Chugai’s Strategy for Drug Discovery Research

Junichi Nezu, PhD
General Manager of Research Division
CHUGAI PHARMACEUTICAL CO., LTD.

December 9, 2019
Important Reminders

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Information regarding pharmaceuticals (including products under development) is included in this presentation, but is not intended as advertising or medical advice.
New Mid-Term Business Plan: 5 Strategies

Create global growth drivers and maximize value

Strategy 1
Value Creation
Realize innovative drug discovery to cure and manage diseases

Strategy 2
Value Delivery
Deliver patient-centric solution to maximize value of growth drivers

Strategy 3
Promote advances in personalized healthcare
Realize the further advancement of PHC and innovate R&D process by utilizing digital technology and data

Strengthen HR and infrastructure that support Chugai’s business

Strategy 4
Human capital and structural reform
Develop high-caliber HR talent that support innovation, and drastically reform costs, systems and processes

Strategy 5
Strengthen sustainable platforms
Simultaneously realize company growth and sustainable social development
Basic Policy of Chugai Drug Discovery Strategy

The fusion of biology and technology generates innovations in drug discovery

Strengths in biology

- Antibody engineering technology
- Middle molecules (cyclic peptides)
- Small molecules (beyond the Rule of 5)

Create unprecedented and overwhelming patient value
### The Fusion of Biology and Technology Generates Innovations in Drug Discover

<table>
<thead>
<tr>
<th>Biology</th>
<th>Technology</th>
<th>Innovation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery of erythrocyte growth factor Erythropoietin</td>
<td>Recombinant DNA technology</td>
<td>エポジン®</td>
</tr>
<tr>
<td>Discovery of neutrophil growth factor G-CSF</td>
<td>Manufacturing biologics using CHO</td>
<td>ノイトロジン®</td>
</tr>
<tr>
<td>Discovery of key immune regulator IL-6</td>
<td>Humanization of antibody</td>
<td>アクテムラ®</td>
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<tr>
<td>Discovery of strong driver oncogene ALK</td>
<td>Kinase inhibitor with high selectivity</td>
<td>アレセンサ®</td>
</tr>
<tr>
<td>Invention of MOA to mimic Factor VIII</td>
<td>Bispecific antibody</td>
<td>ヘムライブラ®</td>
</tr>
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</table>
Measures to Establish Strength in Biology

Collaboration with Academia

- IFReC (Osaka Univ.)
- Center of Innovation, The University of Tokyo
- National Cancer Center

Cultivation of a deep in-house understanding of human disease biology

- Deep understanding of targets and MoA
- Integrated disease database
- Fresh samples derived from human patients

TACTICS

The Autonomous and Constitutive Target Idea Creating System

- Activities organized across the research division to create drug discovery ideas
  - Promotion of unique biological discoveries
  - Sublimation to "invention" that leads to highly effective products
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Collaboration with Academia

**IFReC\(^a\)**
Osaka Univ.

Acquisition of new findings based on world’s top basic immunology

**COI\(^b\)**
Univ. of Tokyo

Search for new targets in rheumatic diseases through GWAS/eQTL analysis

**NCC\(^c\)**
National Cancer Center

Search for new CIT targets through immune cell profiling of tumors

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**On-site Lab.**

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Discovery of new biology insights/targets
Measures to Establish Strength in Biology

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TACTICS

The Autonomous and Constitutive Target Idea Creating System

- Activities organized across the research division to create drug discovery ideas
  - Promotion of unique biological discoveries
  - Sublimation to "invention" that leads to highly effective products
Cultivation of a Deep in-house Understanding of Human Disease Biology

Collection of human disease samples

Evaluation of compound activity using fresh samples
Target validation

Acquisition and integration of comprehensive and multifaceted analysis data on various diseases
Measures to Establish Strength in Biology

**Collaboration with Academia**
- IFReC (Osaka Univ.)
- Center of Innovation, The University of Tokyo
- National Cancer Center

**Cultivation of a deep in-house understanding of human disease biology**
- Deep understanding of targets and MoA
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**TACTICS**
- The Autonomous and Constitutive Target Idea Creating System

- Activities organized across the research division to create drug discovery ideas
  - Promotion of unique biological discoveries
  - Sublimation to "invention" that leads to highly effective products
TACTICS: System for Creating Drug Discovery Ideas Across the Research Division

