EDQM & European Pharmacopoeia: State-of-the-art Science for Tomorrow's Medicines

International Conference organised by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe

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Workshop on the 3Rs and ATMPs

Moderator

Dr Lukas Bruckner, Chair of the Biological Standardisation Programme Steering Committee

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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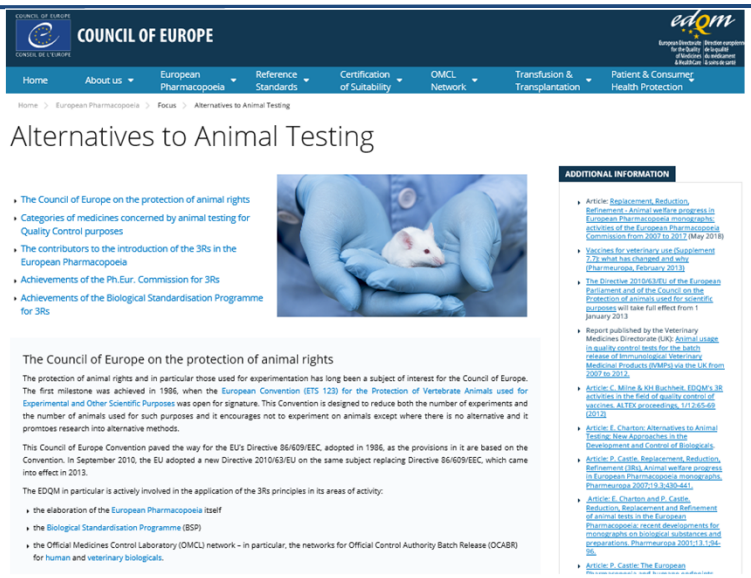
Achievements of the Biological Standardisation Programme and the Ph. Eur. in the field of 3Rs

Alternatives to Animal Testing at EDQM



Why 3R Alternatives?

- Ethical concerns
- Legal obligations
- Variability of *in vivo* results
- Costs
- Advances in analytics and production
- Public pressure

Application of 3Rs is of high importance for Ph. Eur./EDQM

Classic 3Rs - Russell and Burch (1959)

- Refine, Reduce, Replace

Plus important 4th R

- Remove

Application is case specific – all are used

Biological Standardisation Programme (BSP)

- Joint Programme Council of Europe & EU Commission
- Biological Reference Preparations for Standardisation
- Application of 3Rs to QC methods for biologicals
- Started in 1993
- 163 Projects initiated/concluded
- 43 Projects on method development
- 24 Projects on 3Rs methods → Goal = description of method in the European Pharmacopoeia
  Focus on Refine – Reduce- Replace
- 86 Projects on reference standards

Programme succeeds thanks to the collaborative work of experts in the Steering Committee, scientific project leaders, participating laboratories (OMCLs/manufacturers), donators of materials, method developers and dedicated staff

Selected Achievements

Reduce/Refine

- Replacement of lethal challenge test by serological assay (& significant reduction of number of animals) for
  - Tetanus vaccine
  - Diphtheria vaccine
  - Acellular pertussis vaccine
  - Rabies vaccine (vet. use)
  - Swine erysipelas vaccine

Replace

- Replacement *in vivo* test by *in vitro* tests
  - Hepatitis A vaccine
  - Inactivated Poliomyelitis Vaccine (IPV)
  - Histamine sensitisation test (HIST) for acellular pertussis vaccine
  - Newcastle disease vaccine (NDV)
  - Tetanus immunoglobulin
  - Somatropin
  - Clostridium septicum vaccine: *in vitro* test to replace tests in mice to determine antigenicity & toxicity of toxin and toxoid

- Method developed by contributing labs and brought to BSP
- Collaborative validation of principle in large scale BSP study
- Integration into Ph. Eur. texts as appropriate by Ph. Eur. experts

Ongoing and future targets

Current/future BSP projects

- Rabies vaccine (human use): ELISA to replace *in vivo* potency assay
- BINACLE assay: *in vitro* test to replace *in vivo* test for "Absence of toxin" in tetanus toxoid
- Tetanus- & diphtheria vaccine: *in vitro* immuno assay to replace *in vivo* potency assay
- Cell based *in vitro* potency assay for erythropoietin to replace the *in vivo* assay

Challenges – by no means a definitive list

For the studies…..

- Identifying appropriate methods – applicable to all/majority of products in the category
- Securing necessary materials and reagents for public use e.g. specific mAbs, potential need for common reference standards
- Overcoming the 'quest to correlate' against existing *in vivo* methods
- Increase in product specificity /complexity and multiplicity of approach
- Timelines – multiple phases, long and complex progression – start and end conditions may not be the same
- Getting everyone on the same page
- Resources – at OMCLs, at companies, at EDQM and the funders

And after…..

- Implementation and use by laboratories
- Regulatory acceptance of methods for individual products
- Identifying appropriate product specific specifications

Achievements of the Ph. Eur. Commission for 3Rs

- Numerous 3Rs changes introduced in monographs over the years
  - Review of 3Rs activities over the last decade in the article:

Pharmeuropa Bio & Scientific Notes 2018: Replacement, Reduction, Refinement

Replacement, Reduction, Refinement

Animal welfare progress
in European Pharmacopoeia monographs:
activities of the European Pharmacopoeia Commission
from 2007 to 2017

Catherine Lang, Olga Kolaj-Robin, Gwenaël Cirefice,
Laure Taconet, Ellen Pel, Sébastien Jouette, Mihaela Buda,
Catherine Milne, Emmanuelle Charton[1]

ABSTRACT

Since the opening for signature of the European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes in 1986, the European Pharmacopoeia

Recent achievements in the Ph. Eur. – selected examples

- Suppression of ATT
  - An example of Remove (4th R) impacting multiple products, and convergence towards global acceptance
- Replacement of HIST & review of toxicity testing requirements for acellular Pertussis vaccines
  - An example of Replacing an animal test and Removing outdated requirements
- General chapter 5.2.14
  - New guidance on "substitution" of in vivo methods, for vaccines and sera

Abnormal Toxicity Test (ATT)

= General Safety Test (US), Innocuity Test (WHO)

- Principle: inject batches of product into guinea pigs/mice. A batch passes the test if no animal shows any sign of illness, or dies within a defined timeframe
  → Animal suffering
- Considerable usage of animals: e.g. for vaccines, 5 mice and 2 guinea pigs for each batch
- One of the most controversial animal tests in the Ph. Eur.
  → *Priority target for 3Rs!*

Relevance of ATT

- Safety tests in mice and guinea pigs date back to the early 1900s
  - detection of toxic levels of phenol in sera (mice)
  - detection of contamination with tetanus toxin & spores in sera (guinea pigs)
- In 1940s both tests were combined to become a general safety test. ATT largely unchanged since then, despite evolution of analytical techniques, manufacturing processes
- Retrospective analysis concluded: the ATT is neither specific, reproducible, reliable, nor suitable for the intended purpose (*Duchow et al, 1994*)
  - More relevant tests used for testing phenols, toxins
- Deletion as a routine batch release test from >80 monographs in 1998
  - Moved to Production section (development test)
- Use of GMP and stringent QC measures to prevent contamination also puts in question the relevance of the ATT

Converging regulatory agreement on ATT

European Partnership for Alternative Approaches to Animal Testing (EPAA)

- Discussed the ATT issue in depth in a Workshop in 2016 with global stakeholders
- Workshop included review of case studies and data
- Conclusion: the ATT lacks scientific relevance and its omission does not compromise the safety of biologics. *Consensus to strive for deletion of the ATT from regulatory requirements*
- Deletion of ATT should be addressed at a global level
  - A harmonised approach by all regulators across the globe is important for a real and effective deletion

Suppression of ATT from the Ph. Eur. … and beyond!

