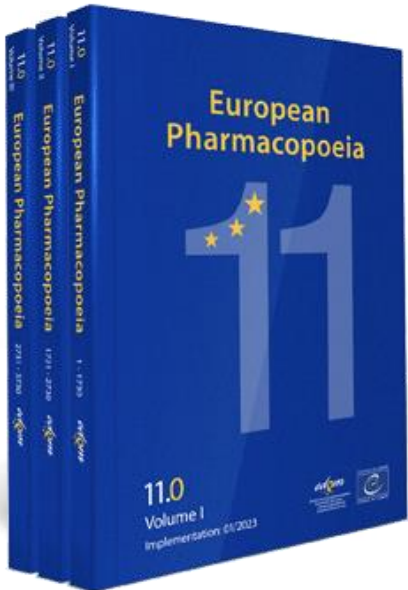
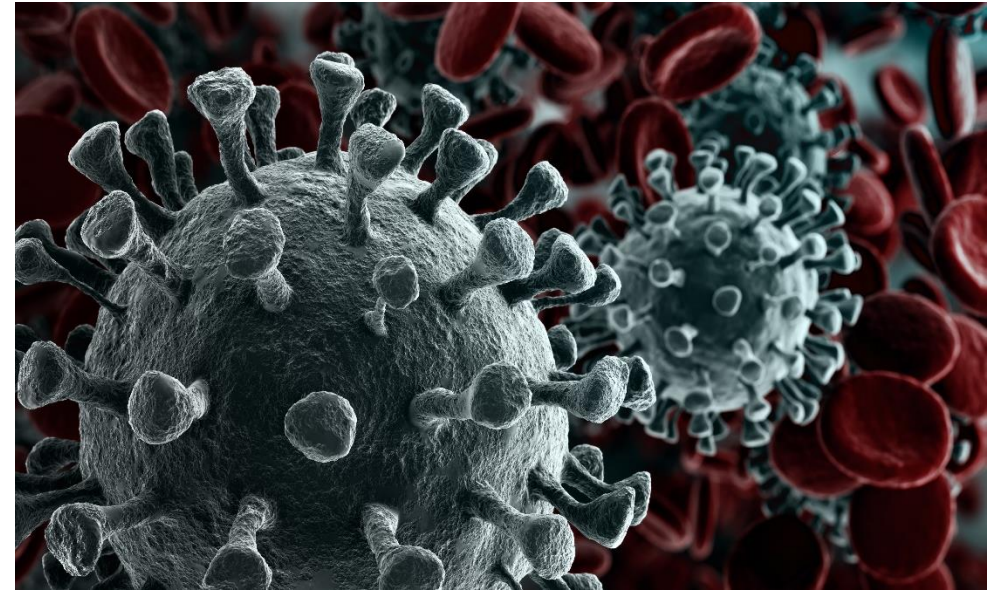


THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)



EDQM/Ph. Eur. Activities and Perspectives on Next Generation Sequencing



USP Biologics Stakeholder Forum, 22 February 2024
Gwenael Cirefice, EDQM

Outline

- ▶ European Pharmacopoeia (Ph. Eur.)
- ▶ Extraneous agents testing applicable to vaccines and viral vectors used as gene therapy products: evolution of the Ph. Eur.
 - ▶ Drivers for revising Ph. Eur. requirements
 - ▶ Evolution of Ph. Eur. 5.2.3 & 2.6.16
 - ▶ Risk assessment to define the testing strategy
 - ▶ Use of molecular methods
 - ▶ The concept of Substitution to replace *in vivo* methods as described in Ph. Eur. 5.2.14
- ▶ Perspectives on HTS and elaboration of a new Ph. Eur. chapter
- ▶ Conclusion

▶ European Pharmacopoeia (Ph. Eur.)

European Pharmacopoeia (Ph. Eur.)



