

Phase 1 Safety and Immunogenicity Evaluation of a Next-Generation DNA-Based Yellow Fever Vaccine

Isabel Leroux-Roels

Center for Vaccinology (CEVAC)

Ilse Coster

Centre for the Evaluation of Vaccination (CEV), Vaccine and Infectious Disease Institute

Lidia Oostvogels

Consultant for Astrivax Therapeutics, MinervaX ApS

Wilfried Dalemans

Astrivax Therapeutics

Kai Dallmeier

KU Leuven

Joke Debruyne

Astrivax Therapeutics

Simon Gussem

Center for Vaccinology (CEVAC)

Brenda Keersmaecker

Astrivax Therapeutics

Sofie Vos

Astrivax Therapeutics

Bart Jacobs

Center for Vaccinology (CEVAC)

Johan Neyts

KU Leuven

Sharon Porrez

Center for Vaccinology (CEVAC)

Bruno Speder

Astrivax Therapeutics

Frederik Stevenaert

Astrivax Therapeutics

Leen Suykens

Centre for the Evaluation of Vaccination (CEV), Vaccine and Infectious Disease Institute

Lien Eyck

Astrivax Therapeutics

Thomas Vercruysse

Astrivax Therapeutics

Gwenn Waerlop

Center for Vaccinology (CEVAC)

Kanchanamala Withanage

Centre for the Evaluation of Vaccination (CEV), Vaccine and Infectious Disease Institute

Karen Yu

Astrivax Therapeutics

Hanne Callewaert

clinical@astrivax.com

Astrivax Therapeutics

Article

Keywords:

Posted Date: October 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10850582/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: Competing interest reported. I.L.-R., S.D.G., B.J., S.P., G.W., all affiliated with CEVAC, and I.D.C., K.W. and L.S., all affiliated with CEV, conducted the SAFYR trial, for which their institution received financial compensation from Astrivax Therapeutics, the sponsor of the trial. H.C. is co-founder and the CEO of Astrivax Therapeutics. Professors J.N. and K.D. are the inventors of the Launch-iT technology, are listed in several patents (applications), are co-founders of Astrivax Therapeutics and act as scientific advisory board members to the company. H.C., K.D. and J.N. are shareholders of Astrivax Therapeutics. W.D., J.D., B.D., S.D.V., L.O., B.S., F.S., L.V.E., T.V. and K.Y. are (former) employees of or consultants for Astrivax Therapeutics, and H.C., W.D., K.D., J.D., B.D., S.D.V., J.N., , B.S., T.V., K.Y. participate in the company's ESOP. L.O. is an employee of MinervaX ApS.

Abstract

Recurrent issues with global yellow fever (YF) vaccine supply have highlighted the need for next-generation YF vaccines that come with simplified and scalable manufacturing processes. Here, we report the first clinical evaluation of Launch-iT YF, a next-generation DNA-based YF vaccine from which live-attenuated YF17D virions are produced upon administration. In this randomized, double-blind, placebo-controlled, dose-escalation trial (SAFYR), healthy adults (18-40 years) received Launch-iT YF 30 µg (N = 12), 100 µg (N = 24), 300 µg (N = 12), or placebo (N = 16). Launch-iT YF had an acceptable safety profile with limited reactogenicity. A single vaccination induced persistent coordinated humoral and cellular immune responses in participants across Launch-iT YF groups, with a response rate up to 8/11 (73%) evaluable participants. These findings supported the development of a second-generation Launch-iT YF to improve seroconversion rates, as well as the utilization of this novel technology as a rapidly adaptable vaccine platform.

ClinicalTrials.gov identifier: NCT06998004; EU CT number: 2024-511194-29

Introduction

Yellow fever (YF) is an acute viral hemorrhagic disease that is transmitted by infected mosquitoes and caused by the yellow fever virus (YFV). Although infection can be asymptomatic or self-limiting, approximately 15% of infected people develop a severe toxic phase characterized by high fever, jaundice, hemorrhage, shock and organ failure, with a case-fatality rate of approximately 50% despite supportive medical care. In the absence of effective treatments and adequate mosquito-control measures, vaccination is the cornerstone of YF prevention and control¹. Vaccines based on the live-attenuated YF17D strain, derived in the 1930s from the original wild-type Asibi isolate², are the only approved products for immunization against YF to date. As these vaccines provide lifelong protection after a single vaccination^{3–7}, they are widely regarded among the most efficacious vaccines ever developed. Nevertheless, YF remains an important public health threat, causing recurrent outbreaks with substantial morbidity and mortality, with an estimated 67 000–173 000 severe YFV infections and 31 000–82 000 deaths per year⁸. Substantial global YF17D vaccine supplies therefore remain necessary for the immunization of new birth cohorts, preventive mass-vaccination campaigns, outbreak response efforts, and vaccination of international travelers^{9,10}.

YF17D vaccine-related manufacturing constraints however lead to recurrent vaccine supply issues¹¹. The vaccines are still produced using traditional practices based on the propagation of the live-attenuated virus in embryonated chicken eggs, a biologically complex, time-consuming and labor-intensive approach that is difficult to scale up rapidly. On top of that, the need to preserve the vaccine strain's genetic and phenotypic characteristics necessitates seed-lot systems, limited passaging, as well as extensive characterization and release testing¹². Even so, YF17D vaccines are not genetically homogenous but consist of populations of closely related viral variants (quasispecies)^{13,14}. In addition,

YF17D vaccine potency depends on preservation of viral infectivity throughout manufacturing, storage, distribution and administration, resulting in the requirement for a stringent cold chain. This not only adds to the logistical complexity and costs, but also complicates vaccine stockpiling efforts and puts constraints on rapid vaccine deployment during outbreak responses. Due to the complexity and the stringent requirements associated with YF17D vaccine manufacturing, as well as the need for WHO prequalification for vaccines used in immunization programs, only a limited number of facilities worldwide are licensed for producing these vaccines. There is hence an urgent need for next-generation YF vaccines that address the manufacturing-related limitations of currently available YF17D vaccines^{10,15}.

We developed a next-generation DNA-based YF vaccine, Launch-iT YF. Launch-iT YF is a plasmid encoding the full YF17D genome. Upon intradermal (ID) administration, Launch-iT YF enters human host cells, where it uses the host transcription and translation machinery to produce replicating, live-attenuated YF17D virions. Launch-iT YF can be produced in bacterial bioreactors using a standard plasmid manufacturing process that consists of two steps (bacterial fermentation and plasmid purification)¹⁶. Launch-iT YF manufacturing is therefore readily scalable, and the process can be rapidly transferred between facilities. In addition, by employing a clonal copy DNA (cDNA) template, Launch-iT YF is genetically stable and hence not subject to the variability associated with conventional YF17D manufacturing¹⁴. Moreover, DNA is intrinsically more physiochemically stable than live viral particles, offering the potential for reduced cold-chain dependence. Launch-iT YF may therefore have the potential to address the real-world challenges faced by the current legacy vaccines, while retaining YF17D as well established immunizing agent. Preclinical data showed that administration of Launch-iT YF to hamsters induced high levels of YFV-specific neutralizing antibodies (nAbs), with ID administration resulting in the highest seroconversion rates (*unpublished data*). Here, we report the results of a first-in-humans dose-escalation Phase 1 trial to evaluate the safety, reactogenicity and immunogenicity of Launch-iT YF in healthy adults without pre-existing immunity against YFV. Three doses of Launch-iT YF were assessed (30 µg, 100 µg and 300 µg), using 3-site ID administration as the primary vaccination regimen (3-s dose groups). For the middle dose (100 µg), 1-site ID administration was also evaluated (1-s dose group).

Results

Trial Participants

A total of 65 participants were randomized in a staggered manner by dose group as described in detail in the Supplementary Materials. One participant allocated to the 100 µg (1-s) group withdrew consent before vaccination and was replaced. Twelve participants were vaccinated in each of the four Launch-iT YF groups. Sixteen participants received placebo, including twelve participants in the Placebo (3-s) group, and four in the Placebo (1-s) group. One participant in the 100 µg (1-s) and one in the Placebo (3-s) group were lost to follow-up after the Month 6 visit. All remaining participants completed all study visits. All vaccinated participants were included in the Safety Analysis Set. Immunogenicity data from

four participants (two in the 30 µg [3-s], one in the 100 µg [3-s] and one in the Placebo [3-s] group) were excluded due to the presence of detectable YFV-specific nAbs at baseline. Two participants were vaccinated against a flavivirus disease during the trial: one participant in the 100 µg (3-s) group received the tick-borne encephalitis vaccine FSME-IMMUN® after the Month 6 visit, and one participant in the 100 µg (1-s) group received the commercial YF vaccine Stamaril® after the Month 2 visit; immunogenicity data collected after these immunizations were excluded. Participant disposition is shown in Fig. 1. Baseline characteristics were generally comparable across study groups (Table 1).

