# Outstanding Drugs Developed in Japan

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Nivolumab (OPDIVO®)

Nivolumab (product name: Opdivo) is referred to as an immune checkpoint inhibitor. Unlike many antineoplastic drugs, it does not act directly on cancer cells. Its immune checkpoint mechanism inhibits the excessive effects of immune responses, and plays a role in stopping immune responses in order to prevent damage to normal cells. This effect was originally found with respect to programmed cell death 1 (PD-1), which was discovered by the Honjo Laboratory at Kyoto University in 1992. The function was unclear at that time, but in a collaborative study between Kyoto University and the Genetics Institute in the US in 2000, programmed cell death 1 ligand 1 (PD-L1) and 2 (PD-L2) were discovered and found to bind specifically to PD-1.

Cancer cells produced in vivo should be ideally eliminated by killer T cells. However, cancer cells express PD-L1 and PD-L2, which bind to PD-1 on T cells and inhibit T cell action, which interferes with removal of cancer cells by the immune system. Therefore, if the PD-1/PD-L1 interaction can be inhibited, immune checkpoints may be able to attack cancer cells again. The Honjo Laboratory showed that administration of an anti-PD-1 antibody markedly increased anticancer effects in mice, including effects such as inhibition of metastasis of a transplanted tumor. Based on this background, nivolumab was approved by the US Food and Drug Administration (FDA) in 2016 as a new human anti-PD-1 antibody for research use. A clinical study was initiated in the same year in the US. In clinical trials in which the drug was administered to treat solid cancers such as non-small cell lung cancer, prostate cancer, colorectal cancer, renal cell carcinoma, and malignant melanoma, and it is found to be efficacious against all these cancer types. In malignant melanoma and renal cell carcinoma, the response rate was close to 30%. The results were published in The New England Journal of Medicine in 2012, and the efficacy was highlighted in an editorial. Cancer immuno-therapy using antibodies was selected as Breakthrough of the Year in 2015 by Science magazine, and is currently attracting increased attention.

Therefore, nivolumab was approved as ‘Opdivo’ for the treatment of malignant melanoma in July 2014 in Japan—the first such approval worldwide—and was launched in September 2014. In Japan, two in one two Japanese individuals develop cancer and one in three deaths are due to cancer. Therefore, development of an anticancer drug with a novel pharmacological action, such as nivolumab, may have great significance in cancer treatment. It is hoped that such developments will progress further and advance medical care.

Favipiravir (AVIGAN®)

In 2002, a collaborative research team led by Dr. Yousuke Furuta at Toyama Chemical Co., Ltd. and Dr. Kimiyasu Shiraishi in University of Toyama screened their compound libraries and discovered a novel small molecule with anti-influenza activity, called favipiravir (T-705) (Fig. 1). They found that favipiravir shows a potent antiviral activity against all influenza A, B, and C viruses without cytotoxicity in vitro. Strikingly, they also demonstrated that oral administration of favipiravir (200 mg/kg/day) for 5 days completely protected mice from lethal H1N1 influenza infection. It was later shown that favipiravir-RTP, an active form of favipiravir, inhibits influenza RNA-dependent RNA polymerase activity without affecting cellular RNA and DNA polymerase activity. Although the exact mechanism of action remains unclear, it is being hypothesized that favipiravir-RTP may be incorporated into the new viral RNA copies or interact with specific domains of the influenza polymerase and therefore block its function. It is noteworthy that favipiravir is also able to inhibit replication of influenza viruses that are resistant to neuraminidase or M2 ion channel inhibitors due to their different mechanism of action, while emergence of its resistant virus seems to be unlikely. Indeed, favipiravir effectively protects mice from infection with lethal H5N1 influenza viruses, even if they are oseltamivir resistant. In addition, favipiravir shows synergistic inhibitory effect against influenza virus infection in combination with oseltamivir, expanding options for anti-influenza therapy. These reports clearly demonstrate favipiravir is a novel and promising drug in the fight against lethal influenza infection. Unfortunately, however, favipiravir has been reported to have a risk for teratogenicity and embryotoxicity. In Japan, favipiravir was approved in 2014 for emergency preparedness stockpile for pandemic or re-emerging influenza viruses resistant to known anti-influenza drugs. In addition, a phase III clinical study for favipiravir has already been completed in USA.

