"FARMACI INNOVATIVI, BIOTECNOLOGICI E TERAPIE STAMINALI: FARMACOLOGIA, FARMACOTERAPIA E NORMATIVE"

Roma, 14 aprile 2015

Farmaci biotecnologici:
Farmacologia e Farmacoterapia

Pierluigi Navarra

Farmaco Biotecnologico: esempi

Ormoni

- Insulina umana ricombinante

Proteine

- Eritropoietina umana ricombinante

Fattori di crescita

- GCSF (Granulocyte colony-stimulating factor) umano ricombinante

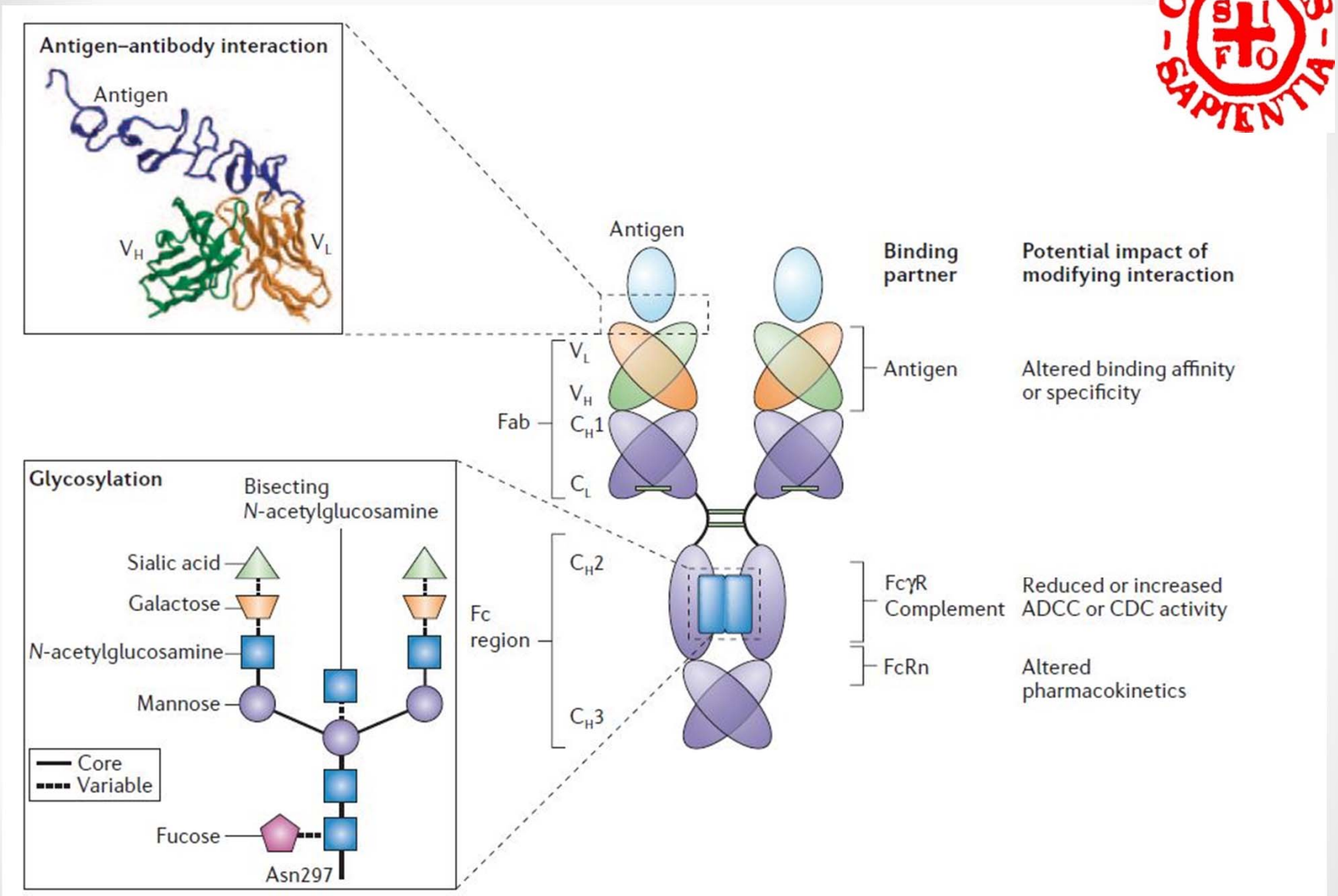
Anticorpi monoclonali

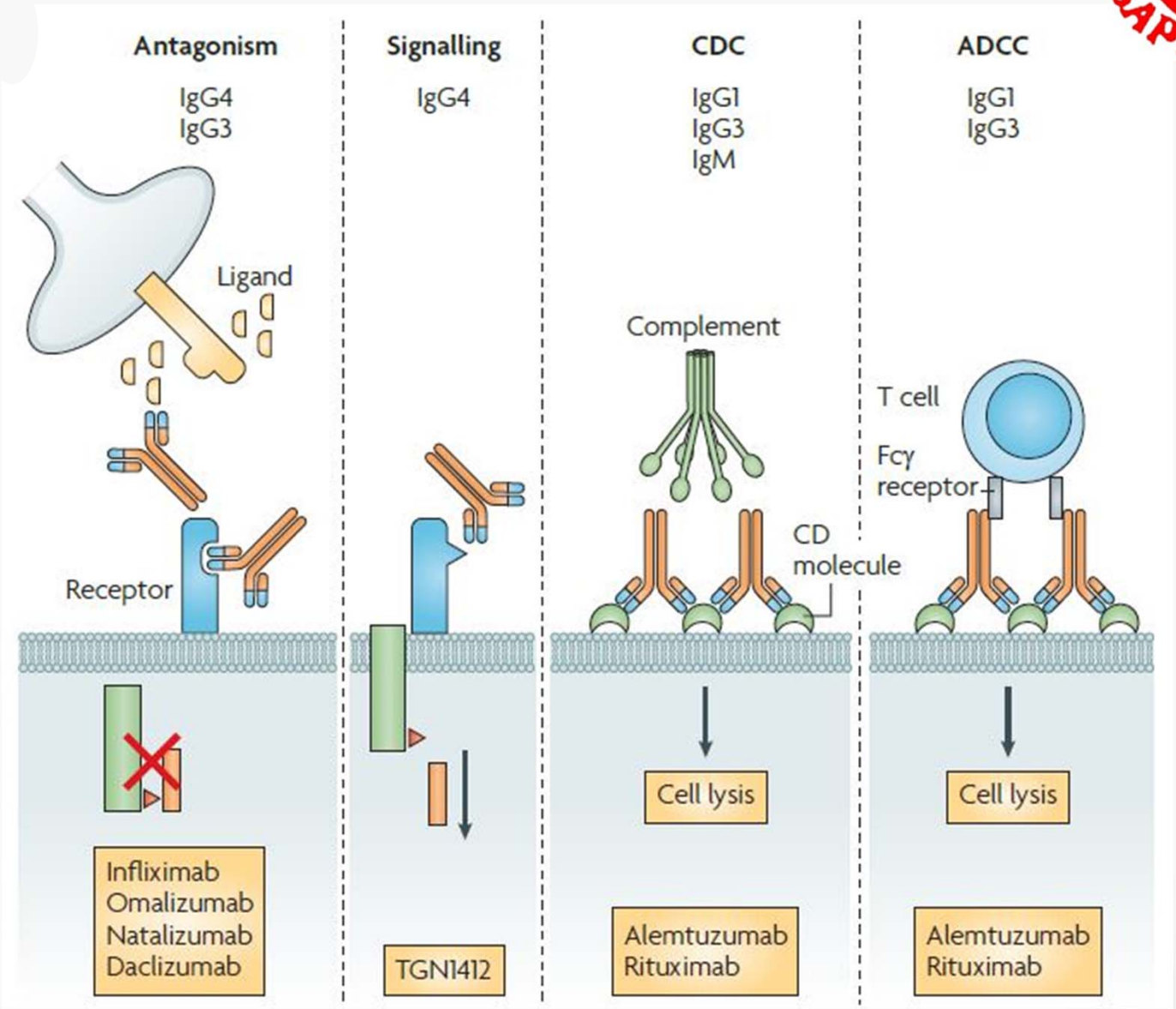
- Infliximab (CHIMERICO)
- Bevacizumab (UMANIZZATO)
- Adalimumab (UMANO)

Proteine di Fusione

- Etanercept

	Chemical	Biologics	
	Small molecule *(e.g. fluoxetine)*	**Simple** *(e.g. omnitrope – hGH)*	**Complex** *(e.g. Herceptin - MAb)*
Characteristics:			
• Size:	• Small	• Large	• Greater likelihood of post-translational modification – Phosphorylation – Glycosylation – Acetylation
• Structure:	• Simple	• Complex	
• Stability:	• Stable	• Unstable	
• Modification:	• Well-defined	• Modifiable	• Impacts 3D structure, and: – Activity / stability – Immunogenicity
• Characterization:	• Fully characterized	• Not fully characterized	
Manufacturing:	• Predictable chemical process that can be copied	• Produced in living organisms – Highly sensitive to manufacturing changes – High fixed investment	• Increased costs/risks: – Complex manufacturing – Regulatory scrutiny – Higher burden of proof
