

Farmaci ottenuti con biotecnologie: aspetti generali e specifici

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Qualità e sicurezza dei farmaci biologici e dei farmaci prodotti con biotecnologie (biotecnologici)
Roma 17 maggio 2011



Doc. Ref. EMEA/74562/2006 Rev. 1

What is a **biological medicine**?

A biological medicine is a medicine whose active substance is made by or derived from a living organism. For example, insulin can be produced by a living organism (such as a bacterium or yeast), which has been given the gene that enables it to produce insulin.



“**Biotechnological/biological products**” refers to any products prepared from cells cultivated from cell banks with the exception of microbial metabolites such as, for example, antibiotics, amino acids, carbohydrates, and other low molecular weight substances. Cell banks used to prepare gene therapy products or vaccines should follow the recommendations presented in this document.

Some biological products, such as certain viral vaccines, are prepared in primary cell cultures derived directly from animal tissues or organs. Primary cells are not banked and therefore are not addressed by this document.”

(CPMP/ICH/294/95)



Farmaci Biotecnologici

Esempi:

citochine

ormoni

anticorpi monoclonali



IntronA is a medicine that contains the **active substance interferon alfa-2b**. It is available as a powder and solvent that are made up into a solution for injection or infusion (drip into a vein), and as a ready-to-use solution for injection in a vial or in a multidose pen.

- IntronA is used for the treatment of many diseases, among those: long-term hepatitis B in adults (aged 18 years and older), long-term hepatitis C in patients aged three years and older, hairy cell leukaemia, chronic myelogenous leukaemia, multiple myeloma, ecc..
- The **active substance in IntronA, interferon alfa-2b**, belongs to the group 'interferons'. The interferon alfa-2b in IntronA is produced by 'recombinant DNA technology': it is made by a bacterium that has received a gene (DNA), which makes it able to produce interferon alfa-2b. The replacement interferon alfa-2b acts in same way as naturally produced interferon alpha

Actrapid is a solution for injection. It is available in a vial, a cartridge (PenFill) or in a prefilled pen (NovoLet, FlexPen or InnoLet).

- Actrapid is used in patients who have diabetes
- Diabetes is a disease in which the body does not produce enough insulin to control the blood glucose. Actrapid is a replacement insulin that is identical to the insulin made by the pancreas. The **active substance** in Actrapid, **insulin human (rDNA)**, is produced by a method known as ‘recombinant technology’: the insulin is made by a **yeast that has received a gene (DNA)**, which makes it able to produce **insulin**.



Humira is a medicine that contains the **active substance adalimumab**. It is available as a solution for injection in a vial, pre-filled syringe or pre-filled pen, all containing 40 mg adalimumab.

- Humira is an anti-inflammatory medicine (severe active rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, active and progressive psoriatic arthritis, severe active ankylosing spondylitis, severe active Crohn's disease, psoriasis)
- The active substance in Humira, **adalimumab**, is a **monoclonal antibody**. A monoclonal antibody is an antibody (a type of protein) that has been designed to recognise and attach to a specific structure (called an antigen) that is found in the body. Adalimumab has been designed **to attach to a chemical messenger in the body called tumour necrosis factor (TNF)**.

Aspetti regolatori affrontati in ISS

- *Valutazione degli aspetti legati alla produzione della DS e del DP (Qualità)
- Valutazione degli studi pre-clinici e clinici
- *Controlli Post Marketing

Aspetti regolatori

- Immissione in commercio
- Variazioni

CTD



- Controlli post marketing (OMCL)

OMCL = Official Medicines Control Laboratories Network

Aspetti regolatori

Immissione in commercio

Procedura centralizzata

- autorizzazione al commercio valida per tutta EU
- un solo nome
- un comune SPC (Summary Product Characteristics)
- per il completamento della procedura max 210 giorni



Procedura centralizzata

Ciò comporta:

- Migliore utilizzazione delle risorse
- Considerazioni scientifiche armonizzate
- Informazioni per i medici ed i pazienti armonizzate
- Accesso alle cure per un maggior numero di persone



