HONG KONG PHARMACEUTICAL JOURNAL

VOL 25 NO 4 Oct - Dec 2018 ISSN 1727-2874

The Pharmaceutical Society of Hong Kong
The Practising Pharmacists Association of Hong Kong
The Society of Hospital Pharmacists of Hong Kong

HONG KONG PHARMACEUTICAL JOURNAL

VOL 25 NO 4 Oct - Dec 2018 ISSN 1727-2874

All communications and enquiries should be directed to:
The Secretary, Hong Kong Pharmaceutical Journal, Room 1303, Rightful Centre, 12 Tak Hing Street, Jordan, Hong Kong.

For all enquiries regarding advertisement, please contact:
Ms. Wanda Kwan (Tel. 61086231) or Ms. Ritchie Kwok (Tel: 96457911)
at the following email address: ***hkpjadv@gmail.com***

INSTRUCTIONS FOR AUTHORS

The Hong Kong Pharmaceutical Journal is a journal of the pharmacists, for the pharmacists and by the pharmacists. Submissions are welcome for the following sections:

- Pharmacy Education & Practice
- Drugs & Therapeutics
- OTC & Health
- Pharmaceutical Techniques & Technology
- Medication Safety
- Herbal Medicines & Nutraceuticals
- Society Activities
- New Products

Comments on any aspects of the profession are also welcome as Letter to the Editor.

There is no restriction on the length of the articles to be submitted. They can be written in English or Chinese. The Editorial Committee may make editorial changes to the articles but major amendments will be communicated with the authors prior to publishing.

It is preferable to have original articles submitted as an electronic file, in Microsoft Word, typed in Arial 9pt. Files can be sent to the following address:

e-mail: ***editor@hkpj.org***
address: ***Room 1303, Rightful Centre, 12 Tak Hing Street, Jordan, Hong Kong.***

For detail instructions for authors, please refer to the first issue of each volume of HKPJ.

Entering a Season of Gratitude

This is the last issue of *Hong Kong Pharmaceutical Journal* (HKPJ) for 2018 and I would like to close the year with my thanks to all the members of the Editorial Committee for their diligent and dedicated work. They have selflessly contributed their efforts and time in putting together each issue. In addition, my thanks go to all the authors who have been very supportive to the Journal.

In this issue, Ceci Yuen and Donald Chong (page 112) wrote an overview and the impact of Prescription (Rx)-to-OTC switch to public health. With the increased interest in self-care and health literacy, Rx-to-OTC switch has become more prominent recently. As discussed in the article, the switch can, directly or indirectly, bring various benefits to the patients and the society. However, it is not without any negative impacts. In fact, pharmacist involvement can greatly enhance safety and more appropriate use of these medications. There is no doubt that pharmacists will be an important part of this movement toward greater patient autonomy and expanded access to health care.

On page 119, Sarah Kong wrote an update on drug treatments for idiopathic pulmonary fibrosis (IPF). IPF, as discussed in the article, is a rare lung disease that results in progressive fibrosis of the lungs. Nintedanib and pirfenidone have been shown to increase progression free survival time. Still the high drug costs of both medications have limited the access to patients who can afford it. Fortunately, a reimbursement scheme has been arranged and pharmacists can help patients in navigating this reimbursement arrangement.

Both Ceci Yuen and Sarah Kong were my students when they were at HKU. Today, I am proud to witness their growth and achievement. What a privilege it was to be part of the journey with them. Keep up with the good work!

As always, you may provide suggestions and give feedbacks on any aspect of the Journal by contacting me or other members of the Editorial Committee. We would very much like to hear your thoughts on any part of the Journal and how we can further develop the Journal. But most importantly, how we can make it more appealing to you, our valued readers.

May PS Lam
Editor-in-Chief
13 January 2019

Prepared by Howard Chan; Chiu Tsz Ching; Bryan Kan; Tommy Lee

Six-month Dual Antiplatelet Therapy Non-inferior to 12-month Treatment after STEMI Drug-eluting Stent Implantation

Date: October 2, 2018

Dual Antiplatelet Therapy (DAPT), a combination of aspirin and $P2Y_{12}$ inhibitor, is usually prescribed after percutaneous coronary intervention (PCI) for ST-elevation myocardial infarction (STEMI) treatment. A trial of six versus 12 months of DAPT after second-generation drug-eluting stent implantation in patients presenting with STEMI (DAPT-STEMI) proposed non-inferiority of a six-month treatment to a prolonged regimen.

In DAPT-STEMI, a prospective, randomized, multicentre, non-inferiority trial, 870 patients on DAPT and had been event-free for six months after zotarolimus-eluting stent implantation were included. They were randomly assigned in 1:1 ratio to either the single antiplatelet therapy (SAPT) group, where only 80-100mg aspirin was continued, or the DAPT continuation group for further six months. Follow-up lasted for 18 months to assess primary endpoint occurrence, which comprises of all-cause mortality, any myocardial infarction (MI), any revascularization, stroke and thrombolysis in MI major bleeding.

With respect to the primary endpoint assessment, percentages of occurrence in SAPT and DAPT were 4.8% and 6.6% respectively (hazard ratio [HR], 0.73; 95% confidence interval [CI], 0.41 to 1.27; p=0.26), with no statistical differences observed for all individual components. Additionally, major secondary endpoint with a composite of bleeding and safety also reflected non-inferiority (HR, 0.75; 95% CI, 0.37 to 1.49; p=0.40).

Having the CI upper limit below prespecified margin of 1.66, non-inferiority was met. Kaplan-Meier curves derived further suggested absence of association between DAPT discontinuation and rebound effect on primary endpoint events; nevertheless, further research is required to investigate potential rebound effects of treatment discontinuation at different timepoints.

Source: www.bmj.com

Reduces Hospitalizations for Heart Failure in Patients with Diabetes

Date: November 10, 2018

A recent study suggested that dapagliflozin, a selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, lowers hospitalization rate for heart failure in patients with type 2 diabetes (T2DM).

In DECLARE-TIMI 58, a randomized, double-blind, multinational, placebo-controlled trial, 17,160 patients with T2DM who had or were at risk for atherosclerotic cardiovascular disease (ASCVD) were randomly assigned to receive either dapagliflozin 10mg daily or matching placebo. The primary safety outcome was a composite of major adverse cardiovascular events (MACE), defined as cardiovascular death, myocardial infarction, or ischaemic stroke. The primary efficacy outcomes were MACE and a composite of cardiovascular death or hospitalization for heart failure. Secondary efficacy outcomes included a renal composite (≥40% decrease in eGFR to <60 mL/min/1.73 m^2, new end-stage renal disease, or death from renal or cardiovascular causes) and death from any cause.

Dapagliflozin met the prespecified criterion for non-inferiority to placebo with respect to MACE (upper boundary of 95% CI <1.3, p<0.0001 for non-inferiority). Dapagliflozin did not significantly reduce rate of MACE (8.8%), as compared to 9.4% in the placebo group (HR, 0.83; 95% CI, 0.84 to 1.03, p=0.17), but did result in a significantly lower rate of cardiovascular death or hospitalization for heart failure (4.9% vs 5.8%; HR, 0.83; 95% CI, 0.73 to 0.95; p=0.005). No difference in cardiovascular death was observed (HR, 0.98; 95% CI, 0.82 to 1.17).

Renal events occurred in 4.3% and 5.6% of the dapagliflozin and placebo group respectively (HR, 0.76; 95% CI, 0.67 to 0.87), while the corresponding occurrence of death from any cause were 6.2% and 6.6% (HR, 0.93; 95% CI, 0.82 to 1.04). Diabetic ketoacidosis was more common with dapagliflozin than with placebo (0.3% vs. 0.1%, p=0.02), as well as the rate of genital infections that were considered as serious adverse effects or led to regimen discontinuation (0.9% vs. 0.1%, p <0.001).

Source: www.nejm.org

FDA Approves New Drug Rifamycin to Treat Travellers' Diarrhoea

Date: November 16, 2018

The United States Food and Drug Administration (FDA) approved Aemcolo (rifamycin), an antibacterial drug indicated for the treatment of adult patients with travellers' diarrhoea caused by non-invasive strains of *Escherichia*

coli (E. coli), not complicated by fever or blood in the stool.

The efficacy of Aemcolo was demonstrated in a randomized, placebo-controlled clinical trial in 264 adults with travellers' diarrhoea in Guatemala and Mexico, with 199 patients in the Aemcolo group and 65 patients in the placebo group. The median time to last unformed stool (TLUS) in the Aemcolo group and placebo group were 46.0 hours and 68.0 hours respectively (difference, −22.0 hours; p=0.0008). The percentage of patients achieving clinical care was 81.4% in the Aemcolo group and 56.9% in the placebo group (difference, 24.5%; 95% CI, 11.3% to 37.7%; p=0.0001).

The most common adverse reactions with Aemcolo include headache and constipation. Aemcolo was not shown to be effective in patients with diarrhoea complicated by fever and/or bloody stool or diarrhoea due to pathogens other than non-invasive strains of *E. coli* and is not recommended for use in such patients. Aemcolo should not be used in patients with a known hypersensitivity to rifamycin, any of the other rifamycin class antimicrobial agents such as rifaximin, or any of the components in Aemcolo.

Source: www.fda.gov

FDA Approves First Biosimilar for Treatment of Non-Hodgkin's Lymphoma

Date: November 28, 2018

The FDA approved Truxima (rituximab-abbs) as the first biosimilar to Rituxan (rituximab) for the treatment of adult patients with CD20-positive, B-cell non-Hodgkin's lymphoma (NHL) to be used as a single agent or in combination with chemotherapy.

Truxima is indicated for the treatment of adult patients with relapsed or refractory, low grade or follicular, CD20-positive B-cell NHL as a single agent; previously untreated follicular, CD20-positive, B-cell NHL in combination with first-line chemotherapy and, in patients achieving a complete or partial response to a rituximab product in combination with chemotherapy, as single-agent maintenance therapy; and non-progressing (including stable disease), low-grade, CD20 positive, B-cell NHL as a single agent after first-line cyclophosphamide, vincristine and prednisone (CVP) chemotherapy.

The most common adverse reactions of Truxima are infusion reactions, fever, lymphopenia, chills, infection and asthenia. Healthcare providers are advised to monitor patients for tumour lysis syndrome, cardiac adverse reactions, bowel obstruction and perforation, and renal toxicity. Patients should not receive vaccinations while in treatment. Pregnant or breastfeeding women should not take Truxima due to its potential harm to a developing foetus or a newborn baby.

Like Rituxan, the labelling for Truxima contains a Boxed Warning regarding increased risks of the following: fatal infusion reactions, potentially fatal severe skin and mouth reactions; Hepatitis B virus reactivation which may trigger serious liver problems; and progressive multifocal leukoencephalopathy, a rare, serious brain infection that can result in severe disability or death.

Source: www.fda.gov

Apixaban for Prevention of Venous Thromboembolism in Patients with Cancer

Date: December 4, 2018

Patients with active cancer, especially those undergoing the first six months of chemotherapy, are subjected to an elevated risk of 9.6% in developing venous thromboembolism (VTE). While parenteral thromboprophylaxis such as low-molecular-weight heparin is available, its association with increased major bleeding risk limits its routine use in practice. A recent randomized, placebo-controlled, double-blind trial suggested that apixaban, an oral factor Xa inhibitor, can significantly reduce the rate of developing VTE within such patient group.

In Apixaban for the Prevention of Venous Thromboembolism in High-Risk Ambulatory Cancer Patients (AVERT) trial, 563 adult patients of intermediate-to-high risk – those with a Khorana score of 2 or greater – were included in the modified intention-to-treat analysis. Participants were randomly assigned to either treatment or control groups in 1:1 ratio; they received 2.5mg apixaban twice a day and matching placebo respectively. The treatment lasted for 180 days and patients were followed up to 210 days or until death.

As far as VTE development is concerned, the occurrence in the apixaban group was significantly lower than that of placebo group (HR, 0.41; 95% CI, 0.26 to 0.65; p<0.001). In terms of safety, percentage of major bleeding episodes was higher with apixaban (HR, 2.00; 95% CI, 1.01 to 3.95; p=0.046). No significant difference was observed in terms of overall survival; since most patients included in AVERT trial had advanced cancer, it was suggested that a distinct large-scale trial design should be adopted to investigate the ideal effect of mortality reduction by venous thromboembolism prophylaxis.

Source: www.nejm.org

Rx-to-OTC Switch – An Overview and its Implications to Public Health

YUEN, Ceci Wing Sze[a]; CHONG, Donald Wing Kit [a]*

*[a]GSK (Consumer Healthcare) HK Limited, 23/F, Tower 6, The Gateway, 9 Canton Road, Tsimshatsui, Kowloon, Hong Kong SAR, China (*Corresponding author)*

ABSTRACT

"Prescription-to-OTC switch" ("Rx-to-OTC switch") is a process allowing the reclassification of prescription-only medications to OTC status and it has become more prominent in recent years. This article provides an overview of the development of the Rx-to-OTC switch process and a review on the positive and negative impacts of Rx-to-OTC switch to public health worldwide. The expected impacts of Rx-to-OTC switch in Hong Kong would be reducing healthcare burden, enhancing involvement of community pharmacists in primary care and risk of misuse of medications. Pharmacists may play a broader role in medication therapy management.

INTRODUCTION

A "Prescription-to-OTC switch" ("Rx-to-OTC switch") is defined as the process of transferring a health authority-approved prescription drug to over-the-counter (OTC) status for the same dosage form, population, and route of administration.[1-3] It is a data-driven, scientifically rigorous and highly-regulated process.[4] Medications may become available in community pharmacies, convenience stores and supermarkets after reclassification to OTC status. The Rx-to-OTC switch was first started in 1976, but it has become more prominent only in recent years. The educational level of the public has been elevated through these years. Increased public health awareness and interest in self-care may be attributable to the rise of Rx-to-OTC switches in recent years.

The criteria for a medicine to undergo Rx-to-OTC switch are evolving. In general, potential drugs for Rx-to-OTC switch possess common features such as a high safety margin, using for medical conditions with easily recognisable symptoms and easy administration.[5] New drugs or new chemical entities are mostly approved and registered as prescription-only medications by health authorities when they are first introduced to the market. A reclassification application to OTC status may be submitted by manufacturer after gathering sufficient data from clinical trials and post-marketing surveillance studies. The World Health Organisation (WHO) has released a guideline for assessing the suitability of medications for use in self-medication. Various factors would be reviewed and considered by health authorities including safety profile, effectiveness and labelling before a drug may be switched to OTC status successfully.[6]

Numerous guidelines have been developed to foster and better regulate the Rx-to-OTC switch process. Currently available switch guidelines are developed by different countries and organisations including the WHO, the European Commission, United Kingdom, Canada, Australia, New Zealand and China. However, there is no Rx-to-OTC switch in Hong Kong, which complicates the switch process.

EVOLUTION & DEVELOPMENT OF RX-TO-OTC SWITCH AND GUIDELINES

The Rx-to-OTC switch first started in 1976 and the process of Rx-to-OTC switch has been evolving since then. More and more drugs of different categories have undergone reclassification to OTC status and the uprising trend in the number of Rx-to-OTC switches is expected to continue in the future.

In the Past

The regulation of OTC medicines has been evolving over the last century from the unrestrained and unregulated "dark ages" through the development of a formal regulatory process known as the OTC review to the appearance of Rx-to-OTC switches.[7] The first Rx-to-OTC switch occurred in 1976 was the switching of two antihistamines, brompheniramine and chlorpheniramine from prescription-only status to OTC

status. Since then, more and more products of different categories such as topical steroids, cough syrups, analgesics and antipyretics have undergone Rx-to-OTC switches. In 1994, it was estimated that up to 30% of the marketed OTC products in the US were from Rx-to-OTC switches.[8]

The process of Rx-to-OTC switch has gradually becomes more widely accepted and used due to the potential benefits brought. The prominent trend of Rx-to-OTC switches leads to the development of initiatives and guidelines for standardization and governing of the process. In 2012, the U.S. Food and Drug Administration (FDA) introduced the Nonprescription Drug Safe Use Regulatory Expansion (NSURE) initiative, which aimed to gain insight through public hearings to address issues pertaining to the Rx-to-OTC switch process, develop new strategies in expanding the scope of Rx-to-OTC switch and safeguard patients' safety on OTC medication use.[9]

At the Present

According to the Consumer Healthcare Products Association (CHPA), Rx-to-OTC Switches have transformed 106 approved ingredients, indications and dosage strengths into 700 OTC products currently on the market since 1976.[2] In the US, nearly 40% of the OTC medications at present were estimated to be transferred from Rx-to-OTC switches.[5]

With reference to the information from FDA, CHPA and other literatures, a list of Rx-to-OTC switch examples from 1976 to 2017 is shown in **Table 1**.[3, 10, 11]

In the Future

There has been a rising trend for Rx-to-OTC switches in recent years and it is anticipated that more and more Rx-to-OTC switches will take place in the future. Studies indicated that the number of Rx-to-OTC switch applications are likely to increase in Europe and the US.[3, 12] Technologies such as the use of smartphone apps will be more widely used to facilitate patient self-care and education for common medical conditions and assist Rx-to-OTC switches.[13-15]

The potential candidates for future Rx-to-OTC switches may include medications for migraine (triptans),

Table 1: Summary of Rx-to-OTC Switches in the US from 1976 to 2017[3, 10, 11]

Year of Rx-to-OTC Switches	Product category first underwent Rx-to-OTC Switch	Ingredients	Product examples
1976	Upper respiratory medications (Antihistamine, nasal decongestant)	Chlorpheniramine maleate, Pseudoephedrine hydrochloride	Chlor-Trimeton, Sudafed
1978	Sleeping aid	Doxylamine succinate	Unisom
1979	Topical steroid	Hydrocortisone	Lanacort
1980	Dental care	Sodium fluoride rinse	Fluorigard
1980	Topical anorectal vasoconstrictor for haemorrhoid	Ephedrine sulphate	Pazo Ointment
1981	Cough syrups	Diphenhydramine hydrochloride	Benylin
1982	Topical antifungals	Miconazole nitrate	Micatin
1982	Oral anaesthetic	Dyclonine hydrochloride	Sucrets Maximum Strength
1984	Pain killer, Antipyretics	Ibuprofen	Advil
1986	Ophthalmic preparations	Oxymetazoline hydrochloride	Ocuclear
1986	Anthelmintic	Pyrantel pamoate	Pin-X
1988	Antidiarrheal	Loperamide	Imodium
1990	Topical pediculicide (Head lice treatment)	Permethrin	Nix
1990	Topical anticandidal for vaginal candidiasis treatment	Clotrimazole	Gyne-Lotrimin
1995	Acid reducer for heartburn and indigestion	Famotidine	Pepcid
1996	Topical hair grower	Minoxidil	Rogaine
1996	Nicotine replacement therapy	Nicotine polacrilex	Nicorette
1997	Topical anti-dandruff	Ketoconazole	Nizoral
1998	Migraine	Aspirin/ Caffeine/ Acetaminophen	Excedrin Migraine
2000	Topical cold sore cream	Docosanol	Abreva cream
2003	Hives relief	Loratadine	Claritin hives relief
2006	Contraceptive	Levonorgestrel	Plan B
2006	Laxative	Polyethylene glycol 3350	MiraLAX
2007	Weight loss aid	Orlistat	Alli
2013	Patch for overactive bladder	Oxybutynin	Oxytrol for Women
2013	Intranasal steroid	Triamcinolone acetonide	Nasacort Allergy 24HR
2016	Topical gel for acne	Adapalene	Differin Gel

benign prostatic hyperplasia and contraception (combined oral contraceptives and progestin-only-pills).[13, 16, 17]

CURRENT SITUATION OF RX-TO-OTC SWITCH IN HONG KONG

The Department of Health governs drug-related regulatory affairs including Rx-to-OTC switch application review in Hong Kong. No Rx-to-OTC switch guideline has been released in Hong Kong, which complicates the application process. Pharmaceutical companies usually refer to in-house archives of some successfully switched cases and other countries' regulatory status of the pharmaceutical product for switch applications. Examples of successful switched products in Hong Kong include loratadine (Claritin®), omeprazole(Losec®), ranitidine (Zantac®) and salbutamol (Ventolin®).

IMPACT OF RX-TO-OTC SWITCH TO THE WORLD

The Rx-to-OTC switch provides benefits to patients and countries including higher accessibility of medications, cost-savings to the healthcare system and other economic benefits. However, the Rx-to-OTC switch might increase risk of errors in self-diagnosis and misuse of medications by patients.

Positive Impact

Greater access to medications

The Rx-to-OTC switch provides a greater access to medications for patients, which is implied by the increase in sales volume of medications after Rx-to-OTC switches. A descriptive study of national sales data from Sweden reported that the sales of 14 out of the 16 drugs undergoing Rx-to-OTC switches from 1980 to 1994 increased immediately after the reclassification and increased by 36% in average two years after reclassification.[18] Greater access to medications offers greater convenience to patients as OTC medications may be bought at convenience stores and community pharmacies and the time and cost travelling to clinics could be avoided. A survey from the US revealed that consumers were supportive to Rx-to-OTC switches as it was more convenient with prescription medications reclassified and available over-the-counter.[7] Self-care and treatment at early stages could also be facilitated by more accessible medications. By carrying out Rx-to-OTC switches enables a more widespread use of drug classes with an overall positive effect of 25%-42% on drug class utilization.[13] In the United States, the number of allergy patients using OTC allergy medications has increased from 66% in 2009 to 75% in 2015 after the reclassification of prescription-only allergy medications.[19] These figures indicate that Rx-to-OTC switch empowers patients to have autonomy in taking control of their own health and patients are more likely to seek for treatment of minor illness with OTC medications at an early stage.

