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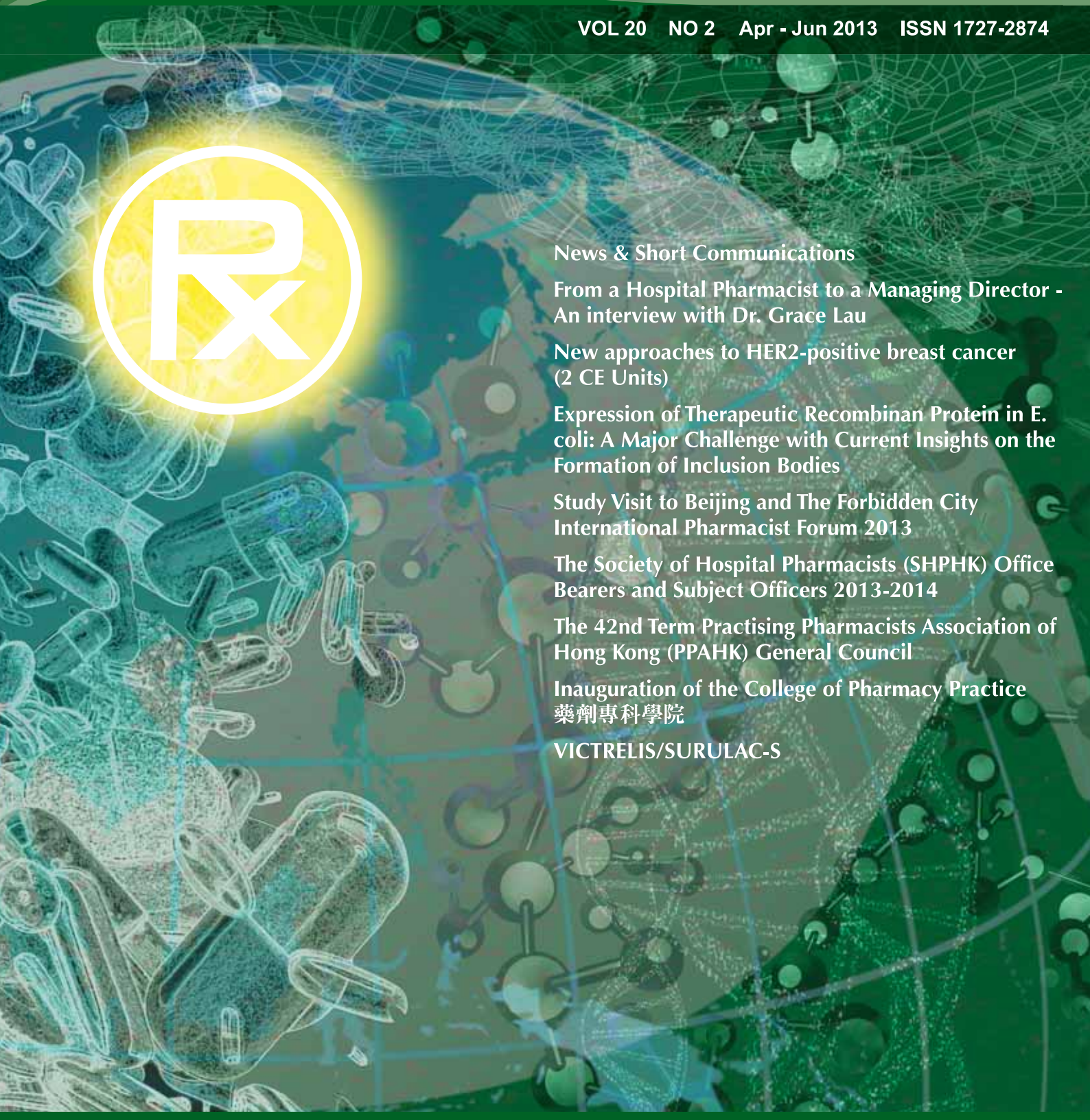

News & Short Communications

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1. Lemoine P, Guilleminault C, Alvarez E. Improvement of subjective sleep in major depressive disorder with a novel antidepressant, agomelatine: randomized, double-blind comparison with venlafaxine. *J Clin Psychiatry*. 2007;68:1723-1732.
2. Kennedy S.H, Rizvi S, Fulton K, et al. A double-blind comparison of sexual functioning, antidepressant efficacy and tolerability between agomelatine and venlafaxine-XR. *J Clin Psychopharmacology*. 2008;28(3):329-333.
3. Goodwin G.M, Emsley R, Rembry S, et al. Agomelatine prevents relapse in patients with major depressive disorder without evidence of a discontinuation syndrome: a 24-week randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry*. 2009;70:1128-1137.

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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
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- 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:

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

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From Biopharmaceuticals to the Need for Specialization of Pharmacists

Over the last 30 years, the advances in recombinant DNA and hybridoma technologies have led to the development of many modern biopharmaceuticals. Biopharmaceuticals approved as therapeutic agents to date include blood factors (Factor VIII and Factor IX), thrombolytic agents (tissue plasminogen activator), therapeutic enzymes, hormones (insulin, glucagon, growth hormone, gonadotropins), haemopoietic growth factors (Erythropoeitin, colony stimulating factors), a number of interferons (Interferons-α, -β, -γ) and interleukins (Interleukin-2). Recombinant vaccines (Hepatitis B surface antigen) and many monoclonal antibodies based products are now also available on the market.

Biopharmaceuticals could be produced from microbial cells (e.g. recombinant Escherichia coli or yeast cultures), mammalian cell lines and plant cell culture in bioreactors of various configurations, including photo-bioreactors.[1] Important issues of concern are the cost of production and microbial contamination. It is desirable to produce a low volume, high purity product that is free from contamination by bacteria, viruses, and mycoplasma. In the article "Expression of Therapeutic Recombinant Protein in E. coli: A Major Challenge with Current Insights on the Formation of Inclusion Bodies" in page 75 of this issue, ALQOUQA and CHEUNG pointed out that in the past three decades, recombinant DNA technology has enabled scientist to produce a diverse range of proteins in microorganisms, those that were previously relatively expensive, or difficult to obtain in large quantities. *E. coli* is one of the most widely used host for the efficient and cost-effective and high-level production of heterologous proteins. There are however problems encountered during their expression, with particular to the formation of insoluble inclusion bodies. This article describes some contemporary approaches and strategies for improving the expression of functional and to enhance the solubility of recombinant proteins (RPs) and it is an up-to-date review.

In recent years, there's been an explosion of life-saving treatment advances against breast cancer. Instead of only one or two options, today there's an overwhelming menu of treatment choices that fight the complex mix of cells in each individual cancer. The decisions — surgery, then perhaps radiation, hormonal (anti-estrogen) therapy, and/or chemotherapy can be overwhelming. In many cases, chemotherapy medicines are given in combination, which means two or three different medicines are given at the same time. In early stage breast cancer, standard chemotherapy regimens lower the risk of the cancer coming back. In advanced breast cancer, chemotherapy regimens make the cancer shrink or disappear in about 30-60% of people treated. More recently, greater emphasis has been placed upon development of monoclonal antibody preparations used to detect or treat various cancers.

Starting from two decades ago, scientists have discovered the underlying patterns of breast cancer, marked the beginning of journey in targeted therapy. Overexpression of HER2, a member of human epidermal growth factor receptor, has been identified to be associated with the more aggressive type of breast cancer.[2] Tumor with two-fold or greater amplification of HER-2 gene is typically classified as HER-2 positive, in which account for 20-30% of all breast cancer cases.[3] The overall survival rate and relapse time for HER-2 positive breast cancer patients are significantly shorter than patients without HER2 overexpression.[4] Therefore, HER2 appears to be an attractive and logical target in cancer management. In the article on "New approaches to HER2-positive breast cancer" in page 64, Truda TSOI and Stella LUK write about trastuzumab and pertuzumab, both monoclonal humanized antibodies indicated for treatment of HER2 positive breast cancer, and a variety of agents engineered to target at HER2 as well as its signaling pathways.

With the advances in therapeutics and increase in clinical and public health needs, there is a need for pharmacist to receive continuing education and be specialized in specific areas. This is the approach adopted by many foreign countries in the management of chronic diseases. In the USA, specialty pharmacies are currently operating in areas such as oncology, geriatrics, diabetes, fertility, HIV, psychiatry, nutrition support and pharmacotherapy. Various pharmacy specialty certifications are granted through organizations such as the American Pharmacists Association, the Board of Pharmacy Specialties, the American Society of Health-System Pharmacists, and the American College of Clinical Pharmacy. In Hong Kong, it is apparent that there is a need for the training of specialty pharmacists. The pharmacy departments in the public and private hospitals have also started to provide specialty services in Oncology, Pediatrics, Diabetics, and Geriatrics. The College of Pharmacy Practice was formed in June 2010 to offer specialty training to pharmacists and accreditation of courses and specialists. The College reported its inauguration ceremony held on 22 June 2013 on page 87. Dr. Sophia Chan, Under Secretary of the Health Food Bureau was the key note speaker at the event. In her keynote speech, she acknowledged and supported the need for specialists training. There are many diverse areas that pharmacists are involved, specialty training is required not only in clinical area but also in drug regulatory, quality assurance, pharmacovigilance, etc. As the Hong Kong Drug Office is targeting to join the Pharmaceutical Inspection Convention in 2015, all the local manufacturers have to proceed hand in hand to comply with the Good Manufacturing Practice requirements of the PIC/S Guide to Good Manufacturing Practice for Medicinal Products. It is necessary to train pharmacists specializing in Good Manufacturing Practice and Quality Assurance. With that, we can envision that the local manufacturers of pharmaceutical products can be raised to world renounced standards in the next few years.

As reported on page 82, it is encouraging to read that the Pharmaceutical Society of Hong Kong has led a group of pharmacists and students to visit the 301 Hospital and Novartis Pharmaceuticals in Beijing and to attend the Forbidden City Conference in May 2013. It is a great opportunity for the students and pharmacists to learn about the advanced practices in the famous 301 hospitals and to keep abreast of the pharmacy practice in China and other countries.

Cheng Mary Catherine
Managing Editor
6 August 2013

References

1. Eva L. Decker und Ralf Reski (2008): Current achievements in the production of complex biopharmaceuticals with moss bioreactor. Bioprocess and Biosystems Engineering 31, 3-9. doi: 10.1007/s00449-007-0151-y
2. Tai, Wanyi, Rubi Mahato, and Kun Cheng. "The Role of HER2 in Cancer Therapy and Targeted Drug Delivery." *Journal of Controlled Release* 146.3 (2010): 264-75.
3. Slamon, D., G. Clark, S. Wong, W. Levin, A. Ullrich, and W. McGuire. "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene." *Science* 235.4785 (1987): 177-82.
4. Engel, RH, and VG Kaklamani. "HER2-positive Breast Cancer: Current and Future Treatment Strategies." *Drugs* 67.9 (2007): 1329-341.

Deregistration of Pharmaceutical Products Containing Combination of Corticosteroid and Nonsteroidal Anti-inflammatory Drug

Date: May 2, 2013

On 2 May 2013, DH announced the decision by the Pharmacy and Poisons (Registration of Pharmaceutical Products and Substances: Certification of Clinical Trial/Medicinal Test) Committee of the Pharmacy and Poisons Board to deregister pharmaceutical products containing the combination of corticosteroid and nonsteroidal anti-inflammatory drug (NSAID) with effect from 1 January 2014, because the benefits of the products no longer outweigh their risks. The Committee's decision was made at its meeting on 30 April 2013, after taking into consideration that the risk of gastro-intestinal bleeding and ulceration associated with NSAID would be increased when it is used in combination with corticosteroid and that DH had received local reports of adverse drug reactions related to such combination of drug ingredients. The Committee noted that patients should not stop using the products abruptly as sudden withdrawal of corticosteroid may lead to serious health consequences. Therefore, it decided that the deregistration would take effect on 1 January 2014, to provide a transition period for doctors to switch to alternative treatments for their patients. DH would issue letters to healthcare professionals to inform them of the Committee's decision and related matters.

Source: www.drugoffice.gov.hk

US: Valproate and related products are contraindicated for pregnant women for prevention of migraine headaches

Date: May 6, 2013

On 6 May 2013, FDA advised healthcare professionals that the anti-seizure medication valproate sodium and related products, valproic acid and divalproex sodium, are contraindicated and should not be taken by pregnant women for the prevention of migraine headaches. This safety communication was based on the final results of the Neurodevelopmental Effects of Antiepileptic Drugs (NEAD) study showing that children exposed to valproate products while their mothers were pregnant had decreased IQs at age 6 compared to children exposed to other anti-epileptic drugs. The difference in average IQ between these two groups varied between 8 and 11 points depending on the drug to which valproate was compared. Stronger warnings about use during pregnancy will be added to the drug labels, and valproate's pregnancy category for migraine use will be changed from "D" (the potential benefit of the drug in pregnant women may be acceptable despite its potential risks) to "X" (the risk of use in pregnant women clearly outweighs any possible benefit of the drug). FDA recommended that valproate products should not be used in pregnant women for the prevention of migraine headaches. Valproate products should be used in pregnant women with epilepsy or bipolar disorder only if other treatments have failed to provide adequate symptom control. In Hong Kong, the Registration Committee of the Pharmacy and Poisons Board decided that the package insert of valproate or related products should be updated to include the appropriate safety information. A letter to healthcare professionals was issued on 7 May 2013

Source: www.drugoffice.gov.hk

US: FDA approves label changes for zolpidem products, including new dosing and a recommendation to avoid driving the day after Ambien CR use

Date: May 14, 2013

FDA announced on 14 May 2013 that they had approved label changes specifying new dosing recommendations for all zolpidem products (Ambien, Ambien CR, and Edluar), which are widely prescribed sleep medications. FDA had approved these changes because of the known risk of next-morning impairment with these drugs. FDA also warned that patients who take the sleep medication zolpidem extended-release should not drive or engage in other activities that require complete mental alertness the day after taking the drug because zolpidem levels can remain high enough the next day to impair these activities. This new recommendation has been added to the Warnings and Precautions section of the physician label and to the Patient Medication Guide for zolpidem extended-release (Ambien CR). The new dosing recommendations stated in FDA's January 2013 Drug Safety Communication were also updated.

In Hong Kong, there are 15 registered pharmaceutical products containing zolpidem which include immediate-release 5mg or 10mg tablets and modified-release 6.25mg or 12.5mg tablets. All of them are prescription only medicines indicated for the treatment of insomnia. Zolpidem is also controlled as psychotropic substance internationally including Hong Kong. A letter to healthcare professionals was issued on 11 January 2013 and the concern was reported in Drug News Issue No. 39.

Source: www.drugoffice.gov.hk

Hope For Rosiglitazone

Date: June 10, 2013

Despite lingering concerns about the cardiovascular safety, a joint Food and Drug Administration advisory panel has voted to allow Rosiglitazone (GlaxoSmithKline as Avandia) a come back.

After the publication of a meta-analysis of 42 randomised trials by Nissen & Wolski in 2007, and the subsequent update in 2010 involving 56 trials, concern on the safety, in particular the increased risk in developing myocardial infarction had been raised with rosiglitazone.

In those meta-analyses, none of the trials included was specifically designed in evaluating the cardiovascular outcomes. In the "Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Gycaemia in Diabetes" (RECORD) trial, it was even found that there was no overall increased risk of cardiovascular events in the rosiglitazone arm when compared against a combination of sulfonylurea and metformins.

In 2010, the FDA advisory committee raised the concerns on the reliability and interpretability of the RECORD trial. At the time that the FDA imposed its restrictions on rosiglitazone it also called for an independent readjudication of RECORD. The result of the readjudication supported the original conclusion of the RECORD trial. Kenneth Mahaffey, the institution's associate director reported that none of the hazard ratios in the readjudication were significant, this included cardiovascular death; myocardial infarction; stroke; and all cause mortality.

Despite of the result of the readjudication not reaching statistical significance, panel members expressed the concern that the trial was conducted in an open label design, its use of active comparators, and the many missing data points could potentially mask the risks of rosiglitazone. Some called for a new larger placebo controlled trial, but many doubted that such a trial would be feasible or ethical.

Panel members are calling for the official to clarify the medical guidance, as it is now shifting the burden of the decisiion making in prescribing rosiglitazone to the physicians; some are feeling comfortable with the current restriction as they do not think rosiglitazone has clear evidence in being superior to the other member in the same class [pioglitazone] or the many other choices of drug classes.

Whether rosiglitazone will make a come back, we shall wait and see, but so far, no timetable has been set for the FDA's decision.

Source: http://www.bmj.com

Should E-Cigarette Be Included As A Drug?

Date: June 14, 2013

From 2016, electronic cigarettes and other electronic products containing nicotine are to be regulated as medicines in the United Kingdom. The use of these products have increased significantly in the UK since the implementation of banning smoking in enclosed public places.

An investigation carried out by the Medicines and Healthcare Products Regulatory Agency (MHRA) is the driving force for such move. This investigation involved a public consultation; a review of the MHRA's own existing commissioned research in the quality, safety, marketing, and use of the products; and an impact analysis on the consequences of regulation. The investigation confirmed that there were safety concerns with the e-cigarettes, by which the nicotine level varied from what was labelled, and also from batch to batch.

The new regulation imposed on e-cigarettes means that they cannot be promoted to people younger than 16 years old and that manufacturers are not allowed to design their packaging and flavouring to attract young smokers. This also ensures that their long term safety is monitored. Further research has been planned by the MHRA to determine how the nicotine from the products is delivered into bloodstream.

Source: http://www.bmj.com

Scientists in England Discover the Secret of Spreading of Cancer

Date: June 17, 2013

Scientists have figured out how cancer metastasizes, and they think the findings could help develop drugs to halt the disease's spread. The study, published in Nature Cell Biology, describes a phenomenon researchers call "chase and run," in which cancer cells and healthy cells follow each other through the body. Though healthy cells try to escape, these scientists explains, they produce small molecules, which in turn attract malignant cells to the healthy cells.

Source: Nature Cell Biology, Vol 15 No 8

Olmesartan May Cause Intestinal Problems

Date: July 3, 2013

The Food and Drug Administration (FDA) warned that olmesartan medoxomil, the blood pressure regulating angiotesin receptor blocker can cause intestinal problems known as sprue-like enteropathy. FDA has approved changes to the labels of these drugs to include this warning.

Symptoms of sprue-like enteropathy include severe, chronic diarrhea with substantial weight loss. The enteropathy may develop months to years after starting olmesartan, and sometimes requires hospitalization. If patients taking olmesartan develop these symptoms and no other cause is found, the drug should be discontinued, and therapy with another antihypertensive started. Discontinuation of olmesartan has resulted in clinical improvement of sprue-like enteropathy symptoms in all patients. No other ARBs have been detected to cause sprue-like enteropathy other than olmesartan.

Patients who are currently taking olmesartan-containing product are advised to contact healthcare professional as soon as possible if they experience severe diarrhoea that do not resolve or significant weight loss.

Source: http://www.fda.gov

Pharmacists Still Cannot Be Replaced By Technology

Date: July 3, 2013

It has been long speculated that as automation and information technology advances, pharmacists may one day, be replaced. However, research results seem to disagree. According to a recent randomised clinical trial, published in the Journal of American Medical Association (JAMA) in July 2013, which studied whether an intervention combining home blood pressure telemonitoring with pharmacist case management improves blood pressure control as compared to usual care, and to also study whether the blood pressure control would be maintained when the intervention is stopped. 450 patients with uncontrolled blood pressure were recruited, and are randomly assigned to clinics that provide usual care practice in blood pressure monitoring, versus clinics that also provide telemonitoring intervention. Patients from the intervention arm received home blood pressure telemonitors and transmitted blood pressure data to pharmacists who adjusted anti-hypertensive therapy accordingly. The study measured the control of blood pressure to <140/90 mmHg (<130/80 mmHg in diabetic patients or chronic kidney disease) at 6 and 12 month during the intervention period; and the changes in blood pressure, patient satisfaction and blood pressure control at 18 month (6 month after intervention period).

Blood pressure was controlled at 6 and 12 month was found to be 57.2% (95% CI, 44.8% to 68.7%) in the intervention arm, versus 30.0% (95% CI, 23.2% to 37.8%) in the control group. At 18 month, 71.8% (95% CI, 65% to 77.8%) of patients' blood pressure was under control in the intervention arm, versus 57.1% (95% CI, 51.5% to 62.6%) in the control group. The study concluded that home blood pressure telemonitoring in combination with pharmacist case management improves blood pressure control than usual care practice, and the effect was able to persist during 6 months of postintervention follow-up. Now pharmacists can be reassured that this shall not happen, not yet!

Source: http://jama.jamanetwork.com

New Evidence On Walking

Date: July 3. 2013

Walking has a lot of benefits, especially for patients suffer from peripheral artery disease (PAD). Clinical guidelines state that there is insufficient evidence to support the advising of patients with PAD to actively participate in a home-based walking exercise programme. A study was published in the Journal of American Medical Association (JAMA) in July 2013, in determining whether PAD patients may benefit from home-based walking exercise programme. The study compares 194 subjects, randomised into 2 parallel arms of home-based walking exercise programme, that adopts a group-mediated cognitive behavioural intervention, incorporating group-support and self-regulatory skills; versus a health educational control group, with or without claudication.

Results show that the intervention group performed significantly better in their 6-minute walk distance, maximal treadmill walking time, accelerometer-measured physical activity over 7 days, Walking Impairment Questionnaire distance and speed score. These results could have implication for the large number of patients suffering from PAD, who may be unable or unwilling to participate in supervised exercise programmes, as a home-based walking exercise programme has significant improvement in walking endurance, physical activity, patient-perceived walking endurance & speed.

Source: http://jama.jamanetwork.com

Dual Anti-platelet Therapy For TIA Is The Way To Go?

Date: July 4, 2013

Patients who have had an acute minor stroke or transient ischaemic attack may benefit from dual anti-platelet therapy, says some researchers. According to the CHANCE (Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Event) study, that compared the use of dual anti-platelets (300mg clopidogrel loading followed by 75mg maintenance dose plus aspirin 75mg) versus single anti-platelet (aspirin 75mg plus placebo) in stroke prevention for patients who suffered from acute minor stroke or transient attack, published in the New England Journal of Medicine in July 2013. Results have shown that subsequent stroke occurred within 90 days in 8.2% patients of the dual anti-platelet therapy arm, versus 11.7% (95% CI, 0.57 to 0.81) in the single anti-platelet arm.

Could this have implication in our current practice? Other researchers argued that the study of the use of dual anti-platelet therapy (clopidogrel + aspirin) in patients with acute coronary syndromes reduces the risk of vascular events recurrence by 20% (95% CI, 10 to 28). However, the use of dual anti-platelet therapy also increases the risk of major bleeding by 38% (95% CI, 13 to 67). Some patients who have had a stroke or transient ischaemic attack (TIA) are at high risk in developing early recurrent stroke, mostly due to arterial thromboembolism. Therefore, by adding clopidogrel to the anti-platelet management in patients with acute ischaemic stroke, may increase their bleeding risk. More research is needed before we change our current practice.

Dual anti-platelet therapy for TIA is the way to go? We will just have to wait and see.

Source: http://www.nejm.org/

The Secrets Of Ageing Revealed

Date: July 8, 2013

Scientists have found a chemical "fingerprint", that could provide clues about someone's health at old age, can be revealed by a simple blood test at birth. Researchers have found 22 metabolites that may be valuable indicators in determining the risk of disease at advanced age. The implication of this important finding is that a blood test could provide clues on the ageing process of a person, and opens the door for potential preventive treatment. Study leader Professor Tim Spector from King's College London found that the concentration of the metabolites are higher in older people.

