出國報告（出國類別：進修）

2019 美國乳癌基礎研究的最新發展—類器官(Organoids)培養模式

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出國期間：2018/08/01-2019/07/31
報告日期：2019/09/02

摘要

過往癌症的基礎研究模式，若非由已建立的癌細胞株(cell line)開始研究，就是從人源化腫瘤異種移植(patient-derived xenograft, PDX)來進行。然而單一癌細胞的研究無法完整表達整個癌症腫瘤的面貌，而 PDX 的培養又需花費大量的時間與金錢，因此類器官(organoids)的培養成了另一種癌症研究的模式。

類器官是一種三維立體結構的細胞培養，其空間組織結構與器官相似。一來相對於二維的癌細胞株培養更能完整表現原始腫瘤的特性，二來類器官培養的成本遠低於 PDX 培養，生長效率也遠勝於 PDX。類器官更能直接從病人端取出的腫瘤組織，直接進行類器官培養。因此可以預先檢測藥物的敏感性與耐受性。

本次美國進修主要為學習如何利用類器官培養模式，來進行乳癌腫瘤對於藥物的敏感性與耐受性，以達到精準醫療之目的。

關鍵字

乳癌，基礎研究，類器官，癌症，腫瘤，藥物測試

目次

一、目的

本次美國進修主要為學習如何利用類器官培養模式及技術，來進行乳癌腫瘤對於藥物的敏感性與耐受性，以達到精準醫療之目的。

二、過程

2018 年 8 月

8 月 1 日正式到美國洛杉磯希望之城國家醫學中心報到。希望之城國家醫學中心為大洛杉磯地區專治癌症及糖尿病之醫學中心。希望之城成立於 1913 年，其組織包含希望之城 Helford 臨床研究醫院(City of Hope Helford Clinical Research Hospital)、貝克曼研究中心(Beckman Research Institute of City of Hope)、生物製藥含遺傳學中心（Center for Biomedicine & Genetics)，以及希望之城研究所（City of Hope Irell & Manella Graduate School of Biological Sciences)。希望之城國家醫學中心聞名因其領先的基礎研究轉譯工作與臨床治療技術，更是全美骨髓移植和遺傳學領域的先驅，已施行了超過 12,000 例骨髓及幹細胞的移植手術。希望之城著名的貢獻即在 1978 年成功合成人類胰島素。美國國家癌症研究(National Cancer Institute）在癌症的治療，研究，預防以及學術指導方面授予希望之城國家醫學中心最高認可。

2018 年 9 月

希望之城有著嚴謹的訓練制度。前三個月必須接受新進人員教育訓練、實驗室安全教育訓練、動物室安全教育訓練以及動物室麻醉手術訓練。課程結束後仍須完成測驗及格才能進入動物室和開始進行實驗。本月所修習的課程名稱如下：1.Rodent Survival Surgery, 2.COH/BRI Animal Care and Use Program & DCM/ARC Orientation, 3.Handling, Restraint & Biomethodology, 4.Anesthesia & Aseptic Techniques

2018 年 10 月

在前三個月基礎訓練期間，即開始學習如何進行類器官培養。

實驗步驟如下：

Procedures

A. Processing solid tissue into fragments

1. Place the solid tumor tissue (size can vary) in a 10 cm petri dish, pouring sterile DMEM medium on the tissue to keep it moist.
2. Cut the tumor into pieces using a scalpel or razor blade, removing necrotic tissue if present.

B. Processing solid tissue into organoids

1. Weigh the tissue.
2. Mince the tissue using a crisscross motion with two disposable scalpels until finely chopped, and
then transfer the minced tissue to a 50 mL conical vial using a cell lifter.
3. Add digestion buffer to a final volume of 10 mL per gram of tissue.

a. For primary human specimens: Add 10× Collagenase Solution and 100× Hyaluronidase Solution
(see REAGENTS AND SOLUTIONS) to a final concentration of 1× each.
a. For tumor graft tissue: Add only 10× Collagenase Solution to a final concentration of 1×.
4. Pipette up and down for ~2-3 minutes to further break up tissue clumps.
5. Take a 25ul sample of the digestion media and dilute 1:10-1:100 in PBS and place 25ul of the
dilution in an empty well of a 24 well standard tissue culture dish. Look for large refractory clusters of epithelial cells, called organoids. More digestion may be appropriate if most of the organoids are
>200um diameter. If the tissue does not digest well, remove all undigested fragments and debris by
straining the digested tissue into a new tube using a 100um cell strainer. Keep the digestion media that passes through the strainer and visualize under a microscope as described above. If organoids are
present, continue protocol with this solution at step 6. Place the strained material in a 50 mL conical
tube, add fresh digestion buffer, then shake at 37 degrees C at 200 rpm (checking frequently) more
hours to recover more digested tissue. Continue with step 6 when most organoids are <200um diameter. (Only perform this step if there are not enough organoid present in previous step)
6. Centrifuge at 530 × g at room temperature for 5 min to pellet the cells.
7. Aspirate the supernatant, which contains fat. If the pellet contains red blood cells (observed as a red layer on top of the pellet), resuspend the pellet in 5 – 10 mL of TAC buffer and incubate 8 min in a 37˚C water bath. Centrifuge at 530 × g at room temperature for 5 min. Repeat this step until the red
blood cells are no longer visible. Steps 8-9 in this protocol function to separate organoids, which
contain most of the tumor epithelium, from stromal cells and debri using a process called differential
centrifugation.
8. Resuspend the pellet in 10 mL of DMEM/F12 medium (with no supplements) and centrifuge at 530× g at room temperature for 10-30 sec. collect both the supernatant (suspension fraction) and pellet(organoid fraction). Resuspend each fraction in 1ml DMEM/F12 medium (with no supplements). Take a 10-25ul sample of the fraction media and dilute 1:10-1:100 in PBS and place 25ul of the dilution in an empty well of a 24 well standard tissue culture dish. Examine the quality of the fraction under a light microscope. The organoid fraction should be more enriched for refractile, solid clusters of tumor cells, termed “organoids,” and few single cells (Figure 3). The suspension fraction

should be enriched in single cells and be relatively free of organoids. Continue fractionating by differential centrifugation as described in step 9.
9. For the organoid fraction: resuspend the pellet in 9 mL of DMEM/F12 medium (with no
supplements) and centrifuge at 530 × g at room temperature for 10-30 sec to pellet the cells. To further enrich organoids from single cells, perform differential centrifugation by repeating this step 3-4 times until the desired organoid enrichment is achieved.
10. Estimate the number of organoids in the final sample and calculate the approximate total number of organoids recovered from the tissue. The 24 well plate containing the 25 μl samples used to assess the fractionation procedure (step 9) can be cultured overnight in 0.5 mL of HBEC medium/well to
determine whether or not the sample is contaminated with bacteria or fungus.

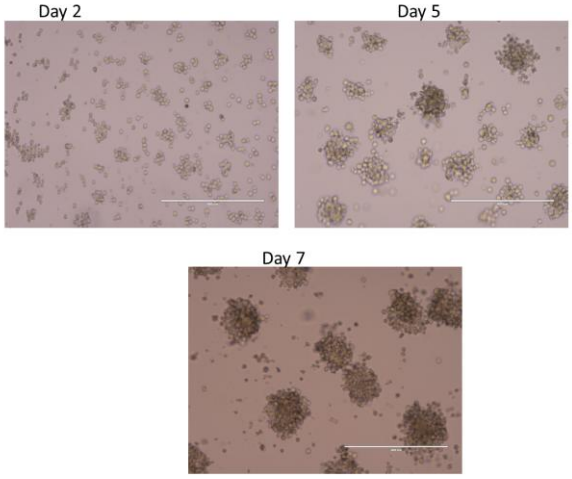
Three-dimensional primary tumor cultures
A. Embedding tumor organoids or aggregates in Matrigel
1. Defrost a bottle or aliquot of frozen growth factor reduced Matrigel at 4°C. This process can take
several hours to overnight. It is very important to keep the Matrigel at 4°C at all times to prevent it
from solidifying.
2. Carefully transfer the organoids/aggregates to a 15 ml conical tube containing 10ml of the modified M87 media that has been pre-warmed to 37°C. Centrifuge the cells for 30 sec at 400 × g at room temperature. Aspirate the supernatant to remove the single cells. Resuspend the pellet by slowly adding 1 ml of modified M87, taking care to prevent breaking up the organoids. Perform a trypan blue exclusion test using a hemocytometer to determine the number of viable organoids/aggregates per ml.
3. Centrifuge the organoids/aggregates at 400 × g for 5 min at room temperature. Remove the
supernatant and place the tube on ice. Determine the number of organoids to be added to each well of a 24-well plate.
4. Add 200uL matrigel very carefully to coat the entire bottom.
5. Carefully suspend the pellet of organoids/aggregates in M87 medium, and then add the desired organoid number/volume to each well over the matrigel.

The drug test was conducted three days after the organoids were cultured. The test method is the ATP
assay. The drug test results were compared with the clinical real response to the sensitivity and
specificity.

