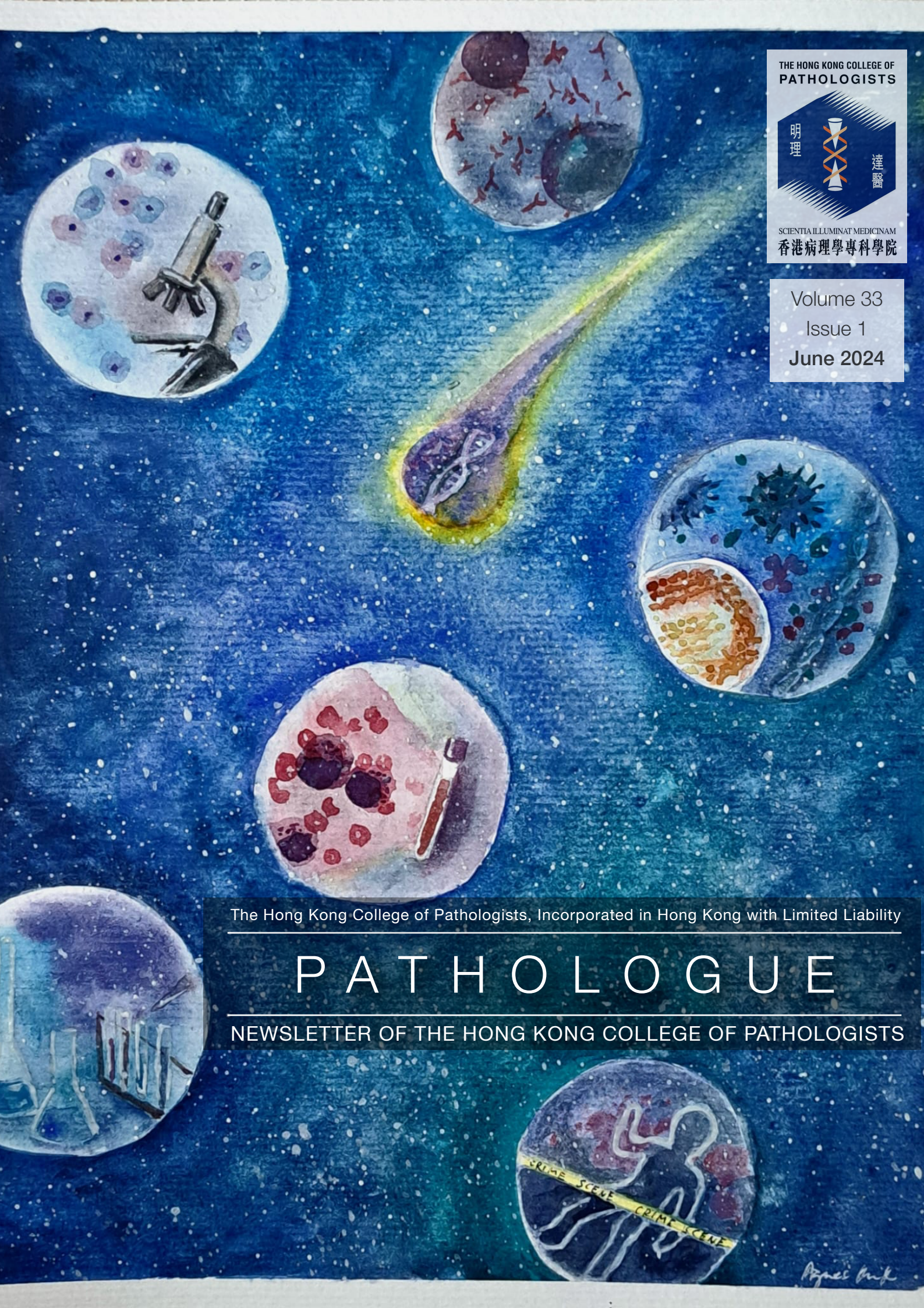


THE HONG KONG COLLEGE OF PATHOLOGISTS

明理 達醫

SCIENTIA ILLUMINAT MEDICINAM

香港病理學專科學院

Volume 33
Issue 1
June 2024

The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability

PATHOLOGUE

NEWSLETTER OF THE HONG KONG COLLEGE OF PATHOLOGISTS

Cover:

Watercolour painting by Dr KWOK Ka Ki titled *'Innovation and Integration'*.

College Secretary:
Ms Dilly YIP

Tel: 2871 8756
Fax: 2871 8755
Email: hkcpath@hkcpath.org

Homepage:
http://www.hkcpath.org/

Address:
College Chamber, Rm. 606, 6/F,
HKAM Jockey Club Building,
99 Wong Chuk Hang Road,
Aberdeen, Hong Kong.

Facebook:
https://www.facebook.com/hkcpath

The Editorial Board

Dr CHEUNG Tin Yan, Elaine
(Chief Editor)
Dr CHEONG Renee Constance Yue-Kew
Dr CHENG Hua Yin, Naomi
Dr CHEUNG Sin
Dr CHU Sin Yan
Dr KWOK Ka Ki
Dr LAM Tit Leung
Dr LEUNG Mei Tik
Dr LO Hui Yin

Table of Contents

03 Message from the President

06 President's activities

12 Topical Update: Screening of DPD Deficiency before Fluoropyrimidine Chemotherapy

18 International Liaison of Pathology Presidents (ILPP) Annual Meeting 2023

20 The 19th Trainee Presentation Session

23 The 32nd Annual General Meeting

25 The 31st Conferment Ceremony

33 The 31st T.B. Teoh Foundation Lecture

34 College Dinner 2023 - Photos

38 The 30th Annual Fellowship Conferment Ceremony of the Hong Kong Academy of Medicine

39 Best Original Research by Trainees (BORT) of the Hong Kong Academy of Medicine 2023

41 Museum of Medical Sciences Fundraising Dinner

42 10th Anniversary Celebration Ceremony of the Hong Kong Jockey Club Innovative Learning Centre for Medicine

43 HKAM Distinguished Young Fellow Award 2023

44 International Pathology Day 2023

45 Regulations on Postgraduate Training and Examinations 2023 – Updates and Implementation

46 Lecture by Professor Noel Woodford

47 Diagnostic Pathology in Nature: Pallas's cat (Manul)

49 Beyond the Microscope: The Art of Money Origami

Message from the President

It is my great pleasure to write my first message in the College newsletter. Following the Annual General Meeting held on 25 November 2023, I have assumed the position of College President. That evening, I had the privilege of sharing a memorable and enjoyable dinner, a long-awaited one after the COVID era, with our esteemed past Presidents, including Dr MAK Wai Ping, Dr LEE Kam Cheong, Prof CHEUNG Nga Yin, Annie, Dr CHAN Ho Ming and Dr CHAN Chak Lam, Alexander. Through these interactions, I gained a deeper understanding of the immense responsibilities that come with being a President, and I am committed to upholding the legacy of our predecessors.



First row, from left to right

Dr CHAN Chak Lam, Alexander, Dr Teresa LI, Dr MAK Wai Ping, Dr LEE Kam Cheong, Prof CHEUNG Nga Yin, Annie

Second row, from left to right

Dr LEUNG Yuk Yan, Rock, Dr LAI Koon Chi, Christopher, Dr CHAN Ho Ming, Dr MAK Siu Ming, Dr AU YEUNG Kwok Him, Rex, Dr Matthew LUK Man Hei

As mentioned in the President's Speech at the Conferment Ceremony, I outlined a number of key priorities for the College. Moving forward, we will focus on the faculty development initiatives aligned with the Academy's vision, and on the implementation of the revised Regulations on Postgraduate Training and Examinations, which shall be led by Dr LEUNG Yuk Yan, Rock, one of our College Vice-Presidents, in the coming days. I am pleased that a successful forum was organized in late April, which was attended by trainers and trainees from various disciplines. The forum provided an invaluable opportunity for us to discuss the changes in the new Regulations and to address questions from the audience. I am grateful to all who contributed to the success of this event, and I believe it further strengthens our commitment to transparency and collaboration.

As we step into 2024, a year filled with both challenges and opportunities, we are confronted with a significant workforce challenge that has persisted over the past decade. This issue is not limited to Hong Kong or Asia but is a global phenomenon. I had the privilege of joining the International Liaison of Pathology Presidents (ILPP) 2023, where I learned about the strategies from around the world aimed at alleviating the manpower gap. Despite various efforts, significant improvements have been elusive. It is time for us to think outside the box and consider innovative approaches. I firmly believe that advocating for the adoption of digital pathology and artificial intelligence can be a potential solution, paving the way for enhanced efficiency and accuracy in patient care.

In addition to addressing the workforce challenge, our Council remains dedicated to enhancing the College's reputation and image, while upholding the standard of practice and fostering collaboration with overseas and local organizations. We will continue our efforts to promote Pathology through International Pathology Day and our College's International Pathology Day Workshop, which usually takes place in early December each year. The International Pathology Day Workshop has already become a flagship program for our younger generation to learn about and engage with Pathology. It is satisfying to witness the enthusiasm and eagerness of our Young Fellows in leading the workshop and promoting our specialty within the society. I am delighted to share that some participants of our previous workshops have chosen Pathology as their career path, a testament to the impact we are making.

Personally, I will continue to fulfil my responsibilities as President, which include working closely with our College Fellows, Members and trainees. I will align our efforts with the Academy's direction, particularly in light of the Position Statement "Position Paper on Postgraduate Medical Education 2023" released earlier in 2023. I aim to foster synergy with the sister Colleges in Hong Kong and maintain strong connections with our overseas counterparts.



Dr MAK Siu Ming

President

May 2024

President's activities



The President attended the Hong Kong Academy of Medicine (HKAM) 30th Anniversary President's Dinner on 7 December 2023 in the Aberdeen Marina Club



30th Annual Fellowship Conferment Ceremony of the HKAM, 2023 David Todd Oration cum Pearl Jubilee Gala Dinner on 8 December 2023

30th Annual Fellowship Conferment Ceremony of the HKAM - Stage Party - 8 December 2023







The President attended the HKAM Dinner on 8 December 2023



The President attended the HKAM Spring Reception on 20 February 2024



The President joined the HKAM Workshop on Expert Witness Report Writing on 9 March 2024



Hong Kong Academy of Medicine 30th Anniversary Fun Day on 10 March 2024

The President joined the HKAM 30th Anniversary Fun Day at the HKU Stanley Ho Sports Centre. It was fortunate to have Council members, young College Fellows and trainees join this memorable event.







