The study listed may include approved and non-approved uses, formulations or treatment regimens. The results reported in any single study may not reflect the overall results obtained on studies of a product. Before prescribing any product mentioned in this Register, healthcare professionals should consult prescribing information for the product approved in their country.

GSK Medicine: GSK 692342

Study Number: 116777 (TUBERCULOSIS-019)

Title: Evaluation of the kinetics of mRNA* expression after two doses of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 in healthy adults.
GSK 692342 (TB): GlaxoSmithKline (GSK) Biologicals' candidate recombinant *Mycobacterium tuberculosis* (*M. tuberculosis*) vaccine, adjuvanted.
**mRNA stands for messenger Ribonucleic Acid (RNA).*

Rationale: The aim of the study was to evaluate the kinetics of mRNA expression after two doses of the TB vaccine in healthy Bacille Calmette-Guérin (BCG)-primed, human immunodeficiency virus (HIV)-negative adults aged 18-50 years when administered according to a 0, 1 month schedule.
The study had an approximate duration of 210 days for all subjects.

Phase: II

Study Period: 21 August 2012 to

- 06 December 2012 (Last Subject Last Visit for the Day 47 time point)
- 24 May 2013 (Last Subject Last Visit for the Day 210 study end timepoint).

Study Design: Open label, mono-centric study

Centres: One centre in Belgium

Indication: TB disease in healthy adults aged 18 to 50 years.

Treatment: The study group was the TB Group. The TB Group included subjects aged 18 to 50 years of age and who received 2 doses of TB vaccine administered at Days 0 and 30. The TB vaccine was administered intramuscularly in the deltoid region of the arm.

Objectives[£]:
*Immunological research**

- To evaluate the kinetics of mRNA expression after two doses of the TB vaccine to identify the most informative time points for collection of whole blood samples.
- To compare the mRNA signatures in whole blood to Peripheral Blood Mononuclear cells (PBMCs) collected at predefined time points by transcriptome microarray analysis.
- To compare the use of transcriptome microarray and RNA sequencing in whole blood for selection of the most informative time point.

Immunogenicity

- To assess the immunogenicity of two doses of TB vaccine.

Safety

- To assess the safety of two doses of TB vaccine.

[£]No categorization was made between primary and secondary objectives in the study protocol, all objectives were classified as primary for this results reporting summary.
**RNA sequencing results were not available at the time of writing this summary. This CTRS will be updated when they become available.*

Primary Outcome Variable[£]:
Immunological research[X]

- Description of mRNA signatures in PBMCs:
  - Determined by transcriptome microarray.
  - Time points: prior to Dose 1 (Day 0) and post Dose 2 (Days 31 and 44).
- Description of mRNA signatures in whole blood samples:
  - Determined by transcriptome microarray analysis.
  - Determined by RNA sequencing*.
  - Time points: prior to Dose 1 (Day 0), post Dose 1 (Day 30) and post Dose 2 (Days 31, 37, 40, 44 and 47).

Immunogenicity

- Evaluation of interferon-γ (IFN-γ) secretion in serum[X]:
  - Determined by IFN-γ-specific antibody concentrations as measured by Enzyme-Linked Immunosorbent Assay (ELISA)[%].
  - Time points: prior to Dose 1 (Day 0), post Dose 1 (Day 30) and post Dose 2 (Days 31, 37, 40, 44 and 47).
- Evaluation of cell-mediated immune (CMI) responses with respect to components of the study vaccine[$]:

- Determined by the frequency of M72-specific Cluster of Differentiation (CD)4+/CD8+ T cells per million cells identified after *in vitro* stimulation, as expressing at least 2 immune markers among interleukin (IL)-2 (IL-2), tumour necrosis factor-α (TNF-α), IFN-γ, cluster of differentiation-40-ligand (CD40L), IL-13 and IL-17.
- Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after *in vitro* stimulation, as expressing any combination of immune markers among IL-2, TNF-α, IFN-γ, CD40L, IL-13 and IL-17.
- Time points: prior to Dose 1 (Day 0), post Dose 2 (Day 60).

Safety

- Occurrence of serious adverse events (SAEs).
  - During the entire study period.
- Occurrence of solicited local and general adverse events (AEs).
  - During the 7-day follow-up period following vaccination (day of vaccination and 6 subsequent days after each vaccine dose).
- Occurrence of unsolicited AEs.
  - During the 30-day follow-up period following vaccination (day of vaccination and 29 subsequent days after each vaccine dose).
- Occurrence of all potential immune-mediated disorders (pIMDs).
  - During the entire study period.

[x]Please note that immunological research and IFN-γ immunogenicity outcomes and results refer to readouts that have not been fully validated according to International Conference of Harmonization (ICH) guidelines.
[£]No categorization was made between primary and secondary outcomes in the study protocol. As such, all outcomes were classified as primary for this results reporting summary.
**RNA sequencing results were not available at the time of writing this summary. This CTRS will be updated when they become available.*
[%]Analysis of IFN-γ-specific antibody concentrations was performed by cytometric bead array (CBA) assay, due to the higher sensitivity as compared to ELISA.
[$]Please note that the evaluation of CMI responses to the TB vaccine did not include assessment of a possible correlation between CMI outcome and protection against tuberculosis.