Ph. Eur.

- Based on this review, the Ph. Eur. Commission decided to embark on the deletion of ATT from 49 monographs
- A detailed evaluation was conducted for each monograph
- Decision to suppress the ATT (Nov 2017)
  - Revised monographs omitting the ATT published in Supplement 9.6 (July 2018)
  - Simultaneous suppression of chapter *2.6.9 Abnormal toxicity*, no longer referenced in any monograph

No More ATT in the Ph. Eur.!
A MAJOR STEP FORWARD FOR 3Rs

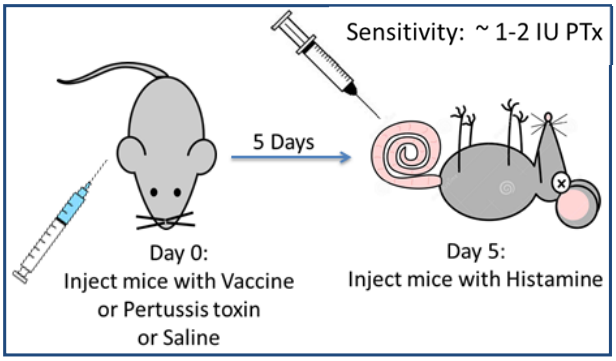
WHO

- WHO's ECBS recommendation to discontinue ATT in guidelines on vaccines and biologicals (Nov 2018) → A further step towards global acceptance

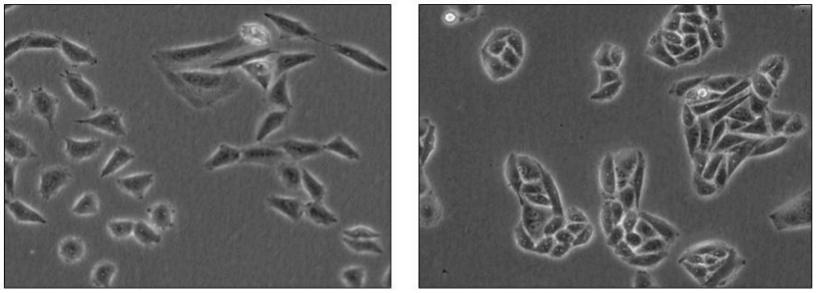
Histamine Sensitisation Test (HIST)

- HIST is used as a release test for acellular Pertussis (aP) vaccines (*test for residual pertussis toxin and irreversibility of toxoid*)
- Large number of animals, high variability, several versions of HIST are required in different regions
- End-point = death, 24h after injection of histamine
- High distress of animals

→ *Replacement considered a priority!*



Replacement of HIST: CHO Cell Clustering Assay



Source: R. Isbrucker, Health Canada

Control (no PTx) + PTx (48 hrs)

Sensitivity: ~ 0.006 IU PTx

- Induction of clusters in non-confluent CHO cell cultures by active pertussis toxin
- Standardised method validated in a collaborative study (*cf. Isbrucker R, Daas A, Wagner L et al. Transferability study of CHO cell clustering assays for monitoring of pertussis toxin activity in acellular pertussis vaccines, Pharmeur Bio Sci Notes 2016:97-114*)

BSP study: validation of alternative method

Rationalising toxicity testing requirements for aP vaccines

Ph. Eur. Expert Group and Commission: rationalise testing requirements

- Test for residual toxin on purified bulk: replacement of HIST by CHO clustering assay
  - Data from BSP study
  - *Standardised CHO assay method described as an example*
- Removal of the Test for irreversibility
  - Data confirming that the pertussis toxoid is stable and reversion is not an issue for marketed aP vaccines (→ *manufacturers & OMCLs survey*)
  - History of safe use of aP vaccines
- Removal of the requirement to test the final lot for residual toxin
  - Testing of the pre-adsorbed bulk is considered the most effective and robust approach

Substitution of in vivo methods for the quality control of vaccines

- The introduction of in vitro methods to replace in vivo methods often prevented due to the properties of in vivo methods (e.g. variability, validation of in vivo methods, different responses measured)

- Demonstration of equivalence may not only be problematic, but also of limited relevance

  → New general chapter 5.2.14

Aim: facilitate the transition from *in vivo* to *in vitro* methods

Substitution of in vivo methods for the quality control of vaccines

- Chapter 5.2.14 provides guidance on how to introduce alternative *in vitro* methods, where a head-to-head comparison is not possible

- Envisages the possibility that the validity of the *in vitro* method be demonstrated without such head-to-head comparison (concept of "substitution") and discusses alternative approaches for replacement

- Focus on the scientific rationale behind the in vitro methods, relative to what is provided with current in vivo methods

→ *More to follow in A. Akkermans' presentation… stay tuned!*

Conclusion and outlook

- Significant achievements in animal welfare made possible by continued collaboration between Ph. Eur./EDQM, EU Commission, regulators/OMCLs and manufacturers
- After 3 decades of the Convention*, the animal tests that remain in the Ph. Eur. are the most difficult to eliminate (e.g. potency assays of rabies vaccines, EPO, botulinum toxin)
- Efforts to Replace, Reduce, and Refine the use of animals need to be sustained, e.g. by supporting studies that will lead to progress in animal welfare
- Continue to review remaining animal tests in monographs to assess their continued relevance and identify opportunities for application of 3Rs, e.g. Remove
- Continue to engage and exchange information with partners outside Europe to foster acceptance of 3Rs advances at a global level

We count on all our partners to help to make progress happen

*European Convention on the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Council of Europe (ETS 123)

Thank you for your attention

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The 3Rs
Perspectives for the Future

Lukas Bruckner, Switzerland

Veterinary Vaccines (Ph.Eur.)
tests during the development

Safety

- Carry out the test for each route and method of administration to be recommended for vaccination and in *animals* of each category for which the vaccine is intended…

Immunogenicity

- A test is carried out for each route and method of administration to be recommended for vaccination using in each case *animals*...
  Vaccinate by a recommended route ... *animals* …
  Maintain …*animals* as controls.
  Challenge each *animal*…
  Observe the *animals*... after challenge...

Veterinary Vaccines (Ph.Eur.)

tests for each batch

Potency

- Immunogenicity test in *animals*
  validated (*in vitro*) alternative

Safety tests

- Application of humane endpoints

Immunogenicity tests

- Evaluation of the number of animals
  - Vaccinated animals
  - Non-vaccinated controls
- Analysis of the need of challenge infection
- Evaluation of challenge infection
- Use of non-clinical signs of infection
  - viraemia
- Evaluaion through clinical signs
  - Use of scoring systems and
  - Application of humane endpoints

RABIES VACCINE (INACTIVATED) FOR VETERINARY USE

Vaccinum rabiei inactivatum ad usum veterinarium

1. DEFINITION

Rabies vaccine (inactivated) for veterinary use is a preparation of a suitable strain of fixed rabies virus, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of animals against rabies.

