Australian Government
Department of Health
Therapeutic Goods Administration

Nonclinical aspects of vaccine development

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Presentation at Vaccine Development Forum (ERA Consulting (Australia) Pty Ltd), Brisbane

29 October 2013

TGA Health Safety Regulation

Vaccine regulatory pathway

Preclinical	*In vitro*, animal immunogenicity, protection, toxicity	
Clinical trials (Concurrent nonclinical[1])	Phase I	20-80 healthy adults (18-50 years old)
	Phase II	Several hundred subjects
	Phase III	3000+ subjects to detect adverse events with incidence of 1/100-1/1000 vaccinated subjects, more for specific issues (70,000+ for Rotateq®)
Registration	All quality, nonclinical and clinical data evaluated for safety and efficacy	
Post-registration	Phase IV	Passive surveillance, phase IV clinical trials (booster, new population), pregnancy register, lot testing

[1]May address formulation change, manufacture change or upscale, new target population, clinical findings.

Nonclinical regulatory guidelines for vaccines

- Nonclinical guidelines for vaccines have been produced since 1997 by the:

- European Medicines Agency (EMA) and the International Conference on Harmonisation ... (ICH) (USA, EU, Japan collaboration) (adopted by TGA[1])
- USA Food and Drug administration (FDA-CBER)
- World Health Organization (WHO)

- [1]For TGA-adopted guidelines, refer to www.tga.gov.au

Nonclinical regulatory guidelines for vaccines

Vaccine type	Guideline
Most vaccines	EMA: Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95, 1997)
All vaccines	WHO: Guidelines on nonclinical evaluation of vaccines (WHO Technical Report Series, no. 927, 2005) WHO: Guidelines on clinical evaluation of vaccines: regulatory expectations (WHO Technical Report Series, no. 924, 2004)
Vaccines for infectious disease, in pregnant women, women of child-bearing potential, men	FDA (CBER) Guidance for industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (2006)
Recombinant DNA protein vaccines	ICH: Preclinical safety evaluation of biotechnology-derived pharmaceuticals S6(R1) (2011)

Nonclinical regulatory guidelines for vaccines (cont.d)

Vaccine type	Guideline
Adjuvanted vaccines	EMA: Guideline on adjuvants in vaccines for human use (2005) WHO: Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (proposed, 2013)
DNA vaccines	FDA: Guidance for industry: Considerations for plasmid DNA vaccines for infectious disease indications (2007) WHO: Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines (Technical Report Series, 941, 2007)
Live viral vectored vaccines	EMA: Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines (2011)
“Pharmaceuticals”	ICH: Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals M3(R2) (2009)

Recommendations for nonclinical vaccine studies

Assessed by scientific criteria on a case-by-case basis, taking into account:

- available nonclinical and clinical data with any related vaccines
- type of vaccine and degree of novelty
- human disease characteristics
- availability of relevant animal models

  -case-by-case does not mean minimal approach

Nonclinical studies aim to:

- provide evidence of proof of concept and safety prior to first clinical trial
- support changes to the vaccine and its use during clinical development
- support registration

Basic nonclinical studies for vaccines[1, 2]

Study type	Guideline recommendations
Primary pharmaco-dynamics	Immunogenicity, protection if animal model of disease available = proof of concept.
Safety pharmacology	CNS, CV, respiratory, temp. measures incorporated in tox. study.
Biodistribution	For live attenuated, DNA vaccines, new adjuvant, excipient, device.
Toxicity	Repeat-dose toxicity study, modelled on clinical regimen.
Local tolerance	Assessed in repeat-dose toxicity study, or independently.
Reproductive toxicity	Need depends on target population.

[1]EMA Note for guidance on preclinical pharmacological and toxicological testing of vaccines (1997).

[2]WHO Guidelines on nonclinical evaluation of vaccines (2005).

Basic nonclinical studies for vaccines[1, 2]

Immunogenicity + protection

- Immunogenicity studies assess responses to each vaccine antigen (antibodies-IgG, IgA, neutralizing, bactericidal, cell mediated immunity, seroconversion rate, memory) relevant to protection.
- Studies guide selection of antigen(s), route of administration, need for adjuvant, clinical dose and dose regimen.
- Protection, if animal model of disease available.
- Immunogenicity measures can be incorporated in toxicity studies, and vice versa.
- Good Laboratory Practice (GLP) compliance not essential.

[1]EMA Note for guidance on preclinical pharmacological and toxicological testing of vaccines (1997).

[2]WHO Guidelines on nonclinical evaluation of vaccines (2005).

