26 March 2026
EMA/107903/2026
Committee for Medicinal Products for Human Use (CHMP)
Committee for Advanced Therapies (CAT)

Assessment report

Adstiladrin

International non-proprietary name: Nadofaragene firadenovec

Procedure No. EMEA/H/C/005856/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AE	adverse event
AUA	American Urological Association
BCG	Bacillus Calmette-Guérin
BLQ	Below the Limit of Quantification
CAR	Chimeric antigen receptor
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CIS	carcinoma in situ
CMA	conditional marketing authorisation
CPE	cytopathic effect
CR	complete response
CTR	clinical trial report
DNA	deoxyribonucleic acid
DoCR	duration of complete response
EAU	European Association of Urology
ECL	electrochemiluminescent assay
ECOG	Eastern Cooperative Oncology Group
EMA	European Medicines Agency
EOT	end-of-text
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
GAG	Glycosaminoglycan
GCP	Good Clinical Practice
GFP	Green fluorescent protein
HG	high grade
IBCG	International Bladder Cancer Group
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IFN	interferon
IFNα	interferon alpha
IFNα2b	interferon alpha-2b
IFNβ	interferon beta

IND	investigational new drug application
INN	international non-proprietary name
KM	Kaplan-Meier
LG	low grade
LLOQ	lower limit of quantitation
LOQ	limit of quantitation
MA	marketing authorisation
MIBC	muscle-invasive bladder cancer
MHC-1	major histocompatibility complex 1
MTD	Maximum Tolerated Dose
N/A	not applicable
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NHP	Non-human primates
NMIBC	non-muscle-invasive bladder cancer
OS	overall survival
PD	pharmacodynamic(s)
PFU	plaque-forming units
PK	pharmacokinetic(s)
QoL	quality of life
qPCR	quantitative polymerase chain reaction
RBC	Red Blood Cell
RC	radical cystectomy
RFS	recurrence-free survival
SAE	serious adverse event
SAT	single-arm trial
SCIo	severe combined immunodeficiency
SNF	serum neutralizing factors
SUO CTC	Society for Urologic Oncology Clinical Trials Consortium
T	tumour
Tis	tumour in situ (carcinoma in situ)
TURBT	transurethral resection of bladder tumour
US	United States of America
UTUC	upper tract urothelial carcinoma

VEGF	Vascular Endothelial Growth Factor
vp	viral particles
vp/mL	viral particles per millilitre

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Ferring Pharmaceuticals A/S submitted on 22 November 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Adstiladrin, through the centralised procedure falling within the Article 3(1) and point 1a of Annex I of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 January 2021.

The applicant applied for the following indication:

ADSTILADRIN is indicated for the treatment of adult patients with high-grade (HG), Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that nadofaragene firadenovec was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision, P/0149/2021 on the granting of a product-specific waiver.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request for consideration

1.5.1. Conditional marketing authorisation

The applicant requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of Regulation (EC) No 726/2004

1.5.2. New active Substance status

The applicant requested the active substance nadofaragene firadenovec contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Scientific advice

The applicant did not seek Scientific advice from the CHMP.

1.7. Steps taken for the assessment of the product

The CAT Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Berendina Maria van den Hoorn CAT Co-Rapporteur: Marcos Timón

The application was received by the EMA on	22 November 2024
The procedure started on	24 December 2024
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	17 March 2025
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	31 March 2025
The CAT Co-Rapporteur's first Assessment was circulated to all CAT and CHMP members on	1 April 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	10 April 2025
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	16 April 2025
The applicant submitted the responses to the CAT consolidated List of Questions on	3 October 2025
The following GMP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GMP inspection at Bioreliance Corp., 9820 Darnestown Rd, Rockville, Maryland, 20850, United States between 10/03/2025 to 14/03/2025. The outcome of the inspection carried out was issued on	23 May 2025
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	11 November 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	27 November 2025
The CAT agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	5 December 2025

The applicant submitted the responses to the CAT List of Outstanding Issues on	16 February 2026
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	5 March 2026
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Adstiladrin on	20 March 2026
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Adstiladrin on	26 March 2026
Furthermore, the CAT and CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product	26 March 2026

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The Applicant initially proposed the following indication:

> "*Adstiladrin is indicated for the treatment of adult patients, Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC).*".

2.1.2. Epidemiology and risk factors

Bladder cancer is the 9th most common cancer type worldwide, with approximately 614,300 cases and 220,596 deaths reported in 2022 (Globocan), of which 224,777 and 70,383, respectively, in Europe. In 2022, the worldwide age-standardised incidence rate (per 100,000 person/years) was 9.3 in men and 2.4 in women (Globocan), and in Europe 21.1 and 5.0, respectively. The median age at time of diagnosis is 73 years (Shariat et al. BJU Int. 2010). The most important risk factor for developing bladder cancer is tobacco smoking, followed by occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons (2022 ESMO Guideline; EAU NMIBC Guidelines).

2.1.3. Biologic features

NMIBC is confined to the urothelium and does not extend beyond the submucosa (lamina propria). It includes Ta and T1 disease, and Tis/CIS (carcinoma in situ) which is always high-grade (HG).

BCG-unresponsive disease is defined as being at least one of the following (EAU NMIBC Guidelines; ESMO Guideline):

- persistent or recurrent CIS alone or with recurrent Ta/T1 disease within 12 months of completion of adequate BCG therapy;
- recurrent HG Ta/T1 disease within 6 months of completion of adequate BCG therapy; or
- HG T1 disease at the first evaluation following an induction BCG course,

with adequate BCG therapy defined as at least one of the following:
- at least 5/6 doses of an initial induction course plus at least 2/3 doses of maintenance therapy; or
- at least 5/6 doses of an initial induction course plus at least 2/6 doses of a second induction course.

2.1.4. Clinical presentation, stage/prognosis

Bladder cancer is classified into two types based on the degree of invasion into the bladder wall, either as NMIBC where the tumour is confined to the urothelium and does not extend beyond the submucosa (lamina propria) or MIBC where the tumour invades the muscularis propria. NMIBC is further classified based on the extent of involvement within the various layers of the urothelium, with Ta and T1 papillary tumours being localised and projecting into the bladder lumen, and CIS not presenting as a papillary growth, but as a flat tumour appearing red and velvety (if visible) and often being a pan-urothelial disease.

The tumour grade describes how cancer cells resemble normal cells under a microscope. Low grade (LG) looks more like normal cells, less likely to recur or progress, and high grade (HG) looks less like normal cells, more likely to recur or progress. While Ta and T1 papillary tumours can be either LG or HG, CIS is always graded as HG. HG papillary tumours (Ta/T1) and CIS have a high risk of progression to MIBC.

2.1.5. Management

The treatment of NMIBC focuses on preventing disease recurrence and halting progression to MIBC and metastasis (EAU NMIBC Guidelines).

Transurethral resection of bladder tumour (TURBT) is recommended to surgically remove all visible tumours in patients with high-risk NMIBC (EAU NMIBC Guidelines). However, due to the clinicopathological features of CIS disease, it is not always visible and therefore cannot be resected completely. For these patients, intravesical BCG therapy is the standard-of-care treatment and is recommended after TURBT to ablate the tumour and reduce the risk of recurrence and progression. For high-risk papillary tumours, the tumours are visible and can all be removed by TURBT. Therefore, the purpose of adjuvant BCG therapy is preventative against future tumour recurrence and progression.

A complete BCG course consists of two phases: an induction phase with instillations scheduled once weekly for 6 weeks, followed by a maintenance phase with instillations scheduled once weekly for 3 weeks every 3 to 6 months, for up to 3 years. However, approximately 40% of patients do not respond to BCG therapy or experience treatment failure (Zlotta et al. Can Urol Assoc J. 2009). BCG-unresponsive high-risk NMIBC is defined as persistent disease despite adequate BCG therapy, disease recurrence after initially achieving a tumour-free state after adequate BCG, or T1 tumours following a single induction course of BCG. Adequate BCG therapy is defined as a minimum of 5 of 6 doses of an induction course (adequate induction) plus 2 of 3 doses of a maintenance course, or 2 of 6 doses of a second induction course (EAU NMIBC Guidelines; FDA guidance). In patients with BCG-unresponsive NMIBC, the likelihood of a positive response to further BCG therapy is low and the risk of invasive or metastatic disease is greatly increased. These patients may be offered off-label bladder-sparing

options such as intravesical immunotherapy, intravesical chemotherapy (single-agent or combination therapy), device-assisted therapy, or combination chemo-immunotherapy. Treating BCG-unresponsive NMIBC patients with these off-label options has been evaluated by the EAU as oncologically inferior compared to radical cystectomy (RC) and the evidence supporting their efficacy was rated as 'weak'. RC, therefore, remains the recommended treatment option for these patients (EAU NMIBC Guidelines). RC, though considered a curative option, is associated with significant risks including a considerable negative impact on quality of life (QoL), significant perioperative morbidity, and a 3 to 5% increased risk of mortality within 90 days of the surgery (Korkes et al. Urol Int. 2023; Knorr et al. Urology. 2021). In addition, elderly patients with comorbidities may not endure the complications associated with cystectomy. As a result, many patients are considered ineligible for RC by their treating physician. Further, some patients are unwilling to undergo the procedure despite the risk of disease progression to muscle-invasive and/or metastatic disease. Therefore, there is a high unmet need for effective and safe alternative non-surgical treatment options for patients with BCG-unresponsive NMIBC.

A bladder-sparing treatment for BCG-unresponsive disease has been recently approved in EU: Anktiva (approved on 17-February-2026).

The current submission concerns nadofaragene firadenovec, a further new pharmacological treatment option for patients with BCG-unresponsive NMIBC for bladder-sparing treatment.

2.2. About the product

Mechanism of action:

Adstiladrin is a non-replicating recombinant type 5 adenovirus vector-based gene therapy containing the human IFNα2b transgene. Intravesical administration of Adstiladrin results in the entry of viral particles into the tumour cells and the urothelium that make up the luminal surface of the bladder, leading to the expression of IFNα2b protein by those cells. In the transduced cells, the viral DNA does not integrate into the genome. Treatment with nadofaragene firadenovec has shown anti-tumour effects in mice with bladder (cancer cell) xenografts.

The **approved indication** for Adstiladrin (nadofaragene firadenovec) is:

"*Adstiladrin is indicated as monotherapy for the treatment of adult patients with Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumours.*".

Intended posology –The recommended dose of nadofaragene firadenovec is 75 mL at a concentration of 3 x 10^{11} viral particles (vp)/mL administered by intravesical instillation every three months.

2.3. Type of Application and aspects on development

Development programme

The nadofaragene firadenovec clinical development programme in NMIBC consists of two phase 1 studies, the supportive phase 2 study rAd-IFN-CS-002, and the pivotal, single-arm study rAd-IFN-CS-003, see *Table 1*. All these studies were conducted in the US (ClinicalTrials.gov).

Scientific Advice (SA) from EMA has not been obtained for nadofaragene firadenovec on the development program for the treatment of patients with HG BCG-unresponsive NMIBC.

In light of the concerns raised during the assessment on the comprehensiveness of the submitted data

set, the applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of Regulation (EC) No 726/2004the, based on the following criteria which express only the view of the Applicant:

- The benefit-risk balance is positive:

  The applicant indicates that nadofaragene firadenovec provided a clinically meaningful complete response rate supported by adequate evidence of durability of response and a risk for progression that appears low and is not higher than historical rates of progression to MIBC at cystectomy, which vary from 27% to 51%. The applicant also reports that the safety profile of nadofaragene firadenovec is considered to be manageable in the urology setting. The majority of the trial drug-related AEs were mild to moderate in severity, localised to the bladder, and transient. Based on this evidence the applicant consider that nadofaragene firadenovec treatment, present a positive benefit-risk balance.

- It is likely that the applicant will be able to provide comprehensive data.

  The sponsor proposed using data generated from the nadofaragene firadenovec monotherapy arm in the ongoing ABLE-22 trial (study 000434) to provide additional clinical efficacy and safety data post-authorisation.

  The ABLE-22 (000434) study (study protocol v7.0) is a phase 3, randomised, multi-centre, open-label trial to evaluate the safety and efficacy of intravesical nadofaragene firadenovec alone or in combination with chemotherapy (gemcitabine and docetaxel) or immunotherapy (pembrolizumab) in subjects with high-grade BCG-unresponsive NMIBC. Approximately 100 patients will be randomised to the nadofaragene firadenovec monotherapy arm. Overall, the applicant considers data from the nadofaragene firadenovec monotherapy arm of the ABLE-22 study (000434) reported by the primary CTR will substantiate the clinical data from the pivotal phase 3 trial (rAd-IFN-CS-003) and will provide the additional data to ensure sufficient comprehensive characterisation of the efficacy and long-term safety profile. It is expected that this additional data will confirm and further substantiate the positive benefit/risk balance of nadofaragene firadenovec under a CMA and fulfil the criteria for transitioning to a full marketing authorisation when data from this commitment has been submitted for assessment.

- Unmet medical needs will be addressed, as, given the limited available options for bladder-sparing management in patients with BCG unresponsive NMIBC, nadofaragene firadenovec provides a new pharmacological treatment option for those patients with carcinoma in situ (CIS) with or without papillary tumours. Recently Anktiva (nogapendekin alfa inbakicept) has been approved under the centralised procedure as a conditional marketing authorisation in a similar indication. Nadofaragene firadenovec and ANKTIVA are both bladder-sparing therapies with the same target population: adult patients with BCG-unresponsive non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumours. Based on the presented efficacy and safety data, the applicant considers that nadofaragene firadenovec addresses the unmet medical need for a bladder-sparing therapy in patients with BCG-unresponsive NMIBC to a similar or greater extent than ANKTIVA in line with the relevant EMA guideline (EMA/CHMP/509951/2006, Rev.1).

  The applicant also claimed that nadofaragene firadenovec provides fewer instillations, shorter dwell time and a ready-to-use presentation compared to Anktiva; in addition, nadofaragene firadenovec is a monotherapy not dependent on BCG. The replication-deficient vector design minimises the potential for replication or systemic exposure. Nadofaragene firadenovec has demonstrated comparable efficacy and safety data to ANKTIVA. Long-term (5-years) efficacy and safety data is consistent with the continuously accumulating post-marketing data, real-world

experience, and supports the use of nadofaragene firadenovec as an effective bladder-sparing therapy.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required:

  As a result of the unmet need in this patient population there is a need for the immediate availability of nadofaragene firadenovec rather than waiting until data from the ongoing ABLE-22 (000434) trial is available. While ABLE-22 is a global trial including EU countries, not all countries in Europe will be included, and not all patients will meet the strict inclusion/exclusion criteria required to participate in the trial. Additionally, trial sites will be limited to specialised centres most suited for running clinical trials. The immediate availability of nadofaragene firadenovec in Europe outside of a clinical trial setting will provide access to a greater number of patients that will likely benefit from treatment. The time from expected marketing authorisation of Adstiladrin (2026) until completion and reporting of the ABLE-22 trial (2029) leading to a conversion to a full marketing authorisation is within a reasonable timeframe considering how conditional marketing authorisation has been used for other medicinal products. In the EMA report on 10 years of experience with CMA, it is stated that the mean time from granting a CMA till conversion to a standard MA is around 4 years. The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. Given the positive benefit/risk and the unmet medical need in the applied indication as described above, this is considered fulfilled.

2.4. Quality aspects

2.4.1. Introduction

Nadofaragene firadenovec is a gene therapy medicinal product, a non-replicating type 5 adenovirus (Ad5) vector encoding for human interferon alpha-2b (IFNα2b).

The finished product is presented as an intravesical suspension containing 3 x 10^{11} viral particles (vp)/mL of Nadofaragene firadenovec as active substance.

Other ingredients are: Syn3NODA ([N-(3-cholamidopropyl)-N-(3-lactobionamidopropyl)]-cholamide), Citric acid monohydrate (for pH adjustment), Sodium citrate (for pH adjustment), Polysorbate 80, Hydroxypropylbetadex, Sodium dihydrogen phosphate dihydrate (for pH adjustment), Trometamol (for pH adjustment), Sucrose, Magnesium chloride hexahydrate, Glycerol, Water for Injections (WfI). Syn3NODA is a novel excipient that functions to enhance the transduction of the urothelium.

The finished product is available in 20 mL of intravesical suspension in a single-dose clear Type I glass vial with a rubber stopper sealed with a tamper-evident aluminum crimp (4 vials x 20 mL).

2.4.2. Active Substance

2.4.2.1. General information

Nadofaragene firadenovec (rAd-IFN) is the INN for the new active biological substance consisting of a non-replicating recombinant type 5 adenovirus vector (Ad5) containing the complementary DNA (cDNA) of the human interferon α2b (IFN-α2b) transgene.

The active substance of nadofaragene firadenovec (rAd-IFN) is a non-replicating recombinant type 5

adenovirus (Ad5) based vector encoding human the Interferon alpha 2b (IFN- α2b) gene. A schematic representation of the vector, the amino-acid sequence of the expressed IFN- α2b, and a tabular overview of the major features of the rAd-IFN expression cassette have been provided. The full sequence of the virus vector is provided.

In terms of biological activity, it is proposed that rAd-IFN will deliver the IFNa2b gene into the urothelium, which is expected to have antitumor effects via direct and indirect actions, including cytotoxicity, apoptosis, angiogenesis inhibition, and immunoregulation. To facilitate the vector's uptake, the excipient Syn3NODA is included in the formulation. Sufficient information the mechanism of action of rAd-IFN/Syn3NODA has been provided.

2.4.2.2. Manufacture, characterisation and process controls

Manufacturers

The sites involved in manufacture the active substance, including sites responsible for manufacture, testing and storage of cell banks and virus seed stocks, and sites involved in testing, are listed.

Active substance manufacturing takes place at FinVector Oy in Kuopio, Finland. Initially a GMP certificate was missing (Major Objection). A valid GMP certificate was provided and this MO was considered resolved.

All the active substance manufacturing sites are GMP compliant.

Manufacturing process

A flow chart and a narrative summary of each part of the manufacturing process have been provided. Briefly, the process consists of an upstream part including cell expansion and infection and a downstream part involving several purification steps. The process is performed using single-use equipment. Information on operational ranges and in-process controls is provided. The information provided is acceptable.

Sufficient information has been provided regarding the batch-numbering system for the commercial manufacturing and the batch scale. The numbering of AS batches is described.

The manufacturing process has been described in sufficient detail. Process parameters and operational ranges are laid down in the narrative of S.2.2 and/or tabular overviews in S.2.4. Criticality of the different process steps has been included in S.2.2. The information provided is adequate and sufficient.

Control of materials

Raw materials

Raw materials cover materials of biological origin as well as non-biological origin. For non-compendial grade material the in-house specifications are provided.

Membranes and resins used in routine production of the AS are stated, and the manufacturing steps where they are used, are specified. Suppliers for critical raw material are specified.

A plan to ensure continuous, uninterrupted manufacture of the product (ensure availability of the cell banks) has been provided and it is explained how the uniformity of the master cell banks (MCBs) is ensured. Testing of cell banks includes tests for identity, microbial and viral safety. Testing of the MCB, WCB, and EOPCs is in general in line with expectations. Stability monitoring of the cell banks is

acceptable. Information on stability of the virus seed stocks was provided. Testing of the different banks and seed stocks is acceptable. Genetic stability of the virus at the production level is sufficiently demonstrated.

Control of critical steps and intermediates

Section S.2.4 includes a summary of the process control strategy, critical quality attributes (CQAs), and tabular overviews of the process control elements (critical material attributes (CMA), critical process parameters (CPP) and in process controls (IPCs)).

CPP and IPC per step of the manufacturing process are listed. IPC and CPP are listed with an acceptance criterion or acceptance ranges (AC/AR) which are in line with the information in S.2.2. The actions following non-accordance of any process control element are sufficiently laid out.

The methods for the IPC testing are sufficiently described. The information provided is adequate.

Process validation

The proposed manufacturing process is supported by adequate data. The manufacturing conditions, controls, sampling and expected outcomes (acceptance criteria) are specified. In general, the PPQ results demonstrate that the manufacturing process consistently yields a product that meets specifications and in general support the operational ranges defined in the dossier. This is acceptable.

Manufacturing Process Development

Process development has been described and divided in three main stages.

Tabular overviews of the main process changes are provided. Comparability studies to support the process changes are performed and summarized in the dossier. No stability data were used for the comparability assessment. This is acceptable as AS is stored frozen.

The provided summary of the comparability evaluation that was initially provided was very concise. Additional data to support the approach to conclude on comparability, a justification of the selection of batches that are included in the comparability studies, and additional data have been provided in response to a Major Objection that was initially raised on comparability. Upon request, the requested additional data provided sufficiently supports comparability during development and this Major Objection was considered solved.

Charactersation

A substantial amount of batches covering the clinical development are included for characterisation. However, for almost all quality attributes characterised, the results of only one AS batch are provided. Additional data have been provided upon request and the information on the manufacturing process that was used for the listed batches has been updated and is sufficient. Process-related and product-related impurities are described and are adequately controlled.

The information provided is sufficient and adequate.

2.4.2.3. Specification

The specifications are provided for production control cells, bulk harvest and active substance. Nadofaragene firadenovec active substance specifications are in line with expectations and covers relevant parameters e.g. general methods, identity, potency, safety. The justification of the specifications provided is generally acceptable. For the control of RCA, additional justification of the

proposed limit was provided. Initially the Applicant has not demonstrated that RCA was adequately controlled. As the control of RCA within clinically justified limits is considered critical a Major Objection was raised.
The applicant provided additional information in response to this MO and agreed to tighten the AS acceptance criterion for RCA in line with the RCA levels detected in clinical batches. This is endorsed.

Taken together, this MO was considered resolved and the acceptance criteria for the active substance were tightened to the clinically justified limits.

Analytical procedures and reference standards

The applicant indicates that compendial and non-compendial methods have, respectively, been qualified and validated. For non-compendial methods, method validation summaries are provided. The information provided is acceptable.

Batch analysis

The batch analysis section contains release results for several batches ranging from phase 1 clinical material to the PPQ batches for the intended commercial process.

All released batches met the acceptance criteria according to the specifications that were in place at the time of release testing.

Reference Standards and Materials

Qualification of new reference standard (RS) and working standard (WS) is sufficiently described. The use of a well-characterised RS was requested for the analysis of the viral proteins and the analysis of identity and genome integrity. The RS information provided RS is sufficient.

Container closure

The active substance is filled into single-use, sterile, containers for storage prior to finished product manufacture. The containers are Animal Derived Components Free (ADCF), certified Transmissible Spongiform Encephalopathies (TSE) or Bovine Spongiform Encephalopathy (BSE) risk free materials. The assessment and control of Extractables and Leachables (E/L) has been described.

The information provided is adequate.

2.4.2.4. Stability

A table with stability batches and tabular overviews of their stability results are provided. For the commercial process stability data from development and PPQ batches are available. The applicant indicates that the container closure system used for stability samples is made from identical materials to that described in 3.2.S.6.

The stability results for the commercial process batches all complied with the specification and support the proposed shelf life.

Based on the stability data provided the claimed shelf-life for the active substance when stored at the recommended storage conditions is acceptable.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

The finished product is presented as an intravesical suspension containing 3 x 10^{11} viral particles (vp)/mL of Nadofaragene firadenovec as active substance.

Other ingredients are: Syn3NODA ([N-(3-cholamidopropyl)-N-(3-lactobionamidopropyl)]-cholamide), citric acid monohydrate (for pH adjustment), sodium citrate (for pH adjustment), polysorbate 80, hydroxypropylbetadex, sodium dihydrogen phosphate dihydrate (for pH adjustment), trometamol (for pH adjustment), sucrose, magnesium chloride hexahydrate, glycerol, water for Injections.

Syn3NODA is a novel excipient that functions to enhance the transduction of the urothelium.

Nadofaragene firadenovec finished product is a ready-to-use presentation for intravesical instillation, supplied as four vials in a secondary container, forming one dose unit.

Each vial is a Type I borosilicate clear glass 30R vial closed with a Type I rubber stopper and sealed with an aluminium crimp cap. Each vial contains 20 mL suspension which is frozen during transportation and long-term storage. Thawed vials are combined in one or two Luer lock syringes for the administration of 75 mL suspension via catheter. There are no overages.

Components of the FP include the active substance, the novel excipient Syn3NODA, and compendial (Ph. Eur. grade) excipients.

Pharmaceutical development

The FP intended for commercialisation is a ready-to-use presentation. The qualitative and quantitative composition of the FP has remained unchanged with the transition to a ready-to-use formulation. The AS is a suspension containing nadofaragene firadenovec virus. The physico-chemical properties of the AS that can impact the performance of the FP are identified.

Descriptions for each excipient are provided and include information on their function in the suspension, their final concentration, their use in other pharmaceutical products, and a short description how their quality is controlled. Excipients initially used during clinical studies are present in the commercial product. Excipients are not of human or animal origin. For the manufacture of Syn3NODA an animal sourced starting material is used. The TSE certificate provided for this starting material is considered acceptable. Full information on the novel excipient Syn3NODA is provided in the dossier.

As polysorbates may cause allergic reactions and Polysorbate 80 (PS80) is present in the FP with a labelled amount of 9.6 mg/vial, a warning is included in the SmPC (section 2 and section 4.4) and in the Package Leaflet in accordance with the Annex to the '*European Commission guideline on Excipients in the labelling and package leaflet of medicinal products for human use*'.

A Quality Target Product Profile (QTPP) is presented which covers the relevant quality characteristics of the FP and associated CQAs that have been identified as having an impact on patient safety and product efficacy. The list of CQAs is endorsed. Despite the transition to the ready-to-use formulation, essentially no changes to the FP composition were made throughout product development. It is therefore considered that the composition of the commercial product is adequately supported by clinical development.

The description of the manufacturing process development is considered sufficiently detailed. All CPPs are provided with limits, these are considered to be adequately justified.

The suitability of the container closure system used for the storage, transportation and use of the finished product has been demonstrated. A brief overview of the controls in place to ensure microbial safety is provided and found sufficient. The integrity of the container closure system is briefly discussed. Container closure integrity testing is performed and the suitability of the container closure system has been demonstrated.

A compatibility study has been undertaken to support the administration procedure for the FP. The studies, in combination with the in-use hold time studies presented, are considered to adequately support the compatible materials and storage conditions as described in section 6.6 of the SmPC.

2.4.3.2. Manufacture of the product and process controls

Manufacturers

The names, addresses, and responsibilities of all the sites involved in the manufacturing and quality control of the finished product are provided in the dossier. All the finished product manufacturing sites are GMP compliant.

Manufacturing process

The FP manufacturing process is a relatively standard formulation-fill-freeze process and is described using flow diagrams and narrative descriptions. All the incoming materials, step principles, and in-process controls are listed and are considered sufficiently detailed.

The information provided is sufficient and acceptable.

Process controls

For each step as applicable, IPCs are listed with analytical principles, acceptance criteria, CPPs/CMAs and CQAs. In addition, descriptions are provided for several of the in-house procedures.

The information provided is adequate.

Process validation

The FP manufacturing process was validated using a traditional approach with the manufacturing of several consecutive production scale batches. It is adequately laid down which solution batches were used for the manufacture of which FP batch, and IPC results and CPP values are provided. Results for IPC testing and FP release testing comply with the acceptance criteria and are consistent between the PPQ batches, demonstrating that the FP manufacturing process is well controlled. Transportation and shipping validation is performed, and results are provided. The approach and results are adequate.

2.4.3.3. Product specification

Nadofaragene firadenovec finished product specifications covers general methods, identity, concentration, potency, purity, safety.
The quality attributes and analytical procedures included in the specification are in line with expectations based on Ph. Eur. 5.14 and Ph. Eur. 5.34. A summary of the risk assessment for elemental impurities in accordance with ICH Q3D is included in the dossier.
A statement is provided in Module 1 confirming that a risk evaluation applying the principles outlined in CHMP article EMEA/H/A-5(3)/1490 has been performed and that no risk of presence of nitrosamines was identified. A summary of the nitrosamine risk evaluation which takes into consideration potential sources of nitrosamine impurities is provided and the conclusion that the risk of nitrosamine

contamination in both the active substance and finished product is very low and unlikely to occur, is agreed.
The acceptance criteria for the quantitative quality attributes are based on batch data. Overall, the release and stability specifications for the FP are considered approvable.

Analytical procedures and reference standards

The method descriptions are sufficiently detailed and no issues were raised.

Brief method validation summaries are provided for the non-compendial methods, which are stated to be performed in line with ICH Q2. The validations of the analytical procedures used for release and stability testing of the FP have been performed. The analytical procedures are considered to be adequately validated.

As there is no reference standard specific for the FP, reference is made to the section S.5 for the assessment of the information on the nadofaragene firadenovec reference standard, and to section A.3.5 for the assessment of the Syn3NODA reference standard. This is acceptable.

Batch analysis

Batch analysis data are provided. Information on the batches provided includes the batch size, AS batch number(s) and date of manufacture and use of the batch. The acceptance criteria are in accordance with the specifications at the time of batch release.

All results comply with the acceptance criteria at the time of testing and the results indicate a consistent quality.

Container closure system

The container closure system consists of a 30R vial of clear Type I (USP/Ph. Eur.) borosilicate glass, a FluroTec coated Type I (USP/Ph. Eur.) bromobutyl rubber stopper, and an aluminium crimp cap with flip-off seal.

The glass vial is Type I borosilicate in accordance with Ph. Eur. 3.2.1 (Glass containers for pharmaceutical use) and the rubber stopper is Type I in accordance with Ph. Eur. 3.2.9 (Rubber closures for containers for aqueous parenteral preparations, for powders and for freeze-dried powders).

Suppliers of the primary container closure components are listed in the dossier and technical drawings with dimensions are provided. The information provided is sufficient.

2.4.3.4. Stability of the product

The proposed shelf-life for the finished product is 48 months when stored at below −60°C, whereof up to 3 months at maximum −20 ± 5°C. In addition, a handling period of 7 days at refrigerated conditions (2-8°C) and 24 hours at room temperature is proposed.

Since the intended long-term storage is below −20°C, the stability studies were not performed according to ICH guidelines. This is acceptable.

Initially a Major Objection was raised since the data provided for comparability between the FP primary stability batches and the commercial FP batches was not very comprehensive and the comparability was not considered satisfactorily demonstrated.

Upon request, stability data for the long-term storage condition was presented for primary stability batches, PPQ batches, and additional FP batches.

Comparability between primary stability and commercial FP materials have been adequately demonstrated and the MO was considered resolved. Based on the comparability data presented in the updated dossier, it is considered demonstrated that the stability data obtained using the FP primary stability batches can be used to establish a shelf-life for the commercial FP, which means that 48 months stability is supported by real-time stability data and is considered endorsed. It can therefore be concluded that the proposed shelf-life and storage conditions, i.e. 48 months when stored at below −60°C, whereof up to 3 months at maximum −20 ± 5°C, without exceeding the original expiry date, and the handling period of 7 days at refrigerated conditions and 24 hours at room temperature are adequately supported by stability data. If unable to administer the suspension shortly after withdrawal, the solution may be stored in syringes for up to 6 hours at room temperature (20-25°C), protected from light.

The stability data and compatibility data presented in the dossier support the information provided in the SmPC with regard to the shelf-life, handling conditions after initiation of vial thawing, and shelf-life after withdrawal from the vial. The photostability study confirms the instruction in the SmPC which states that the FP must be protected from light. The Applicant commits to complete the ongoing studies and to perform annual stability studies.

2.4.3.5. Post approval change management protocol(s)

A post-approval change management protocol (PACMP) is included with the MAA. The proposed protocol is in accordance with the EMA *Questions and answers on post approval change management protocols* (EMA/CHMP/CVMP/QWP/586330/2010) and the changes proposed in the protocol are justified and described in sufficient detail.

2.4.3.6. Adventitious agents

Section 3.2.A contains a discussion of the materials of animal origin, tabular overviews of the test performed on the cell banks and virus seed stocks, a summary of the adventitious agents' methods, and a summary of the virus safety test performed during manufacturing. A focused discussion of the measures taken to avoid and control non-viral adventitious agents (e.g. TSE/BSE, bacteria, mycoplasma) is also provided.

The information provided on these materials is adequate and sufficient.

Cell banks and virus seeds are tested for the presence of virus contamination. The bulk harvest is tested. There are no process steps specifically designed to remove or inactivate viruses, and as no virus validation studies have been performed a Major Objection was initially raised.

Upon request, virus validation studies have been performed. The applicant has performed a viral removal study. The study included 4 representative model viruses. Viral reduction capability of the different steps were evaluated for all 4 virus types. An updated risk assessment has been provided including the information on the virus clearance studies. In addition, the potential sources of virus contamination, the control strategy for cell banks and viral seeds, and the tests performed during manufacturing were considered. The testing of cell banks and seed stocks is in line with requirements, each harvest is tested for the presence of virus contaminants.

Based on the provided information the virus safety of the product is considered sufficiently ensured and the Major Objection raised on the missing virus validation studies is considered solved.

2.4.3.7. GMO

Adstiladrin (nadofaragene firadenovec) is a replication-deficient, non-pathogenic adenoviral vector, and once administered to the patient, it cannot spread in the environment. The molecular characterization of Adstiladrin does not implicate environmental risks. Limited amounts of RCA are accepted in each batch (see 2.5.3, section RCA detection). RCAs present will be lacking a functional E3 and thus are attenuated.

Recombination of the vector *in vivo*, after administration, could theoretically lead to recovery of both the E1- and E3-region. However, in this case the resulting RCA will be similar to the wild-type adenovirus with which the treated individual was already infected. The risk to the environment due to such recombination is negligible, because none of the scenarios will result in recombinants that are more fit than the wildtype virus with which the treated individual was already infected.

The environmental risks regarding formation of RCA during manufacturing or in the patient are considered negligible. Considering all the above, Adstiladrin poses a negligible risk to human health and the environment.

For discussion and conclusions on the environmental risks related to this GMO product see relevant sections in the CHMP/CAT Assessment Report (see non-clinical section for further information).

Novel excipient Syn3NODA

Syn3NODA is a novel excipient with the chemical name ([N-(3-cholamidopropyl)-N-(3-lactobionamidopropyl)]-cholamide.

Physico-chemical properties have been sufficiently described. The appearance is an amorphous hygroscopic, white to off-white powder.

Manufacturers

The names, addresses, and responsibilities of all the sites involved in the manufacturing, quality control, testing of Syn3NODA are provided in the dossier. All sites work fully according to the dossier.

Manufacturing process, process controls and validation

The manufacturing process of Syn3NODA is stated to be in compliance with GMP. The manufacturing process is comprehensive and acceptable described by flows, narrative and the synthetic process has been presented in clear molecule structures and materials/reagents schemes.

Initially essential information concerning the starting materials (SM) was not provided and a Major Objection was raised. Upon request additional information was provided on these SMs and this MO was considered resolved.

The starting materials are acceptable in view of the defined chemical properties and significant structural fragments in the final excipient structure. The specification has been updated for tests and limits on purity and related substances upon request. This is acceptable.

For the reagents, solvents and auxiliary materials specifications and CoAs have been provided which sufficiently address the characteristics of the material and suitability for the intended use including identification test.

IPCs including method descriptions and justified criteria have been provided. Also justified CPPs including justified set point or ranges have been provided.

The specifications of the intermediates are considered limited but the performed tests have been adequately justified, described and validated and the acceptance criteria have been justified by batch data.

Manufacturing process development, Characterisation

The manufacturing process was transferred to the current manufacturer. The transfer of the manufacturing process involved minimal alterations, with starting materials and the synthetic route remaining unchanged. The processes were comparable with minor changes.

The structure of Syn3NODA has been sufficiently demonstrated.

Initially insufficient information and data was provided concerning Syn3NODA impurities and acceptance criteria and therefore a Major Objection was raised. Furthermore, according to ICH Q3A, all related substances above 0.15% should be qualified. Upon request, sufficient information and adequate discussion was provided and this MO was considered resolved.

The possible routes of degradation have been adequately discussed. Also process related impurities and genotoxic impurities have been discussed. The MO concerning Syn3NODA impurities was considered resolved. The impurities discussion is considered sufficient and acceptable.

Specification, analytical procedures and batch analysis

The proposed Syn3NODA specification limits for the impurities are acceptable from a toxicological perspective. Also batch analyses data in support of the proposed specifications has been provided. In addition, an adequate genotoxic risk assessment report and related quantitative structure–activity relationship (QSAR) reports have been provided.

Adequate quality specifications have been established for Syn3NODA.

The controlled attributes are considered sufficient. Justification of the proposed acceptance criteria has been provided. The specification is overall considered acceptable. The analytical procedures have been provided and are considered acceptable.

Batch analysis data results were provided for several excipient batches (full scale manufacturing size). The batches demonstrate compliance to the current proposed specification.

Container system

The Syn3NODA excipient is packaged in bags. Specifications have been provided for the primary packaging container. Furthermore, compliance statements with Ph. Eur. 3.1.4 have been provided.

Stability

The stability study for the novel excipient Syn3NODA has been conducted on several batches under ICH long term conditions and under accelerated conditions. The batches were tested for description, assay and impurities. No clear trends were seen for any of the tested batches or storage conditions. The proposed retest period without special storage conditions is considered acceptable. The storage condition "store in the original package" is acceptable.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Seven Major Objections were initially raised during the evaluation of this MAA for Adstiladrin related to: GMP compliance (MO1); Replication Competent Adenovirus acceptance proposed limits and justification (MO2); comparability (MO3); viral safety (MO4); finished product stability (MO5); and two MOs on the novel excipient Syn3NODA (polyamide surfactant), which is used to enhance an efficient entry of the

adenovirus into the cells. These 2 MOs on the novel excipient concerned missing essential information on starting materials (MO6) and another MO regarding impurities and also the genotoxic risk assessment which had not been provided (MO7).

All these MOs were satisfactorily addressed and were considered resolved: a valid GMP certificate has been provided (MO1); adequate comparability data were provided (MO3), and virus validation studies and risk assessment were performed (MO4).

Concerning the presence of RCAs, the limit for RCA proposed by the applicant is supported by clinical data. Also, it is noted that the product is administered locally in the bladder and removed after treatment. The applicant indicates that no disseminated adenoviral infections or systemic indicated of RCA-related safety signals were observed. Furthermore, an additional control for RCA at the level of the active substance is in place, which ensures that the levels of RCA in the product will remain within the limits that have been clinically qualified. The applicant agreed to tighten the AS acceptance criterion for RCA, in line with the RCA levels detected in clinical batches. Taken together, the justification provided by the applicant and proposed acceptance criterion for RCA is acceptable and this MO was considered resolved (MO2).

Regarding the finished product shelf-life claim based on real-time data for FP primary stability batches, comparability with commercial FP batches was initially not adequately demonstrated (MO5). This has been satisfactorily resolved and the proposed shelf life (48 months at -60ºC whereof 3 months at maximum -20ºC) is endorsed (MO5 resolved).

Concerning the MOs on the novel excipient Syn3NODA, the data initially missing on the starting materials and impurities was provided upon request during the procedure. Additionally, a clear overview and full quantitative structure–activity relationship (QSAR) reports with a full toxicological prediction for Syn3NODA has also been provided as requested and is acceptable. These MOs on the novel excipient were considered resolved (MO6 and MO7).

In conclusion, based on the review of the quality data provided, the marketing authorisation application for Adstiladrin is considered approvable from the quality point of view.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall quality of Adstiladrin is considered acceptable when used in accordance with the conditions as defined in the SmPC. The quality of the active substance and finished product is controlled by adequate test methods and specifications. The manufacturing process of the active substance is adequately described, controlled and validated. The active substance is well characterised and appropriate specifications are set. The manufacturing process of the finished product has been satisfactorily described and validated. Adventitious agents' safety including TSE have been sufficiently assured.

The CHMP endorses the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above. The Applicant agreed to some recommendations for future quality development.

2.4.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommended some points for further investigation. The CHMP endorses the CAT assessment regarding the recommendations for future quality development.

2.5. Non-clinical aspects

2.5.1. Introduction

Nadofaragene firadenovec is a non-replicating recombinant type 5 adenoviral vector-based gene therapy instilled into the bladder to deliver the IFNα2b gene into the urothelium. The excipient Syn3NODA (which is a polyamide surfactant) enhances the entry of the adenoviral vector into the urothelial cells. Transduction of urothelial cells with the adenoviral vector results in the expression of the IFNα2b transgene. The urothelium thus functions as a bioreactor, producing the IFNα2b protein and generating a high local concentration of IFNα2b within the bladder, resulting in various anti-tumour effects.

The non-clinical dossier consists of several *in vitro* and *in vivo* (literature) pharmacodynamic (PD) studies to evaluate the anti-tumour effects of Adstiladrin, including the impact of intermittent treatment, dose level and dose volume, and the role of Syn3NODA. Pharmacokinetic (PK) and toxicokinetic (TK) studies have been conducted to assess the (systemic) exposure, distribution, metabolism and excretion of the vector, transgene protein and excipient. Toxicology studies have been performed to evaluate the local and systemic safety of Adstiladrin or Syn3NODA *in vivo* (general safety in Sprague Dawley (SD) rats and cynomolgus monkeys) or *in vitro* (genotoxicity). An environmental risk assessment has also been provided by the Applicant.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

In vitro primary PD

The Applicant refers to three publications (Ahmed et al. (2001); Benedict et al. (2004); Adam et al. (2007)) in which rAd-IFN (with or without Syn3NODA) has been evaluated in the context of anti-tumour activity. In Ahmed et al. (2001), it was shown that biologically active IFNα2b can be produced from the transgene in rAd *in vitro* (and *in vivo*). Intravenous administration of rAd-IFN results in the transduction of and protein expression by primarily (healthy) liver, spleen and lungs. Although this is not the anticipated clinical route of administration for Adstiladrin, it suggests that these (healthy) tissues were permissive to adenovirus infection and could produce transgene-related IFNα2b.

In Benedict et al. (2004), it was shown that IFNα2b expressed from the transgene in rAd *in vitro* resulted in sustained local protein levels for several days and anti-tumour activity (cytostasis and cytotoxicity), even in IFN-resistant human tumour cells. Due to the secretion of this IFN protein from vector transduced cells, adjacent non-transduced cells could also be targeted and killed (i.e. bystander effect).

In Adam et al. (2007), it appeared that IFNα2b expressed from the transgene in rAd can have an angiostatic effect on tumour microvessels, e.g. by reducing VEGF levels, which likely will contribute to the anti-tumour activity of Adstiladrin.

It also appears from these studies that transgene-derived IFNα2b has considerably higher anti-tumour activity compared to the local administration of recombinant IFNα2b protein (Intron A).

In vivo primary PD

<u>In vivo anti-tumour efficacy (Benedict et al. (2004))</u>

Upon IV administration of rAd-IFN to nude mice that were subcutaneously injected with bladder cancer cell to form xenografts, IFNα2b serum levels and tumour inhibition appeared to be dependent on the dose of rAd-IFN dose and were correlated. This study supports the antitumour effect of rAdIFN on subcutaneously xenografts upon intravenous administration of rAd-IFN.

The xenografts were generated with UMUC3 or KU7 cells. The KU7 cell line employed in the in vitro and in vivo efficacy studies was in 2013 determined to be contaminated and, in fact, composed of HeLa cells (Jäger et al 2013 DOI: 10.1016/j.juro.2013.03.009). The Applicant explained that the clone used in their non clinical work originated from the 1980s and was later confirmed to consist entirely of HeLa cells. Despite the unavailability of original KU7 material for genomic comparison, the HeLa based intravesical model has consistently demonstrated its utility in reproducing key features of NMIBC and in supporting the translation of preclinical findings into clinical benefit. Consequently, additional non-clinical studies are not considered necessary at this time, and the overall body of evidence continues to support the therapeutic efficacy of nadofaragene firadenovec.

Treatment with rAd-IFN of nude mice bearing orthotopic tumour model bladder cancer cell KU7 (expressing GFP), resulted in IFN tissue concentrations up to 100,000 pg/mg that remained high over the 7-day assessment period. Marked regression of human bladder cancer KU7 cell tumour was observed in xenografts of nude mice 21 days after the two days-dosing treatment with rAd-IFNα2α1/Syn3NODA or rAd-IFNα2b/Syn3NODA.

Nude mice with human superficial bladder cancer KU7 cell xenografts treated with intravesical instillation (1 hour on 2 consecutive days) of $1x10^{11}$ rAd-IFN in 1 mg/mL Syn3NODA showed reduced VEGF expression and tumour micro vessel density (evaluated via MECA-32 expression) at day 7 after treatment, indicating that IFNα2b derived from the vector transgene has anti-angiogenic effects that contribute to its anti-tumour activity.

Syn3NODA

Syn3NODA was identified as an excipient to increase adenovirus-mediated gene expression in the bladder epithelium in animal models. It is thought to facilitate adenoviral passage through the protective GAG barrier of the urothelium and permitting attachment of the adenovirus to urothelial cells. Syn3NODA can enhance adenoviral gene transfer and expression to both normal urothelium and superficial bladder tumours.

Treatment with Syn3NODA was very well tolerated in mice (intravesical and intraperitoneal) and rat (intravenous) and no side effects were observed. A more thorough analysis of the local and systemic tolerability of Syn3NODA has been studied in rat and NHP which is reflected later in this report.

For both Syn3NODA and Syn3NODA-DL (Syn3NODA in which lactobionic acid has been substituted for the gluconic acid moiety of Syn3NODA, to increase the solubility in aqueous solution. no adverse effects were observed during the 30 minutes observation time after treatment, or at blood analysis 24 hr post treatment or by histopathology at sacrifice 14 days post treatment up to the highest dose IV administered of 2.5 times the effective dose administered intravesical to enhance rAd transduction to the urothelium (0.08 ml of a 1mg/ml Syn3NODA formulation).

It appeared that 1 mg/mL Syn3NODA as excipient in the formulation seems to be the most optimal concentration resulting in most efficacious IFN expression levels upon rAd-IFN treatment. Another study showed that a larger dose volume also results in more IFN expression from rAd-IFN.

IFN expression in mice

IFNa2b expressed by rAd-IFN was still detectable in urine of immunodeficient mice up to more than 60 days, after administration, whereas this was much shorter in immunocompetent mice, where IFNα2b was only detectable up to 20 days. This suggests that clearance of the viral vector via turnover of

urothelial cells is not the only way to eliminate the virus. Immune mediated clearance might be another mechanism responsible for the loss of rAd-IFN mediated IFNα2b expression. However, as both the adeno virus and the human IFN are immunogenic in the immunocompetent mouse, it is not clear what part of the treatment would result in immune mediated clearance and how this should be translated to human.

