Janssen Vaccines & Prevention B.V. *

Pharmacokinetics Written Summary

MODULE 2.6.4

VAC31518 JNJ-78436735

Prophylactic COVID-19 Vaccine

* Janssen Vaccines & Prevention B.V. is a Janssen pharmaceutical company of Johnson & Johnson and is hereafter referred to as the sponsor.

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LIST OF ABBREVIATIONS

Ad26	adenovirus type 26
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
DNA	deoxyribonucleic acid
E1	early region
EDTA	ethylenediaminetetraacetic acid
EMA	European Medicines Agency
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
(b) (4)	
(b) (4)	
IM	intramuscular
NZW	New Zealand white
OECD	Organization for Economic Co-operation and Development
(b) (4)	
(b) (4)	
q-PCR	quantitative polymerase chain reaction
(b) (4)	
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
(b) (4)	
(b) (4)	
vp	virus particles
WHO	World Health Organization

Vectors and (Candidate) Vaccines

Ad26.COV2.S	Ad26 vector encoding a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Spike protein
Ad26 (b) (4)	Ad26 vector encoding (b) (4)
Ad26 (b) (4)	Ad26 vector encoding (b) (4)

1. BRIEF SUMMARY

Ad26.COV2.S (also known as VAC31518 or JNJ-78436735) is a monovalent, recombinant, replication-incompetent adenovirus type 26 (Ad26) vectored vaccine encoding a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Spike protein. It is being developed for prophylactic immunization against coronavirus disease 2019 (COVID-19), which has spread rapidly and globally since its emergence.

This Pharmacokinetics Written Summary provides an overview of the available pharmacokinetic data in support of the Ad26.COV2.S development. In accordance with the World Health Organization (WHO) Guidelines on Nonclinical Evaluation of Vaccines [6], pharmacokinetic studies are usually not needed for vaccines. However, in line with the European Medicines Agency (EMA) Guideline on quality, nonclinical and clinical aspects of live recombinant viral vectored vaccines [1] and the FDA Guidance on considerations for plasmid DNA vaccines for infectious disease indications [2], biodistribution studies have been conducted to assess the distribution, persistence, and clearance of the Ad26 vector (platform) following intramuscular (IM) injection.

The biodistribution profile of the Ad26 vector platform has been evaluated in the rabbit using an Ad26-based (b) (4) vaccine, i.e., Ad26 (b) (4) (Ad26 vector encoding the (b) (4) and an Ad26-based (b) (4) (b) (4) vaccine, i.e., Ad26 (b) (4) (Ad26 vector encoding (b) (4) (b) (4). An overview of the biodistribution studies is provided in Table 1. No pharmacokinetic or biodistribution studies have been conducted with Ad26.COV2.S specifically.

Table 1: Overview of Biodistribution Studies in Support of the Development of COVID-19 Vaccine Candidate Ad26.COV2.S

Study Type	GLP	Route	Species	Vaccines Administered	Number of Injections	Study No.
Biodistribution	Yes	IM	NZW rabbits	Ad26 (b) (4)	1-dose	(b) (4)
Biodistribution	Yes	IM	NZW rabbits	Ad26 (b) (4)	1-dose	(b) (4)

GLP = Good Laboratory Practice; IM = intramuscular; NZW = New Zealand white

The biodistribution studies for the Ad26 vector platform were conducted in compliance with U.S. FDA GLP Regulations (21 CFR Part 58) and/or the Organization for Economic Cooperation and Development (OECD) Principles of GLP in a country that is part of the OECD Mutual Acceptance of Data Process, and include the appropriate documentation. Information on e.g., study GLP status and test facility identity are provided in the Pharmacokinetics Overview Table in Mod2.6.5.1.

The biodistribution studies with Ad26 (b) (4) and Ad26 (b) (4) were conducted using the IM route, which is also the intended route for use of Ad26.COV2.S in humans.

