Proprietary Innovative Antibody Engineering Technologies in Chugai Pharmaceutical

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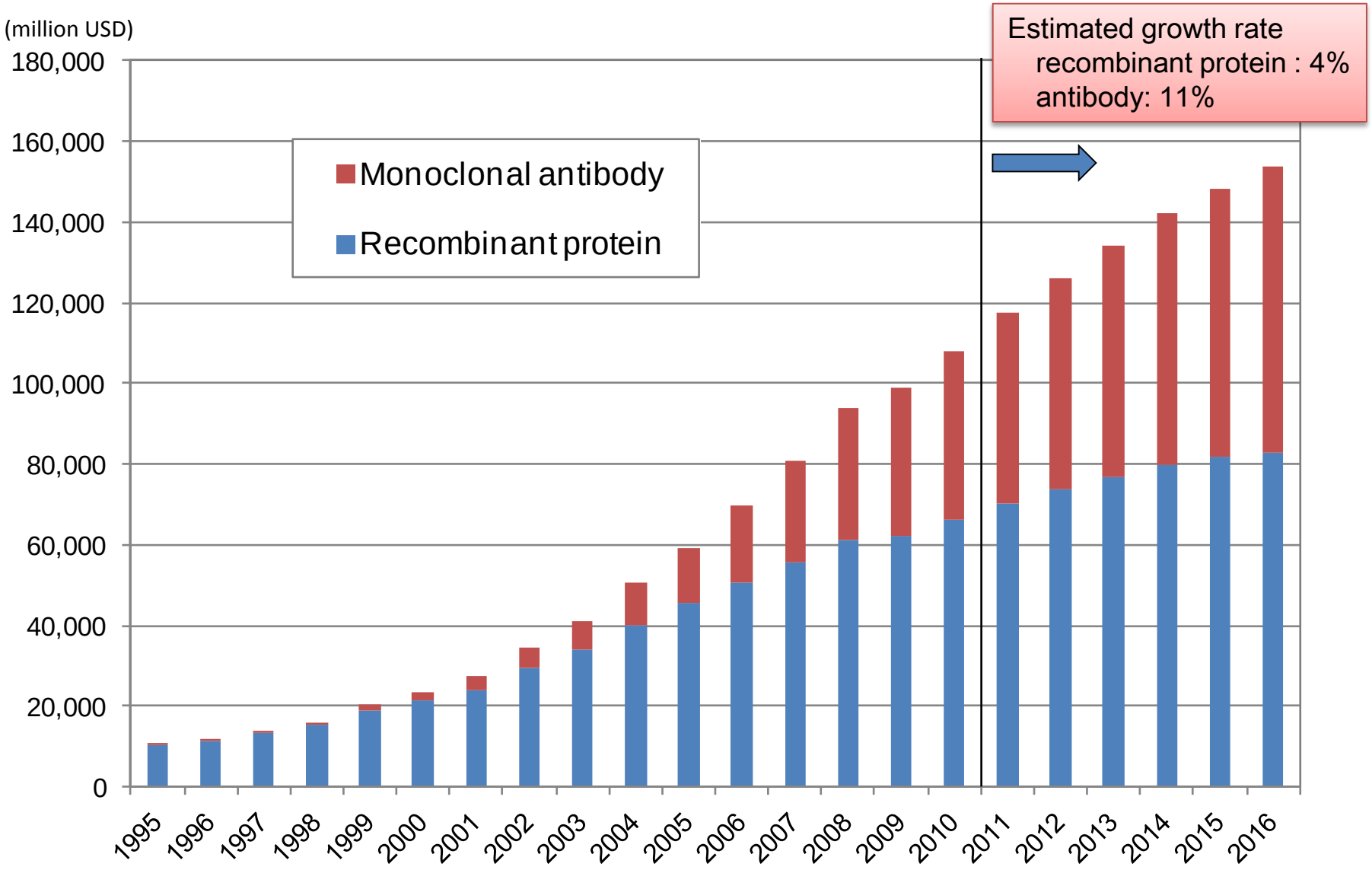
2012.12.18

Forward-Looking Statements

This presentation may include forward-looking statements pertaining to the business and prospects of Chugai Pharmaceutical Co., Ltd. (the “Company”). These statements reflect the Company’s current analysis of existing information and trends. Actual results may differ from expectations based on risks and uncertainties that may affect the Company’s businesses.

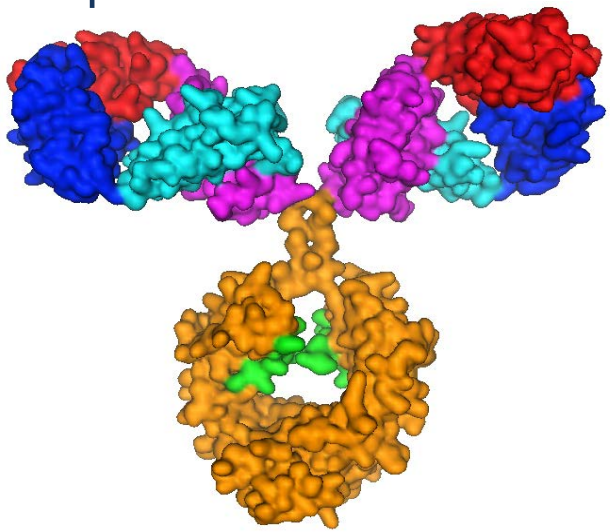
Therapeutic antibodies drive growth of biopharmaceutical market



source: Evaluate Pharma®

Characteristics of antibodies

- High efficacy, less side effect and favorable plasma persistence
  - High specificity and affinity to target antigen
  - High safety due to naturally derived molecule
  - Long duration due to favorable plasma persistence



- Applicability to various drug targets
  - Diverse target antigen
  - Diverse mode of action
- Industrial manufacturing
  - Feasible of engineering and improving by genetic engineering
  - Established manufacturing technologies of recombinant proteins
- Applicability of personalized healthcare (PHC)
  - Antigen itself as a candidate for biomarker
  - Antibody itself as a evaluation tool

Mode of action of antibody therapeutics

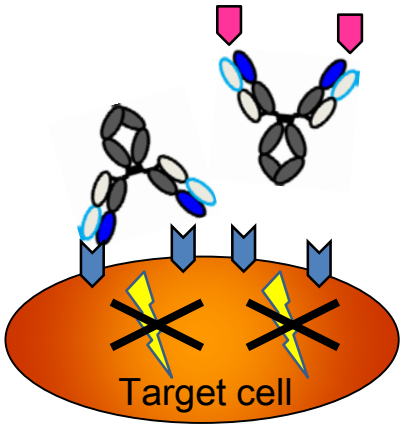
Therapeutic antibodies which blocks the function of the antigen

Therapeutic antibodies which elicits cytotoxicity against target cell

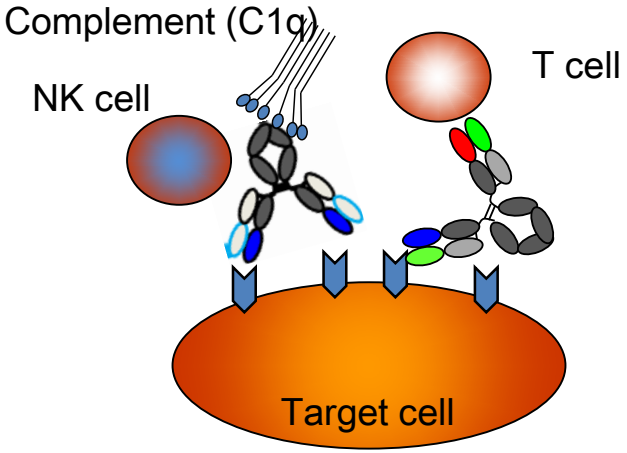
Ligand / receptor Binding blockade

Antibody Dependent Cellular Cytotoxicity(ADCC)
Complement Dependent Cytotoxicity(CDC)
T-cell Dependent Cellular Cytotoxicity

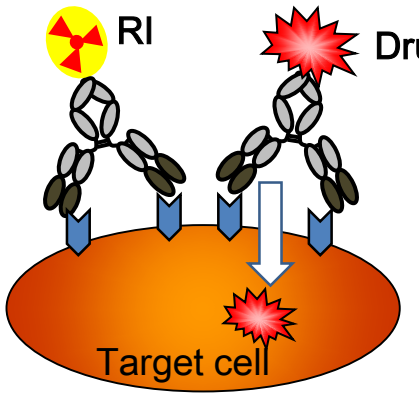
RI-labeled antibody
Antibody Drug Conjugate (ADC)



- tocilizumab
- bevacizumab
- daclizumab
- basiliximab
- abciximab
- efalizumab
- belimumab
- palivizumab
- natalizumab
- ipilimumab
- denosumab
- infliximab
- golimumab
- adalimumab
- panitumumab
- omalizumab
- ranibizumab
- ustekinumab
- eculizumab
- canakinumab



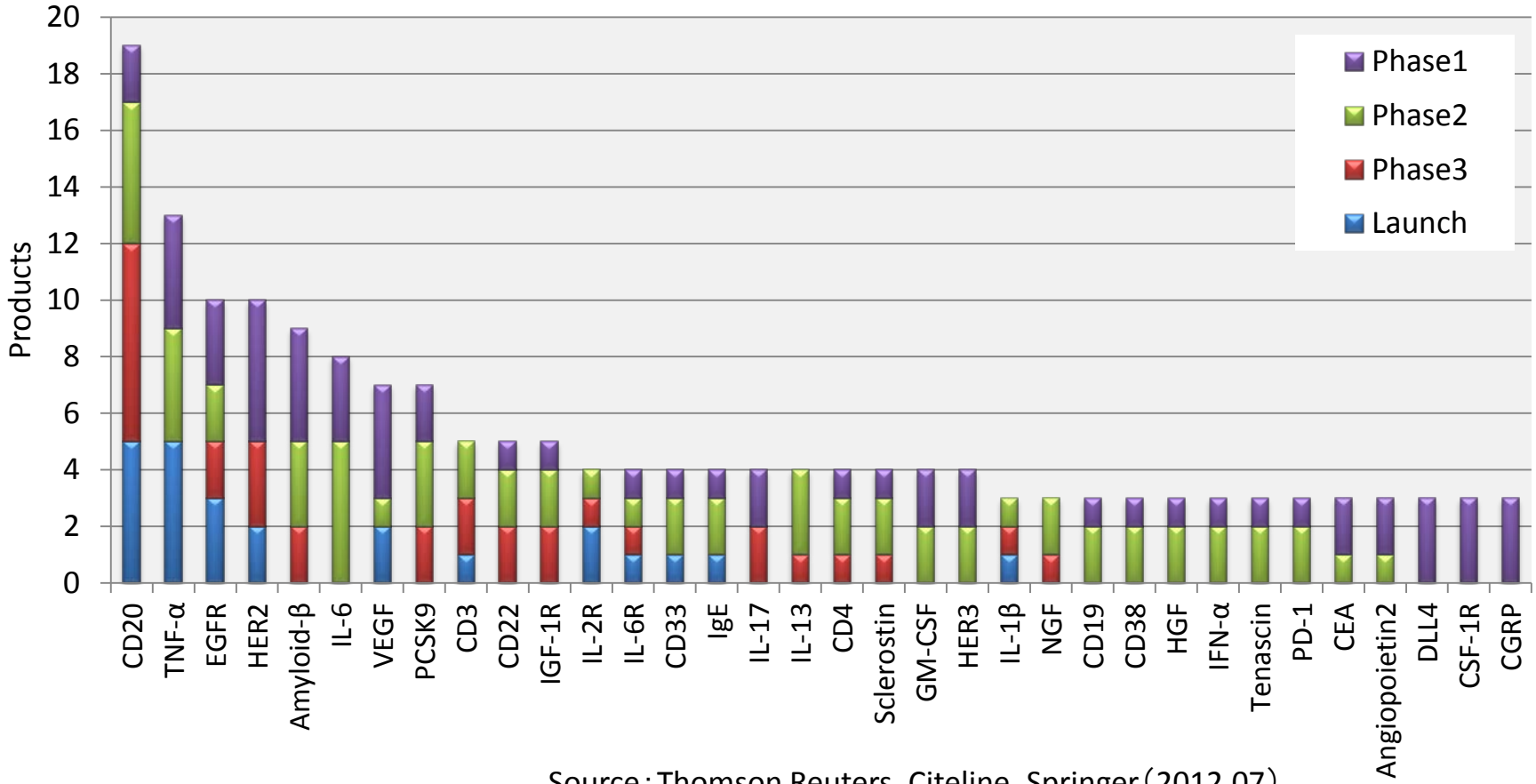
- trastuzumab
- pertuzumab
- cetuximab
- rituximab
- ofatumumab
- alemtuzumab
- mogamulizumab
- catumaxomab



- ibritumomab tiuxetan
- iodine 131 tositumomab
- gemtuzumab ozogamicin
- brentuximab vedotin

Severe competition in the development of therapeutic antibodies

- All major pharma companies focusing on therapeutic antibodies due to the expansion of the market of therapeutic antibodies
- More than 400 antibodies currently under clinical development
- 174 antibodies being developed against 34 promising antigens



Source：Thomson Reuters, Citeline, Springer（2012.07）

Difficult to achieve competitiveness only by conventional technologies

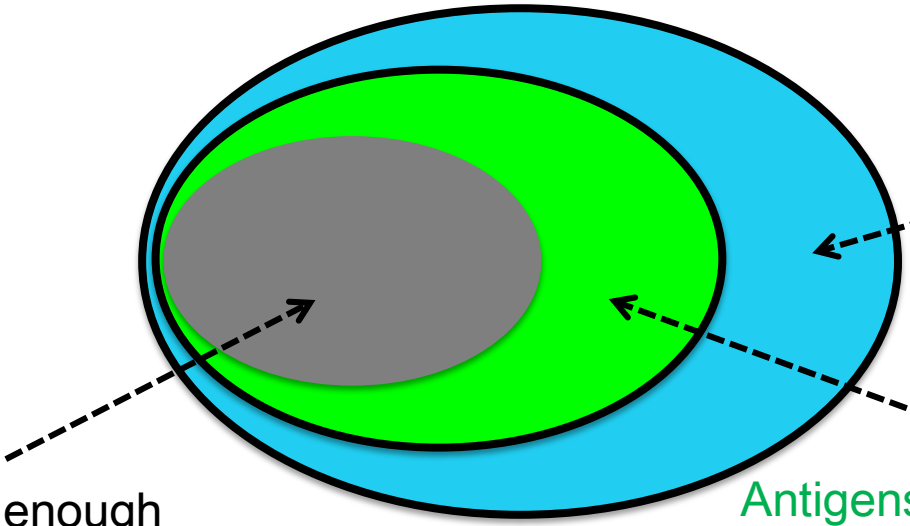
- Technologies for generating therapeutic antibody are fast-improving, and rapidly diffuse and become common
  - High affinity antibody technologies :
    many technologies such as phage display etc.
  - ADCC enhancing technologies :
    many technologies such as Potelligent™, Glycomab™, Xmab™ etc.
  - Half life extending technologies :
    many technologies such as Xtend™, albumin binding, PEGylation etc.
  - Many other technologies are widely used
- Difficult to differentiate by generating antibody drugs utilizing commonly used technologies
- Limited number of targetable antigens utilizing commonly used technologies

- We need to continuously develop proprietary technologies to ensure competitiveness

Effect of innovating technologies

- By developing only one or number one proprietary technologies and ensuring intellectual properties and know-hows, Chugai will create therapeutic antibodies that competitors cannot create

Target antigen space for therapeutic antibodies



Antigens that can be targeted only by using proprietary technologies
⇒First-in-class strategy

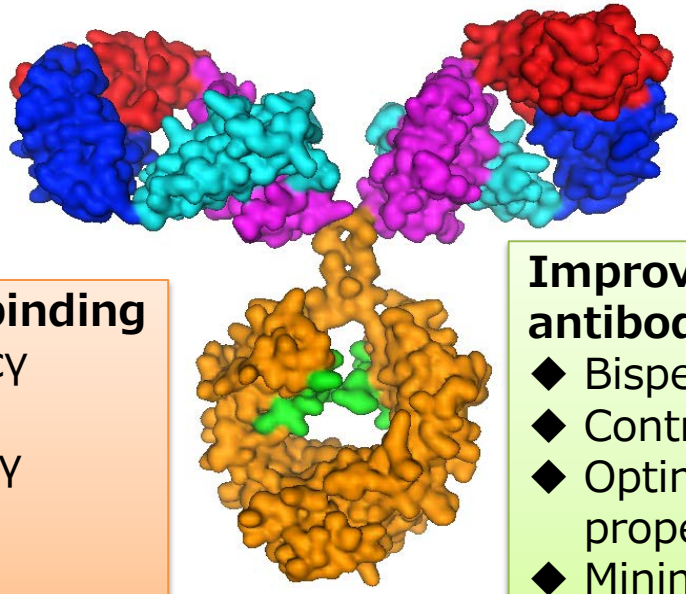
Antigens in which enough therapeutic value can be delivered by conventional technologies
⇒Difficult to differentiate

Antigens in which proprietary technology can provide therapeutic value that cannot be achieved by conventional technologies
⇒Best-in-class strategy

Importance of multidimensional technologies

- Chugai Pharmaceutical will provide therapeutic antibodies with highest quality regarding efficacy, safety and convenience
  - Despite single superior property, unmet needs would not be satisfied in case issues remain in other properties
  - We maintain and reinforce technologies to a highest level required to create therapeutic antibodies

Controlling antigen binding
- Optimizing affinity
- pH dependent antigen binding (recycling antibody technology)



Controlling Fc receptor binding
- Controlling activating Fcγ receptor binding
- Controlling inhibitory Fcγ receptor binding
- Controlling neonatal Fc receptor binding

Improving properties of antibody molecule
- Bispecific antibody
- Controlling pharmacokinetics
- Optimizing physicochemical properties
- Minimizing immunogenicity

Chugai's proprietary antibody technologies introduced today

SMART-Ig (Sequential Monoclonal Antibody Recycling Technology - Immunoglobulin)

- Recycling antibody technology and sweeping antibody technology

ART-Ig (Asymmetric Re-engineering Technology - Immunoglobulin)

- Bispecific antibody technology

ART-Fc (Asymmetric Re-engineering Technology - Fc domain)

- Enhancing binding selectively to activating Fcγreceptor (ADCC enhancing technology)

TRAB (T cell Redirecting AntiBody)

- T-cell redirecting antibody technology

TwoB-Ig (FcγRIIB selective binding technology - Immunoglobulin)

- Enhancing binding selectively to inhibitory Fcγreceptor

ACT-Ig (Antibody Charge engineering Technology - Immunoglobulin)

- Antibody half life extending technology

Technology Introduction of SMART-Ig and its Application to Actemra

CHUGAI PHARMACEUTICAL CO., LTD.
Research Division, Discovery Research Dept.
Team leader, Technology development
Tomoyuki Igawa

2012. 12.18

Summary of characteristic of SMART-Ig

- Conventional antibody derived from known technology, although how high the affinity is,
  - can bind to the antigen only once
  - only binds to the antigen, and cannot eliminate the antigen

  therefore the limitation existed.

- SMART-Ig overcomes this limitation and,
  - can bind to the antigen repeatedly (recycling antibody)
  - can eliminate antigen from plasma (sweeping antibody)

  therefore enables targeting of target antigen and achieving product profile that could not be previously achieved.

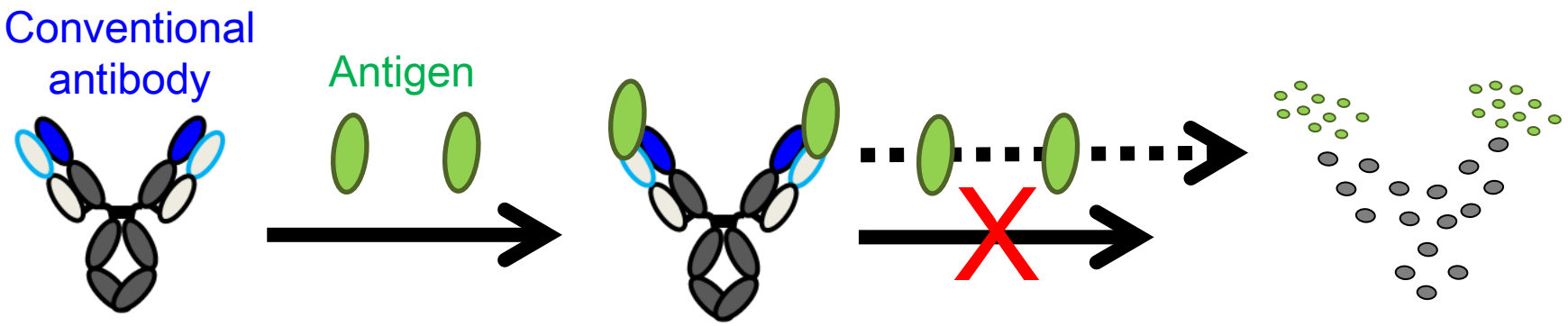
SMART-Ig

Sequential **M**onoclonal **A**ntibody **R**ecycling **T**echnology

Immuno**g**lobulin

Recycling Antibody

Limitation of known technology (conventional antibody)



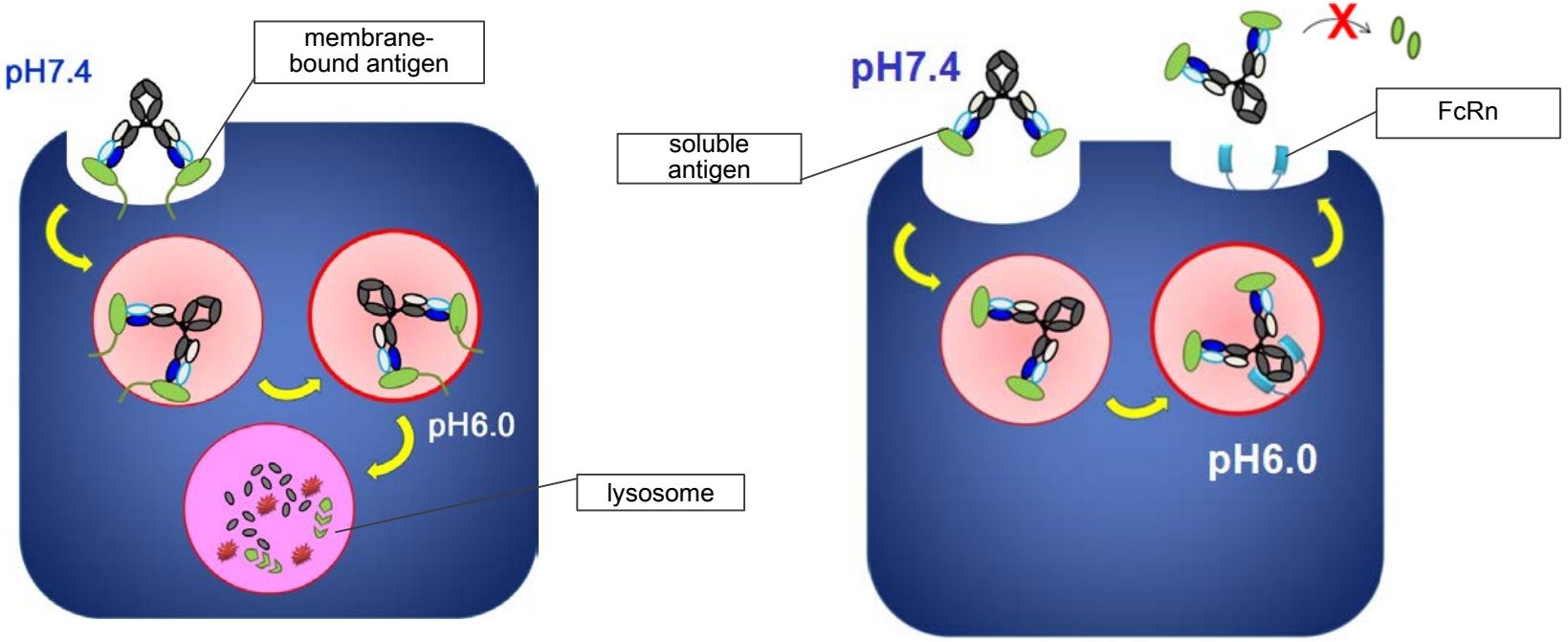
- ✓Antibody can bind to the antigen only once
- ✓Antibody-antigen complex is eventually degraded by lysosome
- ✓Even antibody with infinite affinity can bind to the antigen only once
- ✓Even antibody with infinite half life can bind to the antigen only once

Limitation of conventional antibody

Limitation of conventional antibody against soluble antigen and membrane-bound antigen

Conventional antibody against membrane-bound antigen (receptor, etc.)

