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Edited by Albert Wai-Kit Chan, Ph.D., J.D.

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Volume IV

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Editor's Note

The global environment under which we have put this fourth volume together has changed considerably since we published the last volume of Biotechnology in Hong Kong. There is much uncertainty in today's political and economic climate in many parts of the world, including China and Hong Kong. Many countries are experiencing varying levels of instability, and feuding countries are often resorting to using trade as a weapon of political causes. Despite all that is going on in the world at this point in time, Hong Kong continues to strive towards development of innovation and technology. Many of the features that made Hong Kong an ideal trade center still remain: rule of law, strategic location, steady regulatory system, and free trade policy.

With its Made in 2025 plan where China highlighted a list of technologies that it would focus on, such as artificial intelligence (AI) and genetic engineering, China remains an undeniable market, and Hong Kong maintains its position as its economic complement. In 2018, the Hong Kong Stock Exchange changed its rules and listed half of its total number on the exchange from mainland Chinese companies. Partnering together with neighboring technology hub Shenzhen to create a new innovation and technology center known as the Great Bay Area, Hong Kong is truly the gateway to China.

We're proud to present the fourth volume of Biotechnology in Hong Kong. The publication, as with all the past volumes, is made possible with the help of countless contributors, dedicated scientists and experts working on solutions to make the world a better place. I am ever grateful to our generous authors for contributing their time and effort to our continuous endeavor in advancing this educational dialogue. In several articles, our authors discuss their research in impactful and diverse topics, from nanovesicles to human organoids. Others share their thoughts on such varied topics as precision medicine and new developments like the Great Bay Area. I am also thankful to ARTHUR LIU for the beautiful artwork contribution for our cover, with his lovely and ethereal interpretation of biotechnology. Thanks also to Ms. Julie Lai and Dr. Stephen Chang for their assistance in editing this volume.

My lovely daughter, Alex, finished her college in 2020 January. Chris now is a college junior. Elliot hit middle school and is becoming quite independent. My wife remains beautiful as always. Without their support, this publication could not be accomplished.

Scientific

Chapter 1

HUMAN ORGANOIDS: PAST, PRESENT, AND FUTURE

Martin Chi-Wai Chan[1,2,3*], PhD

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Abstract

Human organoids, also known as mini-organs, were described as one of the Top 10 Breakthroughs of 2013 by Science magazine. Organoids are either made from induced pluripotent stem cells or organ-specific adult stem cells. They are currently being widely used in studying pathogenesis of pathogens in infectious diseases; modelling host-pathogen interactions in microbiome; predicting therapeutic potentials in drug treatment; replacing animal models in drug testing; and developing regenerative organs in bioengineering. In this Chapter, we will first discuss the history of the development of organoids. Next, we will highlight several success stories of local scientists using this revolutionary technology in their biomedical research. Last, but not least, we will discuss the current limitations of the organoid model and its future directions.

Introduction

Human organoids, also known as mini-organs, were described as one of the Top 10 Breakthroughs of 2013 by Science magazine. The term “organoid”, literally means organ-like, has a long history that dates back to the 1960s (*1*). At that time, organoids referred to whole-tissue explants that were used to cultivate viruses and bacteria. For example, *ex vivo* organoids obtained from donor intestinal tissues were used to attempt to grow the Norwalk virus in the 1970s, when the virus was discovered as the cause of nonbacterial gastroenteritis outbreaks (*2*). These organoids are not derived from stem cells, difficult to obtain, and can only remain viable for a few days. Thus, they have very limited use in research. Nowadays, organoids refer to three-dimensional cell culture made from either induced pluripotent stem cells or organ-specific adult stem cells resident in tissues.

The first stem cell-derived organoid was reported in 2009 by Sato and colleague from Han Clevers lab at the Hubrecht Institute, the Netherlands (*3*). They defined a condition with Wnt3A, Noggin, and R-spondin as crucial growth factors to stimulate Lgr5+ adult stem cells, which reside in the crypts of the intestinal epithelium to develop into organoids that contained multiple cell types. Compared with traditional two-dimensional culture using transformed cell lines, organoids are cultured in a three-dimensional matrix. They can be propagated for months or years with very stable genetic composition. They can be cryopreserved for later use. Most importantly, they recapitulate many physiological features in vivo and are thus promising pre-clinical models to study the pathogenesis of pathogens in infectious diseases; to model host-pathogen interactions in microbiome; to predict therapeutic potentials in drug treatment; to replace animal models in drug testing; and to develop regenerative organs in bioengineering. For example, intestinal organoids (also known as enteroids or mini-guts) have been used to study enteric viruses (*4,5*) and to generate hypothesis on the enteric involvement of respiratory viruses (*6*). Airway organoids have recently been shown to produce physiopathology upon infections by human and avian influenza viruses in the same way as in the ex vivo tissue explant model (*7*). Organoids from a wide range of organs are now available, including stomach, intestine, liver, kidney, and brain (*8*); the list is expanding fast. Here, we highlight several success stories of local scientists using this revolutionary organoid technology in their biomedical research with public health impact.

Organoids in Infectious Disease Research

<u>*Intestinal Viral Infections and Norovirus*</u>

Intestinal infections are one of the leading causes of deaths due to infectious agents globally. A large proportion of intestinal infections are diarrheal diseases caused by a variety of foodborne pathogens, leading to a substantial morbidity and mortality in both developed and developing regions. In the 2015 World Health Organization Report on the estimation of global burden of foodborne illness, diarrheal diseases account for nearly half of these cases, in which norovirus is the most common cause (*9*). Norovirus is also the primary cause of deaths among foodborne infections in 5 out of 6 WHO regions (*10*). A recent meta-analysis estimated that norovirus accounts for 18% of sporadic and outbreak cases of acute gastroenteritis (*11*). Norovirus research, especially in the areas of virus-host interaction and molecular pathogenesis, has been severely hampered by the lack of a robust cell culture model since virus discovery in 1972 (*12*). Despite encouraging claims of successful human norovirus cultivation in three-dimensional cultures of intestinal epithelial cells and hematopoietic B lymphocytes in the presence of bacteria (*13,14*), attempts to reproduce these findings have proved challenging (*15-17*). In 2016, Dr. Mary Estes' lab from Baylor College of Medicine (Houston, Texas) announced a long-awaited triumph that human norovirus could finally be cultivated in an adult stem cell-derived human intestinal organoid model (*4*). Since then, studies on evaluating human norovirus infectivity and method of inactivation using the organoid model have been described by several leading norovirus laboratories (18,19), supporting that the model is reproducible and viable. The organoid model has not only solved a nearly 50-year-old challenge to cultivate human norovirus, it also opens a new door to study norovirus-host interaction. A Hong Kong research group is embarking on establishing and utilizing the organoid model to uncover the disease-causing mechanism of human norovirus.

1. <u>Dr. Martin Chi-Wai Chan</u> (Department of Microbiology, Chinese University of Hong Kong) - Chan is a molecular biotechnologist by training and started exploring gastrointestinal virology since his doctoral study at the Chinese University of Hong Kong. In collaboration with Mary Estes from Baylor College of Medicine, Chan's lab has successfully adapted the protocol for making organoids and established a panel of adult stem cell-derived human intestinal organoid lines. An experiment outline is shown in **Figure 1, Panels A-D**. First, intestinal biopsy tissues were obtained from consented volunteers via endoscopy. Tissues were then minced and washed. Intestinal epithelial crypts were isolated after shaking in the presence of cell dissociation agent. Cells were suspended in Matrigel, a nutrient-rich three-dimensional matrix. Adult stem cells were cultured in a complete medium containing essential growth factors (Wnt3A, Noggin, and R-spondin-1) to maintain stemness in organoids. Bright, cystic organoids appeared around 3 days after culture. High density of organoids was achieved after 2 to 3 passages (**Figure 1, Panel E**). The research team

is employing the organoid model to correlate infectivity with cycle threshold value of a diagnostic real-time polymerase chain reaction assay for norovirus in order to estimate virus infectivity in a much simpler and quicker process. Other ongoing organoid-based research projects include the use of genome-wide CRISPR screen to decipher a catalogue of host factors important for norovirus infection and the identification of exact cell types targeted by human norovirus.

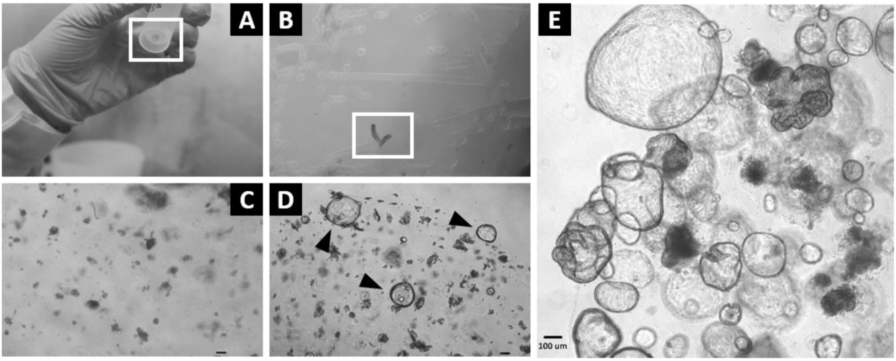

Figure 1. An experiment outline of making human intestinal organoids. **(A)** Intestinal biopsy tissues (white box) were obtained from consented volunteers via endoscopy. **(B)** Tissues were minced and washed. Intestinal epithelial crypts (white box) were isolated after shaking in the presence of cell dissociation agent. **(C)** Cells were suspended in Matrigel, a nutrient-rich three-dimensional matrix. **(D)** Adult stem cells were cultured in complete medium containing essential growth factors (Wnt3A, Noggin, and R-spondin-1) to maintain stemness in organoids. Bright, easily-identifiable cystic organoids (arrows) appeared around 3 days after culture. Medium was refreshed every second day. **(E)** High density of organoids after 2 to 3 passages. (Photos: courtesy of Jenny Chan)

Respiratory Viral Infections

Acute respiratory infections (ARIs) are the leading cause of deaths, causing an estimation of 4.3 million annual deaths globally (*20*) and are associated with substantial morbidity in all age groups (*21*). Viruses are common causes, including influenza A and B viruses, respiratory syncytial viruses, rhinoviruses, adenoviruses, coronaviruses (229E, HKU1, NL63, OC43), human metapneumoviruses, and parainfluenza viruses. It has been estimated that over 300,000 mammalian viruses remain to be discovered (*22*). Such a high diversity of viruses, presumably in animal reservoirs, poses a substantial threat to the public health of mankind if zoonotic transmission occurs. The emergence of bat-originated severe acute respiratory syndrome (SARS) coronavirus that causes severe ARIs in late 2002 in Asia and its subsequent global spread serves as an example of such risk (*23,24*). The recent emergence of a highly pathogenic Middle East respiratory syndrome (MERS) coronavirus of unknown animal origin in 2012 in the Middle East and highly pathogenic influenza A(H7N9) virus from poultry sources in 2013 in China that cause severe ARIs reiterate the imminent threat posed by yet unknown viruses (*25,26*). Due to high pathogenicity of most respiratory viruses, knowledge of the molecular mechanism behind virus pathogenesis and transmission came mostly from studies on small animal models and traditional two-dimensional culture using transformed cell lines. However, these artificial models were restricted by either expressing limited cell types or lack of the specific human receptors to support viral replications. More advanced respiratory models such as virus culture using tissue explants can partly overcome these limitations (*27*), yet obtaining a continuous supply of surgical tissue materials from the same donors is ethically impossible. In contrast, stem cell-derived organoids can be propagated in relatively large quantities for months without loss of in vivo-like three-dimensional cell organization and have been successfully used by three prominent Hong Kong virology research groups since 2017 as a state-of-the-art model to characterize emerging respiratory viruses.

1. Prof. Kwok-Yung Yuen (Department of Microbiology, University of Hong Kong) - Yuen is an internationally renowned clinical microbiologist and most well-known by local members of the general public for his frequent presence in local television and radio channels commenting on infectious disease issues. His team is among the first in Hong Kong to use organoids to study emerging infectious viruses. In his first study published in late 2017, it was revealed that, by using intestinal organoids, human intestinal tract could serve as an alternative route for MERS coronavirus (*6*). The virus was first identified in 2012 in Saudi Arabia and has resulted in more than 2,400 laboratory-confirmed cases with a fatality rate of 34.5% globally (*28*). Although the transmission of MERS coronavirus from dromedary camel to human and human-to-human transmission has been well documented (*29,30*), a large proportion of cases occurred among patients without direct contact with infected camels or individuals (*31*). In addition, despite the common symptoms like pneumonia, gastrointestinal tract symptoms, such as vomiting and diarrhea, were also commonly reported (*29*), raising concerns of an unrecognized transmission route. The team performed in vitro studies on human primary intestinal epithelial cells followed by validating the results with intestine explants, Caco-2 and human intestinal organoids. All of them were highly susceptible to MERS coronavirus and could sustain robust viral replication. Results from the intestinal organoid model prompted the team to further explore the gastrointestinal involvement of MERS coronavirus, and they found the virus was more resistant to fed-state gastrointestinal acids than fasted-state gastric acids, proving virus viability and infectivity in the gastrointestinal tract. Finally, the team performed direct intragastric inoculation of MERS coronavirus in human DPP4 transgenic mice, resulting in a lethal infection. The enteric route of virus inoculation resulted in inflammation, detection of virus-positive cells, and isolation of live viruses in lung tissues, hinting the sequential development of respiratory infections. This study could help to improve current intervention and infection control strategies against MERS. It may also help to investigate the possibility of non-symptomatic individuals shedding MERS coronavirus

in their intestinal tract, which may contribute to the MERS epidemic. The organoid model has become a new, very useful approach in our toolbox to address research questions with immediate clinical and public health impact.

In his second study published in mid-2018, Yuen and colleagues, in collaboration with the Clevers lab of the Hubrecht Institute from the Netherlands, reported the establishment of a long-term human airway organoids (AOs) culture (*32*). These AOs are derived from tissue-specific resident adult stem cells and are differentiated to form four major epithelial cell types, including physiologically relevant and active ciliated cells with beating cilia. The team then functionally evaluated the clinical relevance of AOs in studying influenza viruses. As a proof-of-concept, they selected two influenza virus subtypes, one human seasonal H1N1pdm09 and one avian H7N9, for testing on AOs. The H1N1pdm09 subtype is epidemic in humans, whilst the H7N9 subtype has restricted replication in humans due to the difference in receptor usage. The authors found that human H1N1 subtype replicated consistently to a higher level than the avian H7N9 subtype. This illustrates that AOs can distinguish influenza viruses with different host tropism and highlights the potential application of AOs in influenza research. The fact that AOs can be propagated continuously with high genomic stability and with composition of multiple cell types resembling the corresponding respiratory tissue makes the model a very promising alternative to existing methods to study virus-host interactions.

2. Dr. Michael Chi-Wai Chan (School of Public Health, University of Hong Kong) – Chan was ranked by Essential Science Indicator as a top 1% highly cited researcher. His main research interests are the virus-host interaction and pathogenesis of influenza virus and coronavirus (including those that cause SARS and MERS). In his recent work published in 2018, in collaboration with the Clevers lab from the Netherlands, his team established ex vivo human bronchus explant, and human AOs from lung stem cells obtained from patients who had underwent routine surgical resection (*7*). The team utilized the human AOs and the explant culture to compare the viral replication competence, tissue tropism, cytokine and chemokine responses elicited by human (H1N1) and avian (H5N1, H5N6, and H7N9) influenza virus subtypes. Firstly, for viral replication competence, both cultures illustrated higher titers of virus replication for H1N1 and H7N9, followed by the highly pathogenic H5N1 and H5N6 subtypes with a moderate replication. Secondly, for tissue tropism, they observed that ciliated cells and goblet cells were infected with H1N1, H7N9 and H5N6 viruses in immunohistochemical staining and transmission electron microscopy, while none of the basal cells in either human AOs or ex vivo bronchus explant were infected. As for cytokine and chemokine responses, human AOs infected with H5N1 virus has expressed and secreted higher levels of interleukin 6, interferon β, RANTES (regulated-on-activation, normal T-cell expressed and secreted) than other virus subtypes. The expression of MCP-1

(monocyte chemoattractant protein-1) was also significantly higher in human AOs infected with H5N1 than other virus subtypes. These data proved that highly pathogenic influenza subtypes caused more vigorous immune response and severe pathology, which are observed in naturally infected patients, in both models. Importantly, the group demonstrated that results achieved in human AOs mimicked those observed in ex vivo bronchus explant cultures, suggesting human AOs, thus, may act as a physiologically relevant model to evaluate the pandemic threat of emerging animal influenza viruses to humans. This organoid model may also serve as a robust experimental model for virus-host interaction and pathogenesis research.

3. Dr. Renee Wan-Yi Chan (Department of Pediatrics, Chinese University of Hong Kong) – Chan is a molecular virologist by training and has been working on understanding the pathogenesis of respiratory viruses, especially influenza viruses, for more than 10 years since her doctoral study at the University of Hong Kong. In her recent paper published in 2019, in collaboration with Michael Chan from the University of Hong Kong, Chan compared influenza B virus infections in AOs, ex vivo tissue explant culture, and in vitro primary epithelial cell culture (*33*). Unlike influenza A virus which has been extensively studied due to their ability to cause severe infections and complications, research on influenza B virus has not been very active, although accumulating evidence suggests this virus also causes substantial morbidity and disease burden as influenza A virus does. To answer whether the issue could be examined and addressed in laboratory settings, her research group tested 16 influenza B virus strains, including an ancestral one that dates back to 1940, and monitored virus replication and cell cytokine response. They found influenza B virus replicates to a similarly high level as influenza A virus does in the three models. Using AOs, they further demonstrated that both influenza A and B viruses showed a similar profile of target cell types (basal cells, ciliated cells, club cells, and goblet cells). Although this study was not specifically designed to compare the performance of AOs against other commonly used virus culture models, findings from AOs were in general in high agreement with those from tissue explant culture, further expanding the list of respiratory viruses that can be studied in AOs.

Organoids in Cancer Research

Apart from modelling infectious diseases and studying pathogen-host interactions, organoids are now rapidly being used in cancer research. According to the latest statistics released by the World Health Organization, cancer is ranked second as the cause of deaths globally, attributing to nearly 20% of all-cause deaths (*34*). In Hong Kong, cancer is the most common cause of deaths with about 1 in every 3 people who die are due to cancer malignancy (*35*). Thus, research to improve prognosis, diagnosis, and treatment of cancer is of public health importance, and Hong Kong has been leading in studying cancer types such as gastric and nasopharyngeal malignancies that are prevalent in Asia. While studies in small animal models and in vitro two-dimensional cell culture have substantially enhanced our understanding on cancer biology in the past decades, these simplified systems do not faithfully and sufficiently recapitulate human physiology and thus have been found challenging to translate findings from these systems to impact clinical practice. Patient-specific organoids provide an auspicious technological opportunity to address the gap (*36*).

1. Prof. Suet-Yi Leung (Department of Pathology, University of Hong Kong) – Leung's lab has been pioneering in utilizing advanced molecular approaches to dissect the host genetic network underlying the development of gastric cancer and to improve diagnosis, in hope to ultimately guide treatment options and to increase patient survival. In her team's recent landmark paper, Leung and colleagues described the first organoid biobank for gastric cancer in Hong Kong (*37*). They made organoids from biopsy samples collected via surgery from 34 patients at different stages of gastric cancer. They then proved that these organoids exhibited histopathological features and genetic signatures resembling those of the donor patients. More importantly, these organoids are genetically very stable upon prolonged culture in laboratory conditions, allowing the model to be used for comprehensive testing. In a subsequent screen against cancer drugs, they found organoids responded differently among patients. It remains to be validated whether gastric cancer will respond to therapeutics intervention in patients in the same way as organoids do in vitro. If proven, this will open an uncharted area in personalized cancer therapy in which treatment options can be guided by prior testing in organoids. Such method would be beneficial to patients by avoiding unnecessary treatment, reducing medical expense, and lessening human suffering. Given the cancer burden in Hong Kong, such an organoid biobank can be expanded to include other organs such as colon, rectum, liver, breast, and brain. A territory-wide, central organoid biobank to be coordinated and managed by local health authorities should be seriously considered.

Challenges and Opportunities

Stem cell-derived organoids are rapidly being employed in different areas of biomedical research. Despite the enormous potential of this groundbreaking model, there are areas that need to be overcome before the model could be widely translated into clinical use. First, organoids are cultivated in extracellular matrix derived from animal source. This restricts the implantation of organoids back into human bodies for therapeutic intervention. Second, organoids are slow-growing compared with conventional transformed cell lines. High-throughput screening is therefore technically challenging. Third, though organoids are composed of multiple cell types, lack of vascular structure hampers the feasibility of growing organoids beyond the miniature "lab dish" size which is currently too small to be useful in clinical settings. Fourth, organoids are usually cultured in aseptic conditions. Without microbiota and immune cells, how authentic the response will be in the model remains elusive. Where there are challenges, there are opportunities. The organoid wave has attracted researchers from diverse disciplines, especially those in material science and bioengineering, to tackle these grand challenges. For example, a bioengineering research team from Harvard University recently reported an intestine-on-a-chip co-culture system with bacteria (*38*). Local researchers in infectious diseases are among the first to utilize organoids. However, due to the steep learning curve and demanding resource requirements of establishing and maintaining the organoid platform in individual laboratory, widespread and routine use of organoids remain unlikely in the next five years in Hong Kong. A territory-wide, central repository of organoids prioritized to serve the local research and clinical communities may speed up organoid research and its translational application. A steering consortium convened by local experts of the organoid technology and composed of other stakeholders in medical, regulatory, and commercial sectors could shape a direction and framework that would ultimately lead to the establishment of an organoid biobank in Hong Kong.

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Chapter 2

RESEARCH AND DEVELOPMENT OF ANTI-CANCER MONOCLONAL ANTIBODIES IN CHINA:

AN ANALYSIS BASED ON PATENTS

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Abstract

Monoclonal antibodies (mAbs) are monovalent antibodies generated from a single clone of B-lymphocytes, which bind to specific epitope of the surface of cells. Within approximately three decades of clinical development, mAbs have been successfully transformed from scientific tools to powerful human therapeutics and become a powerful weapon among biopharmaceutical products for treating a variety of diseases, especially cancers. As a result, mAbs have become the fastest growing sector in the biopharmaceutical industry with a global sales revenue of US$98 billion, with an annual growth rate of 18.3% since 2016. This tremendous commercial potential has led to various countries, including China, to actively join in the research and development (R&D) and patent applications of mAbs. By March 2019, 11 locally produced mAbs therapeutic products have been made available and more than 20 mAbs R&D projects are undergoing clinical trials in China.

As the bridge between academia and industry, patents can serve as a useful innovation indicator for observing technology flow and development activities in technological industries, including the biopharmaceutical industry. However, the exponential growth of patent applications in China raises concerns on patent quality from both scholars and government officials. Therefore, by investigating the invention patent application data collected from China National Intellectual Property Administration or CNIPA, this research aims to: 1) outline the technological development of therapeutic mAbs for cancers based on the patent applications in CNIPA, with the emphasis on patents from the mainland and Hong Kong applicants, 2) identify the quality of sample patents by comparing patents from CNIPA across different applicants' countries of origin over different time periods by using patent age as a proxy indicator of patent quality, and 3) explore the significant factors of the quality of anti-cancer mAb patents.

In terms of the importance of patents to scientific research and commercial development, this study may help policy makers, stakeholders of biopharmaceutical industry and financial institutions to comprehend the development of anti-cancer mAbs in China and further to make relevant strategic decisions.

1. Introduction

Monoclonal antibodies (mAbs) are mono-specific antibodies generated from a single clone of B-lymphocytes [1, 2]. These antibodies show great specificity and high affinity by recognizing and targeting specific antigens [3]. As a result, mAbs have successfully transformed from scientific tools to powerful human therapeutics within three decades of clinical development [4]. Nowadays, mAbs are regarded as a powerful weapon among biopharmaceutical products for treating a variety of diseases, especially cancers. In 2013, more than half of the best-selling drugs were mAbs [5]. The global sales revenue for all mAbs reached US$98 billion, with an annual growth rate of 18.3% since 2016 [6]. Emerging countries, such as China, are also taking an increasingly active part in the mAb market, which exceeded US$12.6 billion in sales in 2015 [6]. There is no doubt, mAbs have become the fastest growing sector in biopharmaceutical industry.

This tremendous commercial potential has led various countries, including China, to actively join in the research and development (R&D) and patent applications of mAbs. Chinese domestic mAbs have undergone great breakthroughs in recent years. In December 2018, Junshi Biosciences' Tuoyi (toripalimab) became the first Chinese homegrown programmed cell death-1 (PD-1) inhibitor to gain a conditional market approval under priority review by National Medical Products Administration of China for the treatment of melanoma. Two months later, Henlius' HLX01, which referenced from Rituxan (rituximab) and used in the primarily treatment of non-Hodgkin lymphoma, was approved as the first ever biosimilar drug in China. By March 2019, 11 local produced mAbs have been made available and more than 20 mAb R&D projects are undergoing clinical trials in China [7, 8].

As the bridge between academia and industry, patents can serve as a useful innovation indicator for observing technology flow and development in technological industries, including biopharmaceutical industry. However, the exponential growth of patent applications in China raises concerns on patent quality from both scholars and government officials [9-11]. In 2018, the patent applications received by National Intellectual Property Administration of China (CNIPA) reached 1,381,594, accounting for 43.6% of the whole world's patent applications and more than double the applications received by the United States Patent and Trademark Office (USPTO) [12]. Not only do the quantity but also the quality of patents need to be examined, as China is moving from an 'imitator' to 'innovator'.

Most of the previous studies of mAbs focused on R&D status and provided information about the development of mAbs, collaboration between different parties or the leading countries or enterprises in this field [13, 14]. For studies towards patent analysis of mAbs, they mainly focused on the technological development described within the patents [3, 15]. This study provides a detailed investigation by analyzing the invention patents of oncology mAbs from CNIPA because most of the mAb patents are indicated for treatment of malignant tumors. This research will analyze, not only the application and the after-grant status, but also the post-granted situation and patent life cycle.

Thus, this study aims to: 1) outline the technological development of therapeutic mAbs for cancers based on the patent applications in CNIPA, with the emphasis on patents from the mainland and Hong Kong applicants, 2) identify the quality of sample patents by comparing patents from CNIPA across different applicants' countries of origin over different time periods by using patent age as a proxy indicator of patent quality, and 3) explore the significant factors associated with the quality of anti-cancer mAb patents.

2. Methods

2.1 Data collection

For accessibility and repeatability, this research was performed in a free access database SOOIP to collect the invention patent applications of anti-cancer mAbs in China which were received by CNIPA, the official organization in charge of the application, examination and management of intellectual property, including patents. The search criteria were as follows: International Patent Classification or IPC = (A61K39/395 or A61K39/40 or A61K39/42 or A61K39/44 or C07K16 or C12N5/20 or C12N5/24 or C12P21/08 or G01N33/577) for mAbs and within IPC = A61P35 for anti-cancer drugs, and application year between 1985 and 2018, as the Chinese patent law prosecuted from 1985. A total of 6,910 invention patent applications and 1,398 granted invention patents were collected in this study. According to our research interest, we mainly focused on these 1,398 granted patents. Then the detailed information of these granted patents was run manually through the official CNIPA database and the SOOIP database. During this vetting process, 52 patents were excluded as they were not related to mAbs, and finally 1,346 granted patents of anti-cancer therapeutic mAbs were analyzed in this study.

2.2 Data analysis

This study uses patent renewal as a proxy indicator to evaluate the quality of invention patents of anti-cancer mAbs in China by comparing the patent age across applicants received by CNIPA from five countries, including China, United States, Japan, Switzerland and Germany, over three time periods: (a) 1991-1998, (b) 1999-2001, (c) 2002-2011. Also, patents from Hong Kong will be analyzed, as one of our research purposes is to reveal Hong Kong applicants' patent behaviors in anti-cancer mAb development in China. The basic assumption of this patent quality evaluation is based on a purely economic consideration that the patent holders will only renew their patents if the expected value of their patents in the following year(s) is higher than the renewal cost [16, 17]. As the maintenance fee is required every single year after a patent is granted according to the Chinese patent law, a patent's age, which means the length of time the patent is kept valid, will be positively associated with the patent qualMoreover, patents contain timely and plentiful information of innovation (e.g., technological area, backward and forward citations to other related patents or literature) and they are easily accessible, so patent statistics can be used as a measurement of innovation and R&D output [10, 18]. In this research, different indicators (as shown in Table 1) are constructed and examined by different statistical methods, such as Mann-Whitney U test, Chi-Square test and binary logistic regression to identify whether these indicators are significant in influencing the quality of Chinese domestic mAb patents.

Table 1. Definitions and sources of all collected indicators

Indicators	Definitions	Sources
Patent age	Time lag between the application date and authorization date of the patent, counted in days. Use for patent quality evaluation in this study.	SOOIP
Applicants		SOOIP
Individual	Whether the patent applicant is an individual. (0: no; 1: yes)	
University	Whether the patent applicant is a university. (0: no; 1: yes)	
Research institute	Whether the patent applicant is a research institute. (0: no; 1: yes)	
Biopharmaceutical	Whether the patent applicant is a biopharmaceutical. (0: no; 1: yes)	
Hospital	Whether the patent applicant is a hospital. (0: no; 1: yes)	
In cooperation	More than two applicants within the same patent is considered to be in cooperation. (0: no; 1: yes)	SOOIP
Number of inventors	Number of inventors the patent owns.	SOOIP
Number of backward citations (to other patents)	Number of other patents that are cited by the patent.	CNIPA
Number of backward citations (to non-patents)	Number of non-patents (e.g. scientific literature) that are cited by the patent.	CNIPA
Number of claims	Number of claims the patent owns.	SOOIP
Number of IPC subgroups	Number of IPC subgroups the patent owns.	SOOIP
Family size	Number of patents taken in various countries relating to the same invention [26].	CNIPA
PCT	Whether the patent applies for an PCT. (0: no; 1: yes)	SOOIP
Priority right	Whether a prior application of the same invention the patent contains by the same applicant [26]. (0: no; 1: yes)	SOOIP
Examination time	Time gap between the effective date of substantive examination and the authorization date of the patent, counted in days.	SOOIP
Patent assignment	Whether the patent ownership has been changed or not. (0: no; 1: yes)	CNIPA
Patent pledge	Whether the patent is used as collateral for a loan. (0: no; 1: yes)	CNIPA
Patent licensing	Whether the patent owner gives the right to use his/her patent under license agreement. (0: no; 1: yes)	SOOIP
Patent agent	Whether the patent applicant employ a patent agent to handle the patent application. (0: no; 1: yes)	SOOIP

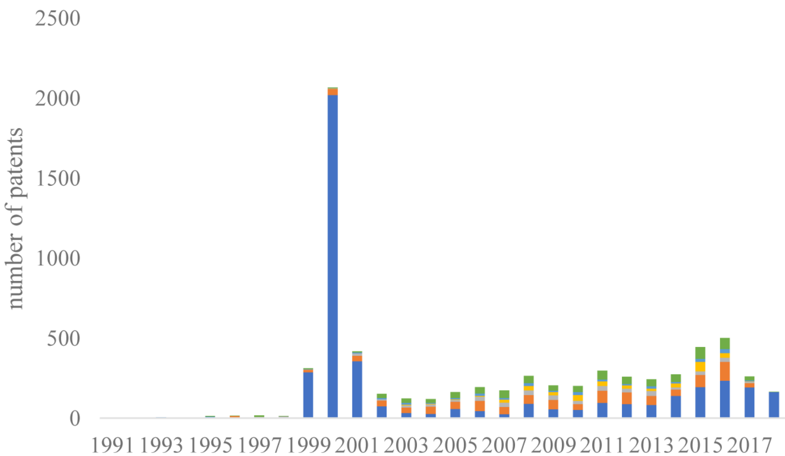
To emphasize, all the patent applications collected in this research were received by CNIPA. We will analyze the sample patents by their applicants' countries of origin. For example, domestic Chinese patents refer to the patents from domestic Chinese applicants. Meanwhile, US patents, or patents originated from the US, refer to the patents from US applicants received by CNIPA in the following contents.

3. Results

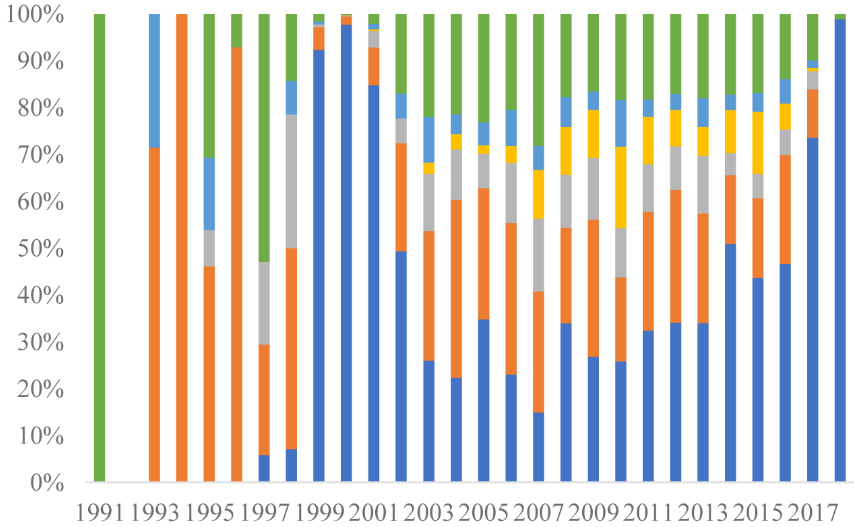
3.1 The overall development of therapeutic mAbs on oncology in China

Figure 1 indicates the annual number of applied and granted invention patents of anti-cancer mAbs and their corresponding distributions by different applicants' countries of origin. Notice, as the average time for an anti-cancer mAb patent to get approval is 5.5 years in China, and there is an 18-month time lag for the publication of a patent application, the number of granted malignant cancer treatment mAb patents between 2014 and 2018 may not be completed. From Figure 1, the development of anti-cancer mAbs in China can be divided into three periods, as revealed by the patents.

(a)



(b)



100%
90%
80%
70%
60%
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20%
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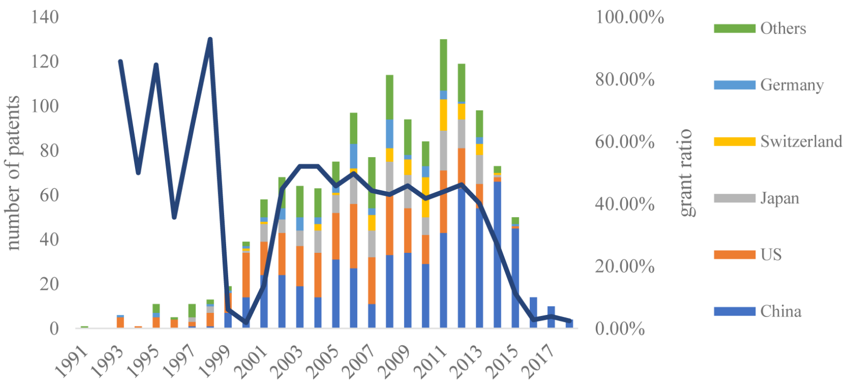
1991 1993 1995 1997 1999 2001 2003 2005 2007 2009 2011 2013 2015 2017

(c)



number of patents
140
120
100
80
60
40
20
0
1991
1993
1995
1997
1999
2001
2003
2005
2007
2009
2011
2013
2015
2017
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
grant ratio
Others
Germany
Switzerland
Japan
US
China

(d)

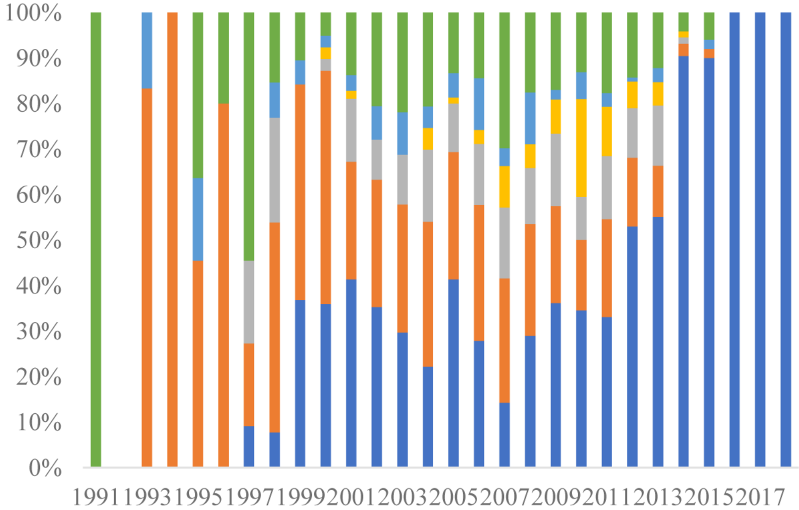


Figure 1. The annual number of application and granted invention patents of anti-cancer mAbs and their corresponding distribution by different countries of origin in CNIPA

(a) The annual number of invention patent applications of therapeutic mAbs in cancer; (b) The distribution of invention patent applications of therapeutic mAbs in cancer from different countries' applicants; (c) The annual number of granted invention patents of therapeutic mAbs in cancer, and the annual grant ratio of applications; (d) The distribution of granted invention patents of therapeutic mAbs in cancer from different countries' applicants.

The first period took place in 1991 to 1998; only 68 patent applications and 48 granted patents were recorded. The vast majority of these patents originated from foreign applicants, such as the United States, Japan and Germany. Only two Chinese domestic patents were recorded in this period. At this moment, the core technology and mAbs R&D were led by foreign biopharmaceuticals and research institutes. In the second period, which was from 1999 to 2001, there was a surge of Chinese domestic patent applications, probably as the result of the second revision of China's patent law. As China joined the World Trade Organization (WTO) in 2001, some amendments, such as the Trade-Related Aspects of Intellectual Property Rights (TRIPS) Agreement, were made in this revision of patent law to fit the requirements of the WTO in 2000. The examination process was simplified and the protection of patent rights was increased [19]. Around 40% of granted anti-cancer mAb patents came from Chinese applicants in this period. However, the rapid growth in domestic patent applications did not reflect the number of granted patents. The grant ratio of applications decreased to 1.89% in 2000, meaning that much of these Chinese domestic anti-cancer mAb patent applications did not satisfy the patent granting criteria of novelty, usefulness or non-obviousness. In the third period, which began in 2002, a steady increase in both the patent applications and granted patents were observed. The grant ratio was kept between 41% and 52%. The participation of Chinese applicants was around 30% on average, and the dominance of the US was decreasing within these years. This meant that the knowledge of mAb technology in treatment of cancers had been spreading to other countries, which also led to a mature and diverse development on mAbs in the treatment of malignant cancer in China.

Technologically, mAbs can be divided into four generations: murine, chimeric, humanized and fully human mAbs. In 1975, Kohler and Milstein first introduced hybridoma technology to produce murine mAbs in vitro, turning mAbs into therapeutic use [20]. However, the development of murine mAbs was restricted due to short half-life in human and severe allergic reactions, such as the immunogenicity of human anti-murine antibody (HAMA), antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) induction [3, 4]. Chimeric mAbs were then developed by replacing the constant domains of mouse into human antibody, making it ~65% human. Later on, humanized mAbs were developed, only the hypervariable regions of humanized mAbs were from mouse, resulting in ~95% human. At last, fully human mAbs were generated by the advance of in vitro phage display technology and transgenic mouse technology [4]. For the technology flow of therapeutic anti-cancer mAbs revealed by Chinese domestic patents, there was not an obvious boundary for the change from murine to fully human mAbs. Actually, since the domestic research of therapeutic mAbs towards cancers started later than their foreign counterparts, murine mAbs were hardly found in our set of data. The Chinese domestic patentees claimed a broad range of protection in the forms of chimeric, humanized and later including fully human for their polypeptide sequence of mAbs. After 2010, the applications of fully human mAbs towards various targets increased, meaning that a technological innovation of Chinese mAbs had caught up with their foreign counterparts.

Various targets were indicated in the patent information towards anti-cancer mAbs by Chinese domestic patents. Particularly, EGFR, CD20, VEGFR, HER2 and CD3 were of much research interest, as these were all well-developed therapeutic targets towards treatment of malignancy. The domestic patent applications of programmed cell death-1 and its ligand (PD1/ PDL1) target first appeared in 2006 and had been increasing since 2013. More than 18 universities and biopharmaceuticals gained patents towards PD1/ PDL1 targets, indicating that PD1/ PDL1 target was a hot spot of research development in recent years.

By the structure of mAbs, the trend of recent researches turned into the patent applications of bispecific and fragments of these antibodies, such as the fragment antigen binding (Fab) and the single chain fragment variable (scFv) mAbs. Composed of fragments of two different mAbs, bispecific mAb can bind to the target antigen on a tumor cell with one arm and to the activating antigen on an immune effector cell with another simultaneously in cancer therapy [21]. Comparing to the whole structure of mAbs (~150kDa), these small structures of Fab and scFv fragments (~15-55KDa) can provide rapid blood circulation in human body, good penetration in target cancer cells, low retention in the kidneys or other non-target organs, low immunogenicity, and are easy and cost-effective for large-scale production [15].

3.2 Primary patent value evaluated by the patent office

The value of patents is mainly realized by the patent law of the country in which the patent has been granted; so it is important for the patent to be granted by the patent office to embody the primary value of the patent [18, 22]. From 1991 to 2018, 23.26% of anti-cancer mAb patent applications were under substantive examination, indicating a continuous development of mAbs (Figure 2). However, a high proportion of patent application withdrawals (43.31%) were recorded, only 21.58% of inventions went through the examination process and gained the patent protection successfully. This situation was mainly contributed by the surge of Chinese domestic applications in 1999-2001. Patent withdrawal means that the patent applicants give up their applications, implicating that even the Chinese applicants themselves are not satisfied with their patent quality as they are most knowledgeable as to the content of their patent applications.

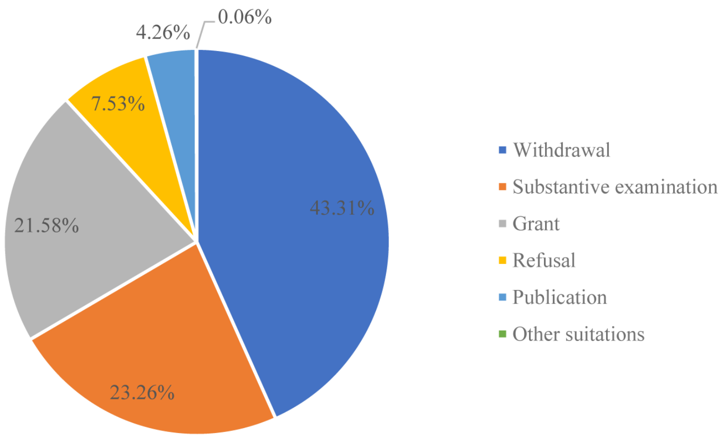


Figure 2. The Legal status of anti-cancer mAb patent applications received by CNIPA

3.3 Origins of patent applicants in China

Figure 3 shows the origins of patent applicants for anti-cancer mAbs obtained by CNIPA from China, the United States, Japan, Switzerland and Germany account for nearly 90% of patent applications and 85% of patent grants. In the 1990s, only an average of 4 countries were involved in anti-cancer mAb patent applications in China per year. After 2000, an average of 18.5 countries took part in the applications in China annually. However, the dominance of domestic Chinese applications dropped from 62.40% in applications to 40.63% in granted patents, indicating that the quality of Chinese mAb patents for cancer treatment was not as high as its foreign counterparts.

