Dartmouth

1. PROTOCOL DAR-PIA

Study Title	A Phase 2, Randomized, Placebo-Controlled, Double-Blind, Study of the Prevention of Infection with Mycobacterium tuberculosis Among Adolescents Who Have Previously Received BCG
Investigational Drug:	DAR-901
IND Number:	15838
EudraCT	Not applicable
Sponsor:	Charles Fordham von Reyn MD Geisel School of Medicine
Protocol Number:	DAR-901-PIAT
Protocol Version:	1.4
Date:	1 January 2018

Protocol Approval Page

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Approved for the Sponsor by:

C. Fordham von Reyn, M.D.
Dartmouth College

1 January 2018

Signature Date

Revision History

Ver. No.	Date	Comment
1.0	17 August 2015	For submission to 4 IRBs and regulatory agencies
1.21	27 February 2016	Incorporates recommendations from MUHAS, NIMRI, and Monitor; also adds new secondary endpoint (1.21 April 10 edit clarifies MUHAS refusal of 2 additional phlebotomies)
1.3	1 September 2016	Clarifies that this is a Phase 2b trial Clarifies replacing subjects lost to follow-up before Visit 2 Specifies concomitant medications which are exclusionary Adds 28 day patient diary Adds extra follow-up and confirmatory IGRA for new positive IGRAs Adds 2.5 mL sample to be co-ordinated with final safety CBC Corrects one item in DSMB charter remaining from prior dose escalation trial Adds immune assay results from DAR-MDES Phase 1 trial in the US Adds Dr. Albert Magohe as Deputy Study Director
1.4	1 January 2018	Adds additional IGRA test with consent/assent (Table 1) Revises time of blinded endpoint analysis (2.4.2) Clarifies efficacy population as ITT population (2.8.1) Specifies active TB as an exploratory endpoint (2.8.3) Defines active TB as an SAE (12.1.3) Clarifies that borderline or invalid 2 month IGRA confers ineligibility (14.3) Revises Clinical and Deputy Study Directors (17)

2. PROTOCOL SYNOPSIS

2.1 Protocol Information

Protocol Number:	DAR- 901- PIAT
Protocol Title	A Phase 2, Randomized, Placebo-Controlled, Double-Blind, Study of the Prevention of Infection with Mycobacterium tuberculosis Among Adolescents Who Have Previously Received BCG
Sponsor:	Dartmouth College, Hanover, NH
Name of Finished Product	DAR-901 for Injection
Name of Active Ingredient	DAR-901
Phase of Development	2b
Indication (Target)	Prevention of infection with *Mycobacterium tuberculosis* in adolescents previously vaccinated with BCG.
Number of Subjects	650 in planned dose groups
Number of Sites	One site in Dar es Salaam, Tanzania

2.2 Study Objectives

2.2.1 Primary Objective

To determine the safety and efficacy of a 3 dose series of DAR-901 for the prevention of infection with *M. tuberculosis* (TB) among healthy adolescents in Tanzania previously immunized with BCG.

2.2.2 Secondary Objectives

To identify risk factors for infection with TB among adolescents in Tanzania.

To identify subject characteristics associated with vaccine induced protection against infection with TB.

2.3 Rationale for the Current Study

Development of an improved vaccine for the prevention of tuberculosis is a major international health priority. BCG (bacillus Calmette-Guerin) is almost universally administered in childhood in countries with endemic tuberculosis including Tanzania. However, vaccine-induced protection against TB wanes in adolescents and young adults. A major current emphasis is the development of a vaccine that would enhance BCG-induced immunity in both these populations. Inactivated *Mycobacterium obuense* DAR-901 represents a candidate vaccine designed to fulfill this "prime-boost" strategy in the prevention of TB.

The natural history of TB proceeds from initial infection, typically asymptomatic and identified by a positive tuberculin skin test or positive interferon gamma release assay (IGRA), to overt symptomatic disease. Among 100 persons with asymptomatic latent TB infection approximately 10% eventually progress or reactivate to develop symptomatic active TB disease. Traditional efficacy trials for the prevention of TB have used active TB disease as the primary trial endpoint. IGRA assays, which are unaffected by most TB vaccines, including BCG, now make it possible to test whether a candidate TB vaccine prevents TB infection. A recent meta-analysis of 9 published studies using IGRAs have shown that BCG prevents TB infection (1). Prevention of Infection (POI) trials are now being considered for evaluating candidate TB vaccines (2) The present trial will be a DAR-901 prevention of infection trial in adolescents.

In previous studies, we have demonstrated the safety, immunogenicity, and efficacy of a 5-dose series of inactivated *Mycobacterium obuense* SRL 172, a whole-cell vaccine derived from a environmental non-tuberculous mycobacterium and prepared using organisms grown on agar and then heat-inactivated. Phase I safety studies were conducted in HIV-negative adults and HIV-positive adults and children in the United States, and Phase II safety and immunogenicity studies in HIV-positive adults in Zambia and in Finland.

An NIH-sponsored Phase III efficacy trial of SRL 172 was initiated in Tanzania in 2001 (hereafter referred to as the "DarDar Trial"). A total of 2013 HIV subjects with prior BCG were randomized 1:1 to receive a 5-dose series of vaccine (1 mg in 0.1 mL) or placebo (buffered saline alone) administered intradermal in the deltoid) at 0, 2, 4, 6, and 12 months. Subjects were followed every 3 months for the development of tuberculosis. The vaccine was safe and well-tolerated with minimal local reactions, 0.3% vaccine site sterile abscesses and 0.4% self-limited generalized rashes. Compared to placebo recipients, vaccine recipients showed significant increases in IFN-γ responses to the vaccine antigen and significant increases in antibody to lipoarabinomannan (LAM). In 2008 the trial was stopped after the DSMB concluded that SRL 172 had shown significant protection against active tuberculosis (defined as "definite" tuberculosis supported by smear or culture evidence of infection) (3).

The agar-based manufacturing method used to prepare inactivated *M. obuense* SRL 172 scaled poorly. A new broth-based manufacturing process was developed at the Aeras (Rockville MD), starting from the Master Cell Bank for SRL 172. The broth-produced product, now designated *M. obuense* DAR-901, completed non-clinical immunogenicity, toxicology and challenge studies. An IND was obtained from FDA in January 2014 (von Reyn, Principal Investigator) and a Phase I multiple- dose-escalation trial (DAR-901 MDES) among 59 HIV-negative and HIV-positive subjects with prior BCG is nearing completion in the United States. DAR-901 MDES has demonstrated that a 3 injection series of the vaccine is safe and well-tolerated in HIV-negative and HIV-positive adults with prior BCG immunization and does not affect results of an IGRA assay. An intradermal dose of 1 mg was selected by the external Dose Review Committee for further trials.

The present Phase II study will obtain preliminary data on the efficacy of a 3-injection booster series of DAR-901 in preventing TB infection among adolescents in Tanzania who received BCG at birth. The trial is designated DAR-PIA for DAR-901 Prevention of Infection in Adolescents.

2.4 Study Design

This is a Phase II 3-injection randomized, controlled trial of DAR-901 to be conducted in 13-15 year old adolescents in Tanzania previously immunized with BCG. The goals are to establish the safety and efficacy of DAR-901 in preventing infection with TB. The 1 mg level corresponds to the dose of SRL 172 used in the successful DarDar Trial.

Doses will be administered by intradermal (ID) injection in the deltoid area at 0, 2 and 4 months. In the Phase 2 study of SRL 172 conducted in Finland, significant immunogenicity was induced by 3 injections [1]. The dose intervals and route of administration are consistent with all previous studies of SRL 172.

All subjects will be screened by the T-spot® IGRA (Oxford Immunotec, Oxford, England) for evidence of TB infection. All screened subjects will have height and weight measured and will have a structured interview to identify risk factors for TB infection (=positive IGRA). IGRA-positive subjects will be referred for further evaluation and will not be entered in the immunization phase of the trial.

It is estimated that 1000 adolescents will need to be screened to enroll a total of 650 IGRA-negative adolescents in the immunization phase of the trial. Subjects will be and randomized 1:1 to DAR-901 or saline control at 0, 2 and 4 months. IGRA testing will be repeated before dose 2, at 14 months, and again at end of study or 24 months, whichever comes first.

The risk factor study will employ a cross-sectional analysis comparing IGRA-positive and IGRA-negative adolescents at baseline (estimated 650 IGRA-negative and 350 IGRA-positive subjects). Risk factor analysis will also be conducted on subjects enrolled in the immunization phase who develop new TB infection during the study.

2.4.1 Enrollment Process

As part of the overall risk management plan enrollment will proceed as follows:

- All adolescent subjects will provide written assent and their parents or guardians will provide written consent. Informed consent and assent forms will be written in Kiswahili and will be explained by a study nurse.
- Urine pregnancy tests will be performed on all female subjects on the day of scheduled immunization. Those with positive tests will be excluded from further immunization but will continue in follow-up.

2.4.2 Potential protocol adjustments

The protocol is based on a sample size calculation which assumes a 7% annual risk of new TB infection in the control group and 50% efficacy in the vaccine group. A blinded endpoint analysis will be conducted by the DSMB after all subjects have had a repeat IGRA test at one year.

2.4.3 Risk Management

- All intradermal injections of study medication (DAR-901, sterile saline) will be administered by trained study personnel.
- All subjects will be observed for at least 30 minutes after each dose
- The trial will be conducted in compliance with the protocol, GCP and applicable human studies and regulatory requirements.
- Participants who consented to Protocol version 1.3 will be provided a diary to record injection site reactions after Dose 3 and monitor adverse events up to 28 days after immunization.

2.5 Subject selection

2.5.1 Inclusion Criteria

To be eligible for this study, a subject must meet ***all*** of the following inclusion criteria:

1. Is age 13 to 15 years, inclusive;
2. Has completed the informed assent procedure including signing and dating the informed assent form;
3. Has had a parent/guardian complete an informed consent procedure including signing and dating the informed consent form;
4. Has received BCG as documented by presence of a scar consistent with immunization or a contemporary medical record;
5. Female subjects must have a negative urine pregnancy test within 24 hours prior to each dose of study drug;
6. Female subjects must agree to prevent pregnancy (strict abstinence or use of ***two*** of the following methods: hormonal contraceptive [oral, injectable, implanted or intravaginal ring], condom, diaphragm, spermicide, or an intra-uterine device) from Dose 1 through the treatment period and for four (4) weeks after the last injection of study drug.

2.5.2 Exclusion Criteria

A person who meets ***any*** of the following exclusion criteria will ***not*** be enrolled in the study:

1. Has a history of active tuberculosis;
2. Has previously received another investigational vaccine against tuberculosis;
3. Has had an illness consistent with acute viral or bacterial infection within the prior (2) two weeks;
4. Has significant medical disease (chronic or active within the past 6 months), including, but not limited to: cardiac disease (e.g., symptomatic congenital heart disease, unstable angina, myocardial infarction, congestive heart failure, ventricular arrhythmia), uncontrolled seizure disorder, liver disease, autoimmune or antibody-mediated diseases (e.g., lupus, rheumatoid arthritis), organ transplantation, chronic infection, uncontrolled diabetes; diseases judged by the Investigator as not clinically significant or as fully resolved will be reviewed with the Medical Monitor;

5. Has received systemic immune suppressive, stimulatory prescription drugs, or anti-retroviral therapy during the prior three (3) months
6. Has abnormal CBC values that are considered clinically significant .
7. Has clinically significant abnormal findings on vital signs;
8. Is expected to have surgery requiring general anesthesia during the study period;
9. In the judgment of the Investigator, not suitable to participate in this clinical study.

2.6 Treatments

All treatments will be administered as scheduled by intradermal injection in the deltoid.

2.6.1 Investigational Treatment

A 1 mg dose of DAR-901 administered in a dose volume of 0.1 mL.

2.6.2 Comparator treatment

Inactive comparator: 0.1 mL of Sterile Saline for Injection (placebo).

2.7 Assessments

The safety and tolerability of DAR-901 will be assessed using reported and observed adverse events.

2.8 Statistical Analyses

2.8.1 Analysis Populations

Analysis will be performed comparing subjects by treatment assignment (DAR-901 vs. Placebo).

- *Safety Population* — all subjects who received at least one injection of study medication.
- *Efficacy Population (ITT population)* — all subjects, as randomized with a negative IGRA at enrollment and at the time of the 2 month (=dose 2) study visit. This is the Intent-to-Treat (ITT) population.

2.8.2 Safety Analyses

Tabulation and descriptive statistics of adverse events, and vital signs.

2.8.3 Efficacy Analyses

The efficacy analysis will include all subjects with a negative IGRA at enrollment and a second negative IGRA at 2 months.

The primary endpoint is the difference in the rate of new TB infection in two treatment groups. New TB infection is defined as conversion from IGRA-negative at 2 months to IGRA-positive at any time later in the study.

The secondary endpoint is the difference in the rate of persistent new TB infection in the two treatment groups. Persistent new TB infection is defined as new TB infection which remains IGRA positive on a second IGRA test 3 or more months later.

Although the rate of active tuberculosis disease is expected to be very low it will be analyzed as an exploratory endpoint.

2.8.4 Risk factor Analyses

Data will be analyzed using descriptive and analytic statistics. Variables will include household or community contact with TB, residence or exposure to congregate settings such as hospitals, correctional facilities, nursing homes, and orphanages; room size; ventilation systems (windows); airflow patterns; use of public transportation (e.g. daladala buses); socioeconomic status, nutritional status, and underlying disease (e.g., diabetes).

2.8.5 Determination of Sample Size

The sample size of 650 subjects (325 in each treatment group) is based on an expected 7% annual IGRA conversion rate among placebo recipients who remain IGRA-negative at 2 months (pre-dose 2; IGRA conversions before dose 2 may represent TB infection acquired prior to the dose 1). The sample size is calculated with 80% power to detect vaccine efficacy of 50% in preventing TB infection. Loss to follow-up is estimated at 5-10% per year. A blinded interim analysis will be conducted after results of the 14 month IGRA are available on 200 subjects. The study duration and number of repeat IGRAs will be increased if needed to detect sufficient endpoints.

2.9 Schedules of Study Events

Table 1. Schedule of Study Events –

Event / Evaluation Visit #	Screen	1				2				3				4	5,6
Day	≤-28	0			+7	60 [a] (46-74)			+7	120 [a] (106-134)			+7	420[b]	720 [c] <1085 [g]
Hour		Pre	0	0.5		Pre	0	0.5		Pre	0	0.5			
Informed Consent/Assent	X														X
Risk factor interview	X													X	X
Examination for BCG scar	X														
Vital signs, height and weight	X	X		X	X	X		X	X	X		X	X	X	X
Examination of injection site [d]					X				X				X		
Complete blood count (CBC)	X					X							X		X
Urine pregnancy test (F only)		X				X				X					
IGRA [e,f,g]	X					X								X	X [f]
RNA expression													X		
Dose administration			X				X				X				

[a] Numbers in parentheses provide range that is acceptable
[b] 14 month visit can be performed at 13-15 months
[c] 24 month visit is end of study (EOS) and can be performed at 22-24 months
[d] Day 7 injection site examination can be performed on day 6-8 after injection.
[e] Blood volume for T-spot IGRA = 6 mL, blood volume for CBC = 4 mL; blood volume for RNA expression = 2.5 mL; total blood volume for study = 42.5 mL (+ or – 20%)
[f] Subjects with a new positive IGRA at the end of study or at Visit 5 will need a repeat IGRA at ≥3 months later
[g] Subjects will be reconsented to obtain an additional IGRA at or before 12 months after Visit 5

Table 2. Study Timeline

Month	0-4	5-8	9-12	13-15	17-20	22-24	24-36
Recruitment	X						
Risk factor analysis	X					X	X
Immunization	X	X					
Interim analysis				X			
IGRA testing	X			X		X	X
Data analysis							X

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4. ABBREVIATIONS

Abbreviation	Definition
ADL	activities of daily living
AE	adverse event
CRF	case report form
CRO	Contract Research Organization
FDA	Food and Drug Administration (U.S.)
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HPF	high-power field
HR	heart rate
ICF	informed consent form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IFN	Interferon
IRB	institutional review board
LFT	liver function test
MCB	master cell bank
PBMC	peripheral blood mononuclear cell
PI	principal investigator
PK	pharmacokinetics
POI	prevention of infection (TB infection)
RBC	red blood cell
SAE	serious adverse event
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
TNF	tumor necrosis factor
ULN	upper limit of normal
WBC	white blood cell

5. BACKGROUND

5.1 Rationale for Investigation of DAR-901

Development of an improved vaccine for the prevention of tuberculosis is a major international health priority. BCG (Bacillus Calmette-Guerin) is almost universally administered in childhood in countries with endemic tuberculosis. However, resistance to active infection wanes in adolescents and young adults and the risk of disease is markedly increased in HIV-infected persons. A major current emphasis is the development of a vaccine that would enhance immunity in these populations. Inactivated *Mycobacterium obuense* DAR-901 represents a candidate vaccine designed to fulfill this "prime-boost" strategy with minimal risk in immunecompromised persons.

