UNIVERSITY FOR DEVELOPMENT STUDIES

T-CELL EXHAUSTION AMONG PULMONARY TUBERCULOSIS CLIENTS IN NORTHERN REGION, GHANA

BY

JACOB NII OTINKORANG ANKRAH
MPHIL CLINICAL MICROBIOLOGY
(UDS/MMB/0003/21)

A THESIS SUBMITTED TO THE DEPARTMENT OF CLINICAL MICROBIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CLINICAL MICROBIOLOGY

APRIL, 2025.

DECLARATION

By submitting this thesis for the Master of Philosophy in Clinical Microbiology, I, JACOB NII OTINKORANG ANKRAH, hereby declare that it is entirely my original work and does not, to the best of my knowledge, contain any material that has already been published by someone else or accepted for the award of any other university degree, except for those cases in which proper textual acknowledgement has been made..

Signature:..... ..... Date: 24th February, 2025

JACOB NII OTINKORANG ANKRAH
(STUDENT, UDS/MMB/0003/21)

SUPERVISOR DECLARATION

Under the project work requirements set forth by the University for Development Studies, I hereby declare that the project work was created and delivered under the supervision.

Signature:..... Date: 24th February, 2025
DR. WILLIAMS WALANA
(SUPERVISOR)

Signature:..... Date: 24th February, 2025
PROF. AKOSUA BONSU KARIKARI
(HEAD OF DEPARTMENT)

ABSTRACT

Pulmonary tuberculosis is commonly caused by *Mycobacterium tuberculosis*. Tuberculosis pathogenesis is based on immune cell depletion and immune system evasion. Peripheral blood mononuclear cells from pulmonary tuberculosis clients were studied for transcription patterns associated with immune cell exhaustion, cellular activity, and inflammatory response at various therapeutic stages of anti-TB therapy at the Chest Clinic of Tamale Teaching Hospital, an experimental study was carried out with twenty-five clients diagnosed with pulmonary tuberculosis and five healthy control clients, making a total of thirty clients. Based on the different stages of the anti-TB treatment, the clients were divided into six groups; namely: newly diagnosed (zero month), intensive phase (≤ 2 months), continuous phase I (>2 & ≤ 5 months), continuous phase II (>5 & ≤ 6 months), completed phased (>6) and healthy control group. Blood specimens were taken from every client in each group. Peripheral blood mononuclear cells were isolated, followed by Ribonucleic acid extraction, then complementary deoxyribonucleic acid synthesis, polymerase chain reaction of the biomarkers of T cells exhaustion, inflammatory cytokines and cellular activities markers. 1% agarose gel electrophoresis was used for the deoxyribonucleic acids bands visualization. The deoxyribonucleic acid gene expression and quantification were carried out using Image J software and Graph pad Prism version 8 statistical tool on the genes of T cell exhaustion, inflammatory cytokine and cellular activities markers. P-values of less than 0.05 were considered statistically significant between the groups.

Programme cell death 1 protein, cytotoxic T lymphocytes associated protein 4, CD244 and lymphocyte activated gene 3 in peripheral blood mononuclear cells predict positive pulmonary tuberculosis prognosis during treatment. Correlation analysis suggests CD244 could be a strong marker for monitoring pulmonary tuberculosis treatment outcomes. Transcription of inflammatory cytokines was restored to normal following the initiation of anti-TB treatment. Cellular activity markers CD69, Ki67, Interleukin 7R, Interleukin 15R, GATA-3, CD4 and CD8 were restored to normal and could be used to monitor PTB treatment outcome. The study demonstrated the potential value of T-cell exhaustion markers, specifically PD-L1, inflammatory markers like Interleukin 2, Interferon-gamma and Tumor necrosis factor-a, as well as cellular activity indicators Kiel 67, CD69, GATA-3, CD4, and CD8 blood-based biomarkers can be used in place of sputum in monitoring treatment progress of pulmonary tuberculosis clients.

DEDICATION

I dedicate this work to the Almighty Lord for His divine supply and health, to my dear wife Dorcas Afia Ankrah, to my lovely boy Dominion Nii Ankrah, Grandma, family and friends who have supported, loved, and cared for me in many ways throughout this journey

ACKNOWLEDGEMENT

First of all, I give thanks to the Almighty God for His kindness and never-ending mercies. To my immediate supervisor, Dr. Williams Walana, who has been an essential source of ideas, counsel, and support throughout this endeavour.

I am sincerely thankful to Nana Boakye Alahaman for his support, encouragement, academic and technical input. I am so grateful to the Head of Department Prof. Akosua Bonsu Karikari for her inspiration, care and guidance throughout my academic period at the department, and to every faculty member, I am very grateful.

I am very grateful to every client who volunteered to take part in this study without whom this project would not have been a success. I thank the Head of the Chest Clinic unit, Madam Felecia Vuu and her entire staff for their support in client identifications, interactions, filling of the consent and questionnaire forms. I am grateful to the management of Tamale Teaching Hospital for granting me site permission at the facility.

I appreciate my study team and everyone who was directly or indirectly involved in the success of this study.

TABLE OF CONTENT

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ACTB	-	Beta Actin
APC	-	Antigen Presenting Cells
cDNA	-	Complementary Deoxyribonucleic Acid
CTLA-4	-	Cytotoxic T-lymphocyte-Associated Protein 4
GAPDH	-	Glyceraldehyde-3-Phosphate Dehydrogenase
GATA-3	-	Binding Proteins-3 that recognizes G-A-T-A nucleotide sequences
GZMB	-	Granzyme B
IL-2	-	Interleukin 2
IL-4	-	Interleukin 4
IL-10	-	Interleukin 10
INF-ɣ	-	Interferon Gamma
Kiel 67	-	Ki 67
LAG-3	-	Lymphocyte Activation Gene 3
MHC	-	Major Histocompatibility Complex
MTB	-	*Mycobacterium tuberculosis*
PBMC	-	Peripheral Blood Mononuclear Cells
PBS	-	Phosphate Buffered Saline
PD-1	-	Programmed Cell Death 1 Protein
RNA	-	Ribonucleic Acid
TB	-	Tuberculosis
T cell	-	Thymus cell
TCR	-	T Cell Receptor
TGF-beta	-	Transforming Growth Factor-Beta
TIM-3	-	T cell Immunoglobulin and Mucin Domain Protein 3
TNF-alpha	-	Tumor Necrosis Factor-Alpha
TTH	-	Tamale Teaching Hospital
WHO	-	World Health Organization

CHAPTER 1
INTRODUCTION

1.0 BACKGROUND

Pulmonary tuberculosis remains a major global health challenge, significantly impacting morbidity and mortality worldwide. Despite advancements in diagnosis, treatment, and prevention, tuberculosis remains among the top infectious disease killers, alongside HIV/AIDS and malaria (H. Yang et al., 2024). According to the World Health Organization Global Tuberculosis Report 2023, an estimated 10.6 million people fell ill with TB in 2022. Of these, around 6 million (57%) were cases of pulmonary TB, the most common and transmissible form of the disease. TB caused approximately 1.3 million deaths among HIV-negative individuals and 167,000 deaths among HIV-positive individuals in 2022. The TB incidence rate is approximately 134 per 100,000 population, varying across regions (Ummah, 2019). According to WHO Over 85% of TB cases occur in 30 high-burden countries, with the highest cases reported in: India (27%), China (7%), Indonesia (10%), Philippines (7%), Pakistan, Nigeria, Bangladesh, and South Africa (each contributing 4-5%) (Raghav et al., 2021)

In Ghana, Pulmonary tuberculosis remains a significant public health concern, with recent data indicating a higher disease burden than previously estimated (Abdul et al., 2020). The 2013 national TB prevalence survey revealed 290 cases per 100,000 population, which was four times higher than the World Health Organization's estimate of 71 per 100,000 for that year. More recent studies have reported a prevalence of bacteriologically confirmed TB at 356 per 100,000 population (Iddrisu et al., 2024). Annually, approximately 44,000 individuals develop TB in Ghana, with an estimated 6,600 cases occurring in children. TB is the seventh leading cause of death in Ghana, responsible for about 4.9% of all mortalities. In 2018, there were over 15,000 TB-related deaths reported (Owusu et al., 2024). In 2023, over 19,000 TB cases were detected, marking an improvement from the 16,500 cases identified in 2022. Despite these efforts, the case notification rate has remained relatively stagnant at around 60 per 100,000 population over the past five years (Kwabla et al., 2025).

TB disproportionately affects low- and middle-income countries due to poverty, malnutrition, overcrowding, and poor healthcare access. TB can lead to long-term disability, economic hardship, and stigma (Hargreaves et al., 2011).

Pulmonary tuberculosis an intracellular pathogen that primarily infects the lungs. The disease progression is determined by complex host-pathogen interactions, where the immune response plays a critical role in controlling or exacerbating the infection. *Mycobacterium tuberculosis* is transmitted via aerosol droplets from an infected person (Moule & Cirillo, 2020). Once inhaled, *Mycobacterium tuberculosis* reaches the alveoli, where it is engulfed by alveolar macrophages. The bacteria can survive intracellularly by inhibiting phagosome-lysosome fusion, preventing its degradation. Macrophages produce pro-inflammatory cytokines (TNF-α, IL-12, IL-6) to recruit immune cells (Maphasa et al., 2021). Neutrophils and dendritic cells contribute to early bacterial control and antigen presentation to T cells. Infected macrophages and immune cells form a granuloma to contain the bacteria. The granuloma consists of macrophages, epithelioid cells, multinucleated giant cells, and T lymphocytes. The bacteria remain dormant inside the granuloma, leading to latent TB infection in 90-95% of cases (Sholeye et al., 2022). In some individuals, granuloma breakdown occurs due to immune suppression, leading to active TB. *Mycobacterium tuberculosis* escapes into the lung parenchyma, causing tissue necrosis, cavitation, and bacterial dissemination, uncontrolled inflammation results in caseous necrosis and lung damage (Elkington et al., 2022).

T cells play a crucial role in immune defense against TB. CD4+ T Cells (Helper T Cells) secrete IFN-γ and TNF-α, activating macrophages to kill *Mycobacterium tuberculosis*. Th1 cells are protective, while Th2 and Th17 responses may contribute to pathology (Dis et al., 2022). CD8+ T Cells (Cytotoxic T Cells) directly kill infected macrophages through perforin and granzymes, contribute to *Mycobacterium tuberculosis* clearance but can be dysfunctional in chronic infection. Regulatory T Cells (Tregs) suppress excessive inflammation but can inhibit effective *Mycobacterium tuberculosis* control. Increased Treg activity is associated with TB progression (Ashenafi & Brighenti, 2022).

T cell exhaustion is a state of immune dysfunction characterized by progressive loss of T cell function due to chronic antigen exposure and persistent inflammation. In TB, chronic infection and high bacterial load lead to T cell exhaustion, reducing the ability to clear *Mycobacterium tuberculosis* (Kahan et al., 2015). Characteristics of T Cell Exhaustion in TB include the exhausted T cells produce less IFN-γ, IL-2, and TNF-α. Increased Expression of Inhibitory Receptors such as Programmed death-1, T cell immunoglobulin and mucin-domain containing-3 Cytotoxic T

lymphocyte-associated protein 4, Lymphocyte activation gene-3, T cell immunoreceptor with Ig and ITIM domains. Reduced ability to expand and kill infected cells (Kahan et al., 2015).

The persistent exposure to *Mycobacterium tuberculosis* antigens leads to sustained T cell activation and eventual exhaustion. Increased levels of IL-10, TGF-β, and IDO (indoleamine 2, 3-dioxygenase) contribute to immune suppression. Myeloid-derived suppressor cells (MDSCs) and exhausted macrophages impair T cell function leading to low oxygen tension in granulomas limits T cell function and survival (Kotzé et al., 2020).

Inflammatory Cytokines play a crucial role in modulating immune responses, either promoting protective immunity or contributing to excessive inflammation. Interferon-Gamma (IFN-γ) produced by Th1 cells, CD8+ T cells, NK cells activates macrophages to enhance phagosome-lysosome fusion (Alspach et al., 2019). Stimulates nitric oxide and reactive oxygen species production, aiding in *Mycobacterium tuberculosis* killing. IFN-γ deficiency leads to uncontrolled bacterial growth and disseminated TB. Excessive IFN-γ activation can cause tissue damage and necrosis. Tumor Necrosis Factor-Alpha (TNF-α) produced by Macrophages, T cells, dendritic cells promotes granuloma formation and maintenance, enhances macrophage activation and apoptosis of infected cells. High TNF-α levels contribute to immune-mediated tissue damage in active TB (Tiwari & Martineau, 2023). Interleukin-6 (IL-6) produced by Macrophages, epithelial cells, T cells Induces acute-phase response and promotes differentiation of Th17 cells, regulates inflammation and recruitment of immune cells. High IL-6 levels correlate with disease severity and poor TB outcomes. Interleukin-1 Beta (IL-1β) produced by Macrophages, neutrophils, dendritic cells enhances neutrophil recruitment to infection sites, stimulates TNF-α and IL-6 production (Grebenciucova & VanHaerents, 2023).

Excessive IL-1β leads to hyperinflammation and tissue necrosis. Interleukin-10 (IL-10) produced by Regulatory T cells (Tregs), macrophages, dendritic cells suppresses Th1 responses, reducing TNF-α and IFN-γ production, limits tissue damage by dampening excessive inflammation (Ng et al., 2013). High IL-10 levels in active TB impair macrophage bactericidal activity, facilitating *Mycobacterium tuberculosis* persistence. TB pathogenesis is driven by a delicate cytokine balance that determines whether the immune response controls or exacerbates disease (Sankar & Mishra, 2023). While pro-inflammatory cytokines (IFN-γ, TNF-α, IL-6) are crucial for bacterial containment, their dysregulation can lead to immune-mediated pathology. Conversely, excessive anti-inflammatory cytokines (IL-10, TGF-β) may promote bacterial persistence (Cicchese, 2018).

The host immune response plays a crucial role in controlling or exacerbating TB, with T cell exhaustion, inflammatory and cellular activity markers being essential for monitoring disease progression and treatment response. These markers can help in evaluating immune activation, inflammation, and bacterial clearance (Ghoshal et al., 2024).

One of the important factors to consider in PTB treatment is monitoring the progress of treatment. However, patients at certain stages of treatment find it difficult to produce sputum for examination, especially in younger patients and the elderly. In view of this, we hypothesize that there could be blood-borne markers to monitor the positive prognosis of PTB treatment. In this regard, this study explored potential T cell exhaustion, inflammatory cytokines and cellular activity markers for monitoring PTB treatment other than sputum.

1.1 PROBLEM STATEMENT AND JUSTIFICATION

The immune system is vital in immune defense against *tuberculosis* infection, which is still a serious public health concern, particularly in low- and middle-income nations. T-cell exhaustion may result from a persistent TB infection. T-cell exhaustion is important in *tuberculosis*, but little is known about how TB causes exhaustion at different stages of anti-TB treatment or how it affects the course of the disease. Given this, it's critical to look at T-cell exhaustion markers at various PTB disease stages and how they relate to the severity of the illness. It is possible that utilizing T cell exhaustion markers can greatly assist *tuberculosis* management. Thus, the goal of the study was to investigate the relationship between *M. tuberculosis* and host immunity, with a particular emphasis on the T cell exhaustion makers, inflammatory cytokines and cellular activity markers.

Investigating and comprehending T-cell exhaustion in PTB patients might offer important insights into the way the immune system reacts to *tuberculosis* infection, which may result in the development of more diagnostic tools and therapeutic strategies., Blood-based biomarkers could be identified as alternatives to or addition to sputum specimens for laboratory diagnosis, especially when sputum production becomes problematic.

1.2 HYPOTHESIS:

In the Northern Region, there are significant differences in T-cell exhaustion makers across different groups of individuals with *Mycobacterium tuberculosis* infection.

1.3 NULL HYPOTHESIS:

Among the many clients in the Northern Region infected with *Mycobacterium tuberculosis*, there is no statistically significant difference in T-cell exhaustion makers.

1.5 RESEARCH QUESTION

i. In individuals with PTB, what is the pattern of T-cell exhaustion biomarkers?

ii. How do T-cell exhaustion biomarker levels in *pulmonary tuberculosis* patients alter at the anti-TB treatment?

iii. What is the relationship, can the levels of T-cell exhaustion biomarkers be predictive of treatment response and differences between the stages of anti-TB therapy (intensive, continuous and cured stages) and the levels of T-cell exhaustion biomarkers in PTB patients?

1.6 AIM

The aim of this study was to investigate T-cells exhaustion among pulmonary tuberculosis clients in Northern Region-Ghana.

1.7 OBJECTIVES

To determine the pattern of T-cells exhaustion markers in patients with pulmonary TB at different stages of anti-TB therapy.

To investigate the inflammatory cytokine expression at different stages of anti-TB therapy.

To investigate the expression of cellular activities and proliferation makers at different stages of anti-TB therapy.

CHAPTER 2
LITERATURE REVIEW

2.0 Introduction

Pulmonary tuberculosis remains a major global health challenge, significantly impacting morbidity and mortality worldwide. Despite advancements in diagnosis, treatment, and prevention, tuberculosis remains among the top infectious disease killers, alongside HIV/AIDS and malaria (H. Yang et al., 2024). According to the World Health Organization Global Tuberculosis Report 2023, an estimated 10.6 million people fell ill with TB in 2022. Of these, around 6 million (57%) were cases of pulmonary TB, the most common and transmissible form of the disease. TB caused approximately 1.3 million deaths among HIV-negative individuals and 167,000 deaths among HIV-positive individuals in 2022. The TB incidence rate is approximately 134 per 100,000 population, varying across regions (Ummah, 2019). According to WHO Over 85% of TB cases occur in 30 high-burden countries, with the highest cases reported in: India (27%), China (7%), Indonesia (10%), Philippines (7%), Pakistan, Nigeria, Bangladesh, and South Africa (each contributing 4-5%) (Raghav et al., 2021)

2.1 Brief overview of tuberculosis

In Ghana, Pulmonary tuberculosis remains a significant public health concern, with recent data indicating a higher disease burden than previously estimated (Abdul et al., 2020). The 2013 national TB prevalence survey revealed 290 cases per 100,000 population, which was four times higher than the World Health Organization's estimate of 71 per 100,000 for that year. More recent studies have reported a prevalence of bacteriologically confirmed TB at 356 per 100,000 population (Iddrisu et al., 2024). Annually, approximately 44,000 individuals develop TB in Ghana, with an estimated 6,600 cases occurring in children. TB is the seventh leading cause of death in Ghana, responsible for about 4.9% of all mortalities. In 2018, there were over 15,000 TB-related deaths reported (Owusu et al., 2024). In 2023, over 19,000 TB cases were detected, marking an improvement from the 16,500 cases identified in 2022. Despite these efforts, the case notification rate has remained relatively stagnant at around 60 per 100,000 population over the past five years (Kwabla et al., 2025).

TB disproportionately affects low- and middle-income countries due to poverty, malnutrition, overcrowding, and poor healthcare access. TB can lead to long-term disability, economic hardship, and stigma (Hargreaves et al., 2011).

It is notable for being among the cause of death related to a single infectious agent, and the majority of deaths and highest incidence are found in developing and low-income nations. The bacteria *Mycobacterium tuberculosis* is the source of TB, a chronic global health issue *Mycobacterium tuberculosis* (Alsayed & Gunosewoyo, 2023). Although it can also affect other organs in the body, the lungs are the primary target of this infectious disease. When someone with *tuberculosis* coughs or sneezes, they release droplets into the air that contain germs. People who breathe in these droplets run the risk of contracting the spread of the bacteria (Bussi & Gutierrez, 2019). The intensity of *tuberculosis* symptoms varies, but frequent ones include fever, weight loss, chronic coughing, and chest pains. Severe instances of *tuberculosis* can result in problems such organ damage and respiratory failure (Bussi & Gutierrez, 2019). Early diagnosis and appropriate treatment are crucial to preventing the spread of the disease and reducing its impact on affected individuals.

