml (≥1.465/<1.465) | 2.28 | 1.36–3.81 | 0.002 |

*Liu L et al. PLoS One. 2014*

# The plasminogen activator system proteins

- Many of these proteins have a role in tumor invasion and metastasis
- Evaluated as potential tumor biomarkers both in cancer tissue specimens and in plasma.




*Schmitt M, et al. Clinical utility of level-of evidence-1 disease forecast cancer biomarkers uPA and its inhibitor PAI-1. Expert review of molecular diagnostics 2010*

Although not utilized in clinics, TISSUE u-PA and PAI-1 are the best-validated prognostic biomarkers for breast cancer

- High uPA and PAI-1 proteins are independent and potent predictors of adverse prognosis in patients with newly diagnosed invasive breast cancer
- uPA and PAI-1 are amongst the best validated prognostic biomarkers currently available for lymph node negative breast cancer.
- High levels of uPA and PAI-1 were also shown to be associated with benefit from adjuvant chemotherapy in patients with early breast cancer.

M.J. Duffy et al. Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM). European Journal of Cancer 75 (2017)

# Clinical use of biomarkers in breast cancer: Updated guidelines from the European Group on Tumor Markers (EGTM)

**14. uPA and PAI-1: EGTM recommendation**

- Levels of PA and PAI-1 protein levels may be combined with established factors for assessing prognosis and identifying ER-positive, HER2-negative and lymph node–-negative breast cancer patients that are unlikely to benefit from adjuvant chemotherapy **(LOE, IA; SOR, A).**
- For clinical use, uPA and PAI-1 should be measured by a validated ELISA (e.g. FEMTELLE, American Diagnostica/Sekisui) using extracts of fresh or freshly frozen breast tumour tissue, either from biopsy or surgical specimen.
- Currently, IHC or PCR should not be used when measuring uPA or PAI-1 for clinical purposes.

**15. uPA and PAI-1: recommendation for further research**

- Future research should aim to establish, validate and standardise a method for measuring uPA and PAI-1 by IHC or other techniques using formalin-fixed and paraffin-embedded tumour tissue.

Immunohistochemistry (**IHC**)
Polymerase chain reaction (**PCR**)

Duffy et al European Journal of Cancer 75 (2017) 284e298

# Pre-operative PLASMA levels uPAR and PAI- 1 can predict poor prognosis in patients with colorectal, ovarian, or breast cancers

**Plasma PAI-1 levels in breast cancer – relationship with clinical outcome**. *Ferroni P et al Anticancer Res 2014*

Elevated plasma PAI-1 level had a negative prognostic impact in terms of relapse-free survival and OS

Table IV. *Logistic regression analysis of predictors of relapse-free and overall survival in 152 patients with breast cancer.*

| | | Relapse-free survival | | Overall survival | |
|---|---|---|---|---|---|
| Variable | Code | OR (95% CI) | *p*-Value | OR (95% CI) | *p*-Value |
| Age, years | ≤60<br>>60 | 0.97 (0.91-1.04) | 0.417 | 0.96 (0.90-1.04) | 0.3066 |
| Estrogen receptor status | Negative<br>Positive | 1.25 (0.41-3.78) | 0.696 | 1.51 (0.43-5.39) | 0.5248 |
| Progesterone receptor status | Negative<br>Positive | 0.78 (0.26-2.39) | 0.670 | 1.52 (0.42-5.51) | 0.5206 |
| Menopausal status | Pre<br>Post | 1.16 (0.28-4.88) | 0.836 | 1.70 (0.35-8.63) | 0.4935 |
| Histological diagnosis | Ductal<br>Lobular<br>Others | 0.88 (0.49-1.60) | 0.678 | 0.55 (0.18-1.63) | 0.2805 |
| Stage of disease | Early<br>Advanced<br>Metastatic | 2.48 (1.33-4.64) | 0.005 | 4.67 (2.22-9.81) | <0.0001 |
| Adjuvant treatment | No<br>Yes | 4.43 (1.50-13.1) | 0.007 | 4.82 (1.34-17.4) | 0.0161 |
| ThromboPath level, PICI% | >81<br>≤81 | 2.20 (0.51-9.42) | 0.289 | 1.06 (0.17-6.81) | 0.9481 |
| PAI-1 level, ng/ml | ≤30<br>>30 | 3.11 (1.37-7.05) | 0.007 | 3.74 (1.46-9.59) | 0.0061 |
| HS D-dimer level, ng/ml | ≤500<br>>500 | 0.27 (0.06-1.26) | 0.096 | 0.74 (0.12-4.45) | 0.7436 |
| CA15.3, U/ml | ≤30<br>>30 | 0.99 (0.36-2.67) | 0.998 | 0.45 (0.13-1.50) | 0.1947 |