Secondary Outcome Variable(s): Not applicable

Statistical Methods: The analyses were performed on the Total Vaccinated cohort and on the According-to-protocol (ATP) cohort for analysis of immunogenicity:

- The Total Vaccinated cohort included all subjects with at least one vaccine administration documented.
- The ATP cohort for analysis of immunogenicity included all evaluable subjects included in the Total Vaccinated cohort who met all eligibility criteria, who received all vaccinations according to protocol procedures within specified intervals, who complied with blood sampling schedules and for whom post vaccination blood samples were available.

mRNA immunological research analysis

The analysis was performed on the ATP cohort for immunogenicity.
The transcriptome microarray analysis data of the PBMC and whole blood (WB) mRNA samples were normalized independently by GC-Robust Multiarray Averaging (GCRMA) normalization. The resulting probesets corresponding to the gene clusters were then used to identify the presence of 2 groups of subjects on available time points post Dose 2 (Days 37, 40, 44, 47). This methodology was applied for the analysis of both PBMC and whole blood (WB) samples. The gene expression intensities of the genes in the clusters on a particular day were made relative to the expression values on Day 0 by subtracting the Day 0 values from the Day x values. Subjects were assigned to one of the 2 groups based on whether the majority of the genes in a cluster were higher or lower than Day 0 in Cluster I, and the inverse (lower or higher than Day 0) in Cluster II. The list of genes per Cluster was tabulated.
Once all subjects were assigned to a group, a one sided Student-T test was performed per Cluster using all the genes in the Cluster and the group assignment information to test whether the groups were significantly different. The validity of this approach was tested via Monte Carlo simulation by repeating the procedure using random data based on the distribution of the expression values relative to Day 0 on time point Day x. Random data were generated, the group assignment was performed and a one sided Student-T test was performed with an α of 0.05. The uncorrected acceptance of the alternative hypothesis of differences between the groups was counted. The Monte Carlo procedure was repeated 2500 times.
Furthermore, the robustness of the approach was tested using a resampling approach. Artificial datasets of the same size as the original dataset were created by randomly selecting subjects with replacement. The group assignment was again performed and a one sided Student-T test was performed with an α of 0.05. The acceptance of the alternative hypothesis of

differences between the groups was counted. The resampling procedure was repeated 2500 times.

Immunogenicity analysis
The analysis was performed on the ATP cohort for immunogenicity.
For IFN-γ secretion in serum, Geometric Mean Concentrations (GMCs) measure by CBA assay and seropositivity rates with 95% confidence intervals (CIs) were tabulated at each schedule time point. A seropositive subject was defined as a subject with IFN-γ concentration by CBA assay ≥ 7047 fg/mL.
Descriptive statistics of the frequency of M72-specific CD4+/CD8+ T-cells expressing at least two different immune markers among IFN-γ and/or IL-2 and/or TNF-α and/or CD40-L and/or IL-13 and/or IL-17 were tabulated at each scheduled time point.
Descriptive statistics of the frequency of M72-specific CD4+/CD8+ T-cells expressing any combination of immune markers among CD40L, IFN-γ, IL-2, TNF-α, IL-13 and IL-17 were tabulated at each scheduled time point.

Safety analysis
The analysis was performed on the Total Vaccinated cohort.
The number and percentage of subjects reporting each individual solicited local (any and grade 3) and general (any, related to vaccination and grade 3) symptom during the 7-day (Days 0-6) follow-up period following vaccination was tabulated with exact 95% CI by dose and across doses.
The number and percentage of subjects with at least one unsolicited AE reported during the 30-day follow-up period (Days 0-29) and classified by Medical Dictionary for Regulatory Activities (MedDRA) preferred terms were tabulated.
The percentage of subjects with SAEs, fatal SAEs and SAEs assessed by the investigator as related to study vaccination, reported during the entire study period and classified by MedDRA preferred terms was tabulated. Tabulation was also performed for the percentage of subjects with pIMDs reported during the entire study period and classified by MedDRA preferred terms.

Study Population: Healthy male and female subjects between, and including, 18 to 50 years of age at the time of study start were enrolled in this study. To be included subjects were to be known as having previously received BCG vaccination or to have a BCG scar, to be seronegative for HIV-1. Female subjects were to be of non-childbearing potential or if of childbearing potential had to practice adequate contraception for 30 days prior to vaccination, had a negative pregnancy test and continued such precautions for the entire duration of the study and for 2 months after completion of the vaccination series. Subjects were excluded from study participation if they had any history of tuberculosis disease, history of reaction or hypersensitivity to any component of the TB vaccine or history of medically confirmed autoimmune disease or a positive Quantiferon™ test result. Written informed consent obtained from subjects prior to any study procedure.

