Revisiting hypoxia therapies for tuberculosis

Stefan H Oehlers

Tuberculosis Research Program at the Centenary Institute, The University of Sydney, Camperdown, NSW 2050, Australia
The University of Sydney, Discipline of Infectious Diseases & Immunology and Marie Bashir Institute, Camperdown, NSW 2050, Australia
Corresponding author stefan.oehlers@sydney.edu.au

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Abstract
The spectre of the coming post-antibiotic age demands novel therapies for infectious diseases. Tuberculosis, caused by Mycobacterium tuberculosis, is the single deadliest infection throughout human history. M. tuberculosis has acquired antibiotic resistance at an alarming rate with some strains reported as being totally drug resistant. Host-directed therapies attempt to overcome the evolution of antibiotic resistance by targeting relatively immutable host processes. Here I hypothesise the induction of hypoxia via anti-angiogenic therapy will be an efficacious host-directed therapy against tuberculosis. I argue that anti-angiogenic therapy is a modernisation of industrial revolution era sanatoria treatment for tuberculosis, and present a view of the tuberculosis
granuloma as a “bacterial tumour” that can be treated with anti-angiogenic therapies to reduce bacterial burden and spare host immunopathology. I suggest two complementary modes of action, induction of bacterial dormancy and activation of host Hypoxia Induced Factor-mediated immunity, and define the experimental tools necessary to test this hypothesis.

1. Introduction

After a period of relative success during the golden age of antibiotics in the second half of the 20th century, tuberculosis (TB) rates climbed dramatically with the HIV pandemic from the 1980s. Although recent advances in HIV treatment have seen a fall in the rates of HIV-related TB mortality, the failure to completely eradicate TB and subsequent emergence of multidrug resistant strains of *M. tuberculosis* has created pockets of endemic TB of global importance.

The emergence of extensively and totally drug resistant TB strains have led to the contemplation of living in a post-antibiotic era with this ancient killer. Given the short lag between the deployment of a new antibiotic and reports of resistance, new efforts are being directed to discover host-directed therapies (HDTs) for TB. This hypothesis will discuss the history and recent evidence supporting the induction of granuloma hypoxia as an intervention for difficult to treat TB.

2. TB therapies in the pre-antibiotic era

The high altitude sanatoria of the pre-antibiotic era sought to strengthen patients, at least for those who were able to afford the fees of a sanatorium, with regimented daily regimes. It is important to note that these sanatoria would not deliver a curative treatment but rather induce convalescence or prolong patient survival [1, 2]. Three elements of these regimes that we recognise as biologically active are better nutrition, daily sun exposure to boost vitamin D levels, and reducing the supply of oxygen to the bacterial lesions.
Epidemiological studies have informed us that the burden of TB mortality, like many other infectious diseases, falls disproportionately on the poor, the young, the frail and the weak. Nutritional supplementation, historically achieved by increasing milk consumption, could directly disrupt the infection-malnutrition cycle in favour of patient recovery [3]. Under nutrition is clearly a predisposing factor for TB that remains to be solved as a public health issue [4, 5].

The increased sun exposure prescribed for city dwellers of industrial Europe also has a modern analogy in the form of vitamin D supplementation. The micronutrient vitamin D can boost anti-TB immunity in pre-clinical models and is often found to be deficient in TB patients [6]. Again however, systemic review of vitamin D supplementation as an interventional strategy has not yielded evidence of a clinical benefit [4].

This leaves the final element of altitude and the availability of oxygen on the outcome of infection. Epidemiological studies in mountainous regions of the Americas and Himalayas have noted a decrease in TB rates in high altitude populations [2, 7-11]. Although the effects of urbanisation and poverty can negate the protective effects of living at high altitude, these observations provide strong evidence for a protective role of hypoxia in TB biology.

Efforts to surgically bring the benefits of a high altitude retreat to the masses resulted in the short-lived lung collapse therapies, which peaked in usage immediately prior to the antibiotic age [12]. Collapse of a lung was hypothesised to rest the organ allowing the body time to contain the infection by fibrosis of the lesions and by starving the bacteria of oxygen [13]. Although successful enough to be widely utilised as a treatment for an otherwise fatal condition, lung collapse surgeries are inherently risky and were rendered obsolete with the appearance of antibiotic therapy.
Conversely, hyperoxia has been identified as a risk factor for TB disease. Analysis of a Taiwanese comorbidity-controlled cohort of hyperbaric chamber users revealed an increased rate of TB implying reactivation of subclinical TB infection by the hyperoxic treatment [14]. The “summering” of sanatoria patients at sea level is anecdotally reported to cause relapse, even those within the consolidation phase of immune control [1]. Experimentally, the pivotal experiments of Rich & Follis and Sever & Youmans in the mid 20th century demonstrated the host-protective effect of hypoxia and a detrimental effect of hyperoxia in experimental TB infection (reviewed in [15]).