The studies were done in rabbits as this is a widely accepted species to assess the nonclinical safety of vaccines. In nonclinical studies, Ad26-based vaccines (including Ad26.COV2.S, Ad26 (b) (4) and Ad26 (b) (4)) were shown to elicit immune responses in the animals, indicating the rabbit as a relevant nonclinical species for these vaccines. In addition, rabbits have sufficient muscle mass to receive a full human vaccine dose via the IM route with a single injection.

In the biodistribution studies with Ad26 (b) (4) (administered at a dose of 5×10^{10} virus particles [vp]) and Ad26 (b) (4) (administered at a dose of 1×10^{11} vp), animals were sacrificed on Days 11, 61, or 91 (Ad26 (b) (4)), and on Days 11, 90, 120 or 180 (Ad26 (b) (4)) following single IM injection. Tissues from these animals were harvested for analysis of Ad26 vector DNA using a quantitative polymerase chain reaction (q-PCR) assay. The Ad26 vector did not widely distribute following IM administration in the animals. Vector DNA was primarily detected at the site of injection, draining lymph nodes and (to a lesser extent) the spleen. Comparing the results from the respective necropsy timepoints, the number of animals with positive tissues and/or the vector copy number present in those positive tissues declined to levels close to, or below the detection limit of the q-PCR methods used, indicating clearance of the Ad26 vector from the animals/tissues. In addition, both Ad26-based vaccines tested in the biodistribution studies showed a similar pattern of (systemic) distribution and clearance when delivered via the IM route in the rabbit, despite carrying different transgene inserts.

The Ad26 vector backbone used for Ad26.COV2.S is identical to the vector backbone of the Ad26-based vaccines that were tested in the available biodistribution studies (i.e., Ad26 (b) (4) and Ad26 (b) (4)). The only difference between the vectors, apart from the encoded antigen transgene, is the insertion of a (b) (4) in the cytomegalovirus (CMV) promoter sequence of the transgene expression cassette of Ad26.COV2.S. This is not considered to impact the biodistribution profile of the Ad26 vector.

In conclusion, the Ad26 vector shows a limited distribution profile following IM injection. Clearance (reflected by a downward trend in number of positive tissues and vector copies over time, to levels close to, or below the detection limit of the q-PCR methods used) of the Ad26 vector was observed, indicating that the vector does not replicate and/or persist in the tissues following IM injection. These platform biodistribution data obtained from Ad26 (b) (4) and Ad26 (b) (4) are considered sufficient to inform on the biodistribution profile of Ad26.COV2.S, for which the same (replication-incompetent) Ad26 vector backbone is used. This position has been confirmed and agreed in a previous Scientific Advice by EMA (b) (4) and CBER (b) (4) (b) (4) It is further noted that the same platform biodistribution data were part of the MAA file for the Ebola vaccine component Ad26.ZEBOV (EU/1/20/1444/001).

2. METHODS OF ANALYSIS

In the biodistribution studies, specific PCR assays were used to detect and quantify Ad26-vector DNA in various tissues collected at specified time points following vector administration.

In GLP study No. (b) (4) (Ad26 (b) (4)), a TaqMan-based q-PCR assay was used to detect a target sequence of the Ad26 (b) (4) vector. The detection limit of this assay was 10 copies of Ad26 (b) (4) µg genomic DNA; the lower limit of quantification was 50 copies of Ad26 (b) (4) µg genomic DNA. A description of the assay is available in the report.

In GLP study No. (b) (4) (Ad26 (b) (4)), a TaqMan-based q-PCR assay was used for quantitation of a specific target sequence of the Ad26 (b) (4) vector. The detection limit of the assay was 7.1 copies/µg genomic DNA; the lower limit of quantification was 28.6 copies/µg genomic DNA. A method validation summary is available in the report.

3. ABSORPTION

Not applicable for vaccines.

