Retraction

# Antibiotic-resistant microbes face dual attack

**Youcef Mehellou & Benjamin E. Willcox**

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In view of the fact that the authors of the article 'IspH inhibitors kill Gram-negative bacteria and mobilize immune clearance' (K. S. Singh *et al. Nature* **589**, 597–602; 2021) are retracting their report, the authors of this News & Views wish to retract their article, which dealt with this study and was based on the accuracy and reproducibility of the data in the study.

# News & views

Drug discovery

# Antibiotic-resistant microbes face dual attack

Youcef Mehellou & Benjamin E. Willcox

Isoprenoid molecules are essential in many disease-causing microorganisms, and intermediates made during their synthesis trigger immune-defence responses by γδ T cells. 'Immunoantibiotics' exploit this dual vulnerability. **See p.597**

The emergence of strains of harmful microorganisms that are resistant to antibiotic treatment is a major global health concern. This has prompted ongoing drug-development efforts, including the identification of possible biological targets linked to essential microbial processes. However, the clinical development of drugs is slow, and the emergence of resistance to newly developed compounds remains a continuing problem. Finding alternative ways to tackle the emergence of antibiotic-resistant bacteria is of paramount importance. On page 597, Singh *et al.*[1] outline a two-pronged strategy to address this challenge.

The authors' approach involved the development of a new class of molecule called immunoantibiotics. These target a key pathway that generates a molecule needed by microbes and, in doing so, stimulate a particular population of immune cells. The cells, from the grouping called γδ T cells, then provide a general (innate-like), potent antimicrobial immune response.

Singh and colleagues focused on inhibition of an enzyme termed IspH (Fig. 1). This catalyses a step in a pathway that makes molecules called isoprenoids. Isoprenoids are key building blocks needed for the synthesis of a diverse range of molecules in prokaryotes (organisms lacking a nucleus, such as bacteria) and eukaryotes (organisms that have a cellular nucleus). However, IspH catalyses a reaction in an arm of the isoprenoid-synthesis pathway, called the methylerythritol 4-phosphate (MEP) pathway, that is found in bacteria and certain protozoa (single-celled eukaryotes), but is absent in animals[2]. Several enzymes in this pathway, which underpins processes such as the synthesis of the bacterial cell wall and energy production, have already attracted interest as potential antimicrobial drug targets[3–7]. These targets include IspH itself[3,6], which is present in a diverse range of disease-causing microorganisms, including Gram-negative bacteria, mycobacteria (a grouping that includes the microbe that causes tuberculosis) and certain protozoa, such as *Plasmodium falciparum* (the microorganism that causes malaria).

Choosing IspH as a therapeutic target provides a benefit that extends beyond its role in generating the compounds needed for isoprenoid synthesis. This is because the microbial molecule that it breaks down, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), is one that the immune system of primates has evolved the ability to detect[8]. Humans detect HMBPP in the body by using immune cells known as Vγ9Vδ2 T cells, which represent approximately 1–10% of T cells in the blood. After Vγ9Vδ2 T cells recognize[9] cells exposed to HMBPP, through a process that relies on the receptors BTN3A1 and BTN2A1 in exposed cells (Fig. 1), they become activated, proliferate and mediate potent defence responses, including the production of molecules capable of killing cells and the release of signalling molecules called cytokines.

Activated Vγ9Vδ2 T cells can also either directly kill human cells exposed to HMBPP (exposed by, for example, intracellular microbial infection) or kill bacterial cells themselves. Singh and colleagues' strategy of focusing on IspH, therefore, combines rational targeting

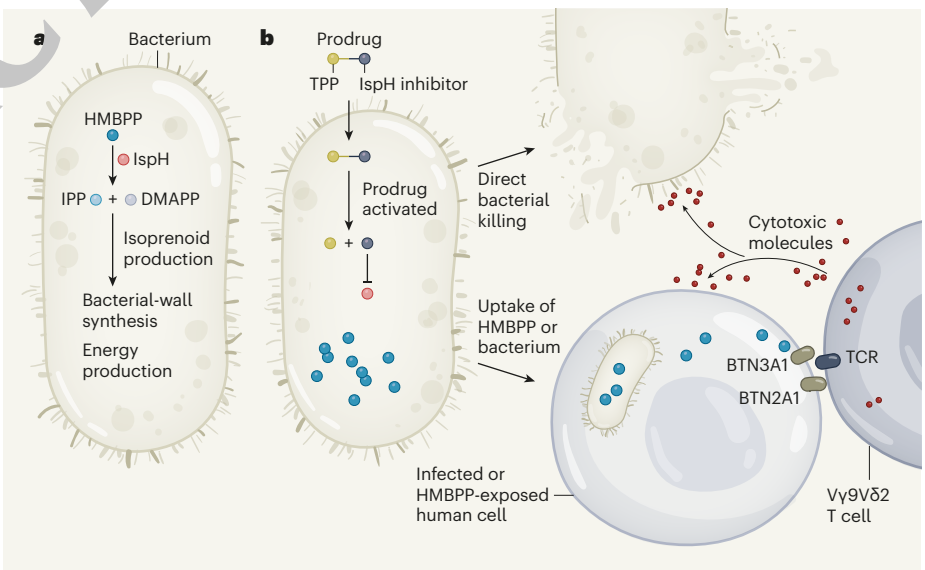


**Figure 1 | Development of an immunoantibiotic. a**, A microbe-specific pathway uses the enzyme IspH to break down the molecule (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). After other steps in the downstream pathway (not shown), isoprenoid molecules are synthesized; these have essential cellular roles. **b**, Singh *et al.*[1] used a screening method to identify IspH inhibitors. To boost entry into cells, the authors generated a prodrug version of the inhibitor, in the form of a triphenylphosphonium (TPP) ester derivative. Inside the bacterium, the inhibitor is separated from the TPP group and targets IspH. IspH inhibition blocks isoprenoid production, eventually killing the bacterium. It also increases HMBPP levels. HMBPP that is taken up by human cells, or that is present in human cells infected with bacteria, is sensed by an immune cell called a Vγ9Vδ2 T cell. This sensing process requires BTN3A1 and BTN2A1 receptors on the target cell and the immune cell's T-cell receptor (TCR). On sensing HMBPP, these T cells become activated and release molecules that can kill infected human cells or bacteria.