Source: http://ije.oxfordjournals.org/

Omega-3 May Increase Risks Of Prostate Cancer

Date: July 10, 2013

Omega-3 is a popular dietary supplement and a big seller in the market, may increase the risk of high-grade prostate cancer by 71%, scientists found. Taking the supplement sourced from oily fish or cod liver oil, have been linked to a 44% increased risk in developing low-grade prostate cancer. Interestingly, the same risks do not apply to those who eat oily fish such as salmon and tuna.

Some researchers criticised that the study was "controversial" and contradicted much some previous advice. In the study, men with the highest blood level of Omega-3 fatty acids were significantly more likely to be diagnosed with prostate cancer than those with the lowest levels. One of the possibilities of the harmful effect to the body would be the conversion of the fatty acids into harmful chemicals that may damage cells and DNA.

Source: http://jnci.oxfordjournals.org/

Insect Repellent Use and Safety in Children

Date: July 26, 2013

On 26 July 2013, The Centre for Health Protection updated the advise for travellers to prevent mosquito bites. Travellers should wear long-sleeved shirts and trousers, stay in air-conditioned accommodation or rooms with mosquito screens or nets; they should use insect repellents such as those with DEET on exposed skin. If travelling to endemic rural areas, they should carry a portable bed-net with permethrin on it as well as on clothings.

The tips for using insect repellants are:
I. Apply DEET containing insect repellents to exposed parts of the body and clothing, in accordance with product instructions on the label.

II. Avoid using DEET containing insect repellents in infants under 6 months of age. Use alternative measures to prevent mosquito bites.

III. When DEET containing insect repellents are to be used in children: use lower concentration of DEET – up to 10%; do not allow children to handle insect repellents or apply it by themselves; apply to adult's own hands and then put it on children; do not apply to young children's hands, around eyes and mouth, or on cut or irritated skin; do not allow children to breathe in or swallow insect repellents, and do not let insect repellents get into their eyes; limit application to the skin and reinforce application to clothing; wash children's treated skin or bathe them after returning indoors; wash the clothes exposed to insect repellents with soap and water.

Source: http://www.chp.gov.hk/en/content/9/24/25044.html

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Reference: 1. Dexilant prescribing information (DEX0912 PIHK). 2. Wittbrodt ET et al., Clin Exp Gastroenterol 2009;2:117-28. 3. Fass R et al., Aliment Pharmacol Ther 2009;29:1261-72. 4. Sharma P et al., Aliment Pharmacol Ther 2009;29:731-41. 5. Wu MS et al., Aliment Pharmacol Ther 2013;38:190-201 6. Metz DC et al., Aliment Pharmacol Ther 2009;29:742-54. 7. Howden CW et al., Aliment Pharmacol Ther 2009;30:895-907. 8. Lee RD et al., Aliment Pharmacol Ther 2009;29:824-33. 9. Lee RD et al., Aliment Pharmacol Ther 2010;31:1001-11. 10. Frelinger AL et al., J Am coll Cardiol 2012;59:1304-11.

*96% of patient on Dexlansoprazole 60mg achieved 24-h heartburn-free days[6]

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From a Hospital Pharmacist to a Managing Director - An interview with Dr. Grace Lau

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INTRODUCTION

Dr. Grace Lau talks about her career journey from hospital pharmacist, to academic researcher, to Managing Director of Merck Sharp & Dohme (Asia) Limited, Hong Kong.

Dr. Grace Lau

Background

After completing her pharmacy preregistration training in the UK, Dr. Grace Lau returned to Hong Kong and started her career as a dispenser at the Prince of Wales Hospital. Two years later, she was promoted to pharmacist. In the meanwhile, she was offered the opportunity to pursue a PhD degree at the Chinese University of Hong Kong (CUHK). After gaining her PhD, Dr. Grace Lau began teaching at the Department of Clinical Pharmacology and the School of Pharmacy, and continued to conduct research projects at the CUHK. A year later, Dr. Grace Lau joined Merck Sharp & Dohme where she began her journey in the pharmaceutical industry. She has held various positions spanning across the Clinical Research, Regulatory Affairs, Medical Affairs, as well as Sales and Marketing. Recently, she progressed from the position of Managing Director to Zone Leader where she oversees the business operation of Taiwan and Hong Kong.

Besides, Dr. Grace Lau was the founding Managing Editor of the Hong Kong Pharmaceutical Journal. She was appointed a member of the Pharmacy and Poisons Board of Hong Kong for two terms.

PJ: Having worked in different fields, which post do you enjoy the most?

Dr. Grace Lau: There are challenges every time switching to a different post. I think learning and development are very important. For me, when I become familiar with my job and it can be done effortlessly, I'll find it not interesting to continue anymore. I'd reach out of my comfort zone and move forward to attempt a different post. Although it's hard when switching to a new position, I'd set a target for the time I need to settle down in the new environment; then follow my own direction and gain support to achieve my goals.

PJ: How did your past experience help you with your current position or future direction?

Dr. Grace Lau: You can't accomplish your goal within a day. You need to have both short-term and long-term planning. During the process you will work together with your manager for your career aspiration and personal development plan; and at the same time you need to meet current position's requirement and expectations.

PJ: Does having a professional pharmacy background assist your career in the industry?

Dr. Grace Lau: Definitely yes, especially in the initial stage. Having a pharmacy background, you'd know how a drug is being invented; you'd recognize the value of the drug to patients and physicians as well as its impact to the world. Working in the industry, you are indeed providing a treatment option to patients so that they can receive proper treatment. Unlike in other consumer industries like papers or cosmetics, in the pharma industry you have a mission in making a drug accessible to patients. Same as the role of a pharmacist, you aim to improve patients' health.

PJ: So far what is your biggest achievement from work?

Dr. Grace Lau: I like to keep moving forward and take risk. I don't like working in a stable position. After achieving my goal in few years time, I'd move forward and progress to a new area. For me, I'd see this as a kind of achievement.

PJ: Have you encountered any challenges at work?

Dr. Grace Lau: There are challenges each time when you pick up a new role. You have to encourage yourself to step out of the area that you are already very used to. Challenges include adapting to a new environment, finding difficult to complete the new task and working with a new manager etc. Nonetheless, if you can easily pick up your new job, I'd say the job is not challenging enough for you.

PJ: What advice would you offer to a newly registered pharmacist?

Dr. Grace Lau: As far as I know, many newly registered pharmacists aim to become a clinical pharmacist or a hospital pharmacist. As I majored in clinical pharmacy during my undergraduate study, I personally also wanted to be a clinical pharmacist at the time I graduated, hoping to offer direct patient care in hospital setting. However, I find that the duty of a hospital pharmacist might not be so clinically related in some hospitals. Therefore, my sharing to newly graduates is that: don't feel that you've left your professional knowledge behind once you're not doing direct patient care. Even if you are working in another field, you still have lots of opportunities to use your professional pharmacy skills through indirect patient care.

PJ: Do you have anything that you'd like to share with the readers?

Dr. Grace Lau: Continuous learning is very important. Don't stop at the point after you've mastered a skill. Keep improving and think of how you can further develop your skills and knowledge just like you are filling a glass with water which will never be fully filled. Develop a learning attitude and always seek for self-growth and development.

CONCLUSION

With the courage to take risk and reach out of her comfort zone, Dr. Grace Lau has shown us that a pharmacist can enjoy wonderful career flexibility.

Author's background

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References:

1. EPAR for TevaGrastim® (European Public Assessment Report. Ref:EMEA/503293/2008).
2. H. Lubenau, P. Bias, A. Maly, K. Siegler, K. Mehltretter. Pharmacokinetic and pharmacodynamic profile of new biosimilar filgrastim XM02 equivalent to marketed filgrastim Neupogen®: single-blind, randomised, cross-over trial. BioDrugs 2009; 23(1): 43-51.
3. Engert A, del Giglio A, Bias P, Lubenau H, Gatzemeier U, Heigener D. Incidence of febrile neutropenia and myelotoxicity of chemotherapy: A meta-analysis of biosimilar G-CSF studies in breast cancer, lung cancer, and Non-Hodgkin-Lymphoma. Onkologie, submitted.

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New approaches to HER2-positive breast cancer

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ABSTRACT

Breast cancer is a devastating disease, it slowly engulfs one's health and appearance, and eventually one's dignity. Starting from two decades ago, scientists have discovered the underlying patterns of breast cancer, marked the beginning of journey in targeted therapy. Overexpression of HER2, a member of human epidermal growth factor receptor, has been identified to be associated with the more aggressive type of breast cancer.[1] Tumor with two-fold or greater amplification of HER-2 gene is typically classified as HER-2 positive, in which account for 20-30% of all breast cancer cases.[2] The overall survival rate and relapse time for HER-2 positive breast cancer patients are significantly shorter than patients without HER2 overexpression.[3] Therefore, HER2 appears to be an attractive and logical target in cancer management. In 1998, the birth of trastuzumab, a monoclonal humanized antibody indicated for treatment of HER2 positive breast cancer, lightened up the world by marking a new page on oncogene-targeted therapy. It is not yet an ultimate curative measure, but merely a start. A variety of agents have been engineered to target at HER2 as well as its signaling pathways ever since. On the way to the light, the profile of HER2 as well as its interactions with other HER family have become clearer. Pertuzumab a new born humanized monoclonal antibody against HER2 uprisen to provide better coverage to the limitations of trastuzumab. On the same track, a variety of novel agents targeting HER2 are getting in shape with promising clinical performance. Their modes of action are also discussed.

Keywords: *HER2; breast cancer; trastuzumab resistance; pertuzumab; T-DM1; afatinib; neratinib*

INTRODUCTION

What is HER2 positive breast cancer?

HER2-positive breast cancer is a type of breast cancer overexpressing a protein called human epidermal growth factor receptor 2 (HER2), usually 2 to 3 fold of normal value, which promotes the growth of cancer cells.[2] Approximately 1 in 5 of every breast cancer patients exhibit elevated HER2 level as a result of gene mutation of HER2/neu proto-oncogene.[2,4] As HER2 is important in promoting cell survival and growth, consequently excessive HER2 will associate with malignant transformation, driving undesired aggressive tumor growth in breast, ovarian, gastric, prostate and other cancers by enhancing and prolonging intracellular signals.[5] This is a gene mutation that occurs only in the cancer cells and is not a type of mutation that can be inherited from a parent.

REVISIT THE ROLE OF HER

HER (human epidermal growth factor receptor) superfamily (including HER1/ ErbB1, HER3/ErbB3 and HER4/ErbB1) plays essential roles in cell growth, survival, and differentiation in intrigued fashion.[6] Each family receptor is composed of an extracellular domain allowing ligand binding to occur, an alpha-helical transmembrane segment and an intracellular protein tyrosine kinase domain (Fig. 1).[7] Receptor dimerization (pairing) is a fundamental requirement for HER function and in turn, initiating activities of these receptors through a complex and tightly controlled array of signaling pathways[8] that drive and regulate many cellular functions, including cell proliferation, and organ development and repair.

Dimerization can occur between two receptors of the same subtype (heterodimerization) or between two receptors of different subtype (homodimerization).[7] Except HER2, HER members normally exist as inactive monomers with the molecules folded in such a way as to prevent dimerization.[9,10] Ligand binding to the extracellular domain initiates a conformational rearrangement by 'opening' up the dimerization domain that forms the core of the dimer interface with another receptor.[11] Thus, binding of growth factors help stabilize the 'open conformation' favoring dimerization.[11] As the story flows, tyrosine kinase segment of the dimer moiety is then transactivated its partner via phosphorylation, which subsequently initiate recruitment and activation of downstream protein, leading to signaling cascade.[7]

The functional activity of each HER member is distinctive, although they all possess the same essential domains. To differentiate, HER1/EGFR and HER4 have active tyrosine kinase domains and known yet different ligands; HER3 can bind to several ligands but intrinsic tyrosine activation is not available; versus HER2 possesses an active tyrosine domain but no direct ligand has been identified.[6]

Structurally interesting feature of HER2 is that it constantly exists in the extended (open) 'active' conformation state, rendering HER2 constitutively available for dimerization.[10] To achieve active conformation, subdomains I and III, the ligand binding sites, are sacrificed for readily available dimerization (Fig. 1). By contrast, HER3 relies on other member for valid pathway signaling, meaning they only form the heterodimers.[12] The identities of the activation ligand and the heterodimer partner are the most crucial factors in determining which of the

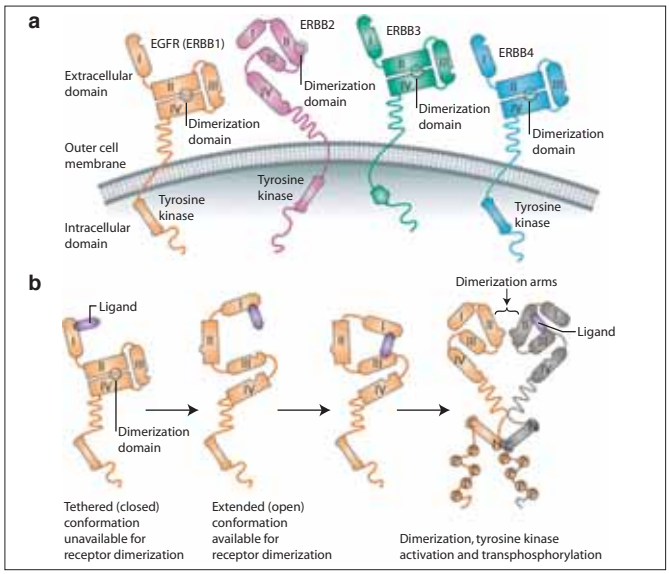

Figure 1.a: *Human Epidermal growth factor (HER) family is composed of four receptors with three functional domains: an extracellular domain responsible for ligand binding; the α-helical transmembrane segment; and the intracellular protein tyrosine kinase domain that also contains motifs and residues that mediate interactions with intracellular signaling molecules. As depicted, HER3 and HER4 exist in a tethered ('closed') conformation in which the dimerization domain is enclosed and unavailable to interact with other receptor moieties without being stabilized by ligand. In contrast, HER2 receptor, with no known identified ligand exists in an active extended ('open') conformation and is permanently available for dimerization.*
b: *Upon ligand binding, a conformational change is included unfolding the dimerization subdomain allowing dimer formation and functional activation of HER receptors. The kinase domain interaction is asymmetric, with the amino-terminal lobe of one tyrosine kinase interacting with the carboxy-terminal lobe of the other.*

vast array of downstream adaptor proteins is engaged and, therefore, which pathway is activated.

DISCUSSION

HER2 - HER3 dimers and PI3K –Akt pathway

To highlight, HER2-HER3 heterodimer is regarded as the most potent oncogenic unit in terms of strength of interaction, ligand-induced tyrosine phosphorylation and downstream signalling and cell survival benefits.[13,14] The significance of this oncogenic unit is that HER2-mediated signalling pathways are responsible for cell survival and proliferation, whereas HER3-mediated signaling pathways are responsible for anti-apoptosis on top of survival.[6] It has been frequently reported that HER2 can translocate to the nucleus as intact, spliced or even with receptor ligands, possibly acting as transcription factors for genes such as Cycline D1, COX2 and p53.[1] Mostly substantiated, nuclear HER2 activates the COX 2 gene promotor, which in turn up-regulates the COX 2 expression in tumor cells that contributes to increased antiapoptotic, proangiogenic, and metastatic potential in cancer cells. What's more, HER2 involved dimers signal more potent transduction pathway among all dimmers formed by the HER family.[15] Such features make HER2 important in participating cell growth, survival, and differentiation, explaining why it has been an appealing target in pharmacological therapy.

Along the same vein, it seems that cancer cells attempt to engage HER3 because of its unique ability to initiate signalling through the PI3K-Akt pathway,[16,17,18] which is particularly important in promoting tumour cell survival and anti-apoptosis. Support from preclinical experiences, HER3 is as crucial as HER2 in maintaining cell proliferation in breast cancer cells that overexpress HER2.[17] In three-dimensional culture and in xenograft breast cancer models, abolishing HER3 function results in an approximate 50% reduction in Akt phosphorylation, followed by rapid tumour regression. In addition, in cells that rely on the HER3 and HER4 ligand heregulin, blocking HER2-HER3 dimerization with HER2 dimerization inhibitor, pertuzumab, demonstrated to inhibit ligand-induced morphogenesis in a three-dimensional culture model and tumor regression in a xenograft model. These effects are not observed upon exposure to trastuzumab, the HER2 subdomain IV targeted antibody.[17] The results hinted the weakness of trastuzumab in anti-tumour activity and how pertuzumab can fill the gap.

Speaking of PI3K pathway, it subsequently activates Akt which then activates mTOR.[6] This intracellular signalling pathway is pivotal in apoptosis and thus cell proliferation, suggesting the significance of blocking HER3 from forming dimerization. Although HER1/EGFR and HER2 can interact with and activate PI3K through the adaptor proteins GRB2 (growth factor receptor bound 2) and GAB1 (GRB2-associated binding protein1), HER3 and HER4 possess direct yet multiple binding sites for the p85 subunit of PI3K, allowing stronger activation of PI3K and its downstream signalling components.[19,20,21]

Through direct activation of PI3K-Akt pathway initiated from HER3, it may underpin the mitogenic potency of the HER2-HER3 dimer, implying the novel therapeutic strategy in pinning HER2 positive cancers.

Targeted therapies in HER2 positive breast cancers

Two targeted therapies have been employed in treating HER2 positive breast cancer patients, trastuzumab the anti HER2 monoclonal antibody and lapatinib the reversible tyrosine kinase inhibitor against HER2 and EGFR/HER1. Trastuzumab is the standard of care, across neoadjuvant, adjuvant and metastatic setting in combination of chemotherapy. In metastatic breast cancer, lapatinib is used in combination with capecitabine when the tumour is not responding to trastuzumab, i.e. trastuzumab resistance. As an orally active small molecule, it is useful against brain metastasis.

Challenges in face

Upon addition of trastuzumab to chemotherapy in HER2-positive early breast cancer, it unleashes patients from suffers with prominent prolongation of disease-free survival.[22] Unfortunately, merely 30% of patients with HER2 overexpressed can benefit from this therapeutic, the rest exhibit either intrinsic or secondary (acquired) resistance to trastuzumab.[23] Similar story, not all HER2 positive tumors respond to lapatinib, and resistance to lapatinib also develops in some patients and/or tumors upon long-term exposure to the drug. Depressing clinical outcomes urge the need to develop new therapeutic agents in response. The mechanisms of trastuzumab and lapatinib resistance are the keys in developing novel anti-HER2 strategies.

The most substantiated mechanisms explaining trastuzumab resistance are outlined as follows. First, truncated HER2 receptors, arisen from either through proteolytic cleavage of its extracellular domain alternative translation-initiation sites of the HER2 protein, are well-known reason that contributes

to the resistance due to loss of trastuzumab-binding site.[23] Second, abnormal activation of downstream signaling pathways due to genetic mutation disrupts cell control. Aberrant PI3k-Akt pathway, the most prevalent example found in breast cancer, contributes to cell proliferation, hence tumorigenesis.[24,25] Thirdly, in the case of the membrane-associated glycoprotein muscin-4 (MUC4) overexpression, the binding between trastuzumab and HER2 subdomain IV is disturbed by MUC4 via steric hindrance. Restoration of trastuzumab binding upon MUC4 siRNA knockdown supported this theory[i]. In the same vein, alternative signalling pathways, namely IGF1R and HER3 downstream signalling, may be activated in response to overcome trastuzumab-HER2 inhibition.[27] In fact, aberrant activation of IGF1R signaling was the first mechanism of trastuzumab resistance to be identified.[28] More importantly, HER3-mediated PI3k-Akt pathway was shown unregulated when treating HER2-positive breast cancer cell lines with tyrosine kinase inhibitors[ii]. As a kinase-inactive receptor, this provides an escape from the targeted therapy. The hypothesis of HER3 being the pivotal mediator is further substantiated by the evidence that pertuzumab, the HER2 dimerization inhibitor, exhibited activity against breast cancer cells resistance to trastuzumab through potent inhibition of HER3 ligand-induced dimerization.[6] Some studies have hypothesized that PTEN deletion and activating mutations of PI3KCA, the gene encoding the p110 catalytic subunit of PI3k, also play major roles in determining tumour inhibition by trastuzumab.[30] Preclinical data showed that reducing PTEN in breast cancer cells by antisense oligonucleotides conferred trastuzumab resistance in vitro and in vivo. Patients with PTEN-deficient breast cancers had significantly poorer responses to trastuzumab-based therapy than those with normal PTEN.[31] Thus, PTEN deficiency is a powerful predictor for trastuzumab resistance. The evidence that PI3K/Akt/mTOR inhibitors[31] rescued PTEN deficiency-induced trastuzumab resistance underpinned such hypothesis.[31]

Clearer picture on the trastuzumab resistance mechanisms has provided scientists directions in engineering new therapeutic weapons. There have been several agents emerging in response to the new discoveries, the upcoming ones include pertuzumab, T-DM1, afatinib, neratinib, everolimus and IGF-1R inhibitors. In highlight, pertuzumab is already approved by FDA and getting ready to be launched in Hong Kong. Thus, details of its profile will be discussed, with key features outlined in table 1.

Table 1. Pertuzumab - At a Glance

Featured indication	
First-line treatment of HER2-positive metastatic breast cancer in combination with trastuzumab and docetaxel	
Mechanism of action	
Prevents dimerization of HER2 with other ligand-activated HER receptors, inhibiting the activation of intracellular signaling pathways associated with the proliferation and survival of cancer cells. Stimulates antibody-dependent cell-mediated cytotoxicity.	
Pertuzumab regimen in the CLEOPATRA trial	
Loading dose	840mg
Maintenance dosage	420mg every 3 wk
Route of administration	Intravenous infusion
Pharmacokinetic profile (420mg in pts with advanced solid tumours)	
Mean maximum serum concentration	150 µg/mL
Mean AUC	2762 µg * h/mL
Mean volume of distribution at steady state	4233 mL
Mean clearance	169 mL/day
Mean elimination half-life	19.10 h
Adverse events (all grades) occurring in ≥5% more	
Diarrhea, rash, mucosal inflammation, febrile neutropenia, dry skin	

EMERGING APPROACH

Pertuzumab (Perjeta®, Roche/Genentech)

Pertuzumab is a humanized monoclonal antibody that binds to an epitope in subdomain II, the dimerization domain of HER2[iii]. Different from Trastuzumab, it targets subdomain IV without interfering dimerization.[33] Having known for long, HER2 is special for its open conformation, which is readily available for dimerization.[34] By inhibiting this site, the subsequent intracellular signaling cascade will not be activated, minimizing cancer cell proliferation and survival.

How is it different from trastuzumab?

These two monoclonal antibodies, trastuzumab and pertuzumab, both targeting at Her2 are proposed to inhibit intracellular signaling pathway by hindering dimerization.[35] Later learned, dimerization involves interaction between receptors in their open-conformation state.[36] In HER family, with exception of HER2, ligand-binding is required to transactivate the dimerization domain from closed to open. In fact, trastuzumab inhibits ligand-independent HER 2-mediated signaling, which is activated through association between HER members without dimerization. Slightly different, pertuzumab inhibits ligand-dependent HER2-mediated signaling, which is activated through dimerization between HER members signaling more long lasting and prominent effect.

