

出國報告(出國類別：開會)

2013 18th International Congress of Cytology
2013 第 18 屆國際細胞學會議

服務機關：國防醫學院三軍總醫院

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摘要

第十八屆國際細胞學會議(The 18th International Congress of Cytology)大會，於一百零二年五月二十四日至五月二十八日，為期共五天在巴黎召開。本人以海報展示，題目為「肺腺癌在轉移過程中導致惡性肋膜腔積水及豐富癌幹細胞特性之研究」，大會宗旨在結合各地病理醫師及細胞醫檢師在基礎醫學研究上的成果和國際學者的演講來帶動主辦細胞病理的進步與學習，如此一方面增進更深一層的學術研究以及發表四年來的成果以綻放出學術智慧的火花及促進新展望。在經由主辦國的會議的安排及在學術研究報告做一相關的整合。此次參展光是海報展示外，還有一系列的四天演講。其內容主要包括了促進子宮頸抹片的精進、乳房篩檢的改良以及相關分子及細胞病理檢驗技術的突破，讓全世界了解醫療知識及未來醫療方向、二方面可同時增進全世界有一致性的細胞病理促進方案。本人榮幸能參加本屆盛大的 2013 年國際細胞學會議，並於會中以海報展示肺癌研究成果，藉此良機汲取醫學新知，獲益匪淺、深深銘感於心。

(參加 2013 年國際細胞學會議)

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壹、目的

奉國防部國醫衛勤字第 1020003293 號令，應邀赴法國巴黎出席 2013 年國際細胞學會議(2013 International Congress of Cytology)大會，出國時間為一百零二年五月二十四日至五月二十八日，為期共五天。本次國際會議以海報展示為主，題目為「肺腺癌在轉移過程中導致惡性肋膜腔積水及豐富癌幹細胞特性之研究」，參加四年一次國際細胞學會議，誠屬學術之饗宴，也是檢驗世界各地細胞病理發展的重要指標與競技場，更是期盼直接經由國際學者的演講和世界各地的壁報展示，彼此觀摩與學習國際最新細胞病理發展的走向，也希望經由參加會議後，再將此國際最新知識帶回國內，並且也可以從他人的報告中獲得其研究構思和最新臨床診斷及技術的重點心得。

國際細胞學會議四年一次由各委員前一屆推舉出著名的城市舉行，國際細胞學會議主要是歐美及澳洲組織，此二屆由歐盟國家和以前的大英國協的國家互相舉辦，四年前於英國愛丁堡舉行，今年在法國巴黎，四年後將在日本橫濱舉行。目的在結合各地病理醫師及細胞醫檢師在基礎醫學研究上的成果和國際學者的演講來帶動主辦細胞病理的進步與學習，如此一方面增進更深一層的學術研究以及發表四年來的成果以綻放出學術智慧的火花及促進新展望。在經由主辦國的會議的安排及在學術研究報告做一相關的整合，才能達到國際會議的主要目的。

此次參展光是海報就有來自世界各地 393 篇的展示，還有一系列的 4 天演講。其內容主要包括了促進子宮頸抹片的精進、乳房篩檢的改良以及相關分子及細胞病理檢驗技術的突破，讓全世界了解醫療知識及未來醫療方向、二方面可同時增進全世界有一致性的細胞病理促進方案。目前國內細胞病理科也希望更多有志者投入參與並竭盡全力朝向此個人化醫療研究及努力。

