XXXVI CONGRESSO NAZIONALE SIFO

IL FARMACISTA PER...

Scelte
Interventi
Futuro
Outcome

Catania,
Centro Congressuale Fieristico
Culturale “Le Ciminiere”
22-25 OTTOBRE 2015
Pierluigi Navarra

Il futuro delle terapie biotecnologiche
## Farmaco Biotecnologico: esempi

<table>
<thead>
<tr>
<th>Ormoni</th>
<th>Anticorpi monoclonali</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulina umana ricombinante</td>
<td>Infliximab (CHIMERICO)</td>
</tr>
<tr>
<td>Eritropoietina umana ricombinante</td>
<td>Bevacizumab (UMANIZZATO)</td>
</tr>
<tr>
<td>Fattori di crescita</td>
<td>Adalimumab (UMANO)</td>
</tr>
<tr>
<td>GCSF (Granulocyte colony-stimulating factor) umano ricombinante</td>
<td><strong>Proteine di Fusione</strong></td>
</tr>
<tr>
<td></td>
<td>Etanercept</td>
</tr>
<tr>
<td>Characteristics:</td>
<td>Chemical</td>
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<tr>
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</tr>
<tr>
<td>Size:</td>
<td>Small</td>
</tr>
<tr>
<td>Structure:</td>
<td>Simple</td>
</tr>
<tr>
<td>Stability:</td>
<td>Stable</td>
</tr>
<tr>
<td>Modification:</td>
<td>Well-defined</td>
</tr>
<tr>
<td>Characterization:</td>
<td>Fully characterized</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Manufacturing:</td>
<td>Predictable chemical process that can be copied</td>
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<td></td>
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</tr>
<tr>
<td>Development:</td>
<td>Limited clinical trials (Phase I PK/PD studies)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Regulation:</td>
<td>Interchangeable status with abbreviated procedures in US/EU</td>
</tr>
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</tr>
</tbody>
</table>

**Chemical**

- Small molecule (e.g. fluoxetine)

**Biologics**

- Simple (e.g. omnitrope – hGH)
- Complex (e.g. Herceptin - MAb)

---

**Chemical Characteristics:**
- Small
- Simple
- Stable
- Well-defined
- Fully characterized

**Biologics Characteristics:**
- Large
- Complex
- Unstable
- Modifiable
- Not fully characterized

**Manufacturing:**
- Predictable chemical process that can be copied
- Produced in living organisms
  - Highly sensitive to manufacturing changes
  - High fixed investment

**Development:**
- Limited clinical trials (Phase I PK/PD studies)
- Significant R&D (cell lines)
- Extensive clinical trials (Phase I and III)

**Regulation:**
- Interchangeable status with abbreviated procedures in US/EU
- Pathway defined by EMEA based on comparable status
- No US pathway under BLA
DISRUPTION OF LIGAND-INDEPENDENT HETERODIMERS

INHIBITED FORMATION OF LIGAND-DEPENDENT HETERODIMERS

INHIBITION OF ErbB1/2 TYROSINE KINASE ACTIVITY

trastuzumab

pertuzumab

lapatinib

tyrosine kinase domain

ErbB2

ErbB3

ErbB2

ErbB2/3/4

ErbB2

ErbB1/3/4

INHIBITION OF LIGAND-INDEPENDENT SIGNALING

INHIBITION OF LIGAND-DEPENDENT SIGNALING

INHIBITION OF LIGAND-DEPENDENT and LIGAND-INDEPENDENT SIGNALING


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Continuous cultures of fused cells secreting antibody of predefined specificity

The manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells\textsuperscript{1,2} and screening procedures have been used to reveal antibody activity in some of them. This, however, is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.

G. Köhler
C. Milstein

*MRC Laboratory of Molecular Biology,*
*Hills Road, Cambridge CB2 2QH, UK*
Lo scale-up alla produzione industriale: “il processo è il prodotto”

KL Karson, Nature biotechnology 2005

<table>
<thead>
<tr>
<th>Fase Operativa</th>
<th>Specificità di Prodotto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Espansione cellulare</td>
<td>Linea cellulare, terreno di coltura, metodo di espansione</td>
</tr>
<tr>
<td>Produzione cellulare nel bioreattore</td>
<td>Linea cellulare, terreno di coltura, condizioni del bioreattore</td>
</tr>
<tr>
<td>Recupero per filtrazione o centrifugazione</td>
<td>Condizioni operative diverse</td>
</tr>
<tr>
<td>Purificazione per cromatografia</td>
<td>Diverse condizioni di legame ed eluzione cromatografica</td>
</tr>
<tr>
<td>Caratterizzazione e stabilità</td>
<td>Differenze di metodi, reagenti e standard di riferimento</td>
</tr>
</tbody>
</table>

Farmaco purificato
A library of phage, each displaying a different peptide sequence, is exposed to a plate coated with the target.

