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Characterizing and Controlling Modes of Action

Critical Requirements for Potency Assays

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Overview of this talk

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- My background
  - PhD/Post-Doc in Biotechnology
  - Quality assessor for biological medicinal products (with focus on therapeutic recombinant proteins) since 12/2021
  - Centralised Procedures & Life Cycle
  - National and European Scientific Advice procedures

- The following presentation will discuss
  - Complexity of Potency Assays - Examples
  - Method validation
  - Regulatory requirements

Definition

Potency

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- Potency (expressed in units) is the quantitative measure of **biological activity** based on the attribute of the product which is linked to the relevant biological properties.
  (ICH Q6B, EMA/CHMP/BWP/532517/2008)

- The measure of the **biological activity** using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product, which is linked to the relevant biological properties. (EMA/CHMP/BWP/271475/2006 rev.1)

- Potency is a measure of a drug's **biological activity** expressed in terms of the dose required to produce a pharmacological effect of given intensity. (Wikipedia)

Description

Potency Assay

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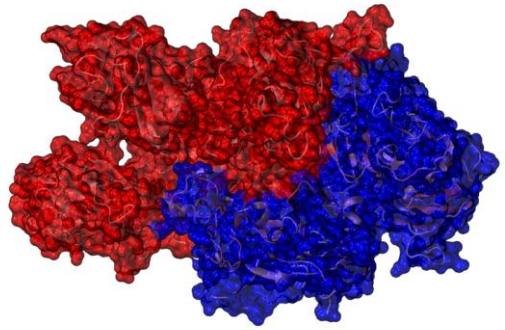
- **Parameters of an optimal assay**
  - Reflective of the (complete) mechanism(s) of action (MoAs) of the product
  - Sensitive to structural changes that impact product safety and efficacy
  - Stability indicating
  - Well controlled (low CV, tight precision, etc.)
  - Usable as a QC release assay

- **Why so important?**
  - Active substance understanding
  - Bridging product activity over development
    (Comparability of batches for clinical study and commercial material)

Case Study 1- Factor VIII

Enzyme based potency assay



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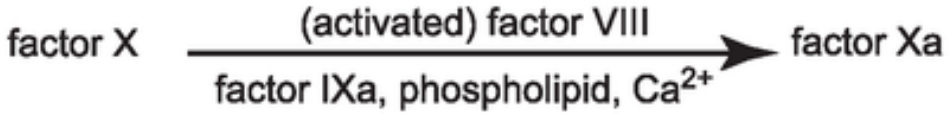
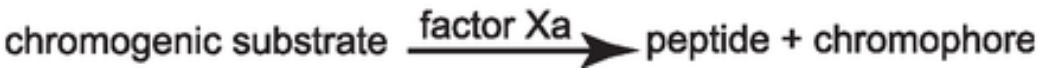
- Mechanism of Action
  1) Interplay of F-VIII together with phospholipids, Ca^{2+} and F-IX
  2) Assembly of Tenase complex on surface of thrombocytes
  3) Activated Tenase complex activates F-X – active blood clotting cascade

- Assay
  - Ph. Eur. 2.7.4
  - (One-stage clotting assay)

Step 1

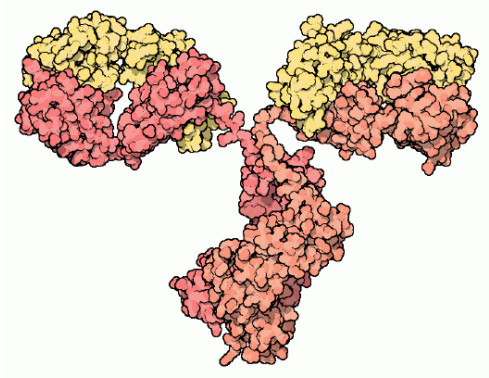


Step 2



Case Study 2 – Bevacizumab

Target binding



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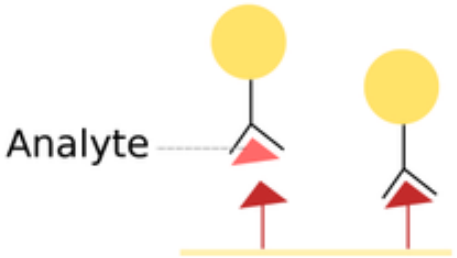
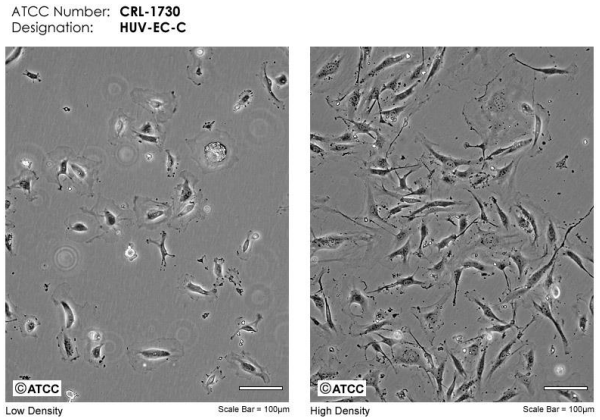
- Mechanism of Action
  1) Binding of mAB (IgG1) to VEGF A
  2) Inhibition of VEGFR dimerization
  3) Cell proliferation/angiogenesis is blocked – treatment of cancer

