



MICROPARTICLES AND FIBRINOLYSIS



Form AH100
03-2009

Jean AMIRAL and Anne Marie VISSAC
Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Micro-Particles and Fibrinolysis

- Are two major biological systems objectivating and regulating the state of body's functions.
- Microparticles, consequence and cause of disease, contribute to its « evolution ».
- Fibrinolysis is a « multi-function » system, of difficult laboratory evaluation, involved in :
brain/knowledge; fertility; malignancy;
thrombosis/reperfusion.

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

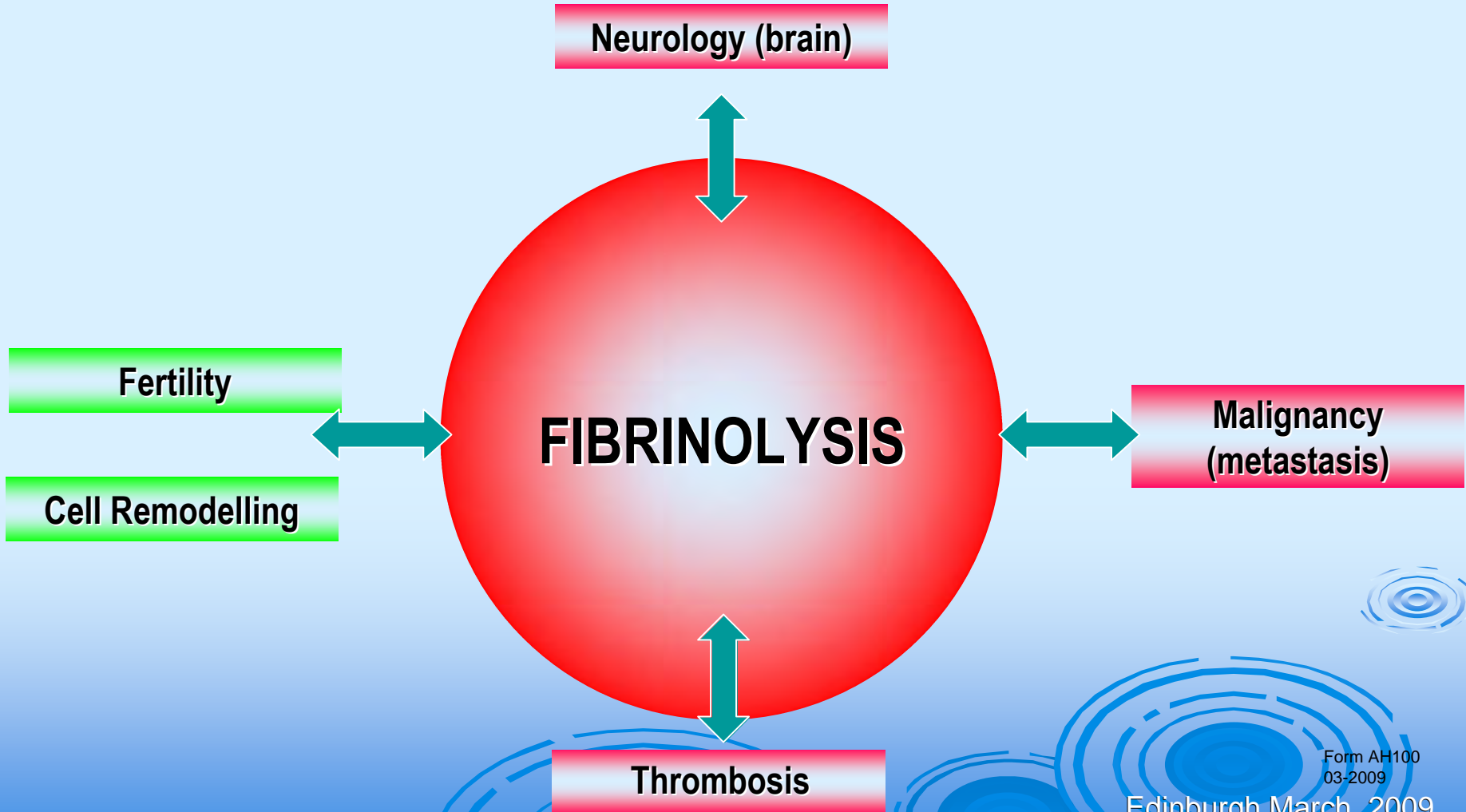
Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Fibrinolysis Functions



Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Fibrinolysis

- Fibrinolysis is a key system in life, probably still under evaluated.
- Important (but occult?) function in regulating many biological functions.
- Diagnostic and prognostic value for the major parameters (tPA, PAI-1, uPA, uPAR...).
- Diagnostic potential of other factors (TAFI, PAI-2, MMPs, TIMPs,.....).

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

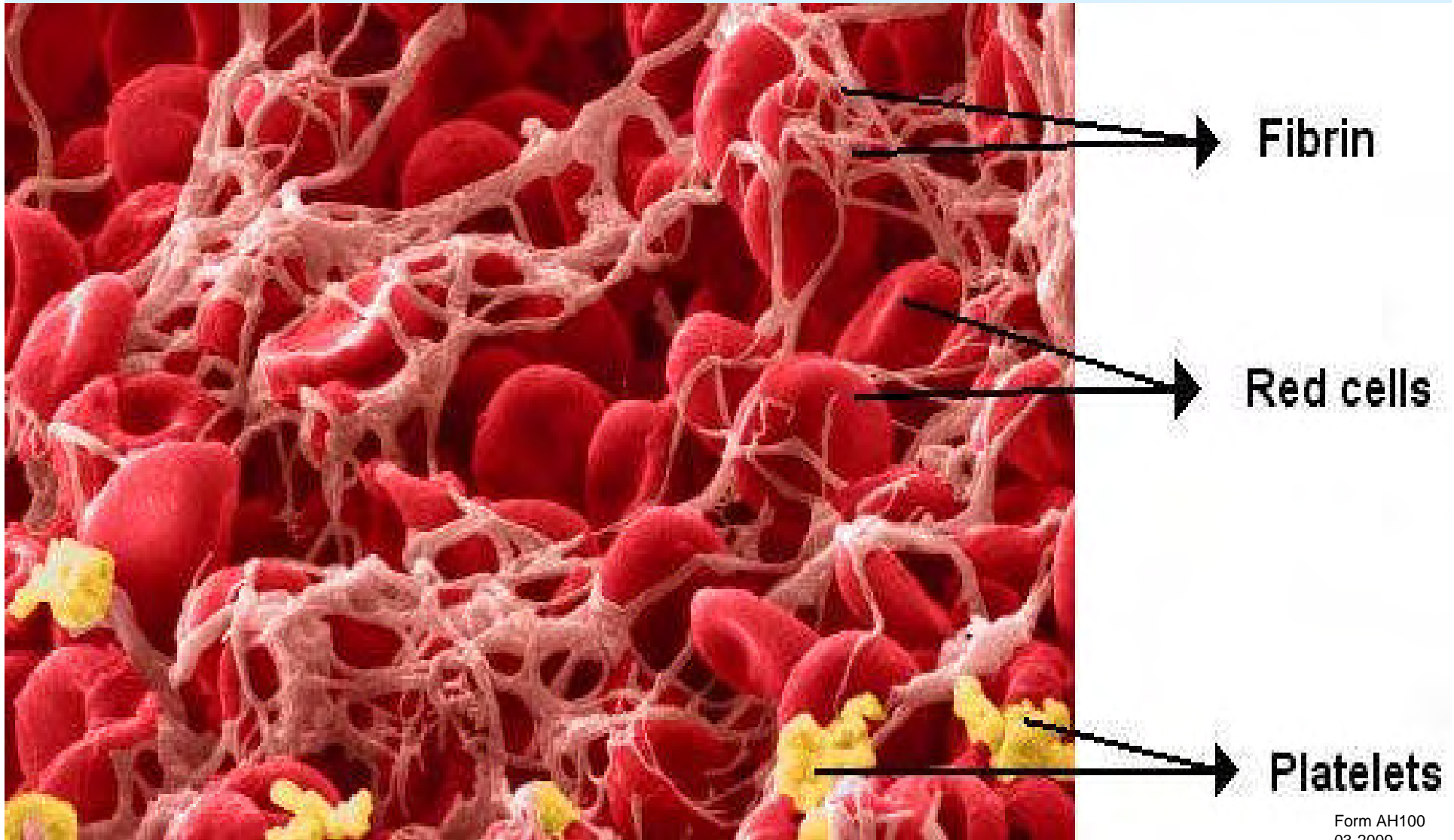
Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Blood Clot: The Fibrinolysis Target