CI: Confidence interval; OR: odds ratio; PICI: protac-induced coagulation inhibition; PAI-1: plasminogen activator inhibitor-1; HS: highly sensitive; CA15.3: cancer antigen 15.3.

# Tissue Factor (TF)

- TF is involved in a variety of biologic processes, and numerous in vitro and in vivo studies have clearly established a central role for TF in cancer progression and spread.
- In addition, TF represents a potential target in the treatment of several malignancies, and different methods of targeting TF have been investigated.

H.H. Versteeg, Tissue Factor: Old and New Links with Cancer Biology, Seminars in thrombosis and hemostasis (2015) 747-55.
W. Ruf, N. Yokota, F. Schaffner, Tissue factor in cancer progression and angiogenesis, Thrombosis research (2010)
M. Cole, M. Bromberg, Tissue factor as a novel target for treatment of breast cancer, The oncologist (2013)

# Tumor tissue-associated TF

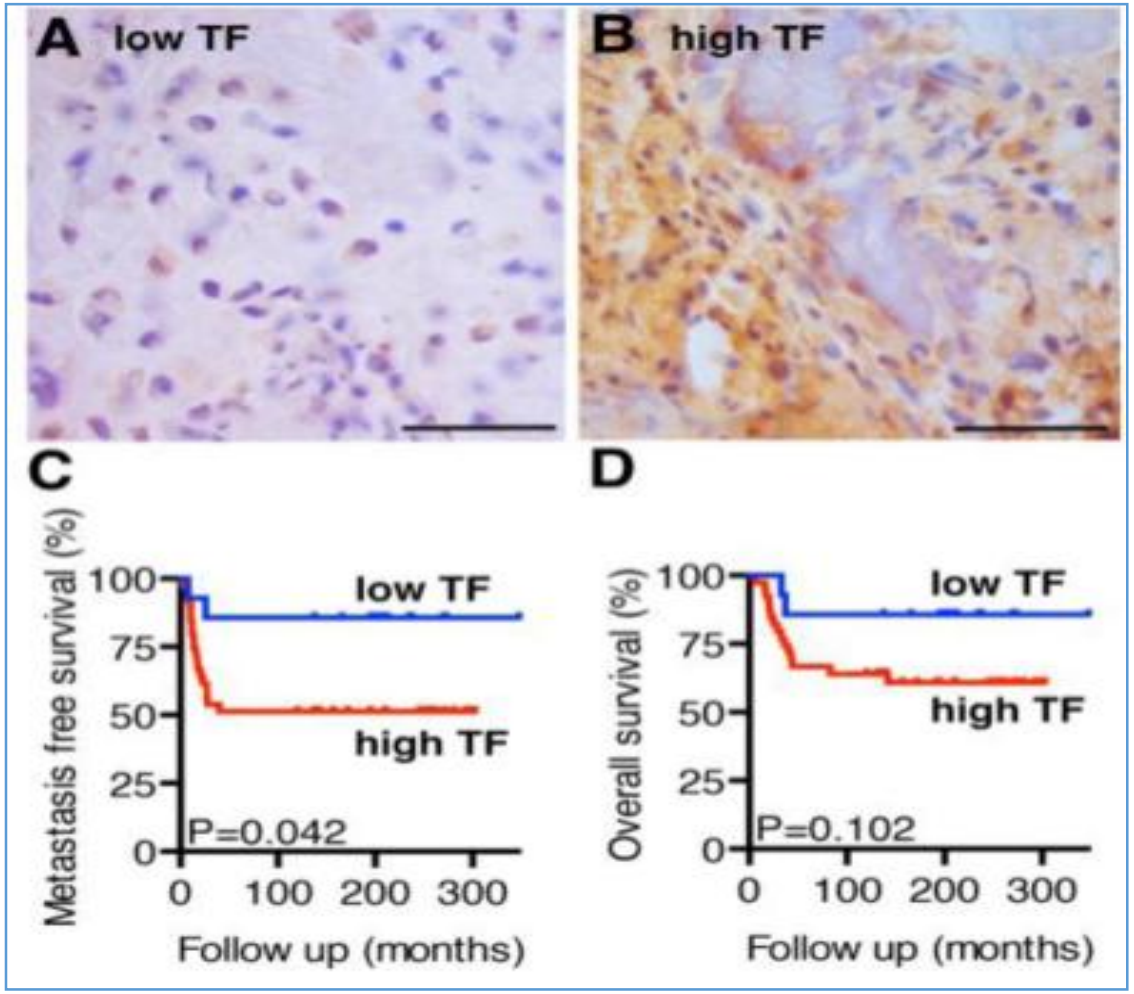
- Tumor TF expression was an independent prognostic indicator for OS in breast cancer and for DFS in osteosarcoma.


