A Splendid Gift from the Earth: The Origins and Impact of the Avermectins

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by Satoshi Ōmura

Kitasato University, Tokyo, Japan.

The origin of one of the world's foremost, revolutionary, versatile yet relatively unknown drugs lies in Japanese soil—literally and metaphorically. Ivermectin, a multipurpose drug derived from a single microscopic organism discovered in Japanese soil, is being taken free of charge annually by over 250 million people—twice as many people as the entire Japanese population. Its impact on improving the overall health and welfare of hundreds of millions of men, women and children, mostly in poor and impoverished communities, remains unmatched. It continues to defy many preconceived concepts, with no drug resistance developing in humans despite years of extensive monotherapy. This has led to it being included on the World Health Organization's "List of Essential Medicines," a compilation of the most important medications needed in any basic health system. Several international public health experts have also taken the unprecedented step of recommending mass administration of ivermectin to all members of often polyparasitised communities in developing countries as a simple, prophylactic and curative public health intervention [1].

Ivermectin, along with its parent compound, avermectin, are both extremely broad-spectrum antiparasitic agents. Ivermectin is among those few compounds, such as penicillin and aspirin, delineated as 'wonder drugs', all, incidentally, originating from natural products. It is also predominantly a drug for the poor. It is

being increasingly used to eliminate intractable tropical diseases, as well to tackle an ever-increasing range of diseases, and is showing promise to provide a solution to hitherto indomitable public health problems. It remains the most potent anti-infective agent in clinical use; the safe single adult dose of around 12 mg once a year comparing favourably with antibiotics like penicillin and tetracycline that require doses of 1000 mg or more per day.

The avermectins, and the derivative ivermectin, were identified in the mid-1970s. The discovery was exceptional, as avermectin represented the world's first 'endectocide', a term specially created to describe the compound which was capable of killing a wide variety of parasitic and health-threatening organisms both inside and outside the body. The avermectins were found to be 2- to 3-fold more potent than compounds in use at the time. Moreover, ivermectin was found to be effective orally, topically or parentally and showed no signs of cross-resistance with commonly used antiparasitic agents [2–4]. Since its discovery, the benefits of ivermectin in terms of global public health and socioeconomic welfare, direct and indirect, have been immeasurable and they continue to accumulate. The discovery occurred at a time when the international community was focussing attention on disregarded and seemingly unconquerable diseases which had been plaguing resource-poor populations throughout the tropics for centuries. The advent of the antiparasitic ivermectin provided a safe, simple and effective solution, now driving several of those seemingly invincible tropical diseases to the brink of eradication.

In science, as elsewhere, it is individuals who are the true agents of change. The discovery of the avermectins was the result of a novel international multidisciplinary research project between a public sector institution (Japan's Kitasato Institute) and a private sector pharmaceutical company (the US-based Merck, Sharp and Dohme). But the successful history of this pioneering public private partnership has been dependent upon the unwavering commitment, ability and quality of scientific and cultural exchanges among the team of exceptional individuals involved, all of whom managed to overcome differences in nationality and working practices and sometimes differing goals.

Likewise, the availability of and access to the drug, its distribution and its enormous and widespread beneficial impact has been dependent upon a combination of an unprecedented drug donation programme plus an exceptional, ground-breaking multifaceted international partnership incorporating, among others, the public and private sectors, multilateral agencies, donor organisations, governments, non-governmental organisations, scientists, health workers and entire disease-affected communities.

IVERMECTIN: PREPARING THE GROUND

In Japan, the Kitasato Institute (KI), founded in 1914 by Shibasaburo Kitasato, known as the father of serotherapy and nominated for the first Nobel Prize in 1901, has long been recognised as a world-leading centre for the discovery of drugs and vaccines, primarily those derived from natural sources. Investigative research and development of chemotherapeutic drugs for practical use is a fundamental core of the work of the institute. Over 100 years ago, in a pioneering breakthrough, Sahachiro Hata, working with Paul Ehrlich, developed salvarsan, a remedy for syphilis, which was a major global health problem at the time. Salvarsan is widely recognised as the world's first chemotherapeutic drug. In the 1930s, Zenjiro Kitasato performed research into plant alkaloids and terpenoids which later led to development of the antitussive compound sapogenin. In the late-1940s, Toju Hata conducted research on antibiotics produced by microbes, leading to discovery of leucomycin in 1953 and the anti-cancer compound mitomycin in 1956.

In the mid-1960s, from a background in studying aspects of fermentation and having garnered significant experience in using the at-the-time novel and nascent Nuclear Magnetic Resonance (NMR) spectroscopy to determine the structure of organic compounds, I was fortunate to join the institute's illustrious alumni, who also include Kiyoshi Shiga and Hideyo Noguchi.

Shortly after joining the KI, having worked on identifying the chemical structure of a handful of compounds, I realised that I could only identify hard-to-find compounds that others had spent a great deal of time, effort and expertise in discovering. Consequently, I decided to challenge myself to actually undertake the discovery process, which was fundamental to identifying new compounds and microbial metabolites, as well as investigate their structure and possible bioactive properties. To that end, coming from a farming family background through which I had developed a profound respect for Nature and its role as a primary source of most of the materials we need for survival, I opted to concentrate on soil microorganisms. Soils often contain 10^9 to 10^{10} microorganisms per gram (dry weight), which possibly represents in excess of 1 million bacterial species [5] and, in my experience, around one third of soil samples tested produce antimicrobial substances. Unfortunately, there is no accepted "Gold Standard" method for isolating and identifying soil bacteria or other microorganisms. A serial dilution and spread-plate method is a reasonably good starting point for isolating bacterial colonies from soil but even at this early stage, the choice of isolation medium is critical and depends on the specific goals. Consequently,

devising mechanisms to cope sensibly with this enormous diversity is essential. As a result I refocussed my research on the search for new antibiotics and other biologically interesting microbial metabolites, such as growth factors, enzymes and enzyme inhibitors, based on my conviction that new and innovative screening systems were the key to discovering new compounds—a belief that I have steadfastly maintained to this day.

In science, knowledge and understanding no longer appear quickly. Time, patience, trial and error are all essential ingredients in any screening process. Most screening systems retain their effectiveness but, over the years, I have devised and implemented one or two new screening mechanisms annually, discarding existing systems when resources did not permit them to be kept in operation. Generally, we now routinely have at least 10 customised screening systems operational.

Although many screens prove successful, others do not yield the results envisaged, although this does not mean they are non-functional. In this matter I have always been guided by the words of Louis Pasteur: 'Chance favours the prepared mind'. I believe that this is the key to investigating and unravelling the mysterious world and secrets of microorganisms. This is the mindset that I have always followed and which has allowed Nature to reveal to me almost 500 microbial metabolites that have unique or useful bioactive properties, several of which have proved of incalculable benefit, direct and indirect, to humankind (Fig. 1).

The painstaking work at the KI involves many of the first steps on a long road to creation of a successful drug or useful chemical reagent. We take samples from Nature that contain microoganisms. We then allow the microbes in the sample to grow on agar media plates, slowly cultivating them to produce a pure strain, making sure that we concentrate on novel types. The organism and strain are

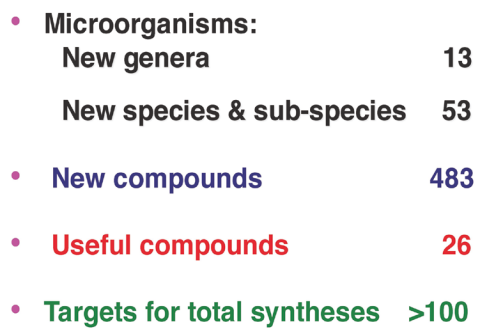


FIGURE 1. Discoveries (1965–2014).

then identified, grown in liquid culture and a culture broth is formed. We carry out initial assays on the broth, including an initial metabolite analysis. In the case of the microbe that was the origin of ivermectin, for example, we identified that it also produced a toxic compound, oligomycin, knowledge that proved to be of great value with respect to explaining toxicity problems during later tests in animal models. Once these initial steps have been completed, we can scale up using a jar fermenter which facilitates clearer identification of the organism and its preservation, as well as purification and structural analysis of any promising compound (Fig. 2). We then conserve all microorganisms and compounds in our libraries for future testing and evaluation, either by KI scientists or others.

Generally, during our routine discovery work, we deliberately select unusual microorganisms with the intent to maximise the chances of finding new compounds. In addition, we generally do not have a single, specific objective, preferring to apply initial screens for a variety of bioactive properties. The characteristics of the microbe that we isolated and cultured at the Kitasato Institute and which produced the avermectins were unique and were critical elements in the discovery process [6].

From the outset of my research, I determined that it was highly useful to identify not just a new compound but also the microbe that produced it, usually

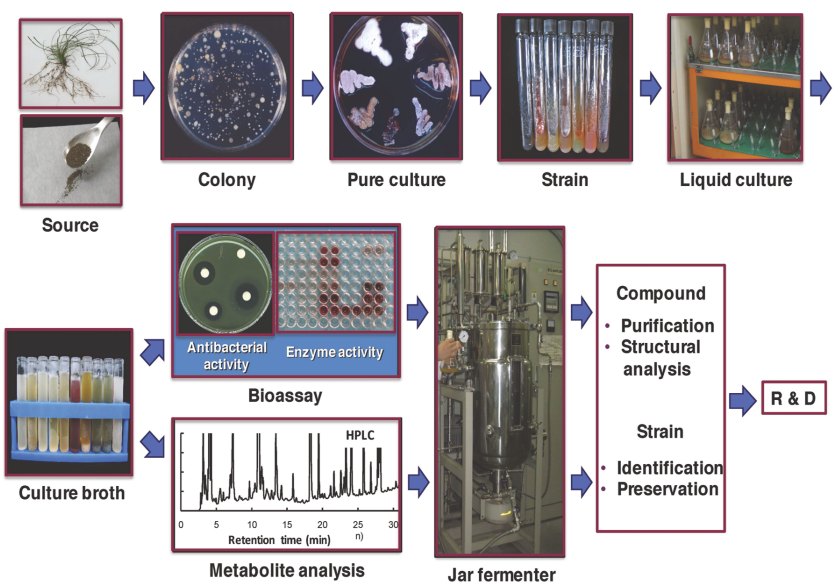


FIGURE 2. Screening for new bioactive compounds.

