
# A zebrafish model of tuberculosis comorbidity and the effects of HIF-activating intervention

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# A zebrafish model of tuberculosis comorbidity and the effects of HIF-activating intervention

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## Abstract

Comorbidities are an important factor in tuberculosis pathophysiology and treatment but are understudied in animal models. Schild, Mohamed, and Wootton *et al*. present a zebrafish model of *Mycobacterium marinum* infection and wound comorbidity that retains responsiveness to protective Hif-1α activation as an example of a host-directed therapy. This platform is a new paradigm for the zebrafish-*M. marinum* infection model and provides a blueprint to test therapeutic interventions on infection and comorbid pathologies.

## Abbreviations

COPD Chronic obstructive pulmonary disease
Hif Hypoxia-inducible factor
HIV human immunodeficiency virus
TB Tuberculosis

## Main

Infections by a single pathogen in a host without comorbidity in the form of chronic or underlying inflammatory diseases are the norm in biomedical infection models. Unfortunately, the prevalence of comorbidities confounding treatment of infectious disease is increasing worldwide and, notwithstanding this year's COVID-19 pandemic, is largely driven by the intersection of improved global living standards and endemic burdens of infectious disease. In the context of tuberculosis (TB), infection by human immunodeficiency virus (HIV) is classically recognised as the most important comorbidity of TB and can be largely blamed for the explosion in drug-resistant TB. Over the last decade of relatively successful antiviral therapies against HIV, diabetes and chronic obstructive pulmonary disease (COPD) have manifested as the most significant non-communicable disease comorbidities of TB [1]. Non-communicable disease comorbidities not only increase the risk of developing TB disease but can reduce the effectiveness of antibiotic therapy thus contributing to the development of antibiotic resistant *Mycobacterium tuberculosis*.

Neutrophils are the most numerous class of leukocyte in human blood and are critical for the acute response to infection [2]. However, there is increasing acceptance that their role in chronic *M. tuberculosis* infection is detrimental. Neutrophilic inflammation correlates with pulmonary inflammation and tissue destruction in advanced TB, and there is convincing evidence that strategies to reduce neutrophil influx is beneficial to the host during chronic TB [3].

Alteration to neutrophil function is a common feature of chronic diseases that are comorbid with TB. Cigarette smoking-induced conditions such as COPD have obvious inflammatory components, while metabolic diseases such as diabetes have more nuanced, but clinically important, effects on

immune responses. An effective immune response to mycobacterial infection requires optimal function of both obvious immune cell subsets such as neutrophils, as well as elements of the infection "stroma" that are particularly affected by diabetes including the haemostatic and vascular systems [4].

Beyond the well-recognised role in coordinating the cellular response to low oxygen tension, hypoxia-inducible factor (HIF in humans, Hif in zebrafish) signalling is critical to the immune response against *M. tuberculosis*, controlling immune cell production of and responsiveness to key cytokines, anti-microbial nitric oxide production, and the ability to engage aerobic glycolysis-dependent bactericidal programs [5, 6]. Conversely, HIF-1α is increased in TB cavitary disease where it drives tissue destruction [7]. Both COPD and diabetes are associated with dysregulation of HIF signalling where increased HIF signalling is implicated in inflammation-mediated lung damage in COPD and excessive neovascularisation resulting in diabetic vasculopathy [8, 9]. Together, these alterations have the potential to affect immunity to *M. tuberculosis* infection (Figure 1).

The zebrafish is an important platform for investigating vertebrate immunity, particularly via live imaging approaches that take advantage of the optical transparency of zebrafish embryos to visualise innate immune cell behaviours. This optical transparency has been instrumental to the application of zebrafish and *Mycobacterium marinum* infection system to decipher the early pathogenesis of TB [10]. Data from the zebrafish platform has demonstrated the value of Hif-centric host-directed therapies that directly stabilise Hif in immune cells or starve granulomas of vasculature, and thus oxygen [11, 12].

In this issue of FEBS J, Schild, Mohamed, and Wootton *et al*. report an elegant mycobacterial infection and wound comorbidity model [13]. They first establish that comorbidity worsens immune control of *M. marinum* infection and increases neutrophilic inflammation at the wound site, as expected from a "split" immune response in a hyperinflammatory background. Using live imaging, they find that when presented with the "choice" of lesions, neutrophils preferentially migrate to sites of *M. marinum* infection over relatively sterile wound sites and that this is driven by microbial signatures preserved in heat-killed *M. marinum* inocula (Figure 2). The authors make elegant use of photoconvertible neutrophil fluorescence to visualise the "redeployment" of wound-experienced neutrophils to *M. marinum* infection, and to demonstrate that neutrophils can have a short attention span as they compute and reprioritise inflammatory stimuli *in vivo*.