TACTICS

*The Autonomous and Constitutive Target Idea Creating System*

![Diagram of TACTICS system]

- TACTICS Committee
- Feasibility study group (FSG)
- Other idea creation groups
- Personal ideas
- R0 proposals
- R1 proposals

The diagram illustrates the flow of drug discovery ideas through the TACTICS system, starting from idea creation groups to feasibility studies, and ultimately leading to R0 and R1 proposals.
Promote Unique Discoveries and Inventions through TACTICS

Ideas → Hypothesis

Hypothesis ← Experiments

Experiments ← Discovery

Discovery ↓ Systematic promotion of unique inventions based on unique discoveries
Paradigm Shift in Drug Discovery
“Era of Discovery → Era of Invention”

Era of Discovery
- Biological discovery

Era of Invention
- Invention of drug discovery idea (MoA)
- Invention of modality technology

Create medical value
HEMLIBRA: Brought by Original Invention

Blood coagulation mechanism by Factor IXa/X

Generally known

Bispecific antibody

Concept: known

Mimic Factor VIII function with a bispecific antibody

Manufacturing: Invention

Invention
Chugai Life Science Park Yokohama (New Research Laboratory)

Summary

A core research facility to be built in Yokohama, Kanagawa Prefecture (planned completion in 2022)
- Building area: 35,210m²
- Total floor area: 119,960m²

- Emphasizing climate change countermeasures, local disaster preparedness, and biodiversity preservation; aiming to acquire LEED Gold certification for environmental performance
- Will reduce our overall environmental footprint (including the consolidation of existing facilities)
- Signed environmental agreement with city of Yokohama, emphasizing coexistence with the local community

- The integration of all functions related to drug discovery research is expected to promote further research efficiency and collaboration.
- Enhanced efforts to promote the fusion of biology and technology
Chugai Pharmabody Research Pte. Ltd. (Singapore)

- Creation of antibody drugs using Chugai's antibody engineering technologies
- Development of new antibody engineering technologies

Opened in 2012, Fully-owned by Chugai
## Chugai’s Mission Statement

~Innovation all for the patients~

### Mission

Dedicate ourselves to adding value by creating and delivering innovative products and services for the medical community and human health around the world

### Core Values

1. **Patient Centric**
   
   Make each patient’s wellbeing our highest priority

2. **Pioneering Spirit**
   
   Pursue innovation by improving ourselves and thinking differently

3. **Integrity**
   
   Maintain the highest standards in all we do to create shared value with society

### Envisioned Future

Become a top innovator for advanced and sustainable patient-centric healthcare, powered by our unique strength in science and technology and the alliance with Roche
Basic Policy of Chugai Drug Discovery Strategy

Strengths in biology

- Antibody engineering technology
- Middle molecules (cyclic peptides)
- Small molecules (beyond the Rule of 5)

The fusion of biology and technology generates innovations in drug discovery

Create unprecedented and overwhelming patient value
Appendix: Characteristics of Each Modality

In addition to antibodies and small molecules, the addition of middle molecules as a drug discovery modality greatly expands the potential for drug discovery.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Small molecule</th>
<th>Middle molecule</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>MW &lt;500</td>
<td>700 &lt; MW &lt;1600</td>
<td>MW 15000</td>
</tr>
<tr>
<td>Oral administration</td>
<td>Available</td>
<td>Available</td>
<td>Not available</td>
</tr>
<tr>
<td>Effects on intracellular targets</td>
<td>Available</td>
<td>Available</td>
<td>Difficult</td>
</tr>
<tr>
<td>Inhibition of protein-protein interaction</td>
<td>Difficult</td>
<td>Available</td>
<td>Available</td>
</tr>
<tr>
<td>Specificity</td>
<td>Low</td>
<td>Mid - High</td>
<td>High</td>
</tr>
<tr>
<td>Dosage interval</td>
<td>Short (daily)</td>
<td>Short (daily)</td>
<td>Long (Every 2 weeks)</td>
</tr>
</tbody>
</table>
Chugai’s Antibody Engineering Technologies for Innovative Drug Discovery

Tomoyuki Igawa Ph.D.
CEO and Research Head
Chugai Pharmabody Research Pte. Ltd.
Singapore