Requisiti per l'immissione in commercio

Qualità, Sicurezza , Efficacia

Un bilancio positivo rischio/beneficio

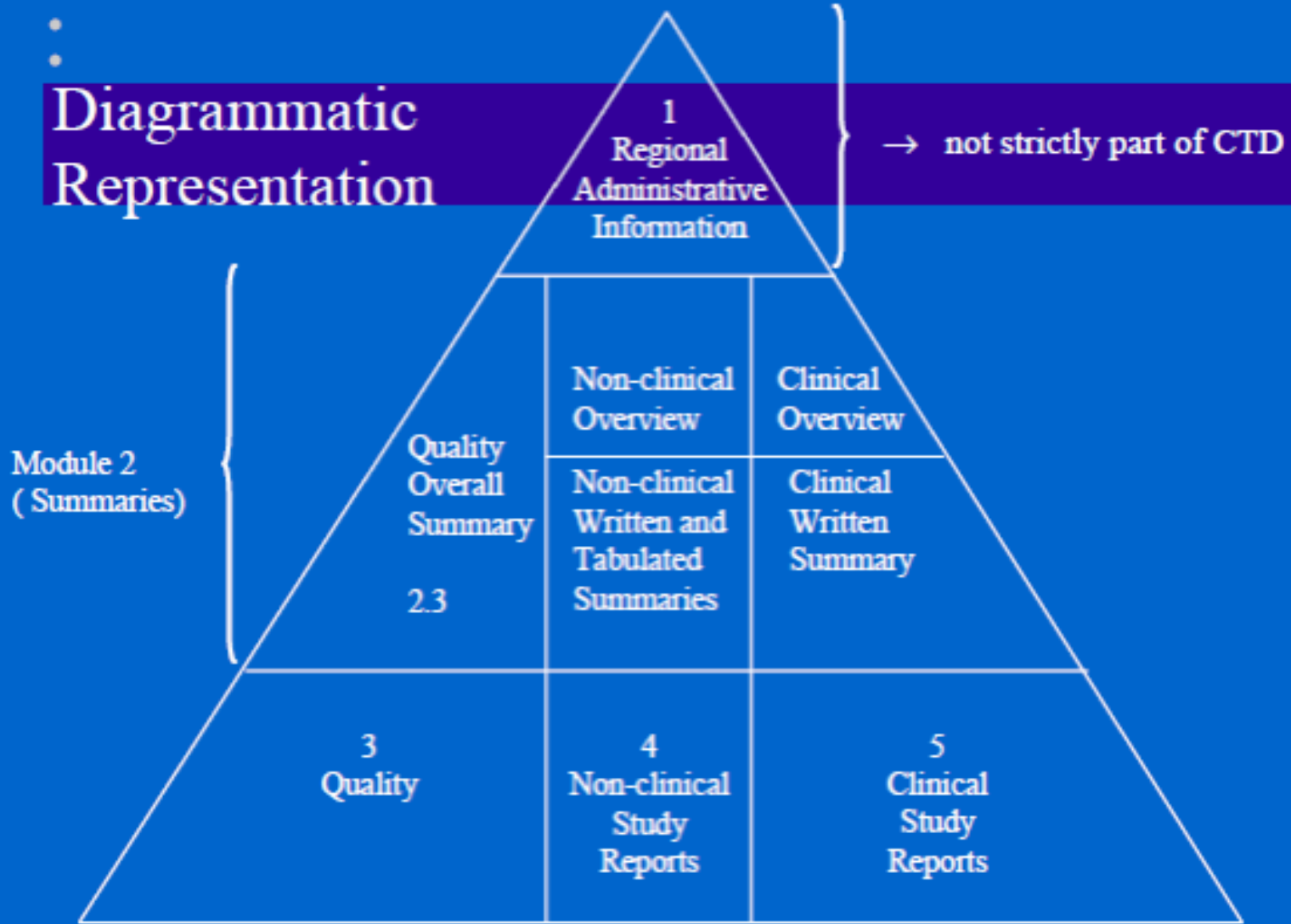


CTD

Common Technical Document



Diagrammatic Representation



CTD-Q basic structure

- **MODULE 1**

Admin and Regional Specific Information

Don't forget molecular structure aspects re: Similarity (1.7)

- although these are outside the main quality/safety/efficacy benefit-risk evaluation for an authorisation.

- **MODULE 2**

CTD Summaries

- Quality Overall Summary (2C) - QOS

- **MODULE 3**

Main body of Quality Data

i.e. The Q dossier will be basically modules 2.3 & 3

Linee guida per la produzione

- **Linee guida (EMA/CHMP/ Biologicals)**

http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000082.jsp&murl=menus/regulations/regulations.jsp&mid=WC0b01ac0580027547

- **Linee guida ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use -**

<http://www.ich.org/home.html>)

- **Farmacopea Europea**

- **Applicazione delle GMP** (*Dott.ssa Salvati*)

