EDA Assessment Report for Biological Medicinal Product

(Scientific Discussion)

Cecolin

Date: October 2024

Unit: Technical Assessment Unit

Assessment report

Cecolin

Administrative information:

Invented name of the medicinal product:	Cecolin
INN (or common name) of the active substance(s):	Human Papillomavirus Type 16L1 Protien (Recombinant) 40 mcg/0.5ml ; Human Papillomavirus Type 18L1 Protien (Recombinant) 20 mcg/0.5ml ;
Marketing Authorization holder	Xiamen Innovax Biotech Co., Ltd., No. 52, Shanbianhong East Road, Haicang District, Xiamen City, Fujian Province 361027, China
Applied Indication(s):	Cecolin is used for preventing the following diseases caused by oncogenic human papillomavirus (HPV) types 16 and/or 18: ● Cervical cancer ● Cervical intraepithelial neoplasia Grade 2 or 3 (CIN2/3) and adenocarcinoma in-situ (AIS) ● Cervical intraepithelial neoplasia Grade 1 (CIN1) And persistent infections of HPV types 16 and/or 18. The use of Cecolin should be in accordance with official recommendations.
Pharmaceutical form(s) and strength(s):	One dose (0.5 ml) of vaccine is filled into a vial which is consist of middle borosilicate glass tubing with a capacity of 2 ml, brominated butyl rubber stoppers and an aluminum-plastic combination cap for vials.

	Each dose (0.5 mL/dose) contains 40 µg (80 µg/mL) of the recombinant HPV16 protein antigen and 20 µg (40 µg/mL) of the recombinant HPV18 protein antigen.
Route of administration	I.M. injection.
Approved Pack(s):	carton of ten single-dose vial (size: 2 mL, type I borosilicaté glass, with a rubber butyl stopper)

List of abbreviations

I.M.	Intramuscular
CTD	Common technical document
SOPs	Standard operating procedures
WHO	World Health Organization
MA	Marketing authorization
HPV	Human Papilloma Virus
CIN	Cervical Intraepithelial Neoplasia
CIN2/3	Cervical intraepithelial neoplasia Grade 2 or 3
CIN1	Cervical intraepithelial neoplasia Grade 1
VLP	virus-like particle
DNA	Deoxyribonucleic Acid
kD	Kilo Dalton
GMP	Good manufacturing practice
TSE	Transmissible spongiform encephalopathy
BSE	Bovine spongiform encephalopathy
IPTG	Isopropyl-β-D-thiogalactopyranoside
CHT	ceramic hydroxyapatite
CD	Circular Dichroism spectroscopy
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
L1	major capsid protein of HPV
PDE	Permitted Daily Exposure
PBS	Phosphate-buffered saline
CHMP	Committee on Human Medicinal Produc

EMA	European medicines agency
GMCs	Geometric mean concentrations
PD	Pharmacodynamics
PK	Pharmacokinetics
SAEs	serious adverse events

Dossier initial submission and evaluation process.

- The product was submitted for registration via 343/2021 ministerial decree.
- The dossier evaluation by the registration administration units was started on 1.11.2023 after providing all the required documents according to the Checklist for documents of new biological products registration file.
- Full CTD along with detailed SOPs were provided.

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1. General introduction about the product including brief description of the AI, its mode of action and indications

-Bivalent HPV vaccine (Escherichia coli) is an adjuvant-containing and non-infectious recombinant vaccine.
-HPV16 and HPV18 are the most common HPV serotypes with high risks. About 70% of cervical cancers are caused by HPV16 or HPV18 infection. In addition, 70% HPV-related anal and vaginal high-grade intraepithelial neoplasia is also caused by the above two viruses.
-This product is suitable for women aged 9-45 years old, and is used for preventing cervical cancer caused by high-risk HPV 16 and HPV 18, grade 2 and grade 3 cervical intraepithelial neoplasia (CIN2/3), adenocarcinoma in-situ, grade 1 cervical intraepithelial neoplasia (CIN1) and persistent infections of HPV types 16 and/or 18.