The efficacy of a single treatment was similar to the effect of two treatments on consecutive days at the high dose of $1x10^{11}$ vp/mL. However, rAd-IFNα2α1 at a concentration of $1x10^{9}$ vp/mL was relatively ineffective at inhibiting the growth of the bladder tumours. It should be noted that the urinary IFN concentrations are within the range of the serum human IFN concentrations, recombinant protein given as a treatment, that inhibit tumour growth in subcutaneous tumour models.

The addition of the Syn3NODA formulation enhanced uptake of rAd-IFN (DNA) by bladder tissue and resulted in the expression of IFN (RNA) that encoded human IFNα2b in bladder tissue in mice.

In female immunocompetent BALB/c mice rAd-IFN in 1 mg/mL Syn3NODA intravesical administration resulted in the detection of urinary IFN up to three weeks post administration.

IFN expression in rats

SD rats intravesically administered with 0.5 mL $1x10^{11}$ vp/mL rAd- IFN in1 mg/mL Syn3NODA had urinary IFNα2b levels of 12,921.51 pg/mL significantly higher than baseline concentrations of IFN in untreated rats (27.11 - 53.71 pg/mL) and in Syn3NODA only treated rats (29.99-50.22 pg/mL) IFN concentrations persist in SD rat urine for approximately one week following intravesical administration of rAd- IFN in Syn3NODA.

IFN expression upon repeated administration in rats

Rats administered with intravesical rAd-IFN in Syn3NODA had urinary peak hIFN levels of 50,000 to 100,000 pg/mL, which was no longer detectable by 7 days. Only minimal systemic exposure to IFN was observed. Short-term redosing marginally improved the IFN urine concentrations, with maximal levels achieved when a second dose was administered 3 days after a first dose. Urinary IFN levels were attenuated when a second dose was given 5 to 7 days after the first treatment but increased again when the interval was increased up to 90 days (as compared to 5,7, 30 or 60 days interval) with measurable urinary IFN levels up to at least 72 h post dose. This suggests that the immune response is initiated between day 3 and 5 after the first dose. Rats dosed with the second dose with an interval of 30 days showed potent urothelial inflammatory infiltrate (24 h after the second dose) suggesting a host immune response. Increase of the interval to 90 days resulted in reduced urothelial inflammatory infiltrate as compared to redosing after shorter time intervals. Thus, the dose interval of 90 days seems most effective, probably because of a decrease in the host immune response. No information was present on the local humoral immunogenic response against rAdIFN or the expression of hIFN.

Another study also investigated the length of the interval between first, second and third administration of rAd- IFN / Syn3NODA. First it was shown that urinary IFN levels correlated with bladder tissue IFN levels. Additionally, it was shown that increasing the interval between doses correlated with increased urinary human IFNα2b concentrations. When the third dose rAd-IFN / Syn3NODA was given 90 days after the second dose to rats that were also treated with a 90 day interval between first and second dose, low but detectable IFN expression in the tissue were detected albeit at 100-fold less than with the first dose and 10-fold less than the second dose, however more than 10-fold more than a single dose of INTRON A® IFN protein (that shows clinical activity).

Repeated administration in Non-Human-Primates

In **study 47457** the urinary IFNα2b levels after (repeated) intravesical administration of rAd-IFN (25 mL of $1x10^{11}$ viral particles/mL) with 1 mg/mL Syn3NODA to three naïve cynomolgus monkeys were

evaluated. Furthermore, pathological evaluation was performed as well as an analysis of rAd-IFN distribution (DNA) and humoral response against the adenovirus.

Peak urinary human IFNα2b concentration was >10,000 – ~50,000 pg/mL in cynomolgus monkeys treated with rAd-IFN /Syn3NODA remaining above background levels for 14 days. Treatment with rAd-IFN without Syn3NODA resulted in much lower urinary IFNa2b levels that soon returned to background level. Repeated dosing resulted in decreased urinary IFNa2b levels. Acute cystitis was observed in the urothelium of all treated animals, which might have been caused by multiple mechanical manipulations or may have resulted from an immune response to rAd-IFN or human IFN expression. Severity and serum neutralizing antibody titre increased with repeated dosing.

Low rAd-IFN DNA was only detected in serum of one monkey 60 minutes postdosing as well as low serum levels of IFNa2b at Day 7 at necropsy (possible due to systemic distribution of the virus). This animal showed elevated anti-adenovirus titres developed within 7 days. Serum IFNa2b levels were detected in one other animal noted on days 1 and 2 post treatment which were likely the result from systemic uptake of IFNa2b from the bladder but not considered the result from systemic presence of the virus. Serum neutralizing anti-adenovirus antibodies were measured before treatment and at the end of the study in animals which were intravesically dosed four times and it is noted that in both animals, the neutralizing antibody titre increased.

The formation or presence of local antibodies again rAd-IFN or human IFN in the bladder was not investigated.

The humoral antibody response of NHP against the adenovirus increased with repeated dosing.

Intravenous administration of rAd-IFN resulted in dose-dependent serum IFN concentrations that persisted 8 - 40 days in rats and rabbits respectively and anti-hIFN antibodies were measured within 5-7 days of intravenous administration. rAd-IFN intravenously administrated to chimpanzees resulted in serum IFN concentration-time profiles like those observed in rats and rabbits without anti-hIFN being detected. Persistence of serum IFN concentrations for over 200 days in beige/SCIo immunodeficient mice was observed due to absence of humoral antibody response.

Rats receiving a second treatment 30 days after the initial dose had potent inflammatory infiltrate in the bladder 24 h after the second dose, which might point towards a host immune response. Redosing after 90 days resulting in reduction of inflammatory infiltrate was observed in the urothelium, correlating with gene expression that persisted up to at least 72 h post treatment. In NHP, acute cystitis was observed in the urothelium of all monkeys that might have been caused by multiple mechanical manipulations or may have resulted from an immune response to rAd-IFN protein expression. Severity increased with repeated dosing.

2.5.2.2. Secondary pharmacodynamic studies

No specific secondary pharmacology studies were conducted.

2.5.2.3. Safety pharmacology programme

Syn3NODA seems to be safe in the non-GLP compliant *in vitro* hERG channel up to a concentration of 3 µM which is far above the clinical systemic concentration (C_{max} 63 ng/mL = 50 nM) upon local administration of the therapy. In a GLP compliant hERG test for SYN3NODA, 4.53 µM (nominally 10 µM) is considered the highest tested concentration of Syn3NODA with no statistically significant effect on hERG tail current, thereby confirming that hERG inhibition by SYN3NODA will not occur upon clinical treatment.

Safety pharmacology studies do not indicate a risk for Syn3NODA in the *in vivo* studies evaluating CNS, cardiovascular and respiratory systems up to a concentration of 5 mg/kg. This is far in excess of the clinical systemic concentration of Syn3NODA after local administration of the therapy. No Syn3NODA related effects on the autonomic nervous system and the gastrointestinal tract were noted.

Renal/Urinary safety was evaluated in the non-clinical toxicology data package. Urinary tract inflammation (temporary), ulcerations (bladder trauma) and local irritation of the urinary bladder/urethra/ureters/kidney was observed following intravesical administration rAd-IFN/Syn3NODA or Syn3NODA alone in rats and NHP.

2.5.2.4. Pharmacodynamic drug interactions

Pharmacodynamic interaction studies are not required for this product as interference with other type of products is not anticipated based on the mechanism of action.

2.5.3. Pharmacokinetics

To assess PK and TK of Syn3NODA, rAD-IFN and IFNα2b, studies involving the intravesical (the intended clinical route of administration) and intravenous (IV) routes of administration in rats and cynomolgus monkeys have been conducted. Rat and cynomolgus monkey have been used as the toxicology species for Syn3NODA and cynomolgus monkeys were used for the toxicological assessment of rAd-IFN/Syn3NODA.

Method validation

The Applicant submitted a number of validation study reports covering all analytical methods used in the non-clinical program.

GLP status and compliance to ICHM10

The required overviews and summaries of GLP status of the performed studies, drug substance batch numbers used in non-clinical studies and summaries in line with M10 recommendations of the main validation endpoints for those validation studies that are considered within the scope of M10 have been provided. All bioanalytical validation work was conducted in accordance with practices commensurate with International Standards of GLP as appropriate for the activities, but no claims with regards to compliance to GLP were made.

Syn3NODA analytical methods

LC-MS/MS

Two validation studies were performed for the analysis of Syn3NODA in rat plasma and cynomolgus monkey plasma. The same Syn3NODA batch was used in both LC-MS/MS validation studies. The calibration range was 5.00 (LLOQ) to 500 ng/mL using 100 µL of plasma. The coagulant used in the plasma samples (both rat and monkey plasma) was EDTA. The results indicate the method to be sensitive, selective, accurate, and reproducible. However, the recovery of Syn3NODA from monkey plasma was low and had a large range (Recovery- extracted versus neat: 56.2% to 117% and Recovery- extracted versus non-extracted: 52.2% to 73.9%). Syn3NODA is stable during storage (144 days at -20°C) and stable in the autosampler for up to 99 hours. Freeze-thaw stability showed Syn3NODA was stable in methanol stock over 5 freeze-thaw cycles. The LC-MS/MS methods validated for Syn3NODA in rat and monkey plasma are fit for purpose and the validation is acceptable.

rAd-IFN related methods

ELISA

An ELISA method was developed to measure anti-rAd-IFN antibody in monkey serum after intravesical administration of rAd-IFN/Syn3NODA to monkeys.

The intra- assay variability can be expected to be less than or equal to 31.1 %CV (coefficient of variation). Intermediate precision was reported to be 25.0 %CV for the assay. Furthermore, samples were stable for six freeze-thaw cycles and seven days when stored at 4°C or at -20°C and up to five months when stored at -80°C. It appears, however, that the stability of anti-adenovirus positive control and anti-adenovirus stability were assessed in other studies (042668 & 046848). Limits of detection were not reported. Only precision, stability and suitability were reported in the study report provided.

CytoFluor

Levels of serum neutralizing factors (SNFs) against rAd-IFN (were measured in cynomolgus monkey serum using a CytoFluor-based assay. GLP status of the validation study was not provided. For the validation of this assay reference was made to other reports. "*CytoFluor-based assays for detecting SNFs for SCH 412499 and SCH 528679 in cynomologous monkey serum have been previously validated following ICH guidelines. Specificity, linearity, precision, sensitivity, accuracy, robustness and system suitability were addressed in the validation reports, #45339 (SCH 412499) and #46856 (SCH 528679).*" However, study report (SN 46856) could not be retrieved and was not provided with this submission.

qPCR

A qualified real-time quantitative polymerase chain reaction (qPCR)-based assay was used to assess rAd-IFN copy number in whole blood and bladder tissue samples from monkeys following intravesical administration of rAd-IFN/Syn3NODA, or placebo control. The same assay was also used for non-GLP determination of rAd-IFN in urine samples.

The R^2 value for linearity was determined to be 0.9867 for the quantitative rAd-lFN PCR assay, which met the acceptance criteria set in the qualification protocol (R2 greater than 0.97). Sensitivity was 10 (LOD) and 100 (LOQ) copies per ug for bladder tissue and $1*10^3$ (LOD) and $1*10^4$ (LOQ) for whole blood. Accuracy and precision were acceptable for rAd-IFN. Interference of Syn3NODA (which will be present in the monkey whole blood and tissue samples) on the analysis of rAd-IFN was not assessed but given the type of assay this is acceptable.

The qPCR method is only validated for the analysis of rAd-IFN in bladder tissue. In a GLP monkey study, it is also used for the analysis of other tissues (liver, kidney, testis, ovary). The method included only validation for liver and kidney tissues. It was assumed that, since liver and kidney tissues are tissues with a high transcription activity, these could be indicative of the other tissues and therefore the method was not validated for testis and ovary tissues.

A non-validated qPCR method was developed to determine rAd-IFN DNA in rat tissues. This method was only developed as research tool.

RCA detection

Detection of replication competent adenovirus (RCA) by a in vitro assay was conducted on rAd-IFN drug substance, which was used to produce the test materials used for the toxicology study (rAd-IFN).

FACS

A fluorescence-activated cell sorting (FACS) bioassay was used to determine the infectivity of rAd-IFN. It was also concluded that Syn3NODA did not interfere with the analysis of infectivity of rAd-IFN.

The FACS assay and RCA detection assay, however, appear to be fit for purpose and adequately validated.

Anti-IFNα2b Antibodies (ADAs) and IFNα2b analysis

BIACore

Monkey serum samples were analysed for anti-IFNα2b antibodies using a BIACore 2000 biosensor assay.

ELISA

A commercially available ELISA was used to measure human IFNα2b protein in urine following intravesical administration of rAd-IFN/Syn3NODA to monkeys. The assay was developed and qualified for GLP use to measure human IFNα2b in Cynomolgus monkey urine. The validation assay had a LLOQ of 20 pg/mL (quantitative range 20 - 2500 pg/mL) and was capable of recovering 100 and 1000 pg/mL IFNα2b reference samples within 20% of the known human IFNα2b concentration spiked into urine (accuracy determination). Inter-assay precision was approximately 20%. Recovery of human IFNα2b from pooled naïve cynomolgus monkey urine was 79% at 1 ng/mL and 107% at 100 pg/mL.

ECL

An electrochemiluminescent assay (ECL) was developed and validated to measure human IFNα2b in serum from monkeys given rAd-IFN/Syn3NODA. The LLOQ for the assay was 5 international units (IU) per mL of serum (IU/mL). The assay used two anti-IFNα2b antibodies, one biotinylated (sheep antibody) and the other labelled with ruthenium tris-bipyridine (murine antibody), targeted to different epitopes of IFNα2b. The linear dose response of the assay ranged from 86% to 110%. Total assay variance for both intra- and inter-assay precision was <9%. The variance of spiked serum samples tested undiluted and 1:10 in the assay was ≤14%. Mean percent recoveries of samples prepared in individual and normal pooled monkey sera ranged from 76% to 114% when tested undiluted, and from 82% to 100% when tested at a 1:10 dilution in the serum. Stability was assessed in the ELISA study and proved to be stable up to at least three freeze-thaw cycles.

In conclusion, the BIACore, ELISA and ECL methods were all developed to measure anti-IFNα2b antibodies and human IFNα2b in serum samples and urine samples from cynomolgus monkeys. The methods described appear to be fit for purpose and sufficiently validated.

Single-dose pharmacokinetics

Rats

After IV injection in female SD rats (n=12) of 0.5 mg/rat ^{14}C-Syn3NODA, there appears to be a rapid decline in ^{14}C-Syn3NODA radioactivity concentrations in plasma, with a $T_{1/2}$ of approximately 2.5 hours (as calculated by the assessor based on the data). In the study report, no PK analysis has been performed and no PK parameters other than mean plasma concentrations per time point are reported. Prior to dosing, rats were anesthetized and then received a single 0.5 mL intravenous dose of 1.0 mg ^{14}C-Syn3NODA/mL solution via tail vein injection. Therefore, the IV pharmacokinetics of Syn3NODA were also assessed in anesthetized animals, as are the animals that are dosed intravesically in other studies, making the results more comparable.

Systemic exposure to Syn3NODA after intravesical dosing was assessed in two studies in female rats, since the procedure cannot be performed in male rats due to physical limitations. In a non-GLP study,

systemic exposure was evaluated after a 0.5 mg/rat dose of ^{14}C-Syn3NODA under anaesthesia with a one-hour dwell period. Syn3NODA radioactivity was detected in blood samples from 6-24 hours. In a GLP study, after intravesical dosing in anesthetized female rats (n = 9/group, n=3 per time point) at doses of Syn3NODA of 0.5 mg/rat and 2.0 mg/rat and a dwell time of 60 minutes in the bladder, Syn3NODA was quantifiable in the systemic circulation. The increase in C_{max} and AUC was greater than dose proportional (approx. factor 10 increase with a dose increase of factor 4). Final quantifiable samples in the systemic circulation were found at 9 hours and 25 hours for the low-dose and high-dose animals respectively, which is in line with the findings from the non-GLP study.

The PK/TK of the rAd-IFN/Syn3NODA combination was not studied in rats.

Monkeys

Two GLP toxicity studies have been performed in monkeys in which TK was measured, covering intravenous dosing and intravesical dosing. In the first GLP-compliant acute toxicity study, the toxicokinetics of Syn3NODA were evaluated in cynomolgus monkeys (n=3 females per group) administered a single intravenous (bolus via saphenous vein) dose of 5 mg/kg. Mean Vd was 56.6 mL/kg and Cl was 8.86 mL/hr/kg. The mean $AUC_{(0-\infty)}$ was 565,000 ng*hr/mL. The half-life was ~4 hours. Comparing this (dose adjusted) AUC with the dose adjusted AUC from the intravesical study in monkeys (see below) the bioavailability of Syn3NODA after intravesical administration would be between ~1-24% dependent on the gender and/or bladder damage.

In the second GLP study, toxicokinetics of rAd-IFN and Syn3NODA were assessed in experimentally naïve cynomolgus monkeys (male and female animals) administered intravesical doses of $2.5x10^{11}$ particles or $1.25x10^{13}$ particles rAd-IFN with 25 mg Syn3NODA or 25 mg Syn3NODA alone on study Days 1 and 91. For the analysis of the TK of Syn3NODA, the animals were not grouped by dose, but by severity of pathological findings in the bladder. Here it is observed that animals with the most pathological findings in the bladder and presence of urine occult blood also had the highest systemic exposure to Syn3NODA. Leakage to the systemic circulation may be higher through damaged tissue. The amount of Syn3NODA is the same in all groups (25 mg). Although the numbers of animals used in this experiment was rather small (3M/3F per group per dosing point), there appear to be substantially more animals with bladder trauma in the highest vector dose group. Also, no bladder trauma was seen in the control animals. Therefore, the trauma was probably less or not a result of the procedure itself. It was noted that the Applicant is of the opinion that the procedure causes at least part of the bladder trauma. Considering the absence of bladder damage in the control group (C1), it is not agreed that the procedure itself causes relevant damage.

There also appears to be a gender difference, where the exposure in the males is lower than in the females. This is apparent from the reported AUCs for the animals in the different histopathological groups, but also from the reported concentration-time profiles for animals per dose group. Furthermore, it appears that at day 1 the AUC of Syn3NODA is not correlated to dosage group (no, low-dose or high-dose of rAd-IFN) and gender. However, at day 91 there appears to be an increasing trend for the Syn3NODA AUC for the females, where a higher AUC is reached in the group receiving the high-dose rAd-INF. For males this trend seems to be a decreasing AUC. This may be due to the large inter-animal differences in the obtained results and the low number of animals used in the study.

Comparing the dose adjusted IV AUC with the dose adjusted AUC from the intravesical study in monkeys, the bioavailability of Syn3NODA after intravesical administration would be between ~1-24% in cynomolgus monkeys dependent on the gender and/or bladder damage.

Presence of rAd-IFN in whole blood was determined after intravesical administration in cynomolgus monkeys by PCR at 0.5 hr and 24 hrs. In placebo animals no rAd-IFN was found and in the low-dose group the levels at 0.5 hr were below LLOQ for the majority of the animals. Quantifiable levels ($1*10^4$

to $3.41*10^6$ copies/mL whole blood) were obtained from the majority of the high-dose animals at 0.5 hr post dose, which remained over a 24hr period. For monkeys dosed a second time at day 91 similar results were obtained. Higher doses of rAd-IFN intravesically seem to increase the exposure to rAd-IFN in the systemic circulation.

Immunogenicity for rAd-IFN adenovirus was assessed in a SNF bioassay and through ELISA. The majority of the monkeys in the high-dose group had quantifiable levels of SNFs to rAd-IFN adenovirus after the first dose, whereas half of the monkeys in the low-dose group had quantifiable levels. Highest levels of SNF were measured on day 98 for the high-dose group and day 105 for the low-dose group. This is shortly after the second dose at day 91. SNF decreases after these timepoints but are still detectable at day 148 in both groups.

Antibody detection by ELISA shows antibodies in both low-dose and high-dose groups, however, the levels reached in the high-dose group are indeed higher than in the low-dose group. At day 148 there are still levels of antibodies detected in both groups at similar levels to the levels after the first dose. It seems that antibody formation is therefore correlated with the dose of rAd-IFN administered and that antibodies are persistent over time.

IFNα2b was detected in the serum following the first intravesical administration in all monkeys in the high-dose group and about half of the monkeys in the low-dose group. The serum levels in the high-dose group had a maximum of 59,417, 20,250 and 5,200 IU/mL at Days 2, 3 and 4, respectively. The serum levels in the low-dose group for these respective days had a maximum of 2,250, 330 and 113 IU/mL at these days. Therefore, serum levels of IFNα2b do appear to increase with increasing dose of rAd-IFN. The number of monkeys with serum IFNα2b levels declined on day 8 and only a few monkeys in each group had detectable serum levels on day 15. Interferon was detected in the serum following the second dose on day 91 in about half of the monkeys in the low- and high-dose groups.

Antibodies against IFNα2b were assessed by BIACore. Antibodies in serum were not detected at any interval in the placebo control group with the exception of one monkey in Week 12. The majority of the monkeys in the high-dose group developed antibodies against IFNα2b after a single dose compared to about half of the monkeys in the low-dose group. There appears to be a positive correlation between IFNα2b protein levels and presence of antibodies against IFN in serum, especially in the high-dose animals.

Repeat-dose pharmacokinetics

Repeat-dose pharmacokinetics in rats have only been studied for Syn3NODA, not for the combination rAd-IFN/Syn3NODA. The pharmacokinetics of rAd-IFN/Syn3NODA repeat dosing in monkeys (2 doses at day 1 and at day 91, respectively) has been assessed under the single-dose pharmacokinetic studies. A pilot study in cynomolgus monkeys with intravesically administered rAd-IFN/Syn3NODA at different dosing intervals was performed to support the choice for the therapeutic dosing interval. This study is discussed under the primary pharmacology section of this AR (section 2.5.2.1).

In male and female SD rats, intravenous doses of 0.5 mg/kg, 1.5 mg/kg or 5 mg/kg Syn3NODA were administered daily for 10 days. Over time, there was no accumulation of Syn3NODA with accumulation ratios just below 1 (0.7-0.9). There was also no substantial (>2-fold) observed sex difference, however, exposure of males was higher at all doses and timepoints measured. The increase in C_0 and AUC was dose proportional across the three tested dose levels.

Another study in male and female SD rats (n = 6/sex/dose) used doses of 0.66 mg/kg and 2.0 mg/kg Syn3NODA were administered intravenously once daily for 14 days. The findings were similar to the previous IV study. There appears to be no accumulation over 14 days and there are no apparent sex differences, yet, exposure in males was consistently higher than in females (<2-fold). Clearance (Cl) was also determined and did not change over time nor by dose. A sex and dose combined mean Cl of

0.038 L/h/kg was established at day 1 and 0.043 L/h/kg at day 14. Mean combined Volume of distribution (Vz) was 0.115 L/kg on day 1 and 0.112 L/kg on day 14. Therefore, the compound is slowly cleared compared to hepatic blood flow and Syn3NODA is not confined to the distribution only in the circulation. Half-life was very stable at ~2 hours.

In cynomolgus monkeys (n = 4/sex/group) daily intravenous administration of 0.5 mg/kg, 1.5 mg/kg and 5.0 mg/kg Syn3NODA for 16-18 days was studied. Kinetics were determined on day 1 and day 15. Overall, the TK in monkeys appear comparable to the TK in rats. No accumulation over 15 days was observed, nor any sex differences. This is in contrast to the TK observed after intravesical dosing, where a sex difference was observed. The increase in C_0 and $AUC_{0\text{-}24hr}$ was dose proportional across the three tested dose levels.

Distribution

Syn3NODA distribution

Plasma protein binding and distribution in blood cells of Syn3NODA have not been established. The Applicant argues that SynNODA plasma protein binding and distribution to blood cells is not considered necessary based on the PK profile and intended clinical use.

Distribution of Syn3NODA was studied in female rats (2 studies) and male rabbits (1 study). The choice for the rabbit as a species for a distribution study is not justified, other than that it was a requirement from the FDA to study the distribution to male reproductive tissues after intravesical dosing (which is not possible in male rats).

In rats, distribution of Syn3NODA was studied in two different non-GLP studies. One study used intravenous dosing in female SD rats (0.5 mg ^{14}C-Syn3NODA/rat) and the other study used intravesical dosing in female SD rats (0.5 mg ^{14}C-Syn3NODA/rat). Whole body autoradiography was used to analyse tissue radioactivity in both studies.

After intravesical dosing with a 1-hour dwell time, highest concentrations were found in the bladder (1 hr post-dose), kidney (3 hrs post-dose) and liver (6 hrs post-dose). Tissue:blood ratios were >1 for bladder (~29.5), fat (~8-9), kidney (~5.5) and liver (~3) at the aforementioned timepoints. In all other tissues the ratios were <1, with the exception of spleen (~1 at 6 hrs post-dose), stomach (~3 at 6 hrs post dose), small intestine (3.8 at 6 hrs post dose) and ovary (~2 at 6 hrs post dose). Syn3NODA was also detected in tissues of the central nervous system (brain, pineal gland and spinal cord). At 96 hours post dose, radioactivity was still present in the lymph nodes, bladder wall, kidney, liver, adrenal gland, thymus, fat tissue, ovary, lung, pancreas, spleen skin and GI tract tissues.

After IV administration of the same dose, a similar distribution in rats was observed, although only 1 hr and 168 hr timepoints were measured. Substantial concentrations of Syn3NODA were seen in blood, lymph node, bladder wall, kidney, liver, adrenal gland, ovary and lung, but tissue:blood ratio was only >1 for liver tissue. Again, Syn3NODA was also detected in the central nervous system tissues, even up to 168 hrs post dose (pineal gland and spinal cord). Other tissues that still had quantifiable amounts of Syn3NODA-related radioactivity at 168 hrs post dose were lymph node, kidney, liver, adrenal gland, pituitary gland, thymus, salivary gland, brown fat, ovary, uterus, spleen, oesophagus and small and large intestine, although no tissue:blood ratios were calculated for the 168 hour time points.

Based on those two studies it can be concluded that Syn3NODA is distributed to different tissues in healthy female rats after both intravenous and intravesical administration. Exposures were understandably higher after intravenous administration (7160 ng eq/g in blood) versus intravesical administration (223 ng eq/g in blood). Bladder wall concentrations were comparable, being 6580 ng eq/g after intravesical dosing and 5910 ng eq/g after intravenous dosing. Concentrations in tissues do not exceed blood concentrations to a large degree, however, at 96 hrs and 168 hrs post-dose

Syn3NODA is still quantifiable in a substantial number of tissues. Syn3NODA is, therefore, relatively persistent.

In a GLP study in rabbits, 2 mg/kg [^{14}C]-Syn3NODA was administered to male Dutch Belted rabbits via intravesical administration, with a dwell time of 60 minutes. The distribution of radioactivity into tissues was examined using Quantitative Whole-Body Autoradiography (QWBA) at 1, 24, 96 and 168 hours after dose administration (n = 1 per time point). At 1 hr, Syn3NODA was measured at quantifiable levels in almost all tissues. Highest concentrations were in the bile duct (4298 ng eq/g), bladder contents (14855 ng eq/g), urethra (5152 ng eq/g) and small intestine (1030 ng eq/g). These concentrations were also much higher than the concentration in blood (77.1 ng eq/g), which is in line with the primary excretion and administration route. Low concentrations (< blood concentrations) were detected in the male reproductive tissues. Concentration in testis:blood was at a ratio of 0.19 at 1 hr. At 24 hrs, Syn3NODA was present mostly in GI tissues, while it was BLQ or not detected in blood or reproductive tissues anymore. At 168 hours, Syn3NODA was still present in caecum contents, large intestine contents, small intestine contents, small intestine mucosa and stomach contents. Distribution to the CNS was limited. Syn3NODA was only present in quantifiable amounts in the choroid plexus at 1 hr post dose. Syn3NODA does not distribute well to pigmented tissues. Concentrations in pigmented skin and non-pigmented skin were comparable, and the concentrations found in the uveal tract in the eye were low and only detected at 1 hr post dose.

Overall, it appears Syn3NODA is distributed to several tissues in male rabbits, comparable to female rats, after intravesical administration.

rAd-IFN distribution

In rats, a pilot study was performed for the distribution of rAd-IFN to blood, liver and kidney. Six female SD rats were intravesically dosed with 0.5 mL rAd-IFN ($1x10^{11}$ viral particles/mL) in 1 mg/mL Syn3NODA. Samples were analysed on day 4 and day 14 by analyzing rAd-IFN DNA with PCR. No rAd-IFN DNA was detected in the blood or liver samples. One kidney sample contained rAd-IFN DNA ($2.88x10^{2}$ copies/mg kidney tissue) at day 14.

In a GLP cynomolgus monkey study, tissue distribution of rAd-IFN was studied following intravesical administration, but only for bladder, liver, kidney and gonad tissue. A full tissue analysis of the presence and persistence of rAd-IFN has not been performed. Moreover, it has not been evaluated which cell types/layers in the bladder were transduced with rAd-IFN. Furthermore, the qPCR method is only validated for the analysis of rAd-IFN in bladder tissue.

In short, rAd-IFN specific DNA was determined in monkey tissue with qPCR. Cynomolgus monkeys were dosed intravesically with a high-dose or low-dose of rAd-IFN/Syn3NODA at day 1 and day 91. Copies of rAd-IFN specific DNA were obtained from liver tissue at day 8 (2/6 animals) and day 98 (3/6 animals), but not day 148 for the high-dose group. In kidney rAd-IFN DNA was found only in the high-dose group at day 8 (2/6 animals) and day 98 (1/6 animals), but not day 148. In the low-dose group kidney values were only obtained at day 148 (1/4 animals). Testes and ovary were only analysed in the high-dose group and were positive for rAd-IFN specific DNA at day 8 only (1/3 animals for both tissues).

Overall, although tissue distribution data is limited, it appears that at least in monkeys rAd-IFN does enter the blood and distribute systemically into several tissues following intravesical administration.

IFNa2b distribution

IFNα2b distribution after subcutaneous administration of ^{125}I- IFNα2b was studied in male SD rats up to 24 hrs after administration by qualitative whole-body autoradiography. Distribution of ^{125}I- IFNα2b was mainly evident in blood and kidneys at a maximum at 1hr post-dose and decreasing at 4hrs and 24 hrs post-dose. Radioactivity was also detected in the GI tract and thyroid. No radioactivity was detected in brain at any timepoint. The distribution of 125I- IFNα2b therefore seems to be limited mainly to blood and kidneys. After 24hrs remaining radioactivity was negligible.

In the above-described intravesical monkey study, IFNα2b levels could be detected in serum from low- and high vector dose animals for several days and subsequently declined. However, it is not clear whether the human IFN protein in serum is all derived from production in the urothelium or also from other tissues.

Metabolism

Syn3NODA metabolism was studied in two studies in female SD rats. ^{14}C-Syn3NODA was dosed at 0.5 mg/rat either via the intravesical administration route (SN-03332) or via the intravenous route (SN-0423). One metabolite was observed after IV dosing, but only in faeces. It is suggested that the metabolite in faeces is formed by gut microorganisms. An *in vitro* metabolism study in hepatocytes, comparing the non-clinical species and human metabolism of Syn3NODA was provided. The main identified component in hepatocytes after 120 minutes of incubation (20uM) was the parent Syn3NODA compound, ranging from 73.5% in male rabbits to 96.2% in human hepatocytes. Least metabolism of Syn3NODA was detected in humans. In non-clinical species (rabbit, mouse, rat minipig, monkey), no major metabolites were detected, with the exception of OxSyn3NODA in male rabbits (19.1%) and male monkeys (11.5%). In human hepatocytes, this metabolite was only 1.14%

In vivo studies in monkeys (and humans) were not performed, which is acceptable from a 3Rs point of view.

Metabolism of rAd-IFN was not studied *in vitro* nor *in vivo*. This can be agreed for the type of product.

Excretion

Syn3NODA

After a single IV dose of 0.5 mg Syn3NODA/rat, excretion in faeces was the primary route of elimination of ^{14}C-Syn3NODA-derived radioactivity with, on average, nearly 79% of the administered dose being excreted over the first 48 hr. Over this same time period, mean urinary excretion was approximately 7% of the administered dose.

Urine samples from rats dosed intravesically with Syn3NODA were collected until 96 hours post dose. The majority of Syn3NODA in rats (after intravesical administration) is excreted in the first 24 hours. The amounts excreted after 24 hours are very small (<5%) compared to the amount excreted in the first 24 hours. There appears to be no renal accumulation after 96 hours of intravesical administration.

IFNα2b

In the GLP study in cynomolgus monkeys (intravesical dosing at two dose levels of rAd-IFN), presence of IFNα2b in urine was assessed and found by ELISA. All animals (also placebo and Syn3NODA control animals) tested negative for human IFNα2b pre-dose. At day 1, day 2, day 3 and day 4 the concentrations of IFNα2b correlated with the dose, with higher concentrations found in the high-dose compared to the low-dose group. (High) levels were also found in a placebo animal at day 3 and at day 15 in the Syn3NODA control group in 2/10 animals, likely due to contamination. At day 91, levels of IFNα2b were still detectable in the low-dose group, but not the high-dose group. After redosing higher levels were again reached at day 91-day94 in the high-dose group compared to the low-dose group. At day 105 no IFNα2b was detected in any animals in any group.

Pharmacokinetic drug interaction & other PK studies
No interaction or other PK studies have been performed for Syn3NODA nor the rAd-IFN/Syn3NODA combination.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

The Applicant conducted several single dose toxicity studies. Two studies with rAd-IFN/Syn3NODA administered intravesically (in rat and NHP respectively), three studies in which Syn3NODA was administered to rats intravesically, IV or IP and one study with Syn3NODA administered IV to NHP.

rAd-IFN/Syn3NODA
In female Sprague Dawley rats, there were no signs of local or systemic toxicity related to treatment with $0.5x10^{11}$ rAd-IFN in 0.5 mg Syn3NODA. This pilot study indicated that IFNα2b could be produced following dosing of rAd vector in the bladder and that this production did not result in rAd-IFN-specific (local) toxicity or considerable systemic distribution/exposure in female rats.

For the study in cynomolgus monkeys, reference is made to the description and assessment below under repeated dose toxicity (Section 2.5.4.2).

Syn3NODA alone

Female Sprague-Dawley rats were intravesically treated once with 0.5, 1 or 2 mg Syn3NODA (in 0.5 ml) for a one hour instillation period. Animals were sacrificed at 4 or 15 days post-treatment. Syn3NODA administered in the bladder resulted in temporary urinary tract inflammation and local irritation of the urinary bladder/urethra/ureters/kidney was observed (with hematological and histopathological analysis) in several rats in all dose groups. Systemic toxicity was not evaluated in this study. The NOAEL for local toxicity of Syn3NODA was < 0.5 mg, based on clinical and histological signs of (temporary) urinary tract inflammation and local irritation.

Following IV administration of 1 or 5 mg/kg Syn3NODA to female Sprague-Dawley rats, the NOAEL for systemic toxicity of Syn3NODA was ≥ 5 mg/kg for males and 1 mg/kg for females, based on the decrease in mean body weight gain. Following intra peritoneal administration of 2.5 to 50 mg/kg Syn3NODA to Sprague-Dawley rats, the NOAEL for systemic toxicity of Syn3NODA was 10 mg/kg. This can be endorsed.

Female cynomolgus monkeys were intravenously treated once with 1 or 5 mg/kg Syn3NODA. Animals were sacrificed at 14 days post-treatment. IV Syn3NODA administration resulted in mild RBC changes with the highest dose level. Nevertheless, the clinical significance of the change in RBC parameters did not seem to be of clinical significance (based on clinical observations), although no necropsy was performed, and the Applicant did not indicate any adversity or a NOAEL. The NOEL for systemic toxicity of Syn3NODA was set at 1 mg/kg. Nevertheless, it is considered that both 1 and 5 mg/kg did not cause significant acute toxicity in cynomolgus monkeys, based on the performed in-life analyses.

2.5.4.2. Repeat dose toxicity

rAd-IFN/Syn3NODA

The Applicant conducted one repeat dose toxicity study with rAd-IFN/Syn3NODA, which is considered pivotal for Adstiladrin safety assessment. Cynomolgus monkeys were intravesically treated with

$2.5x10^{11}$ vp or $1.25x10^{13}$ vp of rAd-IFN in 25 mg Syn3NODA or treated with 25 mg Syn3NODA alone. Animals were instilled in the bladder once (day 1) or twice (day 1 and day 91) for 60 minutes, and sacrificed at day 8 or day 98 (3 animals/sex/group) or after a recovery period at day 148 (2 animals/sex/group) post-treatment.

Intravesical dosing via catheterization resulted in occult blood in the urine of some animals. Urinary tract inflammation (including chronic inflammation in the tunica muscularis), ulceration (bladder trauma), tissue changes (urothelial hyperplasia and cytoplasmic vacuolation and local irritation was observed in several monkeys in all groups dosed once or twice with Syn3NODA, which was exacerbated by the vector. In some animals, this has led to actual bladder damage with increased leakiness (see PK aspects and discussion in section 2.5.3 and 2.5.6). Local signs of toxicity were most severe in the high dosed rAd-IFN/Syn3NODA group, suggesting a dose-relation with the amount of viral infection. Toxicity signs following two doses at least partially regressed after a 2-month recovery period with minimal urothelial inflammation and fibrosis in the lamina propria of the bladder remaining in a few animals.

Nadofaragene firadenovec distributed to ovary in female monkeys and testes in male monkeys after intravesical dosing.

No systemic Syn3NODA- and vector-related toxicity was observed, despite the presence of Syn3NODA, rAd DNA and human IFNα2b protein in the serum and in several tissues (especially in animals with bladder damage, although it was not possible to establish a consistent correlation, likely due to the low number of animals). Anti-adenovirus and anti-hIFN antibodies were induced in treated animals, which remained measurable at the end of the recovery phase.

The NOAEL for local toxicity was not set, because of the treatment-related changes in the urinary tract at all doses. However, 25 mg Syn3NODA and $1.25x10^{13}$ vp rAd-IFN could be considered the highest non-severely toxic dose. As the alterations in the urinary tract were reversible (in the recovery phase), this can be endorsed. The NOAEL for systemic toxicity was also set at 25 mg Syn3NODA and $1.25x10^{13}$ vp rAd-IFN. This can be endorsed.

Syn3NODA alone

Syn3NODA was also tested in repeat dose toxicity studies: in rats and in NHP following IV administration.

Sprague-Dawley rats were intravenously treated daily with 0.5, 1.5 or 5 mg/kg Syn3NODA for two weeks and subsequently sacrificed. This study indicates that Syn3NODA injected intravenously for two weeks in rats resulted in local inflammation at the injection site but was otherwise well tolerated. NOAEL for local toxicity of Syn3NODA was set at < 0.5 mg/kg, based on the irritation at the injection site. The reversibility of the local irritation signs was not investigated.

Cynomolgus monkeys (4 animals/sex/group) were intravenously treated daily for 16-18 days with 0.5, 1.5 or 5 mg/kg Syn3NODA and subsequently sacrificed. There was no recovery period. Although not mentioned by the Applicant, some mild Syn3NODA-related changes were observed in leukocyte blood parameters (↓ neutrophils in females, ↑ eosinophils in females, ↑ monocytes in males). These changes might be related to the local inflammation at the site of injection and were milder than those observed in male rats (see previous study).

Histopathological findings included irritation at the injection site in animals from both the Syn3NODA as well as the Syn3NODA placebo group (as was also observed in the Syn3NODA repeat dose study in rats), with vein intimal degeneration, perivascular inflammation/haemorrhage and thrombosis. The severity of these findings was higher in the Syn3NODA-dosed animals than in the placebo-dosed

animals, especially at doses ≥ 1.5 mg/kg. As such, this local irritation is considered related to both the formulation as well as the excipient itself.

The NOAEL for local toxicity of Syn3NODA in cynomolgus monkeys was 0.5 mg/kg, based on the more severe (Syn3NODA-related) irritation at the injection site at ≥ 1.5 mg/kg. The reversibility of the local irritation signs was not investigated. The NOAEL for systemic toxicity of Syn3NODA was set at ≥ 5 mg/kg for both species. This can be agreed.

2.5.4.3. Genotoxicity

The genotoxicity of Syn3NODA was evaluated *in vitro* in an Ames test for bacterial mutagenicity and in a chromosome aberration study for the potential for clastogenicity. The non-GLP Ames test was negative for bacterial mutagenicity, as there was no increase in the number of revertant colony counts in either of the tested strains (Salmonella typhimurium strains TA1535, TA97a, TA98, TA100, and TA102 and Escherichia coli strain WP2uvrA) with or without rat liver S9 metabolic activation, up to concentrations of 5,000 µg/plate if not limited by cytotoxicity. The GLP Ames test was also negative for bacterial mutagenicity, as there was no increase in the number of revertant colony counts in either of the tested strains (Salmonella typhimurium strains TA1535, TA97a, TA98, TA100, and TA102 and Escherichia coli strain WP2uvrA) with or without rat liver S9 metabolic activation, up to nominal concentrations of 5,000 µg/plate if not limited by cytotoxicity or solubility of the test compound.

The potential for clastogenicity of Syn3NODA was investigated in a GLP-compliant chromosome aberration study using human peripheral blood lymphocytes in the presence and absence of metabolic activation. There was no evidence of induction of chromosome aberrations from exposure to Syn3NODA at concentrations up to 230 µg/mL in the initial assay (4-hour treatment) with and without metabolic activation, and up to 230 µg/mL or 360 µg/mL in the confirmatory assay without (19-hour treatment) or with (4-hour treatment) metabolic activation, respectively. Higher concentrations were not scored for chromosome aberrations since mitotic index reductions of at least 60% were observed.

An GLP-compliant *in vivo* micronucleus study in rats was conducted with Syn3NODA. In the dose-range finding test conducted in male and female rats, 25 mg/kg/day appeared to be the MTD. In the main study, male rats (n=5/group) dosed up to 25 mg/kg/day did not show an increase in the mean frequency of micronucleated polychromatic erythrocytes in bone marrow compared to control animals. All criteria for positive and negative control values were met in this study and Syn3NODA did not have a toxic effect on erythropoiesis.

Absence of genotoxicity assessment of rAd-IFN was justified with literature information on the potential for adenoviral integration in the host genome from (bladder) cells of NMIBC patients treated with Adstiladrin (after treatment with BCG). Although infrequently, random integration into the host genome can occur and may even result in mutagenesis (although very rare), comparable to AAVs. Considering the infrequent biodistribution of rAd-IFN to the systemic in patients, primarily local mutagenesis could be a risk, though very low. However, any new bladder tumour in clinical practice will more likely reflect the natural course of NMIBC rather than insertional mutagenesis. Moreover, the rAd-IFN is intended to induce an anti-tumour immune response, which further decreases the chance of observing clinically relevant oncogenesis following vector integration.

2.5.4.4. Carcinogenicity

No carcinogenicity studies were conducted with Adstiladrin.

2.5.4.5. Reproductive and developmental toxicity

No clinical data are available on the possible effects of nadofaragene firadenovec on fertility, and nonclinical studies have not been conducted

No studies have been conducted with rAd-IFN or Syn3NODA to assess effects upon fertility and general reproductive behaviour, embryo-foetal toxicity, or peri-/post-natal toxicity.

2.5.4.6. Toxicokinetic data

Toxicokinetics are already extensively described and assessed in the Pharmacokinetics part (section 2.5.3) of the report.

2.5.4.7. Interspecies comparison

Syn3NODA was studied in rats and cynomolgus monkeys via intravenous and intravesical administration. Especially for monkeys, however, pharmacokinetic data are limited. After intravesical administration in female rats and in male and female monkeys there is systemic exposure to Syn3NODA. This exposure appears to be driven by bladder histopathological findings (bladder tissue damage for catheterization combined with irritating effects of Syn3NODA) combined with the hight of the rAd-IFN dose, with higher dose causing a higher systemic exposure to Syn3NODA in cynomolgus monkeys. Also, there appears to be a lower exposure to Syn3NODA in male monkeys compared to female monkeys after intravesical, but not intravenous, dosing. There is no information on distribution, metabolism (neither *in vitro* nor *in vivo*) and excretion of Syn3NODA for cynomolgus monkeys.

Metabolism studies on Syn3NODA were performed in hepatocytes, *in vitro*. Overall, no major differences between non-clinical species and humans for the *in vitro* metabolism/degradation of Syn3NODA were observed. No unique human metabolites were observed. Substantial sex differences were also not present.

In patients (n=17) at different doses of vector, but equal doses of Syn3NODA, systemic exposure to Syn3NODA was limited, but observed for all individuals after intravesical dosing with rAd-IFN/Syn3NODA at clinical dose. Interindividual variability is substantial. However, this can be due to the state of the individuals' bladder tissue, where more damage could result in more systemic exposure, in line with finding in cynomolgus monkeys.

Distribution of Syn3NODA after intravesical administration was studied in female rats and male rabbits. Tissue distribution appeared more substantial in female rats compared to male rabbits. Syn3NODA was present in ovary and uterus of female rats and in the sexual organs in the male rabbits. The clinical relevance of this finding is unknown, as no reproductive toxicity studies have been performed.

Regarding excretion, it was shown that after IV administration to rats it is actually excreted via the faeces. However, after intravesical administration it is assumed that most will be excreted by urine.

rAd-IFN & IFNα2b

In humans, systemic exposure to rAd-IFN was very limited. It was studied in patients in a phase 2 trial by qPCR. After the first dose of Adstiladrin no patients had rAd-IFN-derived DNA in the blood. After the second dose only 1 patient ($3*10^{11}$ vp/mL dose group) had a single positive test result at the end of instillation (1hr). Quantifiable IFNα2b protein levels were detected in human serum from 4 of 17 patients (5 rAd-IFN dose levels with 75 mg Syn3NODA); 3 of these 4 patients were at the highest dose level. The extent of exposure was low and transient with a maximum of a 96-hour duration of exposure post-dose compared with up to 10 days duration of exposure in urine. The IFNα2b protein

detected in the patients' serum is likely derived from the protein entering the bloodstream from the transduced bladder epithelium, since no rAd-IFN DNA was detected in any blood sample.

This is in contrast to the findings in monkeys, where rAd-IFN is found systemically after intravesical administration. As a toxicological species, monkeys may be a worst-case model, since systemic toxicity of rAd-IFN will be of very limited relevance to the clinical situation.

Exposure multiples

A calculation of approximate exposure multiples shows a systemic exposure multiple of Syn3NODA based on AUC between non-clinical species and humans of 47x (female rats), 57x (male rats) and 143x (female monkeys). Male monkeys were not included but would result in an exposure multiple of 199000/1604 = 124x.

