Requirements for quality control and registration of influenza vaccines developed using novel biotechnological approaches

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Outline of Presentation:

⇒ **INTRODUCTION TO BASIC REGULATORY REQUIREMENTS:**
General aspects of quality, preclinical and clinical evaluation of vaccines

⇒ **REGULATORY REQUIREMENTS FOR NOVEL INFLUENZA VACCINES:**

- Bacterial expression of immunogens
- Novel cell substrates
- Novel adjuvanting systems
- LAIV and recombinant viral vector based vaccines
- DNA and RNA based vaccines
GENERAL ASPECTS:
Basic Regulatory Requirements
Potential scenarios with increasing “regulatory challenging potential”:

- **LOW:** Pertinent regulatory guidance available / licensed vaccines 😊
- **INTERMEDIATE:** Insufficient specific guidance / similar vaccines evaluated or licensed 😐
- **HIGH:** No or insufficient specific guidance / product represents an absolute novelty 😞

⇒ The underlying regulatory rationale for evaluation of novel vaccines and/or innovative technologies:

“Transfer - as much as/whenever possible – existing knowledge, considerations and decisions made before for similar products or technologies”

**Aim:**
- Consistent, reliable and transparent regulatory requirements for all products
- Scientifically sound decisions to assure safety and efficacy
**Quality Requirements for all (innovative/novel) Vaccines:**

Complete description and definition of all quality-related attributes is key requirement:

- **Manufacturing process:** comprehensive description, all in-process tests and specifications
- **Consistent and robust manufacturing:** validation of process and analytical methods
- **Materials and substrates:** quality, origin and sources (supplier), tests, certificates
- **Developmental steps** of manufacture and materials applied (history)
- **Absence of extraneous agents:** viruses, bacteria, TSE, … (“PCV”!!)
- **Full characterization** of (intermediates) and final products: physicochemical, functional,...
- **Stability** of intermediates and final products
- **Valid potency assay** for batch release – correlation with clinical efficacy

⇒ **To be fully established prior to licensure**

**Even for very innovative products**

Regulatory Guidance:

Numerous guidelines and recommendations for specific quality issues: WHO, Ph. Eur., USP, ICH,...

ICH Q6B: Specifications: Test procedures and acceptance criteria for Biotechnological and biological products

EMA: GL on requirements for quality documentation concerning biological IMP in clinical trials
Basic Preclinical Requirements

**Preclinical Issues:**

- **Relevant animal model(s):** Pharmacodynamics vs Safety evaluation
- **Validated/approved methodology**
- **Pharmacodynamics:** "Proof-of-Principle" investigations
  - **Immunogenicity:** Immune response (adaptive: humoral, cellular; innate,...)
  - **Protection:** Disease symptoms, death, virus replication/shedding ("Correlate of protection")
- **Pharmacokinetics:** normally not needed for vaccines, but: *product-specific-considerations*
- **Safety Pharmacology:** cardiovascular, respiratory effects, ...
- **Toxicology**
  - Single and repeated dose toxicity
  - Local and systemic tolerance
  - Reproduction and developmental toxicity (dependent on claimed indication/target groups)

**WHO guidelines on the nonclinical evaluation of vaccines, 2005**
**EMA NfG on preclinical pharmacological and toxicological testing of vaccines, 1997**
**ICH S6: Preclinical safety evaluation of biotechnology-derived pharmaceuticals (basic considerations)**
**Specific guidance on individual topics: Repeated dose toxicity testing, local tolerance testing...**
**Basic Clinical Requirements**

**CLINICAL ISSUES:**

- **Consider** appropriate target population
- **Pharmacodynamics:**
  - **Immunogenicity:** comprehensive characterisation of immune response (product-specific considerations, eg IgG vs IgA,...)
  - **Protection** from infection, disease (“Correlate of protection”), efficacy and effectiveness,
  - **Dose-finding studies**
- **Safety:** collect data after each dose, monitoring of general AEFI and potential product-specific risks
  - Detect adverse events with frequency between 1/100 and 1/1000
    → Data from app. 3000 subjects (very rough estimate)
    (mostly: much more, eg rotavirus and HPV vaccines; but: pandemic situation!!??)