Form AH100
03-2009

FIBRINOLYSIS IN BODY

➤ Intra-vascular: Mainly triggered by tPA



Plasmin \Rightarrow Clot dissolution

Body defence against thrombosis, Recanalisation, Thrombolytic therapy.

➤ Extra-vascular: Mainly triggered by uPA



Plasmin \Rightarrow MMPs

Matrix degradation and Tissue Remodelling or Neovascularisation (Cancer/Metastasis, Fertility, Cognitive functions of brain).



Form AH100
03-2009

FIBRINOLYSIS REGULATION

➤ Highly regulated biological system

Early Progenitors
release tPA

Cells in later stages
secrete uPA

➤ Equilibrium between Activators and Inhibitors

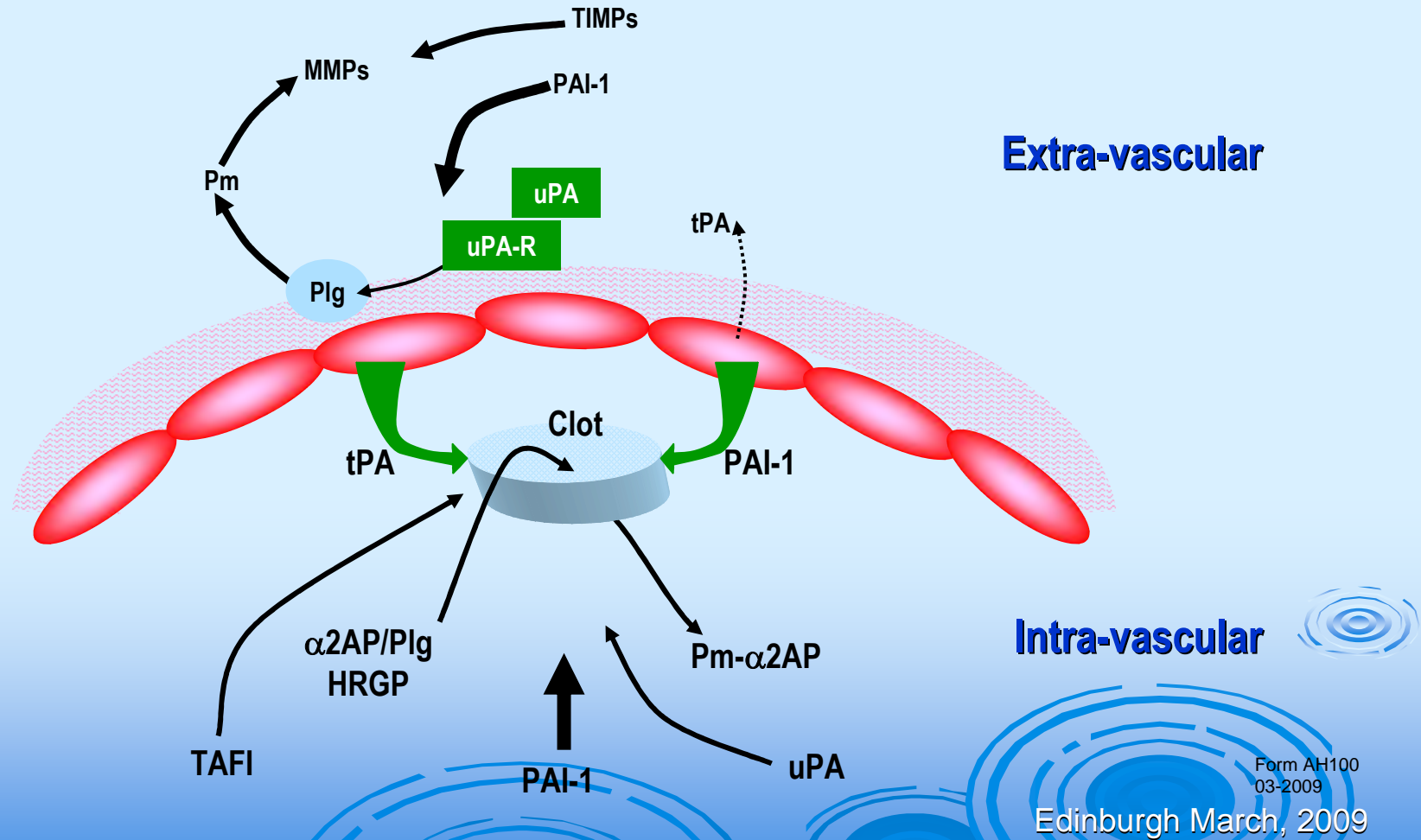
Intravascular
tPA-uPA/PAI-1
Plasminogen/ α 2AP/TAFI/HRGP/
MMP2-MMP-9/TIMP1-2