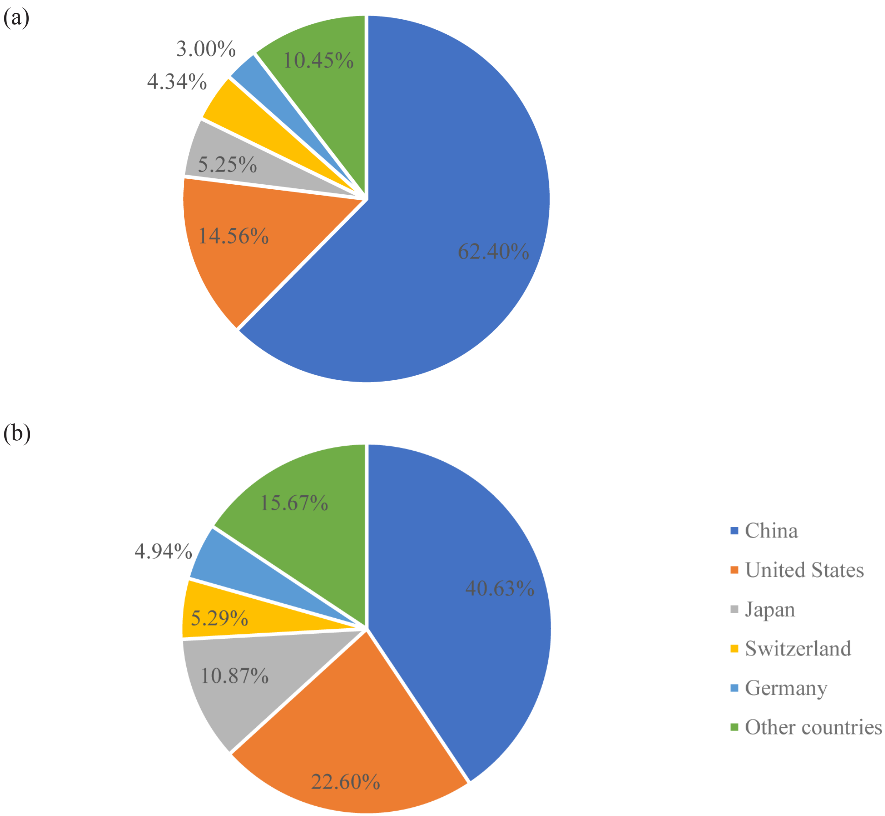


Figure 3. The composition of applicants from different countries in applications and granted patents of anti-tumor mAbs received by CNIPA

(a) Composition of applications; (b) Composition of granted patents.

3.4 Types of Chinese domestic applicants in granted anti-cancer mAb patents

In order to reveal the source of technology, the Chinese domestic applicants of granted oncology mAb patents are analyzed instead of the patent assignees, as some of the patent rights may be transferred before a patent is authorized. In 1991-1998, only two Chinese domestic patents were recorded by this study, both of them were from research institutes. From 1999 to 2001, although research institutes were still dominant, its share among all applicants decreased to 58.73%, followed by biopharmaceuticals, individuals and hospitals. The proportion from universities increased dramatically from 1.19% in 1999-2001 to a quarter in 2002-2018, marking it the third place. Since 2002, biopharmaceuticals became the leader by accounting for over one third of total applicants. Research institutes marked as the second place by a similar share to that of the universities (Figure 4).

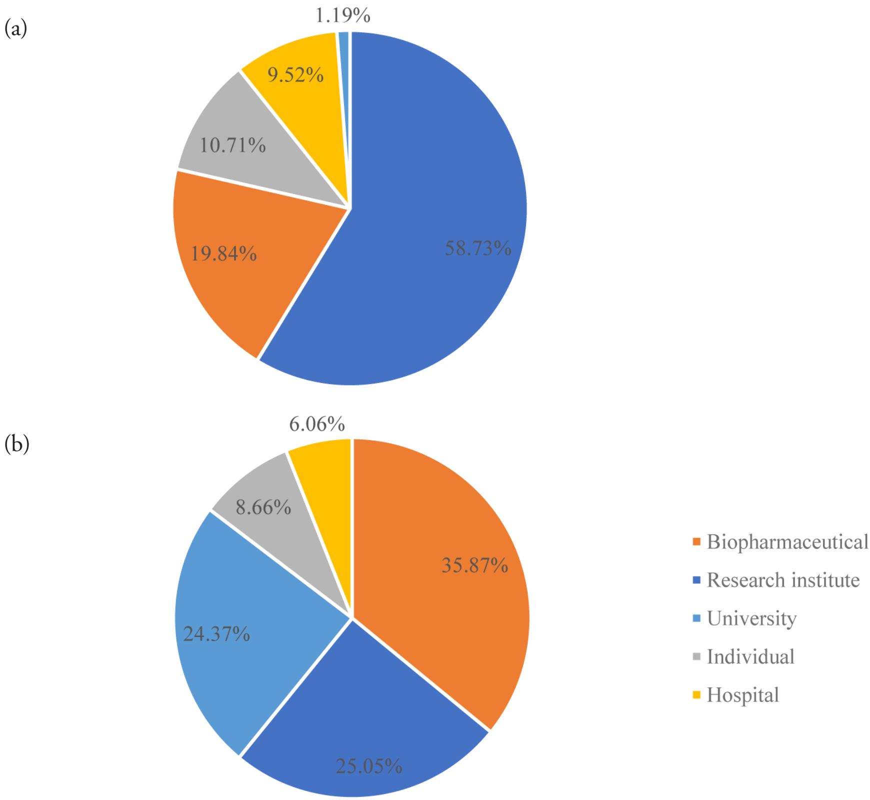


Figure 4. Types of applicants in granted patents of domestic Chinese anti-cancer mAbs

(a) In 1999-2001; (b) In 2002-2018.

Collaboration is critical as knowledge can be diffused between the involved parties [13]. Figure 5 indicates the collaboration activities of mAbs in cancer treatments noted in the patents. Only 11.68% of domestic Chinese patents had collaboration. There were 20 cases of partnership between biopharmaceuticals among 62 instances of collaborations. Partnerships between biopharmaceuticals and research institutes or universities were also the major collaboration types. Five cases of international collaborations were observed by Chinese domestic companies partnered with research institutes in Cuba or biopharmaceuticals in the US. Besides that, it was noteworthy that the rate of collaboration and assignment of Chinese universities only accounted for 13.28% and 7.81% respectively, and those for the research institutes in China were 11.39% and 17.09% respectively. The low cooperation rate and patent assignment rate of universities and research institutes indicate that they should strengthen cooperation with other enterprises in order to transfer the knowledge and technology of anti-cancer mAbs from academia to the market.

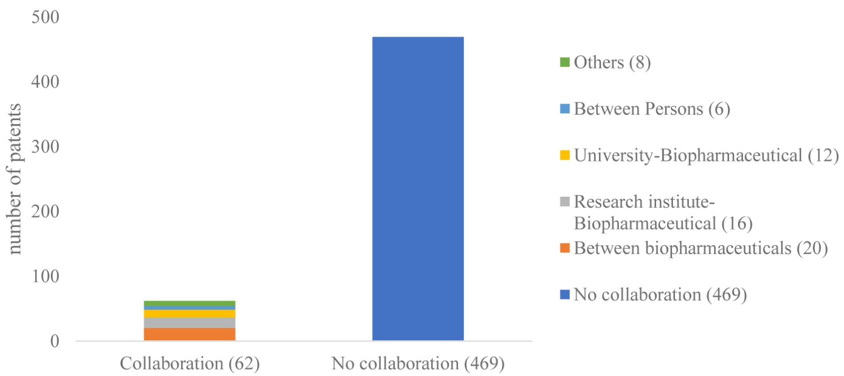


Figure 5. The collaboration activities of mAbs in cancer treatments lying in patents

Note: The number of cases within the category is showed in parentheses.

3.5 Patent quality revealed by the patent age in China

The process of patent registration in China includes: filing application, preliminary examination, publication (18 months from the application date, or earlier by the applicant's request), substantive examination (the substantive examination request should be applied within 3 years after the application date, or it will be counted as withdrawal), and finally grant and registration [23]. The renewal fee should be submitted every year from registration in other to keep the patent right valid according to the Chinese patent law. Since the renewal fee increases in a step-wise function every three years of patent age (which is counted from the application date of a patent), ranging from 900 RMB in the first to third year to 8,000 RMB in the 16th to 20th year of a patent, it is understandable that patents of higher economic value will more likely be maintained for a longer period. For this reason, patent age is used as a proxy parameter to evaluate the patent quality in this study.

Table 2 listed the average examination time and approval time for the Chinese domestic patents and patents from foreign applicants received by CNIPA in the field of anti-cancer mAbs. On average, approximately 5.59 years (2039.92 days) was needed for a patent to be granted. Under the efforts of CNIPA, the examination time had been shortened after 1999, resulting in a huge improvement in approval time from 11.37 years (4151.87 days) during 1991-1998 to 5.24 years (1912.43 days) after 2001.

Table 2. Average examination and approval time for Chinese domestic patents and foreign patents received by CNIPA in anti-cancer mAbs

Time	Origin	Examination time (in days)	Approval time (in days)
1991-1998	All	2307.15 (857.77)	4151.87 (1427.88)
	Chinese	1011.50 (519.72)	1316.00 (287.09)
	Foreign	2364.73 (826.66)	4277.91 (1322.06)
1999-2001	All	1390.75 (697.44)	2515.55 (1148.38)
	Chinese	1020.17 (438.00)	1575.40 (330.27)
	Foreign	1623.06 (731.16)	3096.24 (1087.39)
2001-2018	All	1024.80 (485.37)	1912.43 (949.80)
	Chinese	734.49 (310.15)	1198.48 (638.85)
	Foreign	1226.19 (483.11)	2407.73 (853.08)
Total	All	1099.26 (579.07)	2039.92 (1078.03)
	Chinese	758.13 (331.29)	1228.73 (534.08)
	Foreign	1321.80 (597.66)	2568.43 (1012.73)

Note: Standard deviation is provided in parentheses.

However, we observed a much faster examination and approval time for Chinese domestic patents. The time for a foreign patent to be approved in China almost doubled of their Chinese counterparts. Previous study had also indicated this faster patent grants of Chinese applicants and this may be explained by a 'home advantage effect' that the domestic patent applicants were more familiar with the patent system of their home countries, such as same language and administrative requirements [24]. However, this may also demonstrate that the contents of patent applications by foreign applicants in China are much richer than the Chinese domestic patents, or there are some difficulties in examining the foreign patents by the CNIPA examiners.

Since the time lag for the patent approval is around 5.59 years and time is required for the renewal pattern of therapeutic anti-cancer mAb patents to be observed, we only analyzed our datasets by dividing into three time periods: 1991-1998, 1999-2001 and 2002-2011.

3.5.1 Across different countries

Figure 6 shows the patent renewal rate (by patent age) of anti-cancer mAbs of the top five countries from which applications were received by CNIPA: China, the United States, Japan, Switzerland and Germany. Patents from Hong Kong applicants are also analyzed for our research interest to provide Hong Kong's role in anti-tumor mAbs development.

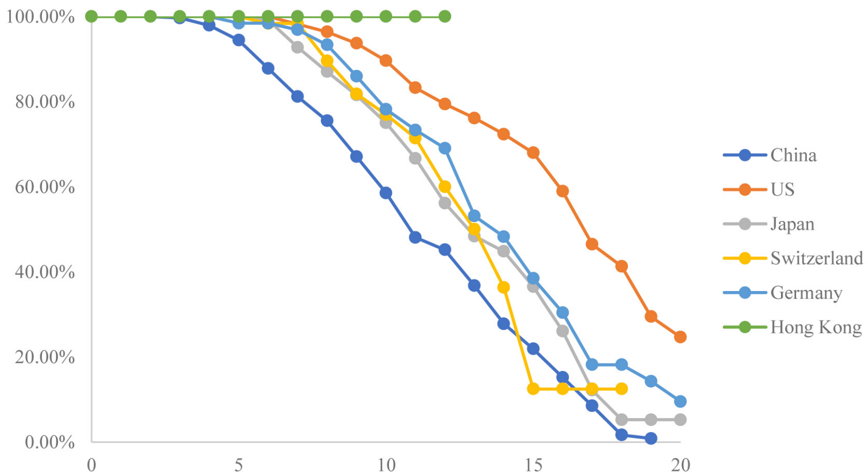


Figure 6. The renewal rate of anti-cancer mAb patents across different applicants' countries of origins

Since the approval time was also calculated into the patent age, almost 100% of patents were kept in force in the first 5 years. The patents from China experienced the fastest decrease in renewal rate among the analyzed countries and regions, while Japan, Switzerland and Germany shared a similar trend of decrease in renewal rate. Only half of the Chinese anti-cancer mAb patents could maintain over 10 years while more than 75% of patents from the other four major countries could survive after 10 years. Patents from the US applicants dropped the slowest within the top five origins of anti-cancer mAb patent applications. Surprisingly, patents from Hong Kong applicants have maintained a 100% update rate in the previous 12 years. However, only nine patents in our data set came from Hong Kong applicants, and only one patent was invalidated in the thirteenth year.

Table 3 describes the percentage of losing patent rights by age of lapse. The 6^{th}, 7^{th} and 9^{th} year was the critical time for a Chinese domestic patent lapse, 11-16% of patents would not be renewed per these years. Patents of domestic applicants in China could not go through a 20-year patent cycle. However, 24.66%, 9.52% and 5.26% of patents from the United States, Germany and Japan could enjoy the whole protection period.

Table 3. The percentage of losing patent rights by age of lapse

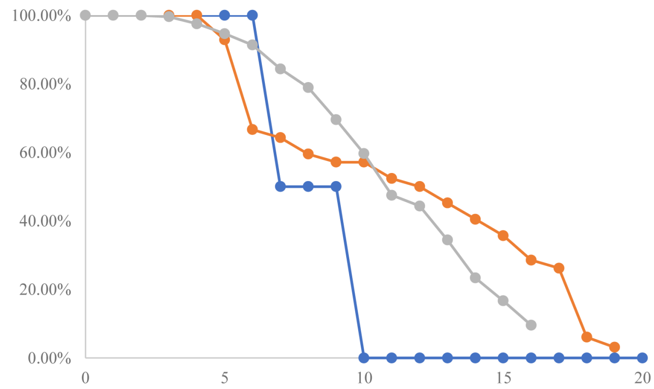
Age of lapse	China		United States		Japan		Switzerland		Germany		Hong Kong	
	%	Cumulative %	%	Cumulative %	%	Cumulative %	%	Cumulative %	%	Cumulative %	%	Cumulative %
1	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
3	0.85%	0.85%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
4	4.24%	5.08%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
5	8.47%	13.56%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.76%	4.76%	0.00%	0.00%
6	**16.10%**	29.66%	0.00%	0.00%	2.63%	2.63%	**14.29%**	14.29%	0.00%	4.76%	0.00%	0.00%
7	**16.10%**	45.76%	6.85%	6.85%	**21.05%**	23.68%	0.00%	14.29%	4.76%	9.52%	0.00%	0.00%
8	6.78%	52.54%	5.48%	12.33%	**13.16%**	36.84%	**57.14%**	71.43%	9.52%	19.05%	0.00%	0.00%
9	**11.86%**	64.41%	8.22%	20.55%	**13.16%**	50.00%	**14.29%**	85.71%	**19.05%**	38.10%	0.00%	0.00%
10	7.63%	72.03%	**10.96%**	31.51%	10.53%	60.53%	0.00%	85.71%	**19.05%**	57.14%	0.00%	0.00%
11	8.47%	80.51%	**13.70%**	45.21%	**13.16%**	73.68%	0.00%	85.71%	0.00%	57.14%	0.00%	0.00%
12	1.69%	82.20%	5.48%	50.68%	10.53%	84.21%	0.00%	85.71%	4.76%	61.90%	0.00%	0.00%
13	6.78%	88.98%	0.00%	50.68%	0.00%	84.21%	0.00%	85.71%	9.52%	71.43%	**100.00%**	100.00%
14	3.39%	92.37%	2.74%	53.42%	0.00%	84.21%	**14.29%**	100.00%	0.00%	71.43%	0.00%	100.00%
15	4.24%	96.61%	2.74%	56.16%	2.63%	86.84%	0.00%	100.00%	4.76%	76.19%	0.00%	100.00%
16	2.54%	99.15%	6.85%	63.01%	2.63%	89.47%	0.00%	100.00%	0.00%	76.19%	0.00%	100.00%
17	0.85%	100.00%	9.59%	72.60%	5.26%	94.74%	0.00%	100.00%	9.52%	85.71%	0.00%	100.00%
18	0.00%	100.00%	1.37%	73.97%	0.00%	94.74%	0.00%	100.00%	0.00%	85.71%	0.00%	100.00%
19	0.00%	100.00%	1.37%	75.34%	0.00%	94.74%	0.00%	100.00%	0.00%	85.71%	0.00%	100.00%
20	0.00%	100.00%	0.00%	75.34%	0.00%	94.74%	0.00%	100.00%	4.76%	90.48%	0.00%	100.00%
No lapse	0.00%	100.00%	**24.66%**	100.00%	5.26%	100.00%	0.00%	100.00%	9.52%	100.00%	0.00%	100.00%

Note: The higher percentage of age of lapsing a patent in each country is **in Bold.**

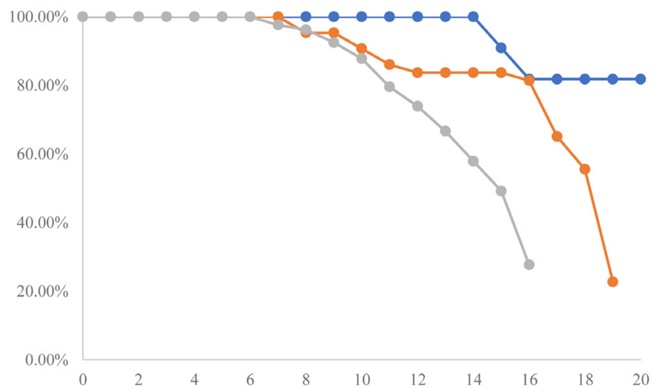
3.5.2 Across different time periods

The renewal rate of anti-cancer mAb patent applicants across different time periods in Figure 7 answers the question of whether the patent quality from the above countries have been improved or not. Since all patent applications from Hong Kong were recorded between 2002 and 2011, a change in time scale could not be evaluated. In these five major countries of patent applicants' origin, only the renewal rate of Chinese domestic patents had improved. The renewal rates decreased at a smoother and gentler pace in recent time periods. The renewal rate of Chinese domestic patents in the 10th year improved from 0% in 1991-1998, to around 60% after 1999. The improvement in Chinese patent quality and the decline in those from foreign applicants indicate the fierce competition that the technology of anti-cancer mAbs has undergone in recent years and the gap between Chinese and foreign mAb technology in therapeutic mAbs in oncology narrowing.

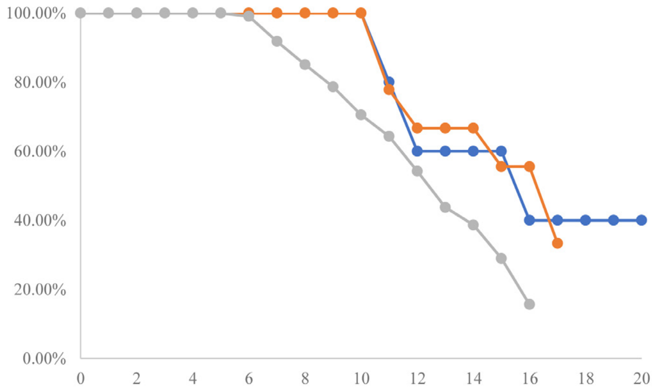
(a)



(b)



(c)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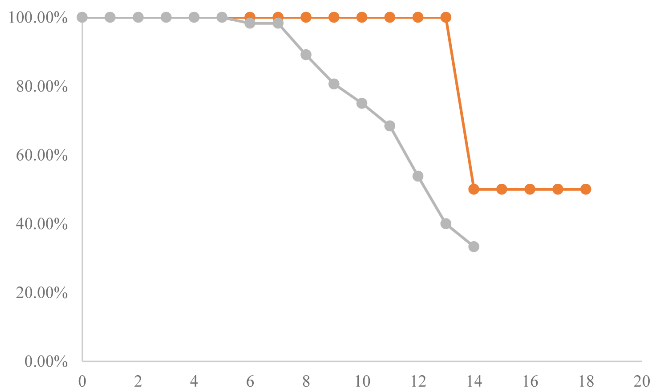
(d)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(e)

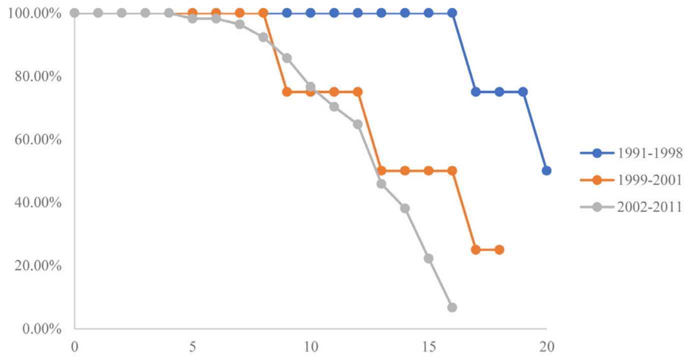

Figure 7. The renewal rate of anti-cancer mAb patents of applicants form 5 major countries of origins across different time periods

(a) China; (b) United States; (c)Japan; (d) Switzerland; (e) Germany.

3.5.3 Times of renewal

As we further analyze the times of renewal behavior in lapse patents, we discovered that almost all patents from different countries' applicants in China concentrated on the first 4 times of renewals, accounting from a cumulative percentage of 58.90% of patents from applicants in United States to 100% of those from Switzerland (Table 4). Chinese applicants' cumulative percentage from 1 to 4 times of renewal decisions were about 61.02%. However, there was a second surge of renewal times lapsing in patent applications from some technologically advanced countries, such as the US, happened in around 10 times, and Germany experienced two after-surge in 7 and 10 times.

Table 4. The percentage of times of renewal

Times of	China		United States		Japan		Switzerland		Germany		Hong Kong	
renewal	%	Cumulative %	%	Cumulative %	%	Cumulative %	%	Cumulative %	%	Cumulative %	%	Cumulative %
1	21.19%	21.19%	13.70%	13.70%	21.05%	21.05%	28.57%	28.57%	9.52%	9.52%	0.00%	0.00%
2	11.86%	33.05%	17.81%	31.51%	28.95%	50.00%	14.29%	42.86%	14.29%	23.81%	0.00%	0.00%
3	12.71%	45.76%	12.33%	43.84%	13.16%	63.16%	14.29%	57.14%	19.05%	42.86%	0.00%	0.00%
4	15.25%	61.02%	15.07%	58.90%	10.53%	73.68%	42.86%	100.00%	19.05%	61.90%	0.00%	0.00%
5	6.78%	67.80%	5.48%	64.38%	18.42%	92.11%	0.00%	100.00%	4.76%	66.67%	0.00%	0.00%
6	5.93%	73.73%	4.11%	68.49%	0.00%	92.11%	0.00%	100.00%	0.00%	66.67%	0.00%	0.00%
7	5.93%	79.66%	2.74%	71.23%	2.63%	94.74%	0.00%	100.00%	14.29%	80.95%	0.00%	0.00%
8	4.24%	83.90%	5.48%	76.71%	2.63%	97.37%	0.00%	100.00%	0.00%	80.95%	0.00%	0.00%
9	5.08%	88.98%	6.85%	83.56%	0.00%	97.37%	0.00%	100.00%	0.00%	80.95%	0.00%	0.00%
10	5.93%	94.92%	8.22%	91.78%	0.00%	97.37%	0.00%	100.00%	14.29%	95.24%	100.00%	100.00%
11	3.39%	98.31%	2.74%	94.52%	0.00%	97.37%	0.00%	100.00%	0.00%	95.24%	0.00%	100.00%
12	1.69%	100.00%	1.37%	95.89%	2.63%	100.00%	0.00%	100.00%	4.76%	100.00%	0.00%	100.00%
13	0.00%	100.00%	2.74%	98.63%	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%
14	0.00%	100.00%	1.37%	100.00%	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%	0.00%	100.00%

3.6 The significant indicators indicating the quality of anti-cancer mAbs patents

From Tables 3 and 4, a surge of patent lapse for Chinese domestic patents happened at the 6th year and the majority of these patents renewed less than 4 times. In consideration of the average approval time of 3.37 years (1228.73 days) for Chinese domestic patents, we set the standard of judging patent quality by dividing these Chinese domestic patents into two groups: patent age of or less than 7 years, and older than 7 years, which the quality of the best 80% and worst 20% domestic patents can be distinguished. Patents from foreign countries are not included in this part of the analysis as we want to focus on the characteristics of the Chinese domestic patents of therapeutic mAbs in oncology. Also, patents that are valid in less than or equal to 7 years of age will be excluded, as it may happen to be in the group of higher value in later years as they are still alive. Finally, 253 domestic patents were analyzed to determine the significant indicators of the quality in Chinese mAb patents in oncology.

As all of the continuous variables are not in normal distribution, Mann-Whitney U Test is used in this study. The Chi-Square Test is performed for the categorical variables. Table 5 revealed the descriptive statistics in mean value and their corresponding standard deviations, and also the results and significances of the Mann-Whitney U Test or Chi-Square Test. Six significant indicators were found in evaluating the quality of Chinese domestic anti-cancer mAb patents, including applicants from individuals, applicants from universities, number of inventors, number of IPC subgroups, family size, and patent assignment, with the significance at 5% level.

Table 5. Descriptive statistics of the indicators dividing by patent age of 7 years, and also the results and significances of the Mann-Whitney U Test or Chi-Square Test

Indicator	Mean (S.D.)		Type of statistical test	z-score (in Mann-Whitney U test) / Chi-square value (in Chi-Square test)
	Patent age ≤7 years	Patent age >7 years		
Applicants				
Individual	0.03 (-)	0.12 (-)	Chi-Square	**4.09***
University	0.32 (-)	0.17 (-)	Chi-Square	**6.89***
Research institute	0.40 (-)	0.47 (-)	Chi-Square	0.74
Biopharmaceutical	0.19 (-)	0.26 (-)	Chi-Square	1.18
Hospital	0.08 (-)	0.06 (-)	Chi-Square	0.42
In cooperation	0.05 (-)	0.10 (-)	Chi-Square	0.30
Number of inventors	3.84 (2.93)	4.36 (2.47)	Mann-Whitney U	**-2.35***
Number of backward citations (to other patents) [a]	3.02 (6.23)	2.72 (2,42)	Mann-Whitney U	-1.25
Number of backward citations (to non-patents) [b]	8.73 (7.34)	8.81 (10.10)	Mann-Whitney U	-1.07
Number of claims	6.85 (3.14)	8.48 (7.17)	Mann-Whitney U	-1.04
Number of IPC subgroups	7.94 (2.84)	7.07 (3.06)	Mann-Whitney U	**-2.23***
Family size [c]	2.55 (2.28)	3.19 (3.42)	Mann-Whitney U	**-2.46***
PCT	0.02 (-)	0.07 (-)	Chi-Square	2.42
Priority right	0.06 (-)	0.08 (-)	Chi-Square	0.13
Examination time	761.19 (291.00)	816.81 (362.02)	Mann-Whitney U	-0.72
Patent assignment	0.02 (-)	0.26 (-)	Chi-Square	**17.55*****
Patent pledge	0.00 (-)	0.04 (-)	Chi-Square	2.34
Patent licensing	0.00 (-)	0.01 (-)	Chi-square	0.65
Patent agent	0.94 (-)	0.88 (-)	Chi-square	1.53

Note: * p<0.05; ** p<0.01; *** p<0.001.

Due to the source of dataset that patents with 0 backward citations may be regarded as missing value, all 0 and blank number of backward citations was not included in this study. In all cases, N=62 and 191 in the group of patent age ≤7 years and >7 years, respectively, except: a. N=54 and 163 for in the group of patent age ≤7 years and >7 years, respectively; b. N=49 and 133 for in the group of patent age ≤7 years and >7 years, respectively; c. Due to 1 missing value in family size in the group of patent age >7 years, N=190.

Then a binary logistic regression is conducted in order to further evaluate the contribution of these significant indicators. Before the binary logistic regression is performed, a partial correlation analysis is performed to ensure that there is no association between the continuous variables. Although there is a moderate correlation between the number of backward citations to patents and non-patents, both of them did not show significance in the Mann-Whitney U test, and thus will not be included into the regression model. All the other variables show a weak correlation, indicating that there is no existing co-linear problem.

Table 6 displays the most fitted model of the binary logistic regression performed by SPSS 22.0. The model is conducted by putting all the six significant indicators mentioned before into binary logistic regression, and using a forward stepwise variable selection method (inclusion criteria: 0.05; exclusion criteria: 0.10). After 3 steps of modelling, 3 variables: applicants from universities, number of IPC subgroups, patent assignment were finally included in the regression model. This model was statistically significant with χ^2=33.18, p<0.001 and did not show significant misfit under Hosmer & Lemeshow Test ($\chi^2(8)$=7.78, p=0.45). The overall percentage accuracy in classification of this model was 75%, showing an acceptable performance of this model.

Table 6. Results of the most fitted binary logistic regression model

Indicator	β	S.E.	Wald χ^2	df	P value (two-tailed)	Exp(β) (odds ratio)
Applicants from universities	-0.92	0.36	6.43	1.00	0.01	0.40
Number of IPC subgroups	-0.12	0.05	4.67	1.00	0.03	0.89
Patent assignment	2.94	1.03	8.22	1.00	0.00	18.90
Constant	1.95	0.48	16.76	1.00	0.00	7.01

Note: N=253. Hosmer & Lemeshow Test: $\chi^2(8)$=7.78, p=0.45 >0.05. Overall percentage accuracy in classification of this model: 75%.

The variable 'patent assignment' showed a great positive relation to patent quality, if the ownership of a patent had been transferred, 18.9 times the possibility of it belonged to the group of patent age >7 would be added. However, whether applicants were from universities and the number of IPC subclasses indicated a negative relation to the patent quality, 60% of the possibility for a patent from university and 11% of the possibility for the addition of an extra IPC class would be decreased in the group of patent age >7. Although the other 3 indicators 'applicants from individuals', 'number of inventors', and 'family size' were not included in this model, all of them showed a positive correlation to patent quality.

4. Discussion

By analyzing the therapeutic anti-cancer mAb patents in China, the first part of our study discovered that the development of this biopharmaceutical technology could be divided into three stages. The first stage occurred in 1991-1998. The core technology and R&D of mAbs was led by foreign biopharmaceuticals and research institutions. Although China began its R&D of mAbs in the 1980s, they did not appear to shift their attention of mAbs into oncology at that moment. The first murine mAb therapeutic agent for treatment and prevention for acute rejection as prognosis of kidney and organ transplant was introduced in 1999 [25], but only 2 mAb patents related to oncology were found in this period. The second stage happened between 1999 and 2001, the applications from Chinese applicants grew exponentially as China joined the WTO and a more open market was formed towards foreign investments, which resulted in about two-fifth of granted patents of anti-cancer mAbs being from domestic Chinese applicants. However, the fluctuating quality of these applications was reflected by the low grant ratio of applications recorded within this period. The third stage, which started in 2002, more countries participated in the R&D of anti-cancer mAb patent activity and the dominance of the US was decreasing, indicating a diverse and mature development of mAbs towards malignancy in China was already taking place.

The second part of our study used patent age as a proxy indicator for evaluation of patent quality. In general, domestic Chinese patents in mAbs of oncology showed a lower quality among the top five applicants' countries of origin in China. However, by analyzing across time periods, an increased quality in Chinese domestic patents could be observed, while patents from the other four major foreign countries' applicants showed a decrease in quality. This may suggest that the gap between Chinese and foreign mAb technology in oncology became narrower and an intense competition began in recent years.

The third part of our research indicated that the following indicators: applicants from individuals; applicants from universities, number of inventors, number of IPC subgroups, family size, patent assignment showed a significant relation to the quality of domestic Chinese anti-cancer mAb patents of classifying whether the patent age was longer than 7 years. The Mann-Whitney U Test revealed that both the number of inventors, number of IPC subgroups and family size were proportional to the patent quality. An increasing number of inventors can enhance the depth and breadth of invention. Number of IPC subgroups can be regarded as the technological scope of patents and is highly used to determine patent value [11]. However, the model performed by binary logistic regression indicated a negative correlation of number of IPC subgroups towards patent quality, this might be caused by the skewed distribution of IPC subgroups. Those from the closer IPC subclasses will be more technologically correlated, so some studies only use the total number of 4-digit IPC classes to calibrate the effectiveness of this indicator [11]. Family size means the number of patents taken in various countries relating to the same invention, a patent of higher value tend to seek protection from more countries [26]. Patent applicants were categorized into individual, university, research institute, biopharmaceutical and hospital to explore the impact on patent quality. Patents from universities and research institutes showed significantly higher value in Branstetter's study by measuring patent citations [27]. However, in our research, patents from universities were less likely to keep their patents valid longer than 7 years. Due to a low assignment and collaboration rate, universities may not be willing to keep their patents for a long period as their patents could not be transferred to the industry and become a product. Patents from individuals showed a positive relation to patent quality. Lastly, patent assignment showed huge significance in our model by increasing 19 times of possibility for keeping the patent over 7 years. The transference of a patent ownership means that it contains much market potential and indicates a high positive relation toward patent quality.

5. Hong Kong's role in anti-cancer mAb development in China

In this study, a total of 11 invention anti-cancer mAb patent applications from Hong Kong were received by CNIPA, and 9 of them got approvals. The development of therapeutic mAbs in Hong Kong started after 2002, a little bit later than mainland China. But they all focused on the R&D on humanized and fully human mAbs, which indicated an advanced and rapid development of anti-cancer mAbs in Hong Kong. These patents could be divided into two categories by their applicant types. The first type was formed by two universities: University of Hong Kong and Chinese University of Hong Kong. All of the patents applied by these two universities had gained the priority rights from the US, and two of them had PCT applications. The second type was conducted by three biopharmaceuticals: Baimaibo Pharmaceutical Co. Ltd., Sinomab Bioscience Limited and Gen Regeneratives Ltd. Baimaibo Pharmaceutical was actually a Chinese domestic pharmaceutical company and its related patent ownerships had been transferred back to its headquarter in Shanghai, China. The patent quality revealed by patent age indicated that Hong Kong had a high patent quality. All of their patents could last for over 10 years. However, there is a similar problem in both Hong Kong and Chinese patent behaviors: the low assignment and collaboration rates. Universities in Hong Kong establish an advanced R&D quality, but few biopharmaceutical companies are found in Hong Kong. A stronger collaboration between Hong Kong's universities and China's biopharmaceuticals is highly recommended for knowledge transfer from academia to industry. Hong Kong's universities can rely on the financial support from Chinese domestic biopharmaceuticals and the rich resources in R&D in China, especially its resources in conducting clinical trials. Chinese biopharmaceuticals can depend on the strength of scientific research in Hong Kong's universities to increase the quality in R&D and shorten the time for new drug development.

6. Conclusion

By analyzing the invention patents of anti-cancer mAbs in China, this study indicates that although the quality of patents authorized by foreign applicants in China is better than those from domestic Chinese applicants, the gap between Chinese and foreign mAb technology has been lessened per the evaluation of quality in Chinese domestic patents in recent decades. A mature and diverse mAb market towards anti-tumor treatments has already occurred in China and the relationship between domestic and foreign players will be increasingly competitive. Moreover, this study has discovered that six indicators, including applicants from individuals, applicants from universities, number of inventors, number of IPC subgroups, family size, and especially patent assignment show significant influence on the patent quality of Chinese domestic mAb patents in oncology. However, stronger collaboration or patent assignments are highly suggested for both mainland China and Hong Kong's universities and research institutes to collaborate with biotech companies to transfer the advanced technology in oncology mAbs from academia to industry.

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Chapter 3

THE RECENT ADVANCE IN DIGITAL MICROFLUIDIC TECHNOLOGIES

Joseph Chow

Microfluidics refers to approaches that enable exploitation of fluids and substance in fabricated nanoscale and microfluidic channels. Digital microfluidics (DMF) is an emerging liquid-handling technology that **utilizes the precise manipulation of droplets in the microliter to nanoliter range to achieve complex analyses.** DMF is often used in integration with other biosensing technologies like mass spectrometry, colorimetry, electrochemical analysis, and chemiluminescence to achieve different analytical purpose. In recent years, A broad range of improvements and innovations in digital microfluidic technology have been advanced in the areas of electronics, machinery, fluidics control and fabrication methods (See Table 1). DMF has been reported and used in enormous applications such as nanoscale detection in medical diagnosis, food industry, security, and health or epidemic prevention.

One of the often-used technique in DMF is droplet manipulation, droplets are dispensed, mixed, moved, reacted, or analyzed on a miniaturized chip-based platform. These miniaturized biochemical analysis systems are often collectively called micro Total Analysis Systems (µTAS) or “Lab-On-a-Chip (LOC)” devices. LOC is a very active and fast-growing field that keeps providing new and creative solutions to improve current diagnostic performance.

Material	Advantage	Disadvantage
Silicon	High precision	High cost
Thermoplastics	Very Low cost	Need molding
Glass	High precision, excellent Transparency	Fragile, difficult production
Paper	Low cost and easy to produce	Not transparent
PDMS	Good Transparency, low cost, elastomeric	Unstable for mass production no stable

Table 1: Comparison of different materials for digital microfluidics. Recent improvement of DMF materials such as plastic and paper provide enormous opportunities in DMF research [49].

The concept of the integrated Lab On Chip (LOC) microfluidic device was introduced by Manz et al. in the early 1990s [1]. Since then, microfluidic devices have led to great advances in many research fields. Over the last decade, a fast-growing interest was noticed for developing microdevices that integrate several functionalities (e.g., from sample preparation to signal detection) onto a single millimeter chip to perform all types of chemical and clinical analysis. Compare to current diagnostic methods, LOC contain several distinctive advantages: 1) Using very low fluid volume of sample, low reagent consumption, usually in nanoliter or picolitres, the use of an extremely tiny volume for biochemical reactions enable more diagnostics parameters from the same samples, which can be analyzed to provide extra treatment information and decision; 2) Large repeat of microfluidic reactors in same reaction enable accurate results, such large repeats facilitate parallelization of reaction than traditional bulk analysis; 3) Low fabrication cost for plastics disposable chips instead of expensive silicon chips; 4) Small size and easy Integration (e.g., from DNA extraction, amplification to detection in one chip). Due to the small size of the system, whole biological process can be integrated and thus a simplified device can be developed into a portable health care system which allows continuous monitoring of health status at home; and 5) Integration of smaller fluid volumes and chemical energies stored on chip makes the reaction much safer, integration of chemical storage allowing companies be able to manufacture their products that easily accessible to the local market [2-8].

Based on these advantages, microfluidic devices have been developing rapidly into many different areas in both fundamental research and actual practice such as:

Applications of Microfluidic integrated Lab-On-Chip

1. Nucleic Acids detection
2. Protein and Exosome detection
3. Single-cell Manipulating and Sorting
4. Rapid microorganism and virus detection
5. Environmental safety and public health
6. GMO and food safety
7. Point-of-care testing (POCT)
8. Clinical and forensic analysis
9. Drug Screening
10. Microfluidic Cell Culture

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a method for DNA amplification invented in the 1980s. In PCR, template DNA will be amplified by several orders (See Figure 1). A single or a very small number of molecules can be amplified into millions of copies with a series of thermal cycles in the presence of necessary PCR component. This technique has revolutionized many aspects of current research, including genetic research, phylogenetics, the discovery and diagnosis of diseases, food and agriculture, forensic science, environmental microbiology, consumer genomics and more.

Modifications in PCR methods have been developed to enhance the utility of this method in diagnostic settings based on their applications. Some of the common types of PCR are Real-Time PCR (qPCR), Nested PCR, Multiplex PCR, Quantitative PCR and Arbitrary Primed PCR. Recently digital PCR (dPCR) and droplet digital PCR (ddPCR) were added to the family.

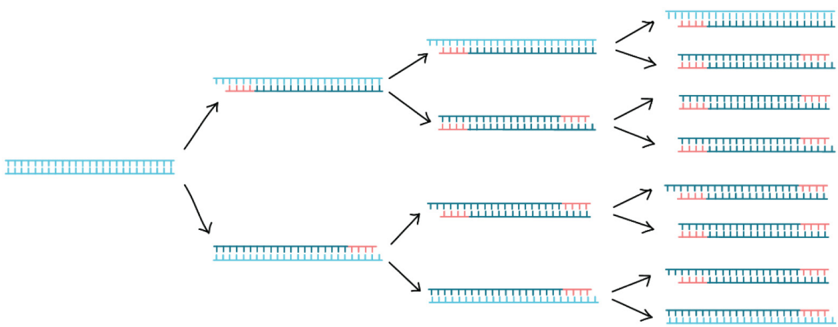

Fig. 1 Polymerase chain reaction (PCR): A single or a very small number of molecules can be amplified into millions of copies with a series of thermal cycles in the presence of necessary PCR component. (Adopted from khanacademy)

Droplet-Based Platforms

Droplet-based microfluidics is one of the techniques that enable manipulating and processing of droplets in their immiscible phase. Droplets are generated using active or passive methods. The active method for generation of droplets involves the use of an external factor such as dielectrophoresis (DEP) and electrowetting on dielectric (EWOD).

In passive generation methods usually employing water-in-oil systems, droplet-based techniques enable the ingredients to assemble and manipulate in small quantities which goes down to the single molecule level. Cross-flowing, co-flowing and flow-focusing methods are examples of passive methods used for generation of droplets. Since the droplets are isolated and protected by the immiscible phase, each droplet can be regarded as a micro-chamber to perform chemical/biological reactions independently. Control and manipulation of such droplets in microfluidic systems can be achieved by hydrodynamics, acoustic waves, optics manipulation, electrical or magnetic control and other techniques [7, 9-10].

Scientists have exploited the use of droplet-based platform in Polymerase Chain Reaction (PCR). The small reaction volumes of water-in-oil droplet system offer fast thermal control, independent isolation of targets which is of benefit to reactions that require thermocycling and absolute quantitation in molecular level (See Figure 2).

One of the most important use of droplet-based microfluidic technology to the clinical diagnosis is digital Polymerase Chain Reaction (dPCR) and droplet digital PCR (ddPCR) [4, 11-13]. The technologies have played a central role in enabling the revolution of digital quantification with the core concept of sample partitioning. It offers increased precision, more reliable measurements and absolute quantification from very small or mixed samples. Lately, the technology has become an indispensable tool in the research and in the clinic field.

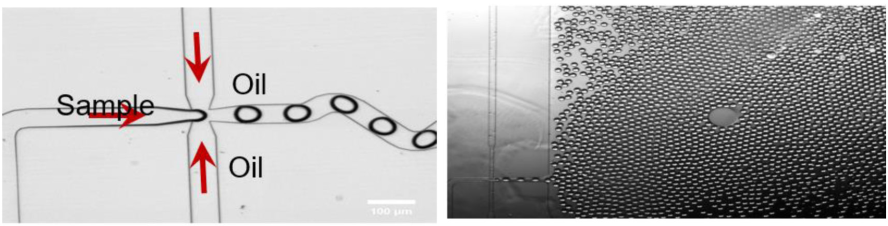

Fig. 2 Droplet generation for droplet digital PCR, droplets encapsulated with sample and PCR reagent are generated by T-junction configuration. The droplets are then dispersed into the single layer chamber for polymerase chain reaction.

The term "digital PCR" was first proposed by Kinzler and Vogelstein in 1999 [14]. The early methodology was described as "single molecule PCR" or "limiting dilution PCR". The main processes involved in dPCR are sample dispersion, thermal cycling, and output detection. In digital PCR or ddPCR, the PCR mixture is compartmentalized into millions of smaller units (See Figure 3). Each partition acts as an individual PCR chamber and undergoes the similar thermal cycles as in the conventional PCR. Partitions containing amplified target fluorescence signals are detected by CCD camera, positive PCR reactions can be identified by the fluorescent signal and scored as '1', while negative fluorescence is considered '0'. dPCR or ddPCR quantification is based on the fact that the random distribution of molecules in the partitions follows Poisson distribution; a distribution function useful for characterizing events with very low probabilities of occurrence within some definite time or space. Hence, the concentration of the target sequence is direct and without a need for calibration [9-10].

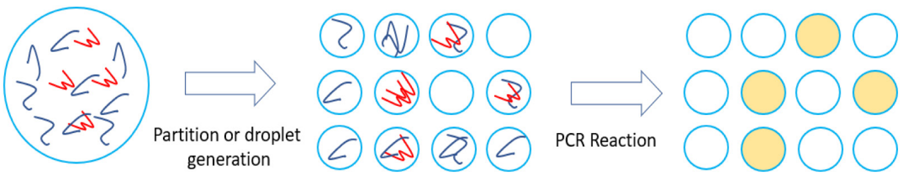

Fig. 3 Principles of digital and droplet PCR. The sample is divided into many independent partitions and undergo PCR reaction. Present of positive fluorescence signals will be counted as "1" and negative signal will be counted as "0".