- ▶ More than **2 800 documentary standards** for the quality control of medicines
 - Cover **the whole** manufacturing process (*e.g. excipients, medicinal products*)
 - All stages of the **life cycle** of a medicine from development through to production and market surveillance
 - **Methods verified & standardised**

- ▶ **About 3000 reference standards shipped to 132 countries**

Binding in the **39** signatory states of the Ph. Eur. Convention and used as a reference worldwide; **31** observers from all continents



European Pharmacopoeia Commission - treaty-based body - and its expert groups



Biological Standardisation Steering Committee

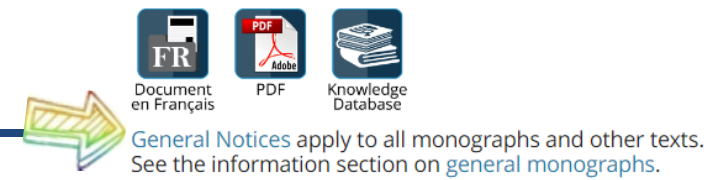


Laboratory, production, storage and distribution

**PUBLIC HEALTH
IMPACT**

- **Ensure equivalent quality and safety of medicinal products throughout Europe and facilitate their free movement in Europe and beyond**

Ph. Eur. content and structure

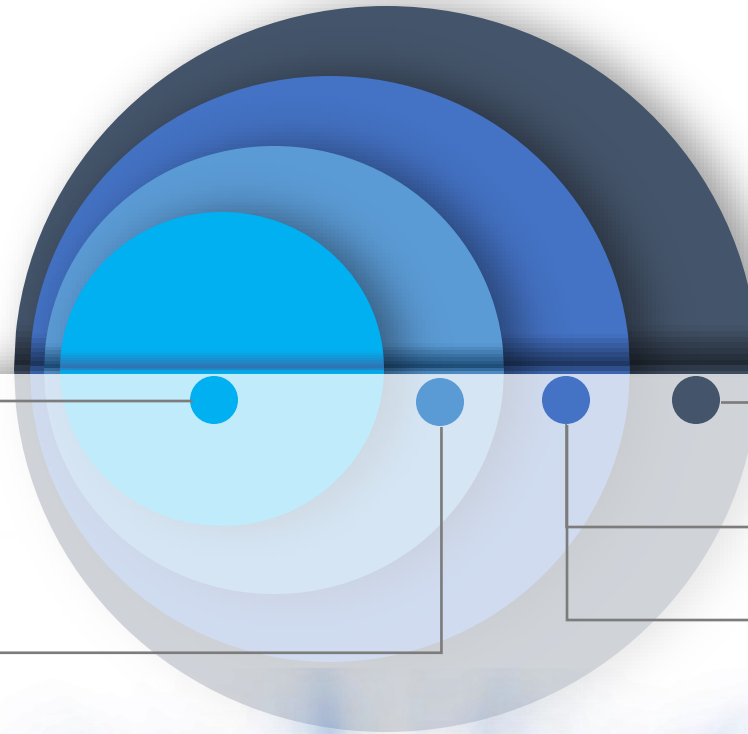


General chapters & general texts

- avoid repeating standard procedures or requirements in each monograph; aspects that cannot be treated in each monograph
- **become mandatory** when referred to in a monograph
- provide standard analytical procedures; guidance

Individual monographs

- Specific but not stand alone
- Analytical procedures and acceptance criteria represent required quality standards
- Based on approved specifications backed up by batch data
- Reliance on manufacturers' feedback (public consultation)



General notices

- **Essential reading**
- Apply to all texts
- Address general topics
- Provide basic information
- Include rules to understand texts, conventional expressions

General monographs

Dosage form monographs

- Classes of substances/medicinal products
- Mandatory for all substances/preparations within the scope of the definition
- Not cross-referenced in individual monographs (exceptions)

-
- ▶ Extraneous agents testing for vaccines and viral vectors used as gene therapy products: evolution of the Ph. Eur.



Extraneous agents testing for vaccines: drivers for change

- **Contamination of a Rotavirus vaccine** by Porcine Circovirus (2010)
 - Victoria *et al.* (Journal of virology): results showed the presence of PCV1 viral sequences using a new high throughput molecular biology method (MPS)
- **Emergence of broad molecular methods** for extraneous agent detection
- **Revised WHO TRS 978 Annex 3** "*Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks*" (adopted in 2010)
 - Risk assessment strategy and new methodologies (e.g. NGS)
- **Convergence with FDA Guidance for Industry** (2010) on testing methodologies
- **3Rs context** in Europe:
 - European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe), EU Directive 2010/63/EU

