NKCP in Cancer Management (removing cancer's camouflage)

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Discovery of Thrombosis and Cancer

- In 1865, Armand Trousseau observed several cases of severe blood clotting in patients with malignancy [1] but was unable to speculate on the underlying mechanism.
- In 1882, Bizzozero first demonstrated that blood platelets, or thrombocytes, adhered to damaged blood vessels and hypothesised that these blood components played a central role in haemostasis and experimental thrombosis [2].
- Subsequently, Riess reported an association between thrombocytosis (defined as a platelet count of >400x109/ L of blood) and cancer death [3].
- Almost a century later, these preliminary observations were revisited and confirmed [4, 5], initiating a renewed interest in a potential role for platelets in cancer metastasis, invasion and angiogenesis [6-10].

https://www.intechopen.com/books/the-non-thrombotic-role-of-platelets-in-health-and-disease/platelets-allies-of-tumour-cells

Thrombosis is the second most common cause of death of cancer patients!

Plasminogen Activator Inhibitor-1 and Thrombotic Cerebrovascular Diseases

Anna Tjärnlund-Wolf, PhD, Helen Brogren, PhD, [...], and Xiaoying Wang, PhD

Additional article information

Alterations in thrombosis and fibrinolysis comprise important parts of stroke pathophysiology. A key step in the fibrinolytic process includes the tissue-type plasminogen activator (tPA)-mediated conversion of the proenzyme plasminogen into the active protease plasmin, which in turn degrades the fibrin structure of intravascular thrombi. There are a number of review articles well summarizing molecular

the fibrinolytic

fibrin

intravascular thrombi. There are a number of review articles well summarizing molecular

the fibrinolytic

fibrin

"... PAI-1 has become recognized as a central molecule linking pathogenesis and progression of thrombotic vascular events ..."

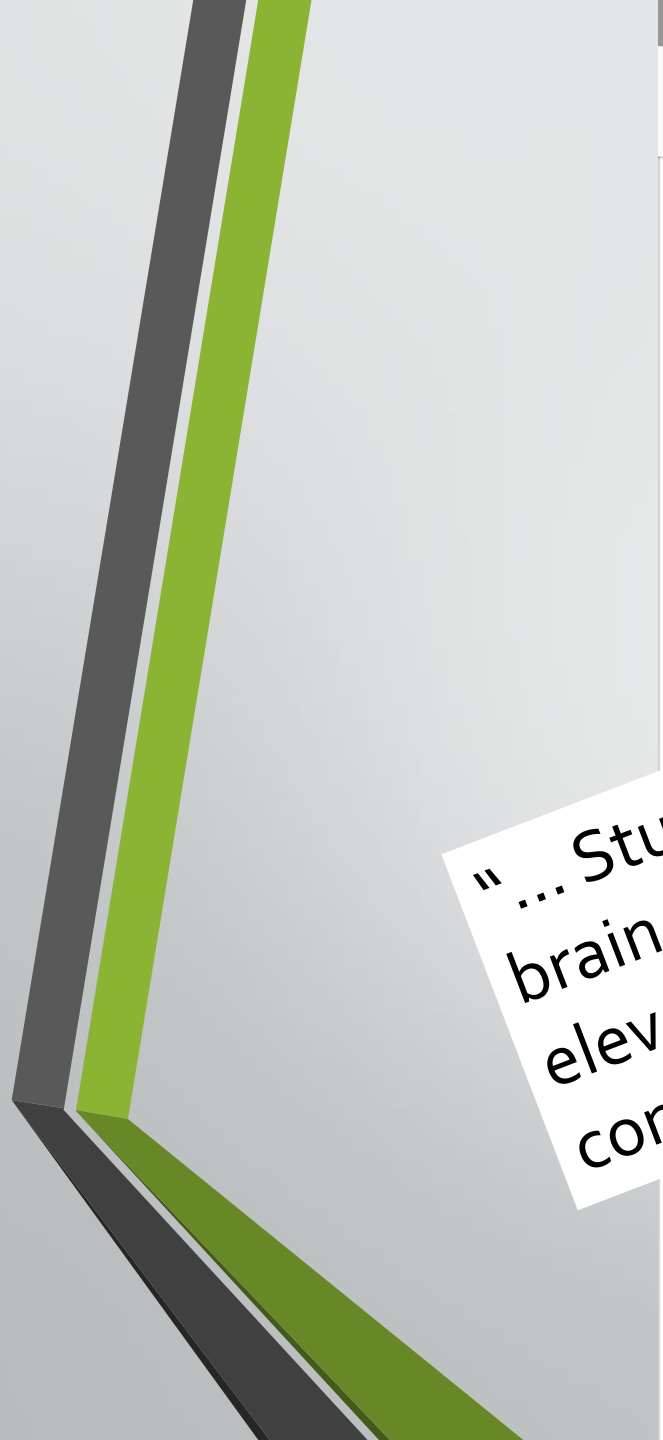
...ecently

...rinolysis

...e level of plasmin, by

...plasmin. The roles of these 3 inhibitors are complementary in thrombolysis.[3]

PAI-1 has become recognized as a central molecule linking pathogenesis and progression of thrombotic vascular events including stroke. As a main endogenous inhibitor of tPA, PAI-1 might be related to reperfusion efficacy and hemorrhagic risk of tPA thrombolytic therapy. Moreover, a clear association has been observed between elevated PAI-1 plasma levels and



Cancer-associated pathways and biomarkers of venous thrombosis.

Hisada Y, et al. Blood. 2017.
Show full citation

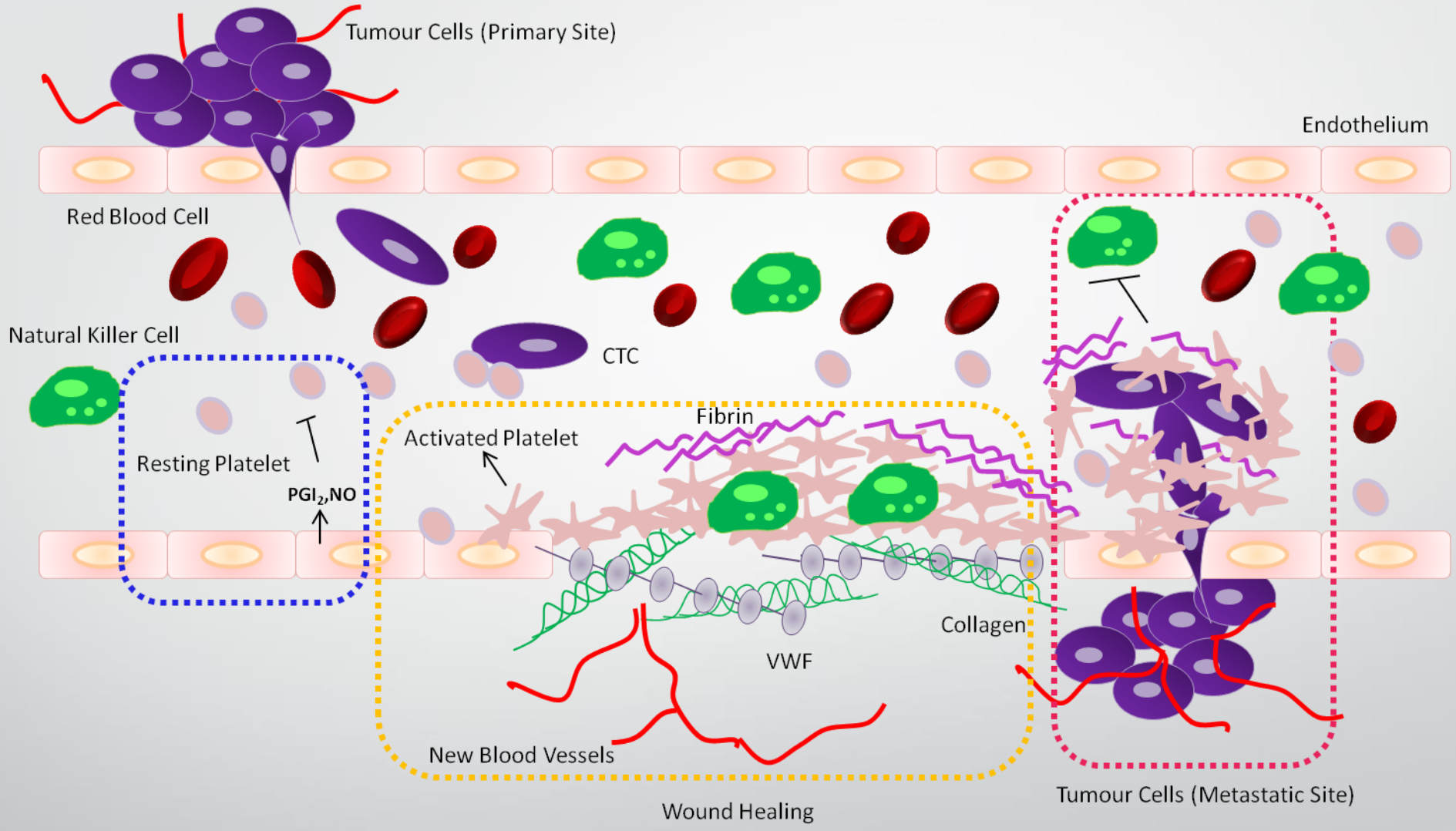
Abstract

Cancer patients have an increased risk of venous thromboembolism (VTE). In this review, we will summarize common and cancer type-specific pathways of VTE in cancer patients, with a focus on the role of leukocytes, platelets and tissue factor (TF+) microvesicles, alone or in [illegible] observed and ovarian cancer the threshold required for -selectin has been identified as a marker of cancer-associated thrombosis in a general cancer population, and may reflect activation of the endothelium. P-selectin expression by the endothelium may enhance VTE by increasing the recruitment of leukocytes. Studies with pancreatic and brain cancer patients suggest that elevated levels of PAI-1 may contribute to VTE. Although elevated levels of TF+ microvesicles have been observed in patients with different types of cancer, association between TF+ microvesicles and VTE has only been observed in pancreatic cancer. Podoplanin expression is associated with VTE in brain cancer patients and may activate platelets. Future studies should measure multiple biomarkers in each cancer type to determine if combinations of biomarkers can be used as predictors of VTE. A better understanding of the pathways that increase VTE in cancer patients may lead to the development of new therapies to reduce the morbidity and mortality associated with thrombosis.