Digital PCR (dPCR) and Droplet Digital PCR (ddPCR)

There are several dispersion methods for dPCR which including Microwell-based, droplet-based, channel-based, printing-based method and etc. (See Figure 4). Microwell-based dispersion in which sample and PCR mixture are filled into thousands of micro-sized reaction chambers made from plastic or silicon chip. The second dispersion method is droplet-based microfluidics in which sample and reaction mix is generated as droplets are dispersed in another immiscible liquid by configurations of T-junction, flow-focusing or co-flow channels [6]. These chips are then covered with oil for thermal cycling reaction and subsequently by signal detection. In both dispersion techniques, the PCR mixture forms immiscible "droplets" in an oil phase and subsequently undergoes thermal cycling. The major advantage of this method is the protection of the PCR mixture from contamination. The oil phase also prevents the droplets of a PCR mixture from evaporation [9-10, 15]

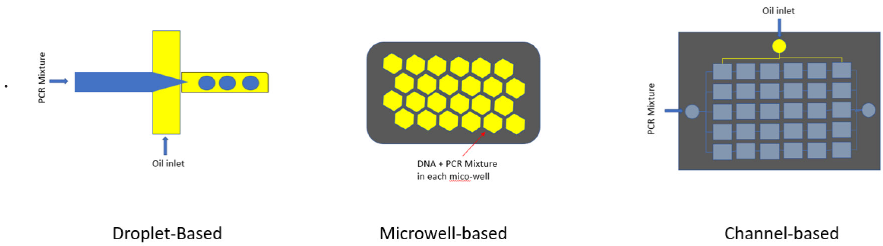

Fig. 4 Types of dispersion methods for dPCR. a) Droplet-based b) Microwell-based c) Channel-based.

The difference between dPCR/ddPCR and Real-time polymerase chain reaction (qPCR) is that, the fluorescence output is monitored throughout in process in qPCR, while dPCR/ddPCR is performed only in the end of 50 PCR reaction cycles for quantification of the total target molecules within the sample (See Figure 5). So far, Digital PCR is the most precise PCR technique compared to qPCR. The technique has demonstrated a supreme sensitivity with qPCR in several potential clinical applications [16-20,48].

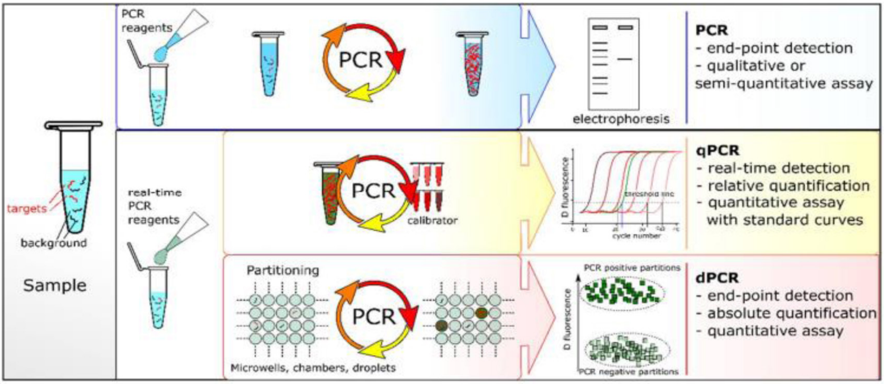

Fig. 5 Different types of PCR comparison. a) convention PCR, b) qPCR, c) dPCR. Adopted from Brouzes [48].

Due to its robustness and high reproducibility, dPCR/ddPCR has grown to become the new standard for nucleic acid quantification in past several years. It has been rapidly adopted into clinical oncology where clinicians are beginning to use the technology for monitoring treatment response in cancer patients by monitoring circulating tumor DNA (ctDNA) quantity [21-23]. In addition, scientists have extended the benefits of reaction partitioning to demonstrate its usefulness in measuring protein levels, enzyme activity and capturing specific cancer cells for genome analysis in a sample, and more [24].

The use of digital PCR and droplet digital PCR in the field

Digital or droplet digital PCR have many advantages over traditional PCR and qPCR. Besides absolute quantification, digital PCR and droplet digital PCR has gained popularity in many other aspects of advantage [25]:

1. Superior sensitivity - DNA template is compartmentalized into smaller units, dPCR/ddPCR reduce "template competition effect" to increase the relative concentration of the target DNA sequence in each unit.

2. ddPCR is reported to show higher resistance to inhibitors compared to qPCR.

3. Absolute quantification of DNA and RNA - the calibration-free nature of dPCR should confer an advantage on assay reproducibility.

4. Good repeatability and reproducibility - reproducibility and coefficient of variation are generally lower in using dPCR due to use of statistically significant number of reactions.

5. The partitioning of a reaction into many smaller reactions can greatly improve the signal-to-noise ratio over bulk reactions.

6. Low reagent consumptions, hence, cost reduction per analysis.

From 2007 onwards, there has been a rapid and exponential increase in the number of publications referring to digital PCR. More and more research results and uses are published in agriculture and plant monitoring, food safety testing, environmental monitoring, marine ecology, early cancer screening, companion diagnostics, non-invasive prenatal testing, virus and bacterial quantification, genetic diseases diagnosis and gene expression analysis.

Future of digital microfluidic (DMF), beyond digital PCR

The use of digital microfluidic devices in the field of discovery has been growing rapidly in the last few years, due to the fact that this new technology can accurately isolate and measure molecules or anything in nano, femto liter scale. On the other hand, DMF technology can be combined with different methods to separate, extract and study the analytes. General detection technology such as optical tweezers, magnetic particles, thermal dynamics or hydrodynamic can be used for expanding the use of DMF in different field.

Several groups have commercialized the use of ddPCR and device but also demonstrated that DMF in immunology will be another useful way to replace traditional enzyme-linked immunosorbent assay (ELISA) method (See Figure 6). Our group encapsulated functionalized magnetic particles that bind to the target analyte in oil and analyze by optical detection. We were able to capture and detect exosomes in breast cancer patient with high accuracy and high detection limited [26-28].

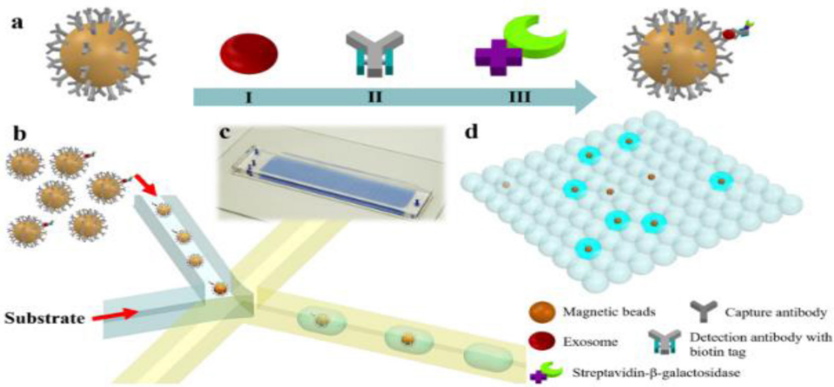

Fig. 6 Schematic showing the droplet digital Exo-ELISA for exosome quantification. (a) Single exosome immunocomplex constructed on a magnetic bead. (b) Substrate and beads are co-encapsulated into microdroplets. (c) Droplet digital Exo-ELISA chip. (d) Fluorescent readout for counting the positive droplets with the target exosomes.

Extraction of biological principles is often difficult because of small sample volumes used in DMF. However, the combination of DMF with macrofluidic systems can bypass this obstacle [3].

Recently researchers and companies commercialized DMF technology to study single cell, screening of immunotherapy clones, encapsulate drug [24,40-41], CRISPR-Cas system into droplets and release them by using magnetic wave, UV, Light or heat [31-32,34-35,44] (See Figures 7 and 8). Some of the attempt is to replace existing expensive testing such as Noninvasive prenatal testing (NIPT) [41-42]. The potential of droplet microfluidics as a powerful tool for single cell studies has been demonstrated since the emergence of the field. DFM to manipulate single cells in microfluidic chips opened up novel ways of revealing individual responses through high-throughput functional imaging. Recent studies show a potential commercial direction with single cell screening assays. Such assays can even be a potential application for the production of monoclonal antibodies from individual hybridoma cell clones. The use of DMF in single cell will become invaluable tools in the near future [3, 30, 33, 35-39].

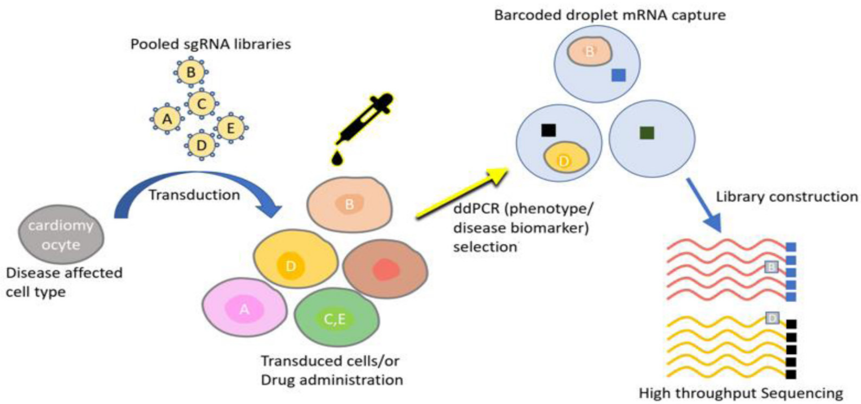

Fig. 7 Single cell screening by droplet encapsulation. Cells are encapsulated by selected hydrogel material. The encapsulated cell and then reverse transcript and amplified by rt-PCR for next generation sequencing analysis.

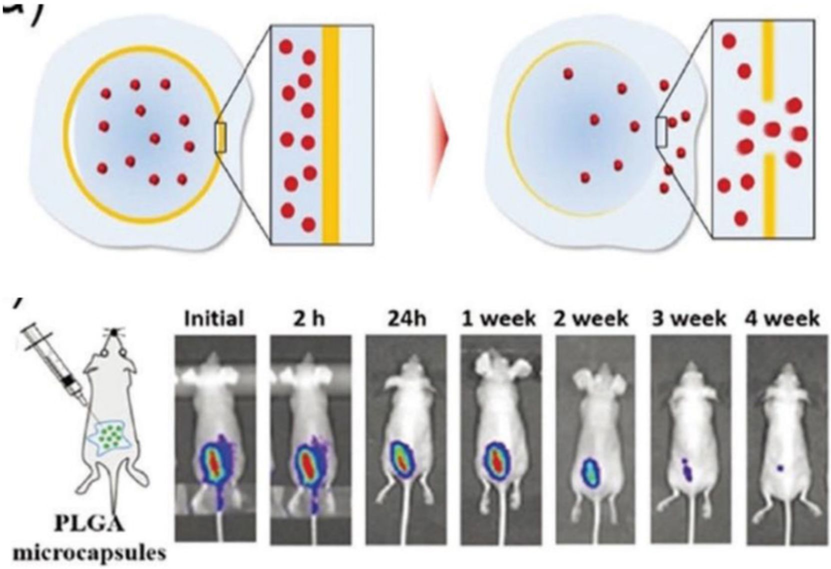

Fig. 8 Drug encapsulation, Drug delivery and Drug release. (Figure adopted from Li [30] with permission.)

Conclusion:

The present paper introduces recent advances in the area of DMF and its broad applicability in various fields of research. Besides the applications mentioned in the article, researchers have also shown that microfluidic technologies demonstrated potential to dramatically improve many aspects of analytical research by providing superior performance when compared to the classical protocols. It has an enormous potential to use automatic DMF for replacing the traditional labor-intensive analytical procedures. The automation process for DMF is also much easier than traditional liquid-handling method since it uses extremely low volume, and is simple and cost-effective. Other interesting applications of using droplet microfluidics include the production of bio-fuel by Algae, the study of crystal growth, small molecule synthesis in microdroplets for pharmaceutical applications and infusion of bio-active food production [40, 44-47]. People are breaking the barrier of using DMF in every field to enhance their efficiency (i.e., High throughput sample processing, record, analysis and predict their data by assist of company program). It is exciting to see methods developed in last few years and hoping to see Digital Microfluidic technology become invaluable tools in the near future.

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Chapter 4

LIFE SCIENCE COMMUNITY COLLECTIVELY AND POSITIVELY ASSERTS THE NECESSARY OVERSIGHT ON THE MERITS OF LIFE SCIENCE AND BIOTECHNOLOGY PUBLICATIONS

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Important Roles the Scientific Community Plays

As pharmaceutical industries reported low reproducibility of published discoveries in life science research[1,2], this news created quite a stir in the scientific world – despite the well-known limitations of the peer review system[3] in scientific publications that have been debated for a great number of years[4, 5, 6]. However, it is obvious that one's judgment on the merits of the peer review system depends on one's view on the roles that the system plays. It is well documented from the various debates and past events that the peer review system is not designed to be a policing tool to eliminate frauds. It is, however, an indispensable and useful tool in ensuring that scientific publications present clear, timely, and definite communications; in exposing and advising readers of potential ethical and conflict of interest issues; and most importantly, in alerting readers of new development in the discipline in a timely manner. The peer review system, foremost, must not stifle paradigm-changing creative concepts, new discoveries, and innovations.

As an analogy, many people augment their home security with various modern electronic and digital means[7,8,9] acknowledging the reality that the doors and windows of their homes are not absolutely secure against potential invaders and other malfeasant activities that can happen. Nonetheless, nobody will consider a home without doors and windows. One should, therefore, view the roles the peer review system in a like manner. It does not provide absolute protection, but it serves certain and specific purposes. In the case of publications, the peer review system serves to communicate and alert readers of new development, although it does not guarantee the accuracy or reproducibility. It should not be viewed as a proving or a policing mechanism. Knowing that the peer review system is not 'fool-proof', then how much trust can one put in any published research results and conclusions drawn therefrom? The key role scientific publications play is to allow timely communications and sharing of novel ideas, research results and conclusions, no matter how speculative or paradigm changing they may be. They do not need to guarantee usefulness, nor functionalities. There is no need for conformity. Such communications serve to alert and make the scientific community aware, and eventually – through further work and research – will render the final judgment on the reproducibility, validity, and eventually the usefulness of the reported data, discoveries, or innovations. The community will, through its own initiative, validate or invalidate such published results. In a sense, the publications recruit the community to play a "self-policing" role in testing how reproducible or accurate the published results are or how valid are the conclusions derived therefrom. This is particularly important in the life science and biotechnology fields since there remains much to explore and elucidate, due to the "evolving" nature[10] of this discipline, in that life forms evolve under the well-studied and generally accepted mechanisms of spontaneous mutation (estimated in higher eukaryotes to be roughly 0.1 to 100 per genome per sexual generation[11]) and selection pressure (natural[12] or experimentally introduced selection bias). Nonetheless, the publishing agencies do play an administrative, although often primary and superficial, role in checking for certain comparatively easier identifiable malfeasance, such as academic misconduct (e.g. plagiarism[13]).

As an illustration of another form of misconduct and how it may evolve with advances in technology, Springer, the Berlin-based publisher of many key life science journals, retracted a record number of publications by a group of Chinese authors after its editorial reviews discovered the use of fake email address for fictitious reviewers to fabricate peer review[14] reports by this group of authors. This type of misconduct would not be easily done in such a scale without the advancement in the Internet and subsequent popular usage of electronic mail. Springer's editorial reviews were mostly performed both by the agency's editorial staff (i.e., check for malfeasant activities) and scientific experts (who are mostly volunteers) in the field within the life science community. This arrangement provides administrative support to editors with scientific expertise in the subject field. This example serves well to illustrate the self-policing power of the life science community in exposing even deliberate fraud. However, self-policing power by the life science community is not limited to exposing deliberate fraud. It also serves to expose the non-reproducibility of research results that may simply be biased by the researchers' wishes or by errors in interpretation. For example, in 2016, a Chinese research group reported a novel gene-editing method (the NgAgo gene-editing method) in the popular life science journal of Nature Biotechnology[15]. That was exciting news in that it suggested an alternative approach that could overcome many limitations of the popular CRISPR-Cas9 gene-editing method. In fact, this author opined positively on the potential of this new method in plant agriculture[16]. However, within months of the original Nature Biotechnology publication, the excitement was overcome by controversies regarding the reproducibility of the initial published results by the original authors, with strings of publications describing mostly unsuccessful attempts by other scientists to use the new NgAgo method[17]. The original authors subsequently retracted the publication[18] although the university cleared the authors of academic misconduct and concluded that the study was simply flawed[19]. In another instance, former professor He Jiankui of South University of Science and Technology of China announced that he had successfully altered the genes of human embryos[20] that gave birth to two girls in the hope of making them more resistant to infection by the Human Immunodeficiency Virus. Within weeks of He's announcement (and pre-summit announcements) at the Second International Summit on Human Genome Editing in Hong Kong[21], the life science community not only pointed out the ethical issues with He's work but also analyzed the data he presented and concluded that He failed to achieve the 32-base-pair deletion to the CCR5 gene that He aimed to accomplish[22]. Analysis of He's data required a level of scientific expertise that only the science community can provide, not by any administrative processes.

Further, the science community in general (including the life science community) has also evolved to establish reliable mechanisms to document and summarize corrective efforts by building the publication "Retraction Watch" database[23]. Note that this database more than just catalogs publications that were retracted by journals, but also provides citation statistics (see, for example, Table 1.) and additional information, including fallouts[24, 25] and explanatory comments, regarding the authors and the underpinning rationales for the retraction. It is accessible and free online. Despite such valiant efforts, however, it often took much time for a journal to retract a published article with an average of ~7.2 years lapsed for the 10 examples presented in Table 1. The retracted journal publications often also were continually cited by later publications[26]. In fact, many retracted science papers had been highly influential in the field (Figure 1). It is not clear why retracted publications were continuously cited[27]. It possibly is due to a lack of awareness by the authors of the citing publications that might have been augmented by a lack of incentives and efforts by the journals to highlight retracted publications. One likely positive possibility is that the retracted publications might also have been cited by subsequent publications to illustrate the fallacy or deficiencies of the results/conclusions put forward in the retracted publications. Nonetheless, the ability of the life science community to self-correct on the merits of research results in publications should not be overlooked nor should it be considered infallible. Although it often takes time, it is nonetheless a reliable mechanism that complements the peer review system and the diligence of the editorial staff well.

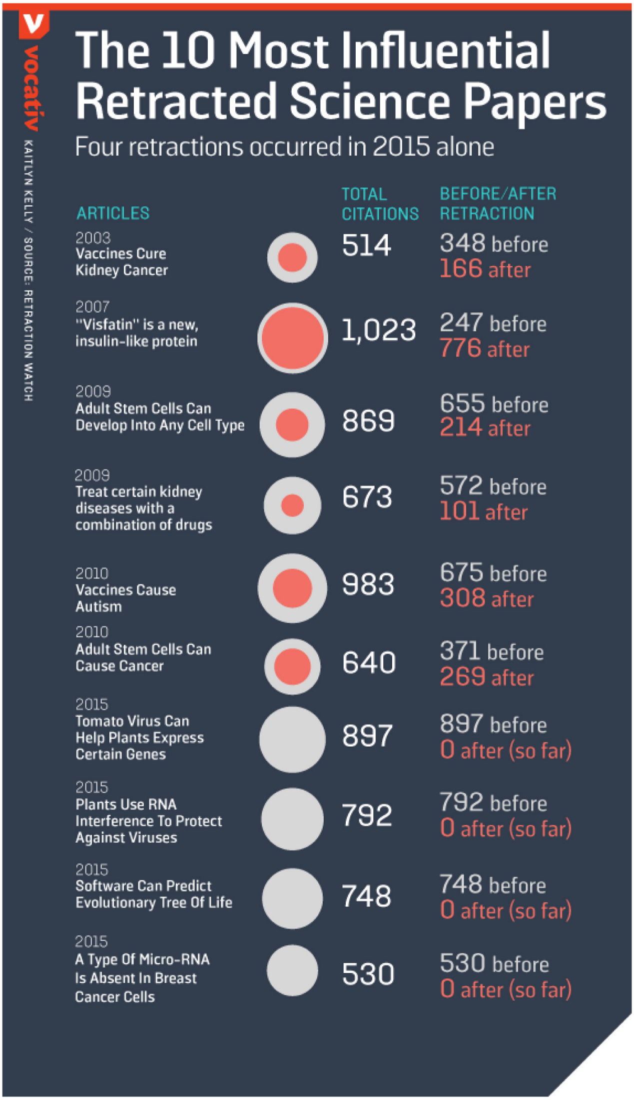
Table 1.

Article published in journal	Publication Year	Retraction Year	Number of citations before retraction	Number of citations after retraction	Total number of citations	Years Lapsed prior to retraction
Science	2005	2007	247	776	1023	2
Lancet	1998	2010	675	308	983	12
Plant	2003	2015	897	0	897	12
Blood	2001	2009	655	214	869	8
EMBO	1998	2015	792	0	792	7
BMC Evol. Biol.	2004	2015	748	0	748	11
Cancer Reserch	2005	2010	572	101	673	5
Lancet	2003	2009	371	269	640	6
Cell	2009	2015	530	0	530	6
Nature Medicine	2003	2003	348	166	514	3

after Quartz/qz.com with data from Retraction Watch

Table 1. Citations of retracted life science publications by journals

Figure 1. The 10 most influential retracted life science papers as of December 31, 2015 listed in chronological order by the time of retraction.



Orange = citations after retraction;
White = citations before retraction.

Since science research often pushes the boundary of current knowledge and paradigm, the challenge is not to summarily reject new ideas, concepts, and new discoveries simply because they do not fit in the mold of the existing paradigm. Reports of results that introduces new paradigms, ideas, concepts, and discoveries are the basis for science progress and should be looked at with an open mind. The peer review system is used in such instances to ensure clear and concise communications reporting the data underpinning them and to highlight to readers of the potential bias and any potential ethical or conflict of interest issues. Then, once the publications are available to the public, allow time for the science community to confirm or to invalidate the reported novelties. Although the process of community validation (or invalidation) may at times not be very timely, it nonetheless works and effectively confirms the merits (or demerits) of results and conclusions in publications. Such unique "self-policing" and "self-validation" mechanisms are inherent in the science community and are especially essential in the "ever-evolving" life science and biotechnology fields.

Not All Non-Reproducible Publications or Publications with Invalid Conclusions Resulted from Misconduct

Often, there is a misconception that when publication results or conclusions are reported to be "non-reproducible" or invalid, the presumption generally is that the author(s) lied or misbehaved. However, in cutting-edge sciences, this "default conclusion" is not necessarily correct. Except for proven "deliberate fraud" like the above cited examples of using fictitious reviewers, "non-reproducible" results in publications or invalid conclusions may be results of genuine errors in the interpretation of data, contaminations that were not detected/realized during the generation of the misleading data, or simply unconscious bias introduced by the "wishful thinking" of the investigator(s) and author(s). Although engaged scientists do have a tendency to have "wishful thinking" in support of their own hypothesis, the scientific community in general does not and will serve well as the unbiased checkpoint for such "wishful thinking". The so-called "Baltimore Case"[28] is a good illustrative example. The case eventually boiled over to the level of Congressional investigation in the US[29] involving Dr. David Baltimore, a prominent scientist and a Noble Laureate in the field of molecular immunology, as a co-author[30]. Here I quote part of his congressional testimony[20] that I personally find quite insightful and reflects well on the messages of this article:

> *"So what is the [misconduct] case against us? That there are legitimate disagreements over the results of our work? This I recognize and* ***encourage*** *[emphasis added]. That we were not as perfect as, in hindsight, we might have been? This I concede. That scientists keep notes in ways that are sometimes orderly and sometimes not? Of course. That we committed fraud, created data, or misrepresented the facts? This I reject categorically. The subcommittee can point to nothing that supports these allegations.*

I should like to reflect on what I have learned from this affair. First, having been forced to think through how scientific data is [sic] verified, I am committed more than ever to the classic mode of ***verification by scientific replication [emphasis and note added: i.e. by the science community]*** *and further development. Second, I realize that, with the increased sensitivity about scientific fraud, it is more crucial than ever that scientists keep records that can easily be referred to, so that the scientific basis for published results is easily determined at any time. Third, it is important that the institutions that support science—the universities, the research institutes, and the NIH—respond quickly to any allegations of irregularities in the scientific process, and in a way that safeguards the reputation of those accused as well as those who accuse…"*

His testimony brought out the inevitable although politically unwelcome truth that administrative oversight (often staffed with scientists and investigators not in the cutting-edge of the subject field and with little expertise), as noble an aim as it carries, alone often is insufficient (author's opinion – but can bring attention to the science community) and can therefore only be supplemental to replications by the science community, which shall serve as the "final judge."

Why Are There Deliberate Frauds?

Since there are deliberate frauds proven throughout the history of science, it is worthy to examine the root cause(s) that induce scientists to engage in deliberate activities such as, plagiarism, fabricating data and/or drawing outrageous and unsupported conclusions etc. in the modern era. In the US as well as in many developed and developing countries, one may point the finger to the evolving academic environment of most research universities. They are currently dominated by a culture of "publish or perish" for promotion and tenure determinations, as well as for funding support decisions by granting agencies. They are mostly based on the number of publications by the individual researcher since they are the most easily quantifiable measures. With only a finite number of senior academic appointments available for promotion and tenure and limited amounts of funding available to support research as grants, the number of publications becomes a key determinant for researchers to obtain each or both; and hence for the career success and survival of the researchers under such an academic environment. They perhaps are two of the reasons why some investigators are incentivized to commit deliberate frauds to increase their publication counts.

One conflicting phenomenon is that while science is increasingly complex and technology convergence is increasingly key to the successful and meaningful deployment of research results for the benefit of mankind, most universities, despite the establishment of many interdisciplinary (or multi-disciplinary) research units (such as research institutes, centers, and programs), still base their promotion & tenure decisions on their researchers' ability to perform "independent research." This perhaps is the key reason why, increasingly, there are more and more publications noting co-authors as with "equal contributions and credits." A survey by Akhabue & Lautenbach[31] comparing the percentage of co-authorships specifying "equal contributions and credits" among co-authors in publications of five high impact general medicine journals [New England Journal of Medicine (NEJM), Journal of the American Medical Association (JAMA), Lancet, Annals of Internal Medicine (AIM), and British Medical Journal (BMJ)] between the periods 2000 and 2009 noted that the percentage of co-authorships specifying such had increased (NEJM from less than 1% in 2000 increased to 8.6% in 2009; JAMA increased from 0% to 7.5%; AIM increased from 0% to 3.8%; Lancet increased from <1% to 3.6%; and BMJ increased from 0% to 1%). The increase in each journal is statistically significant at the p</=0.001 confidence level.

Challenges for Changes

Although the life science community serves well in "self-policing" the merits of published research results and conclusions, it is a slow process that is increasingly failing to keeping pace with the fast development of science. The advance of the Internet and social media helps with the efforts of the community. It appears there is also a need to reconsider the current promotion and tenure and funding support for research decision-making criterion of "publish or perish." Although many universities aim to emphasize undergraduate teaching on their promotion & tenure decisions for professors, such an aim does not seem to garner much traction in research universities and is generally not supported by outcome. Undergraduate teaching is considered a "soft" criterion and is very challenging to qualify or even to quantify as compared to the numbers of publications and the numbers of degrees granted that resulted from research activities from specific research groups headed by professors. Although new technology has afforded new approaches to evaluate undergraduate teaching, such as the "Rate My Professors" websites that are currently being used by over 7,500 universities in the US, UK, and Canada, it is not without inherent problems[32]. Many such websites have slowly devolved into places where uninspired students use to search for easy grades, and where some professors, mindful of their ratings that may end up negatively by failing students on the sites, choose to grant easy grades to all student without demanding students to demonstrate actual command of the subject matter. This is not an issue that concerned educators are unaware of. In fact, the US National Academies of Sciences, Engineering, and Medicine, through the National Academies Press have continuously shared their views on the subject[33]with the public. Other scholars have also openly provided input in the subject matter[34]. Although the debate has been vibrant, a clear and generally accepted common approach or solution is still not yet available. Regarding research support granting decisions, granting agencies are increasingly looking at the result impact on society, which although a good aim, normally takes a much longer time frame than the grant period of most competitive grants. Although in the US, there are now research "career development"[35,36,37,38] category of grants that have longer grant lives with an aim to allow the necessary time to better evaluate research results based on impact.

Opportunities for Hong Kong

Universities in Hong Kong have long been known to be excellent "teaching universities" in the southeastern Pacific region. This, however, has changed since the sovereignty of Hong Kong was transferred from the United Kingdom to China in 1997 as a Special Administrative Region to be governed under the "one country, two systems" regime. Since then, through various favorable policies and incentives, Hong Kong universities have been subtly encouraged to be more involved in research to promote innovations for China's economic growth and global competitiveness. This has led to a change in the universities' mandate and has also increasingly put pressure on the university constituencies. Research that produces quantitative results, such as the number of publications, the number of citations the publications received, and commercial successes as reflected by the number of new business formation, their successful fundraising, and/or market capitalization has become more and more important a criterion for recognition, remuneration, and promotion. This change raises a genuine concern that the universities are evolving to adopt the short-sighted and undesirable "publish or perish" criterion for career development of their members. Currently and fortunately, Hong Kong universities are still emerging "research universities" and have not been deeply ingrained in the "publish or perish" culture. It is, therefore, not too late to implement well thought-out policies and incentives to, instead, encourage "curiosity-driven" activities that may pioneer genuine new discoveries and innovations to bring much more significant long-term impact rather than activities that solely aim to publish for recognition and instant gratification. To come up with such new policies and incentives will be the next challenge for Hong Kong leaders to position universities in Hong Kong as an exciting place for quality pioneering and cutting-edge research that may bring genuine innovations and global competitiveness to bear. This is especially timely for the emerging hot fields of life science and biotechnology where much remains to be discovered and benefit from!

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Chapter 5

DETECTION AND THERAPY OF BRAIN CANCER WITH FLUORESCENT AND RADIOLABELED-SAPC-DOPS NANOVESICLES

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Phospholipids are large fatty molecules, a major part of cell membranes, with both fat soluble (lipophilic) and water soluble (hydrophilic) sections. In normal cells most neutral phospholipids are arrayed on the outer leaflet of the cell membrane while acid (anionic) phospholipids, such as phosphatidylserine (PS) and phosphatidylethanolamine (PE) are, symmetrically, on the inside of the bilayer [1-3]. Moving PS from the interior to the exterior of the cell, eliminating symmetry, is an early process in cell death, apoptosis. In the death of a normal cell the external PS triggers phagocytosis (digestion of unwanted cellular trash), particularly by macrophages, to minimize inflammation [4-5].

The surface of viable cancer cells and their associated tumor blood supply show a high level of PS [6]. However, this external phospholipid is not due to apoptosis. Compared with non-cancerous cells, expression of PS on the cell surface is a consistent biomarker of malignancy in both primary and metastatic cancers [4, 7-13]. Riedl et al. [12], saw increased cell surface PS in cancer cells, particularly in difficult-to-treat primary and metastatic tumors, including metastatic melanoma, glioblastoma, and metastatic lesions

High cell surface PS makes it an ideal target for diagnostic testing with fluorescently-labeled proteins or peptides that bind to PS to mark apoptotic and tumors cells. Annexin A5 [14-15], an endogenous protein, and lactadherin[16], a major glycoprotein in milk that promotes cellular adhesion, are widely used.

Several studies have shown that high PS on the surface of cancer cells can be a therapeutic target [8-9, 12]. Agents that target surface PS have been developed to treat tumors. The PS-targeting antibody, bavituximab [17], and the PS-binding peptide-peptoid hybrid, PPS1D1 [18] have been successfully used to trigger cytotoxicity of tumor-associated endothelial cells or cancer cells, in *vitro* and in *vivo*.

We developed a stable nanovesicle, Saposin C - dioleophosphatidylserine (SapC-DOPS), that specifically binds to PS on tumor cells in the acidic tumor environment, sparing normal cells. Remarkably, SapC-DOPS exhibits therapeutic properties against a variety of cancer types, without cytotoxicity against normal cells. Saposin C (SapC) is a small, multifunctional, fusogenic protein (facilitates cell to cell membrane binding) that is unusually heat-stable and protease-resistant [19-22]. In *vitro*, at low pH, SapC and DOPS spontaneously form nanovesicles with a mean diameter of approximately 200 nm.

The amino- and carboxyl ends (termini) of SapC are lipid soluble helices. They insert into the lipid bilayer of the nanovesicle while the middle, hydrophilic, region is exposed to water. PS and SapC interaction changes SapC conformation, suggesting that PS actually triggers changes in

SapC helix hydrophilic and lipophilic domains [23].

SapC has no enzymatic activity but it activates lysosomal enzymes by binding the PS of intracellular vesicles' membrane; particularly, acid sphingomyelinase and acid beta-glucosidase, which catalyze the breakdown of sphingomyelin and glucosylceramide into phosphocholine and ceramide, and glucose and ceramide, respectively [24 25-26]. Increasing ceramide levels may also result in cancer cell death. Ceramide has been implicated in apoptosis [27], possibly through the actions of caspases [28].

Some tumor cells undergo lysosomal destabilization in response to SapC-DOPS instead of apoptosis [29]. Notably, the cytotoxicity of SapC-DOPS positively correlates to the level of surface PS of cancer cells. Higher external PS increases SapC-DOPS cancer cell binding and triggers a ceramide cascade or lysosomal destabilization [12-13].

SAPC-DOPS SAFETY

By targeting PS-rich domains on cancerous, neoplastic, cell membranes, SapC-DOPS selectively kills various tumor cells, in vivo and in vitro, without off-target toxicity to normal cells and tissues [13, 30 11, 28, 31-33]. SapC-DOPS pharmacologic safety has been evaluated in mice at 12X the typical therapeutic dose of 4 mg/kg of Sap C and 2 mg/kg of DOPS 28. Even at these levels, no acute toxicity or weight loss were seen. No damage or toxic changes were seen in histological examination of vital organs (i.e., lung, liver, spleen, kidney, heart, brain). Chronic toxicity studies were also performed at 2X the therapeutic concentrations of SapC-DOPS weekly for 5 weeks.

THERAPEUTICS

SapC-DOPS

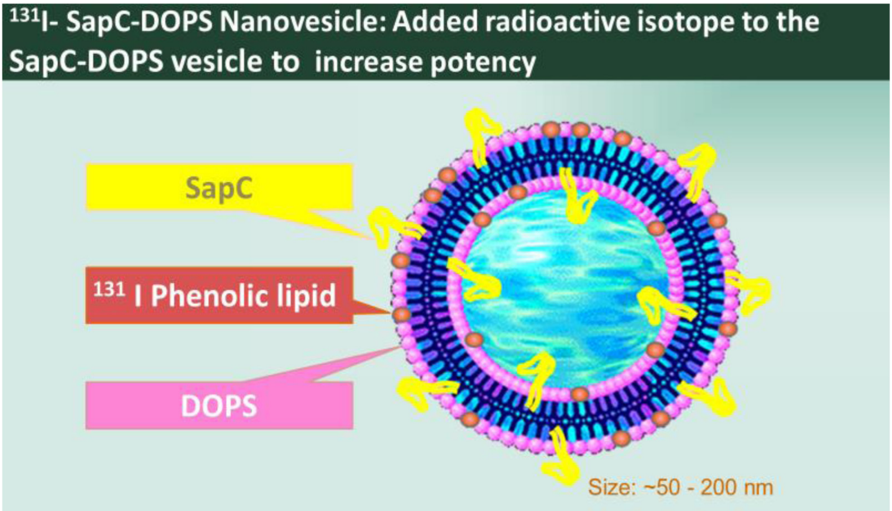
Mice bearing orthotopic pancreatic tumors treated with SapC-DOPS lived significantly longer compared with untreated control groups. All control mice had died by 23 weeks after treatment, while 4 of the 6 SapC-DOPS-treated mice were still alive without detectable tumors [11].

Similar results were obtained with a brain tumor model [33]. Mice with orthotopic glioblastoma multiforme (GBM) that were treated with DOPS alone all died within 20 days. However, 25% of those treated with SapC-DOPS survived at least 350 days. In addition, the tumors were smaller in the SapC-DOPS treated mice. Again, these studies provide preclinical data supporting the safety of systemic SapC-DOPS administration for diagnostic and therapeutic purposes. Although a diagnostic dose of SapC-DOPS has some therapeutic value, multiple doses are generally given to shrink tumors. Again, these studies demonstrated no significant toxicity on histological review of the vital organs listed above [28].

A Phase Ia clinical trial of SapC-DOPS (BXQ-350) was recently completed showing excellent safety profile [36] and no evidence of hypercoagulability or antiphospholipid syndrome [37]. In addition, we demonstrated that SapC-DOPS can enhance cancer therapy in combination with irradiation treatment [38] and chemotherapy [38].

^{131}I SapC-DOPS

Incorporating a custom designed radiotherapeutic iodine on a lipophilic backbone into the SapC-DOPS nanovesicle provided a novel agent with potentially superior efficacy for targeted radionuclide therapy (TRT) of glioblastomas (GBMs). Treatment of 24 mice (3 groups of 8) with 4 doses (61-177µCi) of ^{131}I SapC-DOPS demonstrated a 43% increase in mouse survival over a non-radioactive nanovesicles.



DIAGNOSTICS

The SapC-DOPS nanovesicle can be loaded with fluorescent probes, imaging enhanced contrast agents, and/or radioisotopes for tumor detection or therapy.

Optical Imaging - Fluorescent Probes

Optical imaging of SapC-DOPS distribution has been done with mice orthotopically implanted with various cancer cells, including GBM cells [13]. The fluorescent signal of SapC-DOPS labeled with the far-red fluorescent CellVue-Maroon (CVM from Molecular Targeting Technologies, Inc.) dye, was diffused between 0 and 5 hours, but had accumulated specifically within the tumor region by 24 hours, persisting for up to 100 hours. However, there was no fluorescence signal when uncoupled SapC was administered with DOPS-CVM 28. Similar results were obtained in mouse xenograft models of pancreatic adenocarcinoma and neuroblastoma and a murine rhabdomyosarcoma model [34]. These studies demonstrate the specificity of SapC-DOPS for cells that have undergone neoplastic transformation. This is a new paradigm for improved diagnosis and early detection of malignancy.

MRI - Contrast Agents

Contrast agents can also be loaded into SapC-DOPS for MRI or PET scans. High-resolution MRI scanning was performed for detection of a mouse glioma at 7T. T2* weighted 3D FLASH sequence (TE/TR = 10 ms/20 ms/FA = 10°) were used with a 320 × 320 × 64 matrix and 3.2 × 3.2 × 0.64 cm FOV resulting in an isotropic 100 mm resolution. Negative contrast enhancement was observed 4 hours following SapC-DOPS-USPIO [33].

The paramagnetic contrast agent, gadolinium, was also incorporated into SapC-DOPS vesicles by using the lipophilic gadolinium chelate, gadolinium-DTPA-bis (stearylamide) [35]. These vesicles produced a 9 % increase in the longitudinal relaxation rate (R1) of orthotopic GBM tumors in mice within 10 hours post-injection, but only minimal changes in normal brain tissue; demonstrating improved specificity of tumor detection.

PET - Radioiodine DiD

We have recently used a phenol-substituted lipophilic analog of indodicarbocyanine (DiD from Molecular Targeting Technologies, Inc.) to label SapC-DOPS with ^{124}I, a positron emitter. We then used this labeled SapC-DOPS for MicroPET imaging of mouse glioblastomas. We were able to selectively enhance the intracranial glioblastomas with PET scanning [32]. Concurrent studies with SapC-DOPS labeled with 125I instead of 124I showed that SapC-DOPS specifically targeted the tumor, although some label was detected in the liver and spleen, which are likely disposal routes.

CONCLUSION

All these studies demonstrate that SapC-DOPS crosses the blood brain barrier (BBB) without requiring either alteration of the BBB or direct intracranial administration of the agent [13].

We demonstrated that the novel PS-targeted nanovesicle, SapC-DOPS, can be used to easily, quickly trace hard to detect malignancies and metastases, independent of size or location. Preclinical studies show that these tumor–selective nanovesicles can contribute to earlier, better, more precise cancer diagnosis and treatment. Current studies will support SapC-DOPS as an accurate, sensitive "theranostic" for cancer therapy and detection. an easier, safer tool for detection and treatment of brain cancers and abnormal tissue growth, a characteristic of cancer.

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APPENDIX: AN NIH I-CORPS PROGRAM CASE STUDY: ^{131}I SapC-DOPS

Our study of glioblastoma multiform (GBM) therapy was funded under a National Institutes of Health (NIH) Phase I contract Small Business Innovation Research (SBIR) in 2018. Preparing as we would for any grant, we did a deep dive into the internet and talked to university collaborators, KOLs and a few industry stakeholders about the market.

Multiple national and global health organizations and cancer advocacy groups estimate more than 200K glioblastoma patients worldwide, with 13,000 new cases in the US annually. GBM is a devastating, highly emotional disease. Survival following diagnosis is, typically, 3 months without treatment; 12 to 15 months with treatment. Thirty percent of patients survive 2 years; fewer than 5% last 5 years. Treatment costs can reach $450,000.

The disease exhibits vague symptoms, quick onset, severe decay, elusive hope, and rapid death. The best current treatments only extend survival to 24 months.

Current therapy draws from variants of surgery, radiation and Temozolomide and a few other chemotherapies. We noted some recently approved and innovative chemotherapies, immunotherapies and electrostimulation in trials. In the SBIR application, we were satisfied that a commercial GBM drug was justified and desirable. We had no idea of how naïve we were.

The NIH I-Corps program evolved out of a successful immersion commercialization program run by Steve Blank in Engineering at Stanford University. Program administrators screened SBIRs for likely business successes and trained roughly 25 three-person teams in each of a few cohorts annually. This program began at NSF and NASA and was adopted by NIH in 2015.

It forces developers to clearly define a need then create a detailed business plan based on real world data collected from personal interviews of stakeholders in every element of that plan to fully commercialize a product.

This was as much about retraining us, forcing a culture shift, getting us to accept we weren't the smartest person in the room, as it was learning good business development tools. Our legacy knowledge was clearly skewed toward what we thought was the obvious value of our strong science, not what the market really needed, how they would use it or everything we would need to do to get them to buy it.

Over 8 weeks we were immersed in a program that filled two key gaps in most rudimentary grant commercialization sections: does anyone really need it and what will it really take to get it to market and convince someone to buy it.

Our personal commitment during the 8-week training was mandated and absolute. Program leaders demanded that we interview at least 100 stakeholders, in defined weekly quotas. We had to meet them, face to face. Though video conferences with stakeholders were reluctantly accepted. A few phone calls, in rare cases, were tolerated. We were encouraged to go to advocacy meetings where our targets congregated.

Every week we presented a short slide deck on defined deliverables in a 3- hour video conference with all 24 of our peer groups.

We ultimately did 126 interviews, 60% in person.

In the first 3 days at I-Corps in Boston we spoke to 10 opinion leaders at major cancer centers and the National Brain Tumor Society. To start, forbidden to talk about our product, we asked about stakeholder needs and how a new therapy would be selected and used. We learned the entire chain of decisions and events, from end to end; project funding, user approval, administrative sanction, thought leader endorsement, federal registration, manufacture, distribution and, ultimately, payment.

Data collected in interviews was introduced into a Business Model Canvas (BMC), an online planning matrix. The BMC defines the Value Proposition, Customer Relationships, Customer Segments, Channels, Key Partners, Key Activities, Key Resources, Cost Structure and Revenue Streams. Hypotheses were created for each element then validated or invalidated through interviews. Rarely was a hypothesis validated in a single interview.

By week 6 we clearly defined what we were developing:

^{131} I SapC-DOPS is a targeted radiopharmaceutical indicated for the treatment of adult patients younger than 70 years old with:

- Newly diagnosed glioblastoma multiforme (GBM) as an adjunct to surgery and radiation
- Pancreatic cancer
- Small cell lung cancer

SURPRISES: We engaged patients, the Care Team, hospital staff, researchers, contract service providers, Industry partners, patient welfare advocates, investors, insurers and channel partners.