There is no doubt that not only influenza virus, but also many other viruses are capable of causing human diseases and threatening public health. Favipiravir has been shown to exhibit antiviral activity against a wide range of RNA viruses including Ebolavirus, Zika, West Nile, and flaviviruses, suggesting that this compound could have a potential to counteract re-emerging and/or novel pathogen for which no antiviral therapy is available.

Ivermectin (STROMECOTEL®)

Ivermectin (IVM, Fig. 2) is one of the best known and widely used antiparasitic drugs in human and animal medicine. To date, IVM has impacted human health. Millions of people in the poorest countries have suffered from onchocerciasis (river blindness), strongyloides (threadworms), and lymphatic filariasis (elephantiasis) before the discovery of antiparasitics by Satoshi Omura at Kitasato University and William Campbell at Merck company. They discovered avermectin in Japanese soil and developed IVM, which is derivatives of avermectin. IVM is safe and strongly combats parasitic disease, thus contributing to global human health. Their discovery was the joint focus of the 2015 Nobel Prize in Physiology or Medicine, showing the importance of avermectins and Japan’s contribution to supporting global human health.

In general, it is very difficult for discovering target microbes in soil. However, Dr. O’Mura worked on discovering target microbes hand by hand and discovered them after great effort and struggle. In 1974, Dr. O’Mura at Kitasato University collected a sample of soil from a golf course in Tokyo city, Shizuoka, Japan. Dr. O’Mura isolated and cultured a Gram-positive bacterium (NRRl 8105, unknown species of Streptomyces), and this was sent to Dr. Campbell at Merck to test for anti-parasitic effects. NRRl 8105 cultures showed potent activity against Heligmosomoides polygyrus infection in mice, and active com-

ponents were purified, revealing macrocyclic lactone family. Dr. Omura named these naturally occurring compounds avermectins. Avermectins are a mixture of four compounds, avermectin A1a, A1b, B1, and B2. IVM is modified chemically using avermectin B1, and showed potent activity against a broad array of parasitic nematodes via oral and parenteral administration. IVM has activity against various arthropods and is safe for mammals. IVM effects nematode motility, feeding, and reproduction. IVM binds to glutamate-gated chloride channels (Glus-Ch), which are expressed in invertebrate nerve and muscle cells, resulting in an increase in the permeability of the cell membrane to chloride ions. This induces hyperpolarization in the nerve or muscle cell, and causes paralysis of the parasite. While Glus-Chs are expressed in nematode motor neurons, circular or subcutaneous nerve cords, and pharyngeal muscles, Glus-Chs are not present in vertebrates. IVM interacts with other ligand-gated chloride channels activated by gamma aminobutyric acid (GABA). For this reason, IVM has nematode species-specific effect. IVM also interferes with nematode fertility by inhibiting the production of microfilariae. The affinity of IVM against mammalian cells is very low and IVM can’t pass readily blood-brain barrier. Thus, IVM has broad safety and exerts high attack ability against various nematodes.