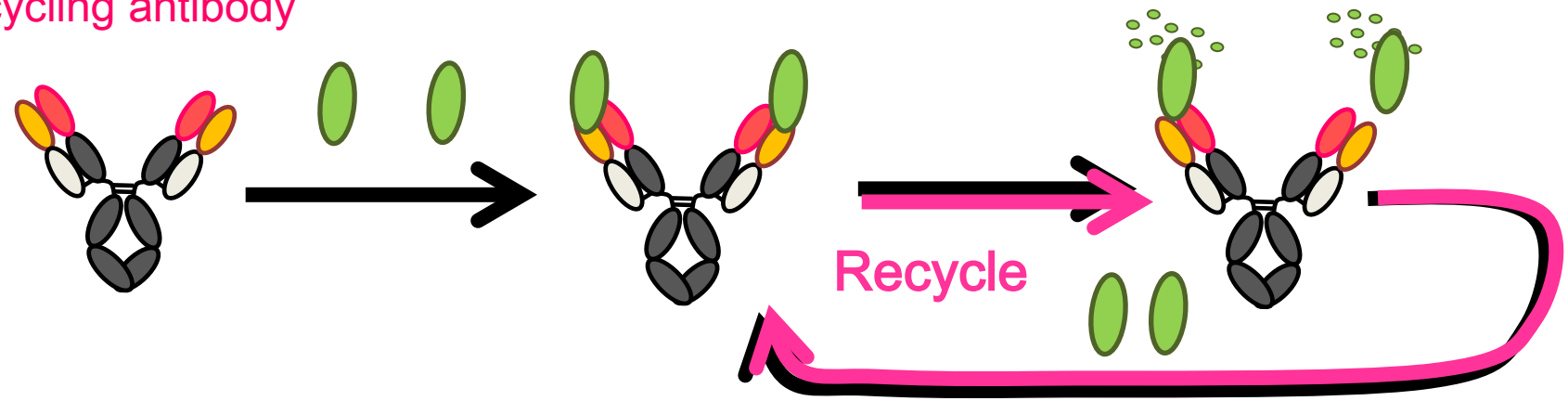
Conventional antibody against soluble antigen (cytokine, etc.)



Conventional antibody can bind to the antigen in both case whether the antigen is membrane-bound or soluble

Concept of recycling antibody

Recycling antibody

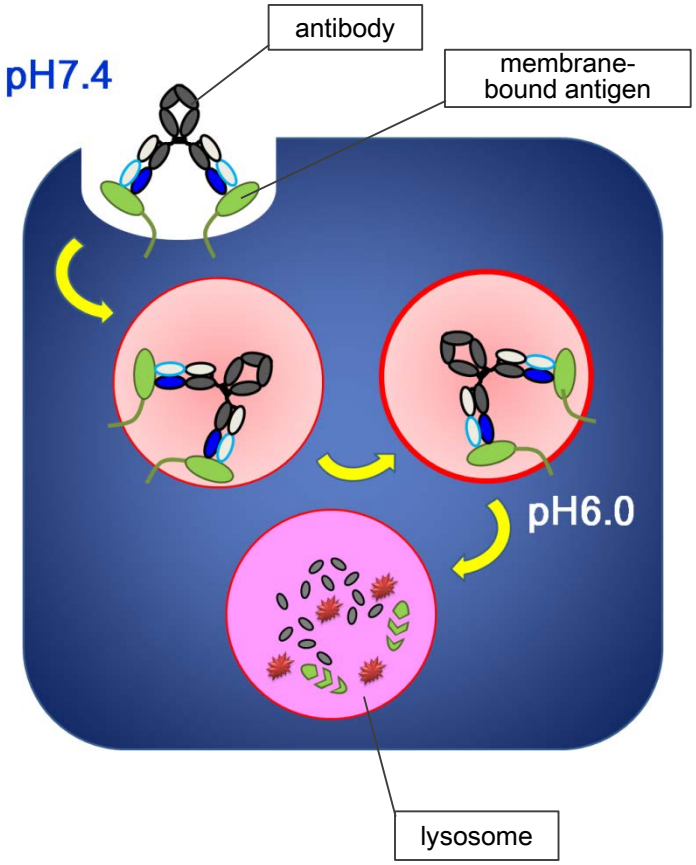


✓Antigen is selectively degraded, while antibody is not
✓Single antibody molecule binds to the antigen multiple times

Can overcome the limitation
of conventional antibody

Issues and limitation of conventional antibody against membrane-bound antigen

Conventional antibody



Antibody can bind to the membrane-bound antigen only once

↓

Antibody bound to antigen is transferred to lysosome and degraded by protease

↓

Antibody bound to membrane-bound antigen is taken up by cells and cleared from plasma

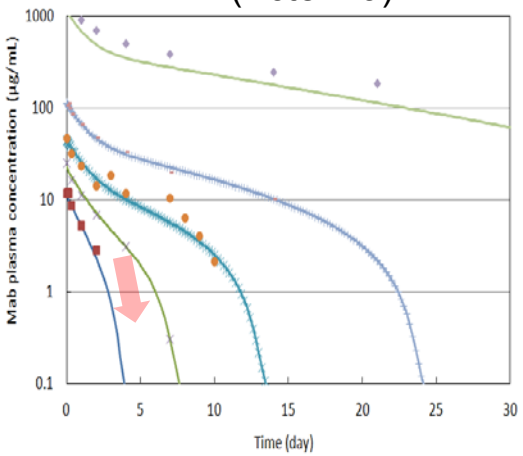
↓

In case target antigen is present in large amount in the body, administered antibody will be degraded very rapidly

Issues of conventional antibody targeting membrane-bound antigen

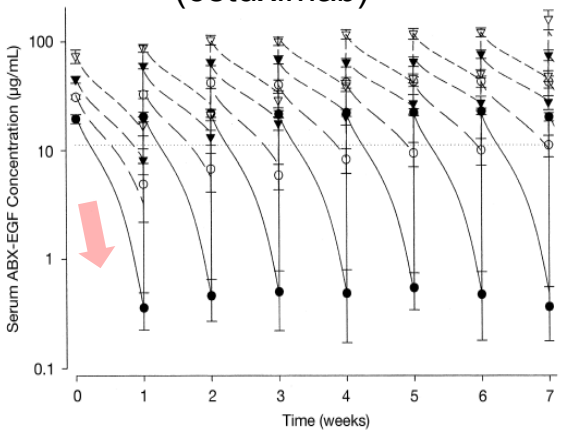
•Pharmacokinetics of antibodies targeting membrane-bound antigen

Anti-IL6 receptor antibody (Actemra)



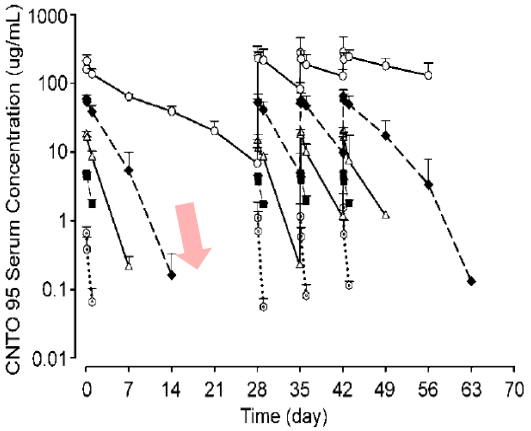
(internal data)

Anti-EGFR antibody (cetuximab)



J Clin Oncol 22:3003-3015. 2004

Anti-α_v integrin antibody



Clin Cancer Res 2007;13:2128-2135.

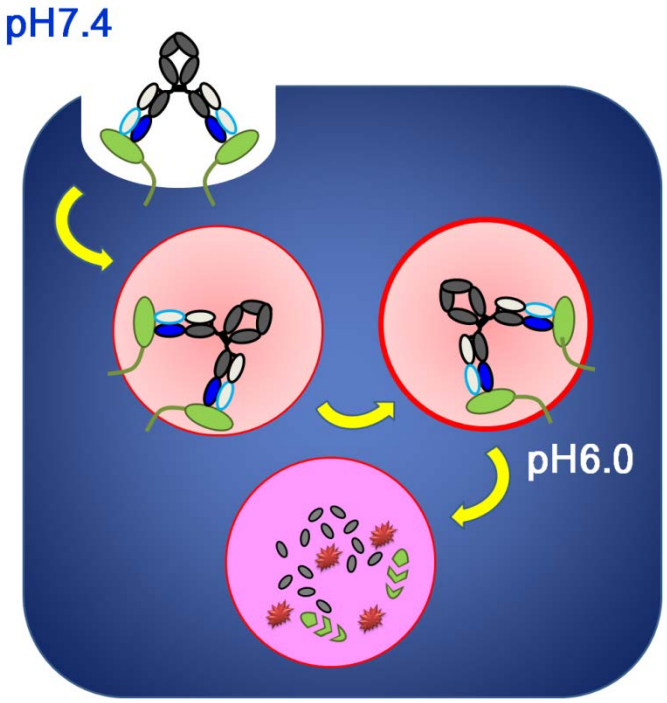
Conventional antibody binds to membrane-bound antigen, internalized into cells and rapidly cleared from plasma

➔Requires large amount of antibody to block the function of antigen for a long term

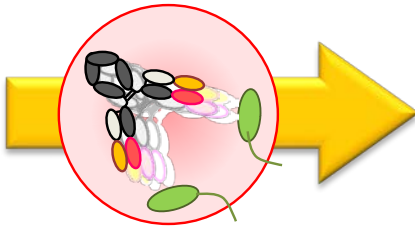
➔Can be overcome by recycling antibody technology

Effect of recycling antibody against membrane-bound antigen

Conventional antibody

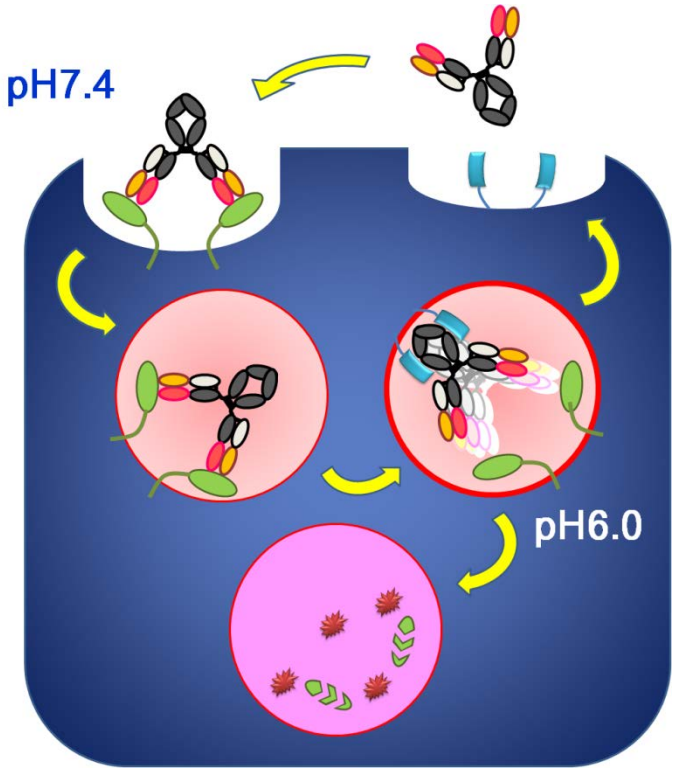


- ✓Antibody can bind to the antigen only once
- ✓Antibody binds to the antigen and rapidly cleared



Engineer antibody to dissociate from the antigen pH-dependently

Recycling antibody



- ✓Antibody can bind to the antigen multiple times
- ✓Can reduce the antibody clearance

Nature Biotechnology. **28**, 1203-7, 2010

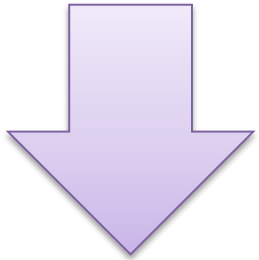
Product concept of recycling antibody SA237

- **Actemra**

  Bind to IL-6 receptor only once and rapidly cleared from plasma

  “Once monthly intravenous injection (approved)”

  “Once weekly or biweekly subcutaneous injection (filed NDA in Japan, and preparing to file overseas)”



Generated SA237 from Actemra by antibody engineering technologies

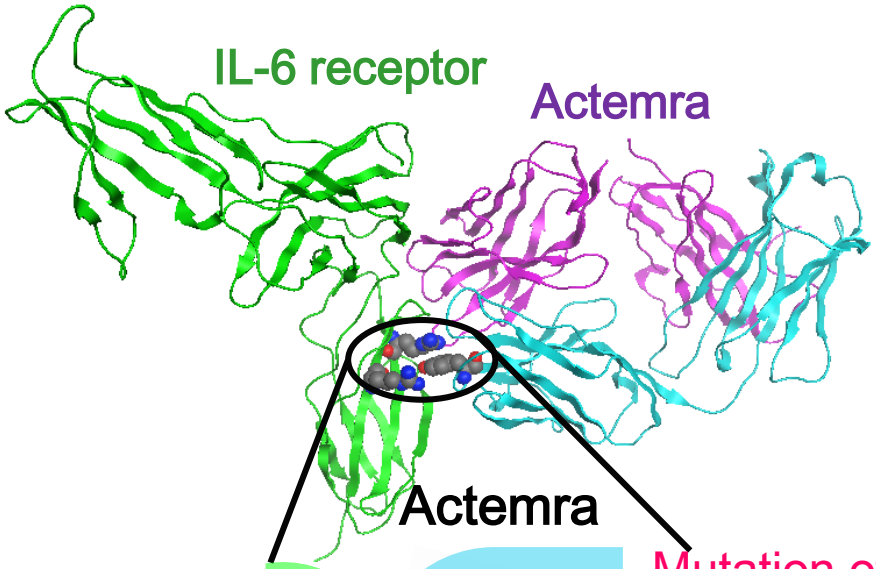
- **SA237**

  Bind to IL-6 receptor multiple times and slowly cleared from plasma

  “Once monthly or less subcutaneous injection”

Improvement of patient’s convenience by once monthly or less dosing subcutaneous formulation

Generation of SA237 by engineering Actemra



Complex crystal structure of Actemra and IL-6 receptor

pH-dependent IL-6 receptor binding

SA237

Neutral (pH7.4)

Acidic (pH6.0)

Mutation of tyrosine in CDR into histidine by genetic engineering

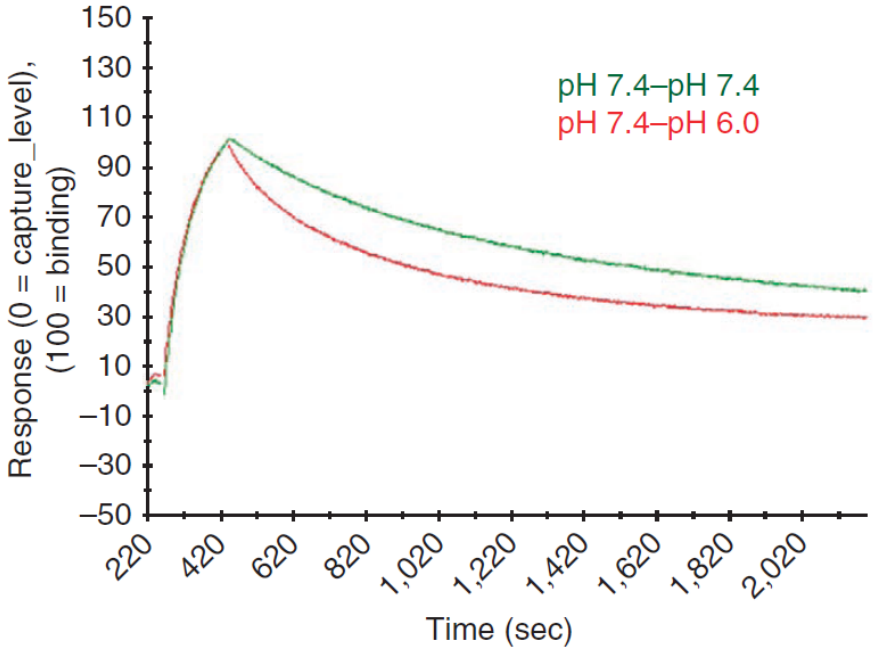
Repulsion with IL-6 receptor by being positively charged within acidic condition

Tyrosine

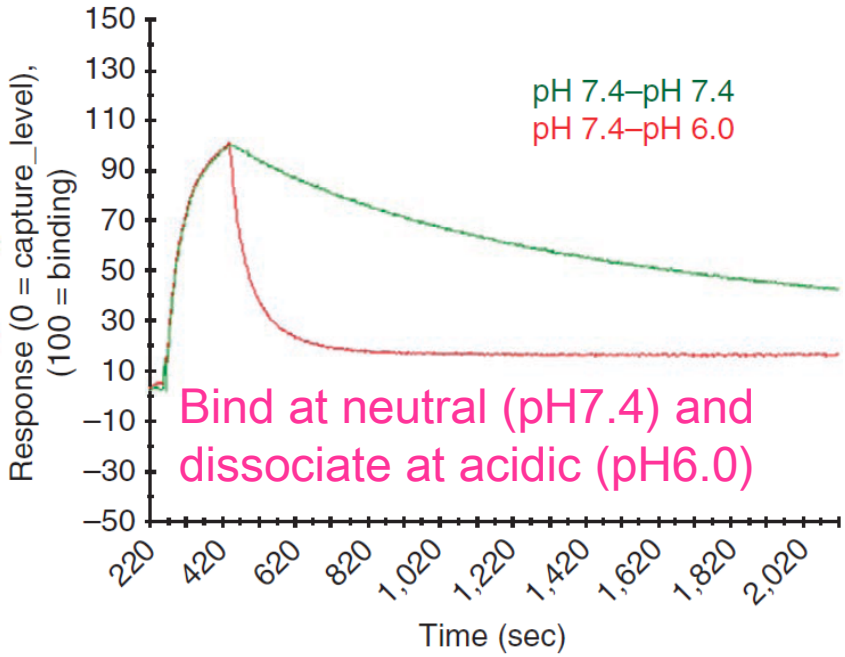
Histidine

Generation of pH-dependent IL-6 receptor binding recycling antibody SA237

Actemra
(conventional antibody)



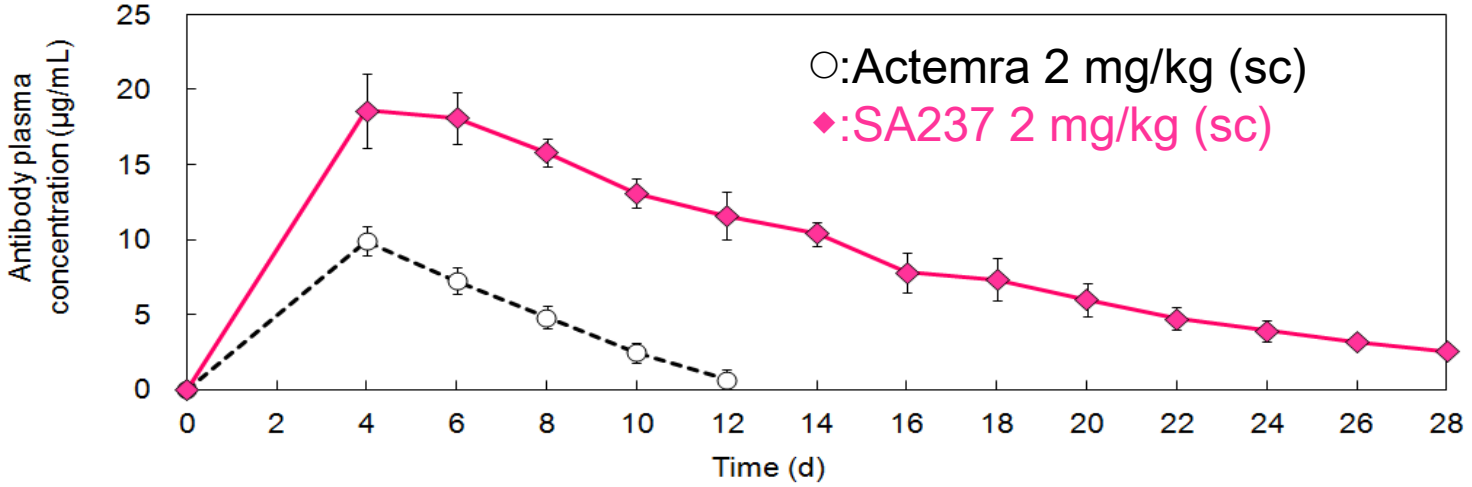
SA237
(recycling antibody)



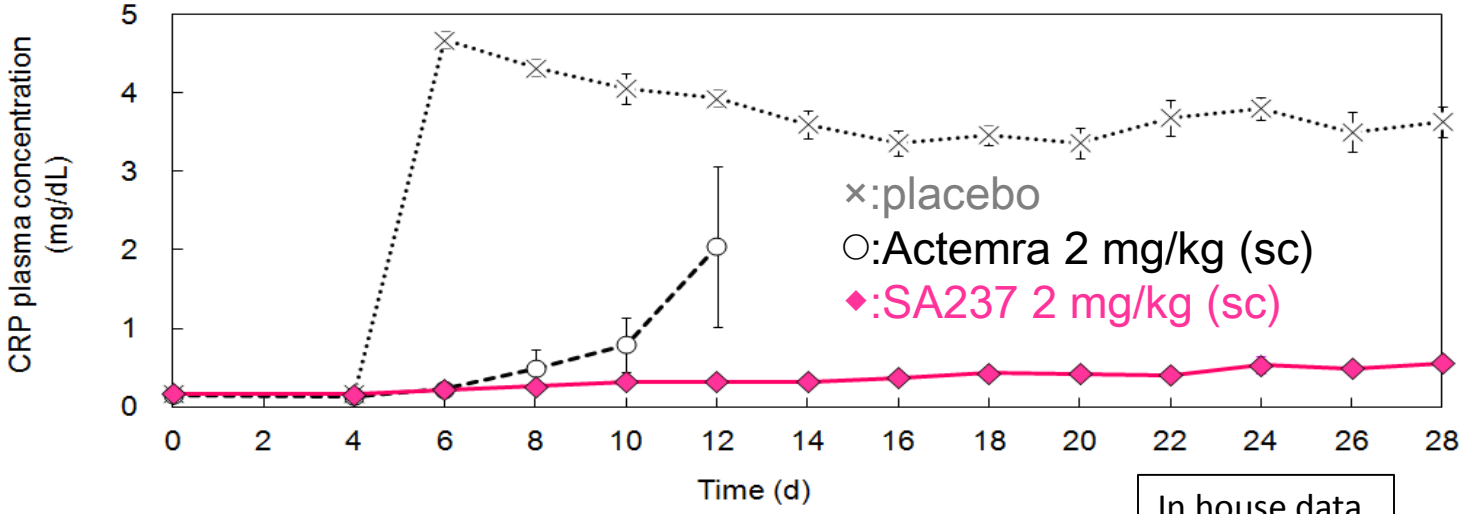
In house data

SA237 shows significantly longer plasma persistence and efficacy compared to Actemra

