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(54) 名稱

C D 2 缺失之非洲豬瘟病毒做為對抗哺乳動物非洲豬瘟之減毒活疫苗或後續滅活之疫苗  
CD2 DEFICIENT AFRICAN SWINE FEVER VIRUS AS LIVE ATTENUATED OR SUBSEQUENTLY  
INACTIVATED VACCINE AGAINST AFRICAN SWINE FEVER IN MAMMALS

(57) 摘要

本發明係關於較佳活的經減毒或後續滅活之非洲豬瘟病毒(African swine fever virus；ASFV)，其包含無功能性基因組 CD2 基因，其中該 ASFV 在其複製中無缺失，亦係關於相應組合物或免疫原性組合物或疫苗、產生方法及用於治療及/或預防哺乳動物、較佳豬科(Suidae)、例如豬，更佳地馴養豬(家豬(*Sus scrofa domesticus*))、野豬(歐洲野豬(*Sus scrofa scrofa*))、疣豬(非洲野豬(*Potamochoerus porcus*))、藪豬(假面野豬(*Potamochoerus larvatus*))、大林豬(巨林豬(*Hylochoerus meinertzhageni*))以及野生豬之非洲豬瘟的用途。

The present invention is directed to a preferably live attenuated or subsequently inactivated African swine fever virus (ASFV), comprising a non-functional genomic CD2 gene, wherein such ASFV is not deficient in its replication, as well as to corresponding compositions or immunogenic compositions or vaccines, methods of production and uses for treating and/or preventing African swine fever in mammals, preferably of the family Suidae, for instance pigs, more preferably domestic pigs (*Sus scrofa domesticus*), wild pigs (*Sus scrofa scrofa*), warthogs (*Potamochoerus porcus*), bushpigs (*Potamochoerus larvatus*), giant forest hogs (*Hylochoerus meinertzhageni*) as well as feral pigs.

指定代表圖：

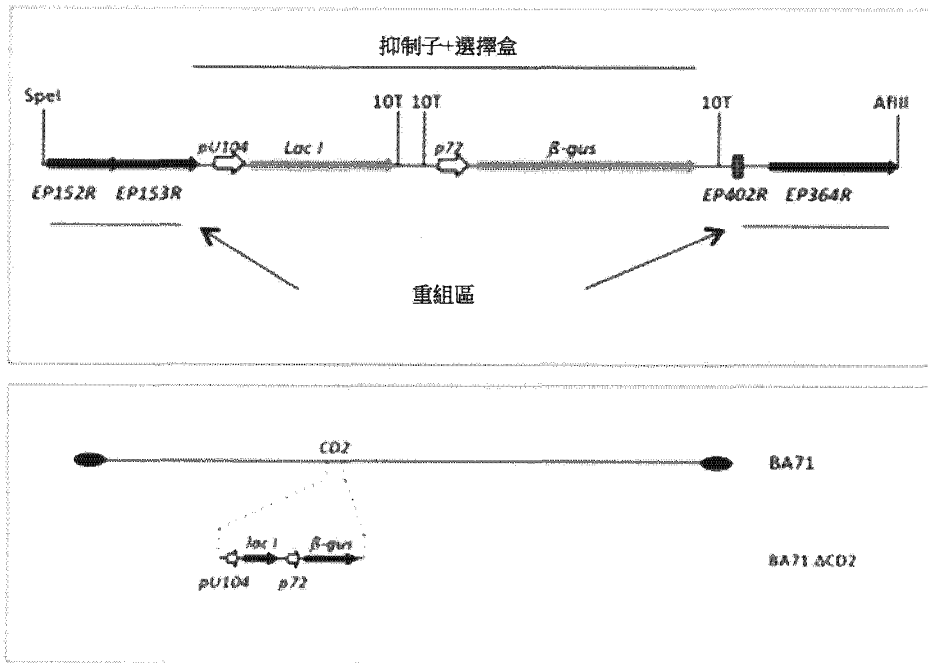


圖 1

# 發明專利說明書

(本說明書格式、順序，請勿任意更動)

## 【發明名稱】

CD2缺失之非洲豬瘟病毒做為對抗哺乳動物非洲豬瘟之減毒活疫苗或後續滅活之疫苗

CD2 DEFICIENT AFRICAN SWINE FEVER VIRUS AS LIVE  
ATTENUATED OR SUBSEQUENTLY INACTIVATED VACCINE  
AGAINST AFRICAN SWINE FEVER IN MAMMALS

## 【技術領域】

本發明係關於醫藥領域，具體而言係關於獸醫醫藥領域。本發明係關於較佳活的經減毒或後續滅活之非洲豬瘟病毒(ASFV)，其包含無功能性基因組CD2基因，其中該ASFV在其複製中無缺失，亦係關於相應組合物、免疫原性組合物或疫苗、產生方法及用於治療及/或預防哺乳動物、較佳豬科、例如豬之非洲豬瘟的用途。

## 【先前技術】

非洲豬瘟(ASF)係世界動物衛生組織之前一A類中所包括之感染馴養豬之高度傳染病。儘管在九十年代中期其已自歐洲大陸根除，但ASF病毒(ASFV) (即ASF之病原體)仍在薩丁及許多亞撒哈拉國家流行，其中其引起巨大經濟損失。在喬治亞州病毒自東非之最近再引入及其朝向俄羅斯國家之傳播已開啟關於ASFV再進入歐洲及亞洲國家(包括中國，世界上豬之主要生產者及消費者)之風險之新問題。該情況因以下事實加重：無對抗ASFV之可用疫苗，因此控制措施僅限於疾病之有效快速診斷及剔除受感染動物；受該疾病影響之最窮困國家完全無力實行該措施。過去進行之工作明確證實，ASFV之滅活之製劑不能賦予針對病毒攻擊之保護(Stone及Hess, 1967；Forman等人，

1982；Mebus 1988)。與之顯著不同，已證實用ASFV之減毒株對豬進行免疫可誘導針對同源病毒攻擊之極堅實保護(Ruiz Gonzalvo等人，1983；Wardley等人，1985)。儘管安全性問題使得減毒病毒不可應用作疫苗(Sanchez Botija, 1963)，但其提供關於參與保護之免疫參數之當今現存之最有用之數據，其包括：

(i) 中和抗體(Onisk等人，1994)及/或能夠活體外抑制ASFV感染之抗體(Ruiz Gonzalvo等人，1986)

(ii) 特異性CD8<sup>+</sup> T細胞反應(Oura等人，2006)。

抗體及T細胞似乎皆不能自身賦予無菌保護，此明確地指示對抗ASFV之理想疫苗應能夠賦予兩種免疫反應。

因同源重組缺失特異性毒力基因導致減毒ASF病毒，但其作為減毒活疫苗之用途之實驗證據至多僅限於處於少數參考文獻；所有該等參考文獻皆係在十多年前公佈(Borca等人，1998；Moore等人，1998；Lewis等人，2000；Neilan等人，2002)。

ASFV之高度複雜性(包含超過150種經編碼抗原)使得選擇亞單位疫苗中包括之最佳抗原之任務困難。九十年代中期進行之工作闡述三種ASFV結構蛋白在桿狀病毒系統中表現且未經進一步純化即與弗式(Freund's)佐劑一起投與時之保護潛能：p54、p30及血球凝集素(HA)(Ruiz-Gonzalvo等人，1996；Gómez-Puertas等人，1998)。儘管保護與分別中和及抑制性抗體之誘導相關，但不應排除細胞反應之誘導。最近，該等研究已擴展至DNA免疫之領域。因此，用編碼p54、p30及血球凝集素(sHA)之細胞外結構域且稠合至泛素之質體進行DNA免疫在不存在抗體下賦予針對致死攻擊之部分保護。與CD8<sup>+</sup> T細胞之擴增相關之保護尤其識別HA內之兩種9肽(Argilagué等人，2012)。

概言之，目前可用之對抗哺乳動物非洲豬瘟之疫苗之缺點包括：無效能(滅活疫苗)、無安全性(可恢復至有毒力之天然減毒活疫

苗)或無可靠實驗證據(包括亞單位疫苗及重組缺失減毒活病毒之重組疫苗)。

最後，基於可誘導病毒之最有前景之策略之一尚未優化用於活體內使用：WO 2012/107164係關於基於複製缺失重組病毒、較佳基於有毒力ASFV株BA71之對抗ASFV的疫苗，其中必需基因(例如pp220、pp62或pB438L)之表現在活體外可誘導且因此在活體內受到抑制。迄今為止，僅使用一種複製缺失ASFV且其測試為候選疫苗：BA71.v220i.TK<sup>-</sup>。此重組株係基於編碼ASFV多聚蛋白pp220之基因之可誘導表現。出於此目的，將Lac I抑制子與β-葡糖醛酸糖苷酶標記基因一起引入病毒基因組之TK基因座中。在活體外非容許性條件下，所得重組株BA71.v220i.TK<sup>-</sup>導致能夠離開受感染Vero細胞之非傳染性二十面體無核粒子裝配(Andrés等人，2002)。然而，利用BA71.v220i.TK<sup>-</sup>之活體內免疫不賦予針對用同源BA71攻擊之保護，此可能係由於儘管TK基因可分配用於在組織培養細胞中生長，但其對於豬巨噬細胞及受感染動物中之病毒複製係必需的(Moore等人，1998)。無保護與BA71.v220i.TK<sup>-</sup>不能在活體內誘導體液或細胞反應相關。

其他先前技術係如下：

Borca MV及同事(J Virol 1998, 72(4): 2881-2889)涉及自ASFV缺失CD2樣基因8-DR，即病原性非洲分離株Malawi Lil-20/1，其影響家豬中之病毒感染。然而，作者報導，儘管CD2缺失，但Malawi突變體株仍具有高度病原性(第2886頁，左手列，倒數第二段，以及第2884頁，表1)且因此不適用於疫苗接種。