In previous studies, we have demonstrated the safety, immunogenicity, and efficacy of a 5-dose series of inactivated *M. obuense* SRL 172, a whole-cell vaccine derived from a environmental non-tuberculous mycobacterium and prepared using organisms grown on agar and then heat-inactivated. Phase I safety studies were conducted in HIV-negative adults and HIV-positive adults and children in the United States, and Phase II safety and immunogenicity studies in HIV-positive adults in Zambia and in Finland.

An NIH-sponsored Phase III efficacy trial of SRL 172 was initiated in Tanzania in 2001 (hereafter referred to as the "DarDar Trial") (3). A total of 2013 HIV-positive subjects with prior BCG were randomized 1:1 to receive a 5-dose series of vaccine (1 mg in 0.1 mL) or placebo (buffered saline alone) administered intradermal in the deltoid) at 0, 2, 4, 6, and 12 months. Subjects were followed every 3 months for the development of tuberculosis. The vaccine was safe and well-tolerated with modest local reactions; the incidence of sterile abscesses at the vaccine site was 0.3% and of self-limited, generalized rashes, 0.4%. Compared to placebo recipients, vaccine recipients showed significant increases in IFN-γ responses to the vaccine antigen and significant increases in antibody to lipoarabinomannan (LAM). In 2008 the trial was stopped after the DSMB concluded that SRL 172 had shown significant protection against active tuberculosis (defined as "definite" tuberculosis supported by smear or culture evidence of infection).

The agar-based manufacturing method used to prepare *M. obuense* SRL 172 scaled poorly. A new broth-based manufacturing process was developed at Aeras, starting from the Master Cell Bank for SRL 172. The broth-produced product, now designated *M. obuense* DAR-901, is similarly a heat-inactivated, whole cell preparation. DAR-901 completed non-clinical immunogenicity, toxicology and challenge studies (detailed in Section 5.3), an IND obtained from the US FDA in January 2014 and a Phase I Multiple Dose Escalation Trial (DAR-901 MDES) initiated in the United States in March 2014. Note that GMP-grade SRL 172 for human use is no longer available.

The Phase I DAR-901 MDES trial has completed enrollment of 53 HIV-negative and 6 HIV-positive adults with prior BCG immunization. The trial, which is proceeding successfully to closure, has shown the safety and tolerability of DAR-901, and has resulted in the selection of a dose of 1 mg for further human trials (see Section 5.4.1.).

The present Phase II trial is designed to obtain preliminary data on the efficacy of DAR-901 in preventing tuberculosis. Traditional efficacy trials for the prevention of TB – including our successful DarDar trial which used the prior SRL-172 vaccine preparation – have used active TB disease as the primary trial endpoint. A major challenge in all such trials is the need for complex algorithms and blinded adjudication committees to achieve consistent assessment of clinical and microbiologic data. IGRA assays, which are unaffected by most TB vaccines, including BCG, now make it possible to objectively define incident cases of TB infection and use that endpoint to determine the efficacy of a candidate TB vaccine. A recent meta-analysis of 9 published studies using IGRAs has shown that BCG prevents TB infection (1). Prevention of Infection (POI) trials are now being considered for evaluating candidate TB vaccines (2). Because rates of TB infection in endemic countries are 5-10 times more common than rates of TB disease, trials using POI as an endpoint can be designed with smaller sample sizes and shorter follow-up periods. The present trial – designated DAR-PIA (DAR-901 for the Prevention of Infection in

Adolescents) – will obtain preliminary data on the efficacy of a 3-injection booster series of DAR-901 in preventing TB infection among adolescents in Tanzania who received BCG at birth.

5.2 Overview of DAR-901

5.3 Nonclinical Studies Conducted with DAR-901

5.3.1 Provenance.

DAR-901 is prepared from GMP stocks used to prepare SRL 172; the designation has been changed because DAR-901 is prepared with a different growth process (broth) by a new manufacturer (Aeras, Rockville, MD). The provenance of DAR-901 is summarized below.

- A strain of non-tuberculous mycobacteria was cultured from soil in Uganda by Dr. John Stanford in 1971 and a stable, rough variant was isolated on subculture (4).
- The rough variant was deposited in 1984 with National Culture Type Collection as NCTC 11659.
- An aliquot of NCTC 11659 was used to prepare a Master Cell Bank, designated MS/01/93 (Public Health England, Porton Down, UK) under a contract with SR Pharma, London, UK.
- MCB MS/01/93 was used by the Centre for Applied Microbiology and Research (CAMR) (Salisbury, UK) to prepare SRL 172 Clinical Trial Material, an agar-grown, heat-inactivated, whole cell vaccine for SR Pharma (London, UK). This was the material investigated in the "DarDar" trial (C. Fordham von Reyn, Principal Investigator).
- MCB MS/01/93 was used by Eden Biodesign Ltd. (Liverpool, UK) to prepare MCB lot C001-07-001.
- Aliquots of MCB lot C001-07-001 were provided to Aeras (Rockville, MD) and used to prepare MCB lot 12-107M-001.
- MCB 12-107M-001 was used by Aeras to prepare DAR-901 Drug Product, a broth-grown, heat-inactivated, whole-cell vaccine, lot 12-107F-001.

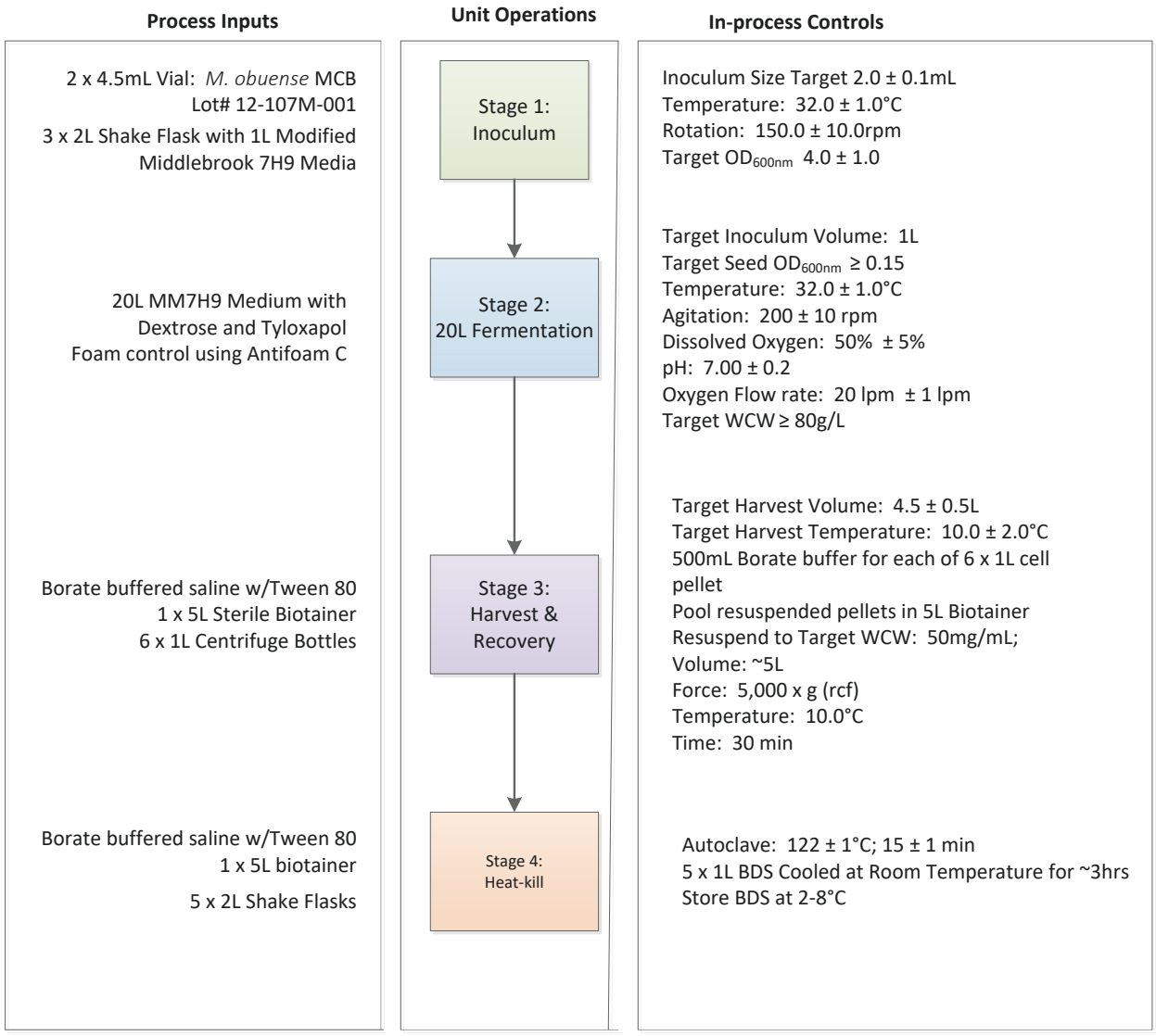
Aeras performed 16S rRNA gene sequencing of MCB lot #12-107M-00 and confirmed it was 100% identical to MCB MS/01/93, MCB C001-07-001, and SRL 172 CTM, which was used in the DarDar trial.

The 16s rRNA gene sequencing indicates that the MS/01/93 MCB, C001-07-001 MCB, and 12-107M-001 MCB have >99.6% identity to the reference 16S rRNA sequence for *Mycobacteria obuense* and <95% with *M. vaccae*.

5.3.2 Preparation.

DAR-901 is manufactured by Aeras by fermentation of the bacterial strain, heat inactivation and distribution of bulk drug substance as a 0.3-0.4 mL suspension into 2 mL vials at a concentration of 1 mg/mL. The manufacturing process is summarized below:

Figure 5-1. DAR-901 Bulk Drug Substance Manufacturing Flow Diagram



5.3.3 Immunogenicity.

5.3.3.1 Immunogenicity study #1 (2013)

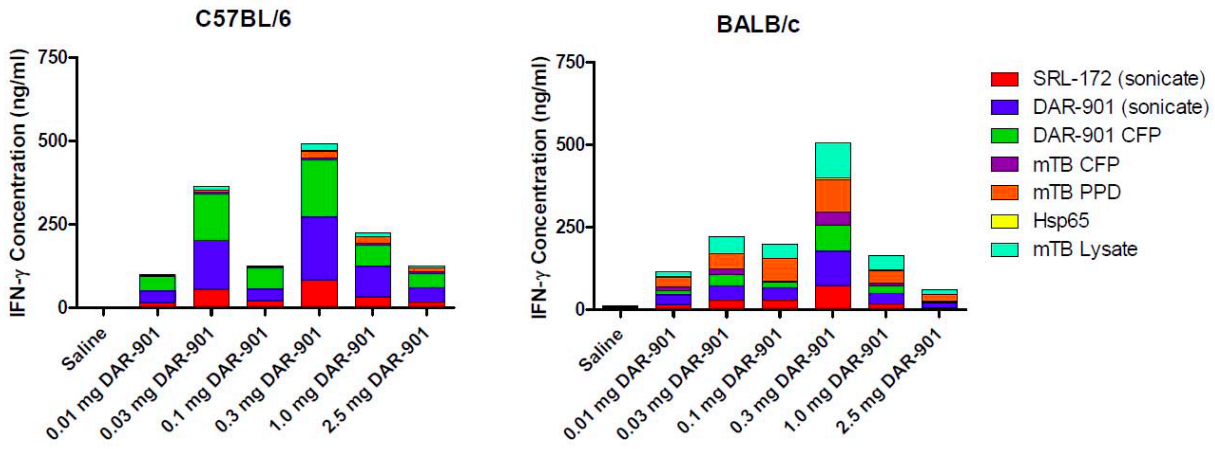
Objective. To determine the immunogenicity of a 3-administration series of DAR-901 at different dose levels in 2 species of mice. The DAR-901 used in the immunogenicity studies was prepared as per the procedures outlined in the vaccine preparation section above (5.3.2)

Design. A total of 105 C57BL/6 and 105 BALB/c mice received 3 administrations of intradermal DAR-901 at 2 week intervals at the following doses (mg): .01, .03, .1, .3, 1.0, 2.5. A total of 70 animals were sacrificed 2 weeks after each dose. Spleens and blood were collected for the following assays: in vitro stimulation assay for IFN-γ, ELISpot for IFN-γ and antibody ELISA. Antigens included DAR-901 sonicate and CFP; SRL-172 lysate and CFP; *M. tuberculosis* lysate and CFP; *M. tuberculosis* PPD and hsp. Antibody to the same antigens and to *M. tuberculosis* lipoarabinomannan (LAM) was also assayed at a single time point 2 weeks after dose 3.

Results. As shown in Figure 5-2 IFN-γ responses to the vaccine antigen, DAR-901 sonicate, were induced after dose 1 and increased progressively with doses 2 and 3 in both mouse species with both IFN-γ assays.

Maximum responses were typically observed at the 0.3 mg dose. IFN-γ responses to the DAR-901 CFP followed a similar pattern. Similar, though lesser magnitude responses were induced by SRL-172. IFN-γ responses to *M. tuberculosis* lysate and CFP were also induced, most notably in BALB/c mice.

Figure 5-2. IFN-γ responses based on in vitro stimulation after 3 immunizations at various dose levels.



Antibody was induced to DAR-901 sonicate, SRL-172 lysate and DAR-901 CFP. There were no detectable responses against LAM.

Conclusions

Mycobacteria-naïve mice showed both cellular and humoral immune responses to a 3-administration series of DAR-901, with increasing responses after each of 3 doses. Responses were maximal at a dose of 0.3 mg.

5.3.3.2 Challenge study #1 (2015)

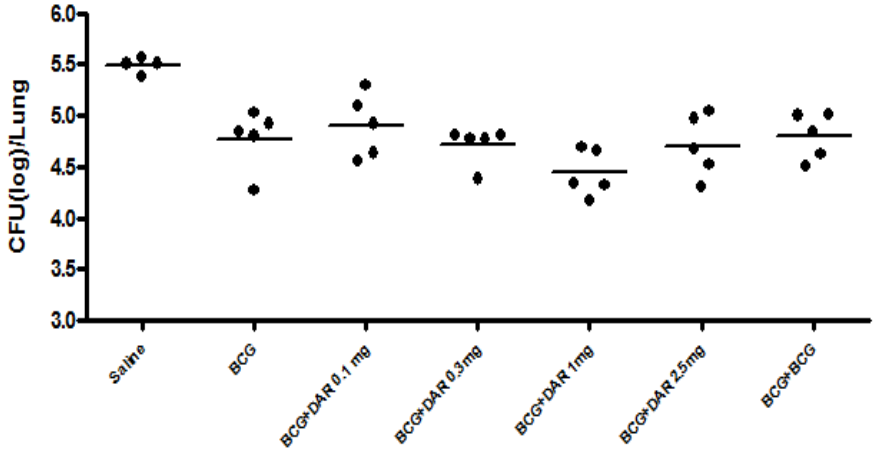
Design

C57BL/6 mice were primed with BCG tice and then boosted starting at week 12 with either a single BCG booster or 3 injections of DAR-901 booster at two week intervals. Mice were challenged at week 20 with 50-100 CFU MTB H37Rv and sacrificed at week 31.