- T. Ueno, et al, Tissue factor expression in breast cancer tissues: its correlation with prognosis and plasma concentration, British journal of cancer (2000).
- C. Tieken et al, Tissue factor associates with survival and regulates tumour progression in osteosarcoma, Thrombosis and haemostasis (2016).

# TF associates with survival and regulates tumor progression in osteosarcoma

Patient Material: Formalin-fixed, paraffin-embedded pre-treatment biopsy tissues were collected from high-grade osteosarcoma patients diagnosed between 1984 and 2003.

TF protein expression was determined in 53 biopsies by immunohistochemical staining.
TF was scored as staining intensity
A score below 4 was considered low TF, 4 or higher was considered as high TF.

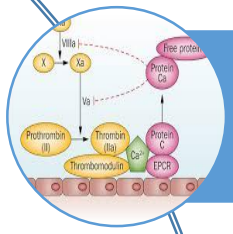



C. Tieken et al, Thrombosis and haemostasis (2016)

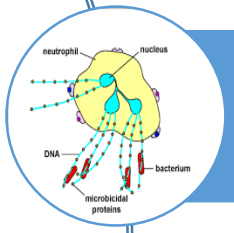
# Circulating TF

- A potential diagnostic value of MP-TF activity has been suggested in women with suspected ovarian cancer
- MP-TF activity represent a biomarker for a poorly differentiated and invasive pancreatic cancer phenotype and poor survival.
- Among the soluble forms of TF, alternatively spliced TF in the plasma of patients with pancreatic cancer may predict aggressive tumor phenotype.

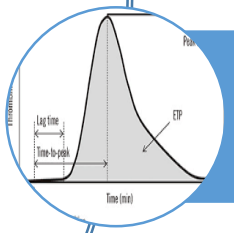

- C. Claussen, et al, Microvesicle-associated tissue factor procoagulant activity for the preoperative diagnosis of ovarian cancer, Thromb Res (2016).
- A. Bharthuar, et al, Circulating microparticle tissue factor, thromboembolism and survival in pancreaticobiliary cancers, Thrombo Res (2013)
- J. Thaler, et al, Microparticle-associated tissue factor activity in patients with pancreatic cancer: correlation with clinicopathological features, EJCI (2013).
- D. Unruh, et al, Levels of Alternatively Spliced Tissue Factor in the Plasma of Patients with Pancreatic Cancer May Help Predict Aggressive Tumor Phenotype, Ann Surg Oncol (2015)



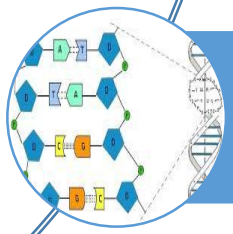

Antithrombin, Protein C and endothelial protein C receptor (EPCR)




Neutrophil extracellular traps (NETs)




Thrombin generation potential



Coagulation gene polymorphisms

# Coagulation gene polymorphisms

**Colorectal cancer**

- Carriers of the antithrombotic FXIII Val34Leu polymorphism showed a 15% reduced risk of developing cancer (OR = 0.85; 95% CI, 0.74 to 0.97) compared with non-carriers. Vossen et al., J Clin Oncol 2011.
- The inherited homozygous CC polymorphism of TFPI (-33T-->C), associated with higher TFPI levels, predicted for improved DFS. Bazzarelli et al. Ann Surg Oncol 2016.