2. Quality aspects:

1.2.1 Introduction

As mentioned in the aforementioned section.

1.2.2Drug Substance (Active ingredient)

- **General information**
  - **HPV16 L1 Protein**

-The antigen is a recombinant HPV-16 L1 protein which is heterologously expressed in Escherichia coli ER2566 strain. Five L1 monomers form a pentamer and 72 pentamers self-assemble into a virus-like particle (VLP).
-The DNA sequence of the E. coli-expressed gene encoding major capsid L1 protein was originally isolated from lesion tissues of patient with cervical cancer. The gene isolated from patient were truncated at N-terminal to optimize protein expression in bacterial system, resulting 502 amino acid sequence of the N-terminal truncated HPV-16 L1 protein, and the theoretical molecular weight is 55.9 kD

  - **HPV18 L1 Protein**

-The antigen is a recombinant HPV-18 L1 protein which is heterologously expressed in Escherichia coli GI698 strain. Five L1 monomers form a pentamer and 72 pentamers self-assemble into a virus-like particle (VLP).
-The DNA sequence of the E. coli-expressed gene encoding major capsid L1 protein was originally isolated from lesion tissues of patient with cervical cancer. The gene isolated

from patient were truncated at N-terminal to optimize protein expression in bacterial system, resulting 504 amino acid sequence of the N-terminal truncated HPV-18 L1 protein, and the theoretical molecular weight is 56.1 kD.

- **Manufacture, process controls and characterization:**
  - **Manufacturer**:

Xiamen Innovax Biotech Co., Ltd. No. 52, Shanbianhong East Road, Haicang District, Xiamen City, Fujian Province, China 361027

  - **Description of Manufacturing Process and Process Controls**

-In commercial manufacture, rHPV16& rHPV18 proteins are produced by fermentation in a 400L fermenter (maximum effective volume is about 500L. One batch of single harvest is purified in one batch to obtain one batch of antigen bulk.
-All process steps and materials controls are well described and controlled.

  - **Control of Materials**

-A list and specifications of raw materials used in the production of purified HPV16 antigen bulk & HPV18 antigen bulk for HPV16/18 bivalent vaccine are provided in the MA file.
-Two animal-derived materials are used in the manufacturing process. Tryptone which used in the fermentation culture medium and IPTG used as inducer in the fermentation are obtained from bovine milk sourced from healthy animals.
-One animal-derived material is used in the manufacturing process of purified HPV18 antigen bulk. Tryptone which used in the fermentation culture medium is derived from bovine milk from healthy animals in the same manner as milk for human consumption, and no other ruminant material are used.
-The TSE/BSE certificate, CoA for tryptone (Thermofisher Scientific) and CoA for IPTG are attached with the MA file.
-The History and origin of both HPV16 L1 & HPV18 L1 genes are well described in the MA file
-both Master and working seeds for both proteins are well characterized.

  - **Controls of Critical Steps and Intermediates**

-Critical Process Parameters of HPV16 & 18 Antigen Bulks Manufacturing Process are mentioned in the MA file with their corresponding acceptance criteria & results of process validation batches (3 batches for each bulk) which found satisfactory.
-According to the manufacturing process of HPV16 antigen bulk and HPV 18 antigen bulk, four intermediates are tested, including antigen single harvest, antigen Butyl loading pool sample, antigen CHT loading pool sample and antigen CHT flow-through pool.

- **Process Validation**

-The whole process is carried out under the strict control of pre-established validation protocols and GMP.
-All the raw materials involved in validations comply with the requirements described in MA file and the equipment, facilities and various tools all conform to GMP requirements.
-No deviation or anomaly occurred during validation.
-The validation results showed that the quality and the yield of the monovalent bulk antigen products met the specified requirements, and the production processes for HPV 16/18 antigen bulk were reproducible, reliable and consistent, and could ensure that the quality of the bulk antigen products conformed to specifications
-the test methods have been fully validated. The test results of purified antigen bulk and intermediates of the batches for process validation meet the proposed specification and acceptance criteria.