* 48 participants were randomized in Part 2 of the trial, in which Launch-iT Rabies (a next-generation rabies vaccine) was evaluated, which is not reported here.

Table 1
Baseline characteristics of the trial participants

Characteristic	30 µg (3-s) (N = 12)	100 µg (3-s) (N = 12)	100 µg (1-s) (N = 12)	300 µg (3-s) (N = 12)	Placebo (3-s) (N = 12)	Placebo (1-s) (N = 4)	Overall (N = 64)
Sex – n (%)							
Female	4 (33.3)	9 (75.0)	5 (41.7)	9 (75.0)	9 (75.0)	3 (75.0)	39 (60.9)
Age (years)							
Mean (SD)	30.8 (5.9)	30.8 (6.3)	29.1 (7.5)	30.0 (6.8)	29.3 (3.8)	23.3 (2.8)	29.5 (6.1)
Min, max	22, 40	20, 40	19, 39	20, 40	23, 35	20, 26	19, 40
Race – n (%)							
White	12 (100)	12 (100)	12 (100)	12 (100)	11 (91.7)	4 (100)	63 (98.4)
Black or African	0	0	0	0	1 (8.3)	0	1 (1.6)
BMI (kg/m²)							
Mean (SD)	23.3 (3.9)	23.6 (4.0)	25.5 (4.2)	25.3 (4.1)	23.4 (2.9)	23.3 (1.8)	24.2 (3.7)
Min, max	18.8, 31.6	19.9, 31.3	19.2, 31.9	19.4, 31.6	20.9, 31.6	21.6, 25.8	18.8, 31.9

1-s, treatment groups vaccinated through 1-site intradermal administration; 3-s, treatment groups vaccinated through 3-site intradermal administration; BMI, body mass index; SD, standard deviation

Safety and Reactogenicity

No vaccine-related serious adverse events (SAEs), adverse events of special interest (AESIs) or adverse events (AEs) leading to trial discontinuation were reported.

Three SAEs occurred in three participants, all of which were deemed unrelated to vaccination. Two occurred in participants in the 100 µg (3-s) group: one participant was hospitalized for intractable vomiting that started over 9 months after vaccination, and another participant was hospitalized more than 3 months after vaccination for tonsillectomy following recurrent tonsillitis. The third SAE occurred in a participant in the 100 µg (1-s) group, who was hospitalized almost 1 year after vaccination for arthroscopic treatment of recurrent right shoulder dislocation.

Overall, 32/48 (67%) participants across the Launch-iT YF groups and 11/16 (69%) participants across the placebo groups reported solicited AEs in the 14-day follow-up period (Fig. 2). There was no evidence of a dose-relationship between the administered Launch-iT YF dose and the occurrence of solicited AEs. All solicited AEs were of mild or moderate severity, except for one severe episode of headache that occurred from Day 8 to Day 9 in a participant in the 100 µg (1-s) group, which was deemed unrelated to vaccination. Pain was the only reported solicited local AE, occurring in 6/48 (13%) participants across the Launch-iT YF groups, and in none of the placebo participants. The most frequent solicited general AEs were fatigue and headache. Fatigue was reported by 24/48 (50%) participants across the Launch-iT YF groups, and by 7/16 (44%) participants across the placebo groups. For 15/48 (31%) participants across the Launch-iT YF groups and 6/16 (38%) participants across the placebo groups, the fatigue was deemed vaccine-related. Headache was reported by 23/48 (48%) participants across the Launch-iT YF groups, and by 7/16 (44%) of the participants across the placebo groups. For 12/48 (25%) participants across the Launch-iT YF groups and 2/16 (13%) participants across the placebo groups, the headache was deemed vaccine-related. Vaccine-related solicited AEs are presented in Supplementary Table 1.

(a) Solicited local AEs. **(b)** Solicited general AEs.

Overall, during the 1-month follow-up period, unsolicited AEs were reported in 29/48 (60%) participants across the Launch-iT YF groups and 10/16 (63%) participants across the placebo groups. Vaccine-related unsolicited AEs were reported in 11/48 (23%) participants across the Launch-iT YF groups and 3/16 (19%) participants across the placebo groups. All vaccine-related unsolicited AEs were of mild or moderate severity. Vaccine-related unsolicited AEs reported in more than one participant were neutropenia (in three participants across the 100 µg groups), neutrophil count decreased (in two participants in the 100 µg [1-s] and one in the Placebo [1-s] group), alanine aminotransferase increased (in two participants in the 100 µg [1-s] group), injection site hemorrhage (in one participant in the 30 µg [3-s] group and one in the Placebo [3-s] group), and feeling feverish (in two participants in the 100 µg [1-s] group). A detailed overview of unsolicited AEs by treatment group is provided in Supplementary Table 2, and of vaccine-related unsolicited AEs by treatment group in Supplementary Table 3.

Humoral Immune Responses

YFV-specific nAbs are generally considered the primary correlate of protection against YF¹⁷. A single vaccination with Launch-iT YF induced YFV-specific nAbs above the seroprotective level (PRNT₅₀ ≥ 10) in participants across all Launch-iT YF groups (Fig. 3).

1-s, group vaccinated through 1-site intradermal administration; 3-s, groups vaccinated through 3-site intradermal administration

The highest seroconversion rate was observed in the 100 µg (3-s) group, in which 8/11 (73%) evaluable participants seroconverted. Seroconversion occurred also in 2/10 (20%), 6/12 (50%) and 5/12 (42%) evaluable participants in the 30 µg (3-s), 100 µg (1-s) and 300 µg (3-s) groups, respectively. No participants in the placebo groups seroconverted (*data not shown*).

Among the participants who seroconverted, the induced YFV-specific nAb titers overlapped across the different Launch-iT YF groups, *i.e.* there was no evident dose-response relationship with respect to the strength of the humoral immune response. Peak nAb titers were generally detected around Month 1, after which they persisted and remained well above the seroprotective threshold throughout the 12-month follow-up.

Cellular Immune Responses

In addition to YFV-specific nAbs, cell-mediated immunity plays a crucial role in short- and long-term protection from YF^{7,18}.

A single vaccination with Launch-iT YF induced YFV-specific IFNγ⁺ CD4⁺ and CD8⁺ T cell responses in participants across all Launch-iT YF groups (Fig. 4). Placebo participants did not show YFV-specific CD4⁺ or CD8⁺ T cell responses (*data not shown*).

(a) Frequency of YFV-specific IFNγ⁺ CD4⁺ cells (% of parent population). (b) Frequency of YFV-specific IFNγ⁺ CD8⁺ cells (% of parent population). Serological responders (participants reaching YFV-specific nAb PRNT₅₀ ≥ 10 at any post-vaccination timepoint) are presented in dark blue (closed circles) and non-responders in light blue (open circles). Non-responders were tested up to Month 6.

1-s, group vaccinated through 1-site intradermal administration; 3-s, groups vaccinated through 3-site intradermal administration

Participants who seroconverted for YFV-specific nAbs generally also mounted detectable YFV-specific CD4⁺ and CD8⁺ T cell responses, as opposed to the other participants. When induced, YFV-specific cellular immune responses generally peaked around Month 1, after which they persisted throughout the 12-month follow-up.

Further characterization showed that Launch-iT YF vaccination induced functionally diverse YFV-specific T cell responses (Fig. 5). YFV-specific CD4⁺ T cells were mostly polyfunctional, expressing different combinations of the evaluated functional markers (IFN γ , TNF α , IL-2 and CD40L) following YFV antigen stimulation, corresponding to a Th1-type functional profile¹⁹. YFV-specific CD8⁺ T cells predominantly produced IFN γ in response to YFV antigen stimulation, indicative for the induction of antigen-specific antiviral effector responses²⁰.