Basic toxicity studies for vaccines[1]

- Repeat-dose toxicity study aims to establish a safe human dose, identify any toxicities to be monitored in trials.
- Test vaccine should be representative of clinical formulation.
- Aim to maximise immune response. Dose number ≥ number of clinical doses.
- 2-3 week dose intervals (compressed schedule) often feasible, depending on kinetics of the immune response[1].
- Protection study may test for paradoxical enhancement of disease[2].
- GLP required (+ regional animal ethics) .

[1]WHO Guidelines on nonclinical evaluation of vaccines (2005).

[2]Polack FP *et al* (2002). A role for immune complexes in enhanced respiratory syncytial virus disease. The *Journal of Experimental Medicine*196(6): 859-865.

Repeat-dose toxicity parameters for a vaccine[1]

Test groups	1. Saline control 2. Vaccine 3. Vaccine recovery – 2-3 weeks
Serology	Pre-test, post-dose, and recovery.
Clinical observations	Bodyweight and food consumption daily then weekly, body temperatures up to 24 h post-dose. Skin injection sites graded (Draize).
Clinical chemistry + haematology	1-3 days after first and last dose, and recovery.
Terminal gross pathology	Termination 2-3 days after last dose or recovery, gross pathology, organs weighed.
Microscopic pathology	~50 tissues preserved, injection sites, pivotal and immune organs examined, incidence and severity of any findings recorded.

[1]WHO Guidelines on nonclinical evaluation of vaccines (2005).

Toxicity studies for vaccines

Dose selection

If possible, the full human dose should be administered, otherwise exceed the human dose on a mg/kg bw basis[1]. Single dose level usually adequate.

Species (adult bw)	Typical IM dose volume	Animal:human dose multiple (mg/kg bw)
Mouse (20 g)	0.05 mL	250
Rat (200 g)	0.1 mL	50
Rabbit (2.5 kg)	0.5 mL	20
Rhesus macaque (5 kg)	0.5 mL	10
Human adult (50 kg)	0.5 mL	1

[1]WHO Guidelines on nonclinical evaluation of vaccines (2005).

Reproductive toxicity studies for vaccines

- Reproductive toxicity studies refer to fertility, embryofetal, and peri-postnatal development.
- Main safety concern is embryofetal development[1, 2].
- Male/female fertility assessed by reproductive organ histology in repeat-dose toxicity study[3].
- Pregnant women (usually excluded) – developmental toxicity studies prior to enrolment.
- Women of childbearing potential (pregnancy prevention) - developmental toxicity studies required, with some exceptions[1], FDA requirement for vaccine licence application[2].
- Men – fertility study prior to phase III[3].

[1]WHO Guidelines on nonclinical evaluation of vaccines (2005).

[2]FDA: Guidance for Industry. Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (2006).

[3]ICH Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals M3(R2) (2009).

Nonclinical studies for adjuvanted vaccines

- The increased/modified immune response with the adjuvanted vaccine should be demonstrated in a relevant animal model[1, 2]. Investigate mechanism of action.
- Toxicity studies with the **adjuvant alone** for adjuvants with no existing toxicology data[1], and/or adjuvant-only group in adjuvanted vaccine repeat-dose toxicity study[2]. Markers of inflammation useful.
- Local tolerance – consider the possibility of late granulomas with particles and mineral oils. Assess local and regional tolerance for intranasal, oral vaccines.
- If the adjuvant itself is immunogenic, tests may be indicated for hypersensitivity[1] *e.g.* passive cutaneous anaphylaxis, active systemic anaphylaxis assays, IgE measures, and dermal sensitization potential.
- Test the adjuvant for pyrogenicity.

[1]EMA. Guideline on adjuvants in vaccines for human use (2005).

[2]WHO. Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (proposed, 2013).

Toxicity studies for DNA vaccines

- Repeat-dose toxicity study (+ local tolerance).
- Assess plasmid biodistribution, persistence (~2-3 months), integration[1, 2].
- Theoretical risks of integration are tumourigenesis, chromosomal instability.
- If persistence of plasmid DNA exceeds threshold[1,2], investigate integration.

[1]FDA: Guidance for industry: Considerations for plasmid DNA vaccines for infectious disease indications (2007).

[2]WHO. Guidelines for assuring the quality and nonclinical safety evaluation of DNA vaccines (2007).

Toxicity studies for live attenuated vaccines

- Toxicity studies should assess degree of attenuation, stability of attenuated phenotype *i.e.* reversion to virulence[1]. Genetic markers of attenuation valuable.
- Assess potential for genetic exchange with non-vaccine strains.
- Biodistribution study should assess tropism, strain shedding, transmission[2].
- May require assessment by the Office of the Gene Technology Regulator.

[1]WHO. Guidelines on nonclinical evaluation of vaccines (2005).