- Antibody plasma concentration time-profile

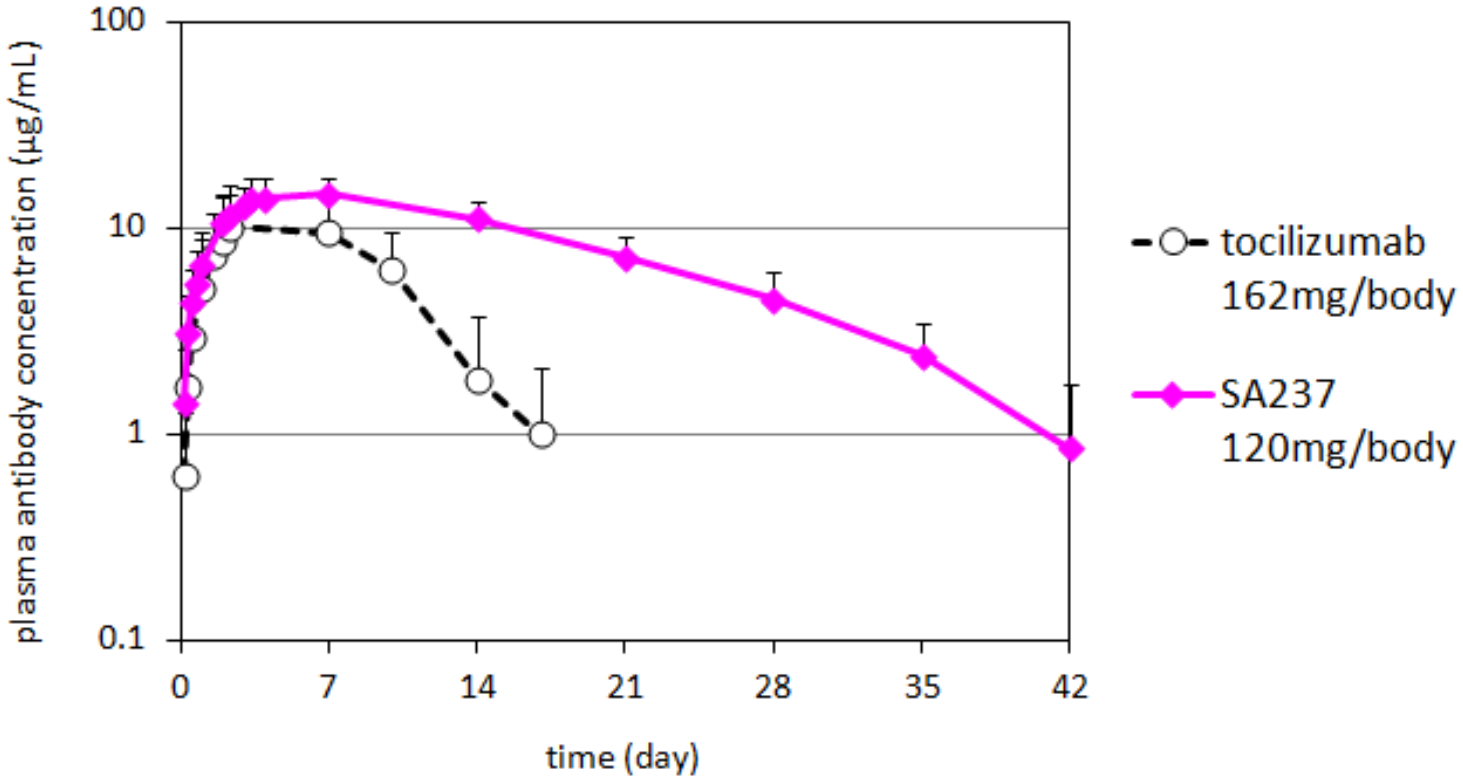


- C-reactive protein plasma concentration time-profile



In house data

Phase 1 clinical study in healthy volunteer : SA237 exhibits longer plasma persistence than Actemra



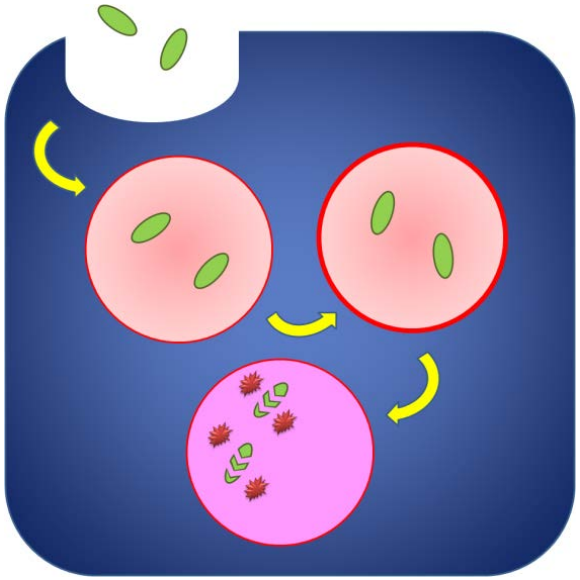
Recycling antibody SA237 120 mg (~2.0 mg/kg) exhibited significant improved duration compared to tocilizumab 162 mg (~2.9 mg/kg)

➔ Clinical proof of concept of recycling antibody technology

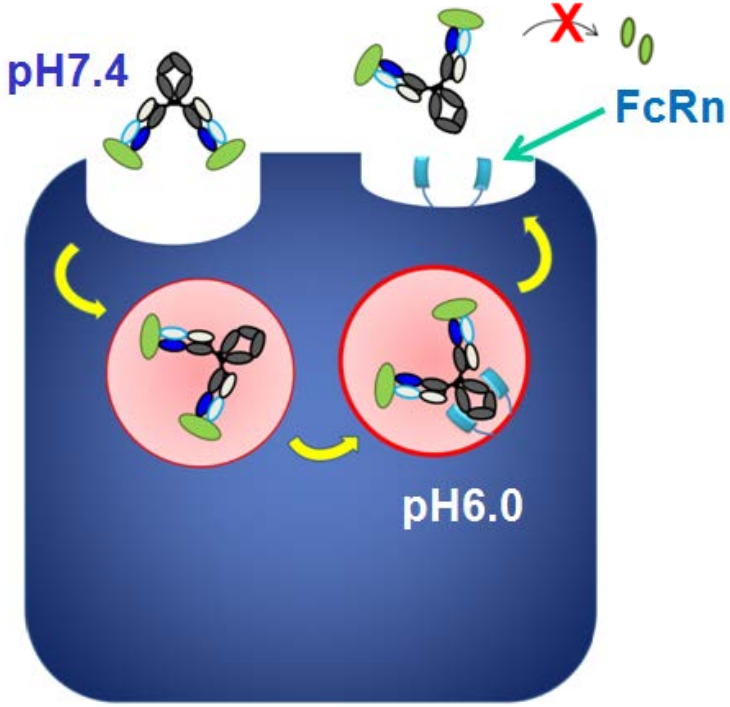
In house data

Issues and limitation of conventional antibody against soluble antigen

Antigen (no antibody)



Conventional antibody



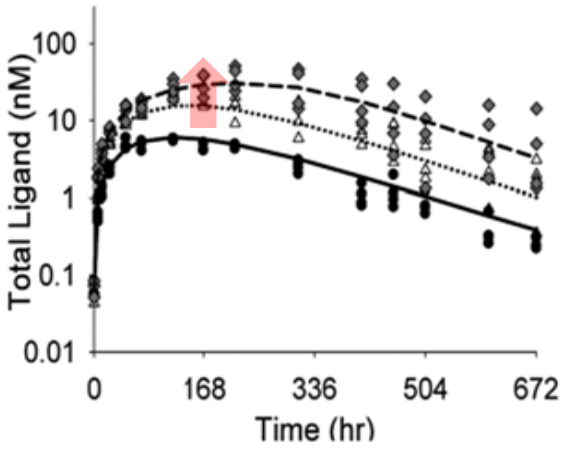
An antibody can bind to the antigen only once

- Antigen persist in plasma without being degraded as an antibody bound form
- Administration of antibodies results in accumulation of antigen, thus increasing the concentration of antigen in the plasma

Issues of conventional antibody targeting soluble antigen

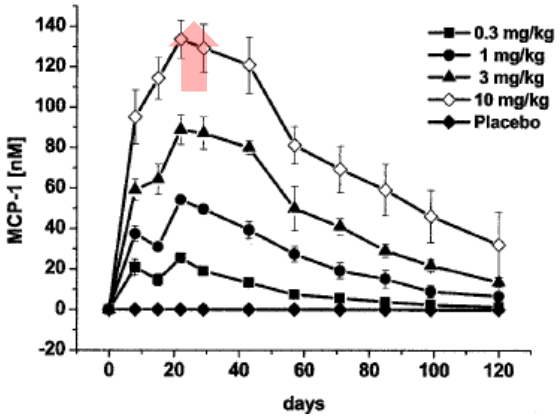
Antigen concentration time-profile after administration of antibody against soluble antigen

Anti-amyloid beta antibody



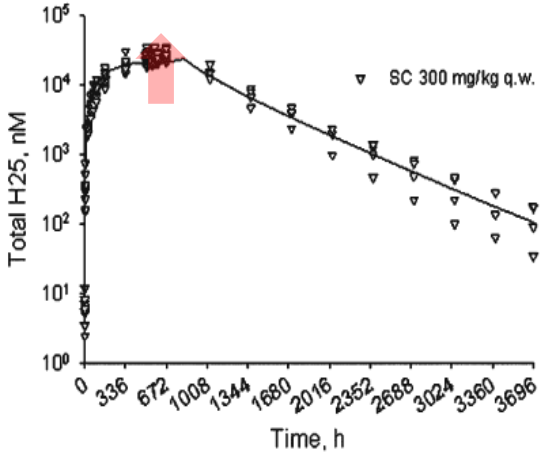
mAbs, 2010, 2:5, 1-13

Anti-MCP1 antibody



ARTHRITIS & RHEUMATISM 2006, 54,2387–92

Anti-hepcidin antibody



AAPS J. 2010, 4, 646-57.

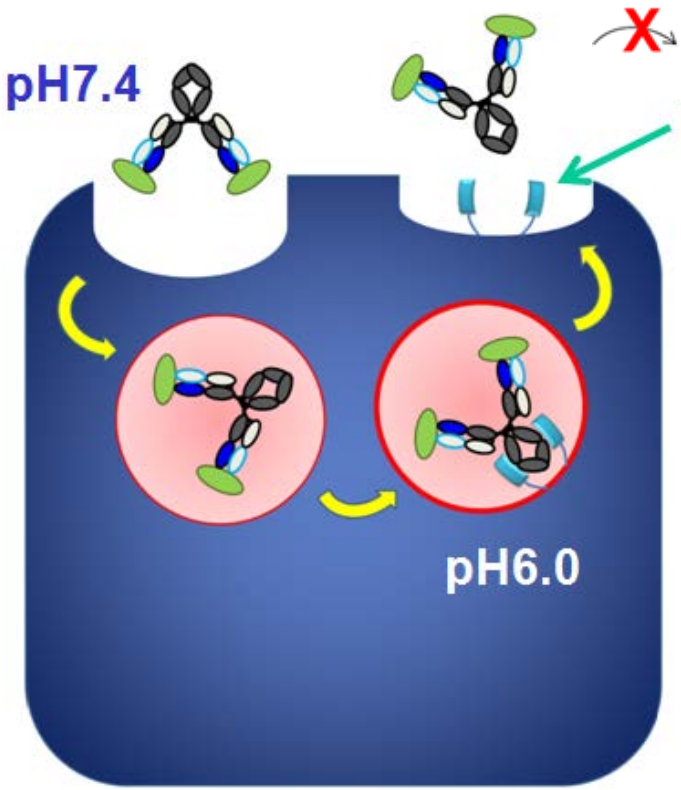
By administering conventional antibody, antigen persists in plasma as an antibody bound form, and antigen concentration increases (accumulates) by more than 1000-fold

➔Requires large amount of antibody to block highly accumulated antigen

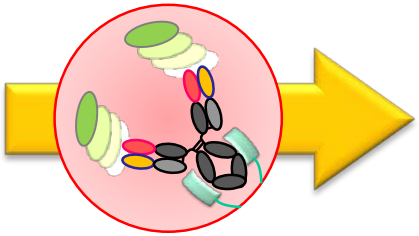
➔Can be overcome by recycling and sweeping antibody technology

Effect of recycling antibody against soluble antigen in plasma

Conventional antibody

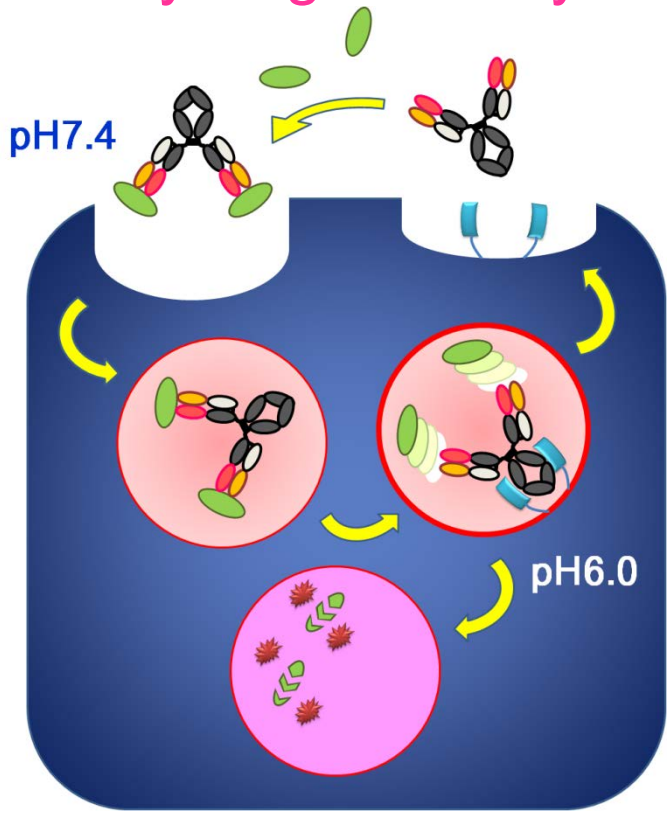


- ✓Antibody can bind to the antigen only once
- ✓Antigen persist in plasma as an antibody bound form, and antigen accumulates in plasma



Engineer antibody to dissociate from the antigen pH-dependently

Recycling antibody



- ✓Antibody can bind to the antigen multiple times
- ✓Prevents from antigen accumulation, by discarding the antigen within the cell

Nature Biotechnology. **28**, 1203-7, 2010

SMART-Ig

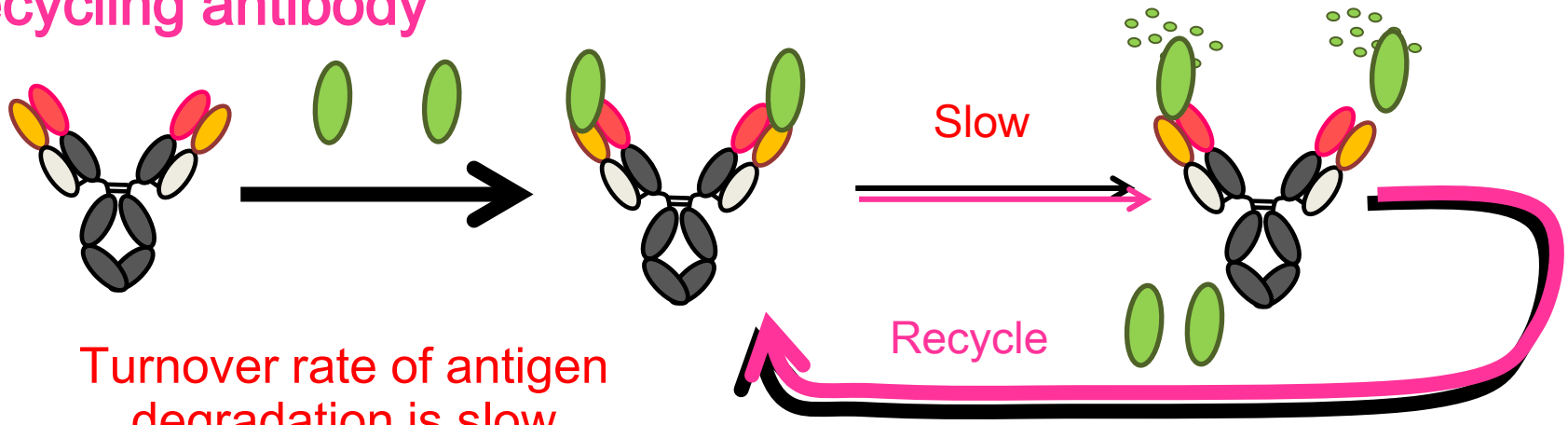
Sequential **M**onoclonal **A**ntibody **R**ecycling **T**echnology

Immuno**g**lobulin

Sweeping antibody

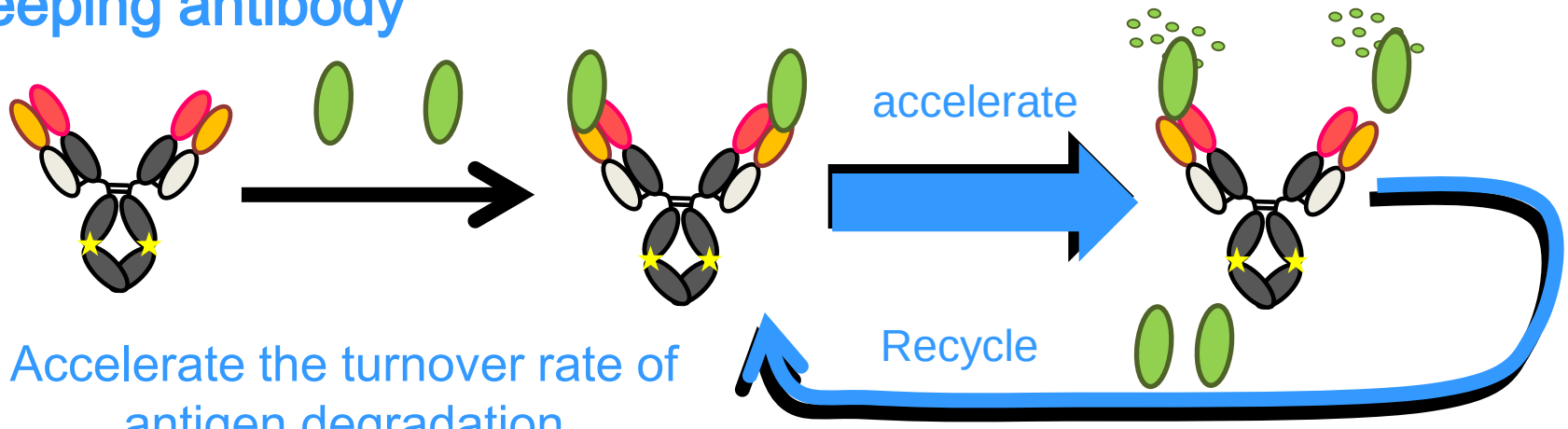
Concept of sweeping antibody

- Recycling antibody



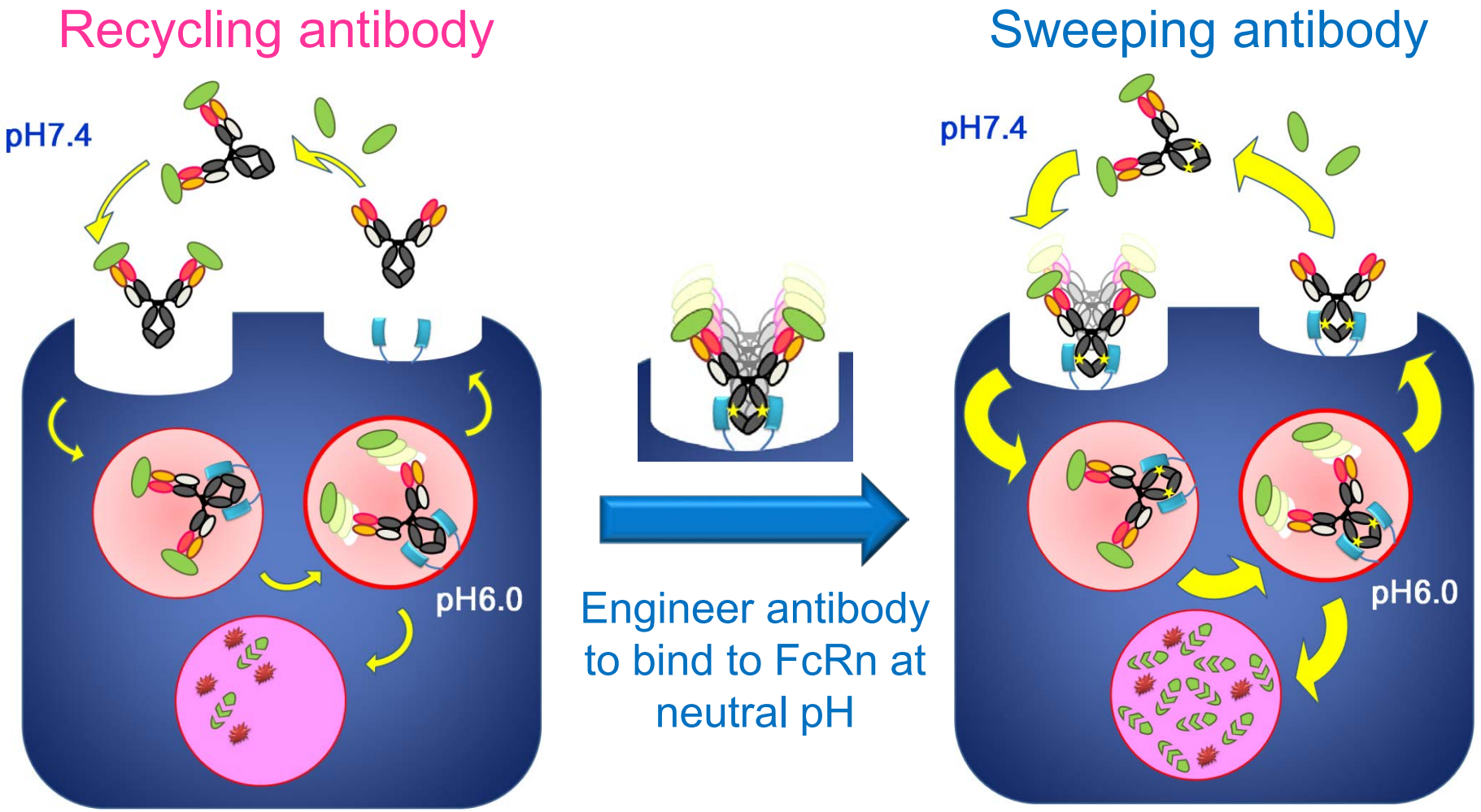
Turnover rate of antigen degradation is slow

- Sweeping antibody



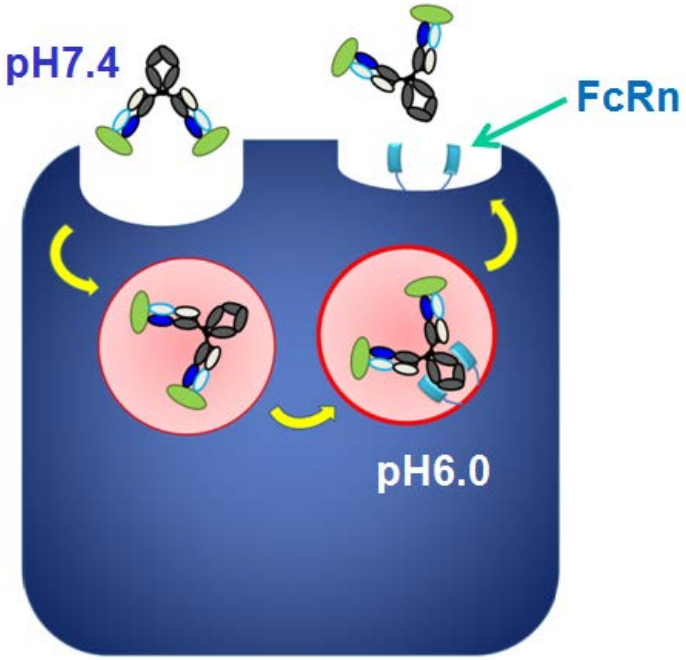
Accelerate the turnover rate of antigen degradation

