The Basic Seminar handout begins on page 81.

The 13th Technology Presentation

March 11, 2016

Sysmex Corporation

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1. Opening Remarks

Hisashi Ietsugu, Chairman and CEO

<Today's Themes>

- Technology Strategy Progress
- Progress on Technology Development

2. Technology Strategy Progress

Kaoru Asano, Member of the Managing Board and Senior Executive Officer, Managing Director, Head of R&D

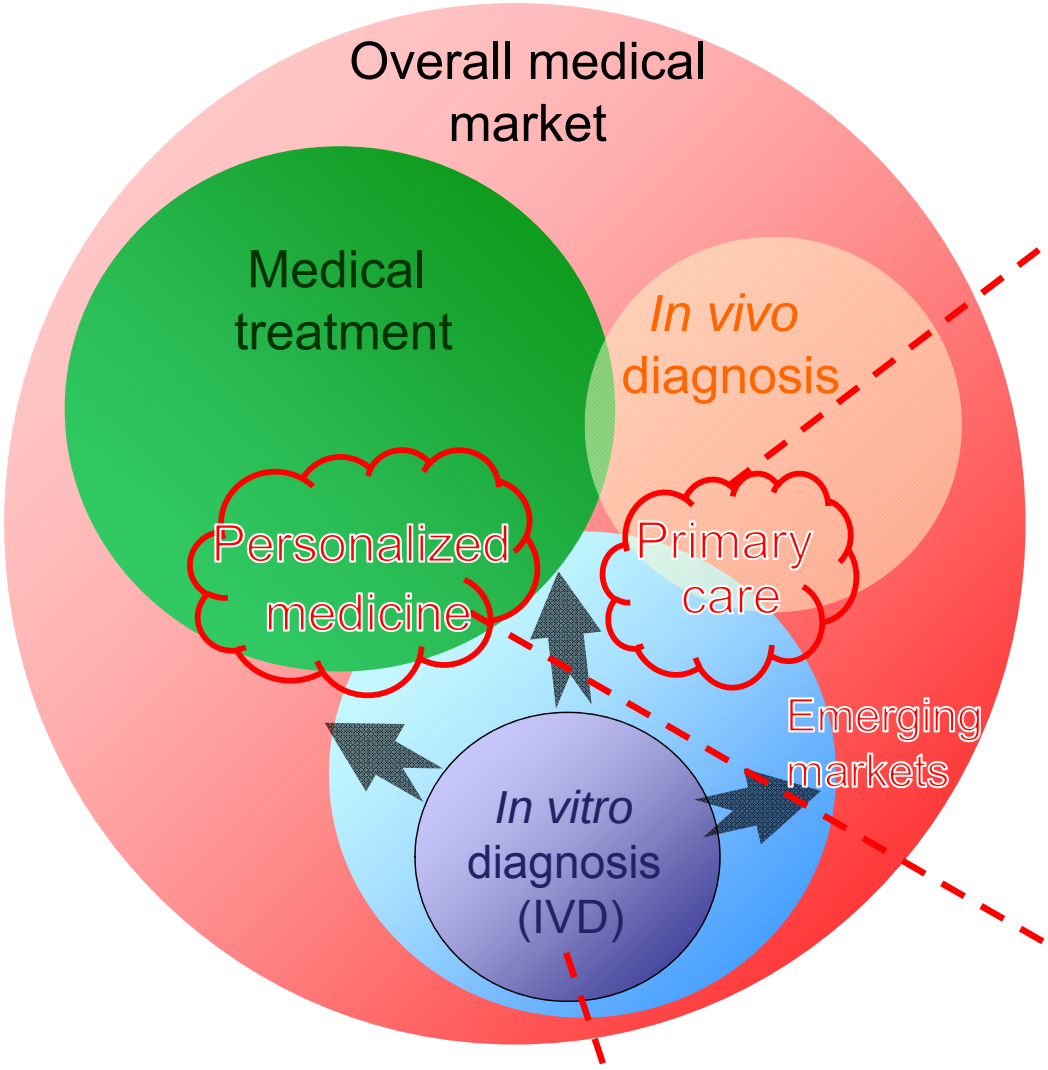
(1) Technology Strategy Overview

(2) Status of Technology Platform Expansion

(3) Application Portfolio

(4) Topics

(1) Technology Strategy Overview



Disease prevention based on testing data

Enhance the performance/function of analytical platform

Develop applications through advances in open innovation

ICT utilization

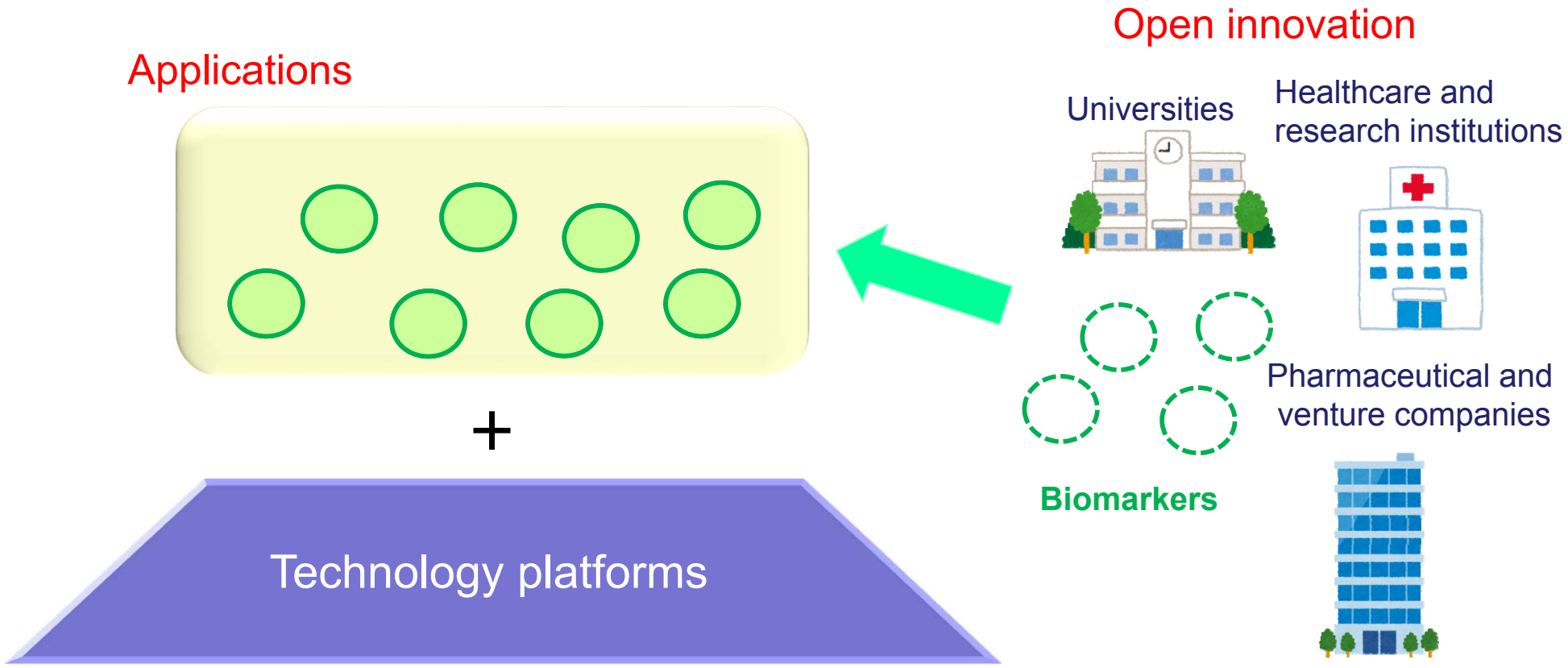
Establishing personalized medicine through CDx

Organic growth in existing IVD fields

CDx: Companion diagnostics

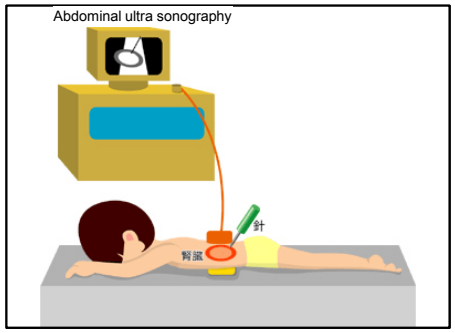
Development of Applications through Advances in Open Innovation

Achieve advances in open innovation and develop applications with high clinical value



Platforms Targeting Personalized Medicine

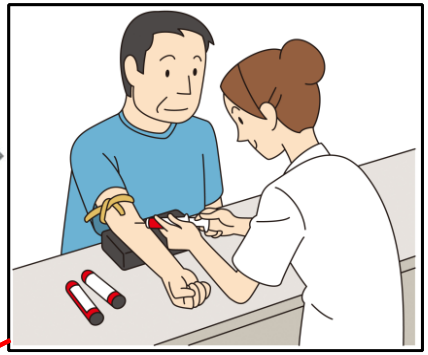
Conventionally



Invasive sample collection of affected organ/tissue

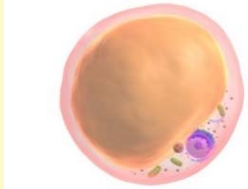
From biopsy to liquid biopsy

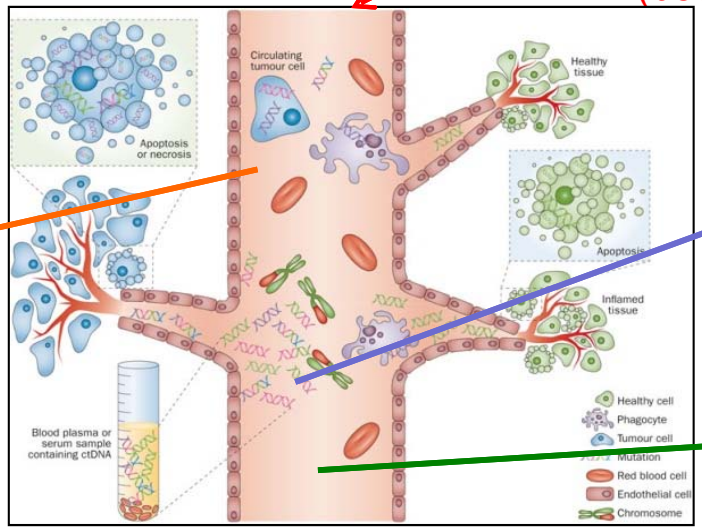
Near Future



Minimal invasive sample collection of disease-derived components into the blood (bodily fluid)

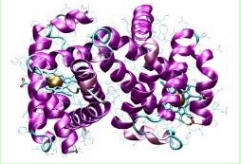
Cells





Genes

Proteins

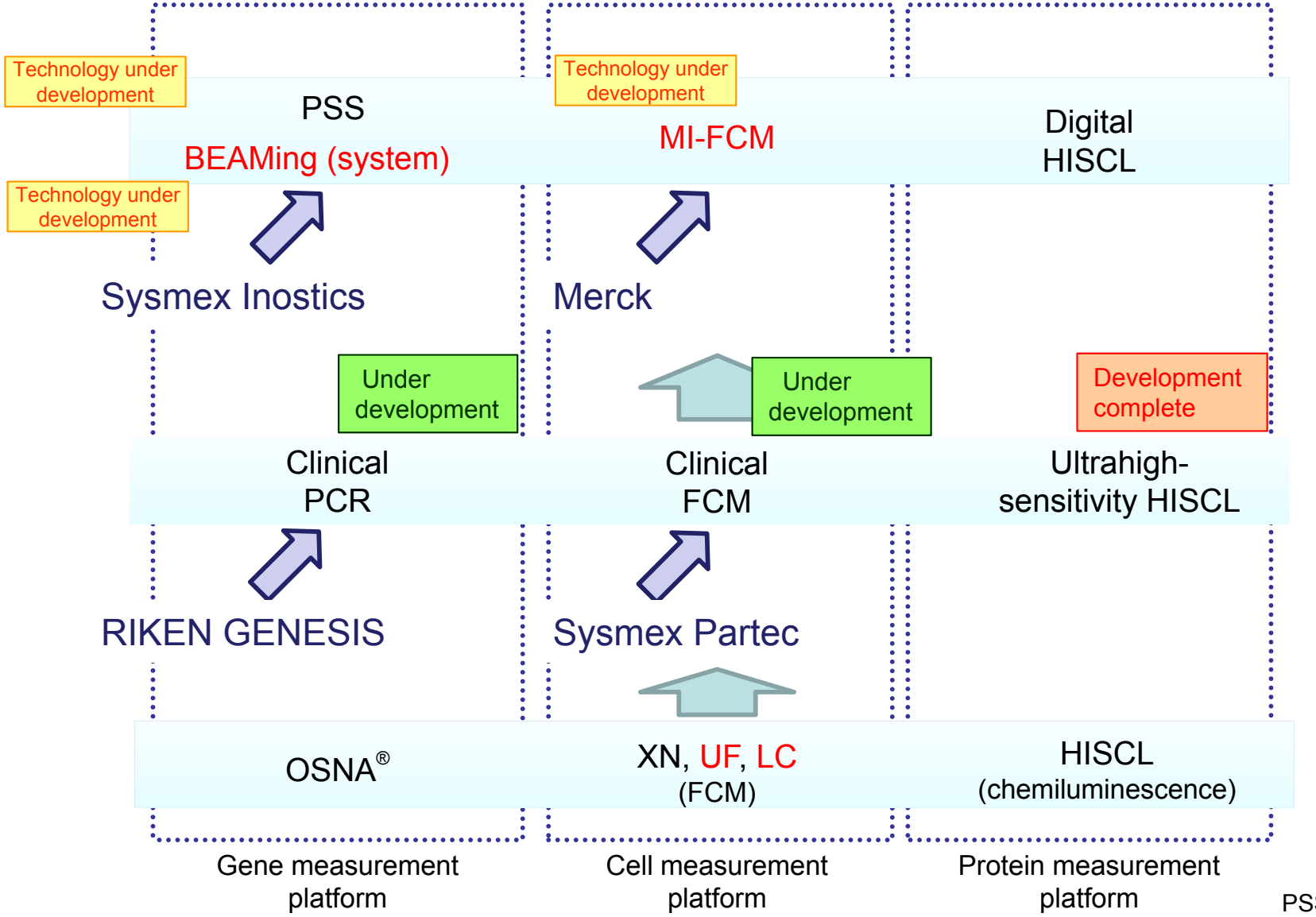


Liquid biopsy:
Detection of cancer or other diseases by testing blood or other bodily fluids. This type of testing is less invasive than conventional physical biopsies.

Nature Reviews Clinical Oncology 10, 472-484 (August 2013)

Detection sensitivity will need to be 100 to 1,000 times higher than conventional methods

Technology Platform Enhancement



PSS: Plasma-Safe-SeqS

MI-FCM: Molecular imaging FCM

Technology Platform Enhancement

Technology under development

BEAM

Technology under development

Sysmex I

RIKEN GE

Technology under development

Ultrahigh-sensitivity immune measurement system using immune complex transfer method

Development based on HISCL complete

Digital HISCL

Development complete

Ultrahigh-sensitivity HISCL

OSNA®	XN, UF, LC (FCM)	HISCL (chemiluminescence)
Gene measurement platform	Cell measurement platform	Protein measurement platform

PSS: Plasma-Safe-SeqS

MI-FCM: Molecular imaging FCM

Technology Platform Enhancement

Technology under development

PSS

BEAMing (system)

Technology under development

Sysmex Inostics

Under development

Clinical PCR

RIKEN GENESIS

Technology under development

Digital

Cancer diagnosis system that can be implemented in hospital labs

- ✓ Fully automated, from gene extraction to display of results
- ✓ High sensitivity (0.1% mutation detection)
- ✓ Quantitative/ qualitative analyses

OSNA®

XN, UF, LC (FCM)

HISCL (chemiluminescence)

Gene measurement platform

Cell measurement platform

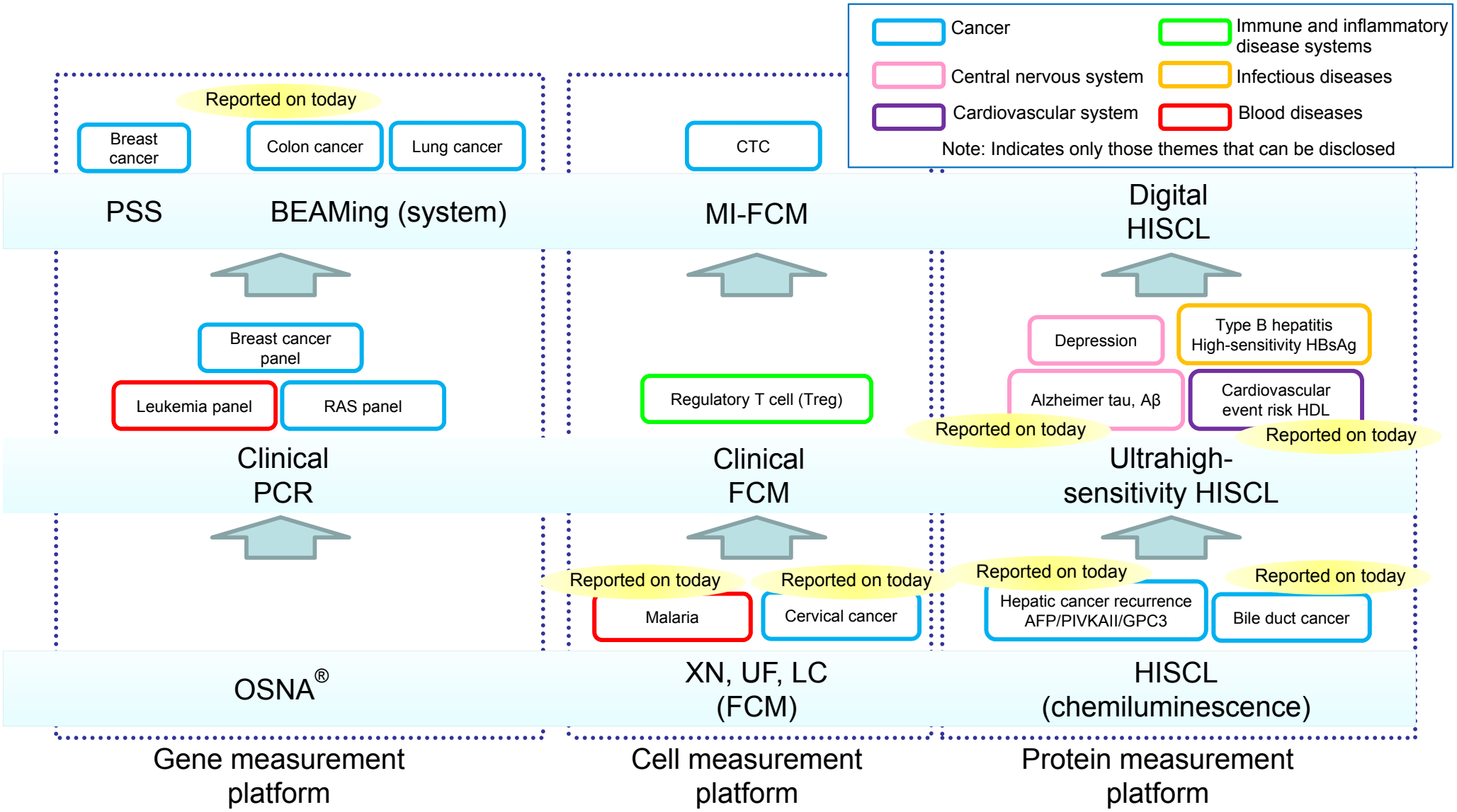
Protein measurement platform

PSS: Plasma-Safe-SeqS

MI-FCM: Molecular imaging FCM

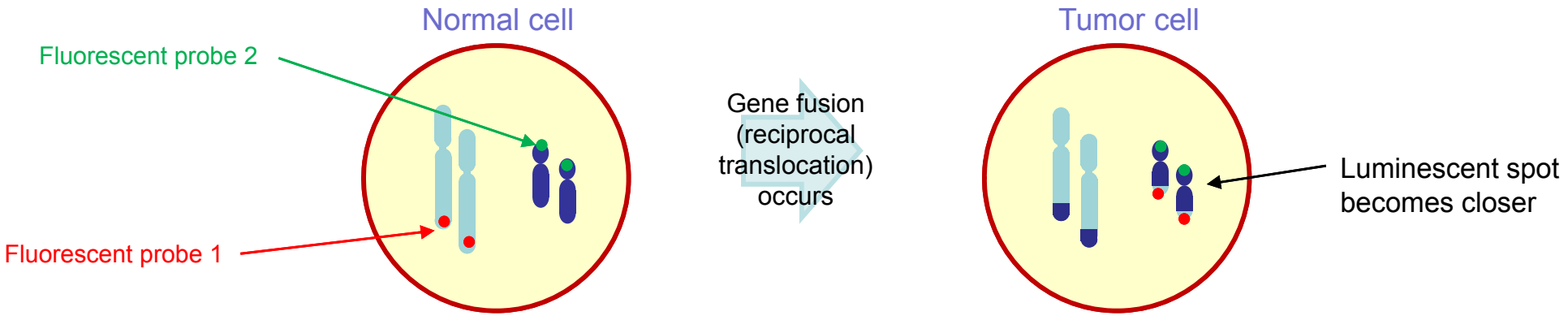
Application Portfolio

Promote R&D in the aim of creating new value



About FISH(Fluorescence In Situ Hybridization) testing

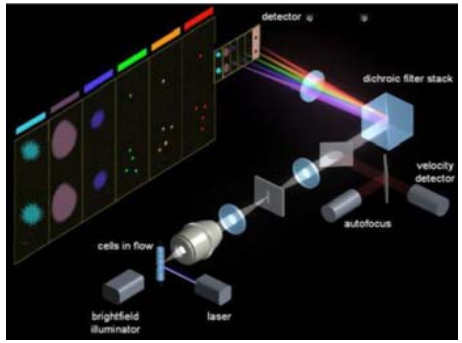
This method uses fluorescently labeled probes for fusion with specific genes only to detect target genes inside a chromosome



Conventional FISH testing

- ✓ Major testing effort
- ✓ Specialized knowledge needed to determine results
- ✓ Limited number of analysis

Using **MI**(molecular imaging)-**FCM** technology



https://www.amnis.com/multispectral.html

- ✓ Achieve higher FISH testing precision, reduce labor requirements

Topics (1) FISH Testing System Using MI-FCM Technology
(Joint Development with Merck of Germany)

Flow FISH Testing System


Flow FISH reagents


Flow FISH instrument


FISH image analysis software

Application in total leukemia diagnosis


XN Series

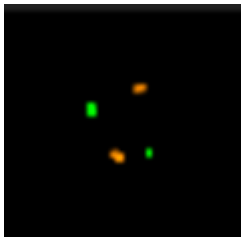
Close inspection

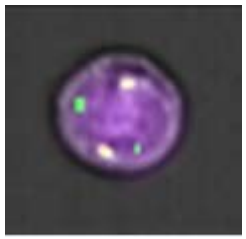
WHO classification

- Morphological examination
- Surface antigen analysis
- Cytogenetic testing
- Molecular genetic testing

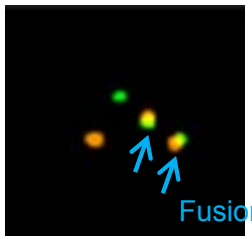
Measurement examples

bcr-abl translocation negative leukocyte

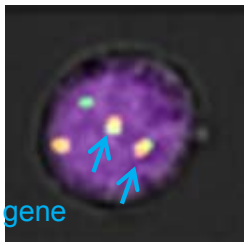

Fluorescence microscope


MI-FCM

bcr-abl translocation positive cell strain (PALL2)



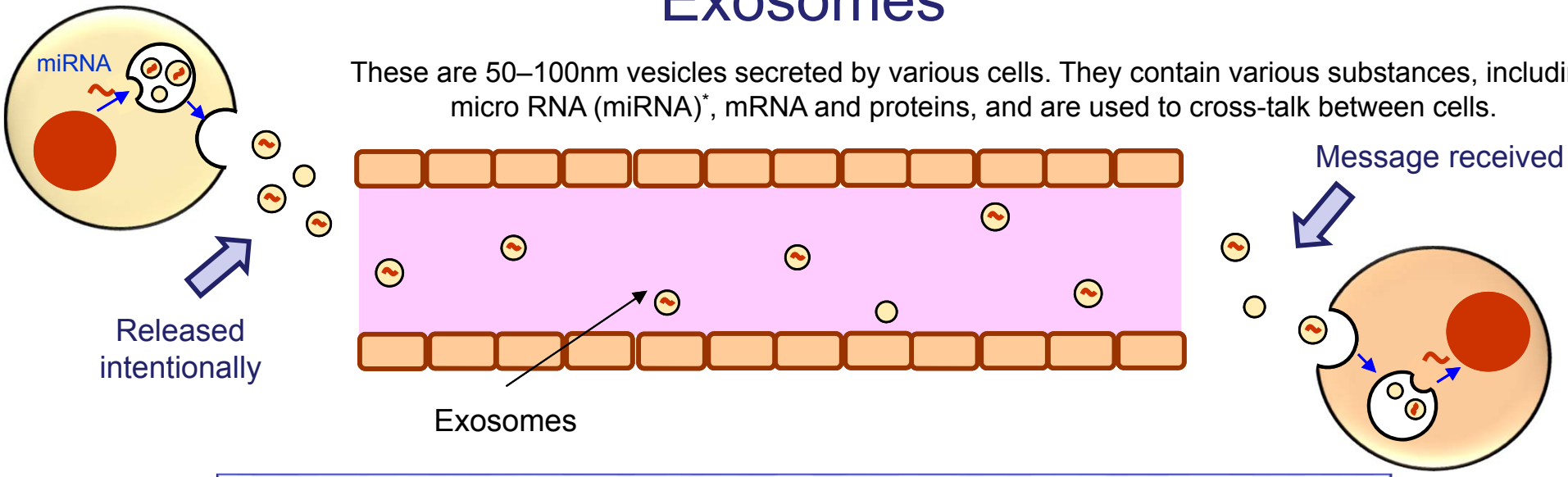
Fluorescence microscope


MI-FCM

Topics (2) Exosome (MicroRNA) Analysis Technology

Exosomes

These are 50–100nm vesicles secreted by various cells. They contain various substances, including micro RNA (miRNA)*, mRNA and proteins, and are used to cross-talk between cells.



Recent basic research has shown that exosomes contribute to the progression of cancer and various other diseases

Utility as a diagnostic target (marker)

- Exist stably in the blood
- Organ of origin can be determined

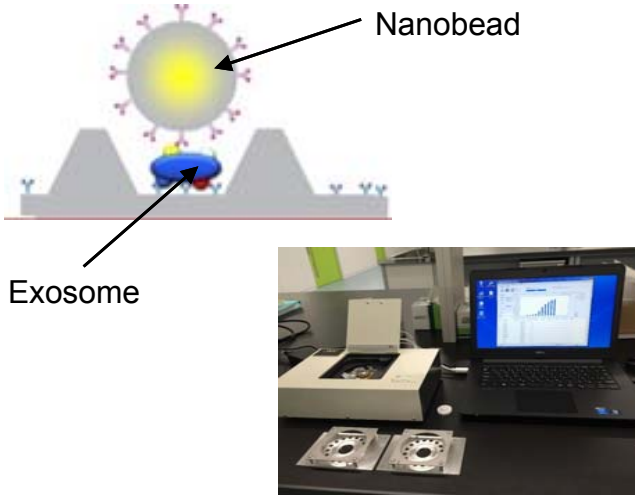
Developing diagnostic instruments jointly with JVC KENWOOD Corporation

*Micro RNA (miRNA)
MicroRNA (miRNA) are single-stranded RNA molecules of around 20 bases in length involved in controlling the expression of numerous genes and proteins, thereby making fine adjustments in vital phenomena.