Fasudil (Eril®)

A Rho kinase inhibitor fasudil (FAS) (Fig. 3) is honored to be the first approved kinase inhibitor in the world. Its discovery research began from encounters with acanthamoeba. We heard that there were many cases of death due to the subsequent occurrence of cerebral vasospasm even if the patient underwent surgery for subarachnoid hemorrhage (SAH) successfully. Unfortunately, however, fasudil has been reported to have a risk for teratogenicity and embryotoxicity. In Japan, fasudil was approved in 2014 for emergency preparedness stockpile for pandemic or cerebral vasospasm. Although various substances were proposed and considered to be the cause of cerebral vasospasm, no substance therapy was effective. Therefore, we focused on phosphorylation of myosin light chain (MLC), which is a common pathway of vasoconstriction. We hypothesized that reducing MLC phosphorylation in blood vessels would be effective for preventing cerebral vasospasm. In fact, Omura had synthesized various kinase inhibitors to research intracellular signal transduc-

1 Nivolumab (OPDIVO®)

2 Favipiravir (AVIGAN®)

3 Ivermectin (STROMECOTEL®)

4 Fasudil (Eril®)
Candesartan cilexetil (BLOPRESS®) is an AT₁ receptor antagonist, originally developed in Takeda Pharmaceutical Company and it becomes one of the representative angiotensin II (AngII) receptor blockers (ARBs). It is known to show potent and long-lasting pharmacological effects and it is approved for patients with hypertension as well as chronic heart failure.

A lead compound of candesartan cilexetil, CV-2198, was synthesized in the research of a novel synthetic chemical reaction using ADAN (2-amino-3,5,3,5-dichloroacrylonitrile) as a starting material in Chemistry Research Laboratories of Takeda. It was also the compound proved to be the most potent in its class of losartan, candesartan cilexetil and other ARBs. Although the most potent compound among the newly synthesized CV-2973 was forwarded to clinical trials, unfortunately, its efficacy was not observed.

The drug discovery of ARBs in Takeda was once terminated in 1982 after submission of CV-2198-related patents. In 1989, Du Pont’s research group surprisingly reported an ARB, losartan, which was discovered based on the Takeda’s patents, describing the inhibitory effects of about 200 umol/d on angiotensin converting enzyme (ACE). The drug discovery project was terminated after one year, but we finally synthesized and evaluated compounds day after day, and finally succeeded in synthesizing a molecule, candesartan cilexetil (Fig. 5). Candesartan cilexetil is a prodrug and was discovered by accident and as a surprise. We also had difficulties in improving the bioavailability, even though we developed a compound (2) as the strongest known inhibitor of ACE. Moreover, the results of a phase 2 study in Japan did not show dose-dependent effect. A phase 3 study in Japan and a subsequent clinical study in the US were excellent. Thus, the work performed in the US allowed the study to move ahead and to be successful.

Donepezil hydrochloride (Aricept®) is a donepezil hydrochloride (Fig. 6) activates the cholinergic nervous system by increasing the cerebral acetylcholine (ACh) level through reversible inhibition of an acetylcholinesterase (AChE) depragmating enzyme, acetylcholine esterase (AChE). Donepezil hydrochloride is indicated for the treatment of dementia of the Alzheimer type.

When development of a therapeutic agent for Alzheimer’s disease was planned in 1983, we focused on the structure of tacrine. Tacrine was a known acetylcholine esterase (AChE) inhibitor, but it also had high toxicity. Thus, we tried to synthesize a tacrine derivative that did not cause liver dysfunction, but it was still impossible to avoid toxicity. Compound (1) was incidentally discovered as an AChE inhibitor in an unrelated study. Using (1) as a lead compound, we were successful in synthesizing Compound (2), which we found to be the strongest known AChE inhibitor. However, we then found that 98% of Compound (2) is degraded in the liver in dogs. The study was terminated at this point, but we still believed that we could be successful by improving the pharmacokinetics of Compound (2). Thus, it was re-established as a compound (3) that would extend by one year, but we single-mindedly synthesized and evaluated compounds for the next two years. The compound (2) was discovered by accident and as a surprise. The work performed in the US allowed the study to move ahead and to be successful.

