Profile of William C. Campbell, Satoshi Ōmura, and Youyou Tu, 2015 Nobel Laureates in Physiology or Medicine

Where new drugs come from: A Nobel tale of ancient Chinese texts and a Japanese golf course

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Nobel Prizes are intended for "those who (...) shall have conferred the greatest benefit to mankind," as stated in Alfred Nobel's will. The 2015 Prize in Medicine, for William C. Campbell and Satoshi Ōmura for one half and Youyou Tu for the other half, for novel therapies against roundworm infections and malaria, respectively, fulfils this criterion particularly well.

Miracle Worm Drug from the Rough of the Golf Course

Nobel Prize winner Satoshi Ōmura says that along with a picture of his wife and daughter, he always carries a small plastic specimen bag wherever he goes. This habit of collecting environmental samples led him in 1970 to collect NRRL 8165, the mold later named *Streptomyces avermectinius*, from the woods close to a Japanese golf course. Extracts from it and other tens of thousands microbes collected by Ōmura and his colleagues at the Kitasato Institute in Japan were sent to the Merck Institute for Therapeutic Research in the United States for testing in the laboratory run by his co-Nobel Prize winner, William C. Campbell. There, the extract of *S. avermectinius* was found to have remarkable effects on eliminating a *Nematospiroides dubius* nematode infection in mice, in an antihelminthic screening test. The extract was fractionated, and macrocyclic lactones were found to be responsible for the activity, with the Avermectin B1a component having the leading activity (1). Campbell's group showed that Avermectin B1 had potency and safety superior to all known antihelminthic therapies at the time, capable of resolving a wide variety of animal nematode (roundworm) infections (2). Merck chemists made a minor chemical modification to Avermectin B1 to create ivermectin, which was marketed in 1981 for animal use. Beyond infections with nematodes, such as heartworm in dogs, and dozens of other worm species in commercial livestock, ivermectin has potent effects on killing ectoparasitic insects, such as fleas and ticks. Ivermectin was and is a "blockbuster drug" in animal medicine, and remains one of the most effective, potent, and safe antiparasite drugs for animal use.

William C. Campbell. Image courtesy of Corbis Images, copyright Brian Snyder/Reuters/Corbis.

Human Side of Ivermectin: Merck's Roy Vagelos Pledges to Do the Right Thing. In addition, ivermectin had great potential for human helminthic infection. After Campbell's group found ivermectin had great activity against *Onchocerca* infection in horses, he hypothesized the compound could also be useful in treating river blindness in humans, caused by *Onchocerca volvulus*. Onchocerciasis, or river blindness, is the second most common cause of blindness, after trachoma. It also causes a debilitating, chronic skin disease due to inflammation caused by microfilaria released from adult worms. Mohammed Aziz of Merck led the clinical trials that confirmed Campbell's hypothesis that ivermectin would be effective for onchocerciasis, and remarkably so. A single dose of ivermectin led

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Satoshi Ōmura. Image courtesy of Satoshi Ōmura.

to a dramatic reduction in microfilaria burden in humans, with amelioration of disease. One drawback was that ivermectin did not kill adult worms, which would resume microfilaria production after about six months; thus, about six to eight repeated doses at six-month intervals are necessary. Nonetheless, no drug came close to ivermectin's safety and efficacy for river blindness. In October of 1987, Roy Vagelos, Merck's Chief Executive Office, made the unprecedented decision to donate as much Mectizan, alias ivermectin, as was needed, for as long as it was needed, to anyone who needs it. Merck still stands behind this decision today, providing ∼300 million doses annually of ivermectin for the control and eventual eradication of river blindness and, more recently, for lymphatic filariasis (elephantiasis), another human helminthic disease that ivermectin treats effectively.

Ivermectin and Elimination Campaigns. The deployment of ivermectin, coordinated by the Carter Center and Pan American Health Organization, has led to river blindness being eliminated from three of six countries in the Americas as certified by the WHO, whereas transmission was halted in a fourth. Today transmission has been restricted to the Amazonas region of Brazil and to Venezuela.* In Africa, where the disease was much more prevalent, rates of infection are down fivefold, and in 2014, most African countries with endemic infection decided to move from river blindness control with annual doses of ivermectin to elimination campaigns with twice-annual dosing (3). Moreover ivermectin has recently been used in elimination campaigns for lymphatic filariasis, greatly reducing the numbers of human cases as well (4).