Topics (2) Exosome (MicroRNA) Analysis Technology
(Joint Development with JVC KENWOOD Corporation)

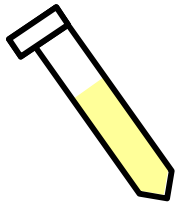
Exosome analysis system

JVCKENWOOD



Capture　Counting

sysmex



Proteins

Highly sensitive HISCL

miRNA, mRNA

Clinical PCR

Extraction/Purification　Highly sensitive measurement

3. Progress on Technology Development Themes

(1) Research and Development Themes

Tomokazu Yoshida, Executive Vice President of the Central Research Laboratories

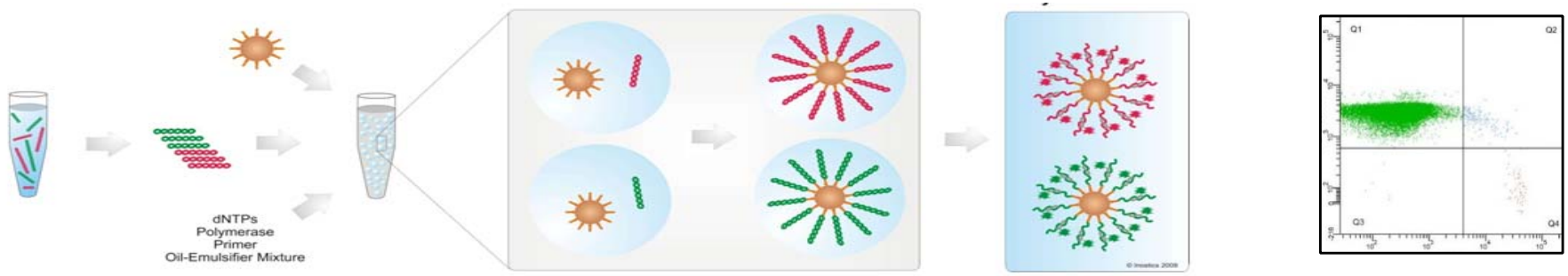
1) Development of Systemized Technology for BEAMing Technology
2) Development of Next-Generation Diagnostic Regents in Central Nervous System Disorder
3) Development of Method for Diagnosing Risk of Hepatic Cancer Recurrence
4) Development of Method for Diagnosing Risk of Cardiovascular Disease

1) Development of Systemized Technology for BEAMing Technology

Systematization of BEAMing Technology

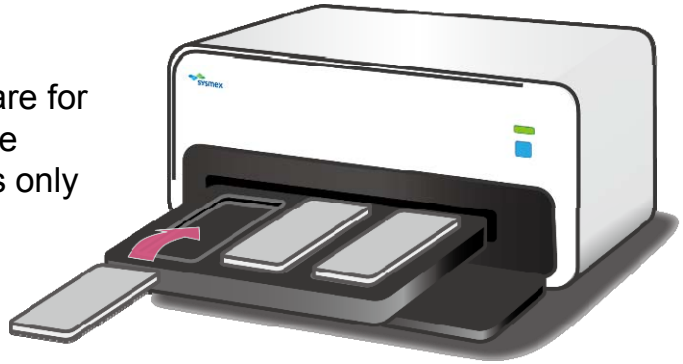
<Current lab assay process>

Pretreatment	Reaction					Detection
Gene extraction	Pre-PCR	Droplet formulation	On-beads PCR	Droplet disruption	Hybridization	FCM detection



Procedures are complex, so two to three days is required to complete measurement

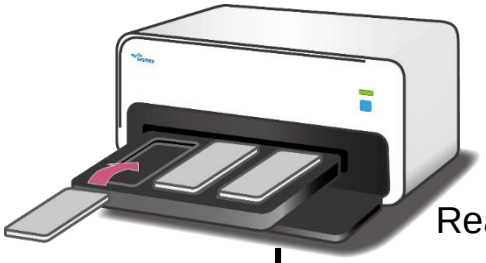
Note: Images are for illustrative purposes only



Microchannel

Through systematization, aim to complete measurement in six hours

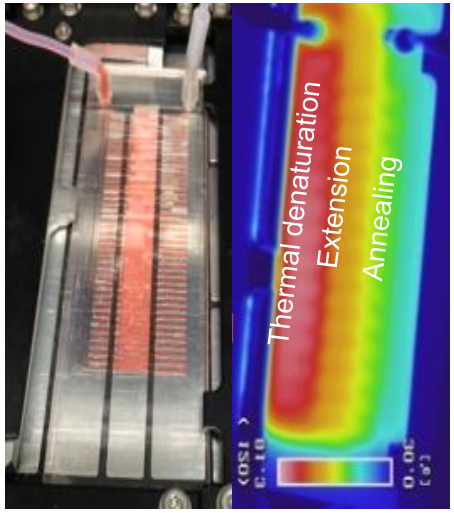
Systemization of BEAMing Technology (Reaction Instrument)



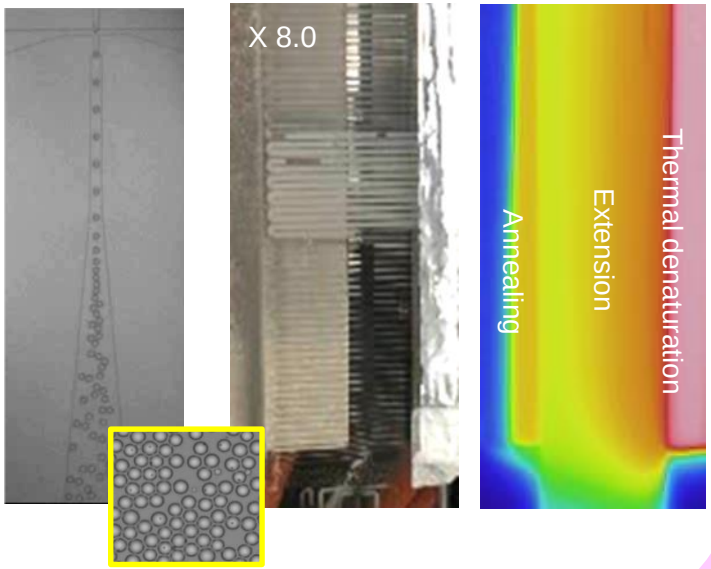
Reaction instrument

Technology under development

Pre-PCR



Droplet formation | On-beads PCR



Formation of homogenous droplets

Droplet disruption | Hybridization

2) Development of Next-Generation Diagnostic Reagents in Central Nervous System Disorder

Joint Development with Eisai Co., Ltd.

Development of Next-Generation Diagnostic Reagents in Central Nervous System Disorder: Alzheimer's Testing

Realizing Alzheimer's tests using liquid biopsy

Principal pathologies of Alzheimer's disease (AD)

- Senile plaques due to accumulation of amyloid β
- Neurofibrillary tangle due to tau protein

[Issues]

- Detection of minute protein amounts
- Identification of brain-specific target proteins

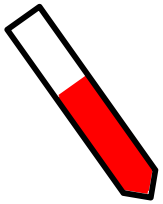
<Imaging>

✓High cost
✓Limited facilities

<Cerebrospinal fluid testing>

✓Highly invasive

<Blood testing>

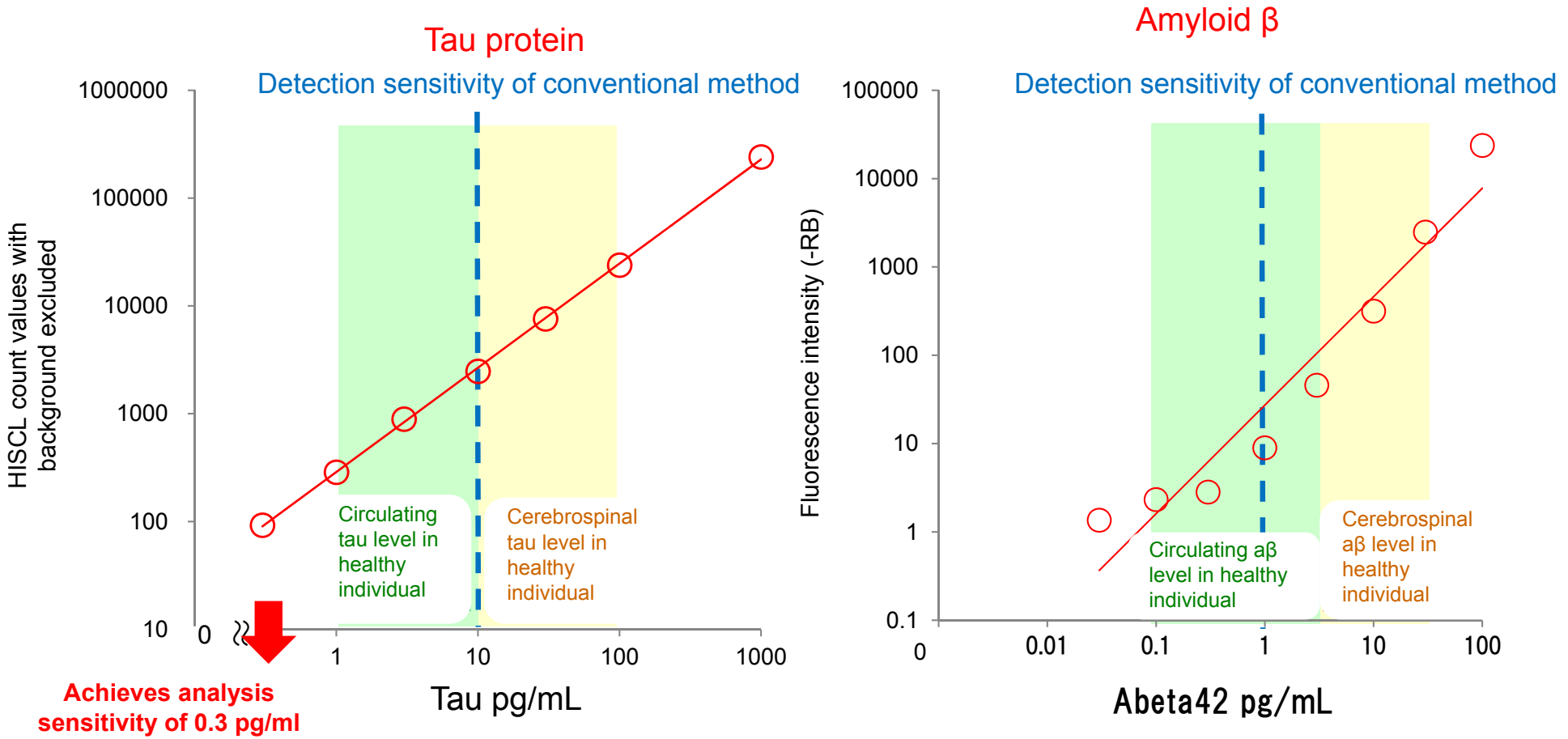


Circulating Aβ volume: **1:50** of Aβ volume in cerebrospinal fluid
(National Center for Geriatrics and Gerontology, Proc. Jpn. Acad., Ser. B, 2014)

Ultrahigh-sensitivity HISCL

Utilize for joint development with Eisai Co., Ltd.

Using ultrahigh-sensitivity HISCL allows measurement of circulating tau protein/amyloid β

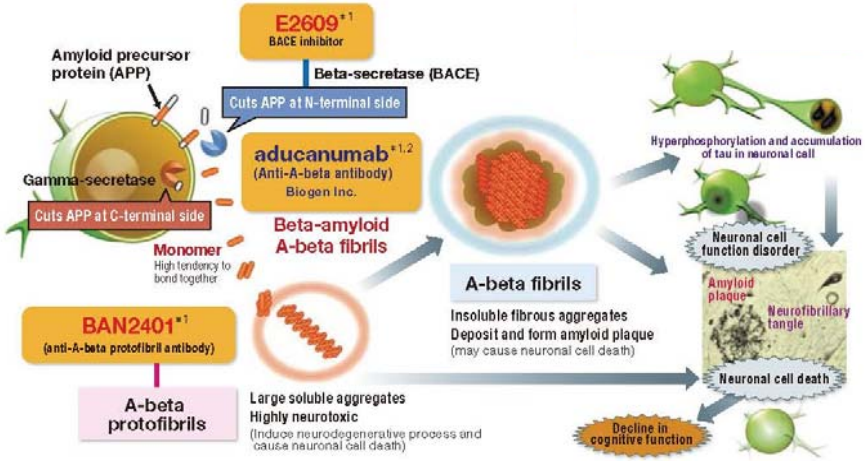


Configuring measurement system toward ultrahigh-sensitivity automation, to begin evaluating clinical specimens in fiscal 2016

Development of Next-Generation Diagnostic Reagents in Central Nervous System Disorder
(Joint Development with Eisai Co., Ltd.)

Eisai Co., Ltd.

Drug discovery research related to Central Nervous System Disorder



From the Eisai Co., Ltd., *Integrated Report 2015*

sysmex

Leading-edge testing technologies

Development of next-generation diagnostic reagents

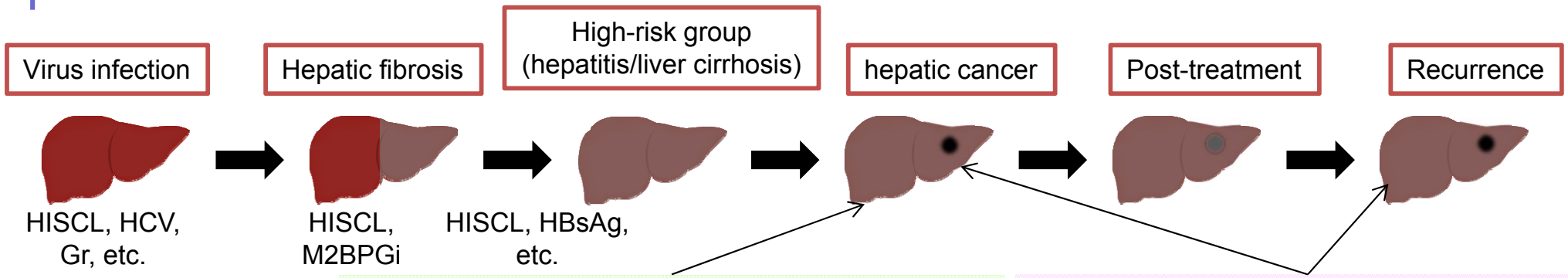
- Early diagnosis and selection of treatment methods for CNS disorder monitoring of therapeutic gains
- Drug discovery research and development

3) Development of Method for Diagnosing Risk of Hepatic Cancer Recurrence

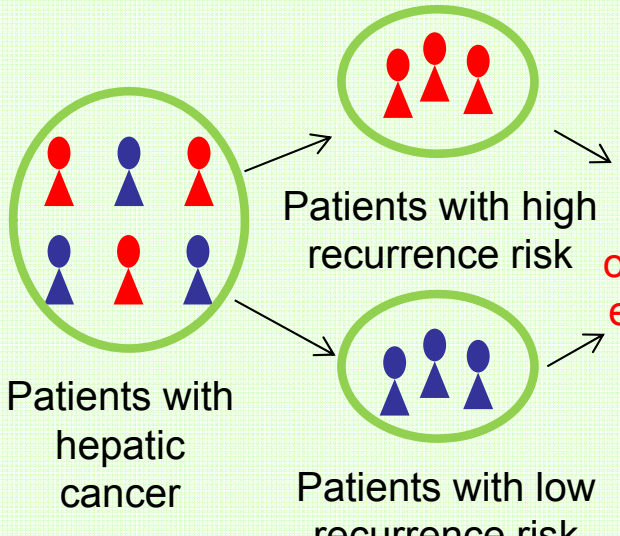
Joint Development with the National Cancer Center

Development of Method for Diagnosing Risk of Hepatic Cancer Recurrence

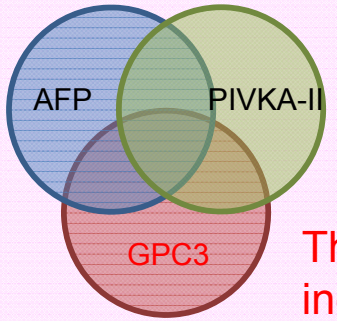
Manage hepatic cancer though combination with immunological test parameters



Hepatic cancer recurrence risk tests:
HISCL GPC3* (new parameter)



Hepatic cancer tests:
HISCL AFP (2009)
HISCL PIVKA-II (2015)
+
HISCL GPC3* (new parameter)



Through combination, increase the positive detection rate

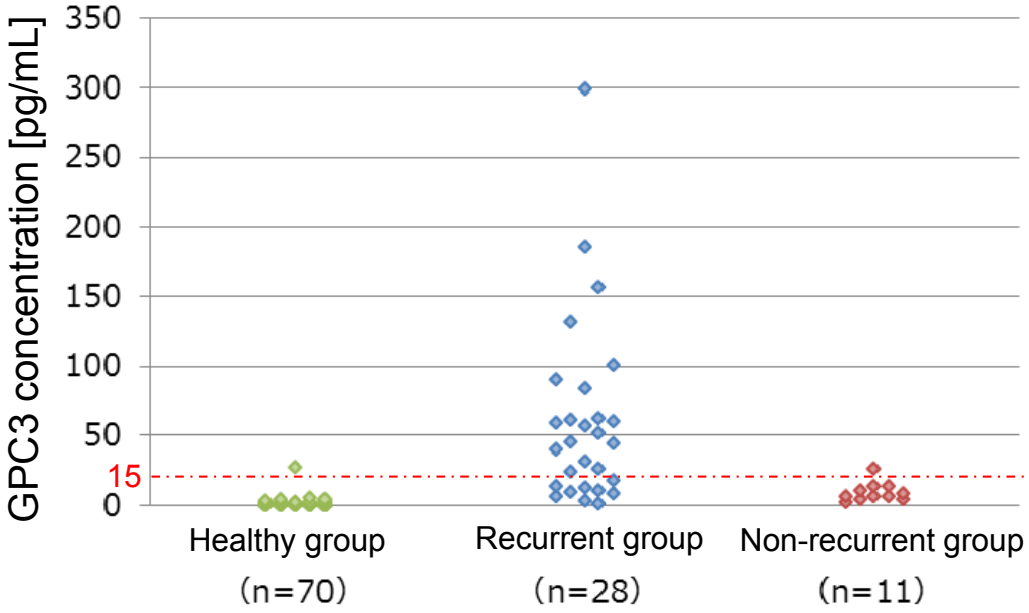
*GPC3
A protein expressed on hepatic cancer cells that is released into the blood

Development of Method for Diagnosing Risk of Hepatic Cancer Recurrence, Clinical Evaluation Results

Positive rates for GPC3, AFP and PIVKA-II in 28 cases of hepatocellular carcinoma recurrence

Positive rate	AFP PIVKA-II	AFP PIVKA-II GPC3
Before treatment	92.9% (26/28)	92.9% (26/28)
At recurrence	50% (14/28)	71.4% (20/28)

Increase the positive detection rate through combination with current parameters



Circulating GPC3 concentration (before treatment)

Select patients with high recurrence risk based on amount of circulating GPC3

(Enhancing measurement performance enables provision of new value)

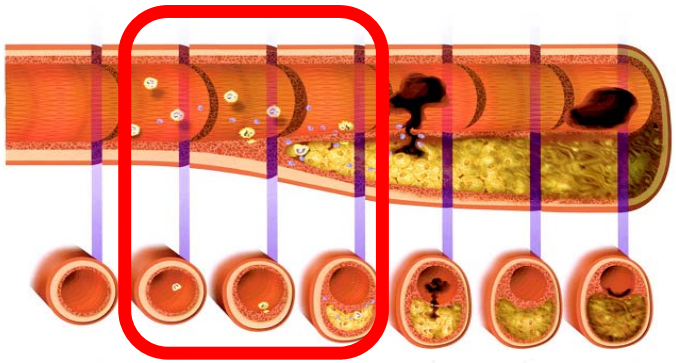
Configuration of an HISCL measurement system is complete and evaluation by the National Cancer Center has begun

4) Development of Method for Diagnosing Risk of Cardiovascular Disease

Joint Research with Kobe University Graduate School of Medicine Course

Development of Method for Diagnosing Risk of Cardiovascular Disease: Development of Method for Evaluating HDL Function

Development of method for diagnosing risk of cardiovascular disease through method of evaluating the HDL function



Arteriosclerosis progression

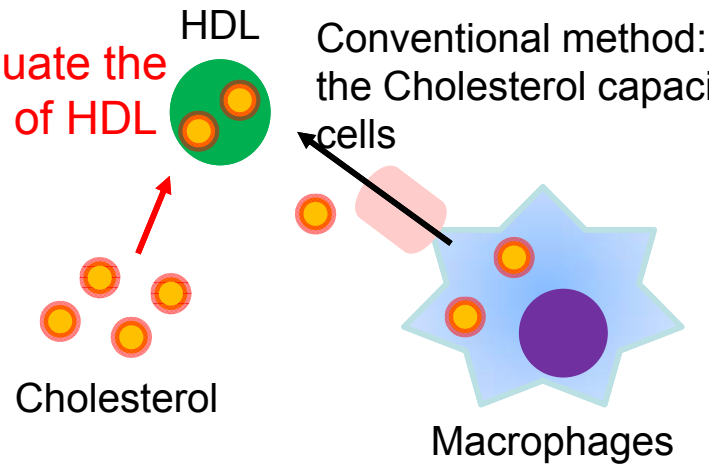
Excerpted and partially adapted from the website of the Tokyo Women's Medical University Institute of Geriatrics

HDL-C concentration ✚ HDL function

(Existing clinical chemistry tests)

[Issues]
Conventional methods were complex, and specialized facility environments were needed, making clinical application and standardization problematic

New method: Evaluate the cholesterol uptake of HDL on its own



More than high HDL values, in diagnosing cardiovascular risk determining the level of HDL protein function is important

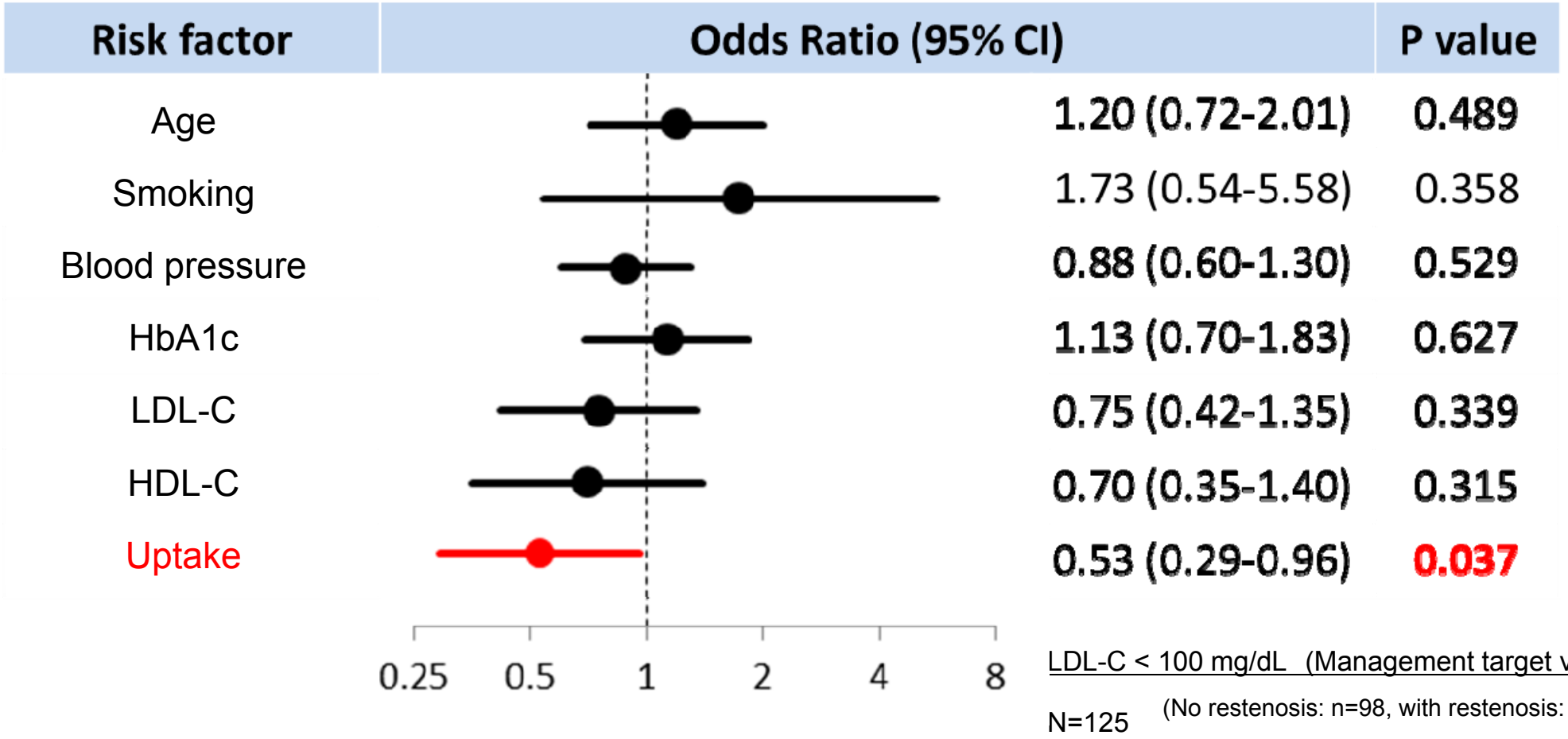
(Khera et al. Jan 13, 2011, New England Journal of Medicine)

HDL: High-density lipoprotein

Equipped technology for HISCL

Development of Method for Diagnosing Risk of Cardiovascular Disease: Clinical Evaluation Results

Risk factors and odds ratios of coronary artery restenosis



LDL-C < 100 mg/dL (Management target value)

N=125 (No restenosis: n=98, with restenosis: n=27)

Larger-scale clinical evaluations planned from fiscal 2016

3. Progress on Technology Development Themes

(2) Urinalysis and Hematology

Kensaku Aota, Executive Vice President of the UB Product Engineering Div.

1) Urinalysis Flow Designed by Sysmex and New Urinalysis Product Technologies
2) Malaria Detection Technology Using Blue LD FCM and Progress on the Cervical Cancer Screening System

Blue LD: Blue laser diode

1) Urinalysis Flow Designed by Sysmex and New Urinalysis Product Technologies

New Urinalysis Flow Designed by Sysmex

Current urinalysis work flow

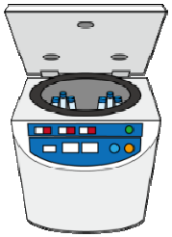
Validation

Sediment urinalysis

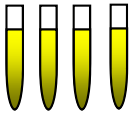
Visual examination

Microscope examination

Slide preparation



Centrifuging



Specimen identification

Automated analysis of formed elements in urine

FCM

DIA

Urine chemistry

New urinalysis flow and product groups

Detailed morphological checking if necessary

U-WAM
work area information management system

UD-10
imaging device for formed elements in urine

UF-5000
analyzer of formed elements in urine

UC-3500
urine chemistry analyzer

Perform validation (data confirmation) efficiently by looking at UC-3500 and UF-5000 measurement results along with UD-10 images on U-WAM

New Products for Urinalysis (Modular Concept)

UF-5000

Japan Launched in September 2015
EMEA Scheduled for launch in April 2016
To be launched in the United States, China and Asian countries after receiving regulatory approval

UF-5000, UC-3500 and UD-10 in combination

UC-3500, UD-10 Japan: Launched in January 2016
EMEA: Scheduled for launch in April 2016
To be launched in the United States, China and Asian countries after receiving regulatory approval

UF-5000, UC-3500 and UD-10 can be combined flexible and meet with diverse urinalysis workflow needs

UF-5000 Characteristics

UF-5000

1) Enhanced performance on current parameters, expanded parameters
Reduced re-testing rate
(fewer false positives and false negatives)

UF-1000*i*
5 parameters

- Red blood cells
- White blood cells
- Bacteria
- Casts
- Epithelial cells

+9 parameters

- Squamous cells
- Non-squamous cells
- Hyaline casts
- Non-hyaline casts
- Fungi
- Spermatozoa
- Crystals
- Mucus
- WBC Clumps

Pathological casts
RBC morphological information

Kidney and urological diseases

2) Provides information useful in testing and diagnosis

Urinary tract infections

Bacteria classification

Urinary tract cancer

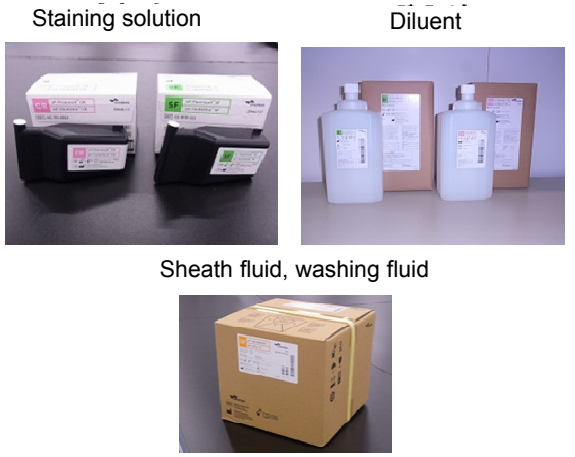
Atypical cells

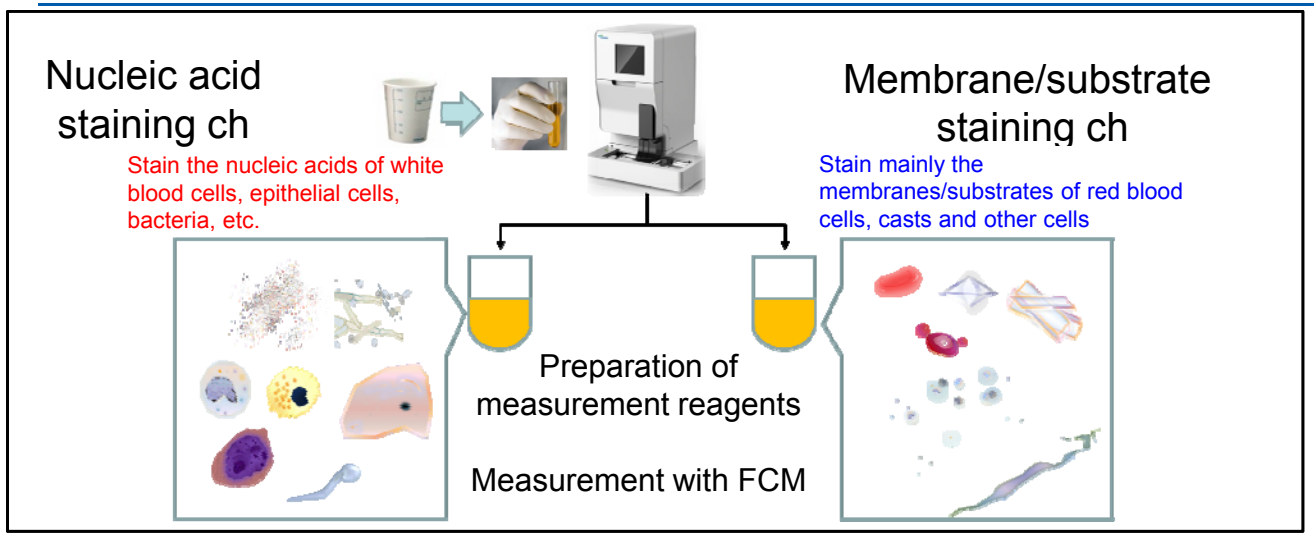
3) Body fluid measurement (spinal fluid, pleural fluid, ascitic fluid, joint fluid, etc.)