[2]Bowen and Payne (2012) Rotavirus vaccine-derived shedding and viral reassortants. *Expert Rev. Vaccines* 11(11): 1311-1314.

Toxicity studies for viral-vectored vaccines

Vectors – pox viruses, adenoviruses, alphaviruses, yellow fever virus ...

- Investigate immune responses to both the vector and antigen(s).
- Heterologous antigen may alter cell tropism – virulence may not reflect vector.
- Biodistribution should be studied in a wide range of tissues, including gonads.
- May require assessment by the Office of the Gene Technology Regulator.

[1]EMA: Guideline on quality, nonclinical and clinical aspects of live recombinant viral vectored vaccines (2011).

Cell and gene therapies – nonclinical guidelines

Scope	Guideline
Gene therapy	EMA: Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (2001).
Gene therapy	EMA: Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (2008).
Gene vectors	EMA : Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (2006).
Cell therapy	EMA: Guideline on human cell-based medicinal products (2008).
Cell + gene therapy	EMA: Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells (2010).
Cell + gene therapy, therapeutic vaccines	FDA Guidance for Industry. Preclinical Assessment of Investigational Cellular and Gene Therapy Products (Nov 2012 draft).

Cell and gene therapies – nonclinical studies

- Risk/benefit often not acceptable for healthy human volunteers.
- Immunotherapies (dendritic cells, adoptive T cell transfer) focus on cancer[1], infectious disease *treatment*.
- Ideally test human cell product, animal models – immunosuppressed, immunodeficient, humanised[2], transgenic, homologous.
- Limited animal models places greater emphasis on *ex vivo* cell studies, genomics, related clinical experience.
- Dose selection often not straightforward – transduction efficiency, lack of potency assay, *in vivo* expansion, engraftment.

[1]Linette GP *et al* (2013). Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 122 (6): 863-871.

[2]Ramer PC (2011). Mice with human immune system components as *in vivo* models for infections with human pathogens. *Immuno & Cell Biol* 89: 408-416.

Gene therapy – insertional mutagenesis

Scope	Guideline
Vector integration (nonclinical models)	EMA: Reflection paper on management of clinical risks deriving from insertional mutagenesis (2012).

- Safety concern is insertional mutagenesis leading to oncogenesis with integrating vectors *e.g.* γ-retroviruses, lentiviruses used for gene transfer.
- Assessed by *in vitro* immortalisation assay, i*n vivo* clonal analysis, animal tumourigenicity.

Gene therapy – delayed adverse events

Scope	Guideline
Gene therapy	EMA: Guideline on follow-up of patients administered with gene therapy medicinal products (2009).
Gene therapy (animal biodistribution)	FDA (CBER) Guidance for industry – Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events (2006).

<u>Preclinical risk assessment</u>

- persistence of viral vector (latency, reactivation)
- prolonged transgene expression
- integration into genome

Clinical follow-up maximum 5+10 years (FDA guidance)

Clinical trials in Australia

- All clinical trials in Australia require review and approval of trial proposal by a Human Research Ethics Committee (HREC).
- Clinical trials using unapproved therapeutic goods are required to use either the Clinical Trial Notification (CTN) or clinical Trial Exemption (CTX) scheme[1, 2].

CTN	CTX
Principal investigator submits proposal to HREC, which reviews Investigator's Brochure and Trial Protocol. Final approval granted by institution at which trial will be conducted. TGA notified, but no review by TGA.	Sponsor submits application to TGA, which reviews Usage Guidelines, quality, nonclinical and clinical data, overseas status (50 working days). TGA delegate no objection/objection, written comment via sponsor to HREC. Final approval by HREC and institution.

[1]The Australian Clinical Trial Handbook. March 2006.
[2]Access to unapproved therapeutic goods - Clinical trials in Australia. October 2004.

Clinical trials of biologicals (cells and tissues) in Australia[1]

Class	Meaning	Risk
1	Defined in Schedule 16 of Therapeutic Goods Regulations (1990)	Low
2	Minimal manipulation, homologous use	Low
3	More complex manipulation, homologous use	Medium
4*	More complex manipulation (gene-modified), non-homologous use	High
*CTX mandatory, unless previous clinical use, or approved by comparable national authority.		

[1]Australian Regulatory Guidelines for Biologicals (June 2011):
Part 1 – Introduction to the Australian Regulatory Guidelines for Biologicals.
Part 2 – Regulatory life cycle for biologicals that are included on the Australian Register of Therapeutic Goods.
*Part 3 – Access to unapproved biologicals.

Vaccines – additional information

Clinical Evaluation Unit 2 (clinical)

Experimental Products Section (clinical trials)

Immunobiology Section (vaccine manufacturing quality)

Biological Science Section (cells and tissues)