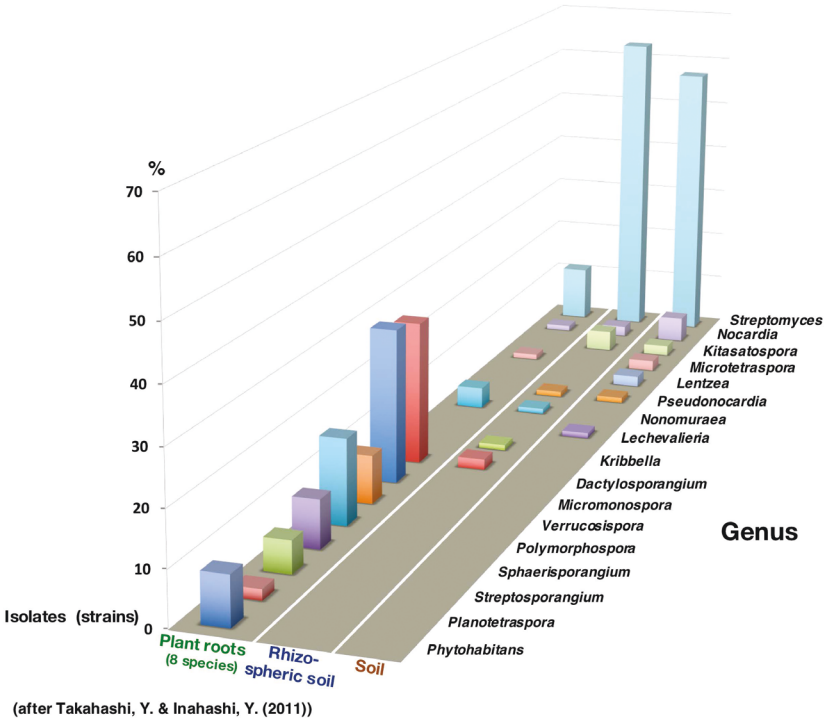
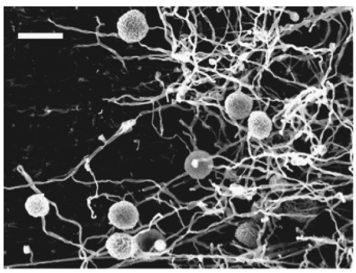


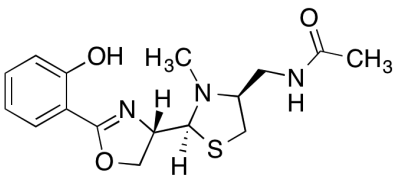
FIGURE 3. Actinomycetes (from plant root and soil samples).

placing both together in a visual presentation, a tradition that I shall maintain in this article. We have attempted to isolate microbes from every kind of natural environment, primarily from soil and latterly from seaweed, plant leaves and plant roots. Figure 3 shows the diversity of microorganisms that have been isolated from plants roots as opposed to soil and provides an indication of how the source can significantly impact the type of microbe found. For example, we have recently identified two new compounds, spoxazomycin (Fig. 4) [7], which displays antitrypanosomal activity, and trehangelin (Fig. 5) [8], a photo-oxidative hemolysis inhibitor from plant root origins.

It goes without saying that all microbes and chemicals are small, well beyond human visual acuity. It therefore seemed sensible to find mechanisms that would clearly signal the presence of something new or potentially useful. Mindful of the fact that, throughout human history, alkaloids, mostly from plant sources, have been the mainstay of traditional medicine, I decided to introduce a new method of 'chemical screening'. This entailed a search and isolation method to identify organic compounds in fermentation broths employing a simple colour-change

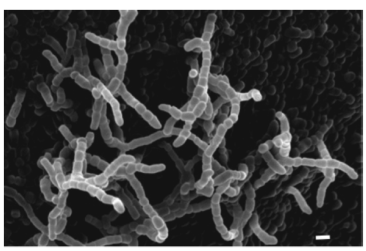


Streptosporangium oxazolinicum K07-0460^T
(Bar: 10 μm)

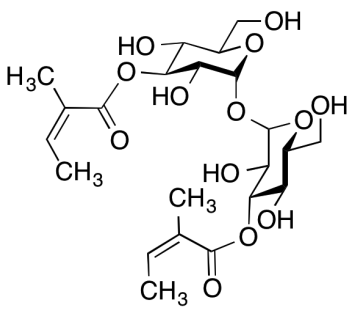


Spoxazomicin A

FIGURE 4.



Polymorphospora rubra K07-0510
(Bar: 1 μm)

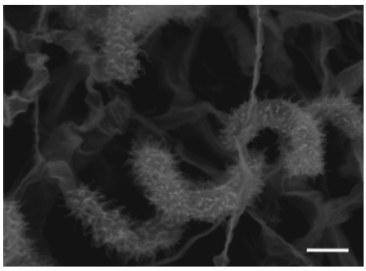


Trehangelin A

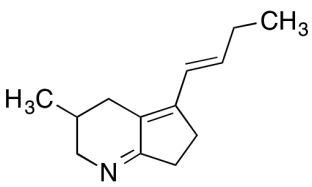
FIGURE 5.

reaction. I decided to utilise a simple mechanism using Dragendorff's reagent. Alkaloids, if present, react with the reagent, which contains bismuth nitrate and potassium iodide, to produce an easily visible orange or orange red precipitate.

We implemented this screening system in 1968, based on my profound belief that microorganisms never engage in futility; it is just our lack of knowledge and vision that prevents us from understanding what they produce, how and for what purpose. The first compound isolated through this chemical screening system was the antimicrobial pyrindicin (Fig. 6) [9]. Of far greater significance, in 1977 we isolated the world's first naturally-occurring indolocarbazole compound, staurosporine, produced by *Streptomyces staurosporeus (Lentzea albida)* (Fig.7) [10, 11]. Nine years later, Dr T. Tamaoki found that staurosporine possessed the ability to potently inhibit the functioning of protein kinase C (PKC), the first such compound identified to do so. PKC is a family of enzymes that cause increased expression of oncogenes, thereby promoting cancer progression [12].

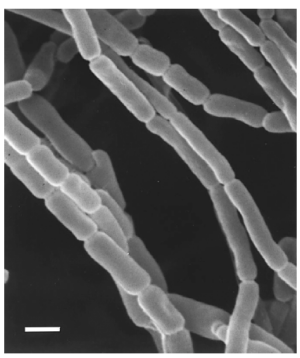


Streptomyces griseoflavus subsp. *pyrindicus* NA-15^T (Bar: 1 μm)

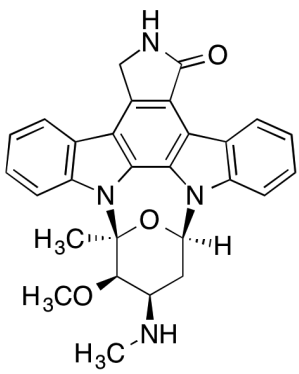


Pyrindicin

FIGURE 6.



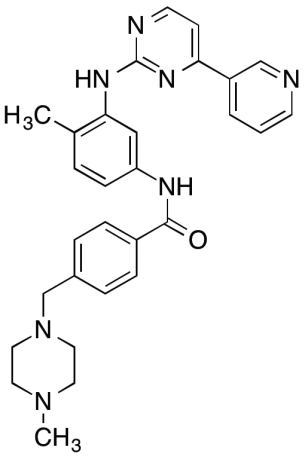
Saccharothrix aerocolonigenes subsp. *staurosporeus* AM-2282^T (*Lentzea albida* AM-2282) (Bar: 1 μm)



Staurosporine

FIGURE 7.

Almost immediately, staurosporine became one of the world's most prominent research reagents of microbial origin and proved to be the forerunner of many of the recently introduced anti-cancer agents. For example, the development of imatinib (Gleevec®) (Fig. 8) has been directed and influenced by the unique chemical structure and biological activity of staurosporine [13]. For me, the discovery of staurosporine was a significant milestone, not just because of its major impact in science and biomedicine, but because it was a vindication of my beliefs that microorganisms offer a virtually unlimited panoply of beneficial products. It is simply a matter of us finding ways to identify and apply them for the good

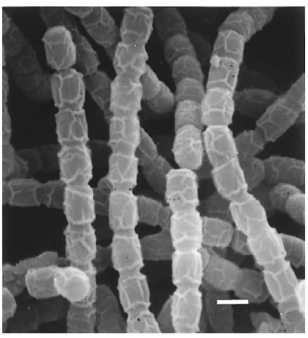


Imatinib (Gleevec®)

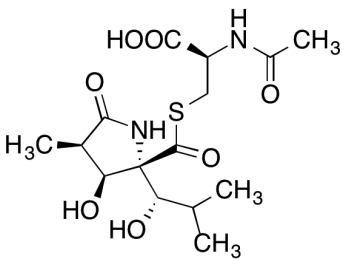
FIGURE 8.

of human society. I also firmly believe that the work that I accomplish and the compounds identified and stored can be taken forward or exploited by others for the good of all.

Another novel screening system led to the discovery of lactacystin (Fig. 9), an inhibitor of proteosomes. Lactacystin was found via a method involving induction of neurite outgrowths in Neuro2a, a cell line of murine neuroblastoma cells [14]. This compound proved to be the forerunner for the anticancer agent bortezomib (Fig. 10) (Velcade®).

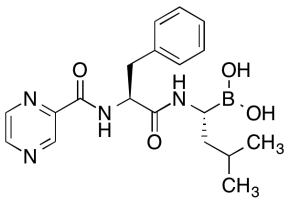


Streptomyces lactacystinicus OM-6519^T
(Bar: 1 μm)



Lactacystin

FIGURE 9.



Bortezomib (Velcade®)

FIGURE 10.

The experience, techniques and knowledge gained at the KI in isolating microorganisms, cultivating them, identifying them and then determining the compounds they produce, analysing the chemical structure and elucidating their biological or chemical properties provided an optimal basis for the discovery of ivermectin. However, although we possessed the skill and expertise to discover novel microorganisms and chemicals we had neither the techniques nor the resources to carry out the requisite research and development essential for taking a promising compound though the extremely expensive and often disappointing drug production pipeline. To accomplish that task requires the commitment and extensive resources of a major commercial partner.