December 9, 2019
Agenda

1. Antibody Drug Discovery Strategy and Platforms
2. Recycling antibody® and Sweeping antibody® Technology
3. Switch Antibody™ Technology
4. Next Generation Bispecific Antibody Technology
5. Summary
From Unmet Medical Needs to Discovery of Innovative Antibody Drug

- **Unmet medical needs**
- **Technology**
  - Only one (No similar technology)
  - Number one (Best in similar technology)
  - Proprietary technology (in-house development)
- **Biology**
  - Understanding of disease biology
  - Proprietary target & MOA
  - Undruggable or difficult target & MOA
- **Platform**
  - Antibody engineering, protein science and pharmacology etc to realize & evaluate the idea
  - Systematic platform (IT, automation, outsourcing etc)

- **Innovative antibody drug**
Discovery of Innovative Antibody Drug

**Target & MOA**

- **Unmet medical needs**
- **Technology**
  - Only one (No similar technology)
  - Number one (Best in similar technology)
  - Proprietary technology (in-house development)
- **Biology**
  - Understanding of disease biology
  - Proprietary target & MOA
  - Undruggable or difficult target & MOA
- **Platform**
  - Antibody engineering, protein science and pharmacology to realize & evaluate the idea
  - Systematic platform (IT, automation, outsourcing etc)
  - TACTICS (Idea creation)
  - Collaboration with academia
  - Deepening human disease biology

**Innovative antibody drug**
Discovery of Innovative Antibody Drug Platform

Unmet medical needs

Technology
- Only one (No similar technology)
- Number one (Best in similar technology)
- Proprietary technology (in-house development)

Biology
- Understanding of disease biology
- Proprietary target & MOA
- Undruggable or difficult target & MOA

Platform
- Antibody engineering, protein science and pharmacology to realize & evaluate the idea
- Systematic platform (IT, automation, outsourcing etc)

Innovative antibody drug
- In-house platform
- Roche and Genentech platform
Chugai’s Four Competitive Platforms Supporting Antibody Drug Discovery

Antibody drug discovery process

- Target validation
- Lead antibody Identification (LI)
- Lead antibody Optimization (LO)
- Clinical candidate Selection (CS)
- CMC and IND-enabling
- IND

- De-immunization
- Multidimensional antibody optimization system (COSMO)
- Rabbit B cell cloning
- Designed antibody phage library
Lead Antibody Identification (LI) Platform

**Rabbit B-cell cloning**
- mAb with high affinity binding against the right epitope

**Designed phage library**
- mAb with unique function which is impossible by animal immunization

- **Immunization**
- **B-cell sorting**
- **B-cell culture**
- **Lead candidates**
- **Gene cloning**
- **HT B-cell screening**

**Animal immunization with DNA, protein or cell followed by automated high-throughput screening system**

**Phage panning**

**Designing library and phage panning (antibody screening) against target protein or cell in automated high-throughput system**

*Source: Chugai Pharmaceutical Co., Ltd.*
Lead Antibody Optimization (LO) Platform

**COSMO**: **C**omprehensive **S**ubstitution for **M**ultidimensional **O**ptimization

- **HTP affinity measurement**
  ~2000 Run/Week
- **Multidimensional evaluation**
  (i.e. stability, solubility, immunogenicity, non-specific binding)
- **HTP antibody purification**
  ~1500 Abs/Day
- **HTP primer design system**
- **HTP Ab construction and transfection**
  ~3000 Abs/week

Source: Chugai Pharmaceutical Co., Ltd.
De-immunization Platform

- Challenge: Increased immunogenicity was the main concern when we “engineer” humanized antibody or human IgG1 sequence.
- Solution: We have established de-immunization platform to minimize immunogenicity risk of our highly engineered antibodies.

**Diagram:**

- **Antibody variants** → **T cell assay** → **Immunogenicity analysis** → **Identify presented peptide sequence** → **Prediction by computer** → **In silico** → **Clinical candidates**

**T cell assay:** In vitro method to predict immunogenicity using human CD4+ T cell

**MAPPPs:** Mass spectrometry method to identify the sequence of peptide presented on MHC class II by dendritic cells

**In silico:** Prediction of binding affinity of peptide to MHC class II
Discovery of Innovative Antibody Drug

In-house technology development

Unmet medical needs

Technology

- Only one (No similar technology)
- Number one (Best in similar technology)
- Proprietary technology (in-house development)

Biology

- Understanding of disease biology
- Proprietary target & MOA
- Undruggable or difficult target & MOA