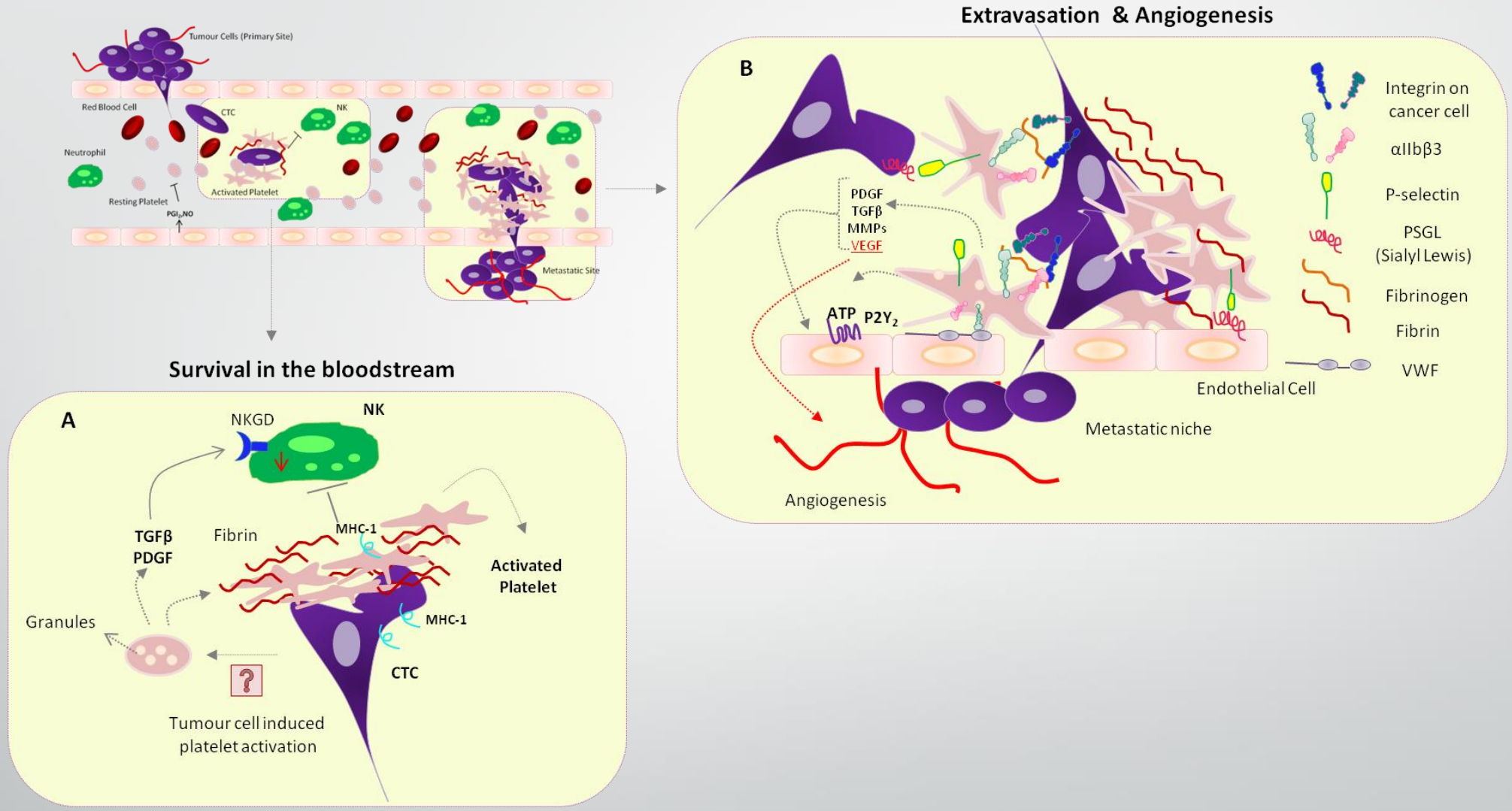
PMID: 28807983 [PubMed - as supplied by publisher]

"... Studies with pancreatic and brain cancer patients suggest that elevated levels of PAI-1 may contribute to VTE. ..."

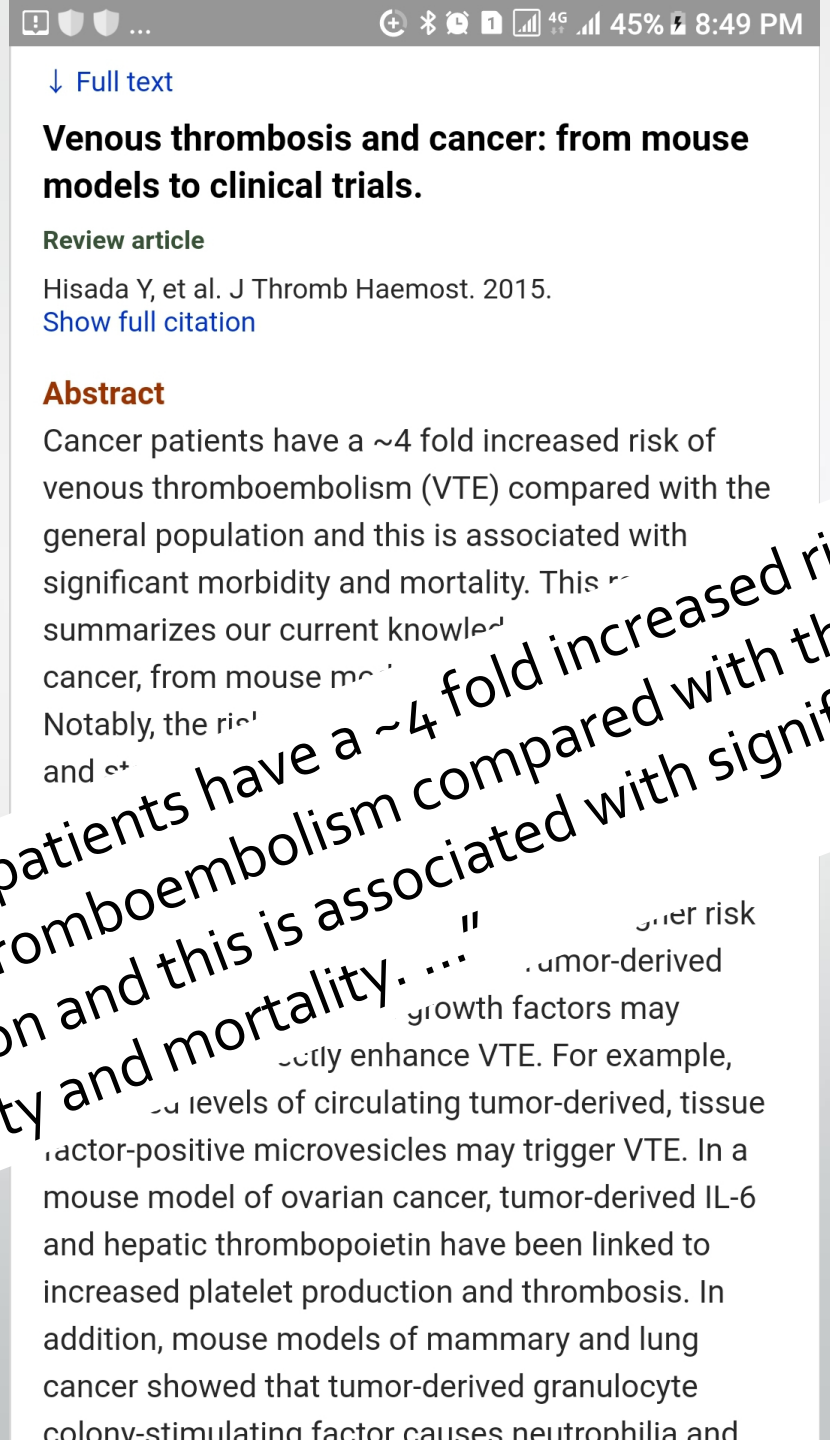
Cancer and Platelets



https://www.intechopen.com/books/the-non-thrombotic-role-of-platelets-in-health-and-disease/platelets-allies-of-tumour-cells



https://www.intechopen.com/books/the-non-thrombotic-role-of-platelets-in-health-and-disease/platelets-allies-of-tumour-cells


45% 8:49 PM
↓ Full text
Venous thrombosis and cancer: from mouse models to clinical trials.
Review article
Hisada Y, et al. J Thromb Haemost. 2015.
Show full citation
Abstract
Cancer patients have a ~4 fold increased risk of venous thromboembolism (VTE) compared with the general population and this is associated with significant morbidity and mortality. This r
summarizes our current knowle
cancer, from mouse mo
Notably, the ris
and st
"... Cancer patients have a ~4 fold increased risk of venous thromboembolism compared with the general population and this is associated with significant morbidity and mortality. ..."
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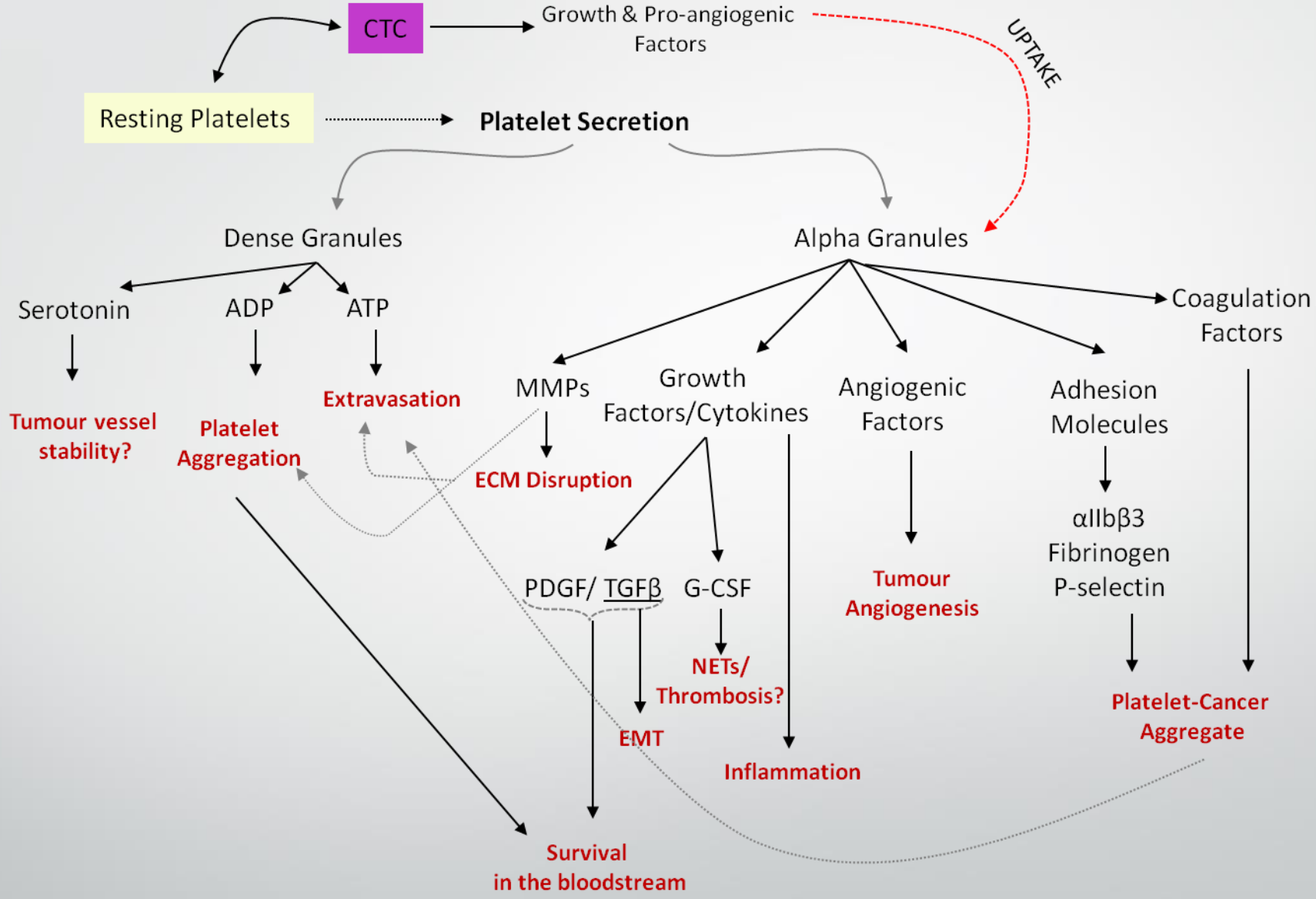
umor-derived
growth factors may
ctly enhance VTE. For example,
d levels of circulating tumor-derived, tissue
factor-positive microvesicles may trigger VTE. In a
mouse model of ovarian cancer, tumor-derived IL-6
and hepatic thrombopoietin have been linked to
increased platelet production and thrombosis. In
addition, mouse models of mammary and lung
cancer showed that tumor-derived granulocyte
colony-stimulating factor causes neutrophilia and