Despite significant progress in understanding TB, challenges exist in combating the epidemic. The emergence of drug-resistant strains of *Mycobacterium tuberculosis* is a significant worry as it presents a significant risk to international health initiatives (Heidary et al., 2022). Drug-resistant TB complicates treatment regimens and requires more specialized and costly medications. Additionally, the intersection of tuberculosis with other health issues, such as HIV, further exacerbates the challenges in managing and controlling the disease (Koch et al., 2018).

Efforts to control TB involves a combination of strategies, including widespread testing, contact tracing, and the administration of appropriate antibiotic treatments (WHO, 2008). Immunizations, with the Bacillus Calmette-Guérin (BCG) vaccine, have been developed to stop severe TB, especially in infants. However, these measures are not foolproof, and ongoing research is essential to improving diagnostic tools, treatment options, and preventive measures (Dara et al., 2014). The WHO and other global health organizations continue to prioritize the fight against TB. International collaboration, research initiatives, and public health campaigns aim to raise awareness and reduce the incidence of TB. A focus on vulnerable people, enhanced access to

diagnosis and treatment, and an improved healthcare infrastructure are all essential elements of global efforts to manage and finally eradicate tuberculosis (WHO, 2022). TB is still a major public health concern, especially in underdeveloped areas (Bai & Ameyaw, 2024). While progress has been made in understanding and addressing the disease, ongoing research and global collaboration are essential to develop more effective prevention, diagnosis, and treatment strategies (Cole et al., 2020). The ultimate goal is to reduce the burden of TB and work towards its eradication on a global scale

2.2 Pathogenesis of pulmonary TB

Pulmonary tuberculosis an intracellular pathogen that primarily infects the lungs. The disease progression is determined by complex host-pathogen interactions, where the immune response plays a critical role in controlling or exacerbating the infection. *Mycobacterium tuberculosis* is transmitted via aerosol droplets from an infected person (Moule & Cirillo, 2020). Once inhaled, *Mycobacterium tuberculosis* reaches the alveoli, where it is engulfed by alveolar macrophages. The bacteria can survive intracellularly by inhibiting phagosome-lysosome fusion, preventing its degradation. Macrophages produce pro-inflammatory cytokines (TNF-α, IL-12, IL-6) to recruit immune cells (Maphasa et al., 2021). Neutrophils and dendritic cells contribute to early bacterial control and antigen presentation to T cells. Infected macrophages and immune cells form a granuloma to contain the bacteria. The granuloma consists of macrophages, epithelioid cells, multinucleated giant cells, and T lymphocytes. The bacteria remain dormant inside the granuloma, leading to latent TB infection in 90-95% of cases (Sholeye et al., 2022). In some individuals, granuloma breakdown occurs due to immune suppression, leading to active TB. *Mycobacterium tuberculosis* escapes into the lung parenchyma, causing tissue necrosis, cavitation, and bacterial dissemination, uncontrolled inflammation results in caseous necrosis and lung damage (Elkington et al., 2022).

2.4 Relevance to TB

TB represents a significant global health challenge, characterized by a wide clinical spectrum and a notable mortality rate (Heidary et al., 2022). It is instigated by *Mycobacterium tuberculosis*, a slow-growing, aerobic, acid-fast bacillus, and can manifest either as pulmonary or extrapulmonary

disease (Alsayed & Gunosewoyo, 2023). Transmission of the disease occurs through the inhalation of MTB droplet nuclei, potentially leading to lung tuberculosis infection (Tuberculosis, 2019). Predominantly affecting the lungs, TB accounts for over 80% of cases related to this infectious disease (Tuberculosis, 2019). Health professionals must grasp the essential aspects of TB, including symptoms, diagnosis, treatment, and prevention methods, to effectively control its dissemination (Cole et al., 2020).

2.5 Importance of the immune response in TB

TB is a contagious ailment instigated by *Mycobacterium tuberculosis*. The management and eradication of TB infections are significantly influenced by the immune response (Sia & Rengarajan, 2019b). The immune system mounts a defense during *Mycobacterium tuberculosis* infection with the goal of containing and eliminating the bacteria (Martino et al., 2019). This response involves multiple immune system components, including T cells, cytokines, and macrophages. Together, these immune components form an intricate network aimed at containing and getting rid of the bacteria (Martino et al., 2019).

The immune response in TB holds importance for several reasons. Firstly, it aids in regulating the initial infection by stimulating macrophages to engulf and obliterate the bacteria. Secondly, the immune response deters bacterial dissemination by generating cytokines that entice and activate other immune cells (Carabalí-Isajar et al., 2023). These immune cells can subsequently construct granulomas, organized structures that confine the TB infection and impede its spread. Additionally, the immune response in TB contributes to shaping the infection's outcome (Cronan, 2022). In particular, a strong and effective immune response may lead to the bacterial containment and elimination, ultimately resulting in the infection's resolution. On the other hand, a weak or dysregulated immune system might result in a chronic infection and the emergence of active *tuberculosis* (Chandra et al., 2022).

An important aspect of tuberculosis infection is the immunological response, which focuses on the development and maintenance of granulomas (Pagán & Ramakrishnan, 2015). Specific cytokines, including TNF-a, play a major role in the immune response to MTB. Phagocytosis, granuloma development, and a complex network of cytokines and chemokines are all part of the early host

immune response against *Mycobacterium tuberculosis* (Yuk et al., 2024). The adaptive immune response, in which T cells and B cells coordinate intricate interactions, is essential to the fight against tuberculosis. Research by Cooper, (2009) emphasizes how important CD4+ T cells especially Th1 cells are in triggering macrophages to regulate *Mycobacterium tuberculosis* replication. For the adaptive immune system to effectively battle the intracellular infection, it must be able to mount a strong and coordinated response. Cytokines serve as key messengers in the immune response against TB. IFN-g, highlighted by Cooper, (2009) emerges as a central player, promoting macrophage activation and antimicrobial mechanisms. Additionally, TNF-a contributes significantly to the immune response, as discussed by (Orme, 2016). During MTB infection, inflammatory cytokines play a pivotal role as mediators, influencing the immunological environment. The innate immune system is responsible for the early detection of tuberculosis and the start of the immunological response against it (Cooper et al., 2011).

The importance of macrophages and dendritic cells—which have Pattern Recognition Receptors (PRRs) that recognize MTB-associated chemical patterns—has been underlined (Mogues et al., 2001). An efficient immune response against *tuberculosis* is facilitated by the interaction between the innate and adaptive components (Shabariah et al., 2022). While an effective immune response is necessary, an imbalance can lead to immunopathology. O'Garra et al.,(2013) emphasizes that TB pathogenesis may be exacerbated by increased inflammation. How the delicate balance between immunopathology and an efficient immune response is crucial in determining the clinical presentation of *tuberculosis* emphasized by Persons et al., (2018).

2.6 T-cell exhaustion

T-cell fatigue is a dysfunctional condition that manifests as poor effector function and persistent expression of inhibitory receptors in cancer and chronic infections (Wherry, 2011). Aspects of tiredness include an increase in inhibitory receptors, a decrease in cytokine production, and a compromise in cytotoxicity and proliferation (Jiang et al., 2015). T-cells subjected to extended antigen stimulation and inflammatory cytokines, particularly in the setting of persistent viral infections, experience T-cell exhaustion, which is characterized by decreased function and responsiveness in T-cells (Wherry & Kurachi, 2016). T-cells eventually lose their capacity to respond to infections and carry out their immunological duties while in this state. This results in a

weakened immune system and reduced ability to fight off diseases. Furthermore, cytokine production gradually declines with T-cell exhaustion, and the expression of inhibitory receptors like CTLA-4 and PD-1 rises and dampens T-cell activation and effector functions (Blank et al., 2019). Hence, T-cell exhaustion is a regulatory mechanism that may compromise immunological responses in order to prevent excessive inflammation and immune-mediated harm (Gao et al., 2022). T-cell exhaustion occurs when T-cells progressively lose their capacity to react to infections and carry out their immunological duties. This leads to a compromised immune system and decreased protective immunity against infections (Brunell et al., 2023).

2.7 Some Exhaustion markers and their significance

Certain indicators of T cell exhaustion include T cell expression of the inhibitory receptor PD-1, which binds to the ligand of antigen-presenting cells, PD-L1. Because TB results in T cell exhaustion, one of the key ways it evades the host immune response is through the interplay between PD-1 and PD-L1 (Ghosh et al., 2021). Another inhibitory receptor that is expressed on T cells and is elevated in chronic *tuberculosis* is called TIM-3. TIM-3 binds to its receptor, galectin-9, to promote T cell exhaustion (Das et al., 2017). T cells produce the surface protein LAG-3, which attaches itself to Major Histocompatibility ComplexClass II molecules on antigen-presenting cells. T cell dysfunction results from an increase in LAG-3 in exhausted T cells during a protracted MTB infection (Receptor, 2020). T cells produce CTLA-4, an inhibitory receptor that competes with CD28 to attach to CD80/CD86 on APC (Sansom, 2000). When an MTB infection persists, CTLA-4 signaling is elevated and causes T cell exhaustion (Kennedy et al., 2023).

2.8 Overview of T-Cell Functions

T cells perform a variety of roles in immunological responses and are a crucial part of the adaptive immune system (Kurd et al., 2017). Mechanical cues exert an influence on T cells, modulating aspects such as adhesion, migration, and the initiation of immune functions Chen (2013). Within the thymus, T cell development takes place, involving a selection process that leads to the expression of diverse antigen receptors (Walker, 2015). These receptors give T cells the ability to recognize and react to particular antigens, which in turn triggers effector T cell responses such cytokine production and target cell lysis (Cells et al., 2019). The diversity within T cell subsets,

encompassing both conventional and nonconventional types, further underscores their critical role in the immune system (Walker, 2015).

2.9 Role of T-Cells in TB

T-cells are essential for the body's reaction to an *Mycobacterium tuberculosis* infection. Th1 and Th17 cells in particular highlight the importance of T-cell subsets in protecting against MTB infection (Abebe, 2014; Lyadova & Panteleev, 2015). These particular T-cell subsets activate macrophages' antimycobacterial properties and start an inflammatory response by releasing cytokines including IFN-g and TNF-a (Damsker et al., 2010). The contribution of T-cell-derived TNF-a to maintaining defense against long-term *Mycobacterium tuberculosis* infection is further highlighted (Allie et al., 2013). However, Hougardy et al., (2007) draws attention to the possibility that regulatory T-cells in active tuberculosis may attenuate immunological responses to protective antigens, suggesting a complex interaction between various T-cell subsets in the immune response to *Mycobacterium tuberculosis* infection.

2.10 Importance of T-Cell response in controlling TB

The control of tuberculosis infection is largely dependent on the T-cell response. Data from animal models confirm the importance of CD8 T cells in limiting *Mycobacterium tuberculosis* spread, highlighting their non-redundant role (Lin & Flynn, 2015). Efficient T-cell responses play a vital role in *tuberculosis* immunity by helping to regulate the steady-state infection in the lungs (Manuscript, 2013). T-cell-produced TNF-a, vital for the sustained control of TB infection, with TNF from myeloid cells facilitating early immune function and T-cell-derived TNF ensuring protection throughout chronic infection (Allie et al., 2013). However, the onset of detectable T-cell responses is delayed in *tuberculosis*, and multiple mechanisms pathogen-induced apoptosis suppression, delayed dendritic cell migration, and regulatory T cell influence affect this delay

2.11 Nutritional status in TB

Numerous studies have brought attention to the elevated prevalence of undernutrition among individuals with TB, with a notable portion experiencing severe undernourishment (Anbese et al., 2021). This state of undernutrition has been linked to unfavorable treatment outcomes and an increased risk of mortality (Pandey, 2019;Shukla, 2019; Bhargava, 2013). Nevertheless, there is

also evidence indicating a shift toward overweight and obesity among TB patients, suggesting a multifaceted relationship between TB and nutritional status (Campos-góngora et al., 2019). These findings emphasize the significance of proper nutritional management and counseling for individuals with TB, along with the necessity for further research to comprehensively comprehend the nutritional status within this population (Id et al., 2021).

2.12 T-cell exhaustion in chronic infections

T-cell exhaustion is a critical feature of chronic infections and it is characterized by decreased effector cell functioning and extended expression of inhibitory receptors (Wherry & Kurachi, 2016). This state of exhaustion results from a combination of active suppression and inherent deficiencies in signaling and metabolism (Wherry & Kurachi, 2016). Promising research in this field focuses on the potential to reverse T-cell exhaustion and rejuvenate immune responses, emphasizing interventions such as inhibitory receptor blockade and metabolic targeting (Saeidi et al., 2018). Our comprehension of this phenomena is further complicated by the complex nature of exhausted T-cells, which includes the identification of a subgroup displaying stem cell-like characteristics (Hashimoto et al., 2018).

2.13 T-cell exhaustion in infectious diseases

T-cell exhaustion, characterized by dysfunction or elimination, is a prevalent feature in chronic infections like HIV, HBV, and HCV (Saeidi et al., 2018). This state is not exclusive to infections; it has also been identified in immune-mediated inflammatory diseases (IMIDs) (Gao et al., 2022). When it comes to HIV, T-cell fatigue results from long-term antigen exposure without CD4 support, which ultimately leads to memory T-cell failure. This exhaustion may result in inadequate immunological response to viral infections, (Fenwick et al., 2019).

2.14 Examples from other infections

Individuals with HIV face an increased risk of *Mycobacterium tuberculosis* infections, with prevalent infections including *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella spp*., and *Pseudomonas aeruginosa* (Rameshkumar, 2018). The transmission of infectious agents between humans and animals, particularly nonhuman primates, is a significant concern, with zoonotic pathogens presenting a particular threat (Rahman et al., 2020). The field of epidemiology

and control of bacterial infections in humans is intricate, involving consideration of a diverse array of pathogens and diseases (Straif-bourgeois & Ratard, 2014). The distinction between classical and host-specific bacteria is important for the diagnosis and management of illnesses in humans, and microorganism quantification is essential to these processes (Casadevall & Pirofski, 2000).

2.15 T-Cell Exhaustion in Pulmonary TB

One important feature of the immune response to pulmonary *tuberculosis* is T-cell fatigue, which is characterized by decreased cytokine production and increased expression of inhibitory receptors (Wong et al., 2018; Jacques 2016). This state of exhaustion can be influenced by the presence of inhibitory molecules like Tim3 and PD1, and blocking them has shown the potential to enhance T-cell function and improve infection outcomes (Sakuishi et al., 2010; Jacques 2016). Checkpoint inhibitors may play a specific role in restoring T-cell fatigue in *tuberculosis*, but this involvement is still unknown (Khan 2017). To lay the foundation for the management of successful immunotherapies, it is critical to understand the underlying mechanisms and investigate potential therapeutic interventions for T-cell exhaustion in tuberculosis (Zebley & Youngblood, 2022).

2.16 Evidence from clinical studies

Research on T-cell exhaustion in pulmonary TB has unveiled a complex association of factors, leading to varied findings. The discovery of minimal T-cell depletion levels within TB lung granulomas implies that additional mechanisms may contribute to the immunological landscape in pulmonary TB (Wong et al., 2018). This underscores the importance of exploring alternative factors that could influence the T-cell response during TB infection. Contrastingly, Kahan et al., (2015) work has shed light on the potential involvement of checkpoint inhibitors in preventing T-cell exhaustion in TB. This highlights the need for more research into the complex regulatory processes controlling T-cell responses during TB infection and points to a potentially effective route for intervention. Understanding the role of checkpoint inhibitors could provide new therapeutic strategies to modulate the immune response and enhance T-cell functionality in TB patientscite here.

The identification of depressed T-cell interferon-gamma responses in pulmonary TB adds another layer to the complexity of T-cell exhaustion. The potential link between depressed interferon-gamma responses and T-cell exhaustion highlights the multifaceted nature of the immune

dysregulation in TB (Hirsch et al., 1999). Since T-cell depletion can be reversed clinically in the context of pulmonary sarcoidosis, more research into the molecular and cellular mechanisms causing these reduced responses may reveal new targets for therapeutic approaches. The finding suggests that there might be dynamic and reversible aspects to T-cell exhaustion, opening possibilities for interventions aimed at restoring T-cell function in TB patients. The correlation between clinical resolution and the reversal of T-cell exhaustion in sarcoidosis implies that similar strategies can be explored in the context of TB to improve treatment outcomes.

2.17 Results Concerning T-Cell Exhaustion

Paley et al., (2013) investigates the collaboration between progenitor and terminal subsets of CD8+ T cells in containing chronic viral infections. The study provides insights into the dynamics of T-cell responses during persistent infections, highlighting the importance of both early progenitor cells and terminally differentiated effector cells in controlling viral replication. Wherry & Kurachi, (2016) an overview of the cellular and molecular pathways underlying T-cell exhaustion is provided by this work. It explored the signaling pathways, transcriptional changes, and epigenetic modifications associated with T-cell exhaustion, shedding light on the complex interplay of factors that contribute to the dysfunction of T cells during chronic infections and cancer. Vansteenkiste et al., (2014) looked at the emerging immunotherapeutic approaches aimed at reinvigorating exhausted T cells, including checkpoint blockade therapies and adoptive cell transfer. The goal was to provide insights into potential interventions for restoring T-cell function. Conversely, however, a study by Mackie, (2017) explores the coregulation of CD8+ T-cell exhaustion by multiple inhibitory receptors during chronic viral infections. The research identifies the existence of a hierarchy of inhibitory receptors that collectively contribute to T-cell exhaustion, providing valuable insights into potential targets for immunotherapy in chronic infections and cancer.

2.18 Diagnostic markers for T-cell exhaustion

Gupta et al., (2015) identified CD39 as a marker indicative of exhausted CD8+ T cells, particularly in the context of chronic viral infections like HCV and HIV. This marker was observed to be co-expressed with PD-1 and showed correlation with viral load. Supporting this, Henao-Tamayo et al., (2011) endorsed the use of exhaustion markers, specifically PD-1 and TIM-3, as potential biomarkers to determine the effectiveness of tuberculosis chemotherapy. Building on these

findings, Riches et al., (2013) demonstrated that T cells from patients with chronic lymphocytic leukemia (CLL) show signs of T-cell exhaustion, including elevated expression of exhaustion markers and the capacity to produce cytokines. Collectively, these investigations highlight the potential of exhaustion markers as useful diagnostic instruments for detecting T-cell exhaustion in various disease conditions.

2.19 Biomarkers associated with exhaustion in TB

Exhaustion in TB, a disease with a complex immunopathology, is manifested by modifications to T cell surface markers on CD8 T cells, namely PD-1, TIM-3, and KLRG-1. (Henao-Tamayo et al., 2011) . These markers serve as indicators of the immune system's state and its response to the infection. Additionally, biomarkers such as SAA1 (Serum Amyloid A1), PCT (Procalcitonin), IL-1β (Interleukin-1 beta), IL-6 (Interleukin-6), CRP (C-Reactive Protein), PTX-3 (Pentraxin 3), and MMP-8 (Matrix Metallopeptidase-8) have been identified as potential tools for monitoring disease severity and evaluating treatment efficacy in TB (Sigal et al., 2017). These biomarkers provide physicians with important insights into the intricate interactions that occur between the pathogen and the human immune response.

2.20 Blood-Based Biomarkers for TB Patient Care

Recent research has brought attention to the exciting possibilities of using immune profiling to manage TB. By analyzing immune molecules, this approach has shown promise in enhancing TB diagnosis, monitoring treatment progress, and predicting outcomes (Thu et al., 2023). In addition, multidimensional omics, including molecular diagnostics and personalized care, have been suggested as ways to improve TB detection and patient well-being (Buzimkic et al., 2021). The application of systems biology techniques, such as genomics, transcriptomics, and immunophenotyping, can further enhance TB management by offering insights into disease mechanisms, identifying biomarkers, and optimizing prevention, diagnosis, and treatment (Kontsevaya et al., 2021).