Results

As shown in Figure 3 animals boosted with 1 mg DAR-901 showed a significantly greater reduction in CFUs than animals boosted with BCG.

Figure 3 CFU of *M. tuberculosis* in spleen of C57BL6 mice primed with BCG and boosted with DAR-901 at increasing doses or boosted with a second dose of BCG then challenged with MTB. Boost with 1 mg DAR-901 shows significantly greater reduction in CFU than boost with BCG (p=0.027).



Conclusion

This study demonstrates that in a prime-boost animal challenge model boosting with DAR-901 is more effective than boosting with BCG.

5.3.4 Toxicology.

Objective. A GLP repeat dose intradermal toxicity study conducted in C57BL/6NHsd mice. The purpose of this study was to investigate the local and systemic toxicity and immunogenicity of DAR-901. The toxicology study exposure exceeded the schedule proposed for the human dose escalation study as follows:

- Number of doses: five (5) in the toxicology study vs three (3) in the human trial
- Dose level: 2.5 mg in the toxicology study vs. in the human trial, the starting dose is 0.1 mg and the maximum dose is 1 mg
- Dose volume per total subject weight: 0.05 mL per 20 gram mouse in toxicology study vs. 0.1 mL per 70 kilogram (average) adult in the human trial
- dosing interval intensity: two (2) week intervals in toxicology study vs eight (8) week intervals in the human trial.

Methods. Two (2) groups of C57BL/6NHsd, specific pathogen free mice (30/sex/group) received a 50 uL intradermal injection of test (Group 2) or control (formulation buffer; Group 1) article on Days 0, 14, 28, 42, and 56. At each interval 2.5 mg of test article was injected in a separate injection site in the back. The DAR-901 used in the repeat dose intradermal toxicity studies was prepared as per the procedures outlined in the vaccine preparation section above (5.3.2)

Toxicity was assessed based on clinical observations, physical exams, administration site evaluation, body weights, hematology (5/sex/group/interval), coagulation (5/sex/group/interval), serum chemistry (5/sex/group/interval), organ weights, and macroscopic and microscopic pathology evaluation. Inflammatory response was assessed by measuring serum fibrinogen levels. Local (injection site) reactions were evaluated using a modified Draize score (Draize et al., 1944). Thirty animals per sex per group were sacrificed at Day 59 (End of Treatment Period) and the remaining animals (thirty per sex per group) were sacrificed at Day 70 (End of Recovery Period).

Results. Confirmation of vaccine take was performed using ELISA for serum immunoglobulin (IgG) to culture filtrate protein (CFP) from the mycobacterial strain from which DAR-901 was prepared. Serology data confirmed induction of an immune response against the test article in Group 2 animals.

Adverse observations related to the test article were restricted to changes at the injection site. These were similar in both treated males and females. The changes include:

- Erythema and/or edema – seen at the majority of injection sites beginning 1 to 2 days post-injection and typically lasting for 4 to 6 days.
- Induration – seen at the majority of injection sites, typically with delayed onset at the sites injected for doses 1, 2, and 3 (days 0, 14, and 28, respectively), with induration first noted at the exams on day 47 to 49. For doses 4 and 5 (days 42 and 56, respectively), the onset of induration of induration was more rapid and was noted as early as 1 week post-injection. Thus, as the study progressed, induration was frequently noted at locations separate from the most recent injection site. The size of the induration was typically 1 to 10 mm; occasional larger induration reactions appeared to represent the confluence of induration at two adjacent injection sites.
- Ulceration – a subset of injection sites developed ulceration, which was described as minimal or moderate except in three animals where ulceration was described as severe at one to three injection sites each.
  - Overall, 20 (33%) of 60 test-article animals had ulceration at one or more injections sites during the course of the study. Most ulcerations resolved by the time of sacrifice.
  - Among 40 animals sacrificed at day 60, 4 (10%) had ulceration at one to four injection sites; in three of the four animals the sites from dose 1 or 2 (day 0 and 14, respectively) were ulcerated.
  - Among the 20 animals sacrificed on day 70, 3 (15%) had ulceration at two or three injections sites; in all cases the involved sites represented doses 3, 4 or 5 (days 28, 42, and 56, respectively).

Minor changes in clinical pathology were noted, consistent with an inflammatory response, but none were considered adverse. Microscopic examination confirmed an inflammatory response at the injection site in animals with cutaneous ulceration.

Conclusions. Injection site reactions were observed in animals who received five (5) administrations of DAR-901 at a dose 2.5x higher than the maximum human dose proposed in Phase I dose escalation study. Most reactions were resolving at the time of necropsy. The reactions at early injection sites were delayed and were more frequent and intense after administration of multiple doses, consistent with the induction of a strong cutaneous delayed hypersensitivity response after multiple immunizations.

These reactions are consistent with observations in human studies involving injection of live or heat-inactivated mycobacterial vaccines. The proposed exposure in the proposed human trial is appreciably less intense with respect to dose, interval and total exposure. Specifically, the starting dose in the human dose escalation trial represents 4% of the dose used in the animal toxicity study; the interval between doses is increased 4-fold; and the maximum number of doses is three. There is provision for careful and regular assessment of injection site reactions both local and systemic, after each dose.

5.4 Clinical Experience with DAR-901

5.4.1 *DAR-901 MDES* (Phase I Multiple-Dose Escalation Study, United States)

This study was initiated on March 30, 2014 and has been completed.

Study overview:

- Subjects: 54 HIV-negative adults and 5 HIV-positive adults with prior BCG
- Treatments: DAR-901 at 3 dose levels or control (saline, BCG)
- Dose schedule: intradermal injection of 0.1 mL at 0, 2, 4 mos (see Table 3)
- Twenty-eight day safety diary kept by subjects after each dose of vaccine

- Clinic visit for measurement of vaccine site reactions 7 days after each dose of vaccine
- Phlebotomy for safety labs (CBC, chem 12, U/A) at baseline, before each dose, and 28 days after dose 3
- Phlebotomy for immune assays at baseline and 6 additional time points during the study
- Study duration: 9 months for each subject, End of Study visit 6 months after dose 3

Table 3. DAR-901 Multiple-dose Escalation Study. Dose groups and numbers of subjects

Dose Group*	HIV Status	IGRA status	Dose DAR-901	DAR-901 x3 (N)	Saline x2 BCG x1 (N)	Saline x3 (N)	Total
A1	Neg	Neg	0.1 mg	10	3	3	16
A2	Neg	Neg	0.3 mg	10	3	3	16
A3	Neg	Neg	1.0 mg	10	3	3	16
A4	Neg	Pos	1.0 mg	5	-	0	5
B1+B2	Pos	Neg+Pos	1.0 mg	6	–	-	6
Total				**41**	**9**	**9**	**59**

* Groups A1, A2 and A3 double-blind; Groups A4, B1 and B2 open label

Dose selection:

- Safety data reviewed by a 3 person external Dose Review Committee (DRC) on dose cohorts A1, A2 and A3
- Based on the safety profile and animal immunogenicity (see 5.3.3) the DRC selected the 1.0 mg dose of DAR-901 for cohorts A4, B1, B2 and further human studies (1.0 mg is the same dose used in the SRL-172 Phase III DarDar Trial)

Safety:

- Immunization well tolerated with no SAEs reported
- Fever: none except transient low grade fever after dose 3 in <9 subjects who had no vaccine site reactions after dose 1 and 2 (and presumably received BCG active control for dose 3)
- Safety labs: no significant abnormalities
- Repeat IGRAs (Quantiferon): performed on 10 subjects in A3 at 2-6 mos after dose 3; all final IGRAs were negative

Vaccine site reactions:

- Injection Site Reactions at Day 7 (ISRs). Median erythema and induration measured in clinic 7 days after each dose of DAR-901 (Table 4).
- Injection site reactions at EOS. Among six A3 subjects who received the selected 1 mg dose and have completed their EOS visit, minor skin discoloration of 2-5 mm was still visible at the dose 1 site in 2 subjects, dose 2 site in 3 subjects and dose 3 site in 2 subjects.

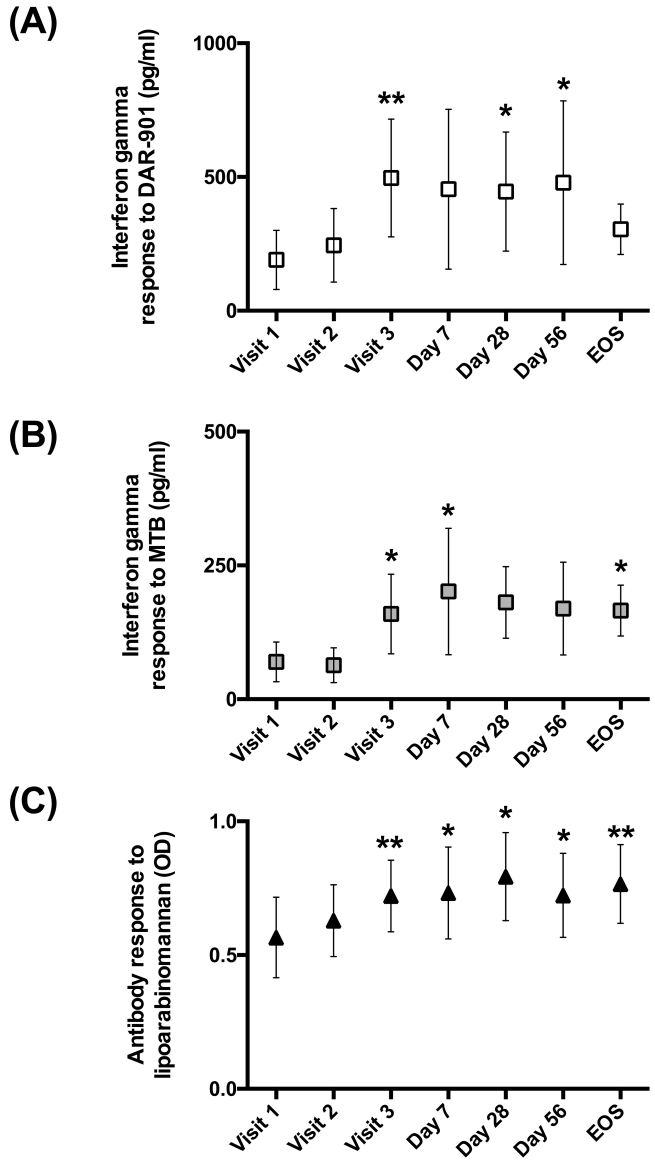
Table 4. DAR-901 Multiple-dose Escalation Study. Injection site reactions (ISRs).

Cohort[a] ISR Type	Dose 1	Dose 2	Dose 3
A1 (0.1 mg, HIV neg, IGRA neg)			
Erythema, mm (range)	5 (0-8)	6 (2-10)	7 (3-13)
Induration, mm (range)	6.5 (0-12)	4.0 (2-8)	3.5 (0-10)
A2 (0.3 mg, HIV neg, IGRA neg)			
Erythema, mm (range)	6 (0-10)	6 (0-10)	7.5 (0-10)
Induration, mm (range)	4 (0-10)	6.5 (2-11)	3 (2-6)
A3 (1.0 mg), HIV neg, IGRA neg			
Erythema, mm (range)	10 (4-20)	8 (0-20)	8 (4-18)
Induration, mm (range)	4.5 (1-16)	6 (0-10)	5 (4-10)
A4 (1.0 mg, HIV neg ,IGRA pos)			
Erythema, mm (range)	8 (5-14)	12 (0-18)	NA
Induration, mm (range)	8 (7-14)	6 (2-10)	NA
B1 (1.0 mg, HIV pos, IGRA neg)			
Erythema, mm (range)	8 (8-12)	10 (0-12)	NA
Induration, mm (range)	6 (4-8)	8 (2-12)	NA

Immunogenicity

Table 6. Immunogenicity of DAR-90 among 10 subjects who received three injections of 1 mg DAR-901 (Cohort A3).

Samples for Visit 1, 2, and 3 were collected 2 months apart and were obtained prior to dose 1, 2 and 3 respectively. (A) Immunization with DAR-901 at the 1 mg dose elicited greater interferon gamma responses (IFN-γ) to DAR-901 lysate at visit 3, day 28 and day 56 compared to pre-vaccination levels. (B) IFN-γ responses to *Mycobacterium tuberculosis* (MTB) whole cell lysate were significantly greater, compared to pre-vaccination levels at visit 3, day 7, and end-of-study. (C) Antibody responses to MTB lipoarabinomannan (LAM) were significantly greater than pre-vaccination responses at visit 3, day 7, day 28, day 56, and end-of-study compared to pre-vaccination responses. Graphs depict mean and standard error responses.



* indicates P<0.05; ** indicates P<0.01

- Results show immune reponses to 1 mg DAR-901 comparable in magnitude to those observed previously with SRL172 in the DarDar Trial

Conclusions

- ISRs to DAR-901 were mild to moderate with no severe reactions or complications
- both erythema and induration increased modestly but consistently with increasing dose and with repeated doses over time
- data from A4 (IGRA-positive) suggest increased responses in subjects with prior TB infection
- data from B1 and B2 (HIV-positive) are comparable to data in HIV-negatives
- immune responses to 1 mg DAR-901 are comparable to those observed with SRL172 and are significant after only 2 doses
- immunization with DAR-901 does not affect IGRA assay results
- collectively these data support the safety and immunogenicity of 1.0 mg DAR-901 and are consistent with the experience with 1.0 mg SRL-172 in the DarDar Trial (see 5.5.6)

5.5 Clinical Experience with Vaccines Derived from the Same Genotypic Strain

As detailed in Section 5.3.1, SRL 172 was a heat-inactivated, whole-cell vaccine derived from a rough variant of an environmental mycobacterium. Lot MV 001 of SRL 172 was used for a series of six human studies described below (Table 5-5). All doses were administered as an 0.1 mL intradermal injection over the deltoid containing 1 mg SRL 172 in borate buffered saline (estimated to represent 10^9 colony forming units based on wet weight). All studies were investigator-initiated, conducted in accordance with applicable regulatory requirements, and published in peer-review journals as indicated.

Table 5-5. Studies conducted using SRL 172 (Lot MV 001)

Study Number	Title	Reference
001	A Phase 1 Study of Five Doses of SRL 172 in Healthy Adults	(5, 6)
002	A Phase 1 Study of Five Doses of SRL 172 in HIV-infected Adults	(7)
003	A Phase 1 Randomized, Controlled, Phase 2 Study of Three Doses of SRL 172 in HIV-infected Children	(8)
004	A Phase 2 Open-Label, Controlled Study of Five Doses of SRL 172 in BCG-positive and BCG-negative, HIV-infected Adults in Zambia	(9)
005	A Phase 2 Randomized, Controlled Trial of Five Doses of SRL 172 in HIV-negative, BCG-positive and HIV- positive, BCG-positive Adults in Finland	(10)
006	A Phase 3, Randomized, Placebo-Controlled Trial of Five Doses of SRL 172 for Protection against Tuberculosis in BCG-primed, HIV-infected Adults in Tanzania (the DarDar Trial)	(3)

Table 5-6. Key characteristics of studies conducted using SRL 172 (Lot MV 001)

Study Number	Period Conducted	HIV status	BCG status	N SRL 172[a]	N PLA	No. Doses	Dosing (months)
001	1994–1995	Neg	Neg[b]	10	–	5[c]	0, 2, 10, 16, 18
002	1995	Pos	Neg	12	–	5[d]	0, 2, 4, 25, 26
003[e]	1994–1997	Pos	Neg	23	12[f]	3	0, 2, 4
004	1996	Pos	Neg	11	11	5	0, 2, 4, 12, 16
		Pos	Pos	11	11		
005	1997	Neg	Pos	10	–	5	0, 2, 4, 6, 12
		Pos	Pos	19	20[f]		
006	2001–2008	Pos	Pos	1006	1007	5	0, 2, 4, 6, 12

a. In all adult studies the dose of SRL 172 was 1 mg / 0.1 mL administered intradermally in the deltoid (dose for children < 5 was 0.05 mL).
b. 1 subject was BCG-positive.
c. 3 subjects received only 3 doses.
d. 4 subjects received only 3 doses.
e. Study 003 was conducted in children (ages 6 mo to 13 yr). All other studies were in adults, ages 18 to 70 yr.
f. Controls received hepatitis B vaccine.