Cost-savings to the healthcare system

The Rx-to-OTC switch can help save billions of dollars to the healthcare system. A study published by the CHPA in 2012 demonstrated that an annual saving of USD 120 billion to the US healthcare system would be brought by the availability of non-prescription medications.[20] A report published by the MacQuarie University also reflected that $10.4 billion could be saved for the Australian healthcare system by consumer self-care.[21] A systemic review of 12 published economic evaluations on Rx-to-OTC switches including 10 budget impact analyses (BIA), 3 cost-utility analyses (CUA) and 2 cost-effectiveness analyses (CEA) from 1995-2010 demonstrates that cost-saving is reported by most of the existing economic evaluations.[22] Cost-savings to healthcare system occurred nearly in all categories of medications after Rx-to-OTC switches, including upper respiratory medications,[23-26] heartburn medications,[4, 27] topical steroids,[28] vaginal antifungals[29] and nicotine replacement therapies.[30] An average saving of $174 per year to consumers and a $750 million saving to the healthcare system in the US after Rx-to-OTC switches of heartburn medications.[4] An estimated total annual savings attributed to the reclassification of nicotine replacement therapy would be be $1.8 to 2 billion dollars.[30]

Cost-savings to the healthcare system could be attributed to the reduction in unnecessary physician visits and laboratory tests after Rx-to-OTC switches as shown by research. An Australian study estimated the impact of Rx-to-OTC switches on physician visit associated costs through two hypothetical scenarios.[21] An additional AUD 3.8 billion would be costed by physician visits if eight common OTC product categories were changed to prescription-only products. It was also estimated that AUD 730 million, AUD 110 million and AUD 300 million would be saved for Medicare, health insurance and consumers respectively if 11 common prescription drug categories were reclassified to OTC status. Gurwitz *et al.* evaluated the effects caused by Rx-to-OTC switch of clotrimazole.[29] It was found that the number of physician visits dropped by 0.66 per 100 persons and an additional $13000 to $ 26000 could be saved by Health Maintenance Organization one year after reclassification of clotrimazole. Lipsky *et al.* also analyzed that the number of physician visits for vaginitis declined with more than $45 million savings annually after the switches of vaginal antifungal preparations.[31] In addition to vaginal antifungal preparations, decrease

in the number of physician visits was shown after the Rx-to-OTC switch of proton pump inhibitor. The number of physician visits for gastro-esophageal reflux disease (GERD) declined significantly after the reclassification of proton pump inhibitors to OTC status, which would result in an annual saving of $5.2 billion by avoiding half of the unnecessary physician visits.[32] In addition, pharmaceutical companies are more willing to lower the price of OTC drug products that are previously prescription-only medications due to larger consumer population after Rx-to-OTC switches.

The Rx-to-OTC switch could give rise to a general improvement in public health, which in turn improves health outcome and lessens healthcare burden in the long term. Earlier treatment and symptomatic relief using OTC medications could reduce healthcare costs pertaining to disease progression to later stage and management, emergency visits and hospitalizations. Apart from earlier disease management, Rx-to-OTC switch also encourages healthy lifestyle changes such as smoking cessation by the enhancement of the access to nicotine replacement therapies. Study data revealed that the sales and use of nicotine replacement therapies increased by 150% to 200% in the first year after reclassificatio.[33] The number of quit attempts using nicotine replacement therapies was estimated to increase from 2.5 million in 1995 before Rx-to-OTC switch to 5.8 million in 1997 after Rx-to-OTC switch based on the sales data[34] while the number of successful smoking cessation cases grows with estimated 17400 to 19700 extra quits from OTC nicotine patches and gums.[30] As a result, the increase in smoking cessation contributes to $1.8 to $2 billion societal benefits, higher quality-adjusted-life years and longer life expectancy for previous smokers.[30, 35]

However, the estimated amount of money saved due to Rx-to-OTC switches might be overestimated, as assumptions were made in the analyses without much supporting such as assumptions on consumer behaviour and OTC-switch rates.[22] Some economic evaluations did not consider certain factors such as additional physician visits caused by patients' misdiagnosis, which may increase healthcare costs.

Other economic benefits to the country or place

The Rx-to-OTC switch could improve labour productivity by reducing absences from work due to physician visits. The Centre for Workforce Health and Performance in the US reported an annual lost-productivity cost of USD $165 billion caused by 26 chronic conditions.[36] Allergies and hay fever, chronic back and neck pain, and heartburn and gastro-esophageal reflux disease were the conditions contributing to the highest lost-productivity costs in the US. Another modelling study from the Association of the European Self-Medication Industry estimated that Rx-to-OTC switches of 5% prescription-only medications would result in annual savings of more than EUR 16 billion in Europe.[37] Not only do OTC drug products for disease treatment improve productivity, but OTC supplements for disease prevention such as vitamin D and folic acid also help improve productivity in high-risk populations.[38]

Increase profitability for pharmaceutical companies

The Rx-to-OTC switch is beneficial to pharmaceutical companies in two ways: increasing sales revenues and extending product lifecycle. While the price for one product with the same composition in OTC status may be lower than that in prescription status, but the Rx-to-OTC switch enhances the availability and consumer accessibility to the OTC drug products and thus sales of the products. A new self-medication category could even be created in some cases after reclassification such as nicotine replacement therapies and hair growth treatment, in which sales may be further increased. Studies showed that OTC brands could generate sales revenues ranging from US$20 million to more than US$200 million per year for manufacturers.[3] Pepcid AC® is another example demonstrating the rise in sales revenues after Rx-to-OTC switches. The sales of Pepcid AC® in the first year after reclassification was more than $200 million.[39] Apart from higher sales revenues, product lifecycle could also be expanded. Pharmaceutical companies could submit for Rx-to-OTC switch when the prescription products are close to patent expiration, so as to extend their product lifecycle and reduce the impact on sales of generic competition.[40]

Negative Impacts

Errors in self-diagnosis

While the Rx-to-OTC switch may enhance self-care, misdiagnosis and improper use of OTC medications may occur. Self-medication relies heavily on patients' own judgement and information on the product label. Errors might arise in self-diagnosis as the symptoms of some diseases like vulvovaginal candidiasis might be difficult to be differentiated from other medical conditions such as bacterial vaginosis and trichomoniasis.[41] Ferris *et al.* highlighted the fact that the sales of antifungal products was nearly doubled after Rx-to-OTC switch, but the incidence of candidal vaginitis remained similar, implying that antifungal OTC products might be used by consumers without candidal vaginitis due to mistakes in initial diagnosis.[42] Misdiagnosis would give rise to multiple consequences including financial expenditure wastage, suboptimal or delayed treatment of underlying conditions, lack of drug efficacy and higher risk of adverse drug reactions in patients.

Misuse and noncompliance of OTC medications

The Rx-to-OTC switch is not without potential risks. The situation of misuse and noncompliance of OTC medications may be worsened by Rx-to-OTC switches. Advertisements of OTC medications are allowed in some countries, which could be misleading if not properly regulated. For instance, the OTC sales of sex enhancer drugs is being advertised using mass media in India[43] and the inappropriate advertisements may exacerbate drug misuse by the public. Misuse and noncompliance of OTC drugs such as exceeding the maximum dose, using multiple preparations and drug abuse are not uncommon. A survey conducted by the American Pharmaceutical Association identified that one third of consumers exceeded maximum dose of OTC products.[44] Consumers were also shown to have a misconception that switch OTC products were more effective than other OTC products. Non-compliance could lead to risk of adverse drug reactions and overdose. Using multiple OTC preparations may result in polypharmacy and drug-drug interactions while the use of OTC analgesics particularly opiate-based analgesics might be abused by some patients. Another concern would be the drug resistance problem associated with Rx-to-OTC switches of antimicrobials. Drug resistance to S pneumoniae and non-typhi salmonella was aggravated by the non-prescription use of penicillins, erythromycin and ciprofloxacin in Thailand.[45] In New Zealand, the incidence of drug-resistant skin infections increased after reclassification of a topical antibiotic mupirocin to OTC status.[46] The proposed reclassification of oral acyclovir for genital herpes treatment was not supported due to drug resistance concerns.[47]

Shifted healthcare costs from insurance companies to consumers

The Rx-to-OTC switches help save money to the overall healthcare system. However, OTC medications are not covered by healthcare and insurance plans and the healthcare costs may be transferred from insurance companies to consumers.[29] Patients may encounter difficulties in obtaining reimbursement for OTC products and have higher resulting out-of-pocket costs, which could impose a financial burden especially to underprivileged individuals.[2, 15, 48] Gianfrancesco et al. examined the consumer OTC drug costs after the Rx-to-OTC switches of cromolyn sodium, tioconazole, ketoconazole, and terbinafine in 2002.[49] It was found that the out-of-pocket costs for consumers increased after reclassification for all four switched products. A retrospective study investigating the prescription records of patients revealed that patients using loratadine were more than twice as likely to change to use another prescription-only antihistamine before the known Rx-to-OTC switch loratadine, in order to avoid the loss of insurance reimbursement for antihistamine.[3]

EXPECTED IMPACTS OF RX-TO-OTC SWITCH IN HONG KONG

There are both positive and negative expected impacts of Rx-to-OTC switches on Hong Kong. Not many Rx-to-OTC switches had taken place in Hong Kong and there are limited local data and studies on the impact of Rx-to-OTC switches in Hong Kong. The studies and statistics about the impacts of Rx-to-OTC switch from other places around the world may be used as references for the expected impacts on Hong Kong, but the data used would be subject to the limitation of regional variations in healthcare systems, economic and social conditions.

Reduction in healthcare burden

The Rx-to-OTC switches is expected to reduce the healthcare burden in Hong Kong by enhancing self-care and accessibility to OTC medications. The healthcare system in Hong Kong operates on a dual-track basis with both public and private sectors.[50] As the public sector acts as the safety net for patients, healthcare services from the public sector have a very low user charges with 95% of the costs subsidised by the Hong Kong government.[50] The burden of the public healthcare sector is huge. The public sector is currently providing medical services to 90% of patients with only 40% of doctors, while the private sector is providing medical services to 10% of patients with 60% of doctors.[51] The Rx-to-OTC switches could provide cost-savings to the healthcare system and reduce unnecessary physician visits as demonstrated by aforementioned studies, which may help lessen the healthcare burden especially in the public sector in Hong Kong.

Enhancing involvement of community pharmacists in primary care

The Rx-to-OTC switches increase the accessibility of medications and encourage the management of minor illnesses using OTC products, which offers more opportunities for pharmacists to involve in providing primary care services to patients in the community pharmacy setting. Pharmacists could play a broader role in medication therapy management such as helping patients to choose the most appropriate OTC medication and monitoring of therapeutic outcome using diagnostic tests.[52] There is no separation of prescribing and dispensing in Hong Kong unlike in other places around the world. Pharmacists are only present at authorised seller of poisons (ASP) with a “Rx” symbol. With more OTC products available and a higher public awareness

of self-care, it is anticipated that the role and participation of community pharmacists in symptoms and disease management could be enhanced.

Risk of multiple and inappropriate medications

More OTC products would be available after Rx-to-OTC switches, which might increase the risk of misuse and self-misdiagnosis. The use of unnecessary drugs increases polypharmacy in patients especially in elderly patients with multiple chronic diseases. Hong Kong has the highest life expectancy for both male and female around the globe,[53] with life expectancy of 81.3 years for male and 87.3 years for female in 2016.[54] The population of Hong Kong is aging with proportion of elders as one in eight now. The proportion of elders is foreseen as one in four by 2030.[50] The risk of multiple and inappropriate medications is expected to be higher to patients particularly elderly patients after the reclassification of medications to OTC status without the need of prescriptions.

CONCLUSION

The Rx-to-OTC switch is a prevalent trend around the world at the present and in the coming years. Numerous benefits are brought by switching appropriate drug candidates from prescription-only to OTC status as shown by studies and the Rx-to-OTC switch is expected to lessen the healthcare burden in Hong Kong. However, it is of essence to lower the concomitant risk of misuse of OTC medications. Technology could be used to develop user-friendly and reliable self-monitoring means and provide health information to patients aside labelling information of OTC products. Pharmacists also play an important role in medication therapy management.

The Rx-to-OTC switch guidelines provide framework for switch application to health authorities and facilitate the Rx-to-OTC switch process. There is no Rx-to-OTC switch guideline in Hong Kong and it is suggested to develop a local switch guideline in Hong Kong with reference to other countries' existing switch guidelines and criteria in differentiating OTC and Rx medications.

Author's background
YUEN, Ceci Wing Sze *is a pharmacy intern of the GSK (Consumer Healthcare) HK Limited. For more information about this article, please contact her through her email address: ceciyuen0314@gmail.com*
CHONG, Donald Wing Kit *is currently the Regulatory Affairs Director of the GSK (Consumer Healthcare) HK Limited. His email address is: donald.w.chong@gsk.com*

References

1. US Food and Drug Administration (FDA). Regulation Overview: Rx-to-OTC Switch. (Available from: https://www.accessdata.fda.gov/scripts/cder/training/OTC/topic3/topic3/da_01_03_0180.htm.)
2. Consumer Healthcare Products Association. FAQs About Rx-to-OTC Switch. (Available from: https://www.chpa.org/switchfaqs.aspx.)
3. Mahecha LA. Rx-to-OTC switches: trends and factors underlying success. Nat Rev Drug Discov. 2006;5(5):380-5.
4. Consumer Healthcare Products Association. Rx-to-OTC Switch. (Available from: https://www.chpa.org/switch.aspx.)
5. Kartha SS, Kulyadi GP, Bhat K, Sathyanarayana MB. Switching Drugs from Rx to OTC status – A Regulatory Perspective. *J Young Pharm.* 2017;9(1):3-7.
6. World Health Organisation (WHO). Guidelines for the Regulatory Assessment of Medicinal Products for Use in Self-Medication. 2000.
7. Soller RW. Evolution of Self-Care with Over-the Counter Medications. *Clin Ther.* 1998;20 Suppl C(C134-140).
8. Newton G, Popovich NG, Pray WS. Rx-to-OTC switches: from prescription to self-care. *J Am Pharm Assoc (Wash).* 1996;NS36(8):488-95.
9. Mehta RS. Nonprescription Drug Safe Use Regulatory Expansion (NSURE). Presented at: FDA 2nd Annual Health Professional Organizations Conference. 2012 (Available from: http://www.fda.gov/downloads/ForHealthProfessionals/UCM330650.pdf.)
10. Food and Drug Administration (FDA). Prescription to Over-the-Counter (OTC) Switch List. 2017 [updated 2017 Sep 20]. (Available from: https://www.fda.gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm106378.htm.)
11. Consumer Healthcare Products Association. Ingredients & Dosages Transferred From Rx-to-OTC Status (or New OTC Approvals) by the Food and Drug Administration Since 1975. 2018.
12. Hemwall EL. Increasing access to nonprescription medicines: a global public health challenge and opportunity *Clin Pharmacol Ther* 2010;87(267-269).
13. Stomberg C, Philipson T, Albaugh M, Sood N. Utilization effects of Rx-OTC switches and implications for future switches. *SciRes*. 2013;5(10):1667-80.
14. Engelberg Center for Health Care Reform. Innovative Technologies and Nonprescription Medications: Addressing Undertreated Diseases and Conditions through Technology Enabled Self-Care 2013. (Available from: https://www.brookings.edu/wp-content/uploads/2013/05/09-innovative-technologies-nonprescription-medications-presentation-slides.pdf.)
15. Baker DE. Nonprescription drug safe use regulatory expansion. *Hosp Pharm* 2013;48(6):448-50.
16. Grossman D, Fuentes L. Over-the-counter access to oral contraceptives as a reproductive healthcare strategy. *Curr Opin Obstet Gynecol.* 2013;25:500-5.
17. Howard DL, Wall J, Strickland JL. Physician attitudes toward over the counter availability for oral contraceptives. *Matern Child Health J.* 2013;17:1737-43.
18. Carlsten A, Wennberg M, Bergendal L. The influence of Rx-to-OTC changes on drug sales. Experiences from Sweden 1980-1994. *J Clin Pharm Ther*. 1996;21(6):423-30.

19. Consumer Healthcare Products Association. Statistics on OTC Use (Available from: https://www.chpa.org/marketstats.aspx.)

20. Consumer Healthcare Products Association. OTC Value (Available from: https://www.chpa.org/OTCValue.aspx.)

21. MacQuarie University The MacQuarie Centre for the Health Economy. The value of OTC medicines in Australia 2014 2014.

22. Cohen J, Millier A, Karray S, Toumi M. Assessing the economic impact of Rx-to-OTC switches: systematic review and guidelines for future development *J Med Econ* 2013;16(6):835-44.

23. Temin P. Realized benefits from switching drugs *J Law Econ*. 1992;35:351-69.

24. Sullivan PW, Nair KV, Patel BV. The effect of the Rx-to-OTC switch of loratadine and changes in prescription drug benefits on utilization and cost of therapy. *Am J Manag Care* 2005;11(6):374-82.

25. Spencer HM. The Rx-to-OTC switch of Claritin, Allegra, and Zyrtec: an unprecedented FDA response to petitioners and the protection of public health. *Am Univ Law Rev.* 2002; 51:999-1050.

26. Northwestern. Over-the-counter drugs could save $4.75 billion annually 2004 (Available from: https://www.northwestern.edu/newscenter/stories/2004/11/upper.html).

27. Kunz K, Arundell E, Cisternas M, A. H. Economic Implications of Self-treatment of Heartburn/Nonulcur Dyspepsia with Nonprescription Famotidine in a Managed Care Setting. *J Manag Care Spec Pharm.* 1996;2(3):263-71.

28. Nonprescription Drug Manufacturers Association. Citizen's Petition on the Rx-to-OTC switch of 1% hydrocortisone Fed Regist 1990;55:6932-51.

29. Gurwitz JH, McLaughlin TJ, Fish LS. The effect of an Rx-to-OTC switch on medication prescribing patterns and utilization of physician services: the case of vaginal antifungal products. *Health Serv Res.* 1995;30:672-85.

30. Keeler TE, Hu TW, Keith A, Manning R, Marciniak MD, Ong M, et al. The benefits of switching smoking cessation drugs to over-the-counter status. *Health Econ*. 2002;11(5):389-402.

31. Lipsky M, Waters T, Sharp L. Impact of vaginal antifungal products on utilization of healthcare services: evidence from patient visits. *J Am Board Fam Pract.* 1999;13(3):178-82.

32. Chang DW, Grotts J, Tseng CH, Brass EP. Time Trends in Physician Visits for Gastroesophageal Reflux Disease Before and After the Rx-to-OTC Switch of Proton Pump Inhibitors. *J Clin Pharmacol.* 2017;57(4):452-8.

33. Shiffman S, Sweeney CT. Ten years after the Rx-to-OTC switch of nicotine replacement therapy: what have we learned about the benefits and risks of non-prescription availability? . *Health Policy*. 2008;86(1):17-26.

34. Shiffman S, Gitchell J, Pinney JM, Burton SL, Kemper KE, Lara EA. Public health benefit of over-the-counter nicotine medications *Tobacco Control* 1997;6(4):306-10.

35. Lawrence WF, Smith SS, Baker TB, Fiore MC. Does over-the-counter nicotine replacement therapy improve smokers' life expectancy?. *Tobacco Control*. 1998;7(4):364-8.

36. Jinett K. Consumerism, Self-Care Trends and the Broader Value of Employee Health: The Center for Workforce Health and Performance; 2016 (Available from: https://www.tcwhp.org/sites/default/files/reports/CWHP%20Report%20August%202016_Consumerism_Self-Care%20Trends_Broader%20Value%20of%20Employee%20Health.pdf.)

37. Association of the European Self-Medication Industry. The economic and public health value of self medication: Association of the European Self-Medication Industry; 2004 (Available from: http://www.aesgp.eu/media/cms_page_media/68/2004study.pdf)

38. Shanahan C, Lorimier RD. Targeted Use of Complementary Medicines: Potential Health Outcomes & Cost Savings in Australia 2014 (Available from: http://www.asmi.com.au/media/14046)final_frost_sullivan_report_photocopy_ready_8_oct_2014.pdf.

39. Brass EP. Changing the status of drugs from prescription to over-the-counter availability. *N Engl J Med*. 2001;345(11): 810-6.

40. Newton GD, Pray WS, Popovich NG. New OTC drugs and devices: a selected review. *Am Pharm*. 1995;NS35(2):35-46.

41. Sobel JD. Vaginitis. *N Engl J Med*. 1997;337:1896-903.

42. Ferris DG, Nyirjesy P, Sobel JD, Soper D, Pavletic A, Litaker MS. Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. *Obstet Gynecol* 2002;99(3):419-25.

43. Debaje SP, Hiremath RC. Over the counter (OTC) sell of sex enhancer drugs: an emerging public health issue in India. *Int J Res Med Sci.* 2014;2(1):198-201.

44. Schulke DG. American Pharmaceutical Association review of literature on prescription to over-the-counter drug switches *Clin Ther* 1998;20(Suppl 3):C124–C33.

45. Morgan DJ, Okeke IN, Laxminarayan R, Perencevich EN, Weisenberg S. Non-prescription antimicrobial use worldwide: a systematic review. *Lancet Infect Dis*. 2011;11(9):692–701.

46. Upton A, Lang S, Heffernan H. Mupirocin and Staphylococcus aureus: a recent paradigm of emerging antibiotic resistance. *J Antimicrob Chemother* 2003;51(3):613-7.

47. Sande MA, Armstrong D, Corey L, Drew WL, Gilbert D, Moellering RC, et al. Perspectives on switching oral acyclovir from prescription to over-the-counter status: report of a consensus panel. *Clin Infect Dis* 1998;26(3):659-63.

48. Pawaskar MD, Balkrishnan R. Switching from prescription to over-the counter medications: a consumer and managed care perspective *Manag Care Interface*. 2007;20:42-7.

