17 October 2024
EMA/509248/2024
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Siiltibcy

Common name: rdESAT-6 / rCFP-10

(*Mycobacterium tuberculosis* derived antigens:

- Recombinant dimer of *Mycobacterium tuberculosis* 6 KDa early secretory antigenic target / rdESAT-6; and

- Recombinant 10 kDa culture filtrate protein of *Mycobacterium tuberculosis*, rCFP-10)

Procedure No. EMEA/H/C/006177/0000

Note

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

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List of abbreviations

ART	Anti-retroviral treatment
BCG	Bacillus of Calmette and Guerin
CFU	Colony forming units
CHMP	Committee for Medicinal Products for Human Use
CPP	Critical process parameters
CQA	Critical quality attributes
DP	Drug product
DS	Drug substance
DSMB	Data Safety Monitoring Board
DTH	Delayed Type Hypersensitivity
EOP	End of Production
EPCB	End of Production Cell Bank
FAS	Full Analysis Set
HCD	Host cell DNA
HCP	Host cell protein
HIV	Human immunodeficiency virus
HMW	High molecular weight
i.d.	intradermal
IGRA	Interferon-Gamma Release Assay
IHRS	In-house reference standard
ISR	Injection site reaction
IV	Intravenous
KPP	Key performance parameter
L. lactis	*Lactococcus lactis*
LMW	Low molecular weight
LTBI	Latent Tuberculosis Infection
MCB	Master Cell Bank
MO	Major objection
MS	Mass spectroscopy
Mtb	*Mycobacterium tuberculosis*
NAS	New Active Substance
NC	Negative Control
NOAEL	No Observed Adverse Effect Level
OC	Other concern
PC	Positive Control
PDCO	Paediatric Committee
PLHIV	People living with HIV
PPD	Purified Protein Derivative

PSUR	Periodic safety update report
PV	Process validation
rCFP-10	Recombinant Culture filtrate Protein 10
rdESAT-6	recombinant dimer Early secretion antigen target 6
SAE	Serious adverse event
SC	subcutaneous
SDS-PAGE	Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis
SIIPL	Serum Institute of India Private Limited
SSI	Statens Serum Institute Denmark
TB	Tuberculosis
TBC	TB microbiologically confirmed
TBD	TB diagnosed (clinically or microbiologically)
TEAE	Treatment-Emergent Adverse Events
TK	Toxicokinetic
TST	tuberculin skin test
WCB	Working Cell Bank

1. Background information on the procedure

1.1. Submission of the dossier

The applicant then called Vakzine Projekt Management GmbH submitted on 6 February 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Siiltibcy, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The initially proposed indication was: *in adults and children aged 28 days and older for diagnosis of infection with Mycobacterium tuberculosis. This medicinal product is for diagnostic use only.*

During the assessment of the dossier, Vakzine Projekt Management GmbH changed its name to Serum Life Science Europe GmbH end of 2023, as communicated with the applicant's responses to the Day 120 List of Questions.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

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

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0188/2016 the agreement of a paediatric investigation plan (PIP) and the granting of product-specific waiver for population below 28 days.

At the time of submission of the application, the PIP P/0188/2016 was completed.

The PDCO issued an opinion on compliance for the PIP P/0188/2016 (EMEA-C-001156-PIP01-11-M07).

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

1.4.2. New active substance status

The applicant requested the active substances (*Mycobacterium tuberculosis* derived antigens rdESAT-6 and

rCFP-10) contained in the medicinal product to be considered as a new active substance in comparison to Tuberculin PPD RT23, previously authorised in the European Union as Tuberkulin PPD RT23 SSI (2 T.E.), as the applicant claimed that *Mycobacterium tuberculosis* derived antigens rdESAT-6 and rCFP-10 differ significantly in properties with regard to molecular structure, nature of source material or manufacturing process and safety and/or efficacy from the already authorised active substance.

1.5. Scientific advice

The applicant received the following scientific advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
21 June 2012	EMEA/H/SA/2332/1/2012/III	Caroline Auriche, Mair Powell
23 July 2015	EMEA/H/SA/2332/2/2015/PED/II	Minne Casteels, Jan Mueller-Berghaus

The scientific advice pertained to the following quality, non-clinical, and clinical aspects:

- CMC data to support MAA including: batch strategy, evaluation of impurities, analytical comparability to support changes in manufacturing, specification and characterisation of drug substances and drug product, potency and stability testing, removal of phenol preservative in the case of a single dose presentation
- Adequacy of the data to support approval in women of childbearing potential and associated SmPC statements
- Agreement that Siiltibcy represents a diagnostic benefit
- Agreement on the assessment of diagnostic performance of Siiltibcy in different populations and more specifically:
    - that the specificity and the sensitivity of Siiltibcy, Quantiferon and PPD can be estimated and compared in the Phase 3 trials by the proposed statistical method, although the true state of infection will remain unknown
    - that the sensitivity and specificity estimates of both Phase 3 clinical trials will apply to all age groups and to HIV-positive as well as HIV-negative individuals
    - that data obtained in South Africa are relevant in the European setting
    - the same dose of Siiltibcy may be applicable for all target groups, irrespective of e.g. age and HIV-status, without additional clinical dose-finding studies in specific target groups
    - that a single cut-off at 5 mm for PPD must be applied for the statistical analysis of the sensitivity and the specificity in the Phase 3 trials TESEC-05 and TESEC-06
- Safety assessments and safety database to support MAA
- Paediatric development strategy to support indication in children and more specifically:
    - concurrence that if the pharmacodynamics are sufficiently similar in children and adults then sensitivity and specificity in the paediatric population may be extrapolated from adult data

- proposed statistical method for validation of the extrapolation concept including definitions of sufficient similar pharmacodynamics and a potential lower age limit for extrapolation from adults to children
- agreement that a demonstrated lower rate of Siiltibcy responses compared to TST in a paediatric subpopulation of BCG-vaccinated negative endemic controls aged 5 – 11 years is supporting the extrapolation of specificity of adult data to all paediatric age groups
- proposal to omit QuantiFERON®-TB Gold In Tube testing in trial participants below the age of five for Siiltibcy registration in the EU which includes infants and children down to 28 days

1.6. Steps taken for the assessment of the product

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

Rapporteur: Maria Grazia Evandri Co-Rapporteur: Jan Mueller-Berghaus

The application was received by the EMA on	6 February 2023
The procedure started on	23 February 2023
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	19 May 2023
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	15 May 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	30 May 2023
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 June 2023
The applicant submitted the responses to the CHMP consolidated List of Questions on	15 January 2024
The following GMP inspections were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product: – A GMP inspection at Serum Institute Of India Private Limited 212/2 Off Soli Poonawala Road Hadapsar Pune 411028 India was carried out between 16 – 23 January 2024. The outcome of the inspection was issued on 20/01/2024 – A GMP inspection at Serum Institute Of India Private Limited S No 105-110 Zone 3 Tal Haveli Manjari Bk Pune 412307 India was carried out between 16 – 23 January 2024. The outcome of the inspection was issued on 23/01/2024	

The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	29 February 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	7 March 2024
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	21 March 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	27 May 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	12 June 2024
The CHMP Rapporteur circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	21 June 2024
The CHMP agreed on a 2nd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	27 June 2024
The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on	14 August 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	4 September 2024
The CHMP Rapporteur circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	12 September 2024
The CHMP agreed on a 3rd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	24 September 2024
The applicant submitted the responses to the CHMP 3rd List of Outstanding Issues on	23 September 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	4 October 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Siiltibcy on	17 October 2024
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	17 October 2024

2. Scientific discussion

2.1. Problem statement

The applicant submitted a dossier to support a marketing authorisation for Siiltibcy, initially proposed to be indicated “in adults and children aged 28 days and older for diagnosis of infection with Mycobacterium tuberculosis”. The proposed route of administration (intradermic Mantoux technique) and dose are the same for all age groups and all populations, including BCG-vaccinated individuals and human immunodeficiency virus (HIV)-positive individuals.

Mycobacterium tuberculosis (Mtb) normally enters the host by inhalation of infectious droplets from a contagious individual. In the lungs, the bacteria are taken up by phagocytic cells, but the bacteria are able to survive and undergo progressive growth inside these cells, which is a troubling condition of Mtb infection. If the infection is not successfully contained by the host, then typical symptoms of active TB disease will develop, including persistent cough (often with blood in sputum), fever, pain in chest, weight loss, night sweats, and loss of appetite. As an approximation, the lifetime risk of infected individuals developing active TB disease is between 5% and 15% (Vynnycky and Fine 2000; WHO 2021). Individuals with HIV infection, or patients under immunosuppressive treatment, are at particular risk of developing TB disease when infected.

In adults and older children (over 5 years), the loss of containment by the host gives rise to typical symptoms, notably a persistent cough with blood in the sputum. As infants and younger children (below 5 years) are less likely to develop these typical symptoms but are at greater risk of a rapidly disseminating disease, clinical diagnosis of TB disease in this age group is more difficult.

2.1.1. Disease or condition

2.1.2. Epidemiology

Diagnosis of infection with virulent mycobacteria such as Mtb continues to be critical as tuberculosis (TBC), caused by infections with Mtb, remains a major cause of morbidity and mortality throughout the world, with most incidences in Africa, South-East Asia, and Western Pacific. According to the World Health Organization (WHO), 10.6 million (9.9 to 11 million) new (incident) TB cases were estimated, and 1.6 million people died from the disease in 2021, including 0.21 million among people with HIV infection (WHO 2022).

Although the number of TB deaths fell by 5.9% between 2015 and 2021, TB is the 13th leading cause of death and the second infectious killer after COVID-19 (above HIV/AIDS) worldwide (WHO 2022). In Europe in 2020, there were 3800 TB deaths reported in the European Union (EU) and European Economic Area (0.8 deaths per 100,000) (ECDC 2022a). An estimated number of 21000 TB death occurred among HIV-positive people in the European Region in 2020, equivalent to 2.3 deaths per 100,000 population (range 2.2 – 2.4) (ECDC 2022b).

Ref:
European Centre for Disease Prevention and Control. Tuberculosis. 2022a. In: ECDC. Surveillance and Monitoring in Europe, https://www.ecdc.europa.eu/sites/default/files/documents/Tuberculosis-surveillance-monitoring-europe-2022_0.pdf.
European Centre for Disease Prevention and Control. Tuberculosis. 2022b. In: ECDC. Annual epidemiological report for 2020. Stockholm. Available online from: https://www.ecdc.europa.eu/sites/default/files/documents/tuberculosis-annual-epidemiological-report-2020_1.pdf
World Health Organization. Global Tuberculosis Report 2022, at https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022.

2.1.3. Aetiology and pathogenesis

Tuberculosis is referred to disease caused by *Mycobacterium tuberculosis*, which has human beings as main reservoir. Related mycobacteria (such as *M. bovis, M. africanum, M. microti*) can sometimes cause a similar disorder.

People with active (respiratory) TB disease, can eject droplets containing a large number of Mtb organisms. These droplets can remain in room air for a while and, thus, healthy subjects can inhale the droplets with their load of Mtb. Patients with more advanced disease, with pulmonary cavitations, can have a large number of Mtb organisms and thus can be more contagious.

Environmental factors have an important role in the infection process: constrained space or rooms with poor ventilation are particularly risky for people living in them (e.g., people in institutions). Frequent contacts with patients with active disease increase the risk of infection (e.g., healthcare workers). It is thus difficult to estimate the risk of infections, but according to a WHO estimation, each untreated patient might infect 10 to 15 people per year.

It is important to keep in mind that most infected people do not develop active disease. However, one a patient is treated, the risk of transmission is rapidly reduced. The risk of reactivation is increased in patients with HIV or other immunosuppressive disease.

After reaching the alveoli, if the bacillum is not defeated by immune response, it can proliferate inside the lung macrophages and can diffuse to other tissues. The lung macrophages produce cytokines which attract other immune cells that at the end produce a nodular lesion (tubercle). The bacterium, if not contained, can survive inside the tubercles for years and can spread to lymphatic stations (lymph nodes) or to the blood.

2.1.4. Clinical presentation and diagnosis

About 5 – 10% of infected individuals develop active disease (half of them in 2 – 3 years after the infection). Most often the reactivation occurs within the first 2 years, but it some cases can occur decades later. The most frequent site of TBC reactivation is the lung (apices); however, any involved tissue can be the site of the reactivation. The probability of reactivation is increased by conditions of impaired cellular immunity (e.g., non-treated HIV). Other important medical conditions increased the likelihood of reactivation (e.g., diabetes mellitus, cancer, some surgeries, immunosuppressants such as in transplanted patients). In areas where TBC is prevalent, another possible cause of reactivation is reinfection.

Primary infection is almost always asymptomatic, but in some cases nonspecific symptoms may occur (mild fever and fatigue). Active pulmonary tuberculosis can have few symptoms (if any) as well; patients can complain of vague symptoms (general symptoms, appetite and weight loss, fatigue, night sweats, mild fever). Cough can be present (typically in the morning) and, if cavitations occur, hemoptysis can be observed. If the parenchymal damage of the lungs is important, dyspnea can be present. Patients with impairment of cell immune response (such as in HIV) can have atypical clinical manifestations (symptoms from other organs).

In people screened positive for pulmonary or extrapulmonary TB a WHO recommended rapid diagnostic test (with or without resistance testing) should be performed[1]. Microscopic culture is recommended to control the treatment.

INF-dependent assays and skin tests are only recommended in low- and middle-income countries to test for latent TB but not for active disease. Two types of these tests are currently available for TB infection: the tuberculin skin test (TST) and the interferon-gamma release assay (IGRA) blood test. Both of them evaluate cell-mediated immunity, but they are very different in terms of technique used, costs and facility requirement. It seems that there is no clear-cut advantage of one of them in order to predict the risk of developing active TB disease in the future.

The TST (administered according to the Mantoux intradermal method) uses purified protein derivative (PPD). The response is given by a skin induration of the arm (where TST is injected) and it is measured 48 to 72 hours after injection. TST may yield false-positive results in patients with nontuberculous mycobacterial infections or who have received the bacille Calmette-Guérin (BCG) vaccine.

IGRA test is a blood test in which patient lymphocytes are exposed to TB antigens, and release interferon gamma if they were previously exposed to TB antigens, suggesting prior contact with the TB germ. IGRA it is more specific than TST since it is not affected by previous BCG vaccination. However, it is a more expensive method compared to TST.

Neither of the above-described tests is able to distinguish between active and latent TB.

2.1.5. Management

First-line drugs for TB isoniazid, rifampin, pyrazinamide, ethambutol. Many TB regimen treatments are available. Many medicinal products are orally available and can cross the blood-brain barrier; moreover, they have different safety profiles. Other antibiotic products are used as second-line therapy. Drug resistance is a recognised problem in TB treatment. It can derive from poor adherence to the treatment, single agent therapy, and acquisition of an already resistant strain from another infected subject.

2.2. About the product

The name of the product is Siiltibcy but, during development, it was first named ‘C-Tb’, then ‘Cy-Tb’; therefore, some tables/data below make reference to these names.

Siiltibcy is an immunological recombinant medicinal product for diagnosing Mtb infection in humans (pharmacotherapeutic group: tuberculosis diagnostics). Siiltibcy contains two recombinant proteins: rdESAT-6 and rCFP-10. ESAT-6 (the monomer) is a potent T-cell antigen secreted during the early phase of infection with Mtb. ESAT-6 and CFP-10 have been identified from Mtb culture filtrate. The genes for ESAT-6 and CFP-10 are encoded in the same Mtb operon and are transcribed together, from the RD-1 region, which is present in virulent mycobacteria, i.e., Mtb and two other more rare mycobacteria species causing human TB disease. Very few atypical mycobacteria express these proteins. BCG is defined through the absence of the RD-1

[1] “People screened positive for TB include adults and children with signs or symptoms suggestive of TB, with a chest X-ray showing abnormalities suggestive of TB, a positive mWRD used as a screening tool or positive C-reactive protein test (>5 mg/L) in PLHIV. A person with a positive mWRD used as a screening tool and a low pretest probability should be clinically assessed and, if deemed a presumptive TB patient, should have a repeat mWRD performed and follow Algorithm 1.” WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detention, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

region, therefore these antigens are not found in any of the BCG vaccine strains used for vaccination worldwide. The proteins form a natural complex in a 1:1 molar ratio.

The Siiltibcy formulation is: rdESAT-6 0.5 micrograms + rCFP-10 0.5 micrograms/mL solution for injection. It is presented as multidose vial containing 10 doses of 0.1 mL each. Phenol is included as preservative.

Siiltibcy is administered directly by intradermic administration to the site of action. Dermis and epidermis of skin are rich in antigen-presenting cells, which make the intradermal route optimal to a more efficient immune responses with smaller amounts of antigens. Given this route of administration, clinical activity is not related to systemic exposure as there should be no significant systemic absorption using the Mantoux injection technique.

Siiltibcy is designed to induce a limited immune response. If the subjects have previously been infected, they will normally have generated an immune response: T-cells proliferate in response to the infection and give rise to T-cells specifically sensitised to antigens from the pathogen. This T-cell "memory response" can enter the bloodstream and circulate for months or years (latent infection). Subsequent re-stimulation of these T-cells with intradermal injection of antigen evokes a local Delayed Type Hypersensitivity (DTH) response directed by cytokines [such as Interferon gamma (IFNg)] in subjects sensitised by prior infection, which is seen as swelling (induration) and redness (erythema) in the skin at the site of injection. Delayed because the reaction becomes evident hours after injection. The area of induration reflects DTH activity and is an easy way to measure response. Mtb infection is recognized by an "induration" (i.e., the appearance of localized thickening and swelling of the skin caused by inflammation) ≥ 5 mm at the injection site 48 to 72 h after injection of Siiltibcy. The stronger the response, the more likely the infection is both recent and active.

Siiltibcy is indicated as a diagnostic aid for detection of *Mycobacterium tuberculosis* infection, including disease, in adults and children aged 28 days or older.

2.3. Type of application and aspects on development

The MAA was submitted according to mandatory scope (Article 3(1) of Regulation (EC) No 726/2004) Annex (1) (Biotech medicinal product) and the legal basis refers to Article 8.3 of Directive 2001/83/EC - complete and independent application.

Siiltibcy has been developed by Statens Serum Institut (SSI) – Denmark, and has been exclusively licensed to Serum Institute of India Pvt. Ltd (SIIPL), Pune for commercialization. The Siiltibcy manufacturing process technology has also been transferred to SIIPL, Pune from SSI, Denmark.

The rationale behind developing the Siiltibcy test is an attempt to combine the strength of IGRAs and the skin test technologies to achieve superior specificity and ease of use.

The clinical development programme for Siiltibcy consists of seven completed clinical studies with Siiltibcy and two completed clinical studies with rdESAT-6, a component of Siiltibcy. In each study, Siiltibcy or rdESAT-6 were administered by i.d. injection using the Mantoux technique into the flexor surface of the forearm at the junction of the upper third with the lower two thirds.

Of the seven clinical studies completed with Siiltibcy, studies TESEC-05, TESEC-06, and TESEC-07 are confirmatory Phase 3 studies, contributing most significantly to the benefit-risk assessment of this diagnostic test. TESEC-05, TESEC-06 and TESEC-07 compared the diagnostic performance of Siiltibcy to PPD and QuantiFERON®-TB Gold In-Tube Test (QFT). Both PPD TST and QFT are widely accepted tests in Europe used

in current medical practice for the detection of latent Mtb infection. Comparison of Siiltibcy with these tests was used by the applicant as benchmark of diagnostic performance.

Two key supporting studies (TESEC-03 and TESEC-04) investigated the specificity and sensitivity of Siiltibcy, respectively. Additionally, studies TESEC-01 and TESEC-02 were Phase 1 studies investigating the safety and dosage of Siiltibcy.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as solution for injection containing 0.5 micrograms of recombinant dimer of *Mycobacterium tuberculosis* early secretory antigenic target (rdESAT-6) and 0.5 micrograms of recombinant culture filtrate protein of *Mycobacterium tuberculosis* (rCFP-10) per ml as active substances.

Other ingredients are: disodium hydrogen phosphate dihydrate, potassium dihydrogen orthophosphate, potassium chloride, sodium chloride, polysorbate 20, phenol, and water for injections.

The product is available in clear multidose glass vial with stopper (bromobutyl rubber) and a plastic flip off cap with aluminium over-seal. Each vial contains 10 doses of 0.1 mL.

2.4.2. Active substance: rCFP-10

2.4.2.1. General information

rCFP-10 (recombinant culture filtrate protein) is a recombinant version of *Mycobacterium tuberculosis* CFP-10 protein. The complete rCFP-10 protein has 103 amino acids, including 4 extra amino acids at the N terminal end. rCFP-10 has a molecular weight of 11.1 kDa and is not glycosylated.

The rCFP-10 active substance is a *M. tuberculosis* specific antigen, which induces a cytokine-mediated inflammatory response in individuals previously exposed to a *M. tuberculosis* infection. The mode of action of the active substance is described. The 103 amino acid sequence of rCFP-10 encoded by the expression construct, pCGJ.SSI.40 is shown below in Figure 1.

DTRSAEMKTDAATLAQEAGNFERISGDLKTQIDQVESTAGSLQGQWRGAAGTAAQAAVVR

FQEAANKQKQELDEISTNIRQAGVQYSRADEEQQQALSSQMGF

Figure 1: Amino acid sequence of rCFP-10

2.4.2.2. Manufacture, characterisation and process controls

The rCFP-10 active substance is manufactured, tested and released at Serum Institute of India Private Limited (SIIPL) at its two sites in India: Hadapsar (212/2 Off Soli Poonawalla Road, Hadapsar, Pune, India) and Manjari (S No 105-110 Zone 3, Tal Haveli, Manjari Bk, Pune, India [MSEZ]). Manufacturing of the active substance is restricted to the Hadapsar site. While Statens Serum Institute (SSI), 5, Artillerivej, 2300, Copenhagen S, Denmark is responsible for the generation of the master cell bank (MCB) and working cell

bank (WCB), cell banks are stored at the SIIPL Hadapsar site. All the sites involved in manufacturing and testing of the active substance have been appropriately GMP authorised.

The rCFP-10 active substance was developed at the SSI site in Denmark, whereafter the technology of rCFP-10 active substance manufacturing was transferred to the current SIIPL manufacturing site in India.

Description of manufacturing process and process controls

The rCFP-10 active substance manufacturing process has been adequately described. rCFP-10 active substance is manufactured by a fermentation process starting from a *Lactococcus lactis* WCB. Main steps are harvest of crude protein after fermentation using microfiltration, concentration by ultrafiltration and precipitation by acidification. A purified protein fraction is obtained by microfiltration followed by concentration and diafiltration. Finally, the active substance is shipped from the manufacturing site in Hadapsar to the Manjari site in India for finished product formulation.

The ranges of critical process parameters and the routine in-process controls (IPCs) along with acceptance criteria, are described for each step. IPCs include bioburden, integrity testing of filters/membranes/cassettes, testing of pH, conductivity, appearance and cell density. Overall, the chosen IPCs are considered adequate to ensure a well-controlled active substance manufacturing process. Based on the process validation runs, acceptable limits/set points were provided for IPCs and process parameters. In-process hold times were tightened according to process validation results. The active substance manufacturing process is considered acceptable.

In the hold time study, bioburden was tested in media/buffers used for rCFP-10 active substance manufacturing. No microbiological contamination was demonstrated over the hold time period, which is satisfactory.

The rCFP-10 active substance is filled into 250 mL DURAN glass bottle consisting of type I glass with a screw cap and pouring ring of polypropylene. Additionally, black (opaque) bags are introduced as secondary packaging instead of clear bags. A certificate of conformity has been provided for the active substance container closure system and compliance with the Ph. Eur. has been declared.

Overall, appropriate extractables, leachables, and delamination studies are presented, which supports the suitability of the container closure system of pharmacopeia quality standard. Thus, the active substance container closure system is found acceptable.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. No human or animal derived materials are used in the active substance manufacturing.

The production strain of the rCFP-10 active substance is *L. lactis* CGJ.SSI.67, which has been transformed with the pCGJ.SSI.40 expression construct encoding rCFP-10, resulting in the strain CGJ.SSI.370. Overall, the source and history of strain is well described. Furthermore, the *L. lactis* strain was transformed with a test plasmid to obtain a mutant with increased secretion before transformation with the rCFP-10 expression construct.

A two-tiered cell banking system is used, and sufficient information is provided regarding testing of MCB and WCB and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age. Cell bank characterization included testing of strain genome, sequence confirmation, integrity of

plasmid, copy number of plasmid, viability, purity, identity, and erythromycin resistance. All characterization results complied with pre-defined acceptance criteria and consistency was shown between the SSI site and SIIPL site. Overall, the MCB and WCB are considered appropriate starting materials for the manufacture of rCFP-10 active substance ensuring a consistent production. Data were provided for whole genome sequences of the MCB. In addition, numbers of generations the end of production (EOP) cell bank was stated and data is provided for the live/dead cell ratio and plasmid stability at the EOP stage.

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the rCFP-10 active substance manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests.

Process validation

To demonstrate a suitable, consistent and well-controlled manufacturing process of the rCFP-10 active substance at commercial scale at the Hadapsar site in India, three process validation (PV) runs have been performed. PV batches are comparable and within predefined acceptance criteria/target ranges for each process parameter, IPCs and release tests. Therefore, process performance was demonstrated to be consistent, reproducible, and robust, and the process yielded product of acceptable quality. The rCFP-10 active substance manufacturing process has been validated adequately.

Manufacturing process development

During clinical development, the first change introduced to the rCFP-10 active substance manufacturing process is before manufacturing of clinical trial Phase 2 batches. This involves the change of filter size for the peptone medium. Before clinical trial Phase 3, the manufacturing scale was changed. After clinical development, the rCFP-10 active substance manufacturing process was transferred from the SSI site to the SIIPL site. Here, different filtration systems but with same cassette/membranes were implemented. The comparability approach used to demonstrate comparability during clinical development and manufacturing transfer between the SSI and SIIPL sites included comparison of release tests between three non-clinical/clinical development batches (non-clinical/Phase 1, Phase 2, Phase 3) and comparison between three clinical Phase 3 batches produced at SSI with three PV batches produced at SIIPL. This comparability approach is endorsed. The provided comparability data between the two manufacturing sites (SSI and SIIPL) are considered overall acceptable. As requested during the procedure, two additional parameters: purity (SE-HPLC) and oxidation impurities (RP-HPLC) were included in the active substance release and stability specification. Accelerated and stress stability data from the SSI and SIIPL sites supporting comparability of active substance batches manufacturing at the SSI site and the SIIPL site have been provided and found acceptable.

Characterisation

The characterisation studies included testing of the molecular weight by mass spectroscopy (MS), primary structure by peptide map analysis using liquid chromatography coupled MS, secondary structure by far UV CD Spectroscopy, tertiary structure by fluorescence spectroscopy, physicochemical properties by immunoblotting and SDS-PAGE, and biological activity using three PV batches manufactured at the SIIPL site and one representative clinical batch manufactured at the SSI site (primary reference standard at SIIPL). The characterization studies adequately cover the relevant structural, physicochemical and biological attributes of rCFP-10. The results of the characterization studies showed that rCFP-10 active substance has the expected

structure with no glycosylation or disulfide bridges as well as the expected features to induce a DTH response to the *Mycobacterium tuberculosis.*

Potential impurities were identified and characterized for the rCFP-10 active substance and categorized into process- or product-related impurities. Process-related impurities included bacterial endotoxin, host cells proteins (HCPs) and DNA arising from the host organism (host cell DNA (HCD)), which is found appropriate. A justification and risk assessment of not having residual peptones as a process-related impurity is provided and found acceptable.

A characterisation of HCP impurities was performed on three PV batches from the SIIPL site and one clinical batch from SSI (reference standard) using a MS-based method. For all batches, 9 proteins were identified from *L. lactis* with a relative abundance. At SIIPL, measured levels of HCP at release using a Sandwich ELISA indicate that the process is capable of consistently reducing the levels of HCP. The provided information and data on HCP for the rCFP-10 active substance gave rise to a line of questions throughout the dossier sections, which were altogether raised as a Major Objection. The issues were all solved.

Product-related impurities were evaluated as truncation, oxidation, deamidation, aggregation, and charge variants of the rCFP-10 active substance. The categorization of impurities is acceptable. Impact of truncation, oxidation, deamidation and aggregation impurities on the potency of the active substance have been addressed.

The rCFP-10 active substance has been sufficiently characterised by physicochemical and biological state-of-the-art methods revealing that the active substance has the expected structure. The analytical results are consistent with the proposed structure. Furthermore, heterogeneity of the active substance was adequately characterised. In summary, the characterization is considered appropriate for this type of molecule.

2.4.2.3. Specification

The specification includes tests for appearance, identity, purity, microbiological control and other general tests.

The specification for rCFP-10 active substance is in accordance with the ICH Q6B Guideline. Additional characterisation tests included impurity testing of HCD by qPCR and biological activity with the *in vivo* Guinea DTH response test, but these tests were proposed to be excluded from the active substance (and finished product) specification.

The *in vivo* biological activity method was discontinued from the specification for routine commercial batches and from the stability programme due to 3R animal welfare. A correlation study has been performed between the *in vivo* biological activity and *in vitro* potency by RP-HPLC for the rCFP-10 and rdESAT-6 active substances and Sandwich ELISA for the finished product. The calculated relative potencies were similar to the rCFP-10 and rdESAT-6 protein concentrations determined from RP-HPLC and sandwich ELISA concentrations, and the applicant concluded that the *in vitro* RP-HPLC and Sandwich ELISA could be used as a measure of biological activity of both the active substances and finished product to avoid the use of animals. The strategy of incorporating 3R animal welfare principles into the release- and stability testing programmes is highly endorsed. Three *in vitro* methods, SEC-HPLC, RP-HPLC and Sandwich ELISA, are included to replace the *in vivo* biological activity method; it has been adequately demonstrated that together the *in vitro* methods can be used to test stability indicating attributes detecting negative trends in the biological activity of the active substances and finished product.

Regarding the proposal of excluding the HCD test in the specifications for both active substances, the applicant received a Scientific advice from EMA, where it was recommended that HCD was included in the active substance specifications. Therefore, the HCD test per in-house qPCR method is included in the rCFP-10 active substance specification and rdESAT-6 active substance specification.

The acceptance criteria for the specification methods are defined and considered well justified and acceptable.

Analytical methods

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines. For the HCP Sandwich ELISA, assay additional information has been provided on the production, characterisation and coverage of anti-HCP antibody reagents.

Release testing for the rCFP-10 active substance is planned to be executed at the SIIPL Manjari site as for the SIIPL Hadapsar site. A subset of active substance release tests was validated at the Manjari site and the validation summery reports for these tests were provided and the results of the validation studies are considered comprehensive for release testing of rCFP-10 active substance batches.

Batch analysis

Batch analysis data on three PV batches of the active substance manufactured at the SIIPL Hadapsar site using the process intended for commercial purposes were provided. Moreover, batch data were provided from three clinical active substance batches, which were produced at the SSI site and used for clinical trials or for method validation of finished product produced at the SIIPL site. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

rCFP-10 internal reference standards are used for the Western blot, RP-HPLC, SDS-PAGE, HCP Sandwich ELISA, biological activity assay, and rCFP-10 Sandwich ELISA. The quality of rCFP-10 active substance is controlled by a two-tiered reference standard approach with a primary reference standard and an in-house reference standard (IHRS). The current primary reference standard, and the first IHRS, are derived at the SSI and SIIPL manufacturing sites, respectively, by full-scale manufacturing processes. The primary reference standard was used for process qualification and was part of the Phase 3 clinical batch campaign. This batch was qualified as primary reference standard at the SSI site using release tests and extended characterization tests for structure, identity and physico-chemical properties. Hereafter, the primary reference standard was transferred to the commercial manufacturing site, SIIPL, where it was qualified using release tests. After transfer of the manufacturing process from the SSI site to the SIIPL site, the rCFP-10 active substance batch was produced and qualified as IHRS at the SIIPL site using release tests and extended characterization tests against the primary reference standard; this IHRS has been discontinued due to a low number of remaining vials. A new IHRS, based on PPQ batch manufactured at SIIPL, has been qualified against the PRS and implemented. Qualification included the added specification parameters purity by SEC-HPLC and oxidation impurities by RP-UPLC and the IHRS is placed on stability.

Overall, the provided qualification data for the primary reference standard and the current IHRS was considered acceptable. Procedure for qualification of future IHRSs is described and found adequate.

The reference standards are stored at ≤ -20 °C and the IHRS is requalified annually according to standard operating procedures, which is satisfactory.

The primary reference standard was placed on stability at SSI and 12 years of available stability data illustrate a stable active substance at ≤ -20 °C. The new in-house reference was placed on stability at the SIIPL site at real time storage conditions (≤ -25 °C).

Acceptable information was provided for the reference standards used for endotoxin and HCD measurements.

2.4.2.4. Stability

The shelf-life proposal for the rCFP-10 active substance is 5 years of storage at -25 °C ± 5 °C, when stored in a USP type I glass bottle.

Stability studies were performed on five supportive clinical or clinical-representative active substance batches manufactured at the SSI site, which are stored at -20 °C ± 5 °C for 12 years or at ≤ -15 °C for up to 36 months, and on three primary PV batches manufactured at the SIIPL site, which are stored on the real-time storage conditions (-25 °C ± 5 °C) for 12 years, accelerated storage conditions (2 °C to 8 °C) for 6 months, and stress storage conditions (25 °C ± 5 °C) for 1 month.

All stability studies included testing for appearance, purity (SDS-PAGE), antigen concentration, biological activity, bioburden, and pH, but reduced testing was performed for the accelerated- and stress stability studies. This approach was found acceptable.

Additional quantitative purity analyses by SEC-HPLC have been included in the release and stability specification; the stability protocols have been updated to include these additional stability parameters.

Primary stability batches from SIIPL

For the primary rCFP-10 stability batches, the applicant provided 24 months of stability data at the long-term storage condition, which all met the pre-defined acceptance criteria.

At accelerated and stressed storage conditions, rCFP-10 active substance was observed to be stable. Inclusion of additional purity tests in the stability programme and active substance specification was requested: purity (SEC-HPLC) and oxidation impurities (RP-UPLC). Comparative stress stability data for clinical representative SSI batches and SIIPL PPQ batches have been provided demonstrating similar stress behaviour for the two additional stability parameters purity by SEC-HPLC and oxidation impurities by RP-UPLC; therefore, the proposed 5-years shelf life for rCFP-10 active substance is acceptable.

Supportive stability batches from SSI

The five supportive batches included in the stability studies performed at the SSI site are clinically representative. It was confirmed that the container closure system used at the SSI site is representative of the one used at commercial scale. Stability data was provided from three batches at commercial scale over 7 years of storage at -20 °C ± 5 °C and from two batches stored for 24 months and 36 months, respectively, at ≥ -15 °C.

All stability data met the acceptance criteria, except for one out of specification, which was detected on the purity in one of the active substance batches at the 24 months timepoint. However, since the purity complies with the acceptance criteria at the 36 months timepoint, this is considered acceptable. Therefore, overall the rCFP-10 active substance manufactured at SSI was shown to be stable with the chosen stability indicating parameters at the storage condition of -20 °C for 7 years and at -15 °C for up to 36 months.

Photostability

Photostability of the rCFP-10 active substance was assessed in accordance with the ICH Guideline Q1B using one PV batch manufactured at the SIIPL site. The active substance was shown not to be light sensitive as determined from tests on appearance, purity, antigen concentration, size exclusion chromatography, absorbance of quinine monohydrochloride dihydrate solution, and pH, upon light exposure.

Overall conclusion

Overall, the shelf-life of 5 years of storage at -25 °C ± 5 °C for the rCFP-10 active substance is considered acceptable based on 24 months of available stability data from the SIIPL manufacturing site supported by 7 years of stability data from the SSI site, since comparability has been demonstrated between the SSI and SIIPL site.

2.4.3. Active substance: rdESAT-6

2.4.3.1. General information

rdESAT-6 (recombinant Early Secretory Antigen Target) is a recombinant dimer of *M. tuberculosis* ESAT-6 protein. The complete rdESAT-6 protein has 196 amino acids, including 5 extra amino acids at the N terminal end. rdESAT-6 has a molecular weight of 20.5 kDa and is not glycosylated. Amino acid sequence of rdESAT-6 is provided in Figure 2: Amino acid sequence of the rdEsat6 hybrid protein. The five amino acids in the N-terminus written in red are not part of the Esat6 protein and the three amino acids written in green are intertwining amino acids and are not part of ESAT6

As for the rCFP-10 active substance, the rdESAT-6 active substance is a *M. tuberculosis* specific antigen, inducing a cytokine-mediated inflammatory response in individuals previously exposed to a *M. tuberculosis* infection.

DTRSMTEQQW NFAGIEAAAS AIQGNVTSIH SLLDEGKQSL TKLAAAWGGS
GSEAYQGVQQ KWDATATELN NALQNLARTI SEAGQAMAST EGNVTGMFAR
SMTEQQWNFA GIEAAASAIQ GNVTSIHSLL DEGKQSLTKL AAAWGGSGSE
AYQGVQQKWD ATATELNNAL QNLARTISEA GQAMASTEGN VTGMFA

Figure 2: Amino acid sequence of the rdEsat6 hybrid protein. The five amino acids in the N-terminus written in red are not part of the Esat6 protein and the three amino acids written in green are intertwining amino acids and are not part of ESAT6

2.4.3.2. Manufacture, characterisation and process controls

The manufacturing sites involved in development, manufacture and testing of the rdESAT-6 active substance are identical to those for the rCFP-10 active substance.

Description of manufacturing process and process controls

The rdESAT-6 active substance manufacturing process has been adequately described. rdESAT-6 active substance is manufactured by the same process as for the rCFP-10 active substance, except for the addition of polysorbate 20, and the use of an ultrafilter with a higher cut-off for diafiltration. The ranges of critical process parameters and the routine in-process controls along with acceptance criteria, are described for each step. IPCs and their acceptance criteria are identical for the two Siiltibcy active substances, except for the IPC acceptance limit for the absorbance of purified rdESAT-6 protein. In addition, process parameters and their

acceptance criteria are identical for the two Siiltibcy active substances. In-process hold times were defined according to process validation results.

The active substance manufacturing process is considered acceptable.

The rdESAT-6 active substance is filled into 250 mL DURAN glass bottle consisting of type I glass with a screw cap and pouring ring of polypropylene, as for the rCFP-10 active substance. The container is stored at -25 °C ± 5 °C. A certificate of conformity has been provided for the active substance container closure system and compliance with Ph. Eur. has been declared.

Overall, appropriate extractables, leachables, and delamination studies are presented, which supports the suitability of the container closure system of pharmacopeia quality standard. Therefore, the active substance container closure system is found acceptable.

Due to light sensitivity of the rdESAT-6 active substance, black (opaque) bags are introduced as secondary packaging instead of clear bags.

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. No human or animal derived materials are used in the active substance manufacturing process.

The production strain of rdESAT-6 is PSM631. This production strain PSM631 consists of the *L. lactis* strain PSM565 transformed with pAMJ752dEsat6 plasmid. Overall, the source and history of strain is well described. Furthermore, the *L. lactis* strain was transformed with a test plasmid to obtain a mutant with increased secretion of the test protein before transformation with the rdESAT-6 expression construct; the mutant was demonstrated cured for this test plasmid.

A two-tiered cell bank system is applied for the rdESAT-6 active substance with a MCB and WCB. Cell bank characterization included testing of strain genome, sequence confirmation, integrity of plasmid, copy number of plasmid, viability, purity, identity, and erythromycin resistance.

All characterization results complied with pre-defined acceptance criteria and consistency was shown between the SSI site and SIIPL site. Overall, the MCB and WCB are considered appropriate starting materials for the manufacture of rdESAT-6 active substance ensuring a consistent production. Data were provided for whole genome sequences of the MCB and for the live/dead cell ratio and plasmid stability of the EOP cell bank.

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the rdESAT-6 active substance manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. Actions taken if limits are exceeded are specified.

Process validation

The rdESAT-6 active substance manufacturing process has been validated adequately. To demonstrate a suitable, consistent and well-controlled manufacturing process of rdESAT-6 active substance at commercial scale at the Hadapsar site in India, three PV runs have been performed. PV batches are comparable and within predefined acceptance criteria/target ranges for each process parameter, IPCs and release tests. Thus,

process performance was demonstrated to be consistent, reproducible, and robust, and the process yielded product of acceptable quality.

In the hold time study, bioburden was tested in media/buffers used for rdESAT-6 active substance manufacturing. No microbiological contamination was demonstrated over the hold time period, which is satisfactory.