5.5.1 SRL 172, Study 001 (United States)

Study overview:

- Subjects: 10 healthy (HIV-negative) adults, age 23-68, in US; 9 BCG negative, 1 BCG positive
- Dose schedule: 0, 2 and 10 months (10 subjects); 16 and 18 month (7 subjects)

Vaccine site reactions: Assessed at 2d, 14d, 2 months (photographs). Vaccine reactions noted over the first three doses (0, 2 and 4 months; 10 subjects) were:

- Erythema and induration: Noted in all subjects after each dose, maximal at 2 days. Median (range) induration at 2d post-dose were: dose one, 9 mm (6-25 mm), dose two, 8 mm (6-13 mm), dose three, 7 mm (4-17 mm).
- Drainage: Three subjects noted scant drainage: in subject 2, after doses one and two but not three; in subject 6 [BCG positive], after dose one [then withdrew because of pregnancy]; in subject 7, after doses two and three.
- Pain: "Sore arm" was reported by 2-5 subjects after each dose.

Other Safety Assessments:

- Temperature was recorded daily at home for 14 days after each dose and at study visits at 2d, 14d, 2 mos after each dose. All recorded temperatures were normal (<38.0°C)
- Laboratory: There was no clinically significant abnormal laboratory tests.
- Patient-reported adverse events: Patients were asked about interim and current symptoms at all visits. 1 feverish sensation and 1 mild malaise were reported after dose one; 1 headache, 1 feverishness and 1 malaise after dose two; 1 headache after dose three.

Three patients were lost to follow-up. Among the 7 patients who received additional doses at 16 and 18 months (total 14 doses administered):

- Median vaccine site induration, 8 mm (range 6-11) after dose 4; 7 mm (range 4-13) after dose 5.
- Fever was self-reported after 2 (14%) of 14 doses.
- "Sore arm" was reported after 2 (14%) of 14 doses.

Immunogenicity:

- Lymphocyte proliferation assay:
  - SRL 172 sonicate: 6 of 7 subjects had stimulation index >2
  - *M. avium* sensitin: No significant change in stimulation indices

- Antibody to *M. tuberculosis* lipoarabinnomannan (MTB LAM): 4 of 10 subjects had >2x increase

5.5.2 SRL 172, Study 002 (United States)

Study overview:

- Subjects: 12 HIV-infected adults, CD4 ≥300 in US; all BCG-negative
- HIV-related characteristics: 3 on ART (1 or 2 drugs) at baseline, 5 on ART by end of study
- Dose schedule: 0, 2, 4 mos (12 subjects); 25 and 26 months (8 subjects)

Vaccine site reactions: Assessed at 2d, 14 d, 2 mos after each dose.

- Induration: maximum at 2 days, median 6 mm

Safety:

- Temperature was recorded daily at home for 14 days after each dose. All were normal,
- No systemic side effects were reported after any dose.
- CD4: Mean change from baseline to post-dose 3 was +28 (range: -137 to +137)
- HIV viral load: Mean $\log_{10}$ change from baseline to post-dose 3 was + 0.4 (range: -0.3 to +1.5)

Immunogenicity:

- Lymphocyte proliferation:
  - SRL 172: Four subjects had stimulation index >2 after dose 3 (baseline not available)
  - *M. avium* sonicate: No increases in response from baseline to post-dose 3.
- Antibody to MTB LAM: No change in antibody titer

Four patients were lost to follow-up. Among the 8 subjects who received 2 additional doses at 25 and 26 months:

- Erythema with or without induration: 4 (50%) of 8 patients (diameter not available).
- No systemic symptoms were reported.
- Stimulation indices to SRL 172 and *M. avium* were generally higher in vaccine recipients than in 7 unimmunized HIV-positive controls.

5.5.3 SRL 172, Study 003 (United States)

Study overview:

- Subjects: 35 HIV-infected children, ages 6 mo to 13 yr
- Subject characteristics: CD4 ≥300, age 1-8; ART encouraged (data not available)
- Treatments: 23, SRL 172; 12, intradermal hepatitis B (control)
- Dose schedule: 0, 2, 4 mos

Vaccine site reactions:

- median induration, 5 mm at 2 days; 3 mm at 14 days; 0 mm at 2 mos; 2 subjects had 4-5 mm induration at end of study (1 at dose one site, 1 at dose two site)
- crusting: present after 2 (3%) of 68 doses
- "sore arm" reported after 19 (28%) of 68 doses

Safety:

- Fever: recorded after 9 (13%) of 68 SRL 172 doses vs 3 (9%) of 35 HB doses
- CD4: median change SRL 172 = -99, HB = + 89 [p=0.50]
- Viral load: median change = - 0.1 $\log_{10}$ in both groups

Immunogenicity:

- 1 SRL 172 recipient had 2x increase in Ab to LAM; no subjects had increased lymphocyte proliferation response to LAM

5.5.4 SRL 172, Study 004 (Zambia)

Study overview:

- Subjects: 44 HIV-infected adults in Zambia, CD4; 22 BCG positive, 22 BCG negative; 31 male, 13 female; ages 21 to 51 yr.
- HIV characteristics: CD4 ≥200; none on ART
- Study design: open label
- Treatment, N, BCG status, and Dose schedule
  - SRL 172: 11 BCG-pos, 11 BCG-neg; 0, 2, 4, 12, 14 mo (5 doses)
  - borate buffered saline (control): 11 BCG-pos, 11 BCG-neg; 12, 14 mo only (2 doses)

Vaccine site reactions:

- induration
  - BCG pos: range over doses one to four: 11-14 mm at 2d, 0-3 mm at 14d; after dose five: median 5 mm at 2d, 3 mm at 14d
  - BCG neg: range over doses one to four: 8-11 mm at 2d, 0-3 mm at 14d; after dose five: median 6 mm at 2d, 3 mm at 14d
- drainage after 3 (3%) of 110 doses
- sore arm after 4 (4%) of 110 doses,

Safety:

- Temperature (measured daily for 15 days by subjects using digital thermometer): not fever noted
- Other symptoms: headache after 3 (3%) of 110 doses; malaise after 1 (0.9%) of 110 doses
- Viral load:
  - BCG neg: pre-dose 4 to post-dose 5: no significant differences compared to controls
  - BCG pos: baseline to post-dose 3, 0.5 log decrease (p=0.007); pre-dose 4 to post-dose 5: no significant differences compared to controls

Immunogenicity:

Table 5-7. Change in median lymphocyte stimulation index to SRL 172 sonicate, pre-dose 4 to post-dose 5

	SRL 172	Control	p-value
BCG negative	incr: 2.3 -> 6.0	decr: 3.7 to 2.6	<0.05
BCG positive	incr: 4.3 -> 8.8	unch: 1.9 to 1.9	<0.001

5.5.5 SRL 172, Study 005 (Finland)

Study overview:

- Subjects: 10 healthy HIV-negative adults, 39 HIV-infected adults with CD4≥200; all BCG-positive, in Finland
- Treatments:
  - SRL 172 at 0, 2, 4, 6, 12 months
  - Control vaccine (CV), hepatitis B vaccine at 0, 2, 12 mo; borate-buffered NaCl at 4, 6 mo (all intradermal)
- The 39 HIV-positive subjects were randomized between the two different treatments. Subject characteristics and outcomes are shown in Table 5-8.
- The 10 HIV-negative subjects all received SRL 172 at 0, 2, 4, 6, and 12 months. The key observations in these subjects were:
  - The five-dose schedule of SRL 172 was safe, well-tolerated and immunogenic.

  - Post-dose 5, LPA both to MTB whole cell lysate and to SRL 172 sonicate both were significantly greater than baseline ($p = 0.02$ and 0.008 respectively).
  - Post-dose 3, IFN□ to SRL 172 sonicate was greater than baseline ($p = 0.06$).
- Discontinuations. Among 29 subjects who received SRL-172 no subject withdrew before dose 3. Two subjects withdrew after dose 3: subject P29 from 30 mm erythema at the injection site, subject P38 because of 25 mm erythema and drainage lasting 5 weeks (this subject had a chest x-ray consistent with prior TB). Three subjects withdrew after dose 4: N1 for arthralgias, P11 for musculoskeletal discomfort and P27 for a sterile abscess.

Table 5-8. SRL 172, Study 005: Characteristics, Safety, and Immunogenicity of HIV-positive Subjects by Treatment Group

Characteristics	SRL 172	Control Vaccine (CV)[a]
N	19[b]	20
Male (N, %)	17 (89%)	18 (80%)
Age (median)	40 yr	41 yr
HIV status	HIV-positive	HIV-positive
Combination anti-retroviral therapy	17	10
CD4 (median)	559 /mm^3	631 /mm^3
Safety		
ISR: induration 2d medians (range)	4-7 mm (0-30)	0-10
ISR: erythema 2d means (range)	10-17 mm (5-26 mm)	0-3 mm (0-10)
ISR: skin breakdown	11 – 37% post each dose	10 – 30% post each dose
ISR: drainage	5 – 11% post each dose	0 – 5% post each dose
ISR: sterile abscess	1 (5%)	0
ISR: "sore arm"	16 – 37% post each dose	10 – 30% post each dose
Fever	5% post each dose	5% post each dose
Adenopathy	5% post each dose	5% post each dose
Malaise	5 – 11% post each dose	5 – 11% post each dose
CD4 count (2 mo after dose 5)	no change from baseline no significant diff c/w CV	no change from baseline
HIV viral load (2 mo after dose 5)	no change from baseline no significant diff c/w CV	no change from baseline
Serious adverse events	0	0
Discontinuations [b]	5	0
After 3 doses	2	
After 4 doses	3	
Immunogenicity		
LPA to SRL 172 sonicate	increased c/w CV post dose 3, dose 5 and 1 year	
LPA to SRL 172 sonicate	post dose 5: median cpm 12,560 vs 22,547 in HIV-neg; p=0.17	
LPA to MTB sonicate	increased c/w CV post dose 3	
IFN-γ to SRL 172 sonicate	increased c/w CV post dose 3, dose 5 and 1 year	
IFN-γ to MTB sonicate	no significant difference c/w CV	

a. CV = intradermal hepatitis B vaccine at 0, 2, and 12 mo; borate buffered saline at 4 and 6 mo.
b. Five subjects withdrew due to adverse events: one each with (a) injection site sterile abscess post dose 4; patient had apical scarring consistent with prior TB; (b) injection site induration and drainage post dose 3; (c) injection site prolonged drainage post dose 3; and (d) musculoskeletal pain, temporally related to immunization after dose 4 and (e) arthralgias after dose 4..

5.5.6 SRL 172, Study 006 (DarDar Trial, Tanzania)

The DarDar Trial of SRL 172 was a 7-year Phase III, randomized, controlled, GCP-compliant trial conducted in Dar es Salaam, Tanzania, initiated in 2001, and sponsored by the National Institutes of Health (von Reyn, Principal Investigator).

Study overview (Table 5-9):

- Subjects: HIV infected adults (age ≥18) with prior BCG (by scar) and CD4 ≥200
- N: 2013; randomized (1:1) to SRL 172 (V) or placebo (P; borate-buffered saline)
- Dose schedule: 0, 2, 4, 6, and 12 mo (5 doses)
- Follow-up: Seen every 3 months; median 3.3 years.

- Endpoints – all reviewed by a blinded 3-person expert panel using pre-defined criteria.
  - Primary endpoint: disseminated tuberculosis with positive blood culture
  - Secondary endpoint: definite tuberculosis (all culture-positive tuberculosis)

Results. SRL 172 was safe, well-tolerated, and induced T cell responses against the vaccine antigen and antibody to lipoarabinomannan. The trial was stopped at 7 years when the Data Safety Board determined that the vaccine was effective based on a significant reduction in all culture positive tuberculosis and a trend in the reduction of disseminated tuberculosis.

Table 5-9. SRL 172, Study 006 (DarDar Trial): Subject Characteristics and Outcomes by Treatment Group

Characteristics	SRL 172	Placebo	P value
N	1006	1007	
Female (%)	774 (77%)	766 (76%)	NS
Age (median)	33 yr	33 yr	NS
CD4 (median)	428 /mm^3	404 /mm^3	NS
History of prior TB	9%	8%	NS
PPD ≥5 mm	31%	32%	NS
Current ART	3%	3%	NS
Safety			
ISR[1,2] induration d+7	6.2 mm (0 – 30)	0.1	
d+28	4.4 mm (0 – 30)	0.1	
ISR[2]: skin breakdown d+7	36 – 58%	1-3%	
d+28	17 – 23%	0-1 %	
ISR[2]: drainage d+7	22 – 49%	0-1 %	
d+28	11 – 20%	0%	
ISR: sterile abscess[3]	3 (0.3%)	0	
Fever (T ≥38°C)	1%	1%	NS
Feverish	7 – 14%	4 – 13%	NS
Headache	5 – 17%	4 – 13%	NS
Malaise	13 – 23%	11 – 18%	NS
CD4 count change[2]	-57	-58	0.76
Log $_{10}$ HIV viral load change[2]	+ 0. 08	+ 0.42	0.01
Serious adverse events[4]	209	232	0.24
Discontinuations[5]	46	31	0.08
By MD	11	1	
By subject	35	30	
Immunogenicity[6]	**Baseline to post-dose 5**	**SRL 172 vs Placebo at post-dose 5**	
LPA to SRL 172 sonicate	Increased	V increased	
LPA to ESAT, Ag85, or MTB whole cell lysate	NSD	NSD	
IFN-γ to SRL 172 sonicate	NSD (trend only)	V increased	
IFN-γ to ESAT, Ag85, or MTB whole cell lysate	NSD	NSD	
Antibody to LAM	Increased	V increased	
Vaccine Efficacy[7,8]			
Disseminated TB	7	13	HR 0.52; p = 0.16
Definite TB	33	52	HR 0.61; p = 0.027
Probable TB	48	40	HR 1.17; p = 0.46

NS, P value not significant; NSD, no significant difference.

1. ISR data are median (range) for all doses at day +28 post-dosing unless otherwise indicated. Maximum reactions typically after dose 3.
2. Data from Substudy A (162 subjects). CD4 and viral load change is from baseline to 2 mos post-dose 5
3. Injection site sterile abscesses occurred after the first dose in one patient and after the third dose in two patients (both with a history of prior TB); all abscesses drained spontaneously and resolved with routine wound care and oral antibiotic therapy.
4. None considered related to immunization.
5. Immunization was discontinued by the MD investigator in 12 (1.2%) subjects because of adverse reactions considered possibly or probably vaccine-related: 3 vaccine site abscesses five other local reactions and four generalized rashes. An additional 65 subjects (35 SRL-172, 30 placebo) withdrew themselves from the trial

before completing the trial citing a switch to alternative medicine, perceived vaccine side effects or inconvenience. All reactions were considered mild or moderate and resolved after discontinuation of immunization.

6. Immunogenicity assay results compare (a) baseline to post-dose 5 among SRL 172 recipients; (b) SRL 172 vs Placebo, post-dose 5. Only significant effects noted, defined as $p \leq 0.05$. NSD, no significant difference
7. Vaccine efficacy endpoints were prospectively defined; data were reviewed and outcomes assigned by blinded expert panel.
8. Disseminated TB, defined as a positive blood culture (Primary Endpoint). Definite TB, stringent laboratory-defined criteria (Secondary Endpoint). Probable TB, lesser laboratory findings or only clinical findings.

6. OBJECTIVES

6.1 Primary:

To evaluate the safety and efficacy of a 3-injection series of DAR-901 booster in preventing tuberculosis infection among healthy adolescents

6.2 Secondary:

To identify risk factors for infection with *M. tuberculosis* among healthy adolescents

To identify subject characteristics associated with vaccine induced protection against infection with TB.

