Second five-year follow-up after a booster vaccination against tick-borne encephalitis following different primary vaccination schedules demonstrates at least 10 years antibody persistence

Jiri Beran [a,b], Maria Lattanzi [c], Fang Xie [d], Luca Moraschini [c], Ilaria Galgani [c,*]

[a] Vaccination and Travel Medicine Centre, Tylovo nábřeží 418/6, 500 02 Hradec Králové, Czech Republic
[b] Department for Tropical, Travel Medicine and Immunization, Institute for Postgraduate Medical Education in Prague, Ruská 2412/85, 100 00 Prague 10, Czech Republic
[c] GSK, Via Fiorentina 1, 53100 Siena, Italy
[d] GSK Vaccines, 14200 Shady Grove Rd, Rockville, MD 20850, USA

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ABSTRACT

Background: Tick borne encephalitis (TBE) endemic zones are expanding. We previously evaluated long term persistence of antibody 5 years after the first booster immunization following different primary immunization schedules with the polygeline-free inactivated TBE vaccine (TBEvac) in adults and adolescents. Here, we report anti-TBE virus (TBEV) antibody persistence from 6 to 10 years post-booster administration.

Methods: This was a phase IV, open-label, single-center, second extension study (NCT01562444), conducted in Czechia. Healthy adults and adolescents ≥12 years who had received 3 different primary vaccination schedules (rapid, conventional and accelerated conventional) in the parent study and a booster dose before (12–18 months post-primary series completion) or at the beginning (3 years post-primary series completion) of the first extension study were screened and enrolled in this study. Blood samples were collected yearly and anti-TBEV antibody response was evaluated by neutralizing test (NT) antibody assays. Analysis was performed overall and per age strata: 15–49 years, ≥50 years, and ≥60 years.

Results: Of 206 screened individuals, 191 completed the study. Overall, 90–100% of participants in the all-screened set and ≥97% in the per-protocol set had the clinically meaningful threshold of protection (NT titers ≥10) across all timepoints, regardless of the primary vaccination schedule. Overall, antibody geometric mean titers (GMTs) varied from 134 to 343 in the all-screened set. Older age groups showed overall lower GMTs, although GMTs remained higher than NT titers ≥10 up to year 10 in all groups.

Conclusion: This study showed long-term persistence of anti-TBEV NT antibodies for up to 10 years after the first booster dose of TBEvac in all age groups, regardless of the primary vaccination schedule.

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1. Introduction

Tick-borne encephalitis (TBE) is caused by the TBE virus (TBEV), a member of the *Flaviviridae* family, which comprises about 70 species including the dengue, yellow fever, Japanese encephalitis and West Nile viruses [1]. The TBEV is transmitted to humans by a bite from an infected tick or, more rarely, from ingesting the unpasteurized milk and milk products of infected animals [2]. In the latter cases, infections occur rapidly and often affect groups of people at the same time [3].

TBE is a serious acute infection of the central nervous system that can lead to death or long-term neurological sequelae [4]. Up to 30% of adults with clinical evidence of infection are believed to develop meningitis, meningoencephalitis or, more rarely, encephalomyelitis/radiculitis, while meningitis is the predominant reported neurological complication in children [5,6]. Case-fatality rates of ≥20% for the Far-Eastern subtype, 6–≤8% for the Siberian subtype, and 1–≤2% for the European subtype [2] have been reported. The incidence of TBE follows a seasonal pattern that corresponds to periods of outdoor activities and tick activity, with peaks in spring, summer and autumn [2]. Climate changes have led to prolonged periods of TBE occurrence in endemic areas and an

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expansion of the TBEV to new foci in previously non-endemic regions [7,8].

Vaccination is considered an effective prophylaxis against TBE, as no specific antiviral exists. Treatment is based on symptomatic measures. The polygeline-free inactivated TBE vaccine TBEvac (*Encepur Adults*, GSK) is licensed for use in adults and adolescents from 12 years of age in Europe. There are several different schedules for primary vaccination against TBE and a first booster dose is generally given at either 12–18 months or 3 years after the completion of primary vaccination, depending on the primary schedule applied [2]. Although the vaccine was shown to be immunogenic and well-tolerated in adults [9] and antibody persistence up to 5 years post-booster vaccination has been demonstrated [10,11], long-term protection afforded beyond this period was not assessed to date. Data collected at 5 years after booster vaccination showed that antibody titers measured by neutralization test (NT) were persistently high (≥10) [10,11]. Therefore, the objective of the current study was to evaluate the persistence of antibody response through 6–10 years after different primary vaccination schedules [12] and a first booster with TBE vaccine at 12–18 months or 3 years following primary vaccination [10].