(a) Number of simultaneous functions of the YFV-specific CD4⁺ T cells. Functions assessed were IFN γ , TNF α , IL-2, and CD40L. **(b)** Number of simultaneous functions of the YFV-specific CD8⁺ T cells. Functions assessed were IFN γ , TNF α and IL-2. Serological responders were defined as participants reaching YFV-specific nAb PRNT₅₀ $\geq$ 10 at any post-vaccination timepoint. Each bar represents one serological responder, at the timepoint at which the highest frequency of YFV-specific CD4⁺ or CD8⁺ T cells was observed. Bar height represents the total frequency of YFV-specific CD4⁺ or CD8⁺ T cells (% of parent population). Colored segments indicate the proportion of cells expressing 1, 2, 3 or 4 simultaneous functions in panel a, and 1, 2 or 3 simultaneous functions in panel b. (*) Data not shown due to quality issues (high background responses).

1-s, group vaccinated through 1-site intradermal administration; 3-s, groups vaccinated through 3-site intradermal administration

Discussion

Recurrent issues with global YF vaccine supply have highlighted the need for next-generation YF vaccines that come with simplified and scalable manufacturing processes. Launch-iT YF is a next-generation DNA-based YF vaccine from which live-attenuated YF17D virions are produced upon administration. Launch-iT YF may therefore have the potential to address the real-world challenges faced by the current legacy vaccines, while retaining YF17D as well established immunizing agent. In this first-in-humans trial, the safety, reactogenicity and immunogenicity of Launch-iT YF were evaluated. The vaccine had an acceptable safety profile with limited reactogenicity. No vaccine-related SAEs, AESIs or AEs leading to trial discontinuation were reported. Non-serious AEs were reported at comparable frequencies in the Launch-iT YF and the placebo groups. Moreover, a single vaccination elicited coordinated humoral and cellular immune responses in a subset of the participants. In those participants, Launch-iT YF induced durable YFV-specific nAb responses above the seroprotective level, in combination with likewise durable YFV-specific polyfunctional CD4⁺ and CD8⁺ T cell responses characterized by consistent Th1-type and antiviral functional profiles, respectively.

In the majority of responders, YFV-specific nAbs were detectable from the Month 1 visit onwards. This is slightly later than what has been reported for commercial YF17D vaccines, the administration of which was followed by a rapid appearance of nAbs with most, if not all, vaccinees seroconverting within 2 weeks after vaccination^{21,22}. The slightly later onset of the immune response observed in the SAFYR trial

would be in line with the Launch-iT YF mechanism of action, which entails an initial phase during which the YF17D virions are produced from the Launch-iT plasmids, followed by viral replication leading to the induction of an adaptive immune response. Comparably delayed seroconversion kinetics have been observed following fractional dosing of YF17D vaccines, without impact on peak nAb titers at 1 month after vaccination²³. Similarly, the peak YFV-specific nAbs titers induced following immunization with Launch-iT YF appeared to be in line with the titers induced by immunization with commercial YF17D vaccines^{23–25}. YFV-specific nAb levels persisted throughout the 1-year follow-up period, which is encouraging and consistent with the durability of the immune response induced by commercial YF17D vaccines^{3–7}.

High frequencies of YFV-specific CD4⁺ and CD8⁺ T cells that persisted throughout the 1-year follow-up period were detected across all Launch-iT YF groups. Overall, humoral and cellular immune responses were coordinated with participants who seroconverted for YFV-specific nAbs generally also mounting detectable YFV-specific CD4⁺ and CD8⁺ T cell responses. YFV-specific CD4⁺ T cells were mostly polyfunctional, expressing different functional markers, corresponding to a robust Th1-type functional profile¹⁹. YFV-specific CD8⁺ T cells predominantly produced IFN γ in response to YFV antigen recall, indicative for the induction of antigen-specific antiviral effector responses²⁰. Even though the intracellular cytokine staining (ICS) panel was designed to assess different functional markers, and did not include markers for cytotoxic function, the data showed that Launch-iT YF can elicit functionally diverse T cell responses, a hallmark of a robust, qualitative immune response strongly linked to antiviral efficacy and durability. Moreover, the profile of the cellular immune response induced by Launch-iT YF appeared to be broadly consistent with what has been reported following immunization with commercial YF17D vaccines^{26–28}.

While YFV-specific immune responses were induced across all Launch-iT YF groups, response rates varied, with the highest response rate in the 100 μ g (3-s) group, in which 8/11 (73%) evaluable participants seroconverted. The underlying cause of the incomplete seroconversion is being studied. One possible explanation is that, under certain circumstances, the activation of innate DNA sensing pathways upon administration of Launch-iT YF could hamper YF17D virion production²⁹. Similarly, the launched YF17D virions themselves could trigger innate DNA sensing pathways, thereby preventing virion replication^{30,31}. Alternatively, there could be a physical effect of Launch-iT YF concentration on the efficiency of plasmid uptake. Any of these factors could prevent the efficient initiation of YF17D replication, which is needed for the induction of an adaptive immune response. To increase seroconversion rates in the future, a second-generation Launch-iT YF with improved launch kinetics is being developed.

This Phase 1 trial had inherent limitations. First, the small sample size limits the detection of rare AEs and warrants cautious interpretation of observed differences between groups, as the trial was not powered to support formal comparative analyses. In addition, the trial population consisted of healthy young adults within a narrow age range (18–40 years) and similar baseline characteristics, which limits

the generalizability to broader populations. Lastly, the absence of an active comparator (commercial YF vaccine) prevents head-to-head benchmarking of both safety and immunogenicity results. Nonetheless, the obtained safety and immunogenicity data appeared to be largely consistent with what has been reported in literature for commercial YF17D vaccines.

In addition to being the commercial vaccine against YF, YF17D has gained attention for its potential use as a powerful viral vector³². The properties of YF17D that render it unique as a viral vector are: 1) its capability of inducing broad immune responses that provide lifelong protection with a single vaccination³⁻⁷, 2) its well-known and favorable long-term safety record³³, and 3) its tolerance for the insertion of heterologous antigen sequences into its genome³². The goal would not be merely the delivery of heterologous antigens, it would also be the leveraging of the powerful immunological adjuvant effect of the YF17D backbone to induce robust immune responses against the heterologous pathogen^{32,34}. The utilization of YF17D as a viral vector has been successfully explored preclinically for a wide range of prophylactic and therapeutic indications, including COVID-19, Lassa fever, Ebolavirus species that are not covered by the current Ebola vaccines, Chagas disease, HIV and malaria³⁵⁻⁴⁵. Likewise, the YF17D backbone is successfully used in the licensed chimeric vaccines IMOJEV® and Dengvaxia®, in which selected viral genes are replaced with the corresponding genes from a heterologous flavivirus^{46,47}. Through the insertion of heterologous antigen sequences into the Launch-iT encoded YF17D genome, the Launch-iT DNA technology could be utilized as a rapidly adaptable platform for the *in situ* production of YF17D viral vectors. As such, this opens avenues for this novel technology to become a platform for the development of a wide range of vaccines, including prophylactic and therapeutic vaccines, for which there is a continuous medical need. Indeed, despite tremendous successes in the vaccinology field, several life-threatening diseases are not yet vaccine-preventable (e.g. HIV, Lassa fever, certain types of Ebola). On top of that, new infectious diseases can emerge any time, as recently shown by the COVID-19 pandemic, and by the Mpox and Hantavirus outbreaks. Moreover, there is an increasing interest in the development of therapeutic vaccines (or immunotherapies) for the treatment of chronic infectious diseases⁴⁸.

In conclusion, the next-generation DNA-based YF vaccine Launch-iT YF was safe and well tolerated in a first-in-humans dose-escalation Phase 1 trial in healthy adults. In a subset of the participants, a single vaccination induced coordinated humoral and cellular immune responses that persisted throughout the 1-year trial period, demonstrating clinical feasibility of this novel technology. Encouraged by these results, a second-generation Launch-iT vaccine is being developed to further increase response rates. Moreover, considering the potential of YF17D to serve as a viral vector, these data form a promising basis for the further development of this novel technology as a rapidly adaptable vaccine platform, which could be utilized for the development of a wide range of vaccines.