7. INVESTIGATIONAL PLAN

7.1 Overall Study Design

Overall study design is presented in Section 2.4, including

- Dose Groups
- Enrollment Process
- Study Structure
- Potential protocol adjustments
- Risk Management

7.2 Rationale for Treatment Regimens

7.2.1 Dose Level

The dose level (1 mg per dose) is based on

- the safety and immunogenicity observed in DAR-901 MDES study (see 5.4.1)
- prior clinical experience with SRL172, a killed whole-cell vaccine prepared from the same genotypic strain grown on agar (see 5.5), and
- mouse immunogenicity studies conducted with DAR-901 (see 5.3.3)

Clinical studies with SRL172. All clinical studies by Dartmouth investigators were conducted with an adult intradermal dose of 1 mg in a volume of 0.1mL (dose in children < 5 = 0.05 mL). This dose was established in initial studies by British investigators using vaccine prepared by SR Pharma (London). In the DarDar Trial of HIV-infected patients this dose was found to be safe, immunogenic, and effective in the prevention of tuberculosis (see Section 5.5.6). This will be the dose of DAR-901 in the present Phase 2 study.

7.2.2 Dose Number and Dosing Interval

The dose number and dosing interval are based on

- the safety and immunogenicity observed in DAR-901 MDES study (see 5.4.1)
- prior experience with SRL172, a killed whole-cell vaccine prepared from the same genotypic strain grown on agar (see 5.5), and
- published experience with other inactivated, whole-cell vaccines

Dose number. Inactivated whole cell vaccines are typically administered in a 2- or 3-dose schedule. A 3 dose schedule was safe and well tolerated in the DAR-901 MDES study and produced injection site reactions comparable to those observed in studies with SRL-172. Studies by Dartmouth investigators with SRL172 have shown safety and immunogenicity with both a 3-dose (Study 005, Table 5-8) and 5-dose schedule (Study 006, Table 5-9). A 3 dose schedule will be employed in the present study.

Dosing interval. Two month intervals between doses was safe and well tolerated in the DAR-901 MDES study and produced injection site reactions comparable to those observed in studies with SRL-172 (see Table 4) Prior studies by Dartmouth investigators with SRL-172 have used 2 month dosing intervals for 3 doses or the first 3 doses (Studies 002-006, Table 5-6). Safety and immunogenicity have been demonstrated with a 3-dose schedule at 0, 2 and 4 mos (Study 005, Table 5-8)

7.3 Rationale for Study Design

The study design – randomized, placebo-controlled, double-blind – is consistent with both the study objectives and current principles for the evaluation of multiple dose courses of investigational treatments. In particular, double-blinding avoids bias by the Investigator and subjects in assessing the subjective aspects of the study, particularly adverse events.

7.4 Maximum Exposures and Maximum Number of Subjects

The maximum exposures to DAR-901, the investigational agent, will be:

- Dose level: 1 mg per dose
- Number of doses: 3 doses over 4 months (120 days)
- Number of subjects: 650 IGRA-negative subjects

7.5 Definitions Applicable to Managing the Study

Treatment-emergent is defined as onset after active engagement in the trial; that is, after the administration of the first injection of study treatment. It is anticipated that treatment-related events may be observed after each injection of study treatment.

7.5.1 Definition of a Dose-Limiting Toxicity (DLT) Event

A DLT *systemic clinical event* is defined as a treatment-emergent systemic clinical event that meets ***all*** of the following criteria:

- is assessed by the investigator as related or possibly related to study drug (see Section 12.3.3);
- is of severity (see Section 12.3.4) Grade 2 or higher;
- is of duration >48 hours.

A DLT *injection site reaction* (ISR) is defined as an event that meets ***both*** of the following criteria:

- occurs at the injection site;
- is of severity Grade 3 or higher.

A DLT *laboratory event* is defined as a confirmed, treatment-emergent laboratory finding that meets ***both*** of the following criteria:

- is ***not*** considered consistent with a concurrent clinical event that is assessed as not related or unlikely related to study drug (e.g., an accidental injury, new ART); ***and***
- based on pre-specified toxicity criteria (see Section xx) represents an increase of two Grades or more compared to pre-treatment baseline value.

A subject who experiences a DLT event will not receive further doses of study drug. Unblinding may be performed to determine if a DLT event has occurred in a subject receiving DAR-901.

7.5.2 Definition of a Completed Patient

A **subject** is considered complete when s/he meets ***either*** of the following criteria:

- received all scheduled doses of DAR-901 and completes the study visit at 24 months, ***or***
- received at least one dose of DAR-901 ***and*** had treatment discontinued due to a DLT event.

7.6 Procedures for Managing the Study

7.6.1 Discontinuing Study Treatment in Individual Patients

Study treatment may be discontinued in an individual patient for ***any*** of the following reasons:

- The subject withdraws from study participation by their own decision (“withdrawal of consent”); this may happen at any time and for any reason without prejudice for their continued care.
- The subject has a DLT event as defined in Section 7.5.1.
- The Investigator determines, based on their judgment, that discontinuation of study treatment is in the subject’s best interest, e.g., due to an adverse event, noncompliance, or any reason, whether or not related to study drug or study procedures.

If study treatment is discontinued prematurely, the reasons will be recorded and, if possible, the EOS visit will be performed as specified (see Section 11). If a subject cannot be seen, attempts will be made to contact the subject by telephone.

7.6.2 Replacement of Subjects

A subject who is not IGRA negative at 2 months (Visit 2) or who is lost to follow-up before Visit 2 will not be part of the efficacy cohort and will be replaced to ensure the requirements for achieving the target sample size are met. A replacement subject will be identified by a distinctive subject number and will receive the same treatment as the subject being replaced.

7.7 Data and Safety Monitoring Board (DSMB)

As part of the comprehensive risk management program, the Sponsor will establish a 3 person Data and Safety Monitoring Board (DSMB) comprising two independent physicians with clinical trials experience (preferably including early phase and/or vaccine studies) and a representative of the Sponsor who has prior experience with SRL-172. One member will be from Tanzania.

The DSMB will serve the following functions:

- Review the Protocol and suggest possible changes prior to study initiation.
- Be notified of and review promptly any Serious Adverse Events. If the DSMB considers that the SAE may be treatment-related, they may request to be unblinded in order to proceed to a recommendation regarding the ongoing conduct of the Study.
- Recommendations regarding the conduct of the study may include, but are not limited to, increasing safety monitoring procedures or tests; terminating study treatment.
- Review blinded safety and efficacy data every 6 months
- Recommendations to the sponsor will be based on a majority vote.

7.8 Discontinuation of the Study

The Sponsor may terminate the study at any time for any reason. Subjects would still be followed for safety. In the event the study is terminated, the IECs and appropriate regulatory authorities will be notified of the decision.

8. SUBJECT SELECTION

8.1 Source of Subjects and Recruitment Methods

Following appropriate Human Studies review and approval, the Investigator may initiate and manage subject recruitment. This will include contact with the Ministries responsible for Education and Health in Tanzania and administrative leaders of secondary schools in Dar es Salaam. To reach an economically and socially diverse population, the study may be announced in newspapers, on the radio, at school functions and on relevant Internet websites.

8.2 Subject Disclosures and Restrictions during the Conduct of the Study

This is a Phase 2 study of healthy adolescents. In the interest of their safety and to facilitate accurate assessment of the data, the subjects will agree to the disclosures and restrictions detailed in Table 8-1 for the duration of their participation in the study, i.e., screening visit to End-of-Study (EOS) visit.

Table 8-1. Subject disclosures and restrictions during the conduct of the study

Item / Activity	Action	Comment
Prescription medication	Disclosure	Prescription medications in use at the time of screening will be reviewed in detail. Medications subsequently prescribed by physicians other than the Investigator will be disclosed promptly.
Over-the-counter medication	Disclosure	Over-the-counter medications in use at the time of screening will be reviewed in detail. Over-the-counter medications subsequently initiated by the subject will be disclosed promptly.
High-dose Vitamins[b]	Prohibited	Vitamins and minerals in doses substantially exceeding recommended daily requirements.
Blood donation	Prohibited	Prohibited until 2 months after Dose 3 (usually 6 months after enrollment)

a. Patients will be instructed about the range of products containing St. John's Wart, other herbals, caffeine or xanthines (including chocolate).
b. Vitamins and minerals in doses substantially exceeding recommended daily requirements.

8.3 Definitions

8.3.1 Non-childbearing potential

Non-childbearing potential is defined as meets ***one*** of the following two criteria:

- documented hysterectomy or bilateral oophorectomy.

8.3.2 Effective birth control (contraception) methods

Effective birth control (contraception) methods means strict abstinence or use of ***two*** of the following methods: hormonal contraceptive (oral, injectable, implanted [e.g., Implanon™], or intravaginal ring), condom, diaphragm, spermicide, or an intra-uterine device.

8.4 Subject Selection Criteria

Subject selection criteria are detailed in Section 2.4, including

- Inclusion and Exclusion Criteria (Sections 2.5.1 and 2.5.2, respectively)

8.4.1 Observed Variances

Subjects meeting inclusion and exclusion criteria at screening may be scheduled for enrollment. If on Day 0 prior to the first dose, clinical variances are noted compared to screening enrollment may proceed with the approval of the Medical Monitor.

9. STUDY TREATMENTS

9.1 Study Treatments to be Administered

Table 9-1. Study treatments

Role in Study	Identity of treatment	Comments
Investigational	DAR-901	Administered by ID injection
Placebo Control	Sterile Saline for Injection	Administered by the same route and in the same dose volume.

9.2 Identity of the Investigational Product

Table 9-2. Physical and Chemical Properties of Active Ingredient (Drug Substance)

Name	DAR-901
Vaccine Class	whole-cell, heat-killed organisms
Appearance	Slightly turbid yellow suspension

Table 9-3. Formulation of DAR-901 for Injection (Drug Product)

Name	DAR-901 for Injection
Active ingredient	DAR-901
Excipients	Borate-buffered 0.9% NaCl
How supplied	Sterile 2 mL vial containing 0.35 ± 0.05 mL of 10 mg/mL DAR-901
Storage	2-8°C
Preparation and handling	Preparation varies by dose level; see Section 9.3 for details.
Administration	The dose is administered as a single intradermal (ID) injection in the deltoid using a fresh sterile needle (approximately 26g).

9.3 Preparation and Handling of DAR-901 for Injection by Dose Level

9.3.1 1 mg DAR-901 for Injection

- Gently agitate the vial to assure an even suspension
- Withdraw 0.1 mL from the vial for administration.

Detailed dose-preparation instructions and flow-sheets will be provided in the Pharmacy Manual.

9.4 Reference and Blinding Therapy

Sterile Saline for Injection is used as the placebo control and for blinding.

9.5 Administration of Study Treatments

Each dose of DAR-901 or placebo will be administered as a single intradermal (ID) injection. The recommended site for injection is the upper deltoid region of the arm, with sequential doses administered in the opposite arm. In the event this recommendation cannot be accommodated due to injury or other issue, the same arm may be used successively, placing the injection at least 5 cm apart.

9.5.1 Variances in Dose Administration Schedule

The protocol permits variance in dose administration of plus or minus two weeks from the nominal scheduled timepoint. Variances that would exceed plus or minus two weeks should be discussed with the Medical Monitor.

9.6 Method of Assigning Patients to Treatment Groups

Subjects will be assigned by computer-generated randomization. Suitable randomization procedures will be established by the CRO data management and clinical services performing the protocol and approved by the Sponsor. The research pharmacist will be provided a list of treatment assignments.

9.7 Selection of Dose Levels in the Study

See Section 7.2.1.

9.8 Selection of Dose Number and Interval

See Section 7.2.2.

9.9 Blinding

Subjects randomized to placebo regimen will receive an injection of Sterile Saline for Injection at the same dose volume as active treatment. The pharmacist is responsible for maintaining the blind, that is, assuring that treatment allocation is not revealed to other study staff or the patients.

9.9.1 Procedures for Unblinding Individual Patients during the Study

There are no specific treatments for the effects of DAR-901; the Investigator should manage patients symptomatically based on any changes observed. Consequently, it is not expected that the treatment allocation for a particular subject will need to be revealed (i.e., unblinded).

If the Investigator needs to unblind the treatment assignment for a particular subject, prior approval by the DSMB should be obtained and the following information entered into the medical record:

- date and time of the last injection,
- reasons study drug was discontinued,
- name of the Medical Monitor who approved unblinding,
- reasons the subject's treatment allocation was unblinded.

In the event of a true medical emergency in which the Investigator judges that the subject cannot be managed safely without unblinding, the Investigator may obtain the treatment allocation directly from the pharmacist at the site. All steps above will be followed, including contacting the DSMB as soon as possible and not more than 24 hours afterwards.

9.10 Prior and Concomitant Therapy

See Section 8.2. Treatments prohibited prior to enrollment are prohibited for the duration of the study.

9.11 Treatment Compliance

All doses will be administered by a study nurse or physician.

9.12 Accountability of Investigational Drug Supplies

The Investigator at each study site will identify trained and experienced personnel to handle the study drug in accordance with the protocol and appropriate GCP and GMP principles. This includes:

- storing the drug in a secure, limited access facility and under the appropriate conditions;
- dispensing and administering study drug only in accordance with the protocol;
- maintaining drug accountability records;
- at the completion of the study, returning or destroying unused study drug in compliance with the written instructions of the Sponsor.

Detailed procedures for accountability of drug product are provided in the SOPs which include requirements for destroying unused drug according to guidelines of the Tanzanian Food and Drugs Authority.

10. STUDY EVALUATIONS

Detailed schedules of evaluations are shown in Table 1.

10.1 Medical Evaluation

The Investigator will assess the general health of the potential subject at the Screening visit; any new findings observed at subsequent scheduled and unscheduled visits will be recorded.

10.2 Vital Signs

Vital signs include temperature, heart rate (HR), respiratory rate (RR) and blood pressure (BP). Where feasible, vital signs should be measured before blood is drawn and after the patient has been sitting comfortably for ~5 min with the BP cuff in place (preferably on the non-dominant arm). BP and HR measurements may be done manually or by automated recorder. Temperature will be obtained using an electronic (rapid reading) device. Respiratory rate will be determined by observation for at least 30 sec.

10.3 Laboratory Studies

The laboratory tests indicated below will be performed by an approved laboratory proposed by the Investigator at each site and approved by the Sponsor. Details of procedures for collecting, processing, storing and shipping the blood samples will be provided in the SOPs.

The Investigator may order additional local laboratory tests consistent with their routine standard of care.

10.3.1 Safety Laboratory Tests

Table 10-1. Complete blood count (CBC)

Hematocrit White blood cell count Platelet count	White blood cell differential (if WBC abnormal and clinically significant) - Neutrophils - Lymphocytes - Monocytes - Eosinophils - Basophils

10.3.2 Pregnancy Tests

For all females, a urine pregnancy test will be conducted as scheduled. A negative pregnancy test result must be reported within 24 hours prior to the first dose of study drug.

10.4 Reporting of Safety Laboratory Tests

The results of Safety Laboratory Tests will be returned to the Investigator as quickly as possible, typically within 48 hours. Reference ranges (lower limit of normal, upper limit of normal; by sex and age, if appropriate) will be provided for the CBC.

Procedures for the investigator assessment of laboratory results are detailed in Section 12.1.5.

10.4.1 Repeating Abnormal Laboratory Tests

A CBC showing abnormal or clinically significant values at screening may be repeated no more than once. After dosing, an abnormal CBC assessed as “clinically significant” values may be repeated as often as deemed clinically necessary by the Investigator until the test values return to clinically acceptable limits or until an explanation other than drug effect is given.

10.5 CBC. A 4 ml phlebotomy will be performed to obtain blood for a CBC, kept in a cooler at 20-22 C and transported to the clinical laboratory for the study.