Extravascular
uPA-uPAR/PAI-1
MMPs/TIMPs

Form AH100
03-2009

Edinburgh March, 2009

FIBRINOLYSIS ACTIONS



Form AH100
03-2009

Edinburgh March, 2009

ANIARA

7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

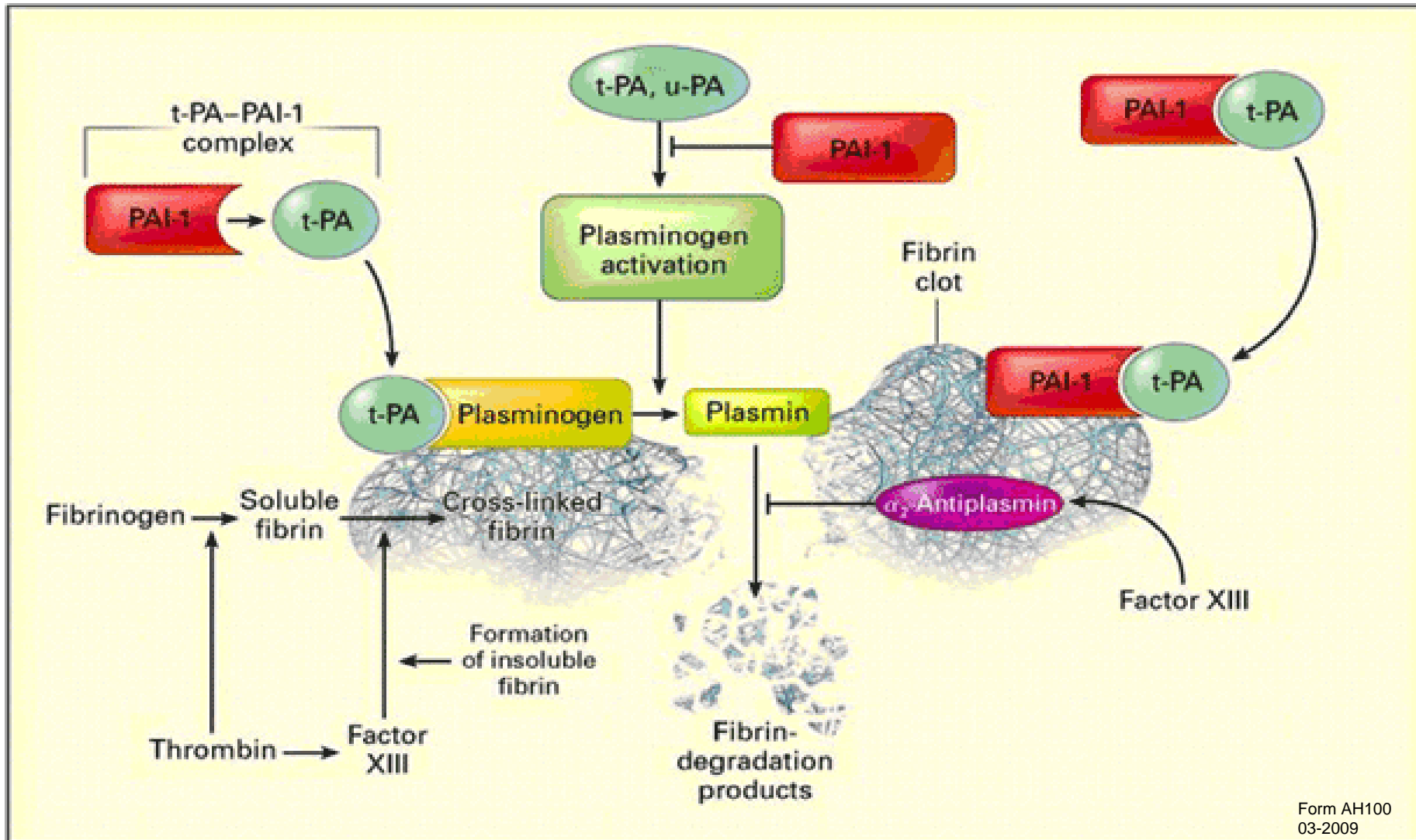
Toll Free: 866.783.3797

Fax: 513.573.9241

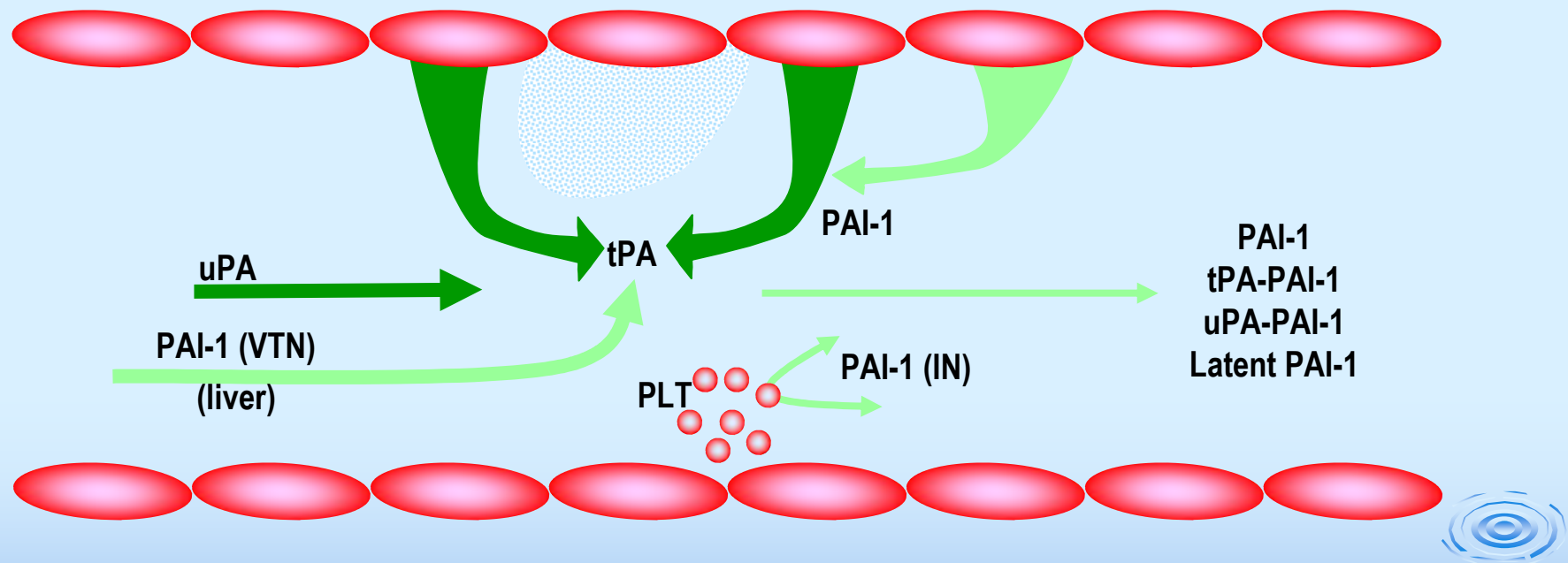
Email: info@aniara.com

www.aniara.com

Schema of Fibrinolysis



PAI-1 in blood vessels



Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

MAJOR DIAGNOSTIC FIBRINOLYSIS ANALYTES

INTRAVASCULAR (PLASMA)

- tPA
- PAI-1
- uPA
- MMP-2
- MMP-9
- TIMP-1

EXTRAVASCULAR (Tissues)

- uPA
- uPA-R
- PAI-1
- MMPs/TIMPs



Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

FIBRINOLYSIS IN BRAIN

- tPA involved in knowledge and protects from Alzheimer disease (tPA knock out mice model).
- When excessive in brain, can contribute to matrix degradation and anevrysm.
- Reactive fibrinolysis to cerebral thrombosis contributes to brain damage in stroke.