Kay-Jackson PC (J General Virol 2004, 85: 119-130)涉及ASFV之CD2蛋白質與肌動蛋白結合銜接體蛋白SH3P7之相互作用。

Boinas FS等人 (J General Virol 2004, 85: 2177-2187)係關於來自

葡萄牙之遊走烏壁虱(*Ornithodoros erraticus*)棲息豬房屋之病原及非病原性非洲豬瘟病毒分離株、尤其ASFV分離株OURT88/1、OURT88/2、OURT88/3及OURT88/4之表徵。

Chapman DAG及同事(*J General Virol* 2008, 89: 397-408)揭示葡萄牙之非病原及病原性ASFV分離株、即非病原性ASFV分離株OURT88/3與西非之高度病原性ASFV分離株Benin 97/1以及組織培養基適應之ASFV分離株BA71V的基因組序列之間的比較。其顯示ASFV分離株OURT88/3在編碼CD2樣(EP402R)及C型凝集素(EP153R)蛋白之開放閱讀框(ORF)中具有中斷(摘要以及第403頁，右手列，倒數第二段，及第406頁，右手列，自頂部第二段)。

King K等人(*Vaccine* 2011, 29(28): 4593-4600)闡述藉由實驗免疫保護歐洲馴養豬免受ASFV之有毒力非洲分離株影響。King及同事顯示，具有葡萄牙之無毒力OURT88/3基因型I分離株、之後緊密相關之有毒力OURT88/1基因型I分離株之豬的實驗免疫可賦予針對利用非洲有毒力分離株(包括基因型I Benin97/1分離株及基因型X Uganda 1965分離株)攻擊的保護。然而，作者報導，在其第二實驗中，僅60%豬倖免於利用Benin 97/1分離株攻擊(第4594頁，右手列，最後一段)。

Abrams CC及Dixon LK (*Virology* 2012, 433(1): 142-148)涉及使用cre/loxP重組系統自在組織培養基適應之非病原性ASFV株上例示之BA71V非洲豬瘟病毒基因組依序缺失基因。然而，該完全非病原性ASFV株不可在活體內感染宿主(即使於高劑量下，例如 $10^7$ 個溶菌斑形成單位)且不可引起各別免疫反應。

Escribano JM及同事(*Virus Research* 2012, 173(1): 101-109)提及抗體介導之ASFV之中和，其迷思及事實。

因此，本發明下之目的係提供用於預防及/或治療哺乳動物非洲豬瘟之用藥，其克服先前技術之問題。

**【發明內容】**

在一個態樣中，本發明之目的藉由提供包含無功能性基因組CD2基因之非洲豬瘟病毒(ASFV)、較佳地非天然重組ASFV驚人地得以解決，前提係該ASFV在其複製中無缺失，其中較佳地，該ASFV係滅毒活ASFV或經由後續物理及/或化學滅活自滅毒活ASFV產生之後續滅活之ASFV。

該物理滅活較佳係藉由用UV輻射、X射線輻射、 $\gamma$ 輻射、冷凍-解凍及/或加熱後續處理滅毒活ASFV來達成。該化學滅活較佳係藉由用一或多種化學滅活劑後續處理滅毒活ASFV來達成，其中更佳地，該一或多種化學滅活劑係選自由以下組成之群： $\beta$ -丙內酯、戊二醛、次乙亞胺、 $\beta$ -次乙亞胺、雙次乙亞胺、乙醯基次乙亞胺、臭氧及/或甲醛。

在本發明上下文中，術語「無功能性基因組CD2基因」係指位於ASFV(較佳地非天然重組ASFV)之基因組中之經修飾CD2基因，例如EP402R，其中與未經修飾之功能性ASFV CD2基因相比，該ASFV CD2基因之該修飾根本不產生ASFV CD2基因產物或產生無生物功能之ASFV CD2基因產物。包括但不限於，該修飾可為(例如)完全或部分缺失基因組ASFV CD2基因及/或修飾一或多個控制及/或編碼相應ASFV CD2基因產物之核苷酸及/或藉由(例如)向ASFV CD2開放閱讀框(ORF)中插入一或多個核苷酸及/或任何其他目前已知或可能之滅活或敲除該ASFV CD2基因之功能表現之方法破壞該ASFV CD2 ORF。藉助該ASFV CD2基因滅活或敲除可生成較佳地活的經滅毒或後續滅活之ASFV。

在本發明上下文中，術語「在其複製中無缺失」係指能夠在活體外及/或活體內複製及/或能夠產生病毒後代但該複製及/或病毒後代產生亦可以稍微降低程度(例如低於現有技術之方法及/或裝置之檢測

限值)發生的ASFV、較佳地**非**天然重組ASFV。因此，情況可為，該ASFV在其活體外複製中、例如在細胞培養基(例如經培養巨噬細胞)中**無**缺失，但在哺乳動物活體內該ASFV在其複製中至少嚴重受損，例如引起複製及/或病毒後代產生低於檢測限值。

在另一態樣中，本發明之目的已藉由提供生成ASFV基因組中之**無**功能性ASFV CD2基因之方法驚人地得以解決，該方法包含以下步驟：

(a) 向ASFV CD2基因中引入一或多個完全或部分缺失及/或修飾一或多個控制及/或編碼相應ASFV CD2基因產物之核苷酸及/或破壞ASFV CD2開放閱讀框(ORF)藉此使得ASFV CD2**無**功能性，該破壞較佳係藉由將Lac I抑制子與 $\beta$ -葡糖醛酸糖苷酶標記基因一起引入ASFV CD2基因座中來進行，從而導致ASFV CD2基因幾乎完全缺失，藉此使得其在活體外及活體內**無**功能性。

在再一態樣中，本發明之目的已藉由提供產生包含**無**功能性基因組CD2基因之**非**天然重組ASFV之方法驚人地得以解決，前提係該ASFV在其複製中**無**缺失，如本文中闡述及/或定義，該方法包含以下步驟：

(a) 根據用於生成ASFV基因組中之**無**功能性ASFV CD2基因之上述方法製備包含**無**功能性基因組CD2基因之**非**天然重組ASFV；

(b) 用步驟(a)之ASFV活體外感染不使病毒滅活之初代豬巨噬細胞及/或易於受ASFV感染之不使病毒滅活之細胞系、較佳地COS-7細胞；

(c) 自步驟(b)之細胞分離ASFV及/或對其進行純化，此較佳地係藉由收集含有細胞外ASFV之培養基、首先以低速對其離心以去除細胞碎屑且隨後以高速離心以使病毒沈積並將其重新懸浮於PBS中來進行，其中在最後將病毒重新懸浮於PBS中之前，視情況藉由在PBS



中之25%蔗糖墊上離心純化此重新懸浮之病毒；

(d) 視情況較佳地藉由形成溶胞溶菌斑滴定步驟(c)之經分離及/或純化之ASFV [ASFV濃度表示為溶菌斑形成單位(pfu)/mL]；

(e) 對於自步驟(c)或(d)獲得之減毒活ASFV，視情況較佳地藉由用UV輻射、X射線輻射、 $\gamma$ 輻射、冷凍-解凍及/或加熱處理物理滅活及/或較佳地藉由用一或多種化學滅活劑處理化學滅活，藉此產生一或多種後續滅活之ASFV，其中更佳地，該一或多種化學滅活劑係選自由以下組成之群： $\beta$ -丙內酯、戊二醛、次乙亞胺、 $\beta$ -次乙亞胺、雙次乙亞胺、乙醯基次乙亞胺、臭氧及/或甲醛。

在再一態樣中，本發明之目的已藉由提供可藉由如本文中闡述及/或定義之方法獲得之非然重組ASFV驚人地得以解決。

在再一態樣中，本發明之目的已藉由提供組合物或免疫原性組合物或疫苗驚人地得以解決，該組合物或免疫原性組合物或疫苗包含治療有效量之一或多種如本文中闡述及/或定義之ASFV，視情況另外包含一或多種醫藥上可接受之賦形劑及/或一或多種醫藥上可接受之載劑，其中較佳地，該一或多種醫藥上可接受之賦形劑及/或一或多種醫藥上可接受之載劑係選自由以下組成之群：溶劑、分散介質、佐劑、穩定劑、稀釋劑、防腐劑、抗細菌劑及抗真菌劑、等滲劑、吸附延遲劑。

在本發明上下文中，術語「*免疫原性組合物*」係指能夠引發細胞及/或體液免疫反應但不必賦予對抗哺乳動物非洲豬瘟之完全或部分免疫保護的組合物。換言之，該免疫原性組合物可根本不導致免疫保護。然而，為免生疑問，該免疫原性組合物可賦予對抗哺乳動物非洲豬瘟之完全或部分保護且此亦係較佳。相比之下，本發明上下文中之「*疫苗*」不賦予針對哺乳動物非洲豬瘟之完全或部分、但至少部分免疫保護。

在再一態樣中，本發明之目的已藉由提供一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗驚人地得以解決，該組合物或免疫原性組合物或疫苗用於治療及/或預防哺乳動物、較佳豬科、例如豬、更佳歐洲之馴養豬(家豬)及野豬(歐洲野豬)、非洲之疣豬(非洲野豬)、藪豬(假面野豬)及大林豬(巨林豬)、以及美洲地區之野生豬(其可能係部分源自歐洲野豬)之非洲豬瘟的方法中。

預防及/或治療有需要之哺乳動物、較佳豬科、例如豬、更佳歐洲之馴養豬(家豬)及野豬(歐洲野豬)、非洲之疣豬(非洲野豬)、藪豬(假面野豬)及大林豬(巨林豬)以及美洲地區之野生豬(其可能係部分源自歐洲野豬)之非洲豬瘟的相應方法及用於製備用於預防及/或治療哺乳動物、較佳豬科、例如豬、更佳歐洲之馴養豬(家豬)及野豬(歐洲野豬)、非洲之疣豬(非洲野豬)、藪豬(假面野豬)及大林豬(巨林豬)以及美洲地區之野豬(其可能係部分源自歐洲野豬)之非洲豬瘟之醫藥組合物/藥劑的用途亦意欲屬本發明之精神。

