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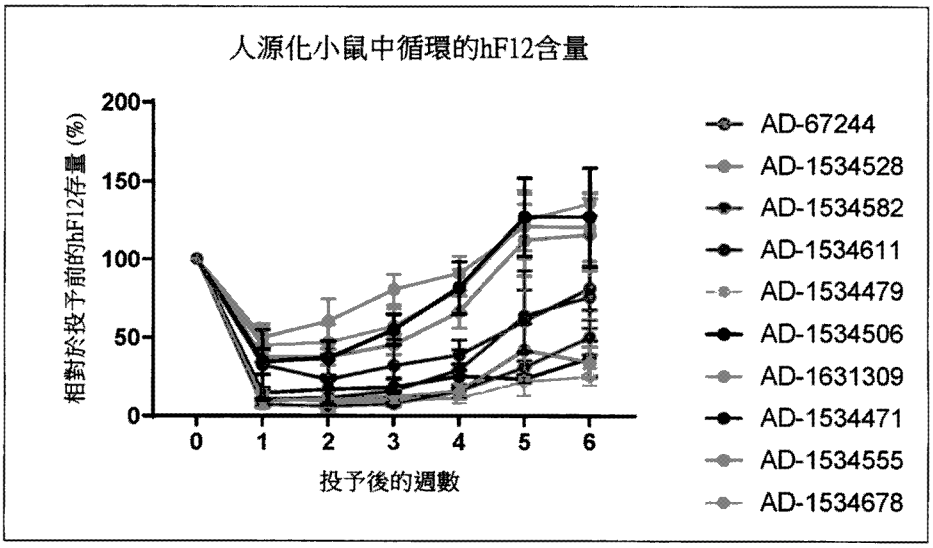
第十二因子（Ｆ１２）ｉＲＮＡ組成物及其使用方法

(57)摘要

本發明關於靶向第十二因子(F12)基因之 iRNA 劑，例如雙股 RNA 劑。本發明也關於使用該 RNAi 劑來抑制 F12 基因的表現之方法，以及預防和治療 F12 相關疾病之方法，例如遺傳性血管水腫(HAE)、前激肽釋放酶缺乏症、惡性原發性高血壓、高血壓、末期腎臟疾病或夫列契因子缺乏症。

The present invention relates to RNAi agents, e.g., double stranded RNA (dsRNA) agents, targeting the Factor XII (F12) gene. The invention also relates to methods of using such RNAi agents to inhibit expression of an F12 gene and to methods of preventing and treating an F12-associated disorder, e.g., heredity angioedema (HAE), prekallikrein deficiency, malignant essential hypertension, hypertension, end stage renal disease, or Fletcher Factor Deficiency.

指定代表圖：


人源化小鼠中循環的hF12含量
相對於投予前的hF12存量 (%)
200
150
100
50
0
0
1
2
3
4
5
6
投予後的週數
AD-67244
AD-1534528
AD-1534582
AD-1534611
AD-1534479
AD-1534506
AD-1631309
AD-1534471
AD-1534555
AD-1534678

【圖1】

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【發明摘要】

【中文發明名稱】 第十二因子(F12) iRNA 組成物及其使用方法

【英文發明名稱】 FACTOR XII (F12) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

【中文】

本發明關於靶向第十二因子(F12)基因之iRNA劑，例如雙股RNA劑。本發明也關於使用該RNAi劑來抑制F12基因的表現之方法，以及預防和治療F12相關疾病之方法，例如遺傳性血管水腫(HAE)、前激肽釋放酶缺乏症、惡性原發性高血壓、高血壓、末期腎臟疾病或夫列契因子缺乏症。

【英文】

The present invention relates to RNAi agents, *e.g.*, double stranded RNA (dsRNA) agents, targeting the Factor XII (F12) gene. The invention also relates to methods of using such RNAi agents to inhibit expression of an F12 gene and to methods of preventing and treating an F12-associated disorder, *e.g.*, heredity angioedema (HAE), prekallikrein deficiency, malignant essential hypertension, hypertension, end stage renal disease, or Fletcher Factor Deficiency.

【指定代表圖】 圖1

【代表圖之符號簡單說明】無

【特徵化學式】 無

【發明說明書】

【中文發明名稱】 第十二因子(F12) iRNA 組成物及其使用方法

【英文發明名稱】 FACTOR XII (F12) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

相關申請

【0001】 本申請案請求於 2021 年 8 月 13 日提交的美國臨時申請案第 63/232,840 號的優先權權益。上述申請的全部內容藉由引用併入本文。

【技術領域】

【0002】 本發明關於第十二因子(F12)iRNA 組成物及其使用方法。本發明尤其關於靶向第十二因子(F12)基因之 iRNA 劑。本發明進一步關於使用該 RNAi 劑抑制 F12 基因表現及預防和治療 F12 相關疾病之方法。

【先前技術】

【0003】 遺傳性血管水腫(hereditary angioedema；簡稱 HAE)是一種罕見的體染色體顯性疾病，其會導致 1/3 的病人的四肢、臉部、咽喉、上呼吸道、腹部、軀幹、生殖器反覆的水腫和腫脹、以及非搔癢性皮疹。未受治療的 HAE 病人平均每個月會經歷一至兩次的血管性水腫，但是發作頻率和嚴重性有顯著不同。水腫腫脹常導致外觀的損害和失能，經常需要住院照護，且病人可能還需要精神方面的照護以治療與疾病相關的焦慮症。腹痛發作可導致嚴重疼痛、噁心、嘔吐，且會導致手術不順利。此外，超過一半的 HAE 病人終其一生也會經歷致命的喉

水腫，需要緊急的氣管造口術來防止窒息。在美國，預估 HAE 影響 6000 至 10000 名屬於不同族群的個體，對病人造成嚴重的經濟衝擊，其佔每年 15000 至 30000 次的就醫次數及 20 至 100 日的病假日數。

【0004】 HAE 由導致 C1INH 蛋白缺乏的 C1 抑制劑(C1INH、SERPING1)之基因突變引起。已顯示有超過 250 種不同 C1INH 突變會導致 HAE 的臨床表現。這些 C1INH 突變通常來自基因遺傳，然而高達 25%的 HAE 案例來自 C1INH 的從頭突變(*de novo* mutation)。第一型 HAE 來自 C1INH 突變，導致較低的截短或錯誤折疊的蛋白之低效率分泌，此佔 HAE 案例約 85%。第二型 HAE 約佔所有病例的 15%，由 C1INH 活性位點附近的突變引起，導致正常量的功能異常 C1INH 蛋白。HAE 第三型則是罕見的第三種型式的疾病，由第十二凝血因子(F12)(哈格曼因子)的功能獲得型突變所導致。

【0005】 C1 抑制劑是絲胺酸家族的絲胺酸蛋白酶抑制劑，且是補體和接觸啟動路徑的蛋白酶主要抑制劑，也是血纖維蛋白分解酶溶解作用的次要抑制劑。這些血漿蛋白酶級聯反應在 HAE 發作的時候被活化，產生增加血管通透性的物質。研究顯示，活化前發炎訊息傳導路徑的舒緩肽(bradykinin)，會擴張血管和誘發嗜中性球的趨化反應，是在 HAE 發作時，藉由與血管內皮細胞上的舒緩肽受體連結從而增加血管通透性的主要物質。血漿內的舒緩肽形成通常需要三種蛋白的交互作用，即 F12、前激肽釋放酶(prekallikrein)及高分子量激肽原，統稱為「接觸活化路徑」或「激肽釋放酶-激肽系統(Kallikrein-Kinin System)」。前激肽釋放酶和高分子量激肽原以雙分子複合物循環著。接觸活化路徑藉由 F12 與帶負電荷的表面(或大分子)結合時啟動，其誘發 F12 的構形改變從而產生活化的 F12

(active F12；簡稱 F12a)。F12a 裂解前激肽釋放酶以產生活性激肽釋放酶，後者反過來活化 F12。活性激肽釋放酶隨後消化高分子量激肽原以釋放舒緩肽。

【0006】 通常，C1INH 抑制 F12 的自動活化、F12a 活化前激肽釋放酶的能力、激肽釋放酶對高分子量激肽原的活化，以及激肽釋放酶對 F12 的回饋活化。因此，導致 C1INH 缺乏或 F12 功能獲得型之突變造成舒緩肽過量產出和 HAE 血管水腫的發作。

【0007】 目前，HAE 可以 17α-烷基化的雄性激素治療，來預防性降低反覆發作的機率，或使用疾病專一性療法來治療急性發作。大約 70%的 HAE 個體是使用雄性激素治療或未接受治療，約 30%接受治療。然而，雄性激素不適合作為急性發作的短期治療，因為需要數天才能產生藥效，且會造成嚴重的副作用並影響生長和發育。因此，雄性激素被用來作為長期性的預防，且通常不會給予懷孕的婦女或孩童。此外，現今用來治療急性發作的療法必須每週靜脈注射許多次，否則可能會產生副作用，其需要給予藥物及後續住院接受監測，從而限制了雄型激素在一般性預防的使用以長期控制疾病。

【0008】 因此，目前沒有安全的、有效的以及更方便的途徑和方案來治療急性血管水腫發作及預防性控制大部分患者(包含孕婦和孩童)反覆發作的方案的情況下，對於患有 HAE 的個體需要替代性的療法。

【發明內容】

【0009】 本發明案提供 iRNA 組成物，其會影響編碼第十二因子(F12)基因的 RNA 轉錄本之 RNA 誘導沉默複合物(RNA-induced silencing complex；簡稱 RISC)介導的裂解。F12 基因可位在細胞內，例如個體體內的細胞，如人類個體。

本發明也提供使用 iRNA 組成物於抑制 F12 基因表現及/或治療個體的方法，該個體能受惠於 F12 基因表現的抑制和降低，舉例而言，該個體為罹患或傾向罹患 F12a 相關疾病諸如遺傳性血管水腫(如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或其他因舒緩肽量升高所致的遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症之個體。

【0010】 因此，在一個面向中，本發明提供雙股 RNA 核糖核酸(double stranded ribonucleic acid；簡稱 dsRNA)劑來抑制細胞內第十二因子(F12)的基因表現，其中，該 dsRNA 劑包括形成雙股區域的有義股和反義股，其中，該有義股包括至少 15 個連續核苷酸，如 15、16、17、18、19、20 或 21 個，其與核苷酸序列 SEQ ID NO:1 相差不超過 0、1、2 或 3 個核苷酸；該反義股包括至少 15 個連續核苷酸，如 15、16、17、18、19、20、21、22 或 23 個，其與核苷酸序列 SEQ ID NO:2 中相對應的部分相差不超過 0、1、2 或 3 個核苷酸。

【0011】 在另一個面向中，本發明提供雙股 RNA 核糖核酸(double stranded ribonucleic acid；簡稱 dsRNA)劑來抑制細胞內第十二因子(F12)的基因表現，其中，該 dsRNA 包括形成雙股區域的有義股和反義股，其中，該有義股包括一段與編碼 F12 的 mRNA 的互補區域，其中，該互補區域包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與表 2 至表 3 之任一者中的任一反義股核苷酸序列相差不超過 0、1、2 或 3 個核苷酸。

【0012】 在一個態樣中，該 dsRNA 劑包括有義股，其包括與表 2 至 3 之任一者中任一有義股的核苷酸序列具有至少 85%的核苷酸同一性之連續核苷酸序列，例如 85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%

或 100%的同一性；該 dsRNA 包括反義股，其包括與表 2 至表 3 之任一者中任一反義股的核苷酸序列具有至少 85%的核苷酸同一性之連續核苷酸序列，例如 85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或 100%的同一性。

【0013】 在一個態樣中，該 dsRNA 劑包括有義股，其包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與表 2 至表 3 之任一者中的任一有義股核苷酸序列相差不超過 3 個核苷酸。該 dsRNA 劑包括反義股，其包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與表 2 至表 3 之任一者中的任一反義股核苷酸序列相差不超過 3 個核苷酸。

【0014】 在另一個態樣中，該 dsRNA 劑包括有義股，其包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與表 2 至表 3 任一者中任一有義股核苷酸序列相差不超過 2 個核苷酸。該 dsRNA 劑包括反義股，其包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與表 2 至表 3 任一者中任一反義股核苷酸序列相差不超過 2 個核苷酸。

【0015】 在另一個態樣中，該 dsRNA 劑包括有義股，其包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與表 2 至表 3 任一者中任一有義股核苷酸序列相差不超過 1 個核苷酸。該 dsRNA 劑包括反義股，其包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與表 2 至表 3 任一者中任一反義股核苷酸序列相差不超過 1 個核苷酸。

【0016】 在另一個態樣中，該 dsRNA 劑包括有義股，其包括選自由表 2 至表 3 任一者中的任一有義股核苷酸序列組成之組的核苷酸序列，以及反義股，其

包括選自由表 2 至表 3 任一者中的任一反義股核苷酸序列組成之組的核苷酸序列。

【0017】 在一個面向中，本發明提供雙股 RNA 核糖核酸(double stranded ribonucleic acid；簡稱 dsRNA)劑來抑制細胞內第十二因子(F12)的基因表現，其中，該 dsRNA 包括形成雙股區域的有義股和反義股，其中，該有義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與核苷酸序列 SEQ ID NO:1 中核苷酸序列 75 至 97、76 至 98、79 至 101、115 至 137、117 至 139、123 至 145、132 至 154、135 至 157、137 至 159、144 至 166、201 至 223 及 1462 至 1484 相差不超過 0、1、2 或 3 個核苷酸，以及其中，該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與 SEQ ID NO:2 中相對應的核苷酸序列相差不超過 0、1、2 或 3 個核苷酸。

【0018】 在一個態樣中，該有義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與核苷酸序列 SEQ ID NO:1 中核苷酸序列 135 至 157、144 至 166、201-223 相差不超過 0、1、2 或 3 個核苷酸，以及該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與 SEQ ID NO:2 中相對應的核苷酸序列相差不超過 0、1、2 或 3 個核苷酸。

【0019】 在一個態樣中，該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與選自由 AD-1534528、AD-1534479、AD-1534582、AD-1534611、AD-1534506、AD-1631309、AD-1534471、AD-1534555、AD-1534678、AD-1534585、AD-1534502 及 AD-153449 組成之組中任一雙鏈體的反義股核苷酸序列相差不超過 0、1、2 或 3 個核苷酸。

【0020】在一個態樣中，該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的反義股核苷酸序列相差不超過 0、1、2 或 3 個核苷酸。

【0021】在一個態樣中，該有義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中的任一雙鏈體的有義股核苷酸序列相差不超過 3 個核苷酸。該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中的任一雙鏈體的反義股核苷酸序列相差不超過 3 個核苷酸。

【0022】在一個態樣中，該有義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的有義股核苷酸序列相差不超過 2 個核苷酸。該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的反義股核苷酸序列相差不超過 2 個核苷酸。

【0023】在一個態樣中，該有義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20 或 21 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的有義股核苷酸序列相差不超過 1 個核苷酸。該反義股包括至少 15 個連續核苷酸，例如 15、16、17、18、19、20、21、22 或 23 個，其與選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的反義股核苷酸序列相差不超過 1 個核苷酸。

【0024】在一個態樣中，該有義股包括選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的有義股核苷酸序列。該反義股包括選自由 AD-1534528、AD-1534479 及 AD-1534582 組成之組中任一雙鏈體的反義股核苷酸序列。

【0025】在一個態樣中，該 dsRNA 劑包括至少一個經修飾的核苷酸。

【0026】在一個態樣中，實質上所有有義股的核苷酸是經修飾之核苷酸；實質上所有反義股的核苷酸是經修飾之核苷酸；或實質上所有有義股的核苷酸和實質上所有反義股的核苷酸是經修飾之核苷酸。

【0027】在一個態樣中，所有有義股的核苷酸是經修飾之核苷酸；所有反義股的核苷酸是經修飾之核苷酸；或所有有義股的核苷酸和所有反義股的核苷酸是經修飾之核苷酸。

【0028】在一個態樣中，至少有一個經修飾之核苷酸是選自由去氧核苷酸、3'-末端去氧胸腺嘧啶核苷酸 (dT)、2'-O-甲基修飾之核苷酸、2'-氟修飾之核苷酸、2'-去氧-修飾之核苷酸、2'-5'-連接的核糖核苷酸(3'-RNA))鎖核苷酸、未鎖核苷酸、構形限定核苷酸、約束性乙基核苷酸、無鹼基核苷酸、2'-胺基修飾之核苷酸、2'-O-烯丙基修飾之核苷酸、2'-C 烷基修飾之核苷酸、2'-羥基修飾之核苷酸、2'-甲氧基乙基修飾之核苷酸、2'-O-烷基修飾之核苷酸、N-嗎啉基核苷酸、磷醯胺酯、含非天然鹼基之核苷酸、四氫吡喃修飾之核苷酸、1,5-脫水己糖醇修飾之核苷酸、環己烯基修飾之核苷酸、包含硫代磷酸酯基之核苷酸、包含甲基磷酸酯基之核苷酸、乙烯基磷酸酯核苷酸、包含 5'-磷酸酯之核苷酸、包含 5'-磷酸酯模擬物之核苷酸、熱去穩定核苷酸、二醇修飾之核苷酸(GNA)、二醇核酸 S-

異構物(S-GNA)、包含 2'-磷酸酯之核苷酸、2-O-(N-甲基乙醯胺)修飾之核苷酸及其組合所組成之組。

【0029】 在一個態樣中，至少有一個經修飾之核苷酸是選自由 LNA、HNA、CeNA、2'-甲氧基乙基、2'-O-烷基、2'-O-烯丙基、2'-C-烯丙基、2'-氟、2'-去氧、2'-羥基、乙二醇及其組合所組成之組。

【0030】 在一個態樣中，至少有一個經修飾之核苷酸是選自由去氧核苷酸、2'-O-甲基修飾之核苷酸、2'-氟修飾之核苷酸、2'-去氧修飾之核苷酸、乙二醇修飾之核苷酸(GNA)，例如 Ggn、Cgn、Tgn、Agn、包含 2'-磷酸酯之核苷酸例如 G2p、C2p、A2p、U2p、包含硫代磷酸酯基之核苷酸，及其組合所組成之組。

【0031】 在一個態樣中，至少有一個經修飾之核苷酸是具熱去穩定核苷酸修飾之核苷酸。

【0032】 在一個態樣中，熱去穩定核苷酸修飾是選自由無鹼基修飾、雙鏈體中互補的核苷酸之錯配、去穩糖修飾、2'-去氧修飾、無環核苷酸、未鎖核酸(UNA)、甘油核酸(GNA)及 2'-5'-連接的核糖核苷酸(3'-RNA)組成之組。

【0033】 在一些實施例中，經修飾之核苷酸包括 3'-末端去氧胸腺嘧啶核苷酸的一段短序列。

【0034】 在一些實施例中，dsRNA 劑進一步包括至少一個硫代磷酸酯核苷酸間鍵聯。在一些態樣中，dsRNA 劑包括 6 至 8 個硫代磷酸酯核苷酸間鍵聯。在一個態樣中，硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯位在其中一股的 3'-末端。視情況地，該股是反義股。在另一個態樣中，該股是有義股。在一個相關的態樣中，硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯是在其中一股的 5'-末端。視情況地，該股是反義股。在另一個態樣中，該股是有義股。在另一個態樣中，硫代磷酸酯

或甲基磷酸酯核苷酸間鍵聯是同時在其中一股的 3'-和 5'-末端。視情況地，該股是反義股。在另一個態樣中，該股是有義股。

【0035】 雙股區域之長度可為 19 至 30 個核苷酸對、19 至 25 個核苷酸對、19 至 23 個核苷酸對、23 至 27 個核苷酸對，或 21 至 23 個核苷酸對。

【0036】 在一個態樣中，各股長度獨立地不超過 30 個核苷酸。

【0037】 在一個態樣中，有義股長度是 21 個核苷酸，反義股長度是 23 個核苷酸。

【0038】 互補區域的長度至少是 17 個核苷酸、長度介於 19 和 23 個核苷酸，或長度為 19 個核苷酸。

【0039】 在一個態樣中，至少一股包括至少 1 個核苷酸 3'端突出。在另一個態樣中，至少一股包括至少 2 個核苷酸 3'端突出。

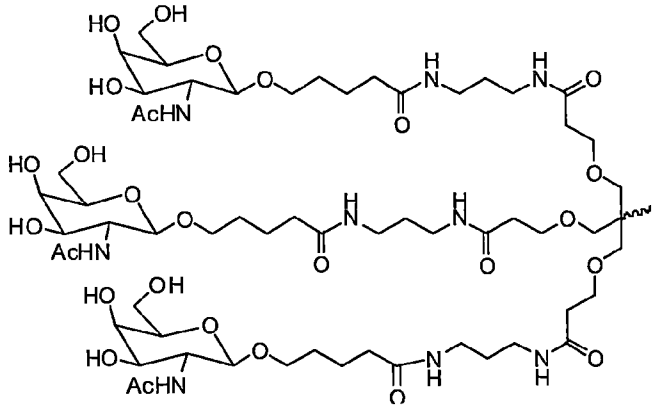
【0040】 在一個態樣中，dsRNA 劑進一步包括配體。

【0041】 在一個態樣中，配體與 dsRNA 劑的有義股 3'末端接合。

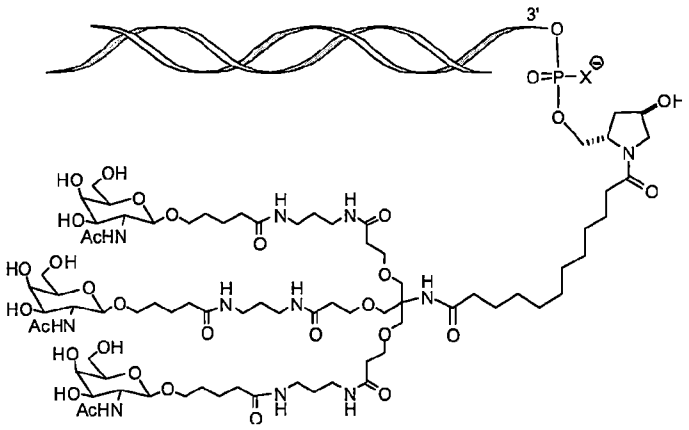
【0042】 在一個態樣中，配體是 N-乙醯半乳胺糖(GalNAc)衍生物。

【0043】 在一個態樣中，配體是藉由單價、二價、三價分支的連接子所附接一個或多個 GalNAc 衍生物。

【0044】 在一個態樣中，配體是



【0045】 在一個態樣中，dsRNA 劑如下所示與配體接合：



且其中，X 是 O 或 S。

【0046】 在一個態樣中，X 是 O。

【0047】 在一個態樣中，dsRNA 劑進一步包括至少一個硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯。

【0048】 在一個態樣中，硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯位在其中一股的 3’-末端，例如反義股或有義股。

【0049】 在另一個態樣中，硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯位在其中一股的 5’-末端，例如反義股或有義股。

【0050】 在一個態樣中，硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯同時位在其中一股的 5’-和 3’-末端。在一個態樣中，該股是反義股。

【0051】 在一個態樣中，鹼基對在雙鏈體的反義股 5’末端的位置 1 是 AU 鹼基對。

【0052】 本發明也提供包含本發明中任一 dsRNA 劑的細胞，和包含本發明中任一 dsRNA 劑的醫藥組成物。

【0053】 本發明的醫藥組成物可包含無緩衝溶液中的 dsRNA 劑，例如生理食鹽水或水，或本發明之醫藥組成物可包含緩衝溶液中的 dsRNA 劑，例如醋酸鹽、檸檬酸鹽、醇溶蛋白、碳酸鹽、磷酸酯或其任意組合，或磷酸鹽緩衝生理鹽水(PBS)。

【0054】 在一個面向中，本發明提供抑制細胞內第十二因子(F12)基因表現的方法。該方法包含將本發明之任一 dsRNA 或本發明之任一醫藥組成物與細胞接觸，從而抑制細胞內 F12 基因表現。

【0055】 在一個態樣中，細胞位在個體體內，例如人類個體，例如罹患第十二因子(F12)相關疾病之個體，諸如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因舒緩肽量升高所致的遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓例如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症。

【0056】 在特定的態樣中，F12 基因表現被抑制至少 30%、40%、50%、60%、70%、80%、90%或 95%。在一個態樣中，抑制 F12 基因表現降低個體血漿中 F12 蛋白質量至少 30%、40%、50%、60%、70%、80%、90%或 95%。

【0057】 在一個面向中，本發明提供一種方法，其能治療罹患能受惠於第十二因子(F12)基因表現量降低的疾病之個體。該方法包括投予個體治療上有效量之本發明之任一 dsRNA 或本發明之任一醫藥組成物，從而治療罹患能受惠於 F12 基因表現量下降之疾病的個體。

【0058】 在另一個面向中，本發明提供在罹患能受惠於 F12 基因表現量下降之疾病個體中，預防至少一種症狀出現之方法。該方法包括投予該個體預防有

效量之本發明任一 dsRNA 或本發明任一醫藥組成物，從而在罹患能受惠於 F12 基因表現量下降之疾病的個體中，預防至少一種症狀出現。

【0059】 在特定的態樣中，該疾病為第十二因子(F12)相關疾病。

【0060】 在一些態樣中，該第十二因子(F12)相關的疾病選自由遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因舒緩肽量升高所致的遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓例如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症組成之組。

【0061】 在一些態樣中，該第十二因子(F12)相關的疾病為 HAE。

【0062】 在其他態樣中，第十二因子(F12)相關的疾病為前激肽釋放酶缺乏症。

【0063】 在其他態樣中，第十二因子(F12)相關的疾病為高血壓如惡性原發性高血壓。

【0064】 在其他態樣中，在其他態樣中，第十二因子(F12)相關的疾病為末期腎臟疾病。

【0065】 在其他態樣中，第十二因子(F12)相關的疾病為夫列契因子缺乏症。

【0066】 在特定的態樣中，給予個體 dsRNA 劑能降低個體體內 F12 蛋白累積。

【0067】 在特定的態樣中，給予個體 dsRNA 劑可降低個體體內：一或多種升高的舒緩肽、前激肽釋放酶缺乏、惡性原發性高血壓、高血壓、末期腎臟疾病、夫列契因子缺乏症；四肢、臉部、咽喉、上呼吸道、腹部、軀幹、生殖器水腫和腫脹或前驅症狀；喉水腫、非搔癢性皮疹、噁心、嘔吐或腹部疼痛。

【0068】在進一步的面向中，本發明也提供抑制個體體內第十二因子(F12)基因表現的方法。該方法包含投予個體治療上有效劑量之本文提供的任一dsRNA，從而抑制細胞內 F12 基因表現。

【0069】在一個態樣中，個體為人類。

【0070】在一個態樣中，給予個體 dsRNA 劑可降低個體體內 F12 蛋白累積。

【0071】在一個態樣中，dsRNA 劑以 0.01mg/kg 至 50mg/kg 之劑量投予至個體。

【0072】在一個實施態樣中，dsRNA 劑利用皮下注射至個體體內

【0073】在一個態樣中，本發明的方法進一步包含判定個體體內樣本的 F12 含量。

【0074】在一個態樣中，個體體內樣本的 F12 量是 F12 蛋白在血液或血清或肝組織樣本中的量。

【0075】在一些態樣中，本發明的方法進一步包含判定個體體內舒緩肽的量、前激肽釋放酶的量或血壓。

【0076】在特定的態樣中，本發明的方法進一步包括給予個體附加治療劑。

【0077】在特定的態樣中，附加治療劑選自由雄性激素，如達那唑(danazol)、氧雄龍(oxandrolone)、Berinert®、Cinryze™、Rhuconest®、伊卡蘭肽(Ecallantide)、Firazyr®、Kalbitor®或上述任意之組合所組成之組。

【0078】本發明也提供套組，其包括本發明之任一 dsRNA 或本發明之任一醫藥組成物，以及視需要的使用說明。在一個態樣中，本發明提供套組來執行

抑制細胞內 F12 基因表現之方法，其藉由將有效量的本發明雙股 RNAi 劑與細胞接觸，以抑制細胞內的 F12 基因表現。該套組包含 RNAi 劑和使用說明，視需要用於投予個體 RNAi 劑。

【0079】 本發明也提供包括本發明的 dsRNA 劑或是本發明的醫藥組成物的小瓶。本發明進一步提供包括本發明 dsRNA 劑或是本發明醫藥組成物的注射器。

【0080】 本發明進一步提供包括本發明中 dsRNA 劑之任一反義股的 RNA 誘導沉默複合物(RISC)。

【0081】 在另一個態樣中，RNAi 劑是其藥學上可接受的鹽。本文中各 RNAi 劑之「藥學上可接受的鹽」包括但不限於，鈉鹽、鈣鹽、鋰鹽、鉀鹽、銨鹽、鎂鹽及其混合物。所屬領域中具通常知識者可以理解 RNAi 劑，當其以聚陽離子鹽提供時，其具有選擇性修飾之磷酸二酯主鏈及/或任一酸性修飾(例如 5'-末端磷酸酯基)的每個游離酸基具有一個陽離子。例如，長度為 n 個核苷酸的寡核苷酸含有 n-1 個選擇性修飾之磷酸二酯，使長度為 21 nt 的寡核苷酸可以具有高達 20 個陽離子(例如 20 個鈉陽離子)之鹽提供。相似地，具有有義股長度為 21 nt 和反義股長度為 23 nt 的 RNAi 劑可以具有高達 42 個陽離子(例如 42 個鈉陽離子)之鹽提供。在先前的示例中，RNAi 劑還包含 5'-末端磷酸酯或 5'-末端乙烯磷酸酯基的情況下，RNAi 劑可以具有高達 44 個陽離子(例如 44 個鈉陽離子)之鹽提供。

【圖式簡單說明】

【0082】 圖 1 描述每週注射單一 0.2g/kg 劑量的所示雙鏈體後，人源化第十二因子小鼠血清中殘餘的人類第十二因子蛋白百分比。殘餘的人類第十二因子蛋白百分比係相對於給藥前個體中人類第十二因子的量。

【實施方式】

【0083】 本發明提供 iRNA 組成物，其會影響第十二因子(F12)基因的 RNA 轉錄本之 RNA 誘導沉默複合物(RISC)介導的裂解。基因可位在細胞內，例如個體(如人類)體內的細胞。使用這些 iRNA 可靶向降解哺乳動物中相應基因(F12)的 mRNA。

【0084】 本發明的 iRNA 設計成靶向人類第十二因子(F12)基因，包括在其他哺乳動物物種的 F12 直系同源物中保守的基因部分。不欲受縛理論，據信，上述特性和特定靶位點的組合或子組合或此類 iRNA 中的特定修飾賦予本發明的 iRNA 改進的功效、穩定性、效力、耐久性和安全性。

【0085】 據此，本發明提供治療和預防第十二因子(F12)相關的疾病之方法，例如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量所致的遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症。該方法使用影響 F12 基因的 RNA 轉錄本之 RNA 誘導沉默複合物(RISC)介導的裂解之 iRNA 組成物。

【0086】 本發明之 iRNA 包含具有長度高達或少於 30 個核苷酸的 RNA 股，例如長度為 19 至 30、19 至 29、19 至 28、19 至 27、19 至 26、19 至 25、19 至 24、19 至 23、19 至 22、19 至 21、19 至 20、20 至 30、20 至 29、20 至 28、20

至 27、20 至 26、20 至 25、20 至 24、20 至 23、20 至 22、20 至 21、21 至 30、21 至 29、21 至 28、21 至 27、21 至 26、21 至 25、21 至 24、21 至 23、或 21 至 22 個核苷酸，其與 F12 基因的 mRNA 轉錄本至少一部份實質上互補。

【0087】 在特定態樣中，本發明之雙股 RNAi 劑其中一股或兩股長度為 66 個核苷酸，例如 36 至 66、26 至 36、25 至 36、31 至 60、22 至 43 或 27 至 53 個核苷酸，其至少 19 個連續核苷酸與 F12 基因的 mRNA 轉錄本至少一部份實質上互補。在一些態樣中，具有較長反義股的該 iRNA 劑可以例如包含長度為 20 至 60 核苷酸之第二個 RNA 股(有義股)，其中，有義股和反義股形成 18 至 30 個連續核苷酸之雙鏈體。

【0088】 使用本發明的 iRNA 能夠靶向降解哺乳動物中相應基因(F12 基因)的 mRNA。使用體外測定，本發明人已經證明靶向 F12 基因的 iRNA 能有效地介導 RNAi，導致 F12 基因表達的顯著抑制。因此，包括此類 iRNA 的方法和組成物可用於治療罹患 F12 相關疾病的個體，例如罹患遺傳性血管水腫(如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量所致的遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症的個體。

【0089】 據此，本發明提供治療個體的方法和合併療法，該個體罹患能受惠於 F12 基因表現量抑制或降低的疾病，例如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任一因升高的舒緩肽量導致的遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症，該方法使用影響 F12 基因的 RNA 轉錄本之 RNA 誘導沉默複合物(RISC)介導的裂解之 iRNA 組成物。

【0090】 本發明亦提供預防個體中的至少一種症狀，該個體罹患能受惠於F12基因表現量抑制或降低的疾病，例如第十二因子(F12)相關疾病，如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量導致的遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症。

【0091】 以下態樣揭示如何製造和使用包含 iRNA 之組成物，以抑制 F12 基因表現，以及治療能受惠於 F12 基因表現之抑制或降低的個體之治療組成物、治療用途和治療方法，例如疑似罹患或已確定罹患 F12 相關疾病之個體。

【0092】 I. 定義

為使本案發明顯而易懂，首先定義特定的術語，須留意的是，不論如何描述數值或數值的範圍，介於所述數值間之數值或數值範圍也屬於本發明的一部份。

【0093】 本文使用的冠詞「一」和「一個」係指一個或多於一個(亦即至少一個)冠詞之語法受詞。作為示例，「一元件」意指一個元件或多於一個元件，例如多個元件。

【0094】 本文使用的術語「包括」指「包括但不限於」，兩者可互相交換使用。

【0095】 本文使用的術語「或」指「及/或」，除非內文明確表示並非如此，否則兩者可互相交換使用。例如「有義股或反義股」可被理解為「有義股或反義股或有義股及反義股」。

【0096】 本文使用的「約」意指介在所屬領域所能容受的數值中的特定範圍。例如，「約」可以被理解為與平均值差距大約 2 個標準差。在特定的態樣中，

大約是平均值±10%。在特定的態樣中，約是平均值±5%。當「約」位在一系列數值或範圍前面時，可以理解「約」可以修飾該系列或範圍內的各個數值。

【0097】 位於數值或一系列數值前之術語「至少」、「不少於」、「或更多」可以被理解為包含數值鄰近於術語「至少」，還有邏輯上可被包含的所有後續數值或整數。例如，核酸分子中的核苷酸數量必須是整數。例如，舉例而言，「21個核苷酸核酸分子之至少 19 個核苷酸」意指 19、20 或 21 個核苷酸具有所述的特性。當「至少」位在一系列數值或範圍前面時，可以理解「至少」可修飾該系列或範圍內的各個數值。

【0098】 如本文所使用，「不超過」或「或少於」可視為數值鄰近於該術語和合乎邏輯的較小的數值或整數，視內文的邏輯而定，最低至 0。例如，一個「不超過 2 個核苷酸」突出之雙鏈體，有 2、1 或 0 個核苷酸突出。當「不超過」位在一系列數值或範圍前面時，「不超過」視為可修飾該系列或範圍內的各個數值。如本文所使用，範圍同時包含上限和下限。

【0099】 如本文所使用，檢測方法可包括判定分析物存在的量低於該方法所測得的值。

【0100】 若指示之標的位點與有義股和反義股的核苷酸序列出現矛盾，以指示之序列為優先。

【0101】 如果序列與其在轉錄本或其他序列上的指定位點之間出現矛盾，，則本文中列舉的核苷酸序列為優先。

【0102】 如本文所使用，「第十二因子(哈格曼因子)」，與術語「第十二凝血因子」、「FXII」、「F12」、「活化的 F12」、「F12a」可互換使用，係指編碼 F12a 酶原形式的天然存在之基因。F12 是絲胺酸蛋白酶(或絲胺酸內胜酶)家族中的酵

素(EC 3.4.21.38)，其會分解前激肽釋放酶以形成激肽釋放素，其接著從高分子量激肽原中釋放舒緩肽並活化 F12。

【0103】 人類 F12 mRNA 轉錄本之序列可以在例如 GenBank Accession No. GI: 1813757520(NM_000505.4; SEQ ID NO:1; 反向互補體，SEQ ID NO: 2)找到。小鼠 F12 mRNA 之序列可以在例如 GenBank Accession No. GI: 805299477 (NM_021489.3; SEQ ID NO:3; 反向互補體，SEQ ID NO:4)找到。大白鼠 F12 mRNA 之序列可以在例如 GenBank Accession No. GI: 62078740 (NM_001014006.1；SEQ ID NO:5；反向互補體，SEQ ID NO: 6)找到。食蟹獼猴 F12 mRNA 之序列可以在例如 GenBank Accession No. GI: 982255999 (XM_015452310.1； SEQ ID NO:7；反向互補體，SEQ ID NO: 8)找到。恆河獼猴 F12 mRNA 之序列可以在例如 GenBank Accession No. GI: 1622948449 (XM_028849951.1；SEQ ID NO:9；反向互補體，SEQ ID NO:10)找到。

【0104】 更多 F12 mRNA 序列的示例可自公開可取得的資料庫獲取，例如 GenBank、UniProt、OMIM 及 *Macaca* 基因體計畫網。

【0105】 F12 進一步的資訊可以自例如 www.ncbi.nlm.nih.gov/gene/?term=F12 獲取。

【0106】 自提交本申請之日起，上述每個 GenBank 登錄號和基因數據庫號的全部內容均藉由引用併入本文。

【0107】 如本文所用，術語 F12 還指 F12 基因的變異，包括 SNP 數據庫中提供的變異。F12 基因內的許多序列變異已被鑑定並可在例如 NCBI dbSNP 和 UniProt 中找到(參見例如，www.ncbi.nlm.nih.gov/snp/?term=F12)，其全部內容自提交本申請之日起藉由引用併入本文。

【0108】 如本文所用，「目標序列」是指在 F12 基因轉錄期間形成的 mRNA 分子的核苷酸序列的連續部分，包括作為初級轉錄產物的 RNA 加工產物的 mRNA。 在一個態樣中，序列的目標部分將至少足夠長，以在 F12 基因轉錄過程中形成的 mRNA 分子的核苷酸序列部分或附近充當 iRNA 定向裂解的受質。

【0109】 目標序列可以形成長度大約為 19 至 36 個核苷酸，例如長度約 19 至 30 個核苷酸、長度為 19 至 30、19 至 29、19 至 28、19 至 27、19 至 26、19 至 25、19 至 24、19 至 23、19 至 22、19 至 21、19 至 20、20 至 30、20 至 29、20 至 28、20 至 27、20 至 26、20 至 25、20 至 24、20 至 23、20 至 22、20 至 21、21 至 30、21 至 29、21 至 28、21 至 27、21 至 26、21 至 25、21 至 24、21 至 23、或 21 至 22 個核苷酸。在特定態樣中，目標序列長度為 19 至 23 個核苷酸，視情況為 21 至 23 個核苷酸。當範圍和長度介於上述列舉的範圍和長度內時亦為本揭示的一部份。

【0110】 本文使用的術語「包含序列的股」係指寡核苷酸包括由標準核苷酸命名法所描述的序列之核苷酸鏈。

【0111】 「G」、「C」、「A」、「T」、「U」通常是核苷酸分別包含鳥嘌呤、胞嘧啶、腺嘌呤、胸腺嘌呤、尿嘧啶作為鹼基的縮寫。然而，術語「核醣核苷酸」或「核苷酸」可以被理解為指經修飾之核苷酸，如進一步詳述於下的或是替代的置換部分(參見例如表 1)。所屬領域的技術人員能夠明白鳥嘌呤、胞嘧啶、腺嘌呤、尿嘧啶可以被其他部分取代，且在實質上不改變包括可以承受該取代部分之寡核苷酸鹼基對的特性。例如，不欲受限，包括肌核苷作為鹼基的核苷酸可與包含腺嘌呤、胞嘧啶、尿嘧啶的核苷酸鹼基配對。因此，包含尿嘧啶、鳥嘌呤、腺嘌呤的核苷酸可在本發明的 dsRNA 核苷酸序列中被取代，例如被包含肌苷的核

苷酸取代。在另一個態樣中，寡核苷酸中任一位置的腺嘌呤和胞嘧啶可以分別被鳥嘌呤和尿嘧啶所取代，來形成 G-U 搖擺鹼基對與標靶的 mRNA。含有此類取代部分的序列適用於本發明的組成物和方法。

【0112】 術語「iRNA」、「RNAi 劑」、「iRNA 劑」、「RNA 干擾劑」於本文中可以互相交換使用，包含 RNA的試劑，已該術語在本文中所定義，且其藉由 RNA 誘導沉默複合物(RISC)路徑介導 RNA 轉錄本的靶向裂解。iRNA 藉由稱為 RNA 干擾(RNA interference；簡稱 RNAi)的過程引導 mRNA 序列之專一性降解。iRNA 調節，例如抑制細胞內 F12 基因的表現，例如個體的肝臟細胞，如哺乳類個體。

【0113】 在一個態樣中，本發明的 RNAi 劑包含單股 RNA，其會與標靶 RNA 序列交互作用，例如 F12 標靶 mRNA 序列來引導標靶 mRNA 裂解。不欲受縛於理論，據信，引入細胞的長雙股 RNA 會由稱為 Dicer(Sharp *et al.*(2001) *Genes Dev.* 15:485)的第三型內切酶分解為 siRNA。Dicer 為一種核糖核酸酶-III 樣酶，將 dsRNA 加工成 19 至 23 個鹼基對的短干擾 RNA，具有特徵性的兩個鹼基 3'突出(Bernstein, *et al.*,(2001) *Nature* 409:363)。然後將 siRNAs 摻入 RNA 誘導的沉默複合物(RISC)，其中一個或多個解旋酶解開 siRNA 雙鏈體，使互補的反義鏈能夠引導目標識別(Nykanen, *et al.*,(2001) *Cell* 107:309)。在與適當的目標 mRNA 結合後，RISC 內的一種或多種核酸內切酶裂解目標以誘導沉默(Elbashir, *et al.*,(2001). *Genes Dev.* 15:188)。因此，一方面，本發明涉及在細胞內產生細胞內產生的單股 RNA(siRNA)，其促進 RISC 複合物的形成以影響標靶基因即 F12 基因的沉默。據此，術語「siRNA」於本文中也用於指上述的 iRNA。

【0114】 在特定態樣中，RNAi 劑可為單股 siRNA(ssRNA)，被送入細胞或器官內來抑制標靶 mRNA。單股 RNAi 劑與 RISC 內切酶 Argonaute 2 結合，，接著裂解目標 RNA。單股 siRNA 通常為 15 至 30 個核苷酸且經化學修飾。單股 siRNA 的設計和測試描述於 U.S. Patent No. 8,101,348 和 Lima *et al.*,(2012) *Cell* 150:883-894，其整體內容透過引用併入本文。任何於本文中描述的反義核苷酸序列均可作為本文描述的單股 siRNA 使用，或透過 Lima *et al.*,(2012) *Cell* 150:883-894 所描述的方法進行化學修飾。

【0115】 在特定態樣中，用於本發明之組成物、用途及方法的「iRNA」為雙股 RNA，且於本文係指「雙股 RNA 劑」、「雙股 RNA(dsRNA)分子」、「dsRNA 劑」或「dsRNA」。術語「dsRNA」指核醣核酸分子複合物，其具有包括兩條反向平行及實質上互補的核酸鏈的雙鏈體構造，指相對於目標 RNA，具有「有義」和「反義」方向，舉例而言，F12 基因。在本發明的一些態樣中，雙股 RNA (dsRNA) 透過本文稱為 RNA 干擾或 RNAi 的轉錄後基因沉默機制觸發靶標 RNA 如 mRNA 的降解。

【0116】 通常地，dsRNA 分子中各股大部分的核苷酸是核醣核苷酸，但在本文所述，各股或是兩股也可包含一個或更多個非核醣核苷酸，例如去氧核醣核苷酸或是經修飾之核苷酸。此外，如本說明書中所使用，「iRNA」可包含具有化學修飾之核糖核苷酸；iRNA 可包括在多個核苷酸處的大量修飾。於本文所使用，術語「修飾之核苷酸」係指獨立地具有修飾的糖部分、修飾的核苷酸間鍵或修飾的核鹼基或其任何組合的核苷酸。因此，術語經修飾之核苷酸包括對核苷間鍵、糖部分或核鹼基的例如官能團或原子的取代、添加或去除。適用於本發明試劑的修飾包括本文公開的或本領域已知的所有類型的修飾。出於本說明書和申請專

利範圍的目的，任何此類修飾，例如 siRNA 類型的分子中使用的，均包含於「iRNA」或「RNAi 劑」之中。

【0117】在本揭示的某些態樣中，若去氧核苷酸存在於 RNAi 劑中，可被認為構成修飾的核苷酸。

【0118】雙鏈體區可以是允許所需目標 RNA 透過 RISC 途徑發生專一性降解的任何長度，目標並且長度範圍可為約 19 至 36 個鹼基對，例如，約為 19 至 30 鹼基對，例如約 9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36 個鹼基對，約 19 至 30、19 至 29、19 至 28、19 至 27、19 至 26、19 至 25、19 至 24、19 至 23、19 至 22、19 至 21、19 至 20、20 至 30、20 至 29、20 至 28、20 至 27、20 至 26、20 至 25、20 至 24、20 至 23、20 至 22、20 至 21、21 至 30、21 至 29、21 至 28、21 至 27、21 至 26、21 至 25、21 至 24、21 至 23、或 21 至 22 個鹼基對。在特定態樣中，雙鏈體區的長度為 19 至 21 個鹼基對，例如 21 個鹼基對。範圍和長度介在上述列舉的範圍和長度內時，也可以是本揭示的一部份。

【0119】形成雙鏈體結構的兩股可能是一個較大 RNA 分子的不同部分，或者它們可能是獨立的 RNA 分子。當兩股是一個更大的分子的一部份，因此由其中一股的 3'-末端和另一股的 5'末端的連續核苷酸鏈相連接，形成雙鏈體構造，連接的 RNA 鏈被稱為「髮夾環圈」。髮夾環圈可包括至少一個未配對的核苷酸。在一些態樣中，髮夾環圈可包括至少 2、3、4、5、6、7、8、9、10、20 或 23 個未配對核苷酸。在一些態樣中，髮夾環圈可以是 10 個或更少個核苷酸。在一些態樣中，髮夾環圈可以是 8 個或更少個未配對核苷酸。在一些態樣中，髮夾環圈可為 4 至 10 未配對核苷酸。在一些態樣中，髮夾環圈可為 4 至 8 個核苷酸。