In preclinical studies, pertuzumab showed significant activity, both in vitro and in vivo, against several breast cancer cells expressing HER ligands, regardless of HER2 expression levels.[37,38] Similar activities were also shown in other human tumour cells, in vitro and in vivo (in human xenograft models), expressing different levels of HER2, ranging from ovarian,[39] prostate,[40] lung[iv] and colorectal cancer cell.[42]

Its ability to interfere cells with lower levels of HER2 expression is proposed to be due to antagonism of the ligand-dependent activation of HER2 through HER3 or HER1/EGFR, which requires the presence of an HER3 or EGFR ligand but not necessarily HER2 overexpression.[6] Unfortunately, such preclinical success does not appear to be as promising in clinical situation, in which limited activity was shown against patients with metastatic breast cancer (MBC) whose tumors expressed low levels of HER2.[43] Yet, the combination of pertuzumab with trastuzumab has been shown to synergistically inhibit the survival of breast tumor cells in patients with HER-2 positive breast cancer.[44]

EFFICACY

Dual anti-HER2 targeted therapy for metastatic breast cancer

Dual inhibition with pertuzumab and trastuzumab has reached an advanced page of development in combination with chemotherapy, yielding optimistic results. A randomized phase III study (CLEOPATRA)[45] evaluated the benefit of adding pertuzumab to the current standard of care, combination of trastuzumab and docetaxel, in previously untreated HER2 positive metastatic breast cancer. Worth-notice is the median PFS (progression free survival) was found to be 18.5 months when treated with three agents

(Pertuzumab group) versus 12.4 months in the control group (trastuzumab and chemotherapy only). The ORR (objective response rate) was found higher in pertuzumab group than the control group (80.2% vs. 69.3%). Less deaths occurred in the pertuzumab group than in the control group [69 (17.2%) vs96 (23.6%)]. These clinical results suggests dual anti-HER2 agents (trastuzumab and pertuzumab) with complementary mechanisms of action offer more comprehensive blockade than treatment with either antibody alone and further evidence the importance of preventing both ligand-independent and ligand-dependent HER2 dimers from forming to turn-off HER2 signaling to the greatest extent possible.

Dual anti-HER2 targeted therapy for the neoadjuvant setting

Three published clinical trials have explored the significance of pertuzumab as an adjunct to trastuzumab in neoadjuvant therapeutic setting on HER2-positive breast. (table 2)[46,47,48]

A phase II Neoadjuvant Study (NeoSphere)[46] evaluated four different combination regimens among pertuzumab, trastuzumab and docetaxel. Patients were randomly assigned to 1 of 4 regimens: trastuzumab and docetaxel (group A); pertuzumab, trastuzumab and docetaxel (group B); pertuzumab and trastuzumab (group C); and pertuzumab and docetaxel (group D). Focusing on the pathologic complete response (pCR), patients in groups B had demonstrated the highest pCR rate among the other groups (45.8% vs A: 29%, C: 16.8%, D: 24.0%). From this clinical trial, ER-negative patients were the most sensitive to dual anti-HER2. Interestingly, dual anti-HER2 therapy alone yielded pCR in almost 17% of patients. This arm hints the potential power of dual anti-HER2 agents in inducing regression.

SAFETY PROFILE

As a single agent, pertuzumab is generally well tolerated. Concluding from two phase I studies, the most frequently reported adverse events (AEs) included asthenia, nausea, vomiting, diarrhea and rash, with the majority being NCI-CTC grade 1 or 2.[49,50] When combining with trastuzumab for the treatment of advanced or metastatic breast cancer, no significant difference to the toxicity profile depicted above was noticed.[51,52]

The phase II Neoadjuvant Study (NeoSphere) mentioned earlier revealed the toxicity profiles in combination regimens. Regimens incorporating with chemotherapy, i.e. group A, B and D, showed similar toxicity profiles, including alopecia, neutropenia, diarrhea, nausea, fatigue, rash and mucosal inflammation, with occurring rate approximately 7-8%. By contrast, the group treated with dual anti-HER2 alone experienced no alopecia, neutropenia nor mucosal inflammation, implying these are docetaxel-related. All in all, these clinical experiences suggest pertuzumab is generally well-tolerated.[53]

Cardiotoxicity

Trastuzumab is well known to induce some degree of cardiotoxicity, in which is attributable to its inhibition of HER2–mediated signaling pathway in heart.[54] Pharmacologically speaking, pertuzumab is presumed to exert similar effect on heart.

The lack-of-thought of whether pertuzumab induces cardiotoxicity, and induces additive risk of cardiotoxicity when combining with trastuzumab therapy, was addressed throughout the pertuzumab clinical trials. During phase I and II monotherapy studies, pertuzumab elicited modest cardiotoxicity similar to that of trastuzumab.[53] Results from early phase II studies had arisen concern for additive cardiotoxicity. Surprisingly, under appropriate monitoring, larger phase III trials (CLEOPATRA) demonstrated no increase in the risk of cardiotoxicity compared with using trastuzumab alone.[45] Such findings further provide confidence in safety applying to real life treatment situation.

NOVEL HER2 TARGETED THERAPEUTIC STRATEGY (Fig.2)

Trastuzumab emtansine/T-DM1 (Kadcyla®, Roche/Genentech)

In early 2013, another novel agent received approval from US FDA - Trastuzumab-mertansine (DM1) (Trastuzumab emtansine; Genentech) pushes the development forward in drug delivery. It is an antibody-drug conjugate (ADC), in which takes advantage of the specificity of antibody to lead the genuine 'bullet' to the intended sites. T-DM1 is integrated by three parts, namely trastuzumab, the monoclonal antibody is conjugated with DM1, an antimicrotubule cytotoxic agent, joined by a nonreducible thioether linkage named MMC (maleimidomethylcyclohexane-1-carboxylate).[55] T-DM1 demonstrated significant improvement in survival among HER2 positive metastatic breast cancer patients comparing with lapatinib and capecitabine combination therapy in a newly released Phase III EMILIA study results.[56]

T-DM1 preferentially delivers highly potent cytotoxic agents to tumor cells through the high specificity against

Table 2. Pathologic complete response (pCR) rates with anti=HER2 therapy in the neoadjuvant setting

Trial	Taxane + trastuzumab (%)	Taxane + pertuzumab (%)	Taxane + lapatinib (%)	Taxane + trastuzmab + pertuzmab (%)	Taxane + trastuzmab + lapatinib (%)	Trastuzumab + pertuzmab (%)
NeoSphere						
Overall	29	24	-	46	-	17
ER+	20	17	-	26	-	6
ER-	37	30	-	63	-	27
NeoALTTO						
Overall	30	-	25	-	51	-
ER+	23	-	16	-	42	-
ER-	27	-	34	-	61	-
CHER-LOB						
Overall	25	-	26.3	-	46.7	-
ER+	25	-	22.7	-	35.7	-
ER-	26.6	-	35.7	-	56.2	-

† ER = estrogen receptor, NeoSPhere Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation. NeoALTTO Neoadjuvant Laptinib and/or Trastuzumab Optimization trial. CHER-LOB Chemotherapy Herceptin and Lapatinib in Operable Breast Cancer

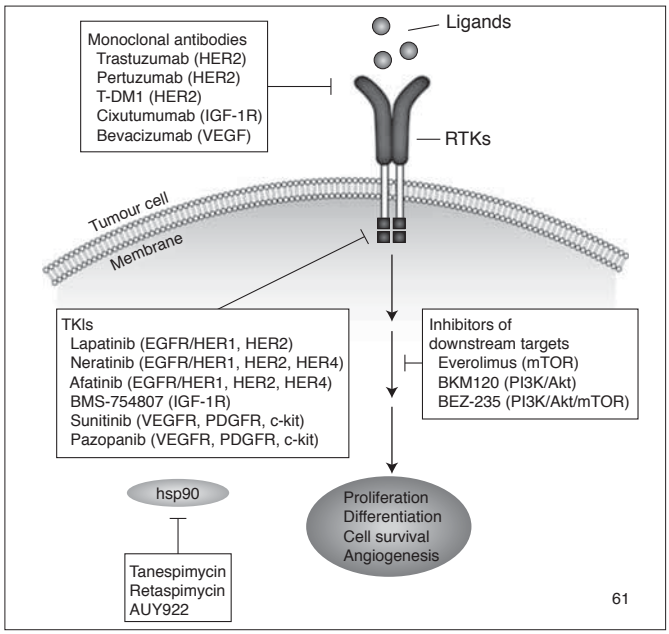

Figure 2: *Targeted agents for HER2-positive MBC. Diagram illustrating the molecular targets of approved and investigationalagents for HER2-positive MBC. Abbreviations: Akt, protein kinase B; c-kit, stem cell factor receptor; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; hsp90, heat shock protein 90; IGF-1R, insulin-like growth factor-1 receptor; MBC, metastatic breast cancer; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide-3-kinase; RTK, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.*

overexpressed cell surface antigens upon internalization of trastuzumab, the cytotoxic agent is thus merely released intracellularly. Along with increasing cytotoxic drug delivery to tumor, it can significantly reduce normal tissue drug exposure, hence, better safety profile.[6,57] Selection of linker is one of the critical considerations involved for successful development of ADC, as it needs to be stable enough to deliver ADC to the target site and release upon the site at right time. In other words, it plays pivotal role in pharmacokinetic activity.

With better safety and efficacy proven in clinical trials, it is suggested to be used as front line therapy for metastatic breast cancer treatment. In this trend, combination of T-DM1 and pertuzumab, without additional cytotoxic agent, is likely to be a promising regimen for better blockade without harming the healthy cells when tackling HER2 positive carcinomas.

HER FAMILY TKIs

Afatinib (Tomtovok®, Boehringer Ingelheim)

Afatinib, an anilino-quinazoline-derieved oral small molecule ErbB family TKI behaves similarly as lapatinib targeting the same sites - EGFR/HER1, HER2 and HER3, yet in irreversible manner.[58,59] It has also exhibited activity in early-phase trials of advanced solid tumors and trastuzumab-refractory HER2-positive breast cancer.

In preclinical studies, it illustrated high potency against EGFR, HER2 and HER4 kinase at low nanomolar concentration and anti-proliferative effects in trastuzumab-resistant HER2 overexpressing cell lines and promising results in phase I development.[60,61] An open label, single-arm phase II study of afatinib in 41 heavily pretreated patients with HER-2 positive MBC following trastuzumab failure yielded 12% response rate, including partial responses in 4 patients and stable disease in 8 patients (maintained for at least 4 cycles).[62]

It shares similar safety profile with lapatinib, gastrointestinal (diarrhea and nausea/vomiting) and dermatologic AEs (rash and dry skin), as well as fatigue, were most common.[59] At a dose of 50mg once daily, grade 3 rash (9.8%) and diarrhoea (22%) were most common.[58] Recently, a global ongoing phase III randomized trial of afatinib (NCT01125566) is evaluating the efficacy of vinorelbine/afatinib v.s. Vinoelbine/trastuzumab in HER2-positive MBC patients following trastuzumab failure.

Neratinib

Neratinib is an irreversible oral small-molecule TKI targeting EGFR/HER1, HER2 and HER4. In preclinical HER2 overexpressing models, anti-proliferative effects along with G1 cell-cycle arrest and decreased downstream signal transduction were observed.[63] In a phase I study of neratinib in advanced HER2-positive breast cancer populations pretreated with trastuzumab, anthracyclines and taxanes, partial response was demonstrated in 8 out of 25 (32%) patients.[64] An open-label, phase II multicenter trials of neratinib as monotherapy in advanced HER2 positive breast cancer, which recruited 66 trastuzumab-refractory and 70 trastuzumab-naive patients, yielded modest clinical activity in both cohorts. Objective response rates of 24% and 56% were seen in the trastuzumab-refractory and trastuzumab-naive groups respectively, with a median pFS of 22.3 and 39.6 weeks.[65]

Similarly as other TKIs, diarrhea was regarded as the dose-limiting toxicity, grade 3/4 at dose of 240mg once daily[66] and 320mg as the maximum tolerated dose.[64] Up to 30% of patients experienced it and required dose reductions and/or symptomatic management.[66] Yet, no cases of grade 3 or 4 cardiotoxicity were observed. Currently, studies of single-agent neratinib (neratinib vs lapatinib/capectabine, NCT00777101) and neratinib combinations (with capecitabine, NCT00445458; trastuzumab, NCT00398567; paclitaxel, NCT00445458; vinorelbine, NCT00706030; and neratinib/paclitaxel vs trastuzumab/paclitaxel, NCT00915018) are under investigation as in HER2 positive MBC.[67] The clinical relevance of neratinib as a 'pan-HER' family inhibitor and it being irreversible is yet to be explored.

PI3K/AKT/mTOR PATHWAY MODULATION

PI3k/mTOR pathway is often dysregulated in breast cancer, with higher frequency in ER- positive and HER2 positive subtypes,[68] as an alternative oncogenic pathway that may underpin trastuzumab resistance. Preclinical studies with PI3k/Akt inhibitors illustrated increased anti-tumour activity in patients with PIK3CA mutations,[69] identified in approximately 20 to 30% of HER2-positive breast cancers.[70,71] Modulation of the PI3K/Akt/mTOR pathway is the most advanced strategy to tackle trastuzumab resistance, in which Everolimus appears to be the most encouraging agent in this area among the class.

Everolimus (Afinitor®, Novartis)

Everolimus, the mTOR inhibitor, has been approved for a few indications in treating various cancers.[72] It is now being evaluated its value in HER2 positive breast cancer, with or without chemotherapy. A recently published phase I/II study

evaluated the chemotherapy-free combination of everolimus and trastuzumab in heavily pretreated HER2-positive MBC patients with prior trastuzumab exposure, which demonstrated ORR of 15%, CRR of 34% and median PFS of 4.1 months.[73] More assuring results yielded when combining with chemotherapy and trastuzumab, evidenced by a phase II multicenter study in heavily pre-treated HER2 positive metastatic breast cancer patients with prior trastuzumab and taxane exposure, in which an ORR of 25%, stable disease (SD) rate of 56% were observed along with acceptable safety profile.[74]

Safety wise, combining with paclitaxel and trastuzumab at the established dose of 10mg per day in MBC patients, grade 3/4 neutropenia were the most common AEs (52%), whereas combining with vinorelbine and trastuzumab, neutropenia (92%) and stomatitis (70%) were the most common toxicities.[71]

Based on these promising early-phase clinical data in HER2-positive MBC, phase-III trials of trastuzumab/ paclitaxel ± everolimus in the first line setting (BOLERO1) and trastuzumab/ vinorelbine ± everolimus in the trastuzumab and taxane refractory setting are under investigation (BOLERO-3; NCT00876395, NCT01007943). The combination of everolimus and lapatinib is also currently being investigated in a phase II study of HER2-positive metastatic breast cancer patients with prior trastuzumab exposure (NCT01283789). The possibility of mTOR inhibitors, deforolimus (AP23573), temsirolimus (CCI779), and PI3K inhibitor, BKM-120, are still under investigation for its potential therapeutic use in HER2 positive breast cancer.

IGF-1R INHIBITORS

As discussed, crosstalk between HER2 and IGF-1R signalling pathways has also been implicated as one of the mechanisms of trastuzumab resistance. This theory is supported by preclinical studies, in which trastuzumab sensitivity is restored by disrupting IGF-1R/HER2 heterodimer using the human anti-IGF-1R antibody, CP-751871[75] and small molecule selective IGF-1R TKI, NVP-AEW541,[76] in models of trastuzumab-resistant, HER2-positive breast cancer. These agents were well-tolerated in general, yet with toxicities of thrombocytopenia and hyperglycemia identified.[77]

To highlight, two IGF-1R inhibitors, Cixutumumab (IMC-A12) and BMS-754807, are currently being further explored in combination with existing HER2-targeted agents in this area.

HEAT-SHOCK PROTEIN INHIBITORS

Heat shock proteins (HSPs) are essential in facilitating conformational maturation and folding of a variety of signaling proteins, including HER2.[6] To be specific, it apparently controls the stability of HER2 as well as its downstream signaling components, namely RAF1 and AKT1.[78] Without proper function of this protein, ubiquitylation and proteasomal degradation of both HER2 and its downstream signaling counterparts are in consequence, hence abolishing the activity of pathways mediated by PI3K, Akt and cyclin D.[79] HSP inhibitors also offer the potential to counter trastuzumab resistance, including truncated p95HER2.[80]

The significance of HSP arises a great interest among scientist to explore its possibility as a therapeutic agent. Its potential is supported by a preclinical study, in which the HSP90 inhibitor tanespimycin (17-AAG), an ansamycin, induced HER2 degradation, growth arrest and apoptosis.[81] It as well translated into promising clinical efficacy with good tolerability in combination with trastuzumab in a phase II study conducted in advanced trastuzumab-refractory HER2-positive breast cancer, yielding an ORR of 24%, CBR of 59%, median PFS of 6 months and median OS of 17months.[82] Encouraging data of tanespimycin warrants further evaluation in phase III clinical trials. It would be of great interest to explore whether trastuzumab is mandatory to obtain maximal clinical efficacy.

Another HSP90 inhibitor, AUY922, is currently under investigated its clinical performance in combination with trastuzumab among patients with trastuzumab-refractory HER2-positive metastatic breast cancer in a phase I/II study (NCT01271920).

BISPECIFIC ANTIBODIES

To further invent strategies against trastuzumab resistance, a new class of agent known as trifunctional bispecific antibody is being developed. MM-111 is another antibody targeting both the HER2/HER3 heterodimer and the HER3 ligand, herogulin. Its efficacy upon combination with trastuzumab in HER2 overexpressing metastatic breast cancer patients with prior trastuzumab exposure is currently underway as phase study (NCT01097460).[83]

FUTURE DIRECTION-NOVELCHEMOTHERAPY-FREE ANTI-HER2 COMBINATIONS

Combinations of novel anti-HER2 agents have been heavily explored, its value is not merely rooted on providing better side effect profile and overcoming trastuzumab resistance by blocking various HER members and their subsequent signalling pathways, but also revealed chemotherapy-free potential by discovering the synergistic effects. The most frequently quoted clinical example is the combination of trastuzumab and lapatinib. Sticking to trastuzumab as the backbone, there have been multifarious dual anti-HER2 therapies being explored. The most promising combination by far is pertuzumab and trastuzumab. Neratinib combined with trastuzumab has also demonstrated similarly promising efficacy. Last but not least, the combination of an mTOR and novel HER-family TKI is worthy of anticipation in due to its strong preclinical rationale and promising early phase activity in tackling trastuzumab resistance.[84] We may keep a close eye on the upcoming novel agents. (Table 3)

LIMITATIONS

Whether drug combination is the key to success remains a challenge. During the course of tumour development, multiple genetic alterations rise that contributes to the process linked to metastatic cancer. Aberrantly activated HER receptors contributed to many of the processes. However, it is very unlikely that inhibiting merely these receptors will block the whole maglignant process. A combination of signal-transduction inhibitors will probably have a stronger inhibitory effect in retarding disease progression. However, cost will be another issue, which is particularly crucial in targeted therapy. It is anticipated to be an extremely costly solution to the patient, health system and third-party payer, making usable therapy unreachable.

CONCLUSION

Tumour management has been evolutionary and revolutionary, we have moved from surgery to chemotherapy, and now we have 'targeted' therapy. Such 'pin-point' approach in removing 'unintended cells' is leading specific and sophisticated. This is pivotal in achieving effectiveness yet minimizing unnecessary damages to innocent cells. Discovery of the role of HER2 is a significant milestone allowing further therapeutic development. The intelligent creation of trastuzumab, a humanized monoclonal antibody, brought a strike to the medicine history and initiated exploration in targeted cancer therapy. Narrowing to HER2 therapy, both biologics and small molecules have been developed to attack HER2 receptor protein or interfere its signaling pathways, making treatment empirical and comprehensive.

Combination regimen has become a growing trend in oncology management to achieve optimal clinical benefits. Incorporating HER2 targeted agents in breast cancer management is recommended by many world leading guidelines, such as NICE and WHO. What is more, those guidelines appear to emphasize the importance of detection technique, hence, more patients can benefit from these therapies.

In the vein of personalized medicine, future efforts should be directed towards biomarker-driven, HER2 directed therapies for optimal selection of therapy for the individual patient. Bispecific and trispecific antibodies that target HER2 and other key proteins responsible for facilitating immunological response against HER-2 overexpressed cells are also under investigation. The beauty of this strategy is to promote cell normalization, leaping from symptomatic relief to disease-modifying.

Nevertheless, the picture in managing cancers is becoming clearer every second. Driven by the concept of pharmacogenetics, what we need is to identify the right patients for appropriate targeted therapy. With all the virtues such advanced targeted therapy may bring, painful is the cost. Not every patient may afford this fortune, making it difficult to be popularized. Biosimilar, a follow-on biologic, is therefore lining in the trend to make advanced therapy accessible.