Effect of recycling antibody against soluble antigen in plasma



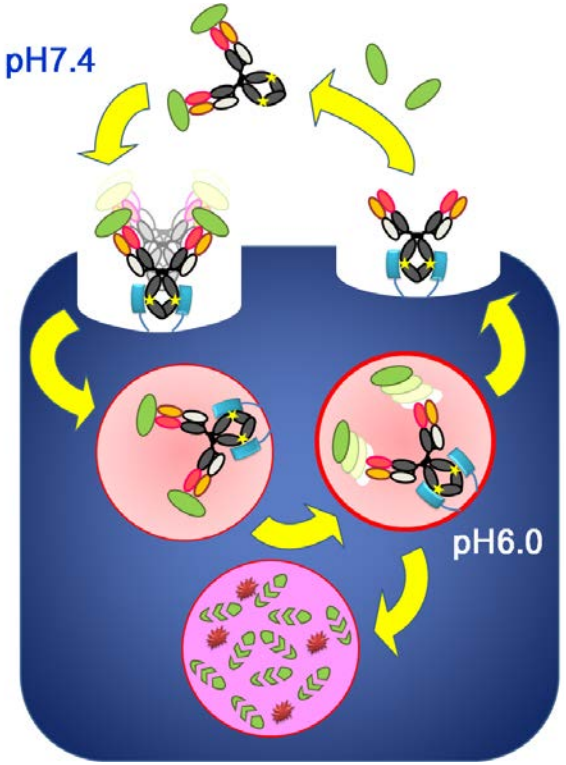
Differences between conventional antibody and sweeping antibody

Conventional antibody



✓Antibody can bind to the antigen only once
✓Antigen persist in plasma as an antibody bound form, and antigen accumulates in plasma

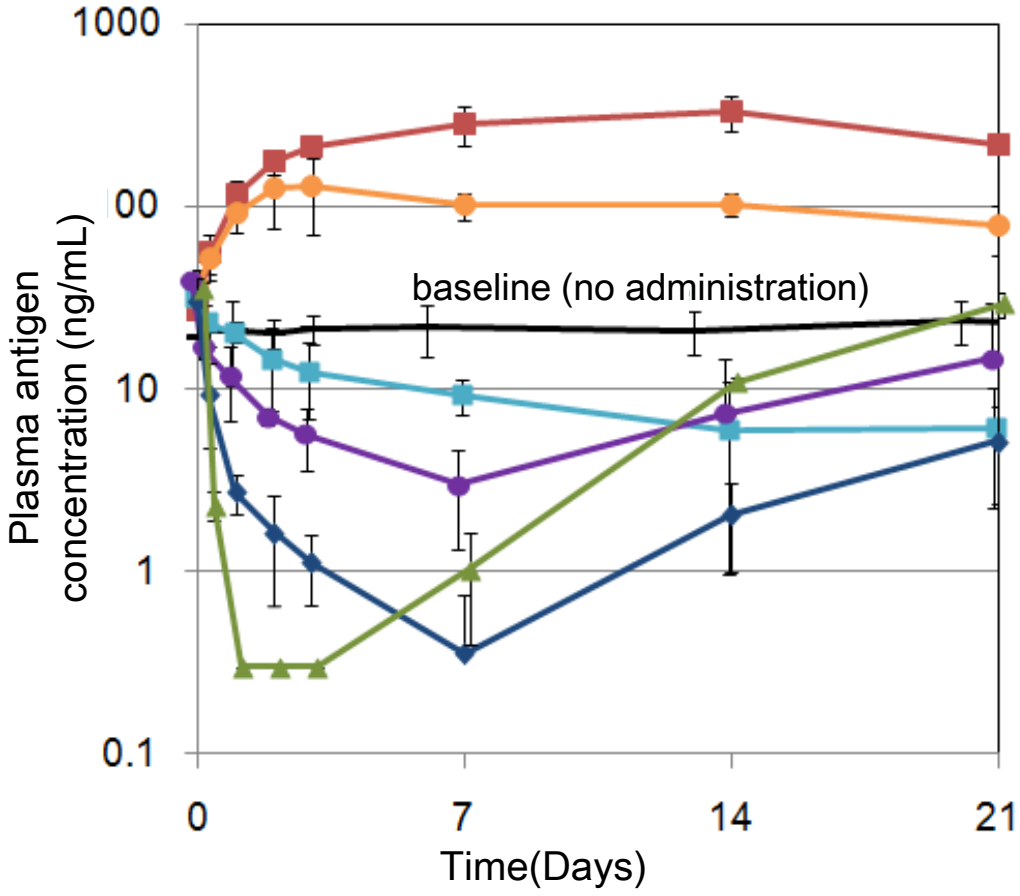
Sweeping antibody



✓Antibody can bind to the antigen multiple times
✓Antibody can actively degrades antigen
✓Antibody can eliminate or sweep antigen from plasma

Various types of sweeping antibody can be generated by controlling binding affinity to FcRn

"Antigen" plasma concentration time profile



In house data

Conventional antibody

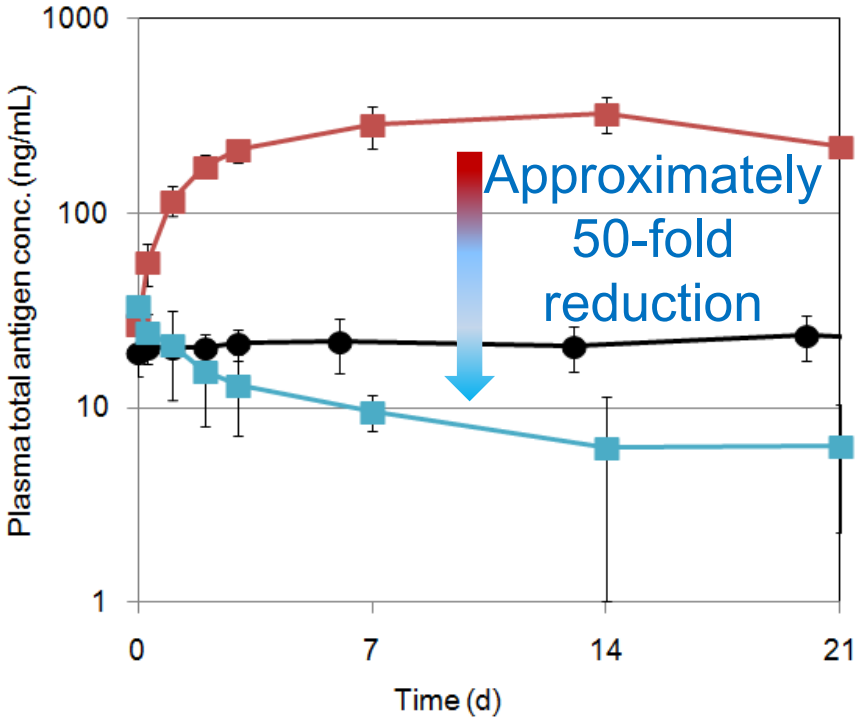
Recycling antibody

Various sweeping antibody

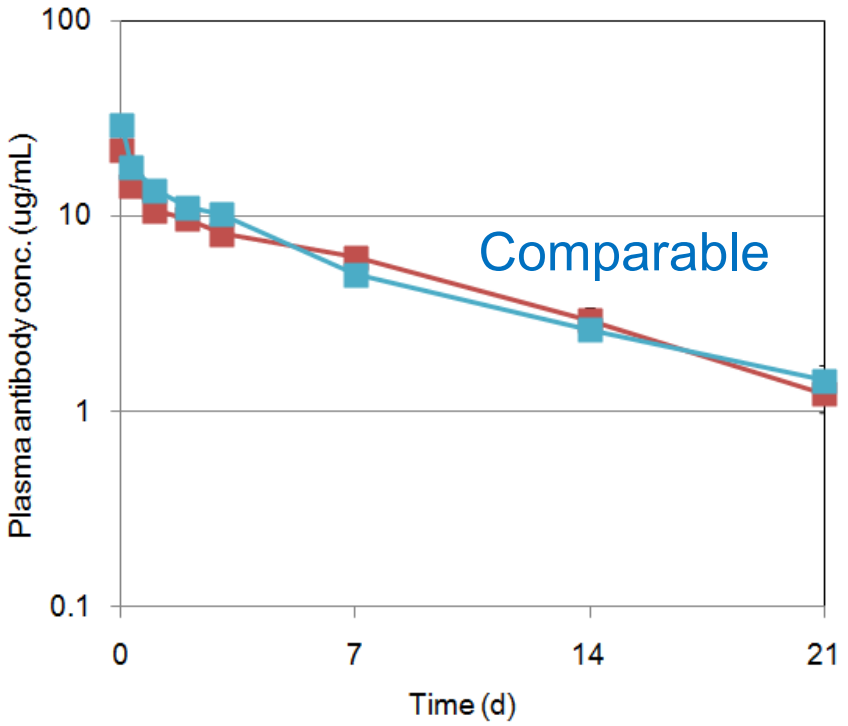
By modulating the binding affinity to FcRn, sweeping antibody having appropriate property depending on the antigen or disease to which it targets

Long-acting sweeping antibody selectively reduces plasma antigen concentration 50-fold in mice model

"Antigen" plasma concentration time-profile



"Antibody" plasma concentration time-profile



● : placebo

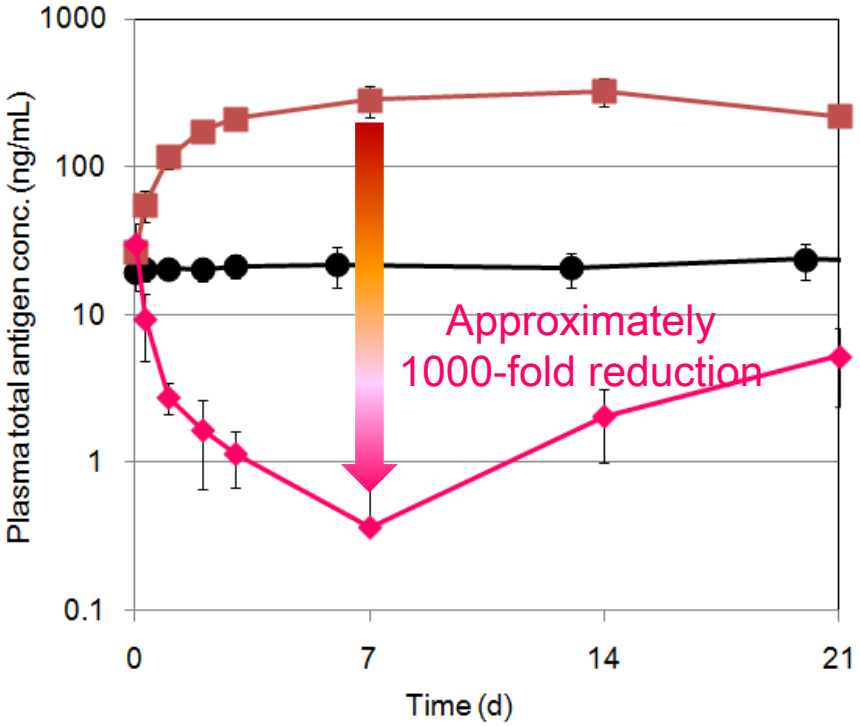
■ : conventional antibody

■ : long-acting sweeping antibody

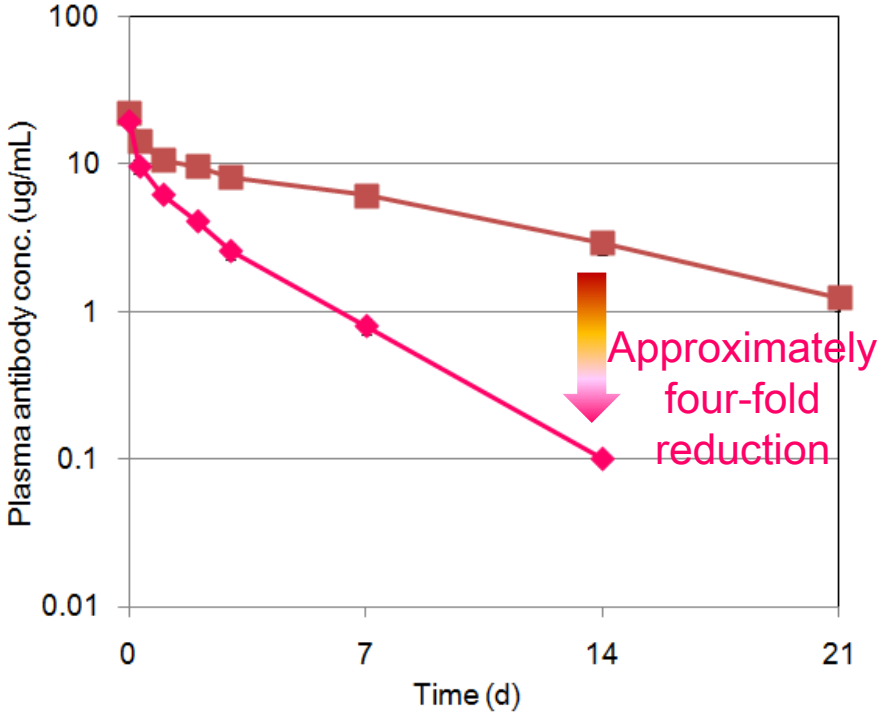
In house data

Rapid-acting sweeping antibody rapidly and transiently reduces plasma antigen concentration in mice model

"Antigen" plasma concentration time-profile



"Antibody" plasma concentration time-profile



● : placebo
■ : conventional antibody
◆ : rapid-acting sweeping antibody

In house data

SMART-Ig

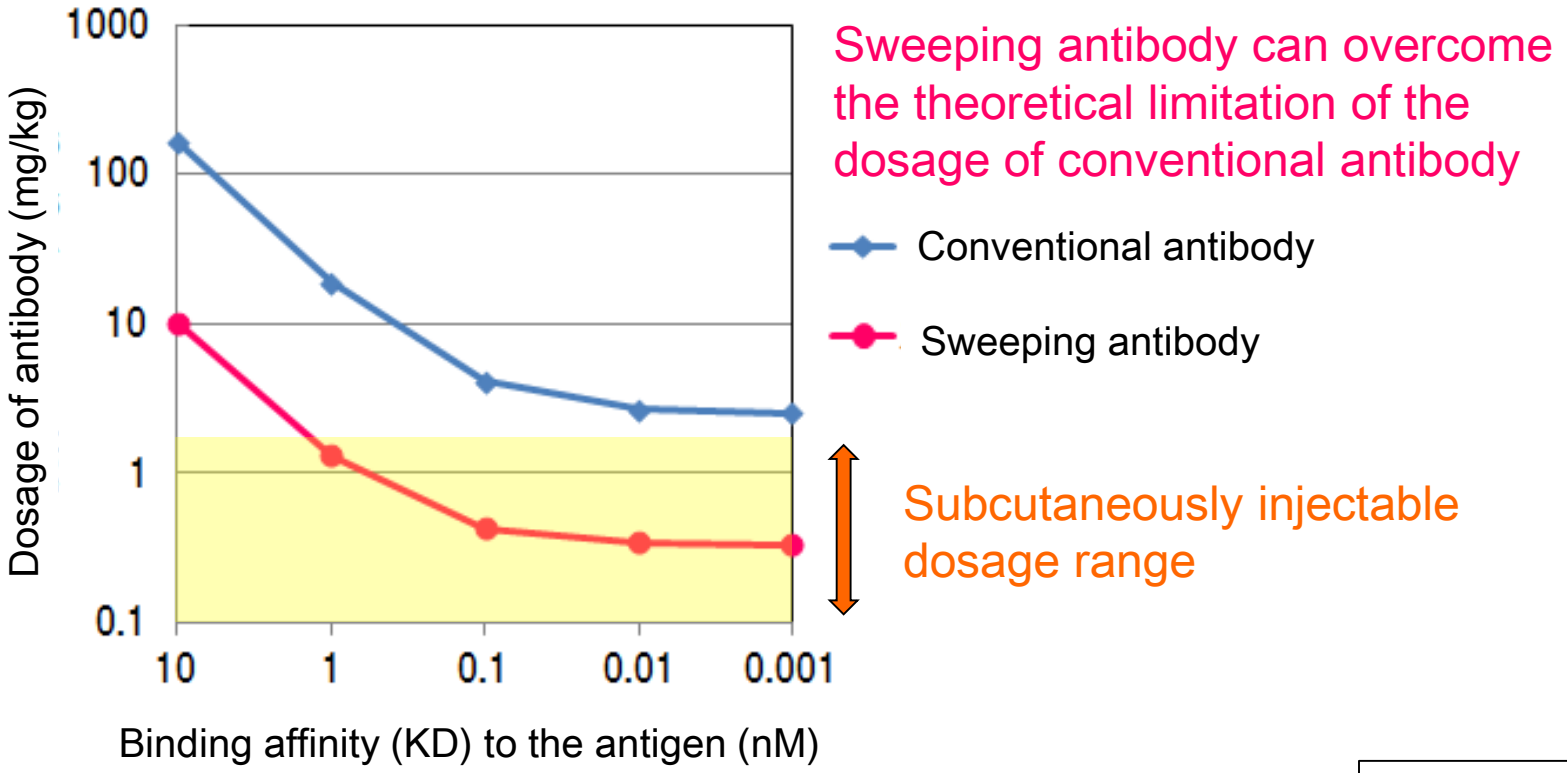
Sequential **M**onoclonal **A**ntibody **R**ecycling **T**echnology
Immuno**g**lobulin

Application to drug discovery

Sweeping antibody enables low dosage which can never be achieved by conventional antibody

Comparison of dosage between conventional and sweeping antibody

Simulation of antibody dosage required for inhibiting the function of antigen X by 90% with once a month dosing

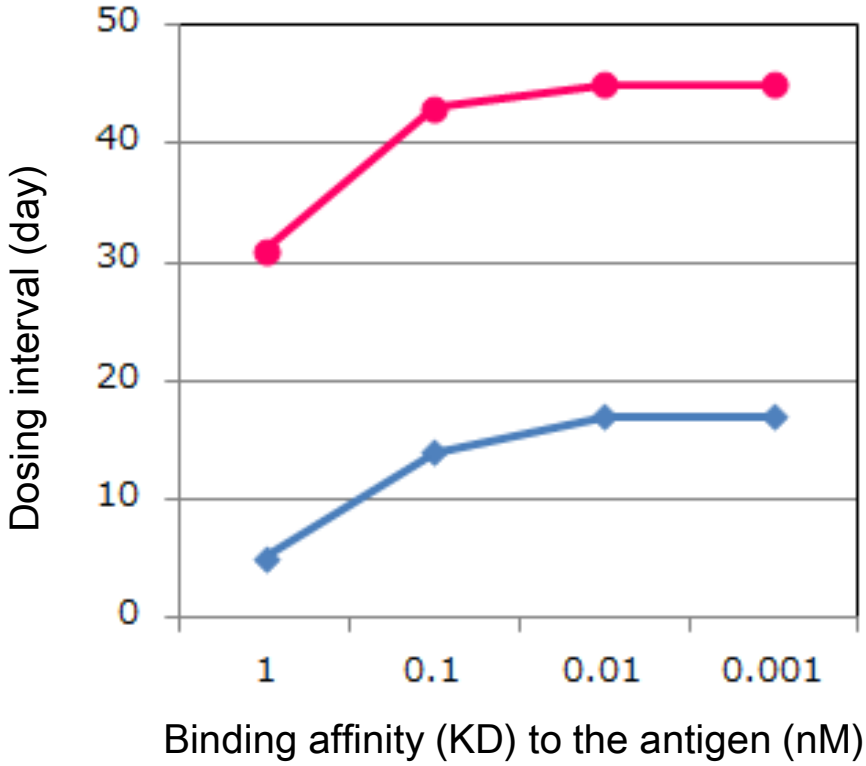


In house data

Sweeping antibody enables long dosing interval which can never be achieved by conventional antibody

Comparison of dosing interval between conventional and sweeping antibody

Simulation of the duration (dosing interval) for inhibiting the function of antigen Y by 90% at a dose of 2 mg/kg

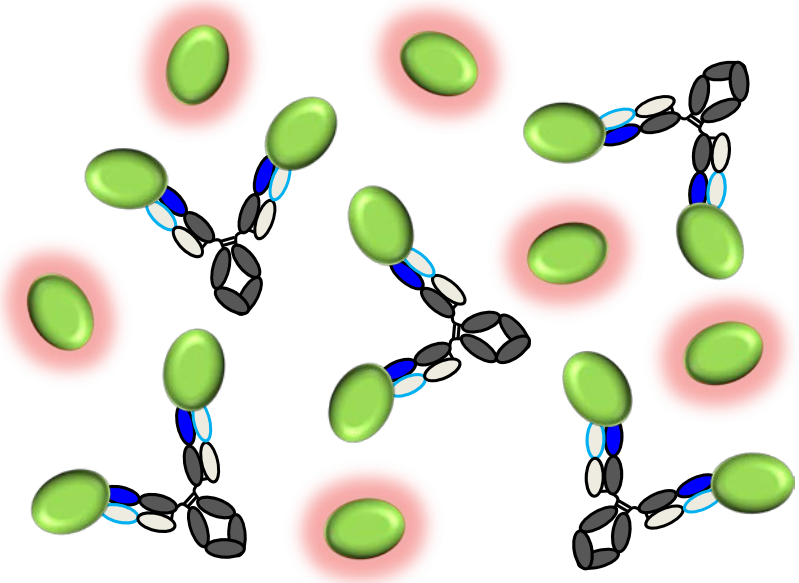


Sweeping antibody can overcome the theoretical limitation of the dosing interval of conventional antibody

In house data

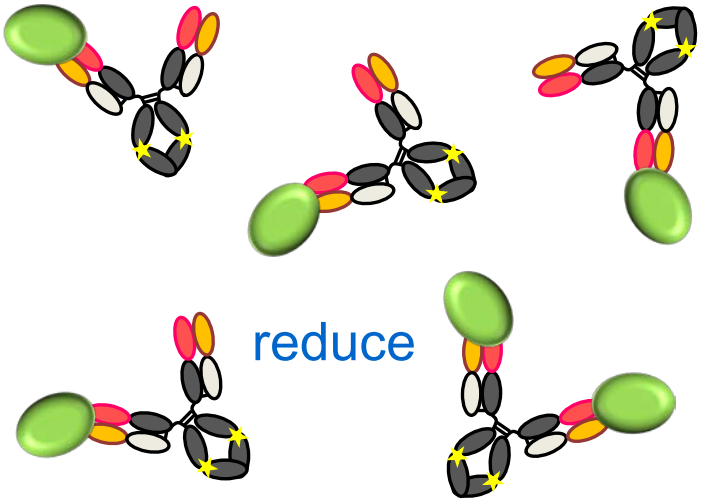
Sweeping antibody can block antigen present in large amount in the plasma

Conventional antibody



Cannot block the antigen present in large amount in plasma at a realistic dosage

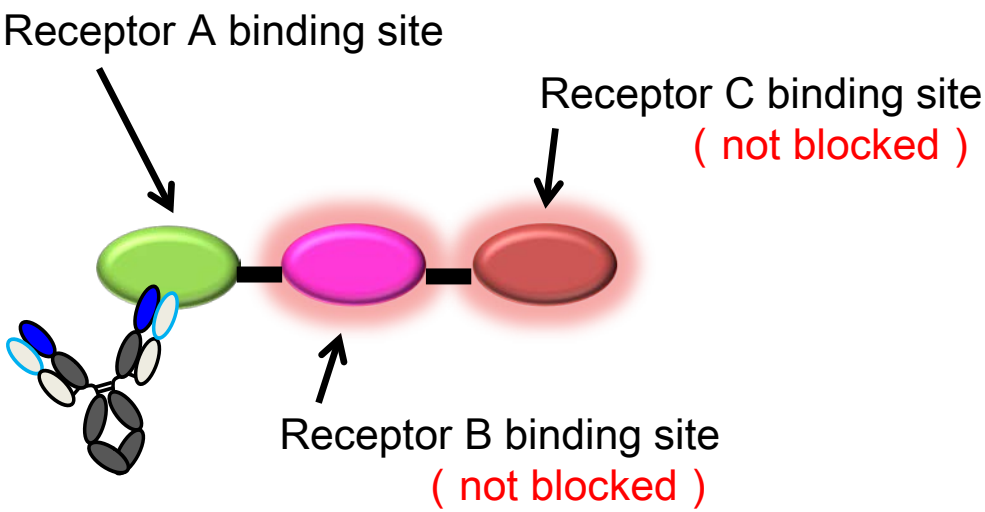
Sweeping antibody



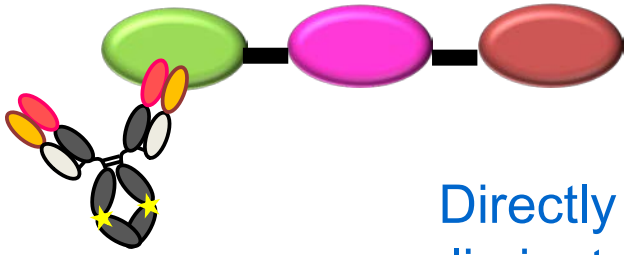
Can block the antigen by reducing antigen concentration at a realistic dosage