Methods

Vaccines

Launch-iT YF is a DNA plasmid encoding the full YF17D genome (YF17D-204 substrain) under the control of transcriptional elements as described in Patent WO2014/174078. The plasmid backbone includes the elements required for its manufacturing as described in Patent WO2021/058722. Launch-iT YF was manufactured under cGMP by Eurogentec (Seraing, Belgium). The vaccine was formulated at a concentration of 1 mg/mL in phosphate buffered saline (PBS) and stored at -20°C. PBS was used as the placebo control.

Three doses were evaluated (30 µg, 100 µg and 300 µg). The starting dose was selected based on preclinical studies in hamsters showing immunogenicity of intradermally-administered doses in this range. For dose-escalation, approximately 3-fold increases were selected to allow the assessment of potential dose-response relationships. Based on the results of the GLP-compliant repeated dose toxicity study, which showed that repeated ID administrations of the maximum feasible dose level (20 µg) were safe and tolerable in hamsters, there was an appropriate safety margin for all clinical doses.

All participants received a single vaccination on Day 1 of the trial. Administration through 3-site ID was the primary administration regimen. This was based on preclinical studies in piglets, which have a skin structure similar to that of humans, in which a trend towards higher seroconversion rates was observed following administration through 3-site ID as compared to 1-site ID. For the middle dose (100 µg), administration through 1-site ID was also evaluated. There were hence 4 dose groups in total. Each injection had a volume of 100 µL and was administered into the volar aspect of the forearm using the Mantoux technique. Dilution in PBS was performed for administration of the 30 µg dose and the 100 µg dose in the 3-site ID regimen.

Trial Design and Participants

This was a randomized, double-blind, placebo-controlled dose-escalation Phase 1 trial. The trial consisted of two parts: Launch-iT YF was evaluated in Part 1, and Launch-iT Rabies (a next-generation rabies vaccine) in Part 2. Here, the results of Part 1 are reported. Part 1 was conducted at two centers in Belgium. Participants vaccinated through 3-site ID were enrolled at the Center for Vaccinology (CEVAC), and participants vaccinated through 1-site ID at the Centre for the Evaluation of Vaccination (CEV). Enrolment started on 16 June 2024, and the last visit took place on 26 January 2026. The trial was conducted in accordance with the Declaration of Helsinki, the International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice (ICH GCP) guidelines, and all applicable regulatory requirements. The trial was approved by an independent Ethics Committee and by the Belgian Federal Agency for Medicines and Health Products (FAMHP) and was registered on the EU Clinical Trials Information System (CTIS) and on ClinicalTrials.gov (identifiers 2024-511194-29 and NCT06998004; registration dates 13 June 2024 and 22 May 2025, respectively).

Within each dose group, vaccination followed a sentinel design, which is described in detail in the Supplementary Materials. Overall, a total of sixteen participants were vaccinated per dose group, twelve with the applicable dose of Launch-iT YF and four with placebo. An external and independent data safety

monitoring committee (DSMB) oversaw participant safety and recommended on dose-escalation decisions. For the 3-s dose groups, dose escalation was only permitted following a favorable DSMB review of at least the Day 15 safety data from all participants in the previous dose group. In addition, the 1-s dose group was allowed to proceed in parallel with the vaccination of the highest 3-s dose group. More information on dose-escalation and the DSMB can be found in the Supplementary Materials.

Eligible participants were healthy women and men aged 18–40 years with a body mass index (BMI) ≥ 18.0 and ≤ 32.0 kg/m². Potential participants were excluded if they had a (confirmed or suspected) history of flavivirus infection, or if they had previously been vaccinated against a flavivirus disease (including YF). The full list of inclusion and exclusion criteria can be found in Supplementary Table 4.

Safety and Reactogenicity Assessments

All vaccinated participants were provided with an electronic diary (eDiary) to record solicited local (pain, redness and swelling) and general (fever, fatigue, headache and generalized myalgia) AEs during 14 consecutive days, starting on the day of vaccination. They were also provided with a paper diary card to record unsolicited AEs up to the Month 1 visit. SAEs and AESIs were collected throughout the 1-year trial period. Monitored AESIs were YF vaccine associated neurotropic disease (YEL-AND) and YF vaccine-associated viscerotropic disease (YEL-AVD), using the CDC and Brighton Collaboration case definitions^{49,50}. Additional safety assessments included hematology (hemoglobin, platelet and differential white blood cell count) and biochemistry (alanine aminotransferase, aspartate aminotransferase and creatinine) parameters at Day 8, Day 15, Month 1 and Month 6, and a complete physical examination at Day 3. All AEs and clinically significantly abnormal hematology / biochemistry parameters were graded for severity as detailed in the Supplementary Materials. For each AE, the maximum severity that occurred for the duration of the event was assigned. All solicited local AEs were deemed vaccine-related. For all other AEs, the investigator determined whether there was a reasonable possibility that the vaccine had caused the event.

Humoral Immune Responses

YFV-specific nAb titers were measured in serum at baseline (Day 1), Day 15, Month 1, Month 2, Month 6 and Month 12, using a validated YF PRNT at VisMederi srl (Sienna, Italy), in accordance with the WHO protocol⁵¹. At Month 12, only samples from participants who had previously seroconverted for YFV-specific nAbs were tested as no *de novo* vaccine-induced immune responses were expected at this timepoint. Seroconversion was defined as the appearance of YFV-specific nAbs (PRNT₅₀ ≥ 10).

Cellular Immune Responses

Peripheral blood mononuclear cells, collected at baseline (Day 1), Day 15, Month 1, Month 6 and Month 12 were stimulated with YFV-specific antigens followed by ICS, at CEVAC, Ghent University (Ghent, Belgium). At Month 12, only the samples from participants who had previously seroconverted for YFV-specific nAbs were tested as no *de novo* vaccine-induced immune responses were expected at this

timepoint. The frequency (%) of YFV-specific CD4⁺ and CD8⁺ T cells producing IFN γ , TNF α , IL-2, and / or CD40L was determined relative to the total CD4⁺ and CD8⁺ T cell populations, respectively.

Polyfunctional CD4⁺ T cells were defined as YFV-specific CD4⁺ T cells positive for at least two out of the four evaluated markers. Polyfunctional CD8⁺ T cells were defined as YFV-specific CD8⁺ T cells positive for at least two out of the following three markers: IFN γ , IL-2 and TNF α . All ICS measurements were background-corrected. Negative values after background subtraction were set to 0.0001% for log-scale graphical displays and to 0% for linear-scale displays. Samples with elevated background responses were excluded. More detailed information on the ICS assay is provided in the Supplementary Materials.

Statistical Methods

All analyses were descriptive in nature, no hypotheses were tested and no formal sample size calculations were performed. Missing data were not imputed. AEs were coded by System Organ Class and Preferred Term using MedDRA Version 27.0. Safety analyses were performed on the Safety Analysis Set, which included all participants with at least one documented injection. Immunogenicity results are presented at the individual participant level. Immunogenicity results from participants with detectable YFV-specific nAbs (PRNT50 $\geq$ 10) at baseline (Day 1) were excluded. In addition, for participants who received a vaccination against a flavivirus disease during the trial, the immunogenicity data collected after that vaccination were also excluded.

Data Availability

The underlying data reported in this paper are available on request from the corresponding author upon reasonable request.

Declarations

I confirm that all participants provided written and dated consent prior to their participation in the trial.

Competing Interests

I.L.-R., S.D.G., B.J., S.P., G.W., all affiliated with CEVAC, and I.D.C., K.W. and L.S., all affiliated with CEV, conducted the SAFYR trial, for which their institution received financial compensation from Astrivax Therapeutics, the sponsor of the trial. H.C. is co-founder and the CEO of Astrivax Therapeutics. Professors J.N. and K.D. are the inventors of the Launch-iT technology, are listed in several patents (applications), are co-founders of Astrivax Therapeutics and act as scientific advisory board members to the company. H.C., K.D. and J.N. are shareholders of Astrivax Therapeutics. W.D., J.D., B.D., S.D.V., L.O., B.S., F.S., L.V.E., T.V. and K.Y. are (former) employees of or consultants for Astrivax Therapeutics, and H.C., W.D., K.D., J.D., B.D., S.D.V., J.N., , B.S., T.V., K.Y. participate in the company's ESOP. L.O. is an employee of MinervaX ApS.