Extraneous agents testing for vaccines: drivers for change

- **EDQM survey** (2012) with Vaccine Manufacturers and CROs regarding contamination cases over a period of 10 years
- **Publications*** highlighting gaps in compendial tests:
 - Evaluation and comparison of the sensitivity of current testing packages for detection of extraneous agents → poor sensitivity of *in vivo* methods, gaps in testing packages

*J Gombold *et al.* *Systemic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products (Vaccine)* 2014

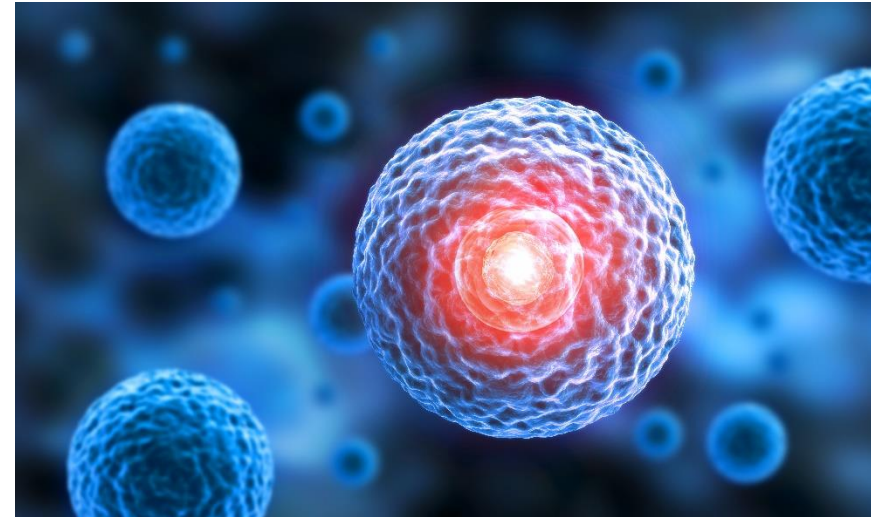
*R Sheets and P Duncan, in *Vaccine Analysis: Strategies, Principles, and Control*, Springer-Verlag Berlin Heidelberg 2015



of Ph. Eur. requirements!

5.2.3 Cell substrates for the production of vaccines for human use

- Scope: diploid cell lines and continuous cell lines used as cell substrates for the production of vaccines
 - Referenced in general monograph 0153 *Vaccines for human use* & chapter 5.14 *Gene transfer medicinal products for human use* (viral vectors used in gene therapy)
- Chapter 5.2.3 revised in 2017 (Suppl. 9.3) to **introduce the risk assessment, allow the use of broad molecular methods (e.g. HTS), and remove an *in vivo* test (test in adult mice)**
- Harmonised with WHO recommendations (WHO TRS 978 Annex 3 "*Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterisation of cell banks*").



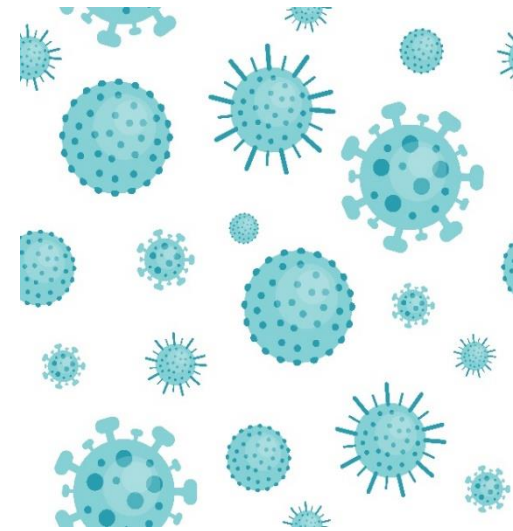
5.2.3 Cell substrates for the production of vaccines for human use

- Infectious extraneous agents: testing strategy is to be based on a risk assessment considering e.g.:
 - Choice of permissive cells: takes into account the origin of the cell substrate and the extraneous agents that may be introduced during production or through the use of animal- or plant-derived raw materials
 - Nature of cell lines (e.g. insect cell lines)
 - Cell lines shown to express endogenous retroviral particles
 - *in vivo* tests to be justified if maintained
- A strategy is given in chapter 5.2.3. Alternative strategies could focus on more extensive testing of the MCB or WCB