A summary contextualizing the results and potential clinical research relevance and impact is displayed in the Focus on Patient Section (Fig. 1) for the benefit of Health Care Professionals.

2. Methods

2.1. Study design and participants

This phase IV, open-label, single-center second extension study (NCT01562444) was conducted between March 2012 and September 2016 in Czechia. In the parent study, 398 healthy adolescents and adults aged ≥12 years had received 3 primary TBE-vac doses according to a licensed (rapid [Group R]; conventional [Group C]; accelerated conventional [Group A]) or an unlicensed (modified conventional [Group M]) vaccination schedule [12] (Fig. 2).

In the first extension study (NCT00387634), 283 previously primed participants received a booster dose of TBEvac 3 years post-primary vaccination, while 40 participants (from Group R in the parent study) received it prior to enrollment at 16–18 months post-primary vaccination (Group R_v); all participants were followed for 5 years [10]. Of these, 206 individuals who had received one of the 3 licensed vaccination schedules (Groups R, C and A) were invited to participate in the second extension study, which evaluated the persistence of antibody response elicited by the booster dose from 6 years up to 10 years post-last vaccination (Fig. 2).

Individuals were excluded from the second extension study if they had a NT titer <10 before the first study visit, had received a TBE or other flavivirus (e.g. yellow fever, dengue fever, Japanese encephalitis) vaccine between the completion of the first extension study and the start of the current trial, had a previous confirmed TBEV infection or were exposed to other flaviviruses, had received any investigational or non-registered product within 28 days prior to enrollment or expected to receive one prior to the completion of the study. Additional exclusion criteria were: any vaccination within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in the study, the presence of a condition which, in the opinion of the investigator, might have interfered with the evaluation of the study objective, chronic immunosuppressive conditions and treatment with immunosuppressants, systemic corticosteroids or immunoglobulins within 4 weeks prior to enrollment or during the study period (except short term use of topical corticosteroids), and receipt of whole blood or plasma derivatives up to 3 months

Focus on the Patient

What is the context?

Tick-borne encephalitis (TBE) is a serious viral disease of the central nervous system associated with potentially life-threatening neurological sequelae. TBE is spread in various areas of Europe and Asia as transmitted by vector ticks and caused by TBE virus strains.

Immunization is considered an important, effective prophylaxis, as no specific antiviral exists and the only curative measures are symptom-based. There are several different schedules for primary vaccination against TBE virus and a first booster dose is generally given at either 12–18 months or at 3 years after the conclusion of primary vaccination, depending on the primary schedule applied.

What is new?

This study demonstrates a long-term antibody response for up to 10 years after the first booster dose of TBEvac in all age groups, regardless of which of the 3 licensed primary vaccination schedules was applied. The results of the present study support the potential extension of following TBEvac booster dose intervals from 5 years (or 3 years for persons 50 years and older) to 10 years in all age groups.

What is the impact?

TBEvac provides at least 10 years protection in individual of all ages when adequately following different primary and a booster vaccination schedule.