Systemic exposure multiples based on dose are lower, with 6.66x for Syn3NODA and 11.12x for nadofaragene firadenovec. The low systemic Syn3NODA exposure multiple based on dose is overruled by the AUC-based exposure multiple. The systemic exposure multiple for nadofaragene firadenovec is considered adequate. The local exposure multiples between monkeys and humans are low with 3.11 for nadofaragene firadenovec and 1.87 for Syn3NODA based on dose. Effects seen in non-clinical monkey studies at a local level can therefore be of clinical relevance.

2.5.4.8. Local tolerance

No local tolerance studies have been conducted.

2.5.4.9. Other toxicity studies

Studies on impurities

In order to assess individual impurities and the degradation product (triamine-bis-cholate, FE 1004306) of Syn3NODA, a 14-day GLP repeat-dose toxicity study in rats was conducted with daily intravenous administration of Syn3NODA levels of 0.66 mg/kg and 2 mg/kg. Based on Syn3NODA - related local reactions consisting of swelling and discolouration observed at the tail of the animals dosed at 2 mg/kg/day, the systemic NOAEL was considered to be 2 mg/kg while the local NOAEL was 0.66 mg/kg. These local reactions were most likely attributed to Syn3NODA itself as these effects were also observed in general repeated dose toxicity studies. Therefore, the impurity levels present at the dose of 2 mg/kg can be considered qualified.

Other toxicity studies, e.g. *in vitro* haemolysis assay, do not indicate clinically relevant safety issues.

2.5.5. Ecotoxicity/environmental risk assessment

Adstiladrin (nadofaragene firadenovec) is a replication-deficient, non-pathogenic adenoviral vector, and once administered to the patient cannot spread in the environment. The molecular characterization of Adstiladrin is not expected to pose environmental risks. Limited amounts of RCA are accepted in each batch (see 2.5.3, section RCA detection). RCAs present will be lacking a functional E3 and thus are attenuated.

Recombination of the vector *in vivo*, after administration, could theoretically lead to recovery of both the E1- and E3-region. However, in this case the resulting RCA will be similar to the wild-type adenovirus with which the treated individual was already infected. The risk to the environment due to such recombination is negligible, because none of the scenarios will result in recombinants that are more fit than the wildtype virus with which the treated individual was already infected.

The environmental risks regarding formation of RCA during manufacturing or in the patient are considered negligible. Considering all the above, market authorization of Adstiladrin poses a negligible risk to human health and the environment. For further details see the discussion (2.5.6 Ecotoxicity/environmental risk assessment).

The ERA documentation and related SmPC sections are complete and clear, including the information provided for health care professionals and patients. Instructions for patients specifying how to disinfect voided urine and to wash their hands after toilet use are available. In addition, information on how to handle in case of accidental exposure are present in the SmPC/PL.

2.5.6. Discussion on the non-clinical aspects

Pharmacology

The Applicant has provided sufficient insight in the mode-of-action of the different components of Adstiladrin primarily based on literature. Following intravesical instillation of Adstiladrin, primarily superficial urothelium is transduced, in agreement with the barrier function of this layer. Because of the high CAR surface expression on healthy cells, normal urothelium will be highly transduced. As the CAR expression on bladder tumour cells is variable, the transduction efficiency of these cells is also variable. The transduction of healthy cells (next to some bladder tumour cells) will allow for IFNα2b secretion that will render the microenvironment more pro-inflammatory. This is considered beneficial for the immune system to combat the viral-infected (and tumour) cells. However, transduced healthy cells will thereby also be attacked by the immune cells and lost. Nevertheless, urothelium can recover well from injury and the Applicant refers to the repeated-dose NHP study in which local inflammation showed (partial) recovery over time, and to patients in which no major bladder toxicities were observed. A plausible course of events would be that transduced normal urothelium cells would get lost (due to the immune response) and restoration of the superficial layer would subsequently be initiated by the proliferation of urothelium progenitor cells.

IFNα2b is secreted from transduced cells, primarily healthy cells (i.e. highly susceptible to transduction). Interferons have an anti-viral function, including blocking viral entry in non-transduced cells. The virus is only able to attach to cells within the hour of instillation, as the bladder is flushed afterwards to remove/diluting residual vector particles. In contrast, IFNα2b production/secretion is only observed after several hours (especially after 24h). It is therefore very unlikely that this IFNα2b will significantly block the entry of non-replicating vectors such as Adstiladrin, as the concentration of these vectors in the bladder lumen is expected to be very low 24-48h post-instillation. The function of Adstiladrin-related IFNα2b secretion is to enhance the anti-viral (and anti-tumour) immune response. Immune cells may be partly present in the bladder wall (as resident cells) but are also attracted to the bladder via the blood stream. However, IFNα2b also has angiostatic activity, reducing the formation of new blood vessels. This may be beneficial in treating the tumour (i.e. reducing blood supply) but may also have an impact on the influx of immune cells. Infiltration of immune cells was apparent in treated mice in which pro-angiogenic factors were reduced, thus concluding that IFNα2b did not impact immune cell influx. Although it is not clear whether immune cell influx could have been higher when IFNα2b would not inhibit angiogenesis, the non-clinical data indicate that the immune response in the bladder is not considerably hampered by this anti-angiogenic status. Literature even suggests that anti-angiogenetic drugs can alleviate the immune suppressive environment in the tumour. Whether this is also the case with IFNα2b has not been described. Insight in the activation of NK and/or T cells was also provided. Adstiladrin can enhance MHC-I expression on tumour cells (making them more vulnerable to T cells) but can also enhance the activity of NK cells (which can kill cells with low/absent MHC-I expression). In addition, both pathways can be enhanced by IFNα2b and that the balance

between both pathways is likely dependent on tumour intrinsic properties. In NMIBC, IFNα2b has thus immunostimulatory properties on way or the other.

As an immune response is initiated locally against the vector, this may hamper repeated dosing. The reduced IFNα2b concentration in urine of animals following redosing may be related to (anti-viral) immunogenicity, but relevant treatment efficacy-decreasing anti-viral antibodies or cellular responses were not observed in patients. Although no definitive answer could be given, the clinical efficacy data are reassuring with regard to the impact of immunogenicity.

The *in vivo* pharmacodynamic data provide proof-of-concept for treatment of bladder cancer xenografts by intravesical rAd-IFN/Syn3NODA. Furthermore, the *in vivo* studies support the choice for the dose of $1x10^{11}$ as well as the choice for use of 1 mg/mL Syn3NODA. In addition, it was shown that a higher instillation volume works better than a lower instillation volume.

In two studies relevant xenograft models were used. Thereby, treatment was intravesical, reflecting the clinical route of administration. The mice were immunocompromised which does not represent the clinical situation reliably, however this is inherent to the use of human bladder cancers cells for the xenograft. Therefore, the use of immunodeficient mice can be accepted, but it might have influenced the results as it is shown that IFN is much longer expressed from rAdIFN in immunodeficient mice as compared to immunocompetent mice. Support for the treatment's anti-cancer efficacy in humans should come from clinical studies.

Rats receiving a second treatment 30 days after the initial dose had potent inflammatory infiltrate in the bladder 24 h after the second dose, which might point towards a host immune response. Redosing after 90 days resulted in reduction of inflammatory infiltrate observed in the urothelium, correlating with gene expression that persisted up to at least 72 h post treatment. Acute cystitis was observed in the urothelium of all monkeys that might have been caused by multiple mechanical manipulations or may have resulted from an immune response to rAd-IFN or hIFNa2b Severity increased with repeated dosing. Both observations might result from a host immune response against the rAd-IFN, which might not occur in human.

Study 54980 and the publication of Connor et al, (2005) are likely the basis for the 90-day dose interval in the clinical setting. A host immune response to the Ad-virus has been suggested as reason for decreased IFN expression at timepoints before the 90-day interval. However, in animals, both rAd-IFN and expressed transgene, human IFN, could result in an immunogenic response. In addition, expression of IFN in rats seems to be shorter than for immunocompetent mice.

It remains unclear why the choice for the 90-days dosing interval was based on rats (studies), whereas mice show in general longer expression, but these animal data are not considered relevant for the prediction of IFNα2b expression levels in human. The immune response to the virus and the immune response to the transgene is likely to determine the declining levels of IFNα2b expression in the rat upon repeated dosing. As in human an immune response against the transgene is not expected, the expression in human may not decline to the same extent as observed in rats. Consequently, clinical IFNα2b expression level may be different in rats and humans upon repeated dosing. In the clinic there is reduced IFNα2b expression, but this is likely not mediated by an antibody response but seems to rather reflect a T-cell mediated reaction against transduced cells or an innate immune anti-viral response.

Pharmacokinetics

The pharmacokinetics of Syn3NODA have been assessed in multiple studies in rats, rabbits and cynomolgus monkeys. For pharmacokinetics of rAd-IFN and IFNa2b monkeys have been studied, and some non-pivotal studies have been done in rats.

Analytical methods

A large variety of analytical methods were used for the analysis of Syn3NODA, rAd-IFN (DNA) and human IFNa2b in plasma, blood, urine and tissues in rats, monkeys and rabbits. For the detection of Syn3NODA in plasma of rats and monkeys, HPLC methods were validated. It was noted that the recovery of Syn3NODA from monkey plasma was low and had a large range (Recovery- extracted versus neat: 56.2% to 117% and Recovery- extracted versus non-extracted: 52.2% to 73.9%). The impact of this large range in recovery on sample analysis was minimized by adding sufficient quality controls and internal standards to the samples.

A qPCR for rAd-IFN DNA was only validated for bladder tissue in monkeys and not the other tissues assessed in the monkey distribution study. Validation data of the analytical method was submitted but included only the liver and kidney tissues, not the reproductive tissues. The liver and kidney are tissues with a high transcription activity and were selected because of this property, however, their function as metabolizing and excretory organs may also result in a highly specific transcription activity compared to other tissues, such as reproductive tissues. The lack of validation of the reproductive tissues is considered an omission in the validation data, but not of such a serious nature it justifies performing new *in vivo* studies. One supporting study report regarding the CytoFluor assay is also missing and could no longer be retrieved by the Applicant. Since it is of a supporting nature this was considered acceptable.

Syn3NODA

For Syn3NODA, it appears in general to be absorbed into the systemic circulation after intravesical absorption in rats, rabbits and monkeys. In rats, there was distribution into tissues where it appears persistent in reproductive tissues in female rats and to a lesser extent in male rabbits. Male rats were not studied due to limitation in the study method regarding the intravesical administration.

Plasma protein binding and distribution in blood cells of Syn3NODA have not been established.

Metabolism of Syn3NODA has not been extensively studied, but an *in vitro* hepatocyte study was performed in rabbit, mouse, rat minipig, monkey and human hepatocytes. Overall, no major differences between non-clinical species and humans for the *in vitro* metabolism/degradation of Syn3NODA were observed. No unique human metabolites were observed. Substantial sex differences were also not present.

Regarding the excretion of Syn3NODA in rats, this appears to be mainly via faeces after intravenous administration. However, since the route of administration is intravesical, it is expected that most Syn3NODA will be excreted via urine.

Syn3NODA & rAd-IFN

In monkeys, pharmacokinetic of Syn3NODA was assessed in a intravesical GLP study using one dose of Syn3NODA combined with two different dose levels of rAd-IFN, where there appeared to be a positive correlation between histopathological findings in the bladder and systemic exposure. It was, however, unclear how monkeys from different dosing groups were correlated to different levels of histopathological findings and there appears to be an unexplained gender difference that is not seen after intravenous dosing of Syn3NODA. However, this is difficult to assess with certainty, since only a limited number of animals were used per group in this study.

Additional data were provided regarding the distribution of animals with and without histopathological bladder damage by treatment group and achieved Syn3NODA Cmax. Actual damage of the bladder was classified by the Applicant as ulceration, haemorrhage or submucosal congestion. Inflammation was not considered damage, likely because inflammation on its own may not increase the leakiness of the bladder. This approach can be agreed.

Based on these data, it appears that none of the placebo control animals had bladder damage, 2 of the Syn3NODA only group (C2) had bladder damage, 3 animals in the low-dose vector group (T1) had bladder damage and 6 animals in the high-dose vector group (T2) had bladder damage, although there was no clear relationship between the severity of the damage and the systemic exposure of Syn3NODA. There was no sex difference, 6 males and 5 females had bladder damage. The dose of Syn3NODA was the same in groups C2, T1 and T2.

Although the numbers of animals used in this experiment was rather small (3M/3F per group per dosing point), there appear to be substantially more animals with bladder trauma in the highest vector dose group. Also, no bladder trauma was seen in the control animals (C1). Inflammation in the bladder and urinary tract observed following treatment with Syn3NODA (with or without vector) indicates that the excipient has caused irritation to the epithelium, which is further enhanced by the vector. As such, patients are at risk of systemic exposure to Syn3NODA, vector and IFNα2b derived from the bladder. Assessment of these exposures and further monitoring/mitigation strategies, where needed, are described clinically.

rAd-IFN & IFNa2b

rAd-IFN pharmacokinetics were mainly studied in monkeys. It is distributed to the systemic circulation and some tissues (liver, kidney, gonads). Extensive tissue distribution was not studied. Although it is agreed that biodistribution data in animals may overestimate tissue exposure compared to humans (when bladder damage due to the procedure would be more severe in animals), such data would at least have allowed for understanding the distribution profile of rAd-IFN and would contribute to the overall interpretation of (pharmacological and toxicological) study findings, in line with ICH S12. This important element of the non-clinical development program is missing in the current dossier. Based on the provided literature data, there is a clear distribution pattern of adenovectors following systemic exposure; adenoviral vector can distribute to many tissues to a greater or lesser extent. Following intravesical administration, rAd-IFN may enter the systemic circulation and distribute to various tissues. However, the impact of limited systemic exposure to this vector on patient safety is anticipated to be low. Moreover, distribution (measured via qPCR of viral DNA) may not be the same as transduction/permissiveness of the tissue (with intact virus within the cells and subsequent transgene expression). Nevertheless, a bulk of viral genomes within a tissue can have considerable impact on the functionality of the cells (especially when an immune response is induced) and thus may impact the safety of a gene therapy product. Therefore, close monitoring of patients for potential adverse events related to systemic vector exposure remains essential.

It was also unclear what the consequence of the distribution (and persistence) of rAd-IFN into tissues is and whether there could be IFNα2b production (detectable in serum) from the rAd-IFN in these tissues or that it can only leak from the bladder. There is a lack of consistent correlation between serum IFNα2b levels and bladder trauma in study SN03245. Individual animal data provided show that while some animals with elevated IFNα2b levels exhibited bladder lesions, others with similar or even higher levels did not.

Vector DNA was still detectable systemically at day 8 following second dosing in NHP, while IFNα2b was no longer detectable by then. As such, presence of vector DNA was not accompanied by IFN transgene expression. This would indicate that IFNα2b in serum would not be derived from production outside of the bladder. Still, local production of IFN in tissues (without leakage to the circulation) cannot be excluded, but this would require IFN mRNA/protein analysis in tissues. Taken together, IFNα2b in serum is likely derived from production in the bladder instead of transgene expression in tissues outside the bladder. Any production outside of the bladder will be minimal and considered of no clinical relevance.

Metabolism and excretion were not assessed extensively, although IFNa2b was found in urine in monkeys. However, in the ELISA study also some control and placebo animals tested positive for human IFNa2b, likely due to contamination.

Since systemic exposure to both Syn3NODA and rAd-IFN after intravesical administration was measured and substantial distribution to tissues was assessed in both rats and monkeys (and male rabbits), these species are deemed suitable for toxicological assessment of Adstiladrin. Compared to humans, the pre-clinical animal species had a higher systemic exposure to both Syn3NODA and rAd-IFN after intravesical administration.

Toxicology

In rats, no GLP-compliant toxicity study has been conducted with rAd-IFN in Syn3NODA. The toxicity of the vector and transgene (together with Syn3NODA) was only evaluated in the GLP-compliant cynomolgus monkey study. For ATMPs, toxicity evaluation in one animal species is considered sufficient. Syn3NODA toxicity was evaluated in GLP-compliant studies in both rats and monkeys. This approach can be endorsed.

In rats, the intravesical route was only evaluated in females because of difficulties with catheterisation of males. This can be endorsed, as intravesical toxicity with Syn3NODA was evaluated in male NHP in the pivotal general toxicity study. In the single dose rat study, the NOAEL for local toxicity of Syn3NODA was < 0.5 mg, based on clinical and histological signs of (temporary) urinary tract inflammation and local irritation. This can be agreed, although the adversity of the local toxicity may be limited considering the observed (partial) reversibility. Nevertheless, the impact of the Syn3NODA-based toxicity on the efficacy and safety of rAd-IFN (Adstiladrin) has not been evaluated in this study and remains therefore unclear. Reference is made to the studies in NHP for further evaluation of intravesical Syn3NODA toxicity and the impact thereof on rAd-IFN efficacy and safety.

The studies provided in the Toxicology part of the dossier have largely been conducted around 2004/2005. Several Syn3NODA and rAd-IFN DS batches have been used for these studies. However, no quality data for these batches have been provided at time of submission of the dossier. Upon request data on some of the batches have been provided. Nevertheless, taken together, not all batches used in the non-clinical PK and toxicity studies are sufficiently representative for the clinical/commercial manufacturing process, but the provided data are considered supportive of the non-clinical profile of the product.

Single and repeated dose toxicity

In a repeat-dose toxicity study with nadofaragene firadenovec, there were no treatment-related macroscopic or histopathologic findings in reproductive tissues of cynomolgus monkeys at exposures up to 11-fold the clinical systemic dose.

The rAd-IFN/Syn3NODA study in rats is only considered supportive, since distribution and toxicity evaluations were limited to bladder, whole blood, liver and kidney and no information on the validity of the used PCR and ELISA methods was presented. Moreover, it was not clarified in the study report how long the product(s) remained in the bladder. Considering the presence of a GLP-compliant rAd-IFN/Syn3NODA study in NHP, no additional study in rats is considered warranted for this ATMP.

Although the single dose IV and IP studies with Syn3NODA in rat were conducted in compliance with GLP, only clinical signs and body weight gain were followed to evaluate systemic toxicity. No necropsy was performed. This is considered very limited. However, considering that Syn3NODA in the currently proposed product (Adstiladrin) is not injected in a vein or surrounding tissue but instilled in the bladder, the clinical relevance of the local toxicity observed in the rats is considered limited, but these

data do provide a 'worst-case scenario' (i.e. maximal systemic exposure). Therefore, no additional Syn3NODA-related toxicity studies are awaited.

In repeat-dose toxicity studies with Syn3NODA, there were no treatment-related macroscopic or histopathologic findings in reproductive tissues of rats (IV study, Syn3NODA only) or cynomolgus monkeys (intravesical study) at exposures up to 143-fold and 124-fold, 47-fold and 57-fold the clinical systemic AUC in female and male monkeys, female rats and male rats, respectively.

In the SYN3NODA study in NHP, reversibility of the local irritation signs was not investigated. However, considering that Syn3NODA in the currently proposed product (Adstiladrin) is not injected in a vein or surrounding tissue but instilled in the bladder, the clinical relevance of the local toxicity observed in the NHP is considered limited, but these data do provide a 'worst-case scenario' (i.e. maximal systemic exposure).

On the basis of the available toxicological and toxicokinetic data, no conclusion can be drawn with regard to the proposed treatment interval of 3 months. The data indicate short-term IFNα2b secretion and adenoviral vector persistence in the bladder, but it is not clear to what extent this is related to animal-specific immunogenicity. The therapeutic interval is therefore only discussed in the Pharmacology section 2.5.2.

Genotoxicity

As Syn3NODA is a novel excipient, a full genotoxicity evaluation as described in ICH S2 (R1) (EMA/CHMP/ICH/126642/2008) is required. The genotoxicity of Syn3NODA was evaluated *in vitro* in an Ames test for bacterial mutagenicity and in a chromosome aberration study in human lymphocytes for potential for clastogenicity. Overall, these studies indicate that Syn3NODA is not genotoxic *in vitro*. Genotoxicity of Syn3NODA was further evaluated an *in vivo* micronucleus study in rats. The representativeness of batches (used in the *in vitro* and in the *in vivo* genotoxicity study) was sufficiently justified with batch data and reference to comparability of Syn3NODA preparation for non-clinical, clinical and commercial batches. For another batch, the Applicant has not been able to locate manufacturing data. Considering that this batch was used for an exploratory, non-GLP study, this is acceptable. It was claimed that there was no evidence of induction of aneuploidy, however, the provided chromosome aberration study is not designed to evaluate aneuploidy (also see OECD 473). Nevertheless, ICH S2 accepts the *in vitro* metaphase chromosome aberration as second *in vitro* genotoxicity test, and it is therefore acceptable.

Carcinogenicity

Carcinogenicity studies have not been conducted with nadofaragene firadenovec.

As the proposed indication (high grade Bacillus Calmette-Guerin unresponsive non-muscle invasive bladder cancer) is not an advanced cancer indication, the omission of carcinogenicity evaluation needed to be supported with a weight-of-evidence (WoE) approach for both the vector and the novel excipient. The approach to determine the carcinogenic potential for Syn3NODA and rAd-IFN was based on the (absence of) pharmacological activity of both Adstiladrin components, transient histopathological features in toxicology studies, considerable exposure margins, limited (bio)distribution beyond the bladder, absence of potential for hormonal perturbation and low genotoxic risks. In summary, the limited exposure due to dosing frequency (not chronic, but once per three months) together with the low systemic levels of Syn3NODA and rAd-IFN/IFNα2b reduce the risk for potential carcinogenicity/tumorigenicity. However, contribution of local treatment-related inflammation to tumour relapse cannot be excluded and will be a matter of benefit:risk assessment at a clinical level.

Reproductive and developmental toxicity

No reproductive and developmental toxicity studies were conducted with rAd-IFN or Syn3NODA.

The omission of these studies was not considered significant, given the clinical route of administration and inability to conduct standard studies by this route, and the infrequent dosing and anticipated limited systemic exposure. Considering the distribution to reproductive organs in non-clinical studies, the fact that systemic exposure cannot be excluded clinically and that novel excipients fall within the scope of the ICH S5 (R3) guideline, a weight-of-evidence approach to estimate the reproductive toxicity risks of rAd-IFN and Syn3NODA was provided. The WoE approach for the excipient was primarily based on Syn3NODA exposure in reproductive tissues after intravesical dosing in rats and rabbits (PK studies) and the absence of histopathological findings in reproductive organs in repeated dose toxicology studies with Syn3NODA in rats and monkeys. Syn3NODA distributed to female reproductive organs in the rat following intravesical dosing, with more persistent levels in the ovary compared to the uterus although levels in both tissues decreased over time. Syn3NODA distribution evaluated in male rabbits showed low transient exposure in the testes and prostate only detectable shortly post-dosing. Repeat-dose toxicity studies with Syn3NODA have not caused any adverse, treatment-related macroscopic or histopathologic findings in reproductive tissues of rats (14-day IV) or cynomolgus monkeys (dosed twice with three months in between, intravesical study). Exposures provided safety margins compared to clinical systemic AUC of ≥143x and ≥124x in female and male monkeys and ≥47x and to ≥57x in female and male rats, respectively.

Based on a literature review, it is concluded that the reproductive and developmental risk of rAd-IFN for NMIBC patients is low, considering the patient population (usually above reproductive age), the infrequent clinical dosing (once every three months), the low systemic exposure of IFNα2b in patients and the absence of histopathological features in gonads of animals.

The lack of reproductive, development and lactation studies is also clearly reflected in the section 4.6 and 5.3 of the SmPC in the relevant sections. Relevant recommendations on contraception, pregnancy, breast-feeding and fertility are included in section 4.6 of the SmPC.

Other toxicity studies

In order to assess individual impurities and the degradation product (triamine-bis-cholate, FE 1004306) of Syn3NODA, a 14-day GLP repeat-dose toxicity study in rats was conducted with daily intravenous administration of Syn3NODA levels of 0.66 mg/kg and 2 mg/kg. The impurity levels present at the dose of 2 mg/kg can be considered qualified. The proposed Syn3NODA specification limits for the impurities Syn3-ox1 Syn3-oxX, Syn3-cholic1 and Syn3-cholic3 and Syn3-acetyl1/2are acceptable from a toxicological perspective. In silico methods were used following the guidance of the EMA draft reflection paper "Reflection paper on the qualification of non-mutagenic impurities" (EMA/CHMP/543397/2024) to evaluate the toxicological properties of Syn3NODA related impurities.

The calculations of the safety margins show an appropriate systemic exposure multiple of Syn3NODA based on AUC between non-clinical species and humans of 47x (female rats), 57x (male rats) and 143x (female monkeys). Male monkeys were not included but would result in a exposure multiple of 199000/1604 = 124x. Systemic exposure multiples based on dose are lower, with 6.66x for Syn3NODA and 11.12x for nadofaragene firadenovec. The low systemic Syn3NODA exposure multiple based on dose is overruled by the AUC-based exposure multiple. The systemic exposure multiple for nadofaragene firadenovec is considered adequate.

The local exposure multiples between monkeys and humans are low with 3.11 for nadofaragene firadenovec and 1.87 for Syn3NODA based on dose. Effects seen in non-clinical monkey studies at a local level can therefore be of clinical relevance.

Ecotoxicity/environmental risk assessment

Risk of shedding of replication deficient vector particles

The adenoviral vector is replication-deficient and has a partial deletion of the E3 gene, making the virus more susceptible to detection and removal by the host immune system compared to wildtype adenovirus. No harmful sequences or (proto)oncogenes are present in Adstiladrin. Accidental exposure to third parties will not to lead to an active infection, as the vector is replication deficient, non-pathogenic and the vector will be eliminated by the immune system. The dose to which non-target individuals would be exposed in a worst case will be orders of magnitude lower than that received by the patient. Overall, the consequences related to shedding of vector particles are negligible.

Risk of formation of replication competent adenovirus during production of Adstiladrin

The viral vector used for Adstiladrin is a non-replicative viral vector; however, during production replication competent virus can be formed. Due to quality control and acceptation criteria only limited amounts of RCA can be present in the medicinal product.

If recombination occurs during production, the E1 gene could be inserted in the rAd-IFN vector without removal of the transgene expression cassette, or the modified E1 region containing the transgene expression cassette could be exchanged for a wildtype E1 region. For the ΔE1ΔE3-AdV vector used, in both cases the formed RCA will be strongly attenuated due to the partial deletion in the E3 region. The E3 deletion prevents a productive infection *in vivo*, because infected cells will be cleared by the immune system. Functional E3 proteins inhibit the antiviral response of the host that is initiated upon infection, among other through actions on Natural Killer cells, Cytotoxic T lymphocytes and TNF-alpha. Adenoviruses lacking the E3 region are only very rarely found in in nature; only one report can be found of a patient infected with Human Adenovirus 7 with a partial E3 deletion (*Su X et al. (2011)).* The patients' immune response will be activated upon administration of the viral vector; thus further reducing the likelihood that any RCA (if present) will replicate within the patient and may be shed to the environment. The amounts of viral vector or RCA that may be shed are orders of magnitude lower than the amount administered to the patient; thereby further reducing the likelihood on negative effects on third individuals. Accidental exposure to third parties is expected not to lead to an active infection, as the vector is replication deficient, non-pathogenic and the vector will likely be eliminated by their immune system. To minimize the chance of third individuals being infected precautionary measures are proposed limiting exposure:

- The precaution provided in the SmPC as to handle the discard of the urine using bleach/virucidal substances reduces the dissemination of the Adeno viral vector (or any RCA, if present) in the environment.
- Direct exposure to health care professionals handling the medicinal product is possible but clear instructions for manipulation is given to reduce as much as possible the risk for the operator and to drastically reduce any spill/distribution in the environment.

The risk to human health and the environment due to the presence of RCA in the medicinal product is negligible, because in the worst case an attenuated RCA, lacking functional E3 can be formed and the likelihood of exposure of third individuals is minimal.

Risk of formation of replication competent adenovirus after administration of Adstiladrin

When a replication-deficient AdV vector is administered to a person, there is a possibility that a cell simultaneously becomes infected with the vector and a related wild-type adenovirus. When co-infection occurs, the lost functions in the vector can be restored by recombination and RCA could be formed. Recombination in the case of co-infection could lead to the recovery of the E1-deletion in the rAd-IFN vector and the formation of a replication-competent vector, that is still attenuated due to its deletion of a large portion of the E3-region. Restoration of only the E3 region will not result in a replication competent virus. Recombination could theoretically lead to recovery of both the E1- and E3-region, in this case the resulting RCA will be similar to the wild-type adenovirus with which the treated individual

was already infected. The likelihood of recombination to occur is considered low when taking into the account the transient presence of the vector in the patient and the location (bladder instillation). Wildtype adenoviral infection primarily occurs in the respiratory tract (although in some cases also the gastrointestinal tract and urinary tract may be infected); thus minimizing the likelihood for such a simultaneous infection to occur and recombination to happen.

The risk to human health and the environment due to recombination is negligible, because none of the scenarios will result in recombinants that are more fit than the wild-type virus with which the treated individual was already infected.

Risk of complementation after administration of Adstiladrin

In addition to recombination, complementation of the adenoviral vector could occur, due to the presence of wild-type adenoviruses (or other viruses) that are simultaneously present in the same cell as the vector. By (trans)complementation of proteins with a similar function as the adenoviral E1 proteins, the ability to replicate the vector can be temporarily restored. In the worst case, complementation could lead to increased shedding of the vector, but this increased shedding will only occur for a limited time. The AdV vector particles that are secreted after complementation are still replication deficient and cannot spread further.

Risk to animals

Wild-type hAdV-5 is known to replicate only in a very limited number of species of animals other than humans, when experimentally infected: Syrian hamsters and cotton rats. Since these both are not found in the wild in Europe, the likelihood of animals being infected with the adenoviral vector is negligible. Thus, the risk is negligible.

Overall risk estimation

Considering all of the above, Adstiladrin poses a negligible risk to human health and the environment.The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.5.7. Conclusion on the non-clinical aspects

Considering the relevant evidence obtained from *in vitro* and *in vivo* studies (as described above) with the relevant consideration about the shortcomings (also described above) which seems to have limited confirmation in the clinical pharmacology, the non-clinical profile of Adstiladrin can be considered approvable.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Table 1. List of clinical study

Country **Phase** **Trial ID** **CTR Location**	**Design** **Primary Objective**	**Treatment / Treatment Regimen**	**Population**	**Number of Patients**
US Phase 1 P03816 (*safety, tolerability, PD, PK*)	Multi-centre, nonrandomised, parallel group, open-label, rising-dose To determine the safety and tolerability of intravesical administration of nadofaragene firadenovec	Single intravesical instillation with ascending doses of nadofaragene firadenovec (3×10^9 vp/mL, 1×10^{10} vp/mL, 3×10^{10} vp/mL, 1×10^{11} vp/mL, or 3×10^{11} vp/mL [75 mL]) If no dose-limiting toxicity, patients were offered to receive a second instillation	BCG-refractory superficial transitional cell carcinoma of the bladder	Total: 17 Treated: 17 Patients/dose level: 3×10^9 vp/mL: 3 1×10^{10} vp/mL: 3 3×10^{10} vp/mL: 3 1×10^{11} vp/mL: 4 3×10^{11} vp/mL: 4
US Phase 1b 2009-0938 (*Tolerability, PD, Efficacy*) Navai et al. Ann Surg Oncol. 2016	Single-centre, single-arm, non-randomised, open-label, conducted under a separate investigator IND To determine whether a second instillation of trial drug on Day 4 improved gene transfer efficacy	Intravesical instillation of nadofaragene firadenovec (3×10^{11} vp/mL) on Day 1 and Day 4 Complete responders were allowed a second treatment cycle 3 months after the initial treatment	BCG-refractory NMIBC patients who refused radical cystectomy	Total: 7 Treated: 7 Patients/dose level: 3×10^{11} vp/mL: 7
US Phase 2 rAd-IFN-CS-002 *(efficacy and safety)*	Multi-centre, randomised, parallel arm, open-label, repeat-dose To evaluate the incidence of high-grade recurrence-free survival at 12 months	Intravesical instillations with nadofaragene firadenovec (1×10^{11} vp/mL or 3×10^{11} vp/mL [75 mL]) every 3 months, if no high-grade disease recurrence[a] (max 4 instillations/ 12 months)	High-grade BCG-refractory or relapsed NMIBC	Total: 43 Treated: 40 Patients/dose level: 3×10^{11} vp/mL: 19 1×10^{11} vp/mL: 21
rAd-IFN-CS-002 Long-term follow-up	3 years long-term follow-up of the rAd-IFN-CS-002 trial To evaluate long-term outcomes of the gene therapy following drug instillation	No treatment during long-term follow-up	All patients who received at least 1 instillation of nadofaragene firadenovec in the rAd-IFN-CS-002 trial	Total: 40

Country Phase Trial ID CTR Location	Design Primary Objective	Treatment / Treatment Regimen	Population	Number of Patients
US Phase 3 rAd-IFN-CS-003 *(efficacy and safety)*	Multi-centre, single-arm, open-label, repeat-dose To evaluate the complete response rate in patients with CIS (with or without concomitant high-grade Ta or T1 papillary disease)	Intravesical instillations with nadofaragene firadenovec (3×10^{11} vp/mL [75 mL]) every 3 months, if no high-grade disease recurrence[a] (max 4 instillations/12 months) Patients with no high-grade disease recurrence at Month 12 were offered continued intravesical instillations with nadofaragene firadenovec (3×10^{11} vp/mL [75 mL]) up to 5 years from first instillation, if no high-grade disease recurrence[b]	High-grade BCG unresponsive NMIBC, defined as either: CIS only Ta/T1 high-grade disease with concomitant CIS Ta/T1 high-grade disease without concomitant CIS	Total: 157 Treated: 157 Patients/dose level: 3×10^{11} vp/mL: 157 Patients/cohort: CIS cohort: 107 Papillary disease cohort: 50

Abbreviations: BCG = Bacillus Calmette-Guérin; CIS = carcinoma in situ; CTR = clinical trial report; IND = investigational new drug application; LTFU = long-term follow-up; NMIBC = non-muscle invasive bladder cancer; PD = pharmacodynamic; PK = pharmacokinetic; US = United States; vp = viral particles.

a) High-grade disease recurrence was assessed up to 2 weeks prior to dosing by cytology, cystoscopy, and biopsy(ies) if clinically indicated (biopsy at Month 12 was mandatory).

b) Up to Month 24, high-grade disease recurrence was assessed up to 2 weeks prior to dosing by cytology, cystoscopy, and biopsy(ies) if clinically indicated. From Month 24 onwards, assessments were performed by the investigator in accordance with usual clinical practice, confirming the suitability of the patient to continue to receive nadofaragene firadenovec prior to each instillation.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Nadofaragene firadenovec is a non-replicating recombinant adenoviral vector containing the interferon alfa-2b (IFNα2b) transgene and an excipient. The vector is administered via intravesical instillation in the bladder. The recommended dose of nadofaragene firadenovec is 75 mL at a concentration of 3 x 10^{11} viral particles (vp)/mL administered by intravesical instillation every three months. A novel excipient, Syn3NODA is included in the formulation. Syn3NODA is a polyamide. Syn3NODA measurements indicate the systemic exposure to the excipient. The pharmacokinetics of SCH 209702 in systemic circulation were assessed after the intravesical administration of five dose levels ($3x10^9$ particles/mL to 3x1011 particles/mL) in humans. Despite the number of subjects per dose level group is very limited (n ranging from 3 to 5), no relevant dose dependency is observed on PK exposure endpoints (Cmax, AUC0-24, AUC0-last or AUC0-inf).

At recommended dose of nadofaragene firadenovec of 75 mL at a concentration of 3 x 10^{11} viral particles (vp)/mL with a concentration of Syn3NODA of 0.95 mg/mL the observed Cmax was 170 ng/ml, with a Tmax around 1 hour. The AUC0-inf was approximately 3160 ng*hr/ml, the volume of distribution in plasma was around 308 L, indicating that Syn3NODA is distributed into tissue. The apparent clearance of Syn3NODA is approximately 25.6 L/h. The half-life of Syn3NODA is approximately 8.4 hours. Following repeated dosing after 3 months apart, no accumulation was observed.

PK measurements of rAd-IFN-derived DNA showed that no rAd-IFN-derived DNA was detectable in any blood samples collected from patients after their first dose of nadofaragene firadenovec. Only 1 out of

23 patients receiving a second dose in the phase 2 trial had measurable rAd-IFN-derived DNA in blood at any timepoint (qPCR LLOQ, 1×10^4 copies/mL). Taken together, data from the phase 1 and phase 2 trials indicate a low likelihood of systemic exposure to rAd-IFN-derived DNA in patients treated with nadofaragene firadenovec.

Measurable amounts of rAd-IFN-derived DNA in urine were detected in both phase 1 and phase 2 trials. In the phase 1 P03816 trial, a greater frequency of detection of urine samples positive for rAd IFN derived DNA by qPCR, and persistence of presence of rAd-IFN-derived DNA, were correlated with increase in dose level. Detectable levels of rAd-IFN-derived DNA in urine persisted up to Day 14 in some patients. In the phase 2 rAd-IFN-CS-002 trial, all patients (100%) had rAd-IFN-derived DNA in their urine at Month 1 Day 2; and these rates steadily declined to 97.5% at Day 4, 84.6% at Day 12, and finally only 13% at Month 4 Day 1. Most patients receiving a second dose (19 [90.5%] patients) had rAd-IFN-derived DNA in urine by Month 4 Day 4; this decreased to 6 (28.6%) patients by Month 4 Day 12. Taken together, data from these trials indicate that rAd-IFN-derived DNA (not necessarily intact viral particles) is likely to be excreted in urine for at least 14 days.

In summary, the available clinical PK data across both phase 1 and phase 2 trials indicate limited systemic exposure to rAd-IFN-derived DNA and transient exposure to Syn3NODA following intravesical instillation of nadofaragene firadenovec.

No interaction studies have been performed.

2.6.2.2. Pharmacodynamics

Mechanism of action

Nadofaragene firadenovec (pharmacotherapeutic group: other antineoplastic agents, antineoplastic cell and gene therapy; ATC code: L01XL10) is a non-replicating recombinant type 5 adenovirus vectorbased gene therapy containing the human IFNα2b transgene. Intravesical administration of nadofaragene firadenovec results in the entry of viral particles into the tumour cells and the urothelium that make up the luminal surface of the bladder leading to the expression of IFNα2b protein by those cells. The excipient Syn3NODA enhances an efficient entry of the adenovirus into the cells. In the transduced cells, the viral DNA does not integrate into the genome but exists as an episome; expression of the transgene results in the secretion of the therapeutic IFNα2b protein that has direct anti-tumour effects and stimulates the patient's own immune response to the tumour. Treatment with nadofaragene firadenovec has shown anti-tumour effects in mice with bladder (cancer cell) xenografts.

Primary and Secondary pharmacology

PD measures of IFN (IFNα2b) protein reflect transgene expression and secretion from transduced urothelial cells. Across these measures, IFNα2b protein concentration in urine is the primary clinical pharmacodynamic marker of nadofaragene firadenovec activity.

Measurable levels of IFNα2b protein in urine were observed in most patients in the phase 1 trial and in all patients in the phase 2 trial and declined over time. Generally, higher IFNα2b levels secreted in urine were observed with increasing doses of nadofaragene firadenovec, with measurable levels observed for approximately 10-12 days, indicating sustained expression of IFNα2b protein in the bladder. In trial 2009-0938, transient peaks in IFNα protein concentration in urine of 3 patients were reported following the first and second doses of nadofaragene firadenovec, but the sustained production of IFNα was not demonstrated and all patients returned to baseline levels by Day 10. Across the phase 1 and phase 2 trials, no correlation was observed between IFNα2b protein levels in urine and clinical response. In the phase 1 study P03816, a limited number of patients were enrolled and neither urinary IFNα2b levels nor urinary IFNα2b persistence correlated directly with clinical response or

durability. In the phase 2 study rAd-IFN-CS-002, the levels of IFNα2b protein were highly variable and did not appear to correlate to efficacy.

Similarly, IFNα2b protein in serum was measurable in some patients in both trials (23.5% of patients in P03816 trial and 30.8% of patients in rAd-IFN-CS-002 trial). Subsequently, IFNα2b protein levels in serum declined over a period of 10-12 days as anticipated.

Immunogenicity

Immunogenicity was evaluated through the measurement of anti-adenovirus type 5 antibody levels in serum samples collected from patients included in the P03816, rAd-IFN-CS-002, and rAd-IFN-CS-003 trials, and through the measurement of anti-IFNα2b antibody levels in serum samples collected from patients included in the P03816 and rAd-IFN-CS-002 trials.

The analysis of anti-adenovirus type 5 antibody levels in serum revealed a significant immunogenic response in 29.4% of patients in trial P03816, 55.0% of patients in trial rAd-IFN-CS-002, and 72.4% of patients in trial rAd-IFN-CS-003. Nevertheless, it has been reported in the phase 3 trial rAd IFN CS-003 that 77.7% of patients with a positive immunogenic response achieved high-grade-recurrence-free survival at Month 3. In a post-hoc analysis of the phase 3 trial rAd-IFN-CS-003, the ability of anti-adenovirus antibody levels to predict the durability of therapeutic response to nadofaragene firadenovec was further investigated. A total of 91 patients with both baseline and post-treatment titre were included in the analysis (47 (52%) remained high-grade recurrence-free at Month 12 [responders], while 44 (48%) developed high-grade recurrence or disease progression at or before Month 12 [nonresponders]). While baseline titres did not predict treatment response, 3-month titres >800 were associated with a higher likelihood of durable response. Peak post-treatment titres >800 were noted in 42 (89%) responders versus 26 (59%) nonresponders. These findings suggest a strong association between clinical outcome and immunogenic response. In addition, no negative impact of an immunogenic response is anticipated.

Regarding anti-IFNα2b antibody measurement, most patients in trials P03816 and rAd-IFN-CS-002 did not have a measurable anti-IFNα2b response, with the exception of 2 patients in trial P03816 who had a positive antibody response and 1 patient in trial rAd-IFN-CS-002 who had a weak 1:20 titre at Month 1 Day 12.

2.6.3. Discussion on clinical pharmacology

Pharmacokinetics

The Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) specifies that classical pharmacokinetic studies based on absorption, distribution, metabolism and excretion (ADME) studies are usually not required for GTMPs. Therefore, the lack of formal PK assessment in this dossier is acceptable for this type of product.

The pharmacokinetics of SCH 209702 in systemic circulation were assessed after the intravesical administration of five dose levels ($3x10^9$ particles/mL to $3x10^{11}$ particles/mL) in humans. Despite the number of subjects per dose level group is very limited (n ranging from 3 to 5), no relevant dose dependency is observed on PK exposure endpoints (Cmax, AUC0-24, AUC0-last or AUC0-inf). Therefore, the impact of dose levels does not lead to a positive trend in the exposure of patients. On the other hand, PK parameters governing drug disposition (CL/F and Vz) were estimated based on linear regression of the observed data. The range of systemic SCH 209702 CL/F and Vz values are 20 to 132 L/H and 209 to 1650 L respectively. In general, parameters do vary across the dose levels tested, suggesting that the PK properties of SCH 209702 are highly dependent on the tissue/disease status. This is also confirmed when analysing the elimination half-life ($t_{1/2}$), which remains very

constant across the dose levels, suggesting that the higher elimination of the drug (CL/F) is associated with a higher distribution of the drug within the body (Vz) and the opposite. No conclusions can be made based on the time-dependency due to the lack of adequate data and the pharmacokinetic analysis should be considered exploratory rather than confirmatory.

Nadofaragene firadenovec is a gene therapy product administered via intravesical instillation, and systemic exposure to the vector is very limited. PK measurements of rAd-IFN-derived DNA showed that no rAd-IFN-derived DNA was detectable in any blood samples collected from patients after their first dose of nadofaragene firadenovec. Only 1 out of 23 patients receiving a second dose in the phase 2 trial had measurable rAd-IFN-derived DNA in blood at any timepoint (qPCR LLOQ, 1×10^4 copies/mL).

In clinical studies P03816 and rAd-IFN-CS-002, rAd-IFN-derived DNA is likely to be excreted in urine in gradually declining numbers. However, the ratio of intact to degraded vector fragments was not analysed and infectivity assessment of PCR-positive samples was not performed. In accordance with EMA´s General Principles to Address Virus and Vector Shedding (EMEA/CHMP/ICH/449035/2009) guideline if the assay used does not distinguish intact from degraded or non-infectious virus / vector, then the data might not be informative as to the potential risk associated with transmission. Therefore, assays such as qPCR may be coupled with the use of infectivity assays.

As nadofaragene firadenovec is a viral vector which is not available in plasma, several pharmacokinetic data are not available, which is acceptable. This includes intra- and inter-individual variability, dose linearity, and intrinsic factors. Additionally, nadofaragene firadenovec is a viral vector and is, therefore, not a substrate, inhibitor or inducer of metabolising enzymes or drug transporters.

Due to the intravesical route of administration, local mechanism of action and low systemic exposure a clinical biodistribution study may not be needed. Based on a non-clinical study in monkeys in which rAd-IFN-specific DNA was detected in the liver, kidney, and gonads, the question was raised during the evaluation whether such distribution could also occur in humans. As nadofaragene firadenovec is a replication-deficient recombinant adenovirus and systemic exposure has been found to rarely occur, the distribution to other tissues beyond the systemic circulation is anticipated to be low and has not been assessed.

Metabolism and elimination

Pharmacokinetic data are too limited to evaluate dose proportionality of Syn3NODA. As only one dose level is proposed, this is acceptable. Accumulation is not likely with a half-life of approximately 8.3 hours.

In conclusion, in the clinical development of nadofaragene firadenovec, PK analysis of rAd-IFN-derived DNA indicate measurements of both systemic exposure and shedding in urine (not necessarily of intact viral particles). Syn3NODA measurements indicate the systemic exposure to the excipient.

There was no detectable systemic exposure of vector-derived DNA in patients in the phase 1 and 2 studies, except for in 1 out of 40 patients in the phase 2 study.

Vector-specific DNA was present in the urine of most of the patients in the phase 1 study and all patients in the phase 2 study. Presence was correlated to the dose level. The vector-specific DNA persisted for at least 14 days in the phase 1 study and at least 12 days in the phase 2 study. 3 out of 23 (13 %) patients in the phase 2 study were positive for vector-specific DNA prior to the second dose.

The excipient Syn3NODA enhances an efficient entry of the adenovirus into the urothelial cells. Systemic exposure of Syn3NODA was assessed in the phase 1 study and was found to be transient with a mean elimination t½ of 8.4 hours with no evidence of retention.