The President attended the Conferment Ceremonies of sister Colleges: the Hong Kong College of Anaesthesiologists on 18 November 2023, Dental Surgeons of Hong Kong on 26 November 2023 and the College of Ophthalmologists of Hong Kong on 10 December 2023.





The President represented the HKCPath and shared our College's experience in competency-based medical education in the first Competency-Based Medical Education Seminar held on 27 January 2024. Our College's committed Fellows also joined the event and shared their thoughts. The second seminar will be conducted in the second half of the year.





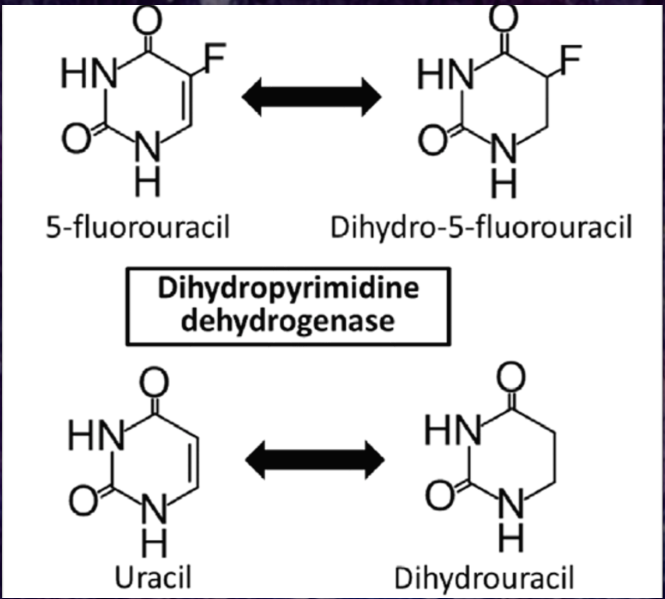


Figure: Metabolic pathway of 5-fluorouracil and uracil. Both are catabolized by dihydropyrimidine dehydrogenase. Adapted from Hishinuma et al. Front Pharmacol. 2022 Jun 15:13:930470.

Editorial note:

Screening of dihydropyrimidine dehydrogenase (DPD) deficiency before systemic fluoropyrimidine chemotherapy can improve safety and prevent the occurrence of the associated toxicity. Both genotyping and phenotyping approaches have been advocated. In this review, Dr Felix Wong compares and contrasts both approaches and explains the upcoming situation in Hong Kong in the near future. We welcome any feedback or suggestions. Please direct them to Dr Esther Hung (email: hly304@ha.org.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Topical Update: Screening of DPD Deficiency before Fluoropyrimidine Chemotherapy

Volume 19, Issue 1, Jan 2024

The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability

Dr WONG Chi Kin, Felix
Consultant Chemical Pathologist
Queen Mary Hospital

Introduction

Pre-emptive testing for dihydropyrimidine dehydrogenase (DPD) deficiency is currently recommended for the prevention of fluoropyrimidine toxicity. Dihydropyrimidine dehydrogenase (DPD), which is encoded by the DPYD gene on chromosome 1, is the major catabolic enzyme of fluoropyrimidines responsible for 80% of their metabolism. Fluoropyrimidines include the chemotherapeutic agents 5-fluorouracil (5-FU) and its prodrugs (capecitabine and tegafur). DPD converts 5-FU to dihydrofluorouracil, which is non-cytotoxic. Genetic variants of the DPYD gene are associated with decreased DPD activity and increased fluoropyrimidine-related toxicity (bone-marrow and gastrointestinal toxicity, even death due to severe toxicity) due to an accumulation of 5-FU and its downstream active cytotoxic metabolites. In recent years, multiple guidelines recommending preemptive *DPYD* testing before fluoropyrimidine chemotherapy from different countries or regions have been published[1–6]. These guidelines are focused on targeted genotyping of the following variants based on studies done in the Caucasian population: c.1905+1G>A (rs3918290, also known as *DPYD**2A, *DPYD*:IVS14 + 1G>A), c.1679T>G (rs55886062, *DPYD**13, p.I560S), c.2846A>T (rs67376798, p.D949V), and c.1129–5923C>G (rs75017182, HapB3 – corresponds to a combination of five genetically linked polymorphisms. It includes four intronic variants and one exon variant, two of which are in complete linkage disequilibrium: the c.1129-5923C>G variant (intronic variant) and the

c.1236G>A variant (exon variant). The c.1129-5923C>G variant introduces a cryptic splice site and the partial production of a non-functional transcript.[5,7]). Nevertheless, other rarer decreased- or no-function DPYD variants exist. Notably, based on multiethnic population databases, e.g. Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/) and multiple studies performed in the East Asian population, it is known that these 4 variants are rare or absent in the East Asian population. For Hong Kong, an international city with a predominantly Chinese population, DPYD genotyping has been mostly performed in a retrospective manner to ascertain the cause in patients who had suffered from severe fluoropyrimidine toxicity[8]. Obviously, this approach does not prevent fluoropyrimidine toxicity or improve patient outcomes. It is uncertain how preemptive *DPYD* testing should be conducted in this locality. Whole DPYD gene sequencing with investigation of DPD enzyme activity for patients with novel variants has been proposed by some local experts[9].

An alternative approach to DPYD genotyping is DPD phenotyping. 5-FU is an synthetic analog of uracil, which is the native substrate of the DPD enzyme located in the liver as part of the pyrimidine catabolic pathway. DPD deficiency is associated with an increase in uracil and decrease in dihydrouracil, the product of the DPD enzymatic reaction. Therefore, a high plasma uracil (U) concentration or a low dihydrouracil (UH2) to uracil ratio (UH2/U ratio) indicates DPD deficiency. DPD phenotyping by plasma uracil measurement is recommended by the European Medicines Agency[10] for the detection of DPD deficiency and has been adopted in France[11] since 2018 using a cutoff of >=16 ng/mL, above which DPD deficiency is diagnosed. France is the first country in the world adopting DPD phenotyping by plasma uracil measurement for the screening of DPD deficiency and there has been an increase in the number of tests and European countries offering the test from 2019 to 2021[12].

Genotyping

The Activity Score system, proposed by Henricks et al[13] and endorsed by the Clinical Pharmacogenetics Implementation Consortium (CPIC)[5], translates DPYD genotype to a predicted DPD phenotype. An Activity Score of 2 represents normal DPD activity and is a sum of a score of 1 for each normal allele that an individual carries. A decreased-function allele is assigned a score of 0.5 and a no-function allele is assigned a score of 0. *DPYD**2A and *13 alleles are assigned a score of 0 (no-function variant) while c.2846A>T and c.1129–5923C>G alleles are assigned a score of 0.5 (decreased-function variant). Based on this system, an Activity Score of 2 translates into DPYD normal metabolizer, while an Activity Score of 1 or 1.5 translates into DPYD intermediate metabolizer and an Activity Score of 0 or 0.5 translates into DPYD poor metabolizer. For example, homozygous DPYD c.1129-5923C>G has an Activity Score of 0.5 + 0.5 = 1, which is equal to the Activity Score of heterozygous DPYD*2A. Dosage recommendations based on the metabolizer status are available, ranging from a reduced starting dose of 50% in intermediate metabolizers to a complete avoidance of fluoropyrimidines for poor metabolizers with an Activity Score of 0. Furthermore, therapeutic drug monitoring of 5-FU is recommended for guiding dosage adjustment following initial dosing. Prospective clinical trials involving upfront testing for these variants with genotype-based dosage adjustments have demonstrated improved patient safety[14,15] and cost-effectiveness[15,16], with no adverse impact on treatment response rates for the reduced fluoropyrimidine dose given to DPYD variant carriers[17]. Nevertheless, it is known that this approach has limited sensitivity (17%) for severe fluoropyrimidine-related toxicity [>=grade 3 according to the Common Terminology Criteria for Adverse Events (CTCAE)] because only 39 – 61% of patients having severe fluoropyrimidine-related toxicity are due to DPD deficiency, and even if DPD deficiency is present, it cannot be always traced back to a genetic alteration in the DPYD gene despite full gene sequencing[18].

On the other hand, multiple studies performed in the East Asian population (Chinese[19–21], Korean[21,22], Japanese[21,23]) have demonstrated the scarcity of the *2A, I560S, D949V and c.1129–5923C>G in the studied populations. Indeed, fluoropyrimidine-induced toxicity appears to be less common in the East Asian population than other populations[24,25]. From the DPYD allele frequencies based on population databases together with a comprehensive evaluation of variants by functional data/in silico predictions, it has been deduced that the East Asian population may have a lower incidence of DPD deficiency than the European population (3.4% and 0.02% of intermediate metabolizer and poor metabolizer, respectively, in East Asians, in contrast to 7.6% and 0.09%, respectively, in non-Finnish Europeans).[26] Table 1 shows a comparison of minor allele frequencies (MAF) in Genome Aggregation Database (gnomAD) of the 4 variants in the European and East Asian populations. It can be deduced that targeted genotyping of these 4 variants in the local Chinese population will result in poor sensitivity of detecting DPD deficiency, and whole DPYD gene sequencing is required for the detection of other deleterious DPYD alleles. Based on the literature review performed by Zhou et al, 49 DPYD variants (*2A, I560S and D949V included) are considered to be pathogenic[26], and by in silico predictions of all observed DPYD variants in the global population in the same study, it was estimated that a large number of DPYD variants (174 variations, which corresponds to only 34.3% of deleterious DPYD variants) have to be tested in order to explain 95% of genetically encoded functional DPD variability in the population, underscoring the presence of significant allelic heterogeneity. DPYD variants with unknown functional impact may be analyzed by in silico prediction tools, which are computational algorithms developed to predict the functional impact of DPYD variants, e.g. DPYD-Varifier[27],

Absorption, Distribution, Metabolism and Excretion (ADME) Prediction Framework[26,28], or evaluated using in vitro functional assays of DPD enzyme activity, which involves expression of DPYD variants in mammalian cells and incubation of radiolabelled 5-FU coupled with the measurement of rate of formation of dihydrofluorouracil from 5-FU[29–32]. It has been suggested that computation algorithms specifically developed for pharmacogenes (e.g. ADME Prediction Framework) or in a gene-specific manner (e.g. DPYD-Varifier) may be preferable to conventional prediction methods for non-pharmacogenes because conventional methods are primarily based on evolutionary conservation while pharmacogenes are poorly conserved[33]. Given the rarity of each individual DPYD variant, in vivo data from clinical studies is mostly unavailable or limited to isolated case reports. Nevertheless, the approach of including additional DPYD deficiency variants with evidence of a deleterious impact on protein function equivalent to the CPIC high level evidence variants has been shown to improve the sensitivity of the prediction of grade 3/4 haematological toxicity while retaining excellent specificity.[34]

Table 1. Minor allele frequencies (MAF) for *2A (c.1905+1G>A), I560S (c.1679T>G), D949V(c.2846A>T) and c.1129–5923C>G. Source: Genome Aggregation Database, accessed 20 December 2023.