Notably, Mabwe et al., (2019) and Adekambi et al., (2015) have identified specific blood-based biomarkers that could assist in diagnosing and monitoring TB treatment. Duffy's research with TruCulture has shown the ability to distinguish active TB cases from latent infections, while

Adekambi's identification of immunological activation markers on CD4+ T cells specific to *Mycobacterium tuberculosis* offers a useful set of instruments for tracking therapy responses. Collectively, these studies emphasize the potential of immune profiling in improving the care provided to TB patients.

2.21 Challenges and Future Directions

The ongoing worldwide health crisis of TB calls for a better comprehension of the immunological assessment. T-cell exhaustion has received attention in relation to tuberculosis. This review of the literature discusses the challenges now facing the area and suggests potential future directions for it. Research has revealed that T-cell exhaustion markers persist during TB infections include PD-1, Tim-3, and Lag-3 (Sakuishi et al., 2010). Despite advancements in the fight against TB infections, challenges exist in understanding the specific mechanisms driving T-cell exhaustion in TB. Limited access to clinical samples, heterogeneity in TB manifestations, and the influence of comorbidities contribute to the complexity of research in this field.

CHAPTER 3
METHODOLOGY

3.0 Ethical approval

The study was approved by the University for Development Studies' (UDS) Institutional Review Board (UDS/RB/125/23). Site permission at the Tamale Teaching Hospital (TTH) was granted by the facility's administration. Also, permission was given by the head of the Chest Clinic Unit. In addition, formal consent from all the study participants was sought.

3.1 Samples collection site

Samples were collected at the Tamale Teaching Hospital located in Tamale in the Northern Region of Ghana, and serves as a referral hospital for the sector (Northern, Savana, North-East, Upper East, and Upper West). The Tamale Teaching Hospital has a bed capacity of 800 and offers general medicine, surgery, obstetrics and gynecology, pediatrics, orthopedics, ophthalmology, dermatology, psychiatry, and other services. The hospital contains specialized departments and sections that are staffed by skilled medical staff and administrative personnel who are dedicated to giving patients high-quality care. In addition to offering clinical services, the Tamale Teaching Hospital is actively engaged in research, which advances knowledge and promotes healthcare.

3.2 Characteristics of patients

A structured questionnaire was used to obtain demographic and clinical information such as age, sex and duration on the anti-TB drugs, which were also confirmed by the TB register 04. In all, 30 clients we recruited for the study. The clients were grouped into apparently healthy control (H-control) not on medication, newly diagnosed i.e. month zero (0T), intensive phase of treatment i.e. within two months on the anti-TB drugs (1T), three months to four months on anti-TB drugs (2T), five to month six on anti-TB drugs (3T), and. completed therapy group. Per the treatment modality for the patients, those in the intensive phase (0 - $\leq$ 2, months), designated 1T were on Rifampicin, Isoniazid, Ethambutol and Pyrazinamide. For the continuation phase of treatment (2T and 3T), the clients were on Rifampicin and Isoniazid. There was no case of multidrug resistance (MDR) among the clients.

3.3 Exclusion criteria

Clients diagnosed with other chronic conditions such as HIV, HBsAg, HCV, Hb SS, Diabetes, hypertension, and asthma were verified from TB register 04 and confirmed by laboratory test were excluded from the study. Also, those on herbal treatment and pregnant mothers were excluded..

3.4 Blood sample collection

Patient charter (Scale, 1998) and infection prevention and control guidelines (Ministry of Health et al., 2015) were strictly adhered to before, during and after blood sample collection. Clients were recruited and bled between 16th October 2023 to 23rd February 2024. 3ml of venous blood sample from the antecubital fossa was collected from each client into labeled K2 Ethylenediaminetetraacetic acid (EDTA) tubes. Samples were arranged according with the questionnaire, packaged carefully and transported on cold-chain immediately to the postgraduate research laboratory for further analysis (Geneva, 2021).

3.5 Peripheral blood mononuclear cells (PBMC) isolation

In a labeled, sterile 15mls falcon tubes (Jiangsu Huida Medical Instruments Co., Ltd.). 2ml of freshly drawn whole blood samples that had been obtained in an EDTA anticoagulant tube were diluted with an equivalent volume of Phosphate buffered saline (PBS). Falcon tubes were caped, and samples were gently mixed by inversion 5 to 8 times. The diluted blood in the falcon tubes was carefully placed in a second, sterile falcon tube with a label, over a 1.5 ml solution of Ficoll-Paque lymphocyte separation medium (Tianjin Haoyang Biological Products Technology, China). Without disturbing the two layers of solution in the falcon tubes, the samples were centrifuged (Centurion scientific centrifuges-UK) at 2,000g for 10 minutes. After centrifugation, three distinct layers were observed. The yellowish top, creamy layer in the middle and reddish layer at the bottom. The creamy layers containing the PBMC were transferred into a clean labelled 1.5ml Eppendorf tube, and more PBS was added to wash the PBMC collected. After carefully mixing the Eppendorf tubes with the PBMC, the samples were centrifuged at 1,000g for 10 minutes. Following centrifugation, the PBMC pellet was left behind after the supernatants were carefully collected and disposed of. The PBMC pellets were suspended in 500µl PRImeZOL RNA isolation reagent (Canvax - Boecillo Technological Park, Spain) for nucleic acid extraction.

3.6 Ribonucleic acid (RNA) isolation using Canvax reagents with spin columns

The PBMC pellets were lysed with 500µl PRImeZOL by passing through the pipette tip several times and incubated at room temperature for 5 minutes. 200µl of chloroform was added per 500µl of PRImeZOL used. The Eppendorf tubes were capped securely and shook vigorously by hand for 15 seconds. The samples were then incubated for 3 minutes at room temperature. They were centrifuged at 13,000Xg for 5 minutes at 4^{O}C. The samples were separated into a red lower organic layer, an interphase, and a colourless upper aqueous layer that contained the total RNA.

500µl chilled isopropyl alcohol was measured and transferred into a new labelled spin column, the aqueous phase was then transferred into the new spin column tube containing the chilled isopropyl alcohol without disturbing the interphase. The samples were incubated for 5 minutes at room temperature, then centrifuged at 13,000Xg for 1 minute. The flow-through was discarded, RNA precipitation was repeated with 500µl chilled isopropyl alcohol, centrifuged at 13,000Xg for 1 minute and discarded the flow-through. 600µl chilled 70% ethanol was added, centrifuged at 13,000Xg for 1 minute and discarded the flow through., another 600µl chilled 70% ethanol was added, centrifuged at 13,000Xg for 4 minutes and discarded the flow through, the columns were transferred onto a new labelled Eppendorf tube, the RNA was eluted with 20µl PCR grade water and centrifuged at 13,000Xg for 3mins. The purity and the concentration of the RNA were checked with Lasany Nanodrop spectrophotometer (Chennai Technologies, India),

3.7 complementary DNA synthesis using Canvax reagents, gene amplification and quantification

New sterile PCR tubes were labelled, and the following molecular reagents were carefully measured per one reaction, as indicated by the kit manufacturer (Canvax reagents). Briefly, 50ng of RNA template was added to a cocktail of oligo dT (1µl), dNTP (1µl), and nuclease-free water to make 12.5µl, then heated for five minutes at 65°C. The mixture was put on ice, and 5X cDNA buffer (4µl), DTT 0.1M (2 µl), RNAse (0.5µl), and reverse transcriptase (1µl) making a total of 20µl. The cDNA synthesis was done in a PCR thermal cycler set for incubation at 50°C for 60 minutes, inactivate the reaction by heating at 85°C for 5 minutes, then to 0°C holding, the cDNA products synthesized was then followed by polymerase chain reaction (PCR).

The cDNA synthesis was followed with PCR with immune cell exhaustion makers namely, PDL-1, CTLA-4, CD244, LAG-3 along with house-keeping gene GAPDH. Also, inflammatory

cytokines makers namely, IL2, INF-g, TNF-a, and Granzyme B, and cellular activity and proliferation makers namely, CD 69, Ki67, IL-7R, IL-15R, GATA-3, CD4, CD8 alongside with GAPDH for genes amplifications (primers shown in **Table 1**). Following the manufacturer's instructions, 1µl of the cDNA was added to the PCR reaction mixture.

3.8 1% Agaro gel electrophoresis

After gene amplification, the visualizations were done with 1% Agarose gel electrophoresis. 0.2g of agarose powder was weighed, poured in 20mls of 1 X TAE buffer, and swiveled to dissolve. The solution was microwaved to completely melt agarose powder. After melting/boiling, 0.5µl Ethidium bromide was added to stain the agarose solution, and then, the agarose solution was gently poured into the casting block. The combs were gently placed into agarose solution for the creation of wells, the gel was allowed to set/solidify. 1 X TAE buffer was poured into the electrophoresis tank. After the gel had been set, the combs were gently removed to see the wells that had been created. The solidified gel was placed into the electrophoretic tank, more 1 X TAE buffer was added to completely submerge the gel. The 10µl of amplified DNA samples and 0.5µl 100 bp molecular ladder were loaded from the negative pole. The negative end of the power pack was connected to the negative end of the electrophoretic tank and the positive end of the power pack to the positive end of the electrophoretic tank. Switch on the power of the power pack was switched on, the voltage and the current were adjusted to 100V and 90A and time 30mins to run.

Table 1**: List of primer sequences used.**

GENE	ACCESS NUMBER	PRIMER DIRECTION	PRIMER QUENCE	PRIMER LENGTH	TM	GC%	PRODUCT LENGTH (BP)
PD-L1 (CD274) Human mRNA	NM_014143	Forward	TGCCGACTACAAGCGAATTACTG	23	61.22	47.83	150
		Reverse	CTGCTTGTCCAGATGACTTCGG	22	61.25	54.55	
Lymphocyte Activation Gene 3 (LAG-3) Human Mrna	NM_002286	Forward	GCAGTGTACTTCACAGAGCTGTC	23	61.4	52.17	143
		Reverse	AAGCCAAAGGCTCCAGTCACCA	22	64.42	54.55	
CD244 Human mRNA	NM_016382	Forward	TCTACTGCCTGGAGGTCACCAG	22	63.14	59	152
		Reverse	GACCAAGCAAGACAGAGCCACT	22	62.77	54.55	
CTLA4 Human mRNA	NM_005214	Forward	ACGGGACTCTACATCTGCAAGG	22	61.79	54.55	121
		Reverse	GGAGGAAGTCAGAATCTGGGCA	22	61.75	54.55	
Ki67 (MKI67) Human mRNA	NM_002417	Forward	GAAAGAGTGGCAACCTGCCTTC	22	61.97	54.55	151
		Reverse	GCACCAAGTTTTACTACATCTGCC	24	60.38	45.83	
CD69 Human mRNA	NM_001781	Forward	GCTGGACTTCAGCCCAAAATGC	22	62.86	54.55	
		Reverse	AGTCCAACCCAGTGTTCCTCTC	22	61.62	54.55	121
Granzyme B (GZMB) Human mRNA	NM_004131	Forward	CGACAGTACCATTGAGTTGTGCG	23	62.23	52.17	122
		Reverse	TTCGTCCATAGGAGACAATGCCC	23	62.23	52.17	
GATA-3 Human mRNA	NM_001002295	Forward	ACCACAACCACACTCTGGAGGA	22	63.23	54.55	132
		Reverse	TCGGTTTCTGGTCTGGATGCCT	22	63.67	54.55	
CD4 Human mRNA	NM_000616	Forward	CCTCCTGCTTTTCATTGGGCTAG	23	61.5	52.17	126
		Reverse	TGAGGACACTGGCAGGTCTTCT	22	63.02	54.55	
CD8 Human mRNA	NM_001768	Forward	ACTTGTGGGGTCCTTCTCCTGT	22	62.99	54.55	106
		Reverse	TGTCTCCCGATTTGACCACAGG	22	62.25	54.55	
IL-2 Human mRNA	NM_000586	Forward	AGAACTCAAACCTCTGGAGGAAG	23	59.67	47.83	153
		Reverse	GCTGTCTCATCAGCATATTCACAC	24	60.03	45.83	
Interferon gamma (IFN-g) Human mRNA	NM_000619	Forward	GAGTGTGGAGACCATCAAGGAAG	23	60.62	52.17	124
		Reverse	TGCTTTGCGTTGGACATTCAAGTC	24	62.79	45.83	
TNF alpha (TNF-a) Human mRNA	NM_000594	Forward	CTCTTCTGCCTGCTGCACTTTG	22	62.27	54.55	135
		Reverse	ATGGGCTACAGGCTTGTCACTC	22	62.33	54.55	
IL7R alpha (IL-7R) Human mRNA	NM_002185	Forward	ATCGCAGCACTCACTGACCTGT	22	62.2	54.55	101
		Reverse	TCAGGCACTTTACCTCCACGAG	22	62.98	54.55	
IL-15RA Human mRNA	NM_002189	Forward	TGGCTATCTCCACGTCCACTGT	22	63.13	54.55	116
		Reverse	CATGGCTTCCATTTCAACGCTGG	23	63.06	52.17	
GAPDH Human mRNA	NM_002046	Forward	GTCTCCTCTGACTTCAACAGCG	22	60.92	54.55	131
		Reverse	ACCACCCTGTTGCTGTAGCCAA	22	64.41	54.55	

3.9 Statistical analysis

The software ImageJ was used to quantify the DNA bands. Microsoft Excel was used to construct the gene expression in relation to GAPDH, which was then exported to Graph Pad Prism version 8 for analysis. Three duplicate ImageJ results were subjected to the Graph Pad column analysis. The groups were compared using a one-way ANOVA, and multiple comparisons were performed using Tukey analysis. Pearson correlation analysis was performed to assess the direction of association among the variables measured. In every instance, P-values of less than 0.05 were deemed statistically significant when comparing groups.

CHAPTER 4

RESULTS

4.1 Demographic characteristics of the study participants

All clients were males with ages ranging from 25 to 88 years. Healthy controls were drawn from qualified blood donors at the Tamale Teaching Hospital blood bank. The clients were grouped into 6 experimental categories based on the stage of anti-TB therapy and the age of the clients as shown in **Table 2**.

Table 2: Experimental groupings of the study participants.

	Healthy Control	Group A (0T)	Group B (1T)	Group C (2T)	Group D (3T)	Group E
EXPERIMENT	PN (Age in years)	PN (Age in years)	PN (Age in years)	PN (Age in years)	PN (Age in years)	PN (Age in years)
Experiment 1	N001 (40)	A001 (58)	B001 (50)	C001 (43)	D001 (49)	E001 (52)
Experiment 2	N002 (30)	A002 (38)	B002 (35)	C002 (43)	D002 (58)	E002 (31)
Experiment 3	N003 (38)	A003 (88)	B003 (71)	C003 (34)	D003 (26)	E003 (70)
Experiment 4	N004 (30)	A004 (25)	B004 (47)	C004 (34)	D004 (40)	E004 (55)
Experiment 5	N005 (35)	A005 (75)	B005 (53)	C005 (45)	D005 (53)	E005 (65)

Key: Participant's number (PN); month zero (0T) yet to start treatment, intensive phase of treatment i.e. within two months on the anti-TB drugs (1T), three months to four months on anti-TB drugs (2T), five to month six on anti-TB drugs (3T), and completed therapy.
Patients' treatment regimen: Intensive phase (0 - ≤ 2, months), 1T; Rifampicin, Isoniazid, Ethambutol and Pyrazinamide. Continuation phase of treatment (2T and 3T); Rifampicin and Isoniazid. There was no case of multidrug resistance (MDR) among the patients.

4.2 CD244 in PBMCs predicts positive PTB prognosis during treatment.

The transcription pattern of T cell exhaustion markers PD-L1, CTLA4, CD244 and LAG3 were assessed in PBMCs from patients at varied stages of PTB treatment. Compared to the control, it was observed that PD-L1 was profoundly expressed in newly diagnosed PTB patients. However, following the onset of treatment, PD-L1 gene expression significantly decreased especially from the second phase of treatment through to the cured state. The pattern of CTLA4 gene expression was significantly low in healthy control but profound during the treatment phases, particularly 1T and diminished in the cured state. The gene expression pattern of CD244 was like that seen in

CTLA-4. Interestingly, an increased transcription of LAG-3 gene was noticed in the healthy control compared to the diseased phase but comparable to the cured persons. Generally, among the T cells exhaustion markers invested in PBMCs from PTB patients, PD-L1 gene produced a consistent pattern suggesting its potential as a marker for monitoring PTB treatment outcome (**Figure 1**). The correlation analysis revealed a strong negative relationship with CD244 expression, particularly between 1T and 2T (r=-0.94; p=0.018), and 3T (r=-0.95; p=0.013), suggesting DC244 as a relatively strong marker for monitoring PTB treatment progress. Comparing 0T and 3T, a negative correlation existed in PD-L1 (r=-0.74) but was statistically insignificant, as seen in CTLA4 and LAG-3 expressions (Figure 4 A to D).

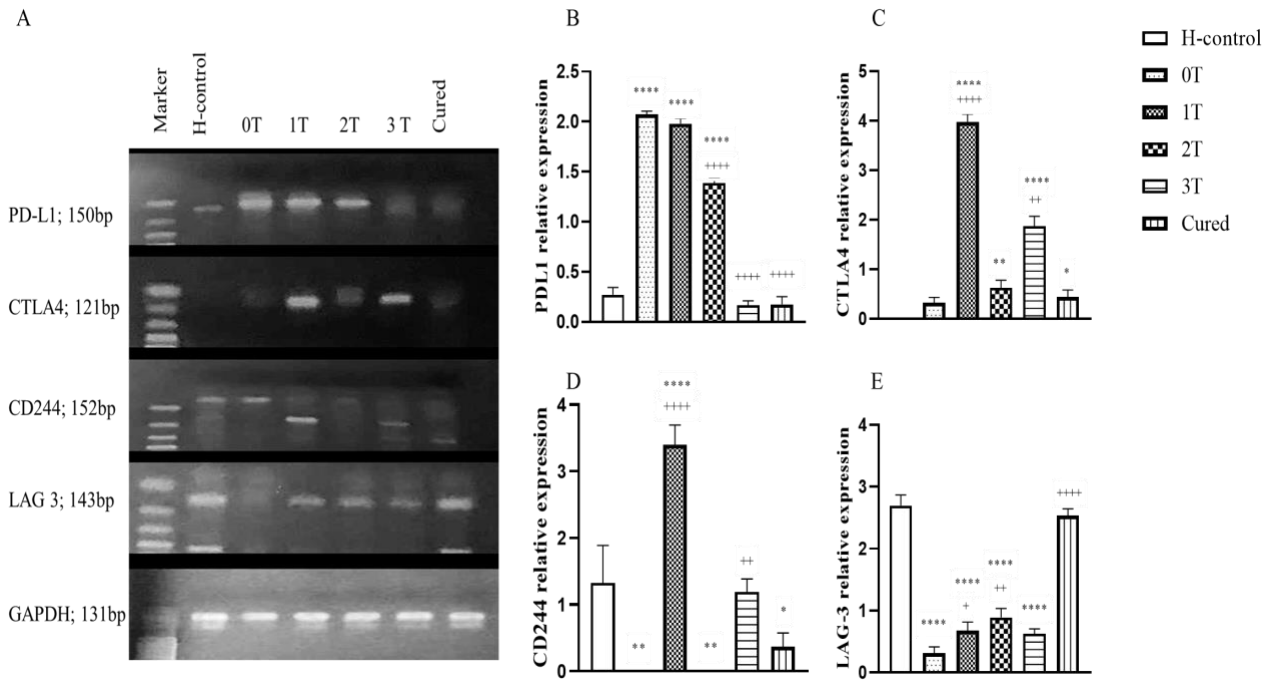


Figure 1*:* The T cell exhaustion gene pattern at different PTB therapy phases

(A) Gel electrophoresis bands of amplicons with their respective base pairs. B, C, D, E The respective relative expression of PD-L1, CTLA4, and LAG-3 to GAPDH. Results are a representation of triplicate experiments. Every data point is shown as mean ± SEM (n = 5). Compared to a healthy control, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. +p <0.05, ++p <0.01, + ++p <0.001, + +++p <0.0001 in comparison to a newly diagnosed patient who has not yet begun treatment. H-control (healthy control), 0T (newly diagnosed yet to start treatment), 1T (first phase of treatment), 2T (second phase of treatment), 3T (third phase of treatment). Cured means those who have completed treatment and tested negative for *Mycobacterium tuberculosis*.4.3 Transcription of inflammatory cytokines is restored to normal following the initiation of PTB treatment.