49. Gianfrancesco F, Manning B, Ruey-Hua W. Effects of prescription to OTC switches on out-of-pocket health care costs and utilization. *Drug Benefit Trends* 2002;14(3):13-30.

50. Ko WM. HK healthcare is a dual-track system 2013 (Available from: http://www.news.gov.hk/en/record/html/2013/04/20130409_190409.shtml.)

51. Tsang E. Inside Hong Kong's public hospital crisis: temporary beds, angry patients, nurses and doctors stretched to breaking point: South China Morning Post; 2016 [updated 2016 Mar 27. (Available from: http://www.scmp.com/news/hong-kong/health-environment/article/1931049/inside-hong-kongs-public-hospital-crisis-temporary.)

52. Barlas S. FDA Considers a New Paradigm For Over-the-Counter Medications. More Power—but More Burdens—for Pharmacists and Pharmacies. P T. 2012;37(5):300-302,305.

53. Hollingsworth J. Hongkongers top life expectancy rankings worldwide for second year in a row South China Morning Post; 2017 [updated 2017 Jul 29]. (Available from: http://www.scmp.com/news/hong-kong/health-environment/article/2104584/hongkongers-top-life-expectancy-rankings-worldwide.)

54. Centre for Health and Protection, Department of Health, HKSAR Government. Major Health Indicators in 2015 and 2016 (Available from: https://www.chp.gov.hk/en/statistics/data/10/27/110.html.)

Update on Drug Treatments for Idiopathic Pulmonary Fibrosis for Hong Kong Pharmacists

KONG, Sarah Hiu Hung[a]
[a] *Grantham Hospital, Hospital Authority, Wong Chuk Hang, Hong Kong SAR, China*

ABSTRACT

About 350 people have idiopathic pulmonary fibrosis (IPF) in Hong Kong. In 2016, two medications, nintedanib and pirfenidone, became available in Hong Kong for the treatment for this rare disease. Clinical trial data and real-world data supports nintedanib and pirfenidone's ability to increase a patient's progression free survival and is shown to be efficacious in Asian patients. The access to IPF through the Hong Kong Alliance for Rare Diseases scheme for nintedanib is discussed. There still needs to be more research on patient groups not included in clinical trials and on new targets for more treatment options for this rare disease.

Keywords: *idiopathic pulmonary fibrosis, nintedanib, pirfenidone, treatment, Hong Kong, Asian*

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive idiopathic interstitial pneumonia that presents with fibrosis and progressive respiratory failure. In Asia, the incidence has been reported approximately 1.3-3.6 per 100,000 population.[1] In Hong Kong, it is estimated that 350 people have this rare condition.[2] It primarily affects older adults, its incidence increasing with age, typically in the sixth and seventh decades with a higher rate in men compared with women.[3] From the onset of symptoms, patients typically have only 3-5 years to live.[4] The 5 year survival rate is about 20%, which is lower than some cancers such as colorectal and breast cancer.[2] The aetiology of IPF remains unknown; however some risk factors associated with the disease include cigarette smoking, gastroesophageal reflux, specific environmental exposures, viral infection and age.[5] Several factors have been associated with shortened survival time: older age at presentation, extensive cigarette smoking, lower body mass index, more severe physiologic impairment, greater radiologic extent of disease, and the development of other complications or conditions (e.g. pulmonary hypertension, emphysema, cardiovascular disease, and bronchogenic cancer).[6] Until recently, without medication, patients could only rely on pulmonary rehabilitation exercise or oxygen therapy to ease symptoms. In 2016, two medications, nintedanib and pirfenidone, became available in Hong Kong for the treatment of IPF. These drugs were first approved in the United States Food and Drug Administration and the European Medicines Agency in 2014.

Clinical Presentation and Diagnosis

Patients with IPF classically present with worsening chronic exertional dyspnea and cough, bibasal end-inspiratory crackles, and may be hypoxemic.[3] The diagnosis of IPF is typically based on the combination of clinical features and typical high-resolution computed tomography (HRCT) appearances, following the exclusion of known causes of interstitial lung disease.[3] On HRCT, usual interstitial pneumonia (UIP) with the following four features: honeycombing, reticular abnormality, sub pleural and basal predominance, and an absence of features inconsistent with a UIP pattern is used as a diagnosing criteria **(Figure 1)**.[3] There is much heterogeneity amongst patients with IPF, which is why it is both a great challenge and an opportunity to personalize care, treatment and outcomes.[1]

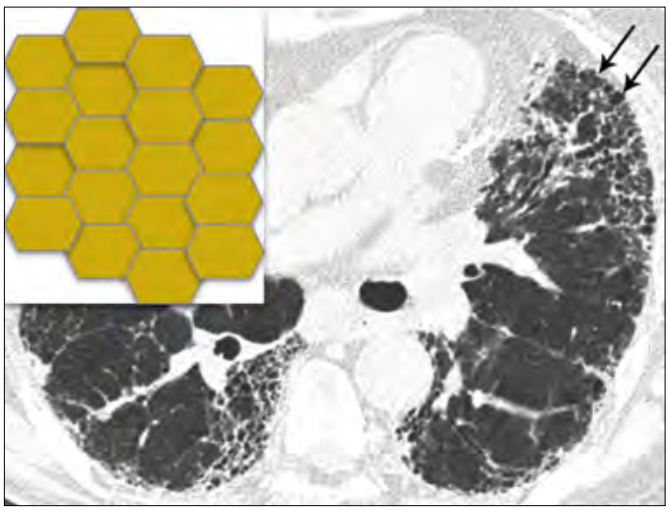

Figure 1. *Classic idiopathic pulmonary fibrosis in 70-year-old man. HRCT shows bilateral sub-pleural reticulation and honeycombing (straight arrows).*
(adapted from Souza, C. et al)[7]

Treatment

Previously IPF has been viewed as a chronic inflammatory disease and that chronic inflammation

precedes and leads to fibroblast activation, remodeling and fibrosis.[8] As a direct result, historical treatment consisted of immunomodulation with corticosteroid therapy or a combination of immunosuppressive agents. Trials of agents such as interferon-γ, prednisolone, N-acetylcysteine (NAC), azathioprine, etanercept and bosentan were eventually found to be non-effacacious.[4] Other randomized controlled trials in IPF that have been found to have negative outcomes can be seen in **Table 1**.[9]

It was later postulated that pro-fibrotic cytokines directly attract myofibroblasts and subsequent matrix formation.[23] This leads to architectural distortion and chronic progressive scarring of the gas-exchanging interstitial tissue of the lung causing pulmonary function decline and ultimately, a fatal outcome.[24] Pirfenidone and nintedanib, as shown in **Table 2**, benefit patients by slowing disease progression and both drugs are now integrated into regular IPF care. A revision of the ATS/ERS/JRS/ALAT (American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association) Clinical Practice Guideline from 2011 was made in 2015 and highlights of changes in recommendations can be found in **Table 3**.[25] A brief summary of pirfenidone and nintedanib can be found in **Table 4**.

Clinical efficacy of pirfenidone

Two key placebo controlled clinical trials led to the approval of pirfenidone. The two trials, "Clinical studies assessing pirfenidone in IPF: research of efficacy and safety outcomes-1" (CAPACITY-1) and "Clinical studies assessing pirfenidone in IPF: research of efficacy and safety outcomes-2" (CAPACITY-2), were carried out across 110 centers in Australia, Europe, and North America.[29] Patients recruited were aged between 40-80 years, with measure FVC ≥ 50% predicted and diffusion capacity for carbon monoxide (DL_{30}) ≥ 35%.[29] They were followed-up for 72 weeks and the primary endpoint was change in percent predicted FVC at week 72.[29] In CAPACITY-1 study, 435 patients were assigned 2:1:2

Table 1: Completed Negative Clinical Trials of Drug Therapy in IPF[9]

Study Drug	Mechanism of Action	Primary Outcome (met/not met)
N0/AZA/CS versus NAC/CS[10]	Immunosuppression/ anti-inflammatory/ antioxidant	Change in FVC and DL_{CO} (met)
NAC/AZA/CS versus NAC verse placebo[11]	Immunosuppression/ anti-inflammatory/ antioxidant	Terminated early (increase deaths and hospitalization)
NAC versus placebo[12]	Antioxidant	Change in FVC (not met)
IFN-g[13]	Immunomodulation	Progression-free survival (not met)
IFN-r[14]	Immunomodulation	Terminated early
Etanercept[15]	Immunomodulation	Change in FVC (not met)
Warfarin[16]	Anticoagulation	Terminated early because of increased mortality in treatment arm
Bosentan[17]	Endothelin receptor antagonist	Change in 6MWD (not met)
Bosentan[18]	Endothelin receptor antagonist	Time to IPF worsening (not met), death (not met)
Macitentan[19]	Endothelin receptor antagonist	Change in FVC (not met)
Ambrisentan[20]	Endothelin receptor antagonist	Terminated early-increase risk of progression and hospitalization in the treatment arm
Sildenafil[21]	PDE-5 Inhibitor	Increase in 6MWD>20% (not met)
Imatinib[22]	TKI	Time to disease progression (not met)

Abbreviations: IPF, idiopathic pulmonary fibrosis; AZA, azathioprine; CS, corticosteroid; NAC, N-acetylcysteine; FVC, forced vital capacity; DL_{co}, diffusion capacity for carbon monoxide; IFN-γ, interferon-γ; 6MWD, 6-minute walking distance; PDE-5, phosphodiesterase-5; TKI, tyrosine kinase inhibitor.

Table 2: Completed Positive Trials of Drug Therapy in IPF[9]

Study Drug [clinical trial]	Mechanism of Action	Primary Outcome (met/not met)
Nintedanib [To improve pulmonary fibrosis with BIBF 1120 (TOMORROW)][26]	TKI	Annual rate of decline in FVC- not met, trend toward reduction of FVC decline
Nintedanib [Efficacy and safety of nintedanib in IPF randomized, double-blind phase 3 trials (INPULSIS-1 and INPULSIS-2)][27]	TKI	Annual rate of decline in FVC (met)
Pirfenidone [Taniguchi][28]	Antifibrotic	Change in VC (met)
Pirfenidone [Clinical studies assessing pirfenidone in IPF: research of efficacy and safety outcomes-1(CAPACITY-1)][29]	Antifibrotic	Change in FVC (met)
Pirfenidone [Clinical studies assessing pirfenidone in IPF: research of efficacy and safety outcomes-2(CAPACITY-2)][29]	Antifibrotic	Change in FVC (not met)
Pirfenidone [Assessment of pirfenidone to confirm efficacy and safety in IPF(ASCEND)][30]	Antifibrotic	Change in FVC (not met)

Abbreviations: FVC, forced vital capacity; TKI, tyrosine kinase inhibitor; VC, vital capacity

Table 3: Revised Recommendations in ATS/ERS/JRS/ALAT IPF Guidelines[25]		
Therapy	2015 Guidelines	2011 Guidelines
New and revised recommendations on usage		
Warfarin	Strong recommendation against[a]	Conditional recommendation against[c]
Prednisone + Azathioprine + N-acetylcysteine	Strong recommendation against[b]	Conditional recommendation against[b]
Ambrisentan	Strong recommendation against[b]	Not addressed
Imatinib	Strong recommendation against[a]	Not addressed
Nintedanib	Conditional recommendation for[a]	Not addressed
Pirfenidone	Conditional recommendation for[a]	Conditional recommendation against[b]
Macitentan	Conditional recommendation against[b]	Strong recommendation against[a]
Bosentan	Conditional recommendation against[b]	Strong recommendation against[a]
Sildenafil	Conditional recommendation against[a]	Not addressed
Unchanged recommendations on usage		
Antacid therapy	Conditional recommendation for[c]	Conditional recommendation for[c]
N-acetylcysteine monotherapy	Conditional recommendation against[b]	Conditional recommendation against[b]
Anti-pulmonary hypertension therapy	Reassessment deferred	Conditional recommendation against[c]
Lung transplant: single vs. bilateral	Formulation of recommendation deferred	Not addressed
[a] moderate confidence in effect estimates; [b] low confidence in effect estimates; [c] very low confidence in effect estimates.		

Table 4: Summary of Nintedanib and Pirfenidone		
	Nintedanib[31,32]	Pirfenidone[33]
Brand Name	Ofev®	Esbriet®
Dose	150mg every 12 hours	Week 1: 267mg 3 times daily Week 2: 534mg 3 times daily Week 3: 801mg 3 times daily Titration to help with adverse reaction tolerance
Renal Impairment	CrCl ≥ 30ml/minute: no initial dosage adjustment necessary CrCl < & end-stage renal disease: no dosage adjustments provided by manufacturer	Use with caution
Hepatic Impairment	Mild (Child-Pugh class A): 100mg every 12 hours Moderate to severe (Child-Pugh Class B or C): Use is not recommended	Use with caution
Mechanism of Action	Inhibits multiple receptor tyrosine kinases and non-receptor tyrosine kinases including platelet-derived growth factor (PDGFR alpha and PDGFR beta); fibroblast growth factor receptor (FGFR1, FGFR2, FGFR3); vascular endothelial growth factor (VEGFR1, VEGFR2, and VEGFR3); and Fms-like tyrosine kinase-3 (FLT3). It also binds competitively to the adenosine triphosphate binding pocket of these receptors and blocks the intracellular signaling which is crucial for the proliferation, migration, and transformation of fibroblasts.	Pleiotropic antifibrotic effect suppressing fibroblast proliferation, reducing the production and activity of fibrosis-associated proteins and cytokines, including the key profibrotic cytokine transforming growth factor-beta
Available Drug Forms (estimation of cost per tablet)	100mg capsule (HK$ 200) 150mg capsule (HK$ 300)	267mg capsule (HK$ 180)
Key Clinical Trials	CAPACITY-1 CAPACITY-2 ASCEND	INPULSIS-1 INPULSIS-2 INPULSIS-ON
Drug Interactions requiring dose adjustments		CYP1A2 inhibitors (fluvoxamine, ciprofloxacin)
Contraindications	Hypersensitivity to nintedanib, peanuts, soya or any of the excipients.	
Adverse Effects	Diarrhea, elevated liver enzyme, cardiac events	Photosensitivity, nausea, dyspepsia, vomiting, rashes, dizziness, elevated liver enzymes, upper respiratory tract infection, anorexia

ratio to pirfenidone 2403mg/day, pirfenidone 1197mg/day, or placebo, whereas in the CAPACITY-2 trial, 344 patients were assigned 1:1 ratio to pirfenidone 2403mg/day or placebo.[29] The primary outcome in the two trials differed, with CAPACITY-1 favoring the treatment arm (FVC -8.0% versus -12.4%), while CAPACITY-2 showed no statistically significant change.[29] The trial group further completed a preplanned pooled analysis of the patients in both trials that suggested an overall benefit from pirfenidone treatment.[26] There was no statistically

significant difference in the risk of death between the treatment and placebo groups.[29]

Due to the discrepancy between the primary outcomes in the two CAPACITY trials, a further randomized placebo-controlled trial "Assessment of pirfenidone to confirm efficacy and safety in IPF" (ASCEND) was designed.[30] The main differences of the ASCEND trial compared with the CAPACITY trials were that diagnosis, spirometry, and decisions regarding deaths were centralized and eligibility criteria was aimed at patients who were at higher risk of disease progression.[30] Patients were recruited from 127 centers across nine countries, inclusion criteria were age between 40-80 years, diagnosis of UIP on HRCT or surgical lung biopsy, FVC 50-90%, DL_{CO} 30-90% predicted, FVC>80%, and 6MWD of > 150 m. The primary endpoint was to measure the change in the percent predicted FVC at the end of 52 weeks.[30] A total of 555 people were enrolled and assigned to placebo or pirfenidone in 1:1 ratio.[30] The groups were well matched and 94.1% completed the study.[30] In the pirfenidone arm (46 patients) compared with placebo arm (88 patients) had a decline in FVC of 10% or more (47.9% reduction with treatment).[30] 63 patients on pirfenidone showed no decline in the percent predicted FVC compared to 27 receiving placebo.[30] Mean decline in FVC was 235 ml in those taking pirfenidone compared with 428 ml in those taking placebo.[30] It further translates into fewer patients showing deterioration in their 6MWD of 50 m or more at the end of the 52-week period.[30] All of the above mentioned results had statistical significance.30 When looking at all-cause mortality, there was no statistically significant difference although it was lower in the pirfenidone group.[30] None of the secondary outcomes showed symptomatic benefits for patients, with no statistically significant difference in their Shortness of Breath Questionnaire scores.[30]

In a separate double-blind randomized trial of pirfenidone consisting of 76 Chinese patients, showed that pirfenidone treatment prolonged the progression-free survival time.[34] Treatment effects were significant at the 24th week but did not persist to the 48th week.[34] Adverse event rates were higher in the treatment group, with rash being the most common adverse event seen in Chinese pirfenidone users.[34]

Adverse effects of pirfenidone

In the CAPACITY studies, 15% of patients discontinued treatment due to side effects.[29] Patients in the treatment arm had a higher incidence of nausea, dyspepsia, vomiting, rashes, and dizziness than the placebo group.[29] A common side effect was photosensitivity,[29] and it is now recommended that patients taking pirfenidone should avoid sun exposure, wear protective clothing and use sunblock with high ultraviolet A and B protection.[35] The prevalence of elevations in alanine aminotransferase and aspartate aminotransferase more than three times the upper limit of normal was 4% in the treated group and <1% in the placebo group.[29]

Most of the patients who completed the CAPACITY trials were enrolled into an open-label extension study (RECAP) to evaluate the long-term safety of pirfenidone.[36] An interim analysis was published, reviewing data from enrollment in September 2008 until August 2013. The median duration of treatment was 163 weeks, with 99% of patients reporting at least one treatment-emergent adverse event.[36] In more than one-third of patients, these included dyspnea, cough, or bronchitis, as well as upper respiratory symptoms of infection or nasopharyngitis.[36] In total, 33% of patients also experienced nausea, with just less than one-third complaining of fatigue and dizziness.[36] Some patients also experienced anorexia.[36] Rash and photosensitivity were less of a problem in RECAP (16% and 9%) compared with CAPACITY trials (32% and 12%), although 45% of patients went on to discontinue treatment due to treatment-emergent adverse events.[29,36]

Pirfenidone's place in therapy

The 2015 ATS/ERS/JRS/ALAT Clinical Practice Guideline conditionally recommends pirfenidone for use.[25] National Institute for Health and Care Excellence (NICE) guidelines recommend that only patients with IPF and FVC of 50-80% predicted should be eligible to receive pirfenidone.[37] Lung function should be monitored and treatment discontinued if the FVC continues to fall by >10% in 12 months.[37] Other things pharmacists could monitor during treatment is liver serum transaminases (prior to initiation, monthly for the first 6 months, then every 3 months) and be alert if values increase to be over 3 times the upper limit; signs of photosensitivity; gastrointestinal events such as diarrhea, nausea, vomiting; and weight loss.

Clinical efficacy for nintedanib

"Efficacy and safety of nintedanib in IPF" (INPULSIS-1 and INPULSIS-2) were two international, multi-centered, randomized, double-blind, placebo-controlled phase III clinical trials which evaluated oral nintedanib in patients with IPF.[27] Both trials had a duration of 52 weeks and a primary endpoint of the annual rate of decline in FVC.[27] Inclusion criteria included FVC ≥ 50% predicted and DL_{CO} 30-79% predicted.[27] Patients included in both INPULSIS studies had a mean FVC of ~80% predicted [27] (indicating a milder severity of disease compared with patients receiving pirfenidone in the CAPACITY and ASCEND trials). 515 patients were randomized in INPULSIS-1 and 551 patients in INPULSIS-2 based on a 1:2 ratio of placebo to nintedanib in both studies.[27] Discontinuation rates were higher in the treatment arm of INPULSIS-1 (25.2% vs. 17.6%) and similar in both arms in INPULSIS-2 (23.7% nintedanib vs. 20.1% placebo).[27]

Reasons for discontinuation was most commonly due to adverse events.[27] Both trials showed a smaller change in FVC in the treatment groups compared to placebo (INPULSIS-1, -114.7 ml/year for nintedanib vs. -239.9 ml/year for placebo and INPULSIS-2, -113.6 ml/year for nintedanib versus -207.3 ml/year for placebo).[27] These were statistically significant differences in both studies.[27] A preplanned pooled analysis revealed a between group difference of -109.9 ml/year change in FVC.[27] In INPULSIS-1, there was no difference in time to first acute exacerbation and St. George's respiratory questionnaire score.[27] INPULSIS-2 showed slight improvements, which were statistically significant, in both of these secondary endpoints.[27]

In a subgroup analysis of Asian versus White patients in the INPULSIS trials of nintedanib in IPF, race did not influence the effect of nintedanib on disease progression.[38] It revealed a consistent treatment effect of nintedanib regardless of whether the patient was male or female, Asian or Caucasian, was older or younger than 65, a smoker or had an FVC less than or greater than 70%.[38] Of the treated patients, 322 were Asian (nintedanib n=194; placebo n=128) and 608 were White (nintedanib n=360; placebo n=248).[38] In Asian patients, the nintedanib versus placebo difference in the adjusted annual rate of decline in FVC was 94.1 mL/year (95% CI: 33.7, 154.6).[38] The treatment effect of nintedanib on the annual rate of decline in FVC in Asian and White patients was similar (P=0.72) and consistent with the overall population.[38] Nintedanib had an equivalent (non-significant) effect on both exacerbation rate and time to first exacerbation regardless of race.[38] The most common adverse event in Asian patients in the nintedanib group was diarrhea (56.2% of patients vs. 15.6% for placebo).[38] It was demonstrated that nintedanib demonstrated equivalent efficacy in terms of lung function and decline and quality of life and similar side effect profile in Asian and Caucasian populations.[38]

In the INPULSIS phase III trials, Patients who completed the INPULSIS trials could receive nintedanib in an open label extension trial "Long-term safety and tolerability of nintedanib in patients with IPF" (INPULSIS-ON). Of 807 patients who completed the INPULSIS trials, 734 continued with INPULSIS-ON (430 continuing nintedanib and 304 initiating nintedanib).[39] In patients with baseline FVC ≤ 50% and > 50% predicted at the start of INPULSIS-ON, the absolute mean change in FVC from baseline to week 48 of INPULSIS-ON was = 62.3 and -87.9mL respectively (n=24 and n=558, respectively).[27] The decline in FVC in INPULSIS-ON in both subgroups by baseline FVC percent predicted was similar to that in INPULSIS suggesting that nintedanib may have a similar effect on disease progression in patients with advance disease as in less advanced disease.[24] It should be noted that the number of patients with FVC ≤ 50% predicted at the start of INPULSIS-ON was small.

