國家科學及技術委員會補助專題研究計畫報告

以病患來源異體移植及類腫瘤培養系統來建立未分化/去分化子宮內膜癌的藥物篩選模式

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本研究具有政策應用參考價值：■否 □是，建議提供機關
（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
本研究具影響公共利益之重大發現：□否 □是

中 華 民 國 113 年 10 月 24 日

中 文 摘 要 ： 子宮內膜癌是婦科癌症中發生率最高，其中未分化/去分化子宮內膜癌(UDEC)是子宮內膜癌中侵襲力最高，最容易發生轉移與復發且對化療最易產生抗性的癌種。真實世界數據顯示UDEC對傳統化放療效果有限，加上目前沒有標準的治療，因此找出適合的治療策略是必須且迫切需要的。基於最近文獻指出UDEC的病患帶有高度PTEN突變，因而造成下游PI3K/AKT /mTOR過度活化、DNA修復與細胞週期異常，因此我們藥物標靶PTEN基因所參與的調控路徑，而給予合併依維莫司(mTOR抑制劑)與愛乳適(細胞週期依賴性激酶4和6抑制劑)標靶藥物於我們目前已經建立的UDEC病患來源異體移植與類器官平台。我們結果顯示合併使用依維莫司與愛乳適於細胞株、病患來源異體移植與類器官模式均具有加成抑制癌細胞增生的效果且對於小鼠模式並沒有產生體重減輕等副作用產生。機制上利用RNA定序與分子路徑分析經藥物處理前後的病患來源異體移植小鼠檢體，顯示生存素具有潛力成為UDEC生物性標記，因此我們的研究顯示合併依維莫司與愛乳適加成抑制UDEC癌細胞生長是經由標靶生存素基因作用機制，而這個研究揭露對於UDEC病患來源異體移植可以合併依維莫司與愛乳適作為臨床前的試驗，並利用生存素表現做為婦癌中特別難治療的UDEC患者的生物性指標。

中 文 關 鍵 詞 ： 未分化/去分化子宮內膜癌、依維莫司、愛乳適、病患來源異體移植、類器官、RNA定序、生存素

英 文 摘 要 ： Endometrial cancer is the most common gynecologic malignancy. Undifferentiated /Dedifferentiated endometrial carcinoma (UEC/DEC, UDEC) is aggressive, metastatic, recurrent, and chemotherapy resistant. There is little experience with novel treatment in case series. Real-world experience is limited to conventional chemotherapy and/or radiotherapy. There is not standard procedure for treatment strategy for UDEC. Based on literature reported that high-frequency mutations of PTEN mutation -related deregulated downstream pathways of PI3K/AKT /mTOR and cell cycle are more common in UDEC. We aimed that treatment with druggable axis in combination of everolimus (mTOR inhibitor) and palbociclib (CDK4/6 inhibitor) has potential to improve clinical outcomes of women with UCEC to preclude single-inhibitor initiated pathway rewiring and limit toxicity in our established patient- derived xenograft (PDX) and organoid models. We found that combination of everolimus and palbociclib exhibited synergistic therapeutic effects against cell lines, PDX and organoid model. Moreover, this combination treatment significantly reduced tumor growth in PDX model without resulting in weight loss in these mice. Mechanically, RNA-sequencing and molecular pathway analyses identified survivin as a significantly differentially expressed gene in PDX treated with a combination of everolimus and palbociclib versus control. Moreover, survivin is a promising predictive marker and a potential

therapeutic target in UDEC. Thus, our findings suggest that the combination of everolimus and palbociclib reduces UDEC progression by targeting survivin. This study provided evidences on the value of PDX models for preclinical testing of everolimus and palbociclib therapy in difficult-to-treat gynecologic malignancies with UDEC.

英文關鍵詞：undifferentiated /Dedifferentiated endometrial carcinoma、everolimus、palbociclib、patient- derived xenograft、organoid、RNA-sequencing、survivin

(二) 中、英文摘要及關鍵詞 (keywords)。

中文摘要

關鍵詞：未分化/去分化子宮內膜癌、依維莫司、愛乳適、病患來源異體移植、類器官、RNA 定序、生存素

本研究計畫為一年期計畫，由於子宮內膜癌是婦科癌症中發生率最高，其中未分化/去分化子宮內膜癌(UDEC)是子宮內膜癌中侵襲力最高，最容易發生轉移與復發且對化療最易產生抗性的癌種。真實世界數據顯示 UDEC 對傳統化放療效果有限，加上目前沒有標準的治療，因此找出適合的治療策略是必須且迫切需要的。基於最近文獻指出 UDEC 的病患帶有高度 PTEN 突變，因而造成下游 PI3K/AKT /mTOR 過度活化、DNA 修復與細胞週期異常，因此我們藥物標靶 PTEN 基因所參與的調控路徑，而給予合併依維莫司(mTOR 抑制劑)與愛乳適(細胞週期依賴性激酶 4 和 6 抑制劑)標靶藥物於我們目前已經建立的 UDEC 病患來源異體移植與類器官平台。我們結果顯示合併使用依維莫司與愛乳適於細胞株、病患來源異體移植與類器官模式均具有加成抑制癌細胞增生的效果且對於小鼠模式並沒有產生體重減輕等副作用產生。機制上利用 RNA 定序與分子路徑分析經藥物處理前後的病患來源異體移植小鼠檢體，顯示生存素具有潛力成為 UDEC 生物性標記，因此我們的研究顯示合併依維莫司與愛乳適加成抑制 UDEC 癌細胞生長是經由標靶生存素基因作用機制，而這個研究揭露對於 UDEC 病患可以合併依維莫司與愛乳適作為臨床前的試驗，並利用生存素表現做為婦癌中特別難治療的 UDEC 患者的生物性指標。

英文摘要及關鍵詞 (keywords)。

英文摘要

keywords：undifferentiated /Dedifferentiated endometrial carcinoma、everolimus、palbociclib、patient- derived xenograft、organoid、RNA-sequencing、survivin

This research project is a one-year project. It focuses on endometrial cancer, which is the most common gynecologic malignancy. Undifferentiated /Dedifferentiated endometrial carcinoma (UEC/DEC, UDEC) is aggressive, metastatic, recurrent, and chemotherapy resistant. There is little experience with novel treatment in case series. Real-world experience is limited to conventional chemotherapy and/or radiotherapy. There is not standard procedure for treatment strategy for UDEC. Based on literature reported that high-frequency mutations of PTEN mutation -related deregulated downstream pathways of PI3K/AKT /mTOR and cell cycle are more common in UDEC. We aimed that treatment with druggable axis in combination of everolimus (mTOR inhibitor) and palbociclib (CDK4/6 inhibitor) has potential to improve clinical outcomes of women with UCEC to preclude single-inhibitor initiated pathway rewiring and limit toxicity in our established patient- derived xenograft (PDX) and organoid models. We found that combination of everolimus and palbociclib exhibited synergistic therapeutic effects against cell lines, PDX and organoid models. Moreover, this combination treatment significantly reduced tumor growth in PDX model without resulting in weight loss in these mice. Mechanically, RNA-sequencing and molecular pathway analyses identified survivin as a significantly differentially expressed gene in PDX treated with a combination of everolimus and palbociclib versus control. Moreover, survivin is a promising predictive marker and a potential therapeutic target in UDEC. Thus, our findings suggest that the combination of everolimus and palbociclib reduces UDEC progression by targeting survivin. This study provided evidences on the value of PDX and organoid models for preclinical testing of everolimus and palbociclib therapy in difficult-to-treat gynecologic malignancies with UDEC.