The transcription of four inflammatory markers were examined in PBMCs from PTB patients at varied levels of treatment. IL-2 was significantly elevated in the 0T group when compared to the healthy control but showed undulating expression pattern during the treatment phase. However, the cured group had similar IL-2 expression when compared to the healthy control group. The transcription of IFN-g significantly diminished in the newly diagnosed (0T) group, but it was restored right at the initiation of treatment and sustained till completely cured. The pattern of TNF-a expression was like that of IFN-g. Even though diminished Granzyme B expression was restored during the early phase of treatment, it remained significantly low throughout the other treatment phases and cure stage. Overall, the inflammatory markers assessed were restored to normancy and sustained through treatment to cure, except for Granzyme B which remained low after the cured stage (**Figure 2**). TNF-a expression in 0T group negatively correlated to 2T (r=-0.10), 3T (r=-0.98), and cured (r=-0.31). IL-2 expression in the 0T group negatively correlated to 1T (r=-0.79), 2T (r=-0.83) and completed phase (r=-0.80), but the p-values (association) were not statistically significant, Figure 4 E and F.

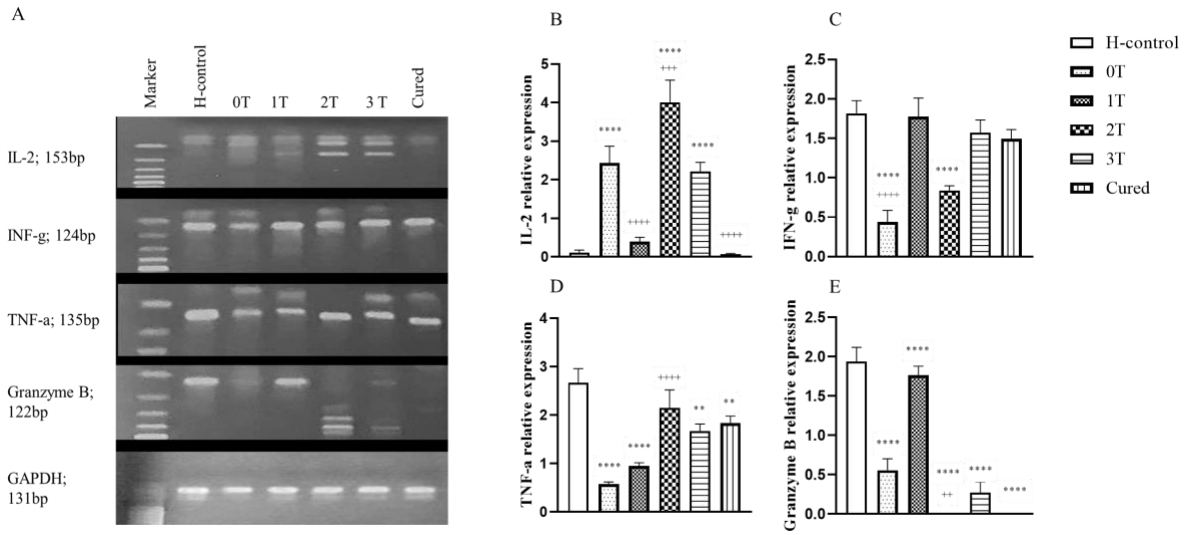


Figure 2**:** Transcription of inflammatory cytokines in various stages of PTB treatment

(A) Gel electrophoresis bands of amplicons with their respective base pairs. B, C, D, E The relative expression of IL-2, IFN-g, TNF-a and Granzyme B to GAPDH. Results are a representation of triplicate experiments. Every data point is shown as mean ± SEM (n = 5). Compared to a healthy control, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. +p <0.05, ++p <0.01, + ++p <0.001, + +++p <0.0001 in comparison to a freshly diagnosed patient who has not yet begun therapy (0T). H-control (healthy control), 0T (newly diagnosed yet to start treatment), 1T (first phase of treatment), 2T (second phase of treatment), 3T (third phase of treatment). Cured means those who have completed treatment and tested negative for *Mycobacterium tuberculosis*.

4.4 Cellular activity markers could be used to monitor PTB treatment outcome.

PBMCs from PTB patients at various stages of treatment were examined for the transcription pattern of cellular activity genes. These genes included CD69, Ki67, IL-7R, IL-15R, GATA-3, CD8 and CD4. The group that was recently diagnosed and has not yet started therapy had significantly higher levels of CD69, IL-7R, and IL-15R expression. However, at the cured stage CD69 expression was comparable to the healthy control. The transcription of Ki67, GATA-3 and CD4 were not detectable in the 0T group but their activities increased once treatment was started, and those of the cured stage were comparable to the healthy control. For CD8, the downregulation observed in the 0T group was significantly elevated during the first treatment phase and reversed to that of the healthy control in the subsequent treatment phase until cure. Possibly, these cellular activity markers could be used to monitor the progress of PTB treatment (**Figure 3**). The correlation investigation revealed relatively strong negative association between 1T and cured for GATRA-3 (r=-0.98), 1T and 3T (r=-0.97) for CD4, 1T and 2T (r=-0.99) for Ki67, but p-values were statistically not significant, (Figure 4 G to I).

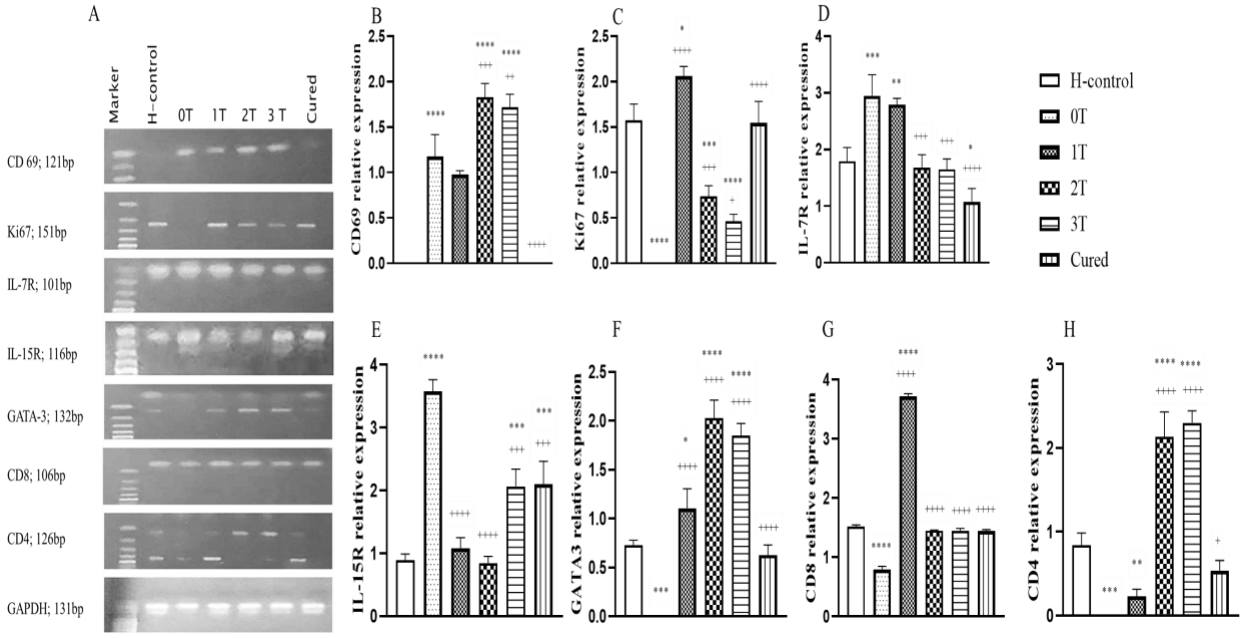


Figure 3**:** Transcription pattern of cellular activity markers in various stages of PTB treatment

(A) Gel electrophoresis bands of amplicons with their respective base pairs. B, C, D, E, F, G and H. The relative expression of CD69, Ki67, IL-7R, IL-15R, GATA-3, CD8, and CD4 respectively, to GAPDH. Results are a representation of triplicate experiments. Every data point is shown as mean ± SEM (n = 5). Compared to a healthy control, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. +p <0.05, ++p <0.01, + ++p <0.001, + +++p <0.0001 in comparison to a freshly diagnosed patient who has not yet begun therapy (0T). H-control (healthy control),

0T (newly diagnosed yet to start treatment), 1T (first phase of treatment), 2T (second phase of treatment), 3T (third phase of treatment). Cured means those who have completed treatment and tested negative for MTB.

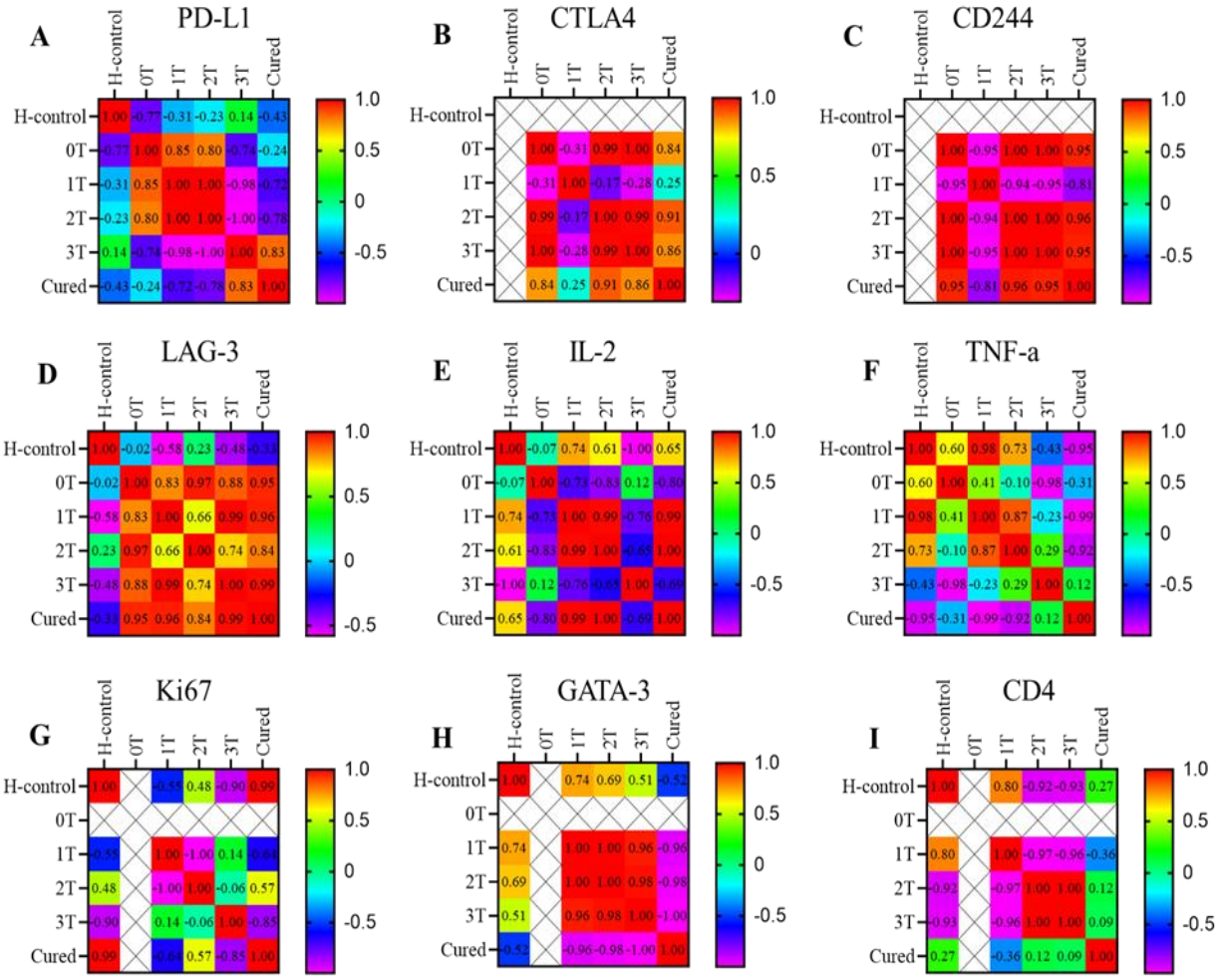


Figure 4. Correlation analysis for expression pattern of markers associated with T cell exhaustion, inflammatory and cellular activity genes. H-control (healthy control), 0T (newly diagnosed yet to start treatment), 1T (first phase of treatment), 2T (second phase of treatment), 3T (third phase of treatment), Cured phase.

CHAPTER 5
DISCUSSION

Even with the significant advancements in the fight against TB in recent decades, *tuberculosis* remains the world's worst communicable disease (WHO, 2023), it suggests that further knowledge of the immunological characteristics of *M. tuberculosis* infection is necessary. The PTB control programs require TB-specific blood-based biomarkers instead of sputum specimens for assessing its progression, predicting reactivation, prognosis and vaccine-induced immunity protection; and differentiating between various stages of PTB infection (Goletti et al., 2016). Since *Mycobacterium tuberculosis* pathogenesis is mostly T-cell dependent, it might be useful to measure these changes in terms of T-cell exhaustion biomarkers, inflammatory cytokines and cellular activities (Goletti et al., 2016). At the gene level, the study investigated exhaustion markers of immune cells (PD-L1, CTLA-4, CD244 and LAG-3), inflammatory cytokine patterns (IL-2, INF-g, TNF-a and Granzyme B) as well as patterns for cellular activities (CD69, Ki67, IL-7R, IL-15R, GATA-3, CD8 and CD4) among PTB patients on anti-TB regimens at different treatment stages, newly diagnosed clients yet to start treatment and healthy control.

Given that PDL-1 expression is comparatively low in healthy individuals, this shows that PDL-1 serves as a biomarker of immunological dysregulation in *tuberculosis*. Blocking PD-1 can increase T cell activation when used to cancer treatment, where PD-1 inhibition is already used as a therapeutic strategy (Jubel et al., 2020). The severity of *tuberculosis* can be decreased in patients by inhibiting the PDL-1 axis and enhancing T-cell immunity. Immune control against *Mycobacterium tuberculosis* can be strengthened by boosting T-cell immunity (M. Ahmed et al., 2022). T-cell activity has restored after the TB therapy began, and the immunological weariness has subsided as seen by the decline in PDL-1 expression. Prior research has demonstrated that T lymphocytes are prevented from eradicating tumor cells within the body when PD-L1 attaches to PD-1. Anti-PD-L1 or anti-PD-1 immune checkpoint drugs function by stopping PD-L1 from sticking to PD-1, enabling T lymphocytes to destroy tumor cells (Tezera et al., 2020). These results demonstrate the potential benefits of PDL-1 targeting as a therapeutic approach to enhance host immunity and improve tuberculosis treatment outcomes.

When it comes to preserving immunological homeostasis, CTLA-4 is essential. It helps avoid the immune system overreacting to a TB infection, which could result in tissue damage. The dynamic

expression of CTLA-4 reflects the balance the immune system strikes between combating *M. tuberculosis* and preventing autoimmunity (Inoue et al., 2011). In this study, the pattern of CTLA-4 gene expression showed significant variations at various stages of anti-TB treatments. T cells express the inhibitory receptor CTLA-4 to suppress immunological responses and avoid autoimmunity. When *tuberculosis* is not present, its expression stays at normal levels (Buchbinder & Desai, 2016). The immune system is more active when *tuberculosis* is active because the body is trying to fight off the *Mycobacterium tuberculosis M. tuberculosis* infection. In order to balance the immune response and avoid excessive inflammation, T cells are activated to combat the *M. tuberculosis* infection, which results in an increase of CTLA-4. The increased synthesis of CTLA-4 provides a regulatory mechanism to regulate immune activation and minimize tissue damage from an overactive *M. tuberculosis* (Sia & Rengarajan, 2019a).

During anti-TB treatment, the TB bacilli load decreases, and the immune system gradually shifts from an active disease-fighting mode to a resolution and healing mode. The (1T) phase of TB treatment might still show significantly elevated CTLA-4 expression as the immune system continues to deal with the residual TB bacilli and inflammation (Tiwari & Martineau, 2023b). As treatment progresses and the patient approaches a cured TB state, the immune system's activation diminishes, leading to a reduction in CTLA-4 expression. By this stage, the immune response has been largely successful, and the regulatory mechanisms of CTLA-4 return to baseline levels as the immune system no longer needs to control *Mycobacterium tuberculosis* (Zhang et al., 2023).

Overall, in this study, the pattern of CTLA-4 gene expression changes in response to the immune system's needs at various stages of *Mycobacterium tuberculosis* and anti-TB treatment. Low expression in healthy controls reflects the absence of immune activation, increased expression during active disease (0T) indicates regulatory mechanisms to control inflammation, and reduced expression in the 2T, 3T and cured TB state signifies the resolution of the infection and return to immune homeostasis (Figure 1C). Therefore, CTLA-4 can be used as a therapeutic strategy to enhance host immunity and improve TB treatment outcomes.

CD244 expression was more expressed in (1T) intensive than in newly diagnosed (0T) PTB patients. It was the only marker that showed significant association and a strong negative

correlation, particularly between 1T and 2T (r=-0.94; p=0.018), and 3T (r=-0.95; p=0.013), suggesting DC244 as a relatively strong marker for monitoring PTB treatment progress. The host's immune response determines how long the TB infection lasts, and CD244's immunological activity influences the emergence of active PTB (Sun et al., 2021). Its expression in newly diagnosed PTB clients was low, this did not support a similar study conducted (Wang et al, 2015). Nevertheless, because TCR signal upon antigenic contact and a variety of signals from costimulatory molecules, including CD244, regulate CD244 activation and effector activities, there was a dramatic increase in CD244 expression during the first tumorous phase of the anti-TB treatment (Wang et al., 2015). As the treatment progresses and the infection is brought under control, this may lead to alterations in CD244 expression. Once the TB infection is successfully treated and the patient enters the cured stage, CD244 expression diminishes (B. Yang et al., 2013).

There was an increased transcription of the LAG-3 gene in the apparently healthy controls compared to (0T) PTB phase. By preventing T cell activation, proliferation, cytokine generation, and cytolytic activity, LAG-3 is thought to support immunological homeostasis (Gertel, 2021). The function of LAG-3 in preserving body immune system hemostasis and preventing the immune system from overreacting to exposure to *M. tuberculosis* in the absence of active disease may be indicated by the increased transcription of LAG-3 in healthy controls (Infections, 2023). During the TB diseased phase, there is an active immune response aimed at controlling and eliminating the *M. tuberculosis*, and LAG-3 expression can be downregulated during this phase (Phillips et al., 2015). Consequently, lower levels of LAG-3 may correlate with the heightened immune activity observed in (0T) PTB disease phase (Phillips et al., 2015). In healthy controls, higher LAG-3 transcription may indicate a more robust regulatory network preventing unwarranted immune activation. In contrast, during active (0T) PTB disease, the immune system prioritizes an aggressive response over regulation, potentially leading to reduced LAG-3 expression (M. Ahmed et al., 2022).