Sweeping antibody can block antigen having multiple functional domain

Conventional antibody



Sweeping antibody



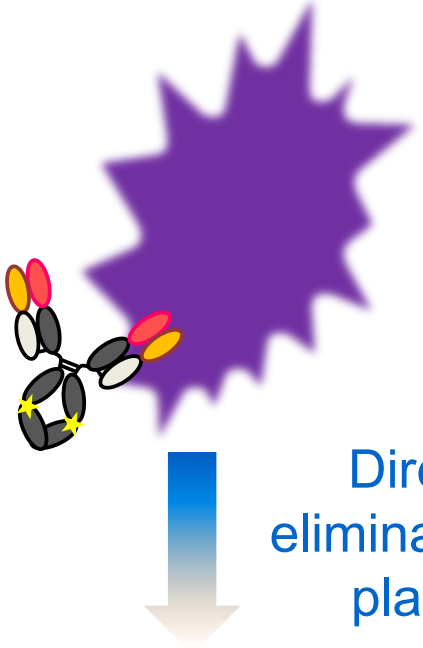
Directly eliminate from plasma

Sweeping antibody can be effective against toxic antigen by directly eliminating from plasma

Conventional antibody

No functional epitope to neutralize its toxicity

Sweeping antibody



Directly eliminate from plasma

Market opportunity of SMART-Ig in the field of therapeutic antibody

- **Second generation of the marketed products**

  IL-6R (tocilizumab), TNF (adalimumab), IgE (omalizumab),
  VEGF (bevacizumab), EGFR (cetuximab), α4β1 integrin (natalizumab),
  RSV (pavilizumab), C5 (eculizumab), IL-1 (anakinra),
  IL-12/23 (ustekinumab), Blys (belimumab), RANKL (denosumab), etc

- **Best-in-class against well validated antigens**

  PSCK9 (hypercholesterolemia), IL-13 (asthma), sclerostin (osteoporosis),
  INFα (SLE), GM-CSF (autoimmune disease), IL-17 (psoriasis),
  DKK1 (osteoporosis), α4β7 integerin (Crohn disease, ulcerative colitis),
  IL-20 (psoriasis), IL-5 (asthma), etc

- **First-in-class against difficult to target antigens by conventional mabs**

  tau protein (Alzheimer disease), oxLDL (atherosclerosis),
  GM-CSFR (autoimmune disease), MCP-1 (cancer etc),
  hepcidin (anemia), CD4 (autoimmune disease), CD23 (asthma), etc

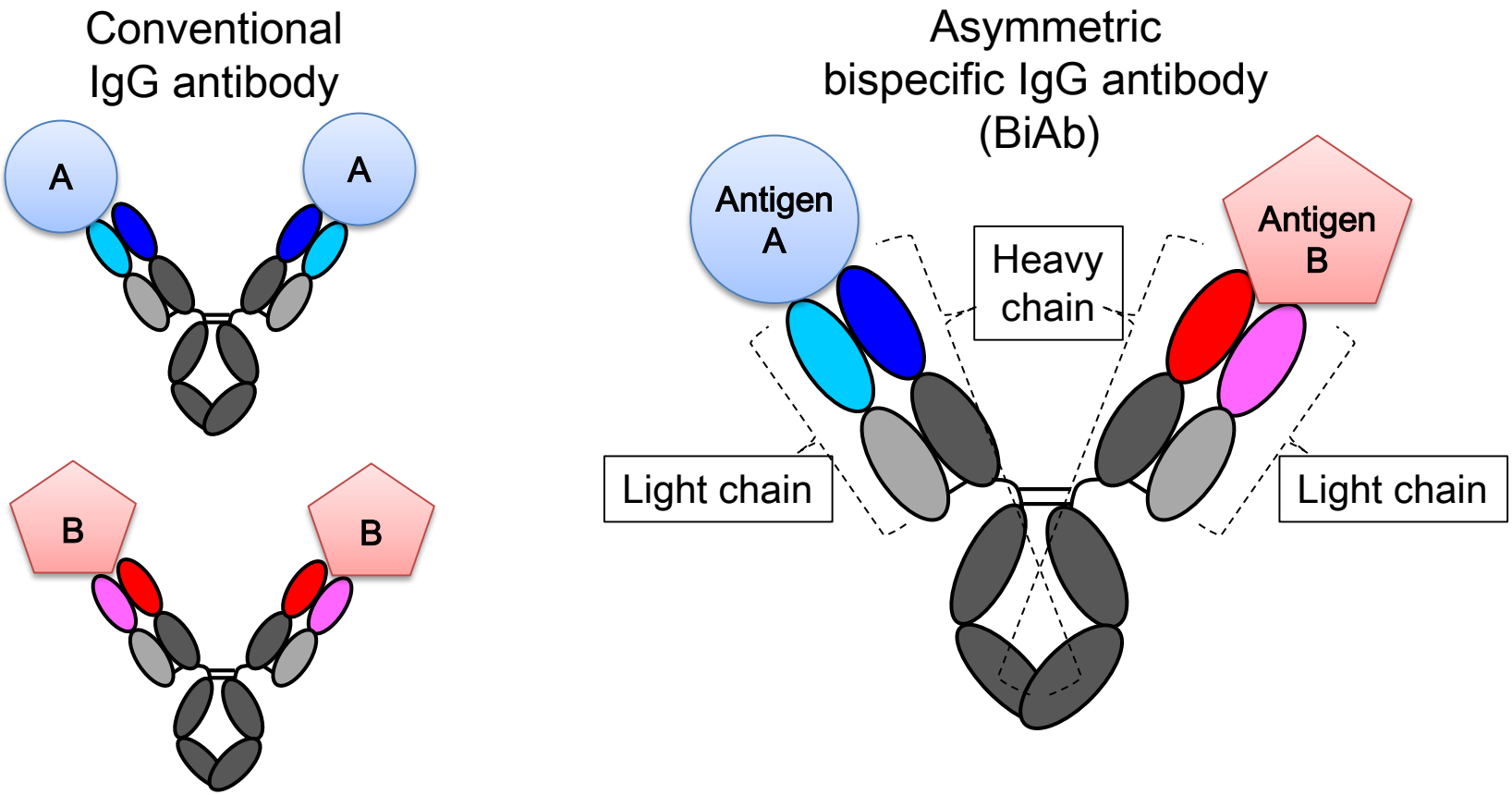
Introduction of ART-Ig and application to hemophilia A treatment

Chugai Pharmaceutical Co., Ltd
Research Division,
Department Manager, Discovery Research Dept.
Kunihiro Hattori

2012. 12.18

What is bispecific antibody (BiAb) ?

- Bispecific antibody (BiAb), consist of 2 different heavy chains and 2 different light chains, has two different antigen binding sites which can respectively bind two different antigens.
- BiAb could provide new mode of action to therapeutic antibodies.

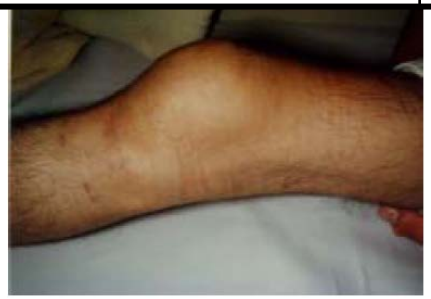


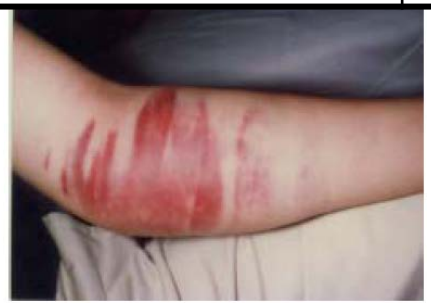
What is hemophilia A?

- Definition
  - Hemophilia A is an inherited deficiency in clotting factor VIII (FVIII) , which causes increased bleeding (bleeding disorder)
- Causes
  - X-linked recessive trait (about 105 in one million males)
- Symptoms
  - Difficult hemostasis of hemorrhages caused by bruise or burden on joint results in large hematoma; difficult hemostasis in case of trauma, surgery and tooth extraction
  - Arthritis as complication reduces QOL of hemophilia patients

	Severe	Moderate	Mild%
% of normal FVIII	< 1 %	1～5 %	5～50 %
Rate of patients	60%	15%	25%
Bleeding frequency	30 times / year	Several times / year	One or twice / year

Joint bleeds (left knee)

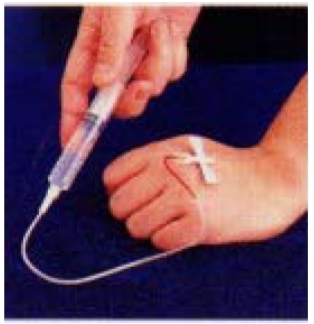




Muscle hemorrhages

Treatment and problems of hemophilia A

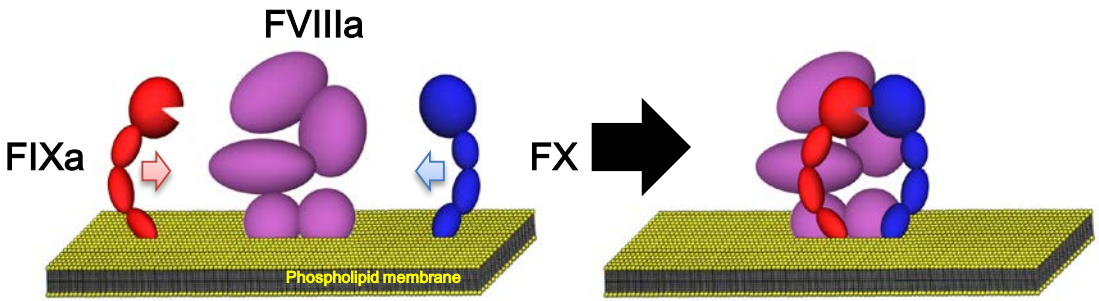
- Treatments
  - Replacement therapy with concentrates of FVIII
    - ✓ Hemostatis for on-going bleeding (on-demand treatment)
    - ✓ Routine supplementation (prevent bleeding and effectively leads to better joint status)
- Issues of current therapy
  - Developing inhibitory antibodies against FVIII (called inhibitors)
    - ✓ Inhibitors make it difficult to control hemorrhaging
    - ✓ Alternative treatment with bypass agents and immune tolerance therapy is not always effective
  - Frequent iv injections in routine supplementation (3 times a week)
    - ✓ Although home treatment for prophylaxis has become common, technical difficulty of venous access especially for infants, forces both physically and psychologically burden

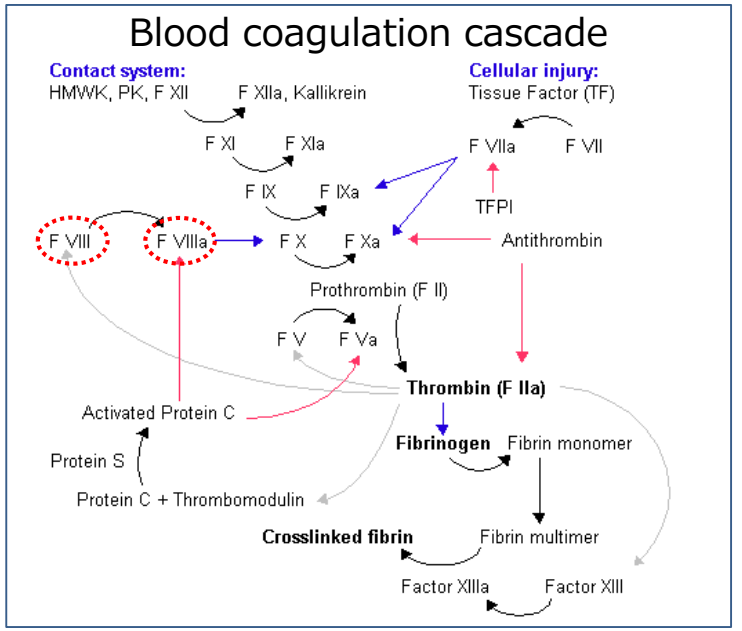


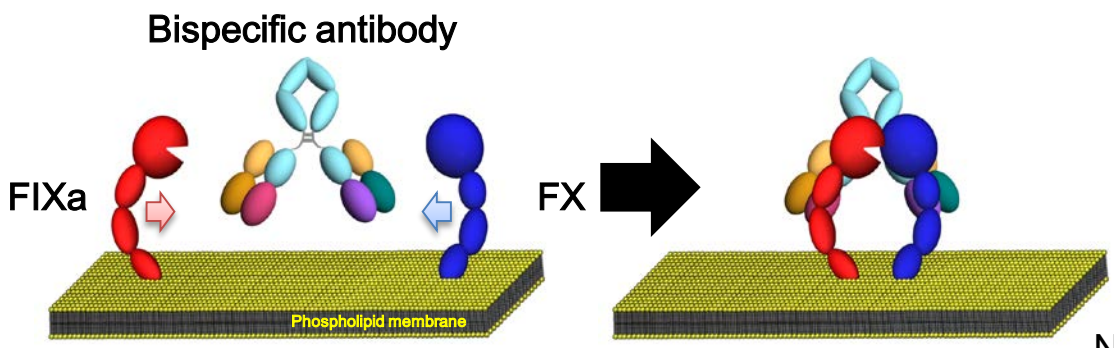
- New drugs having long-acting hemostatic effect even in the presence of inhibitors with subcutaneous delivery

A bispecific antibody (BiAb) mimicking FVIII function

Provide innovative drug which improves QOL of hemophilia patients by bispecific antibody simultaneously recognizing activated FIX and FX and promote FIXa-catalyzed FX activation like FVIII





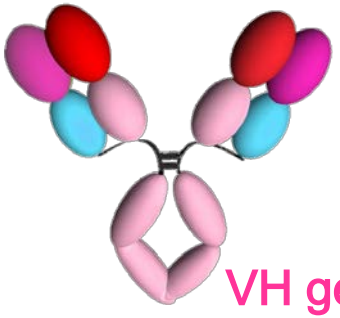


Characteristics of BiAb

- ✓ Convenient injection (SC)
- ✓ Long acting (durable action)
- ✓ No induction of inhibitors
- ✓ Not affected by inhibitors

Nature Medicine **18**, 1570-1574 (2012)

Generation of lead BiAb with FVIIIa activity



Anti-F.IXa

~200 clones

VH genes

VL genes

Expression vectors/ human Ig $\gamma_{2/4}$

Expression vectors/ human Ig κ/λ

Anti-F.X

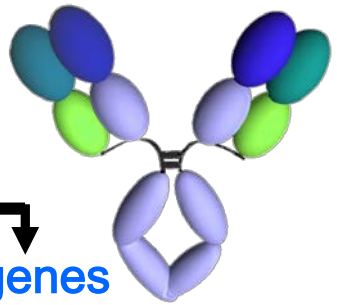
~200 clones

VH genes

VL genes

Expression vectors/ human Ig $\gamma_{2/4}$

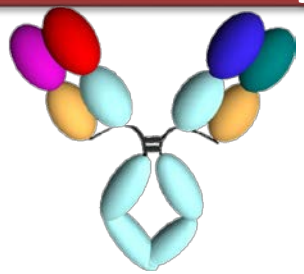
Expression vectors/ human Ig κ/λ



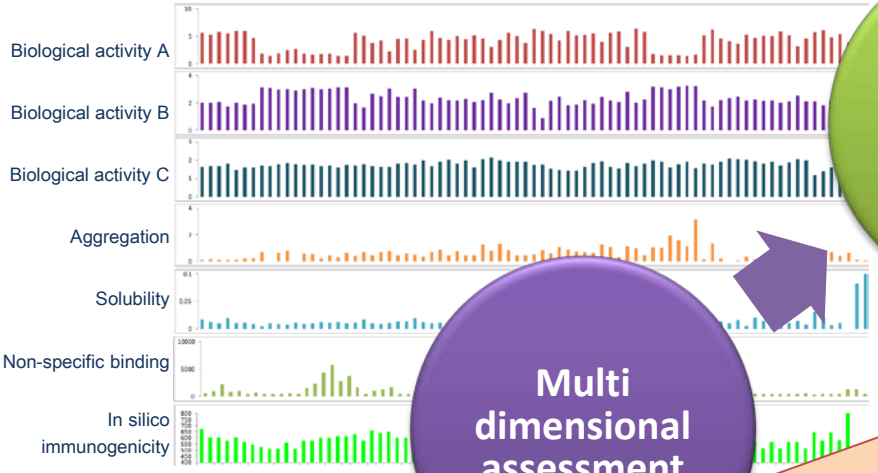
Bispecific IgG (~40,000 combinations)
Transient expression in HEK293 cells

Chromogenic assay with activated FIX and FX

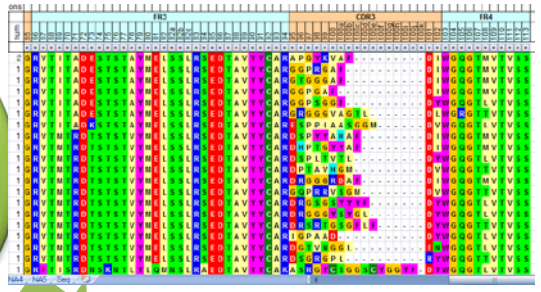
Lead antibody



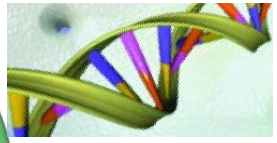
Multidimensional optimization of BiAb



Design of amino acid sequence of BiAb

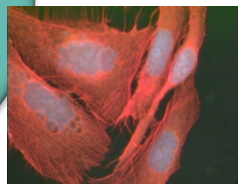


Construction of expression vector

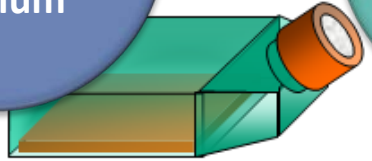


Generation of ACE910 by multidimensional optimization

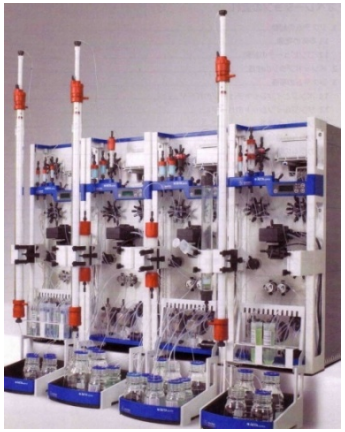
Transfection into cells and culture



Purification of BiAb from culture medium

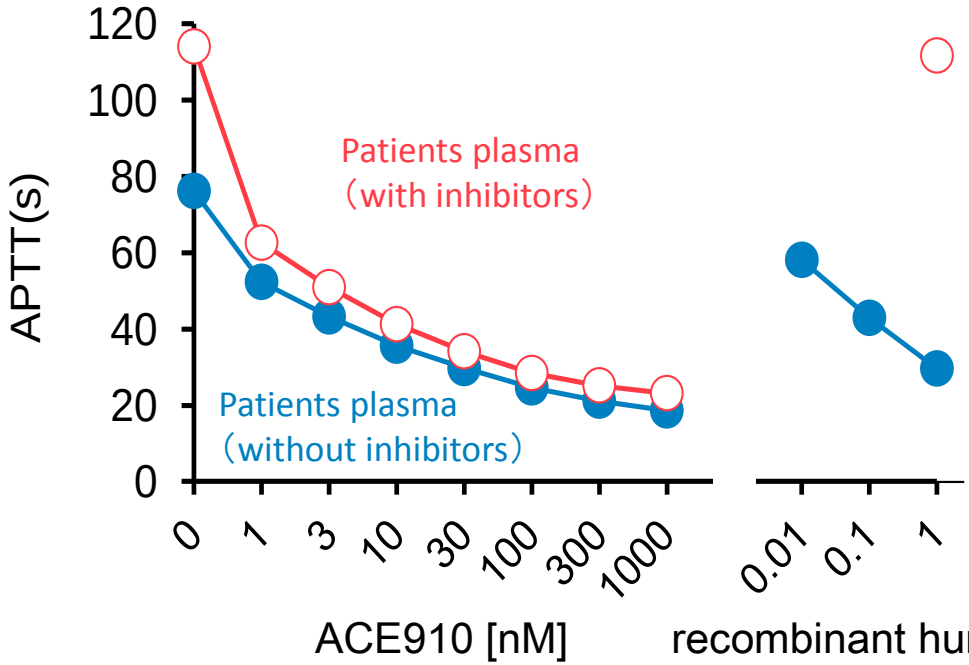


Multi dimensional assessment



In vitro FVIII-mimetic cofactor activity of BiAb

- ACE910 is improved version of hBS23 (Nature Medicine **18**, 2012) with regard to these properties
  - ✓ FVIII-mimetic cofactor activity ✓pharmacokinetics ✓ Immunogenicity
  - ✓ Physicochemical properties (solubility, viscosity)
  - ✓ Manufacturability (productivity, purification)
- Effects of BiAb on APTT of hemophilia patient plasma
  - ACE910 dose-dependently shortened the APTT similar to FVIII
  - Effective even in the presence of inhibitors (anti-FVIII antibodies)

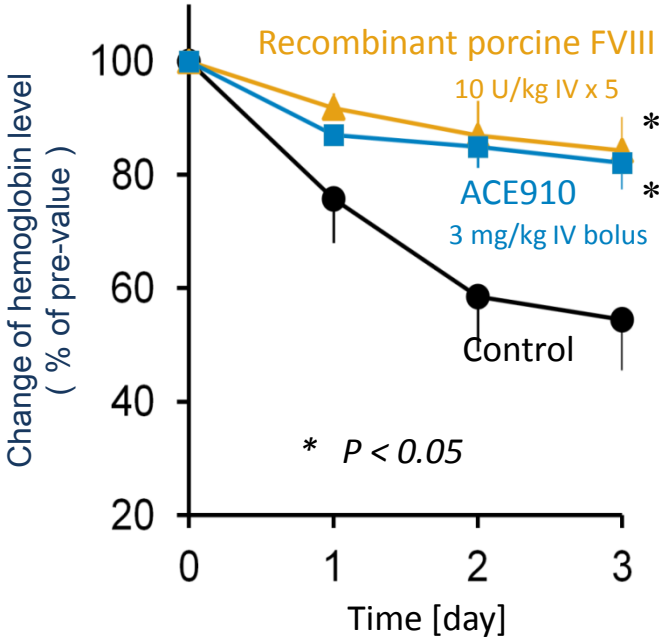


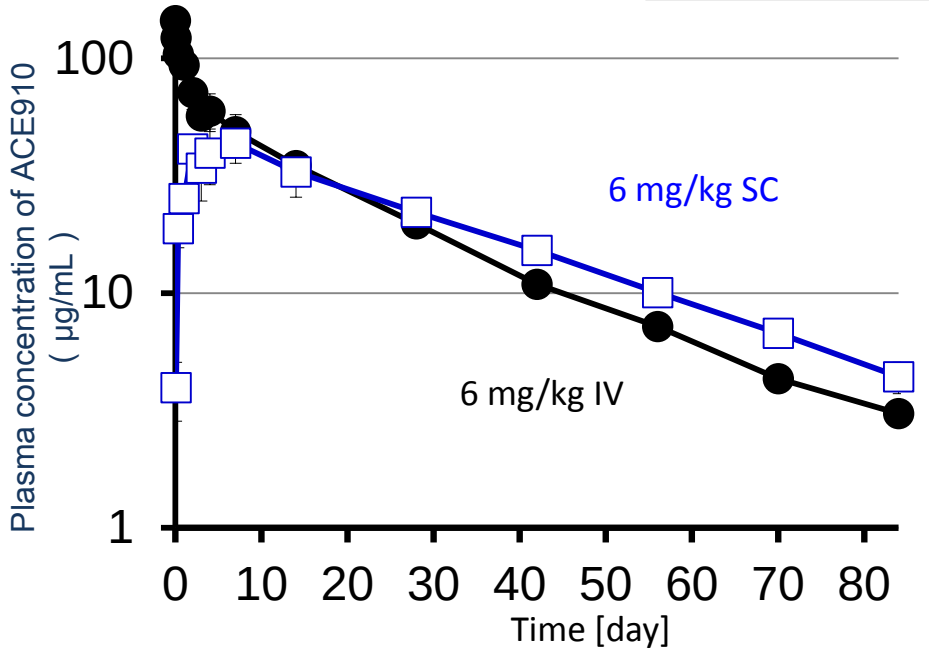
In house data

In vivo activity and plasma concentration of BiAb

- Hemostatic effect in cynomolgus monkey hemophilia A model (Left)
  - ACE910 exhibited an in vivo hemostatic action similar to FVIII
- Pharmacokinetic of ACE910 after iv and sc injection in monkey (Right)
  - Bioavailability after subcutaneous injection was nearly 100%
  - Plasma half life of ACE910 was about 3 weeks