DPYD Variant	MAF (European, non-Finnish)(%)	MAF (East Asian)(%)
c.1905+1G>A (rs3918290, *2A)	2.385	0
c.1679T>G (rs55886062, I560S)	0.06220	0
c.2846A>T (rs67376798, D949V)	0.5163	0.005013
c.1129–5923C>G (rs75017182)	2.102	0.1926

A prospective study was performed from November 2020 to December 2021 in the author's hospital recruiting patients requiring fluoropyrimidine-containing chemotherapy. Polymerase chain reaction followed by Sanger sequencing of the 23 coding exons, their flanking intronic regions and HapB3 (c.1129-5923C>G included) of the *DPYD* gene was performed. 103 patients (97 Chinese, 94%) requiring chemotherapy for colorectal, stomach and other cancers were recruited. 4 heterozygous decreased-function/no-function *DPYD* variants were detected in 4 Chinese patients (heterozygous c.2210C>T, p.Thr737Ile: 2 patients; heterozygous c.220C>T, p.Arg74Ter and heterozygous c.1314T>G, p.Phe438Leu: 1 patient each). In addition, two heterozygous VUS were detected in three patients (heterozygous c.2303C>A, p.Thr768Lys: two Chinese patients; heterozygous c.2528T>C, p.Ile843Thr in a Filipino patient). None of the well-established alleles affecting *DPYD* function were detected in the study population.

Phenotyping

The DPD enzyme activity is mainly located in the liver in vivo. It is also present in peripheral blood mononuclear cells (PBMCs) which can be measured ex vivo and acts as a surrogate marker of the hepatic enzymatic activity[35]. Nevertheless, the assay is not widely available because it is time-consuming, requires a large volume of blood and the use of radiolabeled materials[36]. Of note, only 25% of patients with a decreased DPD enzyme activity carries one of the four DPYD variants[37]. Alternatively, plasma uracil and dihydrouracil measurements by High Performance Liquid Chromatography – Ultraviolet detection (HPLC-UV) or Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) have been studied as surrogate markers of DPD enzyme activity. The theoretical advantage of DPD phenotyping resides in the possibility of identifying more patients with DPD deficiency beyond which can be explained by DPYD genetic variants alone. In a study of 550 patients requiring fluoropyrimidine chemotherapy with pretreatment serum uracil measured, a concentration of >=16 ng/mL strongly associated with global severe toxicity (odds ratio 5.3, p = 0.009). Serum uracil was shown to correlate better with DPD activity in PBMCs than UH2/U ratio and be superior to UH2/U ratio as a predictor of severe toxicity[38]. The same cutoff in another study demonstrated a sensitivity of 67% and specificity of 92% in the prediction of grade 4 capecitabine toxicity with a relative risk of 20.6 (p = 0.021)[39]. The distribution of plasma uracil in the pre-treatment population across studies is shown in Table 2. One of these studies was performed in the Chinese population[40]. Plasma uracil >= 16 ng/mL was observed in approximately 10 – 15% of the population[39,41]. Plasma uracil measurement has been endorsed by the European Medicines Agency (EMA) in 2020 for the detection of DPD deficiency[10]. Detailed guidelines on the detection of DPD deficiency by plasma uracil measurement are available in France (>=16 ng/mL to 150 ng/mL: partial DPD deficiency; >= 150 ng/mL: complete DPD deficiency)[42] and Belgium (>= 14 ng/mL to 100 ng/mL: partial DPD deficiency; >=100 ng/mL: complete DPD deficiency)[43]. Dosage adjustment based on plasma uracil leads to a decreased 5FU toxicity in patients with partial DPD deficiency, while the resulting lower dose of 5FU received by these patients suggests the need for therapeutic drug monitoring of 5FU to uphold treatment efficacy[44]. Plasma uracil is unstable in whole blood samples and strict preanalytical requirements are

required (maximum delay between sample and centrifugation of 1h30 mins if the sample is stored at ambient temperature, and 4h if it is stored at + 4°C, centrifugation preferably at + 4°C then immediate freezing of the resulting plasma, transport respecting the cold chain)[42], otherwise, artefactual elevations may occur[45]. There are known effects of food and circadian rhythm affecting plasma uracil concentration[45,46]. Plasma uracil concentration may be elevated in renal impairment[47,48], abnormal liver function[48] and tumor lysis syndrome[49]. Patients with end stage renal disease on haemodialysis were shown to have a higher uracil concentration pre-analysis (mean = 14 ng/mL) than post-dialysis (mean = 8 ng/mL)[50]. Plasma uracil concentration is also elevated if fluoropyrimidine has already been administered because of the competition between uracil and 5-FU for metabolism by DPD[51].

In the author's laboratory, residual plasma from 74 specimens delivered on ice for plasma renin analysis was used for plasma uracil analysis. The average, median, 5th and 95th percentile of plasma uracil were: 9.8, 9.6, 5.9 and 16.2 ug/L. Therefore, around 5% of samples is expected to show a plasma uracil >=16 ug/L ("Flagging rate"), indicating a positive result for DPD screen. Uracil >= 150 ug/L is expected to be rare (occurrence rate 0.08% in France)[11] and there were no such cases in our 74 specimens.

Table 2. Distribution of plasma uracil concentrations across different studies. LC-MS/MS: liquid chromatography tandem mass spectrometry; HPLC-UV: high performance liquid chromatography – ultraviolet spectroscopy.

Study	Median (ng/mL)	Range (ng/mL)	No. of subjects (n)	Methodology	Diagnosis	Ethnicity
Coudoré et al, 2012[52]	8.4	0.6 – 43.3	26	LC-MS/MS	Advanced or recurrent cancer	French
Etienne-Grimaldi et al, 2017[39]	9.6	3.9 – 75.3	205	HPLC-UV	Advanced breast cancer patients	French
Pan et al, 2017[40]	11.2	5.16 – 120.6	68	HPLC-UV	Colorectal cancer patients	Chinese
Dolat et al, 2020[53]	10.8	3 – 37.6	169	LC-MS/MS	Colorectal, pancreas and stomach cancer	French
Tafzi et al, 2020[41]	10.6	3.9 – 81.6	526	LC-MS/MS	Patients before 5FU treatment	French

Genotyping and Phenotyping

Plasma uracil concentration correlates with the presence of known deleterious DPYD variants *2A, I560S and D949V – such variants were associated with a higher plasma uracil concentration, though not necessarily higher than the cutoff of 16 ng/mL, while this association appears to be weaker for HapB3[38,39,54]. The presence of any of the 4 variants has a sensitivity and specificity of 11% and 95%, respectively, for the detection of DPD deficiency defined as a uracil concentration of > 16 ng/mL[54]. A retrospective study of 472 patients with DPYD targeted genotyping (the 4 DPYD variants recommended by CPIC, plus DPYD*7, a frameshift variant classified to be a no-function allele by CPIC) and phenotyping (plasma uracil and UH2/U ratio) done without fluoropyrimidine dosage being adjusted according to such results (either because the patient has already suffered from toxicity of fluoropyrimidine or because such testing was done in parallel with treatment initiation) demonstrated sensitivities and specificities of targeted genotyping and plasma uracil to be 33%/59% and 95%/84%, respectively, for the prediction of grade 3 or higher fluoropyrimidine toxicity[55].

For the local population who is predominantly Chinese, it was demonstrated that none of the 4 variants recommended for testing by CPIC (c.1905+1G>A, c.1679T>G, c.2846A>T and c.1129–5923C>G) were detected in the author's laboratory, while sequencing of whole *DPYD* gene enabled the detection of other *DPYD* variants enriched in the East Asian population which may lead to DPD deficiency and confer susceptibility to fluoropyrimidine toxicity. Nevertheless, these variants are less well characterized and their interpretation in the local population as decreased or no function variants relies on in vitro or in silico data, i.e. human data is lacking.

According to the CPIC guideline, when two different decreased-/no -function variants are detected in a patient, it is assumed that the two variants are in trans, i.e. located on different alleles, while this may not be true if one of the two detected variants is novel. In the absence of haplotyping techniques, phenotyping may be helpful to differentiate partial from complete DPD deficiency.[5] This approach has been highlighted in a review by Knikman et al. which outlined the possibility of combining phenotyping with genotyping in the same patient. They proposed that while dose modification may be based on genotyping result if one known variant is detected, dose modification should be based on phenotyping results in the event of two variants detected or no variants detected[56]. In fact, even if two deleterious alleles are proven to be in trans by haplotyping techniques, the Dutch Pharmacogenetics Working Group (DPWG) guideline recommends the use of phenotyping to determine the residual DPD activity for guiding the fluoropyrimidine use at significantly reduced doses[2].

Screening of DPD deficiency in Hong Kong

While screening of DPD deficiency by plasma uracil has not been formally studied in the Chinese population, it is assumed to be applicable to all populations because the phenotype of uracilemia is reasoned to a biomarker of DPD deficiency independent of ethnicity[57]. A decision was made to go for the phenotyping rather than genotyping approach and Queen Mary Hospital Chemical Pathology will launch preemptive screening of DPD deficiency by plasma uracil measurement to all patients requiring systemic fluoropyrimidine chemotherapy in the Hospital Authority in 2024 Q1. Plasma uracil is measured by liquid chromatography tandem mass spectrometry, which requires in-house method development and evaluation. We have subscribed to an external quality assurance program based in France (ASQUALAB). Uracil is an unstable analyte in plasma and an unbroken cold chain is required from specimen collection to centrifugation and storage. A turnaround time of less than 10 calendar days, which fulfils the French standard[42], will be implemented to minimize the delay before treatment initiation. Under this service, genotyping is not required and will not be performed, even for patients screened positive for partial or complete DPD deficiency by plasma uracil. While fluoropyrimidines are contraindicated in patients with complete DPD deficiency, patients with partial DPD deficiency may be started on a reduced dose and the option of therapeutic drug monitoring of intravenous infusion of 5-fluorouracil (the topic of which is beyond the scope of this article) will be available as a part of the service to improve the drug safety of fluoropyrimidines. The service of measuring plasma 5FU level will be launched simultaneously with that of plasma uracil. According to an article published in 2023, no preventable toxicity-related death has been declared to the French Pharmacovigilance Network in patients with complete or partial DPD deficiency since 2020[57]. We hope that the same benefits will soon become a reality in Hong Kong.