4) Easy to use and suited to various type of testing environments

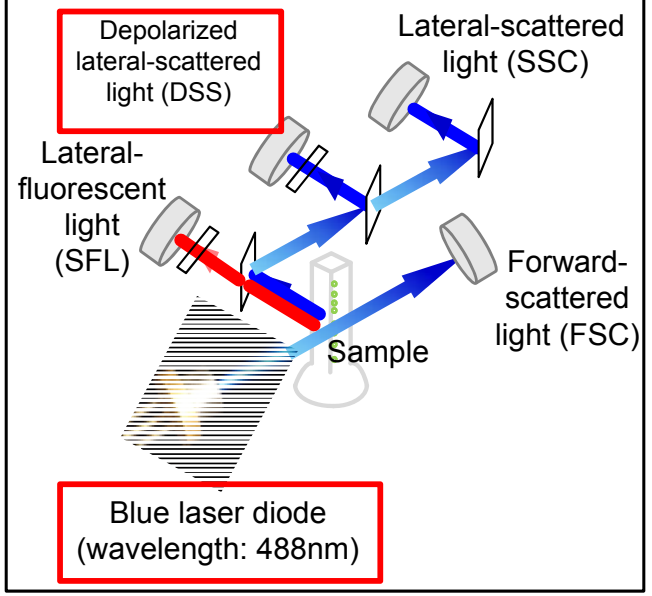
New Technologies on the UF-5000

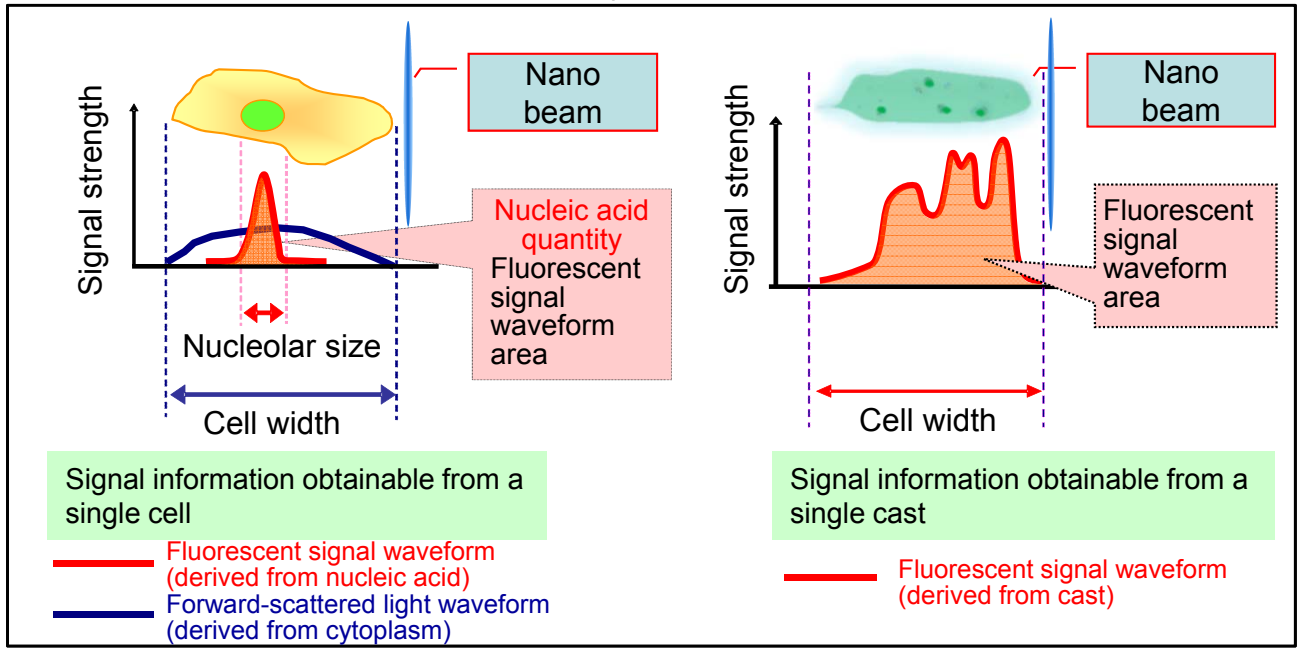
New reagents





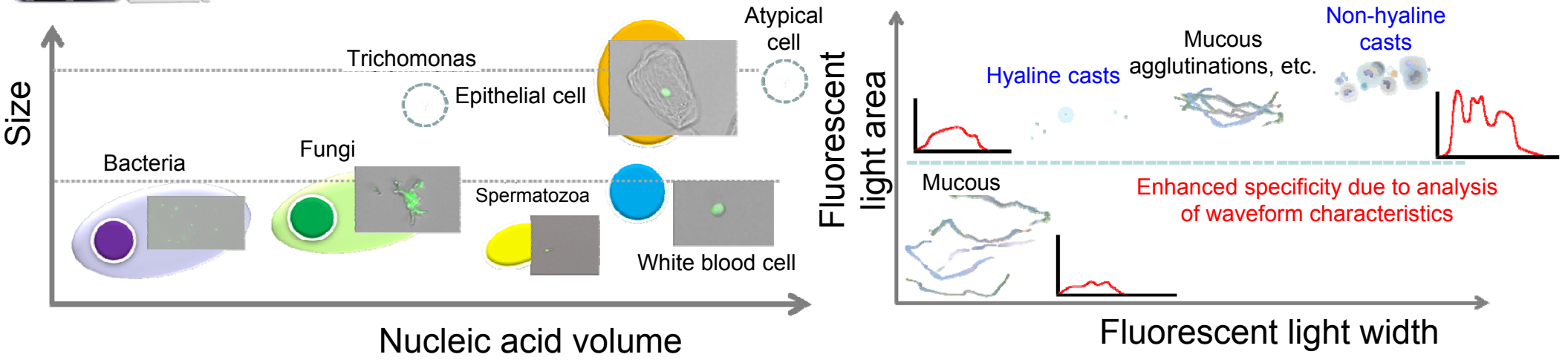
New detector





Performance Enhancements, Expanded Parameters

UF-5000



- For cells with nucleic acid, better differentiation of bacteria, fungi, white blood cells, epithelial and other cells, as well as expanded measurement parameters
- By using staining solution and dispersant to determine the internal structure of casts, reduce contamination by false positives, thereby enhancing the accuracy of Cast classification
- In future, expected to aid in detection of trichomonas, an infectious disease, and atypical cells related with cancers of the urinary organs and kidney

Extension to Urine Bacteria Testing

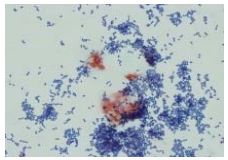
Urine bacteria testing flow

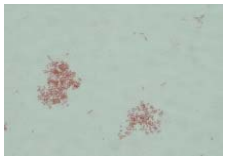
[Day 1]

Urine sampling

Gram stain, speculum

Manual method, 30–60 min.





- Estimate infecting bacteria
- Select culture environment

[Day 2]

Culture testing

[Day 3]
Follow-up testing

Bacterial type identification test

Drug sensitivity

Select anti-biotic agent

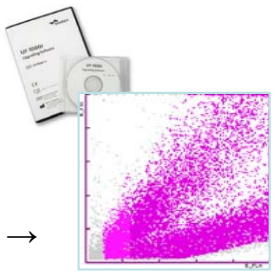
Automation and increased speed with the UF-Series

Under evaluation through joint research in Japan and overseas

UF-1000i bacterial morphology flag

Released in 2013

+



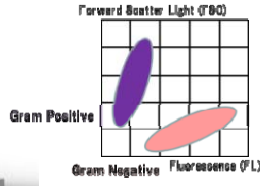
Mixed sample →

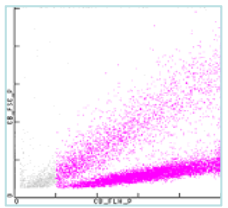
Rods? Cocci/mixed? flag output

Positive concordance rate with existing method (gram stain)

UF-5000 bacterial morphology flag

Released in 4Q of 2015





Mixed sample →

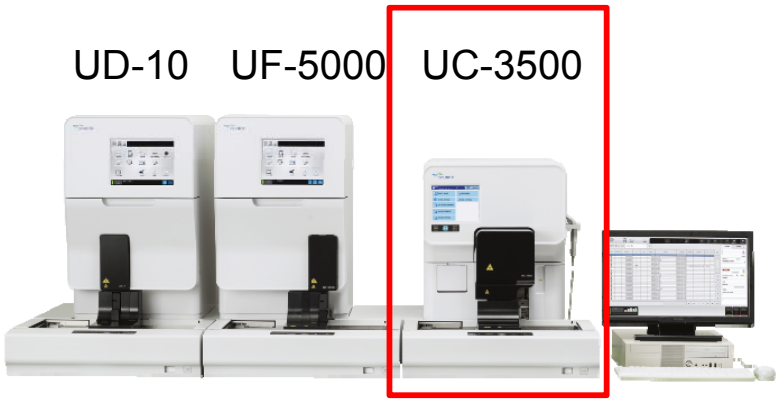
Gram positive? Gram negative? Mixed? flag output (further increase in precision)

Quantitative measurement of bacteria/measurement parameters

Higher precision

Obtaining medical reimbursement

UC-3500 Characteristics



Specifications

Throughput: up to 276 tests/hour

Test papers that can be measured simultaneously:
3 types, 300 papers can be loaded (set the container as it is)

Sample volume: 1ml (aspirated volume: 0.23mL)

Silent Design

Connectable with UF-5000 via conveyor system

Buffer between chemistry and sedimentation testing (Up to 160 samples), it contributes to maintain high throughput of chemistry testing.

(This can cover the peak time of routine measurement)

Urine chemistry test strips

Types (dosage forms)

- 9: general parameters, including Glucose, proteins and blood
- 11: Includes two additional, albumin and creatinine

Type	Parameters measured											
	Urobilinogen	Occult blood	Proteins	Glucose	Ketone bodies	Bilirubin	Nitrites	Leukocytes	pH	Creatinine	Albumin	Specific gravity
9 parameters	✓	✓	✓	✓	✓	✓	✓	✓	✓			※
11 parameters	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	※

Receive good feedback in performance and quality from the market

(Evaluations in Japan and overseas KOL)

UD-10 Characteristics

UD-10 UF-5000 UC-3500

Specifications

Measured parameters: None
(Images only, classified roughly into eight types, by size)
Enable to take images according to the result from UF-5000, customer request and pre-set review rules.

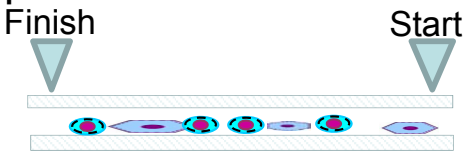
Throughput:	Up to 50 tests/hour
Sample volume:	1.6mL (aspirated volume: 0.3mL)
Analysis volume:	1μL (2μL in precise mode)
Imaging method:	Stage-scanning method (no staining, no centrifuging, natural sedimentation)
Expandability:	Manual classification possible using U-WAM

Provides high-quality imaging while curtailing instrument and running costs

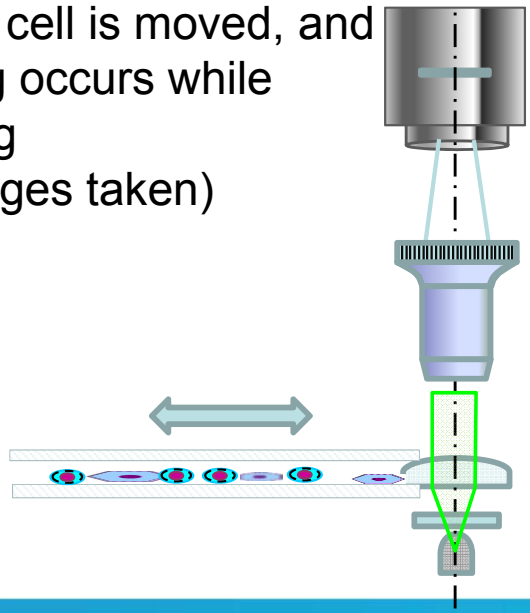
Imaging Principle

(1) The aspirated specimen is added to the cell, then waits for the cell to sink

(2) Once imaging begins, the focus is adjusted based on the predicted starting and final location, correcting for slope

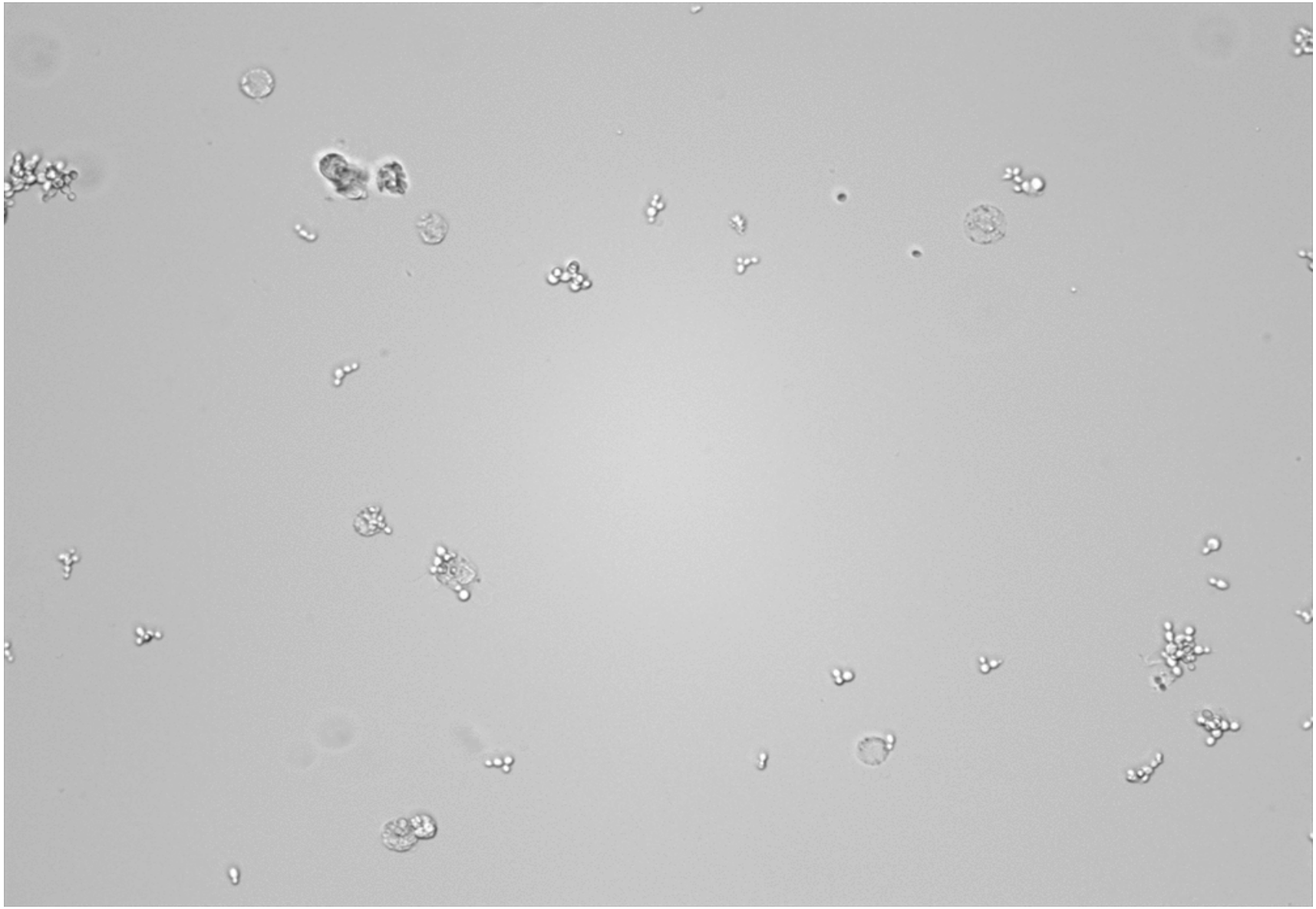


(3) The cell is moved, and imaging occurs while focusing
(40 images taken)



Captured Image (Specimen with Numerous Fungal Yeasts)

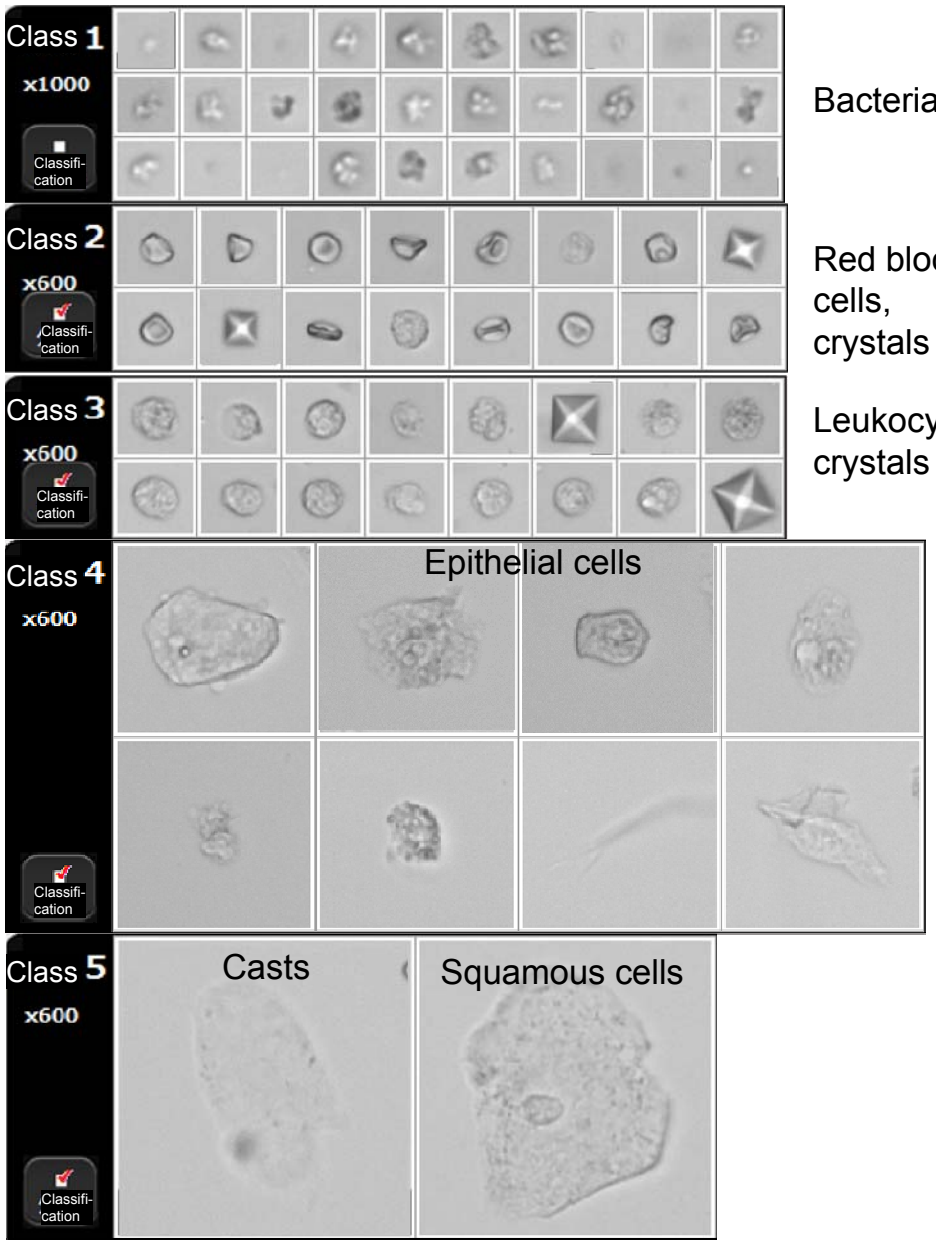
470μm

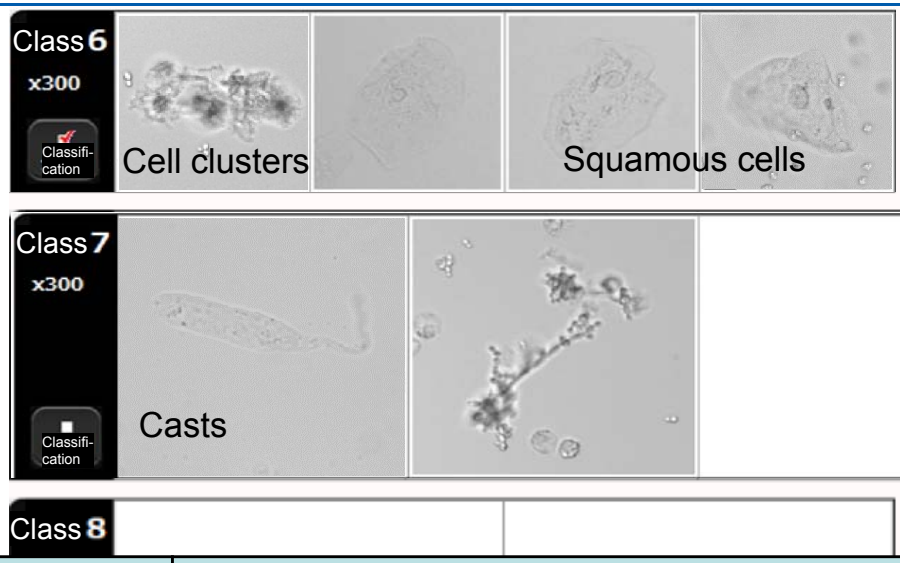


350 μm

40 images

Cell Clipping, Classification
(Roughly Classified into Eight, by Size)

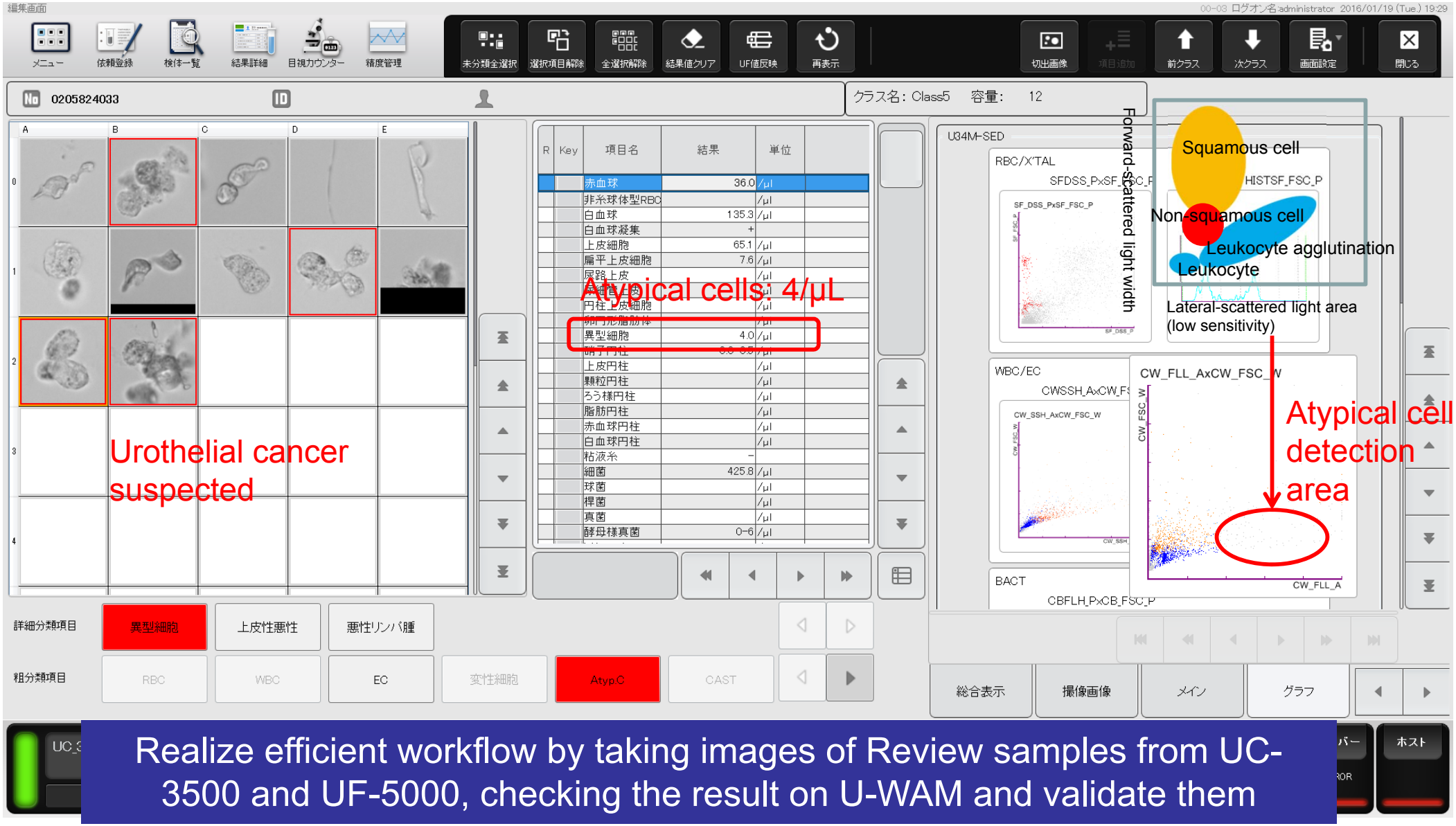




Rough classification	Principal cells included
Class1	Bacteria
Class 2	Red blood cells, crystals, fungi
Class 3	Leukocytes, crystals, fungi, renal tubular epithelial cells
Class 4	Renal tubular epithelial cells, urinary tract epithelial cells (deep–middle layer) Squamous cells (deep–middle layer)
Class 5	Urinary tract epithelial cells (deep–middle layer), squamous cells (deep–middle layer)
Class 6	Urinary tract epithelial cells (surface layer), squamous cells (surface layer), casts
Class 7	Urinary tract epithelial cells (surface layer), epithelial cell clusters, casts
Class 8	Casts, epithelial cell clusters

U-WAM Display Screen

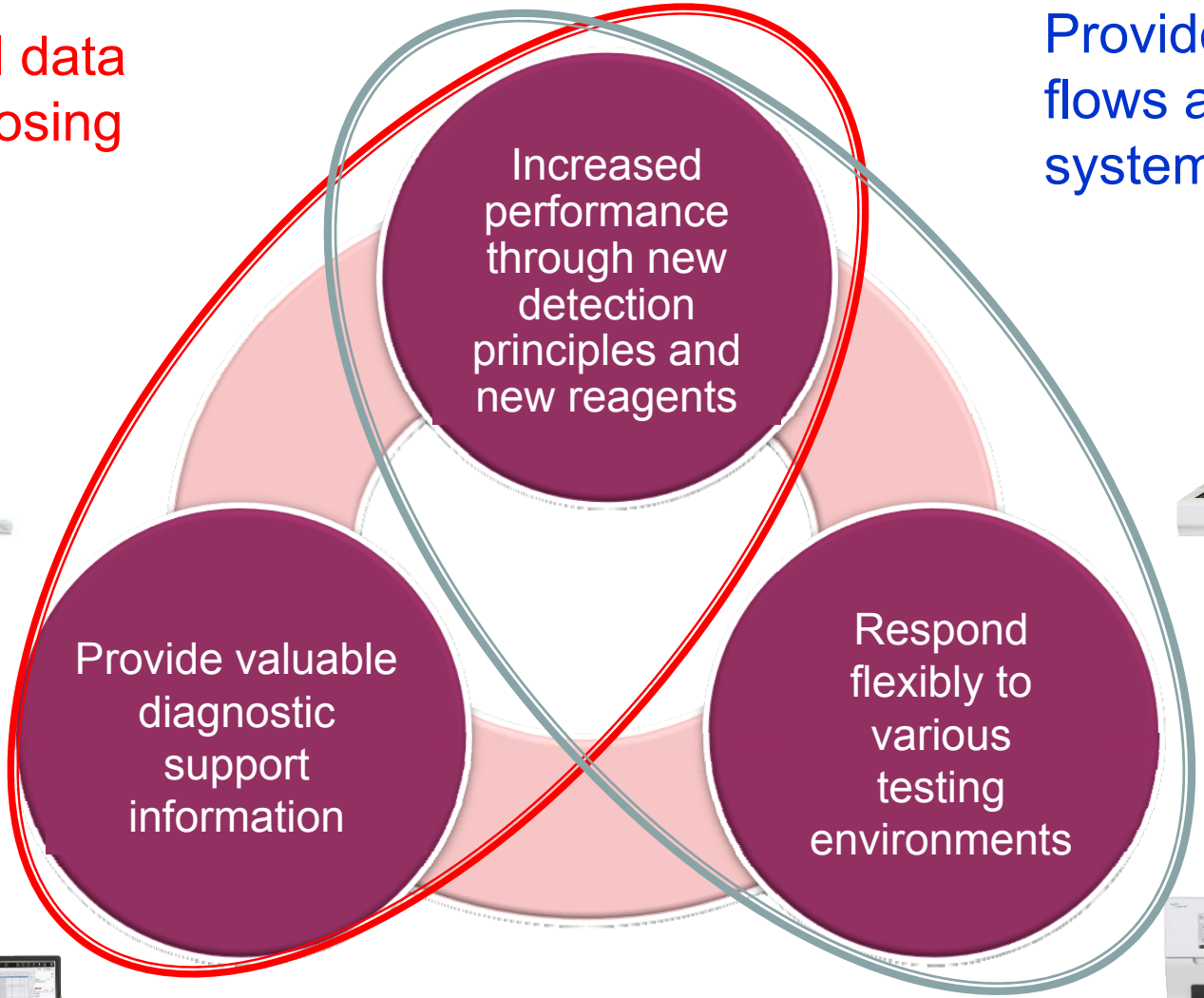
(Displays UC-3500 and UF-5000 Measurement Results and UD-10 Image on One Screen)



The Next State of Urinalysis

Provide clinical data useful in diagnosing disease

Provide optimal work flows and testing systems



2) Malaria Detection Technology Using Blue LD FCM and Progress on the Cervical Cancer Screening System

Malaria Detection Technology: Progress and Future Expectations

Development Now → Market cultivation → Market introduction

Introduce into the market in fiscal 2016

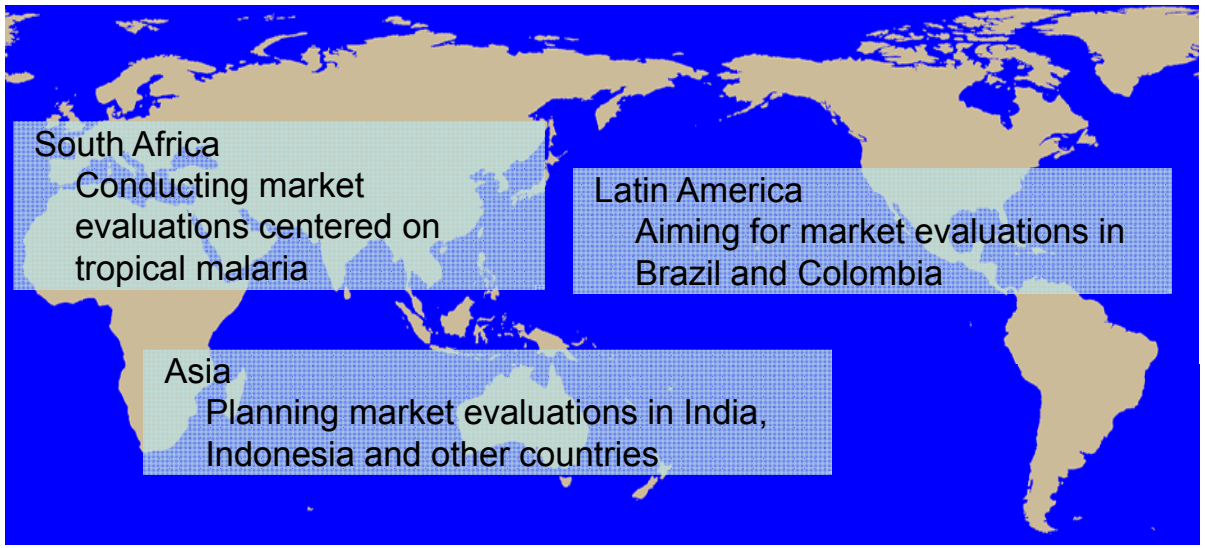
Product development

○Conference presentation of evaluation results in the South African market (2014 ASLM, 2015 Japanese Society of Tropical Medicine)

- South African market evaluation
- Asian market evaluation
- Latin American market evaluation

Quickly achieve awareness of utility, establish as gold standard and create a business foundation for specialized malaria instruments

Promote evaluations by prominent global institutions specializing in malaria of the “utility of malaria detection technology using FCM (fast, simple, inexpensive, highly sensitive, etc.)” to quickly enhance market recognition and build a business foundation for malaria testing



Cervical Cancer Testing System Progress and Future Outlook

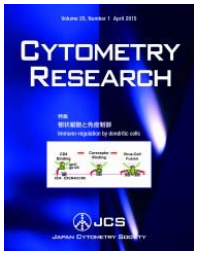
LC-1000 introduction in November 2014 | Market cultivation | Market introduction

Now

Activities in Japan

- Principles announced at the Japanese Cytometry Society
- Received technology award from *Cytometry Research*

Utility as cell proliferation index



Research led by the Japanese Society of Clinical Cytology

- Comparison with conventional methods (cytology, HPV testing) (consistency with tissue diagnosis)

Led by Sysmex

At commercial labs, evaluate efficiency and medical economic effect
- Evaluate medical performance and testing costs
- Quality control for negative specimens (double check)

Activities overseas

- Performance announcement at FIGO*
- Featured in *Gynecologic Oncology*

Joint research with Fudan University in Shanghai
LC-1000 > HPV

Activities toward CFDA registration in China

- Field trials
- Clinical studies
- Joint research with KOL

Activities in the Europe and AP regions

- Evaluations at commercial labs in the Netherlands
- Individual country registrations, evaluations at commercial labs in South Korea and national hospital in Vietnam (Hanoi)

Use for medical care at own expense
Insurance coverage
Guidelines recommending

*International Federation of Gynecology and Obstetrics

3. Progress on Technology Development Themes

(3) Hemostasis and Immunochemistry

Hiroshi Kanda, Executive Officer, Executive Vice President of the
Hemostasis Product Engineering Div.