In healthy individuals, a stable cytokine environment supports the expression of various regulatory molecules, including LAG-3. In (0T) PTB disease, the pro-inflammatory cytokine environment driven by *M. tuberculosis* infection might downregulate LAG-3 expression as part of the immune system's effort to maximize the inflammatory response required to control the infection (Li et al.,

2023). Overall, the immune system's dynamic modification in response to various stages of PTB infection is likely reflected in the enhanced transcription of the LAG-3 gene in healthy TB controls compared to the (0T) TB sick phase. LAG-3 is essential for immune regulation and homeostasis in the healthy state, but in active (0T) PTB disease, the immune system adopts a less controlled and more aggressive response, which changes the expression of the immune checkpoint protein LAG-3.

Furthermore, in the inflammatory cytokine patterns, the observed elevation of IL-2 in (0T) PTB clients suggests a hyperactive immune response, possibly indicative of early-stage inflammation ref. The dynamic changes in IL-2 expression during anti-TB treatment and its normalization upon cured implied its potential as a biomarker for monitoring therapeutic efficacy (De Oyarzabal et al., 2019). The initial elevation in IL-2 levels in the 0T patients may indicate an immune response to an underlying infection, which is not present in the healthy controls. This could be due to an ongoing inflammatory process at the time of baseline measurement (Perry, 2018). These feedback loops may be reflected in the undulating expression pattern observed during anti-TB medication. For instance during treatment, IL-2 can promote Treg cell growth, which in turn releases cytokines that inhibit IL-2 synthesis, causing fluctuations (Sa et al., 2013).

Genetic, environmental, and disease-specific factors can cause large variations in an individual's response to anti-TB medication. These variations can cause differences in IL-2 cytokine production and response to anti-TB treatment, resulting in the observed undulating pattern (Turner et al., 2011). Fluctuations in IL-2 levels could also be attributed to the variations in PTB patient adherence to treatment protocols, or other external factors that influence IL-2 cytokine levels (Tirore et al., 2024). Overall, the undulating expression pattern of IL-2 during the treatment phase is likely due to a combination of treatment effects, immune regulation, individual patient variability, and the natural course of the disease.

IFN-g is secreted mainly by activated T cells and NK cells. It can mediate antiviral and antibacterial immunity, enhance antigen presentation, orchestrate innate immune system activation, coordinate interaction between lymphocytes and endothelium, and regulate Th1/Th2 balance (Davies et al., 2023). IFN-g was significantly expressed in the healthy control compared to the (0T) PTB. The healthy control group may have a more activated immune response against

Mycobacterium tuberculosis compared to those who are newly diagnosed (0T) PTB. This heightened immune response could result in increased production of IFN-g as part of the body's defense mechanism against infections (Scriba et al., 2017). The (0T) PTB group may represent individuals whose immune system have been deleted to the infection with compromised containment of the bacterium (Carabalí-Isajar et al., 2023).

The fluctuation in IFN-g transcription observed in the (0T) PTB group suggests a dynamic interplay between the immune response and the disease progression. Upon initial diagnosis, the immune system might be dysregulated, leading to a decrease in IFN-g transcription (Berns et al., 2022). However, as anti-TB treatment begins, the immune system is activated and responds to the infection, leading to a restoration of IFN-g transcription (Maison, 2022). The body's attempt to fight the TB infection by boosting the activity of immune cells like T cells and NK cells, which release IFN-g as part of the immunological response against *Mycobacterium tuberculosis*, is probably what caused this reaction (Shanmuganathan et al., 2022). The sustained elevation of IFN-g transcription throughout the course of treatment to complete cured indicates the effectiveness of the anti-TB treatment in controlling the TB infection and restoring immune function (Ivashkiv et al., 2019). All things considered, the pattern of IFN-g that has been seen highlights how dynamic the IFN-g immune response is both during TB infection and treatment.

TNF-a is crucial for the immune system's reaction, which includes containing *mycobacteria* in granulomata during PTB infection and managing chronic infection (Harris & Keane, 2010). The significant reduction in TNF-a levels observed in the (0T) PTB group suggests that there may be an initial suppression of this cytokine during active TB infection. However, the subsequent restoration of TNF-a levels upon initiation of anti-TB treatment indicates that this suppression is reversed by the therapeutic intervention (Harris & Keane, 2010). During active TB infection, the immune response may initially suppress TNF-a production as part of the host defense mechanism or due to the immune-modulatory effects of the *Mycobacterium tuberculosis* (Carabalí-Isajar et al., 2023). TNF-a decrease during an active TB infection may be due to the body's attempt to lessen excessive inflammation, which is a defining feature of the pathology associated with TB (Sasindran & Torrelles, 2011).

The first step in treating tuberculosis is to give isoniazid, rifampicin, pyrazinamide, and ethambutol. These drugs directly target the *Mycobacterium tuberculosis* leading to reduced pathogen burden and consequently, normalization of immune responses including TNF-a production (Khadka et al., 2023). As the infection is brought under control, immune cells regain their functionality, including the ability to produce TNF-a. TNF-a levels may return as the course of treatment progresses and the equilibrium between host defenses and pathogen control changes. Overall, the observed pattern of TNF-a transcription in (0T) PTB patients, its restoration at treatment initiation, and sustained levels until cured, likely resulted from a complex interaction between the host immune response, the actions of antibiotics, and the dynamics of *tuberculosis* pathogenesis.

Granzyme B is a caspase-like serine protease that tumor and virus-infected cells are killed by cytotoxic lymphocyte discharge (Lo et al., 2020). Compared to the (0T) PTB group, the healthy control group had higher levels of Granzyme B expression which may indicate a more robust immune response (Shen et al., 2023). Granzyme B expression ranged from low during the (0T) TB treatment phase to high during the (1T) TB treatment phase. Granzyme B is mainly produced by cytotoxic T cells and NK cells, and its expression levels can be lowered when the TB bacilli first avoid the immune response (Dyatlov et al., 2019). During the (1T) phase of TB treatment, which typically involves a combination of potent anti-TB drugs, the TB bacilli load decreases significantly. The body's increased immune system activity may result from this decrease in TB bacilli burden when the body starts to eliminate contaminated cells (Patil et al., 2018).

As a result, immune effector activities are upregulated. Activated cytotoxic T cells and natural killer cells that target infected cells produce more Granzyme B. During active TB disease, there may be a shift towards an anti-inflammatory state, which could dampen the expression of Granzyme B (Llibre et al., 2022). PTB infection can cause tissue damage in the lungs, this damage can affect the local immune response and may result in reduced expression of Granzyme B (Panetti et al., 2022). Granzyme-B's significance in the host defense mechanism is shown by the notable decrease in Granzyme-B from PBMCs of active PTB that was seen following therapy. This suggests that the infection has been successfully controlled, and the immune response has returned to normal.

CD69 serves as a co-stimulatory signal and biomarker for cell activation. Research has demonstrated that CD69+T cells rise in response to IL-12β, IL-18α, and IL-16β mRNA levels. Additionally, the production of IFN-g is promoted in the presence of recombinant IL-12 or IL-18 (Mahdavi et al., 2020).. The expression of CD69 was greatly elevated in the newly diagnosed (0T) TB clients. However, at the cured stage, CD69 expression was similar to the healthy control. In the (0T) TB patients, the immune system is actively responding to the TB bacilli, leading to the upregulation of activation CD69. This elevated expression of CD69 indicates an ongoing immune response against the TB bacteria (Chen et al., 2020). After anti-TB treatment, there was significant downregulation of CD69 expression, as the TB bacilli load diminishes, the antigenic stimulation that drives the activation of immune cells also reduces (Chen et al., 2020).

As a result, the immune system's need to sustain a high degree of activity is reduced, which lowers the expression of CD69. Anti-TB medication reduces *Mycobacterium tuberculosis*, burden which helps to reduce inflammation. As the inflammatory signals decreased, the activation status of immune cells normalized, resulting in lower CD69 expression (Chen et al., 2020). T cells and other immune cells are more activated and proliferate during a (0T) TB infection. An effective anti-TB treatment brought the immune system back into balance, decreased over-activity, and promoted regulatory mechanisms that decreased the activation of CD69 biomarkers (Ahmed, 2020). Overall, once the TB infection is brought under control, the downregulation of CD69 following anti-TB treatment represents the immune system's recovery from antigenic stimulation and inflammation, immunological balance restoration, and overall immune system healing.

Transcription of Ki67 was not detectable in the (0T) PTB patients, but Ki67 biomarker activities detected when anti-TB treatment started, and those of the cured stage were comparable to the healthy control. Because of the tremendous impact that an active *Mycobacterium tuberculosis* infection has on the immune system, the transcription of Ki67 may not be detectable (Díaz-fernández et al., 2022). During (0T) PTB, the immune system is heavily engaged in combating the infection, and T cell proliferation, which is indicated by Ki67 expression, might be suppressed as the immune response is initially exhausted (Marshall et al., 2018). Once anti-TB treatment begins, the TB bacilli load starts to decrease. The immune system can recover and mount a more potent

response thanks to this decrease in the burden of *Mycobacterium tuberculosis* (Kaufmann et al., 2023).

As the immune system begins to recover, T cell proliferation increases, which is reflected by an increase in Ki67 biomarker activities (Bengsch et al., 2017). Ki67 is a biomarker for cell proliferation, so its increased activity indicates that the immune cells are actively proliferating to combat the infection and repair the damage caused by *Mycobacterium tuberculosis*. When TB is cured, the immune system stabilizes to a level similar to that of a healthy person, T cell proliferation returns to normal, and Ki67 biomarker activities are similar to those seen in healthy controls (Díaz-fernández et al., 2022). This implies that the immune system has recovered to a homeostatic state in which normal processes of cell proliferation are occurring.

The PTB patients' (0T) increased expression of IL-7R and IL-15R is suggestive of the immune system's response to the *Mycobacterium tuberculosis*. T-cell proliferation and control are critical for the immune system's defense against *Mycobacterium tuberculosis*, and they are made possible by the vital cytokines IL-15 and IL-7 (Mhatre V. Ho, Ji-Ann Lee & Craik, 2012; Maeurer et al., 2000). The body intensifies its immune response during a (0T) PTB infection, which raises the expression of IL-7R and facilitates the growth and upkeep of T cells that are necessary to fight the infection. IL-15 also encourages T-cells, such as NK cells and CD8+ T-cells, to survive and proliferate. Elevated IL-15R expression reflects the body's need to bolster these immune cell populations to fight the *Mycobacterium tuberculosis* effectively (Schluns & Anthony, 2015).

As the anti-TB treatment progresses, the bacterial load decreases, leading to a reduced antigenic stimulus. This diminishes the need for an intense immune response. With effective anti-TB treatment reducing the infection, the demand for T-cell proliferation and activation above physiological demands decreases. Consequently, as the immune system lowers its heightened state of activation, the expression levels of IL-7R and IL-15R decrease (Rochman et al., 2010). The body then begins to return to a state of immune homeostasis, where the heightened levels of cytokine receptors are no longer necessary. The reduction in IL-7R and IL-15R expression is part of this process, reflecting the resolution of the infection and a return to restoration of immune homeostasis activity.

The transcriptions of GATA-3 and CD4 were not detectable in the (0T) PTB patients, however, GATA-3 and CD4 expression increased when anti-TB treatment started. The expression of these biomarkers reduced at the cured stage comparable to the healthy control. This implies that during the (0T) phase of PTB infection, these indicators are inhibited (Shaukat et al., 2023). This might be because *Mycobacterium tuberculosis* uses immunological evasion techniques. The expression of GATA-3 and CD4 increased after anti-TB medication started. This shows that as the TB bacilli burden drops as a result of anti-TB therapy, the immune system starts to recuperate and react to the infection (Téllez-Navarrete et al., 2021). One transcription factor that is essential for Th2 cell development is GATA-3 (Wilson, M.S. , Metink-Kane, 2012), whereas CD4 is a helper T cell biomarker, which both are crucial for putting up a successful immune response (Luckheeram et al., 2012).

GATA-3 and CD4 expression levels dropped to levels similar to those in healthy controls at the cured stage (Acharya et al., 2023) . This return to normalcy implies that the immune system has been successfully resolved, ending in inflammation and immune activation, and returning to a condition akin to that of healthy regulation. GATA-3 and CD4 may be used as biomarkers to track the development of tuberculosis and the efficacy of treatment (Laufer et al., 2022). Their increased expression can be a sign of a therapeutic response going well. Initially suppressed during active infection, the immune system's recovery during treatment is marked by the resurgence of these biomarkers.

The best host defense after M. tuberculosis infection depends on CD8+ T cells, which has prompted research into the mechanism behind this protective effect. (Woodworth & Behar, 2006). CD8 was downregulated in the (0T) PTB patients, but it was significantly elevated during the first phase (1T) of anti-TB treatment and reversed to that of the healthy control in the subsequent treatment phases (2T, 3T) until the cured stage. In order to avoid being found and eliminated, *Mycobacterium tuberculosis* has the ability to control the host's immune response. The bacilli may suppress the immune response in order to ensure their survival and reproduction within the host, which could lead to the initial downregulation of CD8 T cells (Carabalí-Isajar et al., 2023). Chronic inflammation and extended antigen exposure in (0T) TB patients can cause T cell exhaustion, a

state in which CD8 T cells are downregulated and lose some of their functional ability (Lombardi et al., 2021).

When anti-TB treatment begins, the TB bacilli load decreases, reducing the immunosuppressive effects of *Mycobacterium tuberculosis*. This may result in the immune system being reconstituted, with CD8 T cells being reactivated and multiplying to aid in the removal of the TB infection. Anti-TB treatment reduces immune-suppressive factors such as high levels of regulatory T cells or myeloid-derived suppressor cells, which aids in CD8 T cell recovery (Ayodele et al., 2023). As anti-TB treatment progresses and the TB bacilli burden continues to decrease, the immune system shifts from an active infection response to a more regulated state. The numbers of CD8 T cells return to normal when the immune system is not needed to sustain an elevated response (Katrachanca & Koleske, 2017). The immune system aims to return to homeostasis after the initial phase of combating the infection. This involves regulating CD8 T cell levels back to those similar to healthy controls once *M. tuberculosis* is controlled and eventually eliminated.

CHAPTER 6
CONCLUSION, LIMITATIONS AND FUTURE STUDIES

6.1 CONCLUSION

TB remains a major world health problem, requiring ongoing improvements in management techniques, treatment plans, and diagnostic tools. Immune profile may be crucial for tracking the effectiveness of PTB treatment particularly when sputum production becomes problematic for the clients. It was observed that CD244 expression in treatment naïve PTB patients strongly correlated negatively to 1T, 2T and 3T treatment phases, suggesting its potential as a marker for monitoring the progress of PTB treatment. The adoption and utilization of blood-based biomarkers and exhaustion markers for a comprehensive immune profiling approach represent significant strides in the fight against TB. Possibly, the integration of immuno-omics into TB management could be beneficial to patient care. Expanded immune profiling could provide additional details to unearthing potential immune biomarkers for TB diagnosis, treatment options and treatment outcome monitoring.

6.2 LIMITATIONS AND FUTURE STUDIES

The study's variances may have been influenced by patients' physiological differences, the length of time it took for their illness to manifest before visiting a physician, their use of self-medication prior to consulting a physician, and other confounders that the exclusion criteria were unable to rule out. The initial *M. tuberculosis* load was not captured since diagnosis was via GeneXpert. Therefore, a future study will be designed to follow newly diagnosed patients and will include microscopy to estimate the bacilli load prior to commencement of treatment, then through to completion, and targeting markers at both the transcription level and protein phase at various stages of PTB treatment. Only male participants could be included, and expanded study will pave way for gender inclusion.

REFERENCE:

Abdul, I. W., Ankamah, S., Iddrisu, A. K., & Danso, E. (2020). Space-time analysis and mapping of prevalence rate of tuberculosis in Ghana. *Scientific African*, *7*. https://doi.org/10.1016/j.sciaf.2020.e00307

Abebe, F. (2014). *The Role of T cells In Mucosal Immunity against Mycobacterium tuberculosis (Mtb) Infection : A Review of Current Understanding*. *5*(4). https://doi.org/10.4172/2155-9899.1000254

Acharya, S., Lumley, A. I., Zhang, L., Vausort, M., & Devaux, Y. (2023). GATA3 as a Blood-Based RNA Biomarker for Idiopathic Parkinson's Disease. *International Journal of Molecular Sciences*, *24*(12). https://doi.org/10.3390/ijms241210040

Adekambi, T., Ibegbu, C. C., Cagle, S., Kalokhe, A. S., Wang, Y. F., Hu, Y., Day, C. L., Ray, S. M., & Rengarajan, J. (2015). *Biomarkers on patient T cells diagnose active tuberculosis and monitor treatment response*. *125*(5), 1827–1838. https://doi.org/10.1172/JCI77990.TB

Ahmed, A. (2020). *Emerging patterns of regulatory T cell function in tuberculosis*. 273–287. https://doi.org/10.1111/cei.13488

Ahmed, M., Tezera, L. B., Elkington, P. T., & Leslie, A. J. (2022). The paradox of immune checkpoint inhibition re-activating tuberculosis. *European Respiratory Journal*, *60*(5). https://doi.org/10.1183/13993003.02512-2021

Al, I. K. E. T., Kontsevaya, I., Lange, C., Comella-del-barrio, P., Coarfa, C., Dinardo, A. R., Gillespie, S. H., Hauptmann, M., Leschczyk, C., Mandalakas, A. M., Martinecz, A., Merker, M., Niemann, S., Reimann, M., Rzhepishevska, O., Schaible, U. E., Scheu, K. M., & Schurr, E. (2021). *REVIEW Perspectives for systems biology in the management of tuberculosis*. https://doi.org/10.1183/16000617.0377-2020

Allie, N., Grivennikov, S. I., Keeton, R., Hsu, N. J., Bourigault, M. L., Court, N., Fremond, C., Yeremeev, V., Shebzukhov, Y., Ryffel, B., Nedospasov, S. A., Quesniaux, V. F. J., & Jacobs, M. (2013). Prominent role for T cell-derived Tumour Necrosis Factor for sustained control of Mycobacterium tuberculosis infection. *Scientific Reports*, *3*. https://doi.org/10.1038/srep01809

Alsayed, S. S. R., & Gunosewoyo, H. (2023). Tuberculosis: Pathogenesis, Current Treatment Regimens and New Drug Targets. *International Journal of Molecular Sciences*, *24*(6). https://doi.org/10.3390/ijms24065202

Alspach, E., Lussier, D. M., & Schreiber, R. D. (2019). Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. *Cold Spring Harbor Perspectives in Biology*, *11*(3), 1–20. https://doi.org/10.1101/cshperspect.a028480

Anbese, A. T., Egeta, G., Mesfin, F., & Sadore, A. A. (2021). *Determinants of Undernutrition among Adult Tuberculosis Patients Receiving Treatment in Public Health Institutions in Shashemane Town , Southern Ethiopia*.

Ashenafi, S., & Brighenti, S. (2022). Reinventing the human tuberculosis (TB) granuloma: Learning from the cancer field. *Frontiers in Immunology*, *13*(December), 1–19. https://doi.org/10.3389/fimmu.2022.1059725

Ayodele, S., Kumar, P., van Eyk, A., & Choonara, Y. E. (2023). Advances in immunomodulatory strategies for host-directed therapies in combating tuberculosis. *Biomedicine and Pharmacotherapy*, *162*(December 2022), 114588. https://doi.org/10.1016/j.biopha.2023.114588

Bai, W., & Ameyaw, E. K. (2024). Global, regional and national trends in tuberculosis incidence and main risk factors: a study using data from 2000 to 2021. *BMC Public Health*, *24*(1), 1–14. https://doi.org/10.1186/s12889-023-17495-6

Bengsch, B., Manne, S., Xu, W., & Harmon, S. (2017). *HHS Public Access*. *545*(7652), 60–65. https://doi.org/10.1038/nature22079.T-cell

Berns, S. A., Isakova, J. A., & Pekhtereva, P. I. (2022). *Therapeutic potential of interferon-gamma in tuberculosis*. *10*(1), 63–73.