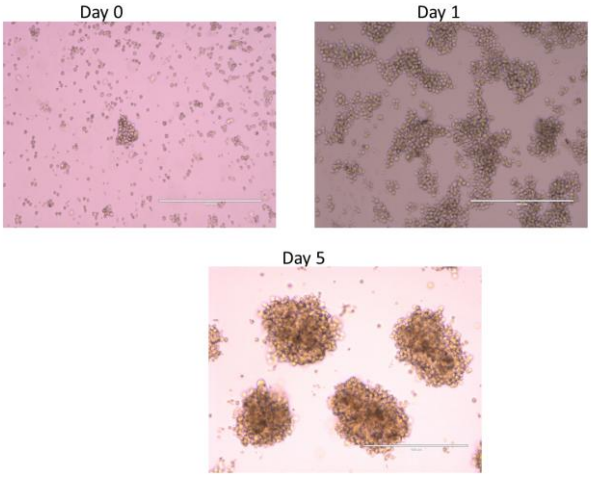
2018 年 11 月

經過四個月進行類器官培養成果如下。

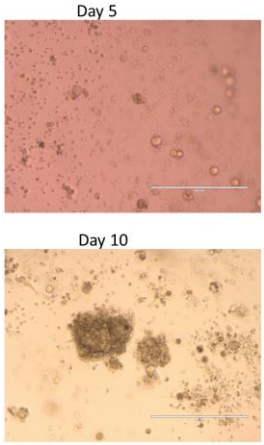
COH 31 (10x)

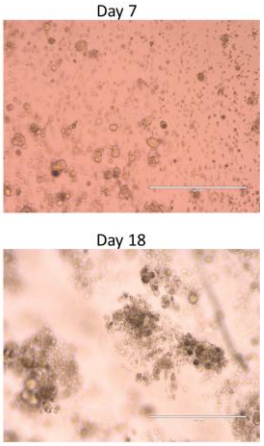


COH 31 frozen tumor piece (10x)

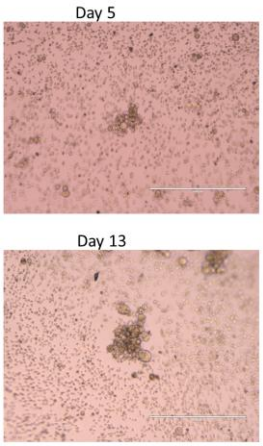


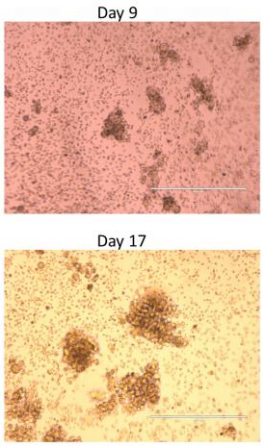
GS 1



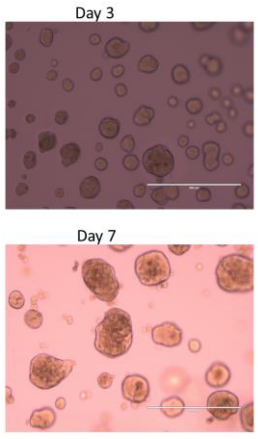


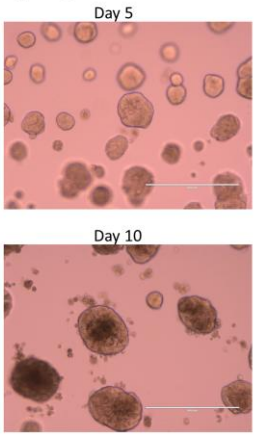
GS 2



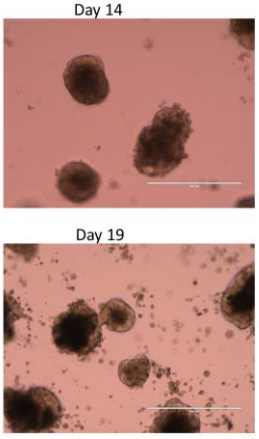


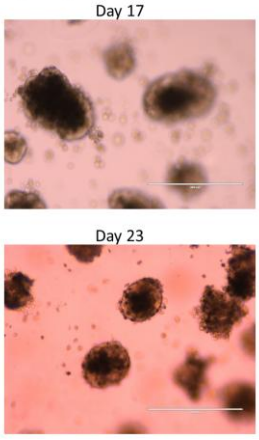
T47D in 3D culture (regular medium)

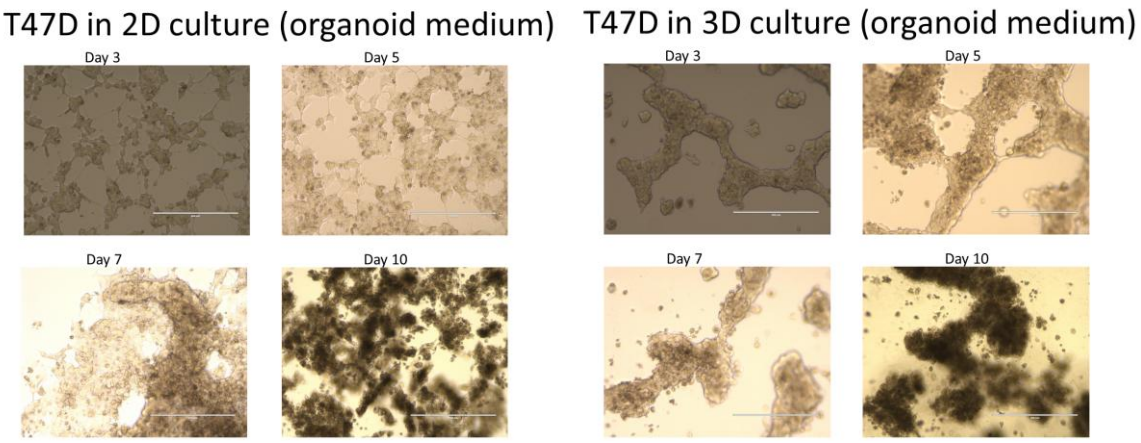




T47D in 3D culture (regular medium)







2018 年 12 月

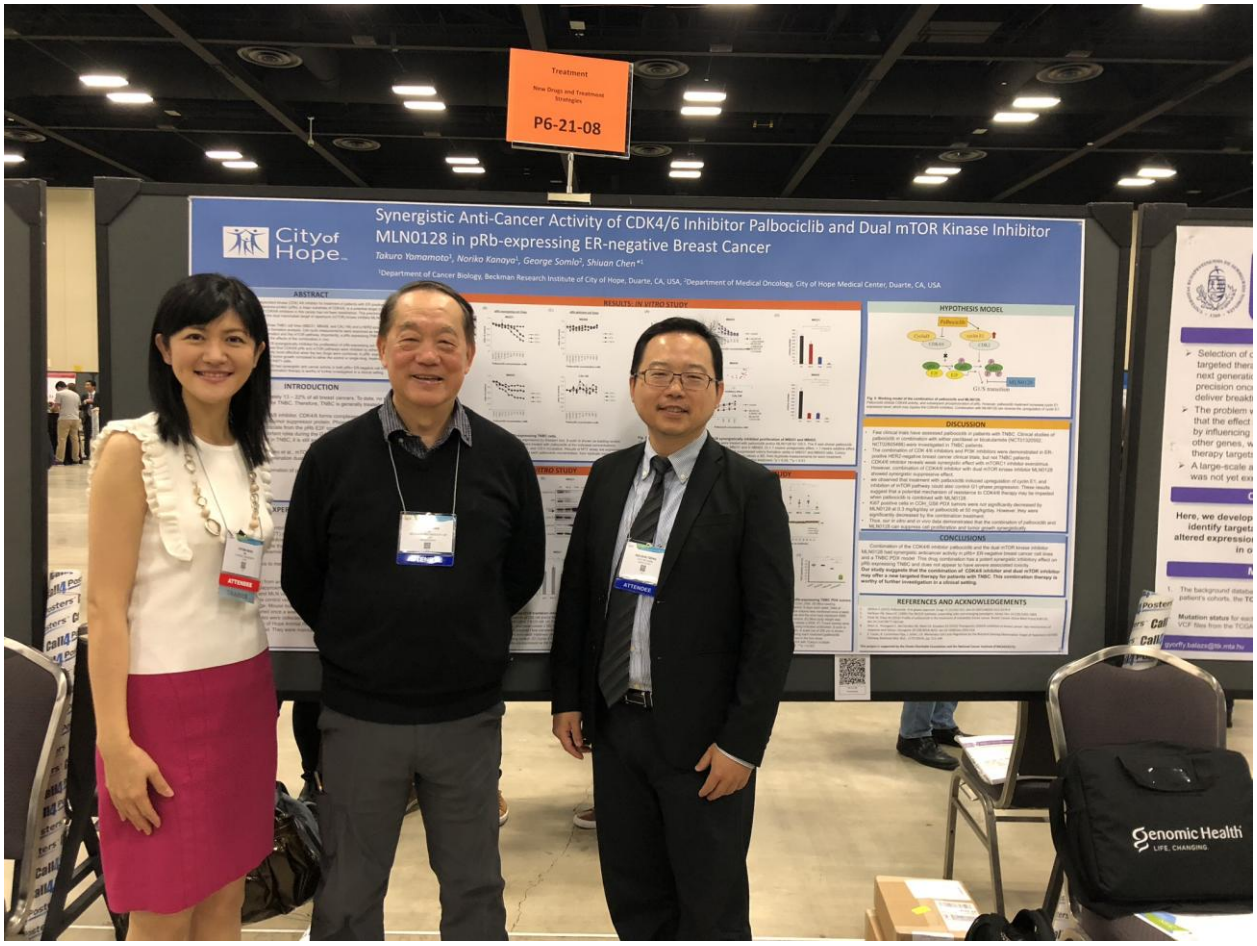
本月 12/4-12/8 參加聖安東尼奧乳癌研討會(San Antonio Breast Cancer Symposium)。

該大會在乳癌治療領域饒富盛名。首次的聖安東尼奧乳癌研討會是在 1978 年 11 月 11 日舉行，原來是由 Cancer Therapy and Research Center (CTRC) 和美國癌症學會(American Cancer Society)及聖安東尼奧的德州大學健康科學中心共同舉行的 Breast Cancer Awareness Week 中之一部份。在 1978 年時獨立出來舉辦了第一屆乳癌研討會議，參加的內外科醫師在當時約有 500 人。到了 1981 年此會議被延長為 2 天之研討會，而且向全世界徵稿，也將口頭報告及海報展示一齊放入此一討論會中，因此此討論會之範圍從此發展成為國際性的研討會。此一研討會常態性的邀請有聲望的學者專家給予充實完整醫學新知的演講，及一些臨床試驗的結果報告。此一研討會的主要目標是對世界各國研究乳癌之醫師學者提供有關乳癌或癌前期之病因學、治療、診斷、預防及生物學研究發展中之知識資訊。