**WHO guidelines on clinical evaluation of vaccines: regulatory expectations, 2004**
*EMA guideline on clinical evaluation of new vaccines, 2006*
*EMA Guideline on strategies to identify and mitigate risks for first-in-man clinical trials..., 2007*
*First-in-human clinical trials with vaccines – what regulators want; Goetz et al., 2010, Nature Biotechnology*
*ICH S8: General considerations for clinical trials, 1997*
*EU clinical trials directive (2001/20/EC) and GCP guidance documents*
Overview to Influenza Virus Immunogenic Components

**Surface Antigens**

- **NEURAMINIDASE NA:** Release of progeny viruses
- **M2 PROTEIN:** Ion channel
- **HEMAGGLUTININ HA:** Receptor binding Fusion mediation

**Internal components**

- **MATRIX PROTEIN M1:** Structural component
- **Ribonucleoprotein (RNP):**
- **NUCLEOPROTEIN (NP):**
- **POLYMERASES PB1, PB2, PA:** 8 RNA segments

**Future concepts/approaches to achieve:**

- **LONG LASTING IMMUNITY:**
  Immunity to T-cell epitopes on viral components: NP, M1, HA stem, (viral polymerases) and LAIV

- **BROADLY PROTECTIVE IMMUNITY:**
  B-cell and T-cell epitopes on (highly) conserved viral proteins: M2, NP, M1, HA stem and LAIV

- **INCLUSION OF NOVEL / MODIFIED VIRAL COMPONENTS AS VACCINE ANTIGENS**
Novel Cell Substrates for Vaccine Manufacture
Prokaryotic Expression Systems

Examples of Vaccine Candidates in (Pre)Clinical Trials: Recombinant and Fusion Proteins

Mono/oligomeric conserved viral protein(domain)
- M2-protein extracellular domain (M2e),
- NP, M1
- HA2-subunit (conserved region)
- combinations: eg HA, NP, M1, M2e

- fused to carriers, or immune-activators
  - HBV-core particles
  - flagellin
  - Glutathion S transferase
  - keyhole limpet hemocyanin

Regulatory Issues and Requirements:
- Description of manufacture, also of all recombinant DNA operations and constructs
- Description and characterisation of all materials used (purity, absence of contaminants)
  → Products from bacterial expression system highly purified – no enhanced risk for contamination
- Stability of DNA constructs and expression profile during manufacture
- Full characterisation of structural/functional properties of expressed proteins
- Established control and release assays (potency!!)
- Note: Bacterial expression system

Ph. Eur.: Vaccine for human use, 2008
Ph. Eur.: Recombinant DNA technology, 2008
Eukaryotic Manufacturing Systems – Quality Aspects

No Novel Continuous Cell Substrate Have Recently Attracted Immense Regulatory Attention:
- Vero cells (simian): Influenza, Japanese Encephalitis, Rabies, IPV, ….
- MDCK (canine): seasonal and pandemic Influenza
- T. ni, SF9 (insect-lepidopteran): HPV vaccine, Influenza
- PER.C6 (human): Influenza

Guiding Principles for the Applicability of Cell Substrates Are Well Established:
- Genetic and phenotypic identification and stability (eop!)
- History of derivation and materials used then and now
- Comprehensive extraneous agents evaluation:
  - Testing in vitro and in vivo plus cell-type specific testing (acc. to source, origin) (“PCV”!!)
  - Susceptibility and replication efficiency of potential contaminants
  - Virus inactivation and removal capacity of manufacturing process (“model virus studies”)
  - TSE safety evaluation (derivation and passaging history!!)
- Tumorigenicity (Vero, MDCK,…)
- Validated or approved methodology (eg PCR detection of potential contaminants)