在再一態樣中，本發明之目的已藉由提供引發動物、較佳豬科、例如豬、更佳地馴養豬(家豬)、野豬(歐洲野豬)、疣豬(非洲野豬)、藪豬(假面野豬)、大林豬(巨林豬)以及野生豬之保護性免疫反應的方法驚人地得以解決，該方法係藉由向該動物投與一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗來進行。

本發明之較佳AFSV株(BA71.ΔCD2)之特徵在於以下優點：病毒CD2基因幾乎完全缺失。唯一剩餘之序列係在基因末端之36個鹼基對序列。此序列可能不包括於任何病毒mRNA中，此乃因所插入盒以10T轉錄終止序列終止。CD2之缺失不影響活體外COS-7細胞中之病毒複製。此特徵與BA71.ΔCD2可在COS-7細胞中生長之事實一起確保

ASFV之高效價原液之產生用於疫苗目的。重要的是注意，野生ASFV分離株在初代豬巨噬細胞中排他地生長，出於商業目的維持並使用以使病毒生長遠更困難且昂貴。

此疫苗之一個額外優點係其在鈍緣蜱屬蜱(即ASFV之非哺乳動物儲存器)中缺失，此乃因CD2係此無脊椎動物中ASFV複製之關鍵毒力因子(Rowland等人，2009)。ASFV可感染鈍緣蜱屬且保持無症狀達一年以上(Boinas等人，2011)，係針對環境之病毒之連續來源且亦係針對重組之離心活體內容器。CD2缺失病毒將在蜱中缺失地傳播之事實避免因與循環ASFV株重組而逆轉之任何風險。此外，如上文闡釋之ASFV株BA71 $\Delta$ CD2、較佳BA71. $\Delta$ CD2之CD2缺失係靶向重組之結果，即無自發逆轉之風險，如(例如)移碼突變、如針對(例如)ASFV株OURT88/3之情形。

對有毒力ASFV株BA71之基因組進行完全測序(基因庫條目：KP055815；在隨同委託之序列表中亦稱作SEQID NO: 4)。自該測序，可推斷基因EP402R (CD2)及EP153R (C型凝集素)具有完全功能且不含任何缺失及/或移碼突變。

換言之，上文提及之ASFV株BA71 $\Delta$ CD2、較佳BA71. $\Delta$ CD2含有編碼C型凝集素蛋白之完全功能EP153R基因。此與ASFV株OURT88/3相反，其不含多個缺失及添加，例如編碼CD2樣蛋白(EP402R)及C型凝集素蛋白(EP153R) ORF中之中斷。

最後，此疫苗之最重要之優點來自本文提供之活體內證據。在用作疫苗時，其係安全、極具免疫原性的且能夠保護對抗同源及異源病毒攻擊。最後效力使得此疫苗獨特，此乃因天然減毒分離株僅限於同源保護。

### 【圖式簡單說明】

圖1： 上圖：重組質體含有抑制子+選擇盒，其由在病毒早期/

晚期啟動子pU104控制下之Lac I抑制基因及在晚期p72啟動子控制下之 $\beta$ -葡糖醛酸糖苷酶( $\beta$ -gus)基因組成。質體亦含有由左側之基因EP152R及EP153R及右側之基因EP364R及最後36 bp之基因EP402R構成的重組區。下圖：藉由BA71與重組質體同源重組獲得之所得ASFV重組株BA71. $\Delta$ CD2。EP402R係編碼CD2之ASFV基因。

圖2： 每一豬之資訊(包括鑑別編號、免疫組及用於攻擊之病毒)之概述。

圖3： ASFV攻擊之實驗設計之概述。

圖4： BA71 $\Delta$ CD2保護對抗同源及異源ASFV攻擊。

圖5： 存活豬對照病毒血症：LAV 8及29分別在BA71及E75攻擊後不顯示病毒血症(箭頭)，而LAV 11之血液中(加重橢圓)較死於感染之豬顯示較短且較低ASFV效價；GEC/ml：基因組當量拷貝/毫升血清(藉由RT PCR量測)

圖6： 存活豬對照發熱：LAV 8及LAV29分別在BA71及E75攻擊後不顯示任何發熱，而LAV11較死於ASFV感染之豬顯示較短且較低發熱峰值(加重橢圓)。

圖7： 存活豬顯示在同源(紅色實線)及異源(紅色虛線)攻擊時可檢測之抗ASFV抗體。

圖8： 保護與在攻擊時存在之ASFV特異性T細胞量之間之關聯。

圖9： BA71 $\Delta$ CD2誘導識別同源及異源ASFV株之多株CD8 T細胞。

圖10： BA71 $\Delta$ CD2保護對抗同源(BA71)及異源(E75)致死攻擊。

圖11： BA71 $\Delta$ CD2以劑量依賴性方式保護。

圖12： BA71 $\Delta$ CD2保護對抗ASFV株Georgia 2007。

圖 13： BA71 $\Delta$ CD2 保護對抗異源 ASFV 株 Georgia 2007 致死攻擊。存活豬對照病毒血症：豬編號(系列) 4、8 及 9 在 Georgia 2007 攻擊後不顯示病毒血症，而豬編號 1、2、3、6 及 7 之血液(加重橢圓)中較死於感染之對照豬(綠色線)顯示較短且較低之 ASFV 效價；GEC/ml：基因組當量拷貝/毫升血清(藉由 RT PCR 量測)

圖 14： 直腸溫度(經疫苗接種豬中之唯一臨床體徵)與病毒血症一致。存活豬對照發熱：豬編號(系列) 4、8、9 及 10 在 Georgia 2007 攻擊後不顯示任何發熱，而豬 1、2、3、6 及 7 較死於感染之對照豬(加重橢圓)顯示較短且較低之發熱峰值。

### 【實施方式】

在進一步詳細闡述本發明實施例之前，應注意，如本文中所使用且如在隨附申請專利範圍中，除非上下文另外明確指明，否則單數形式「一(a)」、「一(an)」及「該(the)」包括複數個指示物。

除非另有定義，否則本文中所使用之所有技術及科學術語皆具有熟習本發明所屬領域技術者一般理解之相同意義。除非另有說明或熟習此項技術者另外已知，否則所有給出之範圍及值均可變化 1% 至 5%，因此，通常在說明及申請專利範圍中省略術語「約」。儘管在本發明之實踐或測試中可使用任何與本文所述之任何方法及材料類似或等效之彼等，但現在闡述較佳方法、器件及材料。本文所提及之所有公開案以引用方式併入本文中以說明並揭示如可與本發明結合使用之公開案中所報導之物質、賦形劑、載劑及方法學。本文中之內容皆不應視為承認本發明沒有資格早於根據先前發明之該揭示內容。

除非另外指明，否則本發明之實踐將採用分子生物學、微生物學、重組 DNA 技術、蛋白質化學及免疫學之習用技術，其均在本領域之技術範圍內。該等技術充分闡釋於有關文獻中。

在本發明上下文中，術語「保護對抗非洲豬瘟」、「保護性免

疫」、「功能免疫」及類似片語意指藉由投與一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗生成之對抗非洲豬瘟(病毒)之反應，其相較暴露於非洲豬瘟(病毒)之未經免疫哺乳動物所預期具較少有害效應。即，在經疫苗接種哺乳動物中會減輕感染有害效應之嚴重程度。在經疫苗接種哺乳動物中可減少、減慢或可能完全預防感染。在本文中，意指完全預防感染之處將特別指出。若未指出為完全預防，則該術語包括部分預防。

相比之下，在本發明上下文中，與功能基因組ASFV CD2基因相關聯及/或由功能性非基因組ASFV CD2基因補充之術語「免疫保護」係指保護宿主中之ASFV免於宿主之細胞免疫反應或對抗該ASFV之細胞及體液免疫反應。

在本發明上下文中，術語「降低臨床體徵之發生率及/或嚴重程度」或「減少臨床症狀」意指(但不限於)與野生型感染相比，減少組中感染哺乳動物之數目、減少或消除展現感染之臨床體徵之哺乳動物之數目或降低一或多種哺乳動物中存在之任何臨床體徵之嚴重程度。舉例而言，其應指病原體載量、病原體脫落之任何減少、病原體傳播之減少或具有非洲豬瘟症狀之任何臨床體徵之減少。較佳地，與未接受一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗且被感染之個體相比，該等臨床體徵在接受一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗之一或多種哺乳動物中減少至少10%。更佳地，臨床體徵在接受一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗之哺乳動物中減少至少20%、較佳至少30%、更佳至少40%且甚至更佳至少50%。

在本發明上下文中，術語「增加之保護」意指(但不限於)哺乳動

物之經疫苗接種組對哺乳動物之未經疫苗接種對照組中一或多種與由野生型ASFV感染相關之臨床症狀在統計上明顯減輕。術語「臨床症狀之統計上明顯減輕」意指(但不限於)在用野生型ASFV攻擊後與未經疫苗接種對照組相比在經疫苗接種哺乳動物組中至少一種臨床症狀之發生頻率低至少10%、較佳20%、更佳30%、甚至更佳50%且甚至更佳70%。

在本發明上下文中，術語「長期保護」應指「改良之功效」持續至少3週，但更佳至少3個月，仍更佳至少6個月。在家畜之情形下，最佳地，該長期保護應持續到直至動物以肉出售之平均年齡。