Fig. 1. Focus on the patient.
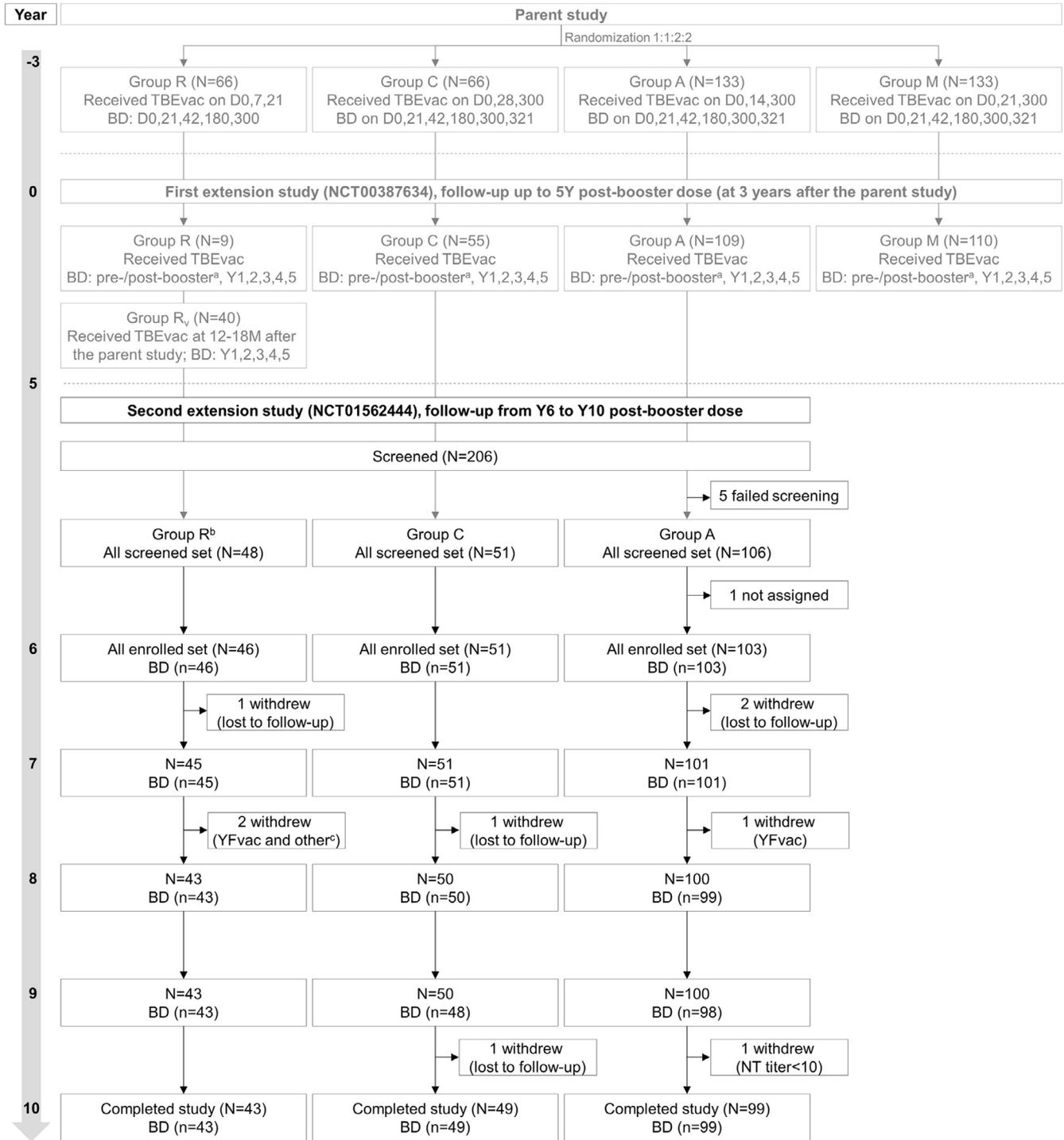
Fig. 2. Participant flowchart. N, number of participants in each group; D, day; M, month; BD, blood draw; Y, year; YFvac, yellow fever vaccination; NT, neutralization test; n, number of participants with blood draw at each timepoint; TBEvac, tick-borne encephalitis vaccine. Note: [a]Pre-/post booster blood samples were drawn immediately before and 28 days after the administration of the booster dose, respectively. [b]Group R (second extension study), includes participants from Group R_v and R (first extension study). [c]Other, administration of forbidden medication.

before enrollment. In addition, all participants were re-assessed for continued eligibility in the study, and a physical examination and documentation of medical history and concomitant medications taken were performed on a yearly basis. Any participants with an NT titer <10 who were discontinued from the trial were offered a booster dose of TBEvac outside the study.

