Bispecific antibody-armed nanoparticles for future theranostic applications

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National Health Research Institutes
Acknowledgements

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Ming-Feng Hou (侯明鋒)
Yun-Ming Wang (王雲銘)

Research Expertise:

• Bioinorganic Chemistry
• Paramagnetic metal complex and bio-activated metal complex for magnetic resonance imaging (MRI)
• Superparamagnetic iron oxide nanoparticle (SPIO) for MRI
• Probe for optical imaging
• Molecular Imaging

Contrast agents and probes development for molecular imaging

Target tumor cell

Optical imaging
Bispecific Antibody Conjugated Manganese-Based Magnetic Engineered Iron Oxide for Imaging of HER2/neu- and EGFR-Expressing Tumors

The manuscript was accepted by Theranostics. (2015/09/22)
The founding of the National Health Research Institutes (NHRI) was first proposed in 1988 by members of Academia Sinica.

In 1994 a preparatory committee was formed.

NHRI was officially established in January 1996.

NHRI relocated to its permanent campus in Zhunan in 2004.
Intramural Research Units

**Institutes**
- Cancer Research
- Cellular and System Medicine
- Population Health Sciences
- Biotechnology & Pharmaceutical Research
- Molecular & Genomic
- Infectious Disease & Vaccinology
- Biomedical Engineering & Nanomedicine

**Divisions/Center**
- Environmental Health & Occupational Medicine
- National Environmental Health Research
- Immunology Research
- Neuropsychiatric Research
PUBLICATIONS from PIs at NHRI:
Please refer to National Health Research Institutes Institutional Repository
Taiwan: Ilha Formosa ~ a Beautiful Island
Please visit us at www.nhri.org.tw
NHRI Intramural capability for new drug discovery

- Considerations of Targets
- Assay Developments
- Drug Screening
- Active Hits
- Pharmacokinetics
- Pharmacology & Animal Models
- Development Candidate
- Pre-formulation
- Pharmaceutical studies
- Lead compounds
- Patent issues
- Lead optimization
- Patent issues
- DMPK (Metabolism & PK)
- Safety Pharmacology & Toxicity
- IND Clinical Trial

NHRI Intramural capability for new drug discovery
Core Technologies of IBPR

IBPR/NHRI

Chemistry

Pharmacokinetics
Drug Metabolism

Pharm. Development

Biology

High throughput screening

Animal pharmacology

Project Management
Preclinical/Clinical Development

Chemistry

Pharmacokinetics
Drug Metabolism

Pharm. Development

Biology

High throughput screening

Animal pharmacology

Project Management
Preclinical/Clinical Development
My Experiences

• BS, Ch.E. NTU 1989
• Army Service, Second Lieutenant
• PhD, Ch.E., Johns Hopkins University 1991-1995
  – Expression of IgG with chaperone and foldase
• Post-Doc at Human Genome Sciences, Inc., 1996 - 1997
  – Learned many fields ranging from HTS cDNA sequencing, protein expression and purification, protein chemistry
• Merck & Co., 1997 - 1998
  – Biopharmaceutics, Live virus vaccine production and formulation
• Nation Health Research Institutes (1998-2015; now 1,400 employees)
  – Established drug discovery and development group
    • ~ 10 patents, > 100 papers
Jeremy Lin, an NBA player (Hornet)
Experiences at Merck

• To improve Varivax
  – Varivax is a live virus vaccine for chickenpox
    (Chickenpox = 水痘 = Ветряная оспа)
  – To improve the storage conditions for Varivax
    • Membrane stability, virus stability, cryobiology, ...
Experiences at Merck

• To improve Varivax
  – Varivax is a live virus vaccine for chickenpox
    (Chickenpox = 水痘 = Ветряная оспа)
  – To improve the storage conditions for Varivax
    • Membrane stability, virus stability, cryobiology, ...
  – To transform Varivax into Zostavax for shingles
    • Zostavax was approved in 2005
      (Shingle (herpes zoster) = 皮蛇 = опоясывающий лишай)
Shingles/Zostavax

Company
Merck & Co. Inc.

Description
Live attenuated zoster virus vaccine

Standard Indication
Varicella zoster virus (VZV)
Prevent shingles (herpes zoster) in adults 60 years or older

Indication Details
Vaccinate against shingles (herpes zoster) in adults ages 50-59
Experiences at Merck

• To improve Varivax
  – Varivax is a live virus vaccine for chickenpox
    (Chickenpox = 水痘 = Ветряная оспа)
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  – To transform Varivax into Zostavax for shingles
    • Zostavax was approved in 2005
    (Shingle (herpes zoster) = 皮蛇 = опоясывающий лишай)
  – To develop a trivalent vaccine for
    • Chickenpox, Measles, Mumps, and Rubella
    • ProQuod: combining Varivax with MMR, approved in 2005
Chickenpox/ProQuod

Company
Merck & Co. Inc.