**Breast cancer**

- SNPs in FV, FX and EPCR are associated with cancer susceptibility.

  Tinholt et al., BMC cancer 2014.
- FV Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms are not associated with DFS.

  Eroglu et al., IJCEM 2015.

# SUMMARY

- The activation of the clotting-fibrinolytic system in cancer patients represents an unfavorable clinical sign.
- A hypercoagulable state is associated with a large tumor burden, clinical progression, low rates of response to chemotherapy, and a poor prognosis.
- A substantial amount of data suggests that hemostatic biomarkers might be of potential utility in predicting cancer outcomes.
- Several publications are focused on specific cancer types, and show a significant relation of these biomarkers with different tumor outcome measures.

# Discussion

- Limitations:
  - Studies are often retrospective and not specifically designed to address the role of hemostatic biomarkers in cancer disease.
  - often mono-institutional, and include small heterogeneous cohorts of patients with different local and systemic treatments.
  - The cut-off values for each biomarker are derived from specific study populations and therefore cannot be easily extrapolated and compared from one laboratory setting to another.
- Indeed, except from tumor-tissue uPA and PAI-1, that have achieved a high level of validation in breast cancer, **thrombotic biomarkers' evaluation need to be standardized at analytical levels, rely on well established cut-off values, and clinically validated by means of prospective clinical trials.**

# The HYPERCAN STUDY

an ongoing prospective Italian, multicenter, observational study, designed in two Projects:

**PROJECT 1 . Assessment of thrombotic markers as a tool for cancer risk prediction in healthy subjects**

**PROJECT 2. Evaluation of thrombotic markers in patients with NEWLY DIAGNOSED cancer in relation with prognosis and VTE**

*Falanga et al, Thromb Res 2016*

**HYPERCAN STUDY** (AIRC 5x1000 grant #12237)
Registered in ClinicalTrials.gov, Identification Number: **NCT02622815**
**Evaluation of thrombotic markers in patients with cancer in relation with prognosis and VTE**

AIRC

# Study subjects accrual

- Project 1
  - 8,125 healthy subjects (blood donors)

- Project 2
  - 3,429 cancer patients (Lung, Breast, Colo-rectal, and Gastric):
    - 1,893 with limited resected tumors,
    - 310 with locally advanced tumors, and
    - 1,226 with metastatic disease

Project 2 prospectively assesses whether hypercoagulability in patients with a newly diagnosed Breast, NSCLC, CRC, or gastric cancer may predict for OS, PFS, RFS, response to therapy, and occurrence of VTE.

**Peripheral venous Blood Samples**

## LIMITED RESECTED DISEASE

- ❑ Enrollment, before starting systemic treatment
- ❑ 1 year
- ❑ 2 years
- ❑ 3 years
- ❑ 4 years
- ❑ at recurrence

## METASTATIC DISEASE

- ❑ Enrollment, before starting anticancer treatment
- ❑ 3 chemotherapy cycles
- ❑ 6 chemotherapy cycles
- ❑ end of treatment
- ❑ at progression

At each time point, information on treatment, recurrence, metastasis, survival and VTE are documented

Samples are stored at the **Biobank** of the Immunohematology and Transfusion Medicine Dept. of Papa Giovanni XXIII Hospital - BERGAMO (Italy)

# Substudy of the cohort of newly diagnosed breast cancer patients, undergoing adjuvant therapy after cancer resection

Evaluating the predictive value of hemostatic biomarkers of early disease recurrence (within 2 years)

**Breast cancer surgery** → **Enrollment** → **Start adjuvant therapy** → **Annual follow-up**

**Limited-resected cancer:**

**- T2-T3 N0 M0**

**- Tx N+ M0**

**Objectively confirmed**

**Disease Recurrence**

## Enrolled patients

| | |
|---|---|
| **N.** | 701 |
| **Age, yrs *** | 52 (29-79) |
| **Gender** | 690 F / 11 M |

*Data are shown as median (min-max)

Poster Session 1. Biomarkers / Hypercoagulabiity.
Saturday April 14th , 14.30-15.30, PO-03.