The second extension study was carried out in accordance with the Declaration of Helsinki and conformed to the International Conference on Harmonization-Good Clinical Practice guidelines, as well

as local regulatory requirements. The study was also approved by a local Ethics Review Committee and written informed consent was obtained from all participants prior to enrollment. The trial is registered at ClinicalTrials.gov (NCT01562444).

2.2. Assessments

Blood samples (approximately 10 ml) were drawn once a year, from year 6 up to year 10 from the first extension study, allowing

Table 1
Demographic and other baseline characteristics (all-screened set).

	Group R (N = 48)	Group C (N = 51)	Group A (N = 106)	Total (N = 205)
Mean age ± SD, years[a]	42.7 ± 15.3	41.8 ± 14.3	42.4 ± 14.4	42.3 ± 14.5
Age strata, n (%)[b]				
15–49 years	35 (67%)	39 (72%)	81 (72%)	155 (71%)
≥50 years	13 (25%)	12 (22%)	25 (22%)	50 (23%)
≥60 years	4 (8%)	3 (6%)	7 (6%)	14 (6%)
Female, n (%)	24 (50%)	32 (63%)	55 (52%)	111 (54%)
White/Caucasian, n (%)	48 (100%)	51 (100%)	106 (100%)	205 (100%)
Mean weight ± SD, kg	81.9 ± 17.3	76.4 ± 15.8	80.1 ± 17.6	79.6 ± 17.1
Mean height ± SD, cm	174.1 ± 9.3	170.6 ± 9.4	173.0 ± 9.3	172.6 ± 9.4
Mean BMI ± SD, kg/m^2	27.0 ± 4.9	26.2 ± 4.9	26.7 ± 4.8	26.6 ± 4.83

Group R, participants who received 3 primary doses at D0, 7, 21 and a booster dose at 12–18 month or 3 years post-primary vaccination; Group C, participants who received 3 primary doses at D0, 28, 300 and a booster dose at 3 years post-primary vaccination; Group A, participants who received 3 primary doses at D0, 14, 300 and a booster dose at 3 years post-primary vaccination.
N, number of participants in each group; SD, standard deviation; n (%), number (percentage) of participants in each category; BMI, body mass index; D, day.
  [a] At the start of the current study.
  [b] At the start of the first extension study.

Table 2
Percentage of participants with antibody titers ≥2 and ≥10 as measured by neutralization test, by timepoint (all screened set).

	Group R (N = 48) % (95% CI)		Group C (N = 51) % (95% CI)		Group A (N = 106) % (95% CI)	
	NT titers ≥2	NT titers ≥ 10	NT titers ≥2	NT titers ≥ 10	NT titers ≥2	NT titers ≥10
Year 6	96 (86–99)	96 (86–99)	100 (93–100)	100 (93–100)	97 (92–99)	97 (92–99)
Year 7	94 (83–99)	94 (82–99)	100 (93–100)	100 (93–100)	95 (89–98)	95 (89–98)
Year 8	90 (77–97)	90 (77–97)	98 (90–100)	98 (90–100)	93 (87–97)	93 (86–97)
Year 9	90 (77–97)	90 (77–97)	94 (84–99)	94 (84–99)	92 (86–97)	93 (86–97)
Year 10	90 (77–97)	90 (77–97)	96 (87–100)	94 (84–99)	93 (87–97)	93 (87–97)

Group R, participants who received 3 primary doses at D0, 7, 21 and a booster dose at 12–18 month or 3 years post-primary vaccination; Group C, participants who received 3 primary doses at D0, 28, 300 and a booster dose at 3 years post-primary vaccination; Group A, participants who received 3 primary doses at D0, 14, 300 and a booster dose at 3 years post-primary vaccination.
N, number of participants in each group; %, percentage of participants with titers above the pre-specified threshold; CI, confidence interval; NT, neutralization test.

for a window of 30 days for the collection of each sample. Immune response to TBEvac was assessed at each timepoint by a validated NT assay [13], which was previously described in detail [14,15]. The same assay was used in the parent and first extension studies. The NT was performed at the GSK Clinical Science Laboratory in Marburg, Germany.