What is it? GBM is highly invasive and rapidly growing, mostly through stem cell distribution in the brain. In addition to forming difficult to excise fingerlike tumors, metastases followed by multiple surgeries are common.

There are more than 20 genotypes of brain tumors, each with different survival characteristics.

Quality of Life is very difficult to define. Radical brain surgery has significantly greater impact on QoL than short-term side effects from medication. Ideally, surgery could be avoided and even a marginal drug would stall tumor growth and extend life long enough for chemo-cocktails to work.

The effect of this disease on the family who watch the rapid physical decline and loss of function of their loved ones is just as poignant.

Advocacy groups like the National Brain Tumor Society are driving accelerated trials strategies and increased federal funding.

How is it treated? Treatment decisions are made by a GBM Care team, a sort of fast track Cancer Board, headed by a Neurosurgeon, Radiation oncologist or Neuro oncologist who owns and personally directs that decision.

Each leader has a preferred treatment approach, some radical, some prophylactic. Some want to beat the disease at all cost, some accept its inevitability and lean toward optimizing Quality of Life. Anyone introducing a new therapy needs to influence early adopters and educate these teams.

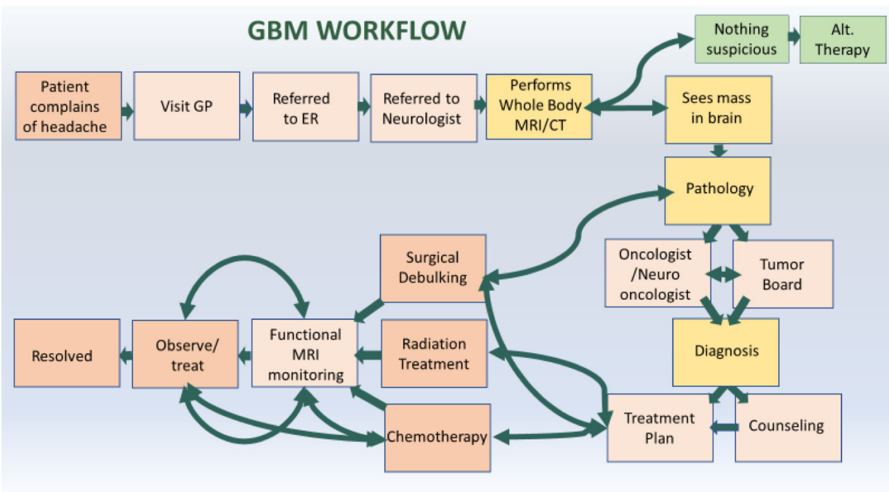
Standard of care for primary cancers always starts with surgery unless complicated by tumor proximity to the spine. Most follow with radiation treatment. There is no Standard of Care for recurring GBM and it almost always recurs.

The greatest improvement in GBM survival in 40 years of development has been improved surgery. Intrasurgical chemotherapy (implanting treated wafers where the tumor was debulked) or Convection Enhanced Delivery (CED) may be better than systemic.

The GBM Care team engages counselors, caregivers and family to help with significant declines in Quality of Life and end of life.

Stakeholders were divided, some preferring a 14-month life extension target, most wanted 24 months for a meaningful therapeutic effect. Our product should target at least 24-month survival.

Young patients survive longer, patients over 70 progress quickly. Our preferred target population is in between.



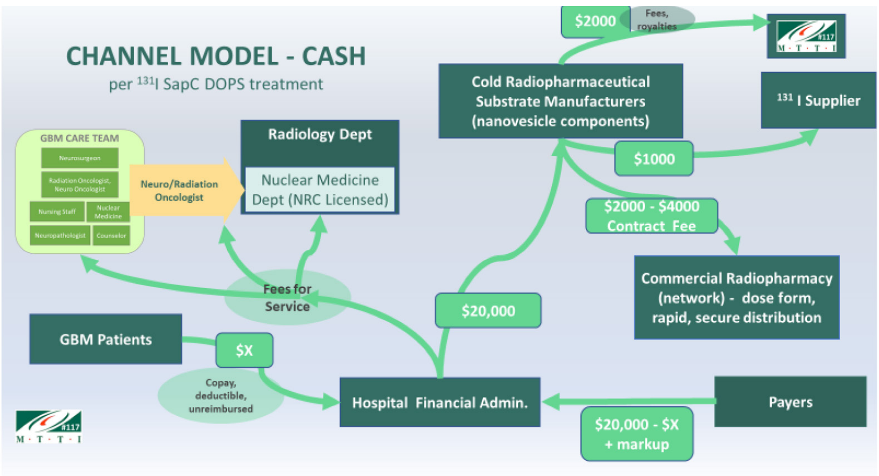
Regulatory. We outlined a regulatory strategy to move the asset through an eIND, obtaining Orphan Drug status, into an IND, obtaining Breakthrough designation and completing accelerated adaptive clinical trials. Glioblastoma drugs are approved after Phase II clinical trials.

Adaptive, accelerated trials. Poor survival and the huge physical and emotional impact on patients and their families prompted multiple high-profile advocate initiatives. Some orchestrated by the National Brain Tumor Society, directed at new federal research funding, regulatory change and innovative trials. With FDA support, brain cancer drugs are moving through expedited, adaptive clinical trials through the newly formed global GBM Agile and the Brain Tumor Consortium.

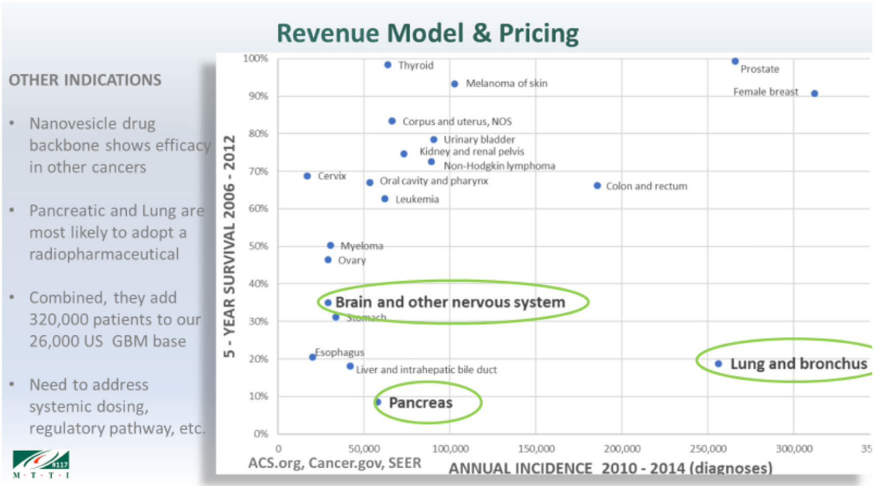
Complex manufacturing. Our complex, 3 component nanovesicle demands multiple unique skill sets, regulatory controls and significant process development. Only a small handful of manufacturers have all these skills in one place. Radiopharmacies (commercial and in-hospital) create and move the finished radiopharmaceutical Just-in-Time. Our kits need to be simple enough for them to create the product quickly then transport and dose safely.

Partnering. Partnering with Bexion, owner/developer of a non-radioactive version of our product, was the best first step toward a productive relationship with someone already familiar with chemical manufacturing, toxicology, clinical development and investor funding. This relationship was established shortly after completing I-Corps.

Cash/Resouces/Information Flow. Once we had a rough handle on how the product was funded, made, priced, purchased, used and reimbursed we developed a messy but telling graphic showing the movement of cash, resources and information among all the stakeholders.



Capturing Value. Though we expect to exit at the end of Phase II, I-Corps mandates a clear understanding of the end game. All this helped us rationalize a two-tiered market approach, recognizing our first target is an asset acquiror and their targets are GBM care groups.



We set a short-term goal of capturing some funding from partnerships. We estimate that an acquiror piloting the asset to full commercialization will take roughly $50 million. At commercial launch after completing Phase II trial we envision the market value of this speculative company to be roughly $450 million.

To enhance value to acquiror we detailed how a developer would socialize the product among their potential Pharma partners who have GBM treatments like Amgen, Merck, Novartis, BMS or smaller firms like Sigma Tau, Alliance Pharma, Corden and BenVenue. Engaging KOLs, building a web presence, maintaining a blog, sponsoring targeted webinars and trade show sessions etc. would expose the market to our progress and raise product credibility.

Through I-Corps we identified the critical path to launch. We know what we need to do, when to do it and roughly what it will cost. Investors meticulously balance risk and reward. By answering "does anyone really need it and what will it really take to get it to market and convince someone to buy it" and assigning a defensible value to every stakeholder in the process; to the patient, neurosurgeon, hospital, manufacturers, service providers, MTTI, partners and the investors, we will easily make a defendable case to potential investors.

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Chapter 6

INDUCED PLURIPOTENT STEM CELL (IPSC)-BASED APPLICATIONS IN REGENERATIVE MEDICINE: A BRIEF REVIEW

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Abstract

Regenerative medicine is a discipline of modern medicine that deals with the restoration of normal body function by repairing, regenerating or replacing damaged cells, tissues or organs. The high degree of similarity between embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), the indefinite renewal capacity of cells, and the absence of ethical concerns all favor the use of iPSC-based approaches in the development of cell-based therapeutics. Yet, several fundamental barriers in the realization of iPSC-based therapy remain to be overcome. In this review, the author will provide a brief introduction of iPSCs, summarize the current applications of iPSC technology in both pre-clinical and clinical areas, and point out several major obstacles limiting the development of iPSC-based therapeutics. The opportunities and challenges presented will be the lighthouses guiding the development of the whole field, no matter overseas or here in Hong Kong.

Introduction

To define it, regenerative medicine is a discipline of modern medicine that deals with the restoration of normal body function by repairing, regenerating or replacing damaged cells, tissues or organs. Two major approaches emerged to realize this objective, namely the stimulation of the endogenous repair mechanism of the body and the expansion of cells or engineered tissues *in vitro* for implantation. For the latter, it is most ideal that materials to be used for regenerative medicine are derived from an autologous source, i.e., the patient himself. However, this approach is hampered by the finite supply of autologous cells and the inability of harvesting certain cell types, for instance, neurons and heart cells, without invasive procedures or the risk of death. An alternative solution to tackle this issue is to derive such cells *in vitro* from stem cells, specifically embryonic stem cells (ESCs).

ESCs are pluripotent stem cells originated from the inner cell mass of an early-stage pre-implantation embryo, which are able to develop into any type of cell in the body except those of the placenta. Despite the usefulness, however, the use of ESCs in regenerative medicine suffers from ethical concerns regarding the use of cells of embryonic origin and the possibility of immune rejection of transplanted cells/tissues by the recipients. A game-changing event took place in 2006 when scientists reported the successful reprogramming of terminally differentiated mouse somatic cells to a pluripotent state by forced expression of only a few transcription factor genes [1]. The same phenomenon was proven successful in human cells by the same group the following year [2]. These reprogrammed cells, named as induced pluripotent stem cells (iPSCs), exhibit a high degree of similarity to ESCs in terms of their morphology, gene expression pattern, epigenetic profile, as well as developmental potential. The indefinite renewal capacity and multi-lineage developmental potential means that the provision of iPSCs and their differentiated derivatives can be unlimited. Without the concern of destroying a developing embryo, iPSCs represent the most appropriate alternative to the use of ESCs in both basic and translational research.

Pre-clinical applications

Since the first report of successful generation of iPSCs, tremendous research efforts around the world have been put forth to improve the efficiency of the iPSC generation process. Also, iPSCs have been utilized for disease modeling *in vitro* by inducing the cells to differentiate into different cell lineages of relevance. Further investigations also led to the establishment of procedures that guide the induction process in a more precise way, through the identification of protein growth factors involved. The discovery of small-molecule chemicals, which can mimic the biological effects of these protein growth factors, enables a more standardized production of various types of differentiated cells at a reduced cost.

(I) Disease modeling

Several seminal research reports described the faithful recapitulation of the respective pathophysiology and clinical manifestations (i.e., disease phenotypes) in vitro in differentiated cells generated from patient-derived iPSCs [3-5]. These successes certainly facilitate a deeper interrogation of disease etiology at the molecular level, an endeavor that has long been limited by the scarce amount of primary tissues which can be obtained from appropriate donors. With the availability of molecular tools to edit genomic DNA (genome editing) at a high degree of accuracy (down to single nucleotide level) [6], scientists can now "correct" genetic mutations and examine the functional restoration from genetic defects on a culture dish (reviewed in [7]). By generating a "disease" iPSC line congenic to the parental "normal" iPSC line (i.e., to introduce a specific genetic defect at its exact genomic locus in an iPSC line derived from a healthy control subject), a more precise evaluation of the biological effect of a genetic defect can be achieved without the influence of genetic makeup differences and line-to-line variation.

(II) Drug development and toxicological studies

A lot of experimental drugs fail in late-stage clinical trials. While the causes can be multi-pronged, drug trial failure owing to inadequate efficacy and safety issue [8] indicates the existence of shortfalls in the current pre-clinical drug candidate evaluation methodologies. For instance, immortalized human cell lines, which are commonly used in early-stage investigation *in vitro*, may not produce a drug response representative of their normal counterparts. While it is essential to understand the pharmacology, pharmacokinetics and safety of experimental drugs in animal models, the intrinsic physiological differences between animal models and human cannot be discounted. Additionally, individual differences owing to genetic makeup may not have been heavily considered in the design of clinical studies. As a result, an experimental drug candidate, even demonstrating a good performance in pre-clinical and early-stage clinical trials, may not survive in later-stage clinical development when more participants are involved. A more reliable prediction system, which is representative of human physiology, of the efficacy and safety of experimental drugs should be implemented at an early stage of drug discovery endeavor.

In view of the above concerns, the use of specific cell lineages deriving from iPSCs offers a unique advantage in drug development process, which is the scalability of sample size and versatility of iPSCs themselves. iPSCs can be derived from somatic cells of multiple individuals of different genders, ages, ethnicities and pathophysiological status. A large cohort of "normal" or "disease" human cell models can be made available for establishing a "clinical-trial-on-a-culture-dish" platform to address the efficacy and safety concerns as well as to screen out inactive entities. Moreover, the experimental readouts may allow further segregation of efficacy and drug responsiveness in regard to specific demographic parameters of the cohort. All of the above analyses can be carried out at an early stage of the drug development process to improve the successful identification of candidate compounds.

The plasticity of iPSCs means that multiple cell lineages can be generated from the same donor's somatic cells. The agglomeration of the respective cell types into 3-dimensional organoids and the assembly of these organoids on the same assay platform (the so-called "organs-on-a-chip") provide the instrument for predicting simultaneously the systemic drug responses in multiple organs. Appropriate design can also be incorporated to recapitulate the complex tissue architecture and mimic the physio-chemical microenvironment in the human body [9].

The integration of the described "normal" and "disease" human cell models, the automated high-throughput drug discovery instrumentation, and the large-scale data analytical capability (the so-called "omics" applications), put together, provides a powerful tool for better prediction of drug response and adverse effect. It will maximize the chance of discovery of "genuine" lead compounds at an early stage of drug development process. It is predictable that the combined efforts would revolutionize the way of planning and performing experimental drug clinical trials by facilitating the stakeholders to make a more informed decision before committing their resources to further studies in animal models and the actual human clinical trials.

By the same token, the same concept can also be applied to toxicological studies, in which toxicants affecting embryonic development or eliciting teratogenicity can be tested or screened on a panel of iPSCs. On the other hand, the evaluation of chemical toxicity (e.g., cardiotoxicity, hepatotoxicity or neurotoxicity) can be fulfilled by testing the target compounds on relevant cell types differentiated from iPSCs.

Clinical applications

The provision of appropriate cell preparations at high quantity (in terms of billions of clinically relevant phenotypic cells, such as neurons, hepatocytes and cardiomyocytes) and a lack of immune rejection are critical to the realization of cell-based therapy. As discussed, the indefinite renewal capacity and multi-lineage developmental potential of iPSCs as well as the autologous origin of donor samples greatly facilitate the development of cell-based therapeutics.

Japan is currently the forerunner in the translation of iPSC-based therapy into clinical applications. The first ever iPSC-based clinical trial was launched in Japan in 2014 for the treatment of age-related macular degeneration (ARMD) with the use of autologous iPSC-derived retinal pigment epithelial cells (iPSC-RPE) [10]. Since then, additional clinical trials have been approved in Japan for the treatment of heart failure [11], Parkinson's disease [12] and spinal cord injury [13]. Another team is preparing to file for a clinical trial to test for the use of iPSC-derived Natural Killer (NK) T-cells in the treatment of head and neck cancer, which is expected to be launched in late 2019 [14].

In the US, biopharmaceutical company Fate Therapeutics, in collaboration with researchers from the University of California, San Diego, has received approval from the Food and Drug Administration (FDA) in November 2018 for a clinical trial with an off-the-shelf iPSC-derived NK cells to treat solid tumors [15]. A separate clinical trial of iPSC-RPE in the treatment of ARMD, led by a research group from the National Eye Institute (NEI) of the National Institutes of Health (NIH), is expected to begin later this year [16]. In Australia and the UK, a Phase I clinical trial (sponsored by Cynata Therapeutics) of the use of allogeneic iPSC-derived mesenchymal stem cells (MSCs) in the treatment of graft-versus-host disease (GvHD) was completed in August 2018 and revealed exceptional safety and efficacy data [17]. In China, a clinical study to test for the treatment of ischemic heart failure with allogeneic iPSC-derived cardiomyocytes has also recently begun [18].

Concerns and technical challenges

(I) Unmatched scale of production and functionality of clinical-grade cell products

While the induction of iPSCs into specific cell lineages has proven successful on an experimental scale, the preparation of such cells on production scale (at a level of ~10 billion cells per preparation at high purity) remains a technical challenge. Additionally, the biochemical characteristics of these differentiated cells often resembled their fetal or embryonic counterparts, suggesting the cells are functionally immature. This phenomenon may be explained by an inability to activate the genome at genetic and/or epigenetic level to attain a mature gene expression signature and cellular functionality. As a result, the differentiated cells display biochemical activities that are inferior to the corresponding adult primary cells. The use of small-molecule chemicals to promote functional maturation of the differentiated cells may allow the perfection of their performance and quality and thus warrants further evaluation.

(II) Autologous or allogeneic treatment?

Reagents designed for the preparation of clinical-grade cell products are mostly available for the development of cell-based therapeutics. The concern of immuno-incompatibility should be minimal in the case of autologous applications, which is a determining factor that favors the adoption of autologous cell therapy. Nonetheless, the cost associated with the provision of dedicated facilities, availability of appropriately trained personnel and a stringent production management system results in a high price tag for the provision of cell-based therapeutics for a single person's use. Allogeneic cell therapy emerges as an alternative to reduce the technical burden and production cost associated with autologous cell therapy.

Allogeneic transplants use cells obtained or derived from a donor whose human leukocyte antigens (HLAs) are acceptable matches to the patient's. Human leukocyte antigens (HLAs; also known as major histocompatibility complexes or MHCs) represent a set of molecular markers expressed on the cell surface which are recognized by the human immune system in the discrimination of "self" from "non-self." A mismatch of HLAs between the recipient and transplanted materials, for instance, cells or tissues differentiated from allogeneic iPSCs, will elicit an immune rejection of the implant unless immunosuppressive drug is applied. Even so, adverse effects and health concerns associated with immunosuppression will follow. The adoption of allogeneic cell therapy, however, may become more feasible if a close match of HLAs between donor materials and recipient can be identified. Such notion forms the basis of banking on iPSCs derived from "HLA super donors": a limited number of lines of iPSCs derived from various HLA-homozygous donors may cover the haplotypes of the majority of the population [19]. This strategy may save time, cost, and permits the preparation of large quantities of transplantable cells as off-the-shelf products in contrast to autologous cell preparation, which is tailored to the donor himself.

Recent research studies discovered that the inactivation of MHC class I and II genes and overexpression of CD47 (a protein found on the surface of many cells that tells circulating immune cells not to eat same cells) in iPSCs may enable them to evade immune rejection in fully MHC mismatched allogeneic recipients and the transplanted cells survived long term without the need of immunosuppression [20]. These approaches may open doors to therapeutic use of allogeneic iPSC-derived cell products without immune rejection concerns and complications.

(III) Lack of defined quality attributes of cell-based therapeutics

Unlike chemical drugs, cell-based therapeutics is less "stable" and their performance may be easily affected by a slight change in environmental conditions. In other words, a quality control and assurance system different from that of chemical drug is required for monitoring the quality of cell-based therapeutics. A consistent and reliable translational model, and the establishment of definition of cellular characteristics representative of a specific iPSC-derived cell product, will form a vital pre-requisite for the development of cell-based therapeutics. However, a consensus remains to be achieved in the field on the acceptable quality attributes of iPSCs, as well as their differentiated derivatives.

(IV) Tumorigenicity of iPSCs

Owing to the capability of unlimited proliferation as well as teratoma (a type of tumor) formation *in vivo* (even under the condition that the recipient is immuno-compromised), the potential of malignant transformation of iPSCs in the recipients cannot be ignored. However, since the cell-based therapeutics intended for clinical applications is likely the differentiated derivatives of iPSCs, the chance of malignant cell formation in the recipients should be largely minimized. The use of small-molecule chemicals targeting specifically to pluripotent stem cells, or the incorporation of a suicidal gene mechanism into iPSCs [21], may allow a selective elimination of these cells from a pool of differentiated cell derivatives before they are administered for transplantation.

Conclusion and outlook

In just over a decade, iPSCs have revolutionized the biomedical field. The ability to recapitulate human physiology on a culture dish provides a more sophisticated model for studying genetic disorders and a more precise prediction of drug response with the consideration of individual differences. The further improvement of genome editing tools may permit the elimination of life-threatening diseases by offering the correction of erroneous genetic message at high precision with no off-target effects in somatic cells. The number of clinical trials with iPSC-derived cells is on the rise. Yet, technical hurdles remain to be tackled to ensure an efficient and cost-effective supply of cell products to patients without compromising their safety. Autologous cell therapy and allogeneic cell therapy both have their own benefits and shortfalls. Currently, the adoption of either approach appears to be determined on the basis of availability of resources instead of scientific merits. With further advancement in cell differentiation and biomaterial fabrication technologies, a better understanding of human immunology and the implementation of "omics" and "big-data" approaches in the validation of cell products' quality, as well as prediction of outcomes in pre-clinical studies, it is optimistic that a lot of the described hurdles will be overcome in the not-too-far future.

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Chapter 7

DIABETES MELLITUS IS MORE THAN JUST A HYPERGLYCEMIC DISEASE: A LOW-CARB, MEDICINE PERSPECTIVE

Richard Z. Cheng, M.D., Ph.D.

Abstract

Type 2 Diabetes mellitus (T2DM) is a major pandemic afflicting hundreds of millions of people worldwide. A low-carb/ketogenic diet for diabetes management has been gaining momentum globally for the last few years, primarily among the public, but also some allied healthcare professionals and physicians. Although well-designed and executed clinical studies convincingly demonstrating the effectiveness of a low-carb/ketogenic diet for diabetes are still few, such clinical studies are beginning to emerge. Many diabetics and healthcare providers seem to be expecting a magic "one-size-fits-all" solution: a low-carb/ketogenic diet will cure all T2DM problems. Albeit very encouraging and exciting to all involved, this may be misleading. While a low-carb/ketogenic diet should be the backbone of diabetes management, we need to understand among ourselves and also educate the public loudly and clearly that hyperglycemia is only a symptom of T2DM, that blood glucose control is the first step towards the ideal diabetes management, but that's not all. It's now generally accepted that the management of T2DM should take a holistic approach to address not only lifestyle modifications (dietary, exercise, relaxation, sleep), but also the underlying contributing pathologies such as elevated oxidative stress (excessive toxins, especially dental toxins, anti-oxidants/nutrient deficiency), mitochondrial dysfunction, nutritional deficiency such as Vit C and D deficiency, hormonal imbalance, etc. T2DM, like most other chronic diseases, is a multifactorial systemic illness, and should be treated as such.

Low-carb/ketogenic diet, a huge step forward in T2DM:

A May 2019 study of 349 people over 2 years concluded that lifestyle changes including a low-carb/ketogenic diet significantly improved multiple health parameters including HbA1c, fasting glucose, fasting insulin, blood lipids and blood pressure [1]. This same study also saw a significant reduction in diabetic drug usage including oral hypoglycemics and insulin. The hallmark of T2DM is hyperglycemia, hence, it should be obvious that a low-carb/ketogenic diet would play a major role in T2DM management. Why mainstream medicine has struggled over this concept for decades and many continue to do so is beyond my comprehension. Although we begin to see some medical associations and clinicians start to take a more positive approach to the low-carb/ketogenic diet, the vast majority of physicians are still on the sidelines. The first step towards ideal diabetes control is blood glucose control. However, T2DM is more than just a hyperglycemic problem.

Increased gastrointestinal permeability:

Digestive system dysfunction (increased gastrointestinal (GI) permeability, or Leaky Gut, and Dysbiosis) plays an important role in most, if not all, chronic diseases, including T2DM [2–8]. The mucosa lining of the gastrointestinal tract and the respiratory system is an extension of our integumentary system that plays a critical role in preventing foreign pathogens and antigens from entering our body, causing diseases. When this barrier function of the GI tract is broken (increased GI permeability), foreign pathogens and antigens can enter the human body, and this has been proposed to cause many diseases, including, not only inflammatory bowel disease and irritable bowel syndrome, but also various other diseases, such as allergies, diabetes mellitus, liver diseases, and collagen diseases. With a thorough review of medical history and physical examination, a physician can learn much about a patient's GI health. With these, plus some lab tests, an experienced physician can arrive at an impression of the GI function status and render proper intervention. Detailed diagnosis and management are beyond the scope of this article.

Elevated oxidative stress:

Life in its essence is electricity. Clinical death is defined by lack of electricity in the heart (ECG) and the brain (EEG). Electricity is the flow of electrons between molecules. Biochemically, electricity is simply a reduction and oxidation reaction, or Redox. All life events are Redox reactions. Normal, balanced Redox reactions are defined as healthy. When this Redox is out of balance, disease ensues. Oxidative stress (OS), defined as total oxidative capacity (all oxidants) divided by anti-oxidant capacity. Elevated OS is a hallmark of many, if not all, chronic diseases.

T2DM is now generally accepted as a result of long-term chronic stress on pancreatic Beta-cells, eventually causing Beta-cell failure to produce insulin, resulting in hyperglycemia. Such Beta-cell stresses manifest as increased oxidative stress, local inflammation, and endoplasmic reticulum (ER) stress, leading to Beta-cell destruction, which further exacerbates the metabolic stress and places further stress on residual Beta-cells. T2DM is associated with significantly elevated oxidative stress, which can result from increased free radical production and/or reduced anti-oxidant defenses [9]. Elevated oxidative stress and mitochondrial damage are implicated to play a significant role in the development and progression of T2DM[10].

Environmental toxins, including both metal and nonmetal chemicals, are a major class of agents causing the elevation of oxidative stress. The two major public health crises, chronic arsenic poisoning and diabetes, are very likely interrelated [11]. Reactive oxygen species (ROS) accumulation is a hallmark of inorganic arsenic (iAs) toxicity. Arsenic is a carcinogen and oxidizing agent and may cause neural, hepatic, cardiovascular, integumentary, and renal damages. Arsenic exposure includes GI, skin and lungs. Groundwater Arsenic contamination is a threat to a significant portion of the global population, affecting ~140 million people worldwide. In some areas, iAs may contribute to as high as 21% of all-cause-mortality [12].

Infections:

Dental toxicity and diabetes: Abundant studies have been done to demonstrate the relationship between endodontic infections and systemic diseases including diabetes and CVD. Root canal treatment, apical periodontitis, and other dental diseases have been proposed to be significant source of inflammation and increased oxidative stress and correlated with systemic diseases including diabetes mellitus [22–28]. These studies provide strong evidence that endodontic infections are associated with increased oxidative stress [21].

Anti-Oxidants:

Increased oxidative stress can be due to increased free radical production, and/or reduced anti-oxidants. Many of the diabetes complications may very well be due to elevated oxidative stress [29,30]. Diabetics have reduced blood levels of Vitamin C [31–35]. Multiple studies show that diabetics have depressed, scurvy-like plasma levels of Vitamin C. Other vitamins, nutrients or trace minerals also play significant roles in diabetes. Manganese deficiency and excess could increase ROS generation [31]. Vitamin D is one of the key controllers of systemic inflammation, oxidative stress, and mitochondrial energy metabolism [32]. Dr. Thomas Levy ([9]) has elegantly reviewed this topic, which I summarize below:

1. Diabetics have depressed, scurvy-like plasma levels of Vitamin C [33–38]. Reduced Vit C levels are associated with elevated oxidative stress which plays an important role in the development of diabetes.

2. Glucose directly competes with Vit C for uptake into the cells [39,40]. Increased blood glucose levels further reduce intracellular Vit C levels, leading to a vicious downward spiral.

3. Insulin helps transport of Vit C into the cells throughout the body [41]. Vit C is structurally very similar to glucose, which explains why insulin aids the cellular uptake of both glucose and Vit C. Hyperglycemia inhibits Vit C cellular uptake, leading to reduced cellular Vit C deficiency and pathology.

4. Hyperglycemia promotes renal excretion of Vit C and inhibits renal Vit C reabsorption [42,43].

5. Hyperglycemia depletes Vit C from monocytes, the while blood cells that are attracted to the sites of inflammation and infection [44]. Depleting monocytes of Vit C directly impairs the defensive mechanisms of the immune system.

6. Vit C plays an essential role in regulating the release of insulin by the pancreas [45–48], demonstrating the important interplay between insulin and Vit C. These studies suggest that Vit C is critical in the optimal management of diabetes.

7. Diabetics are much more likely to develop advanced periodontal disease [49,50].

Mitochondrial dysfunction:

Mitochondrial dysfunction is found in nearly all chronic diseases and in diabetes as well. Diabetes profoundly alters energy metabolism. The two hallmarks of diabetes, insulin deficiency and insulin resistance, are characterized by inefficient mitochondrial coupling and excessive production of ROS[41].

Hormone Balance:

Up to 40% of men with Type 2 diabetes (T2DM) and metabolic syndrome have hypogonadotrophic hypogonadism (HH). Men with HH are at increased risk of cardiovascular and all-cause mortality, as well as of the development of incident T2DM [51]. Hackett recently (2019) reviewed 1,817 articles, including 54 clinical trials and 32 randomized controlled trials (RCTs), spanning 13 years between 2005 and 2018. RCTs of testosterone replacement therapy suggest significant benefits for glycemic control, insulin sensitivity, sexual function, quality of life, anemia, bone density, and fat and lean muscle mass. The greatest benefits are seen in men who reach target testosterone levels and for longer durations.

Insulin resistance, insulinemia and fasting plasma glucose were significantly reduced in a group of postmenopausal women with T2DM receiving hormone replacement therapy (HRT)[52]. Individualized hormone balance (via hormone replacement) should be part of the treatment plan for postmenopausal women [53].

Hormone replacement therapy (HRT) has been around for decades. While HRT clearly provided a plethora of health benefits to women, the Women's Health Initiative (WHI) also raised the dangerous side effects of Premarin and Prempro, 2 nonhuman hormones commonly used in these HRT protocols. Reflecting the current trend of precision medicine, using human hormones (bioidentical vs. synthetic or animal hormone) such as estradiol (E2), estriol (E3) and progesterone (P4) shows individualized Bio-identical Hormone Replacement Therapy (BHRT) is safer physiologically compared to nonhuman HRT, while also maintaining/maximizing the health benefits to postmenopausal women [59–61]. Since the WHI showed significantly increased risk of breast cancer from HRT, the use of HRT has markedly dropped. More and more US women choose individualized BHRT to FDA-approved off-the-shelf HRT and from physicians providing alternative care to conventional care. Currently there is an estimated 1-2.5 million US women who use individually compounded BHRT in America today[58].

Summary:

T2DM is a global pandemic, with ever-increasing health and economic costs. The current global low-carb/ketogenic diet trend is very encouraging in fundamentally changing the landscape in diabetic management. At the same time, we should clearly remind all involved that T2DM is not simply a hyperglycemic disease. Like many other chronic diseases, the elevated oxidative stress (due to ever-increasing environmental toxin excesses and reduced anti-oxidants intake) plays a fundamental role in the genesis of T2DM, which causes mitochondrial dysfunction and is further compounded by hormonal imbalance in mid-aged men and women. Even when blood glucose levels are controlled with a low-carb/ketogenic diet, how do these other factors interplay with each other and affect the final outcome of T2DM? These questions warrant further research. From a practice point of view, a clinician should strive to assess each and all of these factors in a patient and provide an individualized medical treatment plan.

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Chapter 8

THE DEVELOPMENT OF A YEAST-DERIVED ORAL VACCINE AGAINST HEPATITIS B

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Abstract

Hepatitis B is a major human infectious disease, which affects hundreds of millions of people worldwide. The survey demonstrates about 10% of the population in China are hepatitis B virus carriers. Hepatitis B virus (HBV) can cause acute and chronic hepatitis, while hepatitis can develop into more serious diseases, such as liver cirrhosis and liver cancer. Vaccination is the most effective way to prevent and control HBV infection. Surface antigen or core antigen of HBV is an ideal target for vaccine candidate. The commercial HBV subunit vaccines are injectable and manufactured from engineered yeast fermentation. This review summarizes recent advancements in the HBV vaccines, discusses their potentially beneficial and harmful effects, and suggests future guidelines towards the design of a truly protective edible HBV vaccine, as well as the development of vaccines for the prevention of other novel infectious diseases such as COVID-19.

Key words: HBV, Yeast fermentation, Oral HBV vaccine, Edible Vaccine

Introduction

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a major global health issue and can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer. In highly endemic areas, hepatitis B is commonly spread from mother to child at birth (perinatal transmission), or through horizontal transmission (exposure to infected blood), especially from an infected child to an uninfected child during the first 5 years of life [1]. The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days. The virus may be detected within 30 to 60 days after infection and can persist and develop into chronic hepatitis B. The likelihood that infection becomes chronic depends on the age at which a person becomes infected [2]. Children less than 6 years of age who become infected with the hepatitis B virus are the most likely to develop chronic infections. In infants and children: 80–90% of infants infected during the first year of life develop chronic infections, and 30–50% of children infected before the age of 6 years develop chronic infections. In adults: less than 5% of otherwise healthy persons who are infected as adults will develop chronic infections, and 20–30% of adults who are chronically infected will develop cirrhosis and/or liver cancer [3].

The HBV genome is organized as a partially double-stranded, relaxed circular DNA molecule of approximately 3,200 bases encased within viral-specific proteins (Figure 1A). The longer strand of HBV DNA (L strand) is a complete circle with a nick. The complementary strand is shorter (S strand) than the L strand [4]. The four overlapping genes are P for translating polymerase, PreS1/PreS/S for hepatitis B surface antigen (HBsAg), PreC/C for core protein and X for HBx protein. The P protein has reverse transcriptase activity. The C and S open reading frames (ORFs) have extensions at their 5’ ends termed pre-C and pre-S. The pre-S region is divided into pre-S1 and pre-S2 domains, and translation of the S ORF leads to the production of the large (L), medium (M) and small (S) HBsAg. Translation of the pre-C ORF results in a secretory protein, hepatitis B e antigen (HBeAg), which is an accessory protein required establishing chronicity, whereas the C ORF results in the capsid protein. HBx is required for establishment of infection and maintenance of active replication by inhibiting the host nuclear restriction factor, sister chromatid cohesion 5/6 (SMC 5/6). Overexpression of HBx has been shown to result in cellular transformation via promiscuous transactivation [5].

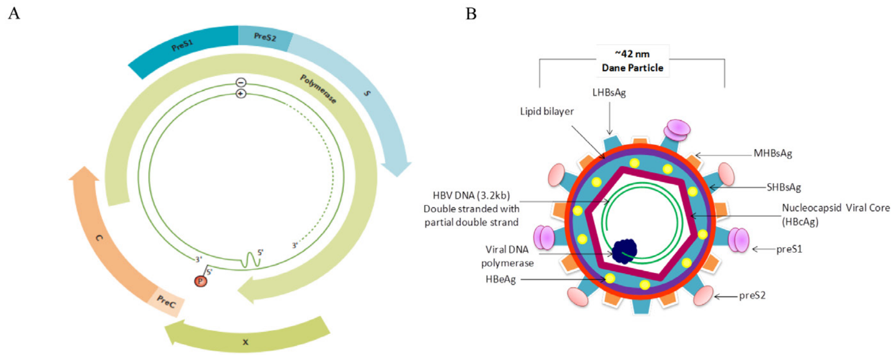

Figure 1. Schematic diagram of genomic structure of HBV. **(A)** Genomic structure of HBV. **(B)** Virus particle of HBV including envelope, S, M, L, with their S, preS2 and preS1 domains in sub-boxes of size proportional to their polypeptide length.

The infectious HBV virion is approximately 42 nm in diameter and is also called the Dane particle **(Figure 1 B)**. The viral DNA genome and endogeneous DNA polymerase that is covalently linked to the 5' end of minus strand DNA is enclosed inside a nucleocapsid core which assembles into a 27 nm icosahedron. The HBV DNA contains four overlapping open reading frames that code 7 distinct proteins [6]. Of importance is HBsAg which is encoded by HBV as three envelope proteins called Large (LHBs, 389–400 aa, 39 kDa), Medium (MHBs, 281 aa, 31 kDa) and Small (SHBs, 226 aa, 24 kDa) surface antigens. The nucleocapsid core is surrounded by morphologically distinct outer capsid core of lipid bilayer (4-5 nm) studded with complexes of viral glycoproteins envelope containing the Hepatitis B surface antigen (HBsAg). The SHBsAg or the HBsAg used in licensed vaccines has a molecular mass of 24 kDa and is predicted to be intimately associated with lipid, having four or five membrane-spanning helices, and is found in two forms, glycosylated and unglycosylated (translation products of the same gene), in approximately equal amounts and which account for more than 90% of the protein contained in HBsAg. SHBsAg glycosylation is a single biantennary glycan at Asn-146 (N-glycosylated) and raises its molecular mass to 27 kDa [7]. About 80% of HBsAg is in the S form, which assembles with M and L forms into both the outer surface of the Dane particle and smaller subviral particles.

Hepatitis B vaccine

HBV infection is the direct cause of hepatitis B. If the disease is not controlled, it may develop into cirrhosis and even primary liver cancer. To date, there is no specific drug for the treatment of hepatitis B [8]. Therefore, injectable hepatitis B vaccine has become an important means to prevent and control the epidemic of hepatitis B. The complete vaccine 3-dose series can induce protective antibody levels in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is probably lifelong. WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 h.

Table 1. HBV Vaccine Approved in the United States

Trade Name	Manufacturer	Antigens Included	Patient Population	Dosage	Route, Schedule
Comvax	Merck	*Hib* capsular polysaccharide + recombinant HBsAg Alum	Infants 6 weeks to 15 months born of HBsAg-negative mothers	7.5 mcg Hib polysaccharide 5 mcg HBsAg	IM 3 dose schedule 2, 4, and 12–14 months
Engerix-B	GSK	Purified recombinant HBsAg from *Saccharomyces cerevisiae* *Alum*	Infants-adults	Infant-19 yrs=10 μg/dose Adults (>19 years)=20 μg/dose Adult hemodialysis=20 μg/dose	IM, 3 dose schedule 0, 1, and 6 months
FENDrix*	GSK	Purified recombinant HBsAg from *Saccharomyces cerevisiae* *AS04 adjuvant (MPL on Al-phosphate)*	patients with renal insufficiency (including pre-haemodialysis and haemodialysis patients) from the age of 15 years onwards	HBsAg – 20 μg/dose AS04 – 50 μg/dose	IM in deltoid region 4 dose schedule at 0, 1, 2 and 6 months
Pediarix	GSK	Diphtheria and tetanus toxoids (DT); acellular pertussis toxoid, filamentous hemagglutinin; HBsAg and inactivated poliovirus (IPV) types 1, 2, 3 *Alum*	Infants born of HBsAg-negative mothers.	6 weeks-6 years	IM 3 dose schedule 2, 4, and 6 months
Recombivax HB	Merck	HBsAg produced in yeast *Alum*	Infants-adults	Infant-19 yrs=10 μg/dose Adults (>19 years)=10 μg/dose Adult hemodialysis=40 μg/dose	IM 3 dose schedule 0, 1, 6 months
Twinrix	GSK	Inactivated hepatitis A (strain HM175) grown in MRC-5 cells with purified HBsAg *Alum*	Persons >18 years	720 units of inactivated hepatitis A virus and 20 μg of HBsAg/dose	IM. Standard 3 dose schedule 0, 1, 6 months; or alternative accelerated 4 dose schedule - days 0, 7, and 21–30 followed by a booster dose at 12 months

At present, the main commercial hepatitis B vaccine products are as follows.

(1) ENGERIX-B. ENGERIX-B [Hepatitis B Vaccine (Recombinant)] is a sterile suspension of noninfectious HBsAg for intramuscular administration. It contains purified surface antigen of the virus obtained by culturing genetically engineered Saccharomyces cerevisiae cells, which carry the surface antigen gene of the hepatitis B virus. The HBsAg expressed in the cells is purified by several physicochemical steps and formulated as a suspension of the antigen adsorbed on aluminum hydroxide. The procedures used to manufacture ENGERIX-B result in a product that contains no more than 5% yeast protein. [9]

(2) RECOMBIVAX HB. RECOMBIVAX HB Hepatitis B Vaccine is a sterile suspension of non-infectious subunit viral vaccine derived from HBsAg produced in yeast cells. A portion of the hepatitis B virus gene, coding for HBsAg, is cloned into yeast, and the vaccine for hepatitis B is produced from cultures of this recombinant yeast strain according to methods developed in the Merck Research Laboratories.RECOMBIVAX HB has been shown to elicit antibodies to hepatitis B virus as measured by ELISA. Antibody concentrations ≥10mIU/mL against HBsAg

are recognized as conferring protection against hepatitis B infection. [10]

(3) Bimmugen. Kaketsuken originally obtained approval for the vaccine in 1988 as a genetic recombinant drug manufactured using proprietary Japanese technology. The main aim of the vaccine is prophylaxis against hepatitis B, and to date the vaccine has been administered to over 4 million people in Japan.

(4) HEPLISAV-B. HEPLISAV-B [Hepatitis B Vaccine (Recombinant), Adjuvanted] is a sterile solution for intramuscular injection. The HBsAg is expressed in a recombinant strain of Hansenula polymorpha yeast. The fermentation process involves growth of the recombinant H.polymorpha on chemically-defined fermentation media containing vitamins and mineral salts. [10]

(5) Gen-hevac-B. Gen-hevac-B is generated from a CHO cell developed by Pasteur Merieux, and it was put on the market in 1988.

(6) Other. FENDrix* was developed by GSK, the recombinant HBsAg purified from Saccharomyces cerevisiae AS04 adjuvant (MPL on Al phosphate). Pediarix was also developed by GSK.

Collectively, two types of hepatitis B vaccine are available: (1) Recombinant or genetically engineered vaccines are manufactured in yeast cell (Saccharomyces cerevisiae) or in mammalian cells which the HBsAg gene has been inserted into chromosome of these hosts. (2) Human plasma-derived hepatitis B vaccine. The purified HBsAg is isolated from the plasma of individuals with chronic HBV infection.

Yeast-based oral or edible vaccines

The technology of HBsAg gene recombinant in yeast (Saccharomyces cerevisiae. Hansenula polymorpha, Pichia pastoris) combined with bioreactor processing facilitated low-cost commercial-scale vaccine production. Although use of whole recombinant yeast expressing the immunogenic protein from pathogenic species is an important and novel strategy (Figure 2), yeast surface display (YPD) appears more important and allows development of oral or edible vaccines. Thus, unicellular yeast can be the right candidate for a live oral vaccine [11, 12]. As a proof of concept, many recent reports showed the encouraging results where yeast display being used as a prophylactic vaccine. For example, an oral vaccine against candidiasis was introduced using molecular display systems with S. cerevisiae [15]. Identification of anti-B7-H4 antibodies using yeast-display library of recombinant antibodies derived from ovarian cancer patients was able to restore antitumor T cell responses [16. In another case, envelope protein E2 of bovine viral diarrhoea virus (BVDV) displayed on budding yeast surface was able to stimulate the production of CXCL-8 in macrophages. The same study also showed that the particulate nature of yeast is also important in efficient stimulation of T cells [17]. Feeding of freeze-dried budding yeast particles expressing the PCV2b Cap protein on their surface provides protection against PCV2b challenge in pigs [18]. Although yeast display is in its infancy stage and requires further research, some of the studies already highlighted in this article clearly showed the impact this technology may have on vaccine development and application. The edible vaccines will be more acceptable because of its oral rather than injectable route of application. In contrast, producing the vaccines in plants could reduce the cost to less than a penny per dose, and simple fast food processing like drying and grinding could create non-perishable preparations without refrigeration [19]. Further, Lam's research team has also recently focus on yeast displayed vaccine which is used to prevent avian influenza infection [20].