2. PRODUCTION

2-1. *PREPARATION OF THE VACCINE*

The vaccine is prepared from virus grown either in suitable cell lines or in primary cell cultures from healthy animals (*5.2.4*). The virus suspension is harvested on one or more occasions within 28 days of inoculation. Multiple harvests from a single production cell culture may be pooled and considered as a single harvest.

The virus harvest is inactivated. The vaccine may be adjuvanted.

2-2. *SUBSTRATE FOR VIRUS PROPAGATION*

2-2-1. **Cell cultures**. The cell cultures comply with the requirements for cell cultures for production of veterinary vaccines (*5.2.4*).

2-3. *CHOICE OF VACCINE COMPOSITION*

The vaccine virus is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the species for which it is intended.

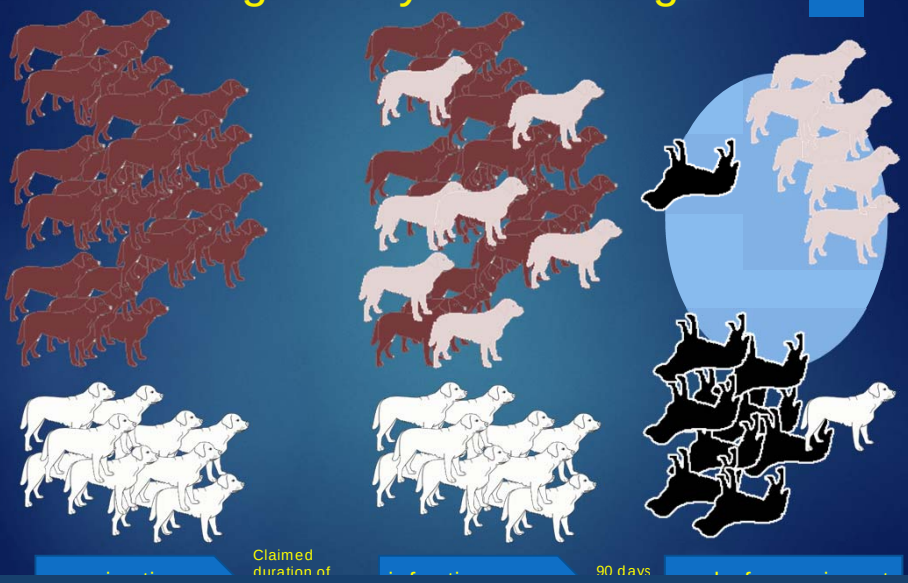
The following tests for safety (section 2-3-1) and immunogenicity (section 2-3-2) may be used during the demonstration of safety and efficacy in cats and dogs.

2-3-2. **Immunogenicity**. Each test is carried out for each route and method of administration to be recommended, using in each case animals of the minimum age to be recommended for vaccination. The vaccine administered to each animal is of minimum potency.

Use for the test not fewer than 35 animals. Take a blood sample from each animal and test individually for antibodies against rabies virus to determine susceptibility. Vaccinate not fewer than 25 animals, according to the schedule to be recommended. Maintain not fewer than 10 animals as controls. Observe all the animals for a period equal to the claimed duration of immunity. No animal shows signs of rabies. On the last day of the claimed period for duration of immunity or later, challenge each animal by intramuscular injection with a sufficient quantity of virulent rabies virus of a strain approved by the competent authority. Observe the animals at least daily for 90 days after challenge. Animals that die from causes not attributable to rabies are eliminated. The test is not valid if the number of such deaths reduces the number of vaccinated animals in the test to fewer than 25 and the test is invalid unless at least 8 control animals (or a statistically equivalent number if more than 10 control animals are challenged) show signs of rabies and the presence of rabies virus in their brain is demonstrated by the fluorescent-antibody test or some other suitable method. The vaccine complies with the test if not more than 2 of the 25 vaccinated animals (or a statistically equivalent number if more than 25 vaccinated animals are challenged) show signs of rabies.

Lukas Bruckner, The 3Rs, Perspectives for the Future
EDQM & European Pharmacopoeia: State-of-the-Art Science for Tomorrow's Medicines, 19/20 June 2019, Strasbourg (F)

Immunogenicity test in dogs



Immunogenicity test in dogs potential for optimization

- # of vaccinated dogs ?
  # of control dogs ?
- Need to infect dogs showing neutralizing antibodies at time of infection ?

  (demonstration of neutralizing antibodies considered suitable for other species than dogs and cats)
- Euthanasia of dogs showing signs of rabies
  - Application of humane endpoints

RABIES VACCINE (INACTIVATED) FOR VETERINARY USE

Vaccinum rabiei inactivatum ad usum veterinarium

Use groups of not fewer than 8 female mice (strain NMRI), each weighing 18-20 g. Prepare a 1 IU/mL suspension of *rabies vaccine (inactivated) for veterinary use BRP* using phosphate-buffered saline (PBS) for dilution. Vaccines with a minimum potency requirement of 1 IU/mL are used without further dilution. Vaccines with a minimum potency requirement of more than 1 IU/mL are diluted with PBS to contain approximately, but not less than, 1 IU/mL. Administer by the intraperitoneal route to each mouse of one group 0.2 mL of the vaccine, diluted where necessary, and to each mouse of another group 0.2 mL of the suspension of *rabies vaccine (inactivated) for veterinary use BRP*. Take blood samples 14 days after the injection and test the sera individually for rabies antibody using a suitable virus neutralisation test, for example the rapid fluorescent focus inhibition test (RFFIT) described for *Human rabies immunoglobulin (0723)* or a suitable validated modification of the RFFIT[4].

Batch potency test

alternatives to the mouse potency test

- Antigen quantification test available for vaccines for humane use
  Test uses specified monoclonal antibodies
  (monoclonals suitable for the detection of various virus strains)
  - Method not suitable for *adjuvanted* vet vaccines

- Antigen quantification test licensed for one adjuvanted veterinary product
  uses different monoclonal antibodies than those used for human vaccines
  - Suitability of the method for other products, when using monoclonal antibodies for human vaccines should be clarified

AUJESZKY'S DISEASE VACCINE (LIVE) FOR PIGS FOR PARENTERAL ADMINISTRATION

Vaccinum morbi Aujeszkyi vivum ad suem ad usum parenteralem

1. DEFINITION

Aujeszky's disease vaccine (live) for pigs for parenteral administration is a preparation of a suitable strain of Aujeszky's disease virus. This monograph applies to vaccines intended for the active immunisation of pigs and for passive protection of their progeny against Aujeszky's disease. The vaccine may be administered after mixing with an adjuvant.

2-3-5. **Immunogenicity**. A test is carried out for each route and method of administration to be recommended for vaccination. The quantity of vaccine virus to be administered to each pig is not greater than the minimum virus titre to be stated on the label and the virus is at the most attenuated passage level that will be present in a batch of vaccine.

2-3-5-2. *Vaccines intended for passive protection*. If the vaccine is intended for use in sows for the passive protection of piglets, the suitability of the vaccine virus for this purpose may be demonstrated by the following method.