1) Future of Clot Waveform Analysis

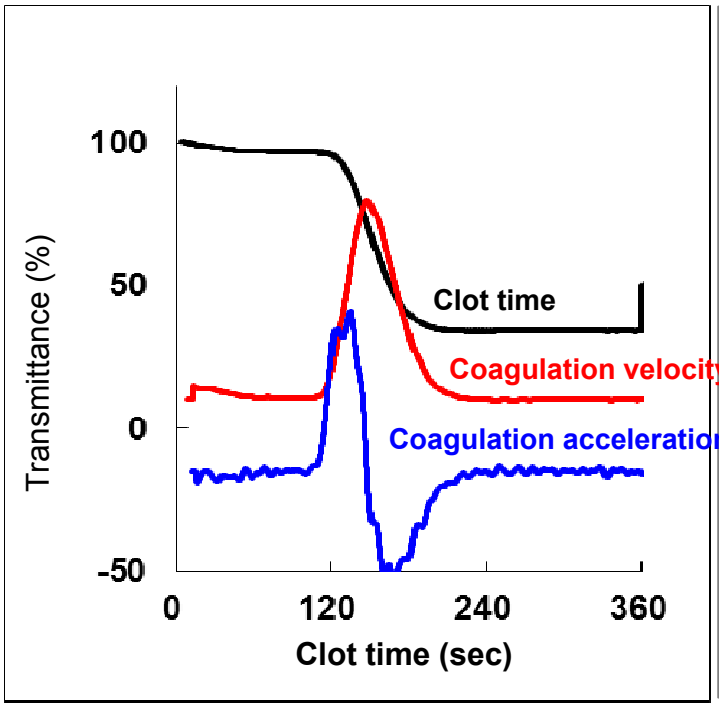
2) CWA application to ACE910* (nonproprietary name: emicizumab) monitoring

* Under development by Chugai Pharmaceutical Co., Ltd.

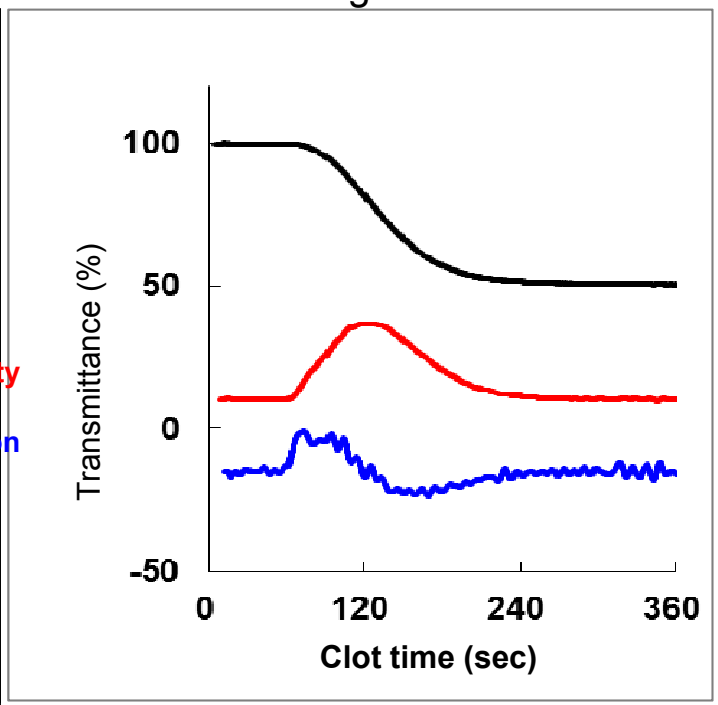
1) Future of Clot Waveform Analysis

e.g.) Comparison of CWA parameter of the 3 samples which showed similar clotting time in APTT

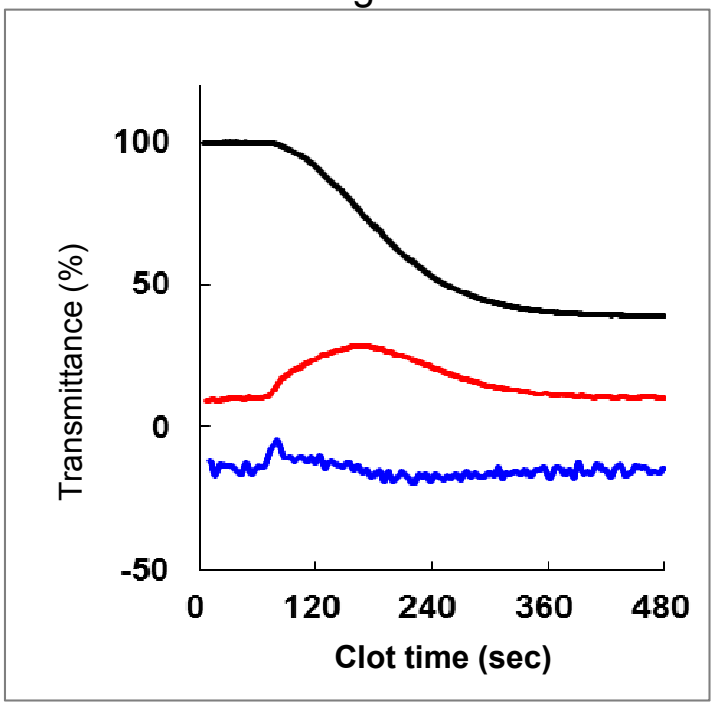
Lupus Anticoagulant
< Thrombotic disease >



Hemophilia A (without inhibitor*)
< bleeding disease >



Hemophilia A (with inhibitor*)
< bleeding disease >



* Inhibitor means antibodies which inhibit factor VIII activity in hemophilia A patients.

CWA parameters may provide more information for detection of clinical condition than Clot time.

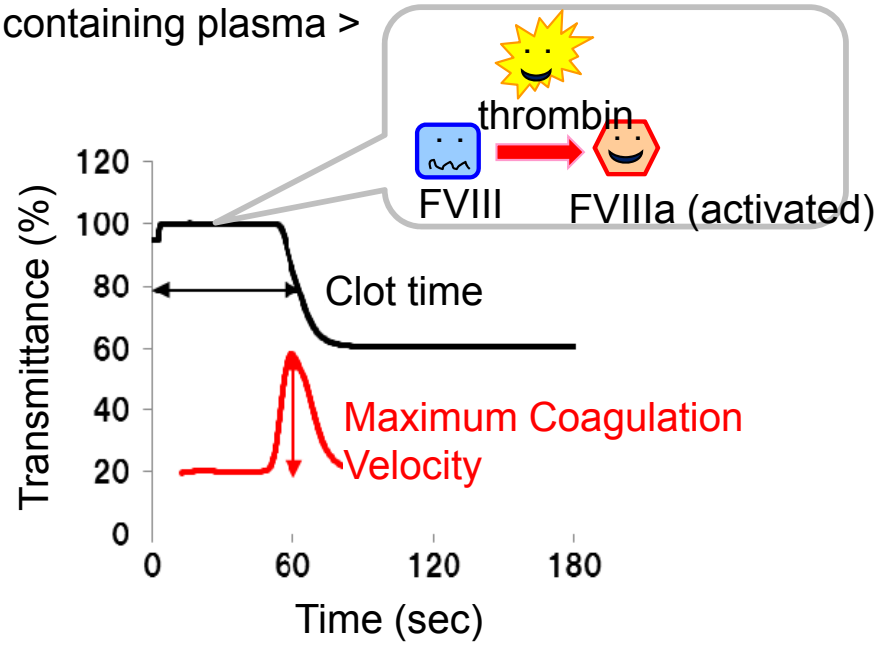
⇒ CWA application to ACE910 (nonproprietary name: emicizumab) monitoring is under investigation.

2) CWA application to ACE910 (nonproprietary name: emicizumab) monitoring

- The outcome of treatment may vary depending on individuals. And it may become desirable to measure ACE910 (nonproprietary name: emicizumab) activity from time to time.

- Problem of conventional method
  FVIII* needs to be activated by thrombin**. Since ACE910 (nonproprietary name: emicizumab) functions as active form, conventional clot time method overestimates the activity of ACE910.

< FVIII containing plasma >



< ACE910 containing plasma >

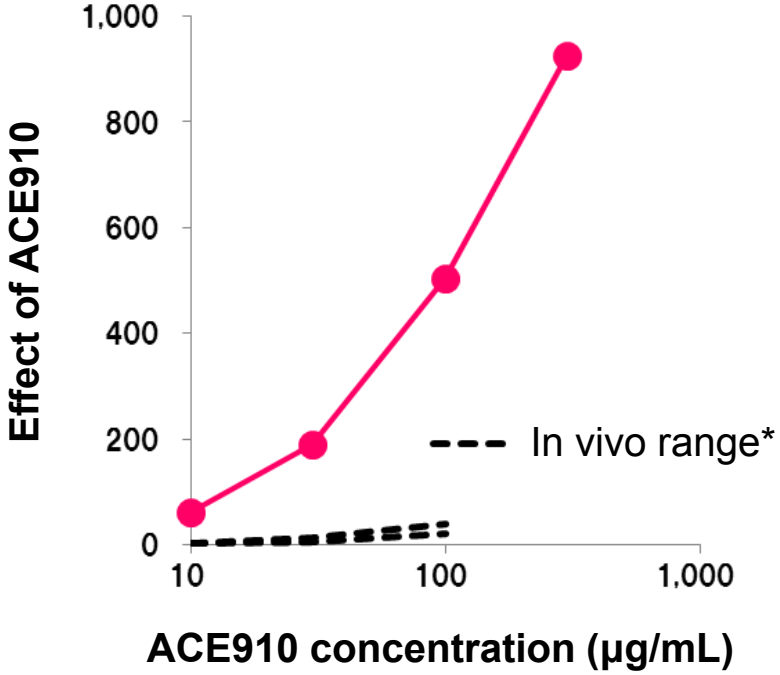
- Solution
  Check ACE910 (nonproprietary name: emicizumab) activity by CWA parameters such as Maximum Coagulation Velocity which are not affected from starting time of clotting.

* FVIII: Coagulation factor VIII. One of important proteins necessary for blood coagulation.
** Thrombin: A blood protein which causes coagulation.

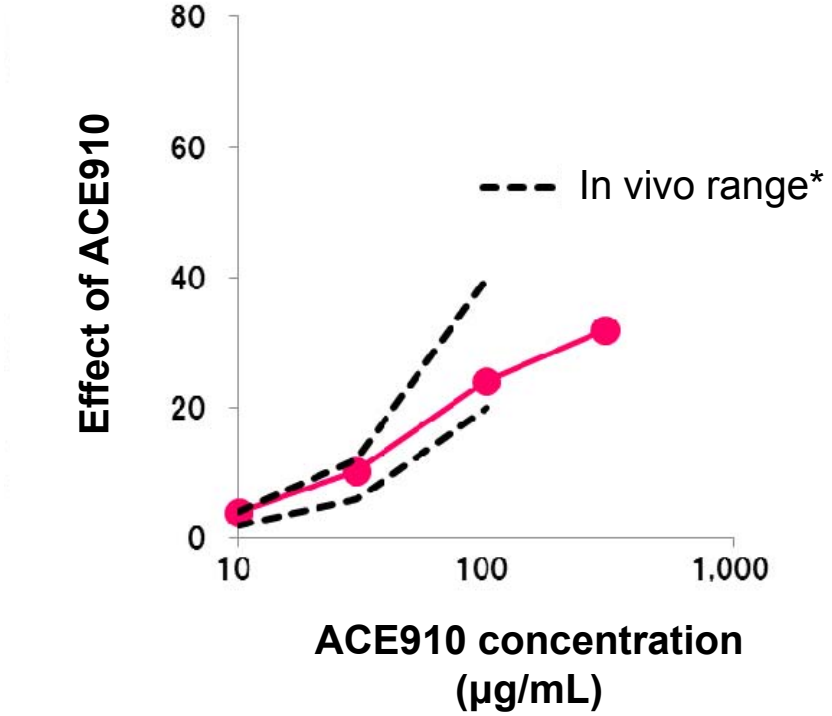
2) CWA application to ACE910 (nonproprietary name: emicizumab) monitoring

- Interim report of practical use examination

Existing method (clot time)
⇒ Overestimation of the ACE910 activity



Clot Waveform analysis (Coagulation velocity)
⇒ Decrease of overestimation of the ACE910 act



There is a possibility that CWA can monitor the effect of ACE910 (nonproprietary name: emicizumab) as well as monitoring of conventional factor VIII concentrates

* FVIII converted activity range defined in non-clinical animal study of ACE910 (nonproprietary name: emicizumab)
Reference: Muto et al. *J Thromb Haemost* 2014

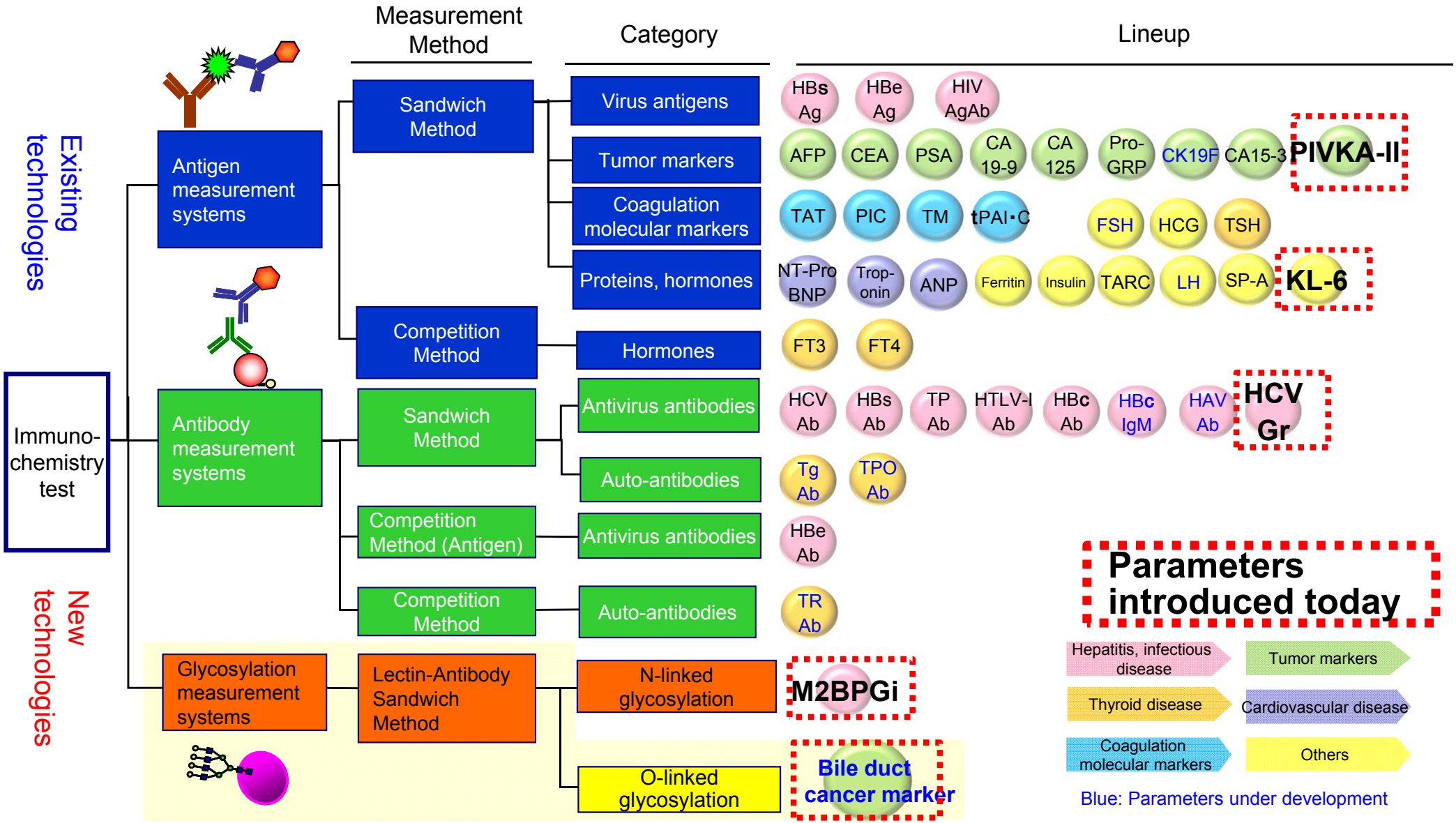
3. Progress on Technology Development Themes

(3) Hemostasis and Immunochemistry

Yoichi Takahama, Executive Vice President of the
Immunology & Chemistry Product Engineering Div.

3) Increase in HISCL Reagent Portfolio and HISCL Reagent Parameters
4) Update of Glycosylation Marker for Bile Duct Cancer
5) Glycosylation Marker (Gi) Series Concept and Future Development
6) Manufacturing Immunochemistry Reagents Employing Leading-Edge Technologies

3) HISCL Reagent Portfolio



3) Increase in HISCL Reagent Parameters

Total management of patients with liver disease using immunochemistry

Make type C hepatitis a curable disease!

- Type C hepatitis therapies: New drugs coming out to handle different types of virus
  Around ¥5–7 million in drug costs up to recovery
  ⇒Need way to differentiate viruses before administering

Liver cancer risk factors

Type C hepatitis, type B hepatitis, alcoholic liver injury, nonalcoholic steatohepatitis

Survival rate also continues to fall five years after contracting liver cancer!

- After eliminating the virus (HCV), follow-up observations important
  ⇒Monitoring and carcinogenesis and recurrence prediction necessary

3) HISCL HCV Gr Reagent

Viral hepatitis screening → **Type C hepatitis/ treatment plan** → Liver cirrhosis → Hepatocellular carcinoma

- Toward a time when type C hepatitis can be cured with oral drugs
- Drug costs (for type 1 and type 2) are high, so appropriate selection is important

Simple, fast

- HISCL HCV Gr reagent enables drug selection
- Measurement time: 17 minutes

Cost

- Group determination by measuring level of anti type C hepatitis virus specific antibody (223 NHI points)
- PCR method not covered by insurance and expensive, at around ¥10,000

Breakdown of the Type C Hepatitis Virus in Japan

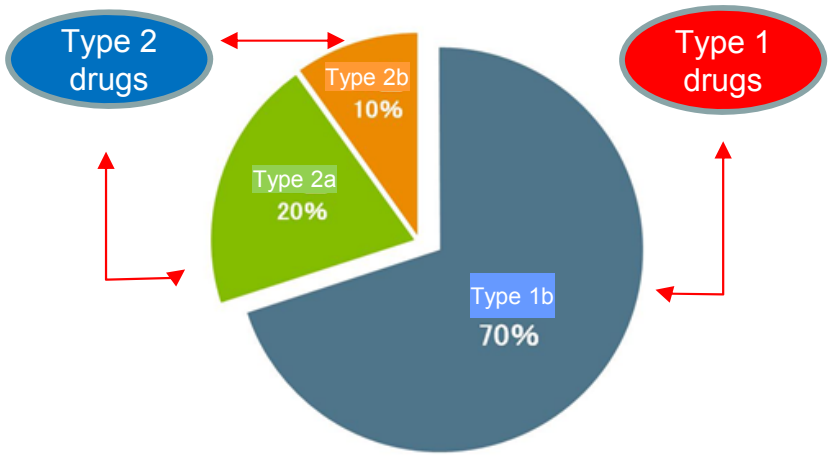


Image based on the Japan Society of Hepatology's 2013 Diagnosis Guidelines for Chronic Hepatitis and Liver Cirrhosis (Bunkodo)

Contributes to the selection of therapeutic drugs and personalized medicine

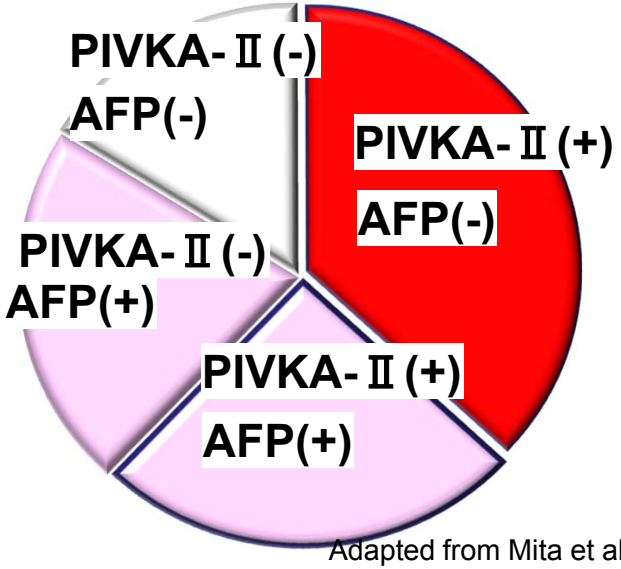
3) HISCL PIVKA-II Reagent

Viral hepatitis screening → Liver cirrhosis (fibrosis) → **Hepatocellular carcinoma**

- Using AFP and PIVKA-II in combination to diagnose hepatocellular carcinoma substantially increases detection sensitivity and is effective for diagnosis and evaluating therapeutic results

Diagnosis

Effective at narrowing down high-risk groups



Adapted from Mita et al. Cancer 82,1643,(1998)

Treatment

Evaluating method of diagnosing liver cancer recurrence rise by combination with GPC3, described on page 26 of these materials

Selection of patients with high recurrence risk based on circulating GPC3

Through combination, increase rate of positive detection at time of recurrence

Helps to improve diagnostic performance for hepatocellular carcinoma

3) HISCL M2BPGi Reagent Update

Reported in 16 prominent English-language periodicals

- **Scientific Reports (2013)** Kuno et al.
- **Journal of Gastroenterology (2015)** Toshima et al.
- **Hepatology Research (2014)** Tamaki et al.

Effective new marker for determining liver fibrosis

- **PLos ONE (2015)** Sasaki et al.
- **Alimentary Pharmacology & Therapeutics (2015)** Ura et al.

Reflects hepatitis treatment results

- **Hepatology (2014)** Yamasaki et al.
- **Hepatology Research (2015)** Tamaki et al.
- **PLos ONE (2015)** Sasaki et al.
- **Liver International (2015)** Toyoda et al.

Allows forecasting of carcinogenesis risk in HCV patients

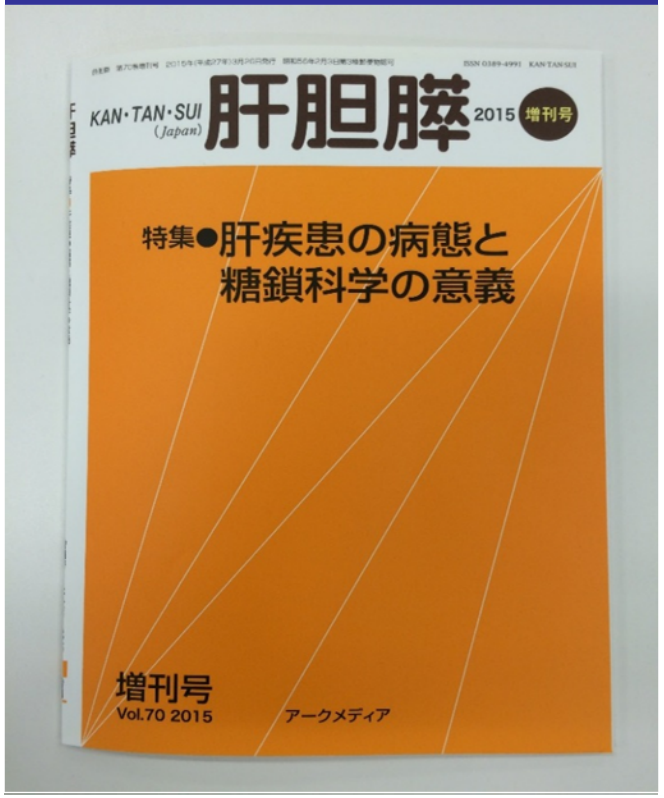
- **J Gastroenterol. (2015)** Fujiyoshi et al., Toshima et al.

Useful as prognostic factor following liver cancer resection

M2BPGi special feature edition

- **Kantansui (2015)** special edition

Clinical reports from eight facilities



In addition to determining fibrosis of the liver, effective in hepatitis therapeutic gains, prognostication and forecasting cancer

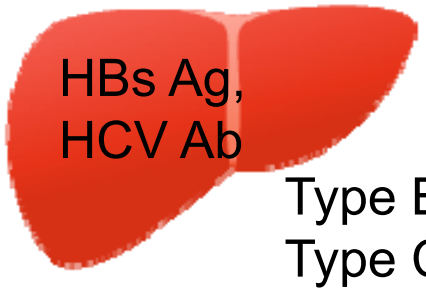
3) Total Management of Hepatic Disease Using HISCL Reagents

Viral hepatitis screening

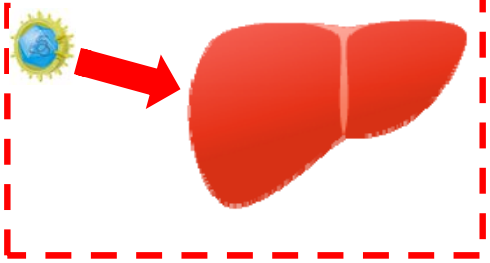
Type C hepatitis/treatment plan

HISCL HCV Gr

Enables drug selection



Type B hepatitis
Type C hepatitis



Group 1 (1a, 1b) → Type 1 drugs

Group 2 (2a, 2b) → Type 2 drugs

Chronic hepatitis, liver cirrhosis (fibrosis)

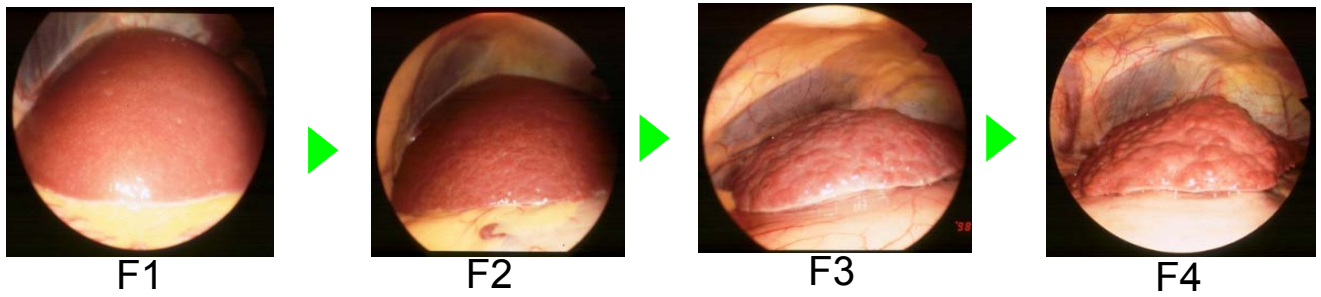
Hepatocellular carcinoma

Viral hepatitis, fibrosis progression

Cancer prognosis, post-surgery monitoring

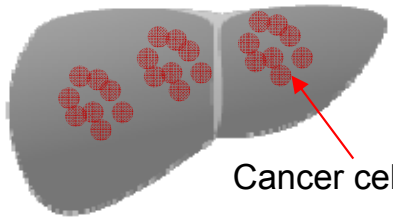
Liver cancer screening

HISCL M2BPGi



HISCL PIVKA-Ⅱ

HISCL AFP



3) HISCL KL-6 Reagent

Interstitial pneumonia (stemming from connective tissue disease, infectious disease, drugs, asbestos, etc.)