目錄

(三)報告內容：包括前言、研究目的、文獻探討、研究方法、結果與討論（含結論與建議、執行計畫過程遇到之困難或阻礙）等。

1. 前言

(1) 子宮內膜癌

依據最新衛生福利部 2019 年癌症登記報告，子宮內膜癌發生率已超越子宮頸癌和卵巢癌，且年齡層有逐漸年輕化的趨勢。子宮內膜癌中，未分化/去分化子宮內膜癌 (undifferentiated/dedifferentiated endometrial carcinoma, UEC/DEC: 合稱 UDEC) 極惡性，容易早期擴散且預後差，近年來有越來越多屬於 UDEC 型態個案出現，因此找出適合的治療策略是必須且迫切需要的 [1]。

UDEC 中的 DEC 是同時存在未分化的子宮內膜癌(undifferentiated endometrial carcinoma)與分化良好的子宮內膜癌(differentiated endometrioid carcinoma) [2] 兩種組織型態。分化良好的子宮內膜癌組織免疫染色，其常見 Estrogen Receptor positive/ Progesterone receptor positive (簡稱 ER+/PR+)；相反的，未分化的子宮內膜癌其組織免疫染色則較多屬於 ER-/ PR-/ p53+ **(Figure B-1)** [3]，因此臨床 UDEC 病患較不採用荷爾蒙相關藥物作為治療。根據我們先前整理所發表的文章，綜合現今文獻來看其治療方法與療效都沒有標準有效的治療[4]，而這類病人臨床發現越來越多案例出現，因此值得我們找出 UDEC 可能機轉與治療方針。

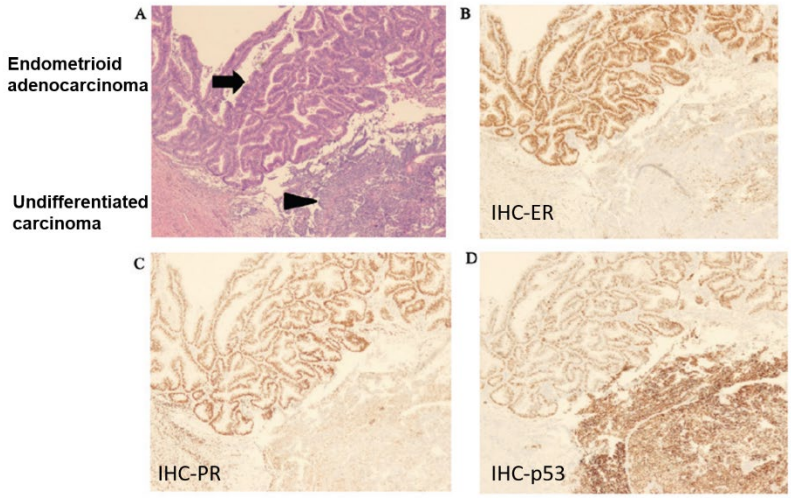

Figure B-1. Pathological findings of dedifferentiated endometrial carcinoma (DEC). The left upper area (arrow) is composed of fused glandular component and is thought to be endometrioid adenocarcinoma grade 1. On the other hand, the right lower area (arrowhead) shows cells with high nuclear/cytoplasmic (N/C) ratio proliferating without any differentiation and is thought to be undifferentiated carcinoma. According to these findings, this endometrial carcinoma is classified as dedifferentiated endometrial carcinoma **(A)**. For immunohistochemistry, endometrioid adenocarcinoma shows ER (+) and PR (+), and p53 (-) (left upper area). Undifferentiated carcinoma shows ER (-) and PR (-), and p53 (++) (right lower area) **(B, C, D)**. Original magnification, x40 [3].

(2) UDEC 基因圖譜

最近研究指出利用標靶基因測序平台定序 UDEC (N=14 位)，基因圖譜顯示突變頻率最高的基因分別是 *PTEN* (71%)， *PIK3CA* (57%) 與 *ARID1A* (43%) [5]，這些基因體異常突變有可能是造成 UDEC 細胞生長異常增生的原因 **(Figure B-2)**。

Figure B-2. The genomic landscape of undifferentiated endometrial carcinoma and undifferentiated component of dedifferentiated endometrial carcinoma. [5].

(3) 針對突變頻率最高與 CNV 變異基因擴增而使用的標靶藥物

UDEC 中，突變頻率最高為抑癌基因 *PTEN*，在癌細胞中 *PTEN* 一旦突變將導致無法抑制癌症增生，*PTEN* 基因中 lipid phosphatase 活性可以阻止 phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR 訊號傳遞路徑，進而抑制癌細胞生長。再者，*PTEN* 在細胞核中的功能包括(1)降低 MAPK，導致下游 cyclin D1 下調，進而導致細胞停在 G0-G1 時期，(2)上調 RAD51 和修復双股 DNA，(3)與 centromere-specific binding protein C (CENP-C)相互作用，增強 centromere 穩定度和促進染色體穩定；因此，一旦 *PTEN* 失去功能將造成基因體不穩定與細胞週期異常增生(**Figure B-3**) [6, 7]。因此在 *PTEN* 缺失的子宮內膜癌中，使用細胞週期 CDK4/6 抑制劑-**Palbociclib** 反應較佳[8]或使用 PI3K 下游 mTOR 的抑制劑-**Everolimus** 對腫瘤細胞抑制效果將更顯著[9]。

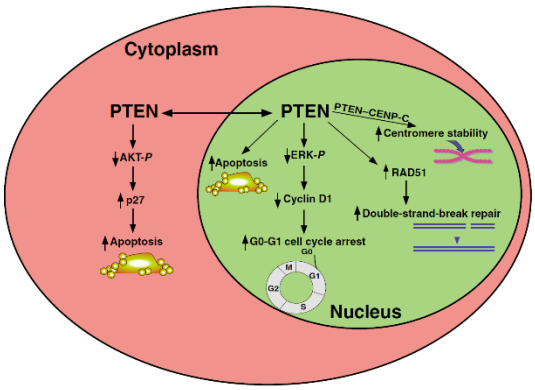

Figure B-3. Nuclear and cytoplasmic PTEN signaling. PTEN localizes to both the cytoplasm and the nucleus and shuttles between each by a variety of mechanisms. PTEN function is, at least in part, determined by its subcellular localization. The 'classic'PTEN function is cytoplasmic and includes downregulation of AKT, which increases p27 levels and thereby leads to apoptosis. By contrast, nuclear PTEN has a variety of functions: downregulation of MAPK (ERK), leading to a decrease in cyclin D1 levels and G0-G1 arrest; upregulation of RAD51 levels and double-stranded-break repair; an interaction with CENP-C, which enhances centromere stability specifically and overall genomic stability; and apoptosis [7].