- Assays
  - Competitive binding ELISA

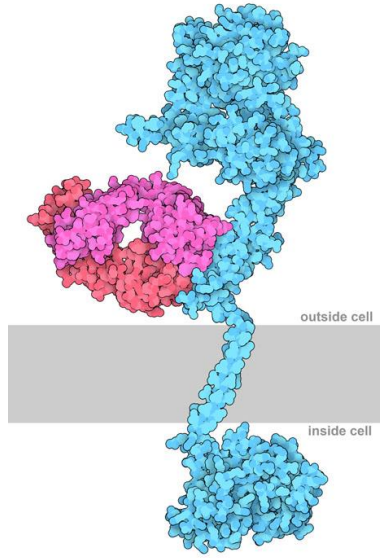


  - HUVEC proliferation inhibition bioassay



Case Study 3 – Trastuzumab

Target binding plus effector functions



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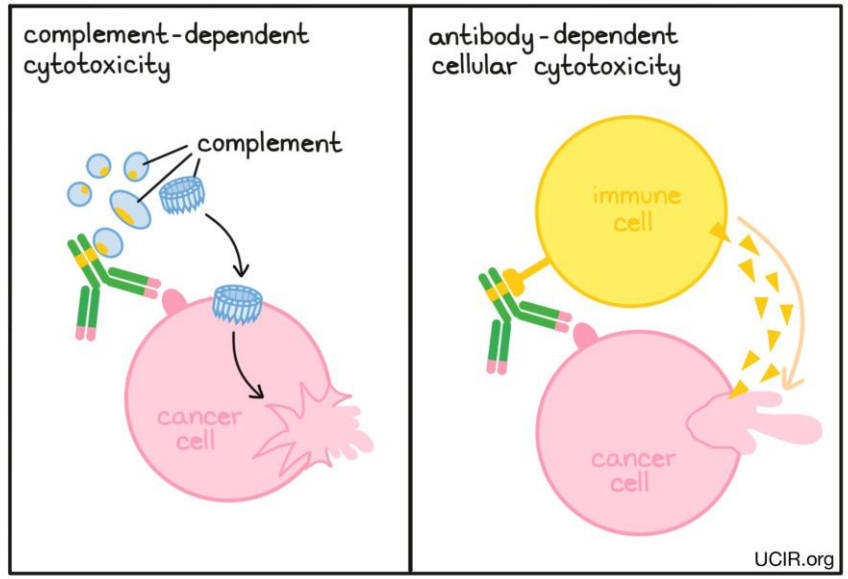
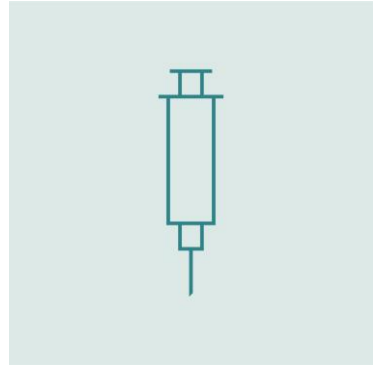
- Mechanism of Action
  1) Binding of mAB (IgG1) to HER2 receptor
  2) Dimerization of HER2 is blocked
  3) Cell proliferation/survival/mobility is inhibited
  4) Activation of ADCC and CDC
  5) Tumor-cell lysis – treatment of cancer

- Assays
  - HER2 binding by ELISA
  - Proliferation inhibition bioassay
  - ADCC/CDC assay



Case Study 4 - Vaccines

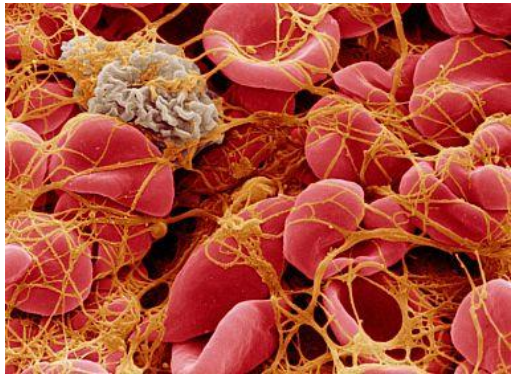
Mechanism of Action - Formation of specific antibodies