Good Manufacturing Practices = GMP

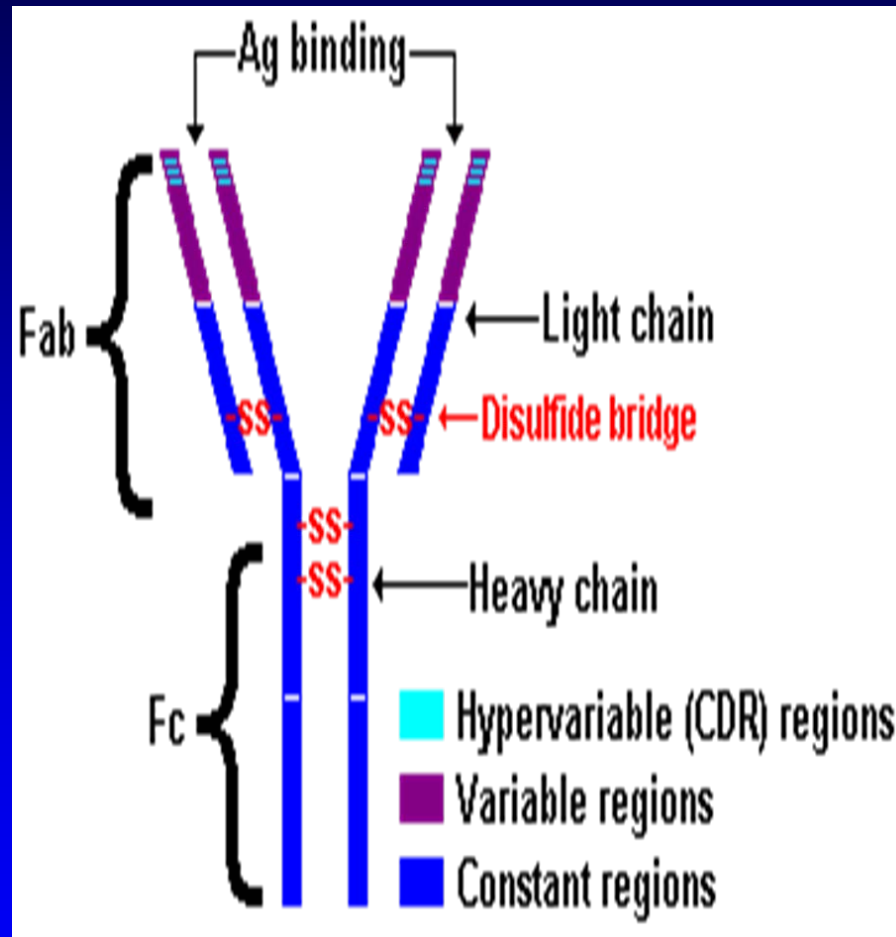


Esempio

Anticorpi monoclonali



ANTIBODIES



ANTIBODIES

POLYCLONAL

Derived from different B Lymphocytes cell lines

Batch to Batch variation affecting Ab reactivity & titre

NOT Powerful tools for clinical diagnostic tests

MONOCLONAL

Derived from a single B cell clone

mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity

Enable the development of secure immunoassay systems.

DS = anticorpo monoclonale purificato



Ad esempio: tecniche di fusione cellulare oppure
**trasfezione di sequenze di interesse in cellule
eucariotiche**

MODULE 3	
CTD	EU CTD (NTA, Vol. 2B, Edition 2001)
3.1	MODULE 3 TABLE OF CONTENTS
3.2	BODY OF DATA
3.2.S	DRUG SUBSTANCE
3.2.S.1	General Information
3.2.S.1.1	Nomenclature
3.2.S.1.2	Structure
3.2.S.1.3	General Properties
3.2.S.2	Manufacture
3.2.S.2.1	Manufacturer(s)
3.2.S.2.2	Description of manufacturing process and process controls
3.2.S.2.3	Control of materials
3.2.S.2.4	Controls of critical steps and intermediates
3.2.S.2.5	Process validation and/or evaluation
3.2.S.2.6	Manufacturing process development
3.2.S.3	Characterisation
3.2.S.3.1	Elucidation of structure and other characteristics
3.2.S.3.2	Impurities
3.2.S.4	Control of drug substance
3.2.S.4.1	Specification
3.2.S.4.2	Analytical Procedures

MODULE 3	
CTD	EU CTD (NTA, Vol. 2B, Edition 2001)
3.2.S.4.3	Validation of analytical procedures
3.2.S.4.4	Batch analyses
3.2.S.4.5	Justification of Specification
3.2.S.5	Reference Standards or Materials
3.2.S.6	Container Closure System
3.2.S.7	Stability

3.2.S.1.2 Structure (name, manufacturer)

Biotech:

The schematic amino acid sequence indicating glycosylation sites or other post-translational modifications and relative molecular mass should be provided, as appropriate.