4. DISTRIBUTION

To assess distribution, persistence, and clearance of the Ad26 viral vector (platform), IM biodistribution studies have been conducted in rabbits using an Ad26-based (b) (4) vaccine, i.e., Ad26 (b) (4) and an Ad26-based (b) (4) vaccine, i.e., Ad26 (b) (4). A comparison of the Ad26 (b) (4) and Ad26 (b) (4) biodistribution data is discussed in Section 9.

4.1. Ad26 (b) (4) (Study (b) (4))

Study Title (Report Date)	Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits (14 September 2007)
Conducting Laboratory, Location	(b) (4)
Sponsor	Beth Israel Deaconess Medical Center, Massachusetts, United States
GLP Compliance	Yes
Study Report	(b) (4)
Tabulated Summary	Mod2.6.5.5A

New Zealand White (NZW) rabbits were administered placebo or Ad26 (b) (4) at $5x10^{10}$ vp via a single IM injection into the right hind thigh muscle on Day 1 (Table 2). Parameters evaluated during the study included clinical and cage-side observations, body weights, and biodistribution. Necropsies were performed on 3 rabbits/sex in the placebo group and 5 rabbits/sex in the Ad26 (b) (4) group on Days 11, 61, and 91 to collect tissues for biodistribution analysis. These timepoints are in line with other biodistribution studies conducted with adenovirus type 5 (Ad5) and type 35 (Ad35) based vaccines [5] and were selected to cover sufficient time to assess clearance of the vector.

The following tissues were collected: blood, ovaries/testes, liver, thymus, heart, lung, kidney, spleen, mesenteric and iliac lymph nodes, bone marrow, brain, and skin, subcutis and muscle at the injection site. All tissues collected on Day 11, 61, and 91 were analyzed for the presence of the Ad26 (b) (4) DNA using a q-PCR method.

Table 2: Experimental Design of Biodistribution Study with Ad26 (b) (4) (Study (b) (4)

Group	Test Article	Dose Level	Dose Volume	Route	Scheduled Sacrifice Timepoint		
					Day 11	Day 61	Day 91
1	Placebo[a]	0	0.5 mL	IM	3/sex	3/sex	3/sex
2	Ad26 (b) (4)	5×10^{10} vp	0.5 mL	IM	5/sex	5/sex	5/sex

[a] (b) (4)
(b) (4)
(b) (4) mL formulated in (b) (4)
(b) (4)
(b) (4) IM = intramuscular; vp = virus particles

A single IM injection of Ad26 (b) (4) in male and female NZW rabbits was well tolerated with no effect on clinical/cage-side observations, or body weights.

For all Group 1 samples collected at Day 11, Day 61 or Day 91, Ad26 (b) (4) vector DNA was below the limit of detection of the assay (<10 copies/µg DNA).

Analysis of Group 2 samples on Day 11 indicated that Ad26 vector DNA was primarily localized in the injection site muscle, draining (iliac) lymph nodes and to a lesser extent the spleen. Ad26 (b) (4) vector DNA was below limit of detection in all other organs, except for one animal that showed a low signal in the injection site skin at a level below the lower limit of quantification (50 copies/µg DNA).

In the animals sacrificed on Day 61, the Ad26 vector DNA was no longer detected in the spleen, while vector DNA in the injection site muscle and iliac lymph nodes was detected at a reduced incidence and quantity compared to Day 11.

On Day 91, detection of the vector was limited to the injection site muscle and iliac lymph nodes in 2 of 10 treated animals and was below the limit of detection in all other examined tissues or animals.

4.2. Ad26 (b) (4) (Study (b) (4))

Study Title (Report Date)	Single Dose Biodistribution Study of Ad26 (b) (4) by Intramuscular Injection in Rabbits with up to 180 Days Observation Period (08 May 2019)
Conducting Laboratory, Location	(b) (4)
Sponsor	Janssen Infectious Diseases-Diagnostics, Belgium
GLP Compliance	Yes
Study Report	(b) (4)
Tabulated Summary	Mod2.6.5.5B

