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Drug discovery for the developing world: progress at the Novartis Institute for Tropical Diseases

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Tropical infectious diseases remain a substantial cause of morbidity and mortality (see Further information), as noted in a recent article on tuberculosis (Advances in the development of new tuberculosis drugs and treatment regimens. *Nature Rev. Drug Discov.* **12**, 388–404 (2013))[1]. For those diseases such as tuberculosis and malaria that have treatments available, the emergence of drug resistance is a continual concern. Other pathogens, such as dengue virus, have no effective drugs at present. In both cases, there is a major need for new therapies.

Tropical diseases also present major hurdles to drug developers. The lack of healthcare infrastructure in some endemic regions necessitates simple and convenient dosing. Compounds should be highly potent, orally bioavailable and not subject to drug–drug interactions. An excellent safety profile is desired, including in children. Ideally, drugs should have pharmacokinetics compatible with a minimal number of doses and a stable formulation even under extremes of heat and humidity, and the active ingredients should be inexpensive and easy to synthesize. Identifying a drug with such characteristics would be a challenge for any disease, but developers tackling neglected infectious diseases may also lack tools, reagents and mechanistic information.

Established as a public–private partnership between Novartis and the Singapore Economic Development Board (EDB), the Novartis Institute for Tropical Diseases (NITD) was founded 12 years ago with the expectation that by applying drug discovery expertise and cutting-edge technologies, these challenges could be confronted. In just over a decade, the NITD has identified two new malaria drugs that are now in early-stage clinical trials, several promising leads for tuberculosis and tool compounds for dengue fever. Here, we highlight this progress and the keys to success: understanding disease biology, applying advanced technologies and partnering with academic experts throughout the world.

In vitro assays and animal models

The high rates of attrition in drug development are well known and due in part to the lack of predictive preclinical models. This is an issue for *Plasmodium vivax*, a widespread malarial species that can remain dormant in the liver as a hypnozoite. The only drugs available to treat the quiescent form have serious adverse effects in some patients and cannot be used in children or pregnant women. The development of alternatives has been hampered by the inability to culture the hypnozoite *in vitro*. The Biomedical Primate Research Centre, the Netherlands, and the Université Pierre et Marie Curie, France, have shown that infection of simian hepatocytes with *Plasmodium cynomolgi* results in non-dividing cells with a drug-susceptibility profile suggestive of hypnozoites[2]. In collaboration with these groups, the NITD conducted the first low-throughput screen against this form of the parasite[3]. This identified KAI407, a compound with an *in vitro* profile similar to the currently used drug primaquine and the ability to prevent parasitaemia in mice (FIG. 1).

Animal models are also important for compound optimization. A difficulty in studying dengue virus is that its complex pathology is not readily reproduced in rodents. The NITD optimized an infection protocol for AG129 mice that results in viraemia, inflammation and antibody induction. Treatment with tool compounds, including the polymerase inhibitor NITD-008, reduced viraemia and inflammatory markers[4,5]. This model has since become the standard *in vivo* efficacy assay for dengue drug discovery.

Characterization of drug targets

For many neglected infectious diseases, the basic biology of potential drug targets has been understudied. For dengue, the efforts of several groups over the past decade have significantly advanced our understanding of its structural biology. The NITD and its collaborators contributed to this work by investigating the viral enzymes. Together with the Novartis Center for Proteomic Chemistry, the NITD solved the first structures of the flavivirus NS3 protease, including the West Nile and dengue enzymes bound to inhibitors[6,7]. The bound form of NS3 with its cofactor NS2B reflects the catalytically competent conformation, which is valuable to aid rational drug design. The NITD also investigated the virus methyltransferase, which uses *S*-adenosylmethionine to methylate the viral RNA cap. A hydrophobic cavity, which is conserved among flaviviruses and does not exist in human enzymes, was identified next to the *S*-adenosylmethionine-binding pocket. Compounds extending into this pocket showed selective activity against the dengue methyltransferase but unfortunately did not have cellular activity or desirable physiochemical properties[8]. Finally, in collaboration with Nanyang Technological University, Singapore, the NITD solved the first structures of the dengue polymerase and helicase[9,10]. These structures provide a basis for rational drug discovery.

Phenotypic screening

When the NITD was founded, a handful of clinically validated targets were known for tuberculosis and malaria. Unfortunately, multiple efforts by the NITD and others to discover and develop compounds against these targets, as well as against other essential genes (Working Group on New TB Drugs), failed to yield tractable leads. Because of this, the NITD turned to phenotypic screening — an approach that automatically selects hits for permeability and minimal cytotoxicity. Importantly, modern omics technologies have facilitated mechanism-of-action studies for these hits, and their targets can be rapidly identified.