Manufacturing process development

During clinical development, the first change introduced to the rdESAT-6 active substance manufacturing process is before manufacturing of clinical trial Phase 2 batches. Before clinical trial Phase 3, the manufacturing scale was changed. After clinical development, the rdESAT-6 active substance manufacturing process was transferred from the SSI site to the SIIPL site. Here, different filtration systems but with same cassette/membranes were implemented. The comparability approach used to demonstrate comparability during clinical development and manufacturing transfer between the SSI and SIIPL sites included comparison of release tests between three non-clinical/clinical development batches (non-clinical/Phase 1, Phase 2, Phase 3) and comparison between three clinical Phase 3 batches produced at SSI with three PV batches produced at SIIPL. The comparability approach is acceptable.

The provided comparability data between the two manufacturing sites (SSI and SIIPL) are considered overall acceptable. As requested, inclusion of two additional parameters purity (SE-HPLC) and oxidation impurities (RP-HPLC) were included in the active substance release and stability specification accelerated and stress stability data from the SSI and SIIPL sites supporting comparability of active substance batches manufacturing at the SSI site and the SIIPL site have been provided and it was found acceptable.

Characterisation

The characterisation study included testing of the molecular weight MS, primary structure by peptide map analysis using liquid chromatography coupled MS, secondary structure by Far UV CD Spectroscopy, tertiary structure by fluorescence spectroscopy, physicochemical properties by immunoblotting and SDS-PAGE, and biological activity on three PV batches manufactured at the SIIPL site and one representative clinical batch manufactured at the SSI site (primary reference standard at SIIPL). The characterization studies adequately cover the relevant structural, physicochemical and biological attributes of rdESAT-6. The results of the characterization studies showed that rdESAT-6 active substance has the expected structure with no glycosylation or disulfide bridges as well as the expected ability to induce a delayed type hypersensitivity response to the *M. tuberculosis*.

Impurities were categorised into process- or product-related impurities. Potential impurities were identified and characterized for the rdESAT-6 active substance. Process-related impurities included bacterial endotoxin, and host cells proteins and DNA arising from the host organism, which is found appropriate.

A characterisation of HCP impurities was performed on three PV batches from the SIIPL site and one clinical batch from SSI (reference standard) using a MS-based method. For all batches, 4 proteins were identified from *L. lactis* with a relative abundance. At SIIPL, measured levels in PV batches at release using a Sandwich ELISA indicate that the process is capable of consistently reducing the levels of HCP.

Product-related impurities were evaluated as truncation, oxidation, deamidation, aggregation, and charge variants of the rdESAT-6 active substance. The categorization of impurities is acceptable. Impact of truncation and oxidation impurities on the potency of the active substance has been addressed.

2.4.3.3. Specification

The specification includes tests for appearance, identity, purity, microbiological control and other general tests.

The specification for rdESAT-6 active substance is in accordance with the ICH Q6B Guideline. Additional characterisation tests included impurity testing of HCD by qPCR and biological activity with the *in vivo* Guinea DTH response test, but these tests were proposed to be excluded from the active substance specification. Refer to the specification section for the rCFP-10 active substance.

The acceptance criteria for the specification methods are defined and considered well justified and acceptable.

Analytical methods

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines.

Release testing for the rdESAT-6 active substance is planned to be executed at the SIIPL Manjari site as for the SIIPL Hadapsar site. A subset of active substance release tests was validated at the Manjari site and the results of the method validation studies are considered comprehensive for release testing of rdESAT-6 active substance batches.

Batch analysis

Batch analysis data has been provided from three PV batches of the rdESAT-6 active substance manufactured at the SIIPL Hadapsar site using the process intended for commercial purposes. Moreover, batch data were provided from three clinical active substance batches, which was produced at the SSI site and used for clinical trials or for method validation of the finished product produced at the SIIPL site.

All release results comply with the specification in place at the time of testing and batch-to-batch consistency is overall demonstrated.

Reference materials

rdESAT-6 internal reference standards are used for the Western blot, RP-HPLC, SDS-PAGE, HCP Sandwich ELISA, biological activity assay, and rdESAT-6 Sandwich ELISA. The quality of rdESAT-6 active substance is controlled by a two-tiered reference standard approach with a primary reference standard and an IHRS. The current primary reference standard, and the first in-house reference standard, are derived at the SSI and SIIPL manufacturing sites, respectively, by full-scale manufacturing processes representative of the late clinical development. The primary reference standard is part of the process validation batches at the SSI site. This batch was qualified as primary reference standard at the SSI site using release testing and extended characterization test for primary structure, identity and physico-chemical properties. Hereafter, the primary reference standard was transferred to the commercial manufacturing site, SIIPL, where it was qualified using release tests. After transfer of the manufacturing process from the SSI site to the SIIPL site, the rdESAT-6 active substance batch was produced and qualified as in-house reference standard at the SIIPL site using release tests and extended characterization tests against the primary reference standard; this IHRS has been discontinued due to a low number of remaining vials. A new IHRS, based on PPQ batch manufactured at SIIPL, has been qualified against the PRS and implemented. Qualification included the added specification parameters purity by SEC-HPLC and oxidation impurities by RP-UPLC and the IHRS is placed on stability.

The extended structural characterization of the primary reference standard included only elucidation of the primary structure of rdESAT-6. The applicant argues that elucidation of the secondary and tertiary structure are redundant in this context, since the biological response of rdESAT-6 is elicited solely by certain amino acid sequence(s) by linear T-cell epitopes. Furthermore, no S-S cross binding is seen for rdESAT-6 due to the absence of cysteines. This is considered acceptable. In addition, it is noted that the chosen primary reference standard batch is shown to be oxidized at one of its oxidation sites. This is, however, considered acceptable, since the active substance of the other process validation batches have similar oxidation patterns. The rdESAT-6 active substance used for clinical trial Phase 3 is, moreover, observed to be oxidized. It is confirmed that the oxidation of the primary reference standard is localized outside the amino acid sequence responsible for the biological activity.

Overall, the provided qualification data for the primary reference standard and the in-house reference standard was considered acceptable. Procedure for qualification of future IHRSs is described and found adequate.

The reference standards are stored at ≤ -20 °C and the in-house reference standard is requalified annually according to standard operating procedures, which is satisfactory.

The primary standard reference was placed on stability for 12 years and 9 years of available stability data illustrate a stable active substance at ≤ -20 °C. The new in-house reference was placed on stability at the SIIPL site at real time storage conditions (≤ -25 °C).

Acceptable information was provided for the reference standards used for endotoxin, polysorbate 20, and HCD measurements.

2.4.3.4. Stability

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container. The shelf-life proposal for the rdESAT-6 active substance is 5 years of storage at -25 °C ± 5°C, when stored in a USP type I glass bottle.

Stability studies were performed on four clinical or clinical-representative active substance batches manufactured at the SSI site at -20 °C ± 5 °C for 12 years or at ≤-15 °C for 36 months, and on three process validation batches manufactured at the SIIPL site, which are stored on real-time storage conditions (-25 °C ± 5°C) for 12 years, accelerated storage conditions (2 °C to 8 °C) for 6 months, and stress storage conditions (25 °C ± 5 °C) for 1 month.

All stability studies included testing for appearance, purity (SDS-PAGE), antigen concentration, biological activity, bioburden, polysorbate 20 concentration, and pH, except for the accelerated and stressed storage conditions where reduced testing was performed. This was found acceptable.

Additional quantitative purity analyses by SEC-HPLC have been included in the release and stability specification; the stability protocols have been updated to include these additional stability parameters.

Primary stability batches from SIIPL

For the primary rdESAT-6 stability batches, the applicant provided 24 months of stability data at the long-term storage condition, which all met the pre-defined acceptance criteria.

rdESAT-6 active substance was observed to be stable upon storage at accelerated and stressed storage conditions. Inclusion of additional purity tests in the stability programme and active substance specification

was requested during the procedure: purity (SEC-HPLC) and oxidation impurities (RP-HPLC). Comparative stress stability data for clinical representative SSI batches and SIIPL PPQ batches have been provided demonstrating similar stress behaviour for the two additional stability parameters purity by SEC-HPLC and oxidation impurities by RP-UPLC; therefore, the proposed 5-years shelf life for rdESAT-6 active substance is acceptable.

Supportive stability batches from SSI

The four supportive batches included in the stability studies performed at the SSI site are clinically representative. Stability data was provided from three batches at commercial scale over 7 years of storage at -20 °C ± 5 °C and from one batch stored for 36 months at ≥-15 °C.

All stability data met the acceptance criteria, except for one of the active substance batches, which was observed to change appearance after 5 years from a clear solution to unclear solution. No deviation report was made, since the clinical trial ended, but the two other rdESAT-6 batches had unaffected appearance after 5 years. Therefore, overall the rdESAT-6 active substance manufactured at SSI is considered stable based on the chosen stability indicating parameters at the storage condition of -20 °C for 7 years and -15 °C for 36 months.

Photostability

Photostability of the rdESAT-6 active substance was assessed in accordance with the ICH Guideline Q1B using one PV batch manufactured at the SIIPL site. The active substance was shown to be light sensitive as determined from changes observed in the size exclusion chromatography results and quinine monohydrochloride dihydrate results upon light exposure of the active substance. This is endorsed. Based on this, black (opaque) bags are introduced as secondary packaging instead of clear bags.

Overall conclusion

An acceptable post-approval stability protocol and a stability commitment were provided.

Overall, the shelf-life of 5 years of storage at -25 °C ± 5 °C for the rdESAT-6 active substance is considered acceptable based on 24 months of available stability data from the SIIPL manufacturing site supported by 7 years of stability data from the SSI site.

2.4.4. Finished medicinal product

2.4.4.1. Description of the product and pharmaceutical development

Siiltibcy is presented as a clear, colourless to pale yellow solution for injection with a pH of 7.2 – 7.6. Siiltibcy is a skin test for diagnosis of Mtb infection. It is a sterile solution formulated with 0.1 µg protein/0.1 mL consisting of equal amounts of rdESAT-6 and rCFP-10 (0.05 µg rdESAT-6 per 0.1 mL and 0.05 µg rCFP-10 per 0.1 mL) active substances with phenol as preservative. It is presented in multidose (ten dose) vials.

The primary packaging is Type I, 2R clear tubular glass vials. The glass vials are stoppered with bromobutyl rubber stoppers and closed using aluminium flip-off seals. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

For formulation, the finished product was designed with rCFP-10 and rdESAT-6 in the ratio of 1:1. With the finished product, 7 clinical trials have been performed in healthy participants, participants with different risk of Mtb infection as well as participants with confirmed TB. Adult and paediatric participants were included.

The formulation development is solely based on the clinical experience gained from TST and clinical studies performed with closely related formulations. The Phase 2 and Phase 3 clinical trials (TESEC-03 to TESEC-07) were performed with the final Siiltibcy formulation.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. or USP-NF standards. There are no novel excipients used in the finished product formulation.

As the finished product is a multidose preparation, the effectiveness of the preservative must be demonstrated according to Ph. Eur. 5.1.3. The Antimicrobial Effectiveness Testing study was performed and evaluated against acceptance criteria that are in accordance with Ph. Eur. The results support that phenol in the finished product Siiltibcy 10 dose/vial is effective against the test organisms and meets the acceptance criteria of log reduction.

The manufacturing process development encompasses upscale, equipment change, reduction in mixing steps and addition of a sterile filtration step before filling. These changes are related to process upscale and the additional sterile filtration is considered as an improvement.

Upon technology transfer to SIIPL, the raw materials, excipients, manufacturing process and process controls as critical process parameters (CPPs) and critical quality attributes (CQAs) have been adopted. A comparability exercise was performed for the batches manufactured at SSI (used in Phase 3 clinical trial) and SIIPL (Development and PV batches). The comparability was made on excipients, container closure system, process equipment and the manufacturing process parameters. The process parameters evaluated during the formulation, filling, stoppering and sealing activities of Siiltibcy finished product were shown to be comparable between the SSI site and SIIPL site.

The comparability of batch release analysis between the Siiltibcy finished product batches manufactured at SSI and SIIPL was also performed and showed adequate comparability of SSI and SIIPL batches.

As the process was further transferred from SIIPL Hadapsar premises (Building No. 5, FF) to SIIPL Manjari premises (MSEZ-3, First Floor (FF), MMA2), a comparability exercise was also performed for process parameters at the two production sites.

The transfer report was reviewed, finished product manufacturing data was evaluated and detailed comparison of raw materials, primary packing materials, facility, equipment, manufacturing process and analytical results of Sending Unit (B.No.5, FF, EOU) against Receiving Unit (MMA-2, MSEZ-3, FF) was carried out. Stability study of the batches manufactured at receiving unit (MMA-2, MSEZ-3) is in-progress. Based on the results obtained, it is evident that manufacturing process can be considered as transferred successfully from the sending unit to receiving unit.

The process was subsequently transferred from the MSEZ-3 FF site to the MSEZ-1 Ground Floor (GF) site at the Manjari premise, which is intended for manufacture of batches for Europe. The major process changes implemented at this facility are the use of an isolator line and the introduction of an online redundant filtration using two filters placed inside the isolator at the aseptic filling step. These changes are considered improvements and found acceptably supported by data. In addition, the capacity of the filling machine is increased, resulting in an increase in filling batch size at the MSEZ-1 GF site. Adequate information about the filters that come in contact with finished product or intermediate should is provided.

A comparability report was provided to demonstrate comparability between finished product manufactured at MSEZ-3 FF and finished product manufactured at MSEZ-1 GF. The comparability analysis included comparison of raw materials, equipment, facility, primary packaging materials, and process parameters used at both sites. Alternate vendors of excipients were successfully qualified during process performance qualification (PPQ) and different equipment (filling machine, closure processing unit used for sterilisation and vacuum drying of rubber stoppers and aluminium seals, vial washing machine, depyrogenation tunnel) was adequately validated. Regarding primary packaging materials, Type I glass 2R vials, bromobutyl fluoro-coated stoppers and flip-off seal aluminium caps are used at both sites. However, at MSEZ-1 GF, the vials are no longer ready to use but washed and sterilised. Overall, process parameters are the same at both sites, except for minor changes which were validated during the PPQ runs.

The comparability exercise also comprised a comparison of analytical results for the intermediate Siiltibcy 100 µg/mL, Siiltibcy final bulk 1 µg/mL and fill finished product manufactured at both sites. Three PV batches manufactured at each site were tested for the following release parameters: appearance, pH, rdESAT-6 concentration, rCFP-10 concentration, phenol content, polysorbate content, extractable volume, subvisible particles, visible particles, microbial bioburden, bacterial endotoxins, and sterility. The data provided showed that all testing results complied with the specifications in place for the intermediate bulk, final bulk and finished product. Due to low concentration of protein and the presence of phenol and polysorbate 20, structural characterisation of finished product is difficult. In addition, as rdESAT-6 and rCFP-10 proteins do not have the disulfide bridges with defined folding pattern, higher order structural analysis may not be most relevant. Therefore, only primary structural analysis by peptide mapping along with deamidation by LC-MS was carried out. This is endorsed. Peptide mapping analysis provided 100% coverage of theoretically known sequence of rdESAT-6 and rCFP-10 protein in each analysed sample and confirmed the presence of both proteins. Global percentage deamidation was found to be comparable for rdESAT-6. The differences are not statistically significant. It is therefore considered that the primary structure of rdESAT-6 and rCFP-10 proteins is similar between materials manufactured at both sites.

In addition, the three batches manufactured at MSEZ-1 GF were placed in the stability program. So far, 6 months stability data at the long-term condition are submitted for MSEZ-1 batches while 24 months data are available for MSEZ-3 batches. Small differences are observed between both materials.

In conclusion, it can be agreed that the batches manufactured at MSEZ-1 GF are of similar quality when compared to the batches manufactured at MSEZ-3 FF. The approach used for the analytical comparability exercise is found adequate and specific comparability acceptance criteria were defined. No comparability testing for purity/impurities was performed. However, with respect to purity/impurities, a different profile is not expected to be seen between materials from both sites. The lack of purity/impurity data in the comparability exercise can therefore be accepted.

The filter validation studies were performed at SSI in Denmark and consist of bacterial retention study, filter integrity test, chemical compatibility and filter extractable study. The same filter is used at the SIIPL site, i.e. 0.2 µm filter. Hence, the filter validation studies are considered applicable for manufacturing at the SIIPL site as the composition and volume of the filtered bulk in SSI and SIIPL are similar.

Extractable volume and multi dose withdrawal study was performed on the batch filled at Hadapsar plant and Manjari plant and the results justify the fill volume of 1.85 ± 0.05 mL/vial (1.0 mL for 10 dose + 0.8 mL dead volume + 0.05 mL overfill) in case of using 1.0mL, short-bevel needle. The information regarding the type of syringe and needle is adequately reflected in the Summary of Product Characteristics.

2.4.4.2. Manufacture of the product and process controls

The batches of the finished product intended for Europe are manufactured at the SIIPL Manjari MSEZ-1 GF site. The responsibility of SIIPL is to manufacture, test and package the finished product. Quality control takes place at the Hadapsar site of SIIPL and SIIPL Manjari MSEZ-2 Second Floor site. EU importer, which is responsible for batch control and release testing, and QP batch certification, is Bilthoven Biologicals B. V., Antonie van Leeuwenhoeklaan 9-13, 3721 MA, Netherlands.

The manufacturing process has been validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate.

The intermediate bulk 100 µg/mL is stored in 5L USP Type I glass bottle. The final bulk 1 µg/mL is stored in sterile single-use 3D Biocontainers, which conform with Ph. Eur. Specifications for intermediate and final bulk are provided and found acceptable.

As demonstrated during the hold time study and supported by the validation batches, the thawed active substance should be held within the predefined storage period. Justification of specifications for intermediate and final bulk are acceptable.

The thawed active substance can be stored at 2 - 8 °C, and final bulk 1 µg/mL at room temperature for a designated time. The proposed hold times are supported by the validation batches.

At the MSEZ-3 FF site, three full scale batches were used for validation study, as according to the guideline EMA/CHMP/CVMP/QWP/BWP/70278/2012-Rev1, Corr.1 Guideline on process validation for finished products - information and data to be provided in regulatory submissions. Studies and protocols used for process validation are considered adequate. In the guideline, it is stated that 3 consecutive batches should be used, however, 3rd batch was excluded from the analysis and 4th batch was used instead. The reason for exclusion was that during filling, total number of filled vials was less than the proposed batch size as per PV protocol. Investigation was performed, a root cause was identified, and correction measures implemented. This is acknowledged and considered justified. It is concluded that, based on the manufacturing data evaluated and from the review of quality control results obtained, the manufacturing process is capable of consistently producing the product meeting with the pre-established quality attributes, and is hence validated. This conclusion can be supported.

At the MSEZ-1 GF site, PPQ studies were performed on three full scale batches manufactured in. Key performance parameters (KPPs), CPPs and IPCs were evaluated at the Formulation stage and at the Aseptic filling, stoppering and sealing stage. Intermediate Siiltibcy 100 µg/mL was tested for rdESAT-6 concentration, rCFP-10 concentration and for bioburden. In addition, formulated blend samples analysed for several time intervals of mixing as well as the filtered formulated blend were tested for the parameters appearance, pH, rdESAT-6 concentration, rCFP-10 concentration, bacterial endotoxins, sterility (only for filtered formulated blend), phenol content and polysorbate 20 content. The batches were further tested at the filling stage, at the start, middle and end of the process, for the parameters rdESAT-6 concentration, rCFP-10 concentration, phenol content, polysorbate 20 content, container closure integrity, extractable volume and subvisible particles. Homogeneity of the filling process is considered adequately demonstrated. Testing of filled unlabelled vials was also performed for relevant CQAs. Acceptance criteria were met in all tested samples. It can therefore be concluded that the manufacturing process performed at MSEZ-1 GF produces a finished product of consistent quality.

For the initial aseptic process validation, three consecutive simulation runs were performed at MSEZ-1 GF. Media fills were executed in the same way as the manufacture of finished product batches and included a comprehensive program of interventions in order to simulate all interventions required during product blending and filling.

At the formulation stage, samples withdrawn for Growth Promotion Test (GPT) and Visual Detection of Microbial Growth (VDMG) complied with the acceptance criteria. In addition, one batch was held in the blending bag after aseptic process simulation before commencing the filling operation. Samples withdrawn after the hold met the acceptance criteria, supporting the proposed hold time.

At the filling stage, a simulation parameter, including assembly and filling, was applied. All withdrawn samples complied with the acceptance criteria.

Overall, it is concluded that the aseptic procedures are adequate to prevent contamination during finished product manufacture.

Autoclave validation and cleaning validation for Manjari premises have been provided. The cleaning procedures are validated using three full scale cleaning validation runs. Since Siiltibcy finished product manufacturing and filling are performed in a multi-product manufacturing facility, change over procedures to prevent cross contamination are followed before switching over to new product. Product change over SOP has been provided.

Filter validation has not been performed at SIIPL, since the filter validation was performed by SSI and reports are provided in the technology transfer report. The applicant argues that the validation at SIIPL is not necessary, since the filters used during manufacturing process of Siiltibcy at SSI and SIIPL are the same. i.e. 0.2 µm filter. As a part of filter validation, bubble point, compatibility of the finished product, extractables challenge and bacterial retention were carried out by SSI. The reports of filter validation tests results are included in the dossier as a separate document. This is accepted. Transport validation has been performed adequately.

No reprocessing steps are performed during the finished product manufacturing process.

Overall, it can be concluded that the PPQ campaign and the additional validation studies demonstrate that the finished product manufacturing process performs as designed and provides a product that consistently meets its predefined quality attributes at the commercial manufacturing site MSEZ-1 GF. The effectiveness of the preservative has been demonstrated by a test discussed in the Pharmaceutical Development part of this report.

2.4.4.3. Product specification

The release specification includes test for appearance, identity, potency, purity, microbiological endotoxin control and sterility other general tests as follows. Visual appearance by visual inspection, identity of rdESAT-6 and rCFP-10 by Western blot, specific reaction and antigen concentration of rdESAT-6 and rCFP-10 by Sandwich ELISA, Bacterial endotoxin by kinetic chromogenic assay, sterility test by membrane filtration, visible particles by visual inspection, subvisible particles by light obscuration, phenol content by spectrophotometry, polysorbate 20 content by spectrophotometry, pH by potentiometry, test for extractable volume and multi dose withdrawal by visual inspection, container closure integrity by dye ingress method and purity by SEC-HPLC.

The same parameters are tested at shelf life except for identity, specific reaction, extractable volume and multi dose withdrawal.

Overall, the parameters included in the finished product specification are found adequate to control the quality of the finished product at release and shelf-life, except from that the applicant was asked to implement a validated analysis for product-related purity and impurities at the finished product level and define clinically justified limits for purity at release and stability (a major objection was raised during the procedure). In order to meet the request, the applicant developed an SDS-PAGE (silver staining); however, the proposed test method for purity and impurities is not considered adequate for a number of reasons, including e.g. lack of specificity and unsatisfactory detection limit rendering the test inconclusive and not suitable for purpose. A SEC-HPLC method to monitor the overall finished product profile and estimation of purity/product-related impurities has subsequently been developed and validated. The proposed SEC-HPLC is not found adequate as it was not demonstrated that the method can detect changes (aggregation and truncation) of the product. However, since the chromatogram profile can be considered a supportive specification parameter, the SEC-HPLC method should remain on the release and stability specifications for the finished product, confirming sample peak profile comparable with reference standard. The applicant has provided additional data showing maintained biological activity and efficacy of aged finished product batches and samples exposed to stressed conditions. Supplemented with the ability of the ELISA analysis to detect changes in samples exposed to forced degradation, the SEC-HPLC method's ability to indicate significant change in product profile, and additional characterisations studies of the finished product performed by silver stained SDS-PAGE reflecting changes in samples exposed to forced degradation, the control of finished product at release and during shelf life can be considered adequate to confirm product purity, efficacy and safety. The data provided was sufficient to resolve the major objection. Three quality recommendations are put forward in this regard:

The applicant committed to re-validate the SEC-HPLC method with the aim of proposing valid acceptance criteria for % purity and impurities (% high molecular weight (HMW) and % low molecular weight (LMW) product). This point is put forward as quality recommendation 1 (REC1). The applicant committed to developing more robust method/s for the estimation of total LMW impurities; this includes exploring innovative techniques such as protein labelling with detection dyes to reduce interference from excipients like polysorbate and estimating extremely low level of protein impurities. This point is put forward as quality recommendation 2 (REC2). The applicant committed to monitor the degradation profile by the SDS-PAGE silver stain method on additional 20 finished product batches. The analyses should include data from both release and stability (end-of-shelf life) testing. Furthermore, the applicant should commit to inform the authorities in case of any unexpected observations. This point is put forward as quality recommendation 3 (REC3).

The applicant states that no new impurities/degradation products are formed during the finished product manufacturing process.

A risk assessment to evaluate the potential for nitrosamine formation and/or contamination during the manufacturing process was performed. The overall risk of a potential release of nitrosamines into the product during production is evaluated as low. The evaluation of the risk of nitrosamine is considered acceptable.

Justification of specification is based on manufacturing experience till date at the SSI site and SIIPL site. Several of the test parameters and acceptance criteria are identical with the tests and acceptance criteria applied for the active substance. The justifications are considered acceptable.

The potential presence of elemental impurities in the finished product has been assessed and it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (as requested) considering all suspected and actual root causes in line with the “Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products” (EMA/409815/2020) and the “Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products” (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Analytical methods

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analysis data for 13 batches of the finished product were provided. Three of the batches are process validation batches. The batch data presented complies with the finished product specification and demonstrates manufacturing consistency. Additional batch analysis data of 3 PV batches manufactured at the MSEZ-1 GF site have been provided. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

Qualified rdESAT-6 and rCFP-10 internal reference standards are used for the identification by Western blot (refer to the respective active substance sections).

For analysis of antigen concentration rdESAT-6 and antigen concentration rCFP-10 by sandwich ELISA in drug product (DP) stage and in-house finished product reference standard is used. The current in-house finished product reference standard was established based on clinically representative batches of rdESAT-6 and rCFP-10 which were qualified as in-house primary reference standards for the respective active substances. A protocol for preparation and establishment of a new in-house finished product reference standard is provided and is considered adequate.

Adequate information is provided on reference standards used in analysis of other tests for Siiltibcy finished product (endotoxin, phenol, polysorbate 20).

2.4.4.4. Stability of the product

Based on available stability data, the shelf-life of 24 months when stored at 2 – 8 °C as stated in the SmPC are acceptable.

The batches of the finished product manufactured at SIIPL for use in process validation study are monitored for real time stability at 2 – 8 °C. These batches are representative of the commercial scale. Clinical Batches manufactured at SSI were also monitored for real time stability. Accelerated stability studies, stress stability studies, photostability and in-use stability studies have been performed on batches produced at SIIPL. The

choice of batches put on stability program as well as frequency of studies follow guidance found in ICH Guideline Q5C. The container used in stability studies is the same which is used for regular storage of the finished product.

In addition, in the in-use stability study, it is observed that Siiltibcy is stable up to 28 days after the vial has been opened. This data is reflected in the SmPC.

Long term stability study

The following batches were put on long-term stability study: two Phase 2 clinical batches (SSI) with 48- and 36-months available data, three process validation batches (SIIPL, MSEZ-3 FF site) with 24 months available data; one developmental batch (SIIPL) with 36 months available data and three process validation batches (SIIPL, MSEZ-1 GF site) with 6 months available data.

The stability studies with process validation batches are ongoing. For the batches manufactured at the MSEZ-1 GF site, the stability protocol has been amended to include the parameters bacterial endotoxins, subvisible particles and visible particles. In addition, as per Ph. Eur. 5.17.2, testing for visible particles should be included in the stability protocol at the accelerated and stress conditions. However, this cannot be implemented as the stress stability study is completed and the accelerated stability study is almost completed.

In the long-term stability study of the clinical batch, finished product is stable for up to 24 months. For the second clinical batch, the concentration of rdESAT-6 antigen was measured until 18 months, while for rCFP-10, it was measured until 36 months. Therefore, the applicant's conclusion that this batch is stable for up to 36 months cannot be supported due to lack of data.

The developmental batch (SIIPL) is stable for up to 36 months in both upright and inverted positions.

All PV batches manufactured at the MSEZ-3 FF site are stable for 24 months. Data obtained so far for the PV batches manufactured at the MSEZ-1 GF site show stability of the finished product. Purity by SEC-HPLC, which was included in the protocol based on Day 180 LoOI, will be monitored from 12 months onwards.

Accelerated stability studies (25 ± 2 °C, 60 ± 5% RH)

The accelerated study using the developmental batch lasted 6 months. The results show that the Siiltibcy finished product quality was stable for less than a month due to the decline of rCFP-10 protein concentration. This decline was not observed for rdESAT-6. All MSEZ-3 PV batches were put on accelerated stability study, which lasted 35 days, and all batches were stable during that study, no trends for decline in antigen concentration were observed. It is not explained however, why the accelerated study for PV batches was designed to last 35 days instead of 6 months.

The PV batches manufactured at the MSEZ-1 GF site were tested for stability under accelerated condition for 6 months. No trend was observed.

Stress stability studies (40 ± 2°C, 75 ± 5% RH)

The PV batches manufactured at the MSEZ-1 GF site were placed under stress conditions for 4 weeks. The study is completed and shows stability of the batches although a small decrease in both rdESAT-6 and rCFP-10 antigen concentrations can be observed.

In-use stability study

The in-use stability study of ten dose presentation of Siiltibcy has been performed on 3 batches produced at SIIPL: 2 PV batches and 1 developmental batch. The product was kept at the intended storage conditions and the in-use study lasted for 28 days. The purpose of in-use stability testing was to establish a time period during which multiple doses of finished product may be used while retaining acceptable quality specifications once the container is opened (after container has been punctured with a needle). The study aimed to justify that the vial stopper withstands 10 maximum number of punctures as the Siiltibcy vial is intended for administration of 10 doses, without impacting the sterility of the product. The conclusion that all batches are stable for up to 28 days when stored at storage condition 2 - 8 °C after opened (needle punctured) for the 10 dose vials of Siiltibcy can be supported based on the data. The tests included in the study are considered well chosen, test for sterility and endotoxin are included, which is endorsed.

Photostability study

The photostability study is carried out according to ICH Guideline Q1B. The applicant concludes that finished product is stable for its intrinsic properties after completion of 1.2 million lux hours, 200-watt hours/square meter when stored at 25±1°C.

Stability commitment

SIIPL commits to continue the ongoing long-term stability studies. One batch of Siiltibcy will be placed on stability each year. Stability studies will be conducted at 2 - 8°C for annual testing and under accelerated conditions if a significant change is made. The applicant commits to inform the Agency of unexpected stability issues in the ongoing studies (including trends and out-of-specifications results) and to propose corrective action as appropriate.

2.4.4.5. Adventitious agents

The applicant provided an adventitious agents safety evaluation. Here, the applicant concluded that there is no risk of potential contamination with adventitious agents and that the safety concern for the finished product is thereby negligible. This is based on the fact that no raw materials or excipients used in the manufacturing process of the active substance, or the finished product are of animal origin. Furthermore, the cell bank starting material is a microbial strain (*L. lactis*), where no cell culture derived components were used in the preparation. The bacterial fermentation process does not support growth of mammalian viruses. The safety evaluation of adventitious agents is endorsed.

2.4.5. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During the procedure, provided information and data on HCP for the rCFP-10 active substance gave rise to a line of questions throughout the dossier sections, which were altogether raised as a Major Objection. The issues were all solved and data provided in response acceptable.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product. These points are put forward and agreed as recommendations for future quality development.

2.4.6. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

The CHMP has identified the following measures necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product.

2.4.7. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The applicant should commit to re-validate the SEC-HPLC method with the aim of proposing valid acceptance criteria for % purity and impurities (% HMW and % LMW product).
2. The applicant should commit to developing more robust method/s for the estimation of total LMW impurities; this includes exploring innovative techniques such as protein labelling with detection dyes to reduce interference from excipients like polysorbate and estimating extremely low level of protein impurities.
3. The applicant should commit to monitor the degradation profile by the SDS-PAGE silver stain method on additional 20 DP batches. The analyses should include data from both release and stability (end-of-shelf life) testing. Furthermore, the applicant should commit to inform the authorities in case of any unexpected observations.

2.5. Non-clinical aspects

2.5.1. Introduction

Siiltibcy immune-based diagnostic is constituted from the Mtb antigens rdESAT-6 and rCFP-10 which are the basis of immunological mechanism of action of TST/PPD (and IGRAs): the intradermal presentation of the antigens to the immune system.

Differently from TST/PPD, Siiltibcy can discriminate between individuals infected by *M. tuberculosis* and vaccinated with *M. bovis* Calmette-Guerin (BCG) vaccine, limiting the negative consequences of false positive results. Indeed, ESAT-6 and CFP-10 are present in very few mycobacteria species causing human TB disease apart from *M. tuberculosis* (i.e., *Mycobacterium bovis* and *Mycobacterium africanum*), are not found in the BCG vaccine and very few atypical mycobacteria express these proteins (*M. kansasii, M. marinum, M. szulgai*).

The entire non-clinical data package in support of the MMA of Siiltibcy is abridged. Due to its proteic nature, its mode of action and its intended clinical use (local effect), Siiltibcy was considered similar to a preventive vaccine (no systemic absorption) and thus, besides ICH S6 guideline, the WHO guideline on non-clinical evaluation of vaccines, Annex 1, TRS No 927, was also followed.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

A number of *in vivo* studies on naïve Guinea pigs and Guinea pigs sensitised by infection with various mycobacteria administered ID with rdESAT-6 or rdESAT-6 + rCFP-10, were carried out, in order to demonstrate dose-response activity and sensitisation potential. No study was carried out with rCFP-10 alone.

Dose-response diagnostic performance/discrimination ability
(Study 01-01a) In a guinea pig disease model of TB infection, rdESAT-6 administered intradermally was able to discriminate the latent infection in animals infected by *M. kansasii* (non-tuberculosis strain) and not in animals infected by BCG (*M. bovis* tuberculosis strain not containing antigens ESAT-6 and rCFP-10).

After 4 weeks from the infection, erythema dimension was dose-proportional up to the highest rdESAT-6 dose tested of 1 ug. Differently, a single dose of PPD (the highest human dose of 10 TU) was not able to discriminate infected and vaccinated animals: however, the skin reaction of PPD was significantly lower in guinea pigs sensitised with *M. kansasii* than with *M. bovis* BCG.

In terms of dimension, erythemas caused by rdESAT-6 were larger than those caused by PPD.

Histological examination of reactions sites revealed an acute inflammation resembling a delayed-type hypersensitivity response (type IV, cell-mediated).

Based on a comparison with single PPD dose of 10 TU, the study director concluded that 0.1 ug rdESAT-6 may be used as a rough estimate of a human dose.

Non-tuberculosis mycobacteria strains represent an alternative model to *M. tuberculosis* infection with the ability to sensitise the animals to give a skin reaction upon challenge with the antigens but without causing disease (e.g., enlargement of the spleen, immunosuppression) which would mask the skin reaction. The *M. kansasii* appeared to be a suitable model: all of the animals became sensitised to rdESAT-6 and PPD and they all remained healthy.

(Study HEA8) When comparing response of intradermal administration of rdESAT-6 (up to 1 ug) and rCFP-10 (up to 10 ug) in guinea pigs infected by *M. tuberculosis* (rdESAT-6 and rCFP-10) and *M. kansasii* (rCFP-10) or BCG (rdESAT-6 and rCFP-10), after 4 weeks, both of the antigens rdESAT-6 and CFP10 gave rise to erythema in *M. tuberculosis* sensitised guinea pigs, even if rdESAT-6 showed an 87-fold higher potency than CFP10. Although the spleen of the animals was significantly enlarged compared to the groups sensitised with BCG and *M. kansasii*, this did not affect the immune-mediated response.

Additionally, in guinea pigs infected with *M. kansasii* (a milder infectious model), the response towards CFP10 was weaker than rdESAT-6 even at the highest dose level (refer to study 01-01a).

According to study director the lower potency of CFP10 compared to rdESAT-6 in both severe and mild infectious models, may be due to its low molecular weight, which is half the size of rdESAT-6. This may, on the other hand, reflect in a lower sensitising risk upon successive injections, as demonstrated in study HEA7.

Although limited, CFP10 and not rdESAT-6 gave a skin reaction in BCG-infected control animals, thus questioning its discrimination ability. This was also recorded in study HEA6 in which a lower dose than that used in study HEA8 (1 ug) CFP10 induced erythema also in guinea pigs infected with BCG, while 1 ug of rdESAT-6 was able to discriminate BCG infection.

(Study 1254) In guinea pigs infected by *M. tuberculosis*, after 4 weeks, intradermal injection of 1 µg rdESAT-6 gave the same response than a mixture of 0.5 µg rdESAT-6 + 0.5 µg CFP10.

This demonstrates that the addition of the low potent CFP10 in a ratio 1:1 in terms of ug, contributes to the activity compensating the reduction of the dose of the high potent rdESAT-6, but no conclusion on the increase sensitivity (number of animals with erythema or size of erythema) from the presence of CFP10 could be draw based on the fact that 1 animal (out of 6) did only respond to the mixture of rdESAT6 + CFP10.

(Study HEA1476) When comparing response in terms of erythema of 2 concentrations 0.05 ug/ml vs 0.5 ug/ml containing different ratios of rdESAT-6 + CFP10 (including rdESAT-6 alone), in guinea pigs infected with *M. tuberculosis* challenged after 4 weeks, the highest response in terms of dimension of erythema, was observed with the higher concentration group (0.5 ug/ml). In this group, considering the lowest erythema dimension of each animal, the applicant concludes that the optimal response is obtained with the antigens ratio 1:1 (0.25 ug + 0.25 ug). Anyway, considering the mean values instead of the lowest response values, ratios 1:1 and 2:1 (0.33 ug + 0.17 ug) gave a very similar response, with a trend better for ratio 2:1.

An increase in the number of rdESAT-6 + rCFP-10 injections (12) did not reduce the response that is quantified by measuring the size of erythema, suggesting that repeated injections of Siiltibcy did not lead to immunological suppression.

Siiltibcy PD studies showed a consistent lower potency of CFP10 than rdESAT-6 in inducing skin reaction (response) in sensibilized animals, both in severe and mild infectious model (*M. tuberculosis* and *M. kansasii*). Also a less ability of CFP10 to discriminate vaccinated animals was observed (Studies HEA8, HEA 6). The potential effect of CFP10 to increase sensitivity (number of animals with erythema or size of erythema) to the diagnostic performance of rdESAT-6 alone, is not clearly demonstrated (Study 1254). Although the use of both antigens in TB diagnostics is recognised and it is intuitive that the combination of the 2 antigens CFP-10 and ESAT-6 can be more efficacious than ESAT-6 alone, non-clinical direct demonstration of the contribution of rCFP-10 in the Siiltibcy remain uncertain. In non-clinical studies rdESAT resulted to be an immunodominant protein, showing approx. 87-fold higher immunogenic potential than CFP10.

With regards to the quantitative Siiltibcy composition, the 1:1 weight ratio of rdESAT-6 (dimer) to CFP-10 corresponds with a 1:1 molar ratio of ESAT-6 (monomer) to CFP-10. Is studies HEA1476 and 1254 the 1:1 ratio in terms of ug was chosen since ESAT-6 and CFP-10 naturally form a complex in a 1:1 -ESAT-6 and CFP-10 are expressed in equimolar amounts *in vivo* and have affinity to each other- even if for the 0.05 µg dose, the optimum ratio was 1:2 (rdESAT-6:rCFP-10).

The applicant clarified that the human dose of 0.05 ug for each antigen was elected starting from non–clinical data in comparison with PPD (human dose 2 tuberculin units). The applicant concluded that the relationship between responses to PPD and rdESAT-6 in humans is not known. Thus, in dose-finding clinical trials with Siiltibcy, 0.01 µg and 0.1 µg Siiltibcy with 1:1 ratio of rdESAT-6 to rCFP-10 were tested (TESEC-01, TESEC-02). TESEC-02 concluded that indurations with 0.1 µg Siiltibcy were similar to those reported previously for PPD.

Sensitisation

(Study 01-01d) Using a similar sensitisation model as the one described for PPD in the European Pharmacopoeia 2000 (potency assay in guinea pigs of tuberculin PPD for human use), 10 ug rdESAT-6 intradermically injected 3 times at intervals of 5 days plus a fourth injection after 2 weeks from the last, sensitised all of the animals giving a mean size of erythema of 25.6 mm in the test group compared to 4.4 mm in the control group 8 (PBS + Tween 20). Since antibody levels were found among the animals with the strongest local reactions (induration and pronounced erythema), the immunological response may involve a type III (IgE) and a type IV reaction (cell-mediated).