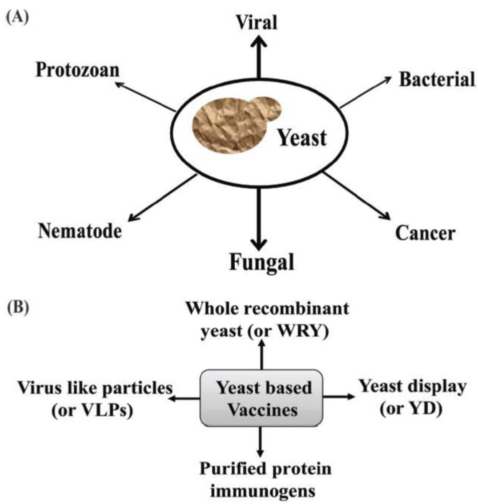

Figure 2. Yeast-based vaccines. (A) Schematic diagram of the application of yeast in expressing antigens against diverse infectious diseases. (B) Various ways in which yeast-based vaccines are or can be used [21].

The fact that many yeast-based vaccines are under different phases of clinical trials, and many of them reached the third phase of clinical trials, showed clear merits associated with these vaccines and use of purified protein from yeast as a peptide vaccine against hepatitis B is an important success story of a yeast-based vaccine. Thus, in the end, it can be concluded that further identification of more protein antigens, their successful expression in yeast could prove instrumental in reducing the time and cost involved in vaccine preparation. These yeast-based vaccines may also be of great value in vaccine development, particularly in those cases where the mass culture of associated pathogens is still not feasible, such as in leprosy and malaria. Taken together, yeast-based vaccines can provide a powerful, rapid and inexpensive platform against both human and animal diseases.

In addition to yeast, many other edible plants and microorganisms have also been used for producing oral vaccines [14, 19, 22, 23, 24]. The concept of oral vaccines produced in edible plants was proposed by Dominic Lam and first executed by him and his colleagues in the early 1990s. They first reported the expression of hepatitis B virus surface antigen (HBsAg) in tomatoes [13, 14]. Since then, there have been a number of reports on expressing HBsAg in different plants with varying degrees of success. In particular, much progress has also been made using maize-produced HbsAg [25].

At the beginning of this century, Dr. A. S. Fauci published a prophetic article entitled "Infectious diseases: considerations for the 21st Century" [27]. The unexpected emergence of the global COVID-19 pandemic at this time demonstrates most vividly the importance of developing vaccines expediently, inexpensively and which are easily applicable in both well- and less-developed countries [28]. In this regard, the development of Oral vaccines might serve as a powerful platform and defense mechanism against such pandemics that affect billions of people in the world.

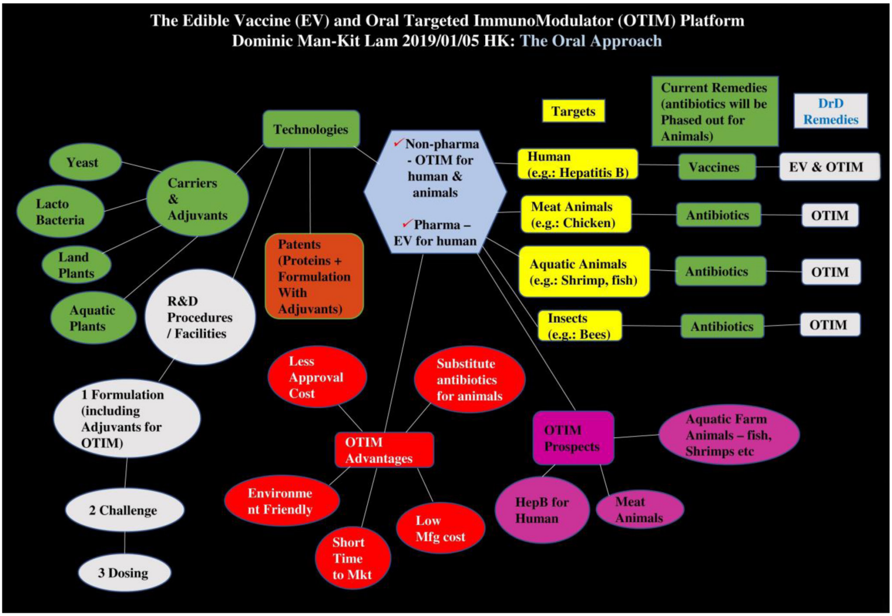

Figure 3. Represents a block diagram to illustrate and summarize the potential of oral vaccines for prevention of infectious diseases in man and other animals.

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Business Development

Chapter 9

STARTING BUSINESS / INVESTMENT IN HONG KONG? WORTH YOUR ATTENTION!

Johnson Lam

Over the past decades, globalization has significantly altered our economies and presented a wealth of opportunities. Amongst other things, businesses have utilized the differentiation of tax and legal systems of various jurisdictions to achieve better tax planning, such as engagement of intra-group intellectual property (IP) transfers and licensing exercises. In extreme cases, over-aggressive tax-avoidance strategies may amount to manipulation and lead to call for international cooperation and scrutiny. It was against this backdrop that the Organisation for Economic Co-operation and Development (OECD) developed the Automatic Exchange of Information ("AEOI") and Common Reporting Standard ("CRS") for tax transparency, and Hong Kong is no exception. Furthermore, the Inland Revenue (Amendment) (No. 6) Ordinance (the "Ordinance") came into force on July 13, 2018[1], which codifies transfer pricing laws in Hong Kong and helps curb over-aggressive international tax-avoidance practices involving Hong Kong.

Despite such regulatory measures, Hong Kong has been rated as world's freest economy for 25 consecutive years since the introduction of the Index of Economic Freedom[2]. Sound legal systems, low taxation, free port trade, mature and vivid financial and capital market, close proximity with the China market, availabilities of and attractiveness to international talents, all have made Hong Kong a fantastic place for starting businesses. The Hong Kong government has also recently introduced some new changes in laws which only add incentives for local investment, including:

- A new chapter 18A to the listing rules to allow biotechnology companies which originally do not satisfy the financial eligibility criteria for listing on the Hong Kong Stock Exchange;

- A new two-tier profit tax regimes, whereby the first two million of profits will be taxed at the low rate of 8.25%; and

- An enhanced research and development tax incentive scheme.

With the above highlighted regulations and incentives, Hong Kong is arguably one of the optimal places in the world for bio-technology businesses and investments.

1 https://www.ird.gov.hk/eng/ppr/archives/18071601.htm
2 https://www.heritage.org/index/country/hongkong

Brief introduction of CRS and AEOI

The AEOI regime, which is a new system that involves the transmission of financial account information from Hong Kong to an overseas jurisdiction, was launched in Hong Kong in 2017[3]. Under the AEOI regime, the type of information and the due diligence to put in place for all financial accounts are defined by CRS. Under the AEOI standard, financial institutions are required to identify financial accounts held by tax residents of reportable jurisdictions. "Tax residents of reportable jurisdictions" refer to those who are liable to tax by reason of residence in the jurisdictions, or in case of a company, the place of incorporation or the place where the central management and control of the entity is exercised.

Tax residency is also becoming more important under the transfer pricing rules. It will be discussed later in this article.

Brief introduction of Transfer Pricing

Obviously, a taxpayer in Hong Kong can employ tax-planning but not tax-avoidance practices. While there are general anti-avoidance rule provisions (for example, sections 9A and 61 of the Inland Revenue Ordinance), before the Ordinance there were no specific legislations in Hong Kong echoing the global standards introduced by Organization for Economic Cooperation & Development in recent years.

Many businesses employ tax planning strategies to engage the discrepancies in tax laws to shift profits to jurisdictions with lower tax rates. One typical way is to use offshore holding companies to house much of the IP value.

The Ordinance together with the AEOI scheme makes Hong Kong a more tax-transparent jurisdiction. In accordance with the Ordinance, a company is required to provide transfer pricing documents relating to master file, local file and country-by-country report if there is a sizeable business in Hong Kong (i.e. hitting two of the three threshold: (i) total annual revenue of HK$400 million; (ii) total assets of HK$300 million; and (iii) employing more than 100 staff). A global business will also need to present a country-by-country report to the Hong Kong authority if the global revenue exceeds HK$6.8 billion. This international collaboration helps different authorities to formulate the big picture so that they can consider if the profits in a certain jurisdiction are fair and justified. In turn, it becomes more difficult to manipulate the pricing of related-party transactions.

3 https://www.ird.gov.hk/eng/tax/dta_aeoi.htm

Another new trend of global scrutiny is that there are more regulations (especially from high-tax jurisdictions) to tie the profits booked to the work done. It becomes more important to obtain Certificate of Tax Resident Status in low-tax jurisdictions such as Hong Kong. If your company holds IP in Hong Kong for global licensing, you should have an office with staff in Hong Kong to create the IP and for daily management. Your central management might probably well be in Hong Kong to justify. In gist, you need to demonstrate that the group is normally managed and/or controlled in Hong Kong.

More tax planning is therefore required to convince the worldwide tax authorities that more profit should be allowed for low-tax jurisdictions. In this connection, Hong Kong has an edge over some other low-tax jurisdictions in establishing a "real" business, due to the other factors (legal system, close proximity with China, availabilities of talents, etc.) as discussed earlier. The latest introduction of IP-friendly and tax-friendly legislations only further attracts investment in Hong Kong.

Initial Public Offering of Biotechnology Companies

Biotechnology companies are magnets for capital, elites and other resources. Coupled with the true necessity for constant financial commitments to technological researches, Initial Public Offering ("IPO") should certainly come into the picture.

In 2018, in recognizing that biotechnology companies may have a legitimate need for capital market for research and developments before making any meaningful profit, the Hong Kong Stock Exchange added a new chapter 18A to the listing rules to allow biotechnology companies, which originally did not satisfy the financial eligibility criteria for listing on the Hong Kong Stock Exchange.

Biotech companies are defined as companies primarily engaged in the research and development, application and commercialization of biotechnological products, processes or technologies. In turn, "Biotech" is defined as the application of science and technology to produce commercial products with a medical or other biological application.

The possible candidate must demonstrate, inter alia, that it has developed at least one core product beyond the concept stage; has primarily engaged in research and development for the purposes of developing this core product; and that it has registered patent(s), patent application(s) and/or other intellectual property in relation to its core product(s). The possible candidate should have also previously received significant third party investment from at least one sophisticated investor before the IPO.

There are still certain financial requirements, such as working capital and expected capitalization.

Although it is expected that the above are no easy hurdles and the Hong Kong Stock Exchange is expected to adopt a conservative approach in examining IPO applications from biotechnology companies, it at least signifies a major step forward for attracting Biotech listings in Hong Kong. This also add fuels for biotechnology companies to invest in Hong Kong.

Recent Tax Deductions in Hong Kong

In 2018, the Hong Kong government introduced certain tax measures to increase its tax competitiveness internationally. There are two notable new measures particularly worth the attention of biotechnology companies.

Firstly, there is a new two-tier profit tax regime[4], whereby the first two million of profits of a designated Hong Kong company will be taxed at the low rate of 8.25%, with the remaining profits to be taxed at the normal rate of 16.5%. This reinforces the low-taxation branding of Hong Kong.

Secondly, an enhanced research and development tax incentive scheme has been introduced[5]. Research and development expenditure will be introduced into two main categories. For certain qualifying expenditure, 300% tax deduction will be allowed for the first two million, and 200% tax deduction will be allowed for the remaining amount. The relevant research activity must be wholly carried out in Hong Kong, and paid to a designated local research institution or an employee who engaged directly in qualifying research and development activities. Unsurprisingly, the conditions that need to be satisfied in order to qualify for an enhanced tax deduction is not straight-forward.

4 https://www.ird.gov.hk/eng/faq/2tr.htm

5 https://www.ird.gov.hk/eng/pdf/2019/dipn55.pdf

Conclusion

Intellectual property is indisputably crucial for the business development of technology-driven biotechnology companies. It could also well be engaged as a useful tool for legitimate tax-planning activities. This article certainly does not serve as a tax advice but it should still be interesting to understand more about the global trend and information.

There is a call for tax-transparency worldwide and global collaboration against exploitation by over-aggressive tax-avoidance strategies and practices. Thanks to the introduction of CRS and AEOI, it will be challenging in creating a proper structure for tax-planning, and the tax residency status of a low-tax jurisdiction such as Hong Kong becomes significant and important. It was discussed in this article, (i) the many factual advantages of starting businesses in Hong Kong; (ii) the latest introduction of tax reductions; and (iii) easing of listing of biotechnology companies. Weighing against all the pros and cons, Hong Kong is certainly not a place you can neglect for starting business or further investments.

Chapter 10

BUILDING AN INTERNATIONAL BIOTECHNOLOGY HUB IN HONG KONG AND THE GREATER BAY: THE CHALLENGES AND OPPORTUNITY

Joseph Wing On Tam, PhD

Abstract

The biotechnology industry in Hong Kong started in the early 80s with the introduction of rDNA technology, the establishment of HKIB, BRI, MBI and the ISF to fund technology innovative projects by the Hong Kong Government in 1994. The proposal on the establishment of the Science Park, Cyberport and the TCM hub in 1997 presented extra incentives for many to engage in technology startups. The Dot.Com upsurge, the HKEX GEM Board established in 1999 had attracted private investors, including local tycoons to provide adequate funding to support the growth of the industry. Unfortunately, the tech bubble busted rapidly because of short-term greed due to the lack of public understanding on the nature of the industry; we failed to capture the tide. Now 20 years later, realizing we are behind many of our neighbors, HKSAR government is taking a top-down approach to offer strong support to boost technology innovation, and the Greater Bay Area Initiative represents a much stronger critical mass and resources to create a second chance to put Hong Kong as a biotechnology hub on the world map. Great challenges lie ahead in creating the right environment for general public participation and building a favorable ecosystem for scientists, entrepreneurs as well as investors to work together to ensure success from bottom-up.

Introduction

Hong Kong has always been one of the top innovative international cities in the world. The industrial and manufacturing sectors had been the major pillar since the 1950s and peaked at 1984 when the sector contributed up to 28% in GDP and 40% in employment. During that period, influx of immigrants provided the much-needed low cost labor essential for the industry to flourish. Hence our industry leaders, 90% Small to Medium Enterprise (SME), could produce top quality products to cater for international customers' needs. Exportation of products in textile, electronics and toys had been a major source of revenues. Consequently, our industrial entrepreneurs had made these products the No.1 in supply and in quality, and Hong Kong became the leader of the "Four Little Dragons" ahead of Singapore, Korea and Taiwan in the last decades of the 20th Century. However, these countries and Shenzhen had subsequently recognized the potential of technology industry and started early. Evidently in the past two decades, these counties and city have made extraordinary progress in developing technology industry that have contributed to the major source for GDP growth (see Figure 1a). The crash of the US real estate market in 2008 created the world's most severe financial crisis in modern history that led to negative growth for most countries worldwide, except a few i.e. United States, Korea, Singapore and China. It was the technology industry, especially the internet-related industry that had helped these countries to recover, while others lagged behind and still remain in their pre-2008 GDP level today (see Figure 1b). Unquestionably the rapid rise of China to become the world's 2nd economic power was mainly stimulated by the government's decision to raise R&D support for science and technology innovation and development, i.e. Hong Kong's neighboring city Shenzhen's R&D budget was raised to 4.1% GDP compared to 0.73% in 2018 for Hong Kong (see Table I).

	R&D Expenditure			Global	GDP (norm)		GDP (ppp)	
	GDP % (Rank)	Total $ [ppp] Rank	$ per Capita (Rank)	Innovation Index (GII)	GDP Rank	GDP per Capita Rank	GDP Rank	per Capita Rank
Hong Kong	0.73 (47)	42	373 (31)	14	34	15	43	9
Shenzhen	4.1(3)	[5]	2146 (1)		33	13	35	16
Singapore	2.186(15)	26	1831 (2)	5	35	7	36	3
Israeli	4.3(1)	21	1361 (10)	11	32	21	51	34
S. Korea	4.29(2)	6	1518 (4)	12	12	28	14	29
TaiWan	3.1(7)	12	1386 (9)		21	35	22	15
China	2.17(17)	2 [1]	321 (35)	17	2	6	1	73
USA	2.744(11)	1[2]	1586 (3)	6	1	8	2	10
UK	1.701(22)	9	692 (24)	4	5	20	9	26

Table 1. Gross Expenditure on RGD by difference countries and cities in 2018

The rapid modernization of China also helped Hong Kong to become the world's 2nd Financial Center. At the same time, because of the rise in labor and land cost, Hong Kong entrepreneurs and industrial leaders moved their factories, including R&D if there were any, into China's special economic zones such as Shenzhen and other cities to take advantage of the China's reform and open policies. On the other hand, the shift diminished Hong Kong's local industry and forced its manufacturing and related work force into service industries. Consequently, the GDP from the manufacturing sector decreased rapidly from 28% to less than 3% according to reports and replaced by re-export trades. This paradigm shift made Hong Kong dependent on import-export trades, finance and tourism; an almost exclusively service international city. The rapid growth in China also helped Hong Kong to become a global center in trade and finance, a location for international companies to setup headquarters because Hong Kong was the only window open to China. Hence, Hong Kong was firmly established as a world's financial center in the late 90s. Unfortunately, at the same time because of the change of real estate policy change in 2004 after the financial crisis in Asia, the real estate property values were pushed up to the world's highest levels, making it unaffordable to most people especially the younger generation. That has started to create the social conflicts as of today. Ironically, Hong Kong's neighboring city Shenzhen has been maximizing the tech industrial development and, in the same period, have resulted in 3-fold increase in GDP per capita and that have made a contrast. Evidently the lack of, or the change in, policy for technology industry development was the direct consequence.

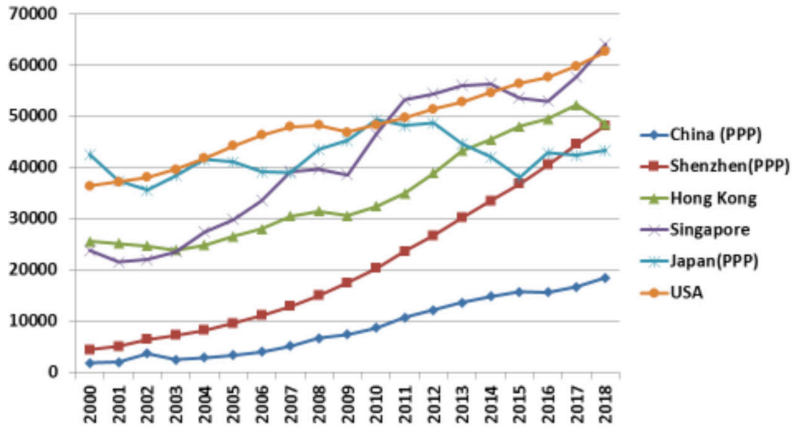

Figure 1a : Past 20 years' Historic GDP per Capita of Countries and Cities (in US$)

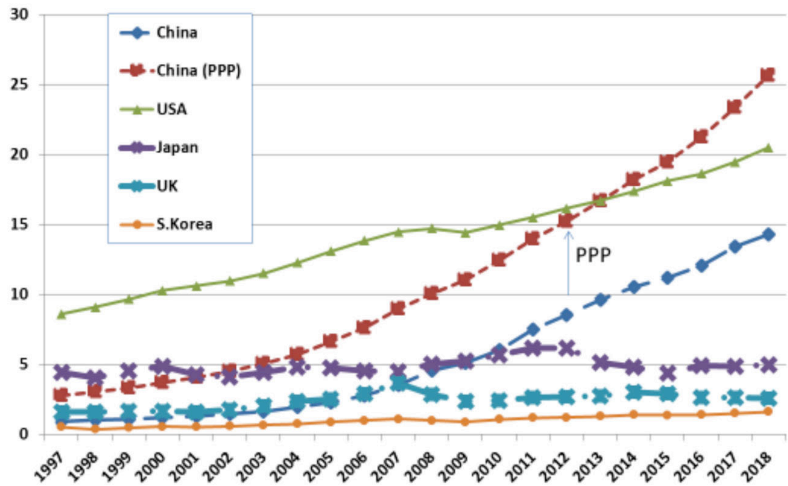

Figure 1b : The Historic GDP of different Countries in the last 2 decades (in US$)

After many difficult years, it was gratifying to know that in 2017 the new Chief Executive Carrie Lam Cheng Yuet-ngor promised to address not only the housing issue but also put forward new policies to put technology innovation, biotechnology and Artificial Intelligence (AI) as the city's top priorities. Together with the Greater Bay Area master plan initiated by China, Hong Kong should have a second chance to become the leader not only in finance but also in technology development. This has created an unprecedented opportunity for the future of Hong Kong. Our challenge as professionals in the field is to find a way to help build the right ecosystem to make this a reality.

The untapped potential: Hong Kong Technology Strength

For decades, Hong Kong as a global city that has acted as a bridge between the West, East and China adopted well with both cultures and constant updates on international information and technology exchanges. From the late 90s to early 2000s, Hong Kong had the first chance to become a tech center soon after the GEM board was established to allow tech startups to raise funds from Initial Public offering (IPO). The DOT.COM upsurge in NASDAQ and fall in the real estate/property market also helped to redirect investors into technology startups. Suddenly, many startup companies were formed and were able to raise funds through IPOs with just 2 years' company history. Such a low entry barrier attracted finance bankers, including local tycoons such as Li Ka Shing to take advantage of it and captured the opportunity to create the Tech Bubble rapidly. The short-lived "Technology Tide" was gone mainly because: (a) the general public did not understand the nature of tech industry and its risks trusted celebrities and followed blindly in investing into companies with unreasonably high valuation and suffered huge loss; and (b) the sudden change in Real Estate policy in 2004 by the Hong Kong Government proposed by the Honorary Mr. Michael Suen (namely the 孫九招) to reduce housing supply, that allowed the few private property developers to monopolize the market. As a result, rapid increase in property values, at the same time investors and people with money turned back to property which led to the drop in investments to support the tech industry. Hence the short-lived Tech Bubble left behind the common slogan in Cantonese: "high-tech Hi-yee, low-tech lo-yee" (loosely translated as "high tech burns, low tech earns"), as investors lost faith in the technology industry. Consequently, the untapped science and technology potential generated by Hong Kong's world-class scientists in Hong Kong's universities were ignored by investors as well as the general public. Ironically, this same feeling also spread among our scientists because of the lack of tech transfer expertise to help them reach the market successfully. In the western society (including China today), most technology companies are built around or nearby University hubs, i.e. in Boston, San Francisco Bay or ChungKwan Village (中關村) in Beijing, etc., because they are the source of innovation and discovery and often the professors themselves and their graduates become the entrepreneurs.

Hong Kong has 10 universities: 5 of them ranked top 50 worldwide; 2 ranked top 10 in AI and we have a Nobel laureate and nominee in science; and therefore should be in the technology world map. Israel is similar in population size and has fewer universities and lower in ranking, yet, has been well recognized as one of the top innovative countries in science and technology development in the world. Similarly, Singapore also moved ahead of Hong Kong in technology development; therefore, GDP per capita also has surpassed Hong Kong over the last two decades. Unquestionably, however, Hong Kong has made its leading contribution in science and technology to date. It was the first in providing molecular diagnostics for genetic diseases in the early 60s, and the first to introduce the cutting-edge rDNA technology and trained scientists in the Far East. Diagnostic tests such as the non-invasive prenatal diagnosis (NIPD) and the liquid biopsy used worldwide to date were discovered by our CUHK professors, just to name a few. In engineering, a good example is the world's first and No.1 drone, produced by DJI, which was invented in Hong Kong and further developed for commercialization in Shenzhen. In fact, Hong Kong has been, and still is, the training center for many of the scientists and engineers in China. Many of them, including some Hong Kong citizens, became entrepreneurs to startup tech companies in China where local policies provide the right environment and incentives to help increase success rate and for long term development sustainability.

The role of Hong Kong in the Greater Bay Area Initiative

The success of economic reform led to prosperity in Shenzhen, China was founded by the rapid establishment of the manufacturing and technology industries. As stated above, Hong Kong has one of the world's best intellectual concentrations in science and technology, top business management (i.e., 3 top-ranked business schools) and financial system. Hence, it should be able to lead in innovation technology and providing funding. For this reason, the central government, recognizing the untapped scientific resources, proposed and implemented the Greater Bay Area master plan to optimize various resources to support the technology industry to the fullest. Indeed at the China Development Forum, in Beijing in March 2019, Hong Kong Chief Executive Carrie Lam Cheng Yuet-ngor said: "In my view, the Greater Bay Area has good potential to become not only the Silicon Valley of the East but also Silicon Valley and Wall Street within the same city cluster." The internationally recognized and well-accepted Common Law that can protect IP gives the added advantage to facilitate international collaborations, making Hong Kong the ideal location for technology companies to establish headquarters and research centers for research and development activities on cutting-edge ideas.

Biotechnology Revolutionizes Medicine and Healthcare

During my lifetime, I have been very fortunate to witness the two advances in technologies that help revolutionized the world: the computer and genome science. Figure II shows the rapid advances in growth of computing power. This has led us to expeditions to the moon and far beyond in outer space. In the very near future, data communication can be close to the speed of light and, very soon, the AI development can perhaps create robots that can think and help bio scientists solve the most complicated biology systems. Similarly, because of the exponential increase in speed of computing power, gene technologies are advancing in unprecedented speed since the past several decades. At the molecular level, we can analyze target molecule down to single quantity with polymerase chain reaction (PCR) for nucleic acids (others with microscopic imaging) and modify gene precisely with the discovery of gene editing technology. Biosynthetic genome is likely to become reality very soon. To date, we can complete the sequencing and analyze our genome in hours instead of years. Moreover, the price of sequencing the whole human genome came down from billions to hundreds of dollars, making it affordable for routine examinations. With the accumulation of enough data in genome, proteome and detailed components in any living organism, from single cell to multi-cellular species, we can perhaps begin to understand details and stages of individual cells and the health of the organism. Hence, such Big Data can be utilized as reference, with test results generated by the advanced analytical and computational tools, to predict the health status and to prescribe appropriate individualized medical management and treatment for any known diseases, leading to the truly "Preventive Healthcare" era. At cellular level, any committed cells can be reverted to other types of stem cells by culture that can subsequently be turned into artificial organs. Thus, through "regenerative medicine," the healthcare profession can provide identical organs for transplantation with no rejection.

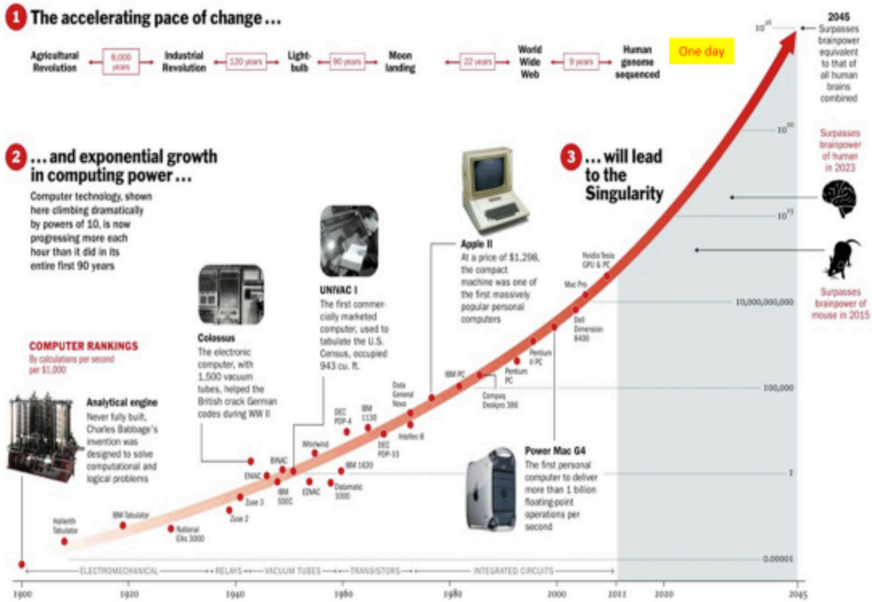

Figure 2. The Exponential Growth of computing power (Nilesh Sharma, Devops Manager/Justproperty.com Team Lead)

Biotechnology Development History

In a broad definition, wine, vinegar and bread are earliest example of biotechnology products produced by fermentation in 5000-10000 BC. However, the first product, acetone – a pure biological component – was made in 1917. To date, most people recognize Genentech, a spinout from Stanford University and UCSF in 1976 by Boyer and Cohen was the first Biotechnology Company because the company successfully used recombinant DNA construct to produce growth hormone for human therapy for growth hormone deficiency. However, Cetus, established in 1971 to start the bioengineering processes right after the discovery of restriction endonucleases in 1970, should have been the very first biotechnology company in the modern sense. It started first on selection of industrial microorganisms for effective production of chemical feedstocks, antibiotics, or vaccine components but soon became the pioneer in the new revolutionary techniques: recombinant DNA, monoclonal antibodies, and gene expression, the foundations of the biotechnology industry. In 1981, the company raised $108 million in an initial public offering (IPO), the largest IPO to that date. The company had successfully produced the beta-interleukin, followed by IL2 through gene expression in culture using rDNA clones and validated for clinical use and submitted for FDA approval. In addition, the most widely used PCR technology was in fact also discovered by Cetus in 1983. Unfortunately, the refusal of FDA approval in 1990 of the recombinant drug IL2 led to Cetus' downfall and acquired by Chiron in 1991. The PCR technology acquired by Roche, for which it paid $300 million USD, made Roche the King of the Molecular Diagnostics today (as just annual royalty income alone tops over billion dollars), yet Cetus went down in history missed by many in the Biotechnology Industry record (see "Biotechnology" and "list of Biotechnology company" in Wikipedia) while other late comers such as Genentech, Biogen, Amgen and Gilead became leaders of the biotechnology industry in the 80s, producing recombinant drugs that revolutionized the healthcare treatment. DNA Sequencing was discovered in 1977, but the first automated sequencer was produced by ABI in 1983 and that provided the means to complete the whole genome project in the late 1990s, which in turn helped to push biotechnology activities to peak with over US$7.8 billion in IPO in 2000. Despite the financial disturbances created by the Dot.com and the real Estate Bubbles and the biotechnology industry continues to grow. In 2015, the number of well-established biotechnology companies was 2,336 in private and 449 publicly listed. The European Commission setup a biotechnology strategy in 2012 targeting to achieve 22 million in employment with 2 trillion Euro revenue target in 2020 (https://europa.eu/rapid/press-release_SPEECH-10-423_en.htm). As a result, the number of biotechnology SME increased drastically.

In the past decade, China's innovation technology policies have been the driving force for growth, with rapid increase in R&D expenditure and biotechnology being on highest priority. In fact, the average venture capital funds raised per round has been higher than the US and well above Europe (John Hodgson: Biotech's baby boom, Nature Biotechnology: Vol 37, May 2019, 502-519) in recent years. The pharmaceutical industry has been one of the fastest growing sectors in the last two decades. Today, the Chinese pharmaceutical market is worth over $110 billion, and already ranked second, only after the US. The country is now the second economic power. Having more than 4 times the US population and the increasing number of returnees of millions of scientists educated abroad should give the blooming industry enough manpower and support to succeed. Under the "One Country, Two Systems" policy, Hong Kong has the total freedom for international exchanges that can tap on worldwide resources and market to become the center of excellence in technology. Unfortunately, despite the recent surge in activities in Science Park, biotechnology is still far behind other major countries. Thus, building up the right ecosystem from all levels is imperative and should move fast.

In previous volume of this series (Vol II, Chapter 15, p215-232) I have addressed in some details on the Hong Kong biotechnology developmental history in Hong Kong and the strong advocating power from a few influential individuals who were instrumental in the formation of Hong Kong Institute of Biotechnology (HKIB) in CUHK, Biotechnology Research Institute (BRI) in UST, Molecular Biology Institute (MBI) in HKU and the establishment of the Innovation and Technology Fund (ITF) in providing the Industry Support Fund (ISF) that formed the basis of nurturing the Biotechnology industry in Hong Kong, moving in line with the rapid increase in Biotechnology activity in the United States. Subsequently our first Chief Executive Mr. Tung's decision to establish the Science Park, CyberPort and Traditional Chinese Medicine (TCM) hub in 1997 gave a further push for many entrepreneurs to start companies. That was indeed the "Golden Age of Biotechnology" for Hong Kong, as proclaimed by Dr. Raymond K.F. Ch'ien and Prof. Yuk Lam Lo in the first volume of Biotechnology in Hong Kong by Dr. Albert Wai-Kit Chan in 1998. The first book not only summarized the background on the subject industry, it also provides positive pitch to promote biotechnology for Hong Kong. Subsequent volumes continued to do the same.

To date, the situation is apparently much more favorable for the tech industry because: (a) Government R&D funding has doubled with extra incentives for Technology startups; (b) the launch of the new Chapter 18A, custom-designed for providing public funding to technology companies; and (c) there are indeed Angel funds for A round funding, and the government also provides matching funds to give extra incentive (see J-F Tremblay, C&EN Vol 97 i22). In addition, the Greater Bay Area master plan provides unlimited human resources, supporting infrastructures, and market potential. Thus now we are presented with unprecedented favorable opportunities. The challenge is for us as professionals in the field to make sure we won't miss out on this second chance.

Building the Ecosystem - Bottom Up Approach

Hong Kong missed the first Biotechnology wave because of some obvious reasons in hindsight. To date, although Hong Kong has excellent world-class academics with ideas and research outputs in science and technologies, there is still a lack of downstream tech transfer experienced executors. The same is true in the finance sector, lacking technology expertise in funding capitalists. Therefore, we must resolve these two critical elements by building up such talents to create the right ecosystem for the industry to flourish. In addition, we must also build up the confidence of the general public participation in the investment market. The immediate solution to both, now initiated by the government, is to import talents; to attract foreign companies to come to help in creating the industry faster; to allow companies with good and solid performance to be listed in IPO to facilitate rebuilding the public confident in technology. However, the more important long-term solutions should be to build up our own talents by: (a) investing into early startups and (b) rebuilding our own local students' interest to science, engineering and mathematics through education in universities.

Now we have good government policies with attractive incentives and apparently ample venture capitals for young entrepreneurs to consider forming startups. The Chapter 18A provides a clear roadmap for companies that, once entering into relatively mature stage, can get more funding to move up. Basic conditions have been created for the technology industry to move forward. However, the number of startups is still minimal compared to well-developed countries or China.

The United States was ranked first by the Organisation for Economic Co-operation and Development (OECD) in biotechnology by the number of firms, R&D spending, and commercial output. In 2015, there were already 11367 biotechnology firms, and 72% of them having 50 or fewer employees. The Boston Greater Area alone has over 1,000 biotechnology companies; San Francisco Bay has more and likewise in Beijing and Shanghai, China. Indeed, "small biotech firms are the rule rather than the exception" for the success of world's best biotechnology hubs. All hubs are built by huge numbers of startups to fuel the industry supply with innovative ideas and work force.

Singapore ranked second by Scientific America in 2010 based on: IP and the ability to protect it; intensity, defined on R&D spending; availability of venture capital and support; availability of expert manpower and supporting infrastructure. Hong Kong, except for expert manpower, have most of the above: the right legal system; R&D increasing trend; good supporting infrastructures nearby in the Greater Bay, and huge capital market if we can successfully change their mindset. Hence, Hong Kong must build up the critical mass quickly through: (a) training and importing more talents and (b) providing seed money to encourage local and outside young entrepreneurs to found startups like what the Science Park has been doing over the years. Government incentive policy alone is not enough though, we need more private participation to build the ecosystem from the public.

Biotechnology, unlike others, has much higher entry barriers: high risk in nature and requires much higher sum of seed money. Hence, very few young talents can afford to start on their own. At the same time, although the return is high, there is a lack of experts to help to give proper project evaluation for capitalists to consider. Hence current venture capitals, including angel funds and even the matching fund provided by government, would not support early startups with innovative ideas and IP before pilot proof on their concept. This could often wipe out some of the brilliant ideas or the entrepreneurial spirit of our young talents to contribute to the industry. As professionals, our immediate challenge is finding ways to minimize the risk to increase the rate of success and continue to educate the public to change the mind-set of investors that the "high risk high return" could indeed work to their advantage. In a small way, this is what I have been doing and would like to see others to join and continue in creating a more favorable environment for the future of the biotechnology industry.

In my generation, the bright students often chose to pursue a career in science. In the past decades though, because of the lack of industrial jobs for science graduates, talented students either went overseas or changed to other subjects, leading to the closure of many university programs that I have witnessed in my teaching career in Hong Kong. Similar phenomena also occurred decades ago in the US for computer subjects; students turned away because the job market did not support their career goals. However, once the career prospect of the industry turned north, students frothed in. In fact, in recent years we are seeing the return movement, biotechnology and related programs have seen increase in activities as job openings continue to increase for local graduates to pursue their industrial career. Hence, the more startups the better and faster to build up the expert manpower for the industry.

The Road Map of DiagCor and Pangenia – building up industrial talents

To take up such a challenge, I decided to accept a very small sum of funding to stay in Hong Kong to startup the biotechnology company after my retirement. My goal was simply to create jobs and a place for local graduates to work with what they have learnt as I truly believe Hong Kong has enough talents and resources to become an international technology hub, i.e., like Israeli, despite most people believing otherwise then and despite no venture funding. I co-founded Hong Kong DNA Ltd.; Medical Gene Center Ltd., and HybriBio Ltd. that employed and trained many before HybriBio decided to move to China. After a cooling period, DiagCor was established in 2006, again for the same reason, to create job for science graduates and to prove that Hong Kong can have high-technology industry and that would be the alternative for making Hong Kong sustainable. To date, DiagCor became part of Pangenia, one of the very first molecular diagnostic laboratory with international standard, and have developed its own niche within diagnostic low-density DNA array kits with patent-protected IP for international sales. Under Pangenia, we have established the one and only independent clinical diagnostic laboratory (ICL) in China that can offer services with Hong Kong-quality standard. In addition, Pangenia owns the biggest scientific distribution company in Hong Kong and an international sales network (see Figure 3). Thus, to date, Pangenia is in a unique position, bridging the East and the West, which can effectively capture the "One-Belt One-Road" potential in molecular diagnosis in Hong Kong.

Pangenia is still a relatively small company compared to many, but it gave a solid proof: a technology company in Hong Kong can indeed survive the severe conditions with will power. The most important byproduct, however, is that over the past decades we have employed and trained hundreds of local graduates. Many of them have since progressed to start their own companies, helped other tech companies, and joined venture funds to provide professional analyses. In the meantime, despite the difficulties we were facing in past years, the numbers of biotechnology companies have increased steadily to keep the industry alive. The Science Park, under the new proactive management in the past few years, has put Hong Kong on the tech world map by providing the venue and incentives to encourage entrepreneurs to come. The number of technology companies is now increasing, creating a more favorable environment for the tech industry. Local startups, e.g. Gene Harbor, Prenetics, Xcelom, etc. are excellent examples to give the public more confidence to support the industry.

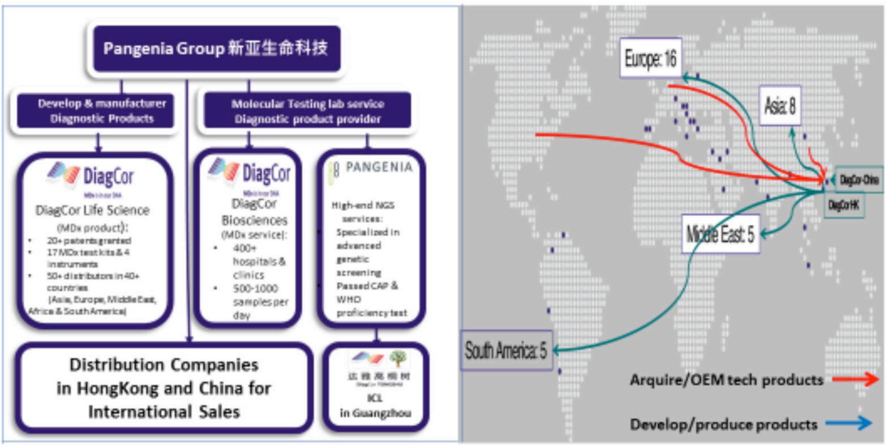

Figure 3: Pangenia Group Company structure and Marketing and Business Network
(As a HK Company WOFE in China Can provide a bridging role between China and the West)

Further Improve the Current Funding Environment: a Scheme for Helping Startups to Reduce Risk

Biotechnology industry is intrinsically risky. Even in the most developed countries like the US, investors realize the success in new drug development is less than 1 in 20 and may take many years. The success rate for medical devices and diagnostics may be lower but still much higher than conventional startups, and the investment time is long. A well-known example: Oxford Nanopore started in 2005, which had raised over US$500m, is still not yet profitable, nor has yet to do an IPO for investors to exit. However, successful companies not only give extraordinary return to investors but also become the major pillar for economic growth. Therefore, the US dominated the world market. The biopharma sector alone has produced over $200 billion of the $446 billion worldwide market. In view of the growing healthcare expenditure (Figure 4) and the favorable policies in Hong Kong and China, we are in the opportune time and place to develop the industry. However, published statistics reveals that 90% of startups fail with only 1 in 2 surviving in 4 years in general, and biotechnology is a very high risk venture. Hence, we must find ways to reduce the risk to increase success rate, to encourage more entrepreneurs to join to make the industry sustainable in Hong Kong.

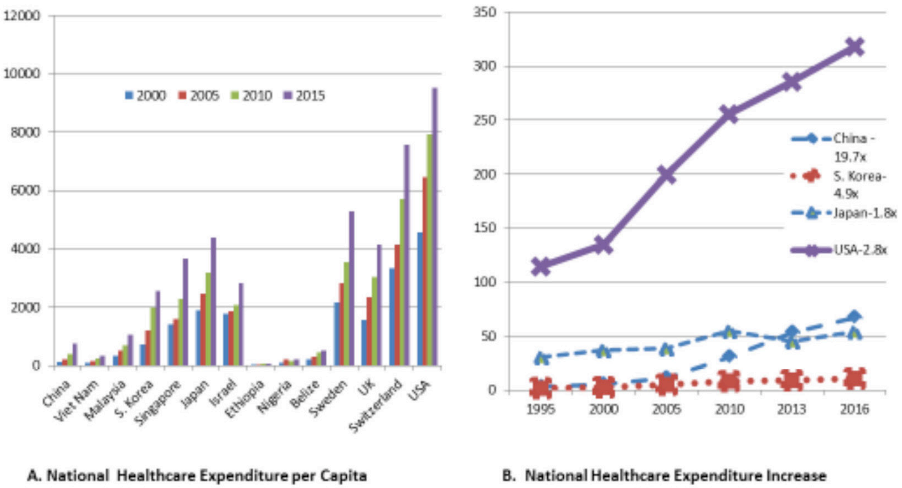

Figure 4 : Growth of Healthcare Market in Developing & Underdeveloped Countries

Realizing the difficulties for young talents to start and the concerns of venture capitalists, I started to move to support some startups that have difficulty in getting seed money and provided expert advice to avoid unnecessary mistakes to reduce failure rate. To make it more effective, I decided to stay in Diagnostics, not only because it is my expertise, but also I truly believe this shall be the future for healthcare as we are indeed entering into the "Era of Preventive Medicine." Soon, in combination of AI and detailed analyses of extracellular or cellular components (i.e., nucleic acids, proteins and metabolites) and ultra-imaging details, prediction of health status will be possible and interpretable for preventive treatments.

For in vitro molecular analyses, sampling technology is most important because all test results depend on the quality and quantity of such isolation. Hence, the strategy is to select target technologies with synergistic linkages so that we can gather talents with different but related expertise so that they can form a good ecosystem. In this case, our selection focus on sampling, screening, diagnostic, prognostic to include drug screening with molecular and/or cellular methods to form a diagnostic expertise pool. The goal is to provide minimum seed money for them to kick off and be able to reach the proof of concept stage to find Angel Funds to support further development.