Beyond Ivermectin. Fortunately, human helminthic resistance to ivermectin has not (yet) emerged despite the large-scale campaigns undertaken, and it remains effective for river blindness and lymphatic filariasis. However, ivermectin resistance has emerged in some animal helminths, prompting agencies such as the Bill & Melinda Gates Foundation, Drugs for Neglected Diseases *Initiative*, and partners to seek new drugs for river blindness actively. In addition, a drug that kills adults (macrofilaricidal) is actively sought because it would simplify elimination campaigns, which now require twice-annual mass administration of ivermectin for at least several years to eliminate infection and transmission.

Rediscovery of a 1,700-Year-Old Long-Forgotten Malaria Cure

On May 23, 1967, China set up secret military Project 523 to identify new medicines that could be used against malaria. This strategic objective was important, not only for the eradication of malaria but also to support troops during the Vietnamese war, who died more frequently from mosquito bites than from bullets. It is noteworthy that many of the other antimalarial medications come from this period, with both the Chinese and the American military investing significantly to protect troops in Southeast Asia. The project started from over 2,000 Chinese herb preparations, which were tested against infected erythrocytes: Continuous culture of parasites did not come until a decade later (5). One of the plant extracts was taken from an ancient text called *Emergency Prescriptions Kept Up One's Sleeve*, from Ge Hong (A.D. 284–346), who recommended to take a handful of *qinghao* (the "blue-green herb," which refers to *Artemisia* species, including *A. annua* or sweet wormwood) immersed in 2 L of water for patients to drink. The initial results were difficult to reproduce, but Tu discovered that cold, not hot, water was to be used for extraction (later replaced by ether). With this extraction method, the team obtained good, reproducible, biological activity. The first (modern) human subject to test qinghao in 1971 was Youyou Tu herself, with preclinical safety testing not being practical during the Chinese cultural revolution. With the safety of the drug shown, its efficacy was proved on patients in Hainan province. Spurred on by this success, the team then went on to purify the active ingredient, known as *qinghaosu*, or artemisinin, and characterized its surprising structure as a sesquiterpene lactone in 1975.

Road to a Medicine. The work was only published in 1977, by the "Collaboration Research Group for Qinghaosu" (listing no individual contributors) in Chinese (6); however, by 1981, it was presented at the WHO working group meeting on chemotherapy and entered the mainstream literature in 1982 (7). By then, the conflict in Vietnam was over, and the control of malaria in Hainan province had also been mainly achieved using older medicines that were based on quinine. Herbal medicine from Pre-Columbian Peru had delivered quinine, but medicinal chemistry improved on quinine to make chloroquine and its followers. The path was long from the scientific breakthrough discovery of artemisinin to a new medicine that would save the lives of millions of children, whereas older antimalarials were lost one by one to resistance (8). New variants of artemisinin were developed, such as the methyl ether artemether, which is soluble in oils. Later, the hemisuccinate ester prodrug of artemisinin, artesunate, was developed. This derivative more easily produces stable formulations, and it is also more soluble than artemether. In 1994, Novartis signed an agreement with the Beijing Academy of Military Medical Sciences to develop artemether-lumefantrine. This process entailed many early clinical trials, and demonstrated that moving from a two-day regimen to a three-day regimen increased efficacy from 83% to well above the 95% seen today. Much work was needed to develop an appropriate formulation; these combinations had to be stable under conditions of high temperature

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and humidity for over two years. This work led to the medicine becoming available, and another landmark agreement with the WHO in 2001, which resulted in over 250 million treatments being delivered over the following seven years. Clearly artemisinin derivatives were a powerful ally, but knowing how readily other treatments had succumbed to resistance, the WHO decided to advise against the use of artemisinin monotherapy, with WHO member states adopting World Health Assembly resolution WHA60.18 in 2007. Artemisinin was to be used in fixed-dose combinations, for its own protection, with other existing medicines, such as lumefantrine and amodiaquine, and, later, mefloquine, piperaquine, and pyronaridine. These medicines have now been developed by the pharmaceutical industry in product development partnerships with the Medicines for Malaria Venture (MMV) and Drugs for Neglected Diseases *Initiative* (reviewed in 9). All these products have now been approved by a stringent regulatory authority and are available at low prices to developing countries, with their quality underscored by the WHO's prequalification program (apps.who.int/prequal/query/ProductRegistry.aspx). A reasonable estimate is that a billion treatments have been produced, and these treatments have had a massive impact on malaria disease-endemic countries. The latest efforts aim at developing more child-friendly formulations, with taste-masking and sweetening to mask the bitter taste of both artemisinin and the partner drug.