- **Manufacturing Process Development**

- The first stage (2009-2014): The process was scaled up and optimized to meet the lot size requirements for clinical trial and ensure product quality. The scale of fermentation process was scaled up from 50L to 100L (fermenter volume). In this stage the purification process was scaled up and optimized accordingly.
- The second stage (2014-2017): The process of the first stage was scaled up to meet the requirements of commercial production. The scale of fermentation process was scaled up from 100L to 500L (the actual working volume is 400L) in this stage. There is no more antibiotics used in the HPV16 fermentation so the whole production process is free of antibiotics. Furthermore, the purification process is scaled up to align with the scaled-up fermentation process.
- The third stage (2017-2019): After the commercial production facility put into use, the scaled-up production process established in the second stage was transferred to the commercial production facility and the production scale, raw materials and excipients, production process, and operation principles of the main equipment are unchanged.
- At each stage of the process development, comprehensive comparability study of the process and product quality before and after the changes was conducted. The comparability study demonstrated the comparable quality of the antigen bulk lots produced at the final commercial scale and the lots produced at the 100L scale process (for Phase III clinical trial).

- **Characterization**

-the N-terminal amino acid sequence, amino acid composition, molecular weight via mass spectrometry, post-translational modifications, peptides coverage and peptide mapping, were used to characterize the primary protein structure;
-CD spectroscopy was used to characterize the secondary protein structure and particle morphology and particle radius were used to characterize the higher order protein structure.

-The biological activity and immunochemical properties of the HPV proteins are evaluated by antigen content.
Other physicochemical characteristics included antigen purity, intact L1 monomer content (SDS-PAGE) and ultraviolet spectroscopy.
-HPV 16 &HPV18 antigen bulk characterization results were summarized in tabulated form in the MA file.
-Besides process related impurities are found to be below the detection limit & no product related impurities are detected.

- **Specification**

-The release specification for the both purified HPV 16 & 18 antigen bulks comprises tests for identity, purity and impurities, potency, quantity, microbiological attributes and general attributes.
-some analytical procedures are in compliance with Chinese pharmacopia and other ones are in-house which fully validated.
-SOPs were provided with the MA file.

- **Batch analysis**

-batch analysis for three commercial consecutive batches for both HPV16 & 18 bulk antigens are provided in the MA file and their results found to be complying with the specifications.

- **Reference Standards or Materials**

-list of both commercial & inhouse reference standards that used for each test are provided in the MA file.
-Their preparation and qualification of these references are well described in the MA file.

- **Container closure system**

-The disposable liquid storage bags and glass bottles are used for storage of purified HPV16/18 antigen bulk.

-The content of most of the dissolved substances is basically low. Majority of them are close to the lower limit of detection of analytical technology, and all identifiable chemical substances can be interpreted as oligomers, polymers or oxidation resistant products obtained from degradation. It can be inferred that these substances would have no impact on the safety and the quality and therefore can be used for storing HPV16/18 bulk.

** **Specification for Disposable Liquid Storage Bags & glass bottles for HPV 16/18 bulk are mentioned in the MA file.**

-The results of the leachable, extractables, adsorption & quality studies for disposable liquid storage bags show that the concentration of possible extractables in the liquid storage bag has been shown to be very low and, has been shown to have no adverse impact on the quality of the purified HPV16/18 antigen bulk product. The liquid storage bag has no significant adsorption effect on the antigen bulk, and the antigen bulk stored in the liquid storage bag meets the specifications for the duration of the study periods under the long-term storage condition of 2-8°C.