Author Contribution

H.C., W.D., K.D., J.D., I.D.C., S.D.V., I.L.-R., J.N., L.O., B.S. and T.V. contributed to the trial design. S.D.V and L.V.E. worked on the methodology. B.D., F.S., L.V.E. and K.Y. supported the data analyses. S.D.V., L.O., L.V.E. supported trial conduct; I.D.C., S.D.G., B.J. and I.L.-R. conducted the trial. I.D.C., S.D.G., B.J., I.L.-R., L.O., S.P., L.V.E. and G.W. generated data. H.C., W.D. and T.V. provided oversight. L.O., B.D., S.D.V. and J.D. drafted the paper, and all authors contributed to the revisions and approved the final version.

Acknowledgement

The authors would like to thank Astrivax Therapeutics' founding institution, the KU Leuven, and especially Lotte Coelmont and Hendrik Jan Thibaut, for their continued support and insights and their essential contribution in shaping the development of the technology at the Virology, Antiviral Drug & Vaccine Research Group. They would like to thank the participants who took part in this trial. They acknowledge the contributions of the staff at CEVAC and CEV, and would also like to thank the teams of the contract research organizations Clinigen (for vaccine packaging and labelling) Harmony (for regulatory submissions and monitoring), Clinfidence (for data management) and Clin-Q (for biostatistics), as well as the laboratory staff of VisMederi (for PRNT analyses) and CEVAC (for CMI analyses). They are grateful to the invaluable input of Astrivax Therapeutics' Scientific Advisory Board (chaired by Emmanuel Hanon), to the members of the DSMB, and to the people who played a key role in the successful start and conduct of the SAFYR trial (Véronique Bodo, Liesbet De Cock, Jan Eelen and Mathieu Peeters). Astrivax Therapeutics NV sponsored this trial. The preclinical research and development activities leading up to this trial were partially funded by Flanders innovation & entrepreneurship (VLAIO; grant HBC.2022.0953) and the Gates Foundation (grant OPP1195179).

Data Availability

The underlying data reported in this paper are available on request from the corresponding author upon reasonable request.

References

- ¹World Health Organization. Vaccines and vaccination against yellow fever. WHO position paper – June 2013. *Wkly Epidemiol Rec.* 88, 269–283 (2013).
- ²Theiler, M. & Smith, H.H. The use of yellow fever virus modified by in vitro cultivation for human immunization. *J Exp Med.* 65, 787–800 (1937).
- ³Lindsey, N.P. et al. Persistence of yellow fever virus-specific neutralizing antibodies after vaccination among US travellers. *J Travel Med.* 25;10.1093/jtm/tay108 (2018).

4. ⁴Lindsey, N.P. et al. Duration of seropositivity following yellow fever vaccination in U.S. military service members. *Vaccine* 38, 8286–8291 (2020).
5. ⁵Monath, T.P. Yellow fever vaccine. *Expert Rev Vaccines* 4,553–574 (2005).
6. ⁶Schnyder, J.L., de Jong, H.K., Bache, B.E., Schaumburg, F. & Grobusch, M.P. Long-term immunity following yellow fever vaccination: a systematic review and meta-analysis. *Lancet Glob Health* 12, e445-e456 (2024).
7. ⁷Wieten, R.W. et al. A Single 17D Yellow Fever Vaccination Provides Lifelong Immunity; Characterization of Yellow-Fever-Specific Neutralizing Antibody and T-Cell Responses after Vaccination. *PLoS One* 11, 10.1371/journal.pone.0149871 (2016).
8. ⁸World Health Organization. Yellow fever. %3C<https://www.who.int/news-room/fact-sheets/detail/yellow-fever>%3E (2025).
9. ⁹World Health Organization, UNICEF, Gavi. Eliminate Yellow Fever Epidemics (EYE): A Global Strategy, 2017–2026. Geneva: World Health Organization (2017).
10. ¹⁰Barrett, A.D.T. Yellow Fever in Angola and Beyond–The Problem of Vaccine Supply and Demand. *N Engl J Med.* 375, 301–303 (2016).
11. ¹¹Barrett, A.D.T. Yellow fever live attenuated vaccine: A very successful live attenuated vaccine but still we have problems controlling the disease. *Vaccine* 35, 5951–5955 (2017).
12. ¹²World Health Organization Expert Committee on Biological Standardization. Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines. Annex 5, TRS No 978 (2013).
13. ¹³Beck, A. et al. Comparison of the live attenuated yellow fever vaccine 17D-204 strain to its virulent parental strain Asibi by deep sequencing. *J Infect Dis.* 209, 334–344 (2014).
14. ¹⁴Kum, B.D. et al. Limited evolution of the yellow fever virus 17d in a mouse infection model. *Emerg Microbes Infect.* 8, 1734–1746 (2019).
15. ¹⁵Hansen, C.A. & Barrett, A.D.T. The Present and Future of Yellow Fever Vaccines. *Pharmaceuticals (Basel).* 14, 891; 10.3390/ph14090891 (2021).
16. ¹⁶Janssen, M. et al. Large vectors and methods for high-yield production. WO patent WO2021/058722 (2021).
17. ¹⁷Staples, J.E., Barrett, A.D.T, Wilder-Smith, A. & Hombach, J. Review of data and knowledge gaps regarding yellow fever vaccine-induced immunity and duration of protection. *NPJ Vaccines* 5, 54; 10.1038/s41541-020-0205-6 (2020).
18. ¹⁸Bassi, M.R. et al. CD8 + T cells complement antibodies in protecting against yellow fever virus. *J Immunol.* 194, 1141–1153 (2015).
19. ¹⁹Zhu, J., Yamane, H. & Paul, W.E. Differentiation of Effector CD4 T Cell Populations* *Annu Rev Immunol.* 28, 445–489 (2010).

20. ²⁰Ruby, J. & Ramshaw, I. The antiviral activity of immune CD8 + T cells is dependent on interferon-gamma. *Lymphokine Cytokine Res.* 10, 353–358 (1991).
21. ²¹Reinhardt, B., Jaspert, R., Niedrig, M., Kostner, C. & L'age-Stehr, J. Development of viremia and humoral and cellular parameters of immune activation after vaccination with yellow fever virus strain 17D: a model of human flavivirus infection. *J Med Virol.* 56,159–167 (1998).
22. ²²Silva, M.L. et al. Characterization of main cytokine sources from the innate and adaptive immune responses following primary 17DD yellow fever vaccination in adults. *Vaccine* 29, 583–592 (2011).
23. ²³Juan-Giner, A. et al. Immunogenicity and safety of fractional doses of yellow fever vaccines: a randomised, double-blind, non-inferiority trial. *Lancet* 397, 119–127 (2021).
24. ²⁴Modjarrad, K. et al. Safety and immunogenicity of a next-generation live-attenuated yellow fever vaccine produced in a Vero cell line in the USA: a phase 1 randomised, observer-blind, active-controlled, dose-ranging clinical trial. *Lancet Infect Dis.* 24, 1393–1402 (2024).
25. ²⁵Desai, S. et al. A phase I clinical study to assess safety and immunogenicity of yellow fever vaccine. *NPJ Vaccines* 7,170. 10.1038/s41541-022-00595-6 (2022).
26. ²⁶Akondy, R.S. et al. The yellow fever virus vaccine induces a broad and polyfunctional human memory CD8 + T cell response. *J Immunol.* 183, 7919–7930. (2009).
27. ²⁷Gaucher, D. et al. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. *J Exp Med.* 205, 3119–3131 (2008).
28. ²⁸Miller, J.D. et al. Human effector and memory CD8 + T cell responses to smallpox and yellow fever vaccines. *Immunity* 28, 710–722 (2008).
29. ²⁹Paludan, S.R. & Bowie, A.G. Immune sensing of DNA. *Immunity* 38, 870–880 (2013).
30. ³⁰Goubau, D., Deddouche, S. & Reis e Sousa, C. Cytosolic sensing of viruses. *Immunity* 38, 855–869 (2013).
31. ³¹Iwasaki, A. A virological view of innate immune recognition. *Annu. Rev. Microbiol.* 66, 177–196 (2012).
32. ³²Sanchez-Felipe, L., Alpizar, Y.A., Ma, J., Coelmont, L. & Dallmeier, K. YF17D-based vaccines - standing on the shoulders of a giant. *Eur J Immunol.* 54; 10.1002/eji.202250133 (2024).
33. ³³Rojas, A., Hachey, W., Kaur, G., Korejwo, J. & Muhammad, R. Enhanced safety surveillance of STAMARIL® yellow fever vaccine provided under the expanded access investigational new drug program in the USA. *J. Travel Med.* 30; 10.1093/jtm/taad037 (2023).
34. ³⁴Draper, S.J. & Heeney, J.L. Viruses as vaccine vectors for infectious diseases and cancer. *Nat Rev Microbiol.* 8, 62–73 (2010).
35. ³⁵Sanchez-Felipe, L. et al. A single-dose live-attenuated YF17D-vectored SARS-CoV-2 vaccine candidate. *Nature* 590, 320–325 (2021).
36. ³⁶Ma, J. et al. Live-attenuated YF17D-vectored COVID-19 vaccine protects from lethal yellow fever virus infection in mouse and hamster models. *EBioMedicine* 83; 10.1016/j.ebiom.2022.104240