Inhibitors of malaria blood and liver stages. With support from the EDB, the Wellcome Trust and the Medicines for Malaria Venture, the NITD and three collaborators (the Genomics Institute of the Novartis Research Foundation (GNF), the Biomedical Primate Research Centre and the Swiss Tropical Public Health Institute) have the ambitious goal of a single-dose cure for *Plasmodium falciparum* malaria. So far, several phenotypic screens have yielded over 500 scaffolds with antimalarial properties (ChEMBL-NTD). One hit was optimized to KAE609, a spiroindolone with potent activity against *P. falciparum* and *P. vivax*, including chloroquine-resistant strains[11]. Genetic and physiological studies suggest that KAE609 inhibits the parasite ATP-dependent sodium channel PfATP4; this causes a lethal

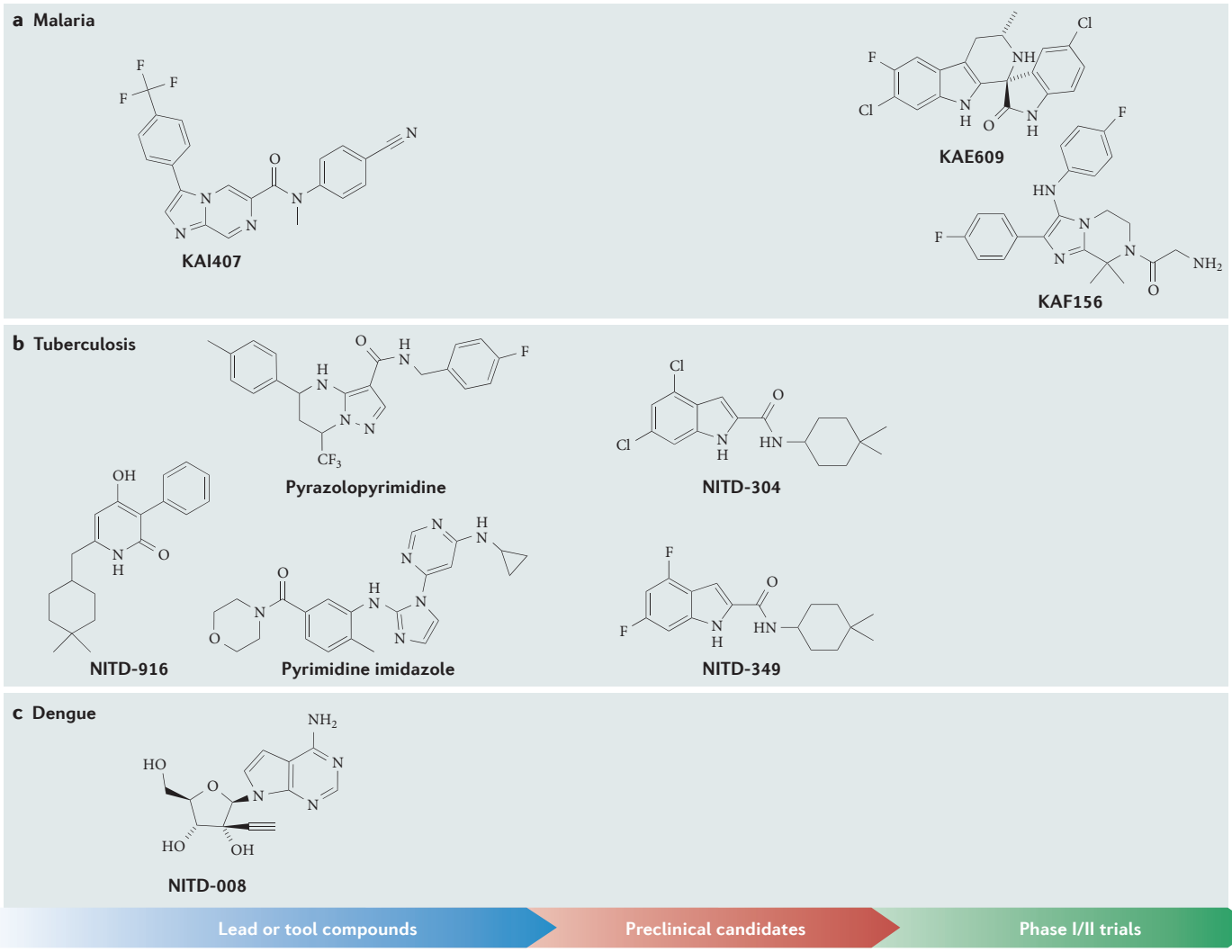

Figure 1 | **Compounds discovered by the Novartis Institute for Tropical Diseases and its partners.** The structures and development status of compounds to target malaria (part **a**), tuberculosis (part **b**) and dengue (part **c**) are shown.