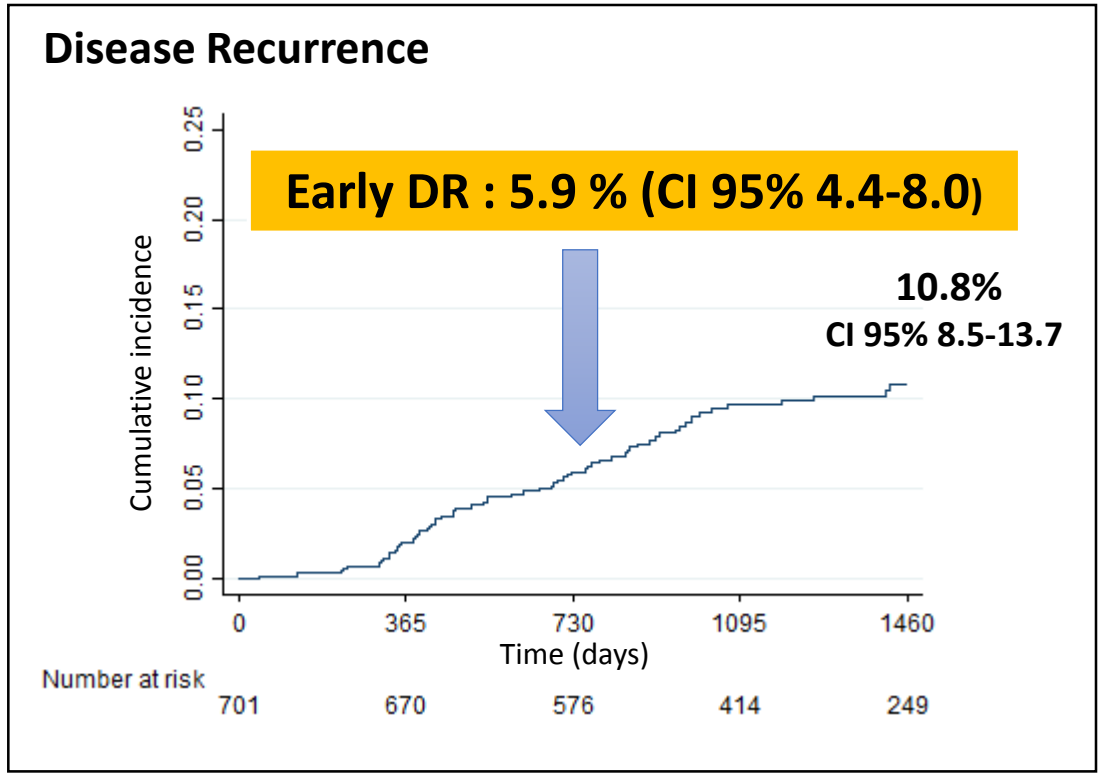
At enrollment (after tumor resection, before starting adjuvant chemotherapy) patients present with a hypercoagulable state

## Hemostatic biomarkers at enrollment

| | Patients | Healthy controls | P |
|---|---|---|---|
| **D-dimer (ng/ml)** | 197 (48-646) | 66 (0-202) | **<0.001** |
| **FVIIa-AT (pM)** | 120 (71-269) | 129 (76-278) | 0.139 |
| **F 1+2 (pmol/l)** | 199 (116-388) | 167 (116-300) | **<0.001** |
| **Fibrinogen (mg/dl)** | 302 (214-486) | 233 (185-332) | **<0.001** |
| **TG ETP (nM*min)** | 1,665 (1150-2300) | 1,516 (1,033-2,119) | **<0.05** |
| **TG peak (nM)** | 354 (220-481) | 237 (126-386) | **<0.001** |

Data are shown as median ($5^{th}$-$95^{th}$)

# Disease Recurrence and possible predictive factors




Median Follow-up = 2.8 yrs

**The study population has been randomly split in derivation and validation cohorts.**

**Multivariate analysis**

**Derivation cohort**

| Variable | HR | 95% CI | Coefficient | P |
|---|---|---|---|---|
| **TG ETP** | 1.001 | (1.000 - 1.002) | **0.001** | **<0.05** |
| **Luminal B HER2-** | 2.791 | (1.232 - 6.321) | **1.026** | **<0.05** |
| **Triple negative** | 3.133 | (1.146 - 8.562) | **1.142** | **<0.05** |
| **Mastectomy** | 2.414 | (1.170 - 4.981) | **0.881** | **<0.05** |