Number of Subjects:	**TB Group**
Planned, N	20
Randomised, N (Total Vaccinated cohort)	20
Completed, n (%)	20 (100)
Total Number Subjects Withdrawn, N (%)	0 (0.0)
Withdrawn due to Adverse Events n (%)	0 (0.0)
Withdrawn due to Lack of Efficacy n (%)	Not Applicable
Withdrawn for other reasons n (%)	0 (0.0)
Demographics	**TB Group**
N (Total Vaccinated cohort)	20
Sex, n (%) Females	13 (65.0)
Males	7 (35.0)
Mean Age, years (SD)	33.7 (11.36)
Median	28.5
Minimum, Maximum	18-50
Race, n (%) African heritage / African American, n (%)	10 (50.0)
White - Caucasian / European heritage, n (%)	9 (45.0)
Asian - East Asian heritage, n (%)	1 (5.0)

Primary Outcome Results: mRNA gene signatures by transcriptome microarray analysis: description of gene clusters (ATP cohort for immunogenicity)

Gene	**Gene Cluster**
ATL2	I
C14orf64	I
C1orf26	I

CCT6P1 /// LOC643180	I
CNPY4	I
DENND4C	I
EXOSC6	I
FBXO9	I
HECTD1	I
IPW	I
LMLN	I
LOC100134017	I
NAP1L5	I
OR2A20P /// OR2A5 /// OR2A9P	I
ORC2L	I
PDCD4	I
PDZK1	I
PLA2G12A	I
PUS7L	I
RAPH1	I
RMND5A	I
TAF1	I
TRIM52	I
TTC33	I
USP40	I
BAG1	II
C19orf63	II
EIF4E2	II
FAM21A /// FAM21B /// FAM21C /// FAM21D	II
FAM53C	II
GADD45B	II
GTF2E2	II
HLA-A	II
HLA-B	II
HLA-C	II
HLA-DMB	II
HSP90B1	II
IDH3G	II
IRF7	II
KLHL6	II
LOC161527 /// PML	II
LOC648998	II
MT1F	II
MT2A	II
MYD88	II
NAPRT1	II
NCAPH2	II
NSD1	II
POLE4	II
PTPN6	II
RBBP6	II
RHBDF2	II
RNASEH2C	II
RNF31	II
SCO2	II
SP110	II
TAX1BP1	II
TNIP2	II

TRIM26	II
WAS	II
WWP2	II
ZFP36	II

Primary Outcome Results: mRNA gene signatures by transcriptome microarray analysis: Statistical evaluation of group assignment for PBMC and WB samples in GCRMA normalized microarray data (ATP cohort for immunogenicity)

Origin of Sample	Day	Cluster	p-value Student T-test	Monte Carlo success (%)	Resampling success (%)
PBMC	44	I	$1.80 \cdot 10^{-5}$	2.32	95.3
PBMC	44	II	$1.51 \cdot 10^{-18}$	2.44	100
WB	37	I	$1.31 \cdot 10^{-1}$	2.24	29.6
WB	37	II	$1.33 \cdot 10^{-12}$	2.68	94.3
WB	40	I	$5.65 \cdot 10^{-1}$	1.68	8.28
WB	40	II	$6.31 \cdot 10^{-35}$	1.80	100
WB	44	I	$9.99 \cdot 10^{-1}$	2.36	0.200
WB	44	II	$1.20 \cdot 10^{-34}$	2.20	100
WB	47	I	$4.05 \cdot 10^{-1}$	1.68	8.20
WB	47	II	$2.77 \cdot 10^{-15}$	2.00	99.4

Sample = mRNA origin PBMCs (Peripheral Blood Mononuclear cells) or whole blood (WB)
Day = study day
p-value Student T-test = p-value associated with the difference between the obtained groups in Clusters I and II. A p-value < $5 \cdot 10^{-2}$ was considered statistically significant.
Monte Carlo success (%) = Percentage of incorrect acceptance of the incorrect alternative hypothesis that there was a difference between the groups based on random data.
Resampling success (%) = Percentage of acceptance of the alternative hypothesis that there was a difference between the groups based on resampled data.

Primary Outcome Results: Seropositivity rates and GMCs for IFN-γ in serum in the TB Group by CBA assay (ATP cohort for immunogenicity)

			≥ 7047 fg/mL				GMC (fg/mL)		
					95% CI			95% CI	
Antibody	Timing	N	n	%	LL	UL	value	LL	UL
IFN-γ protein	PRE	18	9	50.0	26.0	74.0	6279.7	4624.2	8527.7
	PI(D30)	18	10	55.6	30.8	78.5	7035.1	5089.0	9725.5
	PII(D31)	17	16	94.1	71.3	99.9	64288.5	33671.9	122743.5
	PII(D37)	18	17	94.4	72.7	99.9	32710.5	23747.1	45057.0
	PII(D40)	18	10	55.6	30.8	78.5	8851.2	5250.8	14920.4
	PII(D44)	18	10	55.6	30.8	78.5	6755.1	4939.5	9238.0
	PII(D47)	17	11	64.7	38.3	85.8	7245.0	5389.6	9739.3