https://www.intechopen.com/books/the-non-thrombotic-role-of-platelets-in-health-and-disease/platelets-allies-of-tumour-cells

Coagulation/Fibrinolysis System

- Comprised of a series of complicated reactions designed to maintain the balance between healthy circulation and prevention of excess bleeding. Many factors can influence this system, however, it is not easily disrupted.
- In the event that the system shifts towards excess thrombus formation, it is challenging to return the system back to balance. Because it is difficult to lyse a formed thrombus, the emphasis of MOST DRUGS is placed on PREVENTION OF THROMBUS FORMATION rather than on thrombolysis.

Anticoagulants in Cancer Management

- In the average ambulatory cancer population, the overall risk of venous thromboembolism is 4% to 6%; however, this risk is clearly higher with certain tumor types—gastrointestinal cancers, especially gastric and pancreatic disease, along with lung cancer and a few others. Risk is increased for the first 3 months after diagnosis and accumulates over time. Bleeding complications are also increased in cancer patients, both with treatment and with the use of thromboprophylaxis.

http://www.ascopost.com/issues/march-25-2016/anticoagulation-in-patients-with-cancer-understanding-the-complexities-of-prophylaxis-and-management/

Management Guidelines

- There are guidelines from the NCCN [National Comprehensive Cancer Network], ASCO, the ACCP [American College of Clinical Pharmacology], ESMO [European Society for Medical Oncology], and GFTC [Groupe Francophone Thrombose et Cancer].
- All of these guidelines presently state that for cancer patients with new blood clots, treat with low–molecular-weight heparin for the first 3 to 6 months. After that, the guidelines vary somewhat in terms of next-step recommendations.
- (Heparin inactivates thrombin which stops the formation of fibrin thus stopping clot formation. The side effect is bleeding.)

http://www.ascopost.com/issues/march-25-2016/anticoagulation-in-patients-with-cancer-understanding-the-complexities-of-prophylaxis-and-management/

Long-Term Anticoagulation

- An important study that might provide some data was presented at ASH 2015 by **Chatree Chai-Adisaksopha, MD**, and colleagues, demonstrating that some patients can be switched from low–molecular-weight heparin to warfarin after 6 months.[9] The study evaluated data from the RIETE Registry, selecting 1,502 patients who completed 6 months of low–molecular-weight heparin, then either continued low–molecular-weight heparin or switched to warfarin at the treating physician's discretion. They found no statistically significant difference in recurrent venous thromboembolism between these groups (HR = 0.67, 95% CI = 0.44–1.02, *P* = .06) and no difference in major bleeding or total bleeding, although the number of events in both groups was low
- The authors concluded that in patients with cancer-associated thrombosis who complete 6 months of anticoagulation, switching to warfarin does not increase the risk of recurrent venous thromboembolism, major bleeding, or total bleeding as compared to continuing low–molecular-weight heparin in selected patients. It showed that warfarin can be an acceptable alternative anticoagulant in patients who, for one reason or another, do not desire long-term treatment with low–molecular-weight heparin.
- (Warfarin blocks the formation of vitamin k dependent clotting factors. The side effect is bleeding.)

Chai-Adisakspha C. et al. Switching to Warffarin after 6-Month Completion of Anticoagulant Treatment for Cancer-Associated Thrombosis. Blood 2015 126:430

Aspirin and Cancer

- There is convincing evidence that regular low-dose aspirin not only reduces vascular disease incidence and mortality [2,3], but also reduces the incidence and mortality of colorectal and other cancers [4–7]. Furthermore, there is growing evidence which suggests that aspirin, used as an adjuvant treatment following a diagnosis of cancer, may reduce metastatic spread and may increase the survival of patients with cancer.
- It appears likely that low-dose aspirin has a beneficial role as an adjunct treatment of cancer. Reductions in mortality are shown in colon cancer, probably in prostate cancer and possibly in breast and individual studies of several other cancers also suggest benefit. Aspirin benefit in colorectal cancers, and possible other cancers, may be restricted to patients with tumours expressing certain genetic mutations. However, other benefits of low-dose aspirin, including reductions in metastatic spread and in vascular events, including venous thromboembolism appear to be independent of these biomarkers, and so information on aspirin should be given to patients whatever the state of the possible biomarkers.

Elwood P. C. et al., Aspirin in the Treatmentof Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One. 2016; 11(4): e0152402.

Aspirin can cause stomach and intestinal bleeding which is fatal. Although the risk is low.

Is there anything that will dissolve the clot but will not prevent clotting such that there will be no bleeding side effect?

Natto

- **Natto** is a Japanese traditional fermented food made from soybean with growing *Bacillus subtilis* var. *natto*.

NKCP

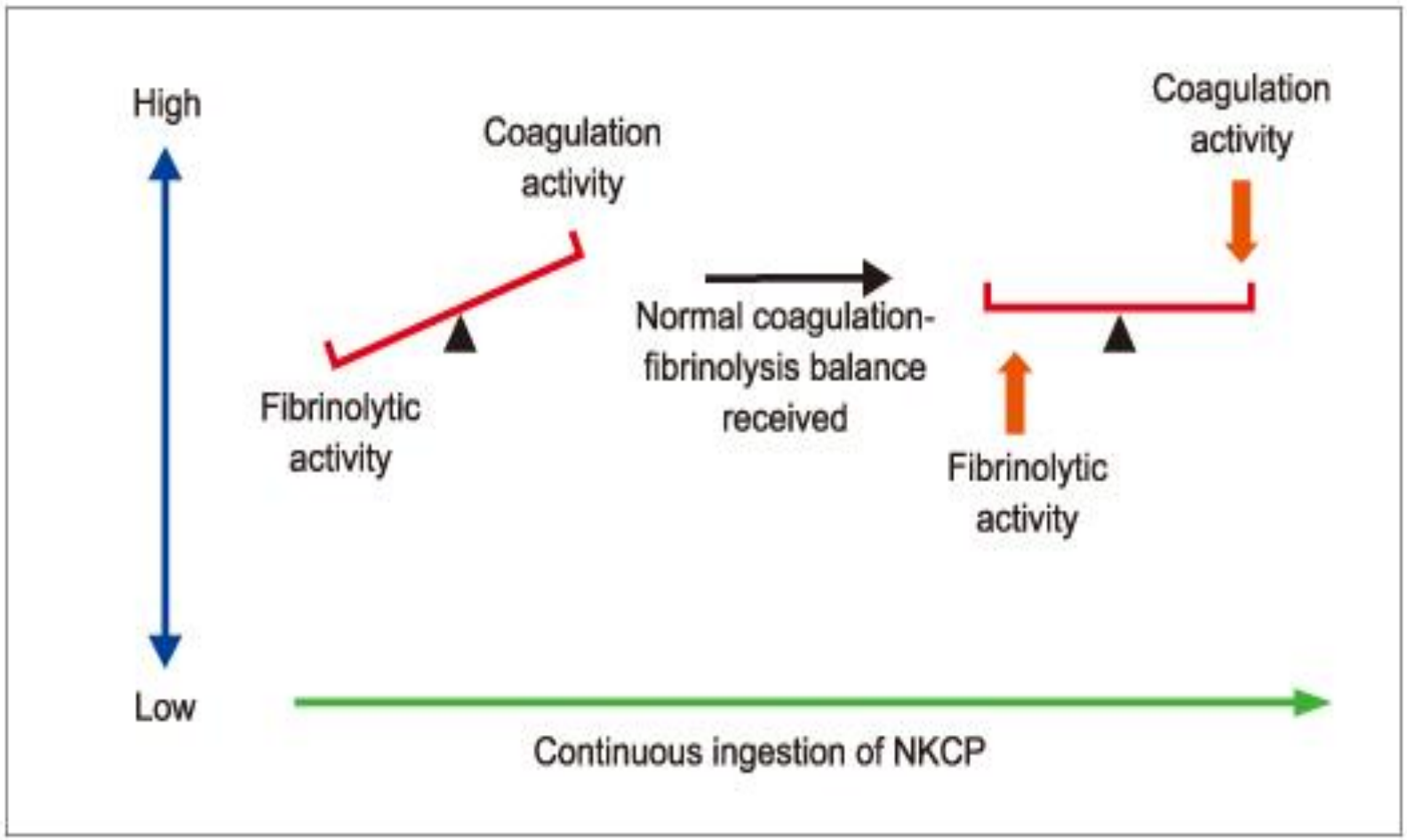
- **Food based extract of "natto", a Japanese traditional fermented food made from soybean.**
- **Purified filtrate of *Bacillus subtilis* var. *natto* culture**
- **Purification**
  - **removes most of distinctive odor of natto and its vitamin K2**
  - **yields an easy-to-eat food that has a wide variety of uses as a functional food.**
- **Contains proteolytic enzymes secreted by *Bacillus subtilis* var. *natto* (*Bacillus subtilis* var. *natto*-produced protein)**
  - **balance clotting mechanisms in the blood.**
- ***In vitro* and clinical studies have demonstrated that the consistent intake of NKCP over a prolonged period helps to maintain normal circulation. The safety of NKCP has been demonstrated in safety studies.**

Single-dose	**LD50＞5,000mg/kg**
Repeated-dose	NOAEL Males: > 1,325mg/kg body weight/day Females: > 1,541mg/kg body weight/day
Mutagenicity	Negative (± metabolic activation)
Antigenicity (guinea pigs)	Negative for active systemic anaphylactic reaction (ASA) and passive cutaneous anaphylactic reaction (PCA)

Effect on bleeding time (rats)	**In rats orally given NKCP, a 0.5mm incision was made in the tail tip after 1 hour to measure bleeding time. NKCP at 300mg/kg did not prolong the bleeding time.**
Interaction with warfarin (rats)	NKCP at 250mg/kg was administered into the duodenum by the in situ loop method in rats, in which bleeding time was delayed by treatment with warfarin, and blood collected after 6 hours was measured for coagulation time. The warfarin treatment significantly prolonged the coagulation time in comparison with the control group, but no added delay of coagulation was observed in the warfarin + NKCP treatment group compared with warfarin treatment group.