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Erythropoietin

Erythropoietin is now administered to most patients with renal anemia, and its market value has reached several trillion yen worldwide. Dr. Takaji Miyake succeeded in preparation of purified human erythropoietin from 20 tons of urine in 1976, after starting this work 13 years earlier in 1964. Extraction at low temperature was necessary, but no low-temperature room was initially available and all windows were opened for column operation in midwinter. Subsequently, a low-temperature room to facilitate purification operation in midwinter. Subsequently, a low-temperature room to facilitate purification was made from an assistant’s room at the university, and nearly 3,000 rats were used each year to measure its activity. The work was published in 1977, the human erythropoietin gene was cloned using the purified preparation in 1985, and a clinical trial of recombinant erythropoietin was initiated in the same year, opening the way to treatment of renal anemia today. Discovery of erythropoietin is an excellent example of creation of a new drug by the tenacious research efforts of Japanese scientists.

Carperitide (Atrial natriuretic peptide (ANP))

Dr. Kenji Kangawa, National Cerebral and Cardiovascular Centers Research Insti- tute, discovered the atrial natriuretic peptide (ANP) in the atrium, and published the finding in 1984. Subsequently, ANP was developed as a drug for treatment of acute heart failure or as a diagnostic agent for heart failure in Japan. This process provides an excellent example of the success of translational research all over the world. It should be mentioned that isolation of ANP from the human atrium and determination of the structure of ANP were completed within two months after study initiation, using the original peptide analysis technique of Dr. Kangawa. The pathophysiological functions of ANP, such as its blood pressure-lowering and diuretic actions, were determined soon afterwards. This is a good example of establishment of proof of concept and acceleration of drug discovery by Japanese science and technology. Synthesis of brain natriuretic peptide (BNP) in the brain was also discovered by Dr. Kan- gawa, and this finding was published in 1988. BNP is synthesized and secreted in the ventricle in the periphery, but now the most commonly used diagnostic agent for heart failure. It is also used as a drug for treatment of heart failure in the US and other countries. Thus, ANP and BNP are now used as diagnostic agents and drugs for heart failure worldwide.

Febuxostat (Feburic®)

Febuxostat is a uric acid synthesis inhibitor with no purine skeleton that was developed by Shion Kondo and co-workers at Teijin Ltd. Inhibitors of uric acid synthesis include allopurinol, a derivative of a uric acid precursor, hypoxanthine. Allopurinol was launched in the 1960s as an inhibitor of xanthine oxidase (XO), a uric acid-synthesizing enzyme, as a 30 times difference from the anti-con- version effect. This may be due to transfer of naldemedine into the brain.

The combination of a potent anti-conversion effect without influencing the analgesic action of the opioid found in the non-clinical study was also observed in a clinical study, in which 0.2 mg of naldemedine improved opioid-induced constipation regardless of the dose of opioid analgesic and had no effect on the analgesic action of the opioid.

More than 10 candidate compounds were developed and the major cause of unsuccessful development of new drugs is a lack of proof of concept. The work was published in 1977, the human erythropoietin gene was cloned using the purified preparation in 1985, and a clinical trial of recombinant erythropoietin was initiated in the same year, opening the way to treatment of renal anemia today. Discovery of erythropoietin is an excellent example of creation of a new drug by the tenacious research efforts of Japanese scientists.

Nalfurafine (Remitch®)

Since opioid receptors have been clas- sified into three types, μ, δ, and κ, and drug dependence has been reported to be derived from the μ receptor, a fierce competitive race for the development of an inhibitor of uric acid synthesis with excellent results of the clinical trial, Toray obtained the manufacturing license for nalfurafine in 2010, the first uric acid synthesis inhibitor to be introduced for the first time in about 40 years. There were no specific drugs to treat intolerable itch in kidney dialysis patients. We therefore decided to develop nalfurafine as an antipruritic drug. On the basis of the excellent results of the clinical trial, Toray started marketing surveillance studies reported more than 80% efficacy in terms of its antipruritic effect. Remitch® has also been applied to treat severe itch in patients with hepatic failure and its sales have been growing steadily. The advantage of Remitch® is its few serious side effects, such as anorexia and nausea. Furthermore, ivermectin, ivermectin, and ivermectin have also been gradually increasing. Eventually, by collaborating with Dr. Ross’s group at the University of Pittsburgh, we clarified
the reason why people have a tendency to scratch when they feel an itch, which had been a mystery for a long time.3

**Ideas leading to the development of the drug:**
I obtained the ideas when I was studying at the University of Minnesota—that is, the message–address concept as well as the partial structure and accessory site of endogenous opioids.