In clinical trials and for routine testing it is not expected that skin testing will be performed with a frequency of 5 days. Further, the applied dose of 10 µg rdESAT-6 corresponding to some 100 human doses was based on a rough estimate and may be artificially high. As PPD is a complex mixture of many different proteins each epitope will appear in a low concentration contrary to the epitopes in rdESAT-6. Thus, the chance of sensitising an individual using a purified protein at a high concentration such as rdESAT-6 may be significant compared to PPD, but the risk may be reduced provided testing is performed at long time intervals. A sensitising ability of the antigens may jeopardise the interpretation of a skin reaction in an individual previously subjected to skin testing with the antigen (give rise to false positive reactions).

(Study HEA7) In a simplified sensitisation model compared to the one described in Ph. Eur. for PPD, 1 ug and 10 ug rdESAT-6 and 10 ug CFP10 intradermal injected were able to sensitize guinea pigs following 2, 4 or 8 weeks from 1 intradermal injection (instead of 3 according to PhEur method). For rdESAT-6, sensitising ability was dose-dependent and higher than CFP10, in line with result in study 01-01d. This may in part be due to its lower molecular weight (less immunogenic, study HEA8).

Heat treatment (autoclaving) of rdESAT-6 did not seem to diminish the sensitising ability. In study HEA5, autoclaving did not diminish the ability of rdESAT-6 up to 1 ug, to induce erythema in guinea pig sensitised with *M. kansasii* 4 weeks before challenging.

The sensitising risk seems to be a function of the antigen, the dose and the time span between succeeding doses.

(Study 1478) In line with studies HEA7 and 01-01d, 2 intradermal injections of 1 ug rdESAT-6 and 1 ug rdESAT-6 + CFP10 (0.5 ug + 0.5ug) given 4 weeks apart, sensitise guinea pigs with similar potency. The substitution of half the rdESAT-6 amount with CFP10 did not seem to increase the size of the erythema.

2.5.3. Pharmacokinetics

The mode of action for Siiltibcy is the intradermal presentation of the antigens to the immune system. As the medicinal product is injected intradermally, directly to the site of action, no bioavailability studies were necessary. Moreover, due to its proteic nature, no standard PK/ADME studies were conducted.

The measure of antibodies towards the most immunogenic antigen ESAT-6 2 observed in some PD primary and repeated-dose toxicity studies, is an indirect sign of systemic exposure.

Foetal anti-ESAT-6 antibodies were detected after maternal exposure during gestation.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

(Study 54903) A single IV injection of 100 µg (0.1 ml) rdESAT-6 to mice followed by 14 days observation resulted in no toxicity. The dose was selected as the highest dose tested in a previous dose-range study. The microscopic examination revealed no systemic or local changes related to the test article. No abnormal findings were detected on the reproductive organs (microscopic evaluation). No toxicokinetics (TK) was evaluated.

(Study 54902) A single SC injection of 1000 ug (1 ml) rdESAT-6 to rats followed by 14 days observation resulted in no systemic toxicity. Local test article related inflammation was observed microscopically at the injection sites on Day 3 after injection. Thus, could be due to the high volume of 1 ml for a SC administration. The changes had resolved on Day 15 after injection. No abnormal findings were detected on the reproductive organs (microscopic evaluation). No TK was evaluated.

2.5.4.2. Repeat dose toxicity

(Study 54904) A SC injection of 0.1 ml rdESAT-6 administered weekly at doses 1, 10, 100 ug to rats for 4 weeks, followed by 3 – 4 days recovery for the main group and 15 days recovery for the recovery group, resulted in no systemic toxicity. Local test article related reactions (granulomatous inflammation and necrosis graded minimal/slight) were observed microscopically at the injection sites of the main study animals treated with 100 ug. The reaction resolved as no changes were observed in the recovery animals, sacrificed 15 days after the last injection. The No Observed Adverse Effect Level (NOAEL) is considered to be 10 ug. No abnormal findings were detected on the reproductive organs (microscopic evaluation). 50% of the recovery animals receiving the highest dose (100 ug) had developed anti-rdESAT-6 antibodies.

(Study V7849) Four repetitive SC injections to rat each with a 7-day interval of either 10 ug rdESAT-6 or 5 ug rdESAT-6+5 ug CFP10 did not result in local or systemic toxicity that was considered to be related to these test substances. Local skin reactions at the sites of application were observed mainly after third and fourth injection, but these were considered to be related to local trauma introduced by the injections itself rather than by treatment items. No abnormal findings were detected on the reproductive organs (microscopic evaluation). The NOAEL is considered to be 10 ug.

Overall, after 4 injections with rdESAT-6 + CFP-10 all rats remained antibody-negative against CFP-10 and 4 out of 10 rats (40%) rats had obtained antibodies against rdESAT-6.

After 4 injections with rdESAT-6, 2 out of 10 rats (2/10) were tested positive for anti CFP-10 and 6 out of 10 rats (60%) were anti rdESAT-6 positive. The 2 rats tested positive for anti CFP-10 may be related to cross reactivity between rdESAT-6 and CFP-10 as the positive values are relatively low.

The number of rats having an antibody response was for rdESAT-6 alone 60% and for rdESAT-6 + CFP-10 40%. The higher immunogenicity potential of rdESAT-6 vs CFP-10 observed in guinea pig studies in terms of skin reaction (erythema/induration), was thus confirmed here. The immunogenicity potential appears to be dose-dependent since 2-fold higher dose of the antigen rdESAT-6 alone induced the highest titre.

In this study, the higher immunogenicity of rdESAT-6 seems not to correlate to a higher toxicity since no differences were found in all the toxicity assessed endpoints between rat treated with rdESAT-6 and rdESAT-6 + CFP-10. Also the contribution of CFP-10 to toxicity is confirmed to be negligible.

(Study 54905) A weekly SC injection of 0.1 ml rdESAT-6 to dogs over a period of 4 weeks at 10 and 100 ug, resulted in no toxicity. No abnormal findings were detected on the male reproductive organs (no female dogs were tested). The electrocardiography examination also revealed no treatment-related effects.

A tendency towards a decrease in the number of neutrophils was observed amongst all animals prior to termination (Day 25). The NOAEL is than considered as 100 ug. Both animals dosed at 100 ug developed antibodies.

2.5.4.3. Genotoxicity

N/A

2.5.4.4. Carcinogenicity

N/A

2.5.4.5. Reproductive and developmental toxicity

No fertility and early embryonic development toxicity studies were carried out, nor prenatal and postnatal development, including maternal function, toxicity studies.

(Study V20365) Embryo-foetal developmental toxicity study with Siiltibcy administered subcutaneously in rats. Four repetitive SC injections of 10 ug Siiltibcy (0.5 ug each antigen) in rat 14 days before mating and on gestation days 0, 6 and 13 of Siiltibcy did not result in maternal or developmental toxicity that was considered to be related to Siiltibcy. Increased local skin reactions as erythema and encrustations were observed at the sites of application mainly after the fourth injection. Sensitisation ability of Siiltibcy after repeated administration was known by guinea pigs PD studies in naive animals ID administered.

Siiltibcy induced formation of ESAT-6 antibodies in some of the pregnant rats after 4 injections of Siiltibcy, of which a positive ESAT-6 antibody level could be detected in some of their litters.

2.5.4.6. Toxicokinetic data

N/A

2.5.4.7. Local Tolerance

No separate local tolerance studies were performed. However, epidermal and dermal reactions by Siiltibcy, are expected since direct manifestation of its mode of action, i.e., the intradermal presentation of the antigens to the immune system.

2.5.4.8. Other toxicity studies

Antigenicity/Immunogenicity in terms of antibody formation vs the 2 antigens rdESAT-6 > rCFP-10 and sensitisation ability, were assessed in primary PD studies in guinea pig administered ID and in repeat-dose toxicity studies in rat administered SC.

2.5.5. Ecotoxicity/environmental risk assessment

Due to the proteic nature of the active substances rdESAT-6 and rCFP-10, according to “Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use”, Siiltibcy was exempted from the obligation to submit an environmental risk assessment. Being proteic in nature and considering its intended use, it is not expected for Siiltibcy to pose risk to the environment.

2.5.6. Discussion on non-clinical aspects

The formulation is multidose vial containing 10 doses of 0.1 mL, each containing 0.1 ug of the combination of rdESAT-6 0.05 ug + rCFP-10 0.05 ug. Phenol 0.5% is included as preservative. Siiltibcy is to be administered as intradermal injection (using the Mantoux technique). Dermis and epidermis of the skin are rich in antigen-presenting cells, which make the intradermal route optimal, rather than to injection to the muscle or subcutaneous tissue, to a more efficient immune responses with smaller amounts of antigens.

Recombinant dESAT-6 + CFP10 are antigens highly specific in *M. tuberculosis* and few mycobacteria species causing human TB disease (i.e., *Mycobacterium bovis* and *Mycobacterium africanum*); they are not found in the BCG vaccine and other very few atypical mycobacteria express these proteins (*M. kansasii, M. marinum, M. szulgai*).

Due to its proteic nature, its mode of action (intradermal presentation of the antigens to the immune system) and its intended clinical use, Siiltibcy was considered similar to a preventive vaccine (no systemic absorption) and thus, besides ICH S6 guideline, the WHO guideline on non-clinical evaluation of vaccines, Annex 1, TRS No 927, was also followed. The non-clinical development was discussed on several occasions between the applicant and EMA; overall, the scientific advice was followed.

All the non-clinical development was carried out in facilities located in Denmark and the Netherlands from 2001 to 2013, in compliance with GLP requirements where requested.

Proof of concept information to support the clinical development of Siiltibcy came from the already approved TST based on PPD in which a mix of crude ESAT-6 and CFP-10 proteic antigens are present, thus no *in vitro* binding affinity or activity on cell-based systems, were carried out.

Pharmacology *in vivo* studies in guinea pigs confirmed the immunological mode of action of ESAT-6 alone and the combination rdESAT-6 + CFP10, i.e., the ability to evoke a local DTH (type IV) response directed by cytokines (such as Interferon gamma, IFNg) produced by T-cells, in animals sensitised by prior infection, which is seen as swelling (induration) and redness (erythema) in the skin at the site of intradermal injection. rdESAT resulted to be an immunodominant protein, showing approx. 87-fold higher immunogenic potential than CFP10.

The ability of the antigen rdESAT-6 to discriminate between infections by *M. tuberculosis* and *M. bovis* Calmette-Guerin (BCG) vaccination, was shown. This was evident by comparing the diagnostic performance (in terms of size of erythema and number of animals with erythema) of challenges of rdESAT-6 vs TST/PPD (highest human dose), in both severe and mild infection model in guinea pig. This is relevant for a diagnostic medicinal product which has been developed to overcome the false positive results obtained with the TST/PPD test on the market.

From PD studies, it is less evident which is the contribution of the less immunogenic antigen CFP10 (possibly due to lower molecular weight) to the diagnostic performance of Siiltibcy and its discriminant capacity. It should be noted that initially, the non-clinical and clinical development was based upon rdESAT-6 alone.

As regards the sensitisation ability (potential false-positive test) in naïve guinea pigs after repeated intradermal injections (PhEur method), both rdESAT-6 alone and rdESAT-6 + CFP10 induced sensitisation which, may also be mediated by antibody induction. Comparing with TST/PPD (a mixture of many different proteins each epitope appearing in a low concentration), purified proteins/epitopes such as rdESAT-6 + CFP10, induced a higher sensitisation. However, the sensitisation risk seems to be a function of the immunogenicity potential of the antigen, the dose and the time span between succeeding doses.

In guinea pigs, sensitisation was still observed with injections given 8 weeks apart. Since the maim skin reaction due to sensitisation is erythema, this can be mis-interpreted as a false positive reaction in an individual previously subjected to skin testing with the antigen. Section 4.2 of the SmPC reads: "*The risk of false-positive test results may increase if Siiltibcy is repeated within 6 weeks. Therefore, an interval of at least 6 weeks should be observed between repeated tuberculosis skin tests.*" Six weeks is the incubation period after exposure to Mtb.

Non-clinical studies have not concerned *L. lactis* derived Host cell protein which are present in Siiltibcy.

The efficient removal of low molecular weight impurities including e.g. medium derived peptone in drug substance (DS) manufacturing for SSI clinical representative PPQ and SIIPL PPQ batches, was demonstrated. No additional toxicological characterisation of the impurity profile of Siiltibcy was performed during product development in agreement with obtained regulatory advice. The widespread presence of *L. lactis* in food and its consequent interaction with the human gastrointestinal immune system likely to promote a state of systemic tolerance that would diminish a response to *L. lactis* antigens, if they are administered in immunogenic form elsewhere in the body. In addition, injection site reactions (ISR) in the negative control group of healthy subjects in the three pivotal TESEC trials were comparable for Siiltibcy and PPD, and the proportion of subjects who experienced at least one ISR after receiving Siiltibcy or PPD was similar across the seven TESEC studies. Thus, no false-positive reactions were observed due to *L. lactis* derived proteins in Siiltibcy or prior sensitization of individuals with *L. lactis* proteins. This is further supported by the result that the positivity rate of Siiltibcy (30.6%) was not higher than of PPD (34.1%) in the BCG-unvaccinated subpopulation of all TESEC trials. In conclusion, so far no indications have been obtained that *L. lactis* proteins present in Siiltibcy yield false-positive DTH reactions caused by prior systemic exposure to *L. lactis* derived HCPs.

No secondary pharmacodynamics studies were conducted with Siiltibcy components, which is acceptable. No off-target effect is expected.

No PD drug interaction studies were conducted; being proteins for local action, this is acceptable.

No stand-alone safety pharmacology studies were conducted with Siiltibcy or any of its components: safety pharmacology endpoints were evaluated in rats and dogs as a part of GLP repeated-dose toxicity studies.

As Siiltibcy is injected intradermally, directly to the site of action, no bioavailability studies were necessary. Moreover, due to its proteic nature, no standard PK/ADME studies, were conducted.

Since the two Siiltibcy antigens rdESAT-6 + CFP10 have been known in their crude form for decades, local deposition studies that would assess the retention of the Siiltibcy component at the site of injection and its further distribution (e.g. to the draining lymph nodes) are considered of poor relevance: Lymphadenopathy (swelling of lymph nodes) is amongst the adverse drug reactions reported in SmPC section 4.8.

Single dose and repeated-dose toxicity studies showed overall no systemic effect, including respiratory and cardiovascular parameters, for both rdESAT-6 alone and the combination rdESAT-6 + rCFP-10 in mouse (IV),

rat and dog (SC) at multiples of human dose. Animal doses of Siiltibcy were normalised per Kg and converted in human equivalent dose at which NOAEL was observed.

The SC administration was the preferred route to reflect the intended ID route of administration in man as closely as possible allowing eventual systemic absorption, as the aim of toxicity studies was not to induce an immune response but to test the toxicity (local and systemic) of Siiltibcy. rCFP-10 alone was never tested in toxicity studies, this is acceptable considering its less immunogenicity/sensitisation ability assessed in guinea pig studies.

As the mode of action of Siiltibcy is based on local (at injection site) induction of cellular immune response, no TK evaluation was performed. However, induction of antibodies was also assessed in pharmacological studies in guinea pig and in repeat-dose toxicity studies, thus their production is a sign of a systemic effect of Siiltibcy. Only anti-rdESAT-6 antibodies were observed since the most immunogenic vs rCFP-10. The higher immunogenicity of rdESAT-6 seems not to correlate to a higher toxicity since no differences were found in all the toxicity assessed endpoints between rat treated with rdESAT-6 and rdESAT-6 + CFP-10.

Foetal anti-rdESAT-6 antibodies were also detected after maternal exposure during gestation.

Across toxicity studies, no abnormal findings were detected on the reproductive female and male organs (microscopic evaluation). This, together the negative results from the embryo-fetal developmental toxicity study testing 10 ug rdESAT-6 + rCFP-10 in its commercial formulation containing phenol 0.5% as preservative, confirmed the negligible risk for pregnant women and women of childbearing potential. It should be noted that the phenol concentration in Siiltibcy is 0.5%, the same as the concentration accepted for PPD RT23 551 that is approved for women of childbearing potential.

Throughout the clinical development program of Siiltibcy, women of childbearing potential have been included. No unintended or serious adverse events related to the active ingredient have been identified.

Pregnant women were excluded in the clinical trials. No *in vitro* or *in vivo* genotoxicity or long-term carcinogenicity studies were conducted with Siiltibcy or any of its components, since it is not expected to interact directly with DNA or other chromosomal material due to its nature and mechanism of action.

No new components such as novel excipients which would deem these studies necessary, are present in the formulation.

Siiltibcy is not expected to pose risk to the environment being proteic in nature and considering the intended use. The mode of action is adequately described in SmPC section 5.1.

2.5.7. Conclusion on the non-clinical aspects

Overall, the non-clinical development was appropriately performed according to received advice from EMA and in compliance with GLP and it is considered adequate to support the approval of the MAA of Siiltibcy in the recommended indication.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The applicant claimed that clinical trials were performed in accordance with GCP.

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

- Table 1: Tabular overview of clinical studies

Trial / Phase / Trial Site	Population	Summary
Clinical Studies with rdESAT-6		
TESAT-01 / Phase 1 / Netherlands	35 healthy or treated TB adult subjects. HIV-negative	First-in-human trial. Assessed the safety and diagnostic potential of four dose levels of rdESAT-6. rdESAT-6 was safe and induced a DTH reaction.
TESAT-02 / Phase 1 / Denmark	31 healthy adult subjects. HIV-negative	Assessed the safety and risk of sensitization when rdESAT-6 was injected twice, 28, 56, or 112 days apart. No sensitization was observed after an interval of 112 days.
Clinical Studies with Siiltibcy		
TESEC-01 / Phase 1 / Denmark	42 healthy adult subjects. HIV-negative	First-in-human trial. Assessed the safety and risk of sensitization when Siiltibcy was injected twice, 6 or 12 weeks apart. Results indicate no risk of sensitization with a time span of minimum 41 days between repeated injections with the Siiltibcy skin test.
TESEC-02 / Phase 1b / UK	38 adult TB subjects. HIV-negative	Provided safety data in TB subjects, identified the optimal Siiltibcy dose of 0.1 µg and confirmed that Siiltibcy testing could be performed in a manner similar to existing TST.
TESEC-03 / Phase 2a / UK	151 healthy unexposed and BCG-vaccinated adult subjects. HIV-negative	Two cut-off point finding trials provided specificity and sensitivity data to enable a ROC curve analysis determining the optimal cut-off point for Siiltibcy to be ≥ 5 mm. They also provided initial comparison between Siiltibcy and QFT or PPD.
TESEC-04 / Phase 2b / South Africa	253 adult TB subjects. 100 HIV-positive and 153 HIV-negative	
TESEC-05 / Phase 3 / South Africa PIVOTAL	1190 subjects including 1090 paediatric and adult subjects with suspected TB disease or exposure to Mtb and 100 healthy subjects (aged 5 to 11 years) with no known exposure to Mtb and no signs or symptoms of TB. 299 HIV-positive, 730 HIV-negative, and 161 unknown HIV status	Provided safety data in all age groups (including paediatric age groups) and provided data to support the use of Siiltibcy in all age groups and in HIV-positive subjects. Data support the use of Siiltibcy in the paediatric population from 28 days of age. The size of Siiltibcy indurations appeared constant among HIV-positive responders with CD4+ T-cell counts above 100 cells/µL. Further, the trial allowed a comparison of the diagnostic performance of Siiltibcy to QFT and PPD.
TESEC-06 / Phase 3 / Spain PIVOTAL	979 subjects in four risk groups: Negative Control (no history of exposure to TB and no signs or symptoms of TB), Occasional Contact; Close Contact; Positive Control (confirmed TB). Aged 6 weeks to 65 years. 7 HIV-positive	Demonstrated that Siiltibcy responder rates correlated with exposure to Mtb and thereby confirmed the overall claim that Siiltibcy diagnoses infection with Mtb. Secondary, the trial provided safety data and efficacy data confirming a superior specificity compared to PPD and similar diagnostic performance as QFT.
TESEC-07 / Phase 2/3 / South Africa PIVOTAL	456 adult TB subjects Siiltibcy: 154 subjects Siiltibcy+PPD : 153 subjects PPD: 149 subjects 92 HIV-positive	Provided data to demonstrate that Siiltibcy induration responses are not affected by simultaneous administration of PPD (in the other arm) immediately one after another. Secondary, the trial provided extended safety data in TB

Trial / Phase / Trial Site	Population	Summary
		subjects and data to support that Siiltibcy has similar sensitivity as QFT.

BCG = Bacillus Calmette-Guérin; CD4 = cluster differentiation 4; HIV = human immunodeficiency virus; Mtb = *Mycobacterium tuberculosis*; PPD = tuberculin purified protein derivative RT 23 SSI; QFT = QuantiFERON® Gold In-Tube Test; rdESAT-6 = recombinant dimer of the 6 kDa early secretory antigen target; ROC = receiver operating characteristics; TB = tuberculosis; TST = tuberculin skin test; UK = United Kingdom

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

As Siiltibcy is injected intradermally, directly to the site of action, no bioavailability studies were necessary. Moreover, due to its proteic nature, its mode of action and its intended clinical use, Siiltibcy was considered similar to a preventive vaccine (biological activity exerted locally, at administration site, intradermic, no systemic absorption).

2.6.2.2. Pharmacodynamics

Mechanism of action

Siiltibcy is an immunological recombinant medicinal product for diagnostic use in humans. It contains two recombinant Mycobacterium tuberculosis specific antigens: rdESAT-6 and rCFP-10. In case of infection with Mycobacterium tuberculosis, Siiltibcy induces a delayed-type hypersensitivity reaction directed by cytokines, which are released by TH1 cells after stimulation by the specific antigens included in Siiltibcy.

This reaction is seen as an induration at the site of injection. The induration reaches its maximum 48 – 72 hours after administration.

2.6.3. Discussion on clinical pharmacology

The nature of the product (injectable proteins) might lead not only to the intended immune reaction but could also cause allergic reactions. A systemic reaction is in that case also possible. However, the “normal” test reaction also needs a local swelling of defined size. Intradermal use is intended.

2.6.4. Conclusions on clinical pharmacology

Local reactivity is intended, potential for systemic (allergic) reactions is expected; no special issues are predicted in comparison with licensed TSTs. Hypersensitivity and local or systemic allergic reactions are contraindications in SmPC section 4.3 and risk of anaphylaxis is described in SmPC section 4.4.

2.6.5. Clinical efficacy

2.6.5.1. Dose response studies

TESEC-01

TESEC-01 was an open, Phase 1 clinical trial on the safety and the risk of sensitisation by escalating doses and repeated injections of the rdESAT-6 + rCFP-10 skin test reagent following intradermal administration to healthy adults.

The primary objective of this study was to assess the safety of three Siiltibcy doses: 0.01 µg/0.1 mL, 0.1 µg/0.1 mL, and 1.0 µg/0.1 mL (due to stability concerns with the 1.0 µg formulation, no subjects were recruited in this group), when injected in healthy subjects, by assessing local and systemic adverse events.

The secondary objective was to assess the risk of sensitisation of two doses (0.01 and 0.1 µg) of Siiltibcy when injected twice 6 or 12 weeks apart (Induration ≥ 6 mm or an IFN-γ response ≥ 0.35 IU/mL after the second injection were defined as possible sensitisation reactions).

In total healthy 42 volunteers (non-black, female/male adults, 39 completed the study) with a negative INF-γ response at inclusion (below 0.35 IU/mL) according to the QuantiFERON®-TB Gold In-Tube test) were included in the trial: 11 volunteers in group A (0.01 µg; 6 weeks), 10 volunteers in group B (0.01 µg; 12 weeks), 10 volunteers in group in group C (0.1 µg; 6 weeks), and 11 volunteers in group D (0.1 µg; 12 weeks).

Safety and Sensitisation results

In total, 58 adverse events (related or unrelated to the trial product) were reported during the trial, 36 after the 1st injection, 20 after the 2nd injection and 2 with start dates unknown to be before or after the 2nd injection. In total, 19 adverse events were reported in group A, 10 in group B, 9 in group C and 20 in group D.

Sixteen (16) adverse events were assessed as at least possibly related to the trial product. Of these, 4 events were reported from group A, 3 from group B, 3 from group C and 6 from group D. 11/16 occurred after the 1st injection and 5/16 occurred after the 2nd injection.

Table 2: Number of AE episodes reported (Relation: 'Possible'or more)

Group	Dose	Delay	First injection	Second injection	All
A	0.01 µg/0.1 mL	6 weeks	1	3	4
B	0.01 µg/0.1 mL	12 weeks	2	1	3
C	0.1 µg/0.1 mL	6 weeks	2	1	3
D	0.1 µg/0.1 mL	12 weeks	6	-	6
All			11	5	16

Injection site reactions:

3/42 volunteers had at least one injection site reaction during the trial: 3/42 after the 1st injection and 1/39 after the 2nd injection.

Table 3: Number of subjects reporting Injection site reactions

Group	Dose	Delay	First inject.	Second inject.	Un-known inject.	Any time	Subj. per Group	Pct first	Pct second	Pct un-known	Pct any
A	0.01 µg/0.1 mL	6 weeks	-	-	-	-	11	-	-	-	-
B	0.01 µg/0.1 mL	12 weeks	1	1	-	1	10	10	10	-	10
C	0.1 µg/0.1 mL	6 weeks	-	-	-	-	10	-	-	-	-
D	0.1 µg/0.1 mL	12 weeks	2	-	-	2	11	18	-	-	18
All			3	1	-	3	42	7	2	-	7

One Volunteer (group D; 0.1 µg) showed a positive skin test reaction after the first injection of Siiltibcy associated with a high QuantiFERON®-TB Gold In-Tube test result of 1.57 IU/mL measured 96 hours after the 1st Siiltibcy injection. At baseline (screening) the QuantiFERON®-TB Gold In-Tube test showed a negative, but high IFN-γ response of 0.24 IU/mL. The volunteer, known to be TST and QuantiFERON negative in 2002, was working as a nurse and had on several occasions travelled to TB high endemic countries. A likely explanation of these observations is that the Siiltibcy test has identified an undiscovered case of latent TB infection. This volunteer was withdrawn and replaced by another.

Non injection site reactions:
Eleven (11/42) volunteers had at least 1 non-injection site reaction possibly related to the trial product after the first and second injection of the Siiltibcy agent. Eight volunteers 8/42 had a non-injection site reaction possibly related to the trial product after the first injection and 4/39 volunteers after the second injection.

Table 4: Number of subjects reporting non-Injection-site reactions with relation 'Possible' or more

Group	Dose	Delay	First inject.	Second inject.	Un-known inject.	Any time	Subj. per Group	Pct first	Pct second	Pct un-known	Pct any
A	0.01 µg/0.1 mL	6 weeks	1	2	-	3	11	9	18	-	27
B	0.01 µg/0.1 mL	12 weeks	1	1	-	2	10	10	10	-	20
C	0.1 µg/0.1 mL	6 weeks	2	1	-	2	10	20	10	-	20
D	0.1 µg/0.1 mL	12 weeks	4	-	-	4	11	36	-	-	36
All			8	4	-	11	42	19	10	-	26

One Volunteer (group C) had an unusual IFN-γ profile as measured by QuantiFERON®-TB Gold: before the first TST his IFN-γ value was 0.02 IU/mL, prior to the second TST it was 10.00 IU/mL and 30 days after the 2nd TST it was 0.02 IU/mL. Additional in-house IGRAs showed no response to neither ESAT-6 nor CFP-10 peptide stimulation in the blood sample drawn 30 days after the second TST, confirming the negative IFN-γ result.

This volunteer had no visible or palpable skin reaction. The unusual IFN-γ profile and the absence of skin reactions suggest that the 10.00 IU/mL value might be the result of a laboratory error.

TESEC-02

TESEC-02 was a Safety and Dose Finding study in Adult Patients Recently Diagnosed with Active TB.

This clinical trial was a single centre Phase 1b open dose adjustment study with respect to the dose of Siiltibcy combined with a double blind randomised, split body comparison of unpreserved Siiltibcy and Siiltibcy preserved with 0.5% phenol (each patient received the unpreserved version in one arm and the preserved version in the other arm).

The Primary Objective of this study was to assess the safety of two dose levels of Siiltibcy (0.01 and 0.1 µg/0.1 mL) when administered intradermally by the Mantoux technique to patients in the acute phase of treatment against active TB. (One of the secondary objectives was to assess the immune response of two doses (0.01 and 0.1 µg/0.1 mL) of Siiltibcy from the size of induration).

Adult subjects without HIV infection who were newly diagnosed with active TB and in treatment for ≤ 60 days were eligible for the study. In total, 38 patients (23 male, 15 female) between 18 and 60 years of age (mean: 33 years) were included.

Safety and Immune Response Results

Data from all 38 included patients were used in the safety evaluation.

The majority of adverse events were itch and pain at the injection site. There was no association between phenol-preserved IMP and pain or itch at the injection site. Itch was reported by 6/12 patients and pain by 2/12 at the site injected with 0.01 µg/0.1 mL Siiltibcy without phenol.

In comparison, 3/12 patients injected with same dose including phenol reported itch and 1/12 reported pain. Injection of 0.1 µg/0.1 mL Siiltibcy without phenol resulted in itch in 17/26 of the cases and pain in 8/26 of the cases. In comparison, the same dose Siiltibcy with phenol resulted in itch (16/26) and pain (6/26) to a similar extent as Siiltibcy without phenol.

Non-Injection Site Reactions are also superimposable between the two group (with/without phenol).

In addition, none of the laboratory findings suggest any adverse effect of Siiltibcy with or without phenol on haematology or biochemistry values.

Immune Response Results

Evaluation of the immune response variables (induration, erythema and QFN) included 11 patients injected with 0.01 µg/0.1 mL Siiltibcy (one patient excluded from analysis,) and 24 patients from the 0.1 µg/0.1 mL Siiltibcy group (two patients excluded from analysis).

The first reading of the responses after injection of 0.01 µg and 0.1 µg Siiltibcy were done after one day. Succeeding readings were performed after two days, after three days and after four days.

Visual inspection showed that the size of induration after the two formulations was similar. This was also supported by a Wilcoxon's Signed Rank test of the difference of induration after Siiltibcy with phenol and induration after unpreserved Siiltibcy for each combination of time point and dose group (Table 5). No indication of a systematic or significant difference was found.

Comparability of the induration response to Siiltibcy and PPD administration was also evaluated. Injection of 0.1 µg Siiltibcy resulted in an induration response similar to the response expected for two tuberculin units (T.U.) PPD, which is normally distributed and peaks at 15 mm.

Table 5

Mean Induration and Erythema Size 48 h After C-Tb Administration (TESEC-02)

Parameter	C-Tb Dose	Formulation Without Phenol	Formulation With Phenol
Induration size (mm)	0.01 µg/0.1 mL	8.5	6.8
	0.1 µg/0.1 mL	14.1	13.1
Erythema size (mm)	0.01 µg/0.1 mL	13.9	9.4
	0.1 µg/0.1 mL	26.2	27.9

Source: TESEC-02 CSR, pages 44-45
CSR = clinical study report

2.6.5.2. Main studies

The following table lists the three PIVOTAL clinical studies supporting the MAA.

Table 6

Trial / Phase / Trial Site	Population
TESEC-05 / Phase 3 / South Africa	1190 subjects including 1090 paediatric and adult subjects with suspected TB disease or exposure to Mtb and 100 healthy subjects (aged 5 to 11 years) with no known exposure to Mtb and no signs or symptoms of TB. 299 HIV-positive, 730 HIV-negative, and 161 unknown HIV status
TESEC-06 / Phase 3 / Spain	979 subjects in four risk groups: Negative Control (no history of exposure to TB and no signs or symptoms of TB), Occasional Contact; Close Contact; Positive Control (confirmed TB). Aged 6 weeks to 65 years, 7 HIV-positive
TESEC-07 / Phase 2/3 / South Africa	456 adult TB subjects, Siiltibcy: 154 subjects, Siiltibcy+PPD : 153 subjects, PPD: 149 subjects, 92 HIV-positive

These three pivotal trials TESEC-05, TESEC-06 and TESEC-07 will be described and analysed separately for each aspect covered herein after.

TESEC-05

A Phase 3 trial in subjects suspected to have tuberculosis, comparing the diagnostic performance of Siiltibcy to QuantiFERON®-TB Gold In-Tube Test, in combination with a double-blind randomised split-body safety assessment of Siiltibcy versus 2 T.U. Tuberculin PPD RT 23 SSI (PPD).

Trial design:

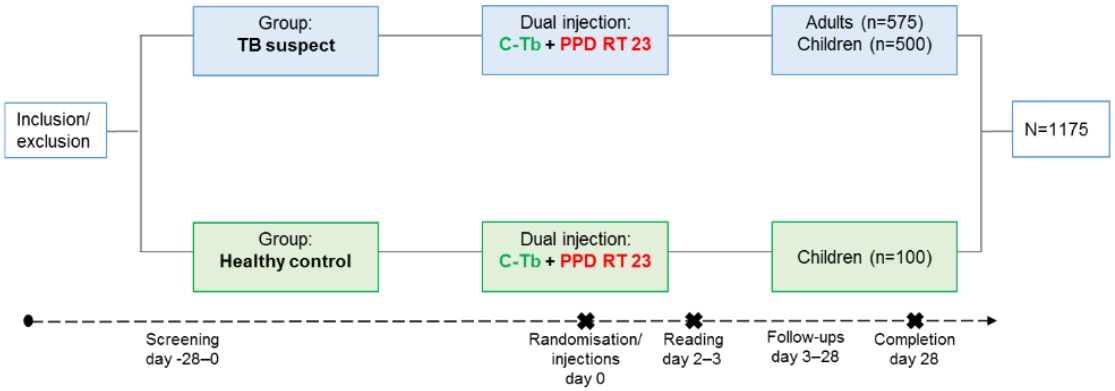

Figure 3

TESEC-06

A Phase 3 contact tracing trial comparing the diagnostic performance of Siiltibcy to QuantiFERON®-TB Gold In-Tube Test, in combination with a double-blind randomized split-body safety assessment of Siiltibcy versus 2 T.U. Tuberculin PPD RT 23 SSI. There were 13 investigational sites, all located in Spain.

Trial design:

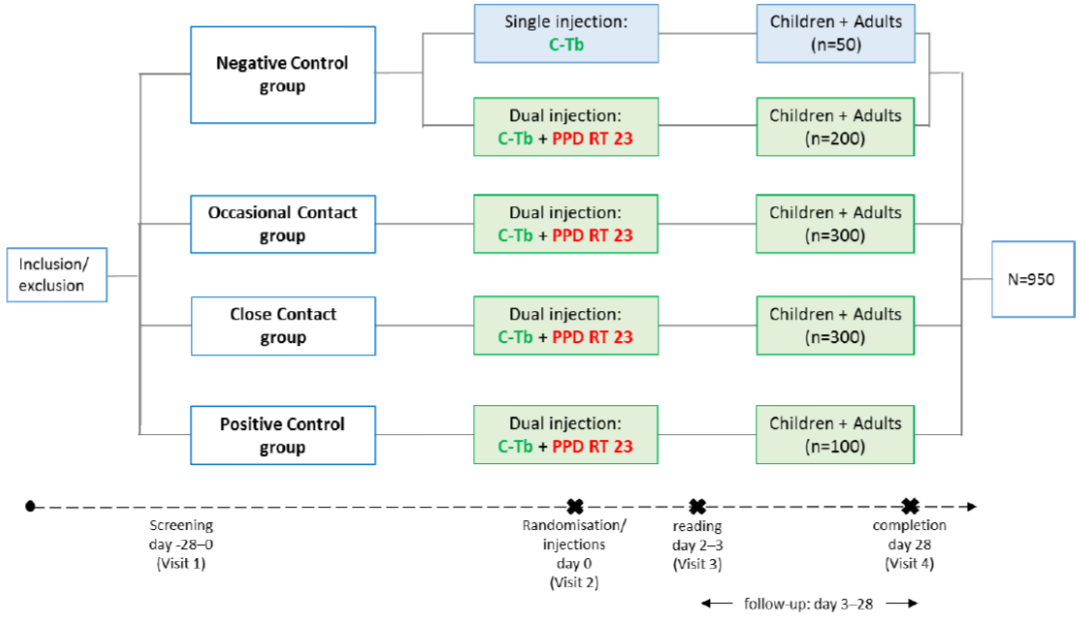

Figure 4

TESEC-07

A double-blind randomised Phase 2/3 trial in adult patients recently diagnosed with active TB, investigating if concomitant injections of the diagnostic agents Siiltibcy and 2 T.U. Tuberculin PPD RT 23 SSI (PPD) affect the induration responses (compared to the administration of a single agent), in combination with a safety assessment of Siiltibcy.

Trial design:

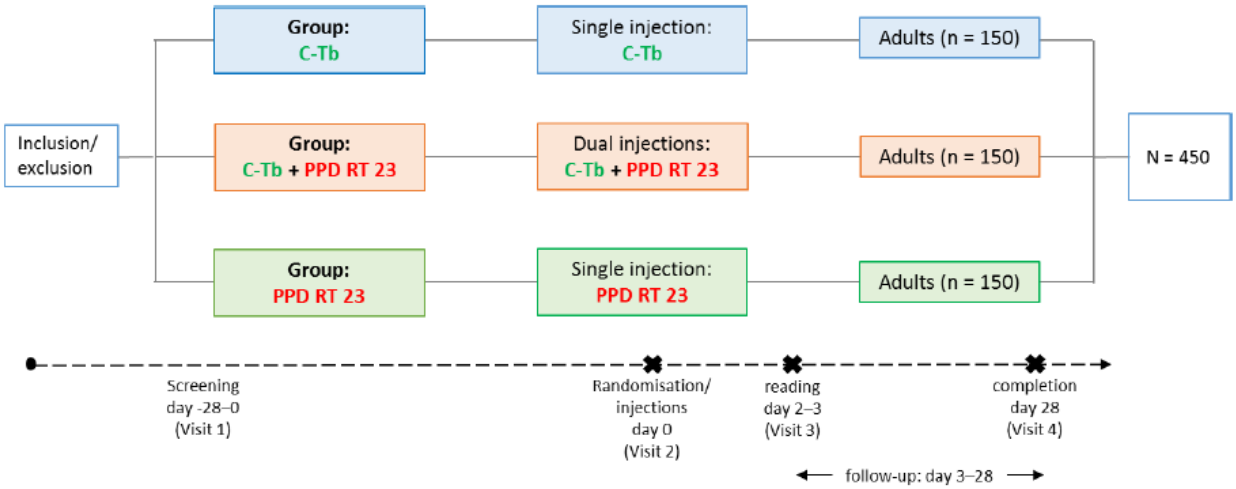

Figure 5: Measurement of IFN-gamma by QFT was performed in all arms.

Methods

- Study Participants

TESEC-05
TESC 05 was a Phase 3 trial in subjects suspected to have tuberculosis, comparing the diagnostic performance of Siiltibcy to QuantiFERON®-TB Gold In-Tube Test, in combination with a double-blind randomized split-body safety assessment of Siiltibcy versus 2 T.U. Tuberculin PPD RT 23 SSI (PPD).

The main inclusion criteria were:

TB suspect: paediatric participants between 28 days – 4 years of age with either symptoms or signs of TB or being in close contact with a smear positive pulmonary TB case, and participants between 5 – 65 years of age suspected to have TB disease.

Negative Control (NC) group: 100 children between 5 – 11 years of age with no TB symptoms or known exposure to Mtb was recruited from an area with a presumed low prevalence of TB infection.

HIV status: confirmatory tests were to be performed on all participants older than 5 years old.

HIV-negative participant:
- Between 5 – 65 years old who attended the TB clinic due to suspicion of TB disease
- Infants, toddlers and children between 28 days – 4 years old who either had symptoms and or signs of TB or was in close contact with a positive pulmonary TB person (more than 6 hours/day for at least 5 days)
- HIV-negative confirmed by 2 rapid tests (children between 28 days – 4 years old could have unknown HIV status and could have received antiretroviral therapy (ART) or have breastfeeding mothers on ART)

HIV-positive participant:
- Between 5 – 65 years old who attended the TB clinic due to suspicion of TB disease
- Infants, toddlers and children between 28 days – 4 years old who either had symptoms and or signs of TB or were in close contact with a positive pulmonary TB person (more than 6 hours/day for at least 5 days)
- Confirmed HIV-positive by either: 2 positive rapid tests or 1 positive rapid test and an additional confirmatory (via enzyme-linked immunosorbent assay test)

- CD4 count measured

Exclusion criteria were:

- The participants were not allowed to have a confirmed diagnosis of TB disease at the screening visit.
- A recent vaccination with live vaccine might suppress the induration response, and, therefore, patients were to be excluded if they had been vaccinated less than 6 weeks before screening.
- Because a recent testing with TST could boost the response of the comparator PPD RT 23 SSI, people who were tested less than 12 months before screening were to be excluded.
- Patients with a known diagnosis of acquired immune deficiency syndrome (AIDS) were not to be enrolled as their immunodeficiency was expected to be at an advanced stage.