【0120】 當實質上互補的兩股 dsRNA 由單獨的 RNA 分子組成時，這些分子不需要但可以共價連接。當兩股是藉由共價連接而非由核苷酸連續鏈中一股的 3'-末端和另一股的 5'末端形成雙鏈體構造，該連接構造稱為「連接子(linker)」。RNA 股可以有相同或不同數量的核苷酸。最大鹼基對的數目是 dsRNA 最短鏈中的核苷酸數目減去雙鏈體中存在的任何突出。除了雙鏈體結構之外，RNAi 還可以包含一個或多個核苷酸突出。在 RNAi 劑的一個態樣中，至少一股包括至少 1 個核苷酸 3'突出。在另一個態樣中，至少一股包括至少 2 個核苷酸 3'突出，例如 2、3、4、5、6、7、9、10、11、12、13、14 或 15 個核苷酸。在其他態樣中，至少 RNAi 劑一股包括至少一個核苷酸 5'突出。在特定態樣中，至少一股包括至少 2 個核苷酸 5'突出，例如 2、3、4、5、6、7、9、10、11、12、13、14 或 15 個核苷酸。在其他態樣中，RNAi 劑其中一股的 3'和 5'末端包括至少 1 個核苷酸突出。

【0121】 在特定態樣中，本發明的 iRNA 劑是 dsRNA，各股包括 19 至 23 個核苷酸，其與目標 RNA 序列交互作用，例如 F12 基因，以引導目標 RNA 的裂解。

【0122】 在一些態樣中，本發明的 iRNA 是具有 24 至 30 個核苷酸的 dsRNA，其會與目標 RNA 序列交互作用，例如 F12 目標 mRNA 序列，以引導目標 RNA 的裂解。

【0123】 於本文使用，術語「核苷酸突出」指至少一個未配對的核苷酸從雙股 iRNA 的雙股構造中突出。例如，當 dsRNA 一股的 3'-末端延伸出另一股的 5'-末端具有核苷酸突出，或反之亦然。dsRNA 可包括至少一核苷酸突出，或者可包括 2 個核苷酸、3 個核苷酸、4 個核苷酸、5 個核苷酸、或更多核苷酸突出。核

苷酸突出可包含或由核苷酸/核苷類似物組成，包括去氧核苷酸/核苷。突出可以位在有義股、反義股、或其任合組合上。此外，突出的核苷酸可出現於 dsRNA 反義股或有義股的 5'-末端、3'-末端，或兩端皆有。

【0124】在一個態樣中，dsRNA 的反義股有 1 至 10 個核苷酸，例如 1、2、3、4、5、6、7、8、9、10 個核苷酸，突出位在 3'-末端或 5'-末端。在一個態樣中，dsRNA 的有義股有 1 至 10 個核苷酸，例如 1、2、3、4、5、6、7、8、9 或 10 個核苷酸，突出位在 3'-末端或 5'-末端。在另一個態樣中，突出中一個或更多個核苷酸由核苷硫代磷酸酯取代。

【0125】在特定態樣中，dsRNA 的反義股有 1 至 10 個核苷酸，例如 0 至 3、1 至 3、2 至 4、2 至 5、4 至 10、5 至 10，例如 1、2、3、4、5、6、7、8、9 或 10 個核苷酸，突出位在 3'-末端或 5'-末端。在一個態樣中，dsRNA 的有義股有 1 至 10 個核苷酸，例如 0 至 3、1 至 3、2 至 4、2 至 5、4 至 10 或 5 至 10，例如 1、2、3、4、5、6、7、8、9 或 10 個核苷酸，突出位在 3'-末端或 5'-末端。在另一個態樣中，突出中一個或更多個核苷酸由核苷硫代磷酸酯取代。

【0126】在特定態樣中，dsRNA 的反義股有 1 至 10 個核苷酸，例如 1、2、3、4、5、6、7、8、9 或 10 個核苷酸，突出位在 3'-末端或 5'-末端。在特定態樣中，dsRNA 的有義股或反義股或兩者的突出，可以包含延伸的長度大於 10 個核苷酸，例如長度為 1 至 30 個核苷酸、2 至 0 個核苷酸、10 至 30 個核苷酸、10 至 25 個核苷酸、10 至 20 核苷酸、或 10 至 15 個核苷酸。在特定態樣中，延伸的突出位在雙股的有義股。在其他態樣中，延伸的突出位在雙股的有義股 3'末端。在特定態樣中，延伸的突出位在雙股的有義股 5'末端。在特定態樣中，延伸的突出位在雙股的反義股。在特定態樣中，延伸的突出位在雙股的反義股 3'末端。在特

定態樣中，延伸的突出位在雙股的反義股 5'末端。在其他態樣中，延伸的突出中一個或更多個核苷酸由核苷硫代磷酸酯取代。在特定態樣中，突出包含自我互補部分，該突出可以形成髮夾結構，其在生理狀況下是穩定的。

【0127】「平的」或「平端」意指在雙股 RNA 劑末端並沒有未配對的核苷酸，亦即沒有核苷酸突出。「平端」雙股 RNA 劑於整個長度中皆是雙股的，亦即在分子任一端皆沒有核苷酸突出。本發明的 RNAi 劑包含沒有核苷酸在其中一端突出的 RNAi 劑(亦即具有突出和平端之劑)或在兩端皆沒有核苷酸突出。大部分時候該分子可以於整個長度中都是雙股的。

【0128】術語「反義股」或「引導股」係指 iRNA 股，例如 dsRNA，其包含實質上與目標序列互補的區域，例如 F12 mRNA。

【0129】於本文中使用，術語「互補區域」係指反義股上的區域在實質上與序列互補，例如本文中定義的 F12 核苷酸序列。當互補區域與目標序列並不完全互補時，可在分子中的中間或末端區域發現錯配。通常地，最可能具有的錯配位在末端區域，例如位於 iRNA 5'-或 3'-末端之 5、4、3 個核苷酸。在一些態樣中，本發明的雙股 RNA 劑包含在反義股的核苷酸錯配。在一些態樣中，本發明的雙股 RNA 劑的反義股包含與目標 mRNA 不超過 4 個錯配，例如反義股包含 4、3、2、1 或 0 個與目標 mRNA 的錯配。在一些態樣中， 本發明的雙股 RNA 劑的反義股包含與有義股不超過 4 個錯配，例如反義股包含 4、3、2、1 或 0 個與有義股的錯配。在一些態樣中，本發明的雙股 RNA 劑包含在有義股的核苷酸錯配。在一些態樣中，本發明的雙股 RNA 劑的有義股包含與反義股不超過 4 個錯配，例如有義股包含 4、3、2、1 或 0 個與反義股的錯配。在一些態樣中，核

苷酸錯配是在例如 iRNA 3'-末端的 5、4 或 3 個核苷酸。在另一個態樣中，核苷酸錯配是在例如 iRNA 3'-末端的核苷酸。在一些態樣中，錯配不位在種子區。

【0130】 因此，本文描述之 RNAi 劑可包含一個或更多個與目標序列之錯配。在一個態樣中，本文描述之 RNAi 劑可以包含不超過 3 個錯配(亦即 3、2、1 或 0 個錯配)。在一個態樣中，本文描述之 RNAi 劑可以包含不超過 2 個錯配。在一個態樣中，本文描述之 RNAi 劑可以包含不超過 1 個錯配。在一個態樣中，本文描述之 RNAi 劑可以包含 0 個錯配。在特定的態樣中，如 RNAi 劑的反義股包含與目標序列之錯配，錯配可視情況限制於互補區域 5'-或 3'-末端最後 5 個核苷酸。例如，在此類態樣中，對於 23 個核苷酸的 RNAi 試劑，與 F12 基因互補的股通常在中央 13 個核苷酸中不含任何錯配。本文所述之方法或本領域習知之方法可用於確定含有與靶標序列錯配之 RNAi 劑是否有效抑制 F12 基因表現目標。考量錯配對於 RNAi 劑抑制 F12 基因表現的療效很重要，尤其若已知 F12 基因中的特定互補區域在群體中具有多型性序列變異。

【0131】 本文使用術語「有義股」或「過客股」，係指 iRNA 的股包含實質上與反義股的區域互補的區域，該術語於本文中定義。

【0132】 於本文中使用，「實質上所有核苷酸已被修飾」係大部分但不是全部都被修飾，且可包含不超過 5、4、3、2 或 1 個未修飾之核苷酸。

【0133】 於本文中使用，術語「裂解區域」係指鄰近裂解位點的區域。裂解位點是目標發生裂解的目標位點。在一些態樣中，裂解區域包含在裂解位點的任一端並緊鄰裂解位點的三個鹼基三個鹼基。在一些態樣中，裂解區包含在裂解位點的任一端並緊鄰裂解位點的兩個鹼基。在一些態樣中，裂解位點專一性地出現

在反義股的核苷酸 10 和 11 所結合的位點，並且裂解區域包含核苷酸 11、12 和 13。

【0134】 於本文中使用，除非有另外指示，當術語「互補」用於描述與第二條核苷酸序列有關的第一條核苷酸序列，係指寡核苷酸或聚核苷酸包括第一條核苷酸序列有能力與寡核苷酸或聚核苷酸包括第二條核苷酸序列來雜交並在特定條件下形成雙股構造，且能夠被所屬領域一般技術者理解。該狀況可以是，例如，具有嚴格的條件，其嚴格條件可以包含：400 mM NaCl、40 mM PIPES pH 6.4、1 mM EDTA、50℃或 70℃下等待 12 至 16 小時，接著沖提(參見例如 Molecular Cloning: A Laboratory Manual, Sambrook, *et al.*(1989) Cold Spring Harbor Laboratory Press)。可以應用其他條件，例如在生物體內可能遇到的生理相關條件。 技術人員將能夠根據雜交核苷酸的最終應用確定最適合測試兩個序列互補性的一組條件。

【0135】 在 iRNA 中的互補序列，例如於本文中描述在 dsRNA 中，包含寡核苷酸或聚核苷酸包括第一條核苷酸序列有能力與寡核苷酸或聚核苷酸包括第二條核苷酸序列於其中一條或兩條核苷酸序列整個長度之鹼基配對。本文中該序列可以係指兩者「完全地互補」。然而，本文中當地一條序列涉及與第二條序列「實質上互補」，兩條序列可以完全地互補，或是可以形成一個或更多個鹼基互補，但通常在高達 30 個鹼基對的雙股之雜交不超過 5、4、3 或 2 個錯配鹼基對，保留其最終應用最相關的條件下雜交之能力，例如在體外或體內抑制基因表現。然而，當兩條寡核苷酸被設計在雜交時形成一個或多個單股突出，該突出不該被認為是判定互補的錯配。例如，dsRNA 包括長度為 21 個核苷酸的寡核苷酸和另一條長度為 23 個核苷酸，其中包括 21 個核苷酸的序列之較長的寡核苷酸

與較短的寡核苷酸完全地互補，出於本文所述的目的，仍可將其稱為「完全互補」。

【0136】 如本文所用，「互補」序列還可以包括或完全由非 Watson-Crick 鹼基對或由非天然和修飾的核苷酸形成的鹼基對組成，只要滿足上述關於它們雜交能力的要求。 此類非 Watson-Crick 鹼基對包括但不限於 G:U Wobble 或 Hoogsteen 鹼基配對。

【0137】 本文使用術語「互補」、「完全地互補」、「實質上互補」可以涉及與 dsRNA 有義股和反義股之間鹼基配對之使用，或兩條寡核苷酸或聚核苷酸之間，如雙股 RNA 劑的反義股和目標序列，正如從其上下文中理解者。

【0138】 於本文中使用，與訊息 RNA(messenger RNA(mRNA))「至少部分實質上互補」的多核苷酸是指與感興趣的 mRNA 的連續部分實質上互補的多核苷酸。例如，如果序列與編碼 F12 基因的 mRNA 的未中斷部分實質上互補，則多核苷酸與 F12 mRNA 的至少一部分互補。

【0139】 據此，在一些態樣中，本文揭示之反義股聚核苷酸與目標 F12 序列完全地互補。在其他態樣中，本文揭示之反義股聚核苷酸與目標 F12 序列在實質上互補，且包括連續核苷酸序列，該核苷酸序列在其整個長度上與任一 SEQ ID NOs：1、3、5、7、9 的核苷酸序列的等效區域，或與任一 SEQ ID NOs：1、3、5、7、9 中任一者的片段至少 80%互補，如約 85%、約 90%、約 91%、約 92%、約 93%、約 94%、約 95%、約 96%、約 97%、約 98%或約 99%互補。

【0140】 在其他態樣中，本文揭示的反義股多核苷酸實質上與目標 F12 序列互補，且包括連續核苷酸序列，其全長與表 2 至表 3 任一者中任一有義股核苷酸序列，或表 2 至表 3 任一者中任一有義股核苷酸序列片段至少 80%互補，如

約 85%、約 90%、約 91%、約 92%、約 93%、約 94%、約 95%、約 96%、大約 97%、約 98%、約 99%或約 100%互補。

【0141】 在一些態樣中，本文揭示的反義多核苷酸實質上與目標 F12 序列之片段互補，且包括連續核苷酸序列，其整段與 SEQ ID NO：1 中選自由核苷酸 75 至 97、76 至 98、79 至 101、115 至 137、117 至 139、123 至 145、132 至 154、135 至 157、137 至 159、144 至 166、201 至 223、或 1462 至 1484 組成之組的片段至少 80%互補，如約 85%、約 90%、約 91%、約 92%、約 93%、約 94%、約 95%、約 96%、約 97%、約 98%或約 99%互補。

【0142】 在一些態樣中，本文揭示之反義多核苷酸在實質上與目標 F12 序列互補，且包括連續核苷酸序列，其整段與 SEQ ID NO: 1 中選自由核苷酸 135 至 157、144 至 166、及 201 至 223 組成之組的片段至少 80%互補，如約 85%、約 90%、約 91%、約 92%、約 93%、約 94%、約 95%、約 96%、約 97%、約 98%或約 99%互補。

【0143】 在一個態樣中，本揭示之 RNAi 劑包含有義股，其在實質上與反義多核苷酸互補，因此，與目標 F12 序列相同，其中有義股聚核苷酸包括連續核苷酸序列，其整段與任一相對應於 SEQ ID NOs：2、4、6、8 或 10 的核苷酸序列或任一 SEQ ID NOs：2、4、6、8 或 10 的片段至少 80%互補，如大約 85%、大約 90%、大約 91%、大約 92%、大約 93%、大約 94%、大約 95%、大約 96%、大約 97%、大約 98%、大約 99%或 100%互補。

【0144】 在一些態樣中，本發明之 iRNA 包含有義股，其在實質上與反義多核苷酸互補，因此，與目標 F12 序列互補，其中有義股聚核苷酸包括連續序列，其整段與表 2 至表 3 任一者中任一反義股核苷酸序列，或表 2 至表 3 任一者

中任一反義股核苷酸序列片段至少 80%互補，如大約 85%、大約 90%、大約 91%、大約 92%、大約 93%、大約 94%、大約 95%、大約 96%、大約 97%、大約 98%、大約 99%或 100%互補。

【0145】 在其他態樣中，有義和反義股選自以下任一雙鏈體：AD-1534528、AD-1534479、AD-1534582、AD-1534611、AD-1534506、AD-1631309、AD-1534471、AD-1534555、AD-1534678、AD-1534585、AD-1534502 或 AD-1534491。

【0146】 在特定態樣中，有義和反義股選自以下任一雙鏈體 AD-1534528、AD-1534479 或 AD-1534582。

【0147】 通常，「iRNA」包含核醣核苷酸具有化學修飾。該修飾可包含各種本文揭示之修飾或為本領域習知之修飾。作為 dsRNA 分子使用，出於本說明書和申請專利範圍之目的，任何修飾均包含於「iRNA」中。

【0148】 在本揭示的特定態樣中，如果存在於 RNAi 試劑中，則包含去氧核苷酸可被視為構成修飾的核苷酸。

【0149】 在本發明一個面向中，本發明的方法和組成物之藥劑的使用是單股反義寡核苷酸分子，其會藉由反義抑制機制來抑制目標 mRNA。單股反義寡核苷酸分子與目標 mRNA 中的序列互補。單股反義寡核苷酸分子可以化學劑量法抑制轉譯，藉由 mRNA 鹼基配對和生理上阻礙轉譯機制，參見 Dias, N. *et al.*,(2002) *Mol Cancer Ther* 1:347-355。單股反義寡核苷酸分子長度大約為 14 至大約 30 個核苷酸，且有與目標序列互補的序列。例如，單股反義寡核苷酸分子可包含與本文所述的任一反義序列至少約 14、15、16、17、18、19 或 20 或更多個連續核苷酸的序列。

【0150】片語「利用 iRNA 接觸細胞」，如本文使用之 dsRNA，包含任何可能接觸細胞的方法。利用 iRNA 接觸細胞包含利用 iRNA 在體外接觸細胞，或是利用 iRNA 在體內接觸細胞。接觸可以是直接地或間接地。因此，iRNA 可被與細胞藉由個別執行方法以生理上的接觸，或是相反地，iRNA 可以被納入一個允許或導致其隨後與細胞接觸的情況。

【0151】在體外接觸細胞可以，例如，藉由將細胞與 iRNA 一起培養。在體內接觸細胞可以，例如，藉由將 iRNA 注射至或鄰近細胞所在的組織，或藉由將 iRNA 注射至其他區域，例如血管或皮下區域，該藥劑可接著抵達欲接觸的細胞所在的組織。例如，iRNA 可包含或連接配體，例如 GalNAc，其引導 iRNA 至感興趣的位置，例如肝臟。結合在體外和在體內接觸的方法是可行的。例如，細胞可與 iRNA 在體內接觸，接著移植至個體體內。

【0152】在特定態樣中，利用 iRNA 接觸細胞包含「引入」或「遞送 iRNA 進入細胞」係藉由促進或影響攝取和吸收至細胞。吸收或攝取 iRNA 可以透過未受幫助的擴散和主動細胞代謝過程，或透過助劑或裝置進行。將 iRNA 進入細胞可在體外或在體內。例如，就體內引入而言，可將 iRNA 注射至組織位置或藉由全身性地注射。在體外引入細胞內包含所屬領域習知的方法，如電穿孔法和脂質轉染法。進一步的方法於本文中描述或為所屬領域習知者。

【0153】術語「脂質奈米製劑」或「LNP」是一種囊泡，其包括脂質層封裝藥學上活性分子，如核酸分子，例如 iRNA 或轉譯出 iRNA 的質體。LNP 描述於，例如，美國專利公開第 6,858,225、6,815,432、8,158,601 和 8,058,069 號，其所有內文以引用方式併入本文。

【0154】 於本文中使用，「個體」是動物，如哺乳類，包含靈長類動物(如人類、非人類靈長類，例如猴子和黑猩猩)、非靈長類動物(如乳牛、豬、馬、山羊、兔子、綿羊、倉鼠、天竺鼠、貓、狗、大白鼠、小鼠)、或是內生性地或異源性地表現目標基因的鳥。在一個態樣中，個體是人類，例如正在治療或評估將受益於 F12 表達減少的疾病或病症的人；或因能受惠於如本文所述的 F12 表達減少而治療的疾病或病症的人。在一些態樣中，個體是女性人類。在其他態樣中，個體是男性人。在一個態樣中，個體是成年人。在另一個態樣中，個體是兒童。

【0155】 於本文中使用，術語「治療(treating)」或「療法(treatment)」係指有益的或目標的結果，如減緩個體 F12 相關至少一個徵象或症狀；減弱不良的 F12 活化和穩定的程度；改善或緩和不良的 F12 活化和穩定的程度。「療法」也可指相較於未治療的情況下，預期的存活之延長。

【0156】 內文中個體 F12 的含量或是疾病標記物或症狀之術語「較低的」，係指統計上顯著降低該量。降低可以是，例如，至少 10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%或更多。在特定態樣中，降低至少 20%。在特定態樣中，降低至少 50%的疾病標記物，例如蛋白質或基因表現量。內文中個體 F12 量「較低的」是降低至沒有罹患該疾病的個體可接受的正常範圍的量。在特定態樣中，「較低的」是降低罹病個體之標記物的量或症狀程度，與個體可接受的正常範圍的量之間的差異。術語「較低的」也可以用於相關於疾病或狀況的症狀之正常化，亦即降低罹患 F12 相關疾病個體之程度，與沒有罹患 F12 相關疾病的正常個體之程度間的差異。於本文中使用，如疾病與症狀程度的提升有關，「正常」被認為是正常的上限。如疾病與症狀程度的降低有關，「正常」被認為是正常的下限。

【0157】 於本文中使用，「預防(prevention)」或「預防(preventing)」當用於提及疾病、病症或其病況時，可透過降低F12基因的表達來治療或改善，藉由，是指個體將出現與此類疾病相關的症狀之可能性的降低，例如 F12 相關疾病的症狀，例如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或其他因升高的舒緩肽量所致之遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症。未能發展疾病、病症或病症，或與此類疾病、病症或病症相關的症狀的發展減少(例如，按該疾病或病症的臨床可接受量表減少至少約 10%)，或延遲出現症狀(例如，延遲數天、數週、數月或數年)被認為是有效的預防。

【0158】 於本文中使用，術語「第十二因子相關疾病」或「F12 相關疾病」，是一個源自或相關於 F12 基因表現或 F12 蛋白質產出的疾病或病症。術語「F12 相關疾病」包含可受惠於 F12 基因表現、複製、蛋白質活性降低之疾病、病症、狀況。在一些態樣中，F12 相關疾病選自由遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或其他因升高的舒緩肽量導致之遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症組成之組。

【0159】 在一個態樣中，F12 相關疾病是 HAE。在另一個態樣中，F12 相關疾病是前激肽釋放酶缺乏症。在另一個態樣中，F12 相關疾病是高血壓，例如惡性原發性高血壓。在另一個態樣中，F12 相關疾病是末期腎臟疾病。在另一個態樣中，F12 相關疾病是夫列契因子缺乏症。

【0160】 本文使用「治療有效劑量」是傾向包含注射致罹患 F12 相關疾病的個體之 RNAi 劑的量是足夠影響疾病的治療(例如減弱、改善、維持已存在的

疾病或一或多個疾病症狀)。「治療有效劑量」可取決於 RNAi 劑、藥劑是如何被投予、疾病和其嚴重程度和病史、年紀、體重、家族病史、基因組成、先前或合併的治療類型(如果有)、和其他待治療的個體之個體特徵。

【0161】本文使用「預防有效劑量」旨在包括當給予罹患 F12 相關疾病的個體足以預防或改善病症或病症的一種或多種症狀之 RNAi 劑的量如四肢、臉部、咽喉、上呼吸道、腹部、軀幹、生殖器水腫腫脹；前驅症狀；喉腫脹；非搔癢性皮疹、噁心、嘔吐或腹部疼痛。改善疾病包含減緩疾病之或降低晚發性疾病的嚴重程度。「預防有效劑量」可取決於 RNAi 劑、藥劑是如何被投予、疾病風險的程度和病史、年紀、體重、家族病史、基因組成、先前或合併的治療類型(如果有)、和其他待治療的病人之個體特徵。

【0162】「治療有效劑量」或「預防有效劑量」還包括以適用於任何治療的合理收益/風險比產生一些期望效果的 RNAi 劑的量。在本發明的方法中使用的 iRNA 可以足夠的量施用以產生適用於此類治療的合理的利益/風險比。

【0163】於本文使用的片語「藥學上可接受」，係指那些在合理的醫學判斷範圍內合與人類個體組織和動物個體組織接觸的化合物、材料(包含鹽)、組成物、劑型，而不會有過量的毒性、發炎、過敏反應、或其他問題或併發症，與合理的效益/風險比例相稱。

【0164】於本文使用片語「藥學上可接受載體」意指藥學上可接受材料、組成物、載體，如液體或固體充填物、稀釋劑、賦形劑、製造助材(例如潤滑劑、硬脂酸鎂/硬脂酸鈣/硬脂酸鋅滑石粉、硬脂酸)、參與攜帶或輸送目標化合物從身體一器官或部分至身體另一個器官或部分之溶劑封裝材料。各載體在與製劑的

其他成分相容的意義上必須是「可接受的」，且對於待治療的個體不具傷害性。該載體在本領域中是已知的。藥學上可接受的載體包括用於注射給藥的載體。

【0165】 本文使用術語「樣本」，包含收集分離自個體之相似的體液、細胞或分離自個體的組織，以及個體內的體液、細胞、組織。生物體液的例子包含血液、血清和漿液、血漿、腦脊髓液、眼液、淋巴液、尿液、唾液、和其類似物。組織樣本可包含來自組織、器官、局部區域的樣本。例如，樣本可衍生自特定器官、部分器官、位於該器官內的液體或細胞。在特定態樣中，樣本可衍生自肝臟(例如整個或部分的肝臟，或肝臟內特定類型的細胞，如肝細胞)。在一些態樣中，「衍生自個體的樣本」係指取自個體之尿液。「衍生自個體的樣本」可係指來自個體的血液或血液衍生之血清或血漿。

【0166】 II.本發明之 iRNA

【0167】 本發明提供抑制 F12 基因表現的 iRNAs。在特定態樣中，iRNA 包含雙股核糖核酸(double stranded ribonucleic acid；簡稱 dsRNA)分子，用於抑制細胞內 F12 基因的表現，如個體體內的細胞，個體例如哺乳類，如疑似罹患 F12 相關疾病的人類；F12 相關疾病例如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或其他因升高的舒緩肽量導致之遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症。dsRNAi 劑包含具有互補區域之反義股，該互補區域與至少部分形成用於表現 F12 基因的 mRNA 互補。互補區域長度大概為 19 至 30 個核苷酸(例如約 30、29、28、27、26、25、24、23、22、21、20、19 個核苷酸)。

【0168】 iRNA 一旦與表現 F12 基因的細胞接觸，iRNA 抑制至少 50%的 F12 基因(例如人類、靈長類、非靈長類、兔子之 F12 基因)的表現，由例如 PCR、以分支 DNA 為主(branched DNA(bDNA)-based) 之方法、以蛋白質為主之方法如免疫螢光分析，使用例如西方點墨法或流式細胞術加以檢驗。在特定態樣中，基因表現的抑制由本文提供的示例中之 qPCR 判定，舉到而言，本文提供的適當的生物體細胞株之濃度為 10 nM 的 siRNA。在特定態樣中，體內基因表現的抑制係透過減弱表現人類基因的齧齒類動物來確定，例如小鼠或表現人類目標基因的 AVV 感染小鼠，例如，當以單一劑量給藥時，例如，在 RNA 表現最低點為 3 mg/kg。

【0169】 dsRNA 包含兩 RNA 股，其可以在 dsRNA 可以被使用的形況下互補和雜交形成雙鏈體結構。其中一股的 dsRNA(反義股)包含一個互補區域，其可以與目標序列在實質上互補和通常地完全互補。目標序列可以衍生自在 F1 基因表現時形成的 mRNA 序列。另一股(有義股)包含與反義股互補的區域，該兩股在適合的條件下雜交形成雙鏈體結構。如本文別處所述和本領域已知者，dsRNA 的互補序列也可以被包含於單個核酸分子的自我互補區域，而不是在單獨的寡核苷酸。

【0170】 通常地，雙鏈體結構長度為 15 至 30 個鹼基對，例如長度為 15 至 29、15 至 28、15 至 27、15 至 26、15 至 25、15 至 24、15 至 23、15 至 22、15 至 21、15 至 20、15 至 19、15 至 18、15 至 17、18 至 30、18 至 29、18 至 28、18 至 27、18 至 26、18 至 25、18 至 24、18 至 23、18 至 22、18 至 21、18 至 20、19 至 30、19 至 29、19 至 28、19 至 27、19 至 26、19 至 25、19 至 24、19 至 23、19 至 22、19 至 21、19 至 20、20 至 30、20 至 29、20 至 28、20 至 27、20 至 26、

20至25、20至24、20至23、20至22、20至21、21至30、21至29、21至28、21至27、21至26、21至25、21至24、21至23、或21至22個鹼基對。在特定態樣中，雙鏈體結構長度為18至25個鹼基對，例如18至25、18至24、18至23、18至22、18至21、18至20、19至25、19至24、19至23、19至22、19至21、19至20、20至25、20至24、20至23、20至22、20至21、21至25、21至24、21至23、21至22、22至25、22至24、22至23、23至25、23至24、或24至25個鹼基對，例如19至21個鹼基對。介於上述所述的範圍和長度之間的範圍或長度也為本揭示的一部份。

【0171】 相似地，與目標序列互補區域長度為15至30個核苷酸，例如為15至29、15至28、15至27、15至26、15至25、15至24、15至23、15至22、15至21、15至20、15至19、15至18、15至17、18至30、18至29、18至28、18至27、18至26、18至25、18至24、18至23、18至22、18至21、18至20、19至30、19至29、19至28、19至27、19至26、19至25、19至24、19至23、19至22、19至21、19至20、20至30、20至29、20至28、20至27、20至26、20至25、20至24、20至23、20至22、20至21、21至30、21至29、21至28、21至27、21至26、21至25、21至24、21至23、或21至22個鹼基對，例如為19至23個核苷酸。介於上述所述的範圍和長度之間的範圍或長度也為本揭示的一部份。

【0172】 在一些態樣中，雙鏈體的結構長度為19至30個鹼基對。相似地，與目標序列互補的區域長度為19至30個核苷酸。

【0173】 在一些態樣中，dsRNA長度大約是19至大約23個核苷酸，或是長度大約25至大約30個核苷酸。通常，dsRNA的長度是足夠作為Dicer酶的受

質。例如，本領域習知，長度大於 21 至 23 個核苷酸的 dsRNAs 可作為 Dicer 的受質。本領域具通常知識者也可理解，RNA 所目標裂解的區域通常為較大的 RNA 分子的一部份，通常是 mRNA 分子。在相關情況下，目標 mRNA 的「部份」是 mRNA 靶標的連續序列，其長度足以使其為 RNAi 定向裂解(即透過 RISC 途徑裂解)的受質。

【0174】 所屬領域一般技術者可以辨認該雙鏈體區域是 dsRNA 主要功能區域，例如雙鏈體區域大約是 19 至大約 30 個鹼基對，例如約 19 至 30、19 至 29、19 至 28、19 至 27、19 至 26、19 至 25、19 至 24、19 至 23、19 至 22、19 至 21、19 至 20、20 至 30、20 至 29、20 至 28、20 至 27、20 至 26、20 至 25、20 至 24、20 至 23、20 至 22、20 至 21、21 至 30、21 至 29、21 至 28、21 至 27、21 至 26、21 至 25、21 至 24、21 至 23、或 21 至 22 個鹼基對。因此，在一個態樣中，就其被加工成例如 15-30 個鹼基對的功能性雙鏈體而言，具有 15 至 30 個鹼基對，其靶向所需目標 RNA 進行裂解，RNA 分子或 RNA 分子複合物具有超過 30 個鹼基對的雙鏈體區域是 dsRNA。因此，所屬領域中具通常知識者可以辨認在態樣中，miRNA 是 dsRNA。在另一個態樣中，dsRNA 並不是自然出現之 miRNA。在另一個態樣中，適用於目標 F12 基因表現的 iRNA 劑並非藉由在目標細胞中裂解較大的 dsRNA 所產生。

【0175】 本文所述之 dsRNA 可以進一步包含一或多個單股核苷酸突出，例如 1 至 4、2 至 4、1 至 3、2 至 3、1、2、3 或 4 個核苷酸。具有至少一核苷酸突出的 dsRNAs 相對於其平端的副本具有較好的抑制特性。核苷酸突出可以包括或由核苷酸/核苷類似物組成，包含去氧核苷酸/核苷。突出可以位於有義股、反

義股、或其任意結合。此外，核苷酸突出可以位於 5'末端、3'末端、或是 dsRNA 的反義股或有義股之兩端。

【0176】 dsRNA 可由所屬領域習知的標準方法合成。本發明之雙股 RNAi 化合物可以由兩步驟的流程被製備。首先雙股 RNA 分子的各股是分別被製備。接著，成份股被黏合。siRNA 化合物的各股可以使用溶液相或固態相有機合成或以上兩者被製備。有機合成提供寡核苷酸股包括非自然或修飾之核苷酸可以簡單地被製備之優勢。相似地，本發明單股寡核苷酸可使用溶液相或固相有機合成或以上兩者加以製備。

【0177】 在一個面向中，本發明的 dsRNA 包含至少兩條核苷酸序列、有義序列或反義序列。有義股是選自由表 2 至 3 中之任一序列組成之組，且與有義股相對應的反義股是選自由表 2 至 3 中之任一序列組成之組。在此面向，兩條序列中的其中一條與另外兩條序列中的一條序列互補，其中一條序列與由 F12 基因表現產生的 mRNA 序列在實質上互補。嚴格地說，在此面向，dsRNA 可包含兩個寡核苷酸，其中第一條寡核苷酸係描述於表 2 至 3 中的任一有義股，第二條寡核苷酸係描述於表 2 至 3 中的任一有義股之相對應的反義股。

【0178】 在特定態樣中，dsRNA 實質上互補序列包含在單獨的寡核苷酸上。在其他態樣中，dsRNA 實質上互補序列包含於單個寡核苷酸。

【0179】 應當理解，雖然例如表 3 中的序列未被描述為修飾的或接合的序列，但是本發明的 iRNA 的 RNA 例如本發明的 dsRNA 可以包含序列組中的任何一個 表 2 至 3 中任一表中所列的未修飾、未接合或與其中所述不同的修飾或接合。換言之，本發明包括表 2 至 3 的 dsRNA，其如本文所述是未修飾的、未接合的、經修飾的或接合的。

【0180】本領域技術人員可以清楚理解，具有大約 20 至 30 鹼基對的雙鏈體結構之 dsRNA，例如 21 個鹼基對，其已裂解，對於誘導 RNA 干擾(Elbashir et al.；EMBO 2001, 20:6877 至 6888)特別有效。然而，其他研究發現較短或較長的 RNA 雙股結構亦有效(Chu 和 Rana(2007) RNA 14:1714 至 1719；Kim et al.(2005) Nat Biotech 23:222-226)。在上述態樣中，憑藉表 2 至 3 中任一者提供的寡核苷酸序列的性質，本文所述之 dsRNAs 可包含至少一條長度最短為 21 個核苷酸的單股。可合理預期具有表 2 至 3 中任一者中的任一序列的較短雙鏈體在一端或兩端僅減去幾個核苷酸時，與上述 dsRNAs 相比同樣有效。因此，具有至少 19、20 或更多個連續核苷酸序列的 dsRNAs 來自表 2-3 中任一序列的任一者，且其抑制 F12 基因表現的能力與包含完整序列的 dsRNA 的抑制能力差異不超過約 5%、10%、15%、20%、25% 或 30%者，被視為在本發明範圍內。

【0181】此外，於表 2 至 3 提供的 RNA 識別了於 F12 轉錄本中易受 RISC 介導的裂解影響的位點。因此，本發明進一步著重於目標向這些位點之一的 iRNA。如本文所用，如果 iRNA 在特定位點內的任何地方促進轉錄本的裂解，則稱 iRNA 靶向 RNA 轉錄本的特定位點內。此類 iRNA 通常將包括至少約 19 個來自表 2 至 3 中任何者中提供的序列中的任一連續核苷酸，其接合至取自與 F12 基因中選定序列相鄰的區域的額外核苷酸序列。

【0182】III.本發明之經修飾的 iRNA

在特定態樣中，本發明的 iRNA 之 RNA，例如 dsRNA，是未經修飾者且不包括，例如，所屬領域習知和本文所述之化學修飾或接合。在特定態樣中，本發明的 iRNA 之 RNA，例如 dsRNA，是經化學修飾來提升穩定性和其他有益特徵。在本發明特定態樣中，本發明 iRNA 的所有核苷酸實質上是經修飾的。在本發明

其他態樣中，iRNA 的所有核苷酸或 iRNA 實質上所有的核苷酸是經修飾的，亦即 iRNA 鏈上存在不超過 5、4、3、2 或 1 個未經修飾的核苷酸。

【0183】 本發明著重的核酸可以由所屬領域習知之方法加以合成和修飾，如在“Current protocols in nucleic acid chemistry,” Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA et al.中描述的方法，而以其整體併入本文。修飾包含，例如末端修飾，如 5’末端修飾(磷酸化、接合、反轉的鍵結)或 3’末端修飾(接合、DNA 核苷酸、反轉的鍵結等)；鹼基修飾，例如替換為穩定的鹼基、不穩定鹼基、與擴張序列的另一方進行鹼基配對的鹼基、移除鹼基(無鹼基核苷酸)、接合鹼基；醣修飾(例如在 2’位置或 4’位置)或取代醣；主鏈修飾，包含修飾或取代磷酸雙脂鍵。本文所述於態樣中特定的有效 iRNA 化合物之示例包含但不限制於含有經修飾的主鍊或不含天然核苷間鍵的 RNA。具有修飾之主鏈的 RNA 包括但不限於主鏈中沒有磷原子者。出於本說明書的目的，且如本領域有時所引用的，在其核苷間主鏈中不具有磷原子的修飾的 RNA 也可以被視為是寡核苷。在一些態樣中，經修飾的 iRNA 在其核苷間主鏈中具有磷原子。

【0184】 經修飾之 RNA 主鏈包含，例如，硫代磷酸酯、手性硫代磷酸酯、二硫代磷酸酯、磷酸三酯、胺基烷基磷酸三酯、甲基和其他烷基膦酸酯，包括 3'-亞烷基膦酸酯和手性膦酸酯、次膦酸酯、胺基磷酸酯，包括 3'-胺基胺基磷酸酯和胺基烷基胺基磷酸酯、硫代胺基磷酸酯、具有正常 3' -5' 連接的硫代烷基膦酸酯、硫代烷基磷酸三酯、它們的 2'-5'-連接類似物，以及具有反轉極性者，其中相鄰的核苷單元對從 3'-5' 連接到 5'-3'或 2'-5' 至 5'-2'。還包括各種鹽、混合鹽、游離酸形式。在本發明一些態樣中，本發明之 dsRNA 劑是游離酸形式。在本發明其他態樣中，本發明之 dsRNA 劑是鹽形式。在一個態樣中，本發明之 dsRNA

劑是鈉鹽形式。在特定態樣中，當本發明之 dsRNA 劑是鈉鹽形式時，鈉離子作為試劑中存在的實質上所有磷酸二酯和/或硫代磷酸酯基團的抗衡離子存在於試劑中。實質上所有的磷酸二酯和/或硫代磷酸酯鍵都具有鈉抗衡離子的試劑包括不超過 5、4、3、2 或 1 個沒有鈉抗衡離子的磷酸二酯和/或硫代磷酸酯鍵。在一些態樣中，當本發明的 dsRNA 劑是鈉鹽形式時，鈉離子作為試劑中存在的所有磷酸二酯和/或硫代磷酸酯基團的抗衡離子存在於試劑中。

【0185】 教示製備上述含磷鍵聯之代表性美國專利包括但不限於：美國專利號 3,687,808、4,469,863、4,476,301、5,023,243、5,177,195、5,188,897、5,264,423、5,276,019、5,278,302、5,286,717、5,321,131、5,399,676、5,405,939、5,453,496、5,455,233、5,466,677、5,476,925、5,519,126、 5,536,821、5,541,316、5,550,111、5,563,253、5,571,799、5,587,361、5,625,050、 6,028,188、6,124,445、6,160,109、6,169,170、6,172,209、6, 239,265、6,277,603、6,326,199、6,346,614、6,444,423、6,531,590、6,534,639、6,608,035、6,683,167、6,858,715、6,867,294、6,878,805、7,015,315、7,041,816、7,273,933 及 7,321,029，以及美國專利第 RE39464 號，各專利之所有內容藉由引用併入本文。

【0186】 其中不包括磷原子的經修飾的 RNA 主鏈具有由短鏈烷基或環烷基核苷間鍵聯、混合雜原子和烷基或環烷基核苷間鍵聯、或一個或多個短鏈雜原子或雜環核苷間鍵聯形成的主鏈。 這些包括具有嗎啉代鍵的那些(部分由核苷的糖部分形成)、矽氧烷主鏈、 硫化物、亞碸和碸主鏈、甲醯基和硫代甲醯基主鏈、亞甲基甲醯基和硫代甲醯基主鏈、含有主鏈的烯烴、胺基磺酸酯主鏈、 亞甲基亞胺基和亞甲基肼主鏈、磺酸鹽和磺醯胺主鏈、醯胺主鏈、以及其他混合了 N、O、S 和 CH2 成分的物質。

【0187】 教示上述寡核苷之製備的代表性美國專利包括但不限於：美國專利號 5,034,506、5,166,315、5,185,444、5,214,134、5,216,141、5,235,033、5,64,562、5,264,564、5,405,938、5,434,257、5,466,677、5,470,967、5,489,677、5,541,307、5,561,225、5,596,086、5,602,240、5,608,046、5,610,289、5,618,704、5,623,070、5,663,312、5,633,360、 5,677,437 及 5,677,439，上述各專利的所有內文藉由引用併入本文。

【0188】 合適的 RNA 模擬物被考慮用於本文提供的 iRNA，其中，核苷酸單元的糖和核苷間鍵，即主鏈，被新基團取代。保持鹼基單位用於與合適的核酸目標化合物雜交。一種如此的寡聚化合物，其中的 RNA 模擬物已顯示具有優異的雜交特性，被稱為肽核酸 (PNA)。在 PNA 化合物中，RNA 的糖主鏈被含有醯胺的主鏈取代，尤其是胺基乙基甘胺酸主鏈。核鹼基被保留並直接或間接地結合到主鏈的醯胺部分的氮雜氮原子上。教示 PNA 化合物之製備的代表性美國專利包括但不限於：美國專利號 5,539,082、5,714,331 及 5,719,262，其中各專利之全部內容藉由引用併入本文。適用於本發明之 iRNA 的其他 PNA 化合物描述於例如 Nielsen *et al.*, *Science*, 1991, 254, 1497-1500 中。

【0189】 本發明著重的一些態樣包含具有的硫代磷酸脂主鏈之 RNA 和具有雜原子主鏈之寡核苷，尤其是上述參照的美國專利第 5,489,677 號中--CH_2—NH--CH_2-、--CH_2--$N(CH_3)$--O--CH_2--[也稱為亞甲基(methylimino)或 MMI 主鏈]、--CH_2--O--$N(CH_3)$--CH_2--、--CH_2--$N(CH_3)$--$N(CH_3)$--CH_2—和--$N(CH_3)$--CH_2--CH_2--，以及上述參照美國專利第 5,602,240 號中的醯胺主鏈。在一些態樣中，本文所描述的 RNA 具有上述美國專利第 5,034,506 號中的 N-嗎啉基主鏈結構。天然磷酸二酯主鏈可表示為 O-P(O)(OH)-OCH2-。

【0190】修飾之 RNA 可以保留一個或更多個取代的糖部分。iRNA，例如於本文中著重的 dsRNA，可以包含在 2'位置中包含以下之一：OH；F；O-、S-、N-烷基；O-、S-、N-烯基；O-、S-、N-炔基；O-烷基-O-烷基，其中烷基、烯基、炔基可以被 C1 至 C10 烷基或 C2 至 C10 烯基和炔基取代或不取代。合適之修飾的示例包含 $O[(CH_2)_nO]_mCH_3$、$O(CH_2)_nOCH_3$、$O(CH_2)_nNH_2$、$O(CH_2)_nCH_3$、$O(CH_2)_nONH_2$，及 $O(CH_2)_nON[(CH_2)_nCH_3)]_2$，其 n 和 m 從 1 至大約 10。在其他態樣中，dsRNA 在 2'位置中包含以下之一：C1 至 C10 低級烷基、經取代之低級烷基、烷芳基、芳烷基、O-烷芳基或 O-芳烷基、SH、SCH_3、OCN、Cl、Br、CN、CF_3、OCF_3、$SOCH_3$、SO_2CH_3、ONO_2、NO_2、N_3、NH_2、雜環烷基、雜環烷芳基、胺烷基胺、聚烷基胺、經取代之矽基、RNA 分解基、信息基團、嵌入劑、改善 iRNA 的藥物動力特性之基團、或是改善 iRNA 的藥效動力特性之基團、和其他聚有相似特性的取代基。在一些態樣中，修飾包含 2'-甲氧乙氧(2'-O--$CH_2CH_2OCH_3$，也稱為 2'-O-(2-甲氧乙氧)或 2'-MOE)(Martin et al.；Helv. Chim. Acta, 1995, 78:486-504)亦即烷氧-烷氧基。另一個修飾之示例是 2'-二甲胺氧乙氧，亦即 $O(CH_2)_2ON(CH_3)_2$ 基，也稱為 2'-DMAOE，與本文以下示例所述，以及 2'-二甲胺乙氧乙氧(於所屬領域也稱為 2'-O-二甲胺乙氧乙基或 2'-DMAEOE)，亦即 2'-O--CH_2--O--CH_2--$N(CH_3)_2$。進一步示例的修飾包含：5'-Me-2'-F 核苷酸、5'-Me-2'-OMe 核苷酸、5'-Me-2'-去氧核苷酸(這三個家族中的 R 和 S 異構體)；2'-烷氧烷基；和 2'-NMA(N-甲基乙醯胺)。

【0191】其他修飾包含 2'-甲氧基(2'-OCH3)，2'-胺丙氧基(2'-$OCH_2CH_2CH_2NH_2$)和 2'-氟(2'-F)。相似的修飾也可以於 iRNA 中的 RNA 其他位置進行，尤其是 3'末端核苷酸的糖之 3'位置或 2'-5' 連結的 dsRNAs 和 5'末端核苷酸

的 5'位置。iRNA 也可以具有糖模擬物如環丁基部分代替呋喃戊糖基糖。教示製備此類修飾糖結構之代表性美國專利包括但不限於：美國專利號 4,981,957、5,118,800、5,319,080、5,359,044、5,393,878、5,446,137、5,466,786、5,514,785、5,519,134、5,567,811、5,576,427、5,591,722、5,597,909、5,610,300、5,627,053、5,639,873、5,646,265、5,658,873、5,670,633、及 5,700,920，上述某些與本申請共同擁有。前述各項的全部內容藉由引用併入本文。