References

1. Hamzic S, Aebi S, Joerger M, Montemurro M, Ansari M, Amstutz U, et al. Fluoropyrimidine chemotherapy: recommendations for DPYD genotyping and therapeutic drug monitoring of the Swiss Group of Pharmacogenomics and Personalised Therapy. Swiss Med Wkly 2020;150:w20375
2. Lunenburg CATC, van der Wouden CH, Nijenhuis M, Crommentuijn-van Rhenen MH, de Boer-Veger NJ, Buunk AM, et al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. Eur J Hum Genet EJHG. 2020 Apr;28(4):508–17.
3. Wörmann B, Bokemeyer C, Burmeister T, Köhne CH, Schwab M, Arnold D, et al. Dihydropyrimidine Dehydrogenase Testing prior to Treatment with 5-Fluorouracil, Capecitabine, and Tegafur: A Consensus Paper. Oncol Res Treat. 2020;43(11):628–36.
4. 5-fluorouracil (intravenous), capecitabine, tegafur: DPD testing recommended before initiation to identify patients at increased risk of severe and fatal toxicity [Internet]. GOV.UK. [cited 2023 Dec 21]. Available from: https://www.gov.uk/drug-safety-update/5-fluorouracil-intravenous-capecitabine-tegafur-dpd-testing-recommended-before-initiation-to-identify-patients-at-increased-risk-of-severe-and-fatal-toxicity
5. Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. Clin Pharmacol Ther. 2018 Feb;103(2):210–6.
6. García-Alfonso P, Saiz-Rodríguez M, Mondéjar R, Salazar J, Páez D, Borobia AM, et al. Consensus of experts from the Spanish Pharmacogenetics and Pharmacogenomics Society and the Spanish Society of Medical Oncology for the genotyping of DPYD in cancer patients who are candidates for treatment with fluoropyrimidines. Clin Transl Oncol. 2022 Mar 1;24(3):483–94.
7. Amstutz U, Farese S, Aebi S, Largiadèr CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. Pharmacogenomics. 2009 Jun;10(6):931–44.
8. Tong CC, Lam CW, Lam KO, Lee VHF, Luk MY. A Novel DPYD Variant Associated With Severe Toxicity of Fluoropyrimidines: Role of Pre-emptive DPYD Genotype Screening. Front Oncol. 2018;8:279.
9. Lam KO, Tong CC, Lee VHF, Luk MY, Lam CW. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy: who and how? Lancet Oncol. 2019 Feb;20(2):e66.
10. EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine | European Medicines Agency [Internet]. [cited 2023 Dec 21]. Available from: https://www.ema.europa.eu/en/news/ema-recommendations-dpd-testing-prior-treatment-fluorouracil-capecitabine-tegafur-flucytosine
11. Méthodes de recherche d'un déficit en dihydropyrimidine deshydrogénase visant à prévenir certaines toxicités sévères associées aux traitements incluant une fluoropyrimidine (5-fluorouracile ou capécitabine) [Internet]. Haute Autorité de Santé. [cited 2023 Dec 21]. Available from: https://www.has-sante.fr/jcms/c_2891090/fr/methodes-de-recherche-d-un-deficit-en-dihydropyrimidine-deshydrogenase-visant-a-prevenir-certaines-toxicites-severes-associees-aux-traitements-incluant-une-fluoropyrimidine-5-fluorouracile-ou-capecitabine
12. De With M, Sadlon A, Cecchin E, Haufroid V, Thomas F, Joerger M, et al. Implementation of dihydropyrimidine dehydrogenase deficiency testing in Europe. ESMO Open. 2023 Apr;8(2):101197.
13. Henricks LM, Lunenburg CATC, Meulendijks D, Gelderblom H, Cats A, Swen JJ, et al. Translating DPYD genotype into DPD phenotype: using the DPYD gene activity score. Pharmacogenomics. 2015;16(11):1277–86.
14. Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol. 2018 Nov;19(11):1459–67.
15. Deenen MJ, Meulendijks D, Cats A, Sechterberger MK, Severens JL, Boot H, et al. Upfront Genotyping of DPYD*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. J Clin Oncol Off J Am Soc Clin Oncol. 2016 Jan 20;34(3):227–34.
16. Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. A cost analysis of upfront DPYD genotype-guided dose individualisation in fluoropyrimidine-based anticancer therapy. Eur J Cancer Oxf Engl 1990. 2019 Jan;107:60–7.

17. Glewis S, Alexander M, Khabib MNH, Brennan A, Lazarakis S, Martin J, et al. A systematic review and meta-analysis of toxicity and treatment outcomes with pharmacogenetic-guided dosing compared to standard of care BSA-based fluoropyrimidine dosing. Br J Cancer. 2022 Mar 19;1–11.

18. Meulendijks D, Cats A, Beijnen JH, Schellens JHM. Improving safety of fluoropyrimidine chemotherapy by individualizing treatment based on dihydropyrimidine dehydrogenase activity - Ready for clinical practice? Cancer Treat Rev. 2016 Nov;50:23–34.

19. Li GY, Duan JF, Li WJ, Liu T. DPYD*2A/*5A/*9A and UGT1A1*6/*28 polymorphisms in Chinese colorectal cancer patients. J Cancer Res Ther. 2016 Jun;12(2):782–6.

20. He YF, Wei W, Zhang X, Li YH, Li S, Wang FH, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in Chinese cancer patients. J Clin Pharm Ther. 2008;33(3):307–14.

21. Shin JG, Cheong HS, Kim JY, Kim LH, Han CS, Kim JO, et al. Screening of Dihydropyrimidine Dehydrogenase Genetic Variants by Direct Sequencing in Different Ethnic Groups. J Korean Med Sci. 2013 Aug;28(8):1129–33.

22. Cho HJ, Park YS, Kang WK, Kim JW, Lee SY. Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. Ther Drug Monit. 2007 Apr;29(2):190–6.

23. Maekawa K, Saeki M, Saito Y, Ozawa S, Kurose K, Kaniwa N, et al. Genetic variations and haplotype structures of the DPYD gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences. J Hum Genet. 2007 Oct;52(10):804–19.

24. Haller DG, Cassidy J, Clarke SJ, Cunningham D, Van Cutsem E, Hoff PM, et al. Potential regional differences for the tolerability profiles of fluoropyrimidines. J Clin Oncol Off J Am Soc Clin Oncol. 2008 May 1;26(13):2118–23.

25. Loh M, Chua D, Yao Y, Soo RA, Garrett K, Zeps N, et al. Can population differences in chemotherapy outcomes be inferred from differences in pharmacogenetic frequencies? Pharmacogenomics J. 2013 Oct;13(5):423–9.

26. Zhou Y, Dagli Hernandez C, Lauschke VM. Population-scale predictions of DPD and TPMT phenotypes using a quantitative pharmacogene-specific ensemble classifier. Br J Cancer. 2020 Dec;123(12):1782–9.

27. Shrestha S, Zhang C, Jerde CR, Nie Q, Li H, Offer SM, et al. Gene-Specific Variant Classifier (DPYD-Varifier) to Identify Deleterious Alleles of Dihydropyrimidine Dehydrogenase. Clin Pharmacol Ther. 2018 Oct;104(4):709–18.

28. Zhou Y, Mkrtchian S, Kumondai M, Hiratsuka M, Lauschke V. An optimized prediction framework to assess the functional impact of pharmacogenetic variants. Pharmacogenomics J. 2019 Apr 1;19.

29. Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic Profiling of *DPYD* Variations Relevant to 5-Fluorouracil Sensitivity Using Real-time Cellular Analysis and *In Vitro* Measurement of Enzyme Activity. Cancer Res. 2013 Mar 15;73(6):1958–68.

30. Offer SM, Fossum CC, Wegner NJ, Stuflesser AJ, Butterfield GL, Diasio RB. Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. Cancer Res. 2014 May 1;74(9):2545–54.

31. Elraiyah T, Jerde C, Shrestha S, Wu R, Nie Q, Giama N, et al. Novel Deleterious Dihydropyrimidine Dehydrogenase Variants May Contribute to 5-Fluorouracil Sensitivity in an East African Population. Clin Pharmacol Ther. 2017;101(3):382–90.

32. Ly RC, Schmidt RE, Kiel PJ, Pratt VM, Schneider BP, Radovich M, et al. Severe Capecitabine Toxicity Associated With a Rare DPYD Variant Identified Through Whole-Genome Sequencing. JCO Precis Oncol. 2020 Jun 12;4:PO.20.00067.

33. Zhou Y, Lauschke VM. Computational Tools to Assess the Functional Consequences of Rare and Noncoding Pharmacogenetic Variability. Clin Pharmacol Ther. 2021;110(3):626–36.

34. Palles C, Fotheringham S, Chegwidden L, Lucas M, Kerr R, Mozolowski G, et al. An Evaluation of the Diagnostic Accuracy of a Panel of Variants in DPYD and a Single Variant in ENOSF1 for Predicting Common Capecitabine Related Toxicities. Cancers. 2021 Jan;13(7):1497.

35. Lu Z, Zhang R, Diasio RB. Dihydropyrimidine dehydrogenase activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients, and clinical implication in 5-fluorouracil chemotherapy. Cancer Res. 1993 Nov 15;53(22):5433–8.

36. Hodroj K, Barthelemy D, Lega JC, Grenet G, Gagnieu MC, Walter T, et al. Issues and limitations of available biomarkers for fluoropyrimidine-based chemotherapy toxicity, a narrative review of the literature. ESMO Open. 2021 Jun;6(3):100125.

37. Coenen MJH, Paulussen ADC, Breuer M, Lindhout M, Tserpelis DCJ, Steyls A, et al. Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time. Curr Ther Res. 2019 Jan 1;90:1–7.

38. Meulendijks D, Henricks LM, Jacobs BAW, Aliev A, Deenen MJ, de Vries N, et al. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. Br J Cancer. 2017 May;116(11):1415–24.