Although there is compelling evidence for a detrimental effect of wounding on control of an existing infection, the mechanism underlying this effect is not clear. Do neutrophils respond to the latest insult when wounding occurs after infection is established, thus depleting the total bactericidal capacity at the site of infection by diluting the focus of the immune system? The visual accessibility of the zebrafish embryo and the cell tracking tools used in this paper presents themselves as key tools to unravel this mechanism in future studies.

In the second half of their paper, Schild, Mohamed, and Wootton *et al*. then address the question of whether stabilisation of Hif-1α is still beneficial in their comorbidity model using a mix of chemical and genetic tools to globally effect Hif-1α activation. Hif-1α has anti-microbial effects and both pharmacological stabilization of Hif-1α and overexpression of an oxygen-stable form of Hif-1α ablated the increase in *M. marinum* burden caused by wounding. However, they found resolution of wound inflammation is delayed in Hif-1α-stabilised embryos regardless of infection status suggesting such an intervention risks exacerbation of the non-infectious pathology, consistent with their prior wound-only studies [14].

Much like the effects of Hif-1α stabilisation on neutrophil function, the simple nature of the zebrafish embryo *M. marinum* infection and tail wound assay is a double-edged sword. The comorbidity assay is clearly a powerful tool to examine leukocyte behaviour by live imaging and test potential therapies. However, the tail wound is an acute and self-limiting comorbidity that likely lacks features of important chronic comorbidities such as COPD and diabetes. Furthermore, *M. marinum* infection of zebrafish embryos has limited use as a model of chronic mycobacterial infection as embryos lack the adaptive immune cells necessary to sustain control of the infection. Fortunately, there is an ever-increasing catalogue of zebrafish disease models that can be "plugged into" this comorbid infection platform, including models of diabetes, and the adult zebrafish-*M. marinum* infection platform has been well described for studies of chronic infection and treatment.

TB remains the single biggest infectious disease killer across the world, assisted largely by comorbidities that exacerbate pathology and impede antibiotic treatment. The zebrafish-*M. marinum* infection system has been applied to study a vast range of fundamental and therapeutic aspects of TB. Schild, Mohamed, and Wootton *et al*. have thrust the zebrafish-*M. marinum* system into fight against TB comorbidities and provide a blueprint for studying the effects of therapeutic interventions on infection and comorbid pathology.

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Figure legends

Figure 1: The double-edged sword of HIF-1α stabilisation on neutrophil function.
Stabilisation of HIF-1α can have positive effects on neutrophil function against acute infection by increasing classical neutrophil functions. However, when these same functions cause collateral damage in the context of chronic inflammatory disease pathogenesis.

Figure 2: Neutrophil decision making in the comorbid model
A. When presented with a comorbid wound and infection, naïve neutrophils preferentially home to mycobacterial infection (red *M. marinum*). Naïve Hif-stabilised (yellow) neutrophils have an exaggerated response to infection, compared to naïve neutrophils, and do a better job of killing *M. marinum* once there,
B. Wound-experienced neutrophils are able to redeploy to a subsequent infection (red *M. marinum*). However, Hif-stabilised (yellow) wound-experienced neutrophils refuse to redeploy and largely remain at the wound site.

