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出國報告（出國類別：其他）

參加 2016 第三屆抗體造影與分子治療研討會出國報告

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摘　要

本次出國公差目的是參加抗體造影與分子治療(Immuno-Imaging and Molecular Therapy)國際研討會，蒐集歐洲最新標靶分子與造影技術發展資訊。

抗體因為具有高標靶性、低免疫基因毒性、高親和性與高專一性以及易於修飾進行生物工程等優勢，快速成為最廣泛使用之分子診斷、造影與治療之藥劑。比利時布魯塞爾自由大學從事奈米抗體研發工作超過 20 年，並有乳癌抗體藥物在臨床試驗階段，是國際最著名的奈米抗體研發中心，每年集合國際抗體研發專家就免疫抗體診療學(immunotheranostics)主題，共同舉辦「抗體造影與分子治療」國際研討會，今年是第三屆，議程涵蓋抗體生物工程、臨床前/臨床核醫造影、藥劑設計、影像處理與重建、放射化學、標靶放射核種治療、光學影像、免疫基因學、巨分子藥物專利保護等。核能研究所同位素組「輻射應用與分子影像技術平台」及「加速肝功能量化正子造影劑之產業化」計畫主持人王美惠博士，為配合分子影像標靶診斷造影藥劑產業化工作之推動，前往參加「抗體造影與分子治療」國際研討會，觀摩與收集歐洲最新標靶奈米抗體造影平台技術與資訊。

此次比利時「抗體造影與分子治療」國際研討會國外公差效益有：

(一) 觀摩國外奈米抗體標靶造影劑診療與轉譯醫學之研發資訊，作為本所分子影像新藥開發之參考。

(二) 研習西方國家分子影像教育訓練模式,作為本所分子影像平台人才培訓之範例。

(三) 結識來自世界各國、不同領域之專家與會，建立良好合作與交流管道，激發產出國際可能創新之藥物研發方向。

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一、目 的

核能研究所同位素組王美惠博士，為配合分子影像標靶診斷造影藥劑產業化工作之推動，前往比利時布魯塞爾分子影像中心參加「抗體造影與分子治療」國際研討會，觀摩與收集歐洲最新標靶奈米抗體造影平台技術與資訊。

比利時布魯塞爾分子影像中心(ICMI, In vivo cellular and molecular imaging Brussels, Vrije University Brussel)應用奈米抗體於腫瘤、心血管與抗發炎藥物之開發已有20年歷史，該單位性質和本所輻射應用與分子影像平台接近，擁有animal SPECT/PET/CT/MR/optical imaging全方位之影像融合實驗室；且一向致力於標靶藥物之開發，與本所加速肝功能量化正子造影劑之產業化研發方向一致，因此，希望藉由實地參加布魯塞爾自由大學分子影像中心所舉辦的國際奈米「抗體造影與分子治療」研討會，了解歐洲標靶分子影像轉譯醫學之經驗，並希望藉由與分子影像專家面對面的接觸，得以對歐洲標靶分子進展有更多的認識。

奈米抗體由於具有高標靶性、低免疫基因毒性、高親和性與高專一性及易於修飾進行生物工程等優勢，快速成為最廣泛使用之分子診斷、造影與治療之藥劑。比利時布魯塞爾自由大學是國際最著名的奈米抗體研發中心，每年集合國際抗體研發專家就免疫抗體診療學(immunotheranostics)主題，共同舉辦「抗體造影與分子治療」國際研討會，今年是第三屆；議程涵蓋抗體生物工程、臨床前/臨床核醫造影、藥劑設計、影像處理與重建、放射化學、標靶放射核種治療、光學影像、免疫基因學、巨分子藥物專利保護等。這個研討會是國際研討會，但自由大學也要求ICMI相關研究之碩博士全程參加，會議結束後有圓桌會議討論，檢討整體會議的優缺點做為來年改進之參考，由於自由大學將此會議列為所有碩博士生教育訓練的一部分。因此，會議結束後每位與會人員皆發予30小時的訓練證明；此會議固定排在四月份舉行，新進分子影像領域的碩博士可以先熟悉實驗室一段時間，在有初淺的概念且明白自己的需求後，再參加此國際研討會，將可以達更大之收益；此國際研討會已被列為ICMI的年度教育訓練，觀摩此一運作模式有助未來計畫實施教育訓練做為範例之參考；再者該國際會議多以奈米抗體為範圍，講述如何由發現潛力藥物到臨床試驗，觀摩此等會議除了可以揭開奈米抗體的診療效果，亦足以用相似模式檢視本所目前開發藥物方向與國際開發方向是否相一致。

比利時布魯塞爾是國際之都，歐洲的心臟，歐盟總部設置在此，其中的自由大學有兩個院區分別是法語區的ULB和荷語區的VUB(Vrije Universiteit Brussel)；此

次前往參加研討會的會場是在荷語區VUB。有名的布魯塞爾分子影像中心設立於此區的藥學與分子生物研究所的ICMI，這個分子影像中心是在最近五年才成立的，它主要是以其20年前發現的高標靶性奈米抗體為主要核心技術走進製藥領域，聚焦在腫瘤、心臟與抗發炎藥物之開發；整個分子影像中心任務除了是朝生技製藥價值鏈方向努力外，還能將研發能量釋放出來服務學研產業界，在今年度該中心首度於網站對外公告服務項目及收費標準；此外，歐洲核醫學會希望這些分子影像中心能對外實施教育訓練；為此，ICMI以其自身奈米抗體研發成果，每年舉辦「抗體診斷與分子治療」研討會，今年是第三屆，共有26位國際學者參加，邀請12位講員傳授分子影像基本原理到如何將研發藥物轉譯到臨床試驗。由於本所加速肝功能量化正子造影劑之產業化計畫正準備將肝受體造影劑推向臨床，因此，歐洲如何將藥物研發成果成功轉譯到臨床試驗之議題，與目前本計畫之努力方向與策略相一致，可以作為本計畫營運模式之參考。

本次比利時布魯塞爾自由大學國外公差目的在參加「抗體造影與分子治療」國際研討會，蒐集歐洲最新奈米抗體標靶分子與造影技術發展資訊，作為計畫補強之參考；同時研習西方國家分子影像教育訓練模式，作為本所分子影像平台人才培訓範例之參考。總而言之，目的有三：

(一).參加「抗體造影與分子治療」國際研討會，蒐集歐洲最新奈米抗體標靶分子與造影技術發展資訊。

(二).觀摩比利時布魯塞爾分子影像中心教育訓練模式，收集國外分子影像營運與技術資訊，作為本所分子影像專業教育訓練之參考。

(三).結識國際知名分子影像專家學者，建立連繫與技術交流管道。

二、過 程

(一)、行程：

本次國外公差奉派參加比利時布魯塞爾自由大學舉行之 2016 年第三屆「抗體造影與分子治療」國際研討會，國外公差日期為 4 月 23 日至 5 月 1 日共計 9 天。行程如下：

(1)4/23 從桃園中正機場出發，搭機經曼谷飛往阿姆斯特丹史基浦機場。

(2)4/23 抵達阿姆斯特丹史基浦機場，轉搭巴士經安衛特普前往布魯塞爾。

(3)4/24 抵達布魯塞爾與整理行李。

(4)4/25-4/29 參加抗體造影與分子治療研討會。

(5)4/30 自布魯塞爾搭巴士經安衛特普前往阿姆斯特丹史基浦機場。

(6)4/30 於阿姆斯特丹史基浦機場搭機，經曼谷飛往台灣桃園中正機場。

(7)5/01 抵達桃園中正機場。

(二)、抗體造影與分子治療研討會議程:

表一為抗體造影與分子治療研討會的議程。

第一天為分子影像概論，包括抗體造影分子設計、造影技術、奈米抗體發現與生物技術應用與臨床核醫概論。

第二天起開始分組熟悉小組彼此成員(分組名單如表二)，並只針對奈米抗體做專題探討，包括奈米抗體臨床前造影、奈米抗體標誌、奈米抗體標靶治療。

第三、四天下午有小組討論課程，針對個別需求分五組，分別為奈米抗體藥物篩選組、奈米抗體藥物生物體分布組、奈米抗體放射標誌組、光學影像組與臨床核醫組。由於分組討論所需花費時間較多，因此每組限制最多8人，每人最多選擇兩組。五個分組中，因為本人對奈米抗體藥物篩選組最不熟，因此，選擇全程待在奈米抗體藥物篩選組學習；雖然如此，我還是儘可能透過會議休息時間與其他學者交換心得，儘可能去了解每一分組的內容

第三、四天上午依舊有專題介紹，第三天介紹奈米抗體螢光造影與其於心血管之應用研究及探討奈米抗體之免疫毒性；

第四天介紹奈米抗體造影劑之轉譯醫學與生物製劑之專利簡介。

第五天上午介紹臨床免疫PET機會與挑戰，以及抗體的阿法放射治療，下午有針對一週來的收穫做評估與交流。會議結束後，大會統一發給與會同仁一份30小時教育訓練證明(圖1)。

(二)、抗體造影與分子治療研討會活動實況報導:

自由大學以奈米抗體聞名於世，已經開發的藥物有Her2奈米抗體腫瘤造影劑，此藥劑已完成臨床第一期試驗，正準備執行第二期臨床試驗；巨噬細胞甘露醣受體發炎造影劑，此藥劑已通過歐洲IND許可，正準備執行第一期臨床試驗；血管細胞黏附分子造影劑，此藥劑正在先導藥物最佳化階段；此次大會共有12位講員，會議內容多是以自由大學這三個研發藥物為主軸。

此次大會主席Nick Devoogdt教授(任教於自由大學)除負責開場致歡迎詞、分組圓桌討論以外，亦擔任奈米藥物設計與臨床前動物試驗的講員；他特別以血管細胞黏附分子造影劑為例，說明如何產製血管細胞黏附分子之奈米抗體與做生化定性。根據統計，全球1/3死因與心血管疾病有關，主要是因為動脈粥腫硬化，危險因子包括肥胖、糖尿病、不健康飲食、酗酒、遺傳、年老、高血壓、抽菸、動太少等等。動脈粥腫硬化的成因，是因為局部血管壁血脂堆積引發連續發炎反應，發炎反應的過程中，一些內皮吸附分子會參與固定發炎反應，加速白血球的滲透，這些內皮吸附分子包括血管細胞黏附分子(VCAM-1)。VCAM-1是一種膜蛋白，存在動脈粥腫硬化與巨噬細胞聚集的內皮細胞上，專司辨認白血球的表面抗原VLA4(very late antigen-4)，人和鼠的VCAM-1在面向細胞外側與訊息分子結合的質域(extracellular domain)有85%的相似性，因此找到對人和鼠的VCAM-1有交互作用的奈米抗體，如此動物試驗使用的奈米抗體就有機會轉譯到人體試驗。

VCAM-1是動脈粥腫硬化的一個標靶分子，因為血管壁發炎(比如因為血脂太高)會誘導血管壁的血管細胞黏附分子(VCAM-1)表現增加，因此，加劇血管表面之發炎反應；早期動脈粥腫硬化症狀是不明顯的，臨床檢查比如血液CK-MB以及運動心電圖或心臟超音波等，靈敏度都不夠高；因此，ICMI開發VCAM-1的奈米抗體，以其高親和性、高專一性、優良溶解度、安定性、高放射標誌產率及快速血液清除速率等優勢，可作為動脈粥腫硬化的造影標記，以及早診斷出動脈粥腫硬化