【0192】iRNA 也可以包含核鹼基(通常於所屬領域僅係指「鹼基」)修飾或取代。於本文中使用，「未修飾」或「原生」核鹼基包含嘌呤鹼基腺嘌呤(A)和鳥嘌呤(G)，以及嘧啶鹼基胸腺嘧啶(T)、胞嘧啶(C)、尿嘧啶(U)。修飾之核鹼基包含其他合成和原生的核鹼基如去氧胸苷(dT)、5-甲基胞嘧啶(5-me-C)、5-羥甲基胞嘧啶、黃嘌呤、嘌呤酮、2-胺腺嘌呤、6-甲基和其他腺嘌呤和鳥嘌呤之烷基衍伸物、2-丙基和其他腺嘌呤和鳥嘌呤之烷基衍伸物、2-硫代尿嘧啶、2-硫代胸腺嘧啶和 2-硫代胞嘧啶、5-鹵代尿嘧啶和胞嘧啶、5-丙炔基尿嘧啶和胞嘧啶、6-偶氮尿嘧啶、胞嘧啶和胸腺嘧啶、5-尿嘧啶(假尿嘧啶)、4-硫代尿嘧啶、8-鹵代、8-胺基、8-硫醇、8-硫代烷基、8-羥基及其他 8-取代的腺嘌呤和鳥嘌呤、5-鹵代、尤其是 5-溴代、5-三氟甲基和其他 5-取代的尿嘧啶和胞嘧啶、7-甲基鳥嘌呤和 7-甲基腺嘌呤、8-氮雜鳥嘌呤和 8-氮雜腺嘌呤、7-去氮雜鳥嘌呤和 7-去氮雜腺嘌呤及 3-去氮雜鳥嘌呤和 3-去氮雜腺嘌呤。核鹼基進一步包含那些於美國專利第 3,687,808 號所揭示的、那些於 Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008 所揭示的修飾之核苷、那些於 The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990 所揭示者、由 Englisch *et al.*, Angewandte Chemie,

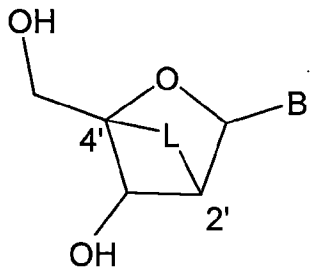
International Edition, 1991, 30, 613 et al.所揭示者、由 Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993 所揭示者。特定核鹼基尤其對於提高本文著重的寡聚化合物的結合親和力有效。這些包含 5-取代的嘧啶、6-氮雜嘧啶和 N-2、N-6 和 0-6 取代的嘌呤，包含 2-胺丙基腺嘌呤、5-丙炔基尿嘧啶和 5-丙炔基胞嘧啶。5-甲基胞嘧啶之取代已顯示提高核酸雙鏈體穩定性從 0.6 至 1.2°C(Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278)，且為示例性的鹼基取代，與 2'-O-甲氧乙氧糖修飾結合尤其提高更多。

【0193】 教示製備上述特定的修飾之核鹼基以及其他修飾之核鹼基之代表性美國專利包括不限於：上述美國專利號 3,687,808、4,845,205、5,130,30、5,134,066、5,175,273、5,367,066、5,432,272、5,457,187、5,459,255、5,484,908、5,502,177、5,525,711、5,552,540、5,587,469、5,594,121、5,596,091、5,614,617、5,681,941、5,750,692、6,015,886、6,147,200、6,166,197、6,222,025、6,235,887、6,380,368、6,528,640、6,639,062、6,617,438、7,045,610、7,427,672、及 7,495,088，各專利所有內容藉由引用併入本文。

【0194】 在一些態樣中，所揭示之 RNAi 劑也可以被修飾來包含一個或更多個雙環糖部分。「雙環糖」是呋喃環由連結兩個碳所形成的環所修飾，不論鄰近或不鄰近。「雙環核苷(bicyclic nucleoside；簡稱 BNA)」是一個具有糖部分包括由連結兩個碳所形成環的核苷之糖環，不論鄰近或不鄰近，從而形成雙環系統。在特定態樣中，鍵聯連接糖環 4'-碳和 2'-碳，可選擇地，透過 2'-非環氧原子。因此，在一些本發明藥劑的態樣中，可包含一或多個鎖核酸(LNA)。鎖核酸

是一個具有修飾之核糖部分包括額外的鍵聯連接的 2'和 4'碳之核苷酸。換言之，LNA 是一個包括雙糖部分包括 4'-CH2-O-2'鍵聯之核苷酸。這個結構有效地在 3'-內式結構構型中「鎖住」核糖。添加鎖核酸至 siRNA 已顯示提高 siRNA 於血清中的穩定性，且降低脫靶效應(Elmen, J. et al.,(2005) Nucleic Acids Research 33(1):439-447；Mook, OR. et al.,(2007) Mol Canc Ther 6(3):833-843；Grunweller, A. et al.,(2003) Nucleic Acids Research 31(12):3185-3193)。本發明之雙核苷使用於聚核苷酸之示例包含不限於包括 4'和 2' 核糖環原子間的鍵聯。在特定態樣中，本發明的反義多核苷酸試劑包括一種或多種包含 4'至 2'橋的雙環核苷。

【0195】 鎖核苷可以由以下結構表示(省略立體化學)



【0196】 其中，B 是核鹼基或修飾之核鹼基且 L 是連結核糖環 2'-碳至 4'-碳的聯結基。該 4'至 2'連結的雙環核苷的示例，包括但不限於：4'-(CH_2)—O-2'(LNA)、4'-(CH_2)—S-2'、4'-$(CH_2)_2$—O-2'(ENA)、4'-$CH(CH_3)$—O-2'(亦指「受限的乙基」或「cEt」)和 4'-$CH(CH_2OCH_3)$—O-2'(及其類似物；參見例如美國專利號 7,399,845)、4'-$C(CH_3)(CH_3)$—O-2'(及其類似物；參見例如美國專利號 8,278,283)、4'-CH_2—$N(OCH_3)$-2'(及其類似物；參見例如美國專利號 8,278,425)、4'-CH_2—O—$N(CH_3)$-2'(參見例如美國專利公開第 2004/0171570 號)；4'-CH_2—N(R)—O-2'，其中，R 是 H、C1-C12 烷基、或氮保護基(參見例如美國專利號 7,427,672)、4'-CH_2—$C(H)(CH_3)$-2'(參見例如 Chattopadhyaya et al., J. Org. Chem.,

2009, 74, 118-134)、4′-CH_2—C(=CH_2)-2′(及其類似物；參見例如美國專利號8,278,426)。各專利所有內文藉由引用併入本文。

【0197】 教示製備鎖核酸核苷酸之額外代表性美國專利包括但不限於：美國專利案號 6,268,490、6,525,191、6,670,461、6,770,748、6,794,499、6,998,484、7,053,207、7,034,133、7,084,125、7,399,845、7,427,672、 7,569,686、7,741,457、8,022,193、8,030,467、8,278,425、8,278,426、及 8,278,283、美國專利公開 US 2008/0039618 及 US 2009/0012281，各專利所有內文藉由引用併入本文。

【0198】 可以製備具有一種或多種立體化學糖構型的任何前述雙環核苷，包括例如 α-L-呋喃核糖和 β-D-呋喃核糖(參見 WO 99/14226)。

【0199】 iRNA 之 RNA 也可以被修飾來包含一個多個約束性乙基核苷酸。於本文中使用，「受限乙基核苷酸」或「cEt」是鎖核酸包括雙環糖部分包括 4'-$CH(CH_3)$-O-2'鍵聯(亦即在前述結構中的 L)。在一個實施方案中，受限乙基核苷酸處於 S 構形，在本文中稱為「S-cEt」。

【0200】 本發明 iRNA 也可包含一個或多個「構形限制性核苷酸(confrmationally restricted nucleotides；簡稱 CRN)。CRN 是核苷酸具有連接核糖 C2'和 C4'碳或核糖 C3 和-C5'碳核苷酸的連接子的類似物。CRN 將核糖環鎖定為穩定的構象，並增加與 mRNA 的雜交親和力。連接子具有足夠的長度以將氧置於穩定性和親和力的最佳位置，從而減少核糖環起皺。

【0201】 教示製備特定上述 CRN 之代表性專利公開包含括但不限於美國專利公開號 2013/0190383 及 PCT 專利公開號 WO 2013/036868，各公開的所有內容藉由引用併入本文。

【0202】在一些態樣中，本發明之 iRNA 包括一個或多個單體，其為 UNA(未鎖核酸)核苷酸。UNA 還包括 C1' -C4' 之間的鍵已被移除的單體(即共價碳-氧-C1' 和 C4' 碳之間的碳鍵)。在另一個例子中，糖的 C2'-C3' 鍵(即 C2' 和 C3' 碳之間的共價碳-碳鍵)已被去除(參見 Nuc . Acids Symp. Series, 52, 133-134 (2008) 和 Fluiter et al., Mol. Biosyst., 2009, 10, 1039 ，以引用方式併入本文)。

【0203】教示製備特定上述 UNA 之代表性美國專利包括但不限於美國專利案號 8,314,227、美國專利公開案號 2013/0096289、2013/0011922 及 2011/0313020，上述專利之所有內容以引用方式併入本文。

【0204】RNA 分子末端潛在地穩定的修飾可包含 N-(乙醯胺 caproyl)-4-羥脯胺酸(Hyp-C6-NHAc)、N-(己醯-4-羥脯胺酸(Hyp-C6)、N-(乙醯-4-羥脯胺酸(Hyp-NHAc)、胸腺嘌呤-2'-O-去氧胸腺嘌呤(乙醚), N-(胺己醯基)-4-羥脯胺酸(Hyp-C6-胺)、2-二十二醯基-尿核苷-3'- 磷酸酯、反向的 2'-去氧-修飾之核糖核酸，如反向的 dT(idT)、反向的 dA(idA)、反向的無鹼基 2'-去氧核糖核酸(iAb)和其他。該修飾之揭示可於 WO 2011/005861 查閱。

【0205】在一個示例中，寡核苷酸的 3'或 5'末端與反向的 2'-去氧-修飾之糖核核酸連結，如反向的 dT(idT)、反向的 dA(idA)、或反向的無鹼基 2'-去氧糖核核酸(iAb)。在一個特定的示例中，反向的 2'-去氧-修飾之糖核核酸與寡核苷酸的 3'末端連結，如本文所述之有義股的 3'-末端，其藉由經由 3'-3'磷酸二酯鍵或 3'-3'-硫代磷酸酯連結。

【0206】在另一示例中，有義股之 3'末端透過 3'-3'-硫代磷酸酯鍵與反向的無鹼基核糖核酸(iAb)連結。在另一示例中，之 3'末端透藉由 3'-3'-硫代磷酸酯鍵與反向的 dA(idA)連結。

【0207】 在一特定的示例中，反向的 2'-去氧-修飾之核糖核酸寡核苷之 3' 末端連結，如本文所述之有義股 3'-末端，其透藉由 3'-3'磷酸二酯鍵或 3'-3'-硫代磷酸酯鍵連結。

【0208】 在另一示例中，有義股之 3'-末端核苷酸是反向的 dA(idA)，且與前述的核苷酸透藉由 3'-3'-鍵聯連結(例如 3'-3'-硫代磷酸酯鍵)。

【0209】 其他本發明 iRNA 之核苷酸修飾包含 5'磷酸酯或 5'磷酸酯模擬物，例如 5'-末端磷酸酯或 iRNA 反義股之磷酸酯模擬物。適合的磷酸酯模擬物於例如美國專利公開 2012/0157511 中揭示，其所有內容藉由引用併入本文。

【0210】 *A.修飾之 iRNAs 包括本發明之模體*

在本發明特定的面向中，本發明之雙股 RNA 劑包含具有所揭示的化學修飾之劑，例如，揭示於 WO2013/075035 中，其公開之所有內容藉由引用併入本文。於本文及 WO2013/075035 中所揭，三個連續的核苷酸的三個相同修飾之一個或多個模體可被引入 dsRNAi 劑中的有義股或反義股，尤其位在或接近裂解位點。在一些態樣中，dsRNAi 劑中的有義股或反義股可以另外的方式被完全地修飾。引入此類模體中斷了有義或反義股之修飾模式(如果存在)。dsRNAi 劑可任選地與 GalNAc 衍生物配體接合，例如在有義股。

【0211】 更具體地，當雙股 RNA 劑的有義股和反義股被完全修飾以在 dsRNAi 劑的至少一股的裂解位點處或附近的三個連續核苷酸具有三個相同修飾的一個或多個模體時，觀察到 dsRNAi 劑的基因沉默活性。

【0212】 據此，本發明提供能在體內抑制目標基因(亦即 F12 基因)的雙股 RNA 劑。RNAi 劑包括有義股和反義股。各 RNAi 劑之股可以是，例如長度為 17 至 30 個核苷酸、長度為 25 至 30 核苷酸、長度為 27 至 30 個核苷酸、長度為 19

至 25 核苷酸、長度為 19 至 23 個核苷酸、長度為 19 至 21 個核苷酸、長度為 21 至 25 核苷酸、長度為 21 至 23 個核苷酸。

【0213】 有義股和反義股通常形成雙股(「dsRNA」)，也於本文中指「dsRNAi 劑」。dsRNAi 劑之雙股區域可以是，例如雙股區域長度為 27 至 30 個核苷酸對、長度為 19 至 25 個核苷酸對、長度為 19 至 23 個核苷酸對、長度為 19 至 21 個核苷酸對、長度為 21 至 25 個核苷酸對、或長度為 21 至 23 個核苷酸對。在另一示例中，雙股區域長度為 19、20、21、22、23、24、25、26 或 27 個核苷酸。

【0214】 在特定態樣中，dsRNAi 劑可包含在 3'-末端、5'-末端、或一或兩股之兩個末端中一個或多個突出區域或封端基。突出可以是，獨立地，長度為 1 至 6 個核苷酸，例如長度為 2 至 6 個核苷酸、長度為 1 至 5 個核苷酸、長度為 2 至 5 個核苷酸、長度為 1 至 4 個核苷酸、長度為 2 至 4 個核苷酸、長度為 1 至 3 個核苷酸、長度為 2 至 3 個核苷酸、或長度為 1 至 2 個核苷酸。在特定態樣中，突出區域可以包含上述提供的延伸突出區域。突出可以是一股長於另一股的結果，或是長度一樣的兩股被交錯之結果。突出可以與目標 mRNA 形成錯配，或其與被靶向的基因序列或可以是其他序列互補。第一股和第二股也可以被連結，例如藉由外加的鹼基形成髮夾形，或藉由非鹼基鍵聯。

【0215】 在特定態樣中，dsRNAi 劑的突出區域中的核苷酸可以各自獨立地是修飾或未修飾的核苷酸，包括但不限於 2'-糖修飾的，例如 2'-F、2'-O-甲基，胸苷 (T)、2`-O-甲胺基乙基-5-甲基尿苷 (Teo)、2`-O-甲胺基乙基腺苷 (Aeo)、2`-O-甲氧基乙基-5-甲基胞苷 (m5Ceo) 及其任意組合。

【0216】 例如，TT 可以是突出序列於任一股之任一末端。突出可以與 mRNA 形成錯配，或其與被目標靶向的基因序列互補或可以是與其他序列互補。

【0217】 位於有義股、反義股、或 dsRNAi 劑的雙股之 5’-或 3’-突出可以被磷酸化。在一些態樣中，突出區域包含兩個核苷酸，於兩個核苷酸間具有硫代磷酸酯，該兩個核苷酸可以相同或不同。在一些態樣中，突出存在於有義股、反義股、或兩股的 3’-末端。在一些態樣中，該 3’-突出呈現於反義股。在一些態樣中，該 3’-突出存在於有義股。

【0218】 dsRNAi 劑可只包含單一突出，其可增強 RNAi 干擾活性而不影響其整體的穩定性。例如，單股突出可以位在有義股的 3’-末端，或者，位在反義股 3'-末端。RNAi 也可具有平端，位在反義股的反義股(亦即有義股的 3’-末端)或反之亦然。通常地，dsRNAi 劑之反義股具有於 3’-末端的核苷酸突出，而 5’-末端為平端。儘管不欲受理論束縛，但反義股 5'-端的不對稱平端和反義股的 3'-端突出有利於將引導股加載到 RISC 過程中。

【0219】 在特定態樣中，dsRNAi 劑是長度為 19 個核苷酸之雙平端，其中有義股包含位在 5’末端位置 7、8、9 三個連續的核苷酸的三個 2’-F 修飾的至少一個模體。反義股包含位在 5’末端位置 11、12、13 三個連續的核苷酸的三個 2’-O-甲基修飾的至少一個模體。

【0220】 在其他態樣中，dsRNAi 劑是長度為 20 個核苷酸之雙平端，其中有義股包含位在 5’末端位置 8、9、10 三個連續的核苷酸的三個 2’-F 修飾的至少一個模體。反義股包含位在 5’末端位置 11、12、13 三個連續的核苷酸的三個 2’-O-甲基修飾的至少一個模體。

【0221】 尚在其他態樣中，dsRNAi 劑是長度為 21 個核苷酸之雙平端，其中有義股包含位在 5’末端位置 9、10、11 三個連續的核苷酸的三個 2’-F 修飾的至少一個模體。反義股包含位在 5’末端位置 11、12、13 三個連續的核苷酸的三個 2’-O-甲基修飾的至少一個模體。

【0222】 在特定態樣中，dsRNAi 劑包括 21 個核苷酸有義股和 23 個核苷酸反義股，包含位在 5’末端位置 9、10、11 三個連續的核苷酸的三個 2’-F 修飾的至少一個模體；反義股包含位在 5’末端位置 11、12、13 三個連續的核苷酸的三個 2’-O-甲基修飾的至少一個模體，其中 RNAi 劑是平端，而另一末端包括 2 個核苷酸突出。在一個態樣中，2 個核苷酸突出是位在反義股的 3’-末端。

【0223】 當 2 個核苷酸突出是位在反義股的 3’-末端，其可於三個核苷酸末端間，其中三個核苷酸中的其中兩個是突出核苷酸，而第三個核苷酸是配對的核苷酸位在突出核苷酸旁邊，具有兩個硫代磷酸酯核苷酸間鍵聯。在一個態樣中，RNAi 劑額外具有兩個硫代磷酸酯核苷酸間鍵聯，介於三個核苷酸末端間，同時位在有義股 5’-末端和反義股 5’-末端。在特定態樣中，每一個 dsRNAi 劑中有義股和反義股的核苷酸，包含模體中一部份的核苷酸是修飾之苷酸。在特定態樣中各殘基與 2’-O-甲基或 3’-氟代，例如於替代的模體中，獨立地被修飾。可選擇地，dsRNAi 劑進一步包括配體(如 GalNAc3)。

【0224】 在特定態樣中，dsRNAi 劑包括有義和反義股，其中有義股長度為 25 至 30 個核苷酸殘基，其中起始於第一股 5’末端核苷酸(位置 1)位置 1 至 23 包括 8 個核糖核苷酸；反義股長度為 36 至 66 個核苷酸殘基，起始於第一股 3’末端核苷酸包括至少 8 個核糖核苷酸，與有義股位置 1 至 23 配對形成雙鏈體；其中反義股的 3'末端核苷酸不與有義股配對，且最多 6 個連續的 3'末端核苷酸不與

有義股配對，從而形成 1 至 6 個核苷酸的 3’單股突出；其中反義股 5'末端包括從 10 至 30 個連續的核苷酸其不與有義股配對，從而形成 10 至 30 個核苷酸單股的 5’突出；其中當有義和反義股對齊以獲得最大化互補性時，至少有義股 5’末端和 3’末端核苷酸與反義股的核苷酸鹼基配對，從而在有義股和反義股之間形成實質上雙鏈的區域；當雙股核酸被引入哺乳類細胞時，反義股與目標 RNA 有效地互補與至少長度為 19 個反義股的核糖核苷酸來降低目標基因的表現；其中有義股包含三個連續的核苷酸的三個 2’-F 修飾的至少一個模體，其至少一個模體出現於或鄰近於裂解位點。反義股三個連續的核苷酸的三個 2’-O-甲基修飾的至少一個模體位於或鄰近於裂解位點。

【0225】 在特定態樣中，dsRNAi 劑包括有義和反義股，其中 dsRNAi 劑包括第一股具有至少長度 25 個核苷酸且最多 29 個核苷酸，第二股具有長度最多 30 個核苷酸與至少 5’末端位置 11、12、13 三個連續的核苷酸的三個 2’-O-甲基修飾的至少一個模體；其中第一股的 3’末端和第二股的 5’末端形成平端，且第二股是 1 至 4 個核苷酸於其 3’末端比第一股長，其中雙股區域，其長度至少為 25 個核苷酸且第二股有效地與目標 mRNA 互補與第二股至少 19 個核苷酸來降低目標基因的表現，當 RNAi 劑引入哺乳類細胞時，其中 dsRNAi 劑之 Dicer 分解導致 siRNA 包括第二股的 3’-末端，因此降低了哺乳類目標基因的表現。可選擇地，dsRNAi 劑進一步包括配體。

【0226】 在特定態樣中，dsRNAi 劑之有義股包含三個連續的核苷酸的三個相同修飾中至少一個模體，其中一個模體出現於有義股的裂解位點。

【0227】 在特定態樣中，dsRNAi 劑之反義股也可以包含三個連續的核苷酸的三個相同修飾的至少一個模體，其中一個模體出現於或鄰近有義股的裂解位點。

【0228】 對於具有長度為 19 至 23 個核苷酸的雙股區域之 dsRNAi 劑，裂解位點通常位於 5'-末端的位置 10、11、12。因此三個相同修飾之模體可出現於反義股位置 9、10、11；位置 10、11、12；位置 11、12、13；位置 12、13、14；位置 13、14、15，由反義股 5'-末端的第一個核苷酸開始計數，或是由反義股 5'-末端的雙股區域第一配對的核苷酸開始計數。反義股的裂解位點也可根據來自 dsRNAi 劑雙股區域的長度而從 5'-末端變化。

【0229】 dsRNAi 劑之有義股也可以在股的裂解位點包含三個連續的核苷酸的三個相同修飾的至少一個模體；反義股可以位在或鄰近股的裂解位點包含三個連續的核苷酸的三個相同修飾的至少一個模體。當有義股和反義股形成 dsRNA 雙鏈體，有義股和反義股也可以如此排列，使得有義股三個連續的核苷酸之一個模體和反義股三個連續的核苷酸之一個模體具有至少一個核苷酸重疊，即有至少一個核苷酸重疊，亦即有義股模體至少三個核苷酸中的一個與反義股模體至少三個核苷酸中的一個形成鹼基配對。額外地，至少兩個核苷酸可以重疊，或是三個核苷酸可以重疊。

【0230】 在一些態樣中，dsRNAi 劑之有義股可以在三個連續的核苷酸三個相同修飾包含多於一個模體。第一個模體可出現於或鄰近於股的裂解位點且另一個模體可以是翼修飾(wing modification)。本文術語「翼修飾」係指出現於股的另一部分的模體，其與模體出現於或鄰近於相同股的裂解位點是獨立的。翼修飾不是鄰近於第一個模體就是被至少一個或多個核苷酸分隔。當模體緊接地鄰

近於彼此時，模體的化學特性與彼此不同，當模體被一個或多個核苷酸分隔時，化學特性是相同或不同。可能存在兩個或多個翼修飾，例如，當存在兩個翼修飾時，各翼修飾可出現於相對於第一個模體的一端，該模體位於或鄰近於裂解位點或是前導模體的任一端。

【0231】 與有義股類似，dsRNAi 劑之反義股可以包含在三個連續的核苷酸三個相同修飾的多於一個模體，至少有一個模體位於或鄰近於股的裂解位點。該反義股還可包含一或多個與可能存在於有義股之翼修飾類似排列的翼修飾。

【0232】 在一些態樣中，dsRNAi 劑的有義股或反義股之翼修飾通常並不包含在股的 3'-末端、5'-末端、或兩末端中的第一或二末端核苷酸。

【0233】 在其他態樣中，dsRNAi 劑的有義股或反義股之翼修飾通常並不包含在股的 3'-末端、5'-末端、或兩末端中雙股區域的第一或二配對的核苷酸。

【0234】 當 dsRNAi 劑的有義股或反義股各包含一個翼修飾，翼修飾可以位在雙股區域相同的模端，且具有一、二、三個核苷酸重疊。

【0235】 當 dsRNAi 劑的有義股或反義股各包含兩個翼修飾，有義股和反義股可以這樣排列，使來自其中一股的兩個修飾各位在雙股區域的一末端，具有一、二、三個核苷酸重疊；來自其中一股的兩個修飾各位在雙股區域的另一末端，具有一、二、三個核苷酸重疊；來自其中一股的兩個修飾位在主要模體的各末端，於雙股區域具有一、二、三個核苷酸重疊。

【0236】 在一些態樣中，dsRNAi 劑的有義股或反義股之每一個核苷酸，包含模體一部份的核苷酸，可以被修飾。各核苷酸可以被相同或不同地修飾，其可以包括一個或兩個非連結磷酸酯的氧或是一個或多個連結磷酸酯的氧之一個或多個修飾；核糖成分的改變，例如核糖的組成部分的改變，例如核糖的 2′-羥基；

全部的將磷酸酯部分以「去磷酸」連接子取代；修飾或取代天然出現的鹼基；以及取代或修飾核糖-磷酸主鏈。

【0237】 因為核酸是次單元的聚合物，許多修飾出現於核酸內重複的位置，例如修飾鹼基、磷酸酯部分、磷酸酯部分非連結的 O。在一些態樣中，修飾會出現於核酸主要位置，但在許多態樣中並不是如此。作為示例，修飾可只出現在 3'-或 5'末端位置，可僅出現在末端區域，例如在末端核酸的位置或在股的最後 2、3、4、5 或 10 個核苷酸。修飾可出現於雙股區域、單股區域、或兩者。修飾可只出現於 RNA 雙股區域或可只出現於 RNA 單股區域。例如在非連結的氧位置之硫代磷酸酯修飾可只出現其中一末端或兩端，可出現在末端區域，例如在末端核苷酸的位置或在股的最後 2、3、4、5 或 10 個核苷酸，或出現於雙股區域和單股區域，尤其在末端。5'-末端或末端可以被磷酸化。

【0238】 例如，為提高穩定性，在單股突出中，包含突出特定鹼基或包含修飾之核苷酸或核苷酸替代物，例如在 5'-或 3'-突出中，或在兩者中。例如，可需要在突出中包含嘌呤核苷酸。在一些態樣中，所有或一些在 3'- 或 5'-突出的鹼基可以被修飾，例如本文所述之修飾。修飾可以包含，例如使用所屬領域習知的修飾在核糖 2'的位置進行修飾，例如使用去氧核糖核苷酸、2'-去氧-2'-氟(2'-F)或 2'-O-甲基修飾而不是核鹼基的核糖，以及磷酸基的修飾，例如硫代磷酸酯修飾。突出不需要與目標序列同源。

【0239】 在一些態樣中，有義股和反義股各殘基是獨立地被 LNA、CRN、cET、UNA、HNA、CeNA、2'-甲氧基乙基、2'- O-甲基、2'-O-烯丙基、2'-C-烯丙基、2'-去氧、2'-羥基、2'-氟所修飾。股可以包含多於一個修飾。在一個態樣中，有義股和反義股各殘基是獨立地被 2'- O-甲基或 2'-氟所修飾。

【0240】 至少兩個不同修飾通常存在於有義股和反義股。該兩種修飾可以是 2'- O-甲基或 2'-氟修飾，或其他。

【0241】 在特定態樣中，N_a或 N_b包括交替模式。本文使用術語「交替模體」係指具有一個或多個修飾的模體，每個修飾出現於一股的交替核苷酸。替代的核苷酸可以指其他所有核苷酸中每隔一個或是每三個核苷酸，或是相似的模式。例如，假設 A、B、C 各自表示核苷酸的一種修飾型態，替代的模體可以是「ABABABABABAB...,」、「AABBAABBAABB...」、「AABAABAABAAB...」、「AAABAAABAAAB...」、「AAABBBAAABBB...」、「ABCABCABCABC...」等。

【0242】 於交替模體中的修飾模式可以是相同或不同的。例如，假設 A、B、C 各表示核苷酸的一種修飾，則交替模式，即每隔一個核苷酸進行修飾，可以是相同的，但各有義股或反義股皆可以選自交替模體中數個修飾可能性，如「ABABAB...」、「ACACAC...」、「BDBDBD...」、「CDCDCD...」等。

【0243】 在一些態樣中，本發明的 dsRNAi 劑包括有義股的交替模體之修飾型態相對於反義股的交替模體之修飾型態是發生移位的。移位可以是該有義股的核苷酸修飾基對應於反義股的核苷酸修飾基，反之亦然。例如，當在 dsRNA 雙股中有義股與反義股鹼基配對時，在雙股區域中有義股的交替模體可從股的 5'至 3'以「ABABAB」開始，而反義股的交替模體可從股的 5'至 3'以「BABABA」起始。作為另一個示例，在雙股區域中有義股的交替模體可從股的 5'至 3'以「AABBAABB」起始，而反義股的交替模體可從股的 5'至 3'以「BBAABBAA」起始，使得有義股和反義股之間具有完全的或部分修飾型態移位。

【0244】 在一個特定的示例中，有義股的交替模體以「ABABAB」從股的 5’至 3，其各 A 是未修飾之核糖核酸且各 B 是 2’-O 甲基修飾之核苷酸。

【0245】 在一個特定的示例中，有義股的交替模體以「ABABAB」從股的 5’至 3，其各 A 是 2’-去氧-2’-氟修飾之核苷酸且各 B 是 2’-O 甲基修飾之核苷酸。

【0246】 在另一個特定的示例中，反義股的交替模體以「BABABA」從股的 5’至 3，其各 A 是 2’-去氧-2’-氟修飾之核苷酸且各 B 是 2’-O 甲基修飾之核苷酸。

【0247】 在一個特定的示例中，有義股的交替模體是從股的 5’至 3 開始的「ABABAB」，且反義股的交替模體是從股的 5’至 3 開始的「BABABA」，其各 A 是未修飾之核糖核酸且各 B 是 2’-O 甲基修飾之核苷酸。

【0248】 在一個特定的示例中，有義股的交替模體是從股的 5’至 3 開始的「ABABAB」，且反義股的交替模體是從股的 5’至 3 開始的「BABABA」，其各 A 是 2’-去氧-2’-氟修飾之核苷酸，且各 B 是 2’-O 甲基修飾之核苷酸。

【0249】 在一些態樣中，dsRNAi 劑包含有義股的 2’-O-甲基修飾和 2’-F 修飾的交替模體的模式，最初相對於 2’-O-的交替模體的模式具有偏移。最初在反義股進行甲基修飾和 2’-F 修飾，即，有義股的 2’-O-甲基修飾的核苷酸與反義股的 2’-F 修飾的核苷酸鹼基配對，反之亦然。有義股的位置 1 可從 2'-F 修飾開始，反義股的位置 1 可從 2'- O-甲基修飾開始。

【0250】 引入三個連續的核苷酸的三個相同修飾之一個或多個模體至有義股或反義股打斷了有義股或反義股存在的初始修飾型態。這種透過在有義或反義股的三個連續核苷酸引入三個相同修飾的一個或多個模體來中斷有義或反義股的修飾模式可以增強針對靶基因的基因沉默活性目標。

【0251】在一些態樣中，三個連續的核苷酸的三個相同修飾之一個模體被引入任一股，模體旁的核苷酸修飾與模體修飾是不同的修飾。例如包含模體的一部份序列是「...N_aYYYN_b...」，其「Y」表示三個連續的核苷酸的三個相同修飾之一個模體的修飾，且「N_a」和「N_b」表示模體「YYY」旁的核苷酸修飾，其與 Y 修飾是不同的，N_a和 N_b可以是相同或不同的修飾。相對地，當翼修飾存在時，N_a和 N_b可以存在或不存在。

【0252】iRNA 可進一步包括至少一硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯。硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯修飾可出現於有義股、反義股、或兩股任一位置中任一核苷酸。例如核苷酸間鍵聯修飾可以出現於有義股或反義股的所有核苷酸；各核苷酸間鍵聯修飾可出現於有義股或反義股上的替代模式；或有義股或反義股可同時包含於替代模式的核苷酸間鍵聯修飾。有義股核苷酸間鍵聯修飾之替代模式可與反義股相同或不同，有義股核苷酸間鍵聯修飾之替代模式可相對於反義股核苷酸間鍵聯修飾之替代模式具有移位。在一個態樣中，雙股 RNAi 劑包括 6 到 8 個硫代磷酸酯核苷酸間鍵聯。在一些態樣中，反義股包括在 5'-末端兩個硫代磷酸酯核苷酸間鍵聯，在 3'-末端兩個硫代磷酸酯核苷酸間鍵聯，有義股在 5'-末端或 3'-末端包括至少兩個硫代磷酸酯核苷酸間鍵聯。

【0253】在一些態樣中，dsRNAi 劑在突出區域包含硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯修飾。例如，突出區域可包含兩個核苷酸，在這兩個核苷酸間具有硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯。核苷酸間鍵聯修飾也可作為在雙股區域將突出核苷酸與末端配對的核苷酸連接。例如，至少 2、3、4 或所有突出核苷酸可經由硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯被連結，可選擇地，可有外加的硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯與突出核苷酸連結與突出核苷酸旁

邊的配對的核苷酸。例如，可至少有兩個硫代磷酸酯核苷酸間鍵聯介於末端三個核苷酸中，其三個核苷酸中的兩個是突出核苷酸，而第三個是位在突出核苷酸旁邊的配對的核苷酸。這些三個末端核苷酸可位於反義股 3'-末端、有義股 3'-末端、反義股 5'-末端或反義股 5'末端。

【0254】 在一些態樣中，2-核苷酸突出是位在反義股 3'-末端，且有兩個硫代磷酸酯核苷酸間鍵聯介在三個末端核苷酸，其中三個核苷酸中的兩個為突出核苷酸，第三個核苷酸是緊鄰突出核苷酸的成對核苷酸。可選擇地，dsRNAi 劑在有義股的 5' 端和反義股的 5' 端的末端三個核苷酸之間可以另外具有兩個硫代磷酸酯核苷酸間鍵。

【0255】 在一個態樣中，dsRNAi 劑包括與目標、雙股內、或其組合的錯配。錯配可出現在突出區域或雙股區域。鹼基對可由其促進解離或解鏈的傾向進行排序(例如，根據特定配對的締合自由能或解離能，最簡單的方法是在各配對基礎上檢查鹼基對，也可以使用藉由近鄰分析或相似分析)。就促解離而言：A:U 優於 G:C；G:U 優於 G:C；I:C 優於 G:C (I=肌核苷)。錯配，例如非典型或其他典型配對(於本文他處所述)之外的配對優於典型(A:T、A:U、G:C)配對；並且包含通用鹼基之配對優於典型配對。

【0256】 在特定態樣中，dsRNAi 劑在雙股區域中包括至少最前面 1、2、3、4、5 鹼基對從反義股 5'-末端獨立地選自由 A:U、G:U、I:C，以及錯配的配對組成之組，例如非典型或其他典型配對或包含通用鹼基之配對，來促進雙股 5'-末端的反義股之解離。

【0257】 在特定態樣中，反義股 5'-末端的雙股區域在位置 1 的核苷酸是選自 A、dA、dU、U、dT。可選地，雙股區域內從反義股 5'-末端開始的 1、2、3

個鹼基對至少一個是 AU 鹼基對。例如，雙股區域中從反義股 5' 端開始的第一個鹼基對是 AU 鹼基對。

【0258】 在其他態樣中，在有義股 3'-末端的核苷酸是去氧胸苷(dT)或反義股 3'-末端的核苷酸是去氧胸苷(dT)。例如，具有去氧胸苷核苷酸短序列，例如，在有義、反義股、或兩者 3'-末端的兩個 dT 核苷酸。

【0259】 在特定態樣中，有義股序列可由化學式(I)表示：

$$5'\ n_p\text{-}N_a\text{-}(XXX)_i\text{-}N_b\text{-}YYY\text{-}N_b\text{-}(ZZZ)_j\text{-}N_a\text{-}n_q\ 3' \qquad (I)$$

其中：

i 和 j 各獨立地為 0 或 1；

p 和 q 各獨立地為 0 至 6；

各 N_a 獨立地表示包括 0 至 25 個修飾之核苷酸的寡核苷序列，各序列包括至少兩個不同修飾之核苷酸；

各 N_b 獨立地表示包括 0 至 10 修飾之核苷酸的寡核苷序列；

各 n_p 和 n_q 獨立地表示突出核苷酸；

其中 N_b 和 Y 不具有相同修飾；且

XXX、YYY、ZZZ 各獨立地表示三個連續核苷酸上的三個相同修飾之模體。在一個態樣中，YYY 是所有 2'-F 修飾之核苷酸。

【0260】 在一些態樣中，Na 或 Nb 包括替代模式之修飾。

【0261】 在一些態樣中，YYY 模體出現在或鄰近有義股裂解位點。例如，當 dsRNAi 劑長度為 17 至 23 個核苷酸之雙股區域，YYY 模體可以出現在或鄰近有義股裂解位點(例如可以出現在位置 6、7、8；7、8、9；8、9、10；9、10、

11；10、11、12；或 11、12、13)，由 5'-末端第一個核苷酸起計數；或可選地，由雙股區域 5'-末端第一個配對的核苷酸起計數。

【0262】 在一個態樣中，i是 1 和 j是 0，或 i是 0 和 j是 1，或 i和 j都是 1。有義股因此可以下列化學式表示：

5' n_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib)；

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic)；或

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id)。

【0263】 當有義股由化學式(Ib)表示，N_b 表示包括 0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、0 個修飾之核苷酸的寡核苷序列。各 N_a 獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0264】 當有義股由化學式(Ic) 表示，N_b 表示包括 0 至 10、0 至 7、0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、0 修飾之核苷酸的寡核苷序列。各 N_a 可獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0265】 當有義股由化學式(Id)表示，各 N_b 獨立地表示包括 0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、0 個修飾之核苷酸的寡核苷序列。在一個態樣中，N_b 是 0、1、2、3、4、5、6。各 N_a 可獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0266】 各 X、Y、Z 可與彼此相同或不同。

【0267】 在其他態樣中，i是 0 和 j是 0，有義股可以下述化學式表示：

5' n_p-N_a-YYY- N_a-n_q 3'　(Ia)。

【0268】 當有義股由化學式(Ia)表示，各 N_a 可獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0269】在一個態樣中，RNAi之反義股序列可以化學式(II)表示：

$$5'\ n_{q'}\text{-}N_a'\text{-}(Z'Z'Z')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(X'X'X')_l\text{-}N'_a\text{-}n_p'\ 3' \quad (II)$$

其中：

k和l各獨立地為0或1；

p'和q'各獨立地為0至6；

各N_a'獨立地表示包括0至25個修飾之核苷酸的寡核苷序列，各序列包括至少兩個不同的修飾之核苷酸；

各N_b'獨立地表示包括0至10個修飾之核苷酸的寡核苷序列；

各n_p'和n_q'獨立地表示突出核苷酸；

其中N_b'和Y'不具有相同的修飾；且

X'X'X'、Y'Y'Y'、Z'Z'Z'各獨立地表示三個連續的核苷酸的三個相同修飾之一個模體。

【0270】在一些態樣中，Na'或Nb'包括替代模式之修飾。

【0271】Y'Y'Y'模體出現於或鄰近於反義股裂解位點。例如，當dsRNAi劑長度為17至23個核苷酸之雙股區域，Y'Y'Y'模體可以出現在反義股位置9、10、11；10、11、12；11、12、13；12、13、14；13、14、15)，由5'-末端第一個核苷酸起計數；或可選擇地，雙股區域5'-末端第一個配對的核苷酸起計數。在一個態樣中，Y'Y'Y'模體出現在位置11、12、13。

【0272】在其他態樣中，Y'Y'Y'模體是所有2'-OMe修飾之核苷酸。

【0273】在其他態樣中，k是1和l是0，或k是0和l是1，或k和l都是1。

【0274】反義股因此可以由以下化學式表示：

5' $n_{q'}$-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'-n_p' 3' (IIb)；

5' $n_{q'}$-N_a'-Y'Y'Y'-N_b'-X'X'X'-n_p' 3' (IIc)；或

5'$n_{q'}$-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_b'-X'X'X'-N_a'-n_p'3' (IId)。

【0275】當反義股由化學式(IIb)表示，N_b'表示包括 0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、0 個修飾之核苷酸的寡核苷序列。各 N_a'獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0276】當反義股由化學式(IIc)表示，N_b'表示包括 0 至 10、0 至 7、0 至 7、0 至 5、0 至 4、0 至 2、或 0 個修飾之核苷酸的寡核苷序列。各 N_a'獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0277】當反義股由化學式(IId)表示，各 N_b'獨立地表示包括 0 至 10、0 至 7、0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、或 0 個修飾之核苷酸的寡核苷序列。各 N_a'獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。在一個態樣中，N_b是 0、1、2、3、4、5、6。

【0278】在其他態樣中，k 是 0 和 l 是 0 且反義股可由下式表示：

5' n_p'-N_a'-Y'Y'Y'-N_a'-n_q' 3'　(Ia)。

【0279】當反義股由化學式(IIa)表示，各 N_a'獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0280】各 X'、Y'、Z'可與彼此相同或不同。

【0281】各有義股和反義股之核苷酸可獨立地被 LNA、CRN、UNA、cEt、HNA、CeNA、2'-甲氧基乙基、2'-O-甲基、2'-O-烯丙基、2'-C-烯丙基、2'-羥基、2'-氟所修飾。例如，各有義股和反義股之核苷酸可獨立地被 2'-O-甲基或 2'-氟

所修飾。各 X、Y、Z、X′、Y′、Z′，尤其是，可表示 2’-O-甲基之修飾或 2’-氟之修飾。

【0282】 在一些態樣中，dsRNAi 劑有義股可包含 YYY 模體出現在位置 9、10、11，當雙股區域是 21nt，由 5’-末端第一個核苷酸起計數；或可選地，由雙股區域 5’-末端第一個配對的核苷酸起計數；Y 表示 2’-F 修飾。有義股可額外地包含 XXX 模體或 ZZZ 模體且翼修飾位在雙股區域相反的末端；XXX 和 ZZZ 各獨立地表示 2’-OMe 修飾或 2’-F 修飾。

【0283】 在一些態樣中，反義股可包含 Y′Y′Y′模體出現在位置 11、12、13，由 5’-末端第一個核苷酸起計數；或可選地，由雙股區域 5’-末端第一個配對的核苷酸起計數；Y 表示 2’-O-甲基修飾。反義股可額外地包含 X′X′X′模體或 Z′Z′Z′模體且翼修飾位在雙股區域相反的末端；且 X′X′X′和 Z′Z′Z′各獨立地表示 2’-OMe 修飾或 2’-F 修飾。

【0284】 由上述化學式(Ia)、(Ib)、(Ic)、(Id)任一者表示的有義股，與化學式(IIa)、(IIb)、(IIc)、(IId)任一者表示的反義股形成雙鏈體。

【0285】 據此，用於本發明方法的 dsRNAi 劑可包括有義股和反義股，各股具有 14 至 30 個核苷酸，iRNA 雙鏈體由式(III)表示：

有義：5' n_p -N_a-(X X X)$_i$ -N_b- Y Y Y -N_b -(Z Z Z)$_j$-N_a-n_q 3'

反義：3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'-n_q' 5'

(III)

其中：

i、j、k、l 各獨立地為 0 或 1；

p、p′、q、q′各獨立地為 0 至 6；

各 N_a 和 N_a'獨立地表示包括 0 至 25 個修飾之核苷酸的寡核苷序列，各序列包括至少兩個不同的修飾之核苷酸；

各 N_b 和 N_b'獨立地表示包括 0 至 10 個修飾之核苷酸的寡核苷序列；

其中各 n_p'、n_p、n_q'及 n_q，其各可能存在或可能不存在，獨立地表示突出核苷酸；且

XXX、YYY、ZZZ、X'X'X'、Y'Y'Y'、Z'Z'Z'各獨立地表示三個連續的核苷酸的三個相同修飾之模體。

【0286】在一個態樣中，i是0和j是0；或i是1和j是0；或i是0和j是1；或i和j都是0；i和j都是1。在另一個態樣中，k是0和l是0；或k是1和l是0；k是0和l是1；或k和l都是0；或k和l都是1。

【0287】示例性的結合有義股和反義股形成 iRNA 之雙鏈體包含以下化學式：

5' n_p - N_a -Y Y Y -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y' -N_a'n_q' 5'

(IIIa)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y'-N_b'-Z'Z'Z'-N_a'n_q' 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

$3'\ n_p'\text{-}N_a'\text{-}X'X'X'\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}Z'Z'Z'\text{-}N_a\text{-}n_q'\ 5'$

(IIId)

【0288】當 dsRNAi 劑是由式(IIIa)表示，各 N_a 獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0289】當 dsRNAi 劑是由式(IIIb)表示，各 N_b 獨立地表示包括 1 至 10、1 至 7、1 至 5、1 至 4 個修飾之核苷酸的寡核苷序列。各 Na 獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0290】當 dsRNAi 劑是由式(IIIc)表示，各 N_b 及 N_b'獨立地表示包括 0 至 10、0 至 7、0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、或 0 個修飾之核苷酸的寡核苷序列。各 N_a 獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。

【0291】當 dsRNAi 劑是由式(IIId)表示，各 N_b 及 N_b'獨立地表示包括 0 至 10、0 至 7、0 至 10、0 至 7、0 至 5、0 至 4、0 至 2、或 0 個修飾之核苷酸的寡核苷序列。各 N_a 及 N_a' 獨立地表示包括 2 至 20、2 至 15、2 至 10 個修飾之核苷酸的寡核苷序列。各 Na、Na'、Nb、Nb'獨立地包括替代模式之修飾。

【0292】式(III)、(IIIa)、(IIIb)、(IIIc)、(IIId)中各 X、Y、Z 可與彼此相同或不同。

【0293】當 dsRNAi 劑由式(III)、(IIIa)、(IIIb)、(IIIc)、(IIId)表示，至少一個 Y 核苷酸可與 Y'核苷酸形成鹼基對。另選地，至少兩個 Y 核苷酸與相對應 Y'核苷酸形成鹼基對；或所有三個 Y 核苷酸與相對應 Y'核苷酸全部形成鹼基對。