No interaction studies have been performed.

Pharmacodynamics

The parameters chosen for the pharmacodynamics assessment can be regarded appropriate to study the function and/or expression of the transgene in the urothelial cells and production of the therapeutic IFN-α2b protein.

The pharmacodynamic marker IFNα2b was present in urine from all patients in the phase 1 and 2 studies except for two patients at the lowest dose level in the phase 1 study (3 × 109 vp/mL). Urine IFNα2b protein was detected up to Day 12 post-dose.

Quantifiable levels of IFNα2b protein in serum were detected in a subset of patients (4 out of 17) in the phase 1 study. The extent of exposure was low and transient with a maximum of a 96-hour duration of exposure post-dose. In the phase 2 study, 12 out of 40 patients had measurable serum IFNα2b protein at month 1 day 2 and 2 out of 40 patients by day 12.

Indeed, the sustained presence of IFNα2b protein in urine supports the mechanism of action and transduction of nadofaragene firadenovec, indicating effective delivery and activity of the adenoviral vector within the bladder. Although there was no direct correlation between PD markers and clinical outcome in either the phase 1 or phase 2 trials, clinical response was observed in both trials.

Immunogenicity

Although anti-adenovirus type 5 antibody levels were measurable in the clinical trials, it appeared to have no negative impact on clinical efficacy.

2.6.4. Conclusions on clinical pharmacology

The available clinical PK data across both phase 1 and phase 2 trials indicate limited systemic exposure to rAd-IFN-derived DNA and transient exposure to Syn3NODA following intravesical instillation of nadofaragene firadenovec. The sustained presence of IFNα2b protein in urine supports the mechanism of action and transduction of nadofaragene firadenovec, indicating effective delivery and activity of the adenoviral vector within the bladder. Although there was no direct correlation between PD markers and clinical outcome in either the phase 1 or phase 2 trials, clinical response was observed in both trials. furthermore, although anti-adenovirus type 5 antibody levels were measurable in the clinical trials, it appeared to have no negative impact on clinical efficacy. In conclusion the pharmacology profile of nadofaragene firadenovec is considered acceptable.

The CHMP endorse the CAT assessment regarding the conclusions on the clinical pharmacology as described above.

2.6.5. Clinical efficacy

2.6.5.1. Dose response studies

The dose level and regimen investigated in the pivotal Study CS-003 are based on data from the non-clinical and clinical development programme of nadofaragene firadenovec drug product. The 3-month administration frequency is based on repeat and re-dose pharmacology studies conducted in rats after intravesical administration (D-47451, D-54980) which indicated that longer time intervals between doses, such as 90 days, increased the potential for sufficient IFNa2b expression upon repeat dosing, which was evaluated for three consecutive administrations. However, in animals IFN expression decreased with each following dose (rat, NHP) which might be due to immunogenicity to rAd-IFN and

expressed human transgene (IFNa2b). The safety of the 3-month administration frequency was evaluated in cynomolgus monkeys after intravesical administration SN 03245).

Furthermore, an instillation volume of 75 mL with a 1-hour dwell time was chosen to ensure that nadofaragene firadenovec is in direct contact with the urothelial cells, allowing adequate transduction of the urothelium. The safety of the 1-hour dwell time was supported by the study conducted in cynomolgus monkeys (SN 03245).

In the phase 1 trial P03816, the initial dose concentration of nadofaragene firadenovec was 3×10^9 vp/mL utilising a 75 mL instillation and 0.95 mg/mL Syn3NODA. The concentration of nadofaragene firadenovec was approximately 30% of the low concentration used in the intravesical administration of rAd-IFN in cynomolgus monkeys in the toxicology study (SN 03245), where animals were intravesically dosed at 1×10^{10} vp/mL (low dose) or 5×10^{11} vp/mL (high dose) of rAd-IFN in 25 mL (containing 1 mg/mL Syn3NODA). In the phase 1 trial P03816, a dose range of 3×10^9 to 3×10^{11} vp/mL in a dose volume of 75 mL was investigated in approximately half-log increments (Table 1).

In the phase 2 Study CS-002, patients were randomised to receive a treatment dose of 75 mL of either 1×10^{11} vp/mL or 3×10^{11} vp/mL, and were to be re-treated every 3 months with the same dose (see Table 1). At all time-points, the incidence of HG recurrence-free survival was numerically higher in the 3×10^{11} vp/mL dose group compared to the 1×10^{11} vp/mL dose group (Table 27). Additionally, a numerical difference was observed in the time to disease progression in the 3×10^{11} vp/mL dose group (11.7 months) compared to the 1×10^{11} vp/mL dose group (3.5 months). Since no differences in relation to safety were identified between dose levels (Table 42), it was decided to use the higher of the two doses in the pivotal study to maximise the potential benefit for patients.

2.6.5.2. Main study

Title of Study

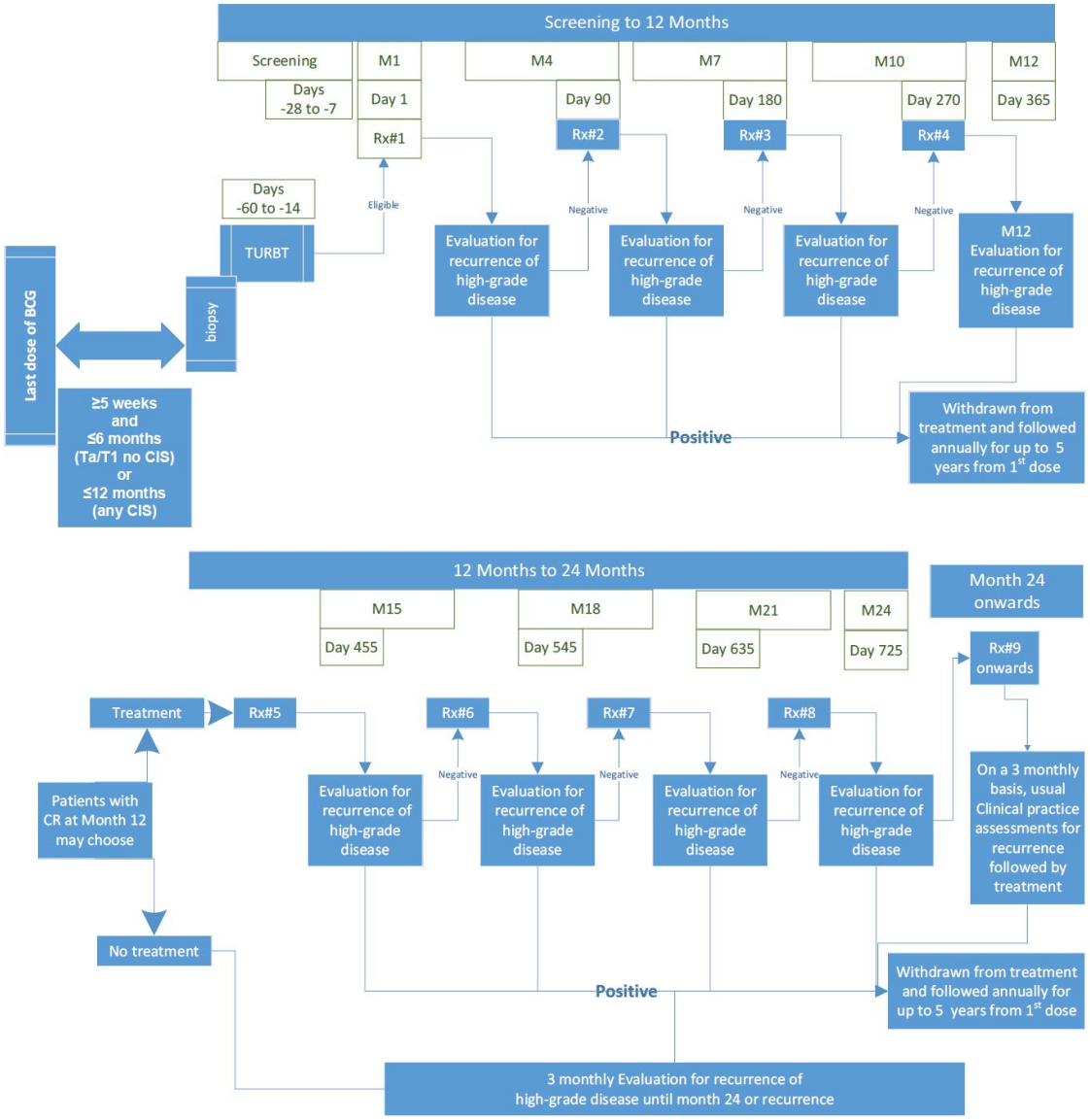
A Phase III, Open-Label Study to Evaluate the Safety and Efficacy of INSTILADRIN (rAd-IFN/Syn3) Administered Intravesically to Patients with HG BCG-Unresponsive Non-Muscle Invasive Bladder Cancer (NMIBC).

Methods

- **Study design**

The pivotal study rAd-IFN-CS-003 (Study CS-003; Boorjian et al. Lancet Oncol. 2021) is an open-label, single-arm trial (SAT) to evaluate the safety and efficacy of nadofaragene firadenovec administered intravesically to patients with HG BCG-unresponsive NMIBC.

Figure 1. Study Design Schematic



Abbreviations: BCG = Bacillus Calmette-Guerin; CIS = carcinoma in situ; CR = complete response; M = Month; Rx = dose; TURBT = transurethral resection of bladder tumour.

Following the initial 12-month treatment period, patients entered a 4-year follow-up period. Patients with no evidence of HG disease recurrence at Month 12 were offered continued treatment with nadofaragene firadenovec up to 5 years from the first instillation if considered appropriate by their treating physician. Patients with evidence of HG disease were discontinued from treatment but were followed up in the trial for collection of long-term safety, cystectomy, and survival data for up to 5 years after the first instillation of nadofaragene firadenovec.
Figure 1 presents the study design schematic.

Assessments for disease progression were performed every three months up to 2 weeks before the retreatment target date at Months 4, 7, and 10. These assessments included cystoscopy and urine cytology, and biopsy(ies) if clinically indicated. i.e., patients with a positive cytology or with findings on cystoscopy that were suspicious for recurrent cancer underwent a biopsy(ies) to confirm/rule out recurrence of HG NMIBC.
Urine cytology was analyzed at the site using local procedures. Biopsy(ies) was (were) analyzed by local pathology, but slides were stored centrally for possible correlation to local pathology.

At 12 months after the first dose of nadofaragene firadenovec (i.e., Month 1 [Day 1] + 365 days), all patients who did not withdraw from dosing underwent cystoscopy, urine cytology, and mandatory biopsies to exclude tumour recurrence. At least five biopsies were obtained (dome, trigone, right and left lateral wall, posterior wall) +/- prostatic urethra in men with positive cytology and prior disease in this region.

At Month 24, treatment continued for patients who were responding to nadofaragene firadenovec; further assessments were performed in accordance with usual clinical practice.

For patients who withdrew from treatment and agreed, long-term follow-up survival data, including information regarding progression to invasive disease and cystectomy (conducted in accordance with usual clinical practice) were collected every 12 months from the date of withdrawal for up to 5 years after the first dose of nadofaragene firadenovec.

- **Study Participants**

Key Inclusion Criteria

- Aged 18 years or older;
- At entry, confirmed by a pathology report:
    - CIS only;
    - HG Ta/T1 disease with concomitant CIS; or
    - HG Ta/T1 disease without concomitant CIS.
- Were "BCG unresponsive" which referred to patients with HG NMIBC who were unlikely to benefit from and who did not receive further intravesical BCG. The term "BCG unresponsive" included patients who did not respond to BCG treatment and had a persistent HG recurrence within 12 months after BCG was initiated, and those who, despite an initial CR to BCG, relapsed with CIS within 12 months of their last intravesical treatment with BCG or relapsed with HG Ta/T1 NMIBC within 6 months of their last intravesical treatment with BCG. The following criteria defined the patients who were eligible for inclusion in the study:
    a. Had received at least 2 previous courses of BCG within a 12-month period. This was defined as at least 5 of 6 induction BCG instillations and at least 2 out of 3 instillations of maintenance BCG, or at least 2 of 6 instillations of a second induction course, where maintenance BCG was not given;
        i. Exception: Those who had T1 HG disease at the first evaluation after induction BCG alone (at least 5 of 6 doses) qualified in the absence of disease progression.
    b. At the time of tumour recurrence, patients with CIS alone or HG Ta/T1 with CIS were within 12 months of last exposure to BCG and patients with HG Ta/T1 without CIS were within 6 months of last exposure to BCG;
    c. No maximum limit to the amount of BCG administered; and
    d. All visible papillary tumours were required to be resected and those with persistent T1 disease on TURBT undergone an additional re-TURBT within 14 to 60 days prior to beginning study treatment. Obvious areas of CIS were also fulgurated.
- Eastern Cooperative Oncology Group (ECOG) status 2 or less;

- Absence of concomitant UTUC or urothelial carcinoma within the prostatic urethra. Freedom from upper tract disease (if clinically indicated) as indicated by no evidence of upper tract tumour by either intravenous pyelogram, retrograde pyelogram, computed tomography scan with or without urogram, or magnetic resonance imaging with or without urogram performed within 6 months of enrolment;
- Patients with prostate cancer on active surveillance at a low risk for progression, defined as prostate specific antigen <10 ng/dL, Gleason Score 6, and cT1 were permitted to be included into the study at the discretion of the investigator; and
- Adequate laboratory values, e.g., an estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m^2.

Key Exclusion Criteria

- Current or previous evidence of muscle invasive (muscularis propria) or metastatic disease presented at Screening. Examples of increased risk of metastatic disease included, but were not limited to:
    - Presence of lymphovascular invasion and/or micropapillary disease as shown in the histology of the biopsy sample; and
    - Patients with T1 disease accompanied by the presence of hydronephrosis secondary to the primary tumour.
- Current systemic therapy for bladder cancer;
- Current or prior pelvic external beam radiotherapy within 5 years of entry;
- Prior treatment with adenovirus-based drugs;
- Symptomatic urinary tract infection or bacterial cystitis (once satisfactorily treated, patients could have entered the study);
- History of malignancy of another organ system within the past 5 years, except treated basal cell carcinoma or squamous cell carcinoma of the skin and ≤pT2 UTUC at least 24 months after nephroureterectomy. Also, patients with genitourinary cancers other than urothelial cancer or prostate cancer that were under active surveillance were excluded;
- Patients who could not hold instillation for 1 hour; and
- Patients who could not tolerate intravesical dosing or intravesical surgical manipulation.

- **Treatments**

Patients received a 75 mL intravesical administration of nadofaragene firadenovec at a dose of 3×10^{11} vp/mL on each dosing day of the study. It was recommended that anticholinergics were used on each dosing day (unless contraindicated) to reduce potential bladder irritation to prevent product leakage. Nadofaragene firadenovec was administered into the bladder through a urinary catheter; 75 mL was instilled and left in the bladder for 1 hour. During the dwell time, the patient was repositioned from left to right, to the back, and to the abdomen to maximize bladder surface exposure. The patient was repositioned approximately every 15 minutes. If the patient exhibited bladder cramping and/or leakage after dosing, turning of the patient was adjusted or discontinued.

The first dose of nadofaragene firadenovec was administered intravesically on Day 1 (Month 1). If no evidence of HG disease recurrence was detected, further doses were administered at Day 90 (Month

4), Day 180 (Month 7), and Day 270 (Month 10). Patients received up to four intravesical administrations of nadofaragene firadenovec over the initial 12-month observed period.
All patients with an absence of high-grade disease recurrence at Month 12 were offered continued treatment every 3 months after Month 12. Beyond Month 24, dosing could continue every 3 months, or 6 months at sites with Sponsor approval, for patients with no evidence of HG disease recurrence up to 5 years from the first instillation if considered appropriate by their treating physician.

Dose interruptions – If any experienced toxicity did not recover to less than Grade 2 in severity, then treatment was delayed for a maximum of 14 days.

Dose reductions – No dose reductions were permitted. Adjustments to the dwell time or splitting of the dose (e.g., 37.5 mL × 2) were made based upon assessments of local tolerability.

Concomitant and rescue therapies – All patients continued to receive appropriate supportive care for the treatment of their cancer, including the use of anticholinergic therapeutic agents prior to the instillation of nadofaragene firadenovec, if required.

No unspecified systemic anticancer therapy or radiotherapy was permitted during the study.

The following concomitant medications were prohibited for the length of time specified prior to Screening or while treatment with nadofaragene firadenovec was ongoing:

- Immunosuppressive therapy:
    - 3 months prior to Screening.
- Investigational drugs:
    - 30 days prior to Screening.
- Intravesical therapy:
    - Intravesical therapy within 8 weeks prior to beginning study treatment except for cytotoxic agents (e.g., Mitomycin C, doxorubicin, and epirubicin) when administered as a single instillation immediately following a TURBT procedure which was permitted between 14 to 60 days prior to beginning study treatment and previous intravesical BCG therapy, which could be given at least 5 weeks before the diagnostic biopsy required for entry into the study.

Use of inhaled corticosteroids was permitted during the study. Patients who received oral or injectable corticosteroids for the treatment of other conditions within 3 months of receiving the first dose of nadofaragene firadenovec were discussed with the Medical Monitor. During the treatment phase, patients who required treatment with corticosteroids were not withdrawn from the study.

- **Objectives**

Primary objective – primary objective of study CS-003 was to evaluate the CR rate in patients with CIS (with or without concomitant HG Ta/T1 papillary disease) at any time after first administration of nadofaragene firadenovec, testing superiority over a null hypothesis of a true response rate of 27%.

The value of 27% was chosen, as it was >50% of the observed CR rate in the phase 2 Study CS-002 (see also below subsection 'Sample size') supported also by the clinical relevance indicted by the Society of Urologic Oncology Clinical Trials Consortium (SUO CTC), when the protocol was written in 2016 and consistent with those used in clinical trials for other products, such as pembrolizumab (Balar

et al. Lancet Oncol. 2021), vicineum (oportuzumab monatox; withdrawal EPAR), and cretostimogene grenadenorepvec (NCT04452591).

Estimand for the primary objective

Estimands have not been implemented. Intercurrent events and their handling is summarised from post-hoc considerations by the Applicant.

Table 2. Estimand for Primary Objective – Complete Response in Patients with CIS

Population	**Adult patients with high-grade, Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) consisting of carcinoma in situ (CIS) with or without Ta/T1 papillary disease**
Treatment condition<s>	At least one instillation of nadofaragene firadenovec, regardless of discontinuation after first instillation, with no use of prohibited medication (anti-cancer therapy, radiotherapy, immunotherapy, intravesical therapy)
Endpoint (variable)	Complete response at any time after first administration before start of new ant-cancer therapy
Population-level summary	Proportion
Intercurrent events and strategy to handle them	
Death	Composite
Premature treatment discontinuation during the primary 12 month treatment period	Treatment policy[a]
Initiation new anti-bladder cancer therapy before disease recurrence	While no anti-cancer therapy was started[b]

[a] The Applicant (in their post-hoc 'Guidance to Assessor' document) considered 'premature discontinuation' not as an intercurrent event for CR, as the included patients were required to have the first instillation (modified intention to treat) and all responses were observed at the month 3 assessment. However, given the intention of 'at any time point', the handling is set by the Assessor to 'treatment policy' for 'premature discontinuation'.
[b] Inferred from the intention of CR. Of note, no new anti-cancer therapy before disease recurrence was observed.

The corresponding clinical question of interest is the complete response rate after at least 1 instillation of nadofaragene firadenovec in adult patients with HG, BCG-unresponsive NMIBC consisting of CIS with or without Ta/T1 tumours.

Secondary objectives – The secondary efficacy objectives of study CS-003 were the following:

- To evaluate the durability of CR in patients with CIS (with or without concomitant HG Ta/T1 papillary disease) who achieved a CR;
- To evaluate the rate of event-free survival, where event-free survival was defined as HG recurrence-free survival (RFS) in patients with HG Ta/T1 papillary disease (without concomitant CIS);
- To evaluate the durability of event-free survival in patients with HG Ta/T1 papillary disease (without concomitant CIS), who had no recurrence of HG Ta/T1 papillary disease. For comparison purposes, this was also evaluated in patients with CIS;
- To determine the incidence of and time to cystectomy;

- To determine the overall survival (OS) in all patients;
- To determine the anti-adenoviral antibody levels for correlation to response rate; and
- To monitor the durability of response during the long-term follow-up period.

Estimands for the secondary objectives

Table 3. Estimand for Key Secondary Objective – Durability of Complete Response in Patients with CIS

Population	**Adult patients with HG, BCG-unresponsive NMIBC consisting of CIS with or without Ta/T1 papillary disease that have reached a CR after first instillation of nadofaragene firadenovec**
Treatment condition<s>	At least one instillation of nadofaragene firadenovec, regardless of discontinuation after first instillation, with no use of prohibited medication (anti-cancer therapy, radiotherapy, immunotherapy, intravesical therapy)
Endpoint (variable)	Duration of CR (DoCR) from the first post-treatment assessment where CR was determined until HG disease recurrence, disease progression, or death, which ever occurred earlier
Population-level summary	Median and probability of duration every 3 months
Intercurrent events and strategy to handle them	
death	Composite
Premature treatment discontinuation during the primary 12 month treatment period	Treatment policy
Initiation new anti-bladder cancer therapy before disease recurrence	While no anti-cancer therapy was started[a]

[a] Inferred from the intention of duration of CR. Of note, no new anti-cancer therapy before disease recurrence was observed.

The corresponding clinical question of interest is the duration of CR (DoCR) until death or recurrence of HG disease, regardless of treatment discontinuation, after at least 1 instillation of nadofaragene firadenovec in adult patients with HG, BCG-unresponsive NMIBC consisting of CIS with or CIS without Ta/T1 tumours.

Table 4. Estimand for Secondary Objective – Incidence of HG RFS in Patients with HG Ta/T1 Papillary Disease (without CIS)

Population	Adult patients with HG, BCG-unresponsive NMIBC consisting of Ta/T1 papillary disease only
Treatment condition<s>	At least one instillation of nadofaragene firadenovec, regardless of discontinuation after first instillation, with no use of prohibited medication (anti-cancer therapy, radiotherapy, immunotherapy, intravesical therapy)
Endpoint (variable)	HG RFS
Population-level summary	Crude proportion at 3, 6, 9, 12 months
Intercurrent events and strategy to handle them	
Death	Composite
Premature treatment discontinuation during the primary 12 month treatment period	Treatment policy
Initiation new anti-bladder cancer therapy before disease recurrence	Treatment policy[a]

[a] As stated in the 'Guidance to Assessor' document. Of note, no new anti-cancer therapy before disease recurrence was observed.

The corresponding clinical question of interest is the proportion alive without documented recurrence of HG disease, after at least 1 instillation of nadofaragene firadenovec in adult patients with only HG, BCG-unresponsive Ta/T1 papillary NMIBC.

- **Outcomes/endpoints**

The efficacy endpoints in study CS-003 are summarized in Table 5 and further described below.

Table 5. Efficacy Endpoints in Study CS-003

Efficacy Endpoint	Type of Endpoint
Whether or not a patient with CIS (with or without concomitant high-grade Ta/T1 papillary disease) responds to treatment, defined as complete response at any time[a] after first administration of nadofaragene firadenovec	Primary
Durability of complete response in patients with CIS (with or without concomitant high-grade Ta/T1 papillary disease) who show a complete response at any time[a] after first administration of nadofaragene firadenovec	Key secondary
Incidence of high-grade recurrence-free survival	Secondary[b]
High-grade recurrence-free survival[c]	Secondary
Incidence of cystectomy	Secondary
Time to cystectomy (cystectomy-free survival)	Secondary
Overall survival	Secondary

Abbreviation: CIS = carcinoma in situ.

a) The first efficacy assessment visit was scheduled 3 months from first instillation of nadofaragene firadenovec where patients were assessed for evidence of low- or high-grade disease recurrence. Therefore, the primary endpoint of complete response was measured at the 3-month visit.

b) The incidence of high-grade recurrence-free survival at Month 12 was the original primary endpoint in Study CS-003 but was amended to a secondary endpoint based on recommendation from the US FDA and in line with FDA's 2018 guidance.

c) This is a time-to-event endpoint.

Primary efficacy endpoint – The proportion of patients with CIS (with or without concomitant HG Ta/T1 papillary disease) achieving CR at any time is presented together with a 2-sided 95% Clopper-Pearson confidence interval (CI).

A patient in the CIS cohort was judged to have achieved a CR where:

- Urine cytology was reported as normal, atypical, degenerative, reactive, inflammatory, or nonspecific; AND
- Cystoscopy was reported as normal or with findings that did not include evidence of LG or HG recurrence;
- Bladder biopsy(ies), if performed (not mandatory), demonstrated an absence of LG or HG recurrence.

A patient in the CIS cohort was also judged to have achieved a CR where:

- Urine cytology was reported as normal, atypical, degenerative, reactive, inflammatory, nonspecific, suspicious, malignant cells, or not done; AND
- Bladder biopsy(ies) had been performed and demonstrated absence of LG or HG recurrence.

A patient in the CIS cohort was judged to have NOT achieved a CR where:

- Urine cytology was reported as suspicious, malignant cells, or not completed; AND
- Bladder biopsy(ies) had NOT been performed; and
- There was HG recurrence, confirmed by bladder biopsy observed at the next scheduled visit OR biopsy was not performed at the next visit but the urine cytology result at the next scheduled visit was reported as suspicious or malignant.

A patient was also deemed NOT to have achieved a CR if there were insufficient data to determine whether or not a complete response has occurred.

Once CR was achieved, treatment failure was defined as recurrence of HG disease confirmed by bladder biopsy or disease progression as assessed by the Investigator or death, whichever occurred earlier.

Key secondary efficacy endpoint – The durability of CR in the CIS cohort (patients with CIS with or without concomitant HG Ta/T1 papillary disease) who showed CR at any time after the first administration of nadofaragene firadenovec was calculated from the first post-treatment assessment where CR was observed to treatment failure, where treatment failure was defined as HG disease recurrence, disease progression, or death, whichever occurred earlier. If none of the 3-monthly assessments showed treatment failure, then durability was censored at the last disease assessment not showing treatment failure.

The date of initial CR was defined as the date of the last efficacy assessment procedure (cytology, cystoscopy, and biopsy) that determined CR at the first post-treatment visit at which CR was achieved.

Other secondary efficacy endpoints – A patient was judged to have achieved HG RFS at a time point (e.g., Months 3, 6, 9, and 12) if the patient was alive and without documented recurrence of HG disease or muscle invasive disease progression as assessed by the Investigator at that time point.

In addition, for the incidence of HGRF survival at the initial Month 3 Efficacy Assessment Visit, similar rules for handling suspicious urine cytology without biopsy results as described for the primary efficacy endpoint were applied. When there was uncertainty in the response assessment at the initial Month 3 Efficacy Assessment Visit (e.g., suspicious urine cytology and no bladder biopsy), the response assessment at the subsequent visit was evaluated to determine whether the patient was truly HG RFS at the Month 3 Efficacy Assessment Visit.

For both the CIS and papillary disease cohorts, a patient was judged to have NOT achieved HG RFS at the initial Month 3 Efficacy Assessment Visit where the following:

- Urine cytology was reported as suspicious, malignant cells, or not done, and no bladder biopsy performed at the Month 3 Efficacy Assessment Visit; AND
- There was HG recurrence, confirmed by bladder biopsy, observed at the next scheduled visit OR biopsy was not performed at the next visit but urine cytology result at the next scheduled visit was reported as suspicious or malignant.

A patient was also deemed NOT to have achieved HG RFS if there were insufficient data to determine whether or not HG RFS has occurred.
It was considered sufficient evidence for HG RFS if a subsequent assessment showed HG RFS and there had been no intermediate treatment for bladder cancer other than nadofaragene firadenovec.

- **Sample size**

The observed CR rate at any time in patients with CIS with or without papillary disease in the phase 2 study CS-002 was equal to 50%. Assuming the pivotal study CS-003 retains 87.5% of that efficacy, the true response rate will be 43.75%. One hundred patients with CIS with or without papillary disease would provide 90% power to reject the hypothesis that the true response rate is 27% at a one-sided alpha of 2.5%. Thirty-seven responding patients at any time (response rate of 37%, two-sided 95% Clopper-Pearson CI = [27.5%, 47.3%]) would be sufficient to reject the hypothesis.

- **Randomisation and Blinding (masking)**

Not applicable, as the study is an open label, single arm study.

- **Statistical methods**

Planned analyses – The analysis populations were the following:

The **Safety Analysis Set** was defined as all patients who received at least 1 dose of nadofaragene firadenovec. This set was used for all safety analyses.

The **Efficacy Analysis Set** was defined as all patients in the Safety Analysis Set with a diagnosis of HG BCG-unresponsive NMIBC. This set was used as the primary analysis set for the analyses of efficacy data.

The **Per-Protocol Analysis Set** was defined as all patients in the Efficacy Analysis Set who had no major protocol violation and either of the following:
- Completed the Month 12 Efficacy Assessment Visit at earliest Day + 357 and at latest Day + 396; OR
- Withdrew before the Month 12 Efficacy Assessment Visit because of disease recurrence or progression, death, an AE related to the disease or treatment, or lack of tolerability.

Efficacy analyses were performed based on the Efficacy Analysis Set. Supportive efficacy analyses were performed for selected efficacy endpoints using the Safety Analysis Set and Per-Protocol Analysis Set.

The primary part of this study was considered complete when all evaluable patients either completed the Month 12 Efficacy Assessment Visit or withdrew from the study and completed a safety assessment.

Two cohorts were defined based on the patient's diagnosis at enrolment:
- The **CIS cohort** comprised patients with CIS (with or without concomitant HG Ta/T1 papillary disease); and
- The **papillary disease cohort** comprised patients with HG Ta/T1 papillary disease (without concomitant CIS).

Cohorts were summarized separately and together, when appropriate.

Planned subgroup analyses – The primary efficacy variable, rate of CR in patients with CIS based on the Efficacy Analysis Set, was analysed for the following subgroups:

- Sex (male or female);
- Age group (<65 or ≥65 years);
- Race group (White or non-White);
- Time from initial diagnosis of bladder cancer (less than the median or greater than or equal to the median);
- Tumour type at study entry (CIS only or CIS with Ta/T1 papillary disease); and
- Positive immunogenic response in anti-adenoviral antibodies at postbaseline (yes or no).

Error probabilities, adjustment for multiplicity and interim analyses – The type I error for the primary endpoint was controlled with a one-sided alpha of 2.5% and/or a two-sided alpha of 5%.

There was no adjustment for multiplicity and, therefore, no type I error control for (any of) the secondary endpoints.

No formal interim analyses were planned.

Changes from protocol-specified analyses – The original SAP was dated 09 May 2016. There were two amendments to the SAP (dated 07 November 2018 and 08 May 2023).

SAP version 2.0 was dated 07 November 2018. The key changes are summarized below:

- The definitions of the secondary efficacy endpoints were clarified;
- The definitions of the 2 patient cohorts were added;
- Baseline disease characteristics were clarified; and
- Detail was added with respect to determining CR in patients with CIS.

SAP version 3.0 was dated 08 May 2023. The SAP was updated to reflect changes made in Protocol Amendment version 8.0 (dated 01 February 2022).

SAP supplement version 1.0 (09 January 2019) was developed to describe the details for the planned statistical analyses to enhance clarity and comprehension. These clarifications did not result in any major changes in the definition of efficacy variables as specified in the SAP. The key changes are summarized below:

- Language regarding urine cytology was added to the determination of CR for patients who were judged to have not achieved CR;
- The definition of treatment failure was clarified;
- The initial date of CR was clarified;
- The definition of HG RFS at Months 3, 6, 9, and 12 was clarified; and
- The definition of HG RFS as a time-to-event variable was clarified.

SAP supplement version 2.0 was dated (08 May 2023). The SAP supplement was updated to reflect changes made in SAP version 3.0 (dated 08 May 2023).

Though incidence of HG RFS at Month 60 is indicated in the SAP, the final collection of HG RFS data was performed at the Month 57 visit. Therefore, the data on HG RFS is presented through Month 57.

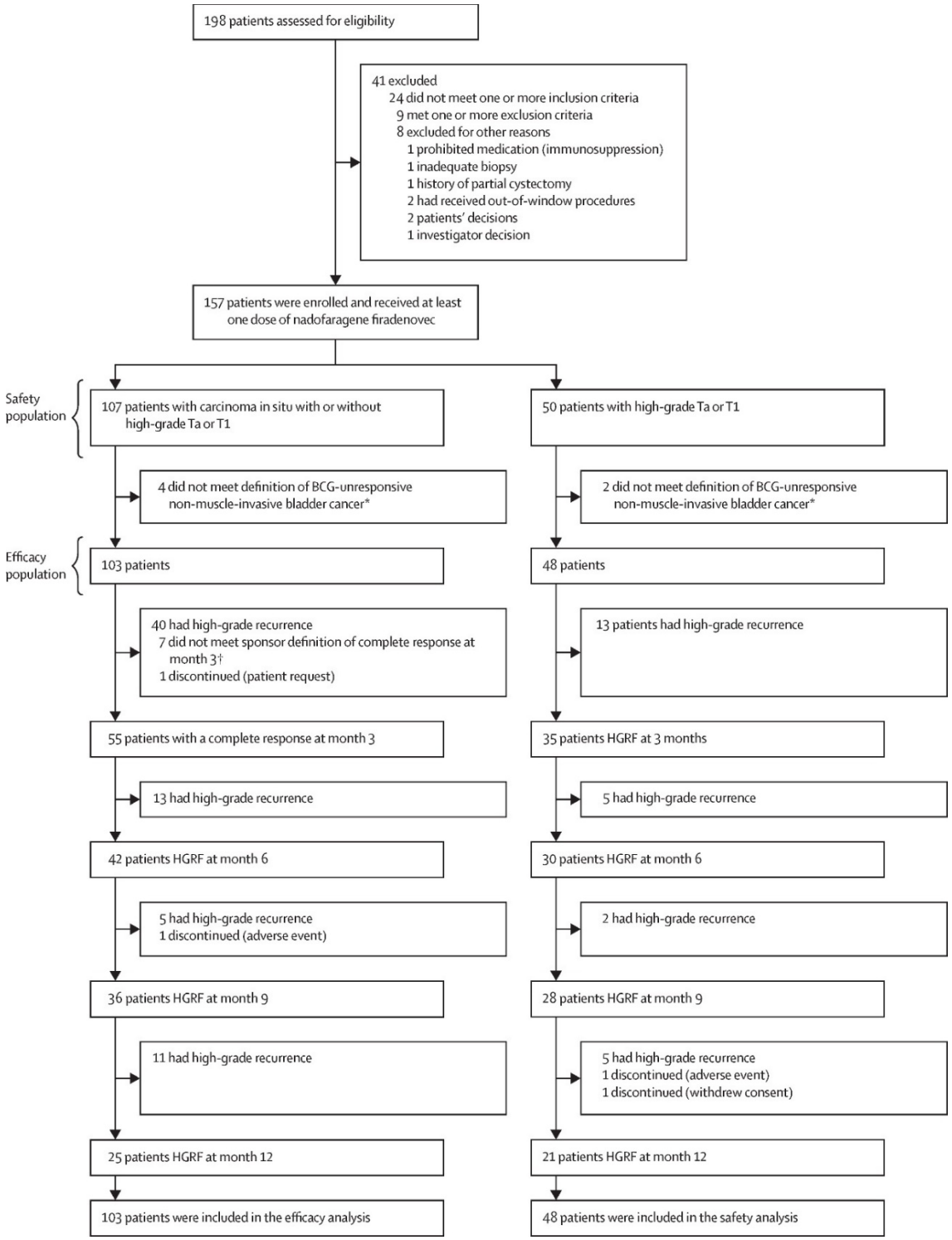
Results

The first CSR for study CS-003, dated 16 September 2019, presented data through 08 July 2019 after all patients had completed their Month 12 Efficacy Assessment Visit or had withdrawn prior. The current CSR addendum (follow-up report) presents data for all patients who have completed the Month 60 long-term follow-up (Narayan et al. J Urol. 2024).

- **Recruitment and Participant flow**

The initiation date for study CS-003 was 12 September 2016. Between then and 24 May 2019, 198 patients were assessed for eligibility at 33 US study sites, and 157 patients were enrolled and received at least 1 dose of nadofaragene firadenovec (107 patients in the CIS cohort and 50 patients in the papillary disease cohort). The completion date for the **main phase of the study** (initial 12-month period) was 24 May 2019 (date of the Month 12 Efficacy Assessment Visit for the last patient) and the database lock was 21 June 2019. The participant flow is shown in Figure 2.

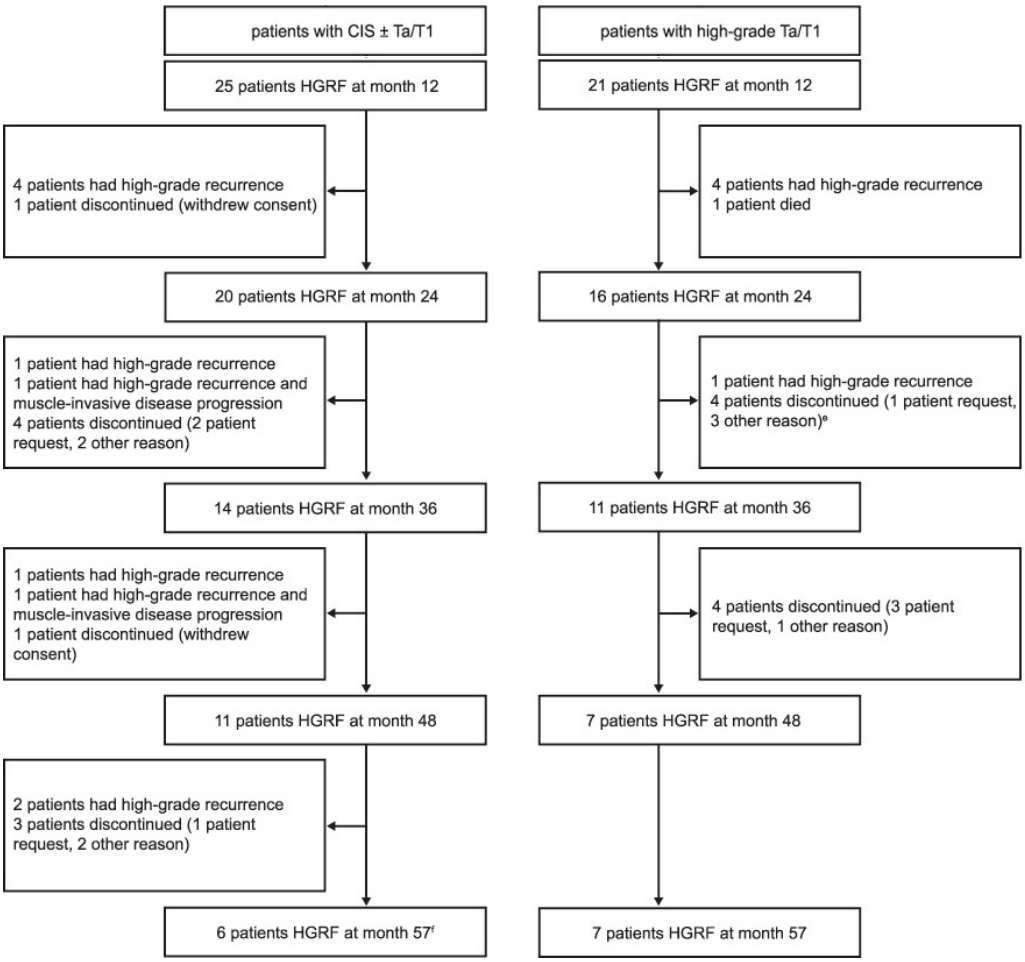
Figure 2. Participant Flow Main Phase of Study CS-003



HGRF=high-grade recurrence-free. *Post-enrolment review of medical records. †Defined in the final Statistical Analysis Plan. Five patients in the carcinoma in situ Ta or T1 cohort and three patients in the high-grade Ta or T1 cohort progressed to detrusor muscle invasion (≥pT2) by data cutoff.

The completion date for the **long-term follow-up** (up to 5 years from the first instillation of nadofaragene firadenovec) was 24 May 2023 and the database lock was 26 June 2023. The participant flow for the long-term follow-up is shown in Figure 3.

Figure 3. Participant Flow Long-Term Follow-Up of Study CS-003



CIS, carcinoma in situ; CR, complete response; HGRF, high-grade recurrence–free; NMIBC, non-muscle-invasive bladder cancer. [e] Of the 3 patients who discontinued for other reasons, all 3 received last treatment at month 9. [f] One patient was subsequently reported to have high-grade recurrence at month 63.
Amended from Narayan et al. J Urol. 2024.

- **Conduct of the study**

Changes in the planned conduct of the study – The original protocol (version 1.1) was dated 16 February 2016 and the last protocol version 8.0 was dated 01 February 2022.

Table 6 shows the key changes from protocol version 4.0 to version 5.0. These significant changes to the design of study CS-003 during its conduct, were implemented based on a request by the US FDA. These changes aimed to align with the FDA 2018 guidance and included a change to the primary endpoint, along with a resulting change to the patient population on which the primary endpoint analysis was based, and the final sample size of the trial.

Table 6. Key Changes from Protocol Version 4.0 to Version 5.0

Protocol version 4.0 (dated 16 August 2017)	**Protocol version 5.0** (dated 29 January 2018)
Primary and Secondary Objectives	
Primary Objectives 1. To evaluate the incidence of Event-Free Survival at 12 months, where event-free survival is defined as High-Grade-Recurrence Free Survival [4] **Secondary Objectives** 1. To evaluate the incidence of Event-Free Survival during the first 12 months of the long term follow up period, where Event-Free Survival is defined as High-Grade-Recurrence Free Survival 2. To evaluate the incidence of Event-Free Survival at 3, 6 and 9 months, where Event-Free Survival is defined as High-Grade-Recurrence Free Survival 3. To determine time to the recurrence of high grade disease 4. To determine the incidence of and time to cystectomy 5. To determine the overall survival in all patients 6. To determine the anti-adenoviral antibody levels for correlation to response rate 7. To evaluate the safety of INSTILADRIN® 8. To monitor durability of response during long term follow-up period after completing the primary objective	**1. Primary Objectives** 1. To evaluate the complete response rate in patients with CIS (with or without concomitant high-grade Ta or T1 papillary disease) **2. Secondary Objectives** 1. To evaluate the durability of complete response in patients with CIS (with or without concomitant high-grade Ta or T1 papillary disease) who achieve a complete response. 2. To evaluate the rate of event-free survival, where event-free survival is defined as high-grade recurrence free survival, in patients with high-grade Ta or T1 papillary disease (without concomitant CIS) 3. To evaluate the durability of event-free survival in patients with high-grade Ta or T1 papillary disease (without concomitant CIS), who have no recurrence of high-grade Ta or T1 papillary disease 4. To determine the incidence of and time to cystectomy 5. To determine the overall survival in all patients 6. To determine the anti-adenoviral antibody levels for correlation to response rate 7. To evaluate the safety of INSTILADRIN® 8. To monitor durability of response during long term follow-up period
Estimation of Sample Size and Operating Characteristics of Design	
16.7 Estimation of Sample Size and Operating Characteristics of Design Both the anticipated and observed response rates in the Phase II study were equal to 35%. Assuming the Phase III study retains 87.5% of that efficacy, the true response rate will be 30.625%. One hundred and thirty-five subjects provide 90% power to reject the hypothesis that the true response rate is 18% at a one-sided alpha of 2.5%. Thirty-four responding subjects (response rate of 25.2%, two-sided 95% Clopper-Pearson CI = [18.1%, 33.4%]) are sufficient to reject the hypothesis.	**16.7. Estimation of Sample Size and Operating Characteristics of Design** The observed complete response rate at any time in CIS +/- papillary subjects in the Phase II study was equal to 50%. Assuming the Phase III study retains 87.5% of that efficacy, the true response rate will be 43.75%. One hundred CIS +/- papillary subjects provide 90% power to reject the hypothesis that the true response rate is 27% at a one-sided alpha of 2.5%. Thirty-seven responding subjects (response rate of 37%, two-sided 95% Clopper-Pearson CI = [27.5%,47.3%]) are sufficient to reject the hypothesis.

Protocol deviations – Protocol deviations were categorized as major or minor at a data review meeting convened by the Sponsor. All protocol deviations presented below were considered Clinical Study Report (CSR) reportable:

- Two patients were in violation of the informed consent process.
- Twenty-three patients had their Month 1 Day 1 Visit conducted outside of the protocol-defined window. One of these patients had a major protocol deviation (Month 1 Day 1 Visit conducted >60 days from TURBT).
- Sixteen patients did not meet the entry criteria. Of these, 6 patients (four from the CIS cohort and two from the papillary disease cohort) were not included in the Efficacy Analysis Set because they did not meet the protocol definition of HG BCG-unresponsive NMIBC. The unmet entry criteria for the remaining 10 patients were determined by the Sponsor to have no impact on the key patient requirements as defined for BCG-unresponsive NMIBC.
- Twelve patients had deviations related to the dosing of nadofaragene firadenovec. Nine patients did not receive the correct dose of nadofaragene firadenovec, two patients received nadofaragene firadenovec within 14 days of a biopsy, and a single patient received treatment with nadofaragene firadenovec following temperature excursions during storage that had not been reported by the site.
- Two patients withdrew before Month 12 due to reasons other than disease recurrence or progression, death, AE related to disease/treatment, or lack of tolerability.
- For five patients (three from the CIS cohort and two from the papillary disease cohort; patients), one or more biopsies were not collected from the protocol-specified locations at the Month 12 visit.
- One patient (from the CIS cohort) did not have biopsies performed at Month 12 in accordance with protocol requirements because the patient withdrew consent for biopsy collection due to their previous history of haematuria. Since this patient did not consent to the biopsies, it was determined that optional dosing at Month 12 and beyond would not continue. This patient was not withdrawn from the study and continued to be seen every 3 months.
- One patient received an excluded medication. A prohibited concomitant medication, methotrexate, was administered during the study due to the patient's medical history of arthritis.

- **Baseline data**

The demographics and baseline characteristics for the Efficacy Analysis Set are presented in Table 7. For the Safety Analysis Set and the Per-Protocol Analysis Set, these were similar (data not shown).