39. Etienne-Grimaldi MC, Boyer JC, Beroud C, Mbatchi L, van Kuilenburg A, Bobin-Dubigeon C, et al. New advances in DPYD genotype and risk of severe toxicity under capecitabine. PloS One. 2017;12(5):e0175998.

40. Pan W, Li Y, Feng Y, Yang F, Liu H. A new sample preparation and separation combination for the precise, accurate, and simultaneous determination of uracil and dihydrouracil in human plasma by reversed-phase HPLC. J Sep Sci. 2017 Oct;40(19):3763–70.

41. Tafzi N, Woillard JB, Fleytoux A, Picard N, Marquet P. Phenotyping of Uracil and 5-Fluorouracil Metabolism Using LC-MS/MS for Prevention of Toxicity and Dose Adjustment of Fluoropyrimidines. Ther Drug Monit. 2020 Aug;42(4):540–7.

42. INAHTA Brief - Screening for dihydropyrimidine dehydrogenase deficiency to decrease the risk of severe toxicities related to fluoropyrimidines (5-fluorouracil or capecitabine) [Internet]. [cited 2023 Dec 21]. Available from: https://www.inahta.org/upload/2019/19011_Screening%20for%20dihydropyrimidine%20dehydrogenase%20deficiency.pdf

43. Casneuf V, Borbath I, Van den Eynde M, Verheezen Y, Demey W, Verstraete AG, et al. Joint Belgian recommendation on screening for DPD-deficiency in patients treated with 5-FU, capecitabine (and tegafur). Acta Clin Belg. 2021 Jan 11;1–7.

44. Laures N, Konecki C, Brugel M, Giffard AL, Abdelli N, Botsen D, et al. Impact of Guidelines Regarding Dihydropyrimidine Dehydrogenase (DPD) Deficiency Screening Using Uracil-Based Phenotyping on the Reduction of Severe Side Effect of 5-Fluorouracil-Based Chemotherapy: A Propension Score Analysis. Pharmaceutics. 2022 Oct 6;14(10):2119.

45. Capiau S, Van Landschoot A, Reyns T, Stepman H. Pre-analytical considerations for the analysis of uracil and 5,6-dihydrouracil in heparin plasma. Clin Chem Lab Med CCLM. 2022 Apr 26;60(5):e112–5.

46. Henricks LM, Jacobs BAW, Meulendijks D, Pluim D, van den Broek D, de Vries N, et al. Food-effect study on uracil and dihydrouracil plasma levels as marker for dihydropyrimidine dehydrogenase activity in human volunteers. Br J Clin Pharmacol. 2018;84(12):2761–9.

47. Burns KE, Chavani O, Jeong SH, Duley JA, Porter D, Findlay M, et al. Comparison of a thymine challenge test and endogenous uracil–dihydrouracil levels for assessment of fluoropyrimidine toxicity risk. Cancer Chemother Pharmacol. 2021 May 1;87(5):711–6.

48. Callon S, Brugel M, Botsen D, Royer B, Slimano F, Feliu C, et al. Renal impairment and abnormal liver function tests in pre-therapeutic phenotype-based DPD deficiency screening using uracilemia: a comprehensive population-based study in 1138 patients. Ther Adv Med Oncol. 2023 Jan 1;15:17588359221148536.

49. Launay M, Guitton J, Balluet R, Moreau A, Phelip JM, Tholance Y, et al. Clinical considerations for DPD deficiency testing in advanced cancer patients: tumor lysis syndrome should be considered as a major interference. Ann Oncol. 2022 Aug;33(8):850-852.

50. Gaible C, Narjoz C, Loriot MA, Roueff S, Pallet N. Pretherapeutic screening for Dihydropyrimidine deshydrogenase deficiency in measuring uracilemia in dialysis patients leads to a high rate of falsely positive results. Cancer Chemother Pharmacol. 2021 Dec 1;88(6):1049–53.

51. Thomas F, Maillard M, Launay M, Tron C, Etienne-Grimaldi MC, Gautier-Veyret E, et al. Artificial increase of uracilemia during fluoropyrimidine treatment can lead to DPD deficiency misinterpretation. Ann Oncol. 2021 Jun;32(6):810–1.

52. Coudoré F, Roche D, Lefeuvre S, Faussot D, Billaud EM, Loriot MA, et al. Validation of an Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometric Method for Quantifying Uracil and 5,6-Dihydrouracil in Human Plasma. J Chromatogr Sci. 2012 Nov 1;50(10):877–84.

53. Dolat M, Macaire P, Goirand F, Vincent J, Hennequin A, Palmier R, et al. Association of 5-FU Therapeutic Drug Monitoring to DPD Phenotype Assessment May Reduce 5-FU Under-Exposure. Pharmaceuticals. 2020 Nov 23;13(11):416.

54. Pallet N, Hamdane S, Garinet S, Blons H, Zaanan A, Paillaud E, et al. A comprehensive population-based study comparing the phenotype and genotype in a pretherapeutic screen of dihydropyrimidine dehydrogenase deficiency. Br J Cancer. 2020 Sep;123(5):811–8.

55. Capitain O, Seegers V, Metges JP, Faroux R, Stampfli C, Ferec M, et al. Comparison of 4 Screening Methods for Detecting Fluoropyrimidine Toxicity Risk: Identification of the Most Effective, Cost-Efficient Method to Save Lives. Dose-Response. 2020 Jul 1;18(3):1559325820951367.

56. Knikman JE, Gelderblom H, Beijnen JH, Cats A, Guchelaar HJ, Henricks LM. Individualized Dosing of Fluoropyrimidine-Based Chemotherapy to Prevent Severe Fluoropyrimidine-Related Toxicity: What Are the Options? Clin Pharmacol Ther. 2021;109(3):591–604.

57. Etienne-Grimaldi MC, Pallet N, Boige V, Ciccolini J, Chouchana L, Barin-Le Guellec C, et al. Current diagnostic and clinical issues of screening for dihydropyrimidine dehydrogenase deficiency. Eur J Cancer. 2023 Mar;181:3–17.

International Liaison of Pathology Presidents (ILPP) Annual Meeting 2023

After a deferral of 3 years, Hong Kong could finally host the International Liaison of Pathology Presidents' annual gathering from 30 to 31 October 2023. The last time that the meeting was held in Hong Kong was in 2010.

The International Liaison of Pathology Presidents (ILPP) brings together Presidents, Presidents-Elect and Chief Executive Officers of Pathology Colleges or Associations of English-speaking countries/regions, to meet face to face annually to discuss matters relevant to Colleges and/or Associations. The Colleges or Associations are involved in pathology service delivery, with a particular emphasis on professional issues such as workforce, training, professional development, quality and safety, and accreditation.

This year's participants included representatives from Hong Kong, UK, USA, Canada, Australia, the European Union, Malaysia and the World Health Organization. The gathering was held immediately after the Fall Congress of the Hong Kong International Academy of Pathology, thus enabling many participants to attend both meetings.

Hot topics during this year's discussions included issues on manpower, the impact of artificial intelligence, and digital pathology. The annual event closed with an afternoon of social activities including a visit to Victoria Peak and a tram ride.

Dr CHEONG Renee Constance Yue-Kew
Treasurer and Editor of Newsletter Editorial Board




evian







The 19th Trainee Presentation Session

The 19th Trainee Presentation Session (TPS) took place successfully on 25th November 2023. There were a total of 28 trainees participating in this fully physical event at the venue Hong Kong Academy of Medicine Building. The topics covered various fields of pathology including forensic pathology and immunology in addition to the other subspecialities. We would like to thank our judges, Dr CHENG Hua Tse, Timothy, Dr CHAN Fuk Woo, Jasper, Dr WONG Hung Fan and Dr CHAU Kwok Fung, Tony, for their generous contribution and invaluable comments; and to thank the Education Committee Chairman, Dr LAI Koon Chi Christopher, the Education Committee members, HKAM/HKCPath staff as well as our TPS candidates and their supervisors for their hard work and support for the event.

Congratulations go to Dr CHENG Shui Kuen, who won the best oral presentation prize. Her topic was on "Antifungal Susceptibility Profile of Candida Species: 5 Years of Experience from a Tertiary Referral Hospital Laboratory in Hong Kong".

Dr YAU Tsz Wai
Vice-Chairman
Education Committee

Below:

Left: Dr LAI Koon Chi, Christopher, Chairman of Education Committee, together with Dr YAU Tsz Wai, Vice-Chairman of the Education Commitee interacting with this year's judges

Right: Dr LAI Koon Chi, Christopher, presenting Certificate of Appreciation to Dr CHENG Shui Kuen, winner of the best oral presentation prize





Left: Judges panel from left to right, upper to lower: Dr CHENG Hua Tse, Timothy, Dr CHAU Kwok Fung, Tony, Dr CHAN Fuk Woo, Jasper and Dr WONG Hung Fan.

Below (multiple): Some participants of the TPS and posters

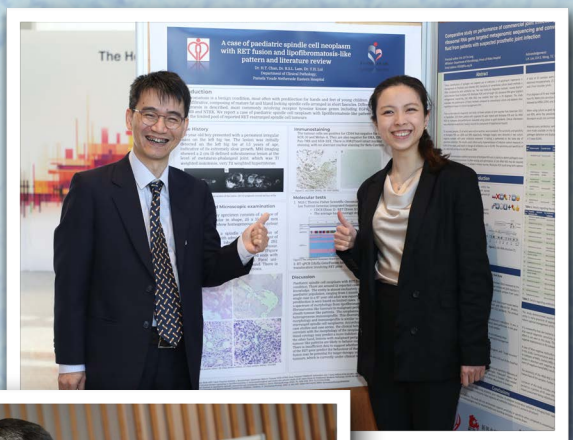




















Abstract of Prize Winner of Best Presentation Award:

Antifungal Susceptibility Profile of Candida Species: 5 Years of Experience from a Tertiary Referral Hospital Laboratory in Hong Kong

Dr CHENG Shui Kuen, Clinical Microbiology & Infection, Department of Pathology, United Christian Hospital

Background: Fungal infections are becoming increasingly common. On top of limited antifungal options, emerging antifungal resistance further compounds this public health threat. However, antifungal susceptibility testing (AFST) is only routinely performed in some laboratories due to technical complexity and cost constraints. Candida species were included by WHO in the Global Antimicrobial Resistance Surveillance System (GLASS) to monitor the resistance. Such data is still scarce at a local level.