10.6 Interferon Gamma Release Assay (IGRA)

A 4-6 mL phlebotomy will be performed to obtain blood for the IGRA (T spot, Oxford Immunotec, Oxford, UK). Blood will be immediately placed in a cooler at 20-22 degrees C and transported within 4 hours to the DarDar Laboratory. Processing will be completed within 32 hours of collection according to

manufacturer's instructions and results will be interpreted as positive or negative according to manufacturer's instructions.

10.7 RNA Expression

A 2.5 mL phlebotomy will be performed 7 days after Dose 3 using a Paxgene tube. Samples frozen at -80C, batched and stored at MUHAS for later processing to compare differences in RNA expression between vaccine and placebo recipients.

11. STUDY EVENTS

Detailed schedules of evaluations are shown Section 2.9. The schedule is presented relative to the day and time of dosing. All Days are relative to day of first injection of study drug, designated Day 0; all times are relative to the most recent injection, designated 0 hr.

Monitoring for adverse events and concomitant medications will be performed on an ongoing basis from screening through End-of-Study visit.

11.1 Screening

The screening evaluation may be performed up to 28 days prior to dosing. The subject will sign an Informed Consent/Assent Form before any study-specific procedures are performed.

The subjects will receive a printed version of the signed informed consent for their records.

11.2 End-of-Study Visits For Subjects who Terminate Prematurely

Subjects who terminate prematurely, for any reason, should have a final safety visit completed at approximately 30 days after the last dose received, or if that timepoint is already passed, as soon as possible. This final visit should include phlebotomy for an IGRA assay. If they cannot complete a visit, safety follow-up should be conducted by phone.

12. SAFETY EVALUATIONS

Clinical trials sponsored by Dartmouth College will be conducted in accordance with Good Clinical Practices for collecting and reporting safety information. Safety and tolerability will be evaluated based on AEs, vital signs, physical exams, laboratory tests and other assessments.

12.1 Definitions

12.1.1 Adverse Event (AE)

An Adverse Event is any untoward medical occurrence temporally associated with the use of a medical product in a subject, ***whether or not*** the event is considered causally related to the medical product.[1] An AE can be a new occurrence or an existing process that increases significantly in intensity or frequency.

An AE in a clinical trial may be ***any*** of the following:

- Unfavorable and unintended *symptom reported by the subject* — subjects will be encouraged to report treatment-emergent AEs spontaneously; general, non-directed questioning may also be used to elicit reports of AEs;
- Clinical *sign detected by the Investigator* — observations by other study personnel will be reported to the Investigator for evaluation;
- Is a treatment-emergent new or increased abnormal result from a *laboratory study* or other *diagnostic procedure.*

12.1.2 Pregnancy

Pregnancy is not an AE. Pregnancy testing will be performed as scheduled. If a female subject becomes pregnant during a study, the Medical Monitor must be notified in writing within five days. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be obtained.

12.1.3 Serious Adverse Event (SAE)

An AE is **serious** when the subject outcome is one or more of the following:

- Death.
- Life-threatening, meaning that the subject was at immediate risk of death from the event at the time that the event occurred. It does not include an event which hypothetically might have caused death if it occurred in a more severe form.
- Hospitalization, initial or prolonged, meaning that a hospital admission and/or prolongation of a hospital stay was required for the treatment of the AE, or occurred as a consequence of the event. It does not include a pre-planned elective hospital admission for treatment or diagnostic procedures, or, in general, a hospital admission of less than 24 hours duration.
- Disability or incapacity that is persistent or significant.
- Congenital anomaly or birth defect.
- Important medical event that, although not immediately life-threatening, requires intervention in order to prevent one of the other serious outcomes listed above. Examples of such events are allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse. For the present study a new case of active tuberculosis will be considered an SAE in this category.

[1] A medical product may be a drug or a device being used either prior to or after regulatory approval. The medical product in this protocol will hereafter be referred to as study drug (synonym: investigational agent).

12.1.4 Suspected, Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is defined as an SAE that meets ***both*** the following criteria with respect to study drug:

- *Suspected* — is assessed as related or possibly related to study drug (see Section 12.3.3);
- *Unexpected* — compared to the study drug-related AEs described in Investigator's Brochure, the event meets ***any*** of the following criteria:
  - The event was not previously described;
  - The event is now characterized as more severe (see Section 12.3.4);
  - The event is now characterized more specifically (e.g., an event of "interstitial nephritis" in a subject receiving an agent previously described as associated with "acute renal failure").

In clinical trials involving ill patients, events considered related to the natural history of the disease under study or to lack of efficacy (that is, the event is considered more likely related to those factors than to other factors, including study drug) are not considered "unexpected". Lack of efficacy is recorded as specified elsewhere in the Protocol.

12.1.5 Investigator Assessment of Safety Laboratory Tests

The Investigator will review the results of the CBC (see Section 10.3.1 and Section 10.3.2) and designate any results outside of the reference range as ***either*** of the following:

- Abnormal, not clinically significant (NCS)
- Abnormal, clinically significant (CS).

In making this judgment, the Investigator will consider all available information, including the patient's clinical condition, all available laboratory results (central and local), and the potential for false positive test results. In addition, laboratory studies that result in the actions specified in Section 12.1.1 will be classified as "abnormal, clinically significant".

Any result assessed as "abnormal, clinically significant" will be recorded as an AE *unless* it is consistent with one or more of the following:

- Process noted in the medical history.
- Ongoing adverse event already recorded;
- Expected course of the primary disease under study (if applicable);

12.2 Collecting and Recording Adverse Events

Procedures for the collection and recording of AEs are as follows:

- At all study visits subjects will questioned using both a scripted checklist to elicit anticipated vaccine-related adverse events, as well as open-ended queries to elicit unanticipated events.
- After the EOS, surveillance will be passive (only events brought to the investigator's attention will be considered) and only events assessed as SUSARs will be recorded.

12.3 Characterizing Adverse Events

For each AE recorded the following characteristics will be noted.

12.3.1 Description of Event

The diagnosis or description will be as specific and complete as possible (i.e., "lower extremity edema", rather than just "edema"). Whenever possible, signs and symptoms due to a common etiology will be reported as an integrated diagnosis; for example, cough, runny nose, sneezing, sore throat and head congestion would be reported as "upper respiratory infection".

12.3.2 Date and Time of Onset

The date and time at which the event was first apparent. Table 12-1 summarizes the basis for reporting the date and time of onset for the different types of AEs described in Section 12.1.1.

Table 12-1. Reporting the Date and Time of Onset of AE for Different Types of Events

Type of Event	Examples	Source of Date and Time of Onset
Symptom	Headache, feverish, paresthesias	When first experienced by the patient
Sign (Finding)	Elevated BP, enlarged liver on physical exam	When first observed by the Investigator or other study staff
Laboratory / diagnostic result	Neutropenia, hyperglycemia, lesions on brain scan	When lab sample was obtained or diagnostic study performed

The time of onset of symptoms may be appreciably earlier than the date and time the Investigator becomes aware of the event. Some events may be apparent to the patient and Investigator independently, and information from each may contribute to the final report. For example, a patient may report the onset of a rash two days before being seen by a physician who makes a diagnosis of herpes zoster based on appearance and laboratory confirmation. In that case, there is a single AE, with the date of onset based on the date of the initial observation by the patient and a specific description (herpes zoster) based on the clinical exam and tests.

12.3.3 Relationship to Study Drug

This determination is based on the Investigator's clinical judgment and the Medical Monitor's clinical judgement regarding the likelihood that the study drug caused the AE and may include consideration of some or all of the following factors:

- Alternative possible causes of the AE, including the subject's underlying disease or co-morbid conditions, other drugs, other host and environmental factors;
- Temporal sequence between the exposure to study drug and the AE;
- Whether the clinical or laboratory manifestations of the AE are consistent with known actions or toxicity of the study drug;
- Whether the AE resolved or improved with stopping the study drug (i.e., dechallenge); or recurred or worsened with re-exposure to the drug (i.e., rechallenge).

The relationship between the study drug and the AE will be described using one of the following categories:

- ***Related*** — the study drug is more likely the cause of the AE than other factors;
- ***Possibly related*** — there is a *reasonable* possibility that the study drug is the cause of the AE, including that the study drug and another factor(s) are equally likely as causes of the AE;
- ***Unlikely related*** — another factor is considered more likely the cause of the AE than the study drug;
- ***Not related*** — another factor is considered to be the cause of the AE.

Related or possibly related AEs may result during the use of the study drug as planned (per protocol), or from abuse, withdrawal or over-dosage of the agent.

12.3.4 Intensity (Severity)

The intensity (synonym: severity) of clinical AEs (i.e., symptoms reported by the patient and/or signs observed by the investigator) will, in general, be assessed by the Investigator using the five-level grading system (Table 12-2; adapted from CTCAE v4.02 (11)). The system reflects the duration of the event, its impact on the subject's activities, the level of medical intervention required, and, for events assessed as related or possibly related to study drug, the action taken with study drug. The Table below is intended to provide guidance; the investigator should use judgment in assigning an intensity grade to an event. In

some instances a single characteristic may determine the grade; in other instances, the overall pattern may be considered more appropriate.

For this purpose, activities of daily living (ADL) are classified into two subsets:

- ***Instrumental ADL*** — e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money;
- ***Self-care ADL*** — e.g., bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

Table 12-2. Guidelines for grading the intensity (severity) of AE

Event Characteristic	Event Grade [a]			
	1 (Mild)	**2 (Moderate)**	**3 (Marked)**	**4 (Extreme) [b]**
Duration of symptoms	Transient, typically <48 hrs	Up to 2 weeks	>2 weeks, reversible	Symptoms / disabilities may be permanent
Impact on ADL	No limitations in ADL;	Some limitations in age-appropriate instrumental ADL	Some limitations in self-care ADL	Limitations in all activities; significant assistance required
Medication intervention	None or only OTC meds	OTC or prescription meds; provide relief	Prescription meds required; relief may be partial	Multiple meds required
Interventions other than medication	Minimal, local, or non-invasive	Minimal, local, or non-invasive	May be hospitalized <24 hr	Hospitalization >24 hr; surgery
Typical action with study drug [c]	No adjustment in study treatment regimen required	Study drug may or may not be continued	Study treatment may be discontinued	Study drug is discontinued

[a]. **Grade 5** is death (fatal) and is reserved for the particular AE that is assessed as the primary cause of death. Alternative terminology in use for Grade 3 includes "Severe"; for Grade 4 includes "Life-Threatening".
[b]. Typically "Life-Threatening," that is, imminent risk of death, urgent or significant intervention required.
[c]. Applicable only if event assessed as related or possibly related to study drug.

12.3.4.1 Relationship Between "Intensity" (Severity) and Seriousness in Characterizing Clinical AEs

Intensity (severity) and seriousness are distinct and independent items, with some interrelationship. By definition, clinical events assessed as SAEs meeting criteria for "death" would be Grade 5 and those meeting criteria for "hospitalization" or "life-threatening" would be Grade 4. Typically, events assessed as SAEs based on associated "significant disability" or being "medically important" would be Grade 3 or Grade 4. However, a clinical event may be assessed as Grade 3 and *not* qualify as an SAE; for example, a patient with a history of migraine headaches could have an episode that restricted them to bed for several hours but responded to the usual treatment, ran its usual course and had no sequelae.

12.3.5 Management of Study Drug

For each AE the Investigator will indicate which one of the following actions regarding the administration of study drug (study treatment) was taken because of that AE:

- ***Drug withdrawn (discontinued)*** — study drug was stopped permanently due to the AE;
- ***Drug interrupted*** — study drug regimen was modified temporarily, including one or more doses were not administered, but drug was not stopped permanently;
- ***Dose not changed (No action taken)*** — no change in the administration of study mediation.

12.3.6 Actions Taken for Management of AE

AEs will be followed and managed by the Investigator, including obtaining any supplemental studies needed to define the nature and/or cause of the event (e.g., laboratory tests, diagnostic procedures, consultation with other health care professionals).

For each AE the Investigator will categorize as follows the actions taken to manage the AE:

- ***Concomitant medication*** — one or more medications (prescription or over-the-counter) were started or increased in dose; non-medication actions may *also* have been ordered.
- ***Other action*** — *only* non-medication action(s) were ordered as management of the AE (e.g., bed placed in Trendelenburg position, warm compresses applied to IV access site).
- ***No action*** — no actions were ordered for management of the AE.

12.3.7 Outcome

Follow-up of AEs. If possible, AEs will be followed until resolved (synonyms: recovered, recuperated, ended) either with or without sequelae, including for subjects who prematurely discontinue study participation. For AEs that are assessed as not drug-related and are not resolved at the End-of-Study visit, follow-up may be limited with the approval of the Medical Monitor.

Outcome of AEs. The outcome of each event will be described using the following categories:

- ***Resolved (recovered) without sequelae*** — the event resolved and subject returned to baseline;
- ***Resolved (recovered) with sequelae*** — the event resolved but the subject is left with residual problems (e.g., functional deficits, pain);
- ***Resolving (recovering)*** — at the last observation, the event was improving;
- ***Not Resolved (not recovered)*** — at the last observation, the event was unchanged;
- ***Death (Fatal)*** — to be used for the *one* AE which, in the judgment of the Investigator, was the *primary* cause of death;
- ***Unknown*** — there were no observations after the onset (initial observation or report) of the event.

Note: Resolving and Not Resolved may also be used for AEs that were unresolved at the time a subject died, but were *not* assessed as the primary cause of death.

12.3.8 Date and Time of Outcome

For each class of outcome as defined above, Table 12-3 indicates the date and time to be recorded. As discussed in detail for date / time of onset (see Section 12.3.2), determining the date / time an event resolved (ended) should reflect the type of event and the source of the information.

Table 12-3. Date and Time of Outcome for AE by Outcome Class

Outcome assigned to AE	Date and Time to be Recorded
Resolved (with or without sequelae)	Date and time event observed or reported as resolved
Death	Date and time of death
Resolving or Not Resolved	Date and time of last observation
Unknown	None (see definition above)

12.4 Reporting of Serious Adverse Events

12.4.1 Where to Report SAEs

SAE reporting forms with detailed instructions will be provided during training. Serious adverse events will be entered immediately into the electronic study record with concomitant notification of all relevant study personnel, including the Medical Monitor. The CRO will work with the Site to collect any additional data needed to further evaluate the SAE. Reports and supporting materials relating to SAEs should be obtained and noted in the CRF.

The Investigator will notify the relevant IRBs and Tanzanian FDA of SAE's based on reporting requirements. Contact information is provided in Section 17.

12.4.2 Procedures for Reporting SAEs to the Sponsor

The ***initial notification*** should be completed by phone or email for each SAE within 24 hours of the time the Investigator (or the Investigator's designee) becomes aware that the event has occurred and will include the following items of information (any items not available should be explicitly noted):

- protocol number, study site, subject number;
- Investigator's name, address, and contact information (phone, fax, email);
- description of the event (i.e., date and time of onset, initial assessment, treatments and course);
- current status of the subject and the event;
- criteria by which the event was assessed as serious;
- date of the first injection of study drug;
- date of the last injection of study drug prior the event;
- assessment of relationship of study drug to the event;
- whether the study drug was discontinued or adjusted as a result of the event.

The ***initial full report***, signed by the Investigator, will be submitted within two days for death and life-threatening events and within four days for all other SAEs; the report will include all of the above information *plus* the following items:

- narrative summary of the event — to include specific information that will assist in understanding the event, e.g., relevant medical history, co-morbid conditions, physical exam, diagnostics, assessment, treatments (including concomitant medications), response to treatment, course, and outcome (if known);
- copy of the completed AE page of the CRF (or completion of online data entry);
- copies of relevant medical reports — including diagnostic procedures (e.g., laboratory, ECG, x-ray), surgical procedures, and consultations.

Thereafter, signed ***supplemental reports*** will be submitted as any additional information (e.g., more definitive outcome regarding events previously reported as ongoing or unknown outcome) becomes available to the Investigator (either directly or as a result of investigation into a query).

12.4.3 Requirements for Expedited and Periodic Reporting of Adverse Events

SUSARs are required to be reported rapidly to the DSMB, regulatory authorities and to IRBs (within seven days for fatal or life-threatening SUSARs; within 15 calendar days for all other SUSARs). There are varying requirements for periodic (annual or semi-annual) reporting of all SUSARs and, in some cases, all SAEs. The Sponsor and the Investigator will work together to meet these reporting requirements.