Wesleyan University
USA (1972)

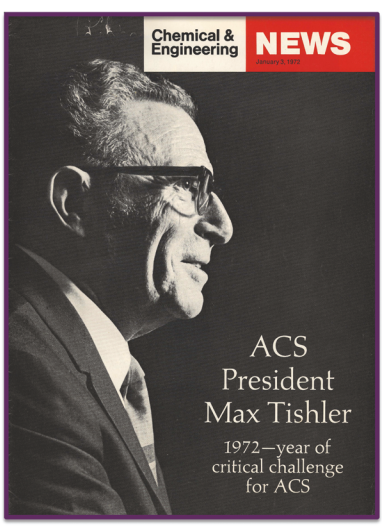
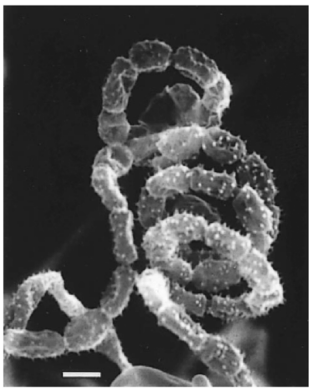


FIGURE 11. Ivermectin: the beginning.

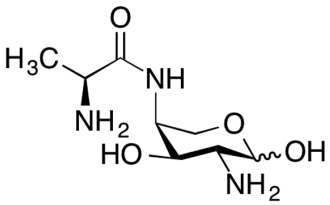
IVERMECTIN: THE BEGINNINGS

In the early 1970s, Professor Yukimasa Yagisawa, General Manager of the Japan Antibiotics Research Association (JARA), encouraged me to exploit the possibilities for research work overseas and the benefits it could provide for both myself and for Japan. He kindly introduced me to key individuals in his network of overseas connections and, as a consequence, in 1971, I was granted a sabbatical that allowed me to take up an invitation from Prof Max Tishler to work as Visiting Research Professor in his newly-formed Chemistry Department at Wesleyan University (Fig. 11.). Max, who almost immediately became President of the American Chemical Society (ACS), had established the department following retirement from his position as President of the Merck Sharp & Dohme Research Laboratory (MDRSL), where he had had a long and distinguished career. My initial work in his laboratory focused on the structural analysis of a new antibiotic, prumycin (Fig. 12)[15] that I had discovered prior to my departure from Japan, as well as on the structure/activity relationships of macrolides [16] and the mode of action of cerulenin (Fig. 13). The contribution that both of these individuals made to my development, as a scientist, educator, and individual, has been inspirational and beyond measure.

My intended stay in the US was curtailed, as I was recalled to head the Research Department at the KI, following the retirement of the then director, and I returned in early 1973. In view of my impending return, and extremely mindful of the critical need to obtain funds to support research work in Tokyo

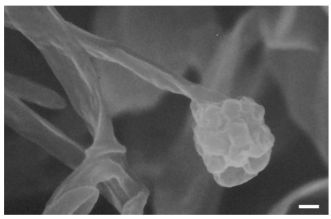


Streptomyces kagawaensis F-1028^{T}
(Bar: 1 μm)

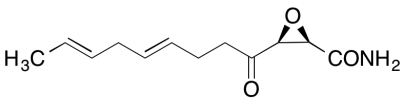


Prumycin

FIGURE 12.



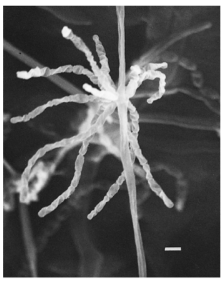
Cephalosporium caerulens KF-140^T
(*Sarocladium oryzae* KF-140)
(Bar: 5 μm)



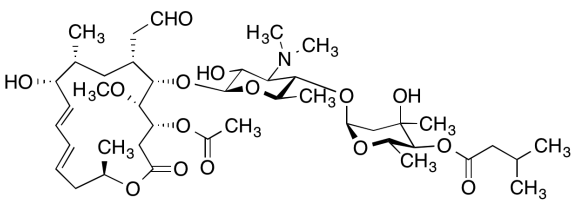
Cerulenin

FIGURE 13.

after I returned, I visited many major US pharmaceutical companies, presenting a proposal for a collaborative research project. I was greatly encouraged because, as I had previously discovered several antibiotics, such as the aforementioned prumycin (an antifungal agent) [17] and cerulenin (an antifungal and inhibitor of fatty acid biosynthesis) [18], as well as leucomycin A_3 (an antimicrobial) (Fig. 14) [19], all of the companies were supportive. At the time, Max, who knew my work and ideas very well, discussed my plan with Dr L.H. Sarett, Max's successor at MSDRL with whom he had worked closely for many years. Max's close connection with Merck and his personal linkage to Dr Lew Sarrett expedited my research collaboration with the MSDRL, which commenced in April 1973. Individuals who played key roles in the alliance are shown in Figure 15. Initially, the goal was to find growth promoting antibiotics suitable for animals, enzyme inhibitors and general purpose antibiotics produced by microorganisms, but the work soon expanded to encompass other targets.



Streptomyces kitasatoensis KA-6^T
(Bar: 1 μm)



Leucomycin A_3

FIGURE 14.

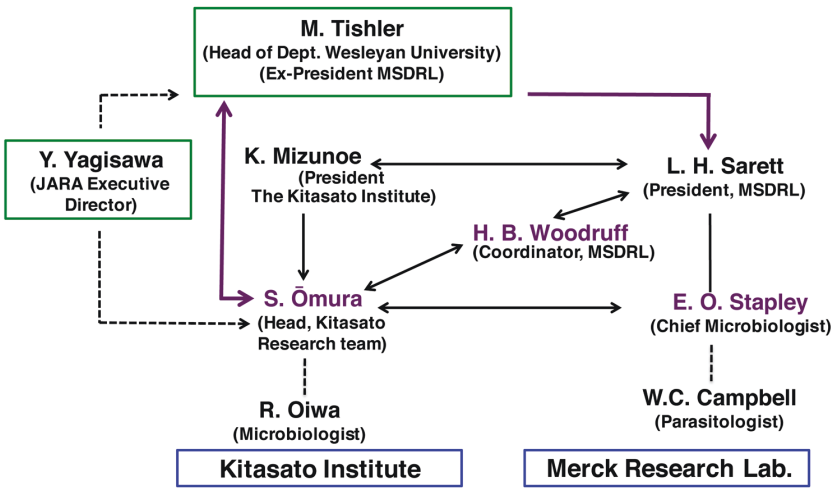


FIGURE 15. Kitasato—MSDRL Collaboration (1973).

IVERMECTIN: THE ADVENT AND USE IN ANIMALS

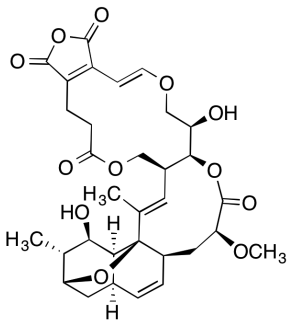
As the basis of the research initiative, the KI carried out isolation of what we identified as extraordinary microorganisms, culturing them and then undertaking preliminary in vitro evaluation of the bioactivity of any compounds we deemed to be of potential interest, prior to sending the most promising, from our existing library and from newly identified specimens, to MSDRL for in vivo testing.

As a result of the collaboration, a variety of compounds were discovered, the majority exhibiting a range of interesting biological activities and structures. These included luminamycin (Fig. 16) [20], an anti-anaerobic bacterial, vineomycin A_1 (Fig. 17) [21] and setamycin (Fig. 18) [22], both of which have unique structures; elasnin (Fig. 19) [23], the first human elastase inhibitor of microbial origin; and factumycin (Fig. 20), a growth promoting antibiotic for veterinary use [24].

Of far greater importance was avermectin. Simply put, avermectin proved to be one of the world's most remarkable biomedical discoveries, being accompanied by a number of world 'firsts' and having an immeasurably beneficial impact on animal and human health worldwide.

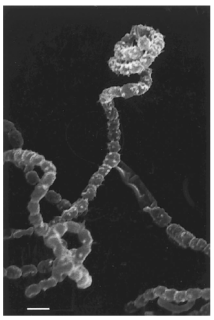
As part of their new in vivo evaluation, and following a suggestion from Max Tishler, the MSDRL introduced a new programme to screen fermentation broths that we identified as being promising [25]. This was done because there was

Streptomyces sp. OMR-59
(Bar: 1 μm)

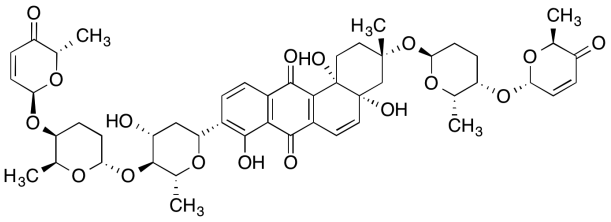


Luminamicin

FIGURE 16.

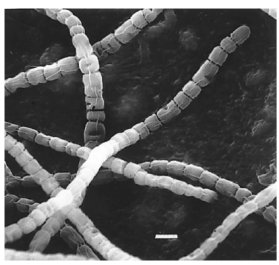


Streptomyces matensis subsp.
vineus OS-4742^T (Bar: 1 μm)

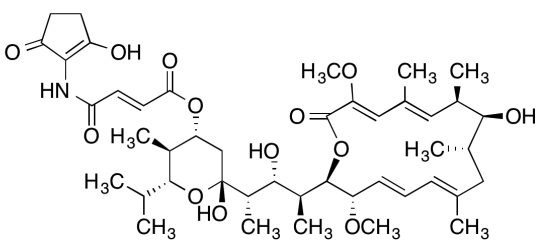


Vineomycin A_1

FIGURE 17.