The lowest limit of detection (LLOD) for the NT assay was 2; NT titers ≥10 were considered a clinical meaningful threshold [16,17]. Immunogenicity results were expressed as the percentage of participants with NT titers ≥2 and ≥10 and geometric mean antibody titers (GMTs) in each group. Within-group geometric mean ratios (GMRs) were also calculated using 2 different baselines as denominators: either the pre-booster (excluding individuals from Group R$_v$ of the first extension study) or the post-booster vaccination GMT (calculated on the first day of the first extension study for Group R$_v$ and 21 days post-booster dose for Groups R, C and A).

No safety endpoints were assessed in this study.

2.3. Statistical analysis

The main analyses for immunogenicity were based on the all-screened set, which included all screening failures and those who withdrew from the study. In this set, missing antibody values were set to half of the LLOD (i.e., an NT titer of 1) and used in the analyses.

Analyses were also performed on the per-protocol set (PPS) at each timepoint, which included enrolled participants with an evaluable serum sample at the relevant timepoint, and not meeting pre-specified exclusion criteria (for example, NT titers <10, confirmed exposure to TBEV or other flaviviruses, documented TBE or *flavivirus* vaccination during the study).

The percentages of individuals with NT titers ≥2 and ≥10 were tabulated by vaccine schedule and timepoint together with their Clopper-Pearson 95%, 2-sided confidence intervals (CIs). GMTs, GMRs and 95% CIs were calculated using an analysis of variance method with logarithmically transformed titers/ratio values.

For each group, analyses were repeated in the following age strata, defined based on the participants' age at the start of the first extension study: 15–49 years, ≥50 years, and ≥60 years (a subset of the ≥50 years age stratum).

The kinetics of long-term antibody titers of the booster response were evaluated by summary statistics only and no tests for group comparisons were planned. All NT results were interpreted descriptively.

3. Results

3.1. Study population

Of the 206 screened individuals, 201 were enrolled and 191 completed the study. Reasons for early termination are listed in Fig. 2. The PPS included 92–96% (190/206–198/206) of the all-screened set across the 5 years of follow-up. The main reasons for exclusions from the PPS were: lost to follow up (n = 5) administration of any medication or concomitant vaccine(s) specified in the protocol (n = 3), missing serological results (n = 4), failure to comply with the blood draw schedule (n = 2) or to meet the entry criteria (n = 6) and NT titer < 10 (n = 1). A higher percentage of females (54%; 111/205) than males were screened and all participants were White/Caucasian. The mean age at enrollment was 42.3 ± 14.5 years. The demographic characteristics were well-balanced among groups (Table 1).

Table 3
Percentage of participants with antibody titers ≥2 and ≥10 as measured by neutralization test, by timepoint (per-protocol set).

	Group R			Group C			Group A		
	N	% (95% CI)		N	% (95% CI)		N	% (95% CI)	
		NT titers ≥2	NT titers ≥ 10		NT titers ≥2	NT titers ≥ 10		NT titers ≥2	NT titers ≥ 10
Year 6	45	100 (92–100)	100 (92–100)	51	100 (93–100)	100 (93–100)	102	100 (96–100)	100 (96–100)
Year 7	43	100 (92–100)	100 (92–100)	51	100 (93–100)	100 (93–100)	100	100 (96–100)	100 (96–100)
Year 8	43	100 (92–100)	100 (92–100)	50	100 (93–100)	100 (93–100)	99	100 (96–100)	99 (95–100)
Year 9	43	100 (92–100)	100 (92–100)	48	100 (93–100)	100 (93–100)	98	100 (95–100)	100 (95–100)
Year 10	43	100 (92–100)	100 (92–100)	49	100 (93–100)	98 (89–100)	99	100 (96–100)	100 (96–100)

Group R, participants who received 3 primary doses at D0, 7, 21 and a booster dose at 12–18 month or 3 years post-primary vaccination; Group C, participants who received 3 primary doses at D0, 28, 300 and a booster dose at 3 years post-primary vaccination; Group A, participants who received 3 primary doses at D0, 14, 300 and a booster dose at 3 years post-primary vaccination.
N, number of participants with available results in each group; %, percentage of participants with titers above the pre-specified threshold; CI, confidence interval; NT, neutralization test.

Table 4
Antibody geometric mean titers (95% CI) as measured by neutralization test, by timepoint.