TESEC-06
This Phase 3 clinical study, conducted in 13 centres in Spain, was an open comparison of the diagnostic performance of Siiltibcy compared to QFT in combination with a double-blind randomized split-body safety assessment with PPD. The subjects were recruited according to four TB risk groups (listed below).

Main inclusion criteria were:

Complied with 1 of the following risk groups:

- Negative Control (NC) group: participants must not have had history of exposure to a TB index case and have no signs or symptoms of TB.
- Occasional Contact group: participants must have been in contact with a pulmonary TB index case (sputum or broncho smear positive, subsequently confirmed by Culture, GeneXpert or PCR) between 6 hours/week and 6 hours/day.
- Close Contact group: participants must have been in close contact with a pulmonary TB index case (sputum or broncho smear positive, subsequently confirmed by Culture, GeneXpert or PCR) for more than 6 hours/day for at least 5 days.
- Positive Control (PC) group: participants must have had TB disease within the last 3 years confirmed by culture, GeneXpert or PCR.
- Was between 6 weeks – 65 years old.

Main exclusion criteria:

- Had been vaccinated with a live vaccine within 6 weeks prior to the day of inclusion
- Had been tuberculin tested less than 12 months prior to randomization

The prevalence of Mtb infection was estimated to be 20% in the Occasional Contact group and 50% in the Close Contact group, while using a fixed prevalence of 1% in the unexposed participants and 100% in TB patients.

TESEC-07
All TESEC-07 trial sites were in South Africa.

Major inclusion criteria:

1. Aged between 18 – 65 years

2. Was HIV-negative (2 negative rapid tests) or HIV-positive (2 positive rapid tests or 1 positive rapid test and an additional confirmatory ELISA). For HIV-positive participants, CD4 count was performed.

3. Had been diagnosed with active pulmonary TB: had a compatible clinical picture of TB according to South African guidelines with the intention-to-treat and one documented positive culture result or one documented positive GeneXpert analysis.

Major exclusion criteria:

1. Had been in treatment for TB for more than 2 weeks.

2. Had a known multi-drug resistant tuberculosis / extremely drug resistant tuberculosis.

- Treatments

Subjects received Siiltibcy, PPD or both, according to the specific trial design (see above).

Dosage: A dose of 0.1 µg Siiltibcy refers to a test solution consisting of 0.05 µg rdESAT-6 and 0.05 µg rCFP-10 per 0.1 mL. Concomitantly 1 injection of Siiltibcy (0.1 µg/0.1 mL) in 1 forearm and 1 injection of the comparator PPD in the other forearm immediately one after another and according to the randomization code (single injection of the Siiltibcy in 50 subjects). Route of administration: intradermal injection using the Mantoux technique.

QFT is a whole blood assay containing the Mtb antigens ESAT-6 and CFP-10 and TB7.7. Blood for QFT analyses was to be sampled before administration of the skin tests (V2) to avoid any possible interference with the skin tests. Blood for QFT analyses was to be collected from all participants age 5 and older.

The QFT cut-off is 0.35 IU/ml. This is the commercial standard. But note that results below 0.35 IU/ml have to be explicitly designated as ‘negative’ in order to be used as ‘negative’.
Indeterminate results are considered as missing.

Concomitant medications live vaccines (e.g., MMR, yellow fever, oral typhoid vaccines) were not allowed 6 weeks before the day of inclusion or during the trial. Tuberculin skin test was not permitted 12 months before the day of inclusion or during the trial.

- Objectives

TESEC-05

Primary objective

The efficacy part of the primary objective is to investigate whether induration sizes and derived test positive rates depend on age and HIV status in a population with a presumed high prevalence of Mtb infection.

The ‘test positivity’ rate of the Siiltibcy diagnostic is defined as the prevalence of subjects in a given sub-group of the trial population who have an induration response above a certain cut-off value.

An induration reading performed 2-3 days after Siiltibcy injection is considered positive if the reading is at least 5 mm: Siiltibcy cut-off = 5mm. This cut-off is used irrespective of patient characteristics.

Primary trial objectives are stated as follows in the protocol:

1) To evaluate the diagnostic performance of Siiltibcy in relation to age, HIV and CD4 counts:
a) To evaluate Siiltibcy induration diameters as a function of age, with emphasis on children
b) To evaluate Siiltibcy induration diameters as a function of HIV status
c) To evaluate Siiltibcy induration diameters as a function of CD4 counts in HIV-positive participants
d) To evaluate Siiltibcy test positivity as a function of age, with emphasis on children, using the above cut-off to define positivity
e) To evaluate Siiltibcy test positivity according to HIV status, using the above cutoff to define positivity
f) To evaluate Siiltibcy test positivity according to CD4 counts in HIV-positive participants using the above cut-off to define positivity

2) To evaluate the clinical safety of Siiltibcy, with emphasis on children and HIV-positive participants.

Secondary objectives

The 'test positivity' rate of the PPD RT23 diagnostic is defined as the prevalence of subjects who have an induration response above a certain cut-off value.

An induration reading performed 2 – 3 days after PPD RT23 injection is considered positive:
• For simultaneously confirmed BCG-vaccinated and known HIV-negative subjects if and only if the reading is at least 15 mm: PPD RT23 cut-off = 15mm.
• For all other subjects if and only if the reading is at least 5mm: PPD RT23 cut-off = 5mm.

Secondary trial objectives are stated as follows in the protocol:
To evaluate the difference in sensitivity, specificity between Siiltibcy and PPD or QuantiFERON®-TB Gold in-Tube in trial participants with confirmed TB diagnosis, overall, and according to age and HIV status.

To compare the diagnostic outcome of Siiltibcy vs PPD and QuantiFERON®-TB Gold in-Tube using a latent class approach.

TESEC-06

The primary objective of TESEC-06 was to demonstrate an increasing trend in Siiltibcy positivity rate across four different TB risk groups with "positivity" defined as an induration diameter ≥ 5 mm.

Secondary objectives included (not limited to):

1) To demonstrate a significantly lower response rate of Siiltibcy as compared to that of PPD in the BCG-vaccinated participants in the Negative Control group with response defined as any induration (> 1 mm) for both agents.

2) To evaluate the difference in sensitivity and specificity, between Siiltibcy and PPD or QFT in the Positive Control group.

3) To compare the diagnostic outcome of Siiltibcy vs PPD or QFT using the latent class approach.

TESEC-07

The primary objectives of TESEC-07, were:

1. To compare the size of induration of Siiltibcy and PPD RT 23 SSI (PPD hereafter) if injected alone or concomitantly in TB participants (HIV-positives and HIV-negatives).

2. To assess if concomitant injections of Siiltibcy and PPD influence the sensitivities of Siiltibcy and PPD in TB participants.

Secondary objectives were:

1. To compare the sensitivity of Siiltibcy with the *in vitro* QuantiFERON®-TB Gold In-Tube assay in blood collected immediately before application of the Siiltibcy skin test.

2. To compare the sensitivity of Siiltibcy with PPD.

3. To assess the safety of Siiltibcy.

- Outcomes/endpoints

TESEC-05

Primary endpoints

1) Diameter of induration at the Siiltibcy injection site measured transversely to the long axis of the forearm 2–3 days after intradermal administration of Siiltibcy (at day 2 – 3 using a pre-defined cut-off of 5 mm).

2) The test positivity of each participant as evaluated by the Siiltibcy induration at day 2 – 3 in conjunction with the above cut-off to define positivity.

Secondary endpoints

– Diameter of induration at the PPD injection site measured transversely to the long axis of the forearm at 2 – 3 days after intradermal administration of PPD

– Positivity of each participant as evaluated by the PPD induration at day 2 – 3 according to the pre-defined cut-off values of 5 and 15 mm that define positivity

– QFT raw test results in IU/ml

– QFT dichotomised test result pre-defined by the standard cut-off values of 0.35 IU/mL

Safety endpoints

– All AEs occurring within 28 days after intradermal administration of Siiltibcy and PPD

– Laboratory safety parameters: haematology and biochemistry in participant ≥ 5 years of age

TESEC-06

Primary endpoint: Positivity of each trial participant as evaluated by the Siiltibcy induration at day 2 – 3 (Visit 3 [V3]) in conjunction with the cut-off value = 5 mm.

Trial criteria for analysis:

– Cut-off value for Siiltibcy: ≥ 5 mm

– Cut-off value for PPD: BCG-vaccinated and HIV-negative/unknown participants: ≥ 15 mm, all other participants: ≥ 5 mm

– PPD6 (alternative cut-off for PPD) ≥ 6 mm

Main secondary endpoints were:

- Siiltibcy induration at day 2 – 3 after injection (V3)
- PPD induration at day 2 – 3 after injection (V3)
- QFT result in IU/mL as well as the dichotomised test result defined by the cut-off value
- Safety: All adverse events (AEs) occurring within 28 days after intradermal administration of Siiltibcy and PPD, Laboratory safety parameters.

TESEC-07

The endpoints for the primary objectives were:

1. The diameter of induration at the injection sites measured transversely to the long axis of the forearm 2 – 3 days after application of Siiltibcy and PPD: the delayed type hypersensitivity reactions, erythema and induration, were independently measured by 2 experienced trial staff at V3 (2 – 3 days after the injection) and V4 (28 days after the injection).

2. Outcome of the QuantiFERON®-TB Gold In Tube assay (QFT, measured in IU/ml).

- Sample size

TESEC-05: Overall sample size for the study program was determined from the goal of an overall exposure of Siiltibcy to approximately 3000 participants. This is based on consideration of the precision of the estimate of the prevalence of rare adverse events. The trial planned to enrol 600 participants. The paediatric group comprised 500 subjects with suspected TB disease (below 18 years of age, close contact to a TB case is considered sufficient) plus 100 children between 5 – 11 in a negative control group. HIV-positive: a target of 300 of the trial population will be HIV-positive. For children below 5 years of age the HIV status is based on historical records. HIV status might therefore be unknown in a certain number of these subjects.

TESEC-06: Overall sample size for the study program was determined from the goal of an overall exposure of Siiltibcy to approximately 3000 participants. This is based on consideration of the precision of the estimate of the prevalence of serious adverse events. For the present trial, extensive simulation was performed to evaluate the sample needed to achieve at least 90% power of the primary analysis to detect a trend in Siiltibcy test positivity across the four risk groups as well as a reasonable precision of the estimated differences in sensitivity and specificity between Siiltibcy, PPD RT23 and QFT using the latent class method. No interim analysis is planned.

TESEC-07: The size of the trial was then determined using the requirement that the precision (half-width of a 95% confidence interval) of the difference in mean induration diameters between subjects with single and double Siiltibcy administration should be at most 4 millimetres. This will be attained using the present 3-treatment-arm design with 150 subjects per arm. The overall study population comprised 450 adult subjects with acute active pulmonary TB, comprising 360 HIV-negative and 90 HIV-positive adults, allocated to 3 trial groups (Siiltibcy and PPD RT 23 given concomitantly; Siiltibcy only; PPD RT 23 only).

- Randomisation and Blinding (masking)

-Randomisation

TESEC-05, -06, -07: The randomization scheme was prepared in advance, following a block randomization method that would have ensured a balance in sample size across group over time.

- Blinding (masking)

TESEC-05, -06, -07: For blinding purposes and to avoid interference from an immune response, Siiltibcy and Tuberculin PPD RT23 SSI were given concomitantly to each participant in the right and left forearms according to a double-blind randomization scheme. This trial is double blind and therefore neither the research staff, sponsor nor the participant will know which arm is injected with the Siiltibcy skin test and which arm is injected with the 2 T.U.

- Statistical methods

TESEC-05

The analysis sets were defined as follows:

- FAS (Full Analysis Set): All enrolled and randomised participants who had been tested with Siiltibcy , PPD or QFT.
- PP (Per-Protocol): All participants who had complied with the protocol and who had non-missing diagnostic read-outs of both Siiltibcy and PPD indurations as well as QFT.
- Safety analysis set: All enrolled and randomised participants who had been tested with Siiltibcy or PPD.

Several distinct sub-populations of the FAS were used for analysis or display purpose: the NC, the confirmed TB group and the diagnosed TB group.

All missing values were left missing and no imputation performed. Multiplicitywas not considered an issue for the present trial, as hypothesis testing was kept to a minimum.

Primary analysis: Concerned with the distribution of Siiltibcy induration diametersand test-positive rates as depending on key factors age, HIV status and sex.

Secondary analyses: Concerned with 1) the distribution of PPD induration diameters and test-positive rates as depending on key factors age, HIV statusand sex, 2) the distribution of QFT test-positive rates as depending on key factors age, HIV status and sex, 3) the relation between Siiltibcy test outcome and the corresponding outcomes for PPD and QFT.

Safety analyses: Safety variables were tabulated. Laboratory safety parameters of haematology and biochemistry in participants from 5 years of age were tabulated descriptively and are presented in shift figures, depictingthe change in a number of out-of-range participants.

TESEC-06

The analysis sets were defined as follows:

Full analysis set (FAS) = all participants enrolled, randomised and tested irrespective of any results obtained.

By design, QFT results were not available in participants < 5 years old

Per protocol set (PP) = participants who had complied with the protocol and who had a non-missing diagnostic read-outs of both Siiltibcy and PPD indurations as well as QFT. By design, QFT results were not available in participants < 5 years old

Safety set = all participants enrolled and randomised irrespective of any results obtained

Primary analysis: Concerned with the distribution of Siiltibcy positivity rates as depending on Mtb risk level defined by the 4 risk groups. It comprised 1) histograms of the Siiltibcy induration diameters distribution across risk groups based on FAS and PP, 2) test positivity rates for Siiltibcy tabulated descriptively in total and split into risk groups and based on FAS and PP and 3) logistic regression model of Siiltibcy positivity (binary response) describing positivity rates as dependent on: risk group, age, gender, BCG-vaccination status

Confirmatory hypothesis corresponds to the risk group factor being of statistically significant importance (5% level). The contrast between risk groups were derived and presented with 95% confidence intervals.

Secondary analysis: First secondary endpoint analysis compared response rate of Siiltibcy with a response rate of PPD in the subgroup of the Negative Control group that had been previously BCG-vaccinated. Positivity rates (response defined as any response ≥ 1 mm) were compared using:

As for the primary analysis, a FAS and a PP version were performed.

Second secondary analysis was concerned with the change in Siiltibcy induration diameters across the 4 risk groups. Linear analysis of covariance model of Siiltibcy test-induration diameters, describing diameters as dependent on the risk group, age, gender and BCG-vaccination status.

Confirmatory hypothesis corresponds to the risk group factor being of statistically significant importance (5% level). The contrast between risk groups was derived and presented with 95% confidence intervals. The analysis was based on the FAS as well as the PP population.

Sensitivity of Siiltibcy was compared to the sensitivities of QFT and PPD, respectively in the Positive Control group using paired within-participant design of the trial and the (paired) McNemar test.

Specificity of Siiltibcy was compared to the specificity of PPD and QFT respectively in the Mtb-negative Control group, with ‘negative Siiltibcy test’ defined as a Siiltibcy induration below the cut-off value and correspondingly for PPD and QFT. The specificities were compared using the paired within-participant design of the study, and the standard McNemar test was used for this purpose.

The 50 BCG-negative participants from the Negative Control group (who were given a single injection of Siiltibcy) were compared directly to the remaining dual injection BCG-negative participants in terms of Siiltibcy induration diameters and Siiltibcy positivity rates. In the first case, the two-sample Hodges-Lehman estimator with 95% confidence limits was derived, in the second case, the 2 specificity estimates were compared using the Fisher test.

Direct estimation of Siiltibcy/PPD/QFT sensitivity and specificity was done using the latent class approach.

Missing values for the diagnostic outcome was likely to occur in particular for the QFT test, where some

indeterminate results were expected. Furthermore, this test was not taken in children below 5 years of age. Influence of missing values on the estimates of sensitivity and specificity was evaluated using a multiple imputation approach.

TESEC-07

The analysis sets were defined as follow:

Full analysis set (FAS): FAS consisted of all enrolled and randomised (to Siiltibcy and PPD concomitantly or as single injections) participants who had been tested with either Siiltibcy or PPD. The exclusion from the FAS was considered in the case of complete lack of data post-randomisation or severe violation of inclusion/exclusion criteria

Per protocol analysis set (PP): PP consisted of all participants who had complied with the protocol and who had a non-missing diagnostic read-out of both Siiltibcy and PPD. A missing QFT measurement did not lead to the exclusion from the PP set

Safety analysis set: The safety analysis population consisted of all enrolled and randomised participants who had been administered either Siiltibcy or PPD, irrespective of any results obtained. It was the administration of the skin test that defined the population

Special populations: The HIV status of certain participants could turn out to be a matter of decision where their status was left out (i.e. missing). Only positive (confirmed) cases were considered for alternative PPD cut-offs (PPD5/10/PPD5/15)

All missing values were left missing and no imputation performed. This was in particular relevant for a substantial fraction of QFT results.

The primary analyses concerned the distribution of Siiltibcy and PPD induration diameters in participants who received a single injection compared to those who received concomitant injections. The trial aimed to describe and possibly detect a shift in induration diameter distribution, either upwards or downwards when comparing the 2 skin tests.

The diagnostic outcome of QFT was compared to Siiltibcy in terms of sensitivity. Likewise, the sensitivities of Siiltibcy and PPD were compared in concomitant administration participants using the pairing of measurements.

Some analyses involved the derivation of Cohen's kappa (κ) coefficient as a measure of concordance level.

Results

- Participant flows

TESEC-05

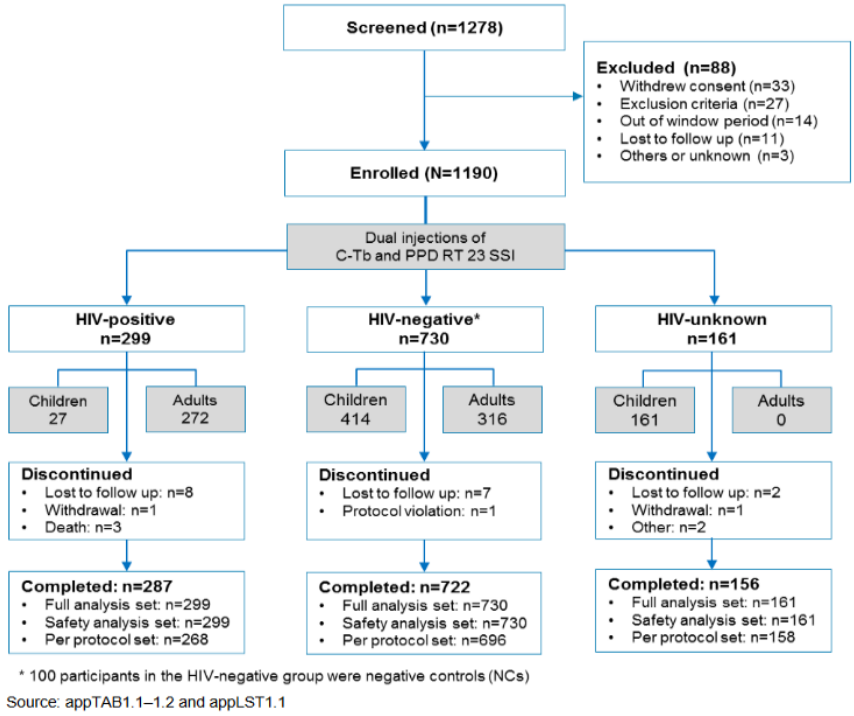

Figure 6

TESEC-06

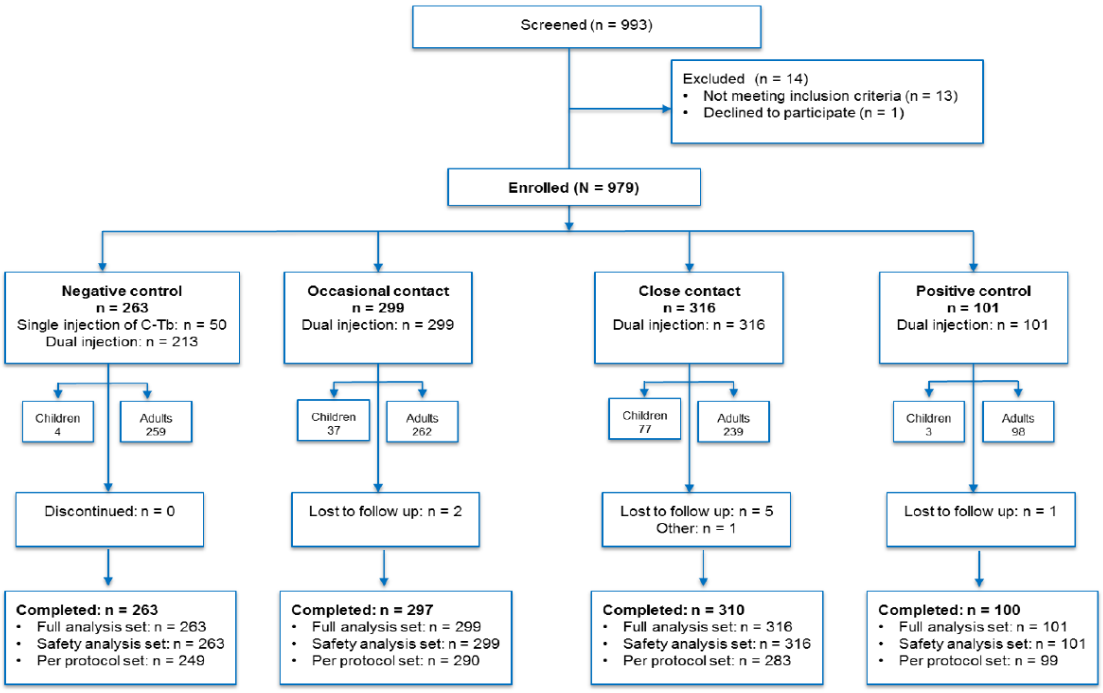

Figure 7

TESEC-07

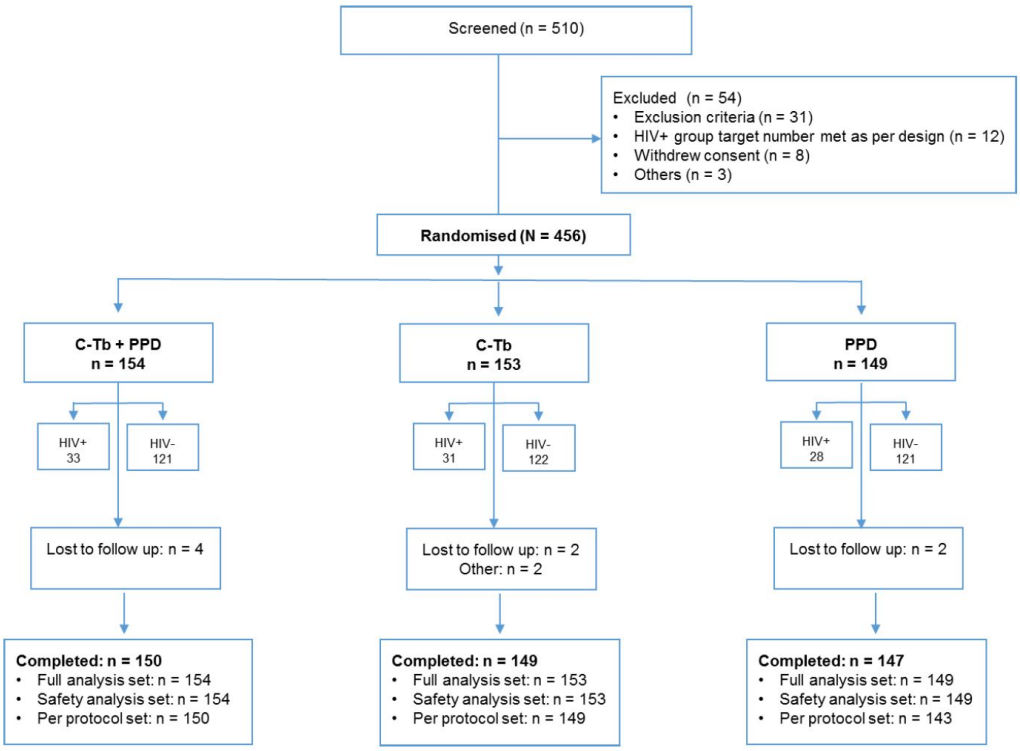

Figure 8

- Recruitment

TESEC-05, -06, -07: Start year for all the studies was 2012 and the end of enrolment was in 2014.

- Conduct of the studies

TESEC-05, -06, -07: a low percentage of major protocol deviations occurred; not impacting the quality of the study conduct. Two GCP violations occurred in as many study sites, in South Africa, described below.

In clinical trial TESEC-05, which took place in parallel to TESEC-07 (both carried out in sites located in South Africa), there was a GCP breach at one site (from here on referred to as Site A), a site participating in both TESEC-05 and TESEC-07 trials. The breach regarded a participant that was considered as 'lost to follow-up' when in fact the participant died due to natural causes. The case was evaluated by an appointed committee and data from the site were checked for validity at the blinded data review meeting. Therefore, it was considered that the data of Site A were valid and could be used to support the MAA. No further concerns were raised during the assessment regarding GCP compliance of Site A.

With their D121 responses, the applicant informed about another GCP critical issue on data verification (premature destruction of Investigator Site Files) from a different site (from here on referred to as Site B), again located in South Africa, enrolling subjects in studies TESEC-05 and TESEC-07. Site B was the lowest recruiting site and the subjects involved by the GCP breach were n = 187 (15% of the total) enrolled in TESEC-05 and n = 27 (6% of the total) enrolled in TESEC-07. In order to verify the impact of this GCP breach, further efficacy analyses excluding subjects impacted were requested to the applicant: the baseline characteristics of the subjects excluded from the analyses (i.e. coming from Site B) were similar to the

overall trial population, with not major differences considered able to substantially bias the results of the analysis conducted excluding the affected site. The results of the analysis excluding Site B showed, overall, no major differences between the sensitivity excluding and including the respective site (see clinical section).

Specificity results were not impacted by the GCP violation. No further concerns were raised during the assessment regarding GCP compliance of Site B.

Although South Africa study sites enrolled the majority of the subjects (1646 vs 979 in Spain) across pivotal studies, as clarified by EMA, there is a long and frequent GCP inspection experience in South Africa for centralised applications. Moreover, the SIILTICBY studies were carried out under the supervision of the sponsor Statens Serum Institut (Denmark) with knowledge on the EU GCP requirements. Although in both cases clinical studies TESEC-05 and -07 were impacted by GCP breach at sites A and B, considering the nature of the two GCP violations (particularly the one for Site B, not strictly critical for data anomaly), the existence of other clinical studies, and the limited impact in terms of subjects involved and efficacy outcome, no EU GCP inspection was deemed needed.

- Baseline data

Main demographic and baseline characteristics are listed below:

Table 7: TESEC-05

Demographic characteristics according to HIV status (FAS)

Characteristics		HIV-negative N=730	HIV-positive N=299	HIV-unknown N=161	Total N=1190
Age (year), n (%)	0–1	32 (4.4)	-	67 (41.6)	99 (8.3)
	2–4	44 (6.0)	-	93 (57.8)	137 (11.5)
	5–11	255 (34.9)	14 (4.7)	1 (0.6)	270 (22.7)
	12–17	83 (11.4)	13 (4.3)	-	96 (8.1)
	18–39	142 (19.5)	169 (56.5)	-	311 (26.1)
	40–65	174 (23.8)	103 (34.4)	-	277 (23.3)
Gender, n (%)	Female	336 (46.0)	176 (58.9)	77 (47.8)	589 (49.5)
	Male	394 (54.0)	123 (41.1)	84 (52.2)	601 (50.5)
Race, n (%)	African origin	503 (68.9)	283 (94.6)	123 (76.4)	909 (76.4)
	Other	227 (31.1)	16 (5.4)	38 (23.6)	281 (23.6)
BCG status, n (%)	Vaccinated	557 (76.3)	165 (55.2)	160 (99.4)	882 (74.1)
	Not vaccinated	153 (21.0)	111 (37.1)	-	264 (22.2)
	Unknown	20 (2.7)	23 (7.7)	1 (0.6)	44 (3.7)

N=number of participants in FAS; n(%)=number of participants (percentage) with observation; Source: appTAB1.3–1.5 and appLST3

Table 4 Demographic characteristics according to age groups (FAS)

Characteristics	Category/Age (years)	0–4* N=236	5–11 N=270	12–17 N=96	18–39 N=311	40–65 N=277	Total N=1190
Gender, n (%)	Female	114 (48.3)	137 (50.7)	49 (51.0)	160 (51.4)	129 (46.6)	589 (49.5)
	Male	122 (51.7)	133 (49.3)	47 (49.0)	151 (48.6)	148 (53.4)	601 (50.5)
BCG status, n (%)	Vaccinated	233 (98.7)	250 (92.6)	82 (85.4)	167 (53.7)	150 (54.2)	882 (74.1)
	Not vaccinated	1 (0.4)	20 (7.4)	14 (14.6)	117 (37.6)	112 (40.4)	264 (22.2)
	Unknown	2 (0.8)	-	-	27 (8.7)	15 (5.4)	44 (3.7)

*28 days–4 years; N=number of participants in FAS; n(%)=number of participants (percentage) with observation; Source: appTAB1.3–1.5.

Table 8: TESEC-06

Demographic characteristics (FAS)

Characteristics	Category	Negative N' = 263	Occasional N' = 299	Close N' = 316	Positive N' = 101	Total N = 979
Age	Mean (SD)	24.1 (7.9)	31.5 (14.3)	32.9 (17.7)	37.3 (11.2)	30.6 (14.5)
Gender, n (%)	Female	188 (71.5)	150 (50.2)	146 (46.2)	40 (39.6)	524 (53.5)
	Male	75 (28.5)	149 (49.8)	170 (53.8)	61 (60.4)	455 (46.5)
Race, n (%)	Other[1]	0	12 (4.0)	49 (15.5)	23 (22.8)	84 (8.6)
	White	263 (100.0)	287 (96.0)	267 (84.5)	78 (77.2)	895 (91.4)
BCG status, n (%)	No	154 (58.6)	167 (55.9)	151 (47.8)	37 (36.6)	509 (52.0)
	Unknown	1 (0.4)	31 (10.4)	52 (16.5)	20 (19.8)	104 (10.6)
	Yes	108 (41.1)	101 (33.8)	113 (35.8)	44 (43.6)	366 (37.4)

1=Other includes Asian and African origins; N=number of participants in FAS; N'=number of participants in each risk group; n (%)=number (%) of participants with observation; SD=standard deviation; Source: appTAB1.2, LST3

Table 9: TESEC-07

Demographic characteristics (FAS)

Characteristics	Category	C-Tb + PPD N' = 154	C-Tb N' = 153	PPD N' = 149	Total N = 456
Age	Mean (SD)	35.3 (11.5)	37.2 (12.7)	36.3 (11.8)	36.2 (12.0)
Gender, n (%)	Female	59 (38.3)	56 (36.6)	48 (32.2)	163 (35.7)
	Male	95 (61.7)	97 (63.4)	101 (67.8)	293 (64.3)
Race, n (%)	African origin	99 (64.3)	100 (65.4)	96 (64.4)	295 (64.7)
	Coloured	43 (27.9)	38 (24.8)	37 (24.8)	118 (25.9)
	Other[1]	12 (7.8)	15 (9.8)	16 (10.8)	43 (9.4)
BCG status, n (%)	Unvaccinated	50 (32.5)	43 (28.1)	45 (30.2)	138 (30.3)
	Vaccinated	94 (61.0)	97 (63.4)	93 (62.4)	284 (62.3)
	Unknown	10 (6.5)	13 (8.5)	11 (7.4)	34 (7.5)
HIV status, n (%)	Positive	33 (21.4%)	31 (20.3%)	28 (18.8%)	92 (20.2%)
	Negative	121 (78.6%)	122 (79.7%)	121 (81.2%)	364 (79.8%)

1=Other includes Afrikaans, Coloured, Mixed and Xhosa; N=number of participants in PP; N'=number of participants in each group; n (%)=number (percentage) of participants with observation; SD=standard deviation; Source: appTAB1.2, appLST3.0

- Numbers analysed

TESEC-05, -06, -07: The FAS and safety analysis set consisted of all 1190, 979, 456 respectively.

- Outcomes and estimation

TESEC-05

- Primary endpoint

Siiltibcy induration: the diameter (mm) of induration at the Siiltibcy injection site measured transversely to the long axis of the forearm at 2 to 3 days after intradermal administration of Siiltibcy

99.3% had a Siiltibcy induration reading, but 8 (1%) had missing Siiltibcy induration reading.

The overall Siiltibcy induration diameter mean (SD) was 9.4 (13.7) mm, ranging from 0 to 100 mm.

Criterion for defining 'Responders': participants who did respond to the skin tests with an induration diameter ≥ 1 mm for the respective agent Siiltibcy or PPD.

Criterion for defining 'Response rate': represent the percentage of participants tested who responded to the skin test with an induration ≥ 1 mm.

There were more Siiltibcy non-responders (55.8%) than there were Siiltibcy responders (44.2%).

The median induration among responders was 20 mm (range 1 – 100 mm).

Analysis of the Siiltibcy induration among all participants suspected of TB disease (excluding NC) did not show a significant statistical difference between females and males. However, the comparison of Siiltibcy indurations between BCG-vaccinated and unvaccinated participants showed a significant difference (Table 10 and 11).

Table 10: Linear analysis of CTb induration – Full Analysis Set minus NC

Contrast	Estimate	95% CI	P-Value
Age (per year)	0.184	(0.130, 0.238)	0.0000
Female-Male	1.228	(-0.373, 2.829)	0.1325
BCG Not vaccinated - BCG Unknown	-9.740	(-14.00,-5.483)	0.0000
BCG Not vaccinated - BCG Vaccinated	-5.073	(-7.123,-3.023)	0.0000
BCG Unknown - BCG Vaccinated	4.668	(0.553, 8.783)	0.0262
HIV Negative - Positive	4.971	(3.022, 6.920)	0.0000
HIV Negative - Unknown	4.894	(2.325, 7.463)	0.0002
HIV Positive - Unknown	-0.077	(-3.173, 3.019)	0.9610

Table 11: Siiltibcy induration according to age limited to responders

...3 C-Tb induration at V3 among responders according to age (FAS)

Population	Age group (year)	< 2	2–4	5–11	12–17	18–39	40–65
FAS	N	99	136	270	96	307	274
	Responders, n (%)	24 (24.2)	43 (31.6)	81 (30.0)	51 (53.1)	165 (53.7)	159 (58.0)
	Responders median, mm	10.5	18.0	20.0	23.0	20.0	20.0
	Responders mean (SD), mm	9.8 (7.5)	15.6 (8.3)	21.5 (12.0)	24.4 (17.2)	21.4 (12.0)	23.0 (13.7)
FAS minus NC	N	99	136	170	96	307	274
	Responders, n (%)	24 (24.2)	43 (31.6)	64 (37.6)	51 (53.1)	165 (53.7)	159 (58.0)
	Responders median, mm	10.5	18.0	20.5	23.0	20.0	20.0
	Responders mean (SD), mm	9.8 (7.5)	15.6 (8.3)	21.8 (12.7)	24.4 (17.2)	21.4 (12.0)	23.0 (13.7)

Response ≥ 1 mm; N=number of participants in with C-Tb induration reading; n(%)=number of participants (percentage) with observation; Source: appTAB2.3

Siiltibcy induration according to HIV status

The HIV status is divided into HIV-negative, HIV-positive and HIV-unknown. Note that 99.4% of HIV-unknowns were represented by children 28 days – 4 years of age.

The Siiltibcy responder rate was the highest among the HIV-negative compared to HIV-positive and HIV-unknown. The Siiltibcy induration median among responders was similar between HIV-negatives and HIV-positive. The same trend occurred when the NC group was left out.

Table 12

4 C-Tb induration at V3 according to HIV status (FAS)

Population	HIV status	HIV-negative	HIV-positive	HIV-unknown
FAS	N	725	296	161
	Responders, n (%)	349 (48.1)	127 (42.9)	47 (29.2)
	Responders median, mm	21.0	19.0	17.0
	Responders mean (SD), mm	22.4 (13.1)	20.4 (13.6)	14.2 (8.3)
FAS minus NC	N	625	296	161
	Responders, n (%)	332 (53.1)	127 (42.9)	47 (29.2)
	Responders median, mm	21.0	19.0	17.0
	Responders mean (SD), mm	22.5 (13.3)	20.4 (13.6)	14.2 (8.3)

Response ≥ 1 mm; N=number of participants in with C-Tb induration reading; n(%)=number of participants (percentage) with observation; Source: appTAB2.7

Analysis of C-Tb induration at V3 according to HIV status (FAS minus NC)

Contrast	Estimate	95% CI	P value
HIV-negative vs. HIV-positive	4.971	3.022, 6.920	<0.0001
HIV-negative vs. HIV-unknown	4.894	2.325, 7.463	0.0002

Contrast	Estimate	95% CI	P value
HIV-positive vs. HIV-unknown	-0.077	-3.173, 3.019	0.9610

P value derived from linear analysis; CI=confidence interval; Source: appTAB2.18

Apart from a decreased response rate of Siiltibcy for CD4+ count below 100 cells/µL, the Siiltibcy response rate seemed fairly constant irrespective of the CD4+ count.

Table 13

C-Tb induration at V3 according to CD4 count in HIV-positive responders (FAS)

CD4 count (cell/µL)	<100	100–199	200–299	300–399	400–499	500–599	>600
N	40	40	49	36	38	35	48
Responders, n (%)	9 (22.5)	18 (45.0)	25 (51)	17 (47.2)	17 (44.7)	19 (54.3)	18 (37.5)
Responders median, mm	15.0	17.0	16.0	25.0	19.0	20.0	19.5
Responders mean (SD), mm	13.6 (7.3)	18.2 (13.9)	21.2 (18.5)	23.6 (14.7)	19.7 (10.8)	19.8 (8.0)	22.4 (14.9)

Response ≥ 1 mm; N=number of participants with CD4 count; n(%)=number of participants with observation; SD=standard deviation; Source: appTAB2.26

Siiltibcy positivity measured at V3 (2 – 3 days after injection).

'Test-positive' for Siiltibcy represent the participants with an induration size ≥ 5 mm. For PPD, the cut-off corresponds to ≥ 15 mm for PPD for participants who were BCG-vaccinated with negative or unknown HIV status and ≥ 5 mm for all other participants.

476 (40.3%) participants were tested Siiltibcy positives with induration ≥ 5 mm. All induration ≥ 5 mm were considered Siiltibcy test-positives.

Analysis of the Siiltibcy induration among the test-positive TB suspects (excluding NC) did not show statistically significant difference between females and males. The comparison between BCG-statuses were statistically significantly different.

Siiltibcy positivity according to age

Siiltibcy test-positive rate showed a steady increase with age among the suspect TB participants, which may indicate an increasing risk of infection with age.

Table 14. C-Tb positivity at V3 according to age (FAS)

Population	Age (year)	< 2	2–4	5–11	12–17	18–39	40–65
FAS	N	99	136	270	96	307	274
	Positivity, n (%)	14 (14.1)	35 (25.7)	75 (27.8)	45 (46.9)	153 (49.8)	154 (56.2)
FAS minus NC	N	99	136	170	96	307	274
	Positivity, n (%)	14 (14.1)	35 (25.7)	58 (34.1)	45 (46.9)	153 (49.8)	154 (56.2)

N=number of participants with C-Tb induration; n(%)=number of participants (percentage) with observation; Source: appTAB2.3

Siiltibcy positivity according to HIV status

The Siiltibcy positive rate was the highest among the HIV-negative group and lowest among the HIV-unknown group. The same trend occurred when the NC participants were taken out.

It should be noted that HIV-unknown group was represented by children ≤ 5 years of age.

Table 15. C-Tb positivity at V3 according to HIV status (FAS)

Population	HIV status	HIV-negative	HIV-positive	HIV-unknown
FAS	N	725	296	161
	Positivity, n (%)	327 (45.1)	113 (38.2)	36 (22.4)
FAS minus NC	N	625	296	161
	Positivity, n (%)	310 (49.6)	113 (38.2)	36 (22.4)

N=number of participants in FAS; n(%)=number of participants (percentage) with observation; Source: appTAB2.7

Analysis of Siiltibcy positivity among participants suspected TB (excluding NC) showed significant statistical difference among HIV-negative participants having double the odds of being Siiltibcy-positive compared to HIV-positive participants and those with unknown HIV status.