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Table 3. Ongoing clinical trials of novel HER2-directed therapies in HER2-positive breast cancer (source: ClinicalTrials.gov)

Trial	Development phase (Protocol no.)	Study population (Sample size/planned enrollment)	Intervention
Pertuzumab - Dimerization inhibitor (Roche/Genentech)			
CLEOPATRA	Randomized phase III (NCT00567190)	MBC, 1st line (n = 808)	Pertuzumab + Trastuzumab + Docetaxel vs. Placebo + Trastuzumab + Docetaxel
BO25126	Randomized phase III (NCT01358877)	Adjuvant, EBC (n = 3806)	Chemotherapy + Trastuzumab + Placebo vs. Chemotherapy + Trastuzumab + Pertuzumab
NeoSphere	Randomized phase II (NCT00545688)	Neoadjuvant, stage II/III EBC (n = 417)	Pertuzumab + Trastuzumab + Docetaxel vs. Pertuzumab + Docetaxel vs. Trastuzumab + Docetaxel vs. Pertuzumab + Trastuzumab
T-DM1 – Antibody-drug conjugate (Roche/Genentech)			
MARIANNE	Randomized phase III (NCT01120184)	MBC, 1st (n = 1092)	T-DM1 + Pertuzumab vs T-DM1 + placebo vs Trastuzumab + taxane
BO22857	Single-arm phase II (NCT01196052)	Neoadjuvant/adjuvant, stage I-III after anthracyclines (n = 135)	T-DM1
Afatinib (BIBW-2992) – TKI (Boehringer Ingelheim)			
LUX – Breast 1	Randomized phase III (NCT01125566)	MBC, 2nd line or above, prior trastuzumab (n = 780)	Afatinib + Vinorelbine vs Trastuzumab + Vinorelbine
Neratinib(HK-272) –TKI (Pfizer)			
NEFERTT	Randomized phase II (NCT00915018)	MBC, 1st line (n=480)	Neratinib + Paclitaxel vs Trastuzumab + Paclitaxel
ExteNET	Randomized phase III (NCT00878709)	Adjuvant, node-positive, stage II-III, completed trastuzumab (n = 3850)	Neratinib monotherapy v.s. Placebo
FB-7	Randomized phase II (NCT01008150)	Neoadjuvant, stage IIB-IIIC (n = 120)	Neratinib + Paclitaxel vs Trastuzumab + Paclitaxel
AUY922 - Heat – shock protein 90 inhibitor (Novartis)			
CAUY922A2109	Single arm phase II (NCT01271920)	MBC, 2nd line or above, prior trastuzumab (n=9)	AUY 922 + Trastuzumab
Everolimus (RAD001)- mTOR inhibitor (Novartis)			
BOLERO-1	Randomized phase III (NCT00876395)	MBC, 1st line (n = 717)	Everolimus + Trastuzumab + Paclitaxel vs Placebo + Trastuzumab + Paclitaxel
BOLERO-3	Randomized phase III (NCT01007942)	MBC, 2nd line or above, prior trastuzumab + taxane	Everolimus + Vinorelbine + trastuzumab vs Placebo + Vinorelbine + trastuzumab
12418	Single arm phase II (NCT01283789)	MBC, 2nd line or above, prior trastuzumab (n = 45)	Everolimus + Lapatinib
Temsirolimus (CCI-779) - mTOR inhibitor (Wyeth)			
10-005	Single-arm phase I/II (NCT01111825)	MBC, 2nd line or above, prior trastuzumab (n = 45)	Temsirolimus + Neratinib
BKM120 – PI3K inhibitor Novartis			
CBKM120X2107	Single-arm phase I/II (NCT01132664)	MBC, 2nd line or above, prior trastuzumab (n = 70)	BKM120 + Trastuzumab
Cixutumumab IGF-1R monoclonal antibody (Eli Lilly)			
CDR0000596070	Randomized phase II (NCT00684983)	MBC, 2nd line or above, prior trastuzumab, anthracycline and/ or taxane (n = 154)	Lapatinib + Capecitabine + Cixutumumab vs Lapatinib + Capecitabine

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References

1. Tai, Wanyi, Rubi Mahato, and Kun Cheng. "The Role of HER2 in Cancer Therapy and Targeted Drug Delivery." *Journal of Controlled Release* 146.3 (2010): 264-75.
2. Slamon, D., G. Clark, S. Wong, W. Levin, A. Ullrich, and W. McGuire. "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene." *Science* 235.4785 (1987): 177-82.
3. Engel, RH, and VG Kaklamani. "HER2-positive Breast Cancer: Current and Future Treatment Strategies." *Drugs* 67.9 (2007): 1329-341.
4. Siegel, P. M., D. L. Dankort, W. R. Hardy, and W. J. Muller. "Novel Activating Mutations in the Neu Proto-oncogene Involved in Induction of Mammary Tumors." *Mol. Cell. Biol.* 14.11 (1994): 7068-077.
5. Rubin, I., and Y. Yarden. "The Basic Biology of HER2." *Ann. Oncol* 12 (2001): S3-S8.
6. Baselga, José, and Sandra M. Swain. "Novel Anticancer Targets: Revisiting ERBB2 and Discovering ERBB3." *Nature Reviews Cancer* 9.7 (2009): 463-75.
7. Olayioye, MA, RM Neve, HA Lane, and NE Hynes. "The ErbB Signaling Network: Receptor Heterodimerization in Development and Cancer." *EMBOJ* 19 (2000): 3159-167.
8. Ferguson, Kathryn M., Mitchell B. Berger, Jeannine M. Mendrola, Hyun-Soo Cho, Daniel J. Leahy, and Mark A. Lemmon. "EGF Activates Its Receptor by Removing Interactions That Autoinhibit Ectodomain Dimerization." *Molecular Cell* 11.2 (2003): 507-17.
9. Cho, HS, and Et Al. "Structure of the Extracellular Region of HER3 Reveals an Interdomain Tether." *Science* 297 (2002): 1330-333.
10. Burgess, Antony W., Hyun-Soo Cho, Charles Eigenbrot, Kathryn M. Ferguson, Thomas P.J Garrett, Daniel J. Leahy, Mark A. Lemmon, Mark X. Sliwkowski, Colin W. Ward, and Shigeyuki Yokoyama. "An Open-and-Shut Case? Recent Insights into the Activation of EGF/ErbB Receptors." *Molecular Cell* 12.3 (2003): 541-52.
11. Ogiso, Hideo, Ryuichiro Ishitani, Osamu Nureki, Shuya Fukai, Mari Yamanaka, Jae-Hoon Kim, Kazuki Saito, Ayako Sakamoto, Mio Inoue, and Mikako Shirouzu. "Crystal Structure of the Complex of Human Epidermal Growth Factor and Receptor Extracellular Domains." *Cell* 110.6 (2002): 775-87.
12. Berger, Mitchell B., Jeannine M. Mendrola, and Mark A. Lemmon. "ErbB3/HER3 Does Not Homodimerize upon Neuregulin Binding at the Cell Surface." *FEBS Letters* 569.1-3 (2004): 332-36.
13. Tzahar, E., and Et Al. "A Hierrarchical Network of Interreceptor Interactions Determines Signal Transduction by Neu Differentiation Factor/neuregulin and Epidermal Growth Factor." *Mol. Cell. Biol.* 16 (1996): 5276-287.
14. Pinkas-Kramarski, R. "Diversification of Neu Differentiation Factor and Epidermal Growth Factor Signaling by Combinatorial Receptor Interactions." *EMBO J.* 15 (1996): 2452-467.
15. Wang, SC. "Binding at and Transactivation of the COX-2 Promotor by Nuclear Tyrosine Kinase Receptor ErbB-2." *Cancel Cell* 6.3 (2004): 251-61.
16. Hsieh, A. C., and M. M. Moasser. "Targeting HER Proteins in Cancer Therapy and the Role of the Non-target HER3." *British Journal of Cancer* 97.4 (2007): 453-57.
17. Lee-Hoeflich, S. T., L. Crocker, E. Yao, T. Pham, X. Munroe, K. P. Hoeflich, M. X. Sliwkowski, and H. M. Stern. "A Central Role for HER3 in HER2-Amplified Breast Cancer: Implications for Targeted Therapy." *Cancer Research* 68.14 (2008): 5878-887.
18. Hynes, Nancy E., and Heidi A. Lane. "ERBB Receptors and Cancer: The Complexity of Targeted Inhibitors." *Nature Reviews Cancer* 5.5 (2005): 341-54.
19. Prigent, S.A., and W.J. Gullick. "Identification of C-erbB-3 Binding Sites for Phosphoinositide 3'-kinase and SHC Using an EGF Receptor/c-erbB-3 Chimera." *EMBO J.* 13 (1994): 2831-841.
20. Soltoff, S.P., K.L. Carraway, and S.A. Prigent. "ErbB3 Is Involved in Activation of Phosphatidylinositol 3-kinase by Epidermal Growth Factor." *Mol. Cell. Biol.* 14 (1994): 3550-558.
21. Hellyer, N.J., K. Cheng, and J.G. Konald. "ErbB3 (HER3) Interaction with the P85 Regulatory Subunit of Phosphoinositide 3-kinase." *Biochem. J.* 333 (1998): 757-63.
22. "Genentech: Herceptin® (trastuzumab) - Information for Patients." *Genentech: Herceptin® (trastuzumab) - Information for Patients.* N.p., n.d. Web. 31 Mar. 2013.
23. Arribas J, Baselga J, Pedersen K, et al. p95HER2 and breast cancer. Cancer Research, 2011: 71(5) pp. 1515-1519
24. Serra, V., B. Markman, M. Scaltriti, P. J.A. Eichhorn, V. Valero, M. Guzman, M. L. Botero, E. Llonch, F. Atzori, S. Di Cosimo, M. Maira, C. Garcia-Echeverria, J. L. Parra, J. Arribas, and J. Baselga. "NVP-BEZ235, a Dual PI3K/mTOR Inhibitor, Prevents PI3K Signaling and Inhibits the Growth of Cancer Cells with Activating PI3K Mutations." *Cancer Research* 68.19 (2008): 8022-030.
25. Berns, Katrien, Hugo M. Horlings, Bryan T. Hennessy, Mandy Madiredjo, E. Marielle Hijmans, Karin Beelen, Sabine C. Linn, Ana Maria Gonzalez-Angulo, Katherine Stemke-Hale, Michael Hauptmann, Roderick L. Beijersbergen, Gordon B. Mills, Marc J. Van De Vijver, and René Bernards. "A Functional Genetic Approach Identifies the PI3K Pathway as a Major Determinant of Trastuzumab Resistance in Breast Cancer." *Cancer Cell* 12.4 (2007): 395-402.
26. Nagy, P., E. Friedländer, M. Tanner, and Et Al. "Decreased Accessibility and Lack of Activation of ErbB2 in JIMT-1, a Herceptin-resistant, MUC4-expressing Breast Cancer Cell Line." *Cancer Research* 65.2 (2005): 473-82.
27. Wong, Andrea L.A., and Soo-Chin Lee. "Mechanisms of Resistance to Trastuzumab and Novel Therapeutic Strategies in HER2-positive Breast Cancer." *International Journal of Breast Cancer* 415170th ser. 2012 (2012): 1-13.
28. Lu, Y., X. Zi, Y. Zhao, D. Mascarebhas, and M. Pollak. "Insulin-like Growth Factor-I Receptor Signaling and Resistance to Trastuzumab (Herceptin)." *Journal of the National Cancer Institute* 93.24 (2001): 1852-857.
29. Sergina, Natalia V., Megan Rausch, Donghui Wang, Jimmy Blair, Byron Hann, Kevan M. Shokat, and Mark M. Moasser. "Escape from HER-family Tyrosine Kinase Inhibitor Therapy by the Kinase-inactive HER3." *Nature* 445.7126 (2007): 437-41.
30. Serra, V., B. Markman, M. Scaltriti, P. J.A. Eichhorn, V. Valero, M. Guzman, M. L. Botero, E. Llonch, F. Atzori, S. Di Cosimo, M. Maira, C. Garcia-Echeverria, J. L. Parra, J. Arribas, and J. Baselga. "NVP-BEZ235, a Dual PI3K/mTOR Inhibitor, Prevents PI3K Signaling and Inhibits the Growth of Cancer Cells with Activating PI3K Mutations." *Cancer Research* 68.19 (2008): 8022-030.
31. Nagata, Yoichi, Keng-Hsueh Lan, Xiaoyan Zhou, Ming Tan, Francisco J. Esteva, Aysegul A. Sahin, Kristine S. Klos, Ping Li, Brett P. Monia, and Nina T. Nguyen. "PTEN Activation Contributes to Tumor Inhibition by Trastuzumab, and Loss of PTEN Predicts Trastuzumab Resistance in Patients." *Cancer Cell* 6.2 (2004): 117-27.
32. Franklin, Matthew C., Kendall D. Carey, Felix F. Vajdos, Daniel J. Leahy, Abraham M. De Vos, and Mark X. Sliwkowski. "Insights into ErbB Signaling from the Structure of the ErbB2-pertuzumab Complex." *Cancer Cell* 5.4 (2004): 317-28.
33. Adams, CW, DE Allison, K. Flagella, and Et Al. "Humanization of a Recombinant Monoclonal Antibody to Produce a Therapeutic HER Dimerization Inhibitor, Pertuzumab." *Cancer Immunol Immunother* 55.6 (2006): 717-27.
34. Di Fiore PP, erbB-3 is a potent oncogene when overexpressed in NIH/3T3 cells. Science 1987; 237 (4811): 178-82
35. Park JW, Unraveling the biologic and clinical complexities of HER2. Clin. Breast Cancer 2008; 8 (5) : 382-401
36. Ogiso, Hideo, Ryuichiro Ishitani, Osamu Nureki, Shuya Fukai, Mari Yamanaka, Jae-Hoon Kim, Kazuki Saito, Ayako Sakamoto, Mio Inoue, and Mikako Shirouzu. "Crystal Structure of the Complex of Human Epidermal Growth Factor and Receptor Extracellular Domains." *Cell* 110.6 (2002): 775-8
37. Agus, D.B., and et al. "Targeting Ligand Activated ErbB2 Signaling Inhibits Breast and Prostate Tumour Growth." *Cancer Cell* 2 (2002): 127-37.
38. Schaefer, G., V.D. Fitzpatrick, and M.X. Sliwkowski. "Gamma-heregulin: A Novel Heregulin Isoform That Is an Autocrine Growth Factor for the Human Breast Cancer Cell Line, MDA-MB-175." *Oncogene* 15 (1997): 1385-394.
39. Yap TA, Vidal &, Adam J, Stephens P Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors.
40. T. Hickish, D. Wheatley, and N. Lin, "Use of BIBW 2992, a novel irreversible EGFR/HER1 and HER2 tyrosine kinase inhibitor to treat patients with HER2-positive metastatic breast cancer after failure of treatment of trastuzumab," Cancer Research, vol. 69, supplement 24, 2009, Abstract 5060
41. Rabindran, S. K. "Antitumor Activity of HKI-272, an Orally Active, Irreversible Inhibitor of the HER-2 Tyrosine Kinase." *Cancer Research* 64.11 (2004): 3958-965.
42. Mann, M., H. Sheng, J. Shao, C. Williams, P. Pisacane, M. Sliwkowski, and R. Dubois. "Targeting Cyclooxygenase 2 and HER-2/neu Pathways Inhibits Colorectal Carcinoma Growth." *Gastroenterology* 120.7 (2001): 1713-719.
43. Burstein, H. J., Y. Sun, L. Y. Dirix, Z. Jiang, R. Paridaens, A. R. Tan, A. Awada, A. Ranade, S. Jiao, G. Schwartz, R. Abbas, C. Powell, K. Turnbull, J. Vermette, C. Zacharchuk, and R. Badwe. "Neratinib, an Irreversible ErbB Receptor Tyrosine Kinase Inhibitor, in Patients With Advanced ErbB2-Positive Breast Cancer." *Journal of Clinical Oncology* 28.8 (2010): 1301-307.
44. Baselga, J., and Et Al. "Objective Response Rate in a Phase II Multicenter Trial of Pertuzumab, a HER2 Dimerization Inhibiting Monoclonal Antibody in Combination with Trastuzumab in Patients with HER2-positive Metastatic Breast Cancer Which Has Progressed during Treatment with Trastuzumab." *J. Clin. Oncol.* 25 (Suppl) (2007): 1004.

45. Baselga, J., and et al. “Pertuzumab plus Trastuzumab plus Docetaxel for Metastatic Breast Cancer.” *N Eng J Med* 366.2 (2012): 109-19.

46. Gianni, L., and Et Al. “Efficacy and Safety of Neoadjuvant Pertuzumab and Trastuzumab in Women with Locally Advanced, Inflammatory, or Early HER2-positive Breast Cancer (NeoSphere): A Randomized Multicentre, Open-label, Phase 2 Trial.” *Lancet Oncol* 13.1 (2012): 25-32.

47. Baselga, J., and Et Al. “Lapatinib with Trastuzumab for HER2-positive Early Breast Cancer (NeoALTTO): A Randomized, Open-label, Mulricentre, Phase 3 Trial.” *Lancet* 379.9816 (2012): 633-40.

48. Guarneri, Valentina, Antonio Frassoldati, Federico Piacentini, Gordana Jovic, Simona Giovannelli, Cristina Oliva, and PierFranco Conte. “Preoperative Chemotherapy plus Lapatinib or Trastuzumab or Both in HER2-Positive Operable Breast Cancer (CHERLOB Trial).” *Clinical Breast Cancer* 8.2 (2008): 192-94.

49. Agus, D. B. “Phase I Clinical Study of Pertuzumab, a Novel HER Dimerization Inhibitor, in Patients With Advanced Cancer.” *Journal of Clinical Oncology* 23.11 (2005): 2534-543.

50. Yamamoto, N., Y. Yamada, Y. Fujiwara, K. Yamada, Y. Fujisaka, T. Shimizu, and T. Tamura. “Phase I and Pharmacokinetic Study of HER2-targeted RhuMAb 2C4 (Pertuzumab, RO4368451) in Japanese Patients with Solid Tumors.” *Japanese Journal of Clinical Oncology* 39.4 (2009): 260-66.

51. Cortes, J., P. Fumoleau, G.V. Bianchi, and Et Al. “Pertuzumab Monotherapy after Trastuzumab-based Treatment and Subsequent Reintroduction of Trastuzumab: Activity and Tolerability in Patients with Advanced Human Epidermal Growth Factor Receptor 2-positive Breast Cancer.” *J Clin Oncol* 30.14 (2012): 1594-600.

52. Baselga, J., K. A. Gelmon, S. Verma, A. Wardley, P. Conte, D. Miles, G. Bianchi, J. Cortes, V. A. McNally, G. A. Ross, P. Fumoleau, and L. Gianni. “Phase II Trial of Pertuzumab and Trastuzumab in Patients With Human Epidermal Growth Factor Receptor 2-Positive Metastatic Breast Cancer That Progressed During Prior Trastuzumab Therapy.” J*ournal of Clinical Oncology* 28.7 (2010): 1138-144

53. Smith, M.B., J. Reardon, and E.M. Olson. “Pertuzumab for the Treatment of Patients with Previously Untreated HER2-positive Metastatic Breast Cancer.” *Drugs of Today* 48.11 (2012): 713-22.

54. Ozcelik C, Erdmann B, Pilz B, et al. “Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy.” *Proc Natl Acad Sci U S A.* 2002 Jun 25;99(13):8880-8885.

55. Lewis Phillips, G. D., G. Li, D. L. Dugger, L. M. Crocker, K. L. Parsons, E. Mai, W. A. Blattler, J. M. Lambert, R. V.J. Chari, R. J. Lutz, W. L. T. Wong, F. S. Jacobson, H. Koeppen, R. H. Schwall, S. R. Kenkare-Mitra, S. D. Spencer, and M. X. Sliwkowski. “Targeting HER2-Positive Breast Cancer with Trastuzumab-DM1, an Antibody-Cytotoxic Drug Conjugate.” *Cancer Research* 68.22 (2008): 9280-290.

56. “2012 ASCO Annual Meeting | ASCO Daily News | LBA1.” *2012 ASCO Annual Meeting | ASCO Daily News | LBA1*. N.p., n.d. Web. 06 Apr. 2013.

57. Austin, C. D. “Endocytosis and Sorting of ErbB2 and the Site of Action of Cancer Therapeutics Trastuzumab and Geldanamycin.” *Molecular Biology of the Cell* 15.12 (2004): 5268-282.

58. Hickish, T., D. Wheatley, N. Lin, LA Carey, S. Houston, DS Mendelson, F. Solca, M. Uttenreuther-Fischer, and E. Winer. “Use of BIBW 2992, a Novel Irreversible EGFR/HER1 and HER2 Tyrosine Kinase Inhibitor To Treat Patients with HER2-Positive Metastatic Breast Cancer after Failure of Treatment with Trastuzumab.” *Cancer Research* 69 (2009): 2191-194.

59. Yap, T. A., L. Vidal, J. Adam, P. Stephens, J. Spicer, H. Shaw, J. Ang, G. Temple, S. Bell, M. Shahidi, M. Uttenreuther-Fischer, P. Stopfer, A. Futreal, H. Calvert, J. S. De Bono, and R. Plummer. “Phase I Trial of the Irreversible EGFR and HER2 Kinase Inhibitor BIBW 2992 in Patients With Advanced Solid Tumors.” *Journal of Clinical Oncology* 28.25 (2010): 3965-972.

60. Tsang, R. Y., and R. S. Finn. “Abstract.” *National Center for Biotechnology Information*. U.S. National Library of Medicine, 01 Mar. 2012. Web. 30 Mar. 2013.

61. Lu, C.-H., S. L. Wyszomierski, L.-M. Tseng, M.-H. Sun, K.-H. Lan, C. L. Neal, G. B. Mills, G. N. Hortobagyi, F. J. Esteva, and D. Yu. “Preclinical Testing of Clinically Applicable Strategies for Overcoming Trastuzumab Resistance Caused by PTEN Deficiency.” *Clinical Cancer Research* 13.19 (2007): 5883-888.

62. Morrow, P. K., G. M. Wulf, J. Ensor, D. J. Booser, J. A. Moore, P. R. Flores, Y. Xiong, S. Zhang, I. E. Krop, E. P. Winer, D. W. Kindelberger, J. Coviello, A. A. Sahin, R. Nunez, G. N. Hortobagyi, D. Yu, and F. J. Esteva. “Phase I/II Study of Trastuzumab in Combination With Everolimus (RAD001) in Patients With HER2-Overexpressing Metastatic Breast Cancer Who Progressed on Trastuzumab-Based Therapy.” *Journal of Clinical Oncology* 29.23 (2011): 3126-132.

63. Rabindran, S. K. “Antitumor Activity of HKI-272, an Orally Active, Irreversible Inhibitor of the HER-2 Tyrosine Kinase.” *Cancer Research* 64.11 (2004): 3958-965.

64. Wong, K.-K., P. M. Fracasso, R. M. Bukowski, T. J. Lynch, P. N. Munster, G. I. Shapiro, P. A. Janne, J. P. Eder, M. J. Naughton, M. J. Ellis, S. F. Jones, T. Mekhail, C. Zacharchuk, J. Vermette, R. Abbas, S. Quinn, C. Powell, and H. A. Burris. “A Phase I Study with Neratinib (HKI-272), an Irreversible Pan ErbB Receptor Tyrosine Kinase Inhibitor, in Patients with Solid Tumors.” *Clinical Cancer Research* 15.7 (2009): 2552-558.

65. Brachmann, S. M., I. Hofmann, C. Schnell, C. Fritsch, S. Wee, H. Lane, S. Wang, C. Garcia-Echeverria, and S.-M. Maira. “Specific Apoptosis Induction by the Dual PI3K/mTor Inhibitor NVP-BEZ235 in HER2 Amplified and PIK3CA Mutant Breast Cancer Cells.” *Proceedings of the National Academy of Sciences* 106.52 (2009): 22299-2304.

66. Burstein, H. J., Y. Sun, L. Y. Dirix, Z. Jiang, R. Paridaens, A. R. Tan, A. Awada, A. Ranade, S. Jiao, G. Schwartz, R. Abbas, C. Powell, K. Turnbull, J. Vermette, C. Zacharchuk, and R. Badwe. “Neratinib, an Irreversible ErbB Receptor Tyrosine Kinase Inhibitor, in Patients With Advanced ErbB2-Positive Breast Cancer.” *Journal of Clinical Oncology* 28.8 (2010): 1301-307

67. Saal, L. H. “PIK3CA Mutations Correlate with Hormone Receptors, Node Metastasis, and ERBB2, and Are Mutually Exclusive with PTEN Loss in Human Breast Carcinoma.” *Cancer Research* 65.7 (2005): 2554-559.

68. Saal, L. H. “PIK3CA Mutations Correlate with Hormone Receptors, Node Metastasis, and ERBB2, and Are Mutually Exclusive with PTEN Loss in Human Breast Carcinoma.” *Cancer Research* 65.7 (2005): 2554-559.

69. Brachmann, S. M., I. Hofmann, C. Schnell, C. Fritsch, S. Wee, H. Lane, S. Wang, C. Garcia-Echeverria, and S.-M. Maira. “Specific Apoptosis Induction by the Dual PI3K/mTor Inhibitor NVP-BEZ235 in HER2 Amplified and PIK3CA Mutant Breast Cancer Cells.” *Proceedings of the National Academy of Sciences* 106.52 (2009): 22299-2304.

70. Saal, L. H. “PIK3CA Mutations Correlate with Hormone Receptors, Node Metastasis, and ERBB2, and Are Mutually Exclusive with PTEN Loss in Human Breast Carcinoma.” *Cancer Research* 65.7 (2005): 2554-559.

71. Stemke-Hale, K., A. M. Gonzalez-Angulo, A. Lluch, R. M. Neve, W.-L. Kuo, M. Davies, M. Carey, Z. Hu, Y. Guan, A. Sahin, W. F. Symmans, L. Pusztai, L. K. Nolden, H. Horlings, K. Berns, M.-C. Hung, M. J. Van De Vijver, V. Valero, J. W. Gray, R. Bernards, G. B. Mills, and B. T. Hennessy. “An Integrative Genomic and Proteomic Analysis of PIK3CA, PTEN, and AKT Mutations in Breast Cancer.” *Cancer Research* 68.15 (2008): 6084-091.

72. *AHFS Drug Information.* Web. 7 Apr. 2013.

73. Morrow, P. K., G. M. Wulf, J. Ensor, D. J. Booser, J. A. Moore, P. R. Flores, Y. Xiong, S. Zhang, I. E. Krop, E. P. Winer, D. W. Kindelberger, J. Coviello, A. A. Sahin, R. Nunez, G. N. Hortobagyi, D. Yu, and F. J. Esteva. “Phase I/II Study of Trastuzumab in Combination With Everolimus (RAD001) in Patients With HER2-Overexpressing Metastatic Breast Cancer Who Progressed on Trastuzumab-Based Therapy.” *Journal of Clinical Oncology* 29.23 (2011): 3126-132.