【0294】 當 dsRNAi 劑由式(IIIb)及(IIId)表示，至少一個 Z 核苷酸可與 Z'核苷酸形成鹼基對。另選地，至少兩個 Z 核苷酸與相對應 Z'核苷酸形成鹼基對；或所有三個 Z 核苷酸與相對應 Z'核苷酸全部形成鹼基對。

【0295】 當 dsRNAi 劑由式(IIIc)及(IIId)所表示，至少一個 X 核苷酸可與 X'核苷酸形成鹼基對。另選地，至少兩個 X 核苷酸與相對應 X'核苷酸形成鹼基對；或所有三個 X 核苷酸與相對應 X'核苷酸全部形成鹼基對。

【0296】 在特定態樣中，Y 核苷酸之修飾與 Y'核苷酸之修飾不同，Z 核苷酸之修飾與 Z'核苷酸之修飾不同，或 X 核苷酸之修飾與 X'核苷酸之修飾不同。

【0297】 在特定態樣中，當 dsRNAi 劑由式(IIId)表示，N_a 修飾是 2'-O-甲基或 2'-氟修飾。在其他態樣中，當 RNAi 劑由式(IIId)表示，N_a 修飾是 2'-O-甲基或 2'-氟修飾，達 $n_p'>0$ 且至少一個 n_p' 藉由硫代磷酸酯與鄰近核苷酸連接。還在其他態樣中，當 RNAi 劑由式(IIId)表示，N_a 修飾是 2'-O-甲基或 2'-氟修飾，$n_p'>0$ 且至少一個 n_p' 藉由硫代磷酸酯與鄰近核苷酸連接，有義股藉由經由雙價或三價分支連接子(於以下描述)與一個或多個 GalNAc 衍生物接合。當 RNAi 劑由式(IIId)表示，N_a 修飾是 2'-O-甲基或 2'-氟修飾，$n_p'>0$ 且 n_p' 經由硫代磷酸酯與鄰近核苷酸連接，有義股藉由經由雙價或三價分支連接子與一個或多個 GalNAc 衍生物接合。

【0298】 在一些態樣中，當 dsRNAi 劑由式(IIIa)表示，Na 修飾是 2'-O-甲基或 2'-氟修飾。np'>0 且至少一個 np'藉由硫代磷酸酯與鄰近核苷酸連接。有義股包括至少一個硫代磷酸酯，有義股藉由經由雙價或三價分支連接子與一個或多個 GalNAc 衍生物接合。

【0299】 在一些態樣中，dsRNAi 劑是包含至少兩個由式(III)、(IIIa)、(IIIb)、(IIIc)和(IIId)表示的雙鏈體的多聚體，該雙鏈體由連接子連接。連接子可以是可裂解或不可裂解的。可選擇地，多聚體還包括配體。各雙鏈體可以目標向相同基因或兩個不同的基因；或各雙鏈體可目標向相同基因的兩個不同靶位點目標。

【0300】 在一些態樣中，dsRNAi 劑是含有三個、四個、五個、六個或更多個由式(III)、(IIIa)、(IIIb)、(IIIc)和(IIId)表示的雙鏈體的多聚體，該雙鏈體由連接子連接。連接子可以是可裂解或不可裂解的。可選擇地，多聚體還包含配體。每個雙鏈體可以靶向相同的基因或兩個不同的基因；或者每個雙鏈體可以在兩個不同的靶位點靶向相同的基因。

【0301】 在一個態樣中，兩個 dsRNAi 劑由至少式(III)、(IIIa)、(IIIb)、(IIIc)、(IIId)中其中一個表示，與彼此在 5’末端連接，以及其中一 3’末端或兩個 3’末端，且可選擇地與配體接合。各劑可以靶向相同基因或是兩個不同的基因；或各劑可以靶向在不同目標位點的相同基因。

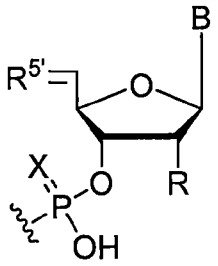
【0302】 在特定態樣中，本發明之 RNAi 劑可包含少量含有 2’-氟修飾的核苷酸，例如，10 或更少個具有 2’-氟修飾之核苷酸。例如，RNAi 劑可包含 10、9、8、7、6、5、4、3、2、1、0 個具有 2’-氟修飾之核苷酸。在特定的態樣中，本發明之 RNAi 劑包含 10 個具有 2’-氟修飾之核苷酸，例如，在有義股具有 2’-氟修飾之 4 個核苷酸和在反義股中具有 2’-氟修飾之 6 個核苷酸。在另一個特定的態樣中，本發明之 RNAi 劑包含 6 個具有 2’-氟修飾之核苷酸，例如，在有義股具有 2’-氟修飾之 4 個核苷酸和在反義股中具有 2’-氟修飾的 2 個核苷酸。

【0303】 在其他態樣中，本發明之 RNAi 劑可包含少量的含有 2’-氟修飾的核苷酸，例如，2 個或更少的包含 2’-氟修飾的核苷酸。例如，RNAi 劑可包含 2、

1、0 個具有 2'-氟修飾之核苷酸。在特定的態樣中，RNAi 劑可包含具有 2'-氟修飾之 2 個核苷酸，例如，在有義股中具有 2'-氟修飾之 0 個核苷酸和在反義股中具有 2'-氟修飾之 2 個核苷酸。

【0304】 各種出版物描述了可用於本發明方法的多聚體 iRNA。此類出版物包括 WO2007/091269、美國專利號 7,858,769、WO2010/141511、WO2007/117686、WO2009/014887、和 WO2011/031520，每個專利的全部內容其中的文獻藉由引用併入本文。

【0305】 在特定態樣中，本揭示之組成物和方法包括本文所述的 RNAi 劑之磷酸乙烯酯(vinyl phosphonate；簡稱 VP)修飾。在示範的實施例中，本揭示的 5'-磷酸乙烯酯修飾之核苷酸具有以下結構：



其中，X 是 O 或 S；R 是氫、羥、氟、C1-20 烷氧(例如甲氧基或 n-十六烷氧基)；

R5'是=C(H)-P(O)(OH)2 且介於 C5'碳和 R5'中的雙鍵是 E 或 Z 定向(例如 E 定向)；且

B 是核鹼基或修飾之核鹼基，可選擇地其 B 是腺嘌呤、鳥嘌呤、胞嘧啶、胸腺嘧啶、尿嘧啶。

【0306】 在一個態樣中，R5'是=C(H)-P(O)$(OH)_2$，且介於 C5'碳和 R5'中的雙鍵是 E 定向。在另一個態樣中，R 是甲氧基和 R5'是=C(H)-P(O)$(OH)_2$，且介在

C5'碳和 R5'中的雙鍵是 E 定向。在另一個態樣中，X 是 S、R 是甲氧基、R5'是 =C(H)-P(O)(OH)$_2$，且介在 C5'碳和 R5'中的雙鍵是 E 定向。

【0307】 本揭示之磷酸乙烯酯可與本揭示 dsRNA 之反義股或有義股連接。在其他態樣中，本揭示之磷酸乙烯酯與 dsRNA 之反義股連接，可選擇地在 dsRNA 反義股之 5'末端。

【0308】 磷酸乙烯酯修飾也可預期作為本揭示之組成物和方法。示例性的磷酸乙烯酯結構包含前面所述之結構，其 R5'是=C(H)-OP(O)(OH)2，且介於 C5'碳和 R5'中的雙鍵是 E 或 Z 定向(例如 E 定向)。

【0309】 於以下描述更多細節，包含與 iRNA 接合一個或多個碳水部分之 iRNA 可以優化一個或多個 iRNA 之特性。在許多案例中，碳水部分可以與修飾之 iRNA 次單元連接。例如，iRNA 中一個或多個核糖核酸次單元之核糖可以被其他部分取代，例如，與碳水配體連接的非碳水化合物(如環狀)載體。次單元的核糖已被取代之核糖核酸次單元於本文係指核糖取代修飾次單元(ribose replacement modification subunit；簡稱 RRMS)。環狀載體可以是碳環系統，亦即，所有環原子是是碳原子，或雜環系統，即， 一個或多個環原子可以是雜原子，例如，氮、氧、硫。環載體可以是單環系統，或可包含兩個或更多個環，例如融合環。環狀系統可以是完全飽和環系統，或可包含一個或更多個雙鍵。

【0310】 配體可藉由經由載體與聚核苷酸連接。載體包含(i) 至少一個「主鏈連結點」，如兩個「主鏈連結點」和(ii) 至少一個「聯繫連結點」。本文使用之「主鏈連結點」係指官能基，例如羥基，或通常地，可使用的鍵聯，其是適合將載體整合於主鏈，例如，磷酸酯或修飾之磷酸酯，例如，核糖核酸含硫主鏈。在一些態樣中的「繫帶連結點(tethering attachment point；簡稱 TAP)」係指環載體之

組成環原子，例如，碳原子或雜原子(與提供主鏈連結點的原子不同)，其連接選擇的部分。部分可以是，例如，碳水化合物，例如單碳醣、雙碳醣、三碳醣、四碳醣、寡醣、多醣。可選擇地，選擇的部分藉由干擾的繫帶與環載體連結。因此，環載體通常可包含官能基，例如，胺基，或通常地，提供適合整合或連繫其他化學，例如組成環的配體之鍵聯。

【0311】 iRNA 可藉由載體與配體接合，其中載體可以是環族或非環族。在一個態樣中，環族可以選自吡咯啶基、吡唑啉基、吡唑啶基、咪唑啉基、咪唑啶基、哌啶基、哌嗪基、[1,3]二氧雜環戊烷、噁唑啶基、異噁唑啶基、嗎啉基、四氫噻唑基、異四氫噻唑基、喹噁啉基、吡嗪酮基、四氫呋喃甲基、十氫化萘。在一個態樣中，非環族是絲胺醇主鏈或二乙醇胺主鏈。

【0312】 *i. 熱去穩定修飾*

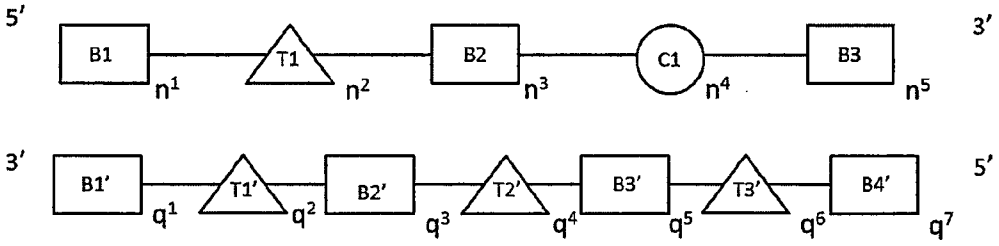
在其他態樣中，dsRNA 分子可以被優化作為 RNA 干擾藉由在義股之種子區域(亦即，反義股 5'-末端位置 2 至 9 或是反義股 5'-末端位置 2 至 8)整合熱去穩定修飾來降低或抑制脫靶基因沉默。

【0313】 術語「熱去穩定修飾」包含會導致相對於沒有該修飾之 dsRNA 修飾融熔溫度(melting temperature；簡稱 Tm)整體較低的 dsRNA Tm 之修飾。例如，熱去穩定修飾可以降低 dsRNA 的 1 至 4 °C 之 Tm，如攝氏 1、2、4 度。且，術語「熱去穩定核苷酸」係指核苷酸包含一個或多個熱去穩定修飾。

【0314】 已發現具有反義股 dsRNA 包括從反義股 5'末端起計數，至少在雙股最前面 9 個核苷酸位置之熱去穩定修飾，以降低脫靶基因沉默活性。據此，在一些態樣中，反義股包括在反義股 5'區域中雙股最前面 9 個核苷酸位置至少一個(例如 1、2、3、4、5、或更多)熱去穩定修飾。在一些態樣中，一個或多個

雙股熱去穩定修飾位在位置 2 至 9，如反義股 5’末端位置 4。在一些進一步的態樣中，雙股熱去穩定修飾是位在從反義股 5’末端起計數的位置 6、7、8。在一些進一步的態樣中，雙股熱去穩定修飾是位在從反義股 5’末端起計數的位置 7。在一些態樣中，雙股熱去穩定修飾是位在從反義股 5’末端起計數的位置 2、3、4、5、9。

【0315】 iRNA 劑包括有義股和反義股，各股具有 14 至 40 個核苷酸。RNAi 劑可由式(L)表示：

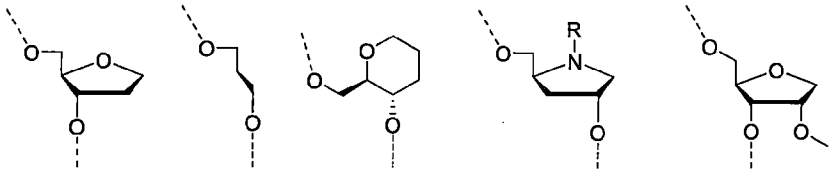


(L)。

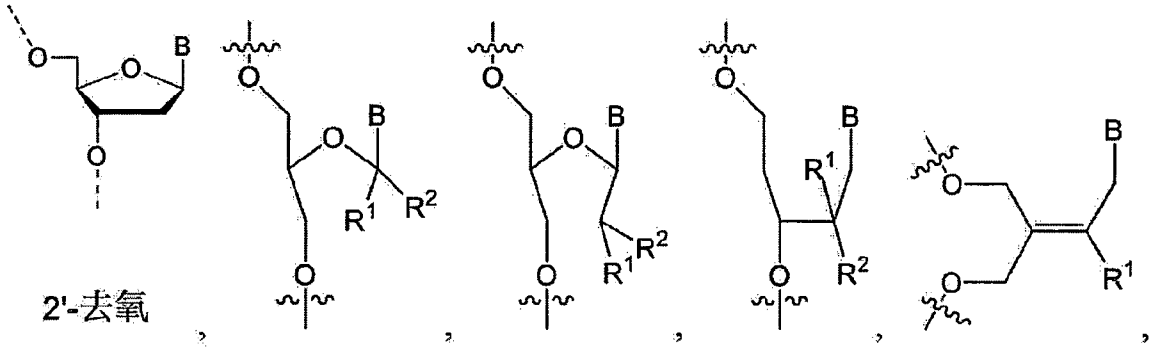
【0316】 在化學式(L)中，B1、B2、B3、B1’、B2’、B3’、B4’各自是獨立地包含選自由 2’-O-烷基、2’-取代的烷氧、2’-取代的烷基、2’-鹵、ENA、BNA/LNA 的修飾組成之組的核苷酸。在一個態樣中，B1、B2、B3、B1’、B2’、B3’、B4’各自包含 2’-OMe 修飾。在一個態樣中，B1、B2、B3、B1’、B2’、B3’、B4’各自包含 2’-OMe or 2’-F 修飾。在一個態樣中，B1、B2、B3、B1’、B2’、B3’、B4’中至少一個包含 2'-O-N-甲基乙醯胺基(2'-O-NMA, 2’O-CH2C(O)N(Me)H)修飾。

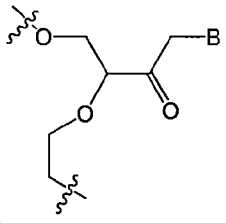
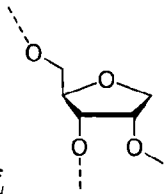
【0317】 C1 是一種熱不穩定核苷酸，位於與反義股種子區域相對的位點(即反義股 5’-末端位置 2 至 8 或是反義股 5’-末端位置 2 至 9)。例如，C1 是位在與反義股 5’-末端位置 2 至 8 核苷酸配對之有義股。在一個示例中，C1 位在有義股 5’-末端位置 15。C1 核苷酸可帶有熱去穩定修飾，其可包含無鹼基修飾；與雙股相對的核苷酸錯配；和糖修飾如 2’-去氧修飾或無環核苷酸，例如，未鎖核酸

(UNA)或甘油核酸(GNA)。在一個態樣中，C1 具有選自由以下組成之組的熱去穩定修飾：i) 與反義股相對的核苷酸錯配；ii) 選自由以下組成的組的無鹼基修飾：



；且 iii) 選自由以下組成的組的糖修飾：



及 ，其中，B 是修飾之或未修飾之核鹼基，R^1 和 R^2 獨立地為 H、鹵素、OR_3、烷基；且 R_3 是 H、烷基、環烷基、芳香基、芳烷基、砸芳香基或糖。在一個態樣中，在 C1 中的熱去穩定修飾是選自由以下組成之組的錯配：G：G、G：A、G：U、G：T、A：A、A：C、C：C、C：U、C：T、U：U、T：T、U：T；且可選擇地，在錯配對中至少一個核鹼基是 2'-去氧核鹼基。在一個示例中，在 C1 中的熱去穩定修飾是 GNA 或 。

【0318】 T1、T1'、T2'、T3 各獨立地表示核苷酸包含修飾的核苷酸，該修飾為核苷酸提供小於或等於 2'-OMe 修飾空間體積的空間體積。空間體積係指修

飾之空間效應之總和。用於確定核苷酸修飾的空間效應的方法是本領域技術人員已知的。修飾可以在核苷酸的核糖的 2'位置，或對非核糖核苷酸、無環核苷酸或與核糖的 2'位置相似或等同的核苷酸主鏈的修飾，並為核苷酸提供小於或等於 2'-OMe 修飾的空間體積的空間體積。例如，T1、T1'、T2'、T3'各獨立地選自 DNA、RNA、LNA、2'-F、2'-F-5'-甲基。在一個態樣中，T1 是 DNA。在一個態樣中，T1'是 DNA、RNA、LNA。在一個態樣中，T2'是 DNA 或 RNA。在一個態樣中，T3'是 DNA 或 RNA。

【0319】 n^1、n^3及 q^1長度獨立地為 4 至 15 個核苷酸。

【0320】 n^5、q^3、q^7長度獨立地為 1 至 6 個核苷酸。

【0321】 n^4、q^2、q^6長度獨立地為 1 至 3 個核苷酸；相對地，n^4是 0。

【0322】 q^5長度獨立地為 0 至 10 個核苷酸。

【0323】 n^2和 q^4長度獨立地為 0 至 3 個核苷酸。

【0324】 相對地，n^4長度為 0 至 3 個核苷酸。

【0325】 在一個態樣中，n^4可以是 0。在一個示例中，n^4是 0，且 q^2和 q^6是 1。在另一示例中，n^4是 0，且 q^2和 q^6是 1，具有位在有義股位置 1 至 5(從有義股 5'-末端起計數)之兩個硫代磷酸酯核苷酸間鍵聯修飾，位在有義股位置 1 和 2 之硫代磷酸酯核苷酸間鍵聯修飾，以及位在有義股位置 18 至 23(從有義股 5'-末端起計數)之兩個硫代磷酸酯核苷酸間鍵聯修飾。

【0326】 在一個態樣中，n^4、q^2及 q^6各為 1。

【0327】 在一個態樣中，n^2、n^4、q^2、q^4及 q^6各為 1。

【0328】在一個態樣中，當有義股長度為 19 至 22 個核苷酸且 n^4 是 1 時，C1 是位在有義股 5’-末端之位置 14 至 17。在一個態樣中，C1 是位在有義股 5’-末端之位置 15。

【0329】在一個態樣中，T3’是從反義股 5’-末端起計數之位置 2。在一個示例中，T3’是位在反義股 5’-末端起計數之位置 2，且 q^6 等於 1。

【0330】在一個態樣中，T1’是從反義股 5’-末端起計數之位置 14。在一個示例中，T3’是位在反義股 5’-末端起計數之位置 14，且 q^2 等於 1。

【0331】在一個示範的實施例中，T3’是從反義股 5’-末端起計數之位置 2，且 T1’ 是從從反義股 5’-末端起計數之位置 14。在一個示例中，T3’是從反義股 5’-末端起計數之位置 2，且 q^2 等於 1，以及 T1’是從反義股 5’-末端起計數之位置 14，且 q^2 等於 1。

【0332】在一個態樣中，T1’和 T3’間隔為長度 11 個核苷酸(即並沒有計算 T1’和 T3’核苷酸)。

【0333】在一個態樣中，T1’起始於反義股 5’-末端之位置 14。在一個示例中，T1’起始於反義股 5’-末端之位置 14，且 q^2 等於 1，以及位在 2’位置之修飾或位在非核糖、非環、或提供相較於 2’-OMe 核糖較少的空間體積之主鏈。

【0334】在一個態樣中，T3’起始於從反義股 5’-末端之位置 2。在一個示例中，T3’起始於反義股 5’-末端之位置 2，且 q^2 等於 1，以及位在 2’位置之修飾，或位在非核糖、非環、或提供相較於 2’-OMe 核糖較少或相等的空間體積之主鏈。

【0335】在一個態樣中，T1 是位在有義股之裂解位點。在一個示例中，當有義股長度為 19 至 22 個核苷酸且 n^2 是 1 時，T1 是位在有義股 5’-末端之位置

11。在一個示例性態樣中，當有義股長度為 19 至 22 個核苷酸且 n^2 是 1 時，T1 是位在從有義股 5’-末端之位置 11 之有義股裂解位點。

【0336】 在一個態樣中，T2’起始於反義股 5’-末端之位置 6。在一個示例中，T2’起始於有義股 5’-末端之位置 6 至 10，且 q^4 是 1。

【0337】 在一個示範的實施例中，T1 是位在有義股之裂解位點。例如，當有義股長度為 19 至 22 個核苷酸且 n^2 是 1 時，T1’是位在從反義股 5’-末端之位置 14 且 q2 是 1，和 T1’修飾是位在核糖 2’位置或位在非核糖、非環、或提供相較於 2’-OMe 核糖較少的空間體積之主鏈；T3’是位在從反義股 5’-末端之位置 6 至 10 且 q^4 是 1；T3’是位在從反義股 5’-末端之位置 2 且 q^6 是 1；以及 T3’修飾是位在 2’位置，或位在非核糖、非環、或提供相較於 2’-OMe 核糖較少或相等的空間體積之主鏈。

【0338】 在一個態樣中，T2’起始於反義股 5’-末端之位置 8。在一個示例中，T2’ 起始於反義股 5’-末端之位置 8，且 q^4 是 2。

【0339】 在一個態樣中，T2’起始於反義股 5’-末端之位置 9。在一個示例中，T2’ 起始於從反義股 5’-末端之位置 9，且 q^4 是 1。

【0340】 在一個態樣中，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 1，B3'是 2'-OMe 或 2'-F，q^5 是 6，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1；在有義股的位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。

【0341】在一態樣中，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是1，B3'是2'-OMe或2'-F，q^5是6，T3'是2'-F、q^6是1，B4'是2'-OMe，且q^7是1；在有義股的位置1至5內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的5'-末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鍵結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的5'-末端計數)。

【0342】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-OMe，且q^7是1。

【0343】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-OMe，且q^7是1；在有義股位置1至5內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的5'-末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鍵結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的5'-末端計數)。

【0344】在一態樣中，B1是2'-OMe或2'-F，n^1是6，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是7，T1'是2'-F，q2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-OMe，且q^7是1。

【0345】在一態樣中，B1是2'-OMe或2'-F，n^1是6，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是7，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-OMe，且q^7是1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鏈結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的5’ -末端計數)。

【0346】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是1，B3'是2'-OMe或2'-F，q^5是6，T3'是2'-F，q^6是1，B4'是2'-OMe，且q^7是1。

【0347】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是1，B3'是2'-OMe或2'-F，q^5是6，T3'是2'-F，q^6是1，B4'是2'-OMe，且q^7是1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鏈結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的5’ -末端計數)。

【0348】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是5，T2'是2'-F，q^4是1，B3'是

2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，且 q^7是 1；視需要地，在反義股 3'-末端至少具有 2 個額外的 TT。

【0349】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 5，T2'是 2'-F，q^4是 1，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，且 q^7是 1；視需要地，在反義股 3'-末端中至少具有 2 個額外的 TT；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。

【0350】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe,n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1。

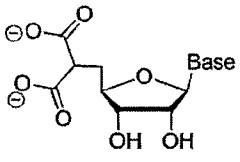
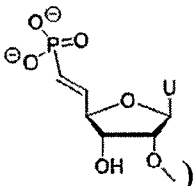
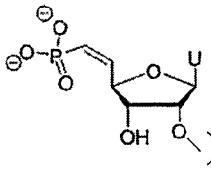
【0351】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe,n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從 5’-末端計數)。

【0352】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，且q^7是1。

【0353】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，且q^7是1；在有義股位置1至5內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的5'-末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鏈結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的5'-末端計數)。

【0354】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，q^4是0，B3'是2'-OMe或2'-F，q^5是7，T3'是2'-F，q^6是1，B4'是2'F，且q^7是1。

【0355】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，q^4是0，B3'是2'-OMe或2'-F，q^5是7，T3'是2'-F，q^6是1，B4'是2'F，且q^7是1；在有義股位置1至5內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的5'-末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鏈結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的5'-末端計數)。

【0356】該 RNAi 劑可在有義股或反義股的 5'-末端包含含磷基團。該 5'-末端含磷基團可以是 5'-末端磷酸(5'-P)、5'-末端硫代磷酸酯(5'-PS)、5'-末端二硫代磷酸酯(5'-PS_2)、5'-末端乙烯基膦酸酯(5'-VP)、5'-末端甲基膦酸酯(MePhos)或 5'-去氧-5'-C-丙二醯基()。當 5'-末端含磷基團是 5'-末端乙烯基膦酸酯(5'-VP)時，該 5'-VP 可以是 5'-E-VP 異構體(即，反式-乙烯基膦酸酯，)、5'-Z-VP 異構體(即，順式-乙烯基膦酸酯，)或其混合物。

【0357】在一態樣中，該 RNAi 劑在有義股的 5'-末端包含含磷基團。在一態樣中，該 RNAi 劑在反義股的 5'-末端包含含磷基團。

【0358】在一態樣中，該 RNAi 劑包含 5'-P。在一態樣中，該 RNAi 劑在反義股中包含 5'-P。

【0359】在一態樣中，該 RNAi 劑包含 5'-PS。在一態樣中，該 RNAi 劑在反義股中包含 5'-PS。

【0360】在一態樣中，該 RNAi 劑包含 5'-VP。在一態樣中，該 RNAi 劑在反義股中包含 5'-VP。在一態樣中，該 RNAi 劑在反義股中包含 5'-*E*-VP。在一態樣中，該 RNAi 劑在反義股中包含 5'-*Z*-VP。

【0361】在一態樣中，該 RNAi 劑包含 5'-PS_2。在一態樣中，該 RNAi 劑在反義股中包含 5'-PS_2。

【0362】在一態樣中，該 RNAi 劑包含 5'-PS_2。在一態樣中，該 RNAi 劑在反義股中包含 5'-去氧-5'-C-丙二醯基。

【0363】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，且 q^7是 1。該 RNAi 劑還包含 5'-PS。

【0364】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，並且 q^7是 1。該 RNAi 劑還包含 5'-P。

【0365】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，且 q^7是 1。該 RNAi 劑還包含一個 5'-VP。該 5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0366】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe 且 q^7是 1。該 RNAi 劑還包含 5'-PS_2。

【0367】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1。該 RNAi 劑還包含 5'-去氧-5'-C-丙二醯基。

【0368】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-P。

【0369】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-PS。

【0370】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe,n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-VP。5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0371】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-PS_2。

【0372】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至

23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-去氧-5'-C-丙二醯基。

【0373】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q5 是 7，T3'是 2'-F，q6 是 1，B4'是 2'OMe，且 q^7是 1。該 RNAi 劑還包含 5'-P。

【0374】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9。T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1。該 dsRNA 劑還包含 5'-PS。

【0375】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1。該 RNAi 劑還包含 5'-VP。該 5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0376】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1。該 RNAi 劑還包含 5'-PS_2

【0377】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，q^4是0，B3'是2'-OMe或2'-F，q^5是7，T3'是2'-F，q^6是1，B4'是2'OMe，且q^7是1。該RNAi劑還包含5'-去氧-5'-C-丙二醯基。

【0378】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，q^4是0，B3'是2'-OMe或2'-F，q^5是7，T3'是2'-F，q^6是1，B4'是2'OMe，且q^7是1；在有義股位置1至5內有兩個硫代磷酸酯核苷酸間鏈結修飾(從5'-末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鏈結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鏈結修飾(從5'-末端計數)。該RNAi劑還包含5'-P。

【0379】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，q^4是0，B3'是2'-OMe或2'-F，q^5是7，T3'是2'-F，q^6是1，B4'是2'OMe，且q^7是1；在有義股位置1至5內有兩個硫代磷酸酯核苷酸間鏈結修飾(從5'-末端計數)，及在反義股的位置1和2有兩個硫代磷酸酯核苷酸間鏈結修飾及位置18至23內有兩個硫代磷酸酯核苷酸間鏈結修飾(從5'-末端計數)。該RNAi劑還包含5'-PS。

【0380】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B^3是2'-OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，q^4是0，B3'是2'-OMe或

2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)。該 RNAi 劑還包含 5'-VP。該 5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0381】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n2 是 3，B2 是 2'-OMe，n3 是 7，n4 是 0，B3 是 2'-OMe,n5 是 3,B1'是 2'-OMe 或 2'-F,q1 是 9,T1'是 2'-F,q2 是 1,B2'是 2'-OMe 或 2'-F,q3 是 4，q4 是 0，B3'是 2'-OMe 或 2'-F，q5 是 7，T3'是 2'-F，q6 是 1，B4'是 2'OMe，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)。該 RNAi 劑還包含 5'-PS_2。

【0382】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B^3是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)。該 RNAi 劑還包含 5'-去氧-5'-C-丙二醯基。

【0383】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是

2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，且q^7是1。該RNAi劑還包含5'-P。

【0384】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，且q^7是1。該RNAi劑還包含5'-PS。

【0385】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，且q^7是1。該RNAi劑還包含5'-VP。5'-VP可以是5'-*E*-VP、5'-*Z*-VP或其組合。

【0386】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，且q^7是1。該dsRNAiRNA劑還包含5'-PS_2。

【0387】在一態樣中，B1是2'-OMe或2'-F，n^1是8，T1是2'F，n^2是3，B2是2'-OMe，n^3是7，n^4是0，B3是2'OMe，n^5是3，B1'是2'-OMe或2'-F，q^1是9，T1'是2'-F，q^2是1，B2'是2'-OMe或2'-F，q^3是4，T2'是2'-F，q^4是2，B3'是2'-OMe或2'-F，q^5是5，T3'是2'-F，q^6是1，B4'是2'-F，並且q^7是1。該RNAi劑還包含5'-去氧-5'-C-丙二醯基。

【0388】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-F，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-P。

【0389】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-F，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-PS。

【0390】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，T2'是 2'-F，q^4 是 2，B3'是 2'-OMe 或 2'-F，q^5 是 5，T3'是 2'-F，q^6 是 1，B4'是 2'-F，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內

有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-VP。該 5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0391】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-F，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-PS_2。

【0392】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMe 或 2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-F，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-去氧-5'-C-丙二醯基。

【0393】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B^3是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或

2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1。該 RNAi 劑還包含 5'-P。

【0394】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1。該 RNAi 劑還包含 5'-PS。

【0395】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1。該 RNAi 劑還包含 5'-VP。該 5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0396】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1。該 RNAi 劑還包含 5'-PS_2。

【0397】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1。該 RNAi 劑還包含 5'-去氧-5'-C-丙二醯基。

【0398】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，q^4 是 0，B3'是 2'-OMe 或 2'-F，q^5 是 7，T3'是 2'-F，q^6 是 1，B4'是 2'F，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-P。

【0399】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，q^4 是 0，B3'是 2'-OMe 或 2'-F，q^5 是 7，T3'是 2'-F，q^6 是 1，B4'是 2'F，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-PS。

【0400】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，q^4 是 0，B3'是 2'-OMe 或 2'-F，q^5 是 7，T3'是 2'-F，q^6 是 1，B4'是 2'F，且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5'-VP。該 5'-VP 可以是 5'-*E*-VP、5'-*Z*-VP 或其組合。

【0401】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-PS_2。

【0402】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'F，且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5'-去氧-5'-C-丙二醯基。

【0403】在一態樣中，B1 是 2’-OMeor2’-F，n^1是 8，T1 是 2’F，n^2是 3，B2 是 2’-OMe，n^3是 7，n^4是 0，B3 是 2’-OMe，n^5是 3，B1’是 2’-OMe 或 2’-F，q^1是 9，T1’是 2’-F，q^2是 1，B2’是 2’-OMeor2’-F，q^3是 4，T2’是 2’-F，q^4是 2，B3’是 2’-OMeor2’-F，q^5是 5，T3’是 2’-F，q^6是 1，B4’是 2’-OMe，且 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。該

RNAi 劑還包含 5'-P 及靶向配位子。在一態樣，該 5'-P 是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0404】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMeor2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMeor2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，及 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5' -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5' -末端計數)。該 RNAi 劑還包含 5'-PS 及靶向配位子。在一態樣中，該 5'-PS 是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0405】 在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMeor2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMeor2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，及 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5' -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5' -末端計數)。該 RNAi 劑還包含 a5'-PS 及靶向配位子。該 RNAi 劑還包含 5'-VP(例如，5'-*E*-VP，5'-*Z*-VP 或其組合)，及靶向配位子。

【0406】 在一態樣中，該 5'-VP 是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0407】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMeor2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMeor2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，及 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5'-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5'-末端計數)。該 RNAi 劑還包含 5'-PS_2及靶向配位子。在一態樣中，該 5'-PS_2是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0408】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMeor2'-F，q^3是 4，T2'是 2'-F，q^4是 2，B3'是 2'-OMeor2'-F，q^5是 5，T3'是 2'-F，q^6是 1，B4'是 2'-OMe，及 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5'-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5'-末端計數)。該 RNAi 劑還包含 5'-去氧-5'-*C*-丙二醯基及靶向配位子。在一態樣中，該 5'-去氧-5'-*C*-丙二醯基是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0409】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMeor2'-F，q^3是 4，q^4是 0，B3'是 2'-OMeor2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'OMe，及 q^7是 1；在有義股位

置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)。該 RNAi 劑還包含 5’-P 及靶向配位子。在一態樣中，該 5’-P 是在反義股 5’-末端，該靶向配位子是在有義股 3’-末端。

【0410】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1 是 8，T1 是 2’F，n^2 是 3，B2 是 2’-OMe，n^3 是 7，n^4 是 0，B3 是 2’-OMe，n^5 是 3，B1’是 2’-OMe 或 2’-F，q^1 是 9，T1’是 2’-F，q^2 是 1，B2’是 2’-OMe 或 2’-F，q^3 是 4，q^4 是 0，B3’是 2’-OMe 或 2’-F，q^5 是 7，T3’是 2’-F，q^6 是 1，B4’是 2’-OMe，及 q^7 是 1;;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)。該 RNAi 劑還包含 5’-PS 及靶向配位子。在一態樣中，該 5’-PS 是在反義股 5’-末端，該靶向配位子是在有義股 3’-末端。

【0411】 在一態樣中，B1 是 2’-OMe 或 2’-F,n^1 是 8，T1 是 2’F，n^2 是 3，B2 是 2’-OMe，n^3 是 7，n^4 是 0，B3 是 2’-OMe，n^5 是 3，B1’是 2’-OMe 或 2’-F，q^1 是 9，T1’是 2’-F，q^2 是 1，B2’是 2’-OMe 或 2’-F，q^3 是 4，q^4 是 0，B3’是 2’-OMe 或 2’-F，q^5 是 7，T3’是 2’-F，q^6 是 1，B4’是 2’-OMe，及 q^7 是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從 5’ -末端計數)。該 RNAi 劑還包含 5’-VP(例如，5’-*E*-

VP、5'-*Z*-VP 或其組合)及靶向配位子。在一態樣中，該 5'-VP 是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0412】在一態樣中，B1 是 2'-OMe 或 2'-F,n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，q^4 是 0，B3'是 2'-OMe 或 2'-F，q^5 是 7，T3'是 2'-F，q^6 是 1，B4'是 2'OMe，及 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從 5' -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從 5' -末端計數)。該 RNAi 劑還包含 5'-PS_2 及靶向配位子。在一態樣中，該 5'-PS_2 是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0413】在一態樣中，B1 是 2'-OMeor2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，q^1 是 9，T1'是 2'-F，q^2 是 1，B2'是 2'-OMe 或 2'-F，q^3 是 4，q^4 是 0，B3'是 2'-OMe 或 2'-F，q^5 是 7，T3'是 2'-F，q^6 是 1，B4'是 2'-OMe，及 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從 5' -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從 5' -末端計數)。該 RNAi 劑還包含 5'-去氧-5'-*C*-丙二醯基及靶向配位子。在一態樣中，該 5'-去氧-5'-*C*-丙二醯基是在反義股 5'-末端，且該靶向配位子是在有義股 3'-末端。

【0414】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1 是 8，T1 是 2'F，n^2 是 3，B2 是 2'-OMe，n^3 是 7，n^4 是 0，B3 是 2'-OMe，n^5 是 3，B1'是 2'-OMe 或 2'-F，

q^1是 9，T1’是 2’-F，q^2是 1，B2’是 2’-OMe 或 2’-F，q^3是 4，T2’是 2’-F，q^4是 2，B3’是 2’-OMe 或 2’-F，q^5是 5，T3’是 2’-F，q^6是 1，B4’是 2’-F 且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5’-P 及靶向配位子。在一態樣中，該 5’-P 是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0415】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1是 8，T1 是 2’F，n^2是 3，B2 是 2’-OMe，n^3是 7，n^4是 0，B3 是 2’-OMe，n^5是 3，B1’是 2’-OMe 或 2’-F，q^1是 9，T1’是 2’-F，q^2是 1，B2’是 2’-OMe 或 2’-F，q^3是 4，T2’是 2’-F，q^4是 2，B3’是 2’-OMe 或 2’-F，q^5是 5，T3’是 2’-F，q^6是 1，B4’是 2’-F 且 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5’-PS 及靶向配位子。在一態樣中，該 5’-PS 是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0416】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1是 8，T1 是 2’F，n^2是 3，B2 是 2’-OMe，n^3是 7，n^4是 0，B3 是 2’-OMe，n^5是 3，B1’是 2’-OMe 或 2’-F，q^1是 9，T1’是 2’-F，q^2是 1，B2’是 2’-OMe 或 2’-F，q^3是 4，T2’是 2’-F，q^4是 2，B3’是 2’-OMe 或 2’-F，q^5是 5，T3’是 2’-F，q^6是 1，B4’是 2’-F 且 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鍵結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鍵結修飾及位置 18

至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5’-VP(*例如*，5’-*E*-VP、5’-*Z*-VP 或其組合)及靶向配位子。在一態樣中，該 5’-VP 是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0417】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1是 8，T1 是 2’F，n^2是 3，B2 是 2’-OMe，n^3是 7，n^4是 0，B3 是 2’-OMe，n^5是 3，B1’是 2’-OMe 或 2’-F，q^1是 9，T1’是 2’-F，q^2是 1，B2’是 2’-OMe 或 2’-F，q^3是 4，T2’是 2’-F，q^4是 2，B3’是 2’-OMe 或 2’-F，q^5是 5，T3’是 2’-F，q^6是 1，B4’是 2’-F 且 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5’-PS_2 及靶向配位子。在一態樣中，該 5’-PS_2 是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0418】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1是 8，T1 是 2’F，n^2是 3，B2 是 2’-OMe，n^3是 7，n^4是 0，B3 是 2’-OMe，n^5是 3，B1’是 2’-OMe 或 2’-F，q^1是 9，T1’是 2’-F，q^2是 1，B2’是 2’-OMe 或 2’-F，q^3是 4，T2’是 2’-F，q^4是 2，B3’是 2’-OMe 或 2’-F，q^5是 5，T3’是 2’-F，q^6是 1，B4’是 2’-F 且 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’-末端計數)。該 RNAi 劑還包含 5’-去氧-5’-*C*-丙二醯基及靶向配位子。在一態樣中，該 5’-去氧-5’-*C*-丙二醯基是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0419】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'-F 且 q^7是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5'-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5'-末端計數)。該 RNAi 劑還包含 5'-P 及靶向配位子。在一態樣中，該 5'-P 是在反義股 5'-末端且該靶向配位子是在有義股 3'-末端。

【0420】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'-F 且 q^7是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5'-末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5'-末端計數)。該 RNAi 劑還包含 5'-PS 及一靶向配位子。在一態樣中，該 5'-PS 是在反義股 5'-末端且該靶向配位子是在有義股 3'-末端。

【0421】在一態樣中，B1 是 2'-OMe 或 2'-F，n^1是 8，T1 是 2'F，n^2是 3，B2 是 2'-OMe，n^3是 7，n^4是 0，B3 是 2'-OMe，n^5是 3，B1'是 2'-OMe 或 2'-F，q^1是 9，T1'是 2'-F，q^2是 1，B2'是 2'-OMe 或 2'-F，q^3是 4，q^4是 0，B3'是 2'-OMe 或 2'-F，q^5是 7，T3'是 2'-F，q^6是 1，B4'是 2'-F 且 q^7是 1;在有義股位置 1

至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5’-VP(*例如*，5’-*E*-VP、5’-*Z*-VP 或其組合)及靶向配位子。在一態樣中，該 5’-VP 是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0422】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1 是 8，T1 是 2’F，n^2 是 3，B2 是 2’-OMe，n^3 是 7，n^4 是 0，B3 是 2’-OMe，n^5 是 3，B1’是 2’-OMe 或 2’-F，q^1 是 9，T1’是 2’-F，q^2 是 1，B2’是 2’-OMe 或 2’-F，q^3 是 4，q^4 是 0，B3’是 2’-OMe 或 2’-F，q^5 是 7，T3’是 2’-F，q^6 是 1，B4’是 2’-F 且 q^7 是 1；在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含 5’-PS_2 及靶向配位子。在一態樣中，該 5’-PS_2 是在反義股 5’-末端且該靶向配位子是在有義股 3’-末端。

【0423】 在一態樣中，B1 是 2’-OMe 或 2’-F，n^1 是 8，T1 是 2’F，n^2 是 3，B2 是 2’-OMe，n^3 是 7，n^4 是 0，B3 是 2’-OMe，n^5 是 3，B1’是 2’-OMe 或 2’-F，q^1 是 9，T1’是 2’-F，q^2 是 1，B2’是 2’-OMe 或 2’-F，q^3 是 4，q^4 是 0，B3’是 2’-OMe 或 2’-F，q^5 是 7，T3’是 2’-F，q^6 是 1，B4’是 2’-F 且 q^7 是 1;在有義股位置 1 至 5 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從有義股的 5’ -末端計數)，及在反義股的位置 1 和 2 有兩個硫代磷酸酯核苷酸間鏈結修飾及位置 18 至 23 內有兩個硫代磷酸酯核苷酸間鏈結修飾(從反義股的 5’ -末端計數)。該 RNAi 劑還包含

5'-去氧-5'-*C*-丙二醯基及靶向配位子。在一態樣中，該 5'-去氧-5'-*C*-丙二醯基是在反義股 5'-末端且該靶向配位子是在有義股 3'-末端。

【0424】 在一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括透過三價分支鍵聯連結的三個 GalNAc 衍生物；且

(iii) 位置 1、3、5、7、9 至 11、13、17、19 及 21 之 2'-F 修飾和位置 2、4、6、8、12、14 至 16、18 及 20 之 2'-OMe 修飾(從 5' 末端計數)；且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3、5、9、11 至 13、15、17、19、21 及 23 之 2'-OMe 修飾和位置 2、4、6 至 8、10、14、16、18、20 及 22 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 dsRNA 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0425】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括透過三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1、3、5、7、9 至 11、13、15、17、19 及 21 之 2'-F 修飾和位置 2、4、6、8、12、14、16、18 及 20 之 2'-OMe 修飾(從 5' 末端計數)；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3、5、7、9、11 至 13、15、17、19 及 21 至 23 之 2'- OMe 修飾和位置 2、4、6、8、10、14、16、18 及 20 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0426】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1 至 6、8、10 及 12 至 21 之 2'-OMe 修飾、位置 7 以及 9 之 2'F 修飾、位置 11 之去氧核苷酸(例如 dT)(從 5' 末端計數)；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3、7、9、11、13、15、17 及 19 至 23 之 2'-OMe 修飾和位置 2、4 至 6、8、10、12、14、16 及 18 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端其中。

【0427】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1 至 6、8、10、12、14 及 16 至 21 之 2'-OMe 修飾和位置 7、9、11、13 及 15 之 2'F 修飾；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、5、7、9、11、13、15、17、19 及 21 至 23 1 之 2'-OMe 修飾和位置 2 至 4、6、8、10、12、14、16、18 及 20 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0428】 在另一個特定的態樣中，本發明 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1 至 9 及 12 至 21 之 2'-OMe 修飾和位置 10 以及 11 之 2'F 修飾；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5’ 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3、5、7、9、11 至 13、15、17、19 及 21 至 23 之 2’-OMe 修飾和位置 2、4、6、8、10、14、16、18 及 20 之 2’F 修飾(從 5’ 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5’ 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3’-末端，和一個平端位在反義股 5’-末端。

【0429】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3’-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1、3、5、7、9 至 11 及 13 之 2’-F 修飾和位置 2、4、6、8、12 及 14 至 21 之 2’-OMe 修飾；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5’ 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3、5 至 7、9、11 至 13、15、17 至 19 及 21 至 23 之 2'-OMe 修飾和位置 2、4、8、10、14、16 及 20 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0430】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1、2、4、6、8、12、14、15、17 及 19 至 21 之 2'-OMe 修飾和位置 3、5、7、9 至 11、13、16 及 18 之 2'F 修飾(從 5' 末端計數)；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

且

(b) 反義股具有：

(i) 長度為 25 個核苷酸；

(ii) 位置 1、4、6、7、9、11 至 13、15、17 及 19 至 23 之 2'-OMe 修飾、位置 2、3、5、8、10、14、16 及 18 之 2'F 修飾、位置 24 和 25 之去氧核苷酸(例如 dT)(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 RNAi 劑具有 4 個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0431】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1 至 6、8 及 12 至 21 之 2'-OMe 修飾和位置 7 及 9 至 11 之 2'F 修飾；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3 至 5、7、8、10 至 13、15 及 17 至 23 之 2'-OMe 修飾和位置 2、6、9、14 及 16 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0432】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 21 個核苷酸；

(ii) 連結於 3'-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1 至 6、8 及 12 至 21 之 2'-OMe 修飾和位置 7 及 9 至 11 之 2'-F 修飾；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5' 末端計數)；