在本發明上下文中，術語「免疫反應」(「*immune response*」或「*immunological response*」)意指(但不限於)對一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗發生細胞及/或抗體介導之免疫反應。通常，免疫反應(*immune*或*immunological response*)包括(但不限於)一或多種下述效應：製造或激活特異性地針對一或多種如本文中闡述及/或定義之ASFV或如本文中闡述及/或定義之組合物或免疫原性組合物或疫苗中所包括之一或多種抗原之抗體、B細胞、輔助性T細胞、抑制性T細胞及/或細胞毒性T細胞。較佳地，宿主會呈現治療性或保護性免疫(記憶)反應以增強對新感染之對抗及/或降低疾病之臨床嚴重程度。該保護可藉由以下來證實：與野生型ASFV感染相關之症狀數目減少、症狀嚴重程度降低或一或多種症狀消失、病毒血症發作延遲、病毒持續時間縮短、總病毒載量降低及/或病毒分泌減少。

在本發明上下文中，術語「醫藥上可接受或獸醫學上可接受之載劑」包括任何及全部溶劑、分散介質、包衣劑、佐劑、穩定劑、稀釋劑、防腐劑、抗細菌劑及抗真菌劑、等滲劑、吸收延遲劑及諸如此類。在一些較佳實施例中，且尤其彼等包括凍乾免疫原性組合物者，

用於本發明中之穩定劑包括凍乾或冷凍-乾燥用穩定劑。在一些實施例中，本發明之免疫原性組合物含有佐劑。如本文所用「佐劑」可包括氫氧化鋁及磷酸鋁；皂苷，例如Quil A, QS-21 (Cambridge Biotech公司，Cambridge MA)、GPI-0100 (Galenica Pharmaceuticals公司，Birmingham, AL)；油包水乳液；水包油乳液；水包油包水乳液。乳液可具體而言基於輕質液體石蠟油(歐洲藥典類型(European Pharmacopea type))；類異戊二烯油，例如角鯊烷或角鯊烯；由烯烴(具體而言異丁烯或癸烯)寡聚產生之油；含有直鏈烷基之酸或醇之酯，更具體而言植物油、油酸乙酯、丙二醇二-(辛酸酯/癸酸酯)、甘油三-(辛酸酯/癸酸酯)或丙二醇二油酸酯；具支鏈脂肪酸或醇之酯，具體而言異硬脂酸酯。油與乳化劑組合使用以形成乳液。乳化劑較佳係非離子型表面活性劑，具體而言為山梨醇酐、二縮甘露醇(例如無水甘露醇油酸酯)、二醇、聚甘油、丙二醇與油酸、異硬脂酸、蓖麻油酸或羥基硬脂酸之視情況經乙氧基化之酯，以及聚氧丙烯-聚氧乙烯共聚物嵌段，具體而言Pluronic產品，尤其L121。

佐劑之又一實例係選自丙烯酸或甲基丙烯酸之聚合物及馬來酸酐與烯基衍生物之共聚物之化合物。較佳之佐劑化合物係丙烯酸或甲基丙烯酸之聚合物，其尤其與糖類或多元醇之聚烯基醚交聯。此等化合物被稱為術語卡波姆(carbomer) (Pharmeuropa，第8卷，第2冊，1996年6月)。熟習此項技術者亦可參考美國專利第2,909,462號，其闡述與聚羥基化合物交聯之該等丙烯酸系聚合物，該聚羥基化合物具有至少3個(較佳不超過8個)羥基，至少3個羥基之氫原子由具有至少2個碳原子之不飽和脂肪族基團置換。較佳基團係彼等含有2個至4個碳原子者，例如乙烯基、烯丙基及其他烯系不飽和基團。不飽和基團自身可含有諸如甲基等其他取代基。以名稱Carbopol (BF Goodrich, Ohio, USA)出售之產品尤其適合。其可與烯丙基蔗糖或與烯丙基異戊



四醇交聯。其中可提及者係Carbopol 974P、934P及971P。最佳使用Cabopol 971P。共聚物EMA (Monsanto)屬馬來酸酐與烯基衍生物之共聚物，其為馬來酸酐與乙烯之共聚物。將該等聚合物溶解於水中會產生酸性溶液，較佳將其中和至生理pH以得到佐劑溶液，將免疫原性、免疫或疫苗組合物本身納入該佐劑溶液中。

其他適宜佐劑尤其包括(但不限於) RIBI佐劑系統(Ribi公司)、嵌段共聚物(CytRx, Atlanta GA)、SAF-M (Chiron, Emeryville CA)、單磷醯脂質A、阿夫立定(Avridine)脂質-胺佐劑、來自大腸桿菌(重組或其他性質)之不耐熱腸毒素、霍亂毒素、IMS 1314或胞壁醯二肽、或天然或重組之細胞因子或其相似物或者內源細胞因子釋放之刺激物。

預計佐劑可以每劑量約100 µg至約10 mg之量、較佳地以每劑量約100 µg至約10 mg之量、更佳地以每劑量約500 µg至約5 mg之量、甚至更佳地以每劑量約750 µg至約2.5 mg之量且最佳地以每劑量約1 mg之量添加。另一選擇為，以最終產物之體積計，佐劑之濃度可為約0.01%至50%，較佳濃度為約2%至30%，更佳濃度為約5%至25%，仍更佳濃度為約7%至22%，且最佳濃度為10%至20%。

在本發明上下文中，術語「稀釋劑」可包括水、鹽水、右旋糖、乙醇、甘油及諸如此類。等滲劑可尤其包括氯化鈉、右旋糖、甘露醇、山梨醇及乳糖。穩定劑尤其包括白蛋白及乙二胺四乙酸之鹼金屬鹽。

在本發明上下文中，術語「減弱」意指減輕病原體之毒力。在本發明中，減毒ASFV係毒力已降低從而不會引起非洲豬瘟感染之臨床體徵但能夠在目標哺乳動物中誘導免疫反應者，但亦可意指與感染未減毒(野生型) ASFV且未接受減毒ASFV之動物「對照組」相比在感染減毒ASFV之動物中臨床體徵之發生率或嚴重程度降低。在此上下文中，術語「降低(reduce/reduced)」意指與如上文所定義之對照組相

比降低至少10%、較佳25%、甚至更佳50%、仍更佳60%、甚至更佳70%、仍更佳80%、甚至更佳90%且最佳100%。因此，減毒ASFV株係適於納入包含一或多種如本文中闡述及/或定義之ASFV之免疫原性組合物中者。

在本發明上下文中，術語「有效劑量」意指(但不限於)抗原引發或能夠引發免疫反應而使投與抗原之動物臨床症狀減輕的量。

在本發明上下文中，術語「有效量」意指免疫原性組合物能夠在動物中誘導免疫反應而降低感染或疾病事件之發生率或減輕其嚴重程度的量。具體而言，有效量係指每劑量之溶菌斑形成單位(pfu)。另一選擇為，在療法之上下文中，術語「有效量」係指療法足以減少或改善非洲豬瘟或其一或多個症狀之嚴重程度或持續時間、預防該疾病進展、使該疾病消退、預防與該疾病相關之一或多個症狀復發、發展、發作或進展或增強或改良另一療法或治療劑之預防或治療的量。

在較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中無功能性基因組CD2基因(若具有功能或由功能性非基因組CD2基因補充)賦予宿主中之該ASFV之免疫保護，即保護該ASFV免於宿主對抗該ASFV之細胞免疫反應或細胞及體液免疫反應。

在本發明上下文中，術語「由功能性非基因組CD2基因補充」係指(但不限於)藉由引入表現構築體(例如編碼功能性CD2基因、較佳地功能性ASFV CD2基因之載體及/或質體)補充無功能性基因組ASFV CD2基因。

在較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中無功能性基因組CD2基因包含根據SEQ ID NO 1:AATATTTTCGCTTATTCATGTAGATAGAATTATTTAA之核酸序列或較佳地由其組成。

此序列對應於EP402R ORF (基因庫條目：L16864.1)之編碼ASFV

CD2之最後36個核苷酸。

在較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中該ASFV僅包含無功能性基因組CD2基因且不包含任何其他無功能性基因組基因。

在又一較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中該ASFV包含無功能性基因組CD2基因(較佳地EP402R)及功能性基因組C型凝集素基因(較佳地EP153R)。

在較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中該ASFV包含無功能性基因組CD2基因及另外一或多種其他無功能性基因組基因。

在本發明上下文中，結合如本文中闡述及/或定義之ASFV之術語「任何其他無功能性基因組基因」係指除位於ASFV(較佳地非天然重組ASFV)之基因組中之CD2外之一或多種經修飾基因，其中與給出之未經修飾之功能性ASFV基因相比，該等ASFV基因之該修飾根本不產生ASFV基因產物或產生無生物功能之ASFV基因產物。包括但不限於，該修飾可為(例如)完全或部分缺失基因組ASFV基因及/或修飾一或多個控制及/或編碼相應ASFV基因產物之核苷酸及/或藉由(例如)向ASFV開放閱讀框(ORF)中插入一或多個核苷酸及/或任何其他目前已知或可能之滅活或敲除該ASFV基因之方法破壞各別ASFV ORF。

在較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中該ASFV係有毒力及/或減毒之歐洲或非洲ASFV株。較佳地，該ASFV係選自由以下組成之群之有毒力ASFV株：BA71、E70、E75、E75L、Malawi Lil-20/1、OURT 88/1、OURT 88/3、Benin 97/1、Georgia 2007/1、Pretorisuskop/96/4,3、Warthog、Warmbaths、Mkuzi 1979、Tengani 62、Kenya 1950；更佳地BA71。

在較佳實施例中，提供如本文中闡述及/或定義之ASFV，其中該

ASFV係ASFV株BA71 $\Delta$ CD2，較佳地BA71. $\Delta$ CD2 [於2014年3月14日在鑑別參考文獻「BA71. $\Delta$ Fx」下以登錄號CNCM I-4843存放於Collection Nationale de Cultures de Microorganisms (CNCM) of the Institut Pasteur, Maria Luisa Salas, worker of Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC) in its Centro de Biología Molecular Severo Ochoa, 地址為Nicolás Cabrera, 1, 28049 Madrid (Spain)]。