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

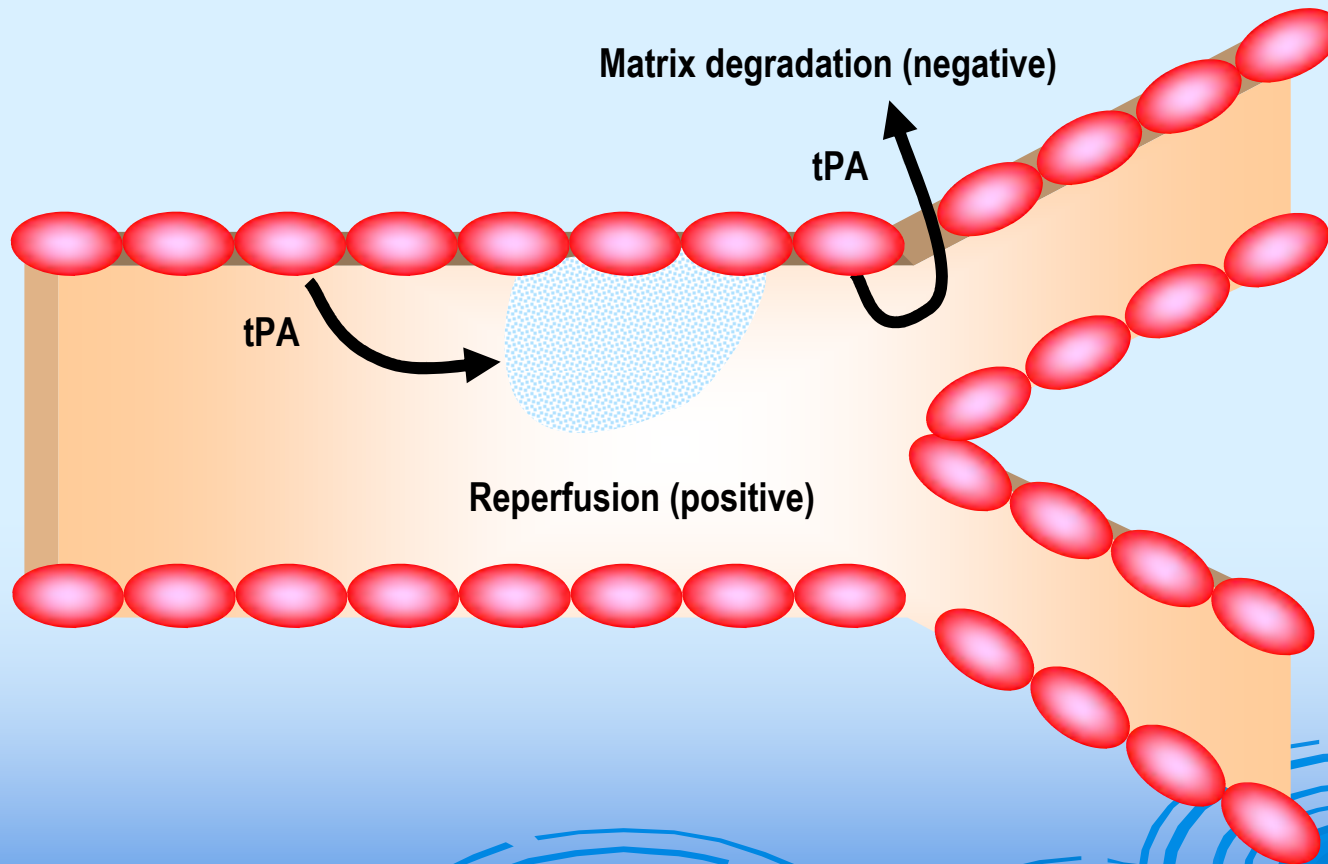
Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Yin and Yan effect of tPA in brain



Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Clinical applications of Fibrinolysis

- **Metabolic Syndrome (X-Syndrome)**
- **Diabetes, Type II (not affected by Type I)**
- **Cardiovascular diseases (predictive value of tPA, PAI-1?, ...)**
- **Malignancy (Breast Cancer, ...), etc ...**

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

ISSUES IN EVALUATING FIBRINOLYSIS

- It is a site targeted activity, promptly inhibited out of this location.
- Promoted and inhibited by locally secreted factors, present at high concentrations « only at these sites ».
- Very low residual active factors in blood circulation, and at low concentrations.

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

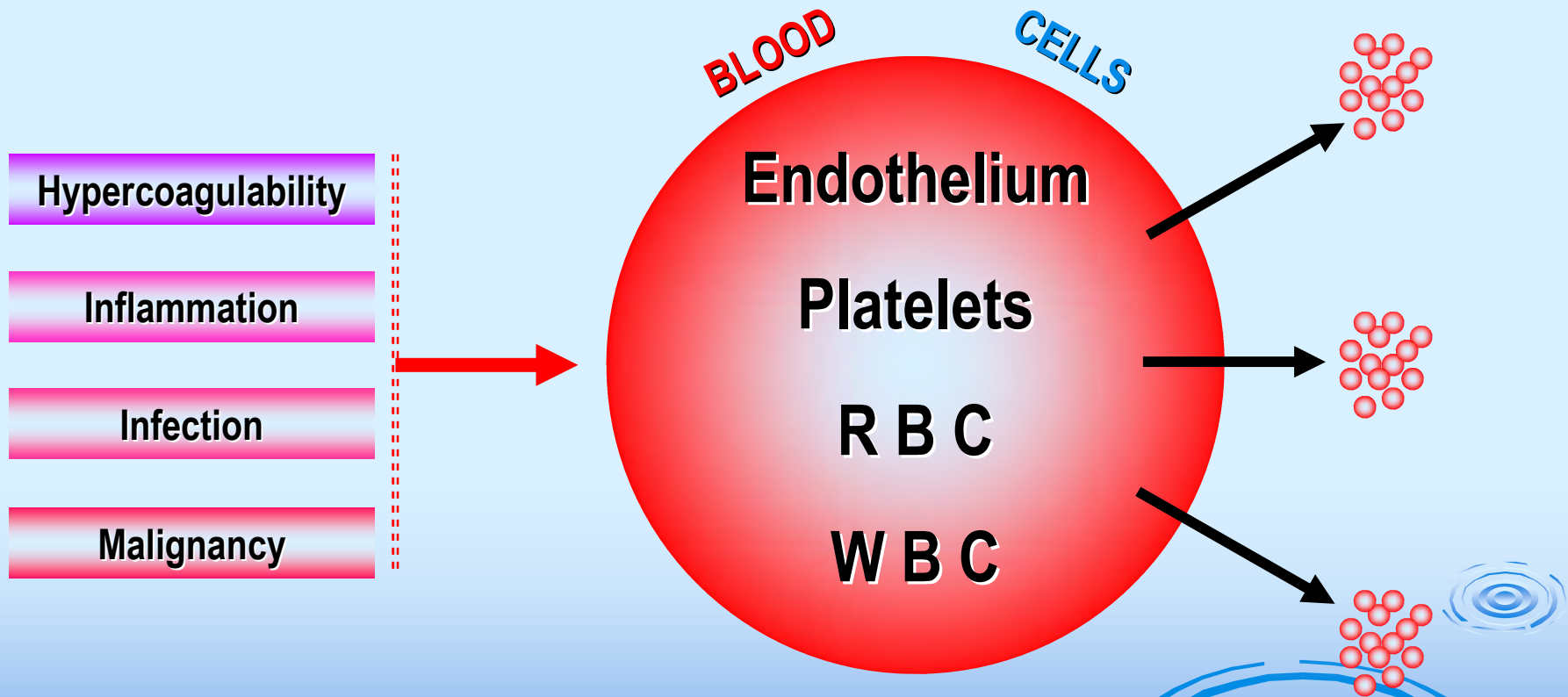
Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Microparticles as diagnostic markers



Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Microparticles

- **Long shelf life** (\approx 6 days)
- **Bind to Annexin V**
- **Released from many blood cells**
- **Bear CDs, TF, TM, GP IIb-IIIa, ...**

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

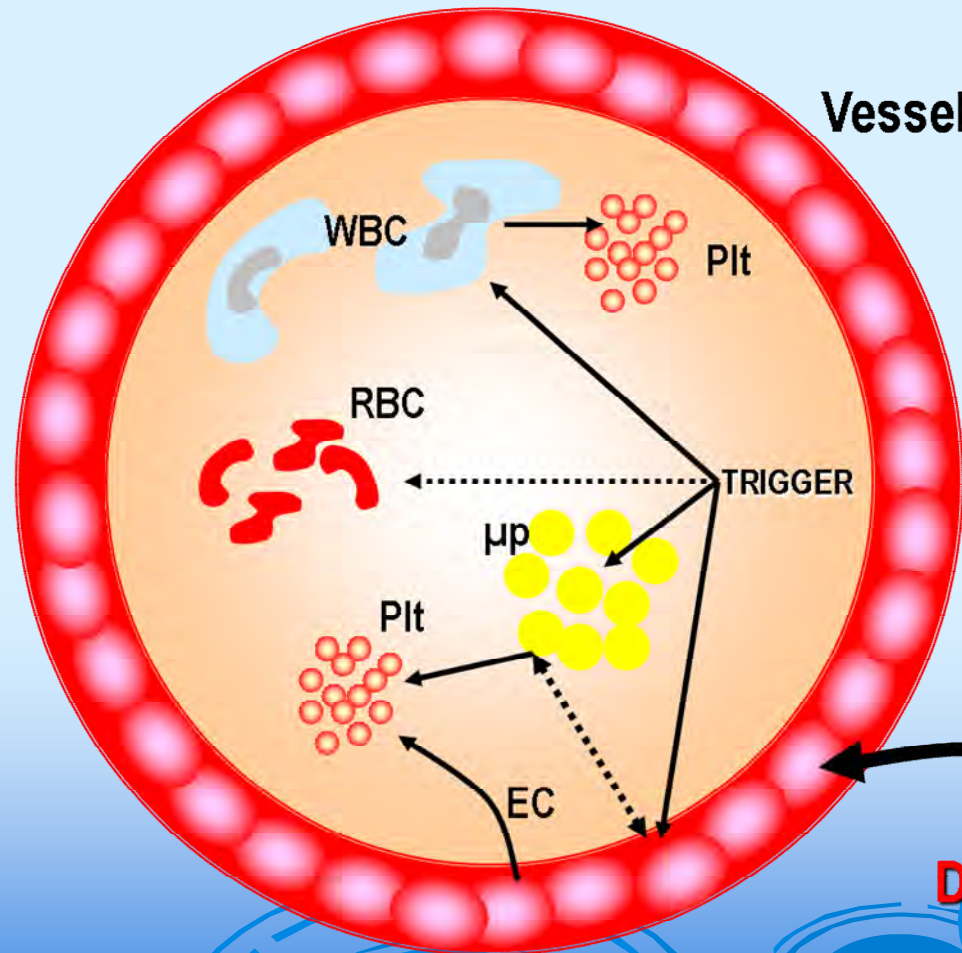
Cellular origin of microparticles

- **Platelets** (activation of coagulation)
- **Endothelial cells** (auto-immune diseases, TTP, activation of coagulation)
- **Monocytes** (inflammation, infection, ...)
- **Leucocytes** (inflammation, ...)
- **Lymphocytes** (diabetes mellitus, ...)
- **Tumoral cells**