Siiltibcy positivity according to CD4+ count

109 (37.7% of the HIV-positive with CD4 count value) of the total HIV-positive participants were Siiltibcy test-positives. Apart from a low positivity rate of Siiltibcy for CD4 counts below 100 cells/µL, the Siiltibcy response rate and induration median seemed fairly constant irrespective of the CD4 count.

Table 16 **2 C-Tb positivity at V3 according to CD4 count (FAS)**

CD4 count (cell/µL)	<100	100–199	200–299	300–399	400–499	500–599	>600
N	40	40	49	36	38	35	48
Positivity, n (%)	7 (17.1)	16 (40.0)	20 (40.8)	15 (39.5)	16 (42.1)	19 (54.3)	16 (33.3)

N=number of participants in CD4 count group; n(%)=number of participants (percentage) with observation; Source: appLST8.1

Secondary objectives

The secondary objectives were to evaluate the:
- diagnostic performance of PPD and QFT (see Clinical AR).
- sensitivity and specificity of Siiltibcy vs. QFT and Siiltibcy vs. PPD
- diagnostic outcome comparison of Siiltibcy vs. PPD and QFT using the latent class model

Sensitivity

Sensitivity (also known as the true positive rate) is defined as the positivity rate among individuals who has the condition (here being Mtb-infected).

The paired McNemar test was used to assess differences in positivity and the kappa coefficient (κ) with 95% CI was used to present the degree of agreement within Siiltibcy vs. PPD and Siiltibcy vs. QFT.
The estimated sensitivity in this context was defined as the relative frequency of participants meeting the positivity cut-off values among the confirmed TB diagnosis cases (TBC). All TBC cases were confirmed by either a sputum smear microscopy, culture, or PCR / Gene X-pert.

A total of 127 (10.7%) participants were newly diagnosed with TB during the trial, of which 75 were confirmed by laboratory means (i.e., TBC), 50 were diagnosed by clinical evaluations, and 2 were diagnosed by unspecified methods and thus were not included in the analysis. These two subjects were Siiltibcy and PPD non-responders and their QFT results were either negative or indeterminate. Thus, the combined number of newly diagnosed (i.e., TBD) was 125 (75 confirmed and 50 with TB symptoms).

Siiltibcy vs. PPD sensitivity
The sensitivity of the Siiltibcy and PPD was analysed in TBC alone (n = 75) and all newly diagnosed participants, i.e., TBD, (n = 125). The sensitivity of Siiltibcy was comparable to that of PPD.

Table 17 **3 C-Tb vs. PPD sensitivity at V3 (FAS)**

TBC		C-Tb +	C-Tb -	Σ		
PPD	+	49	9	58	C-Tb sensitivity: 72.0%	P value = 0.4240
	-	5	12	17	PPD sensitivity: 77.3%	
Σ		54	21	75		
TBD		C-Tb +	C-Tb -	Σ		
PPD	+	84	18	102	C-Tb sensitivity: 74.4%	P value = 0.1221
	-	9	14	23	PPD sensitivity: 81.6%	
Σ		93	32	125		

TBC=TB confirmed by laboratory means; TBD=TBC and TB diagnosed by clinical symptoms; P value derived from McNemar exact test; Test-positive=PPD ≥ 15 mm for BCG-vaccinated with negative/unknown HIV status and ≥ 5 mm all others; Source appTAB2.42

The small difference was not statistically significant (p > 0.05). The agreement between the 2 tests was moderate (κ=0.5084 [CI 0.2863; 0.7305]) among the TBC population and fair (κ=0.3753 [CI 0.1864; 0.5643]) among TBD participants (i.e. TBD).

Table 18 C-Tb vs. PPD sensitivity at V3 according to age (FAS)

TBC	0–17		C-Tb +	C-Tb -	Σ	
	PPD	+	10	0	10	C-Tb sensitivity: 86.7% / PPD sensitivity: 66.7% — P value = 0.2500
		-	3	2	5	
	Σ		13	2	15	
	18–65		C-Tb +	C-Tb -	Σ	
	PPD	+	39	9	48	C-Tb sensitivity: 68.3% / PPD sensitivity: 80.0% — P value = 0.0654
		-	2	10	12	
	Σ		41	19	60	
TBD	0–17		C-Tb +	C-Tb -	Σ	
	PPD	+	19	6	25	C-Tb sensitivity: 75.8% / PPD sensitivity: 75.8% — P value = 1.0000
		-	6	2	8	
	Σ		25	8	33	
	18–65		C-Tb +	C-Tb -	Σ	
	PPD	+	65	12	77	C-Tb sensitivity: 73.9% / PPD sensitivity: 83.7% — P value = 0.0352
		-	3	12	15	
	Σ		68	24	92	

TBC=TB confirmed by laboratory means; TBD=TBC and TB diagnosed by clinical symptoms; P value derived from McNemar exact test; Test-positive=PPD ≥ 15 mm for BCG-vaccinated with negative/unknown HIV status and ≥ 5 mm all others; Source: appTAB2.42

Siiltibcy seemed to have a higher sensitivity than PPD among children in TBC and a lower sensitivity among adults but the number were too few to show a statistical difference (p > 0.05). The level of agreements between the 2 skin tests were moderate, k=0.3753.

The sensitivity of Siiltibcy approached 75% when all TB diagnosed participants (TBD) were included.

Table 19 .J C-Tb vs. PPD sensitivity at V3 according to HIV status (FAS)

TBC	HIV neg		C-Tb +	C-Tb -	Σ	
	PPD	+	26	5	31	C-Tb sensitivity: 75.0%
		-	4	5	9	PPD sensitivity: 77.5%
	Σ		30	10	40	
	HIV pos		C-Tb +	C-Tb -	Σ	
	PPD	+	22	4	26	C-Tb sensitivity: 67.7%
		-	1	7	8	PPD sensitivity: 76.5%
	Σ		23	11	34	
	HIV unk		C-Tb +	C-Tb -	Σ	
	PPD	+	1	0	1	C-Tb sensitivity: 100.0%
		-	0	0	0	PPD sensitivity: 100.0%
	Σ		1	0	1	
TBD	HIV neg		C-Tb +	C-Tb -	Σ	
	PPD	+	46	10	56	C-Tb sensitivity: 77.6%
		-	6	5	11	PPD sensitivity: 83.6%
	Σ		52	15	67	
	HIV pos		C-Tb +	C-Tb -		
	PPD	+	30	4	34	C-Tb sensitivity: 70.5%
		-	1	9	10	PPD sensitivity: 77.3%
	Σ		31	13	44	
	HIV unk		C-Tb +	C-Tb -		
	PPD	+	8	4	12	C-Tb sensitivity: 71.4%
		-	2	0	2	PPD sensitivity: 85.7%
	Σ		10	4	14	

TBC	P value = 1.0000
	P value = 0.3750
	-
TBD	P value = 0.4545
	P value = 0.3750
	P value = 0.6875

TBC=TB confirmed by laboratory means; TBD=TBC and TB diagnosed by clinical symptoms; P value derived from McNemar exact test; Test-positive=PPD ≥ 15 mm for BCG-vaccinated with negative/unknown HIV status and ≥ 5 mm all others; Source: appTAB2.42

In the TBC population, the sensitivity comparison between Siiltibcy and PPD did not show a statistical difference among the HIV-negative and HIV-positive participants. The level of agreement between the 2 skin tests was fair to moderate. In the TBD population, the sensitivity of Siiltibcy and PPD was slightly higher.

Siiltibcy vs. QFT sensitivity

The sensitivity of the Siiltibcy and QFT was analysed in the TBC (n = 56) and TBD (n = 50) populations. Similar to the comparison between Siiltibcy and PPD, there was no major difference in the sensitivity between Siiltibcy vs. QFT ($p > 0.05$); the agreement level between the 2 tests was moderate (κ=0.5632 [CI 0.3238; 0.8026]) among the TBC population and fair among all when TB diagnosed participants were included (κ=0.4132 [CI 0.1935; 0.6329]).

Table 20

Siiltibcy		QFT		Difference in sensitivity
n	Sensitivity in % (95% CI)	n	Sensitivity in % (95% CI)	Siiltibcy – QFT in % (95% CI)
75	72.0 (61.8; 82.2)	70	58.6 (47.0; 70.1)	13.4 (-3.3; 30.2)

Siiltibcy showed similar sensitivities to QFT in children as well as in adult irrespective the comparison was based on TBC or TBD.

Table 21

2 C-Tb vs. QFT sensitivity according to age (FAS)

			C-Tb +	C-Tb -	Σ		
TBC	0–17	QFT +	9	0	9	C-Tb sensitivity: 81.8%	P value = 1.0000
		QFT -	0	2	2	QFT sensitivity: 81.8%	
		Σ	9	2	11		
	18–65	QFT +	26	6	32	C-Tb sensitivity: 66.7%	P value = 0.7539
		QFT -	4	9	13	QFT sensitivity: 71.1%	
		Σ	30	15	45		
TBD	0–17	QFT +	10	1	11	C-Tb sensitivity: 76.9%	P value = 1.0000
		QFT -	0	2	2	QFT sensitivity: 84.6%	
		Σ	10	3	13		
	18–65	QFT +	46	8	54	C-Tb sensitivity: 75.7%	P value = 0.8145
		QFT -	10	10	20	QFT sensitivity: 73.0%	
		Σ	56	18	74		

C-Tb reading at V3; QFT result at V2; TBC=TB confirmed by laboratory means; TBD=TBC and TB diagnosed by clinical symptoms; P value derived from McNemar exact test; Source: appTAB2.43

In the TBC population, the sensitivity of Siiltibcy and QFT did not show a statistical difference among the HIV-positive participants (69.6% vs. 65.2% respectively). QFT sensitivity was about 10%-point higher than Siiltibcy sensitivity among HIV-negative participants; this was however not significant ($p > 0.05$) (Table below) Both analyses showed a fair to moderate agreement between the 2 skin tests.

Siiltibcy and QFT sensitivity improved when all TB diagnosed participants were included.

Table 22

.3 C-Tb vs. QFT sensitivity according to HIV status (FAS)

			C-Tb +	C-Tb -	Σ		
TBC	HIV neg	QFT +	22	4	26	C-Tb sensitivity: 69.7%	P value = 0.3750
		QFT -	1	6	7	QFT sensitivity: 78.8%	
		Σ	23	10	33		
	HIV pos		C-Tb +	C-Tb -	Σ		
		QFT +	13	2	15	C-Tb sensitivity: 69.6%	P value = 1.0000
		QFT -	3	5	8	QFT sensitivity: 65.2%	
		Σ	16	7	23		
TBD	HIV neg		C-Tb +	C-Tb -	Σ		
		QFT +	38	7	45	C-Tb sensitivity: 75.0%	P value = 0.5488
		QFT -	4	7	11	QFT sensitivity: 80.4%	
		Σ	42	14	56		
	HIV pos		C-Tb +	C-Tb -	Σ		
		QFT +	18	2	20	C-Tb sensitivity: 77.4%	P value = 0.2891
		QFT -	6	5	11	QFT sensitivity: 64.5%	
		Σ	24	7	31		

C-Tb reading at V3; QFT result at V2; TBC=TB confirmed by laboratory means; TBD=TBC and TB diagnosed by clinical symptoms; P value derived from McNemar exact test; Source: appTAB2.43

Specificity

Specificity (also known as true negative rate) is estimated by the rate of test-negativity of a diagnostic test among individuals who do not have the condition.

Siiltibcy vs. PPD specificity

Both skin tests showed a specificity above 80%, which was not significantly different. The agreement between the 2 tests was good with a κ=0.7026 [CI 0.5097; 0.8956]). 12% of the NC participants were positive with both tests indicating a high prevalence of Mtb infections even among young children 5 – 11 years of age from a high endemic area.

Table 23

.5 C-Tb vs. PPD specificity at V3 (FAS)

NC		C-Tb +	C-Tb -	Σ		
PPD	+	12	3	15	C-Tb specificity: 83.0%	P value = 0.7266
	-	5	80	85	PPD specificity: 85.0%	
Σ		17	83	100		

Test-positive=PPD ≥ 15 mm for BCG-vaccinated with negative/unknown HIV status and ≥ 5 mm all others; P value derived from McNemar exact test; Source: appTAB2.44

The diagnostic outcomes of Siiltibcy and PPD tests were cross-tabulated for NC participants with non-missing data against children of the same age suspected of TB disease with non-missing data.
Both skin tests showed a higher negativity rate among the NC group than among the suspected TB participants (Non-NC group).

Table 24

.4 C-Tb and PPD outcome at V3 in children age 5–11 years old (FAS)

Skin test	Contrast	NC	Non-NC	P value
C-Tb	N	100	170	-
	Negativity, n (%)	83 (83.0)	112 (65.9)	-
	Positivity, n (%)	17 (17.0)	58 (34.1)	-
	Fishers test	-	-	0.0030
	Kolmogorov-Smirnov test	-	-	0.0093
	Wilcoxon test	-	-	0.0005
PPD	N	100	170	-
	Negativity, n (%)	85 (85.0)	119 (70.0)	-
	Positivity, n (%)	15 (15.0)	51 (30.0)	-
	Fisher test	-	-	0.0055
	Kolmogorov-Smirnov test	-	-	0.0854
	Wilcoxon test	-	-	0.0096

Test-positive=PPD ≥ 15 mm for BCG-vaccinated with negative/unknown HIV status and ≥ 5 mm for all others; N=number of participants in the subgroup; n(%)=number of participants (percentage) with observation; Source: appTAB2.15

Siiltibcy vs. QFT specificity

Table 25

Siiltibcy		QFT		Difference in specificity
n	Specificity in % (95% CI)	n	Specificity in % (95% CI)	Siiltibcy – QFT in % (95% CI)
100	83.0 (75.6; 90.4)	98	71.4 (62.5; 80.4)	11.6 (-1.0; 24.2)

The diagnostic outcomes of Siiltibcy and QFT tests were cross-tabulated for NC participants with non- missing data against children of the same age suspected of TB disease with non-missing data.
Both tests had about a 16%-points higher negativity rates among the NC group than the suspected TB participants. The difference between the 2 groups were statistically significant via the Fishers test.

Table 26: QFT IFN-y values by risk group for TESEC-05

	Negative Control (N=100)	Suspected TB (N=1090)*	Total (N=1190)
QFT IFN-y value (IU/mL)			
n	97	782	879
Mean (SD)	0.5 (1.92)	1.1 (2.16)	1.0 (2.14)
Median	0.04	0.16	0.13
Min; Max	-4.4; 9.2	-5.1; 9.9	-5.1; 9.9

*75 subjects with TB confirmed during the trial are included

TESEC-06

The primary endpoint was positivity of each trial participant as evaluated by the Siiltibcy induration at day 2 – 3 (Visit 3 [V3]) in conjunction with the cut-off value = 5 mm.

Siiltibcy positivity rate increased with increasing exposure to Mtb (Table below). The positivity rates ranged between 3.4% and 68.0% among the 4 risk groups (Table 27 below).

Table 27: Siiltibcy, PPD, and QFT Positivity Rates (TESEC-06)

Test	Positivity Rates	NC Group	Occasional Contact Group	Close Contact Group	PC Group
Siiltibcy	N	263	299	316	101
	Positivity, n (%)	9 (3.4)	49 (16.4)	136 (43.0)	68 (68.0)
PPD	N	213	299	316	100
	Positivity, n (%)	14 (6.6)	57 (19.1)	140 (44.3)	81 (81.0)
QFT	N	263	284	288	101
	Positivity, n (%)	10 (3.8)	57 (20.1)	122 (42.4)	82 (81.2)

Source: TESEC-06 CSR, Table 11-1 and Table 11-5
CSR = clinical study report; N = number of analysed subjects in each risk group; n (%) = number (percentage) of subjects who were Siiltibcy/PPD/QFT positive; NC = negative control; PC = positive control; PPD = tuberculin purified protein derivative RT 23 SSI; QFT = QuantiFERON®-TB Gold In-Tube Test

The main secondary endpoints are listed in the following section.

The sensitivity was positivity rate in the Positive Control group.

Siiltibcy vs. QFT sensitivity

The objective was to evaluate the difference in sensitivity between Siiltibcy and QFT among TB patients in the Positive Control group.

The sensitivity of Siiltibcy was significantly (McNemar P < 0.01) lower than that of QFT in the Positive Control group.

Table 28: Sensitivity of Siiltibcy vs. QFT (Positive Controls)

		C-Tb +	C-Tb -	Σ		
QFT	+	64	18	82	C-Tb sensitivity: 68.0%	P = 0.0043
	-	4	14	18	QFT sensitivity: 82.0%	
Σ		68	32	100		

Note: Participants with QFT indeterminate values (n=4) were not included in this analysis; C-Tb was measured at V3 and QFT at V2; P value was calculated using McNemar test; Source: appTAB2.35

Siiltibcy vs. PPD sensitivity

The objective was to evaluate the difference in sensitivity between Siiltibcy and PPD in Positive Control group.

The sensitivity of Siiltibcy was significantly P < 0.05) lower than that of PPD in the Positive Control group Table 29 below).

Table 29

7 Sensitivity of C-Tb vs. PPD - V3 (Positive Controls)

		C-Tb +	C-Tb -	Σ		
PPD	+	62	19	81	C-Tb sensitivity: 68.0%	P = 0.0146
	-	6	13	19	PPD sensitivity: 81.0%	
Σ		68	32	100		

PPD cut-off: BCG-vaccinated and HIV-negative/unknown participants: ≥ 15 mm, all other participants: ≥ 5 mm; P value was calculated using McNemar test; Source: appTAB2.33

The specificity of Siiltibcy was similar to that of QFT (96.6 vs 96.2%, p = 1.00) and to that of PPD (95.8 vs 93.4%, p = 0.1797) in the Negative Controls.

The performance of Siiltibcy was similar to that of PPD and QFT also in the other risk groups enrolled in TESEC-06: close and occasional contacts. In particular, no significant difference in positivity between Siiltibcy and QFT was observed among Occasional Contact group and the Close Contact group as shown in the next table 30.

Table 30: Siiltibcy vs. QFT diagnostic tests (FAS)

		C-Tb +	C-Tb -	Σ		
QFT	+	43	14	57	C-Tb positivity: 17.3%	P = 0.1153
	-	6	221	227	QFT positivity: 20.1%	
Σ		49	235	284		

Occasional Contact group

		C-Tb +	C-Tb -	Σ		
QFT	+	113	9	122	C-Tb positivity: 43.8%	P = 0.5235
	-	13	153	166	QFT positivity: 42.4%	
Σ		126	162	288		

Close Contact group

Note: QFT indeterminate (n = 4) values were not included in this analysis; Note: C-Tb was measured at V3 and QFT at V2; C-Tb cut-off ≥ 5 mm; Σ=sum; P values were calculated using McNemar test; Source: appTAB2.35

The positivity rate of Siiltibcy was not significantly different from the positivity rate of PPD in close and occasional contacts as shown in the table 31 below.

Table 31: Siiltibcy vs. PPD diagnostic tests - V3 (FAS)

		C-Tb +	C-Tb -	Σ		
PPD	+	42	15	57	C-Tb positivity: 16.4%	P = 0.1338
	-	7	235	242	PPD positivity: 19.1%	
Σ		49	250	299		

Occasional Contact group

		C-Tb +	C-Tb -	Σ		
PPD	+	118	22	140	C-Tb positivity: 43.0%	P = 0.6358
	-	18	158	176	PPD positivity: 44.3%	
Σ		136	180	316		

Close Contact group

Σ=sum; PPD cut-off: BCG-vaccinated and HIV-negative/unknown participants: ≥ 15 mm, all other participants: ≥ 5 mm; P values were calculated using McNemar test; Source: appTAB2.33

BCG-vaccinated participants

Among BCG-vaccinated participants in the Negative Control group, Siiltibcy showed a significantly lower response rate of 7.4% compared to PPD (54.6%) 2 – 3 days after injections (V3) among the Negative Controls (Table 32 below).

Table 32: Response rates in BCG-vaccinated participants - V3 (Negative Controls)

		C-Tb +	C-Tb -	Σ		
PPD	+	6	53	59	C-Tb response rate: 7.4%	P < 0.0001
	-	2	47	49	PPD response rate: 54.6%	
Σ		8	100	108		

C-Tb and PPD responders ≥ 1 mm; P value was calculated using McNemar test; Source: appTAB2.29

Among BCG-vaccinated participants in the Negative Control group, the specificity of Siiltibcy (96.3%) was significantly higher than that of PPD6 (66.7%) as shown in the table 33 below.

Table 33: Specificity of Siiltibcy vs. PPD6 in BCG-vaccinated - V3 (Negative Controls)

		C-Tb +	C-Tb -	Σ		
PPD	+	3	33	36	C-Tb specificity: 96.3%	P < 0.0001
	-	1	71	72	PPD6 specificity: 66.7%	
Σ		4	104	108		

Σ=sum; PPD6 cut-off: ≥ 6 mm fixed for all participants; Source: appTAB2.32

The following table 34 shows the induration diameter with Siiltibcy and PPD in the risk group.

Table 34

4 Magnitude of C-Tb and PPD indurations and response rates (FAS)

Diagnostic test		Negative N = 263	Occasional N = 299	Close N = 316	Positive N = 101
C-Tb	Responder diameter median (min, max), mm	6.0 (1.0, 55.0)	20.0 (2.0, 65.0)	20.0 (3.0, 46.0)	17.0 (4.0, 35.0)
	Responder mean (SD), mm	15.2 (16.5)	18.7 (10.1)	20.1 (7.9)	17.7 (6.4)
	Response rate, n (%)	14 (5.3)	54 (18.1)	139 (44.0)	69 (69.0)
PPD	Responder diameter median (min, max), mm	7.0 (1.0, 35.0)	14 (2.0, 55.0)	17.0 (3.0, 48.0)	17.0 (2.0, 25.0)
	Responder mean (SD), mm	9.5 (7.6)	13.9 (8.4)	16.9 (7.3)	15.8 (5.3)
	Response rate, n (%)	65 (30.5)	90 (30.1)	169 (53.5)	91 (91.0)

N=number of participants in each risk group; n(%)=number (percentage) of participants who were test positive; C-Tb/PPD response=induration diameter ≥ 1 mm; SD=Standard deviation; Source: appTAB2.1

Table 35: QFT IFN-y values by risk group for TESEC-06

	Negative Control (N=263)	Occasional Contact (N=299)	Close Contact (N=316)	Positive Control (N=101)	Total (N=979)
QFT IFN-y value (IU/mL)					
n	263	286	290	101	940
Mean (SD)	0.2 (1.10)	1.3 (2.99)	2.5 (4.11)	4.0 (4.55)	1.7 (3.45)
Median	0.06	0.08	0.24	2.02	0.10
Min; Max	0.0; 11.6	0.0; 16.4	0.0; 19.2	0.1; 19.8	0.0; 19.8

Source: Table 14.1.3.1 (t_ema_qft_06) 08Dec2023

TESEC-07

The assessment of efficacy mainly concerned the induration size and positivity rate of Siiltibcy and PPD when injected alone or concomitantly. A Siiltibcy and PPD 'responder' was defined as an individual with an induration diameter ≥ 1 mm at day 2 – 3 (V3). The "responder rates" for Siiltibcy and PPD were defined as the prevalence of responders, in a given subgroup of the trial population.

The 'test-positive' participant was defined as an individual with an observation at day 2 – 3 (V3) after the injection above the cut-off value of:

- Siiltibcy: induration diameter ≥ 5 mm
- PPD6: induration diameter ≥ 6 mm
- QFT: outcomes derived based on manufacture algorithm involving the following 3 components: Tb - Antigen, NIL and Mitogen
- PPD5/10: induration diameter ≥ 5 mm for HIV-positive participants and ≥ 10 mm for all others
- PPD5/15: induration diameter ≥ 5 mm for HIV-positive participants and ≥ 15 mm for all others.

The 'test-positivity' rate was defined as the prevalence of test positives, in a given subgroup of the trial population of interest at the time of test reading (V3, 2 – 3 days after the injection). During this trial, 153 participants received a single injection of Siiltibcy and 149 received a single injection of PPD and 154 received concomitant injections.

Since PPD was assessed using varying cut-off in this section, for convenience, the presentation of the term 'PPD' was done along with the cut-off used when test-positivity results are presented (PPD6, PPD5/10 and PPD5/15).

The primary analysis presented in this report are based on the FAS and PP sets.

Primary endpoint

The median induration diameters of Siiltibcy and PPD were unaffected when injected alone or concomitantly as shown in the table 36 below.

Table 36: Siiltibcy and PPD induration sizes: single and concomitant – V3 (FAS)

Skin test	Population	Response	Concomitant	Single
C-Tb	Overall	N	154	153*
		Median diameter, mm	20.0	19.0
		Min, Max, mm	0, 94.0	0, 115.0
		Mean (SD) diameter, mm	18.5 (14.0)	19.2 (16.4)
	Responders	Median diameter, mm	21.0	21.0
		Min, Max, mm	3.0, 94.0	5.0, 115.0
		Mean (SD), mm	23.3 (11.6)	24.8 (14.5)
PPD	Overall	N	154	149
		Median diameter, mm	20.0	20.0
		Min, Max, mm	0, 90.0	0, 64.0
		Mean (SD) diameter, mm	20.7 (11.0)	20.9 (10.4)
	Responders	Median diameter, mm	20.0	20.0
		Min, Max, mm	3.0, 90.0	4.0, 64.0
		Mean (SD) diameter, mm	21.9 (10.1)	22.2 (9.2)

*Two participants did not have observation; N=number of participants in each group; n(%)=number (percentage) of participants with observation; C-Tb/PPD responder=induration diameter ≥ 1 mm; SD=Standard deviation; Source: appTAB2.1

The Linear analysis model showed that the estimated overall mean of Siiltibcy induration diameters was comparable between the single and concomitant injections groups (19.23 vs. 18.48) in the FAS (P > 0.05; Table 37 below).

Table 37: Linear analysis of Siiltibcy: single vs. concomitant injections (FAS)

Comparison	Variable	N	Estimate	Standard error	SD	95% CI	P value
Single	Mean values	151	19.23	1.24	16.43	16.79, 21.67	< 0.0001
Concomitant	Mean values	154	18.48	1.23	13.99	16.06, 20.90	< 0.0001
Single vs. Concomitant	Linear analysis	-	0.75	1.75	-	-2.69, 4.19	0.6674
Single vs. Concomitant	Hodges-Lehmann	-	0	-	-	-2.00, 2.00	0.9995

CI=confidence interval; N=number of participants in each group with observation; SD=standard deviation; Source: appTAB2.9

A similar observation was made with PPD, where PPD injected alone or concomitantly with Siiltibcy did not affect (P > 0.05) the mean induration diameters (20.90 vs. 20.72; Table 38 below).

Table 38: Linear analysis of PPD: single vs. concomitant injections (FAS)

Comparison	Variable	N	Estimate	Standard error	SD	95% CI	P value
Single	Mean values	149	20.90	0.88	10.35	19.18, 22.62	< 0.0001
Concomitant	Mean values	154	20.72	0.86	11.00	19.03, 22.42	< 0.0001
Single vs. Concomitant	Linear analysis	-	0.18	1.23	-	-2.24, 2.59	0.8845
Single vs. Concomitant	Hodges-Lehmann	-	0	-	-	-1.00, 2.00	0.7712

CI=confidence interval; N=number of participants in each group with observation; SD=standard deviation; Source: appTAB2.21

Siiltibcy and PPD6 sensitivities: single vs. concomitant

The objective was to assess if Siiltibcy and PPD6 sensitivities are influenced by concomitant injections of both skin tests compared to if Siiltibcy and PPD are injected alone. Sensitivity was defined as the probability that the Siiltibcy and the PPD6 test positive, given the participant has microbiologically confirmed Mtb infection. All participants in this trial had active TB and therefore the positivity rate represented the sensitivity.

Overall, the sensitivity of Siiltibcy and PPD6 were similar in the single and concomitant injections groups. However, the sensitivity of both Siiltibcy and PPD6 in the HIV-positive group was lower than in the HIV-negative group (Table 39 below).

Table 39: Siiltibcy Sensitivity: single vs. concomitant according to HIV status (FAS)

Skin test (HIV status)	Test result	Concomitant	Single
C-Tb (All)	N	154	151
	Positive, n (%)	121 (78.6)	117 (77.5)
	Negative, n (%)	33 (21.4)	34 (22.5)
C-Tb (HIV-positive)	N	33	31
	Positive, n (%)	22 (66.7)	22 (71.0)
	Negative, n (%)	11 (33.3)	9 (29.0)
C-Tb (HIV-negative)	N	121	120
	Positive, n (%)	99 (81.8)	95 (79.2)
	Negative, n (%)	22 (18.2)	25 (20.8)

N=number of participants who received C-Tb and had a reading; n(%)=number (percentage) of participants with observation; C-Tb positivity=induration diameter ≥ 5 mm; Source: appTAB2.1

The sensitivity of PPD6 in HIV-negative participants who received concomitant injections was 100% (Table 40 below).

Table 40: PPD6 Sensitivity: single vs. concomitant according to HIV status (FAS)

Skin test (HIV status)	Test result	Concomitant	Single
PPD6 (All)	N	154	149
	Positive, n (%)	145 (94.2)	139 (93.3)
	Negative, n (%)	9 (5.8)	10 (6.7)
PPD6 (HIV-positive)	N	33	28
	Positive, n (%)	24 (72.7)	23 (82.1)
	Negative, n (%)	9 (27.3)	5 (17.9)
PPD6 (HIV-negative)	N	121	121
	Positive, n (%)	121 (100.0)	116 (95.9)
	Negative, n (%)	0	5 (4.1)

N=number of participants who received PPD and had a reading; n(%)=number (percentage) of participants with observation; PPD6 positivity=induration diameter ≥ 6 mm; Source: appTAB2.1

Sensitivity: Siiltibcy vs. QFT

The objective was to evaluate the difference in sensitivity between Siiltibcy and QFT. Participants with indeterminate QFT results were not included in this analysis.

The sensitivity of Siiltibcy versus that of QFT among all participants and also among those who were HIV-positive and HIV-negative is shown in the table below. The agreement between the 2 tests was fair among all participants (κ = 0.285 [95% CI: 0.127, 0.443]), among HIV-positives (κ = 0.261 [95% CI: -0.047, 0.569]) and among HIV-negatives (κ = 0.293 [95% CI: 0.110, 0.476]).

Table 41: Sensitivity of Siiltibcy vs. QFT (PP)

			C-Tb +	C-Tb -	Σ		
All	QFT	+	172	24	196	C-Tb sensitivity: 83.2%	P = 0.7660
		-	21	15	36	QFT sensitivity: 84.5%	
	Σ		193	39	232		
			C-Tb +	C-Tb -	Σ		
HIV+	QFT	+	35	8	43	C-Tb sensitivity: 76.5%	P = 0.3877
		-	4	4	8	QFT sensitivity: 84.3%	
	Σ		39	12	51		
			C-Tb +	C-Tb -	Σ		
HIV-	QFT	+	137	16	153	C-Tb sensitivity: 85.1%	P = 1.0000
		-	17	11	28	QFT sensitivity: 84.5%	
	Σ		154	27	181		

Note: C-Tb was measured at V3 (2–3 days after injection) and QFT at V2 (day 0); P value was calculated using McNemar test; Source: appTAB2.34

Table 42: Sensitivity of QFT with and without indeterminate results

Study	Handling of indeterminate results	QFT sensitivity % (95% CI)
TESEC-05	Including QFT indeterminates	60.6 (48.8; 72.4)
	Excluding QFT indeterminates	76.9 (65.5; 88.4)
TESEC-06 (no indeterminate results for QFT in positive control group)	Including QFT indeterminates	81.2 (73.6; 88.8)
	Excluding QFT indeterminates	81.2 (73.6; 88.8)
TESEC-07	Including QFT indeterminates	69.7 (65.3; 74.1)
	Excluding QFT indeterminates	86.6 (82.9; 90.2)

Site B subjects from TESEC-05 and TESEC-07 have been excluded.

Sensitivity: Siiltibcy vs. PPD6

The objective was to evaluate the difference in sensitivity between Siiltibcy and PPD6. The sensitivity of Siiltibcy was significantly lower ($P < 0.01$) than that of PPD6 among all participants. There was no significant difference ($P > 0.05$) in sensitivity between Siiltibcy and PPD6 among HIV-positives (see table below). The agreement between the 2 tests was fair among all participants ($\kappa = 0.264$ [95% CI: 0.088, 0.440]) and moderate among HIV-positives ($\kappa = 0.566$ [95% CI: 0.260, 0.871]).

Table 43: Sensitivity of Siiltibcy vs. PPD6 – V3 (PP)

			C-Tb +	C-Tb -	Σ		
All	PPD6	+	115	26	141	C-Tb sensitivity: 78.0%	P < 0.0001
		-	2	7	9	PPD6 sensitivity: 94.0%	
	Σ		117	33	150		
			C-Tb +	C-Tb -	Σ		
HIV+	PPD6	+	19	4	23	C-Tb sensitivity: 65.6%	P = 0.6875
		-	2	7	9	PPD sensitivity: 71.9%	
	Σ		21	11	32		
			C-Tb +	C-Tb -	Σ		
HIV-	PPD6	+	96	22	118	C-Tb sensitivity: 81.4%	-
		-	0	0	0	PPD sensitivity: 100%	
	Σ		96	22	118		

HIV+=HIV-positive participants; HIV-=HIV-negative participants; PPD6 cut-off=induration diameter ≥ 6 mm; P value was calculated using McNemar test; Source: appTAB2.31

Table 44: PPD outcome in negative control BCG-vaccinated subjects at different cut-offs – pooled TESEC-05 and TESEC-06*

n (%)	BCG-vaccinated (N=300)				
Cut-off	6 mm		15 mm		
Age in years	Pos. n (%)	Neg. n (%)	Pos. n (%)	Neg. n (%)	Total (%)
0 - ≤ 5	2 (12.5)	14 (87.5)	0 (0)	16 (100)	16 (100)
> 5 - ≤14	20 (25)	60 (75)	12 (15)	68 (85)	80 (100)
> 14 - ≤ 18	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)
> 18 - ≤ 40	28 (29.8)	66 (70.2)	3 (3.2)	91 (96.8)	94 (100)
> 40	8 (66.6)	4 (33.3)	6 (50)	6 (50)	12 (100)

Overall	58 (28.4)	146 (71.6)	21 (10.3)	183 (89.7)	204 (100)

*Site B of TESEC-05 was excluded from the calculations

Analyses requested after GCP breach

After a GCP breach was identified at Site B, the applicant was asked to re-analyse the data excluding the subjects involved in the GCP violation (involving studies TESEC-05 and 07). The data are presented in the following section.

Table 45: Characteristics of subjects excluded and included at analysis of studies TESEC-05 and TESEC-07

	TESEC-05 (N = 1190)		TESEC-07 (N = 456)		Total (N = 1646)	
	Site B	Subjects not excluded	Site B	Subjects not excluded	Site B	Subjects not excluded
Number of subjects	187	1003	27	429	214	1432
Female	103	486	10	153	113	639
Male	84	517	17	276	101	793
Negative controls (No TB)	0	100	0	0	0	100
Positive controls (TB positive)	4	71	27	429	31	500
BCG immunisation	173	709	22	262	195	971
No BCG immunisation	14	250	5	133	19	383
Unknown BCG immunisation status	0	44	0	34	0	78
HIV-negative	125	605	16	348	141	953
HIV-positive	44	255	11	81	55	336
HIV-unknown	18	143	0	0	18	143

Table 46: Sensitivity analysis - individual studies

	Siiltibcy		QFT		PPD		Difference in sensitivity	
Clinical trial	n	Sensitivity in % (95% CI)	n	Sensitivity in % (95% CI)	n	Sensitivity in % (95% CI)	Siiltibcy – QFT in % (95% CI)	Siiltibcy – PPD in % (95% CI)
Analysis including site B subjects								
TESEC-05	75	72.0	70	58.6	75	77.3	13.4	-5.3

		(61.8; 82.2)		(47.0; 70.1)		(67.9; 86.8)	(-3.3; 30.2)	(-20.6; 9.9)
TESEC-07	305	78.0 (73.4; 82.7)	446	69.5 (65.2; 73.8)	303	87.1 (83.4; 90.9)	8.5 (1.9; 15.1)	-9.1 (-15.4; -2.8)
Analysis excluding site B subjects								
TESEC-05	71	74.6 (64.5; 84.8)	66	60.6 (48.8; 72.4)	71	76.1 (61.1; 86.0)	14.0 (-3.0; 31.0)	-1.4 (-17.0; 14.2)
TESEC-07	288	79.2 (74.5; 83.9)	419	69.7 (65.3; 74.1)	286	87.8 (84.0; 91.6)	9.5 (2.8; 16.2)	-8.6 (-15.0; -2.2)

Source: Table 14.2.1.5 (t_ema_sens2_05) 08Dec2023; Table 14.2.1.7 (t_ema_sens2_07) 08Dec2023 ; Table 14.2.1.5.b (t_ema_sens2_05_b) 22Apr2024; Table 14.2.1.7.b (t_ema_sens2_07_b) 22Apr2024

Table 47: Pooled sensitivity analysis of all TESEC studies

TESEC-01 – TESEC-07	n	Siiltibcy	n	QFT	n	PPD	Difference Siiltibcy – QFT in % (95% CI)	Difference Siiltibcy – PPD in % (95% CI)
Analysis with site B subjects of TESEC-05 and TESEC-07								
Sensitivity % (95% CI)	808	74.4 (71.1; 77.2)	905	71.2 (68.2; 74.1	780	85.8 (83.3; 88.2)	3.0 (-1.4; 7.3)	-11.6 (-15.7; -7.6)
Analysis without site B subjects of TESEC-05 and TESEC-07								
Sensitivity % (95% CI)	787	74.7 (71.7; 77.8)	874	71.5 (68.5; 74.5)	759	85.9 (83.4; 88.4)	3.2 (-1.2; 7.6)	-11.2 (-15.2; -7.1)

Source: Table 14.2.1.1 (t_sens) 14Dec2023; Table 14.2.1.1.a (t_sens_a) 22Apr2024

Table 48: Sensitivity of pooled TESEC-05/-07 studies

Siiltibcy		QFT		PPD		Difference in sensitivity	
n	Sensitivity in % (95% CI)	n	Sensitivity in % (95% CI)	n	Sensitivity in % (95% CI)	Siiltibcy – QFT in % (95% CI)	Siiltibcy – PPD in % (95% CI)
Analysis with site B subjects of TESEC-05 and TESEC-07							
380	76.8 (72.6; 81.1)	516	68.0 (64.0; 72.0)	378	85.2 (81.6; 88.8)	8.8 (2.7; 14.9)	-8.3 (-14.2; -2.5)
Analysis without site B subjects of TESEC-05 and TESEC-07							

359	78.3 (74.0; 82.5)	485	68.5 (64.3; 72.6)	357	85.4 (81.8; 89.1)	9.8 (3.6; 16.0)	-7.2 (-13.1; -1.3)

Source: Table 14.2.5.1 (t_sens_05_07) 14Dec2023 ; Table 14.2.5.1.a (t_sens_05_07_a) 22Apr2024

As shown above, the removal of site B from the pooled analysis did not substantially impact the results: importantly for sensitivity the 95% CI remained within the -15% limit; specificity results of the three tests are unaffected by exclusion of site B data as no negative control participants were enrolled by this site for the two studies. Therefore, it was agreed that the evaluation of diagnostic performance (sensitivity and specificity) be based on the overall data submitted.

- Ancillary analyses

None

- Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections). The key results for describing diagnostic accuracy of Siiltibcy should be considered those related to the test sensitivity and specificity of Siiltibcy compared to PPD and QFT. These are therefore reported below for all the 3 pivotal trials and the different populations of interest.