在較佳實施例中，提供一或多種如本文中闡述及/或定義之ASFV或免疫原性組合物或疫苗，其中一或多種ASFV欲以 $10$ 至 $10^8$ 溶菌斑形成單位(pfu)、較佳 $10$ 、 $10^2$ 、 $10^3$ 、 $10^4$ 、 $10^5$ 、 $10^6$ 、 $10^7$ 或 $10^8$  pfu、更佳 $10^3$  pfu之劑量直接或作為組合物或免疫原性組合物或疫苗之一部分投與。較佳地，一或多種ASFV欲以單一劑量或以若干劑量直接或作為組合物或免疫原性組合物或疫苗之一部分投與。

在本發明上下文中「*pfu*」定義為「溶菌斑形成單位」，即溶胞病毒之量化之標準值，其在於量化由在感染在半固體介質中生長之細胞單層時病毒誘發之溶胞溶菌斑。在該等條件下，每一病毒溶菌斑皆係源自僅一種親本病毒粒子。

在較佳實施例中，提供一或多種如本文中闡述及/或定義之ASFV或組合物或免疫原性組合物或疫苗，其中一或多種ASFV欲在單次或多次投與另一免疫原性組合物或疫苗之前、同時或之後(較佳地在投與DNA疫苗、更佳ASFV-DNA疫苗之前、同時或之後)直接或作為組合物或免疫原性組合物或疫苗之一部分投與。較佳地，一或多種ASFV欲在單次或多次投與ASFV-DNA疫苗之後(較佳地在兩次投與ASFV-DNA疫苗之後)直接或作為組合物或免疫原性組合物或疫苗之一部分投與。

## 佐劑

為進一步增加如本文中闡述及/或定義之含有一或多種如本文中闡述及/或定義之ASFV之免疫原性組合物及/或疫苗的免疫原性，亦可包含一或多種佐劑。

佐劑可藉由前文所述或業內已知之任何技術純化。較佳純化技術係矽膠層析，具體而言「急驟」(快速)層析技術。然而，可使用其他層析方法(包括HPLC)來純化佐劑。結晶亦可用於純化佐劑。在一些情形下，因自合成直接獲得分析純產物而無需純化。

如本文中闡述及/或定義之免疫原性組合物及/或疫苗係藉由在適當無菌條件下根據用以產生輔佐組合物之已知技術物理混合佐劑與如本文中闡述及/或定義之ASFV製得。

預計佐劑可以每劑量約100  $\mu\text{g}$ 至約10 mg之量、較佳地以每劑量約100  $\mu\text{g}$ 至約10 mg之量、更佳地以每劑量約500  $\mu\text{g}$ 至約5 mg之量、甚至更佳地以每劑量約750  $\mu\text{g}$ 至約2.5 mg之量且最佳地以每劑量約1 mg之量添加。另一選擇為，以最終產物之體積計，佐劑之濃度可為約0.01%至75%，較佳濃度為約2%至30%，更佳濃度為約5%至25%，仍更佳濃度為約7%至22%，且最佳濃度為10%至20%。

### 生理上可接受之媒劑

如本文中闡述及/或定義之免疫原性組合物及/或疫苗可使用類似於用於其他醫藥組合物之技術之技術調配。因此，佐劑及一或多種如本文中闡述及/或定義之ASFV可以凍乾形式儲存且在投與之前於生理上可接受之媒劑中重構以形成懸浮液。或者，佐劑及一或多種如本文中闡述及/或定義之ASFV可儲存於媒劑中。較佳媒劑係無菌溶液，具體而言，無菌緩衝溶液，例如磷酸鹽緩衝鹽水。在媒劑中組合佐劑及一或多種如本文中闡述及/或定義之ASFV之任何方法使得免疫原性組合物之改良之免疫有效性適當。

如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗

之單一劑量之體積可變但通常將在常用於習用疫苗中之範圍內。單一劑量之體積以一或多種如本文中闡述及/或定義之ASFV及上述佐劑之濃度較佳地介於約0.1 ml與約3 ml之間，較佳地介於約0.2 ml與約1.5 ml之間，更佳地介於約0.2 ml與約0.5 ml之間。

如所闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗可藉由任何便捷方式投與。

## 調配物

本發明之調配物包含有效免疫量之如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗及生理上可接受之媒劑。疫苗包含有效免疫量之如本文中闡述及/或定義之免疫原性組合物及生理上可接受之媒劑。調配物應適合投與方式。

如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗(若期望)亦可含有微量潤濕劑或乳化劑或pH緩衝劑。如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗可為液體溶液、懸浮液、乳液、錠劑、丸劑、膠囊、持續釋放調配物或粉劑。口服調配物可包括標準載劑，例如醫藥級甘露醇、乳糖、澱粉、硬脂酸鎂、糖精鈉、纖維素、碳酸鎂等。

## 有效劑量

如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV可以治療有效劑量投與哺乳動物以治療非洲豬瘟。劑量將取決於接受疫苗之宿主以及諸如宿主之體型、體重及年齡等因素。

欲用於調配物中之如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV之精確量將取決於投與途徑及個體之性質(例如物種、大小、疾病階段/程度)，且應根據從業者之判斷及每一哺乳動物之情況根據標準臨床技術決定。有效免疫量係足以

治療及/或預防哺乳動物非洲豬瘟感染之該量。有效劑量亦可自源自動物模型測試系統之劑量-反應曲線外推，且可自0.001 mg/kg至100 mg/kg變化。

如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV之毒性及治療效能可藉由標準醫藥程序在細胞培養基或實驗動物中測定，例如用於測定LD<sub>50</sub> (對50%群體致死之劑量)及ED<sub>50</sub> (在50%群體中治療有效之劑量)。毒性效應與治療效應間之劑量比係治療指數且可表示為比率LD<sub>50</sub>/ED<sub>50</sub>。展現大的治療指數之如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV較佳。儘管可使用如本文中闡述及/或定義之展現毒性副作用之組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV，但應小心設計將如本文中闡述及/或定義之該等組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV靶向受侵襲組織之位點之遞送系統，以使對未感染細胞之潛在損害最小化，且藉此減少副作用。

自細胞培養分析及動物研究獲得之數據可用於調配用於哺乳動物之劑量之範圍。此等化合物之劑量較佳在具有較少毒性或沒有毒性之循環濃度(包括ED<sub>50</sub>)範圍內。端視所用劑型及所用投與途徑而定，劑量可在此範圍內變化。對於如本文中闡述及/或定義之用於本發明方法中之任何組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV，可最初自細胞培養分析估計治療有效劑量。可在動物模型中調配劑量以達成循環血漿濃度範圍(包括IC<sub>50</sub>，即達成症狀一半最大抑制之測試化合物之濃度)，如在細胞培養中所測定。此資訊可用於更精確地測定哺乳動物中 useful 之劑量。可藉由(例如)高效液相層析量測在血漿中之含量。

組合物之免疫原性可藉由利用業內已知之任一免疫分析監測測

試個體在用該組合物免疫後之免疫反應來測定。體液(抗體)反應及/或細胞介導之免疫性之產生可視為免疫反應之指示。測試個體之免疫反應可藉由各種方法分析，例如：所得免疫血清對免疫原性組合物之反應性，如藉由已知技術分析，例如酶聯免疫吸附分析(ELISA)、免疫印跡、免疫沈澱、ELISPOT、淋巴組織增殖分析等；或藉由保護經免疫宿主免於由病原體感染及/或由於經免疫宿主中由病原體感染減弱症狀，如藉由業內已知之任一方法所測定，用於分析感染物之含量、例如病毒ASFV含量(例如，藉由培養個體之試樣)，或業內已知之其他技術。感染物之含量亦可藉由量測免疫球蛋白所對抗之抗原之量來測定。傳染性致病因子之量降低或傳染性疾病之症狀改善指示組合物有效。

可於在活體內用於動物之前在活體外測試本發明治療劑之預期治療或預防活性。舉例而言，可用於確定指示是否投與具體治療劑之活體外分析包括活體外細胞培養分析，其中將來自細胞系或自患有特定疾病或病症之個體培養之細胞之適當細胞暴露於或經其他方式投與治療劑，並觀察該治療劑對細胞之效應。

另一選擇為，可藉由以下方式來分析治療劑：使治療劑接觸易於由傳染性致病因子感染但未感染該傳染性致病因子之細胞(自個體培養或來自經培養細胞系)，將該等細胞暴露於傳染性致病因子，且隨後確定與治療劑接觸之細胞之感染率是否低於未與治療劑接觸之細胞之感染率。可藉由業內已知之任一方法來分析傳染性致病因子對細胞之感染。

在使用本發明之方法及組合物對哺乳動物疫苗接種ASFV後，可使用業內已知之任一結合分析來評定所得抗體與特定分子間之結合。亦可實施該等分析來選擇對特定抗原呈現更高親和性或特異性之抗體。



## 投與

較佳投與途徑包括(但不限於)鼻內、經口、皮內及肌內。最佳呈單一劑量之於飲用水中投與係合意的。熟習此項技術者應認識到，如本文中闡述及/或定義之組合物及/或免疫原性組合物及/或疫苗及/或一或多種ASFV亦可以一個、兩個或更多個劑量以及藉由其他投與途徑投與。舉例而言，該等其他途徑包括皮下、皮內、靜脈內、血管內、動脈內、腹膜腔內、鞘內、氣管內、皮內、心臟內、肺葉內(intralobally)、髓內、肺內及陰道內。端視治療所期望之持續時間及有效性而定，本發明組合物可投與一次或數次，亦可間歇投與，例如以每日計投與數天、數週或數月且以不同劑量投與。