Development:	• Limited clinical trials (Phase I PK/PD studies)	• Significant R&D (cell lines) • Extensive clinical trials (Phase I and III)	• Higher costs reduce number of potential market entrants – Revenue potential increases with complexity
Regulation:	• Interchangeable status with abbreviated procedures in US/EU	• Pathway defined by EMEA based on comparable status • No US pathway under BLA	


Antigen–antibody interaction
Antigen
V_H
V_L
Glycosylation
Bisecting
N-acetylglucosamine
Sialic acid
Galactose
N-acetylglucosamine
Mannose
Core
Variable
Fucose
Asn297
Antigen
Fab
V_L
V_H
C_H1
C_L
Fc
region
C_H2
C_H3
Binding partner
Potential impact of modifying interaction
Antigen
Altered binding affinity or specificity
FcγR
Complement
Reduced or increased ADCC or CDC activity
FcRn
Altered pharmacokinetics


Antagonism
IgG4
IgG3
Ligand
Receptor
Infliximab
Omalizumab
Natalizumab
Daclizumab
Signalling
IgG4
TGN1412
CDC
IgG1
IgG3
IgM
Complement
CD molecule
Cell lysis
Alemtuzumab
Rituximab
ADCC
IgG1
IgG3
T cell
Fcγ receptor
Cell lysis
Alemtuzumab
Rituximab

Key-words importanti: ‘hybridoma’

Nature Vol. 256 August 7 1975

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[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.

