NEW STRATEGIES TO COMBAT TUBERCULOSIS

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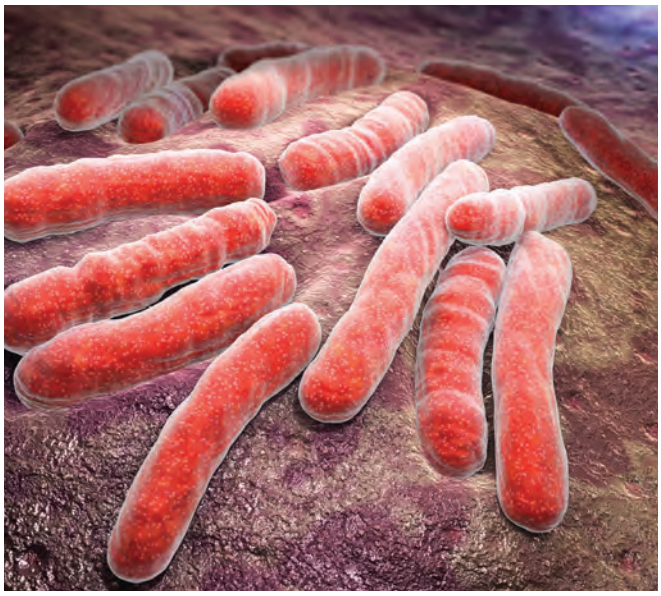
Tuberculosis (TB) is one of the most significant infectious diseases in the world today. According to the 2014 World Health Organization (WHO) annual report, there were an estimated 9.6 million new cases and 1.5 million fatalities in that year, and one-third of the world's population is now infected with the etiological agent *Mycobacterium tuberculosis*. Due to successful control strategies in the 1980s, the current risk of Australians contracting this disease is low, but we are not totally immune to it, with around 1500 new cases diagnosed in Australia in 2014. Of more immediate concern is that TB remains a major threat to our nearest neighbours, including Papua New Guinea, Indonesia, Thailand and Vietnam.