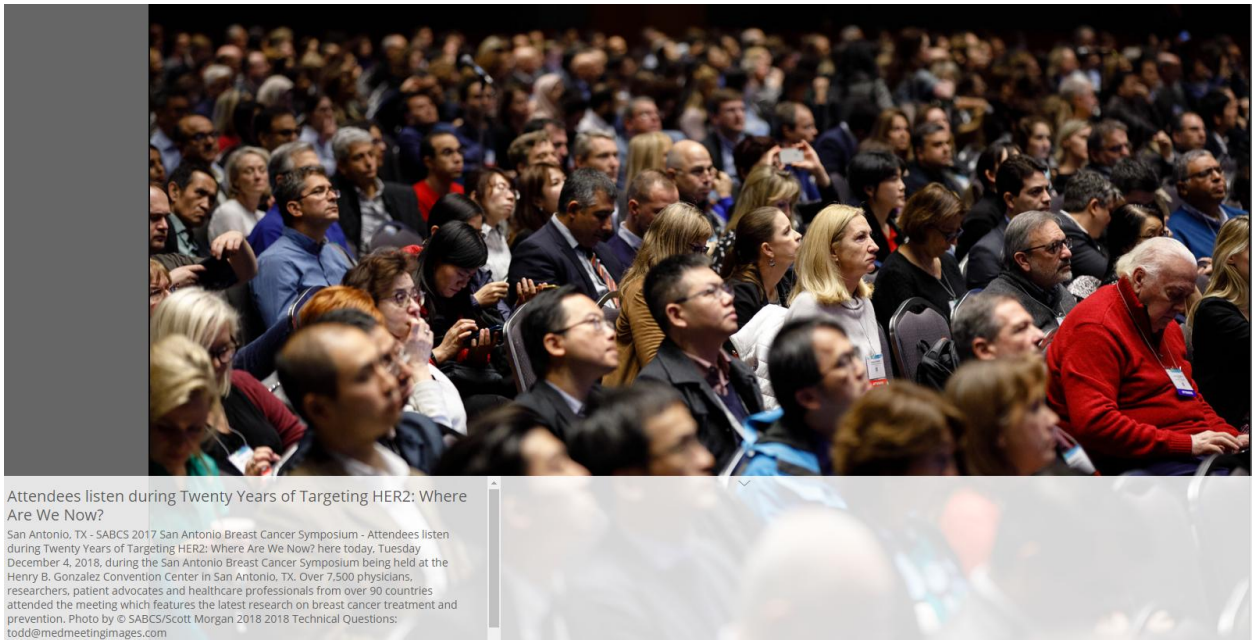
從 2008 年開始，美國癌症研究學會(American Association of Cancer Research, AACR)也參與成為此研討會的重要主辦者之一，此一研討會就吸引更多有高品質分量的研究論文，不論是在臨床基礎或轉譯醫學之研究在此一場合發表，因而成為乳癌治療中較完整的討論場所。對參與之年輕研究人員提供了一個極優良的教育訓練及知識交換之平台，而由於經過這些轉譯及學習的新知識，整合了過去各別研究的力量，將各方的研究成果經過知識的傳播與討論，促使近年來乳癌照護與治療的快速進展。

因希望之城實驗室有投稿海報於本次大會，實驗室主持人陳教授指派本人協助整理資料並張貼海報。

本次大會中由九州大學實驗室刊登的海報
由左至右為日本九州大學Dr. Makoto Kubo、日本九州大學Dr.Hitomi Mori、美國希望之城Prof. Shiuan Chen、台北榮總血庫主任劉峻宇主任及本人。



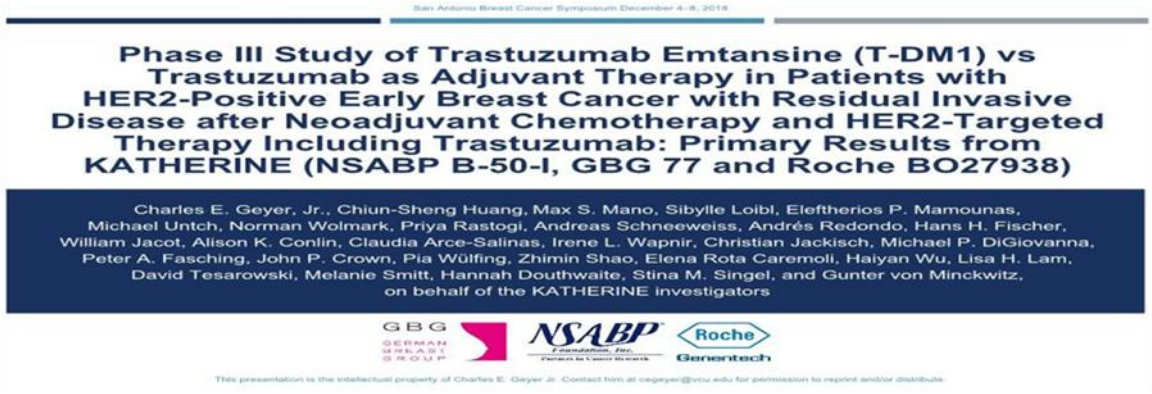
本次大會中由陳教授實驗室刊登的海報
日本九州大學Dr.Hitomi Mori、美國希望之城Prof. Shiuan Chen、本人

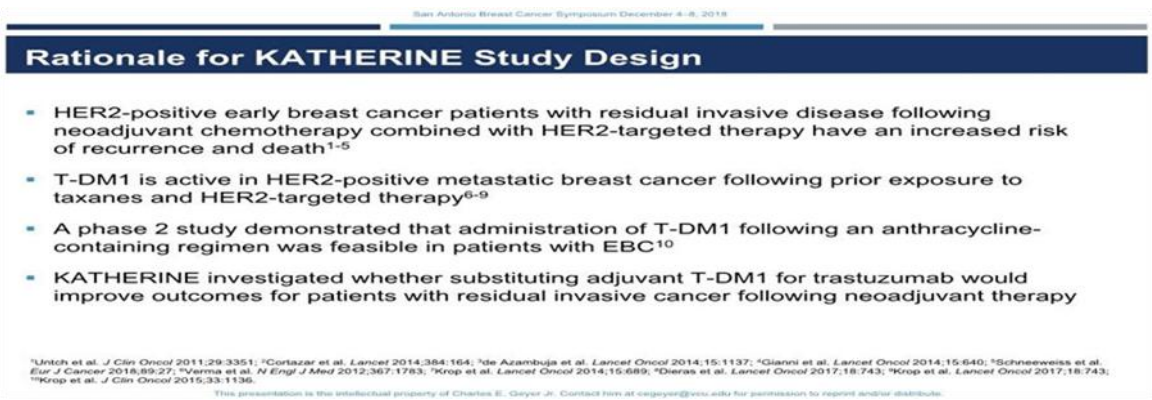


聖安東尼奧乳癌大會刊登出本人與北榮血庫主任劉峻宇主任於演講中仔細聆聽之照片。

本次大會於乳癌治療有幾點重要發表，節錄如下：

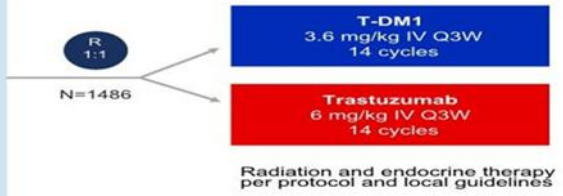
1.針對 HER2 陽性乳癌治療上都以術前雙標靶治療為建議首選治療。雖然反應率可以達到八成以上，但仍有近兩成的病患經過治療之後無法使腫瘤縮小。因此就有此臨床試驗來看看術後的輔助治療，T-DM1 與 Trastuzumab 來比較何者對病患有幫助。臨床試驗的結果發現給予病患 T-DM1 在癌症復發率與遠端腫瘤復發綠都明顯的比 Trastuzumab 來的好。也因著這個重要結果刊登在新英格蘭雜誌(NEJM)上。





KATHERINE Study Design

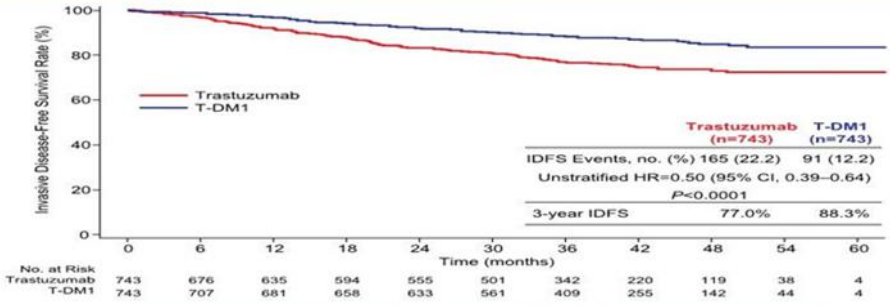
- cT1-4/N0-3/M0 at presentation (cT1a-b/N0 excluded)
- Centrally confirmed HER2-positive breast cancer
- Neoadjuvant therapy must have consisted of
  - Minimum of 6 cycles of chemotherapy
    - Minimum of 9 weeks of taxane
    - Anthracyclines and alkylating agents allowed
    - All chemotherapy prior to surgery
  - Minimum of 9 weeks of trastuzumab
    - Second HER2-targeted agent allowed
- Residual invasive tumor in breast or axillary nodes
- Randomization within 12 weeks of surgery



Radiation and endocrine therapy per protocol and local guidelines

Stratification factors:
- Clinical presentation: Inoperable (stage cT4 or cN2–3) vs operable (stages cT1-3N0-1)
- Hormone receptor: ER or PR positive vs ER negative and PR negative/unknown
- Preoperative therapy: Trastuzumab vs trastuzumab plus other HER2-targeted therapy
- Pathological nodal status after neoadjuvant therapy: Positive vs negative/not done