Table 7. Demographic and Baseline Characteristics - Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Age (years)			
Median	71.0	71.0	71.0
Minimum; Maximum	44; 89	39; 87	39; 89
Age (group), n (%)			
<65 years	24 (23.3)	13 (27.1)	37 (24.5)
>=65 years	79 (76.7)	35 (72.9)	114 (75.5)
Sex, n (%)			
Male	91 (88.3)	34 (70.8)	125 (82.8)
Female	12 (11.7)	14 (29.2)	26 (17.2)
Race, n (%)			
White	95 (92.2)	45 (93.8)	140 (92.7)
Black or African American	6 (5.8)	2 (4.2)	8 (5.3)
Asian	2 (1.9)	1 (2.1)	3 (2.0)
ECOG status, n (%)			
0	93 (90.3)	41 (85.4)	134 (88.7)
1	7 (6.8)	6 (12.5)	13 (8.6)
2	3 (2.9)	1 (2.1)	4 (2.6)

Abbreviations: CIS = carcinoma in situ; ECOG = Eastern Cooperative Oncology Group; N = number of patients.
Note: Percentage was calculated using the number of patients in the column heading as the denominator. Baseline was defined as the last observation before the first dose of trial medication.

Table 8 summarizes disease history characteristics (not including BCG therapy) for the Efficacy Analysis Set. For the Safety Analysis Set and the Per-Protocol Analysis Set, these were similar (data not shown).

Table 8. Disease History – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Time from initial diagnosis of bladder cancer (months) [a]			
Median	19.38	14.72	18.04
Min, max	6.0, 134.0	6.2, 124.4	6.0, 134.0
Number of previous TURBT (n, %)			
1	8 (7.8)	3 (6.3)	11 (7.3)
2	19 (18.4)	7 (14.6)	26 (17.2)
3	27 (26.2)	13 (27.1)	40 (26.5)
4	36 (35.0)	17 (35.4)	53 (35.1)
>=5	13 (12.6)	8 (16.7)	21 (13.9)
Time from the last TURBT/endoscopic resection or fulguration (months) [a]			
Median	1.61	1.38	1.54
Min, max	0.7, 4.9	0.5, 2.1	0.5, 4.9
Current status at entry, n (%)			
Refractory	55 (53.4)	34 (70.8)	89 (58.9)
Relapsed	48 (46.6)	14 (29.2)	62 (41.1)
Current stage at entry, n (%)			
CIS only	79 (76.7)	0 (0.0)	79 (52.3)
Ta	0 (0.0)	33 (68.8)	33 (21.9)
Ta + CIS	19 (18.4)	0 (0.0)	19 (12.6)
T1	0 (0.0)	15 (31.3)	15 (9.9)
T1 + CIS	5 (4.9)	0 (0.0)	5 (3.3)
Prior surgery resection (non-TURBT resection), n (%)			
Yes	4 (3.9)	0 (0.0)	4 (2.6)
No	99 (96.1)	48 (100.0)	147 (97.4)
Prior radiotherapy, n (%)			

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Yes	4 (3.9)	1 (2.1)	5 (3.3)
No	99 (96.1)	47 (97.9)	146 (96.7)
Prior cancer therapy/medication regimen, n (%) [b]			
Yes	31 (30.1)	7 (14.6)	38 (25.2)
No	72 (69.9)	41 (85.4)	113 (74.8)
Type of most recent prior cancer therapy/medications, n (%) [b]			
Chemotherapy	24 (23.3)	7 (14.6)	31 (20.5)
Small molecule targeted therapy	2 (1.9)	0 (0.0)	2 (1.3)
Other	5 (4.9)	0 (0.0)	5 (3.3)

Abbreviations: BCG = Bacillus Calmette-Guérin; CIS = carcinoma in situ; N = number of patients; SD = standard deviation; TURBT = transurethral resection of bladder tumour.
Percentage was calculated using the number of patients in the column heading as the denominator.
a) Time from diagnosis/resection (months) was calculated as (date of first dose – date of diagnosis/resection + 1)/30.4375.
b) Prior non-BCG cancer therapy/regimen. BCG history is presented in Table 9.

Table 9 shows the BCG history for the Efficacy Analysis Set. For the Safety Analysis Set and the Per-Protocol Analysis Set, this was similar (data not shown).

Table 9. BCG History – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Number of courses of BCG administered, n (%)			
1	0 (0.0)	4 (8.3)	4 (2.6)
2	43 (41.7)	27 (56.3)	70 (46.4)
3	28 (27.2)	12 (25.0)	40 (26.5)
4	12 (11.7)	2 (4.2)	14 (9.3)
5	6 (5.8)	2 (4.2)	8 (5.3)
6	7 (6.8)	0 (0.0)	7 (4.6)
7	0 (0.0)	0 (0.0)	0 (0.0)
8	3 (2.9)	0 (0.0)	3 (2.0)
≥9	4 (3.9)	1 (2.1)	5 (3.3)
Total number (induction + maintenance) of previous BCG doses [a]			
Median	12.0	12.0	12.0
Min, max	8, 18	5, 18	5, 18
Time from last exposure to BCG (months) [b]			
Median	4.93	5.24	4.93
Min, max	2.0, 13.1	2.3, 13.1	2.0. 13.1

Abbreviations: BCG = Bacillus Calmette-Guérin; CIS = carcinoma in situ; N = number of patients.
Note: Percentage was calculated using the number of patients in the column heading as the denominator.
a) Note: Number of induction doses and number of maintenance/additional induction doses were collected as >=9 when the number of doses are equal to or above 9. For the calculation of summary statistics, they were imputed as 9.
b) Time from last exposure (months) was calculated as (date of first dose – date of last exposure + 1)/30.4375.

Prior and concomitant medications – Two patients in the papillary disease cohort received intravesical chemotherapy within 8 weeks (allowed per protocol) prior to initiation of trial treatment (mitomycin was given 52 days prior to trial treatment in 1 patient and gemcitabine was given post TURBT 55 days prior to trial treatment in 1 patient).

Concomitant medication was systematically collected until disease recurrence during the first 24 months. All patients continued to receive appropriate supportive care for the treatment of their cancer. No initiation of a new bladder cancer therapy were observed prior to disease recurrence during the first 24 months of the trial.

- **Numbers analysed**

Table 10. Analysis Populations – All Enrolled Patients

	CIS n (%)	Papillary Disease n (%)	Total n (%)
Patients enrolled (received first dose) [1]	107 (100.0)	50 (100.0)	157 (100.0)
Efficacy Analysis Set	103 (96.3)	48 (96.0)	151 (96.2)
Safety Analysis Set	107 (100.0)	50 (100.0)	157 (100.0)
Per-Protocol Analysis Set	100 (93.5)	47 (94.0)	147 (93.6)

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.
Percentage was calculated using the number of treated patients in each cohort as the denominator.
1. Patients enrolled included all patients who signed the ICF, were enrolled in a cohort, and received the first dose.
CIS = carcinoma in situ; ICF = informed consent form.

Of note, three patients completed the Month 12 Efficacy Assessment Visit outside of the specified range, but were still included in the Per-Protocol Analysis Set because they had a subsequent Month 15 Follow-up Visit that included sufficient evidence to include them.

- **Outcomes and estimation**

Primary endpoint – Incidence of CR

Table 11. Incidence of Complete Response at Any Time in Patients With CIS – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)
Patients who achieved CR [1]	
By Month 3 (n, %)	55 (53.4)
95% CI [%] [2]	[43.3, 63.3]
During Months 4 to 6 (n, %)	0 (0.0)
95% CI [%] [2]	[0.0, 3.5]
During Months 7 to 9 (n, %)	0 (0.0)
95% CI [%] [2]	[0.0, 3.5]
During Months 10 to 12 (n, %)	0 (0.0)
95% CI [%] [2]	[0.0, 3.5]
Total (n, %)	55 (53.4)
95% CI [%] [2]	[43.3, 63.3]

Note: CIS = CIS only, Ta + CIS, and T1 + CIS.
Percentage was calculated using the number of patients in the column heading as the denominator.
1. Patients who achieved CR comprised CIS patients (with or without papillary disease) who had CR reported by the Investigator at any time after the administration of nadofaragene firadenovec. Excluded were patients who had CR reported by the Investigator but were judged to have not achieved CR per the definition in the SAP.
2. A 95% exact binomial CI for rate of CR was calculated using the Clopper-Pearson method.
CI = confidence interval; CIS = carcinoma in situ; CR = complete response; nadofaragene firadenovec = rAd-IFN/Syn3; SAP = Statistical Analysis Plan.

Table 12. Patients with CIS with or without Complete Response – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)
Patients with complete response, n (%) [95% CI]	55 (53.4%) [43.3,63.3]
Patients without complete response, n (%)	48 (46.6%)
Recurrence of high-grade disease	46 (44.7%)
Recurrence of high-grade disease and muscle-invasive progression	1 (1.0%)
Not evaluable	1 (1.0%)

N = number of patients, CI=confidence interval, CIS = CIS Only, Ta+CIS, T1+CIS
Percentage (%) is calculated based on all CIS patients in the efficacy analysis set as denominator.
Patients who have achieved complete response (CR) include CIS patients (+/- papillary disease) who had a CR reported by the investigator at any time after first administration of nadofaragene firadenovec, but excluding those patients who had a CR reported by the investigator but were judged to have not achieved CR per the definition in the SAP (i.e., urine cytology was reported as suspicious, malignant cells, or not done, and biopsy were not performed at the initial CR, and there was a high-grade recurrence,

confirmed by biopsy, observed at the next visit for response assessment OR no biopsy was performed at the next response assessment but the urine cytology result at the next response assessment was reported as suspicious or malignant).
CIs are 95% exact binomial CI.

The lower bound (43.3%) of the 2-sided 95% CI of the incidence of CR at any time in patients with CIS exceeded 27%. The null hypothesis specified in the protocol of a true response rate of 27% was, therefore, rejected and the primary endpoint was met.

Similar results were obtained for the Per-Protocol Analysis Set (54/100 patients [54.0%] achieved CR [95% CI: 43.7, 64.0]) and the Safety Analysis Set (56/107 patients [52.3%] achieved CR [95% CI: 42.5, 62.1]).

Key secondary endpoint – Durability of CR

Table 13. Duration of Complete Response in Patients with CIS - Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)
Patients who have achieved complete response, n	55
Patients with treatment failure after complete response, n (%)	41 (74.5%)
Documented recurrence of high-grade disease	38 (69.1%)
Muscle-invasive disease progression	0 (0.0%)
Documented recurrence of high-grade disease and muscle-invasive disease progression	3 (5.5%)
Death	0 (0.0%)
Patients censored, n (%)	14 (25.5%)
Duration of complete response (months)[a]	
Kaplan-Meier estimate[b]	
Median [95% CI]	9.72 [9.17, 23.95]

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; N = number of patients; NE = not estimable.
Note: Percentage was calculated using 55 as the denominator, i.e., the number of patients who have achieved complete response.
a) Duration of complete response was defined as the time from first observed complete response to documented treatment failure. Patients without treatment failure were censored at the last disease assessment not showing treatment failure.
b) Median is Kaplan-Meier estimate. The 2-sided CI for median was computed using the method of Brookmeyer and Crowley.
c) Probability of duration was the Kaplan-Meier estimate of survivor function at each specific time point. The 95% CI for probability of duration was calculated using Greenwood's formula with a log-log transformation.

Table 14. Durability of Complete Response in Patients with CIS - Study CS-003 (Efficacy Analysis Set) (post-hoc analysis)

	CIS (N=55)	
	n (%)	95% CI
Durability of complete response in patients with CIS at		
Month 3	55 (100.0)	[93.5, 100.0]
Month 6	42 (76.4)	[63.0, 86.8]
Month 9	36 (65.5)	[51.4, 77.8]
Month 12	25 (45.5)	[32.0, 59.4]
Month 15	24 (43.6)	[30.3, 57.7]
Month 18	22 (40.0)	[27.0, 54.1]
Month 21	21 (38.2)	[25.4, 52.3]
Month 24	20 (36.4)	[23.8, 50.4]
Month 36	14 (25.5)	[14.7, 39.0]
Month 48	11 (20.0)	[10.4, 33.0]
Month 57	6 (10.9)	[4.1, 22.2]

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; N = number of patients.
Note: Percentage is calculated using the number of patients in the column heading as the denominator. CIs are 95% exact binomial confidence intervals.

The actual probability of duration of CR for at least 6, 12, 18, 24, 36, and 48 months (i.e., as percentage of patients form the CIS cohort at each specific time point) was 40.1%, 24.2%, 21.4%, 19.4%, 13.6%, and 10.7%, respectively.

A KM curve presenting the duration of CR in patients with CIS is shown in Figure 4.

Figure 4. Duration of Complete Response in Patients with CIS - Study CS-003 (Efficacy Analysis Set)

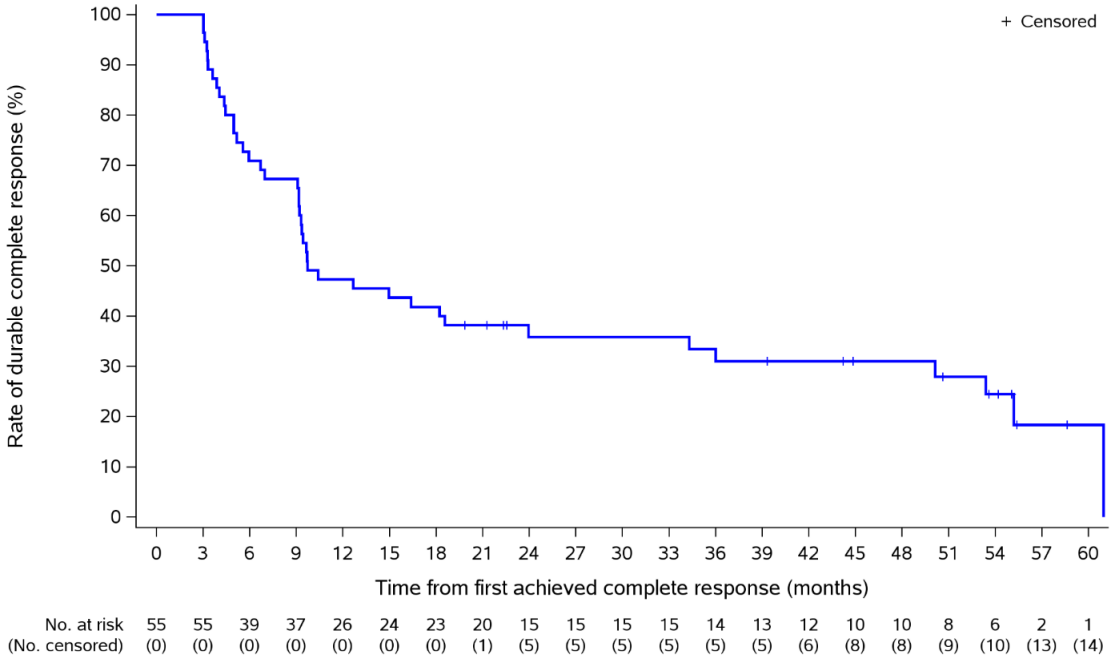
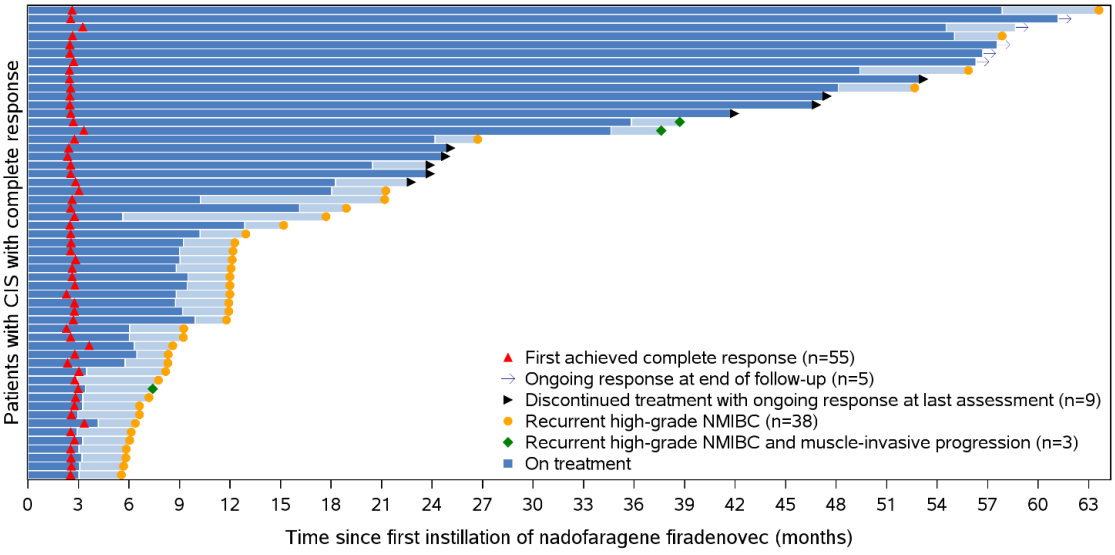


Figure 5 shows the time to CR and recurrence of HG disease in the 55 patients with CIS with a CR.

Figure 5. Time to Complete Response and Recurrence of High-Grade Disease in Patients with CIS with Complete Response – Study CS 003 (Efficacy Analysis Set)



Abbreviation: CIS = carcinoma in situ; NMIBC = non-muscle-invasive bladder cancer.
Note: 'On treatment' includes the time from first to last instillation of nadofaragene firadenovec.

Similar results were obtained for the Per-Protocol Analysis Set (median duration of CR 9.71 months [95% CI: 9.17, 18.56]) and the Safety Analysis Set (median duration of CR 9.71 months [95% CI: 9.17, 18.56]).

Comparable results were also observed in a supplementary KM analysis assessing the durability of CR, in which treatment discontinuations that occurred prematurely during the primary 12-month treatment period of the trial, for reasons other than disease recurrence, were classified as treatment failures (median DoCR 9.69 months [95% CI: 9.10, 23.95]). Notably, only one patient with CIS who achieved a CR prematurely discontinued trial treatment due to an AE, with the last dose administered on Month 7, Day 1. This resulted in a difference of 1.8 percent points lower KM estimate up to month 12 and equal KM estimates from month 18 onwards.

Other secondary endpoints – Incidence of HG RFS

Table 15 summarizes the incidence of HG RFS for the Efficacy Analysis Set, per protocol and SAP reported for the CIS cohort, the papillary disease cohort, and both cohorts combined.

Table 15. Incidence of High-Grade Recurrence-Free Survival – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)		Papillary Disease (N=48)		Total (N=151)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
Patients who achieved high-grade recurrence-free survival at						
Month 3	55 (53.4)	[43.3, 63.3]	35 (72.9)	[58.2, 84.7]	90 (59.6)	[51.3, 67.5]
Month 6	42 (40.8)	[31.2, 50.9]	30 (62.5)	[47.4, 76.0]	72 (47.7)	[39.5, 56.0]
Month 9	36 (35.0)	[25.8, 45.0]	28 (58.3)	[43.2, 72.4]	64 (42.4)	[34.4, 50.7]
Month 12	25 (24.3)	[16.4, 33.7]	21 (43.8)	[29.5, 58.8]	46 (30.5)	[23.2, 38.5]
Month 15	24 (23.3)	[15.5, 32.7]	19 (39.6)	[25.8, 54.7]	43 (28.5)	[21.4, 36.4]
Month 18	22 (21.4)	[13.9, 30.5]	16 (33.3)	[20.4, 48.4]	38 (25.2)	[18.5, 32.9]
Month 21	21 (20.4)	[13.1, 29.5]	16 (33.3)	[20.4, 48.4]	37 (24.5)	[17.9, 32.2]
Month 24	20 (19.4)	[12.3, 28.4]	16 (33.3)	[20.4, 48.4]	36 (23.8)	[17.3, 31.4]
Month 36	14 (13.6)	[7.6, 21.8]	11 (22.9)	[12.0, 37.3]	25 (16.6)	[11.0, 23.5]
Month 48	11 (10.7)	[5.5, 18.3]	7 (14.6)	[6.1, 27.8]	18 (11.9)	[7.2, 18.2]
Month 57	6 (5.8)	[2.2, 12.2]	7 (14.6)	[6.1, 27.8]	13 (8.6)	[4.7, 14.3]

Abbreviations: CI = confidence interval, CIS = carcinoma in situ; N = number of patients.
Note: Percentage was calculated using the number of patients in the column heading as the denominator. CIs are 95% exact binomial confidence intervals.

Table 16 summarizes the durability of HG RFS for the Efficacy Analysis Set.

Table 16. Summary of Durability of High-Grade-Recurrence-Free Survival – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Patients with recurrence of high-grade disease (n, %)	88 (85.4)	31 (64.6)	119 (78.8)
Earliest contributing event			
Documented recurrence of high-grade disease	84 (81.6)	29 (60.4)	113 (74.8)
Muscle-invasive disease progression	0 (0.0)	0 (0.0)	0 (0.0)
Documented recurrence of high-grade disease and muscle-invasive disease progression	4 (3.9)	1 (2.1)	5 (3.3)
Death	0 (0.0)	1 (2.1)	1 (0.7)
Patients censored (n, %)	15 (14.6)	17 (35.4)	32 (21.2)
HGRF survival (months) [1]			
Kaplan-Meier estimate [2]			
Median [95% CI]	5.95 [3.38, 8.31]	12.35 [6.67, 20.27]	7.31 [5.68, 11.93]
Q1, Q3	2.89, 15.18	4.09, NE	3.09, 26.71
Minimum, maximum	0.0+, 63.6	2.5, 60.7+	0.0+, 63.6
Probability of duration of HGRF survival for at least [3]			

3 months (%)	73.5	89.6	78.7
95% CI [%]	[63.8, 81.0]	[76.8, 95.5]	[71.2, 84.4]
6 months (%)	50.0	68.7	56.0
95% CI [%]	[40.0, 59.2]	[53.6, 79.8]	[47.7, 63.5]
9 months (%)	38.2	62.5	46.0
95% CI [%]	[28.9, 47.5]	[47.3, 74.5]	[37.9, 53.7]
12 months (%)	30.4	55.8	38.4
95% CI [%]	[21.8, 39.4]	[40.6, 68.6]	[30.7, 46.2]
18 months (%)	23.5	40.2	28.8
95% CI [%]	[15.8, 32.1]	[26.1, 53.8]	[21.8, 36.3]
24 months (%)	20.6	35.7	25.4
95% CI [%]	[13.4, 28.9]	[22.3, 49.4]	[18.7, 32.6]
36 months (%)	19.3	32.7	23.6
95% CI [%]	[12.2, 27.6]	[19.5, 46.6]	[17.0, 30.8]
48 months (%)	16.7	32.7	21.6
95% CI [%]	[10.1, 24.9]	[19.5, 46.6]	[15.2, 28.8]
57 months (%)	13.2	32.7	19.0
95% CI [%]	[6.9, 21.5]	[19.5, 46.6]	[12.6, 26.4]

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.
Percentage was calculated using the number of patients in the column heading as the denominator.

1. HGRF survival was defined as the time from the first dose to the first recurrence of high-grade disease (including muscle-invasive disease progression and death due to any cause). Patients without high-grade disease recurrence were censored at the last disease assessment not showing high-grade disease recurrence.
2. Median, Q1, and Q3 are Kaplan-Meier estimates. The 2-sided CI for median was computed using the method of Brookmeyer and Crowley. Minimum and maximum included the censored observations where using "+" after the value indicates censoring.
3. Probability of duration was the Kaplan-Meier estimate of survivor function at each specific time point. The 95% CI for probability of duration was calculated using Greenwood's formula with a log-log transformation.

CI = confidence interval; CIS = carcinoma in situ; HGRF = high-grade-recurrence-free; log = logarithmic; NE = not estimable; Q1 = first quartile; Q3 = third quartile.

Only four patients prematurely discontinued trial treatment during the primary 12-month treatment period of the trial before experiencing any recurrence of disease. Two patients discontinued due to patient request (1 patient with CIS, whose last treatment was administered at Month 1 [Day 1], and 1 patient with papillary disease, whose last treatment was administered on Month 7 [Day 1]). The remaining two patients (1 with CIS and 1 with papillary disease) discontinued due to AEs, both with their last treatment administered on Month 7 (Day 1). All four patients were counted as non-responders for the incidence of HG RFS at all subsequent visits due to missing disease response assessments following these intercurrent events. Additionally, these four cases account for the only missing response assessments within the first 24 months of the trial.

At the time of first recurrence, 5 (3.3%) patients (i.e., 4 [3.9%] in the CIS cohort and 1 [2.1%] in the papillary disease cohort) had a documented recurrence of HG disease and **muscle-invasive disease** progression.

The additional post-hoc analysis under the hypothetical scenario that death did not occur (death censored) showed better survival (more than 20 percent points better at Month 3, more than 10% better at Month 6, and more than 5% at Month 12, 36, 48, and 57.

Other secondary endpoints – Incidence of and time to cystectomy

Table 17 shows the incidence of cystectomy for the CIS cohort, the papillary disease cohort, and both cohorts combined.

Table 17. Incidence of Cystectomy – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	**Papillary Disease (N=48)**	**Total (N=151)**
Patients undergoing cystectomy within			
12 months (n, %) [95% CI (%)]	27 (26.2) [18.0, 35.8]	7 (14.6) [6.1, 27.8]	34 (22.5) [16.1, 30.0]
2 years (n, %) [95% CI (%)]	35 (34.0) [24.9, 44.0]	10 (20.8) [10.5, 35.0]	45 (29.8) [22.6, 37.8]
5 years (n, %) [95% CI (%)]	42 (40.8) [31.2, 50.9]	14 (29.2) [17.0, 44.1]	56 (37.1) [29.4, 45.3]

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; N = number of patients.
Note: Percentage was calculated using the number of patients in the column heading as the denominator.
CIs for proportions of patients undergoing cystectomy are 95% exact binomial confidence intervals.

Table 18 shows the time to cystectomy for the CIS cohort, the papillary disease cohort, and both cohorts combined.

Of note, the median (range) time from disease recurrence to cystectomy was 6.44 months (0.0 to 57.6) for the CIS cohort, 2.66 months (0.9 to 32.0) for the papillary cohort, and 3.78 months (0.0 to 57.6) for both cohorts combined.

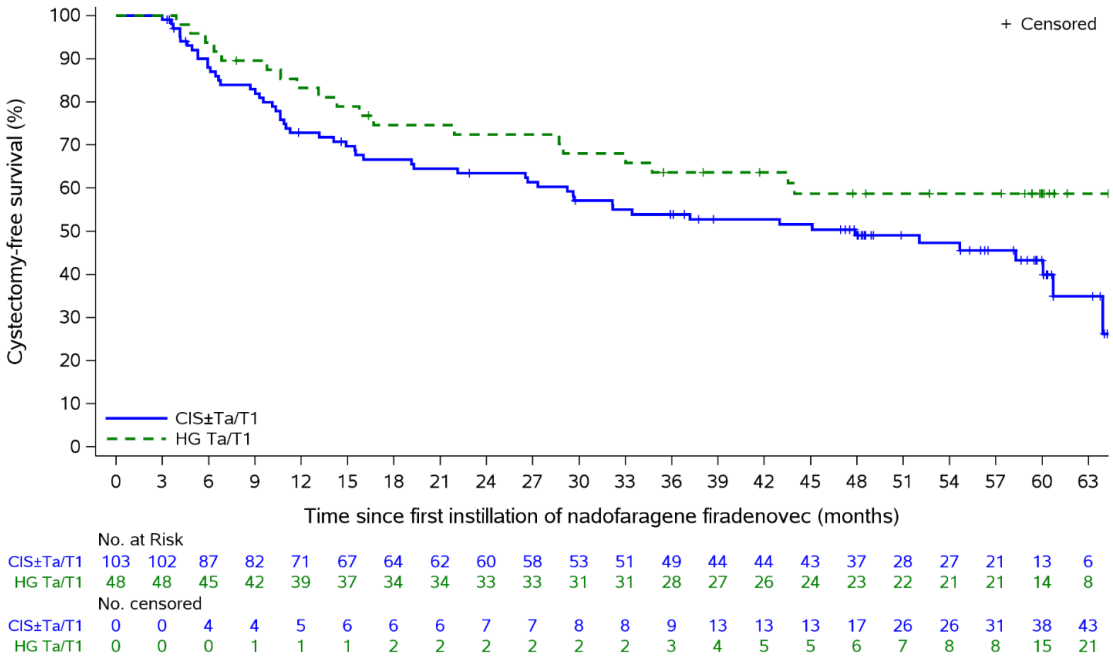
Table 18. Cystectomy-Free Survival – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Patients with cystectomy event (n, %)	55 (53.4%)	19 (39.6%)	74 (49.0%)
Cystectomy	44 (42.7%)	14 (29.2%)	58 (38.4%)
Death	11 (10.7%)	5 (10.4%)	16 (10.6%)
Patients censored (n, %)	48 (46.6%)	29 (60.4%)	77 (51.0%)
Cystectomy-free survival (months) Kaplan-Meier estimate[a]			
Median	47.90	NE	58.28
95% CI for median	[27.33, 60.71]	[34.73, NE]	[33.02, NE]
Cystectomy-free survival rate by[b], % [95% CI]			
12 months	72.8% [62.9%, 80.5%]	83.2% [69.2%, 91.2%]	76.2% [68.4%, 82.3%]
24 months	63.5% [53.1%, 72.1%]	72.4% [57.2%, 83.0%]	66.4% [58.1%, 73.4%]
36 months	53.9% [43.5%, 63.2%]	63.6% [48.1%, 75.6%]	57.0% [48.5%, 64.7%]
48 months	49.0% [38.6%, 58.7%]	58.7% [43.1%, 71.4%]	52.2% [43.6%, 60.1%]
60 months	43.2% [32.2%, 53.7%]	58.7% [43.1%, 71.4%]	48.8% [40.0%, 57.1%]

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; N = number of patients; NE = not estimable.
Note: Percentage was calculated using the number of patients in the column heading as the denominator.
a) Median is Kaplan-Meier estimate. The 2-sided CI for median was computed using the method of Brookmeyer and Crowley.
b) Cystectomy-free survival rate is Kaplan-Meier estimate of survivor function at each specific time point. The 95% CI was calculated using the Greenwood's formula with a log-log transformation.

Figure 6 presents the KM plot of cystectomy-free survival for the CIS cohort and the papillary disease cohort.

Figure 6. Cystectomy-Free Survival – Study CS-003 (Efficacy Analysis Set)



Of the 107 patients with CIS in the Safety Analysis Set, 46 (43%) patients underwent cystectomy, including 16 patients who initially achieved CR at Month 3, and 30 patients who did not achieve an initial CR at Month 3 (Figure 10**Figure 9**). Of the 46 patients with CIS who underwent cystectomy, pathologic data were available for 39 patients (Table 19).

Table 19. Summary of Stage at Cystectomy in CIS Patients in Study CS-003 (Safety Analysis Set)

Cystectomy in patients with CIS, N (%)	**CIS (N=107)**
Yes	46 (43.0)
No	61 (57.0)
All	107 (100.0)
Patients by worst stage, n (%)	
pT0	3 (6.5)
pTis	25 (54.3)
pT1	5 (10.9)
pT2	2 (4.3)
pT2a	1 (2.2)
pT2b	1 (2.2)
pT3a	1 (2.2)
pT3b	1 (2.2)
Missing	7 (15.2)
All	46 (100.0)
Regional lymph nodes, n (%)	
pN0	36 (78.3)
pN1	1 (2.2)
pN2	2 (4.3)
Missing	7 (15.2)
All	46 (100.0)

Abbreviations: N, Number of subjects; n, Number of subjects with observation; %, Percentage of subjects with observation.

Of the 107 patients with CIS in the Safety Analysis Set, 7.5% (n = 8) progressed to muscle-invasive (pT2 or greater) and/or lymph node metastatic (pN+) bladder cancer (Table 20), including 4 during the treatment period. Four patients had progression during treatment at time of first recurrence with a median time from first dose to progression of 686 days (range: 76-1178). The remaining four patients were upstaged at the time of cystectomy with a median time from persistence or recurrence of CIS to cystectomy of 235 days (range: 64-335).

Table 20. Patients with CIS with Progression to muscle-invasive and/or lymph node metastatic bladder cancer at First Recurrence and/or Cystectomy – Study CS-003 (Safey Analysis Set)

Recurrence						Cystectomy			
Subject ID	CR	Time [a] (days)	Stage	MIBC	Tumour category	Event	Time [b] (days)	Stage	Time from recurrence (days)
Xxx1	Y	1145	II	Y	T2	Alive with no cystectomy performed	1456	NA	312
Xxx2	N	84	0IS	NA	TIS	Cystectomy	308	pT2b N1	225
Xxx3	N	81	0IS	N	TIS	Cystectomy	324	pT2 N0	244
Xxx4	Y	201	0IS	N	TIS	Cystectomy	265	pTIS N2	64
Xxx5	N	76	II	Y	T2	Cystectomy	113	pT3a N0	38
Xxx6	Y	253	0IS	N	TIS	Cystectomy	587	pT2 N2	335
Xxx7	Y	226	II	Y	T2	Cystectomy	807	pT3b N0	582
Xxx8	Y	1178	II	Y	T2	Cystectomy	1373	pT2a N0	196

Abbreviations: CIS, carcinoma in situ; CR, complete response; MIBC, Muscle-invasive bladder cancer; NA, not applicable
Note: Table was amended by assessor
[a] Time is from first instillation of nadofaragene firadenovec to recurrence of high-grade disease
[b] Time is from first instillation of nadofaragene firadenovec to cystectomy and includes last date/time of known status being alive and without cystectomy performed for subjects without cystectomy.

Other secondary endpoints – OS

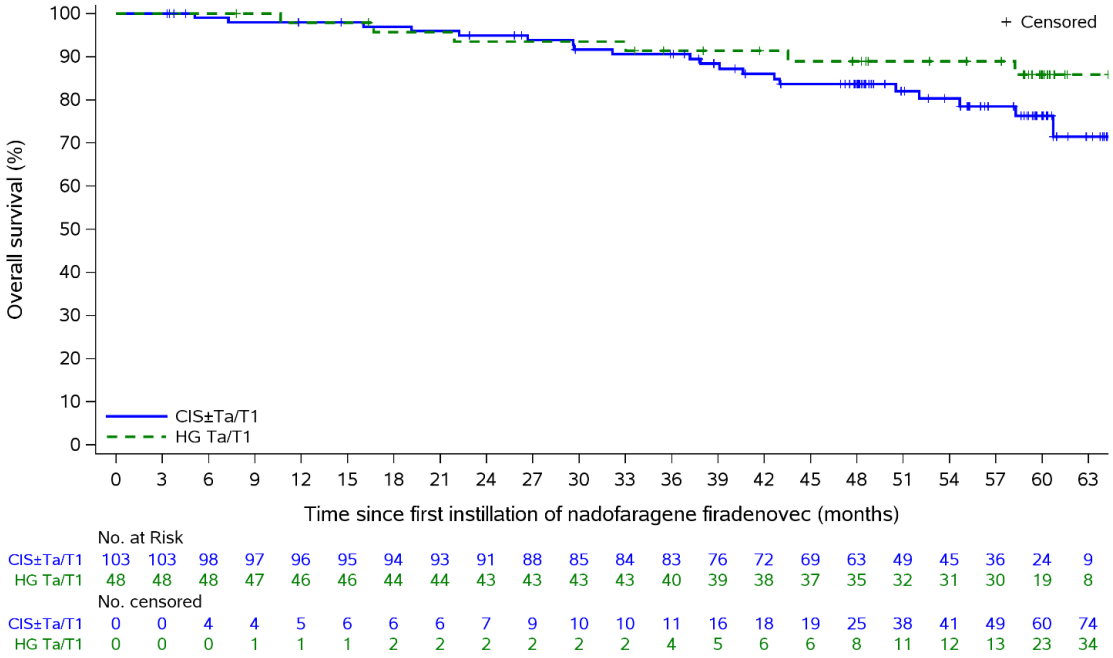
Table 21. Overall Survival – Study CS-003 (Efficacy Analysis Set)

	CIS (N=103)	Papillary Disease (N=48)	Total (N=151)
Patients who died, n (%)	20 (19.4%)	6 (12.5%)	26 (17.2%)
Patients censored, n (%)	83 (80.6%)	42 (87.5%)	125 (82.8%)
Overall survival (months) Kaplan-Meier estimate[a]			
Median	NE	NE	NE
95% CI for median	[NE, NE]	[NE, NE]	[NE, NE]
Overall survival rate at[b], % [95% CI]			
12 months	98.0% [92.2%, 99.5%]	97.9% [85.8%, 99.7%]	98.0% [93.8%, 99.3%]
24 months	94.9% [88.1%, 97.8%]	93.5% [81.2%, 97.9%]	94.5% [89.2%, 97.2%]
36 months	90.6% [82.7%, 95.0%]	91.3% [78.6%, 96.7%]	90.9% [84.8%, 94.6%]
48 months	83.6% [74.3%, 89.8%]	88.9% [75.4%, 95.3%]	85.4% [78.3%, 90.3%]
60 months	76.3% [64.6%, 84.5%]	85.9% [70.9%, 93.5%]	79.7% [71.0%, 86.0%]

Abbreviations: CI = confidence interval; CIS = carcinoma in situ; N = number of patients; NE = not estimable.
Note: Percentage was calculated using the number of patients in the column heading as the denominator
a) Median is Kaplan-Meier estimate. The 2-sided CI for median was computed using the method of Brookmeyer and Crowley.
b) Overall survival rate is a Kaplan-Meier estimate of the survivor function at each specific time point. The 95% CI was calculated using the Greenwood's formula with a log-log transformation.

KM curves presenting the OS for the CIS cohort and the papillary disease cohort are shown in Figure 7.

Figure 7. Overall Survival – Study CS-003 (Efficacy Analysis Set)



- **Ancillary analyses**

Pre-defined subgroup analyses – Table 22 and Figure 8 present the incidence of CR at any time after the first administration of nadofaragene firadenovec in patients with CIS by subgroup for the Efficacy Analysis Set.

Table 22. Incidence of Complete Response in Patients With CIS by Subgroup – Study CS-003 (Efficacy Analysis Set)

Subgroup	CIS (N=103)	
	n/N' (%)	**95% CI [1]**
Sex		
Male	48/91 (52.7)	[42.0, 63.3]
Female	7/12 (58.3)	[27.7, 84.8]
Age group		
<65 years	13/24 (54.2)	[32.8, 74.4]
≥65 years	42/79 (53.2)	[41.6, 64.5]
Race group		
White	52/95 (54.7)	[44.2, 65.0]
Non-White	3/8 (37.5)	[8.5, 75.5]
Time from initial diagnosis of bladder cancer		
<Median	26/44 (59.1)	[43.2, 73.7]
≥Median	29/59 (49.2)	[35.9, 62.5]
Tumour type at study entry		
CIS only	43/79 (54.4)	[42.8, 65.7]
CIS with Ta/T1	12/24 (50.0)	[29.1, 70.9]
Positive immunogenic response in anti-adenoviral antibodies post baseline [2]		
Yes	43/62 (69.4)	[56.3, 80.4]
No	8/24 (33.3)	[15.6, 55.3]
Unknown	4/17 (23.5)	[6.8, 49.9]

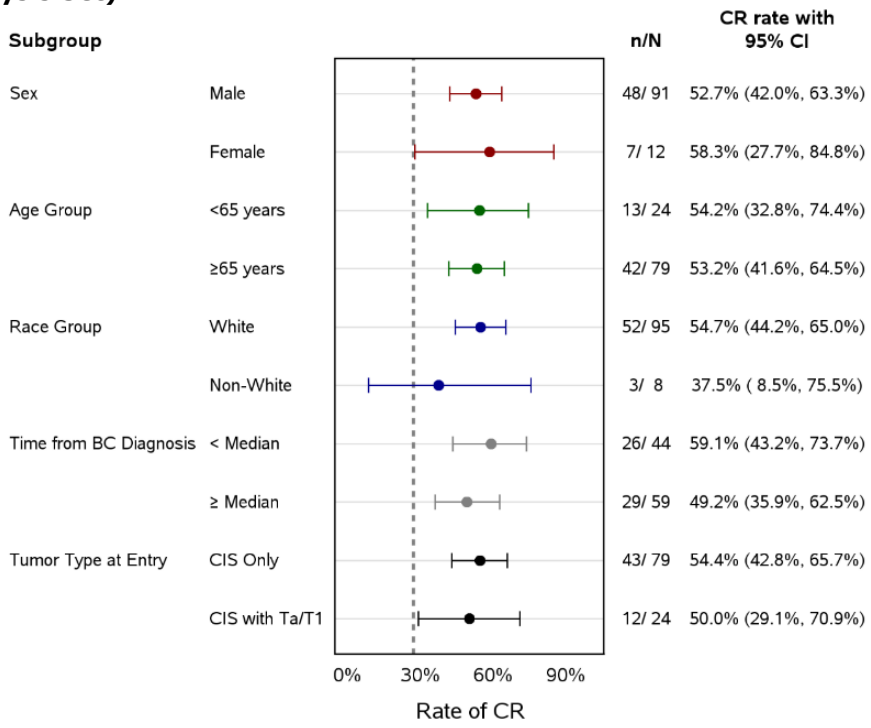
Note: CIS = CIS only, Ta + CIS, and T1 + CIS.
Percentage was calculated using N' as the denominator, where N' was the number of patients in the specified subgroup. Patients who achieved CR comprised CIS patients (with or without papillary disease) who had CR reported by the Investigator at any time after the administration of nadofaragene firadenovec. Excluded were patients who had CR reported by the Investigator but were judged to have not achieved CR per the definition in the SAP.

1. A 95% exact binomial CI for rate of CR was calculated using the Clopper-Pearson method.

2. A patient was considered to have a positive immunogenic response in anti-adenoviral antibodies if a post-baseline titration demonstrated a greater than 2-fold dilution increase from baseline.

CI = confidence interval; CIS = carcinoma in situ; CR = complete response; nadofaragene firadenovec = rAd-IFN/Syn3; SAP = Statistical Analysis Plan.

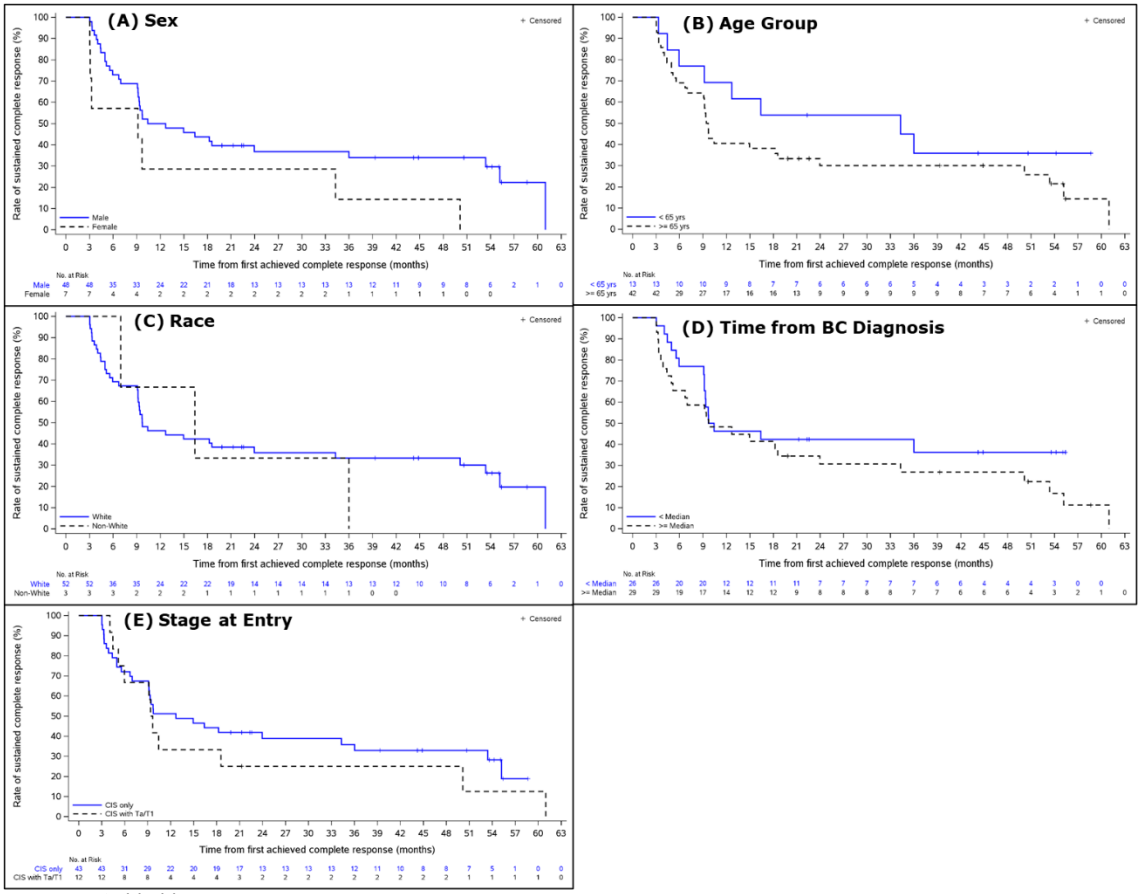
Figure 8. Incidence of Complete Response in Patients with CIS by Subgroup – Study CS-003 (Efficacy Analysis Set)



Abbreviations: CI = confidence interval; CIS = carcinoma in situ; CR = complete response; N = number of patients.
Note: Percentage was calculated using N' as the denominator, where N' was the number of patients in the specified subgroup. Patients who achieved CR comprised CIS patients (with or without papillary disease) who had CR reported by the Investigator at any time after the administration of nadofaragene firadenovec. Excluded were patients who had CR reported by the Investigator but were judged to have not achieved CR per the definition in the statistical analysis plan.
95% exact binomial CI for rate of complete response was calculated using the Clopper-Pearson method.