3. A “bacterial tumour”: Tuberculosis granulomas are structurally and molecularly similar to tumours

Granulomas formed during TB are remarkably similar to solid tumours (Figure 1). Both lesions can form in every organ system of the body, cause chronic diseases requiring lengthy treatment, and are prone to relapse or reactivation. The lesions can even be difficult to differentiate by modern clinical imaging techniques as both take the form of inflamed, metabolically active nodules in fluorodeoxyglucose PET scans. This consumption of glucose is caused by the engagement of aerobic glycolysis in both lesions [16]. Cellular-level similarities such as a encapsulation of a nodule, the development of a necrotic core during disease progression, an ineffective inflammatory response that exacerbates disease [17, 18], and vascularisation are characteristic of both lesions [19]. Below this cellular similarity are molecular similarities including hypoxia and hypoxia induced factor (HIF) signalling [20], and the destruction of surrounding tissue by matrix metalloproteinases [20, 21].

Metabolic syndrome, encompassing diabetes and obesity, is an important co-morbidity of TB, cancer, and other diseases driven by inflammation. Elevated blood glucose and cholesterol associated with metabolic syndrome provide core cellular stressors in TB and tumours. Poorly controlled diabetes impairs protective immunity and exacerbates immunopathology [22, 23], while
cholesterol can be preferentially utilised by mycobacteria as a carbon source, contributes to mycobacterial evasion of host immunity, and encourages the survival of transformed cells in tumours [24, 25]. Interestingly, control of either blood glucose by metformin or cholesterol metabolism by statins has positive effects on immune control of TB [24, 26].

T-cell immunity is essential for immune control of TB and tumours as evidenced by the impact of HIV infection comorbidity. Two important shared aspects of immune dysfunction that are characteristic of both diseases are the appearance of immune exhaustion and lack of lesion penetration that reduces protective T-cell immunity (reviewed in [27]). The immune exhaustion phenotype is characterised by the expression of surface molecules such as PD-L1 and cytokines such as IL-10 that suppress immunity. Immune checkpoint inhibitors targeting CTLA-4 or the PD-1/PD-L1 axis have had remarkable success in stimulating protective immunity against a range of tumours and a similar approach has been proposed for the treatment of TB where a similar immunosuppressive profile can develop [28, 29]. Tumours and granuloma-resident mycobacteria are both adept at feeding off inflammation. In respect to T cells, it is penetration of T cells into tumour masses or into granuloma cores is an important correlate of protective immunity, as the number of T cells distal to the lesion does not affect immune control [30-32].

The conserved vascular pathologies of TB granulomas and tumours have also been well described, and open the door to “Robin Hood” well-known vascular-targeting strategies from the cancer field into HDTs for TB. At a molecular level, both lesions produce the vasoactive cytokines VEGF and ANG-2 [33-35]. VEGF is the canonical pro-angiogenic signalling molecule and acts through the cognate receptor VEGFR on endothelial cells to trigger endothelial proliferation and direct migration. ANG-2 is also produced under similar conditions to VEGF and acts as a context-dependent antagonist of the TIE-2 receptor to antagonise the vascular-stabilizing effects of physiological ANG-1/TIE-2 signalling [36]. These molecular similarities underpin the tumour-like
vasculature seen around mycobacterial granulomas: angiogenesis [19, 37-39], permeability and inconsistent perfusion [34, 35], and haemostasis [40-42].

4. Angiogenesis fuels the growth of mycobacterial granulomas

The observation that tumours must recruit vasculature to continue growing beyond a critical threshold when oxygen can no longer permeate into their core has led to decades of research targeting tumour angiogenesis as a universal cancer therapy [43]. The VEGF-VEGFR signalling axis-driven is required for angiogenesis and inhibition of the axis by neutralising VEGF, blockading the VEGF binding site on VEGFR, or inhibiting the tyrosine kinase activity of VEGFR indeed slows the growth of many tumours in in vivo models, however efficacious translations to human oncology has been limited.