Reference CPMP Guidelines: "Chemistry of the New Active Substance" and "Chemistry of the Active Substance"

3.2.S.1.3 General Properties (name, manufacturer)

A list should be provided of physicochemical and other relevant properties of the drug substance, including biological activity for Biotech.

Reference CPMP-ICH Guidelines: “Specifications – Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products – Chemical Substances” and “Specifications – Test Procedures and Acceptance criteria for Biotechnological, Biological products”

Attività biologica



3.2.S.2 Manufacture (name, manufacturer)

3.2.S.2.2 Description of Manufacturing Process and Process Controls (name, manufacturer)

The description of the drug substance manufacturing process represents the applicant's commitment for the manufacture of the drug substance. Information should be provided to adequately describe the manufacturing process and process controls. For example:

Biotech:

Information should be provided on the manufacturing process, which typically starts with a vial(s) of the cell bank, and includes cell culture, harvest(s), purification and modification reactions, filling, storage and shipping conditions.

Batch(es) and scale definition

An explanation of the batch numbering system, including information regarding any pooling of harvests or intermediates and batch size or scale should be provided.

Cell culture and harvest

A flow diagram should be provided that illustrates the manufacturing route from the original inoculum (e.g. cells contained in one or more vials(s) of the Working Cell Bank up to the last harvesting operation. The diagram should include all steps (i.e., unit operations) and intermediates. Relevant information for each stage, such as population doubling levels, cell concentration, volumes, pH, cultivation times, holding times, and temperature, should be included. Critical steps and critical intermediates for which specifications are established (as mentioned in 3.2.S.2.4) should be identified.

A description of each process step in the flow diagram should be provided. Information should be included on, for example, scale; culture media and other additives (details provided in 3.2.S.2.3); major equipment (details provided in 3.2.A.1); and process controls, including in-process tests and operational parameters, process steps, equipment and intermediates with acceptance criteria (details provided in 3.2.S.2.4). Information on procedures used to transfer material between steps, equipment, areas, and buildings, as appropriate, and shipping and storage conditions should be provided. (Details on shipping and storage provided in 3.2.S.2.4.)

Purification and modification reactions

A flow diagram should be provided that illustrates the purification steps (i.e., unit operations) from the crude harvest(s) up to the step preceding filling of the drug substance. All steps and intermediates and relevant information for each stage (e.g., volumes, pH, critical processing time, holding times, temperatures and elution profiles and selection of fraction, storage of intermediate, if applicable) should be included. Critical steps for which specifications are established as mentioned in 3.2.S.2.4 should be identified.

A description of each process step (as identified in the flow diagram) should be provided. The description should include information on, for example, scale, buffers and other reagents (details provided in 3.2.S.2.3, major equipment (details provided in 3.2.A.1), and materials. For materials such as membranes and chromatography resins, information for conditions of use and reuse also should be provided. (Equipment details in 3.2.A.1; validation studies for the reuse and regeneration of columns and membranes in 3.2.S.2.5.) The description should include process controls (including in-process tests and operational parameters) with acceptance criteria for process steps, equipment and intermediates. (Details in 3.2.S.2.4.).

Reprocessing procedures with criteria for reprocessing of any intermediate or the drug substance should be described. (Details should be given in 3.2.S.2.5.).

Information on procedures used to transfer material between steps, equipment, areas, and buildings, as appropriate, and shipping and storage conditions should be provided (details on shipping and storage provided in 3.2.S.2.4.).

Aspetti valutati per definire la qualità dei farmaci Biotecnologici

- Controllo del materiale di partenza (3.2.S.2.3)
- Controllo del processo di produzione
- Controllo della DS
- Controllo del prodotto finito (DP)

Controllo del materiale di partenza utilizzato per la preparazione di anticorpi monoclonali

- Analisi dettagliata del costrutto
- Valutazione dello stato del costrutto (integrato, extracromosomale, numero di copie)
- Analisi delle banche di cellule (MCB* e WCB**)
- valutazione della stabilità della MCB e WCB
- valutazione delle cellule parentali, delle cellule partner della fusione e di eventuali feeder
- Qualità dei mezzi di coltura (TSE)

*MCB = *Master Cell Bank*

** WCB = *Working Cell Bank*



MCB : a culture of fully characterized cells processed together to ensure uniformity and stability and used to prepare the working cell banks for production.