Platform

- Antibody engineering, protein science and pharmacology to realize & evaluate the idea
- Systematic platform (IT, automation, outsourcing etc)

Innovative antibody drug
Continuous Evolution of Proprietary Antibody Engineering Technologies

Maximize the value of drug target

Create drug against undruggable target and MOA

2005~
Engineering to create best-in-class antibodies

2008~
Engineering to create antibodies with unique mode of action

2012~
Engineering to confer disease tissue/cell specificity

2018~
Engineering to expand sites of action

1990~
Humanized antibodies

- Stability improvement
- Pharmacokinetic improvement
- Deimmunization beyond humanization
- ADCC/ADCP enhancement

- Switch Antibody™
- Second generation TRAB®
- etc

- Bispecific antibody
- Recycling antibody®
- Sweeping antibody®
- FcyRlb selective Fc
- T cell redirecting antibody (TRAB®)
Mission of Chugai Pharmabody Research
Maximize the value of Chugai’s antibody engineering capability

Drug discovery
(from 2012 to present):
Generate clinical candidates based on Chugai’s established proprietary antibody engineering technologies.

Technology to create antibodies with unique mode of action
Technology to create best-in-class antibodies

Technology to confer disease tissue/cell specificity

Technology to expand sites of action

Technology development
(from 2017 to present):
Establish novel antibody engineering technologies to create drug against undruggable target and MOA.

Drug discovery
(from 2012 to present):
Generate clinical candidates based on Chugai’s established proprietary antibody engineering technologies.

Technology to create antibodies with unique mode of action
Technology to create best-in-class antibodies

Technology to confer disease tissue/cell specificity

Technology to expand sites of action

Technology development
(from 2017 to present):
Establish novel antibody engineering technologies to create drug against undruggable target and MOA.

CPR 2012~
CPR 2017~
CPR 2018~
Recycling antibody® and Sweeping antibody® Technology
Recycling antibody®
Enables antibody to bind to target multiple times

- Satralizumab (anti-IL6R Recycling antibody®)
  - Confirmed recycling effect against **membrane** antigen in human
  - Positive phase 3 data in NMOSD patients
    SAkuraSky Study

- Crovalimab (anti-C5 Recycling antibody®)
  - Confirmed recycling effect against **soluble** antigen in human
  - Positive phase 1/2 data in PNH patients
    COMPOSER Study interim report at ASH2018

- AMY109 (Recycling antibody®)
  - Phase 1 study in endometriosis patients
    JapicCTI183841
Sweeping antibody®
Eliminates soluble antigen from plasma

Elimination of soluble antigen from plasma by accelerated endosomal delivery and lysosomal degradation of the antigen can be expected.

Constant region engineering: Enhance cellular uptake via Fc receptor

Variable region engineering: pH dependent antigen binding and dissociation (Recycling antibody®)

Nature Biotechnology, 2010, Igawa et al
PLOS One, 2013, Igawa et al
Biochim Biophys Acta, 2014, Igawa et al
(All of the above, author is an employee of Chugai Pharmaceutical Co., Ltd.)
pH Dependent Antibody Can be Generated from Any Lead Antibody by COSMO

Lead antibody

pH7.4 $\rightarrow$ pH7.4

pH7.4 $\rightarrow$ pH6.0

pH7.4 $\rightarrow$ pH7.4

pH7.4 $\rightarrow$ pH5.8

Antigen A

Histidine substitution

Antigen B

Histidine substitution

Antigen C

Histidine substitution

COSMO: Comprehensive Substitution for Multidimensional Optimization

Binding analysis by surface plasmon resonance

In-house data
pH Dependent Antigen Binding Antibody
Release the Soluble Antigen in Endosome

In vitro confocal microscope

Biochim Biophys Acta, 2014, Igawa et al
(Author is an employee of Chugai Pharmaceutical Co., Ltd.)
Challenges in First Generation FcRn Mediated Sweeping antibody® Technology

- FcRn mediated first generation Sweeping antibody® showed modest sweeping in mouse (~50-fold reduction of antigen)

However, sweeping was inefficient in cynomolgous monkey — Effective sweeping in monkey is required for human translatability
TwoB-Ig®: Enhancing FcγRIIb Mediated Uptake of Antibody-Antigen Complex