- the results of the extractables and migration tests of the changed bulk storage containers for Glass Bottle, the extractables and leachable did not exceed 30% PDE, indicating that the bulk storage bottle system can be used for purified HPV16/18 antigen bulk storage.

- **Stability of drug substance**

Based on available stability data

- ✓ **Approved Shelf Life:**

- **HPV 16 purified antigen bulk:** 4 weeks
- **HPV 18 purified antigen bulk:** 2 months

- ✓ **Approved Storage Conditions:** Store at 5 ± 3°C

2.2.3 Drug product:

- **Description and Composition of the Drug Product:**

-The Recombinant Human Papillomavirus Bivalent (Types 16, 18) Vaccine (*Escherichia coli*) is manufactured by mixing purified HPV16 antigen bulk and purified HPV18 antigen bulk with aluminum adjuvant, to obtain bivalent antigen adsorbed bulk. The finished drug product is a milky-white suspension, stratified precipitates may form which can be dispersed on shaking, and no clumps is found on shaking.

-Each dose (0.5 mL/dose) contains 40 μg (80 μg/mL) of the recombinant HPV16 protein antigen and 20 μg (40 μg/mL) of the recombinant HPV18 protein antigen.

-One dose (0.5 ml) of vaccine is filled into a vial which is consist of middle borosilicate glass tubing with a capacity of 2 ml, brominated butyl rubber stoppers and an aluminum-plastic combination cap for vials.

➢ **Pharmaceutical Development**

- **Components of drug product**

- HPV16 bulk (or HPV18 bulk, respectively) is a colorless or light blue, clear and transparent liquid, and its active ingredient is the recombinant HPV16 protein (or HPV18 protein, respectively).
-HPV16 and HPV18 proteins are virus-like particles (VLPs) that are assembled by the purified recombinant L1 structural proteins of human papillomavirus (HPV) types 16 and 18 expressed in Escherichia coli, which shows a highly similar structure to the native virus particles.
-HPV16 and HPV18 antigen bulk drug substances contain PBS as buffer system and polysorbate 80 as the stabilizer of HPV16 and HPV18 VLP.
-all excipients are pharmacopial.

➢ **Formulation Development**

-The formulation of the drug product was determined based on the data obtained from pre-clinical studies which was then used to produce the clinical batches.
-The drug product used in the Phase I and II clinical studies were made with the formulation described previously. however, the content of the drug substance was different.
-According to the results of Phase I and Phase II studies, the content of the drug substance in final formulation was determined to be that described previously.

➢ **Manufacturing Process Development**

-From the clinical studies to commercial production, the product has undergone three process stages as previously mentioned.
-The changes in the production process (including the changes in batch size, equipment and process parameters) in different process stages are shown in MA file.
-After marketing in China, the formulation of HPV16 and HPV18 adsorbed monovalent antigen bulk was scaled up from “not more than 20L” to “not more than 50L” respectively, while the formulation of final bulk was scaled up from of “not more than 40L” to “not more than 100L”, respectively. The specific formulation process and process parameters remain unchanged. A comparability study was conducted to prove that the product quality was comparable before and after the scale-up and providing supporting data for this variation mentioned in the MA file.

- **Microbiological Attributes**

-The product is a sterile suspension for injection and free of preservatives. The sterility of products during storage is mainly achieved by the sealing of the container. The seal integrity of the vials used for this product was validated and verified by the biological challenge testing
-The study results demonstrated that the packaging materials for this vaccine have a good sealing effect and can meet the requirements.

- **Compatibility**

-The compatibility studies of the packaging materials that directedly contact with the product are mainly expected to investigate the safety of the packaging materials and the interaction between the product and the packaging materials. The compatibility studies include extraction study and leachable study.
-Firstly, three batches of vials and rubber stoppers used for production of the vaccine were selected to conduct an extraction study in different solvents to detect the potential interfering substances from vials and rubber stoppers. Then, based on the detected interfering substances from the extraction study, the leachable study was performed in three batches of the finished product to detect the potential substances and the amount that might be leached from packaging materials into the product.
-The compatibility studies were tested at the following time points and storage conditions: 12 months, 24 months, and 36 months at 2 to 8°C, and 3 months and 6 months at 25 ± 2°C.
-In addition, a toxicological assessment was conducted to evaluate the safe exposure level and the impact of packaging materials on the product, and the quality changes of drugs in long-term contact with packaging materials are monitored.