Use for the test not fewer than 12 sows that do not have antibodies against Aujeszky's disease virus. Vaccinate not fewer than 8 sows, according to the schedule to be recommended. Maintain not fewer than 4 sows as controls. At 6-10 days of age, challenge the piglets from the sows with a sufficient quantity of virulent Aujeszky's disease virus. Observe the piglets at least daily for 21 days.

The test is not valid if the average number of piglets per litter for each group is less than 6.

The vaccine complies with the test if not less than 80 per cent protection against mortality is found in the piglets from the vaccinated sows compared to those from the control sows.

Immunogenicity test: Vaccines intended for passive protection

potential for optimization

- Need to infect suckling piglets?
  - Demonstration of protecting antibody levels in colostrum of the vaccinated sows
- Euthanasia of piglets showing signs of disease
  - Application of humane endpoints

CANINE DISTEMPER VACCINE (LIVE)

Vaccinum morbi Carrei vivum ad canem

1. DEFINITION

Canine distemper vaccine (live) is a preparation of a suitable strain of distemper virus. This monograph applies to vaccines intended for the active immunisation of dogs against canine distemper.

2-3-3. **Immunogenicity**. A test is carried out for each route and method of administration to be recommended for vaccination using in each case dogs 8-16 weeks old. The quantity of vaccine virus to be administered to each dog is not greater than the minimum virus titre to be stated on the label and the virus is at the most attenuated passage level that will be present in a batch of vaccine.

Use for the test not fewer than 7 dogs that do not have antibodies against canine distemper virus. Vaccinate not fewer than 5 dogs according to the schedule to be recommended. Maintain not fewer than 2 dogs as controls. Challenge each dog after 20-22 days by the intravenous route with a sufficient quantity of a suspension of virulent canine distemper virus. Observe the dogs at least daily for 21 days after challenge. Dogs displaying typical signs of serious infection with canine distemper virus are euthanised to avoid unnecessary suffering.

The test is not valid if during the observation period after challenge, fewer than 100 per cent of the control dogs die or show notable signs of canine distemper.

The vaccine virus complies with the test if during the observation period after challenge, all the vaccinated dogs survive and show no signs of disease.

Immunogenicity test in dogs potential for optimization

- Need to infect dogs?
  - Demonstration of protecting antibody levels in serum
- Signs of disease after challenge
  - Viraemia as criterion

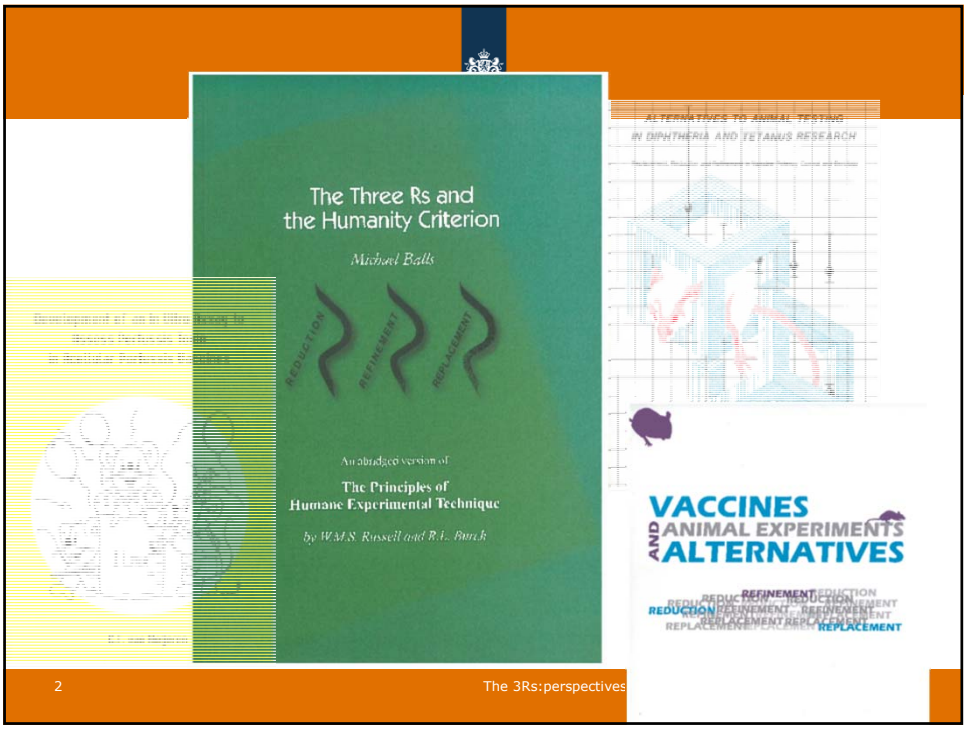
Thank you for your attention

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

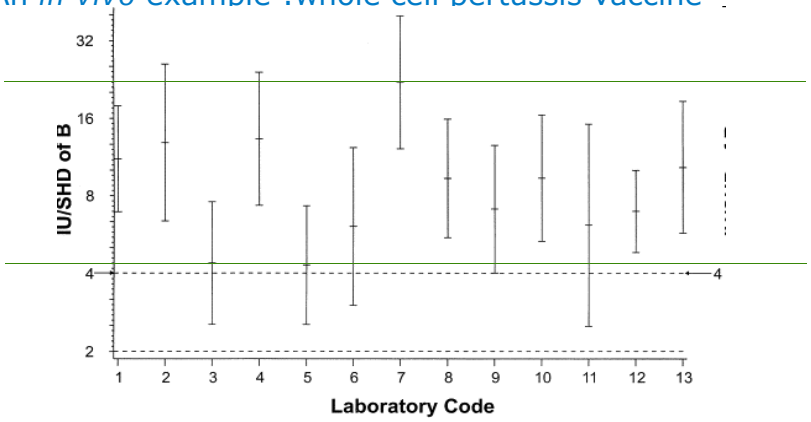
The 3Rs:perspectives for the future

Arnoud Akkermans

Member of Group 15
Ph.Eur.



An *in vivo* example :whole cell pertussis vaccine



Laboratory testing of whole cell pertussis vaccine: a WHO proficiency study using the Kendrick test. D Xing, R.Gaines Das, T O'Neill, M Corbel, N Dellepiane, J Milstien

The 3Rs:perspectives for the future

- the development and implementation of *in vitro* testing in the quality control of vaccines
- highlighting the main messages of the 5.2.14. chapter
- the importance of consistency in vaccine production
- the benefit that more precise *in vitro* test methods

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

In vitro tests support consistency control

- "The test methods used for routine quality control of vaccines are intended to monitor production **consistency** and to ensure comparability of the **quality attributes** between commercial batches and those batches originally found to be safe and efficacious in clinical studies or in the target species for veterinary vaccines."
- "an in vivo test for a given product is to be replaced with an in vitro test, the attribute(s) of the product will likely be **assessed differently.**"
- "Regardless, the in vitro method(s) or testing strategy must provide at least the **same confidence that the key quality attributes**, required to ensure the consistency of a product's safety and effectiveness, are adequately controlled."

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

PURPOSE

The purpose of this general chapter is to facilitate the implementation of *in vitro* methods as substitutes for existing *in vivo* methods, where typical one-to-one assay comparison is not appropriate for reasons unrelated to the suitability of an *in vitro* method(s). This general chapter will not discuss the details of assay validation as such, since those principles are described elsewhere.