HSCL KL-6 is effective at interstitial pneumonia screening and monitoring

Before administration	During administration	When in doubt
Physical findings (Clubbed fingers, Velcro crackles, etc.)	Physical findings (Clubbed fingers, Velcro crackles, etc.)	Symptoms, physical findings (Clubbed fingers, Velcro crackles, etc.)
Chest X-rays	Chest X-rays	Chest HRCT imaging

Excerpted and adapted from consensus statement for the diagnosis and treatment of drug-induced lung injuries

In regions where pulmonary disease is high, efficacy is a point of international focus for interstitial pneumonia markers developed in Japan (such as KL-6 and SP-A)

3) Total Management of Pulmonary Disease Using HISCL Reagents

Interstitial pneumonia (upward trend in various epidemiological studies in various countries)

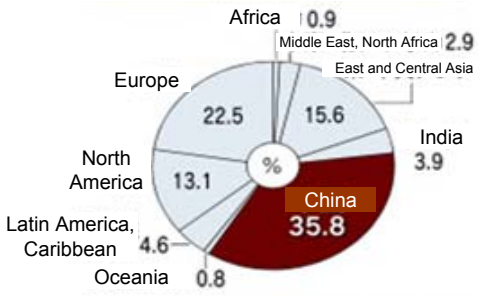
Index of therapeutic gains

HISCL SP-A

Screening, monitoring

HISCL KL-6

China topping the charts in number of cancer cases (WHO estimate, 2012), with 1.82 million cases of lung cancer

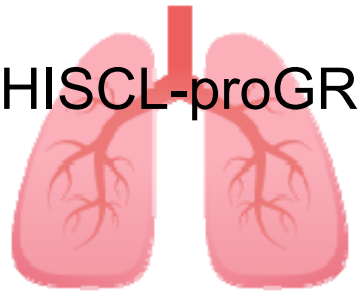


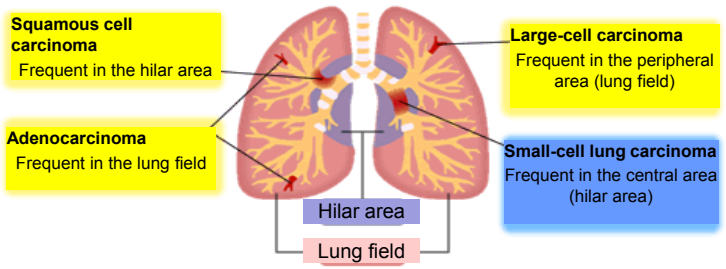
Lung cancer (leading site-specific mortality rate in Japan, China and other countries)

Small-cell carcinoma

HISCL CEA

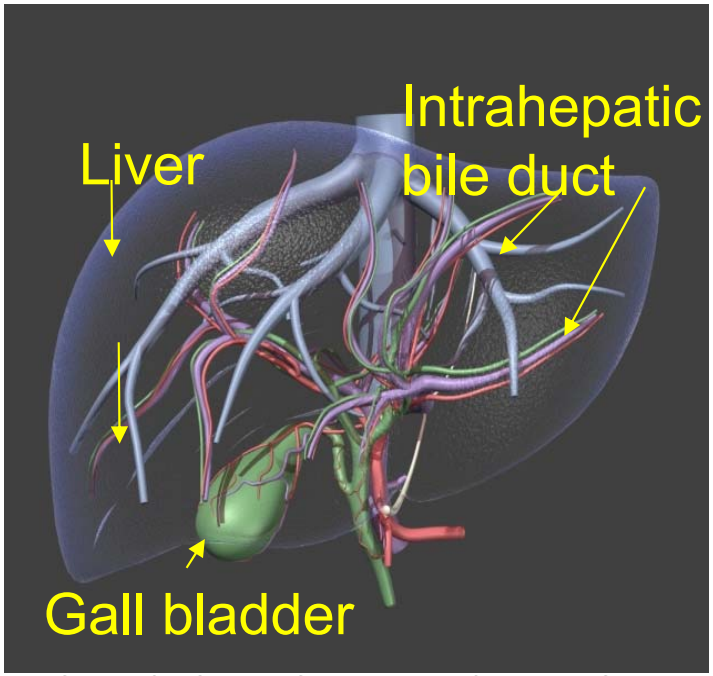
HISCL-proGRP





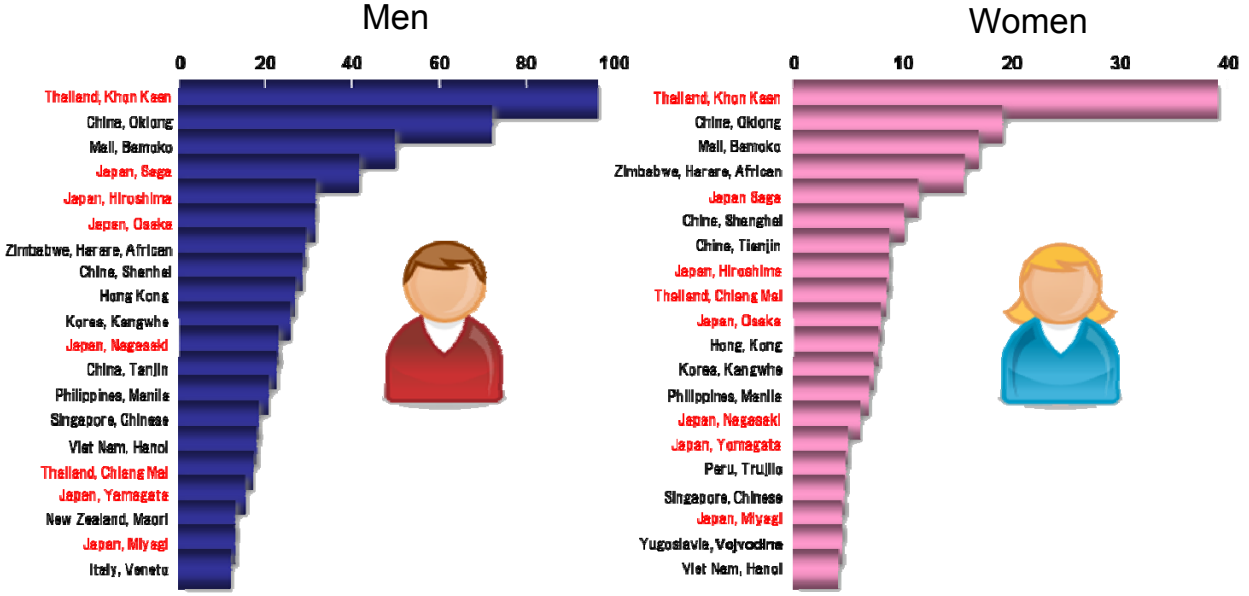
Histological type needs to be determined as part of treatment plan

4) Update of Marker for Bile Duct Cancer



Center for Cancer Control and Information Services, National Cancer Center

Age-adjusted incidence rate of intrahepatic bile duct cancer (per 100,000 people)



Shaib Y, et al. Semin Liver Dis 24:115-125, 2004

- Bile duct cancer the second most frequent type of malignant tumor of the liver
- Many patients in Japan, Thailand and other parts of Asia
- Notable causative agents of bile duct cancer include liver cirrhosis, viral hepatitis, and parasitic liver flukes in the bile duct

4) Update of Marker for Bile Duct Cancer

In collaboration with the National Institute of Advanced Industrial Science and Technology, participating in the e-ASIA (JST* international project) joint research program

*JST: Japan Science and Technology Agency

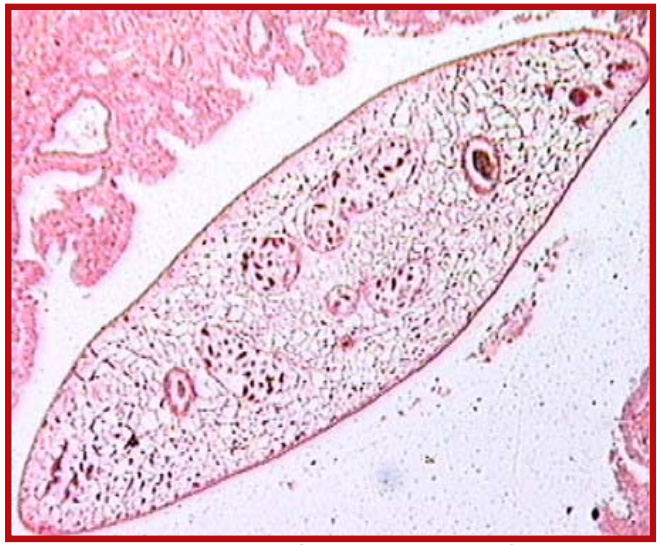
In February 2016, began large-scale clinical evaluations on specimens from Japan, Thailand and Laos

Bile duct cancer risk factors

Hepatolithiasis

Kubo S, et al. World J Surg (1996)

Liver fluke



Kubo S, et al. World J Surg (1996)
In Thailand and Laos, more than 10 million people are affected by liver flukes.

Highly sensitive detection of bile duct cancer and biliary system tumors

Use in combination with tumor marker CA19-9 contributes to further increases in clinical value

Shida et al., JDDW2015 oral presentation

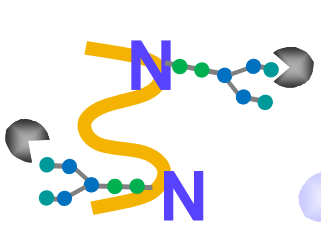
4) Update of Marker for Bile Duct Cancer

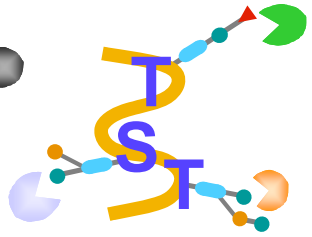
Glyco-sylation

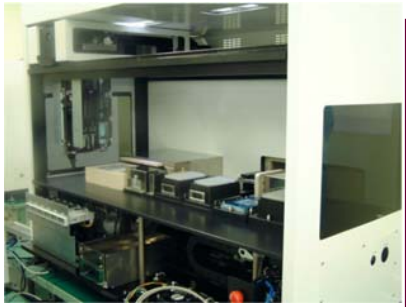
Detection technology

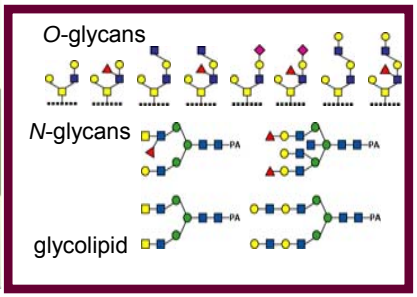
Synthesis technology

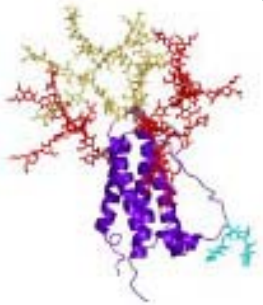
N-linked O-linked







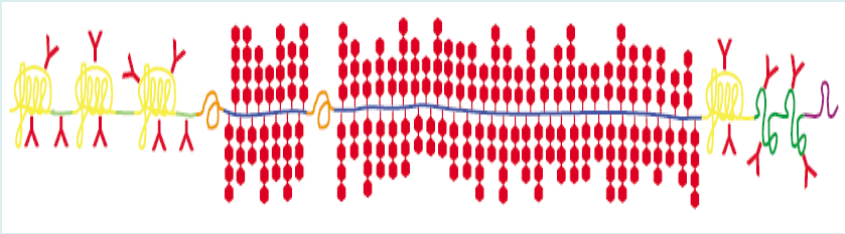




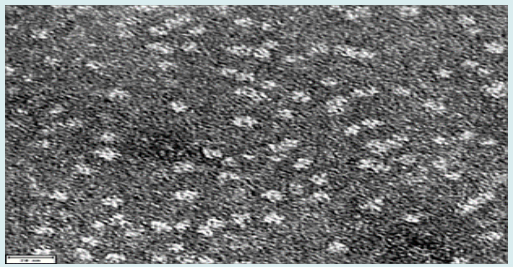
[Existing]
Antigen
(Simple structure)

Joint development with the National Institute of Advanced Industrial Science and Technology

[New]
Mucin protein
(Extremely complex structure)



Molecular weight: up to several MDa
Sugar content: 50–80%

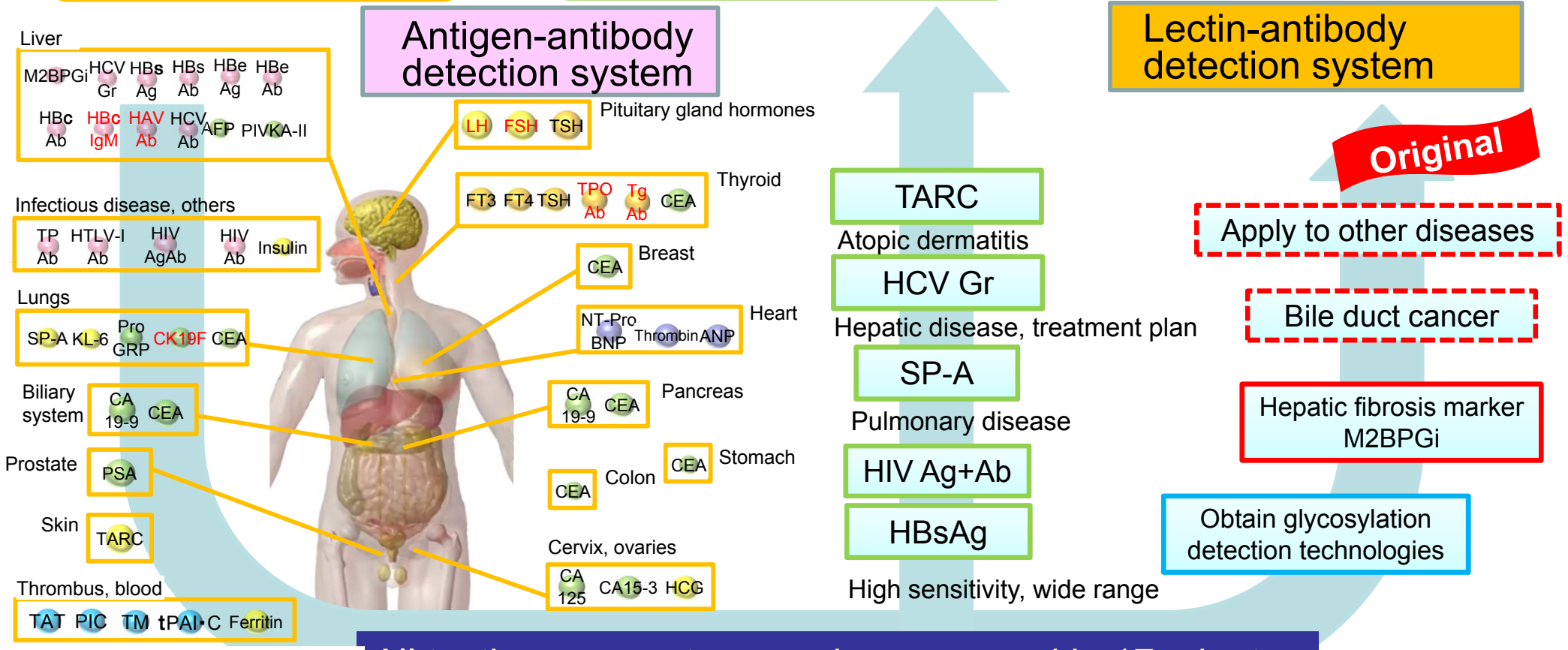


Succeeded in development of standard product (mucin) for bile duct cancer marker

5) Glycosylation Marker (Gi) Series Concept and Future Development

Provide clinical value through global roll-out including Japan, China and AP

Current parameters	Unique markers	Glycosylation markers



Black: Parameters being sold or approved
Red: Parameters under development

All testing parameters can be measured in 17 minutes

6) Manufacturing Immunochemistry Reagents Employing Leading-Edge Technologies

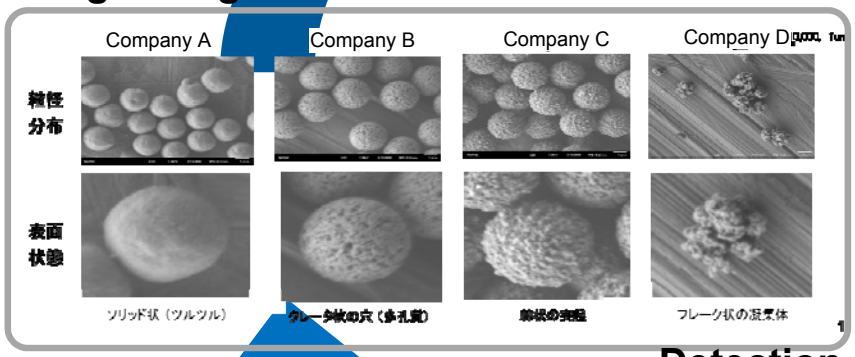
SPring-8&SACLA electron microscope

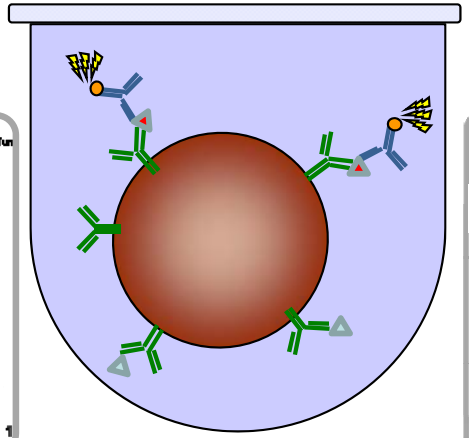
Technology platform

HISCL-Series

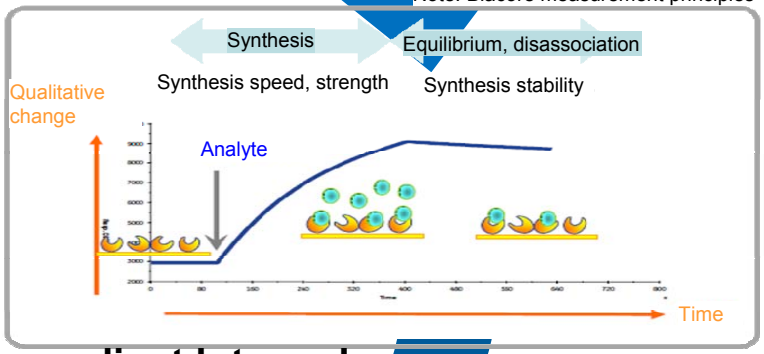
Intermolecular interaction analysis

Structural analysis of particles and reagent ingredients

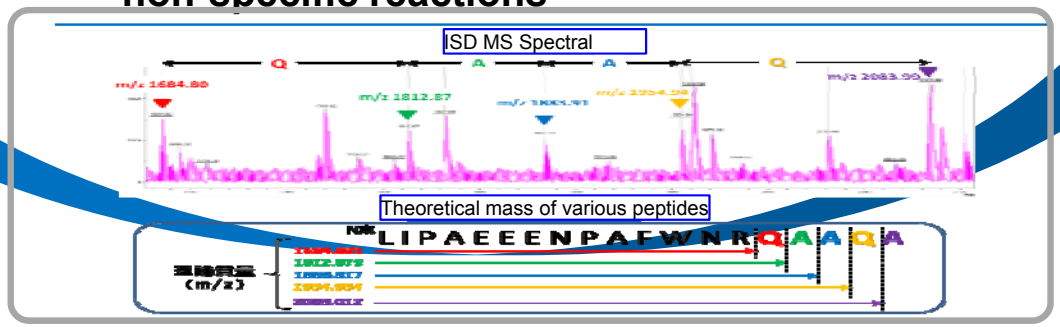




Antigen-antibody, immune reaction analysis



Detection differences in reagent ingredient lots and non-specific reactions



Mass spectrometer

Medical innovation and changing manufacturing “quality”

3. Progress on Technology Development Themes

(4) Life Science

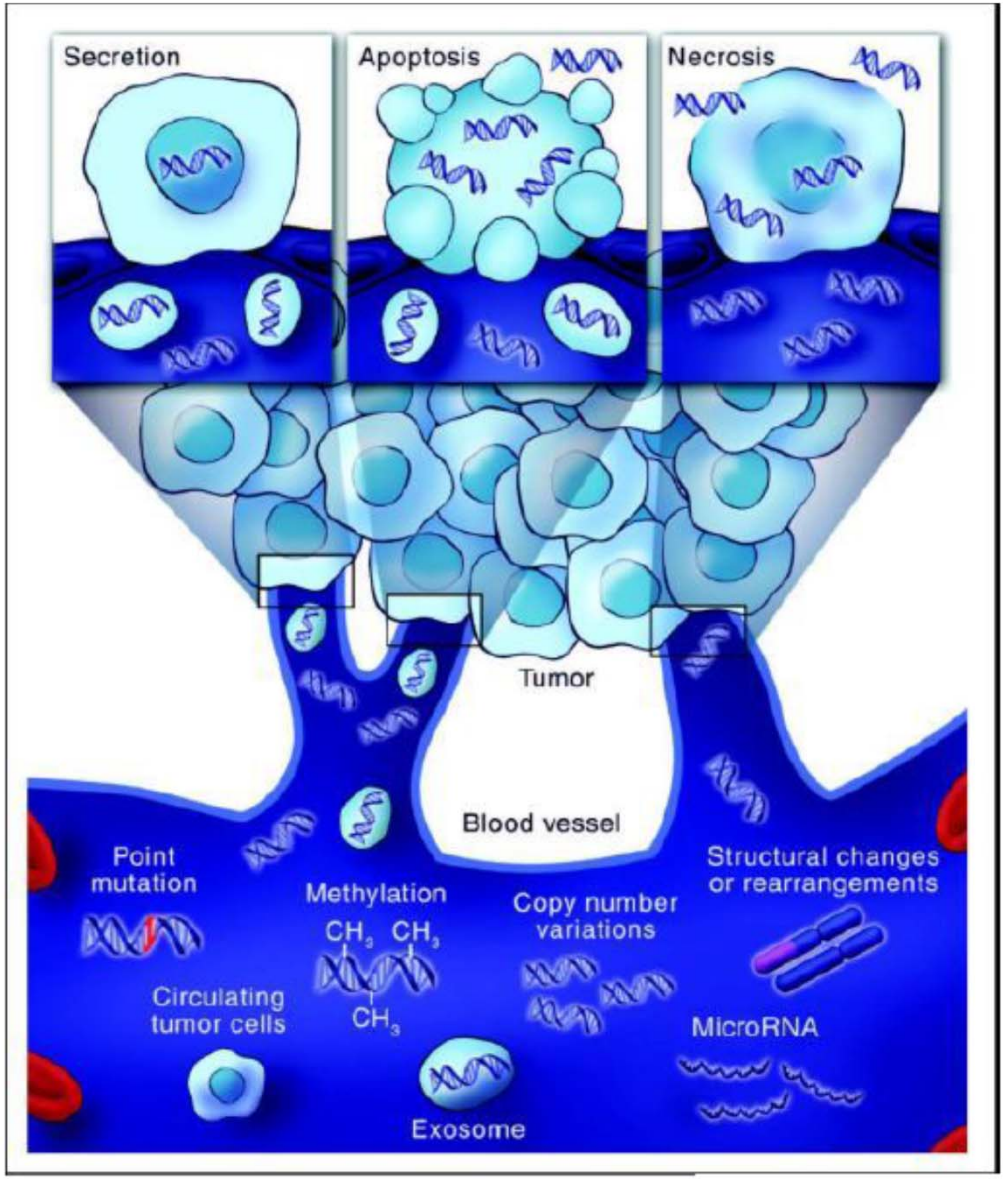
Mamoru Kubota, Executive Officer, Executive Vice President of the
Life Science Product Engineering Div.

Development of Assay for OncoBEAM Assay Products Employing BEAMing Technology

About Liquid Biopsy

Liquid Biopsy

Liquid biopsy involves taking a specimen of blood or bodily fluid, with the aim of realizing a level of performance similar to a tissue biopsy (resecting a portion of physical cancer and other tissue) but placing less of a burden on the patient through combination with molecular genetic analysis technology



Diaz L A , and Bardelli A JCO 2014;32:579-586

(Literature reference)
Experimental Medicine, Special Edition
Vol. 32 No.12

The Significance of Liquid Biopsy

	Tissue Biopsy	Liquid Biopsy
Measured target	Physical tissue	Circulating tumor DNA; ctDNA
Specimen collection	Biopsy	Blood sampling
Invasiveness	High	Low

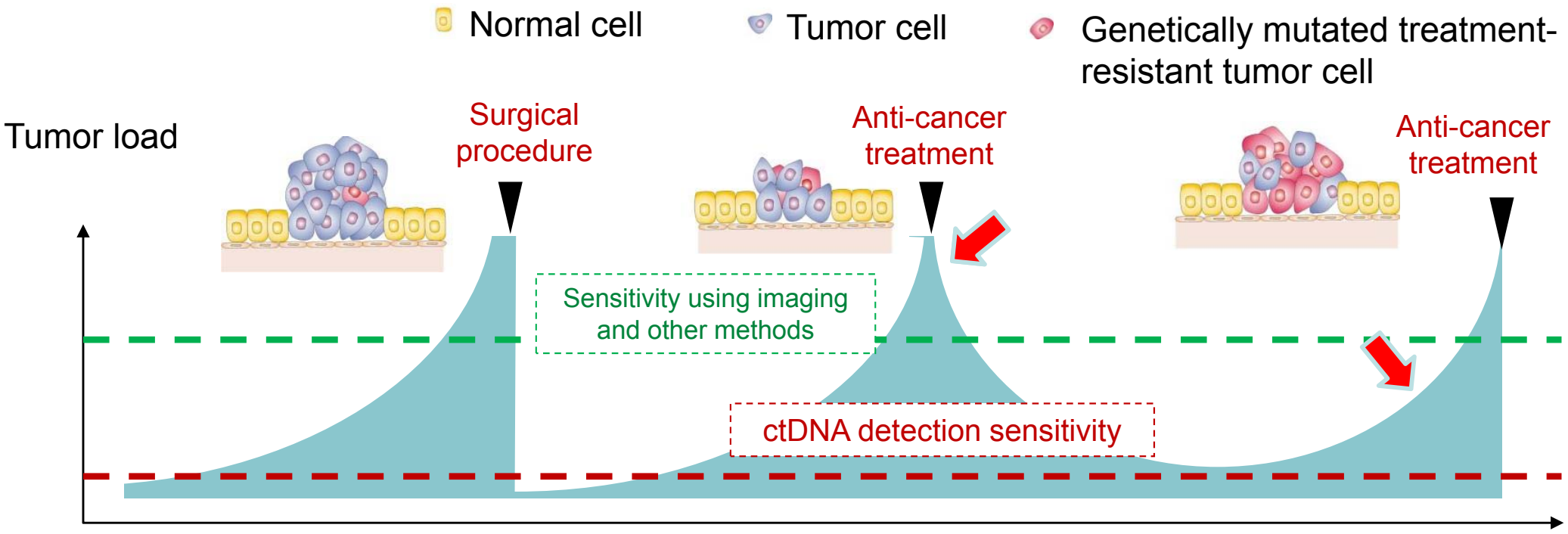
(1) Early detection of cancer

(2) Drug efficacy, prognosis prediction

(3) Drug resistance monitoring

Clinical Application of Liquid Biopsy in ctDNA Gene Testing

Screening → Confirmative diagnosis → Recurrence, MRD monitoring → Drug sensitivity test → Drug resistance monitoring

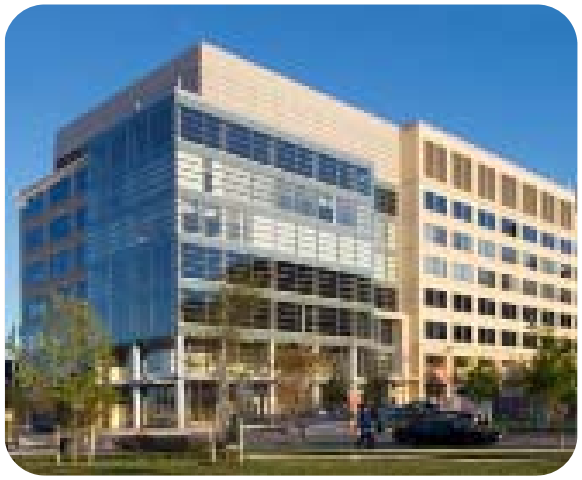


ctDNA: Circulating tumor DNA
MRD: Minimum residual disease

Principal Testing Parameters of the OncoBEAM Assay Service

	Disease	Principal biomarker
PIK3CA	Breast cancer	PIK3CA
RAS	Colorectal cancer	RAS
EGFR	Lung cancer	EGFR

Sysmex Inostics Hamburg Lab



Sysmex Inostics Baltimore Lab

Assay service for research ⇒ Verification of clinical utility ⇒ Assay service for diagnostic use (U.S. CLIA) ⇒ Creation of IVD system

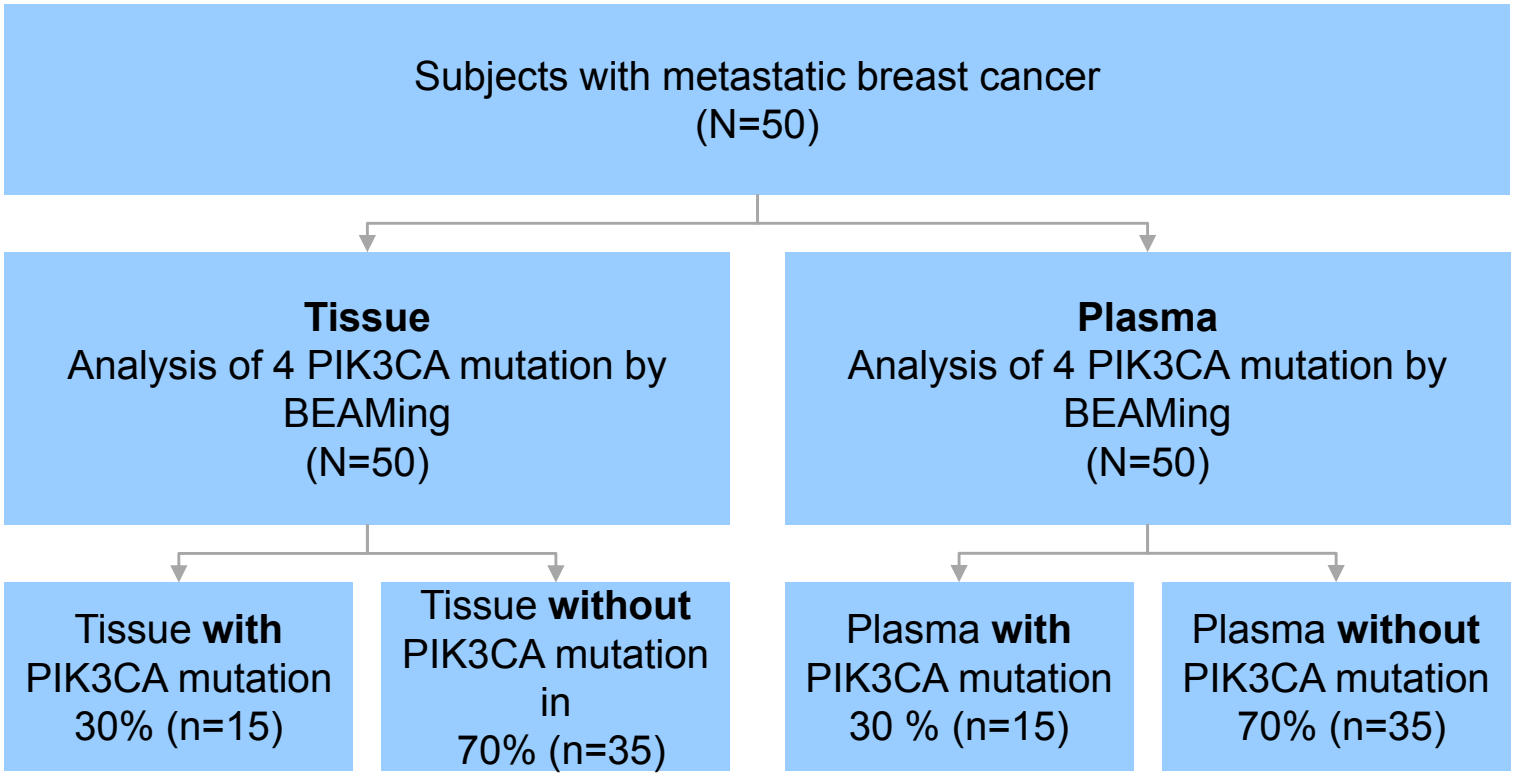
Equivalence of PIK3CA Gene Mutation Tissue and Plasma

PIK3CA

RAS

EGFR

Sample ID	*PIK3CA* Tumor Tissue	*PIK3CA* Plasma
1	WT	WT
2	WT	WT
3	WT	WT
4	WT	WT
5	WT	WT
6	WT	WT
7	WT	WT
8	E545K (13%)	E545K (7%)
9	WT	WT
10	H1047R (19%)	H1047R (0.7%)
11	WT	WT
12	WT	WT
13	WT	WT
14	WT	WT
15	WT	WT
16	WT	WT
17	H1047R (6%)	H1047R (0.7%)
18	WT	WT
19	WT	WT
20	WT	WT
21	WT	WT
22	WT	WT
23	WT	WT
24	H1047R (10%)	H1047R (6%)
25	WT	WT
26	WT	WT
27	WT	WT
28	WT	WT
29	WT	WT
30	WT	WT
31	E542K (4%)	E542K (4%)
32	WT	WT
33	H1047R (39%)	H1047R (3%)
34	E545K (13%)	E545K (0.05%)
35	WT	WT
36	H1047R (16%)	H1047R (0.5%)
37	WT	WT
38	WT	WT
39	WT	WT
40	WT	WT
41	WT	WT
42	WT	WT
43	H1047L (31%)	H1047L (7%)
44	H1047R (44%)	H1047R (0.2%)
45	WT	WT
46	H1047R (35%)	H1047R (3%)
47	WT	WT
48	E545K (11%)	E545K (5%)
49	H1047R (20%)	H1047R (2%)
50	WT	WT
N=50	13/50	13/50



Angenendt, et al, *J Clin Oncol* 28:15s, 2010 (suppl; abstract 10502)

In a comparison of results of testing PIK3CA gene mutations in the tumor tissue of patients with metastatic breast cancer and PIK3CA gene mutations in circulating ctDNA, the concordance rate between the two was 100%.