Description
Live vaccine against measles, mumps, rubella and varicella viruses

Indication Details
Prevent measles, mumps, rubella and varicella infection
Two recombinant baculoviruses were constructed to encode P1 and 3CD of enterovirus 71 (EV71), respectively. The expressed 3CD successfully cleaved P1 in vitro and in vivo.

Biotechnology Letters, June 2003, Volume 25, Issue 12, pp 919-925
In collaboration with Prof. YC Hu
The Virus Images

Influenza viruses

Polio viruses

Rotaviruses
Recombinant Proteins Core

- Protein engineering/expression and purification
- To Support drug discovery programs
Mission of IBPR

To establish, conduct and support research on new medicines and biotechnology that will lead to better human health and quality of life.
Top 10 Drugs by Worldwide Sales 2003

**Lipitor**
- Pfizer
- Treats: Cholesterol
- $10.3 billion

**Zocor**
- Merck
- Treats: Cholesterol
- $6.1 billion

**Zyprexa**
- Eli Lilly
- Treats: Antipsychotic
- $4.8 billion

**Norvasc**
- Pfizer
- Treats: Hypertension
- $4.5 billion

**Procrit/Eprex**
- Johnson & Johnson
- Treats: Anemia
- $4.0 billion

**Prevacid**
- Abbott & Takeda
- Treats: Stomach acid
- $4.0 billion

**Nexium**
- AsstraZeneca
- Treats: Stomach acid
- $3.8 billion

**Plavix**
- Bristol Myers
- Treats: Cardiovascular Disease
- $3.7 billion

**Latus Solostar**
- GlaxoKlineSmith
- Treats: Asthma
- $3.7 billion

**Zoloft**
- Pfizer
- Treats: Antidepressant
- $3.4 billion
<table>
<thead>
<tr>
<th>Drug</th>
<th>Treats</th>
<th>Sales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>Atypical antipsychotic</td>
<td>$7.2 b</td>
</tr>
<tr>
<td>Adalimumab&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Rheumatoid arthritis</td>
<td>$6.3 b</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>Proton pump inhibitor</td>
<td>$6.3 b</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>Cholesterol</td>
<td>$5.6 b</td>
</tr>
<tr>
<td>Etanercept&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Rheumatoid arthritis</td>
<td>$5.0 b</td>
</tr>
<tr>
<td>Fluticasone propionate and</td>
<td>Asthma, allergic rhinitis, nasal polyps</td>
<td>$5.0 b</td>
</tr>
<tr>
<td>salmeterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sofosbuvir</td>
<td>HCV</td>
<td>$4.4 b</td>
</tr>
<tr>
<td>Infliximab&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Crohn's disease; rheumatoid arthritis</td>
<td>$4.3 b</td>
</tr>
<tr>
<td>Latus Solostar</td>
<td>Diabetes</td>
<td>$3.8 b</td>
</tr>
<tr>
<td>Pegfilgrastime</td>
<td>Neutropenia</td>
<td>$3.6 b</td>
</tr>
</tbody>
</table>
Top 10 Drugs by Worldwide Sales 2014

Aripiprazole
- Atypical antipsychotic
- $7.2 billion

Esomeprazole
- Proton pump inhibitor
- $6.3 billion

Rosuvastatin
- Cholesterol
- $5.6 billion

Fluticasone propionate and salmeterol
- Asthma, allergic rhinitis, nasal polyps
- $5.0 billion

Adalimumab
- Rheumatoid arthritis
- $6.3 billion

Etanercept
- Rheumatoid arthritis
- $5.0 billion

Sofosbuvir
- HCV
- $4.4 billion

Infliximab
- Crohn’s disease; rheumatoid arthritis
- $4.3 billion

Latus Solostar
- Diabetes
- $3.8 billion

Pegfilgrastime
- Neutropenia
- $3.6 billion
Anti-TNF Protein Drugs

<table>
<thead>
<tr>
<th>Brand name (generic name)</th>
<th>Nature of agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remicade® (infliximab)</td>
<td>Chimeric human–mouse monoclonal antibody</td>
</tr>
<tr>
<td>Enbrel® (etanercept)</td>
<td>Soluble TNF p75 receptor–IgG fusion protein</td>
</tr>
<tr>
<td>Humira® (adalimumab)</td>
<td>Human monoclonal antibody</td>
</tr>
</tbody>
</table>
Bispecific antibody-armed nanoparticles for future theranostic applications
Antibody structure and types of antibodies