Unbound phage are washed away.

Specifically-bound phage are eluted with an excess of a known ligand for the target, or by lowering pH.

After 3 rounds, individual clones are isolated and sequenced.

Key-words importanti: ‘phage display library’
Categorie di anticorpi monoclonali

- “-omab”
- “-ximab”
- “-zumab”
- “-mumab”

Mouse hybridoma

In vitro antibody libraries
Transgenic mouse
Human hybridomas

Genetic engineering
V gene cloning
CDR grafting
Eukaryotic expression

Nature Reviews | Drug Discovery
Anticorpi completi e frammenti..
Un esempio di un frammento diventato famoso
Unconventional antibodies and derived fragments

Courtesy Dr. Mourad Tayebi D.V.M (Hons) M.Sc Ph.D
Senior Lecturer; University of Surrey / Founder and CSO; PrioCam LLC (USA)
PrP-specific transmigration of camelid antibodies across the BBB *in vitro*

**siRNA**

- PrioV (25µg)
- siRNA (20µM)

**PI-PLC**

- PrioV (25µg)
- PI-PLC (0.2 unites/ml)

Courtesy Dr. Mourad Tayebi D.V.M (Hons) M.Sc Ph.D
Senior Lecturer; University of Surrey / Founder and CSO; PrioCam LLC (USA)
Bio distribution of camelid antibodies in brain and tissues (i.p.)

David et al. J Neuroimmunol 2014

Courtesy Dr. Mourad Tayebi D.V.M (Hons) M.Sc Ph.D
Senior Lecturer; University of Surrey / Founder and CSO; PrioCam LLC (USA)
## Differenze tra anticorpi convenzionali e a singolo dominio

<table>
<thead>
<tr>
<th>Anticorpi a singolo dominio</th>
<th>Anticorpi convenzionali</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piccoli, un solo dominio della catena pesante (VHH) ~13 kDa</td>
<td>Grandi, entrambe le catene leggere e pesante ~120-150 kDa</td>
</tr>
<tr>
<td>Richiedono una sola subunità monomérica VHH per il legame con l’antigene</td>
<td>Richiedono entrambe le catene per il legame con l’antigene e la stabilità</td>
</tr>
<tr>
<td>Altamente suscettibili di processi di ingegnerizzazione a valle</td>
<td>Relativamente poco flessibili all’ingegnerizzazione per via della complessa struttura</td>
</tr>
<tr>
<td>Mantengono stabilità ed efficacia anche a temperature e pH estremi</td>
<td>Non sono stabili a pH e temperature estreme</td>
</tr>
<tr>
<td>Diverse vie di somministrazione</td>
<td>Somministrati per iniezione, non possono essere somministrati per via orale</td>
</tr>
<tr>
<td>Facili da produrre in sistemi microbici e fungini</td>
<td>Impegnativi e costosi da produrre</td>
</tr>
</tbody>
</table>

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**Note:**

- **Anticorpi a singolo dominio**
  - Piccoli, un solo dominio della catena pesante (~13 kDa)
  - Richiedono una sola subunità monomérica VHH per il legame con l’antigene
  - Altamente suscettibili di processi di ingegnerizzazione a valle
  - Mantengono stabilità ed efficacia anche a temperature e pH estremi
  - Diverse vie di somministrazione
  - Facili da produrre in sistemi microbici e fungini

- **Anticorpi convenzionali**
  - Grandi, entrambe le catene leggere e pesante (~120-150 kDa)
  - Richiedono entrambe le catene per il legame con l’antigene e la stabilità
  - Relativamente poco flessibili all’ingegnerizzazione per via della complessa struttura
  - Non sono stabili a pH e temperature estreme
  - Somministrati per iniezione, non possono essere somministrati per via orale
  - Impegnativi e costosi da produrre
Onartuzumab