Adverse effects of nintedanib

The most common adverse effect for nintedanib seen in INPULSIS-1 and INPULSIS-2 was diarrhea, in which only 14 patients (4.5%) discontinued due to this reason.[27] In both trials, patients in the nintedanib arm had a higher incidence of derangement of hepatic enzymes (aspartate aminotransferase and/or alanine aminotransferase.[27] There was <2% incidence of cardiac events, with five patients in each nintedanib group having a myocardial infarction compared to one in each placebo group.[27] Deaths were reported from the pooled analysis rather than the individual trials with no statistically significance differences for all-cause mortality or death from a respiratory cause.[27]

Nintedanib place in therapy

The 2015 ATS/ERS/JRS/ALAT Clinical Practice Guideline conditionally recommends nintedanib for use.[25] NICE guidelines recommend that only patients with IPF and an FVC of 50-80% predicted should be eligible to receive nintedanib.[40] Lung function should be monitored and treatment discontinued if the FVC continues to fall by >10% in 12 months.[40] Other things pharmacist can monitor during treatment include liver function tests (prior to treatment monthly for 3 months and every 3 months thereafter or as clinically indicated); gastrointestinal events such as diarrhea, nausea, and vomiting; arterial thromboembolic events; bleeding; and gastrointestinal perforation. During treatment, antidiarrheal and antiemetic therapy may be indicated and it is important to educate patient on the importance of not smoking while taking nintedanib.

Real-World Data

Data from extension trials indicate that nintedanib continues to slow disease progression for up to 3 years and is similarly effective in patients with mild and severe impairment of lung function.[5] It reduces the risk of acute exacerbations and combined analysis of data from clinical trials shows a trend towards a reduction in mortality.[5] The mean total exposure for patients treated with nintedanib in both INPULSIS and INPULSIS-ON was 40.7 months, with a maximum exposure of 63.1 months.[5] Nintedanib is well tolerated and has been shown to be safe for up to 51 months with diarrhea being the main adverse event due to treatment.[5] Another interesting point to note about nintedanib is its pharmacokinetics influenced by age, body weight and Asian race (with different effect size in different Asian subpopulations including Korean, Japanese, Chinese, Indian, Taiwanese and Others).[41] Asian subpopulations (all except Koreans) had significantly higher exposures (up to 50%) of medication and it could not be explained

by differences in body weight.[42] It should also be noted that patients taking full-dose anticoagulant therapy or high-dose antiplatelet therapy were exclude from INPULSIS trials and therefore nintedanib should be used cautiously in these patients.[27] The mechanism of action of nintedanib and its effect on vascular endothelial growth factor has raised concerns regarding cardiovascular risk; however real world data have not demonstrated any adverse cardiovascular signals.[42,43]

When analyzing patients in clinical trials, we must keep in mind that patients are often younger and have less co-morbidities compared with the real-world setting.[42] The average age for patients presenting with IPF from registry data is 68.7-73.5 years, and 18% of patients are over the age of 80 years.[44] In the pirfenidone trials, the upper age limit was 80 years and the mean age was 68 years, while in the INPULSIS trials the mean age for the nintedanib population was younger, at 66.7 years.[44] Post hoc analysis has suggested that there is no difference in the efficacy of antifibrotics with respect to age.[44] Patients are more likely to discontinue anti-fibrotic therapy due to adverse effects and disease burden if they are aged 70 years or above.[43]

On the basis of the available trial data, no superiority has been established for pirfenidone or nintedanib.[45] For pirfenidone, a positive effect was shown on mortality in pooled (pre-specified) analysis of the three RCTs, whereas for nintedanib the mortality effect was marginal but a positive effect on prevention of adjudicated acute exacerbations was observed.[45] The effects of both drugs in reducing FVC decline are similar but formal comparison would be flawed because the diagnostic HRCT and pulmonary function criteria and variations in the mode of analysis of FVC change, the primary outcomes measure for both drugs are not the same.[45]

There currently is not enough data on combination therapy of pirfenidone and nintedanib. As they have postulated different mechanisms of actions, it is unknown whether their additive effects would be efficacious or not. There is also no data on switching between the two therapies if a patient fails one agent. There has been one case report of a Caucasian male patient with IPF treated with both pirfenidone and nintedanib following 2 years of treatment with pirfenidone monotherapy.[46] The safety, tolerability and pharmacokinetics of concomitant therapy with pirfenidone and nintedanib have previously been evaluated in Japanese patients with IPF in a randomized, double-blind phase II study in which 21 of 50 randomized patients were treatment with concomitant therapy;[47] only 9 patients received pirfenidone and nintedanib at the full recommended dosage. The follow up periods were 14 days in two cohorts and 28 days in one cohort. This trial showed that the addition of nintedanib to chronic background pirfenidone treatment resulted in a trend to lower nintedanib plasma levels, but there was no effect on the pharmacokinetics of pirfenidone. Gastrointestinal toxicity was the most common adverse event and seemed to be more frequent with concomitant treatment.[46] However, this trial has small sample size and duration is short and more studies need to be done to investigate concomitant therapy.

In a meta-analysis of ten papers (3847 IPF patients; 2254 treated; 1593 placebo) for pirfenidone, nintedanib and NAC, the review showed that both pirfenidone and nintedanib, but not NAC were significantly effective in reducing FVC decline and the risk of FVC ≥ 10% decline in percent predicted over 12 months.[47] In this meta-analysis, it showed that nintedanib significantly protected against the risk of acute exacerbation and mortality.[47] Pirfenidone and nintedanib showed a similar and good safety profile, whereas NAC provided a signal for increased adverse events.[47] Furthermore, a subset analysis was done on the currently approved doses of pirfenidone (2403 mg/day) and nintedanib (300 mg/day) for treatment of IPF as multiple doses were considered in several RCTs.[47] In this sub-analysis, it was found that nintedanib has a greater influence in terms of preventing the FVC decline, with an analogous safety profile compared to pirfenidone.[47]

In view that both IPF medications pirfenidone and nintedanib will be offered to patients, factors that may be considered would include contraindications, dosing regimens, side effect profiles, pill burden, tolerability etc. Many adverse effects are tolerable or can be managed by ensuring antifibrotics are not taken on an empty stomach, though dose reduction or with the addition of other treatments e.g. antiemetics or antidiarrheal treatment.[44] Pharmacists can play a role in providing adequate pharmaceutical care to patients, counseling on drug adverse effects and prevention.

Access to IPF medication in Hong Kong

Initiated by the Hong Kong Alliance for Rare Diseases (HKARD) in partnership with other groups, a scheme to target patients suffering from IPF was launched in March 2018.[48] Neither pirfenidone nor nintedanib are included in the Hospital Authority's drug formulary which means the government will not subsidize drug costs for IPF patients. The HKARD scheme's objective is to facilitate early access to nintedanib at a lower drug cost for IPF patients, and to enable them to have an accurate prediction of the treatment cost. Patients currently have to pay about HK$20,000 a month for nintedanib. Patients with the disease who are prescribed nintedanib by a public doctor or a private specialist in respiratory medicine would be eligible to join the scheme. But people eligible for civil service medical and dental benefits will be excluded.

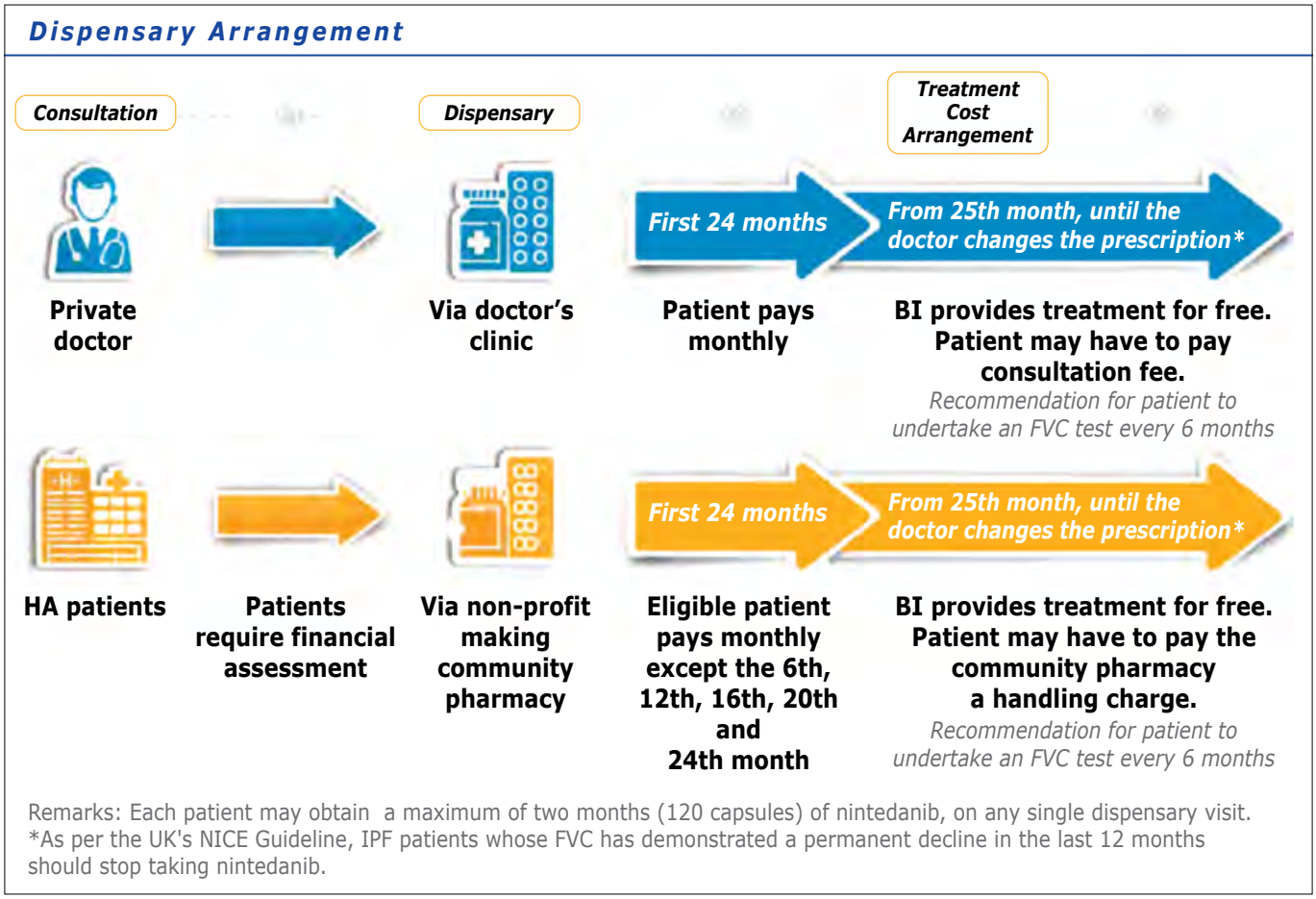

Figure 2. *Dispensary arrangement for HKARD scheme nintedanib patients. (adapted from HKARD)*[48]

Patients would receive free drugs after paying for the first two years.[48] The free medication would run until the patient's doctor changes the prescription which is based on clinical assessment and the NICE treatment guideline, which recommends that nintedanib treatment is discontinued if the disease progresses (i.e. a confirmed decline in percent predicted FVC of 10% or more) in any 12 month period.[48] The doctor has to perform lung function test every 6 months and attach a copy of test results for the last 12 months for the free treatment at month 24 and every sixth month thereafter.[48]

Public patients earning less than HK$40,000 a month would have the treatment cost waived for five months during the two-year period, meaning they would still have to pay about HK$380,000 before receiving free treatment.[2] While private patients can get the drugs from their own specialists, public patients have to bring the prescription and visit any one of the designated community pharmacies under the three charities involved, namely the Hong Kong Pharmaceutical Care Foundation, HKSKH Lady MacLehose Centre and St James' Settlement.[48]

Keeping in mind that most IPF patients usually only have 3-5 years to survive and nintedanib data only supports patient slowing progression free survival up to 3 years so far, it is a huge financial burden for the patient to have to pay for the first 2 years of their treatment as it is likely close to their end of life. Also, it is more likely that IPF disease progression happens after 2 years making patient ineligible to benefit from the free treatment after the two years period. Another point to note is that there is no defined FVC that needs to be met for patients to initiate treatment. This allows doctors and patients to decide together at which point in the disease to start treatment as it may benefit patients throughout all stages of IPF, although there needs to be more clinical trials to supply more concrete evidence on when is the best time to initiate therapy.

Currently there is no scheme to help patients with the cost of pirfenidone. Pirfenidone if taken at full dose (3 tablets 3 times daily) would cost approximately HK$50,000 per month.

Towards the future

Further research for the treatment of IPF is warranted for the agents currently available, for new research targets or for future biomarkers that can provide guidance on predicting individualized treatment responses to specific

agents. Further investigation of the role of gene variants, epigenetic alterations and other molecular biomarkers reflecting disease activity and behavior will hopefully help support the development of novel agents for personalized treatment of IPF.[49] Currently, research shows that patients expressing a Mucin 5B minor allele genotype had significantly lower bacterial load compared with IPF patients without this genotype and a further variant rs35705950 of Mucin 5B allele have shown better survival.[49] Moreover, two large genome-wide association studies have identified common genetic variants, crucial for epithelial integrity, as risk factors of IPF.[50,51] The studies identified the potential importance of telomere biology (TERT, TERC, OBRC1), host defense (MUC5B, ATPase phospholipid transporting 11A-ATP11A, toll interacting protein-TOLLIP), and cellular barrier function (desmoplakin-DSP, dipeptidyl peptidase 9-DPP9) for the development of the disease. Epigenetics, any process that modifies gene activity without changing the underline genetic code, also plays a central role in IPF.[49] Cigarette smoking and ageing are the main effectors of epigenetic modifications through DNA methylation.[49]

The concept of "mild' disease based purely on FVC measurement also needs to be challenged.[44] An FVC of ≥ 80% would traditionally be classified as 'mild' disease, however lung function alone does not appear to be sufficient at predicting the clinical course of IPF.[44] Clinical scoring systems such as the Gender, Age and Physiology (GAP) index, which take into consideration both clinical and physiological variables, may allow better prognostic stratification.[52] As such, limiting prescription of anti-fibrotic therapy to patients base on FVC alone, may be short sighted. In theory, in a disease with otherwise extremely poor outcomes, starting anti-fibrotic therapy in early disease to have the maximum benefit may also provide significant survival benefit, although further robust data are needed to support this.[44]

CONCLUSION

IPF is a rare heterogeneous disease with limited treatment options. Pirfenidone and nintedanib have data to support their safe use as treatment in Asian patients and may increase a patient's progression free survival. Due to high drug costs, access to both medications in Hong Kong is still limited to those who can afford it. There still needs to be more research on patient groups not included in clinical trials and on new targets for more treatment options for this rare disease.

Author's background

KONG, Sarah Hiu Hung *graduated from The University of Hong Kong. She is currently a pharmacist practicing at Grantham Hospital in Hong Kong. Grantham Hospital specializes in tuberculosis and chest medicine. Her e-mail address is khh407@ha.org.hk*

References

1. Chambers, D. Nintedanib for idiopathic pulmonary fibrosis: an Asian perspective. *Respirology*. 2016;21(8):1342-1343.
2. Cheung, E. Free drugs for Hong Kong patients with rare lung disease, after two years. SCMP [newspaper on the Internet]. 2018 March 7 [cited 2018 Sept 17]. Available from: https://www.scmp.com/news/hong-kong/health-environment/article/2136201/free-drugs-hong-kong-patients-rare-lung-disease
3. Oku, H., Shimizu, T., Kawabata, T., *et al.* Antifibrotic action of pirfenidone and prednisolone: different effects on pulmonary cytokines and growth factors in bleomycin-induced murine pulmonary fibrosis. *Eur J Pharmacol.* 2008;590(1-3):400-408.
4. Antoniou, K. *et al.* Medical therapy in idiopathic pulmonary fibrosis. *Semin Respir Crit Care Med.* 2016;37:368-377.
5. Rodriguez-Portal, J. Efficacy and safety of nintedanib for the treatment of idiopathic pulmonary fibrosis: An Update. *Drugs R D.* 2018;18:19-25.
6. King, T. Jr., Tooze, J., Schwarz, M., et al. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med.* 2001;164:1171.
7. Souza, C., Muller, N., Flint, J., Wright, J., and A. Churg. Idiopathic pulmonary fibrosis: spectrum of high-resolution CT findings. *American Journal of Roentgenology.* 2005;185(6):1531-1539.
8. Strieter, RM. Inflammatory mechanisms are not a minor component of the pathogenesis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2002;165(9):1206-1207.
9. Idiopathic Pulmonary Fibrosis Clinical Research Network; Martinez, F., de Andrade, JA., Anstrom, K., King, T. Jr., Ragu, G. Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med.* 2014;370:2093-3101.
10. Demedts, M., Behr, J., Buhl, R., *et al.* High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med.* 2005;353:2229-2242.
11. Idiopathic Pulmonary Fibrosis Clinical Research Network; Raghu, G., Anstom, KJ., King, T. Jr., *et al.* Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. *N Engl J Med.* 2012;366:1968-1977.
12. Idiopathic Pulmonary Fibrosis Clinical Research Network; Martinez, F., de Andrade, JA., Anstrom, K., King, T. Jr., Ragu, G., Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med.* 2014;370:2093-3101.
13. Raghu, G., Brown, K., Bradford, W., *et al.* A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N Engl J Med.* 2004;350:125-133.
14. King, T. Jr., Albera, C., Bradford, W., *et al.* Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicenter, randomized, placebo-controlled trial. *Lancet*. 2009; 374:222-228.
15. Raghu, G., Brown, K., Costabel, U., *et al.* Treatment of idiopathic pulmonary fibrosis with etanercept: an exploratory, placebo-controlled trial. *Am J Respir Crit Care Med.* 2008;178:948-955.
16. Noth, I., Anstrom, K., Calvert, S., *et al.* The Idiopathic Pulmonary Fibrosis Clinical Research Network (IPFnet). A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2012; 186:88-95.

17. King, T., Behr, J., Brown, K., *et al.* BUILD-1: a randomized placebo controlled trial of bosentan in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;177:75-81.

18. King, T., Behr, J., Brown, K., *et al.* BUILD-3: a randomized, controlled trial of bosentan in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2011;184:92-99.

19. Raghu, G., Million-Rousseau, R., Morganti, A., *et al.* Macitentan for the treatment of idiopathic pulmonary fibrosis: the randomized controlled MUSIC trial. *Eur Respir J.* 2013;42:1622-1632.

20. Raghu, G., Behr, J., Brown, K., *et al.* Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. *Ann Intern Med.* 2013;158:641-649.

21. Zisman, D., Schwarz, M., Anstom, K., *et al.* Idiopathic Pulmonary Fibrosis Clinical Research Network. A controlled trial of sildenafil in advanced idiopathic pulmonary fibrosis. *N Engl J Med.* 2010;363:620-628.

22. Daniels, C., Lasky, J., Limper, A., *et al.* Imatinib treatment for idiopathic pulmonary fibrosis: randomized placebo-controlled trial results. *Am J Respir Crit Care Med.* 2010;181:604-610.

23. Selmen, M., King, T. and Pardo, A. American Thoracic Society; European Respiratory Society; American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med.* 2001;134(2):136-151.

24. Trawinska, M., Rupesinghe R., and Hart S. Patient considerations and drug selection in the treatment of idiopathic pulmonary fibrosis. *Dovepress*. 2016;12:563-574.

25. Raghu, G., Rochweg, B., Zhang, Y., *et al.* An Official ATS/ERS/JRS/ALAT Clinical practice guideline: treatment of idiopathic pulmonary fibrosis: an update of the 2011 clinical practice guidelines. *Am J Respir Crit Care Med Vol.* 2015; 192:2;e3-e19.

26. Richeldi, L, Costabel, U., Selman, M., *et al.* Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. *N Engl J Med.* 2011;365:1079-1087.

27. Richeldi, L., du Bois, R., Raghu, G., *et al.* INPULSIS Trial Investigators. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2071-2082.

28. Taniguchi, H, Ebina, M., Kondoh, Y., *et al.* Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J.* 2010;35:821-829.

29. Noble, PW., Albera, C., Bradford, WZ., *et al.* CAPACITY Study Group. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomized trials. *Lancet*. 2011;377(9779):1760-1769.

30. King, T., Bradford, W., Castro-Bernardini, S., *et al.* ASCEND Study Group. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med.* 2014;370(22):2083-2092.

31. Nintedanib [Dosing]. *Lexi Drugs Multinational*. 2017.

32. OFEV. Summary of Product Characteristics – January 2016. Boehringer Ingelheim International GmbH.

33. Pirfenidone[Dosing]. *Lexi-Drugs Multinational*. 2017.

34. Huang, H., Dai, HP., Kang, J., Chen, BY., Sun, TY., and ZJ. Xu. Double-blind randomized trial pirfenidone in Chinese idiopathic pulmonary fibrosis patients. *Medicine*. 2018;94:42.