The general chapter applies primarily to human and veterinary vaccines, however the principles described may also apply to other biologicals such as sera.

"in vivo safety and potency assays for vaccines were generally shown to be fit for purpose and have historically proven their value in ensuring the efficacy and safety of vaccines."

GENERAL CONSIDERATIONS

One of the consequences associated with the inherent variability of *in vivo* assays is the problem this poses with their replacement by the more consistent *in vitro* methods, which typically requires one-to-one assay comparison. This can be a challenge in some cases as repeated efforts through multicentre international collaborative studies can fail due to the variability inherent in the *in vivo* methods. Another consideration is that many of the legacy *in vivo* safety and potency assays for vaccines were generally shown to be fit for purpose and have historically proven their value in ensuring the efficacy and safety of vaccines. However, this was in an era when validation requirements, such as ICH Q2 (R1) guideline, were not in place, making a formal one-to-one comparison challenging or even impossible in some cases. Since precision, reproducibility, limits of detection and quantification were not established for the *in vivo* method, the comparability of 1 method to another becomes difficult to evaluate. It should also be noted that because pharmacopoeial methods are considered validated under the General Notices of the Ph. Eur., it is not only impractical and excessively costly to now undertake a retrospective ICH validation of these methods but it would also be unethical given the above mentioned convention on animal use in Pharmacopoeial testing.

- "As a consequence, a demonstration of agreement between the 2 methods is generally **not scientifically justified** and **should not always be expected**. Even where pass/fail results from the 2 test procedures are in agreement, the correlation between 2 quantitative methods across the assay range may still be low. Regardless, the in vitro method(s) or testing strategy must provide **at least the same confidence that the key quality attributes, required to ensure the consistency of a product's safety and effectiveness, are adequately controlled**. While the focus of this general chapter is on the replacement of existing methods for approved products, it is important to consider the use of in vitro methods for quality control during product development and to understand that the use of in vivo assays is not mandatory."

REQUIREMENT FOR PERIODIC REVALUATION OF *IN VIVO* TESTING

It is essential to continually challenge the scientific value and relevance of *in vivo* test methods. When *in vivo* tests are found to be of limited or no value, it is imperative to eliminate such testing given the ethical considerations and the obligations under the relevant conventions.

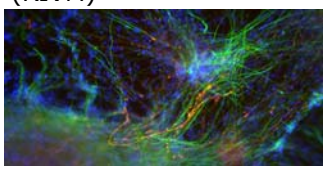
Future of 3R's

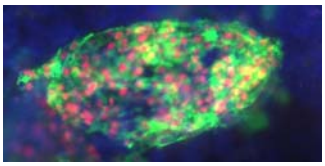
- 5.2.14 can give a push to innovation
- Surpass the *in vivo* testing
- State of the art **science based** monitoring of production
- current project VAC2VAC is implementing this new approach
  - Joint effort of industry, OMCL's and regulators
  - Concerns human and veterinary vaccines
  - long time experience with the manufacturing process and its control (consistency)
  - post-approval characterisation of the drug substance and drug product and intermediates
  - "Reverse characterisation"

vac2vac

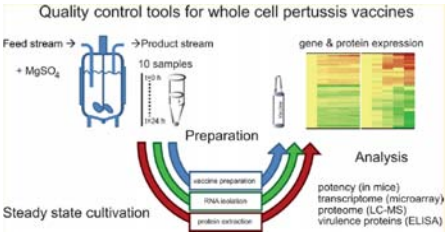
New technology available for inclusion in testing

- iPSC – induced pluripotent stem cells; neuron, beating heart cells (RIVM)





- gene & protein expression (Intravacc & RIVM)
- Big data & AI



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VETERINARY VACCINE TESTING IN MODERN TIMES

3R challenges from industry perspective

MODERN TIMES FOR VETERINARY VACCINES

Characterised by	A globalised world, global and local manufacturers
	Technology available
	GMP (documentation and control in the whole production chain)
Expectation from the public	Science should be driver no 1 for new medicines
	Authorities should approve medicines based on science-informed decisions
	Companies should produce medicines (= and test veterinary vaccines) based on science-informed decisions

→ How does animal testing fit into this?

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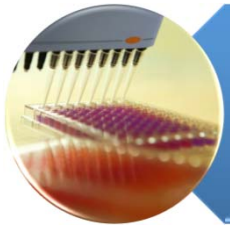
BETWEEN HISTORIC AND MODERN TIMES

Historic times

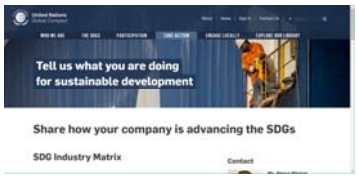
- → Severe diseases: vaccines developed decades ago
- Little technology at those times (=animals)
- Established requirements
  - For potency
  - For absence of toxicity, etc.
- → Consequence: many (Ph. Eur./ worldwide) monographs still with/ only animal tests (despite evident issues)!



Modern times

- Newly developed vaccines are rather different
- → Avoid animal-based release testing since beginning
- → Enrich monographs with *in vitro* tests as one option

VISION AND NEEDS OF TODAY



The 2030 Agenda for Sustainable Development, adopted by United Nations in 2015.

- The 17 Sustainable Development Goals: urgent call for action by all countries - developed and developing - in a global partnership.
- The 1st time in history also companies are directly asked to support and play an important role in it.

VETERINARY VACCINES – DIFFERENCES TO HUMAN

Variety

>> 100 different antigens (diseases/ pathogens/ strains)

>> 100 different antigen production processes (historical origins)

>>> 250 different final products

Sales price

Vet: between approx few cents to €,

compared to approx15 – 100 €/hum[illegible]e

Production

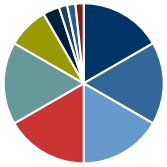
Costs need to be somehow in relation with revenues

Manufacturers

Examples here for multi-national companies

Same principles apply for local manufacturers!

Veterinary vaccines for…



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REAL CHALLENGES → DRIVERS OF 3R

Not all of the >>> 250 different final products are QC-tested on animals, but the old (inactivated) vaccines

→ Enormous QC logistics!

→ Poor reliability of animal-test-values, so (historic) over-formulation needed

→ Strong drivers for modernisation

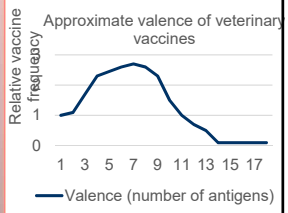
Complexities for *in-vitro* test development:

1) Purification not like for human vaccines: matrix effects!

2) Multivalency: frequent & different combinations (record: 18-valent!)