就可及早做治療與預防，大幅減低中風死因的發生。

圖2是Nick教授對anti-VCAM-1奈米抗體藥物開發的策略。ICMI在這個研究目的有三，包括產製與定性對mouse與human VCAM-1有交叉反應的奈米抗體；建立Tc-99m放射標誌；在ApoE剃除鼠(ApoE$^{-/-}$)看到不同於正常鼠的動脈粥腫硬化造影。所需應用到的技術包括奈米抗體製造、定性，所用技術包括流式細胞術、溫度穩定性、表面電漿共振、放射標誌、體外結合度試驗、動物模式、生物體分布、體內競爭試驗、藥物效力學、血液動力學、體內造影、自體放射顯影術等。

抗體產製與定性的方法，係以鼠和人類VCAM-1的重組蛋白免疫駱駝6周，每周序列稀釋血清，以酵素免疫分析術分析確定免疫抗體產生的濃度，當確定血清中有高濃度抗體產生後，再以蛋白質西方墨點免疫分析術確認IgG的次群組分類，確認是否主要是IgG1及3，因為只有IgG1,3和抗原結合後才能引發補體反應將抗原清除，同時必須確認該抗體與發炎細胞有反應(以流式細胞術確認)，且主要是IgG1。

取得抗血清後，將血液中的淋巴球分離出來，抽取其mRNA，以RT-PCR放大基因量，放入噬菌體載體中，感染到大腸桿菌中培養，建立噬菌體展現系統，以VCAM-1及酵素免疫分析術做多次生物掏洗，生物掏洗越多次，帶有anti-VCAM1抗體的菌株數就越多(可以流式細胞術來確定能與發炎細胞反應的菌株數)。基因定序則是以限制性片段長度多樣性分析術，找出特殊的片段做定序。

接著必須找到對mouse VCAM1和human VCAM1皆有反應的奈米抗體，亦即奈米抗體必須和mouse VCAM1及human VCAM1做交叉比對，找出既對mouse VCAM1也對human VCAM1有交叉反應的奈米抗體。日後就以這菌株做大量表現與純化，就可獲得適合做臨床轉譯用的anti-VCAM1奈米抗體。此外，可以表面電漿共振術得到抗體抗原複合物解離常數(K_D)，另外，也可以表面電漿共振術找出相對應的抗原決定區。以上抗體產製與定性試驗，只需class II(一般實驗室等級)環境進行，且再現性高；Nick教授表示他們的抗體產製實驗室空間不大，但距離遙遠，由於時間有限，今年沒有安排參訪。

Christian Van Hove教授(來自比利時根特大學)，講述各種造影儀器的原理與應用，包括SPECT, PET, CT, optical imaging。Serge Muyldermans教授(自由大學)是免疫學專家，專長在做抗血清，主要介

紹奈米抗體的發現。1993年左右，他讓各位博士研究生以各種不同物種從事抗血清研究，因而讓現在是教授的Hamers Raymond(圖3)發現了駱駝抗血清之特殊性，他們發現駱駝抗體只有heavy chain，大小只有10~15 kDa;有別於由兩個heavy chain 跟兩個light chain所組成的一般抗體。再者駱駝抗體沒有雙硫鍵，不會有雙硫鍵一旦還原就會失去活性的困擾；使用駱駝型抗體可以滿足分子量小、單一結構、與穩定性高之優勢。(回國後搜尋網路資料，據了解比利時已經將奈米抗體的產製專利技轉給Ablynx公司；國內台灣微脂體公司和Ablynx簽署合作契約。)

Marleen Keyaerts教授(自由大學)是臨床醫師，介紹臨床核醫造影的現在與未來。歐洲核醫藥物的產製必須遵循cGRPP(現行優良核子藥學規範)。診斷方法包括有轉運體機制法(腦部造影劑很多採此法)、細胞吞噬機制(肝受體造影劑)、微血管捕捉造影(以放射標誌微球做肺部灌注造影)、compartment localization(blood pool using Tc-99m HAS)、心肌灌注(Tc-99m Sestamibi)、化學趨骨性造影(Tc-99m MDP)、受體造影(比如I123-IBZM研究dopamine D2 receptor，In-111 octreotide神經母細胞瘤造影)、抗原抗體結合術等。目的為功能造影(比如肺臟灌流造影、骨造影術、心肌灌流造影、腎臟造影)，或分子影像(比如多巴胺轉運體造影、葡萄糖代謝造影、抗體造影等)或挑選合適做放射治療的病患。在個人化醫療上，傳統是以生物切片做確診，但容易有取樣誤差，若能有合適標靶造影標記，非侵略性的分子影像造影術，可以獲得全身性的生物體分布，得到精確分子層次之資訊。

Catarina Xavier教授(自由大學)，介紹奈米抗體的放射標誌技術。奈米抗體的放射標誌主要透過輔基(NHS或SFB)或雙功能螯合劑來和放射性同位素結合，放射奈米抗體造影劑是未來開發精準醫療策略的重要工具。精準醫療是國際診療的新趨勢，係以標靶造影分子配合治療策略，可以更精準判別疾病期別與掌握治療療效。分子影像的優勢在非侵略性、可提供全身生物體分布，與避開切片取樣誤差等。放射抗體示蹤劑因具有良好標靶性，是很好的造影標記。然而傳統的抗體較大(160kD)，由肝臟代謝，為了得到較佳的對比影像，限制個體所接受的輻射量，有必要使用較小的抗體分子，自由大學所發展的單體功能性質域抗體片段，沒有補體結合片段(Fc region)，且分子量較小，約

15kD，有較快的血液清除率。有關放射性標誌，一般都是標誌在離胺酸基上，但通常都沒有方向性，接上幾個，接在哪裡也都不一定，雖然方法簡單，但是批次間異質性太高，有時若接在離抗原決定區塊太近的位置，還會因此影響抗體活性。這種隨機鍵結的異質性不僅影響批次製造的恆定性，也可能導致藥動結果的不一致性。接在cysteine上可能會比較能固定標誌的數量與位置，但是它常是以還原的反應條件來進行，但因為傳統抗體都有內雙硫鍵，因此，這樣的反應常要小心不可影響到蛋白質功能性質域間雙硫鍵，以免影響抗體活性。目前已知的抗體結構，只有駱駝產製的單質域抗體片段不具內雙硫鍵又具有抗原活性。但駱駝抗體序列可能不具cysteine胺基酸殘基，因此，自由大學是採C端外加一個cysteine的方式，再以thioether鍵結方式接上maleimide-DTPA，就可接上放射性同位素In-111(圖4)。另一個方法，是在抗體基因設計讓它做出來的抗體末端六個histidine前，有一段LEPTGG胺基酸序列；利用Sortase酵素使抗體-LEPT與GGGYK-DTPA接合，如此一來，亦可把DTPA接上奈米抗體(圖5)。自由大學Tc-99m 標誌抗體方法，為Tricarbonyl method(J Nucl Med 2008; 49:788-795.)。

Matthias D'Huyvetter教授(自由大學)分享Her2標靶抗體治療。乳癌是西方女性最嚴重的疾病，2012年在歐洲新增167萬病例，危險因子有年齡和家族史；20-25%的乳癌為Her2過度表現，而這些病患常規治療常還是有復發現象，需要系統性輔助治療。圖6是自由大學以Her2奈米抗體針對Her2 (+)腫瘤造影的結果，只在腫瘤、腎臟與膀胱有聚積，標靶性非常好(圖6)。因為是放射標靶治療，在設計上還需考量減低腎臟毒性於最低。自由大學係以增加血液滲透壓並改變蛋白序列，減少末端鹼性胺基酸數量方式成功降低腎臟吸收(圖7)。自由大學是使用Gelofusin來增加血液滲透壓，它是琥珀酸膠類血漿代用品，可以維持循環血容量體積，減少藥物於腎臟之吸收，如此可以減低腎臟的毒性。在生物體分布後120h，Tumor/blood比值奈米抗體可達900，一般抗體則只有1.33(表三)，這是奈米抗體作為標靶治療藥物的最大優勢。

Sophie Hernot教授(自由大學)提出已進入臨床試驗的螢光造影劑及巨噬細胞造影劑(針對巨噬細胞膜上的甘露醣受體)，於動脈粥腫硬化診斷的可行性，在她的研究顯示在ApoE-/-鼠，並沒有顯著發現有巨噬細胞造影劑之聚積。所以，先前理論動脈粥腫硬化會有巨噬細胞的聚

積說法，還需要進一步的確定。

Chloe Ackaert博士(自由大學)談論藥物開發時，不想發生的免疫反應(immunogenicity)是藥物開發失敗的其中之一原因。Immunogenicity是臨床試驗需要檢測的一個項目，目的在看是否有anti-drug antibody。自由大學測定有無免疫反應是以酵素免疫分析術，將奈米抗體固定在反應盤上，加上血清看有無抗奈米抗體之抗體，若有則是個體有免疫反應發生。布魯塞爾自由大學目前Her2奈米抗體正要進入臨床試驗II期，而巨噬細胞甘露醣受體奈米抗體則正在預備執行臨床I期；截至目前為止，奈米抗體是很安全的藥劑，並沒有發現有immunogenicity之毒性反應發生。

奈米造影劑的臨床轉譯是由Marleen Keyaerts教授所負責，包括Her2奈米抗體進入臨床二期及甘露醣受體奈米抗體進入臨床一期。轉譯醫學要注意幾件事情，包括體內要有標靶確據，方便製造、純化與標誌，避免含有非哺乳類的成分(比如His-tag或cys-tag)，要能符合GMP規範；針對放射藥劑，還要符合放射性物質之法規管制。藥劑方面法規和台灣相一致，就是要有化學製程品質一致性、不具藥理與毒理作用、能用於臨床診療。要有主持人手冊，包括分析步驟，詳載藥品基本資訊。圖8-10是Her2奈米抗體化學製程品質必須繳交的資訊目錄。歐洲法規規定奈米抗體造影劑不得超過100ug，需有14天延伸性急毒性數據，包含臨床病理與組織病理。Phase I的目標在看安全性、生物體分布、輻射劑量，因此，自由大學Her2奈米抗體造影劑臨床試驗計畫書設計為比較0.01, 0.1和1mg的生物體分布，並且看它在腫瘤病患的腫瘤標靶性。病患則選定肝腎功能正常但帶有侵襲性或轉移性乳癌且>18y之女性，每一個劑量至少5位病患。此外，若有奈米抗體治療計畫，因為會有多劑量給藥計畫，則必須另外加做immunogenicity試驗。自由大學Her2奈米抗體造影劑Phase II的規劃，在評估該藥劑作為乳癌診斷之靈敏度、專一性與再現性。巨噬細胞甘露醣受體造影劑第一階段臨床試驗計畫書的內容在評估安全性、生物體分布、輻射劑量、腫瘤造影標靶性。病患必須>18 y，腫瘤大於1 cm，乳癌、肺癌、頭頸癌不拘。這些試驗結果，也可用以評估未來巨噬細胞甘露醣受體奈米抗體可否用於癌症標靶治療。