NZW rabbits were administered placebo or Ad26 (b) (4) at $1x10^{11}$ vp via a single IM injection into the right thigh. In one study arm, Ad26 (b) (4) was dosed in combination with 150 µg (b) (4) protein as a single injection (Table 3). The following parameters and end points were evaluated in this study: clinical signs, body weights, gross necropsy findings and biodistribution using q-PCR analysis. Tissues for q-PCR analysis were collected from 3 rabbits/sex in the placebo group and 5 rabbits/sex in the Ad26 (b) (4) groups on Days 11, 90, 120, and 180. To assess the clearance of the vector beyond 90 days (i.e., the last sampling

timepoint in the previous study with Ad26 (b) (4) , two additional later timepoints, Day 120 and 180, were included.

The following tissues were collected: blood, ovaries/testes, liver, thymus, heart, lung, kidney, spleen, mesenteric, iliac, and popliteal lymph nodes, bone marrow, brain, skin with subcutis at the injection site, and muscle at the injection site. All tissues collected from euthanasia on Days 11, and 90 from all groups were analyzed for the presence of Ad26 vector DNA using q-PCR. From the animals sacrificed on Day 120, only iliac lymph node, injection site skin, spleen, popliteal lymph node and injection site muscle were analyzed; from the animals sacrificed on Day 180, only spleen and iliac lymph node were analyzed. From Day 120 onwards, no Group 1 samples were analyzed given that they were negative on Day 11 and Day 90.

Table 3: Experimental Design of Biodistribution Study with Ad26 (b) (4) (Study (b) (4))

Group	Test Article	Dose Level	Dose Volume	Route	Scheduled Sacrifice Timepoint			
					Day 11	Day 90	Day 120	Day 180
1	Reference item[a]	0	1 mL	IM	3/sex	3/sex	3/sex	3/sex
2	Ad26 (b) (4) [b]	1×10^{11} vp	0.5 mL	IM	5/sex	5/sex	5/sex	5/sex
3	Ad26 (b) (4) [b] + (b) (4) [c]	1×10^{11} vp + 150 µg	1 mL (of mixture)	IM	5/sex	5/sex	5/sex	5/sex

[a] Reference item: 0.9% Sodium Chloride for Injections, USP
[b] Ad26 (b) (4) 2x10[11] vp/mL (in (b) (4))
[c] (b) (4) 0.3 mg/mL (in (b) (4) (b) (4))

IM = intramuscular; vp = virus particles

There were no Ad26 (b) (4) related changes noted in clinical observations, or body weights, and there were no treatment-related gross necropsy findings.

All samples collected from Group 1 at Day 11 and Day 90 had Ad26 (b) (4) vector DNA results below the limit of detection of the assay (<7.1 copies/µg DNA).

In samples collected from Group 2 and 3 on Day 11, Ad26 (b) (4) vector DNA was primarily detected in the skin at the injection site, iliac lymph nodes, and spleen. The skin at the injection site and the iliac lymph nodes presented the highest number of vector copies. The popliteal lymph node showed a low signal (around or below the lower limit of quantification of 28.6 copies/µg DNA) in 2 animals from Group 2. One animal from Group 3 showed a signal in the liver at a level below the lower limit of quantification.

In Group 2 and 3 animals sacrificed on Day 90, Ad26 (b) (4) vector DNA was detected only in the skin at the injection site and in the iliac lymph nodes, but at a reduced incidence, as well as a lower maximum quantity of vector DNA than on Day 11.

On Day 120, Ad26 (b) (4) vector DNA was only detected at a low vector copy number (close to, or below the lower limit of quantification) in a single spleen sample, and iliac lymph nodes in 3 of 10 treated animals from Group 2.

On Day 180, detection of the vector was limited to the iliac lymph nodes in 3 of 10 treated animals in Group 2 and 2 out of 10 animals in Group 3 at a level close to, or below the lower limit of quantification and was below the limit of detection in all other examined tissues or animals.

Animals from Group 2 and Group 3 showed a similar distribution pattern.

5. METABOLISM

Not applicable for vaccines.