- Established from a single clone
- Represents a cell reserve “Frozen in Time”
 - Preserves characteristics
 - Prevents contamination and deterioration

WCB: cells used in pharmaceutical production grown from those maintained in a master cell bank so that their stability and uniformity are well characterized.

- La WCB deriva dalla MCB
- MCB e WCB: sono nuove linee cellulari derivate da linee cellulari già esistenti o da un clone iniziale.



3.2.S.2.3 Control of Materials (name, manufacturer)

Materials used in the manufacture of the drug substance (e.g., raw materials, starting materials, solvents, reagents, catalysts) should be listed identifying where each material is used in the process. Information on the quality and control of these materials should be provided. Information demonstrating that materials (including biologically-sourced materials, e.g., media components, monoclonal antibodies, enzymes) meet standards appropriate for their intended use (including the clearance or control of adventitious agents) should be provided, as appropriate. For biologically-sourced materials, this can include information regarding the source, manufacture, and characterisation. (Details in 3.2.A.2 for both NCE and Biotech)

Biotech:

Control of Source and Starting Materials of Biological Origin

Summaries of viral safety information for biologically-sourced materials should be provided. (Details in 3.2.A.2.)

Source, history, and generation of the cell substrate

Information on the source of the cell substrate and analysis of the expression construct used to genetically modify cells and incorporated in the initial cell clone used to develop the Master Cell Bank should be provided as described in CPMP-ICH Guidelines Q5B and Q5D.

Cell banking system, characterisation, and testing

Information on the cell banking system, quality control activities, and cell line stability during production and storage (including procedures used to generate the Master and Working Cell Bank(s)) should be provided as described in CPMP-ICH Guidelines Q5B and Q5D.

Reference CPMP-ICH Guidelines: “Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin”, “Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA

Derived Protein Products”, “Quality of Biotechnological Products: Stability Testing of Biotechnological/ Biological Products”, “Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/ Biological Products”

(CPMP/ICH/139/95)

**NOTE FOR GUIDANCE ON QUALITY OF BIOTECHNOLOGICAL
PRODUCTS:
ANALYSIS OF THE EXPRESSION CONSTRUCT IN CELL LINES
USED FOR
PRODUCTION OF
r-DNA DERIVED PROTEIN PRODUCTS**



March 1998
CPMP/ICH/294/95

ICH Topic Q 5 D
NOTE FOR GUIDANCE ON
QUALITY OF BIOTECHNOLOGICAL PRODUCTS: DERIVATION AND
CHARACTERISATION OF CELL SUBSTRATES USED FOR
PRODUCTION OF
BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS
(CPMP/ICH/294/95)



Aspetti valutati per definire la qualità dei farmaci Biotecnologici

- Controllo del materiale di partenza
- Controllo del processo di produzione della DS
- Controllo della DS
- Controllo del prodotto finito (DP)

Produzione biotecnologici: Dalla WCB alla Drug Substance

- Definizione dei processi di fermentazione
 - single harvest, multiple harvest and extended population
- Procedimento di purificazione
 - Intermedi avanzati e prodotti finiti ben caratterizzabili
 - convalide di processo e specifiche per le impurezze quali, ad esempio, proteine e acidi nucleici dell'ospite
 - inattivazione/rimozione virale

Aspetti valutati per definire la qualità dei farmaci Biotecnologici

- Controllo del materiale di partenza
- Controllo del processo di produzione
- **Controllo della DS (3,2,S.4.4)**
- Controllo del prodotto finito (DP)

DS

- **Ripartizione**

- **Conservazione**

→ • **Stabilità**

DS = anticorpo monoclonale purificato



DP* ripartito nel contenitore finale = DS diluita +
eccipienti e/o conservanti

* *Asepsi (Dott.ssa Salvati)*

3.2.P	DRUG PRODUCT
3.2.P.1	Description and composition of the drug product
3.2.P.2	Pharmaceutical Development
3.2.P.2.4	Controls and critical steps and intermediates
3.2.P.3	Manufacture
3.2.P.3.1	Manufacturer(s)
3.2.P.3.2	Batch formula
3.2.P.3.3	Description of Manufacturing Process and Process Controls
3.2.P.3.4	Controls of critical steps and intermediates
3.2.P.3.5	Process validation and / or evaluation
3.2.P.4	Control of excipients
3.2.P.4.1	Specifications
3.2.P.4.2	Analytical procedures
3.2.P.4.3	Validation of analytical procedures
3.2.P.4.4	Justification of specifications
3.2.P.4.5	Excipients of human or animal origin
3.2.P.4.6	Novel Excipients (<i>ref to A 3</i>)
3.2.P.5	Control of drug product
3.2.P.5.1	Specification(s)
3.2.P.5.2	Analytical Procedures
3.2.P.5.3	Validation of Analytical Procedures
3.2.P.5.4	Batch analyses
3.2.P.5.5	Characterisation of Impurities
3.2.P.5.6	Justification of specification(s)
3.2.P.6	Reference Standards or Materials
3.2.P.7	Container Closure System
3.2.P.8	Stability