	Group R		Group C		Group A	
	N	GMT (95% CI)	N	GMT (95% CI)	N	GMT (95% CI)
All-screened set						
Year 6	48	292 (184–463)	51	293 (187–458)	106	221 (162–302)
Year 7	48	295 (180–484)	51	343 (212–555)	106	254 (182–355)
Year 8	48	134 (79–227)	51	211 (127–352)	106	155 (109–221)
Year 9	48	211 (119–375)	51	214 (122–374)	106	194 (131–285)
Year 10	48	166 (94–295)	51	245 (140–428)	106	180 (122–265)
Per-protocol set						
Year 6	45	363 (242–544)	51	293 (200–428)	102	263 (201–344)
Year 7	43	396 (265–591)	51	343 (237–496)	100	335 (257–435)
Year 8	43	237 (159–355)	50	235 (162–341)	99	222 (170–289)
Year 9	43	393 (262–588)	48	299 (204–438)	98	298 (228–389)
Year 10	43	301 (193–470)	49	307 (202–466)	99	260 (194–349)

Group R, participants who received 3 primary doses at D0, 7, 21 and a booster dose at 12–18 month or 3 years post-primary vaccination; Group C, participants who received 3 primary doses at D0, 28, 300 and a booster dose at 3 years post-primary vaccination; Group A, participants who received 3 primary doses at D0, 14, 300 and a booster dose at 3 years post-primary vaccination.
N, number of participants in each group (all screened set)/number of participants with available results (per-protocol set); GMT, geometric mean titer; CI, confidence interval.

3.2. Immunogenicity

In the all-screened set, the percentage of individuals with NT titers ≥2 varied from 96 to 100% at Year 6, and from 90 to 96% at Year 10, among all groups. At all timepoints, all individuals with NT ≥2 also had NT ≥10, with 1 exception in Group A at Year 8 (60 years of age at booster dose) and 1 exception in Group C at Year 10 (36 years at booster dose) (Table 2).

Results for the PPS were consistent with those observed in the all-screened set. The percentages of participants with NT titers ≥10 were ≥98% in the PPS in each of the groups (Table 3). Only 2 participants presented NT titers <10 during the study, one in the 15–49 years age stratum (Group A) and one in the ≥60 years age stratum (Group C).

Within the same group, the percentage of participants with NT titers ≥10 across all timepoints did not seem to differ with age (Fig. S1).

During the 5 years of follow up, NT GMTs varied between 134 and 343 and between 222 and 396 across the 3 groups in the all-screened set and PPS, respectively (Table 4). Overall, GMTs remained similar across groups and within the assay variability (Table 4). NT GMT values seemed to decrease with increasing age, but remained similar within the same age strata at each time-point, although the number of individuals was small in the ≥60 years age group (3–7 individuals per vaccine group). In the all-screened set, GMTs were ≥151, ≥98 and ≥57 until Year 10 in participants aged 15–49 years, ≥50 years and ≥60 years, respectively (Fig. S2).

At Year 10, across all groups GMR values ranged from 0.76 to 1.08 and from 1.12 to 2.89 in the all-screened set and the PPS, respectively, when calculated relative to pre-booster antibody levels, and were lower when computed relative to antibody levels at 21 days post-booster dose (Table S1). Similar trends were observed when GMRs were analyzed by age stratum (Tables S2, S3).

4. Discussion

This study is the first to assess the persistence of antibody levels from 6 up to 10 years post-booster vaccination with TBEvac in individuals who had previously received either the conventional, accelerated conventional, or rapid primary vaccination schedules [12], and expands the results of the previous study assessing antibody persistence up to 5 years post-booster vaccination [10].

Overall, ≥90% of individuals in the all-screened set having received primary vaccination and a booster dose of TBEvac, and ≥98% in the PPS still had NT titers ≥10 10 years post-booster, regardless of the primary immunization schedule received. These results confirm the long-term persistence of an immune response against TBEV, as measured by the percentage of participants with NT titers above a chosen clinically meaningful threshold.