(4) 病患來源異體移植 (patient-derived xenograft : PDX) 動物模型

PDX 是可以連結基礎研究與落實臨床治療的模式，因為 PDX 可以反映病人真實的模式[10]，因為子宮內膜癌為實體腫瘤，而實體腫瘤具有高度異質性 (intratumoral heterogeneity)。先前我們發表文獻已經成功建立 DEC-PDX 模式，並根據定序結果找尋藥物標靶的突變位點而給予 PDX 藥物治療[11]，然而並無法廣泛性適用所有 UDEC 患者，因此在這個計劃下，我們預期建構更多 UDEC-PDX 作為我們全面探討 UDEC 於抗癌標靶治療之轉譯醫學研究，並標靶臨床可用的藥物進行測試與機制探討(**Figure B-4**)。

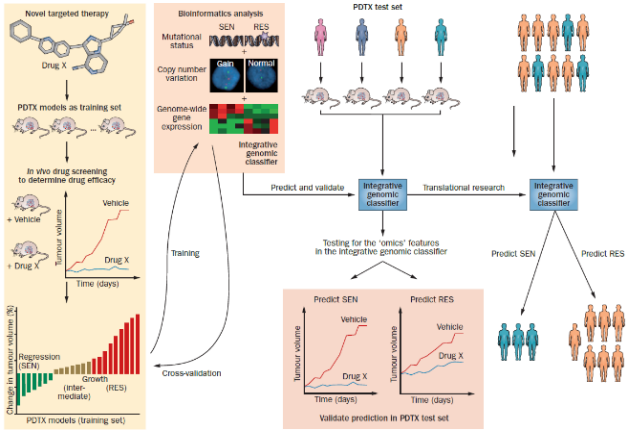

Figure B-4. Predictive biomarker development strategy in PDX models [12]

(5) 類器官(organoid)

類器官培養系統過程來自於病人衍生類器官培育，一種 3D 多細胞體外組織培養的結構體，在這系統中保有人類多功能幹細胞或癌症幹細胞，且類器官技術具有成本低、反應快，更貼近人類體內真實環境等優勢是用來研究癌化過程重要利器[13]。因此，我們將第一線取自於臨床病人組織，將病人檢體進行細胞培養成 organoid 模式，全面系統性探討 UDEC 病人適合治療模式。**(Figure B-5)**。

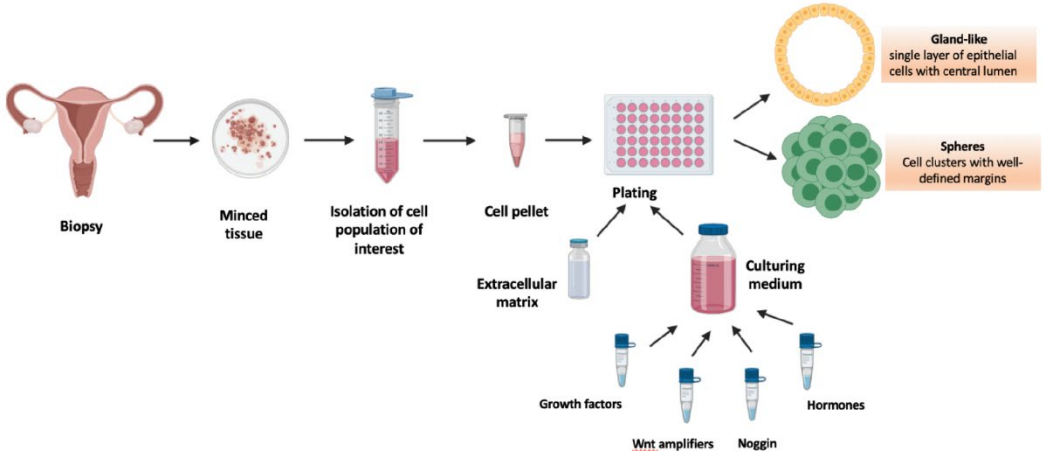

Figure B-5. Workflow of female genital tract organoid culture establishment. Successful formation and long-term maintenance of organoid models relies on starting cell population and two fundamental exogenous cues, the extracellular matrix and culturing medium. To set up a female genital tract organoid culture, a tissue biopsy is obtained, minced and the cell population of interest is isolated. The generated cell pellet is seeded on a culture plate with the addition of extracellular, and medium supplemented with a cocktail of growth factors, Wnt and BMP (Noggin) signalling molecules and hormones, which manipulate developmental pathways and ensure culture prosperity by substituting the endocrine and paracrine signals that cells receive in their natural environment, enabling tissue development, homeostasis and regeneration in cases of tissue damage [14].

因此，本計畫將直接取臨床檢體，部分檢體做為建立 PDX，部分檢體做為類器官平台建立，進行藥物測試，並回饋給臨床醫師作為第一病人的使用(Figure B-6)。

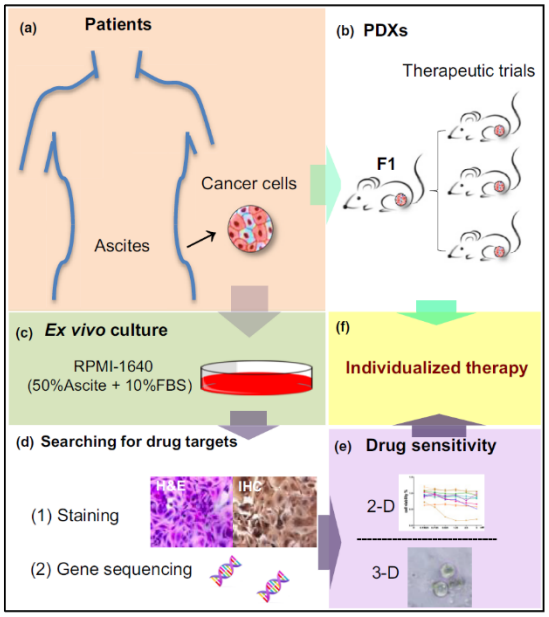

Figure B-6. A reliable platform for cancer individualized medicine. The ex vivo culture of ascites-derived tumor cells and in vivo models were developed for predicting potential effective treatment regimens. All methods ended in a recommendation for a specific therapy for a patient with malignant ascites on an individualized basis. Gene sequencing and immunohistochemistry (IHC) staining were part of a comprehensive approach to precise matching of novel therapies to patients.[13]

2. 研究目的

(1) UDEC 雖然少見，但是近年來發生率卻越來越高，目前臨床並沒有針對此族群有特定藥物治療[4]；我們與共同主持人湯雲心醫師合作，於 2022 年間在 IRB-202002059B0 與動委會 2020112603 同意書支持下，前瞻性收集 UDEC 病患，並養成 PDX 與類器官的培養，而 UDEC-PDX 的確對傳統藥物反應差，因此我們目的是對於這類病人找出適合 UDEC 治療策略。