Seropositive rate = percentage of subjects whose IFN-γ concentration by CBA assay ≥ 7047 fg/mL
GMC = geometric mean antibody concentration calculated on all subjects
N = number of subjects with available results
n/% = number/percentage of subjects with concentration equal to or above specified value
95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit
PRE = Prior Dose 1 (Day 0)
PI(D30) = Post Dose 1 (Day 30)
PII(D31) = Post Dose 2 (Day 31)
PII(D37) = Post Dose 2 (Day 37)
PII(D40) = Post Dose 2 (Day 40)
PII(D44) = Post Dose 2 (Day 44)
PII(D47) = Post Dose 2 (Day 47)

Primary Outcome Results: Descriptive statistics for the frequency of M72-specific CD4+ T-cells expressing at least 2 immune markers among CD40L, IL-2, TNF-α, IFN-γ, IL-13 and IL-17 in the TB Group (ATP cohort for immunogenicity)

Immune marker	Timing	N	Nmiss	Mean	SD	Min	Q1	Median	Q3	Max
CD4.polypositives CD40L + IL-2 + TNF-α + IFN-γ + IL-17 + IL-13	PRE	13	5	253.7	363.4	1.0	1.0	97.0	224.0	1045.0
	PII(M2)	12	6	6905.3	5019.7	485.0	3384.5	5200.0	10781.5	15302.0

N = number of subjects with available results
Nmiss = number of subjects with missing results

SD = standard deviation
Q1, Q3 = first and third quartiles
Min/Max = minimum/maximum
PRE = Prior Dose 1 (Day 0)
PII(M2) = Post Dose 2 (Day 60)

Primary Outcome Results: Descriptive statistics for the frequency of M72-specific CD8+ T-cells expressing at least 2 immune markers among CD40-L, IL-2, TNF-α, IFN-γ, IL-13 and IL-17 in the TB Group (ATP cohort for immunogenicity)

Immune marker	Timing	N	Nmiss	Mean	SD	Min	Q1	Median	Q3	Max
CD8.polypositives CD40L + IL-2 + TNF-α + IFN-γ + IL-17 + IL-13	PRE	13	5	502.4	1023.2	1.0	2.0	95.0	230.0	3510.0
	PII(M2)	12	6	763.3	1460.2	1.0	54.0	134.0	635.5	4866.0

N = number of subjects with available results
Nmiss = number of subjects with missing results
SD = standard deviation
Q1, Q3 = first and third quartiles
Min/Max = minimum/maximum
PRE = Prior Dose 1 (Day 0)
PII(M2) = Post Dose 2 (Day 60)

Primary Outcome Results: Descriptive statistics of the frequency of M72-specific CD4+ T-cells expressing any combination of immune markers among CD40L, IL-2, TNF-α, IFN-γ, IL-17 and IL-13 in the TB Group (ATP cohort for immunogenicity)

Immune marker	Timing	N	Nmiss	Mean	SD	Min	Q1	Median	Q3	Max
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(+)+IL-2(+)+TNFα(+)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	16.69	26.03	1	1.0	1.0	26.0	82
	PII(M2)	12	6	25.08	36.17	1	1.0	14.0	28.5	126
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	22.50	19.78	1	7.5	21.5	28.5	70
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	114.23	120.63	1	43.0	96.0	115.0	441
	PII(M2)	12	6	995.25	677.36	88	517.5	990.0	1344.5	2399
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	2.08	3.75	1	1.0	1.0	1.0	14
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	13.85	20.33	1	1.0	1.0	14.0	54
	PII(M2)	12	6	17.83	23.77	1	1.0	7.5	24.0	70
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	2.00	3.61	1	1.0	1.0	1.0	14
	PII(M2)	12	6	111.25	166.28	1	13.5	28.5	152.0	489
CD4.CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(-)+IL-13(-)	PRE	13	5	29.15	64.17	1	1.0	1.0	14.0	232
	PII(M2)	12	6	2624.42	2422.93	143	1084.5	1830.5	3845.5	8049
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	1.92	3.33	1	1.0	1.0	1.0	13
	PII(M2)	12	6	3.33	8.08	1	1.0	1.0	1.0	29
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	6.58	12.23	1	1.0	1.0	7.5	42
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	6.08	8.81	1	1.0	1.0	13.0	29
	PII(M2)	12	6	161.50	115.73	14	86.0	142.5	228.0	431
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	4.15	8.14	1	1.0	1.0	1.0	28
	PII(M2)	12	6	5.75	15.52	1	1.0	1.0	1.5	55
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	13.69	16.35	1	1.0	14.0	17.0	56
	PII(M2)	12	6	9.17	15.37	1	1.0	1.0	13.5	52
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	47.42	79.72	1	1.0	7.0	55.5	257
CD4.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(-)	PRE	13	5	22.23	23.51	1	1.0	15.0	42.0	68
	PII(M2)	12	6	1306.67	985.61	14	561.5	1202.5	1827.5	3348
CD4.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1