Geert Raes博士介紹歐洲專利法規定，專利申請18個月後就自動公

開揭露其內容。可以從https://worldwide.espacenet.com/和www.google.com/patents進行搜尋檢索。針對前案檢索必須有新穎性與進步性，還需要有產業可利用性。自由大學奈米抗體雖曾申請過歐洲與美國專利，但都已經超過專利有效期，包括缺乏light chain的免疫抗體(EP20130818過期)，V_{HH}奈米抗體產品專利（美國專利20160206到期），奈米抗體製程美國專利（20150206到期），噬菌體展現系統美國專利（20150109到期），以及抗原篩選噬菌體展現抗體庫美國專利（20130818到期）等。

Geraldine Gebhart醫師博士(來自Bordet Institute Brussels)介紹臨床抗體PET造影技術。抗體於臨床的應用可以是抗體直接誘發細胞傳導使細胞凋亡；也可以是誘發補體反應發動體內免疫反應使細胞被殺死；也可以是攜帶抗癌藥物來殺死癌細胞。直到2013年，抗體於臨床的應用已有50個單株抗體在臨床試驗階段，有18個獲FDA/EMA許可，包括13個單株抗體，3個抗體藥物複合體，以及2個放射抗體治療造影劑(表四&五)。臨床試驗階段的50個單株抗體中有8個為診斷用抗體造影劑，但可觀察到若為傳統的單株抗體有很強的心臟毒性，肝/骨頭/肺臟/腦的輻射吸收也高，但若是奈米抗體除了腫瘤和膀胱以外，沒有看見於主要器官有副作用(表六)(Marleen Keyaerts, Catarina Xavier, Johannes Heemskerk, Nick Devoogdt, Hendrik Everaert, Chloé Ackaert, Marian Vanhoeij, Francois P. Duhoux, Thierry Gevaert, Philippe Simon, Denis Schallier, Christel Fontaine, Ilse Vaneycken, Christian Vanhove, Jacques De Greve, Jan Lamote, Vicky Caveliers, and Tony Lahoutte Phase I Study of ^{68}Ga-HER2-Nanobody for PET/CT Assessment of HER2 Expression in Breast Carcinoma. J Nucl Med 2016 57:27-33.)

Sture Lindegren教授介紹標靶alpha治療(來自瑞典Gothenberg大學)，他們過去有過很多種標誌方法，但發現At-211標誌非常困難，傳統雙官能基螯合技術不適合它，傳統鹵素結合技術也不適合它，提出最好的標誌方法如附圖11。

會議結束後最後一天，大會提出6個問題，沒有標準答案，也不公布答案，謹供大家自我評估參考用，如下:

1. What is the major toxicity-limiting organ for radiolabeled small molecules? What are the mechanisms? Which

bioengineering and radiochemical solutions could you propose to come up with a solution?

2. You have a nanobody produced with a hexa-histidine tag and you want to label the protein without making any modifications of the protein. Where can you label and which isotope would you use? Explain.
3. Which biochemical reactions can you propose to conjugate a bifunctional ligand to a nanobody? Which bifunctional ligand would you choose for Ga-68?
4. After the selection and validation of a lead compound based on preclinical experiment, which other steps are essential before you can test this compound in a clinical study?
5. What are the major limitations of in vivo fluorescence imaging? What is the consequence for clinical translation?
6. What are the three types of irradiation that can be used for targeted radionuclide therapy? Give examples and the advantages/disadvantages of each application?

圖 12 是各分組圓桌座談的主席，分別是Nick Devoogdt教授是生物學家也是免疫學家，在分組論時段負責先導藥物篩選與定性；Catarina Xavier教授是化學家也是放射藥學家，在分組時段負責放射化學標誌(以Tc-99m tricarbonyl method為例，有關標誌步驟和檢驗成績書如附錄一&二)；Sophie Hernot教授是生物工程學家，在分組討論負責螢光細胞之應用；Matthias D’Huyvetter教授是生物工程學家，在分組討論負責影像圈選定量提供活體生物體分布數據(如附錄三&四)；Marleen Keyaerts是醫師，在分組討論負責參觀迴旋加速器與醫院運作。

三、 心得

(一)、比利時自由大學顧名思義是一個極其強調自由思考的學校，教學策略則以新穎創新、轉譯醫學、產業價值與合作研究為主軸，不在乎學員的年紀，但非常注重學員學習動機與業界的合作，因此，它有很多外籍生(約佔 1/3 強)，特別歡迎有工作經驗的學者參與碩博士之研究，但若是完全沒有相關工作經驗的學員，自由大學也會儘可能讓他們參與 Eramus Mundus 計畫，那是一個跨國跨領域的產學聯盟研究計畫，學員在就學期間有時有必要要到其他國家做交換學生，透過跨領域或跨國際化的學習，來激發新創意的產出，而且產出結果務求有商業價值與臨床需求性。在組織管理上雖然有 top-down 的管理，比如每位博士有一定的畢業門檻，且都要有出書，但教學研究方法上主要採取 bottom-top 策略；圖 3 就是一個很好的例子，指導教授在指導學生製造抗體時，並沒有限定一定要由老鼠或兔子製造抗體，有人做斑馬魚抗體，有人做駱駝抗體，因而意外發現自然界駱駝可以產製分子量小單功能性的質域抗體(15kD)，並經由一系列的定性分析，確認它的絕佳標靶性與低毒性，並因而成為自由大學分子影像中心賴以營運規劃的核心技術，是近 20 年來最豐碩的成果，自由大學並因此成立分子影像中心，目前已有 3 個新穎有潛力成功的分子影像造影劑在開發，其中 Her2 奈米抗體正朝向 phase II 臨床試驗邁進，而其目標放在疾病偵測靈敏度、專一性與檢驗再現性。

(二)、比利時自由大學強調，每一個藥物研發開始都要先考量所選擇造影技術的優勢與限制?想法有無臨床需求性?每一個階段有無可以驗證正確與否的方法?可否接受臨床轉譯的挑戰，換言之，藥劑可否量產?需求劑量可否達到病患使用?每個病人需要多少成本?這些都是他們在計畫執行時再三考量的關鍵。

(三)、比利時自由大學強調跨領域與跨國際合作，成立歐洲分子影像博士學程(European Molecular Imaging Doctoral School)，博士生沒有固定實驗室，可以也必須是跨國際學習，鼓勵多參加國際研討會，多提出問題討論；因此，他們從不設限只要某種人才，而是強調您想要學習甚麼，只要您有動機，符合產業需求，可以找到評估每個環節

的定性方法，不限環境限制，可以到處去學習；在過去不會有人想到要做駱駝抗體，或做鯊魚抗體，但因為有學生有興趣，就加以鼓勵，反而找出一條別人未走過的新藥創新之路，自由大學後來也因此引進產學聯盟，目前他們的研究仍舊繼續，經費改由產業提供，而自由大學則掌握發表論文的先機；而這些論文也成為後續藥物通過臨床試驗的良好佐證，對藥廠的藥物開發有直接助益；未來上市藥廠可獲利，而自由大學仍可持續獲得技轉金做相關研究，是很好的雙贏策略與良性循環。長遠來看，產學聯盟是很好的規劃，但在這以前，國內需有合適立法或制度；目前生技醫藥國家型計畫有一些規則可依循，但在國內學界並不一定全然適用。本所應有技轉與產學合作專責人員協助制訂方便可行之法則，讓研發單位人員有勇於創新又不致觸法之優質環境，方能使產學聯盟之路更加順暢。

(四)、每個單位都有其特定專長的人員,比利時自由大學以生物人才為主，物理人才比較少；因此，一開始他們就以新穎、合作、跨領域、轉譯為策略。它在儀器製造方面的能力不若德國明斯特大學來得有經驗，因此，他們的設備主要是買來的，甚至臨床淘汰下來的設備，經過與他國或儀器商跨領域合作改裝為臨床可用的設備。目前它們的造影儀器在分子影像中心，主要是以 Siemen SPECT/CT 和 Milab SPECT/CT 為主；至於 PET/CT 則因為核種半衰期短，主要是放在醫院，放射化學實驗室也在醫院，集中管理，也方便標誌後立即造影。他們在生物方面就相對地有較多資源，此外，他們也運用先前約翰霍普金斯大學李教授提出的聚合效應，將先前產製的駱駝型單質域抗體，進一步產製成雙體的駱駝型單質域抗體或雙體的駱駝型兩質域抗體；結果發現這種雙體的駱駝型抗體，和原本單體的駱駝型單質域抗體相比，並和抗原有較佳的結合能力，且能進到腫瘤的比率更高，更有效率地將放射性同位素帶進腫瘤；這也是一種跨國際領域的理論結合。

(五)、由於生技醫藥研發從事人員已漸漸明白分子影像在生技醫藥品研發上的重要性，近幾年本所輻射應用與分子影像技術平台計畫審查人員，也期許本計畫能有對外施以教育訓練的機會。參考國外模式，本所或許可以結合科技部資源，辦理相關講座，或許可以先重用一些原本就有擔任所外講師之人才，先以小規模方式進行教育訓練，一方面強化

所內分子影像人才，一方面提供所外更多人了解分子影像所能提供之技術與應用，同時設立一些合適合作研發管道，刺激更多研發靈感與構想注入本所，擴張本所研發境界。

(六)、奈米抗體的低毒性，確實是未來發展標靶藥劑的一大利器；由於它已經過了專利期，本所若要產製這般抗體，理論上是沒有侵權之虞。但若要有新穎性，還是要在地毯式地做專利檢索與分析，務使產品可以朝向新穎藥劑與新適應症做保護。自由大學三個藥物分別是抗癌、抗發炎與心血管疾病，因為這三類藥物的市場最大；但若是針對一些罕見疾病，在國外亦有快速通關之優惠法案；國內亦可考慮，也許市場沒那麼大，但能節省很多社會成本，如此也能提升人民健康福祉，相對亦能提升經濟產能。

(七)、國外公差雖然是以參加研討會、充實新知與技術交流為主，但順道參訪著名研究機構與人脈建立也是非常重要；雖然機構參訪對它單位都是一個負擔，但國際上基於學術分享與建立人脈的利基，很多單位都是至表歡迎，如本次國際會議，也是過去參加國際會議時經過比利時參訪而後得知，參訪也互留名片，這也是建立人脈與良好互動關係的一種管道;尤其 2018 年國際分子影像學會將在台北主辦，過去很多時候我們是以邀請特定華籍人士來台，未來有望多邀請這些新接觸的分子影像學者專家來台與會，傳授軟硬體與新穎造影劑研發經驗。

(八)、活體組織器官藥物動力學定量與分析是國際趨勢，確實有必要觀摩國外在此方面之經驗，作為計畫補強的參考；而在硬體方面，本所若有研發能力或許亦能培育加強其維修能力，既提升影體開發能量也能減少對廠商的依賴，如此節省的經費可以轉為研發經費，對本所分子影像平台之永續經營將有直接之助益。

(九)、自由大學儀管物理人才較少，但也是有的，他們是集合物理、生物、化學、標誌人才在同一個團隊，本所分子影像中心，可能有必要將與藥物研發的人才集中在同一個功能組，本所現在維修能力多仰賴廠商，但廠商要價偏高，如果自己有研發能力，可以大幅降低成本。而本所在化學組執行藥物開發的人員，若能納編同位素組，在管理方式一致，目標一致下將研發人員統籌運用，將更容易溝通協調，使時間與人力更能聚焦在研發工作上。這次參與國際會議的人員專