Objectives: To review the antifungal susceptibility profile of Candida species in a tertiary referral hospital laboratory in Hong Kong

Method: Candida isolates submitted to the Microbiology Laboratory of United Christian Hospital for AFST from June 2018 to May 2023 were identified on the Laboratory Information System (LIS). The minimal inhibitory concentrations (MICs) were determined by colorimetric assay, Sensititre YeastOne (SYO). The MICs were interpreted according to species-specific categorial endpoints as per CLSI guidelines

Results: A total of 296 Candida isolates were retrieved, including 85 C. albicans, 92 C. glabrata, 8 C. krusei, 70 C. parapsilosis complex and 32 C. tropicalis. Among all isolates, 265 (89.5%) were from normally sterile sites. 184 (62.2%) isolates were recovered from blood, of which 29 (15.8%) were from ICU patients. C. glabrata and C. albicans were the most prevalent bloodstream isolates. Approximately 44% of bloodstream isolates were either non-susceptible/ non-wild-type (NWT) or known to exhibit intrinsically low susceptibility to fluconazole. Resistance to anidulafungin, micafungin, and caspofungin were 1.7%, 1.4% and 2.0% respectively among all Candida species with interpretive breakpoints. Notably, 65.6% of C. tropicalis isolates were classified as NWT if their MICs generated by SYO were interpreted using the epidemiological cutoff value (ECV) of posaconazole.

Conclusion: Antifungal susceptibility data is essential in epidemiological surveillance to combat resistant fungi and direct treatment decisions. ECVs from CLSI should be used with caution to evaluate the MICs generated by colorimetric method.

Experience of participation in the 19th Trainee Presentation Session

The Trainee Presentation Session organized by the Hong Kong College of Pathologists is certainly one of the peaks in our training journey. It inspires us trainees to refine the necessary skills to assume the title of fellow and strive for academic excellence in the complex landscape of clinical service. The experience of exchanging original perspectives on various topics in the diverse field of pathology opens up the world to us. It is also a proud moment for us to showcase to esteemed panel of judges and distinguished audience our countless hours of hard work and passion for pathology. Being awarded the accolade of Best Trainee Presentation is definitely an unexpected and motivating bonus for me.

Personally, the most challenging step I find in the preparation of a high-quality trainee presentation is to select one single topic that holds significant value across a multitude of subjects and can be efficiently delivered within the given time limit. Antifungal susceptibility testing is an essential component of successful antifungal stewardship to combat the ever-increasing fungal infections. I believe sharing of local epidemiology and antibiogram of Candida species can engage the audience and pique their interest in this public health concern. By highlighting pitfalls in the interpretation of data using commercial antifungal susceptibility testing method, it is hoped that the audience can gain practical tips in their daily routine.

Again, I am extremely honoured to receive the Best Trainee Presentation Award. This recognition is not just a testament to my personal effort, but to the high commitment of my educational supervisors and fellow colleagues in supporting training and development in a bustling workplace. My heartfelt appreciation goes to the College and all the participants. The Trainee Presentation Session would not have been such a great success without the unwavering dedication and contribution by each and every one. Looking forward to another successful College event in the near future!



The 32nd Annual General Meeting

The 32nd Annual General Meeting (AGM) was held in the afternoon of 25th November 2023. Eight Councillors were elected. Six of them were in previous Council 2022/23: Dr MAK Siu Ming was elected as President; Dr LEUNG Yuk Yan, Rock was elected as Vice-President; Dr AU YEUNG Kwok Him, Rex was elected as Registrar; Dr WONG Lap Gate, Michael was elected as Deputy Registrar; Dr LEUNG Ying Kit were elected as Council Member; while Dr WONG Chi Kin, Felix was re-elected as Council Member. Two new Council Members were elected: Dr CHEN Pak Lam, Sammy and Dr FUNG Ngai Sheung. We would like to take this opportunity to thank outgoing President Dr CHAN Chak Lam, Alexander and outgoing Council Member Dr CHAN Kui Fat for their contributions to College Council.







Members of College Council 2023/24

Front row from left to right:

1. Dr AU YEUNG Kwok Him, Rex **(Registrar)**
2. Dr LAI Koon Chi, Christopher **(Vice-President)**
3. Dr CHAN Chak Lam, Alexander **(Immediate Past-President)**
4. Dr MAK Siu Ming **(President)**
5. Dr LEUNG Yuk Yan, Rock **(Vice-President)**
6. Dr CHEONG Renee Constance Yue-Kew **(Honorary Treasurer)**
7. Dr WONG Lap Gate, Michael **(Deputy Registrar)**

Back row from left to right:

1. Dr NG Hoi Yan, Joshua
2. Dr POON Wai Ming
3. Dr Siddharth SRIDHAR
4. Dr WONG Chi Kin, Felix
5. Dr CHEN Pak Lam, Sammy
6. Dr FUNG Ngai Sheung
7. Dr CHENG Shui Ying
8. Dr LEUNG Ying Kit



The 31st Conferment Ceremony

Fellows and Members admitted to the College in 2023 AGM were invited to this Conferment Ceremony. In 2023 AGM, 19 Fellows and 9 Members were admitted to the College. 4 Fellows passed the Fellowship Assessment in Genetic and Genomic Pathology in 2023.

Honourable guests included Prof LEUNG Ka Kit, Gilberto, President, Hong Kong Academy of Medicine; Dr LI Mun Pik, Teresa, Deputy Director of Health, Department of Health, HKSAR; Dr Hon LAM Tzit Yuen, David, Legislative Councillor (Medical and Health Services), Legislative Council, HKSAR; Dr LAW Chun Bon, Cluster Chief Executive, Kowloon West, Hospital Authority, HKSAR.







Congratulations!











What a joyous occasion!


The Hong Kong College of Pathologists
香港病理學專科學院


The Hong Kong College of Pathologists
香港病理學專科學院









Many thanks to our distinguished guests for their support!



Snapshots of our President presenting certificates to new Fellows of the College!



CHAN Ka Yin



CHAN Tina Yee Ching



CHEUNG Kevin Ka-Chun



LAI Shui Wun



LAM Ki



LAM Wing Kit



LEE Lok Hang



LEUNG Ho Wai



LI Wai Yan, Jamilla



LI Xin



LING Tsz Ki



MOK Ka Kin



TONG Hok Fung



TSANG Cheuk Ho



WONG Tak Siu



WONG Yuen Sze, Sivia



YUEN Ka Wai, Karen

Welcome, new Members of the College! Congratulations also to Fellows who successfully passed the Fellowship Assessment in Genetic and Genomic Pathology!



CHAN Hoi Tung



CHENG Shui Kuen



LEE Chung Ho



LU Jianlin



YUET Kam Ting



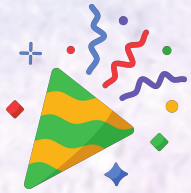
AU Yuen Ling, Elaine



LEUNG Mei Tik



Congratulations & Thank You



We would like to express our gratitude to our College Secretary Ms Dilly YIP for her support in organizing the event. A big thank you to Dr Victoria TSE for being the Mistress of Ceremonies at the AGM and Conferment Ceremony. We would also like to thank our photographer Mr Kevin KU for his professional work, and our helpers Ms YEUNG Yuen Mei, Ms NG Wing Ka and Ms IP Ka Fun for their help.





The 31st T.B. Teoh Foundation Lecture

The 31st T.B. Teoh Foundation Lecture was delivered by Professor LAU Chak Sing, Dean of the Li Ka Shing Faculty of Medicine, The University of Hong Kong on the topic "Everything Immunology, from diagnosis to treatment". Prof LAU shared his insights and experiences on the mechanisms of autoimmnue diseases, recent advances in therapy, and the latest development of clinical allergy service in Hong Kong. The lecture was very informative, and sheds light on the importance of collaboration between pathologists and our clinical colleagues, as well as how the pathology laboratory helps in the management of patients with immunological diseases.

Above left:

Prof LAU delivering his lecture

Above right:

Prof LAU and his attentive audience

Below left:

Our President, Dr MAK Siu Ming, presenting a souvenir to Prof LAU

Below right:

Dr LAI Koon Chi, Christopher, introducing the speaker





College Dinner 2023








7













What a wonderful gathering!

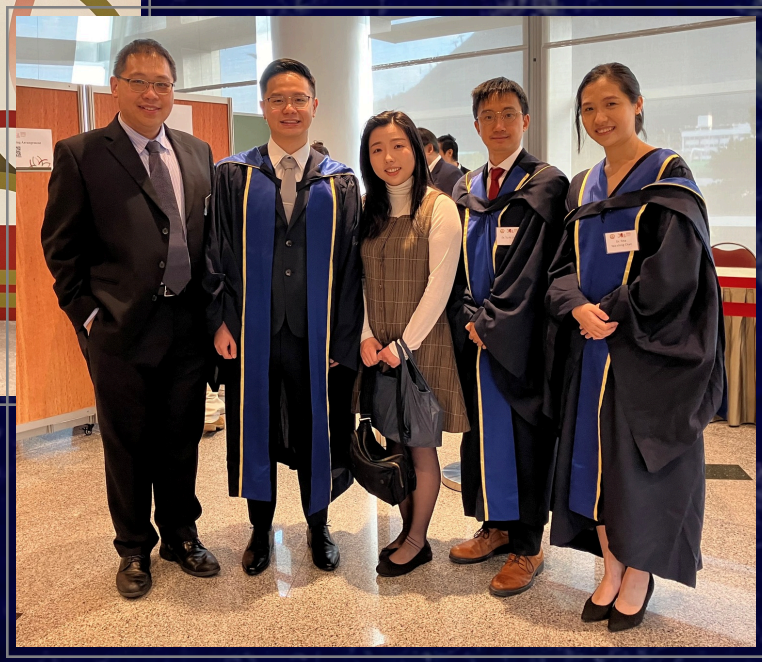


The 30th Annual Fellowship Conferment Ceremony of the Hong Kong Academy of Medicine

We are thrilled to join a momentous occasion of the HKAM Annual General Meeting, 30th Annual Fellowship Conferment Ceremony and 2023 David Todd Oration cum Pearl Jubilee Gala Dinner at the Academy building. This is a time to celebrate their hard work, perseverance, and the incredible journey the new Fellows have undertaken to become dedicated pathologists. The College is immensely proud of their achievements and knows that they will continue to make significant contributions to the field of healthcare.