Manufacture of the drug product:
Description of manufacturing process and process controls along with manufacturers and responsibilities.

- **Manufacturer**:

The commercial production of the drug product is carried out by Innovax in the facility located at the following address:
Xiamen Innovax Biotech Co., Ltd.
No. 52, Shanbianhong East Road, Haicang District, Xiamen City,
Fujian Province, China 361027

- **Control of critical steps and intermediates**

-The critical steps of the Optivate drug product manufacturing process along with the associated in-process tests and acceptance criteria are listed in the dossier.

- **Process validation and / or evaluation**

-The HPV16/18 bivalent vaccine (vial) is a non-terminally sterilized product that must be protected from microbial contamination as much as possible during preparation, filling and other exposure operations. In order to ensure the reliability and adaptability of the aseptic manufacturing process system, we use the media filling test to prove the reliability of the aseptic process. The medium simulated filling verification was performed according to the non-final sterilization injection production process before the process validation.
- Based on that the results of performance qualification of 50L storage tank and 100Lstorage tank and validation of medium filling simulation were all acceptable, Innovax performed the process validation for three consecutive batches
-The production process and test results both met the requirements, demonstrating that products meeting the predetermined requirements could be produced persistent and stable.

- **Product specification:**

-some specifications proposed for release and stability testing of the finished product comply with Chinese pharmacopia
-Detailed SOPs ,validation protocols & reports are provided for the in-house methods
-The specifications include general characteristics, biological& general safety tests, potency &identity tests
-Justification of the drug product specifications at the release and during stability studies are provided.
-All excipients used are in compliance with Chinese,United States & Europian pharmacopia requirements.

- **Reference Standards or Materials.**

-as mentioned previously in the Drug substance section

- **Container closure system**
  - **Primary & Secondary packaging:**

-For the final HPV16/18 bivalent vaccine finished product, the vaccine is filled into a 2mL injection vial made of middle borosilicate glass tubing (referred to as vial).
-After filling, the vial is sealed with a bromobutyl rubber stopper, and then capped with an aluminum-plastic combination cap. After visual inspection, labeling, attaching package insert and boxing, the box is packed into an outer box.
-the finished products in boxes are packed in cartons, then the packaging is completed.
-Information on types, sources and supporting documents of packaging materials is shown in the MA file.

- **Stability of the drug product**

-Based on available stability data,
approved Shelf Life : 36 months

- **approved Storage Conditions:**

-Stored at 2°C to 8°C (36ºF and 46ºF)
-Protected from light.
-Do not freeze.
-Discard if vaccine has been frozen**.**

- **Adventitious agents:**

-Prior to the formulation of the finished product, the purified monovalent antigen bulks are performed sterility test and bacterial endotoxin test in release tests to ensure the absence of microbial contaminants. In addition, the microbial contaminants are also controlled in the finished product by sterility test and bacterial endotoxin test in release tests.

- **Viral Adventitious Agents**

-Not applicable. The drug product is not derived from a mammalian source.

- **Materials of Biological Origin**

-The materials of animal origin used in the manufacture of the vaccine drug substance are IPTG1 (VWR Chemicals LLC, Company) and tryptone (Thermofisher Scientific). The Certificate of Analysis and a BSE/TSE Certification and COA for IPTG are provided in the MA file
-No excipients from human or animal origin used in manufacturing of Cecolin®.