12.4.4 Notification of SAEs to the Investigator by the Sponsor

In accordance with regulatory requirements, the Sponsor will notify the Investigator of the occurrence of SUSARs reported by other Investigators in this or in other studies involving the study drug. The Investigator will promptly inform his/her IEC of such communications from the Sponsor and will document that notification in the Investigator's Regulatory Binder.

12.5 Sponsor Guidance for Grading of Injection Site Reactions

As detailed in Section 5.5 and 5.4.1, the adverse events associated with intradermal injection of SRL 172 and DAR-901 have been primarily injection site reactions similar to, but generally milder than, reactions to BCG vaccination, which has been used world-wide for over 70 years. Further, DAR-901 is a heat-inactivated vaccine with no living organisms, so the rare invasive BCG complications of lymphangitic or hematogenous spread are not under consideration, even in HIV-infected persons.

12.5.1 Definitions of the Most Commonly Expected Systemic Vaccine-related Symptoms

- Fever: elevated temperature documented by any route during a visit or by the subject at home.
- Feverish: subjective fever reported by the subject, but not documented.

- Malaise, myalgia, "flu-like" symptoms: will be defined consistent with routine clinical practice.

12.5.2 Definitions of the Most Commonly Expected Injection Site Symptoms

- Tenderness: discomfort elicited when the area is touched either intentionally or accidently.
- Pain: discomfort or unpleasant feeling (e.g., headache, stubbed toe) experienced while at rest or with activity; in addition to location, the patient's description may include intensity as well a distinctive quality (e.g., burning, stabbing). In the SRL 172 trials (see Section 5.5), these events were reported as "sore arm."
- Pruritus (itch): an unpleasant sensation that evokes the desire or reflex to scratch. (In contrast, pain and tenderness evoke a reflex to withdraw.)

12.5.3 Definitions of the Most Commonly Expected Injection Site Findings

- Erythema: reddening of the skin.
- Desquamation: skin coming off in scales, often patchy or circumferential; maximum linear diameter will be recorded only if the area of involvement is a continuous patch.
- Induration: an area of skin that is thicker, firmer than usual. Will be used to include both the related terms papule / nodule (a solid raised lesion with distinct borders, <1 cm diameter) and plaque (papule-like lesion, >1 cm), since diameter will be recorded independently.
- Vesicle / Blister: a sub-epidermal collection of clear fluid
- Pustule: a sub-epidermal collection of white or yellow fluid up to 2.5 cm diameter that based on appearance is presumed to be "pus", i.e., to contain neutrophils. Commonly seen after intradermal injection of BCG or heat-killed mycobacteria, but rarely infected with pyogenic bacteria.
- Erosion: the loss of the surface of the skin; typically results in a shallow moist or crusted lesion. In the studies with SRL 172 (see Section 5.5), this process was reported as "skin breakdown".
- Ulceration: full thickness loss of epidermis, with erosion into dermal or deeper tissue; commonly crusted or with granulation.
- Crust: dried material covering an erosion or ulceration; may be white or colorless if composed of plasma or exudate, or darker if small amounts of blood are present.
- Eschar: hard dry plaque covering an ulcer, implying underlying tissue necrosis
- Abscess: a sub-epidermal collection of white or yellow fluid greater than 2.5 cm diameter that based on appearance is presumed to be "pus", i.e., to contain neutrophils. If closed, the lesion is typically fluctuant, that is yields to palpation consistent with containing fluid. Abscesses may open and drain spontaneously or may be incised and drained by the investigator. Abscesses will be classified as "Sterile" or "Infected (Pyogenic)" (see below).
  - If an abscess is incised by the investigator, a fresh culture should be obtained. Open lesions have a high likelihood of contamination and culture is generally only useful if there are other findings, such as surrounding cellulitis or acute systemic symptoms.
  - The investigator may prescribe topical or systemic antibiotics based on their judgment of the risk of current or potential pyogenic involvement. The expected pathogens would be *Staphylococcus aureus* or β-hemolytic streptococci.
- Sterile Abscess: In the DarDar trial, sterile abscess was observed in 0.3% of HIV-infected adults who received SRL 172. The trial did not report any pyogenic abscesses. Sterile abscesses are typically not accompanied by surrounding erythema, warmth, or marked tenderness, or by fever or regional lymphadenopathy (12).
- Infected Abscess: An abscess due to pyogenic bacteria, typically *Staphylococcus aureus*. In addition to documented positive cultures, pyogenic abscess is expected to be accompanied by at least one of the following: surrounding erythema, warmth, or marked tenderness. Infected abscess also often present with fever and regional lymphadenopathy.
- Scar: an area of fibrous tissue that replaces normal skin after injury; a natural sequela of wound repair and healing. Often associated with mild discoloration.

12.5.4 Visit Assessment of Injection Site Reactions and Other Post-Dosing Events

At each visit specified in Sections 2.9 and 12.2, the subject will have vital signs obtained, and be interviewed by study personnel regarding symptoms and other events.

Treatment-emergent changes in vital signs will be graded using the criteria shown in Table 18-2, which are taken from FDA Guidance (13).

Subjects will be explicitly asked about systemic and local symptoms (see Sections 12.5.1 and 12.5.2, respectively), any interference with daily activities, and any treatment required. Symptoms will be graded using the functional criteria shown in Table 12-4.

Study personnel (RN or MD) will examine all of the prior injection sites and then:

- Record the presence or absence of the physical findings defined in Section 12.5.3;
- For any findings present, record the maximum linear diameter in mm;
- Pain, erythema, and induration will be graded as shown in Table 12-5, taken from FDA Guidance (13); other ISRs characteristics will be graded using the functional criteria shown in Table 12-4.
- Considering *all* the findings present, a study MD will grade the *overall* intensity (severity) of the ISR as per Table 12-4.
- This grade should be "static", that is, based upon the impact and management of the ISR at the time, without reference to previous observations for the subject.
- Subjects will be asked for permission to photograph the ISR if it is assessed as Grade 2 or higher, is accompanied by systemic symptoms, is managed with prescription medication (e.g., systemic antibiotics or prescription analgesics) or is judged by the Investigator to warrant documentation. Photographs are completely optional and may be declined by the subject without impacting any other aspects of the protocol.

Table 12-4. Grading of Vaccine-Related Adverse Events

Characteristic	Grade 1	Grade 2	Grade 3
Impact on ADL	No limitations in ADL	Some limitations in age-appropriate instrumental ADL	Some limitations in self-care ADL
Medication intervention	None or self-medication with OTC meds	Prescription meds offered; provide relief	Requires prescription meds; relief may be partial
Interventions other than medication	Minimal, local, or non-invasive	Minimal, local, or non-invasive	Requires hospital facilities for <24 hr

This Table is intended to provide guidance; the investigator should use judgment in assigning an intensity grade to an ISR. In some instances a single characteristic may determine the grade; in other instances, the overall pattern may be considered more appropriate.

Table 12-5. Grading of Common Injection Site Reactions

Local Injection Site Reaction	Grade 1	Grade 2	Grade 3	Grade 4
Pain	Present but does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours ***or*** interferes with activity	Any use of narcotic pain reliever ***or*** prevents daily activity	Emergency room (ER) visit ***or*** hospitalization
Erythema[a]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	[c]
Induration/Swelling[b]	2.5 – 5 cm ***and*** does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	[c]

a. Erythema (Redness) should be measured at the maximal diameter and the measurement should be graded and also recorded as a continuous variable.
b. Induration (Swelling) should be measured at the maximal diameter and the measurement will also be recorded as a continuous variable; the event should be graded using the functional scale as well as the actual measurement.
c. Note that Erythema and Induration, in and of themselves, are not "life-threatening (Grade 4)" events; however, they may progress to new events, such as exfoliative dermatitis or necrosis, that should be recorded and graded separately.

12.6 Grading of Specific Laboratory Safety Tests for Reporting and Analysis

For specific laboratory safety tests shown in Table 18-1 all abnormal results will be graded using the criteria shown, which are taken from the "FDA Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. September 2007." (13).

The grading will be used both in reporting AEs and in the data presentation and analysis of laboratory results. Specifically, the data listings will indicate the appropriate Grade and treatment-emergent changes in these laboratory tests will be summarized as "shift tables" using these grades (see Section 0). This process will assure that the final study report contains complete and consistent analyses of these laboratory safety results.

Treatment-emergent abnormal laboratory results for analytes not shown in Table 18-1 will be reported as AEs using the procedures and criteria detailed in Section 12.1.5.

13. DATA QUALITY ASSURANCE

13.1 Compliance

The Sponsor and the Investigator will conduct the study in accordance with:

- The protocol — as approved by applicable regulatory authorities;
- Ethical standards and procedures — as detailed in Section 15;
- "Good Clinical Practices" and "Good Manufacturing Practices" — as detailed in documents issued by the International Committee on Harmonization (ICH);
- Applicable Tanzanian regulations — e.g., in the US, 21 CFR.

13.2 Training and Qualifications of Site Personnel

All site personnel involved in the study will be trained regarding the protocol and the study drug. This includes, but is not limited to, pharmacy, nursing and medical personnel involved in handling and administering the study drug, monitoring the subjects and collecting clinical data. Staff will also be trained and certified in either GCP or GLP as appropriate.

The Sponsor (or designee) will provide formal training sessions either off-site (e.g., Investigators Meeting) or on-site (e.g., site initiation visit). Topics covered will include, but not be limited to, background of the investigational drug, the protocol, study events, study procedures, data collection and recording, expedited and routine reporting of adverse events, and regulatory requirements. It is the responsibility of the Investigator to notify the Sponsor of any new study personnel and to work with the Sponsor to ensure that they receive adequate training.

13.3 General Procedures for Completing Data Collection

All data will be collected on CRFs designed for the study and will be entered in an electronic database, justified and corrected as necessary following GCP procedures.

13.4 Case Report Forms

The Sponsor will provide structured forms for reporting study data to a central facility holding the trial database. The Investigator (or qualified sub-Investigator approved by the Sponsor) will review all CRFs and indicate their concurrence by either a manual or electronic signature, as appropriate. The Sponsor will provide detailed procedures for the system used in the study.

13.5 Source Documents

Source documents are the originals of any documents used by the Investigator, hospital, or institution that verify the existence of the subject and substantiate the integrity of the data collected during the trial. Unless otherwise specified by the Sponsor, source documents will be available to support all the data recorded on the CRF and SAE forms. Source documents forms created exclusively for the purpose of this study (e.g., screening logs, study procedures worksheets) must be reviewed by the Sponsor prior to use.

Source documents may include, but are not limited to, the following:

- the informed consent form, signed and dated by the subject;
- information obtained from the subject's personal physicians or other third parties regarding the subject's medical history or prior physical condition;
- screening logs;
- recorded data and reports from automated instruments (e.g., ECGs, cardiac monitors, vital signs), including annotations of abnormal findings;
- laboratory reports (e.g., hematology, clinical chemistry, urinalysis, urine microscopy), including annotation of abnormal results;
- concomitant medication prescription and administration records;

- medical records relating to scheduled and unscheduled study visits, including, but not limited to, results of examinations, observations relating to AEs, and concomitant medications.

In addition to the practices noted for CRFs (see Section 13.3), source documents must also meet the following requirements:

- Be prepared at the time of the events or activities described (i.e., contemporaneously);
- Indicate both the date and time recorded;
- Identify the source of all recorded information (e.g., the subject, direct observations of the recorder, lab reports, external / historical sources).

13.6 Protocol Deviations

Conduct of the study will be monitored to ensure that protocol deviations are minimized. A protocol deviation is defined as an event in which the Investigator or site personnel did not conduct the study according to the Protocol, including compliance requirements and agreements.

For protocol deviations relating to individual subjects, the event and relevant circumstances will be recorded on source documents and on the appropriate CRF; reported to the Sponsor in a timely manner; and presented in the Clinical Study Report.

Deviations that are not subject-specific (e.g., unauthorized use of an investigational agent outside the protocol, either human administration or laboratory use; non-compliant actions involving another study by site personnel also involved in both this protocol) will be reported to the Sponsor in writing and copies placed in the Trial Master File.

Deviations that can be anticipated should, if possible, be discussed with the Sponsor before being implemented.

13.7 External Review of the Study Conduct at Participating Sites

All study-related materials at the site are subject to external review to ensure the safety of the subjects, the integrity of the study data, and compliance with all applicable regulatory and oversight requirements.

There are several different classes of review:

- Monitoring — review by the Sponsor or authorized representatives, typically from the CRO coordinating the clinical conduct of the trial;
- Audits — independent review by the quality assurance department of the Sponsor or authorized representatives, potentially from an organization not involved in the clinical conduct of the study;
- Regulatory review — performed by representatives of regulatory authorities with responsibility for oversight of the trial or approval of the investigational agent. These authorities may be from the country where the site is located or from another country.

Activities during these on-site reviews may include, but are not limited to:

- Inspection of the facilities (e.g., clinical and administrative areas, pharmacy, laboratory);
- Review of the site trial master file, including documentation related to the protocol, the Investigator, and other study site personnel; correspondence to and from the IRB, the Sponsor, and their representatives;
- Review of standard operating procedures and current practices relating to clinical and pharmacy activities, data handling, the IEC oversight and the informed consent process;
- Review of source documents supporting all data collected during the study (e.g., inclusion/exclusion criteria, informed consent forms, HIPAA authorizations, adverse events records, expedited event reporting, efficacy endpoints);
- Resolution of any discrepancies noted.

Monitoring and auditing visits on behalf of the Sponsor will be scheduled with the Investigator in advance and will be conducted at a reasonable time. To facilitate these visits, the Investigator will assure that the following are available:

- appropriate space, facilities and access to all source documents (including access to computerized records either electronically or as complete print outs);
- consent forms, CRFs, SAE forms, and medical records for all screened and enrolled subjects;
- timely access to site personnel, including the Investigator, sub-Investigator(s), and other study personnel on the day of the visit to resolve any questions that arise.

Regulatory authorities may visit and review the site and/or Investigator during or after the study and may or may not notify the Investigator or the Sponsor in advance. The Investigator will fully cooperate with regulatory audits conducted at a reasonable time in a reasonable manner. The Investigator will notify the Sponsor immediately of any contact by or communication from regulatory authorities regarding the study.

13.8 Resolution of Deficiencies

The Investigator agrees to take promptly any reasonable steps requested by the Sponsor to resolve any deficiencies identified as a result of monitoring, audits, inspections, protocol deviations or review of any other study documentation. Failure to take adequate remedial action can result in suspension or termination of the study at the site.

13.9 Study Closeout

The study will be considered complete when all of the following have occurred:

- All treated subjects have completed all scheduled visits plus any unscheduled follow-up required by AEs;
- All CRFs have been completed, submitted and all queries resolved;
- The trial database has been locked.

The Sponsor or designee will then conduct a study closeout visit, which may include, but is not be limited to, any of the following:

- Review the site Trial Master File to assure all required regulatory documents are current and complete;
- Resolve any open issues from prior monitoring, audit or inspection visits;
- Review the site's provisions for meeting the requirements for retention study records;
- Discuss possible future site audits;
- Review the Sponsor's publication policy;
- Confirm compliance with requirements for notifying the IRB of study events, including closure;
- Collect any unused study materials for either return to the Sponsor or disposal in a manner approved by the Sponsor.

13.10 Record Retention

All study-related materials at the site (e.g., source documents, CRFs, Trial Master File) will be retained according to ICH guidelines and applicable regulations.

The study drug is being developed under a U.S. Investigational New Drug (IND) application; regulations require all study-related materials be retained for ***at least 2 years after*** one of the following events:

- approval of a New Drug Application based on this study;
- notification by the Sponsor that no further application will be filed.

The Investigator will use the following procedures regarding retained records:

- Contact the Sponsor ***before*** destructing any records pertaining to the study;
- Provide the Sponsor an opportunity to collect the records;
- Obtain written permission from the Sponsor to destroy the records;
- Notify the Sponsor if the Investigator plans to leave the institution so that arrangements can be made for the transfer of records;

Clinical and laboratory samples that are unstable may be disposed with the written approval of the Sponsor.