WHO: Recommendations for evaluation of cell substrates for manufacture of biological..., 2010
Ph. Eur. 5.2.3: Cell substrates for the production of vaccines for human use
Ph. Eur. 5.1.7: Viral safety; Ph. Eur. 2.6.16: Extraneous agents testing in viral vaccines for human use
ICH Q5A: Viral safety evaluation of products from cell lines...; ICH Q5D: Derivation ... of cell substrates...
EMA: NfG on virus inactivation and removal validation studies
TSE safety regulations (WHO, EMA,...)
Most Recently Licensed in the US:

- Trimeric HA expressed in SF9 Insect cell ("FluBlok"): seasonal trivalent vaccine containing 45µg HA (of each subtype)

(Pre)Clinical Evaluation:

- VirusLikeParticles: VLP (with VLP-inducers, eg HIV or HBV core proteins)
  M2e, HA stalk domain or “headless HA”, NA, M1 (or combinations)

  Expression in:  - Insect cells (“licensed” before)
  - 293 cells (human embryonic kidney) (not registered)
  - Plants/Plant cells (Tabac) (not registered)
  (- also in bacterial systems)

Important Regulatory Aspects:

- Expression system (Q): transfected plasmids, recombinant baculoviruses
- Consistent manufacture and testing regime (Q): purity, yields, Process and assay validation, cell banking system, virus seeds
- Characterisation/confirmation of structure/function relation (Q): VLP Relevant validated assays (potency)
- Proof of principle, pharmacodynamics, safety in preclinical models
- Clinical evaluation for safety and efficacy (immunogenicity)
  - Reactogenicity, local tolerance due to cell residuals
  - B-/T-cell responses, cross-reactivity
Vaccines with Novel Adjuvanting Systems

⇒ Still one of the most promising and effective approaches for
  ✓ Antigen sparing (eg in pandemic situation)
  ✓ Priming and boosting of potent immune response

!! TO BE USED BASED ON VERY THOROUGH BENEFIT-RISK EVALUATION!!
### Novel Adjuvanting Systems – Quality Aspects

**Examples of established and upcoming novel adjuvants:**

- **Oil-in-Water adjuvants:** AS03, MF59, AF03 → registered with influenza vaccines
- **Bacterial cell wall components:** MPL → registered with HPV and HepB vaccines
- **Bacterial-like oligonucleotides:** CpG
- **Saponins / “ISCOMS”:** QS21
- **Liposomes / Virosomes**
- **Polysaccharides:** Inulin

### Complete Quality Data Package Required for Licensure:

**Adjuvant alone:**

- **Composition and manufacture** (starting materials, validated process, IP-testing,...)
- **Characterization:** specific parameters and assays (chemical, physical, functional, purity,...)
- **Stability evaluation**

**Combination of antigen and adjuvant:**

- **Mode of association / interaction, combination procedure**
- **Comprehensive testing regimen, validated assays, specifications**
- **Stability of combination**
Novel Adjuvanting Systems – (Pre)Clinical Aspects

Due to diverse nature and multiple functional aspects: Extensive (pre)clinical evaluation
⇒ Use of adjuvant to be clearly justified by (pre)clinical data

**Preclinical:**
- Suitable/relevant animal model(s): pharmacology vs safety
  - Pharmacodynamics /Mode of action:
    - “Proof of concept” studies (effect, elicited immunity,... protection – *ferret model*)
- Local tolerance, repeat dose tox (route of administration)
- Toxicity (reprotox, hypersensitivity, pyrogenicity,...)
- Pharmacokinetics (dependent on nature of adjuvant, biodistribution, metabolism, excretion,...)

**Clinical:**
- Consider effect in different population/age groups
- Dose finding studies
- Comprehensive analysis of immune response (humoral IgG vs IgA, cellular Th1 vs Th2, cytokines,...)
- Pharmacokinetics for totally new substances
- Full safety evaluation, taking into account specific adjuvant features
  (at least ≈ 3000 subjects enrolled, can be much more depending on specific potential risks)

*EMA: Guideline on adjuvants in vaccines for human use, 2005
Overview ... regulatory toxicol. requirements for vaccines and adjuvants; Sun et al., 2012, J. pharm. toxic. Methods
WHO guidance in preparation*
Live Attenuated Vaccines and Viral Vectors