*Observations = 259*

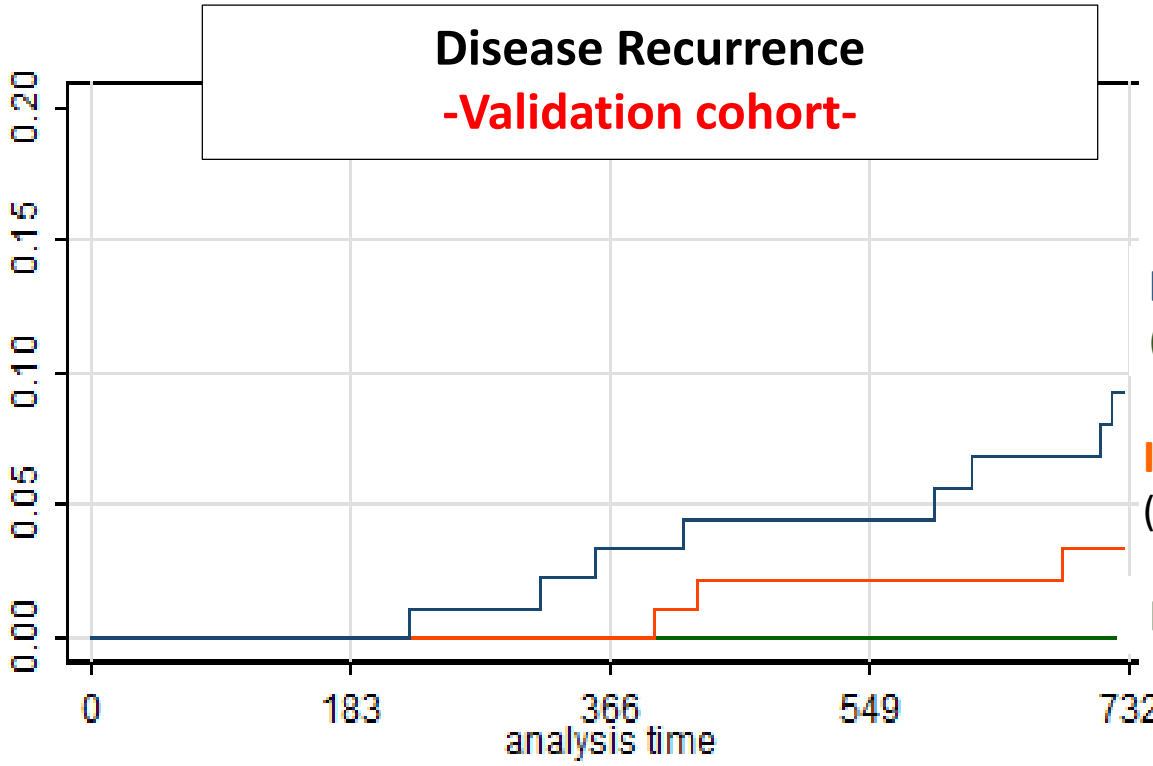
**Multivariate analysis in derivation cohort detected TG ETP value, triple negative and Luminal B HER2- molecular subtypes, and mastectomy, as independent risk factors for disease recurrence.**

From the multivariate analysis on the derivation cohort, a global score for the identification of patients at high risk of early DR has been defined:

**SCORE = (TG ETP*0.001) + 1.026 *(if luminal B HER2-)* + 1.142 (*if triple negative*) + 0.881 (*if mastectomy*)**

**The performance of the global score has been tested in the validation cohort**

# Global score performance in the validation cohort at 2 year-follow-up




**High risk : 8.9 %**
(95% CI 4.5-17.0)

**Intermediate risk: 3.3 %**
(95% CI 1.1-9.8)

**Low risk : 0.0 %**

**No recurrence occurred among patients classified at low risk.**

**CONCLUSION:** In limited-resected breast cancer patients, measurement of TG before starting SAC, together with molecular subtype and type of surgery, is determinant to generate a score for risk prediction of early DR. **The score** can help to tailor a risk-adapted adjuvant treatment**.**

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