CD4.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	3.00	4.88	1	1.0	1.0	1.0	14
	PII(M2)	12	6	6.25	14.58	1	1.0	1.0	1.0	51
CD4.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	10.17	13.47	1	1.0	1.0	15.5	42
CD4.CD40L(+)+IL-2(-) +TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	45.15	107.12	1	1.0	13.0	14.0	388
	PII(M2)	12	6	342.33	275.02	14	94.5	300.0	529.5	837
CD4.CD40L(+)+IL-2(-) +TNF-α(+)+IFN-γ(-) +IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(+)+IL-2(-) +TNF-α(+)+IFN-γ(-) +IL-17(+)+IL-13(-)	PRE	13	5	11.23	13.82	1	1.0	1.0	14.0	42
	PII(M2)	12	6	25.17	23.69	1	13.5	18.5	28.0	86
CD4.CD40L(+)+IL-2(-) +TNF-α(+)+IFN-γ(-) +IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	18.25	30.34	1	1.0	1.0	28.0	86
CD4.CD40L(+)+IL-2(-) +TNF-α(+)+IFN-γ(-) +IL-17(-)+IL-13(-)	PRE	13	5	18.62	22.40	1	1.0	1.0	43.0	58
	PII(M2)	12	6	753.75	597.57	43	230.5	666.5	1220.5	1861
CD4.CD40L(+)+IL-2(-) +TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	2.08	3.75	1	1.0	1.0	1.0	14
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	16.15	25.14	1	1.0	1.0	14.0	74
	PII(M2)	12	6	117.17	106.84	1	40.5	87.0	179.5	347
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	6.46	13.78	1	1.0	1.0	1.0	45
	PII(M2)	12	6	10.67	18.22	1	1.0	1.0	14.0	65
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	13.83	22.54	1	1.0	1.0	22.5	71
CD4.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(-)	PRE	13	5	284.00	494.11	1	1.0	1.0	227.0	1590
	PII(M2)	12	6	1508.00	1355.78	1	80.5	1198.5	2845.5	3373
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	3.00	4.88	1	1.0	1.0	1.0	14
	PII(M2)	12	6	3.17	5.06	1	1.0	1.0	1.0	14
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	9.69	14.76	1	1.0	1.0	17.0	45
	PII(M2)	12	6	46.58	51.48	1	1.0	28.0	78.0	159
CD4.CD40L(-)+ IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	10.00	18.68	1	1.0	1.0	14.0	66
	PII(M2)	12	6	4.25	5.88	1	1.0	1.0	7.5	14
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	3.17	5.06	1	1.0	1.0	1.0	14
CD4.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(-)+IL-13(-)	PRE	13	5	9.92	17.89	1	1.0	1.0	14.0	66
	PII(M2)	12	6	141.42	167.31	1	20.0	69.0	225.5	531
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	4.15	11.37	1	1.0	1.0	1.0	42
	PII(M2)	12	6	3.75	9.53	1	1.0	1.0	1.0	34
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	5.15	6.50	1	1.0	1.0	14.0	16
	PII(M2)	12	6	17.67	38.26	1	1.0	1.0	14.0	136
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1

γ(-)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	8.23	8.90	1	1.0	1.0	14.0	28
γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	11.67	10.85	1	1.0	13.5	18.0	29
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	4.25	5.88	1	1.0	1.0	7.5	14
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	25.46	62.15	1	1.0	1.0	1.0	223
γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	82.50	92.68	1	1.0	66.0	121.0	279
CD4.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	9.08	15.82	1	1.0	1.0	13.0	54
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	2.08	3.75	1	1.0	1.0	1.0	14
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	59.38	129.64	1	1.0	1.0	28.0	371
γ(+)+IL-17(-)+IL-13(-)	PII(M2)	12	6	127.75	178.68	1	29.0	55.5	184.0	629
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	11.77	22.65	1	1.0	1.0	13.0	82
γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	26.42	24.66	1	7.0	28.0	36.0	88
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	2.00	3.61	1	1.0	1.0	1.0	14
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	2.08	3.75	1	1.0	1.0	1.0	14
CD4.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	60.46	62.89	1	1.0	48.0	90.0	196
γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	213.33	176.99	1	75.5	163.0	383.0	517
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	2.33	4.62	1	1.0	1.0	1.0	17
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	4.38	12.20	1	1.0	1.0	1.0	45
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	7.67	10.84	1	1.0	1.0	13.5	29
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(+)	PII(M2)	12	6	4.50	6.37	1	1.0	1.0	7.5	17
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	48.00	59.98	1	6.0	28.0	56.0	194
γ(+)+IL-17(-)+IL-13(-)	PII(M2)	12	6	72.25	100.69	1	1.0	15.0	145.5	278
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(-	PRE	13	5	18.85	26.88	1	1.0	13.0	16.0	82
)+IL-17(+)+IL-13(-)	PII(M2)	12	6	25.83	32.61	1	1.0	20.5	34.0	111
CD4.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(-	PRE	13	5	21.77	30.74	1	1.0	1.0	25.0	83
)+IL-17(-)+IL-13(+)	PII(M2)	12	6	23.42	35.08	1	1.0	1.0	50.0	85

N = number of subjects with available results
Nmiss = number of subjects with missing results
SD = standard deviation
Q1, Q3 = first and third quartiles
Min/Max = minimum/maximum
PRE = Prior Dose 1 (Day 0)
PII(M2) = Post Dose 2 (Day 60)