Table 49: Siiltibcy, PPD, and QFT Sensitivity (Controlled Clinical Efficacy Studies)

Study ID	Subjects	N	Siiltibcy n (%)	PPD n (%)	N	Siiltibcy n (%)	QFT n (%)
TESEC-05	Subjects with microbiologically confirmed TB	75	54 (72.0)	58 (77.3)	56	39 (69.6)	41 (73.2)
	Subjects with microbiologically confirmed TB + TB confirmed by clinical symptoms	125	93 (74.4)	102 (81.6)	87	66 (75.9)	65 (74.7)
TESEC-06	PC	100	68 (68.0)	81 (81.0)	100	68 (68.0)	82 (82.0)
TESEC-07	Subjects diagnosed with active TB	150	117 (78.0)	121 (80.7)	232	193 (83.2)	196 (84.5)

Sources: TESEC-05 CSR, Tables 2.1 (confirmed TB) Siiltibcy vs PPD, 11-41 (confirmed TB Siiltibcy vs QFT), 11-38; TESEC-06 CSR, Tables 11.7, 11-6; TESEC-07 CSR, Table 11-24, 11-15
Test-positive=PPD ≥ 15 mm for BCG-vaccinated with negative/unknown HIV status and ≥ 5 mm all others.
CSR = clinical study report; ID = identifier; N = total number of study subjects; n (%) = number (percentage) of subjects with positive test; PC = positive control; PPD = tuberculin purified protein derivative RT 23 SSI; QFT = QuantiFERON®-TB Gold In-Tube Test; TB = tuberculosis

Subjects with QFT unknown or indeterminate status were excluded from the calculation.

Table 50: Siiltibcy, PPD, and QFT Specificity (Controlled Clinical Efficacy Studies)

Study ID	Subjects	N	Siiltibcy n (%)	PPD n (%)	N	Siiltibcy n (%)	QFT n (%)
TESEC-05	NC	100	83 (83.0)	85 (85.0)	93	76 (81.7)	70 (75.3)
TESEC-06	NC	212	203 (95.8)	198 (93.4)	262	253 (96.6)	252 (96.2)

Sources: TESEC-05 CSR, Tables 11-45, 11-47; TESEC-06 CSR, Tables 11-9, 11-8

CSR = clinical study report; ID = identifier; N = total number of study subjects; n (%) = number (percentage) of subjects with negative test; NC = negative control; PPD = tuberculin purified protein derivative RT 23 SSI; QFT = QuantiFERON®-TB Gold In-Tube Test

Subjects with QFT unknown or indeterminate status were excluded from the calculation.

Table 51: Siiltibcy, PPD, and QFT Positivity Rates

Study ID	Subjects	Siiltibcy N	Siiltibcy n (%)	PPD N	PPD n (%)	QFT N	QFT n (%)
TESEC-05	All	1182	476 (40.3)	1184	523 (44.2)	916	391 (42.7)
	NC	100	17 (17.0)	100	15 (15.0)	98	23 (23.5)
	Suspected TB	1082	459 (42.4)	1084	508 (46.9)	818	368 (45.0)
TESEC-06	All	977	262 (26.8)	928	292 (31.5)	940	271 (28.8)
	NC	263	9 (3.4)	213	14 (6.6)	263	10 (3.8)
	Occasional contact	299	49 (16.4)	299	57 (19.1)	284	57 (20.1)
	Close contact	316	136 (43.0)	316	140 (44.3)	288	122 (42.4)
	PC	101	68 (68.0)	100	81 (81.0)	101	82 (81.2)
TESEC-07	All (TB diagnosed)	305	238 (78.0)	303	252 (83.2)	443	308 (69.5)

Sources: TESEC-05 CSR, Tables 2.1 (All), 2.7 (NC), and 2.3 (Suspected TB); TESEC-06 CSR, Tables 11.1 (Siiltibcy), 115 (PPD, QFT); TESEC-07 CSR, Table 2.1 (All)
BCG = Bacillus Calmette-Guérin; CSR = clinical study report; ID = identifier; N = number of subjects with induration reading; n (%) = number (percentage) of subjects with observation; NC = negative control; PC = positive control; PPD = tuberculin purified protein derivative RT 23 SSI; QFT = QuantiFERON®-TB Gold In-Tube Test; TB = tuberculosis
Notes: TESEC-05 and TESEC-06: PPD positivity rate = diagnostic outcome using PPD cut-off of 15 mm for HIV-negative and BCG-positive subjects and 5 mm otherwise. TESEC-07: PPD positivity rate = diagnostic outcome using PPD cut-off of 5 mm for HIV-positive subjects and 15 mm otherwise

2.6.5.3. Clinical studies in special populations

An extrapolation study has been performed with the main objective to extrapolate sensitivity and specificity from adults to children 28 days or older in order to provide sufficient information on the diagnostic performance of Siiltibcy in the paediatric population.

The central extrapolation concept is that the pharmacodynamics of the Siiltibcy skin test responses in adults and children are sufficiently similar to allow estimates for sensitivity and specificity in the paediatric population to be extrapolated from adult data.

The development of the extrapolation plan relies on the three assumptions as per GL and are discussed/justified here below by the applicant:

- Similarity of disease: children above 5 years can be infected with Mtb exactly as adults, and the same general considerations apply on how to respond to a positive test for Mtb infection, including initial ruling out active TB disease, however, different considerations must be taken into account in smaller children. First, active TB disease is more difficult to rule out as most infants and young children have paucibacillary disease and diagnosis will have to rely on discrete Chest X-ray changes, documented exposure and immunologic evidence of Mtb infection. Secondly, infants and young children are at a higher risk of disseminated and severe TB disease than are older children and adults wherefore TB therapy may be initiated without confirmed diagnosis.

- Similarity of drug disposition and effect: the immune response will be a delayed-type hypersensitivity reaction Type IV. The pharmacokinetic of Siiltibcy antigens is simple. These are deposited intradermally and have local effect at the injection site. The antigens are not distributed systemically. The pharmacodynamic of Siiltibcy antigens elicits itself as an immunological skin response. The reaction is therefore conditioned by the presence of previously sensitized T-cells specific to the antigens in the skin test. Infants and young children are known to be at high risk of progression to severe pulmonary or disseminated active TB disease once infected. This is thought to be due to an immature immune system where T-cell function to some degree is

impaired (Perez-Velez CM 2012). It is unclear to what extent such an immaturity of the immune system affects the pharmacodynamics of a skin test.

- Similarity of clinical endpoints: Siiltibcy is a diagnostic skin test and has no treatment effects. The expected diagnostic benefit of Siiltibcy is the ease of use compared to the IGRAs and the improved specificity compared to available TSTs.

Extrapolation report: As very few paediatric trial participants are recruited in control populations, the data generated from TESEC-05 and TESEC-06 cannot support direct estimates of sensitivity or specificity in such population. Instead, the data generated in the target population have been used to validate the two hypotheses of the extrapolation concept, in the context of a full extrapolation of sensitivity and specificity from adults to the paediatric population. The extrapolation report is based on data from two Phase 3 clinical trials that investigated Siiltibcy as a diagnostic test for *M. tuberculosis* infection (Mtb): TESEC-05 and TESEC-06.

The first validation analysis of the extrapolation concept aims to demonstrate that induration sizes are similar at all ages and between adults and children.

The second validation analysis of the extrapolation concept aims to demonstrate a significantly lower occurrence of responses to Siiltibcy compared to TST in the paediatric TESEC-05 subpopulation consisting of 100 healthy BCG vaccinated endemic negative controls aged 5 – 11 years (specificity).

Sensitivity analysis the primary analysis of the extrapolation of induration diameters in responders (sensitivity) from adults to children was based on a mixed cohort children both suspects of TB and asymptomatic contacts from the TESEC-05 trial. The Siiltibcy test positivity rate was associated with age. Test positivity rate is 56% in the > 40 years old age group, 50% in 18 - 39 years old age group and 47% in the 12 – 17 years old age group. The Siiltibcy test positivity declined further in the group of children 5 – 11 years of age (34%), in the 2 – 4 years old children (26%) and in the 28 days – 23 months old (14%).

The primary analysis focused on the complete TESEC-05 trial and assessed the association between age and reactivity diameters across the ages, in participants who had a non-zero Siiltibcy response (1 mm or above). The median and quartile curves appear constant in ages 65 down to approximately 5 – 7 years of age, thereafter a slow but consistent decline in the induration size curves is observed. The effect of age on induration size becomes evident in children below age 6. However, the median is retained at the adult level to approximately 4 years of age. As the similarity in induration size in responders cannot be demonstrated between children and adults, a lower age limit for the extrapolation was established. Using the pre-specified criteria (i.e. the lower 95% CI of the 20% quartile curve where this lies constantly above the 5 mm cut-off) lower age limit for the extrapolation was estimated to be 3.7 years.

Repeating the extrapolation analysis for PPD on the complete TESEC-05 trial, a similar trend was observed.

TESEC-05 comprises a mixed group of TB suspects and contacts and as mentioned above, the Siiltibcy test performed differently in those included as contacts compared to those included based on TB symptoms, and for this reason, the extrapolation analysis was repeated in the two groups separately.

In the symptomatic TB suspects, there was a pronounced age effect whereas this appeared not to be the case for the contacts. Applying the pre-specified cut-point, the lower limit of the extrapolation was 7.8 years for TB suspects and 2.6 years for the contacts.

The supportive analysis repeats the primary analysis but focus on contacts only (the main target population of the test) and subsequently using the cohort of combined TB contacts from the TESEC-05 and TESEC-06 trials. A joint median regression was performed including the same median predictor structure as above, but with a trial-level included in the model to assess a possible systematic difference between the trials in median induration level. The applicant concludes that there is no indication of an association between age and induration size among the cohort of contacts only, however the lower point wise 95% CI of the 20% quartile curve crosses the predefined 5 mm cut-point defining the lower age limit for the extrapolation at 8 months (sparse data at very low age).

Specificity analysis: the second validation analysis aimed to demonstrate a significantly lower occurrence of responses to Siiltibcy compared to PPD in the paediatric TESEC-05 subpopulation consisting of 100 healthy BCG vaccinated endemic controls aged 5–11 years with no signs of active TB.

The specificity analysis compared the distribution of Siiltibcy and PPD responders (> 0 mm) in 100 endemic controls aged 5–11 without active TB. Seventeen (17%) participants had an induration reaction to Siiltibcy whereas a significantly higher number of 28 (28%) participants responded to PPD (p = 0.001). Interestingly, all the 17 Siiltibcy responders also responded to PPD (all with a Siiltibcy induration > 5mm, indicating true infection), whereas the remaining 11 PPD responders were all non-responders to Siiltibcy. Increasing the cut-off to 5 mm Siiltibcy remained more specific (p < 0.01), but at 10 mm or 15 mm cut-off for PPD the tests performed with comparable specificity.

Table 52: Pooled positivity rates analysis by paediatric age groups

TESEC-05/ -06/-07	Siiltibcy		QFT		PPD	
Age group	N	Positivity rate in %	N	Positivity rate in %	N	Positivity rate in %
0 to <1 years	45	17.8%	n.a.	n.a.	45	17.8%
1 to < 2 years	70	14.3%	n.a.	n.a.	70	11.4%
2 to <5 years	155	24.5%	n.a.	n.a.	156	29.5%
5 to 10 years	373	28.2%	269	30.5	273	24.5%

2.6.5.4. Analysis performed across trials (pooled analyses and meta-analysis)

The applicant submitted, upon request, the following pooled analysis for Siiltibcy diagnostic performance:

Table 53: Pooled sensitivity and specificity analysis of all TESEC studies

TESEC-01 – TESEC-07	n	Siiltibcy	n	QFT	n	PPD	Difference Siiltibcy vs QFT in % (95% CI)	Difference Siiltibcy vs PPD in % (95% CI)
Sensitivity % (95% CI)	808	74.4 (71.1; 77.2)	905	71.2 (68.2; 74.1	780	85.8 (83.3; 88.2)	3.0 (-1.4; 7.3)	-11.6 (-15.7; -7.6)
Specificity % (95% CI)	513	94.7 (92.8; 96.7)	512	92.6 (90.3; 94.8)	463	91.1 (88.6; 93.7)	2.2 (-1.0; 5.3)	3.6 (0.2; 7.0)

Table 54: Specificity on BCG status of pooled TESEC-05/-06 studies

	Siiltibcy		QFT		PPD		Difference in specificity	
BCG status	n	Specificity in % (95% CI)	n	Specificity in % (95% CI)	n	Specificity in % (95% CI)	Siiltibcy – QFT in % (95% CI)	Siiltibcy – PPD in % (95% CI)
Overall	363	92.8 (90.2; 95.5)	361	89.5 (86.3; 92.6)	313	90.7	3.4	2.1

						(87.5; 93.9)	(-1.0; 7.8)	(-2.4; 6.6)
BCG vaccinated	204	91.2 (87.3; 95.1)	202	85.1 (80.2; 90.1)	204	89.7 (85.5; 93.9)	6.0 (-0.7; 12.8)	1.5 (-4.7; 7.7)
BCG unvaccinated	154	97.4 (94.9; 99.9)	154	96.8 (94.0; 99.6)	104	96.2 (92.5; 99.8)	0.6 (-3.8; 5.1)	1.2 (-4.0; 6.5)

Table 55: Positivity rates on close contact groups of pooled TESEC-05/-06 studies

Pooled TESEC-05 and TESEC-06 close contact group	n	Pos. rate %	95% CI	kappa
Siiltibcy	316	43.0	37.6; 48.5	0.74
PPD	316	44.3	38.8; 49.8	
Siiltibcy	290	43.8	38.1; 49.5	0.84
QFT	290	42.1	36.4; 47.8	

Linear regression of PPD induration diameter to IFN-γ values

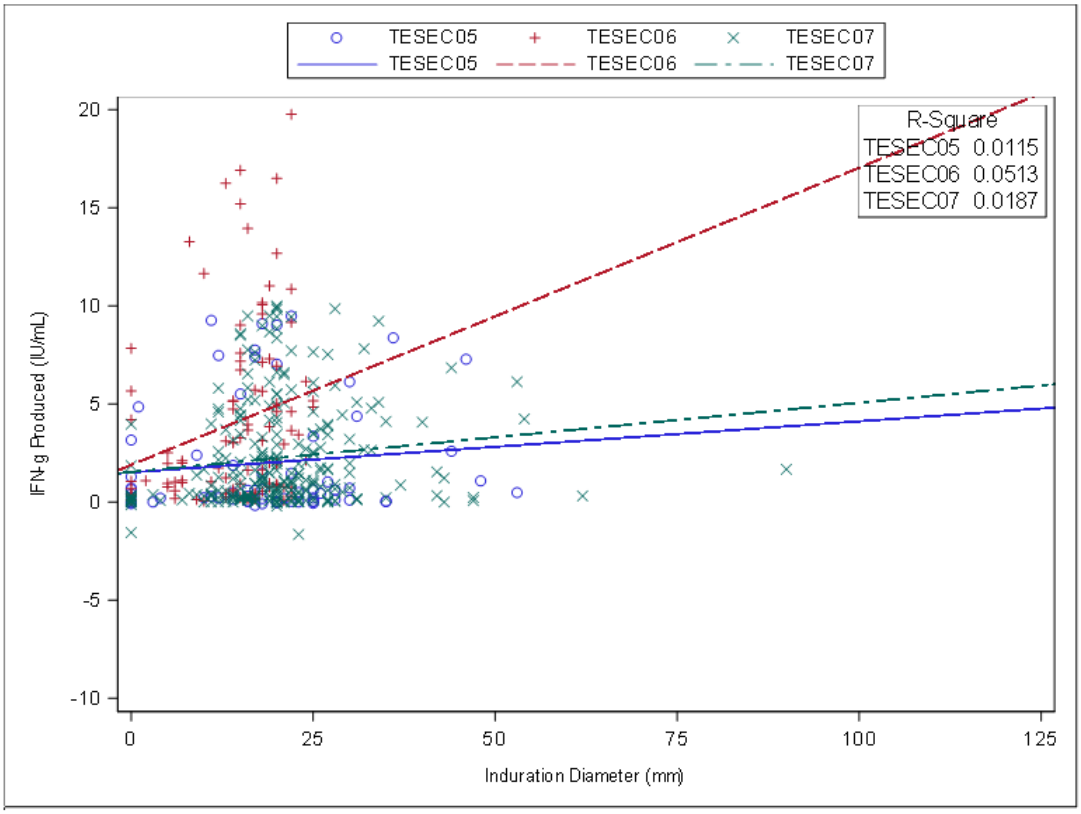

Figure 9

Linear regression of Siiltibcy induration diameter to IFN-y values

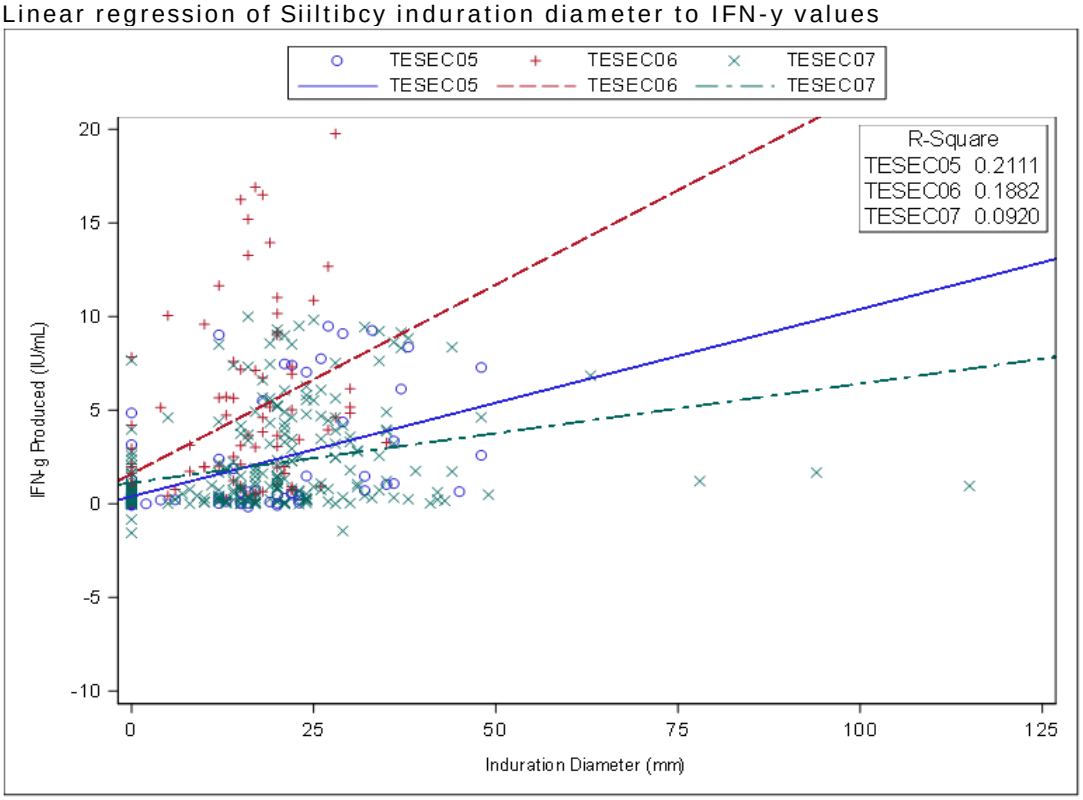

Figure 10

Table 56: Post test probabilities based on different TB prevalences

Prevalence	% (95% CI)	Siiltibcy	PPD	QFT
Low: 5%	PPTP	36.1 (28.0; 48.6)	32.6 (25.6; 43.6)	25.4 (19.7; 34.0)
	NPTP	1.3 (1.0; 1.6)	0.9 (0.6; 1.1)	1.8 (1.6; 2.1)
Medium: 20%	PPTP	72.8 (64.9; 81.8)	69.7 (62.0; 78.6)	61.8 (53.9; 71.0)
	NPTP	5.9 (4.7; 7.1)	3.9 (2.9; 5.0)	8.2 (7.0; 9.4)
High: 50%	PPTP	91.5 (88.1; 94.7)	90.2 (86.7; 93.6)	86.6 (82.4; 90.7)
	NPTP	20.0 (16.5; 23.3)	14.0 (10.7; 17.4)	26.3 (23.2; 29.4)

2.6.5.5. Supportive studies

TESEC-03

TESEC-03 was a Phase 2 trial with the primary objective to assess the specificity of the Siiltibcy test in a population of healthy BCG vaccinated adults in the United Kingdom. The specificity of the Siiltibcy test was evaluated as a function of the cut-off value.

In the table below the specificities of Siiltibcy and PPD RT 23 are shown at cut-off levels of 0, 5, 6, 10 and 15 mm, where 6 mm is the cut-off level for the comparator PPD RT 23. For the specificities of Siiltibcy and PPD RT 23 at any cut-off level ranging from the lowest measured induration diameter of 0 mm to the highest diameter of 22 mm.

Table 57

Diameter of induration, days 2-3, mm	Specificity C-Tb n/N; % [95 % CI]	Specificity PPD RT23 n/N; % [95 % CI]
0	143/147; 97.3 [93.2; 99.3]	89/147; 60.5 [52.2; 68.5]
5	146/147; 99.3 [96.3; 100.0]	92/147; 62.6 [54.2; 70.4]
6	146/147; 99.3 [96.3; 100.0]	97/147; 66.0 [57.7; 73.6]
10	147/147; 100.0 [97.5; 100.0]	113/147; 76.9 [69.2; 83.4]
15	147/147; 100.0 [97.5; 100.0]	135/147; 91.8 [86.2; 95.7]

n = numbers of subjects with negative test outcome and N = total number of subjects in per protocol population. Specificity (%) of Siiltibcy and PPD RT 23 at cut-off levels 0, 5, 6, 10 and 15 mm induration measured day 2 – 3 with 95% confidence intervals [95% CI]

The specificity of Siiltibcy at cut-off levels of 0, 5, 6, 10 and 15 mm induration was 97.3, 99.3, 99.3, 100 and 100 %, where the corresponding specificity of PPD RT 23 was 60.5, 62.6, 66.0, 76.9 and 91.8 %.

TESEC-04

TESEC-04 clinical trial was a Phase 2b trial with the primary objective to assess the sensitivity of Siiltibcy test as a function of the cut-off value (i.e., the smallest size of induration measured in mm resulting in a positive outcome of the Siiltibcy test) in a population of subjects with a recent diagnosis of active TB (including both HIV-negative and HIV-positive subjects) in South Africa.

Immune response results

The sensitivity of HIV-positive and HIV-negative subjects were compared for the cut-off values 1, 5, 6, 10 and 15 mm. There was no statistically significant difference in Siiltibcy sensitivity between the groups for any of these values (Fisher's exact test, $p = 0.295$ for cut off ≥ 1 mm; $p = 0.232$ for cut off ≥ 5 mm and ≥ 6 mm; $p = 0.242$ for cut off ≥ 10 mm; and $p = 0.121$ for cut off ≥ 15 mm).

Table 58 **C-Tb Sensitivity by HIV Status at Various Cut-off Points (TESEC-04)**

Induration Diameter (mm)	C-Tb Sensitivity			
	HIV-negative N = 146 n (%) [95% CI]	HIV-positive N = 95 n (%) [95% CI]	All N = 241 n (%) [95% CI]	Comparison of HIV-positive and HIV-negative Subjects*
≥ 1	112 (76.7) [69.0 – 83.3]	67 (70.5) [60.3 – 79.4]	179 (74.3) [68.3 – 79.7]	p = 0.295
≥ 5	112 (76.7) [69.0 – 83.3]	66 (69.5) [59.2 – 78.5]	178 (73.9) [67.8 – 79.3]	p = 0.232
≥ 6	112 (76.7) [69.0 – 83.3]	66 (69.5) [59.2 – 78.5]	178 (73.9) [67.8 – 79.3]	p = 0.232
≥ 10	110 (75.3) [67.5 – 82.1]	65 (68.4) [58.1 – 77.6]	175 (72.6) [66.5 – 78.1]	p = 0.242
≥ 15	105 (71.9) [63.9 – 79.0]	59 (62.1) [51.6 – 71.9]	164 (68.0) [61.8 – 73.9]	p = 0.121

Source: TESEC-04 CSR, Table 5; Section 10.1.1 (p values)
CI = confidence interval; CSR = clinical study report; HIV = human immunodeficiency virus; n (%) = number (percentage) of subjects with positive test; N = total number of subjects in PP population; PP = per protocol
Note: Induration diameters were measured 48 to 72 h after administration of C-Tb; cut-off points were 1, 5, 6, 10, and 15 mm.
* Fisher's exact test

There was no statistically significant difference between the HIV-negative and HIV-positive group in mean or median Siiltibcy diameter as tested by two-sample t test for means (p = 0.613) and Wilcoxon test for medians (p = 0.5038).

The sensitivity of HIV-negative and HIV-positive subjects were compared for the cut-off values 1, 5, 6, 10 and 15 mm. Sensitivity for the HIV-negative group was statistically significantly higher than the HIV-positive group for all the tested cut-off values (Fisher's exact test, p = 0.007 for cut off ≥ 1 mm, ≥ 5 mm and ≥ 6 mm; p = 0.004 for cut off ≥ 10 mm; and p = 0.002 for cut off ≥ 15 mm).

2.6.6. Discussion on clinical efficacy

Since Siiltibcy intended use is as aid tool for diagnosis of Mtb infection, and not a stand-alone diagnostic, the recommended indication wording is:

"Siiltibcy is indicated as diagnostic aid for detection of *Mycobacterium tuberculosis* infection, including disease, in adults and children aged 28 days or older."

Details on interpretation of test results and warnings/precautions for use have been reflected in the SmPC sections 4.2 and 4.4.

The 28 days lower age limit is supported by a partial extrapolation of sensitivity and specificity of Siiltibcy test down to subjects aged 8 months. Limitations of the main extrapolation assumptions and of sufficient data in the target population to validate the extrapolation concept hamper the reliability this approach. However, although no additional analyses have been provided to validate the extrapolation concept, additional observed data have been submitted to support the diagnostic performance of Siiltibcy in the youngest children (below 8 months) overall showing a lower sensitivity in the youngest group as compared to older subjects (a common issue to all TB tests due to the immature immune system) but importantly similar positivity rates between Siiltibcy and PPD test.

Moreover, several recommendations support the use of skin tests in the paediatric range (especially < 5 years old) and Siiltibcy use could cover an unmet need in children younger than 5 years and BCG-vaccinated

(i.e. QFT cannot be used in subjects below 5 years of age and PPD could perform worse with respect to specificity). The generalizability of Siiltibcy use in subjects older than 65 years (excluded from clinical trials) is supported by the use in elderly of the other tests (PPD and QFT) and by observed data showing similar trend in positivity rate across tests. However, it is known that immunosenescence could affect the immune responses as reported in other TB tests. The limited available information in elderly has been properly reflected in the 4.2 section of the SmPC.

In support of the claimed indication, the applicant submitted three pivotal Phase 3 trials so called TESEC-05, TESEC-06 and TESEC-07 mainly contributing to the benefit-risk assessment of this diagnostic test. Moreover, two supporting studies (TESEC-03 on healthy volunteers, BCG-vaccinated, HIV-negative and TESEC-04, TB subjects, HIV-positive and -negative subjects) investigated the specificity and sensitivity of Siiltibcy and identified the optimal threshold for induration diameter at 5 mm. In dose-finding studies, the dose of 0.1 µg of Siiltibcy was well tolerated and safe and resulted in an induration response similar to that expected for PPD.

For diagnostic agents the relevant regulatory guidance is the "Guideline on Clinical Evaluation of Diagnostic Agents" (CPMP/EWP/1119/98/Rev. 1; EMA GL) stating that the clinical benefit of a diagnostic agent may be evaluated by assessing its *diagnostic performance* as well as its *technical performance*.

'Diagnostic performance' involves sensitivity (positive test in subjects with Mtb infection) and specificity (negative test in subjects not infected with Mtb). Hence, it represents the performance of the diagnostic agent itself and should be minimally influenced by disease prevalence in a studied population.

In the latent TB context, a standard of truth cannot be established. However, as recognized by EMA GL, "if there is a well-documented comparator available, "concordance" in a cross-over study can be used as outcome measure". Therefore, in the Mtb infection setting a way to assess the diagnostic performance of a new test is by testing for (at least) non-inferiority versus a widely available and used test. The applicant chose PPD and QFT as reference comparators and surrogate of standard of truth; this is seen as an acceptable way forward and is recommended also by the WHO (Framework for the evaluation of new tests for tuberculosis infection).

The effective presence of Mtb can be proven only by microbiological confirmation in patients with an active TB disease. On the contrary, in subjects latently infected with *M. tuberculosis* the microbiological confirmation is not possible. Similarly, in children with active tuberculosis disease the microbiological diagnosis could be difficult.

As main outcome measures for evaluation of diagnostic performance the applicant used: a) 'test positivity rate' (i.e., number of positive tests divided by the total number of diagnostic tests administered in the population); b) induration diameter as proxy of magnitude of skin test response.

'Technical performance' which involves precision and reproducibility, comparing Siiltibcy to the other reference tests.

Design and conduct of clinical studies

The three pivotal (Phase 2/3 and 3) studies share a common design: double-blind, randomized, split-body study with a follow-up of approximately 30 days. Each participant had to receive a dual injection: Siiltibcy (0.1 µg/0.1 mL) in one forearm and PPD in the other forearm using the Mantoux technique; contemporarily blood drawing for QTF test was planned. The study design allowed for a direct within-subject comparison and is preferred as instrumental to reduce inter-individual variability.

Moreover, the TESEC-06 study included an additional study arm of subjects to which Siiltibcy was administered alone (i.e. no co-administration to the same subject) and the TESEC-07 study included two further trial arms in which either Siiltibcy or PPD was administered alone (i.e. only one of the two was administered to each subject); this approach was used in order to assess whether the co-administration could alter the immune response and/or the safety. However, due to the short follow-up, the studies do not allow

excluding Siiltibcy induced sensitization potentially relevant in the long-term (i.e., further future TB test in at-risk subjects).

Comparators: PPD and blood based QFT are validated, commercially available and widely used products and thus are acceptable as comparators.

PPD contains a mix of antigens, including antigens from non-tubercular mycobacteria (NTM) and from BCG vaccine, thus false positive reactions to PPD have been described; the two Siiltibcy antigens are included in PPD.

QFT is an interferon-gamma release assays (IGRA) based on whole-blood ELISA, containing specific Mtb antigens (QFT-GIT: ESAT-6, CFP-10 and TB7.7); the two Siiltibcy antigens are included in QFT. As there is no gold-standard method for screening of Mtb infection, the WHO states that either the PPD or IGRAs can be used, recommending the two tests as equivalent options (WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection).

Target populations: The relevant EMA guideline (GL) states that patients included in confirmatory trials should be representative of the population in which the diagnostic agent is intended to be used. According to the applicant, the intended use of Siiltibcy is for routine testing, for TB contact tracing, for screening of people coming from higher prevalence of TB or atypical mycobacteria, for periodic testing of healthcare workers and other workers at risk. Siiltibcy is claimed to be applicable for all ages, including children from 28 days of age, and in immunocompromised subjects.

In TESEC-05 and TESEC-06 studies paediatric subjects have been enrolled. Inclusion of infants, toddlers and children below 5 years of age is supported since are at high risk of developing TB disease following infection. In this age, TB diagnosis is based on symptoms or signs of TB and a positive skin test or being close contact with a TB case. In pivotal trials, children below 5 years of age were excluded from QFT testing; this could be acceptable since there is limited experience. In provided studies, Siiltibcy test results were analysed according to age to check if test performance could be impacted by immune system immaturity and also to support extrapolation to lower age ranges.

To assess diagnostic performance in relation to specific patient populations, different subject characteristics (age groups), risk levels for Mtb infection based on exposure history, and comorbid conditions (HIV infection, CD4+ cell count strata) were investigated and are provided.

TESEC-05: with the main aim to investigate whether induration sizes and test positive rates depend on age and HIV status in a population with a presumed high prevalence of MTb infection, it was conducted in South Africa and enrolled more than 1000 subjects (including paediatric ones) with suspected TB disease or exposure to Mtb, and 100 healthy paediatric subjects (aged 5 to 11 years) with no known exposure to Mtb and no signs or symptoms of TB.

Moreover, about 300 subjects were HIV-positive, and more than 700 were HIV-negative (plus other 160 with unknown HIV status). Inclusion of HIV-positive subjects is supported, since they are at higher risk of developing TB and due to impaired cellular immune response, the accuracy of the diagnostic test could be negatively impacted.

Overall, the target population of TESEC-05 could be considered representative of a population with high prevalence of Mtb infection, such as specific subpopulations in the EU (migrants, IVDU, etc) or the African setting in which the diagnostic agent could have a use.

TESEC-06: the principal scope was to test whether Siiltibcy responder rates correlated with exposure to Mtb, by assessing a trend in Siiltibcy positivity rate across four different risk groups for Mtb infection: Negative Control (with no history of exposure to TB and no signs or symptoms of TB), Occasional Contact, Close Contact, Positive Control (confirmed TB). It was conducted in the EU (all 13 sites in Spain), and thus, differently from TESEC-05 and TESEC-07, within a low TB prevalence setting. It enrolled almost 1000 subjects (aged 6 weeks to 65 years). Overall, the target population of TESEC-06 study could be considered representative of the different risk categories (i.e., clinical settings) in which the test could have a use.

TESEC-07: the main aim was to provide data to demonstrate that Siiltibcy induration responses were not affected by simultaneous administration, in the other forearm, of PPD (immediately before or after Siiltibcy).

It was conducted in South Africa, enrolled 456 adult TB subjects, of which 90 HIV-positive.

Overall, the target population of TESEC-07 could be considered adequate for the aim of testing the effects of concomitant administration of the two skin tests, and also for the estimation of sensitivity (including in HIV-positive subjects).

Methodological considerations: no formal and clear *a priori* null hypothesis of (at least) non-inferiority was included in study protocols, and estimated sample size calculation across the 3 pivotal trials was not sized to evaluate Siiltibcy diagnostic performance and to compare with reference comparators; this was not considered consistent with what was expected according to the relevant the relevant EMA "Guideline on Clinical Evaluation of Diagnostic Agents" (CPMP/EWP/1119/98/Rev. 1) in case of absence of a standard of truth.

Across the 3 studies, the applicant did not define sensitivity/specificity as co-primary endpoints but selected different primary endpoints for each pivotal study, within different populations, providing complementary information about the response to Siiltibcy. Sensitivity and specificity have been included among secondary endpoints. For comparison of Siiltibcy with PDD and QFT, the difference (at least non-inferiority) in sensitivity and specificity should have been included in the protocols. Instead, only a merely descriptive analysis was provided within each SAP, and no assumptions were made about the estimand strategy. In light of these methodological limitations the applicant was asked to provide further, although post-hoc, analysis to overall confirm the diagnostic performance of Siiltibcy.

Efficacy data and additional analyses

TESEC-05: the primary endpoints of Siiltibcy induration diameter (by age, HIV infection and CD4+ cells count) and test positivity rate showed in the primary population (FAS) that there were 55.8% of non-responder vs 44.2% of responder subjects with a mean induration diameter of 9.4 mm (SD +/- 13.7). Results by age showed smaller induration diameters with lower age. The reason could be the lower TB prevalence in children as well as the ability of mounting an immune response in the age below 2 years. A further analysis restricted to suspected TB disease confirmed that induration size is impacted by HIV status. A decreased response rate of Siiltibcy for CD4+ count below 100 cells/µL was noted and reflected in the SmPC.

The sensitivity of Siiltibcy was similar compared to PPD: in TBC (75 subjects with confirmed TB) 74.6% for Siiltibcy versus 76.1% for PPD (difference of 1.4 percentage points (pp)). When TBD (TBC plus TB diagnosed by clinical symptoms) is considered (125 subjects) the sensitivity was 74.4% versus 81.6% (difference of 7.2 pp), respectively for Siiltibcy and PPD. When compared to QFT, Siiltibcy had similar sensitivity (75.9% versus 74.7%) and no major differences were seen between the two groups.

Therefore, the diagnostic performance in terms of sensitivity of Siiltibcy was overall comparable, in this trial, compared to PPD and similar to QFT, with concordance between Siiltibcy and the two tests ranging from fair to moderate depending on the considered population (TBC or TBD).

Siiltibcy specificity was similar (FAS) compared to PPD (83% and 85%, respectively) and concordance between the two tests was good (k = 0.7). Siiltibcy specificity was slightly higher (81.7%) compared to QFT (75.3%) although the difference was not nominally statistically significant.

TESEC-06: the primary objective showed that the test positivity rate of Siiltibcy increased consistently with increasing exposure to Mtb, as expected (ranging from 3.4% in the lowest risk group to 68.0% in the highest one). However, the positivity rate in the positive control group (i.e. sensitivity) of Siiltibcy was lower (68%) compared to both comparators: PPD (81%, nominal p=0.0146) and QFT (82%, nominal p=0.0043). The positivity rates in the other risk groups are in line with what observed with the other two tests, potentially suggesting that the poorer performance of Siiltibcy could be limited to the positive control population (TB

disease). The applicant justified the observed lower sensitivity of Siiltibcy (compared to PPD) in the EU population of study TESEC-06 (i.e. Spain) due to TB treatment received by subjects leading to a lower immune reaction to the test antigens; however, this explanation is not fully supported, given the observed higher mean value of IFN-gamma at QFT in close or occasional contacts of TESEC-06 compared to TB suspected in TESEC-05. Instead, a lower or similar IFN-gamma values would have been expected in TESEC-06 than in TESEC-05. Results regarding the distribution of IFN-gamma values plotted against the skin induration diameters, show different patterns for Siiltibcy in TESEC-06 vs TESEC-05/07, but this difference is also observed with PPD, hence corroborating the idea that extrinsic (geographical) and not intrinsic factors to the test could explain most of the difference. Moreover, there seems to be no strong biological rationale per a different performance of Siiltibcy in the European population *per se*; it is, however, agreed that the very different epidemiology, and maybe also regional clinical practice, could play a role making difficult to draw any firm conclusions about the lower sensitivity of Siiltibcy observed in study TESEC-06 compared to PPD.

The specificity of Siiltibcy was comparable to that of QFT and PPD tests (about 95%). In the subgroup of negative control subjects vaccinated with BCG, the specificity of Siiltibcy was higher compared to PPD (96.3 vs 66.7%, nominal p<0.0001), as expected since PPD contains antigens that are present in the BCG vaccine but absent from the Siiltibcy formulation.

TESEC 07: the primary endpoint showed that there was no change in induration diameter when Siiltibcy was administered alone or concomitantly with PPD (thus reassuring about the interpretation of data coming from study arms in which the two products were administered to the same subjects). However, in all the patients (disregard to their HIV status) Siiltibcy sensitivity (79.2%) was lower compared to PPD (94.2%, nominal p < 0.0001) when the PPD cut-off of 6 mm was used in all patients. However, when the usual PPD cut-offs were used (5 mm in HIV-positive, 15 mm all the others), Siiltibcy sensitivity was lower than PPD (79.2% vs 87.8%; difference of inferior limit of the 95% CI was -15.0 percentage points). The sensitivity of Siiltibcy compared to QFT was similar in the FAS population, however, in the HIV-positive subgroup it was numerically lower (76.5% vs 84.3%). The responder rate in HIV-positive patients by CD4+ cells count was low in patients with CD4+ count <100 cell/mmc, similarly to PPD.

Results from pooling

To support the clinical benefit of Siiltibcy, according to EMA guideline requirements, the applicant was requested to provide post-hoc estimates for differences in sensitivity and specificity between Siiltibcy, PPD and QFT for each individual pivotal study for the subpopulations with true TB status (high level of probability to be positive or negative). Moreover, a pooled analysis was to be performed on all patients with true status for sensitivity and specificity, and other risk groups for positivity rate. Even if this analysis is a *post-hoc*, not a formal, nor pre-specified non-inferiority analysis, for the sake of comparison, a lower limit of -15% for the 95% confidence interval of the differences is used.

Although these studies were not explicitly designed for non-inferiority testing regarding sensitivity and specificity, the differences with their 95%CI presented by the applicant, Siiltibcy exhibits lower sensitivity compared to PPD as shown by the individual studies (TESEC-05: Siiltibcy 77.3%, 95% CI: 67.9, 86.8; difference with PPD: -5.3%, 95% CI: -20.6, 9.9; TESEC-06: 81.0%, 95% CI: 73.3, 88.7; difference: -13.0%, 95% CI: -25.9, -0.1; TESEC-07: 78.0%, CI: 73.4%, 82.7%; difference -9.1% 95% CI: -15.4, 2.8).

In the individual study analyses and the pooled analysis on TESEC-01-TESEC-07, the lower limit of the 95% confidence interval of the difference between Siiltibcy and PPD sensitivity falls below -15%; however, it must be taken into account the small sample size of the single studies not designed for non-inferiority testing and the heterogeneity of their populations.

Results coming from the requested post-hoc analysis with pooling of all TESEC studies (without regarding to the true status) only aimed to increase the sample size, showed statistical differences in terms of sensitivity between Siiltibcy and PPD (74.4% vs 85.8%; difference -11.6% 95% CI: -15.7; -7.6) whereas Siiltibcy sensitivity was comparable to QFT (74.1% vs 71.2%; difference 3.0 95% CI: -1.4; 7.3). Although the population was not optimal for sensitivity calculation, these results showed a trend of improved Siiltibcy sensitivity with the lower limit of the 95% confidence interval of the difference between Siiltibcy and PPD

sensitivity just slightly below the -15% threshold. The performance of Siiltibcy compared to QFT is comparable or slightly better.