Long-term administration (humans)	**Twenty-three healthy adults were given NKCP at 250mg/day for 12 weeks, and no clinically significant adverse events were observed. There were no statistically significant changes in hematological or biochemistry tests.**
	Five healthy adults were given NKCP at 750mg/day for 6 consecutive weeks to study and observe changes in laboratory test values (hematological tests, biochemistry tests, and blood coagulation/fibrinolysis parameters) and adverse events. As a result, ELT shortened, t-PA decreased, and thromboplastinogen activity test (TAT) increased but all values were within normal range. In addition, no adverse events were observed, suggesting NKCP safety.
High dose administration (humans)	Eight healthy adults were given NKCP at 1,250mg/day for 7 consecutive days. Observation of clinical signs and laboratory tests were utilized to evaluate NKCP safety. There were no clinically significant adverse events. There were no abnormal changes in hematological or biochemistry tests.

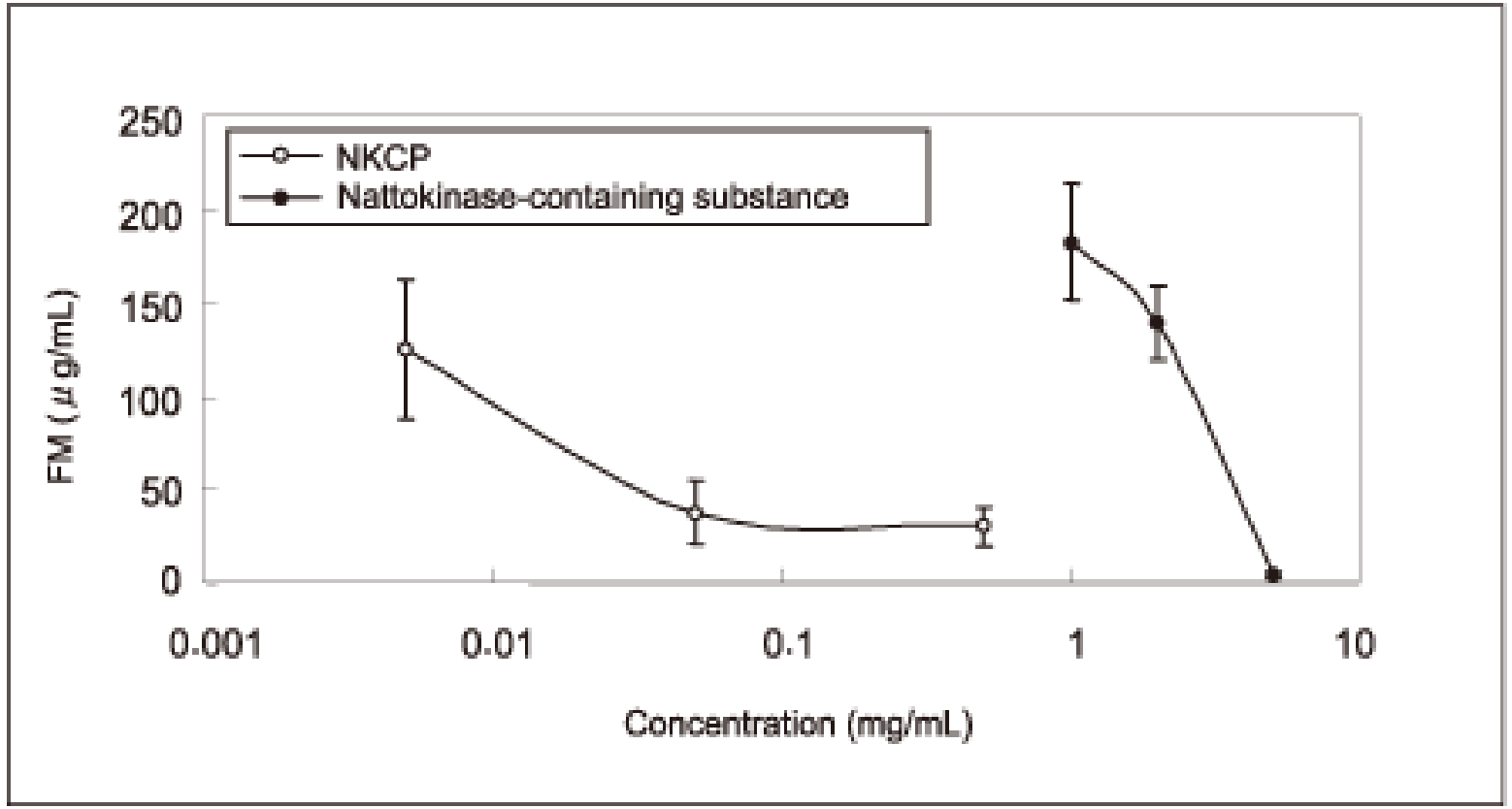
Effect of continuous intake of NKCP



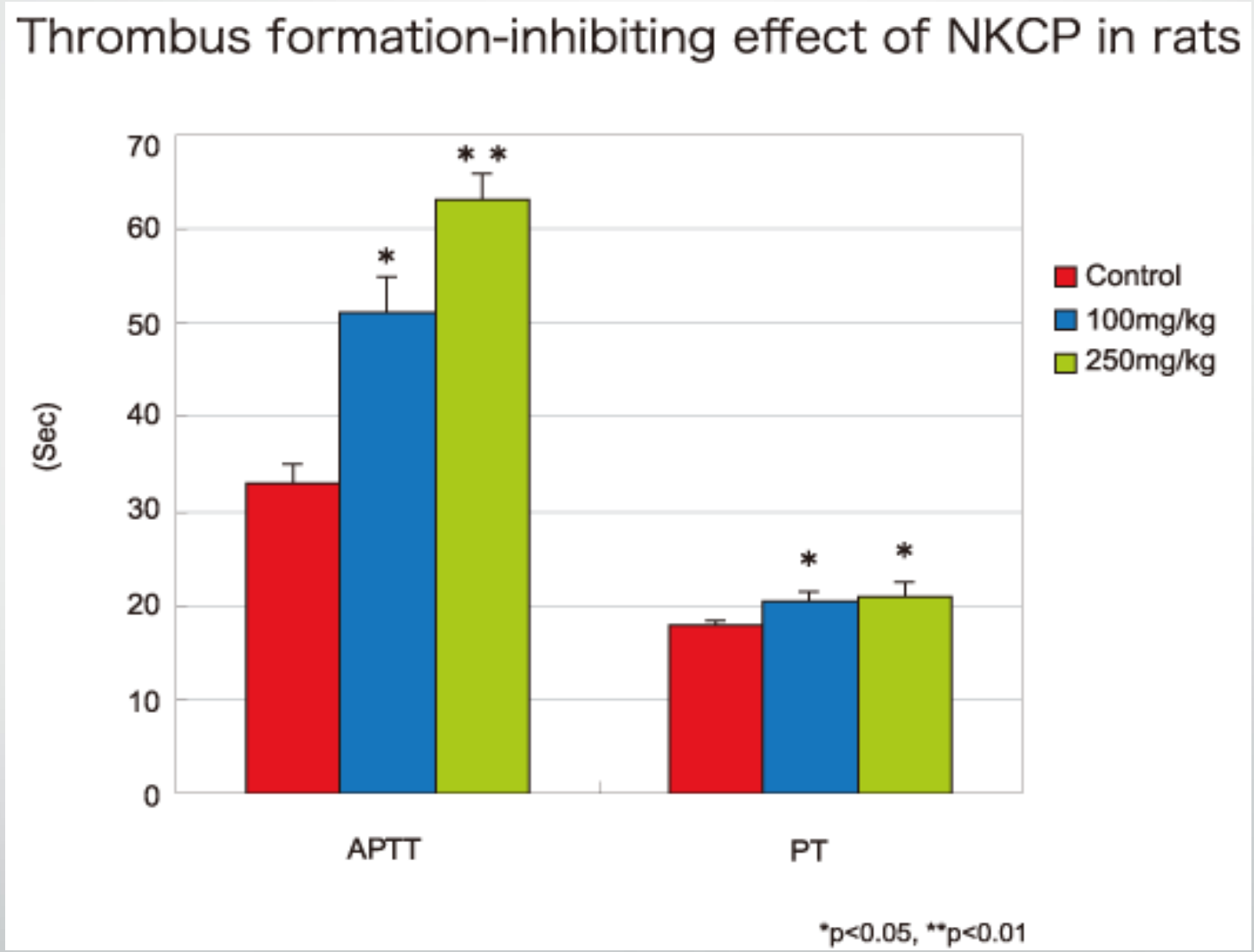
Mechanism of Action

- Inhibiting thrombus formation *in vitro* and *in vivo*
- Decreasing the viscosity of blood *in vitro* and *in vivo*
- Lysing thrombi *in vitro* and *in vivo*