(2) 雖然目前已有基因檢測公司根據基因檢測結果，配合其資料庫尋找合適的化學藥物或標靶藥物，並提供相關的文獻或臨床試驗資料。然而並非每位病人都有能力負擔基因檢測以及標靶藥物治療的醫療負擔，因此如果我們可以提供 UDEC 臨床指引，讓這類病人標靶藥物納入健保，將造福 UDEC 婦女。

(3) UDEC 患者 *PTEN* 突變頻率最高，再加上文獻指出子宮內膜癌細胞中，*PTEN* 功能有喪失的子宮內膜癌細胞株對 Everolimus [9]或 Palbociclib [8]對腫瘤細胞抑制效果更顯著，因而使用 Everolimus 與 Palbociclib 作為這次試驗藥物。

(4) 由於臨床顯示單用一種藥物易產生抗性，例如臨床試驗對象針對轉移的乳癌患者(Hormone Receptor- Positive, Human Epidermal Growth Factor 2-Negative；簡稱 HR+/ HER2-)，先前接受過 Palbociclib 與荷爾蒙治療藥物治療一段時間後，腫瘤細胞產生抗藥性，分析其腫瘤細胞發現參與 PI3K/AKT/mTOR 路徑之基因發生突變，因而造成 p-AKT 過度活化 (ClinicalTrials.gov Identifier: NCT02871791)，以及挑選對 Palbociclib 產生抗藥性的乳癌細胞株，分析其蛋白表現後，發現 PI3K/AKT/TOR 路徑異常上調，因而證實合併使用 Everolimus 與 Palbociclib 效果比單一藥物效果更顯著[15]，然而目前並沒有針對 UDEC 的標準治療，因此我們策略是合併 Everolimus 與 Palbociclib 作為 UDEC 治療方向。

3. 參考文獻

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4. 研究方法

(1) 臨床組織、腹水(Ascites)與胸腔積液(Pleural Effusion): **前瞻性**收集來自**共同主**持人湯雲心醫師臨床 UDEC 病人的組織，進行動物實驗與類器官培養。

(2) 動物實驗: 為了合併藥物治療實驗，我們會使用病人檢體種植于 NPG 小鼠進行 PDX 模式研究。我們會在經過認證的動物中心進行動物實驗，當小鼠狀態不佳或者體重變化減輕 20%或腹水造成體重>20%將提早進行犧牲，所有執行與運作符合「動物保護法」及相關法規之規定。

(3) 建立卵巢癌類器官模式；採取病人手術後的臨床組織立即進行組織細胞培養，新鮮組織由共同主持人湯雲心醫師手術開刀取下的檢體做為檢體來源。我們所使用的 3D 培養材料與方法是採用 Kopper 等人於 2019 年發表於 Nature medicine 國際期刊 [16]，材料如下表:

Name	Stock conc.		50ml
Wnt3a	50ug/ml, 1000x	1	ul
Noggin	100ug/ml, 1000x	2	ul
Rspo1	200ug/ml, 1000x	50	ul
B27	50x	1000	ul
N-Acetylcyste	500mM, 400x	125	ul
Primocin	50mg/ml, 500x	100	ul
Nicotinamide	1M, 100x	500	ul
A83-01	50mM, 10000x	0.5	ul
FGF10	100ug/ml, 10000x	5	ul
Heregulin	100ug/ml	18.75	ul
Y27632	100mM, 20000x	2.5	ul
EGF	500ug/ml, 100000x	0.5	ul
Forskolin	10mM, 1000x	50	ul
Hydrocortisor	250ug/ml, 500x	100	ul
E2	100uM, 1000x	50	ul
Glutamax	100x	0.5	ml
HEPES	1M, 100x	0.5	ml
P-S	100x	0.5	ml
AdDE+++		46.4	ml
AdDE+++			
Advanced DMEM/F12 contiaming 1x Glutamax, 10mM HEPES and 1% P/S			

(4) 細胞存活率分析：使用不同藥物後，細胞株經由(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide，簡稱 MTT)染色[17] ： organoid 細胞則使用 CCK-8 用於測量細胞的活性試驗，並利用 CompuSyn software (ComboSyn Inc., Paramus, NJ, USA) 計算合併指數 combination index (CI)作為計算藥物有無加成效果的依據。

(5) 目標蛋白分析: 將細胞株進行藥物處理或者針對目標蛋白抑制，收取細胞進行蛋白分析與西方轉漬實驗 [16]。

(6) RNA 萃取: 收取腫瘤細胞後，進行低溫磨碎並用 Trizol 試劑進行 RNA 萃取，送至基因體核心實驗室進行 RNA -library 製備與定序 Library-poly A RNA，2*150bp，20G data package，資料分析將與共同主持人-基因體核心實驗室顧問李御賢教授合作分析，再針對有差異的基因進行驗證。

(7) 免疫組織分析(immunohistochemistry, 簡稱 IHC): 類器官檢體進行包埋，並利用特定蛋白進行染色，並搭配 H&E 染色作為對照。

(8) ELISA 分析: 利用 ELISA 分析分泌性 survivin 的表現是否會受到藥物作用而降低分泌量。

(9) Survivin 啟動子建構: 作為探討藥物對 Survivin 調控機轉用。

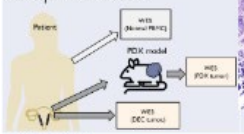
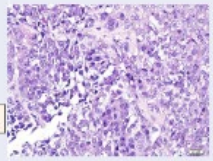
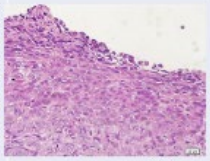
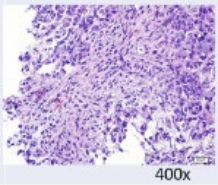
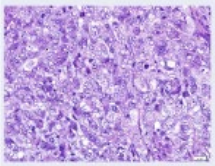
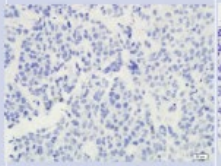
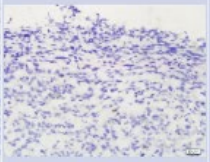
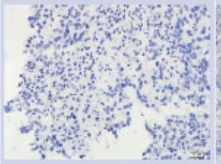
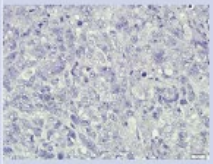
(10) 統計分析:利用 SPSS (version 22.0, SPSS, Inc., IBM, USA)分析，p 值小於 0.05 為具統計顯著性。