Mycobacterial granulomas are known to be highly vascularised during the active stages of infection across a range of host and bacterial species [19, 20, 34, 38]. In TB, extensive angiogenesis has been observed in active granulomas but is mainly absent in the immune-controlled latent form of the disease [19]. Furthermore, serum VEGF levels correlate with active disease and may even constitute a biomarker for disease progression [33].

Mycobacteria intrinsically induce VEGF expression by innate immune cells and subsequent angiogenesis through recognition of cyclopropanated trehalose dimycolate, a cell wall component, leading to activation of innate immune cells [44-46]. Furthermore, mycobacterial ESX-1-dependent macrophage aggregation concentrates pro-angiogenic macrophages to direct the growth of blood vessels towards nascent granulomas [38]. This requirement of multiple bacterial effectors, conserved across species of mycobacteria, to elicit the angiogenic response suggests host angiogenesis is an important core function of mycobacterial virulence.
The correlation between angiogenesis and bacterial growth state prompted us to investigate a mechanistic link using the zebrafish-Mycobacterium marinum infection model [38]. Using small molecule inhibitors of VEGFR, we found inhibition of angiogenesis reduced growth of *M. marinum*, reduced bacterial dissemination away from primary granulomas, and increased survival of drug-treated animals. VEGFR inhibition increased granuloma hypoxia in infected zebrafish but was ineffective when granulomas formed in highly vascularised tissues in oxygen permeant tissues of the embryo suggesting a mechanism of action dependent largely on the ability to induce hypoxia within granuloma. Additional work by Walton *et al.* used a dominant negative VEGF isoform to elegantly demonstrate the necessity of the VEGF ligand in the VEGF-VEGFR axis that promotes mycobacterial growth [46].

Interestingly, we observed an increased rate of granuloma conversion from a highly cellular phenotype to cuffed acellular core with low-burden phenotype was observed following VEGFR inhibition in zebrafish (Figure 2). This histological shift is reminiscent of the fibrotic walling off of granulomas seen after lung collapse therapy and may represent a conserved aspect of natural host containment of mycobacterial infection.

An important paper by Polena *et al.* utilised SCID mice to show 1) human macrophage-derived *M. tuberculosis* granulomas recruit vasculature *in vivo*, and 2) reduced *M. tuberculosis* dissemination in animals treated with VEGFR-blockading antibodies [39]. This important advance showed for the first time that experimental *M. tuberculosis* infection of mammals could be controlled by targeting infection-induced angiogenesis.

Complementary to the vascular-directed effects of anti-VEGF therapy, there is an emerging body of literature addressing the non-angiogenic effects of VEGF blockers in mycobacterial infection. It has become apparent from the cancer literature that immune cells are responsive to VEGF and respond in an immunosuppressive manner. The VEGFR inhibitors pazopanib and VEGFR2 kinase inhibitor
I (SU 5408) were detected as protective in a macrophage-based *in vitro* infection screen, independent of any effect on the vasculature [47]. A recent paper by Harding *et al.* has elegantly demonstrated a role for granuloma-derived VEGF in promoting harmful granulomatous inflammation in several mouse-*M. tuberculosis* models [48]. Together these papers provide evidence that targeting the VEGF-VEGFR axis has a net positive effect on the outcome of infection.

### 5. Case report evidence for the efficacy of anti-angiogenic drugs against TB

Anti-angiogenic therapies for cancer aim to induce sufficient tumour hypoxia to cause growth arrest. Technologies have largely focused on inhibiting the VEGF-VEGFR axis by neutralising VEGF, blockading the VEGF binding site on VEGFR, and inhibiting the tyrosine kinase activity of VEGFR. Ocular inflammation-induced angiogenesis can cause loss of vision and is typically treated by oral anti-inflammatories such as steroids or intravitreal injection of VEGF-neutralising agents such as bevacizumab (Avastin) and ranibizumab. Ocular TB thus presents an important manifestation of disseminated TB where TB treatment overlaps with anti-angiogenic therapy.

A total of 11 case reports have utilised VEGF neutralisation during TB. Injection of bevacizumab, or the derivative ranibizumab, has been safely used as an adjunctive treatment to improve vision in conjunction with antibiotics [49-52], to clear granuloma-associated angiogenesis after successful antibiotic treatment [53, 54], as a component of treatment for paradoxical TB-associated immune reconstitution inflammatory syndrome [55, 56], and even as monotherapy [57-59].