2. Non–clinical aspect:

Cecolin® is a bivalent non-infectious recombinant HPV vaccine made by purifying and recombining the L1 structural proteins of recombinant HPV 16 and 18 and indicated for preventing the diseases caused by oncogenic HPV as Cervical cancer, Cervical intraepithelial neoplasia and persistent infections of HPV types 16 and/or 18 in persons aged 9 years and older. WHO prequalified it for use on 14/10/2021.

- **Pharmacology:** The immunogenicity studies in rodents and primates demonstrated that the vaccine was highly immunogenic and can elicit a robust and persistent humoral immune response.
- **Pharmacokinetics:** As described in the WHO Guideline on non-clinical testing of vaccines and Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004), ADME (absorption, distribution, metabolism and excretion) was not conducted.
- **Toxicology:** The results of single and repeated toxicology studies carried out in rodents demonstrated that Cecolin® is well-tolerated and the observed clinical findings were non-adverse and reversible. The vaccine is not a reproductive toxicant in animals and does not appear to induce an allergic response.
- **Overall conclusion:** Based on the pharmacology and the toxicology data, the nonclinical evaluation of Cecolin® supports its clinical use in the proposed patient population

3. Clinical aspect:

- **Clinical Pharmacology conclusion.**

Clinical PKS & PDS studies are not required according to EMEA/CHMP/VWP/164653/05 Rev. 1 guideline

- **Clinical Efficacy conclusion**

These vaccines have shown an excellent efficacy profile in numerous clinical trials as a part of immunogenicity

- **Clinical Safety conclusion**

Overall, the safety profile of Cecolin vaccine not have safety concerns throughout the trials.
In the Phase I trial, there were 38 volunteers received the test vaccine (HPV16/18) who occurred (84.2%) adverse reactions that included solicited adverse reactions and unsolicited adverse events, all the adverse events were Grade 1-2 in severity.
the Common adverse reactions were reported pain at the injection site (44.8%) and fever (29.0%)
and no serious adverse events (SAEs) were reported in test group.
In the Phase II trial involving 1594 subjects, most solicited adverse reactions were Grade 1 severity, with no statistically significant differences between test and control groups
In the Phase III trial with 7372 subjects, no significant differences in adverse reactions were found between test and control groups.
The solicited adverse reactions mostly were mild (Grade 1-2) that occurred as pain at the vaccination site and fever and no Grade 4 solicited reactions were recorded.

In the Bridging study involving 979 subjects that had fluctuations in adverse events were observed across groups, with younger participants experiencing more adverse reactions compared to older participants.
The adverse reactions mostly were Grade 1, and no serious adverse events related to the vaccine were reported

- **Clinical immunogenicity conclusion**

In Phase II, III, and Bridging studies, the HPV16/18 bivalent vaccine's immunogenicity was evaluated across different doses and age groups.
In Phase II, doses of 30 µg, 60 µg, and 90 µg were assessed in healthy women aged 18-25.
In Phase III focused on the 60 µg dose in healthy women aged 18-45, evaluating peak response, immune persistence, and lot consistency.
The Bridging study compared immunogenicity in girls aged 9-17 and 9-14 to women aged 18-26year.
Across all studies, the positive conversion rates of neutralizing and IgG antibodies against HPV16/18 were consistently high (close to 100%) across age groups (9-45) year and significantly higher than controls group
Geometric mean concentrations (GMCs) of antibodies were consistently higher in the vaccine groups compared to controls that received Recombinant Hepatitis E vaccine
.
In the Bridging study, the antibody levels of HPV16/18 in younger age groups receiving either the 2-dose or 3-dose schedule were not inferior to levels in the older control group.

- **General Conclusion**

In conclusion the overall benefit/risk of HPV16/18 bivalent vaccine is favorable in female subjects 9 to 45 years of age that indicated for preventing cervical cancer, Grade 2 and Grade 3 cervical intraepithelial neoplasia and adenocarcinoma in-situ, Grade 1 cervical intraepithelial neoplasia and persistent HPV type 16 and type 18 infections