本人榮幸能參加本屆盛大的 2013 年國際細胞學會議，並於會中以海報展示肺癌研究成果，藉此良機汲取醫學新知，深深銘感於心。

貳、會議緣起

國際細胞學會議主旨係承促進國際間細胞病理學之學術交流，聯絡病理醫師及細胞醫檢師之彼此情誼，延聘許多國際知名的學者蒞臨演講，以提供目前醫學新資訊及研究導向。

每四年皆選定國際中各大新興發展城市為會場，今年訂在巴黎為會議主要城市，會期為期五日。

參、會議過程

此次四年一度的細胞學國際醫學會議，誠屬學術之饗宴，也是檢驗世界各地細胞病理發展的重要指標與競技場，更是期盼直接經由國際學者的演講和世界各地的壁報展示，彼此觀摩與學習國際最新細胞病理發展的走向。會議議程為期五天，前三天是註冊報到的時段，而會議的地點位於巴黎的世貿中心，交通便捷是大眾交通地鐵的交會處，世界各地的病理及細胞學者紛紛參與會議，包括口頭報告與海報展示。大會還安排許多參展的廠商，展現新穎的儀器設備。本人參觀的過程中，發現傳統的細胞抹片製作的過程費時且不穩定，品質不如現在帶領潮流的液相抹片，這種液相的抹片純粹由自動化的機器製作。一來可以避免傳統抹片人為的疏失，達到較為一致的良好品質。二來可以集中細胞數量並去除干擾物，使抹片的背景很乾淨，達到較好的診斷品質。大會展示的兩項機器包括：(1)BD SurePath 自動化液相儀，來自英國，其中尚有自動閱片的功能，可提供細胞學實驗室做品質管制或品質保證。(2)Thin Prep 薄層細胞儀，來自美國，抹片亦較傳統製作者為佳，機器花費較貴。除了見識到新穎的細胞病理儀器外，另有一展示口腔癌篩檢的設計毛刷，並提供口腔前期癌病變白紅斑之刷抹偵測染液；這對近年來台灣漸漸上升的口腔癌病變似乎有可幫助。與廠商接洽商談並留下聯絡方式以便日後互相交換心得。本人於大會議裡的第二天進入會場報到後，並積極與國際相關研究學者交流，表達自己的海報內容，此次大會有另一項新的創舉為所有 393 篇海報都可以電子檔方式呈

現，任何有興趣參觀學者都可透過大會安排的電腦將有興趣之海報編號輸入電腦後可放大投影至大螢幕上，更清楚顯示其海報的真實內容。

大會海報展示的論文中，與本人類似或相關的報告亦有多篇，其中最令人激賞的一篇是來自土耳其學者的論文報告，有關肺癌末期中導致惡性積水後，研究體液中惡性細胞之 EGFR 及 KRAS 之相對應關係，積極找出為何針對 KRAS 突變之病例，用 anti-EGFR 標靶藥物效果不彰的原因。利用運用免疫組織及細胞化學染色法來鑑定惡性積水中的可疑細胞並分離凝聚細胞團塊，進一步做成細胞蠟塊(Cell block)的分離技術讓人印象深刻。

一、題目：

「肺腺癌在轉移過程中導致惡性肋膜腔積水及豐富癌幹細胞特性之研究」。
(Pulmonary Adenocarcinoma in Malignant Pleural Effusion Enriches Cancer Stem Cell Properties during Metastatic Cascade)

二、研究背景：

惡性腫瘤轉移是一連串之步驟包括侵襲血管新生、淋巴血路滲透及建立轉移的地盤。肺腺癌在轉移過程中導致惡性肋膜腔積水通常表示極不佳的預後，其潛在的機轉至今尚不十分清楚。因此也激發本實驗室研究的動機，研究轉移的過程中包括上皮-間葉轉型、抗凋亡、癌幹細胞特性的發展以及與微環境的關係。

三、研究方法與步驟

20 例肺腺癌導致惡性積水以免疫組織化學染色研究上皮-間葉轉型及癌幹細胞的腫瘤標記表現，並以原組織細胞培養觀察其形成球狀的能力。

四、結果

20 例中約有 15-90%的比例呈現或多或少癌幹細胞標記的表現，其中以 Oct-4 最爲特別明顯。Oct-4 過度表現表示臨床已趨向末期及預後極不佳的狀況。原組織培養 8 例中有 5 例可養出球狀幹細胞的特性。