Our yearly screening and assessments seem to indicate that no participant was infected with TBEV during the study, as convalescent samples would have been detected by spikes in antibody levels. However, it is uncertain whether the participants in our study were simply not exposed to TBEV infection or if the data pro-

vide evidence for the effectiveness of TBEvac. Reports from Austria serve as the most reliable data source concerning the effectiveness of TBE vaccination [18]. The mass vaccination program initiated more than 30 years ago has resulted in a profound drop in TBE cases when compared to Czechia, where endemic areas are similar and TBE surveillance is excellent but vaccination coverage is low (23%) [19,20].

Increasing age is the most important predictive parameter for lower antibody titers and has been associated with a stronger decline in anti-TBEV post-vaccination antibody levels [21]. In line with other reports [21–23], overall lower GMTs were observed in the current extension study in individuals ≥50 and ≥60 years of age compared to younger adults. Nevertheless, it is noteworthy that NT GMTs were ≥57 across all groups until Year 10 and that, in contrast to published data [24], the decline was not stronger with increased age. Although the sample size was small for this age group, this suggests a long-term immunity against TBE of up to 10 years, which may point towards an extension of the booster intervals for elderly individuals, as well.

Only 2 participants could not maintain NT titers ≥10 from 6 to 10 years post-booster vaccination and 1 additional individual had NT titers <10 during the first extension study [10]. The latter was screened and included in the all-screened set of the present extension study, but not enrolled. Only one participant was ≥60 years old, while the other belonged to the 15–49 years age group, suggesting that age was not the only factor for the lower titers observed.

This study enabled the long-term monitoring of NT titers with high compliance rate, as a high percentage of participants from the parent study were retained over the course of 15 years and 191 out of 213 participants who received the first booster dose completed this 10-year follow-up. This, together with the use of the all-screened set to assess the long-term persistence of anti-TBEV antibodies, constitutes the main strengths of our study. In antibody persistence studies, un-observable data due to dropouts or early terminations may lead to biased estimates, as these missing data are missing not at random (MNAR). PPSs generally exclude participants with these MNAR missing data, so analysis using PPS over-estimates the true persistence. Therefore, in this study, we also presented the persistence results in the all-screened set using an imputation of missing MNAR data which assumed any un-observable antibody results as below the LLOQ, thus under-estimating true antibody persistence. We observed that immune response to TBEvac (NT titers ≥10) persists in an estimated 90 and 98% of vaccinees in the all-screened set and PPS, respectively, following different immunization schedules.

A recent study also evaluated seropersistence of anti-TBEV antibodies in adults 10 years after a first booster vaccination [25]. The percentage of participants demonstrating NT titers ≥10 was lower (85%) in the PPS [25] following administration of TicoVac (FSME-Immun, Pfizer), another widely used vaccine against TBE, and GMTs also declined more significantly than what was observed in this and other TBEvac persistence studies [10,11]. In addition, only 68% of individuals in the TicoVac study completed 10-year follow-up without booster dose [25], whereas only 3 out of 216 individuals in the all-screened set from this study had confirmed NT antibody levels <10 and received the second TBEvac booster dose during the course of the study. Therefore, the findings from our study should be considered as vaccine-specific, and cannot be extended to other TBE vaccines.

Limitations of the study include the relatively small sample sizes for some of the age-stratified data analyses in older adults (≥60 years), which might preclude generalization of our findings for this age subset.

In conclusion, based on data from a continuous 15-year follow-up, participants are protected against TBE, regardless of the schedule used and the age at immunization. The results of this second 5-year extension study are similar to those of the 5-year follow-up post-booster dose, suggesting that the conventional, accelerated conventional or rapid schedules are all appropriate primary vaccination schedules and that 4 doses of TBEvac administered as a 3-dose primary vaccination and a booster dose afford protection for at least 10 years.

Trademark statement

Encepur Adults is a trademark owned by the GSK group of companies. *FSME-IMMUN* is a trademark of Pfizer.

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Conflict of Interest

ML, IG, LM and FX are employees of the GSK group of companies. JB reports grants from Novartis Vaccines and Diagnostics during the conduct of the study.

Authors' contribution

JB, ML and FX were involved in the conception or design of the study. JB, ML, IG and LM participated in the collection or generation of the data. JB, ML and LM performed the study. All authors were involved in the analysis and interpretation of the data.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2017.12.081.

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