After two years, the preliminary results are very encouraging. Three of the selected startups have presented beautiful score sheets with substantial increase in the company valuations. These companies are all in various stages of commercialization with imminent products or services ready to be launched. Although in reality, they are still too early to be considered success stories, but the risk of early failure has indeed been reduced.

Ideally, the portfolio should expand to at least 10 companies to form a group loosely linked together (as shown in Figure 5) to provide a good environment for young entrepreneurs to form a critical mass for effective brainstorming in problem solving, share experiences and failures to ensure future success. Indeed, failure for startups is part of the process no matter how good the scheme is. However, this learning process is a must to build the talent pool. To date there are many good candidates that fit the above criteria to support, but the limited fund will make it difficult to expand the portfolio alone by myself. Nevertheless, I hope this model set for the diagnostics as an example could present a new scheme, new way for investors and professionals to consider in nurturing the talent pool in different areas of the biotechnology industry which can rapidly build the right ecosystem for Hong Kong biotechnology industry from private participation. This "bottom up approach" in parallel to the now favorable policy in HKG and the Greater Bay Area is strongly advocated.

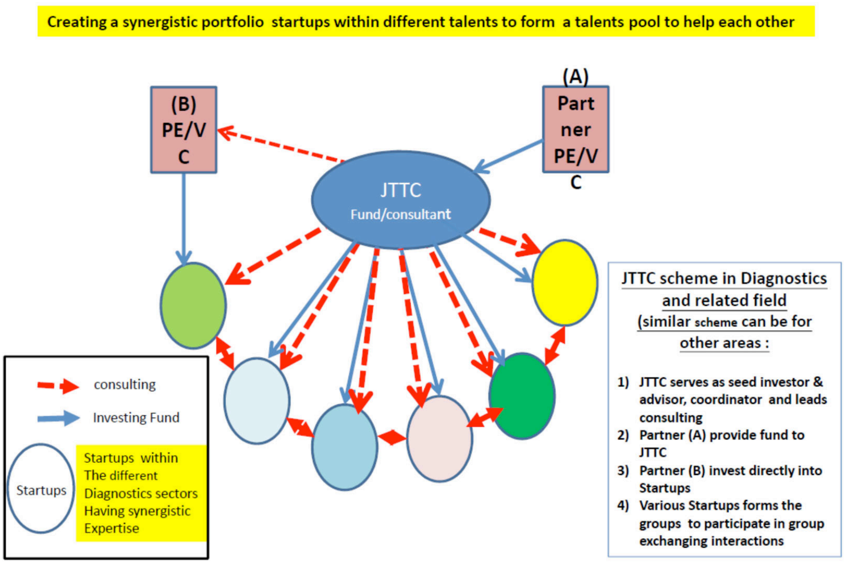

Figure 5. JTT's Scheme for creating a portfolio for investment.

Epilogue

Hong Kong has developed into the world's best international metropolitan city, well-integrated with different races and cultures by taking advantage of both political systems and moving closely with the world's advanced countries. In technology, because of some initial failures of startup in tech industry, most of the ITC public money had been channeled back to either academic institutions or semi-government run centers. Hence, de facto, the lack of support in promotion in the private industry by the previous administrations contributed to the setback, despite the technology policy set two decades ago still being in place. In retrospect, the government has indeed given tremendous supports to technology in Hong Kong through the Innovation and Technology Commission (ITC). Science Park has made tremendous progress in recent years. Under the new management, the biotech industry has been the fastest growing sector in attracting local startups and companies from overseas, producing the critical environment to support the new policy going forward. After 4 decades, I finally can see the twilight of my dream come true. We now have the will and the policies from the top level from Hong Kong and China to make a biotechnology hub in Hong Kong a reality. We are in the *Opportune Time* and the *Right Place*; I just hope that people can truly work together to make Hong Kong a better future.

Acknowledgement

Throughout my early life, I was fortunate to have met great teachers and advisors, without them I would still be an illiterate, a vagrant. The best way to thank them is to follow in their footsteps to help our next generation, and that has been my way of life. In my teaching career, HKU gave me the freedom to do just that, i.e., other than to Hong Kong students, I can help the Chinese students and scientists in learning some of the advances in technologies I learned in my limited capacity; for that I am grateful. My industrial life after retirement from HKU came from our students and encouraged by Dr. Kit Chan, the editor of this series, and Prof. Yuk Lam Lo, both great and persistent advocates on Biotechnology for Hong Kong. The success in gaining acceptance of Biotechnology today belong to many of the silent majority scientists and workers in the field devoting long years of hard-working hours. To my working associates and young talents, I thank them for their dedicated devotion to make things possible and my life enjoyable. I would like to thank Dr. H Y Poon for helping to put this in place. Finally, my thanks go to my wife and my family for their patience and love throughout my life.

Chapter 11

BIOMEDICAL TECHNOLOGY DEVELOPMENT OPPORTUNITIES AND CHALLENGES IN THE GREATER BAY

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Abstract
Since 2017, the Hong Kong government has injected about 1 trillion Hong Kong Dollars into innovation and technology (I&T) development, benefiting the academia and industry. This investment has included the conversion of a piece of land of about 85 acres, at the border between Shenzhen and Hong Kong, into a technology park as a solution to ease the space limitations in Hong Kong. By February 2019, a draft plan for developing the Greater Bay Area (GBA) was announced by the Chinese government. With an area of 66,000km2, population of about 70 million and an estimated GDP of 1.1 trillion USD, the GBA represents a huge development opportunity for a broad range of businesses ranging from travel, entertainment, education, R&D, manufacturing and investments, especially for Hong Kong. This is because, among the 11 cities in the GBA, Hong Kong is uniquely positioned as the international gateway city targeted to connect technology and investment alike between the GBA and the rest of the world. Building on its strength, here we specifically take a look at the development opportunities and the challenges for the biomedical industry in the GBA surrounding three particular areas: genomic medicine, advanced therapies (i.e., cell therapy), and medical device. While the land availability, large market (10 times that of Hong Kong), and lower labor costs are clear advantages for doing businesses in the GBA, differences in culture and regulatory systems for biomedicines, education, and hospital systems create unprecedented challenges during the development and eventual practice in the biomedical technology field. With patience, open communication, and support from the various levels of government, industry, investors and academia, a prosperous GBA will be in sight.

1. Introduction

Hong Kong attempts to catch up with the big shots in technology and innovation development

For decades, Hong Kong has been inarguably reputed as a financial metropolitan. Most would not bat an eyelash at the thought of Hong Kong as an international hub for innovation and technology. With the upsurge on developing diversified economies, biotechnology has become a prominent player in healthcare, agricultural, environmental, chemical and other sectors. It is projected by the Organization for Economic Co-operation and Development (OECD) that bio-based products would constitute at least 2.7% of GDP among the OECD member countries by 20301. It is undeniable, however, that Hong Kong needs to overcome a few hurdles before she can catch up with other countries leading in this arena. In spite of this, Hong Kong also has her own competitive edge in terms of education, hospital systems and a fast-growing industry in genomic medicine, cell therapy and medical device, which will be further elaborated in this chapter. Realizing reindustrialization was key to revitalizing Hong Kong's economy, the government established the Innovation & Technology Bureau in 2015 to drive high-level initiatives and provide funding resources to the industry. Various funding schemes were made available to the public sector; in addition to that, other crucial infrastructures and initiatives were also introduced to further develop the I&T sector.

Announced in the 2018/19 Government Budget, a total of HKD $50 Billion was allocated to accelerate development of the I&T sector, which is illustrated in Diagram 1 below. It is worthwhile to note, that of the $50 Billion budget, $10 billion has been allocated to 'upgrade facilities of the Science Park and enhance support for enterprises in the Park'. This is in reference to the key infrastructures that Hong Kong is currently lacking, in order to bridge the gap between bench work research and downstream commercialization. These key infrastructures are namely Good Manufacturing Practice (GMP) grade cell processing facilities, Good Laboratory Practice (GLP) grade biobank, centralized biomedical data platform, and GLP grade Drug Safety Center. Their establishment is vital to the success of the biotechnology industry in Hong Kong because infrastructures with respective international accreditations do not exist in Hong Kong or nearby regions, and current practice involves acquiring specimens from abroad to conduct specific experiments, which is both time-consuming and costly.

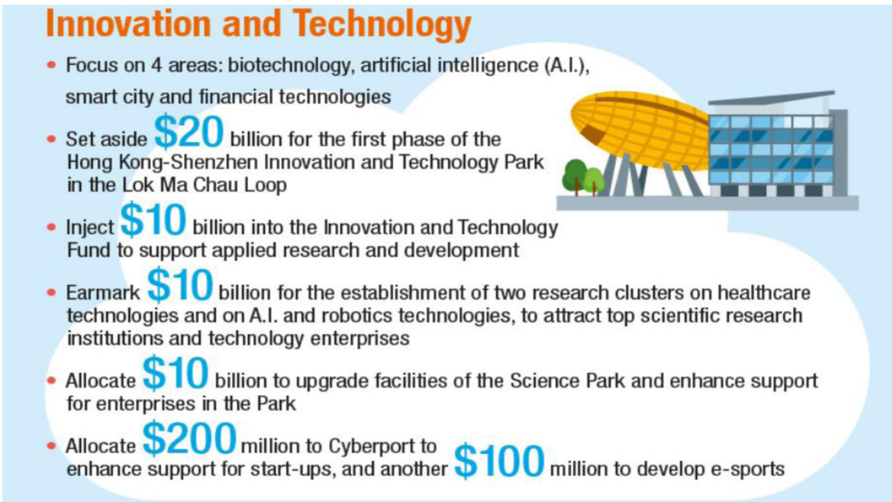

Diagram 1 - Government Budget 2018/19 on Innovation and Technology
Source: https://www.budget.gov.hk/2018/eng/ec.html

Other than the additional funding as mentioned above, the government has also put forth various support schemes to complement the financial support in the I&T sector. This includes the following:

1. Tax deduction for R&D expenses to encourage private investments in the R&D sector: 8.25% tax for the first HKD $2Million and the rest at 16.5%;

2. Talent recruitment support: HKD $500M to fund enterprises to recruit postdoctoral talent for R&D;

3. Housing support: development of "InnoCell", a housing project to provide accommodation for R&D personnel; and

4. Innovation & Technology Venture Fund: HKD $2 Billion for the establishment of a co-investment fund with selected Venture Capitalists to co-invest in startups with a ratio of 1:2 (government/VC).

While it is encouraging to see such a drastic shift in terms of financial support coming from the government and sophisticated investors from the private sector (such as real estate tycoons, investment banks, and the Big 4 accounting firms) all geared up to support the biomedical technology industry, a regulatory framework and other measures are required to be in place for Hong Kong to excel in the highly regulated field of biomedical technology.

Despite the current challenges and gaps that Hong Kong has to overcome, there are ample features that Hong Kong can leverage, such as her favorable geographic location at the doorsteps of China facing the external world and the 1.4 Billion population in China serving as the market base. Hong Kong's proximity to China has granted her an advantage that neighboring countries like Singapore cannot enjoy – the perfect position as a gateway to the Chinese market for innovation and enterprises. By just targeting the Greater Bay Area, we are already looking at a total area of 56,000 km2 along with a total population of around 70 million at the end of 2017.

Despite previous and ongoing debates and political differences, China has provided unwavering support to Hong Kong in terms of biotechnology development since 2017. As witnessed by President Xi Jinping, the National Development and Reform Commission and the governments of Guangdong, Hong Kong and Macao signed the 'Framework Agreement on Deepening Guangdong-Hong Kong-Macao Cooperation in the Development of the Greater Bay Area in Hong Kong' on July 1 2017. The Framework Agreement laid down the goals and principles of cooperation, and key cooperation areas are established in the development of the Greater Bay Area. Since then, the relevant Central Government departments and the three governments have worked closely together, striving for policy breakthroughs with an innovative and open mind, in pursuit of jointly driving forward the development of the Greater Bay Area.

This is further elucidated in the 'Outline Development Plan for the Guangdong-Hong Kong-Macao Greater Bay Area' where it was publicly announced on the 18th of February 2019 that Hong Kong is to be positioned as the international I&T hub for the Greater Bay Area. Within the selected 11 cities that made up the Greater Bay Area, Hong Kong is identified as one of the core cities to be one of the central engines for regional development. Other than leveraging Hong Kong's well-established information technology infrastructure, free economic system and a wealth of high-end talents with extensive knowledge on global markets, one of the key development focuses for Hong Kong is to develop innovation and technology industries and strengthen her status as a global offshore Renminbi (RMB) business hub. Compared to other key node cities and core cities, Hong Kong differentiates herself as an internationally reputed city for businesses and trade given its credible legal system and other supplementary infrastructures, including free flow of data. Conversely, the inability to transfer bio-samples and data outside of China has been a deterring factor that has hindered international enterprises wishing to tap into the blooming China market. It is promising to see that, after years of driving cross-border transfer of genetic materials between Hong Kong and Guangzhou, one focus of development in the Outline Plan is to enhance the management of cross-boundary use of medical data and bio-samples necessary for R&D collaboration projects. This offers great hope in positioning Hong Kong as an I&T hub, especially in the biotechnology sector.

2. How Hong Kong can contribute to GBA development

As the famous French proverb goes, 'Rome was not built in a day.' The same applies to the biomedical technology ecosystem in Hong Kong. In fact, Hong Kong possesses the fundamentals to position herself as a biomedical technology hub if steered towards the right path. For one, Hong Kong does not lack the talent required to build a vibrant biotech ecosystem. For another, the well-established electronic health record systems of over 20 years have been something in which Hong Kong has taken pride. The free flow of medical data allows for facilitation of longitudinal data for better patient profiling, providing a fertile ground for the development of genomic medicine, cell therapy and medical devices - with the latter sectors being hot commodities that have been highly sought after by investors globally.

Education and Industry

Talent is the core of innovation. Hong Kong has high quality academic institutions of which three of them are ranked top 20th globally by QS World University rankings, capable of nurturing a sustainable talent pool for the development of innovation and technology. In 2017, the New England Journal of Medicine has selected an exclusive collection of 10 important studies, of which two of the top 10 articles are from two local researchers, namely Prof. Dennis Lo and Prof. Tony Mok, both from CUHK. Such prestige is an unprecedented milestone for both the researchers and the institute they represent. Another exciting development is in the study of Alzheimer's disease. Alzheimer's disease has been an alarming issue that has been growing in Hong Kong, given its increasing ageing population. Prof. Nancy Ip from HKUST is a renowned expert in the area, and her Alzheimer's research has been pivotal; it alludes to potential therapeutics and advanced diagnostics with her discovery of the protein interleukin-33 (IL-33), which ameliorates cognitive decline and Alzheimer's disease-like pathology.

The industry has also shown some other promising results as of late. A homegrown biologics company, BioCancer Treatment Inc, has developed a new drug, which is a site-directed PEGylated recombinant human arginase 1 that can lead to arginine depletion. In 2017, the company announced the first complete remission of a post-immunotherapy melanoma patient in their US FDA IND study, which is an encouraging indication for safety performance and inhibition (e.g., for melanoma, acute myeloid leukemia, and pediatric cancer). A local medical device company, Belun has developed a smart biosensor capable of measuring heart rate, level of SpO2, heart rate variability and respiratory event index to monitor sleep disorder and respiratory related diseases, which is great as a home screening tool. Founded in 2016, their product (Belun ring) obtained FDA clearance in 2018, making it a medical-grade product for better market penetration. Other successes like the merger of homegrown company Cirina with the global diagnostic giant Grail, has been a widespread news since 2017.

Noting the wealth of intellectuals thriving in Hong Kong, the Innovation and Technology Bureau established two research clusters within InnoHK on healthcare technologies [Health@Inno], A.I., and robotics [AIR@Inno], with the goal to develop Hong Kong as the hub for fostering global research collaboration (See Diagram 1). The idea of this major initiative is to have top-notch researchers from all over the world converge to conduct world-class and impactful translational research, ranging from personalized medicine, bioinformatics and alternative medicine to chemical biology, to enrich the ecosystem and nurture local talent development. Ultimately, the goal is for the research projects to be commercialized, generating spin-off companies and create more job opportunities for young local talents in pursuit of a career in biotechnology.

With the tremendous support from the Central Government and recent financial support from the Hong Kong government, Hong Kong needs to seize the opportunities at hand and act fast in order to capitalize her current strengths and legal framework before it is too late to do so. Hence, Hong Kong needs to work on its existing regulatory framework, especially in regards to cell therapy, to ensure quality protocols and validated data are utilized for the generation of potential innovative therapies for patients.

3. Hurdles for developing biomedical technology in the GBA

A. Education and talents

The GBA has vast resources in terms of land area and population. A casual scan of several successful bay areas in the world revealed that 'land area' and 'population' are not necessary the deciding factor towards their success or GDP output, whereas likely the brain power, the high quality and value to the output programs and products may be the major factors.

Talent training and education is undoubtedly, a key to success as they produce the brain power and skilled labors behind all the technological development. A clear sore spot is the lack of international students among the universities in China, and especially so for those in the GBA. The top ranked university in the entire GBA, Sun Yat-Sen University in Guangzhou, is only ranked 301 in the Times Higher Education Ranking and 295 in the Top Universities Ranking based on two separate systems in 2019, clearly demonstrating a gap in producing top quality students and talents that may feed into the downstream industries, enterprises and academia alike. For Life Sciences and Medical Schools, again, the highest-ranking Sun Yat-Sen University is ranked 295 in the world, according to the QS Ranking 2019. Thus, it is questionable if the GBA universities could produce a sustainable supply of quality talents to fuel the innovation and technology development. It will be essential to have close ties with the rest of the world in order to facilitate collaborations and be alert of new advances and breakthroughs. This renders international exposure and language communication abilities, as additional features that the next generation of talents needs to possess in order to become a key driver for success of the GBA.

Ranking	GDP (2015)	Billion USD
1	United States	18,037
2	China	11,226
3	Japan	4,382
4	Germany	3,365
5	United Kingdom	2,863
6	France	2,420
7	India	2,088
8	Italy	1,826
9	Brazil	1,801
10	Canada	1,553
11	Korea	1,383
12	Russia	1,366
	GDHKMC Bay Area	**1,300**
13	Australia	1,230
14	Spain	1,194
15	Mexico	1,151

The Bay Area vs. major metropolitans in the world

GDHKMC Bay Area	Greater Tokyo Area
Area: 66,000 sq. km **GDP:** 1.3 trillion USD **Population:** 66.72 million **Administrative units included:** 9 cities+2 SARs*	**Area:** 36,800 sq. km **GDP:** 1.8 trillion USD **Population:** 43.84 million **Administrative units included:** 1 capital + 7 counties
San Francisco Bay Area	**New York City**
Area: 17,900 sq. km **GDP:** 0.8 trillion USD **Population:** 7.6 million **Administrative units included:** 9 counties	**Area:** 783.84 sq. km **GDP:** 1.7 trillion USD **Population:** 8.6 million **Administrative units included:** 5 counties

* Special Administrative Regions

Data source: Wenweipo, Takungpao, Zijin Magazine, IMF, Statistical Communique of China, China Statistical Yearbook, Guangdong Statistical Yearbook, Census and Statistics Department of Hong Kong, Marine department of Hong Kong, Statistics and Census Service of Macau

B. Technology and Knowledge Gap

The GBA has been known for its agricultural importance and manufacturing plants producing consumer goods in general. High-value and high-end products, especially in the biomedical technology field, are less frequently manufactured – albeit several are located in the area such as OrbusNeich, Twenty-Two and Me and BGI. The lack of quality talents and education opportunities could be a negative feedback. Thus, even if new technologies were available, there may not be the proper talent to further grasp the opportunity and develop that into enterprises to fully realize the associated commercial potentials. For the biomedical technology field to be successful, the presence of advanced and well-equipped hospitals is critical. In Shenzhen, the presence of the University of Hong Kong Shenzhen Hospital may partially address this concern by offering professional training to the clinicians but inadequate in providing quality training to the young doctor students from the very beginning.

Especially for the biomedical applications in the clinics and hospitals involving genomic-based early diagnostics, advanced therapies and new diagnostics/treatments that offer unprecedented life-saving clinical solutions, the GBA may lag behind in accessing these technologies and practices, limiting the variety of quality clinical services being offered to the patients and the commercial opportunities associated with such in the GBA.

C. Regulatory and Intellectual Property Hurdles

Since biomedical products for application in patients are highly regulated, to bring forth such advanced therapeutics and technologies into the GBA, the products have to be vigorously inspected and the benefits in humans verified. The candidate products or diagnostics tests have to undergo vigorous clinical trials, whereby the results will be studied by regulatory agencies before they are approved for human applications. In the GBA, such an agency is the National Medical Products Administration (NMPA). The NMPA has its own sets of rules and guidelines for approving therapies and diagnostics that may be distinct from that in the US FDA and European EMA. Although in recent years, NMPA is striving to synchronize its requirements with that of the international circles, both in the speed of approval and the quality of the approved entity. As Hong Kong SAR adopts a one country two systems practice, the regulatory guidelines and approval requirements are quite different from that of NMPA. If this regulatory hurdle were resolved, it will facilitate technologies and treatments to launch in the GBA, presenting the GBA as an innovative hub for testing and launching of new medical solutions.

Likewise, the sharing of quality medical database would be an enormous asset for the medical practitioners and researchers. However, the current rule in China inhibits the sharing of human genetic materials and medical data with parties outside of China, inclusive of Hong Kong. This severely hampers the biological and medical comparison of results and longitudinal monitoring of patient outcome, as well as hinders medical advancement. Especially in this era of genomic medicine when genomic sequencing can predict disease manifestations and administration of effective drugs with less side effects, the lack of consolidated databases and comparisons with the external data will limit the GBA innovation development. The same is true for engineered cells where blood cells are manipulated to battle cancers and other conditions; such cells, being of human origin, may have great difficulty in transporting in and outside of China, affecting the effective launch of cell and regenerative therapy in the GBA.

D. Taxation, Finance and Cultural Differences with International Practices

An inhibitory consideration for Hong Kong residents to move into the GBA for business or for work is the discrepancy in the taxation systems. Hong Kong has a flat maximum personal tax rate of 16.5%, while GBA can amount to over 30%. In addition, the Hong Kong residents working in the GBA may not be able to enjoy any social benefits despite paying a high tax to the government. To encourage the talents, especially the younger generation to venture into the GBA, the governments have devised new measures to attract the talents by announcing 'Hong Kong tax' to be imposed on Hong Kong residents working in the GBA, and the GBA cities would provide social benefits to Hong Kong residents who work and reside in those areas. These privileges may stimulate the Hong Kong professionals and talents to work in the GBA, hence building the talent pool and preparing the region to excel.

The finance system is still a strength for Hong Kong compared to the GBA cities. The free flow of monetary funds in a global fashion is unique and will be maintained. Thus, Hong Kong can easily serve as the finance and business fund raising center, fueling the activities in the GBA.

As described above, there are inherent differences between the regulatory principles of Hong Kong and the GBA that can be extended into bioethics and cultural practices. These may impose extra reluctance for the interflow of work forces and professionals in the two areas, stemming from daily routines to professional practices.

4. Solutions and Measures to Enhance GBA Success

To ascertain a sustained success of the GBA, a continuous supply of talents and skilled workers is a prerequisite. In the biomedical technology field, these talents mostly fall into the clinical professionals' category, ranging from doctors, nurses, pharmacists, radiologists, surgeons, neurologists, orthopedic surgeons, ophthalmologists, medical technologists, physiotherapists, dentists and different professional specialists, each category taking years and decades in the training. Separately, researchers rely on the hospital setting to conduct translational medicine research, transforming research results into clinical practices. While it is apparent that the hospital is a central training and practicing ground for all these professionals, the medical school would provide relentless and quality training of these professionals from multiple disciplines. Taken with the fact that the GBA has few reputable medical schools, it is pertinent to build a high standard medical school for the GBA to train an ample supply of clinical practitioners. Not only would it support the healthcare system in the GBA, the medical school would also develop innovation and derive new solutions that would allow the GBA to become a leading medical innovation place in southern China. To this end, a solution perhaps is to partner with one of the leading medical schools in order to leverage on the experience and the expertise to help build a top-class medical school in the GBA.

The GBA cities may collaborate with, for example, The Chinese University of Hong Kong to build a new medical school. This medical school may follow the medical curricula of the existing Chinese University of Hong Kong Medical School, and the teaching language will be English. Faculty will be hired globally to fulfill this mission without breaching into the Hong Kong government funded medical teaching staff at the Chinese University of Hong Kong. The program could be a six-year program, followed by internships, and the graduates will be granted a Bachelor of Clinical Medicine degree, eligible for clinical practice in the GBA. Students will be recruited primarily from the entire China including Hong Kong. Limited places may be offered in the first couple of years before the medical school and teaching hospital were built. To facilitate clinical teaching, existing hospitals in Shenzhen and the GBA may be considered and fine-tuned to become suitable for teaching, as well as to cope with the high-quality Hong Kong medical school standards. This exciting venture will provide the GBA with a continuous supply of clinical practitioners of high standards and ethics, similar to that in Hong Kong, serving the residents of the GBA, elevating the medical school standards in China and be responsible to become a powerhouse in developing medical innovations for the region. While the medical school will specifically train medical practitioners and researchers in the biomedical technology sector, professionals in other disciplines such as business studies, finance and marketing etc. are being trained by several Hong Kong universities' Shenzhen or Guangzhou sister organizations. In this way the superior education systems from Hong Kong are fully exploited to help train talents in the GBA in preparation for future success.

Separately, the medical database containing valuable information that could be used in comparative or longitudinal studies would allow new and better treatment solutions to be uncovered. Yet, the data in China cannot be transported outside of the country, hence limiting its applications and value. Reluctance for sharing these invaluable data was because of concerns around the credibility of the user and the fear of compromising data privacy. With the establishment of a Medical School in Shenzhen, this could become a pilot-testing site for medical data to be transferred to the Hong Kong medical school and vice versa, whereby comparative studies can be effectively performed, data being tracked and used by credible clinicians in a university setting. Similar arrangement can be made with the University of Hong Kong Shenzhen hospital and the Hong Kong teaching hospital. Thus, effective sharing medical data across the border, undertaken by credible academics, would become a reality benefiting research and clinical practices. The practical value and commercial opportunities thereof would be huge.

As reiterated, the biomedical technology profession is highly regulated; the same applies to alternative medicine such as Traditional Chinese Medicine (TCM) and even medical device. For TCM, it may be effective to unite various entities in the GBA to certify the contents of the TCM herbs; thus, ensuring quality of the materials and reducing fraud. Such centers are readily found in Hong Kong academia, Macau and likely in other GBA universities, especially those in Guangzhou. Together there may be a chance to really modernize TCM and rejuvenating the industry and benefiting many.

The same is true for medical device. Although there are regulations and guidelines for approving medical device in the GBA/China, there is a lack of CE mark certification center in the region. Together with external organizations, academia and industry partnerships, such certification center could be established in the GBA, allowing new medical devices to meet the international quality standards and be available for commercialization with a clear path.

5. Closing statement

The GBA is filled with unexplored opportunities, especially with regard to the biomedical technology industry. The population of 70 million represents a huge market and an invaluable Chinese ethnicity database that would be attractive to healthcare and biotech companies. The launch of a new medical school in the GBA would revolutionize biomedical technology in the region and spearhead new developments. To capitalize on this opportunity, governments, industries, academia and investors must work in concert with open dialogue, in order to bring forth the best option for future development in technology and innovation. Building a GBA not just by size and population, but by value and impact of its products, services and provisions, it will allow the residents and visitors to enjoy this great piece of land in southern China speaking one language of Cantonese.

Reference:

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Chapter 12

GOLDEN PHEASANT HOOTING - THOUGHTS ABOUT PRECISION MEDICINE

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Keywords: precision medicine, human genome,

quantitative Chinese medical big data, targeted therapy

Abstract

In the beginning of 2017, the author reflected on the state and progress of precision medicine for the past several decades, and also elaborated on some preliminary assumptions and assessments for the spirit of collaborative discussion. Traditional Chinese medicine (TCM) is an indispensable part of the human health system. From the macro concept of "harmony of nature and man", traditional Chinese medicine uses simple philosophical ideas to develop a medical system driven by the accumulation of a large number of practical experiments and the syndrome differentiation-based treatment approach. The big question remains how should we further integrate valuable medical cases and countless practical experiences into the big data of human life through modern science and technology and innovative testing methods. The challenge is to use quantifiable data and mathematics models that meet the requirements of Chinese and western medicine. We need to lay a foundation for the emerging intelligent health medicine, to make ecology, health and medical treatment highly integrated, and to create a more harmonious relationship between human and nature.

Birth, aging, illness, and death in human life are the most important processes for the continuous development of all earthly creatures. At the end of the last century, the United States launched the "The Apollo program" in human health, which took a few billions of US dollars to initiate the sequencing of the whole human genome (Human Genome Project, HGP) [1]. The great program has been completed in the early of this century. The importance, necessity, and feasibility of precision medicine to human's health, in particular, cancer treatment, were recognized.

Only when gene mutations causing certain cells to become cancerous are diagnosed at the molecular genetic level and the target sites are subsequently identified, a good therapeutic effect can be achieved by using the targeted therapies (especially biological drugs) for an accurately directorial administration. At present, almost 100 developed targeted anticancer drugs are being used in clinic globally [2].

Of course, till now, there are still many different opinions towards the concept of Precision Medicine, and it is still being perfected. A new medical system can only be formed when an intensive concentration occurs across disciplines. This system comprises the sequencing technology of human molecular genes, scientific and comprehensive analysis of individual's genes and big data, precise personalized treatment, and etc. All technologies are synergically added to the application in clinical diagnosis and treatment, linking omics and diseases, health and environment. Apparently, simply seeing precision medicine as gene sequencing and targeted therapy is incomprehensive.

Precision medicine is a term recently proposed by the United States. Its Chinese translation can only be used as a proper noun. "precision medicine" and precise medicine as an adjective have different connotations. By as of today, in view of Chinese, precision medicine has a meaning more focusing on the sequencing of molecular genes. However, as disease occurred, a large volume of information other than DNA that could cause changes in molecules and cells at all levels is still fully detected or monitored yet. Indeed, the word "precision" is now especially popular in China. In Chinese, it represents precision and accuracy and is synonymous with the highest level. Therefore, the term "precision" is flying around for a time, e.g., precision accounting, precision home design, precision decoration, precision surgery ... has become the most favorably-used fashion term representing modernization.

Most professionals in the field of medicine will concentrate on molecular genetic level to solve the disease. As the "second-generation sequencing" undoubtedly opened up a large window, it contributes significantly by leading people to discover quite a number of genetic information on mutations at the DNA level, and the target sites for treatment. However, here comes new challenge, i.e., whether these sequencing can well represent the overall result of precision medicine. According to an Academician Chen Run-sheng, a Chinese scholar who has fortunately participated the worldwide human genome sequencing project in person, the important hints are: through the completion of the sequencing of the entire human genome, the traditional gene encoding a protein accounted for only 3% of the human genetic code, and the other 97% and the major laws contained therein have not yet been discovered [3]. In other words, we now know only about 3% of the human genetic information, and 97% of the non-coded part that we still do not know about is termed as hidden information. Therefore, it should be clearly understood that interpreting disease merely by using the DNA information is way from sufficiency, that; other hidden information or parameters at all levels such as RNA and protein that involve in transmitting messages, and others should also be considered so as to co-interpret various phenomena of life according to the rules of life and understand the dynamics of organisms' biological physiology and pathological changes.

Of course, for various key parameters, quite a number of various key parameters are yet-known hidden information or yet-incorporated into various disease diagnosis and treatment. We also lack the methods and means for detection of hidden information and these new parameters, and a lot of unsolved mysteries exist. The word precision should be give special attention. It is a huge challenge that we are facing. There is also a great opportunity accompanying it!

Recently, more and more scholars have implemented the modern medical 5P model: (1) Preemptive; (2) Predictive; (3) Personalized; (4) Participatory; and (5) Precision, i.e., which usually refers to the genetic and molecular level. This 5P medical model constitutes a more comprehensive concept of precision medicine (Precision Medicine) [4]. It is not difficult to tell that the Chinese medicine in China is the pioneer and ancestor of (1) preventive, (3) individualized, (4) participatory of the 5P.

For thousands of years, observation, auscultation and olfaction, inquiry, pulse feeling and palpation have been used to every patient according to traditional Chinese medicine, in together with personalized, individual participation of medical treatment through every patient. There are often different approaches to the cultivation, terms of collection, the method of brewing, and time for cooking of medicines, etc. Even if it is just a simple cold, it may vary from person to person and the symptoms can be categorized as cold, febrile, deficiency and excess. The patient should also provide one's inner feelings to the practitioner after each medication, which would be served as a criterion to add or subtract prescriptions accordingly. In the case of acupuncture, it is necessary to get the patient's assistance to obtain "de-qi" for adjusting the depth and direction of the needles. The actual personal relevant feelings as provided in accordance to the treatment would also partly help the doctor to adjust prescription of medications and add or subtract dosage. Direct participation in the interactive treatment process highlights the advantages of personalization and participation.

In the molecular genetic therapy of cancer, Western medicine emphasizes on aiming at the infected direct target sites, which are commonly result from big data analysis based upon the obvious information of DNA sequencing. Usually, the formation of an infected target site is a reflection of the phenomenon that the binding site of the molecular chain is prone to mutate. For example, an abnormality in the methylation around the DNA can lead to the degradation of DNA, leading to the occurring of protein mutations. Once the DNA's self-repairing ability fails, this kind of error can only be remedied by DNA editing, which is usually referred as gene therapy today. However, the factors within the body that cause methylation disorders may not be resolved [5].

As for the therapies of traditional Chinese medicine, Chinese medicine emphasizes on adjusting the overall balance of yin, yang and the five elements in the patient's body. Through yin, yang, and the five elements, the complex contradiction of the human body's internal diversification and multidimensionality (whether it can also be understood as a hierarchical "target site") is vividly expressed. There are also criss-cross situations between the mutual generation and prevailing over; and the situation between coexistence and symbiosis. And let this balance extend from the whole to drive partial to form a new dynamic balance (this is a big black box), also known as reinforcing the vital essence and strengthening the primordial Qi. This "essence" and "qi" has a great deep knowledge, involving the driving force of life. Whether it can be explained by biological "entropy" remains to be further explored and studied in the future. Judgments on diseases in Chinese medicine may begin with numerous comprehensive information including the dark information. It is also necessary to use the long-term medical experience accumulated by the practitioners, the analysis of the complex philosophical thinking, is somewhat like an integrated balance in the black box. Therefore, different practitioners often bring very different interpretation results to patients, and the treatment effect is certainly not the same. For thousands of years, it has been a pity for the lack of accurate mathematical data to summarize the patient's symptoms and treatment on the use of Chinese Medicine. Some people say that Chinese people are smart and highly savvy. From Laozi, Mozi, Zhuangzi and Confucius, this big group of clever people have accumulated a lot of philosophical theories that have persuaded western scholars, so as to influence and produce this simple harmony between the universe and humans, and the yin, yang and five elements of Chinese medicine theories (and probably the most important benefit from the precious and countless years of practice). However, for a long time, traditional Chinese medicine suffers from the lack of sophisticated detection techniques and tools, lacks mathematical data for the accurate measurement of the patients' symptoms and diagnosis, and reliable results of repeated experiments, thus failing to form a modern Chinese medical system. The essence of many successful cases is also inevitably lost in the passage of time and the alternation of old and new.

The "Qi" and "field of Qi" in Chinese medicine can reflect the interpretation towards the micro-dynamics of life. However, till now, it is not supported by accurate quantitative information or measurable data. How can Chinese medicine big data be achieved and scientific systematization be realized? Who can break an opening for such challenge!

According to recent reports, non-coding nucleic acids are also referred to as hidden substance, which was also proved to be able to regulate the diseased cells. Among the three major elements of the laws of life, i.e., DNA, RNA and protein, the first two are the transmitters of genetic information, while proteins are important products that directly affect the changes of functions in various aspects. The role of these three key elements, either as individual or in coordination, play a key regulatory role in changing cells. All will provide a very broad space for disease diagnosis and treatment, and it will also become an important source for innovation and a focus of attention in the future. Whether it may be worthy of our serious consideration: historically, there are quite a number of examples in Chinese and Western medicine showing that the same disease (of course not all diseases) can be cured by the respective train of thoughts and methods of Chinese and Western medicine, like reaching the same goal by different ways. So can there be places or intersections with scientific commonality in the fight against diseases?

The ancients said the universe and men are integrated. "Universe" contains all the material foundations of nature, while these substances are "captured" and have experienced millions of years of all kinds of information and numerous intricate exchanges of energy. It promotes the diversified inheritance of nature and natural evolution. "Human" is the highest, most mature and substantial evolutionary result of various biological evolutions as occurred in all substances of life.

In the past century, there have been fierce debate between the Morgan Stanley Mendelian theory and the Michurin doctrine in biology. The discovery of genetic material and the genetic code have led to remarkable progress in the theory of genes, and lead to the leap development of molecular biology. However, many practical cases in the Michurin doctrine have not found material evidence or systematically supported by the obvious information for years. In addition, the "Darwinian theory of evolution", which has been widely accepted by the world for a long time, has also been considered by some scholars as lacking a powerful direct argument, especially on evidence based on the level of molecular genetics and DNA.

During the fight for survival in life for both Chinese and Western medicines, whether or not they have certain rules or intersection point that are in common and potentially in unity, and whether there are some "language" barriers that are not yet linked till now. There are many of the world's actual unsolved scientific mysteries and examples of different methods leading to the same result. Only by continuously resolving the small black boxes, the mystery in the big black box can be further revealed step by step.

Thinking of the lead of the top 3 treasures among 13 million treasures from the world in the British Museum: This is a defective Rosetta Stone from Egypt [6]. Its preciousness lies in being the world's only stele engraved with inscriptions with already extinguished ancient Greek texts, ancient Egyptian texts, and human-readable texts while the content is the same. It allows linguists and archeologists to decode the character structures of these ancient civilizations, and becomes a bridge of communication in the translation of various languages. It is of great merit and its contribution to the history of human evolution is immeasurable.

Nowadays, among the massive amount of big data of life science information, how to grasp the key points and grasp the transformation point of information, especially by closely monitoring various receptors, enzymes, ion channels, and, proteins related to the maintenance of immunity during various changes of functions. In other words, all key parameters of each change should be identified corresponding to the molecular structure within the cell and the target sites of various functioning therein. Through these expression languages that may be non-common (or non-homogeneous), the bridge for information exchange and the approach for information transformation would be exploited. For sure, over-accumulation of large amounts of big data in in some fields should be prevented when collecting all the big data. It may cause the "blinds touch elephant" in the new era and mislead the emergence of the truth. The complex structure of organisms and its various target sites should be well known. All parameters and information sources for representative changes must be objectively and comprehensively identified. Doing so would allow human beings truly obtain the natural habitat where the universe and men are integrated [7], receive the love and care for the precise personalized medical system, and stop diseases at the earliest stage.

Let human beings and nature become more harmonious, and life more pleasurable!

【1】 Translated from Baidu Baike: The human genome project (HGP) was first proposed by American scientists in 1985 and officially launched in 1990. Scientists from the United States, Britain, France, Germany, Japan, and China joined this $3 billion human genome project. As originally planned, the coding of about 25,000 genes in the human body should be completely revealed, and the map of human genes should be ready by 2005. In other words, it is to unlock the secret of 3 billion base pairs that make up 25,000 genes in the human body. The Human Genome Project, known as the "moon landing plan" of life sciences, are regarded as three major science programs together with the Manhattan Atomic Bomb Program and the Apollo Program.

【2】 https://www.mycancergenome.org/content/molecular-medicine/overview-of-targeted-therapies-for-cancer

【3】 Zhao Y, Li H, Fang S, Kang Y, Wu W, Hao Y.. Chen R. NONCODE 2016: an informative and valuable data source of long non-coding RNAs. Nucleic Acids Res. 2016；44:D203-208.

【4】 Chen Jin Xiong October 2015, “Hulianwang + yiliao jiankang: maixiang 5P yixue shidai” [Internet + Medical Health: Towards the 5P Medical Era]. ISBN: 9787121272240.

【5】 Loss of gene methylation is an important cause of carcinogenesis: it will provide a new way to treat cancer. 2017, 02, 27. 10:20:40 Sina summarized the report that published on “Nature” journal on 2017, 02.

【6】 (a) Gaston Wiet , december 1799: Journal historique du Capitaine Bouchard: Editions de la Revue du Caire Lachute d'El-Arich,1945.

(b) Philadelphia. Report of the committee appointed by the Philomathean Society of the Univesity of Peunsylvania to translate the inseription on the Rosetta stone University of Pennsylvania, 1858.

(C) Adkins, Lesley, The keys of Egypt: The obsession to decipher Egyptian hieroglyphs, Harpercollins, 2000.

【7】 "Tianrenheyi [Integration of universe and men]" See Baidu Baike Encyclopedia item: The concept of the integration of man and universe was first developed by the Taoist thinker Zhuangzi as a philosophical system of harmony between universe and man and thus constructed the main body of Chinese traditional culture. The universe and nature constitute the big world, and people constitute the small world. People and nature are intrinsically connected.