- **Monovalent (one-armed), monoclonal antibody** designed to bind to MET and inhibit Hepatocyte Growth Factor / Scatter Factor (HGF/SF) binding
- Traditional bivalent antibodies to MET potentially activate, rather than inhibit, MET signaling by inducing MET dimerization.
- In contrast, the monovalent design of **onartuzumab inhibits HGF/SF binding** without inducing MET dimerization.
- **Onartuzumab has demonstrated antitumor activity in preclinical analyses and under evaluation in phase III clinical trials for NSCLC and GC**
Next Generation Antibodies: Future Market Prospect

2013
US$501 million

2017
US$2.12 billion

2021
US$5.67 billion

- Engineered antibodies
- ADCs
- Multispecific antibodies
- Non-immunoglobulin ligands

Nature Reviews | Drug Discovery

Courtesy Dr. Mourad Tayebi  D.V.M (Hons) M.Sc Ph.D
Senior Lecturer; University of Surrey / Founder and CSO; PrioCam LLC (USA)
Schema generale dello sviluppo di un MoAB

1. Approved antibody drug
   - Clinical trials
   - Preclinical development
     - Antibody clinical candidate
     - Antibody optimization
     - In vivo test of therapeutic concept

2. Clinically validated target
   - Antibody-based therapeutic concept
   - Antibody design specifications
     - Reagent and assay development
     - Antibody generation
     - In vitro evaluation

3. Early-stage target
Un esempio di ‘clinically validated target’

**T-Cells:**
- Originate in the bone marrow, mature in the thymus
- Migrate to peripheral lymphoid tissue
- Activated in peripheral lymphoid tissue
- Activated T-cells re-circulate via the lymphatic system to sites of inflammation

Un esempio di ‘clinically validated target’
Un esempio di ‘clinically validated target’
Un esempio di ‘clinically validated target’: il meccanismo d’azione di vedolizumab

- Lymphocytes adhere to endothelial cells using integrins when homing to sites of inflammation.
  - The $\alpha_4\beta_7$ integrin is expressed on a subset of T-Cells that specifically interacts with MAdCAM-1 in the gut
- Adhesion facilitates lymphocyte migration into the GI tissue.
- These lymphocytes recruit and activate additional inflammatory cells

### Vedolizumab vs Natalizumab selectivity

<table>
<thead>
<tr>
<th>Binds to integrin:</th>
<th>Vedolizumab</th>
<th>Natalizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>α_{4}β_{1}</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>α_{4}β_{7}</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>α_{E}β_{7}</td>
<td>✓</td>
<td>✓</td>
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</table>

<table>
<thead>
<tr>
<th>Antagonizes:</th>
<th>Vedolizumab</th>
<th>Natalizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAdCAM-1</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blocks migration in:</th>
<th>Vedolizumab</th>
<th>Natalizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CNS</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Other</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Vedolizumab as Induction and Maintenance Therapy for Ulcerative Colitis

Brian G. Feagan, M.D., Paul Rutgeerts, M.D., Ph.D., Bruce E. Sands, M.D., Stephen Hanauer, M.D., Jean-Frédéric Colombel, M.D., William J. Sandborn, M.D., Gert Van Assche, M.D., Ph.D., Jeffrey Axler, M.D., Hyo-Jong Kim, M.D., Ph.D., Silvio Danese, M.D., Ph.D., Irving Fox, M.D., Catherine Milch, M.D., Serap Sankoh, Ph.D., Tim Wyant, Ph.D., Jing Xu, Ph.D., and Asit Parikh, M.D., Ph.D., for the GEMINI 1 Study Group

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RESEARCH PAPER

Pharmacological characterization of PF-00547659, an anti-human MAdCAM monoclonal antibody

N Pullen¹, E Molloy², D Carter¹, P Syntin³, F Clemo⁴, D Finco-Kent⁴, W Reagan⁴, S Zhao⁴, T Kawabata⁴ and S Sreckovic¹

¹Phizer Global Research and Development, Sandwich, Kent, UK, ²J&w PRD, High Wycombe Bucks, UK, ³Novartis Vaccines and Diagnostics Via Fiorentina, Siena, Italy, and ⁴Phizer Global Research and Development, Groton, CT, USA
A Study Of PF-00547659 In Patients With Moderate To Severe Ulcerative Colitis (TURANDOT)

**This study is currently recruiting participants.** *(see Contacts and Locations)*

- **Verified August 2015 by Pfizer**

- **Sponsor:** Pfizer

- **Information provided by (Responsible Party):** Pfizer

**ClinicalTrials.gov Identifier:**

- NCT01620255

- First received: June 13, 2012
- Last updated: August 30, 2015
- Last verified: August 2015
- History of Changes
XXXVI
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