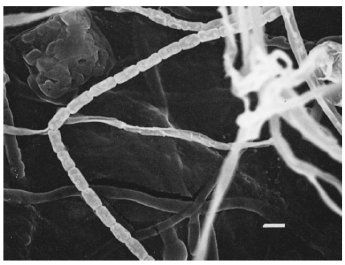


Kitasatospora setae KM-6054^T
(Bar: 1 μm)

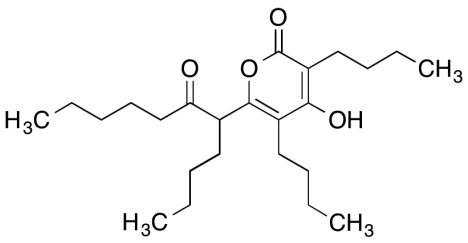


Setamycin

FIGURE 18.

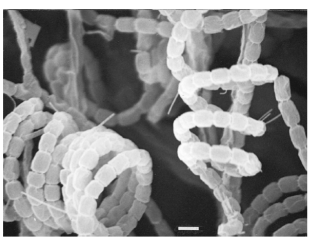


Streptomyces noboritoensis KM-2753
(Bar: 1 μm)

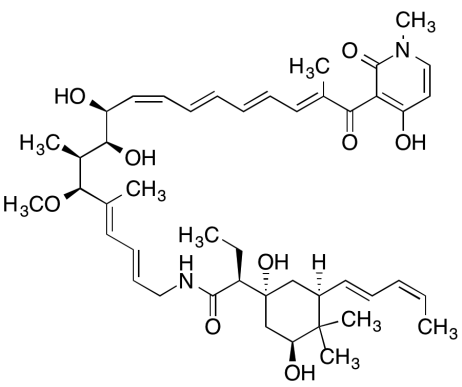


Elasnin

FIGURE 19.



Streptomyces lavendulae OS-4369
(Bar: 1 μm)



Factumycin

FIGURE 20.

confidence that our broths likely contained interesting compounds. In addition, adding a fermentation broth to the feed of a single animal means it can be tested simultaneously for both efficacy and toxicity, often with results appearing in a week rather than the weeks or months that are usually needed using in vitro tests.

MSDRL researchers screened our microorganisms, which were produced according to our description of the necessary fermentation conditions, the fermentation broths being tested in a novel model of helminth (parasitic worm) infection in which mice were infected with the nematode worm *Nematospiroides dubius (Heligmosomoides polygyrus bukeri)* [26, 27]. In one of the first 50 specially selected microorganisms we sent in 1974, Dr William Campbell and his team found an actinomycete, strain MA-4680, which produced a compound possessing excellent anthelmintic activity with little or no toxicity. The unpurified broth killed all intestinal worms and removed all signs of parasite eggs from the animal's faeces.

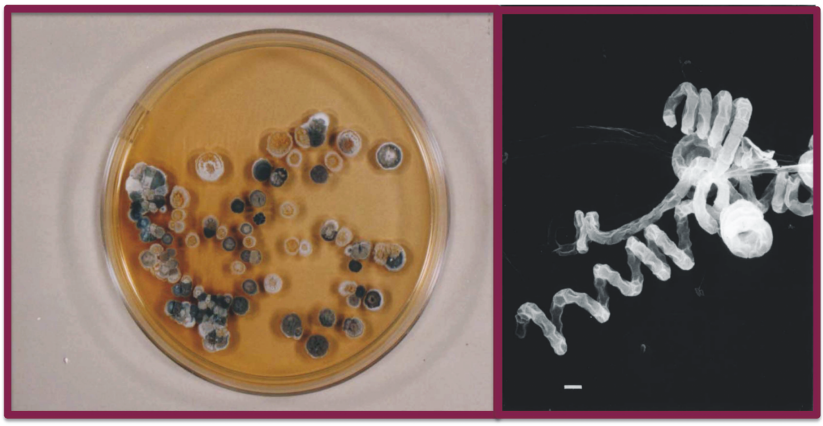
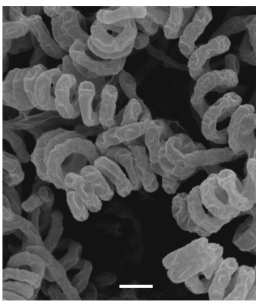


FIGURE 21. The avermectin producing strain.

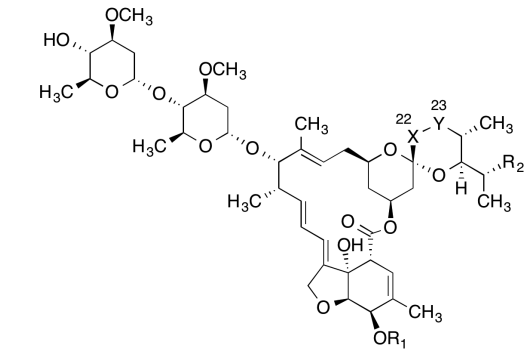
The producing microorganism (Fig. 21) was originally named *Streptomyces avermitilis* MA-4680 but, in 2002, based on characterization of the original strain and morphological and phylogenetic comparisons, including 16S rDNA sequencing, with closely related members of the genus *Streptomyces*, it was proposed that the organism was in fact a new species and renamed *Streptomyces avermectinius* [28].

After a few trials to confirm the bioactivity findings, isolation chemists were engaged to identify the causal entity. The active ingredient of the broth was identified and named avermectin, which MSDRL chemists found to be a complex mixture of 16-membered macrocyclic lactones, fermentation of *S. avermectinius* producing a mixture of eight avermectin compounds (A1a, A1b, A2a, A2b, B1a, B1b, B2a and B2b) (Fig. 22). Compounds of the B-series containing a 5-hydroxyl group are markedly more active than those of the A-series, which contain a 5-methoxyl group. The four main components, avermectin A1a, A2a, B1a and B2a, constituted 80% of the mixture, with the rest composed of four lower homologs A1b, A2b, B1b and B2b. The structure of the compound was also swiftly elucidated and it was fast-tracked for development [29].

In 1979, the first papers on the avermectins were published, describing the chemicals as a series of macrocyclic lactone derivatives possessing extraordinarily potent anthelmintic properties [30–32]. Up until that time, only a handful of the several thousand microbial fermentation products discovered exhibited



Streptomyces avermectinius (*S. avermitilis*) MA-4680^T (Bar: 2 μm)



Avermectin A_{1a} [$R_1 = CH_3$; $R_2 = C_2H_5$; X-Y = ⌒]
A_{1b} [$R_1 = R_2 = CH_3$; X-Y = ⌒]
A_{2a} [$R_1 = CH_3$; $R_2 = C_2H_5$; X-Y = CH_2-CH(α-OH)]
A_{2b} [$R_1 = R_2 = CH_3$; X-Y = CH_2-CH(α-OH)]
B_{1a} [$R_1 = H$; $R_2 = C_2H_5$; X-Y = ⌒]
B_{1b} [$R_1 = H$; $R_2 = CH_3$; X-Y = ⌒]
B_{2a} [$R_1 = H$; $R_2 = C_2H_5$; X-Y = CH_2-CH(α-OH)]
B_{2b} [$R_1 = H$; $R_2 = CH_3$; X-Y = CH_2-CH(α-OH)]

Ivermectin: mixture of dihydroderivatives of
B_{1a} [$R_1 = H$; $R_2 = C_2H_5$; X-Y = CH_2-CH_2]
B_{1b} [$R_1 = H$; $R_2 = CH_3$; X-Y = CH_2-CH_2]

FIGURE 22.

any anthelmintic characteristics. Although structurally similar to macrolide antibiotics and antifungal macrocyclic polyenes, the avermectins did not demonstrate any antibacterial or antifungal activities.

An interdisciplinary team at MSDRL, headed by William Campbell, further investigated the eight active compounds, of which avermectins B1a and B1b were found to have the highest activity. Reduction of the C22–C23 double bond of B1a and B1b compounds with Wilkinson's catalyst improved both the spectrum of activity and safety and the resulting 22,23-dihydro B1 complex (as a mixture of 80% B1a and 20% B1b) was selected for further commercial development under the generic, non-proprietary name ivermectin [33].

The avermectins proved to be effective against roundworms of the intestinal and respiratory tracts as well as filarial parasites [34] and demonstrated biocidal activity against a diverse range of nematodes, insects and arachnids. The mode of action turned out to be both unique and robust, and was 25 times more potent than all currently available anthelmintics. Further analysis revealed that ivermectin was highly efficacious against mite, tick and botfly ectoparasites, organisms that cause massive economic losses in the livestock industry. MSDRL researchers also observed that the compound had remarkable activity against external and internal parasites in horses, cattle, pigs and sheep, effective against, among others, gastrointestinal roundworms, lungworms, mites, lice and

hornflies. It was also found to be successful in treating larval heartworms in dogs, but not adult worms, and could be used to treat mange and other conditions in canines. However, no activity was found against flatworms, protozoa, bacteria or fungi [35–38].

The avermectins' broad spectrum of activity, wide therapeutic index, and novel mode of action resulted in them being introduced onto the animal health market in 1981. Two years after their introduction, avermectin-derivative products became the international veterinary sector's biggest seller, accruing annual sales income of around $1 billion, a position maintained for a quarter of a century, the ivermectin-based parasiticide products reportedly becoming MSD's fifth best-selling product group [39].

MSDRL research staff and others around the world have exhaustively searched since the original discovery but no other avermectin-producing organism has ever been found. The strain that we isolated from a single soil sample collected near a golf course bordering the ocean at Kawana in Ito City in the Shizuoka region of Japan remains the only avermectin-producing organism ever found.

Dr Boyd Woodruff of MSDRL was appointed to work alongside our team at the KI in Tokyo, and I am convinced that his personal commitment and expertise were significant factors in making the collaboration such a great success.

IVERMECTIN: MODE OF ACTION

The avermectins potentiate neurotransmission by boosting the effects of glutamate at invertebrate-specific glutamate-gated chloride channels, with minor effects on gamma-aminobutyric acid (GABA) receptors.

In parasites, neurotransmission inhibition occurs via glutamate-gated chloride channels in nerve and muscle cells, preventing their closure [40]. This leads to hyperpolarisation of the neuronal membrane, inducing paralysis of the somatic muscles, particularly the pharyngeal pump, killing the parasite [41, 42]. GABA-related (chloride) channels are commonplace in nematodes, insects and ticks [43–45]. In mammals, GABA receptors and neurons only occur in the central nervous system (CNS) and are thus not accessible [46], ivermectin being safe for vertebrates as it cannot cross the blood-brain barrier. Initial fears that ivermectin was contra-indicated in children under the age of five or who weighed less than 5 kg, where the drug might be able to cross the as yet not fully developed blood/brain barrier, were proven to be unfounded [47].