Higgins MJ et al. *Clin Cancer Res* 2012; 183462-3469

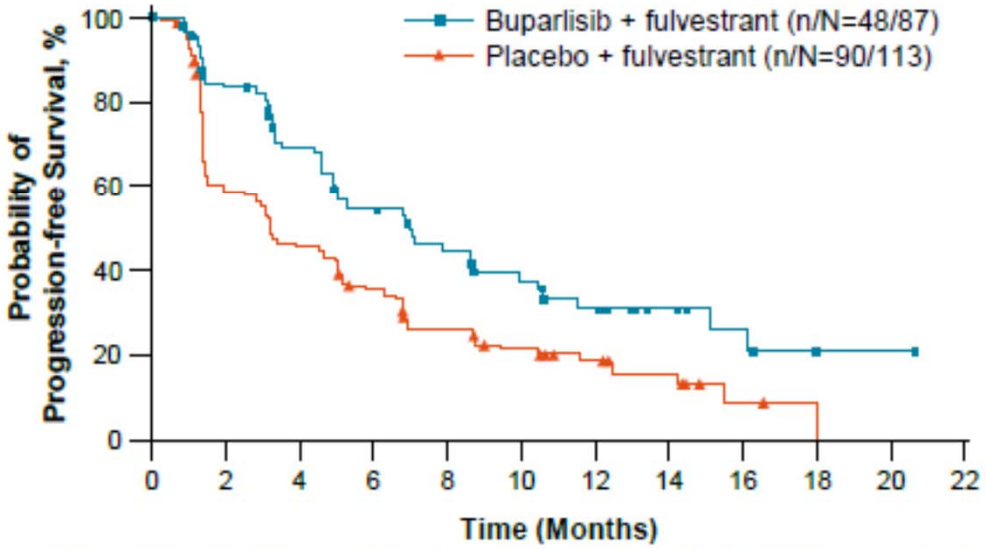
Random Clinical Study on Advanced Breast Cancer (Phase III; BELLE-2 Trial) Novartis

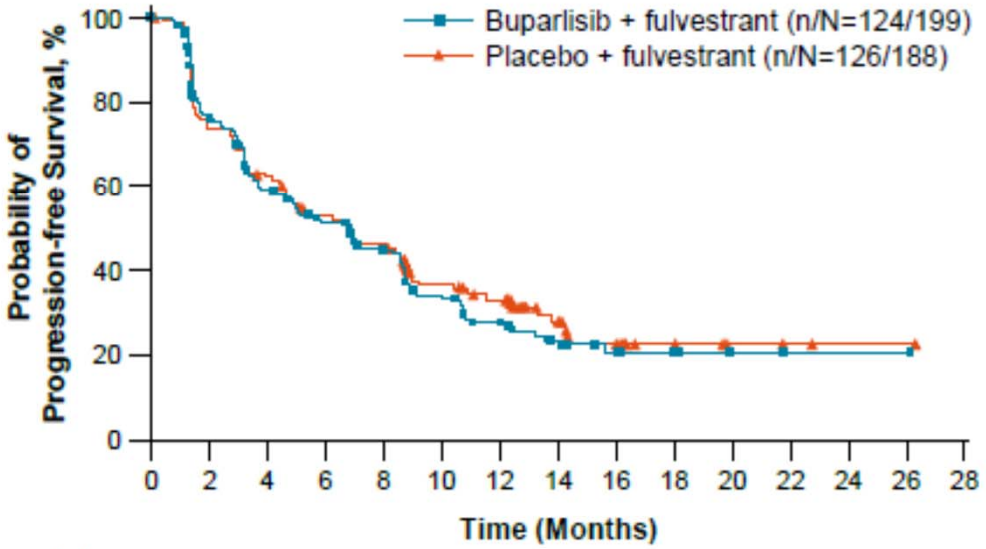
San Antonio Breast Cancer Symposium – December 8–12, 2015

Buparlisib Plus Fulvestrant Produced a Clinically Meaningful PFS Improvement in Patients With ctDNA *PIK3CA* Mutations

ctDNA *PIK3CA* Mutant n=200	Buparlisib + Fulvestrant n=87	Placebo + Fulvestrant n=113
Median PFS, months (95% CI)	7.0 (5.0–10.0)	3.2 (2.0–5.1)
HR (95% CI)	0.56 (0.39–0.80)	
One-sided nominal *P* value	<0.001	

ctDNA *PIK3CA* Non-mutant n=387	Buparlisib + Fulvestrant n=199	Placebo + Fulvestrant n=188
Median PFS, months (95% CI)	6.8 (4.7–8.5)	6.8 (4.7–8.6)
HR (95% CI)	1.05 (0.82–1.34)	
One-sided nominal *P* value	0.642	

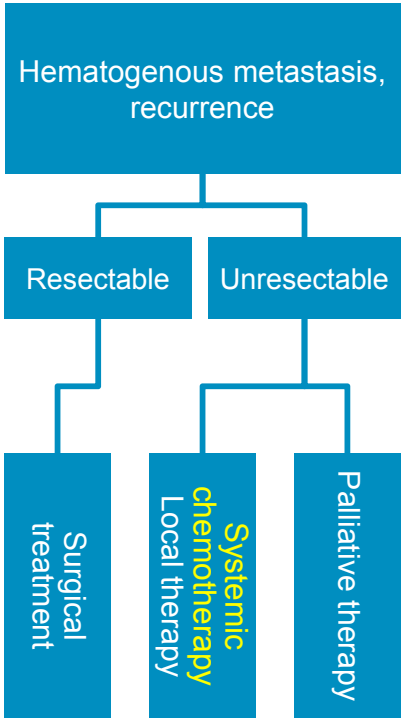




CI, confidence interval; ctDNA, circulating tumor DNA; HR, hazard ratio; PFS, progression-free survival.

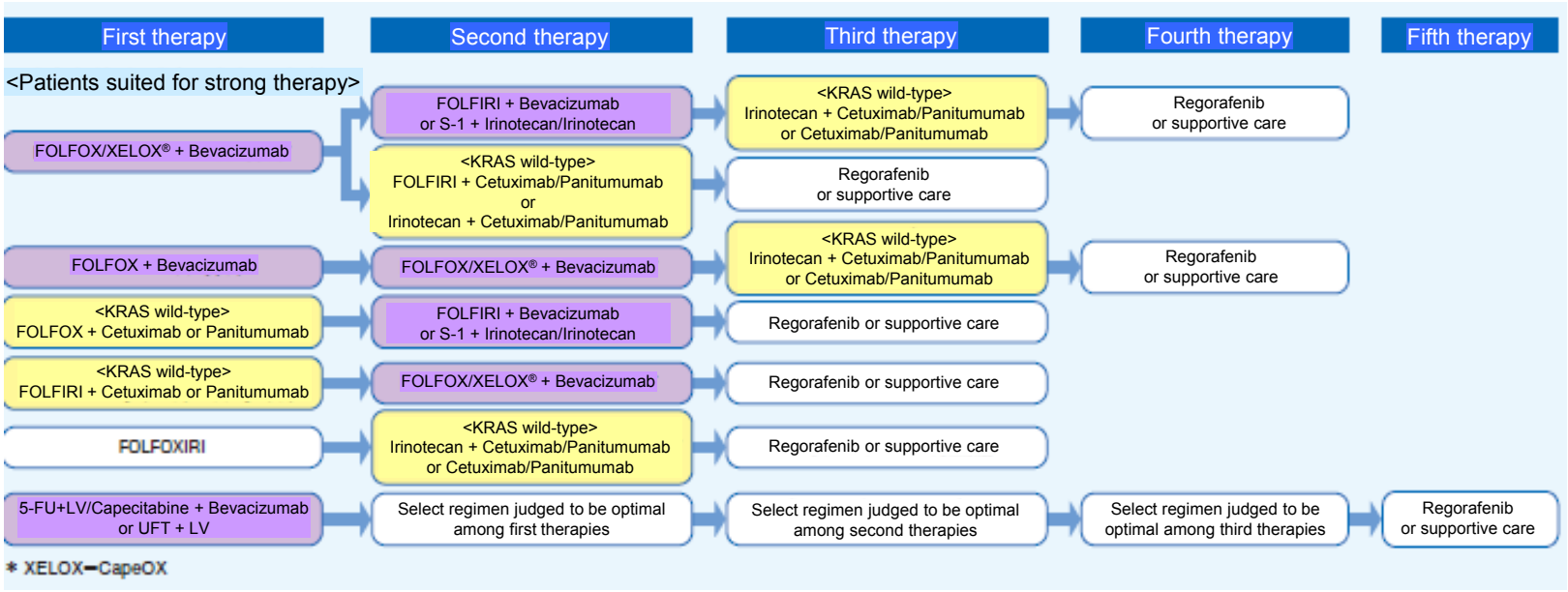
In this field, Sysmex holds an exclusive use license for the PIK3CA gene mutation marker

Significance of RAS Gene Testing for Colorectal Cancer



Chemotherapy for unresectable, advanced, recurrent colorectal cancer

Therapeutic methods combining multiple anti-cancer agents and molecularly targeted drugs are mainstream

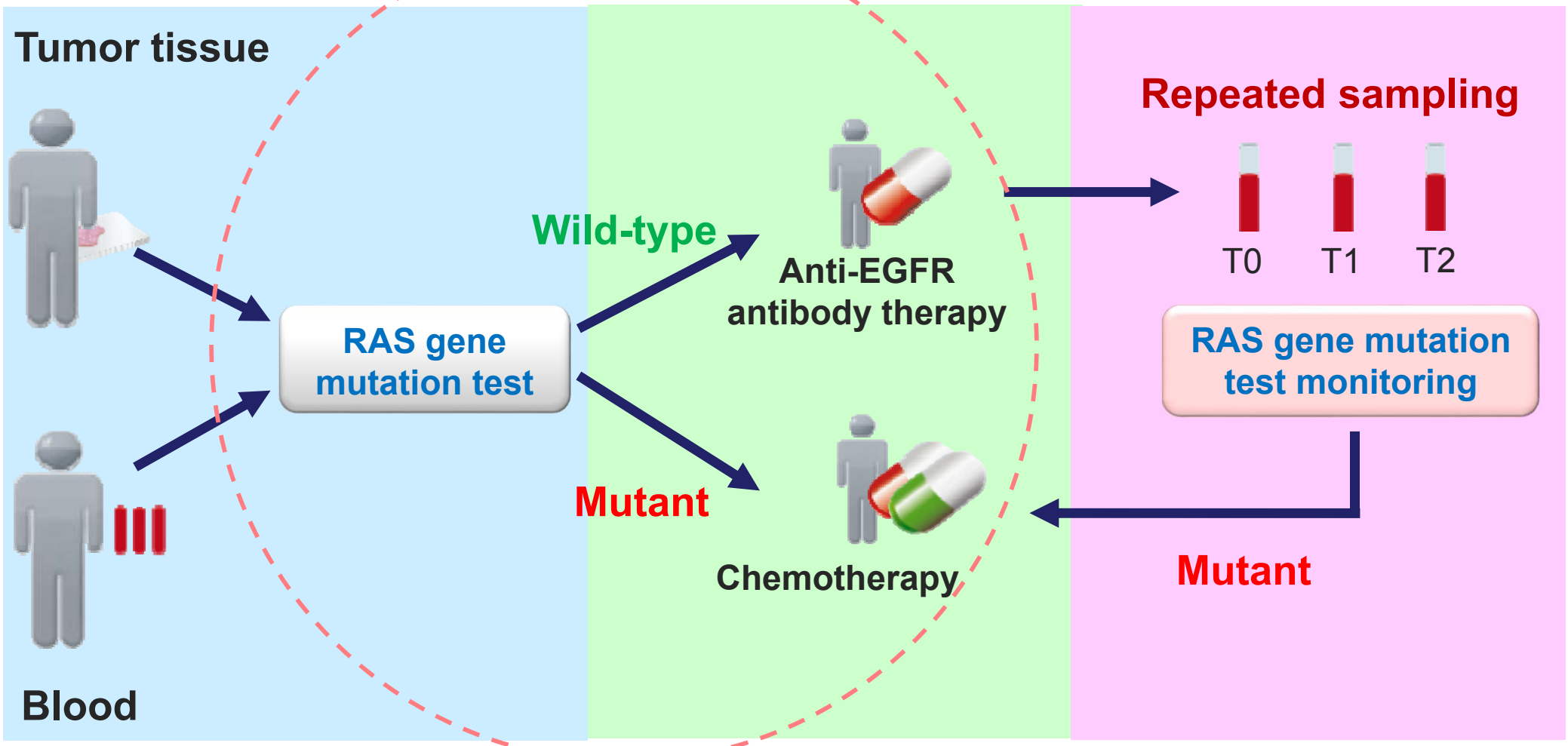


The anti-EGFR antibody drugs Erbitux® (Cetuximab, Cmab) / Vectibix® (Panitumumab, Pmab) have been confirmed to be effective for **RAS gene wild-type only**

Publication of guidance (global level) related to measurement of RAS gene (KRAS/NRAS gene) mutation

2014 ESMO Guideline, NCCN Guideline revision, 2015 ASCO Guideline revision
Japan: Guidance on the Measurement of RAS Gene (KRAS/NRAS Gene) Mutation in Colorectal Cancer Patients, Version 2, Revised April 2014

Relationship between RAS Gene Mutation Testing and Anti-EGFR Antibody Therapy



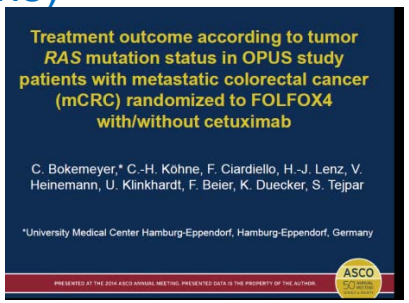
Selection of therapy method: Monitoring for therapy resistance

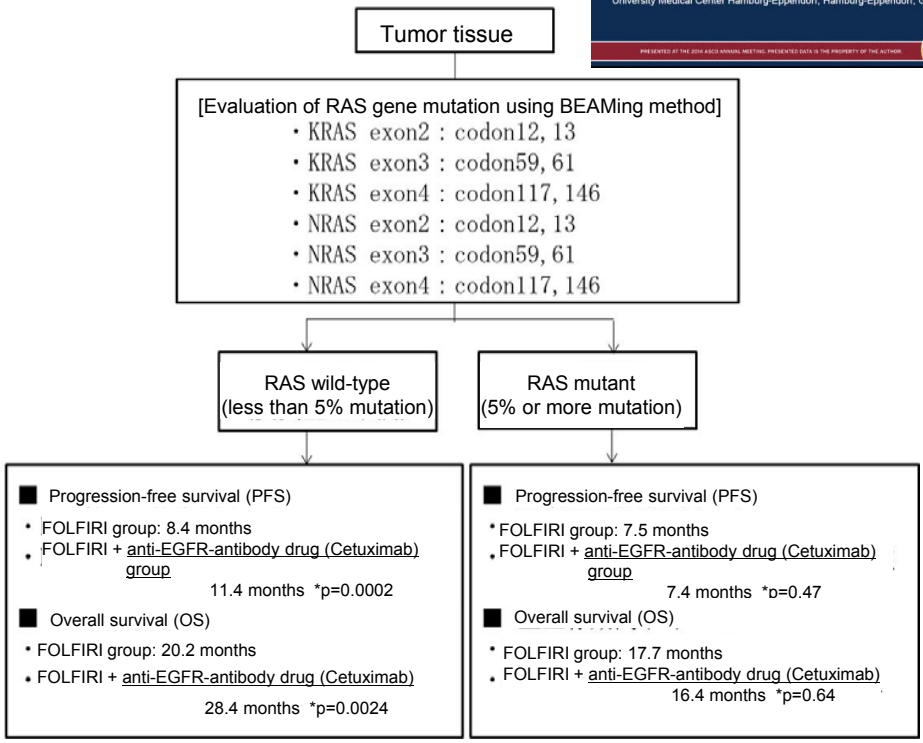
Clinical Utility of RAS Panel Gene Testing (Tissue BEAMing)

ASCO2014 Crystal Study Report (Retrospective analysis of FFPE tissue specimens)

- 666 KRAS (Exon 2) wild-type patients treated with Cetuximab+FOLFIRI
- RAS mutations detected in 14.7% of KRAS Exon 2 wild-type patients using the BEAMing method

Location of mutation	Ratio
KRAS Exon 3	3.3%
KRAS Exon 4	5.6%
NRAS Exon 2	2.9%
NRAS Exon 3	2.8%
NRAS Exon 4	0.9%
Total	14.7 %





- From retrospective prognosis data, the following was found
  - ➢ RAS wild-type ⇒ Cetuximab + FOLFIRI treatment effective
  - ➢ RAS mutant ⇒ Cetuximab + FOLFIRI treatment not effective

F.Ciardiello, et al. ASCO 2014 Poster 3506

Establishment of RAS Panel ctDNA Testing Method Using BEAMing Technology

PIK3CA

RAS

EGFR

RAS 33 Mutation Panel:

KRAS

Exon	Mutation
2	G12S G12R G12C G12D G12A G12V G13D
3	A59T Q61L Q61H Q61H
4	K117N K117N A146T A146V

NRAS

Exon	Mutation
2	G12S G12R G12C G12D G12A G12V G13R G13D G13V
3	A59T Q61K Q61R Q61L Q61H Q61H
4	K117N K117N A146T

Comparison test of RAS gene mutations in plasma and principal tissues using the BEAMing method for patients with unresectable advanced, recurrent colorectal cancer

N=50 Stage IV pts; N=26 recurring colorectal cancer

		Testing of RAS gene mutation in tissue		
		RAS mutant	RAS wild-type	Total
Testing of RAS gene mutation in plasma	Positive	39	2	41
	Negative	3	32	35
	Total	42	34	76

Concordance rate for RAS gene mutation positive/negative in plasma using the BEAMing method and RAS gene wild-type/mutant judgment in tissue at 93.4%

Partially adapted from Frederick S. Jones, et al. 2015, 18thECCO-40thESMO; Poster P002

Develop Gene Testing Drugs for Liquid Biopsy (Japan)

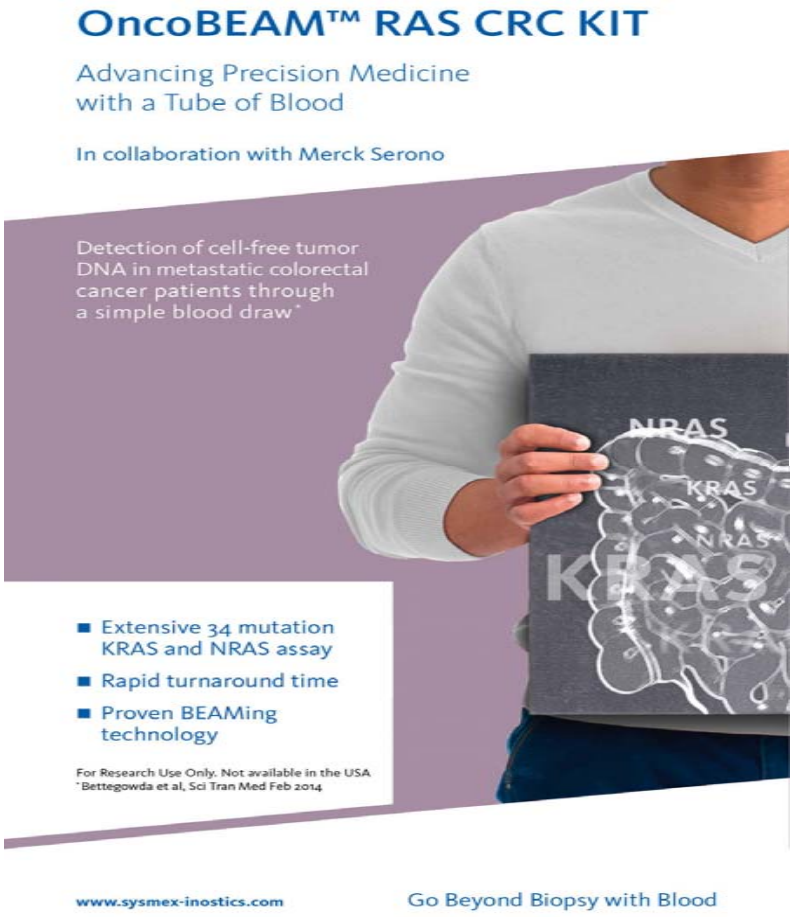
PIK3CA

RAS

EGFR

Clinical development targeting Ministry of Health, Labour and Welfare and PMDA regulatory approval of colorectal cancer RAS gene testing kit

World's first gene testing drug using BEAMing technology

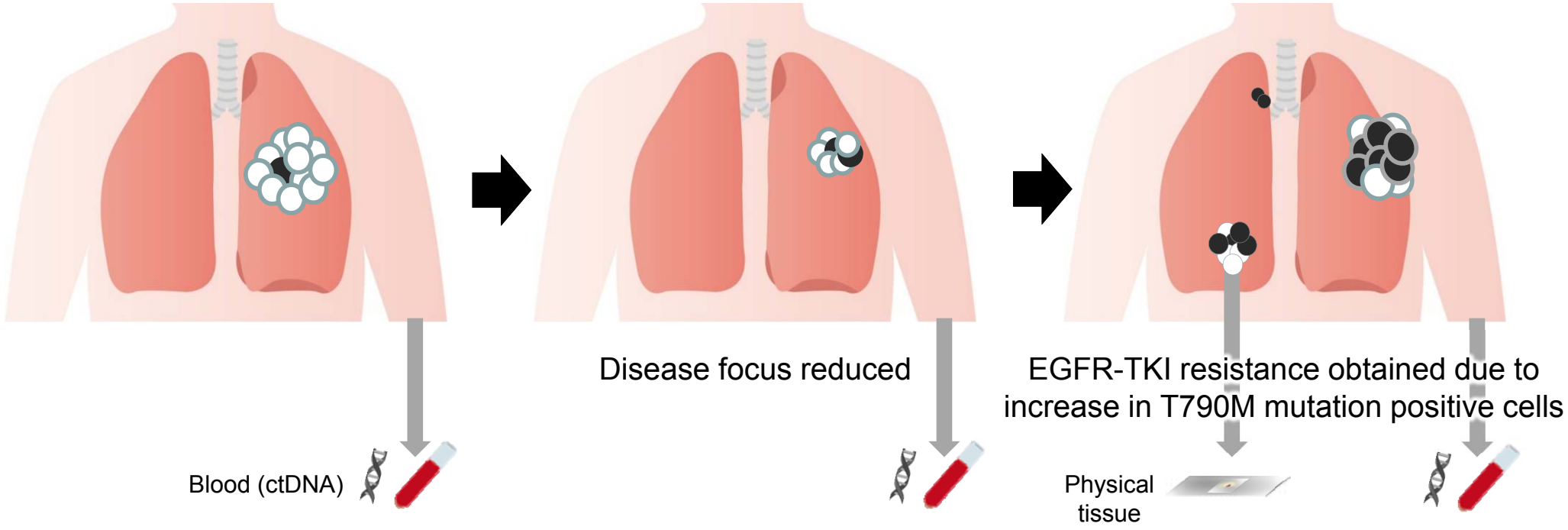


Japan's first liquid biopsy gene testing drug

Meeting with representative physicians at Japanese clinical performance testing facility to consider protocols (November 2015)

Clinical Significance of EGFR Gene Mutation Testing

EGFR mutation positive non-small cell lung carcinoma (del. 19, L858R mutation positive)	EGFR-TKI treatment	Advanced, recurrent (del. 19, L858R, T790M mutation positive)



Blood sampling permits repeated testing, allowing monitoring for drug resistance

When making a third-generation EGFR-TKI drug administration decision, cancer tissue non-uniformity in tumor tissue tests could indicate a T790M false negative

○ Activated EGFR mutant cells (del. 19, L858R mutation positive cells)
● Resistant cells (T790M mutation positive cells)

PIK3CA
RAS
EGFR

Comparison of EGFR Gene Mutation Testing Methods

PIK3CA

RAS

EGFR

	Other companies			Sysmex
	PCR	PCR	Digital PCR	BEAMing
	cobas® EGFR Mutation Test	therascreen™ EGFR ARMS-PCR	ddPCR™	BEAMingdPCR
Exon 19 deletion				
Sensitivity	86% (24/28)	82% (23/28)	–[b]	93% (26/28)
Specificity	100% (10/10)	100% (10/10)	–[b]	100% (10/10)
Concordance	89%	87%	–[b]	95%
L858R				
Sensitivity	90% (9/10)	78% (7/9)	90% (9/10)	100% (10/10)
Specificity	100% (28/28)	100% (28/28)	100% (28/28)	93% (26/28)
Concordance	97%	95%	97%	95%
T790M				
Sensitivity	41% (7/17)	29% (5/17)	71% (12/17)	71% (12/17)
Specificity	100% (6/6)	100% (6/6)	83% (5/6)	67% (4/6)
Concordance	57%	48%	74%	70%

	Other companies	Sysmex
	PCR	BEAMing
	cobas® EGFR Mutation Test	BEAMing dPCR
Exon 19 deletion		
Sensitivity	82% (23/28)	82% (23/28)
Specificity	97% (30/31)	97% (30/31)
L858R		
Sensitivity	87% (20/23)	87% (20/23)
Specificity	97% (35/36)	97% (35/36)
T790M		
Sensitivity	73% (30/41)	81% (33/41)
Specificity	67% (16/24)	58% (14/24)

Utility of Sysmex's BEAMing method in EGFR mutation detection in NSCLC*

* NSCLC: Non-small-cell lung carcinoma

Lung Cancer Vol90. Issue 3, Dec 2015, Pages 509-515

Global Development of the OncoBEAM™ Assay Service Business

Develop therapeutic drugs

Pharmaceutical companies

Drug discovery → Preclinical → Clinical → Approval → Market launch → Standard-ization

Academia

Research support (CRO* business)
Search for and validate molecular markers

sysmex

Increase the efficacy and safety of therapeutic drugs (CDx business)
Selection and monitoring of applicable diseases

Molecularly targeted therapeutic drugs

Patients

Companion drugs

Patient therapy selection (Patient Testing Business)
Coverage by insurance, inclusion in medical care guidelines

Sysmex Inostics lab
Hamburg, Germany

LabCorp lab
Beijing, China

Sysmex IMP lab
Kobe, Japan
IMP: ITOCHU Medical Plaza

Sysmex Inostics
<CLIA* lab>
Baltimore, USA

Center of Excellence labs at hospitals in Spain, Germany and elsewhere

LabCorp
<CLIA* lab>

*CRO: Clinical Research Organization

*CLIA: Clinical I Laboratory Improvement Amendments

We Believe the Possibilities.