to inhibit the isoprenoid pathway, and thereby block a source of crucial microbial molecules, with the stimulation of an immune response due to the resulting accumulation of HMBPP, which is a highly potent signal that drives the activation of Vγ9Vδ2 T cells.

The authors took a structure-directed, *in silico* screening approach to identify possible IspH inhibitors, and tested around ten million compounds. Strikingly, 2 of the 24 most promising compounds inhibited IspH with high potency (at nanomolar concentrations) when tested *in vitro*. Further optimization of the molecular structures of these compounds improved their affinity for IspH compared with the affinity of IspH for its natural substrate, HMBPP.

However, the physical characteristics of the inhibitors were expected to limit their entry into bacteria. To circumvent this, Singh *et al.* adopted a strategy previously used to enable drugs to pass through membranes. This method generates what is called a prodrug — an inactive version of the drug (in this case, an ester derivative of the inhibitor) that can be taken up easily by cells and then metabolized into the active version. Crucially, unlike previous work[3,6] that described IspH inhibitors, this prodrug approach allowed such inhibitors to successfully enter bacteria. The authors confirmed that the drugs inhibited enzyme breakdown of HMBPP, hindering essential microbial processes, and that this resulted in the killing of a range of different bacteria, including *Escherichia coli*, without notable signs of drug toxicity to mammalian cells.

In keeping with the ability to inhibit HMBPP breakdown by IspH, prodrug use also led to the *in vitro* activation and proliferation of HMBPP-responsive Vγ9Vδ2 T cells during bacterial infection of samples of human peripheral blood mononuclear cells. This result indicates the potential of such prodrugs to act as dual-action immunoantibiotics. When tested *in vivo* in mice, the prodrugs induced direct antimicrobial effects and controlled bacterial infection through a process mediated by γδ T cells.

Singh *et al.* explored two key aspects of the potential of these new compounds to combat antimicrobial resistance. First, the researchers present *in vitro* and *in vivo* data indicating direct bactericidal effects on a variety of clinically isolated harmful bacteria that are resistant to current antibiotics, including multidrug-resistant microbes. The authors observed that the IspH inhibitors had greater ability to kill multidrug-resistant microbes than do the current best-in-class antibiotics. Second, using an *in vitro* model system, Singh and colleagues showed that bacteria did not acquire resistance to the IspH inhibitors in the presence of γδ T cells. But in the absence of these T cells, drug resistance occurred over a similar timescale to that observed for conventional antibiotics. These results emphasize the potential advantage that immunoantibiotics might have for tackling the emergence of drug resistance.

Singh and colleagues' study is a highly promising proof-of-concept that a new class of antimicrobial can be developed with a dual mechanism of action. Leveraging Vγ9Vδ2 T cells is appealing because of the therapeutic advantages offered by harnessing this approach. These cells, present in humans from early in life, are capable of highly potent defence functions[10], and, unlike many other types of T cell, don't depend on the recognition of major histocompatibility complex (MHC) molecules, which differ between individuals. Encouragingly, the pathway containing IspH is shared by a diverse range of clinically relevant disease-causing microorganisms, suggesting that such antimicrobial drugs could have broad applicability.

Antibiotic approaches using monotherapy (a single type of drug) have often resulted in the emergence of drug resistance, whereas combination therapies using multiple drugs, operating through different mechanisms of action, have been more fruitful and have met with relatively fewer resistance problems. This 'two-in-one' mechanism underpinning Singh and colleagues' strategy might, therefore, allow the targeting of existing multidrug-resistant microbes, as well as decrease the chances of resistance emerging. Although the subsequent steps on the road to drug development can often be challenging, the progress of this exciting class of compound towards clinical application will undoubtedly be followed with interest.

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Experimental physics

# Helium nucleus measured with record precision

**Wilfried Nörtershäuser**

The size of the helium nucleus has been determined using exotic helium atoms in which one electron has been replaced with its heavier cousin, a muon. The result sheds light on a decade-old puzzle regarding the proton radius. **See p.527**

Helium is the second most abundant element in the Universe, after hydrogen. The nucleus of its most common isotope, helium-4, consists of two protons and two neutrons and is called the α-particle. This particle is more compact than other light nuclei — for instance, it is about 20% smaller than the nucleus of the hydrogen isotope deuterium[1], which contains only one proton and one neutron. The exact size of the α-particle is of particular interest because of a decade-old experiment that suggested the radius of the proton is considerably smaller than had been thought[2]. This result led to much speculation about possible missing pieces in the standard model of particle physics[3]. On page 527, Krauth *et al.*[4] report a determination of the α-particle size that strongly restricts such explanations and provides a benchmark for nuclear-structure theory.

The authors measured the α-particle size using a technique known as laser spectroscopy. This approach is based on the fact that atoms can emit and absorb light only at discrete frequencies, which are determined by the details of the atomic structure — namely, the interaction of the negatively charged electrons with the positively charged nucleus and with each other. Protons make up the charged component of the nucleus. The number of protons dictates the element, and their spatial extent is characterized by a property called