Best Original Research by Trainees (BORT) of the Hong Kong Academy of Medicine



Clinical laboratories are facing the challenges of manpower shortages as well as increasing workloads from new specialized tests and an aging population. In α-thalassaemia screening, the commonly used Haemoglobin H (HbH) inclusion test is cumbersome and labour-intensive, involving a manual search for a low number of HbH inclusion bodies within the blood film. Additionally, the HbH inclusion test has a limited sensitivity of 70-80% in diagnosing α-thalassaemia trait carrying the reproductively significant SEA type deletion, prevalent in the Chinese population. Through serendipity, I learnt about a potential alternative method to diagnose α-thalassaemia traits by using the early eluting peaks in high-performance liquid chromatography.

In my project, we described the presence of an early eluting peak present in patients with α-thalassaemia trait that is not seen in patients without α-thalassaemia trait. This was a novel finding not described before in the medical literature. We named the peak the "α-thalassaemia early eluting peak (αEEP)". We demonstrated that the αEEP is more sensitive and specific than the HbH inclusion test for diagnosing α-thalassaemia traits with the SEA type deletion.

Shortly after completing the study, I received an email from the Hong Kong Academy of Medicine (HKAM) about the Best Original Research by Trainees (BORT) 2023. The BORT provided an excellent opportunity to share our research findings with colleagues from diverse backgrounds and learn from their innovative ideas. I was honoured to be shortlisted as a finalist and present our work at the final presentation session. The discussion section of the final presentation was particularly valuable, as it allowed me to receive constructive feedback and insights from esteemed judges who are experts in research. I was impressed by the high quality of research presented by the other finalists, showcasing the breadth and depth of research being conducted by trainees in Hong Kong. After the presentation session, all finalists received a splendid souvenir, either a tie or scarf made for the 30th anniversary of HKAM.

It was a breathtaking moment when I was announced to be the winner of the Silver Medal of BORT 2023 during the HKAM annual dinner. It was a perfect conclusion to my six years of specialist training in haematology, as well as an excellent start for my new career stage as a new fellow. I am indebted to my supervisor, Dr YIP Sze Fai, for his unwavering support, and to Dr Eudora CHOW for making this collaboration possible. I would also like to thank the other seniors and colleagues for their invaluable support and assistance throughout the project.

Finally, I am grateful to the College for the invitation to share my experience in the Newsletter of the College. I would encourage other pathology trainees to embrace research and participate in competitions like BORT. These experiences not only enhance our professional development but also contribute to the advancement of medical knowledge and patient care.

Dr LAM Wing Kit

Winning article - A novel early eluting peak in high-performance liquid chromatography as a more sensitive and specific marker for diagnosis of α-thalassaemia trait in Chinese population

Wing Kit Lam[1], Winnie Yim Fong Law[1], Tsz Fung Wong[1], Vivian Hoi Kei Woo[1], Aves Hui Hsuan Wu[2], Eudora Yu De Chow[2], Ka Ngai Lau[1], Alice Ching Ching Wong[1], Sze Fai Yip[1]

[1]Department of Clinical Pathology, Tuen Mun Hospital, Hong Kong

[2]Department of Pathology, United Christian Hospital, Hong Kong

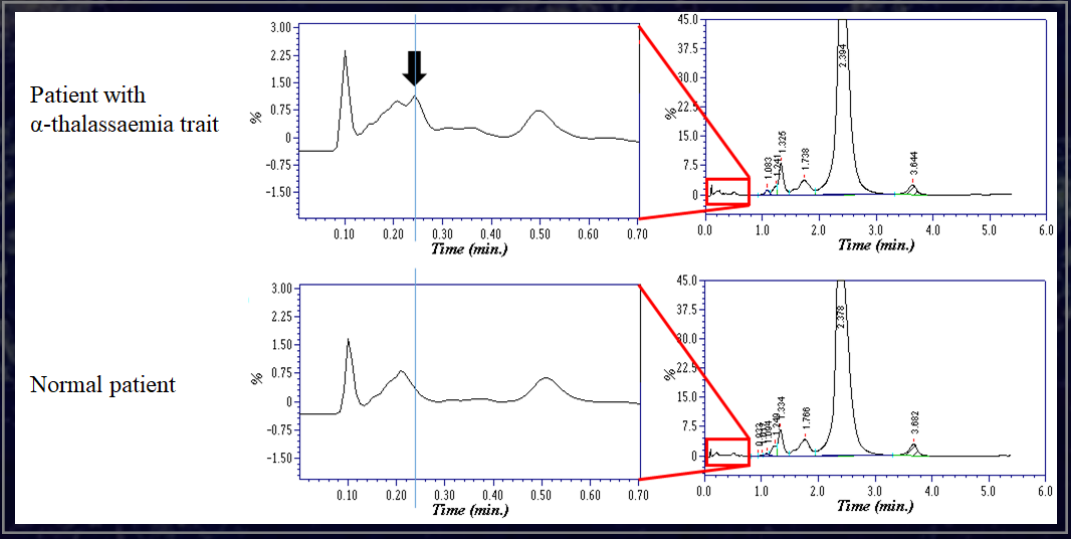
Objective:
The α-thalassaemia screening is commonly based on Haemoglobin H (HbH) inclusion test which is labour-intensive and time-consuming. We aim to investigate the use of a novel α-thalassaemia early eluting peak (αEEP) observed at the retention time of 0.24 min generated by Variant II high-performance liquid chromatography (indicated by the arrow in the Figure) as a potential alternative for α-thalassaemia screening compared with HbH inclusion test in our population.

Study design:
303 archived blood samples for haemoglobin pattern study were analysed for the presence or absence of αEEP and HbH inclusions with blinded observers. Discordant results between αEEP and HbH inclusion test were tested with the genotyping study. The performance characteristics of αEEP and HbH inclusion test were compared.

Results:
The concordance rate of αEEP and HbH inclusion body test was 96.0% (κ=0.921, p<0.001). 6 cases with negative HbH inclusion test but positive αEEP showed heterozygous Southeast Asian (SEA) type deletion by genotyping study. Among the 4 cases with negative αEEP but positive HbH inclusion test, 2 cases showed heterozygous SEA type deletion,1 case showed nondeletional HbQS mutation and 1 case showed no mutation. The performance of αEEP for detecting SEA deletion (sensitivity 93.8%, specificity 100%) was superior to the HbH inclusion test (sensitivity 81.3%, specificity 95.2%). Inter-observer assessment of the αEEP showed perfect agreement. Staff operation time of the αEEP (10 minutes per 100 cases) was significantly shorter than the HbH inclusion test (1800 minutes per 100 case).

Conclusion:
The novelly-described αEEP was found to be a more sensitive and specific method than the HbH inclusion test in detecting the reproductively significant α-thalassaemia trait with SEA type deletion. The αEEP could be a potential alternative of HbH inclusion test for α-thalassaemia screening, with over 98% reduction in reagent and labour cost.

This work has been published in the journal Pathology (https://www.sciencedirect.com/science/article/pii/S0031302523001678?via%3Dihub)



Hong Kong Museum of Medical Sciences Fundraising Dinner - 21 November 2023

The Hong Kong Museum of Medical Sciences is one of Hong Kong's few independent museums. The museum benefits Hong Kong's youth and the local community through various educational programs and exhibitions. We had the pleasure of attending the Hong Kong Museum of Medical Sciences Fundraising Dinner on 21st November 2023. The event featured inspiring speech, live entertainment and auction! A great chance to network with other medical professionals and advocates!









10th Anniversary Celebration Ceremony of the Hong Kong Jockey Club Innovative Learning Centre for Medicine

Our College President, together with two Vice-Presidents, attended the 10th Anniversary Celebration Ceremony of the Hong Kong Jockey Club Innovative Learning Centre for Medicine (HKJC ILCM) at the Academy building. The Innovative Learning Centre for Medicine (HKJC ILCM) was inaugurated in December 2013 to promote innovative learning and research among healthcare professionals to deliver safe and quality patient care. One of the major roles of HKJC ILCM is faculty development for simulation based medical education.



Dr LAI Koon Chi, Christopher (Vice-President, Dr MAK Siu Ming (President) and Dr LEUNG Yuk Yan, Rock (Vice-President) at the Celebration Ceremony

The Hong Kong Academy of Medicine Distinguished Young Fellows Award 2023

Heartfelt congratulations to Dr CHENG Hua Tse, Timothy on being selected as one of the "Distinguished Young Fellows" in 2023! This award recognises outstanding achievements of young Fellows by Hong Kong Academy of Medicine. This is a well-deserved award for Dr CHENG, in recognition of his exceptional contributions to the field of chemical pathology, particularly in molecular diagnostics, and his dedication to education and College affairs.

Dr Timothy CHENG obtained his Bachelor of Medicine and Bachelor of Surgery from the Chinese University of Hong Kong in 2009. In 2010, Dr Cheng was awarded a Rhodes Scholarship to pursue doctoral studies in cancer genetics at the University of Oxford, where he was a Junior Research Fellow and Junior Dean at Pembroke College. His research interest includes molecular and population genetics.



Professor Gilberto LEUNG (President of the Hong Kong Academy of Medicine), Dr Timothy CHENG and Dr MAK Siu Ming (President of our College)

International Pathology Day 2023

International Pathology Day is an annual awareness day dedicated to promoting the importance of pathology in healthcare. The theme of "How Pathologists Save Lives!" was adopted for the annual workshop this year. The workshop took place in the Pathology Student Laboratory, 1/F, Lui Chi Woo Clinical Sciences Building, Prince of Wales Hospital on 16th December 2023 to engage the future generation to the meaningful roles of pathology disciplines in medicine. Around 150 students from 48 local secondary schools attended the occasion. After a warm welcome and brief introduction of pathology by our College Vice-President, Dr Leung Yuk Yan, Rock, attendees were divided into seven groups to learn about the practice in the seven disciplines of pathology by rotation. From the lively demonstrations and hands-on practical sessions, the attendees interacted with pathologists from various disciplines to learn about discipline-specific technologies and have a preview of the work nature of pathology.