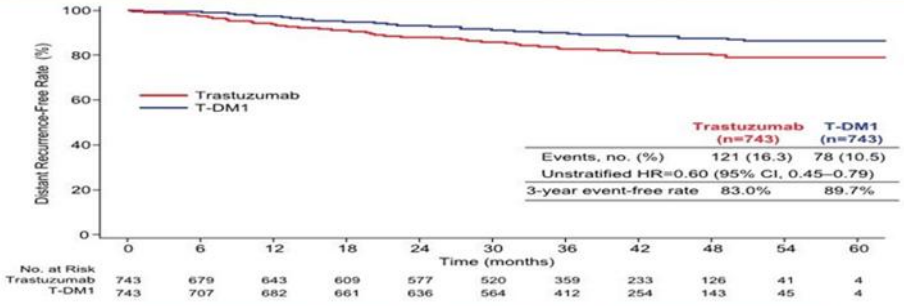
Invasive Disease-Free Survival



	Trastuzumab (n=743)	T-DM1 (n=743)
IDFS Events, no. (%)	165 (22.2)	91 (12.2)
Unstratified HR=0.50 (95% CI, 0.39–0.64)		
P<0.0001		
3-year IDFS	77.0%	88.3%

No. at Risk											
Trastuzumab	743	676	635	594	555	501	342	220	119	38	4
T-DM1	743	707	681	658	633	561	409	255	142	44	4

Distant Recurrence



	Trastuzumab (n=743)	T-DM1 (n=743)
Events, no. (%)	121 (16.3)	78 (10.5)
Unstratified HR=0.60 (95% CI, 0.45–0.79)		
3-year event-free rate	83.0%	89.7%

No. at Risk											
Trastuzumab	743	679	643	609	577	520	359	233	126	41	4
T-DM1	743	707	682	661	636	564	412	254	143	45	4

KATHERINE Summary and Conclusions

- Adjuvant T-DM1 demonstrated both a statistically significant and clinically meaningful improvement in IDFS compared with trastuzumab
  - Unstratified HR=0.50; 95% CI 0.39–0.64; *P*<0.0001
  - 3-year IDFS rate improved from 77.0% to 88.3% (difference=11.3%)
- Benefit of T-DM1 was consistent across all key subgroups including HR status, extent of residual invasive disease, and single or dual HER2-targeted neoadjuvant therapy
- The safety data were consistent with the known manageable toxicities of T-DM1, with expected increases in AEs associated with T-DM1 compared to trastuzumab
- Additional follow-up will be necessary to evaluate the effect of T-DM1 on OS
- The KATHERINE data will likely form the foundation of a new standard of care in this population and increase the use of neoadjuvant therapy in HER2-positive EBC

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer

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2.乳癌的免疫療法一直以來都沒有好的成果，然而今年有一重大突破！發現針對三陰性乳癌，合併有 PDL-1 IC+的病患，使用 Atezolizumab+nab-paclitaxel 會比單獨 nab-paclitaxel 治療效果好。不僅有無並存活期的好處，更有全體存活率的好處。因此該重要臨床試驗同樣發表於新英格蘭雜誌(NEJM)。

San Antonio Breast Cancer Symposium® December 4-8, 2018

IMpassion130: Efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, Phase III study of atezolizumab + *nab*-paclitaxel in patients with treatment-naive, locally advanced or metastatic triple-negative breast cancer

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TNBC background

- TNBC has the worst breast cancer subtype–specific outcomes,[1] and single-agent taxane or anthracycline chemotherapy remains the typical 1L treatment for advanced disease[2,3]
  - Estimates of median OS vary but are generally ≈ 18 months or less[4-6]
- No targeted agents have demonstrated definitive OS benefit
  - Bevacizumab in combination with chemotherapy is an approved treatment for mBC in several countries outside the United States[7]
  - PARP inhibitors for *BRCA1/2*-mutant, HER2-negative mBC have been approved in several countries[8]
- IMpassion130 is the first Phase III study to demonstrate a benefit with immunotherapy in mTNBC[9]
  - Atezolizumab + *nab*-paclitaxel (vs placebo + *nab*-paclitaxel) resulted in a statistically significant PFS benefit in the ITT and PD-L1+ populations (ITT HR, 0.80 [95% CI: 0.69, 0.92] and PD-L1+ HR, 0.62 [95% CI: 0.49, 0.78])
  - At this first interim OS analysis, clinically meaningful improvement in OS with atezolizumab + *nab*-paclitaxel was observed in the PD-L1+ population, with an HR of 0.62 [95% CI: 0.45, 0.86] and a median OS improvement from 15.5 to 25.0 months[9]

PD-L1+: PD-L1 on ≥ 1% of IC (as % of tumor area).
1. den Brok *BCRT* 2017. 2. NCCN 2018. 3. Cardoso *Ann Oncol* 2018. 4. Gobbini *EJC* 2018. 5. Yardley *Ann Oncol* 2018. 6. Miles *Ann Oncol* 2013. 7. AVASTIN SmPC 2017. 8. Lynparza USPI 2018. 9. Schmid *N Engl J Med* 2018.

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IMpassion130 study design: Prespecified analyses in the ITT and PD-L1 IC+ population

Phase III study IMpassion130[a]

Previously untreated metastatic or inoperable locally advanced TNBC[b]
N = 902 patients randomized

Double blind; no crossover

R 1:1

Stratification factors:
1. *Prior taxane use*
2. *Liver metastases*
3. ***PD-L1 on IC[c]***

Atezo + nab-P arm[d]
ITT population: n = 451
PD-L1 IC+ patients: n = 185 (41%)

Plac + nab-P arm[d]
ITT population: n = 451
PD-L1 IC+ patients: n = 184 (41%)

Key study endpoints
- Co-primary: PFS (ITT and PD-L1 IC+)
  OS (ITT and PD-L1 IC+)
- Secondary: ORR and DOR
- Safety and tolerability

[a] NCT02425891. [b] Locally evaluated per ASCO-CAP guidelines. Prior chemotherapy in the curative setting, including taxanes, allowed if treatment-free interval ≥ 12 mo. [c] Centrally evaluated per VENTANA SP142 IHC assay (double blinded for PD-L1 status. PD-L1+: PD-L1 on ≥ 1% of IC). [d] Atezolizumab or placebo 840 mg IV on days 1 and 15 + *nab*-paclitaxel 100 mg/m² IV on days 1, 8 and 15 of 28-day cycle until RECIST v1.1 PD. 1. Schmid *N Engl J Med* 2018.

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IMpassion130 primary analysis[1,2]: Clinically meaningful PFS and OS benefit in the PD-L1+ population

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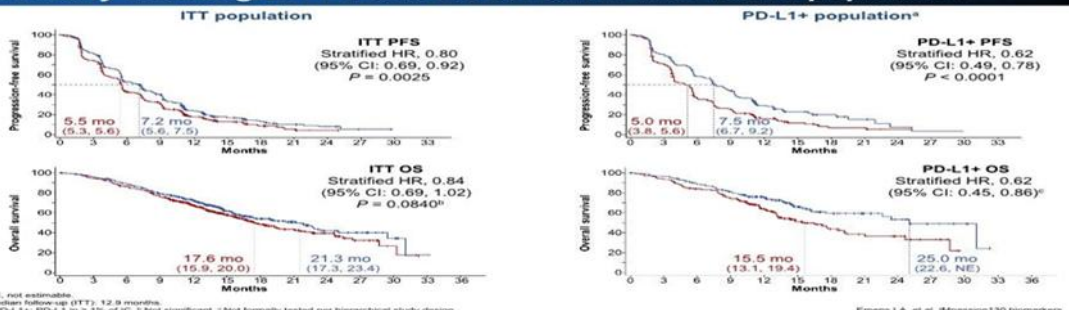

NE, not estimable.
Median follow-up (ITT): 12.9 months.
[a] PD-L1+: PD-L1 in ≥ 1% of IC. [b] Not significant. [c] Not formally tested per hierarchical study design.
1. Schmid *N Engl J Med* 2018. 2. Schmid ESMO 2018 [LBA1_PR].

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IMpassion130 biomarker analyses

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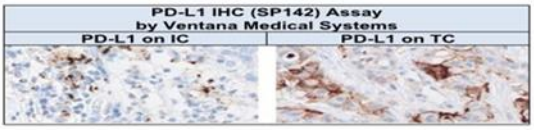
- Pre-existing immune biology, including PD-L1 expression on TC, CD8+ T cells and stromal TILs, has also been associated with clinical benefit from anti–PD-L1/PD-1[1,2]
- In this exploratory analysis, we sought to evaluate whether this immune biology and *BRCA1/2* mutation status were associated with clinical benefit from atezolizumab + *nab*-paclitaxel
- Biomarkers were centrally analyzed in pre-treatment biopsies
  - PD-L1 on IC and TC by VENTANA SP142 IHC assay[a]
  - Intratumoral CD8+ T cells by IHC (Dako clone C8/144B) and stromal TILs by H&E[b]
  - *BRCA1/2* mutation status by FoundationOne assay



H&E, hematoxylin and eosin staining; IHC, immunohistochemistry.
[a] PD-L1 scoring: IC0: < 1%; IC1: ≥ 1% and < 5%; IC2: ≥ 5% and < 10%; IC3: ≥ 10%; TC−: < 1% PD-L1 on tumor cells; TC+: ≥ 1% PD-L1 on tumor cells.
[b] Pre-specified cutoffs for CD8 IHC and stromal TILs are based on references 1 and 2.
1. Adams *JAMA Oncol* 2018. 2. Denkert *Lancet Oncol* 2018.