長化學、標誌、生物、物理、機電都有，有的人就是直接和老師說，他對這個領域有興趣就得以參加。事實上 ICMI 也是將分子影像研究計畫的人集中在一起，他們並沒有化學組、物理組獨立，而是將這些專長人員散在各個計畫中，因為轉譯研究的確是需要化學、標誌、生物、物理、機電等人才密集地討論，集中在同一個計畫或功能組管理較為務實，在管理上宜採迴旋加速器專案計畫過去編制之制度為宜。本所在國家政策中，佔居生技醫藥開發第二棒重要責任，分子影像儀器設計與維修、融合定量軟體開發、藥物開發等儀電、物理、醫工、化學、標誌、生物、獸醫、藥理等人才的培育與集中，我認為有其必要。

(十)、聚合效應無論在本所醣質藥物及國際奈米抗體都獲得驗證有其應用潛力，針對其他組織器官的特定受體或轉運體，配合多聚醣的技術，也是有開發標靶藥物的契機，這方面的努力值得投入與關切。

四、建 議 事 項

(一)、加強所內外合作，積極開發標靶正子藥物:

分子影像最大優勢在可提供全身生物體分布，標靶確效及為非侵襲性。因此，有無標靶與可能毒性，以分子影像時能及早有效看出未來轉譯成功之潛力；此外，本所在最近五年除本身有一些研發成果，於執行學研界的合作研究之餘，也有產出一些具潛力的分子影像造影劑，足以與學研界藥物達精準醫療之策略要求。將透過專利保護與論著發表，繼續深耕本所之研發能量，提升本所的學術威望與加深本所對學研界直接或間接之有感價值。未來亦宜持續加強對外合作研究管道，讓學研界感到本所研發利器對整體國家科技之貢獻，加速國家醫藥品之產業化方向努力。

(二)、**藥物研發首重新穎創新，宜加強專利佈局與申請:**

自由大學發現駱駝型單質域抗體，是意外的發現；所謂意外發現科學常見；因此，策略上鼓勵創新，研發上時時灌注新點子與方法，且在發現新穎性時立即申請專利保護，是非常有助於新穎藥物之開發。我相信自由大學一開始申請駱駝型抗體時也沒有想到開發造影劑，但就是先把奈米抗體專利保護起來，隨著研發資訊的累績，會更清楚未來創新路途之方向。尤其是本所有分子影像技術，能及早配合篩選技術看出藥物的有效性與安全性，透過善加運用技術資源，人力集中使用，將有機會及早開發具潛力藥物。

(三)、**分子影像平台應考量永續經營之策略與作法，有效降低維護負擔:**

國內外許多單位都相繼成立分子影像中心，不但供應自己研發，也將能量貢獻出來提供服務；既然已知分子影像是轉譯醫學的最佳利器，相關儀器關鍵技術一直掌握在少數廠商是最大劣勢，目前所內 SPECT 和 PET 都已老舊，與其花高額費用維護，不如以維護費抵採購費。但在每年支出費用上做合理的營運規劃，使全成本收支可以達到平衡；此外，儀器品質也是重點，本所宜好好評估可永續經營之策略與做法，妥善維護分子影像平台品質，使所用平台既能幫助本所使用亦能拓寬供學研及產學使用，讓社會

有感，以提升本所國際形象。

表 一、2016 第三屆抗體造影與分子治療研討會議程

Day 1	09h00 - 09h15	Welcome
	09h15 - 10h45	Lecture: Prof. Dr. Nick Devoogdt (VUB, Belgium) - Do’s and don’ts in Immunotracer Design
	10h45 - 11h00	Coffee Break
	11h00 - 12h30	Lecture: Prof. Christian Van Hove (UGent, Belgium) - Overview of Imaging Modalities
	12h30 - 14h00	Lunch Break
	14h00 - 15h30	Lecture: Prof. Dr. Serge Muyldermans (VUB, Belgium) - Nanobody Discovery and Biotechnological Applications
	15h30 - 15h45	Coffee Break
	15h45 - 17h00	Lecture: Dr. Marleen Keyaerts (VUB/UZ Brussel, Belgium) - Overview of Clinical Nuclear Medicine
Day 2	09h00 - 10h30	Welcome & Round Table Introduction
	10h00 - 11h30	Lecture: Prof. Dr. Nick Devoogdt (VUB, Belgium) - Imaging with Nanobodies in Preclinical Models
	11h30 - 13h00	Lunch Break
	13h00 - 14h30	Lecture: Dr. Catarina Xavier (VUB, Belgium) - Radiolabeling Techniques for Nanobodies
	14h30 - 14h45	Coffee Break
	14h45 - 16h15	Lecture: Dr. Matthias D’huyvetter (VUB, Belgium) - Targeted Radionuclide Therapy: Nanobodies as an Example
	16h30	Social Event in the evening: Brussels Visit & Dinner
Day 3	09h00 - 10h30	Lecture: Dr. Sophie Hernot (VUB, Belgium) - Fluorescence and Cardiovascular Applications of Nanobodies
	10h30 - 10h45	Coffee Break
	10h45 - 12h15	Lecture: Dr. Chloé Ackaert (VUB, Belgium) - Immunogenicity of Proteins and Nanobodies
	12h15 - 13h30	Lunch Break
	13h30 - 17h00	Hands-on Session
Day 4	09h00 - 10h30	Lecture: Dr. Marleen Keyaerts (VUB/UZ Brussel, Belgium) - Clinical Translation of Nanobody-tracers
	10h30 - 10h45	Coffee Break
	10h45 - 12h15	Lecture: Prof. Dr. Geert Reas (VUB/VIB, Belgium) - Intellectual Property of Biologics
	12h15 - 13h30	Lunch Break
	13h30 - 17h00	Hands-on Session
Day 5	09h00 - 10h30	Lecture: Dr. Géraldine Gebhart & Dr. Zéna Wimana (Bordet Institute Brussels, Belgium) - Clinical Immuno-PET
	10h30 - 10h45	Coffee Break
	10h45 - 12h15	Lecture: Prof. Dr. Stüre Lindegren & Prof. Dr. Tom Bäck (Gothenburg University, Sweden) - Alpha-therapy with Antibodies
	12h15 - 16h00	Round Table Discussion & Workshop Evaluation

表 二、抗體造影與分子治療研討會小組分組名單

	Analysis of Nanobody In vivo Biodistribution Data in Mice	Nanobody Lead Selection Analysis	Visit and Operations of Hospital Nuclear Medicine Department	Optical Imaging in Mice	Nanobody Radiochemistry Techniques
	Dr. Matthias D'huyvetter *Ahmet Krasniqi* *Cindy Peleman*	*Prof. Dr. Nick Devoogdt* *Janik Puttemans*	*Dr. Marleen Keyaerts* *Prof. Dr. Vicky Caveliers* *Dr. Ilse Vaneycken*	*Prof. Dr. Sophie Hernot*	*Dr. Catarina Xavier*
Capacity	*Max 8*			*Max 6*	
Wednesday April 27 2016	- Sandra van Tiel - Peter Covens - Bart Roman - Lara Struelens - Kim De Veirman - Magdalena Bialkowska	- Yannick Regin - Mei-Hui Wang - Nahid Yusufi - Pedro Mendes Fontes - Marti Boss - Mijke Buitinga - Florent Antoni	- Wendy Kucma - Elina Van Langenhoven - Nicky Hoevenaars	- Leentje De Ceuninck - Marius Regin - Joke Mertens - Stijn Piessens - Jordy Stichelmans - Mohammed Zourob	- Danielle Berus - Clarita Saldarriaga Vargas - Joao Correia - Inge Hansen
Thursday April 28 2016	- Wendy Kucma - Elina Van Langenhoven - Danielle Berus - Clarita Saldarriaga Vargas - Mei-Hui Wang - Nahid Yusufi - Pedro Mendes Fontes - Marti Boss	- Leentje De Ceunick - Nicky Hoevenaars - Joao Correia - Inge Hansen - Bart Roman - Lara Struelens - Kim De Veirman	- Marius Regin - Joke Mertens - Stijn Piessens - Magdalena Bialkowska		- Jordy Stichelmans - Mohammed Zourob - Sandra van Tiel - Yannick Regin - Mijke Buitinga - Florent Antoni

表 三、奈米抗體與傳統抗體的輻射劑量評估

Tissue	Lu-177 DTPA Her 2 nanobody	Lu-177 DTPA-Trastuzumab
Lung	0.01	1.55
Heart	0.01	0.98
Liver	0.05	1.72
Kidney	0.90	1.22
Spleen	0.02	1.60
Muscle	0.01	0.41
Bone	0.03	0.77
Intestine	0.01	0.44
Blood	0.001	4.18
Tumor	0.90	5.55

Dosimetry data are expressed as Gy/MBq

Ref:

D'Huyvetter M, Vincke C, Xavier C, Aerts A, Impens N, Baatout S, De Raeve H, Muyldermans S, Caveliers V, Devoogdt N, Lahoutte T. Targeted Radionuclide Therapy with A ^{177}Lu-labeled Anti-HER2 Nanobody. *Theranostics* 2014; 4(7):708-720. doi:10.7150/thno.8156. Available from http://www.thno.org/v04p0708.htm

表 四、用於血液疾病受核准的抗體藥物

Generic name	Indications	Target
Rituximab	CLL, NHL	CD20 (B cells)
Ibritumomab tiuxetan; ^{90}Y-ibritumomab tiuxetan	NHL	CD20 (B cells)
Tositumomab; ^{131}I-tositumomab	NHL	CD20 (B cells)
Ofatumumab	CLL	CD20 (B cells)
Obinutuzumab	CLL	CD20 (B cells)
Brentuximab vedotin	HL, anaplastic large cell lymphoma	CD30 (B & T cells)
Gemtuzumab ozogamicin	AML	CD33 (myeloid cells)
Alemtuzumab	CLL	CD52 (lymphoid cells)

AML: acute myeloid leukemia; CLL: chronic lymphocytic leukemia; HL: hodgkin lymphoma; NHL: non-hodgkin lymphoma

資料來源:2016 年第三屆抗體造影與分子治療研討會

表 五、用於顆粒性腫瘤受核准的抗體藥物

Generic name	**Indications**	**Target**
Trastuzumab	Breast cancer	HER2 (tumor cell membrane)
Pertuzumab	Breast cancer	HER2 (tumor cell membrane)
Trastuzumab emtansine	Breast cancer	HER2 (tumor cell membrane)
Panitumumab	Colorectal cancer	EGFR(tumor cell membrane)
Cetuximab	Colorectal cancer	EGFR (tumor cell membrane)
	Head and neck cancer	
Ipilimumab	Melanoma	CTLA-4 (T-cells)
Pembrolizumab	Melanoma	PD-1 (T cells)
MPDL3280A	Bladder cancer	PDL1 (tumor cell membrane)
Ramucirumab	Gastric cancer	VEGFR2 (microenvironment)
Bevacizumab	Colorectal cancer	VEGF (microenvironment)
	Renal cell cancer	
	Non small cell lung cancer	