Anticoagulant effects of NKCP and nattokinase-containing substance

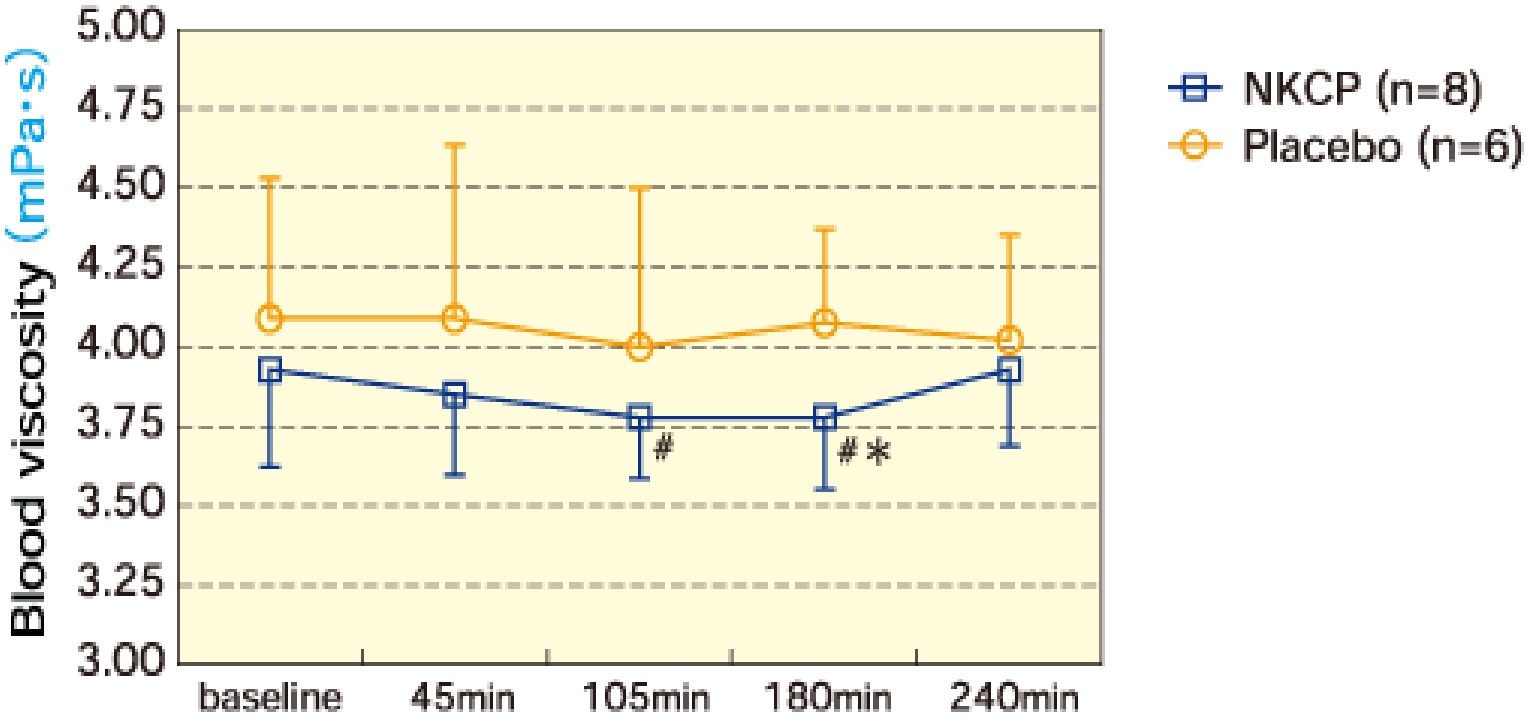


The 54th Study Meeting of Rheology 2006;
Department of Legal Medicine, Dokkyo Medical University School of Medicine



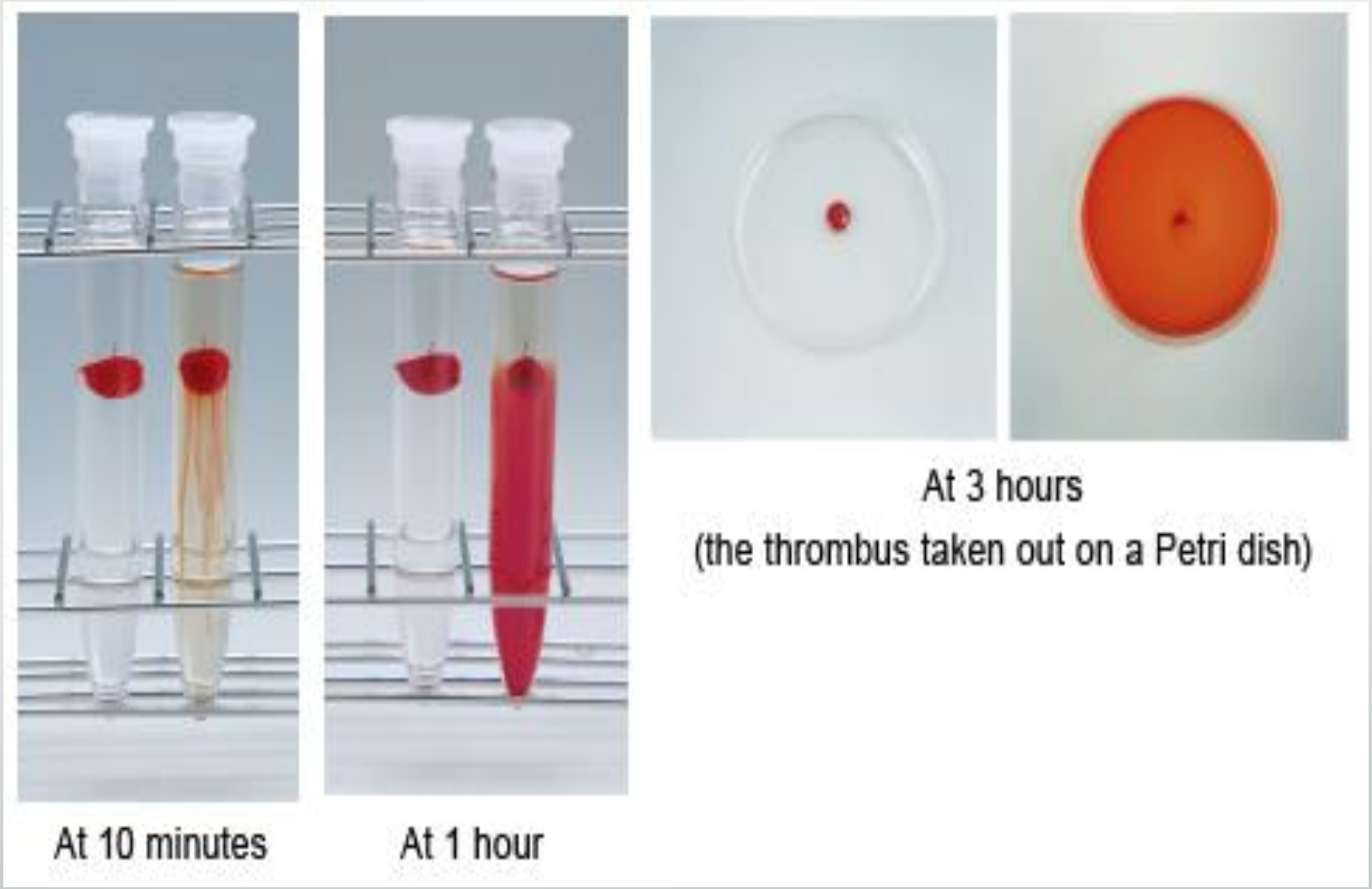
Research and Development Department, Daiwa Pharmaceutical Co., Ltd., 2001

Changes in blood viscosity after NKCP ingestion



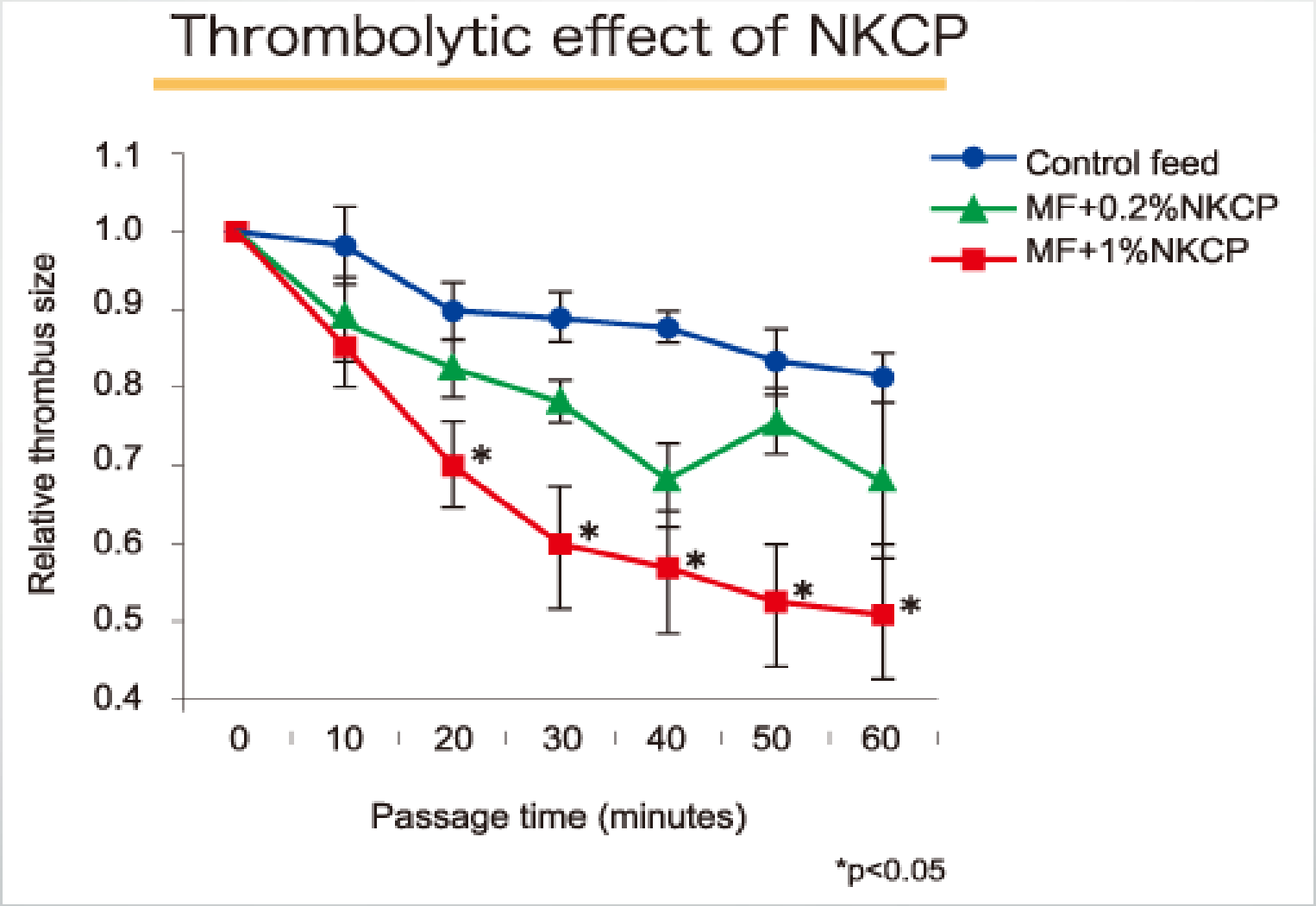
#: Difference from baseline by Duncan s multiple test, p<0.05
*: Difference between NKCP vs placebo by paired t test, p<0.05

Department of Legal Medicine, Dokkyo Medical University School of Medicine



J. Pharmacol Sci 2005; 99: 247-251

Thrombolytic effect of NKCP



Pathophysiol Haemost Thromb 2003; 33: 138-143

Table 1: Enzymes extracellularly secreted by *Bacillus subtilis*

Protease	Gene	Properties
Bacillopeptidase F	*bpr*	Molecular weight by SDS-PAGE: 47kDa [1], 48kDa [2] Secreted as a 92kDa protein and converted into 80kDa and 48kDa proteins [2]. It has high esterase activity as well as proteinase activity [3].
Subtilisn (alkaline) protease	*apr*	Molecular weight by SDS-PAGE: 20kDa[4], 28kDa[5] The structure is similar to that of nattokinase. Its casein decomposing activity and direct fibrinolytic activity have been confirmed. It is applied as protease in laundry detergent. The ability to decompose and inactivate plasminogen activator inhibitor type 1 (PAI-1) has also been reported [5].
Neutral protease	*npr*	A major protease like Subtilisin (alkaline) protease.
Extracellular protease	*epr*	Molecular weight by SDS-PAGE: 40-34kDa[6]
Metallo protease	*mpr*	Molecular weight by SDS-PAGE: 28kDa[1] Expression of the activity requires a metal atom.