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In IMpassion130, PD-L1 in TNBC is expressed mainly on tumor-infiltrating immune cells

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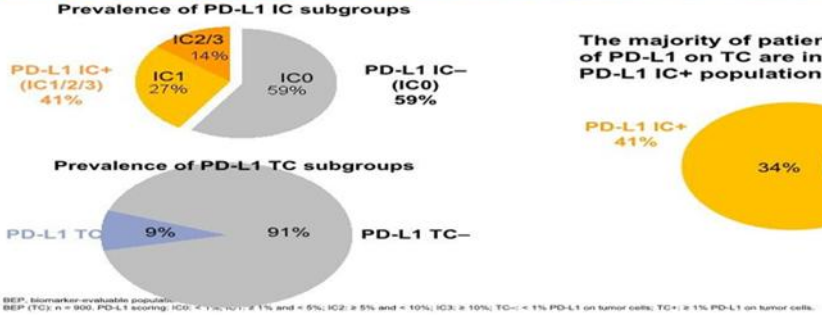

BEP, biomarker-evaluable population.
BEP (TC): n = 900. PD-L1 scoring: IC0: < 1%; IC1: ≥ 1% and < 5%; IC2: ≥ 5% and < 10%; IC3: ≥ 10%; TC−: < 1% PD-L1 on tumor cells; TC+: ≥ 1% PD-L1 on tumor cells.

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7

PD-L1 IC status (positive vs negative) predicts PFS benefit with atezolizumab + *nab*-paclitaxel

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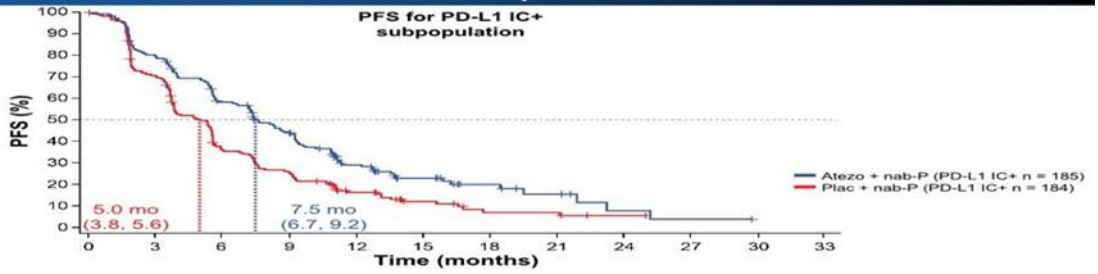

Median PFS durations (and 95% CIs) are indicated on the plot. Stratified HRs are shown. All *P* values except for PD-L1 IC+ PFS are nominal *P* values. Data cutoff: April 17, 2018.

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8

PD-L1 IC status (positive vs negative) predicts PFS benefit with atezolizumab + *nab*-paclitaxel

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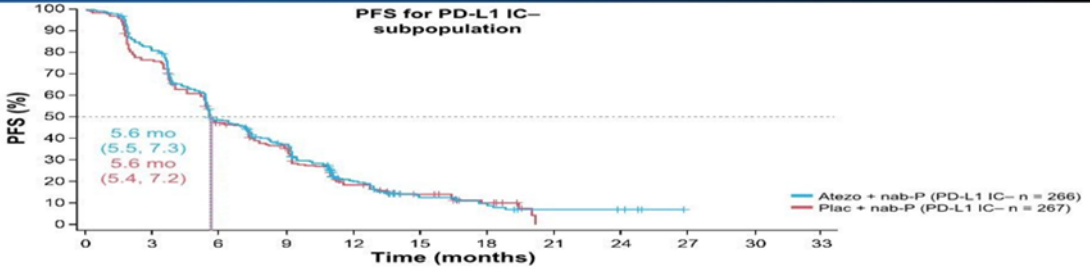

Median PFS durations (and 95% CIs) are indicated on the plot. Stratified HRs are shown. All *P* values except for PD-L1 IC+ PFS are nominal *P* values. Data cutoff: April 17, 2018.

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9

PD-L1 IC status (positive vs negative) predicts PFS benefit with atezolizumab + *nab*-paclitaxel

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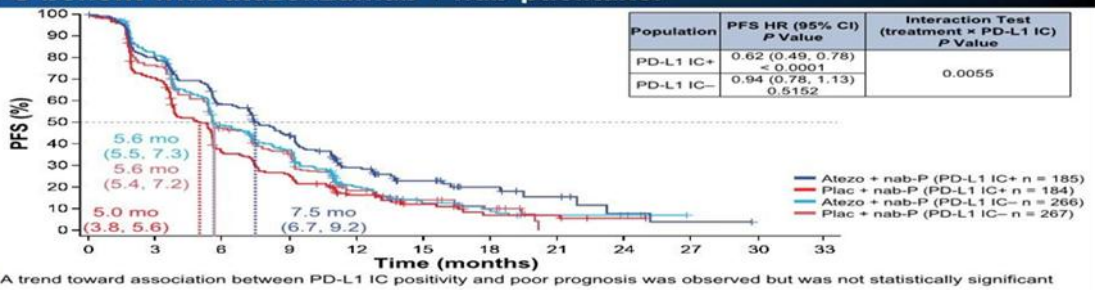

Population	PFS HR (95% CI) *P* Value	Interaction Test (treatment × PD-L1 IC) *P* Value
PD-L1 IC+	0.62 (0.49, 0.78) < 0.0001	0.0055
PD-L1 IC–	0.94 (0.78, 1.13) 0.5152	

- A trend toward association between PD-L1 IC positivity and poor prognosis was observed but was not statistically significant
- PD-L1 IC positivity was predictive of PFS and OS benefit with atezolizumab + *nab*-paclitaxel

Median PFS durations (and 95% CIs) are indicated on the plot. Stratified HRs are shown. All *P* values except for PD-L1 IC+ PFS are nominal *P* values. Data cutoff: April 17, 2018.

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10

PD-L1 IC status (positive vs negative) predicts OS benefit with atezolizumab + *nab*-paclitaxel

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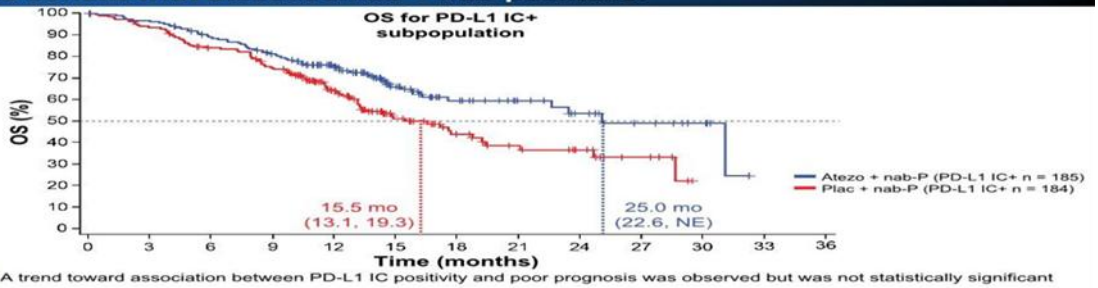

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11

PD-L1 IC status (positive vs negative) predicts OS benefit with atezolizumab + *nab*-paclitaxel

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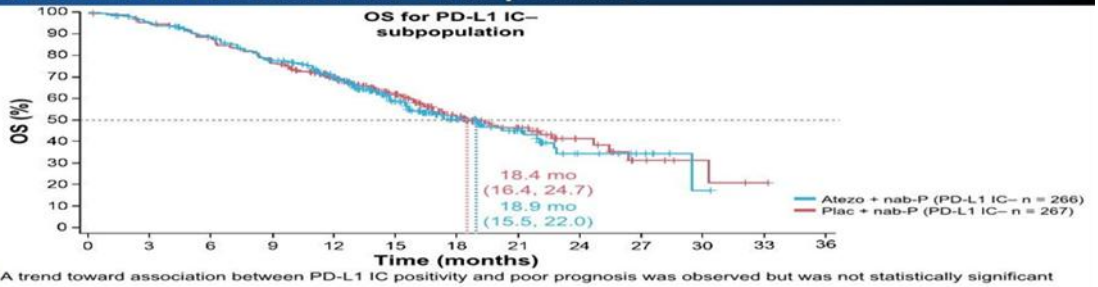

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Median OS durations (and 95% CIs) are indicated on the plot. Stratified HRs are shown. All *P* values are nominal. Data cutoff: April 17, 2018.

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12

PD-L1 IC status (positive vs negative) predicts OS benefit with atezolizumab + *nab*-paclitaxel

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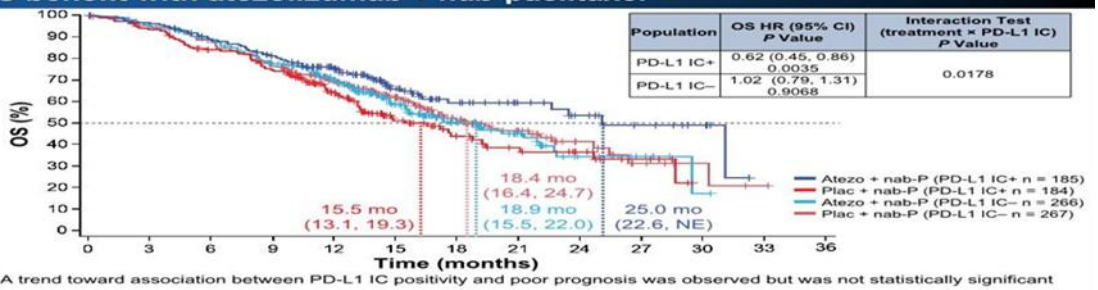

Population	OS HR (95% CI) *P* Value	Interaction Test (treatment × PD-L1 IC) *P* Value
PD-L1 IC+	0.62 (0.45, 0.86) 0.0035	0.0178
PD-L1 IC–	1.02 (0.79, 1.31) 0.9068	

- A trend toward association between PD-L1 IC positivity and poor prognosis was observed but was not statistically significant
- PD-L1 IC positivity was predictive of PFS and OS benefit with atezolizumab + *nab*-paclitaxel

Median OS durations (and 95% CIs) are indicated on the plot. Stratified HRs are shown. All *P* values are nominal. Data cutoff: April 17, 2018.