## 實例

以下實例用於進一步闡釋本發明；但同樣不應視為限制本文所揭示之本發明之範疇。

### 實例1

對於藉由同源重組之重組之構築，使用圖1之上圖中所示之重組質體。此質體含有抑制子+選擇盒，其由在ASFV早期/晚期啟動子pU104控制下之Lac I抑制基因及在晚期p72啟動子控制下之標記 $\beta$ -葡糖醛酸糖苷酶基因組成。質體亦含有由左側之基因EP152R及EP153R基因及右側之EP364R基因及編碼ASFV CD2之基因EP402R末端處之36個鹼基對區組成的重組區。

BA71. $\Delta$ CD2 (圖1，下圖)係藉由COS-7細胞中重組質體與BA71之同源重組獲得。藉由在COS-7細胞中連續形成溶菌斑純化重組病毒，選擇經X-Gluc (即 $\beta$ -葡糖醛酸糖苷酶之受質)染色之藍色溶菌斑，直至僅檢測到藍色溶菌斑為止。藉由在COS-7細胞中生長使ASFV擴增。

為產生大量病毒重組BA71. $\Delta$ CD2用於活體內接種研究，以0.1個溶菌斑形成單位(pfu)/細胞之感染倍數用BA71. $\Delta$ CD2感染COS-7細胞

之預鋪滿單層(25個P150板)。在觀察到嚴重細胞病變效應後，收集含有細胞外病毒之培養基並以低速離心以去除細胞碎屑且隨後以高速離心以使病毒沉降。在藉由形成溶胞溶菌斑滴定後，將沉降物重新懸浮於PBS中並用於保護實驗。在較早實驗中，在重新懸浮於PBS中後，藉由在PBS中之25%蔗糖墊上離心純化病毒。將所得沉降重新懸浮於PBS中並如上述滴定。病毒濃度表示為溶菌斑形成單位(pfu)/ml。

## 實例2

BA71.ΔCD2在獲得後不管是否經純化皆用於疫苗目的幫助以下實驗設計：

- 在BSL3設備中將二十四(24)頭Landrace x Pietrain商業豬(四週齡，雄性)寄宿於兩個盒子中(每個盒子12頭豬)：盒子A及盒子B。
- 將每一盒子分成2個免疫組(每一組中6頭豬)：
- 對照組(C)：經PBS肌內免疫
- 減毒活病毒(LAV)：經 $10^3$  pfu ASFV CD2 缺失突變體 BA71ΔCD2肌內免疫
- 用致死劑量之 $10^3$ 血球吸附單位(HAU<sub>50</sub>)之有毒力BA71 ASFV株肌內攻擊盒子A中之所有豬(20 LD<sub>50</sub>同源攻擊)
- 用致死劑量之 $10^4$  HAU<sub>50</sub>之有毒力E75 ASFV株肌內攻擊盒子B中之所有豬(20 LD<sub>50</sub>異源攻擊)

圖2概述每一豬之資訊，包括鑑別編號、免疫組及用於攻擊之病毒。

不存在ASFV CD2基因使得不可根據血球吸附單位(HAU<sub>50</sub>)滴定BA71.ΔCD2，而其對COS細胞之適應容許使用標準化溶菌斑分析量化病毒原液(以溶菌斑形成單位(pfu)表示)。相反，E75僅在初代豬巨噬細胞中生長，不形成溶菌斑；因此根據血球吸附單位(HAU<sub>50</sub>)滴定E75 [1 pfu等於1 HAU<sub>50</sub>且等於1個基因組當量拷貝(GEC)]。

*ASFV*致死攻擊：

在BA71 $\Delta$ CD2接種後28天，用致死劑量之ASFV\*肌內攻擊豬：

- 用 $10^3$  HAU<sub>50</sub>之有毒力BA71 (即BA71 $\Delta$ CD2之親代株)攻擊盒子A中之豬(20 LD<sub>50</sub>同源病毒)
- 用 $10^4$  HAU<sub>50</sub>之有毒力E75攻擊盒子B中之豬(20 LD<sub>50</sub>之異源病毒)。

\*請注意，為使用相同致死劑量(20 LD<sub>50</sub>)進行ASFV攻擊，需要不同病毒劑量：對於BA71為 $10^3$  HAU<sub>50</sub>且對於E75為 $10^4$  HAU<sub>50</sub> (多10倍病毒)。在使用該等病毒及攻擊劑量時，病毒及死亡動力學類似。亦記住，該等天然病毒分離株必須在初代巨噬細胞中生成，因此藉由血球吸附及/或RT-PCR量化。

在BA71 $\Delta$ CD2接種及致死攻擊後及在BA71 $\Delta$ CD2疫苗接種後之不同時刻[疫苗接種後(pv) 2、8、14、21及28天]及在致死攻擊後[攻擊後(pc)4、7、14及23天]收集血清及/或總血樣。每日記錄直腸溫度。

實驗設計示意性概述於圖3中。

## 分析測定：

病毒檢測：藉由使用對ASFV絲胺酸蛋白激酶基因具有特異性之特製即時PCR量化病毒血症。簡言之，使用NucleoSpin血液套組(Macherey-Nagel)自血清獲得病毒核酸並使用正向 5'-CCTTTCCACCTTTGCTGTAGGA 及 反向 5'-GTCCAGGCCGGAACAACA 引子量化 SybrGreen qPCR (Applied Bioscience)，以使來自高度保守之ASFV絲胺酸蛋白激酶基因(R298L)之85 bp擴增。一式三份地分析所有試樣，包括陰性及陽性對照。使用含有全長R298L ORF ( $10^2$  -  $10^{10}$  GEC/ml)之純化p-R298實施標準曲線。該技術之檢測限值(100%置信)係1,000個基因組當量拷貝/毫升血清(GEC/ml)。一個GEC等於一個血球凝集素單位(HAU)，係檢測複雜

試樣中之傳染性活病毒的經典方式。對RT-PCR呈陰性之所有試樣亦在血球凝集素分析中呈陰性(數據未顯示)。

- 抗體檢測：使所有血清經受OIE批准之ELISA用於ASF診斷。以1:100稀釋測試所有血清試樣。

- T細胞檢測：使在疫苗接種及/或感染後不同天時自豬分離之PBMC經受：

- IFN- $\gamma$  ELISPOT分析以檢測免疫後及感染後不同天時血液中特异性T細胞之存在。使用 $10^5$  HAU<sub>50</sub>/百萬PBMC之有毒力BA71株或相同量之E75株將PBMC用活的ASFV活體外刺激過夜(O/N) (與所用病毒無關，獲得類似結果)。

- 對於一些試樣，再次使用BA71或E75作為5天特定刺激實施羧基二乙酸螢光黃琥珀醯亞胺基酯(CFSE)增殖分析以評估血液中交叉反應性CD8<sup>+</sup> T細胞之存在。

藉由使用此實驗方法，獲得以下結果：

**BA71 $\Delta$ CD2保護豬免於同源及異源致死攻擊**：如所預計，在用致死劑量之 $10^3$  HAU<sub>50</sub>之有毒力BA71株攻擊後(pc)第7日之前，所有對照豬(C)皆死亡(圖4，黑色實線)。屆時(pc第7天)，接受 $10^3$  HAU<sub>50</sub> BA71 $\Delta$ CD2之6頭豬中之3頭仍活著且更重要的是，其中之2者存活(圖4，紅色實線)。

與無藉由用典型減毒E75CV1株免疫觀察之交叉保護(數據未顯示)相反，BA71 $\Delta$ CD2能夠保護對抗E75異源致死攻擊。在此情景下，經 $10^3$  pfu BA71 $\Delta$ CD2疫苗接種之6頭豬中之1頭倖免於E75致死攻擊(不連續紅線)。如針對E75攻擊所預計，所有對照豬(C)在pc 7天與8天之間皆死亡。

**BA71 $\Delta$ CD2在豬中安全且在同源或異源致死攻擊後可減輕或防止病毒血症**：肌內接種 $10^3$  pfu BA71 $\Delta$ CD2之豬既不顯示任何可檢測病毒

血症(圖5)，亦不顯示任何其他與ASF相容之臨床體徵(包括發熱)(圖6)。對照豬(黑色線)在BA71或E75攻擊後很快有病，此顯示截至攻擊後第4天病毒血症之程度極高且在死亡時達到其最大值(pc第7天)。相比之下，經BA71 $\Delta$ CD2疫苗接種之大多數豬較對照豬顯示較低程度之病毒血症，截至感染後第4天差異變得更明顯(圖5，紅線)。足夠令人感興趣地，三頭存活豬中之兩頭(LAV8及LAV29)在感染後之任何時間皆不顯示可檢測之病毒血症，而另一存活者(LAV11)顯示較對照豬更低且更短之病毒血症峰值(圖5，黑色虛線橢圓)。首次使用減毒活疫苗，針對豬LAV29觀察到對抗異源攻擊之消除性免疫。

病毒血癥結果(認為低於 $10^3$  GEC/ml為0值，所施用檢測方法之限值)與直腸溫度記錄匹配。因此，儘管死於ASF之豬在攻擊後很早即患有高熱發作直至死亡之時間，但存活之豬(若存在)顯示與減輕或不可檢測之病毒血症(圖5，紅線)一致之較短且較輕微之發熱峰值(圖6，豬LAV11中之橢圓)。LAV8及LAV 29不顯示任何發熱或對ASF典型之其他臨床體徵。此與病毒不可藉由即時PCR或藉由病毒分離自鼻拭子檢測之事實一起證實其滅菌保護(假定認為檢測方法之限值係 $10^3$  GEC/ml)。

**BA71 $\Delta$ CD2誘導ASFV特異性抗體：**在已證實BA71 $\Delta$ CD2賦予對抗同源及異源致死攻擊之完全保護的能力後，欲回答之關鍵問題係為何並非所有動物皆相同地受保護。旨在回答此問題，藉由使用特異性ELISA監測貫穿實驗在每一經免疫動物中誘導之特異性抗體反應([www.oie.int](http://www.oie.int))。