1)*Journal of Bacteriology* 1990; 172: 1019-1023
2)*The Journal of Biological Chemistry* 1990; 265: 6845-6850
3)*Journal of Bacteriology* 1990; 172: 1470-1477
4)*Experientia* 1987; 43: 1110-1111
5)*The Journal of Biological Chemistry* 2001; 276: 24690-24696
6)*Mol Gen Genet* 1990 May; 221(3): 486-490

Changes in fibrinolysis/coagulation parameters with NKCP intake (n=23)

Parameters	Normal values	Before intake	At 1 month	At 2 months
ELT [1)]	6 – 12 hrs.	9.0±1.3	8.1±1.5**	8.0±1.5**
t-PA [2)]	≦10 ng/mL	5.4±2.6	5.8±2.8	6.4±2.2*
FDP [3)]	≦4 μg/mL	3.0±0.7	2.0±0.6*	3.0±0.7

Figure represents mean±S.D.

Duncan's multiple range test: *p<0.05, **p<0.01

Changes in subjective symptoms with NKCP intake

Symptom	Conditions	Before intake	At 1 month	At 2 months
Shoulder stiffness	Severe	5	1	1
	Moderate	10	9	10
	No Symptom (including mild headache)	8	9	11
	Remarkable improvement	-	4	1
	Shirley-Williams multiple test	-	P<0.05	P<0.05

Figures represent the number of patients experiencing symptoms.

Significant difference by Multiple Range Test.

Journal of the Japanese Society of Biorheology 2004; 18 (1)

Mechanism of Action

- Inhibiting thrombus formation *in vitro* and *in vivo*
- Decreasing the viscosity of blood *in vitro* and *in vivo*
- Lysing thrombi *in vitro* and *in vivo*

Does NKCP reduce PAI-1 levels?

Monitoring the effect of combined Lentin plus and NKCP with liquid biopsy for circulating tumor cells (representative cases)

Case: Pancreatic Cancer

St. Luke's Medical Center
Quezon City

CT-SCAN

1409137659 Sep-16-1931 83 year/s M

SPECIMEN NO.

ROOM/BED	REFERRING PHYSICIAN	DATE/TIME OF EXAM	DATE OF REQUEST	OR/CI No.
428/428	SANTOS, DANILO SANTOS M.D. (809N)	10 17 2014 10:39 PM	10 16 2014 05:48 PM	0069141007762096

EXAMINATION: WHOLE ABDOMEN CT-SCAN

HISTORY

Diagnosed with pancreatic adenocarcinoma

COMPARISON

CT scan of the abdomen (done in another institution) dated August 29, 2014

TECHNIQUE

Non-contrast and contrast enhanced axial 256-Multislice CT scan of the whole abdomen and dynamic CT scan of the pancreas using 3 x 1.5 mm slices with coronal and sagittal reconstructions were done. 90 ml per IV of iopamidol (Iopamiro) were given as contrast medium after a negative test dose. No oral contrast as per request.

FINDINGS

There is a heterogeneously enhancing lobulated mass with cystic spaces in the pancreatic head measuring 7.4 x 6.5 x 6.6 cm (previously 7.3 x 6.4 x 5.5 cm). It is abutting the proximal superior mesenteric vein with no filling defect. The rest of the pancreas is mildly atrophic with ductal dilatation.

The liver is not enlarged and exhibits a smooth outline. Two subcentimeter enhancing foci are seen at segment VI of the liver. The intrahepatic ducts are not dilated. The hepatic vasculatures are patent.

The gallbladder is surgically absent. The common duct is slightly dilated measuring 1.2 cm in diameter.

The spleen and both adrenal glands are normal.

The RIGHT kidney shows a calcific density in the middle calyx measuring 0.2 x 0.2 x 0.2 cm (232 HU). Subcentimeter bilateral renal cysts are noted.

No enlarged lymph node and ascites.

WILSON T. TAN, M.D.
RESIDENT

CURSILL P. IBAY, M.D.
RADIOLOGIST

This report has been electronically validated. No signature is required.

PRINT DATE/TIME: 10 17 2014 03:17 PM
TRANSCRIBED BY: DELA CRUZ, MARICEL

DOCUMENT NO.: 00691014477701

St. Luke's Medical Center
Quezon City

CT-SCAN

1409137659 Sep-16-1931 83 year/s M

SPECIMEN NO.

ROOM/BED	REFERRING PHYSICIAN	DATE/TIME OF EXAM	DATE OF REQUEST	OR/CI No.
428/428	SANTOS, DANILO SANTOS M.D. (809N)	10 17 2014 10:39 PM	10 16 2014 05:48 PM	0069141007762096

The aorta and both common iliac arteries are atherosclerotic.

There is a fat containing umbilical/RIGHT paraumbilical hernia. The visualized gastrointestinal tract is grossly unremarkable.

The urinary bladder is well distended without intraluminal abnormal density. No filling defect noted. The wall is not thickened. The prostate gland is enlarged measuring 5.2 x 4.7 x 5.7 cm (72 grams) with parenchymal concretions.

There are hypertrophic change in the visualized osseous structures with levoscoliosis of the thoraco-lumbar spine.

Incidental note of reticular densities in the imaged lower lung zones. Subcentimeter non calcified subpleural nodules are also noted in the lingula and LEFT lower lobe. Cardiomegaly is appreciated.

IMPRESSION

HETEROGENEOUS PANCREATIC HEAD MASS WITH SLIGHT INTERVAL INCREASE IN SIZE SINCE AUGUST 29, 2014 WITH NO VASCULAR INVOLVEMENT

NO ENLARGED LYMPH NODE

SUBCENTIMETER HEPATIC ENHANCING FOCI AT SEGMENT VI MAY REPRESENT HEMANGIOMA

STATUS-POST CHOLECYSTECTOMY

NON OBSTRUCTIVE RIGHT NEPHROLITHIASIS AND BILATERAL RENAL CYSTS

ATHEROSCLEROTIC AORTO-ILIAC VESSELS

FAT CONTAINING UMBILICAL/PARAUMBILICAL HERNIA

ENLARGED PROSTATE GLAND WITH CONCRETIONS

WILSON T. TAN, M.D.
RESIDENT

CURSILL P. IBAY, M.D.
RADIOLOGIST

This report has been electronically validated. No signature is required.

Lung Center of Philippines
Department of Pathology
Molecular Diagnostics and Cellular Therapeutics Laboratory
Cell Culture Report

PATIENT NAME:	
Age:	83
Gender:	Male
Diagnosis:	Pancreatic Mass
Specimen:	Blood
Sample Submitted:	November 11, 2014
Sample Processed:	November 11, 2014

Culture Description: Blood buffy coat was extracted from the blood sample by centrifugation and incubated with Anti-Epithelial Cell Adhesion Molecule (EpCAM) magnetic beads. Captured circulating tumor cell (CTC) count was determined using light microscopy. CTC's were confirmed by fluorescence microscopy using DAPI, cytokeratin markers.

	Cells/mL	Date Determined
CTC Count	601	11-11-14

Prepared by:

Marlon Dave J. Echavez
Jennifer P. Panuelos,RMT
Research Analyst

Noted by:

Francisco M. Heralde III, R.N., PhD.
Visiting Consultant
Molecular Biology and Biotechnology

Approved by:

Ma. Teresa A. Barzaga, M.D., FPSP
Medical Specialist IV
Head-MDCTL

Lung Center of Philippines
Department of Pathology
Molecular Diagnostics and Cellular Therapeutics Laboratory
Cell Culture Report

PATIENT NAME:	[redacted]
Age:	**83**
Gender:	**Male**
Diagnosis:	**Pancreatic Mass**
Specimen:	**Blood**
Sample Submitted:	**February 23, 2015**
Sample Processed:	**February 23, 2015**

Culture Description: Blood buffy coat was extracted from the blood sample by centrifugation and incubated with Anti-Epithelial Cell Adhesion Molecule (EpCAM) magnetic beads. Captured circulating tumor cell (CTC) count was determined using light microscopy. CTC's were confirmed by fluorescence microscopy using DAPI, cytokeratin markers.

	Cells/mL	Date Determined
CTC Count	2	02-23-15

Prepared by:

Marlon Dave J. Echavez
Jennifer P. Panuelos, RMT
Research Analyst

Noted by:

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Molecular Biology and Biotechnology

Approved by:

Ma. Teresa A. Barzaga, M.D., FPSP
Medical Specialist IV
Head-MDCTL

Lung Center of Philippines
Department of Pathology
Molecular Diagnostics and Cellular Therapeutics Laboratory
Cell Culture Report

PATIENT NAME:	[redacted]
Age:	83
Gender:	Male
Diagnosis:	Pancreatic Mass
Specimen:	Blood
Sample Submitted:	June 11, 2015
Sample Processed:	June 11, 2015

Culture Description: Blood buffy coat was extracted from the blood sample by centrifugation and incubated with Anti-Epithelial Cell Adhesion Molecule (EpCAM) magnetic beads. Captured circulating tumor cell (CTC) count was determined using light microscopy. CTC's were confirmed by fluorescence microscopy using DAPI, cytokeratin markers.

	Cells/mL	Date Determined
CTC Count	1	06-11-15

Prepared by:

Marlon Dave J. Echavez
Research Specialist

Jennifer P. Panuelos, RMT
Medical Technologist I

Noted by:

Francisco M. Heralde III, R.N., PhD.
Visiting Consultant
Molecular Biology and Biotechnology

Approved by:

Ma. Teresa A. Barzaga, M.D., FPSP
Medical Specialist IV
Head-MDCTL

Lung Center of Philippines
Department of Pathology
Molecular Diagnostics and Cellular Therapeutics Laboratory
Cell Culture Report

PATIENT NAME:
Age: 84
Gender: Male
Diagnosis: Pancreatic Mass
Specimen: Blood
Sample Submitted: July 14, 2016
Sample Processed: July 14, 2016

Culture Description: Blood buffy coat was extracted from 7.5ml of blood sample by centrifugation and incubated with Anti-Epithelial Cell Adhesion Molecule (EpCAM) magnetic beads. Captured circulating tumor cell (CTC) count was determined using light microscopy. CTC's were confirmed by fluorescence microscopy using DAPI, cytokeratin markers.