Primary Outcome Results: Descriptive statistics of the frequency of M72-specific CD8+ T-cells expressing any combination of immune markers among CD40L, IL-2, TNF-α, IFN-γ, IL-17 and IL-13 in the TB Group (ATP cohort for immunogenicity)

Immune marker	**Timing**	**N**	**Nmiss**	**Mean**	**SD**	**Min**	**Q1**	**Median**	**Q3**	**Max**
CD8.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	2.50	5.20	1	1.0	1.0	1.0	19
CD8.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	12.38	20.18	1	1.0	1.0	23.0	66

γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	23.58	34.86	1	1.0	1.0	42.0	113
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	4.77	13.59	1	1.0	1.0	1.0	50
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	2.92	6.93	1	1.0	1.0	1.0	26
γ(+)+IL-17(-)+IL-13(-)	PII(M2)	12	6	8.08	13.39	1	1.0	1.0	10.0	36
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	2.92	6.93	1	1.0	1.0	1.0	26
γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(+)+IFN-	PRE	13	5	2.23	4.44	1	1.0	1.0	1.0	17
γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.08	0.28	1	1.0	1.0	1.0	2
γ(+)+IL-17(-)+IL-13(-)	PII(M2)	12	6	4.83	13.28	1	1.0	1.0	1.0	47
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	2.08	3.30	1	1.0	1.0	1.0	13
γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(+)+IL-2(-)+TNF-α(-)+IFN-	PRE	13	5	62.15	83.01	1	1.0	6.0	128.0	212
γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	42.33	81.89	1	1.0	1.0	43.5	259
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	2.42	4.91	1	1.0	1.0	1.0	18
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	48.31	133.63	1	1.0	1.0	1.0	485
γ(+)+IL-17(-)+IL-13(-)	PII(M2)	12	6	52.67	151.66	1	1.0	1.0	22.5	532
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	6.69	14.73	1	1.0	1.0	1.0	50
γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	2.75	6.06	1	1.0	1.0	1.0	22
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1

CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	4.46	12.48	1	1.0	1.0	1.0	46
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	28.31	58.50	1	1.0	1.0	1.0	194
	PII(M2)	12	6	73.00	143.66	1	1.0	1.0	61.5	405
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	3.62	6.70	1	1.0	1.0	1.0	23
	PII(M2)	12	6	7.33	15.80	1	1.0	1.0	1.0	52
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	4.92	9.68	1	1.0	1.0	1.0	30
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(+)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(-)	PRE	13	5	41.31	84.28	1	1.0	1.0	62.0	299
	PII(M2)	12	6	79.00	126.76	1	1.0	14.5	110.5	421
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	3.00	6.93	1	1.0	1.0	1.0	25
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	428.15	914.92	1	3.0	32.0	160.0	3028
	PII(M2)	12	6	590.33	1253.36	1	1.0	40.5	339.5	4028
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(+)+IFN-γ(-)+IL-17(-)+IL-13(-)	PRE	13	5	72.15	118.77	1	4.0	38.0	69.0	448
	PII(M2)	12	6	108.33	178.59	1	1.0	10.0	189.5	559
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	2.31	4.42	1	1.0	1.0	1.0	17
	PII(M2)	12	6	7.67	16.83	1	1.0	1.0	1.0	56
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	732.23	1481.08	1	1.0	66.0	333.0	4310
	PII(M2)	12	6	1252.92	2372.01	1	1.0	109.0	1432.5	7753
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(+)+IL-13(-)	PRE	13	5	24.23	32.48	1	1.0	1.0	31.0	102
	PII(M2)	12	6	13.75	30.25	1	1.0	1.0	13.0	105
CD8.CD40L(-)+IL-2(-)+TNF-α(-)+IFN-γ(-)+IL-17(-)+IL-13(+)	PRE	13	5	46.00	68.88	1	1.0	1.0	101.0	200
	PII(M2)	12	6	40.50	58.10	1	1.0	13.5	64.5	185
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	3.58	8.95	1	1.0	1.0	1.0	32
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(+)+IL-13(-)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(+)+IL-17(-)+IL-13(-)	PRE	13	5	17.85	41.88	1	1.0	1.0	1.0	149
	PII(M2)	12	6	42.75	48.59	1	1.0	45.0	57.5	163
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-γ(-)+IL-17(+)+IL-13(+)	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1

γ(-)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(+)+IFN-	PRE	13	5	6.92	18.06	1	1.0	1.0	1.0	66
γ(-)+IL-17(-)+IL-13(-)	PII(M2)	12	6	6.58	13.53	1	1.0	1.0	1.0	43
CD8_CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	2.69	6.10	1	1.0	1.0	1.0	23
γ(+)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(+)+IL-13(-)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1
CD8_CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(+)+IL-17(-)+IL-13(-)	PII(M2)	12	6	3.08	7.22	1	1.0	1.0	1.0	26
CD8_CD40L(+)+IL-2(+)+TNF-α(-)+IFN-	PRE	13	5	1.00	0.00	1	1.0	1.0	1.0	1
γ(-)+IL-17(+)+IL-13(+)	PII(M2)	12	6	1.00	0.00	1	1.0	1.0	1.0	1