資料來源:2016 年第三屆抗體造影與分子治療研討會

表 六、各種放射抗體造影劑的臨床發現整理

Type of probe	PET or SPECT Probe (dose)	Patient population (N)	Optimal timing for imaging	Findings	Reference
Intact mono-clonal antibodies	^{111}In-trastuzumab 185 Mbq with variable amount of trastuzumab	Advanced disease (10)	not mentioned	Ability to predict cardiotoxicity and response to trastuzumab	Behr et al. (2000)
	^{111}In-trastuzumab (100-150 Mbq) after the first therapeutic dose of trastuzumab	Advanced disease (15)	up to 7 days after injection	Low tumor detection rate No ability to predict trastuzumab-induced cardiotoxicity	Perik et al. (2006)
	^{89}Zr-trastuzumab (37 Mbq + either 10 or 50 mg trastuzumab)	Advanced disease (14) on/off trastuzumab therapy	day 4-5 after injection	Excellent tumor uptake Visualization of metastasis in liver/bone/lung/ brain 50 mg trastuzumab needed if patients off trastuzumab	Dijkers et al. (2010)
	^{64}Cu-trastuzumab (130 Mbq, no unlabeled trastuzumab)	Early and advanced disease (6)	48 hours after injection	No "cold" trastuzumab used Suboptimal visualisation of liver lesions Primary tumors/Brain metastasis seen	Tamura et al. (2013)
	^{64}Cu-trastuzumab (364–512 MBq, 5 mg of trastuzumab preceded by trastuzumab infusion (45 mg))	Advanced disease (8) off trastuzumab therapy for ≥ 4 months	48 hours after injection	Mapping of lesions very close to the one obtained by ^{18}F-FDG-PET Some lesions only seen on ^{64}Cu-trastuzumab PET 45 mg cold trastuzumab needed	Mortimer et al. (2014)
Affibody molecules	^{111}In or ^{68}Ga-labeled ABY-002 (80–90 mg with activity ranging from 110-267 Mbq)	Advanced disease (3) on/off trastuzumab therapy	3 hours after injection	High detection rate of known lesion on ^{18}F-FDG-PET High level of liver/kidney uptake is problematic	Baum et al. (2010)
	^{111}In-ABY-025 (100 mg with a mean activity of 142.6 MBq, range, 131–154 MBq)	Advanced disease (7) with 5 HER2+ and 2 HER2 - tumors; some on trastuzumab therapy	24 hours after injection	Visualization of HER2 metastasis including liver/brain	Sörensen et al. (2014)
Nano-bodies	^{68}Ga-antiHER2-nanobody (53-174 Mbq-average 107Mbq)	Early and advanced disease	90 min after injection (allowing decreased uptake in the liver)	No adverse reaction to tracer 4.6 mSv/patient Critical organ: bladder Visualization of HER2 metastasis Primary lesions were more variable in tracer accumulation.	Keyaerts et al. (2015)

資料來源:2016 年第三屆抗體造影與分子治療研討會

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Workshop CERTIFICATE

Vrije Universiteit Brussel

I, undersigned, Nick Devoogdt, Coordinator of the training course

Immuno-Imaging and Molecular Therapy

held at Brussels, Belgium

certify that

MEI-HUI WANG

has attended the 5-day EMIDS Workshop

from 25/04/2016 to 29/04/2016
(ie. 30 hours)

Contents :
- *D1 : Welcome by Nick Devoogdt (30min)*
- *D1 : Do's and Don'ts in Immunotracer Design by Nick Devoogdt (1h30)*
- *D1 : Overview of Imaging Modalities by Christian Van Hove (1h30)*
- *D1 : Nanobody Discovery and Biotechnological Applications by Serge Muyldermans (1h30)*
- *D1 : Overview of Clinical Nuclear Medicine by Marleen Keyaerts (1h30)*
- *D2 : Welcome and Round-Table Introduction by Nick Devoogdt (1h)*
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- *D3 : Fluorescence and Cardiovascular Applications of Nanobodies by Sophie Hernot (1h30)*
- *D3 : Immunogenicity of Proteins and Nanobodies by Chloé Ackaert (1h30)*
- *D3 : Hands-on Session (4h)*
- *D4 : Clinical Translation of Nanobody Tracers by Marleen Keyaerts (1h30)*
- *D4 : Intellectual Property of Biologics by Geert Raes (1h30)*
- *D4 : Hands-on Session (4h)*
- *D5 : Clinical Immuno-PET by Géraldine Gebhart and Zéna Wimana (1h30)*
- *D5 : Alpha-Therapy with Antibodies by Stüre Lindegren (1h30)*
- *D5 : Round Table Discussion (1h)*

Done at Brussels, on 29/04/2016

Course leader :

圖 **1**、大會提供之抗體造影與分子治療研討會 **30** 小時訓練證明

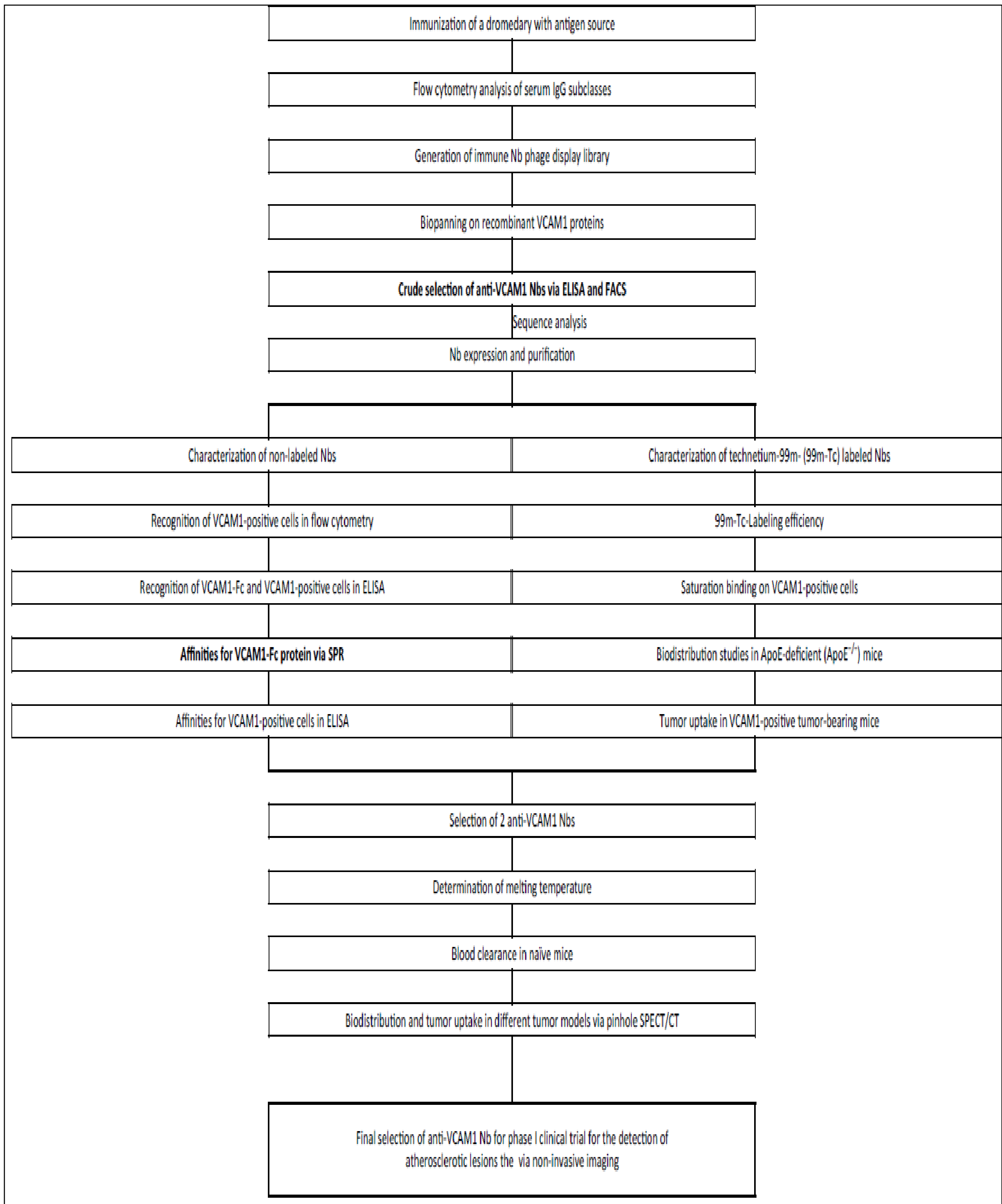


圖 2、ICMI 對開發 anti-VCAM-1 之研發策略

Courtesy from Prof Dick Devoogdt

圖 3、奈米抗體發現的早期研究者(箭頭為主要發現者 Hamers Raymond)
Courtesy from Prof Serge Muyldermans

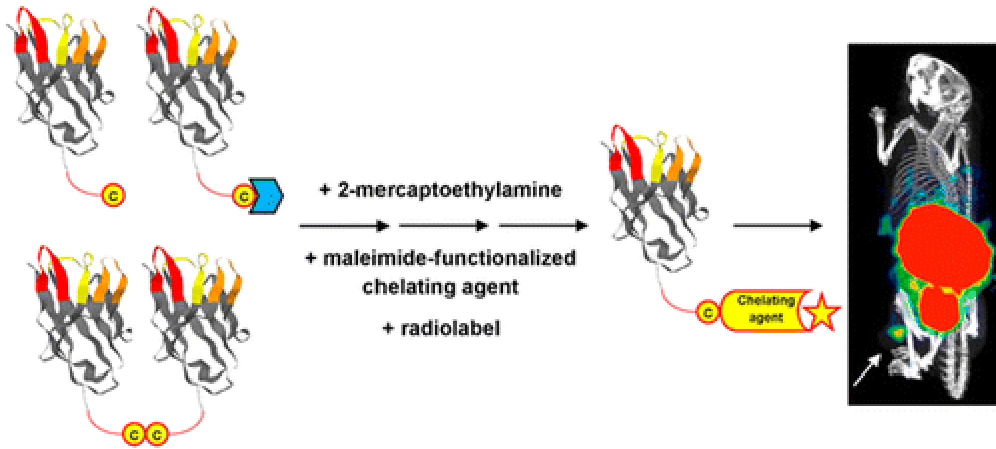


圖 4、C-terminal 定位鍵結 Maleimide-functionalized chelating agent 的策略

Ref:

Sam Massa, Catarina Xavier, Jens De Vos, Vicky Caveliers, Tony Lahoutte, Serge Muyldermans, and Nick Devoogdt. Site-Specific Labeling of Cysteine-Tagged Camelid Single-Domain Antibody-Fragments for Use in Molecular Imaging. *Bioconjugate Chem.*, 2014, 25 (5), pp 979–988.