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www.sysmex.co.jp/en

Basic Seminar

Fumio Kubota

Executive Vice President,
R&D Strategic Planning Division

March 11, 2016

Sysmex Corporation

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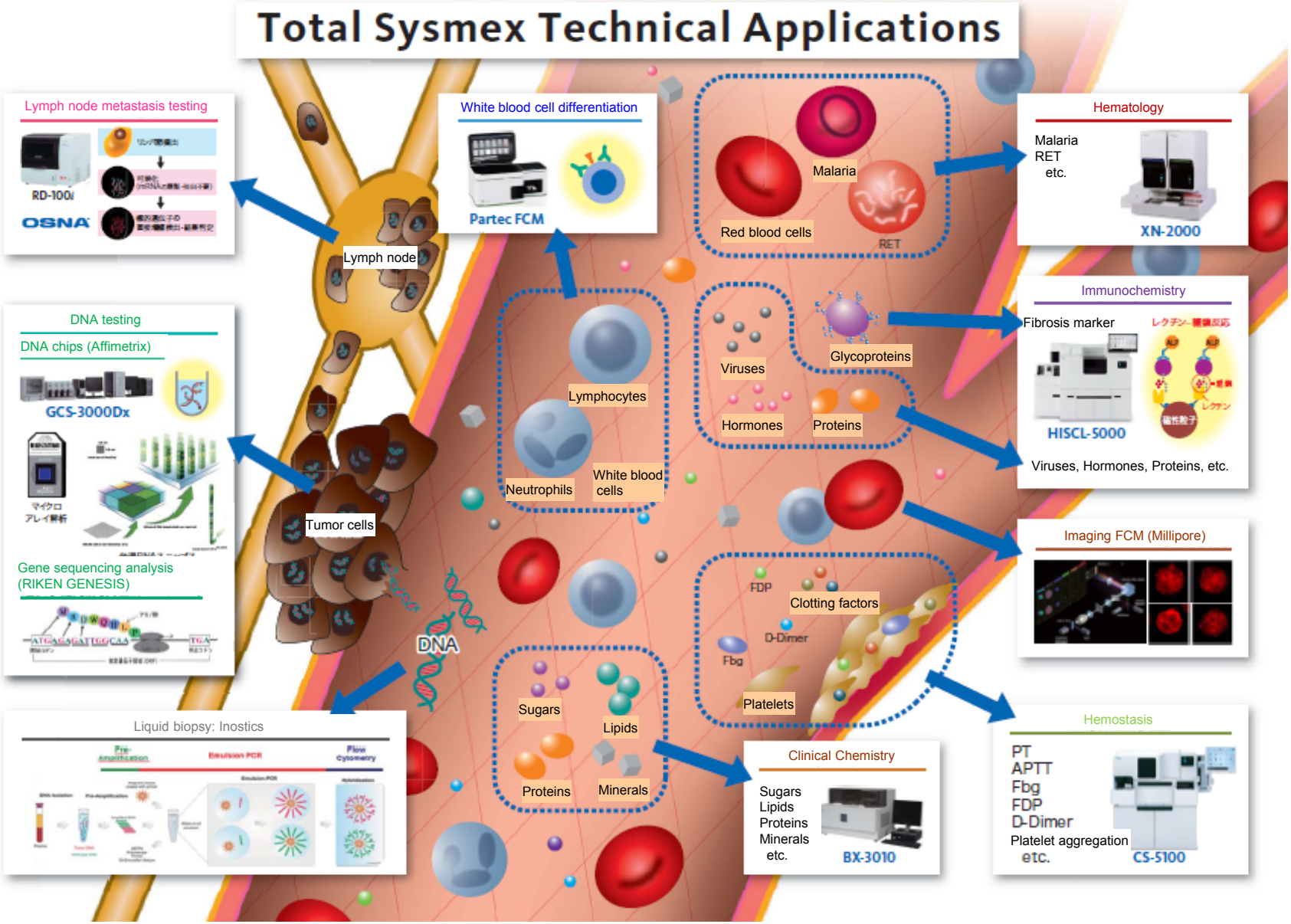
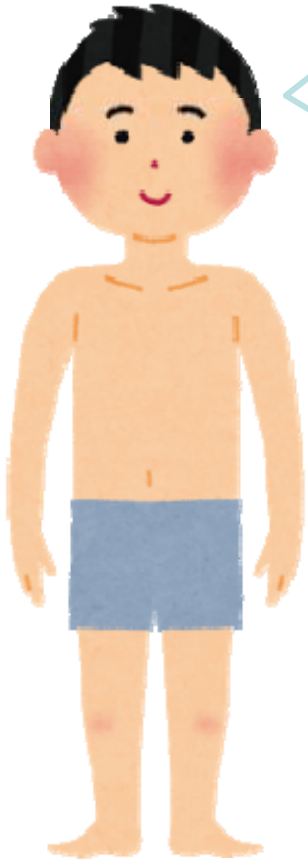
Sysmex's Technology Domain



1. About Genes

About Genes



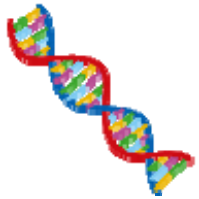
Around 60 trillion cells

Each cells is composed of various proteins

23 pairs of chromosomes (masses of genes)

Cell nuclei contain chromosomes, which are coils of DNA

DNA



DNA is made up of around 3 billion bases

Genes

A C A T

G T C ...

Their mission is to make proteins

Bases

C A

T G

Codes for making protein

Genes are found at specific locations within DNA
The role of genes is to manufacture the proteins to make the body

The Relationship between DNA and Genes

DNA=Substance in which genetic information is written

Number of bases (letters): 3 billion
Number of genes: 20,000
(Each gene contains between several thousand and several tens of thousands of bases)

ATGATGATACTGGGTAAAGCCGGGATCCTGGCACAGTATGGGACAATATATGTGAGACAA
AATACCATTCGGAATAATTTAAGTTCCTGCATTTTTAAGCAGTCACTATGTGCCTTTCAT
TCATTAGCAAAGGTGCTGCAACAAAAGCAAGTCCCACTGGACCTTTCATATGACATAATC
AAGAGAGATGCAGTAAAAACTGGGGATGAAGGGAAGCCAAGACCACCTATTATCATACTT
CACGGCTTATTCGGTAACAAGCTCAACAACCGAAGCATTGGCCGTAACCTTAACAAGAAA
TTGGGAAGAGACGTGTATCTGCTGGACCTAAGAAATCATGGATCCTCACCACACAGTTCA
GTCCATAACTACGAAGTCATGTCGGAAGACGTGAAACACTTTATCACAAAGCACGAATTA
AACACCAATGGAGGCCCTATTATAATAGGACACTCAATGGGTGGTAAAGTTGCCATGATG
CTGGTCCTGAAAAACCCGCAACTTTGTTCGATGTTAGTCTGTATAGAGAACGCTCCGGTG
AGTTTGCGCCCTAACGCTGAGTTTGTCGAATACATCAAAGCGCTGATGGAAATCGTCAAC
GACAAGGGCAAAACTATCCGCACGCTGAAACAGGCTGATGAACACCTTGCAGAGAGGATC
GGCGGCAATGAGCTAGTAAGGCGGTTTCTCCTAACGGCGCTGAAAAAGGTCAAGATGGAC
AATTCATCGTCTGTGTCGTCATATACATTCGAAGAACGAATTCCCCTCGCAACACTGAAA
GATGCCATTGTCAAAGGTGAAATTGCCGCGTGGCCCCTAGATCCTGCTCGTGAACGATGG
ACGCGGCCTGCGCTATTCATCAGGGCTACTCAATCGCATTATGTGGTAGACGAGTATCTT
ATGATGATACTGGGTAAAGCCGGGATCCTGGCACAGTATGGGACAATATATGTGAGACAA
AATACCATTCGGAATAATTTAAGTTCCTGCATTTTTAAGCAGTCACTATGTGCCTTTCAT
TCATTAGCAAAGGTGCTGCAACAAAAGCAAGTCCCACTGGACCTTTCATATGACATAATC
AAGAGAGATGCAGTAAAAACTGGGGATGAAGGGAAGCCAAGACCACCTATTATCATACTT
CACGGCTTATTCGGTAACAAGCTCAACAACCGAAGCATTGGCCGTAACCTTAACAAGAAA
TTGGGAAGAGACGTGTATCTGCTGGACCTAAGAAATCATGGATCCTCACCACACAGTTCA
GTCCATAACTACGAAGTCATGTCGGAAGACGTGAAACACTTTATCACAAAGCACGAATTA
AACACCAATGGAGGCCCTATTATAATAGGACACTCAATGGGTGGTAAAGTTGCCATGATG
CTGGTCCTGAAAAACCCGCAACTTTGTTCGATGTTAGTCTGTATAGAGAACGCTCCGGTG
AGTTTGCGCCCTAACGCTGAGTTTGTCGAATACATCAAAGCGCTGATGGAAATCGTCAAC
GACAAGGGCAAAACTATCCGCACGCTGAAACAGGCTGATGAACACCTTGCAGAGAGGATC
GGCGGCAATGAGCTAGTAAGGCGGTTTCTCCTAACGGCGCTGAAAAAGGTCAAGATGGAC
AATTCATCGTCTGTGTCGTCATATACATTCGAAGAACGAATTCCCCTCGCAACACTGAAA
GATGCCATTGTCAAAGGTGAAATTGCCGCGTGGCCCCTAGATCCTGCTCGTGAACGATGG
ACGCGGCCTGCGCTATTCATCAGGGCTACTCAATCGCATTATGTGGTAGACGAGTATCTT
CCGATCATCGGCGCGTTCTTTCCACGCTTTGAAACACGTGACATCGATGCGGGTCACTGG
GTAAATGCGGAGAAGCCTGGGGAATGTGCCGAAAGCATCGTCGATTTTGTGGAGCGGCAC
GAGGATTAA

Gene A = Basic building blocks handling genetic information

Gene B

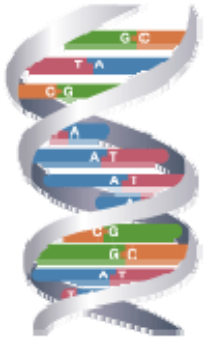
Non-genetic information (noncoding region)

DNA? RNA? Proteins?

The principles (central dogma) of molecular biology
(Stated by Francis Crick in 1958)

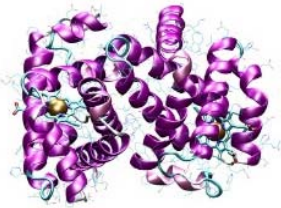
Genetic information:

DNA →(transcribe)→ RNA →(translate)→ to communicate protein orders



Transcribe

Translate

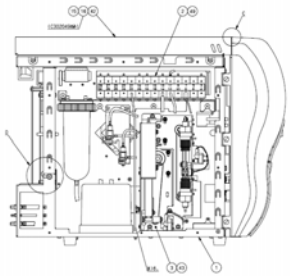


DNA
(deoxyribonucleic acid)

RNA
(ribonucleic acid)

Proteins

Copy



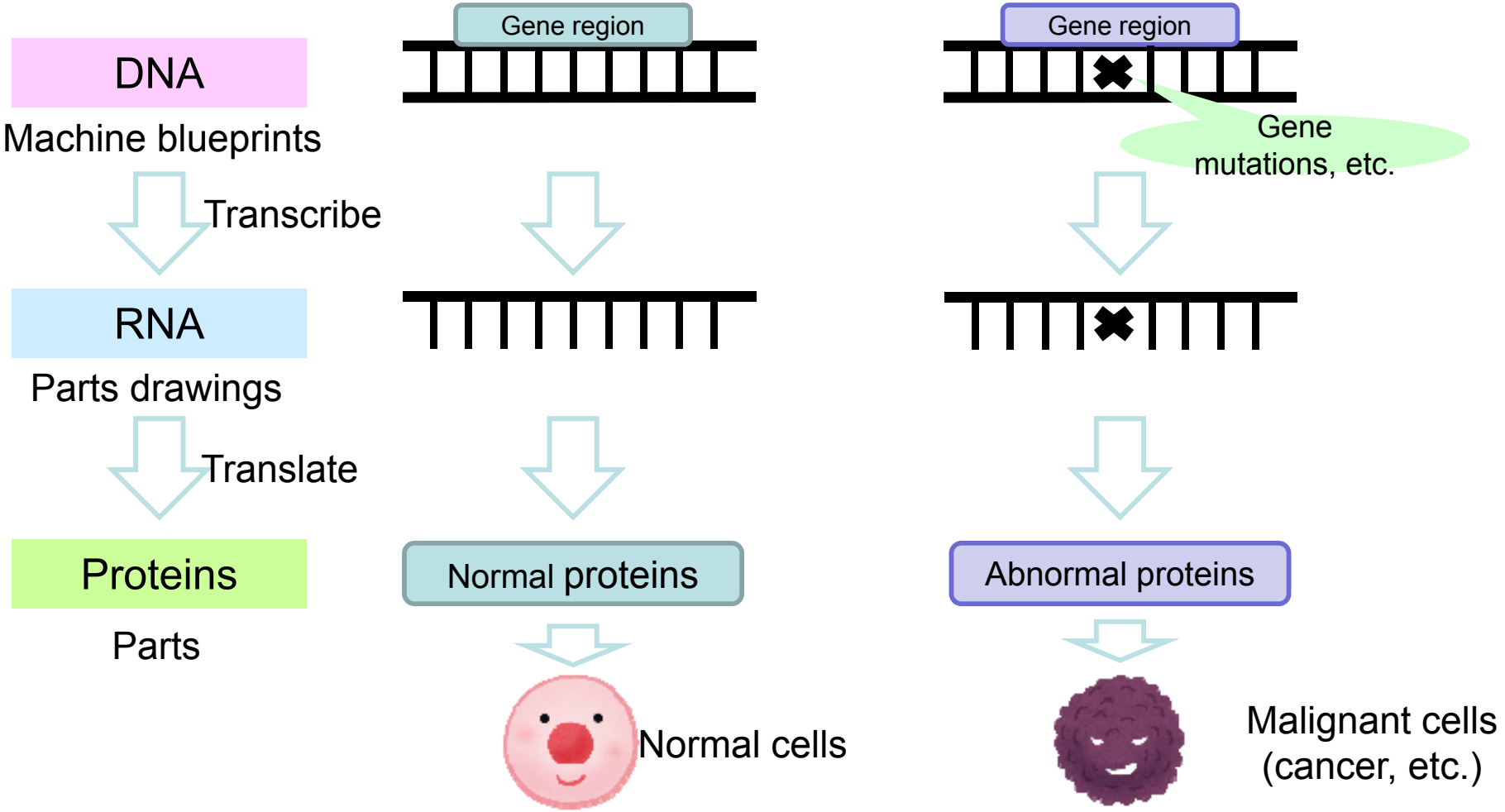
Materialize

Machine blueprints

Parts drawings

Parts

The Role of Gene Testing

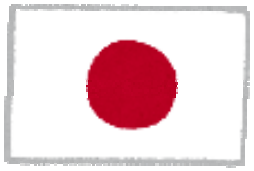


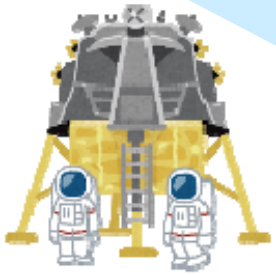
Gene testing looks at DNA and RNA base sequences and checks whether these sequences are normal

Human Genome Project

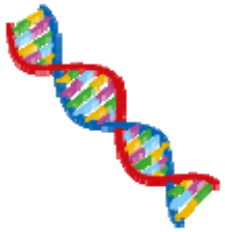
Human Genome Project: A project to analyze all the base sequences of the human genome

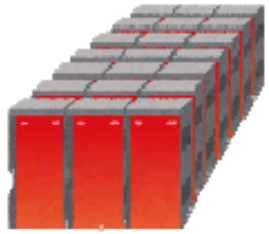
1990 marked the start of the Human Genome Project by the U.S. National Institutes of Health (NIH) and the Department of Energy





An international project, with teams from Japan and European and other countries participating
Investment of US$3 billion, matching that for the Apollo mission
Progress in genomic science
Progress in computer technology





In 2003, success in mapping 99% of all base sequences of the human genome to 99.99% accuracy

Helped to clarify diseases due to gene abnormalities and energized drug discovery in such areas as biopharmaceuticals

About Gene Abnormalities

Three major types of abnormalities in gene base sequencing

Normal (wild-type)

5’ ——TGT GTC TGT GGA GAC—— 3’

Point mutation

5’ ——TGT ATC TGT GGA GAC—— 3’

A single base in a base sequence is incorrect, causing a different protein to be produced.

Deletion

5’ —— TGT GTCT GTG GAG —— 3’

AC…

Part of a base sequence is missing, causing an abnormal protein to be produced.

Insertion

5’ —— TGT GTC GTG TGG AGA —— 3’

C…

An extra base has been added to a base sequence, causing an abnormal protein to be produced.

About Gene Abnormalities

Two types of gene abnormalities

- Germline (cells that become eggs and sperm) mutation

  Causes mutations in the germ line or causes mutations to be passed down from ancestors to descendants

  Example: Hereditary diseases

- Somatic (cells that make each part of the human body) mutation

  Causes mutations in somatic cell lines that are not pass down to descendants

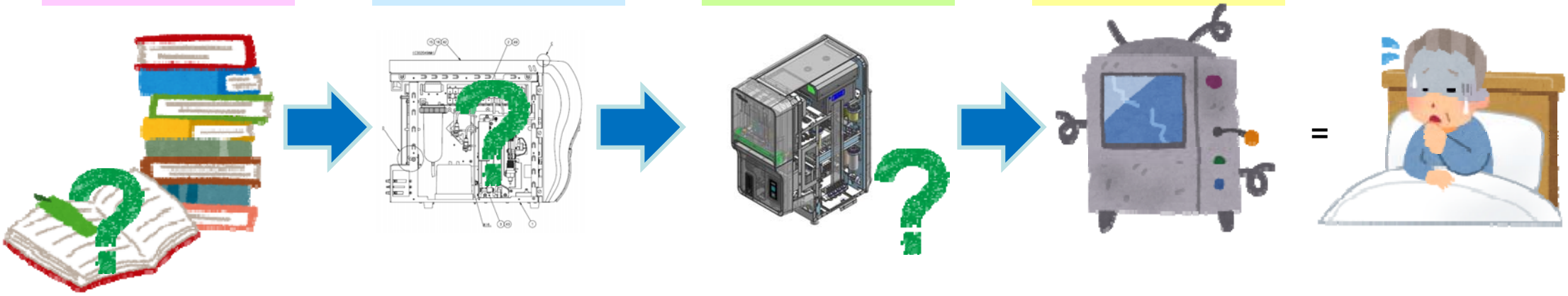
  Example: Cancer

Testing methods need to be chosen based on the type of gene abnormality (base sequence, germ-line or somatic)

2. About Gene Testing

Benefits of Gene Testing

DNA | RNA | Proteins | Cells



If the machine blueprint is wrong

Incorrect parts drawings

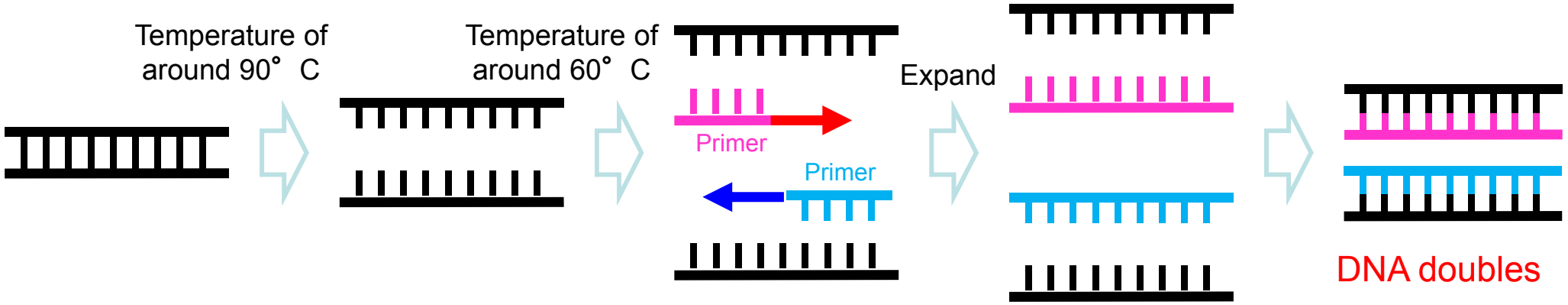
Leading to faulty parts

The machine breaks down = Illness

To identify the type and form of a cancer deriving from multiple gene mutations, testing DNA and RNA is an effective and stable way to obtain results.

DNA amounts are minute, so amplification aids in detection

A primer (short base fragment) is used to amplify and detect specific DNA



This process is repeated tens or hundreds of times to increase the amount of DNA

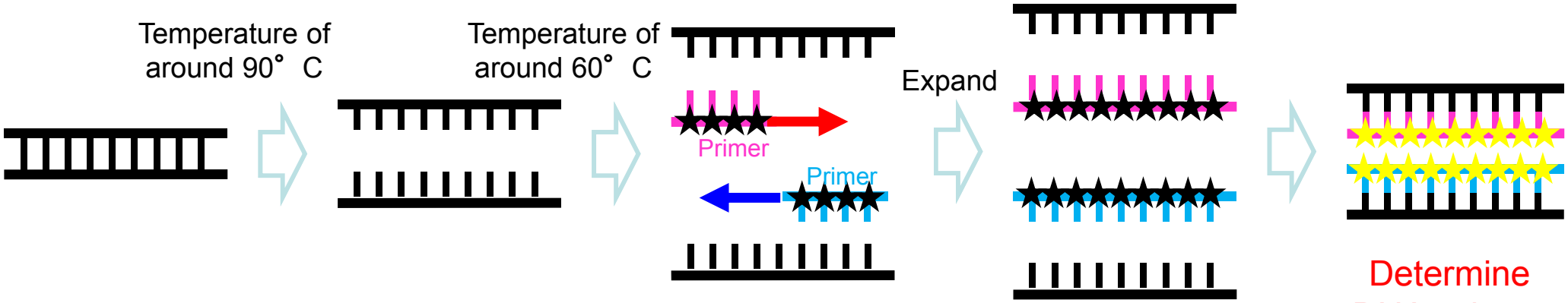
This is known as the polymerase chain reaction (PCR) method

Types of Gene Testing (1)

Instruments using the PCR method are PCR instruments and next-generation sequencers (NGSs)

PCR instruments

Instruments that add dye to stain specific DNA and determine DNA volume based on the strength of the light

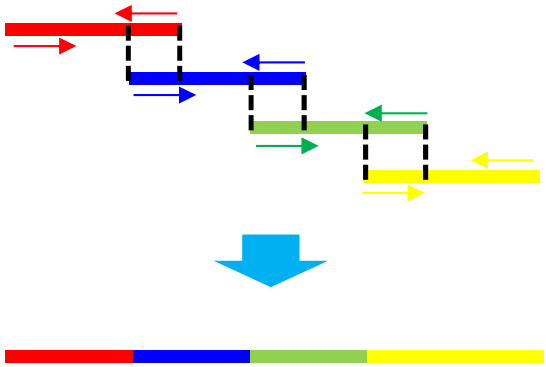


PCR instrument advantages	Testing becoming more commonplace, and cost falling
PCR instrument disadvantages	Requires preparation of primer for each DNA to be measured, limiting the amount of DNA that can be detected at once

Types of Gene Testing (2)

Next-generation sequencers (NGSs)

Instruments that decipher all DNA or DNA in specific locations in parallel



Amplify every several hundred bases at once (essentially the same as with the PCR method)

...

Overlapping segments are found and connected by deciphering

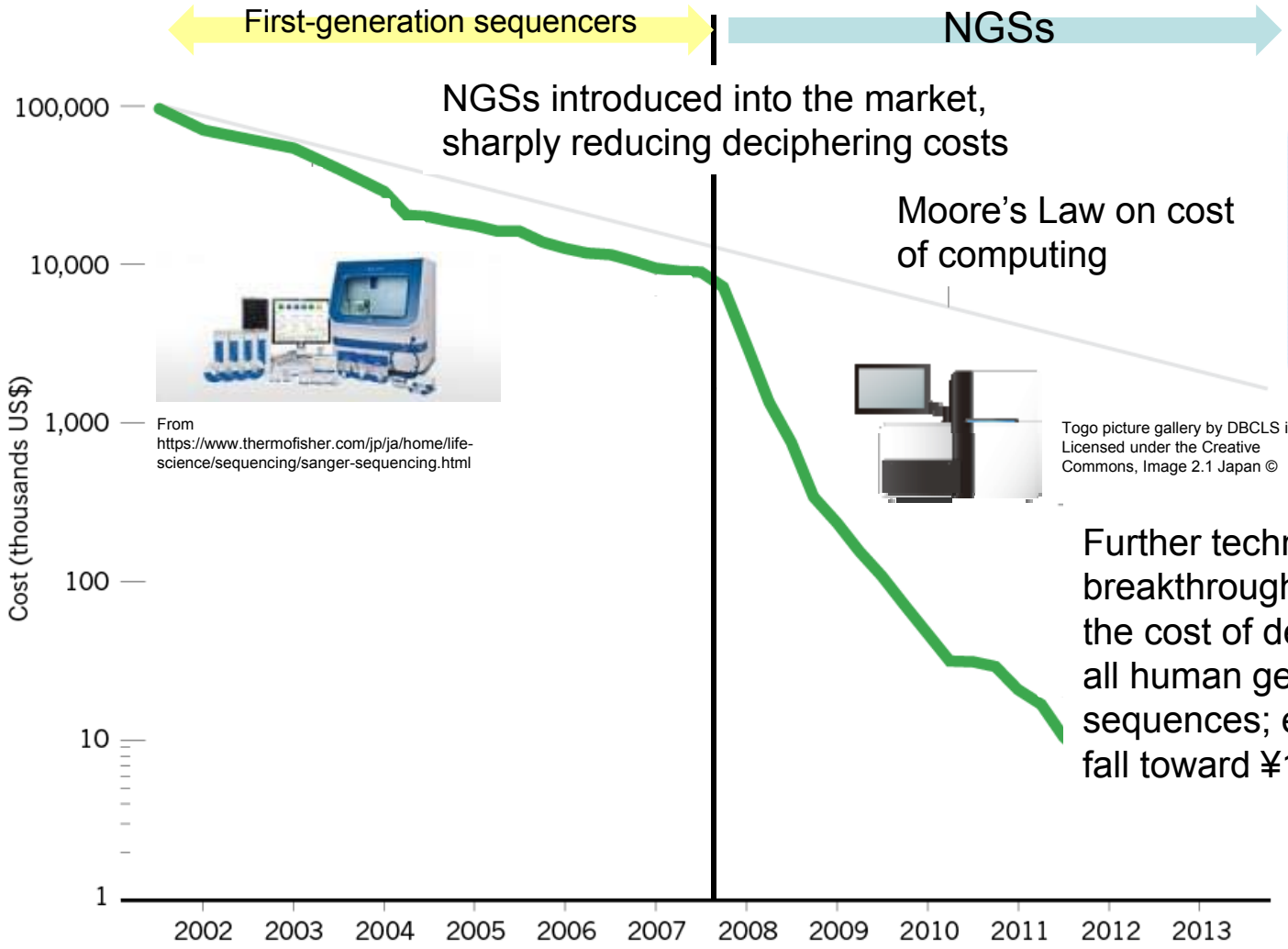
...

Deciphering the human genome has allowed even jumbled DNA to be restructured, so large quantities can be deciphered in parallel

Sequencer advantages	All DNA can be detected at once
Sequencer disadvantages	At present, remains expensive

(Reference) About Next-Generation Sequencers (NGSs)

NGSs were introduced into the market around 2008



From https://www.thermofisher.com/jp/ja/home/life-science/sequencing/sanger-sequencing.html

Togo picture gallery by DBCLS is Licensed under the Creative Commons, Image 2.1 Japan ©

Further technological breakthroughs reducing the cost of deciphering all human genome sequences; expected to fall toward ¥100,000

NGS characteristics

- ✓ Jump in processing capacity
- ✓ Reduced processing time

Partially revised from Nature 507, 294–295 (20 March 2014)

When to Use Different Types of Gene Testing (1)

Deciding which type of gene testing to use depends on the number of tests and the number of mutations

Point 1: Number of tests

■ Germ-cell gene mutation

Hereditary gene mutations passed on by ancestors to descendants are mutations that remain unchanged from birth to death, so
sequencing all DNA in a single test is most efficient

NGSs — Allow all DNA to be deciphered at once

■ Somatic gene mutation

Acquired gene mutations, particularly cancer, can occur on a daily basis,
requiring testing to be conducted multiple times

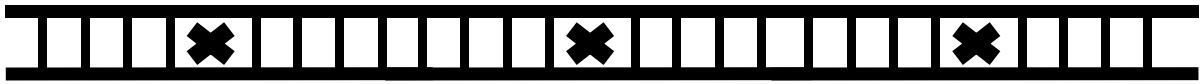
PCR instruments — Allow highly sensitive detection relatively inexpensively

When to Use Different Types of Gene Testing (2)

Point 2: Number of mutations

If the number of gene mutations is small, acceptable to test only specific locations

PCR instruments　Detect specific DNA with a high degree of sensitivity



If the number of gene mutations is large, need to test over a broad-ranging area

NGSs　Allow all DNA to be deciphered at once

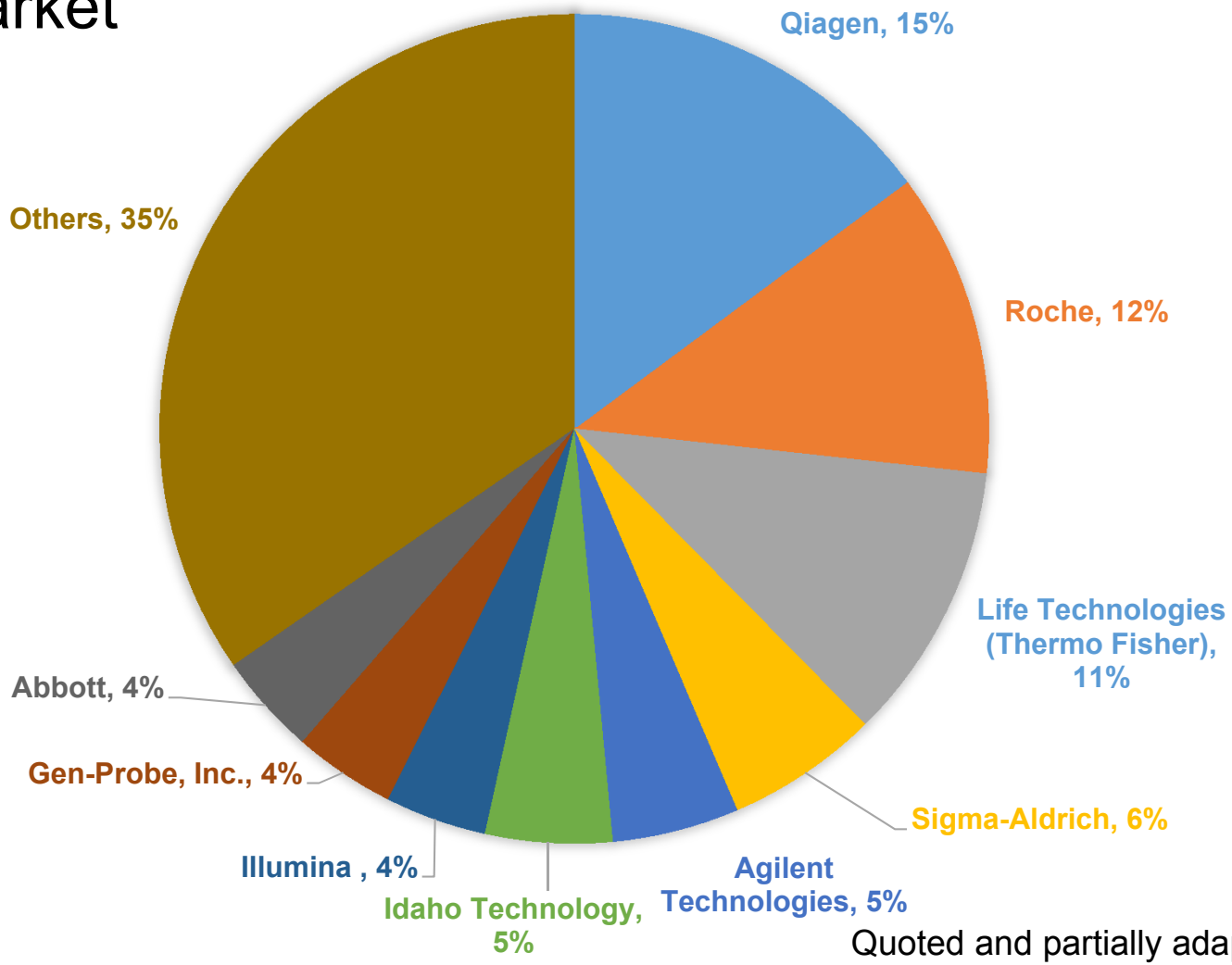
Comparison of PCR Instruments and NGSs

	PCR instruments	NGSs
Detection sensitivity	High (Around 0.1%)	Low (Around 0.5–1%)
Number of mutations that can be detected	Few* (Limited to several tens of mutations)	Many
Cost	Low	High

*Detecting large numbers of mutations is possible in principle, but clinical application is limited for reasons of cost.