The three monotherapy reports of VEGF neutralisation leading to ocular disease remission provide the clearest evidence yet for an anti-TB effect of anti-angiogenic therapy in human TB. The 4 case reports of anti-angiogenic therapy used as an adjunct to antibiotics demonstrate that adjunctive use does not impair antibiotic efficacy, albeit only in the context of ocular infection. Together, these
reports provide some evidence of the efficacy of anti-angiogenic therapy in human TB and demonstrate safety of anti-angiogenic therapy during active ocular TB.

6. Molecular level protective effects of hypoxia

6.1. Effects on the bacterium

The most obvious effect of hypoxia is that the lack of oxygen starves the mycobacteria and stops proliferation. Indeed, low oxygen pressures do halt the growth of *M. tuberculosis* *in vitro*, causing the bacteria to enter a relatively metabolically dormant lifestyle that is also observed in experimental models and clinically in non-progressing latent infection [60]. Hypoxia causes the build-up NADH, an indicator of cellular stress, in mycobacteria. This metabolic block exposes the bacterial electron transport chain as an antibiotic target in the absence of cellular division [61].

*M. tuberculosis* responds to hypoxic conditions through activation of the DosR regulon (well-reviewed in [62]). Expression of DosR regulon genes is exquisitely correlated with hypoxia *in vivo* and is maximal in the hypoxic granulomas characteristic of the latent TB classification [60]. Mutants lacking components of the DosR regulon are unable to established latent infection in animal models that form granulomas with significant hypoxia but remain relatively virulent in mouse models that do not recapitulate a hypoxic infection environment [63, 64]. Thus induction of granuloma hypoxia is likely to drive a non-replicative bacterial state that can be at least partially controlled by host immunity.

The intrinsic resistance of dormant mycobacteria to antibiotics compounds the hypoxia-induced shift of mycobacteria to a dormant phenotype. It is conceivable that in drug-sensitive TB cases, adjunctive hypoxia-inducing therapy could counterproductively promote drug resistance by intrinsically limiting the sensitivity of TB to antibiotics.
6.2. Host protective effects of hypoxia

Granuloma hypoxia may also stimulate host immunity to mycobacterial infection. Complementary to the findings of reduced TB incidence in humans living at altitude, whole blood restriction of mycobacterial growth was increased in donors living at high altitude [65]. Furthermore, macrophages cultured in low oxygen show increased bactericidal activity compared with macrophages cultured in atmospheric oxygen [66-68]. Recent analyses of host-mycobacterium interactions have revealed how exposure to hypoxia may augment anti-TB immunity at the molecular level.

IFNgamma activation is required for optimal macrophage killing of mycobacteria and loss of IFNgamma signalling is responsible for the heritable cluster of diseases termed Mendelian Susceptibility to Mycobacterial Diseases in humans [69]. HIF1alpha signalling has recently been demonstrated to potentiate IFNgamma-dependent immunity against TB [70]. Important anti-TB HIF1alpha-dependent immune pathways include effectors that modulate further immune activation such as PGE2, and directly bactericidal compounds such as nitric oxide [70, 71]. While HIF1alpha-dependent immune pathways have been shown to be directly induced by mycobacterial infection [71], we have found anti-angiogenic therapy increases HIF1alpha activation in mycobacterial granulomas in vivo [38].

Another recently appreciated anti-mycobacterial killing mechanism is activation of the Warburg effect in mycobacteria-infected macrophages resulting in a switch to glycolysis at the expense of oxidative phosphorylation [72]. This metabolic shift produces sufficient ATP to fuel antibacterial effector activity, glucose-derived metabolites that are toxic to M. tuberculosis under hypoxic conditions, and, in a positive feedback loop, stabilises HIF1alpha to further enhance cellular immunity [73].
Together, this potentiation of anti-mycobacterial immunity by hypoxia and HIF1alpha signalling demonstrate a molecular pathway linking anti-angiogenic therapy to reduced mycobacterial burden.