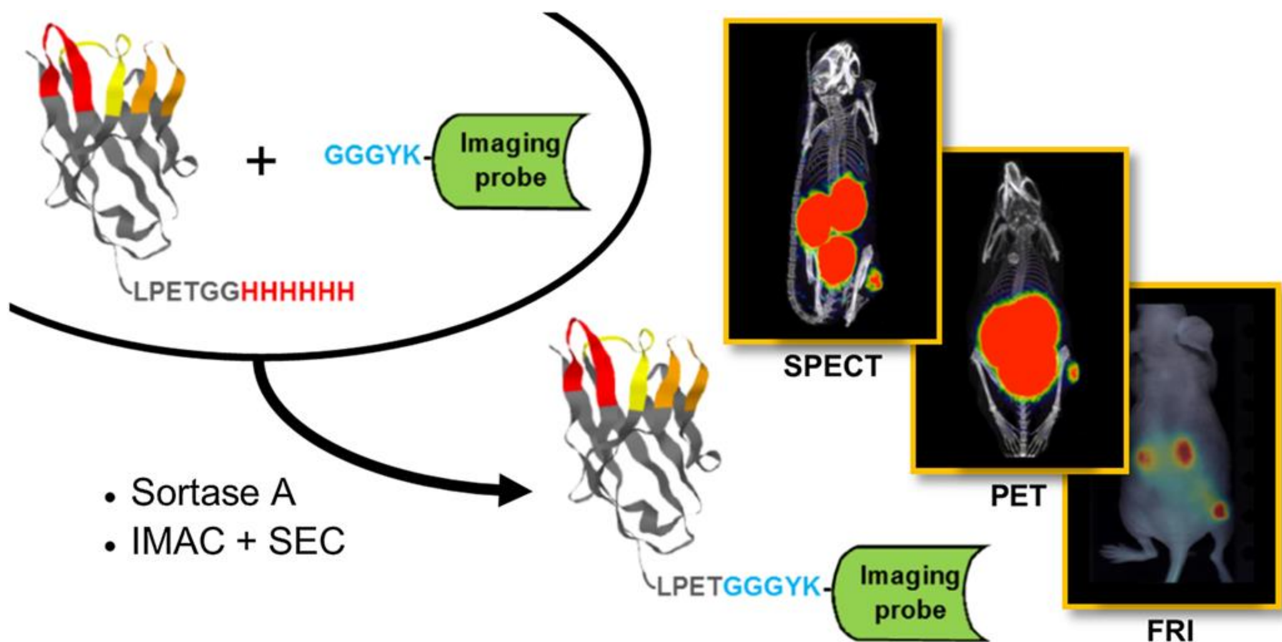
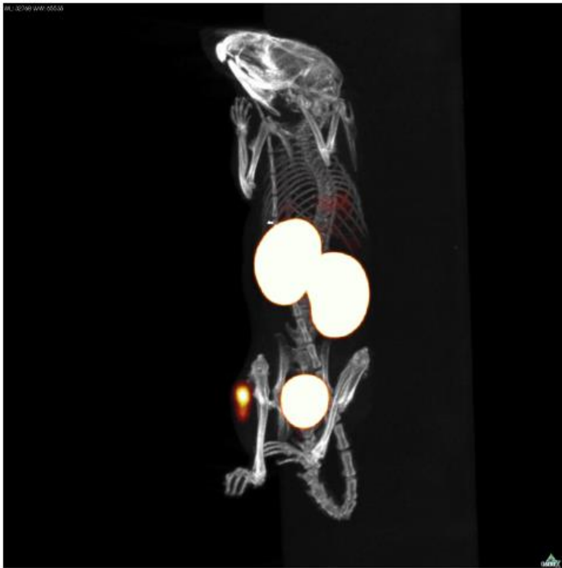


圖 5、C-terminal 定位鍵結接造影示蹤劑的策略

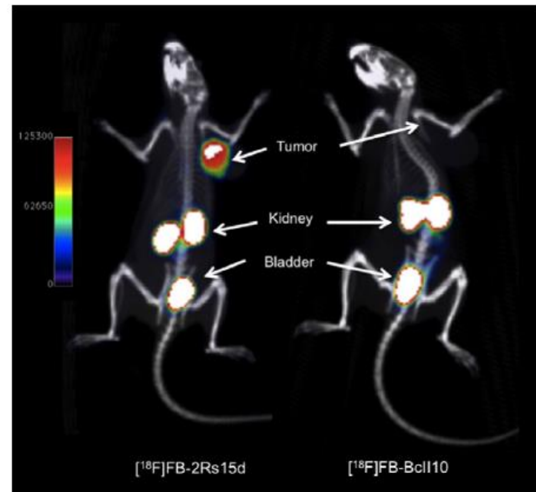
Ref:
Sam Massa, Niravkumar Vikani, Cecilia Betti, Steven Ballet, Saskia Vanderhaegen, Jan Steyaert, Benedicte Descamps, Christian Vanhove, Anton Bunschoten, Fijs W. B. van Leeuwen, Sophie Hernot, Vicky Caveliers, Tony Lahoutte, Serge Muyldermans, Catarina Xavier and Nick Devoogdt. Sortase A-mediated site-specific labeling of camelid single-domain antibody-fragments: a versatile strategy for multiple molecular imaging modality.Contrast Media and Molecular Imaging (2016) DOI: 10.1002/cmmi.1696.

Preclinical SPECT/CT imaging, 1h p.i.



^{99m}Tc-labeled HER2-nanobody

Preclinical PET/CT imaging, 1h p.i.



^{18}F-labeled HER2-nanobody

圖 6、Tc-99m Her2 奈米抗體和 F-18 Her2 奈米抗體的動物造影圖

資料來源:2016 年第三屆抗體造影與分子治療研討會

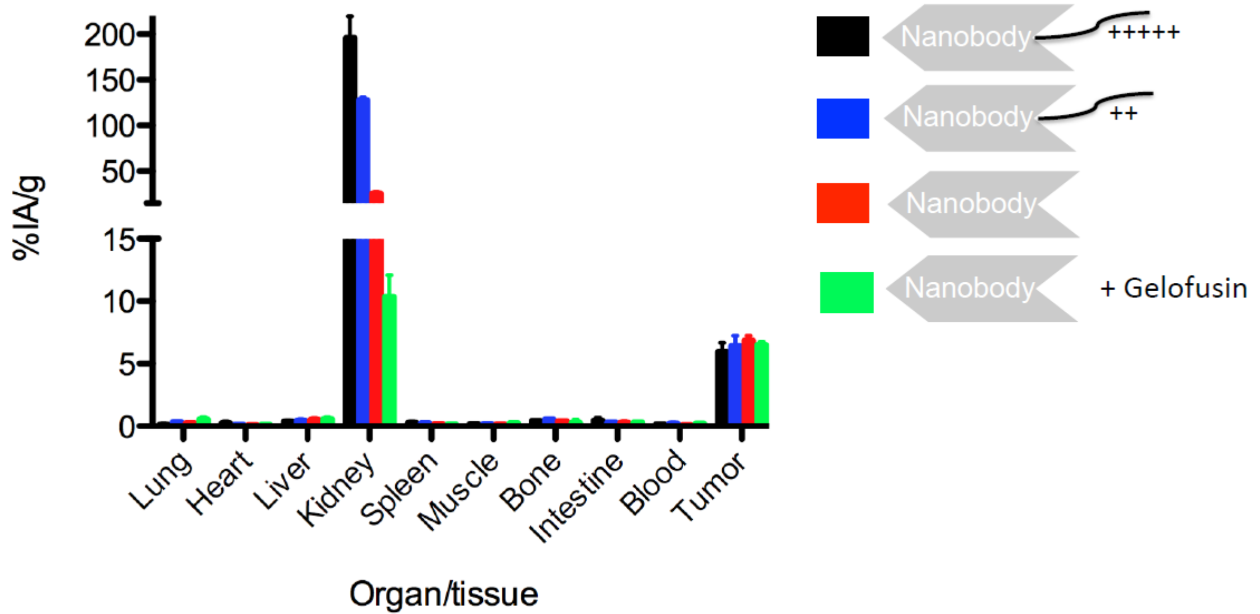


圖 7、蛋白序列和加血漿代用品對生物體分布之影響

Ref:

D'Huyvetter M, Vincke C, Xavier C, Aerts A, Impens N, Baatout S, De Raeve H, Muyldermans S, Caveliers V, Devoogdt N, Lahoutte T. Targeted Radionuclide Therapy with A ^{177}Lu-labeled Anti-HER2 Nanobody. *Theranostics* 2014; 4(7):708-720. doi:10.7150/thno.8156. Available from http://www.thno.org/v04p0708.htm

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圖 8、臨床試驗化學製程管控所需提報資料

資料來源:2016 年第三屆抗體造影與分子治療研討會

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圖 9、臨床試驗化學製程管控所需提報原料藥部分的資料

資料來源:2016 年第三屆抗體造影與分子治療研討會

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圖 10、臨床試驗化學製程管控所需提報成品部分的資料

資料來源:2016 年第三屆抗體造影與分子治療研討會

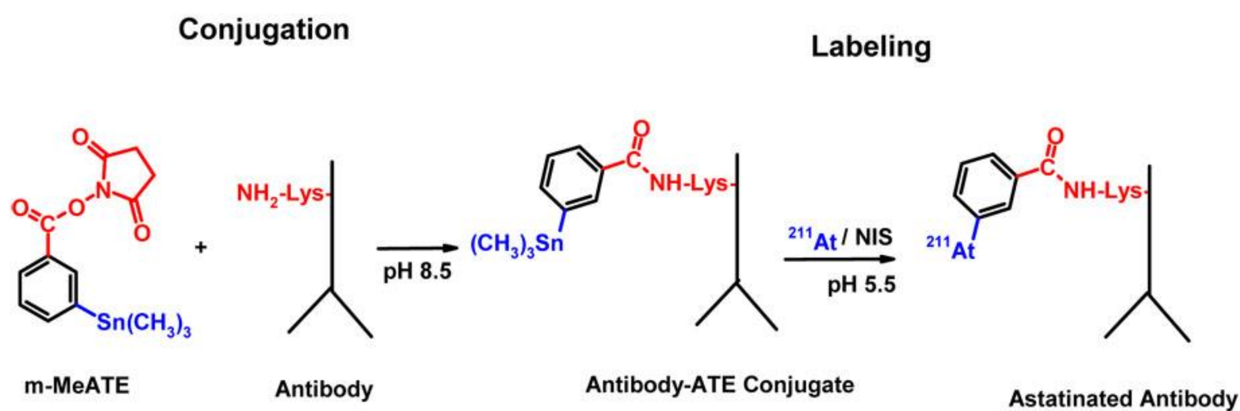

圖 11、Conjugation of antibody with the labeling reagent N-succinimidyl 3-(trimethylstannyl)benzoate (m-MeATE) followed by labeling of immunoconjugate with ^{211}At

Ref:

Kim YS, Brechbiel MW.Tumour Biol. 2012 Jun;33(3):573-90. doi: 10.1007/s13277-011-0286-y. Epub 2011 Dec 6.

Nick Devoogdt; Biologist, Immunologist

Catarina Xavier; Chemist, Radiopharmacist

Sophie Hernot (left) Matthias D'Huyvetter(right); Bioengineer

Marleen Keyaerts, MD

圖 12、分組討論的主席(也是 ICMI 的核心講員)

附錄一、自由大學Tc-99m標誌奈米抗體標準程序書(Tricarbonyl Method)

Site-specific labeling of His-tagged nanobodies with ^{99m}Tc: a practical guide

1. Materials

1.1. Preparation of ^{99m}Tc-Tricarbonyl Precursor

1.1.1. Lyophilized kit (IsoLink™, Covidien, St Louis, USA) containing 4.5 mg of sodium boranocarbonate, 2.85 mg of sodium tetraborate.10H_2O, 8.5 mg of sodium tartrate.2H_2O, and 7.15 mg of sodium carbonate, pH 10.5

1.1.2. Hydrochloric acid (HCl): 1 M solution in water.

1.1.3. ^{99}Mo/^{99m}Tc generator (Drytec; GE Healthcare).