Monoclonal antibody-based cancer therapeutic strategies

- **a** Tumour-specific IgG
- **b** Angiogenesis inhibition
- **c** Checkpoint blockade
- **d** Radioimmunotherapy
- **e** Antibody–drug conjugate therapy
- **f** Bispecific antibody therapy
- **g** CAR T cells

**b** Angiogenesis inhibition:
- VEGFR
- VEGF
- CTLA4
- PD1
- PDL1
- Ipilimumab
- Nivolumab

**c** Checkpoint blockade:
- T cell
- PDL1-specific

**d** Radioimmunotherapy:
- Complement

**e** Antibody–drug conjugate therapy:
- Effector cell

**f** Bispecific antibody therapy:
- CD3

**g** CAR T cells:
- Tumour cell
- Tumour antigen

**a** Tumour-specific IgG:
- Receptor
- Complement

Immunoonjugates

Antigen-based retargeting of cellular immunity

Traditional antibodies and antibody fragments vs nanobodies

**Canonical, full-length antibody (160 kDa)**

**Antibody fragments**

**Fab (55 kDa)**

**scFv (28 kDa)**

**Single-domain antibody/Nanobody® (Nb or VHH or V_{NAR}) (15 kDa)**

**Camelid HCAb**

**Shark HCAb**

Heavy-chain only antibodies (HCAbs)
**Next-generation antibodies**

1. Antibody–drug conjugates (ADCs)
2. Engineered antibodies
3. Multispecific antibodies

![Diagram showing the therapeutic window and drug dose relationship between chemotherapy and ADCs.](image)

**Figure 1** Market potential for NGA products, 2013–2021. Source: company reports.

ADC, antibody–drug conjugate; NGA, next-generation antibody.
Bispecific antibody development timeline

Hybridoma technology (1975) → Chemical conjugation


Recombinant technologies (1982) →

- Humanised antibodies (1986)
- Human antibodies (1992)
- Single variable domain antibodies
- Novel scaffolds

IgG-like BsAbs →

- Knob-in-hole IgG
- IgG-scFv
- scFv-Fc-scFv
- DVD-Ig
- Fab-scFv
- taFv-Fc

Small BsAbs →

- taFv, BiTE
- Db, scDb, DART, tandAb

Structure of Catumaxomab (Removab™), the first bispecific antibody to achieve approval in EU

Catumaxomab was approved in the European Union for the intraperitoneal treatment of patients with malignant ascites. (2009)
Mechanism of action of the novel bispecific antibody Blinatumomab (Blincyto™)

US FDA Approves Blinatumomab for B-Cell ALL (2014)
Antibody Fragment Configurations

- Whole IgG ~ 150kDa
- (F\text{Ab})_2 ~ 110kDa
- Light chain
- Heavy chain
- (F\text{Ab})_2 ~ 110kDa

- F\text{Ab} ~ 55kDa
- VL ~ 30kDa

- Triabody ~ 75kDa
- Diabody ~ 50kDa

- Bi Specific Antibody ~ 100kDa
- Minibody ~ 75kDa

- 2x scFv

https://fusionantibodies.com/services/antibody-engineering/antibody-fragments/
FDA Approval of Antibodies and Antibody-Like Molecules

49 molecules approved from 1986 to December 2014
<table>
<thead>
<tr>
<th>International non-proprietary name</th>
<th>Trade name</th>
<th>Target; Format</th>
<th>Indication first approved or reviewed</th>
<th>First EU approval year</th>
<th>First US approval year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idarucizumab</td>
<td>Praluent</td>
<td>Dabigatan; Humanized Fab</td>
<td>Reversal of dabigatran-induced anticoagulation</td>
<td>In review</td>
<td>In review</td>
</tr>
<tr>
<td>Alirocumab</td>
<td>Repatha</td>
<td>PCSK9; Human IgG1</td>
<td>High cholesterol</td>
<td>In review</td>
<td>In review</td>
</tr>
<tr>
<td>Mepolizumab</td>
<td>Unituxin</td>
<td>GD2; Chimeric IgG1</td>
<td>Severe eosinophilic asthma</td>
<td>In review</td>
<td>In review</td>
</tr>
<tr>
<td>Necitumumab</td>
<td>Cosentyx</td>
<td>IL-17a; Human IgG1</td>
<td>Non-small cell lung cancer</td>
<td>In review</td>
<td>In review</td>
</tr>
<tr>
<td>Evolocumab</td>
<td>Opdido</td>
<td>PD1; Human IgG4</td>
<td>High cholesterol</td>
<td>EC decision pending</td>
<td>In review</td>
</tr>
<tr>
<td>Dinutuximab</td>
<td>Blinicyto</td>
<td>CD19, CD3; Murine bispecific tandem scFv</td>
<td>Acute lymphoblastic leukemia</td>
<td>In review</td>
<td>2014</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Keytruda</td>
<td>PD1; Humanized IgG4</td>
<td>Melanoma, non-small cell lung cancer</td>
<td>EC decision pending</td>
<td>2014</td>
</tr>
<tr>
<td>Blinatumomab</td>
<td>Cyramza</td>
<td>VEGFR2; Human IgG1</td>
<td>Gastric cancer</td>
<td>2014</td>
<td>2014</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Entyvio</td>
<td>α4β7 integrin; humanized IgG1</td>
<td>Ulcerative colitis, Crohn disease</td>
<td>2014</td>
<td>2014</td>
</tr>
<tr>
<td>Siltuximab</td>
<td>Sylvant</td>
<td>IL-6; Chimeric IgG1</td>
<td>Castleman disease</td>
<td>2014</td>
<td>2014</td>
</tr>
</tbody>
</table>