⇒ **LICENSED SEASONAL VACCINE „FLUMIST“ (US), FLUENZ (EU)**
Live attenuated influenza vaccines (LAIV): Classical virus reassortants between circulating strains and cold-adapted, temperature-sensitive, attenuated acceptor ("Masaab") strains

⇒ Fully characterized, highly stable viral acceptor strains with long-standing history
⇒ Comprehensive (pre)clinical data base available
Selected examples of potential novel developments:

- **Live attenuated** *ΔNS1 influenza virus* (deleted or truncated NS1 protein) – by reverse genetics
  NS1 protein inhibits host immune response ⇒ stronger immune response when inactivated

- **Recombinant Adeno virus vectors (AdV)** expressing influenza virus protein(s):
  Replication-defective AdV5 with HA and NP grown in susceptible/helper cell lines

- **Recombinant Poxviral vectors** expressing influenza virus protein(s):
  Highly attenuated „Modified vaccinia virus Ankara“ (MVA) expressing NP and M1
  grown in chicken eggs, BHK21 cells

- **Recombinant Sendai virus** vector expressing M2

**REGULATORY REQUIREMENTS:**
Novel viruses/vectors with limited safety data base: comprehensive examination mandatory

**QUALITY:** in addition to general requirements
- Characterisation of construct and all manipulations conducted – sequences
- Cell substrate (banked) and all materials used for propagation (Bovine serum: TSE)
- Identity, genetic stability of viruses and vectors – stability of attenuated phenotype
- Expression profile
- Potency assay (validation)
**PRECLINICAL:** in addition to general requirements

- **Pharmacodynamics:** Complete evaluation of Immune response (appropriate methodology) against heterologous antigens and viral vector (repeated use of vector)
  - Protection from disease, death (ferret), shedding of virus, transmission

- **Safety evaluation:** Stable attenuation in animal, (virulence), tropism of viruses, biodistribution
  - Crossing of blood-brain barrier (neurotropism, -virulence)
  - Reprotox and developmental toxicity (for use in pregnancy)
  - Germline transmission

**CLINICAL:** in addition to general requirements

- **Immune response:** humoral, cellular response; protection (expressed antigens and vector)
  - Pre-existing immunity to vector??
  - Interference of response to expressed proteins and vector

- **Safety evaluation:** Genotypic stability, attenuated phenotype, tropism and biodistribution (viremia?)
  - Virulence, recombination or reassortment with with circulating infectious agents, shedding and transmission to contacts, genetic integration, autoimmune disease

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**EMA:** Points to consider on the development of live attenuated influenza vaccines, 2003
**EMA:** Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines, 2010
**EMA:** NfG on quality, preclinical and clinical aspects of gene transfer medicinal products, 2005
**Ph. Eur. 5.14:** Gene transfer medicinal products for human use, 2010
**EMA:** Environmental risk assessment for medicinal products containing... GMOs, 2005
DNA / RNA Vaccines
Vaccines Based on DNA or RNA Constructs

Concept of DNA vaccination not new: more than 20 years of experience
- Immune response to defined (conserved) viral epitopes and combinations
- Intradermal application: antigen presenting cells
- T-cell immunity
- Potential co-administration with selected adjuvant (eg cytokines)

But: clinical data are not too “encouraging” so far

Common approaches:
Plasmids expressing HA, NA, M, NP and combinations of these

Quite recently: RNA constructs for vaccination
Stabilized mRNA for high level expression of viral proteins (HA, NA, NP,...)

Regulatory aspects: specific aspects to consider

Quality:
Derivation process and identity of construct, materials used, purification, genetic stability, expression profile, potency assay, ... (adjuvant??)

Preclinical:
Potential integration, expression profile, genetic stability, immunogenicity and protection, Cross-immunity, cross-protection, plasmid persistence

Clinical:
Potential integration, stability, immunogenicity („broader“?) and efficacy, plasmid persistence

WHO: Guidelines for assuring the quality and non-clinical safety evaluation of DNA vaccines, 2005
EMA: Concept paper on guidance for DNA vaccines
That’s All...

Thank You for Your Attention!!