- FcγRIIb plays a major role in clearing antibody-antigen complexes from body through liver sinusoidal endothelial cells (LSEC)
- TwoB-Ig® variant is applied to selectively increase FcγRIIb binding
  - Enhance FcγRIIb-mediated uptake of antibody-antigen complex by LSEC
  - Reduced FcγRIIa/IIIa binding to prevent platelet activation and ADCC activity

<table>
<thead>
<tr>
<th>FcγR type</th>
<th>TwoB-Ig®</th>
<th>Relative binding compared with normal IgG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRI</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>FcγRIIa (H)</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>FcγRIIa (R)</td>
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<td></td>
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<tr>
<td>FcγRIIb</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>FcγRIIIa (V)</td>
<td>N.D.</td>
<td></td>
</tr>
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</table>

Protein Eng Des Sel, 2013, Mimoto et al  
(Author is an employee of Chugai Pharmaceutical Co., Ltd.)

pI-Fc™: Positively Charged Fc to Enhance the Uptake of Antibody–Antigen Complex

• Introducing positive charge to the Fc domain

   IgG1  Fc surface change  Introduction of pI-Fc™ surface change

   Blue: positively charged surface  Red: negatively charged surface

• Positively charged Fc enhance cellular uptake of the complex
Combination of TwoB-Ig® and pI-Fc™ Achieved Strong Antigen Sweeping in Monkey

**Conceptual illustration**

**Antigen Sweeping effect in Cynomolgus monkey**

**In-house data**
Anti-latent myostatin Sweeping antibody®

- Control progression of loss in muscle strength by latent myostatin inhibition for neuromuscular disease
  - Myostatin is autocrine/paracrine protein secreted from skeletal muscles as an inactive form (latent and pro-myostatin) and complete inhibition of biological activity of mature myostatin is understood as a very hard challenge.

Elimination of latent myostatin by the Sweeping antibody® for effective inhibition of myostatin
Sweeping antibody® Reduced Plasma Latent Myostatin by >1000-fold

Sweeping antibody® eliminated latent myostatin from plasma while antibody pharmacokinetics was similar to conventional antibody.
Sweeping antibody® against latent myostatin is Superior to High Affinity Neutralizing Antibody in Mouse

**Delta muscle mass**

- **Day 0-28**
  - Mean ± SE, N = 6

- **Sweeping antibody®**
- **High affinity antibody**

**Delta grip strength**

- **Day -4-27**
  - Mean ± SE, N = 6

- **Sweeping antibody®**
- **High affinity antibody**

*Sweeping antibody® is not GYM329*

**In-house data**

Sweeping antibody® against latent myostatin is highly effective in increasing both muscle mass and muscle function of SCID mouse.

Mouse in vivo study
Recycling antibody® and Sweeping antibody®
Summary

- Recycling antibody® technology was validated clinically.
- Sweeping antibody® technology was established by combination of TwoB-Ig® and pI-Fc™ technology.
  - Confirmed sweeping effect in cynomolgous monkey
- Sweeping antibody® against latent myostatin reduced total antigen concentration by >1000-fold and improved maximum pharmacological efficacy.

- 4 project utilizing these technologies in clinical development.
  - Satralizumab (anti-IL6R Recycling antibody®)
  - Crovalimab (anti-C5 Recycling antibody®)
  - GYM329/RG6237 (anti-latent myostatin Sweeping antibody®)
  - AMY109 (Recycling antibody®)

- 2 project utilizing these technologies in discovery stage.
Switch-Ig® / Switch Antibody™ Technology
On-target Toxicity is One of the Remaining Challenges of Antibody Therapeutics

- **Anti-CD44v6 antibody drug conjugate**
  - Systemic killing of CD44v6+ cells
  - Kill CD44v6 positive cancer cell
  - Fatal skin toxicity as side effect (clinical development terminated)

- **Anti-EGFR Ab for colorectal cancer**
  - Systemic neutralization of EGFR
  - Kill EGFR dependent cancer cell
  - Severe skin toxicity as side effect

- **Anti-CTLA4 antibody for melanoma**
  - Systemic neutralization of CTLA4
  - Activate tumor infiltrating CTLs
  - Severe autoimmune as side effect

- **Anti-4-1BB agonist antibody**
  - Systemic activation of 4-1BB+ immune cells
  - Activate tumor infiltrating 4-1BB+ T cells
  - Fatal hepatic toxicity as side effect (clinical development terminated)

- **Engineered T cell (CAR-T etc) therapy**
  - Systemic killing of antigen expressing cells
  - Kill antigen positive cancer cell
  - Severe side effect by attacking normal cells expressing the target antigen
Switch-Ig®
Disease microenvironment Switch Antibody™ technology

“Switch Antibody™” binds to the antigen only in the presence of tumor specific small molecule metabolite (switch molecule).