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Conclusions

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- In the Phase III IMpassion130 study, PD-L1 expression on IC is a predictive biomarker for selecting patients who clinically benefit from first-line atezolizumab + *nab*-paclitaxel treatment for mTNBC
  - PFS and OS benefit was observed in patients with a PD-L1 IC of ≥ 1% (by VENTANA SP142 IHC assay)
  - A treatment effect was not seen for adding atezolizumab to chemotherapy in the PD-L1–negative subgroup
- PD-L1 expression on TC did not provide additional information beyond PD-L1 IC status
  - Prevalence of tumor-cell PD-L1 expression was low, and the majority of these tumors were also PD-L1 IC+
- PD-L1 IC expression was the best predictor of clinical benefit as the patient subgroups with tumor-infiltrating immune cells (stromal TILs+) or cytotoxic T cells (CD8+) derived clinical benefit with atezolizumab + *nab*-paclitaxel if their tumors were also PD-L1 IC+
- PFS and OS results were consistent regardless of *BRCA1/2* mutation status
- Patients with newly diagnosed metastatic and unresectable locally advanced TNBC should be routinely tested for PD-L1 IC status to determine whether they might benefit from atezolizumab + *nab*-paclitaxel

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18

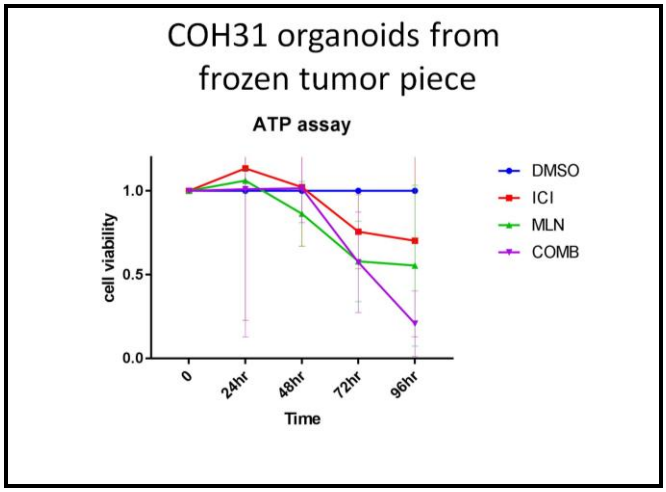
ORIGINAL ARTICLE

Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer

P. Schmid, S. Adams, H.S. Rugo, A. Schneeweiss, C.H. Barrios, H. Iwata, V. Diéras, R. Hegg, S.-A. Im, G. Shaw Wright, V. Henschel, L. Molinero, S.Y. Chui, R. Funke, A. Husain, E.P. Winer, S. Loi, and L.A. Emens, for the IMpassion130 Trial Investigators*

2019 年 1 月

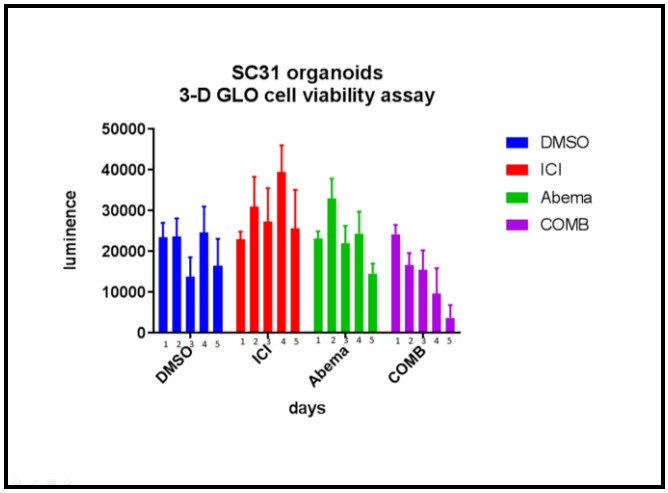
培養中的 organoids 開始進行藥物測試

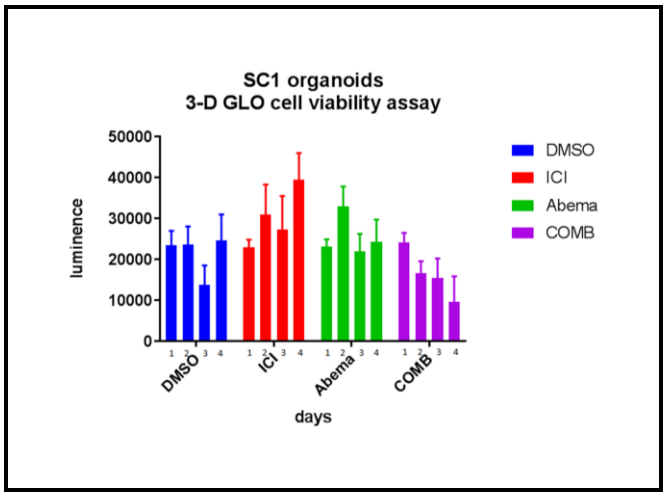


Organoids 比較適合的測試為 ATP assay。本實驗發現 COH31 organoids 接受 ICI 及 MLN 皆有抑制的效果。假如兩藥合成後藥效更佳，有加成的效果。

2019 年 2 月

針對賀爾蒙陽性 HER2 陽性的腫瘤給予新藥 CDK 4/6 inhibitor 測試藥物反應。

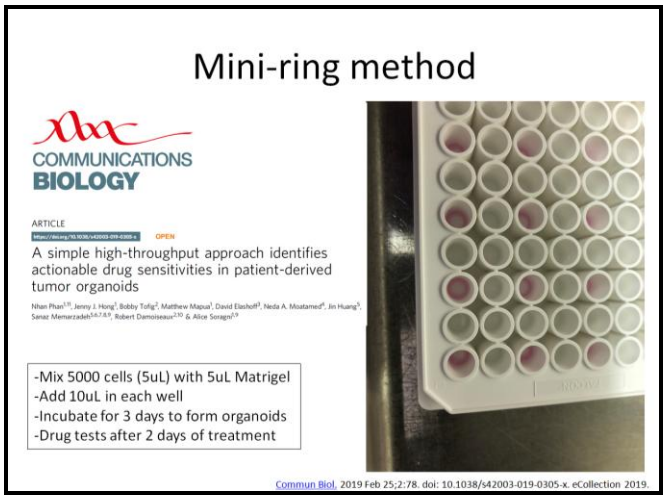


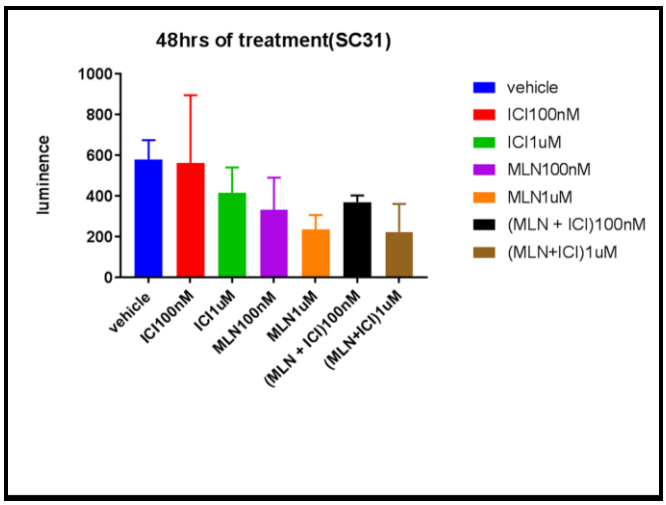


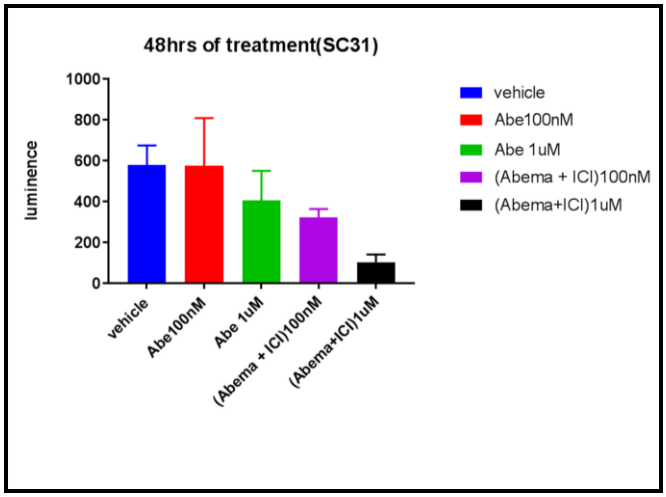
該實驗發現 abemaciclib 與 ICI 皆有抑制此兩癌細胞的效果。然而將兩藥合併可以產生更好的抑制效果。