- Plaque-forming units
  - Smallpox vaccines Ph. Eur. 04/2022:0164
- ELISA
  - Antibody titer after immunisation of mice/guinea-pigs for Pertussis vaccine Ph. Eur. 2.7.16
- Quantification of antigen
  - Saccharide content for Pneumococcal polysaccharide vaccine Ph. Eur. 01/2019:2150
  - Haemagglutinin content for Influenza vaccine Ph. Eur. 07/2022:0869
- Animal challenge assay
  - Tick-borne encephalitis vaccine (mouse), Ph. Eur. 01/2019:1375
    PD_{50}, high variability, 3 R´s

Case Study 5 – ATMP

Factor IX AAV gene therapy



NIH, doi: 10.1038/nmat4066

- Mechanism of Action
  1) F-IX converted to its active form F-IXa by F-XIa
  2) together with phospholipids, Ca^{2+} and F-VIII
  3) build Tenase complex activating F-Xa (active form of F-X) – active blood clotting cascade

- Assay
  - expression assay
  - amount of F-Xa directly proportional to activity of F-IX expressed
  - amount of F-Xa determination through hydrolysis of chromogenic F-Xa substrate

Case Study 6 – ATMP

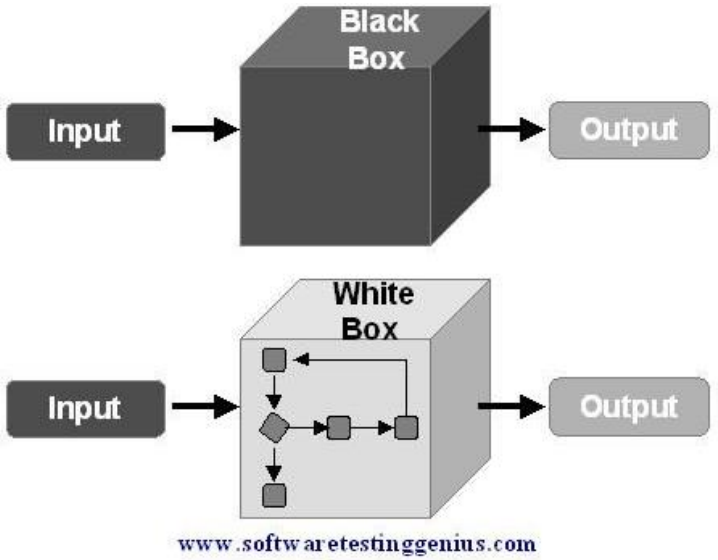
AAV gene therapy - unfavourable potency assay

- Usage of AAV product carrying gene needed for mitochondrial respiratory chain activity

- Assay
  - mRNA level, vg titer/infectious titer

  (complete) mechanism(s) of action (MoAs) not reflected

  - Compromise for complex MoA via EMA-SA
  - Black box testing
    oxygen consumption rate (mitochondrial respiration), extracellular acidification rate (glycolysis)



Method validation

General guidance

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- ICH Q2 (R2) Validation of analytical procedures (under revision)
  Validation principles covering a wider range of analytical procedures (spectroscopic/multivariate statistical analyses/biological products)
  (EMA/CHMP/ICH/82072/2006)

- ICH Q14 Analytical procedure development (under public consultation)
  Science and risk-based approaches for developing and maintaining analytical procedures (EMA/CHMP/ICH/195040/2022)

- ICH M10 Bioanalytical method validation
  Focus on chromatography and ligand binding assay validation parameters
  (EMA/CHMP/ICH/172948/2019)

Method validation

Considerations

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- Qualification term not defined but used for instrument/facilities
- Early suitability needed
- Limited sample availability, sample stability
- ATMPs: often pivotal PhI/II clinical studies
- Acceptance criteria can be adjusted during development
- Broad acceptance criteria better than „report results“ for essential parameters
- Selection of Reference Standard

Regulatory requirements

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- (Full) validation not mandatory for clinical trial approval (except methods related to safety)
  → sufficient product control required before start of clinical studies
- Validated functional assay required at time of MAA
- Comparability of (clinical) batches to intended commercially manufactured batches must be demonstrated
- ATMPs: “pivotal” PhI/II clinical studies - MAA considerations apply early (early interaction with regulatory agencies recommended)
- Stability data
- Comply with Ph. Eur. standard methods
- Characterisation/Establishment Reference standard system

Tack så mycket!

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Thank you!