- (2022).
37. ³⁷Bredenbeek, P.J. et al. A recombinant Yellow Fever 17D vaccine expressing Lassa virus glycoproteins. *Virology* 345, 299–304 (2006).
 38. ³⁸Jiang, X. et al. Yellow fever 17D-vectored vaccines expressing Lassa virus GP1 and GP2 glycoproteins provide protection against fatal disease in guinea pigs. *Vaccine* 29, 1248–1257 (2011).
 39. ³⁹Kelchtermans, L. et al. CD4 + T cell-mediated immunity protects from VSV-SUD lethal challenge in a mouse model of Sudan virus infection. *Nat Immunol.* 27, 1224–1236 (2026).
 40. ⁴⁰Lemmens, V. et al. YF17D-vectored Ebola vaccine candidate protects mice against lethal surrogate Ebola and yellow fever virus challenge. *NPJ Vaccines* 8; 10.1038/s41541-023-00699-7 (2023).
 41. ⁴¹Nogueira, R.T. et al. Recombinant yellow fever viruses elicit CD8 + T cell responses and protective immunity against *Trypanosoma cruzi*. *PLoS One* 8; 10.1371/journal.pone.0059347 (2013).
 42. ⁴²Nogueira, R.T. et al. Biological and immunological characterization of recombinant Yellow Fever 17D viruses expressing a *Trypanosoma cruzi* Amastigote Surface Protein-2 CD8 + T cell epitope at two distinct regions of the genome. *Virol J.* 8; 10.1186/1743-422X-8-127 (2011).
 43. ⁴³Franco, D. Evaluation of yellow fever virus 17D strain as a new vector for HIV-1 vaccine development. *Vaccine* 28, 5676–5685 (2010).
 44. ⁴⁴Stoyanov, C.T. et al. Immunogenicity and protective efficacy of a recombinant yellow fever vaccine against the murine malarial parasite *Plasmodium yoelii*. *Vaccine* 28, 4644-52 (2010).
 45. ⁴⁵Tao, D. et al. Yellow fever 17D as a vaccine vector for microbial CTL epitopes: protection in a rodent malaria model. *J Exp Med.* 201, 201–209 (2005).
 46. ⁴⁶Appaiahgari, M.B. & Vrati, S. IMOJEV(®): a Yellow fever virus-based novel Japanese encephalitis vaccine. *Expert Rev Vaccines* 9, 1371–1384 (2010).
 47. ⁴⁷Tully, D. & Griffiths, C.L. Dengvaxia: the world's first vaccine for prevention of secondary dengue. *Ther Adv Vaccines Immunother.* 9; 10.1177/25151355211015839 (2021).
 48. ⁴⁸Park, P.G. et al. Current development of therapeutic vaccines for the treatment of chronic infectious diseases. *Clin Exp Vaccine Res.* 13, 21–27 (2024).
 49. ⁴⁹Staples, J.E., Gershman, M. & Fischer, M. Centers for Disease Control and Prevention (CDC). Yellow fever vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm. Rep.* 59, 1–27 (2010).
 50. ⁵⁰Gershman, M.D. et al. Viscerotropic disease: case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine* 30, 5038–5058 (2012).
 51. ⁵¹World Health Organization. Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses. %3C<https://iris.who.int/handle/10665/69687>%3E (2007).

Figures

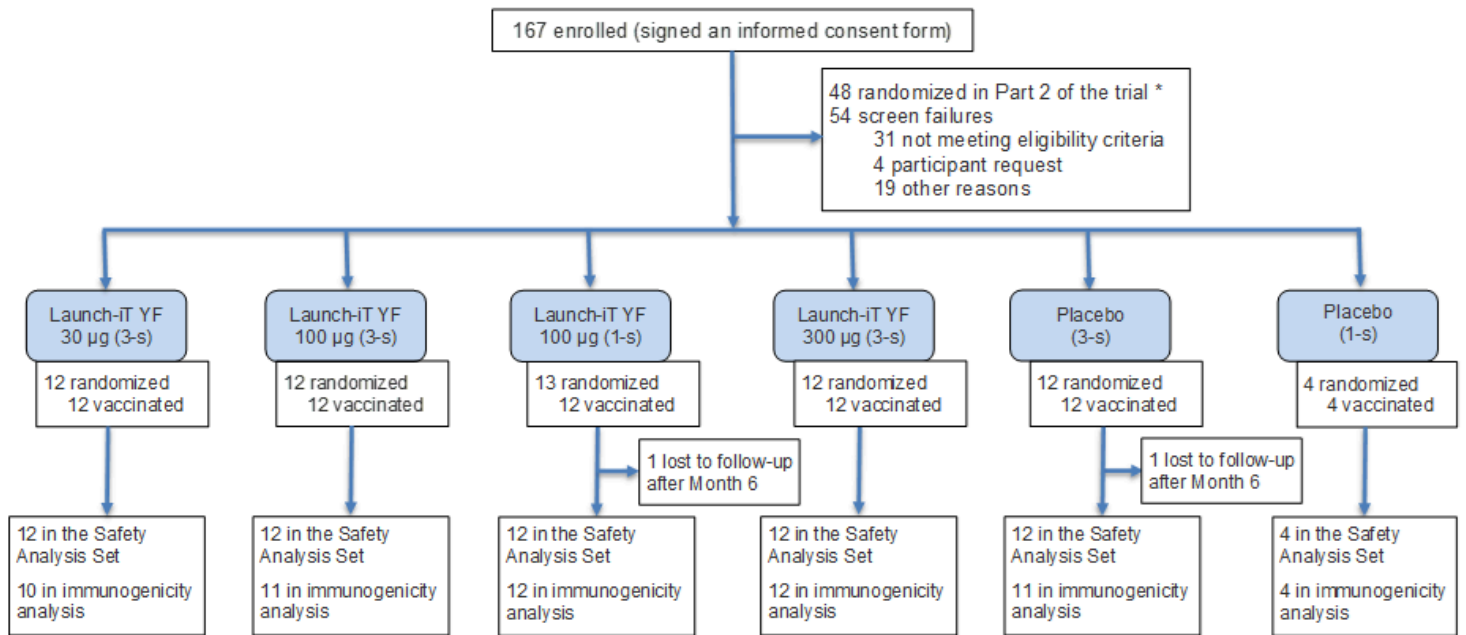


Figure 1

CONSORT flow diagram of participant disposition.

* 48 participants were randomized in Part 2 of the trial, in which Launch-iT Rabies (a next-generation rabies vaccine) was evaluated, which is not reported here.