5. 結果與討論

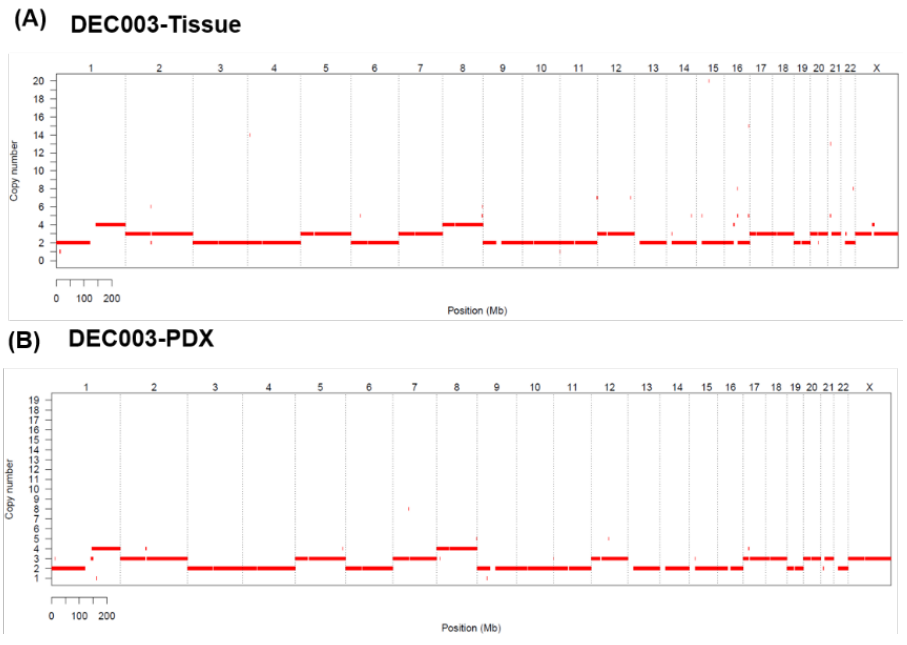
(1) UDEC-PDX 的建立

2022 年期間已經建構完成 4 例的 UDEC-PDX，4 例皆屬於 UDEC 中的 dedifferentiated endometrial carcinoma (DEC)患者，其病人基本特性如下(**Table 1)**，目前對於 UDEC 治療策略 carboplatin 與 paclitaxel，且常常會合併放療，但往往效果還是不如預期，例如:編號 DEC-001 在傳統治療期間，同時建立 PDX 半年期間，病人不幸於 2020/1/18 死亡；編號 DEC-002 於 2022 年 6 月診斷，2022 年 12 月復發，使用 Lenvatinib 加放療，但仍於 2023/3/24 死亡；編號 DEC-003 因為帶有 Mismatch repair deficiency (MMR-d)，2023/8/16 使用 neoadjuvant pembrolizumab 合併 carboplatin 與 paclitaxel 治療後，於 2024/1/10 死亡；編號 DEC-004 目前使用 carboplatin 與 paclitaxel 無效，因為病人無法負擔基因檢測與標靶藥物費用，而使用傳統化療加放療無效後，病人於 2024/4/3 死亡。

Table 1. Preliminary patients baseline demographics, disease characteristics and representative hematoxylin & eosin (H&E) staining histology images of PDX.

Case	DEC-001	DEC-002	DEC-003	UEC-004
Age	48 years old	68 years old	51 year old	53 year old
Stage	IVB 2020/1/18死亡	IB 2023/3/24死亡	IV 2024/1/10死亡	IIIA 2023/4/3死亡
MMR	MMR-p	MMR-p	MMR-d (MLH1/PMS2-loss)	MMR-p
PDX-HE staining	PDX	PDX	PDX	PDX
Schematic overview of sample collection 	 400x	 400x	 400x	 400x
IHC-PAX8	PAX8-  400x	 400x	 400x	 400x

雖然這 4 位都順利養成 PDX，但卻因目前標準治療先使用 carboplatin 與 paclitaxel 再合併放療的方式治療無效，造成病情惡化，最後病人都相繼死亡。因此對於 UDEC 病人，極需提供治療指引方向。因此我們先選擇 DEC003 和 DEC004 進行 WES 分析 **(Figure 1)**，如預期所示，病人檢體與 PDX-CNV 結果相當，代表 PDX 與檢體來源一致。



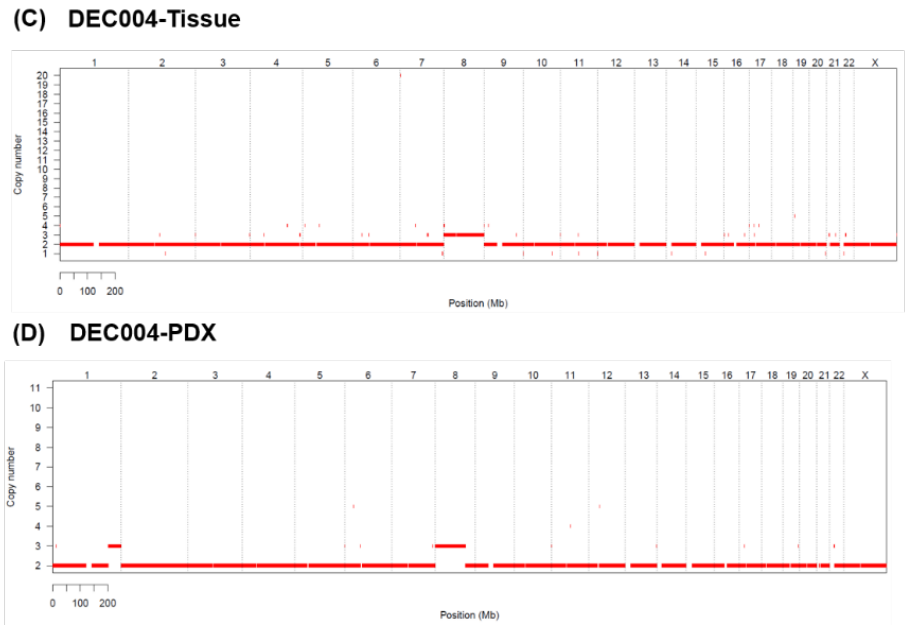


Figure 1. Comparison of copy number varients (CNVs) according to the results of WES.

(2) 類器官(Organoid)建立

我們取病人檢體進行類器官培養系統(3D/Organoid, Organoid)，並將其 organoid 進行包埋與染色，以確保與原先組織具有相同特性，基於文獻指出由於未分化的子宮內膜癌其組織免疫染色常不表現 epithelial markers: pan-keratin, E-cadherin, PAX8 [18, 19]，因此我們先將 organoid 進行 PAX8 免疫組織染色，證實此 organoid 不表現 PAX8；另外，我們也將另一位病人病理分型屬於子宮內膜亮細胞癌(endometrial clear cell carcinoma)的檢體所培養的 organoid，其臨床表現 PAX8 蛋白作為陽性對照組，如 **Figure 2** 所示，DEC-003-organoid 屬於 PAX(-)，endometrial clear cell carcinoma 屬於 PAX(+)，因此 organoid 仍保有原先組織檢體的特性