In humans, ivermectin exerts a peculiar and singular effect that remains poorly understood. The immune response to filarial infection is complex, involving Th2-type systems which counter infective L3 larvae and microfilariae,

whereas a combination of Th1 and Th2 pathways are involved in resisting adult worms. It is believed that female adult worms are able to manipulate the immunoregulatory environment to ensure the survival of their microfilarial offspring [48]. Ivermectin treatment of Onchocercal filarial infection causes microfilariae to quickly disappear from the peripheral skin lymphatics. The effect is long lasting, while adult female worms are prevented from releasing microfilariae [49]. Dermal microfilarial loads are reduced by 78% within two days, and by some 98% two weeks after treatment, remaining at extremely low levels for about 12 months. Female worms slowly resume release of microfilaria 3–4 months post-treatment, but at a mere 35% of original production [50]. Regular treatment consequently decreases incidence of infection, interrupts transmission and reduces morbidity and disability. However, the actual mechanism by which ivermectin exerts its effect on microfilariae remains unclear [51].

The half-life of ivermectin in humans is 12–36 hours. The lowest levels of dermal microfilariae occur well after this timeframe, meaning that not all microfilariae are killed in the early days, and microfilariae are known to migrate into deeper dermal layers, sub-cutaneous fat, connective tissue and lymph nodes following ivermectin administration [52]. It is now believed that ivermectin somehow prevents microfilariae from evading the immune system, resulting in the host's own immune response killing the immature worms [53, 54].

Ivermectin does not kill adult worms but suppresses the production of microfilariae by adult female worms, thereby reducing transmission. As the adult worms can continue to produce microfilariae until they die naturally, ivermectin has to be taken once annually for the 16–18 year adult worm lifespan in order to stop transmission.

Th2 responses instil protective immunity against both L3 infective larvae and the microfilaria stage but parasites are able to avoid these responses, which may help explain why drug resistance in parasites in humans has not yet appeared.

IVERMECTIN: DEVELOPMENT FOR HUMAN USE

In the mid-1970s, the global community mobilised itself to address the major problems of neglected tropical diseases. Following the setting up of the Onchocerciasis Control Programme in West Africa (OCP) in 1974, the UN-based Special Programme for Research & Training in Tropical Diseases (TDR) was established in 1975. Onchocerciasis and lymphatic filariasis were two filarial infections among TDR's eight target diseases, with onchocerciasis, at the time, being a major public health problem affecting 20–40 million people in endemic areas, predominantly in Africa (Fig. 23).

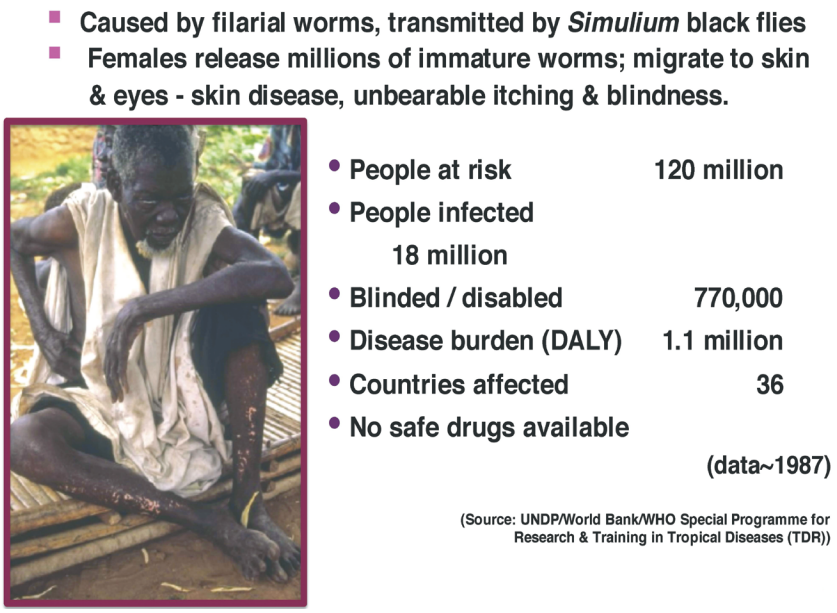


FIGURE 23. Human health goals: Onchocericiasis (River blindness).

Historically found primarily in 30 countries in sub-Saharan tropical Africa, onchocerciasis is caused by a nematode, *Onchocerca volvulus*, which lives for up to 15 years in the human body, female worms continually producing several millions of microfilaria during their lifetime, with the worms being transmitted to humans via the bite of a blood-feeding blackfly.

At the time, there were no safe and acceptable drugs available to treat onchocerciasis, which had plagued Africa for centuries, and nobody was interested in developing anti-*Onchocerca* drugs, as there was no apparent commercial market. Consequently, the OCP based its operations on expensive aerial spraying of insecticides to kill riverine vector fly larvae.

MSDRL scientists soon realised that the anthelmintic potency of ivermectin could help to conquer filarial diseases in humans and joined forces with WHO, nongovernmental organisations, international donors, governments and affected communities to drive forward evaluation of the drug [55].

Meanwhile, with respect to research needs, TDR identified that discovery of effective chemotherapeutic agents was the highest priority, with a macrofilaricide (capable of killing adult worms) substantially preferable to a microfilaricide (which would target immature worms) [56]. Research was hampered by the fact that *Onchocerca* species would not develop to maturity in any rodents, making it impossible to screen compounds against the target organism in a suitable animal

model. TDR established a tertiary screen, using cattle, for compounds showing positive results in any secondary screen, the screen being the best predictor of what a compound would do in humans, with well over 10,000 compounds being screened [57, 58].

In reality, ivermectin's role in human medicine began in 1978 inside the MSDRL, with William Campbell being the driving force behind the investigation of the potential for human use. Receiving very positive results after submitting ivermectin to the Australian cattle screen, he subsequently reported to MSDRL management that "an avermectin could become the first means of preventing the blindness associated with onchocerciasis" [59, 60].

In 1981, MSDRL's Mohammed Aziz, previously of the WHO, undertook a small clinical trial of ivermectin in patients with safety paramount. Commencing with a very low dose of 5 μg/kg, he found that a single dose of 30 μ/kg substantially decreased skin microfilariae and confirmed that the effect lasted for at least 6 months, with no serious adverse events. His tests concluded that doses up to 200 μg/kg were safely tolerated [61, 62].

Ivermectin proved to be ideal for combatting Onchocerciasis, which has two main manifestations, dermal damage resulting from microfilariae in the skin and ocular damage arising from microfilariae in the eye. Ivermectin proved to slightly increase microfilariae in the eye upon treatment, followed by a gradual reduction, reaching to near zero within six months. This meant little or no ocular damage. The large ivermectin molecule cannot cross the blood/aqueous humour barrier, stopping it entering the anterior chamber and directly killing or paralysing microfilariae [63]. This made ivermectin a perfect intervention for patients with ocular involvement.

Similarly, evaluation of the impact ivermectin on dermal microfilariae confirmed that it caused almost complete clearance within two days after treatment, reducing the load to virtually zero within eight days. Ivermectin also produces long-term suppression of circulating microfilariae, making it an ideal treatment for patients with dermal involvement [64].

Merck received approval from French authorities in 1987 allowing human use of ivermectin. In a hitherto unprecedented gesture, immediately following registration, ivermectin (branded as Mectizan®) was donated free of charge by Merck & Co. Inc., under the direction of Roy Vagelos (Fig. 24), for the treatment of Onchocerciasis (River Blindness), with KI foregoing all royalties. The donation was for as long as the drug was required, in the amounts that were needed. This represented the first such large-scale drug donation initiative and it has resulted in the world's largest, longest-running and most successful drug donation programme.

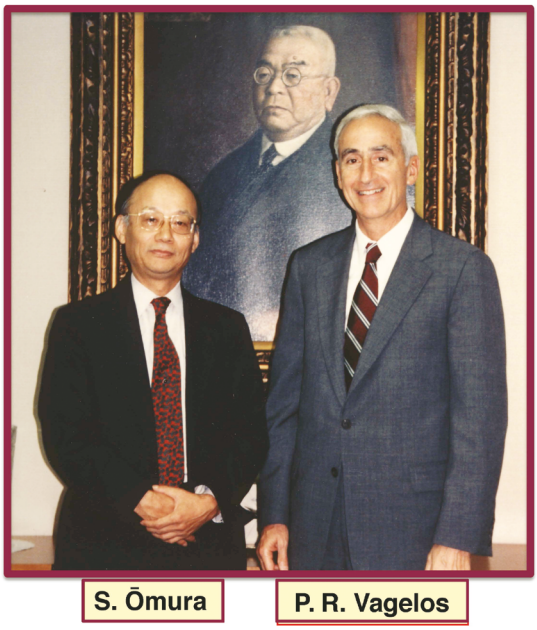


FIGURE 24. Ivermectin—world's most effective drug donation.

Introduced for use in the 11-nation OCP, ivermectin was not a cure. It did not kill adult parasites, a single annual dose simply suppressing symptom-causing onchocercal microfilaria in the skin and eyes and preventing the disease from progressing [65]. To prevent transmission, every eligible member of an affected community needed to take the drug. Ivermectin only kills immature worms, so entire communities in disease endemic areas have to take it for up to 15 years, until the adult female worms die naturally.

Massive clinical trials in Africa proved ivermectin to be a highly effective and safe microfilaricide, which need not be given more frequently than once annually, and showed that it has few side effects, which were dose-dependent, mild and short-lived, with no severe ophthalmological adverse events [66–68]. Ivermectin is very safe and can be given orally in the field by non-medical staff, meaning the drug is ideal for mass treatment programmes.

The African Programme for Onchocerciasis Control (APOC), established in 1995, built on the success of the OCP and extended community-wide mass drug administration (MDA) of ivermectin to 19 other African nations. APOC is recognised as a cost-effective, large-scale public health intervention of enormous significance, preventing an estimated 17.4 million years' worth of healthy

FIGURE 25. Ivermectin distribution.

life from being lost and freeing all African children taking ivermectin from the dangers of onchocercal blindness and skin disease [69].