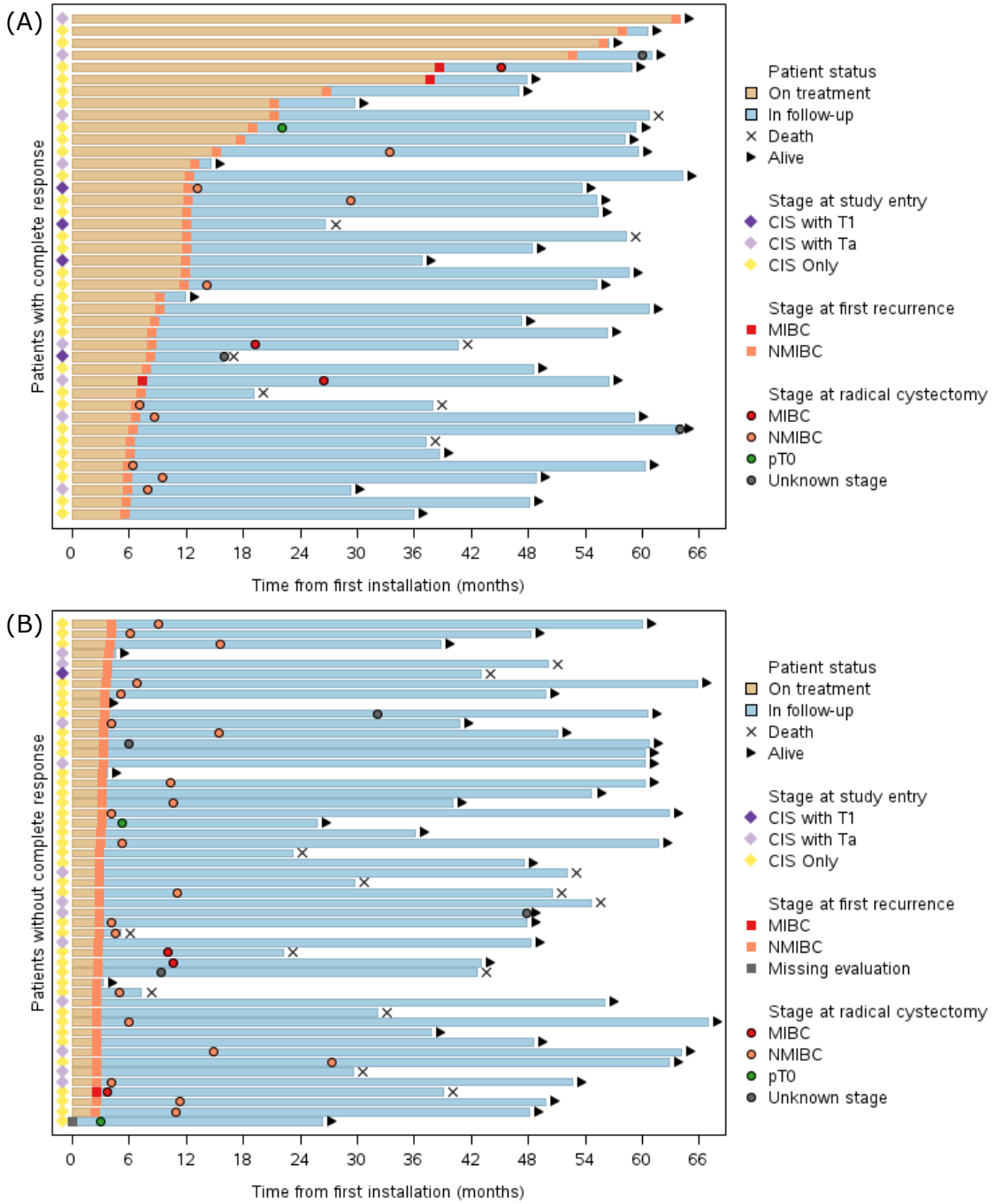
Post-hoc subgroup analyses – Figure 9 shows a post-hoc subgroup analysis for the secondary endpoint of DoCR.

Figure 9. Kaplan-Meier Plot of Durability of Complete Response in CIS patients by (A) Sex (B) Age Group (C) Race (D) Time from Initial Diagnosis of Bladder Cancer (E) Subgroups With and Without Concomitant Ta/T1 – Study CS-003 (Efficacy Analysis Set)



Abbreviations: BC, bladder cancer.
Note: Durability of complete response is defined as the time from first observed complete response to documented treatment failure defined as documented recurrence of high-grade disease, including muscle-invasive disease progression and death due to any cause.
Patients without treatment failures are censored at the last disease assessment not showing high-grade disease recurrence.
Rate of sustained complete response is a Kaplan-Meier estimate of the survival function at each specific time point.

Figure 10. Time from First Instillation to Death or Last Evaluable Time Point and Disease Stage at Study Entry, Recurrence and Cystectomy in Patients with CIS with or without Papillary Tumours
(A) Patients with Initial CR who had Subsequently Evidence of Recurrence or Progression (N=42)
(B) Patients without an Initial CR (N=51) – Study CS-003 (Safety Analysis Set)



Abbreviations: CIS, Carcinoma In Situ; CR, complete response; NMIBC, non-muscle invasive bladder cancer; MIBC, muscle-invasive bladder cancer; T, tumour

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 23. Summary of Efficacy for trial CS-003

Title: A Phase III, Open Label Study to Evaluate the Safety and Efficacy of INSTILADRIN® (rAd-IFN/Syn3) Administered Intravesically to Patients with High-Grade, BCG-Unresponsive Non-Muscle Invasive Bladder Cancer (NMIBC)			
Study identifier	Protocol number: rAd-IFN-CS-003 NCT number: NCT02773849		
Design	A pivotal, multi-centre, open-label, single-arm trial		
	Duration of main phase: Duration of long-term follow-up:	Initial 12-month period Up to 5-year follow-up from first instillation of nadofaragene firadenovec	
Hypothesis	Superiority over a pre-specified minimal important efficacy threshold of 27% for complete response		
Treatment group	Nadofaragene firadenovec 3×10^{11} vp/mL (75 mL)	157 patients were enrolled and received at least 1 instillation of nadofaragene firadenovec Treatment was administered every 3 months up to 5 years from first instillation	
Disease cohorts	Carcinoma in situ (CIS) cohort	107 patients with CIS (± Ta/T1) were enrolled and treated	
	Papillary disease (PD) cohort	50 patients with high-grade Ta/T1 (without CIS) were enrolled and treated	
Endpoints and definitions	Primary: Complete response (CR) at any time in patients with CIS	CR	Binary endpoint of having achieved CR assessed at any time after first instillation of nadofaragene firadenovec
	Key secondary: Durability of CR (DoCR) in patients with CIS	DoCR	Time-to-event (TTE) endpoint defined as time (in months) from first achieved CR to recurrence of high-grade disease, muscle-invasive disease progression, or death due to any cause, whichever occurs first
Database lock	Main phase: 21 June 2019; Long-term follow-up: 26 June 2023		
Results and Analysis			
Analysis description	**Primary analysis: CR at any time in patients with CIS**		
Analysis population (disease cohort) and time point description	Efficacy analysis set (CIS patients) *The efficacy analysis set is a modified full analysis set defined as all treated patients who are BCG-unresponsive high-grade NMIBC* Month 3 (from first instillation of nadofaragene firadenovec)		
Descriptive statistics and estimate variability	Treatment group	Nadofaragene firadenovec 3×10^{11} vp/mL (75 mL)	
	Disease cohort	CIS	
	Number of patients	103	
	CR Rate [95% confidence interval (CI)]	 53.4% [43.3, 63.3]	

Notes	For the primary endpoint to be successful, the lower limit of the 95% CI must be >27%	
Analysis description	**Key secondary analysis: Durability of CR in patients with CIS**	
Analysis population (disease cohort) and time point description	Efficacy analysis set (CIS patients with CR) From first achieved CR and up to Month 57	
Descriptive statistics and estimate variability	Treatment group	Nadofaragene firadenovec 3×10^{11} vp/mL (75 mL)
	Disease cohort	CIS patients with CR
	Number of patients	55
	DoCR (months) Median [95% CI]	 9.72 [9.17, 23.95]

2.6.5.3. Clinical studies in special populations

Due to the intravesical route of administration, local mechanism of action, and low systemic exposure of nadofaragene firadenovec, no formal trials have been conducted to assess efficacy in patients with hepatic or renal impairment.
Similarly, as NMIBC primarily occurs in the elderly, no trials in the paediatric population have been conducted. A product-specific paediatric waiver was agreed in Apr 2021 for the condition of malignant bladder neoplasms (P/0149/2021).

Table 24. Clinical Studies in Special Populations

	Controlled Trials	Non-controlled Trials
Renal impairment* patients (Subjects number /total number)	0	0
Hepatic impairment** patients (Subjects number /total number)	0	0
Paediatric patients <18 years (Subjects number /total number)	0	0
Age 65-74 (Subjects number /total number)	19/57	64/157
Age 75-84 (Subjects number /total number)	18/57	47/157
Age 85+ (Subjects number /total number)	4/57	8/157
Other (age <65) (Subjects number /total number)	16/57	38/157

* Renal impairment is defined as having CKD Stage 3b, 4 or 5 (KDIGO definition)
** Hepatic impairment is defined as having Child-Pugh score B or C
Note: Data from completed investigational clinical trials included in the dataset: rAd-IFN-CS-003, rAd-IFN-CS-002, and P03816. rAd-IFN-CS-002 and P03816 were controlled based on dose regimen.
A phase 1b trial (trial id: 2009 0938) included an additional 7 patients; 2 in the age range 65-74 and, 5 in the age range 75-84.

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable

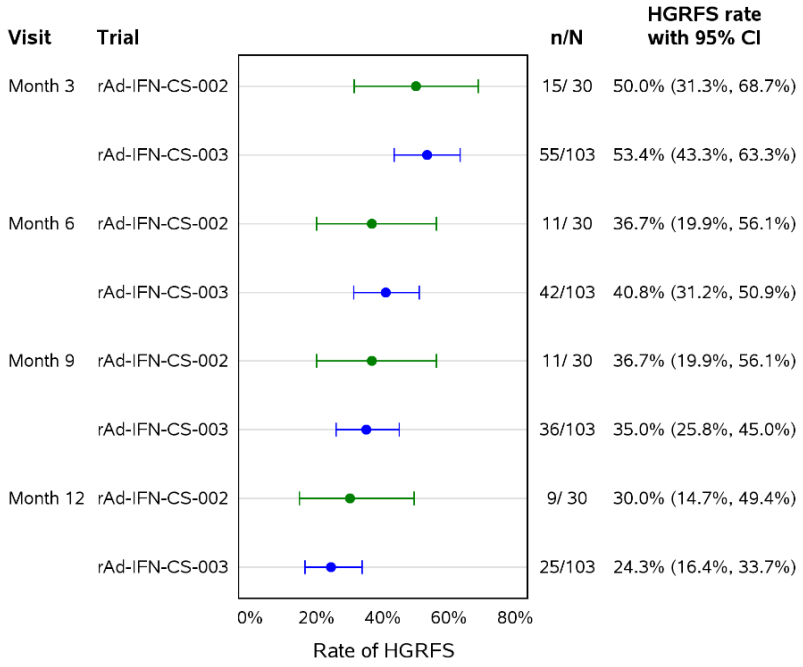
2.6.5.5. Analysis performed across trials

A post-hoc subgroup analysis of incidence of HG RFS by disease cohort (CIS [n=30] and papillary disease [n=10]) was conducted for the phase 2 Study CS-002 for a comparison of the same endpoints in the pivotal study CS-003. Of note, HG RFS at Month 3 for the CIS cohort in study CS-002 corresponds to the primary endpoint of complete response in study CS-003 as CIS per definition is always HG. When interpreting the results for both trials, it is considered that there are no substantial differences in the design of the initial 12-month treatment period or in the patient populations studied (taking into account the evolution in terminology regarding BCG failure as described in Table 25).

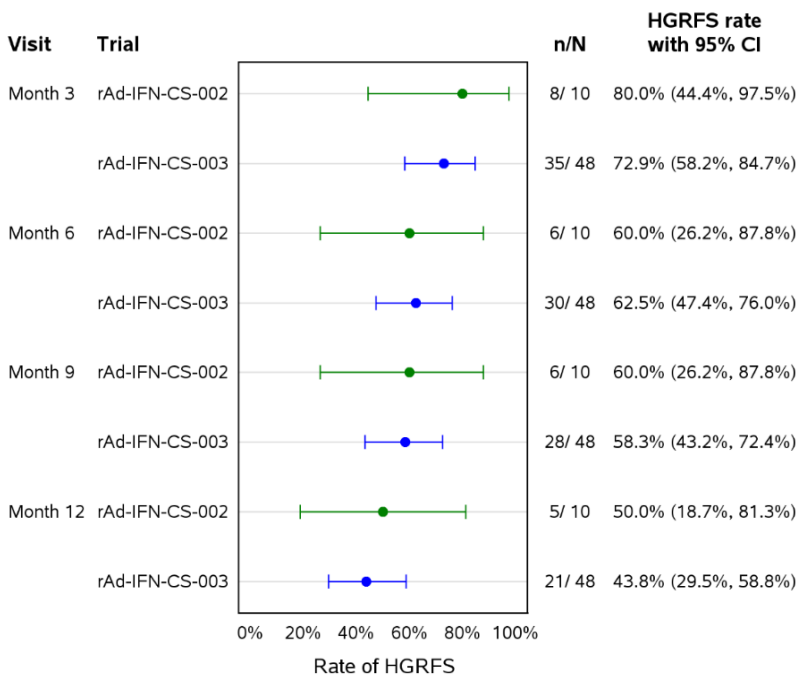
As indicated by the forest plot in Figure 11, the estimated rates of achieving HG RFS at different time points (Months 3, 6, 9, and 12) and by disease cohort (CIS and papillary disease) are consistent across the pivotal study CS-003 and the supportive study CS-002.

Figure 11. Comparison of Supportive and Pivotal Study Data on the Incidence of High-Grade Recurrence-Free Survival in Patients with CIS (A) and Patients with Papillary Disease (B) – Studies CS-002 and CS-003

A:



B:



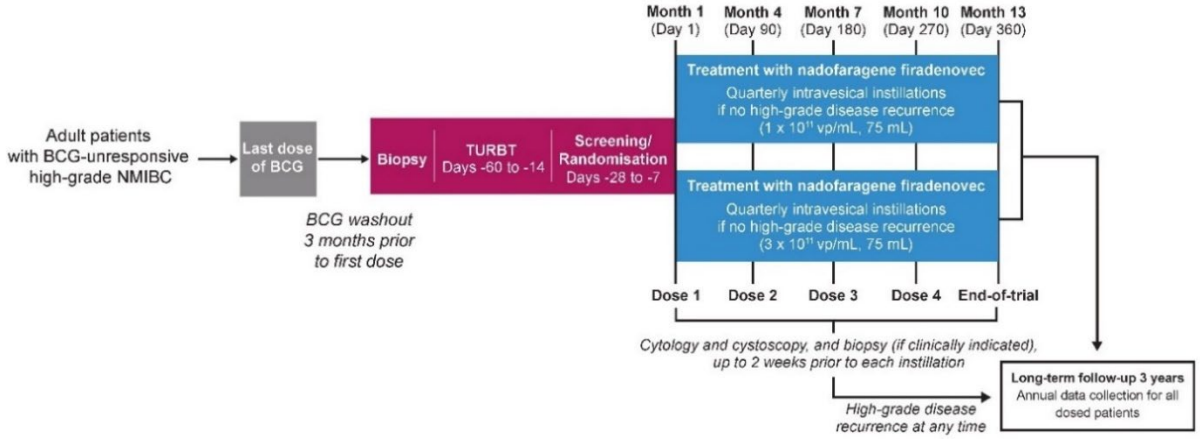
Abbreviations: CI = confidence interval; CIS = carcinoma in situ; HGRFS = high-grade recurrence-free survival.
CIS = CIS only, Ta + CIS, and T1 + CIS.
The rate of patients achieving HGRFS was calculated using N as the denominator, where N was the number of patients in the efficacy analysis set. CIs are 95% exact binomial CIs.

2.6.5.6. Supportive study(ies)

- **Phase 2 study rAd-IFN-CS-002 (study CS-002)**

Study design – The phase 2 study rAd-IFN-CS-002 (study CS-002; Shore et al. J Clin Oncol. 2017) was a randomised, open-label study to evaluate the safety and efficacy of nadofaragene firadenovec in patients with HG BCG-refractory or relapsed NMIBC. A schematic overview of the trial is provided in Figure 12. Final efficacy evaluation was performed at the visit scheduled at 12 months from first instillation (Day 360) and all patients who had not previously been discontinued from trial treatment underwent cystoscopy, cytology, and a mandatory biopsy. The trial was complete when all patients had either completed the Day 360 assessments or had been discontinued from trial treatment and undergone safety assessments.
All patients who had received at least 1 instillation of nadofaragene firadenovec as part of study CS-002 were included in a 3 year long-term follow-up to collect survival data and information regarding cystectomy and muscle-invasive disease progression (trial rAd-IFN-CS-002 LTFU).

Figure 12. Overview of Phase 2 Study CS-002



Abbreviations: BCG: Bacillus Calmette-Guérin; NMIBC: non-muscle-invasive bladder cancer; TURBT: transurethral resection of bladder tumour; vp: viral particles.
Note: The long-term follow-up was a separate trial (Trial rAD-IFN-CS-002 LTFU).
Note: Efficacy assessment visits to evaluate the recurrence of high-grade disease occurred 14 days prior to each dosing visit and is referred to as Month 3, Month 6, Month 9 and Month 12.

Eligibility criteria – Study CS-002 included male and female patients aged 18 years or more with HG BCG-refractory or relapsed NMIBC (CIS only, CIS and Ta/T1 tumours, or HG Ta/T1). Patients were required to have an absence of concomitant UTUC and adequate laboratory values.
BCG-refractory was defined as failure to achieve a disease-free state at 6 months after adequate induction of BCG therapy with either maintenance or re-induction at 3 months. Adequate induction was defined as a minimum of 5 out of 6 induction doses and adequate maintenance was defined as a minimum of 2 out of 3 doses of treatment.
BCG relapse was defined as recurrence within 1 year after complete response to BCG treatment.

Due to an evolution in terminology, there was a slight difference with respect to the definition of BCG failure in study CS-002 (BCG-refractory or relapsed) compared with the definition of BCG-unresponsive used in the pivotal study CS-003 (Table 25). The latter being based on the proposal of Lerner *et al.* in 2015 (Lerner et al. Bladder Cancer. 2015), which was also reflected in the EU clinical guidelines (EAU NMIBC Guidelines; ESMO Guideline).

Table 25. Patient Population in Clinical Trials with Nadofaragene Firadenovec

Trial ID	Disease	Prior BCG Therapy

rAd-IFN-CS-002
 	
Phase 2
	High-grade non-invasive papillary carcinomas (Ta) and patients with high-grade tumours that invade sub-epithelial connective tissue (T1), or CIS only, or CIS and Ta or T1 tumours	• BCG-refractory or -relapsed:
		  • Refractory was defined as failure to achieve a disease-free state at 6 months after adequate induction of BCG therapy with either maintenance or re-induction at 3 months
		Adequate induction was defined as a minimum of 5 out of 6 induction doses and adequate maintenance is defined as a minimum of 2 out of 3 doses of treatment
		  ○ *Relapse was defined as recurrence within 1 year after complete response to BCG therapy*

rAd-IFN-CS-003
 	
Phase 3
	CIS only, or
	Ta/T1 high-grade disease with concomitant CIS or without concomitant CIS
		• BCG-unresponsive - includes patients:
		  ○ Unlikely to benefit from and who will not be receiving further intravesical BCG
		  ○ Who did not respond to BCG therapy and have a persistent high-grade recurrence within 12 months after BCG was initiated
		  ○ Who despite an initial complete response to BCG, relapse with CIS within 12 months of their last intravesical treatment with BCG or *relapse with high-grade Ta/T1 NMIBC within 6 months of their last intravesical treatment with BCG*
		• Prior BCG therapy requirements:
		  ○ Receipt of at least 2 previous courses of BCG within a 12-month period – defined as at least 5 of 6 induction BCG instillations and at least 2 out of 3 instillations of maintenance BCG, or at least 2 of 6 instillations of a second induction course, where maintenance BCG is not given
		Exception: those who have T1 high-grade disease at first evaluation after induction BCG alone (at least 5 of 6 doses) may qualify in the absence of disease progression
		  ○ At the time of tumour recurrence, patients with CIS alone or high-grade Ta/T1 with CIS should be within 12 months of last exposure to BCG and *patients with high-grade Ta/T1 without CIS should be within 6 months of last exposure to BCG*
		  ○ No maximum limit to the amount of BCG administered

Abbreviations: BCG = Bacillus Calmette-Guérin; CIS = carcinoma in situ; NMIBC = non-muscle-invasive bladder cancer.
Note: The slight difference between the definition of BCG failure in both studies is highlighted in yellow.

Statistical methods – The primary efficacy endpoint was the incidence of HG recurrence-free survival (RFS) at 12 months. A patient had achieved HG RFS if at 12 months the patient was alive and a cystoscopic examination showed either (a) no evidence of CIS, Ta or T1 lesions or (b) showed evidence of Ta or T1 lesions which on biopsy were evaluated as LG. The proportion of patients achieving HG RFS at 12 months was reported for each dose group, together with an exact 90% confidence interval for the proportion. The HG RFS rate at 3, 6 and 9 months was reported similarly. Duration of HG grade RFS were summarized by KM methods. It was planned to enrol approximately 40 patients, to achieve 20 evaluable patients in each of the two treatment groups. A group size of 20 patients was sufficient to give an 80% probability of rejecting a reference HG RFS rate of 10% with an exact 5% one-sided test when the true HG RFS rate was 35%.

Study population – A total of 43 patients were randomised 1:1 to receive an intravesical instillation with nadofaragene firadenovec at a dose of 1×10^{11} vp/mL or 3×10^{11} vp/mL [75 mL], and 40 patients received at least 1 intravesical instillation (21 patients in the 1×10^{11} vp/mL dose group and 19 in the 3×10^{11} vp/mL dose group).
The median age of these 40 patients at trial inclusion was 70.5 years (range 52 to 91 years). Most of

the patients were male (82.5%) and White (95.0%). Most patients had an ECOG performance status of 0 (85.0%), and 15.0% had an ECOG status of 1. The key disease history for these patients is presented in Table 26.

Table 26. Key Disease History – Study CS-002

	1×10^{11} vp/mL (N=21)	3×10^{11} vp/mL (N=19)	Total (N=40)
Current status, n (%)			
Relapsed	10 (47.6)	9 (47.4)	19 (47.5)
Refractory	11 (52.4)	10 (52.6)	21 (52.5)
Primary tumour classification, n (%)			
T1 and CIS	2 (9.5)	3 (15.8)	5 (12.5)
T1	2 (9.5)	4 (21.1)	6 (15.0)
Ta and CIS	3 (14.3)	1 (5.3)	4 (10.0)
Ta	2 (9.5)	2 (10.5)	4 (10.0)
CIS	12 (57.1)	9 (47.4)	21 (52.5)

Abbreviations: CIS = carcinoma in situ; CTR = clinical trial report; Max = maximum; Min = minimum; N = number of patients; SD = standard deviation; vp = viral particles.
Percentage was calculated using the number of patients in the column heading as the denominator.

A total of 17 (42.5%) patients completed the 12-month trial period (8 patients in the 1×10^{11} vp/mL dose group and 9 in the 3×10^{11} vp/mL dose group).

Efficacy results – A total of 14 (35.0%) patients were HG recurrence-free at Month 12 (Table 27). The CI shows that the null hypothesis of a HG RFS rate of less or equal to 10% (used for the sample size calculation) can be rejected on a 5% 1-sided level of significance. The incidence was comparable between dose groups, with 7 patients (33.3%; 90% CI: 16.8, 53.6) in the 1×10^{11} vp/mL dose group and 7 patients (36.8%; 90% CI: 18.8, 58.2) in the 3×10^{11} vp/mL dose group.

Table 27. Incidence of High-Grade Recurrence-Free Survival by Dose Group – Study CS-002

	1×10^{11} vp/mL (N=21)		3×10^{11} vp/mL (N=19)		Total (N=40)	
	n (%)	90% CI	n (%)	90% CI	n (%)	90% CI
Patients who achieved high-grade recurrence-free survival at						
Month 3	10 (47.6)	[28.6, 67.2]	13 (68.4)	[47.0, 85.3]	23 (57.5)	[43.3, 70.8]
Month 6	8 (38.1)	[20.6, 58.3]	9 (47.4)	[27.4, 68.0]	17 (42.5)	[29.2, 56.7]
Month 9	8 (38.1)	[20.6, 58.3]	9 (47.4)	[27.4, 68.0]	17 (42.5)	[29.2, 56.7]
Month 12[a]	7 (33.3)	[16.8, 53.6]	7 (36.8)	[18.8, 58.2]	14 (35.0)	[22.6, 49.2]

Abbreviations: CI = confidence interval; N = number of patients; vp = viral particles.
Note: Percentage was calculated using the number of patients in the column heading as the denominator. 90% CIs are based on the exact binomial method.
a) Primary endpoint.

At all time points, the incidence of HG RFS was numerically higher in the 3×10^{11} vp/mL dose group compared with the 1×10^{11} vp/mL dose group (Table 27).

A post-hoc analysis of the incidence of HG RFS by disease cohort (CIS with or without papillary disease [n=30] vs papillary disease only [n=10]) showed that of the first cohort 15 patients (50.0%) with CIS with or without papillary disease had achieved HG RFS at Month 3.

A total of 25 patients had HG recurrence of disease during the study. The overall median time to HG recurrence was 6.51 months (90% CI: 3.52, 12.78). The time to recurrence was numerically higher in the 3×10^{11} vp/mL dose group with 11.73 months (90% CI: 5.88, NE) compared to the 1×10^{11} vp/mL dose group with 3.52 months (90% CI: 3.02, 12.78).

- **Bladder-sparing treatments for BCG unresponsive NMIBC: A systematic literature review and meta-analysis**

A systematic literature review and meta-analysis of bladder sparing treatments for BCG-unresponsive NMIBC has been provided. For brevity, only the 'Clinical question' and the 'Overall conclusion' of this systematic literature review are provided below.

Clinical question

The primary objective of this systematic literature review was to identify and summarize data on the clinical efficacy of interventions reported in published studies involving patients with BCG-unresponsive high-risk NMIBC, defined as HG Ta; all T1 and/or CIS.

In addition, the review explored the following research objectives:

- To evaluate the clinical efficacy of treatments in studies that adhered to the guidelines for defining BCG-unresponsiveness.
- To assess the clinical efficacy of bladder-sparing therapies in BCG-unresponsive NMIBC by pre-defined subgroups (cancer staging and the number of prior BCG treatments).
- To compare the efficacy outcomes of bladder-sparing treatments for BCG-unresponsive NMIBC between prospective and retrospective studies.

Overall conclusion

The result of this systematic review highlights the difficulty in selecting alternative bladder-sparing treatments due to the lack of robust clinical data for this patient population. Most studies in the category of "≥2 prior courses of BCG, patient populations with ≥50% CIS, and adherence to the definition of BCG-unresponsiveness" were small and experimental, and included therapies lacking established benefit-risk profiles. Only two of the included therapies, nadofaragene firadenovec (Adstiladrin), and ALT-803 (Anktiva), have established benefit-risk profiles and are approved for BCG-unresponsive disease in the european setting.

The investigation into emerging therapies for BCG-unresponsive NMIBC has highlighted the challenges in making accurate comparisons of treatment efficacy. While single-arm trials supported by the FDA are necessary for testing new therapies in BCG-unresponsive NMIBC, they introduce a risk of bias and pose challenges for comparing treatment efficacy due to patient heterogeneity and variations in study designs. Broader inclusion criteria may improve trial enrolment but can lead to inconsistent clinical outcomes, especially when compared to the stricter FDA definition of BCG-unresponsive NMIBC. Additionally, differences in cancer staging, particularly the proportion of CIS patients, significantly affect perceived treatment efficacy. Therefore, treatment comparisons should be made cautiously, especially in high-risk populations.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

It is acceptable that all clinical studies with nadofaragene firadenovec were conducted in the US (*Table 1*), as US and EU patients with BCG-unresponsive NMIBC are considered similar regarding patient and disease characteristics and treatment landscape.

No dedicated dose response study(ies) have been conducted and the posology used in the pivotal study CS-003 is based on data from the non-clinical and clinical development programme. Although certainly not ideal, this can be acceptable.

In the supportive study CS-002, no clear differences in relation to safety were identified between the 1×10^{11} vp/mL (75 mL) and the 3×10^{11} vp/mL (75 mL) dose levels (Table 42). In conclusion the efficacy data reported for BCG-unresponsive NMIBC patients treated every 3 months with nadofaragene firadenovec (3×10^{11} vp/mL [75 mL]) demonstrated clinically relevant efficacy with an acceptable safety profile that is manageable in the urology setting. Intravesical instillation of nadofaragene firadenovec at a dose level of 3×10^{11} vp/mL in a dose volume of 75 mL, administered every 3 months, is therefore the proposed dosage regimen for commercialisation.

Design of pivotal study – The pivotal study CS-003 (NCT02773849; Boorjian et al. Lancet Oncol. 2021) is a single-arm trial (SAT).

It is acknowledged that SATs can be considered appropriate for assessment of therapies for patients with BCG-unresponsive disease (CIS *with or without* resected papillary disease) because no effective medical therapies were available at the time of study design and the only alternative was radical cystectomy (RC).
A randomized trial using an investigator-choice comparator could, however, also be feasible for this population (Kamat et al. J Clin Oncol. 2016), with as investigator-choice comparator off-label bladder-sparing options such as intravesical immunotherapy, intravesical chemotherapy (single-agent or combination therapy), device-assisted therapy, or combination chemo-immunotherapy. The EAU considers these off-label options as oncologically inferior compared to RC, though, rating the evidence supporting their efficacy as 'weak' (EAU NMIBC Guidelines).
A full justification why the Applicant chose a SAT design was not provided, nevertheless considering the choice of the study population, the primary objective/endpoint and the observed results, the single-arm design is deemed acceptable. However, the clinical efficacy (and safety) data submitted are not considered comprehensive.

Study population – The inclusion and exclusion criteria are considered acceptable, even though there were some changes in these criteria during the course of the study. It is acknowledged that the used definition of BCG-unresponsive disease in the end aligned with the guideline definition (EAU NMIBC Guidelines; ESMO Guideline).

It is noted that the study enrolled patients with CIS *with or without* resected HG Ta/T1 (papillary) disease, and patients with resected HG Ta/T1 (papillary) disease only. This has implications for the efficacy objectives/endpoints, as mentioned below.

The posology used in study CS-003, i.e. 3×10^{11} vp/mL, 75 mL every 3 months, can be acceptable. The recommendation for the duration of treatment included in section 4.2 of the SmPC is in line with this posology and it is clearly stated that treatment with nadofaragene firadenovec should be discontinued if there is evidence of HG recurrence or unacceptable toxicity, and that patients' response should be measured right before the administration of the next dose to confirm that they are still eligible for treatment. For clarity, it is agreed that per the study CS-003 protocol there was not a limited treatment duration for nadofaragene firadenovec. Therefore, no recommendation to stop treatment at a certain time point is included in section 4.2 of the SmPC. This is accepted, even though less than 10 patients (in the CIS cohort) were treated beyond 48 months and there is a complete lack of data beyond 60 months.

The 3-monthly efficacy assessments with cytology and cystoscopy, and biopsy(ies) when clinically indicated are clinically validated, as they are in line with recommendations in clinical guidelines (EAU NMIBC Guidelines). These are, therefore, considered sufficient for detecting recurrences of HG NMIBC. The choice for mandatory biopsies at Month 12 is considered a strength of the study, as it limits investigator bias/assessment error.
Then again, to prevent missing recurrent disease and incorrectly defining a CR, ideally the mandatory

biopsies would have been at the same time point at which the primary endpoint was measured, i.e., at 3 months. Nevertheless, the performed study assessments are considered acceptable.

Primary objective/endpoint – The primary endpoint of CR rate in patients with CIS (with or without concomitant HG Ta/T1 papillary disease) at any time could in principle be acceptable. It is, however, noted that the primary endpoint was changed during the conduct of study CS-003. Moreover, the assessment of the primary endpoint was conducted at the first efficacy assessment visit, scheduled at 3 months. If patients did not have a CR at that time point, they were discontinued from treatment and considered non-complete responders. Therefore, the primary endpoint should in fact be interpreted as 'CR at 3 months', instead of 'CR at any time'.
According to EMA Reflection paper - Considerations on evidence from single-arm trials (EMA/CHMP/458061/2024), if after treatment intervention a 'cure' is achieved that would not be achievable without treatment, isolation of the treatment effect seems plausible. This is applicable for patients with CIS (with or without concomitant HG Ta/T1 papillary disease), as CIS cannot be resected completely and, without treatment, will persist (and progress). As such, the primary endpoint seems to be in line with the recommendations of the IBCG (Kamat et al. J Clin Oncol. 2016; Kamat et al. J Clin Oncol. 2023).

Justification for null hypothesis – Although the Applicant's rationale for the null hypothesis value of 27% can be understood, the clinical relevance of the 27% threshold remains uncertain. This will, however, not be further pursued since the observed efficacy in the CIS cohort of study CS 003 is considered clinically relevant.

The assumption (in the sample size calculation) for the true CR rate of 43.75% is that 87.5% of the efficacy observed in the phase 2 Study CS-002 is retained. Such a true CR rate of 43.75% can, indeed, be considered in the range of what was considered clinically relevant in 2016, as the IBCG recommended an initial CR rate of 50% at 6 months as a clinically meaningful magnitude of effect for patients with BCG-unresponsive CIS (Kamat et al. J Clin Oncol. 2016).

The Applicant defined intercurrent events and their handling post hoc.

The primary objective estimand seems able to isolate a causal treatment effect.

The key secondary objective of durability of CR in patients with CIS (with or without HG Ta/T1 papillary disease) is agreed. The clinical relevance of a (complete) response is dependent on its duration and, therefore, (complete) response duration should always be reported when reporting a (complete) response rate endpoint.
As the course of BCG-unresponsive CIS is predictable, a large treatment effect on CR *and* DoCR in patients with CIS (with or without HG Ta/T1 papillary disease) might be considered to translate in clinical benefit as per the EMA Guideline on the clinical evaluation of anticancer medicinal products (EMA/CHMP/205/95 Rev. 6).

In combination with the primary objective estimand (CR), DoCR is also able to isolate a treatment effect (in those patients who had reached a CR) and considered acceptable.

Secondary objectives– For patients with HG Ta/T1 papillary disease (without CIS), the endpoint HG RFS is the 'primary' efficacy outcome since they are not part of the primary endpoint analysis population (see above). The clinical relevance of this endpoint is, however, uncertain since patients with papillary disease only (without CIS) do not have active disease at baseline, since this was resected before enrolment, and treatment is thus administered in the adjuvant setting to prevent recurrence. For assessing efficacy in the adjuvant NMIBC setting, a RCT design using RFS is, however, required since RFS cannot isolate a treatment effect in a SAT being a time-to-event endpoint.

The above critique is applicable to all other secondary endpoints, i.e., their clinical relevance is uncertain since the interpretation of their results is severely hampered by the SAT design. Consequently, the results of these other secondary endpoints can provide some support/context for the efficacy of nadofaragene firadenovec but are not considered essential for the benefit-risk (B/R) assessment.

Additionally, although results of cystectomy-related endpoints are considered relevant in terms of the benefit that patients could obtain from treatment, it is also noted that these endpoints are highly subjective, since they are impacted by the patient's willingness to undergo a cystectomy. Therefore, although informative, they cannot be considered as conclusive.
Regarding OS, this is not the most relevant endpoint in NMIBC since there is a curative option (RC) which can be conducted prior to disease progression to MIBC (and thus the disease becoming lethal).

The SAP is considered adequate to support the testing of the hypothesis.

The analysis methods for the proportions (CR, HG RFS as milestones) and time-to-event endpoints are standard and, therefore, agreed.

Missing data in the CR and thus in the duration of CR were handled conservatively (i.e., as non-response), which is agreed. Missing data to establish the presence of recurrence or not in HG RFS at a visit were handled as recurrence except if the next visit showed no recurrence and no other treatment than nadofaragene firadenovec had been applied. This is considered reasonable.

The analysis sets were based on patients who received at least one instillation, and therefore, not an intention-to-treat. However, all enrolled patients had also received at least one instillation.

There was no adjustment for multiplicity and, therefore, no type I error control for (any of) the secondary endpoints. Moreover, it may still be challenged whether type I error control was retained for the primary endpoint, given that it was changed during the trial (although explained to be based on FDA request). However, given that the original primary endpoint was also met, this issue is not further relevant.

Efficacy data and additional analyses

With protocol version 5.0 (dated 29 January 2018), there were significant changes to the design of study CS-003 during its conduct, i.e., a change to the primary endpoint, along with a resulting change to the patient population on which the primary endpoint analysis was based, and the final sample size of the trial. Plus, a new (key) secondary endpoint was added and there were revisions to other secondary endpoints (Table 6).

The changes were implemented based on a request by the FDA and were aimed to align the study objectives with the FDA's 2018 guidance. Such major changes to an ongoing study are considered as an uncertainty and to mitigate the impact on the final data the Applicant performed post-hoc efficacy analyses for the 77 CIS patients enrolled prior to the protocol v5.0 update vs the 26 CIS patients after. The results of the performed post-hoc efficacy analyses do not raise reasons for concern as they indicate that the changes were not driven by available internal data.

Nevertheless, such significant changes to a pivotal study during its conduct warrant independent replication of the study results. This is particularly relevant since the current submission is based on a single pivotal study which has to be compelling with respect to internal and external validity, clinical relevance, statistical significance, data quality, and internal consistency as per the EMA Points to consider on application with one pivotal study (CPMP/EWP/2330/99). In the context of a conditional marketing authorisation the applicant will submit as specific obligation the results of the ongoing study ABLE-22 to further consolidate the benefit-risk of the product.

It is agreed that the 6 patients (four from the CIS cohort and two from the papillary disease cohort) who did not meet the protocol definition of HG BCG-unresponsive NMIBC were excluded from the Efficacy Analysis Set. The CIS cohort Efficacy Analysis Set is thus comprised of 107 – 4 = 103 patients.

Overall, the reported protocol deviations are not considered to have any relevant impact on the results of study CS-003 and/or the interpretability.

The main phase of study CS-003 (initial 12-month period; Figure 2) is considered key for the B/R assessment, one of the reasons being the mandatory biopsies at Month 12 (as these limit investigator bias/assessment error).

Overall, it is considered that the study population (i.e., the CIS cohort Efficacy Analysis Set of n=103) sufficiently reflects the final agreed indication target population.

The primary endpoint of CR rate in patients with CIS (with or without concomitant HG Ta/T1 papillary disease) at any time was met, noting that this should in fact be interpreted as 'CR at 3 months'. The achieved incidence of CR at 3 months was 53.4% (n=55) and the 95% CI (43.3, 63.3) exceeded the protocol-specified null hypothesis of 27%, which is considered clinically relevant and in line with the IBCG which seem to recommend an initial CR rate of 50% either at 3 or 6 months as a clinically meaningful magnitude of effect for patients with BCG-unresponsive CIS (Kamat et al. J Clin Oncol. 2016; Kamat et al. J Clin Oncol. 2023).

The durability of the CRs in patients with CIS (with or without concomitant HG Ta/T1 papillary disease) reports a median duration of 9.72 months with a 3 and 61 range (Table 13 and Figure 4).

Overall, the observed efficacy in the CIS cohort of study CS 003 is considered clinically relevant, with an achieved CR rate of 53.4% at 3 months in combination with a median duration of these CRs of 9.7 months.

Notably, the originally planned primary endpoint of Study CS-003 (Table 6) was also met since the incidence of HG RFS at Month 12 was 30.5% (95% CI: 23.2, 38.5) for both cohorts combined, with the lower bound of the 95% CI exceeding the pre-specified limit of 18% (Table 15).

It is acknowledged that the presence of low-grade recurrence would not affect the decision regarding cystectomy because these patients would endure another TURBT. For completeness, the Applicant clarified that no CIS patients had a low-grade recurrence after having achieved a CR at Month 3, which is considered reassuring.

For the papillary disease cohort (and both cohorts combined), however, it cannot be concluded that the observed incidence of HG RFS demonstrates an effect, as the interpretation of the results is problematic due to its time-to-event nature.

– The interpretability of the incidence of and time to cystectomy endpoint is severely hampered by the SAT design of study CS-003. For example, other studies in this disease setting, (Balar et al. Lancet Oncol. 2021; van der Heijden et al. J Clin Oncol. 2022 [abstract]), only enrolled patients who were ineligible for or declined to undergo radical cystectomy. This was not an inclusion criterion for study CS-003. Such patients may, nevertheless, have been enrolled and thus included in the Efficacy Analysis Set, which would have an impact on this endpoint.

Summarized pathologic data for patients who underwent cystectomy were provided. Cystectomy pathology reports were retrospectively collected for CIS patients with a 36-month cut-off date. This is acceptable. Of the 107 CIS patients in the Safety Analysis Set, 46 (43%) patients underwent cystectomy, including 16 patients that initially achieved CR at Month 3 and 30 patients that did not (Figure 10). Pathologic data were available for 39 of these 46 patients and data were missing in 7 patients (Table 19). The data are further discussed below.

Of the 44 (42.7%) patients in the Efficacy Analysis Set who underwent a cystectomy, there were 15 patients who achieved a CR at Month 3 and 29 patients who did not achieve a CR at Month 3.

The interpretability of overall survival (OS) is severely hampered by the SAT design of study CS-003. There were no deaths during the treatment phase of study CS-003: all reported deaths occurred during the follow-up phase of the study.

Unfortunately, progression to muscle-invasive disease at the end of the 5-year follow-up was not collected as part of the trial conduct.

It was clarified that information on subsequent anticancer therapies (after disease recurrence) was not systematically collected per protocol.

Due to its SAT design, study CS-003 cannot determine the risks associated with patients who are eligible for RC, but choose to defer surgery in favour of bladder-preserving treatment with nadofaragene firadenovec. Consequently, the study cannot address the concern that postponing RC may allow the disease to progress to a stage at which curative surgery is no longer feasible, thereby causing patients to lose the window of opportunity for potentially curative treatment and leading to a possible shorter survival.

This, therefore, remains an uncertainty. Of the 107 patients with CIS treated with Adstiladrin in study CS-003, 8 (7.5%) progressed to muscle invasive (pT2 or greater) and/or lymph node metastatic (pN+) bladder cancer. Four patients had progression during treatment at time of first recurrence with a median time from first dose to progression of 686 days (range: 76-1178). The remaining 4 patients were upstaged at the time of cystectomy with a median time from persistence or recurrence of CIS to cystectomy of 235 days (range: 64-335). Therefore, if patients with CIS who are eligible for cystectomy do not have a complete response to treatment after 3 months or if CIS recurs, cystectomy should be considered. The risk of developing muscle invasive or metastatic bladder cancer increases the longer cystectomy is delayed in the presence of persisting CIS. The EAU NMIBC Guidelines also warn that in patients with BCG-unresponsive tumours a delay in RC may lead to decreased disease-specific survival (Raj et al. J Urol. 2007). In conclusion, the Applicant is proposing an indication covering also patients who are eligible for RC. To inform a decision on whether to undergo RC (yet), a reliable and sufficiently precise estimate of the risk of disease progression to an incurable state when delaying RC, is required. An appropriately worded warning on the risk of muscle invasive and metastatic bladder cancer with delayed cystectomy has been included in section 4.4 of the SmPC.

Results of subgroup analyses were provided for the primary endpoint of incidence of CR in the CIS cohort and it was similar for most subgroups (Table 22 and Figure 8). For example, the 54.4% incidence of CR in the subgroup of patients with CIS *only* (without concomitant papillary disease) was similar to the 50.0% incidence of CR in the subgroup of patients with CIS *with* concomitant Ta/T1 papillary disease. It is, therefore, considered acceptable to also include the latter subgroup in the indication target population which would thus comprise of patients with (HG BCG-unresponsive NMIBC with) CIS *with or without* concomitant papillary disease.
Additionally requested, post-hoc subgroup analyses for the secondary endpoint of DoCR for the CIS cohort (Figure 9) also demonstrated generally consistent results, with no relevant differences needing further investigation.

The rationale for not conducting any formal studies in patients with hepatic or renal impairment due to local administration, local mechanism of action and low systemic exposure is agreed. As well considering that NMIBC primarily occurs in the elderly, no trials in the paediatric population have been conducted. The lack of above specific studies is acceptable. Relevant wording on these aspects has been implemented in section 4.2 of the SmPC.

Supportive phase 2 Study CS-002 (Shore et al. J Clin Oncol. 2017)

Similar to the pivotal study CS-003, study CS-002 has a SAT design, and the limitations of this design discussed for study CS-003 are thus also applicable to study CS-002.

An important limitation of study CS-002 for providing support for study CS-003 is that the primary endpoint of this study (HG RFS at 12 months for all patients) is different from the primary endpoint of study CS-003 (CR at any time for patients with CIS [with or without papillary disease]). The primary endpoint of study CS-002 is thus not aligned with the 2016 IBCG recommendations (Kamat et al. J Clin Oncol. 2016), which makes it very difficult to conclude on a treatment effect for nadofaragene firadenovec from the study results.
Moreover, two nadofaragene firadenovec dose levels were used and less than half of the study patients received the proposed 3×10^{11} vp/mL dose level, i.e., 19 of 40 patients (Table 26).

In conclusion, the results of study CS-002 could - at most – be considered to provide some support for the results of the pivotal study CS-003, but this support is limited to the 13 patients with CIS in study CS-002 who received the proposed nadofaragene firadenovec dose level of 3×10^{11} vp/mL (Table 26).

It is acknowledged that the literature-based contextualisation of the clinical data, focusing on CIS and complete response, could be helpful, although it is considered supportive evidence at most.

Wording of the indication

The initially proposed indication wording was not considered acceptable, as the population included in such indication was broader than the one enrolled in the study CS-003 primary analysis population of patients with CIS with or without concomitant HG Ta/T1 papillary disease. As a consequence the indication is, restricted to the study CS-003 primary analysis population of patients with (BCG-unresponsive NMIBC with) CIS with or without concomitant papillary disease. Notably, as CIS is always HG, it was no longer necessary to specify this in the indication.

In addition "*as monotherapy*" was requested to be added to the indication wording, in line with current EMA guidance on Wording of therapeutic indication (EMA/CHMP/483022/2019).

In conclusion, the indication wording was amended to:

> "*ADSTILADRIN is indicated as monotherapy for the treatment of adult patients with Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumours.*".

Healthcare provider engagement

Upon invitation from the CHMP, the EORTC shared their healthcare professional experience of and/or relevant perspectives on the treatment of adult patients with HG BCG-unresponsive NMIBC (the originally proposed indication), which they summarized as follows:

- "*RC remains the most effective treatment for HG, BCG-unresponsive NMIBC, offering the highest oncological control. However, its invasive nature, associated morbidity, and significant impact on quality of life make it unsuitable for many patients, especially the elderly or those with comorbidities.*
- *Key objectives for novel treatments:*
    - *Bladder-preserving alternatives are required for patients who cannot undergo or decline RC. These therapies must provide durable oncological control while improving quality of life and improve survival outcomes that are at least non-inferior to RC.*
    - *Optimize safety, tolerability, and ease of administration to reduce patient burden.*

- *Integrate predictive biomarkers to personalize therapy and enhance treatment effectiveness.*

- *Future Directions: Advances in innovative intravesical agents, sustained-release therapies, and cost-effective, widely accessible options are essential. A patient-centred approach, balancing oncological outcomes with quality of life, will be important for transforming the treatment landscape of HG, BCG-unresponsive NMIBC."*

The input of the EORTC is welcomed and the above summary is agreed.