1.1.4. Well-ventilated hoods and lead shielding.

1.1.5. Water bath or dry heating block.

1.2. Assessment of Radiochemical Purity of ^{99m}Tc-Tricarbonyl Precursor

1.2.1. HPLC-system equipped with a radiometric γ-detector.

1.2.2. HPLC column: PLRP-S 300 Å, 5 μm, 250 x 4.6 mm (Agilent).

1.2.3. HPLC solvents: 0.1% trifluoracetic acid (TFA) in H_2O (solvent A) and 0.1% TFA in acetonitrile (solvent B).

1.3. Labeling of His-Tagged Nanobodies with ^{99m}Tc-Tricarbonyl

1.3.1. Nanobody: 1 mg/ml in phosphate buffered saline pH 7.4. (*see* **note 1**)

1.3.2. *fac*-[$^{99m}Tc(CO)_3(H_2O)_3]^+$: 0.74 - 3.7 GBq/ml

1.3.3. Eppendorf tubes.

1.3.4. Water bath/dry bath (52 °C)

1.3.5. Disposable NAP-5 columns (GE Healthcare, Diegem, Belgium), equilibrated with 10 mL phosphate buffered saline pH 7.4.

1.3.6. 0.22 μm Millipore filter (4 mm, Millipore, Brussels, Belgium).

1.4. Assessment of Radiochemical Purity of ^{99m}Tc-Tricarbonyl Nanobody by HPLC analysis

1.4.1. HPLC-system equipped with a UV and a radiometric γ-detector connected in series.

1.4.1.1. HPLC column: PLRP-S 300 Å, 5 μm, 250 x 4.6 mm (Agilent).

1.4.1.2. HPLC solvents: 0.1% trifluoracetic acid (TFA) in H_2O (solvent A) and 0.1% TFA Acetonitrile (solvent B).

1.5. Assessment of Radiochemical Purity of ^{99m}Tc-Tricarbonyl Nanobody by ITLC analysis

1.5.1. Instant Thin Layer Chromatography (ITLC) using silica gel impregnated glass fiber sheets (Pall Corporation, Life Sciences).

1.5.2. ITLC eluent: acetone.

1.5.3. Dose calibrator or gamma counter.

2. Methods

2.1. Preparation of ^{99m}Tc-Tricarbonyl Precursor

2.1.1. Add 1-1.5 mL of the $^{99m}TcO_4^-$ solution (^{99}Mo/^{99m}Tc generator eluate; 0.74 – 3.7 GBq) to the IsoLink™ kit.

2.1.2. Incubate the mixture at 100 °C for 20 min.

2.1.3. Cool the reaction mixture in water.

2.1.4. Add HCl 1 M until pH 6.5-7.4

2.2. *Assessment of Radiochemical Purity of ^{99m}Tc-Tricarbonyl Precursor*

2.2.1. For HPLC analysis, inject ^{99m}Tc-Tricarbonyl (5-10 μCi) into the injection loop. Run the following HPLC gradient, at 1 mL/min:

- 0 -5 min: 25% solvent B; 5 - 7 min: linear gradient of 25% to 34% solvent B ; 7 – 10 min: linear gradient of 34% to 100% solvent B; 10 - 25 min: 100% solvent B.

2.2.2. The ^{99m}Tc-tricarbonyl precursor shows a retention time of 5 - 6 min, whereas unreacted $^{99m}TcO_4^-$ shows a retention time of 4 min. Typical purity of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (^{99m}Tc-tricarbonyl) is > 95 %.

2.3. Labeling of His-Tagged Nanobodies with ^{99m}Tc-Tricarbonyl

2.3.1. Mix 50 μL (50 μg; 1 mg/mL) of purified nanobody with 500 μL of *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ at pH 7.4.

2.3.2. Incubate at 52 °C for 60 - 90 min (*see* **note 2**).

2.3.3. Separate the labeled nanobody from free ^{99m}Tc-Tricarbonyl and $^{99m}TcO_4^-$ by gel filtration methods such as the NAP-5 column using phosphate buffered saline (*see* **note 3**).

2.3.4. Pass the purified solution through a 0.22 μm Millipore filter to eliminate possible aggregates.

2.3.5. Evaluate radiochemical purity by RP-HPLC (see 2.4) and/or by iTLC (*see* 2.5). *See* **note 4**.

2.4. Assessment of Radiochemical Purity of ^{99m}Tc-Tricarbonyl Nanobody

HPLC analysis

2.4.1. Inject ^{99m}Tc-Tricarbonyl Nanobody (3 – 10 μCi) into the injection loop. Run the following HPLC gradient, at 1 mL/min:

2.4.1.1. The *^{99m}Tc-Tricarbonyl Nanobody* shows a retention time of about 13 min. The ^{99m}Tc-tricarbonyl precursor shows a retention time of 5 - 6 min, and $^{99m}TcO_4^-$ has a retention time of 4 min.

2.5. ITLC analysis

2.5.1. Spot 2 μL of ^{99m}Tc-Tricarbonyl Nanobody solution on a 10mm x 100 mm silica gel impregnated glass fiber sheet.

2.5.2. Develop the chromatogram in acetone.

2.5.3. Analyze the distribution of radioactivity by scanning with a γ-radiation TLC scanner or counting the strip cut in 3 parts (application point, middle, solvent front) in a dose calibrator or gamma counter. The ^{99m}Tc-Tricarbonyl precursor and the $^{99m}TcO_4^-$ reveal a Rf (retention factor) of 1 and ^{99m}Tc-Tricarbonyl-nanobody a Rf of 0.

3. Notes

3.1. The nanobody solution should be free of imidazole as this substance will interfere with the labeling procedure.

3.2. Temperature of incubation depends on the thermostability of the Nanobody, if possible always determine the melting temperature (Tm) of Nanobody to be labeled.

3.3. If the labeled Nanobody is more lipophilic, there might be some ^{99m}Tc-Tricarbonyl nanobody activity sticking on the NAP-5 column.

3.4. Radiochemical purity before gel fitration, as determined by either method, usually ranges from 90 to 98 %, and depends on protein concentration. At 0.1 mg/mL final concentration, labeling will be complete after 60 min. After gel filtration and microfiltration, radiochemical purity should be >98% before *in vivo* assessment.

4. *References*

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20. De Groeve, K., et al., Nanobodies as tools for in vivo imaging of specific immune cell types. J Nucl Med, 2010. 51(5): p. 782-9.

附錄二、自由大學Tc-99m 標誌奈米抗體檢驗成績書空白表格

^{99m}Tc labeling

Date:

Nb: *(clone, mw, coupling date and fraction, concentration)*

Labeling conditions:

μl Nb (@ mg/ml); moles = (MW = 13452.9 g/mol)

μl $[^{99m}Tc(CO)_3(H_2O)_3]^+$

Final concentration:		M Nb in	μl total reaction volume
Activity:	mCi/MBq		
pH:			
Reaction time:			
Temperature:			

ITLC AP:

M:

SF: → labeling yield:

NAP-5 buffer exchange and purification

Activity before:

Remain in eppendorf:

Remain on NAP-5:

Eluate:

0.22 μm filtration

Remain on filter:

After filtration:

Quality Control:

ITLC (before Purification)

AP:

M:

SF: → labeling yield:

ITLC (after Purification)

AP:

M:

SF: RCP:

RP-HPLC (after Purification)

Radiochemical purity =

Calculate Radiochemical Yield

Radiochemical yield = % (Activity of Purified product/Activity at starting of reaction)

Non-decay corrected (not taking into account the decay of ^{99m}Tc)

Decay corrected (taking into account the decay of ^{99m}Tc)

$A = A_0 * e^{-\lambda t}$

t = time in seconds

^{99m}Tc

$t1/2 = \ln 2/\lambda$

t1/2 = 6*60*60 = 21600 s

λ = 3.21E-05 s-1

Simple Radioactive Decay: Half-life and Decay Constant

Radioactive decay is a random process. As such, one cannot state with certainty when an unstable nuclide will decay. The probability that an atom will decay during the time $\mathrm{d}t$ is given by $k\mathrm{d}t$ where k is the constant of proportionality known as the decay constant. In a system where there are $N(0)$ atoms present initially, the number of atoms decaying in time $\mathrm{d}t$ is given by $-\mathrm{d}N = kN\mathrm{d}t$. In the limit of very small time intervals, this can be expressed as

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -kN\,.$$

Integration with respect to time gives the number of atoms present at any time t, i.e.

$$N(t) = N(0)\mathrm{e}^{-kt}\,.$$

The half-life, τ, is used to denote the time at which the number of atoms has decreased to half the initial value, i.e. $\frac{1}{2} = \mathrm{e}^{-k\tau}$. Hence the half-life is related to the decay constant through the relation

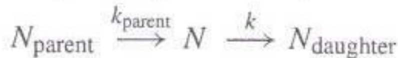
$$\tau = \frac{\ln 2}{k} \approx \frac{0.693}{k}\,.$$

Activity

The number of decays per unit time interval, i.e. the activity A, is defined by

$$A = -\frac{\mathrm{d}N}{\mathrm{d}t} = kN\,.$$

It should be noted in this definition that it is assumed that N is decreasing due to decay. In general the rate equation contains a term for decay (removal) to the daughter and in-growth (production) from the parent, i.e.



$$N_{\mathrm{parent}} \xrightarrow{k_{\mathrm{parent}}} N \xrightarrow{k} N_{\mathrm{daughter}}$$

for which the rate equation becomes

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -kN + k_{\mathrm{parent}}N_{\mathrm{parent}}\,.$$

A situation could arise in which $kN = k_{\mathrm{parent}}N_{\mathrm{parent}}$ and thus N is constant, i.e. $\mathrm{d}N/\mathrm{d}t = 0$. Clearly the activity is not zero. In the definition of A above only decay is considered. In the general case where decay and growth occur, A is given by $A = kN$. Hence A is the number of disintegrations per second even though N may be constant. The unit of activity is the Becquerel, i.e. 1 Bq = 1 disintegration per second.

4.2 Law and Energy of Radioactive Decay

Radioactive decay follows the laws of statistics. If a sufficiently great number of radioactive atoms are observed for a sufficiently long time, the law of radioactive decay is found to be

$$-\frac{dN}{dt} = \lambda N \tag{4.1}$$

where N is the number of atoms of a certain radionuclide, $-dN/dt$ is the disintegration rate, and λ is the disintegration or decay constant (dimension s^{-1}). It is a measure of the probability of radioactive decay. The law of radioactive decay describes the kinetics of the reaction

$$A \rightarrow B + x + \Delta E \tag{4.2}$$

where A denotes the radioactive mother nuclide, B the daughter nuclide, x the particle emitted and ΔE the energy set free by the decay process, which is also called the Q-value. Eq. (4.2) represents a first-order reaction and is in the present case a mononuclear reaction.

Radioactive decay is only possible if $\Delta E > 0$. ΔE can be determined by comparison of the masses. According to the relation found by Einstein (eq. (2.8)),

$$\Delta E = \Delta M\, c^2 = [M_A - (M_B + M_x)]c^2 \tag{4.3}$$

By calculation of ΔE it can be decided whether a decay process is possible or not.

Even if $\Delta E > 0$, the question of the probability of a radioactive decay process is still open. It can only be answered if the energy barrier is known. The energetics of radioactive decay are plotted schematically in Fig. 4.1. The energies of the mother nuclide and the products of the mononuclear reaction differ by ΔE. But the nuclide A has to surmount an energy barrier with the threshold energy E_S. The nuclide may occupy discrete energy levels above ground level. However, only if its excitation

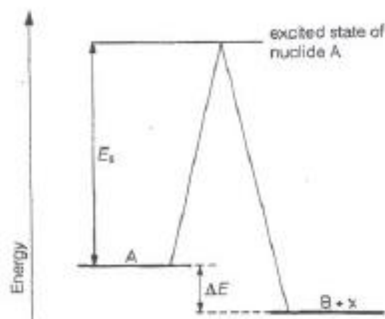


Figure 4.1. Energy barrier of radioactive decay.

energy is high enough can decay occur. The energy barrier must either be surmounted or crossed by quantum mechanical tunnelling.