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金雞啼鳴 - 關於精準醫學的遐思

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關鍵詞：精準醫療，人類基因組，量化的中醫學大數據，靶向治療。

【摘要】2017年年農曆雞年初，筆者就精準醫學的現狀和過去幾十年的發展作出回顧，並就一些初步的假設和設想進行延伸討論，以作拋磚引玉。中醫是人類大健康醫療體系中不可缺少的重要組成部分。中醫從宏觀的“天人合一”的理念，運用了樸素的哲理思維發展成以辨證論治作指導的有豐富實踐經驗的醫療體系。最重要的問題仍然是如何通過現代精準科學技術和測試方法，進一步將寶貴的醫療案例和無數實踐經驗融入到人類生命的大數據裡。如何找出符合中，西方醫學要求的量化數據或有共性的數學模式，是對我們的一大挑戰。我們需要為新生的智能健康醫學打下基礎，使生態，健康和醫療高度結合，從而使人類和大自然之間更加和諧。

人類生命中的生，老，病，死是地球生物不斷向前發展的最重要的過程。上世紀末，由美國啟動了人類健康的“登月計劃”，花了數十億美元開始了人類全基因的測序（Human Genome Project，HGP）【1】，並在本世紀初完全的完成了這一壯舉。人們從中認識到精準醫療對人類健康，特別是癌症治療上的重要性，必要性和可行性。

只有當由基因突變導致的某些細胞癌變在分子基因的層面得到診斷，再找到靶點，才能夠透過使用針對性的靶向藥物（特別是生物藥）進行準確指導性給藥，來達到良好的治療效果。目前國際上已有近百種被開發出的靶向性抗癌藥物在臨床上應用【2】。

當然，至今人們對精準醫學（Precision Medicine）的概念仍然存在很多不同的看法，同時精準醫學也還在不斷完善之中。新醫療體系只有在跨學科的領域里高度濃縮才能形成。這個體系包括了人類分子基因測序的技術，個體基因與大數據信息科學的綜合分析，精確的個性化治療等，並協同加入到臨床診治的應用，把組學與疾病，健康與環境都互相聯繫起來。看來，只把精準醫學簡單看作基因測序和靶向治療是不夠全面的。

美國近年來所提出的精準醫學在中文翻譯上，只能用精準醫學來作為專有名詞。這樣，“精準醫學”和作為形容詞的“精準的”醫學在內涵有相當不同。Precision Medicine（精準醫學）目前在國人裡比較多把目光注視在分子基因的測序方面，但疾病發生時DNA以外能引起分子和細胞中各個層次變化的大量信息，我們還是未能完全監測出來。的確，“精準”這字在國內現在是特別的吃得開，在中文裡是代表精細，準確，是最高級的代名詞。因此什麼精準會計，精準家內設計，精準裝修，精準手術……　“精準”兩個字一時間到處滿天飛，也成了現代化的代表和最喜歡使用的時髦名詞。

而醫學界方面大部分的工作者也會把解決疾病的著眼點集中在分子基因的層面上。因為“二代測序”無疑為人們打開了很大的一面窗戶，給人們找到了不少DNA層面的突變的遺傳信息和治療的靶點，功不可沒。但問題又來了，這些測序是否就能代表精準醫學的總體性結果呢。根據有幸親自參加了世界人類基因組測序研究的國人學者陳潤生院士重要的提示：通過完成了全人類基因組的測序，在人類的遺傳編碼中，編碼蛋白質的傳統基因只佔3%，另外97%中蘊含的主要規律以今為止還沒有被發現。也就

是說現在我們只是讀懂了人類基因信息其中的3%，而有97%被我們稱之為暗信息的非編碼部分還未被解讀。因此我們要清淅地理解到：我們如只是通過DNA的信息來解讀疾病還是遠遠不夠，我們還需要根據生命的法則把同時參與傳遞信息的RNA及蛋白質以及其他的各種多面的暗信息或各層面的參數來共同解讀各種的生命現象，去理解生物的自然生理和病理變化的動態過程【3】。當然各種關鍵的參數，可能不少是目前我們尚未掌握的暗信息或尚未整合到各種疾病的診治過程中，我們還缺少檢測暗信息和這些新參數的方法和手段，存在大量未解之迷。所以我們對精準這個字也要格外小心。也說明擺在我們面前的又是多麼巨大的挑戰！這樣，機遇難道不是也伴隨在其中嗎！

近來越來越多的學者推行近代醫學5P模式：（1）Preemptive- 預防性;（2）Predictive - 預測性;（3）Personalized - 個體化;（4）Participatory - 參與性; 再加上 5）Precision - 精準性，也就是通常所說的精確到基因和分子的層面。這5P醫學模式組成了比較完整的精準醫學概念（精準醫學）【4】。在這當中，我們不難看出中國 的中醫正是5P中（1）預防性，（3）個體化，（4）參與性的先行者和老祖宗。

上千年來，傳統的中醫把望、聞、問、切實施到每一位患者，並把個性化，個體參與醫治過程貫徹到每一位病者身上。也把藥的種植，藥的採集節令，泡製的方法，服藥的時辰等都常有不同的做法。那怕只是簡單的傷風感冒，卻有因人而異的寒、熱、虛、實的症候之分。患者也要把每次服藥後個人的內在的感受提供給醫者，以便作方劑的加減依據。而在針炙時要得到患者協助提供針炙所需的“得氣”，來調整扎針的深淺及方向。病人通過治療，再提供實際個人的相關感受亦部分幫助醫生調整方劑用藥和加減劑量。直接參與和醫生互動治療的過程，突出了個性化和參與性的優勢。

在癌症的分子基因治療上，西藥講究對準病患的直接靶點，這個靶點目前一般是從DNA的測序分析的明信息中，通過大數據的分析所直接得到的結果。往往靶點的形成反映出該處分子鏈上的結合部易於突變的現象，其中例如DNA周圍甲基化的的失常，會引起DNA的退化發生，導致蛋白的變異。一旦DNA自我修復能力失效時，這種錯誤只能應用DNA編緝來作補救治療（也就是現在通常講的基因治療，但那些導致甲基化失常的體內因素並不見得因此而解決）【5】。

但在中醫治療上，中藥講究調節病患者體內的陰陽五行總體平衡。並通過陰陽五行形像地表達出人體內部多元化、多維性（是否也可以理解為多層面的“靶點”）複雜

的矛盾 ：其中還有相生、相剋的交錯; 而又共存、共生的態勢。並讓這個平衡可以從總體延伸到帶動局部形成新的動態平衡（這是一個大黑箱），也稱作固固培　元。這個“本”和“元”是大有深度的學問，牽涉到生命中的原動力。是否能用生物的“熵”來解釋，還有待將來更深入的探討和研究。中醫對於許多病症的判斷可能始於眾多綜合的信息，其中也包括暗信息。更要運用醫者長期積累的醫案經驗，複雜哲理思維的分析，有點像在黑箱裡的綜合平衡。因此不同的醫者往往給患者帶來很不一樣的解讀結果，治療的效果當然也不一樣。可惜的是，數千年來中醫對病患的症狀和醫治缺乏可以準確量化的數學數據作總結。有人說中國人聰明，悟性高。從老子、墨子、莊子、孔子一大批高人中已積累很多使西方學者折服的哲學理論，從而影響並產生了這種樸素的天人合一，陰陽五行的中醫理論（也可能最重要是得益於幾千年無數實踐的寶貴結晶）。但由於長期以來，中醫中藥苦於沒有足夠精細的檢測技術和工具，對病患的症狀和診治缺乏可準確測量的數學數據和重複實驗的可靠結果，因而未能形成現代化中醫學的體系。不少成功的個案精華也難免在歲月的流逝和新舊交替中痛失。

中醫的“氣”和“氣場”是可以體現出對生命的微動力學的解讀，但至今仍無法得到準確量化的信息和可測性數據來支撐。中醫藥如何能取得大數據，以及如何實現科學系統化？這個挑戰到底誰能打開缺口！

根據近來的報導，非編碼核酸也被稱為暗物質，也證明是可以調控病變細胞。在生命法則中的三大要素DNA、RNA和蛋白質中，前二者是遺傳信息的傳遞者，蛋白質則是直接影響各方面功能變化的重要產物。這三個關鍵要素的各自作用或協調作用對細胞的改變都起著關鍵的調控作用。全部都會對疾病的診斷和治療提供了非常廣闊的空間，也將是未來創新的重要泉源和要關注的重點。我們是否值得認真的思索：在中西醫過往的發展中，有不少的實例都向人們展現了相同的病症（當然不是所有的疾病）能根據中西醫各自的思路和方法同樣得到治愈，有點異途同歸之感。所以在對疾病的鬥爭中是否能找到有科學共性的地方或相交點？

古人云：“天人合一”。“天”含有大自然所有的物質基礎，當這些物質在“獲能”後再經歷了億萬年的各種信息和能量的無數複雜的交流，促進了自然界多元化的傳承與進化。“人”則是生命所有物質中產生的各種生物進化體中的最高級，也是最成熟偉大的進化結果。

上世紀生物科學界曾發生過摩爾根－孟德爾學說和米丘林學說兩大學派激烈的爭論遺傳物質和遺傳密碼的發現使基因的學說得到長足的發展，也領引分子生物學飛躍地發展。但是，米丘林學說中的很多實踐案例多年來一直未找到物質證據或系統性的明信息支撐。另外長久以來，已被世人較廣泛的接受的"達爾文進化論"也被部分學者認為 缺乏有力的直接的論證，特別是在分子基因和DNA層面的證據。

中西醫藥在求生的鬥爭中，某些法則是否有共同法則或交匯點，是否有統一的可能性，它們之間又是否存有目前還未相通的"語言"障礙。世間中很多實際存在未解的科學迷團和各種異途同歸的實例，都有待通過不斷解決當中一個個小黑盒，而一步步揭示大黑箱其中的奧秘。

聯想到英國倫敦大英博物館來自全世界1300萬的珍藏品中的三件鎮館寶物之首：這是一塊缺損的埃及羅塞塔石碑（Rosetta Stone）【6】，其珍貴之處在於這個石碑是世上唯一在上面同時刻有已失傳的古希臘文字、古埃及文字，以及現在人能讀懂的文字三種銘文文字，而且內容相同，使語言學家和考古學家能找到破解這些古代文明的文字結構，成為各種語言轉譯中溝通的橋樑，居功至偉，在人類進化史的貢獻是無法估量的。

現在，在生命科學信息的海量大數據裡，如何抓重點，抓信息轉化點，特別在各類功能變化中緊盯著各種受體、酶、離子通道，和維持免疫功能的相關蛋白質換。句話說，就是同時對細胞裡的分子結構和其中各種功能的靶點都找出各主要變化的參數，通過這些可能是非共性（或非同種）的表達語言去尋找各種不同信息交匯的橋樑和轉譯方式。當然，在收集所有的大數據時，特別要防止某些領域中過多堆積海量的大數據。那是有可能會引起新時代的"盲人摸象"而誤導了真相的浮現。當我們要弄清生物體複雜的結構和其中各種"靶點"，就一定要客觀的、全面地找出有代表性變化的各種參數和信息來源。使人類真正得到天人合一【7】的自然生態和精準個性化醫療體系的關愛，在最早的階段攔截疾病。

讓人類和大自然更加和諧，生命更為康樂！

【1】見百度百科：人類基因組計劃（human genome project，HGP）是由美國科學家於1985年率先提出，於1990年正式啟動的。美國、英國、法國、德國、日本和我國科學家共同參與了這一預算達30億美元的人類基因組計劃。按照這個計劃的設想，在2005年，要把人體內約2.5萬個基因的密碼全部解開，同時繪製出人類基因的圖譜。換句話說，就是要揭開組成人體2.5萬個基因的30億個鹼基對的秘密。人類基因組計劃與曼哈頓原子彈計劃和阿波羅計劃並稱為三大科學計劃。被譽為生命科學的“登月計劃”。

【2】https://www.mycancergenome.org/content/molecular-medicine/overview-of-targeted-therapies-for-cancer

【3】Zhao Y, Li H, Fang S, Kang Y, Wu W, Hao Y.. Chen R. NONCODE 2016: an informative and valuable data source of long non-coding RNAs. Nucleic Acids Res. 2016；44:D203-208.

【4】作者：陳金雄，互聯網叢書“互聯網十醫療健康”-邁向5P醫學時代。出版時間2015-10. ISBN:9787121272240.

【5】基因甲基化缺失是致癌重要成因：將為治療癌症提供新途徑.2017,02,27。 10:20:40新浪綜合這項研究結果刊登在2017，02出版的《自然》雜誌上。

【6】(a) Gaston Wiet , December 1799: Journal historique du Capitaine Bouchard: Editions de la Revue du Caire Lachute d'El-Arich,1945.

(b) Philadelphia. Report of the committee appointed by the Philomathean Society of the Univesity of Peunsylvania to translate the inseription on the Rosetta stone University of Pennsylvania, 1858.

(C) Adkins, Lesley, The keys of Egypt: The obsession to decipher Egyptian hieroglyphs , Harpercollins, 2000.

【7】“天人合一”見百度百科詞條：天人合一的思想概念最早是道家思想家莊子發展為天人合一的哲學思想體係並由此構建了中華傳統文化的主體。宇宙自然是大天地，人則是一個小天地。人和自然在本質上是相通的。

鄭勳華 博士 草於2017年1月12日廣州，

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Chapter 13

健港生命科技国际有限公司

Kaizhi Chen, Shyun-hua Zheng, Keith K.H. CHAN

海納百川，有容乃大

人类繁演生息、生命的延续受到地球上生物物种的自然残酷竞争。大自然及人类之间的种种灾难有甚如瘟疫的肆意横行。在数千年的人类发展史 中，中医药始终伴随着龙的传人，把国人的生命长久维持在全球第一。这其中传承的中医学和养生原理非常值得探讨，其中的精华值得发扬光大并融入到现代医学科学的大家庭中去，从而为普罗大 众提供更多安全有效的治病和养生之道。

健港生命科技国际有限公司（简称"健港公司"或"健港"，英文名称Main Harbour Biotech International Limited, 缩写为MHBIL）是专业从事药物研发，特别是植物药开发、转化的公司，其使命是为人类的大健康服务。为了该使命，健港公司重视国际上志同道合的各种专业人才和各种专业的科学家，大家汇聚在一起、拿出智慧和一生的成就、为共同的目标努力。发展是第一要务，人才是 第一资源，创新是第一动力。强大要靠创新，创新要靠人才，健港有"海纳百川，有容乃大 " 的胸襟。健港就象一个国际大港，可以容得下各类的专业人员，热情欢迎志同道合的人才。如今，健港公司拥有越来越多国际一流的科学家，自有的知识产权多而强大。健港的 项目在稳步推进，合作伙伴越来越多，在不久的将来会有规模地出现在国际生 物科技领域。

在公司高层管理、生物研究、专利保护、法律顾问等方面，健港公司现已配置完善。我们是研究开发药物（特别是植物药），以及天然植物为主的健康食品的生物科技公司。首先是研究并发现有生物活性成份的药源，其次要做临床前期的各种安全性研究，然后申报新药临床试验申请 (Investigational New Drug, IND) 并获得批准后进入更为艰难、风险更大的试验阶段，其后是药物上市审批的申报流程。众所 周知，一个药物从研发到成功上市是非常耗时、耗资、风险极大的。我们是否准备好了？是否有充足的信心面对未来的挑战？ ！

健港公司拥有人才优势、研发实力优势、众多独立自主的知识产权优势，有在国际、中国的人脉和社会资源优势。

2004 年，美国食品药品管理局 (Food and Drug Administration, FDA) 成立了植物处方药的审批渠道，至今十六年间只有两个药品获得了上市批准。中药最大的困难是品质管控 (Chemistry, Manufacturing and Control, CMC)，原因是其有效成分不明、药物一致性难以控制，所以很难设定质量标准。要把中药的地位提升到西药相同的标准、一样的 认同，新的研究手段是必要的。

西药的特点之一是有效成分的定质定量。中药的品质一定要达到有效成分的定质定量才能够获得国际认可。针对这个目标，我们的研究团队在 2007 年发明了研究药物的药学 平台科技 (Pharmaceutical Platform Technology, PPT)。我们的核心技术药学平台发明专利是目前世界上唯一的可以在复杂的混合物（如中药植物）中找到一组有效成

分（并且有一定比例和质量）的手段。

西药的开发是由药效驱使的，但是有许多有效的品种往往不能够开发为好的药物。一个有用的品种必须具有优化的药动性能，例如适当的吸收、分布、代谢和排泄，才能够容许药物在人身内有足够的停留时间和浓度，从而建立疗效。随着科技的进展，药效和药动都演变成开发西药的核心指标。

西药的研发往往集中在一个单体和一个靶点上。但是，把西药的科技套用到中药开发会有一定的难度，因为中药的特点是讲究多靶点和多种有效成份之间的相互作用。一个棘手的问题是，现在还没有适合的理论或计算方法来解决多个有效成份的相互作用。例如，假设一个中药包括 100个化合物（对于中药来说这是一个小数目），里面就会有 5,050 个双体相互作用、161,700 个三体相互作用、376,437,600 个四体相互作用。这个例子尚未计 算五体相互作用，就已经有近四亿的相互作用数据。

上述数据量实在惊人！一个简单的中药开发，资源和时间的消耗不一定合算， 而且数据处理也是一个难题。因此，有些科学家觉得中医药现代化是不可能的。我们以开发西药的药效和药动技术为基础，发明了一个开发中药的技术，称之为药学平台。这个药学平台利用体外、硅片和统计的旋回筛选方法，使我们可以在大量的中药化合物中找到多靶点的多种有效成份。

在中国，中药已经有了几千年的历史，中医药产业也是中国的传统优势产业。作为中国医药大健康产业链中重要的一环，中医药产业在国家一系列扶持政策驱动下不断发展壮大，逐步凸显出了战略地位。

我们的一个技术团队从植物中发现了用于治疗癌症的活性成分，可以在抗癌药物领域中开发具有新治疗机制的、低毒性的治疗药物，并开发出解决癌细胞抗药性的 一线药物。

我们有超过 20年的新药开发经验，强项是发现、鉴定、分离、改善和合成抗癌皂甙化合物。我们已鉴定了近 100,000 个新化合物，并建立了进一步有效合成 和鉴定候选药物的平台。我们拥有 30 余项专利，并将会在研发数百种新化合物的进程中不断产生更多的新专利。

我们已有三个全天然植物药正在准备向美国 FDA 申报 IND 。

（一）卵巢癌的抗癌药物（发明专利已授权）：卵巢癌是女性发病死亡率最高的癌症，被称为“沉默的杀手”。卵巢癌在盆腔里，发病前没有征兆，80%的患者被发现时已到了晚期，失去了手术的机会。经一线药物治疗后的复发率亦是最高。 我们在美国德州的团队从数以万计的天然皂苷中筛选出有效分子治疗癌病，最终从一种灌木植物的萃取物中发现了天然的抗癌皂苷，其中一个化合物 (代号 MH-5962-OC) 对卵巢癌

具有明显的抗癌细胞作用。这个天 然皂苷提取物 (MH-5962-OC)可以抑制卵巢癌细胞增长，阻止肿瘤血管生成以杀死癌细胞。我们的研究结果表明，该天然皂苷提取物能降低卵巢癌细胞至少80%的侵略性。接种了 肿瘤的小鼠用药十天后，肿瘤明显缩小了45%。其作用机理是通过增加血管生成素 (angiopoietin-2, ANGPT2)的合成，抑制了肿瘤血管的生成。实验证明，天然皂苷提取物 (MH-5962-OC) 可使 ANGPT2增 加 300%，抑制肿瘤血管的生成。

（二）粘连蛋白的抑制剂（发明专利已授权，代号 MH-3031-GC）：癌细胞转移是恶性肿瘤的主要特征，是癌症患者防不胜防、引发死亡的首要因素。癌细胞转移涉及癌细胞所处的微环境中的复杂通路，其中最重要的是癌细胞制造的粘连蛋白质在表达过程中加强了自我转移扩散的有利环境。因此，克制癌细胞粘连蛋白质的表达来防治癌细胞转移是最新的针对治疗方法。

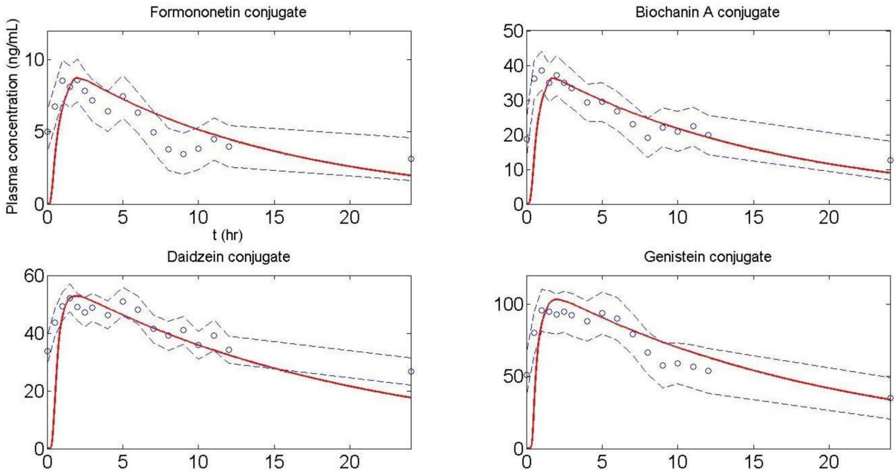
国际知名的药企葛兰素公司 (GSK) 和罗氏药业 (Roche) 都投入巨资试图攻克这一难题，葛兰素史克的临床药物通过抑制粘连蛋白质来治疗胃癌转移扩散，其A轮融资数额达千万美元，投资者包括罗氏药业和其它国际大型药企。从这些大型药企的开发动向，我们更能肯定这个方向正确。

我们开发的粘连蛋白质抑制剂效能更广，可有效抑制 (FAK 蛋白)， 并可更有效地抑制 (纤维连接蛋白 (fibronectin)) ，为混合治疗建立基础。葛兰素史克和罗氏药业开发的是化学药，而我们开发的是天然皂苷药物，可应用在多类产品。 粘连蛋白质抑制剂拥有庞大的 市场需求，因其不受癌症种类限制，有广泛的适应症。

再者，目前用于治疗癌细胞转移的药物(例如 Cisplatin、Docettaxel、Doxorubicin、Hydroxyurea等) 都有非常强的毒性，会对患者产生无法恢复的损伤。因此，我们愈发认为开发天然皂 苷药物刻不容缓。

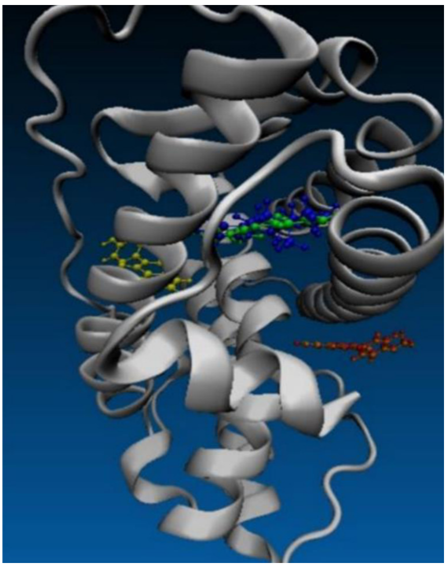
（三）药物代号MH-1341-OD，针对治疗妇女绝经后子宫萎缩、干枯（发明专利已授权）：

我们对 MH-1341-OD的信心建立在几个关键的发现。其中之一是吸收率太低，病人服用药物后不仅血液浓度低而且参差不齐、效果不一、疗效率低。另外一点是我们成功地预测了病人的有效成分血液浓度 (见图一)；因此，我们可以准确预测有效成分的剂量，该数据对临床试验非常重要。天然的有效成份比例跟相互相成的配比是不一样的，数据显示 SCI-1341的一组有效成份会比天然配比更加有效。所以，要把 MH-1341-OD 开发好，就需要重新设计新的剂型和输送渠道。



图一：PPT的药动模型预测病人服药后有效成份的血液浓度（红线）。O是病人 的有效成分的血液浓度。

经过初步研究， MH-1341-OD 不单没有致癌的副作用，还有治癌的疗效（见图二）。这个特性是 MH-1341-OD 的优势。



图二：有效成份跟女性荷尔蒙的受体结合不 一样，所以下游的作用就不相同了。

药物MH-1341-OD针对适应症为子宫萎缩、干枯、防癌病变。健港公司技术团队已经带领专家组工作，预计 2021年上半年通过美国 FDA 的 IND 认可。成功开发 MH-1341-OD并获得美国 FDA 的草本处方药许可后，该药物可用来开发多种相关的妇女收经药品。治疗妇女收经的药物全球市场份额约为 220 亿美元。

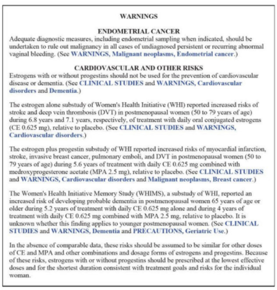
我们计划首先开发治疗阴道萎缩的产品，其全球市场份额约 16 亿美金。目前市场上的产品有毒性，缺乏安全性 (见图三)。现有竞争者的产品副作用是导致乳癌和子宫癌。在MH-1341-OD的第一个品种成功后，可继续开发四个有关更年期妇女收经的处方药，全部产品国际市场总份额约225 亿美元，经济效益更高。

我们有强大的市场合作空间，“药物平台+CRO” 是我们发展创新药、扩大与外界合作的最新科技。在中国，中药治疗有几千年的历史，中国人对植物药有深度的认识和经验。遗憾的是，日本汉 方药占世界市场 90%，而中国仅占 5%。我们没有理由不去努力改变这个现状，这是很重要的 责任。

关于中药二次开发，2019 年10 月的报导钟南山等 4名院士与白云山药业合作，十七年共同研究再开发扳蓝根药物，对其中四个物类比例作了改变，使药效提高了5至 6倍。据钟南山院士说，中药二次开发是中药走向现代化的一种重要的方法。

健港的“药物平台+CRO”正是开发新型植物药、科学化健康食品、做好中药二次开发的最有效的科学技术。中国的中成药、各种饮片、各种健康食品每年的市场份客达几千亿，但目前均存在质量问题。中国药监局停用了大量的中药针剂，每年造成一千多亿的市场损失。历史的原因造成许多中药针剂的药物数据、临床数据无法完善，至今都无法解决。这些也是下一步的发展机遇，是健港的科学家运用核心科技取得成果的机会。

倍美力阴道药膏
US$500M (2015)



WARNINGS

ENDOMETRIAL CANCER

Adequate diagnostic measures, including endometrial sampling when indicated, should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. (See **WARNINGS, Malignant neoplasms, Endometrial cancer**.)

CARDIOVASCULAR AND OTHER RISKS

Estrogens with or without progestins should not be used for the prevention of cardiovascular disease or dementia. (See **CLINICAL STUDIES** and **WARNINGS, Cardiovascular disorders** and **Dementia**.)

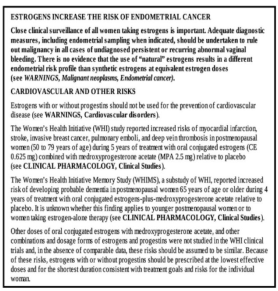
The estrogen alone substudy of Women's Health Initiative (WHI) reported increased risks of stroke and deep vein thrombosis (DVT) in postmenopausal women (50 to 79 years of age) during 6.8 years and 7.1 years, respectively, of treatment with daily oral conjugated estrogens (CE 0.625 mg), relative to placebo. (See **CLINICAL STUDIES** and **WARNINGS, Cardiovascular disorders**.)

The estrogen plus progestin substudy of WHI reported increased risks of myocardial infarction, stroke, invasive breast cancer, pulmonary emboli, and DVT in postmenopausal women (50 to 79 years of age) during 5.6 years of treatment with daily CE 0.625 mg combined with medroxyprogesterone acetate (MPA 2.5 mg), relative to placebo. (See **CLINICAL STUDIES** and **WARNINGS, Cardiovascular disorders** and **Malignant neoplasms, Breast cancer**.)

The Women's Health Initiative Memory Study (WHIMS), a substudy of WHI, reported an increased risk of developing probable dementia in postmenopausal women 65 years of age or older during 5.2 years of treatment with daily CE 0.625 mg alone and during 4 years of treatment with daily CE 0.625 mg combined with MPA 2.5 mg, relative to placebo. It is unknown whether this finding applies to younger postmenopausal women. (See **CLINICAL STUDIES** and **WARNINGS, Dementia** and **PRECAUTIONS, Geriatric Use**.)

In the absence of comparable data, these risks should be assumed to be similar for other doses of CE and MPA and other combinations and dosage forms of estrogens and progestins. Because of these risks, estrogens with or without progestins should be prescribed at the lowest effective doses and for the shortest duration consistent with treatment goals and risks for the individual woman.

雌二醇阴道片
US$450M (2015)



ESTROGENS INCREASE THE RISK OF ENDOMETRIAL CANCER

Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures, including endometrial sampling when indicated, should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is no evidence that the use of "natural" estrogens results in a different endometrial risk profile than synthetic estrogens at equivalent estrogen doses (see *WARNINGS, Malignant neoplasms, Endometrial cancer*).

CARDIOVASCULAR AND OTHER RISKS

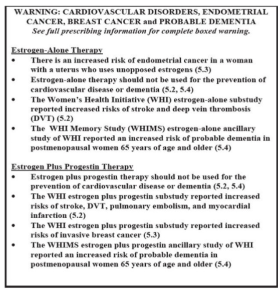
Estrogens with or without progestins should not be used for the prevention of cardiovascular disease (see **WARNINGS, Cardiovascular disorders**).

The Women's Health Initiative (WHI) study reported increased risks of myocardial infarction, stroke, invasive breast cancer, pulmonary emboli, and deep vein thrombosis in postmenopausal women (50 to 79 years of age) during 5 years of treatment with oral conjugated estrogens (CE 0.625 mg) combined with medroxyprogesterone acetate (MPA 2.5 mg) relative to placebo (see **CLINICAL PHARMACOLOGY, Clinical Studies**).

The Women's Health Initiative Memory Study (WHIMS), a substudy of WHI, reported increased risk of developing probable dementia in postmenopausal women 65 years of age or older during 4 years of treatment with oral conjugated estrogens-plus-medroxyprogesterone acetate relative to placebo. It is unknown whether this finding applies to younger postmenopausal women or to women taking estrogen-alone therapy (see **CLINICAL PHARMACOLOGY, Clinical Studies**).

Other doses of oral conjugated estrogens with medroxyprogesterone acetate, and other combinations and dosage forms of estrogens and progestins were not studied in the WHI clinical trials and, in the absence of comparable data, these risks should be assumed to be similar. Because of these risks, estrogens with or without progestins should be prescribed at the lowest effective doses and for the shortest duration consistent with treatment goals and risks for the individual woman.

雌二醇阴道药膏
US$420M (2015)



WARNING: CARDIOVASCULAR DISORDERS, ENDOMETRIAL CANCER, BREAST CANCER and PROBABLE DEMENTIA

See full prescribing information for complete boxed warning.

Estrogen-Alone Therapy

- **There is an increased risk of endometrial cancer in a woman with a uterus who uses unopposed estrogens (5.3)**
- **Estrogen-alone therapy should not be used for the prevention of cardiovascular disease or dementia (5.2, 5.4)**
- **The Women's Health Initiative (WHI) estrogen-alone substudy reported increased risks of stroke and deep vein thrombosis (DVT) (5.2)**
- **The WHI Memory Study (WHIMS) estrogen-alone ancillary study of WHI reported an increased risk of probable dementia in postmenopausal women 65 years of age and older (5.4)**

Estrogen Plus Progestin Therapy

- **Estrogen plus progestin therapy should not be used for the prevention of cardiovascular disease or dementia (5.2, 5.4)**
- **The WHI estrogen plus progestin substudy reported increased risks of stroke, DVT, pulmonary embolism, and myocardial infarction (5.2)**
- **The WHI estrogen plus progestin substudy reported increased risks of invasive breast cancer (5.3)**
- **The WHIMS estrogen plus progestin ancillary study of WHI reported an increased risk of probable dementia in postmenopausal women 65 years of age and older (5.4)**

图三: 市场竞争产品及其市场。每个 产品的年销量大概是五亿美元。

我们的科学家团队分别于 2018 年、2019 年到达广州、贵州、云南等地参与科技路演和交流。贵州医科大学、贵州苗药研究中心、云南科技厅都组织了生物科技研 究所和多家药企业， 一起分享了我们的科技成绩。当地药企对于合作项目、研究开发药物和健康食品的意愿十分強烈。我们发现很多药企投资大、花费时间长，但是产品的科技性差、效果不明显。他们希望和我们共同合作帮助深度的开发现在的中成药和健康食品，提升品质和药效，让产品更加科学化。在与各研究机构、药企交流的过程中，他们表达了产品需要更加完善的知识产权保护。知识产权保护是健港公司的优势：健港不仅可以参与共同研发已有知识产权保护的产品，还可以教导科技研发，协助撰写和提交中国和国际的知识产权申请。陈伟杰律师（病毒学博士、法学博士）是我们的外部法律顾问，他在云南知识产权局做了演讲，同时我们向当地赠送了一批关于知识产权保护的书籍。健港有能力为国内药企提供全面的服务，在合作中成长为最受欢迎的合作者。

研究植物药，基础性的第一步是标准化种植（GACP）。中药研究的核心问题之一是保证药物的一致性，即保证质量可控（CMC），这是目前中药走向世界、科学化的最关键问题。选种 培育、种植、收割、提取技术等一系列环节的科学化是至关重要的。日本的一个汉方药企在中国拥有七十多个标准化种植中药基地，相比之下，中国的同仁堂只有七个基地。标准化药材种植是科学化的前提：标准化可提供合格的原材料，能保证药物的安全性、有效性。健港的第一步行动会是药物的标准化种植。在这个领域，我们有国际上知名的、富有经验的专家支持， 并已经初步展开工作。

中国管理科学研究院（由企业管理创新研究所与健港生物科技有限公司共建）授权成立“健港生物研究中心”，对健港是极大的肯定与支持！我们将把这个中心办成国际 化的生物研究中心，将会邀请更多的国际一流科学家和生物研究机构，为我们带来一批世界高端生物研究项目。我们会定期在不同地点组织生物科技研讨会。相信会有更多媒体、网络关注报道，更多的资金进入支持我们的研究项目。健港的生物科技会得到大发展。

在大健康的产物开发中，我们希望能为人类多找出更多纯天然的保健品。为此，我们已经做出完整的、独立自主的国际专利的保护，受保护的国家和地区包括美国、欧洲、中国、日本、和亚 太地区。

小结

十数年磨一剑！我们这些炎黄子孙有着龙的血脉，历练数十载，把世界先进的科学集合在一起，结合到人类大健康的伟业。以妇女大健康天然产品作为主导的核心目标，通过 两个植物药、中药开发领域的关键技术平台，进而发展一系列的新药，把大湾区健港作为新的 起点。

我们张开双臂欢迎所有致力于生物研究、致力于为世界人民健康服务的人才加入我们，一同为中国乃至世界人民贡献科学成果，奉献我们的爱心。

健港生命科技国际有限公司
(MAIN HARBOUR BIOTECH INTERNATIONAL LIMITED)

陈开枝董事长 (Mr. Kaizhi Chen, Chairman)

郑勋华（博士）首席执行官 (Sherman Zheng, MD, PhD)

陈桂恒（博士）首席全球战略官 (Keith H. Chan, PhD, FAAPS)

附：核心领导团队 和关键人员照片

Sherman Zheng, MD, PhD

Albert Wai-Kit Chan, PhD, JD

Keith H.Chan, PhD, FAAPS

贵州路演

云南东川考察种植

云南省科技厅路演

Legal Regulation

Chapter 14

CHINESE MEDICINE IN HONG KONG MARKET:

A CHAPTER ON PRODUCT REGISTRATION

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Abstract

The industry of Chinese Medicine (CM) supported the living of many generations of "Old Hong Kong" till now. Both providers and customers of Chinese medicine are benefited from this industry. In Hong Kong, the common usage of CM played a role of supplementary and alternative medicine on top of the main stream Western medicine. As compared to China, the US and Europe, the regulations for CM practice and registration are comparatively loose in Hong Kong. However, as there are more and more health problems that cannot be resolved effectively by using modern pharmacological treatments, e.g. chronic disease due to aging population, many patients are seeking alternative therapies. Obviously, the obsolete standards in quality control for CM could not match up with the new expectations by these new group of patients. In this review, we are focusing our discussion on the current situation of the CM market in Hong Kong, as well as the standards, the registration and the limitation for CM development in Hong Kong and China.

Keywords: Chinese medicine, proprietary Chinese medicine, Hong Kong Chinese Materia Medical Standards

Introduction

In Hong Kong, Chinese Medicine (CM) is commonly used to cure various kinds of diseases or health problems having a long history of record. Although CM usually plays a supplementary role in the local medical and health system, the Chinese Medicine sector comprises of: (i) manufacture of Chinese Medicines; (ii) distribution of Chinese Medicines, including import/export, wholesale and retail trades; and (iii) practicing Chinese Medicine, including Chinese Medicine practitioners (CMPs) of general practice, acupuncture and bone-setting. These industries are classified in accordance with the Hong Kong Standard Industrial Classification (HSIC) Version 2.0.

Manufacture and Distribution of Chinese Medicines

The current establishments engaged in the distribution of CM are classified into three industries according to the nature of distributive services, i.e., (i) import/export of Chinese Medicines; (ii) wholesale of Chinese Medicines; and (iii) retail of Chinese Medicines. In 2015, the number of establishments in the industry of primary manufacture of CM was estimated to be about 210; while the number of persons engaging in the business was 2,240. The total sales of the manufacturing industry amounted to a total of HK$3.92 billion. However, the value added, the summation of compensation of employees and gross operating surplus every year, of this CM industry amounted to HK$1.90 billion, up significantly by 37.1% as compared to the previous year (**Table 1**). In the industry of import/export of CM, the number of establishments was about 540 in 2015. The number of persons engaging in this business was 1,840. The total sales, as well as value added of the industry, amounted to HK$10.64 billion and HK$0.96 billion, respectively, up by 2.0% and 8.0% increase as compared to the previous year (**Table 1**). In 2015, the number of establishments in the industry of CM wholesale was 330, and the persons engaged was 1,270. The total sales of CM wholesale amounted to HK$5.62 billion in 2015, and the value added was estimated to be HK$0.63 billion.

In HK$ million

Industry	Year	Sales	Value added
Manufacture	2013	3,652.1	1,196.6
	2015	3,922.8	1,898.3
Import/export	2013	12,985.7	836.9
	2015	10,637.4	964.5
Wholesales	2013	5,426.3	568.7
	2015	5,621.1	631.9
Retail	2013	6,948.2	665.1
	2015	7,105.8	625.9
All industries	**2013**	**29,012.3**	**3,868.7**
	2015	**27,287.1**	**4,798.9**

Table 1. Manufacture and distribution of CM industry in Hong Kong

Hong Kong Market on Proprietary Chinese Medicine

Currently, the majority of CM products being exported in Hong Kong are low value-added products, such as the CM raw herbs, herbal extracts and other pharmaceutical raw materials. In the past, the development of CM industry was dependant on the raw materials in exchange for a surplus output; however, it will not be the way forward in the future. Today, different countries, including those of the European Union and the US, have increased the quality standards of importing raw materials of CM and, in many cases, the standards are too high to be attained by Hong Kong CM industries. As a result, the upgrading of our standard requirements for exporting CM herbal products should be the inevitable choice for the sustainable development.

The proprietary Chinese Medicine (pCM) is able to achieve much larger profit in general. According to the Chinese Medicine Ordinance in Hong Kong, the pCM means any proprietary product of: (i) composed solely of the following as active ingredients, e.g. CM from herbs or animals; (ii) formulated in a finished dose form; and (iii) known or claimed to be used for the diagnosis, treatment, prevention or alleviation of any disease or any symptom of a disease in human beings, or for the regulation of the functional states of the human body. The total trades of CM products (e.g. pCM, raw herbs, herbal health care products etc.) in the global market has amounted to about US$ 135 billion in 2018. As a major trading hub of CM in the world, Hong Kong plays an important role in CM trading. According to statistics in 2011, values of imports and re-exports of CM in Hong Kong have amounted to HK$ 23.5 billion and HK$ 9.5 billion, respectively.

Trading of Chinese Medicines

Most of the CM handled by local manufacturers and distributors are imported, amounting to HK$3.15 billion. A significant portion of the imported CM was consumed locally. In 2016, the value of retained imports of CM amounted to HK$2.02 billion, down by 21.9%, as compared with that in 2015, at HK$2.59 billion. In 2016, the value of re-exports of CM amounted to HK$1.12 billion, down by 5.6% compared with that in 2015, at $1.19 billion. In 2016, Canada was the top supplier of Hong Kong's imports of CM. The share of imports from Canada was 50.9%, followed by the mainland of China (18.9%), US (7.4%), Taiwan (6.0%) and Korea (4.2%). In 2016, Taiwan took up 19.6% of the value of Hong Kong's re-exports of Chinese herbal medicines, followed by Vietnam (15.6%), Korea (12.3%), US (11.9%) and Mainland China (10.8%) (**Figure 1**).

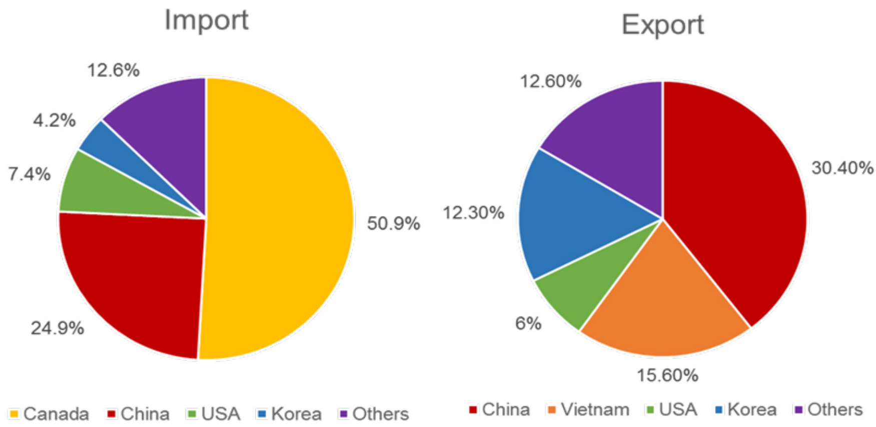


Figure 1. *Percentage distribution of import and export of CM in Hong Kong*

The figure illustrates the trading of CM in Hong Kong market at 2016. CM pure extracts, CM supplements and proprietary Chinese medicines (pCM) are included. Canada supplied most of Hong Kong's import of CM, sharing a percentage of 50.9%. The second largest supplier was China (19.9%),US (7.4%), Taiwan (6.0%) and Korea (4.2%). For export of M, most of the goods (30.4%) were exported to China, 15.6% to Vietnam, 12.3% to Korea, 6% to US and other countries (12.6%).

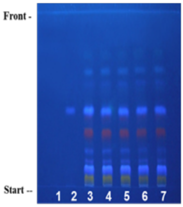
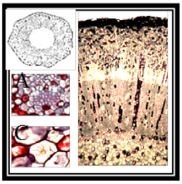
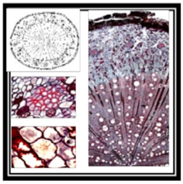
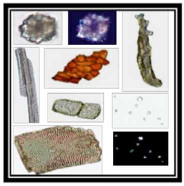
Regulation on crude CM

Due to the global increased usage of CM, the Hong Kong Special Administrative Region (HKSAR) Government has emphasized the quality and safety of Chinese Materia Medica (CMM) being sold in Hong Kong. The Department of Health launched the Hong Kong Chinese Materia Medica (HKCMM) Project in 2002 with the purpose of setting international standards, in terms of safety and quality, for the CMM commonly used in Hong Kong. The HKCMM Standards project covered the CMM with high economic value in the local market, as well as addressing international concern over their safety and quality. In particular, the priority is given to those CM being listed in the Chinese Medicine Ordinance (Cap. 549).

The aims of the development of HKCMM Standards are: (i) to ensure the safety and quality of CMM through providing reference standards to CM industry; (ii) to promote the research on CM; and (iii) to expedite the modernization and internationalization of CM, as well as to facilitate the global trade of CM. The HKCMM Standards can be used by the CM industry as reference for standards of safety and quality of CMM. For example, manufacturers in pCM can employ the HKCMM Standards as a guide for quality control of raw materials, and the CM traders can make reference to the HKCMM Standards for the procurement of CMM. Moreover, HKCMM Standards provides a reliable foundation for the testing and certification industry, in order to facilitate its development of testing services on CMM. The Hong Kong Accreditation Service has already launched the accreditation service to laboratories performing CMM testing and identification by microscopic examination, chemical and physicochemical testing according to the HKCMM Standards.

In accordance with the establishment of HKCMM Standards, the Hong Kong Productivity Council is establishing a Product Certification Scheme for CMM based on HKCMM Standards, which is supported by the panel in promoting testing and certification services in CM trade under Hong Kong Council for Testing and Certification. With the release of the "HKCMM Standards Volume 9" in December 2018, nine editions of the HKCMM Standards covering the standards for a total of 299 CMM have been published today.

In the HKCMM Standards, various tests are employed to explore well-rounded information about the commonly used CMM. In the monograph of CMM, the information includes the morphological characteristics, official name, medicinal part, microscopic illustrations, active compounds etc. **Figure 2** demonstrates an example of monograph that is found in HKCMM Standards. In this example, the CMM adopted is Polygonum Cuspidati Rhizoma et Radix, which is a traditional CMM for the treatment of inflammation, favus and scaldings.

<table>
<tr><td rowspan="4"></td><td>Official Name:</td><td colspan="2">Polygoni Cuspidati Rhizoma et Radix</td></tr>
<tr><td>Chinese Name</td><td colspan="2">虎杖</td></tr>
<tr><td>Species</td><td colspan="2">Polygonum cuspidatum Sieb. et Zucc. (Polygonaceae) .</td></tr>
<tr><td>Medicinal part</td><td colspan="2">the dried rhizome and root</td></tr>
<tr><td>TLC</td><td colspan="3">Microscopic identification</td></tr>
<tr><td>
</td><td>
Radix</td><td>
Rhizoma</td><td>
Powder</td></tr>
</table>

Tests		**Extractives**	
Heavy Metals	Meet the requirements	Water-soluble extractives	not less than 11.0% (cold extraction method)
Pesticide Residues	Meet the requirements		
Mycotoxins	Meet the requirements	Ethanol-soluble extractives	not less than 12.0% (cold extraction method):
Foreign Matter	**Not more than 1.0%**		
Water Content	Oven dried method: not more than 12.%	**Assay**	
Ash	Total ash: not more than 4.5% Acid-insoluble ash: not more than 1.0%	Polydatin+resveratrol ≥1.1%	

Figure 2. *Monograph of Chinese medicine Polygonum Cuspidati Rhizoma et Radix in the Hong Kong Chinese Materia Medica standards. The figure shows a classic monograph found in HKCMM Standards, including official name, Chinese name, species, medicinal part, macroscopic and microscopic identification, thin layer chromatography and HPLC.*

In the HKCMM Standards, the recognized macroscopic and microscopic identification laboratories, chemical laboratories and DNA laboratories are the major places performing the CMM testing. In the macroscopic and microscopic identification laboratories, it is equipped with microscope and stereo-microscope with digital camera system and is mainly employed in the study and record of morphological characteristics of CMM. In the chemistry laboratories, a wide range of chemical analytical equipment can be found. This kind of laboratory is capable of performing qualitative and quantitative analysis of chemical markers in CMM. The high-end chemical analysis technologies, e.g. High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Liquid Chromatography – Mass Spectrometry (LC-MS), act to enhance the accuracy and sensitivity of analytical results. Chemical analysis is a complementary approach for the identification of CMM since it helps to ensure the quality of pCM to an internationally recognized level, such as identifying the active compounds existing in a certain CMM. **Figure 3** illustrates HPLC fingerprinting results of Polygonum Cuspidati Rhizoma et Radix in HKCMM Standards. Polydatin and resveratrol are being identified as the active compounds found in this CMM.