74. Dalenc, F., M. Campone, P. Hupperets, and D. Gajria. “Everolimus in Combination with Weekly Paclitaxel and Trastuzumab in Patients (pts) with HER2-overexpressing Metastatic Breast Cancer (MBC) with Prior Resistance to Trastuzumab and Taxanes: A Multicenter Phase II Clinical Trial.” *Journal of Clinical Oncology* 28 (2010): Suppl 15s.

75. Cohen, B. D. “Combination Therapy Enhances the Inhibition of Tumor Growth with the Fully Human Anti-Type 1 Insulin-Like Growth Factor Receptor Monoclonal Antibody CP-751,871.” *Clinical Cancer Research* 11.5 (2005): 2063-073.

76. García-Echeverría, Carlos, Mark A. Pearson, Andreas Marti, Thomas Meyer, Juergen Mestan, Johann Zimmermann, Jiaping Gao, Josef Brueggen, Hans-Georg Capraro, and Robert Cozens. “In Vivo Antitumor Activity of NVP-AEW541—A Novel, Potent, and Selective Inhibitor of the IGF-IR Kinase.” *Cancer Cell* 5.3 (2004): 231-39.

77. Weroha, S. John, and Paul Haluska. “IGF-1 Receptor Inhibitors in Clinical Trials—Early Lessons.” *Journal of Mammary Gland Biology and Neoplasia* 13.4 (2008): 471-83.

78. Citri, A., B.S. Kochupurakkal, and Y. Yarden. “The Achilles Heel of ErbB-2/HER2: Regulation by the Hsp90 Chaperone Machine and Potential for Pharmacological Intervention.” *Cell Cycle* 3 (2004): 51-60.

79. Citri, A., and et al. “Drug-induced Ubiquitylation and Degradation of ErbB Receptor Tyrosine Kinases: Implications for Cancer Therapy.” *EMBO J.* 21 (2002): 2407-417.

80. Chandarlapaty, S., M. Scaltriti, P. Angelini, Q. Ye, M. Guzman, C. A. Hudis, L. Norton, D. B. Solit, J. Arribas, J. Baselga, and N. Rosen. “Inhibitors of HSP90 Block P95-HER2 Signaling in Trastuzumab-resistant Tumors and Suppress Their Growth.” *Oncogene* 29.3 (2009): 325-34.

81. Münster, P.N., D.C. Machion, A.D. Basso, and N. Rosen. “Degradation of HER2 by Ansamycins Induces Growth Arrest and Apoptosis in Cells with HER2 Overexpression via a HER3, Phosphatidylinositol 3’-kinase-AKT-dependent Pathway.” *Cancer Research* 62 (2002): 3132-137.

82. Modi, S., A. Stopeck, H. Linden, D. Solit, S. Chandarlapaty, N. Rosen, G. D’Andrea, M. Dickler, M. E. Moynahan, S. Sugarman, W. Ma, S. Patil, L. Norton, A. L. Hannah, and C. Hudis. “HSP90 Inhibition Is Effective in Breast Cancer: A Phase II Trial of Tanespimycin (17-AAG) Plus Trastuzumab in Patients with HER2-Positive Metastatic Breast Cancer Progressing on Trastuzumab.” *Clinical Cancer Research* 17.15 (2011): 5132-139.

83. Higgins, M.J., N.Y. Gabrail, K. Miller, and D. Anderson. “A Phase I/II Study of MM-111, a Novel Bispecific Antibody That Targets the ErB2/ErB3 Heterodimer, in Combination with Trastuzumab in Advanced Refractory HER2-positive Breast Cancer.” *Journal of Clinical Oncology* 29 (2011): Abstract 119

84. Gajria, D., T.A. King, and H. Pannu. “Combined Inhibition of MTORC1 with Temsirolimus and HER2 with Neratinib: A Phase I Study in Patients with Metastatic HER2-amplified Breast Cancer.” *Journal of Clinical Oncology* 29 (2011): Abstract 574.

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

New approaches to HER2-positive breast cancer

1. ***What are the key features of HER2?***
   - i. It is no identified ligand
   - ii. It always exist in 'open' conformation state
   - iii. It only forms heterodimers

   A i only
   B i and ii
   C ii and iii
   D All of above

2. ***Why is HER2/HER3 dimer regarded as the most oncogenic unit?***
   - i. HER2-mediated signaling pathways promote cell survival and proliferation, whereas HER3-mediated signaling pathways are responsible for antipoptosis
   - ii. HER2 involved dimers signal more potent transduction pathways
   - iii. HER3 is pivotal in activating PI3k-Akt pathway

   A i only
   B i and ii
   C ii and iii
   D All of above

3. ***Which of the following are the reasons underpin trastuzumab resistance?***
   - i. Truncated HER2 receptors have no recognition site for trastuzumab
   - ii. PTEN deficiency
   - iii. Alternative pathways are activated

   A i only
   B i and ii
   C ii and iii
   D All of above

4. ***What is the difference between trastuzumab and pertuzumab?***
   - A. Trastuzumab is HER2-directed, versus pertuzumab is HER3-directed
   - B. Trastuzumab inhibits ligand-depending dimerization, whereas pertuzumab inhibits ligand-independent dimerization
   - C. Trastuzumab inhibits ligand-independing dimerization, whereas pertuzumab inhibits ligand-dependent dimerization
   - D. Trastuzumab is monspecific antibody, versus pertuzumab is a bispecific antibody

5. ***What are the common adverse events associated with pertuzumab?***
   - i. Hypertension
   - ii. Mucosal inflammation
   - iii. Rash

   A i only
   B i and ii
   C ii and iii
   D All of above

6. ***What are the components of T-DM1?***
   - i. Trastuzumab
   - ii. Mertansine
   - iii. Maleimidomethylcyclohexane-1-carboxylate

   A i only
   B i and ii
   C ii and iii
   D All of above

7. ***What is most important advantage of T-DM1 over trastuzumab?***
   - A. It overcomes trastuzumab resistance.
   - B. It has better pharmacokinetic profile with extended half life.
   - C. It directly delivers the cytotoxic agent to the HER2-overexpressing cells without interfering the normal tissues.
   - D. It significantly improves penetration of trastuzumab to the HER2-overexpressing cells without harming the normal tissues.

8. ***How is afatinib different from lapatinib?***
   - A. Afatinib targets EGFR/HER1, HER2 and HER4, versus lapatinib EGFR/HER1, HER2 and HER3.
   - B. Afatinib has better safety profile than lapatinib does.
   - C. Afatinib has higher potency than lapatinib does.
   - D. Afatinib is an irreversible TKI, versus lapatinib is reversible.

9. ***What are the targets of Neratinib?***
   - A. Pan HER
   - B. HER2, HER3
   - C. EGFR/HER1, HER2, HER4
   - D. EGFR/HER1, HER2, HER3

10. ***What are the limitations of HER2 targeted therapy?***
    - i. Only applicable to small population with HER2 overexpressing.
    - ii. Targeting HER2 is not sufficient to halt the entire malignant process.
    - iii. The treatment is available but may not be affordable.

    A i only
    B i and ii
    C ii and iii
    D All of above

Answers will be released in the next issue of HKPJ.

CE Questions Answer for 201(D&T)

Overview of Targeted Therapies for Renal Cell Carcinoma

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

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References: 1. National Institute for Health and Clinical Excellence. Quick reference guide, 2010. Neuropathic pain: The pharmacological management of neuropathic pain in adults in non-specialist settings. Available at: http://www.nice.org.uk. Accessed October 18 2010. **2.** Dubinsky RM, et al. Practice parameter: treatment of postherpetic neuralgia: an evidence-based report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2004;63:959-965. **3.** Attal N, et al. EFNS guidelines on pharmacological treatment of neuropathic pain. Eur J Neurol 2006;13:1153-1169. **4.** Institute for Clinical Systems Improvement (ICSI). Assessment and management of chronic pain. Bloomington (MN): Institute for Clinical Systems Improvement (ICSI) 2008:84. **5.** Moulin DE, et al. Pharmacological management of chronic neuropathic pain – consensus statement and guidelines from the Canadian Pain Society. Pain Res Manag 2007;12:13-21. **6.** Suarez L. New Guidelines Boast Benefits for Patients Suffering with DPN pain. Diabetic Microvascular Complications Today 2006;May/June:21-22. **7.** Dworkin RH, et al. Pregabalin for the treatment of postherpetic neuralgia: A randomized, placebo-controlled trial. Neurology 2003;60:1274-1283. **8.** Siddall PJ, et al. Pregabalin in central neuropathic pain associated with spinal cord injury. A placebo-controlled trial. Neurology 2006; 67:1792-1800.

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LYRICA ABBREVIATED PACKAGE INSERT 1. TRADE NAME: LYRICA **2. PRESENTATION:** Each Lyrica hard capsule contains 25mg, 50 mg, 75 mg, 150 mg, 225mg or 300 mg of pregabalin. (not all strengths may be marketed). **3. INDICATIONS:** Treatment of peripheral and central neuropathic pain in adults; As adjunctive therapy in adults with partial seizures (epilepsy) with or without secondary generalisation; Treatment of Generalised Anxiety Disorder (GAD) in adults. For the management of fibromyalgia. **4. DOSAGE:** 150 to 600 mg/day to be taken in two or three divided doses with or without food. For neuropathic pain: start at 150 mg/day, increase to 300 mg/day after 3 to 7 days, if needed, then to a maximum of 600 mg/day after an additional 7-day interval. For epilepsy: start with 150 mg/day, increase to 300 mg/day after 1 week if needed, then to a maximum of 600 mg/day after an additional week. For GAD: start with 150 mg/day, increase to 300 mg/day after 1 week if needed, then increase to 450mg/day following an additional week if needed, then to a maximum of 600 mg/day after an additional week. For fibromyalgia, recommended dose is 300 to 450 mg/day, dosing should begin at 75 mg BID (150mg/day) and may be increased to 150mg BID (300 mg/day) within one week based on efficacy and tolerability. Patients who do not experience sufficient benefit with 300 mg/day may be further increased to 225 mg BID (450 mg/day). Renal impairment: daily dose should be adjusted based on renal function. Elderly may require a dose reduction. Discontinuation of pregabalin should be done gradually over a minimum of 1 week independent of indication. **5. CONTRAINDICATIONS:** Hypersensitivity to the pregabalin or to any of the excipients. **6. WARNINGS & PRECAUTIONS:** Avoid in patients with galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption. Adjust hypoglycaemic medications if weight gain occurs in diabetic patients. Use with caution in patients with severe congestive heart failure. Withdrawal symptoms may occur after discontinuation of short-term and long-term treatment. May cause dizziness and somnolence, which could increase the occurrence of accidental injury (fall) in the elderly population and influence the ability to drive or use machinery. The incidence of adverse events especially somnolence may be increased in the treatment of central neuropathic pain due to spinal cord injury which may be attributed to the additive effect from concomitant medication for the condition. **7. INTERACTIONS:** Oxycodone, ethanol and lorazepam. **8. PREGNANCY AND LACTATION:** Should not be used during pregnancy unless in the opinion of the physician, the potential benefit outweighs the potential risk. Effective contraception must be used in women of child bearing potential. Breast-feeding is not recommended. **9. SIDE EFFECTS:** Dizziness, somnolence, appetite increased, euphoric mood, confusion, libido decreased, irritability, ataxia, disturbance in attention, coordination abnormal, memory impairment, tremor, dysarthria, paraesthesia, vision blurred, diplopia, vertigo, dry mouth, constipation, vomiting, flatulence, erectile dysfunction, fatigue, oedema peripheral, feeling drunk, oedema, gait abnormal, weight increased, disorientation, insomnia, balance disorder, amnesia, sedation, lethargy, abdominal distension, feeling abnormal. **Reference:** HK PI (Mar 2009) **Date of preparation:** May 2010 **Identifier number:** LYR0510 **FULL PRESCRIBING INFORMATION IS AVAILABLE UPON REQUEST.**

LYR11010P

Expression of Therapeutic Recombinant Protein in *E. coli*: A Major Challenge with Current Insights on the Formation of Inclusion Bodies

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ABSTRACT

***Escherichia coli* is one of the most widely used host for the efficient, cost-effective and high-level production of heterologous proteins. In order to express the foreign gene, several components have to be used. However, some problems during their expression, with particular attention to the formation of insoluble inclusion bodies (IBs) always occur. In this review article, these problems are addressed and some contemporary approaches are described. Strategies to enhance and maximize the solubility of recombinant proteins (RPs) are also discussed.**

Keywords: *E. coli; recombinant protein; solubility; chaperones; inclusion bodies.*

INTRODUCTION

In the past three decades, recombinant DNA technology has enabled scientist to produce a diverse range of proteins in microorganisms, that were previously unavailable, relatively expensive, or difficult to obtain in large quantities.[1] While the expression of foreign genes has been reported in a number of microorganisms and cell lines, most works utilize *E. coli* as host for the expression of foreign genes. Production of foreign protein involves cloning of appropriate gene into expression vector under the control of inducible promoter.[2] The expression of recombinant protein (RP) in cells that do not occur naturally is termed heterologous protein production. Bacterial expression systems are commonly used for the production of heterologous gene products of both eukaryotic and prokaryotic origin.[3] The expression of heterologous protein in *E. coli* is most widely and routinely used. A number of therapeutically important proteins are now produced in *E. coli.* The first heterologous protein employed clinically was human insulin produced in *E. coli.* It was first approved in 1982 in UK, West Germany, the Netherland, and USA.[4] The use of *E. coli* for RP production, however, has encountered several disadvantages. For example, many of the post-translational modifications found in eukaryotes such as N- and O-glycosylation, amidation, hydroxylation, palmitation, or sulfation, are absent in *E. coli*.[5] Because of lacking these modification functions, its expressed products have limits in application.

On top of this, the high expression levels of RP often leads to accumulation of aggregated insoluble protein, resulting in inclusion-body formation in the cytoplasm of the bacteria.[6] High translation rate can be a serious problem when the target protein is a heterologous molecule. Thus, the soluble expression and native purification of the target protein in *E. coli* remains an important bottleneck in the production area of RP. Nevertheless, if the protein to be expressed is cytoplasmic, lacks the above-mentioned post-translational modifications, possesses few disulfide bonds, and does not present a multi-domain composition, the use of the *E. coli* as the host is the recommended choice for protein production.[7] Production of RP in *E. coli,* whether for bio-chemical analysis, therapeutics, or structural studies, requires the successful operation of two crucial steps: (a) soluble expression of the target protein; and (b) purification and stabilization of a functional molecule.

Expression of RP in *E. coli*

Reasons why *E. coli* is widely used as the host for the expression of heterogeneous protein are due to several advantages including ease and fast growth; availability of dozens of vectors and host strains that have been developed for maximizing expression; a well-known genetics and physiology of *E. coli*; a well-established fermentation technology; potentially unlimited supplies of recombinant protein and economically attractive.[8]

RP expression is normally induced by expression plasmid vector. A number of essential components should be taken into consideration when one thinks for a strategy design for PR in *E. coli.*

Replicon

The replicon of a plasmid contains origin of replication (ORI) that function not only, for replication but also, as a regulatory element for plasmid copy number.[9] The copy number of common *E. coli* expressed plasmid ranges from low (2 to 20) to high (20 to 40).[10] Most often, high copy number is required particularly when maximal gene expression is needed, however, it can result in metabolic burden that negatively affect the biomass and the final yield. The expression plasmid vector commonly replicate by either Col1E1 (high copy) or p15A (low copy).[11] It is also worth to mention that the origin of

replication is important when expressing protein from different plasmid. In such cases, each vector may contain a different origin of replication because plasmids with the same origin are mutually exclusive in the same bacterial host.[12]

Selection of marker

The antibiotic resistant marker gene is essential in isolation and selection of clones based on their resistance to commonly used antibiotics such as ampicillin, kanamycin, chloramphenicol or tetracycline. Plasmid mediated resistance to ampicillin is occurred by expression of β-lactamase from the bla gene. This enzyme is secreted to catalyze hydrolysis of the β-lactam ring whereas kanamycin, chloramphenicol, and tetracycline interfere with protein synthesis by binding to critical areas of the ribosome. For instance, Kanamycin is inactivated by aminoglycoside phosphotransferases meanwhile Chloramphenicol by the cat gene product, chloramphenicol acetyl transferase and various genes confer resistance to tetracycline.[13]

Promoter

The promoter sequence is a central element that affects the strength and duration of transcription, and in turn, protein yield inasmuch as, it is the regulatory region of DNA located upstream of ribosome binding site. Protein induction relies on promoter types that can be either induced thermally or chemically, of which the chemical inducer isopropyl-β-D-thiogalactopyranoside (IPTG; a nonhydrolyzable lactose analogue) is the most commonly used.[10,14,15] It is well known that, the promoter contains specific DNA sequences recognized by proteins known as transcription factors. The function of these factors is bind to specific promoter sequence, and thus recruiting RNA polymerase, to synthesize the RNA from the coding sequence of specific gene. *E. coli* has σ70-one main factor, which equips RNA polymerase to recognize most promoters. For every promoter, there are three regions called the —35 and the —10 box and the spacer region separating both boxes.

T7 promoter

The T7-based pET expression system is by far the most used system for recombinant expression in *E. coli*.[15,16] It is based on the highly selective T7 RNA polymerase from phage T7 to drive RP production. This polymerase only transcribes genes under the control of the T7 promoter and it has been shown that it can transcribe eight times faster than *E. coli* polymerases, producing a high yield of protein. The T7 promoter is considered a strong promoter and RP could reach up to 50% of the total cell proteins. Because *E. coli* lacks this polymerase, some strains, such as BL21 (DE3), have been developed that contain a chromosomal copy of the T7 polymerase gene, under the control of the lac promoter derivative lacUV5. The lacUV5 promoter contains point mutations that increase the promoter strength and make it less sensitive to catabolite repression. In this way, the promoter is only controlled by the lac repressor, LacI, which allows induction with IPTG, even in the presence of glucose. The addition of IPTG releases the repression caused by the binding of LacI to the lac operator, resulting in the expression of T7 polymerase, which in turn transcribes the target gene with the concomitant production of the RP.[12,17,18]

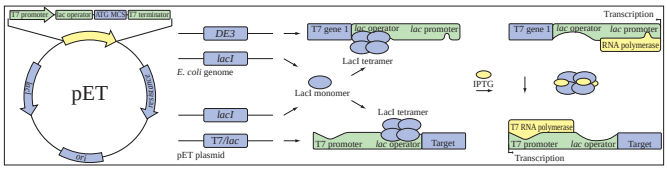

Figure 1: *Recombinant expression mechanisms. A general pET plasmid configuration is shown. The macromolecular situations prior to and after induction are shown.*[11]

Temperature-controlled promoters

Promoters such as pL/pR phage lambda are induced after increasing the culture temperature. In this system, the pL (leftward) and pR (rightward) strong promoters are regulated by the temperature sensitive mutant cI857 repressor of bacteriophage λ.[19] At low temperatures (usually 28–32°C), transcription is inhibited by the binding of cI857 to the pL or pR promoters. After increasing the temperature above 37°C (usually 40–42°C), cI857 binding is released from the promoter and gene expression is induced.[19,20]

Choice of cellular compartment for protein expression

The decision to target the expressed protein to a specific cellular compartment, that is, to the cytoplasm, periplasm or the culture medium, rests on balancing the advantages and disadvantages of each compartment as clearly illustrated in Fig 2.

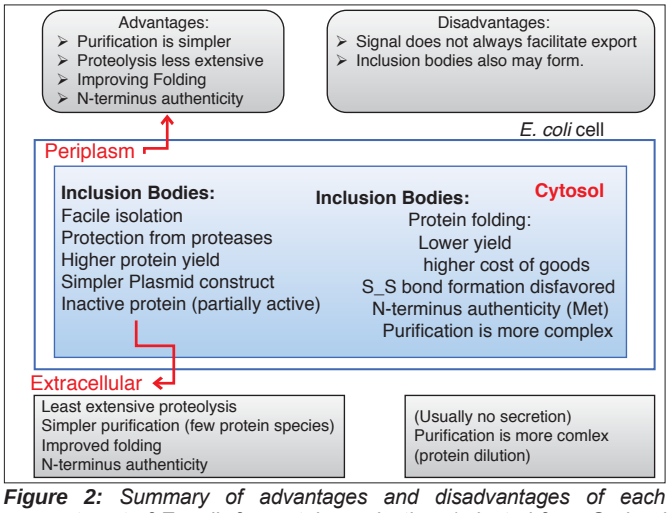

Figure 2: *Summary of advantages and disadvantages of each compartment of E. coli for protein production. (adopted from Gerhard and Savvas,1998)*[21]

Cytoplasmic quality control machinery

Under stress conditions, such as high temperature and RP overproduction, the protein quality control machinery is stimulated. This represents natural cellular defense devoted to prevent protein misfolding and accumulation of the aggregating proteins, based mainly on the activity of chaperones and proteases.

Chaperones

Molecular chaperones are a group of structurally diverse proteins highly conserved in all three kingdoms of life which form a comlex network to assist proper protein folding, prevent their deposition and dissolve deposits of misfolded proteins.[22-24] Even though chaperones are constitutively expressed under

physiological conditions, many of them are up regulated under stress conditions; this regulation is mainly due to the σ32 sigma factor encoded by rpoH gene.[25] Since chaperone abundance increases in cells upon thermal stress, these molecules have been traditionally named heat shock proteins (Hsps).[26] However, despite this historical link, it should be clarified that not all chaperones are heat shock proteins and not all heat shock proteins are chaperones. Molecular chaperones (Fig. 3) can be divided into 3 functional subclasses based on their mechanism of action as illustrated in Figure 3.

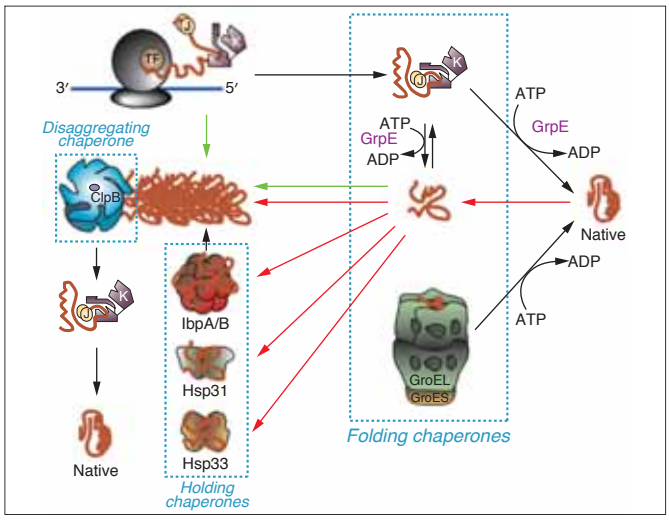

Figure 3: *Chaperone-assisted protein folding in the cytoplasm of E. coli. Trigger factor (TF) binds to nascent polypeptides, KJE (DNak, DnaJ (J)andGrpE) and GroESL(GroEL and GroES) systems assist protein intermediates to reach their native form. The small heat shck protein IbpA and IbpB, toghether with ClpB, cooperate with KJE in the disaggregation process.*[27]

The term "misfolding" is used to describe the process that results in a protein acquiring a sufficient number of persistent non-native interactions to affect its overall architecture and/or its properties in a biologically significant manner.[28] Misfolded and incompletely folded molecules are susceptible to aggregate, due to the exposure of hydrophobic regions that are buried in the native state. To avoid aggregation, cells of living organisms have auxiliary factors, including folding catalysts that accelerate rate-limiting steps and molecular chaperones that assist protein folding. Moreover, the cell quality control mechanism targets for destruction any protein molecule that has not folded correctly (Fig. 4).

Strategies for improving the expression of functional and soluble protein

Much effort has been invested to minimize IB formation during the production process itself, aiming to improve the yield of soluble protein species. Therefore, Minimization or prevention of inclusion body formation is attractive to obtain a high degree of accumulation of soluble protein in the bacterial cell. Nevertheless, protein stability and solubility cannot be predicted in advance and the used strategies have not shown the same degree of success for different polypeptides. The main strategies used to minimize inclusion bodies are those mentioned in the following in Fig. 5.