7. Host-detrimental effects of hypoxia

While HIF1alpha activation leads to an enhancement of anti-mycobacterial immunity, it also drastically induces the production of tissue-destructive matrix metalloproteinases (MMPs) [20, 74]. MMPs are produced as part of inflammatory responses and are central to the pathogenesis of many inflammatory diseases when over produced, and their production in mycobacterial infection aids granuloma formation by remodelling infected tissue [21, 75]. The collagenase activity of MMPs aids the growth and dissemination of mycobacteria, facilitating the escape of mycobacteria away from areas of growth-limiting hypoxia. This effect could be analogous to the pro-metastatic effects of hypoxia on tumours (Figure 1).

The switch to glycolysis is a double-edged sword as prolonged engagement of glycolysis leads to the accumulation of intracellular lipid droplets and the transformation of macrophages into foam cells laden with triacylglycerols (Figure 1). Foam cells are deficient in anti-bacterial function and are thought to provide a ready carbon source for mycobacterial growth in the granuloma core [76].

Hypoxia may inhibit the function of T cells and exclude T cells from mycobacterial granulomas. It is evident from the cancer literature that hypoxia-induced cytokine profiles create a microenvironment conducive to Treg retention at the expense of anti-tumour effector T cell types [77]. It is unknown if a similar mechanism functions in TB granulomas.

It has also been shown that inhibition of lymphangiogenesis by VEGFC reduces systemic T cell responses to infection [78]. If a VEGF family-targeting therapeutic is used to induce hypoxia in TB granulomas, it is conceivable that it could affect inflammatory lymphangiogenesis and the priming
of adaptive immune responses. An important distinction between lung collapse or high altitude environmental hypoxia and anti-angiogenic therapy is that the former methods of inducing hypoxia do not prevent blood flow to the granuloma. Hypoxia induced by anti-angiogenic therapy may thus have compounding effects on protective adaptive immunity.

Mycobacterial granulomas are naturally resistant to penetration by antibiotics, and this is a major therapeutic hurdle for antibiotic delivery [79]. There is thus a valid concern that anti-angiogenesis therapy will create avascular granulomas that could be impenetrable to antibiotics. Vascular normalisation experiments provide a contrasting intervention that decreases granuloma hypoxia by facilitating circulation to the granuloma neovascular network. Work in the rabbit TB model by Datta et al. utilised a short duration of treatment with the monoclonal antibody bevacizumab to neutralise infection-induced VEGF and normalise the granuloma vasculature resulting in better delivery of a small molecule dye to the granuloma core [34]. Xu et al. next demonstrated that inhibiting matrix metalloprotease activity normalised the vasculature and improved antibiotic delivery to mouse granulomas [80]. These two studies highlight the importance of vascular perfusion to delivering antibiotics into granulomas and suggest anti-angiogenic therapies could hinder antibiotic delivery.

8. Necessary elements of a preclinical model to test the hypothesis

TB is a disease that exacerbates economic inequity at personal and international levels. Despite the efficacious application of anti-VEGF biologics to small numbers of ocular TB patients, I believe small molecule VEGFR inhibitors are the most feasible intervention to advance. From an economics standpoint, small molecules can be chemically synthesised as generic drugs as patents expire. Importantly, small molecule VEGFR inhibitors have been formulated for delivery by tablets and capsules extending their delivery range into areas lacking sufficient infrastructure to handle biologics.
Mouse models of TB infection are the gold standard small animal models for testing new therapies. The ideal mouse TB model would replicate:

1. Angiogenic and hypoxic granulomas
2. A natural ability to control or sterilise infection
3. The pharmacokinetics of antibiotic delivery into granulomas

Most mouse strains do not develop hypoxic granulomas following mycobacterial infection like that seen in the zebrafish-\textit{M. marinum} model and in human TB infection \cite{37, 81}. This has recently changed with the characterisation of the C3HeB/FeJ mouse strain that recapitulates necrotic granulomas with areas of hypoxia following pulmonary infection with \textit{M. tuberculosis} \cite{82-84}. The reproduction of hypoxic granulomas is essential to determine if hypoxia-inducing anti-angiogenic therapies are effective in treating \textit{M. tuberculosis} infection in mammals \cite{81}. Although granuloma angiogenesis has not been directly described in this strain, it is likely to recapitulate this phenotype as mycobacterial infection-induced angiogenesis is highly conserved across species pairings.