In referring to the international efforts to tackle Onchocerciasis in which ivermectin is now the sole control tool, the UNESCO World Science Report concluded, "the progress that has been made in combating the disease represents one of the most triumphant public health campaigns ever waged in the developing world" [70].

The success of the campign to overcome Onchocerciasis is due to the sterling efforts and long-term commitment of a truly international, multidisciplinary coalition, some key partners of which are shown in Figure 25.

EFFECTIVENESS AGAINST OTHER FILARIAL DISEASES

Lymphatic filariasis, also known as elephantiasis, is another devastating, highly debilitating disease that threatens over 1 billion people in more than 80 countries (Fig. 26). An estimated 120 million people in tropical and subtropical regions are infected, 40 million of whom are seriously incapacitated. The disease results from infection with filarial worms, *Wuchereria bancrofti*, *Brugia malayi* or *B. timori*. The parasites are transmitted to humans through the bite of an infected mosquito and develop into adult worms in the lymphatic vessels, causing severe damage and swelling (lymphoedema). Adult worms are responsible for the major

disease manifestations, the most outwardly visible forms being painful, disfiguring swelling of the legs and genital organs. Around 25 million men have genital disease (most commonly hydrocoele) and almost 15 million, mostly women, have lymphoedema or elephantiasis of the leg. The psychological and social stigma associated with the disease is immense, as are the economic and productivity losses it causes.

With respect to the use of ivermectin for lymphatic filariasis, again MSDRL took the initial lead. In the mid-1980s, well before ivermectin was approved for human use to treat onchocerciasis, MSDRL scientists were undertaking trials of ivermectin to measure its impact against lymphatic filariasis and to find optimal treatment dosages [71]. Meanwhile, TDR was carrying out multicentre field trials in Brazil, China, Haiti, India, Indonesia, Malaysia, Papua New Guinea, Sri Lanka and Tahiti to evaluate ivermectin, the existing treatment drug, diethylcarbamazine (DEC), and combinations of the two. The results showed that single-dose ivermectin and single-dose DEC worked as well as each other. The combination, even at low dose, proved even more effective, decreasing microfilarial density by 99% after one year and 96% after two years [72–75].

Despite these findings, ivermectin remained unregistered for treatment of lymphatic filariasis until 1998 when approval was granted by French authorities.

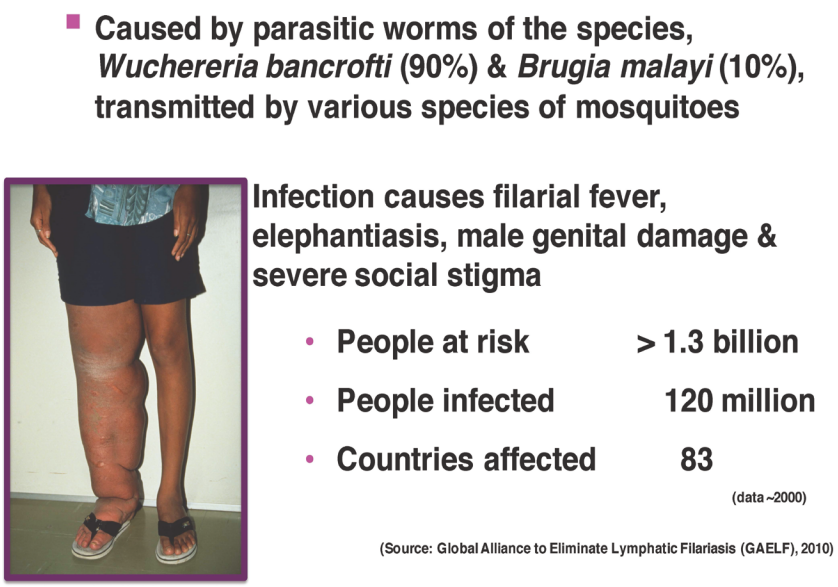


FIGURE 26. Human health goals: Lymphatic filariasis (elephantiasis).

Several years earlier another drug, albendazole, produced by SmithKlineBeecham (now GlaxoSmithKline—GSK) had also been shown to be effective in killing both immature and adult worms. Indeed, field trials had confirmed that once-yearly combinations of albendazole plus DEC or ivermectin were 99% effective in ridding the blood of microfilariae for at least a year after treatment. The primary goal of treating affected communities thus became elimination of microfilariae from the blood of infected individuals so that transmission of infection is interrupted. This opened up the prospect of actually eliminating the disease, something that was made eminently possible thanks to GSK agreeing to donate albendazole. In late-1998, following registration of the drug for lymphatic filariasis, Merck extended its ivermectin donation programme to cover lymphatic filariasis in areas where it co-existed with Onchocerciasis. Subsequently, in 1999/2000, the WHO launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF).

The sheer scale of these disease elimination enterprises is staggering. During the first decade of this century, some 300 million people, roughly the population of the United States, were taking ivermectin tablets annually. In 2014, 328 million ivermectin treatments were requested by disease endemic country governments and approved by the Mectizan Donation Committee. Of this total, 73 million were for combined onchocerciasis/lymphatic filariasis treatments, meaning that around 255 million people were due to receive ivermectin treatment during the year (Fig. 27). In total, 1.4 billion ivermectin treatments have been donated for onchocerciasis (1987–2014) and 1.2 billion for lymphatic filariasis (2000–2014). The goal of elimination of onchocerciasis in Latin America by 2015 has virtually

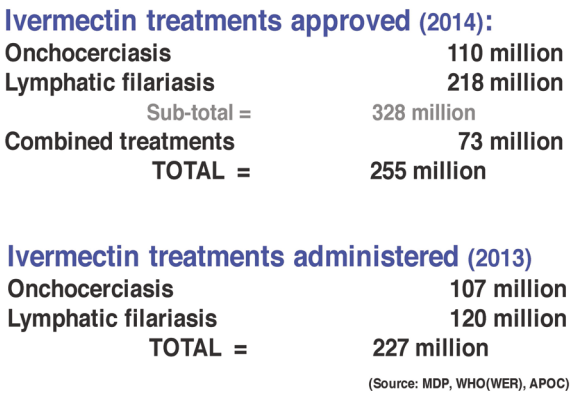


FIGURE 27. Ivermectin treatments.

been accomplished, with just one endemic area remaining on the border between Brazil and Venezuela in remote Yanomami Indian communities where some transmission is still occurring [76].

Today, despite enormous advances in the fight to conquer onchocerciasis in Africa, and with the elimination target date fast approaching, an estimated 172 million people are still in need of treatment [77].

COMMERCIAL IVERMECTIN

Besides donated ivermectin being the sole or primary tool in the two global disease elimination programs, commercial for-profit preparations of ivermectin-based drugs are also being put to ever increasing uses. Ivermectin is being used ever more widely as a remedy for strongyloidiasis (an intestinal infection which afflicts 30–100 million people worldwide) and to treat and prevent scabies (a skin infestation of which 300 million cases are reported each year). Each year, more uses for the avermectins, and ivermectin in particular, are being found in human and animal health [78].

Donated Mectizan® is the primary agent for elimination programmes for onchocerciasis and lymphatic filariasis (in combination with albendazole). At-cost ivermectin has also now become:

1. The drug of choice to treat strongyloidiasis, although it is not available in all nations where the disease is endemic [79].
2. Increasingly used to treat scabies (which afflicts around 130 million people worldwide at any one time). Oral ivermectin has been used since 1993 to treat both common scabies and crusted scabies, particularly to control outbreaks in nursing homes where whole-body application of topical agents is impractical [80]. Recently, topical ivermectin lotions were approved and ivermectin is promising to become the future drug of choice for treating scabies [81].
3. The drug of choice for difficult-to-treat *Pediculosis capitis* (head lice infestation), the most common parasitic condition among children worldwide [82]. Oral ivermectin has high efficacy and tolerability and is more effective than topical malathion lotion [83–87]. Topical application is also effective [88].
4. An option for the intestinal infection ascariasis. Although ivermectin is not recommended for human soil transmitted helminth treatment, except for strongyloidiasis, it has activity against ascariasis, hookworm and trichuriasis. Relatively few trials have examined the use of ivermectin

in this respect. A study to compare the three drugs found that ivermectin was as good as albendazole against ascariasis but that combination therapy provided slightly better results [89]. Another study looked at single-dose ivermectin and found it to be as good as 3-day albendazole treatment [90]. Currently, concern is growing about increasing resistance to albendazole and other anthelmintics [91], emphasising the need for new control tools [92].

5. The best option for the food-borne parasitic infection gnathostomiasis. Albendazole and ivermectin are the preferred treatments but ivermectin is more preferable as it can be given in a single dose [93].
6. An option for the parasitic infection mansonellosis. Ivermectin is highly effective against *Mansonella streptocerca*, with a single dose causing long-term suppression of microfilariae [94]. However, it has demonstrated little or no effect against *Mansonella perstans*. Although there is no consensus on the best therapy, the most commonly used drug, DEC, is often ineffective and it is likely that combination therapy will be the best option [95].
7. Used widely 'off-label' (e.g., to kill skin mites in salmon farming). Toxicity in a range of nontarget animals has been reported, including mice, chicken, rhesus monkeys, bats and turtles [96–100].

HOLISTIC HEALTH, WELFARE AND SOCIOECONOMIC IMPACT

Ivermectin is increasingly being viewed as even more of a 'wonder drug' in human health, as it has also been improving the nutrition, general health and wellbeing of billions of people worldwide ever since it was first used to treat onchocerciasis in humans in 1988. It is ideal in many ways, being multipurpose, highly effective and broad-spectrum, safe, well tolerated and can be easily administered (a single, annual oral dose).

Over the 25-year period that communities in Africa and Latin America have been taking ivermectin to combat river blindness and elephantiasis, anectdotal reports of secondary and non-target benefits have been burgeoning. The benefits described range from an increase in the libido of men to the ability of the tablets to kill termites. Research is accelerating to explore the veracity of these perceived additional benefits and to try and quantify the true overall impact that ivermectin may provide in communities undergoing MDA.