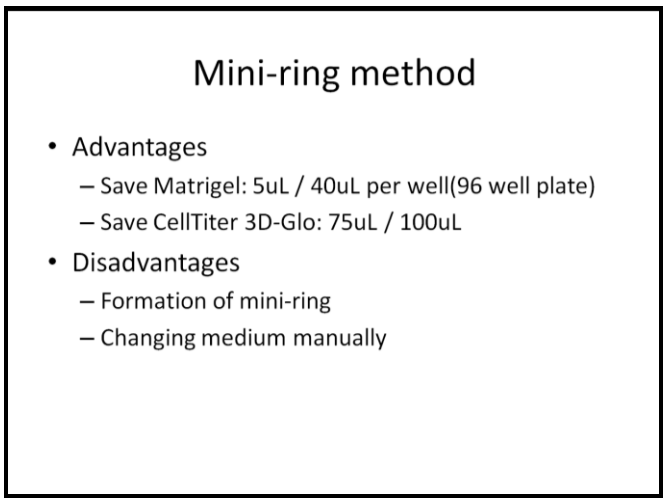
2019 年 3 月

本月嘗試不同培養與測試 organoids 的方式。



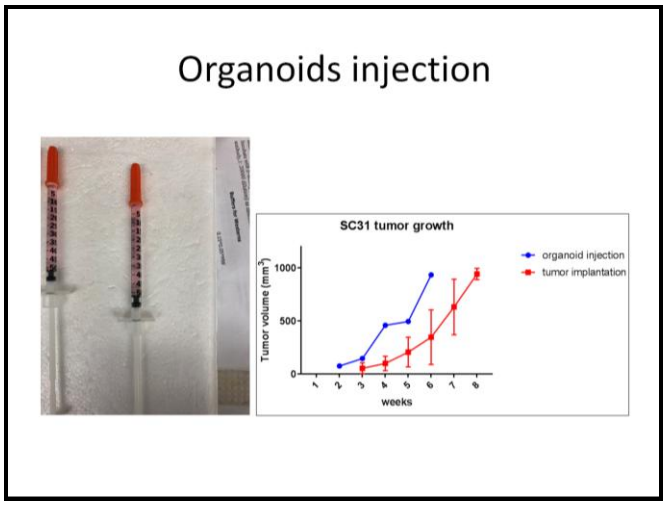






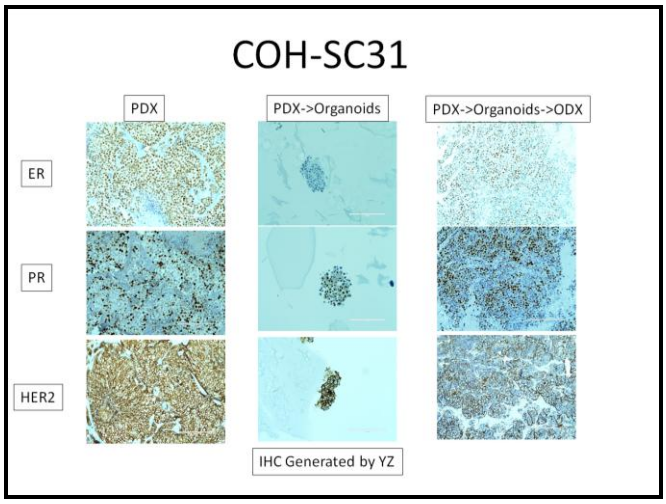
2019 年 4 月

本月嘗試將培養出的 organiods 打回小鼠並觀察其生長情況。



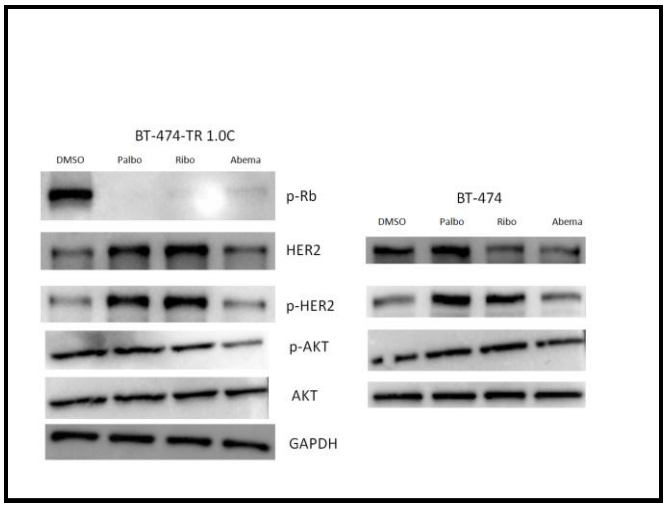
2019 年 5 月

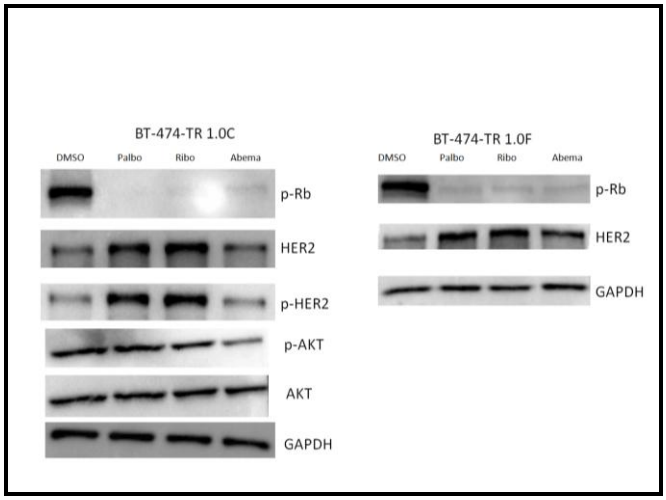
將 organoid 經由福馬林固定過後，染色來確定是否保留與原腫瘤一樣的特性。由 COH-SC31 可以發現，不論是 PDX->organoids 或是 PDX->organoids->PDX，其組織免疫染色皆與原 PDX 腫瘤相同，即代表 organoids 並不會改變腫瘤的原貌與特性。

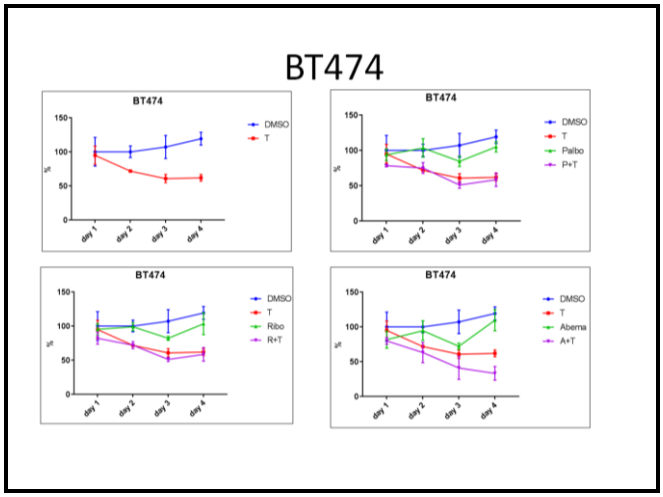


2019 年 6 月

本月研究以觀察 CDK 4/6i 對於 HER2 的影響。由西方點墨法可以發現，三陽性乳癌細胞接受 CDK4/6i 治療過後，HER2 會上升，因此 CDK4/6i 合併 trastuzumab 能達到加成的效果。