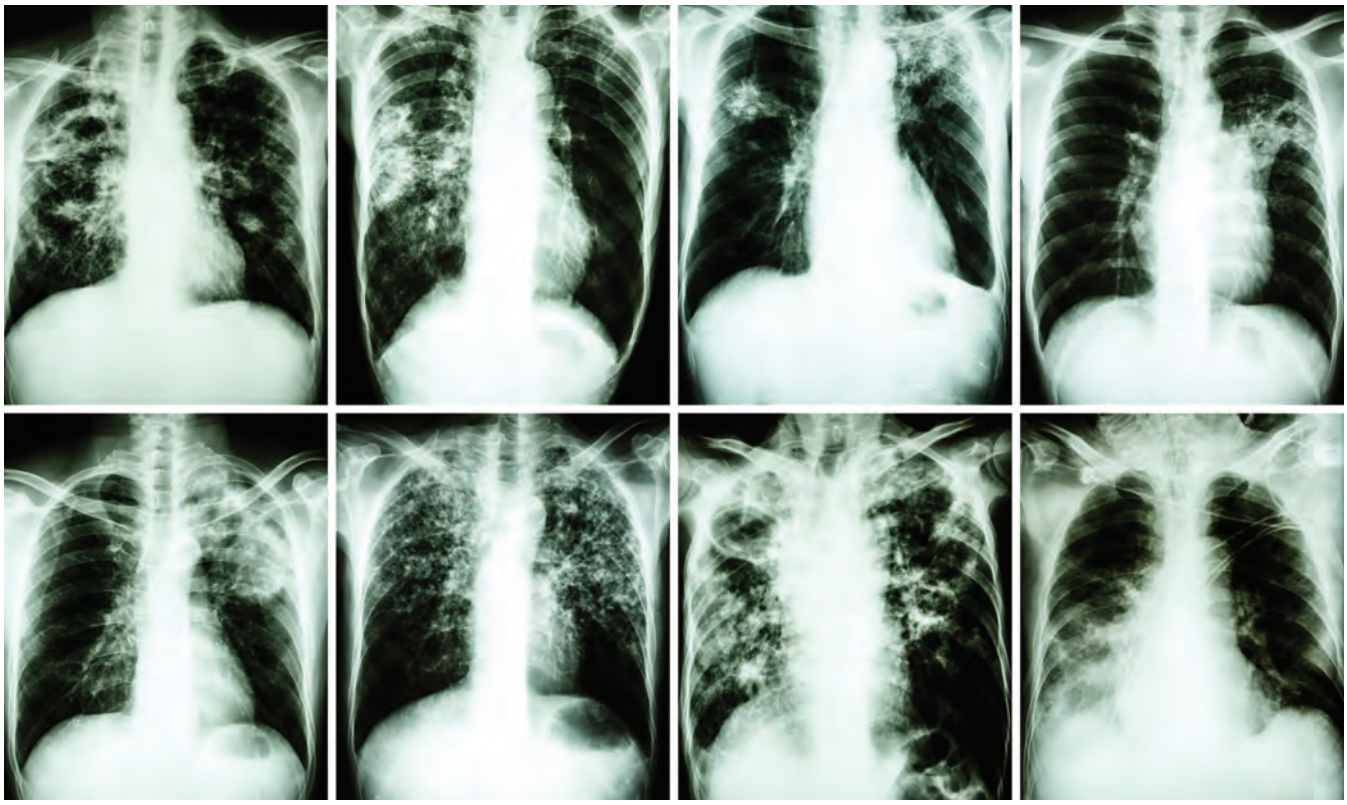
Bacterial infection tuberculosis

The current frontline therapeutics for TB include isoniazid and rifampicin, and second-line drugs that include pyrazinamide, ethambutol kanamycin, capreomycin, ethionamide, fluoroquinolone and rifabutin. It is of concern that in recent years there has been an increasing occurrence of resistance to these drugs, so much so that strains of TB can be identified as multi-drug-resistant TB (MDR-TB), where there is resistance to both isoniazid and rifampicin; extensively drug-resistant TB (XDR-TB), where there is resistance to at least one of the second-line drugs; and totally drug-resistant TB (TDR-TB).

There are other concerns associated with the use of these drugs as therapeutic treatments for TB. In particular, administration is often required over a period of at least six months to keep the disease under control. The cost of the second-line drugs, and the fact that many of these have serious side effects, are other factors that make these treatments less than ideal.

Encouragingly, several pharmaceutical companies have shown an interest in developing new anti-TB agents, and they have met with some success. Recently, Johnson & Johnson developed bedaquiline (an ATP synthase inhibitor), the first new TB drug in 40 years. Novartis has also discovered an experimental drug, PA-824, although its broad efficacy and safety are yet to be established. Thus, while progress is being made to combat this disease, it is unclear how effective these new therapeutics will be in the long term.

Drug resistance is a problem that cannot be overcome easily; however, one of the most effective strategies for infection control is to have available



Chest X-rays exhibiting pulmonary tuberculosis

multiple antibiotics that target a range of metabolic processes essential to the survival of the pathogen. This approach reduces the reliance on mainstay therapeutics, thus prolonging their usefulness. In this respect, enzymes from the branched-chain amino acid (BCAA) biosynthesis pathway offer an exciting and promising new avenue to develop novel anti-TB drug leads.

The primary reason for targeting this pathway is that its activity appears to be absolutely essential to the survival of *M. tuberculosis*. This conclusion stems from a transposon mutagenesis study that has shown that the activity of each of the seven enzymes in the BCAA pathway is essential for the growth of *M. tuberculosis* in cell culture. A second critical factor that authenticates the BCAA pathway as a promising drug target for TB is the fact that this pathway is absent in humans; therefore, specific inhibitors of any of the seven enzymes would be expected to possess low toxicity if given to humans.

Inhibitors of the first two enzymes in the BCAA pathway, acetohydroxyacid synthase (AHAS) and ketol-acid reductoisomerase (KARI), have been tested for their effects on the growth of *M. tuberculosis* cells in culture. One class of AHAS inhibitor, known as the sulfonylureas, have MIC values in the 8–16 micrograms per millilitre range in *M. tuberculosis* susceptibility assays.

Thus, these compounds, which were initially developed as herbicides targeting plant AHAS, also appear to be promising anti-TB agents. Similarly, a KARI inhibitor (N-hydroxy-N-isopropyloxamate, or IpOHA for short), which also acts as a herbicide, has an MIC of 64 micrograms per millilitre in *M. tuberculosis* susceptibility assays. Thus, there is strong experimental data in support of the BCAA pathway as an anti-TB drug target.

To begin the process of rational structure-based drug discovery, our group has recently determined the crystal structure of *M. tuberculosis* KARI at 1.0. This high-quality structure provides new insights into the architecture of the catalytic centre of this enzyme, thus providing an ideal starting point for the rational design of new *M. tuberculosis* KARI inhibitors. Similar studies are in progress for the enzyme AHAS from that organism.

Gerhard Schenk will be speaking at the 17th International Biotechnology Symposium (IBS 2016).