且

(b) 反義股具有：

(i) 長度為 23 個核苷酸；

(ii) 位置 1、3 至 5、7、10 至 13、15 及 17 至 23 之 2'-OMe 修飾和位置 2、6、8、9、14 及 16 之 2'F 修飾(從 5' 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 21 及 22 之間以及核苷酸位置 22 及 23 之間的硫代磷酸酯核苷酸間鍵聯(從 5’ 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3’-末端，和一個平端位在反義股 5’-末端。

【0433】 在另一個特定的態樣中，本發明之 RNAi 劑包括：

(a) 有義股具有：

(i) 長度為 19 個核苷酸；

(ii) 連結於 3’-末端之 ASGPR 配體，其中該 ASGPR 配體包括藉由三價分支鍵聯連結的三個 GalNAc 衍生物；

(iii) 位置 1 至 4、6 及 10 至 19 之 2’-OMe 修飾和位置 5 及 7 至 9 之 2’F 修飾；且

(iv) 核苷酸位置 1 及 2 之間以及核苷酸位置 2 及 3 之間的硫代磷酸酯核苷酸間鍵聯(從 5’ 末端計數)；

且

(b) 反義股具有：

(i) 長度為 21 個核苷酸；

(ii) 位置 1、3 至 5、7、10 至 13、15 及 17 至 21 之 2’-OMe 修飾和位置 2、6、8、9、14 及 16 之 2’F 修飾(從 5’ 末端計數)；且

(iii) 核苷酸位置 1 及 2 之間、核苷酸位置 2 及 3 之間、核苷酸位置 19 及 20 之間以及核苷酸位置 20 及 21 之間的硫代磷酸酯核苷酸間鍵聯(從 5’ 末端計數)；

其中 RNAi 劑具有兩個核苷酸突出位在反義股 3'-末端，和一個平端位在反義股 5'-末端。

【0434】 在某些態樣中，用於本發明方法的 iRNA 是選自表 2-3 中任一個所列之劑的藥劑。該等藥劑可以進一步包括配體。

【0435】 III. iRNAs 接合於配體

本發明的 iRNA 之 RNA 另一種修飾包含化學連結 iRNA 一個或多個配體、部份體、接合體，可提升 iRNA 之活性、細胞分布、細胞攝取例如進入細胞。該部份體包含但限制於脂質部份如膽固醇部份(Letsinger et al., Proc. Natl. Acid. Sci. USA, 1989, 86： 6553-6556)。在其他態樣中，配體是膽酸(Manoharan et al., Biorg. Med. Chem. Let., 1994, 4：1053-1060)、硫代乙醚，例如，己基-S-三苯甲基硫醇(Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660：306-309； Manoharan et al., Biorg. Med. Chem. Let., 1993, 3：2765-2770)、硫基膽固醇(Oberhauser et al., Nucl. Acids Res., 1992, 20：533-538)、脂肪鏈，例如，十二烷二醇或十一烷殘基(Saison-Behmoaras et al., EMBO J, 1991, 10：1111-1118； Kabanov et al., FEBS Lett., 1990, 259：327-330； Svinarchuk et al., Biochimie, 1993, 75：49-54)、磷脂，例如，二-十六烷基-rac-甘油或三乙基-銨 1, 2-二-O-十六烷基-rac-甘油-3-磷酸酯(Manoharan et al., Tetrahedron Lett., 1995, 36：3651-3654； Shea et al., Nucl. Acids Res., 1990, 18：3777-3783)、聚胺或聚乙二醇鏈(Manoharan et al., Nucleosides & 核苷酸, 1995, 14：969-973)、或金剛烷乙酸(Manoharan et al., Tetrahedron Lett., 1995, 36：3651-3654)、棕櫚基部分(Mishra et al., Biochim. Biophys. Acta, 1995, 1264：229-237)、或十八烷基胺或己胺基-羰氧基膽固醇部分(Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277：923-937)。

【0436】在某些態樣中，配體改變其摻入的 iRNA 劑的分佈、靶向、或生命期。在一些態樣中，配體對所選目標提高的親和力(例如，分子、細胞或細胞型)、腔室(例如，身體內之細胞或器官腔室、組織、器官或身體區域)，例如，相較於缺乏該配體的物種。在一些態樣中，配體在雙股核酸中並沒有參與雙股配對。

【0437】配體可以包含自然存在的物質，如蛋白質(例如，人血清蛋白(human serum albumin；簡稱 HSA)、低密度脂蛋白(low-density lipoprotein；簡稱 LDL)、血蛋白)；醣(例如，葡聚糖、聚三葡萄糖、幾丁質、幾丁聚醣、菊糖、環糊精、N-乙醯葡萄糖胺、N-乙醯半乳胺糖、 玻尿酸)；或脂質。配體也可以是重組的或合成的分子，如合成聚合物，例如，合成聚胺基酸。聚胺基酸的示例包含聚胺基酸是聚離胺酸(polylysine；簡稱 PLL)、聚 L-天冬胺酸、聚 L-麩胺酸、苯乙烯-順丁烯二[酸]酐共聚物、聚(L-乳酸-共-乙醇酸)共聚物、雙乙烯基醚-順丁烯二酸酐共聚物、N-(2-羥丙基)甲基丙烯醯胺共聚物(HMPA)、聚乙二醇(polyethylene glycol；簡稱 PEG)、聚乙烯醇(polyvinyl alcohol；簡稱 PVA)、聚胺甲酸酯、聚(2-乙基丙烯酸)、氮異丙基丙烯醯胺聚合物、聚磷腈。聚胺的示例包含：聚乙烯亞胺、聚離胺酸(PLL)、精胺、精胺酸、聚胺、偽肽-聚胺、擬肽聚胺、樹枝狀聚胺、精胺酸、脒、原胺類、陽離子脂質、陽離子卟啉、聚胺之四級鹽、α 螺旋肽。

【0438】配體也可包含靶向基團，例如細胞或組織靶向劑，例如凝集素、醣蛋白、脂質、蛋白質，例如連結於特定的細胞型如腎臟細胞之抗體。靶向基團目標可以是促甲狀腺素、黑促素、凝集素、醣蛋白、表面蛋白 A、黏蛋白醣、多價乳糖、多價半乳糖、N-乙醯-半乳胺糖、N-乙醯-葡萄糖胺多價甘露糖、多價岩

藻醣、糖化聚胺基酸、多價半乳糖、運鐵蛋白、雙磷酸酯、聚麩胺酸、聚天門冬胺酸、脂質、膽固醇、類固醇、膽酸、葉酸、維他命 B12、維他命 A、生物素、RGD 肽或 RGD 肽擬肽物。在特定態樣中，配體是多價半乳糖，例如 N-乙醯-半乳胺糖。

【0439】 其他配體的示例包含色素、嵌入劑(例如吖啶)、交聯劑(例如補骨脂素及絲裂黴素 C)、卟啉(TPPC4、德克薩卟啉、噻呋啉)、多環芳香烴(例如啡吩、二氫啡吩)、人工核酸內切酶(例如 EDTA)、親脂性分子，例如膽固醇、膽酸、金剛烷乙酸、1-芘丁酸、二氫睾固酮、1,3-Bis-氧(十六烷基)甘油、香葉氧己基、十六烷基甘油、冰片(borneol)、薄荷醇、1,3-丙二醇、十七烷基、棕櫚酸、十四酸(myristic acid)、O3-(油醯基)石膽酸、O3-(油醯基)膽烯酸、二甲氧三苯甲基、或啡噁吩及肽複合物(例如觸角足肽、Tat 肽)、烷化劑、磷酸酯、胺、氫硫基、PEG(例如 PEG-40K)、MPEG、[MPEG]2、聚胺、烷基、取代之烷基、放射性標誌標記物、酵素、半抗原(例如生物素)、運輸/吸收促進劑(例如阿斯匹靈、維他命 E、葉酸)、合成核糖核酸酶(例如咪唑、聯咪唑、組織胺、咪唑團簇、吖啶-咪唑接合體、大環四胺基 Eu3+錯合物)、二硝苯基、HRP、AP。

【0440】 配體可以是蛋白質，例如醣蛋白或肽，例如具有特定親和力作為共配體之分子或抗體，例如連結特定細胞型如肝細胞之抗體。配體也可已包含激素和激素受體。其也可包含非肽種，如脂質、凝集素、醣、維他命、輔因子、多價乳糖、多價半乳糖、N-乙醯-半乳胺糖、N-乙醯-葡萄糖胺多價甘露糖、多價岩藻醣。配體可以是例如脂多醣、p38 MAP 激酶活化子、NF-κB 活化子。

【0441】 配體可以是物質，例如可以提高細胞 iRNA 劑攝取之藥劑，例如，藉由破裂細胞之細胞骨架，例如藉由破裂細胞之微管、微絲、中間絲狀體。藥劑

可以是，例如紫杉醇、長春花新鹼、長春花鹼、細胞遲緩素、諾考達唑、促進微絲聚合劑、微絲解聚劑、毒傘素、紅海海綿抗菌素、茚酮衍生物、邁爾素(myoservin)。

【0442】在一些態樣中，配體連結於本文所述之 iRNA 作為藥物動力學調節劑(PK modulator)。PK 調節劑包含親脂體、膽酸、類固醇、磷脂類似物、胜肽、蛋白質結合劑、PEG、維他命等。示範性 PK 調節劑包含，但不限至於膽固醇、脂肪酸、膽酸、石膽酸、二烷基甘油酯、二醯基甘油酯、磷脂、神經鞘脂、萘普生、伊布洛芬、維他命 E、生物素。包括幾個硫代磷酸酯鍵之寡核苷酸也被習知可以與血清白蛋白結合，因此短寡核苷酸，例如，5 個鹼基、10 個鹼基、15 個鹼基、20 個鹼基的寡核苷酸，包括多個在主鏈中的硫代磷酸酯鍵被本案接受作為配體(例如作為 PK 調節配體)。此外，與血液成分結合的適配體(例如血清白蛋白)也適合在本文所述中作為 PK 調節配體。

【0443】本發明的配體接合的 iRNAs 可以透過使用帶有側接反應功能的寡核苷酸來合成，例如衍生自連接分子連接到寡核苷酸上的功能(如下所述)。該反應性寡核苷酸可直接與市售配體、合成的帶有任何多種保護基團的配體或具有與其連接的連接部分的配體反應。

【0444】在本發明中使用於接合體中的寡核苷酸可透過習知的固相合成技術方便地及常規地製備。用於這種合成的設備由幾個供應商出售，包括，例如 Applied Biosystems®(Foster City, Calif.)。所屬領域習知的用於該合成的任一其他方法可加成地或替代地被使用。還已知使用類似技術來製備其他寡核苷酸，例如硫代磷酸酯和烷基化衍生物。

【0445】 在本發明的配體接合的 iRNA 和帶有序列專一性連接的核苷的配體分子中，寡核苷酸和寡核苷可以在合適的 DNA 合成儀組裝，使用標準核苷酸或核苷前體，或已經帶有已經具有配體分子的連接部分、配體-核苷酸或核苷-接合物前體，或不帶有核苷配體的結構單元。

【0446】 當使用已具有連接部分的核苷酸-接合物前體，特定序列的核苷之合成通常已完成，且該配體分子接著與連接部分反應來形成配體接合的寡核苷。在一些態樣中，本發明中的寡核苷酸或連接的核苷由自動化合成儀合成，其係使用衍生自除了市售和常規地用作寡核苷合成的標準亞磷醯胺和非標準的亞磷醯胺之外的配體-核苷接合物之亞磷醯胺。

【0447】 *A. 脂質接合體*

在其他態樣中，配體或接合體為脂質或基於脂質的分子。在一個態樣中，脂質或基於脂質的分子與血清白蛋白結合，例如，人血清蛋白(HSA)。HSA 結合配體容許接合體分配至目標組織，例如，體內非腎臟目標組織。舉例而言，該目標組織可以為肝臟，包含肝臟的實質細胞。其他可以與 HSA 結合的分子也可被用作配體。例如，萘普生或阿斯匹靈可以被使用。脂質或基於脂質的配體可以(a)提高抗接合體之降解、(b)提高靶向或遞送至目標細胞或細胞膜或(c)可以被用作調整與血清白蛋白的結合，例如 HSA。

【0448】 基於脂質的配體可以用作抑制，例如，控制接合體與目標組織的結合。例如，與 HSS 結合較強的脂質或基於脂質的配體較不常以腎臟為目標，因此較不常自體內被清除。與 HSA 結合較弱的脂質或基於脂質的配體可被用作以腎臟的接合體為目標。

【0449】 在特定態樣中，該基於脂質的配體與 HSA 結合。在一個態樣中，其與 HSA 以足夠的親和力結合，所以該接合體會被分配至非腎臟組織。然而，傾向親和力不要太強以至 HSA-配體無法脫離。

【0450】 在其他態樣中，該基於脂質的配體與 HSA 很弱地結合或完全不結合。在一個態樣中，該接合體戶被分配至腎臟。其他以腎臟細胞為目標的部分也可以被用作取代基於脂質的配體或除了基於脂質的配體之外。

【0451】 在其他面向中，配體為一個部分，例如，維他命，其係由目標細胞吸收，例如，增生的細胞。這些尤其地有利於治療歸納為不良細胞增生的疾病，例如惡性或非惡性類型，例如，癌症細胞。示例性的維他命包含維他命 A、E 及 K。其他示範的維他命包含 B 維他命，例如，葉酸、B12、核黃素、生物素、吡哆醛或其他維他命或營養素由目標細胞所吸收，如肝臟細胞。也包含 HSA 和低密度脂蛋白(LDL)。

【0452】 *B. 細胞滲透劑*

在另一個面向中，配體是細胞滲透劑，如螺旋細胞滲透劑。在一個態樣中，該劑是雙極性的。示例性之劑是胜肽如 Tat 肽或觸足肽。假使該劑是胜肽，其可被修飾，包含肽模擬物(peptidylmimetic)、反轉異構體、非胜肽或假胜肽鍵聯及使用 D-胺基酸。在一個態樣中，螺旋藥劑為 α-螺旋藥劑，其 HSA 為親油和疏油相。

【0453】 配體可以為胜肽或擬肽。擬肽(於本文也係指寡擬肽)為能夠像天然胜肽摺疊成三維結構的分子。胜肽和擬肽與 iRNA 劑之附接會影響 iRNA 的藥物代謝動力學分布，如藉由提升細胞辨識和吸收。該胜肽或擬肽部分為大約 5 至 50 胺基酸長，例如，大約 5、10、15、20、25、30、35、40、45 或 50 胺基酸長。

【0454】 胜肽或擬肽可以為，例如，細胞滲透胜肽、陽離子胜肽、雙極性胜肽或疏水性胜肽(例如，主要包含 Tyr、Trp 或 Phe)。該 胜肽部分可以是樹枝狀聚合物胜肽、約束胜肽或交聯胜肽。在另一個替代中，該胜肽部分可包含疏水膜異位序列(Membrane translocation sequence；簡稱 MTS)。示例性的疏水包含 MTS 的胜肽是 RFGF，其具有胺基酸序列 AAVALLPAVLLALLAP(SEQ ID NO： 14)。RFGF 類似物(例如，胺基酸序列 AALLPVLLAAP(SEQ ID NO：15))包含疏水 MTS 也可以是目標部分。胜肽部分可以為「遞送」胜肽，其可以攜帶大極性分子包含胜肽、寡核苷酸及蛋白質穿過細胞膜。例如，來自 HIV Tat 蛋白質之序列(GRKKRRQRRRPPQ(SEQ ID NO：16))及果蠅屬觸足蛋白質(RQIKIWFQNRRMKWKK(SEQ ID NO：17))已被發現能夠用於遞送胜肽。胜肽或擬肽可以被隨機的 DNA 序列編碼，如由噬菌體展示庫(phage-display library)或一珠粒一化合物(one-bead-one-compound；簡稱 OBOC) 組合庫(Lam et al., Nature, 354：82-84, 1991)所識別的胜肽。藉由整合的單體單元作為細胞目標目的，聯繫於 dsRNA 劑之胜肽或擬肽為精胺酸-甘胺酸-天冬胺酸(RGD)-胜肽，或 RGD 模擬物。胜肽部分可以涵蓋長度從大約 5 個胺基酸至大約 40 個胺基酸。胜肽部分可以具有結構修飾，如作為提高穩定性或直接構形的特性。任一於以下描述的結構修飾可以被使用。

【0455】 RGD 胜肽作為本發明所使用的組成物和方法可以是線性的或環狀的，且可以被修飾，例如，醣基化的或甲基化的，來促進目標特定的組織。包含 RGD 的胜肽和肽模擬物可包含 D-胺基酸，以及合成的 RGD 模擬物。除了 RGD 之外，可以使用其他目標整合蛋白配體，例如，PECAM-1 或 VEGF 的其他部分。

【0456】「細胞滲透肽」能夠滲透一個細胞，例如微生物細胞，如細菌或真菌細胞，或哺乳動物細胞，如人類細胞。微生物細胞滲透肽可以是，例如，α-螺旋線性肽(例如 LL-37 或 Ceropin Pl)、含二硫鍵的肽(例如 α-防衛肽、β-防衛肽或牛白細胞抗菌肽)或僅含一種或兩種主要胺基酸的肽(例如 PR-39 或吲哚裡西啶(indolicidin))。細胞滲透肽也可以包含核定位信號(Nuclear localization signal；簡稱 NLS)。例如，細胞滲透肽可以是二重雙極性肽，如 MPG，其源自 HIV-lgp41 的融合物肽結構域和 SV40 大 T 抗原的 NLS(Simeoni et al.，Nucl .Acids Res. 31:2717-2724,2003)。

【0457】*C. 碳水化合物接合體*

在本發明組成物和方法的一些實施例中，iRNA 進一步包括一種碳水化合物。碳水化合物接合的 iRNA 對於核酸的遞送連同適合於體內治療用途的組成物而言是有利的，如本文所述。如本文所使用，「碳水化合物」係指一種化合物，其是一種本身由具有至少 6 個碳原子的一個或多個單糖單位構成的碳水化合物(其可以是線性的、支鏈的或環狀的)，其中氧、氮或硫原子結合至每一碳原子；或是一種具有其一個碳水化合物部分作為其一部分的化合物，該碳水化合物由具有至少六個碳原子的一個或多個單糖單位構成(其可以是線性的、支鏈的或環狀的)，其中氧、氮或硫原子結合至每一碳原子。代表性的碳水化合物包括糖(單糖、二糖、三糖及包含從大約 4、5、6、7、8 或 9 個單糖單元寡聚糖)，以及多糖如澱粉、糖原、纖維素以及多糖膠。特定單糖包含 C5 以及以上的(例如，C5、C6、C7 或 C8)糖；二糖以及三糖包含具有兩個或三個單糖單元的糖(例如，C5、C6、C7 或 C8)。

【0458】 在某些態樣中，用於在本發明的組成物以及方法中使用的碳水化合物接合體 一種單糖。

【0459】 在某些態樣中，該單醣是 N-乙醯半乳胺糖(GalNAc)。GalNAc 接合體，其包括一個或多個 N-乙醯半乳胺糖(GalNAc)衍生物，如所述，例如，於 US 8,106,022 之中，其所有內文以參考資料的方式整合於本文。在一些態樣中，該 GalNAc 接合體作為配體來目標 iRNA 至特定的細胞。在一些態樣中，該 GalNAc 接合體目標 iRNA 至肝細胞，例如，作為配體用於肝細胞(例如，肝細胞(hepatocytes))之去唾液酸糖蛋白受體。

【0460】 在一些態樣中，碳水化合物接合體包括一個或多個 GalNAc 衍生物。該 GalNAc 衍生物可以藉由一個連接子被附接，例如，二價或三價分支的連接子。在一些實施例 GalNAc 接合體是與有義股 3’末端接合的。在一些態樣中，該 GalNAc 接合體是藉由連接子，例如本文所述的連接子，與 iRNA 劑接合的(例如，有義股 3’末端)。在一些實施例 GalNAc 接合體是與有義股 5’末端接合的。在一些態樣中，該 GalNAc 接合體是藉由連接子，例如，如本文所述的連接子，與 iRNA 劑接合的(例如有義股 5’末端)。

【0461】 在本發某些態樣中，該 GalNAc 或 GalNAc 衍生物藉由單價連接子附接於本發明的 iRNA 劑。在一些態樣中，該 GalNAc 或 GalNAc 衍生物藉由二價連接子附接於本發明的 iRNA 劑。在本發明上還有其他實施例中，該 GalNAc 或 GalNAc 衍生物藉由三價連接子附接於本發明的 iRNA 劑。在本發明其他態樣中，該 GalNAc 或 GalNAc 衍生物藉由四價連接子附接於本發明的 iRNA 劑。

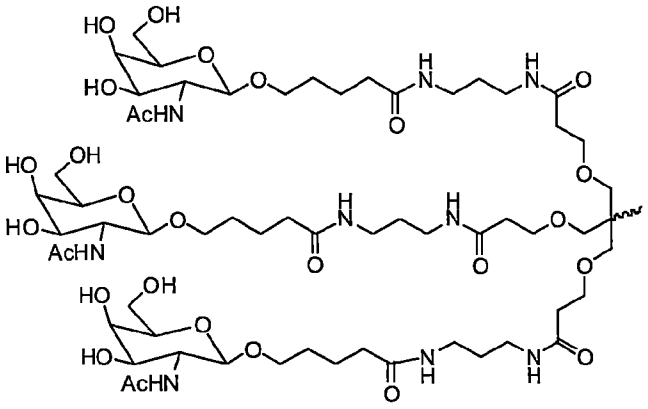
【0462】 在某些態樣中，本發明的雙股 RNAi 劑包括一個 GalNAc 或 GalNAc 衍生物附接於該 iRNA 劑。在其他態樣中，本發明該雙股 RNAi 劑包括

數個(例如 2、3、4、5 或 6)GalNAc 或 GalNAc 衍生物，各自獨立地藉由數個單價連接子附接於數個雙股 RNAi 劑的核苷酸。

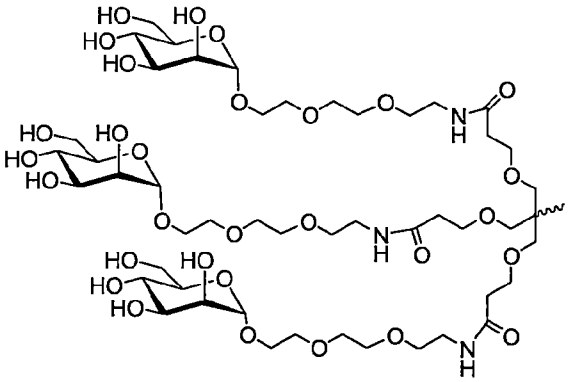
【0463】 在一些態樣中，例如，當本發明的 iRNA 劑之兩股是一個較大分子的一部份，其由核苷酸的連續鏈介於其中一股的 3'-末端和分別的另一股的 5'-末端所連結，形成一個髮夾環圈，包括數個未配對的核苷酸，各位在髮夾環圈內未配對的核苷酸可獨立地包括藉由單價連接子附接的 GalNAc 或 GalNAc 衍生物。髮夾環圈也可由雙股中其中一股的延伸突出所形成。

【0464】 在一些態樣中，例如，當本發明的 iRNA 劑之兩股是一個較大分子的一部份，其由核苷酸的連續鏈介在其中一股的 3'-末端和分別的另一股的 5'-末端所連結，形成一個髮夾環圈，包括數個未配對的核苷酸，各位在髮夾環圈內未配對的核苷酸可獨立地包括藉由單價連接子附接的 GalNAc 或 GalNAc 衍生物。髮夾環圈也可由雙鏈體中其中一股的延伸突出所形成。

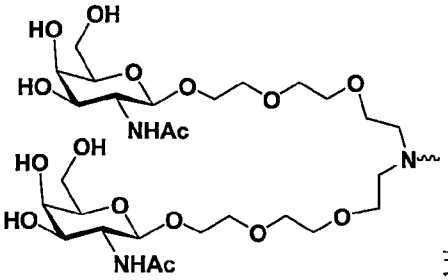
【0465】 在一個態樣中，用於本發明的組成物和方法的碳水化合物接合體是選自由以下組成之組：



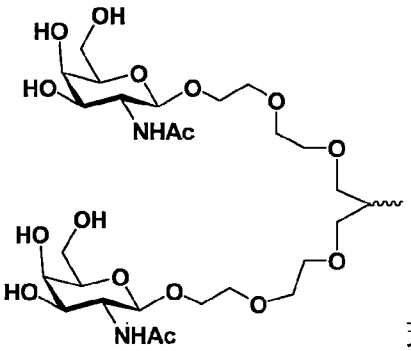
式 II、



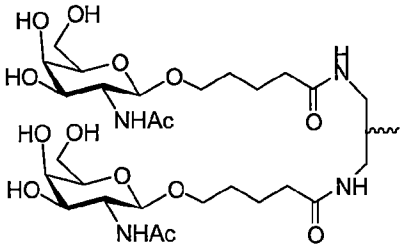
式 III、



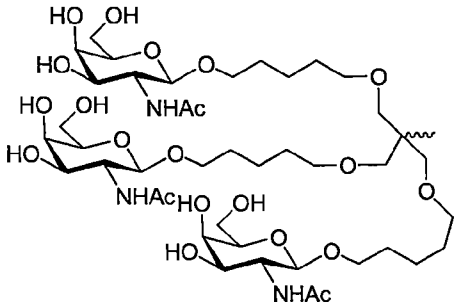
式 IV、



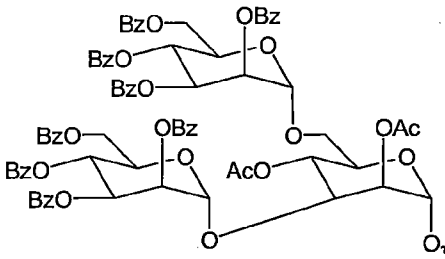
式 V、



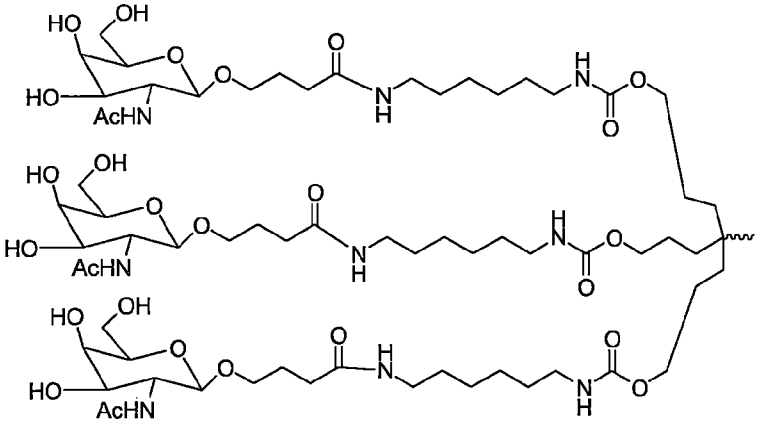
式 VI、



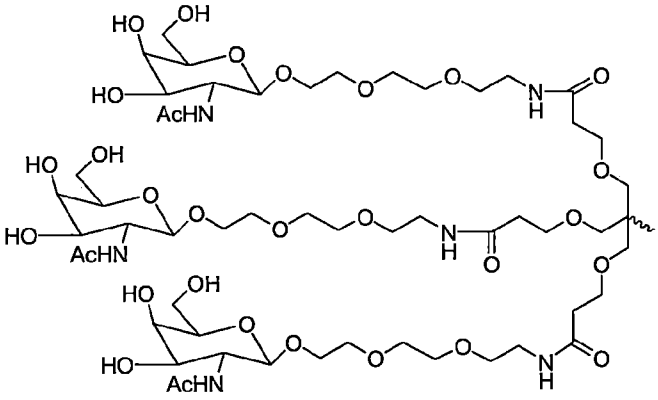
式 VII、



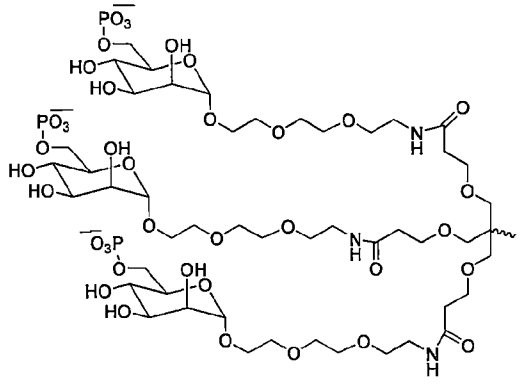
式 VIII、



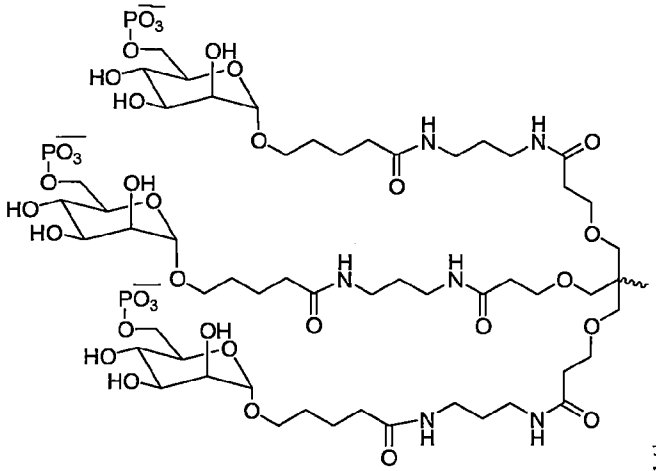
式 IX、



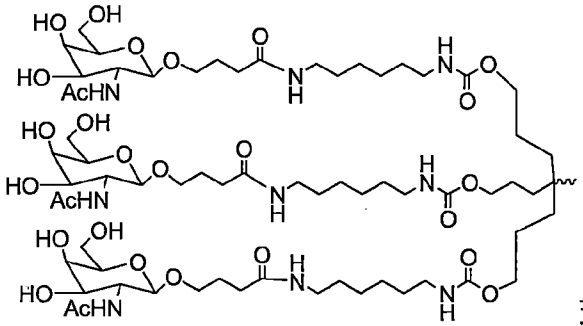
式 X、



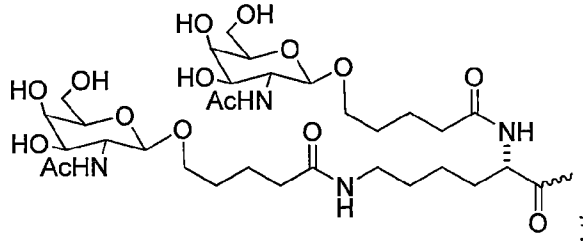
式 XI、



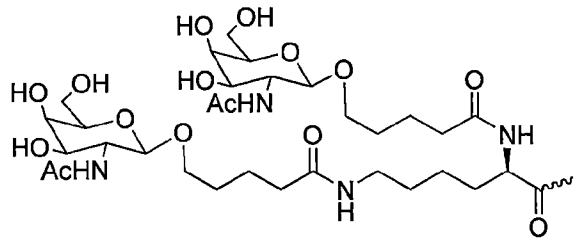
式 XII、



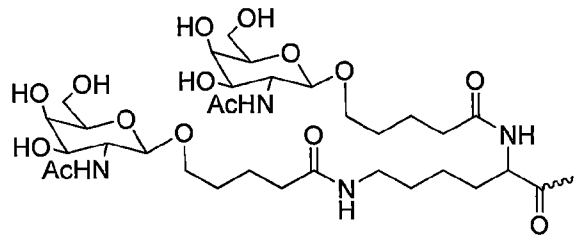
式 XIII、



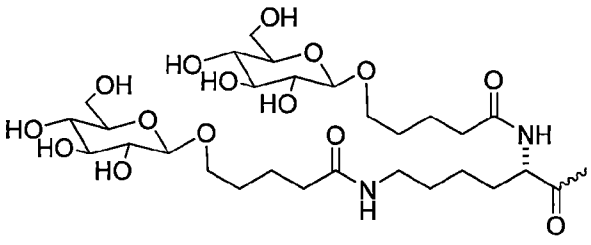
式 XIV、



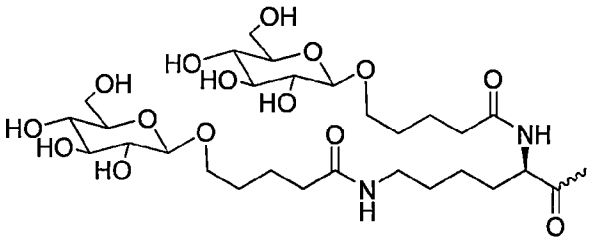
式 XV、



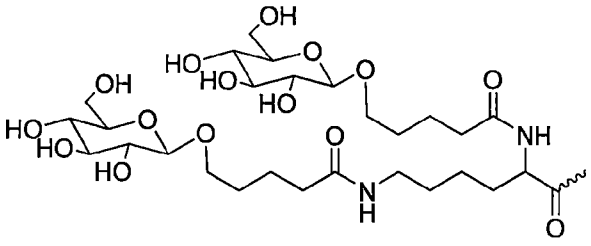
式 XVI、



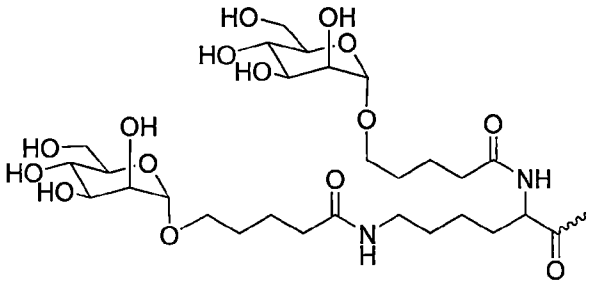
式 XVII、



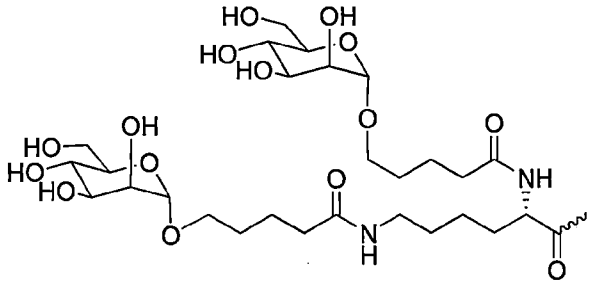
式 XVIII、



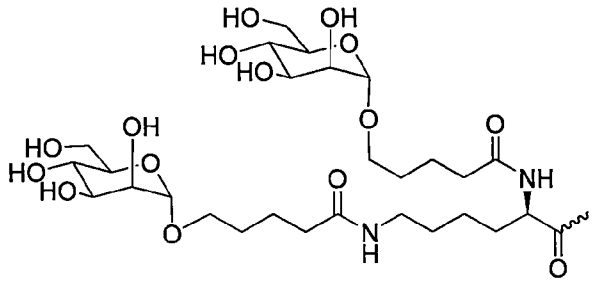
式 XIX、



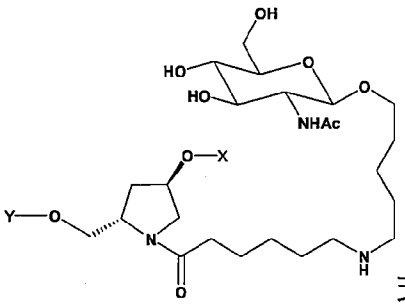
式 XX、



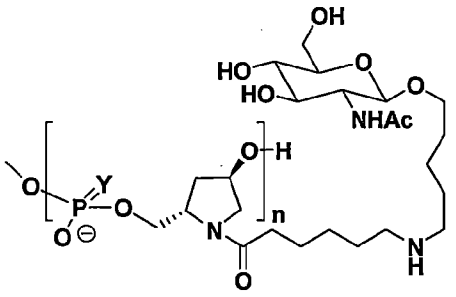
式 XXI、



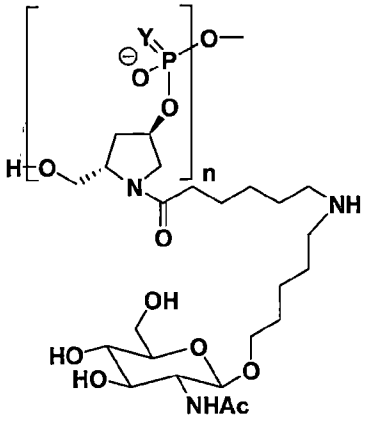
式 XXII、



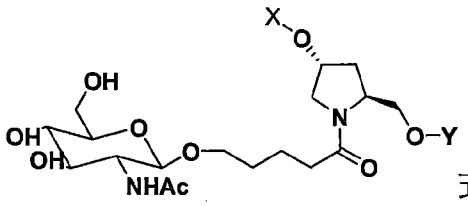
式 XXIII；



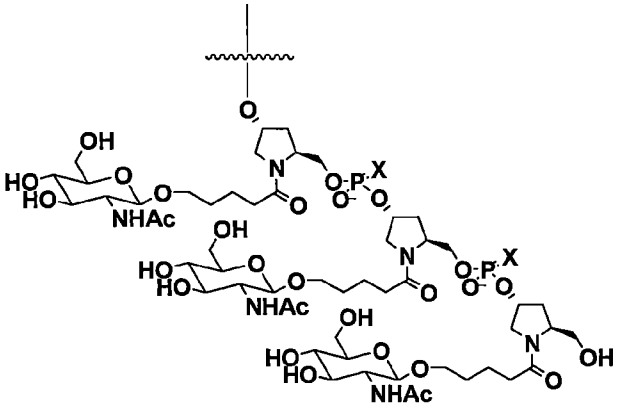
，其中，Y 是 O 或 S，且 n 是 3 至 6(式 XXIV)；



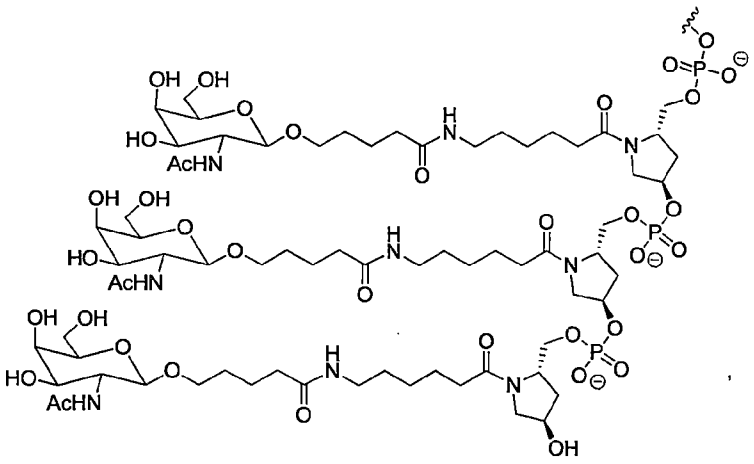
，其中，Y 是 O 或 S 且 n 是 3 至 6(式 XXV)；

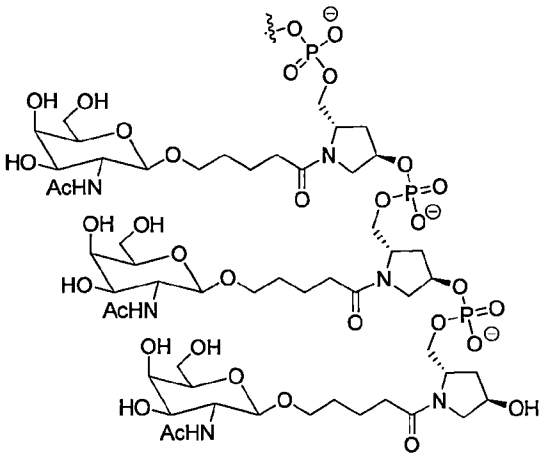


式 XXVI；

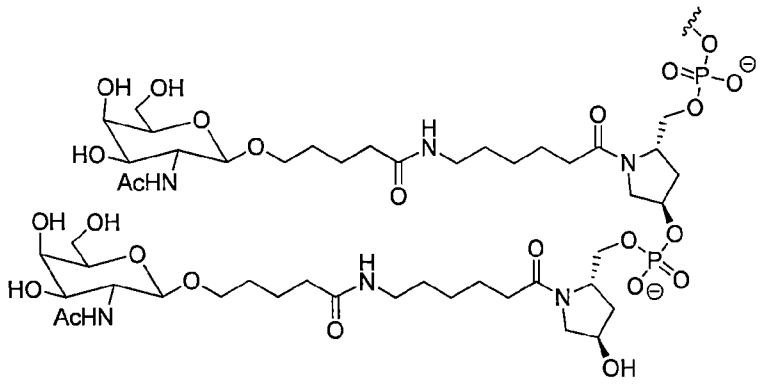


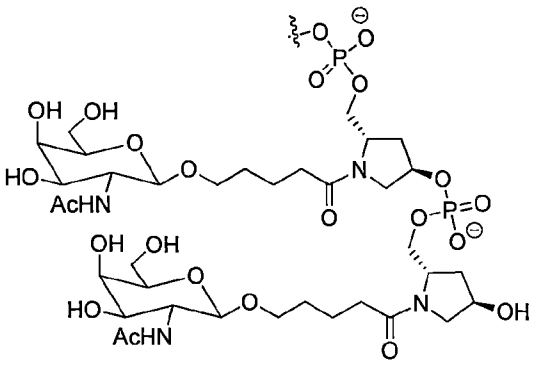
，其中，X 是 O 或 S(式 XXVII)；





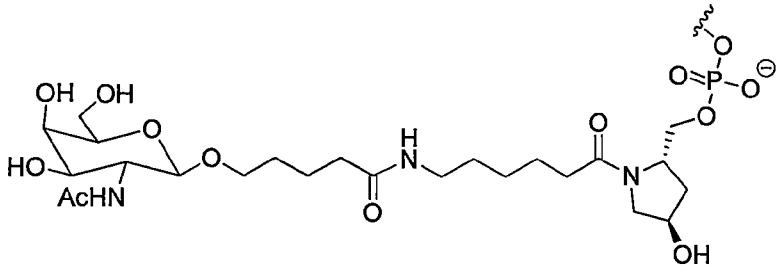
式 XXVII； 式 XXIX；



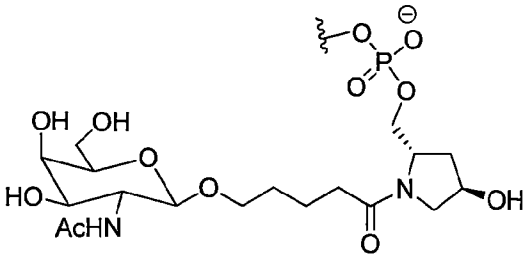


式 XXX；

式 XXXI；

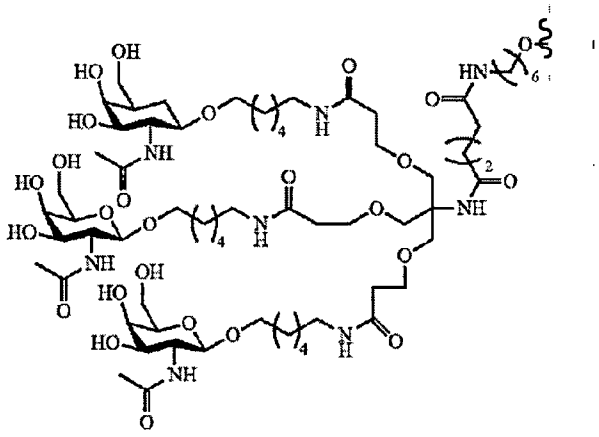


，及



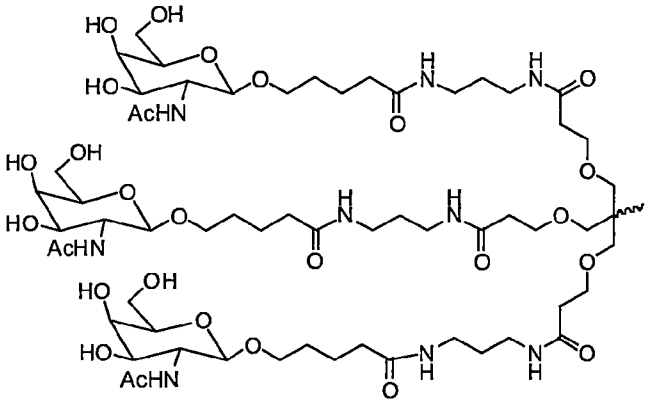
式 XXXII；

式 XXXIII.