When QFT sensitivity was calculated without indeterminate results, an improvement of QFT sensitivity was seen in both TESEC-05 and TESEC-07 studies (in TESEC-06 no indeterminate results were observed).

To refine sensitivity calculation, the applicant was requested to analyse a pooling of studies TESEC-05 and 07, using only subpopulations with true status of disease, thus reflecting a population apter to sensitivity estimation. The results showed that, in subjects with active disease, Siiltibcy was less sensitive than PPD (76.8% vs 85.2%; difference vs PPD: -8.3, 95% CI: -14.2; -2.5) and more sensitive than QFT (76.8% vs 68.0%; difference vs QFT 8.8, 95% CI: 2.7; 14.9). In this pooled analysis, the lower limit of the 95% confidence interval of the difference between Siiltibcy and PPD sensitivity falls slightly above -15% (i.e. -14.2%), showing a slightly improved performance.

Importantly, in the subpopulation better reflecting the targeted population, i.e. pool of subjects at high risk (close contacts) from TESEC-05 and TESEC-06, Siiltibcy positivity rate was comparable to PPD and QFT (43.0% vs 44.3% vs 42.1%, respectively). Results of positivity rates show a good concordance of Siiltibcy with PPD (kappa 0.74) and with QTF (kappa 0.84). The positivity rate evaluated in the target population (close contact at high risk of tuberculosis infection), could better describe the diagnostic accuracy of all tests and highlight the real clinical role of the tests in the diagnosis of tuberculosis infection. Therefore, in the population of interest for the sought indication Siiltibcy showed a performance not too much different from PPD and from QFT which could be overall acceptable.

According to European Centre for Disease prevention and control, the BCG vaccination is available in 22 European Country and mandatory in several of them. Siiltibcy as well as IGRA could only identify the tuberculosis infected subjects. If the aim of the pre-vaccination test is to identify a pre-existing immunity to *M. tuberculosis,* Siiltibcy as well as IGRA could be used. In the less frequent case that the aim of the pre-vaccination test is to identify the sensitized subjects to all mycobacteria (to ensure efficacy and safety of BCG vaccination), Siiltibcy use is not adequate.

Regarding Siiltibcy specificity pooling all TESEC pivotal studies, a slightly better performance was observed as compared to PPD (94.7% vs 91.1%; difference 3.6% 95% CI: 0.2; 7.0) and QFT (94.7% vs 92.6%; difference 2.2 95% CI: -1.0; 5.3).

Even if PPD positive rates could have been impaired by BCG vaccination, the different studies (with population coming from areas with different contact risk and vaccination policies) do not allow a robust assessment of the impact of BCG vaccination on IGRA tests performance, and thus do not strongly show the putative advantage of Siiltibcy over PPD in BCG vaccinated subjects.

In TESEC 06, thus EU population, the major advantage of Siiltibcy is evident when used on BCG-vaccinated subjects (Siiltibcy: 96.3% vs PPD: 66.7%). Using a 6 mm cut-off for PPD, the specificity of Siiltibcy is much higher than PPD in BCG-vaccinated negative control participants. When the specificity is calculated on the overall population (disregarding on BCG vaccination status) with classical 15 mm cut-off for the BCG vaccinated HIV-negative population and > 5 mm for all other participant, this better performance is less evident but still present (Siiltibcy: 95.8% vs PPD: 93.4%).

The tuberculin skin test interpretation is based on the application of different cut-off according to the type of population. A high cut-off could overcome the interference due to BCG vaccination. To note that a high cut-off could also misses positive results in paediatric population. From the data submitted, it can be seen that PPD positivity is affected by BCG vaccination status and by the chosen reading cut-off, therefore making slightly more complicated to read the result with PPD compared to Siiltibcy where a single cut-off is used.

Moreover, some further additional data, on technical performance has been provided by the applicant overall providing some reassurance on reproducibility of test results.

The applicant has provided sufficient support on Siiltibcy impact on diagnostic thinking in different prevalence scenario. For instance, the applicant highlighted the importance of Siiltibcy when used in screening people coming from high TB prevalence countries; if a skin test is positive in a BCG-vaccinated

subject, subsequent IGRA test should be performed to rule out the confounding effect of BCG vaccination (not always possible, for example in children younger than 5 years old). In contrast, Siiltibcy, like IGRA, should not be affected by BCG vaccination and Siiltibcy could be used in the testing of immigrants without the need of PPD and of a subsequent confirmatory IGRA in case of a positive result. Moreover, Siiltibcy results are not affected by previous exposure to non-tubercular mycobacteria and thus its positive result should be more reliable than PPD in identifying TB infection.

Moreover, IGRA tests are currently not recommended for use in children below 5 years old; on the contrary Siiltibcy can be used in this subpopulation. However, Siiltibcy is not able to identify subjects with previous exposure to non-tuberculous mycobacteria or BCG vaccination, which is reflected in the SmPC.

Regarding the possible QFT test results classified as “indeterminates”, if one subject would have positive PPD and indeterminate QFT results, there would not be a prompt and valid method to assess the immunological status to Mtb, since the subject could have one of the three following situations: Mtb infection, BCG vaccination or exposure to environmental mycobacteria; in this particular case (not uncommon: indeterminate estimated 0% to 20.7% from Diel 2010) only Siiltibcy would be able to identify correctly the patient with true Mtb infection; thus Siiltibcy has, at least, a niche in which no other available tests can be fruitfully used.

From a public health perspective, the availability of a further immunological TB skin test from a different manufacturer could be of help in shortage situations.

2.6.7. Conclusions on the clinical efficacy

Siiltibcy test represents an alternative to other widely used tests for diagnosis of Mtb infection. Potential advantages in terms of technical performance are easiness of use and lower costs as compared to QFT, whereas versus PPD possible advantages are use of a unique threshold for positivity in all subjects and a higher specificity in the BCG vaccinated subpopulation only. The clinical benefit of Siiltibcy, as reflected by diagnostic performance (sensitivity and specificity) and technical performance (precision), has been sufficiently demonstrated in respect to chosen comparators (PPD and QFT).

2.6.8. Clinical safety

Considering the nature of the medicinal product (diagnostic), the overall safe profile of Siiltibcy, and the limited number of subjects affected by the GCP violation (n = 187 subjects from TESEC-05 and n = 27 subjects from TESEC-07) against the totality of treated subjects, it is not expected that the exclusion of those subjects would substantially impact the safety profile of Siiltibcy. Moreover, their exclusion is considered appropriate in terms of establishing accuracy of the ADRs type and their frequency. Thus, the following data includes the complete submitted safety dataset.

The safety of Siiltibcy has been evaluated in seven clinical studies. The data comprise two Phase 1 studies (TESEC-01 and TESEC-02), two Phase 2 studies (TESEC-03 and TESEC-04), one Phase 2/3 study (TESEC-07), and two Phase 3 studies (TESEC-05 and TESEC-06).

In addition, data from two Phase 1 studies performed with a diagnostic skin test containing only rdESAT-6 as single recombinant protein (studies TESAT-01 and TESAT-02) are included in this safety analysis.

2.6.8.1. Patient exposure

In the confirmatory studies (TESEC-05, TESEC-06 and TESEC-07), the total number of subjects exposed to Siiltibcy (0.1 µg/0.1 mL) was 2473. To this figure, subjects exposed in the key supporting studies (TESEC-03,

TESEC-04) that were another 404, and in Phase 1 studies (TESEC-01, TESEC-02) that were 80 have to be added.

The main sources of safety data are summarised in the table 59.

Table 59: Summary of Exposure to Siiltibcy in Clinical Studies Contributing to the Safety Evaluation of Siiltibcy

Study ID	Age at Testing	Study Population	Study Drug Active Comparator	Safety Population
Confirmatory Studies				
TESEC-05	28 days to 65 years	Female/male HIV-negative and HIV-positive subjects with suspicion of TB disease and HIV-negative children (5 – 11 years) with no symptoms of TB as negative control. Paediatrics: 28 days – 17 years of age Adults: 18 – 65 years of age	Siiltibcy 0.1 µg/0.1 mL + PPD (Single injections)	1188 [a]
TESEC-06	6 weeks to 76 years	Female/male HIV-negative and HIV-positive subjects belonging to 1 of the following risk groups: Negative control Occasional contact to TB index case Close contact to TB index case Confirmed TB disease (positive control) Paediatrics: 6 weeks – 17 years of age Adults: 18 – 65 years of age	Siiltibcy 0.1 µg/0.1 mL + PPD (Single injections)	928 [b]
			Siiltibcy 0.1 µg/0.1 mL (Single injection)	50
TESEC-07	18 to 67 years	Female/male HIV-negative and HIV-positive adults diagnosed with acute Mtb infection.	Siiltibcy 0.1 µg/0.1 mL (Single injection)	153
			Siiltibcy 0.1 µg/0.1 mL + PPD (Single injections)	154
Key Supporting Studies				
TESEC-03	18 to 65 years	Female/male BCG-vaccinated adults with negative IFN-γ result at inclusion (< 0.35 IU/mL) measured by QFT.	Siiltibcy 0.1 µg/0.1 mL + PPD (Single injections)	151
TESEC-04	18 to 64 years	Female/male HIV-negative and HIV-positive adults with a recent diagnosis of active TB and in treatment for acute Mtb infection.	Siiltibcy 0.1 µg/0.1 mL + PPD (Single injections)	253
Phase 1 Studies				
TESEC-01	18 to 55 years	Healthy, nonblack, female/male adults with negative INF-γ response at inclusion (< 0.35 IU/mL) measured by QFT.	Siiltibcy 0.01 µg/0.1 mL (2 injections, 6 or 12 weeks apart)	21[c]
			Siiltibcy 0.1 µg/0.1 mL (2 injections, 6 or 12 weeks apart)	21[c]

Study ID	Age at Testing	Study Population	Study Drug Active Comparator	Safety Population
TESEC-02	18 to 60 years	Female/male adults without HIV who were newly diagnosed with active TB and in treatment for ≤ 60 days at the time of inclusion. The subjects had positive test results with either sputum smear microscopy, microbial culture, PCR or QFT.	Siiltibcy 0.01 µg/0.1 mL (2 injections ± 0.5% phenol)	12
			Siiltibcy 0.1 µg/0.1 mL (2 injections ± 0.5% phenol)	26
Total				2957

BCG = Bacillus Calmette-Guérin; HIV = human immunodeficiency virus; IFN-γ = interferon gamma; Mtb = *Mycobacterium tuberculosis*; PCR = polymerase chain reaction; QFT = QuantiFERON®-TB Gold In-Tube test; TB = tuberculosis

[a] 2 subjects received PPD but did not receive Siiltibcy as scheduled. They are not included here as they did not receive Siiltibcy but are included in the safety population as they received one dose of PPD as blinded randomised treatment.

[b] 1 subject received PPD but did not receive Siiltibcy as scheduled. The subject is not included here as they did not receive Siiltibcy but are included in the safety population as they received one dose of PPD as blinded randomised treatment

[c] 1 subject in the 0.01 µg/0.1 mL 6 weeks apart group and 2 subjects in the 0.1 µg/0.1 mL 12 weeks apart group did not receive a second injection

Demographic characteristics of subjects enrolled in the seven completed clinical studies with Siiltibcy are summarised in the Table below.

The majority of subjects received Siiltibcy and PPD injections immediately after each other in different forearms. This allowed direct comparison of the diagnostic performance and the tolerability of the 2 skin tests.

The total safety population includes 2957 subjects who received at least 1 dose of Siiltibcy. According to the applicant, the total safety population is representative of the target indication, including data from similar numbers of males and females, subjects from a broad age range (from 6 weeks to 65 years), subjects from a wide range of races, and those with HIV-positive and HIV-negative status. Subjects in the Siiltibcy clinical study program included healthy (negative control) subjects and those with occasional contact with TB subjects, close contact with TB subjects (suspected TB), and those with diagnosed TB (positive control).

The confirmatory studies were conducted in South Africa and Spain, key supporting studies were conducted in the United Kingdom and South Africa, and Phase 1 studies were conducted in Denmark, the United Kingdom, and Netherlands.

Table 60: Summary of Baseline Demographic Characteristics of Subjects in Clinical Studies Included in the Safety Evaluation of Siiltibcy (Safety Population)

	Siiltibcy N = 283	Siiltibcy + PPD N = 2677	PPD N = 149	Total N = 3109 [a]
Age group				
0 to 1 years	-	115	-	115
2 to 4 years	-	156	-	156
5 to 11 years	-	312	-	312
12 to 17 years	3	137	-	140
≥ 18 years	280	1957	149	2386
Sex				
Female	139	1332	48	1519
Male	144	1345	101	1590
Race				
African origin	100	1008	96	1204
White	50	980	-	1030
Black	-	204	-	204
Asian	-	9	-	9
Other	95	476	53	624
Unknown	38	-	-	38
HIV status				
HIV-positive	31	439	28	498
HIV-negative	218	1346	121	1685
Unknown	34	892	0	926
BCG vaccination				
Yes	97	1525	93	1715
No	93	803	45	941
Unknown	93	349	11	453
Severity of TB disease				
Healthy / negative control	92	464	-	556
Occasional contact	-	299	-	299
Close contact / suspected TB	-	1406	-	1406
Diagnosed TB / positive control	191	508	149	848

BCG = Bacillus Calmette-Guérin; HIV = human immunodeficiency virus; N = total number of subjects; n = number of subjects meeting criterion; TB = tuberculosis

[a] Three subjects (2 in study TESEC-05 and 1 in study TESEC-06) did not receive a dose of Siiltibcy but are included in the safety population as they received one dose of blinded randomised study treatment [PPD])

The disposition of subjects included in the confirmatory studies is presented in the table below. Across the three confirmatory studies, the proportion of subjects completing the study was 97.8% or higher. The main reason for discontinuation was lost to follow-up.

Table 61: Subject Disposition in the Confirmatory Studies

Category	TESEC-05	TESEC-06	TESEC-07
Sub-category	n (%)		
Screened	1278	993	510
Enrolled	1190 (100)	979 (100)	456 (100)
Received at least one study test injection	1190 (100)	979 (100)	456 (100)
Completed study	1165 (97.9)	970 (99.1)	446 (97.8)
Discontinued from study	25 (2.1)	9 (0.9)	10 (2.2)
Lost to follow-up	17 (1.4)	8 (0.8)	8 (1.8)
Withdrawal	2 (0.2)	0	0
Protocol violation	1 (0.1)	0	0
Death	3 (0.3)	0	0
Other	2 (0.2)	1 (0.1)	2 (0.4)

2.6.8.2. Adverse events

Siiltibcy is a skin test administered as an injection using the Mantoux method. Most subjects received single injections of Siiltibcy, PPD, or the two skin tests concomitantly in separate forearms. A small proportion received multiple doses of the skin tests. Accordingly adverse events are summarized in two main categories as follows:

Injection-site reactions (ISRs):

- In accordance with the trial protocols, Siiltibcy and PPD were administered separately in pre-specified forearms. Therefore, it was possible to assign each ISR to the respective skin test.
- All injection site reactions were considered to be related to the assigned skin test.
- The data are presented according to the actual skin test received.
- ISRs are reported separately and not included in the systemic Treatment-Emergent Adverse Events (TEAE) data.

Systemic treatment-emergent adverse events (TEAEs):

- Systemic TEAEs do not include local ISRs (see above).
- Where subjects received both Siiltibcy and PPD, any systemic event was assumed to be associated with Siiltibcy as a conservative “worst case scenario.”
- Treatment-related systemic TEAEs were those considered by the investigator to be at least possibly related to the skin test.

Data for the ISRs and systemic TEAEs have been pooled for the two Phase 3 studies (TESEC-05 and TESEC-06) and the Phase 2/3 study (TESEC-07). This provides a larger database with the potential to identify rare adverse events.

In total, 3109 subjects participated in the seven clinical studies performed with Siiltibcy and/or PPD. Of these, 149 subjects received a single administration of PPD only, 283 subjects received Siiltibcy alone, and 2674 subjects received both Siiltibcy and PPD immediately after each other in different forearms. The total population exposed to Siiltibcy is 2957 subjects.

Injection-site reactions (ISRs)

During the clinical development of Siiltibcy, ISRs have been the main focus of the safety profile. The most frequently observed ISRs were injection site pruritus, injection site pain, and injection site rash. Haematomas were also observed as ISRs, although not as frequently as the ISRs mentioned above. Pruritus seemed to be associated with delayed-type hypersensitivity reaction, whereas haematomas may have been induced by the needle during the injection of the skin test agents.

No injection site haematomas were reported in the first two studies performed in Denmark and England, which included both phenol-preserved and unpreserved Siiltibcy. In the subsequent studies, mild haematomas at the injection site were common in the European Studies TESEC-03 (healthy BCG-vaccinated subjects) and TESEC-06 (contact tracing study including a healthy control group) but remained unreported with Siiltibcy in the South African studies TESEC-04, TESEC-05, and TESEC-07 with the exception of two events, both of which were related to PPD.

The table below reports the number of subjects experiencing at least 1 AE in the main studies.

Table 62: Incidence of Injection Site Adverse Reactions and Systemic Adverse Events in Study Subjects Assigned to Receive Siiltibcy (Safety Population)

Study ID	Number of subjects with at least 1 event/Total number in group (%)		
	ISRs		Systemic TEAEs
	Siiltibcy	PPD	
TESEC-01	3/42 (7.1)	NA	29/42 (69.0)
TESEC-02	NR	NA	31/38 (81.6) [a]
TESEC-03	48/151 (31.8)	31/151 (20.5)	80/151 (53.0)
TESEC-04	120/253 (47.8)	150/253 (59.3)	98/253 (38.7)
TESEC-05	282/1188 (23.7)	290/1190 (24.4)	338/1190 (28.4)
TESEC-06	288/978 (29.4)	182/929 (19.6)	317/979 (32.4)
TESEC-07	163/307 (53.1)	205/303 (67.7)	147/456 (32.2)

Sources: TESEC-01 CSR, Section 10.3.2; TESEC–02 CSR, Section 10.3.2; TESEC-03 CSR, Table 5 and Section 11.2.4; TESEC-04 CSR, Table 6 and Section 12.2.2.2; TESEC-05 CSR, Table 12-2 and Table 12-5; TESEC-06 CSR, Table 12-2 and Table 12-3; TESEC-07 CSR, Table 12-2 and Table 12-3.
CSR = Clinical Study Report; ID = identification; ISR = injection site adverse reaction; NA = not applicable (subjects did not receive PPD); NR = not reported "by subject" in the CSR; TEAE = treatment-emergent adverse event.
a Total number of TEAEs including skin reactions and systemic events.

An overview of adverse events in the confirmatory studies is provided in the table below. Across the confirmatory studies, the proportion of subjects who experienced at least one ISR ranged from 19.6% to 67.7%. The highest were in study TESEC-07, where subjects had been recently diagnosed with active TB. All ISRs were defined as related to the skin test in the study protocols. There were no serious ISRs and the great majority were mild or moderate in severity. The highest proportion of severe ISRs was in trial TESEC-07 with little difference between the Siiltibcy and PPD injection sites.

Across the confirmatory studies, the proportion of subjects who experienced at least one systemic TEAE ranged from 28.4% to 32.4%, and those who had a systemic TEAE considered to be related to the skin test ranged from 2.8% to 8.3%. Most of the systemic TEAEs were mild to moderate in intensity with only a few being severe. Similarly, the incidence of systemic serious adverse events (SAEs) was low.

Table 63: Overview of Injection Site Reactions and Systemic Adverse Events in the Confirmatory Studies

Category Sub-Category	TESEC-05		TESEC-06		TESEC-07	
	n (%)					
Injection Site Reactions	Siiltibcy N = 1188	PPD N = 1190	Siiltibcy N = 978	PPD N = 929	Siiltibcy N = 307	PPD N = 303
All ISRs [a]	282 (23.7)	290 (24.4)	288 (29.4)	182 (19.6)	163 (53.1)	205 (67.7)
Serious ISRs	0	0	0	0	0	0
ISR severity						
Mild/moderate	271 (22.8)	282 (23.7)	287 (29.3)	180 (19.4)	153 (49.8)	194 (64.0)
Severe	11 (0.9)	8 (0.7)	1 (0.1)	2 (0.2)	10 (3.3)	11 (3.6)
Systemic TEAE	N = 1190		N = 979		N = 456	
All systemic TEAEs	338 (28.4)		317 (32.4)		147 (32.2)	
Serious systemic TEAEs	15 (1.3)		1 (0.1)		10 (2.2)	
Test-related systemic TEAEs	99 (8.3)		27 (2.8)		35 (7.7)	
Systemic TEAE severity						
Mild	299 (25.1)		263 (26.9)		116 (25.4)	
Moderate	52 (4.4)		91 (9.3)		41 (9.0)	
Severe	14 (1.2)		18 (1.8)		15 (3.3)	

Sources: TESEC-05 CSR Table 3.1, Table 3.3, Table 12-2, Table 12-5, Table 3.7, TESEC-06 CSR Table 12-1, Table 12-2, Table 123, Table 3.6, Table 3.7, TESEC-07 CSR, Table 12-2, Table 12-5, Table 3.1, Table 3.3.
[a] All injection site reactions were considered related to the skin test as defined in the study protocols.

CSR = Clinical Study Report; ISR = injections site reaction; N = number of subjects at risk; n = number of subjects with event; TEAE = treatment-emergent adverse event.

The data for ISRs has been pooled from the 3 confirmatory studies and is summarized by skin test site in the table below. The overall frequency of ISRs was similar for the Siiltibcy and PPD test sites. The most common ISRs were injection site pruritis, injection site pain, injection site rash, and injection site haematoma. The frequency of ISR preferred terms was similar for the Siiltibcy and PPD injection sites with the exception of Injection site haematoma where a larger number of patients reported this event at the Siiltibcy site compared with the PPD site (5.4% versus 0.8%, respectively).

Table 64: Summary of Injection Site Adverse Reactions – Studies TESEC-05, TESEC-06, TESEC-07 (Safety Population)

System Organ Class Preferred Term	Siiltibcy N = 2476 n (%)	PPD N = 2422 n (%)
General Disorders and Administration Site Conditions	733 (29.6)	677 (28.0)
Injection site pruritus	474 (19.1)	522 (21.6)
Injection site pain	182 (7.4)	165 (6.8)
Injection site rash	121 (4.9)	144 (5.9)
Injection site haematoma	133 (5.4)	20 (0.8)
Injection site vesicles	58 (2.3)	73 (3.0)
Injection site induration	25 (1.0)	14 (0.6)
Injection site erythema	10 (0.4)	13 (0.5)
Injection site ulceration	3 (0.1)	5 (0.2)
Injection site swelling	5 (0.2)	5 (0.2)
Injection site discolouration	3 (0.1)	5 (0.2)
Injection site haemorrhage	11 (0.4)	4 (0.2)
Injection site paraesthesia	0	1 (0.0)
Injection site papule	1 (0.0)	1 (0.0)
Injection site exfoliation	0	1 (0.0)
Injection site scar	0	1 (0.0)
Injection site movement impairment	0	1 (0.0)
Injection site scab	0	1 (0.0)
Injection site anaesthesia	2 (0.1)	0
Injection site oedema	1 (0.0)	0
Injection site urticaria	1 (0.0)	0
Injection site nodule	1 (0.0)	0

Source: SSI/TESEC/TESEC-06 - tab_ir_ISS_t5_t6_t7.sas/tab_ir_iss.txt/05dec2022
N = Number of injection sites, n = number of injection sites having an event.

Systemic treatment-emergent adverse events (TEAEs)

The data for systemic TEAEs have been pooled for studies TESEC-05, TESEC-06 and TESEC-07 (total population = 2476 subjects). As subjects received both Siiltibcy and PPD, the applicant assumed that any

systemic event was to be associated with Siiltibcy as a conservative “worst case scenario”; thus no comparative analysis of Siiltibcy versus PPD is provided.

A summary of the most common events (occurring in ≥ 1.0% of subjects) and their treatment-relatedness are presented in the Table below.

The majority of TEAEs were reported by fewer than 1% of subjects. The most frequent systemic TEAE (≥ 5.0%) was Headache (10.8%). The systemic TEAEs reflected common ailments in the general population and there were no trends or patterns to suggest any tolerability of safety issues with the administration of Siiltibcy or PPD skin tests.

The relationship of a TEAE to the skin test was assessed by the investigator who was blinded to the randomization code. In total, 142/2476 (5.7%) subjects had at least one skin test-related systemic TEAE (Table below). The most frequent skin test-related systemic TEAEs were Headache, Pyrexia, and Dizziness, which are commonly recognized as general side effects of injections.

Table 65: Summary of Common (≥ 1.0%) Treatment-Emergent Adverse Events and Treatment-Related Assessment in the Confirmatory Studies

System Organ Class Preferred Term	Safety population (Studies TESEC-05, TESEC-06, TESEC-07) (N = 2476)	
	Number (%) of subjects with TEAE	Number (%) of subjects with skin test-related TEAE
Subjects with any TEAE	746 (30.1)	142 (5.7)
Gastrointestinal disorders	106 (4.3)	8 (0.3)
Diarrhoea	32 (1.3)	4 (0.2)
General disorders and administration site conditions	111 (4.5)	31 (1.3)
Pyrexia	51 (2.1)	16 (0.6)
Infections and infestations	182 (7.4)	6 (0.2)
Nasopharyngitis	41 (1.7)	0
Influenza	24 (1.0)	1 (0.0)
Nervous system disorders	311 (12.6)	72 (2.9)
Headache	267 (10.8)	59 (2.4)
Dizziness	37 (1.5)	13 (0.5)
Reproductive system and breast disorders	37 (1.5)	1 (0.0)
Dysmenorrhoea	26 (1.1)	0
Respiratory, thoracic and mediastinal disorders	82 (3.3)	1 (0.0)
Cough	27 (1.1)	0

Sources: Pooled Data Tables 14.3.1 and 14.3.2.
N = total number of subjects in the analysis; TEAE = treatment-emergent adverse event.

2.6.8.3. Serious adverse event/deaths/other significant events

Deaths

Overall, three (n = 3) deaths were reported across all seven studies. These were all reported in study TESEC-05, which included subjects that had been diagnosed with HIV at inclusion/randomisation. All three cases were assessed as not related to the skin tests by both the investigator and sponsor. One subject (from here on referred to as Subject 1) was diagnosed with HIV with very low CD4+ T-cell count and was found dead. The other two subjects (from here on referred to as Subjects 2 and 3) died from advanced AIDS. All the 3 deaths occurred in subjects receiving both tests, Siiltibcy and PPD. All deaths are summarised in the Table 66.

Table 66: Listing of Deaths

Study	Anonymised subject ID	Dose	Diagnosis	Cause of Death	Other Medications	Other Medical Conditions	Location of Narrative Description
TESEC-05	1	Left: Siiltibcy 0.1 µg/0.1 mL Right: 2 T.U. PPD	Suspected to have TB	Most probably a result of a low $CD4^+$ T-cell count	Antiretroviral treatment started 1 day before death	HIV-positive at inclusion	CSR, Section 14
TESEC-05	2	Left: Siiltibcy 0.1 µg/0.1 mL Right: 2 T.U. PPD	Symptoms of pulmonary TB; sputum smear negative	Advanced AIDS	Not described	HIV-positive	CSR, Section 14
TESEC-05	3	Left: 2 T.U. PPD Right: Siiltibcy 0.1 µg/0.1 mL	Cough; sputum smear negative	Natural causes, advanced AIDS	Not described	HIV-positive	CSR, Section 14

Source: TESEC-05 CSR, Listing 4.0, Listing 9.1, and Listing 9.2.
AIDS = acquired immunodeficiency syndrome; HIV = human immunodeficiency virus; ID = identification; TB = tuberculosis; T.U. = tuberculin unit
Note: All deaths that occurred during the study period up to the follow-up visit are described in this table.

Subject 1: Subject 1 died approximately 2 weeks after receiving the skin tests. The subject was diagnosed with HIV-positive at V1. At V2 the subject's CD4+ count was low. Subject 1 started on anti-retro virus treatment one day before death. The following day Subject 1 had collapsed and passed away. The investigator assessed the death to be not related to the skin tests, but most probably a result of a low CD4+ count.

Subject 2: Subject 2 experienced AIDS approximately 2 days after the skin tests were administered. The subject was seen at V1, 19 days before the subject's death, where the subject appeared wasted and had symptoms of pulmonary TB but fully mobile. Subject 2 was confirmed to be HIV-positive and found to be sputum negative for pulmonary TB. At the day of administration of the skin tests the subject looked ill but was fully mobile and was eating and drinking. Subject 2 was administered the skin tests as the administrating physician felt it was clinically indicated and did not see any clinical contraindications. Subject 2 did not come for V3 and the site was informed that the subject had passed away the same afternoon. The reporting physician stated that the participant died from advanced AIDS and the death was unrelated to the skin tests.

Subject 3: Subject 3 developed CD4+ lymphocytes decreased, acute renal failure and liver function tests raised 8 days before death from natural causes. The skin tests were administered 15 days before the subject's death. The subject was HIV-positive with a CD4+ count of 43x10e6/L. Subject 3 had acute renal failure with hyperkalaemia and was admitted to the hospital. At the time of referral to hospital, the subject's lab tests were as follows: creatinine 1268 µmol/L and urea 49,2 mmol/L. Liver function tests were raised: ALT 46 U/L (normal range: 10 – 40 IU/L), AST 43 U/L (normal range: 15 – 40 U/L), ALP 276 U/L (normal range: 53 – 128 U/L) and GGT 477 U/L (normal range: 0 – 60 U/L). Subject 3 died at the hospital. Investigator assessed all events as not related to investigational product.

SAEs

A total of 36 SAEs were reported for 32 of 3109 (1.0%) subjects included in the seven clinical studies performed with Siiltibcy and/or PPD (Table 67).

Table 67: Summary of Incidence of Serious Adverse Events in Clinical Studies Contributing to the Safety Evaluation of Siiltibcy

Study	N	n (%)	Number of Related SAEs According to the Investigator
TESEC-01	42	0	0
TESEC-02	38	1 (2.6)	0
TESEC-03	151	0	0
TESEC-04	253	5 (2.0)	0
TESEC-05	1190	15 (1.3)	0
TESEC-06	979	1 (0.1)	0
TESEC-07	456	10 (2.2)	0
TESAT-01	35	0	0
TESAT-02	31	0	0
Total	3175	32 (1.0)	0

N = number of subjects in the safety set; n = number of subjects with a serious adverse event; SAE = serious adverse event.

Details of all SAEs including causality, severity, and outcome are summarised in next Table. None were considered related to the skin tests by the investigators. However, the sponsor (Statens Serum Institut [SSI]) disagreed on two of the SAEs, both in study TESEC-05, which they judged to be possibly related to the skin tests. These two events (described below) were unexpected and hence, represented SUSARs. Both subjects had recovered before the end of the study.

No SAEs were reported in the clinical studies performed with rdESAT-6 alone.

Table 68: Summary of All Serious Adverse Events Reported in Clinical Studies Contributing to the Safety Evaluation of Siiltibcy

Study / Location of Narrative Description	Treatment	SAE Preferred Term	Causality Assessment by Investigator	Severity	Outcome
TESEC-02 / Section 10.3.2	Siiltibcy	Blurred vision	Not related	Mild	Unknown
TESEC-04 CSR, Section 16.3.1	Siiltibcy and PPD	Hyperglycaemia	Not related	Severe	Recovered with sequelae
	Siiltibcy and PPD	Jaundice cholestatic	Not related	Severe	Recovered with sequelae
	Siiltibcy and PPD	CD4 lymphocytes decreased	Not related	Severe	Unknown
	Siiltibcy and PPD	Pleural effusion	Not related	Severe	Unknown
	Siiltibcy and PPD	Hepatitis	Not related	Severe	Not yet recovered
TESEC-05 / CSR, Section 14	Siiltibcy and PPD	Gastroenteritis	Not related	Severe	Recovered
	Siiltibcy and PPD	Atrial fibrillation	Not related	Moderate	Not recovered
	Siiltibcy and PPD	Pneumocystis jiroveci pneumonia	Not related	Severe	Recovered
	Siiltibcy and PPD	Disseminated tuberculosis	Not related	Severe	Recovered with sequelae
	Siiltibcy and PPD	Grand mal convulsion	Not related	Moderate	Not recovered
	Siiltibcy and PPD	Cryptococcosis[a]	Not related[b]	Severe	Recovered
	Siiltibcy and PPD	Sepsis	Not related	Severe	Not recovered
	Siiltibcy and PPD	Tuberculosis	Not related	Severe	Recovered
	Siiltibcy and PPD	Pneumonia[a]	Not related[b]	Severe	Recovered
	Siiltibcy and PPD	Death	Not related	Severe	Fatal
	Siiltibcy and PPD	End stage AIDS	Not related	Severe	Fatal
	Siiltibcy and PPD	Haemoptysis	Not related	Severe	Unknown
	Siiltibcy and PPD	Upper respiratory tract infection	Not related	Moderate	Recovered
	Siiltibcy and PPD	Death	Not related	Severe	Fatal
	Siiltibcy and PPD	Febrile convulsion	Not related	Severe	Recovered
	Siiltibcy and PPD	Malnutrition	Not related	Severe	Recovered
	Siiltibcy and PPD	Lobar pneumonia	Not related	Moderate	Recovered

Study / Location of Narrative Description	Treatment	SAE Preferred Term	Causality Assessment by Investigator	Severity	Outcome
TESEC-06 / CSR, Section 14	Siiltibcy and PPD	Aspartate transferase increased	Not related	Moderate	Not recovered
TESEC-07 / CSR, Section 14	Siiltibcy and PPD	Thrombocytopenia	Not related	Severe	Not recovered
	Siiltibcy and PPD	Intentional self-injury	Not related	Severe	Recovered
	Siiltibcy and PPD	Hallucination	Not related	Severe	Recovered with sequelae
	Siiltibcy	Pneumothorax	Not related	Severe	Recovered
	Siiltibcy	Pleural effusion	Not related	Severe	Recovered
	PPD	Liver function test abnormal	Not related	Severe	Outcome is unknown
	Siiltibcy	Liver function test abnormal	Not related	Severe	Recovered
	PPD	Treatment noncompliance	Not related	Moderate	Not recovered
	PPD	Cellulitis	Not related	Severe	Not recovered
	PPD	Pneumothorax	Not related	Severe	Not recovered
	PPD	Bronchopleural fistula	Not related	Severe	Not recovered
	Siiltibcy	Tubulointerstitial nephritis	Not related	Severe	Outcome is unknown

AIDS = acquired immunodeficiency syndrome; SAE = serious adverse event; SSI – Statens Serum Institut; SUSAR = suspected unexpected serious adverse reaction

[a] Reported as SUSAR

[b] Was judged as possibly related to the skin tests by the sponsor (SSI)

The narratives of the two subjects with SAEs that were considered "not related" by the Investigator but "possibly related" by the applicant are in the following paragraphs.

The first subject, had the following AEs: Tuberculosis aggravated-worsening of TB related symptoms, pleural infection, renal function abnormal, sepsis, pneumonia, vomiting, fever, chest pain, tachycardia, oesophagitis and anaemic.

The subject experienced tuberculosis aggravated (start 34 days after skin test administration), pleural infection (start 7 days after skin test administration), renal function abnormal, sepsis, and pneumonia (start 10 days after skin test administration) vomiting, fever, chest pain, and tachycardia (start 34 days after skin test administration) and oesophagitis plus anaemic on an unknown start date. The subject was referred to a pulmonology clinic for a pleural biopsy after the subject was found to have a pleural effusion on clinical examinations and chest radiograph, with a negative sputum auramine stain and GenXpert. Based on the result of the pleural biopsy the subject was diagnosed with pleural cryptococcosis and admitted to hospital 7 days after skin test administration for treatment and further investigations. The subject has a background of advanced retroviral disease (HIV disease with low CD4+ count) and was not on antiretroviral therapy. The subject's course in hospital was complicated by drip-side sepsis and renal dysfunction secondary to IV amphotericin B. Since a month the subject received 2 tablets of cotrimoxazole daily. The subject was discharged after 24 days in the hospital on their own request, and before the treating staff was completely satisfied with the subject's condition. During the days after discharge the subject was vomiting, had

retrosternal chest pain on eating and swallowing. The subject had a fever of 38.2 degrees and tachycardia (110/min). Chest examination revealed slight decreased breath sounds at right base, normal heart sounds and soft abdomen with no palpable organomegaly. Neurological examination was normal. The clinical concern was possible occult sepsis and oesophagitis/gastritis. Investigator indicated that the differential in a patient with profound immunosuppression was wide, including several infections of the upper gastrointestinal tract. The subject was readmitted to hospital 4 days after discharge for investigations and gastroscopy, unresolved drip-side sepsis and hospital acquired pneumonia from his previous admission. The subject was subsequently diagnosed with tuberculosis after pleural biopsy. The subject was further found to be anaemic and received a transfusion of 2 units of blood. Response to treatment was good and the subject was discharged 11 days later. The investigators considered the condition to be unrelated to the skin tests as it was a pre-existing condition of which signs were present at V1. Sponsor cannot exclude a possible relationship regarding the worsening of TB related symptoms and hence represented this case as a SUSAR. At the time of reporting, he had recovered.

The second subject had tuberculosis aggravated-worsening of TB related symptoms and community acquired pneumonia.

The subject experienced tuberculosis aggravated and pneumonia, approximately 7 days after the skin tests were administered. The subject was hospitalised quickly with worsening respiratory symptoms and fever. A chest X-ray showed a bilateral nodular infiltrate and a small right pleural effusion. The admitting diagnosis was community acquired pneumonia. The subject was treated with IV broad-spectrum antibiotics, and was investigated for active pulmonary tuberculosis. The GeneXpert result taken as part of the trial on the day of the skin test administration was positive for Mtb with rifampicin resistance not detected. A subsequent GeneXpert test showed a positive result on the day after hospitalisation and the subject was subsequently started on TB treatment. The subject responded well to treatment and was discharged after 3 days in hospital. At the time of reporting, the subject was on TB treatment and recovering at home. Afterwards the subject moved and was therefore lost to follow-up. The investigator stated that the SAE is not thought to be related to the investigational product. SSI cannot exclude a possible relationship and hence this case was represented as a SUSAR. At the time of reporting, the subject had recovered from pneumonia and the outcome for TB was unknown because the subject was lost to follow-up.

Possible Koch's reactions

For three SAEs (all in study TESEC-05), the Data Safety Monitoring Board (DSMB) raised concerns of involvement of a possible Koch's reaction.

A Koch reaction describes the development of immunopathology in a person or animal with TB, when an exaggerated immune response to Mtb is stimulated. It was described in subjects with TB disease when Robert Koch performed his original studies employing mycobacteria as a type of therapeutic vaccination. It has later been demonstrated in the mouse model of therapeutic vaccination (Taylor et al. 2003). Available animal data suggest that these reactions do not occur in mice latently infected with Mtb, suggesting that such reactions may correlate with high bacterial load.

In the Siiltibcy studies, the hypothetic mechanism was that an existing (known or unknown) Mtb caused inflammation process may be accentuated by dual skin testing (Siiltibcy and PPD). In the three cases identified by the DSMB, this would link the skin testing to the observed TEAEs making the TEAEs related.

One patient developed seizures 14 days after the skin test injections. The DSMB requested that the TB meningitis should be investigated and other likely causes for the seizures should be found before it could be concluded that there was no association with administration of PPD/Siiltibcy.

The second patient, a HIV-positive subject, developed pleural effusion 14 days after administration of the skin tests. Cryptococcus infection was diagnosed but the patient was still not well. It was not described whether TB was sufficiently ruled out. Again, a boosting with immunogenic antigens might have resulted in an inflammatory pulmonary response and pleural exudate.

The third patient was admitted with a potential diagnosis of community-acquired pneumonia. This patient most likely suffered from severe pulmonary TB. The DSMB queried if the worsening of his condition after immune stimulation by PPD/Siiltibcy could have contributed to the clinical deterioration.

The sponsor (SSI) requested and subsequently received additional information regarding these SAE cases from the national principal investigators in South Africa. Based on these responses, SSI decided that in two of the cases ("Cryptococcosis" and "Pneumonia"), an involvement of Koch's reaction could not be ruled out, given that both subjects were shown to have an active TB disease. SSI reported both cases as "possibly related" even though the site investigators maintained the "unrelated" assessment (see description above).