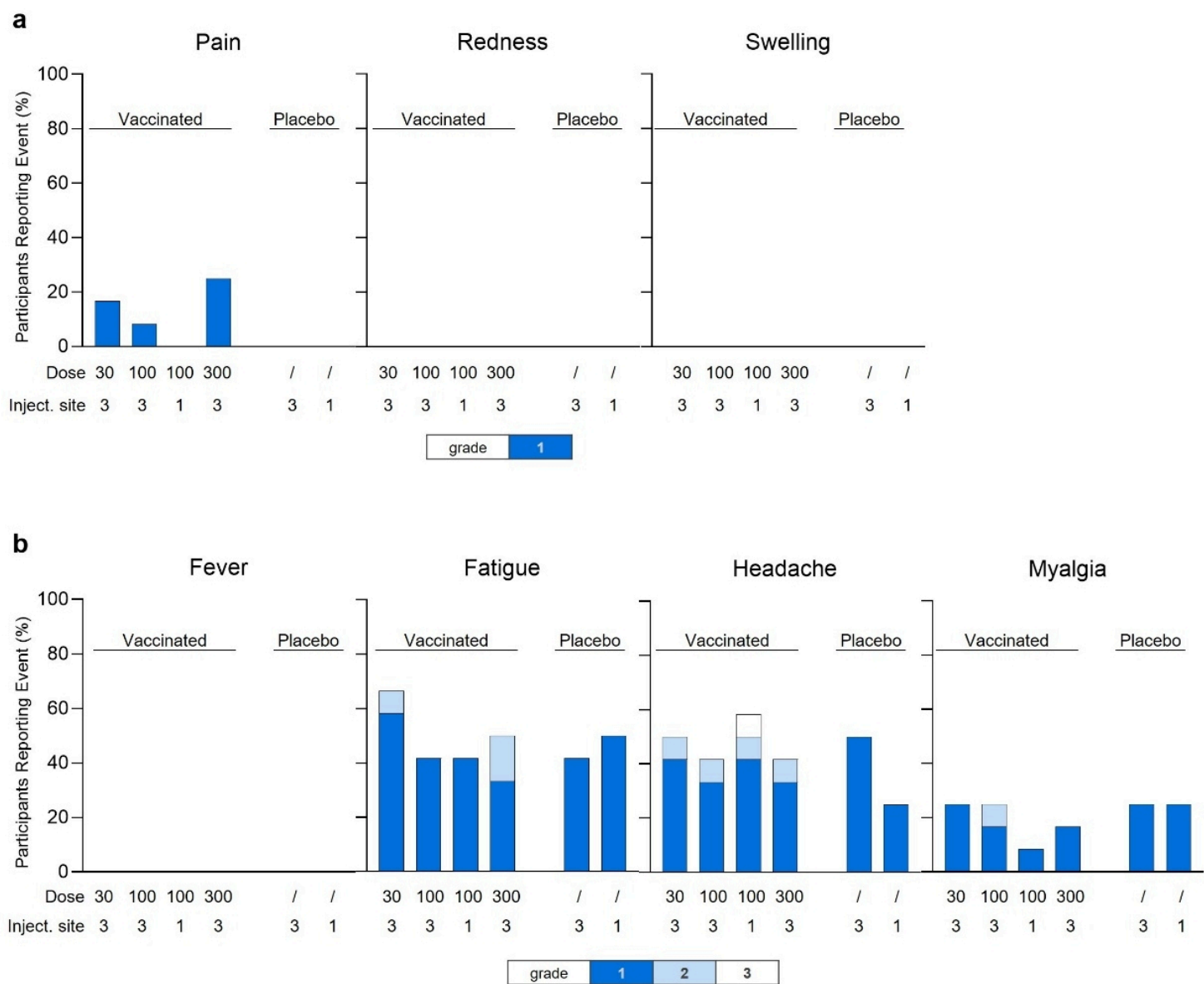


Figure 2

Solicited AEs in the 14 days following the vaccination, by severity and by treatment group.

(a) Solicited local AEs. **(b)** Solicited general AEs.

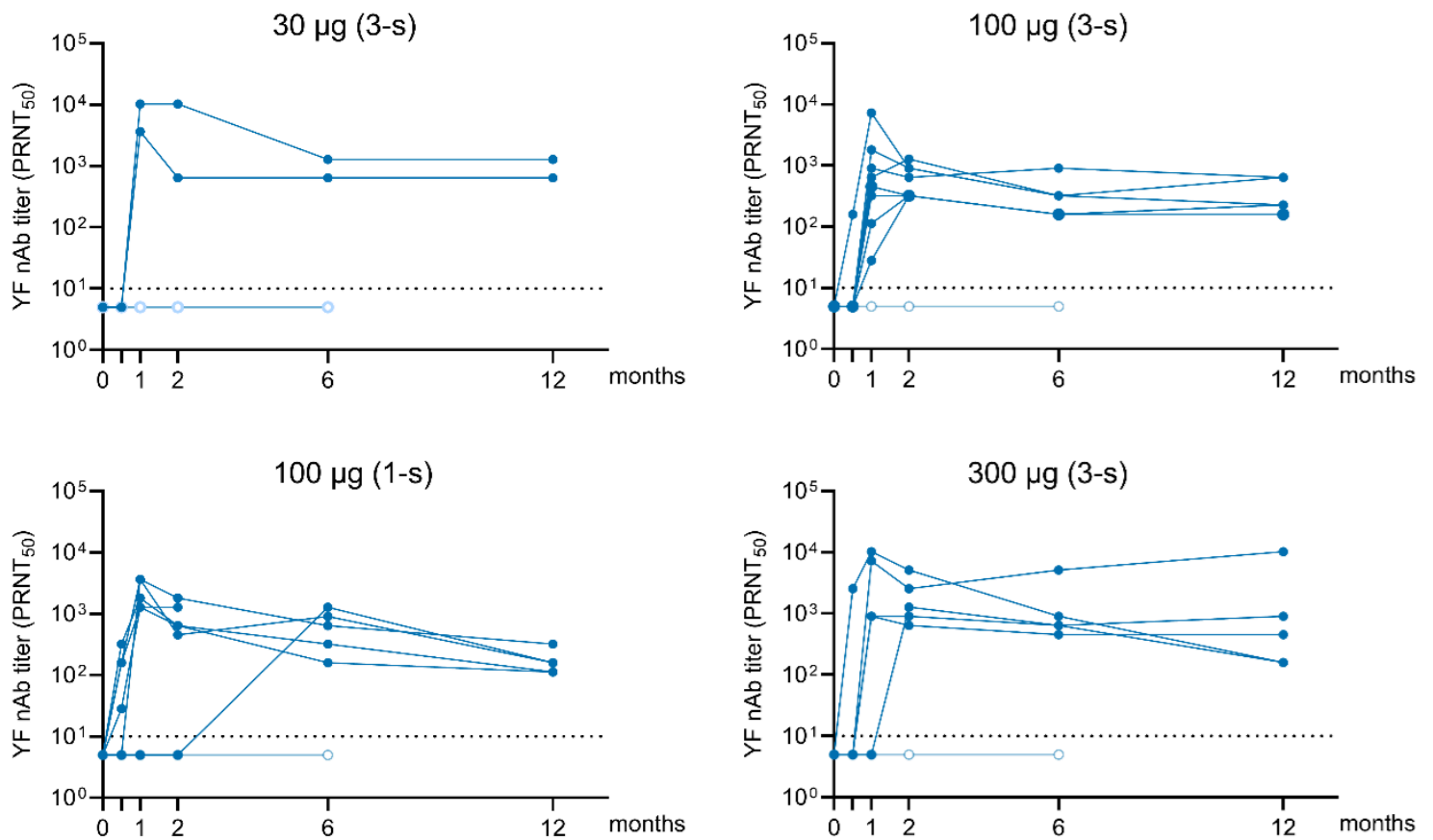


Figure 3

YFV-specific nAb titers (PRNT₅₀) over time, individual participant data, by Launch-iT YF group.

Serological responders (participants reaching PRNT₅₀ ³ 10 at any post-vaccination timepoint) are presented in dark blue (closed circles) and non-responders in light blue (open circles). Non-responders were evaluated up to Month 6. The dotted lines indicate the seroprotective threshold (PRNT₅₀ = 10). PRNT₅₀ values below the limit of detection are plotted as PRNT₅₀ = 5.