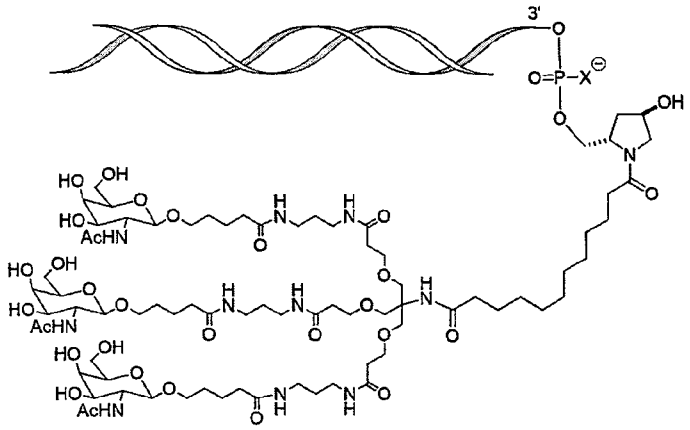
式 XXXIV。

【0466】在另一個態樣中，用於本發明的組成物和方法的碳水化合物接合體是單醣。在一個態樣中，該單醣是 N-乙醯半乳胺糖，如

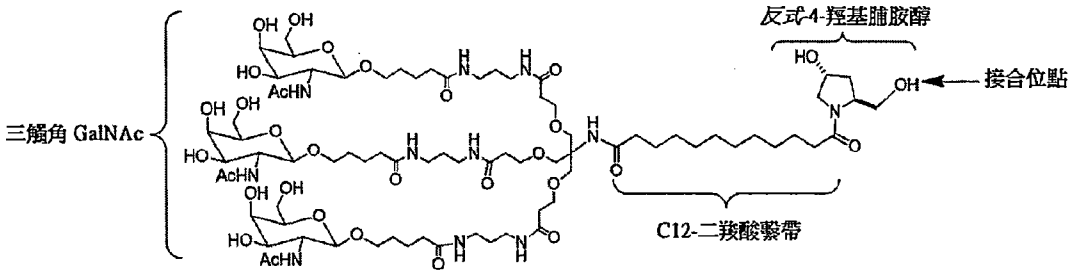


式II。

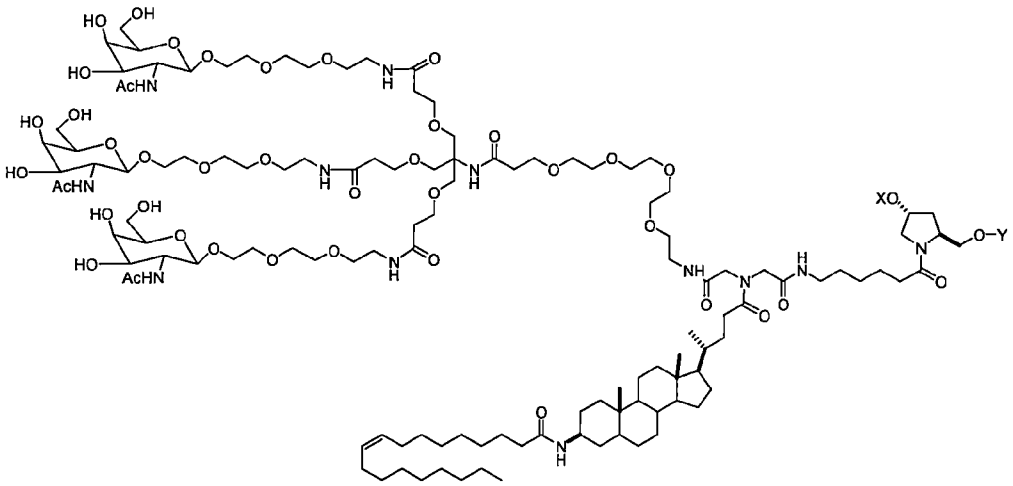
【0467】在一些態樣中，該RNAi劑是如下所示經由連接子附接於碳水化合物接合體，其中，X是O或S。



【0468】在一些態樣中，該RNAi劑如表1所定義與L96接合並示於以下：

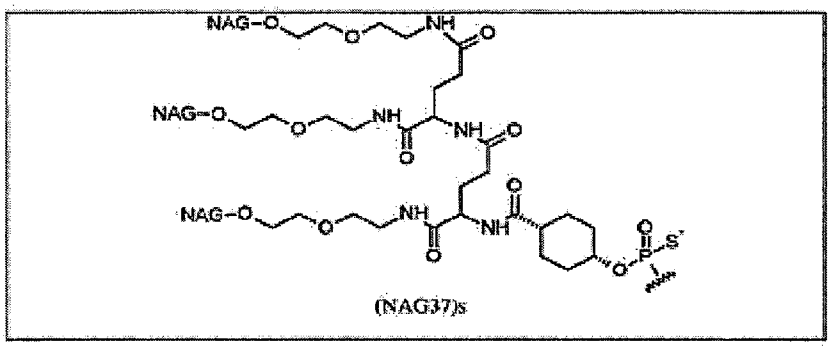


【0469】用於本文所述實施例的另一個示例性碳水化合物接合體包括但不限於，



(式 XXXVI)，當 X 或 Y 其中一個為寡核苷時，另一個是氫。

【0470】在一些態樣中，一個適合的配體為 WO 2019/055633 所揭示的配體，所有內文藉由引用方式併入本文。在一個態樣中，配體包括下結構：



【0471】在本發明某些態樣中，該 GalNAc 或 GalNAc 衍生物是藉由單價連接子附接於本發明的 iRNA 劑。在一些態樣中，該 GalNAc 或 GalNAc 衍生物是藉由二價連接子附接於本發明的 iRNA 劑。又在本發明另一態樣中，該 GalNAc 或 GalNAc 衍生物是藉由三價連接子附接於本發明的 iRNA 劑。

【0472】在一個態樣中，本發明該雙股 RNAi 劑包括一個或多個與 iRNA 劑附接的 GalNAc 或 GalNAc 衍生物。該 GalNAc 可藉由有義股或反義股上的連接子附接於任一核苷酸。該 GalNac 可附接於有義股之 5'-末端、有義股之 3'-末

端、反義股之 5'-末端或反義股之 3'-末端。在一個態樣中，該 GalNac 附接於有義股之 3'-末端，例如藉由三價連接子。

【0473】 在其他態樣中，本發明該雙股 RNAi 劑包括數個(例如 2、3、4、5 或 6)GalNAc 或 GalNAc 衍生物，各獨立地藉由數個連接子，例如單價連接子，與雙股 RNAi 劑數個核苷酸。

【0474】 在一些態樣中，例如，當本發明的 iRNA 劑之兩股是一個較大分子的一部份，其由核苷酸的連續鏈介在其中一股的 3'-末端和分別的另一股的 5'-末端所連結，形成一個髮夾環圈包括，數個未配對的核苷酸時，各位在髮夾環圈內未配對的核苷酸可獨立地包括藉由單價連接子附接的 GalNAc 或 GalNAc 衍生物。

【0475】 在一些態樣中，該碳水化合物接合體進一步包括一個或多個如上所述的額外的配體，如，但不限於 PK 調解劑或細胞滲透胜肽。

【0476】 適合用於本發明的額外的碳水化合物接合體和連結子包含 PCT 公開號 WO 2014/179620 和 WO 2014/179627 所述，所有內文以參考資料的方式併入本文。

【0477】 *D. 連接子*

在一些態樣中，本文所述的接合體或配體可以各式各樣的可裂解或不可裂解的連接子附接於 iRNA 寡核苷。

【0478】 術語「連接子」或「連接基團」意指一種有機部分，其連接一個化合物的兩個部分，例如，共價地附接一個化合物的兩個部分。連接子典型地包括一種直接的鍵或一種原子，如氧或硫，一種單位如 NR8、C(O)、C(O) NH、SO、SO_2、SO_2NH 或者一種原子鏈，如，但不限於，經取代或未經取代的烷基、經取

代或未經取代的烯基、經取代或未經取代的炔基、芳基烷基、芳基烯基、芳基炔基、雜芳基烷基、雜芳基烯基、雜芳基炔基、雜環烷基、雜環烯基、雜環炔基、芳基、雜芳基、雜環基、環烷基、環烯基、烷基芳基烷基、烷基芳基烯基、烷基芳基炔基、烯基芳基烷基、烯基芳基烯基、烯基芳基炔基、炔基芳基烷基、炔基芳基烯基、炔基芳基炔基、烷基雜芳基烷基、烷基雜芳基烯基、烷基雜芳基炔基、烯基雜芳基烷基、烯基雜芳基烯基、烯基雜芳基炔基、炔基雜芳基烷基、炔基雜芳基烯基、炔基雜芳基炔基、烷基雜環烷基、 烷基雜環烯基、烷基雜環炔基、烯基雜環烷基、烯基雜環烯基、烯基雜環炔基、炔基雜環烷基、炔基雜環烯基、炔基雜環炔基、烷基芳基、烯基芳基、炔基芳基、烷基雜芳基、烯基雜芳基、炔基雜芳基，其中一個或多個亞甲基可以被以下中斷或封端：O、S、S(O)、SO_2、N(R8)、C(O)、經取代或未經取代的芳基、經取代或未經取代的雜芳基或經取代或未經取代的雜環基；其中，R8 是氫、醯基、脂肪族的或經取代的脂肪族的。在一個態樣中，該連接子是在約 1 至 24 個原子、2 至 24、3 至 24、4 至 24、5 至 24、6 至 24、6 至 18、7 至 18、8 至 18、7 至 17、8 至 17、6 至 16、7 至 17 或 8 至 16 個原子之間。

【0479】 可裂解連接基是一種基團，其在細胞外部充分穩定，但是一旦進入目標細胞時則被裂解以釋放由連接子固定在一的兩個部分。在一個示例性的態樣中，可裂解連接基在目標細胞中或在第一參照條件(其可以，例如被選為模擬物或代表細胞內條件)下的裂解，比在個體的血液中或在第二參考條件下(其可以，例如被選為模擬物或代表在該血液或血清中發現的條件)快至少約 10 倍、20 倍、30 倍、40 倍、50 倍、60 倍、70 倍、80 倍、90 倍或更多，或至少約 100 倍。

【0480】 可裂解的連接基團易受裂解劑的影響，例如 pH、氧化還原電位或降解分子的存在。通常，裂解劑在細胞內部比血清或血液中更普遍或以更高的含量或活性存在。此類降解劑的例子包括：針對特定受質選擇的或沒有受質專一性的氧化還原劑，存在於細胞中的氧化或還原酶或還原劑，例如硫醇，可以藉由還原降解氧化還原可裂解的連接基團；酯酶；可產生酸性環境的核內體或試劑，例如導致 pH 值為 5 或更低的那些；可以透過充當一般酸來水解或降解酸可裂解連接基團的酶、肽酶(可以是受質專一性的)，及磷酸酶。

【0481】 可裂解鍵基團，如二硫鍵可對 pH 敏感。人類血清的 pH 是 7.4，而平均的細胞內 pH 略微更低，範圍從大約 7.1 至 7.3。胞內體具有一個更酸的 pH，範圍在 5.5 至 6.0，且溶酶體具有一個甚至更酸的 pH，在 5.0 左右。一些連接子具有可裂解連接基，這些基團在一個選定的 pH 被裂解，從而將陽離子脂質從細胞內配體釋放，或釋放進入目標細胞區室。

【0482】 連接子可以包含由特定酶可裂解的可裂解連接基。併入連接子的可裂解連接基類型可以取決於有待被目標的細胞。例如，肝臟目標向配體可以藉由一種包含酯基團的連接子而連接至陽離子脂質。肝細胞富含酯酶，且因此與不富含酯酶的細胞相比，該連接子將在肝細胞中被更有效地裂解。富含酯酶的其他細胞類型包括肺、腎皮質和睾丸的細胞。

【0483】 包含肽鍵的連接子可以用於目標向富含肽酶的細胞類型，如肝臟細胞和滑膜細胞。

【0484】 總體而言，一種候選可裂解連接基的適合性可以藉由測試降解劑(或條件)裂解候選的連接基的能力來進行評估。還希望測試候選可裂解連接基在血液中或當與其他非目標組織接觸時抵抗裂解的能力。因此，可以判定在一個第

一條件與一個第二條件之間進行裂解的相對敏感性，其中該第一條件被選擇為指示在一個目標細胞中的裂解並且該第二條件被選擇為指示在其他組織或生物體液中的裂解，例如，血液或血清。評估可以在無細胞系統、細胞、細胞培養物、器官或組織培養物或整個動物中進行。在特定的態樣中，有用的候選化合物在細胞中(或在選擇為模擬細胞內條件的體外條件下)的裂解比在血液或血清(或在被選擇為模擬細胞外條件的體外條件下)中的裂解快至少大約 2、4、10、20、30、40、50、60、70、80、90 或 100 倍。

【0485】 *i. 氧化還原可裂解連接基*

在某些態樣中，可裂解連接基是一種氧化還原可裂解連接基，其在還原或氧化時被裂解。還原可裂解連接基的實施例是二硫連接基(-S-S-)。為了確定一個候選可裂解連接基是否是一種適合的「還原可裂解連接基」，或者例如是否適合於與一種特定的 iRNA 部分以及具特定目標劑一使用，可以參考本文所述的方法。例如，可以藉由與二硫蘇糖醇(DTT)或使用所屬領域習知的試劑的其他還原劑進行培養來評估候選物，其模擬了會在細胞內(例如在目標細胞內)觀察到的裂解速率。該候選物也可以在選擇為模擬血液或血清條件的條件下進行評估。在一個態樣中，候選化合物在血液中被裂解最多大約 10%。在其他態樣中，有用的候選化合物在細胞中(或在被選擇為模擬細胞內條件的體外條件下)的降解比在血液中(或在選擇以模擬細胞外條件的體外條件下)中的降解快至少大約 2、4、10、20、30、40、50、60、70、80、90 或 100 倍。可以使用標準酶動力學測定法在選擇為模擬胞內介質的條件下且與選擇為模擬胞外介質的條件相比，判定候選化合物的裂解速率。

【0486】 *ii. 基於磷酸酯的可裂解連接基*

在其他態樣中，可裂解連接子包括一種基於磷酸酯的可裂解連接基。基於磷酸酯的可裂解連接基藉由降解或水解該磷酸酯基團的劑而被裂解。一種在細胞中裂解磷酸酯基團的劑的實施例是酶，如細胞中的磷酸酶。基於磷酸酯的連接基的實例是-O-P(O)(ORk)-O-、-O-P(S)(ORk)-O-、-O-P(S)(SRk)-O-、-S-P(O)(ORk)-O-、-O-P(O)(ORk)-S-、-S-P(O)(ORk)-S-、-O-P(S)(ORk)-S-、-S-P(S)(ORk)-O-、-O-P(O)(Rk)-O-、-O-P(S)(Rk)-O-、-S-P(O)(Rk)-O-、-S-P(S)(Rk)-O-、-S-P(O)(Rk)-S-、-O-P(S)(Rk)-S-，其中，在各個基於磷酸酯連接基中的出現可以獨立地為 C1-C20 烷基、C1-C20 鹵烷基、C6-C10 芳香基或芳烷基。示例性的實例是-O-P(O)(OH)-O-、-O-P(S)(OH)-O-、-O-P(S)(SH)-O-、-S-P(O)(OH)-O-、-O-P(O)(OH)-S-、-S-P(O)(OH)-S-、-O-P(S)(OH)-S-、-S-P(S)(OH)-O-、-O-P(O)(H)-O-、-O-P(S)(H)-O-、-S-P(O)(H)-O、-S-P(S)(H)-O-、-S-P(O)(H)-S-以及-O-P(S)(H)-S-。在特定的實施例是-O-P(O)(OH)-O-。可以使用與以上所述的那些相似的方法評估這些候選物。

【0487】 *iii. 酸可裂解連接基*

在其他態樣中，可裂解連接子包括一種酸可裂解連接基。酸可裂解連接基在酸性條件下被裂解的一種連接基。在特定的實施例中，酸可裂解連接基在一個具有大約 6.5 或更低(例如，大約 6.0、5.5、5.0 或更低)的 pH 的酸性環境中被裂解，或者被多種劑如可以作為一種廣義酸的酶裂解。在細胞中，特定低 pH 胞器，如胞內體和溶酶體可以為酸可裂解連接基提供裂解環境。酸可裂解連接基的實施例包括但不限於腙、酯和胺基酸酯。酸可裂解基團可以具有通式-C=NN-、C(O)O 或-OC(O)。示例性的實例是與酯的氧附接的碳(烷氧基)是芳香基、取代的烷基或叔烷基，如二甲基戊基或第三-丁基時的情況。可以使用與以上所述的那些相似的方法評估這些候選物。

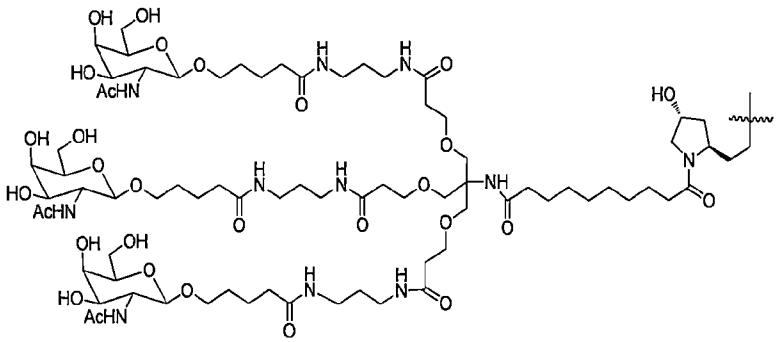
【0488】 *iv. 基於酯的連接基*

在其他態樣中，可裂解連接子包括一種基於酯的可裂解連接基。基於酯的可裂解連接基被酶如細胞中的酯酶與醯胺酶裂解。基於酯的可裂解連接基的實施例包括但不限於亞烷基、亞鏈烯基和亞炔基的酯。酯可裂解連接基具有通式-C(O)O-或-OC(O)-。可以使用與以上所述的那些相似的方法評估這些候選物。

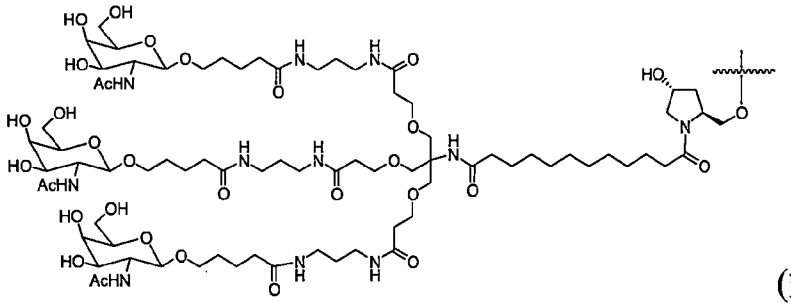
【0489】 *v. 基於胜肽的連接基*

在其他態樣中，可裂解連接子包括一種基於胜肽的可裂解連接基。基於胜肽的可裂解連接基被酶裂解如細胞中的肽酶與蛋白酶。基於胜肽的可裂解連接基是在胺基酸之間形成肽鍵，以產生寡肽(例如，二肽、三肽等)以及多肽。基於肽的可裂解基團不包括醯胺基(-C(O)NH-)。醯胺基可以在任一亞(伸)烷基、亞(伸)鏈烯基或亞(伸)炔基之間形成。肽鍵是在胺基酸之間形成，以產生肽和蛋白的特殊類型的醯胺鍵。基於胜肽的可裂解基團總體上局限於在胺基酸之間形成產生肽以及蛋白的肽鍵，並且不包含整個醯胺官能團。基於肽的可裂解連接基具有通式－NHCHRAC(O)NHCHRBC(O)-，其中 RA 與 RB 是這兩個鄰接胺基酸的 R 基團。可以使用與以上所述的那些相似的方法評估這些候選物。

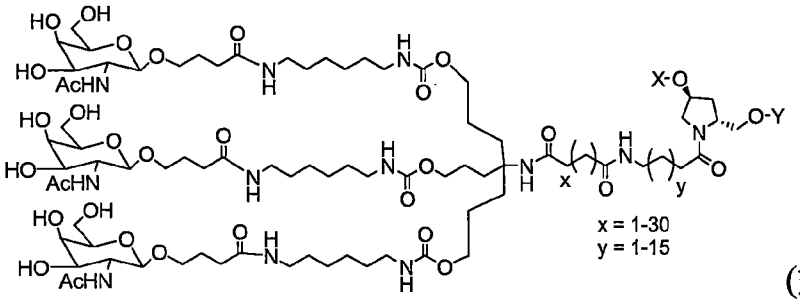
【0490】 在一些態樣中，本發明的 iRNA 藉由一種連接子被接合至一種碳水化合物。具有本發明的組成物與方法的連接子的 iRNA 碳水化合物接合體的非限制性實施例包括但不限於，



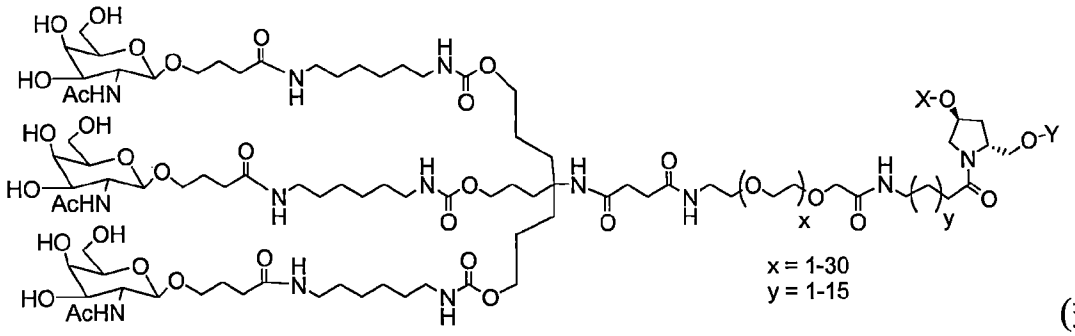
(式 XXXVII)、



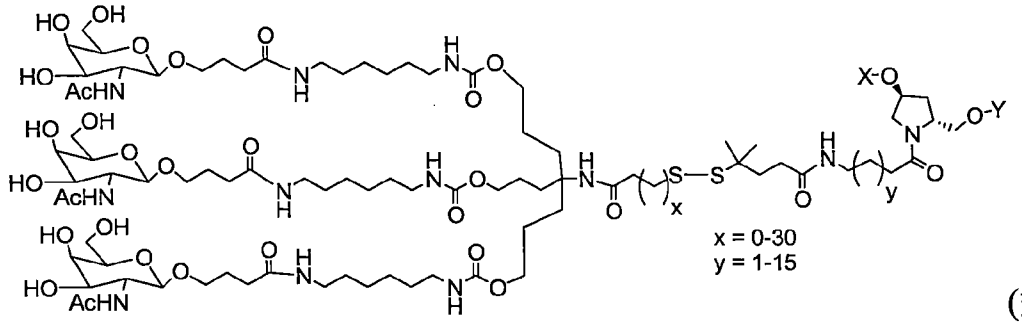
(式 XXXVIII)、



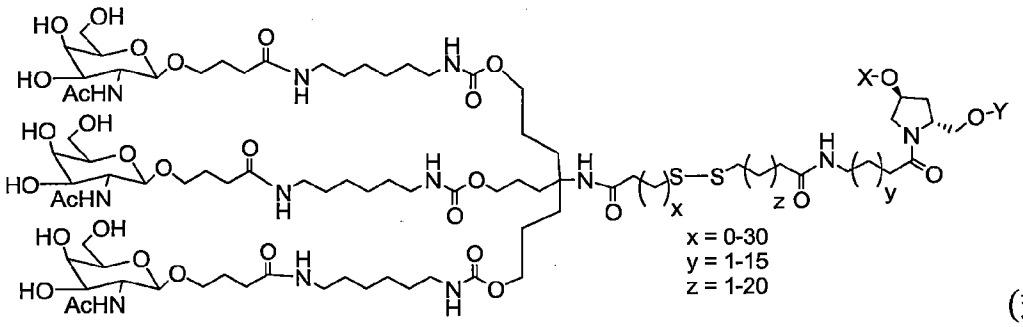
(式 XXXIX)、



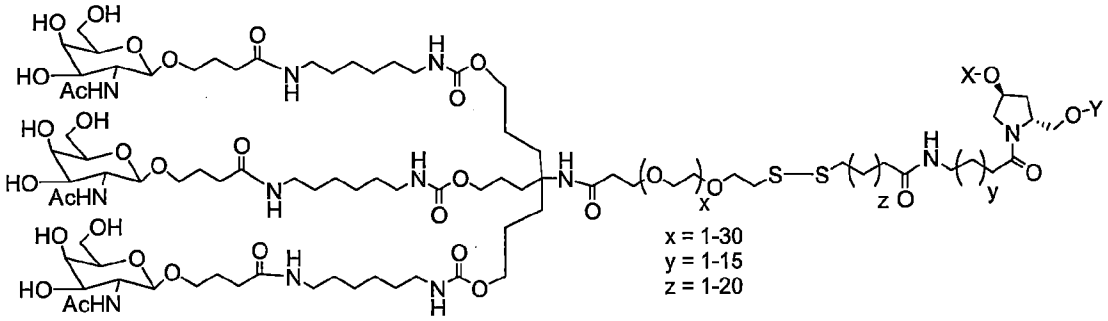
(式 XL)、



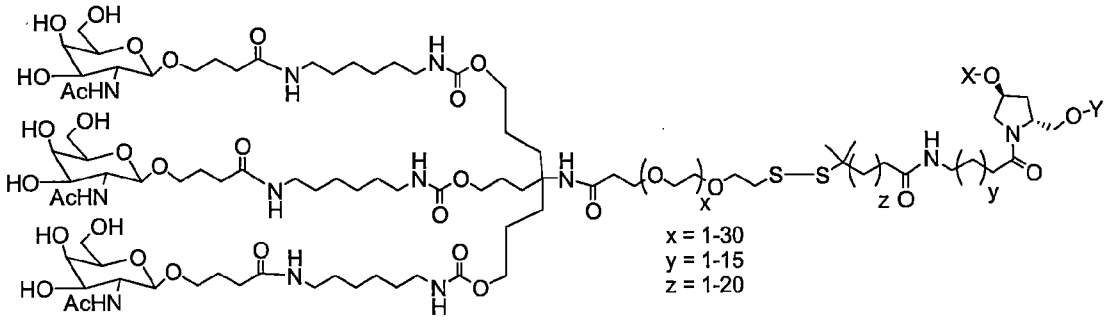
(式 XLI)、



(式 XLII)、



(式 XLIII)，以及

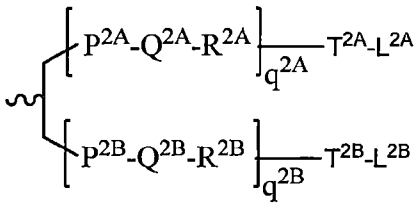


(式 XLIV)，其中當 X 或 Y 其中一個為寡核苷時，另一個為氫。

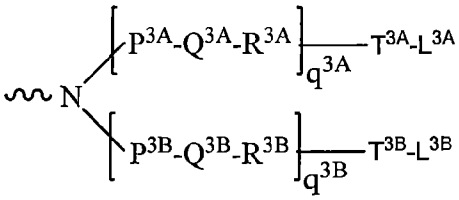
【0491】 在本發明的組成物和方法的某些態樣中，配體是藉由二價或三價分支連接子而附接的一個或多個 GalNAc(N-乙醯半乳糖胺)衍生物。

【0492】 在一個態樣中，本發明的 dsRNA 接合至一種二價或三價分支連接子，其選自由式(XLV)－(XLVI)的任一者中所示的結構組成的組

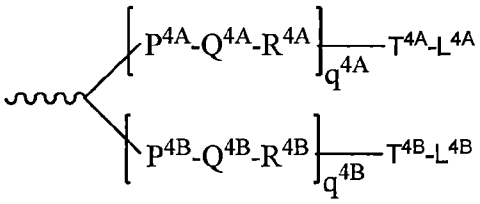
式 XLV　　式 XLVI



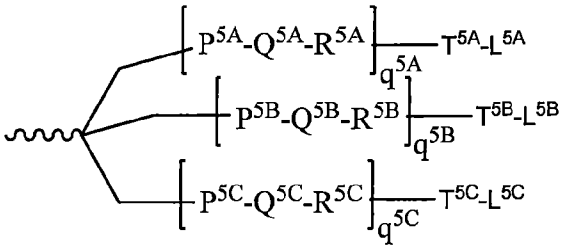
、



、



、或



；

式 XLVII　　式 XLVIII

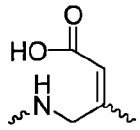
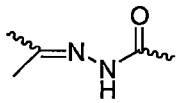
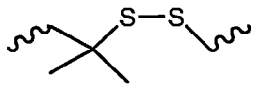
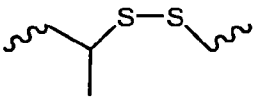
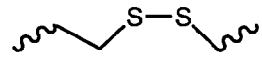
其中：

每次出現時，q2A、q2B、q3A、q3B、q4A、q4B、q5A、q5B 及 q5C 各自獨立表示 0 至 20，且其中該重複單元可以是相同或不同的；

每次出現時，P^{2A}、P^{2B}、P3A、P^{3B}、P^{4A}、P^{4B}、P^{5A}、P^{5B}、P^{5C}、T^{2A}、T^{2B}、T^{3A}、T^{3B}、T^{4A}、T^{4B}、T^{4A}、T^{5B}、T^{5C} 各自獨立地不存在、為 CO、NH、O、S、OC(O)、NHC(O)、CH_2、CH_2NH 或 CH_2O；

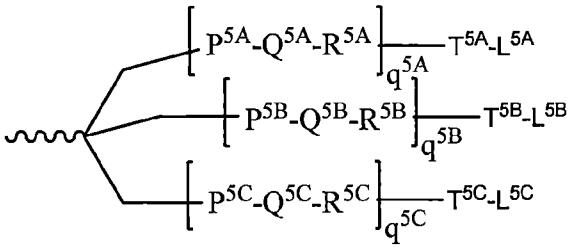
每次出現時，Q^{2A}、Q^{2B}、Q^{3A}、Q^{3B}、Q^{4A}、Q^{4B}、Q^{5A}、Q^{5B}、Q^{5C} 各自獨立地不存在、為亞(伸)烷基、為取代的亞(伸)烷基，其中，一個或多個亞甲基可以被一個或多個 O、S、S(O)、SO_2、$N(R^N)$、C(R')=C(R")、C≡C 或 C(O)中斷或中止；

【0493】每次出現時，R^{2A}、R^{2B}、R^{3A}、R^{3B}、R^{4A}、R^{4B}、R^{5A}、R^{5B}、R^{5C} 獨立地不存在、為 NH、O、S、CH_2、C(O)O、C(O)NH、$NHCH(R^a)C(O)$ 、-C(O)-

CH(R^a)-NH-、CO、CH=N-O、、、、、或雜環基；

【0494】 L^{2A}、L^{2B}、L^{3A}、L^{3B}、L^{4A}、L^{4B}、L^{5A}、L^{5B}及L^{5C}表示配體；亦即每次出現時獨立地為單醣(如 GalNAc)、雙醣、三醣、四醣、寡醣或多醣；且R^a為 H 或胺基酸支鏈。三價接合的 GalNAc 衍生物特別適用於與 RNAi 劑一使用以抑制靶基因的表達，例如式(XLIX)的那些：

式 XLIX



，其中，L^{5A}、L^{5B}以及 L^{5C}表示單醣，如 GalNAc 衍生物。

【0495】 適合的二價和三價分支連接基團接合 GalNAc 衍生物的實例包括但不限於以上所述式 II、VII、XI、X 以及 XIII 的結構。

【0496】 教示製備 RNA 接合體的代表性美國專利包括但不限於：美國專利號 4,828,979、4,948,882、5,218,105、5,525,465、5,541,313、5,545,730、5,552,538、5,578,717、5,580,731、5,591,584、5,109,124、5,118,802、5,138,045、5,414,077、5,486,603、5,512,439、5,578,718、5,608,046、4,587,044、4,605,735、4,667,025、4,762,779、4,789,737、4,824,941、4,835,263、4,876,335、4,904,582、4,958,013、5,082,830、5,112,963、5,214,136、5,082,830、5,112,963、5,214,136、5,245,022、5,254,469、5,258,506、5,262,536、5,272,250、5,292,873、5,317,098、5,371,241、

5,391,723、5,416,203、5,451,463、5,510,475、5,512,667、5,514,785、5,565,552、5,567,810、5,574,142、5,585,481、5,587,371、5,595,726、5,597,696、5,599,923、5,599,928、5,688,941、6,294,664、6,320,017、6,576,752、6,783,931、6,900,297、7,037,646、及 8,106,022，以上各個專利的所有內容藉由藉由引用併入本文。

【0497】給定化合物中的全部位置不必要經統一修飾，且實際上可以在單個化合物中或甚至在 iRNA 內部的單個核苷處併入多於一個前述修飾。本發明也包含作為嵌合化合物的 iRNA 化合物。

【0498】本發明內文的「嵌合體的」iRNA 化合物或「嵌合體」是 iRNA 化合物，如 dsRNA，其包含兩個或更多個化學上不同的區域，每者由至少一個單體單元構成，亦即在 dsRNA 化合物的情況下的一種核苷酸。這些 iRNA 典型地包含至少一個區域，其中 RNA 經修飾，從而賦予 iRNA 增加的核酸酶降解抗性、增加的細胞攝取或增加的目標核酸結合親和力。iRNA 的額外區域可以作為能夠裂解 RNA: DNA 或 RNA: RNA 雜交分子的酶的受質。例如，RNA 酶 H 是裂解 RNA: DNA 雙螺旋的 RNA 鏈的細胞內切酶。因此，RNA 酶 H 的活化導致目標 RNA 的裂解，從而大大提升 iRNA 抑制基因表現的效率。因此，與雜交至相同目標區域的硫代磷酸酯脫氧 dsRNA 相比，可以在使用嵌合 dsRNA 時，經常用更短的 iRNA 獲得可比較的結果。可以藉由凝膠電泳，並且如果需要，以所屬領域習知的相關核酸雜交技術常規地檢測目標 RNA 的裂解。

【0499】在某些態樣中，iRNA 的 RNA 可以藉由非配體基團來進行修飾。多個非配體分子已經被接合至 iRNA 以增強該 iRNA 的活性、細胞分布或細胞攝取，並且用於進行此類接合程序在科學文獻中是可得的。這類非配體部分包括脂質部分，如膽固醇(Kubo, T. *et al., Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-

61; Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553)、膽酸(Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 1994, 4:1053)、硫醚，例如，己基-S-三苯甲基硫醇(Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 1993, 3:2765)、疏基膽固醇(Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533)、脂族鏈，例如，十二醇殘基或十一烷殘基(Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49)、磷脂，例如，二鯨錯基-外消旋-甘油或三乙基銨 1，2-二-O-鯨蠟基-外消旋-甘油-3-膦酸酯(Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777)、多胺或聚乙二醇鏈(Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969)、或金剛烷乙酸(Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651)、棕櫚基部分(Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229)，或十八烷基胺或己基胺基-羰醯-氧基膽固醇部分(Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923)。教示製備這類 RNA 接合體的代表性美國專利已經在上文列出。典型的接合方案涉及在序列的一個或多個位置具有胺基連接子的 RNA 的合成。胺基接著與分子反應，使用適當的接合或活化試劑接合所述分子。接合反應可以與固相支撐物結合或在裂解 RNA 後處於溶液相的 RNA 進行。典型地，透過 HPLC 純化 RNA 接合體以提供純的接合體。

【0500】 IV. 本發明的 iRNA 之遞送

本發明的 iRNA 靶向細胞，例如個體體內的細胞，如人類個體(例如有其需要的個體，如傾向或罹患 F12 相關疾病的個體，例如遺傳性血管水腫(HAE)(第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何

因提升的舒緩肽量導致的任何遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。體內的細胞中的遞送可按多種不同的方式達成。例如，遞送可透藉由用本發明的 iRNA 體外或在體內接觸細胞進行。還可以藉由向個體給予包括 iRNA 的組合物，例如 dsRNA，直接進行體內遞送。可替代地，可透過給予編碼和指導 iRNA 表現的一種或多種載體間接地進行體內遞送。這些替代方案於以下進一步討論。

【0501】 通常地，遞送核酸分子(體外或體內)的任一方法可適於隨本發明的 iRNA 一同使用(參見例如 Akhtar S. and Julian RL.(1992) *Trends Cell. Biol.* 2(5)：139-144 and WO94/02595，所述參考資料藉由之全部內容以引用併入本文)。對於體內遞送，遞送 iRNA 分子的因素包含，例如，所遞送分子的生物學穩定性、防止非特異性作用以及所遞送分子在目標組織中的積累。RNA 干擾也已經成功顯示藉由直接注射至 CNS(Dorn, G., *et al.*(2004) *Nucleic Acids* 32:e49; Tan, PH., *et al*(2005) *Gene Ther.* 12:59-66; Makimura, H., *et al*(2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al*(2004) *Neuroscience* 129:521-528; Thakker, ER., *et al*(2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al*(2005) *J. Neurophysiol.* 93:594-602)。RNA 或醫藥載體的修飾也可以允許目標 iRNA 至目標組織並避免不欲之脫靶效應。iRNA 分子可以透過化學接合至親脂性基團，如膽固醇，以增強細胞攝取和防止降解。例如，將與親脂性膽固醇部分接合的直接針對 ApoB 的 iRNA 全身注射至小鼠，並且導致肝臟和空腸二者中 apoB mRNA 的減弱(Soutschek, J., *et al*(2004) *Nature* 432:173-178)。

【0502】 在一個替代態樣中，可以使用藥物遞送系統，例如奈米顆粒、樹狀物、聚合物、脂質體或陽離子遞送系統遞送 iRNA。帶正電荷的陽離子遞送系

統促進(帶負電荷的)iRNA 分子的結合並且也在帶負電荷的細胞膜增強交互作用以允許 iRNA 由細胞有效攝取。陽離子脂質、樹狀物或聚合物可以與 iRNA 結合或被誘導以形成包裝 iRNA 的囊泡或膠束(參見例如 Kim SH, *et al*(2008) *Journal of Controlled Release* 129(2):107-116)。囊泡或膠束的形成進一步防止全身給予時 iRNA 的降解。用於製造和給予陽離子-iRNA 複合物的方法完全在所屬領域具通常知識者的能力範圍內(參見例如 Sorensen, DR, *et al*(2003) *J. Mol. Biol* 327:761-766; Verma, UN, *et al*(2003) Clin. *Cancer Res.* 9:1291-1300; Arnold, AS *et al*(2007) *J. Hypertens.* 25:197-205，所述參考資料透過引用以其全文併入本文)。對於 iRNA 的全身遞送 iRNA 的遞送系統的一些非限制性示例包括 DOTAP(Sorensen, DR., *et al*(2003), supra; Verma, UN, *et al*(2003), *supra*)、「固體核酸脂質顆粒」(Zimmermann, TS, *et al*(2006) *Nature* 441:111-114)、心磷脂(Chien, PY, *et al*(2005) *Cancer Gene Ther.* 12:321-328; Pal, A, *et al*(2005) *Int J. Oncol.* 26:1087-1091)、聚乙烯亞胺(Bonnet ME, *et al*(2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A.(2006) *J. Biomed. Biotechnol.* 71659)、Arg-Gly-Asp(RGD)肽(Liu, S.(2006) *Mol. Pharm.* 3:472-487)、及聚醯胺型胺類(Tomalia, DA, *et al*(2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al*(1999) *Pharm. Res.* 16:1799-1804)。在一些態樣中，iRNA 與環糊精形成用於全身給藥的複合物。用於給藥的方法與 iRNA 和環糊精的醫藥組成物可見於美國專利號 7,427,605 中，該專利透過引用以其全文併入本文。本發明的特定面向與降低細胞內 F12 的表現之方法有關，包括利用本文揭示的雙股 RNAi 劑接觸所述細胞。在一個態樣，細胞是肝臟的細胞，視需要是肝細胞。在一個態樣中，細胞是肝外細胞。

【0503】 *A. 本發明編碼 iRNA 的載體*

靶向 F12 基因的 iRNA 可以從插入 DNA 或 RNA 載體中的轉錄單元表現(參見，例如 Couture, A, *et al.*, *TIG.*(1996), 12:5-10；Skillern, A, *et al.*, 國際 PCT 公開號 WO 00/22113、Conrad, 國際 PCT 公開號 WO 00/22114 以及 Conrad, 美國專利號 6,054,299)。取決於使用的特定構建體和目標組織或細胞類型，表現可以是瞬時的(在小時至周數量級上)或持久的(數周至數月或更長時間)。可以將這些轉基因作為線性構建體、環狀質體或可以是整合或非整合載體的病毒載體引入。該轉基因也可以被構建以允許其作為染色體外的質體進行遺傳(Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA*(1995) 92:1292)。

【0504】 可以隨本文所述的方法和組成物一使用的病毒載體系統包括但不限於(a)腺病毒載體；(b)逆轉錄病毒載體，包括但不限於慢病毒載體、莫洛尼鼠白血病病毒等；(C)腺聯病毒載體；(d)單純皰疹病毒載體；(e)SV40 載體；(f)多瘤病毒載體；(g)乳頭瘤病毒載體；(h)小 RNA 病毒載體；(i)痘病毒載體如正痘病毒，例如，痘苗病毒載體或鳥類痘病毒，例如金絲雀痘病毒或雞痘病毒；及(j)輔助病毒依賴性或無腸腺病毒。複製缺陷型病毒也可以有利的。不同的載體併入或不併入細胞的基因組中。如希望，構建體可包含病毒序列以用於轉染。可選地，構建體可併入能夠發生附加體型複製的載體中，例如 EPV 和 EBV 載體。用於重組表現 iRNA 的構建體通常需要調節元件，例如，啟動子、增強子等，以確保 RNA 在目標細胞中表現。針對載體和構建體考慮的其他面向為所屬領域所習知。

【0505】 V. 本發明的醫藥組成物

本發明還包括醫藥組成物和配製品，其包含本發明的 iRNA。在一個實施例中，本文提供包含如本文所述的 iRNA 和藥學上可接受的載體的醫藥組成物。包含該 iRNA 的醫藥組成物對於預防和治療 F12 相關疾病是有用的，例如遺傳性

血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因提升的舒緩肽量導致的任一遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。

【0506】基於遞送模式配製這類醫藥組成物。一個態樣是經配製用於藉由腸胃外遞送，例如，透過皮下(SC)、肌肉內(IM)、靜脈內(IV)或用於皮下遞送來全身給藥的組成物。本發明的醫藥組成物可以按足以抑制 F12 基因表現的劑量投予。

【0507】在一些態樣中，本發明的醫藥組成物是無菌的。在另一個態樣中，本發明的醫藥組成物是無熱原的。

【0508】本發明的醫藥組成物可以按足以抑制 F12 基因表現的劑量投予。通常，本發明 iRNA 適合的劑量處於每日每千克接受者體重大約 0.001 至大約 200.0 毫克範圍內，通常為每日每千克體重約 1 至 50 mg 範圍內。典型地，本發明的適合的 dsRNA 劑量為大約 0.1 mg/kg、大約 5.0 mg/kg，如大約 0.3 mg/kg 以及大約 3.0 mg/kg 的範圍內。一種重複劑量方案可包含以既定間隔為基礎投予醫藥有效量的 iRNA，如每個月、每 3 至 6 個月或每年一次。在特定的態樣中，大約每個月至大約每 6 個月投予該 iRNA。

【0509】在初始治療方案後，治療可以更少的頻率基礎給藥。治療的持續時間可依據疾病的嚴重性決定。

【0510】在其他態樣中，醫藥組成物的單一劑量可以是長久持續的，如劑量以不超過 1、2、3 或 4 個月的間隔給藥。在本發明一些實施例中，本發明醫藥組成物的單一劑量每個月給藥一次。在本發明其他態樣中，本發明醫藥組成物的

單一劑量每季給藥一次(即大約每三個月)。在本發明其他態樣中，本發明醫藥組成物的單一劑量每年給藥兩次(即大約每 6 個月)。

【0511】 所屬領域中具通常知識者將理解，某些特定的因素可以影響有效治療一個個體所需的劑量和時間安排，這些因素包括但不限於該個體體內出現的突變、先前的治療、該個體的總體健康或年齡、及其他存在的疾病。另外，用適合的預防或治療有效量的組成物治療個體可以包括單次治療或一系列治療。

【0512】 取決於希望局部還是全身治療，以及取決於有待治療的區域，可將本揭示的醫藥組成物按多種方式給藥。給藥可以是局部的(例如眼部內的、陰道內的、直腸內的、鼻內的以及經皮的)、經口的或腸胃外的。腸胃外給藥包括靜脈內的、動脈內的、皮下的、腹膜內的或肌內的注射或輸注；皮下的，例如經由植入性裝置；或顱內的，例如透過腦實質內、鞘內的或腦室內給藥。

【0513】 iRNA 可以按如此方式遞送以靶向特定組織，如肝臟。

【0514】 用於局部給藥的醫藥組成物以及製劑可以包含透皮貼劑、軟膏、洗劑、乳膏、凝膠劑、滴劑、栓劑、噴霧劑、液體以及粉末。習知的醫藥載劑、水、粉末或油基、增稠劑等可以是必要的或所欲的。塗層避孕套、手套等也可以是有用的。適合的局部用製劑包含其中本揭示中表徵的 iRNA 與局部用遞送劑，例如脂質、脂質體、脂肪酸、脂肪酸酯、類固醇、螯合劑和界面活性劑混合的那些。適合的脂質和脂質體包含中性的(例如，二油醯磷脂醯 DOPE 乙醇胺、二肉豆蔻醯磷脂醯膽鹼 DMPC、二硬脂醯磷脂醯膽鹼)、陰性的(例如，二肉豆蔻醯磷脂醯甘油 DMPG)和陽離子的(例如，二油醯四甲基胺基丙基 DOTAP 和二油醯磷脂醯乙醇胺 DOTMA 複合物)。本發明的 iRNA 可以封裝在脂質體中或可以形成與脂質體特別是陽離子脂質體的複合物。可選地，RNAi 劑可以與脂質，特別是

與陽離子脂質複合。適合的脂肪酸與酯包括但不限於花生四烯酸、油酸、花生酸、月桂酸、羊脂酸、羊蠟酸、肉豆蔻酸、棕櫚酸、硬脂酸、亞油酸、亞麻酸、二癸酸酯、三癸酸酯、甘油單油酸酯、甘油二月桂酸酯、1-單癸酸甘油酯、1-十二烷基氮雜環庚-2-酮、醯基肉毒鹼、醯基膽鹼、或 C1-20 烷基酯(例如，異丙基肉豆蔻酸酯 IPM)、甘油一酯、甘油二酯或其藥學上可接受的鹽。局部用製劑在美國專利號 6,747,014 中詳細描述，該專利藉由引用併入本文。

【0515】 在一個態樣中，本發明雙股 RNA 劑之 siRNA，由局部給藥路徑以醫藥組成物投予至細胞內。在一個態樣中，醫藥組成物可包含一種與局部遞送劑混合的 siRNA 化合物。局部遞送劑可以是數個微囊泡。該微囊泡可以是脂質體。在一些實施例中脂質體是陽離子脂質體。

【0516】 在另一個態樣中，dsRNA 劑是與局部增滲劑混合。在一個態樣中，該局部增滲劑是脂肪酸。該脂肪酸可以是花生四烯酸、油酸、月桂酸、羊脂酸、羊蠟酸、肉豆蔻酸、棕櫚酸、硬脂酸、亞油酸、亞麻酸、二癸酸酯、三癸酸酯、甘油單油酸酯、甘油二月桂酸酯、1-單癸酸甘油酯、1-十二烷基氮雜環庚-2-酮、醯基肉毒鹼、醯基膽鹼、或 C1-10 烷基酯、甘油一酯、甘油二酯或其藥學上可接受的鹽。

【0517】 在另一個態樣中，該局部增滲劑是膽鹽。該膽鹽可以是膽酸、去氫膽酸、去氧膽酸、葡糖膽酸、甘胺膽酸、甘胺去氧膽酸、牛磺膽酸、牛磺去氧膽酸、鵝去氧膽酸、熊去氧膽酸、牛磺-24,25-二氫夫西地酸鈉(sodium tauro-24,25-dihydro-fusidate)、二氫夫西地酸鈉(sodium glycodihydrofusidate)、聚氧乙烯-9-月桂基醚(polyoxyethylene-9-lauryl ether)或其藥學上可接受的鹽。