Cell growth characteristic have a marked effect on the RP expression. Here, we will focus on some parameters, including temperature, medium, and inducer effect.

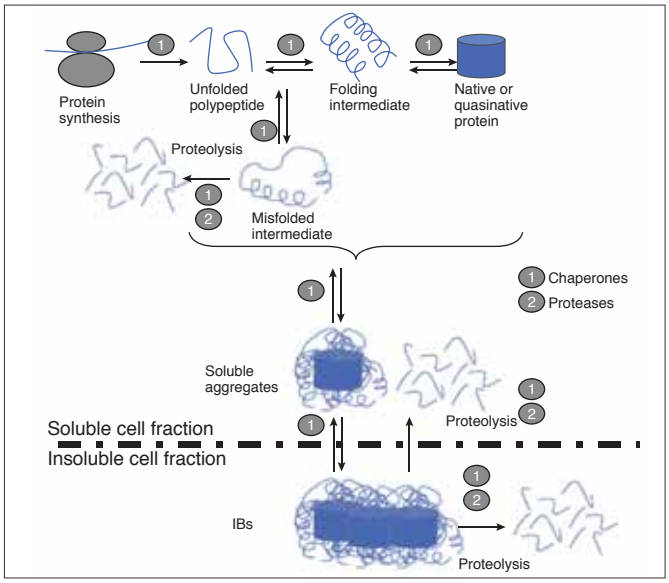

Figure 4: *Model of protein folding, aggregation and proteolysis in the E. coli cytoplasm. Several conformational versions of newly synthesized polypeptides, including those reaching native or native-like forms, can interact to form soluble aggregates, the putative precursors of inclusion bodies. Both soluble aggregates and inclusion bodies are then expected to be heterogeneous regarding protein folding status. The formation of insoluble inclusion bodies is highly favoured at high concentrations of RP. Chaperones (1) regulate aggregation and disaggregation but also protease (2)-mediated digestion of both soluble and insoluble protein.*[29]

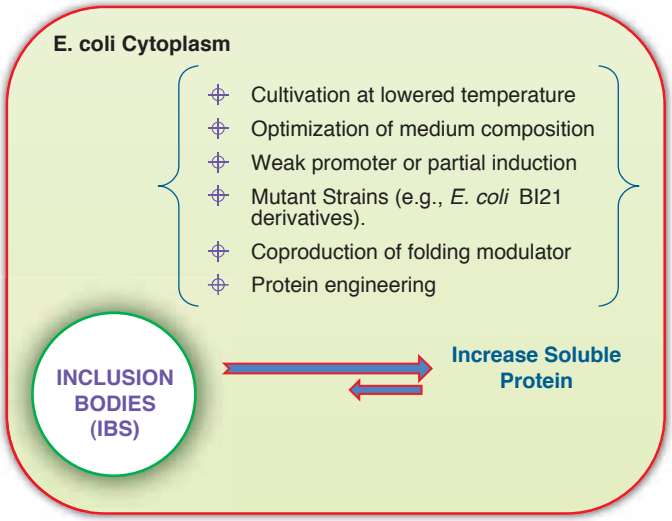

Figure 5: *Strategies to decrease inclusion body formation. Adopted from (Sorensen and Mortensen 2005)*[30]

RP production at lowered temperature

It is a well-known technique to limit in vivo aggregation of RP. A wide number of soluble proteins have been produced following this strategy.[31,32] The advantage of this procedure can be attributed to the fact that lower temperature decrease protease activity and thus substantially reduced protein degradation. Lowering temperature can also reduce the hydrophobic interactions that contribute to protein misfolding and aggregation. Consequently the dual actions contribute for expression of more stable properly folded protein at suboptimal temperature for *E. coli* growth.[33,34] Nevertheless, disadvantages of this procedure may be limited to slow growth of *E. coli*, so longer induction time is needed (e.g., overnight may be necessary to obtain sufficient amount of RP The culture at a lower temperature slow the rate replication, transcription and translation and this result in reduced of RP.[35]

Weak promoter or partial induction of strong promoter

Solubility can be enhanced by using weak promoter or using strong promoter under partial condition of partial induction. Low induction levels have been found to result in higher amounts of soluble protein.[36,37] This is a result of the reduction in cellular protein concentration which favors folding. However, bacterial growth is decreased, thus resulting in a decreased amount of biomass. For lac promoter, IPTG inducer below 100μM is suitable for partial induction. A weaker or partially induced result in higher amounts of soluble protein.[36] This is a result of the reduction in cellular protein concentration which favors folding. However, under these circumstances, growth is decreased, thus lead to a reduction in biomass yield.

Modification of cultivation strategies to obtain soluble protein

The composition of growth media can also minimize inclusion body formation. Through optimizing growth media composition, reduced expression times, increased soluble fraction yield and enhance biological activity of the enzyme have been achieved. Folding of some proteins require the existence of a specific cofactor. Addition of such cofactors or binding partners to the cultivation media may increase the yield of soluble protein dramatically. On the other hand, selection of convenient cultivation strategy can also improve soluble protein production. For example, in fed batch cultivations, the concentration of energy sources can be adjusted according to the rate of consumption. Several other factors can also be regulated in order to obtain the maximal production level in terms of target protein per biomass. The formation of inclusion bodies can be followed in fed batch cultivations by monitoring changes in intrinsic light scattering by flow cytometry.[38] This allows for real time optimization of growth conditions as soon as inclusion bodies are detected even at low levels and inclusion body formation can potentially be avoided.[39] On the contrary, Batch culture despite of its simplicity, it is considered not suitable as fed batch, due to its limitation to control growth and changes in the growth medium such as changes in pH, dissolved oxygen concentration, substrate depletion, as well as inhibitory products of various metabolic pathways. Folding of some proteins require the existence of a specific Cofactor. This was demonstrated for a recombinant mutant of hemoglobin for which the accumulation of soluble product was improved when heme was in excess.[40] Similarly, a 50% increase in solubility was observed for gloshedobin when *E. coli* recombinants were cultivated in the presence of 0.1 mM Mg^{2+}.[41] In addition, solubility up to 170-fold of the expressed mycobacterial proteins was noticed in the presence of a dipeptide, glycylglycine, in the range of 100 mM to 1 M in the medium.[42] Consequently, an important factor in soluble expression of RPs is media composition and optimization. Although this is attained mostly by trial and error, it nevertheless may be beneficial.

E. coli genetically modified strains

Different commercial *E. coli* strains are available that facilitate the soluble production of heterologous proteins. The selection of the expression strain is based on the characteristics of the target protein, such as whether the protein contains disulfide bonds, is highly toxic, or contains rare codons caused by the heterologous taxonomic origin of the target protein. In this context, different strains could be grouped as described below:

Protease-deficient strains

The *E. coli* BL21 and its derivatives are most commonly used for RP expression. BL21 is deficient in the adenosine triphosphate (ATP)-dependent protease Lon and in the outer-membrane protease OmpT, thus reducing the degradation of the target protein and improving the yield. The BL21(DE3) derivative is deficient in OmpT/Lon proteases and contains a chromosomal copy of the T7 RNA polymerase under the control of the lacUV5 promoter for the expression of recombinant under the control of the T7 promoter.[12]

Stringent repression of RP expression

Since transcription of the T7 polymerase even its minimal basal production, results in a leaky expression of the target gene prior to induction. This could be detrimental for the host if the target protein is toxic or even prevent the establishment of the plasmid carrying the toxic gene. To reduce this basal level of expression, several host strains have been developed that contain plasmid coding for the natural inhibitor of T7 polymerase, the bacteriophage T7 lysozyme. Usually pLysS and pLysE plasmids are commercially available as BL21 (DE3) pLysS and BL21 (DE3) pLysE (Novagen).[12]

Fusion Tags

Fusion tags offer several advantages, such as prevention of inclusion-body formation, improved folding characteristics, limited proteolysis and generic protein-purification schemes (Table 1)

Table 1 Commonly used tags for solubility enhancement

Tag	Protein	Solubility enhancement (Y/N)	Affinity (purification) (Y/N)
MBP	Maltose-bindingprotein	Y	Y
GST	Glutathione-S-transferase	Y	Y
Trx	Thioredoxin	Y	N
NusA	N-Utilizationsubstance	Y	N
SUMO	Smallubiquitin-modifier	Y	N
SET	Solubility-enhancingtag(synthetic)	Y	N
DsbC	DisulfidebondC	Y	N
Skp	Seventeenkilodaltonprotein	Y	N
T7PK	PhageT7proteinkinase	Y	N
GB1	ProteinGB1domain	Y	N
ZZ	ProteinAIgGZZrepeatdomain	Y	N
His6	Hexahistidinetag	N	Y
FLAG	FLAGtagpeptide	N	Y
BAP	Biotinacceptorpeptide	N	Y
Strep-II	Streptavidin-bindingpeptide	N	Y

Adopted from [29]

Co-expression of folding modulators

Coproduction of folding modulators such as molecular chaperones was extensively used strategy to reduce inclusion body formation. This strategy is attractive but there is no guarantee that chaperones improve RP solubility. *E. coli* encode chaperones some of which drive folding attempts, whereas others prevent protein aggregation. As soon as newly synthesized proteins leave the exit tunnel of the *E. coli* ribosome they associate with the trigger factor chaperone.[43] Exposed hydrophobic patches on newly synthesized proteins are protected from unintended inter or intramolecular interactions by association with trigger factor. Thus preventing premature folding (Fig. 3).[44] Proteins can start or continue their folding into the native state after release from trigger factor. Proteins trapped in non-native and aggregation prone conformations are substrate for aggregation and have been found associated with inclusion bodies.[45,46] Simultaneous over-expression of

chaperone encoding genes and recombinant target proteins proved effective in several instances. Co-over expression of trigger factor in recombinants prevented the aggregation of mouse endostatin, human oxygen-regulated protein ORP150, human lysozyme and guinea pig liver transglutaminase.[47,48] Soluble expression was further stimulated by the co-over expression of the GroEL-GroES and DnaK-DnaJ-GrpE chaperone systems along with trigger factor.[48] The chaperone systems are cooperative and the most favorable strategies involve co-expression of combinations of chaperones belonging to the GroEL, DnaK, ClpB and ribosome associated trigger factor families of chaperones.[49,50]

Marco et al (2007) demonstrated the effects of combined overproduction of entire network of major cytosolic chaperones in *E.coli* on the solubility of RPs. A two-step procedure was found to show the strongest enhancement of solubility. In a first step, the four chaperone systems GroEL/GroES, DnaK/DnaJ/GrpE, ClpB and the small HspsIbpA/IbpB, were coordinately co-overproduced with RPs to optimize de novo folding. In a second step, protein biosynthesis was inhibited to permit chaperone mediated refolding of misfolded and aggregated proteins in vivo. This strategy, which has been patented, increased the solubility of 70% of 64 different heterologous proteins tested up to 42-fold. Interestingly, that strategy was efficient as most of the constructs used encoded proteins that were difficult to be produced insoluble form.[51]

INCLUSION BODIES: STRUCTURE AND MORPHOLOGY

IBs are protein aggregates with spherical or ovoid shapes, formed either in the cytoplasm or the periplasm. They are observed as refractile particles (usually one or two per cell) by optical microscopy and as electro-dense masses by transmission electron microscopy.[52,53] Soluble polypeptides can be extracted in vitro from IBs by denaturation and refolding sequential procedures[54] that permit to obtain soluble protein species through protein-tailored protocols. Interestingly, the arrest of protein synthesis in recombinant bacteria promotes the fast disintegration of IBs proving that they result from an unbalanced equilibrium between protein deposition and cell mediated protein removal, in which both chaperones and proteases are involved. This fact considered when designing in vivo protein recovery protocols.[51] Classical proteomics of IBs showed them to be relatively homogeneous in composition and mainly formed by the RP itself. Although occurring in variable proportions, the recombinant product can reach more than 90% of the total embedded polypeptides, which is a convenient protein supply for further in vitro refolding. The remaining material includes proteolytic fragments of the RP, traces of membrane proteins, phospholipids and nucleic acids, at least some of these being contaminants retained during the IB purification procedures. In *E. coli* IBs, the small heat-shock proteins IbpA and IbpB have been identified in addition to the main chaperones DnaK and GroEL.[55] The morphology of cytoplasmic inclusion bodies and its localization are illustrated (Fig. 6)

Inclusion Bodies: Amyloid-like Properties

It has been mentioned that bacterial inclusion bodies has amyloid like properties that mimic amyloid fibrils implicated in human associated diseases such as Alzheimer's disease, Parkinson disease, diabetes mellitus type II. Those aggregates have showed β sheet secondary structure that can react with amyloid specific dye such as congo red (CR). In addition

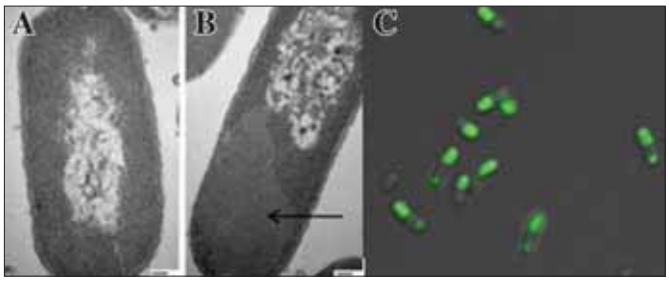

Figure 6. *Intracellular localization of the ELK16 fusion proteins in E. coli BL21 (DE3) cells. (A) Native Aspergillus fumigates amadoriase II, AMA. (B) AMA-ELK16.(ELK16-the peptide terminally fused to AMA). Arrow shows the newly formed inclusion body in the AMA-ELK16 cell. Size bars, 100 nm. (C) Intracellular localization of GFP-ELK16 in E. coli BL21, Size bars, 5 μm [56]*

to CR, it can react with more specific dye i.e., thioflavin S. Recently, the application of flow cytometry to detect thioflavin fluorescence provided fast, robust quantitative, noninvasive method to screen the presence of amyloid like aggregates with a great potential application in analysis of human aggregate diseases.[57-59] Functional and structural coincident traits of inclusion bodies resembling those of amyloid fibrils are shown (Table 2).

Table 2. Functional and structural coincident traits of inclusion bodies resembling those of amyloid fibrils.

Feature
High purity of the aggregates[60]
Aggregation mainly from folding intermediates [61,62]
Sequence specific aggregation[61,63]
Amyloid-tropic dye binding[61]
Seeding –driven aggragation[61]
Chaperones –modulated aggregation[64,65]
Aggregation propensities strongly affected by point mutations[66-68]
Reduced aggregation by stabilization of the native structure[69,70]
Aggregation hot spots[71]
Intermolecular, cross B-sheet organization or in general enrichment of β-structure of a fraction of IB protein species)[61,72]
Fibril like organization (of soluble protein aggregate)[73]
Enhanced proteolytic resistance (IB fraction)[74,75]
Protection from cytotoxicity[76]

Inclusion bodies: A potential vehicles of RP

Inclusion bodies may contain biologically active protein as reported recently.[77] Therefore, it is considered as novel type of RP delivery machines with potential applications in medicine. Recent study provided evidence that a polymeric cytoskeletal protein may be produced in the form of inclusion bodies and used in this form to supplement epithelial cells lacking it. The production was cost effective (cheap), non-toxic , and no particular purification step was needed.[78] Other studies conducted recently, revealed that inclusion bodies (50–500 nm) in recombinant bacteria, can be used to form tailored nanopills containing functional proteins. The nanopills were able to act as a novel natural protein delivery system for sustained drug release and advanced cellular therapy. These studies showed an affinity for biological membranes, and a high cellular penetrability, allowing the functional proteins to be efficiently internalized by mammalian cells (Fig. 7). This work has already been patented.[79-81]

Current insights into "IBS"

Recent studies[79-82] indicated new insights regarding molecular architecture of IBs. Here we would summarize the most recent

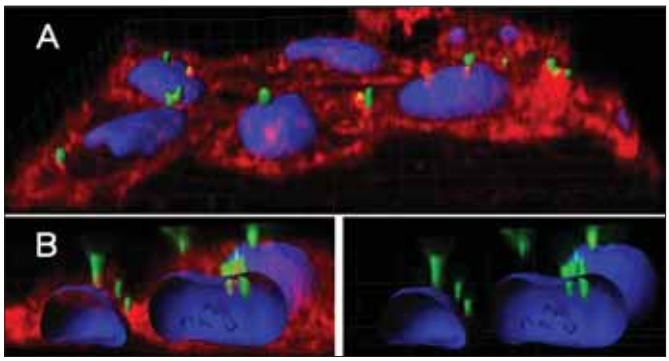

Figure 7. *A 40-Section confocal xyz stack of HeLa cells exposed to GFP IBs, showing IB cell penetrability. Cell membrane is labeled in red and the nuclear material is seen in blue. IBs are observed under their natural green fluorescence as discrete particulate entities. B. GFP IBs embedded or crossing the nuclear membrane are shown in two stack versions, in which the cell membrane is either shown (left) or hidden (right) for clarity.*[81]

issues concerning IBs:

- Inclusion bodies are highly dynamic and contain functional protein. Two important issues were recently mentioned (i) protein deposition in recombinant cells is fully reversible under certain circumstances and (ii) a relevant fraction of IB proteins is actually functional.
- Inclusion bodies are mechanically stable nanoparticulate materials
- Inclusion bodies can be used as naturally immobilized enzymes in biocatalysis
- IBs may be utilized as nanopills for drug delivery.
- IBs act as amyloid bionanomaterials model for the study of the human conformational diseases.

CONCLUSION

Despite, many alternative organisms and expression systems are now available for recombinant protein production, bacteria such as *E. coli* is still one of the most attractive host for the production of heterologous proteins. Todays, certain post-translational modification cannot be achieved in *E. coli*. New strategies for production of complex eukaryotic proteins in a prokaryotic expression system might become available in the near future.

Inclusion bodies that are still considered the major bottleneck for many therapeutic recombinant production in bacterial system. However recently it was found that a fraction of recombinant protein can be recovered from IBs in a functional form, and this is greatly varied from one protein to another. Although, it was reported that some soluble protein (in reality are soluble aggregates) with minimum activity but asa general term higher activity related to higher solubility. Despite inclusion bodies might be accepted to be used widely in near future as biocatalyzer and therapeutic nanopills, functional and soluble protein in appreciable quantities is still essential for therapeutic proteins and enzymes as well as for functional and structural studies.

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References

1. Devasahayam M. (2007). Factors affecting the expression of recombinant glycoproteins. *Indian J Med Res,* 126(1): 22-27.
2. Schumann W, Ferreira L (2004). Production of recombinant proteins in Escherichia coli. *G.a.M.B.,* 27(3):442–453.
3. Glick B, Whitney G (1987) F.a.t.e.o.f.p.i.E.*c. Journal of Industrial Microbiology*, 1(5):277–282.
4. Crowl R, et al. (1985). Versatile expression vectors for high-level synthesis of cloned gene products in Escherichia coli. *Gene*, 38(1-3): 31-38.
5. Brondyk WH (2009). Selecting an appropriate method for expressing a recombinant protein. *Methods Enzymol,* 463:131-147.
6. Widmann M, Christen P (2000). Comparison of folding rates of homologous prokaryotic and eukaryotic proteins. *J Biol Chem,* 275(25): 18619-18622.
7. Dyson MR, et al. (2004). Production of soluble mammalian proteins in Escherichia coli: identification of protein features that correlate with successful expression. *BMC Biotechnol,* 4: 32.
8. Brown TA, G.C.A.I., Wiley-Blackwell, 4th ed, 1995.
9. Baneyx F (1999). Recombinant protein expression in Escherichia coli. *Curr Opin Biotechnol,* 10(5): 411-421.
10. Francis DM, Page R (2010). Strategies to optimize protein expression in *E. coli. Curr Protoc Protein Sci,* 5(5):1-29.
11. Sørensen HP, Mortensen KK (2005). Advanced genetic strategies for recombinant protein expression in Escherichia coli. *Journal of Biotechnology,* 115(2):113-128.
12. Correa A, Oppezzo P (2011). Tuning different expression parameters to achieve soluble recombinant proteins in E. coli: Advantages of high-throughput screening. *Biotechnology Journal,* 6(6): 715-730.
13. Connell SR, et al. (2003). Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother,* 47(12): 3675-3681.
14. Graslund S, et al. (2008). Protein production and purification. *Nat Methods,* 5(2): 135-146.
15. Hannig G, Makrides SC (1998). Strategies for optimizing heterologous protein expression in *Escherichia coli. Trends Biotechnol,* 16(2): 54-60.
16. Xiao R, et al. (2010). The high-throughput protein sample production platform of the Northeast Structural Genomics Consortium. *J Struct Biol,* 172(1): 21-33.
17. Pan SH, Malcolm BA (2000). Reduced background expression and improved plasmid stability with pET vectors in BL21 (DE3). *Biotechniques,* 29(6): p. 1234-8.
18. Sorensen HP, Mortensen KK (2005). Advanced genetic strategies for recombinant protein expression in Escherichia coli. *J Biotechnol,* 115(2): 113-128.
19. Valdez-Cruz NA, et al. (2010). Production of recombinant proteins in E. coli by the heat inducible expression system based on the phage lambda pL and/or pR promoters. *Microb Cell Fact*, 9:18.
20. Menart V, et al. (2003). Constitutive versus thermoinducible expression of heterologous proteins in *Escherichia coli* based on strong PR,PL promoters from phage lambda. *Biotechnol Bioeng*, 83(2):181-190.
21. Hannig G, M.S.S.f.o.h.p. and e.i.E.c.T.B. 1998.
22. Kazemi-Esfarjani P, Benzer S (2000). Genetic suppression of polyglutamine toxicity in Drosophila. *Science*, 287(5459): 1837-1840.
23. Mogk A, et al. (1999). Identification of thermolabile *Escherichia coli* proteins: prevention and reversion of aggregation by DnaK and ClpB. *EMBO J,* 18(24): 6934-6949.
24. Motohashi K, et al. (1999). Heat-inactivated proteins are rescued by the DnaK.J-GrpE set and ClpB chaperones. *Proc Natl Acad Sci USA,* 96(13): 7184-7189.

25. Straus DB, Walter WA, Gross CA (1987). The heat shock response of E. coli is regulated by changes in the concentration of sigma 32. *Nature*, 329(6137): 348-351.

26. Lemaux PG, et al., *Transient rates of synthesis of individual polypeptides in E. coli following temperature shifts*. Cell, 1978. 13(3): p. 427-34.

27. Baneyx, F. and M. Mujacic, *Recombinant protein folding and misfolding in Escherichia coli.* Nat Biotechnol, 2004. 22(11): p. 1399-408.

28. Dobson, C.M., *Principles of protein folding, misfolding and aggregation.* Semin Cell Dev Biol, 2004. 15(1): p. 3-16.

29. Elena Garcìa-Fruitòs, et al., *Systems Biology and Biotechnology of Escherichia coli,* in *Systems-Level Analysis of Protein Quality in Inclusion Body-Forming Escherichia coli Cells* S.Y. Lee, Editor. 2009, Springer Link.

30. Sørensen HP, M.K.S.e.o.r.p.i.t. and c.o.E.c.M.C.F. 4;4(1):1.

31. Niiranen, L., et al. (2007). Comparative expression study to increase the solubility of cold adapted Vibrio proteins in *Escherichia coli. Protein Expr Purif,* 52(1): 210-218.

32. Vera A, et al. (2007). The conformational quality of insoluble recombinant proteins is enhanced at low growth temperatures. *Biotechnol Bioeng*, 96(6): 1101-1106.