Blank, C. U., Haining, W. N., Held, W., Hogan, P. G., Kallies, A., Lugli, E., Lynn, R. C., Philip, M., Rao, A., Restifo, N. P., Schietinger, A., Schumacher, T. N., Schwartzberg, P. L., Sharpe, A. H., Speiser, D. E., Wherry, E. J., Youngblood, B. A., & Zehn, D. (2019). Defining 'T cell exhaustion.' *Nature Reviews Immunology*, *19*(11), 665–674. https://doi.org/10.1038/s41577-019-0221-9

Brunell, A. E., Lahesmaa, R., Autio, A., & Thotakura, A. K. (2023). Exhausted T cells hijacking the cancer-immunity cycle: Assets and liabilities. *Frontiers in Immunology*, *14*(April), 1–13. https://doi.org/10.3389/fimmu.2023.1151632

Buchbinder, E. I., & Desai, A. (2016). *CTLA-4 and PD-1 Pathways*. *39*(1), 98–106. https://doi.org/10.1097/COC.0000000000000239

Bussi, C., & Gutierrez, M. G. (2019). Mycobacterium tuberculosis infection of host cells in space and time. *FEMS Microbiology Reviews*, *43*(4), 341–361. https://doi.org/10.1093/femsre/fuz006

Buzimkic, A., Heck, D. E., & Kim, H. D. (2021). *Risk management of infectious disease using multidimensional Omics : Molecular diagnostic and personal care of tuberculosis*. *15*(01), 1–4.

Campos-góngora, E., López-martínez, J., Huerta-oros, J., & Arredondo-, G. I. (n.d.). *Original Article Nutritional status evaluation and nutrient intake in adult patients with pulmonary tuberculosis and their contacts*. https://doi.org/10.3855/jidc.11267

Carabalí-Isajar, M. L., Rodríguez-Bejarano, O. H., Amado, T., Patarroyo, M. A., Izquierdo, M. A., Lutz, J. R., & Ocampo, M. (2023). Clinical manifestations and immune response to tuberculosis. *World Journal of Microbiology and Biotechnology*, *39*(8), 1–26. https://doi.org/10.1007/s11274-023-03636-x

Casadevall, A., & Pirofski, L. (2000). *MINIREVIEW Host-Pathogen Interactions : Basic Concepts of Microbial Commensalism , Colonization , Infection , and Disease*. *68*(12), 6511–6518.

Cells, C. A. R. T., Benmebarek, M., Karches, C. H., Cadilha, B. L., Lesch, S., Endres, S., & Kobold, S. (2019). *Killing Mechanisms of Chimeric Antigen Receptor*. https://doi.org/10.3390/ijms20061283

Chandra, P., Grigsby, S. J., & Philips, J. A. (2022). Immune evasion and provocation by Mycobacterium tuberculosis. *Nature Reviews Microbiology*, *20*(12), 750–766. https://doi.org/10.1038/s41579-022-00763-4

Chen, W. (2013). <*Imr12122.Pdf*>. *256*, 160–176.

Chen, Z., Wang, L., Gu, L., Qu, R., Lowrie, D. B., Hu, Z., Sha, W., & Fan, X. (2020). *Decreased Expression of CD69 on T Cells in Tuberculosis Infection Resisters*. *11*(August), 1–11. https://doi.org/10.3389/fmicb.2020.01901

Cicchese, et al. (2018). HHS Public Access. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1111/imr.12671.Dynamic

Cole, B., Nilsen, D. M., Will, L., Etkind, S. C., Burgos, M., & Chorba, T. (2020). Essential Components of a Public Health Tuberculosis Prevention , Control , and Elimination Program. *CDC Morbidity and Mortality Weekly Report*, *69*(7), 1–32. https://www.cdc.gov/mmwr/volumes/69/rr/rr6907a1.htm#:~:text=Ensuring access to laboratory and,disease and move toward elimination

Cooper, A. M. (2009). Cell-mediated immune responses in tuberculosis. *Annual Review of Immunology*, *27*, 393–422. https://doi.org/10.1146/annurev.immunol.021908.132703

Cooper, A. M., Mayer-Barber, K. D., & Sher, A. (2011). Role of innate cytokines in mycobacterial infection. *Mucosal Immunology*, *4*(3), 252–260. https://doi.org/10.1038/mi.2011.13

Cronan, M. R. (2022). In the Thick of It: Formation of the Tuberculous Granuloma and Its Effects on Host and Therapeutic Responses. *Frontiers in Immunology*, *13*(March), 1–15. https://doi.org/10.3389/fimmu.2022.820134

Damsker, J. M., Hansen, A. M., & Caspi, R. R. (2010). *Author Manuscript*. 211–221. https://doi.org/10.1111/j.1749-6632.2009.05133.x.Th1

Dara, M., Acosta, C. D., Rusovich, V., Zellweger, J. P., Centis, R., & Migliori, G. B. (2014). Bacille Calmette-Guérin vaccination: The current situation in Europe. *European Respiratory Journal*, *43*(1), 24–35. https://doi.org/10.1183/09031936.00113413

Das, M., Zhu, C., & Kuchroo, V. K. (2017). Tim-3 and its role in regulating anti-tumor immunity. *Immunological Reviews*, *276*(1), 97–111. https://doi.org/10.1111/imr.12520

Davies, L. R. L., Smith, M. T., Cizmeci, D., Fischinger, S., Lee, J. S., Lu, L. L., Layton, E. D., Grant, A. D., Fielding, K., Stein, C. M., Boom, W. H., Hawn, T. R., Fortune, S. M., Wallis, R. S., & Churchyard, G. J. (2023). Articles IFN- γ independent markers of Mycobacterium tuberculosis exposure among male South African gold miners. *EBioMedicine*, *93*, 104678. https://doi.org/10.1016/j.ebiom.2023.104678

De Oyarzabal, E., García-García, L., Rangel-Escareño, C., Ferreyra-Reyes, L., Orozco, L., Herrera, M. T., Carranza, C., Sada, E., Juárez, E., Ponce-De-León, A., Sifuentes-Osornio, J., Wilkinson, R. J., & Torres, M. (2019). Expression of USP18 and IL2RA Is Increased in Individuals Receiving Latent Tuberculosis Treatment with Isoniazid. *Journal of Immunology Research*, *2019*. https://doi.org/10.1155/2019/1297131

Díaz-fernández, S., Villar-hernández, R., Stojanovic, Z., Fernández, M., Tolosa, G., Romero, I., Sabrià, J., Prat, C., Domínguez, J., & Latorre, I. (2022). *and Ki-67 immune profiles for the characterization of active tuberculosis , latent infection and end of treatment*. *July*, 1–12. https://doi.org/10.3389/fmicb.2022.885312

Dis, E. Van, Fox, D. M., Morrison, H. M., Fines, D. M., Babirye, J. P., McCann, L. H., Rawal, S., Cox, J. S., & Stanley, S. A. (2022). IFN-γ-independent control of M. tuberculosis requires CD4 T cell-derived GM-CSF and activation of HIF-1α. *PLoS Pathogens*, *18*(7), 1–23. https://doi.org/10.1371/journal.ppat.1010721

Dunlap, M. D., Prince, O. A., Rangel-Moreno, J., Thomas, K. A., Scordo, J. M., Torrelles, J. B., Cox, J., Steyn, A. J. C., Zúñiga, J., Kaushal, D., & Khader, S. A. (2020). Formation of Lung Inducible Bronchus Associated Lymphoid Tissue Is Regulated by Mycobacterium tuberculosis Expressed Determinants. *Frontiers in Immunology*, *11*(June), 1–15. https://doi.org/10.3389/fimmu.2020.01325

Dyatlov, A. V., Apt, A. S., & Linge, I. A. (2019). B lymphocytes in anti-mycobacterial immune responses: Pathogenesis or protection? *Tuberculosis*, *114*(August 2018), 1–8. https://doi.org/10.1016/j.tube.2018.10.011

Elkington, P., Polak, M. E., Reichmann, M. T., & Leslie, A. (2022). Understanding the tuberculosis granuloma: the matrix revolutions. *Trends in Molecular Medicine*, *28*(2), 143–154. https://doi.org/10.1016/j.molmed.2021.11.004

Fenwick, C., Joo, V., Jacquier, P., Noto, A., Banga, R., Perreau, M., & Pantaleo, G. (2019). *T-cell exhaustion in HIV infection*. *November*, 149–163. https://doi.org/10.1111/imr.12823

Gao, Z., Feng, Y., Xu, J., & Liang, J. (2022). T-cell exhaustion in immune-mediated inflammatory diseases: New implications for immunotherapy. *Frontiers in Immunology*, *13*(September), 1–13. https://doi.org/10.3389/fimmu.2022.977394

Geneva, W. H. O. (2021). Guidance on regulations for the Transport of Infectious Substances the Transport of Infectious Substances. In *Oms* (Issue January).

Gertel, S. (2021). *3 + T cells are decreased in active psoriatic arthritis patients and their restoration in vitro is mediated by TNF inhibitors*. *April*, 173–183. https://doi.org/10.1111/cei.13646

Ghosh, C., Luong, G., & Sun, Y. (2021). A snapshot of the PD-1/PD-L1 pathway. *Journal of Cancer*, *12*(9), 2735–2746. https://doi.org/10.7150/JCA.57334

Ghoshal, A., Verma, A., Bhaskar, A., & Dwivedi, V. P. (2024). The uncharted territory of host-pathogen interaction in tuberculosis. *Frontiers in Immunology*, *15*(January), 1–16. https://doi.org/10.3389/fimmu.2024.1339467

Goletti, D., Petruccioli, E., Joosten, S. A., & Ottenhoff, T. H. M. (2016). Tuberculosis biomarkers: From diagnosis to protection. *Infectious Disease Reports*, *8*(2), 24–32. https://doi.org/10.4081/idr.2016.6568

Grebenciucova, E., & VanHaerents, S. (2023). Interleukin 6: at the interface of human health and disease. *Frontiers in Immunology*, *14*(September), 1–10. https://doi.org/10.3389/fimmu.2023.1255533

Gupta, P. K., Godec, J., Wolski, D., Adland, E., Yates, K., Pauken, K. E., Cosgrove, C., Ledderose, C., Junger, W. G., Robson, S. C., Wherry, E. J., Alter, G., Goulder, P. J. R., Klenerman, P., Sharpe, A. H., Lauer, G. M., & Haining, W. N. (2015). CD39 Expression Identifies Terminally Exhausted CD8+ T Cells. *PLoS Pathogens*, *11*(10), 1–21. https://doi.org/10.1371/journal.ppat.1005177

Hargreaves, J. R., Boccia, D., Evans, C. A., Adato, M., Petticrew, M., & Porter, J. D. H. (2011). The social determinants of tuberculosis: from evidence to action. *American Journal of Public Health*, *101*(4), 654–662. https://doi.org/10.2105/AJPH.2010.199505

Harris, J., & Keane, J. (2010). How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clinical and Experimental Immunology*, *161*(1), 1–9. https://doi.org/10.1111/j.1365-2249.2010.04146.x

Hashimoto, M., Kamphorst, A. O., Im, S. J., Kissick, H. T., Pillai, R. N., Ramalingam, S. S., Araki, K., & Ahmed, R. (2018). *CD8 T Cell Exhaustion in Chronic Infection and Cancer : Opportunities for Interventions*. 301–318.

Heidary, M., Shirani, M., Moradi, M., Goudarzi, M., Pouriran, R., Rezaeian, T., & Khoshnood, S. (2022). Tuberculosis challenges: Resistance, co-infection, diagnosis, and treatment. *European Journal of Microbiology and Immunology*, *12*(1), 1–17. https://doi.org/10.1556/1886.2021.00021

Henao-Tamayo, M., Irwin, S. M., Shang, S., Ordway, D., & Orme, I. M. (2011). T lymphocyte surface expression of exhaustion markers as biomarkers of the efficacy of chemotherapy for tuberculosis. *Tuberculosis*, *91*(4), 308–313. https://doi.org/10.1016/j.tube.2011.04.001

Hirsch, C. S., Toossi, Z., Othieno, C., Johnson, J. L., Schwander, S. K., Robertson, S., Wallis, R. S., Edmonds, K., Okwera, A., Mugerwa, R., Peters, P., & Ellner, J. J. (1999). Depressed T-cell interferon-γ responses in pulmonary tuberculosis: Analysis of underlying mechanisms and modulation with therapy. *Journal of Infectious Diseases*, *180*(6), 2069–2073. https://doi.org/10.1086/315114

Hougardy, J. M., Place, S., Hildebrand, M., Drowart, A., Debrie, A. S., Locht, C., & Mascart, F. (2007). Regulatory T cells depress immune responses to protective antigens in active tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, *176*(4), 409–416. https://doi.org/10.1164/rccm.200701-084OC

Id, Z. S., Tezera, R., Mariam, D. H., Collins, J., & Ali, H. (2021). *in Addis Ababa , Ethiopia : An ambi-directional cohort study*. *184*, 1–14. https://doi.org/10.1371/journal.pone.0247945

Iddrisu, A., Amikiya, E. A., & Bukari, F. K. (2024). *Spatio-temporal characteristics of Tuberculosis in Ghana [version 4 ; peer review : 1 approved , 3 approved with reservations]*. 1–30.

Infections, R. V. (2023). *Respiratory Viral Infections*. 1–15.

Inoue, S., Bo, L., Bian, J., Unsinger, J., Chang, K., & Hotchkiss, R. S. (2011). Dose-dependent effect of anti-CTLA-4 on survival in sepsis. *Shock*, *36*(1), 38–44. https://doi.org/10.1097/SHK.0b013e3182168cce

Ivashkiv, L. B., Program, T. D., Rosensweig, D. Z., Program, M. P., & Medicine, W. C. (2019).

HHS Public Access. *18*(9), 545–558. https://doi.org/10.1038/s41577-018-0029-z.IFN

Jiang, Y., Li, Y., & Zhu, B. (2015). T-cell exhaustion in the tumor microenvironment. *Cell Death and Disease*, *6*(6), 1–9. https://doi.org/10.1038/cddis.2015.162

Jubel, J. M., Barbati, Z. R., Burger, C., Wirtz, D. C., & Schildberg, F. A. (2020). The Role of PD-1 in Acute and Chronic Infection. *Frontiers in Immunology*, *11*(March), 1–15. https://doi.org/10.3389/fimmu.2020.00487

Kahan, S. M., Wherry, E. J., & Zajac, A. J. (2015). T cell exhaustion during persistent viral infections. *Virology*, *479–480*, 180–193. https://doi.org/10.1016/j.virol.2014.12.033

Katrachanca, S. M., & Koleske, A. J. (2017). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1007/s00281-015-0490-8.CD8

Kaufmann, S. H. E., Aiello, A., Naja, S., Goletti, D., Gutie, L. H., & Hilligan, K. L. (2023). *Initial immune response after exposure to Mycobacterium tuberculosis or to SARS-COV-2 : similarities and differences*. *August*, 1–24. https://doi.org/10.3389/fimmu.2023.1244556

Kennedy, A., Robinson, M. A., Hinze, C., Waters, E., Williams, C., Halliday, N., Dovedi, S., & Sansom, D. M. (2023). The CTLA -4 immune checkpoint protein regulates PD-L1 : PD -1 interaction via transendocytosis of its ligand CD80 . *The EMBO Journal*, *42*(5), 1–18. https://doi.org/10.15252/embj.2022111556

Kerantzas, C. A., & Jacobs, W. R. (2017). Origins of combination therapy for tuberculosis: Lessons for future antimicrobial development and application. *MBio*, *8*(2), 1–10. https://doi.org/10.1128/mBio.01586-16

Khadka, P., Dummer, J., Hill, P. C., Katare, R., & Das, S. C. (2023). A review of formulations and preclinical studies of inhaled rifampicin for its clinical translation. *Drug Delivery and Translational Research*, 1246–1271. https://doi.org/10.1007/s13346-022-01238-y

Koch, A., Cox, H., & Mizrahi, V. (2018). Drug-resistant tuberculosis: challenges and opportunities for diagnosis and treatment. *Current Opinion in Pharmacology*, *42*, 7–15. https://doi.org/10.1016/j.coph.2018.05.013

Kotzé, L. A., Young, C., Leukes, V. N., John, V., Fang, Z., Walzl, G., Lutz, M. B., & du Plessis, N. (2020). Mycobacterium tuberculosis and myeloid-derived suppressor cells: Insights into caveolin rich lipid rafts. *EBioMedicine*, *53*. https://doi.org/10.1016/j.ebiom.2020.102670

Kurd, N., Robey, E. A., & Biology, C. (2017). *perspective*. *271*(1), 114–126. https://doi.org/10.1111/imr.12398.T

Kwabla, M. P., Amuasi, J. H., Krause, G., Dormechele, W., Takramah, W., Kye-Duodu, G., Osei, E., Der, J., Baiden, F., Adusi-Poku, Y., Binka, F. N., & Castell, S. (2025). Completeness of tuberculosis case notification in Ghana: record linkage and capture-recapture analysis of three TB registries. *BMC Infectious Diseases*, *25*(1), 1–10. https://doi.org/10.1186/s12879-025-10622-1

Lara-Espinosa, J. V., Santana-Martínez, R. A., Maldonado, P. D., Zetter, M., Becerril-Villanueva, E., Pérez-Sánchez, G., Pavón, L., Mata-Espinosa, D., Barrios-Payán, J., López-Torres, M. O., Marquina-Castillo, B., & Hernández-Pando, R. (2020). Experimental

pulmonary tuberculosis in the absence of detectable brain infection induces neuroinflammation and behavioural abnormalities in male balb/c mice. *International Journal of Molecular Sciences*, *21*(24), 1–32. https://doi.org/10.3390/ijms21249483

Laufer, N., Ben, G., Ameri, D., Gonzalez, D., Parodi, L. M., Giavedoni, L. D., Maidana, P., Fabre, B., Mesch, V., Sued, O., Quiroga, M. F., Aires, D. B., Program, I., National, S., & Antonio, S. (2022). *HHS Public Access*. 1–27. https://doi.org/10.1016/j.tube.2020.102045.Immune

Li, L. S., Yang, L., Zhuang, L., Ye, Z. Y., Zhao, W. G., & Gong, W. P. (2023). From immunology to artificial intelligence : revolutionizing latent tuberculosis infection diagnosis with machine learning. *Military Medical Research*, 1–37. https://doi.org/10.1186/s40779-023-00490-8

Lin, P. L., & Flynn, J. L. (2015). *CD8 T cells and Mycobacterium tuberculosis infection*. 239–249. https://doi.org/10.1007/s00281-015-0490-8

Llibre, A., Smith, N., Rouilly, V., Musvosvi, M., Nemes, E., Posseme, C., Mabwe, S., Charbit, B., Mbandi, S. K., Filander, E., Africa, H., Saint-André, V., Bondet, V., Bost, P., Mulenga, H., Bilek, N., Albert, M. L., Scriba, T. J., & Duffy, D. (2022). Tuberculosis alters immune-metabolic pathways resulting in perturbed IL-1 responses. *Frontiers in Immunology*, *13*(December), 897193. https://doi.org/10.3389/fimmu.2022.897193

Lo, D., Mantel, Y., & Lo, D. (2020). *Granzyme B Attenuates Bacterial Virulence by Targeting Secreted Factors Killer lymphocyte Secre on systems proteins Granzyme B Attenuates Bacterial Virulence by Targeting Secreted Factors*. https://doi.org/10.1016/j.isci.2020.100932

Lombardi, A., Villa, S., Castelli, V., Bandera, A., & Gori, A. (2021). T-cell exhaustion in mycobacterium tuberculosis and nontuberculous mycobacteria infection: Pathophysiology and therapeutic perspectives. *Microorganisms*, *9*(12). https://doi.org/10.3390/microorganisms9122460

Luckheeram, R. V., Zhou, R., Verma, A. D., & Xia, B. (2012). CD4 +T cells: Differentiation and functions. *Clinical and Developmental Immunology*, *2012*. https://doi.org/10.1155/2012/925135

Lyadova, I. V, & Panteleev, A. V. (2015). *Th1 and Th17 Cells in Tuberculosis : Protection , Pathology , and Biomarkers*. *2015*. https://doi.org/10.1155/2015/854507

Mabwe, S., Jaxa, L., Charbit, B., Musvosvi, M., Necker, H., Alcover, A., Aschard, H., Astrom, K., Santo, J. Di, Dromer, F., Eberl, G., Enninga, J., & Epfl, J. F. (2019). *active disease*.