Aspetti valutati per definire la qualità dei farmaci Biotecnologici

- Controllo del materiale di partenza
- Controllo del processo di produzione
- Controllo della DS
- Controllo del prodotto finito (DP)

Controllo del prodotto biotecnologico

- Caratterizzazione fisico chimica
 - peso molecolare, punto isoelettrico
- Analisi strutturale
- Modifiche post traduzionali
 - glicosilazione, acetilazione, idrossilazione, deaminazione, ossidazione, ecc.
- Dati conformazionali
 - light scattering, spettroscopia UV, CD e spettrometria di massa
- Attività biologica



Esempi di metodi

- Aminoacid sequence
- Aminoacid composition
- Terminal amino acid sequence
- Peptide map
- Sulfhydryl groups and disulfide bridges
- Carbohydrate structure
- MW (HPLC, SDS-PAGE, mass spectrometry)
- Isoforms (IEF)
- Extinction coefficient (UV, visible)
- Electrophoresis
- Liquid chromatography
- CD, NMR

Controllo del prodotto biotecnologico

- Sicurezza virale (Dott.sse Wirz e Luciani)
- TSE
- Conservanti
- Stabilità

TSE

I Prioni sono un buon esempio di rischio che non può essere facilmente valutato

http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000082.jsp&murl=menus/regulations/regulations.jsp&mid=W00b01ac0580027547



TSE e biotecnologici

Le problematiche legate la TSE riguardano:

- culture media (growing of cells, media fill)
- gelatine (capsules)
- tallow derivatives (stearic acid)
- lanoline
- Milk

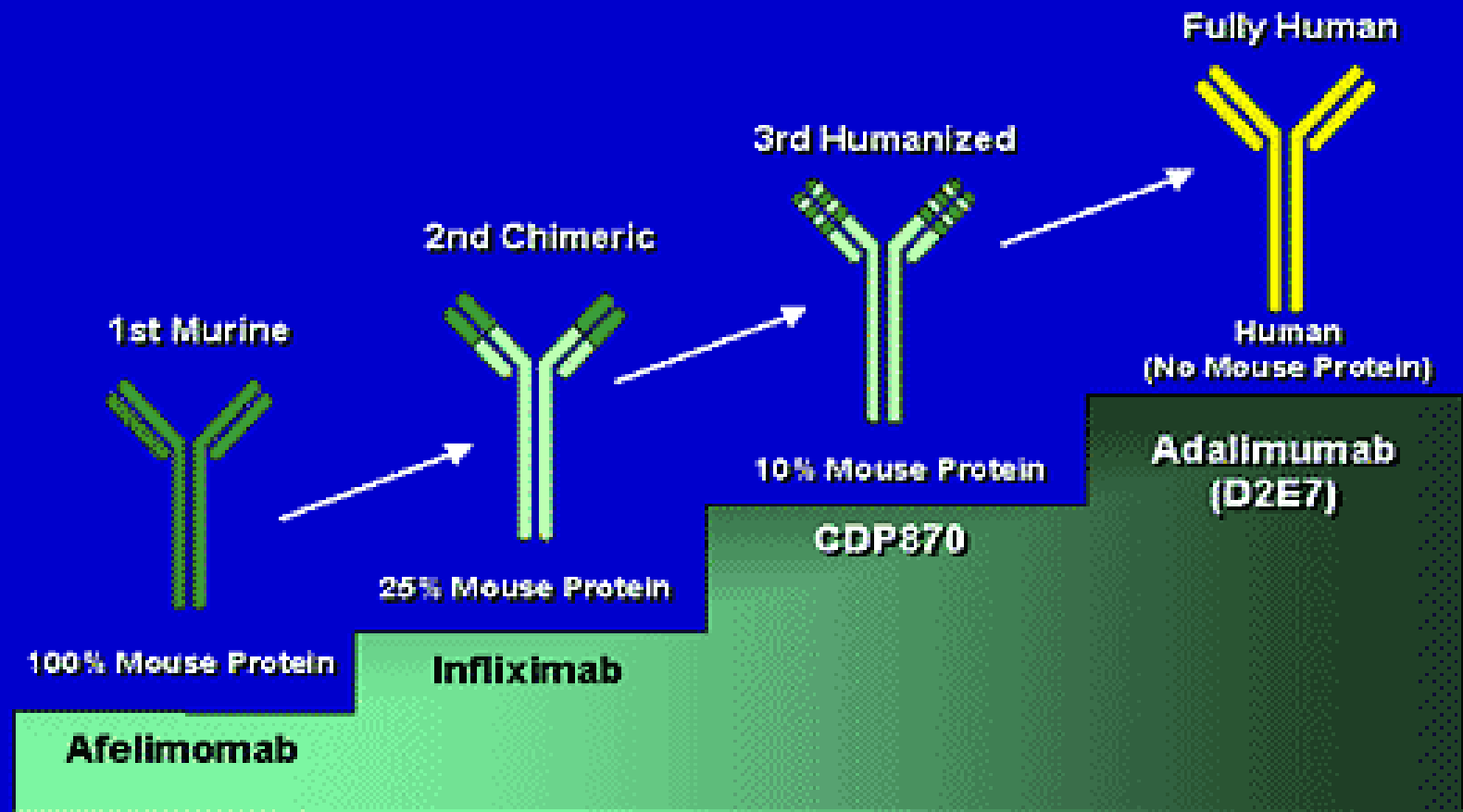
Cioè tutti i prodotti che originano da animali ruminanti