Conceptual illustration
Extracellular ATP Selectively Elevated in Tumor Microenvironment as Switch Molecule

- Intracellular ATP (adenosine triphosphage) is 5-8 mM, and extracellular ATP in normal tissue and plasma is tightly regulated at around ~30 nM.
- Within solid tumor microenvironment, intracellular ATP is released from necrotic, apoptotic and stressed cancer cells.
- >100 μM extracellular ATP is accumulated in solid tumor in mice tumor.

Mouse in vivo study

Switch Antibody™ Binds to the Target Antigen Only under the Presence of ATP

Normal tissue (low ATP)

Non-target cell

Solid tumor (high ATP)

Target cell

Conceptual illustration
Designed Phage Library with ATP-binding Motif for ATP Switch Antibody™ Generation

The Fab library is displayed on phage for ATP-dependent binding antibody selection

In-house data
Demonstrating the Concept of Switch Antibody™ Using Model Antigen and Animal

- Model antigen
  - Antigen: Human IL-6 receptor (hIL-6R)
  - Goal: Generate ATP dependent **anti-hIL-6R Switch Antibody™**

- Mouse model
  - Mouse: Transgenic mouse systemically overexpressing hIL-6R in normal tissues, and bearing hIL-6R expressing solid tumor
  - Goal: **Switch Antibody™ does not bind to hIL-6R in normal tissue, but bind to hIL-6R expressed on cancer cell and exert anti-tumor activity**

*Non-switch Antibody (=Conventional Ab)*

*Switch Antibody™*

*Conceptual illustration*
Switch Antibody™ Demonstrates ATP Dependent hIL-6R Binding and ADCC Activity

**Non-switch Antibody**

0 μM ATP  
\( K_D = 17 \text{ nM} \)

**Switch Antibody™**

0 μM ATP  
N.D.

1 μM ATP  
\( K_D = 2400 \text{ nM} \)

10 μM ATP  
\( K_D = 320 \text{ nM} \)

100 μM ATP  
\( K_D = 48 \text{ nM} \)

**Binding affinity (\( K_D \))**

![Graph showing binding affinity values for different ATP concentrations for both non-switch and switch antibodies.]

**ADCC reporter assay**

![Graph showing ADCC reporter activity for different ATP concentrations for both non-switch and switch antibodies.]

*In-house data*
ATP Located in Between Switch Antibody™ and the Antigen Serving as a Switch

X-ray structural analysis of Switch Antibody™/ATP/antigen ternary complex

In-house data
Switch Antibody™ was Similarly Distributed to hIL-6R Expressing Tumor as Non-switch Antibody

hIL-6R transgenic mouse with hIL-6R expressing Hepa 1-6 mouse tumor
(all three antibodies were labelled in green)

Collaboration with Department of Immunology and Cell Biology, Graduate School of Medicine, Osaka university

In-house data
Switch Antibody™ was not Distributed to hIL-6R Overexpressing Liver

hIL-6R transgenic mouse with hIL-6R expressing Hepa 1-6 mouse tumor
(all three antibodies were labelled in green)

Collaboration with Osaka university

In-house data

Mouse in vivo study
Intravital imaging

Conceptual illustration
Switch Antibody™ was Selectively Distributed to Tumor but not to Liver

hIL-6R transgenic mouse with hIL-6R expressing Hepa 1-6 mouse tumor

Mouse in vivo study
Intravital imaging
Tukey's multiple comparison test

Innovation Beyond Imagination

Collaboration with Osaka university

In-house data
Switch Antibody™ Demonstrated Tumor Growth Inhibition

hIL-6R transgenic mouse with hIL-6R expressing Hepa 1-6 mouse tumor

Mouse in vivo study
Tukey's multiple comparison test

Conceptual illustration

In-house data
Making Undruggable Target Druggable

Undruggable by conventional antibody

Druggable by Switch Antibody™

Minimize side effect (Switch OFF)

Maximize efficacy (Switch ON)

Normal tissue

Disease lesion with high switch molecule
Switch Antibody™ Summary

- Switch-Ig® specifically binds to the target antigen in the tumor microenvironment without detectable binding to the antigen in plasma and normal tissue.
- Switch-Ig® technology transforms undruggable target into druggable target, and enables more effective and safer antibody therapeutics in oncology field.