Dr IP Ho Wan
Secretary, Professional and General Affairs Committee





Upper left:

Vice-President of the Hong Kong College of Pathologists, Dr Leung Yuk Yan, Rock, welcomed the attendees

Lower left:

Attendees took turns to rotate through all the seven stations of pathology disciplines, learning from clinical scenarios and interactions with our pathologists

Lower right:

Workshop poster outlining the seven disciplines of pathology and their discussion topics in the workshop



Regulations on Postgraduate Training and Examinations 2023 – Updates and Implementation

The Forum "Regulations on Postgraduate Training and Examinations 2023 – Updates and Implementation", organized by the Training and Examinations Committee (TEC), took place in the evening of 30 April 2024. More than 30 Educational Supervisors, Trainers and Trainees from different training centres and disciplines attended the event. After an introduction delivered by the President Dr Mak Siu Ming, the Chairman of TEC and Vice President Dr Rock Leung described the history of past Regulations and gave an overview of the new Regulations on Postgraduate Training and Examinations 2023 ("2023 Regulations"), highlighting the need for workplace-based assessment, assessment on ethics and professionalism and the unified minimum Trainer-to-Trainee ratio of 1:3 across all disciplines. This is followed by Chief Examiners or their delegates speaking on discipline-specific updates in Regulations and/or logbooks. Dr Felix Wong, TEC secretary, announced the effective date of the 2023 Regulations to be 13 May 2024 (Monday) and the administrative procedures required for trainees registered before 13 May 2024 to adopt 2023 Regulations if they so wish. Trainees registered on or after the effective date will follow the 2023 Regulations. The event was concluded with a Q&A session with lively discussions and exchange of ideas between the speakers and the audience.

Dr WONG Chi Kin, Felix
Secretary, Training and Examinations Committee





From left to right, top to bottom:

1. Dr MAK Siu Ming, President;
2. Dr LEUNG Yuk Yan, Rock, Vice-President and TEC Chairman;
3. Q&A session;
4. Group photo





Lecture by Professor Noel Woodford

We were enlightened by a lecture given by Professor Noel Woodford, Director of the Victorian Institute of Forensic Medicine (VIFM) on 16th April 2024. He gave a potted history of the VIFM and the development of forensic pathology services in Melbourne, and shared VIFM quality assurance practices. He also discussed some recent developments in the field including the application of various imaging modalities in Coronial death investigation. Professor Noel Woodford's lecture was truly captivating, leaving the audience in awe of the profound knowledge and insightful perspectives. College Council Members also had dinner with him on 17th April 2024 in the Hong Kong Academy of Medicine Building.









Dinner with Prof Noel WOODFORD

Front row (from left to right): Dr LAI Koon Chi, Christopher; Dr MAK Siu Ming; Prof Noel WOODFORD; Dr LEUNG Yuk Yan, Rock
Back row (from left to right): Dr POON Wai Ming; Dr CHAN Chak Lam, Alexander; Dr WONG Chi Kin, Felix; Dr CHEN Pak Lam, Sammy; Dr AU-YEUNG Kwok Him, Rex

Pallas's cat (Manul)

KEY FACTS

By Dr LO Hui Yin

TERMINOLOGY

- 兔猻
- *Otocolobus manul*
- *Otocolobus*: Ugly-eared in Greek
  Manul: wild cat in Mongolian
- *Pallas*: after Prussian naturalist Peter Simon Pallas

DISTRIBUTION AND CONSERVATION STATUS

- Distribution: Central and Western Asia including China, and southern Russia
- Conservation status: Least concern

BEHAVIOUR AND ECOLOGY

- Solitary
- Females give birth to 2-6 kittens
- Adult size by around 6 months, sexually mature at around 1 year old
- Lifespan: around 6-10 years
- Diet: small mammals e.g. rodents, small birds, and insects

MACROSCOPIC

- Size of a domestic cat
- 46-65 cm long, with a 21-31 cm long tail
- Weighs 2.5-4.5 kg
- Low set and round ears
- Round pupils with yellowish iris
- Grey fur with black stripes on the back
- Grey tail with black rings and black tip

DIFFERENTIAL DIAGNOSES

- It should be a spot diagnosis!

(Left) *Pallas's cats have black spots over the top of their head and forehead*
(Right) *They have two black lines on their cheeks, from the corner of eyes to angle of jaws*





Pallas's cat (Manul)

(Left) *They have thin black stripes on their back* **(Right)** *Their fur is light grey in colour, with pale yellowish reddish hues*



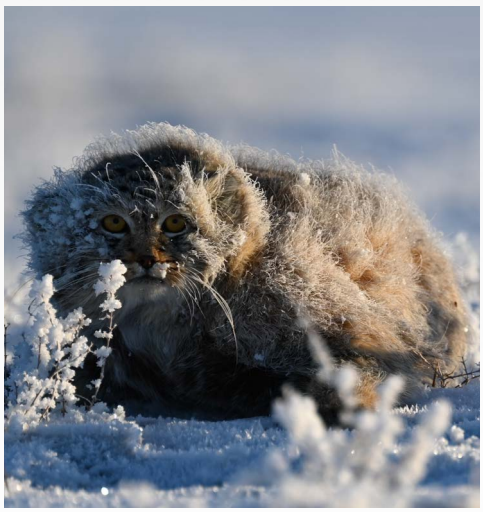


(Left) *They have grey fluffy tail with seven black rings and a black tip* **(Right)** *They have short legs and are slow for cats*





(Left) *They have long and fluffy fur, especially in winter, measuring up to 7 cm long* **(Right)** *They use caves, rock crevices and burrows as shelters and dens*





Photos were taken in the Mongolian Steppe.

From left to right, top to bottom: ***Figure 1:*** *Pot plant series. From left to right: daisy (modified from Herman Lau's design), cactus, rose, sprout and sunflower (modified from Pham Hoang Tuen's design). These are my very first money origami artworks. Each pot is labeled with the amount printed on the banknote.* ***Figure 2:*** *Bonsai. This is a more complex design based on the sprout's design in figure 1.* ***Figure 3:*** *Cactus with spines. This is based on Robert J Lang's design and folded by me with one uncut 50 Hong Kong dollar banknote.* ***Figure 4:*** *Buddha on lotus. This is designed and folded by me to celebrate the Buddha's birthday.*



Figure 1



Figure 2



Figure 3



Figure 4

Beyond the Microscope: The art of money origami – Exploring creative ways to fold currency

Dr CHEUNG Chun Kei

"Is this folded from one piece of paper?" "Have you made any cuts?" These are the questions I often get asked. All the artworks you see in this article adhere the modern rules of origami! Each artwork is folded with a piece of uncut Hong Kong dollar banknote.

Origami is the Japanese art of paper folding. Although the word origami is Japanese, its origin is likely to be in

From left to right, top to bottom: ***Figure 5A & B:*** *Male and female lions in Qing dynasty. Both are designed and folded by me.* ***Figure 6:*** *The Lion King. My inspiration came from Disney's movie.* ***Figure 7A, B, C & D:*** *Travel series - Lion in USA, Egypt, Mexico & Iceland. These are some of my designs from the travel series. I want the HSBC lion to wear different costumes from different countries.* ***Figure 8:*** *Lion with a mask. I designed and folded this during the COVID-19 pandemic.*



Figure 5A



Figure 5B



Figure 6



Figure 7A



Figure 8



Figure 7B



Figure 7C



Figure 7D

China, after the invention of paper. I find origami fascinating. By applying different types of folds, one can transform an ordinary piece of paper into almost anything. I started doing origami when I was small, but I only got serious about origami when I was in medical school. During that time, I have folded numerous complex models from different artists, which laid a good foundation for future designing.

There are several reasons why I choose to fold using a banknote. Firstly, there aren't many designs using rectangular paper, so I need to

From left to right, top to bottom: ***Figure 9:*** *Taxi. I used the Shang Hai building as the grille of the taxi.* ***Figure 10****: Tissue box. This is modified from Francis Ow's design and folded by me with one uncut 50 Hong Kong dollar. The white part of the banknote is used as tissue.* ***Figure 11A, B, C & D:*** *Nostalgic item series - Gramophone, typewriter, telephone and alarm clock. All four artworks are designed and folded by me.* ***Figure 12 A & B:*** *The kitchen series - Fried egg on a pan & gas stove. Figure 11A is a good example of using different colors on the banknote. The egg white and yolk use the white and gold areas of the banknote, while the remaining part of the banknote is used to fold the pan.* ***Figure 13:*** *Pineapple. Using a similar concept as the cactus in figure 1 and 3. I designed and folded this 3D pineapple.*



Figure 9



Figure 10



Figure 11A



Figure 11B



Figure 11C



Figure 12D



Figure 12A



Figure 12B



Figure 13

force myself to create my own design. Secondly, the time to design a model won't be too long, as the size of banknote is restricted. Lastly, I can use the printed patterns and figures on the banknote to make an unique artwork that a plain piece of paper couldn't achieve.

The paper quality of the banknote is quite ideal for folding. It is tough and durable enough to withstand numerous folds without breaking. Although it is not the thinnest paper, it is thin enough to fold most designs. Having said that, folding a banknote has some drawbacks. Due to its

From left to right: ***Figure 14A & B:*** *The Olympic series - Lion with a gold medal & woman with the Olympic torch. Both are designed and folded by me to celebrate Hong Kong's first gold medal in Tokyo Olympic Games 2020.* ***Figure 15 A & B:*** *The Chinese zodiac series - the Year of the Dragon (Loong) & Ox. The models are designed by eagle and Lien Quoc Dat respectively.*



Figure 14A



Figure 14B



Figure 15A



Figure 15B

limited size, folding an extremely complex model is almost impossible. In addition, shaping the paper can be quite challenging, as the folded banknote doesn't hold its shape easily, especially in complex designs.

Banknotes from different countries have different sizes. The Hong Kong dollar banknote is made up of two squares, forming a rectangle. The Hong Kong dollar is quite unique as it is issued by the Government of Hong Kong Special Administrative Region or by three different banks, namely the Hongkong and Shanghai Banking Corporation Limited (HSBC), the Standard Chartered Bank (Hong Kong) Limited, and the Bank of China (Hong Kong) Limited. Banknotes issued by different banks and governments have different designs and patterns. My personal favorite is the HSBC lion on the 2010 series banknotes. Many of my designs are based on it. If I have to design something involving a human figure, I tend to use the opera lady on the 100 Hong Kong dollar banknote of the 2018 series issued by the Bank of China (Hong Kong) Limited. Most of Hong Kong banknotes are made from paper, except the ten-dollar note which can be a polymer note. I have tried to fold with a polymer note and it's a nightmare.

My inspiration usually comes from what I like and what is happening around me. It often takes several hours to days, through trial and error, to finalize my design. Folding a complex design using a banknote is not an easy task. For delicate folds, fingers are just too big and I often have to use a pair of tweezers to complete the job. Some of the most complex designs require numerous folds and precise shaping to capture the details. These designs often challenge my skills and patience, but the end results are rewarding. These complex designs pushed the boundaries of what could be achieved with a banknote, showcasing the versatility and artistic potential of origami.