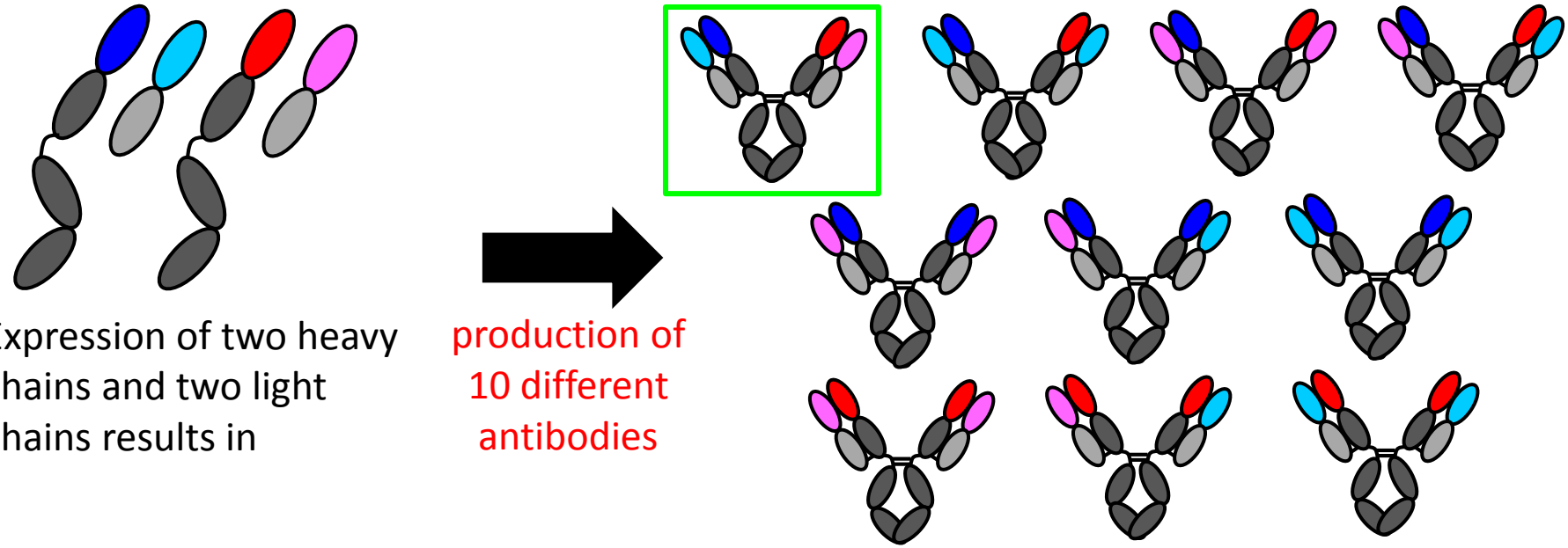
In house data





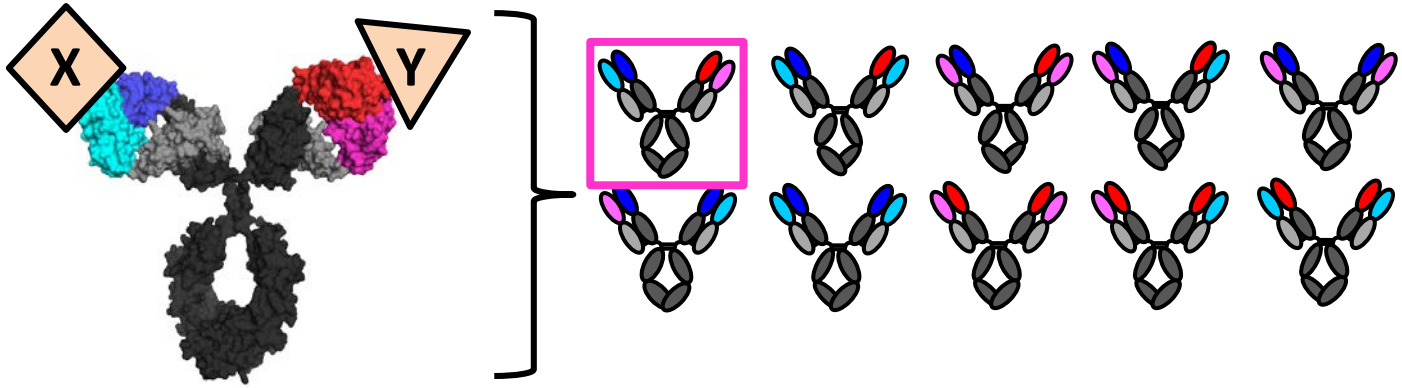
ACE910 is expected to be an innovative treatment for hemophilia A having superior in vivo duration and effectiveness even for inhibitor patients.

Issue of BiAb is manufacturability



- If two heavy chains and two light chains are expressed, 10 heavy and light combinations would occur.
- Incorrect 9 combinations are not merely impurities, but also inhibit the action of BiAb.
- Because these 10 different antibodies have similar physicochemical properties, purification of the target BiAb from other species is nearly impossible.

ART-Ig: facilitating manufacturing of BiAb by protein engineering



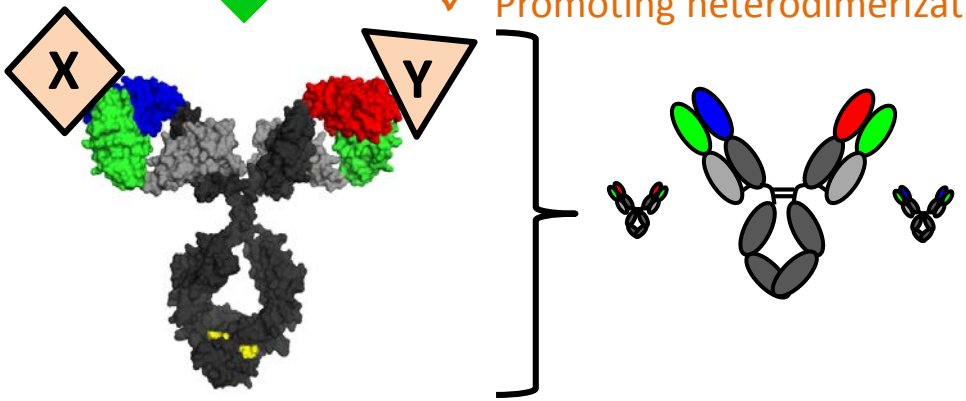
Manufacturing not feasible

ART-Ig

①**Identification of** common light chain
- ✓ Commonizing light chain by CDR shuffling

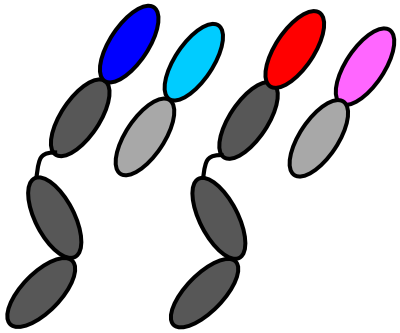
②Facilitate purification of BiAb
- ✓ Introducing difference in the charges of two heavy chains

③Facilitate expression of BiAb
- ✓ Promoting heterodimerization by electrostatic steering



Offers highly efficient manufacturing of BiAb by conventional IgG production platform

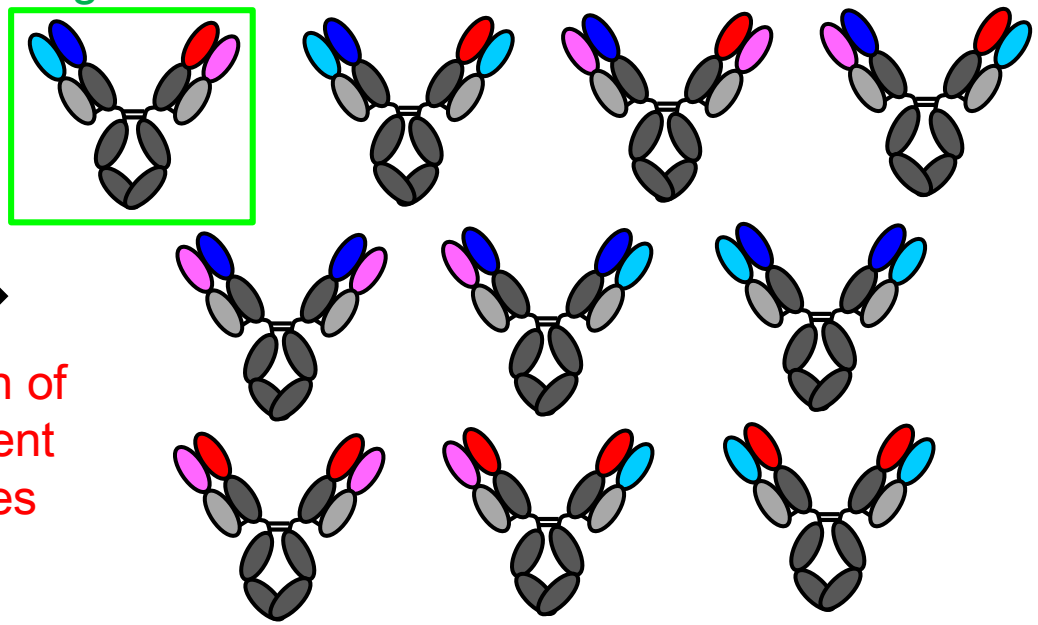
ART-Ig ①:
Common light chain for two heavy chain



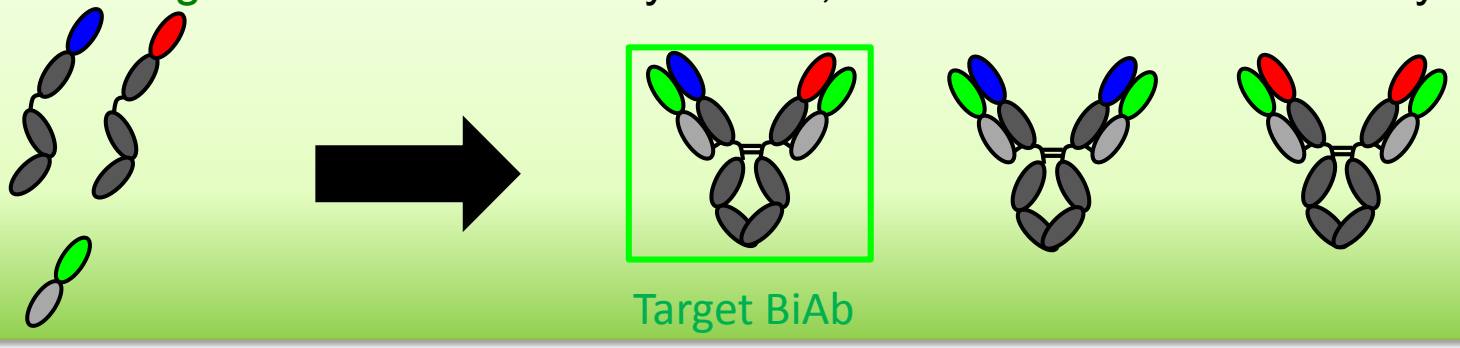
Expression of two heavy chains and two light chains results in

production of ten different antibodies

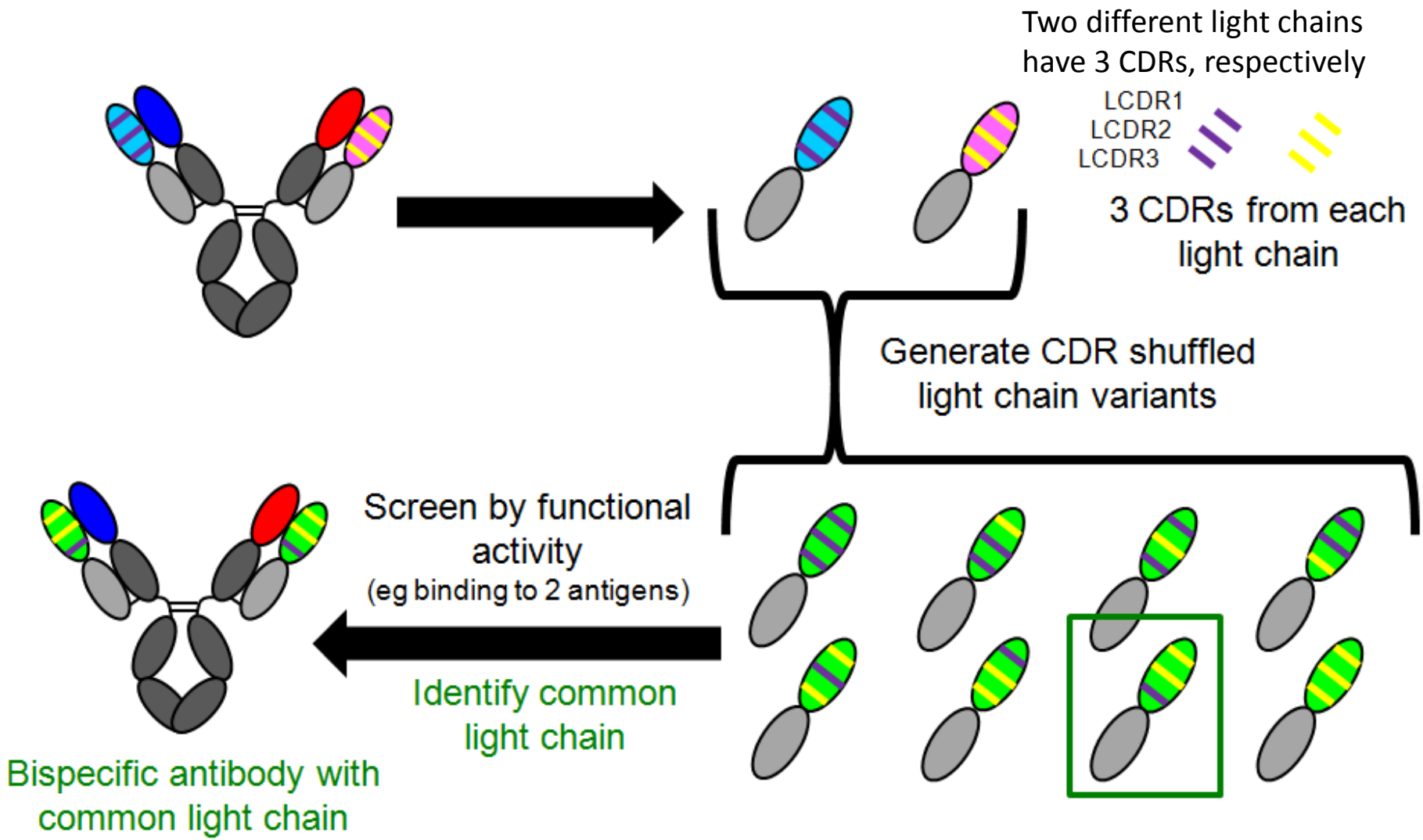
Target BiAb



Using common light chain for two heavy chains, combinations becomes only three.

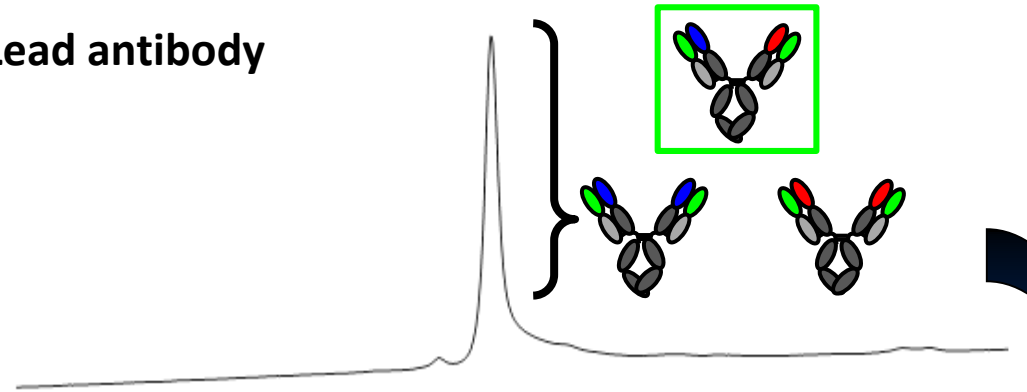


ART-Ig ①: Identification of common light chain

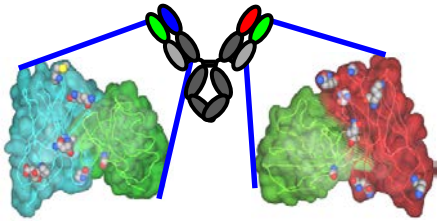


ART-Ig ②:facilitating purification of BiAb

Lead antibody



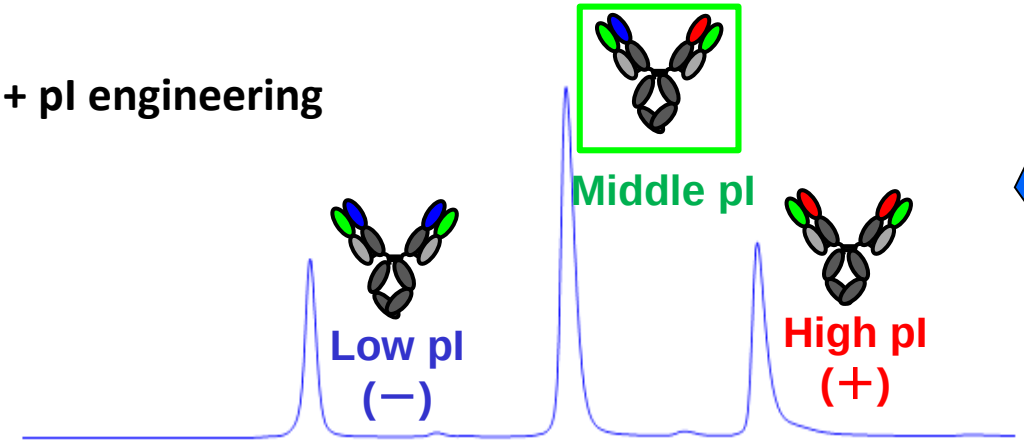
No separation by ion exchange chromatography (IEC)



Low pI (−) **High pI (+)**

Engineered each heavy chain variable region to have different charges

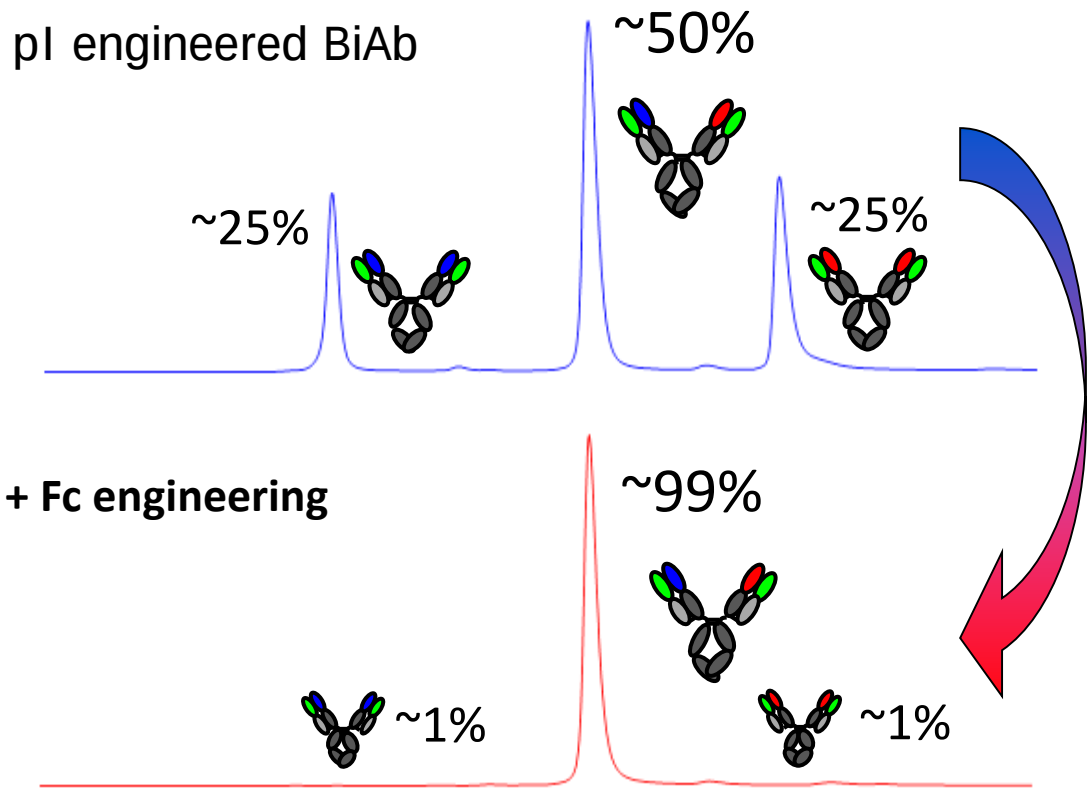
+ pI engineering



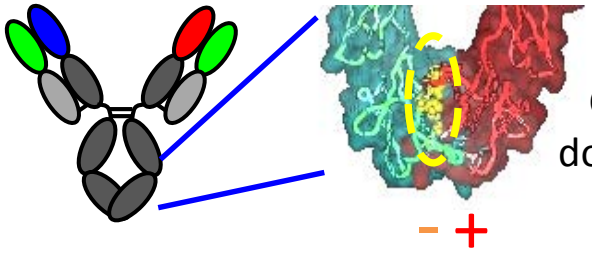
Complete separation of bispecific antibody

In house data

ART-Ig ③:facilitating heterodimerization of two heavy chains



Theoretical efficiency of BiAb expression if 50% of the whole antibody



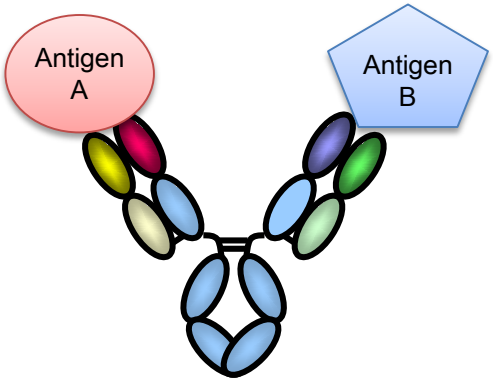
Engineered heavy chain interface (CH3) to promote heterodimerization promoted highly selective bispecific expression

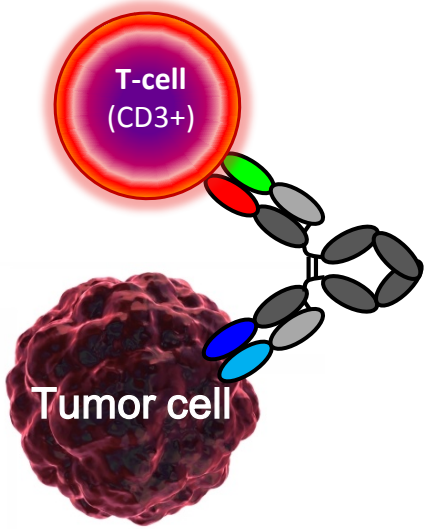
In house data

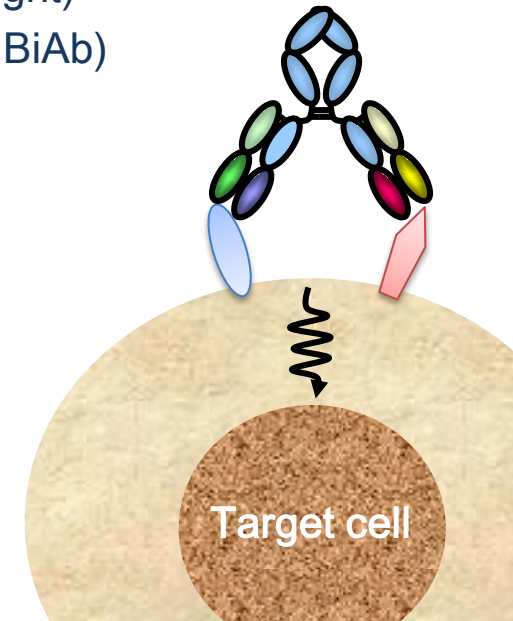
ART-Ig (①+②+③) enabled achievement of 2500 liter scale manufacturing of bispecific antibody with high productivity and purity similar to conventional antibody.