13.11 Data Management

A detailed Data Management Plan will be prepared separately and approved by the Sponsor.

14. STATISTICAL METHODS

The sections below indicate the overall structure and approach to the analysis of this study. A detailed Statistical Analysis Plan (SAP) incorporating these sections below will be prepared separately and approved by the Sponsor. The SAP will define populations for analysis, outline all data handling conventions, including software, and specify additional statistical methods to be used for analysis of safety, efficacy and pharmacokinetics.

14.1 Power and Sample Size

Our data from Tanzania indicate that rates of TB infection defined by a positive tuberculin skin test increase from 0% at birth to approximately 70% in adulthood (14). Data from South Africa using an IGRA to define TB infection indicate that approximately 30% of 12-14 year olds already have TB infection, and that 7-14% develop new TB infection each year (15-17). Since adult rates of TB infection as measured by tuberculin skin test surveys are similar in Tanzania and South Africa, we estimate conservatively that Tanzanian adolescents have an annual rate of new TB infection of 7% per year as defined by conversion of an IGRA from negative to positive. We hypothesize that a DAR-901 booster regimen will be 50% effective in preventing new TB infection among adolescents in Tanzania.

We anticipate identifying 650 IGRA-negative 13-15 year olds by screening 1000 subjects in this age range. Assuming a 7% annual rate of infection, and 5% loss to follow-up per year, 650 subjects randomized to vaccine or placebo and followed for an average of 1.75 years will provide 80% power to detect vaccine efficacy of 50%.

14.2 Analysis Populations

- *Safety population* – all subjects who received at least one dose of the study medication.
- *Efficacy population (ITT)* – all subjects, as randomized, with a negative IGRA at enrollment and at the time of the 2 month (=dose 2) study visit. This is the Intent-to-Treat (ITT) population.

14.3 Statistical Methods

The statistical analysis is divided into two parts.

1) Risk factors for TB infection. All screened subjects will be included in this analysis which will compare subjects who test positive for IGRA at baseline and those who test negative. Demographics, behavioral and epidemiologic risk factors, and other questionnaire items will be compared. Pearson chi-square, Fisher's exact, t-test and Wilcoxon-Mann-Whitney tests will be used as appropriate.

2) Effect of DAR-901 vaccine on preventing new TB infection. New TB infection is defined as conversion from a negative IGRA at 2 months after enrollment (first dose of vaccine) to a positive IGRA at any time thereafter. Subjects who convert from IGRA negative at screening to positive indeterminate or invalid at 2 months (administration of study dose #2) will be assumed to represent TB infection acquired before administration of study dose #1 and will be excluded from the endpoint analysis. All other subjects who complete the visit for dose #2 and remain IGRA negative will be included. The primary endpoint is time to new TB infection (= new IGRA-positivity), subject to right censoring. The primary test statistic will be a log-rank test comparing the two study arms (intention-to-treat), with $p < 0.05$ defined as significant. The proportion converting over time will be calculated using Kaplan-Meier statistic. To account for the interval censoring in the capture of IGRA conversion, we will apply methods for discrete time-to-events. The secondary endpoint is time to persistent new TB infection (= new IGRA-positivity which remains positive on a second IGRA at ≥3 months later) and analysis will be conducted using the same methods described above.

14.4 Safety Assessments

The primary endpoint will be a comprehensive evaluation of AEs and/or toxicity based on:

- subject reports;
- investigator observations of the subject (history and physical examination);
- vital signs;
- safety laboratory tests (CBC)
- need for concomitant medications.

14.5 Identification of Study Event Days and Times

Study events will be recorded using the calendar date and (where applicable) the time to the nearest minute.

For purposes of post-study analysis (e.g., tables and listings), study days will be designated as follows:

- Day 0 is defined as the calendar day of the first injection of study drug.
- The days prior to Day 0 are designated Day –1, Day –2, etc; there is no Day 0.
- The days following the day of the first injection of study drug are designated Day 1, Day 2, etc.
- The day of the last injection of study drug is indicated by adding the suffix "L", e.g., if the last injection is administered on Day 22, it will be displayed as "Day 22L".
- The days following the last injection of study drug are designated Day 1P, Day 2P, etc.

The times of events related to dosing of study drug will be designated as minutes or hours before or after the time of dosing (i.e., the subcutaneous injection of study drug), which is designated as t = 0 (zero). Thus, 15 minutes prior to dosing is t = −15 min; 2 hour after dosing is designated t = 2 h.

14.6 Handling Missing Data

In general, missing data will not be imputed. Further details for handling of missing, duplicated or unscheduled data will be given in the Statistical Analysis Plan.

14.7 Changes in the Planned Analyses

If changes are made to the Statistical Analysis Plan, then these will be listed in the Clinical Study Report, along with an explanation as to why they occurred.

15. ETHICAL CONSIDERATIONS

15.1 Independent Ethics Committee (IEC)

Prior to initiating the study, the Investigator will submit the following to the relevant institutional IECs[2] for approval:

- Study protocol;
- Investigator's Brochure;
- Informed Consent Form and any other written documents to be given to the subject;
- details of any compensation to subjects;
- any other requested document(s).

The study will not commence until the IECs have issued a letter of approval signed and dated by the IEC chair or authorized person which includes the following items:

- protocol number, full title, version number and date;
- version date of the Informed Consent Form;
- version date of the applicable Investigator's Brochure;
- date the protocol and consent form were reviewed and approved by the IEC.

The Sponsor or designee will be provided copies of all correspondence between the Investigator and the IEC. In addition, prior to study initiation, the Sponsor will be provided *one* of the following to verify that the IEC was appropriately qualified to approve the protocol:

- Documentation that on the date of the approval, the IEC met all currently applicable regulatory requirements for policies and procedures (e.g., membership, quorum, and approval procedures);
- A memo listing the voting members of the IEC who were present at the meeting the protocol was approved, including their titles, occupations, and institutional affiliations.

The Investigator will submit to the IEC, at least annually, a report of the study's progress.

15.2 Ethical Conduct of the Study

The study will be conducted in accordance with:

- the current version of "Ethical Principles For Medical Research Involving Human Subjects" as adopted by the World Medical Association (WMA);[3]
- local laws and regulations for the use of investigational therapeutic agents.

15.3 Subject Information, Consent and Assent

The Informed Consent/Assent Form (IC/AF) submitted to the IEC must be (a) based on a master document provided by the Sponsor and (b) reviewed and approved by the Sponsor prior to submission to the IEC. The Sponsor must also review and approve any changes requested by the IEC prior to the ICF or AF being used. The IC/AF will be written in both Kiswahili and English. Subjects will be permitted to chose whichever language is preferred (this will usually be Kiswahili).

The IC/AF will be signed by the parent or guardian and the adolescent subject.

[2] ICH E6, which specifies GCP, requires "an independent body (a review board or a committee, institutional, regional, national or supernational) …. whose responsibility it is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial…" In this protocol, the body performing this function will be referred to as the IEC (Independent Ethics Committee); in practice, many alternative designations are used, e.g., Institutional Review Board (IRB).

[3] This document, commonly referred to as the "Declaration of Helsinki", was issued in 1964 and has been amended or clarified at subsequent WMA Assemblies. Only the current document is considered official by WMA. The most recent version was approved in October 2008 (59th WMA General Assembly, Seoul, Korea).

Both informed consent and assent will be obtained prior to conducting any study procedures that are not part of the subject's routine medical care. During the consent process, each parent/guardian and subject will:

- Be advised of the nature and risk of the study by the Investigator or designated study personnel;
- Be given sufficient opportunity to read the IC/AF, to ask any questions, and to consider whether to participate;
- Provide informed consent or assent voluntarily.

The IC/AF will be signed and dated by the parent or guardian and the adolescent subject. A copy of the signed ICF and Assent will be provided to the parent or guardian and the subject; the originals will be retained by the Investigator as a source document. The informed consent process will be noted in the source documents.

The parent/guardian and subject will be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. Communication of this information to the parent/guardian and the subject will be noted in the source documents.

15.3.1 Obtaining Informed Consent from Subjects Who Are Not Literate

Subjects not literate in English or Kiswahili will not be eligible for enrollment.

15.3.2 Special Informed Consent Situations Not Applicable to This Protocol

Subjects may not be enrolled if they meet ***any*** of the following conditions which require specific provisions and approvals not provided for in this protocol:

- Are not able to provide informed consent (e.g., are acutely or permanently impaired);
- Are at increased risk of coercion (e.g., prisoners, institutionalized persons);

15.4 Protection of Subject Information

The identity and collected data of each subject ("protected health information") will be kept confidential and will be protected in accordance with applicable local regulations.

Methods to be used to protect the data will include the following:

- Each subject will be assigned a unique subject number, which will be used on the CRF in place of the subject's name.
- Computer systems for collecting and analyzing the data will have restricted access.
- In publications, aggregate data will be used wherever possible; any individual data will be redacted of unique identifying characteristics.

The informed consent process will comply with local requirements relating to (a) disclosure of the data to be collected and (b) authorization for its use. When permitted, these issues will be included in the ICF and Assent. In the event a separate form is required, the following will apply:

- The Sponsor must review and approve the separate form.
- The forms will be signed and dated by, and copies provided to, the required parties.
- A completed copy of the forms will be placed in the trial files with the completed ICF.

16. STUDY ADMINISTRATION

16.1 Registration of Study

The Sponsor abides by applicable US regulatory requirements and the guidelines of the International Committee of Medical Journal Editors (ICMJE) regarding registration of controlled clinical trials ("clinically directive trials").

16.2 Changes in the Conduct of the Study

After the Protocol has been approved by the governing IEC and regulatory authority, substantial changes in the conduct of the study will only be made as formal protocol revisions, which must be reviewed and approved by the Sponsor and the Investigator prior to submission to the applicable IEC and regulatory body. Changes will only be implemented after the revised protocol is approved as required.

Changes to contract information or designated study personnel (Section 17) may be handled administratively.

16.3 Confidentiality

This protocol, the applicable Investigator's Brochure, the results of the study and other related information provided by the Sponsor represent confidential and proprietary material of the Sponsor. They will be available only to the Investigator, personnel directly involved in the study, and authorized members and staff of the applicable IEC. These parties agree not to disclose these materials to others.

16.4 Financial Disclosure

In compliance with U.S. 21 CFR 54.4, any listed or identified Investigator or sub-investigator (including the spouse and any dependent children of said individuals) directly involved in the treatment or evaluation of research patients will disclose the following information for the time period during which the Investigator is participating in the study and for 1 year following completion of the study:

- Any financial arrangement between Dartmouth College and the Investigator in which the value of the compensation to the Investigator for conducting the study could be impacted by the outcome of the study.
- Payments (exclusive of the costs of conducting this or other clinical studies) by Dartmouth College totaling >$10,000, including, but not limited to, grants to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation, or honoraria.
- Any proprietary interest held by the Investigator in the product being evaluated.

16.5 Communication (Publication) Policies

Dartmouth College recognizes the importance of communicating the results of scientific studies, including clinical trials, and, therefore, encourages their publication in reputable scientific journals and presentation at seminars or conferences. Dartmouth also has legitimate responsibilities, including, but not limited to, protecting confidential information about its proprietary products and obtaining patent protection for its intellectual property.

Therefore, the following procedures apply to any communication (including written, oral, or electronic; manuscript, abstract, other publication, or presentation) of results or information arising from this study (including any ancillary studies involving trial subjects) to any third parties:

- The proposed communication will be prepared in collaboration with the Sponsor.
- The final proposed version must be submitted to Dartmouth for review and comment at least 30 days prior to presentation, submission for publication or other dissemination.
- In the event Dartmouth reasonably determines that a proposed communication contains confidential or patentable material, they may require *either* of the following:
  - The material be removed from the communication;

- The communication be delayed for up to 60 additional days to permit filing the appropriate intellectual property protection.

These procedures apply regardless of whether the study is completed as planned or is terminated prematurely for any reason.

The publication on vaccine efficacy from this study is expected to be a summary of all protocol results, jointly produced by the Sponsor and the participating Investigators. A publication on risk factors for TB infection will be published by the collaborators involved in that phase of the study.

16.5.1 Authorship and Acknowledgement

All publications will give MUHAS, Dartmouth College, and TMDU and/or their personnel appropriate credit (i.e., authorship or acknowledgement) for any direct contribution made by them.

Authorship will be decided jointly by the Investigators and the Sponsor. Manuscripts will conform to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, including, but not limited to, the standards for authorship contained therein.

17. CONTACT INFORMATION

Contacts for Expedited Reporting (see Section 12.4):

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18. APPENDICES

Table 18-1. Criteria for Grading Abnormal Results of Specific Laboratory Safety Tests

Analyte[1,2]	AE Term	Units	Grade 1	Grade 2	Grade 3	Grade 4[4]
Hb (decr) (F)	Anemia – if baseline WNL	g/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hb (decr) (F)	Anemia – if baseline <LLN decreased from baseline [any]	g/dL	Any decr – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hb (decr) (M)	Anemia – if baseline WNL	g/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hb (decr) (M)	Anemia – if baseline <LLN decreased from baseline [any]	g/dL	Any decr – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC (incr)	increased	cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC (decr)	decreased	cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes	decreased	cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils	decreased	cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils	increased	cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets	decreased	cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000

decr., decreased; Hb, Hemoglobin; WBC, White Blood Cells.

[1] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[2] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as "Potentially Life Threatening" (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

Table 18-2. Criteria for Grading Abnormal Vital Signs

Vital Signs[a]	AE Term	Units	Grade 1	Grade 2	Grade 3	Grade 4
Temperature [b]	Fever	°C °F	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Heart Rate (incr)	Tachycardia	beats/min	101 – 115	116 – 130	> 130	ER visit ***or*** hospitalization for arrhythmia
Heart Rate (decr)	Bradycardia[c]	beats/min	50 – 54	45 – 49	< 45	
BP systolic (incr)	Systolic hypertension	mm Hg	141 – 150	151 – 155	> 155	ER visit ***or*** hospitalization for malignant hypertension
BP diastolic (incr)	Diastolic hypertension	mm Hg	91 – 95	96 – 100	> 100	
BP systolic (decr)	Hypotension	mm Hg	85 – 89	80 – 84	< 80	ER visit ***or*** hospitalization for hypotensive shock
Respiratory Rate	Tachypnea	breaths/min	17 – 20	21 – 25	> 25	Intubation

a. Subject should be at rest for all vital sign measurements.
b. Oral temperature; no recent hot or cold beverages or smoking.
c. When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.
BP, Blood pressure;

19. INVESTIGATOR AGREEMENT

I have read the foregoing protocol (DAR-901-PIAT, version Draft, 10 August 2015) and agree to the following:

- The protocol contains all necessary details for carrying out this study.
- I will conduct the study as detailed in the protocol and will abide by all its provisions.
- I will conduct the study in compliance with ICH Guidelines for Good Clinical Practice, the requirements of the IEC and all applicable government regulations.
- I will train and supervise all individuals delegated to assist me in conducting this study, including providing copies of the protocol and all pertinent information and discussing the material with them to ensure they are fully informed regarding the investigational drug, the protocol and their responsibilities and obligations.
- I will use only the current informed consent form approved by the Sponsor (or their designee) and by the IRB/IEC responsible for this study.
- I will fulfill all requirements for submitting pertinent information to the IEC and to the Sponsor, including reportable serious adverse events.
- I will complete all case report forms, including resolution of queries, in a timely manner.
- I will provide the Sponsor (or their designee) with access to any source documents from which case report form information may have been derived.
- I will provide the Sponsor with complete, signed statements of financial disclosure as required.
- I understand that the information in this protocol and the referenced Investigator's Brochure is confidential and that its disclosure to any third parties (other than those approving or conducting the study) is prohibited. I will take the necessary precautions to protect this information from loss, inadvertent disclosure or access by third parties.

January 1, 2018

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January 1, 2018

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