五、 結論

由以上結果顯示藉本實驗模型之建立可揭露癌症轉移中肋膜積水的機轉及奧妙。Oct-4 可作爲惡性積水之標記及治療的標靶。

肆、會議心得（對應會議過程）

此次參展光是海報就有來自世界各地 393 篇的展示，還有一系列的 4 天近 50 多場的演講。其內容主要包括了促進子宮頸抹片的精進、乳房篩檢的改良以及相關分子及細胞病理檢驗技術的突破，讓全世界了解醫療知識及未來醫療方向、二方面可同時增進全世界有一致性的細胞病理促進方案。此次大會有另一項新的創舉爲所有 393 篇海報都可以電子檔方式呈現，任何有興趣參觀學者都可透過大會安排的電腦將有興趣之海報編號輸入電腦後可放大投影至大螢幕上，更清楚顯示其海報的真實內容。此種方式使海報展示者無須千里迢迢帶著海報至會場展示，也可避免海報格式的不一致。這次海報展示論文主要是著重在臨床的個案分析及罕見病例的報告，另外還有跟偵測 HPV 病毒的濃度及類別相關的論文報告也有二十餘篇，此外亦有多篇 HPV 疫苗製作及疫苗後的追蹤成果報告，口腔癌的篩檢相關的論文報告也是此次大會展示的主題，至於免疫細胞組織化學染色及分子病理相關的報告也有數篇。本人以海報展示發表之文獻爲主要基於肺腺癌惡性胸水發生率在台灣及全世界都有著顯著上升的趨勢。癌症幹細胞的理論解釋了許多我們在研究中或臨床上所發現的問題，如治療抗性、侵襲轉移、癌症復發等。大量關於癌症幹細胞的研究在近幾年興起，因此建立一個有效可靠

的實驗模式是目前十分重要的課題。回顧文獻，目前分離癌症幹細胞方法皆有其限制的地方，並且所篩選出的抗藥性細胞與癌症幹細胞是否劃上等號也需經由實驗進一步驗證。有鑑於上述使用之癌症幹細胞分離培養技術在使用上皆有所限制，因此我們希望發展新的實驗方法提供現今癌症幹細胞研究在實驗技術上一種快速且經濟的選擇。基於以上種種原因也激發本人研究肺癌幹細胞的興趣，並建立以肋膜腔惡性積水為研究肺癌幹細胞的模型，因此引發本實驗室強烈研究之動機並研發提供一個有效之研究模型。根據先前研究指出，當肺癌細胞生長於肋膜腔懸浮狀態時，會逐漸聚集形成 3D 立體球狀，並透過肋膜腔微環境可以激發肺癌幹細胞的特質成為研究肺癌幹細胞的模式。以臨床蠟塊免疫組織化學染色分析原癌細胞株及惡性胸水細胞其癌症幹細胞表面標記(surface markers)表現情形。發現在惡性胸水細胞中癌症幹細胞相關轉錄因子 Oct4、Nanog 及 CD133 在惡性胸水細胞株表現比例明顯上升。抽取惡性胸水進行細胞培養並分析癌症幹細胞相關轉錄因子 Oct4、Nanog、CD133，相較於原癌細胞株其 mRNA 表現量在惡性胸水細胞中均大量表現。經由螢光染色，可見惡性胸水細胞內部各標記表現情形不一致呈非均勻分布，可見惡性胸水細胞具有異質性。由實驗結果驗證，肋膜腔惡性積水充滿著被激發的癌症幹細胞特性，為研究肺癌幹細胞相當好的模型。本研究結果已撰寫成文章，並投稿於 PLOS one 最近已接受刊登。

伍、建議事項

這次 393 篇的海報展示論文主要是著重在臨床的個案分析及罕見病例的報告，另外還有跟偵測 HPV 病毒的濃度及類別相關的論文報告也有二十餘篇，此外亦有多篇 HPV 疫苗製作及疫苗後的追蹤成果報告，口腔癌的篩檢相關的論文報告也是此次大會展示的主題，至於免疫細胞組織化學染色及分子病理相關的報告也有數篇。返回科部後初將擇時間向同仁們口頭報告外，並將此次會議中學習的心得以細胞刷抹製作抹片來偵測初期的口腔癌，連絡院內相關科部包括口腔外科及耳鼻喉科部等共襄盛舉，