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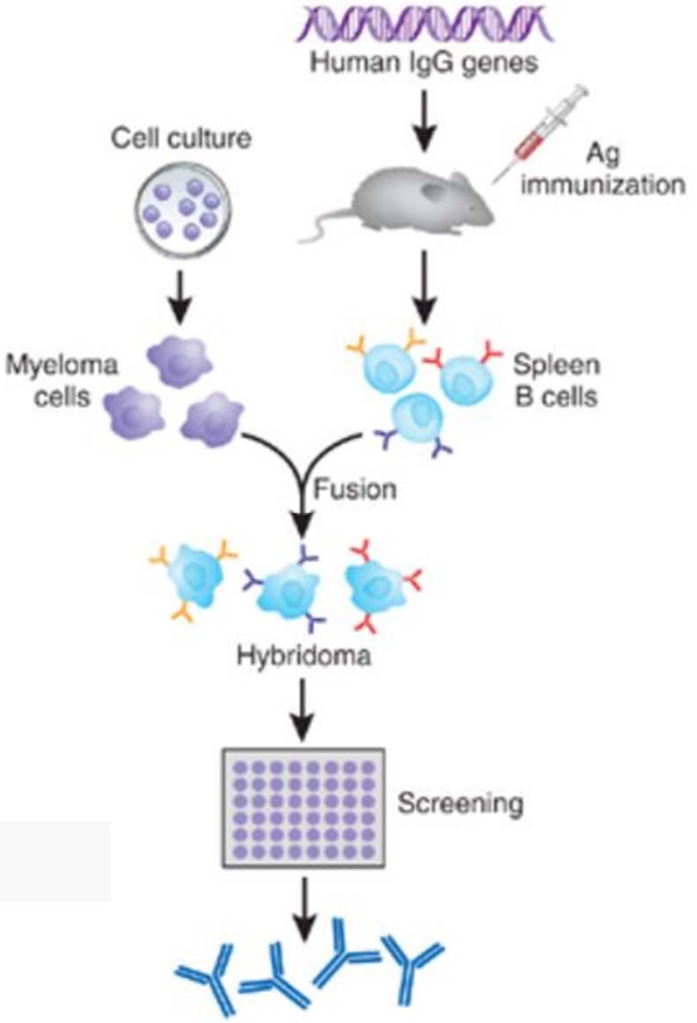
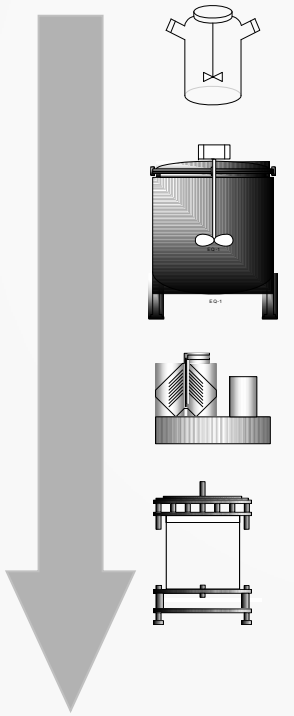


Lo scale-up alla produzione industriale:

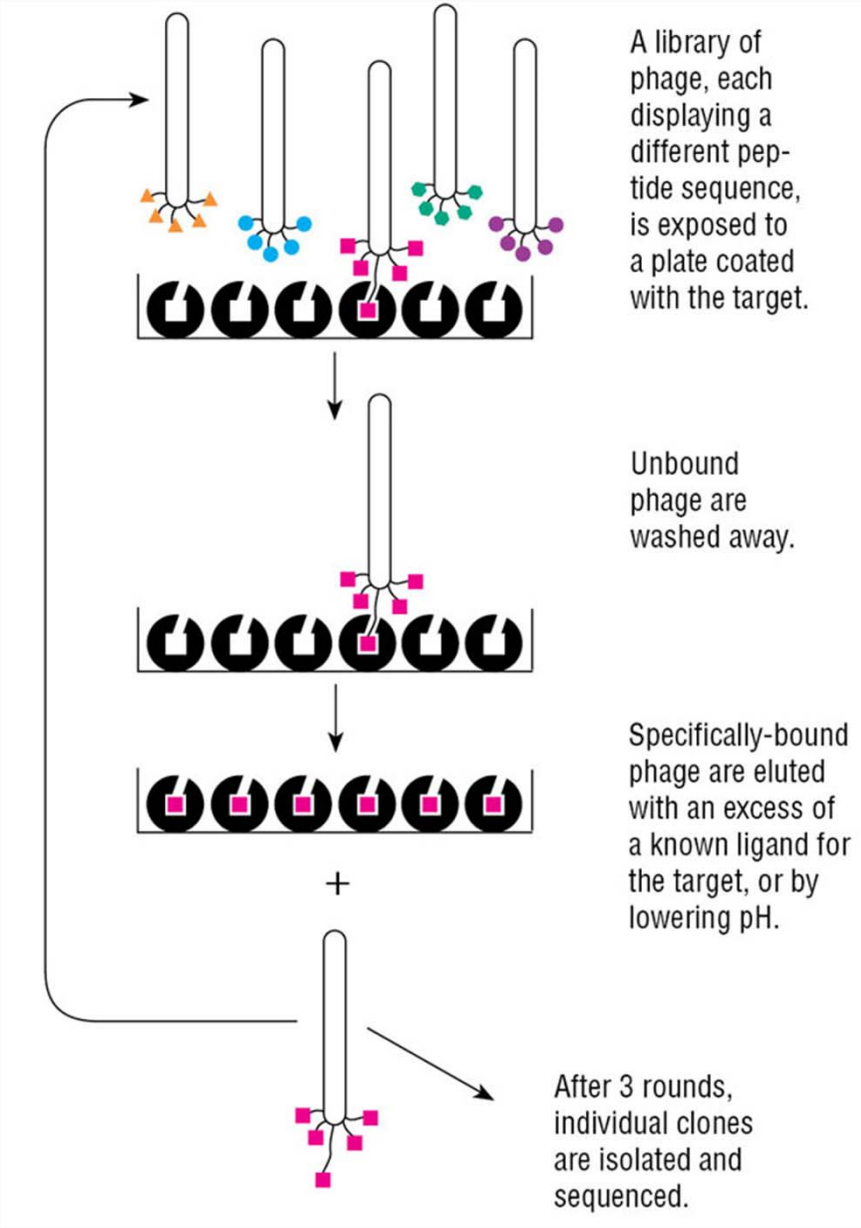
“il processo è il prodotto”