【0518】 在另一個態樣中，該增滲劑是螯合劑。該螯合劑可以是 EDTA、檸檬酸、水楊酸鹽、膠原質的 N-醯基衍生物、月桂醇聚醚-9 和 β-二酮的 N-胺基醯基衍生物或其混合物。

【0519】 在另一個態樣中，增滲劑是界面活性劑，例如離子或非離子界面活性劑。該界面活性劑可以是月桂醇硫酸鈉、聚氧乙烯基-9-月桂基醚和聚氧乙烯基-20-鯨蠟基醚、氟碳化合物之乳化液或其混合物。

【0520】 在另一個態樣中，增滲劑可以被選擇自包括不飽和環狀脲、I-烷基-和 1-烯基氮雜環-烷酮衍生物和類固醇抗發炎藥以及其混合物。在仍其他實施例中，增滲劑可以是乙二醇、吡咯、氮酮或萜類組成之組。

【0521】 在一個面向中，本發明以包含 siRNA 化合物的以可注射劑量形式的醫藥組成物為特色，例如雙股 siRNA 化合物或 ssiRNA 化合物(例如前驅物，例如可以進展成 ssiRNA 化合物的較大的 siRNA 化合物，或編碼 siRNA 化合物的化合物，例如雙股 siRNA 化合物或 ssiRNA 化合物，或其前驅物)。在一個態樣中，醫藥組成物的可注射劑量形式包含無菌水溶液或分散體以及無菌粉末。在一些實施例中，無菌溶液可以包含稀釋劑如水；生理食鹽水溶液；固定油、聚乙二醇、丙三醇或丙二醇。

【0522】 本發明的 iRNA 分子可以包括在醫藥組成物內。這樣的組成物通常包含一個或多個種類的 iRNA 和藥學上可接受載劑。於本文所使用的用語「藥學上可接受的載劑」意圖包含任何和所有溶劑、分散介質、塗層、抗菌劑和抗真菌劑、等滲及吸收延遲劑及其類似物，相較於細胞與醫藥注射劑相容，例如肝臟細胞。此項技術中習知用於醫藥活性物質之該等介質及藥劑的用途。除非任一習

知介質或藥劑與活性化合物不相容，亦預期其在組成物中之用途。補充活性化合物亦可併入組合物中。

【0523】 本發明的醫藥組成物包括但不限於溶液劑、乳劑以及包含脂質體的製劑。這些組合物可以從包括但不限於預制液體、自乳化固體和自乳化半固體的多種組分產生。製劑包含那些靶向肝臟者。

【0524】 本發明的藥物製劑，其可以便利地以單位劑量形式提供，可以根據製藥業中習知的常規技術製備。此類技術包含使活性成分與一種或多種醫藥載劑或賦形劑結合的步驟。通常，透過以下使活性成分與液體載體均勻且密切地結合的方式製備製劑。

【0525】 *A. 額外的製劑*

【0526】 *i.乳劑*

可以將本發明的組成物製備和配製為乳劑。乳劑典型地是一種液體以液滴形式分散在另一種中的非均勻系統，且通常直徑超過 0.1 μm(參見例如 Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins(8th ed.), New York, NY； Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199； Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245； Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335； Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301)。乳劑經常是包括相密切混合和彼此分散的兩種不溶混液的雙

相系統。通常，乳劑可以為油包水(w/o)或水包油(o/w)種類。當水相精細分至本體油相中並作為微小液體分散至本體油相中時，所產生的組成物稱作油包水(w/o)乳劑。可替代地，當油相精細分至本體水相中並作為微小液體分散至本體水相中時，所產生的組成物稱作水包油(o/w)乳劑。除了分散相和活性藥物外，乳劑還可以包含額外成分，活性藥物可以作為在水相、油相中的溶液，抑或其自身作為獨立相。如果需要，也可以存在藥物賦形劑，如乳化劑、穩定劑、染料和抗氧化劑。醫藥乳劑還可以為包括多於兩種相的多重乳劑，例如像油包水包油(o/w/o)和水包油包水(w/o/w)乳劑的情況。這類複合製劑經常提供簡單雙元乳劑所不具備的特定優點。其中 o/w 乳劑的單個油液滴包住小水滴的多重乳劑構成 w/o/w 乳劑。同樣地，在油質連續相中穩定的水小球中包住的油液滴的系統提供 o/w/o 乳劑。

【0527】 乳劑以低或無熱動力穩定性為特徵。經常，乳劑的分散相或不連續相充分分散於外部相或連續相中並且藉由乳化劑或配製品黏度以這種形式維持。其他穩定乳劑的手段需要使用乳化劑，這些乳化劑可以合併進入到乳劑的任一相中。乳化劑可以大致分成 4 類：合成性介面活性劑、天然存在的乳化劑、吸收基質和精細分散的固體(參見例如 Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins(8th ed.), New York, NY； Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199)。

【0528】 合成界面活性劑，也稱作介面活性試劑，已經發現廣泛應用於乳劑的配製並且已經在文獻中綜述(參見例如 Ansel's Pharmaceutical Dosage Forms

and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins(8th ed.), New York, NY； Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285； Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199)。界面活性劑典型地是兩性的並且包括親水部分和疏水部分。界面活性劑的親水對疏水性質的比率已經稱作親水/親油平衡值(Hydrophile/lipophile balance；簡稱 HLB)並且是在製備製劑時界面活性劑分類和選擇的有價值工具。界面活性劑可以基於親水基團的性質：非離子、陰離子、陽離子和兩親分成不同類別(參見例如 Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins(8th ed.), New York, NY Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285)。

【0529】 多種非乳化材料也包括於乳劑配製品中並且對乳劑的特性有貢獻。這些包括脂肪、油、蠟、脂肪酸、脂肪醇、脂肪酯、濕潤劑、親水膠體、防腐劑和抗氧化劑(Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335； Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199)。

【0530】 經由皮膚途徑、口途徑和腸胃途徑應用乳劑配製品和製造它們的方法已經在文獻中綜述(參見例如 Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott

Williams & Wilkins(8th ed.), New York, NY； Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199)。

【0531】 *ii. 微乳劑*

在本發明的一個實施例中，將 iRNA 和核酸的組成物配製為微乳劑。可以將微乳劑定義為水、油和兩親分子的系統，所述系統是單一光學各向同性和熱動力學穩定的單一液態溶液(參見例如 Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins(8th ed.), New York, NY； Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker(Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245)。典型地，微乳劑的製備系統是藉由先在界面活性劑水溶液中分散油，接著添加足夠量的第四成分，通常是中等鏈長度的醇，以形成透明系統。因此，微乳劑也已經被描述成由介面活性分子的界面膜穩定化的兩種不能混合的液體的熱力學穩定的各向同性的澄清分散體(Leung and Shah, in：Controlled Release of Drugs：Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215)。

【0532】 *iii. 微粒*

本發明的 iRNA 可併入一種顆粒，例如一種微粒中。微粒可藉由噴霧乾燥產生，但還可能藉由其他方法，包括凍乾法、蒸發、流化床乾燥、真空乾燥、或這些技術的組合產生。

【0533】 *iv. 增滲劑*

在一個實施例中，本發明採用不同增滲劑來實現向動物皮膚有效遞送核酸，尤其是 iRNA。大多數藥物在溶液中以離子化和非離子化形式存在。然而，通常僅脂溶性或親脂性藥物容易地跨過細胞膜。已經發現如果用增滲劑處理欲通過的膜，甚至連非親脂藥物都可以通過細胞膜。除輔助非親脂性藥物通過細胞膜擴散之外，增滲劑還增強親脂性藥物的通透性。

【0534】 可以將增滲劑劃分為屬於 5 大類之一，亦即界面活性劑、脂肪酸、膽鹽、螯合劑和非螯合性非界面活性劑(參見例如 Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002； Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92)。以上所述各分類的增滲劑以及其製造醫藥組成物的用途和醫藥劑的遞送為所屬領域所習知。

【0535】 *v. 賦形劑*

與載劑化合物相比，「醫藥載劑」或「賦形劑」是用於向動物遞送一種或多種核酸的藥學上可接受的溶劑、懸浮劑或任一其他藥學上惰性的載體。賦形劑可以是液體或固體，當與核酸和給定的醫藥組成物的其他組分組合時，參考所想的計劃給予方式，對賦形劑進行選擇以提供希望的容積、稠度等。該藥劑習知於所屬領域。

【0536】 *vi.其他成分*

本發明的組成物額外地可以包含其他在醫藥組成物中常規發現的添加劑成分，按其技術已建立的使用程度。因此，例如這些組成物可以包含額外的、可相容的藥學上有活性的材料，如例如止癢劑、收斂劑、局部麻醉劑或抗發炎劑，或者可以包含對本發明的組成物的不同劑型的物理配製有用的額外材料，如染料、芳香劑、防腐劑、抗氧化劑、遮光劑、增稠劑和穩定劑。然而，當添加時，此類

材料不應當過度干擾本發明組成物的成分之生物活性。可以將這些製劑進行滅菌，並且如果希望的話，與助劑，例如潤滑劑、防腐劑、穩定劑、濕潤劑、乳化劑、用於影響滲透壓的鹽、緩衝液、著色物質、芳香物質或芬芳物質等進行混合，其不與該製劑中的一種或多種核酸發生有害的相互作用。

【0537】 水性懸浮液可以包含增加該懸浮液的黏度的物質包含，例如羧甲基纖維素鈉、山梨醇或葡聚糖。該懸浮液還可以包含穩定劑。

【0538】 在一些態樣中，本發明的醫藥組成物包含(a)一個或多個 iRNA 以及(b)一個或多個藉由一種非 RNAi 機制發揮功能的並在治療 F12 相關疾病中是有用的劑，例如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因提升的舒緩肽量導致的任一遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。

【0539】 可以藉由標準藥學程序在細胞培養物或實驗動物中確定此類化合物的毒性和預防功效，例如，確定 LD50(致死群體 50 %的劑量)和 ED50(在 50 %群體中預防有效的劑量)。毒性效果和治療效果之間的劑量比是治療指數並且它可以表示為 LD50/ED50 比。展現出高治療指數的化合物為較佳者。

【0540】 從細胞培養物測定法和動物研究中獲得的數據可以在配製人類中使用的劑量範圍時使用。在本發明中在本文的組成物劑量總體上處於毒性低或無毒性情況下，包含 ED50 的循環濃度的範圍內，如 ED80 或 ED90。該劑量可以取決於所採用的劑型以及利用的給予途徑而在該範圍內變化。對於任一在本發明的方法中使用的化合物，預防上有效的劑量可以從細胞培養測定來進行初始估計。可以在動物模型中配製一個劑量以實現化合物的或(當適當時)目標序

列的多肽產物的循環血漿濃度範圍(例如，實現所述多肽濃度降低)，其中所述血漿濃度包括如在細胞培養物中所確定的 IC50(亦即，實驗化合物的實現症狀的半數最大抑制的濃度)。這類資訊可以用來更精確地確定人類中的有用劑量。可以測量血漿中的含量，例如，藉由高效液相層析法。

【0541】 除了上述給藥之外，如以上所討論的，還可以將在本發明的 iRNA 與在預防或治療 F12 相關的疾病有效的其他已知藥劑組合給藥，例如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因提升的舒緩肽量導致的任一遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。在任何情況下，基於使用本領域習知或在本文所述的標準功效量值所觀察到的結果，給予醫師可以調整 iRNA 給予的量和時間安排。

【0542】 VI. 抑制 F12 表現的方法

本發明也提供在細胞內抑制 F12 基因表現的方法。該方法包含使用有效抑制細胞內 F12 表現的含量之 RNAi 劑接觸細胞，例如雙股 RNA 劑，從而抑制細胞內 F12 的表現。在本揭示的一些態樣中，F12 基因的抑制較佳地在肝臟(例如肝細胞)。

【0543】 使用 iRNA，例如雙股 RNA 劑，可以在體外或體內接觸細胞。使用 iRNA 在體內接觸細胞，包含在使用 iRNA 在個體體內接觸一個細胞或一群細胞，例如，人類個體。結合在體外和在體內些處係包的方法也是可行的。如以上所述，接觸細胞可以是直接的或間接的。此外，可藉由靶向配體達成接觸細胞，包含任一本文所述的或所屬領域習知的配體。在一些態樣中，目標靶向配體是碳

水化合物部分，例如 $GalNAc_3$ 配體，或任一其他直接導向 RNAi 劑至目標位置的配體。

【0544】 如本文所使用，術語「抑制」可與「減少」、「沉默」、「下調」、「制止」以及其他相似的術語互換使用並包括任一量級的抑制。

【0545】 片語「抑制 F12 的表現」係指抑制任一 F12 基因(例如，例如小鼠 F12 基因、大鼠 F12 基因、猴 F12 基因或人 F12 基因)連同 F12 基因的變體或突變體。因此，該 F12 基因可以是野生型 F12 基因、突變 F12 基因或在基因調控的細胞、一群細胞或生物體內的基因轉殖的 F12 基因。

【0546】「抑制 F12 基因的表現」包含任一程度上的抑制 F12 基因，例如至少部分抑制 F12 基因的表現。F12 基因的表現可以任一與 F12 基因相關的變化之表現的程度或改變的程度來評估，例如 F12 mRNA 量級或 F12 蛋白質量級量。該量級可在單獨的細胞或一群細胞中評估，包含取自個體的樣本。

【0547】 藉由一種或多種與 F12 表現量相關的變化，相較於控制組量級降低的絕對或相對量量級來評估抑制作用。控制組量級的量可以是所屬領域使用的任一類型的控制組量級量，例如前劑量量級或自相似的未經控制處理或經控制處理的個體、細胞或樣本(舉例而言，如只有緩衝液的控制組或不活化劑控制組)所判定的量級量。

【0548】 在本發明方法的一些態樣中，F12 基因的表現被抑制至少 50%、55%、60%、65%、70%、75%、80%、85%、90%或 95%，或低於檢驗偵測的量級量。在一些態樣中，F12 基因的表現被抑制至少 70%。可以進一步理解在特定組織，例如肝臟中 F12 表現量的抑制；在其他組織，例如大腦，無顯著的表現抑

制，可以是理想的。在一些態樣中，表現量是使用實施例 2 所提供的檢驗方法，利用在合適的種類物種匹配細胞細胞株中使用 10 nM siRNA 濃度加以判定。

【0549】 在某些態樣中，藉由減弱表現人類基因齧齒類的人類基因，例如表現目標人類基因(亦即 F12)的 AAV 感染的小鼠，例如當投予單一劑量，例如在 RNA 表現的最低點中的 3 mg/kg，來判定體內表現之抑制。在模式動物系統中的內生性基因表現之減弱也可以被判定，例如在 RNA 表現的最低點中投予 3 mg/kg 之單一劑量後。當人類基因和模式動物基因的核酸序列非常相近時，如人類 iRNA 有效提供模式動物基因的減弱，該系統是有用的，在肝臟中 RNA 的表現是使用實施例 2 所提供的 PCR 方法判定。

【0550】 F12 基因的表現之抑制可藉由第一個細胞或一群細胞(該細胞可以在，例如取自個體的樣本中表現)中 mRNA 表現量的降低來顯示，其中 F12 基因轉錄且其中已被處理或未被處理(例如，藉由將本發明的 iRNA 與一個細胞或多個細胞，或是藉由投予本發明的 iRNA 至個體中在現在或過去有表現的細胞)，相較於與第一細胞或一群細胞在在某種程度上相同的第二細胞或一群細胞，但並未被如此的處理(未利用 iRNA 處理的控制組細胞或未被目標基因的 iRNA 處理)，該 F12 基因的表現被抑制。在一些態樣中，藉由實施例 2 所提供的方法評估抑制，且使用在物種匹配細胞株中的 10 nM siRNA 濃度以及在經處理的細胞中表現 mRNA 的含量，如同在控制組細胞中 mRNA 和含量的比例，使用下式：

$$\frac{(\text{在控制組細胞中的 mRNA}) - (\text{在經處理的細胞中的 mRNA})}{(\text{在控制組細胞中的 mRNA})} \times 100\%$$

【0551】 在其他態樣中，F12 基因的表現的抑制可根據在功能上與 F12 基因表現相關的參數降低來評估，例如，個體血液或血清中的 F12 蛋白質含量。

F12 基因沉默可在任一表現 F12 的細胞中判定，來自表現建構體的內源性或異源性，以及藉由任何所屬領域習知的檢驗。

【0552】 F12 蛋白質的表現之抑制可藉由一個細胞、一群細胞或個體樣本中表現的 mRNA 含量的降低顯示(例如取自個體的血液樣本中蛋白質含量)。如以上所述，為了評估 mRNA 抑制，經處理細胞或細胞群中蛋白質表達量的抑制可以類似地表示為對照細胞或細胞群中蛋白量的百分比，或在個體樣本中的蛋白量之改變，例如取自其血液或血清。

【0553】 一個對照細胞、一群細胞或個體樣本可以被使用來評估 F12 基因的表現之抑制，包含一個細胞、一群細胞或還未與本發明 RNAi 劑接觸的個體樣本。例如，一個對照細胞、一群細胞或個體樣本可在個體接受 RNAi 劑或適合的配對的族群控制組治療前，取自單獨的個體(例如人類或動物個體)。

【0554】 由一個細胞或一群細胞表現的 F12 mRNA 量可使用任一所屬領域中習知作為評估 mRNA 表現的方法來判定。在一個態樣中，在樣本中 F12 的表現量是由偵測轉錄的多核苷酸或其部分，例如 F12 基因的 mRNA 來判定。RNA 可使用 RNA 萃取技術包含例如酸苯酚/異硫氰酸胍萃取(acid phenol/guanidine isothiocyanate extraction)(RNAzol B； Biogenesis)、RNeasy™ RNA 製備套組(Qiagen®)或 PAXgene™(PreAnalytix™, Switzerland)自細胞中萃取。使用核醣核酸雜交的典型檢驗形式包含核連綴測定(nuclear run-on assays)、RT-PCR、RNA 酶保護測定、北方墨點法、原位雜交以及微陣列分析。

【0555】 在一些態樣中，使用一種核酸探針判定 F12 的表現含量。如本文所使用，術語「探針」係指能夠選擇性結合到一種特定 F12 的任一分子。探針可以由所屬領域具通常知識者合成或取自適合的生物製備。探針可以特定地經設

計成被標記的。可以用作探針的分子的實施例包括但不限於 RNA、DNA、蛋白質、抗體以及有機分子。

【0556】 可以在包括但不限於南方或北方墨點法分析、聚合酶連鎖反應(PCR)分析以及探針陣列中使用分離的 mRNA。一種用於判定 mRNA 含量的方法涉及使該分離的 mRNA 與可以與 F12 mRNA 雜交的一核酸分子(探針)接觸。在一個實施例中，mRNA 被固定在固體表面，並與探針接觸，例如，藉由使分離的 mRNA 在瓊脂凝膠運行，使 mRNA 從凝膠轉移至膜，如硝基纖維素。在一個替代性實施例中，該探針被固定在固體表面，且該 mRNA 例如在 Affymetrix 基因芯片陣列中與該探針接觸。所屬領域具通常知識者可以容易地使已知 mRNA 檢測方法適用於判定 F12 mRNA 的含量。

【0557】 用於判定樣本中的 F12 的表現含量的替代性方法涉及該樣本中的例如 mRNA 的核酸擴增和/或逆轉錄酶(以製備 cDNA)的過程，例如藉由 RT-PCR(the experimental embodiment set forth in Mullis, 1987, 美國專利第 4,683,202 號)、連接酶鏈式反應(Barany(1991) *Proc. Natl. Acad. Sci. USA* 88：189-193)、自主序列複製(Guatelli *et al.*(1990) *Proc. Natl. Acad. Sci. USA* 87：1874-1878)、轉錄擴增系統(Kwoh *et al.*(1989) *Proc. Natl. Acad. Sci. USA* 86：1173-1177)、Q-β 複製酶(Lizardi *et al.*(1988) *Bio/Technology* 6：1197) 、滾環擴增(Lizardi *et al.*, 美國專利號 5,854,033)或任一其他核酸擴增方法，接著使用所屬領域具通常知識者所習知的技術檢測擴增的分子。如果這樣的分子以極低數目存在，那麼這些檢測方案尤其可用於檢測這些核酸分子。在本發明的特定面向，藉由定量螢光 RT-PCR(亦即 TaqMan™ 系統)判定 F12 的表現量。

【0558】 可以使用膜印跡(如使用於雜交分析中者，如北方墨點法、南方墨點法、斑點等)或微孔、樣品管、凝膠、珠粒或纖維(或包含結合核酸的任一固體載體)監測 F12 mRNA 的表現量。參見美國專利第 5,770,722、 5,874,219、5,744,305、5,677,195 和第 5,445,934 號，這些美國專利透過引用併入本文。還可以包括使用溶液中的核酸探針判定 F12 表現量。

【0559】 在一些態樣中，使用支鏈 DNA(bDNA)測定或實時 PCR(qPCR) 評估 mRNA 表現量。這些方法的使用描述並且例證於本文所示的實施例中。在一些態樣中，表現量是由實施例 2 所提供，使用在物種匹配細胞株中 10 nM siRNA 濃度來判定。

【0560】 可使用所屬領域習知用於測量蛋白量的任意方法，判定 F12 蛋白表現量。此類方法包含例如電泳、毛細管電泳、高效液相層析法(HPLC)、薄層層析法(TLC)、超擴散層析、流體或凝膠沉澱素反應、吸收光譜法、比色測定、分光光度測定、流式細胞術、免疫擴散(單向或雙向)、免疫電泳、西方墨點法、放射免疫測定(RIA)、酶聯免疫吸附測定(ELISA)、免疫螢光測定及電化學發光測定等。

【0561】 在一些態樣中，本發明之方法的效果由 F12 mRNA 或蛋白量(例如在肝臟切片中)的下降來評估。

【0562】 在一些態樣中，本發明方法的效果可由偵測或監測 F12 相關的疾病的症狀減少來監測，例如四肢、臉部、咽喉、上呼吸道、腹部、軀幹及生殖器水腫之降低或前驅症候之降低；喉水腫之降低；非搔癢性皮疹之降低；噁心之降低；嘔吐之降低或腹部疼痛之降低。藉由量測任意參數或任意參數的組合，所屬領域具通常知識者於能力範圍內可監測該方法的效果。

【0563】 在本發明方法的一些實施例中，給予個體該 iRNA，使該 iRNA 被遞送到該個體內的特定位置。可使用取自個體特定體液或組織的樣本(例如肝臟或血液)中的 F12 mRNA 或 F12 蛋白量或其改變之測量值，來評估對 F12 表現之抑制。

【0564】 如本文所用，可以理解術語偵測或判定分析物的含量為執行該步驟來判定一種材料，例如蛋白質和 RNA 是否存在。如本文所使用，偵測或判定之方法包含偵測或判定低於方法所使用的偵測含量之分析物含量。

【0565】 VII. 本發明的預防和治療方法

本發明也提供使用本發明 iRNA 或包含本發明 iRNA 的組成物來抑制 F12 表現之方法，從而預防或治療 F12 相關疾病，例如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量所致的遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓例如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。在本發明的方法中，細胞可與 siRNA 在體外或體內接觸，即細胞可位在個體體內。

【0566】 使用本發明方法適合作為治療的細胞可以是任意表現 F12 基因的細胞，例如肝臟細胞。適用於本發明方法的細胞可以是哺乳類細胞，例如靈長類細胞(如人類細胞，包含在嵌合的非人類動物或非人類靈長類細胞中的人類細胞株，例如猴細胞或大猩猩細胞)或非靈長類細胞。在特定態樣中，該細胞是人類細胞，例如人類肝臟細胞。在本發明方法中，F12 表現在細胞內被抑制至少 50%、55%、60%、65%、70%、75%、80%、85%、90%或 95%，或比檢驗中測得的量更低之量。

【0567】本發明的體內方法可包含投予個體包含 iRNA 的組成物，其中 iRNA 包含與至少部分哺乳類 F12 基因的 RNA 轉錄本互補的核苷酸序列，其 RNAi 劑被投予。該組成物可以面向本領域習知的任意方式投予，包括但不限於經口、腹膜內或腸胃外途徑，包括顱內(例如腦室內、腦實質內以及鞘內)、靜脈內、肌肉內、皮下、經皮、氣道(氣溶膠)、經鼻、直腸以及眼部(例如眼周、結膜、球囊下、玻璃體內、眼部、前或後近鞏膜、視網膜下、結膜下、眼球後或管內注射)、靜脈內、肌肉內、皮下、經皮、氣道(氣溶劑)以及局部(包含口頰和舌下)注射。

【0568】在某些態樣中，化合物經由靜脈輸注或注射投予。在特定態樣中，化合物經由皮下注射投予。在特定態樣中，化合物經由肌肉內注射投予。

【0569】投予的模式可根據希望是局部抑或全身性治療，以及根據治療的部位選擇。投予的途徑和位置可經選擇以提升靶向性。

【0570】在一個面向中，本發明也提供用於抑制哺乳類 F12 基因表現的方法。該方法包含投予哺乳類包含目標哺乳類細胞內 F12 基因的 dsRNA 組成物，於哺乳類中維持一段充足的時間以獲得 F12 基因的 mRNA 轉錄本降解，從而抑制細胞內 F12 基因表現。基因表現的降低可由所屬領域習知的任何方法及步驟評估，如本文實施例 2 中所述的 qRT-PCR。蛋白質產出可藉由所屬領域習知的任何方式評估，例如 ELISA。在特定態樣中，用肝臟穿刺的組織切片樣本作為組織材料以偵測 F12 基因或蛋白質表現降低。在其他態樣中，用血液樣本作為個體樣本以偵測 F12 蛋白表現的降低。

【0571】本發明進一步提供治療有其需要的個體之方法，例如被診斷罹患 F12 相關疾病，如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺

傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量導致的任何遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。

【0572】 本發明進一步提供對有其需要的個體預防性投藥(prophylaxis)的方法。本發明的治療方法包含投予本發明的 iRNA 至個體，例如能受惠於以預防有效劑量的 dsRNA 目標 F12 基因或包含目標 F12 基因的醫藥組成物，以使 F12 表現量降低的個體。

【0573】 在一個面向中，本發明提供治療罹患能受惠於 F12 表現降低之疾病的個體之方法，例如 F12 相關疾病，如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量導致的任何遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。

【0574】 能受惠於 F12 基因表現降低及/或抑制的治療包括治療性療法(例如罹患 F12 相關疾病的個體)以及預防性療法(例如無罹患 F12 相關疾病的個體或具有發生 F12 相關疾病風險的個體)。

【0575】 在一些態樣中，該 F12 相關疾病選自由遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量導致的任何遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症組成之組。

【0576】 在一些態樣中，該 F12 相關疾病是 HAE。

【0577】 在其他態樣中，該 F12 相關疾病是前激肽釋放酶缺乏症。

【0578】在其他態樣中，該 F12 相關疾病是高血壓，例如惡性原發性高血壓。

【0579】在其他態樣中，該 F12 相關疾病是末期腎臟疾病。

【0580】在其他態樣中，該 F12 相關疾病是夫列契因子缺乏症。

【0581】在一些態樣中，投予一有效抑制個體細胞內 F12 表現的 RNAi 劑至個體。可使用上述方法評估有效抑制個體細胞內 F12 表現量，包含涉及抑制 F12 RNA、F12 蛋白質或相關變異體之評估方法，如降低 F12 相關疾病的症狀之嚴重程度，例如四肢、臉部、咽喉、上呼吸道、腹部、軀幹及生殖器水腫的嚴重程度之降低；喉水腫嚴重程度之降低；非搔癢性皮疹嚴重程度之降低；噁心嚴重程度之降低；嘔吐嚴重程度之降低或腹部疼痛嚴重程度之降低。

【0582】iRNA 可以作為「游離 iRNA」投予。游離 iRNA 是在醫藥組成物不存在下進行投予。該裸 iRNA 可以處於合適的緩衝溶液中。該緩衝溶液可包括醋酸鹽、檸檬酸鹽、醇溶蛋白、碳酸鹽或磷酸酯，或其任一組合。在一個實施例中，該緩衝溶液是磷酸酯緩衝鹽水(PBS)。可將含有該 iRNA 的緩衝溶液的 pH 和容積滲透濃度(osmolarity)進行調整，使其適合投予至個體。

【0583】視情況地，本發明的 iRNA 可作為醫藥組成物而投予，如 dsRNA 脂質體製劑。

【0584】能受惠於 F12 基因表現的抑制之個體為疑似罹患或罹患 F12 相關疾病的個體，如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因提升的舒緩肽量導致的任何遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病或夫列契因子缺乏症。在一個態樣中，該方法包含投予本文所述的組成物，如

約1、2、3、4、5、6、1至6、1至3或3至6個月投予一次，而使目標F12基因表現降低。在特定態樣中，該組成物是每3至6個月投予一次。

【0585】在一個態樣中，該iRNAs對於本文所述方法和組成物是有用的，特別是目標F12基因的目標RNA(原始或經處理的)。使用iRNA作為抑制該些基因表現的組成物和方法可如本文所述的方式製備和執行。

【0586】根據本發明的方法投予iRNA可預防或治療F12相關疾病，例如遺傳性血管水腫(HAE)(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽含量導致的任何遺傳性血管水腫)、前激肽釋放酶缺乏症、高血壓如惡性原發性高血壓、末期腎臟疾病、或夫列契因子缺乏症。可投予個體一治療量的iRNA，如約0.01 mg/kg至約200 mg/kg。

【0587】在一個態樣中，該iRNA是經由皮下投予，亦即，藉由皮下注射投予。一或多種注射可被用於遞送目標劑量的iRNA至個體。該注射可在一段時間後重複。

【0588】該注射可在常規的基礎上被重複。在特定態樣中，在初始治療方案後，該治療可以在較不頻繁的基礎上被投予。重複劑量方案可包含在常規基礎上投予治療量的iRNA，如每個月一次至每年一次。在特定態樣中，該iRNA大約每個月一次至大約每三個月一次，或大約每三個月至大約每六個月投予一次。

【0589】本發明進一步提供iRNA劑的方法和使用或其醫藥組成物作為治療能受惠於降低及/或抑制F12基因表現的個體，例如罹患F12相關疾病的個體，並與其他醫藥和/或治療方法結合，例如與已知的醫藥和/或已知的治療方法，例如目前用於治療該些疾病的方法。

【0590】 由以上可知，在本發明的一些面向中，包括投予本發明的 iRNA 劑的方法進一步包含投予個體一或多種附加治療劑。例如在特定態樣中，合併投予目標 F12 的 iRNA 與例如治療 F12 相關疾病有用的藥劑。示例性的額外治療劑及用於治療 F12 相關疾病的療法包含雄性激素，如達那唑(danazo)或氧雄龍(oxandrolone)、Berinert®、Cinryze™、Rhuconest®、伊卡蘭肽(Ecallantide)、Firazyr®、Kalbitor®及上述任意之組合。

【0591】 該 iRNA 和該附加治療劑可同時及/或以相同組合投予，例如經腸胃外，或是該附加治療劑可作為獨立的組成物的一部份，或在分別的時間和/或藉由所屬領域習知的或本文所述方法投予。

【0592】 該 iRNA 劑和附加治療劑及/或治療可以同時及/或以相同組合投予，例如經腸胃外，或該附加治療劑可作為獨立的組成物的一部份，或在分別的時間及/或藉由所屬領域習知或本文所述的方法投予。

【0593】 VIII. 套組

在某些面向，本揭示提供套組，，其包括合適的容器，該容器含有 siRNA 化合物的藥物製劑，例如雙股 siRNA 化合物，或 siRNA 化合物(例如前體，如較大 siRNA 化合物，其可被加工成 siRNA 化合物，或編碼 siRNA 化合物的 DNA，例如雙股 siRNA 化合物,、或 ssiRNA 化合物、或其前體)。

【0594】 該套組包含一種或多種 dsRNA 劑以及使用說明，例如，用於投予預防性或治療性有效量的 dsRNA 劑之說明。該 dsRNA 劑可盛裝於小玻璃瓶或載藥注射器中。該套組任選地進一步包括投予 dsRNA 劑的方法(例如注射裝置，如預填充注射器)，或量測 F12 的抑制之方法(例如量測 F12 mRNA、F12 蛋白質和/或 F12 活性的抑制的方法)。此類用於測量 F12 抑制的手段可包括用於從個體

獲取樣本的手段，例如血漿樣本。本發明的套組可任選地進一步包括用於確定治療有效量或預防有效量的方法。

【0595】 在某些態樣中，醫藥製劑的個別組分可以在一個容器中提供，例如小玻璃瓶或載藥注射器。或者，可能需要以兩個或更多的容器分別地提供醫藥製劑的組分，例如一個容器用於 siRNA 化合物製備，以及至少另一個容器盛裝載劑化合物。該套組可以以多種不同的配製加以包裝，如一個或多個容器裝在一個盒子裡。可以組合不同的成分，例如根據該套組所提供的說明。可根據本文所述的方法組合組分，例如，以製備和投予醫藥組成物。該套組也可包含遞送裝置。

【0596】 本發明進一步由以下實施例說明，不應將其解釋為限制性的。本文中引用的所有參考文獻、專利以及公開的專利申請的內文，連同非正式序列表和圖式，均透過引用併入本文。

[實施例]

【0597】 實施例 1. iRNA 合成

【0598】 *試劑的來源*

當本文未具體給出試劑來源時，此類試劑可以從分子生物學試劑的任意供應商獲得，其品質/純度標準符合分子生物學應用。

【0599】 *siRNA 設計*

使用客製化的 R 和 Python 腳本語言設計目標人類第十二因子(F12)基因(human： NCBI refseqID NM_000505.4, NCBI GeneID： 2161)的 siRNA。人類 NM_000505.4 REFSEQ mRNA 的鹼基長度為 2036。

【0600】 未經修飾之 F12 有義和反義股核苷酸序列的說明列示於表 2。經修飾之 F12 有義和反義股核苷酸序列的說明列示於表 3。

【0601】 可以理解在本案中，不帶小數點的雙鏈體名稱等同於帶小數點的雙鏈體名稱，小數僅作為雙鏈體的批號參照，例如 AD-959917 等同於 AD-959917.1。

【0602】 *siRNA 合成*

使用所屬領域習知的方法設計、合成以及製備 siRNA。

【0603】 簡言之，在固體撐體(solid support)上使用 Mermade 192 合成儀(BioAutomation)與亞磷醯胺合成法(phosphoramidite chemistry)以 1 μmol 規模合成 siRNA 序列。該撐體為可控孔徑玻璃(controlled pore glass)(500-1000 Å)裝載自訂 GalNAc 配體(3'-GalNAc 接合體)、通用撐體(AM Chemicals)或第一個目標核苷酸。輔助合成試劑(Ancillary synthesis reagents)和 standard 2-氰基乙基亞磷醯胺(2-cyanoethyl phosphoramidite)單體(2'-去氧-2'-氟、2'-O-甲基、RNA、DNA)自 Thermo-Fisher(Milwaukee, WI)、Hongene(China)或 Chemgenes(Wilmington, MA, USA)獲得。額外的亞磷醯胺單體購自市售供應商、於內部製備或使用來自各種 CMO 的自訂合成採購。在濃度為 100 mM 之下於乙腈抑或 9：1 之乙腈：DMF 製備亞磷醯胺，以及以 400s 的反應時間使用 5-(乙基硫基)-1H-四唑(5-Ethylthio-1H-tetrazole ；簡稱 ETT；0.25 M 在乙腈之中)組合。使用 100 mM 的 3-(二甲胺基-亞甲基) 胺基)-3H-1,2,4-二噻唑-3-硫酮(3-(Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione；簡稱 DDTT；自 Chemgenes(Wilmington, MA, USA)獲得)於無水乙腈/吡啶(9：1 v/v)中以產生硫代磷酸酯鍵。氧化時間是 5 分鐘。所有序列均在最終移除 DMT 基("DMT-Off")的情況下合成。

【0604】 在完成固相合成期間，在 96 孔盤內於室溫以 300 μL 甲胺(含水 40%)處理撐體的寡核糖核苷酸大約 2 小時，以提供撐體的剪切以及接著移除所

有額外的鹼基不穩定保護基。對於包含任何以第三丁基二甲矽基(tert-butyl dimethyl silyl；簡稱 TBDMS)保護的自然的核糖核酸鍵聯之序列，使用 TEA.3HF(三乙胺三氫氟酸鹽)進行第二去保護步驟。添加 200 μL 二甲基亞碸(Dimethyl sulfoxide；DMSO)和 300 μLTEA.3HF(三乙胺三氫氟酸鹽)至每種寡核苷酸的甲胺水溶液，且將該溶液在 60℃下培養約 30 分鐘。在培養之後，允許培養皿進入室溫中且藉由添加 1 mL 9：1 之乙晴：乙醇或 1：1 之乙醇：異丙醇使原寡核苷酸沉澱。培養皿接著在 4℃離心 45 分鐘，且借助多通道移液吸管小心地移出上清液。將寡核苷沉澱物重新懸浮於 20 mM NaOAc 中，接著利用配備自動取樣器、UV 偵測器、導電度計以及分液收集器的 Agilent LC 系統使用 HiTrap 大小排阻柱(5 mL, GE Healthcare)脫鹽。在 96 孔盤中收集脫鹽後的樣本，接著分別由 LC-MS 和 UV 光譜學分析，以確認同一性(identity)和定量材料的含量。

【0605】 由 Tecan 液體分注機器人執行使單股形成雙鏈體。有義和反義單股以等莫耳濃度之最終濃度為 10 μM 在 1x PBS 的 96 孔盤中結合，96 孔盤密封，在 100℃之下培養 10 分鐘，接著被允許緩慢地返回室溫 2 至 3 小時。確認各雙鏈體的濃度和身份作為隨後在體外篩查檢定使用。

【0606】 實施例 2. 在體外篩查方法

【0607】 *細胞培養和 384 孔轉染*

初代人類肝細胞(Primary human hepatocytes；簡稱 PHH)和初代食蟹獼猴肝細胞(初代食蟹獼猴肝細胞；簡稱 PCH)(ATCC, Manassas, VA)在被胰蛋白酶分解釋放前，於補充 10% FBS(ATCC)的伊格爾最低限度必需培養基(Eagle's Minimum Essential Medium)(Gibco)、5% CO_2 中、及 37℃下生長至接近的匯合(confluence)。藉由在 384 孔盤中各孔中的 2.5 μl siRNA 中添加 7.5 μl Opti-MEM 外加 0.1 μl

Lipofectamine RNAiMax(Invitrogen, Carlsbad CA. cat # 13778-150)進行轉染。混合物接著在室溫中培養 15 分鐘。接著將包含約 1.5 x10^4 個細胞的 40 µl 無抗生素之完全生長培養基接添加至 siRNA 混合物中。在 RNA 純化前，將細胞培養 24 小時。在 10 nM、1 nM 及 0.1 nM 最終雙鏈體濃度下，在 PHH 和 PCH 中進行單一劑量實驗。

【0608】 *自由攝取轉染*

還透過自由攝取評估雙鏈體中的一個子集。將在 PBS 中 5 µl 各雙鏈體與 4X10^4 新鮮解凍的初代食蟹猴肝細胞混合，再重新重新懸浮於 96 孔盤中的每孔中的 95 µl In Vitro Gro CP 培養基(In Vitro Technologies- Celsis, Baltimore, MD)中。將混合物在 37℃、5% CO2 的大氣下培養約 24 小時。於 250 nM、100 nM、10 nM 及 1 nM 最終雙鏈體濃度下進行單一劑量實驗。

【0609】 *DYNABEADS mRNA 分離套組(Invitrogen™, part # : 610-12)*

【0610】 細胞在每孔中含有 3 µl 粒珠的 75 µl 裂解/結合緩衝液(Lysis/Binding Buffer)中裂解，並且置於靜電震盪器(electrostatic shaker)混合 10 分鐘。使用磁孔盤撐體(magnetic plate support)於 Biotek EL406 中自動進行洗滌步驟。粒珠在 Buffer A(90 µl)中洗滌 1 次、在 Buffer B(90 µl)中洗滌 1 次以及在 Buffer E(90 µl)洗滌 2 次，且在洗滌步驟間包含廢液抽吸步驟(aspiration step)。如以下所述，在最終的廢液抽吸之後添加完全的 10 µl RT 混合物至各孔中。

【0611】 *使用 ABI 高產量 cDNA 反轉錄試劑盒(ABI High capacity cDNA reverse transcription kit)(Applied Biosystems, Foster City, CA, Cat #4368813)合成 cDNA*

在每孔中添加每反應的 1μl 10X 緩衝液、0.4 μl 25X dNTPs、1 μl 隨機引子(Random primers)、0.5 μl 反轉錄酶(Reverse Transcriptase)、0.5 μl RNA 酶抑制劑(RNase inhibitor)以及 6.6 μl H_2O 之預混溶液(master mix)。密封孔盤並於至於靜電振盪器混勻 10 分鐘，接著在 37 度 C 之下培養 2 小時。在此之後，孔盤在 80 度 C 混勻 8 分鐘。

【0612】 *實時 PCR*

在 384 孔盤(Roche cat # 04887301001)之每孔含有 0.5μl 人類 GAPDH TaqMan 探針(4326317E)、0.5μl 人類 F12、2 μl 無核酸酶水以及 5μl Lightcycler 480 探針預混溶液之預混溶液中，加入 2μl cDNA。於 LightCycler480 實時 PCR 系統(Roche)進行實時 PCR。

【0613】 為計算相對倍數變化，使用 ΔΔCt 方法分析數據並將其正規化為用 10nM AD-1955 轉染的細胞或模擬轉染細胞進行的測定。使用 XLFit 之 4 參數擬合模型(4 parameter fit model)計算 IC_{50}s，並將其正規化為用 AD-1955 轉染或模擬轉染的細胞。AD-1955 的有義和反義股為：有義 cuuAcGcuGAGuAcuucGAdTsdT(SEQ ID NO： 11)以及反義 UCGAAGuACUcAGCGuAAGdTsdT(SEQ ID NO： 12)。

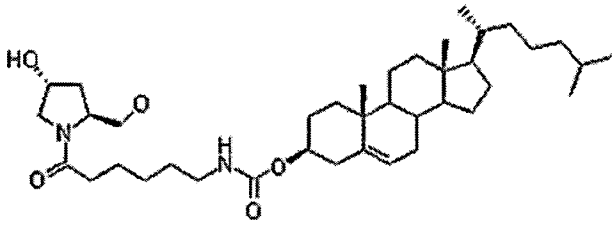
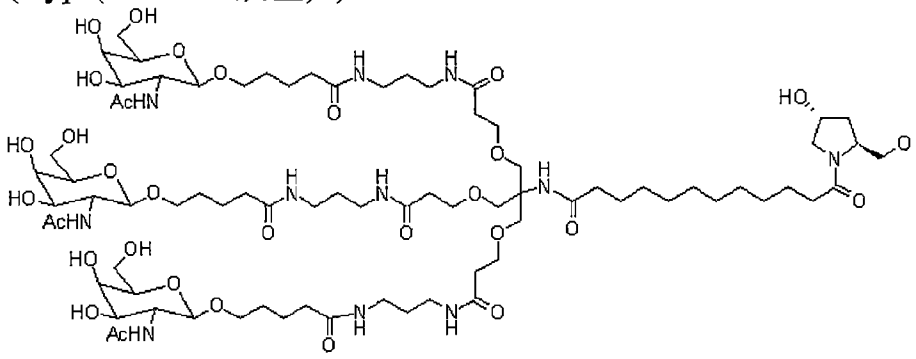
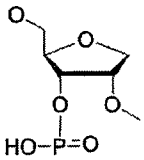
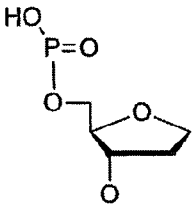
【0614】 表 2 和 3 中所列的 dsRNA 劑於 PHH 細胞內的單一劑量篩查結果顯示於表 4。

【0615】 表 2 和 3 中所列的 dsRNA 劑於 PCH 細胞內的單一劑量轉染篩結果查顯示於表 4。

【0616】 表 2 和 3 中所列的 dsRNA 劑於 PCH 細胞內的單一劑量自由攝取篩結果查顯示於表 4。

表 1. 代表核酸序列的核苷酸單體的縮寫。可以理解當這些單體位在寡核苷之中時，是共同地以 5'-3'-磷酸二酯鍵聯連結；可以理解當核苷酸包含 2'-氟修飾時，則氟取代了親代核苷酸中羥的位置(亦即其為 2'-去氧-2'-氟核苷酸)。

縮寫	核苷酸
A	腺苷-3'-磷酸酯
Ab	β-L-腺苷-3`-磷酸酯
Abs	β-L-腺苷-3`-硫代磷酸酯
Af	2'-氟腺苷-3'-磷酸酯
Afs	2'-氟腺苷-3'-硫代磷酸酯
As	腺苷-3'-硫代磷酸酯
C	胞苷-3'-磷酸酯
Cb	β-L-胞苷-3`-磷酸酯
Cbs	β-L-胞苷-3'-硫代磷酸酯
Cf	2'-氟胞苷-3'-磷酸酯
Cfs	2'-氟胞苷-3'-硫代磷酸酯
Cs	胞苷-3'-硫代磷酸酯
G	鳥苷-3'-磷酸酯
Gb	β-L-鳥苷-3`-磷酸酯
Gbs	β-L-鳥苷-3`-硫代磷酸酯
Gf	2'-氟鳥苷-3'-磷酸酯
Gfs	2'-氟鳥苷-3'-硫代磷酸酯
Gs	鳥苷-3'-硫代磷酸酯
T	5'-甲基尿核苷-3'-磷酸酯
Tf	2'-氟-5-甲基尿核苷-3'-磷酸酯
Tfs	2'-氟-5-甲基尿核苷-3'-硫代磷酸酯
Ts	5-甲基尿核苷-3'-硫代磷酸酯
U	尿核苷-3'-磷酸酯
Uf	2'-氟尿核苷-3'-磷酸酯
Ufs	2'-氟尿核苷 -3'-硫代磷酸酯
Us	尿核苷 -3'-硫代磷酸酯
N	任意修飾之或未修飾之核苷酸
a	2'-O-甲基腺苷-3'-磷酸酯
as	2'-O-甲基腺苷-3'- 硫代磷酸酯
c	2'-O-甲基胞苷-3'-磷酸酯
Cs	2'-O-甲基胞苷-3'- 硫代磷酸酯
g	2'-O-甲基鳥苷-3'-磷酸酯