35. Kreuter, M. Pirfenidone: an update on clinical trial data and insights from everyday practice. *Eur Respir Rev*. 2014;23(131):111-117.

36. Costabel, U., Albera, C., Fagan, E., *et al.* Long-term safety of pirfenidone in RECAP, an open-label extension study in patients with idiopathic pulmonary fibrosis, interim results. *Eur Respir J.* 2014:44(suppl):58.

37. National Institute for Health and Care Excellence. [webpage on the Internet]. *Pirfenidone for Treating Idiopathic Pulmonary Fibrosis Technology Appraisal Guidance*. 2013. Available from: http://www.nice.org.uk/guidance/ta282. Accessed April 13, 2017.

38. Taniguchi, H., Xu, Z., Azuma, A., Inoue, Y., Li, H., Fujimoto, T., Bailes, Z., Schlenker-Herceg, R., Kim, DS. Subgroup analysis of Asian patients in the INPULSIS(R) trials of nintedanib in idiopathic pulmonary fibrosis. *Respirology*. 2016; 21:1425-1430/

39. Wuyts, W., Kolb, M., Stowarsser, S., *et al.* First data on efficacy and safety of nintedanib in patients with idiopathic pulmonary fibrosis and forced vital capacity of ≤50% of predicted value. *Lung*. 2016;194:739-743.

40. National Institute for Health and Care Excellence. [webpage on the Internet]. *Nintedanib for treating idiopathic pulmonary fibrosis technology appraisal guidance*. 2013. Available from: http://www.nice.org.uk/guidance/ta379. Accessed April 13, 2017.

41. Schmid, U., Doege, C., Dallinger, C., Freiwalk, M.. Population pharmacokinetics of nintedanib in patients with idiopathic pulmonary fibrosis. *Pulmonary Pharmacology & Therapeutics*. 2018;48:126-143.

42. Hughes, G. Toellner, H. Morris H., *et al.* Real world experiences: pirfenidone and nintedanib are effective and well tolerated treatment for idiopathic pulmonary fibrosis. *J Clin Med*. 2016;5(9):1-12

43. Galli, JA., Pandya A., Bega-Olivio, M., *et al.* Pirfenidone and nintedanib for pulmonary fibrosis in clinical practice: tolerability and adverse drug reactions. *Respirology*. 2017;22(6):1171-8.

44. Hayton C., and N. Chaudhuri. Managing idiopathic pulmonary fibrosis: which drug for which patient? *Drugs Aging*. 2017;34:647-653.

45. Antoniou, K., Wuyts, W., Wijsenbeek, M., Wells, A. Medical therapy in idiopathic pulmonary fibrosis. *Semin Respir Crit Care Med*. 2016;37:368-377.

46. Hagmeyer, L. *et al.* Successful concomitant therapy with pirfenidone and nintedanib in idiopathic pulmonary fibrosis: a case report. *Respiration*. 2016;91:327-332.

47. Ogura, T. *et al.* Safety and pharmacokinetics of nintedanib and pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* 2015;45:1382-1392.

48. Programme on early access to treatment for idiopathic pulmonary fibrosis (IPF) in Hong Kong. (2018). [ebook] Hong Kong: Hong Kong Alliance for Rare Diseases, pp 1-5. Available at: http://www.hkard.org/database/newsletter/finalipftreatmentearlyaccessprogrammeenglishprogrammeoutline.pdf [access 19 September 2018]

49. Sgalla, Giacomo, *et al.* Idiopathic pulmonary fibrosis: pathogenesis and management. *Respiratory Research*. (2018) 19:32.

50. Noth, I., Zhang, Y., Ma, SF., Flores, C., Barber, M., Huang, Y., *et al.* Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet Respir Med.* 2013;1:309-17.

51. Fingerlin, TE., Murphy, E., Zhange, W., Peljto, AL., Kevin, K., Steele, MP., *et al.* Loci for pulmonary fibrosis. *Nat Genet*. 2013;45:613-20.

Questions for Pharmacy Central Continuing Education Committee Program

(Please be informed that this article and answer sheet will be available on PCCC website concurrently. Members may go to PCCC website (www.pccchk.com) to fill in their answers there.)

2 CE Units

Idiopathic pulmonary fibrosis (IPF): 10 points to take home

1. ***Which of the following feature(s) is not found on high resolution computed tomography appearances to diagnose IPF?***
   A. Honeycombing
   B. Reticular abnormality
   C. Sub pleural and basal predominance
   D. None of the above

2. ***Which of the following adverse effects are not caused by pirfenidone?***
   A. Photosensitivity
   B. Anorexia
   C. Diarrhea
   D. None of the above

3. ***Which of the following do not influence the effect of nintedanib on disease progression?***
   (i) Race
   (ii) Age
   (iii) Smoking
   A. (i) and (ii)
   B. (ii) and (iii)
   C. (i) and (iii)
   D. (i), (ii) and (iii)

4. ***Which of the following statement(s) regarding pirfenidone is/are CORRECT?***
   A. Its mechanism of action is a pleiotropic antifibrotic effect suppressing fibroblast proliferation, reducing the production and activity of fibrosis-associated proteins and cytokines.
   B. No titration of dose is necessary
   C. There are no significant drug interactions requiring dose adjustments
   D. All of the above

5. ***Which of the following statements are TRUE according to Hong Kong Alliance for Rare Diseases scheme to target patients suffering from IPF?***
   (i) Lung function test must be done every 6 months for free treatment.
   (ii) Disease progression is the confirmed decline in percent predicted FVC of 15% or more
   (iii) Patients would receive free drugs after paying for the first two years.
   A. (i) and (ii)
   B. (ii) and (iii)
   C. (i) and (iii)
   D. (i), (ii) and (iii)

6. ***While patients are taking pirfenidone, which of the following should be monitored?***
   (i) Liver serum transaminases.
   (ii) Signs of photosensitivity
   (iii) Weight gain
   A. (ii) only
   B. (i) and (ii)
   C. (ii) and (iii)
   D. (i), (ii) and (iii)

7. ***Which of the following is a recommendation by NICE guidelines regarding the use of nintedanib?***
   A. Only in patients with IPF and an predicted FVC of 50-80%
   B. There is no need to monitor lung function
   C. Treatment should be discontinued if FVC continues to fall by >15% in 12 months
   D. Patients must go through genetic testing before treatment

8. ***Drugs that have completed negative clinical trials and have been found to be non-efficacious in IPF include:***
   (i) N-acetylcysteine
   (ii) theophylline
   (iii) warfarin
   A. (i) and (ii)
   B. (i) and (iii)
   C. (ii) and (iii)
   D. (i), (ii) and (iii)

9. ***According to the 2015 ATS/ERS/JRS/ALAT IPF guidelines, which of the following treatment recommendations is TRUE?***
   A. Strong recommendation for single lung transplant
   B. Strong recommendation against warfarin
   C. Conditional recommendation against antacid therapy
   D. Conditional recommendation for sildenafil

10. ***Which factor(s) has/have been identified by the Genome-wide association studies to be important for the development of IPF?***
    (i) telomere biology (TERT, TERC, OBRC1)
    (ii) host defense (MUC5B, ATPase phospholipid transporting 11A-ATP11A, toll interacting protein-TOLLIP)
    (iii) cellular barrier function (desmoplakin-DSP, dipeptidyl peptidase 9-DPP9)
    A. (iii) only
    B. (i) and (ii)
    C. (i) and (iii)
    D. (i), (ii) and (iii)

Answers will be released in the next issue of HKPJ.

CE Questions Answer for 253(D&T)

Management Approach of Hemophilia A and B

1. B 2. C 3. B 4. A 5. D 6. C 7. B 8. D 9. B 10. D

Application of Various DNA Sequences for Establishing Phylogenetic Relationship of Endophytic Fungi Isolated from *Tripterygium wilfordii* Hook f.

LEUNG, Fo-Man; FENG, Junli; WONG, Hung-Ngan; CHEUNG, Hon-Yeung*
Research Group for Bioactive Products, Department of Biomedical Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon Tong, Hong Kong SAR, China
(Corresponding author. E-mail: cheung.honyeung@cityu.edu.hk; Tel: +852 3442 7746)*

ABSTRACT

***Tripterygium wilfordii* Hook f., a traditional Chinese medicinal plant, has been used to treat various autoimmune disorders since ancient times. Many studies reported that *T. wilfordii* hosts abundant endophytic fungi; some of them may produce bioactive metabolites possessing immunosuppressive effects. In this study, a total of 266 endophytic fungi were isolated from various parts of *T. wilfordii*. Based on the morphological characteristics and ITS sequences, 94 different isolates were identified into 13 fungal taxa at the genus level; namely Genus *Colletotrichum sp.*, *Glomerella sp.* and *Diaporthe sp.* In general, fungi diversity were found more in flower than in other tissues. However, the dominant fungal species in leaves and xylems of *T. wilfordii*, were the *Phomopsis sp*. Phylogenetic analyses based on the ITS, histone H4 and Beta-tubulin sequences revealed that none of these three genes could resolve *C. gloeosporioides*, *Diaporthe sp.* and *Phosmopsis sp.* into monophyletic respective clades. But using the combined database of Beta-tubulin gene and ITS, the genera *Diaporthe sp.* and *Phomopsis sp*. were clearly separated. We believe that these findings will help us better understanding the diversity of endophytic fungi in *T. wilfordii.* However, the application of DNA barcodes for differentiation of species should be thoughtfully interpreted.**

Keywords: *Tripterygium wilfordii Hook f.; Endophytic fungi; Sequence divergence; Species identification; Phylogenetic study*

INTRODUCTION

Tripterygium wilfordii Hook. f. (Celastraceae), a traditional Chinese medicinal herb, has been widely used in clinical treatment of autoimmune diseases and rheumatoid arthritis.[1,2] *T. wilfordii* is distributed mainly in Eastern and Southern China, Korea, and Japan.[1] Up to date, hundreds of effective components have been isolated from *T. wilfordii*, these include dihydroagarofuran sesquiterpene polyesters,[3-5] sesquiterpene alkaloids,[6-10] abietane diterpenoids,[11-14] triterpenoids,[15] and flavonoids.[16,17]

Endophytic fungi are microbes that inhabit inside healthy plant tissues without causing visible disease symptoms on host.[18] It has been reported that endophytic fungi play a major role in plant community health by providing resistance to hosts against different biotic and abiotic stresses.[19] Indeed, some endophytic fungi from Chinese medicinal plants are able to produce bioactive compounds similar to their host.[20,21] Therefore, the interest to screen endophytic fungi as potential producers of novel bioactive products has increased.[22] In the case of *T. wilfordii*, a lot of studies reported the isolation of endophytic fungi with anti-inflammatory, antimicrobial, or anticancer activities.[23,24] There is great genetic diversity of these endophytic fungi, yet the phylogenetic relationships of these endophytic fungi were seldom studied.

It has been suggested to place microbe/host in an evolutionary and ecological context, in understanding the costs and benefits to hosts harboring fungi in their tissues.[25] Therefore before investigating their potential medicinal values, fundamental knowledge is needed regarding endophyte diversity at the levels of genotype, species, and clades. For this purpose, 266 fungal colonies clustered into 94 morphotypes were isolated from *T. wilfordii* in this study, and their internal transcribed spacer (ITS) region of ribosomal DNA (rDNA, histone H4 and partial Beta-tubulin genes were amplified by PCR. Based on the sequencing data, genera *Glomeralla* (32%), *Colletotrichum* (29%), *Diaporthe* (13%), and *Phomopsis* (9%) were the most abundant endophytes isolated. Morover, genera *Glomeralla* and *Colletotrichum* were found prevalently in leaves, whereas *Diaporthe* and *Phomopsis* existed mainly in xylems of *T. wilfordii*. Phylogenetic analysis indicates that combined gene datasets may be a good alternative to obtain more reliable phylogenetic tree than individual gene sequences.

MATERIALS AND METHODS

Plant Material

The leaves, stems, bark and flowers of *T. wilfordii* were collected in Taiwan. All samples were put in sterile polythene bags, identified by Dr. B. Chen and then transported to the laboratory in City University of Hong Kong on the same day. The samples were kept at 4 °C, and processed for isolation of endophytic fungi within 48 h.

Fungi Isolation and Identification

Plant samples were washed thoroughly in running tap water for 10 min to remove debris. All samples were surface sterilized by dipping in 75% ethanol for 1 min, followed by soaking in 3.25% hypochlorite solution for 30 s, and then immersed in 75% ethanol for 30 s. Finally, the sterilized samples were washed three times using double distilled water, and blotted with sterile tissue paper to remove excessive water.

Four discs of 10 mm in diameter were cut from surface-sterilized leaf by using a sterile hole-punch. Twigs were cut into 2 cm-long segment, and bark of each segment was removed to obtain xylem. Flowers were treated as a separate entity, and 28 individual flowers were taken from each inflorescence. These plant samples were then transferred to potato dextrose agar (PDA) plates supplemented with streptomycin (250 mg/L), and incubated for 20 days at 25 ± 2 °C. Tissues were observed for fungal growth at alternate day intervals for 20 days. Actively growing fungal tips emerging from plant tissues were sub-cultured on PDA plates for identification and enumeration. The fungi isolates were numbered and grouped according to their morphological features.

Fungal Genomic DNA Extraction and PCR

Fungal mycelium was collected, and washed three times by double distilled water. 0.3 g dry mycelium was then transferred to a sterilized mortar and pestle for vigorous grinding under liquid nitrogen until powder form was obtained. The grinded power was immediately transferred into 1.5 mL Eppendorf tube, genomic DNA was extracted using a DNA Extraction Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol.

The fungal ITS region of rDNA, Beta-tubulin gene and histone H4 genes were downloaded from NCBI database, and PCR primer sets were designed according to this sequence using Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, CA). The designed primer sequences were shown in **Table 1**, and were synthesized by Tech Dragon Ltd (Hong Kong, China).

PCR was performed in 50 µL volume of reaction mixture containing 50 ng of template DNA, 25 µL of 2x reaction buffer (Fermentas), and 0.5 µM of each each primer. The reactions were performed by incubation at 95 °C for 10 min, followed by 10 touchdown cycles of 95 °C for 1 min, 67-57 °C for 1 min (decreased 1°C for every cycle), and 72 °C for 1 min. And then followed by 30 cycles of 95 °C for 1 min, 56 °C for 1 min, 72 °C for 1.5 min and a final extension at 72°C for 10 min. A 5 µL aliquot of each PCR products was subjected to 1.5% agarose gel electrophoresis, and their lengths were estimated. The successful PCR reactions were subsequently purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany).

Table 1. Details of PCR primers used in this study

Name	Oligonucleotide sequence (5′→3′)
ITS-FP	GGAAGTAAAAGTCGTAACAAGG
ITS-RP	TCCTCCGCTTATTGATATGC
Tub-FP	AAGGGTCACTACACTGAGGGTG
Tub-RP	ATGGACGAGATGGAGTTCAC
H4-FP	TCAA(A/C)ATG(A/T)CTGGACGTAAGT
H4-RP	TTAACCACCGAAACCGTAGA

Nucleotide Sequencing

The purified PCR products were ligated into the pDrive cloning vector (Qiagen) or pCR cloning vector (Invitrogen, Carlsbad, CA, USA), and transformed into competent cells of Escherichia coli DH5a. Recombinant clones containing the inserts of correct size were identified by restriction enzyme digestion of purified plasmids. Three to five independent positive clones were selected and sequenced (Tech Dragon). Fungal sequences obtained in this study have been deposited in the GenBank database under accession numbers MH 930403-MH 930434, MH 937340-MH937371, and MH 986671- MH 986702.

Phylogenetic analysis

For the phylogenetic analysis, sequences from the present study and those from previous studies, obtained from the GenBank database, were used. The multiple sequence alignment was carried out using CLUSTALX version 1.83. Phylogenetic analysis of the aligned sequences was performed with the MOLECULAR EVOLUTIONARY GENETICS ANALYSIS (MEGA) program version 5.0.[26,27] The genetic distance matrix was calculated according to Kimura's two-parameter model,[28] and was then used to construct a phylogenetic tree using the neighbour-joining method (NJ)[29] with the interior branch tests of 2000 replicates.[30]

RESULTS

Features of the Endophytic Fungi

A total of 266 endophytic fungal species were isolated from *T. wilfordii.* Among them, 83 fungi (31.2%) were isolated from barks, 67 (25.2%) were isolated from stems, 61 (22.9%) were isolated from flowers, 54 (20.3%) were isolated from leaves, 1 (0.3%) were isolated from roots.

All the fungi showed different growing patterns on PDA, and their colony size, color, texture, spores color

and pigment secretion were varied. Their macroscopic and microscopic features were compared by naked eyes and magnified 400 times, respectively **(Figure 1)**.

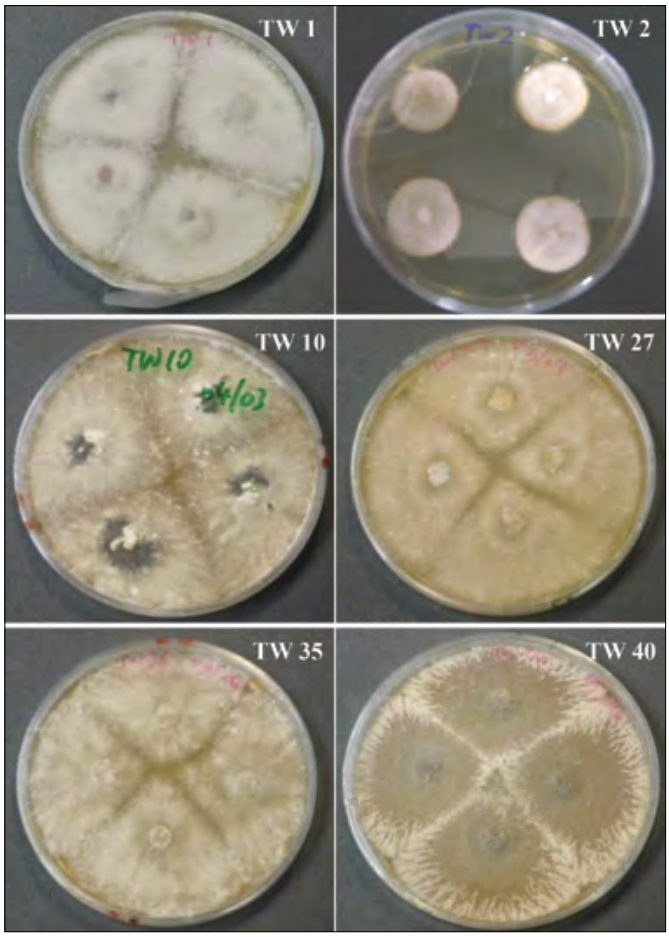

Figure 1. Macroscopic appearance of selected 6 fungal isolates.

Polymerase Chain Reaction Amplification of the ITS region, Beta-tubulin and Histone H4 Genes of Endophytic Fungi

Using the designed primers, the ITS region, Beta-tubulin and histone H4 genes of endophytic fungi were successfully amplified from 94 isolates. Then, their sequence data were deposited in the GenBank database, and blasted to known fungal sequences to make identification at genus or species level. Finally, these endophytic fungal isolates were recovered and identified into 13 taxa at genus level **(Table 2**). Among them, eight isolates belonging to *Diaporthe* (TW32), *Phaeosphaeria* (TW97) and *Phomopsis* (TW16, TW24, TW25, TW29, TW35, TW43) could not be identified into species level. As shown in **Table 2**, species of *Colletotrichum* and *Glomerella* were only present in leaves and flowers but were not found in stems and roots of *T. wilfordii.* Whereas species of *Diaporthe* and *Phomopsis* only existed in flower and stems but not found in leaves and roots. In roots of *T. wilfordii,* only two species *Phaeosphaeria* (TW97) and *Talaromyces trachyspermus* (TW110) were identified. Compared to leaves and stems, the fungal populations in flowers were more diversified.

Next, the amplified sequences from fungal endophytes were specially analyzing. For ITS fragment, the obtained sequences were around 600 bps except TW110 (1069 bps, nearly two times larger than the others). Genetic comparison among these ITS regions revealed that 18S, 5.8S and 28S rDNA regions were highly conserved among species, whereas the variations were observed in ITS1 and ITS2 regions **(Figure 2)**.

The obtained histone H4 sequences varied from 440 bp to 633 bp, and the variations among them appeared greater than ITS sequences. Except *Phomopsis vaccinii* (TW21) which contained only one intron, all other histone H4 genes contained three exons where two introns sandwiched in between exons. The lengths of corresponding exons irrespective of fungal species were unique. All were 19 bps, 138 bps and 164 bps for exon1, exon 2 and exon 3 respectively. In contrast, there was a considerable heterogeneity of intron lengths among the species. And length of intron 1 was greater than intron 2 for all isolates, details of which were shown in **Figure 3** and **4**.

With respect to Beta-tubulin gene, the size of obtained sequences varied from 920 bp in TW97 to 982 bp in TW10. As shown in **Figure 5**, the lengths of exon 1 and exon 2 in all of the tested fungal species were 642 and 275 bps respectively without exception, whereas intron length varied from 3 bps to 65 bps. And the length of intron, to some extent, appeared correlative to the fungal species. For example, intron lengths of Beta-tubulin gene in *Colletotrichum acutatum*, *C. boninense* and *Glomerella cingulata* were 50 bps, 56 bps and 58 bps respectively. When the obtained Beta-tubulin sequences were aligned with each other, we found the coding sequences (exon) of Beta-tubulin were highly conserved among the isolates. However, there were considerable indel substitutions happened in the intron region. For TW94 *(Phaeosphaeria sp),* which was isolated from root of *T. wilfordii*, the length of intron was only 3 bps which made a great contrast to others under alignment.