3) New tests for „x:1 substitution" or „1:1 replacement" must be convenient for QC

4) Define a suitable range for the modern higher precision tests



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3R CHALLENGES FROM INDUSTRY PERSPECTIVE

Some in-vitro tests really made it (or toxicity tests showed not useful):
- Used in production! (or ATT/TABST toxicity tests could be skipped)
- Taken up in Ph. Eur. (have been/will be; or toxicity tests taken out)
- Consider still: multivalent combinations and possible matrix effects

Can animal test be abolished in EU? Unfortunately, no since:
- Continued requirement of historic animal-tests world-wide (even for non-useful animal tests such as some toxicity tests)
- Approval per combination & country!
- Transition time needed (safe harbour principle of parallel testing)

World-wide blockades:
- Different settings between multinational and local manufacturers (animal-tests perceived as beneficial by some locals despite test reliability issues for both manufacturers and authorities!)
- Pseudo-"gold standard" discussion (variability disables correlation old vs new)
- "One fits all" discussion (focus not on suitable, but best global single method)

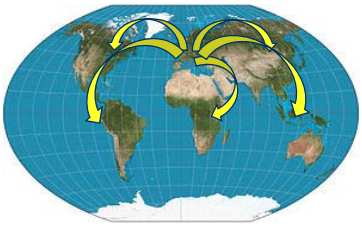
Solution??? → Include *in vitro* tests as <u>one option</u>

EU PROGRESS IS GOOD, BUT… WE NEED PROGRESS THROUGHOUT THE WORLD

Progress has been made (merci EDQM ☺ and USDA!), but
- Far not enough to fully avoid the historic animal tests: the world is larger
- International harmonisation is absolutely crucial

→ Spread progress and knowledge vice versa to and from EU

→ Innovation should be universal no matter the origin



→ If science is the driver of innovation and economic growth, do benefit and participate from available data!

OBSTACLES AND LEARNINGS HOW TO MOVE FORWARD

Obstacles and hesitance observed:

1. Pseudo-"gold standard" discussion – even if correlation is not/hardly possible neither justified
2. "One fits all" discussion - focus not on a suitable method, but on the best global single method
3. NO questions to statistically "failed" animal methods – and deep questioning as soon as animal-free

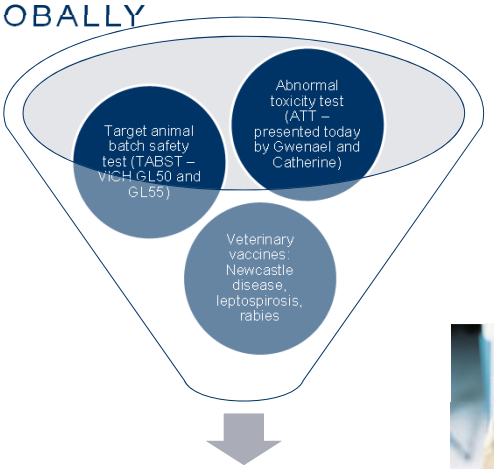
Learnings for new regulation:

→ Be careful: not automatically impose historic tests → unreliability will hit

Solutions/ideas how to overcome obstacles and hesitance:

1. Regarding pseudo-"gold standard": trust the process, it works (for decades!) → New test comes in here
2. Regarding "one fits all": No need (for precision, time, costs, ethics, options in Ph. Eur. general chapter) neither feasibility; different handling of different diseases (rabies)?
3. Use „science-informed decisions", ie: check publications: animal-test variability and *in vitro* options
   World-wide context: Consistency is anchored in key quality attributes such as:
   1) growth, 2) harvest, 3) inactivation time & conditions defined?
   → If applied, vaccine can benefit from *in vitro*
4. Do progress step-wise: **Include *in vitro* (ELISA, cell-based assay, other) as one option**

SCIENCE-INFORMED DECISIONS CAN BE TAKEN GLOBALLY



Next steps: apply progress made!
No matter whether coming from vet or human, nor country of origin

NEWCASTLE DISEASE VACCINE

Viral inactivated vaccine, correlate of protection known

Potency test Ph. Eur.:

1) Historic: chicken challenge
2) Serology
3) Since years also: ELISA

→ Monograph 0870, 2-5-2-1: relative antigen content determined by comparing haemagglutinin-neuraminidase antigen with reference

Ph. Eur. chain of events:

https://www.edqm.eu/medias/fichiers/bsp055_newcstle_serol_p1.pdf

→ „excellent reproducibility of the proposed *in vitro* method"

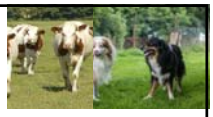
SUPPLEMENT 5.6

→ "An alternative in vitro potency test, which is a control of the antigen, has been added. If this test is performed, tests for the adjuvant must also be performed on each batch."

Next steps:

→ Consider inclusion internationally

LEPTOSPIROSIS VACCINE



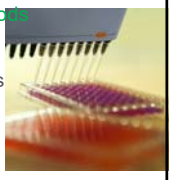
Bacterial inactivated vaccine, correlate(s) of protection not known

Potency test Ph. Eur.:

1) Historic: hamster challenge
2) Serology
3) Since few years also: ELISA

→ Monographs 0447 and 1993, 2-3-2-1:◀ For each serovar determine the content of antigenic components which are indicators of protection and serovar-specific.▶ Methods using lipopolysaccharide (LPS)-based antigen quantification have been shown to be suitable.

→ = Consistency ELISA: if level of LPS is within acceptance range, this demonstrates consistent production, so batch can be released



Ph. Eur. chain of events:

https://extranet.edqm.eu/4DLink1/4DCGI/Web_View/mono/1939

EDITION 9.0

→ "Further to the EDQM workshop 'Alternatives to the leptospirosis batch potency test' held on 26-27 January 2012, the monograph has been revised […]"

Next steps:

→ Consider inclusion internationally

RABIES VACCINE

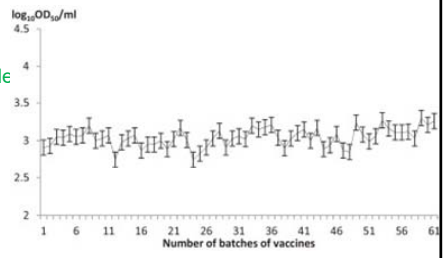
Viral inactivated vaccine, correlate of protection is known

Potency test Ph. Eur.:

1) Mouse challenge: NIH test: highest-ever variability: 25-400% confidence interval → vaccine must be overformulated to pass
2) Serology
3) Since last year at manufactureralso: ELISA

→ Published: Sigoillot-Claude, C et al: Vaccine. 2015 Jul 31;33(32):3843-9. doi: 10.1016/j.vaccine.2015.06.091. Epub 2015 Jul 2

→ ELISA is much more precise than animal test



→ Not yet in Ph. Eur.

→ Correct reading of Ph. Eur. general chapter is neede

A suitable + validated + approved test can be implemented

Next steps:

→ Consider inclusion into Ph. Eur. and internationally

HOW TO MOVE FORWARD?

Define common goals
- *In vitro* is an option

Keep these always present
- Science-informed decisions
- Guide to win-win legislation and medicine

Partnership for the goals
- Connect 3R initiatives throughout the world
- Development sharing
- Knowledge exchange!

EDQM´s contribution and global outreach is so valuable
- Ph. Eur. observer option
- BSP studies with global participation option

Include *in vitro* besides historic tests in worldwide pharmacopoeias:
- Enable getting trust by vaccine batches used in your home country

PARTNERSHIP FOR THE GOALS

Projects and platforms in EU, US, BR, KR and elsewhere

Among others: VAC2VAC

imi innovative medicines initiative 2

1. *In vitro* method development → 2. Method validation → 3. Regulatory promotion

Boehringer Ingelheim

THANK YOU FOR YOUR ATTENTION!