33. Sahdev S, Khattar SK, Saini KS (2008). Production of active eukaryotic proteins through bacterial expression systems: a review of the existing biotechnology strategies. *Mol Cell Biochem,* 307(1-2): 249-264.

34. Khow O, Suntrarachun S (2012). Strategies for production of active eukaryotic proteins in bacterial expression system. *Asian Pacific Journal of Tropical Biomedicine*, 2(2):159-162.

35. Hunke S, Betton JM (2003). Temperature effect on inclusion body formation and stress response in the periplasm of Escherichia coli. *Mol Microbiol*, 50(5): 579-589.

36. Weickert MJ, et al. (1996). Optimization of heterologous protein production in *Escherichia coli. Curr Opin Biotechnol,* 7(5): 494-499.

37. Sorensen HP, Mortensen KK (2005). Soluble expression of recombinant proteins in the cytoplasm of *Escherichia coli. Microb Cell Fact,* 4(1): 1.

38. Fouchet P, et al. (1994). Flow cytometry studies of recombinant Escherichia coli in batch and continuous cultures: DNA and RNA contents; light-scatter parameters. *Appl Microbiol Biotechnol*, 41(5):584-590.

39. Lewis G, et al. (2004). The application of multi-parameter flow cytometry to the study of recombinant *Escherichia coli* batch fermentation processes. *J Ind Microbiol Biotechnol,* 31(7):311-322.

40. Weickert MJ, et al. (1999). A mutation that improves soluble recombinant hemoglobin accumulation in *Escherichia coli* in heme excess. *Appl Environ Microbiol*, 65(2): 640-647.

41. Yang Q, et al. (2003). High-level expression of a soluble snake venom enzyme, gloshedobin, in E. coli in the presence of metal ions. *Biotechnol Lett,* 25(8): 607-610.

42. Ghosh S, et al. (2004). Method for enhancing solubility of the expressed recombinant proteins in *Escherichia coli. Biotechniques*, 37(3): 422-423.

43. Deuerling E (2003). Trigger factor and DnaK possess overlapping substrate pools and binding specificities. *Mol. Microbiol,* 47: 1317-1328.

44. Mogk A, Mayer MP, Deuerling E (2002). Mechanisms of protein folding: molecular chaperones and their application in biotechnology. *Chembiochem*, 3(9): 807-814.

45. Kuczynska-Wisnik D, et al. (2002). The *Escherichia coli* small heat-shock proteins IbpA and IbpB prevent the aggregation of endogenous proteins denatured in vivo during extreme heat shock. *Microbiology*, 148(Pt 6):1757-1765.

46. Kitagawa M, et al. (2002). *Escherichia coli* small heat shock proteins, IbpA and IbpB, protect enzymes from inactivation by heat and oxidants. *Eur J Biochem,* 269(12): 2907-2917.

47. Nishihara K, et al. (2000). Overexpression of trigger factor prevents aggregation of recombinant proteins in *Escherichia coli. Appl. Environ. Microbiol.*, 66:884-889.

48. Ikura K, et al. (2002). Co-overexpression of folding modulators improves the solubility of the recombinant guinea pig liver transglutaminase expressed in *Escherichia coli. Prep Biochem Biotechnol,* 32(2):189-205.

49. Nishihara K, et al. (1998). Chaperone coexpression plasmids: differential and synergistic roles of DnaK-DnaJ-GrpE and GroEL-GroES in assisting folding of an allergen of Japanese cedar pollen, Cryj2, in *Escherichia coli*. *Appl. Environ. Microbiol.*, 64:1694-1699.

50. Amrein KE, et al. (1995). Purification and characterization of recombinant human p50csk protein-tyrosine kinase from an *Escherichia coli* expression system overproducing the bacterial chaperones GroES and GroEL. *Proc Natl Acad Sci U S A,* 92(4):1048-1052.

51. de Marco A, et al. (2007). *Chaperone-based procedure to increase yields of soluble recombinant proteins produced in E. coli. BMC Biotechnol,* 7: 32.

52. Carrio MM, Villaverde A (2005). Localization of chaperones DnaK and GroEL in bacterial inclusion bodies. *J Bacteriol,* 187(10): 3599-3601.

53. Bowden GA, Paredes AM, Georgiou G. (1991). Structure and morphology of protein inclusion bodies in *Escherichia coli*. *Biotechnology* (N Y), 9(8):725-730.

54. Vallejo LF, Rinas U (2004). Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins. *Microb Cell Fact*, 3(1):11.

55. Ventura S, Villaverde A (2006). Protein quality in bacterial inclusion bodies. *Trends Biotechnol,* 24(4):179-185.

56. Wu W, et al. (2011). Active protein aggregates induced by terminally attached self-assembling peptide ELK16 in *Escherichia coli. Microb Cell Fact,* 10:9.

57. Upadhyay AK, et al. (2012). Kinetics of inclusion body formation and its correlation with the characteristics of protein aggregates in *Escherichia coli. PLoS ONE*, 7(3): 29.

58. Espargaro A, Sabate R, Ventura S (2012). Thioflavin-S staining coupled to flow cytometry. A screening tool to detect in vivo protein aggregation. *Mol Biosyst,* 8(11): 2839-2844.

59. Wang L (2009). Towards revealing the structure of bacterial inclusion bodies. *Prion*, 3(3):139-145.

60. Carrio MM, Corchero JL, Villaverde A (1998). Dynamics of in vivo protein aggregation: building inclusion bodies in recombinant bacteria. *FEMS Microbiol Lett*, 169(1): 9-15.

61. Carrio MM, et al. (2005). Amyloid-like properties of bacterial inclusion bodies. *J Mol Biol,* 347(5):1025-1037.

62. Chrunyk BA, et al. (1993). Inclusion body formation and protein stability in sequence variants of interleukin-1 beta. *J Biol Chem*, 268(24):18053-18061.

63. Speed MA, Wang DI, King J (1996). Specific aggregation of partially folded polypeptide chains: the molecular basis of inclusion body composition. *Nat Biotechnol,* 14(10):1283-1287.

64. Carrio MM, Villaverde A (2003). Role of molecular chaperones in inclusion body formation. *FEBS Lett.,* 537:215-221.

65. Thomas JG, Ayling A, Baneyx F (1997). Molecular chaperones, folding catalysts, and the recovery of active recombinant proteins from *E. coli.* To fold or to refold. *Appl Biochem Biotechnol*, 66(3):197-238.

66. Izard J, et al. (1994). A single amino acid substitution can restore the solubility of aggregated colicin A mutants in *Escherichia coli. Protein Eng,* 7(12):1495-1500.

67. Wetzel R, Perry LJ, Veilleux C 91991). Mutations in human interferon gamma affecting inclusion body formation identified by a general immunochemical screen. *Biotechnology*, 9(8):731-737.

68. Nieba L, et al. (1997). Disrupting the hydrophobic patches at the antibody variable/constant domain interface: improved in vivo folding and physical characterization of an engineered scFv fragment. *Protein Eng,* 10(4):435-444.

69. Yan G, et al. (2003). A single residual replacement improves the folding and stability of recombinant cassava hydroxynitrile lyase in *E. coil. Biotechnol Lett,* 25(13):1041-1047.

70. Chan W, et al. (1996). Mutational effects on inclusion body formation in the periplasmic expression of the immunoglobulin VL domain REI. *Fold Des,* 1(2):77-89.

71. Tenidis K, et al. (2000). Identification of a penta- and hexapeptide of islet amyloid polypeptide (IAPP) with amyloidogenic and cytotoxic properties. *J Mol Biol,* 295(4):1055-1071.

72. Przybycien TM, et al. (1994). Secondary structure characterization of beta-lactamase inclusion bodies. *Protein Eng,* 7(1):131-136.

73. Schrodel A, de Marco A (2005). Characterization of the aggregates formed during recombinant protein expression in bacteria. *BMC Biochem,* 6:10.

74. Carrio MM, Cubarsi R, Villaverde A (2000). Fine architecture of bacterial inclusion bodies. *FEBS Lett,* 471(1):7-11.

75. Cubarsi R, Carrio MM, Villaverde A (2001). *In situ* proteolytic digestion of inclusion body polypeptides occurs as a cascade process. Biochem *Biophys Res Commun,* 282(2):436-441.

76. Gonzalez-Montalban N, et al. (2005). Bacterial inclusion bodies are cytotoxic in vivo in absence of functional chaperones DnaK or GroEL. *J Biotechnol,* 118(4):406-412.

77. Peternel S, Komel R (2010). Isolation of biologically active nanomaterial (inclusion bodies) from bacterial cells. *Microb Cell Fact,* 9:66.

78. Liovic M, et al. (22012). Inclusion bodies as potential vehicles for recombinant protein delivery into epithelial cells. *Microb Cell Fact,* 11:67.

79. Vazquez E, et al. (2012). Functional inclusion bodies produced in bacteria as naturally occurring nanopills for advanced cell therapies. *Adv Mater,* 24(13):1742-1747.

80. Villaverde A (2012). Bacterial inclusion bodies: an emerging platform for drug delivery and cell therapy. *Nanomedicine* (Lond), 7(9):1277-1279.

81. Villaverde A, et al. (2012). Packaging protein drugs as bacterial inclusion bodies for therapeutic applications. *Microb Cell Fact,* 11(1):76.

82. Garcia-Fruitos E, et al. (2012). Bacterial inclusion bodies: making gold from waste. *Trends Biotechnol,* 30(2):65-70.

Study Visit to Beijing and The Forbidden City International Pharmacist Forum 2013

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On 9-May-2013, the Pharmaceutical Society of Hong Kong (PSHK), led by Mrs. Mary Cheng, President of the Pharmaceutical Society of Hong Kong, and a delegation of 12 pharmacists and 12 students attended the Forbidden City International Pharmacist Forum in Beijing. This year, we had 6 pharmacists attending as speakers who were Ms. Chiang Sau Chu, Mr. Philip Kwok, Ms. Ritchie Kwok, Prof. Vivian Lee, Mr. Peter Suen and Dr. Keary Zhou. Mrs. Mary Catherine Cheng and Mr. Benjamin Kwong acted as moderators for several sessions. Since 2011, PSHK has sponsored pharmacy students partially to attend the conference to broaden their vision on pharmacy practice outside Hong Kong. Whenever possible, PSHK will arrange visits to hospitals and industry sites in Beijing. The delegation arrived Beijing in the evening of 9-May-2013 and were driven to the Conference venue, at Jiu Hua Resort and Convention Centre.

Before departure

Ms. Chiang Sau Chu as speaker

Mr. Benjamin Kwong as moderator

Mr.Philip Chiu as speaker

Dr. Keary Zhou, Ms. Ritchie Kwok & Prof. Vivian Lee are speakers at the Forum

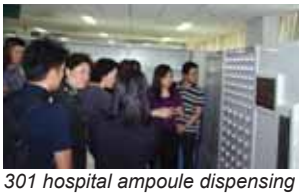
Ms. Caroline Ung, Prof. Vivian Lee, Mr. Ivan Ng & Mr. Peter Suen

VISIT TO THE 301 HOSPITAL

On 10-May-2013, the group visited the General Hospital of the People's Liberation Army, which is conventionally known as the 301 hospital. Founded in 1953, it has been developed into a first-class large-scale modern hospital with 125 departments with state-of-the-art equipment, and 4000 patient beds. It serves medical care, research and education purposes. We were met by Ms. Guo Dai Hong, the head of the Department of Pharmaceutical Care and her colleagues, all in army uniforms, to visit the outpatient and inpatient pharmacy dispensaries.

The drug distribution system of the 301 hospital is more advanced than that in Hong Kong in the sense that informatics and automations are incorporated and integrated as an inseparable system. Take the inpatient service as an example, the physician enters an electronic prescription in the ward after rounding. The prescription is subject to preliminary vetting by nurses and is then sent down to the centralised inpatient pharmacy and checked by a pharmacist. Oral medications are packed in a unit dose (air-tight plastic packaging) with barcode labels on them. Ampoules and vials are dispensed by an automated dispensing machine. Different medications of the same patient are put into the same box container labeled with a number which corresponds to the patient bed number. The box containers with medications, in turn, are aligned in a larger tray in an orderly manner. Finally, after scanning the barcodes and verifying all the medications, a trolley helps deliver the correct drugs to the correct ward. The Pharmacy intravenous admixture services (PIVAS) has been established in the 301 hospital. They have established a system to ensure the quality and safety of the PIVAS. We were all very impressed by the usage of information technology in the 301 hospital.

301 hospital-looking at records of drug administration



301 hospital ampoule dispensing

301 hospital inpatient dispensing

After the visit to the 301 Hospital , we had lunch at The Auspicious Restaurant(大宅門) in Changping. It is nicely decorated with Chinese furniture and the food was excellent and reasonably priced. We spent about REM120 each. The address is: 23, Fuxue Road, Changping.

Auspicious Restaurant

Lunch at Auspicious Restaurant

VISIT TO BEIJING NOVARTIS PHARMACEUTICAL CO., LTD.

Beijing Novartis Pharmaceutical Co., Ltd. was established in 1987 in Beijing. It is one of the first and largest foreign pharmaceutical companies in China. Novartis products in China covers cardiovascular, endocrine, anti-infective, oncology, transplantation immunology, rheumatic pain, bone metabolism, eye, central nervous system and other 9 major fields.

In the afternoon of 10-May-2023, we visited Novartis factory in Changping Science and Technology Park. The Beijing Novartis factory covers an area of 28,000 square meters. It is an international GMP standard clean plant equipped with a full set of advanced production equipment and facilities from Europe, with oral solid dosage forms (tablets, capsule) production line, ointments production line, equipped with aluminum-aluminum, aluminum-plastic packaging production line, automatic purified water preparation facilities and rotating biological wastewater treatment facilities. It has the annual production capacity of 2 billion (capsules) and 12 million Latex agent. The Beijing Novartis factory is one of Novartis global standardization GMP factories, and is monitored by the uniform Novartis global pharmaceutical technology department of technology and quality management. It is a good opportunity for the pharmacists and students to visit and learn about the GMP factory.

Group photo at Beijing Novartis

THE FORBIDDEN CITY INTERNATIONAL PHARMACIST FORUM

The Forbidden City International Forum officially began on 10-May-2013. There were over 100 specialists as speakers and this year 2400 pharmacists from 16 countries and regions including USA, Japan, Germany, Sweden, Malaysia, India, China, Taiwan, Hong Kong and Macao participated. Many pharmacists from China participated in the Conference which became a platform for them to learn from international pharmacists and a chance to practice their presentation skills. It commenced with a short speech from the different leaders of the Organization Committee. As a token of appreciation for the effort, support and hard work of leaders of societies, associations, magazines and academic members, this year, there was a "Special Contribution Award" presented to 6 pharmacists selected by the Organization Committee. We are proud that Mrs. Mary Catherine Cheng, President of the Pharmaceutical Society of Hong Kong was one of the 6 pharmacists to receive the award, along with Professor Dale E. English from US North East Ohio Medical University, Professor Tong Rong-Sheng from Sichuan Provincial People's Hospital, Professor Hao Lizhi Miyun from County Hospital Pharmacy, Professor Takao ORII from NTT East Kanto Hospital and Professor Xie Youwen from Affiliated Hospital of China Medical University.

Mary's Special Contribution award

After the opening ceremony, there were four keynote presentations before lunch. The parallel sessions started in the afternoon. At the first night of the Conference, there was a cocktail and welcome dinner for all participants. A variety of Beijing dishes were served. Pharmacists from different countries and regions gave a performance including dancing, singing, kung fu, etc. It was full of fun and laughter. The delegation from Hong Kong sang two songs: "The Pearl of the Orient" and "Do not hesitate" by Beyond. We have made a DVD as background showing familiar faces of Hong Kong pharmacists from the episodes of the past Hong Kong Pharmacy Conferences. We dressed in red and black, and sang and danced to the music. It was a relaxed atmosphere. We were able to meet pharmacists from all over the world.

Prof. Zhao Zhi-gang (Conference Chair), Mrs. Cheng Mary (President, Pharmaceutical Society of HK) & Hu xin (Director of Dept of Pharmacy, Beijing Hospital)

Gruop photo at Conference dinner

The theme of the Forbidden City Forum this year is "Pharmacist. Team medication. Society contribution". The topics and discussions were centred around "The Value of Pharmacists in Drug Safety Evaluation and Management, The Value of Pharmacists in the Drug Therapy Safety and Management of Chronic Diseases, The Value of Pharmacists in TDM, Pharmacogenomics and Personalized Medicine, The Value of Pharmacists in the Rational Use of Traditional Chinese Medicine, The Implementation Path of Pharmaceutical Services and Value of Pharmacists in Community Pharmacies, The Pharmaceutical Care Information and Automated Pharmacy, The Value of Pharmacists in the Rational Use and Management of Medicines in Primary Care Institutions. There were three to four parallel sessions for both days, which made it rather difficult to choose which session to attend. The students distributed themselves to attend different sessions so that they can write a summary of the presentation. The following are brief summaries of the sessions attended.

Using the Risk Evaluation and Mitigation Strategies (REMS) Program in Patient Care

While it has been clear that medication safety is an essential issue in healthcare provision, the way to achieve an appropriate standard of safety requires a lot of investigation, judgment and planning. In the session, Mr. L. Arneson from the US introduced some risk management approaches applicable in health care, giving a glance at how risk in pharmacy practice can be evaluated and mitigated.

Risk can be regarded as "detectable hazard" – something predictable. And the "hazards" very often arise from single point weaknesses, which can be, in general, classified into 4 categories along the whole drug-use process: prescribing, dispensing, administration and monitoring. Mr. Arneson also divides the causes into 7 modes – ineffective or inefficient process, variation in process, communication gap, staffing limitation, policy defect, equipment failure and knowledge gap. These imply that a complete risk management approach may require a huge amount of work, since accidents can arise from tiny errors of various modes.

Unfortunately, due to limited time and resources, only some risks can be mitigated. To access the necessity to attenuate a certain risk, the severity of its consequence and the probability of failure may be compared. Severe consequences associated with high probabilities must not be accepted, while mild consequences with small chances may be accepted. Besides, catastrophic consequences cannot be accepted even with a very low probability. To draw conclusion from such comparison, effort put into quantifying or scaling severity and probability is required.

Once a risk is recognized, a risk management strategy will be launched. There are a number of models listing the steps in risk management. Generally, they are: defining the topic → team assembling → risk analysis → risk evaluation → action plan → strategies to mitigate risk → risk reduction and strategy re-evaluation → risk acceptance (since risk cannot be completely eliminated). The process may be repeated until the risk is acceptable. During the process, to assess the severity and probability of a particular event, methods like direct observation, surveillance and voluntary reporting systems can be utilized. However, there are some common hurdles against risk management including avoidance, denial, nonchalance, responsibility denial and procrastination. These are some intrinsic factors due to human behavior which are more difficult to get rid of.

Trying to support the value of risk management, Mr. Arneson also mentioned *The FDA Amendment Act of 2007*, which gave FDA the authority to require a *Risk Evaluation and Mitigation Strategies (REMS)* report from drug manufacturers to ensure that the benefit of the drug or biological product outweighs its risk. It has been proved effective in controlling the quality of the drug products.

Although risk management skills seem not closely related to drug knowledge, it plays a big role in pharmacy as a profession. Therefore, pharmacists should make use of their unique experiences in their own positions to help reduce the risk involved in daily practices, in order to promote rational drug use to ensure patient safety.

Roles of Pharmacists in Primary Health Care – Experience from Macao

The value of pharmacists in the rational use and management of medicines in primary care institutions has long been an issue in Hong Kong. As neighbours separated only by a strip of water, the other special administrative region, Macau, faces the same problem as Hong Kong does. After listening to the speech delivered by Carolina UNG, we can have a more comprehensive view on the problems that Macau is facing, which may help to facilitate the system in Hong Kong.

Currently, there are around 300 pharmacists in Macau. More than half of them are working in community pharmacies and the pharmacist-to-patient is about 1:1800. This ratio seems to be fine at first glance. However, some of the pharmacists act as businessmen, rather than health care providers due to the current situation. In 2008, in response to the surging need of pharmacy services, the "three hundred meters" ordinance was cancelled by the Health Bureau of Macau. This contributes to the severe competition among pharmacies in Macau, and more critically, the services

provided by pharmacists become more fluctuating since pharmacists have to spare their thoughts on their business. Pharmacists become more like businessmen, rather than health care providers.

Another problem that pharmacists in Macau come across is that pharmacists do not have adequate reputation among citizens. T e role of pharmacists is very limited as a result. To combat the problem, an outreach program cooperated with an emergency call centre was established in 2010. About 20 pharmacist volunteers conducted home drug safety evaluations for elderly patients. This undoubtedly raises the reputation of pharmacists.

In the future, cross cooperation among health care providers has to be strengthened and pharmacists must take a more active role in direct patient care. Perhaps Hong Kong and Macau can learn from each other and strive for the better future together.

The value of pharmacists in the rational use of traditional Chinese medicine

The role of pharmacist in Chinese medicinal use has been quite well established in Mainland China. Just like system in western medicine, there are TCM clinical pharmacy, TCM pharmacovigilance programme and even analysis on TCM injection. It is clearly understood that combination use of TCM and western medicine in treatment or prophylaxis is becoming more and more prevalent in both HK and Mainland China. Hence, more drug-drug interaction are expected. However, sector of TCM pharmacists are not well developed in HK. The development of this in the Mainland could be a good role model for Hong Kong.

The value of pharmacists in TDM, pharmacogenomics and personalized medicine

Pharmacogenomics has become more and more important in therapeutic drug monitoring nowadays and it can be applied as genetic biomarker for drug response. For example, the therapeutic efficacy of clopidogrel depends on CYP2C19 gene expression, which is the key enzyme that converts the prodrug to its active metabolite. If the patient has inactive gene expression of CYP2C19, the drug will have less or even no effect on patient which could worsen the disease state, causing thrombosis. Therefore, it is important to do the gene test before prescribing this kind of drugs.

To promote its clinical use, it is suggested that pharmacogenomics alert could be incorporated into the computerized drug prescription system when prescribed. In addition, it is recommended to sample all the patients on certain genetic information so that they can be prescribed with these drugs with known genotype immediately, instead of waiting for the gene test result. It is more pre-emptive. However, it is not practical at the current situation as the cost is too high.

Warfarin patient education before discharge

Continuous monitoring and comprehensive patient education by pharmacists to patients taking warfarin are required worldwide due to its narrow therapeutic index. In Taiwan, the China Medical University Hospital provided a series of warfarin patient education and counseling since August 2010. Like Hong Kong, pharmacists in Taiwan warfarin clinic also assess the electronic medical records and provide counseling upon referral by primary care nurses. As for the training of internship pharmacists, warfarin information, EMRs, patient education and counseling processes are trained and the training outcome is evaluated by exercises and exams.

The role of pharmacists in ensuring safety use of warfarin is of high importance. Across the border, mainland and Taiwan also set up warfarin clinics alike to provide patient counseling and education. In Taiwan, the training of pharmacy students in warfarin use is more emphasized and comprehensive. This reflects that the involvement of clinical aspects by pharmacists in Taiwan is more significant than here in HK, and HK can take the practice of other places as reference.

Combined Use of TCM and Western Medicine

It is a common phenomenon that people in Hong Kong used to consult both western medicine specialists and Chinese medicine practitioners, bringing a great concern for drug safety of combination of TCM and western medicine. As such combination may vary the therapeutic effect and toxicity, it can be both beneficial and dangerous to the patients.

This is why the talk “Role of Pharmacist in the Safety of Combination of TCM and Western Medicine”, conducted by Dr. Yang Li-Ping from Beijing have drawn my interest. Dr. Yang had mentioned the drug nature is classified by the favours in Chinese Medicine perspective, which can be cofounded to active ingredients carrying these favours, e.g. “sour” favour is co-related with acidic ingredients while “bitter” favour is co-related with volatile oil substance in herbs. This classification is different from the concept of Western medicines we focus on during study. The speaker also suggested that interactions between TCM and western medicine may be predicted by their drug natures. It implies acquiring fundamental concept and knowledge of Chinese Medicine would help identifying interactions.