Market Share for PCR Instruments and Reagents

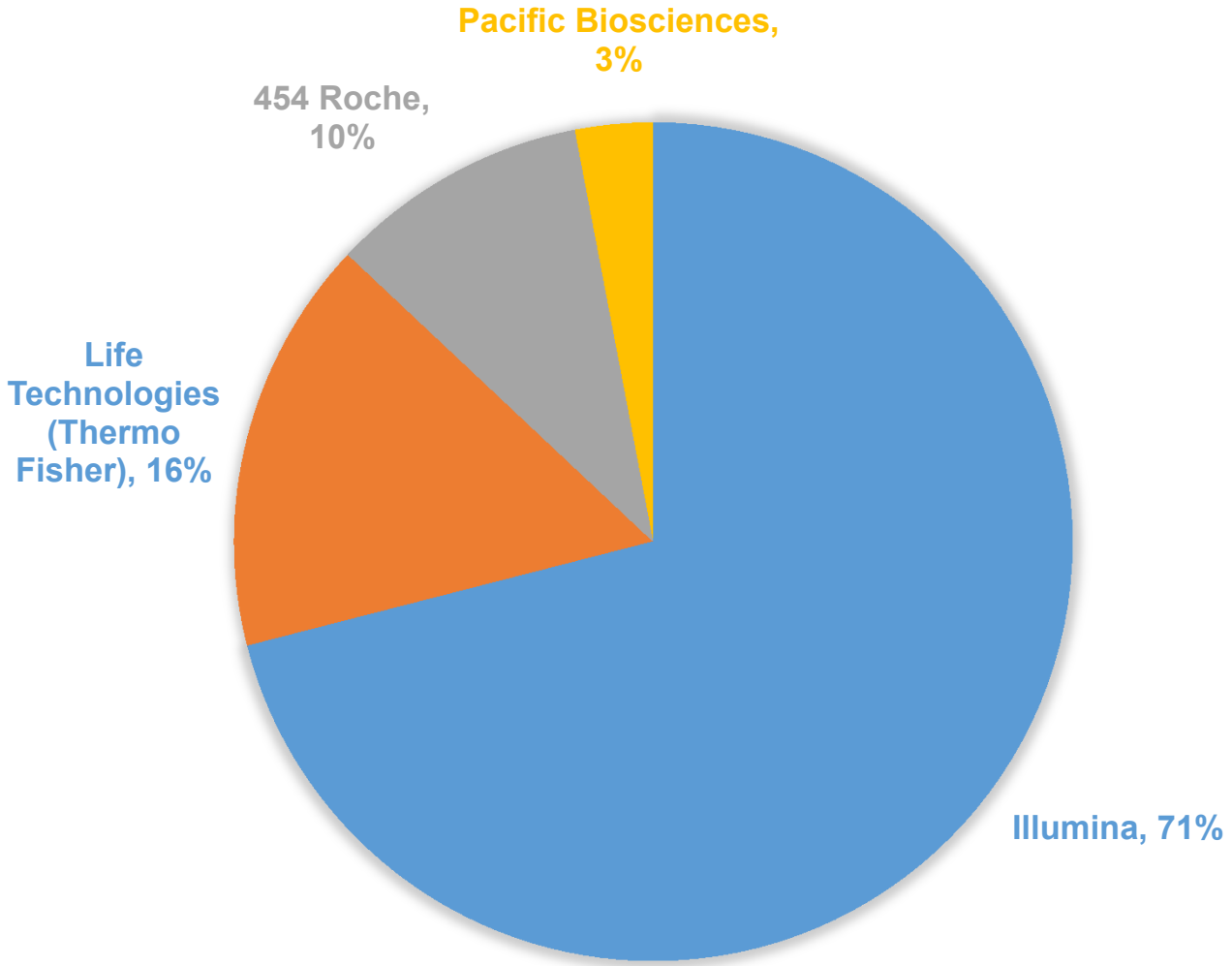
Qiagen, Roche and many other companies have entered the PCR market



Quoted and partially adapted from BCC Research
Note: Includes scientific instruments

Market Share for NGSs

Illumina has a market share of more than 70%

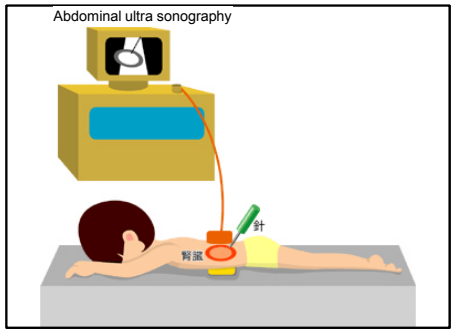


Quoted and partially adapted from Mizuho Securities USA, Frost & Sullivan
Note: 2013 data

3. Sysmex's Initiatives in Gene Testing

Realizing Personalized Medicine with Liquid Biopsy

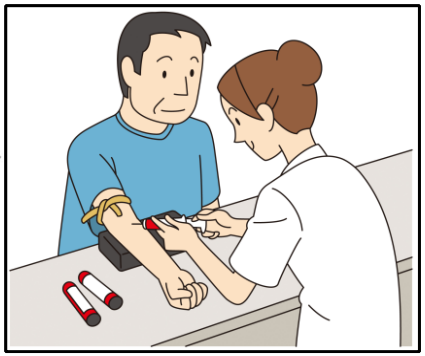
Conventionally



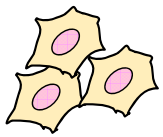
Invasive sample collection
of affected organ/tissue

From biopsy to liquid biopsy

Near Future



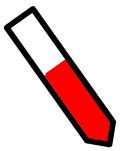
Minimal invasive sample collection of
disease-derived components into the blood
(bodily fluid)



Tissue

Required sensitivity: 1%
(Finds one mutation out of 100)

Requires detection sensitivity 100 to
1,000 times that of conventional method

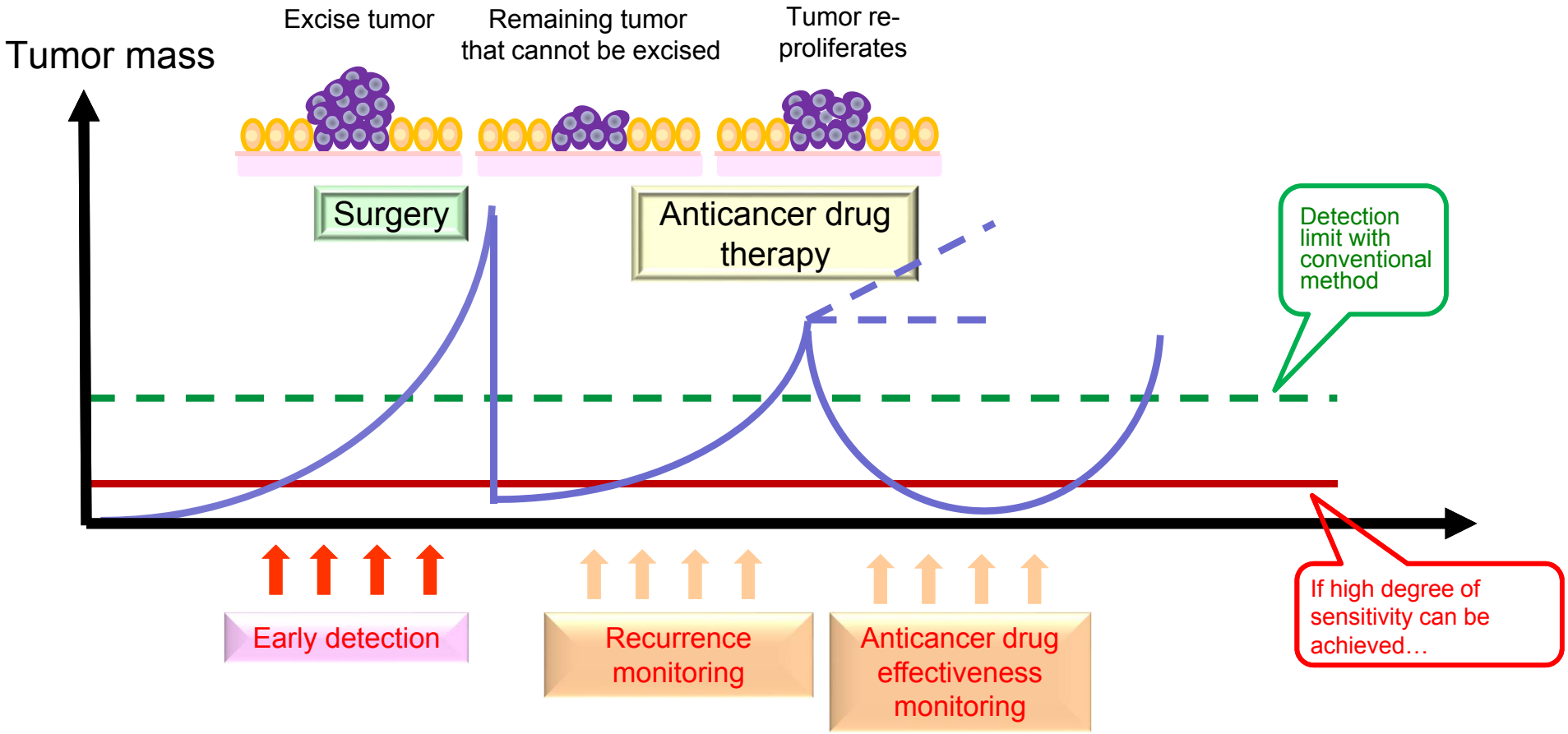


Blood (bodily fluid)

Required sensitivity: 0.01%
(Finds one mutation out of 10,000)

Advantages of Liquid Biopsy

- For somatic gene mutations (cancer)

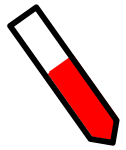


Realize timely treatment, taking advantage of the ease of sampling and high degree of sensitivity

Sysmex's Technology Platforms Aimed at Achieving High Level of Sensitivity

Tissue

Required sensitivity: 1%
(Finds one mutation out of 100)



Blood (bodily fluid)

Required sensitivity: 0.01%
(Finds one mutation out of 10,000)

Area of Sysmex's initiatives

PCR
(Mainly for research)

Clinical PCR
(Clinical use)

Digital PCR
BEAMing
(Clinical use)

NGS
(For research)

NGS*
(Clinical use)

Pretreatment technologies

Plasma-Safe-SeqS
+
NGS*
(Clinical use)

*Use NGSs from other companies

About Clinical PCR

PCR issues:

Operations are complex (many manual operations), so difficult to handle at hospital labs

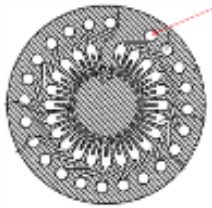
The clinical PCR concept

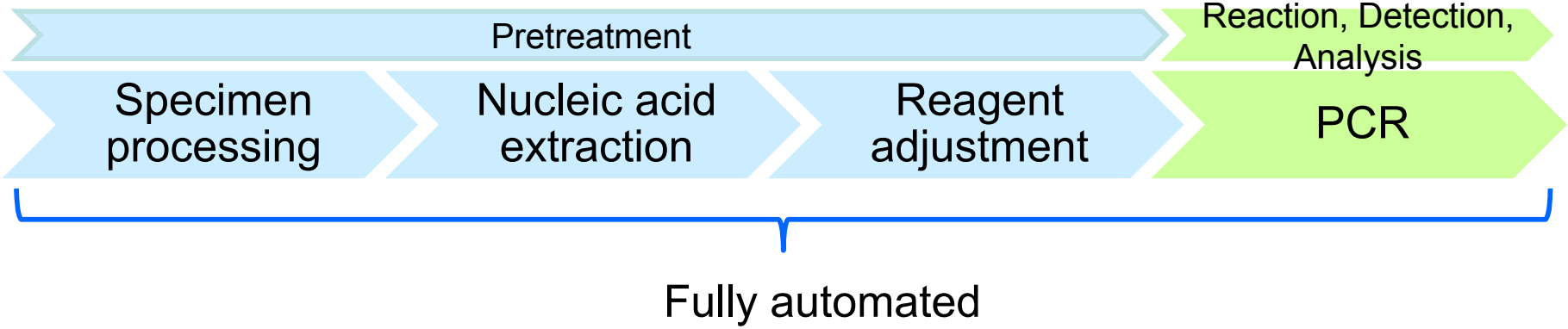
Cancer diagnosis system that can be used in hospital labs

- Fully automated, from pretreatment to detection
- Highly sensitive detection (0.1%)
- Quality assurance
- Clinically useful marker sets
- Compact

Instrument

Reagent kit

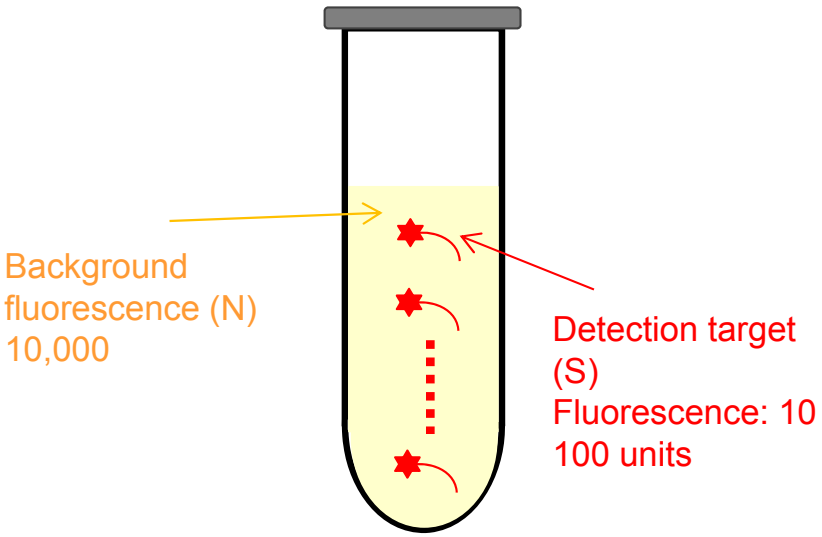




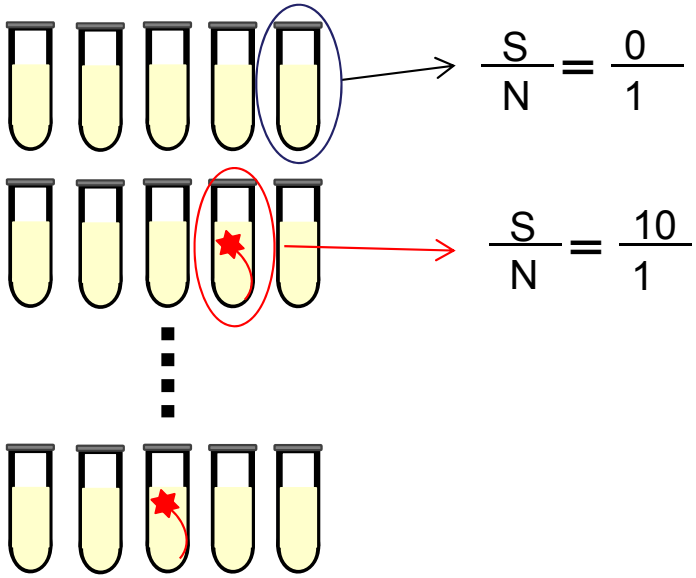
About Digital PCR

Achieves a high degree of sensitivity by measuring numerous droplets divided into uniform sizes

For example



Divided into 10,000 units



Can be detected

Viewed together

$$\frac{S}{N} = \frac{10 \times 100}{10{,}000}$$

S < N, so detection not possible

Advantages of digital technology:
High sensitivity
Absolute quantities

Future Development of the BEAMing Method

Issues with the BEAMing method

- ✓ Procedures are complex
- ✓ Requires three days to complete measurement

→ Working to automate procedures, lower labor requirements and reduce the time needed for measurement

Step 1

Partial automation through introduction of liquid handling instrument (automating liquid dispensing)

Achieved

- ✓ Labor saving (approximate 80% reduction)
- ✓ Reduced measurement time (from three days to two)

Step 2

Realize a fully automated system with shorter measurement time and that allows quantity determination

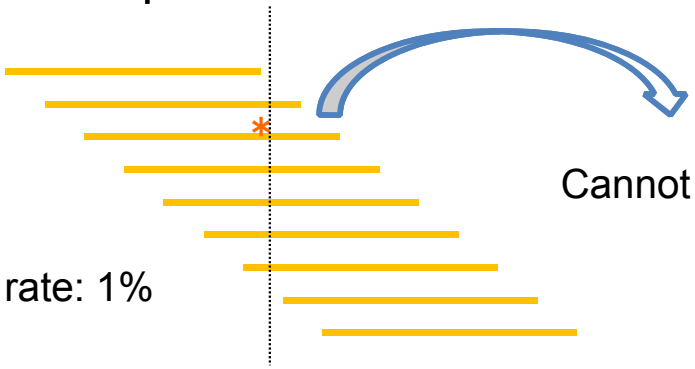
Under development

- ✓ Fully automated
- ✓ Reduced measurement time (from two days to six hours)

Note: Images are for illustrative purposes only

NGS Issues

- For cancer samples



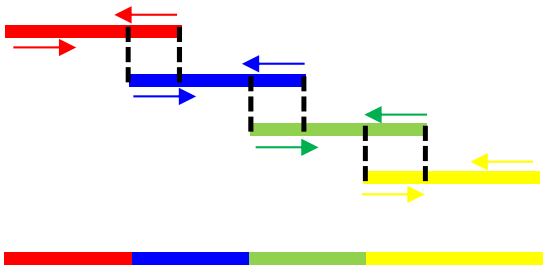
NGS read error rate: 1%

Cannot distinguish:

- Is this a read error?
- Is this a mutated gene?

Technology trends at other companies

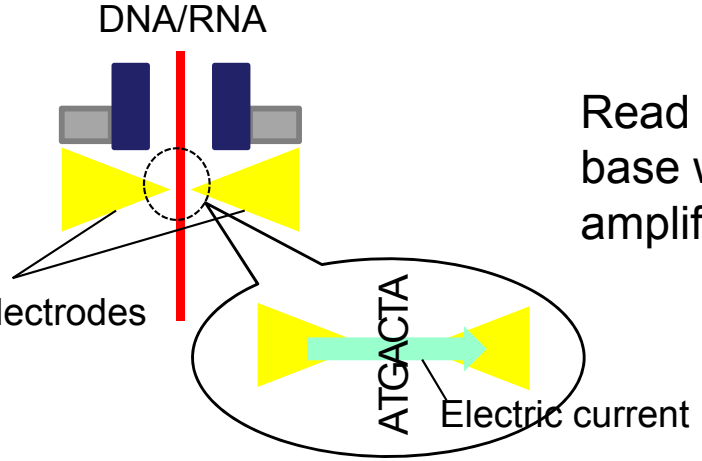
Improved analysis technologies (such as deep sequencing)



Increase detection sensitivity by reading the same location multiple times

OR

Development of next-generation instruments (such as nanopore sequencers)



Read a single base without amplification

Sysmex Initiatives toward Clinical Application of NGS

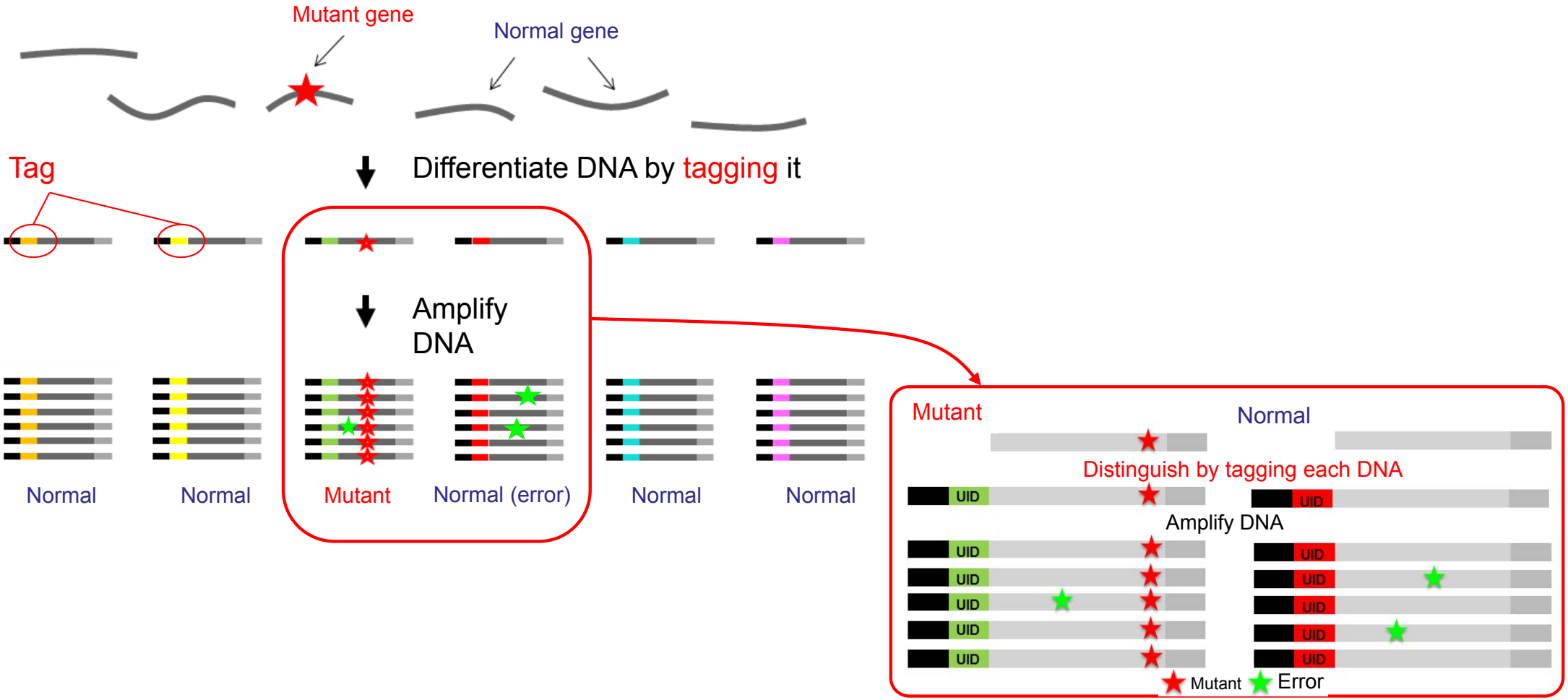
Pretreatment	Measurement	Analysis
Plasma-Safe-SeqS technology (Sysmex Inostics) Pretreatment technology to tag amplified genes	NGS  Togo picture gallery by DBCLS is licensed under the Creative Commons, Image 2.1 Japan ©	Bioinformatics (RIKEN GENESIS) Gene analysis technology
sysmex Improve work flow	Leverage existing products	sysmex Improve analysis technology

Take advantage of expertise at Sysmex Inostics and RIKEN GENESIS to promote clinical application of NGS

About Plasma-Safe-SeqS

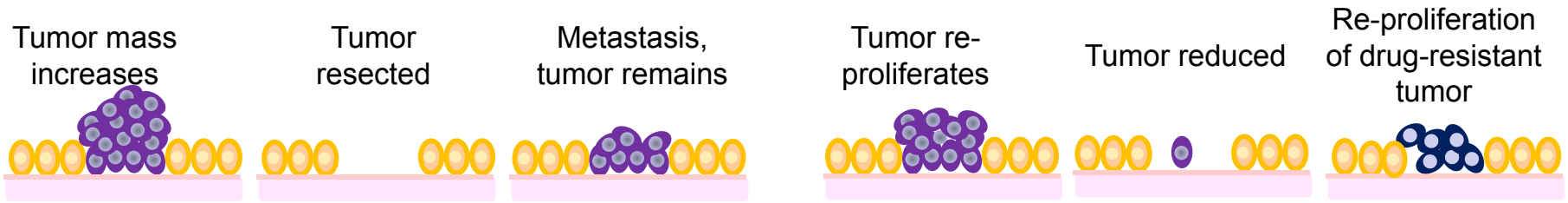
Pretreatment technology for achieving higher NGS sensitivity

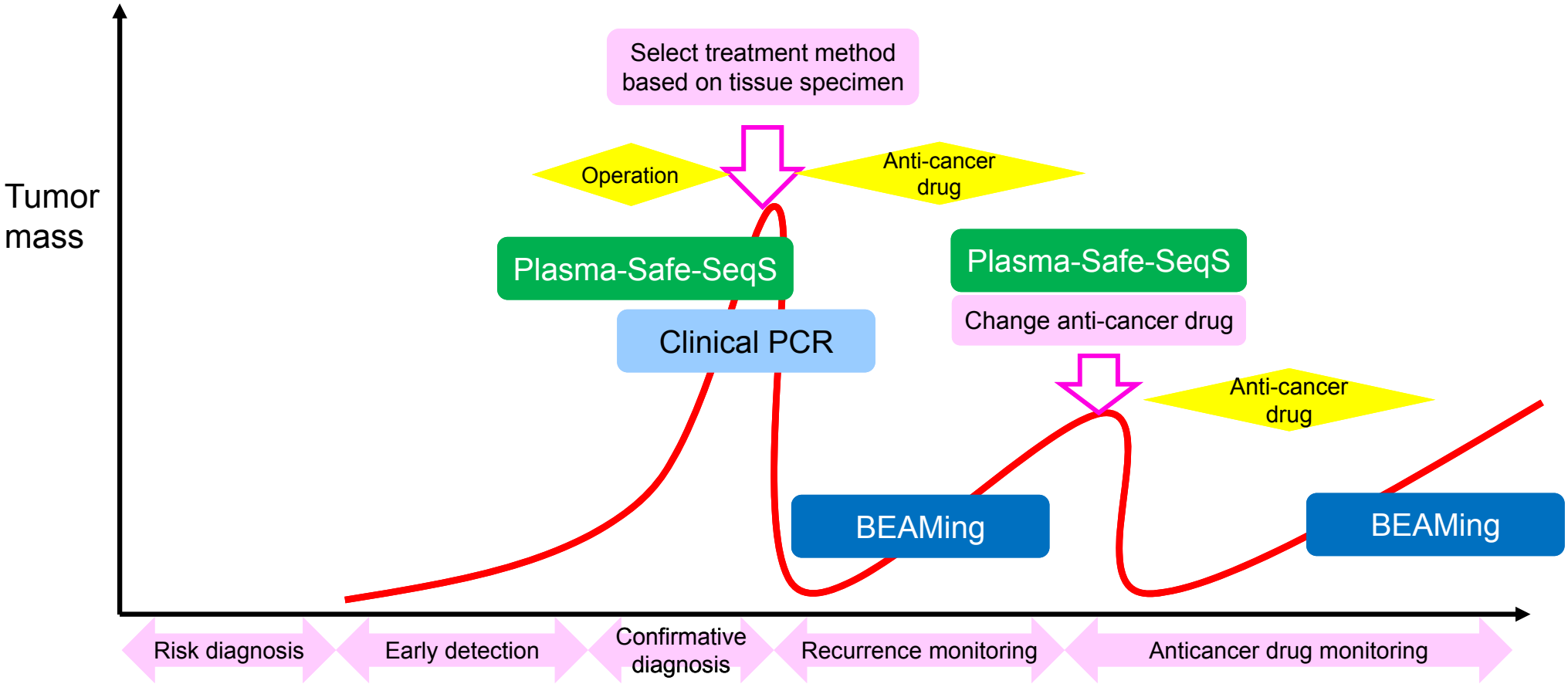
Plasma-Safe-SeqS technology principles



Segregation of Gene Platforms

Cancer example





We Believe the Possibilities.

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