	Cells/ml	Date Determined
CTC Count	1	07-18-2016

Prepared by:

Jennifer P. Panuelos, RMT
Medical Technologist I

Noted by:

Francisco M. Heralde III, R.N., PhD.
Visiting Consultant
Molecular Biology and Biotechnology

Approved by:

Ma. Teresa A. Barzaga, M.D., FPSP
Department Manager III
Head-MDCTL

MAKATI MEDICAL CENTER

Cellular Therapeutics Laboratory
Makati Medical Center
No. 2 Amorsolo Sreet,
Legaspi Village, Makati City,
Philippines 1229
Tel.No.: (02) 888-8999

Circulating Tumor Cell Assay Result

Patient Information

Name: [redacted]
Date of Birth: September 16, 1931
Diagnosis: Pancreatic Cancer
Attending Physician: Dr. Romulo S. De Villa

PIN: N/A
Age/Sex: 83/M
Date of Assay: March 10, 2015
Date of Release: March 12, 2015

Assay Information

Sample type: Whole Blood
Method of Assay: To evaluate relative values of circulating tumor antigens by Polymerase Chain Reaction.

Assay Result

Tumor Markers	Normalized Values (Mar. 12, 2015)		Normalized Values (Nov. 26, 2014)	
	GAPDH	ACTB	GAPDH	ACTB
CEA	2.135	1.826	1.543	1.282
LIVIN	1.955	1.671	1.442	1.198
MAGE-1	Below detection limit	Below detection limit	1.431	1.189
MUC-1	1.628	1.392	1.290	1.072
RECOVERIN	Below detection limit	Below detection limit	1.429	1.187
SURVIVIN	1.914	1.637	1.350	1.089
WNT-1	2.443	2.089	1.626	1.351

Interpretation

Overall, the general trend exhibited a reduction of expression levels of the circulating tumor cell markers relative to the two housekeeping genes or endogenous controls, GAPDH and B-Actin. The expression levels of MAGE-1 and Recoverin for the assay performed March 10, 2015 are below the detection limit. An average decrease of 39.3% relative to GAPDH and 43.4% relative to B-Actin was observed. Correlate this clinically.

Released by:

Melvin E. See
Stem Cell Technologist

Miguel M. de Jesus, RCh
Stem Cell Technologist

Noted by:

Francisco S. Chung Jr., Ph.D.
Co-director

Cases: Breast Cancer

MAKATI MEDICAL CENTER

Cellular Therapeutics Center
Makati Medical Center
No. 2 Amorsolo Street,
Legaspi Village, Makati City,
Philippines 1229
Tel.No.: (02) 888-8999

Circulating Tumor Cell Assay Result

Patient Information

Name:
Date of Birth: December 13, 1953
Diagnosis: Stage IB Breast Cancer
Attending Physician: Dr. Romulo De Villa

PIN: N/A
Age/Sex: 62/Female
Date of Assay: February 22-24, 2016
Date of Release: February 24, 2016

Assay Information

Sample type: Whole Blood
Method of Assay: To evaluate relative values of circulating tumor antigens by Polymerase Chain Reaction.

Assay Result

Tumor Markers	Normalized Values (February 24, 2016)		Normalized Values (October 21, 2015)	
	GAPDH	ACTB	GAPDH	ACTB
CEA	1.377	1.660	1.342	1.360
LIVIN	1.492	1.799	1.259	1.276
MAGE-1	1.307	1.576	1.089	1.081
MUC-1	1.312	1.582	1.305	1.295
RECOVERIN	1.599	1.928	1.324	1.342
SURVIVIN	1.338	1.613	1.125	1.140
WNT-1	1.465	1.767	1.419	1.237
EPCAM	1.398	1.685	1.266	1.257

Interpretation

Compared to the most recent visit (October 21, 2015), the overall trend exhibited a decreased level of expression for all the genes with an average decrease of 11.9% relative to GAPDH and 36.6% relative to B-actin. Correlate this clinically.

Released by:

Melvin Floyd E. See
Stem Cell Technologist

Camille V. Trinidad, RCh
Stem Cell Technologist

Noted by:

Francisco S. Chung Jr., Ph.D.
Co-director

1304003114 DATE OF BIRTH : 03-APR-1976 AGE : 37 year/s F

ROOM/BED	REFERRING PHYSICIAN	DATE/TIME OF EXAM	DATE/TIME OF REQUEST	OR/CI No.
/ OutPatient	PAGDANGANAN, MARIA CECILIA MAGLAYA M.D.	11-FEB-2014 01:18 PM	11-FEB-2014 12:25 PM	0127140205346778

EXAMINATION : 2D BREAST ULTRASOUND

HISTORY

S/P CORE NEEDLE BIOPSY, LEFT BREAST – DUCTAL CARCINOMA IN SITU (LOW GRADE)

COMPARISON/ CORRELATION

FOLLOW-UP STUDY TO APRIL 18, 2013

INTERPRETATION AND FINDINGS

Breast ultrasound revealed the following:

LEFT BREAST

1. solid nodule at 10A position measuring 0.5 x 0.3 x 0.6 cm.
2. complex nodule at 10A position measuring 0.7 x 0.6 x 0.7 cm.
3. cyst at 6A position measuring 0.7 x 0.4 x 0.6 cm.
4. cyst at 6A position measuring 0.6 x 0.5 x 0.5 cm.
5. complex nodule at 6A position measuring 1.1 x 0.8 x 1.2 cm.
6. cyst at 6A position measuring 0.6 x 0.5 x 0.6 cm.
7. cyst at 6A position measuring 0.7 x 0.5 x 0.6 cm.
8. complicated cyst at 3A position measuring 0.8 x 0.4 x 1.0 cm.
9. solid inhomogeneous and irregular at 12-3A position measuring 3.8 x 1.4 x 3.9 cm. (previously 3.2 x 3.9 x 1.0 cm.)
10. inhomogeneous solid nodule at 3A position measuring 1.7 x 0.6 x 1.8 cm.

Read By :

MA. THERESA S. BUENAFLOR, M.D. (Radiologist)

St. Luke's Medical Center Global City
We love life.

Breast Center

1304003114 DATE OF BIRTH : 03-APR-1976 AGE : 37 year/s F

ROOM/BED	REFERRING PHYSICIAN	DATE/TIME OF EXAM	DATE/TIME OF REQUEST	OR/CI No.
/ OutPatient	PAGDANGANAN, MARIA CECILIA MAGLAYA M.D.	11-FEB-2014 01:18 PM	11-FEB-2014 12:25 PM	0127140205346778

RIGHT BREAST

1. cyst at retroareolar area measuring 0.9 x 0.6 x 0.9 cm.
2. cyst at retroareolar area measuring 0.6 x 0.4 x 0.7 cm.
3. cyst at retroareolar area measuring 1.6 x 0.6 x 2.1 cm.
4. cyst at retroareolar area measuring 0.5 x 0.4 x 0.6 cm.
5. cluster of cysts at 10A position measuring 1.7 x 0.5 x 1.0 cm.
6. solid nodule at 10a position measuring 0.5 x 0.3 x 0.4 cm.
7. cyst at 10B position measuring 0.7 x 0.6 x 0.7 cm.
8. cluster of cysts at 10B position measuring 0.7 x 0.7 x 0.6 cm.
9. cyst at 8A position measuring 0.5 x 0.4 x 0.5 cm.
10. complicated cyst at 6A position measuring 0.7 x 0.4 x 0.7 cm.
11. cyst at 5A position measuring 0.9 x 0.6 x 0.8 cm.
12. cyst at 5A position measuring 0.7 x 0.3 x 0.7 cm.
13. cyst at 4B position measuring 0.5 x 0.4 x 0.5 cm.
14. cyst at 4A position measuring 1.3 x 0.3 x 0.7 cm.
15. cyst at 4A position measuring 0.6 x 0.5 x 0.7 cm.
16. cyst at 4A position measuring 0.7 x 0.3 x 0.9 cm.
17. cyst at 2A position measuring 0.4 x 0.3 x 0.4 cm.
18. cyst at 2A position measuring 0.4 x 0.3 x 0.4 cm.
19. solid nodule at 12A position measuring 0.6 x 0.4 x 0.6 cm.

Axillary lymph nodes with fatty hila are seen **bilaterally**.

IMPRESSION:

There is marked interval increase in size of the **left** breast focus corresponding to the known malignancy. CLINICAL CORRELATION is recommended.

BIRADS CATEGORY: 6

Read By :

MA. THERESA S. BUENAFLOR, M.D. (Radiologist)

MAKATI MEDICAL CENTER

Cellular Therapeutics Laboratory
Makati Medical Center
No. 2 Amorsolo Sreet,
Legaspi Village, Makati City,
Philippines 1229
Tel.No.: (02) 888-8999

Circulating Tumor Cell Assay Result

Patient Information

Name:
Date of Birth: April 3, 1976
Diagnosis: Stage Zero Breast Cancer
Attending Physician: Dr. Resurrecion

PIN: N/A
Age/Sex: 39/F
Date of Assay: July 14, 2015
Date of Release: July 16, 2015

Assay Information

Sample type: Whole Blood
Method of Assay: To evaluate relative values of circulating tumor antigens by Polymerase Chain Reaction.

Assay Result

Tumor Markers	Normalized Values (July 16, 2015)		Normalized Values (February 20, 2015)	
	GAPDH	ACTB	GAPDH	ACTB
LIVIN	1.466	1.610	1.233	1.250
MAGE-1	1.702	1.869	Below threshold level	Below threshold level
MUC-1	1.489	1.635	1.107	1.122
RECOVERIN	1.406	1.544	1.233	1.240
SURVIVIN	1.278	1.404	1.090	1.105
WNT-1	1.687	1.853	1.065	1.080
EPCAM	1.502	1.649	1.266	1.283

Interpretation

Overall, the trend exhibited a reduction of expression levels of the circulating tumor cell markers relative to the two housekeeping genes as endogenous controls, GAPDH and β-Actin. The expression level of MAGE-1 was below the detection limit and not included in the comparison. An average decrease of 26.9 % relative to GAPDH and 37.7 % relative to β-Actin was observed. Correlate this clinically.

Released by:

Melvin E. See
Stem Cell Technologist

Noted by:

Francisco S. Chung Jr., Ph.D.
Co-director

THE MEDICAL CITY
Where patients are partners

RADIOLOGY DEPARTMENT

Patient Name:
Patient ID: 887779
File Number: PLA4567001361035
Charge Account:

Age: 56Y **Sex:** F
DOB: 23-Nov-1959
Unit: CTS

Ordering Physician: TAMAYO, MA. BELEN EVANGELISTA
Ordering Department: ONCOLOGY
Procedure: CT SCAN - CHEST - REGULAR W/ CONTRAST
Clinical Indications: CHECK-UP

Findings:

CT SCAN OF THE CHEST

Clinical Data: Known case of Breast Cancer St 1; status post partial mastectomy (RIGHT), status post radiation and chemotherapy, with RIGHT axillary pain.
Comparison: None available at the time of examination.
Technique: Multiplanar plain and intravenous contrast-enhanced CT images of the chest were obtained.
IV Contrast: Iopamidol (Scanlux™) 100 mL. No immediate adverse contrast reaction.