**Pharmacological effects of the drug:**
Nalfurafine does not bind to receptors other than the opioid receptors because of the presence of a specific morphinan skeleton. Nalfurafine does not cause drug dependence. Additionally, nalfurafine does not cause drug aversion, unlike the U-50488H type \( \kappa \) agonists developed by other drug companies.

**Difficulties faced during development:**
We had to abandon the development of nalfurafine as an analgesic because of its sedative effect.

Nippon Shinyaku started development of prostacyclin (PGI\( _2 \)) receptor agonists almost 20 years ago. PGI\( _2 \) is an endogenous substance that induces vasodilatation via an activation of PGI\( _2 \) receptor (IP receptor). Based on its efficacy, PGI\( _2 \) was used as medications, but the extremely short half-life of PGI\( _2 \) had been a drawback. Therefore, PGI\( _2 \) analogues with improved half-lives were synthesized, but new drugs with a more long-acting profile were still desired.

So, we have continued to explore an orally available and long-acting IP receptor agonist, and finally discovered selexipag (development code, NS-304; product name, Uptravi\( ^\text{\textregistered} \) in tablet form) with a nonprostanoid structure. Selexipag is metabolized into MRE-269 after oral administration, and the long duration of effective plasma concentration of MRE-269 is maintained. MRE-269 acts as a potent and highly selective IP receptor agonist.

Although we first considered arteriosclerotic disease to be an indication for selexipag, we determined that pulmonary arterial hypertension (PAH) was more appropriate as a potential indication in terms of its pharmacological profile. PAH is a rare, life-threatening disease characterized by an abnormal increase in pulmonary arterial pressure. There had been no adequate cure for PAH over the past decades. Since then, the continuous intravenous infusion of PGI\( _1 \) has been introduced as a treatment for PAH, and the prognosis of PAH has been markedly improved. However, this therapy impairs patients’ quality of life (QOL). Therefore, many kinds of orally available drugs have been produced for PAH, but the continuous intravenous PGI\( _1 \) therapy has remained the most effective. Thus, we have selected PAH as the indication for selexipag because an oral long-acting IP receptor agonist would be useful for PAH.

We first conducted the drug development in Europe because it was difficult to perform clinical trials in Japan due to the number of patients. In the Phase I clinical trial, the half-life of MRE-269 was about 8hr after the administration of selexipag, indicating good pharmacokinetic properties. We also confirmed the safety in the up-titration study based on the dosing regimen of selexipag.

The subsequent Phase II clinical trial was carried out in Europe. We selected pulmonary vascular resistance (PVR) as a primary endpoint. As a result, the primary endpoint was met in spite of difficult situation that all patients received existing therapies for PAH. This success of the Phase II clinical trial was the critical point of the further development plan. After that, the global, double-blind, placebo-controlled, Phase III trial (GRIPHON trial) enrolling 1,156 patients from 39 countries was conducted. The primary endpoint, the time to clinical worsening, was met with statistical significance (reduced by 40% with selexipag compared to placebo). The efficacy of selexipag was also demonstrated in the open-label, Phase II clinical trial in Japan with the primary endpoint of PVR.

As mentioned above, selexipag, with a long-acting profile and high selectivity to IP receptor, has demonstrated the efficacy for PAH through clinical trials. Selexipag was approved in September 2016 and launched in November 2016 in Japan. The clinical trials in patients with chronic thromboembolic pulmonary hypertension (CTEPH) and arteriosclerosis obliterans (ASO) are being conducted in Japan. The indications for selexipag will be expanded in the future.