Schematic diagram of tetravalent bispecific antibody (Bis-Ab)
Background

• A highly specific contrast agent system comprising an iron oxide nanoparticle core coated with poly(ethylene glycol) was previously developed (MnMEIO).

• The tetravalent bispecific antibody may facilitate detection of HER2/neu- and EGFR-expressing tumors.
Bispecific Antibody Conjugated Manganese-Based Magnetic Engineered Iron Oxide for Imaging of HER2/neu- and EGFR-Expressing Tumors
TEM images and schematic representation of MnMEIO NPs conjugated with or without mPEG, CyTE777 and Bis-Ab
Binding specificity of Ab-armed NPs to HER2/neu- and EGFR-expressing tumor cells

KD (EGFRv3): ~10 nM
KD (HER2/neu): ~3 nM
Histological analyses of Colo-205 and SKBR-3 tumor tissue specimens acquired 96 hrs after injection of Ab-armed NPs

Sections were stained with Prussian blue.
Prussian blue staining of SKBR-3, A431, and Colo-205 cells treated with Ab-armed NPs

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Bispecific</th>
<th>Herceptin</th>
<th>Erbitux</th>
<th>Erb+Her</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR-3</td>
<td><img src="image1" alt="Blank" /></td>
<td><img src="image2" alt="Bispecific" /></td>
<td><img src="image3" alt="Herceptin" /></td>
<td><img src="image4" alt="Erbitux" /></td>
<td><img src="image5" alt="Erb+Her" /></td>
</tr>
<tr>
<td>A431</td>
<td><img src="image6" alt="Blank" /></td>
<td><img src="image7" alt="Bispecific" /></td>
<td><img src="image8" alt="Herceptin" /></td>
<td><img src="image9" alt="Erbitux" /></td>
<td><img src="image10" alt="Erb+Her" /></td>
</tr>
<tr>
<td>Colo-205</td>
<td><img src="image11" alt="Blank" /></td>
<td><img src="image12" alt="Bispecific" /></td>
<td><img src="image13" alt="Herceptin" /></td>
<td><img src="image14" alt="Erbitux" /></td>
<td><img src="image15" alt="Erb+Her" /></td>
</tr>
</tbody>
</table>
**In vitro** optical imaging of Ab-armed NPs treated SKBR-3, A431, and Colo-205 cells

**Blocking study of Bis Ab-armed NPs with tumor cells at 37 °C**
The bivalent binding modes of a Bis-Ab

Either or both antigens at high density but variably distributed

\[ Y + \xrightarrow{k_{on}} Y \xrightarrow{k_{off}} Y \]

The *in vitro* T2-weighted images of SKBR-3, A431, and Colo-205 tumor cells after treatment with Ab-armed NPs.
Pharmacokinetics of MnMEIO-CyTE777-(Bis)-mPEG and control NPs (MnMEIO-CyTE777-mPEG NPs) in tumor-bearing mice
*In vivo* optical images of tumor-bearing mice after intravenous injection of NPs w/wo Bis-Ab

NPs (10 mg/kg)
T2-weighted MR images (7.0 T) of tumor-bearing mice before (Pre), and 2 hrs and 24 hrs after injection of Ab-armed NPs
Conclusions

• Using a bispecific Ab for nanoparticle conjugation not only simplifies conjugation process but also overcome the diverse expression patterns of individual receptors.

• Multifunctional nanoparticles (MnMEIO-CyTE777-(Bis)-mPEG NPs) can be applied to optical imaging, dual targeting effect and MRI contrast agent for early diagnoses of HER2/neu- or EGFR-overexpressing cancers.

• Bispecific antibody-armed nanoparticles can be applied to future theranostic applications.
Acknowledgements
National Health Research Institutes

Спасибо

спасибо

Кан-Хье