Even though the cost of a medicine to treat a child is between $0.25 and $0.60, this cost is still far higher than the cost of earlier generation medicines, such as chloroquine. Two directions have been taken to reduce the bulk price below its long-term average of $400 per kilogram. First is the generation of semisynthetic artemisinin: although de novo synthesis is still too complex to be competitive, despite progress in this area (10), the precursor, artemisinic acid, can be made in genetically engineered yeast cells (11) and converted into artesunate. Although this process is not cheaper than producing the drug from cultured *Artemisia* plants, it means that the lead time to generate large batches of material can now be measured in weeks rather than the usual 18 months. This drug availability should lead to the end of the wild price swings in raw material costs over the past decade. An alternative approach was to design and synthesize new synthetic endoperoxides, originally pioneered at Roche in the 1990s (12), leading to the development of OZ277 and OZ439 in an academic collaboration coordinated by the MMV (13). OZ277 has been launched in India as part of Synriam by Ranbaxy; OZ439 is in clinical trials (14).

Severe Malaria, Chinese Medicine from China. Artemisinin has an extremely rapid mode of action, and so it clearly also had potential for treating severe malaria, where the blockade of brain capillaries with infected erythrocytes may result in coma and death. Two major clinical studies showed that i.v. artesunate was superior to i.v. quinine in terms of lives saved, both in Africa and Asia (15, 16). The challenge back in 2009 was to ensure a high-quality producer of artesunate for injection. Guilin Pharmaceuticals took on the challenge, and for the past five years, it has been the world's sole producer of artesunate for injection for the treatment of severe malaria. With over 30 million vials delivered over the past four years, potentially saving almost 200,000 lives, Chinese manufacturing has therefore come to the aid of a Chinese product, completing the cycle.

So How Does It Work? Using MS, Meshnick et al. (17) discovered that artemisinin generates heme adducts, which may interfere with the malaria parasite's detoxification/polymerization of heme following hemoglobin digestion. Several other molecular targets have been proposed, including *Pf*ATP6 (18), and it is likely that artemisinins have multiple modes of action associated with their free radical-generating capabilities. This pleiotropic activity likely contributes toward the drug's resilience against the emergence of resistance. Nevertheless, in 2008, the first reports of resistance to artemisinin came in from the Thai-Cambodian border region (19, 20), although later longitudinal studies show that resistant *Plasmodium* strains already existed almost a decade earlier (21). It took several years before genetic markers could be found that correlate with resistance, all of which, so far, occur in one genetic locus, Kelch13 (22). The phenotype of resistance is still subtle, resulting in a twofold increase in the time artemisinin takes to clear the parasite in patients. This fact makes the task of linking the genetics to the molecular mechanism of resistance a considerable task, and one that is still to be completely solved (23).

In addition to its activity against malaria, artesunate and dihydroartemisinin may have anticancer activities. An effect on tumor cells has been described by many authors (24), although only a few have followed this reported effect up with clinical trials (25). Two researches, Krishna and Kumar of St. George's University in London, have recently initiated a crowd-sourcing campaign to test artesunate in bowel cancer.

The path to the discovery of artemisinin illustrates several important points. It emphasizes the importance of early testing of decoctions for their activity in patients. This concept was suggested in 1952 by Chen Guofu, labeled *dao-xing-ni-shi*, or "acting in the reversed order"; namely, clinical and then animal studies, structure elucidation, resynthesis, and retesting, followed by structure optimization (26). The idea that a simple active ingredient could be identified from traditional Chinese medicine required being able to see the world from both the traditional Chinese perspective and a Western approach, and holding those two ideas in tension.

Youyou Tu. Image courtesy of Han Haidan/CNSPHOTO/ChinaFotoPress via Getty Images.

Linking both discoveries, the "wonder drug" ivermectin, incredibly, is a double-edged sword that also kills malaria-transmitting mosquitoes that feed on individuals who have taken the medicine, preventing transmission of the disease (27, 28). Due to this activity as an endectocide, the drug has been proposed for use in vector control and malaria elimination (29).

The omitted text in the earlier citation from Nobel's will adds "...during the preceding year. . .," a clause that is wisely ignored by Nobel Committees. Even with 40–50 years of hindsight since the discovery of these two drug classes, it is difficult to quantify their impact precisely in terms of millions lives saved, and cases of blindness and other misery averted. A recent analysis estimated that in Africa alone, artemisinin combination therapies have averted 146 million malaria cases since 2000 (30). Nobel Prizes are rarely scored by impact, but were there ever to be a popular vote, this year's Prize in Medicine would be outranked by few others.

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