- 1 project utilizing Switch-Ig® planned to enter into clinical development next year.
- 6 projects utilizing Switch-Ig® in discovery stage.
Next Generation
Bispecific Antibody Technology
First Generation Bispecific Antibody

Asymmetric bispecific IgG antibody with common light chain

Antigen A

Antigen B

ART-Ig® technology successfully applied to create Hemlibra®

emicizumab

Conceptual illustration
ERY974: T cell Redirecting AntiBody (TRAB®)
Anti-GPC3/CD3 bispecific antibody

- TRAB® is Chugai’s proprietary platform with TA(tumor antigen)/CD3 bispecific IgG antibody engineered not to bind to FcγR.
- ERY974 is Chugai’s first TRAB® being tested in phase 1 study.

Applied ART-Ig® with common light chain for bispecific antibody manufacturing.

(Author is an employee of Chugai Pharmaceutical Co., Ltd.)
Next Generation Bispecific Antibody

- Second generation

  NXT007
  Second generation emicizumab

- Third generation

  Novel mode of action by controlled binding to two antigens
  (not just binding to two different antigens)
Second Generation Bispecific Antibody
Non-common Lch asymmetric bispecific antibody

- First generation: **ART-Ig®** using common light chain

  ![Conceptual illustration](image)
  
  **Advantage** Easiness for manufacturing  
  
  **Disadvantage** Engineering freedom is limited

- Second generation: **FAST-Ig™** with non-common light chain

  ![Conceptual illustration](image)

  Freedom to engineer two light chains independently, and allows design of bispecific antibody with complex mode of action
FAST-Ig™

Four-chain Assembly by electrostatic Steering Technology

- Controlled heavy and light chain assembly by charge engineering

Wild type IgG1 (HHLL)

FAST-Ig™ (HHLL)

Ion exchange chromatography analysis of protein A purified samples

In-house data
NXT007
Anti-FIXa/FX bispecific antibody

Example of enhancing activity with non-common Lch

**FAST-Ig™**

**ACT-Fc**

**NXT007 Target Profile**

- Achieve normal level of hemostatic ability
  - Further optimization of emicizumab variable region enabled by FAST-Ig™
- Improved convenience in administration
  - Achieved by ACT-Fc and application of administration device etc

*This molecule is not NXT007

*In-house data

In vitro study

Conceptual illustration
**ACT-Fc: FcRn Binding Enhancing Mutation Improves the Pharmacokinetics of Emicizumab**

- **Increased affinity to FcRn by ACT-Fc**

**Pharmacokinetics in cynomolgus monkey**

- **Emicizumab**
  - $T_{1/2}$ (day): 19.4
  - CL (mL/day/kg): 3.69

- **Emicizumab + ACT-Fc**
  - $T_{1/2}$ (day): 54.5
  - CL (mL/day/kg): 1.70

- *dose of emicizumab was 6 mg/kg and emicizumab+FcRn was 0.6 mg/kg, and plasma concentration was normalized to be 6 mg/kg in the graph*

**Conceptual illustration**

ACT-Fc is also applied to crovalimab, AMY109 and GYM329
T cell Redirecting AntiBody (TRAB®) Drug Discovery Strategy

1. Bringing multiple TRAB® projects targeting various tumor antigens (TAs) into discovery research pipeline
   - CD3 bispecific antibodies against novel tumor antigens
   - FAST-Ig™ with non-common light chain accelerates the research

2. Combining costimulatory signal to improve anti-tumor efficacy of TRAB®
   - Combination of costimulatory signal with TA/CD137 bispecific

3. Incorporating Switch Antibody™ technology to improve the safety profile of TRAB®
   - Tumor antigen expressed in normal tissue leads to toxicity
   - ATP dependent tumor antigen binding to avoid normal tissue toxicity
TA(tumor antigen)/CD137 Bispecific Antibody to Induce Costimulatory Signal to T cell

TA/CD137 bispecific induces signal 2 by clustering CD137 costimulatory receptor

TRAB® induces signal 1 by clustering CD3ε

TA/CD137 bispecific antibody

Cancer cell

T cell

Full activation and improved survival by combining signal 1 and 2 mimics the natural process of T cell activation
Synergistic Anti-tumor Effect by Combining TA/CD3 and TA/CD137 Bispecific Antibodies

In TRAB resistant mouse tumor model, combination of TA/CD137 bispecific antibody significantly improved anti-tumor efficacy and IFNγ production.