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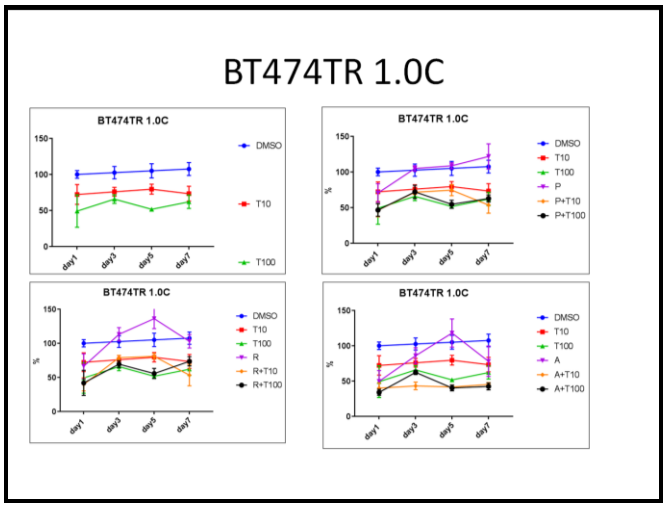
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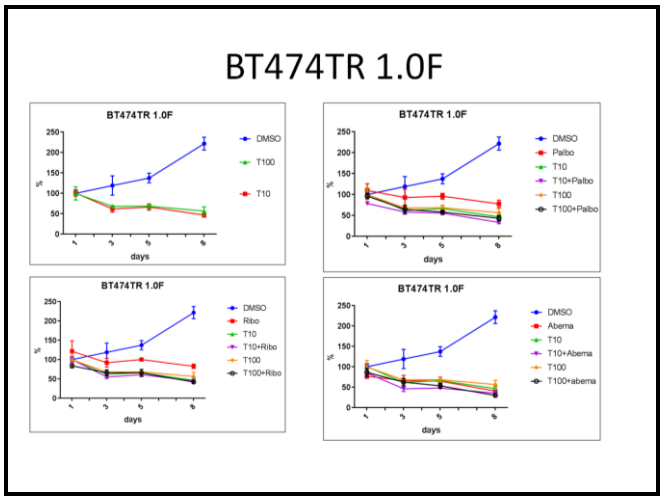
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Palbo
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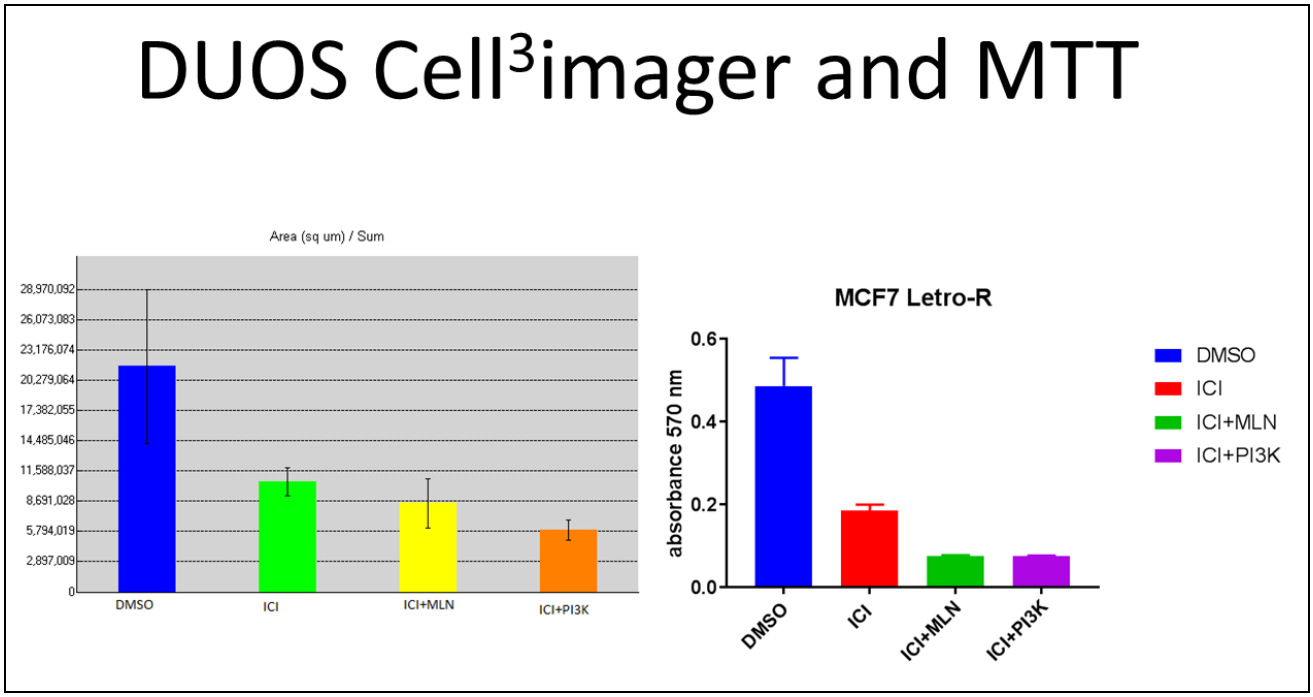
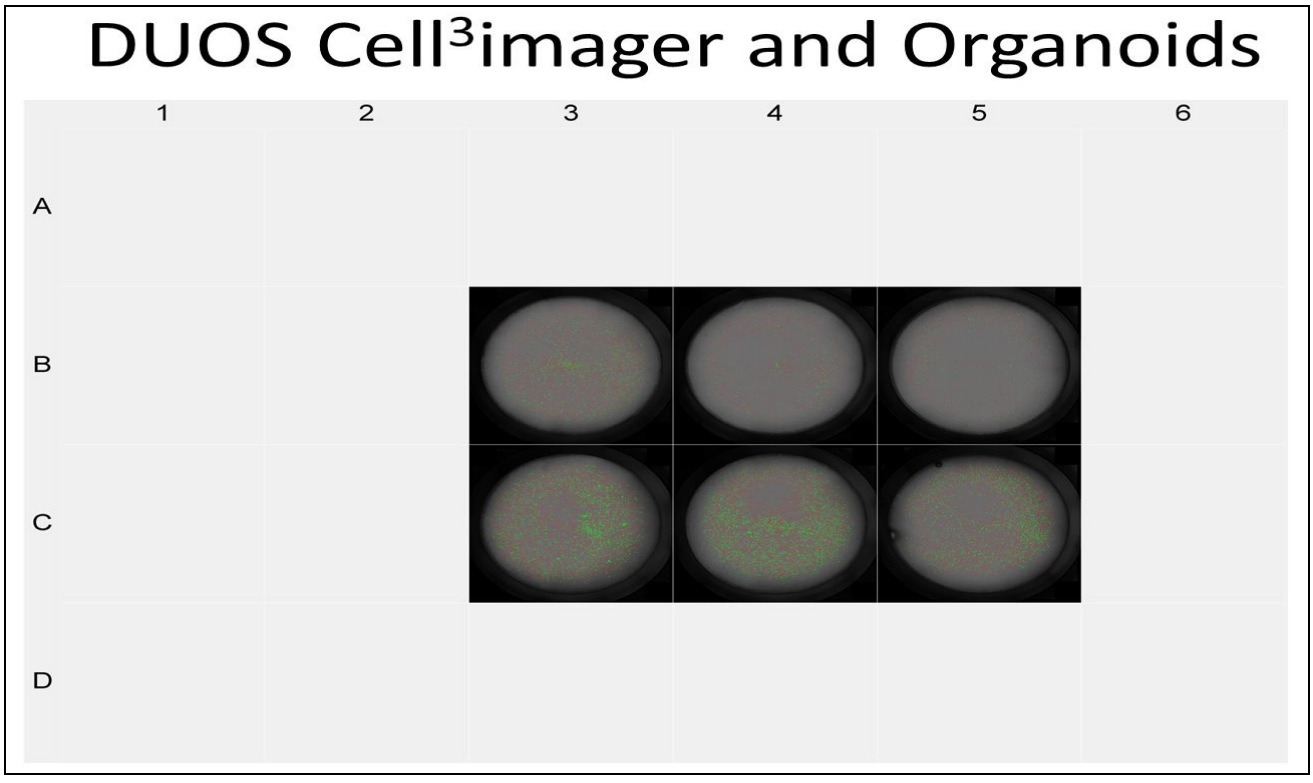
T10+Palbo
T100
T100+Palbo
BT474TR 1.0F
DMSO
Ribo
T10
T10+Ribo
T100
T100+Ribo
BT474TR 1.0F
DMSO
Aberna
T10
T10+Aberna
T100
T100+aberna
%
days

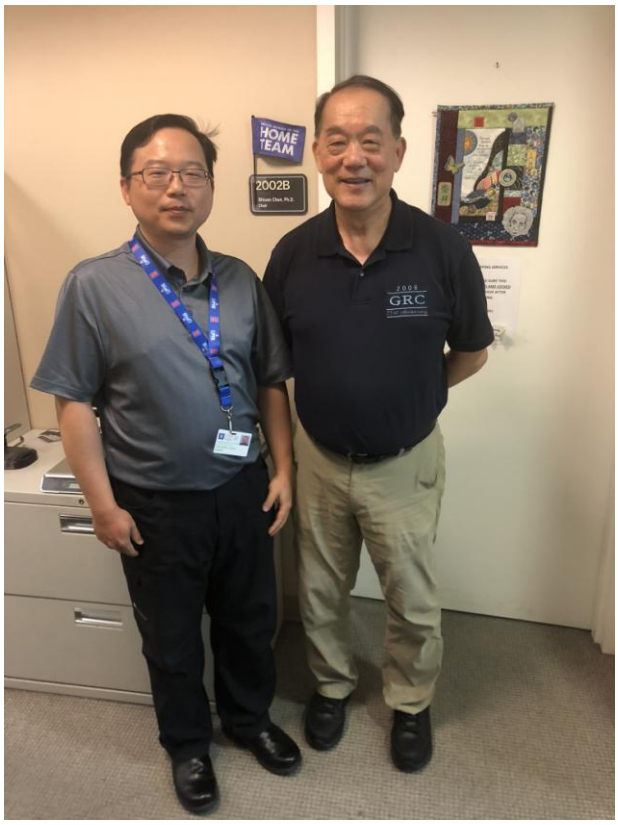
2019 年 7 月

本月實驗室添購一重要 3D 掃描儀器—Cell^{3}imager duo。過往如需要定量類器官在培養皿中的數量，都是非常的困難。能使用的辦法只有運用溶劑測量類器官的 ATP 數量來推斷細胞生長曲線。這樣只能做一次性的檢測，對於連續性檢測不適用。因此在多番討論之後，實驗室在 City of Hope 的支持之下添購了這台掃描儀器，其功能可以做全培養皿的掃描，掃描之後會針對細胞大小及數量測量出客觀的數值。一開始我們使用 2D 細胞來測試，發現其結果與 MTT assay 結果相近。在測量類器官的結果也非常令人滿意。活細胞與死細胞很清楚的可以分得出來。將來如果醫院要發展類器官培養模式，如能添購此一儀器相信勢必能對於類器官研究有顯著的幫助。





本月為最後一個月在 City of hope 學習。感謝陳教授開放實驗室讓一個沒有經驗的學員從頭開始學習，也感謝院方能提供這樣的機會與資源讓資淺的醫師能開開眼界。



三、心得及建議

本次出國進修的目標為學習美國頂尖研究醫院的基礎研究技術。在勤奮不懈之下，終於習得類細胞培養技術。此技術雖與過往單純細胞培養相比確實稍微複雜且有難度，但是在經費消耗上比 PDX(人源化腫瘤異種移植)來的便宜，而且細胞培養所需花費的時間也比 PDX 來的短。因此在基礎研究上為一創新的研究模式，對於基礎研究能有長足進展。

在國外學習的過程中，發掘在美國有幾點現象值得我們學習。首先是美國在投入癌症研究的資源之豐厚。以單一實驗室為例，年度計畫皆為一年百萬美元起跳，然而有一些實驗與基因檢測費用也相對高額。二來是全世界不同國家的醫師/研究員匯集在美國加入研究的行列。以 City of Hope 實驗室為例，除了本人來自台灣之外，更有韓國、日本、中國等國家醫師及研究員一齊參與研究團隊。觀察其他實驗室，幾乎也都是亞洲人為主。由此可見在美國的研究環境與風氣之盛。第三是醫院與研究單位的合作。City of Hope 本身為癌症醫院，然而其研究中心 Beckman Research Center 是與醫院平起平坐的。臨床與基礎研究本應該彼此合作。雙方各有自己的專長，彼此各司其職方能將研究的領域推得更廣。

本人感到非常榮幸，在長官的支持下，以及退輔會提供了補助來學習。本人更期待能貢獻所學來提升高雄榮總的研究風氣。本次所進修學習的乳癌基礎研究技術一類細胞培養，本技術在全台灣仍僅於起步階段。類器官培養技術可以應用於癌症病患的藥物測試敏感性與抗藥性。以現今精準醫療世代來看十分重要。目前已有提出研究計畫，待後續 IRB 計畫通過之後，將開始著手進行由病患的細胞來進行培養類細胞。接著給予藥物測試來提前知曉病患癌細胞對於藥物的敏感度。然而這樣的計畫仍需要多方面的合作。設置實驗室和尋找合作的基礎研究員更是重要的一環。如有機會能在高雄榮總發展這樣技術，期待能有教研部的老師們一同參與，也期待院部長官於研究經費部分能給予更充足的資源。期盼將來這個技術能成為高榮之光。

最後，感謝院長及長官們對於進修人員在財務上的補助，尤其特別在返國後還能補助績效的部分，對於有心出國進修的人員來說真的非常重要。依照公務人員進修法的補助，確實在國外很難生存。往往都是靠著過去幾年下來的積蓄在國外省吃儉用來度日。但是因著醫院的這項政策，年輕的主治醫師們將不在對於出國進修卻步。有越多醫師將國外所學貢獻於高雄榮總，對醫院來說才是福氣啊！