Form AH100
03-2009

Edinburgh March, 2009

GENERATION OF MICROPARTICLES



DISEASE

Form AH100
03-2009

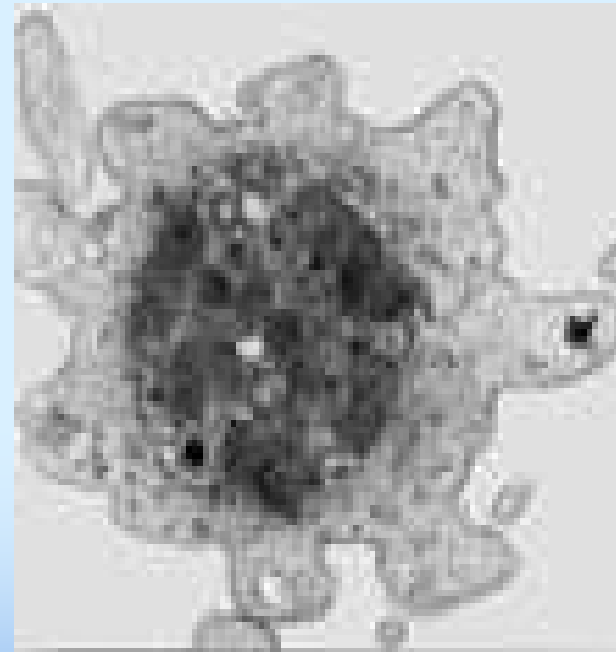
Edinburgh March, 2009

Platelet activation

Resting



Activated



Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

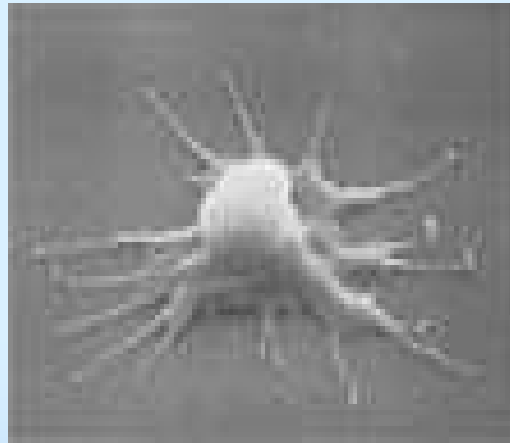
Email: info@aniara.com

www.aniara.com

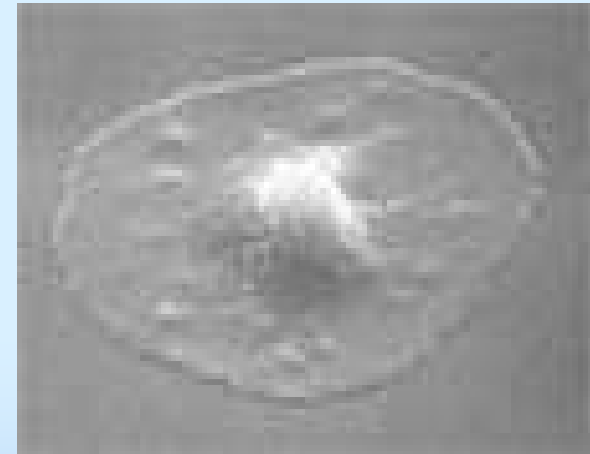
Platelet activation



Resting Platelet



Activation



Adhesion and spreading

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

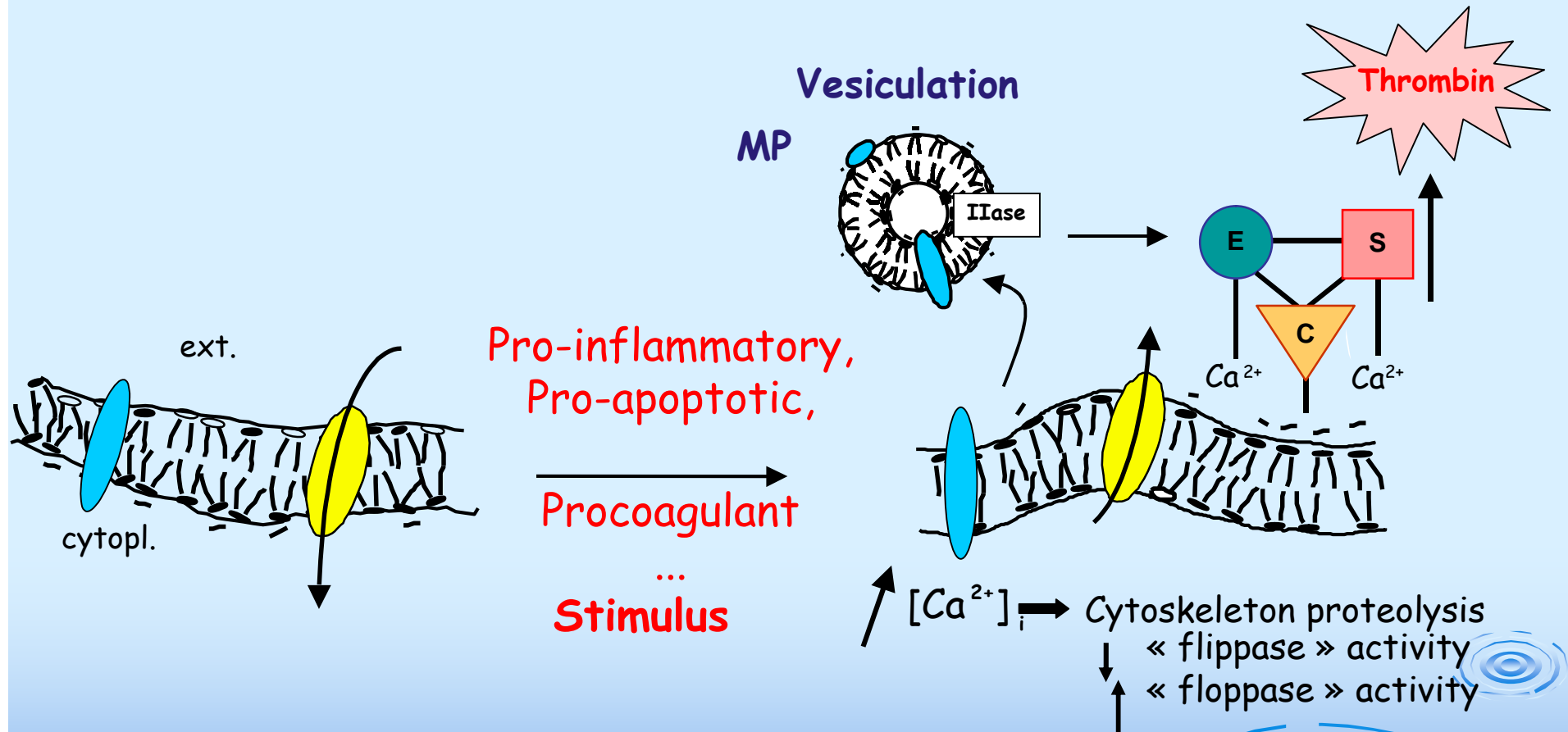
Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Haemostasis and cell membrane remodeling