Graphical abstract

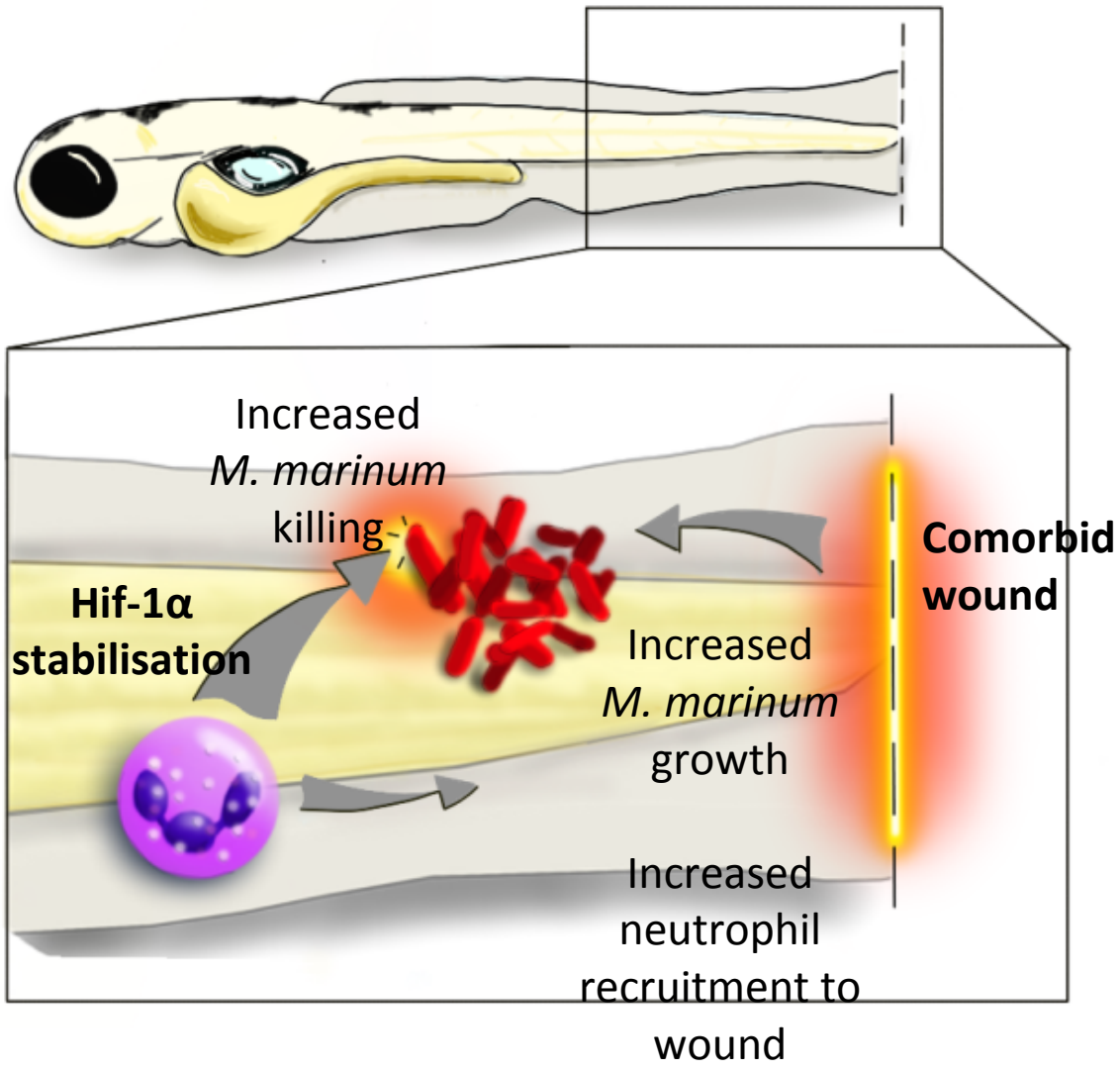
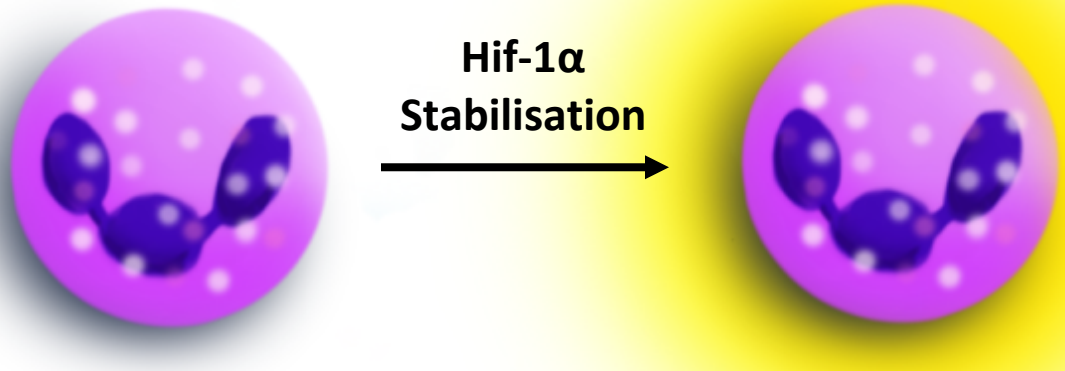