2.6.16 Tests for extraneous agents in viral vaccines for human use

- Applies to starting materials and substrates used for production and control of viral vaccines (virus seed lots, virus harvests, control cells/eggs)
 - Referenced in general monograph 0153 *Vaccines for human use* & chapter 5.14 *Gene transfer medicinal products for human use* (viral vectors used in gene therapy)
- Chapter 2.6.16 revised in 2017 (Suppl. 9.3) to **introduce the risk assessment, allow the use of broad molecular methods** (e.g. HTS), **and remove two *in vivo* tests** (tests in adult mice, guinea pigs)
- Panel of *in vivo* and *in vitro* methods
 - Cell culture methods
 - *In vivo* tests (suckling mice, fertilised eggs): to be justified if maintained
 - **Molecular methods** (for specific extraneous agent or broad virus detection)



2.6.16 Tests for extraneous agents in viral vaccines for human use

- **Testing strategy** (package of suitable tests) is to be built **based on a risk assessment**
 - Includes a package of tests able to detect different families of extraneous agents that may infect the source of virus strains
 - Takes into account the capacity of the process to remove / inactivate viruses

- **The list of tests is to be adapted depending on the extraneous agents that have the potential to contaminate the product**

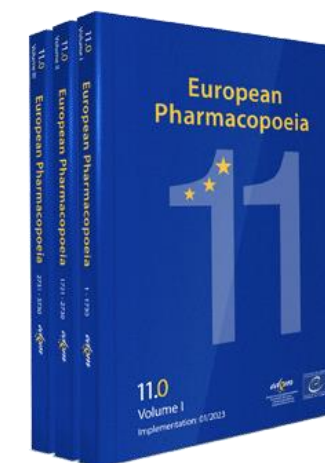


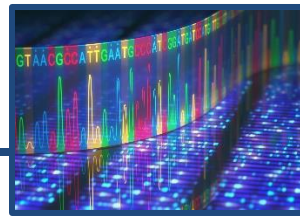
HTS in the Ph. Eur.



	Ph. Eur. Chapter 5.2.3	Ph. Eur. Chapter 2.6.16	Ph. Eur. Chapter 5.2.14
Scope	Testing of cell substrates (including extraneous agent testing)	Extraneous agent testing of viral seed lots/harvests	Concept of Substitution to replace <i>in vivo</i> methods
Test and method	Tests for specific viruses by NAT (e.g. PCR)		Test for extraneous agents: Considerations for the substitution of <i>in vivo</i> methods by broad molecular methods
	Tests for viruses using broad molecular methods (e.g. HTS) - As an alternative to <i>in vivo</i> tests and specific NAT, or - In addition/as an alternative to <i>in vitro</i> cell culture tests		

→ The use of molecular methods is foreseen in the Ph. Eur.!





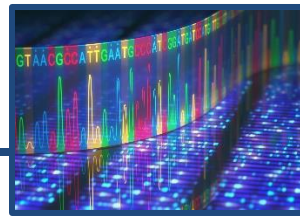
Extract chapter 5.2.3 (cell substrates):

New, sensitive molecular techniques with broad detection capabilities are available, including massive parallel sequencing (MPS) methods, degenerate polymerase chain reaction (PCR) for whole virus families or random-priming methods (associated or not with sequencing), hybridisation to oligonucleotide arrays and mass spectrometry. These methods may be used either as an alternative to *in vivo* or specific NAT tests or as a supplement/alternative to *in vitro* culture tests, in agreement with the competent authority.

Tests for specific viruses. The list of specific viruses to be tested is defined based on a viral contamination risk assessment in accordance with the principles detailed in general chapter 5.1.7. *Viral Safety*, and takes into account (but is not limited to) the origin of the cells and the potential sources of viral contamination (e.g. raw material of animal or plant origin). NAT tests (2.6.21) are carried out with or without prior amplification in cells. For cell lines of rodent origin, NAT (2.6.21) or antibody production tests in mice, rats or hamsters are used to detect species-specific viruses.