N = number of subjects with available results
Nmiss = number of subjects with missing results
SD = standard deviation
Q1, Q3 = first and third quartiles
Min/Max = minimum/maximum
PRE = Prior Dose 1 (Day 0)
PII(M2) = Post Dose 2 (Day 60)

Primary Outcome Results: Number (%) of subjects with solicited local symptoms reported during the 7-day (Days 0-6) post-vaccination period following each dose and across doses (Total Vaccinated cohort)

		TB Group				
					95% CI	
Symptom	**Intensity**	**N**	**n**	**%**	**LL**	**UL**
Dose 1						
Pain	Any	20	18	90.0	68.3	98.8
	Grade 3	20	3	15.0	3.2	37.9
Redness	Any	20	4	20.0	5.7	43.7
	> 50.0 mm	20	0	0.0	0.0	16.8
Swelling	Any	20	4	20.0	5.7	43.7
	> 50.0 mm	20	0	0.0	0.0	16.8
Dose 2						
Pain	Any	17	15	88.2	63.6	98.5
	Grade 3	17	1	5.9	0.1	28.7
Redness	Any	17	4	23.5	6.8	49.9
	> 50.0 mm	17	1	5.9	0.1	28.7
Swelling	Any	17	3	17.6	3.8	43.4
	> 50.0 mm	17	0	0.0	0.0	19.5
Across Doses						
Pain	Any	20	18	90.0	68.3	98.8
	Grade 3	20	3	15.0	3.2	37.9
Redness	Any	20	4	20.0	5.7	43.7
	> 50.0 mm	20	1	5.0	0.1	24.9
Swelling	Any	20	5	25.0	8.7	49.1
	> 50.0 mm	20	0	0.0	0.0	16.8

N= number of subjects with at least one documented dose
n/%= number/percentage of subjects reporting the symptom at least once
95%CI= Exact 95% confidence interval; LL = lower limit, UL = upper limit
Any = Incidence of any particular symptom regardless of intensity grade
Grade 3 pain = Pain that prevented normal, everyday activity

Primary Outcome Results: Number (%) of subjects with solicited general symptoms reported during the 7-day (Days 0-6) post-vaccination period following each dose and across doses (Total Vaccinated cohort)

		TB Group				
					95% CI	
Symptom	**Intensity/Relationship**	**N**	**n**	**%**	**LL**	**UL**
Dose 1						
Fatigue	Any	20	8	40.0	19.1	63.9
	Related	20	8	40.0	19.1	63.9
	Grade 3	20	0	0.0	0.0	16.8
Gastrointestinal symptoms	Any	20	3	15.0	3.2	37.9
	Related	20	3	15.0	3.2	37.9
	Grade 3	20	0	0.0	0.0	16.8
Headache	Any	20	6	30.0	11.9	54.3
	Related	20	6	30.0	11.9	54.3
	Grade 3	20	0	0.0	0.0	16.8
Malaise	Any	20	7	35.0	15.4	59.2
	Related	20	7	35.0	15.4	59.2
	Grade 3	20	0	0.0	0.0	16.8
Myalgia	Any	20	3	15.0	3.2	37.9
	Related	20	3	15.0	3.2	37.9
	Grade 3	20	0	0.0	0.0	16.8
Temperature (axillary)	≥ 37.5° C	20	2	10.0	1.2	31.7
	Related	20	2	10.0	1.2	31.7
	> 39.5 °C	20	0	0.0	0.0	16.8
Dose 2						
Fatigue	Any	17	11	64.7	38.3	85.8
	Related	17	11	64.7	38.3	85.8
	Grade 3	17	2	11.8	1.5	36.4
Gastrointestinal symptoms	Any	17	4	23.5	6.8	49.9
	Related	17	4	23.5	6.8	49.9
	Grade 3	17	0	0.0	0.0	19.5
Headache	Any	17	11	64.7	38.3	85.8
	Related	17	10	58.8	32.9	81.6
	Grade 3	17	2	11.8	1.5	36.4
Malaise	Any	17	9	52.9	27.8	77.0
	Related	17	9	52.9	27.8	77.0
	Grade 3	17	2	11.8	1.5	36.4
Myalgia	Any	17	8	47.1	23.0	72.2
	Related	17	7	41.2	18.4	67.1
	Grade 3	17	2	11.8	1.5	36.4
Temperature (axillary)	≥ 37.5° C	17	7	41.2	18.4	67.1
	Related	17	5	29.4	10.3	56.0
	> 39.5 °C	17	0	0.0	0.0	19.5
Across Doses						
Fatigue	Any	20	14	70.0	45.7	88.1
	Related	20	14	70.0	45.7	88.1
	Grade 3	20	2	10.0	1.2	31.7
Gastrointestinal symptoms	Any	20	6	30.0	11.9	54.3
	Related	20	6	30.0	11.9	54.3
	Grade 3	20	0	0.0	0.0	16.8
Headache	Any	20	13	65.0	40.8	84.6
	Related	20	13	65.0	40.8	84.6
	Grade 3	20	2	10.0	1.2	31.7
Malaise	Any	20	11	55.0	31.5	76.9
	Related	20	11	55.0	31.5	76.9
	Grade 3	20	2	10.0	1.2	31.7
Myalgia	Any	20	10	50.0	27.2	72.8