The law governing radioactive decay (eq. (4.1)) is analoguous to that of first-order chemical kinetics. The excited state on top of the energy barrier corresponds to the activated complex, and E_S is equivalent to the activation energy.

Integration of eq. (4.1) gives

$$N = N_0 e^{-\lambda t} \tag{4.4}$$

where N_0 is the number of radioactive atoms at the time $t = 0$. Instead of the decay constant λ, the half-life $t_{1/2}$ is frequently used. This is the time after which half the radioactive atoms have decayed: $N = N_0/2$. Introducing the half-life $t_{1/2}$ in eq. (4.4), it follows that

$$t_{1/2} = \frac{\ln 2}{\lambda} = \frac{0.693}{\lambda} \tag{4.5}$$

and

$$N = N_0 \left(\frac{1}{2}\right)^{t/t_{1/2}} \tag{4.6}$$

From this equation it is seen immediately that the number of radioactive atoms has decreased to one-half after one half-life, to 1/128 (less than 1%) after 7 half-lives, and to 1/1024 (about 0.1%) after 10 half-lives. If the time t is small compared with the half-life of the radionuclide ($t \ll t_{1/2}$), the following approximation formula may be used:

$$\begin{aligned} e^{-\lambda t} &= 1 - \lambda t + \frac{(\lambda t)^2}{2} - \cdots \\ &= 1 - (\ln 2)\left(\frac{t}{t_{1/2}}\right) + \frac{(\ln 2)^2}{2}\left(\frac{t}{t_{1/2}}\right)^2 - \cdots \end{aligned} \tag{4.7}$$

The average lifetime τ is obtained by the usual calculation of an average value

$$\tau = \frac{1}{N_0}\int_0^\infty N\,dt = \int_0^\infty e^{-\lambda t}\,dt = \frac{1}{\lambda} \tag{4.8}$$

From eq. (4.4) it follows that after the average lifetime τ the number of radioactive atoms has decreased from N_0 to N_0/e ($\tau = t_{1/2}/(\ln 2)$).

Generally, the half-life of a radionuclide does not depend on pressure, temperature, state of matter or chemical bonding. However, in some special cases in which low-energy transitions occur, these parameters have been found to have a small influence (section 10.2).

The activity A of a radionuclide is given by its disintegration rate:

$$A = -\frac{dN}{dt} = \lambda N = \frac{\ln 2}{t_{1/2}} N \qquad (4.9)$$

The dimension is s^{-1}, and the unit is called becquerel (Bq): $1\,Bq = 1\,s^{-1}$. An older unit is the curie (Ci). It is still used sometimes, related to the activity of 1 g of ^{226}Ra, and defined as $1\,Ci = 3.700 \cdot 10^{10}\,s^{-1} = 37\,GBq$. Smaller units are 1 millicurie (mCi) = 37 MBq, 1 microcurie (μCi) = 37 kBq, 1 nanocurie (nCi) = 37 Bq, and 1 picocurie (pCi) = 0.37 Bq. 1 Ci is a rather high activity which cannot be handled directly but needs special installations, such as hot cells. Activities of the order of several mCi are applied in medicine for diagnostic purposes, activities of the order of 1 μCi are usually sufficient for the investigation of the behaviour of radionuclides, and activities of the order of 1 nCi are measurable without special efforts.

As the activity A is proportional to the number N of radioactive atoms, the exponential law, eq. (4.4), holds also for the activity:

$$A = A_0 e^{-\lambda t} \qquad (4.10)$$

The mass m of the radioactive atoms can be calculated from their number N and their activity A:

$$m = \frac{N \cdot M}{N_{Av}} = \frac{A \cdot M}{N_{Av}\lambda} = \frac{A \cdot M}{N_{Av} \ln 2} t_{1/2} \qquad (4.11)$$

where M is the nuclide mass and N_{Av} is Avogadro's number $(6.022 \cdot 10^{23})$.

In laboratory experiments with radionuclides, knowledge of the mass of the radioactive substances is very important. For example, the mass of 1 MBq of ^{32}P ($t_{1/2} = 14.3$ d) is only about 10^{-10} g, and that of 1 MBq of ^{99m}Tc ($t_{1/2} = 6.0$ h) is only about $5 \cdot 10^{-12}$ g. If there is no carrier present in the form of a large excess of inactive atoms of the same element in the same chemical state, these small amounts of radionuclides may easily be lost, for instance by adsorption on the walls. Whereas in the case of radioisotopes of stable elements the condition of the presence of carriers is often fulfilled due to the ubiquity of most stable elements, it is not fulfilled in case of short-lived isotopes of radioelements, and extraordinary behaviour may be observed (section 13.3).

The ratio of the activity to the total mass m of the element (the sum of radioactive and stable isotopes) is called the specific activity A_s:

$$A_s = \frac{A}{m} [Bq/g] \qquad (4.12)$$

Sometimes high or well-defined specific activities are required, for instance in the case of the application of radionuclides or labelled compounds in medicine, or as tracers in other fields of research.

Basic SOP AMIDE Milabs SPECT-CT images

1) **Import SPECT**:

File -> import file (specify) -> NIFTI via (X)MedCon -> open SPECT image (= for example: SPECT_REG_2016000209_v02ss2it4-0-ac.nii)

Remark: A window with a warning will appear. Just click OK.

2) **Import CT**:

File -> import file (specify) -> NIFTI via (X)MedCon -> open CT image (= for example: CT_2016000209.nii)

Remark: A window with a warning will appear. Just click OK.

3) Right mouse click on the SPECT (white frame left):

Basic info

- *Data Set Name*: Here you can change the SPECT name
- *Interpolation Type*: 2^e^ type
- *Conversion Type – Direct – Scaling Factor:* 1

Colormap/Treshhold

- Here you can change the scale and its color

Remark: Never rotate the SPECT image in the rotate window of the SPECT. If you do so, you will only rotate the SPECT image and you will lose your fusion with the CT. If you still did it, you will have to redo the whole procedure to have your fusion again

4) Right mouse click on CT(white frame left):

Basic info

- *Data Set Name*: Here you can change the CT name
- *Interpolation Type*: 2^e^ type
- *Conversion Type – Direct – Scaling Factor:* 1

Colormap/Treshhold

- Here you can change the scale and its color

Remark: Never rotate the CT image in the rotate window of the CT. If you do so, you will only rotate the CT image and you will lose your fusion with the SPECT. If you still did it, you will have to redo the whole procedure to have your fusion again.

5) **Orientatation**

Right mouse click on "Study" (white frame left):

Basic info:

- Name: Here you can change the name of the whole image (this name will then be used when you save your image)

Rotate:

- Rotate the image with the transverse cursor until the spine of the animal is at the bottom on the transverse image, if this is not yet the case.

6) **Drawing ROI's:**

- Edit -> Add ROI -> ellipsoid
- A window appears where in you have to give a name to your ROI
- Draw a ROI on the desired area of the image
- A window appears where you can fill in the size of the ROI (this can also be changed later on if you do right mouse click on the name of the ROI in the white frame on the left of the image)
- Adjusting ROI: orientation, size,... (if you place your mouse on the ROI in the image, you can see below in the white frame left of the image, what you can do with it)
    - ➔ To make several ROI's, just apply the protocol above again

7) **Calculate ROI statistics:**

- Click on the name of the CT (white window left) so that the CT image isn't shown anymore (if you do not do that, you will get also the statistics of the CT image which you don't need for SPECT analyses)
- Tools -> Calculate ROI Statistics
- A window appears:
    - Select: On selected data sets, selected ROIS
    - Chose: Calculate over all voxels (normal) (unless you want to use a threshold)
    - Execute
- A window appears with all your statistics of your ROI('s)
    - ➔ Save as -> name.xls instead of name.tsv -> Ok

8) **Save image:**

File -> Save as (uses the name you chose in point 5, but you can still change it if necessary, just save it as .xif)

If you want to reopen your saved image: File -> Open -> xif file

Basic SOP AMIDE Siemens SPECT-CT images

1) **Import SPECT**:

File -> import file (guess) -> open SPECT image (= for example: 2013000685_3phos_nuac_sc3_phorbit_225mmRoR_sensmap_1.5_4_5_16_0.5_1000_1.5_0_0_1_1_685_764-764-1256_26954.h33)

Remark: Open the .h33 file and not the .i33, but the .h33 and .i33 have to be in the same folder.

2) **Import CT**:

File -> import file (guess) -> open CT image (= for example: 685_764-764-1256.h33)

Remark: Open the .h33 file and not the .i33, but the .h33 and .i33 have to be in the same folder.

3) **Right mouse click on the SPECT (white frame left):**

Basic info

- *Data Set Name*: Here you can change the SPECT name
- *Interpolation Type*: 2^e type
- *Conversion Type – Direct – Scaling Factor:* Chose the scaling factor that suits your scans (see scaling factor file)

Colormap/Treshhold

- Here you can change the scale and its color

Remark: Never rotate the SPECT image in the rotate window of the SPECT. If you do so, you will only rotate the SPECT image and you will lose your fusion with the CT. If you still did it, you will have to redo the whole procedure to have your fusion again

4) **Right mouse click on CT(white frame left):**

Basic info

- *Data Set Name*: Here you can change the CT name
- *Interpolation Type*: 2^e type
- *Conversion Type – Direct – Scaling Factor:* 1

Colormap/Treshhold

- Here you can change the scale and its color

Remark: Never rotate the CT image in the rotate window of the CT. If you do so, you will only rotate the CT image and you will lose your fusion with the SPECT. If you still did it, you will have to redo the whole procedure to have your fusion again.

5) **Orientatation**

Right mouse click on "Study" (white frame left):

Basic info:

- Name: Here you can change the name of the whole image (this name will then be used when you save your image)

Rotate:

- Y: Click on "invert axis"
- If the image is upside down -> rotate the image with the coronal cursor until the image is the right way up
- Rotate the image with the transverse cursor until the spine of the animal is at the bottom on the transverse image

6) **Drawing ROI's:**

- Edit -> Add ROI -> ellipsoid
- A window appears where in you have to give a name to your ROI
- Draw a ROI on the desired area of the image
- A window appears where you can fill in the size of the ROI (this can also be changed later on if you do right mouse click on the name of the ROI in the white frame on the left of the image)
- Adjusting ROI: orientation, size,... (if you place your mouse on the ROI in the image, you can see below in the white frame left of the image, what you can do with it)
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7) **Calculate ROI statistics:**

- Click on the name of the CT (white window left) so that the CT image isn't shown anymore (if you do not do that, you will get also the statistics of the CT image which you don't need for SPECT analyses)
- Tools -> Calculate ROI Statistics
- A window appears:
  - Select: On selected data sets, selected ROIS
  - Chose: Calculate over all voxels (normal) (unless you want to use a threshold)
  - Execute
- A window appears with all your statistics of your ROI('s)
  - ➔ Save as -> name.xls instead of name.tsv -> Ok

8) **Save image:**

File -> Save as (uses the name you chose in point 5, but you can still change it if necessary, just save it as .xif)

If you want to reopen your saved image: File -> Open -> xif file