Boehringer Ingelheim

ATMP: HOW CAN EUR.PH. FULFIL ITS ROLE FOR TOMORROW MEDICINES? VIEWPOINT OF OMCL/REGULATOR

Maria Cristina Galli, B.Sc., Ph.D.

National Centre for Control and Evaluation of Medicines

Istituto Superiore di Sanità, Roma, Italy

EDQM and European Pharmacopoeia:
State-of-the-art Science for Tomorrow's Medicines
Strasburg, June 20, 2019

DISCLAIMER

I attend this conference as an individual expert

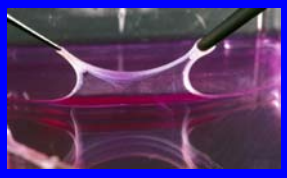
The views expressed here are my personal views, and may not be understood or quoted as being made on behalf or reflecting the position of the European/Italian Medicines Agency or one of its committees or working parties

ADVANCED THERAPIES
EU Regulation 1394/2007

Gene Therapy Medicinal Products

Cell Therapy Medicinal Products

Tissue Engineering Medicinal Products



LEGAL FRAMEWORK FOR ATMP IN EU

ATMP are regulated as medicinal products:

- clinical development under EU Dir 2001/20 (near future: EU Reg 536/2014)
- European marketing authorization granted on the basis of quality, safety and efficacy criteria
- single assessment, authorization (or refusal) across EU
- specialized committee within EMA: the Committee for Advanced Therapies (CAT)
- specific GMP, traceability and pharmacovigilance obligations
- Art 28: hospital exemption
- E.P. texts

EU DEFINITION OF GTMP

Directive 2009/120/EC :

Gene therapy medicinal product means a **biological** medicinal product:

- (a) which contains an active substance which contains or consists of a **recombinant nucleic acid** used in or administered to human beings with a view to **regulating, repairing, replacing, adding or deleting a genetic sequence**;
- (b) its therapeutic, prophylactic or diagnostic effect relates **directly** to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products **shall not include vaccines against infectious diseases.**

GTMP IN EU

viral vectors (e.g. ADV, AAV, HSV, RV, LV)
oncolytic viruses
non viral vectors (e.g. plasmids or liposomes carrying plasmids)
genetically modified bacterial cells

- ***close to classical biologicals e.g. vaccines***

genetically modified cells (autologous, allogeneic)

- ***new pharmaceutical entities***

Gene transfer acceptable only in SOMATIC cells

Germ line cells transduction unacceptable (EU dir. 2001/20, EU Reg.536/2014) → germ line manipulation (e.g. by means of CRISPR technology) not acceptable in EU

ATMP ON EU MARKET

***Chondrocelect** (2009) MACI (2013) **Glybera** (2012) Provenge (2013)*

Holoclar (2015) →TEP corneal tissue with autologous limbal stem cells for cornea regeneration

Imlygic (2015) → **GTMP** oncolytic virus for melanoma

Strimvelis (2016) → **GTMP** autologus CD34+ cells with a retroviral vector encoding human ADA cDNA sequence, for treating ADA-SCID children

Zalmoxis (2016) →CTMP allogeneic T cells genetically modified with HSV-TK for treating GVHD within a haploidentical BM transplant

Spherox (2017) →TEP spheroids of chondrocytes to repair knee cartilage defects

Alofisel (2017) →CTMP allogeneic fat stem cells for treating complex anal fistulas in adults with Crohn's disease

Yescarta (2018) → **GTMP** autologous CD19 CAR-T cell for B cell lymphoma

Kymriah (2018) → **GTMP** autologous CD19 CAR-T cell for B-ALL

Luxturna (2018) → **GTMP** (AAV-RPE65 for retinal disease)

Zynteglo (2019) → **GTMP** (autologous $CD34^{+}$ cells encoding $\beta^{A\text{-}T87Q}$-globin gene for beta thalassemia) **Pending final EC decision**

EUROPEAN PHARMACOPEA TEXT ON GTMP

5.14 GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

5.2.12 RAW MATERIALS FOR THE PRODUCTION OF CELL-BASED AND GENE THERAPY MEDICINAL PRODUCTS

Published for information:

→**not legally binding** but reflecting the E.P. authorities consensus

GTMP ON EU MARKET

Viral vectors:

Glybera (2012): **AAV**-LLP

Luxturna (2018): **AAV**-RPE65

Imlygic (2015): **oncolytic HSV**

Genetically modified cells:

Strimvelis (2016): **genetically modified autologus CD34+ cells** encoding human ADA cDNA sequence

Yescarta (2018): **genetically modified autologous CAR T** cells

Kymriah (2018): **genetically modified autologous CAR T** cells

Zynteglo (2019)**: genetically modified autologus CD34+ cells** encoding human $\beta^{A\text{-}T87Q}$-globin gene

Eu.Ph. GTP WP

A WP for Gene Therapy Products was established, that produced the chapter 5.14

The chapter was written and revised 13 and 11 years ago, respectively, **when no GTMP was market approved in EU**

5.14 GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

Recombinant Vectors
Genetically Modified Cells (**very short, not informative**)
Plasmid vectors for human use
Bacterial cells for the manufacture of plasmid vectors for human use
Adenovirus vectors for human use
Poxvirus vectors for human use
Retroviridae derived vectors for human use
Adeno-associated virus vectors for human use

No information on oncolytic virus
No information on autologous genetically modified cells

MCG 2019

GTP WP WORKPLAN FOR 2019-2020

To revise the chapter in light of development in the field made over the years

- General revision to consider recent developments in the field, e.g., to introduce, expand or revise sections on Autologous genetically modified human cells, Adeno-associated-virus vectors, Oncolytic herpes simplex virus, Retroviridae-derived vectors and Genetically modified bacterial cells with possible revision of remaining sections of the chapter and introduction of additional sections e.g. on allogeneic genetically modified cells or gene editing tools

MCG 2019

GENERAL REVISION OF CHAPTER 5.14

1) On the basis of the GTP categories presently available on the EU market:

- **revision** of the *Genetically modified cells* subsection **to cover autologous** genetically modified human cells;
- **update** of the *Adeno-Associated-Virus vectors for human use* subsection;
- **creation** of a subsection on *Oncolytic herpes simplex virus for human use.*

2) On the basis of other types of GTP most frequently used in clinical trials:

- **revision** of *Retroviridae-derived vectors for human use* subsection;
- **creation** of a subsection on genetically modified bacterial cells.

3) Continuous review of GTP developments to identify the basis for revision of remaining and creation of new sections in the chapter

MCG 2019

THE OMCL GENE THERAPY WORKING GROUP

OMCLs with activities in the field of Gene Therapy Products

Focus on technical aspects of GTP quality control tests/assays

Launched in 2008

Meets once per year

Currently 11 OMCLs involved: AGES (AT), ANSM (FR), DKMA (DK), HC (CA), ISS (IT), NIBSC (UK), PEI (DE), Sciensano (BE), Swissmedic (CH), MPA (SE), T-FDA (TW)

THE OMCL GENE THERAPY WORKING GROUP

Establishment of standard analytical methods for GTPs

- General methods – not product specific
- Easily transferable
- Validated through collaborative studies

Establishment of reference standards for these methods (and others) as needed

Define a common work program & set priorities (technologies, proximity of products to market)

Share information, know-how, resources, materials and work

Centers of excellence with high level of technical and scientific expertise

TAKE HOME MESSAGE

ATMP represent today medicines that are evolving fast

Among ATMP, Gene Therapy has fulfilled some of the promises, being now a cure for diseases that had no other treatment options before

Gene therapy Working Groups of Eur.Ph. and OMCL network will continue to work aiming at establishing a common ground for marketed GTMP

In doing so they will help developing ATMP to their full potential for the benefit of patients.