透過以往婦產科篩檢子宮頸抹片的經驗。與臨床醫師溝通並配合收集及取樣推廣口腔癌的篩檢。每次參加國際醫學會議的感想，就是敬佩當地政府的重視和支持，加上國際工作人員的協助與對大會的準備工作，在會議中場地標示清楚，工作人員態度表現佳，讓外國來的我們感到溫馨，就是他(她)們對事務的投諸很多的心力。台北國際會議廳及各大飯店亦可在台北提供很好的場地，但更殷切需要的是籌辦國際會議的心思及人力灌注。希望有關學術單位能適時的爭取主辦權。更期盼上級多能鼓勵屬下參加類似的國際醫學會議，嶄露頭角，並增廣見聞。並可透過舉辦國際會議，增加台灣的曝光度及知名度，及增進台灣的醫療資訊，進而及時跟上世界潮流。本人榮幸能獲參加本屆盛大的 2013 年第十八屆國際細胞學會議。以海報向國際醫界發表研究成果，同時藉此良機汲取醫學新知，銘感於心，並深切瞭解醫學界之國際脈動及潮流。

陸、附件資料

一、2013 年國際細胞學會議出席證明



P-097

MALIGNANT GASTROINTESTINAL STROMAL TUMOR (GIST) IN ASCID FLUID

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Objectives: Although gastrointestinal stromal tumors (GISTs) are uncommon, these tumors are one of the most common mesenchymal tumors of the gastrointestinal tract. These tumors have in common certain clinical, molecular and immunocytochemical features. About 60% of GISTs are of spindle cell type. These tumors can be recognized in aspiration biopsies. The presence of elongated spindle shaped malignant cells with large abnormal nuclei and nucleoli are characteristic features.

Materials and Methods: 64 years old male patient was treated and followed up for the rectal adenocarcinoma during the last two years. The patient has nausea, loss of weight and abdominal pain for two months. The abdominal magnetic resonance angiography was shown a big mass starting from the level of the cardia region of stomach extending along the posterior wall fundus to the antrypyloric region and filled to the omentum (22x20x10 cm sized). Minimal ascid fluid was detected radiologically and fine needle aspiration was performed. The microscopic examination of Papanicolaou and giemsa stained smears demonstrated the presence of malignant cell groups in the background with red blood cells and rare mesothelial cells. These elongated, spindle shaped malignant cells were found to the have enlarged, pleomorphic, hyperchromatic nucleoli, prominent nucleoli and scanty cytoplasm. These features were reminiscent of the mesenchymal lesions.

Results: We were diagnosed this smear as "malignant mesenchymal tumor, suspicious for the gastrointestinal stromal tumor". Thereafter, the patient underwent abdominal mass excision. Histopathological examination of the huge mass confirmed the "Malignant gastrointestinal stromal tumor, high risk category".

Conclusion: In ascitic fluid, GISTs morphologically resemble adenocarcinomas. Therefore, the cytopathological examination is very important.

References:

Ann Clin Lab Sci 2009; 39(4): 367-371.

Disclosure of Interest: None Declared

Keywords: Ascid fluid, GIST

P-098

CYTOLOGY IS A USEFUL TOOL FOR THE RAPID DIAGNOSIS OF HERPES SIMPLEX VIRUS (HSV) INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS

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Objectives: In recipients of solid organ transplantation, HSV infection most commonly results from reactivation of latent virus. Clinical manifestations are often atypical and may lead to delay in diagnosis, highly prejudicial in cases of disseminated infection. This case illustrates the value of cytology for the rapid diagnosis of HSV infection in solid organ transplant recipients.

Materials and Methods: A 38-year-old man underwent a kidney transplantation for IgA nephropathy. Pre-transplantation serologies were negative, including HSV and CMV. Three weeks after transplantation, the patient was hospitalized for influenza-like syndrome with fever at 40°C. There were no mucocutaneous lesions but a discrete hepatic cytolysis that quickly increased at J5 (AST 523 IU / L, ALT 669 IU / L) associated with liver failure (TP 49%, factor V 56%) and pancytopenia, leading to perform blood PCR HSV1 and HSV2 (Herpes consensus ARGENT) and peritoneal lavage. Cytospins were stained with May Grunwald Giemsa, and Papa or kept for immunocytochemical study (HSV1 and 2).

Results: The peritoneal washing was highly inflammatory with 1000 leucocytes/mm³. Cytological examination revealed the presence of multinucleated cells with ground glass nuclei and nuclear moulding. These cells were specifically labeled by direct fluorescent anti-HSV1 antibody. Labeling of these cells for anti-CKS/6 and anti-calretinin confirmed their mesothelial nature. These cytological and immunocytochemical results allowed the diagnosis of HSV1 peritonitis.