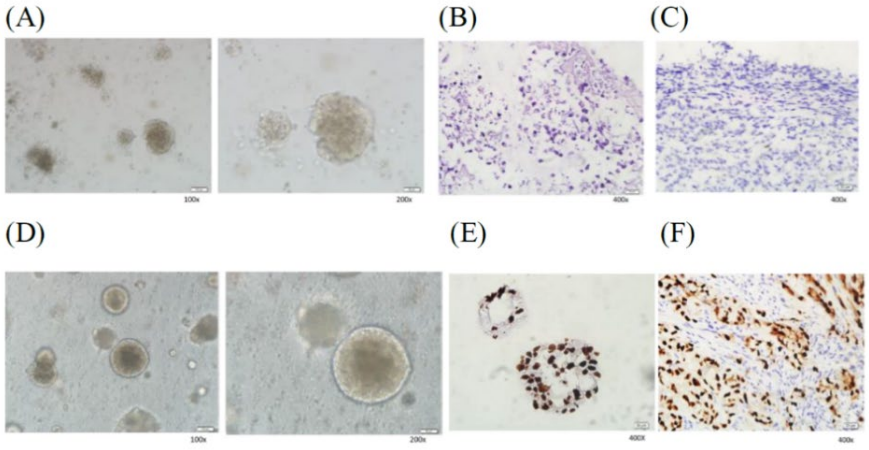


Figure 2. Morphology and immunohistochemistry of organoid and patient's tissue. **(A~C)** The patient with DEC-003. **(D~F)** The patient with endometrial clear cell carcinoma. **(A, D)** Phase-contrast for organoid. **(B, E)** Immunohistochemistry of PAX8(1:100) for organoid. **(C, F)** Immunohistochemistry of PAX8 (1:100) for

patient's tissue.

我們 DEC-003 的 organoid、PDX 檢體與病人檢體進行小型 panel (72 genes)次世代定序[20]，利用 NGSCheckMate tool [21]確定 organoid 與原來組織基因圖譜 99.18%相似，PDX 也與原來組之 99.07%相似，代表培養 organoid 過程仍保有原來組織特性**(Figure 3)**。

(A)

sample_ID	FFPE	PDX	3D/Organoid	Normal
FFPE	100.00%	99.07%	99.18%	97.98%
PDX	99.07%	100.00%	99.92%	97.81%
3D/Organoid	99.18%	99.92%	100.00%	97.84%
Normal	97.98%	97.81%	97.84%	100.00%

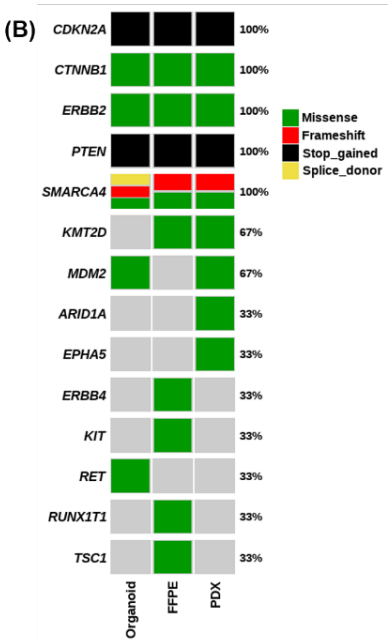


Figure 3. Gene's mutations profile comparison between organoid, PDX and formalin-fixed, paraffin-embedded (FFPE) tumor tissue by using the QIAseq targeted DNA panel DHS-005z that targeted 72 cancers associated. **(A)** The genetic identity of DEC-003 patient's organoid, PDX and FFPE tissue. **(B)** Oncoplots of gene mutations identified in DEC-003 patient's organoid, PDX and FFPE tissue. Only variants that were called by a VMT > 4 and VAF > 5% was considered significant.

(3) 合併藥物於 PDX 與類器官模式

基於 *PTEN* 缺失的子宮內膜癌中，使用細胞週期 CDK4/6 抑制劑-**Palbociclib** 反應較佳[8]或使用 PI3K 下游 mTOR 的抑制劑-**Everolimus** 對腫瘤細胞抑制效果將更顯著[9]而合併使用 Everolimus 與 Palbociclib 於 DEC-003 的 PDX 模式進行試驗，如 **Figure 4** 所示，合併使用 Everolimus 與 Palbociclib 具有加成抑制腫瘤生合成，同樣於類器官模式可以觀察到一致的現象**(Figure 5)**。

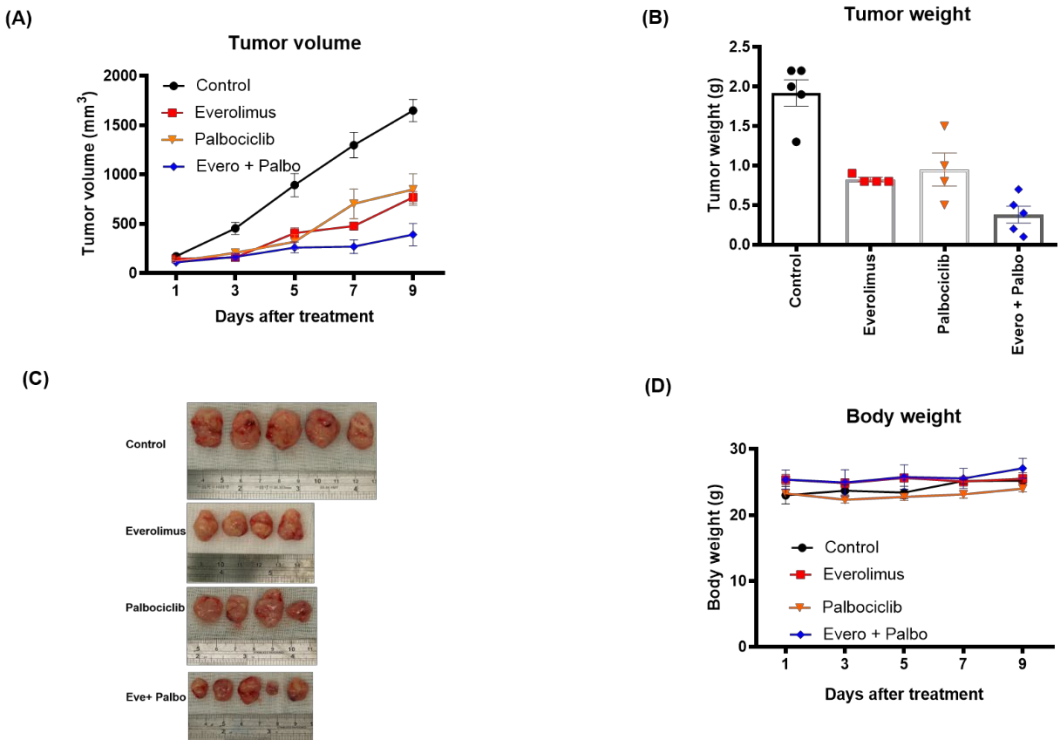


Figure 4. identification of druggable molecular targets in PDX model. PDX mice were randomized (n=4~5 per group) to receive everolimus (2.5mg/kg, given orally five days per week), palbociclib (50 mg/kg, given orally five days per week) and a combination. Control animals were left untreated. **(A)** tumor volume, **(B)** tumor weight. **(C)** gross tumors, and **(D)** body weight.