	Related	20	9	45.0	23.1	68.5
	Grade 3	20	2	10.0	1.2	31.7
Temperature (axillary)	≥37.5° C	20	8	40.0	19.1	63.9
	Related	20	6	30.0	11.9	54.3
	>39.5 °C	20	0	0.0	0.0	16.8

N= number of subjects with at least one documented dose
n/%= number/percentage of subjects reporting the symptom at least once
95%CI= exact 95% confidence interval; LL = lower limit, UL = upper limit
Any = Incidence of any particular symptom regardless of intensity grade or relationship to vaccination.
Grade 3 = Incidence of a particular symptom that prevented normal, everyday activity
Related = general symptom assessed by the investigator as causally related to the study vaccination

Primary Outcome Results: Number (%) of subjects reporting pIMDs during the entire study period (Total Vaccinated cohort)

pIMDs	**TB Group N = 20**
Subjects with any pIMD(s), n (%)	0 (0.0)

Primary Outcome Results: Please refer to the Safety Results section below for unsolicited AEs and SAEs results.

Secondary Outcome Results: Not Applicable.

Safety Results: Number (%) of subjects reporting unsolicited AEs within the 30-day (Days 0-29) post-vaccination period (Total Vaccinated cohort)

Most frequent adverse events - On-Therapy (occurring within Days 0-29 following vaccination)	**TB Group N = 20**
Subjects with any AE(s), n (%)	12 (60.0)
Feeling hot	2 (10.0)
Sinusitis	2 (10.0)
Upper respiratory tract infection	2 (10.0)

Counting rule applied: as there were less than 30 subjects/treatment group, only AEs that occurred in more than one patient are listed

Safety Results: Number (%) of subjects with SAEs during the entire study period (Total Vaccinated cohort)

Serious adverse event, n (%) [n considered by the investigator to be related to study medication]

All SAEs	**TB Group N = 20**
Subjects with any SAE(s), n (%) [n assessed by the investigator as related]	1 (5.0) [0]
Alcohol abuse	1 (5.0) [0]

Fatal SAEs	**TB Group N = 20**
Subjects with fatal SAE(s), n (%) [n assessed by the investigator as related]	0 (0.0) [0]

Conclusion:

mRNA immunological research

In the gene expression microarray analysis of the Peripheral Blood Mononuclear cells (PBMC) samples, significant differences between gene expression levels in the identified groups were found for both gene Clusters I and II on Day 44 (p-value ≤ 0.05). In comparison, in the analysis of the whole blood (WB) samples, significant differences (p-value ≤ 0.05) between gene expression levels in the identified groups were identified for Cluster II but not for Cluster I.

The Monte Carlo analysis showed for the PBMC and WB analysis, for all Clusters, and for all time points that the approach led to slightly higher acceptance rate of the false alternative hypothesis of a difference between the groups of subjects. The resampling analyses were in concordance with the results obtained with the Student-T test.

For Gene Cluster II, informative time points for WB sampling for gene expression analysis were days 37, 40, 44 and 47; p-values for all these time points were below the statistical significance threshold of 0.05. For Gene Cluster I, no informative time point for WB sampling for gene expression analysis was identified; p-values for all analysed points were above the statistical significance threshold of 0.05.

Cell-mediated immunity

The TB vaccine was immunogenic inducing M72-specific CD4+ T-cell responses (mainly CD40-L/IL-2/TNF-α triple positive, CD40-L/IL-2 double positive, CD40-L single positive and CD40-L/IL-2/TNF-α/IFN-γ quadruple positive CD4+ T-cells). A low level CD8+ T-cell response was observed after 2 doses.

Serum IFN-γ

At Days 0 and 47, 50% and 64.7% of subjects had INF-γ concentrations ≥ 7047 fg/mL, respectively. At Days 0 and 47, GMCs for IFN-γ in serum were 6279.7 and 7245.0 fg/mL, respectively. The majority of subjects had serum IFN-γ concentrations above the assay cut-off post Dose 2 (94.1% of subjects at Day 31 and 94.4% of subjects at Day 37) with a maximum GMC of 64288.5 fg/mL observed at Day 31. GMCs returned back to baseline values at Day 40.

Safety

During the 7-day (Days 0-6) post-vaccination period, pain was the most frequently reported solicited local symptom, by 90% of subjects while fatigue was the most frequently reported solicited general symptom, by 70% of subjects.

During the 30-day (Days 0-29) post-vaccination period, at least one unsolicited AE was reported by 12 (60%) subjects.

No pIMDs were reported during the entire study period.

During the entire study period, from study start to Month 7, one SAE was reported by one (5%) subject. This SAE was not fatal and was assessed by the investigator as not causally related to vaccination.

Date updated: 30-July-2014