KL Karson, Nature biotechnology 2005

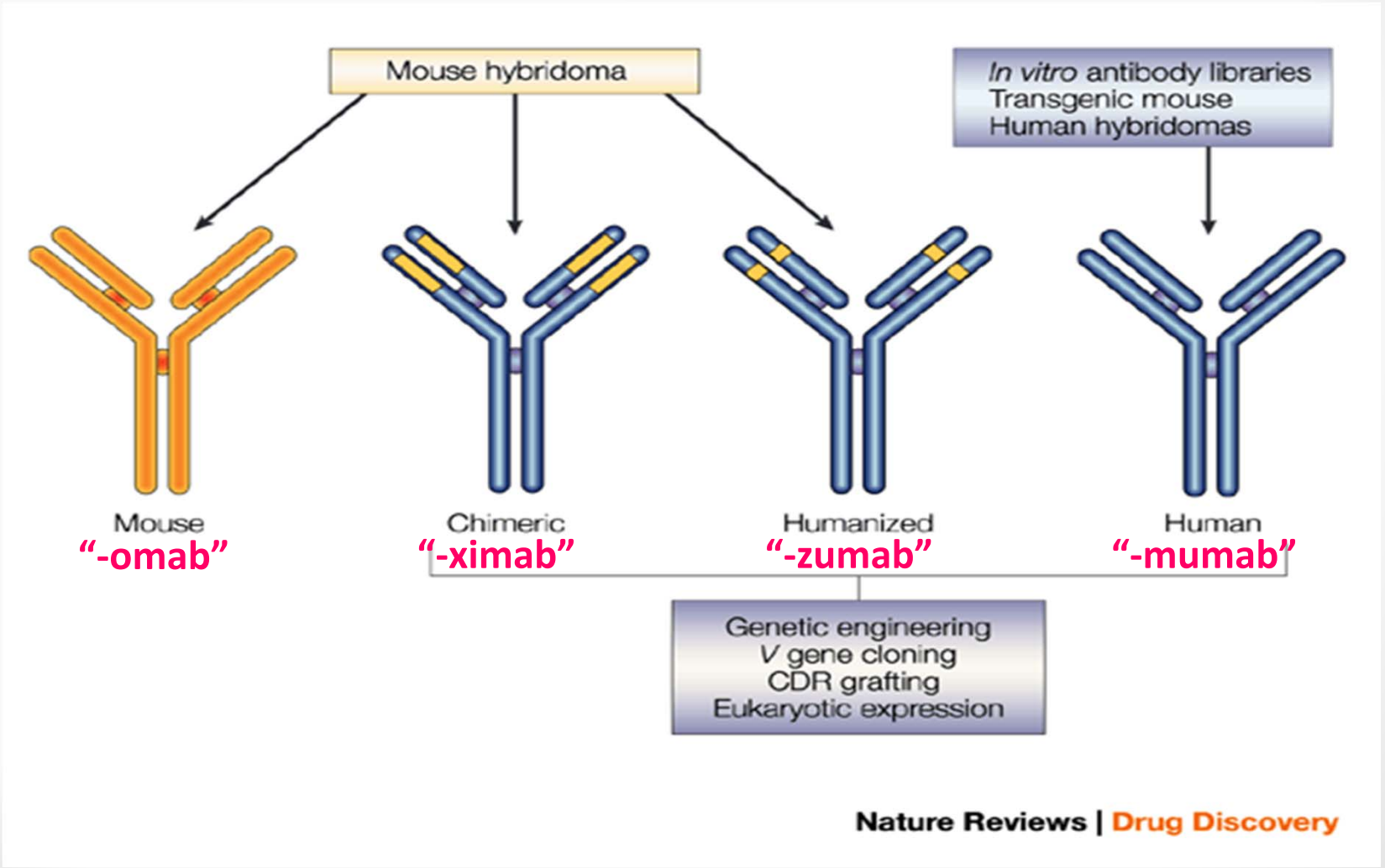


Fase Operativa	Specificità di Prodotto
Espansione cellulare	Linea cellulare, terreno di coltura, metodo di espansione
Produzione cellulare nel bioreattore	Linea cellulare, terreno di coltura, condizioni del bioreattore
Recupero per filtrazione o centrifugazione	Condizioni operative diverse
Purificazione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

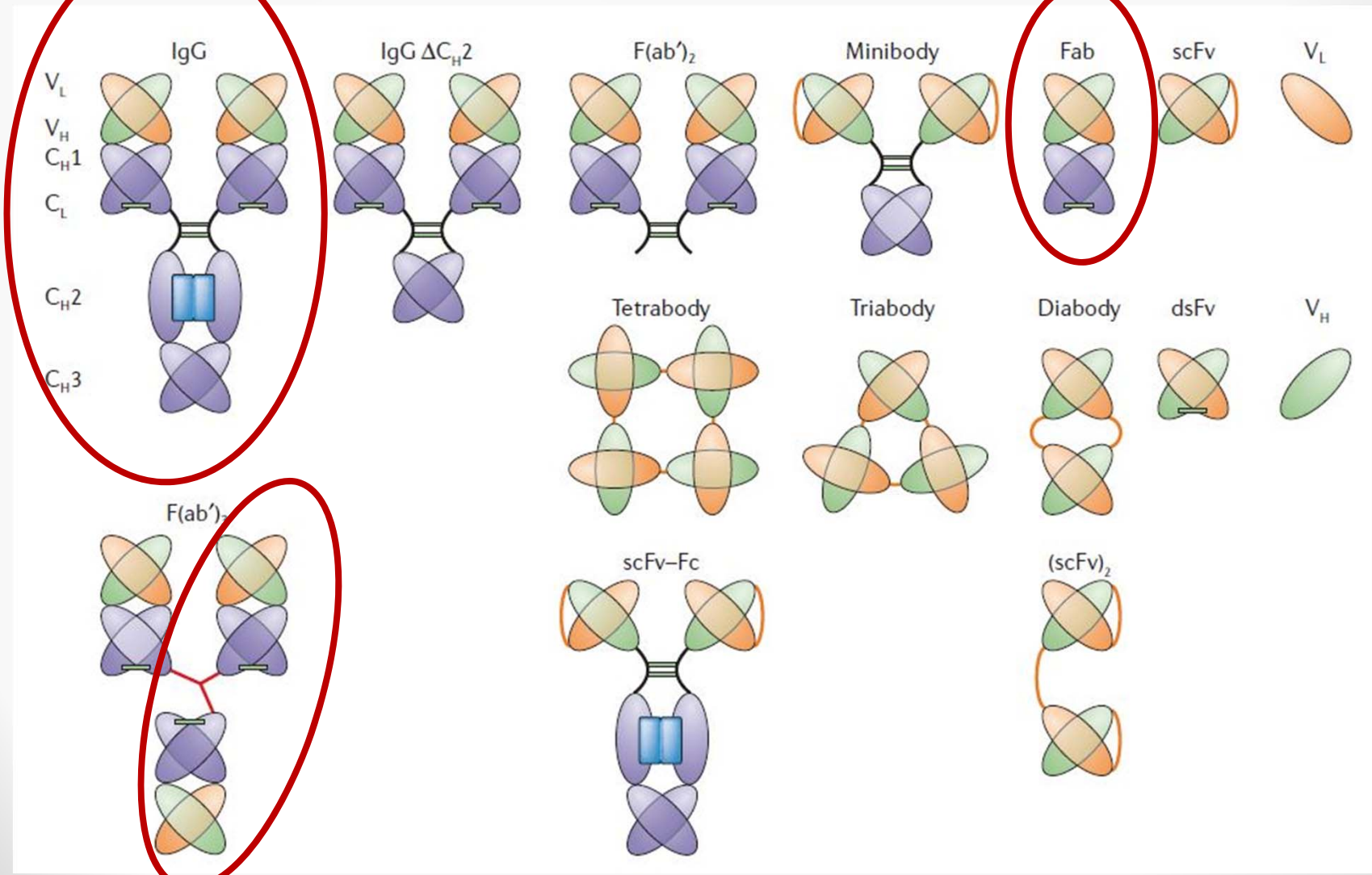
Key-words importanti: ‘phage display library’