From a purely medical standpoint, ivermectin is known to kill a range of intestinal parasitic worms. The outcome is a visible and tangible sign, people observing worms in their stools. Consequently, owing to this outward manifestation,

villagers feel better and are simultaneously encouraged to continue complying with the drug regime.

Work in Brazil investigating the overall health impact of ivermectin in MDA communities indicates that after two standard doses of ivermectin given 10 days apart, intestinal worm burdens are decimated. Infestations with Strongyloides, Enterobius, and Ascaris were completely cured, whereas other worm burdens were cut to 50–85% of original levels. With regard to external parasites, 99% of pediculosis was cured, compared with scabies (88%) and tungiasis (64%) [101, 102]. Another analysis showed that children in a community that underwent 17 years of ivermectin treatment showed markedly reduced prevalence and intensities of *Trichuris trichiuria* infections and that even children not eligible for treatment displayed reductions, indicating that ivermectin benefitted all members of the community by helping to reduce transmission [103].

In a survey of 3,125 community members in Nigeria who had been receiving ivermectin MDA, the results were also diverse and impressive. Among those treated, with regard to onchocerciasis, there was an 18.5% reduction in body itching, along with reduced skin rash (17.3%), reports of 11.7% better vision, and a 6.6% darkening of 'leopard skin'. Moreover, in addition to the targeted improvements, 24.6% of individuals reported being dewormed, 22.3% said their appetite had increased, 7.9% felt that they had experienced a noticeable reduction in arthritic or other musculoskeletal pain, 6.6% of men declared their libido had improved, 4.5% of community members said their head lice had disappeared, and 4.5% of women described a reversal of secondary amenorrhea [104].

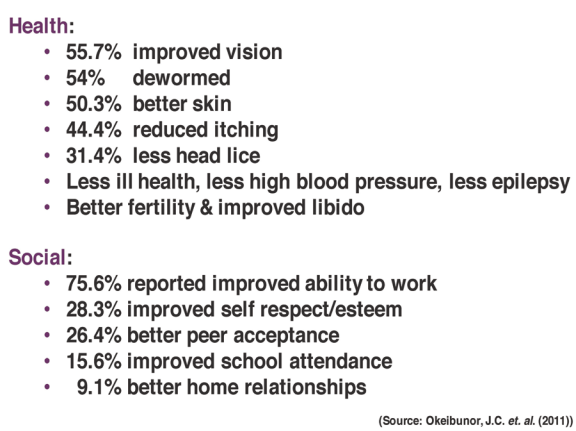


FIGURE 28. Ivermectin mass drug administration secondary benefits: Africa (4-country study).

In a subsequent comprehensive four-country study of MDA patients in Africa, diverse health and social impacts and perceptions were quantified (Fig. 28). Overall, 84.7% felt ivermectin had provided multiple and substantial health and social welfare benefits. All patients reported being better able to sleep at night and were of the opinion that the MDA had improved their social, psychological, and economic wellbeing, with both food productivity and food security being improved [105].

BENEFITS IN JAPAN

The discovery of avermectin has contributed greatly towards improving the lives and living standards of billions of people around the world, as well as to improving the health of livestock and pets. Development, donation and distribution of the drug have been associated with many highly beneficial precedents. The substantial royalties earned by the Kitasato Institute on sales of ivermectin in animal health have also been used wisely and beneficently. They have funded a great deal of highly-focused research, have been used to obtain 27 hectares of land at Kitamoto City in Saitama Prefecture and to construct a vaccine production facility as well as a 440-bed district general hospital and a nursing college. At present, over 1,000 patients per day visit the hospital, which covers a catchment area that was previously grossly underserved with medical facilities. We placed a ceramic plate of a scanning electron micrograph of *S. avermectinius* at the entrance hall of the Kitamoto hospital to illustrate the true foundations on which the building has been constructed and to remind us all of the bounty that still lies hidden in soil, in Japan and elsewhere, awaiting discovery.

GENETICS OF *S. AVERMECTINIUS* AND AVERMECTIN BIOSYNTHESIS

Soon after its use became widespread in animal health, ivermectin resistance began to appear, at first in small ruminants but also more significantly in cattle parasites, especially *Cooperia* spp. [106]. It is well known that high-level resistance to ivermectin appears in free-living *Caenorhabditis elegans* [107]. Thankfully, despite over 30 years of constant worldwide use, there have been no reports of resistance in canine heartworms or among equine *Strongyloides* parasites. More importantly, despite some 25 years of constant monotherapy in humans, no convincing evidence of resistance in *Onchocerca volvulus* has yet been found, although there are indications that resistance may be starting to develop and that resistant parasites are being selected [108, 109].

Chemists have achieved the total synthesis of the avermectins. However to fully understand the biosynthesis of the avermectins, and to allow us to manipulate *S. avermectinius* into producing modified analogues, we mapped the entire genome of the microorganism.

Our work in terms of mapping of biosynthetic genes, elucidation of biosynthetic pathways and overall genome analysis of the avermectin-producing microorganism, *S. avermectinius* MA-4680^{T} allowed us to create mutant organisms in which avermectin biosynthesis was blocked. Thorough stepwise analysis allowed identification of single-point mutations, elucidating the structures of biosynthetic intermediates produced by each mutant and determination of their locations in the biosynthetic pathways. Moreover, the information taken from these blocked mutants became the basis for the cloning of gene clusters for avermectin biosynthesis.

In 1999, we reported that 17 genes of *S. avermectinius* encode enzymes that are involved in avermectin biosynthesis (Fig. 29) [110–113]. Of these, those encoding four type-I polyketide synthases are concerned with lactone formation, via 12 cycles and 53 steps. The remainder act on pathway-specific regulation,

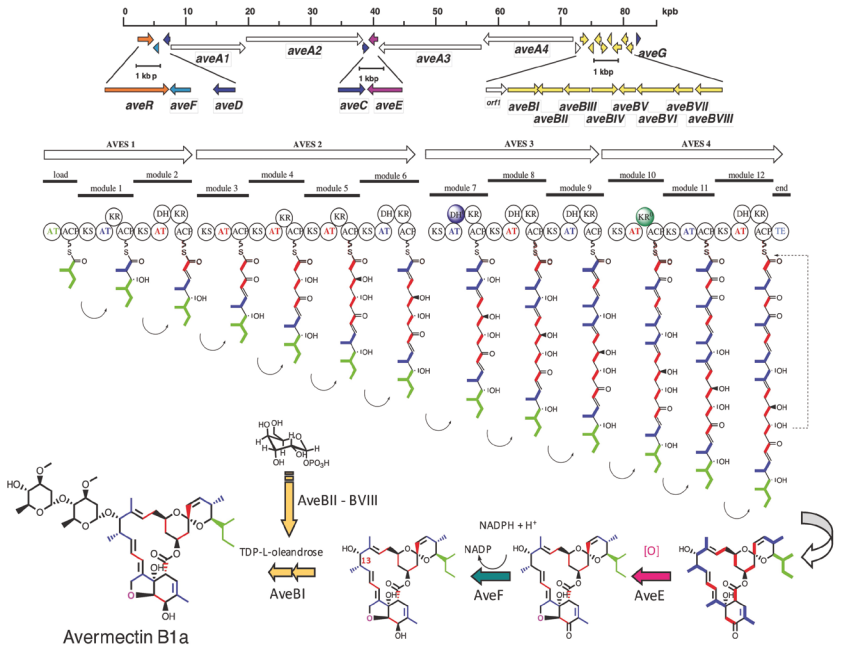


FIGURE 29. *S. avermectinius*: avermectin biosynthesis.

with 12 genes being involved in modification of the lactone ring, biosynthesis of oleandrose and its glycosylation.

The functions of the 17 genes were analysed by cloning. As shown in Figure 29, four genes, *aveA1*, *aveA2*, *aveA3*, and *aveA4*, are involved in the biosynthesis of the basic skeleton of the aglycone moiety. AVES1/AVES4, whose synthesis is governed by these four genes, are multifunctional proteins composed of 3973, 6239, 5532, and 4681 amino acids, respectively.

There are a total of 12 modules in these four large, multifunctional proteins. The AT (acyltransferase) domain transports acyl groups necessary for acyl-chain elongation, one after another, to the ACP (acylcarrier protein) domain present in each module. The acyl groups are then condensed by the catalytic action of the KS (β-oxoacyl-ACP synthase) domain. The resultant β-oxoactyl-ACP is reduced by the KR (β-oxoacyl-ACP reductase) domain and β-hydroxyacyl-ACP is further dehydrated by the DH (dehydratase) domain. The chain elongation reactions and lactonisation at the final step by TE (thioesterase) domain form the basic skeleton of lactone and the nascent lactone is further modified by cytochrome P450 (AveE: CYP171A1) and C5- ketoreductase (AveF) to form avermectin aglycones. Through reaction of the *aveB1-aveBVII* gene's products, namely AveBIIwAveBIII, L-oleandrose is synthesised from glucose-1-phosphate as TDP-L-oleandrose and linked to the aglycone-lactone, completing avermectin biosynthesis. The presence of the hydroxyl group at position 13, which allows the binding of L-oleandrose, is extremely important, as the presence of two L-oleandroses produces the potent antinematode activity of avermectin. The DH domain in module 7 at AVES3 is originally involved in the C13-OH dehydration reaction, but when histidine is substituted for tyrosine in its catalytic active center (consensus motif: HxxxGxxxxP/S), the domain becomes dysfunctional. Subsequently, biosynthesis progresses, while the hydroxyl group at position 13 remains, forming lactone. This single-point mutation, which has resulted in huge health benefits for humankind, allows the sugar (L-oleandrose) binding and subsequent biosynthesis of avermectin, which has superior anthelmintic activity compared to metabolites without the sugar moiety, such as milbemycin and nemadectin (FIg. 29) [114].