Evolutionary Analysis of Endophytic Fungi Isolated from *T. wilfordii*

Estimates of average evolutionary divergence or distance over ITS, histone H4 and Beta-tubulin sequences within fungal genera were conducted in MAGA 5 by using Maximum Composite Likelihood model. In general, the histone H4 appeared to be more parsimony information than ITS and Beta-tubulin sequence in *Colletotrichum sp.*, *Glomerella sp.* and *Diaporthe sp.*, whereas ITS contained more parsimony information in *Phomopsis sp* **(Table 3)**. Therefore, the H4 and ITS were used for subsequently phylogenetic analysis.

Phylogenetic analysis of endophytic fungi isolated from *T. wilfordii*

Phylogenetic analysis of the endophytic fungi isolated from *T. wilfordii* was carried out based on the ITS and histone

Table 2. Summary on recovered endophytic fungi isolated from leaves, flowers, stems and roots of *Tripterygium wilfordii* Hook f, and their sequence features of ITS, histone H4 and Beta-tubulin genes.

Taxa	Code	Isolation site	No. of isolate	Length (bp) ITS	Histone H4	Beta-tubulin
Colletotrichum acutatum	TW2	Leaf	10	618	596	
Colletotrichum acutatum	TW3	Leaf	4	618	596	975
Colletotrichum boninense	TW5	Leaf	6	818	596	
Colletotrichum boninense	TW12	Flower	3	608	440	967
Colletotrichum boninense	TW13	Flower	1	608	440	967
Colletotrichum boninense	TW6	Leaf	1	618	596	975
Colletotrichum gloeosporioides	TW17	Flower	2	599	595	972
Colletotrichum gloeosporioides	TW18	Leaf	5	602	603	972
Colletotrichum gloeosporioides	TW19	Leaf	7	599	595	973
Colletotrichum gloeosporioides	TW22	Flower	1	599	595	
Diaporthe caulivora	TW10	Flower	4	601	498	982
Diaporthe phaseolorum	TW9	Flower	2	600	532	
Diaporthe phaseolorum	TW11	Flower	3	601	633	980
Diaporthe phaseolorum	TW27	Stem	1	604	498	982
Diaporthe phaseolorum	TW28	Stem	1	601	502	982
Diaporthe phaseolorum	TW31	Stem	1			981
Diaporthe phaseolorum	TW33	Stem	1	603	498	982
Diaporthe phaseolorum	TW40	Stem	1			979
Diaporthe phaseolorum	TW41	Stem	1	601	530	982
Diaporthe phaseolorum	TW42	Stem	2	601	530	982
Diaporthe sp.	TW32	Stem	2	602	530	982
Glomerella cingulata	TW1	Leaf	8	598	595	973
Glomerella cingulata	TW4	Leaf	7	599	595	973
Glomerella cingulata	TW7	Leaf	2	599	595	973
Glomerella cingulata	TW8	Leaf	1	599	595	973
Glomerella cingulata	TW20	Leaf	1	599	595	973
Phaeosphaeria sp.	TW97	Root	1			920
Phomopsis liquidambari	TW15	Flower	2	600	500	982
Phomopsis phyllanthicola	TW23	Flower	1	602	496	982
Phomopsis sp.	TW16	Flower	2	602	500	982
Phomopsis sp.	TW21	Flower	1	598	445	980
Phomopsis sp.	TW24	Stem	1	600	498	982
Phomopsis sp.	TW25	Stem	1	598	496	980
Phomopsis sp.	TW29	Stem	2	600	530	980
Phomopsis sp.	TW35	Stem	2			982
Phomopsis vaccinii	TW43	Flower	2			982
Talaromyces trachyspermus	TW110	Root	1	1069	530	

Table 3. Estimates of average evolutionary divergence over sequence pairs within groups. The analysis involved 95 nucleotide sequences. Evolutionary analyses were conducted in MEGA 5.

Fungal genus	Evolutionary Distance		
	ITS	Histone H4	Beta-tubulin
Glomerella sp.	0.004	0.016	0.009
Colletotrichum sp.	0.065	0.081	0.072
Diaporthe sp.	0.054	0.062	0.034
Phomopsis sp.	0.054	0.032	0.035

H4 sequences by the NJ method, using *Saccharomyces cerevisiae* as an outgroup. Other reference fungal species were retrieved from the GenBank database.

As shown in **Figure 6**, the endophytic fungi studied in this paper clustered into four obvious clades when the ITS sequences were used to construct the phylogenetic tree. The *D. phaseolorum, Phomopsis sp., P. vaccinii, P. phyllanthicola, P. liquidambari,* and *D. caulivora* were clustered to form the first clade, with 56% bootstrap value support. *T. trachyspermus* (TW110T) and the reference group were clustered correctly with logical taxonomic placement. *C. acutatum* and *C. boninense* were clustered to form the third clade. And finally, the *C. gloeosporioides* and *G. cingulata* were clustered to form the forth clade.

Phylogenetic trees constructed based on histone H4 are shown in **Figure 7**. Histone H4 successfully separate *C. acutatum* into monophyletic clade. The *C. gloeosporioides* and *G. cingulata* were clustered to form the second clade. *C. boninense* were clustered together to form the third clade. And the other species such as *D. caulivola, D. phaseolorum, P. phyllanthicola, Phomopsis sp., P. liquidambari,* and *P. vaccinii* were formed the fourth clade.

When **Figure 6** and **7** were compared, both of them had excellent taxonomic resolution to delineate all

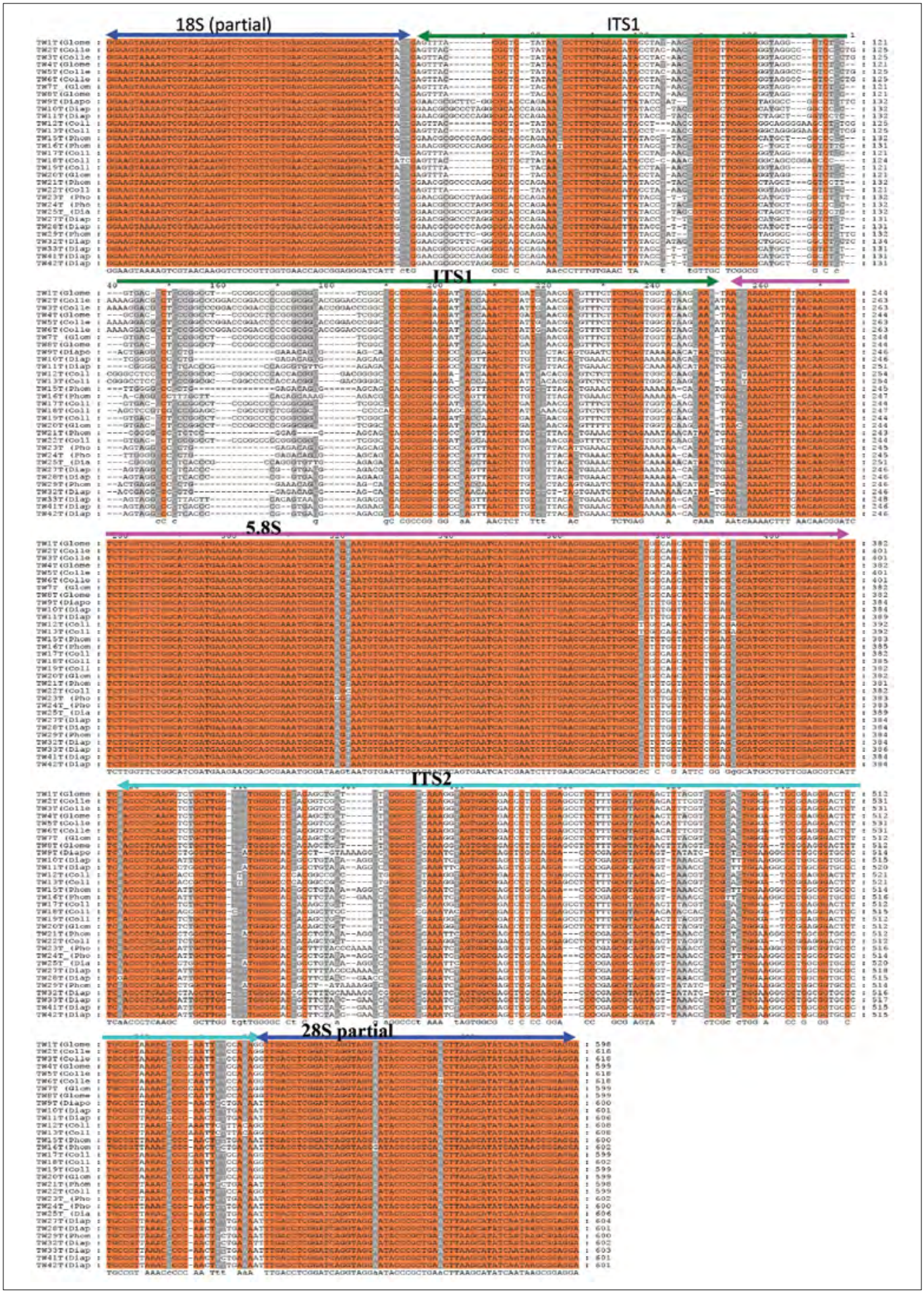

Figure 2. Genetic comparison of ITS1-5.8S-ITS2 nucleotide sequences among 32 fungal endophytes isolated from TwHf.

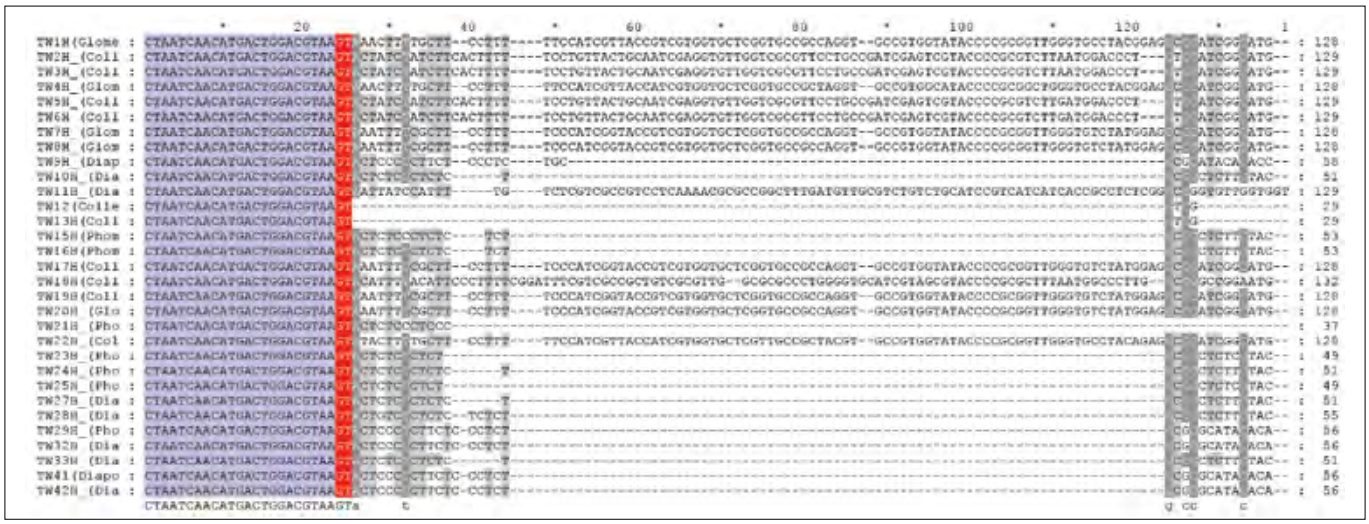

Figure 3. Boundary of exon 1 *(shaded in purple)* ***and intron 1*** *(non-shaded)* ***of aligned histone H4 sequences.*** *Sequences of exon 1 are identical while intron 1 are highly variable. Note the intron always starts with GT sequences (shaded in red).*

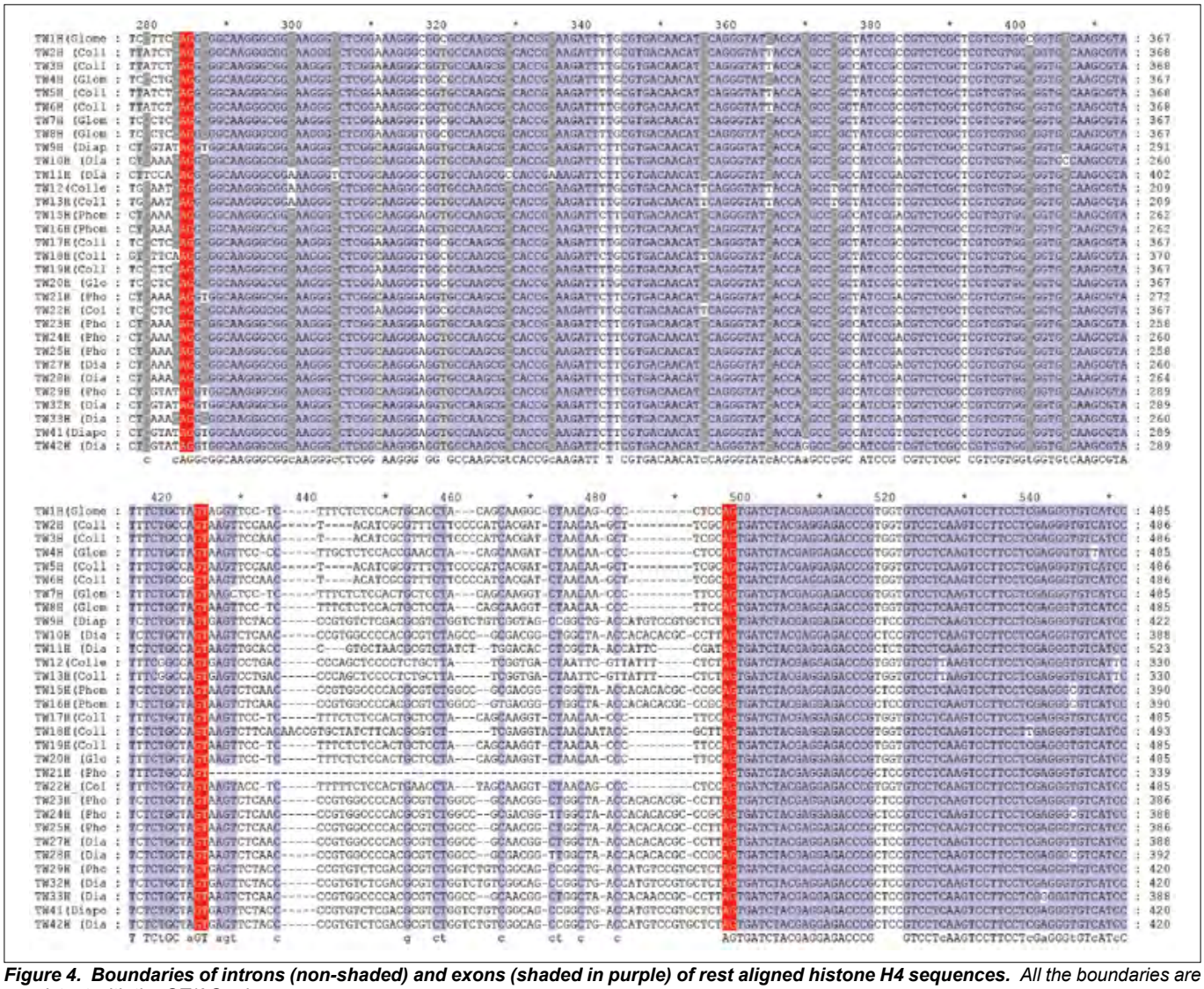

Figure 4. Boundaries of introns (non-shaded) and exons (shaded in purple) of rest aligned histone H4 sequences. *All the boundaries are consistent with the GT/AG rule.*

C. boninense, G. cingulata, C.acutatum into separate monophyletic Clade. However, neither of them could resolve *C. gloeosporioides*, *Phosmopsis* species and *Diaporthe* species into monophyletic respective clades.

Phylogenetic Analyses by Combined Dataset

It has been reported that a single gene loci may be insufficient to contain all evolutionary information of constructing a high taxonomic resolution phylogenetic

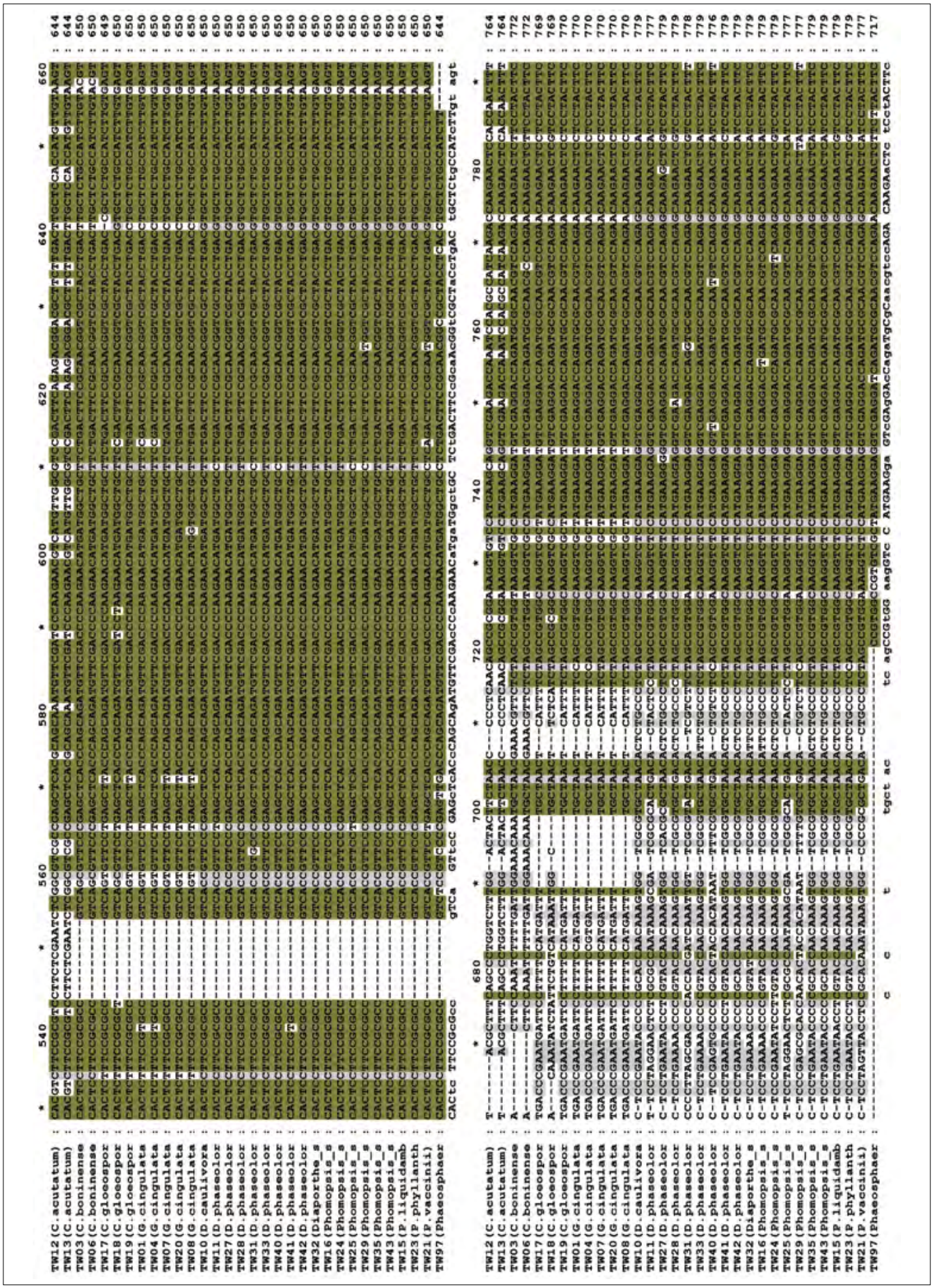

Figure 5. Alignment of Beta-tubulin gene sequences of 32 fungal isolates. *Shaded area indicates existence of consensus nucleotides largely within exon. White strips indicate nucleotide difference among the isolates.*

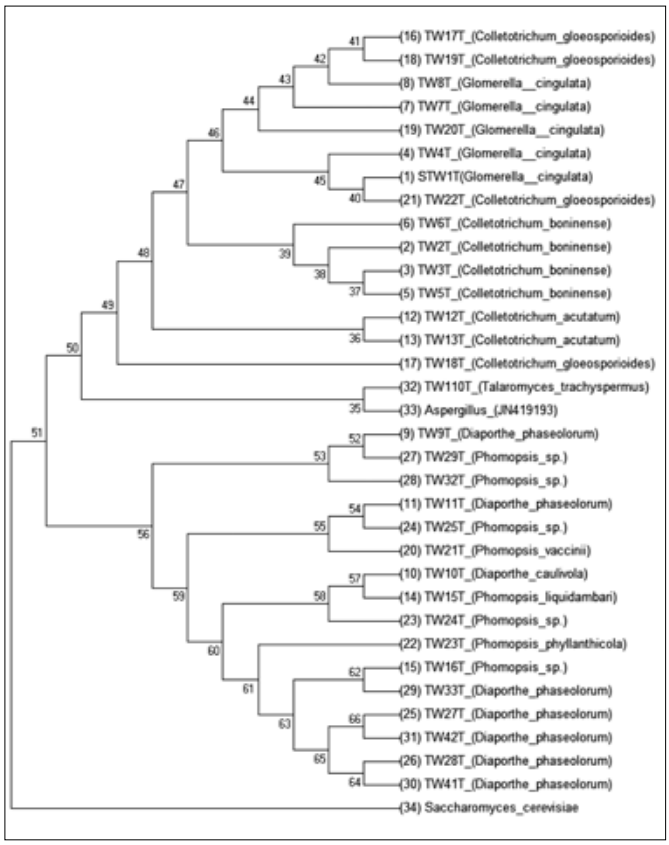

Figure 6. Phylogenetic tree of fungal endophytes based on ITS sequences by using Neighbor-Joining (NJ) method.