1-s, group vaccinated through 1-site intradermal administration; 3-s, groups vaccinated through 3-site intradermal administration

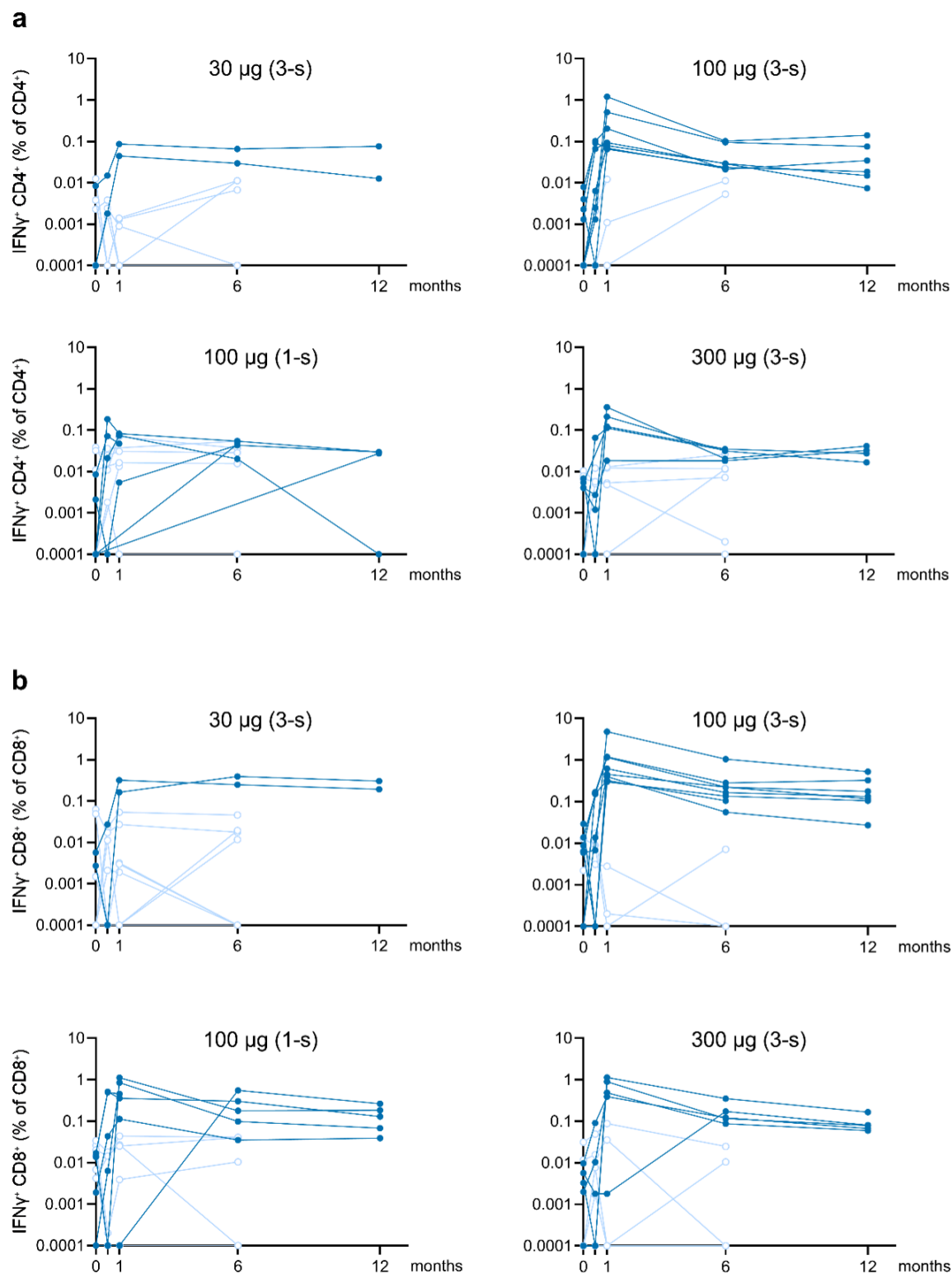


Figure 4

Frequency of YFV-specific IFN γ ⁺ CD4⁺ and CD8⁺ T cells over time, individual participant data, by Launch-iT YF group.

(a) Frequency of YFV-specific IFN γ ⁺ CD4⁺ cells (% of parent population). **(b)** Frequency of YFV-specific IFN γ ⁺ CD8⁺ cells (% of parent population). Serological responders (participants reaching YFV-specific

nAb PRNT₅₀³ 10 at any post-vaccination timepoint) are presented in dark blue (closed circles) and non-responders in light blue (open circles). Non-responders were tested up to Month 6.

1-s, group vaccinated through 1-site intradermal administration; 3-s, groups vaccinated through 3-site intradermal administration

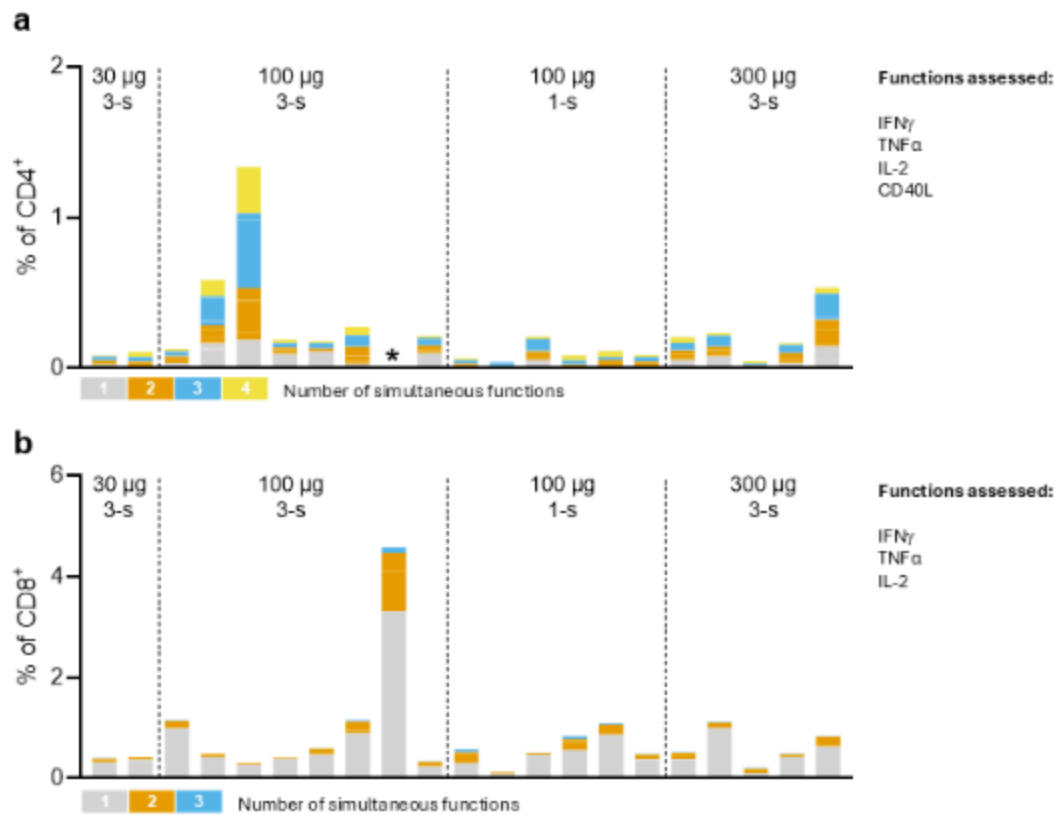


Figure 5

Functionality of the YFV-specific CD4⁺ and CD8⁺ T cells, in serological responders, by Launch-iT YF group.

(a) Number of simultaneous functions of the YFV-specific CD4⁺ T cells. Functions assessed were IFN γ , TNF α , IL-2, and CD40L. (b) Number of simultaneous functions of the YFV-specific CD8⁺ T cells. Functions assessed were IFN γ , TNF α and IL-2. Serological responders were defined as participants reaching YFV-specific nAb PRNT₅₀³ 10 at any post-vaccination timepoint. Each bar represents one serological responder, at the timepoint at which the highest frequency of YFV-specific CD4⁺ or CD8⁺ T cells was observed. Bar height represents the total frequency of YFV-specific CD4⁺ or CD8⁺ T cells (% of parent population). Colored segments indicate the proportion of cells expressing 1, 2, 3 or 4 simultaneous functions in panel a, and 1, 2 or 3 simultaneous functions in panel b. (*) Data not shown due to quality issues (high background responses).

1-s, group vaccinated through 1-site intradermal administration; 3-s, groups vaccinated through 3-site intradermal administration

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [LaunchiTYFmanuscriptsupplements.pdf](#)
- [floatimage1.jpeg](#)