Tests for viruses using broad molecular methods. In agreement with the competent authority, broad molecular methods (e.g. High Throughput Sequencing) may be used either as an alternative to *in vivo* tests and specific NAT or as a supplement or alternative to *in vitro* culture tests based on the risk assessment.

For both NAT (2.6.21) and broad molecular methods, the stage at which testing is to be conducted (e.g. MCB, WCB, EOPC/ECB) is also based on the risk assessment and depends on the steps where viral contaminants may be introduced. In case of positive results with either broad molecular methods or NAT tests, a follow-up investigation must be conducted to determine whether detected nucleic acids are due to the presence of infectious extraneous agents and/or are known to constitute a risk to human health.



Extract chapter 2.6.16 (viral seed lots/harvests):

New, sensitive molecular methods with broad detection capabilities are available. These new approaches include high-throughput sequencing (HTS) methods, nucleic acid amplification techniques (NAT) (e.g. polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), product-enhanced reverse transcriptase (PERT) assays) for whole virus families or random-priming methods (associated or not with sequencing), hybridisation to oligonucleotide arrays, and mass spectrometry with broad-spectrum PCR. These methods may be used either as an alternative to *in vivo* tests and specific NAT or as a supplement/alternative to *in vitro* culture tests based on the risk assessment and with the agreement of the competent authority.

Tests for specific viruses by NAT. Based on a risk assessment related to the manufacturing process, each virus seed lot and each virus harvest may be tested by NAT (2.6.21) for specific viruses that are not detected by conventional *in vivo* or cell culture assays.

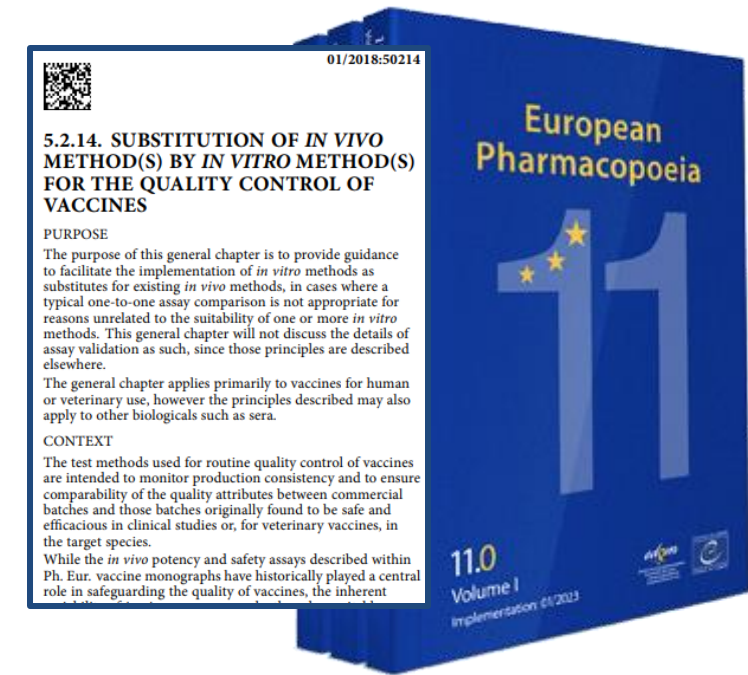
Test for viruses using broad molecular methods. With the agreement of the competent authority, broad molecular methods (e.g. ▶ HTS ◀) may be used either as an alternative to *in vivo* tests and specific NAT, or as a supplement/alternative to *in vitro* culture tests based on the risk assessment.

Both NAT (2.6.21) and broad molecular methods are carried out with or without prior amplification in suitable permissive cells. In cases of positive results with either broad molecular methods or NAT, a follow-up investigation must be conducted to determine whether detected nucleic acids are due to the presence of infectious extraneous agents and/or are known to constitute a risk to human health.