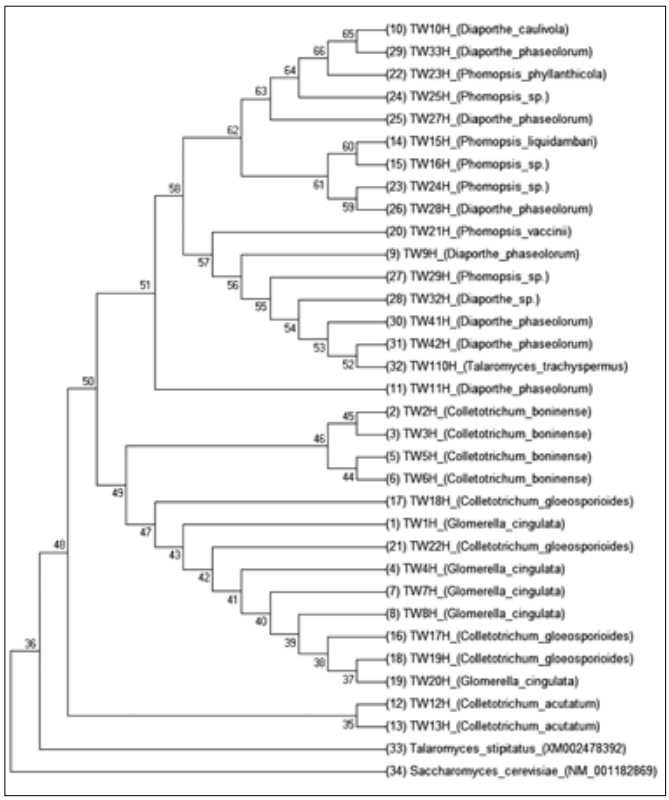

Figure 7. Phylogenetic tree of fungal endophytes based on combined dataset of sequences by using NJ.

tree, especially when species under study are closely related. Thus, it sounds quite logical to use multiple gene analysis conducted simultaneously to obtain a much more reliable phylogenetic tree.[31] In this aspect, Beta-tubulin gene and ITS were combined to form a dataset for phylogenetic analyses. The phylogenetic tree constructed by the combined dataset is shown in **Figure 8,** which indicated an improvement of taxonomic resolution, as the genera *Diaporthe sp*. and *Phomopsis sp.* were clearly separated.

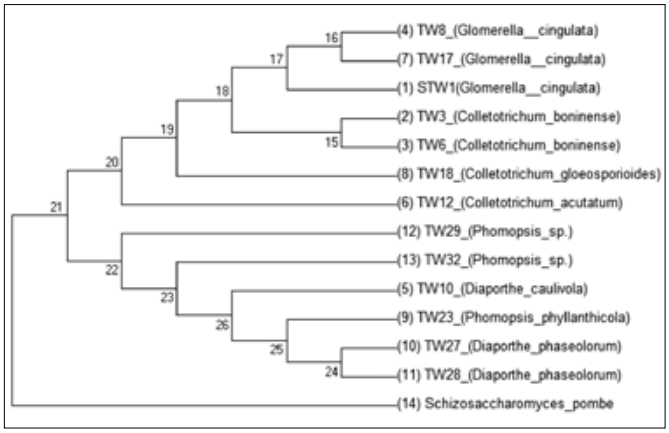

Figure 8. Phylogenetic tree of fungal endophytes based on combined dataset of sequences by using NJ.

DISCUSSION

Diversity of isolated fungal endophytes

A total of 266 fungal endophytes were isolated from various parts of *T. wilfordii*. They were preliminarily classified based on their macroscopic and microscopic traits. And then, the identification of fungal isolates was relied mainly on ITS nucleotide sequences. The results shown fungal endophytes isolated from leaves belong to genera of *Colletotrichum* and *Glomerella*. Whereas in stems, the fungal endophytes isolated belong to genera *Diaporthe* and *Phomopsis*.

According to literature, *Colletotrichum sp.* and *Glomerella sp.* were reported to isolate from a wide variety of plants, including but not limited to, *Livistona chinensis*,[32] *Citrus jambhiri*,[33] *Camptotheca acuminate*.[34] Consistent with our findings, all of the above literatures reported that *Colletotrichum sp./ Glomerella sp.* were mainly isolated from leaves.[35] Similarly, *Diaporte sp./Phomosis sp.* were reported mainly isolated from xylems of *T. wilfordii* and *Garcinia atroviridis*.[35,36] Therefore, *Colletotrichum sp./Glomerella sp.* and *Diaporte sp./Phomopsis sp.* are tissue-specific in lieu of host-specific as revealed in this study.

Another finding in this study is the flowers of *T. wilfordii* contains biggest variety of fungal diversity. This might be explained by the vertical fungal transmission in plants. Namely, some fungal endophytes are transmitted through seeds to next generations.[37,38] If vertical transmission are used for fungal transmissions, the prerequisite criterion is to infect flowers. Thus, it is suspected that *Colletotrichum sp. Glomerella sp* and *Diaporthe sp. Phomopsis sp.* may make use of vertical transmission to increase likelihood of infection to other plants.

Recently, Xie et al.[39] also reported the isolation of 5 *Phomopsis sp.* and 1 *Diaporthe sp*. from stem of *T. wilfordii.* And they found several isolates belong to *Phomopsis sp.* and *Diaporthe sp.* had anti-microbial and anti-tumor activity *in vitro*. In our previous study, *Colletotrichum sp., Glomerella Cingulata* and *Phomopsis sp.* were also isolated from organs of *T. wilfordii.* Moreover, the ethylacetate extracts of *Colletotrichum sp.* and *Phomopsis sp.* had stimulatory effects on proliferation of human peripheral blood mononuclear cells at concentration of 3.9-15 μg/ml, and inhibitory effect at higher concentration.[40] These data suggested that some endophytic fungi isolated from *T. wilfordii* could produce bioactive compounds which might contribute to the medicinal value of its host.

Phylogenetic Information on ITS, Histone H4 and Beta-tubulin Genes

The phylogenetic information was constructed for the fungal isolates. **Table 3** indicated that H4 may be a good phylogenetic marker on average. It contains sufficient parsimony information for taxonomic resolution for wider range of species over ITS and Beta-tubulin gene. This is largely attributed to the added value of intron regions where nucleotide sequences are highly variable. The finding is in consistency to the study conducted by Bernhard and Schlegel.[41]

According to **Figure 6** and **7**, both ITS and histone H4 cannot resolve *C. gloeosporioides, Phosmopsis* species and *Diaporthe* species into monophyletic respective clades. This is one of the reasons why there is a tendency to use multi-genes for phylogenetic applications.[42] Our data also indicated an increased overall evolution distance and improvement of taxonomic resolution when combined data were used. Nevertheless, this method has its own limitations, one of which is not easy to find common outgroup and correct reference sequences.

CONCLUSION

A total of 266 endophytic fungi were isolated from various parts of *T. wilfordii* in this study. Based on ITS sequences, 94 isolates were finally recovered and identified into 13 fungal taxa at genus or species level. Among them, the genus *Colletotrichum sp./ Glomerella sp.* and *Diaporthe sp./Phomopsis sp.* are tissue specific in leaves and xylems respectively. Their evolutionary rate and phylogenetic analyses were also weighted based on ITS, histone H4 and Beta-tubulin sequences. But phylogenetic analyses simply based on one gene cannot resolve *C. gloeosporioides, Diaporthe sp.* and *Phosmopsis sp.* into monophyletic respective clades. Using the combined database of Beta-tubulin gene and ITS, the improvement of taxonomic resolution in phylogenetic were observed. The current study will undoubtedly widen our insight into fungal communities in *T. wilfordii*, and furtherly their role in host metabolites.

ACKNOWLEDGEMENTS

This work was supported by the R & L Fund of Hong Kong Chinese Materia Medica Standard, Department of Health, Hong Kong Government SAR, China.

Author's background

***Mr. LEUNG Fo-Man**, who is a government employee, was a part-time MPhil student in CityU of Hong Kong. This original report was his research works for partial fulfilment of requirements for his MPhil degree under Dr. HY Cheung's supervision. **Dr. FENG Junli** is a molecular biologist by training and is an Associate Professor of the Institute of Seafood, Zhejiang Gongshang University, China. She is currently working as a visiting scholar in Dr. Cheung's laboratory. **Mr WONG Hung-Ngan**, was an undergraduate student majoring in Applied Biology. He joined Dr. Cheung's lab to get involved in this research work as his final year project in order to obtain his honour degree. He is now a Section Manager of Wah-Tak Genome Sequencing Co. Ltd. in Hong Kong. **Dr. CHEUNG Hon-Yeung**, who was an Associate Professor of Pharmaceutical Microbiology & Biotechnology at the City University of Hong Kong. Has been appointed as a Research Fellow in the Department of Biomedical Sciences since retired in July, 2017. He is a Manufacturing Pharmacist and Biotechnologist. He has more than 450 publications and received many awards for both of his research and academic works.*

References

1. Ma JS, Brach AR, Liu QR (1999). A revision of the genus *Tripterygium (Celastraceae).* ***Edinburgh J Botany,*** 56(1):33-46.
2. Wong KF, Yuan Y, Luk JM (2012). *Tripterygium wilfordii* bioactive compounds as anticancer and anti-inflammatory agents. ***Clin Exp Pharmacol P.,*** 39(3):311-320.
3. Duan HQ, Takaishi Y, Jia YF, Li D (2001). Sesquiterpene polyol esters from *Tripterygium wilfordii.* ***Phytochemistry*** 56(4): 341-346.
4. Wang X, Gao W, Yao Z, Zhang S, Zhang Y, et al. (2005). Immunosuppressive sesquiterpenes from *Tripterygium wilfordii*. ***Chem Pharm Bull.***, 53(6): 607-610.
5. Xu J, Lu J, Sun F, Zhu H, Wang L, et al. (2011). Terpenoids from *Tripterygium wilfordii*. ***Phytochemistry***, 72(11-12):1482-1487.
6. Gao C, Huang XX, Bai M, Wu J, Li JY, et al. (2015). Anti-inflammatory sesquiterpene pyridine alkaloids from *Tripterygium wilfordii*. ***Fitoterapia***, 105:49-54.
7. Li BL, Shen Q, Jin MN, Duan HQ (2010). Two new terpenes from *Tripterygium wilfordii*. ***Chinese Chem Lett.***, 21(7): 827-829.
8. Luo Y, Zhou M, Ye Q, Pu Q, Zhang G (2012). Dihydroagarofuran derivatives from the dried roots of *Tripterygium wilfordii.* ***J Nat Prod.***, 75(1):98-102.
9. Luo Y, Pu X, Luo G, Zhou M, Ye Q, et al. (2014). Nitrogen-containing dihydro-β-agarofuran derivatives from *Tripterygium wilfordii.* ***J Nat Prod.***, 77(7):1650-1657.

10. Wu CM, Zhou LM, Chai YF, Wu YT, Fan GR (2010). Three new sesquiterpene alkaloids from the root of *Tripterygium wilfordii*. ***Chinese Chem Lett***., 21(7):830-833.

11. Gao C, Wang D, Zhang Y, Huang XX, Song SJ (2016). Kaurane and abietane diterpenoids from the roots of *Tripterygium wilfordii,* and their cytotoxic evaluation. ***Bioorg Med Chem Lett***., 26(12): 2942-2946.

12. Li HM, Wan DW, Li RT (2015). New abietane-type diterpene glycosides from the roots of *Tripterygium wilfordii*. ***J Asian Nat Prod Res***., 17(7):761-766.

13. Lin S, Yu XY, Que HQ, Chen Z, Xie DL, et al. (2005). Diterpenes constituents of *Tripterygium wilfordii* (II). ***Acta Pharm Sin***., 46(8):942-945.

14. Liu J, Wu Q, Shu J, Zhang R, Liu L (2017). Three new abietane-type diterpene glycosides from the roots of *Tripterygium wilfordii*. ***Fitoterapia***, 120:126-130.

15. Zhou LM, Du J, Wu CL (2010). A new triterpenoid from the roots of *Tripterygium wilfordii*. ***Chinese Chem Lett***., 21(5):600-602.

16. Chen Y, Zhao J, Qiu Y, Yuan H, Khan SI, et al. (2017). Prenylated flavonoids from the stems and roots of *Tripterygium wilfordii*. ***Fitoterapia***, 119:64-68.

17. Zeng F, Wang W, Wu Y, Dey M, Ye M, et al. (2010). Two prenylated and C-methylated flavonoids from *Tripterygium wilfordii*. ***Planta Med***., 76(14):1596-1599.

18. Sun ZL, Zhang M, Zhang JF, Feng J (2011). Antifungal and cytotoxic activities of the secondary metabolites from endophytic fungus *Massrison sp.* ***Phytomedicine***, 18:859-862.

19. Gond SK, Verma VC, Mishra A, Kumar A, Kharwar RN (2010). Role of fungal endophytes in plant protection. In: Arya A, Perello´AE (eds) ***Management of Fungal plant pathogens***. CAB International, Wallingford, pp183-197.

20. Stierle A, Strobel G, Stierle D (1993). Taxol and taxen production by Taxomyces andreanae an endophytic fungus of pacific yew. ***Science*** 260:214-216.

21. Strobel GA, Hess WM, Ford E, Sidhu RS, Yang X (1996). Taxol from fungal endophytes and the issue of biodiversity. ***J Ind Microbiol***., 17(5-6):417-423.

22. Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiology, ***Microbiol Mol Biol R***., 67(4):491-502.

23. Kumar DS, Lau CS, Wan JM, Yang D, Hyde KD (2005). Immunomodulatory compounds from pestalotiopsis leucothës, an endophytic fungus from *Tripterygium wilfordii*. ***Life Sci***., 78(2):147-156.

24. Li SJ, Zhang X, Wang XH, Zhao CQ (2018). Novel natural compounds from endophytic fungi with anticancer activity. ***Eur J Med Chem*** 156: 316-343.

25. Arnold AE (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. ***Fungal Biol Rev***., 21(2):51-66.

26. Kumar S, Dudley J, Nei M, Tamura K (2008). MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. ***Brief Bioinform*** 9: 299-306.

27. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. ***Mol Biol Evol***., 28(10):2731-2739.

28. Kimura M (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. ***J Mol Evol***.,16:111-120.

29. Saitou N, Nei M (1987.) The neighbor-joining method: a new method for reconstructing phylogenetic tree. ***Mol Biol Evol***., 4:406-425.

30. Sitnikova T, Rzhetsky A, Nei M (1995). Interior-branch and bootstrap tests of phylogenetic trees. ***Mol Biol Evol***.,12:319-333.

31. Nuytinck J, Verbeken A, Miller SL (2007). Worldwide phylogeny of *Lactarius* section *Deliciosi* inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. ***Mycologia***, 99(6): 820-832.

32. Guo LD, Hyde KD, Liew EC (2001). Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. ***Mol Phylogenet Evol***., 20(1):1-13.

33. Araujo WL, Maccheroni WJr, Aguilar-Vildoso CI, Barroso PA, Saridakis HO, et al. (2001). Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootstocks. ***Can J Microbiol*** 47(3):229-236.

34. Ding T, Jiang T, Zhou J, Xu L, et al. (2010). Evaluation of antimicrobial activity of endophytic fungi from *Camptotheca acuminata* (Nyssaceae). ***Genet Mol Res.***, 9(4): 2104-2112.

35. De Silva DD, Crous PW, Ades PK, Hyde KD, Taylor PWJ (2017). Life styles of *Colletotrichum* species and implications for plant biosecurity. ***Fungal Biol Rev***., 31(3):155-168.

36. Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006). Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. ***FEMS Immunol Med Mic***., 48(3): 367-372.

37. Grube M, Gaya E, Kauserud H, Smith AM, Avery SV, et al. (2017). The next generation fungal diversity researcher. ***Fungal Biol Rev***., 31(3):124-130.

38. Hartley SE, Gange AC (2009). Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. ***Annu Rev Entomol***., 54(1):323-342.

39. Xie SD, Chen XD, Wang H, Wei HX, Pan Y, et al. (2018). Isolation and bioactivity studies of endophytic fungi from *Tripterygium wilfordii* Hook. f. ***Microbiol China***, 45(2):413-419. (in Chinese)

40. Kumar DSS, Cheung HY, Lau CS, Chen F, Hyde KD (2004). In vitro studies of endophytic fungi from *Tripterygium wilfordii* with anti-proliferative activity on human peripheral blood mononuclear cells. ***J Ethnopharmacol***., 94:295-300.

41. Bernhard D, Schlegel M (1998). Evolution of histone H4 and H3 genes in different ciliate lineages. ***J Mol Evol*** 46(3):344-354.

42. Kawasaki M, Anzawa K, Ushigami T, Kawanishi J, Mochizuki T (2011). Multiple gene analyses are necessary to understand accurate phylogenetic relationships among Trichophyton species. ***Med Mycol***., 52(3):245-254.

SHPHK – 2018 Wrap Up

*Reported by **Vienna Leung***
Pharmacist of the Society of Hospital Pharmacists of Hong Kong

2018 has been a fruitful year for the Society of Hospital Pharmacists of Hong Kong (SHPHK)!

As we come to the end of the year, it is time for us to look back at the activities organised by the Society in 2018!

SHPHK Activities: 2018 in Review

January	Symposium on Clinical Update in Haematology and Oncology: The Evolving Treatment Landscape in Breast Cancer, Myeloproliferative Neoplasm and Iron Overload
April	SHPHK Annual General Meeting
	Seminar on Pain Management in Cancer Patients
May	Hiking Trip to Tung Lung Island
	Sharing Session: Road to Success - How to Prepare for a Job Interview
October	Seminar on Management of Chemotherapy-Induced Nausea and Vomiting (CINV) in Oncology Patients
November	Seminar on Updates on the Evolving Role of Biosimilars (co-organised with PCCC)
	Seminar on Innovation in Chronic Heart Failure Treatment
December	Official Visit to Hospital Conde S. Januário, Kiang Wu Hospital and Farmácia Popular in Macao;
	Meeting with the Committee Members of the Pharmaceutical Society of Macao

Learning Activities in Q4 2018

1. Seminar on Updates on the Evolving Role of Biosimilars

The Society welcomes collaborations with different healthcare professional bodies. For example, an educational seminar was co-organised by the Society and the Pharmacy Central Continuing Education Committee (PCCC) on 6th November 2018. We were honoured to have Prof. Frank van den Hoogen, Specialist in Rheumatology, Radboud University, The Netherlands to deliver an amazing seminar on biosimilar products to our fellow pharmacists. The Society would continue to foster communication between pharmacists of different sectors through the organisation of various events and activities in the coming year. Members are highly encouraged to engage in professional learning by joining different continuing professional development programmes to advance their clinical knowledge.

From left: Dr. Celeste EWIG, Chairlady of the Pharmacy Central Continuing Education Committee, and Prof. Frank van den HOOGEN, Specialist in Rheumatology, Radboud University, The Netherlands.

2. Symposium on 'Innovation in Chronic Heart Failure Treatment'

Learning never stops! There was another educational symposium on chronic heart failure organised by the Society on 30th November 2018. At the symposium, the roles of clinical pharmacists in chronic heart failure were discussed. Audience were equipped with the latest clinical knowledge of treatment options for heart failure.

Official Visit to Macao

The General Committee Members had an official visit to Hospital Conde S. Januário, Kiang Wu Hospital and Farmácia Popular on 1st December 2018 to learn about the latest advances in pharmacy practice in Macao. A special thank you to the Pharmaceutical Society of Macao for their hospitality and introduction of the health system of Macao to the group. It was a very intensive, yet productive day trip, and the SHPHK Committee is hoping to have more collaborations with the fellow pharmacists in Macao in the future.

Photo taken at Farmácia Popular, Macao. From left: Hon. Assoc. Prod. William C M CHUI, President of the Society of Hospital Pharmacists of Hong Kong, and Ms. Carolina UNG, Secretary of the Pharmaceutical Society of Macao.

What's Coming Up in 2019?

The Society is currently revamping its official website. It is expected that the revamp will be completed by Q2 2019. In the future, members can renew their SHPHK membership by just logging into their personal SHPHK account online and connect with other members through the SHPHK forum. The forum is an interactive online platform to allow members to share their clinical experience with other fellow pharmacists, and to provide peer support for one another. Stay tuned!

Furthermore, the Society will be organising a symposium on Multiple Myeloma on 18th January 2019 at the Cityview Hotel, Yau Ma Tei. This is a taster event, therefore, it is also FREE for non-members who work at the hospital! Non-members are highly encouraged to join as member on-site to enjoy the full benefits of the Society. Places are limited! Do not miss the chance to book your first CE event in 2019!

SHPHK would like to take this opportunity to offer its sincerest appreciation to the General Committee Members who are serving the members all year round, and also thank all members for their support to the Society. In 2019, the Society will continue to work closely with its members and different parties to *promote, improve and assist the advancement of hospital pharmacy practice* in Hong Kong!

We wish you all a Merry Christmas and a Happy New Year!

You are most welcome to follow the Society's Facebook page (@SHPHK) to know more about the Society's development and activities. You may also visit the Drug Education Resources Centre (DERC) Website: www.derc.org.hk to keep abreast of the latest news and development of drugs in Hong Kong. Join us now as new member or to renew your membership at the Society's website: www.shphk.org.hk.