In-house data
ON-target OFF Tumor Side Effect is the Major Challenge of TRAB®
Switch Antibody™ is Applicable to TRAB® and Various Cancer Immunotherapies

Conceptual illustration

No cytotoxicity

Normal cell

Cytotoxicity

Cancer cell
Switch TRAB® Shows Strong T cell Activation Only under the Presence of ATP

In vitro reporter cell assay

100 μM ATP

No ATP

In-house data
Third Generation Bispecific Antibody

*Dual specific mutually competitive bispecific Fab*

Conventional IgG antibody

Asymmetric bispecific IgG antibody

Antibody with mutually competitive bispecific Fab

Enables novel mode of action by **controlled** binding to two antigens
Novel Bispecific Antibody Can Bind to Two Different Antigens but not at the Same Time

When antigen A is bound, antigen B cannot bind

controlled binding

binding analysis by surface plasmon resonance

in-house data
Next Generation Bispecific Summary

- **Second generation bispecific antibody**
  - FAST-Ig™ removes common light chain restriction to manufacture bispecific antibody, and enables complex engineering of two arms of bispecific antibody.
    - 1 project utilizing FAST-Ig™ in clinical development.
      - NXT007 (anti-FIXa/FX bispecific antibody)
    - 4 projects utilizing FAST-Ig™ in discovery stage.

- **Third generation bispecific antibody**
  - Mutually competitive bispecific Fab enables novel mode of action by controlled binding to two antigens and antibody with unique mechanism action can be designed.
    - 5 projects utilizing bispecific Fab in discovery stage.
Summary (1)

Druggable target with conventional antibody

Conventional antibody

Only druggable target with new technology

Recycling antibody®

Sweeping antibody®

Switch Antibody™

Switch Antibody™

Next-gen Bispecific antibody

Unique MOA invention with new technology

New antibody engineering technologies enables expansion of druggable target and invention of unique modes of action
Summary (2)

Recycling antibody®
Sweeping antibody®
etc

Satralizumab
Nemolizumab
SKY59 (crovalimab)
AMY109
GYM329/RG6237

Bispecific antibody (1st, 2nd and 3rd generation)

ERY974
NXT007

Switch Antibody™

NEW technology etc

Discovery ➔ Preclinical ➔ Clinical ➔ Launched
Licensable Antibody Engineering Technologies

**SMART-Ig®**
Creates the Recycling Antibody®, which is designed to achieve a longer duration of action than conventional antibodies by binding to an antigen multiple times.

**SMART-Fc®**
Creates the Sweeping Antibody®, which eliminates soluble antigens from plasma.

**ACT-Ig®**
Reduces clearance from plasma.

**ART-Fc®**
Expected to enhance the antibody-dependent cellular cytotoxicity (ADCC) activity and/or antibody-dependent cellular phagocytosis (ADCP) activity by improving the binding activity of the antibody to specific type of FcγRs. Potential applications in the oncology field.

**ART-Ig®/FAST-Ig™**
Enable large-scale production of bispecific IgG antibodies which bind to two different antigens. Eliminates complex downstream process and enables highly efficient manufacturing process.

**ΔGK™**
Makes manufacturing process less complex. Removes heavy chain C-terminal amino acids (glycine and lysine). This technology reduces the heterogeneity of IgG antibody and can be widely applicable to IgG antibodies.

**TRAB®**
Activates T cells in an antigen-dependent manner to specifically kill cancer cells without non-specific FcγR dependent T cell activation.

**TwoB-Ig®**
Increases binding selectivity of the Fc region to inhibitory Fcγ receptor IIb. Potential applications in autoimmune diseases and other areas.

**pI-Fc™**
Improves agonistic activity or efficiency of soluble antigen elimination from plasma through the facilitation of Fc-FcγR interaction. Enhances the potency when used in combination of SMART-Fc®/TwoB-Ig®

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