Mackie, K. and H.-C. L. (2017). Coregulation of CD8+ T cell exhaustion during chronic viral infection by multiple inhibitory receptors. *Physiology & Behavior*, *176*(1), 139–148. https://doi.org/10.1038/ni.1679.Coregulation

Maeurer, M. J., Trinder, P., Hommel, G., Walter, W., Freitag, K., Atkins, D., & Sto, S. (2000). *Interleukin-7 or Interleukin-15 Enhances Survival of Mycobacterium tuberculosis -Infected Mice*. *68*(5), 2962–2970.

Mahdavi, A., Hajighasemi, S., Kiaie, N., Mohammad, S., Hayat, G., Jamialahmadi, T., Johnston,

T. P., & Sahebkar, A. (2020). The pivotal role of CD69 in autoimmunity. *Journal of Autoimmunity*, *March*, 102453. https://doi.org/10.1016/j.jaut.2020.102453

Maison, D. P. (2022). Journal of Clinical Tuberculosis and Other Mycobacterial Diseases Tuberculosis pathophysiology and anti-VEGF intervention. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, *27*(January), 100300. https://doi.org/10.1016/j.jctube.2022.100300

Manuscript, A. (2013). *Early T-cell responses in tuberculosis immunity*. *2*, 1–25. https://doi.org/10.1111/j.1600-065X.2008.00693.x.Early

Maphasa, R. E., Meyer, M., & Dube, A. (2021). The Macrophage Response to Mycobacterium tuberculosis and Opportunities for Autophagy Inducing Nanomedicines for Tuberculosis Therapy. *Frontiers in Cellular and Infection Microbiology*, *10*(February), 1–22. https://doi.org/10.3389/fcimb.2020.618414

Marshall, J. S., Warrington, R., Watson, W., & Kim, H. L. (2018). An introduction to immunology and immunopathology. *Allergy, Asthma & Clinical Immunology*, *14*(s2), 1–10. https://doi.org/10.1186/s13223-018-0278-1

Martino, M. De, Lodi, L., Galli, L., & Chiappini, E. (2019). *Immune Response to Mycobacterium tuberculosis : A Narrative Review*. *7*(August), 1–8. https://doi.org/10.3389/fped.2019.00350

Mhatre V. Ho, Ji-Ann Lee, and K. C. M., & Craik, C. (2012). 基因的改变NIH Public Access. *Bone*, *23*(1), 1–7. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

Ministry of Health, Ghana Health Service, USAID, & Systems for Health. (2015). *National Policy and Guidelines for Infection Prevention and Control in Health Care Settings*. 1–143.

Mogues, T., Goodrich, M. E., Ryan, L., LaCourse, R., & North, R. J. (2001). The relative importance of T cell subsets in immunity and immunopathology of airborne Mycobacterium tuberculosis infection in mice. *Journal of Experimental Medicine*, *193*(3), 271–280. https://doi.org/10.1084/jem.193.3.271

Moule, M. G., & Cirillo, J. D. (2020). Mycobacterium tuberculosis Dissemination Plays a Critical Role in Pathogenesis. *Frontiers in Cellular and Infection Microbiology*, *10*(February), 1–12. https://doi.org/10.3389/fcimb.2020.00065

Ng, T. H. S., Britton, G. J., Hill, E. V., Verhagen, J., Burton, B. R., & Wraith, D. C. (2013). Regulation of adaptive immunity; the role of interleukin-10. *Frontiers in Immunology*, *4*(MAY), 1–13. https://doi.org/10.3389/fimmu.2013.00129

O'Garra, A., Redford, P. S., McNab, F. W., Bloom, C. I., Wilkinson, R. J., & Berry, M. P. R. (2013). The immune response in tuberculosis. In *Annual Review of Immunology* (Vol. 31). https://doi.org/10.1146/annurev-immunol-032712-095939

Orme, I. M. (2016). Immunology of Tuberculosis. *Encyclopedia of Immunobiology*, *4*, 26–33. https://doi.org/10.1016/B978-0-12-374279-7.13005-X

Owusu, A. A., Poku, P. A., Ayim, A., Arhin, J. K., Armah, R. N., & Yawson, A. E. (2024).

Evaluation of tuberculosis surveillance system in a municipality in Ghana during the COVID-19 pandemic: a cross-sectional study. *Pan African Medical Journal*, *48*. https://doi.org/10.11604/pamj.2024.48.170.40303

Pagán, A. J., & Ramakrishnan, L. (2015). Immunity and immunopathology in the tuberculous granuloma. *Cold Spring Harbor Perspectives in Medicine*, *5*(9), 1–19. https://doi.org/10.1101/cshperspect.a018499

Paley, M. A., Kroy, D. C., Odorizzi, P. M., Johnnidis, J. B., Dolfi, V., Barnett, B. E., Bikoff, E. K., Robertson, E. J., Georg, M., Reiner, S. L., & Wherry, E. J. (2013). *NIH Public Access*. *338*(6111), 1220–1225. https://doi.org/10.1126/science.1229620.Progenitor

Pandey, S. (2019). *Indian Journal of. January*. https://doi.org/10.4103/IJCFM.IJCFM

Panetti, C., Kao, K. C., & Joller, N. (2022). Dampening antiviral immunity can protect the host. *FEBS Journal*, *289*(3), 634–646. https://doi.org/10.1111/febs.15756

Patil, K., Bagade, S., Bonde, S., Sharma, S., & Saraogi, G. (2018). Recent therapeutic approaches for the management of tuberculosis: Challenges and opportunities. *Biomedicine and Pharmacotherapy*, *99*(January), 735–745. https://doi.org/10.1016/j.biopha.2018.01.115

Perry, J. S. A. (2018). *The effects of interleukin-2 on immune response regulation*. *35*(1), 79–119. https://doi.org/10.1093/imammb/dqw021.The

Persons, H.--coinfected, Esmail, H., Riou, C., Bruyn, E., Lai, R. P., Harley, Y. X. R., Meintjes, G., Wilkinson, K. A., & Wilkinson, R. J. (2018). *The Immune Response to Mycobacterium tuberculosis in*. *January*, 1–36.

Phillips, B. L., Mehra, S., Ahsan, M. H., Selman, M., Khader, S. A., & Kaushal, D. (2015). LAG3 Expression in Active Mycobacterium tuberculosis Infections. *The American Journal of Pathology*, *185*(3), 820–833. https://doi.org/10.1016/j.ajpath.2014.11.003

Raghav, K., Anand, S., Gothwal, A., Singh, P., Dasari, A., Overman, M. J., & Loree, J. M. (2021). Underreporting of race/ethnicity in COVID-19 research. *International Journal of Infectious Diseases*, *108*, 419–421. https://doi.org/10.1016/j.ijid.2021.05.075

Rahman, T., Sobur, A., Islam, S., Ievy, S., & Ashour, H. M. (n.d.). *Zoonotic Diseases : Etiology , Impact , and Control*.

Rameshkumar, R. (n.d.). *Drug-Resistant Bacterial Infections in HIV Patients*. https://doi.org/10.5772/intechopen.78657

Receptor, C. (2020). *LAG-3.targeting*. 1–23. https://doi.org/10.1016/j.smim.2019.101305.Lymphocyte-Activation

Riches, J. C., Davies, J. K., McClanahan, F., Fatah, R., Iqbal, S., Agrawal, S., Ramsay, A. G., & Gribben, J. G. (2013). T cells from CLLpatients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood*, *121*(9), 1612–1621. https://doi.org/10.1182/blood-2012-09-457531

Rochman, Y., Spolski, R., & Leonard, W. J. (2010). *Id # 13-08 evaluation of*. *9*(7), 1–23. https://doi.org/10.1038/nri2580.New

Sa, Q., Woodward, J., Suzuki, Y., & Alerts, E. (2013). *This information is current as of December 2, 2013.* https://doi.org/10.4049/jimmunol.1202256

Saeidi, A., Zandi, K., Cheok, Y. Y., Saeidi, H., & Wong, W. F. (2018). *T-Cell Exhaustion in Chronic Infections : Reversing the State of Exhaustion and Reinvigorating Optimal Protective Immune Responses*. *9*(November), 1–12. https://doi.org/10.3389/fimmu.2018.02569

Sakuishi, K., Apetoh, L., Sullivan, J. M., Blazar, B. R., Kuchroo, V. K., & Anderson, A. C. (2010). Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *Journal of Experimental Medicine*, *207*(10), 2187–2194. https://doi.org/10.1084/jem.20100643

Sankar, P., & Mishra, B. B. (2023). Early innate cell interactions with Mycobacterium tuberculosis in protection and pathology of tuberculosis. *Frontiers in Immunology*, *14*(October), 1–21. https://doi.org/10.3389/fimmu.2023.1260859

Sansom, D. M. (2000). CD28, CTLA-4 and their ligands: Who does what and to whom? *Immunology*, *101*(2), 169–177. https://doi.org/10.1046/j.1365-2567.2000.00121.x

Sasindran, S. J., & Torrelles, J. B. (2011). Mycobacterium tuberculosis infection and inflammation: What is beneficial for the host and for the bacterium? *Frontiers in Microbiology*, *2*(JAN), 1–16. https://doi.org/10.3389/fmicb.2011.00002

Scale, S. R. (1998). *Ghana health*. *575*(2), 3–7. http://lacelula.udl.es/aprendre/casos/pdf/bipsi.pdf

Schluns, K. S., & Anthony, S. (2015). Emerging roles for IL-15 in the activation and function of T-cells during immune stimulation. *Research and Reports in Biology*, 25. https://doi.org/10.2147/rrb.s57685

Scriba, T. J., Coussens, A. K., & Fletcher, H. A. (2017). *Human Immunology of Tuberculosis*. https://doi.org/10.1128/microbiolspec.TBTB2-0016-2016.Correspondence

Shabariah, R., Farsida, Hatta, M., Patellongi, I., Idris, I., Santoso, A., Islam, A. A., Natzir, R., Wahyudin, B., Warsinggih, & Sanjoyo. (2022). Correlation between innate and adaptive immunity response in TB children post BCG vaccination. Is it effective or not?: Cross-sectional study. *Annals of Medicine and Surgery*, *77*(March), 103586. https://doi.org/10.1016/j.amsu.2022.103586

Shanmuganathan, G., Orujyan, D., Narinyan, W., Poladian, N., Dhama, S., Parthasarathy, A., Ha, A., Tran, D., Velpuri, P., Nguyen, K. H., & Venketaraman, V. (2022). *Role of Interferons in Mycobacterium tuberculosis Infection*. 788–796.

Shaukat, S. N., Eugenin, E., Nasir, F., Khanani, R., & Kazmi, S. U. (2023). Identification of immune biomarkers in recent active pulmonary tuberculosis. *Scientific Reports*, *13*(1), 1–15. https://doi.org/10.1038/s41598-023-38372-7

Shen, X., Wu, T., Ji, X., Yang, K., Wang, L., Peng, Y., Huang, G., Shen, H., & Sha, W. (2023). Mycobacterium tuberculosis infection depressed cytotoxic T cells activity owing to decreasing NKG2C and increasing NKG2A expression. *Molecular Immunology*, *162*(April), 133–142. https://doi.org/10.1016/j.molimm.2023.08.014

Sholeye, A. R., Williams, A. A., & Loots, D. T. (2022). *Tuberculous Granuloma : Emerging Insights From Proteomics and Metabolomics*. *13*(March), 1–16. https://doi.org/10.3389/fneur.2022.804838

Sia, J. K., & Rengarajan, J. (2019a). Immunology of Mycobacterium tuberculosis Infections . *Microbiology Spectrum*, *7*(4), 3–22. https://doi.org/10.1128/microbiolspec.gpp3-0022-2018

Sia, J. K., & Rengarajan, J. (2019b). Immunology of mycobacterium tuberculosis infections. *Gram-Positive Pathogens*, 1056–1086. https://doi.org/10.1128/9781683670131.ch64

Sigal, G. B., Segal, M. R., Mathew, A., Jarlsberg, L., Wang, M., Barbero, S., Small, N., Haynesworth, K., Davis, J. L., Weiner, M., Whitworth, W. C., Jacobs, J., Schorey, J., Lewinsohn, D. M., & Nahid, P. (2017). Biomarkers of Tuberculosis Severity and Treatment Effect: A Directed Screen of 70 Host Markers in a Randomized Clinical Trial. *EBioMedicine*, *25*, 112–121. https://doi.org/10.1016/j.ebiom.2017.10.018

Straif-bourgeois, S., & Ratard, R. (2014). *Infectious Disease Epidemiology*. https://doi.org/10.1007/978-0-387-09834-0

Sun, L., Gang, X., Li, Z., Zhao, X., Zhou, T., Zhang, S., & Wang, G. (2021). *Advances in Understanding the Roles of CD244 (SLAMF4) in Immune Regulation and Associated Diseases*. *12*(March), 1–10. https://doi.org/10.3389/fimmu.2021.648182

Téllez-Navarrete, N. A., Ramon-Luing, L. A., Muñoz-Torrico, M., Preciado-García, M., Medina-Quero, K., Hernandez-Pando, R., & Chavez-Galan, L. (2021). Anti-tuberculosis chemotherapy alters TNFR2 expression on CD4+ lymphocytes in both drug-sensitive and -resistant tuberculosis: however, only drug-resistant tuberculosis maintains a pro-inflammatory profile after a long time. *Molecular Medicine*, *27*(1). https://doi.org/10.1186/s10020-021-00320-4

Tezera, L. B., Bielecka, M. K., Ogongo, P., Walker, N. F., Ellis, M., Garay-Baquero, D. J., Thomas, K., Reichmann, M. T., Johnston, D. A., Wilkinson, K. A., Ahmed, M., Jogai, S., Jayasinghe, S. N., Wilkinson, R. J., Mansour, S., Thomas, G. J., Ottensmeier, C. H., Leslie, A., & Elkington, P. T. (2020). Anti-PD-1 immunotherapy leads to tuberculosis reactivation via dysregulation of TNF-α. *ELife*, *9*, 1–23. https://doi.org/10.7554/eLife.52668

Thu, V. T. A., Dat, L. Da, Jayanti, R. P., Trinh, H. K. T., Hung, T. M., Cho, Y. S., Long, N. P., & Shin, J. G. (2023). Advancing personalized medicine for tuberculosis through the application of immune profiling. *Frontiers in Cellular and Infection Microbiology*, *13*(February), 1–20. https://doi.org/10.3389/fcimb.2023.1108155

Tirore, L. L., Ersido, T., Handiso, T. B., & Areba, A. S. (2024). *Non-adherence to anti-tuberculosis treatment and associated factors among TB patients in public health facilities of Hossana town , Southern*. *March*, 1–9. https://doi.org/10.3389/fmed.2024.1360351

Tiwari, D., & Martineau, A. R. (2023a). Inflammation-mediated tissue damage in pulmonary tuberculosis and host-directed therapeutic strategies. *Seminars in Immunology*, *65*(December 2022), 101672. https://doi.org/10.1016/j.smim.2022.101672

Tiwari, D., & Martineau, A. R. (2023b). Seminars in Immunology Inflammation-mediated tissue damage in pulmonary tuberculosis and host-directed therapeutic strategies. *Seminars in*

Immunology, *65*(November 2022), 101672. https://doi.org/10.1016/j.smim.2022.101672

Tuberculosis, S. M. O. (n.d.). *Transmission and Pathogenesis of Tuberculosis*.

Turner, A. K., Begon, M., Jackson, J. A., Bradley, J. E., & Paterson, S. (2011). *Genetic Diversity in Cytokines Associated with Immune Variation and Resistance to Multiple Pathogens in a Natural Rodent Population*. *7*(10). https://doi.org/10.1371/journal.pgen.1002343

Ummah, M. S. (2019). No析Title. In *Sustainability (Switzerland)* (Vol. 11, Issue 1). http://scioteca.caf.com/bitstream/handle/123456789/1091/RED2017-Eng-8ene.pdf?sequence=12&isAllowed=y%0Ahttp://dx.doi.org/10.1016/j.regsciurbeco.2008.06.005%0Ahttps://www.researchgate.net/publication/305320484_SISTEM_PEMBETUNGAN_TERPUSAT_STRATEGI_MELESTARI

Vansteenkiste, J. F., Cho, B., Vanakesa, T., De Pas, T., Zielinski, M., Kim, M. S., Jassem, J., Yoshimura, M., Dahabreh, J., Nakayama, H., Havel, L., Kondo, H., Mitsudomi, T., Zarogoulidis, K., Gladkov, O. A., Spiessens, B., Brichard, V., Debruyne, C., Therasse, P., & Altorki, N. (2014). Magrit, a Double-Blind, Randomized, Placebo-Controlled Phase III Study to Assess the Efficacy of the Recmage-A3 + As15 Cancer Immunotherapeutic As Adjuvant Therapy in Patients with Resected Mage-A3-Positive Non-Small Cell Lung Cancer (Nsclc). *Annals of Oncology*, *25*(4), iv409. https://doi.org/10.1093/annonc/mdu347.1

Walker, J. M. (n.d.). *T-Cell Development IN Series Editor*.

Wang, Y., Zhong, H., Xie, X., Chen, C. Y., Huang, D., Shen, L., Zhang, H., & Chen, Z. W. (2015). *Long noncoding RNA derived from CD244 signaling epigenetically controls CD8 + T-cell immune responses in tuberculosis infection*. https://doi.org/10.1073/pnas.1501662112

Wherry, E. J. (2011). T cell exhaustion. *Nature Immunology*, *12*(6), 492–499. https://doi.org/10.1038/ni.2035

Wherry, E. J., & Kurachi, M. (2016). *HHS Public Access*. *15*(8), 486–499. https://doi.org/10.1038/nri3862

WHO, T. W. G. (2008). *Policy brief: Policy guidelines for collaborative TB and HIV services for injecting and other drug users*.

Wilson, M.S. , Metink-Kane, M. M. (2012). NIH Public Access. *Bone*, *23*(1), 1–7. https://doi.org/10.1111/j.1399-0004.2010.01592.x.Nature

Wong, E. A., Joslyn, L., Grant, N. L., Klein, E., Lin, L., & Kirschner, D. E. (2018). *crossm Low Levels of T Cell Exhaustion in Tuberculous Lung Granulomas*. *86*(9), 1–18.

Woodworth, J. S. M., & Behar, S. M. (2006). Mycobacterium tuberculosis-specific CD8+ T cells and their role in immunity. *Critical Reviews in Immunology*, *26*(4), 317–352. https://doi.org/10.1615/critrevimmunol.v26.i4.30

World Health Organization (WHO). (2022). *Tuberculosis Action Plan for the WHO European Region 2023–2030*. *September*, 1–16.