Microparticles = in vivo cell activation markers

Form AH100
03-2009

Edinburgh March, 2009

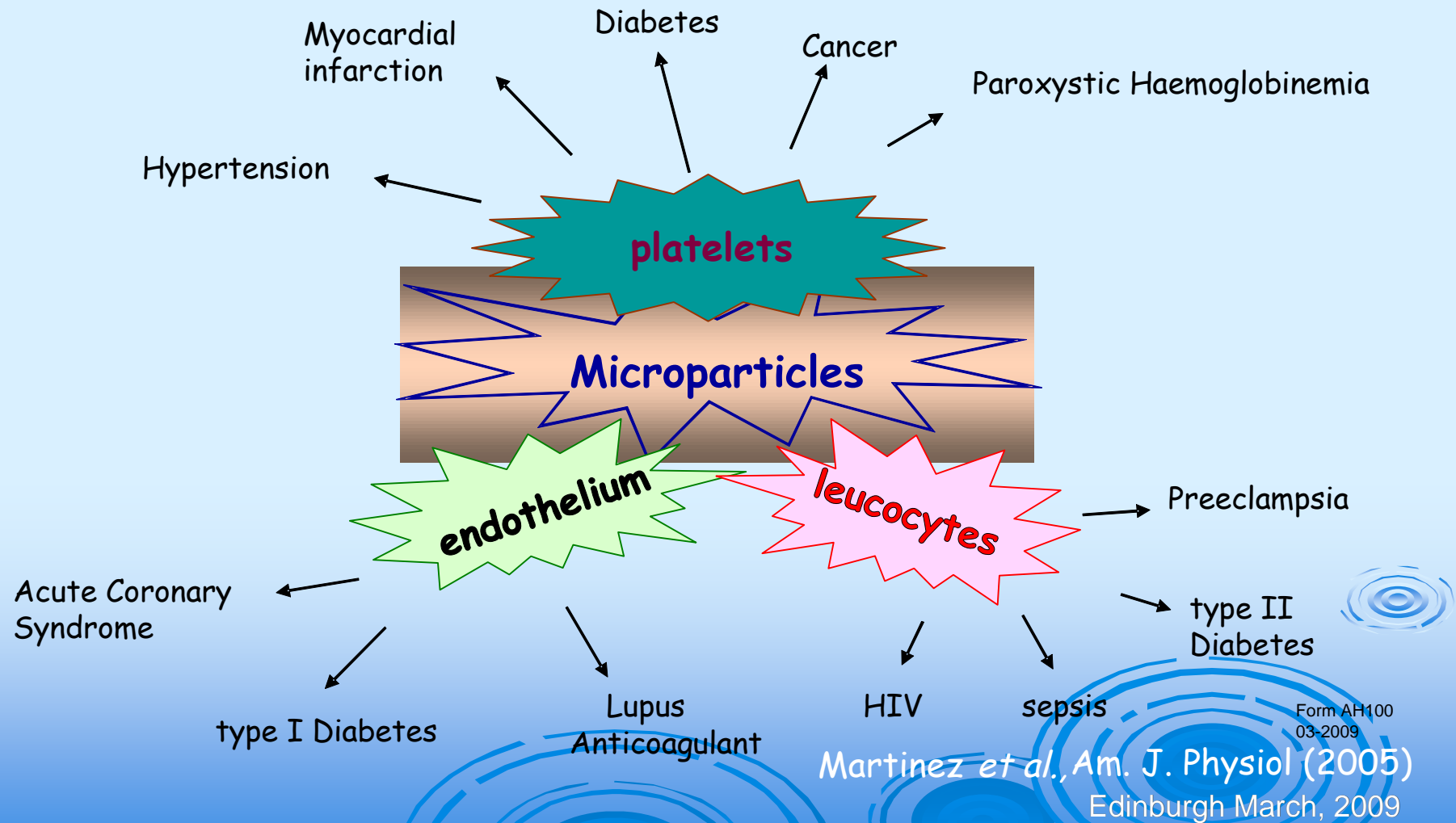
Characteristics of MPs

- The general consensus is that MPs are small: 0.1 to 1 μ m.
- Microparticle membranes consist mainly of lipids and proteins.
- Expose the anionic phospholipids: PS.
- Express membrane antigens that reflect their cellular origin and the cellular processes triggering their formation.

Edinburgh March, 2009

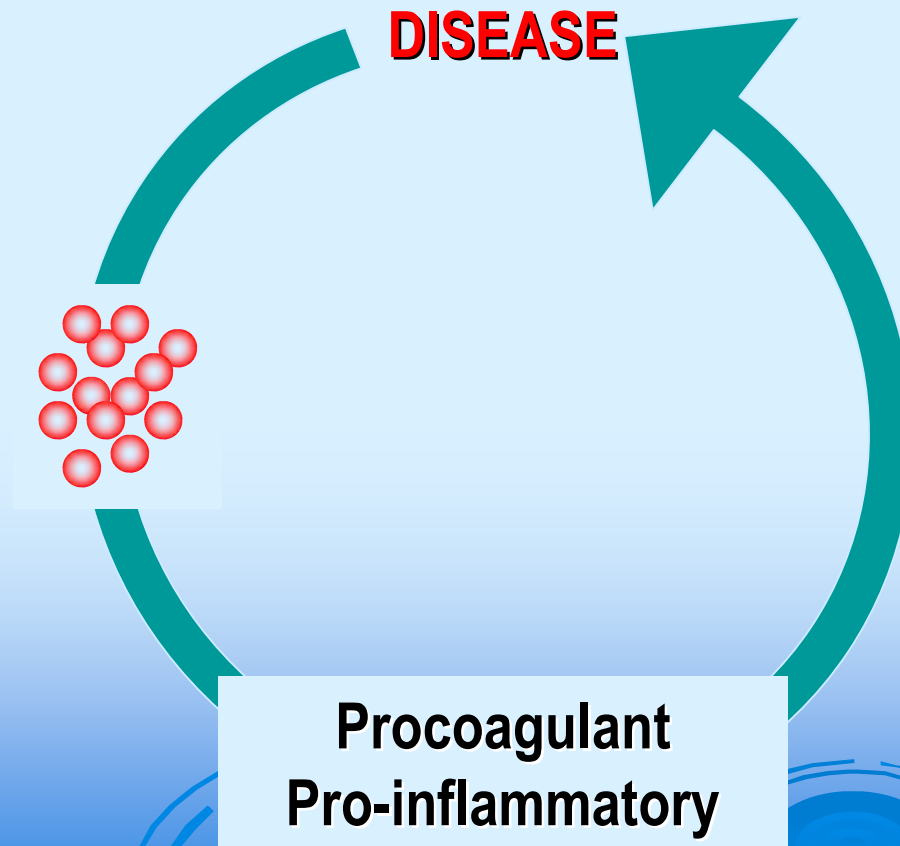
Form AH100
03-2009

PATHOLOGICAL MICROPARTICLES



Microparticles

Cause and consequence of disease states



Edinburgh March, 2009

Form AH100
03-2009

Clinical usefulness of MPs study

- **Modulate the Hemostatic balance and can cause its disruption.**
- **Procoagulant MPs in Immune-mediated Thrombosis.**
- **Procoagulant MPs in Atherothrombosis.**
- **Angiogenesis and MPs.**
- **Circulating MPs : Effectors in the Tuning of Thrombotic Propensity Associated with Cardiovascular Risk.**
- **Pharmacological Modulation of Circulating MPs.**

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Clinical applications of MPs

- Prognosis of myocardial infarction.
- Follow-up and therapy monitoring of patients with myocardial infarction.
- Prognosis of recurrence risk.
- Diabetes, Malignancy, Pregnancy.