Categorie di anticorpi monoclonali

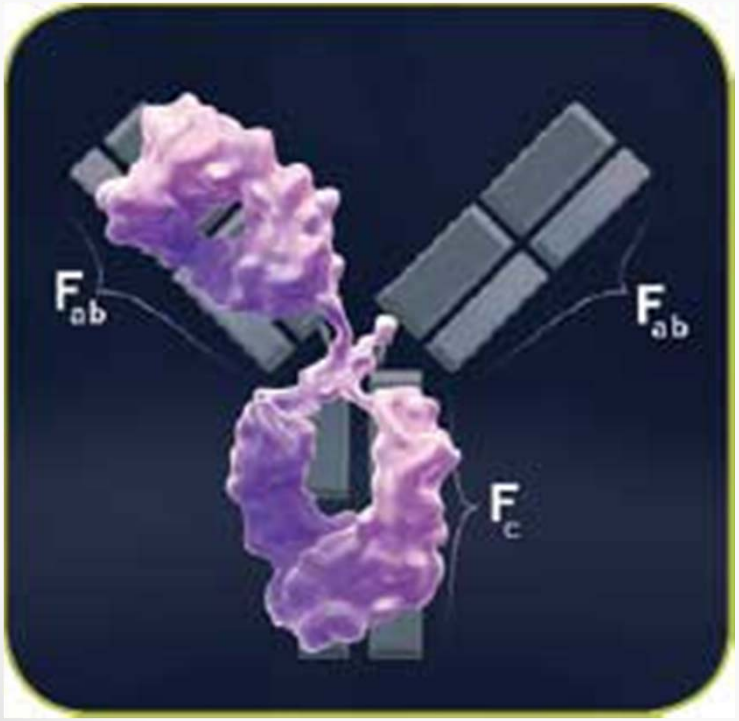


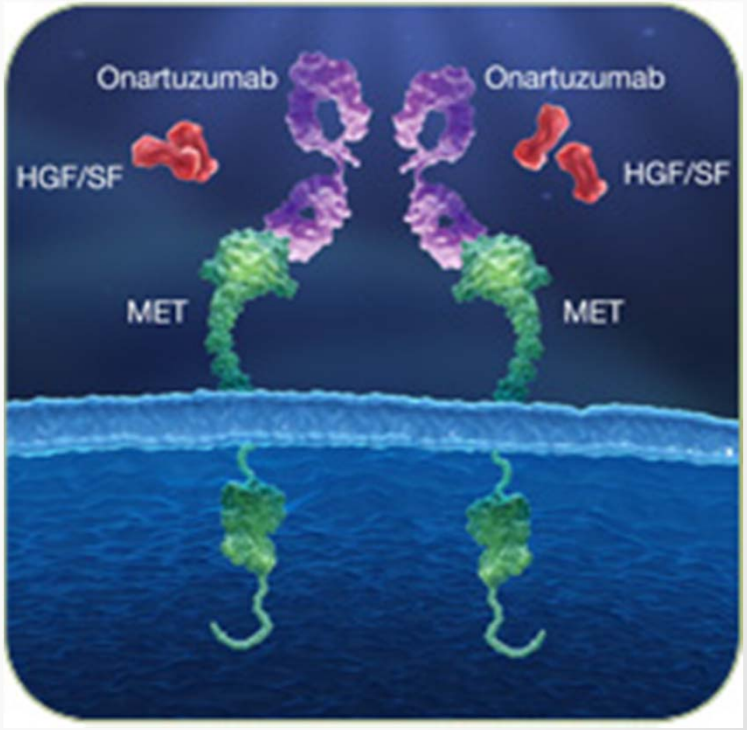
Anticorpi completi e frammenti..