THANK YOU FOR YOUR ATTENTION!








Challenges with Testing Genetically Modified Cell Therapy Products

Mehrshid Alai-Safar, PhD

Kite
A GILEAD Company

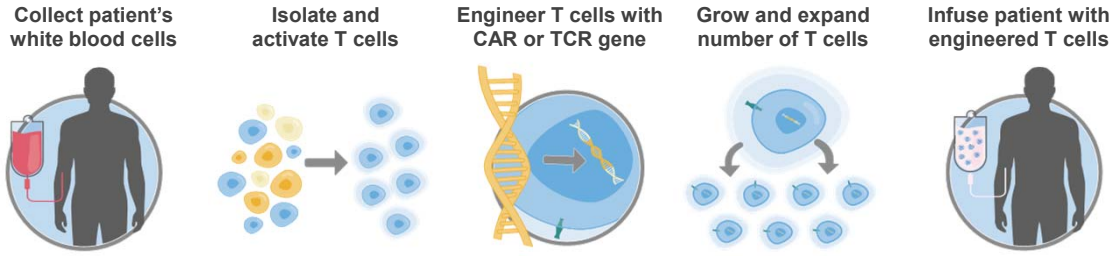
Need for a Paradigm Shift with Cell Therapy

Traditional Biologics	CAR-T Cell Therapy
▪ Decades of ecosystem in recombinant protein and mAb ▪ Bulk manufacturing, staged operations (drug substance, fill and finish) ▪ Long manufacturing cycle time & shelf life 1-5 years ▪ Make to stock ▪ Mostly 2-8°C cold chain ▪ Long lead-time, high capital investment	▪ Standards are being established ▪ Continuous process, each batch is personalized for one patient ▪ Rapid manufacturing and quality release cycle time ▪ Make to order ▪ Complex cold chain ▪ Autologous products: tracking and labeling, chain of identity ▪ Manufacturing facility quickly scalable

Kite
A GILEAD Company

CAR-T Manufacturing Process

CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY



Apheresis — Manufacturing Process — Infusion

***Ex Vivo* Cell Engineering** + **Genetic Manipulation**

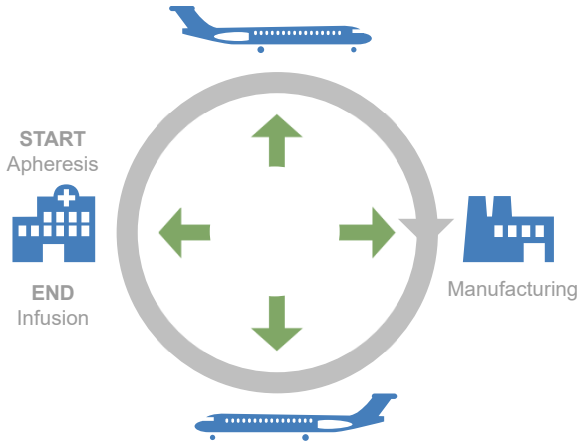
Cell Therapy Production Is Fundamentally Different from Traditional Biologic Products

Cell Therapy Production

Traditional Biologics

- Starting material from patient
- Each patient is one unique batch
- Processing equipment off-the-shelf, disposable, and single use
- Continuous processing from Apheresis to Final Product
- Requires chain of custody throughout the process
- Timing and scheduling are critical to supplying patient

Supplying patients: Coordination of departments and integration of Quality Systems



Quality System applies to Integration of

- Health Care Centers
- Patient scheduling and Treatment
- Quality release
- Traceability
- Courier shipment tracking

Target Rapid Turnaround of Patient Cells

Final Cell Product Undergoes Rigorous QC Testing

Release category	Type of Tests
Identity	Appearance
	Identity
Potency	Dose
	Potency
Purity/safety	Purity
	Microbiological tests
	Other tests

Challenges with the Cell Therapy Assays

- Complex methods
- Variable reagents (cell lines); co-culture methods, etc…
- Flow-cytometry and gating
- Sampling point for testing
- Stability of samples

Product Variability

- **Largest degree of variability comes from patient material**
  - **Manufacturing process is controlled**
  - **Single use materials help**
  - **Growth parameters between patients are variable, but split apheresis manufactured at different sites demonstrated that the final product is comparable**
  - **The patient condition may result in a value that is Out of Specification (OOS)**
  - **Specifications are set based on clinical experience**
  - **For some patients the product may be the only possible treatment option**

Recent EU GMP guidelines for ATMPs allows for making OOS product available to the patient (section 11.5):

> *"**When the request of the treating physician is received, the manufacturer should provide the treating physician with its evaluation of the risks and notify the physician that the out of specification product is being supplied to the physician at his/her request. The confirmation of the treating physician to accept the product should be recorded by the manufacturer**."*

Suggestions for Ph Eur

KILL Assay

Potency assays that are QC friendly

FDA/ EMA

Harmonization between assays and requirements

A global VCN assay

A harmonized method with a defined acceptable limit

Standards for Viability

Visual Inspection

Standards for CoC/CoI

requirements specific to these types of products

More aggressive rapid methods:

- Shorter sterility tests
- Shorter viral tests
- Quick vector titer methods

Impurities

What are acceptable limits for a one-time dose?

Import or External Testing

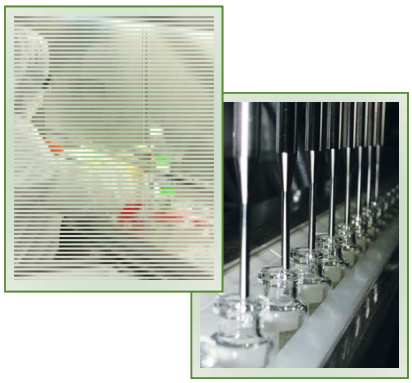
- Section 11.17 of EU GMP for ATMPs

"*It may be justified to rely on testing performed in the third country in cases where the limited amount of material available (e.g. autologous products) or the short shelf-life impedes double release testing.*

In such cases, the testing in the third country should be conducted in GMP-certified facilities (in the case of authorised ATMPs) or under GMP conditions equivalent to those applicable in the EU (in the case of investigational ATMPs)."

Product is limited in quantity and there is an urgent need for the product. Additional samples will add to production time and additional testing could prolong the release of product.

Interpreting Aseptic Processing Regulations for Gene & Cell Therapy



Each Dose is Sterility Tested

- *Challenges*
- Multiple number of workstations per suite
- Multiple manual aseptic manipulations
- High number of single use materials

Kite's Media Simulation Program

- Aseptic Process Validation per Suite
- Aseptic Operator Qualification and Requalification

Patient need drives optimal lot release cycle time in Oncology

- Each patient lot release is targeted within days of manufacture
- Single patient dose manufactured and tested
- Rapid methods used
- Deviation management and QP disposition cycle time must be streamlined and adapted to meet patient needs