The same day, the HSV1 DNA was detected by PCR in the blood (10 log₁₀ copies / mL). **Conclusion:** HSV peritonitis, although very rare, must be considered in ascitis or peritoneal washing. These virus-infected cells may be misinterpreted as malignant cells. This is the first reported case of HSV1 peritonitis. It illustrates the importance of cytological and immunocytochemical examinations for a sensitive, specific and rapid diagnosis of HSV infection, in addition to PCR which remains the gold standard. In this case, the graft may have been the source of infection. The outcome was favorable after rapid administration of intravenous acyclovir.

Disclosure of Interest: None Declared

Keywords: herpes, peritoneal, transplant

P-099

SOLUBLE MESOTHELIN-RELATED PEPTIDES DETECTION IN MESOTHELIOMA PATIENT SERUM AND PLEURAL EFFUSION: A CONTRIBUTION TO CYTOLOGY

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Objectives: The sensitivity of cytology (Cyt) for the diagnosis of Malignant Pleural Mesothelioma (MPM) is low (about 30%). Indeed, the investigation of levels of several soluble tumour biomarkers, such as Soluble Mesothelin (SM), in serum and/or pleural effusion (PE), have been proposed to improve MPM diagnosis. In this study, we assessed the contribution of SM detection to the diagnosis and the routine screening of MPM.

Materials and Methods: Cyt was evaluated on fixed smears stained by Papanicolaou's method. Cyt, PE-SM and serum-SM levels were simultaneously evaluated for each patient. We studied 43 patients with MPM (26 epithelioid (Ep), 9 sarcomatoid (Sr), 4 biphasic (Bp), 2 desmoplastic (Ds), 2 papillary (Pp)), 36 patients with pleural benign lesions (BNG) and 23 patients with non-MPM pleural metastasis (MTS). SM levels were detected by the MesoMark ELISA kit.

Results: By the Youden's index we found that the SM cut-off level for diagnosis of MPM was 1.08 nM in serum and 12.70 nM in PE. Serum-SM levels were > cut off in 20/43 (sensitivity%=46.5) MPM (11 Ep, 4 Sr, 4 Bp, 1 Pp), in 3/36 (8.3%) BNG and in 6/23 (26.1%) MTS, (specificity%=84.7). In contrast, PE-SM levels were > cut-off in 30/43 (sensitivity%=69.8) MPM (19 Ep, 5 Sr, 2 Pp, 4 Bp), in 3/36 (8.3%) BNG and in 4/23 (17.4%) MTS, (specificity%=88.1).

Cyt allowed the diagnosis in 11/43 (sensitivity%=25.6) MPM (6 Ep, 3 Sr, 1 Bp, 1 Pp) and in 11/23 (47.8%) MTS while it was negative in 100% of BNG. Comparison between serum-SM and Cyt demonstrated discrepancy for diagnosis in 41/102 (40.2%) cases. The serum-SM positive/Cyt negative cases were: 16/43 (37.2%) MPM (8 Ep, 4 Sr, 3 Bp, 1 Pp), 3/36 (8.3%) BNG and 5/23 (21.7%) MTS. In contrast, the serum-SM negative/Cyt positive cases were: 7/43 (16.3%) MPM (3 Ep, 3 Sr, 1 Pp), 0/36 (0.0%) BNG and 10/23 (43.5%) MTS. Comparison between PE-SM and Cyt demonstrated discrepancy for diagnosis in 43/102 (42.2%) cases. The effusion-SM positive/Cyt negative cases were: 23/43 (53.5%) MPM (15 Ep, 4 Sr, 3 Bp, 1 Pp), 3/36 (8.3%) BNG and 3/23 (13.0%) MTS. The PE-SM negative/Cyt positive cases were: 4/43 (9.3%) MPM (2 Ep, 2 Sr), 0/36 BNG and 10/23 (43.5%) MTS. Finally PE-SM showed better diagnostic performances than serum-SM in Cyt negative patients (Sensitivity%=71.9 vs 50.0; Odd ratio=17.9 vs 5.0).

Conclusion: SM detection in serum and/or PE may be an adjunct to Cyt for the routine screening of MPM but it cannot replace it. Our data also suggest that detection of PE-SM can contribute to improve the diagnosis of Cyt in MPM more than detection of serum-SM.