如所預計，對照豬直至pc第7天屍檢時不顯示任何特異性反應，此時其顯示對抗ASFV之低但可檢測之抗體(圖7)，與用於攻擊之病毒無關：BA71(黑色實線)或E75(黑色虛線)。相比之下，大比例之BA71 $\Delta$ CD2免疫之豬(75%或12頭豬中之9頭)早至疫苗接種後(pv) 8天

發生可檢測之特異性抗體反應並在攻擊時(d 0pc或28 dpv)達成其最大效價。該等結果證實即使在不存在可檢測之病毒血症下，BA71 $\Delta$ CD2亦能夠誘導ASFV特異性抗體(圖5)。因此，在攻擊時特異性抗體之存在係成功免疫之良好指示。然而，在ASFV攻擊時(d0pc)存在之抗體之量與保護之間似乎不存在明確關聯。作為闡釋性實例，儘管在攻擊時在LAV7之血液中顯示與LAV8 (完全保護免於同源攻擊之豬)類似之抗體量，但LAV7不對抗同源BA71-攻擊。相反，儘管在攻擊時循環中存在相對較低之抗體量，但LAV11倖免於同源攻擊，在攻擊之同一天(d0pc)在後一情形中首次可檢測(圖7)。

與上述結果無關，由BA71 $\Delta$ CD2完全保護之豬經由感染既不顯示病毒血症亦不顯示任何ASF特異性臨床體徵，在ASFV-攻擊時顯示高特異性抗體量且在ASFV感染後立刻加強(LAV8及LAV29)。

存活豬在ASFV致死攻擊時具有大量循環ASFV特異性T細胞：旨在於保護與特異性T細胞反應之誘導之間確立關聯，在同源或異源病毒攻擊之前及之後使用IFN- $\gamma$  ELISPOT分析追蹤經免疫豬之血液中之ASFV特異性T細胞之存在(圖8)。如所預計，對照豬在能夠顯示任何可檢測T細胞反應之前死亡。與之顯著不同，大部分經BA71 $\Delta$ CD2免疫之豬自pv第14天起發生可檢測之特異性T細胞反應。兩頭顯示>600個特異性T細胞/百萬PBMC之豬完全受保護(LAV8及LAV29)，此明確地證實此組免疫反應在針對ASF之保護中之相關性。足夠有趣的是，並非所有在攻擊時顯示適當高之特異性反應之豬皆受保護。因此，儘管顯示200個特異性T細胞/百萬PBMC之LAV11存活，但許多其他儘管在其血液中顯示類似特異性T細胞但亦不存活。

由BA71 $\Delta$ CD2誘導之T細胞反應係多株且針對其他ASFV株交叉反應：前述結果(數據未顯示)證實用典型減毒ASFV株(例如E75CV1)感染誘導針對僅極少顯性表位之極窄T細胞譜。事實上，賦予活體內

保護對抗同源而非異源ASFV分離株之能力與其CD8<sup>+</sup> T細胞因應同源病毒限制排他地識別並在活體外增殖相關。旨在使該等活體外參數與由BA71ΔCD2提供之保護相關，實施以下實驗。將在ASFV攻擊之前自每一動物分離之PBMC用CFSE標記並在活體外用BA71或E75刺激五天。令人感興趣地，由BA71ΔCD2誘導之CD8<sup>+</sup> T細胞能夠因應同源BA71及異源E75病毒在活體外增殖(圖9)，由此與由對抗該兩種病毒之疫苗提供之活體內保護相關。相比之下，由典型減毒E75CV1誘導之CD8<sup>+</sup> T細胞僅識別同源病毒(數據來自前述實驗；圖9)。

### 實例3

為證實BA71ΔCD2誘導之保護之劑量依賴性效應，將豬用 $3.3 \times 10^4$  pfu或 $10^6$  pfu BA71ΔCD2免疫(在實例2中，豬接受 $10^3$  pfu ASFV CD2缺失突變體BA71ΔCD2)。

如所預計，經 $10^4$  HAU<sub>50</sub> (GEC) E75攻擊之所有對照豬(5頭中之5頭)或經 $10^3$  HAU<sub>50</sub> (GEC) BA71攻擊之所有對照豬(5頭中之5頭)皆在攻擊後(pi)第9天之前死亡。與之顯著不同，100%經BA71ΔCD2疫苗接種之豬(24頭中之24頭)倖免於致死攻擊，與所用疫苗劑量或所實施攻擊無關(同源BA71或異源E75；圖10)。此首次證實該兩種ASFV株之總交叉保護。保護與特異性抗體及T細胞反應之誘導相關，從而與所提供之保護相關。

用 $3.3 \times 10^4$  pfu或 $10^6$  pfu BA71ΔCD2免疫對於動物係安全的，其中12頭豬中僅一頭直至疫苗接種後(pi)第7天顯示低病毒血症及稍微發熱(圖11，上圖；豬編號1465，環繞)。接受BA71ΔCD2之剩餘豬(24頭中之23頭)不顯示任何顯著病毒血症或與ASF相容之臨床體徵，此慮及產物之安全性。另外，保持於相同室中之對照(原始)動物皆不受感染，由此顯示可忽略病毒分泌及傳播(若存在)。

相反，用 $10^6$  pfu BA71ΔCD2疫苗接種賦予對抗BA71致死攻擊之

整體保護，且在用同源BA71攻擊或異源E75攻擊(分別圖11之上圖及下圖中之紅線)感染後之任何時間動物皆不顯示ASFV臨床體徵或病毒血症。由較低疫苗劑量(豬接受BA71 $\Delta$ CD2之1/30)提供之保護儘管亦極為堅實，但並非完全消除，此乃因12頭經免疫豬(2頭/攻擊組)中之4頭顯示對應於極低病毒血症峰值(pick) (病毒較對照豬低4-5 log)且接近所施用RT-PCR技術之檢測限值之稍微發熱(圖11中之圈)。

#### 實例4

儘管E75及BA71係異源，但其屬相同基因型且二者與高加索(Caucasus)中之目前循環ASFV株在種系發生上相差極遠。因此，設計該等實驗以證實對抗Georgia 2007 ASFV株之保護性能力。設計兩個活體內實驗。第一實驗旨在活體內滴定Georgia 2007 ASFV株且第二實驗經設計以測試BA71 $\Delta$ CD2對抗Georgia 2007致死攻擊之保護性潛能。

在圖12中，正如對於BA71及E75所發生，顯示BA71 $\Delta$ CD2賦予對抗Georgia 2007致死攻擊之100%保護(劑量為 $10^3$  GEC)。因此，儘管5頭對照豬中之5頭在攻擊後第11天之前死亡，但經 $10^6$  pfu BA71 $\Delta$ CD2疫苗接種之所有豬倖免於相同致死劑量。此組實驗之主要結論係如下：

(i) 即使於較低測試劑量 $2.23 \times 10^3$  GEC (基因組當量拷貝，如藉由RT-PCR驗證)下，可用之Georgia 2007病毒原液在不到10天內殺死所有豬，且顯示ASFV之典型急性體徵(包括高燒及高病毒血症效價[截止感染後(pi)第7天至多 $10^9$  GEC])

(ii) 100%經 $10^6$  pfu BA71 $\Delta$ CD2免疫之豬(9頭中之9頭)倖免於致死Georgia 2007攻擊(經 $10^3$  GEC肌內免疫)，同時如所預計，所有對照豬(PBS接種)在攻擊後第11天死亡。

(iii) 所提供之保護與針對同源攻擊獲得之保護相當，此乃因經



免疫豬中之5頭之血液在測試之任何時間無病毒，而其他四頭顯示病毒血症之極有限峰值；較對照豬低4至5個10倍對數(圖13)。

(iv) 直腸溫度記錄確認僅利用4只動物獲得之結果顯示對應於病毒血症之溫度之峰值，而剩餘動物保持正常(圖14)。令人感興趣地，所有動物皆不顯示任何其他與ASF相容之臨床體徵，而對照豬發生在pc第11天之前死亡之疾病之急性臨床體徵。發熱係唯一記錄之體徵。

該等結果明確地證實2007年歐洲重新引入減毒活ASFV株BA71 $\Delta$ CD2針對ASFV之保護性潛能且證明交叉保護係可能的。

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在序列表中：

SEQ ID NO: 1 對應於編碼ASFV CD2之EP402R ORF之最後36個核苷酸(基因庫條目：L16864.1)；

SEQ ID NO: 2 對應於正向ASFV絲胺酸蛋白激酶基因(R298L) PCR引子；

SEQ ID NO: 3 對應於反向ASFV絲胺酸蛋白激酶基因(R298L) PCR引子；

SEQ ID NO: 4 對應於非洲豬瘟病毒，有毒力株BA71，完全基因組序列。

#### 【符號說明】

無

## 【序列表】

- <110> 德商百靈佳殷格翰維美迪加股份有限公司  
西班牙高等科學研究委員會
- <120> CD2 缺失之非洲豬瘟病毒做為對抗哺乳動物非洲豬瘟之滅  
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## 發明摘要

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## 【發明名稱】

CD2缺失之非洲豬瘟病毒做為對抗哺乳動物非洲豬瘟之減毒活疫苗或後續滅活之疫苗

CD2 DEFICIENT AFRICAN SWINE FEVER VIRUS AS LIVE

ATTENUATED OR SUBSEQUENTLY INACTIVATED VACCINE

AGAINST AFRICAN SWINE FEVER IN MAMMALS

## 【中文】

本發明係關於較佳活的經減毒或後續滅活之非洲豬瘟病毒 (African swine fever virus ; ASFV)，其包含無功能性基因組CD2基因，其中該ASFV在其複製中無缺失，亦係關於相應組合物或免疫原性組合物或疫苗、產生方法及用於治療及/或預防哺乳動物、較佳豬科(Suidae)、例如豬，更佳地馴養豬(家豬(*Sus scrofa domesticus*))、野豬(歐洲野豬(*Sus scrofa scrofa*))、疣豬(非洲野豬(*Potamochoerus porcus*))、薮豬(假面野豬(*Potamochoerus larvatus*))、大林豬(巨林豬(*Hylochoerus meinertzhageni*))以及野生豬之非洲豬瘟的用途。

## 【英文】

The present invention is directed to a preferably live attenuated or subsequently inactivated African swine fever virus (ASFV), comprising a non-functional genomic CD2 gene, wherein such ASFV is not deficient in its replication, as well as to corresponding compositions or immunogenic compositions or vaccines, methods of production and uses for treating and/or preventing African swine fever in mammals, preferably of the family Suidae, for instance pigs, more preferably domestic pigs (*Sus scrofa domesticus*), wild pigs (*Sus scrofa scrofa*), warthogs (*Potamochoerus porcus*), bushpigs (*Potamochoerus larvatus*), giant forest hogs (*Hylochoerus meinertzhageni*) as well as feral pigs.