Application of ART-Ig for therapeutics

- Bispecific antibody contributes to providing new mode of action and expansion of the target antigen space
  - Enhancing the efficacy by binding two soluble factors
    - ✓ Blockade of two different disease mediating antigens (left)
      - Combination of two tumor growth factors or immune related factors etc
    - ✓ Blockade of two different epitopes on same antigen
  - Providing new pharmacology by bridging two antigens
    - ✓ Bridging two different cells for new mode of action (center)
    - ✓ Bridging two antigens on same cell for new mode of action (right)
    - ✓ Bridging two different protein for new action (FVIII-mimicking BiAb)







Technology Introduction of ART-Fc, TRAB, TwoB-Ig, ACT-Ig and its Application

Chugai Pharmaceutical Co., Ltd
Research Division, Discovery Research Dept.
Group Manager
Hiroyuki Tsunoda

2012. 12.18

Antibody technology for application to Oncology area

ART-Fc

(Asymmetric Re-engineering Technology-Fc region)

TRAB

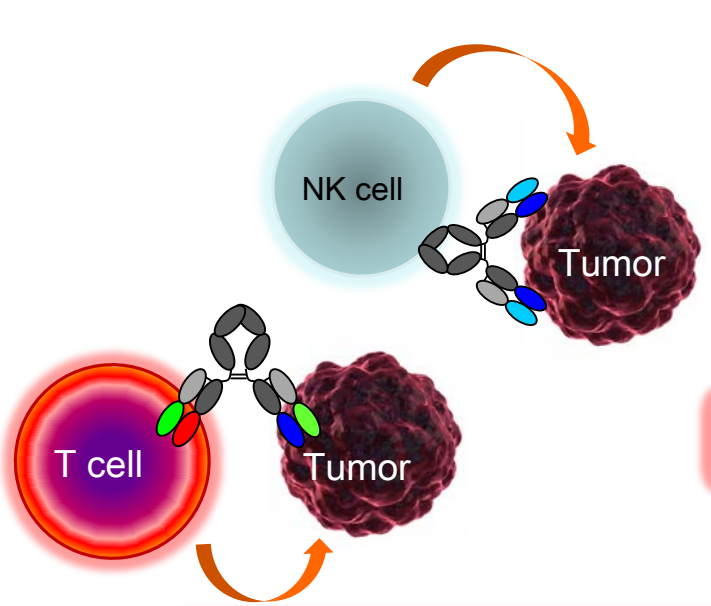
(T cell Redirecting AntiBody)

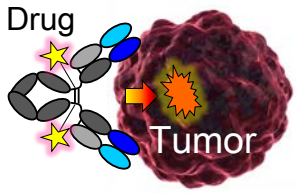
Different antibody technology for different numbers of tumor-specific antigen expression is required

Number of tumor-specific antigen expressed on single tumor cell

10^3 10^4 10^5 10^6

Low High





Conventional antibody

Antibody-drug conjugate

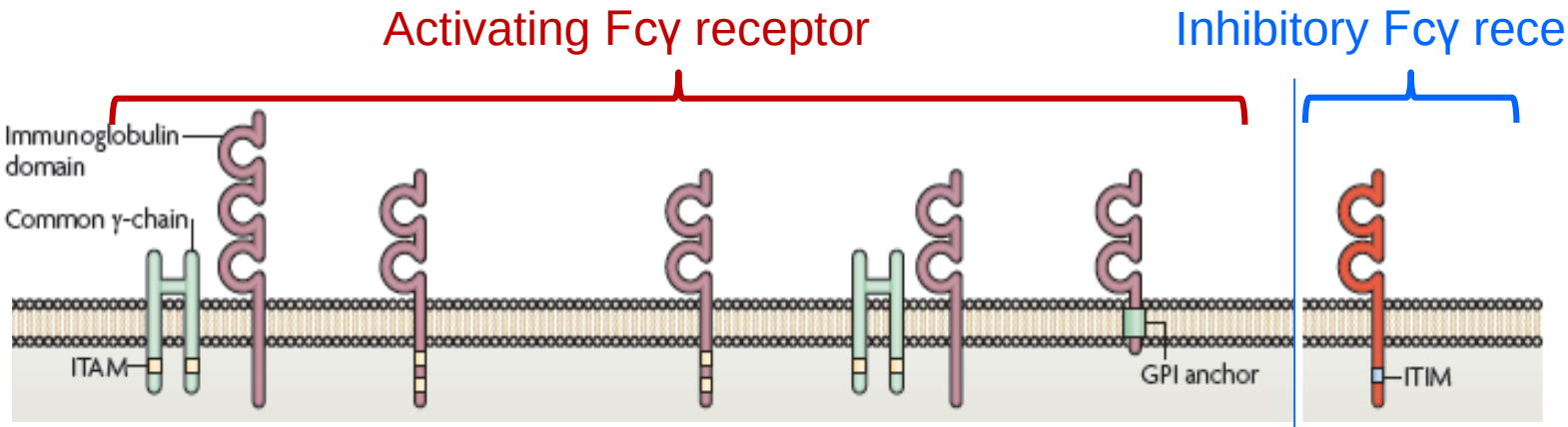
ADCC enhancing technology

ART-Fc

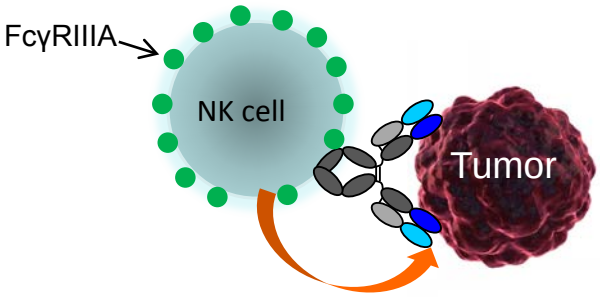
T cell redirecting antibody technology

TRAB

Structure and expression profile of human Fcγ receptors



	FcγR IA (CD64)	FcγR IIA (CD32A)	FcγR IIC (CD32C)	FcγR IIIA (CD16A)	FcγR IIIB (CD16B)	FcγR IIB (CD32B)
Expressing cells	Macrophage, Monocyte Eosinophile, Neutrophil Dendritic cell	Macrophage, Platelet, Neutrophil Monocyte, Dendritic cell	NK cell	**NK cell** Macrophage, Monocyte, Dendritic cell	Eosinophile, Neutrophil Mast cell	B cell, Plasma cell Macrophage, Monocyte Eosinophile, Neutrophil Dendritic cell , Mast cell



Cytotoxicity against tumor cell

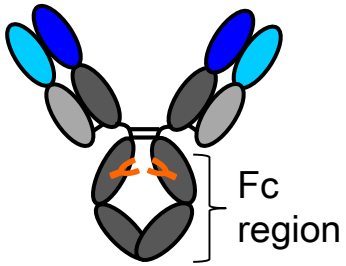
Activation of NK cell by binding to antibody

Cytotoxicity against target cell (Antibody-Dependent Cellular Cytotoxicity : ADCC)

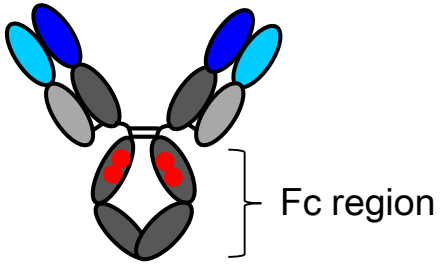
Nat. Rev. Immunol. (2010)

Previous ADCC enhancing technology by increasing binding affinity to FcγRIIIA

- Engineering of glycosylation in the Fc region (removing fucose)
  - Roche / Glycart：Glycomab™
  - Kyowa Hakko Kirin/ BioWa：Potelligent™



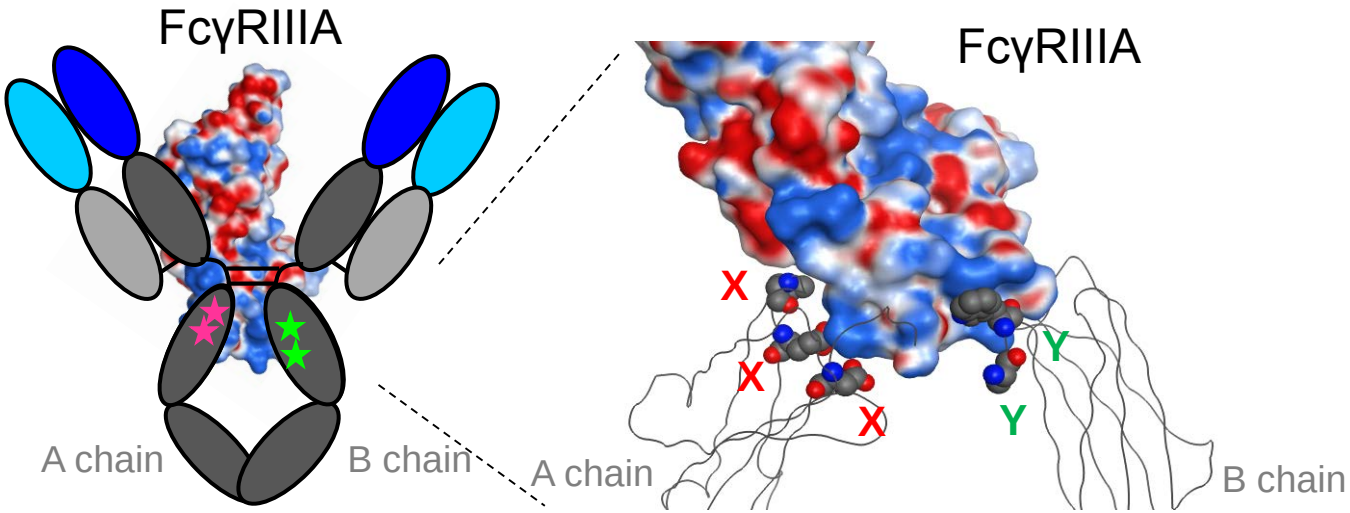
- Engineering of amino acid in the Fc region
  - Xencor : XmAb™
  - MacroGenics



Development of more potent ADCC enhancing technology by applying **ART-Ig**

Chugai's ADCC enhancing technology by ART-Ig

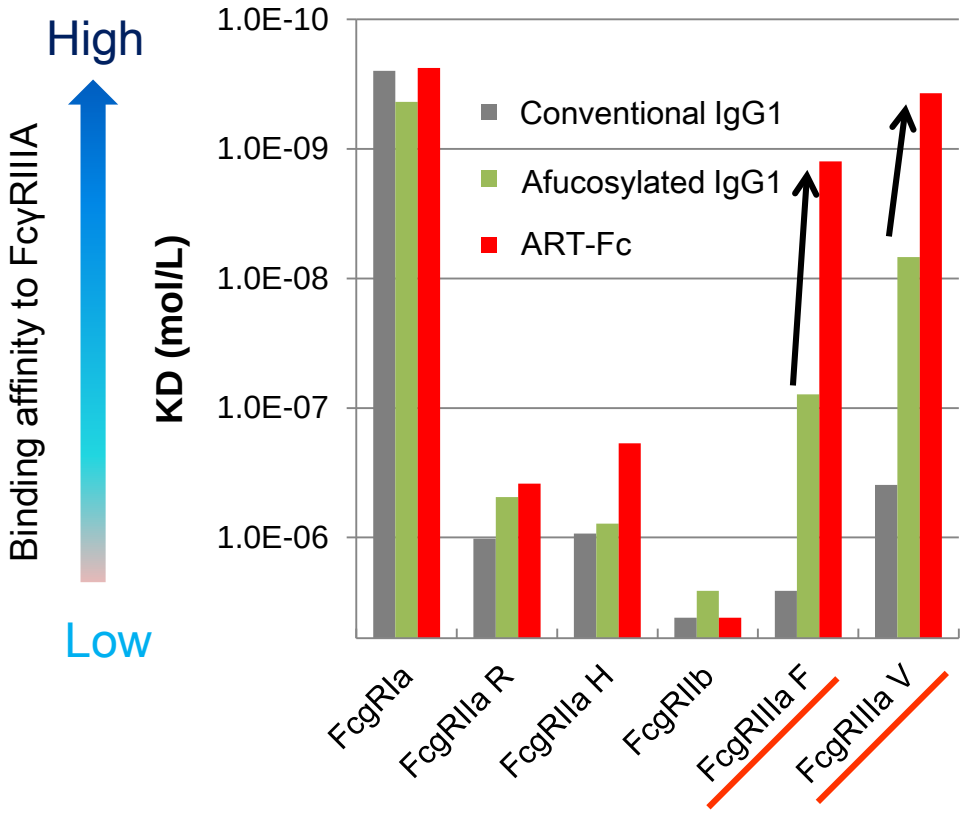
- Focusing on binding pattern of FcγRIIIA to IgG Fc region
  - Symmetric Fc recognizes FcγR asymmetrically or differently from both side
  - ➡ Engineering Fc asymmetrically, instead of symmetrically, is preferable to optimize asymmetric FcγRIIIA binding (Use of ART-Ig)
  - Interface between FcγRIIIA and Fc was comprehensively mutated
  - ➡ Over 1000 mutants were analyzed regarding binding affinity to FcγRs and stability



ART-Fc : FcγRIIIA binding affinity

- **ART-Fc*** achieved stronger binding affinity to both FcγRIIIA F and V type than afucosylated IgG

  * **ART-Fc**: **A**symmetric **R**e-engineering **T**echnology-**Fc**



ART-Fc demonstrated strong binding affinity against even FcγIIIA F type

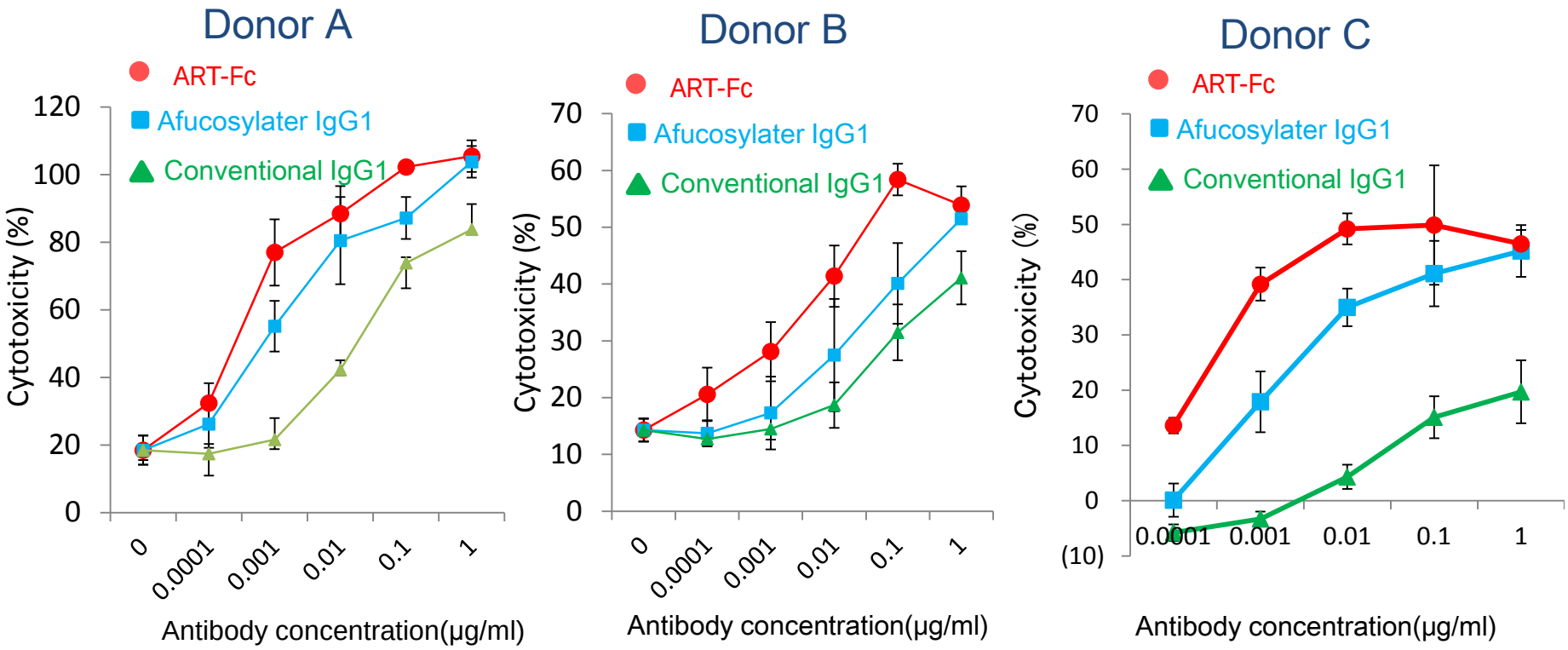
Potential to induce strong anti-tumor effect regardless of allotype of FcγIIIA

In house data

ART-Fc : in vitro ADCC activity

ADCC activity using human PBMC (E/T=50)

In house data



We generated Fc region with highly potent ADCC activity by drastically increasing affinity to FcγRIII compared to previous technology.

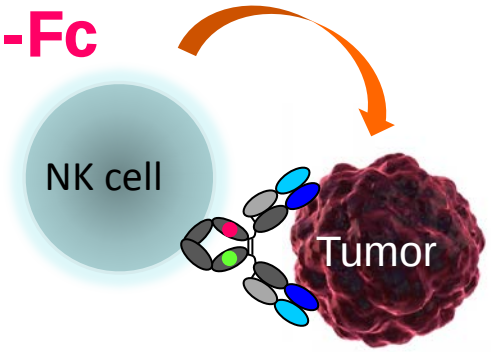
TRAB: T cell redirecting antibody technology using ART-Ig

Number of tumor-specific antigen expressed on single tumor cell

10^3 10^4 10^5 10^6

Low — High

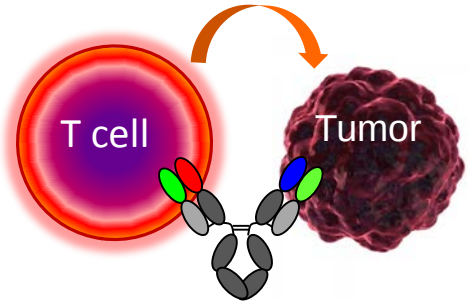
ART-Fc



ADCC enhancing technology

T cell redirecting antibody technology

TRAB



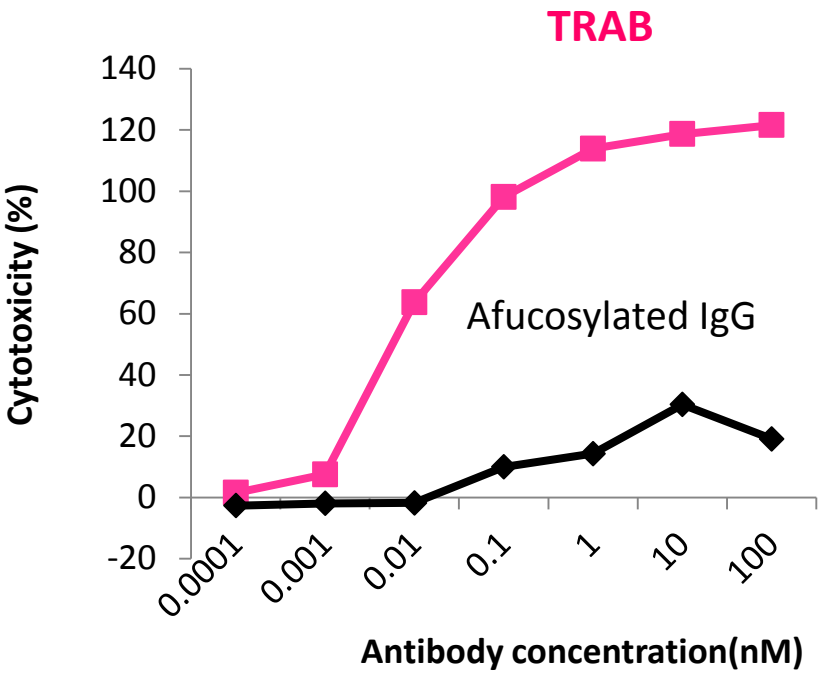
TRAB binds tumor cell specific antigen by one arm, and CD3 on T cell by the other arm, and thereby induce cytotoxicity against tumor cell by bridging these two cells

TRAB : in vitro cytotoxicity activity

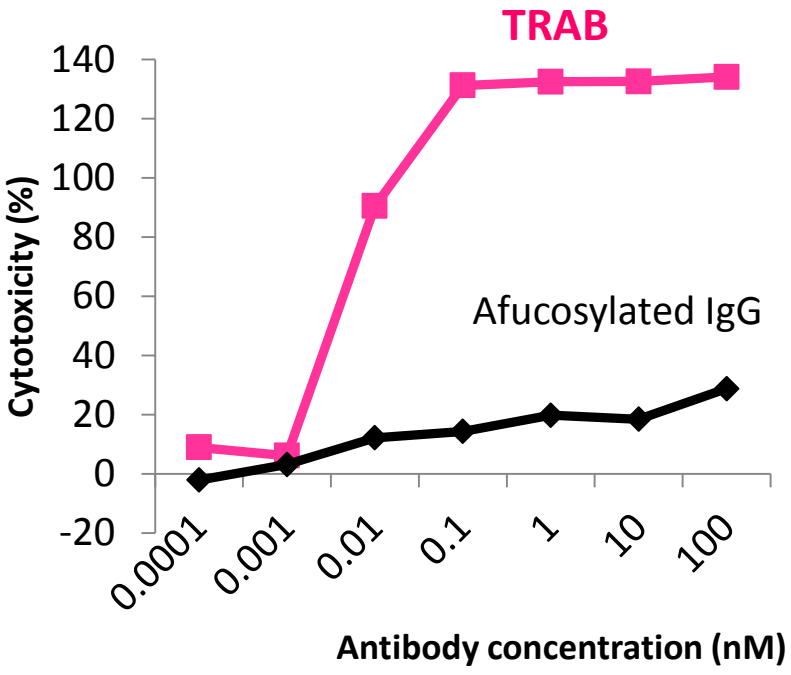
Tumor cytotoxicity activity using human PBMC