Disclosure of Interest: None Declared

Keywords: cytology, mesothelioma, pleural effusion, soluble mesothelin

P-100

PULMONARY ADENOCARCINOMA IN MALIGNANT PLEURAL EFFUSION ENRICHES CANCER STEM CELL PROPERTIES DURING METASTATIC CASCADE

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Objectives: Metastasis occurs in a series of discrete steps involving invasion, angiogenesis, lymphovascular space permeation, and establishment of secondary tumors. Malignant pleural effusion (MPE), a type of tumor metastasis, is usually a poor prognostic sign for patients with pulmonary adenocarcinoma, although its underlying mechanism has received less attention than other types of metastases have. The objective of the current study was to confirm whether cancer stem cells (CSCs) in MPE contribute to the "metastatic cascade" through the epithelial-mesenchymal transition (EMT), anoikis, and adaptation in the microenvironment.

Materials and Methods: Pulmonary tissue and corresponding cell blocks of MPE samples from 20 patients with primary adenocarcinoma were analyzed by immunohistochemical staining with CSC-representative markers (CD133, Nanog, and OCT-4) and EMT-associated markers (E-cadherin and vimentin). Correlations between these variables and clinico-pathological parameters were analyzed. Primary cultures from eight cases of MPE were investigated to characterize the CSC properties, including marker expression, sphere formation, and differentiation.

Results: Expressions of CSC-representative markers 20 cases of MPE cell blocks were quite diverse and variable ranging from 15% to 90%. Stronger expression of CSC-representative markers and alteration of EMT-associated markers, were found at the invasive fronts and in MPEs compared with the expression in primary pulmonary tumor tissues. The expression of OCT-4 in MPEs significantly related to distant metastasis and stage, as well as inversely correlated with patient survival. Primary cultures confirmed the CSC properties in MPE. Five of eight cases of MPE yielded adequate cell clusters, which also showed variable expressions of CSC markers in addition to sphere formation and the ability for differentiation and metastasis.

- P-094** THE ROLE OF IMMUNASSAYING IN CONFIRMING THE SQUAMOUS ORIGIN OF MALIGNANT CELL IN EFFUSION FLUID - A CASE REPORT
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Eleni Ntola ^{1,*}, Evangelos Zacharis ¹, Maria Vrontaki ¹, Eleni Gklisti ¹, Dimitrios Klis ¹, Olena Ignatova ¹, Dastamani ¹, Anastasia Alexiadou ¹
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Mathieu Talagas ^{1,*}, Françoise Charles-Petillon ¹, Arnaud Uguen ², Sebastian Costa ², Christopher Pagan ³, Cornec-Le Gall ⁴, Anne Grall-Jezequel ⁵, Jean Amice ¹, Marc De Braekeleer ¹
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- P-099** SOLUBLE MESOTHELIN-RELATED PEPTIDES DETECTION IN MESOTHELIOMA PATIENT SERUM AND PLEURAL EFFUSION: A CONTRIBUTION TO CYTOLOGY
Franco Fedeli ^{1,*}, Pier Aldo Canessa ¹, Paola Ferro ¹, Enrico Battolla ¹, Antonella Vignani ¹, Donatella Innocenzi ¹, Paolo Dessanti ¹, Luigi Chiaffari ¹, Bartolomeo Bacigalupo ¹, Vincenzo Fontana ², Maria Cristina Franzosini ³, Pia Pistillo ², Silvio Rancella ¹
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- P-100** PULMONARY ADENOCARCINOMA IN MALIGNANT PLEURAL EFFUSION ENRICHES CANCER STEM CELL POPULATION DURING METASTATIC CASCADE
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Hiroyuki Miura ^{1,*}, Eiji Nakajima ¹, Yujin Kudo ¹, Hidenobu Takahashi ¹, Norihiko Ikeda ², Hiromi Sawamura ³, Miura ¹, Yoshie Yakatsuki ⁴, Yume Tanabe ³
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Ji Shin Lee ^{1,*}, Young Kim ¹, Joe Hyuk Lee ¹, Jong Hee Nam ¹, ¹Chonnam National University Hwasun Hospital, Jeollanam-do, Republic of Korea
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