Additional efficacy data needed in the context of a conditional MA

The current clinical data set is not regarded as comprehensive due to the single arm trial design of the pivotal study CS-003 and the limited number of patients. It is agreed that efficacy has been demonstrated, however durability of the CRs seems somewhat limited in the follow-up related to the provided pivotal study. Furthermore, uncertainties remain regarding the possible progression of disease while in treatment and the missing window of opportunity for the RC surgical treatment of some of the patients. The SAT design of study CS-003 severely hampers the interpretability of the (other) secondary endpoints of: incidence of HG RFS, incidence of cystectomy, and OS. Moreover, there were significant changes to the design of study CS 003 during its conduct which add additional uncertainty. This warrants independent replication of the study results. Therefore, additional data should be generated post authorisation as part of study ABLE 22, an ongoing phase 3, randomised, open-label trial to evaluate the safety and efficacy of intravesical nadofaragene firadenovec alone or in combination with chemotherapy or immunotherapy in patients with HG BCG unresponsive NMIBC (i.e., CIS with or without Ta/T1 papillary disease). The monotherapy arm 1 of the study will be the part of the study expected to provide relevant reassurance on efficacy for Adstiladrin as specific obligation.

2.6.7. Conclusions on the clinical efficacy

In the pivotal single-arm study CS-003, the primary endpoint of CR rate at any time (that should in fact be interpreted as 'CR at 3 months') was met in the primary endpoint analysis population of patients with CIS with or without concomitant HG Ta/T1 papillary disease. The achieved CR rate of 53.4% at 3 months could be considered clinically relevant, depending on the durability of the CRs. The durability of the CRs in these patients (key secondary endpoint) is acceptable, with a median duration of 9.72 months. All in all, the observed efficacy is considered clinically relevant in the clinical setting of the final indication taking also into consideration the necessary clinical decision for radical cystectomy for each patient before undergoing the treatment.

The efficacy of Adstiladrin has therefore been demonstrated, however, the current application is mainly based on the results of a pivotal SAT in a limited number of patients and in light of the uncertainties discussed, including the uncertainty on the risk of disease progression that would render patients ineligible to radicalcystectomy, the provided clinical efficacy data package is not considered comprehensive and the product is approvable under the frame of a conditional marketing authorisation.

The CAT considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

In order to confirm the efficacy and safety of Adstiladrin in adult patients with BCG-unresponsive NMIBC with CIS with or without papillary tumours, the MAH shall submit the primary clinical trial report results of study ABLE-22, an ongoing phase III randomised, multi-centre, open-label trial, including from the nadofaragene firadenovec monotherapy Arm 1: i) the CR rate at month 3 (excluding re-induction) and duration of these responses; and ii) safety data.

The CHMP endorses the CAT conclusion on clinical efficacy as described above.

2.6.8. Clinical safety

A total of 4 clinical trials has been completed evaluating the safety and efficacy of nadofaragene firadenovec (*Table 1*).

Common terminology criteria for adverse events version 4.03 (CTCAE v4.03) were used in the pivotal trial rAd-IFN-CS-003 (study CS-003). The following safety evaluations were included: Adverse events (AEs), clinical laboratory assessments, vital signs, physical examinations, resting 12-lead electrocardiogram (ECG), subjective symptomatology, and measurement of the levels of anti-adenoviral antibodies. Patients who completed the trial schedule every 3 months up to Month 24 were followed as per standard clinical practice with AE collection procedure performed every 3 months. In accordance with the protocol, all patients who received at least one dose of nadofaragene firadenovec during the treatment phase of the trial were included in a long-term follow-up where only SAEs considered as trial drug-related by the investigator to nadofaragene firadenovec were collected annually for an additional 3 years.

2.6.8.1. Patient exposure

In total, 221 patients who received nadofaragene firadenovec in any of the completed trials evaluating the safety and efficacy of nadofaragene firadenovec for the proposed indication have been included in this Clinical Summary of Safety. The overall exposure to nadofaragene firadenovec is provided in Table 28 and by the individual trials in Table 29.

Table 28. Overall Exposure to Nadofaragene Firadenovec

	Nadofaragene firadenovec dose (vp/mL)					
Number of instillations	**3×10^{9} N=3 n (%)**	**1×10^{10} N=3 n (%)**	**3×10^{10} N=3 n (%)**	**1×10^{11} N=25 n (%)**	**3×10^{11} N=187 n (%)**	**Total N=221 n (%)**
1	3 (1.4)	2 (0.9)	1 (0.5)	14 (6.3)	71 (32.1)	91 (41.2)
2	-	1 (0.5)	2 (0.9)	3 (1.4)	31 (14.0)	37 (16.7)
3	-	-	-	-	14 (6.3)	14 (6.3)
4	-	-	-	8 (3.6)	29 (13.1)	37 (16.7)
≥5	-	-	-	-	42 (19.0)	42 (19.0)
All patients	3 (1.4)	3 (1.4)	3 (1.4)	25 (11.3)	187 (84.6)	221 (100%)

Abbreviations: N = number of patients; n = number of patients with observation; % = percentage of patients with observation; vp = viral particles.

Table 29. Exposure to Nadofaragene Firadenovec by Trial

	Nadofaragene firadenovec dose (vp/mL)					
Number of instillations	**3×10^{9} N=3 n (%)**	**1×10^{10} N=3 n (%)**	**3×10^{10} N=3 n (%)**	**1×10^{11} N=25 n (%)**	**3×10^{11} N=187 n (%)**	**Total N=221 n (%)**
Trial rAd-IFN-CS-003						
1	-	-	-	-	57 (36.3)	57 (36.3)
2	-	-	-	-	24 (15.3)	24 (15.3)
3	-	-	-	-	14 (8.9)	14 (8.9)
4	-	-	-	-	20 (12.7)	20 (12.7)
≥5	-	-	-	-	42 (26.8)	42 (26.8)
All patients	-	-	-	-	157 (100.0)	157 (100.0)
Trial rAd-IFN-CS-002						
1	-	-	-	11 (27.5)	6 (15.0)	17 (42.5)
2	-	-	-	2 (5.0)	4 (10.0)	6 (15.0)

3	-	-	-	0 (0.0)	0 (0.0)	0 (0.0)
4	-	-	-	8 (20.0)	9 (22.5)	17 (42.5)
All patients	-	-	-	21 (52.5)	19 (47.5)	40 (100.0)
Trial P03816						
1	3 (17.6)	2 (11.8)	1 (5.9)	3 (17.6)	3 (17.6)	12 (70.6)
2	0 (0.0)	1 (5.9)	2 (11.8)	1 (5.9)	1 (5.9)	5 (29.4)
All patients	3 (17.6)	3 (17.6)	3 (17.6)	4 (23.5)	4 (23.5)	17 (100.0)
Trial 2009-0938						
1	-	-	-	-	5 (71.4)	5 (71.4)
2	-	-	-	-	2 (28.6)	2 (28.6)
All patients	-	-	-	-	7 (100.0)	7 (100.0)

Abbreviation: N = number of patients; n = number of patients with observation; % = percentage of patients with observation; vp = viral particles.

Patient disposition

Study CS-003

Overall, 157 patients were enrolled and treated with nadofaragene firadenovec at Month 1, Day 1: 107 patients in the CIS cohort and 50 patients in the papillary disease cohort. The patient disposition is shown in Table 30.

Table 30. Patient Disposition – Study CS-003 (All Enrolled Patients)

	CIS (N = 107) n (%)	Papillary Disease (N = 50) n (%)	Total (N = 157) n (%)
Patients enrolled (received first dose) [1]	107 (100.0)	50 (100.0)	157 (100.0)
Patients treated			
At Month 4 Day 1 [2]	63 (58.9)	35 (70.0)	98 (62.4)
At Month 7 Day 1 [3]	43 (40.2)	31 (62.0)	74 (47.1)
At Month 10 Day 1 [4]	36 (33.6)	26 (52.0)	62 (39.5)
At Month 12 [5]	23 (21.5)	17 (34.0)	40 (25.5)
At Month 15	23 (21.5)	15 (30.0)	38 (24.2)
At Month 18	22 (20.6)	13 (26.0)	35 (22.3)
At Month 21	19 (17.8)	12 (24.0)	31 (19.7)
At Month 24	18 (16.8)	9 (18.0)	27 (17.2)
At Month 27	14 (13.1)	8 (16.0)	22 (14.0)
At Month 30	15 (14.0)	11 (22.0)	26 (16.6)
At Month 33	14 (13.1)	7 (14.0)	21 (13.4)
At Month 36	13 (12.1)	10 (20.0)	23 (14.6)
At Month 39	12 (11.2)	8 (16.0)	20 (12.7)
At Month 42	10 (9.3)	7 (14.0)	17 (10.8)
At Month 45	12 (11.2)	7 (14.0)	19 (12.1)
At Month 48	10 (9.3)	6 (12.0)	16 (10.2)
At Month 51	7 (6.5)	7 (14.0)	14 (8.9)
At Month 54	7 (6.5)	7 (14.0)	14 (8.9)
At Month 57	5 (4.7)	7 (14.0)	12 (7.6)
At Month 60 [6]	1 (0.9)	1 (2.0)	2 (1.3)
Patients who completed the Month 12 efficacy assessment	36 (33.6)	27 (54.0)	63 (40.1)
Patients who did not complete the Month 12 efficacy assessment	71 (66.4)	23 (46.0)	94 (59.9)
Disease recurrence	68 (63.6)	21 (42.0)	89 (56.7)
Adverse event	2 (1.9)	1 (2.0)	3 (1.9)
Patient request	1 (0.9)	1 (2.0)	2 (1.3)
Patients who completed the 12-month treatment period [7]	33 (30.8)	25 (50.0)	58 (36.9)
Patients who did not complete the 12-month treatment period	74 (69.2)	25 (50.0)	99 (63.1)
Disease recurrence	71 (66.4)	23 (46.0)	94 (59.9)
Adverse event	2 (1.9)	1 (2.0)	3 (1.9)
Patient request	1 (0.9)	1 (2.0)	2 (1.3)
Patients who completed treatment at end of trial	5 (4.7)	6 (12.0)	11 (7.0)
Patients who discontinued trial treatment	102 (95.3)	44 (88.0)	146 (93.0)

	CIS (N = 107) n (%)	Papillary Disease (N = 50) n (%)	Total (N = 157) n (%)
Disease recurrence	88 (82.2)	32 (64.0)	120 (76.4)
Other	6 (5.6)	4 (8.0)	10 (6.4)
Patient request	4 (3.7)	5 (10.0)	9 (5.7)
Adverse event	2 (1.9)	2 (4.0)	4 (2.5)
Withdrawal of consent	2 (1.9)	1 (2.0)	3 (1.9)
Patients who completed the trial follow-up period	61 (57.0)	34 (68.0)	95 (60.5)
Patients who discontinued the trial follow-up period	46 (43.0)	16 (32.0)	62 (39.5)
Death	21 (19.6)	5 (10.0)	26 (16.6)
Lost to follow-up	8 (7.5)	3 (6.0)	11 (7.0)
Other	7 (6.5)	4 (8.0)	11 (7.0)
Withdrawal of consent	6 (5.6)	3 (6.0)	9 (5.7)
Patient request	4 (3.7)	1 (2.0)	5 (3.2)

Abbreviations: CIS = carcinoma in situ; HGRF = high-grade-recurrence-free; ICF = informed consent form; n = number of patients with observation; % = percentage of patients with observation; SAP = Statistical Analysis Plan.
Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.
Percentage was calculated using the number of treated patients in each cohort as the denominator.

1. Patients enrolled included all patients who signed the ICF, were enrolled in a cohort, and received the first dose.
2. Patients achieved HGRF survival at Month 3, but did not receive trial drug at Month 4 Day 1.
3. 1 patient achieved HGRF survival at Month 6, but did not receive trial drug at Month 7 Day 1.
4. 2 patients achieved HGRF survival at Month 9, but did not receive trial drug at Month 10 Day 1.
5. 6 patients achieved HGRF survival at Month 12, but did not receive trial drug at Month 12.
6. Data collection stopped at Month 57. Before this decision was made, 2 patients had treatment data entered for Month 60.
7. An additional 5 patients completed the Month 12 Efficacy Assessment Visit but were marked as not having completed the 12 Month treatment period by sites. The sponsor noted that these 5 patients should have been considered as completed the 12 month treatment period per the protocol and SAP.

Trial Completion or Discontinuation

Data are reported in Table 30. Overall, 95 (60.5%) patients completed the follow-up period. The median duration of follow-up was 51 months for the total trial population: 49 months for the CIS cohort and 59 months for the papillary disease cohort. In total, 62 (39.5%) patients did not complete the trial follow-up period. The reasons for discontinuing early from trial follow-up included death (26 [16.6%] patients), lost to follow-up (11 [7.0%] patients), other (11 [7.0%] patients), withdrawal of consent (9 [5.7%] patients), and patient request (5 [3.2%]).

Trial Treatment Completion or Discontinuation

A total of 62 (39.5%) patients received 4 doses of nadofaragene firadenovec during the initial 12-months treatment period. Of note, one patient received trial drug at Month 7 Day 1 too close to Month 10 Day 1, therefore the patient skipped the Month 10 Day 1 dose.

In total, 11 (7.0%) patients completed treatment at the end of trial (during the follow-up): 5 (4.7%) patients in the CIS cohort and 6 (12.0%) patients in the papillary disease cohort. A total of 146 (93%) patients discontinued trial treatment at end of trial. Disease recurrence was the most common reason for discontinuing trial treatment (120 [76.4%] patients). Other reasons for discontinuation of treatment included 'other' (10 [6.4%] patients), patient request (9 [5.7%] patients), adverse events (4 [2.5%] patients), and withdrawal of consent (3 [1.9%] patients).

2.6.8.2. Adverse events

Study CS-003

An overview of adverse events is provided in Table 31.

Table 31. Overview of Adverse Events - Study CS-003 (Safety Analysis Set)

Adverse Event Category	CIS (N=107) n (%)	Papillary Disease (N=50) n (%)	Total (N=157) n (%)
AEs/SAEs			
Patients with ≥1 AE	101 (94.4)	45 (90.0)	146 (93.0)
Severity of AEs[a]			
Grade 1	30 (28.0)	11 (22.0)	41 (26.1)
Grade 2	49 (45.8)	22 (44.0)	71 (45.2)
Grade 3	21 (19.6)	10 (20.0)	31 (19.7)
Grade 4	1 (0.9)	2 (4.0)	3 (1.9)
Grade 5	0 (0.0)	0 (0.0)	0 (0.0)
Patients with ≥1 SAE	10 (9.3)	9 (18.0)	19 (12.1)
Trial drug-related AEs/SAEs			
Patients with ≥1 drug-related AE[b]	77 (72.0)	34 (68.0)	111 (70.7)
Severity of drug-related AEs[a]			
Grade 1	41 (38.3)	22 (44.0)	63 (40.1)
Grade 2	32 (29.9)	9 (18.0)	41 (26.1)
Grade 3	3 (2.8)	3 (6.0)	6 (3.8)
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)
Grade 5	0 (0.0)	0 (0.0)	0 (0.0)
Patients with ≥1 drug-related SAE[b]	0 (0.0)	1 (2.0)	1 (0.6)
Discontinuation of trial drug due to			
Any AE	2 (1.9)	2 (4.0)	4 (2.5)
Trial drug-related AE[b]	2 (1.9)	1 (2.0)	3 (1.9)
Deaths due to AE	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: AE = adverse event; CIS = carcinoma in situ; SAE = serious adverse event; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; vp = viral particles.
a) For individual patients, the severity used for analysis was according to the most severe AE experienced.
b) As per investigator assessment. For individual patients, the relationship used for analysis was according to the most related AE experienced.
Note: Percentage was calculated using the number of patients in the column heading as the denominator.

All Adverse events

Table 32 summarises the most frequently reported AEs (≥10% of patients) by SOC and PT observed across the pivotal trial (study CS-003) in either cohort in the Safety Analysis Set. The AEs are tabulated according to SOC by decreasing order of frequency and to preferred term by decreasing order of frequency within each SOC.

Table 32. Common Adverse Events by SOC and PT (in ≥10% of Patients) – Study CS-003 (Safety Analysis Set)

System Organ Class Preferred Term	CIS (N = 107) n (%)	E	Papillary Disease (N = 50) n (%)	E	Total (N = 157) n (%)	E
Patients with any AE	101(94.4)	775	45(90.0)	393	146(93.0)	1168
General disorders and administration site conditions	**71(66.4)**	**211**	**29(58.0)**	**80**	**100(63.7)**	**291**
Instillation site discharge	37(34.6)	87	15(30.0)	33	52(33.1)	120
Fatigue	29(27.1)	42	8(16.0)	11	37(23.6)	53
Pyrexia	18(16.8)	22	7(14.0)	11	25(15.9)	33
Chills	18(16.8)	27	6(12.0)	7	24(15.3)	34
Renal and urinary disorders	**67(62.6)**	**215**	**32(64.0)**	**81**	**99(63.1)**	**296**
Bladder spasm	22(20.6)	53	9(18.0)	12	31(19.7)	65
Micturition urgency	20(18.7)	41	9(18.0)	10	29(18.5)	51

System Organ Class Preferred Term	CIS (N = 107) n (%)	E	Papillary Disease (N = 50) n (%)	E	Total (N = 157) n (%)	E
Haematuria	19(17.8)	21	7(14.0)	8	26(16.6)	29
Dysuria	17(15.9)	26	8(16.0)	14	25(15.9)	40
Pollakiuria	9(8.4)	9	6(12.0)	7	15(9.6)	16
Infections and infestations	**31(29.0)**	**66**	**22(44.0)**	**41**	**53(33.8)**	**107**
Urinary tract infection	11(10.3)	24	12(24.0)	17	23(14.6)	41
Bronchitis	1(0.9)	1	5(10.0)	5	6(3.8)	6
Nervous system disorders	**30(28.0)**	**44**	**14(28.0)**	**25**	**44(28.0)**	**69**
Headache	16(15.0)	22	8(16.0)	10	24(15.3)	32
Dizziness	8(7.5)	9	6(12.0)	8	14(8.9)	17
Gastrointestinal disorders	**25(23.4)**	**59**	**18(36.0)**	**34**	**43(27.4)**	**93**
Diarrhoea	10(9.3)	15	7(14.0)	8	17(10.8)	23
Abdominal pain	2(1.9)	2	5(10.0)	5	7(4.5)	7
Musculoskeletal and connective tissue disorders	**23(21.5)**	**38**	**16(32.0)**	**27**	**39(24.8)**	**65**
Myalgia	7(6.5)	8	5(10.0)	5	12(7.6)	13

Abbreviations: AE = adverse event; CIS = carcinoma in situ; E = number of observations; PT = preferred term; SOC = system organ class N = number of patients; n = number of patients with observation; % = percentage of patients with observation.

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.

Percentage was calculated using the number of patients in the column heading as the denominator.

If a patient had more than 1 adverse event within a PT, the patient was counted once in that PT. If a patient had more than 1 adverse event within an SOC, the patient was counted once in that SOC.

Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

Trial drug-related adverse events

Table 33 summarizes the number of patients with AEs considered trial drug-related as per investigator assessment (≥10% of patients in either cohort) by SOC and PT for the Safety Analysis Set.

Table 33. Common Trial Drug-Related Adverse Events (in ≥10% of Patients) by SOC and PT – Study CS-003 (Safety Analysis Set)

System Organ Class Preferred Term	CIS (N = 107) n (%)	E	Papillary Disease (N = 50) n (%)	E	Total (N = 157) n (%)	E
Patients with any trial drug-related AE	77(72.0)	358	34(68.0)	157	111(70.7)	515
General disorders and administration site conditions	**53(49.5)**	**153**	**23(46.0)**	**65**	**76(48.4)**	**218**
Instillation site discharge	26(24.3)	62	13(26.0)	29	39(24.8)	91
Fatigue	25(23.4)	36	6(12.0)	9	31(19.7)	45
Chills	13(12.1)	20	5(10.0)	6	18(11.5)	26
Pyrexia	11(10.3)	15	5(10.0)	9	16(10.2)	24
Renal and urinary disorders	**49(45.8)**	**138**	**23(46.0)**	**48**	**72(45.9)**	**186**
Bladder spasm	19(17.8)	35	7(14.0)	10	26(16.6)	45
Micturition urgency	18(16.8)	36	7(14.0)	8	25(15.9)	44
Dysuria	11(10.3)	16	8(16.0)	12	19(12.1)	28
Pollakiuria	7 (6.5)	7	5(10.0)	5	12 (7.6)	12
Gastrointestinal disorders	**11(10.3)**	**17**	**10(20.0)**	**15**	**21(13.4)**	**32**
Diarrhoea	4 (3.7)	4	6(12.0)	7	10 (6.4)	11
Nervous system disorders	**13(12.1)**	**20**	**8(16.0)**	**11**	**21(13.4)**	**31**
Headache	9 (8.4)	12	5(10.0)	7	14 (8.9)	19

Abbreviations: AE = adverse event; CIS = carcinoma in situ; E = number of observations; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; PT = preferred term; SOC = system organ class.

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.

Percentage was calculated using the number of patients in the column heading as the denominator.

Treatment-emergent adverse events were defined as adverse events that occurred or worsened in severity at or after the first dose of the trial drug. If a patient had more than 1 adverse event within a PT, the patient was counted once in that PT. If a patient had more than 1 adverse event within an SOC, the patient was counted once in that SOC.

Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

An overview of the ADRs is provided in Table 34. Tabulated list of adverse reactions.

Unless otherwise stated, the frequencies of adverse reactions are based on all-cause adverse event frequencies identified in 157 patients exposed to nadofaragene firadenovec during a median treatment duration of 3.4 months in clinical study CS-003. The adverse reaction frequencies in clinical study CS-003 are based on all-cause adverse event frequencies, where a proportion of the events for an adverse reaction may have other causes than the drug, such as the disease, the instillation procedure, other medication or unrelated causes.

Table 34. Tabulated list of adverse reactions

System Organ Class	Adverse Reactions (%)
Infections and infestations	Urinary tract infection (14.6)
Blood and lymphatic system disorders	Thrombocytopenia (1.3) Neutropenia (0.6)
Metabolism and nutrition disorders	Decreased appetite (3.2)
Psychiatric disorders	Restlessness (0.6)

Nervous system disorders	Headache (15.3)
	Syncope (1.3) Dizziness (8.9) Paraesthesia (0.6)
Vascular disorders	Hypertension (7.0) Hot flush (0.6)
Gastrointestinal disorders	Diarrhoea (10.8) Abdominal pain (9.6)
	Nausea (7.6) Vomiting (3.8) Defaecation urgency (0.6) Gastrointestinal pain (0.6)
Skin and subcutaneous tissue disorders	Night sweats (2.5) Hyperhidrosis (1.9) Dermatitis allergic (0.6)
Musculoskeletal and connective tissue disorders	Myalgia (7.6) Arthralgia (6.4) Pain in extremity (1.9) Muscular weakness (0.6) Musculoskeletal stiffness (0.6)
Renal and urinary disorders	Bladder spasm (19.7) Micturition urgency (18.5) Haematuria (16.6) Dysuria (15.9) Lower urinary tract pain (10.8) Pollakiuria (9.6)
	Urinary incontinence (6.4) Nocturia (4.5) Urinary retention (4.5) Haemorrhage urinary tract (2.5) Urine odour abnormal (1.9) Cystitis noninfective (0.6)
Reproductive system and breast disorders	Vulvovaginal discomfort (1.3)
General disorders and administration site conditions	Instillation site discharge (33.1%), Fatigue (23.6%), Pyrexia (15.9%), Chills (15.3%) Pain (7%), Influenza like illness (5.1%), Malaise (3.8%), Drug intolerance (0.6%)
Investigations	Urine output increased (1.3%)

Duration of adverse events

Ad-hoc duration summaries were performed for selected trial drug-related AE PTs occurring in ≥5% of the population.

In total, the majority of trial drug-related AEs as per investigator assessment had a short median duration (≤2.0 days). However, fatigue (31 [19.7%] patients) and pollakiuria (12 [7.6%] patients) had a median duration of 11.0 and 54.5 days, respectively.

The median durations of the most common trial drug-related AEs as per investigator assessment for the overall trial population were 1 day for instillation site discharge, 11 days for fatigue, 1 day for bladder spasms, 1 day for micturition urgency, and 2 days for dysuria.

In total, the majority of trial procedure-related AEs as per investigator assessment had a short median duration (≤1 day). However, haematuria (10 [6.4%] patients) had a median duration of 4 days.

Adverse events by severity

Severity of the experienced adverse event is summarized in Table 31.

The most frequently reported Grade 3 AE was hypertension (4 [2.5%] patients). The 3 Grade 4 AEs, consisting of 2 events of sepsis and 1 event of anaphylactic reaction, were considered to be trial drug-unrelated; although sepsis for the first patient was considered trial procedure-related, while the sepsis reported for the other patient was conservatively recorded post Month 24 despite being trial drug-unrelated and trial procedure-unrelated. Anaphylactic reaction was suspected by the investigator to be due to Cysview® that had been instilled preoperatively prior to a planned TURBT procedure.

Trial drug-related Grade 3 AE are summarised in Table 31. The most common trial drug-related Grade 3 AE was micturition urgency (2 [1.3%] patients, all Grade 3). No Grade 4 or 5 AEs were considered trial drug-related. Of the 6 patients with 6 trial drug-related Grade 3 AEs, 5 AEs were non-serious and the causality was only according to investigator's assessment. 1 (0.6%) patient had a trial drug-related SAE (syncope) as assessed by the investigator, but trial drug-unrelated by the sponsor: no patients in the CIS cohort and 1 (2.0%) patient in the papillary disease cohort.

2.6.8.3. Serious adverse event/deaths/other significant events

AEs of special interest

No AEs of special interest were identified.

Serious adverse events (SAEs)

In study CS-003, a total of 19 (12.1%) patients experienced (a) SAE(s): 10 (9.3%) patients in the CIS cohort and 9 (18.0%) patients in the papillary disease cohort. In total, 30 SAEs were reported: 11 SAEs in the CIS cohort and 19 SAEs in the papillary disease cohort. The SAEs by PT are listed in Table 35.

The reported SAEs were all of Grade 1-3, with the exception of 2 Grade 4 SAEs (anaphylactic reaction and sepsis) in the CIS and papillary disease cohort, respectively. Both SAEs were considered unrelated to trial drug and did not result in discontinuation of trial drug.

Table 35. Overview of Serious Adverse Events by SOC and PT - Study CS-003 (Safety Analysis Set)

System Organ Class Preferred Term	CIS (N = 107) n (%)	E	Papillary Disease (N = 50) n (%)	E	Total (N = 157) n (%)	E
Patients with Any SAE	10(9.3)	11	9(18.0)	19	19(12.1)	30
Cardiac disorders	**5(4.7)**	**5**	**2(4.0)**	**5**	**7(4.5)**	**10**
Coronary artery disease	1(0.9)	1	1(2.0)	1	2(1.3)	2
Acute coronary syndrome	1(0.9)	1	0(0.0)	0	1(0.6)	1
Arrhythmia	1(0.9)	1	0(0.0)	0	1(0.6)	1
Atrial fibrillation	1(0.9)	1	0(0.0)	0	1(0.6)	1
Atrial flutter	1(0.9)	1	0(0.0)	0	1(0.6)	1
Cardiac failure	0(0.0)	0	1(2.0)	1	1(0.6)	1
Myocardial infarction	0(0.0)	0	1(2.0)	1	1(0.6)	1
Pericarditis	0(0.0)	0	1(2.0)	2	1(0.6)	2
Nervous system disorders	**2(1.9)**	**2**	**2(4.0)**	**2**	**4(2.5)**	**4**
Syncope	1(0.9)	1	1(2.0)	1	2(1.3)	2
Brain oedema	1(0.9)	1	0(0.0)	0	1(0.6)	1
Transient global amnesia	0(0.0)	0	1(2.0)	1	1(0.6)	1
Infections and infestations	**1(0.9)**	**1**	**2(4.0)**	**2**	**3(1.9)**	**3**
Sepsis	1(0.9)	1	1(2.0)	1	2(1.3)	2
Pneumonia	0(0.0)	0	1(2.0)	1	1(0.6)	1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**1(0.9)**	**1**	**2(4.0)**	**4**	**3(1.9)**	**5**
Lung Cancer metastatic	0(0.0)	0	1(2.0)	1	1(0.6)	1
Lung neoplasm malignant	0(0.0)	0	1(2.0)	1	1(0.6)	1
Pancreatic carcinoma	0(0.0)	0	1(2.0)	1	1(0.6)	1
Transitional cell cancer of the renal pelvis and ureter	0(0.0)	0	1(2.0)	1	1(0.6)	1
Transitional cell carcinoma	1(0.9)	1	0(0.0)	0	1(0.6)	1
Metabolism and nutrition disorders	**1(0.9)**	**1**	**1(2.0)**	**1**	**2(1.3)**	**2**
Dehydration	1(0.9)	1	0(0.0)	0	1(0.6)	1
Hypoglycemia	0(0.0)	0	1(2.0)	1	1(0.6)	1
General disorders and administration site conditions	**0(0.0)**	**0**	**1(2.0)**	**1**	**1(0.6)**	**1**
Pyrexia	0(0.0)	0	1(2.0)	1	1(0.6)	1
Hepatobiliary disorders	**0(0.0)**	**0**	**1(2.0)**	**1**	**1(0.6)**	**1**
Bile duct stone	0(0.0)	0	1(2.0)	1	1(0.6)	1
Immune system disorders	**0(0.0)**	**0**	**1(2.0)**	**1**	**1(0.6)**	**1**
Anaphylactic reaction	0(0.0)	0	1(2.0)	1	1(0.6)	1
Injury, poisoning and procedural complications	**0(0.0)**	**0**	**1(2.0)**	**1**	**1(0.6)**	**1**
Corneal abrasion	0(0.0)	0	1(2.0)	1	1(0.6)	1
Renal and urinary disorders	**1(0.9)**	**1**	**0(0.0)**	**0**	**1(0.6)**	**1**
Haematuria	1(0.9)	1	0(0.0)	0	1(0.6)	1
Skin and subcutaneous tissue disorders	**0(0.0)**	**0**	**1(2.0)**	**1**	**1(0.6)**	**1**
Subcutaneous emphysema	0(0.0)	0	1(2.0)	1	1(0.6)	1

Abbreviations: CIS = carcinoma in situ; SAE = serious adverse event; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; E = number of events; PT = preferred term; SOC = system organ class.

Note: CIS = CIS Only, Ta+CIS, T1+CIS ; Papillary Disease = Ta & T1.

Percentage is calculated using the number of patients in the column heading as the denominator.

If a patient experienced more than one adverse event within a PT, the patient is counted once in that PT. If a patient experienced more than one adverse event within an SOC, the patient is counted once in that SOC.

Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

Deaths

In study CS-003, no patient had an AE leading to death. Overall, 28 deaths were reported in the long-term follow-up period occurring at least 4 months after the last administration of the trial drug. None of the deaths were considered related to trial drug and all patients had been discontinued from trial treatment.

2.6.8.4. Laboratory findings

Laboratory findings

The clinical laboratory evaluations included in study CS-003 were clinical chemistry, haematology, and urinalysis.

Notable changes in laboratory parameters included transient increases in the blood levels of glucose, triglycerides, and creatinine; and transient decreases in blood levels of phosphate and haemoglobin. These changes in laboratory parameters were primarily not reported as AEs. The increase in glucose and triglycerides levels as well as the decrease in haemoglobin levels was due to pre-existing or underlying clinical conditions and risk factors, and there is no evidence to suggest that nadofaragene firadenovec contribute to the test abnormalities or abnormal changes in these laboratory parameters.

A single patient had an SAE of hypoglycaemia and 1 patient had an SAE of haematuria. No other laboratory abnormalities were considered as SAEs or resulted in discontinuation from the trial.

Anti-Adenoviral Antibody Status

A patient was considered to have a positive immunogenic response in anti-adenoviral antibodies if a post-baseline titration demonstrated a greater than two-fold dilution increase from baseline. For 134 patients both baseline and post-baseline samples were available. A positive immunogenic response in anti-adenoviral antibodies has been demonstrated in 97 out of 134 patients in the pivotal study CS-003.

Anti-IFNα2b antibodies

Anti-IFNα2b antibody titres were evaluated in the phase 1 (P03816) and phase 2 (rAd-IFN-CS-002) trials. In the phase 1 trial, 1 patient had a positive antibody level at the pre-dose assessment and only 1 patient had a positive antibody level at the Week 12 assessment. In the phase 2 trial, 1 patient only had a weak anti-IFNα2b antibody titre following the first instillation of nadofaragene firadenovec.

Other findings

The vital signs and other observations related to safety evaluated in study CS-003 were systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature, weight, physical examination, and ECG.

Overall, no notable changes were observed in vital signs, 12-lead ECGs, physical examinations, and urinary symptoms across either cohort in the Safety Analysis Set. The majority of abnormal 12-lead ECG findings for all enrolled patients were not clinically significant.

One SAE of pyrexia was reported in the papillary disease group.

2.6.8.5. Safety in special populations

The safety in special populations is shown in Table 36 and Table 37.

Table 36. AEs by Age Range

	Active				Comparator			
MedDRA Terms	Age <65 n (%)	Age 65-74 n (%)	Age 75-84 n (%)	Age 85+ n (%)	Age <65 n (%)	Age 65-74 n (%)	Age 75-84 n (%)	Age 85+ n (%)
Total AEs	34 (89.5)	62 (96.9)	42 (89.4)	8 (100.0)	NA	NA	NA	NA
Serious AEs – Total	3 (7.9)	9 (14.1)	5 (10.6)	2 (25.0)	NA	NA	NA	NA
- Fatal	0	0	0	0	NA	NA	NA	NA
- Hospitalization/prolong existing hospitalization	3 (7.9)	9 (14.1)	5 (10.6)	2 (25.0)	NA	NA	NA	NA
- Life-threatening	0	0	0	0	NA	NA	NA	NA
- Disability/incapacity	0	0	0	0	NA	NA	NA	NA
- Other (medically significant)	0	1 (1.6)	2 (4.3)	0	NA	NA	NA	NA
AE leading to drop-out	1 (2.6)	1 (1.6)	1 (2.1)	0	NA	NA	NA	NA
Psychiatric disorders	2 (5.3)	6 (9.4)	3 (6.4)	1 (12.5)	NA	NA	NA	NA
Nervous system disorders	12 (31.6)	19 (29.7)	10 (21.3)	3 (37.5)	NA	NA	NA	NA
Accidents and injuries	2 (5.3)	4 (6.3)	4 (8.5)	1 (12.5)	NA	NA	NA	NA
Cardiac disorders	1 (2.6)	7 (10.9)	3 (6.4)	0	NA	NA	NA	NA
Vascular disorders	4 (10.5)	9 (14.1)	1 (2.1)	0	NA	NA	NA	NA
Cerebrovascular disorders	0	0	0	0	NA	NA	NA	NA
Infections and infestations	11 (28.9)	27 (42.2)	14 (29.8)	1 (12.5)	NA	NA	NA	NA
Anticholinergic syndrome	2 (5.3)	9 (14.1)	1 (2.1)	2 (25.0)	NA	NA	NA	NA
Quality of life decreased	0	0	0	0	NA	NA	NA	NA
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	1 (2.6)	3 (4.7)	2 (4.3)	1 (12.5)	NA	NA	NA	NA
<other AE appearing more frequently in older patients>	NA	NA	NA	NA	NA	NA	NA	NA

NA= not applicable

Note: Data from completed investigational clinical trials included in the dataset: rAd-IFN-CS-003.

Total AEs shows the number and percentage of subjects within the given age ranges that reported at least 1 AE.

Table 37. AE by Special Population

	Active				Comparator			
MedDRA Terms	Hepatically impaired* n (%)	Renally impaired** n (%)	Pregnant n (%)	Other n (%)	Hepatically impaired* n (%)	Renally impaired** n (%)	Pregnant n (%)	Other n (%)
Total AEs	0	0	0	0	NA	NA	NA	NA
Serious AEs – Total	0	0	0	0	NA	NA	NA	NA
- Fatal	0	0	0	0	NA	NA	NA	NA
- Hospitalization/prolong existing hospitalization	0	0	0	0	NA	NA	NA	NA
- Life-threatening	0	0	0	0	NA	NA	NA	NA
- Disability/incapacity	0	0	0	0	NA	NA	NA	NA
- Other (medically significant)	0	0	0	0	NA	NA	NA	NA
AE leading to drop-out	0	0	0	0	NA	NA	NA	NA

NA= not applicable

* Hepatic impairment is defined as having Child-Pugh score B or C

** Renal impairment is defined as having CKD Stage 3b, 4 or 5 (KDIGO definition)

Note: Data from completed investigational clinical trials included in the dataset: rAd-IFN-CS-003

In study CS-003, safety analyses in subgroups (age, sex, race, height and weight) were performed as exploratory analyses.

The majority of patients in this trial were males (129 [82.2%]). However, the tolerability of nadofaragene firadenovec was clinically comparable between male patients (120 [93.0%]) and female patients (26 [92.9%]), and between patients aged 65 years and above, and those younger than 65 years (see below).

Table 38 summarises the number of patients aged ≥65 years with AEs (≥10% of patients in either cohort) by SOC and PT for the Safety Analysis Set.

Table 38. Common Adverse Events (in ≥10% of Patients) by SOC and PT – Study CS-003 (Safety Analysis Set: Age ≥65 Years)

System Organ Class Preferred Term	CIS (N = 82) n (%)	E	Papillary Disease (N = 37) n (%)	E	Total (N = 119) n (%)	E
Patients with any AE	78(95.1)	594	34(91.9)	317	112(94.1)	911
General disorders and administration site conditions	**59(72.0)**	**166**	**22(59.5)**	**68**	**81(68.1)**	**234**
Instillation site discharge	32(39.0)	72	11(29.7)	29	43(36.1)	101
Fatigue	23(28.0)	32	8(21.6)	11	31(26.1)	43
Pyrexia	13(15.9)	17	6(16.2)	10	19(16.0)	27
Chills	11(13.4)	18	5(13.5)	6	16(13.4)	24
Renal and urinary disorders	**50(61.0)**	**149**	**23(62.2)**	**53**	**73(61.3)**	**202**
Bladder spasm	18(22.0)	47	4(10.8)	6	22(18.5)	53
Haematuria	15(18.3)	17	5(13.5)	6	20(16.8)	23
Micturition urgency	13(15.9)	20	7(18.9)	8	20(16.8)	28
Dysuria	13(15.9)	16	6(16.2)	12	19(16.0)	28
Pollakiuria	7(8.5)	7	5(13.5)	6	12(10.1)	13
Infections and infestations	**24(29.3)**	**54**	**18(48.6)**	**33**	**42(35.3)**	**87**
Urinary tract infection	10(12.2)	23	10(27.0)	12	20(16.8)	35
Bronchitis	1(1.2)	1	5(13.5)	5	6(5.0)	6
Gastrointestinal disorders	**21(25.6)**	**52**	**17(45.9)**	**32**	**38(31.9)**	**84**
Diarrhoea	9(11.0)	14	7(18.9)	8	16(13.4)	22
Abdominal pain	1(1.2)	1	4(10.8)	4	5(4.2)	5
Nervous system disorders	**21(25.6)**	**33**	**11(29.7)**	**22**	**32(26.9)**	**55**
Headache	10(12.2)	15	7(18.9)	9	17(14.3)	24
Dizziness	7(8.5)	8	5(13.5)	7	12(10.1)	15
Musculoskeletal and connective tissue disorders	**17(20.7)**	**31**	**13(35.1)**	**22**	**30(25.2)**	**53**
Myalgia	5(6.1)	5	5(13.5)	5	10(8.4)	10
Arthralgia	5(6.1)	7	4(10.8)	4	9(7.6)	11
Respiratory, thoracic and mediastinal disorders cough	**6(7.3)**	**12**	**8(21.6)**	**13**	**14(11.8)**	**25**
Cough	0(0.0)	0	4(10.8)	4	4(3.4)	4
Skin and subcutaneous tissue disorders	**9(11.0)**	**9**	**10(27.0)**	**12**	**19(16.0)**	**21**
Night sweats	0(0.0)	0	4(10.8)	4	4(3.4)	4

Abbreviations: AE = adverse event; CIS = carcinoma in situ; E = number of observations; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; PT = preferred term; SOC = system organ class.

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.

Percentage was calculated using the number of patients in the column heading as the denominator.

Treatment-emergent adverse events were defined as adverse events that occurred or worsened in severity at or after the first dose of the trial drug. If a patient had more than 1 adverse event within a PT, the patient was counted once in that PT. If a patient had more than 1 adverse event within an SOC, the patient was counted once in that SOC.

Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

Table 39 summarises the number of patients aged <65 years with AEs (≥10% of patients in either cohort) by SOC and PT for the Safety Analysis Set.

Table 39. Common Adverse Events (in ≥10% of Patients) by SOC and PT – Study CS-003 (Safety Analysis Set: Age <65 Years)

System Organ Class Preferred Term	CIS (N = 25) n (%)	E	Papillary Disease (N = 13) n (%)	E	Total (N = 38) n (%)	E
Patients with any AE	23(92.0)	181	11(84.6)	76	34(89.5)	257

System Organ Class Preferred Term	CIS (N = 25) n (%)	E	Papillary Disease (N = 13) n (%)	E	Total (N = 38) n (%)	E
Renal and urinary disorders	**17(68.0)**	**66**	**9(69.2)**	**28**	**26(68.4)**	**94**
Bladder spasm	4(16.0)	6	5(38.5)	6	9(23.7)	12
Micturition urgency	7(28.0)	21	2(15.4)	2	9(23.7)	23
Dysuria	4(16.0)	10	2(15.4)	2	6(15.8)	12
Haematuria	4(16.0)	4	2(15.4)	2	6(15.8)	6
Bladder pain	1(4.0)	1	2(15.4)	3	3(7.9)	4
Urinary retention	0(0.0)	0	2(15.4)	2	2(5.3)	2
General disorders and administration site conditions	**12(48.0)**	**45**	**7(53.8)**	**12**	**19(50.0)**	**57**
Instilation site discharge	5(20.0)	15	4(30.8)	4	9(23.7)	19
Chills	7(28.0)	9	1(7.7)	1	8(21.1)	10
Fatigue	6(24.0)	10	0(0.0)	0	6(15.8)	10
Pyrexia	5(20.0)	5	1(7.7)	1	6(15.8)	6
Malaise	1(4.0)	1	2(15.4)	3	3(7.9)	4
Pain	3(12.0)	4	0(0.0)	0	3(7.9)	4
Nervous system disorders	**9(36.0)**	**11**	**3(23.1)**	**3**	**12(31.6)**	**14**
Headache	6(24.0)	7	1(7.7)	1	7(18.4)	8
Infections and infestations	**7(28.0)**	**12**	**4(30.8)**	**8**	**11(28.9)**	**20**
Upper respiratory tract infection	3(12.0)	3	1(7.7)	1	4(10.5)	4
Urinary tract infection	1(4.0)	1	2(15.4)	5	3(7.9)	6
Vascular disorders	**2(8.0)**	**2**	**2(15.4)**	**6**	**4(10.5)**	**8**
Hypertension	2(8.0)	2	2(15.4)	6	4(10.5)	8

Abbreviations: AE = adverse event; CIS = carcinoma in situ; E = number of observations; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; PT = preferred term; SOC = system organ class.

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.

Percentage was calculated using the number of patients in the column heading as the denominator.

Treatment-emergent adverse events were defined as adverse events that occurred or worsened in severity at or after the first dose of the trial drug. If a patient had more than 1 adverse event within a PT, the patient was counted once in that PT. If a patient had more than 1 adverse event within an SOC, the patient was counted once in that SOC.

Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

2.6.8.6. ADRs in special populations

Table 40 summarises the number of patients aged ≥65 years with trial drug-related AEs (≥10% of patients in either cohort) by SOC and PT for the Safety Analysis Set.

Table 40. Common Trial Drug-related Adverse Events (in ≥10% of Patients) by SOC and PT – Study CS-003 (Safety Analysis Set: Age ≥65 Years)

System Organ Class Preferred Term	CIS (N = 82) n (%) E			Papillary Disease (N = 37) n (%) E			Total (N = 119) n (%) E		
Patients with any trial drug-related AE	62	(75.6)	269	25	(67.6)	122	87	(73.1)	391
General disorders and administration site conditions	**44**	**(53.7)**	**120**	**17**	**(45.9)**	**55**	**61**	**(51.3)**	**175**
Installation site discharge	22	(26.8)	48	9	(24.3)	25	31	(26.1)	73
Fatigue	20	(24.4)	28	6	(16.2)	9	26	(21.8)	37
Chills	8	(9.8)	15	5	(13.5)	6	13	(10.9)	21
Pyrexia	7	(8.5)	11	4	(10.8)	8	11	(9.2)	19
Renal and urinary disorders	**38**	**(46.3)**	**94**	**14**	**(37.8)**	**32**	**52**	**(43.7)**	**126**
Bladder spasm	16	(19.5)	30	3	(8.1)	5	19	(16.0)	35
Micturition urgency	12	(14.6)	18	5	(13.5)	6	17	(14.3)	24
Dysuria	9	(11.0)	10	6	(16.2)	10	15	(12.6)	20
Pollakiuria	6	(7.3)	6	4	(10.8)	4	10	(8.4)	10
Gastrointestinal disorders	**10**	**(12.2)**	**16**	**9**	**(24.3)**	**14**	**19**	**(16.0)**	**30**
Diarrhoea	4	(4.9)	4	6	(16.2)	7	10	(8.4)	11
Nervous system disorders	**9**	**(11.0)**	**14**	**7**	**(18.9)**	**10**	**16**	**(13.4)**	**24**
Headache	6	(7.3)	8	5	(13.5)	7	11	(9.2)	15
Musculoskeletal and connective tissue disorders	**5**	**(6.1)**	**11**	**4**	**(10.8)**	**5**	**9**	**(7.6)**	**16**
Myalgia	3	(3.7)	3	4	(10.8)	4	7	(5.9)	7

Abbreviations: AE = adverse event; CIS = carcinoma in situ; E = number of observations; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; PT = preferred term; SOC = system organ class.

Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.
Percentage was calculated using the number of patients in the column heading as the denominator.
Treatment-emergent adverse events were defined as adverse events that occurred or worsened in severity at or after the first dose of the trial drug. If a patient had more than 1 adverse event within a PT, the patient was counted once in that PT. If a patient had more than 1 adverse event within an SOC, the patient was counted once in that SOC.
Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

Table 41 summarises the number of patients aged <65 years with trial drug-related AEs (≥10% of patients in either cohort) by SOC and PT for the Safety Analysis Set.

Table 41. Common Trial Drug-related Adverse Events (in ≥10% of Patients) by SOC and PT – Study CS-003 (Safety Analysis Set: Age <65 Years)

System Organ Class Preferred Term	CIS (N = 25) n (%)		E	Papillary Disease (N = 13) n (%)		E	Total (N = 38) n (%)		E
Patients with any trial drug-related AE	15	(60.0)	89	9	(69.2)	35	24	(63.2)	124
Renal and urinary disorders	**11**	**(44.0)**	**44**	**9**	**(69.2)**	**16**	**20**	**(52.6)**	**60**
Micturition urgency	6	(24.0)	18	2	(15.4)	2	8	(21.1)	20
Bladder spasm	3	(12.0)	5	4	(30.8)	5	7	(18.4)	10
Dysuria	2	(8.0)	6	2	(15.4)	2	4	(10.5)	8
Bladder pain	0	(0.0)	0	2	(15.4)	2	2	(5.3)	2
General disorders and administration site conditions	**9**	**(36.0)**	**33**	**6**	**(46.2)**	**10**	**15**	**(39.5)**	**43**
InstilLation site discharge	4	(16.0)	14	4	(30.8)	4	8	(21.1)	18
Chills	5	(20.0)	5	0	(0.0)	0	5	(13.2)	5
Fatigue	5	(20.0)	8	0	(0.0)	0	5	(13.2)	8
Pyrexia	4	(16.0)	4	1	(7.7)	1	5	(13.2)	5
Nervous system disorders	**4**	**(16.0)**	**6**	**1**	**(7.7)**	**1**	**5**	**(13.2)**	**7**
Headache	3	(12.0)	4	0	(0.0)	0	3	(7.9)	4

Abbreviations: AE = adverse event; CIS = carcinoma in situ; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; PT = preferred term; SOC = system organ class.
Note: CIS = CIS only, Ta + CIS, and T1 + CIS; papillary disease = Ta and T1.
Percentage was calculated using the number of patients in the column heading as the denominator.
Treatment-emergent adverse events were defined as adverse events that occurred or worsened in severity at or after the first dose of the trial drug. If a patient had more than 1 adverse event within a PT, the patient was counted once in that PT. If a patient had more than 1 adverse event within an SOC, the patient was counted once in that SOC.
Data are presented for AEs occurring in ≥10% of patients in either cohort by SOC and PT.

2.6.8.7. Immunological events

See section 2.6.8.4. 'Laboratory and other findings'.

2.6.8.8. Safety related to drug-drug interactions and other interactions

No formal trials have been conducted to evaluate the potential for drug-drug interactions. Nadofaragene firadenovec is administered by the intravesical route, transducing cells of the urothelium, resulting in local expression of the IFN protein. No systemic exposure to vector is expected. Drug-drug interactions are therefore not anticipated and there has been no clinical evidence indicative of drug-drug interactions in the clinical development programme for nadofaragene firadenovec.

2.6.8.9. Discontinuation due to adverse events

Discontinuation due to adverse events

Study CS-003

A total of 4 (2.5%) patients experienced an AE leading to discontinuation of the trial drug: 2 (1.9%) patients in the CIS cohort (bladder spasms and instillation site discharge, Grade 3 and 2, respectively) and 2 (4.0%) patients in the papillary disease cohort (benign neoplasm of bladder and sepsis, Grade 2 and 4, respectively). All AEs were non-serious and considered related to drug by the investigator except from sepsis that was an SAE considered to be trial drug-related by the investigator.

2.6.8.1. Supportive study(ies)

Phase 2 study rAd-IFN-CS-002 (study CS-002

Safety results – For completeness, an overview of AEs is presented here (Table 42).

Table 42. Overview of Adverse Events – Study CS-002

	Nadofaragene Firadenovec (vp/mL)		
Adverse Event Category	**1 x 10^{11} (N=21) n (%)**	**3 x 10^{11} (N=19) n (%)**	**Overall (N=40) n (%)**
AEs/SAEs			
Patients with ≥1 AE	20 (95.2)	19 (100.0)	39 (97.5)
Severity of AEs[a]			
Grade 1	6 (28.6)	5 (26.3)	11 (27.5)
Grade 2	8 (38.1)	11 (57.9)	19 (47.5)
Grade 3	6 (28.6)	3 (15.8)	9 (22.5)
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)
Grade 5	0 (0.0)	0 (0.0)	0 (0.0)
Patients with ≥1 SAE	3 (14.3)	2 (10.5)	5 (12.5)
Trial drug-related AEs/SAEs			
Patients with ≥1 drug-related AE[b]	18 (85.7)	16 (84.2)	34 (85.0)
Severity of drug-related AEs[a]			
Grade 1	12 (57.1)	9 (47.4)	21 (52.5)
Grade 2	5 (23.8)	5 (26.3)	10 (25.0)
Grade 3	1 (4.8)	2 (10.5)	3 (7.5)
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)
Grade 5	0 (0.0)	0 (0.0)	0 (0.0)
Patients with ≥1 drug-related SAE[b]	1 (4.8)	1 (5.3)	2 (5.0)
Discontinuation of trial drug due to			
Any AE	0 (0.0)	1 (5.3)	1 (2.5)
Trial drug-related AE[b]	0 (0.0)	0 (0.0)	0 (0.0)
Deaths due to AE	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: AE = adverse event; SAE = serious adverse event; N = number of patients; n = number of patients with observation; % = percentage of patients with observation; vp = viral particles.
a) For individual patients, the severity used for analysis was according to the most severe AE experienced.
b) As per investigator assessment. For individual patients, the relationship used for analysis was according to the most related AE experienced.
Note: Percentage was calculated using the number of patients in the column heading as the denominator.

2.6.8.2. Post marketing experience

Nadofaragene firadenovec became commercially available in the US on 06 September 2023. As of 03 August 2024, a total of 569 patients were estimated to have been cumulatively exposed to nadofaragene firadenovec. Cumulatively, 65 ADR (26 cases) have been reported for nadofaragene firadenovec, of which 2 were serious (cystitis and pyrexia). The system organ class 'general disorders

and administration site conditions' comprised the majority of the adverse drug reactions (29 of 65 [45%]). No new trends, safety signals, or risks were identified during review of the adverse drug reactions. No new significant safety findings were identified from the post marketing data.

2.6.9. Discussion on clinical safety

Patient exposure

Patients received up to 4 intravesical administrations of nadofaragene firadenovec over the initial 12-month period in study CS-003. Forty-two patients received more than 5 intravesical administrations of nadofaragene firadenovec and have been treated longer than 52 weeks (Table 29). Overall, 95 (60.5%) patients completed the four-year follow-up period. The median duration of follow-up was 51 months for the total trial population: 49 months for the CIS cohort and 59 months for the papillary disease cohort.

Overall, 58 (36.9%) patients completed the 12-month treatment period as assessed by the investigator (Table 30). Additional long term safety data will be collected in Arm 1 (nadofaragene firadenovec monotherapy) of the ongoing study ABLE-22.

Adverse Events

Overall, 146 (93.0%) patients experienced at least 1 AE during the trial. The most frequently reported AEs (≥10% of patients) by PT were instillation site discharge (52 [33.1%] patients), fatigue (37 [23.6%] patients), bladder spasm (31 [19.7%] patients), micturition urgency (29 [18.5%] patients), haematuria (26 [16.6%] patients), pyrexia (25 [15.9%] patients), dysuria (25 [15.9%] patients), chills (24 [15.3%] patients), headache (24 [15.3%] patients), urinary tract infection (23 [14.6%] patients), and diarrhoea (17 [10.8%] patients).

The causality assessment of the drug-related AEs (Table 33) is impaired due to the single-arm trial design.

In total, the majority of trial drug-related AEs as per investigator assessment had a short median duration (≤2.0 days) following the drug instillation. However, fatigue (31 [19.7%] patients) and pollakiuria (12 [7.6%] patients) had a median duration of 11.0 and 54.5 days, respectively.

Overall, 31 (19.7%) patients experienced at least 1 Grade 3 AE: 21 (19.6%) patients in the CIS cohort and 10 (20.0%) patients in the papillary disease cohort (Table 31). In total, 6 (3.8%) patients had a trial drug-related Grade 3 AE: 3 (2.8%) patients in the CIS cohort and 3 (6.0%) patients in the papillary disease cohort. The most common trial drug-related Grade 3 AE was micturition urgency (2 [1.3%] patients, all Grade 3). No Grade 4 or 5 AEs were considered trial drug-related. The AE profile of the intravesical administration of nadofaragene firadenovec resembles the expected safety profile of a locally administered immunostimulatory therapy in the bladder. Most AEs were related to the local and systemic activation of the immune system. Overall, the majority of AEs were limited to Grade 1 or 2. The duration of the AEs was in general limited to a short period following the drug instillation.

Adverse Drug Reactions (ADRs)

No AEs or ADRs of special interest were defined in the study protocol. ADRs information has been identified per Treatment emergent adverse drug reactions with an incidence of at least 10% by System Organ Class and Preferred Term in trial rAd-IFN-CS-003 - Safety Analysis Set. According to this information, ADRs with an incidence of at least 10% by SOC and PT were reported in 111 (70.7%) patients (77 [72.0%] in CIS cohort; 34 [68.0%] in papillary disease cohort): instillation site discharge in 39 (24.8%) patients (26 [24.3%] in CIS cohort, 13 [26.0%] in papillary disease cohort); fatigue in 31 (19.7%) patients (6 [12.0%] in the CIS cohort; 25 [23.4%] in the papillary disease cohort);

bladder spasm in 26 (16.6%) patients (19 [17.8%] in the CIS cohort; 7 [14.0%] in the papillary disease cohort); micturition urgency in 25 (15.9%) patients (18 [16.8%] in the CIS cohort, 7 [14.0%] in the papillary disease cohort); dysuria in 19 (12.1%) patients (11 [10.3%] in the CIS cohort; 8 [16.0%] in the papillary disease cohort); chills in 18 (11.5%) patients (13 [12.1%] in the CIS cohort; 5 [10.0%] in the papillary disease cohort); pyrexia in 16 (10.2%) patients (11 [10.3%] in the CIS cohort; 5 [10.0%] in the papillary disease cohort). In addition, the ADRs proposed for inclusion in the SmPC are derived from the study drug-related TEAEs observed in the phase 3 trial (rAd-IFN-CS-003). ADRs with a reasonable possibility of a causal relationship with nadofaragene firadenovec were included after thorough medical evaluation and subsequent review by the applicant's safety management team, labelling management team, and labelling committee in accordance with EU SmPC guidelines.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

The most common severe adverse reactions (NCI CTCAE Grade ≥3) were micturition urgency (1.3 %), syncope (0.6 %), hypertension (0.6 %), bladder spasm (0.6 %), and urinary incontinence (0.6 %)

Syncope (0.6 %) was reported as an adverse reaction with an onset of 4 days from treatment. A fall resulting from the loss of consciousness led to injuries requiring urgent medical care. The syncope resolved 3 days after the onset and did not recur with subsequent treatments.

The frequency of treatment discontinuation due to adverse reactions was 1.3 %. The most common adverse reactions leading to treatment discontinuation were instillation site discharge (0.6 %) and bladder spasm (0.6 %).

The frequency of dose interruption due to adverse reactions was 34.4 %. The most common adverse reactions leading to dose interruption were instillation site discharge (24.2 %), micturition urgency (8.3 %), bladder spasm (8.3 %), and urinary incontinence (2.5 %).

Serious Adverse Events (SAEs)

The type of SAEs according to the preferred term was heterogeneous. SAEs occurred across several SOCs (Table 35). The number of patients with a SAE was relatively low.

Deaths

There were 28 deaths in the pivotal 3 study. All deaths were reported in the long-term follow-up period, occurring at least 4 months after the last administration of the investigational product. None of the deaths were considered as related to the investigational medicinal product.

Discontinuations

The majority of patients who discontinued study treatment was due to disease recurrence (120 [76.4%]), followed by "other" (10 [6.4%]), patient request (9 [5.7%]), adverse event (4 [2.5%]) and withdrawal of consent (3 [1.9%]).

In total, 87 (73.1%) patients aged ≥65 years experienced at least 1 trial drug-related AE: 62 (75.6%) patients in the CIS cohort and 25 (67.6%) patients in the papillary disease cohort. The most common trial drug-related AEs that occurred in most patients (aged ≥65 years) were instillation site discharge (31 [26.1%] patients), fatigue (26 [21.8%] patients), bladder spasm (19 [16.0%] patients), and micturition urgency (17 [14.3%] patients). In total, 24 (63.2%) patients aged <65 years had at least 1 trial drug-related AE: 15 (60.0%) patients in the CIS cohort and 9 [69.2%] patients in the papillary disease cohort. The most common trial drug-related AEs that occurred in most patients (aged <65 years) were micturition urgency (8 [21.1%] patients), instillation site discharge (8 [21.1%] patients), and bladder spasm (7 [18.4%] patients).

It is considered acceptable that no formal trials have been conducted to evaluate the potential for drug-drug interactions. Nadofaragene firadenovec is administered by the intravesical route, transducing cells of the urothelium, resulting in local expression of the IFN protein. No systemic exposure to vector is expected. Drug-drug interactions are therefore not anticipated and there has been no clinical evidence indicative of drug-drug interactions in the clinical development programme for nadofaragene firadenovec.

Vector DNA shedding

Vector DNA shedding has been reported in the pharmacodynamic section 2.6.2.2. Measurable amounts of rAd-IFN-derived DNA in urine were detected in both phase 1 and phase 2 trials.

The use of natural micturition metrics to predict the shedding of intact vector in urine has been identified as a conservative approach based on the fact that the adenoviral vector is replication deficient (as the adenoviral E3 gene has been partially deleted from nadofaragene firadenovec) and consequently there is no loss by transduction of the urothelium neither any vector degradation in the urine whereby the number of intact viral particles can be predicted with each void. However, it is recognised that intact vector voided in urine may carry a risk of transducing human cells with the IFN gene. Moreover, the risk of horizontal transfer is further minimised by the likely presence of pre-existing adenoviral antibodies, and if that were to be the case the amount which would be transferred (for example from aerosol, splashing or fomites) will be "of a magnitude lower than that received by patients". A warning has been included in section 4.4 of the SmPC

Regarding the presence of DNA fragments detected in blood by PCR, systemic exposure would rarely occur based on the phase 1 and the phase 2 trials where rAd-IFN-derived DNA was measured, only 1 patient (out of 57 treated with at least 1 dose) receiving a second dose in the phase 2 trial had measurable rAd-IFN-derived DNA in blood (LLOQ, 1×104 copies/mL) 1h post dosing. However, there is no data from the phase 3 study.

A warning on contraception measures in males and females has been included in section 4.4 of the Summary of Product Characteristics. Furthermore, a warning that patients treated with nadofaragene firadenovec must not donate blood, organs, tissues, and cells for transplantation is included in section 4.4 of the SmPC.

Immunogenicity

For 134 patients, both baseline and post-baseline samples for anti-adenoviral antibody status were available. A positive immunogenic response in anti-adenoviral antibodies has been demonstrated in 97 out of 134 patients. The TEAE incidence was higher in patients with positive immunogenicity than in patients with a negative response. The TEAEs in the patients with positive immunogenicity were still primarily limited to transient, non-serious bladder events without apparent systemic toxicity, or they were related to the instillation procedure.

Anti-IFNα2b antibody titres were evaluated in the phase 1 (P03816) and phase 2 (rAd-IFN-CS-002) trials. In the phase 1 trial, 1 patient had a positive antibody level at the pre-dose assessment and only 1 patient had a positive antibody level at the Week 12 assessment. In the phase 2 trial, 1 patient only had a weak anti-IFNα2b antibody titre following the first instillation of nadofaragene firadenovec, consistent with the expectation that human IFNα2b resulting from adenovirus-mediated transgene expression is not immunogenic.

12-Lead Electrocardiogram

The majority of abnormal 12-lead ECG findings for all enrolled patients were not clinically significant.

A detailed QT/QTc study was not performed because of the lack of systemic exposure of nadofaragene firadenovec and this is consistent with EMA guidance which note that the absence of thorough studies can be justified if a product has a highly localised distribution. However, a possible low level systemic exposure cannot be ruled out.

In addition, further justification based on the non-clinical data has been provided. The available non-clinical data in vivo on Syn3NODA alone and rAd-IFN/Syn3NODA does not indicate any effect on heart rate, ECG intervals (RR, PR, QRS, QT) or blood pressures (systolic, diastolic or mean). In vitro Syn3NODA had no effect on hERG current at concentrations up to 3 µM.

Moreover, a GLP-compliant hERG assay (study 32036131) designed according to ICH S7B guidelines was conducted and no statistically significant inhibition of hERG tail current was observed at any concentration.

Relevant recommendations on contraception, pregnancy, breast-feeding and fertility are included in section 4.6 of the SmPC.

Adstiladrin has no or negligible influence on the ability to drive and use machines.

There has been no experience with overdose of Adstiladrin in clinical studies. In the event of a suspected overdose, the patient should be closely monitored for signs or symptoms of adverse reactions, treated symptomatically, and supportive measures instituted as required.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics

Additional safety data needed in the context of a conditional MA

Considering that a limited number of patients have been exposed to treatment beyond one year, the long-term safety profile has not been comprehensively characterised. Data on this aspect will be provided by monotherapy arm 1 of study ABLE-22 an ongoing phase 3, randomised, open-label trial to evaluate the safety and efficacy of intravesical nadofaragene firadenovec alone or in combination with chemotherapy or immunotherapy in patients with HG BCG unresponsive NMIBC (i.e., CIS with or without Ta/T1 papillary disease).

2.6.10. Conclusions on the clinical safety

The safety data of nadofaragene firadenovec is based on the population who received, at least, one dose treatment in either of the 4 clinical studies performed on patients with high-grade, Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) (n= 221). However, the main source of evidence is the pivotal study rAd-IFN-CS-003, in which 157 patients (107 in CIS cohort and 50 in papillary disease cohort) received treatment at the proposed dosing schedule. The single-arm design, in addition to the low number of dosing instillations received by the patients and the fact that less than a half of patients (36.9%) completed the 12-month pre-established initial treatment period, hamper any strong conclusion regarding the safety profile of nadofaragene firadenovec.

Additionally, despite evidence of the limited system distribution in the clinical data, there were reported AEs that are not common for a medicinal product which is administered as an intravesical instillation, which suggested that unexpected systemic exposure to nadofaragene firadenovec cannot be ruled out.

In conclusion, and taking into consideration the uncertainties reported above, nadofaragene firadenovec safety data, is considered limited for a proper characterisation of the safety profile.

Additional long term safety data will, however, be collected from Arm 1 (nadofaragene firadenovec monotherapy) in study ABLE 22 as part of the SOB for the CMA (see section 5.7.3.). This will add to the safety database.

The CAT considers the following measures necessary to address the missing safety data in the context of a conditional MA:

In order to confirm the efficacy and safety of Adstiladrin in adult patients with BCG-unresponsive NMIBC with CIS with or without papillary tumours, the MAH shall submit the primary clinical trial report results of ABLE-22, an ongoing phase III randomised, multi-centre, open-label trial, including from the nadofaragene firadenovec monotherapy Arm 1: i) the CR rate at month 3 (excluding re-induction) and duration of these responses; and ii) safety data.

The CHMP endorses the CAT conclusion on clinical safety as described above.

2.7. Risk Management Plan

2.7.1. Safety concerns

• **Summary of safety concerns**	
• Important identified risks	• None
• Important potential risks	• Disseminated adenovirus infection
• Missing information	• Long-term safety

2.7.2. Pharmacovigilance plan

No additional pharmacovigilance activities

2.7.3. Risk minimisation measures

Safety concern	**Risk minimisation measures**	**Pharmacovigilance activities**
• ***Important identified risks***		
• None		
• ***Important potential risks***		
• Disseminated adenovirus infection	• **Routine risk communication:** • The risk related to disseminated adenovirus infection is presented in Section 4.4 of the EU-SmPC.	• **Routine pharmacovigilance activities beyond adverse reactions**

	• **Routine risk minimisation activities recommending specific clinical measures to address the risk:** • Instructions that healthcare professionals who are immunosuppressed or immune-deficient should not prepare, administer, or come into contact with Adstiladrin are provided in Sections 4.2, 4.4 and 6.6 of the EU-SmPC and in Section 2 of the EU-PL. Instructions that immunocompromised patients should not come into contact with Adstiladrin are also provided in Section 4.4 of the EU-SmPC. • Instructions that urinary tract infection should be excluded before each bladder instillation are provided in Section 4.4 of the EU-SmPC and in Section 2 of the EU-PL. • Instructions that patients should add virucidal agent to the toilet bowl are provided in Sections 4.2, 4.4, and 6.6 of the EU-SmPC and in Section 3 of the EU-PL. • Instructions that male patients should use a barrier protection contraceptive method during sexual intercourse during treatment with Adstiladrin and for 3 months following the last dose are provided in Sections 4.4 and 4.6 of the EU-SmPC and in Section 2 of the EU-PL. Male patients should also not donate sperm during this period (Section 2 of the EU-PL). • Instructions that female patients of childbearing potential should use an effective (double) contraceptive method during sexual intercourse during treatment with Adstiladrin	**reporting and signal detection:** • None proposed. • **Additional pharmacovigilance activities:** None proposed.

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	and for 6 months following the last dose are provided in Sections 4.4 and 4.6 of the EU-SmPC and in Section 2 of the EU-PL. This applies also to female partners of childbearing potential of male patients. • Instructions that patients treated with Adstiladrin should not donate blood, organs, tissues, and cells for transplantation are provided in Section 4.4 of the EU-SmPC and in Section 2 of the EU-PL. • **Other routine risk minimisation measures beyond the product information:** • Adstiladrin will only be available by restricted medical prescription. • Adstiladrin will be administered in highly specialised clinical settings.	
• ***Missing information***		
• Long-term safety	• **Other routine risk minimisation measures beyond the product information:** • Adstiladrin will only be available by restricted medical prescription. • Adstiladrin will be administered in highly specialised clinical settings.	• **Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** • None proposed. • **Additional pharmacovigilance activities:** • None proposed.

• EU: European Union; PL: package leaflet; SmPC: summary of product characteristics

2.7.4. Conclusion

The CAT considers that the risk management plan version 0.3 is acceptable.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 16 December 2025. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request to omit certain particulars from the labelling as per Art.63.3 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The group accepted the proposal to use minimum particulars in the 20mL vial label. No comments were made to the proposal.

The particulars to be omitted as per the QRD Group decision described above will however be included in the Annexes published with the EPAR on EMA website and translated in all languages but will appear in grey-shaded to show that they will not be included on the printed materials.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Adstiladrin (Nadofaragene firadenovec) is included in the additional monitoring list as:

- It contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU;
- It is approved under a conditional marketing authorisation, under Article 14-a of Regulation (EC) No 726/2004]

Therefore, the summary of product characteristics and the package leaflet include a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of

new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

The agreed indication reflecting the data evaluated is:

Nadofaragene firadenovec as monotherapy is indicated for the treatment of adult patients with Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumours.

3.1.1. Disease or condition

Bladder cancer is the 9th most common cancer type worldwide, with approximately 614,300 cases and 220,596 deaths reported in 2022 (Globocan), of which 224,777 and 70,383, respectively, in Europe.

Bladder cancer is classified into two types based on the degree of invasion into the bladder wall, either as muscle**CIS** (carcinoma in situ) which **is always graded as HG**.

3.1.2. Available therapies and unmet medical need

The treatment of NMIBC focuses on preventing disease recurrence and halting progression to MIBC and metastasis (EAU NMIBC Guidelines). HG papillary tumours (Ta/T1) and CIS have a high risk of progression to MIBC. Intravesical BCG is the standard of care treatment for patients with HG NMIBC.

Transurethral resection of bladder tumour (TURBT) is recommended to surgically remove all visible tumours in patients with (high-risk) NMIBC (EAU NMIBC Guidelines). CIS, however, cannot be resected completely. For these patients, BCG therapy is recommended after TURBT to ablate the tumour and reduce the risk of recurrence and progression. For high-risk papillary tumours, the tumours are visible and normally can all be removed by TURBT. Therefore, the purpose of adjuvant BCG therapy is preventative against future tumour recurrence and progression.

The category of BCG-unresponsive disease is defined in guidelines to be able to specify a subgroup of patients with NMIBC where additional BCG is unlikely to provide benefit, i.e., a subgroup of patients suitable for single-arm trials (SATs) (2018 FDA guidance, EAU NMIBC Guidelines; ESMO Guideline). Until recently, patients with BCG-unresponsive NMIBC could in the EU only be offered off-label bladder-sparing options such as intravesical immunotherapy, intravesical chemotherapy (single-agent or combination therapy), device-assisted therapy, or combination chemo-immunotherapy. On 17 February 2026, a conditional marketing authorisation was issued for the medicinal product Anktiva for the treatment of adult patients with BCG-unresponsive NMIBC with CIS with or without papillary tumours, in combination with BCG (Anktiva SmPC). Radical cystectomy (RC) still is the recommended treatment option for these patients (EAU NMIBC Guidelines). RC is, however, associated with significant risks including a considerable negative impact on QoL. As a result, many patients are considered ineligible for RC by their treating physician or may be unwilling to undergo the procedure. There still is, therefore, a high unmet need for effective and safe alternative non-surgical treatment options for patients with BCG-unresponsive NMIBC.

3.1.3. Main clinical studies

The pivotal study rAd-IFN-CS-003 (study CS-003; NCT02773849; Boorjian et al. Lancet Oncol. 2021) was an open-label, SAT to evaluate the safety and efficacy of nadofaragene firadenovec administered intravesically to adult patients with HG BCG-unresponsive NMIBC.
The primary objective of the trial was to evaluate the complete response (CR) rate in patients with CIS (with or without papillary disease; the CIS cohort), with secondary objectives including duration of CR and incidence of HG recurrence-free survival (RFS) in patients with papillary disease only (without CIS; the papillary disease cohort). Enrolled patients could receive up to four intravesical instillations of nadofaragene firadenovec at 3-month intervals during the primary 12-month part of the trial, provided there was no recurrence of HG disease. Each instillation was administered at the same dose level of 3×10^{11} vp/mL (75 mL). Every three months patients were evaluated for evidence of HG disease recurrence by cytology and cystoscopy, and biopsies were performed if clinically indicated (but mandatory at Month 12).
Following the initial 12-month treatment period, patients entered a 4-year follow-up period. Patients with no evidence of HG disease recurrence at Month 12 were offered continued treatment with nadofaragene firadenovec up to 5 years from the first instillation if considered appropriate by their treating physician. Patients with evidence of HG disease were discontinued from treatment but were followed up in the trial for collection of long-term safety, cystectomy, and survival data for up to 5 years after the first instillation of nadofaragene firadenovec.

3.2. Favourable effects

Primary endpoint – Incidence of CR – In the CIS cohort Efficacy Analysis Set (n=103), 55 (53.4%) patients (95% CI: [43.3, 63.3]) achieved a CR at Month 3, with the lower bound of the 95% CI exceeding 27%. The null hypothesis specified in the protocol of a true response rate of 27% was, therefore, rejected and the primary endpoint was met.

The incidence of CR in the CIS cohort was similar for most subgroups, e.g., the 54.4% incidence of CR in the subgroup of patients with CIS *only* (without papillary disease) was similar to the 50.0% incidence of CR in the subgroup of patients with CIS *with* papillary disease.

Key secondary endpoint – Durability of CR – The median duration of CR was 9.7 months (95% CI: 9.2, 24.0) measured from time initial CR was achieved (at Month 3). The actual probability of duration of CR for at least 6, 12, 18, 24, 36, and 48 months (i.e., as percentage of patients form the CIS cohort at each specific time point) was 40.1%, 24.2%, 21.4%, 19.4%, 13.6%, and 10.7%, respectively.

3.3. Uncertainties and limitations about favourable effects

The pivotal study CS-003 is a SAT which limits the ability to contextualise the efficacy results and to avoid bias. The achieved primary endpoint of CR rate at any time (that should in fact be interpreted as 'CR at 3 months', with no mandatory biopsy at the time) allows the isolation of a treatment effect that could not occur without an active treatment. There was no adjustment for multiplicity and, therefore, no type I error control for (any of) the secondary endpoints. The SAT design of Study CS-003 severely hampers the interpretability of the (other) secondary endpoints of incidence of HG RFS, incidence of cystectomy, and OS. The above reported limitations will be addressed in the context of a specific obligation (SOB) to submit the results from arm 1 of study ABLE-22.

Due to its SAT design, study CS 003 cannot determine the risks associated with patients who are eligible for RC, but choose to defer surgery in favour of bladder-preserving treatment with nadofaragene firadenovec. Consequently, the study cannot address the concern that postponing RC

may allow the disease to progress to a stage at which curative surgery is no longer feasible, thereby causing patients to lose the window of opportunity for potentially curative treatment. Data provided, however, justify an estimate of this risk based on the Adstiladrin clinical study program as discussed in the efficacy evidence. In total, 46 (43%) of the 107 CIS patients in the Safety Analysis Set underwent cystectomy, including 16 patients that initially achieved CR at Month 3 and 30 patients that did not. Pathologic data were available for 39 of these 46 patients. An appropriately worded warning has been included in section 4.4 of the SmPC.

With protocol version 5.0 (dated 29 January 2018), there were significant changes to the design of the pivotal study CS-003 during its conduct, i.e., a change to the primary endpoint, along with a resulting change to the patient population on which the primary endpoint analysis was based, and the final sample size of the trial. Furthermore, a new (key) secondary endpoint was added and there were revisions to other secondary endpoints. Such major changes to an ongoing single pivotal study add additional uncertainty of heterogeneity in the data. The results of performed post-hoc efficacy analyses performed on 77 CIS patients enrolled prior to the protocol change do not raise reasons for concern as they indicate that the changes were not driven by available internal data. Furthermore, comprehensive efficacy data, addressing the above reported uncertainties, will be provided from the ongoing confirmatory study ABLE-22 as SOB.

3.4. Unfavourable effects

The Safety Analysis Set consisted of 157 patients treated with nadofaragene firadenovec at a dose regimen of 3×10^{11} vp/mL (75 mL) every 3 months in the pivotal Study CS-003. Forty-two patients continued to receive more than 5 intravesical administrations of nadofaragene firadenovec and have been treated longer than 52 weeks.

Overall, 146 (93.0%) patients experienced at least 1 AE during the trial. The most frequently reported AEs (≥10% of patients) by PT were instillation site discharge (52 [33.1%] patients), fatigue (37 [23.6%] patients), bladder spasm (31 [19.7%] patients), micturition urgency (29 [18.5%] patients), haematuria (26 [16.6%] patients), pyrexia (25 [15.9%] patients), dysuria (25 [15.9%] patients), chills (24 [15.3%] patients), headache (24 [15.3%] patients), urinary tract infection (23 [14.6%] patients), and diarrhoea (17 [10.8%] patients).

Overall, 31 (19.7%) patients experienced at least 1 **Grade 3 AE**: 21 (19.6%) patients in the CIS cohort and 10 (20.0%) patients in the papillary disease cohort. Almost 20% patients (19.7%) experienced Grade 3 AEs, being hypertension the most frequently reported (2.5%). Overall, 19 (12.1%) of the patients reported SAEs most of them were not related with the route of administration except for haematuria being systemic effects. Ten SAEs in 7 patients were cardiac disorders (coronary artery disease, atrial flutter, arrhythmia, pericarditis, cardiac failure). In total, 3 (1.9%) patients had a **Grade 4 AE**, and no patients had a Grade 5 AE.

The most **frequently reported trial drug-related AEs** as per investigator assessment (in ≥10% of patients in either cohort) by PT were installation site discharge 39 [24.8%] patients), fatigue (31 [19.7%] patients), bladder spasm (26 [16.6%] patients), micturition urgency (25 [15.9%] patients), dysuria (19 [12.1%] patients), chills (18 [11.5%] patients), pyrexia (16 [10.2%] patients), headache (14 [8.9%] patients), pollakiuria (12 [7.6%] patients), and diarrhoea (10 [6.4%] patients).

In total, the majority of trial drug-related AEs as per investigator assessment had a short **median duration** (≤2.0 days). However, fatigue (31 [19.7%] patients) and pollakiuria (12 [7.6%] patients) had a median duration of 11.0 and 54.5 days, respectively.

A total of 19 (12.1%) patients experienced **SAE(s)**. The reported SAEs were all of Grade 1-3, with the exception of two Grade 4 SAEs (anaphylactic reaction and sepsis).

The most common trial drug-related AEs that occurred in most patients (**aged ≥65 years**) were installation site discharge (31 [26.1%] patients), fatigue (26 [21.8%] patients), bladder spasm (19 [16.0%] patients), and micturition urgency (17 [14.3%] patients).

The most common trial drug-related AEs that occurred in most patients (**aged <65 years**) were micturition urgency (8 [21.1%] patients), installation site discharge (8 [21.1%] patients), and bladder spasm (7 [18.4%] patients).

Laboratory abnormalities were observed for 28 of the subjects in the phase 3 study, including elevated glucose, high triglycerides level, increased ALT, AST and abnormal amounts of leukocytes and nitrites in urine, and abnormal presence of occult blood in the urinalysis.

3.5. Uncertainties and limitations about unfavourable effects

The pivotal study (rAd-IFN-CS-003) was a single-arm trial which makes it difficult to establish a causal relationship between nadofaragene firadenovec and reported adverse events. Moreover, more than a half of patients (63.1%) did not complete the initial 12-month treatment period, and the median number of dosing instillations received was limited (2.0; range: 1.0 to 21.0) for the overall population.

Even though the long-term safety database is considered somehow limited at this stage, the available safety data do allow for a preliminary characterization of the safety profile of nadofaragene firadenovec in the context of a conditional MA. Additional safety data including comparative data will be provided as part of study ABLE 22 (SOB).

3.6. Effects Table

Table 43. Effects Table for nadofaragene firadenovec for the treatment of adult patients with high-grade (HG), Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) (data cut-off for main phase of Study CS-003: 21 June 2019; and for long-term follow-up: 26 June 2023).

Effect	Short Description	Unit	Treatment	Uncertainties/ Strength(s) of evidence	References
Favourable Effects					
Incidence of CR (at Month 3)	In the CIS cohort (n=103), i.e., patients with CIS with or without papillary disease	%	53.4 (95% CI: 43.3, 63.3)	Strength(s): - eligibility criteria and primary endpoint (analysis population) aligned with relevant guidance Uncertainties: - SAT design - limited number of patients - significant changes to design during study - achieved CR rate with limited durability	Table 11; Table 12**Table 12**
Median duration of CR		months	9.7 (95% CI: 9.2, 24.0)		Table 13; Figure 4

Effect	Short Description	Unit	Treatment	Uncertainties/ Strength(s) of evidence	References
Unfavourable Effects					
AEs Most frequent AEs	All causes (drug related) Instillation site discharge Fatigue Bladder spasm Micturition urgency Dysuria	%	93.0 (70.7) 33.1 (24.8) 23.6 (19.7) 19.7 (16.6) 18.5 (15.9) 15.9 (12.1)	Uncertainties: - SAT design impairs causality assessment - limited number of patients	Table 31; Table 32; Table 33
Grade ≥3 AEs	All causes (drug related)	%	21.6 (3.8)		Table 31
Serious AEs	All causes (drug related)	%	12.1 (0.6)		Table 31; Table 35

Abbreviations: AE: adverse event; CIS: carcinoma in situ; CR: complete response; HG: high-grade; RFS: recurrence-free survival, SAT: single-arm trial.
Notes: For the 'Unfavourable effects', the Study CS-003 Safety Analysis Set (n=157) was used.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The current application is mainly based on the results of pivotal single-arm study CS-003 in a limited number of patients. The observed effect on the (primary endpoint of) rate of CR at 3 months in the primary endpoint analysis population of patients with CIS (with or without papillary disease; the CIS cohort) is considered clinically relevant, although the durability of the CRs is somewhat limited. All in all, however, the observed activity is considered clinically relevant.

In the frame of the development of Adstiladrin, uncertainties remain regarding the risk of disease progression for some of the patients opting for bladder preserving treatment, that would prevent them from being eligible to curative treatment through RC. Due to the SAT design, study CS 003 cannot provide an answer to this important uncertainty.

Reassurance has been provided and supported by an estimate of the risk of disease progression to an incurable stage when radical cystectomy (RC) is delayed, based on data from the Adstiladrin clinical study program. Nevertheless, a warning has been added to section 4.4 of the SmPC advising that each case be carefully evaluated clinically and that patients be closely monitored during treatment with Adstiladrin, so that timely bladder surgery can be considered if an adequate treatment response is not achieved. The SAT design of Study CS-003 severely hampers the interpretability of the (other) secondary endpoints of incidence of HG RFS, incidence of cystectomy, and OS. Moreover, there were significant changes to the design of Study CS-003 during its conduct which add additional uncertainty. Furthermore, the safety profile of the intravesical administration of nadofaragene firadenovec resembles the expected safety profile of a locally administered immunostimulatory therapy in the bladder. Most AEs were related to the local and systemic activation of the immune system. Overall, the majority of AEs were limited to Grade 1 or 2. The duration of the AEs was in general limited to a short period following the drug instillation. Also given the limited frequency of four intravesical administrations per year, the safety profile is generally considered to be acceptable. However, an insufficient number of patients have been exposed to treatment beyond one year. Therefore, the long-term safety profile has not been comprehensively characterised.

With the efficacy and safety limitations described above, the dossier submitted is therefore not considered to be comprehensive. In order to confirm the efficacy and safety of nadofaragene firadenovec in the claimed indication, the applicant will submit the results of the study ABLE-22 imposed as a SOB in the context of a CMA, see section 3.7.3 for further details.

3.7.2. Balance of benefits and risks

The benefit/risk balance of Adstiladrin is positive.

3.7.3. Additional considerations on the benefit-risk balance

Discussion on the non-comprehensiveness of data in the context of a Conditional Marketing Authorisation

In regard to the request for a conditional marketing authorisation, the CHMP considers that the following is to be accounted in terms of non-comprehensiveness of the dossier:

Considering that i) the current application is mainly based on the results of a pivotal SAT in a limited number of patients, ii) durability of the CRs seems somewhat limited in the follow up related to the provided study, iii) uncertainties remain in regard to the possible progression of disease while in treatment and the missing window of opportunity for the RC surgical treatment of some of the patients, iv) significant changes to the design of Study CS 003 during its conduct added additional uncertainty and v) only a limited number of patients have been exposed to treatment beyond one year, the provided clinical data package is not considered comprehensive.

The applicant initially applied for a standard MA but in view of the above, this was not considered appropriate. The applicant acknowledged the non-comprehensive nature of the clinical dossier and provided a justification (see section 2.3 above) to support the eligibility of Adstiladrin for a conditional marketing authorisation (CMA).

CAT/CHMP Conclusions and recommendation on Conditional Marketing Authorisation

As comprehensive data on the product are not available, as discussed above, a conditional marketing authorisation was proposed by the CAT during the assessment, after having consulted the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it is indicated for the treatment of a seriously debilitating and life-threatening disease considering the possible progression towards cystectomy with the relevant impact this have on the quality of life and possible post-surgery complications.

Furthermore, the CAT considers that the product fulfils the requirements for a conditional marketing authorisation:

- the benefit-risk balance of the medicine is positive;

From a clinical point of view, the observed efficacy in the CIS cohort of Study CS 003 is considered clinically relevant with an acceptable CR rate supported by a duration of response and matched by a safety profile which, despite a short follow up can be considered acceptable, as described above.

- it is likely that the applicant will be able to provide comprehensive data (post authorisation);

The applicant proposed to submit, as a Specific Obligation (SOB) to the conditional marketing authorisation, data generated from the nadofaragene firadenovec monotherapy Arm 1 in ABLE-22 (Trial 000434; NCT06545955).

ABLE-22 is an ongoing phase 3, randomised, open-label trial to evaluate the safety and efficacy of intravesical nadofaragene firadenovec alone (arm 1) or in combination with chemotherapy (gemcitabine/docetaxel) (arm 2) or immunotherapy (pembrolizumab)(arm3) in patients with HG BCG-unresponsive NMIBC (i.e., CIS with or without Ta/T1 papillary disease). Patients will be randomised in a 2:2:1 ratio to each of the three treatment arms, with approximately 100 patients randomised to the nadofaragene firadenovec monotherapy Arm 1. In patients who do not achieve a CR at Month 3 (and have an absence of T1 or progression to T2) re-induction is allowed. The primary endpoint is CR at any time. In contrast to the definition of CR in Study CS-003, LG recurrence is not considered a treatment failure in study ABLE-22. A pre-specified supplementary analysis of CR at Month 3 will, however, be performed based on the study CS-003 CR definition. The secondary endpoints include CR at Month 3 and Month 6, and durability of CR. For DoCR, a sensitivity analysis will be performed that is in line with EMA guidance, i.e., not censoring patients/events after missing 2 or more consecutive assessment visits and/or initiation of additional anti-cancer treatment.
The main part of the trial is from the first nadofaragene firadenovec instillation up to month 12 (with patients receiving four doses of nadofaragene firadenovec), with the expected last patient last visit in 30 September 2028. Optional treatment can continue up to Month 24, with the expected last patient last visit Q1 2031. The initial primary clinical trial report will be prepared once all patients are evaluable for the secondary endpoint CR at Month 6 and is estimated to be available on 31 March 2029. This is considered an appropriate timeframe for the provision of comprehensive data.

The safety data from the nadofaragene firadenovec monotherapy Arm 1 will also add to the safety database. Study ABLE 22 will gather evidence on the potential risk and long-term safety given its 36-month duration including follow-up. As such, study ABLE-22 is included in the RMP under Part IV (post authorisation efficacy study) justifying the presence of the missing information 'long-term safety' in the RMP. Thereby, the positive B/R of nadofaragene firadenovec could be confirmed.

Given the CR rate at Month 3 in Study CS-003 of 53.4%, the overall patient population for the evaluation of efficacy in the indication's target population is expected to increase to over 150 patients, i.e., 103 from study CS-003 and approximately 53 patients from the nadofaragene firadenovec monotherapy Arm 1 in study ABLE-22. For the evaluation of safety, it is recommended that the patients who do receive re-induction could also be used. Either way, over 200 patients will be available for the safety evaluation, i.e., 157 from Study CS-003 and either ~50 or 100 patients from the nadofaragene firadenovec monotherapy Arm 1 in study ABLE-22. In totality, these efficacy and safety data could be considered comprehensive.

- the fulfilment of unmet medical need (UMN) or provide major therapeutic advantage over existing treatments;

Patients with BCG-unresponsive NMIBC are at high risk for disease progression to MIBC. In the EU, until recently no medicinal product was approved for treating these patients, and patients were treated off-label with bladder-sparing options (e.g. systemic immunotherapy, intravesical chemotherapy) which have been though considered of limited value. On 17 February 2026, the medicinal product Anktiva was approved for a similar indication (Anktiva SmPC) as Adstiladrin. Nevertheless, surgery (RC) is/remains to be clinically discussed with patient among the options for their treatment of NMIBC even though it has associated risks, morbidity, and a negative impact on QoL (EAU NMIBC Guidelines). Furthermore, not all patients with NMIBC may be candidate/eligible for RC and there still is, therefore, a high unmet need for effective and safe alternative non-surgical treatment options for these patients.

It is therefore agreed that Adstiladrin, in view of the demonstrated activity and manageable safety profile, is a bladder sparing treatment which addresses an unmet medical need in the EU considering the above reported limitations of radical cystectomy and its associated significant clinical impact.

Furthermore, the target populations of Adstiladrin and Anktiva are considered overlapping. Anktiva has been granted a CMA, and Adstiladrin is expected to address the UMNs to a similar or greater extent than Anktiva (EMA/CHMP/509951/2006, Rev.1) considering that efficacy and safety data for Adstiladrin are generally comparable to those of Anktiva, when taking into account aspects such as i) the limitations of cross-study comparisons; ii) the limitations of the single-arm pivotal studies of both medicinal products; iii) the differences between the primary endpoints in both pivotal studies; iv) the wide CIs of the CR rate and DoCR of both medicinal products.

As a result, Adstiladrin could also be granted a CMA in its final indication since it is expected to address the UMN i) of the specific selected clinical population over the available treatments (Radical cystectomy) and ii) addressing the UMN to a similar or greater extent compared to the recently approved CMA for Anktiva.

- the benefits to public health of the immediate availability of the medicinal product outweigh the risks inherent in the fact that additional data are still required.

Considering the positive B/R and that an unmet medical need will be addressed in the applied indication as described above, the immediate availability of Adstiladrin outweighs the risks inherent to the fact that additional data are needed.

The CHMP endorses the CAT conclusion on conditional marketing authorisation as described above.

3.8. Conclusions

The overall benefit/risk balance of Adstiladrin is positive in the finally agreed indication, subject to the conditions stated in section 'Recommendations'.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit- risk balance of Adstiladrin is favourable in the following indication(s):

Adstiladrin is indicated as monotherapy for the treatment of adult patients with Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumours.

The CAT therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Adstiladrin as monotherapy in the treatment of adult patients with Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumours, is favourable and therefore recommends the granting of the conditional marketing authorisation subject to the following conditions:

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of PSUR for this medicinal product are set out in Article 9 of Regulation (EC) No 507/2006 and accordingly, the marketing authorization holder (MAH) shall submit PSURs every 6 months.

The requirements for submission of PSURs for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder (MAH) shall submit the first PSUR for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	**Due date**
In order to confirm the efficacy and safety of Adstiladrin in adult patients with BCG-unresponsive NMIBC with CIS with or without papillary tumours, the MAH shall submit the primary clinical trial report of ABLE-22, an ongoing phase III randomised, multi-centre, open-label trial, including from the nadofaragene firadenovec monotherapy Arm 1: i) the CR rate at month 3 (excluding re-induction) and duration of these responses; and ii) safety data.	31 March 2029

The CHMP endorses the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation as described above.

New Active Substance Status

Based on the review of available data on the active substance, the CAT considers that nadofaragene firadenovec is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorses the CAT conclusion on the new active substance status claim.