disruption of sodium homeostasis[12]. Unlike similar doses of chloroquine, artesunate and mefloquine, a single dose of KAE609 eliminated *Plasmodium berghei* infection in mice and demonstrated pharmacological properties compatible with once-daily dosing[11]. In a Phase II clinical trial, the drug proved to be effective against artemisinin-resistant parasites[13]. In a little over 6 years from screening to clinical proof of concept, KAE609 is the first antimalarial with a novel mechanism of action in over two decades.

Like the majority of malaria therapies, KAE609 inhibits the blood stage of the parasite. Before replication in erythrocytes, however, *Plasmodium* sporozoites infect hepatocytes. Targeting both the blood and liver stages is important for prophylaxis and complete cure. Unfortunately, discovery of liver-stage inhibitors has been delayed by technical hurdles, including the need to isolate sporozoites from mosquito salivary glands and poor infection of hepatic cell lines. With the Scripps Research Institute and GNF, the NITD used high-content imaging to measure *Plasmodium yoelii* sporozoite replication in cultured hepatocytes[14]. Screening over 5,600 compounds with known activity against blood-stage *P. falciparum* identified an imidazolopiperazine series that also inhibited sporozoites[14]. The optimized compound, KAF156, demonstrated excellent efficacy in rodent models of blood-stage malaria and prevented sporozoite challenge[15]. KAF156, which is currently being evaluated in patients with uncomplicated malaria, appears to target a second transmembrane channel, PfCarl[14]. This is the second malaria drug with a new mechanism of action to be identified from a phenotypic screen.

Additional malaria targets have also been identified. For example, imidazopyrazines block phosphatidylinositol-4-OH kinase: the first target that is essential throughout the life cycle of the parasite in vertebrates[16].

Inhibitors of tuberculosis metabolism. Historically, most approved antibiotics have been discovered through whole-cell screening. The NITD has carried out three phenotypic screens against *Mycobacterium* spp., which have culminated in five promising series: pyrimidine imidazoles[17], imidazopyridines[18], pyrazolopyrimidines[19], indolcarboxamides[20] and pyridones[21].

The first hits to be explored were the pyrimidine imidazoles[17]. Several compounds had excellent bioavailability in mice but, surprisingly, did not show efficacy. Mechanistic analysis found that their activity relied on

accumulation of a glycerol metabolite that is toxic to *Mycobacterium tuberculosis*. Whereas glycerol is readily available *in vitro*, it is not required by bacteria proliferating in the mouse lung. This underscored the importance of assay conditions for screening and evaluating antimicrobials.

Additional tuberculosis hits are more promising. A pyridone, NITD-916, inhibits the clinically validated target InhA but, unlike the long-used antibiotic isoniazid, does not require activation by catalase-peroxidase KatG[21]. Two indolcarboxamide analogues, NITD-304 and NITD-349, are potent against drug-sensitive and multidrug-resistant isolates. Encouragingly, both compounds exhibited good pharmacokinetics and were efficacious in mouse tuberculosis models, with favourable safety margins[20]. Novartis has recently signed an exclusive worldwide licensing agreement with the Global Alliance for TB Drug Development (TB Alliance) to further develop these candidates. The University of Cape Town Drug Discovery and Development Centre is also evaluating and optimizing the tuberculosis hits.

Similarly to malaria screens, tuberculosis phenotypic screens revealed several new targets. For example, cyclomarin A inhibits the Clp protease, imidazopyridines target cytochrome *bc1*, and lipiarmycin revealed a pocket in the RNA polymerase distinct from that which binds rifampicin. Pyrazolopyrimidines and indolcarboxamides both target a transporter (MmpL3) that is essential for cell wall biosynthesis.

Conclusions

In its first 12 years, the NITD has, with its partners, brought 2 malaria drugs into clinical trials; and it has licensed several anti-tuberculosis candidates to the TB Alliance to advance. KAE609 and KAF156 will potentially be the first antimalarial drugs in 2 decades with entirely novel mechanisms of action — a significant step forward as resistance spreads to artemisinin, which is at the core of current first-line treatments for *P. falciparum* malaria. In addition, the NITD has discovered a plethora of novel drug targets that will constitute a long-lasting legacy towards a sustainable drug discovery pipeline. The NITD continues to focus on malaria and dengue and has recently expanded its activities to include human African trypanosomiasis. By understanding disease biology, applying innovative methods and fostering collaborations, the NITD will continue to exemplify the mission of Novartis to improve global health and deliver accessible medicines to patients.