Yang, B., Wang, X., Jiang, J., & Cheng, X. (2013). *Involvement of CD244 in Regulating CD4 +*

T Cell Immunity in Patients with Active Tuberculosis. *8*(4). https://doi.org/10.1371/journal.pone.0063261

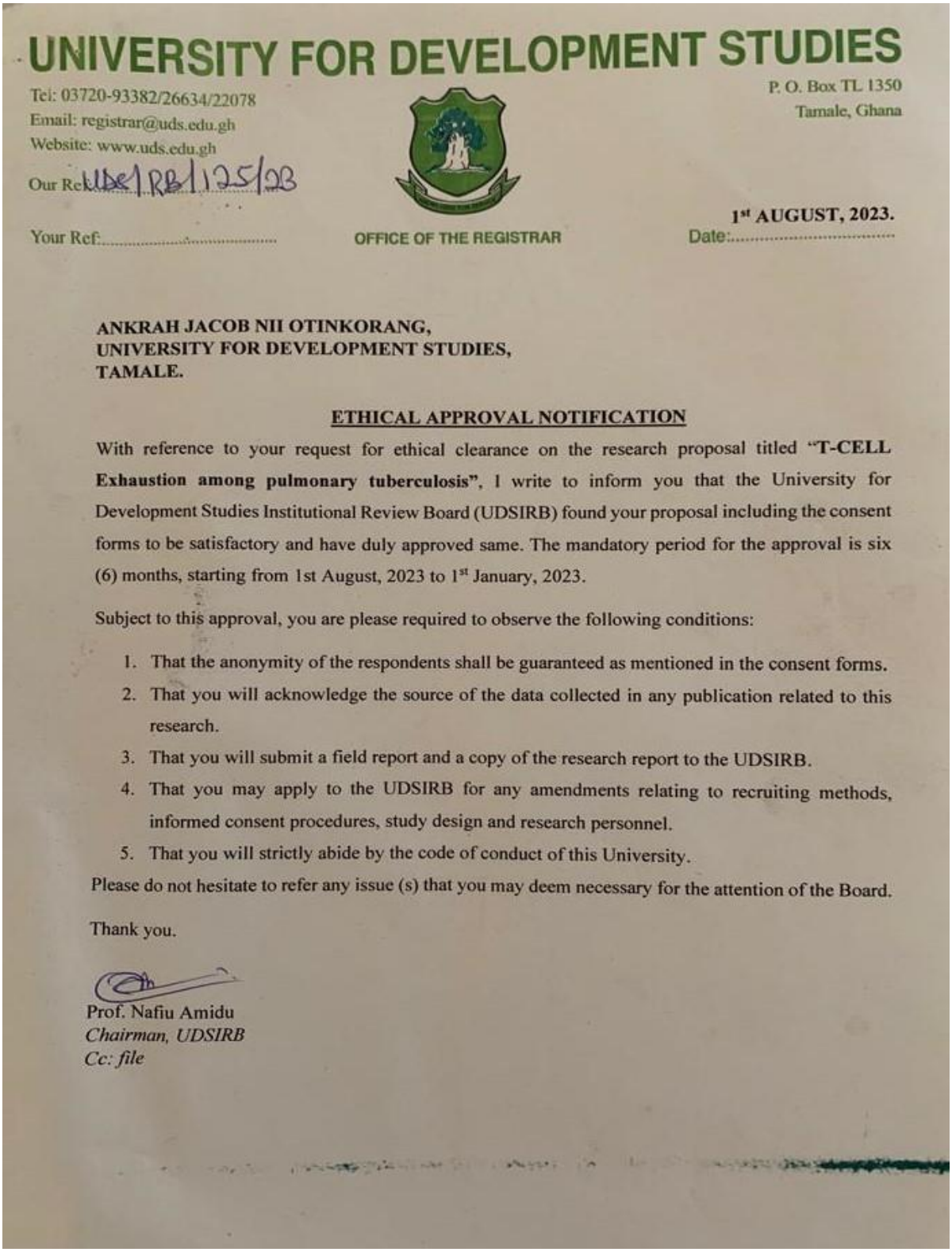
Yang, H., Ruan, X., Li, W., Xiong, J., & Zheng, Y. (2024). Global, regional, and national burden of tuberculosis and attributable risk factors for 204 countries and territories, 1990-2021: a systematic analysis for the Global Burden of Diseases 2021 study. *BMC Public Health*, *24*(1), 3111. https://doi.org/10.1186/s12889-024-20664-w

Yuk, J. M., Kim, J. K., Kim, I. S., & Jo, E. K. (2024). TNF in Human Tuberculosis: A Double-Edged Sword. *Immune Network*, *24*(1), 1–19. https://doi.org/10.4110/in.2024.24.e4

Zebley, C. C., & Youngblood, B. (2022). Mechanisms of T cell exhaustion guiding next-generation immunotherapy. *Trends in Cancer*, *8*(9), 726–734. https://doi.org/10.1016/j.trecan.2022.04.004

Zhang, H., Mi, J., Xin, Q., Cao, W., Song, C., Zhang, N., & Yuan, C. (2023). *Recent research and clinical progress of CTLA-4- based immunotherapy for breast cancer*. *2023*(October), 1–15. https://doi.org/10.3389/fonc.2023.1256360

APPENDIX I: ETHICAL APPROVAL



UNIVERSITY FOR DEVELOPMENT STUDIES

Tel: 03720-93382/26634/22078
Email: registrar@uds.edu.gh
Website: www.uds.edu.gh
Our Ref: UDS/RB/125/23

P. O. Box TL 1350
Tamale, Ghana

Your Ref:.....

OFFICE OF THE REGISTRAR

Date: 1st AUGUST, 2023.

ANKRAH JACOB NII OTINKORANG,
UNIVERSITY FOR DEVELOPMENT STUDIES,
TAMALE.

ETHICAL APPROVAL NOTIFICATION

With reference to your request for ethical clearance on the research proposal titled **"T-CELL Exhaustion among pulmonary tuberculosis"**, I write to inform you that the University for Development Studies Institutional Review Board (UDSIRB) found your proposal including the consent forms to be satisfactory and have duly approved same. The mandatory period for the approval is six (6) months, starting from 1st August, 2023 to 1st January, 2023.

Subject to this approval, you are please required to observe the following conditions:

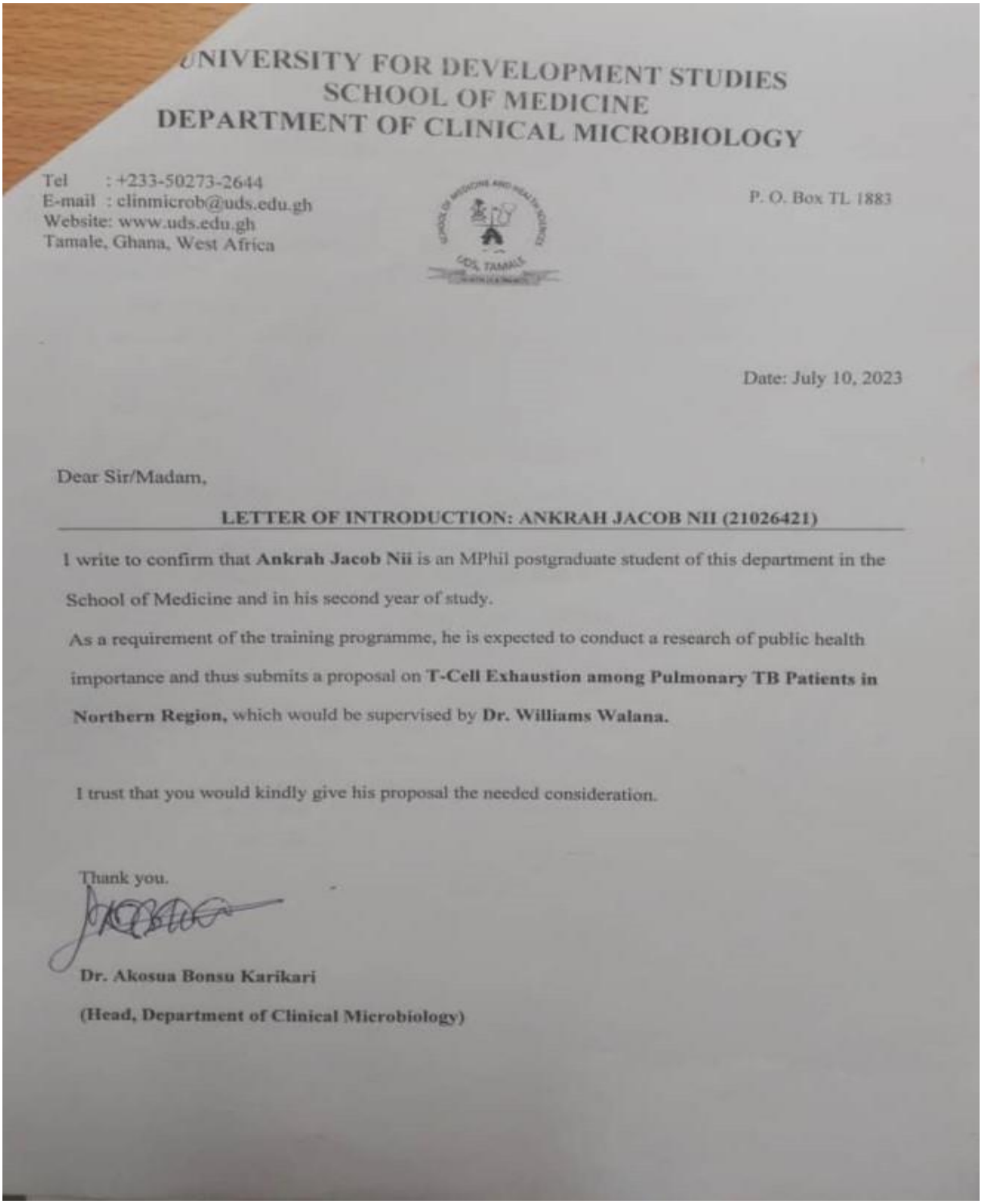
1. That the anonymity of the respondents shall be guaranteed as mentioned in the consent forms.
2. That you will acknowledge the source of the data collected in any publication related to this research.
3. That you will submit a field report and a copy of the research report to the UDSIRB.
4. That you may apply to the UDSIRB for any amendments relating to recruiting methods, informed consent procedures, study design and research personnel.
5. That you will strictly abide by the code of conduct of this University.

Please do not hesitate to refer any issue (s) that you may deem necessary for the attention of the Board.

Thank you.

Prof. Nafiu Amidu
Chairman, UDSIRB
Cc: file

APPENDIX I1: INTRODUCTORY LETTER



UNIVERSITY FOR DEVELOPMENT STUDIES
SCHOOL OF MEDICINE
DEPARTMENT OF CLINICAL MICROBIOLOGY

Tel : +233-50273-2644
E-mail : clinmicrob@uds.edu.gh
Website: www.uds.edu.gh
Tamale, Ghana, West Africa

P. O. Box TL 1883

Date: July 10, 2023

Dear Sir/Madam,

LETTER OF INTRODUCTION: ANKRAH JACOB NII (21026421)

I write to confirm that **Ankrah Jacob Nii** is an MPhil postgraduate student of this department in the School of Medicine and in his second year of study.

As a requirement of the training programme, he is expected to conduct a research of public health importance and thus submits a proposal on **T-Cell Exhaustion among Pulmonary TB Patients in Northern Region,** which would be supervised by **Dr. Williams Walana.**

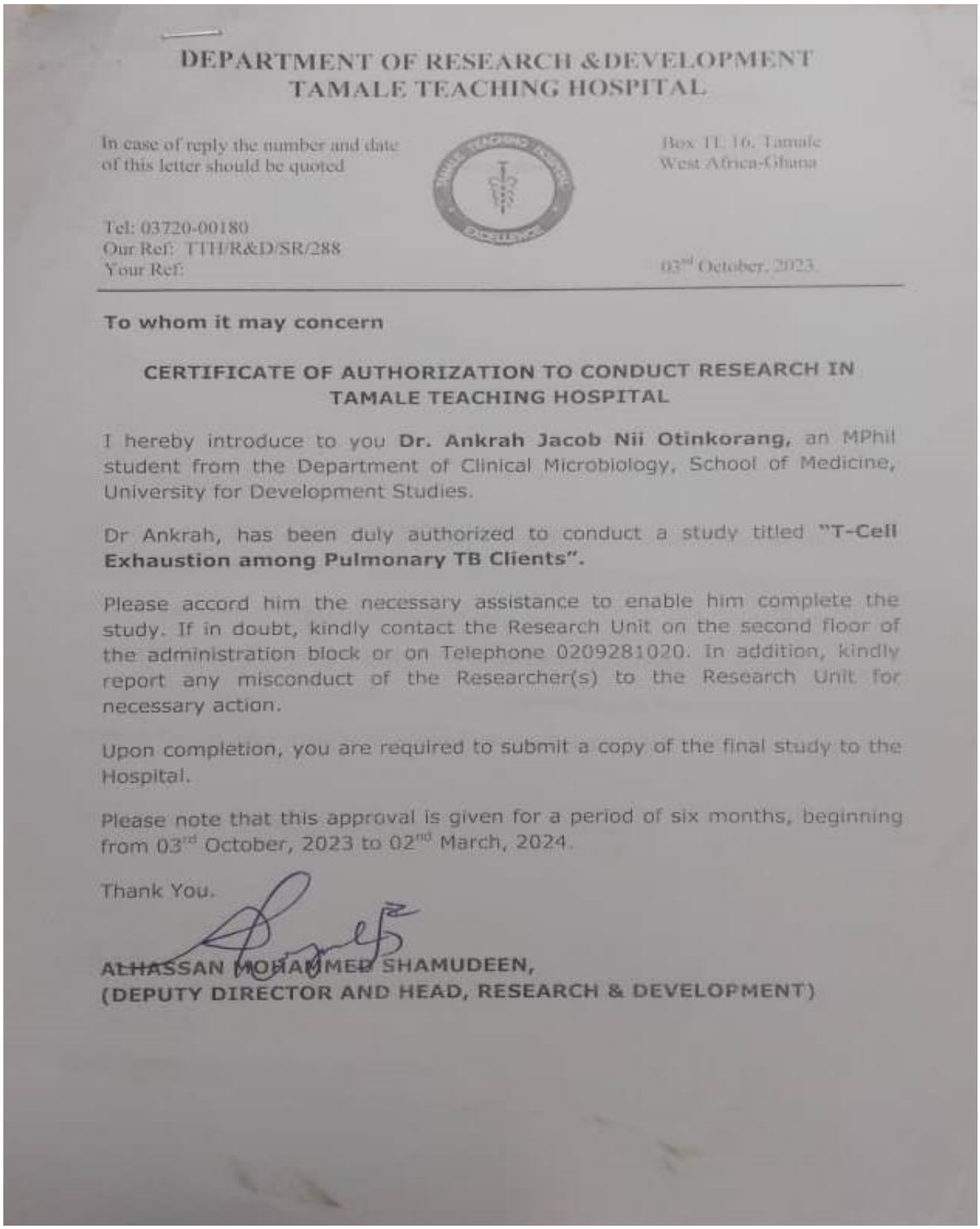
I trust that you would kindly give his proposal the needed consideration.

Thank you.

Dr. Akosua Bonsu Karikari

(Head, Department of Clinical Microbiology)

APPENDIX I1I: SITE PERMISSION



DEPARTMENT OF RESEARCH &DEVELOPMENT
TAMALE TEACHING HOSPITAL

In case of reply the number and date of this letter should be quoted

Box TL 16, Tamale
West Africa-Ghana

Tel: 03720-00180
Our Ref: TTH/R&D/SR/288
Your Ref:

03rd October, 2023.

To whom it may concern

CERTIFICATE OF AUTHORIZATION TO CONDUCT RESEARCH IN TAMALE TEACHING HOSPITAL

I hereby introduce to you **Dr. Ankrah Jacob Nii Otinkorang,** an MPhil student from the Department of Clinical Microbiology, School of Medicine, University for Development Studies.

Dr Ankrah, has been duly authorized to conduct a study titled **"T-Cell Exhaustion among Pulmonary TB Clients".**

Please accord him the necessary assistance to enable him complete the study. If in doubt, kindly contact the Research Unit on the second floor of the administration block or on Telephone 0209281020. In addition, kindly report any misconduct of the Researcher(s) to the Research Unit for necessary action.

Upon completion, you are required to submit a copy of the final study to the Hospital.

Please note that this approval is given for a period of six months, beginning from 03rd October, 2023 to 02nd March, 2024.

Thank You.

ALHASSAN MOHAMMED SHAMUDEEN,
(DEPUTY DIRECTOR AND HEAD, RESEARCH & DEVELOPMENT)

APPENDIX IV: CONSENT FORM FOR STUDY PARTICIPANTS

Form number……..

Project Title: T cell exhaustion among pulmonary tuberculosis clients in Northern Region-Ghana

Name and Address of Principal Investigator

Ankrah Jacob Nii Otinkorang, Department of Clinical Microbiology, School of Medicine and Health Sciences, University for Development Studies, Tamale.

Introduction

I am a student from the department of clinical microbiology, school of medicine and health science, university for development studies-Tamale conducting research on cell exhaustion among pulmonary tuberculosis clients in Northern Region. All information collected will be treated as confidential and no one will be able to trace any information back to you.

Procedure

The study is targeted at patients with pulmonary tuberculosis. Participation is voluntary. Participants will be made to complete a questionnaire and return it to the principal investigator. Participants will also be required to give a 5mL blood sample to the principal investigator.

Risks and Benefits

Participants may feel uncomfortable with some of the questions and also during the sample collection period, however, they will be helpful for the purpose of the research and may contribute to evidence that has the ability to alter treatment regimen for patients with chronic hepatitis b infection.

Right to refuse

Your consent to participate in this study is voluntary, you are not under any obligation to participate, and you are at liberty to withdraw from this study at any point in time. I will however appreciate it, if you could stay on till the completion of the study.

Anonymity and confidentiality

I assure you that any information given will be used purely for the purpose of this academic research. All information given will not be disclosed to anyone.

Voluntary agreement form for study participants

I have read and understood the content of this consent form. I have been given an opportunity to ask question(s) about the research. I agree to participate as a participant.

Name:……..……..

Signature: …… Date:……

Interviewer's statement

I ……., the undersigned, have explained to the subject in the language he/she understand and the participant has agreed to take part in the study.

Signature: …… Date:…….

APPENDIX V: QUESTIONNAIRE

Questionnaire on **T-Cell Exhaustion among pulmonary tuberculosis clients** in Northern Region-Ghana

Instructions

Please note that by completing this questionnaire you are voluntarily agreeing to participate in this research. You will remain anonymous and your data will be treated confidentially at all times. You may withdraw from this study at any time. Please complete the questionnaire in full. Mark in the appropriate response with a cross or write in the space provided. (√ means YES, X means NO)

Path. Number:…… Age:…….. Sex:… Date:……..

Duration of treatment

1. Yet to start treatment: YES { }, NO { }
2. ≤ 2 months on treatment (Intensive phase): YES { }, NO { }
3. >2 months and ≤ 5 months on treatment: YES { }, NO { }
4. 5 months & ≤ 6 months: YES { }, NO { }
5. >6 months (Cured): YES { }, NO { }
6. Weight: ……..kg
7. Height: ……..m
8. Waist Circumference: ..... cm

Other known Conditions

1. Hypertensive: YES { }, NO { }
2. Asthmatic : YES { }, NO { }
3. Diabetes: YES { }, NO { }
4. Sickle cell positive: YES { }, NO { }
5. Pregnancy: YES { }, NO { }
6. HBsAg: YES { }, NO { }
7. HCV: YES { }, NO { }
8. Retro: YES { }, NO { }
9. Others:……