6. EXCRETION

Not applicable for vaccines.

7. PHARMACOKINETIC DRUG INTERACTIONS

Not applicable for vaccines.

8. OTHER PHARMACOKINETIC STUDIES

Other pharmacokinetic studies were not performed.

9. DISCUSSION AND CONCLUSIONS

Pharmacokinetic or biodistribution studies have not been conducted with Ad26.COV2.S. To assess the distribution, persistence, and clearance of the Ad26 vector (platform), the biodistribution profile of the Ad26 vector has been evaluated using Ad26 (b) (4) and Ad26 (b) (4) following IM injection in the rabbit.

The Ad26 vector contains deletions in the early region (E1) of the Ad26 genome, rendering it replication-incompetent. Ad26-based vaccines require recombinant E1 complementing cell lines, like the PER.C6 (b) (4) cells, for virus replication. Outside of these specific cellular environments, Ad26-based vaccines cannot replicate or reproduce and are therefore expected to show a limited distribution and persistence following administration. This is confirmed by the biodistribution studies in rabbits in which the distribution, persistence and clearance of Ad26-based vaccines against (b) (4) (Ad26 (b) (4) study No. (b) (4) Section 4.1) and (b) (4) (Ad26 (b) (4) study No. (b) (4) Section 4.2) have been evaluated following IM administration. As a general pattern, both Ad26 vectors showed a similar and limited biodistribution profile, as they were primarily detected at the site of injection, regional (iliac) lymph nodes and (to a lesser extent) the spleen. No Ad26 vector DNA was detected in the gonads or in the brain.

Comparing the various necropsy timepoints following IM administration (Days 11, 61, and 91 for Ad26 (b) (4) Days 11, 90, 120 and 180 for Ad26 (b) (4) Table 4), a downward trend in number of positive tissues, and/or vector copies was observed, to levels close to, or below the respective limits of detection of the q-PCR assay used, indicating clearance of the Ad26 vector from the tissues. These data further indicate that the Ad26 vector does not replicate and/or persist in the tissues following IM injection.

Comparing the injection site tissues, in study (b) (4) vector DNA was mostly detected in the injection site muscle, while in study (b) (4) vector DNA was mostly detected in the injection site skin. Nevertheless, no clear differences in the systemic distribution and clearance profile of the Ad26 vector were observed between the two studies. Therefore, despite differences in the transgene insert, it can be concluded that both Ad26 vectors showed a similar pattern of (systemic) biodistribution and clearance when delivered via the IM route at full human doses in the rabbit.

The Ad26 vector backbone used for Ad26.COV2.S is identical to the vector backbone of the Ad26-based vaccines that were tested in the available biodistribution studies (i.e., Ad26 (b) (4) and Ad26 (b) (4). Ad26.COV2.S contains a (b) (4) in the CMV promoter sequence of the transgene expression cassette. This (b) (4) was not present in Ad26 (b) (4) and Ad26 (b) (4) Insertion of the (b) (4) is not considered to impact the biodistribution profile of the Ad26 vector. Adenoviruses are non-enveloped viruses whose cell entry, and therefore tropism, is dictated via interactions of structural capsid proteins (mainly the fiber and penton base) with specific cellular receptors [4]. The adenoviral capsid is a highly complex and organized structure [3] which does not easily allow for the introduction or exchange of other proteins. The transgene expression cassette, which is inserted into the site where the early E1 gene was previously located, is thus not considered to impact on the formation or the composition of the Ad26 vector capsid, and hence tropism of the vector. As a consequence, the biodistribution profile of the Ad26 vector is considered independent of the antigen transgene/expression cassette, which is supported by the comparable distribution profile observed for Ad26 (b) (4) and Ad26 (b) (4) Therefore, the biodistribution profile observed for Ad26 (b) (4) and Ad26 (b) (4) is considered sufficient to inform on the biodistribution profile of the Ad26.COV2.S construct when administered via the same route of administration (IM).