Contenitore primario

Stabilità del DP

3.2.P.7	Container Closure System
3.2.P.8	Stability

EVOLUTION OF MONOCLONAL ANTIBODY



Some monoclonal antibodies that have been introduced into human medicine

To suppress the immune system

- **Muromonab-CD3 (OKT3)** and two humanized anti-CD3 monoclonals. Bind to the CD3 molecule on the surface of T cells. Used to prevent acute rejection of organ, e.g., kidney, transplants. The humanized versions show promise in inhibiting the autoimmune destruction of beta cells in Type 1 diabetes mellitus.
- **Omalizumab (Xolair®)**. Binds to IgE thus preventing IgE from binding to mast cells. Shows promise against allergic asthma.
- **Daclizumab (Zenapax®)**. Binds to part of the IL-2 receptor exposed at the surface of activated T cells. Used to prevent acute rejection of transplanted kidneys. Has also showed promise against T-cell lymphoma.



To kill or inhibit malignant cells

- **Rituximab** (Rituxan®). Binds to the CD20 molecule found on most B-cells and is used to treat B-cell lymphomas.
- **Cetuximab** (Erbix®). Blocks HER1, a receptor for **epidermal growth factor** (EGF) that is found on some tumor cells (some breast cancers, lymphomas).
- **Lym-1** (Oncolym®). Binds to the HLA-DR-encoded histocompatibility antigen that can be expressed at high levels on lymphoma cells.
- **Ipilimumab** (Yervoy®) that acts to enhance the body's own immune response to tumors

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EPAR (European Public Assessment Reports) <http://www.ema.europa.eu>