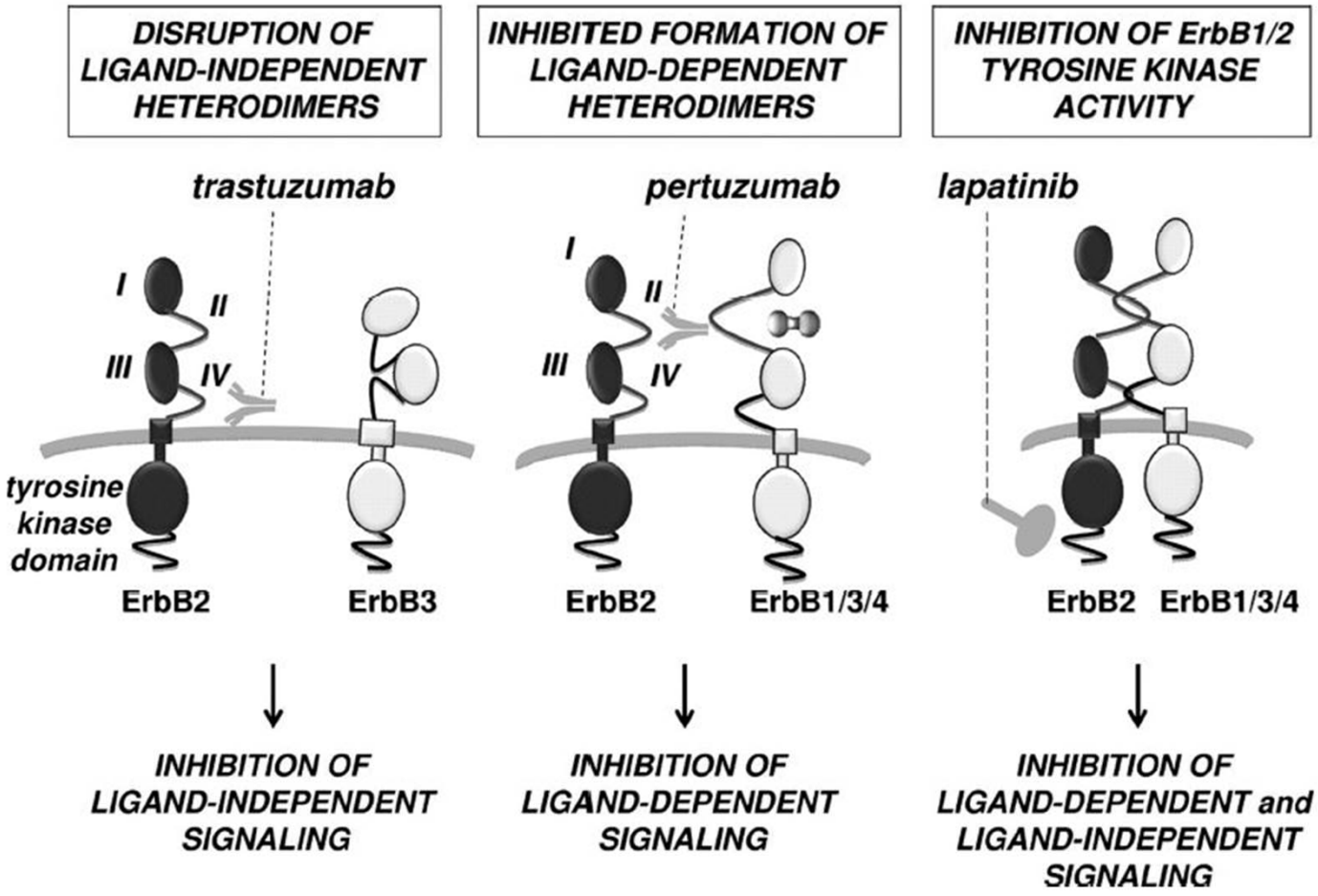


Un esempio di un frammento diventato famoso…

- **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**



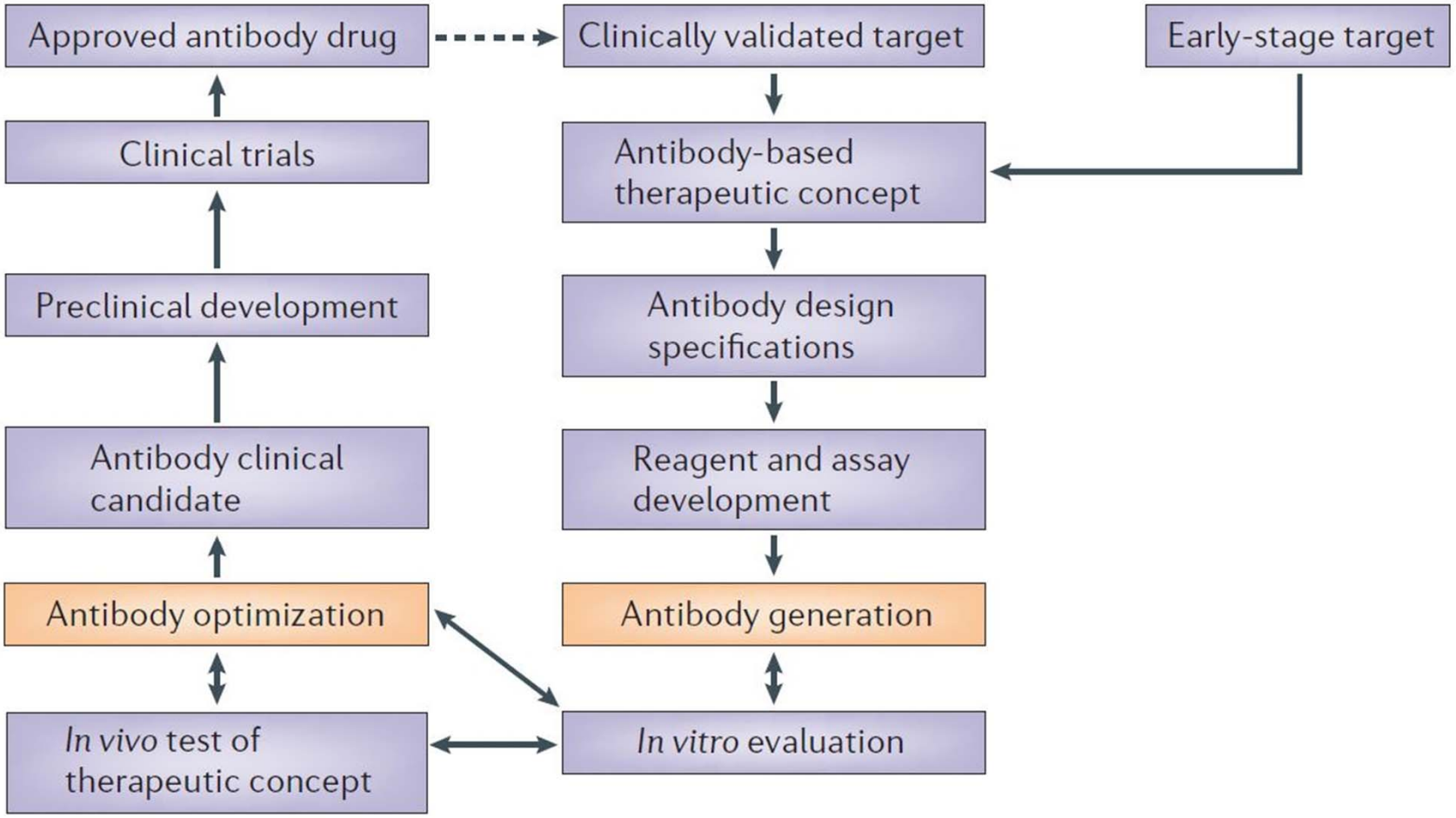




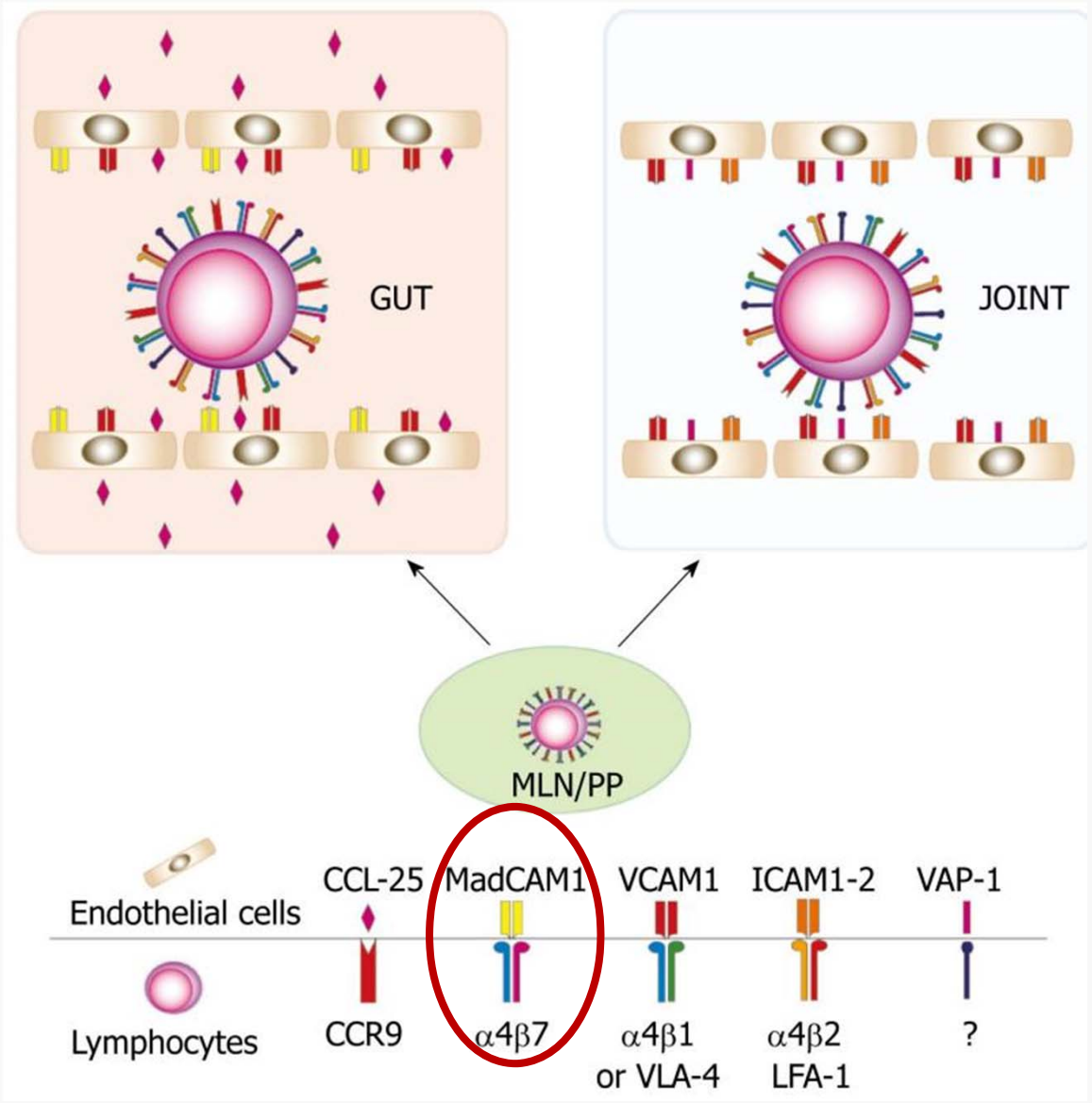
De Keulenaer G W et al. Circulation Research. 2010;106:35-46

American Heart Association.

Schema generale dello sviluppo di un MoAB



Un esempio di '*clinically validated target*'



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ORIGINAL ARTICLE

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