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Pathological variations of microparticles

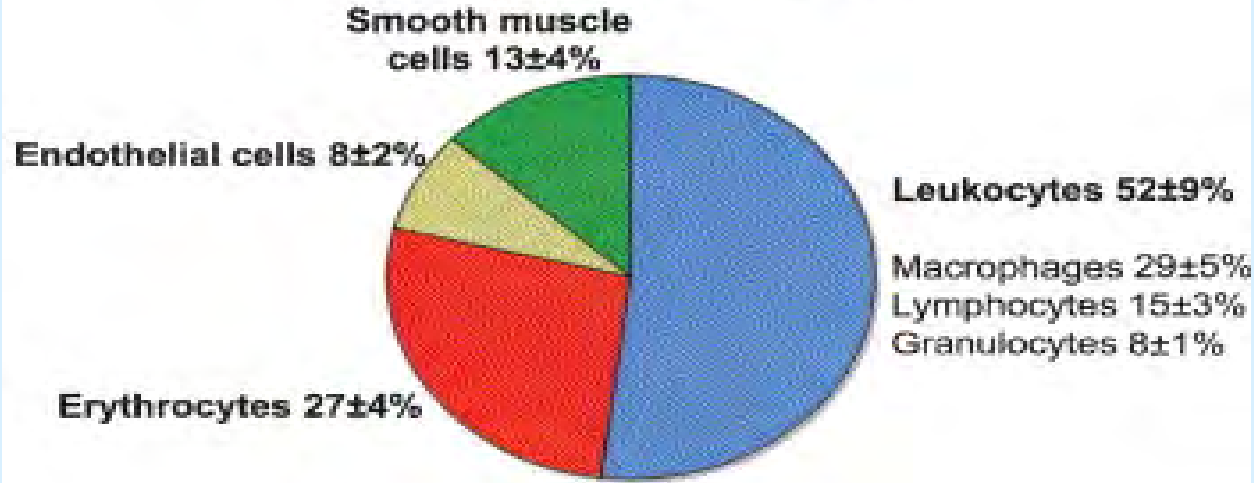
- **Elevated in M.I. (x2 to x10)**
- **Elevated in cancer (predictor of metastasis?)**
- **When Elevated, can predict vascular complications in diabetes**
- **Elevated in haemophilia (x10)**
- **During Novoseven (Vlla) therapy**
- **Correlates with severity of hypertension**

Note: responds to therapy efficacy

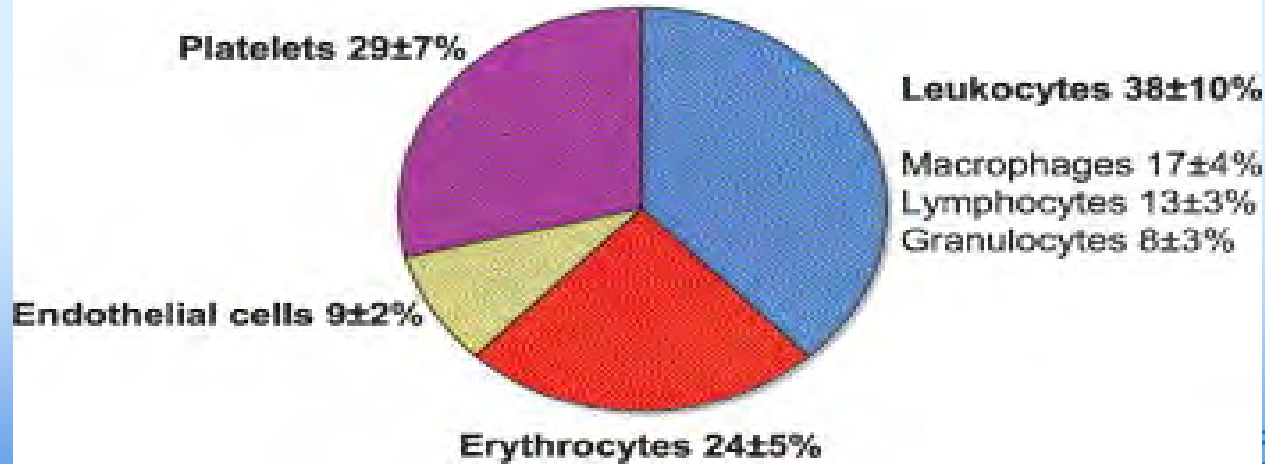
Form AH100
03-2009

Edinburgh March, 2009

Atherosclerotic plaque



Venous blood



MPs can be measured in atherosclerotic plaques...

... and in Blood!

Edinburgh March, 2009

Leroyer AS, et al. J Am Coll Cardiol 2007;49:772

Form AH100
03-2009

Evaluation of Microparticles

➤ **Flow Cytometry:**

- Only « large microparticles ($> 0.4 \mu$) are measured (size, content).
- Characterised by antibody/label used.

➤ **Activity/Immuno-Assay:**

- All MPs are measured (including < 0.4 or 0.1μ)
- Measurement of associated procoagulant activity (PS equivalent).
- Identification of cell origin with MoAbs.

Edinburgh March, 2009

Form AH100
03-2009

Microparticle measurement

Different methodologies are available for MPs determination:

- Flow cytometry relies on the antigenic composition of MPs and allows them to be enumerated according to their cellular origin.
- ELISA capture with Annexin V or antibody and determination of procoagulant activity of MPs.

Edinburgh March, 2009

Form AH100
03-2009

Microparticles in Fibrinolysis

- Elevated PAI-1 induces an important release of endothelial MPs with procoagulant activity.
- Cancer cells release microparticles exposing TF, or uPAR-uPA.
- Chemotherapy generates microparticles from tumoral cells, possibly inducing fibrinolysis (ovarian, prostatic malignancies, acute promyelocytic leukemia,...) and/or thrombosis.

Form AH100
03-2009

Edinburgh March, 2009



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Endothelial MPs



HUYEC + FCS

2 μ m

Taraboletti et al. *Am J Pathol* 2002



7768 Service Center Drive • West Chester OH 45069

Phone: 513.770.1991

Toll Free: 866.783.3797

Fax: 513.573.9241

Email: info@aniara.com

www.aniara.com

Fibrinolytic Markers and Microparticles in Cancer

- Cancer cells promote fibrinolysis for migrating, producing metastasis (uPAR-uPA and MMPs mediated).
- Fibrin protects from tumor growth, but also protects malignant cells from host defence.
- Fibrin attracts EC and favors angiogenesis.
- Cancer cells generate TF and MPs exposing TF, inducing « hypercoagulability ».

Form AH100
03-2009

Edinburgh March, 2009

Emerging markers of tumor invasion

They bring complementary information on disease activity to usual cancer markers:

➤ **Measured in tissue extracts:**

uPA

PAI-1

uPA-PAI-1 complexes



Breast Cancer, many tumors

TF



Many Tumors (lung, pancreatic, gastric,....)

➤ **Measured in plasma:**

MMP-2

MMP-9

TIMP-1

MMP-9-TIMP complexes



Tumors invasiveness



Form AH100
03-2009

Edinburgh March, 2009

Conclusions

- Fibrinolysis and Micro-Particles are emerging or «rediscovered» body's functions with multiple impacts and implications in diseases.
- Their laboratory exploration can contribute to management of pathology and therapy monitoring.
- Understanding their mechanisms of action is useful for new drug developments.
- High potential, in clinical practice, provided that pre-analytical variables are well controlled.

Form AH100
03-2009

Edinburgh March, 2009