In house data

Number of tumor antigen expressed on single tumor cell（10^3 order）



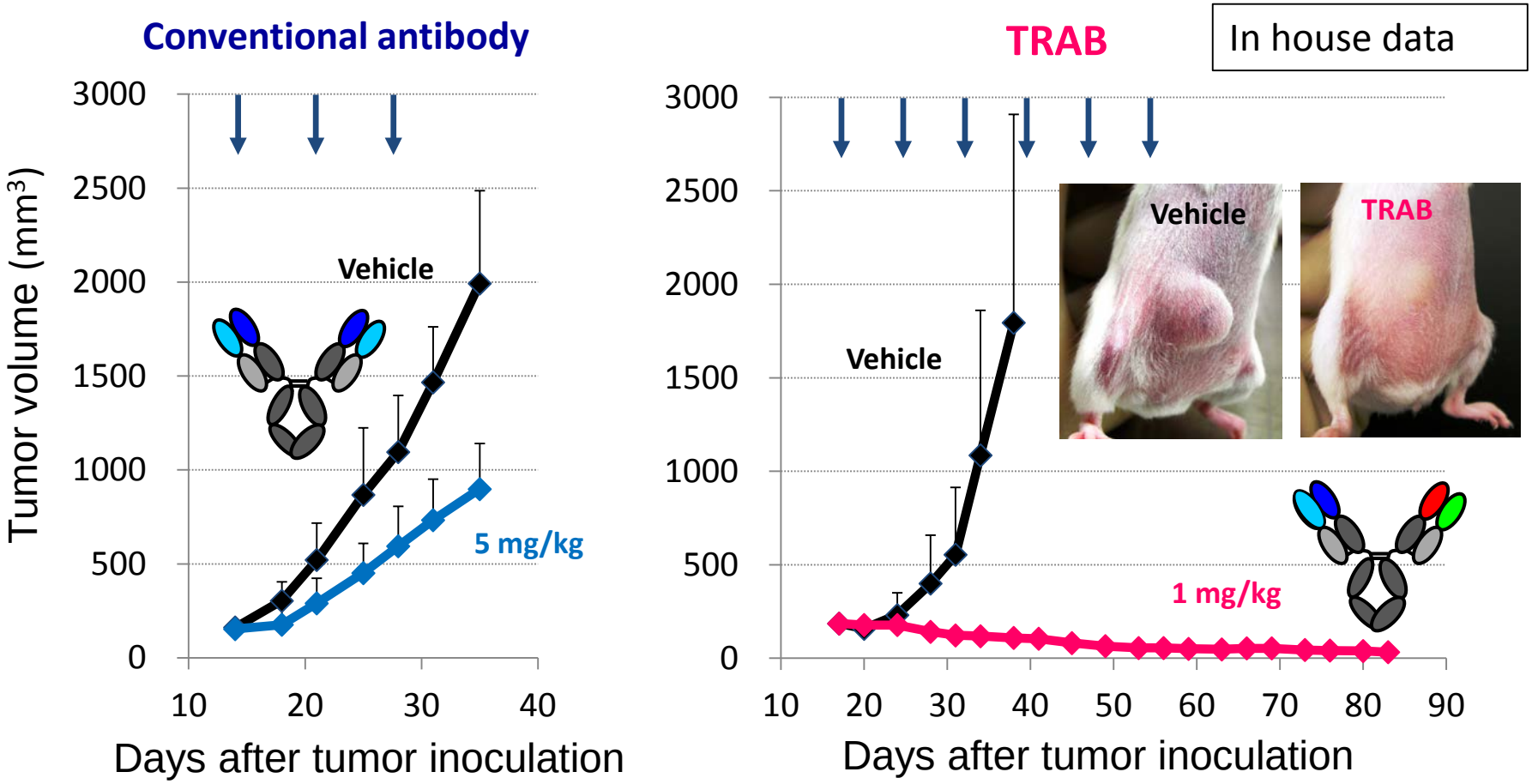
Number of tumor antigen expressed on single tumor cell（10^4 order）



TRAB demonstrated stronger anti-tumor efficacy than afucosylated IgG, and was efficacious even in tumor cell with low antigen expression

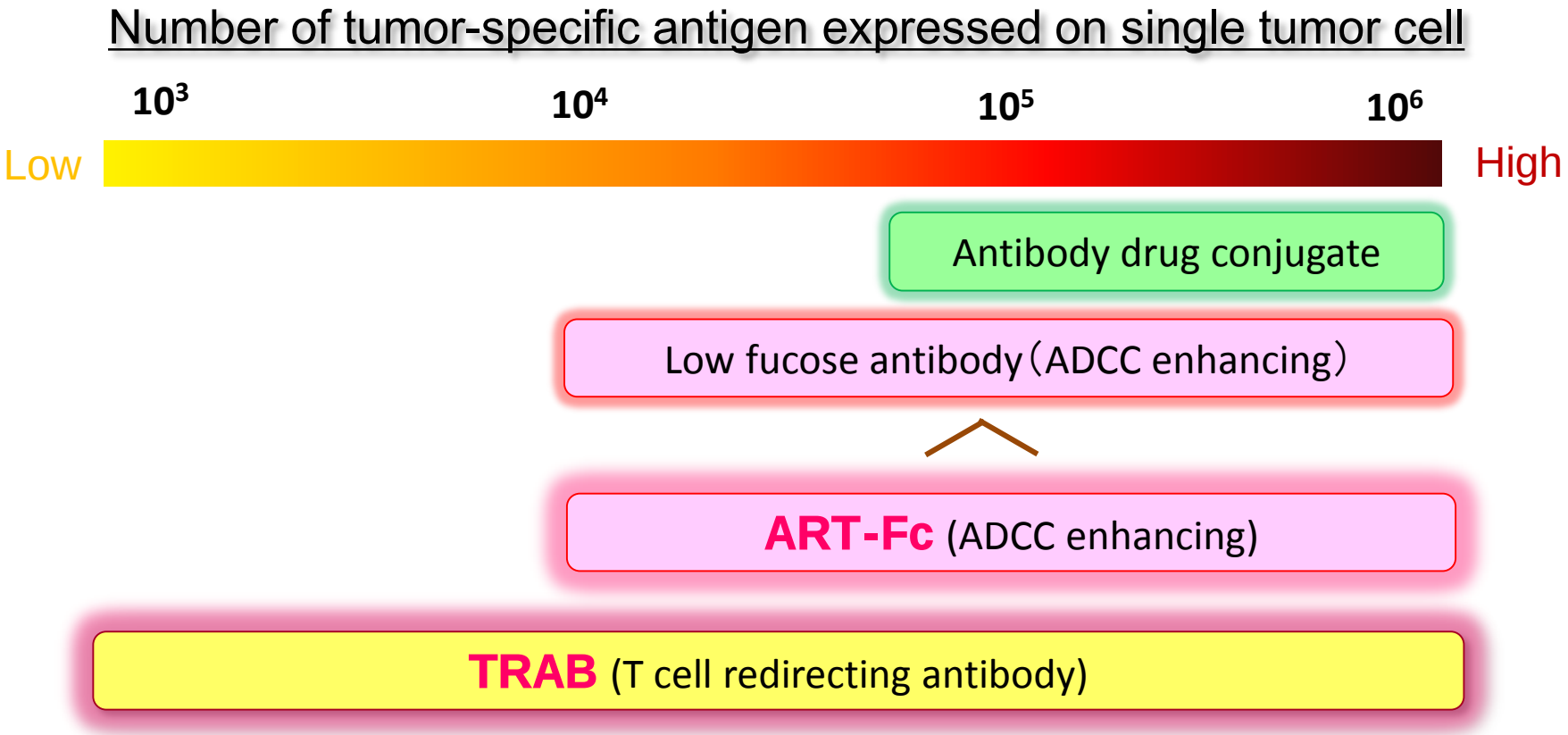
TRAB : in vivo anti-tumor efficacy

In house data



TRAB showed remarkable efficacy even at lower dosage than conventional antibody, completely eliminating the xenografted tumor

Chugai's next generation antibody technology for oncology area



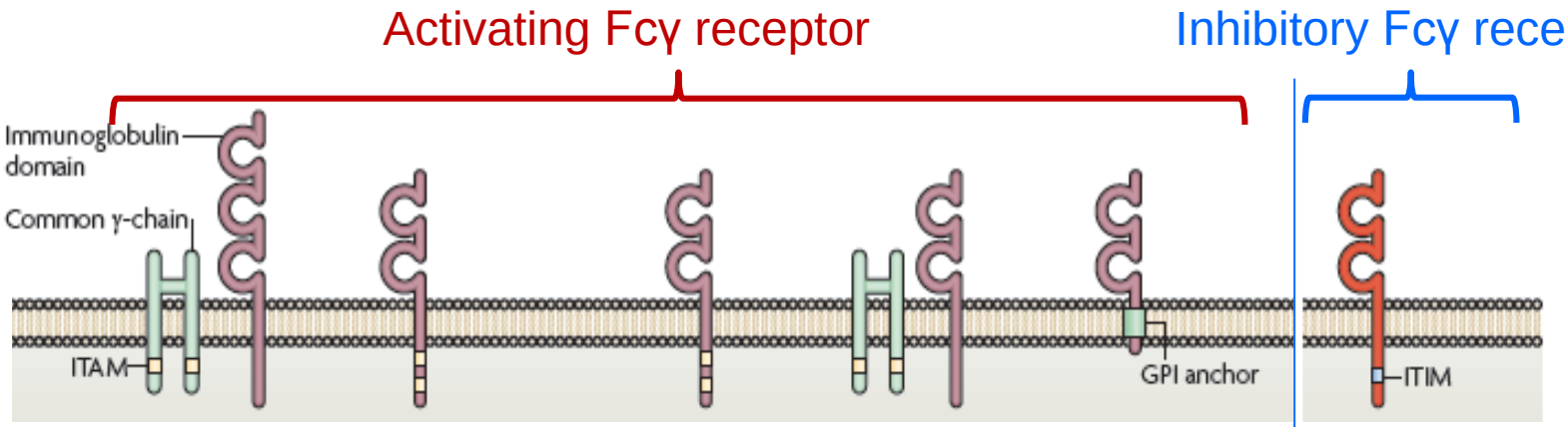
Established novel antibody technologies demonstrating cytotoxicity even against tumor cells with low antigen expression using proprietary **ART-Ig**.
Enables generating therapeutic antibodies with strong anti-tumor efficacy against antigens which were previously unable to target.

Antibody technology for application to Autoimmune diseases

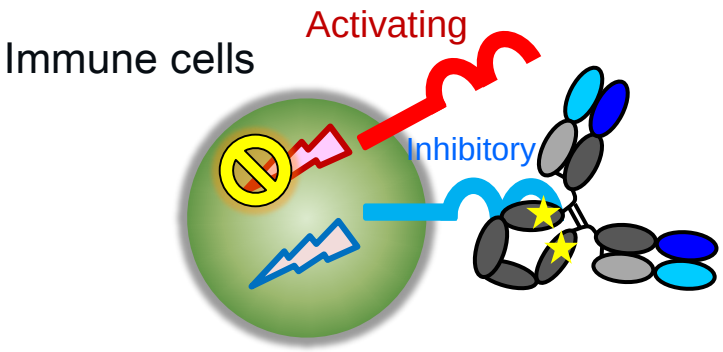
TwoB-Ig

(FcγRIIB selective binding technology-Immunoglobulin)

Structure and expression profile of human Fcγ receptors



	FcγR IA (CD64)	FcγR IIA (CD32A)	FcγR IIC (CD32C)	FcγR IIIA (CD16A)	FcγR IIIB (CD16B)	FcγR IIB (CD32B)
Expressing cells	Macrophage, Monocyte Eosinophile, Neutrophil Dendritic cell	Macrophage, Platelet, Neutrophil Monocyte, Dendritic cell	NK cell	NK cell Macrophage, Monocyte, Dendritic cell	Eosinophile, Neutrophil Mast cell	B cell, Plasma cell Macrophage, Monocyte Eosinophile, Neutrophil Dendritic cell , Mast cell

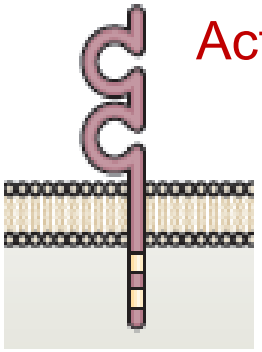
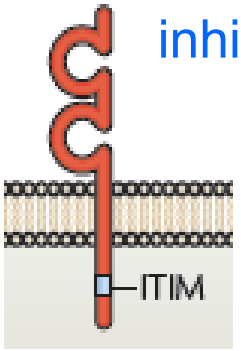


Induction of inhibitory signals into immune cells (FcγRIIB-expressing cells) by antibody binding

Nat. Rev. Immunol. (2010)

Fc engineering to selectively enhance FcγRIIB binding has not been reported

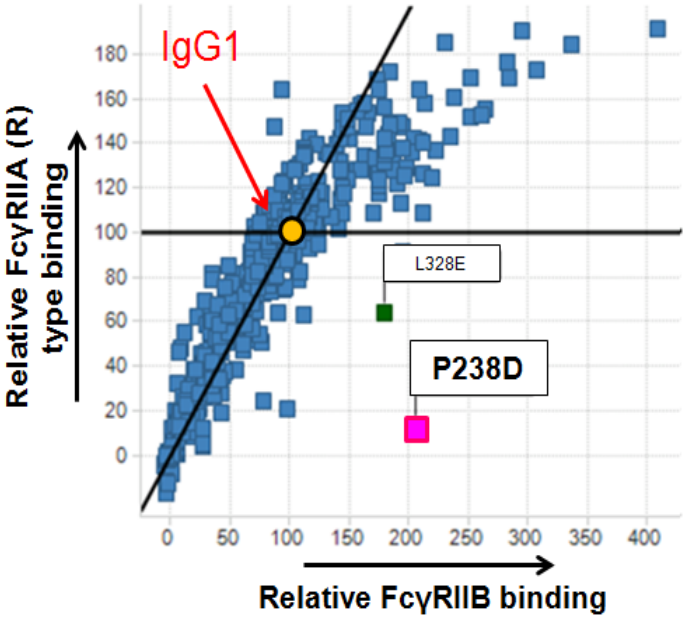
- Due to high homology between inhibitory FcγRIIB and activating FcγRIIA, no successful Fc engineering to *selectively* enhance FcγRIIB binding has been reported.
  - Since FcγRIIA is expressed on platelet, antibody could cross-link platelets by binding to FcγRIIA expressed on platelet, and has a risk of inducing thrombosis

	FcγRIIA	FcγRIIB
	Activating	inhibitory
		
Expressing cells	Macrophage, **Platelet**, Neutrophil Monocyte, Dendritic cell	B cell, Plasma cell Macrophage, Monocyte Eosinophile, Neutrophil Dendritic cell , Mast cell

Generation of novel Fc variant (TwoB-Ig) with selective enhancement of FcγRIIB binding

- More than 1000 variants were evaluated, and successfully identified TwoB-Ig having selectively increased binding affinity to FcγRIIB

  * TwoB-Ig: FcγRIIB selective binding technology-Immunoglobulin



FcγR	IIA(H)	IIA(R)	IIB
Human IgG1	1	1	1
TwoB-Ig	0.1	1.6	**130**

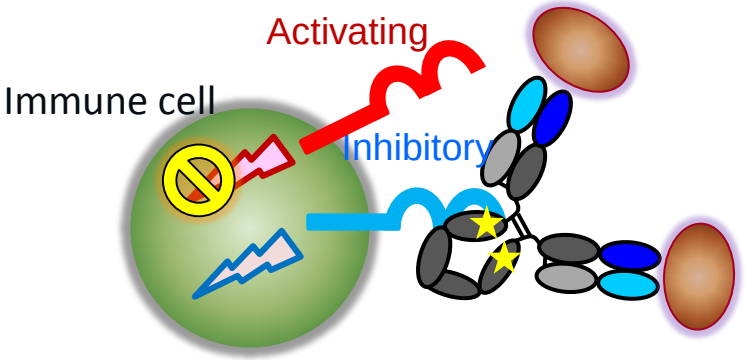
Successfully generated Fc with selectively increased binding to inhibitory FcγRIIB while not increasing binding to FcγRIIA

In house data

TwoB-Ig is an antibody technology applicable for autoimmune disease area

- Autoimmune diseases
  - Systemic lupus erythematosus
  - Type 1 diabetes
  - Ulcerative colitis
  - Rheumatoid arthritis
  - Myasthenia gravis
  - Crohn's disease
  - Psoriasis
  - Pemphigus

Cytokine and immune-activating receptor



TwoB-Ig could increase efficacy by inducing inhibitory signal into immune cell with strong FcγRIIB binding, in addition to neutralization of immune-activating antigen

Antibody technologies available for licensing opportunities

ART-Ig (Asymmetric Re-engineering Technology - Immunoglobulin)

- Bispecific antibody technology

TwoB-Ig (FcγRIIB selective binding technology - Immunoglobulin)

- Enhancing binding selectively to inhibitory Fcγ receptor

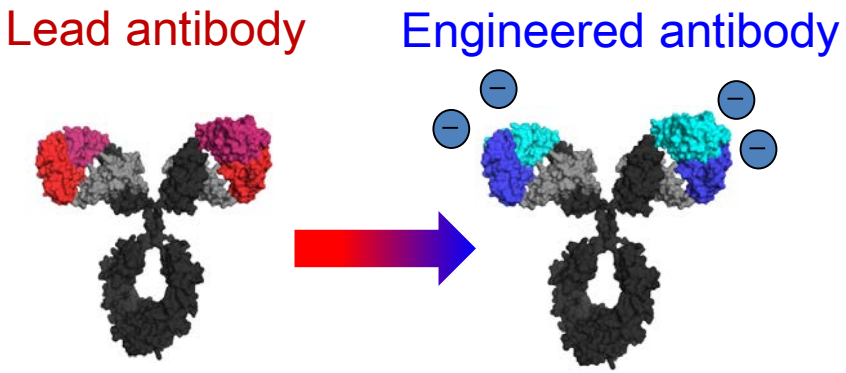
ACT-Ig (Antibody Charge engineering Technology -Immunoglobulin)

- Antibody half life extending technology

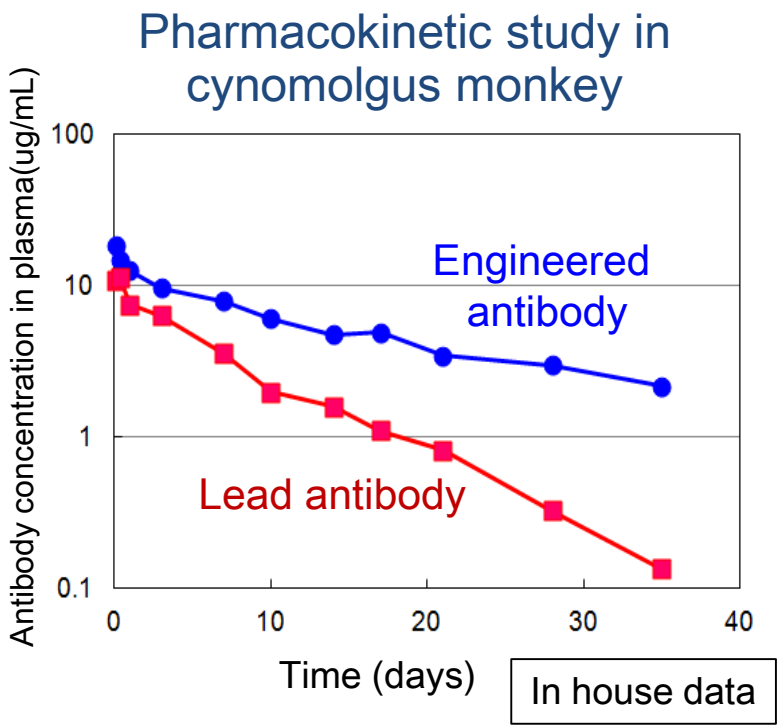
ACT-Ig: Antibody half-life extending technology

Antibody Charge engineering Technology-Immunoglobulin

- pI lowering engineering of the variable region improves antibody PK by repulsion with negatively-charged vascular endothelial cell surface
  - Half-life extension of antibody
  - Confirmed to be generally applicable to IgG antibodies

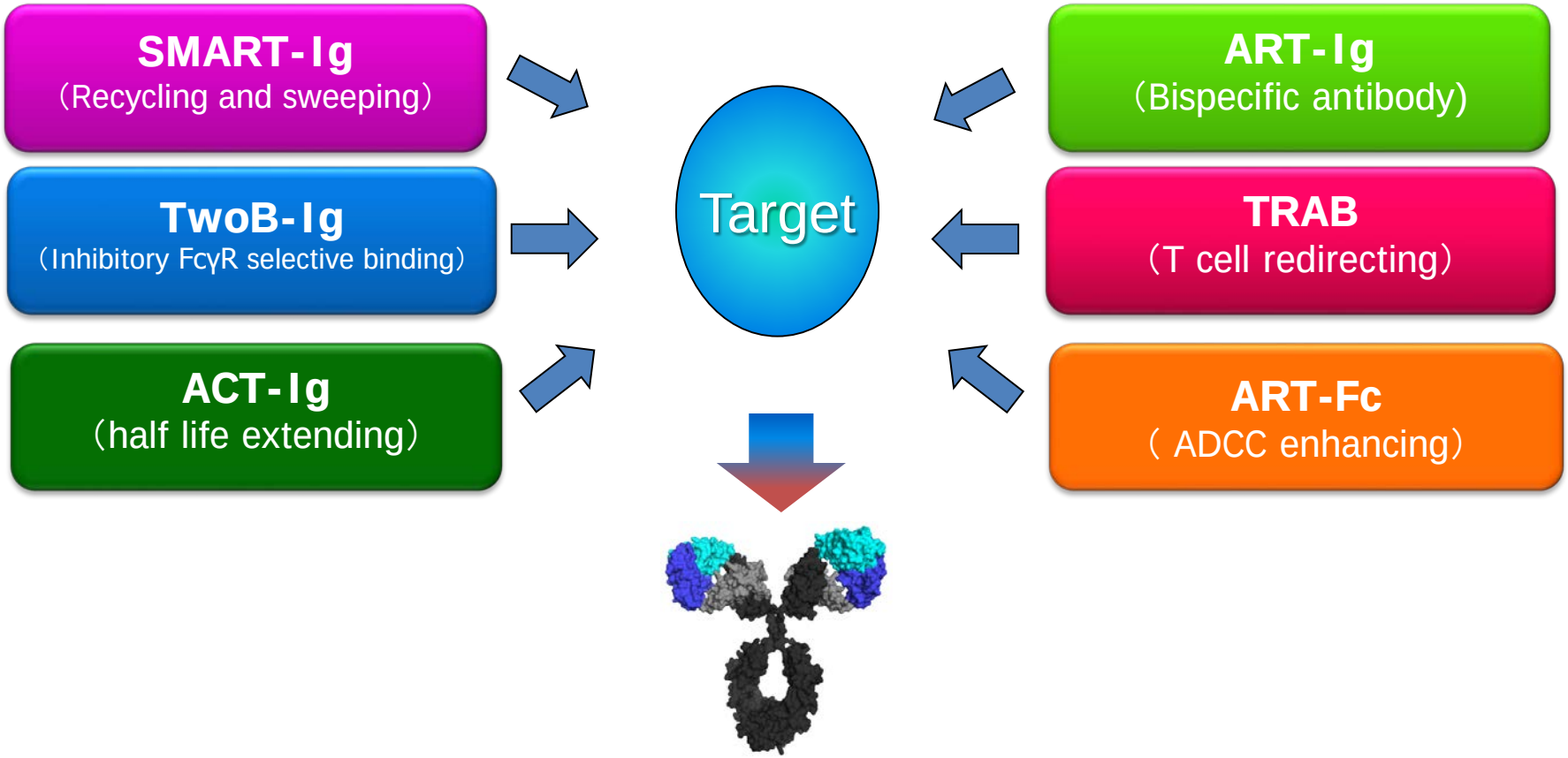


Engineering to lower the pI of variable regions without loss of binding affinity and stability



Chugai will create innovative antibody drug using competitive proprietary technology

Chugai will create innovative antibody drug for the benefit of the medical community and human health around the world by using proprietary only one and number one technologies



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