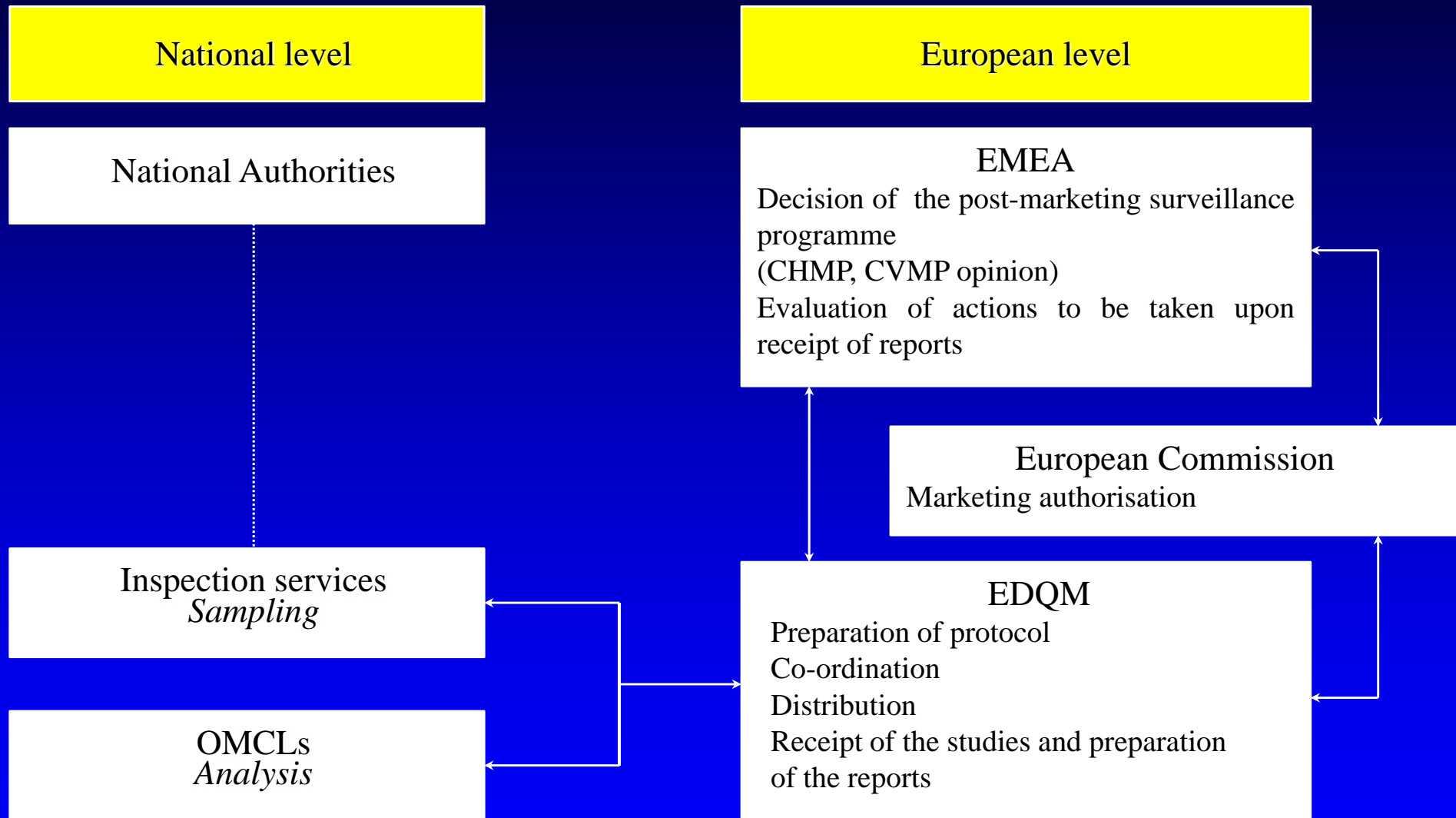


Post-Marketing Surveillance CAP

Centrally Authorised Products



CAP Procedure Scheme



Esempio: Anticorpo monoclonale

On the 25th October 2005, the European Commission issued a Marketing Authorisation for the medicinal product **Xolair, 150mg, Powder and solvent for solution for injection**. This product was selected for the 2010 Sampling and Testing CAP and tested within the EEA Network of Official Medicines Control Laboratories (EEA OMCLs Network).

Omalizumab (Xolair®). Binds to IgE thus preventing IgE from binding to mast cells.



Una terapia biotecnologica richiede tecniche di produzione e processi distributivi complessi e specialistici. I medicinali biotecnologici derivano infatti da fonti naturali: per questo motivo sono spesso meno stabili delle molecole sintetiche e pertanto necessitano di un trattamento e di una distribuzione speciali.

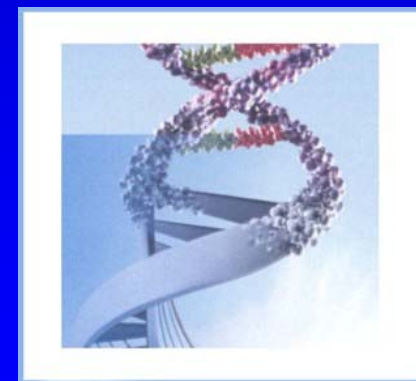


Occorrono molti anni per identificare una molecola target (responsabile di una patologia), determinarne la sequenza genica ed elaborare un processo per produrre un trattamento stabile, attivo biologicamente e riproducibile.



Circa il 50% di tutti i nuovi farmaci in sviluppo originano dalle biotecnologie, e la proporzione cresce nei trattamenti più innovativi come: ormoni della crescita, fattori di crescita ricombinanti, vaccini, anticorpi monoclonali per il trattamento di tumori e malattie infiammatorie/infettive, terapia cellulare ecc.

Il settore delle biotecnologie per la cura della salute cresce ogni anno di circa il 15%, più del doppio rispetto alla farmaceutica tradizionale.



Grazie per l'attenzione