**【代表圖】**

**【本案指定代表圖】**：第（1）圖。

**【本代表圖之符號簡單說明】**：

無

**【本案若有化學式時，請揭示最能顯示發明特徵的化學式】**：

無

## 申請專利範圍

1. 一種非洲豬瘟病毒(African swine fever virus ; ASFV)，較佳地非天然重組ASFV，其包含無功能性基因組CD2基因，前提係該ASFV在其複製中無缺失，其中較佳地，該ASFV係減毒活ASFV或經由後續物理及/或化學滅活從該減毒活ASFV產生之後續滅活之ASFV。
2. 如請求項1之ASFV，其中該無功能性基因組CD2基因係EP402R及/或包含SEQ ID NO 1之核酸序列或較佳地由該核酸序列組成。
3. 如請求項1或2之ASFV，其中該ASFV僅包含無功能性基因組CD2基因，且不包含任何其他無功能性基因組基因。
4. 如請求項1或2之ASFV，其中該ASFV包含無功能性基因組CD2基因、較佳地EP402R，及功能性基因組C型凝集素基因、較佳地EP153R。
5. 如請求項1或2之ASFV，其中該ASFV係有毒力歐洲ASFV株及/或有毒力非洲ASFV株。
6. 如請求項5之ASFV，其中該ASFV係選自由以下組成之群之有毒力ASFV株：BA71、E70、E75、E75L、Malawi Lil-20/1、OURT 88/1、OURT 88/3、Benin 97/1、Georgia 2007/1、Pretorisuskop/96/4,3、Warthog、Warmbaths、Mkuzi 1979、Tengani 62、Kenya 1950；更佳地BA71。
7. 如請求項1或2之ASFV，其中該ASFV係ASFV株BA71 $\Delta$ CD2，較佳地BA71. $\Delta$ CD2 (鑑別參考文獻，「BA71. $\Delta$ Fx」，登錄號CNCMI-4843)。
8. 一種生成ASFV基因組中之無功能性ASFV CD2基因之方法，其包含以下步驟：

(a) 向該 ASFV CD2 基因中引入一或多個完全或部分缺失，及/或修飾一或多個控制及/或編碼相應 ASFV CD2 基因產物之核苷酸，及/或破壞 ASFV CD2 開放閱讀框 (open reading frame ; ORF)，藉此使得該 ASFV CD2 無功能性，該破壞較佳係藉由將 Lac I 抑制子與  $\beta$ -葡糖醛酸糖苷酶標記基因一起引入 ASFV CD2 基因座中來進行，從而導致 ASFV CD2 基因幾乎完全缺失，藉此使得其在活體外及活體內無功能性。

9. 一種產生如請求項 1 至 7 中任一項之包含無功能性基因組 CD2 基因之非天然重組 ASFV 之方法，前提係該 ASFV 在其複製中無缺失，該方法包含以下步驟：

(a) 根據請求項 8 製備包含無功能性基因組 CD2 基因之非天然重組 ASFV；

(b) 用步驟 (a) 之該 ASFV 活體外感染不會使該病毒滅活之初代豬巨噬細胞及/或易於受 ASFV 感染之不會使該病毒滅活之細胞系、較佳地 COS-7 細胞；

(c) 自步驟 (b) 之該等細胞分離該 ASFV 及/或對其進行純化，此較佳地係藉由收集含有細胞外 ASFV 之培養基，首先以低速將培養基離心以去除細胞碎屑且隨後以高速離心以使該病毒沈積並將病毒重新懸浮於 PBS 中，其中在最後將該病毒重新懸浮於 PBS 中之前，視情況藉由在 PBS 中之 25% 蔗糖墊上離心純化此重新懸浮之病毒；

(d) 視情況較佳地藉由形成溶胞溶菌斑滴定步驟 (c) 之經分離及/或純化之 ASFV [ASFV 濃度以溶菌斑形成單位 (pfu)/mL 表示]；

(e) 對於自步驟 (c) 或 (d) 獲得之該滅毒活 ASFV，視情況較佳地藉由用 UV 輻射、X 射線輻射、 $\gamma$  輻射、冷凍-解凍及/或加熱處理之物理滅活及/或較佳地藉由用一或多種化學滅活劑處理之化學

滅活，藉此產生一或多種後續滅活之ASFV，其中更佳地，該一或多種化學滅活劑係選自由以下組成之群： $\beta$ -丙內酯、戊二醛、次乙亞胺、 $\beta$ -次乙亞胺、雙次乙亞胺、乙醯基次乙亞胺、臭氧及/或甲醛。

10. 一種可藉由如請求項9之方法獲得之非天然重組ASFV。
11. 如請求項1、2及10中任一項之ASFV，其用於治療及/或預防哺乳動物、較佳地豬科、例如豬、更佳地馴養豬(家豬(*Sus scrofa domestica*))、野豬(歐洲野豬(*Sus scrofa scrofa*))、疣豬(非洲野豬(*Potamochoerus porcus*))、藪豬(假面野豬(*Potamochoerus larvatus*))、大林豬(巨林豬(*Hylochoerus meinertzhageni*))以及野生豬之非洲豬瘟。
12. 如請求項11之ASFV，其中該ASFV欲以 $10$ 至 $10^8$ 溶菌斑形成單位(pfu)、較佳地 $10$ 、 $10^2$ 、 $10^3$ 、 $10^4$ 、 $10^5$ 、 $10^6$ 、 $10^7$ 或 $10^8$  pfu、更佳地 $10^3$  pfu之劑量直接或作為組合物或免疫原性組合物或疫苗之一部分投與，其中較佳地，該ASFV欲以單一劑量或以若干劑量直接或作為組合物或免疫原性組合物或疫苗之一部分投與。
13. 如請求項11之ASFV，其中該ASFV欲在單次或多次投與另一組合物或免疫原性組合物或疫苗之前、同時或之後、較佳地在投與DNA疫苗、更佳ASFV-DNA疫苗之前、同時或之後直接或作為組合物或免疫原性組合物或疫苗之一部分投與，其中較佳地，該ASFV欲在該單次或多次投與ASFV-DNA疫苗之後、較佳地在兩次投與ASFV-DNA疫苗之後直接或作為組合物或免疫原性組合物或疫苗之一部分投與。
14. 一種免疫原性組合物，其包含治療有效量之一或多種如請求項1至7及10中任一項之ASFV，視情況另外包含一或多種醫藥上可接受之賦形劑及/或一或多種醫藥上可接受之載劑，其中較佳地，

該一或多種醫藥上可接受之賦形劑及/或一或多種醫藥上可接受之載劑係選自由以下組成之群：溶劑、分散介質、佐劑、穩定劑、稀釋劑、防腐劑、抗細菌劑及抗真菌劑、等滲劑、吸附延遲劑。

15. 如請求項14之免疫原性組合物，其用於治療及/或預防哺乳動物、較佳地豬科、例如豬、更佳地馴養豬(家豬)、野豬(歐洲野豬)、疣豬(非洲野豬)、藪豬(假面野豬)、大林豬(巨林豬)以及野生豬之非洲豬瘟。
16. 如請求項14之免疫原性組合物，其中該ASFV欲以 $10$ 至 $10^8$ 溶菌斑形成單位(pfu)、較佳地 $10$ 、 $10^2$ 、 $10^3$ 、 $10^4$ 、 $10^5$ 、 $10^6$ 、 $10^7$ 或 $10^8$  pfu、更佳地 $10^3$  pfu之劑量直接或作為組合物或免疫原性組合物或疫苗之一部分投與，其中較佳地，該ASFV欲以單一劑量或以若干劑量直接或作為組合物或免疫原性組合物或疫苗之一部分投與。
17. 如請求項14至16中任一項之免疫原性組合物，其中該ASFV欲在單次或多次投與另一組合物或免疫原性組合物或疫苗之前、同時或之後、較佳地在投與DNA疫苗、更佳ASFV-DNA疫苗之前、同時或之後直接或作為組合物或免疫原性組合物或疫苗之一部分投與，其中較佳地，該ASFV欲在單次或多次投與ASFV-DNA疫苗之後、較佳地在兩次投與ASFV-DNA疫苗之後直接或作為組合物或免疫原性組合物或疫苗之一部分投與。
18. 一種如請求項1至7或10中任一項之ASFV或如請求項11之免疫原性組合物的用途，其用於製造用於引發哺乳動物、較佳地豬科、例如豬、更佳地馴養豬(家豬)、野豬(歐洲野豬)、疣豬(非洲野豬)、藪豬(假面野豬)、大林豬(巨林豬)以及野生豬之保護性免疫反應的藥劑。