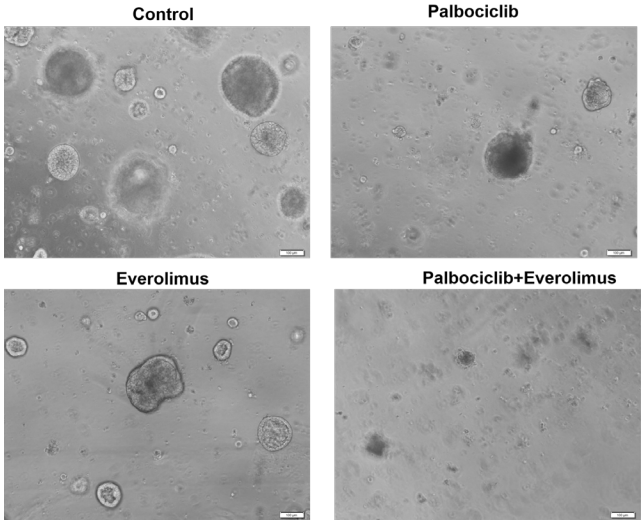


Figure 5. Combined everolimus and palbociclib inhibited DEC-003-PDX tumor growth ex vivo. Representative images of tumoroids derived from DEC-003-PDX tumors treated with vehicle, everolimus (10 μM), palbociclib (10 μM) or both (10 μM) after 14 days. Scale bar represents 100 μm.

(4) 經由 RNA-seq 找出藥物標靶基因 survivin

為了探究合併兩者藥物所調控可能機轉，我們將 DEC-003-PDX 經 everolimus 和 palbociclib 藥物處理後的檢體進行次世代 RNA-seq，交集任一藥物降低的 17 個基因中，找到與細胞凋亡相關基因 BIRC5 又名 survivin **(Figure 6)**.，此基因被報導在子宮內膜癌中，survivin 越高其病人預後越差，是具有潛力作為子宮內膜癌重要預後因子[22]，因此我們假說是否在子宮內膜癌中，UDEC 病人又特別高表現 survivin，造成病人預後差，當我們給予 everolimus 和 palbociclib 藥物，可以標靶 survivin，進而抑制 UDEC 癌細胞生長。

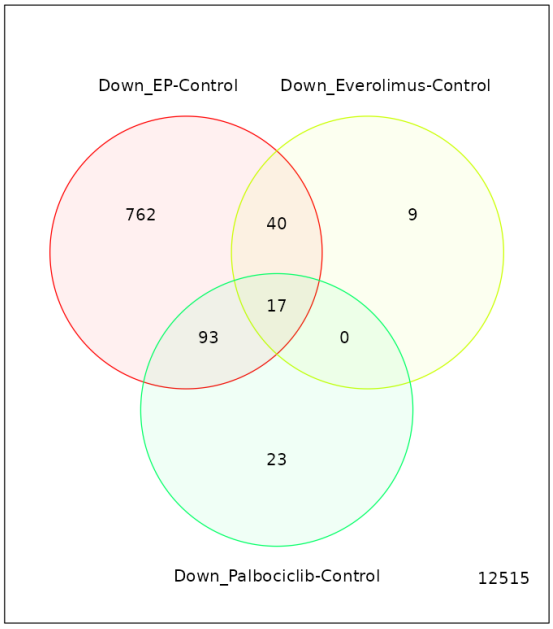


Figure 6. Venn diagram showing numbers of differentially expressed genes in each of the two groups, everolimus/control, palbociclib/control, everolimus+palbociclib/control by RNA sequencing.

(5) Everolimus 和 Palbociclib 對細胞內外的 Survivin 都具有抑制作用

為了證明 UDEC-003-PDX 經 everolimus 和 palbociclib 藥物處理後的檢體的確有效抑制 survivin 表現，我們分析 DEC-003 和 DEC-004 經藥物處理後的檢體與 UDEC 細胞株 AN3CA[23]與 KLE[24]，的確如預期 everolimus 和 palbociclib 抑制 survivin 的 RNA 的表現**(Figure 7)**，同樣 survivin 蛋白也有一致趨勢**(Figure 8)**，我們進一步分析小鼠血液中，游離 survivin 一樣到藥物抑制**(Figure 9)**。

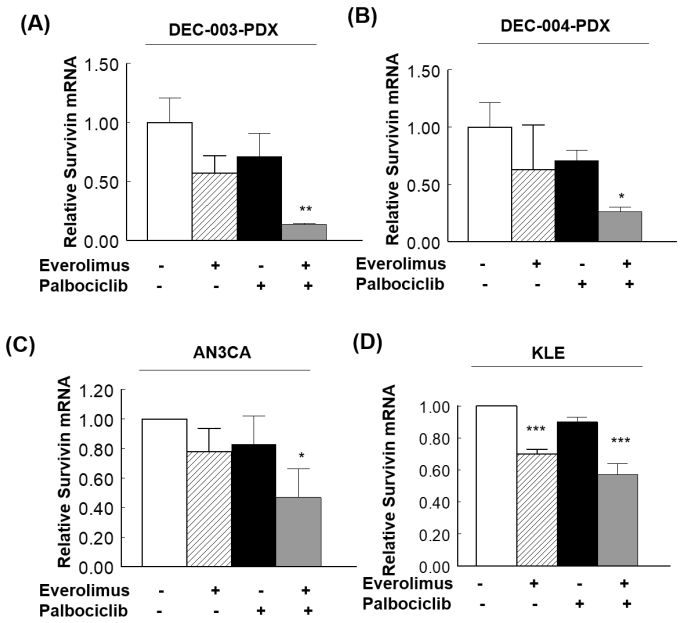


Figure 7. The effect of everolimus and palbociclib on survivin mRNA expression in DEC-PDXs and UDEC cell lines. **(A, B)** Tissues from the DEC-003-PDX and DEC-004-PDX models treated with everolimus and palbociclib or their combination were collected. **(C, D)** AN3CA and KLE cell lines were treated with everolimus (10uM) and palbociclib (10uM) and both, lysates were than analyzed lysates were than analyzed by qPCR.

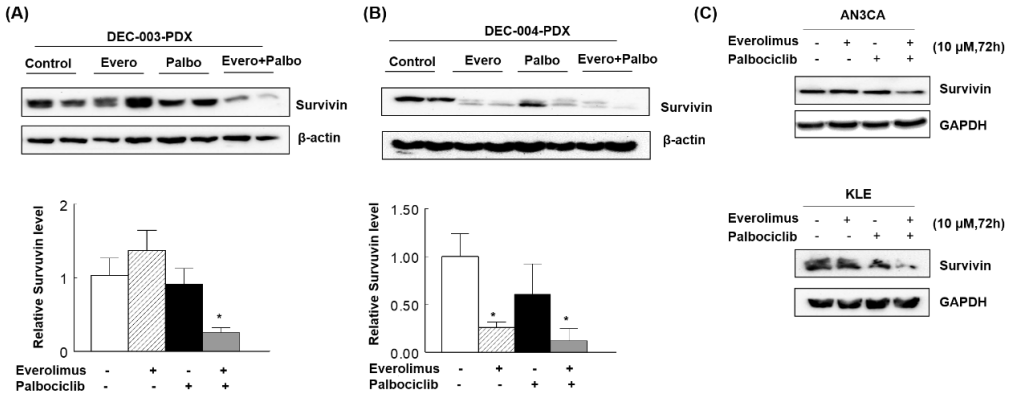


Figure 8. The effect of everolimus and palbociclib on survivin protein expression in DEC-PDXs and UDEC cells. **(A, B)** Tissues from the DEC-003-PDX and DEC-004-PDX models treated with everolimus and palbociclib or their combination were collected. **(C)** AN3CA and KLE cell lines were treated with everolimus (10uM) and palbociclib (10uM) and both, lysates were than analyzed by western blot. Everolimus: Eve, Palbociclib: Palbo, Everolimus plus Palbociclib: Eve+Palbo.