5.2.14 Substitution of *in vivo* methods for the QC of vaccines



- The introduction of *in vitro* methods to replace *in vivo* methods often prevented due to the characteristics of *in vivo* methods (e.g. variability, validation status of *in vivo* methods, product attributes assessed differently)
- Demonstration of equivalence may not only be problematic, but also of limited relevance
 - General chapter 5.2.14
 - Chapter elaborated to facilitate the transition to *in vitro* methods (e.g. HTS)

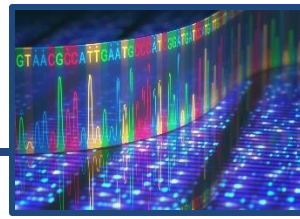


5.2.14 Substitution of *in vivo* methods for the QC of vaccines



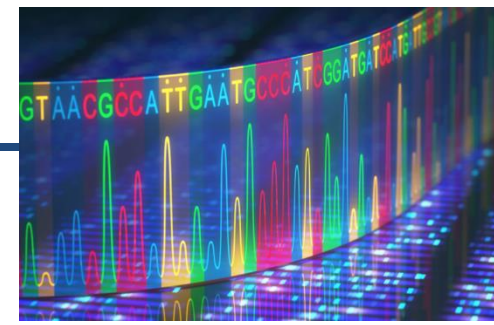
- Chapter 5.2.14 provides guidance on how to introduce alternative *in vitro* methods, where a head-to-head comparison is not possible
- Envisages the possibility that the relevance and performance of the *in vitro* method be demonstrated without such head-to-head comparison: concept of “substitution” as an alternative approach for replacement
- Focus on the scientific rationale behind the *in vitro* methods and the validation package

-
- ▶ Perspectives on HTS and elaboration of a new Ph. Eur. chapter



- Ph. Eur. chapters 5.2.3 & 2.6.16 mention HTS and foresee its use as part of the testing strategy for extraneous agents
- However, **no description** of these methods **or any guidance for their validation is provided.**
- The availability of regulatory standards including validation guidelines in the Ph. Eur. will serve as a reference for regulators and manufacturers, while:
 - HTS was recently introduced in the **revised ICH Q5A** guideline (*Viral safety evaluation of biotechnology products*) (*revision R2 adopted in Nov 2023*)
 - A panel of model viruses developed by **FDA** was also recently adopted by WHO as **WHO international reference panel for HTS**

Elaboration of a Ph. Eur. chapter on HTS



- *"High Throughput Sequencing for the detection of extraneous agents in biological products (2.6.41)"*
- Non-binding general chapter
- Content: **description of the technology/methods** and of the HTS workflow, **guidelines for validation of HTS methods**
- Under elaboration by Ph. Eur.'s **HTS Working Party** (international group of regulators, OMCLs and industry from Europe, US, Canada)
- Draft chapter planned to undergo **public consultation in April**



Elaboration of a Ph. Eur. chapter on HTS – overview

- Draft chapter will describe **HTS methodologies** used for the **detection of viral extraneous agents** in biological products including e.g. vaccines, recombinant proteins, viral vectors used for gene therapy, and cell-based preparations for cell therapy
- It outlines the different steps of the **HTS workflow**, the **design of the method**, **analysis approaches**, and the **controls** used in the routine test
- It also provides **guidelines for HTS method validation**, including recommendations for the selection of the spiking material for validation and the evaluation of the relevant performance characteristics for HTS

Elaboration of a Ph. Eur. chapter on HTS – draft outline

Description of method(s)

- Sample pre-treatment
- Extraction of nucleic acids
- Post-nucleic acid extraction treatment
- Library preparation
- Sequencing
- Bioinformatics analysis
- Scientific evaluation of the results
- Follow-up investigation
- Controls

Elaboration of a Ph. Eur. chapter on HTS – draft outline

Validation (guidelines for method validation)

- General method validation
 - “Modular” approach
 - Validation parameters of the analytical method
- Selection of appropriate spiking material for validation
- Product-specific validation

► Conclusion



Conclusion

- HTS has been successfully introduced in the Ph. Eur. for vaccines and gene therapy viral vectors
- The concept of substitution in Ph. Eur. chapter 5.2.14 can be applied to the replacement of *in vivo* tests for the detection of extraneous agents by HTS without a head-to-head comparison
- The future Ph. Eur. chapter 2.6.41 on NGS will provide a detailed description of the technology together with validation guidelines, to support users implementing this new technology



The draft chapter is planned to undergo public consultation in Pharmeuropa 36.2 (April 2024) (<https://pharmeuropa.edqm.eu/app/phpa/search/>)

→ Stay tuned!

Thank you for your attention



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