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1. Zumla, A., Nahid, P. & Cole, S. T. Advances in the development of new tuberculosis drugs and treatment regimens. *Nature Rev. Drug Discov.* **12**, 388–404 (2013).
2. Dembele, L. *et al.* Towards an *in vitro* model of *Plasmodium* hypnozoites suitable for drug discovery. *PLoS ONE* **6**, e18162 (2011).
3. Zeeman, A. M. *et al.* KAI407, a potent non-8-aminoquinoline compound that kills *Plasmodium cynomolgi* early dormant liver stage parasites *in vitro*. *Antimicrob. Agents Chemother.* **58**, 1586–1595 (2014).
4. Schul, W., Liu, W., Xu, H. Y., Flamand, M. & Vasudevan, S. G. A dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs. *J. Infect. Dis.* **195**, 665–674 (2007).
5. Yin, Z. *et al.* An adenosine nucleoside inhibitor of dengue virus. *Proc. Natl Acad. Sci. USA* **106**, 20435–20439 (2009).
6. Erbel, P. *et al.* Structural basis for the activation of flaviviral NS3 proteases from dengue and West Nile virus. *Nature Struct. Mol. Biol.* **13**, 372–373 (2006).
7. Noble, C. G., Seh, C. C., Chao, A. T. & Shi, P. Y. Ligand-bound structures of the dengue virus protease reveal the active conformation. *J. Virol.* **86**, 438–446 (2012).
8. Lim, S. P. *et al.* Small molecule inhibitors that selectively block dengue virus methyltransferase. *J. Biol. Chem.* **286**, 6233–6240 (2011).
9. Yap, T. L. *et al.* Crystal structure of the dengue virus RNA-dependent RNA polymerase catalytic domain at 1.85Å resolution. *J. Virol.* **81**, 4753–4765 (2007).
10. Xu, T. *et al.* Structure of the Dengue virus helicase/nucleoside triphosphatase catalytic domain at a resolution of 2.4 Å. *J. Virol.* **79**, 10278–10288 (2005).
11. Rottmann, M. *et al.* Spiroindolones, a potent compound class for the treatment of malaria. *Science* **329**, 1175–1180 (2010).
12. Spillman, N. J. *et al.* Na^+ regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. *Cell Host Microbe* **13**, 227–237 (2011).
13. White, N. J. *et al.* Spiroindolone KAE609 for falciparum and vivax malaria. *N. Engl. J. Med.* **371**, 403–410 (2014).
14. Meister, S. *et al.* Imaging of *Plasmodium* liver stages to drive next-generation antimalarial drug discovery. *Science* **334**, 1372–1377 (2011).
15. Kuhen, K. L. *et al.* KAF156 is an antimalarial clinical candidate with potential for use in prophylaxis, treatment, and prevention of disease transmission. *Antimicrob. Agents Chemother.* **58**, 5060–5067 (2014).
16. McNamara, C. W. *et al.* Targeting *Plasmodium* PI(4)K to eliminate malaria. *Nature* **504**, 248–253 (2013).
17. Pethe, K. *et al.* A chemical genetic screen in *Mycobacterium tuberculosis* identifies carbon-source-dependent growth inhibitors devoid of *in vivo* efficacy. *Nature Commun.* **1**, 57 (2010).
18. Pethe, K. *et al.* Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nature Med.* **19**, 1157–1160 (2013).
19. Yokokawa, F. *et al.* Discovery of tetrahydropyrazolopyrimidine carboxamide derivatives as potent and orally active antitubercular agents. *ACS Med. Chem. Lett.* **4**, 451–455 (2013).
20. Rao, S. P. *et al.* Indolcarboxamide is a preclinical candidate for treating multidrug-resistant tuberculosis. *Sci. Transl Med.* **5**, 214ra168 (2013).
21. Manjunatha, U. H. *et al.* Direct inhibitors of InhA are active against *Mycobacterium tuberculosis*. *Sci. Transl Med.* **7**, 269ra263 (2015).

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Competing interests statement

The authors declare competing interests: see Web version for details.

FURTHER INFORMATION

ChEMBL-NTD: https://www.ebi.ac.uk/chemblntd
Working Group on New TB Drugs:
http://www.newtbdrugs.org/targets.php
World Health Organization:
http://www.who.int/topics/tropical_diseases/en/

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