ISENTRESS®

Prepared By Lucilla Leung

Active Ingredient:
Each film-coated tablet contains 600 mg of raltegravir (as potassium).

Presentation:
Film-coated tablet.

Indications:
ISENTRESS 600 mg film-coated tablets is indicated in combination with other anti-retroviral medicinal products for the treatment of human immunodeficiency virus (HIV-1) infection in adults, and paediatric patients weighing at least 40 kg.

Posology
ISENTRESS should be used in combination with other active anti-retroviral therapies (ARTs).

Adults and paediatric population
In adults and paediatric patients (weighing at least 40 kg), the recommended dosage is 1,200 mg (two 600 mg tablets) once daily for treatment-naïve patients or patients who are virologically suppressed on an initial regimen of ISENTRESS 400 mg twice daily.

Additional formulations and strengths available
ISENTRESS is also available as a 400 mg tablet for twice daily use in HIV infected adults or children and adolescents at least 25 kg. The 400 mg tablet should not be used to administer 1,200 mg once daily regimen (please refer to the 400 mg Summary of Product Characteristics).

Elderly
There is limited information regarding the use of raltegravir in the elderly. Therefore, ISENTRESS should be used with caution in this population.

Renal impairment
No dosage adjustment is required for patients with renal impairment.

Hepatic impairment
No dosage adjustment is required for patients with mild to moderate hepatic impairment. The safety and efficacy of raltegravir have not been established in patients with severe underlying liver disorders. Therefore, ISENTRESS should be used with caution in patients with severe hepatic impairment.

ISENTRESS 600 mg film-coated tablet formulation should not be used in children weighing less than 40 kg.

Method of administration
Oral use.
ISENTRESS 600 mg tablets can be administered with or without food as a 1,200 mg once daily dose. The tablets should not be chewed, crushed or split due to anticipated changes in the pharmacokinetic profile.

Contraindications
Hypersensitivity to the active substance or to any of the excipients.

Precautions:
Patients should be advised that current anti-retroviral therapy does not cure HIV and has not been proven to prevent the transmission of HIV to others through blood contact. While effective viral suppression with antiretroviral therapy has been proven to substantially reduce the risk of sexual transmission, a residual risk cannot be excluded. Precautions to prevent transmission should be taken in accordance with national guidelines.

Raltegravir has a relatively low genetic barrier to resistance. Therefore, whenever possible, raltegravir should be administered with two other active ARTs to minimise the potential for virological failure and the development of resistance.

In treatment-naïve patients, the clinical study data on use of raltegravir are limited to use in combination with two nucleotide reverse transcriptase inhibitors (NRTIs) (emtricitabine and tenofovir disoproxil fumarate).

Depression
Depression, including suicidal ideation and behaviours, has been reported, particularly in patients with a pre-existing history of depression or psychiatric illness. Caution should be used in patients with a pre-existing history of depression or psychiatric illness.

Hepatic impairment
The safety and efficacy of raltegravir have not been established in patients with severe underlying liver disorders. Therefore, raltegravir should be used with caution in patients with severe hepatic impairment.

Patients with pre-existing liver dysfunction including chronic hepatitis have an increased frequency of liver function abnormalities during combination anti-retroviral therapy and should be monitored according to standard practice. If there is evidence of worsening liver disease in such patients, interruption or discontinuation of treatment should be considered.

Patients with chronic hepatitis B or C and treated with combination anti-retroviral therapy are at an increased risk for severe and vpotentially fatal hepatic adverse reactions.

Osteonecrosis
Although the aetiology is considered to be multifactorial (including corticosteroid use, alcohol consumption,

severe immunosuppression, higher body mass index), cases of osteonecrosis have been reported particularly in patients with advanced HIV disease and/or long-term exposure to combination anti-retroviral therapy. Patients should be advised to seek medical advice if they experience joint aches and pain, joint stiffness or difficulty in movement.

Immune reactivation syndrome
In HIV-infected patients with severe immune deficiency at the time of institution of combination anti- retroviral therapy (CART), an inflammatory reaction to asymptomatic or residual opportunistic pathogens may arise and cause serious clinical conditions, or aggravation of symptoms. Typically, such reactions have been observed within the first weeks or months of initiation of CART. Relevant examples are cytomegalovirus retinitis, generalised and/or focal mycobacterial infections and pneumonia caused by *Pneumocystis jiroveci* (formerly known as *Pneumocystis carinii*). Any inflammatory symptoms should be evaluated and treatment instituted when necessary.

Autoimmune disorders (such as Graves' disease) have also been reported to occur in the setting of immune reactivation: however, the reported time to onset is more variable and these events can occur many months after initiation of treatment.

Atazanavir
Co-administration of raltegravir 1,200 mg once daily with atazanavir resulted in increased raltegravir plasma levels; therefore, co-administration is not recommended.

Tipranavir/ritonavir
Co-administration of raltegravir 1,200 mg once daily with tipranavir/ritonavir could result in decreased raltegravir trough plasma levels; therefore, co-administration is not recommended.

Antacids
Co-administration of raltegravir 1,200 mg once daily with calcium carbonate and aluminium/magnesium containing antacids resulted in reduced raltegravir plasma levels; therefore, co-administration is not recommended.

Strong inducers of drug metabolizing enzymes
Strong inducers of drug metabolizing enzymes (e.g., rifampicin) have not been studied with raltegravir 1,200 mg once daily, but could result in decreased raltegravir trough plasma levels; therefore, co-administration with raltegravir 1,200 mg once daily is not recommended.

Myopathy and rhabdomyolysis
Myopathy and rhabdomyolysis have been reported. Use with caution in patients who have had myopathy or rhabdomyolysis in the past or have any predisposing issues including other medicinal products associated with these conditions.

Severe skin and hypersensitivity reactions
Severe, potentially life-threatening, and fatal skin reactions have been reported in patients taking raltegravir, in most cases concomitantly with other medicinal products associated with these reactions. These include cases of Stevens-Johnson syndrome and toxic epidermal necrolysis. Hypersensitivity reactions have also been reported and were characterised by rash, constitutional findings, and sometimes, organ dysfunction, including hepatic failure. Discontinue raltegravir and other suspect agents immediately if signs or symptoms of severe skin reactions or hypersensitivity reactions develop (including, but not limited to, severe rash or rash accompanied by fever, general malaise, fatigue, muscle or joint aches, blisters, oral lesions, conjunctivitis, facial oedema, hepatitis, eosinophilia, angioedema). Clinical status including liver aminotransferases should be monitored and appropriate therapy initiated. Delay in stopping raltegravir treatment or other suspect agents after the onset of severe rash may result in a life-threatening reaction.

Rash
Rash occurred more commonly in treatment-experienced patients receiving regimens containing raltegravir and darunavir compared to patients receiving raltegravir without darunavir or darunavir without raltegravir.

Lactose
ISENTRESS film-coated tablets contain lactose. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine.

Drug Interaction
In vitro studies indicate that raltegravir is not a substrate of cytochrome P450 (CYP) enzymes, does not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A, does not inhibit UDP glucuronosyltransferases (UGTs) 1A1 and 2B7, does not induce CYP3A4 and does not inhibit P-glycoprotein-mediated transport. Based on these data, raltegravir is not expected to affect the pharmacokinetics of medicinal products that are substrates of these enzymes or P-glycoprotein.

Based on *in vitro* and *in vivo* studies, raltegravir is eliminated mainly by metabolism via a UGT1A1-mediated glucuronidation pathway.

Considerable inter- and intra-individual variability was observed in the pharmacokinetics of raltegravir.

Effect of raltegravir on the pharmacokinetics of other medicinal products
In drug interaction studies performed using raltegravir 400 mg twice daily, raltegravir did not have a clinically meaningful effect on the pharmacokinetics of etravirine, maraviroc, tenofovir disoproxilfumarate, hormonal contraceptives, methadone, midazolam or boceprevir. These findings can be extended to raltegravir 1,200 mg once daily and no dosage adjustment is required for these agents.

In some studies, co-administration of raltegravir 400 mg tablets twice daily with darunavir resulted in a modest

but clinically insignificant decrease in darunavir plasma concentrations. Based on the magnitude of effect seen with raltegravir 400 mg tablets twice daily, it is expected that the effect of raltegravir 1,200 mg once daily on darunavir plasma concentrations is likely to be not clinically meaningful.

Effect of other medicinal products on the pharmacokinetics of raltegravir

Inducers of drug metabolizing enzymes

The impact of medicinal products that are strong inducers of UGT1A1 such as rifampicin on raltegravir 1,200 mg once daily is unknown, but co-administration is likely to decrease raltegravir trough levels based on the reduction in trough concentrations observed with raltegravir 400 mg twice daily; therefore, co-administration with raltegravir 1,200 mg once daily is not recommended. The impact of other strong inducers of drug metabolizing enzymes, such as phenytoin and phenobarbital, on UGT1A1 is unknown; therefore, co-administration with raltegravir 1,200 mg once daily is not recommended. In drug interaction studies, efavirenz did not have a clinically meaningful effect on the pharmacokinetics of raltegravir 1,200 mg once daily; therefore, other less potent inducers (e.g., efavirenz, nevirapine, rifabutin, glucocorticoids, St. John's wort, pioglitazone) may be used with the recommended dose of raltegravir.

Inhibitors of UGT1A1

Co-administration of atazanavir with raltegravir 1,200 mg once daily significantly increased plasma levels of raltegravir; therefore, co-administration of raltegravir 1,200 mg once daily and atazanavir is not recommended.

Antacids

Co-administration of raltegravir 1,200 mg once daily with aluminium/magnesium and calcium carbonate containing antacids are likely to result in clinically meaningful reductions in the plasma trough levels of raltegravir. Based on these findings, co-administration of aluminium/magnesium and calcium carbonate containing antacids with raltegravir 1,200 mg once daily is not recommended.

Agents that increase gastric pH

Population pharmacokinetic analysis from ONCEMRK (Protocol 292) showed that co-administration of raltegravir 1,200 mg once daily with PPIs or H2 blockers did not result in statistically significant changes in the pharmacokinetics of raltegravir. Comparable efficacy and safety results were obtained in the absence or presence of these gastric pH-altering agents. Based on these data, proton pump inhibitors and H2 blockers may be co-administered with raltegravir 1,200 mg once daily.

Additional considerations

No studies have been conducted to evaluate the drug interactions of ritonavir, tipranavir/ritonavir, boceprevir or etravirine with raltegravir 1,200 mg (2 x 600 mg) once daily. While the magnitudes of change on raltegravir exposure from raltegravir 400 mg twice daily by ritonavir, boceprevir or etravirine were small, the impact from tipranavir/ritonavir was greater (GMR Ctrough=0.45, GMR AUC=0.76). Co-administration of raltegravir 1,200 mg once daily and tipranavir/ritonavir is not recommended.

Previous studies of raltegravir 400 mg twice daily showed that co-administration of tenofovir disoproxil fumarate (a component of emtricitabine/tenofovir disaproxil fumarate) increased raltegravir exposure. Emtricitabine/tenofovir disaproxil fumarate was identified to increase raltegravir 1,200 mg once daily bioavailability by 12%, however its impact is not clinically meaningful. Therefore, co- administration of emtricitabine/tenofovir disaproxil fumarate and raltegravir 1,200 mg once daily is permitted.

All interaction studies were performed in adults. Comprehensive drug interaction studies were performed with raltegravir 400 mg twice daily and a limited number of drug interaction studies were performed for raltegravir 1,200 mg once daily.

Table 1 displays all available interaction study data along with recommendations for co-administration.

Table 1: Pharmacokinetic Interaction Data

Medicinal products by therapeutic area	**Interaction** (mechanism, if known)	**Recommendations concerning co-administration**
ANTI-RETROVIRAL		
Protease inhibitors (PI)		
atazanavir /ritonavir (raltegravir 400 mg Twice Daily)	raltegravir AUC ↑ 41% raltegravir C_{12hr} ↑ 77% raltegravir C_{max} ↑ 24% (UGT1A1 inhibition)	No dose adjustment required for raltegravir (400 mg twice daily).
atazanavir (raltegravir 1,200 mg single dose)	raltegravir AUC ↑ 67% raltegravir C_{24hr} ↑ 26% raltegravir C_{max} ↑ 16% (UGT1A1 inhibition)	Co-administration of raltegravir (1,200 mg once daily) is not recommended.
tipranavir /ritonavir (raltegravir 400 mg Twice Daily)	raltegravir AUC ↓ 24% raltegravir C_{12hr} ↓ 55% raltegravir C_{max} ↓ 18% (UGT1A1 induction)	No dose adjustment required for raltegravir (400 mg twice daily).
	Extrapolated from 400 mg twice daily study	Co-administration of raltegravir (1,200 mg once daily) is not recommended.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)		
efavirenz (raltegravir 400 mg Single Dose)	raltegravir AUC ↓ 36% raltegravir C_{12hr} ↓ 21% raltegravir C_{max} ↓ 36% (UGT1A1 induction)	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily).
efavirenz (raltegravir 1,200 mg single dose)	raltegravir AUC ↓ 14% raltegravir C_{24hr} ↓ 6% raltegravir C_{max} ↓ 9% (UGT1A1 induction)	
etravirine (raltegravir 400 mg Twice Daily)	raltegravir AUC ↓ 10% raltegravir C_{12hr} ↓ 34% raltegravir C_{max} ↓ 11% (UGT1A1 induction) etravirine AUC ↑ 10% etravirine C_{12hr} ↑ 17% etravirine C_{max} ↑ 4%	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or etravirine.
Nucleoside/tide reverse transcriptase inhibitors		
tenofovir disoproxil fumarate (raltegravir 400 mg Twice Daily)	raltegravir AUC ↑ 49% raltegravir C_{12hr} ↑ 3% raltegravir C_{max} ↑ 64% (mechanism of interaction unknown) tenofovir AUC ↓ 10% tenofovir C_{24hr} ↓ 13% tenofovir C_{max} ↓ 23%	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or tenofovir disoproxil fumarate.
emtricitabine and tenofovir disoproxil fumarate (raltegravir 1,200 mg (2 x 600 mg) Once Daily)	Population PK analysis showed that the effect of emtricitabine/tenofovir disaproxil fumarate on raltegravir pharmacokineticswas minimal (12% increase in relative bioavailability), and was not statistically or clinically significant. (Mechanism of interaction unknown)	
CCR5 inhibitors		
maraviroc (raltegravir 400 mg Twice Daily)	raltegravir AUC ↓ 37% raltegravir C_{12hr} ↓ 28% raltegravir C_{max} ↓ 33% (mechanism of interaction unknown) maraviroc AUC ↓ 14% maraviroc C_{12hr} ↓ 10% maraviroc C_{max} ↓ 21%	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or maraviroc.
HCV ANTIVIRALS		
NS3/4A protease inhibitors (PI)		
boceprevir (raltegravir 400 mg Single Dose)	raltegravir AUC ↑ 4% raltegravir C_{12hr} ↓ 25% raltegravir C_{max} ↑ 11% (mechanism of interaction unknown)	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or boceprevir.
ANTIMICROBIALS		
Antimycobacterial		
rifampicin (raltegravir 400 mg Single Dose)	raltegravir AUC ↓ 40% raltegravir C_{12hr} ↓ 61% raltegravir C_{max} ↓ 38% (UGT1A1 induction)	Rifampicin reduces plasma levels of raltegravir. If co-administration with rifampicin is unavoidable, a doubling of the dose of raltegravir (400 mg twice daily) can be considered.
	Extrapolated from 400 mg twice daily study	Co-administration of raltegravir (1,200 mg once daily) is not recommended.
SEDATIVE		
midazolam (**raltegravir** 400 mg Twice Daily)	midazolam AUC ↓ 8% midazolam C_{max} ↑ 3%	No dosage adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or midazolam These results indicate that raltegravir is not an inducer or inhibitor of CYP3A4, and raltegravir is thus not anticipated to affect the pharmacokinetics of medicinal products which are CYP3A4 substrates.
METAL CATION ANTACIDS		
aluminium and magnesium hydroxide antacid (raltegravir 400 mg Twice Daily)	raltegravir AUC ↓ 49% raltegravir $C_{12\ hr}$ ↓ 63% raltegravir C_{max} ↓ 44% 2 hours before raltegravir raltegravir AUC ↓ 51% raltegravir $C_{12\ hr}$ ↓ 56% raltegravir C_{max} ↓ 51%	

	2 hours after raltegravir raltegravir AUC ↓ 30% raltegravir $C_{12\ hr}$ ↓ 57% raltegravir C_{max} ↓ 24% 6 hours before raltegravir raltegravir AUC ↓ 13% raltegravir $C_{12\ hr}$ ↓ 50% raltegravir C_{max} ↓ 10% 6 hours after raltegravir raltegravir AUC ↓ 11% raltegravir $C_{12\ hr}$ ↓ 49% raltegravir C_{max} ↓ 10% (chelation of metal cations)	Aluminium and magnesium containing antacids reduce raltegravir plasma levels. Co-administration of raltegravir (400 mg twice daily and 1,200 mg once daily) with aluminium and/or magnesium containing antacids is not recommended.
aluminium/magnesium hydroxide antacid (raltegravir 1,200 mg single dose)	12 hours after raltegravir raltegravir AUC ↓ 14% raltegravir $C_{24\ hr}$ ↓ 58% raltegravir C_{max} ↓ 14% (chelation of metal ions)	
calcium carbonate antacid (raltegravir 400 mg Twice Daily)	raltegravir AUC ↓ 55% raltegravir $C_{12\ hr}$ ↓ 32% raltegravir C_{max} ↓ 52% (chelation of metal cations)	No dose adjustment required for raltegravir (400 mg twice daily).
calcium carbonate antacid (raltegravir 1,200 mg single dose)	raltegravir AUC ↓ 72% raltegravir $C_{24\ hr}$ ↓ 48% raltegravir C_{max} ↓ 74% 12 hours after raltegravir raltegravir AUC ↓ 10% raltegravir $C_{24\ hr}$ ↓ 57% raltegravir C_{max} ↓ 2% (chelation of metal ions)	Co-administration of raltegravir (1,200 mg once daily) is not recommended.
H2 BLOCKERS AND PROTON PUMP INHIBITORS		
omeprazole (raltegravir 400 mg Twice Daily)	raltegravir AUC ↑ 37% raltegravir $C_{12\ hr}$ ↑ 24% raltegravir C_{max} ↑ 51% (increased solubility)	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily).
famotidine (raltegravir 400 mg Twice Daily)	raltegravir AUC ↑ 44% raltegravir $C_{12\ hr}$ ↑ 6% raltegravir C_{max} ↑ 60% (increased solubility)	
gastric pH altering agents: proton pump inhibitors (e.g. omeprazole), H2 blockers (e.g. famotidine, ranitidine, cimetidine) (raltegravir 1,200 mg)	Population PK analysis showed that the effect of gastric pH altering agents on raltegravir pharmacokinetics was minimal (8.8% decrease in relative bioavailability), and was not statistically or clinically significant. (Increased drug solubility)	
HORMONAL CONTRACEPTIVES		
Ethinyl Estradiol Norelgestromin (raltegravir 400 mg Twice Daily)	Ethinyl Estradiol AUC ↓ 2% Ethinyl Estradiol C_{max} ↑ 6% Norelgestromin AUC ↑ 14% Norelgestromin C_{max} ↑ 29%	No dosage adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or hormonal contraceptives (estrogen- and/or progesterone-based).
OPIOID ANALGESICS		
methadone (raltegravir 400 mg Twice Daily)	methadone AUC ↔ methadon C_{max} ↔	No dose adjustment required for raltegravir (400 mg twice daily and 1,200 mg once daily) or methadone.

Side Effects

Summary of the safety profile

In randomised clinical trials raltegravir 400 mg twice daily was administered in combination with fixed or optimised background treatment regimens to treatment-naïve (N=547) and treatment-experienced (N=462) adults for up to 96 weeks. A further 531 treatment-naïve adults have received raltegravir 1,200 mg once daily with emtricitabine and tenofovir disoproxil fumarate for up to 96 weeks.

The most frequently reported adverse reactions during treatment were headache, nausea and abdominal pain. The most frequently reported serious adverse reactions were immune reconstitution syndrome and rash. The rates of discontinuation of raltegravir due to adverse reactions were 5% or less in clinical trials.

Rhabdomyolysis was an uncommonly reported serious adverse reaction in post-marketing use of raltegravir 400 mg twice daily.

Forensic Classification: P1S1S3

『肝之清』

仙鶴草

專利 護肝配方

專利號：CN102988517B

護肝・強效四合一

◇ ◇ ◇ ◇

修降清護
復醇肝肝

仙鶴草真葉圖

仙鶴草又名脫力草（《滇南本草圖譜》），是薔薇科植物龍牙草 ***Agrimonia pilosa Ledeb.*** 的乾燥地上部分。《百草鏡》曰：仙鶴草「下氣活血，理百病」。有收斂止血，解毒等功效，是應用廣泛的傳統民間草藥（1-2）。現代科學研究顯示，仙鶴草中的活性成分具有強大的肝臟保護作用，能幫助肝臟細胞對抗多種因素的傷害（3）。

(1) 摘錄《中藥大辭典》：仙鶴草
(2) 摘錄《中華本草》：仙鶴草
(3) Dae-Sik Hah, et. al. Toxicol. Res Vol. 25, (4), 243-251

了解更多
仙鶴草的故事

本地大學研發

90粒
標準價$638/盒

Made in U.S.A
美國製造

FDA
註冊證書

美國國家衛生
基金會國際認證

藥品優良
製造規範

素食配方

此產品沒有根據《藥劑業及毒藥條例》或《中醫藥條例》註冊。為此產品作出的任何聲稱亦沒有為進行該等註冊而接受評核。
此產品並不供作診斷、治療或預防任何疾病之用。本產品不能代替藥物，需配合均衡飲食。