2.6.8.4. Laboratory findings

Generally, blood and urine samples for haematology, biochemistry, and urinalysis were collected at baseline and at the follow-up visit approximately 28 days after the skin test injections (after the second injection in studies TESEC-01 and TESAT-02). An additional sample four days (96 hours) after skin test administration was collected in study TESAT-01.

Since the protocols allowed investigators to enrol subjects with clinically insignificant abnormal haematology, biochemistry, and urinalysis results at baseline, the important information is therefore not whether a value is outside the reference intervals but clinically relevant changes from baseline values.

Blood samples were not collected from children below the age of five years in studies TESEC-05 and TESEC-06 due to ethical considerations.

TESEC-05: a total of 13 subjects had laboratory abnormalities subjects that were considered clinically significant. All were graded as mild or moderate and the majority were assessed as not related to the skin test. One laboratory test abnormality (transaminases increased) was assessed as possibly related to the skin tests. No abnormalities were considered serious.

TESEC-06: a total of four subjects who all received dual injections had laboratory abnormalities that were considered clinically significant. These cases are reported as systemic TEAEs. Of these, one subject had an SAE (Aspartate transferase increased) which was not considered by the investigator to be related to the skin test. The other clinically significant laboratory results, all graded as mild included: Transaminases increased (close contact group), which was assessed as probably related to the skin tests, and decreased blood iron (negative control group), and glucose increase (negative control group), which were assessed as not related to skin tests.

TESEC-07: in this study, any out-of-range laboratory values that were considered clinically significant were reported as systemic TEAEs. Two subjects had laboratory test abnormalities reported as SAEs (Liver function test abnormal and Thrombocytopenia).

2.6.8.5. Safety in special populations

Age

Siiltibcy is intended to be used as a diagnostic skin test in subjects of all age groups from the age of 28 days upwards. The dose of Siiltibcy will be 0.1 µg/0.1 mL, irrespective of age. Thus, the safety of Siiltibcy in different age groups is of special interest.

The occurrence of TEAEs according to different age groups was investigated mainly in study TESEC-05 (with some data also coming from TESEC-06) and a summary of the findings is shown in the next Tables 69 and 70.

Table 69: Incidence of Injection Site Adverse Reactions and Systemic Adverse Events by Age Group - Study TESEC-05 (Safety Population)

Age group		0 to 4 years N = 236 n (%)	5 to 17 years N = 366 n (%)	18 to 65 years N = 588 n (%)
ISR	Siiltibcy	21 (8.9)	100 (27.3)	161 (27.4)
	PPD	23 (9.7)	96 (26.2)	171 (29.1)
Systemic TEAE		71 (30.1)	61 (16.7)	206 (35.0)

Source: Study TESEC-05 CSR, Table 12-4
CSR = Clinical Study Report; ISR = injection site adverse reaction; N = number of subjects in the age group; n = number of subjects with the event; TEAE = treatment-emergent adverse event

Table 70: Incidence of injection site adverse reactions and systemic adverse events by age group – study TESEC-06 (safety population)

Age group		**0 to < 2 years N = 16 n (%)**	**2 to 4 years N = 19 n (%)**	**5 to 11 years N = 42 n (%)**	**12 to 17 years N = 44[a] n (%)**	**18 to 39 years N = 589[b] n (%)**	**40 to 65 years N = 267 n (%)**	**> 65 years N = 2 n (%)**
ISR	C-Tb	2 (12.5)	5 (26.3)	14 (33.3)	18 (40.9)	162 (27.5)	86 (32.2)	1 (50.0)
	PPD	1 (6.3)	1 (5.3)	9 (21.4)	12 (29.3)	89 (16.4)	69 (25.8)	1 (50.0)
Systemic TEAE		4 (25.0)	7 (36.8)	12 (28.6)	14 (31.8)	204 (34.6)	76 (28.5)	-

Sources: Study TESEC-06 CSR Table 3.2 and Table 3.7
[a] N = 41 for PPD ISRs; [b] N = 542 for PPD ISRs
CSR = Clinical Study Report; ISR = injection site adverse reaction; N = number of subjects in the age group; n = number of subjects with the event; TEAE = treatment-emergent adverse event

HIV status

In some countries with a high occurrence of TB, HIV infection rates are also high. As HIV infection influences a person's immune response, subjects with HIV-positive status are an especially vulnerable population. Thus, it is of critical importance to understand whether the HIV status of subjects influences the safety profile of the diagnostic skin test Siiltibcy.

In study TESEC-05, substantially more subjects with a HIV-negative status reported ISRs than HIV-positive subjects for both Siiltibcy and PPD arms (almost 10 percentage points of difference). The frequency rates of ISR were lower among subjects with unknown HIV status (for children less than 5 years of age, no blood sample for HIV determination was collected). It should be noted that all subjects with unknown HIV status with at least one ISR were children under the age of 5 years old.

Substantially more subjects with HIV-positive status reported systemic TEAEs than those with HIV-negative status (an almost 10% difference) as summarised in the Table 71.

Table 71: Incidence of Injection Site Adverse Reactions and Systemic Adverse Events by HIV Status - Studies TESEC-04 and TESEC-05 (Safety Population)

		HIV-negative n (%)	HIV-positive n (%)	HIV-unknown n (%)
TESEC-04		N = 153	N = 100	
ISRs	Siiltibcy	76 (49.7)	44 (44.0)	-
	PPD	97 (63.4)	53 (53.0)	-
Systemic TEAEs		54 (35.3)	44 (44.0)	-
TESEC-05		N = 730	N = 299	N = 161[a]
ISRs	Siiltibcy	212 (29.0)	57 (19.1)	13 (8.1)
	PPD	224 (30.7)	52 (17.4)	14 (8.7)
Systemic TEAEs		189 (25.9)	103 (34.4)	46 (28.6)

Sources: TESEC-04 CSR, Table 17 and Table 21; TESEC-05 CSR, Table 3.1 and Table 3.7.
CSR = Clinical Study Report; HIV = human immunodeficiency virus; ISR = injection site adverse reaction; N = number of subjects in the age group; n = number of subjects with the event; TEAE = treatment-emergent adverse event
[a] Subjects with unknown HIV status were predominantly children less than 5 years of age, from whom no blood sample for HIV determination was collected

No serious ISRs occurred in studies TESEC-04 and TESEC-05.

In study TESEC-05, 96.6% of all ISRs were graded as mild or moderate, and 14/1190 subjects (1.2%) had a severe ISR; the remainder were mild or moderate. The majority of systemic TEAEs were also graded as mild or moderate with only 14/1190 (1.2%) subjects experiencing at least 2 systemic TEAEs graded as severe. The frequency of sever systemic TEAEs was 0.5% (4/730 subjects) in the HIV-negative group, 2.7% (8/299 subjects) in the HIV-positive group, and 1.2% (2/161 subjects) in the HIV-unknown group.

In TESEC-04, the majority of ISRs were graded as mild or moderate. The severity of ISRs was similar in HIV-negative and HIV-positive subjects. At the Siiltibcy injection site, 1.6% (4/253 subjects) of ISRs were graded as severe and this was the same at the PPD injection site (1.6%: 4/253 subjects). In the HIV-negative group, 1.3% (2/153) of ISRs were graded as severe at the Siiltibcy injection site and this was the same at the PPD injection site 86.3% (1.3%:2/153). The corresponding results in the HIV-positive group were 2.0% (2/100 subjects) for the Siiltibcy injection site and the same for the PPD injection site (2.0%: 2/100).

Risk category of TB infection

Study TESEC-06 investigated the diagnostic performance of Siiltibcy in subjects with different exposure to TB index cases. The subjects were allocated to four different risk groups. Subjects in the negative control group had no history of exposure to a TB index case and had no signs or symptoms of TB. Subjects in the occasional contact group were in contact with a pulmonary TB index case between six hours/week and six hours/day. Subjects in the close contact group were in close contact with a pulmonary TB index case for more than six hours/day for at least five days. Subjects in the positive control group had confirmed TB disease within the last three years.

The table below summarises the incidences of ISRs and systemic TEAEs in relation to the different risk groups. The proportion of subjects with ISRs increased with increasing risk of Mtb infection and thus with increasing positive skin test outcome; the frequency of ISRs was 25.5% in the negative control group, 34.4% in the occasional contact group, 38.0% in the close contact group, and 50.5% in the positive control group. However, the proportion of subjects with systemic TEAEs in the negative control group was similar to the positive control group (49.8% and 46.5%, respectively) with a lower incidence of systemic TEAEs in the two contact groups (37.1% in the occasional contact group and 26.3% in the close contact group).

Almost all systemic TEAEs (95.3%) and ISRs (99.5%) were graded as mild or moderate. Overall, approximately 6% of systemic TEAEs were assessed as related to the skin tests. All ISRs were presumed to be related to the skin test.

Table 72: Incidence of Injection Site Adverse Reactions and Systemic Adverse Events by TB Risk Group - Study TESEC-06 (Safety Population)

	Negative Control N = 263 n (%)	Occasional Contact N = 299 n (%)	Close Contact N = 316 n (%)	Positive Control N = 101 n (%) e
ISR	67 (25.5)	103 (34.4)	120 (38.0)	51 (50.5)
Systemic TEAE	131 (49.8)	111 (37.1)	83 (26.3)	47 (46.5)

ISR = injection site adverse reaction; N = number of subjects in the TB risk group; n = number of subjects with the event; TEAE = treatment-emergent adverse event

Pregnancy

Siiltibcy has not been tested in pregnant women. Pregnant individuals were excluded from all clinical studies via inclusion/exclusion criteria.

Possible effects of Siiltibcy on pregnant female rats and the development of the embryo and foetus were analysed in the embryo-foetal development toxicity study V20365. Four repetitive subcutaneous injections of either Siiltibcy, using doses 100-fold stronger than a human dose, or a placebo formulation, 14 days before mating and on gestation days 0, 6 and 13, at 4 different locations on the back of the animals did not result in maternal or developmental toxicity that were considered to be related to Siiltibcy.

In total, 26 and 28 females in vehicle control and Siiltibcy treated groups, respectively, were pregnant at caesarean section. All dams had viable foetuses. No treatment-related differences were observed for the mean number of corpora lutea, implantation sites, pre and post implantation losses, alive and dead foetuses, early and late resorptions or affected implants. The sex ratio was similar among the two groups.

No statistically significant differences in the weights of the gravid uterus, carcass (terminal body weight minus gravid uterus weight), net weight change from Day 0 (carcass weight minus body weight on Day 0 of gestation), empty uterus weight, and ovaries weight were observed between the vehicle control and Siiltibcy group. Placental and foetal weights were similar in all groups.

No treatment-related effects were observed on visceral or skeletal malformations and variations.

Lactation

There is no information from clinical studies or literature regarding the presence of Siiltibcy in human milk, the effects on the breast-fed infant, or the effects of Siiltibcy on milk production.

2.6.8.6. Immunological events

None reported.

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

Interactions in general

No drug-drug or drug-food interaction studies have been performed with Siiltibcy. As the diagnostic skin test is applied locally into the skin, interactions with food and other orally applied drugs are considered very unlikely and therefore, such studies are considered unwarranted by the applicant.

Interaction with PPD

Although Siiltibcy will always be used alone in routine medical practice, the safety of concurrent administration of Siiltibcy and PPD in relation to the effect on local and systemic reactogenicity and the induration zone were assessed in the Phase 3 study TESEC-07 in alignment with scientific advice received. In this study, subjects were allocated in a 1:1:1 ratio to receive either Siiltibcy alone, Siiltibcy + PPD, or PPD alone. In subjects who received both tests, these were administered into different forearms.

When considering local and systemic adverse events, in subjects who received a single injection, more ISRs (68.5% and 51.0%, respectively) and more systemic TEAEs (37.6% and 32.0%, respectively) were reported in the PPD group compared with the Siiltibcy group. Overall, more subjects who received both injections or PPD alone (71.4% and 68.5%, respectively) reported ISRs compared to subjects who received Siiltibcy alone (51.0%). Fewer subjects who received both injections or Siiltibcy alone (27.3% and 32.0%, respectively) reported systemic TEAEs compared to subjects who received PPD alone (37.6%).

2.6.8.8. Discontinuation due to adverse events

Patients who discontinued from study were 25 (2.1%) in TESEC-05, 9 (0.9%) in TESEC-06 and 10 (2.2%) in TESEC-07.

2.6.8.9. Post marketing experience

The applicant stated that use of Siiltibcy is authorised in India, but no post-marketing data were provided as part of the MAA dossier.

2.6.9. Discussion on clinical safety

The safety of Siiltibcy was evaluated based on the totality of data available from all completed clinical studies. The safety database included seven clinical studies: two Phase 1 studies (TESEC-01 and TESEC-02), two Phase 2 studies (TESEC-03 and TESEC-04), one Phase 2/3 study (TESEC-07), and two Phase 3 studies (TESEC-05 and TESEC-06). In addition, data from two Phase 1 studies performed with a diagnostic skin test containing only rdESAT-6 as single recombinant protein (studies TESAT-01 and TESAT-02) are included in this safety analysis.

The safety dataset comprises all subjects enrolled and randomised to receive Siiltibcy who received at least one dose of study drug. The total number of subjects exposed to Siiltibcy, at the dose intended for marketing, is approximately 3000 (of which 2400 coming from the confirmatory trials TESEC-05, -06 and -07, representing the primary safety population). The age spanned from 6 weeks to 76 years, an acceptable range considered the sought indication. In particular, more than 100 subjects were 0 – 1 years old; most of the subjects were > 18 years old (n = 2386) and the subjects < 12 years were more than 550. Females and males were equally represented.

Most subjects identified themselves as of African ancestry (n = 1204), whereas those identifying as of white origin were 1030. HIV-positive subjects were 498, versus 1685 HIV-negative; however, a high number of subjects had an unknown HIV status (n = 926). Most subjects were vaccinated with the BCG (n = 1715) vs 941 without vaccine and 453 of unknown vaccinal status.

Regarding risk of Mtb exposure, there were 556 subjects classified as negative controls/healthy, 299 occasional contacts, 1406 as close contacts/suspected TB and 848 diagnosed with TB/positive controls. Only few patients discontinued the studies.

Since the skin test (Siiltibcy or PPD) was administered intradermally in the forearm, the applicant subdivided the AEs in local (ISRs) and systemic. Most subjects received both tests, Siiltibcy and PPD (each one on a different forearm).

Regarding the ISRs with Siiltibcy, the prevalence of these reactions ranged, among the studies, between 23.7% in TESEC-05 and 53.1% in TESEC-07. There was no evident and consistent difference in the ISR prevalence between Siiltibcy and PPD: in study TESEC-06, ISRs were more frequent with Siiltibcy compared to PPD (29.4 vs 19.6%) but in TESEC-07 the opposite was observed (53.1 vs 67.7%), whereas in TESEC-05 the prevalence was similar (23.7 vs 24.4%). The majority of ISRs were classified mild/moderate and only few were severe in intensity.

The most frequent ISR was pruritus, with a similar prevalence between Siiltibcy and PPD (19.1 vs 21.6%), followed by pain (7.4 vs 6.8%) and rash (4.9 vs 5.9%). Overall, thus, the prevalence of the main types of ISRs was similar between Siiltibcy and PPD. Only hematoma showed a substantial difference and was more frequent with Siiltibcy than PPD (5.4 vs 0.8%).

All systemic AEs were considered by the applicant due to Siiltibcy; this is an endorsed conservative approach, but it obviously might overestimate the number of AEs due to Siiltibcy. Systemic AEs had an overall similar prevalence across the three main studies (in term of subjects with event): from about 28% in TESEC-05 to 32% in TESEC-06 and -07. Those considered related to the study drug ranged from 2.8% in TESEC-06 to 8.3% in TESEC-05. Most were of mild intensity. The number of subjects with severe systemic AEs ranged from 4.14% in TESEC-05 to 10.20% in TESEC-07. The incidence of systemic AEs increased with higher risk of TB infection (as expected) and there was no significant difference in incidence between Siiltibcy and PPD.

The number of subjects (in the pooled pivotal trials database, n=2476) with any AE was 30.1%; subjects with AEs considered related to the skin test were the 5.7%. When analysed by SOC: Gastrointestinal AEs were 4.3% (0.3% considered related), mostly diarrhoea (1.3%); general disorders occurred in 4.5% of subjects (1.3% related), mostly were pyrexia (2.1%); infections and infestations were reported in 7.4% of subjects (0.2% related), mostly were Nasopharyngitis (1.7%) and Influenza (1.0%). Nervous system disorders AEs were particularly frequent and observed in 12.6% of subjects (2.9% considered related); they were mostly Headache (10.8% overall; 2.4% related) and Dizziness (1.5%). Dysmenorrhoea was observed in 1.5% of subjects (none related) and Respiratory, thoracic and mediastinal disorders AEs were present in 3.3% of subjects (but only in 1 subject was considered related).

Death occurred in 3 subjects, all in the study TESEC-05: all received Siiltibcy (2 a few days prior to death and 1 one month before). All were HIV-positive and in relatively poor general clinical conditions, with low CD4+ lymphocyte count. Cause of death was suspected TB in two out of three cases; clinical description of the events does not rise any convincing evidence for a causative role of the investigational product.

The number of subjects with SAEs was overall comparable among the main clinical studies (about 1 – 2%). Only in TESEC-06 (conducted in Spain) the prevalence was lower (0.1%, n = 1 out of 979). All the SAEs were considered not related to the study drug by the Investigator. However, for three SAEs, the applicant postulates occurrence of an excessive immune reaction to the Siiltibcy. In general, this tuberculosis-immune reconstitution inflammatory syndrome (TB-IRIS) may occur during or after completion of anti-TB therapy, and especially in HIV-infected subjects with low CD4+ cell counts who recently started antiretroviral treatment. Studies of T-cell responses to Mtb antigens in patients with TB-IRIS suggest that IFN-γ+ T-cells reactive with PPD may contribute to the immunopathogenesis of this condition. By analogy, it seems at least theoretically possible that immune stimulation due to the skin test might exacerbate or precipitate a systemic reaction in subjects with full-blown TB disease.

Regarding laboratory findings (noting that only a routinary blood assessment was performed, as expected given the nature of the products), in some subjects an increase in transaminases (AST or ALT) was observed; in few instances, these increases were deemed related to the skin tests (Siiltibcy or PPD or both). Overall, 36 liver enzyme events were reported in 35 (1.13%) of the 3109 subjects who participated in the seven TESEC trials; the events were mainly mild or moderate in severity and a potential relationship was identified only in few cases. These events are included on the 4.8 section of the SmPC and classified as rare.

The prevalence of the site reactions, according to age, in TESEC-05 was similar between Siiltibcy and PPD (about 27% of subjects experienced AEs in the age range 5 – 65 years). Systemic AEs were observed in 35%

of adult subjects. In the age group 0 – 4 years, the prevalence of systemic AEs was higher than that of local reactions (30.1% vs 8.9%).

HIV-positive subjects experienced less local reaction to either Siiltibcy or PPD. On the contrary, these subjects experienced more systemic reactions.

When studied by Mtb risk level, local ISRs frequency increased with the risk of being Mtb-infected (from 25.5% in Negative Control to 50.5% in Positive Control). This trend was also evident for systemic severe and serious AEs (see TESEC-07).

Pregnant women were excluded by all human studies of Siiltibcy. Animal studies have not shown alterations to reproductive organs and the foetus (see Non-Clinical AR). The applicant does not anticipate any effects during pregnancy, since systemic exposure to Siiltibcy is negligible. It should be noted that the phenol concentration in Siiltibcy is 0.5%, the same as the concentration accepted for PPD that is approved for women of childbearing potential.

Regarding lactation, no data are available on the presence of Siiltibcy in human milk and the possible effects on the breast-fed infant, or on milk production. Nevertheless, based upon the same reasoning as for pregnant women, the applicant does not foresee significant effects. As such, no particular safety risks with the administration of Siiltibcy to healthy pregnant women (or lactating women) are expected.

Since Siiltibcy is injected locally into the skin and the systemic exposure is expected to be very low, there should not be interactions with other products.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

2.6.10. Conclusions on the clinical safety

Overall, the safety profile of Siiltibcy, in particular the frequency of local injection reactions, is similar to that of its comparators TST and predicted to be manageable with routine pharmacovigilance measures. No noteworthy safety issues have been seen in the clinical trials. The SUSARs reported are not very likely related to the use of the product and can be probably attributed to the underlying conditions. Additionally, there are no different safety profiles depending on age of HIV or TB status.

Rare occurrence of an excessive immune reaction to the Siiltibcy, such as tuberculosis-immune reconstitution inflammatory syndrome (TB-IRIS) during or after completion of anti-TB therapy, and especially in HIV-infected subjects with low CD4+ cell counts who recently started antiretroviral treatment, was not observed in clinical trials, but the hypothesis may not be ruled out in subjects with full-blown TB disease.

In subjects with immune-related disease, the test might encounter performance issues (false-negative results) rather than safety issues. This has been reflected in the SmPC.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns
The applicant proposed the following summary of safety concerns in the RMP:

Important Identified Risk	• None

Important Potential Risk	• None
Missing information	• None

2.7.1.1. Discussion on safety specification

The applicant initially proposed to include anaphylactic reactions and medication errors as important potential risks in the RMP. For anaphylactic reactions the applicant's rational was that evidence from clinical and non-clinical studies, published literature and reports of anaphylaxis exist for PPD tuberculin skin tests. However, no cases of acute hypersensitivity reaction were reported in the clinical trials of Siiltibcy, nor anaphylaxis was reported in non-clinical studies.

The applicant's ground for including medication errors was the inaccuracy of the test results in case Siiltibcy is inadvertently administered subcutaneously or intramuscularly. There is the possibility of sensitisation to Siiltibcy as a result of repeat testing administered < 6 weeks apart, leading to a false positive test result, causing a lack of efficacy of the test. As per proposed PI, Siiltibcy is administered via intradermal injection: 0.1 mL of Siiltibcy are administered using a 1 mL syringe with a short-bevel 26-gauge size needle in the middle-third of the forearm. No cases of medication errors were observed in the clinical trials.

As per GVP mod. V rev 2 definition, the important potential risks to be included in the RMP are those that, if confirmed, would have an impact on the B/R balance of the medicinal product and would usually require further evaluation as part of the pharmacovigilance plan. For the risks of anaphylaxis and medication errors there are no grounds for the inclusion in the RMP safety specifications at this stage. The risk of medication errors is related to a potential lack of efficacy rather than a safety issue, and the administration technique is not deemed of such complexity that it requires specific minimisation measures beyond the PI. The risk of medication errors will be specifically revised in a dedicated section of the periodic safety update reports (PSURs), and this is considered sufficient. The risk of anaphylaxis has an impact on the b/r, but in consideration of the evidence collected so far (evidence from PPD, lack of it from Siiltibcy clinical and non-clinical trials), it does not seem to require specific additional pharmacovigilance activities nor risk minimisation measures beyond the wording in the PI. The applicant has been requested to consider Anaphylaxis as important potential risk for the scope of the PSUR, so as to periodically revise the collected post-marketing evidence and to provide a cumulative review in the due PSURs.

"Use in immunocompromised population and patients treated with immunosuppressants" and "Use in pregnant and breastfeeding women" were initially proposed from the applicant as missing information in the RMP. Population who had within 3 months prior to the day of inclusion been in treatment with a product which is likely to modify the immune response (e.g., immunoglobulin, systemic corticosteroids, methotrexate, azathioprine, cyclosporine or blood products) except for HIV treatment was an exclusion criterion in the clinical trials. The rational was that immunocompromised participants may have impaired immune responses and might affect the ability to form an induration response to Siiltibcy, thus causing an efficacy issue. Also, pregnant, breastfeeding, or planning to become pregnant during the study was an exclusion criterion.

As per GVP mod V rev.2 definition, missing information relevant for inclusion in the RMP refers to gaps in knowledge about the safety of a medicinal product for certain anticipated utilisation or for use in particular patient populations, for which there is insufficient knowledge to determine whether the safety profile differs from that characterised so far. The absence of data itself (e.g. exclusion of a population from clinical studies) does not automatically constitute a safety concern. The risk management planning should focus on situations

that might differ from the known safety profile. At this stage there are no sound grounds to include the use in pregnant or breastfeeding women as missing information, since there are no findings from non-clinical studies suggesting possible concerns in this special population. Animal studies have not apparently shown alterations of the foetus; moreover, systemic exposure to Siiltibcy is negligible. No data are available on the presence of Siiltibcy in human milk but, in consideration of the low systemic exposure, also for lactating women no important impact on breast-fed infant or on milk production is anticipated. No additional pharmacovigilance activities are deemed necessary to characterise the use in immunocompromised population, in patients treated with immunosuppressants and in pregnant and breastfeeding women at this stage. Routine pharmacovigilance is considered sufficient, and missing information can be removed from the safety concerns. The SmPC adequately instructs on the use of Siiltibcy in pregnant women.

2.7.1.2. Conclusions on the safety specification

No important identified or potential risk, nor missing information have been identified.

2.7.2. Pharmacovigilance plan

Routine pharmacovigilance activities are considered sufficient to monitor the safety profile of the product.

Based on the various available clinical studies, Siiltibcy was found to be safe and well tolerated.

The pooled safety analysis of Siiltibcy, including data on 3109 participants from 07 clinical trials (Phase 1 – Denmark and UK, Phase 2 – UK and South Africa and USA and Phase 3 – Spain and South Africa), did not report any safety concern.

Therefore, no additional pharmacovigilance activities were proposed such as non-clinical, clinical or epidemiological (non-interventional or interventional) studies that are imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation (key to benefit risk), specific obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances or required activities by the competent authority.

The PRAC, having considered the data submitted, was of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product.

2.7.3. Risk minimisation measures

The PRAC, having considered the data submitted was of the opinion that Routine risk minimisation activities as described in Part V.1 of the RMP are sufficient to manage the safety concerns of the medicinal product.

2.7.4. Conclusion

The CHMP considers that the risk management plan version 4.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant requested alignment of the PSUR cycle with the international birth date (IBD), which is 09.05.2022. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Siiltibcy (rdESAT-6 / rCFP-10) is included in the additional monitoring list as it is a new biotechnological active substance.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-risk balance

3.1. Therapeutic context

3.1.1. Disease or condition

The recommended indication of Siiltibcy is “as diagnostic aid for detection of Mycobacterium tuberculosis infection, including disease, in adults and children aged 28 days or older. This medicinal product is for diagnostic use only.”

Mtb normally enters the host by inhalation of infectious droplets from a contagious individual. In the lungs, the bacteria are taken up by phagocytic cells, but the bacteria are able to survive and undergo progressive

growth inside these cells, which is a troubling condition of Mtb infection. If the infection is not successfully contained by the host, then typical symptoms of active TB disease will develop, including persistent cough (often with blood in sputum), fever, pain in chest, weight loss, night sweats, and loss of appetite. As an approximation, the lifetime risk of infected individuals developing active TB disease is between 5% and 15% (Vynnycky and Fine 2000; WHO 2021). Individuals with HIV infection, or patients under immunosuppressive treatment, are at particular risk of developing TB disease when infected.

In adults and older children (over 5 years), the loss of containment by the host gives rise to typical symptoms, notably a persistent cough with blood in the sputum. As infants and younger children (below 5 years) are less likely to develop these typical symptoms but are at greater risk of a rapidly disseminating disease, clinical diagnosis of TB disease in this age group is more difficult.

3.1.2. Available therapies and unmet medical need

Current TB diagnostic recommendations by the WHO are as follows: in people screened positive for pulmonary or extrapulmonary TB a WHO recommended rapid diagnostic test (with or without resistance testing) should be performed[2]. Microscopic culture is recommended to control the treatment.

Rapid diagnostic test for latent TB but not for active disease, can be divided into skin tests such as PPD, which however does not discriminate between subjects vaccinated with BCG and Mtb infected, and IGRA methods, which usually have higher costs and requirements in terms of facilities, not adequate for in low- and middle-income countries.

Given the high prevalence of Mtb infection (especially in some geographical regions), the availability of screening test is pivotal and functional to the early diagnosis and treatment. Ideally, a screening test should have high sensitivity, specificity and easiness of use/access with low cost.

Siiltibcy can be used to detect past or present MTB infection and distinguish it from past BCG vaccination/contact that has so far been a diagnostic interference with TSTs. How the test is then used in the EU remains the decision of local recommendations.

Siiltibcy cannot be used as stand-alone tool for diagnosis of active tuberculosis disease. Interpretation of skin test results should consider the specific context of use and risk assessment, and could be complemented by radiography and other diagnostic evaluations.

3.1.3. Main clinical studies

The clinical development of Siiltibcy is based upon several clinical trials: the TESEC studies -05, -06 and -07 were Phase 2/3 or Phase 3 studies and are considered as the pivotal clinical trials to support the MAA.

The TESEC-05 was conducted in South Africa and enrolled more than 1000 subjects (including paediatric ones) with suspected TB disease or exposure to Mtb and 100 healthy paediatric subjects (aged 5 to 11 years) with no known exposure to Mtb and no signs or symptoms of TB. About 300 subjects were HIV-positive, and more than 700 were HIV-negative (plus other 160 with unknown HIV status). The main aims of TESEC-05

[2] "People screened positive for TB include adults and children with signs or symptoms suggestive of TB, with a chest X-ray showing abnormalities suggestive of TB, a positive mWRD used as a screening tool or positive C-reactive protein test (>5 mg/L) in PLHIV. A person with a positive mWRD used as a screening tool and a low pretest probability should be clinically assessed and, if deemed a presumptive TB patient, should have a repeat mWRD performed and follow Algorithm 1." WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detention, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.

were to provide safety data and support the use of Siiltibcy in all age groups and in HIV-positive subjects, and a comparison of the diagnostic performance of Siiltibcy to QFT and PPD.

The TESEC-06 was conducted in Spain, thus within a different epidemiological context and provided a EU sub-population. It enrolled almost 1000 subjects (aged 6 weeks to 65 years) in four different risk groups: Negative Control (with no history of exposure to TB and no signs or symptoms of TB), Occasional Contact; Close Contact; Positive Control (confirmed TB). The main scopes were to test weather Siiltibcy responder rates correlated with exposure to Mtb, providing evidence for the applicant's claim that Siiltibcy diagnoses infection with Mtb. Secondly, the trial provided safety data and efficacy data for comparison with PPD and QFT.

TESEC-07, conducted in South Africa, had 456 adult TB subjects, of which 90 HIV-positive. The main scope was to provide data to demonstrate that Siiltibcy induration responses are not affected by simultaneous administration of PPD (in the other arm, immediately one after another). Secondly, the trial provided safety data in TB subjects and data to compare Siiltibcy sensitivity with QFT.

3.2. Favourable effects

The positivity rate of Siiltibcy increased consistently with increasing exposure to Mtb, as expected. Siiltibcy sensitivity ranged from 68% to 83% across the studies.

The totality of evidence coming from the 3 pivotal studies identified as an advantage of Siiltibcy its easiness of use compared to QFT; a minor advantage compared to PPD (that is used with different thresholds based upon subjects' characteristics such as BCG vaccination and HIV status) is that Siiltibcy has a unique threshold for positivity in all subjects; a higher specificity compared to PPD has been shown only in the BCG vaccinated subpopulation.

In TESEC-06, a higher specificity of Siiltibcy compared to PPD is observed when used on BCG vaccinated subjects (Siiltibcy: 96.3% vs PPD: 66.7%).

In TESEC-05, the primary endpoint (Siiltibcy induration diameter in mm by age, HIV infection and CD4+ cells count) in the primary population (FAS) showed that the mean Siiltibcy induration diameter was 9.4 mm. Induration/responder rate is impacted by age (lower induration diameters with lower age), by HIV status (higher response in HIV-negative) and CD4+ count (decreased response rate of Siiltibcy for CD4+ count below 100 cells/µL).

A post-hoc analysis, performed after pooling appropriate populations from the 3 pivotal trials, showed that Siiltibcy diagnostic performance is overall comparable to PPD and QFT, thus demonstrating Siiltibcy clinical benefit. In particular, data confirm that Siiltibcy sensitivity is lower than PPD (in the comparison vs PPD, the lower limit of the 95% confidence interval of the difference between Siiltibcy and PPD sensitivity was slightly above -15%, i.e. -13.1%) and better than QFT, and Siiltibcy specificity could be better than both comparators. The concordance of Siiltibcy with both PPB and QFT was good. The positivity rates of Siiltibcy and PPD and QFT in the intended population (contacts of TB cases) were similar, further reassuring on Siiltibcy performance.

3.3. Uncertainties and limitations about favourable effects

The age cut-off in the indication wording is supported, in particular extrapolation of Siiltibcy diagnostic accuracy from adults to children (down to 28 days) and generalizability of Siiltibcy use in subjects older than 65 years of age.

Uncertainties remain due to the heterogenous population enrolled and to more limited data coming from the EU population.

In the HIV-positive subgroup the sensitivity of Siiltibcy compared to QFT was slightly lower. In the same population, Siiltibcy was less sensitive than PPD in patients with low CD4+ cells count.

When Siiltibcy specificity is calculated on the overall population (disregarding BCG vaccination status), the performance is comparable to PPD (Siiltibcy: 95.8% vs PPD: 93.4%), limiting the advantage of Siiltibcy over PPD to the BCG vaccinated subpopulation. However, a better performance in terms of specificity values of Siiltibcy versus PPD in the BCG-vaccinated is of limited magnitude thus difficult to be translated in terms of clinical relevance.

Evaluation of technical performance, as per relevant guideline should include observer's concordance of the induration diameter outcome carried out by two independent skilled investigators; this is lacking. However, acceptable data have been provided regarding reproducibility.

3.4. Unfavourable effects

The total number of subjects exposed to Siiltibcy, at the dose intended for marketing, is approximately 3000 (of which 2400 coming from the pivotal trials representing the primary safety population). The age spanned from 6 weeks to 76 years, which seems an acceptable range considered the sought indication. Almost all subjects enrolled completed the study.

Overall, the safety profile was favourable and adverse reactions are expected to be manageable with routinary pharmacovigilance measures.

Injection site reactions (ISRs) to Siiltibcy ranged from 23.7% in TESEC-05 to 53.1% to TESEC-07. The most frequent ISR was pruritus with similar prevalence between Siiltibcy and PPD, followed by pain and rash. Hematoma was more frequent with Siiltibcy than PPD. Most local reactions were mild and moderate in severity.

The prevalence of local reactions, according to age, in TESEC-05 was similar between Siiltibcy and PPD (about 27% of subjects experienced AEs in the age range 5 – 65 years). AEs by TB risk showed that local ISRs increased with the risk of being TB-infected (from 25.5% in Negative Control to 50.5% in Positive Control), as expected. This trend was not evident for systemic AEs.

Systemic AEs had an overall similar prevalence across the three main studies: from 28% in TESEC-05 to 32% in TESEC-06 and -07. Most AEs were of mild intensity, and the number of subjects with severe systemic AEs ranged from 4.14% in TESEC-05 to 10.2% in TESEC-07. Systemic AEs were observed in 35% of adult subjects. In the age group 0 – 4 years, the prevalence of systemic AEs was higher than that of local reactions (30.1% vs 8.9%). HIV-positive subjects experienced more systemic reactions (44% vs 35% in TESEC-04). Most systemic TEAEs were mild, and all related systemic TEAEs (about 5.7%) were considered by the applicant due to Siiltibcy.

The frequency of subjects with SAEs was comparable in TESEC-05 and -07 (about 1 – 2%), and very low in TESEC-06 (0.1%). All SAEs were considered not related to the study drug by the Investigator.

Across the three studies, three patients died (TESEC-05): all received Siiltibcy (2 a few days prior to death and 1 one month before). All were HIV-positive and in relatively poor general clinical conditions, with low CD4+ lymphocyte count. Cause of death was 'suspected TB' in two out of three cases; clinical description of the events does not raise any evidence for a causative role of the investigational product.

3.5. Uncertainties and limitations about unfavourable effects

Duration of the pivotal studies was short (approximately 30 days). However, given the nature of the products (single intra-dermal administration), this is considered adequate. Long-term effects are not deemed relevant for this product class.

For three SAEs, the applicant postulates occurrence of an excessive immune reaction to the Siiltibcy. In general, the tuberculosis-immune reconstitution inflammatory syndrome (TB-IRIS) may occur during or after completion of anti-TB therapy, and especially in HIV-infected subjects with low CD4+ cell counts who recently started antiretroviral treatment. By analogy, it seems at least theoretically possible that immune stimulation due to Siiltibcy might exacerbate a systemic reaction in subjects with full-blown (symptomatic) TB disease.

In pregnant and lactating women, no specific safety problems are expected from Siiltibcy and the similar PPD tests have proven to be safe in this population.

3.6. Effects table

Effects Table for Siiltibcy indicated in adults and children aged 28 days and older for diagnosis of infection with *Mycobacterium tuberculosis.*

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Sensitivity	Vs PPD	%	74.6	76.1		TESEC-05
Sensitivity	Vs QFT	%	74.6	70.6		TESEC-05
Sensitivity	Vs PPD	%	68	81		TESEC-06
Sensitivity	Vs QFT	%	68	82		TESEC-06
Sensitivity	Vs PPD	%	79.2	87.8		TESEC-07
Sensitivity	Vs QFT	%	79.2	69.7		TESEC-07
Sensitivity	Vs PPD	%	78.3	85.4		Pooled (TESEC-05 and -07)
Sensitivity	Vs QFT	%	78.3	68.5		Pooled (TESEC-05 and -07)
Specificity	Vs PPD	%	83	85		TESEC-06
Specificity	Vs QFT	%	81.7	75.3		TESEC-06
Specificity	Vs PPD	%	95.8	93.4		TESEC-07
Specificity	Vs QFT	%	96.6	96.2		TESEC-07
Unfavourable Effects						

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
ISRs	Injection site reaction	% of subjects	29.4	19.6	Range 23.7 - 53.1	TESEC-06
pruritus	Injection site	% of sub.	19.1	21.6		pivotal
pain	Injection site	% of sub.	7.4	6.8		
Any TEAE	systemic	% of sub.	30.1			pivotal
Headache		% of sub.	10.8			pivotal
SAEs		% of sub.	1 - 2			pivotal
Deaths		n	3			pivotal

Abbreviations: sub: subjects; n: number; vs: versus.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Siiltibcy skin test represents an alternative to other widely used tests for diagnosis of *M. tuberculosis* infection. A potential advantage is its easiness of use compared to QFT, whereas versus PPD possible advantages are a unique induration threshold for positivity in all subjects and higher specificity in the BCG-vaccinated subpopulation and eliciting a specific *M. tuberculosis* T-cell response excluding the nontuberculous mycobacteria.

Even if the initial statistical plan was not designed to calculate the sensitivity and specificity of Siiltibcy vs PPD and QFT as a primary endpoint, a post-hoc analysis, performed after pooling appropriate populations from the 3 pivotal trials, showed that Siiltibcy has a lower sensitivity than PPD but with a greater specificity, the latter being very useful in EU to test people coming from geographical regions at higher prevalence of TB or of environmental mycobacteria.

Overall, the safety profile of Siiltibcy was favourable and is predicted to be manageable, most local reactions were mild and moderate in severity, and most systemic TEAEs were mild. The dataset analysed was adequate for detection of non-rare events. Some concern is raised by very few cases of SAEs whose relationship with the study drug could not be ruled out (possible excessive immune stimulation).

3.7.2. Balance of benefits and risks

The clinical benefit of Siiltibcy based on diagnostic performance (sensitivity and specificity) with respect to reference comparators (PPD and QFT) is sufficiently supported. The safety profile was favourable and comparable to PPD in the target population.

3.8. Conclusions

The overall benefit/risk balance of Siiltibcy is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus decision that the benefit-risk balance of Siiltibcy is favourable in the following indication:

> Siiltibcy is indicated as a diagnostic aid for detection of *Mycobacterium tuberculosis* infection, including disease, in adults and children aged 28 days or older. This medicinal product is for diagnostic use only.

The CHMP therefore recommends the granting of the marketing authorisation, subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- Risk Management Plan (RMP)

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New active substance status

Based on the review of data on the quality, non-clinical and clinical properties of the active substances, the CHMP considers that, in comparison to Tuberculin PPD RT23 previously authorised as a medicinal product in

the European Union, both *Mycobacterium tuberculosis* derived antigens rdESAT-6 and rCFP-10 are to be qualified as a new active substances as they differ significantly in properties with regard to safety and/or efficacy (diagnostic performance) from the previously authorised substance on the bases of a combination of the following elements: molecular structure, nature of the source material, manufacturing process (rdESAT-6, indent 1) and source material, non-clinical and clinical evidence (rCFP-10, indent 3).

Paediatric data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan PIP P/0188/2016 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

5. Appendix

5.1. CHMP AR on New Active Substance (NAS) dated 17 October 2024