Findings:

Lungs: Fine reticular densities with tubular bronchiectasis are seen in the periphery of the anterior segment of the right upper lobe, lateral segment of the RIGHT middle lobe and anterior basal segment of the RIGHT lower lobe. Linear densities with bronchiectatic changes are noted in the superior segment of the LEFT lower lobe. No pulmonary nodules seen.

Trachea and main bronchi: Unremarkable

Pleura: Unremarkable

Heart: Not enlarged

Pericardium: No pericardial effusion

Vessels: The aorta is minimally segmentally calcified. No aortic dissection or aneurysm.

Mediastinum and hila: Unremarkable

Esophagus: Unremarkable

Chest wall and lower neck: Skin thickening is seen in the right breast. No enlarged lymph nodes or masses are seen.

Bones: Spurs line the endplates of the thoracic vertebrae.

Included upper abdomen: Splenorenal varices are detected.

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Philippines 1229
Tel.No.: (02) 888-8999

Circulating Tumor Cell Assay Result

Patient Information

Name:
Date of Birth: November 23, 1959
Diagnosis: Stage IA Breast Cancer (Right)
Attending Physician: Dr. Romulo S. De Villa

PIN: N/A
Age/Sex: 55/F
Date of Assay: June 1, 2015
Date of Release: June 2, 2015

Assay Information

Sample type: Whole Blood
Method of Assay: To evaluate relative values of circulating tumor antigens by Polymerase Chain Reaction.

Assay Result

Tumor Markers	Normalized Values (Jun. 2, 2015)		Normalized Values (Nov. 18, 2014)	
	GAPDH	ACTB	GAPDH	ACTB
CEA	1.186	1.147	1.160	0.889
LIVIN	1.348	1.304	1.352	1.037
MAGE-1	1.784	1.725	1.548	1.187
MUC-1	1.402	1.356	1.424	1.092
RECOVERIN	1.335	1.291	1.361	1.043
SURVIVIN	1.260	1.219	1.121	0.860
WNT-1	1.579	1.527	1.672	1.281
EPCAM	1.497	1.448	n/a	n/a

Interpretation

Overall, the general trend exhibited a slight reduction of expression levels of the circulating tumor cell markers relative to the two housekeeping genes or endogenous controls, GAPDH and B-Actin. The expression levels of MUC-1, Recoverin, and WNT-1 increased slightly relative to GAPDH but decreased relative to B-Actin. An average decrease of 2.4% relative to GAPDH and 22.6% relative to B-Actin was observed. Correlate this clinically.

Released by:

Melvin E. See
Stem Cell Technologist

Miguel Francisco M. de Jesus, RCh
Stem Cell Technologist

Noted by:

Francisco S. Chung Jr., Ph.D.
Co-director

Case: Colon Cancer

St. Luke's Medical Center
Global City

SURGICAL PATHOLOGY
ONE LARGE TISSUE/ORGAN

PATIENT'S NAME (Last, First Middle Name)				DATE/TIME OF EXAM
				Mar-26-2016 08:45 AM
PIN	BIRTHDATE	AGE/GENDER	ROOM/BED	REQUESTING PHYSICIAN
1603044731	Jan-25-1937	79Y/M	N1127/N1127B	NONATO, ROMMEL TALAMAYAN M.D. (718)

Specimen No.: GC16-2029

Clinical Diagnosis: Acute abdomen secondary to ruptured viscus

Specimen: Rectosigmoid with ruptured diverticulitis

Date and Time Received: 3/24/2016 0050H

Diagnosis:

Rectosigmoid with ruptured diverticulitis, ex-lap Hartmann's procedure:
- ADENOCARCINOMA, MODERATELY DIFFERENTIATED, ARISING IN AN ADENOMA AND ASSOCIATED WITH A RUPTURED DIVERTICULUM.
- TUMOR SIZE: 3 CM. IN WIDEST DIMENSION.
- TUMOR INVADES MUSCULARIS PROPRIA.
- NO DEFINITE LYMPHOVASCULAR INVASION.
- NO TUMOR DEPOSIT.
- PROXIMAL AND DISTAL LINES OR RESECTION: NEGATIVE FOR TUMOR.
- FOUR OF SIXTEEN PERICOLIC LYMPH NODES: POSITIVE FOR METASTASIS.
- AJCC pT2N2aMx (AJCC 7th edition).
- OTHER FINDINGS:
  > DIVERTICULOSIS.
  > ACUTE SEROSITIS.

COMMENT: Dr. Rolando A. Lopez concurs with the diagnosis.

Gross/Microscopic Description

GROSS: Received in formalin is a specimen labeled as "rectosigmoid with ruptured diverticulitis", which consists of a segment of colon measuring 16.3 cm. in length, 6.8 cm. in circumference at one line of resection (inked green) and 7 cm. in circumference at the opposite line of resection (inked black). There is a cream tan, polypoid, firm mass (3x2.8x2.7cm.), which partly obstructs the colonic lumen by about 80% and appears to invade the muscularis propria. The mass is located 10.5 cm. from the line of resection inked green and 5.4 cm. from the line of resection inked black. The mass

GLENDA LYN Y. PUA, M.D.
PRC#0096123
PATHOLOGIST

This result is best interpreted by your attending physician in correlation with your clinical data, imaging and other laboratory results.
This report has been electronically validated. No signature is required.

Rizal Drive corner 32nd Street and 5th Avenue, Taguig City, Philippines * Tel. No.: (632) 789-7700

Lung Center of the Philippines
Molecular Diagnostics and Cellular Therapeutics Laboratory
Quezon Avenue Extension, Quezon City, Philippines
Six-gene Panel Test

DOCTOR'S COPY

Patient's Name:
Diagnosis: COLON CARCINOMA

AGE: 79
GENDER: MALE

Run Date: July 5, 2016
Specimen: Peripheral Blood

Results:

GENE	DESCRIPTION	FOLD VALUE	REMARKS
GAPDH	House Keeping Gene		Valid
CD133	Cancer Stem Cell	0.03	Down-regulated
KRT19	Tumor Detector	18.02	Up-regulated
MUC1	Tumor Anti-apoptosis	0.38	Down-regulated
p53	Tumor Suppressor Related	1.01	Up-regulated
CA125	Advancing Tumorigenesis	1.04	Up-regulated
CEA	Metastatic	0.08	Down-regulated

Note:
Fold Change of 1 is considered normal
Fold value/s ≥1 is considered *up-regulated (promotes tumor proliferation)*
Fold value/s at 0.50-0.99 is considered *down-regulated (on-going tumor obliteration)*
Fold value/s <0.50 is considered *negative (no detectable tumor marker)*

Prepared by:

Mary Suzette A. Angeles, RCh
Research Specialist

Ma. Teresa A. Barzaga, MD
Department Manager III

Noted by:

Francisco M. Heralde III, PhD, RN
Consultant - Molecular Biologist

REF. NO.: MPC-RGQ-2016-057

Lung Center of the Philippines
Molecular Diagnostics and Cellular Therapeutics Laboratory
Quezon Avenue Extension, Quezon City, Philippines
Six-gene Panel Test

DOCTOR'S COPY

Patient's Name:
Diagnosis: COLON CARCINOMA II

AGE: 79
GENDER: MALE

Run Date: October 24, 2016
Specimen: Peripheral Blood

Results:

GENE	DESCRIPTION	FOLD VALUE	REMARKS
GAPDH	House Keeping Gene		Valid
CD133	Cancer Stem Cell	0.19	Negative
KRT19	Tumor Detector	0.00	Negative
MUC1	Tumor Anti-apoptosis	0.05	Negative
p53	Tumor Suppressor Related	1.35	Up-regulated
CA125	Advancing Tumorigenesis	0.19	Negative
CEA	Metastatic	0.00	Negative

Note:
Fold Change of 1 is considered normal
Fold value/s ≥1 is considered *up-regulated (promotes tumor proliferation)*
Fold value/s at 0.50-0.99 is considered *down-regulated (on-going tumor obliteration)*
Fold value/s <0.50 is considered *negative (no detectable tumor marker)*

Prepared by:

Mary Suzette A. Angeles, RCh
Research Specialist

Ma. Teresa A. Barzaga, MD
Department Manager III

Noted by:

Francisco M. Heralde III, PhD, RN
Consultant - Molecular Biologist

REF. NO.: MPC-RGQ-2016-109

MAKATI MEDICAL CENTER

Cellular Therapeutics Center
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No. 2 Amorsolo Street,
Legaspi Village, Makati City,
Philippines 1229
Tel.No.: (02) 888-8999

Circulating Tumor Cell Assay Result

Patient Information

Name:
Date of Birth: January 25, 1937
Diagnosis: Colon Cancer s/p Right Colon Resection
Attending Physician: Romulo S. De Villa, MD, PhD

MRN: 420837
Age/Sex: 79/M
Date of Assay: July 4-8, 2016
Date of Release: July 8, 2016

Assay Information

Sample type: Whole Blood
Method of Assay: To evaluate relative values of circulating tumor antigens by Polymerase Chain Reaction.

Assay Results

Tumor Markers	June 22, 2016		Reference Range	
	GAPDH	ACTB	GAPDH (±0.02-0.04)	ACTB (±0.02-0.05)
MAGE-1	1.32	1.26	1.21-1.74	1.19-1.91
EPCAM	1.32	1.26	1.18-1.66	1.21-1.80
MUC-1	1.57	1.49	1.36-1.84	1.45-1.85
WNT-1	1.37	1.31	1.30-1.65	1.32-1.82
LIVIN	1.53	1.46	1.33-1.90	1.37-2.02
RECOVERIN	1.69	1.61	1.38-1.66	1.46-1.77
SURVIVIN	1.54	1.47	1.24-1.63	1.23-1.70
CEA	1.65	1.57	1.38-1.64	1.37-1.78

Note: This is a baseline assay. A repeat run is highly recommended after 4-6 months. Correlate this result clinically.

Released by:

Melvin Floyd E. See
Stem Cell Technologist

Noted by:

Francisco S. Chung Jr., Ph.D.
Co-Director

Conclusion

- NKCP is a safe way to remove the fibrin clot protecting cancer cells from NK cell attack and help reduce the burden of cancer cells circulating in the blood where metastasis begins.

In the light of evidence that NKCP (able dissolve clots that camouflage cancer cells) combined with Lentin plus (that activate NK cells) can reduce cancer cells circulating in the blood, it is now time to investigate whether NKCP/Lentin plus combination can reduce cancer metastasis, reduce recurrence and prolong survival of cancer patients similar to aspirin.