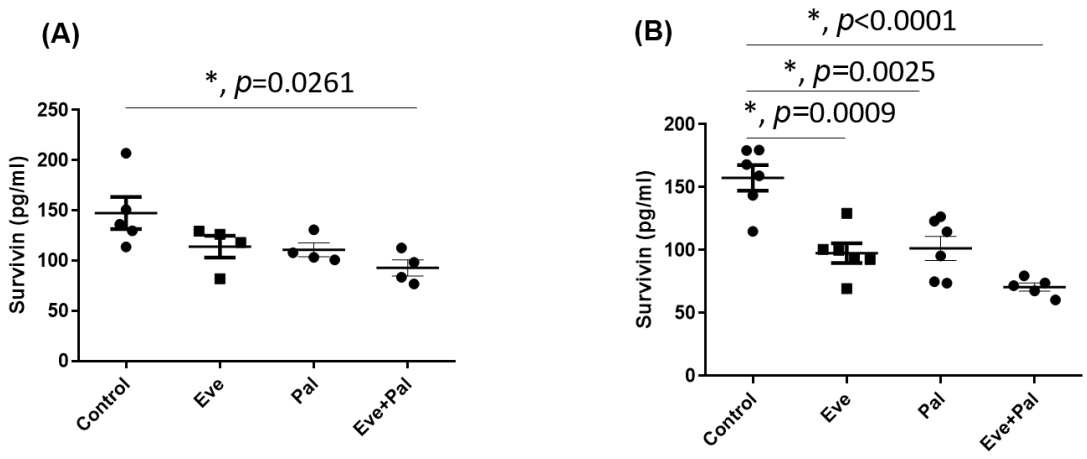
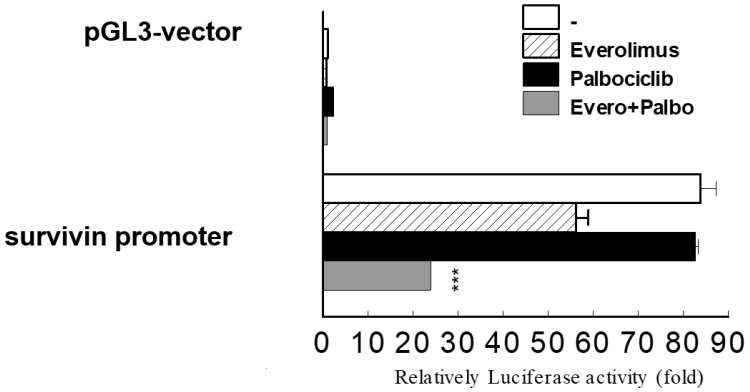


Figure 9. The serum expression levels of survivin in PDX mice treated with everolimus and palbociclib were determined using an ELISA assay. The results are represented for two distinct PDX models. **(A)** DEC-003-PDX. **(B)** DEC-004-PDX

(6)調控 Survivin 的可能機制

由於 Everolimus 和 Palbociclib 藥物抑制經由 Survivin 轉錄調控，因此我們分析藥物是否對 Survivin promoter 進行調控，如 **Figure 10A** 所示，合併兩者於 AN3CA 細胞具有加成抑制，為了找出藥物透過何者基因或訊號傳遞路徑調控 survivin，我們進一步分析 Survivin promoter 上的轉錄因子，包括 NF-**κ**B、Sp1 和 STAT3 binding site **(Figure 10B)**[25]，因此當我們使用 siRNA 標靶 NF-**κ**B 和 Sp1 基因降低 NF-**κ**B 和 Sp1 表現後，發現降低 NF-**κ**B 後，Survivin promoter 活性隨之降低，而這樣的現在在降低 Sp1 基因中沒有看到**(Figure 10C)**，因此我們假設藥物影響 NF-**κ**B 的表現，造成 NF-**κ**B 對 Survivin 調控降低，最後導致 UDEC 癌細胞生長。

(A)



(B)

(C)

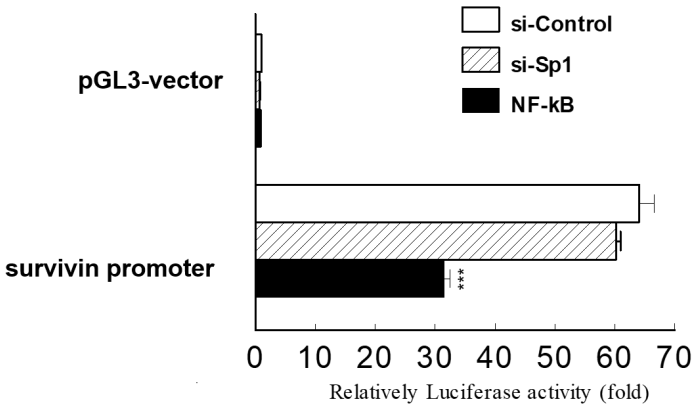


Figure 10. Everolimus and palbociclib treatment reduced survivin promoter activity through in UDEC cells. **(A)** AN3CA cells were transiently cotransfected with 1 μg of survivin promoter reporter and 0.2 μg of β-galactosidase plasmid, and subsequently treated with everolimus (10μM), palbociclib (10μM) or both. Forty-eight hours later, cells were lysed, and protein lysates were assayed for luciferase and β-galactosidase activities. Relative promoter activity was normalized to β-galactosidase activities. **(B)** The schematic diagram shows the promoter regions [25]. **(C)** AN3CA cells were transiently cotransfected with 1 μg of survivin promoter reporter, 0.2 μg of β-galactosidase plasmid, and 10 μM siRNA. Forty-eight hours later, cells were lysed, and protein lysates were assayed for luciferase and β-galactosidase activities. Relative promoter activity was normalized to β-galactosidase activities.

因此，本研究利用 PDX 與類器官證實使用 everolimus 和 palbociclib 可以有效抑制 UDEC 腫瘤生長，機制上是經由 NF-***κ***B 調控 Survivin 進而導致 UDEC 癌化機轉。

六、結論:

綜合以上結果揭露對於 UDEC 病患可以合併 everolimus 與 palbociclib 作為臨床前的試驗，並利用 Survivin 表現做為婦癌中特別難治療的 UDEC 患者的生物性指標。目前，論文正在撰寫中。