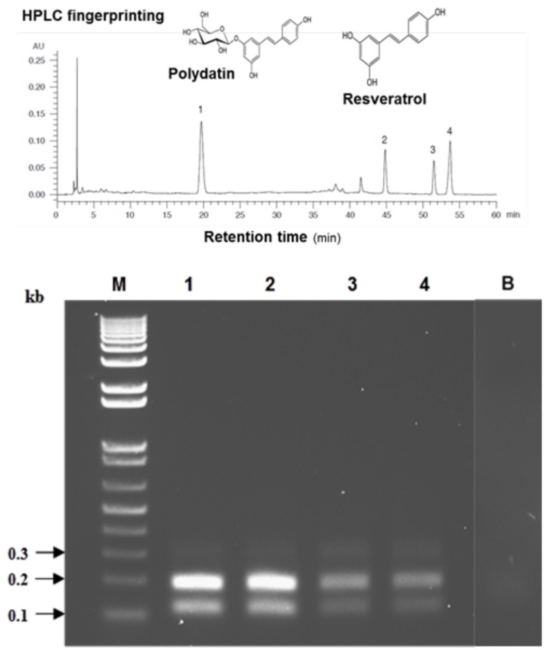


Figure 3. *HPLC chemical fingerprinting and DNA barcoding in HKCMM Standards. HPLC fingerprinting of Polygonum Cuspidati Rhizoma et Radix is being listed in HKCMM Standards (upper panel). The chemical markers of Polygonum Cuspidati Rhizoma et Radix are polydatin and resveratrol, as shown here. The PCR-RFLP pattern of ITS1 region digested with Sma I. M: 1 kb DNA ladder ; B: Blank; 1, 2: Fritillaria unibracteata Hsiao et K. C. Hsia; 3, 4: Fritillaria cirrhosa D. Don (lower panel).*

DNA technologies have been employed to verify the biological and genetic traceability between CMM and their plant/animal species origins in order to ensure the efficacy and safety of the clinical application. In some circumstances, CM does not have specific chemical markers or the CM is deriving from multiple species, therefore DNA analysis is one of the most reliable and suitable methods for species authentication. The herb Fritillaria being listed in HKCMM Standards is an example showing the usage of DNA for authentication (**Figure 3**). The genetic composition of each individual is unique; therefore molecular markers have the advantages in CM authentication, as they will not be affected by age, physiological conditions, environmental factors, harvest, storage and processing. Using a Polymerase Chain Reaction (PCR) technique, nano-gram quantities of DNA can be amplified and yield sufficient amount of template DNA for molecular genetic analysis. After amplification of the region(s) of interest in the genome, subsequent gel electrophoresis is performed to size the PCR products. To date, a variety of PCR-based methods have been developed for the use in CM authentication, including Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP), Random Amplification of Polymorphic DNA (RAPD).

Regulations for pCM in Hong Kong

In order to protect public health and consumers' rights, as well as to ensure the professional standard of CM practitioners and the traders in a "self-regulated" manner, the Chinese Medicine Council in Hong Kong was established under the Chinese Medicine Ordinance (CMO) in 1999. The registration and market activities of pCM are under the surv eillance of the Council and regulated by the Chinese Medicine Ordinance. Before pCM could be imported, manufactured and sold in Hong Kong, they must be registered in the Chinese Medicines Boards.

The arrangement of pCM registration is divided into 2 categories, including transitional registration of pCM, as well as registration of pCM. The former one, with an accordance with sector 128 of the CMO, is applicable to the pCM that is being manufactured, sold or supplied for sale in Hong Kong, on and before March 1, 1999, as denoted with a header of "HKP" (transitional registrated) followed by serial numbers. While the latter one is made in accordance with section 121 of the CMO. For the formal registration of pCM as "HKC", it includes: (1) pCM's Chinese and English name; (2) its dose form; (3) the name and quantity of each of it active ingredient; (4) the name and quantity of each of its excipient (if any); (5) its specification; (6) its indication (if any); (7) its dosage and method of usage; (8) each of its labels to be attached or printed on its package; (9) the package insert to be supplied for its sales inside Hong Kong; (10) each of the package inserts to be supplied for its sales outside Hong Kong (if any); (11) the name and address of each of its manufacturer; and (12) its function or pharmacological action. If there is any alteration in the first three particulars, a new application for the pCM would be required. However, in certain circumstances, the registration of a pCM could be exempted, such as when it is for the purposes of education or scientific research, and for the purpose of clinical trials or medicinal tests and so on. The procedures involved in pCM registration application are illustrated in the flow chart as in **Figure 4.**

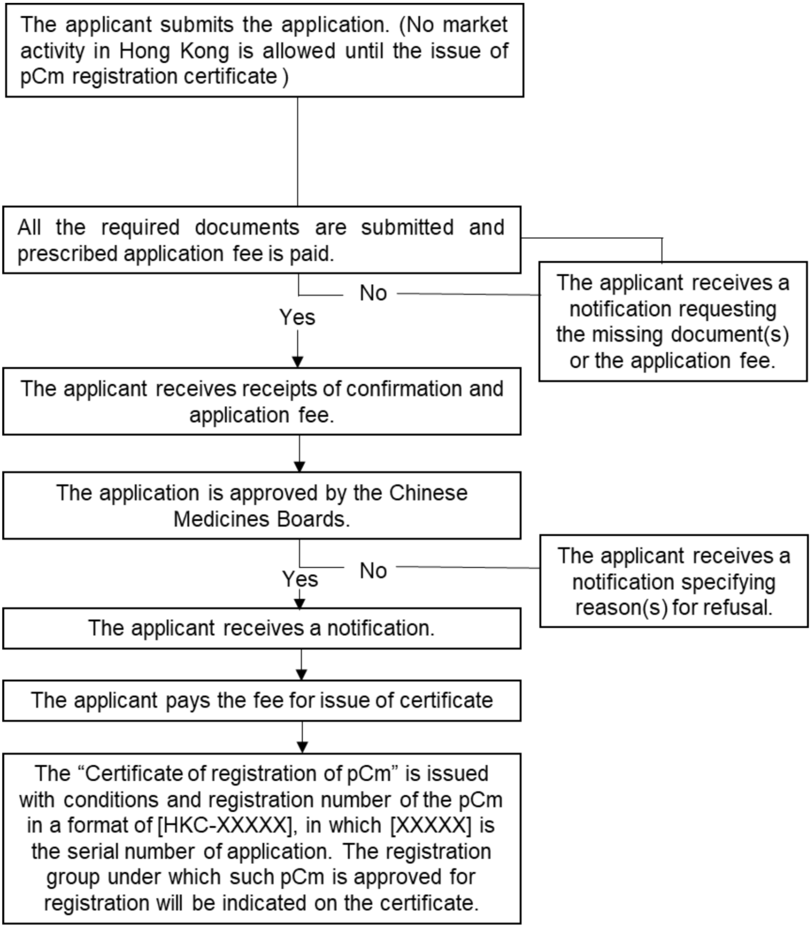


Figure 4. *Flow chart for application for registration of proprietary Chinese medicine (pCM).*

For pCM registration, the pCM should not contain heavy metals, toxic elements, pesticide residues and microbes that are exceeding the limits. In addition, the adulteration with western medicine is not allowed, and it should be complied with other legal requirements. The documents that are required for registration of pCM are as follows:

I. General documents

a. Completed Application Form & appropriate checklist
b. Application fee
c. Personal information of the person-in-charge of the company
d. Documentary proofs of manufacture or sales history of the product
e. Copy of manufacturing authorization issued by the country of origin (if applicable)
f. Copy of free sale documentation issued by the country of origin (if applicable)
g. Product sample and prototype sales pack
h. Label & package insert that have complied with the laws
i. Master formula

II. Product safety documents

a. Heavy metals and toxic elements test report
b. Pesticide residues test report
c. Microbial limit test report
d. Acute toxicity test report
e. Long-term toxicity test report
f. Local toxicity test report
g. Mutagenicity test report
h. Carcinogenicity test report
i. Reproductive and development toxicity test report
j. Summary report on product safety documents

III. Product efficacy documents

a. Interpretation and principle of formulating a prescription

b. Reference materials on product efficacy

c. Principal pharmacodynamic studies report

d. General pharmacological studies report

e. Clinical trial protocol and summary report

f. Summary report on product efficacy documents

IV. Product quality documents

a. Manufacturing method

b. Physicochemical properties of crude drugs

c. Product specification, method and certificate of analysis

d. Accelerated stability test report or general stability test report

e. Real-time stability test report

The pCM registration is managed by a regulatory framework consisting pre-market regulation and post-market monitoring. During the pre-market phase, it requires a full dossier submission of the listed required documents reporting the safety profile, quality and efficacy of the pCM. Several technical guidelines are available for reference in these requirements, including Product Safety Documents Technical Guidelines, Product Quality Documents Technical Guidelines, and Product Efficacy Documents Technical Guidelines. As for the post-market monitoring phase, adverse drug response (ADR) reporting system and public hotline of complaint and enquiry should be set up for public report. Besides, market surveillance and media monitoring should reinforce the strength of surveillance.

Limitation for CM registration

In Hong Kong, comparing to the vast demand on pCM registration, the number of successful cases of registration was quite disappointing. Up to June 30, 2018, there were a total of 18,141 pCM applications; however, more than half, i.e. 9,343, cases failed to pass. Among the successful cases, more than 80% categorized as HKP (transitional registration only). For HKC, a regular official registration, only 1,539 cases passed, which is less than 10% to the all application. Actually, the failure of pCM registration in Hong Kong has reflected the hidden limitation and threats existing in the CM industry. The main problems of CM and pCM in Hong Kong are as follows:

1) Instability of quality control, lack of internationally recognized quality standards and having the high frequency of safety incidents.

2) Active ingredients in pCM are not clear, lack of scientific explanation for action mechanism, insufficient support from basic research, defective legal system makes patent protection difficult.

3) Lag of the modernization in CM processing, disjoint of CM research and production, and having a very slow progress of industrialization.

4) Lack of relevant professional training for personnel, insufficient of high standard employees in CM industries.

Despite the adversity of our current situation in CM development, the vast demand on the quality of health and longevity still provides the driving force for the evolution of CM and other alternative cures. For the sake of world population health, with the ceaseless support from both China and local governments and the high reputation of the local universities, Hong Kong will soon become the trading centre for quality assured CM.

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About the Contributing Authors

Gallant KL Chan is a Research Associate of the Division of Life Science and Center for Chinese Medicine at the Hong Kong University of Science and Technology (HKUST) and Research Assistant Professor of HKUST Shenzhen Research Institute. He has strong enthusiasm in developing quality assurance standards in valuable Traditional Chinese Medicine (TCM) and nutraceuticals. He has been trained as a molecular biologist during his study of undergraduate and Master of Philosophy at City University of Hong Kong. After that, he has applied molecular authentication on TCM materials for more than 15 years. During the years he studied in HKUST for this doctorate, Dr. Chan equipped himself with frontier technology of quality control including the application of LC-MS/MS, macroscopic and microscopic identification of TCM and different cellular and pharmaceutical assays. His expertise is using multi-disciplinal quality assurance methods on a single specific subject. This style has been fully revealed on his Ph.D. thesis about the quality assurance study on cubilose/edible bird's nest (EBN). After he obtained his Ph.D. in 2013, Dr. Chan focuses his research in cosmetics science and development of nutraceuticals products. In need of seeking new targets for product development, Dr. Chan also has been keen on developing high-throughput screening technology for drug screening. Up to now, he has been involved in managing several government or non-government funded projects (UIM/214, UIM/254, UIM/288, UIM/302, UIM/340, UIT/137, TUYF15SC01, TUYF16SC01, TUYF19SC02 and Shenzhen Basic Research). Six of them have been successfully closed (UIM/214, UIM/254, UIM/288, UIM/302, TUYF15SC01, and TUYF16SC01). Dr. Chan has published 33 papers on international scientific journals and 6 awarded conference papers in international TCM symposium. He has published 24 patents, all filed in China and Hong Kong intellectual property departments, from which 14 patents have been granted. His is now the CEO of two biotechnology companies (CR#1945934, CR#2771494) registered in Hong Kong.

Dr. Ging Chan is an Associate Professor of the Institute of Chinese Medical Sciences, University of Macau. He had done research at Lilly Research Laboratory, Indianapolis, IN, USA and University of Chicago, IL, USA. His researches involved Growth Hormone-Releasing Hormone activities, human sex determining Region of Y gene, Doublesex DNA-binding domain of Drosophila melanogaster and the RNA-binding domain of the phage lambda N anti-termination factor. He had extensive experience on big data research at Bank of America, USA. His current research focuses on application on Artificial Intelligence and big data techniques on Traditional Chinese Medicine development.

Ms. Jenny C.M. Chan obtained her B.Sc. (Hons) in Virology from the University of Glasgow, U.K., where she was fascinated by molecular virology. Her research interests center around virus-host interaction and the approaches employed by different viruses to cause diseases. She is currently a final year M.Phil. candidate working in the laboratory of Dr. Martin Chi-Wai Chan at the Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China. Her current project is to study the immune response mediated under human norovirus infection utilizing the human intestinal enteroid model.

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Ms. Ieok Leng Chio received her B.Sc. degree of biomedical engineering from National Yang Ming University (Taiwan, R.O.C). Currently, she is a M.Sc. student of Medicinal Administration at the Institute of Chinese Medical Sciences, University of Macau. Her research interest is pharmaceutical patent quality evaluation.

Dr. Joseph Chow specializes in technology transfer of advance technology to the market. He commenced his career as a medical technologist at Singapore General Hospital, where he received extensive training in the field of Hematology. While obtaining his master's degree, Dr. Chow co-developed and commercialized a hybridization-based diagnostic technology for a recently listed company. Dr. Chow worked in the industrial biotech field for 19 years and his last position in a Hong Kong/China based company was as General Manager. Dr. Chow led a team of 150 staff members. He and his staff developed over 20 international standard diagnostic instrument and diagnostics kits, established over 50 clinical diagnostic services, and own patents for various inventions. Dr. Chow was invited as a professor by the Biotechnology Research Institute, Chinese Academy of Agricultural Sciences in 2014, specializing in technology translational development. He is now working in mainland China focusing on advising foreign companies on how to establish in China, helping them bring their product and technology to the Chinese market using medical technology experience. His is now helping several companies to establish in China.

Dr. Harold W. Davis runs a Translation Research Laboratory in the Division of Hematology/Oncology at the University of Cincinnati Medical Center (OH, USA). Dr. Davis completed his doctoral studies in Physiological Chemistry at The Ohio State University and conducted post-doctoral research in Pharmacology at the University of Connecticut Health Center and in Pathology at Washington University School of Medicine, USA. He then served as Faculty in Internal Medicine at Indiana University School of Medicine, USA, and at the University of Cincinnati Medical Center before assuming his current position. He has authored over 40 publications.

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Dr. Brian Gray is Senior Vice President of Product Development at Molecular Targeting Technologies, Inc. He is a serial innovator and entrepreneur with over 30 years of experience of research and development in the biotechnology industry. He started his industrial career at SmithKline and then helped co-found the drug delivery company, Zynaxis, in 1980, which went public and was subsequently acquired by PTI Research. Dr. Gray's work has resulted in the commercialization of a series of novel fluorescent CellVue® dyes for labeling of live cell membranes, NeuroVue® dyes for neuronal tract tracing, PSVue® (for detection of phosphatidylserine (PS) rich membranes and duramycin probes for the detection of phosphatidylethanolamine (PE) rich membranes. He has also successfully managed several multidisciplinary NIH-funded SBIR projects involving collaborations with academic researchers and has authored over 50 peer-reviewed papers. Drs. Pak and Gray have worked together on various therapeutic and diagnostic projects at MTTI for over 12 years. Dr. Gray obtained his Ph.D. in synthetic organic chemistry from the University of Dundee, Scotland, and conducted post-doctoral research at Oregon State University, USA.

Ms. Maggie Suisui Guo is a first-year Ph.D. student in Life Science, at The Hong Kong University of Science and Technology (HKUST). She finished her BBiomedSc degree in The University of Hong Kong (HKU) and M.Sc. degree in HKUST. During her study in HKU, she participated in several research projects supervised by Dr. Martin C.H. Cheung. Those projects applied avian embryos as model to study developmental biology. The topic of her final year project was "Characterization of the novel role of PHF5a in early stage of neural tube in avian trunk". Those research experiences had cultivated her interests in biotechnology. After graduation from HKU, Ms. Guo pursued her research interest and enrolled in M.Sc. program for biotechnology in HKUST. She joined Prof. Karl W.K. Tsim's lab as a research project student. The one-year lab attachment has equipped her with the modern technologies in Traditional Chinese Medicine (TCM) research, as well as helping her to move forward to the field of dermatology and regeneration science. Currently, Ms. Guo is focused in the research of the application of TCM and autogenous exosomes for skin regeneration and elucidating the mechanisms behind.

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Dr. Daniel H.S. Lee is a clinical biochemist by training and obtained his Ph.D. in Pathology at the University of Hong Kong. He has serviced the pharmaceutical industry for over 25 years including JNJ, Biogen, GSK and lately served as General Manager and Head of site for Roche Pharma R&D China in Shanghai from 2010. He has broad experience in the drug discovery and development business, familiar in both small molecule and biologic drug discovery and development covering therapeutic areas from CNS, infectious diseases, diabetes complications to oncology and inflammation. Over the years he has published over 50 papers, actively engaged in teaching, grant/manuscript reviews, and named as a co-inventor for 15 patents. He served as Head of Biomedical Technology Cluster, Hong Kong Science and Technology Parks and engaged in repositioning the biomedical technology industry in Hong Kong. He has been consulting for several institutes and companies, holds Adjunct Professorship of Division of Life Science, Hong Kong University of Science and Technology, and Honorary Professorship of Faculty of Medicine, University of Hong Kong. He is currently AVP and Chief Innovation and Enterprise Officer, Office of Strategic Development of the The Chinese University of Hong Kong.

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Dr. Koon Y (Chris) Pak is Chairman, President, and CEO of Molecular Targeting Technologies, Inc. He was formerly associated with Centocor, a top-tier biopharmaceutical company and Mallinckrodt Inc. Dr. Pak's recent awards include the Lifetime Achievement Award from the Chinese American Society of Nuclear Medicine and Molecular Imaging, the Distinguished Visiting Alumni Award from the State University of New York at Plattsburgh, SUNY Honor Roll of Alumni, the Ben Franklin Emerging Business Award, the Philadelphia Business Journal Innovation Award and the Outstanding Asian American Business Award. Formerly, Dr. Pak was the President of the Chinese American Society of Nuclear Medicine, the former Chairman and Co-founder of the Chinese Entrepreneur Association and the former Vice Chairman of the Global Monte Jade Science and Technology Association. Dr. Pak holds many patents in the diagnostic imaging and therapy field and has authored more than 80 articles covering the use of small molecules and antibodies for cancer and cardiovascular disease.

Dr. Alan Paau is a former Vice Provost at Cornell University from January 1, 2007 to February 28, 2015. Until August 2014, he was for seven and a half years also President of the Cornell Research Foundation, Inc. and Executive Director of the Cornell Center for Technology Enterprise & Commercialization. Previously, he was Assistant Vice Chancellor and Director of the technology transfer program at The University of California-San Diego ("UCSD"). Prior to UCSD, he was Executive Director of the Iowa State University Foundation, Inc. and the Office of Intellectual Property and Technology Transfer of Iowa State University. He supervised the execution of over 1,800 intellectual property licensing transactions to introduce useful products and services to society and provided the technology basis for the formation of over 150 new businesses to create high-paying jobs that contributed to the economic vitality of the regions. Prior to heading the academic technology transfer efforts, he was Associate Director of the Ohio State Biotechnology Center (Columbus, OH; 1992-1994) and was a scientist, senior scientist, and manager at the Cetus and the W.R. Grace & Co. organizations for twelve years (1981 – 1992) engaging in product R&D activities that resulted in two commercial products. While at Ohio State and at Iowa State, he held adjunct faculty appointments in the departments of Microbiology; Plant Pathology; Preventive Medicine, Microbiology & Immunology; and Zoology & Genetics. He currently serves on the advisory board of several biotechnology companies, academic programs, and non-profit organizations.

Dr. Paau holds a B.Sc., a M.Sc., and a Ph.D. degree in Biological Sciences; an MBA degree; and attended law school. He has been a Certified Licensing Professional since 2014 and currently is a lifetime honorary professor at the Southwest Jiao Tong University (Chengdu, China) since 2012.

He was elected a Fellow of the American Association for the Advancement of Science in October 2015 for his "distinguished contributions in the translation of a broad spectrum of scientific discoveries into real-world applications through technology commercialization, development, and management". He was earlier also recognized by the US Northeast Chapter of the BayHelix Group for his outstanding service in 2014; by the Sino-American Biotechnology and Pharmaceutical Professionals Association with its "San Diego Bio-Pharma Award" in 2006; by the San Diego Chinese Association with its "Vision Award" in 2005; by the San Diego Union-Tribute daily newspaper as one of the "People to Watch" in the business community in 2002; by the San Diego T-Sector Magazine's "Power 20" listing as one of the "people whose efforts go beyond their own positions to influence the entire local tech and life sciences industry" in 2002; by the University of California San Diego with its "UCSD Community Champion Campus-Wide Award" in 2002; and by the Southern Section of the American Society of Plant Physiologists with its "Outstanding Graduate Student Award" in 1974.

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Dr. Xiaoyang Qi was awarded his Ph.D. degree from Oklahoma State University, Oklahoma, USA in 1992 and joined the Faculty of Department of Pediatrics, Cincinnati Children's Hospital Medical Center, USA. Dr. Qi is now a professor and Co-Division Chief for Basic Science Research in the Division of Hematology-Oncology, Department of Internal Medicine, University of Cincinnati College of Medicine. He is the Director for the Brain Tumor Modeling Program in the Gardner Neuroscience Institute, University of Cincinnati, U.S. He discovered that saposin C (SapC) combined phospholipid (dioleoylphosphatidylserine, DOPS) nanovesicles have tumor-selective targeting and anti-cancer properties. His effort has partially focused on development of new anticancer nanovesicles for commercialization. Dr. Qi has published over 150 peer reviewed papers, book chapters, and scientific abstracts. Dr. Qi invented and patented a disease-targeting and therapeutic system (17 issued and >30 pending patents). These patents have been licensed to start-up companies in the USA and China. Bexion Pharmaceuticals (USA) has received Orphan Drug designation for saposin C, the active ingredient in BXQ-350 (SapC-DOPS) for glioblastoma multiforme from the USFDA. Bexion completed a first-in-human phase 1a clinical trial using BXQ-350 for the treatment of cancer patients. This study showed that BXQ-350 was well tolerated at all five doses tested with no dose limiting toxicities observed and with no serious adverse events attributed to the therapy.

Dr. Michael Silvon is Senior Vice President, Business Development, at Molecular Targeting Technologies, Inc. (USA). Before MTTI, Dr. Silvon consulted for a range of new and established life sciences companies on global strategy, business development, operations and M&A. Previously, he was VP Global Biopharmaceutical Services for Charles River Labs, providing worldwide cGMP and GLP molecular biology, virology, microbiology and viral clearance services for large molecule developers and manufacturers from five research sites. Earlier, he was VP Corporate Planning and Development at Bioanalytical Systems, a contract research organization providing clinical, preclinical and analytical services to small molecule drug developers. Dr. Silvon operated two business units, orchestrated five acquisitions, participated in their IPO and led corporate investor and financial management initiatives. He is a member of the Board of Directors of Claro Laboratories (Denver, CO, USA), an advanced diagnostics healthcare services company. He holds a Ph.D. in Physical Organic Chemistry and an MBA, with postdoctoral fellowships at Penn State University in organometallic chemistry and as a von Humboldt fellow at the Technischen Universität München (Germany) in inorganic chemistry.

Dr. Alan Lap-Yin Pang obtained both his bachelor and doctoral degrees from the Department of Biochemistry of School of Life Sciences, at The Chinese University of Hong Kong, Hong Kong. After that, Alan spent more than a decade in the US to pursue biomedical research excellence. He was Senior Research Fellow and then Biologist of the Laboratory of Clinical Genomics, at the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, USA, where he studied the biological function of murine testis-specific genes and epigenetic regulation of gene expression. Later, Alan became Laboratory Supervisor of the Molecular Genomics Laboratory (the next-generation DNA sequencing core facility) of the same institute to manage the operation of massive parallel sequencing services for investigators. Attracted by the opportunity of translating scientific knowledge into biomedical applications, Alan returned to Hong Kong in 2015 and joined a local biotech startup, TGD Life Company Limited, as Chief Executive Officer to develop stem cell and regenerative medicine R&D business at the Hong Kong Science Park. Alan also provides advice to local education sector on life science curriculum development.

Dr. Joseph Wing On Tam, Founder & Chairman of JT Technology and Consultant Ltd. (JTT), received his Ph.D. in Chemistry. He was Founder, Secretary and President of the Hong Kong Biochemistry Association. Dr. Tam had been Visiting Professor at UCSF, BMC and Augusta College of Medicine and Universities in China; Visiting Scientist of NIH and R&D Director, EY Laboratories Inc. Honorary Assoc. Professor, University of Hong Kong (HKU); and Founder & Chairman, Pangenia Group. Dr. Tam's interests spread from protein to molecular biology, and he has published in PNAS, Blood and other journals in genetic disease, DNA diagnosis, DNA fingerprinting and applied genomics. His laboratory in HKU embarked on rDNA technology in 1978 and he cloned the first human thalassemia recombinant libraries and in 1981 he introduced rDNA technology to train regional scientists in 1983. His laboratory has since cloned many genes, uncovered mutations including 2 of the 8 known beta-thalassaemia mutations among the Chinese. He is the inventor of many patents. The US patented Flow-Through DNA Hybridization Process is being applied in DNA macro and microarray development worldwide. Dr. Tam founded biotechnology companies namely HybriBio (凱普), DiagCor and Pangenia after he retired from HKU in dedication to establishing platforms for the Hong Kong science graduates to work and excel. JTT is formed as a pre-angel investor, by investing in and advising early-stage startups in biotechnology and life science to help them build value to increase the odds of success.

Prof. Karl W.K. Tsim received his B.Sc. and M.Phil. from The Chinese University of Hong Kong, and Ph.D. in Molecular Neurobiology from the University of Cambridge, UK. After his post-doctoral training at Stanford University, USA, he joined The Hong Kong University of Science and Technology in 1992. Currently, he is a Chair Professor of Division of Life Science, and the Director of Center for Chinese Medicine R & D at the university. Prof. Tsim has published over 350 scientific papers and serves as editors for many scientific journals internationally. He also serves as an advisor/consultant/member to various organizations, both nationally and internationally, in the standardization of Chinese herbs, which include World Health Organization and HKSAR Government in Testing and Certification of Chinese herbs. He is an active entrepreneur and is a founding director of few biotech companies. His inventions on Chinese medicine have been transformed into products in the market. He also serves as Independent Non-executive Director of Lee's Pharmaceutical Holding Ltd. (HK stock # 950).

Ms. Samantha Yung has been working in the biotechnology industry and related field since 2014, when she moved back to Hong Kong from Colorado, U.S., where she was trained as a biologist with a focus on biomedical and pre-veterinary sciences at Colorado State University. Before joining Hong Kong Science and Technology Parks, she dedicated her time in treating marine mammals and advocating animal welfare at Hong Kong Ocean Park and HKSPCA. At the same time, Samantha also started a business venture targeting the robust biomedical startup community, provocatively seeking innovative biomedical solutions to bring about technological advancement in our daily lives. The company has secured orders with clients like the Hong Kong Jockey Club and other veterinary clinics within the first quarter of establishment. Previously, Samantha worked as a Project Officer at the Biomedical Technology (BMT) Cluster in Hong Kong Science & Technology Parks Corporation, where she helps manage molecular diagnostics and therapeutic company accounts, direct and lead projects for biomedical events and conferences, support business development for bio-incubates and provide other administrative and business support services. With the latest government incentives and the launching of new biomedical initiatives, Samantha was promoted to a new role where she leads the international collaborations & investment team in the BMT Cluster, actively bringing in disruptive technology from overseas while establishing resourceful partnerships with MNCs and various academic institutions while advising park companies on fund raising strategies. She has also been involved with project management on a few key BMT infrastructure projects and submission of proposals for different government initiatives. After a few years at Hong Kong Science Park, Samantha recently accepted a position to work at the Chinese University of Hong Kong, focusing on enterprise innovation under the Office of Innovation and Enterprise, with a goal to deliver solutions and educational programs for innovation and entrepreneurship development.

Dr. Sherman Zheng 郑勋华博士1946年出生於中国，1970年广州中山医学院本科毕业，并在中国有十多年外科医生临床工作经验。八十年代早期到美國深造，于1991年获得美国纽约大学(NYU)生物化学博士学位，并继续博士后的研究工作，重点在蛋白质生物学、免疫学、和分子基因工程的研究。1989年到1994年在美国長島UBI （United Biomedical Inc) 公司作为研究员从事人工合成基因来发展艾滋病疫苗的研发工作，同时也研制对各种外源性病菌的临床分子诊断药箱。1995 年回到美國纽约大学医学中心血液病理， 分子诊断实验室任负责人，用分子生物学的技术检测血液病中癌症的特征和分型以及癌前期各种主要的标记物。特别在淋巴瘤和白血病的基因重组及癌变的研究。是美国早期运用核糖核酸扩增方法从事血液肿瘤临床分子诊断的专家之一，并一直在该医院进行临床第一线的诊疗和科研工作了二十多年，也经常参与美国临床分子病理医生的培训 工作。2015年受香港新亚医学科技有限公司(PANGENIA LIFESIENCES LTD) 和广州百皋医学检验所有限公司聘请为资深顾问，现任香港健港生命科技国际有限公司科技总监。

Richard Cheng, M.D., Ph.D., is the founder and director of Dr. Cheng Integrative Health Center and Doctor's Anti-Aging and Weight Loss Center of Columbia, South Carolina since inception in 2003. Dr. Cheng and his colleagues take an integrative approach to help patients with chronic diseases including diabetes, obesity, cardiovascular diseases and cancer. Dr. Cheng also serves as consultant to Shenzhen BaoAn Central Hospital and Shenzhen Medical Association, Shenzhen, Guangdong, China. Dr. Cheng is also a consultant to SuHa International Hospital, Suzhou, Jiangsu, China. Dr. Cheng serves on the editorial board of Journal of Nutritional Oncology, China and Orthomolecular Medicine News Service. Dr. Cheng is a Fellow and board certified anti-aging physician by the American Academy of Anti-Aging Medicine (A4M) and also a Fellow and board certified in A4m Integrative Cancer Therapy. Dr. Cheng completed his medical school at Shanghai Medical University in Shanghai, China, and completed his Ph.D. degree at the University of Arkansas for Medical Sciences in biochemistry and molecular biology.

Han Lei, Ph.D., is an Associate Professor at College of Medicine, Southwest Jiaotong University. As an executive-level research scientist distinguished in microbiology and vaccine research, he currently focuses on cancer, bacterial and viral vaccines.

Tong Gao is a postgraduate student at College of Medicine, Southwest Jiaotong University. Her major is Pharmacy, and her research interests lie in viral vaccine.

Dr. Y.K. Hamied is Non-Executive Chairman of Cipla, and represents the second generation of Cipla's founding family. A world-renowned scientist, Dr. Hamied obtained his Ph.D. in organic chemistry in 1960 from the University of Cambridge under the tutelage of the Nobel laureate Lord Alexander Todd. He joined Cipla in the same year as an R&D Officer. Dr. Hamied was appointed Managing Director of Cipla in 1976 and became Chairman of Cipla in 1989. He retired as Managing Director on 31st March 2013 and has continued to be the Chairman in a non-executive role since 1st April 2013. For close to 60 years, Dr. Hamied has been an insightful R&D leader, a courageous industry captain, and an outspoken statesman of global pharma. From affordable drugs in HIV to enabling one of the world's largest portfolio of drugs and devices in inhalation therapy, his pioneering work and immense contribution to healthcare have been celebrated around the world. For his distinguished service to the pharmaceutical industry, in 2005, Dr. Hamied was awarded the Padma Bhushan by the Government of India. In 2013, Dr. Hamied was named by NDTV as one of India's 25 Greatest Global Living Legends, and in 2014, the University of Cambridge awarded him a D.Sc, the highest honour that the University can bestow. In 2017, Columbia University's Mailman School of Public Health conferred the 'Public

Health Hero Award' on Dr. Hamied, and in 2018, the India Today publication named him one of '20 Global Indians' who have challenged convention and created history. Dr. Hamied has been the recipient of several lifetime achievement awards, he is an Honorary Fellow of the Royal Society of Chemistry, and is a frequent presence on high-level international panels on healthcare

Dr. John Howard currently serves as President of Applied Biotechnology Institute (ABI). ABI is focused on using its proprietary technology with unique advantages to produce recombinant proteins. These are used in a variety of applications including therapeutics and vaccines. He is the author/inventor on 200 publications and patents involving the expression of recombinant proteins and subsequent applications. Prior to ABI, Dr. Howard has led biotechnology groups in two Fortune 500 companies as well as a previous startup company. He received his Ph.D. In Biochemistry from the University of California at Riverside, CA.

Dr. Jie Liu, Senior Investigator, Chief, Lab. Of Infectious Diseases and Vaccine and Director, Biosafety Core, is an immunologist, whose primary interests are viral pathogenesis, immunity, and vaccine development. His work focuses on respiratory syncytial virus (RSV), influenza virus, SARS-Cov-2, molluscum contagiosum virus (MCV), TB, and other emerging viral diseases. After graduating from Luzhou Medical College, Luzhou, Sichuan, he obtained his Master of Medical Science from the West China School of Medicine in 1990, and his MD. & Ph.D. from the Yamanashi University of Medical Science, Japan, in 2001. He completed postdoctoral training in Immunology and parasitology at the Yamanashi University of Medical Science, a fellowship of Japan Society for the Promotion of Science, and his second postdoctoral training in Immunology, technology in flowcytometry, and viral pathogenesis at the Vaccine Research Center, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA, where he was trained as Postdoctoral Fellow, Research Fellow, and promoted to Staff Scientist and Lab Manager. In 2016, he became the founding investigator of his current lab and core facility. He oversees the development of vaccine candidates. He also serves as a consultant for organizations involved in vaccine development for HIV, TB, MCV, RSV, Cystic echinococcosis (CE), and emerging viral pathogens. His laboratory investigates basic mechanisms by which T cells affect viral clearance and immunopathology, explores mechanisms of antibody-mediated viral neutralization, and develops vaccine approaches against respiratory virus infections and emerging viral diseases.

Robert Davidson, M.P.H., M.A., M.Sc., Ph.D., is founder and CEO of CURE Pharmaceutical. Prior to his role at CURE, he was CEO at InnoZen Inc., Gel Tech LLC, and Bio Delivery Technologies Inc. and served on multiple corporate boards. Dr. Davison has over 23 years of experience in commercial brand extensions and development of drug formulation and delivery technologies in which he owns a multitude of patents. He has worked with brands such as Chloraseptic™, Suppress™, and Pediastrip™. Dr. Davidson received a B.S. Degree in Biological Life Sciences, a Master's Certificate in Applied Project Management from Villanova University, a Master's Degree of Public Health from American Military University, Virginia, and a Master's Degree in Health and Wellness from Liberty University, Virginia. His passion in sustainability in healthcare led him to earn a post-graduate degree in Sustainability Leadership with specialty in Healthcare and Agriculture from the University of Cambridge, UK with letter of commendation.

Raymond Tong, Ph.D., MBA, is the CEO of DrD Novel Vaccines Limited. Born in Hong Kong, he was educated in Canada, where he was qualified as a Medical Doctor in 1983. He also received degrees of Ph.D. in Neurophysiology and MBA from the University of Toronto. Dr. Tong began his career in Canada, where he founded a chain of medical clinics in the province of Ontario, and served as its Medical Director & Chief Physician. He returned to Hong Kong in 1989, where he spent the next two decades in corporate appointments with multinationals in the medical and pharmaceutical industries. In 2000, Dr. Tong moved to join CITIC Pacific and led its Healthcare Investment Division, responsible for a portfolio of over US $100 million, across businesses such as bio-pharmaceuticals, hospital services and IVD businesses. In 2008, Dr. Tong founded Eton Corporation, a private consultancy and investment company. Currently, he is an independent Director of Shanghai CP Guojian Pharmaceutical, the largest bio-pharmaceutical manufacturer, as well as being the Chairman of Shanghai Kedu Healthcare Group, one of the largest medical equipment distributor and third-party service provider in China, representing products from GE, Philips, Siemens and Kodak.

Yee O. Lam, J.D., began her work on edible vaccines with her brother Fong Lam, M.D., in the laboratory of their father Dr. Dominic Lam while they attended high school in The Woodlands, Texas, This research resulted in the first publication (Adv. In Sci. Res. 1:1-16, 1994) establishing the feasibility of producing an edible vaccine (against Hepatitis B0 in tomato fruit. After high school Yee Lam pursued a double major in Biology and English at the University of Texas. In 2002, Yee Lam graduated cum laude from Pepperdine University School of Law. Yee Lam currently practices law in Los Angeles, California.

Fong Wilson Lam, M.D., is a Pediatric Critical Care Physician at Texas Children's Hospital and Associate Professor of pediatrics at Baylor College of medicine. As a Pediatric Intensivist, he cares for many patients with severe infection (sepsis) and inflammation. His clinical and research interests lie in immunity as well as the complex interplay between inflammation and thrombosis.

Dr. Dominic Man-Kit Lam was born in Swatow and grew up in Hong Kong. He obtained his bachelor's, master's, and doctorate degrees by age 22, studied under two Nobel Laureate (Profs. T. Wiesel and D. Hubel) at Harvard Medical School before joining the Harvard Faculty and subsequently became Professor of Ophthalmology and Director of Baylor Center for Biotechnology in Houston. In 1982, Lam and Professor David Paton, Founder of Project Orbis, the charitable ophthalmic airplane, visited China for the first time. In 1985, Lam started the first biotech company in Texas and subsequently took it public in U.S. and was named "The Father of Texas Biotechnology". In 1988, Lam became the founding director of Hong Kong Institute of Biotechnology. In 1989, Lam received the U.S. High Tech Entrepreneur of the Year Award, and the U.S. Presidential Medal of Merit. He was also appointed a member of the U.S. President's Committee on the Arts and Humanities. In 1991, Lam was named Asia Society "Man of the Year". In 1993, he founded LifeTech Group to develop and market healthcare products. In 1999, he was selected as one of 99 most accomplished and influential artists in China in the 20th century. On December 18, 1999, Lam founded World Eye Organization (WEO) to prevent and treat eye diseases for the poor. In 2001, Lam's patent on "Oral Vaccine" was named by MIT as one of "five patents that will transform business and technology," and by Time magazine as one of the ten most important inventions in the 21st century. In 2017, Lam was awarded an Asian Social Caring Leadership Award by United Nations and Nobel Foundation.

Mr. Kaizhi Chen 陈开枝先生1970年毕业于北京理工大学。在光机电产品研究、设计、生产以及国际贸易领域有几十年从业经验，对先进技术的引进、发展和应用有丰富的经验。有中国国内和海外数十个自主专利。现任香港健港生命科技国际有限公司董事长，香港夏娃生命科技国际有限公司董事长，中国管理科学研究院特约研究员，企业管理创新研究所兼职副所长及学术委员会副主任，健港生物研究中心主任，和中国科学家论坛理事会理事。2016年被中国科学家论坛授予科技创新创业先进个人奖，其所带领团队也被授予科技创新创业先进单位。

Dr. Keith K.H. CHAN, Ph.D., is currently Senior Advisor of Cornerstone IP Foundation and adjunct professor of Graduate Institute of Technology Innovation and Intellectual Property Management, National Chengchi University, Taiwan. His past experience includes Manager of drug development at Novartis, Director of Generic drugs in US FDA, Professor of Pharmaceutical Sciences at the University of Minnesota and the University of Maryland in the US. In the last decade, he is teaching at the National Chengchi University and National Yang Ming University in Taiwan well as helping Asian pharmaceutical companies in developing biotech and pharmaceutical products for the world market.

About the Publisher

Started in 1998 by Dr. Albert Wai-Kit Chan, United States-China Intellectual Property Institute (USCIPI) is dedicated to the education of intellectual property concepts and legal matters of both China and the United States. Hoping to serve as a bridge between the two countries, USCIPI supports and hosts meetings, training courses, seminars, and conferences that are open to all participants, including government officials, scholars and business people. Another major focus is producing relevant literature as a medium to propagate information and knowledge. After a period of dormancy, USCIPI reactivated with the publication of a second volume of Biotechnology in Hong Kong and has continued with other volumes since.

OUR MISSION

United States-China Intellectual Property Institute (USCIPI) is a non-profit organization devoted to promoting the mutual understanding of intellectual property practices in the U.S. and China. Our philosophy is "We Educate. We Serve. We Bridge." We hope our work will help minimize intellectual property disputes between the U.S. and China in order to bring about both countries' innovative excellence and maximize collaborative opportunities.

OUR METHOD

We are dedicated to educating those interested in learning about intellectual property concepts and legal matters of both countries. Hoping to serve as a bridge between the U.S. and China, we invite government officials, scholars and business people of both countries to come together at our meetings, training courses, seminars, and conferences.

About the Editor

Dr. Albert Wai-Kit Chan is a former research scientist in molecular biology. Born and raised in Hong Kong, graduated from The Chinese University of Hong Kong, he was awarded his Ph.D. in virology at Baylor College of Medicine in Houston, Texas. Dr. Chan then completed his postdoctoral training at Cold Spring Harbor Laboratory in New York as an American Cancer Society postdoctoral fellow. With the emerging legal needs of the biotechnology industry in the late 1980s, Dr. Chan organically began his career in law, focusing on intellectual property and using his background in science as a solid foundation for his practice. He later received his J.D. degree from Columbia University School of Law in New York.

Through the years, Dr. Chan has handled all areas of intellectual property law, including technology transfer, patents, trademarks, copyrights, business transactions, and trade secrets. He is well-versed in all aspects of prosecution and litigation and is experienced in licensing, technology transfer and the evaluation of intellectual property portfolios. Dr. Chan has served as an adjunct professor of law at The City University of New York School of Law, where he taught intellectual property law, patent law, technology transfer, Internet and the law, food and drug law, and international business law. He is currently adjunct associate professor in the School of Life Sciences at The Chinese University of Hong Kong and has adjunct professorship in the Department of Health Technology and Informatics at The Hong Kong Polytechnic University.

Dr. Chan has worked extensively with all constituents of the intellectual property protection process in the U.S., China and other international jurisdictions. The importance of intellectual property protection for, not only businesses but also the overall national economy, became apparent. With all the IP and transactional work he has done in China, which includes facilitating joint ventures and contracts between East and West companies and building up clients' intellectual property portfolios, one thing became clear: Intellectual Property was a mystery to many people. Furthermore, differences in cultures and constant changes in local laws and practices made it difficult for even those who are considered experts in the field to navigate. There was a need to gather information, communicate, share perspectives, and educate. With this in mind, the United States-China Intellectual Property Institute was founded.

Dr. Albert Wai-Kit Chan is a partner of the Law Offices of Albert Wai-Kit Chan, PLLC in New York. Dr. Chan also heads Albert Wai-Kit Chan Intellectual Property Limited in Hong Kong.