With respect to human-like immune control, the CC001/UncJ mouse strain was identified from a screen of mouse strains as more able to reduce \textit{M. tuberculosis} burden between 3 and 6 weeks post infection \cite{85}. The ability of a mouse model to restrain TB infection is unusual and suggests an effective immune response against \textit{M. tuberculosis} that could be leveraged to examine the effect of hypoxia-inducing therapy in the background of successful immune-mediated control of infection.

As addressed in section 7, anti-angiogenesis therapy could reduce antibiotic penetration into granulomas. Testing of anti-angiogenic therapy in conjunction with conventional antibiotics will determine if anti-angiogenic therapy should be used to treat antibiotic-sensitive TB or only as a “last line” therapy for antibiotic resistant infections.
9. Conclusion

While hypoxia in cancer has been suggested to ultimately lead to more aggressive malignancies, hypoxia in mycobacterial infection may fit a converse paradigm where lack of oxygen slows bacterial growth and improves anti-TB immunity. The observations and the findings discussed here demonstrate potential adjunctive, host-directed therapies and may open new avenues to treating recalcitrant TB infections, including multi-drug resistant disease. However concerns of the wider effects of these treatments on antibiotic susceptibility call for further pre-clinical testing and compassionate use of anti-angiogenic therapies in antibiotic resistant infections.

While induction of hypoxia appears to be effective in slowing the growth of mycobacteria in vivo, this approach is probably not sterilising given the low “cure” rate (5-10%) compared with “improved” status (~50%) measured for collapse therapy patients [12], and the highly variable improvement rate of sanatoria from 90% to 5% declining with initial severity of disease and with an average 9 year fitness rate of 60% [1]. These observations could be due to the conversion of active lesions to more latent lesions with a reduced but not sterile load of mycobacteria. While this is certainly preferable to the high mortality rate seen in the pre-antibiotic era it is unsatisfactory compared to the 86% cure rate for drug susceptible TB recorded by the WHO (http://www.who.int/gho/tb/epidemic/treatment/en/). Where hypoxia-inducing therapies may find a niche is in the treatment of MDR TB (50% cure rate) and XDR TB (23% cure rate).

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Figure legends

Figure 1: The “bacterial tumour”: similarities between the TB granuloma and solid tumours.

(Top left) Both lesions are hypoxic and drive the formation of a leaky and haemostatic vasculature through the production of VEGF and ANG-2. The lack of sufficient perfusion prevents the penetration of drugs into each lesion resulting in phenotypic insensitivity. Activation of haemostasis is a common strategy to subvert the immune response. The hypoxic conditions activate a HIF1-mediated transcriptional program that also feed into metabolism and tissue destruction.

(Top right) There are shared immunological markers of disease prognosis. A robust type 1 immune response with spatial penetration of lymphocytes is associated with lesion apoptosis and immune containment. A skewing to a type 2 response with insufficient lymphocyte penetration is associated with poor immune control and a worse prognosis.

(Bottom right) Matrix metalloprotease (MMP)-mediated remodelling and destruction of the surrounding stromal tissue is essential to facilitate lesion spread from primary foci through surrounding tissue and eventually facilitating haematogenous spread. MMP activity is increased by hypoxia and the HIF1-mediated transcriptional program.

(Bottom left) Metabolic syndrome-associated diseases diabetes and dyslipidaemia are important comorbidities for both diseases. At a molecular level, these comorbidities feed lesions with glucose and lipid. In the TB granuloma this results in loss of effective immune control and fuels bacterial growth, while in cancer the malignant cells use the nutrients.

Figure 2: VEGFR inhibition drives a granuloma maturation phenotype in zebrafish

(A) Representative image of an organised cellular zebrafish-M. marinum granuloma.

(B) Representative image of an organised necrotic zebrafish-M. marinum granuloma.

(C) Quantification of granuloma necrosis in 6 week-post infection zebrafish adults treated with 1 µM pazopanib as indicated. Each dot represents a single granuloma.
Vascular dysfunction: weak perfusion

Drug insensitivity

Vascular dysfunction: thrombosis

Hypoxia / HIF1alpha

O2

Diabetes
Dyslipidemia

Aerobic glycolysis

Citrate / acetyl CoA

Lipid accumulation

Granuloma caseation
Tumour survival

M1
TNF, IFN gamma

Good prognosis
Lesion apoptosis

M2
Th2
IL-10, PD-L1

Poor prognosis
Immune evasion

MMP-mediated matrix destruction

Cancer dissemination

TB dissemination

Tissue pathology

Metabolism

Immunology