Figure 1



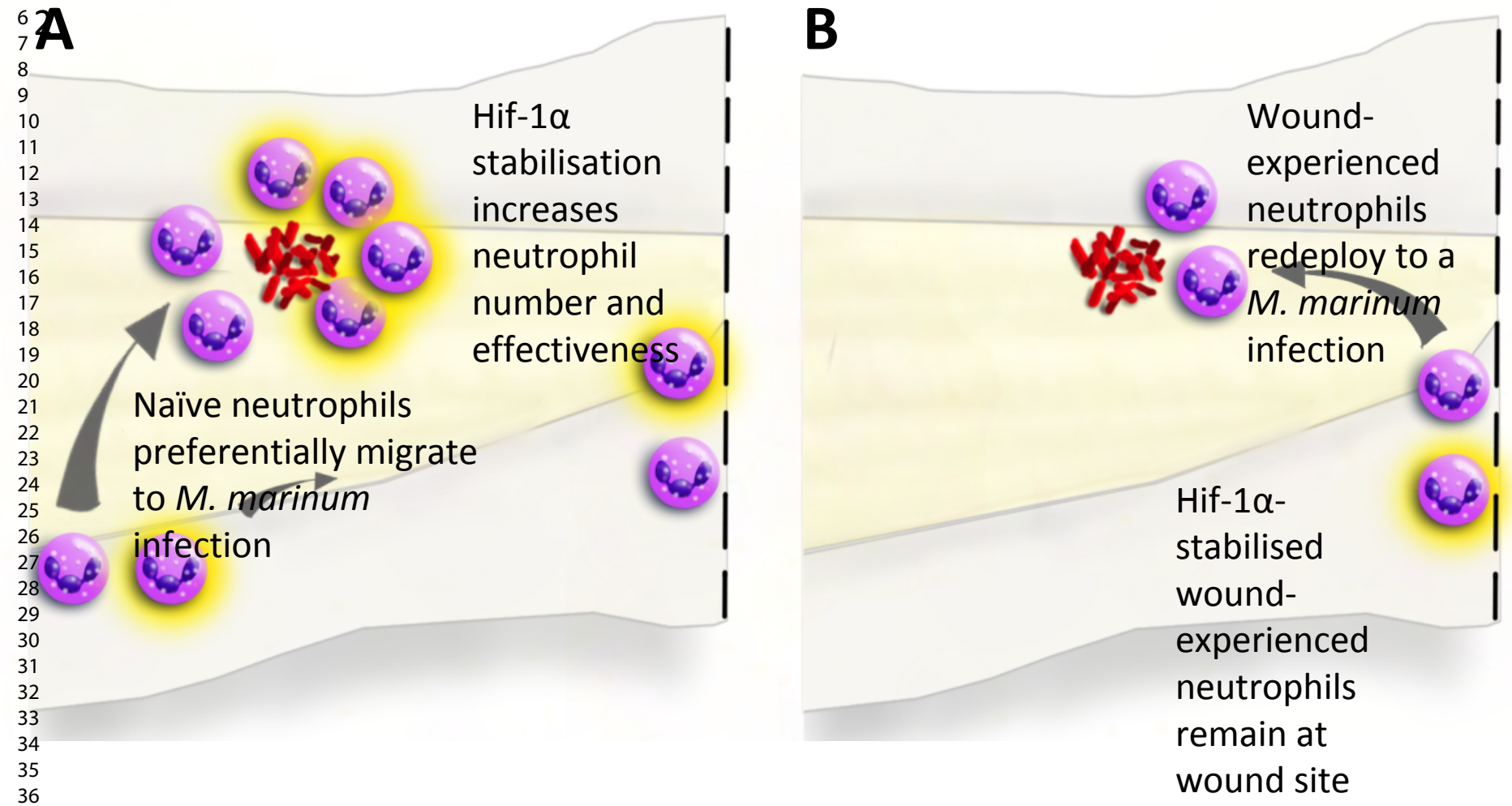

**Antimicrobial vs infection**

- Increased NO production
- Increased lifespan
- Increased aerobic glycolysis (energy for immune function)

**Collateral damage vs normal tissue**

- Delayed inflammatory resolution
- Increased neutrophil recruitment
- Increased neutrophil lifespan
- Increased degradative enzymes

Figure 2


A
Hif-1α stabilisation increases neutrophil number and effectiveness
Naïve neutrophils preferentially migrate to *M. marinum* infection
B
Wound-experienced neutrophils redeploy to a *M. marinum* infection
Hif-1α-stabilised wound-experienced neutrophils remain at wound site