Our group completed analysis of the entire genome (9,025,608 bases) of *S. avermectinius* MA-4680^{T}) in 2003 [115, 116]. The information obtained, which represented the first genome analysis of an industrially important actinomycete, provided a major boost for research of secondary metabolites of microorganisms. We initially estimated that there were 32 such clusters, finally determining that there are 37 clusters involved (Fig. 30) [117]. The production of oligomycin,

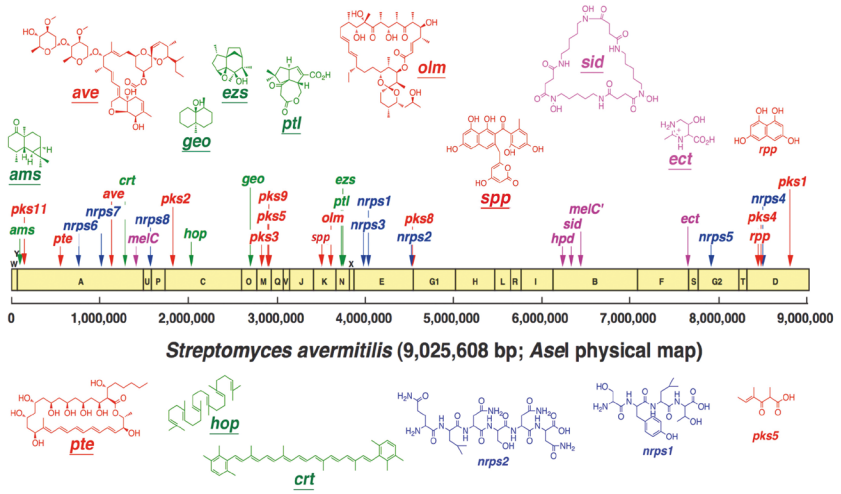
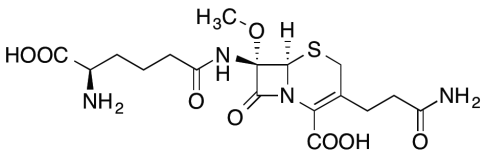


FIGURE 30. Distribution of gene clusters for secondary metabolite biosyntheses in *Streptomyces avermectinius* (*avermitilis*).

along with avermectin, was already known but production of 10 secondary metabolites, including the polyene macrolide, filipine III (*pte*), carotene (*crt*), pentalenolactone (*ptl*), geosmin (*geo*), and nocardamin (*sid*) were all predicted by the genetic analysis, and later confirmed by isolating each metabolite from a fermentation broth of *S. avermectinius*. This created a new research mechanism, whereby production of compounds with specific structures can be predicted by gene analysis and later confirmed through actual production and isolation. The mechanism by which secondary metabolites are produced in *S. avermectinius* has now been fully clarified and work is progressing to engineer the producer microorganism to manufacture yet more potent 'designer' compounds.

We have created an improved strain of *S. avermectinius*, which contains only 80% of the original genome, by removing sequences unnecessary for compound



Cephamycin C

FIGURE 31.

production by the site-specific and homologous recombination technique. Using the genome-minimised strain, the heterologous expression of gene cluster for cephamycin C (Fig. 31) biosynthesis from a *S. clavuligerus* genomic library, was attempted, resulting in astonishing production of bioactive compounds [118]. This is a highly innovative foray in biotechnology, which should provide clues to guide applied research on genetic manipulation and customised culturing systems to facilitate productivity of a range of useful compounds.

IVERMECTIN: THE FUTURE

In addition to the gradual appreciation of the diverse health and socioeconomic benefits that ivermectin does provide, research is beginning to shed light on the promise of ivermectin and the prospects of it combatting a range of diseases and for killing vectors of disease-causing parasites. Box 1 provides an indicator of the potential that has been identified thus far, particularly against diseases of the poor, and provides an insight into the wide spectrum of benefits of ivermectin that may yet lie undiscovered and unexploited.

CONCLUDING REMARKS

Ivermectin has continually proved to be astonishingly safe for human use. Indeed, it is such a safe drug, with minimal side effects, that it can be administered by non-medical staff and even illiterate individuals in remote rural communities, provided that they have had some very basic, appropriate training. This fact has helped contribute to the unsurpassed beneficial impact that the drug has had on human health and welfare around the globe, especially with regard to the campaign to fight onchocerciasis.

In reality, the renewed interest in fighting tropical diseases, including the involvement of the pharmaceutical industry, which has become increasingly evident over the past four decades, and which has saved lives and improved the welfare of billions of people, notably the poor and disadvantaged in the topics, can be traced back to the 1987 introduction of ivermectin for use in humans. The remarkable and unparalleled donation of ivermectin can rightly be seen to be the origin of this philanthropic largesse.

Today, ivermectin is being increasingly used worldwide to combat other diseases in humans, and new and promising properties and uses for ivermectin and other avermectin derivatives are continuing to be found. Of perhaps even greater significance is the evidence that the use of ivermectin has both direct and

indirect beneficial impact on improving community health. Above all, ivermectin has proved to be a medicine of choice for the world's rural poor.

According to many experts, a post-antibiotic era—in which common infections and minor injuries can kill—is a very real possibility, with WHO Director General Dr Margaret Chan declaring "the rise of antibiotic resistance is a global health crisis, and governments now recognise it as one of the greatest challenges for public health today."

My work has always been guided by five fundamental creeds: the almost unlimited abilities of microorganisms to produce novel compounds; the crucial need to establish 'gold-standard' screening systems; recognition that screening is not just a routine exercise; the major contribution of basic research; and the need to assign the highest value to maintaining human relationships and partnerships.

As science advances and our knowledge improves, it is clear to me that the elucidation of suitable targets for medicines, and our expectations for finding remedies to treat both known and as-yet unknown diseases and conditions, will not only improve but also accelerate. Genomic mapping and identification of lead compounds have progressed significantly since the turn of the century, as evidenced by the mapping of the human genome. As mentioned above, research is also expected to develop substantially based on the findings of biosynthetic studies and from the investigation of naturally-occurring substances that boast hitherto unseen structures. I firmly believe that Nature's microbes produce metabolites offering unmatched promise toward meeting our needs, although the introduction of novel screening methods will be key to achieving optimal results. Thus, success will only be restricted by our vision and our innovation—or lack of it. Fortunately, we have access to some of the innovation we need through genetic engineering and the number of non-natural compounds obtained is increasing rapidly to supplement the never-ending stream of novel compounds that Nature can supply.

For 50 years, I have worked alongside specialised researchers in fields such as Biochemistry, Microbiology, and Clinical medicine. My approach has always been influenced by the tenet "One encounter, one chance." This encompasses the deep reverence that is an essential part of the Tea Ceremony (or Chanoyu), which is held in the highest esteem in Japanese culture. As well as the certain fact that exact circumstances at any point in time will never happen again, I believe it is important to seize opportunities as and when they arise. And to maintain profound respect and consideration for all my colleagues—as well as for Nature and the microorganisms I work with. Such sentiments form the fundamental basis for all good scientific research and discovery.

BOX 1. POTENTIAL USES OF IVERMECTIN

- **Streptocerciasis:** occurs in Central Africa due to infection with the nematode *Dipetalonema streptocerca* transmitted by the bite of insects of the genus *Culicoides*. Ivermectin kills the disease-causing microfilaria [B1].
- **Trichinosis:** globally, 11 million individuals are infected with Trichinella roundworms, which can be killed by ivermectin [B2].
- **Myiasis:** infestation by fly larvae that grow inside the host. It is a relatively common affliction of people in poor, rural tropical communities. Surgical removal of the parasites is often the only remedy. Oral myiasis has been successfully treated with ivermectin [B3].
- **Vector control:** The avermectins are toxic to almost all insects, causing water balance difficulties, as well as disruption of moulting and metamorphosis, death occurring from between 1 and 30 days [B4]. Ivermectin kills a wide variety of insects [B5, B6] and is highly effective against bedbugs, capable of eradicating or preventing bedbug infestations [B7].
- **Malaria:** Mosquitos (*Anopheles gambiae*) that transmit *Plasmodium falciparum*, the most dangerous malaria-causing parasite in Africa, can be killed by the ivermectin present in the human bloodstream after a standard oral dose [B8–B10]. At sub-micromolecular levels, ivermectin inhibits the nuclear import of polypeptides of the signal recognition particle of *P. falciparum* (PfSRP), killing the parasites. This raises the possibility that ivermectin could become a useful, novel malaria transmission control tool [B11, B12].
- **Leishmaniasis:** Ivermectin kills sandflies (*Phlebotomus papatasi*) that transmit the parasites that cause leishmaniasis and has been suggested as a means to help control them [B13, B14]. Ivermectin also kills various stages of the disease-causing parasite, *Leishmania major* [B15, B16].
- **Trypanosomiasis:** Ivermectin has promise as a systemic drug against the tsetse fly vectors of African trypanosomiasis (Sleeping Sickness) [B17, B18]. There is scope for investigating the use of ivermectin in the treatment of trypanosomiasis from several aspects [B19].
- **Schistosomiasis:** A research collaboration was established between the Kitasato Institute and the Oswaldo Cruz Institute (Fiocruz) in Brazil in 2008 to test ivermectin analogues and compounds from the chemical libraries of each institute in screening systems being operated in the two institutions. Promising results were immediately found with regard to the impact of ivermectin on the intermediate host snails responsible

for maintaining the schistosomiasis re-infection cycle, offering the prospect of using ivermectin to help control one of the world's major neglected diseases [in press].

- **Antiviral:** Ivermectin is a broad-spectrum inhibitor of importin a/b nuclear import, demonstrating potent antiviral activity towards HIV-1 and dengue viruses [B20].

  Ivermectin also strongly inhibits replication of several flaviviruses (yellow fever dengue, Japanese encephalitis, and tick-borne encephalitis) [B21, B22].
- **Antibacterial:** Ivermectin prevents *Chlamydia trachomatis* infection [B23]. It is also reported to be bactericidal against a range of mycobacterial species, including *Mycobacterium tuberculosis* [B24] and *M. ulcerans* [B25].
- **Anticancer:** Ivermectin promotes cell death in leukaemia cells and ME-180 cervical cancer cells [B26–B28]

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I would like to convey my sincerest gratitude to all those concerned in my being chosen as a recipient of the 2015 Nobel Prize in Physiology or Medicine, which I humbly receive on behalf of everyone who has helped me on every step of a wonderful journey of discovery.

I would also like to express my particular and profound thanks to all the people who have supported me and my research on the discovery, development and deployment of the avermectins/ivermectin, to Professor Andy Crump for his invaluable cooperation in the preparation of this account and, of course, to my family for their unwavering and paramount support.

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