It is noted that for the Ad26 (b) (4) and Ad26 (b) (4) biodistribution studies, the Ad26 vector was formulated in different buffer formulations. The difference in formulation buffer between Ad26 (b) (4) (formulated in (b) (4)) and Ad26 (b) (4) (formulated in (b) (4)) did not impact the overall (systemic) distribution profile of the Ad26 vector. The Ad26.COV2.S vaccine is formulated in the same buffer as Ad26 (b) (4)

Overall, the biodistribution data obtained with Ad26 (b) (4) and Ad26 (b) (4) show a limited distribution profile and indicate clearance over time of the Ad26 vector following IM injection. The biodistribution results obtained with Ad26 (b) (4) and Ad26 (b) (4) are considered sufficient to inform on the biodistribution profile of Ad26.COV2.S, for which the same (replication-incompetent) Ad26 vector backbone is used. This position has been confirmed and agreed in a previous Scientific Advice by EMA (b) (4) and CBER (b) (4). It is further noted that the same

platform biodistribution data were part of the MAA file for the Ebola vaccine component Ad26.ZEBOV (EU/1/20/1444/001).

Table 4: Comparative Table of Biodistribution Data with Ad26-based Vaccines

Vector	Tissue[a]	Range[b] (No. of animals >LLOQ) [No. of animals >LOD and <LLOQ] [c]				
		Day 11	Day 61	Day 90/91	Day 120	Day 180
Ad26 (b) (4) (study (b) (4)	Inj Site Muscle	61-11,981 (7) [1]	<LLOQ [2]	120 (1) [1]	NA	NA
	Inj Site Skin	<LLOQ [1]	-	-	NA	NA
	Iliac Ln	119-8676 (10)	84-1400 (4) [1]	50-1807 (2)	NA	NA
	Spleen	50-116 (4) [5]	-	-	NA	NA
Ad26 (b) (4) (study (b) (4)	Inj Site Muscle	<LLOQ [1]	NA	-	-	-
	Inj Site Skin	39.1-6304.3 (4) [2]	NA	280.1 (1)	-	-
	Iliac Ln	63.6-387.6 (9) [1]	NA	48.1 (1) [3]	37.9-53.4 (2) [1]	37.6 (1) [2]
	Popliteal Ln[d]	29 (1) [1]	NA	-	-	-
	Spleen	37.3-118.6 (6) [4]	NA	-	<LLOQ [1]	-
Ad26 (b) (4) (b) (4) (study (b) (4)	Inj Site Skin	88.6-272.6 (2) [3]	NA	42.7-175.1 (2)	-	-
	Iliac Ln	108.6-347.4 (9)	NA	25.9-35.3 (2) [3]	-	<LLOQ [2]
	Spleen	26.4-75.6 (7) [2]	NA	-	-	-
	Liver	<LLOQ [1]	NA	-	-	-

(a) Only tissues with vector DNA levels above limit of detection (LOD) are listed. All other tissues collected had vector DNA results below the LOD of the assay at all time points
(b) Range in copies/µg genomic DNA
(c) No. of animals out of 10 animals per group
(d) Popliteal Ln was not sampled in Ad26 (b) (4) study [study (b) (4)
\- Levels for all animals were below LOD at this time point
NA Not available
LLOQ Lower limit of quantification (50 copies/µg DNA [study (b) (4) ; 28.6 copies/µg DNA [study (b) (4)])
LOD Limit of detection (10 copies/µg DNA [study (b) (4) ; 7.1 copies/µg DNA [study (b) (4))

10. TABLES AND FIGURES

Supplemental tables and figures are included at appropriate points throughout the summary within the text; additional information is provided within the Pharmacokinetic Tabulated Summaries, located in Mod2.6.5.

11. LIST OF LITERATURE CITATIONS

Literation citations are located in Mod4.3.

1. EMA Guideline on quality, nonclinical and clinical aspects of live recombinant viral vectored vaccines (CHMP/VWP/141697/2009).

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