There are many concerns about the combined use of TCM and western medicines. Pharmacists play an important role in discussing both its potential value and to safeguard patient safety. A more in-depth study in traditional Chinese Medicine would be very helpful.

Evaluate TCM injection from Scientific Perception

TCM injection is popular in Mainland China. It is not difficult to see TCM injection in any Chinese hospitals as Chinese Medicine is one of the major approaches of treatment in Mainland China. After the appearance of the first TCM injection in 1940, the development remains rapid and drastic. However, the development of such injection is not as advanced as expected. According to Wang Wan-Long, a speaker from Tianjin, most injections are still the alcohol or water extractions of the herbs. The exact active ingredients may still be unknown. Only 6 types of TCM injections are pure active ingredients of the herbs. From the above statistic, we can see that the TCM injections are still full of unknowns.

The speaker mentioned an important point that worth discussing – is injection form really necessary for all types TCM? Injection can bring quick relief to patients and it is useful in emergencies. However, in recent years, the development has been going in a wrong direction. The speaker demonstrated that it is not worthy to bare risk of severe side effects of injections such as allergy in exchange for a quicker release of 2-3 hours. So many TCM injections have now been considered as "unnecessary". The only necessary ones are those which cannot be an oral drug as they may degrade rapidly in GI tract, or those that are really needed in emergencies. TCM injection is a breakthrough in Chinese Medicine. But it is time to adjust its direction of development to benefit the patients in a greater extent.

CONCLUSION

The three days passed by very quickly. The Forbidden City International Forum enables us to learn about the development of the Pharmacy Practice in Mainland China and other countries. There are certain areas that we are more advanced than our China counterparts, such as pharmaceutical care and clinical pharmacy. But on the other hand, there are also areas that they are leaping quickly ahead of us such as the use of informative technology in drug delivery and the use of TCM and western medicines for the benefit of patients. We believe that closer partnership and exchange of ideas will enable better pharmacy practice in both Hong Kong and Mainland China for better health outcomes of patients.

The Society of Hospital Pharmacists (SHPHK) Office Bearers and Subject Officers 2013-2014:

Appointment of Office Bearers and Subject Officers

President	CHUI Chun Ming William
Vice President	CHAN Wing Lam Phoebe
Secretary	CHU Man Wa Amy
Treasurer	LAI Oi Lun Ellen

Subject Officer

Member Relations	LING Ho Ming Michael
Clinical Forum	CHAN Wing Lam Phoebe KWOK Ching Chi Ritchie
DERC	LAM Po Yu Daisy SO Yiu Wah
Pharmaceutical Journal	WONG Sze Ho Johnny
Membership	HUI Hoi Yun Helen
PCCC	CHUNG Wing Fai Kenneth CHUI Chun Ming William
IT	NG Man Keung
Publication (Newsletter)	WONG Kai Chung Vincent CHUNG Wing Fai Kenneth KWOK Ching Chi Ritchie

The 42nd Term Practising Pharmacists Association of Hong Kong (PPAHK) General Council

President:	Chang, Iris Jacqueline
First Vice-President:	CHAN, Lok Hang Tom
Second Vice-President:	LAW, Chun Kelvin
Honorary Treasurer:	Yung, Wai Lan Anna
Honorary Secretary:	Tang, Kin Ching Bernard

Council Members:	
CHAN, Ka Chung Jacky	HO, Yin Chung James
CHAN, Cho Hung Philip	YIU, Vilma M.
SUNG, Kai Wing Christy	CHUNG, Wai Kei Rachel
CHAN, Siu Tong Yvonne	WONG, Hing Mang Mathew
POON, Poe Meng Steve	FONG, Ka Man Carmen

Inauguration of the College of Pharmacy Practice
藥劑專科學院

Reported by The College of Pharmacy Practice

Witnessed by guests from the government, Hospital Authority, universities and patient groups, the College of Pharmacy Practice ("CPP") is inaugurated on 22nd June 2013. The ceremony was officiated by the guest of honor, Professor Sophia Chan, the Under Secretary for Food and Health.

Professor Chan congratulated the CPP and delivered a very inspirational keynote speech. She shared her views on the rising public expectation towards pharmaceutical care delivery and the importance of specialization of pharmacy practice. Specialization in specific disease and practice areas with structured continuing education would be essential to raise the confidence of patients, physicians and other stakeholders. Professor Chan also remarked that specialized knowledge is not only limited in clinical pharmacy service, but also in community pharmacy, drug manufacturing and distribution, drug regulatory, teaching and research, etc. Professor Chan believed that the development of pharmacy specialties would be crucial in achieving the goal of ensuring the delivery of the most cost-effective, focused and safe drug treatment to patients.

The CPP announced in the ceremony that Dr. Benjamin Lee is elected as the new chairman of CPP. Professor Vincent Lee of the Chinese University of Hong Kong, who is also the founding chairman of the CPP, and Professor Ian Wong of the University of Hong Kong are bestowed with the Honorary Fellowships. The CPP also awarded twelve founding members with the Founding Fellowships and eleven distinguished pharmacists as the first batch of Fellows of the CPP.

History and Background of the CPP

Under the visionary leadership of Professor Vincent Lee and Professor Kenneth Lee, the CPP was formed as a working group in year 2009. It aimed to investigate ways for advancing the scope of practice of local pharmacists in a formal and systematic way. With the hard work of a group of dedicated pharmacists, the CPP was formally established in September 2010.

The CPP is established as a non-profit, independent professional body of pharmacists that missioned to ensure the highest possible professional standard of pharmacy practice in Hong Kong by providing formal accreditation of specialist training. The ultimate goal of the CPP would be legislative change rendering the CPP to be recognized as an independent accreditation and recognizable professional qualifications granting statutory body for the advanced practice of the pharmacy profession in Hong Kong.

Having collected the views of Hong Kong pharmacists from an open forum and on-line surveys, the CPP has formulated the accreditation framework for its members to become pharmacist specialists or fellows of the CPP. The CPP has also kicked-off with the "Advanced Pharmacotherapy Workshop", a therapeutics review course that facilitates local pharmacists' preparation for the certification examination provided by the US Board of Pharmacy Specialties.

Making Enquiry to the CPP

For membership issue or other enquiries, please visit the website of College of Pharmacy Practice http://www.cpp.org.hk or email to admin@cpp.org.hk.

Distinguished guests

From Left to Right: Mr Ivan NG, Dr Wai-sin CHAN, Dr Pak-yin LEUNG and Mr Patrick HO

Professor Sophia Chan

Officiating Guests and First Batch of Fellows of CPP

Officiating Guests, Honorary Fellows and Founding Fellows of CPP

BARIÉDERM CREAM

Barrier & reconstructive cream for HAND DERMATITIS

Formulated with

Poly-2p

Indication
Acute & chronic irritative dermatitis, allergic dermatitis

Composition
Uriage Thermal Water 10%, Poly-2p complex 2% (Phosphorylcholine polymer + Pyrrolidone polymer), Plant Squalane 1%, Plant Sterols 0.5% & Glycerin 2%

Product mechanism and features
Patented **Poly-2p** uniquely forms an air-permeable, waterproof film over the hand skin, against invasion of allergens (e.g. water, alcohol, latex). The film also protects and reconstructs hand skin. This barrier film is intact even after 10 rinses.

Uriage Thermal Water 10% is clinically proven effective to have hydrating, soothing, anti-pruritus, anti-inflammatory and healing effects on dry, sensitive & atopic skin

Plant Squalane restores the hydrolipidic surface of hand skin. **Plant Sterols** strengthens the intercorneocyte cement. **Glycerin** moisturizes & soothes all dryness symptoms

Hypoallergenic – Fragrance free – Paraben free – Non-comedogenic

Dosage
Apply as often as necessary on the area of the skin to be isolated, protected or repaired. Suitable for both children and adults – Hand, face and body areas.

Manufacturer & origin
Product of Laboratoires Dermatologiques d'Uriage, France.
Made in France.

Reference
1. Bariéderm cream in the management of contact dermatitis. Toni Marius A. Ionesco, MD, PhD, Agnès GOUGEROT, MD. Saint-Louis Hospital, Paris. Saint Denis Hospital. Laboratoires Dermatologiques d'Uriage
2. Barrier cream in a series of 20 cases of chronic contact dermatitis. Prof Dominique Tennstedt, MD, PhD, Bruxelles. Dermatology Congress SIDAPA, Rome, October 2005

Distributor:

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Product Enquiry: 2774 8385

Ad.Bariederm cream.Hand.Wellmark.130301

VICTRELIS
(MERCK SHARP & DOHME (ASIA) LIMITED)

Prepared by Eric Lee and Lucilla Leung

Product Name:
VICTRELIS 200mg Capsule

Presentation:
Each capsule contains 200mg of boceprevir

Pharmacology:
Boceprevir is an inhibitor of the HCV NS3 protease. Boceprevir covalently, yet reversibly, binds to the NS3 protease active site serine (Ser139) through a (alpha)-ketoamide functional group to inhibit viral replication in HCV-infected host cells.

Indications:
Treatment of chronic hepatitis C (CHC) genotype 1 infection, in combination with peginterferon alfa and ribavirin, in adult patients with compensated liver disease who are previously untreated or who have failed previous therapy.

Dosage and Administration:
Treatment with Victrelis should be initiated and monitored by a physician experienced in the management of chronic hepatitis C.

Posology
Victrelis must be administered in combination with peginterferon alfa and ribavirin. The Summary of Product Characteristics of peginterferon alfa and ribavirin (PR) must be consulted prior to initiation of therapy with Victrelis.

The recommended dose of Victrelis is 800 mg administered orally three times daily (TID) with food (a meal or light snack). Maximum daily dose of Victrelis is 2,400 mg. Administration without food could be associated with a net loss of efficacy due to sub-optimal exposure.

Patients without cirrhosis who are previously untreated or who have failed previous therapy, all cirrhotic patients and null responders
Dosing recommendations and treatment duration differ for each group or some subgroups. Please refer to product circular of Victrelis for details.

Missed doses
If a patient misses a dose and it is less than 2 hours before the next dose is due, the missed dose should be skipped.
If a patient misses a dose and it is 2 or more hours before the next dose is due, the patient should take the missed dose with food and resume the normal dosing schedule.

Dose reduction
Dose reduction of Victrelis is not recommended.
If a patient has a serious adverse reaction potentially related to peginterferon alfa and/or ribavirin, the peginterferon alfa and/or ribavirin dose should be reduced. Victrelis must not be administered in the absence of peginterferon alfa and ribavirin.

Special populations
Renal impairment
No dose adjustment of Victrelis is required in patients with any degree of renal impairment.

Hepatic impairment
No dose adjustment of Victrelis is required for patients with mild, moderate or severe hepatic impairment. Victrelis has not been studied in patients with decompensated cirrhosis.

Paediatric population
The safety and efficacy of Victrelis in children aged below 18 years have not yet been established. No data are available.

Elderly
Clinical studies of Victrelis did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other clinical experience has not identified differences in responses between the elderly and younger patients.

Method of administration
To obtain the capsules the foil of the blister should be peeled off. Victrelis is to be taken orally with food (a meal or light snack).

Contraindications:
Victrelis, in combination with peginterferon alfa and ribavirin, is contraindicated in:

- Patients with hypersensitivity to the active substance or any of its excipients.
- Patients with autoimmune hepatitis.
- Co-administration with medicines that are highly dependent on CYP3A4/5 for clearance, and for which elevated plasma concentrations are associated with serious and/or life-threatening events such as orally administered midazolam and triazolam, bepridil, pimozide, simvastatin, lovastatin, lumefantrine, halofantrine, tyrosine kinase inhibitors, and ergot derivatives (dihydroergotamine, ergonovine, ergotamine, methylergonovine).
- Pregnancy.

Warnings:
Anaemia
The onset of anaemia has been reported with peginterferon alfa and ribavirin therapy by Treatment Week 4. The addition of Victrelis to peginterferon alfa and ribavirin is associated with an additional decrease in haemoglobin concentrations of approximately 1 g/dl by Treatment Week 8 compared to standard of care. Complete blood counts should be obtained pretreatment, Treatment Week 4, Treatment Week 8, and thereafter, as clinically appropriate. If haemoglobin is < 10 g/dl (or < 6.2 mmol/l) management of anaemia may be warranted.

Neutropenia
The addition of Victrelis to peginterferon alfa–2b and ribavirin resulted in higher incidences of neutropenia and Grade 3-4 neutropenia compared with peginterferon alfa–2b and ribavirin alone.

The frequency of severe or life threatening infections tends to be higher in Victrelis-containing arms than the control arm. Neutrophils counts should therefore be evaluated before treatment initiation and regularly thereafter. Prompt evaluation and treatment of infections is recommended.

Combined use with peginterferon alfa–2a as compared to alfa–2b:
As compared to the combination of Victrelis with peginterferon alfa–2b and ribavirin, the combination of Victrelis with peginterferon alfa–2a and ribavirin was associated with a higher rate of neutropenia (including grade 4 neutropenia) and a higher rate of infections.

Drospirenone-containing medicines
Caution should be exercised in patients taking drospirenone-containing medicines with conditions that predispose them to hyperkalaemia or patients taking potassium-sparing diuretics. Alternative contraceptives should be considered

Use in prior null responders
Based on a retrospective analysis performed with requalifying on the basis of their on treatment virologic response at treatment week 4 (using the peginterferon alfa/ribavirin lead in period) as compared to baseline, null responders might gain some benefit in adding Victrelis to the bitherapy. However, this cannot be reliably quantified from the retrospective analysis. Moreover, the optimal management of null responders remains to be established and might in the future require antiviral combination.

HCV protease monotherapy
Based on results of clinical studies, Victrelis must not be used alone due to the high probability of increased resistance without combination anti-HCV therapies. It is unknown what effect therapy with Victrelis will have on the activity of subsequently administered HCV protease inhibitors, including re-treatment with Victrelis.

Use in patients with HIV co-infection
The safety and efficacy of Victrelis alone or in combination with peginterferon alfa and ribavirin for the treatment of chronic hepatitis C genotype 1 infection have not been established in patients co-infected with Human Immunodeficiency Virus (HIV) and HCV.

Use in patients with an organ transplant
The safety and efficacy of Victrelis alone or in combination with peginterferon alfa and ribavirin for the treatment of chronic hepatitis C genotype 1 infection in liver or other organ transplant recipients have not been studied.

Use in patients having HCV genotypes other than genotype 1
The safety and efficacy of Victrelis alone or in combination with peginterferon alfa and ribavirin for the treatment of chronic hepatitis C genotypes other than genotype 1 have not been established.

Use in patient who have previously failed treatment with an HCV protease inhibitor
The safety and efficacy of Victrelis alone or in combination with peginterferon alfa and ribavirin for the treatment of chronic hepatitis C genotype 1 infection has not been studied in patients who have failed previous therapy with Victrelis or other HCV protease inhibitors.

Potent CYP3A4 inducers
The concomitant use of Victrelis with potent CYP3A4 inducers (rifampicin, carbamazepine, phenobarbital, phenytoin) is not recommended.

Use in patients with rare hereditary disorders
Victrelis contains lactose. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine.

Proarrhythmic effects:
Caution in patients at risk of QT prolongation (long congenital QT, hypokalaemia).

Interaction:
Victrelis is a strong inhibitor of CYP3A4/5. Medicines metabolized primarily by CYP3A4/5 may have increased exposure when administered with Victrelis, which could increase or prolong their therapeutic and adverse reactions (see Table 2). Victrelis does not inhibit or induce the other enzymes of the CYP450.

Boceprevir has been shown to be a P-gp and breast cancer resistant protein (BCRP) substrate in vitro. There is potential for inhibitors of these transporters to increase concentrations of boceprevir; the clinical implications of these interactions are not known.

Victrelis is partly metabolized by CYP3A4/5. Co-administration of Victrelis with medicines that induce or inhibit CYP3A4/5 could increase or decrease exposure to Victrelis.

Victrelis, in combination with peginterferon alfa and ribavirin, is contraindicated when co-administered with medicines that are highly dependent on CYP3A4/5 for clearance, and for which elevated plasma concentrations are associated with serious and/or life-threatening events such as orally administered midazolam and triazolam, bepridil, pimozide, simvastatin, lovastatin, lumefantrine, halofantrine, and tyrosine kinase inhibitors, and ergot derivatives (dihydroergotamine, ergonovine, ergotamine, methylergonovine).

Rosuvastatin is not recommended to use with boceprevir.

Boceprevir is primarily metabolized by aldoketo reductase (AKR). In medicine interaction trials conducted with AKR inhibitors diflunisal and ibuprofen, boceprevir exposure did not increase to a clinically significant extent. Victrelis may be co-administered with AKR inhibitors.

The concomitant use of Victrelis with rifampicin or anticonvulsants (such as phenytoin, phenobarbital or carbamazepine) may significantly reduce the plasma exposure of Victrelis. No data are available, therefore, the combination of boceprevir with these medicines is not-recommended.

Caution should be exercised with medicines known to prolong QT interval such as amiodarone, quinidine, methadone, pentamidine and some neuroleptics.

Pregnancy and lactation:
Use in pregnancy: Victrelis in combination with ribavirin and peginterferon alfa is contraindicated in women who are pregnant. There are no data on the use of Victrelis in pregnant women.

Treated patients and their partners must use two effective forms of contraceptive methods when boceprevir is used in combination with peginterferon alfa and ribavirin.
Use in lactation: It is not known whether boceprevir is excreted in human breast milk. A risk to the newborns/infants cannot be excluded.
A decision must be made whether to discontinue breastfeeding or to discontinue/abstain from therapy with Victrelis taking into account the benefit

Effects on ability to drive and use machines:
Combination therapy of Victrelis, peginterferon alfa and ribavirin may influence some patients' ability to drive and use machines. Patients should be informed that fatigue, dizziness, syncope, blood pressure fluctuations and blurred vision have been reported.

Side effects:
The most frequently reported adverse reactions in clinical trials were fatique, anaemia, nausea, headache, and dysgeusia.

Other very common (≥ 1/10) and common (≥ 1/100 to < 1/10) adverse reactions in combination with Victrelis with peginterferon alfa-2b and ribavirin reported during clinical trials are listed as follows:

Infections and infestation: *Common:* Bronchitis*, cellulitis*, herpes simplex, influenza, oral fungal infection, sinusitis.

Blood and lymphatic system disorders: *Very common:* Anaemia*, neutropenia*. *Common*: Leukopenia*, thrombocytopenia*.

Endocrine disorders: *Common:* Goitre, hypothyroidism.

Metabolism and nutrition disorders: *Very common:* Decreased appetite*. *Common:* Dehydration*, hyperglycaemia*, hypertriglyceridaemia, hyperuricaemia.

Psychiatric disorders: *Very common:* Anxiety*, depression*, insomnia, irritability. *Common:* Affect lability, agitation, libido disorder, mood altered, sleep disorder.

Nervous system disorders: *Very common:* Dizziness*, headache*. *Common:* Hypoaesthesia*, paraesthesia*, syncope*, amnesia, disturbance in attention, memory impairment, migraine, parosmia, tremour, vertigo

Eye disorders: *Common:* Dry eye, retinal exudates, vision blurred, visual impairment.

Ear and labyrinth disorders: *Common:* Tinnitus.

Cardiac disorders: *Common:* Palpitations.

Vascular disorders: *Common:* Hypotension*, hypertension.

Respiratory, thoracic and mediastinal disorders: *Very common:* Cough*, dyspnoea*. *Common:* Epistaxis, nasal congestion, oropharyngeal pain, respiratory tract congestion, sinus congestion, wheezing.

Gastrointestinal disorders: *Very common:* Diarrhoea*, nausea*, vomiting*, dry mouth, dysgeusia. *Common:* Abdominal pain*, abdominal pain upper*, constipation*, gastrooesophageal reflux disease*, haemorrhoids*, abdominal discomfort, abdominal distention, anorectal discomfort, aphthous stomatitis, cheilitis, dyspepsia, flatulence, glossodynia, mouth ulceration, oral pain, stomatitis, tooth disorder.

Skin and subcutaneous tissue disorders: *Very common:* Alopecia, dry skin, pruritus, rash. *Common:* Dermatitis, eczema, erythema, hyperhidrosis, night sweats, oedema peripheral, psoriasis, rash erythematous, rash macular, rash maculo-papular, rash papular, rash pruritic, skin lesion.

Musculoskeletal and connective tissue disorders: *Very common:* Arthralgia, myalgia. *Common:* Back pain*, pain in extremity*, muscle spasms, muscular weakness, neck pain

Renal and urinary disorders: *Common:* Pollakiuria

Reproductive system and breast disorders: *Common:* Erectile dysfunction.

General disorders and administration site conditions: *Very common:* Asthenia*, chills, fatigue*, pyrexia*, influenza-like illness. *Common:* Chest discomfort*, chest pain*, malaise*, feeling of body temperature change, mucosal dryness, pain.

* Includes adverse reactions which may be serious as assessed by the investigator in clinical trial subjects.

Forensic Classification:
P1S1S3

Prepared by Ivy Chan

Active Ingredient:
Bisacodyl and calcium sennoside

Presentation:
40 tablets per pack
Each enteric-coated tablet contains bisacodyl 5 mg and calcium sennoside 13.33 mg (5.27 mg as sennoside A & B).

Pharmacological Properties:
Bisacodyl acts on the membrane of the colon to strengthen peristaltic movement of the digestive tract and stimulate excretion.
The active ingredient is of calcium sennoside is natural senna. It is metabolised by the intestinal bacteria residing in the colon to give a laxative action.

Indications:
Constipation and relief of the following symptoms accompanying constipation: dull headache, dizziness, loss of appetite (decrease in appetite), skin problems, acne, bloating, abnormal intestinal fermentation, hemorrhoids

Dosage & Administration:
For adults (aged 15 or above), take single dose (1 to 3 tablets) once a day. Take before bed or on an empty stomach. Use the lowest recommended dose first, then increase or decrease the dosage depending on your stool and symptoms.

Forensic Classification:
Non-Poison

ACPE

Asian Conference on Pharmacoepidemiology

8th Asian Conference On Pharmacoepidemiology

Applying Pharmacoepidemiology to Improve Health Care in Asia

25th Oct 2013

Educational Workshop

Conference and Exhibition Centre
Cyberport, Hong Kong

26-27th Oct 2013

Conference

The University of Hong Kong
Hong Kong

Keynote Speakers

- **Bernard MY Cheung** (Hong Kong)
- **Til Stűrmer** (USA)

Speakers

- **Thomas Abraham** (Hong Kong)
- **Bruce Carleton** (Canada)
- **Derrick DC Chan** (Taiwan)
- **Vincent CC Cheng** (Hong Kong)
- **Kenneth Hartigan-Go** (Philippines)
- **Ellis KL Hon** (Hong Kong)
- **Suzanne McCarthy** (Ireland)
- **Debra Rowett** (Australia)
- **Mike Sharland** (UK)
- **Miriam Sturkenboom** (Netherlands)
- **Chunhua Tian** (China)
- **Fandian Zeng** (China)

Early Bird Deadline on/before 30 Aug 2013

	Educational Workshop	Conference	Gala Dinner (on 26th Oct at 7pm)
Student	USD 60	USD 150	
Academic / Government / Hospital / Health Care Professional	USD 90	USD 200	USD 110
Industry	USD 130	USD 300	

Host:

International Society for Pharmacoepidemiology (ISPE)

The University of Hong Kong (HKU)

The Pharmaceutical Society of Hong Kong (PSHK)

Chinese Pharmaceutical Association (CPA)

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