縮寫	核苷酸
gs	2'-O-甲基鳥苷-3'- 硫代磷酸酯
t	2'-O-甲基-5-甲基尿核苷-3'-磷酸酯
ts	2'-O-甲基-5-甲基尿核苷-3'-硫代磷酸酯
u	2'-O-甲基尿核苷-3'-磷酸酯
us	2'-O-甲基尿核苷-3'-硫代磷酸酯
s	硫代磷酸酯鍵聯
L10	N-(膽固醇基醯胺己醯基)-4-羥脯胺醇(Hyp-C6-Chol) 
L96	N-[tris(GalNAc-烷基)-醯胺基癸醯基)]-4-羥脯胺醇 (Hyp-(GalNAc-烷基)3) 
Y34	2-羥甲基-四氫呋喃-4-甲氧基-3-磷酸酯(無鹼基 2'-OMe 呋喃糖) 
Y44	反向的無鹼基 DNA(2-羥甲基-四氫呋喃-5-磷酸酯) 
(Agn)	腺苷-二醇核酸(GNA) S-異構物
(Cgn)	胞苷-二醇核酸(GNA) S-異構物
(Ggn)	鳥苷-二醇核酸(GNA) S-異構物
(Tgn)	胸腺嘧吟-二醇核酸(GNA) S-異構物
P	磷酸酯

縮寫	核苷酸
VP	磷酸乙烯酯
dA	2`-去氧腺苷-3`-磷酸酯
dAs	2`-去氧腺苷-3`-硫代磷酸酯
dC	2`-去氧胞苷-3`-磷酸酯
dCs	2`-去氧胞苷-3`-硫代磷酸酯
dG	2`-去氧鳥苷-3`-磷酸酯
dGs	2`-去氧鳥苷-3`-硫代磷酸酯
dT	2`-去氧胸苷 -3`-磷酸酯
dTs	2`-去氧胸苷-3`-硫代磷酸酯
dU	2`-去氧尿核苷
dUs	2`-去氧尿核苷-3`-硫代磷酸酯
(C2p)	胞苷-2`-磷酸酯
(G2p)	鳥苷-2`-磷酸酯
(U2p)	尿核苷-2`-磷酸酯
(A2p)	腺苷-2`-磷酸酯
(Chd)	2'-O-十六烷基-胞苷-3'-磷酸酯
(Ahd)	2'-O-十六烷基-腺苷-3'-磷酸酯
(Ghd)	2'-O-十六烷基-鳥苷-3'-磷酸酯
(Uhd)	2'-O-十六烷基-尿核苷-3'-磷酸酯

表 2. F12 dsRNA 劑之未修飾之有義及反義股序列

雙鏈體名稱	5'至 3'之有義股序列	SEQ ID NO：	NM_000505.4 之中的範圍	5'至 3'之反義股序列	SEQ ID NO：	NM_000505.4 之中的範圍
AD-1534462	AAAGAGAAGUGCUUUGAGCCU	21	431-451	AGGCTCAAAGCACUUCUCUUUCU	285	429-451
AD-1534463	AAAUGUACCCACAAGGGCCGU	22	215-235	ACGGCCCUUGUGGGUACAUUUGU	286	213-235
AD-1534464	AACCCGGACAACGACAUCCGU	23	839-859	ACGGAUGUCGUTGUCCGGGUUCC	287	837-859
AD-1534465	AACUCAAUAAAGUGCUUUGAU	24	2008-2028	ATCAAAGCACUTUAUUGAGUUCC	288	2006-2028
AD-1534466	AACUGAGCAAGCAGCUGUGGU	25	493-513	ACCACAGCUGCTUGCUCAGUUCU	289	491-513
AD-1534467	AAGAAUGAGAUAUGGUAUAGU	26	473-493	ACUATACCAUATCUCAUUCUUGU	290	471-493
AD-1534468	AAGAGCACACAGUCGUUCUCU	27	138-158	AGAGAACGACUGUGUGCUCUUCA	291	136-158
AD-1534469	AAGCAGCUGUGGCCAGAUGCU	28	501-521	AGCATCTGGCCACAGCUGCUUGC	292	499-521
AD-1534470	AAGCUGCUAUGAUGGCCGCGU	29	682-702	ACGCGGCCAUCAUAGCAGCUUGC	293	680-702
AD-1534471	AAGUACAAAGCUGAAGAGCAU	30	125-145	AUGCTCTUCAGCUUUGUACUUAU	294	123-145
AD-1534472	AAGUGCUUUGAGCCUCAGCUU	31	437-457	AAGCTGAGGCUCAAAGCACUUCU	295	435-457
AD-1534473	AAUAAAGUGCUUUGAAAAUGU	32	2013-2033	ACAUTUTCAAAGCACUUUAUUGA	296	2011-2033
AD-1534474	ACAAAUGUACCCACAAGGGCU	33	213-233	AGCCCUTGUGGGUACAUUUGUGG	297	211-233
AD-1534475	ACACAGUCGUUCUCACUGUCU	34	144-164	AGACAGTGAGAACGACUGUGUGC	298	142-164
AD-1534476	ACACCGAUGUGGCCUACUACU	35	1833-1853	AGUAGUAGGCCACAUCGGUGUAG	299	1831-1853
AD-1534477	ACACUUUCGAUUCCACCUUGU	36	86-106	ACAAGGTGGAATCGAAAGUGUUG	300	84-106
AD-1534478	ACACUUUCGAUUCCACCUUGU	37	86-106	ACAAGGTGGAAUCGAAAGUGUUG	301	84-106
AD-1534479	ACAGUCGUUCUCACUGUCACU	38	146-166	AGUGACAGUGAGAACGACUGUGU	302	144-166
AD-1534480	ACAUCGCCGCGCUGUACUGGU	39	1194-1214	ACCAGUACAGCGCGGCGAUGUAG	303	1192-1214
AD-1534481	ACCAGCACGACCUGGCUCUGU	40	1410-1430	ACAGAGCCAGGTCGUGCUGGUAG	304	1408-1430
AD-1534482	ACCCGGACAACGACAUCCGCU	41	840-860	AGCGGATGUCGUUGUCCGGGUUC	305	838-860
AD-1534483	ACCCUGCAAGGCAUCAUCAGU	42	1772-1792	ACUGAUGAUGCCUUGCAGGGUGA	306	1770-1792
AD-1534484	ACCGCAACAAGCCAGGCGUCU	43	1812-1832	AGACGCCUGGCTUGUUGCGGUCA	307	1810-1832
AD-1534485	ACCUGGCUCUGUUGCGCCUUU	44	1419-1439	AAAGGCGCAACAGAGCCAGGUCG	308	1417-1439
AD-1534486	ACGUUCAGCCGGUGUGCCUGU	45	1479-1499	ACAGGCACACCGGCUGAACGUAA	309	1477-1499
AD-1534487	ACUACCUGGCCUGGAUCCGGU	46	1848-1868	ACCGGATCCAGGCCAGGUAGUAG	310	1846-1868
AD-1534488	ACUCAAUAAAGUGCUUUGAAU	47	2009-2029	ATUCAAAGCACTUUAUUGAGUUC	311	2007-2029

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534489	ACUGAGCAAGCAGCUGUGGCU	48	494-514	AGCCACAGCUGCUUGCUCAGUUC	312	492-514
AD-1534490	AGAGAAGUGCUUUGAGCCUCU	49	433-453	AGAGGCTCAAAGCACUUCUCUUU	313	431-453
AD-1534491	AGAGCACACAGUCGUUCUCAU	50	139-159	AUGAGAACGACUGUGUGCUCUUC	314	137-159
AD-1534492	AGAGUCUGUCUUCGAUGACCU	51	1131-1151	AGGUCATCGAAGACAGACUCUUG	315	1129-1151
AD-1534493	AGAUGCCAGUGCAAGGGUCCU	52	515-535	AGGACCCUUGCACUGGCAUCUGG	316	513-535
AD-1534494	AGCACGACCUGGCUCUGUUGU	53	1413-1433	ACAACAGAGCCAGGUCGUGCUGG	317	1411-1433
AD-1534495	AGCCAGGCGUCUACACCGAUU	54	1821-1841	AAUCGGTGUAGACGCCUGGCUUG	318	1819-1841
AD-1534496	AGCCAGGCGUCUACACCGAUU	55	1821-1841	AAUCGGTGUAGACGCCUGGCUUG	319	1819-1841
AD-1534497	AGCCCAAGAAAGUGAAAGACU	56	306-326	AGUCTUTCACUUUCUUGGGCUCC	320	304-326
AD-1534498	AGCUGAAGAGCACACAGUCGU	57	133-153	ACGACUGUGUGCUCUUCAGCUUU	321	131-153
AD-1534499	AGCUGCUAUGAUGGCCGCGGU	58	683-703	ACCGCGGCCAUCAUAGCAGCUUG	322	681-703
AD-1534500	AGCUGUACCACAAAUGUACCU	59	204-224	AGGUACAUUUGUGGUACAGCUGC	323	202-224
AD-1534501	AGGAGCAUAAGUACAAAGCUU	60	117-137	AAGCTUTGUACTUAUGCUCCUUG	324	115-137
AD-1534502	AGGAGCAUAAGUACAAAGCUU	61	117-137	AAGCTUTGUACUUAUGCUCCUUG	325	115-137
AD-1534503	AGGAUGGCGCAGGAACUCAAU	62	1995-2015	AUUGAGTUCCUGCGCCAUCCUGG	326	1993-2015
AD-1534504	AGGCGUCUACACCGAUGUGGU	63	1825-1845	ACCACATCGGUGUAGACGCCUGG	327	1823-1845
AD-1534505	AGGCUUCAUGUCCCACUCAUU	64	965-985	AAUGAGTGGGACAUGAAGCCUAG	328	963-985
AD-1534506	AGUCAACACUUUCGAUUCCAU	65	81-101	ATGGAATCGAAAGUGUUGACUCC	329	79-101
AD-1534507	AGUCAACACUUUCGAUUCCAU	66	81-101	AUGGAATCGAAAGUGUUGACUCC	330	79-101
AD-1534508	AUAAAGUGCUUUGAAAAUGCU	67	2014-2034	AGCATUTUCAAAGCACUUUAUUG	331	2012-2034
AD-1534509	AUCCGCCCGUGGUGCUUCGUU	68	854-874	AACGAAGCACCACGGGCGGAUGU	332	852-874
AD-1534510	AUCGGGCUGUGGUGACCGCAU	69	1798-1818	AUGCGGTCACCACAGCCCGAUCC	333	1796-1818
AD-1534511	AUGCCAGUGCAAGGGUCCUGU	70	517-537	ACAGGACCCUUGCACUGGCAUCU	334	515-537
AD-1534512	AUGCUCUGCGCAGGGUUCCUU	71	1673-1693	AAGGAACCCUGCGCAGAGCAUGC	335	1671-1693
AD-1534513	AUGUACCCACAAGGGCCGGCU	72	217-237	AGCCGGCCCUUGUGGGUACAUUU	336	215-237
AD-1534514	AUUGCUCAGGGACUCAUCUUU	73	1885-1905	AAAGAUGAGUCCCUGAGCAAUCA	337	1883-1905
AD-1534515	CAAAGCUGAAGAGCACACAGU	74	130-150	ACUGTGTGCUCUUCAGCUUUGUA	338	128-150
AD-1534516	CAAAUGUACCCACAAGGGCCU	75	214-234	AGGCCCTUGUGGGUACAUUUGUG	339	212-234

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534517	CAAGAAAGUGAAAGACCACUU	76	310-330	AAGUGGTCUUUCACUUUCUUGGG	340	308-330
AD-1534518	CAAGAGUCUGUCUUCGAUGAU	77	1129-1149	ATCATCGAAGACAGACUCUUGCG	341	1127-1149
AD-1534519	CAAGGAGCAUAAGUACAAAGU	78	115-135	ACUUTGTACUUAUGCUCCUUGGG	342	113-135
AD-1534520	CACAAAUGUACCCACAAGGGU	79	212-232	ACCCTUGUGGGTACAUUUGUGGU	343	210-232
AD-1534521	CACCCUGCAAGGCAUCAUCAU	80	1771-1791	AUGATGAUGCCUUGCAGGGUGAG	344	1769-1791
AD-1534522	CACCGAUGUGGCCUACUACCU	81	1834-1854	AGGUAGTAGGCCACAUCGGUGUA	345	1832-1854
AD-1534523	CACGACCUGGCUCUGUUGCGU	82	1415-1435	ACGCAACAGAGCCAGGUCGUGCU	346	1413-1435
AD-1534524	CACUGUCUCUGUCCACAACAU	83	389-409	AUGUTGTGGACAGAGACAGUGGG	347	387-409
AD-1534525	CACUUUCGAUUCCACCUUGGU	84	87-107	ACCAAGGUGGAAUCGAAAGUGUU	348	85-107
AD-1534526	CAGAUGCCAGUGCAAGGGUCU	85	514-534	AGACCCTUGCACUGGCAUCUGGC	349	512-534
AD-1534527	CAGCACGACCUGGCUCUGUUU	86	1412-1432	AAACAGAGCCAGGUCGUGCUGGU	350	1410-1432
AD-1534528	CAGCUGUACCACAAAUGUACU	87	203-223	AGUACATUUGUGGUACAGCUGCC	351	201-223
AD-1534529	CAGCUGUGGCCAGAUGCCAGU	88	504-524	ACUGGCAUCUGGCCACAGCUGCU	352	502-524
AD-1534530	CAGGAACUCAAUAAAGUGCUU	89	2004-2024	AAGCACTUUAUUGAGUUCCUGCG	353	2002-2024
AD-1534531	CAGGAUGGCGCAGGAACUCAU	90	1994-2014	AUGAGUTCCUGCGCCAUCCUGGC	354	1992-2014
AD-1534532	CAGGCGUCUACACCGAUGUGU	91	1824-1844	ACACAUCGGUGTAGACGCCUGGC	355	1822-1844
AD-1534533	CAGGGACUCAUCUUUCCCUCU	92	1891-1911	AGAGGGAAAGATGAGUCCCUGAG	356	1889-1911
AD-1534534	CAGUGCAAGGGUCCUGAUGCU	93	521-541	AGCATCAGGACCCUUGCACUGGC	357	519-541
AD-1534535	CCACAAAUGUACCCACAAGGU	94	211-231	ACCUTGTGGGUACAUUUGUGGUA	358	209-231
AD-1534536	CCACAAAUGUACCCACAAGGU	95	211-231	ACCUTGTGGGUACAUUUGUGGUA	359	209-231
AD-1534537	CCAGAUGCCAGUGCAAGGGUU	96	513-533	AACCCUTGCACTGGCAUCUGGCC	360	511-533
AD-1534538	CCAGGCGUCUACACCGAUGUU	97	1823-1843	AACATCGGUGUAGACGCCUGGCU	361	1821-1843
AD-1534539	CCCAAGAAAGUGAAAGACCAU	98	308-328	AUGGTCTUUCACUUUCUUGGGCU	362	306-328
AD-1534540	CCCGGACAACGACAUCCGCCU	99	841-861	AGGCGGAUGUCGUUGUCCGGGUU	363	839-861
AD-1534541	CCCGGACAACGACAUCCGCCU	100	841-861	AGGCGGAUGUCGUUGUCCGGGUU	364	839-861
AD-1534542	CCCUGCAAGGCAUCAUCAGCU	101	1773-1793	AGCUGATGAUGCCUUGCAGGGUG	365	1771-1793
AD-1534543	CCGAUGUGGCCUACUACCUGU	102	1836-1856	ACAGGUAGUAGGCCACAUCGGUG	366	1834-1856
AD-1534544	CCGCAACAAGCCAGGCGUCUU	103	1813-1833	AAGACGCCUGGCUUGUUGCGGUC	367	1811-1833

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534545	CCGCAAGAGUCUGUCUUCGAU	104	1126-1146	ATCGAAGACAGACUCUUGCGGAG	368	1124-1146
AD-1534546	CCGGAACCCGGACAACGACAU	105	835-855	ATGUCGTUGUCCGGGUUCCGGCA	369	833-855
AD-1534547	CCGGAACCCGGACAACGACAU	106	835-855	AUGUCGTUGUCCGGGUUCCGGCA	370	833-855
AD-1534548	CCUGCAGGAGGCGCAGGUACU	107	1591-1611	AGUACCTGCGCCUCCUGCAGGAA	371	1589-1611
AD-1534549	CCUGUCGCCUUACGUUCAGCU	108	1468-1488	AGCUGAACGUAAGGCGACAGGAG	372	1466-1488
AD-1534550	CCUGUCGCCUUACGUUCAGCU	109	1468-1488	AGCUGAACGUAAGGCGACAGGAG	373	1466-1488
AD-1534551	CCUUACGUUCAGCCGGUGUGU	110	1475-1495	ACACACCGGCUGAACGUAAGGCG	374	1473-1495
AD-1534552	CGCAACAAGCCAGGCGUCUAU	111	1814-1834	ATAGACGCCUGGCUUGUUGCGGU	375	1812-1834
AD-1534553	CGCAGGAACUCAAUAAAGUGU	112	2002-2022	ACACTUTAUUGAGUUCCUGCGCC	376	2000-2022
AD-1534554	CGCCUUACGUUCAGCCGGUGU	113	1473-1493	ACACCGGCUGAACGUAAGGCGAC	377	1471-1493
AD-1534555	CGCUCCUGUCGCCUUACGUUU	114	1464-1484	AAACGUAAGGCGACAGGAGCGCG	378	1462-1484
AD-1534556	CGGAACCCGGACAACGACAUU	115	836-856	AAUGTCGUUGUCCGGGUUCCGGC	379	834-856
AD-1534557	CGGCUCCGCAAGAGUCUGUCU	116	1121-1141	AGACAGACUCUUGCGGAGCCGCU	380	1119-1141
AD-1534558	CGUCUACACCGAUGUGGCCUU	117	1828-1848	AAGGCCACAUCGGUGUAGACGCC	381	1826-1848
AD-1534559	CGUGCCAGGGUGAUUCCGGAU	118	1710-1730	ATCCGGAAUCACCCUGGCACGCA	382	1708-1730
AD-1534560	CUACAUCGCCGCGCUGUACUU	119	1192-1212	AAGUACAGCGCGGCGAUGUAGGG	383	1190-1212
AD-1534561	CUACCUGGCCUGGAUCCGGGU	120	1849-1869	ACCCGGAUCCAGGCCAGGUAGUA	384	1847-1869
AD-1534562	CUCAAUAAAGUGCUUUGAAAU	121	2010-2030	ATUUCAAAGCACUUUAUUGAGUU	385	2008-2030
AD-1534563	CUCAAUAAAGUGCUUUGAAAU	122	2010-2030	AUUUCAAAGCACUUUAUUGAGUU	386	2008-2030
AD-1534564	CUCAGGGACUCAUCUUUCCCU	123	1889-1909	AGGGAAAGAUGAGUCCCUGAGCA	387	1887-1909
AD-1534565	CUCCUGUCGCCUUACGUUCAU	124	1466-1486	ATGAACGUAAGGCGACAGGAGCG	388	1464-1486
AD-1534566	CUCUGUUGCGCCUUCAGGAGU	125	1425-1445	ACUCCUGAAGGCGCAACAGAGCC	389	1423-1445
AD-1534567	CUGAAGAGCACACAGUCGUUU	126	135-155	AAACGACUGUGTGCUCUUCAGCU	390	133-155
AD-1534568	CUGAUUGCUCAGGGACUCAUU	127	1882-1902	AAUGAGTCCCUGAGCAAUCAGGA	391	1880-1902
AD-1534569	CUGCAAGGCAUCAUCAGCUGU	128	1775-1795	ACAGCUGAUGATGCCUUGCAGGG	392	1773-1795
AD-1534570	CUGCCGGAACCCGGACAACGU	129	832-852	ACGUTGTCCGGGUUCCGGCAGAA	393	830-852
AD-1534571	CUGUACCACAAAUGUACCCAU	130	206-226	AUGGGUACAUUUGUGGUACAGCU	394	204-226
AD-1534572	CUGUCGCCUUACGUUCAGCCU	131	1469-1489	AGGCTGAACGUAAGGCGACAGGA	395	1467-1489

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534573	CUGUCUCUGUCCACAACACCU	132	391-411	AGGUGUTGUGGACAGAGACAGUG	396	389-411
AD-1534574	CUGUGGUGACCGCAACAAGCU	133	1804-1824	AGCUTGTUGCGGUCACCACAGCC	397	1802-1824
AD-1534575	CUUACGUUCAGCCGGUGUGCU	134	1476-1496	AGCACACCGGCTGAACGUAAGGC	398	1474-1496
AD-1534576	CUUCGAUGACCCGCGUCGUUU	135	1140-1160	AAACGACGCGGGUCAUCGAAGAC	399	1138-1160
AD-1534577	CUUGGAGUCAACACUUUCGAU	136	76-96	ATCGAAAGUGUTGACUCCAAGCU	400	74-96
AD-1534578	CUUUGAGCCUCAGCUUCUCCU	137	442-462	AGGAGAAGCUGAGGCUCAAAGCA	401	440-462
AD-1534579	GAAAGUGAAAGACCACUGCAU	138	313-333	AUGCAGTGGUCUUUCACUUUCUU	402	311-333
AD-1534580	GAACCCGGACAACGACAUCCU	139	838-858	AGGATGTCGUUGUCCGGGUUCCG	403	836-858
AD-1534581	GAACUCAAUAAAGUGCUUUGU	140	2007-2027	ACAAAGCACUUTAUUGAGUUCCU	404	2005-2027
AD-1534582	GAAGAGCACACAGUCGUUCUU	141	137-157	AAGAACGACUGTGUGCUCUUCAG	405	135-157
AD-1534583	GACCGCAACAAGCCAGGCGUU	142	1811-1831	AACGCCTGGCUUGUUGCGGUCAC	406	1809-1831
AD-1534584	GAGCACACAGUCGUUCUCACU	143	140-160	AGUGAGAACGACUGUGUGCUCUU	407	138-160
AD-1534585	GAGCAUAAGUACAAAGCUGAU	144	119-139	AUCAGCTUUGUACUUAUGCUCCU	408	117-139
AD-1534586	GAGCCUCAGCUUCUCCGGUUU	145	446-466	AAACCGGAGAAGCUGAGGCUCAA	409	444-466
AD-1534587	GAGUCUGUCUUCGAUGACCCU	146	1132-1152	AGGGTCAUCGAAGACAGACUCUU	410	1130-1152
AD-1534588	GAUGCCAGUGCAAGGGUCCUU	147	516-536	AAGGACCCUUGCACUGGCAUCUG	411	514-536
AD-1534589	GAUGGCGCAGGAACUCAAUAU	148	1997-2017	AUAUTGAGUUCCUGCGCCAUCCU	412	1995-2017
AD-1534590	GAUUGCUCAGGGACUCAUCUU	149	1884-1904	AAGATGAGUCCCUGAGCAAUCAG	413	1882-1904
AD-1534591	GCAAGAGUCUGUCUUCGAUGU	150	1128-1148	ACAUCGAAGACAGACUCUUGCGG	414	1126-1148
AD-1534592	GCACACAGUCGUUCUCACUGU	151	142-162	ACAGTGAGAACGACUGUGUGCUC	415	140-162
AD-1534593	GCACGACCUGGCUCUGUUGCU	152	1414-1434	AGCAACAGAGCCAGGUCGUGCUG	416	1412-1434
AD-1534594	GCACGACCUGGCUCUGUUGCU	153	1414-1434	AGCAACAGAGCCAGGUCGUGCUG	417	1412-1434
AD-1534595	GCAGCUGUGGCCAGAUGCCAU	154	503-523	AUGGCATCUGGCCACAGCUGCUU	418	501-523
AD-1534596	GCAGGAACUCAAUAAAGUGCU	155	2003-2023	AGCACUTUAUUGAGUUCCUGCGC	419	2001-2023
AD-1534597	GCCAGAUGCCAGUGCAAGGGU	156	512-532	ACCCTUGCACUGGCAUCUGGCCA	420	510-532
AD-1534598	GCCAGGCGUCUACACCGAUGU	157	1822-1842	ACAUCGGUGUAGACGCCUGGCUU	421	1820-1842
AD-1534599	GCCCAAGAAAGUGAAAGACCU	158	307-327	AGGUCUTUCACUUUCUUGGGCUC	422	305-327
AD-1534600	GCCUUACGUUCAGCCGGUGUU	159	1474-1494	AACACCGGCUGAACGUAAGGCGA	423	1472-1494

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534601	GCGCAGGAACUCAAUAAAGUU	160	2001-2021	AACUUUAUUGAGUUCCUGCGCCA	424	1999-2021
AD-1534602	GCGCCUUCAGGAGGAUGCGGU	161	1432-1452	ACCGCAUCCUCCUGAAGGCGCAA	425	1430-1452
AD-1534603	GCGGCUCCGCAAGAGUCUGUU	162	1120-1140	AACAGACUCUUGCGGAGCCGCUG	426	1118-1140
AD-1534604	GCUCAGGGACUCAUCUUUCCU	163	1888-1908	AGGAAAGAUGAGUCCCUGAGCAA	427	1886-1908
AD-1534605	GCUCCGCAAGAGUCUGUCUUU	164	1123-1143	AAAGACAGACUCUUGCGGAGCCG	428	1121-1143
AD-1534606	GCUCCUGUCGCCUUACGUUCU	165	1465-1485	AGAACGUAAGGCGACAGGAGCGC	429	1463-1485
AD-1534607	GCUCUGCGCAGGGUUCCUCGU	166	1675-1695	ACGAGGAACCCUGCGCAGAGCAU	430	1673-1695
AD-1534608	GCUCUGCGCAGGGUUCCUCGU	167	1675-1695	ACGAGGAACCCUGCGCAGAGCAU	431	1673-1695
AD-1534609	GCUCUGUUGCGCCUUCAGGAU	168	1424-1444	ATCCTGAAGGCGCAACAGAGCCA	432	1422-1444
AD-1534610	GCUCUGUUGCGCCUUCAGGAU	169	1424-1444	AUCCTGAAGGCGCAACAGAGCCA	433	1422-1444
AD-1534611	GCUGAAGAGCACACAGUCGUU	170	134-154	AACGACTGUGUGCUCUUCAGCUU	434	132-154
AD-1534612	GCUGCUAUGAUGGCCGCGGGU	171	684-704	ACCCGCGGCCATCAUAGCAGCUU	435	682-704
AD-1534613	GCUGGUGUGUGAGGACCAAGU	172	1735-1755	ACUUGGTCCUCACACACCAGCGG	436	1733-1755
AD-1534614	GCUGUGGUGACCGCAACAAGU	173	1803-1823	ACUUGUTGCGGTCACCACAGCCC	437	1801-1823
AD-1534615	GCUGUGGUGACCGCAACAAGU	174	1803-1823	ACUUGUTGCGGUCACCACAGCCC	438	1801-1823
AD-1534616	GCUUCAUGUCCCACUCAUGCU	175	967-987	AGCATGAGUGGGACAUGAAGCCU	439	965-987
AD-1534617	GCUUUGAGCCUCAGCUUCUCU	176	441-461	AGAGAAGCUGAGGCUCAAAGCAC	440	439-461
AD-1534618	GGAACUCAAUAAAGUGCUUUU	177	2006-2026	AAAAGCACUUUAUUGAGUUCCUG	441	2004-2026
AD-1534619	GGAGCAUAAGUACAAAGCUGU	178	118-138	ACAGCUTUGUACUUAUGCUCCUU	442	116-138
AD-1534620	GGAGCCCAAGAAAGUGAAAGU	179	304-324	ACUUTCACUUUCUUGGGCUCCAA	443	302-324
AD-1534621	GGAGUCAACACUUUCGAUUCU	180	79-99	AGAATCGAAAGTGUUGACUCCAA	444	77-99
AD-1534622	GGCGUCUACACCGAUGUGGCU	181	1826-1846	AGCCACAUCGGTGUAGACGCCUG	445	1824-1846
AD-1534623	GGCGUCUACACCGAUGUGGCU	182	1826-1846	AGCCACAUCGGUGUAGACGCCUG	446	1824-1846
AD-1534624	GGCUCCGCAAGAGUCUGUCUU	183	1122-1142	AAGACAGACUCTUGCGGAGCCGC	447	1120-1142
AD-1534625	GGCUCUGUUGCGCCUUCAGGU	184	1423-1443	ACCUGAAGGCGCAACAGAGCCAG	448	1421-1443
AD-1534626	GGGUCCUGAUGCCCACUGCCU	185	529-549	AGGCAGTGGGCAUCAGGACCCUU	449	527-549
AD-1534627	GUCAACACUUUCGAUUCCACU	186	82-102	AGUGGAAUCGAAAGUGUUGACUC	450	80-102
AD-1534628	GUCAACACUUUCGAUUCCACU	187	82-102	AGUGGAAUCGAAAGUGUUGACUC	451	80-102

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534629	GUCGCCUUACGUUCAGCCGGU	188	1471-1491	ACCGGCTGAACGUAAGGCGACAG	452	1469-1491
AD-1534630	GUCGCCUUACGUUCAGCCGGU	189	1471-1491	ACCGGCTGAACGUAAGGCGACAG	453	1469-1491
AD-1534631	GUCGUUCUCACUGUCACCGGU	190	149-169	ACCGGUGACAGTGAGAACGACUG	454	147-169
AD-1534632	GUCUGUCUUCGAUGACCCGCU	191	1134-1154	AGCGGGTCAUCGAAGACAGACUC	455	1132-1154
AD-1534633	GUCUUCGAUGACCCGCGUCGU	192	1138-1158	ACGACGCGGGUCAUCGAAGACAG	456	1136-1158
AD-1534634	GUGCAAGGGUCCUGAUGCCCU	193	523-543	AGGGCATCAGGACCCUUGCACUG	457	521-543
AD-1534635	GUGGUGACCGCAACAAGCCAU	194	1806-1826	AUGGCUTGUUGCGGUCACCACAG	458	1804-1826
AD-1534636	GUUGCGCCUUCAGGAGGAUGU	195	1429-1449	ACAUCCTCCUGAAGGCGCAACAG	459	1427-1449
AD-1534637	GUUGCGCCUUCAGGAGGAUGU	196	1429-1449	ACAUCCTCCUGAAGGCGCAACAG	460	1427-1449
AD-1534638	UAAGUACAAAGCUGAAGAGCU	197	124-144	AGCUCUTCAGCTUUGUACUUAUG	461	122-144
AD-1534639	UAAGUACAAAGCUGAAGAGCU	198	124-144	AGCUCUTCAGCUUUGUACUUAUG	462	122-144
AD-1534640	UACAUCGCCGCGCUGUACUGU	199	1193-1213	ACAGTACAGCGCGGCGAUGUAGG	463	1191-1213
AD-1534641	UACCUGGCCUGGAUCCGGGAU	200	1850-1870	ATCCCGGAUCCAGGCCAGGUAGU	464	1848-1870
AD-1534642	UACUACCUGGCCUGGAUCCGU	201	1847-1867	ACGGAUCCAGGCCAGGUAGUAGG	465	1845-1867
AD-1534643	UCAACACUUUCGAUUCCACCU	202	83-103	AGGUGGAAUCGAAAGUGUUGACU	466	81-103
AD-1534644	UCACCCUGCAAGGCAUCAUCU	203	1770-1790	AGAUGATGCCUUGCAGGGUGAGC	467	1768-1790
AD-1534645	UCAGCCGGUGUGCCUGCCAAU	204	1483-1503	AUUGGCAGGCACACCGGCUGAAC	468	1481-1503
AD-1534646	UCAGGAGGAUGCGGACGGCAU	205	1438-1458	AUGCCGTCCGCAUCCUCCUGAAG	469	1436-1458
AD-1534647	UCAGGGACUCAUCUUUCCCUU	206	1890-1910	AAGGGAAAGAUGAGUCCCUGAGC	470	1888-1910
AD-1534648	UCCGCAAGAGUCUGUCUUCGU	207	1125-1145	ACGAAGACAGACUCUUGCGGAGC	471	1123-1145
AD-1534649	UCCUGUCGCCUUACGUUCAGU	208	1467-1487	ACUGAACGUAAGGCGACAGGAGC	472	1465-1487
AD-1534650	UCGCCUUACGUUCAGCCGGUU	209	1472-1492	AACCGGCUGAACGUAAGGCGACA	473	1470-1492
AD-1534651	UCUCUGUCCACAACACCUCAU	210	394-414	AUGAGGTGUUGUGGACAGAGACA	474	392-414
AD-1534652	UCUGUUGCGCCUUCAGGAGGU	211	1426-1446	ACCUCCTGAAGGCGCAACAGAGC	475	1424-1446
AD-1534653	UGAAGAGCACACAGUCGUUCU	212	136-156	AGAACGACUGUGUGCUCUUCAGC	476	134-156
AD-1534654	UGAGCCUCAGCUUCUCCGGUU	213	445-465	AACCGGAGAAGCUGAGGCUCAAA	477	443-465
AD-1534655	UGAGCUGGGAGUACUGCGACU	214	891-911	AGUCGCAGUACUCCCAGCUCAGC	478	889-911
AD-1534656	UGAUGGCCGCGGGCUCAGCUU	215	691-711	AAGCTGAGCCCGCGGCCAUCAUA	479	689-711

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1534657	UGCAAGGCAUCAUCAGCUGGU	216	1776-1796	ACCAGCTGAUGAUGCCUUGCAGG	480	1774-1796
AD-1534658	UGCAAGGCAUCAUCAGCUGGU	217	1776-1796	ACCAGCTGAUGAUGCCUUGCAGG	481	1774-1796
AD-1534659	UGCAAGGGUCCUGAUGCCCAU	218	524-544	AUGGGCAUCAGGACCCUUGCACU	482	522-544
AD-1534660	UGCCAGUGCAAGGGUCCUGAU	219	518-538	AUCAGGACCCUUGCACUGGCAUC	483	516-538
AD-1534661	UGCCAUGAGGGCUCUGCUGCU	220	34-54	AGCAGCAGAGCCCUCAUGGCAUC	484	32-54
AD-1534662	UGCGCCUUCAGGAGGAUGCGU	221	1431-1451	ACGCAUCCUCCTGAAGGCGCAAC	485	1429-1451
AD-1534663	UGCUCAGGGACUCAUCUUUCU	222	1887-1907	AGAAAGAUGAGTCCCUGAGCAAU	486	1885-1907
AD-1534664	UGCUCAGGGACUCAUCUUUCU	223	1887-1907	AGAAAGAUGAGUCCCUGAGCAAU	487	1885-1907
AD-1534665	UGGAGUCAACACUUUCGAUUU	224	78-98	AAAUCGAAAGUGUUGACUCCAAG	488	76-98
AD-1534666	UGGCCAGAUGCCAGUGCAAGU	225	510-530	ACUUGCACUGGCAUCUGGCCACA	489	508-530
AD-1534667	UGGCCUACUACCUGGCCUGGU	226	1842-1862	ACCAGGCCAGGTAGUAGGCCACA	490	1840-1862
AD-1534668	UGGCUCUGUUGCGCCUUCAGU	227	1422-1442	ACUGAAGGCGCAACAGAGCCAGG	491	1420-1442
AD-1534669	UGGUGACCGCAACAAGCCAGU	228	1807-1827	ACUGGCTUGUUGCGGUCACCACA	492	1805-1827
AD-1534670	UGUACCACAAAUGUACCCACU	229	207-227	AGUGGGTACAUUUGUGGUACAGC	493	205-227
AD-1534671	UGUCUCUGUCCACAACACCUU	230	392-412	AAGGTGTUGUGGACAGAGACAGU	494	390-412
AD-1534672	UGUGGCCAGAUGCCAGUGCAU	231	508-528	AUGCACTGGCAUCUGGCCACAGC	495	506-528
AD-1534673	UUACGUUCAGCCGGUGUGCCU	232	1477-1497	AGGCACACCGGCUGAACGUAAGG	496	1475-1497
AD-1534674	UUCAUGUCCCACUCAUGCCCU	233	969-989	AGGGCATGAGUGGGACAUGAAGC	497	967-989
AD-1534675	UUCGAUGACCCGCGUCGUUGU	234	1141-1161	ACAACGACGCGGGUCAUCGAAGA	498	1139-1161
AD-1534676	UUGAGCCUCAGCUUCUCCGGU	235	444-464	ACCGGAGAAGCTGAGGCUCAAAG	499	442-464
AD-1534677	UUGGAGCCCAAGAAAGUGAAU	236	302-322	AUUCACTUUCUUGGGCUCCAAAC	500	300-322
AD-1534678	UUGGAGUCAACACUUUCGAUU	237	77-97	AAUCGAAAGUGTUGACUCCAAGC	501	75-97
AD-1534679	UUUGAGCCUCAGCUUCUCCGU	238	443-463	ACGGAGAAGCUGAGGCUCAAAGC	502	441-463
AD-1631272	AAGAGCACACAGUCGUUCUCU	239	138-158	AGAGAACGACUGUGUGCUCUUCU	503	136-158
AD-1631273	AAUAAAGUGCUUUGAAAAUGU	240	2013-2033	ACAUTUTCAAAGCACUUUAUUCU	504	2011-2033
AD-1631274	ACAUCGCCGCGCUGUACUGGU	241	1194-1214	ACCAGUACAGCGCGGCGAUGUCU	505	1192-1214
AD-1631275	ACCAGCACGACCUGGCUCUGU	242	1410-1430	ACAGAGCCAGGTCGUGCUGGUCU	506	1408-1430
AD-1631276	ACCCUGCAAGGCAUCAUCAGU	243	1772-1792	ACUGAUGAUGCCUUGCAGGGUCU	507	1770-1792

雙鏈體名稱	5'至3'之有義股序列	SEQ ID NO：	NM_000505.4之中的範圍	5'至3'之反義股序列	SEQ ID NO：	NM_000505.4之中的範圍
AD-1631277	ACCGCAACAAGCCAGGCGUCU	244	1812-1832	AGACGCCUGGCTUGUUGCGGUCU	508	1810-1832
AD-1631278	ACUACCUGGCCUGGAUCCGGU	245	1848-1868	ACCGGATCCAGGCCAGGUAGUCU	509	1846-1868
AD-1631279	AUUGCUCAGGGACUCAUCUUU	246	1885-1905	AAAGAUGAGUCCCUGAGCAAUCU	510	1883-1905
AD-1631280	CAGGGACUCAUCUUUCCCUCU	247	1891-1911	AGAGGGAAAGATGAGUCCCUGCU	511	1889-1911
AD-1631281	CCACAAAUGUACCCACAAGGU	248	211-231	ACCUTGTGGGUACAUUUGUGGCU	512	209-231
AD-1631282	CCGCAAGAGUCUGUCUUCGAU	249	1126-1146	ATCGAAGACAGACUCUUGCGGCU	513	1124-1146
AD-1631283	CCGGAACCCGGACAACGACAU	250	835-855	ATGUCGTUGUCCGGGUUCCGGCU	514	833-855
AD-1631284	CCUGUCGCCUUACGUUCAGCU	251	1468-1488	AGCUGAACGUAAGGCGACAGGCU	515	1466-1488
AD-1631285	CGCCUUACGUUCAGCCGGUGU	252	1473-1493	ACACCGGCUGAACGUAAGGCGCU	516	1471-1493
AD-1631286	CGUGCCAGGGUGAUUCCGGAU	253	1710-1730	ATCCGGAAUCACCCUGGCACGCU	517	1708-1730
AD-1631287	CUACCUGGCCUGGAUCCGGGU	254	1849-1869	ACCCGGAUCCAGGCCAGGUAGCU	518	1847-1869
AD-1631288	CUCAGGGACUCAUCUUUCCCU	255	1889-1909	AGGGAAAGAUGAGUCCCUGAGCU	519	1887-1909
AD-1631289	CUUCGAUGACCCGCGUCGUUU	256	1140-1160	AAACGACGCGGGUCAUCGAAGCU	520	1138-1160
AD-1631290	GAAGAGCACACAGUCGUUCUU	257	137-157	AAGAACGACUGTGUGCUCUUCCU	521	135-157
AD-1631291	GAGCCUCAGCUUCUCCGGUUU	258	446-466	AAACCGGAGAAGCUGAGGCUCCU	522	444-466
AD-1631292	GCCAGAUGCCAGUGCAAGGGU	259	512-532	ACCCTUGCACUGGCAUCUGGCCU	523	510-532
AD-1631293	GCCUUACGUUCAGCCGGUGUU	260	1474-1494	AACACCGGCUGAACGUAAGGCCU	524	1472-1494
AD-1631294	GCGCAGGAACUCAAUAAAGUU	261	2001-2021	AACUTUAUUGAGUUCCUGCGCCU	525	1999-2021
AD-1631295	GCGCCUUCAGGAGGAUGCGGU	262	1432-1452	ACCGCATCCUCCUGAAGGCGCCU	526	1430-1452
AD-1631296	GCUCAGGGACUCAUCUUUCCU	263	1888-1908	AGGAAAGAUGAGUCCCUGAGCCU	527	1886-1908
AD-1631297	GCUCUGCGCAGGGUUCCUCGU	264	1675-1695	ACGAGGAACCCTGCGCAGAGCCU	528	1673-1695
AD-1631298	GCUCUGUUGCGCCUUCAGGAU	265	1424-1444	ATCCTGAAGGCGCAACAGAGCCU	529	1422-1444
AD-1631299	GCUUUGAGCCUCAGCUUCUCU	266	441-461	AGAGAAGCUGAGGCUCAAAGCCU	530	439-461
AD-1631300	GGAGUCAACACUUUCGAUUCU	267	79-99	AGAATCGAAAGTGUUGACUCCCU	531	77-99
AD-1631301	GGCUCUGUUGCGCCUUCAGGU	268	1423-1443	ACCUGAAGGCGCAACAGAGCCCU	532	1421-1443
AD-1631302	GUCGCCUUACGUUCAGCCGGU	269	1471-1491	ACCGGCTGAACGUAAGGCGACCU	533	1469-1491
AD-1631303	GUCUUCGAUGACCCGCGUCGU	270	1138-1158	ACGACGCGGGUCAUCGAAGACCU	534	1136-1158
AD-1631304	GUUGCGCCUUCAGGAGGAUGU	271	1429-1449	ACAUCCTCCUGAAGGCGCAACCU	535	1427-1449

雙鏈體名稱	5'至 3'之有義股序列	SEQ ID NO：	NM_000505.4 之中的範圍	5'至 3'之反義股序列	SEQ ID NO：	NM_000505.4 之中的範圍
AD-1631305	UCGCCUUACGUUCAGCCGGUU	272	1472-1492	AACCGGCUGAACGUAAGGCGACU	536	1470-1492
AD-1631306	UGAGCCUCAGCUUCUCCGGUU	273	445-465	AACCGGAGAAGCUGAGGCUCACU	537	443-465
AD-1631307	UGCGCCUUCAGGAGGAUGCGU	274	1431-1451	ACGCAUCCUCCTGAAGGCGCACU	538	1429-1451
AD-1631308	UGCUCAGGGACUCAUCUUUCU	275	1887-1907	AGAAAGAUGAGTCCCUGAGCACU	539	1885-1907
AD-1631309	UGGAGUCAACACUUUCGAUUU	276	78-98	AAAUCGAAAGUGUUGACUCCACU	540	76-98
AD-1631310	UGGCCUACUACCUGGCCUGGU	277	1842-1862	ACCAGGCCAGGTAGUAGGCCACU	541	1840-1862
AD-1631311	UUCGAUGACCCGCGUCGUUGU	278	1141-1161	ACAACGACGCGGGUCAUCGAACU	542	1139-1161
AD-1631312	UUGAGCCUCAGCUUCUCCGGU	279	444-464	ACCGGAGAAGCTGAGGCUCAACU	543	442-464
AD-85525	ACUCAAUAAAGUGCUUUGAAA	280	2009-2029	UUUCAAAGCACUUUAUUGAGUUC	544	2007-2029
AD-85531	UCAAUAAAGUGCUUUGAAAAU	281	2011-2031	AUUUUCAAAGCACUUUAUUGAGU	545	2009-2031
AD-85537	CUCAAUAAAGUGCUUUGAAAA	282	2010-2030	UUUUCAAAGCACUUUAUUGAGUU	546	2008-2030
AD-85541	GAACUCAAUAAAGUGCUUUGA	283	2007-2027	UCAAAGCACUUUAUUGAGUUCCU	547	2005-2027
AD-67246	AAUAAAGUGCUUUGAAAACGU	284	2013-2030	ACGUUUUCAAAGCACUUUAUUGA	548	2011-2030

表 3. F12 dsRNA 劑之修飾之有義及反義股序列

雙鏈體名稱	5'至 3'有義股序列	SEQ ID NO：	5'至 3'反義股序列	SEQ ID NO：	5'至 3'mRNA 目標序列	SEQ ID NO：
AD-1534462	asasagagaaGfUfGfcuuugagccuL96	549	asGfsgcdTc(Agn)aagcacUfuCfucuuuscsu	813	AGAAAGAGAAGUGCUUUGAGCCU	1077
AD-1534463	asasauguacCfCfAfcaagggccguL96	550	asdCsggdCcdCuugudGgGfuacauuusgsu	814	ACAAAUGUACCCACAAGGGCCGG	1078
AD-1534464	asascccggaCfAfAfcgacauccguL96	551	asdCsggdAudGucgudTgUfccggguuscsc	815	GGAACCCGGACAACGACAUCCGC	1079
AD-1534465	asascucaauAfAfAfgugcuuugauL96	552	asdTscadAadGcacudTuAfuugaguuscsc	816	GGAACUCAAUAAAGUGCUUUGAA	1080
AD-1534466	asascugagcAfAfGfcagcuguggguL96	553	asdCscadCadGcugcdTuGfcucaguuscsu	817	AGAACUGAGCAAGCAGCUGUGGC	1081
AD-1534467	asasgaaugaGfAfUfaugguauaguL96	554	asdCsuadTadCcauadTcUfcauucuusgsu	818	ACAAGAAUGAGAUAUGGUAUAGA	1082
AD-1534468	asasgagcacAfCfAfgucguucucuL96	555	asdGsagdAadCgacudGuGfugcucuuscsa	819	UGAAGAGCACACAGUCGUUCUCA	1083
AD-1534469	asasgcagcuGfUfGfgccagaugccuL96	556	asGfscadTc(Tgn)ggccacAfgCfugcuusgsc	820	GCAAGCAGCUGUGGCCAGAUGCC	1084
AD-1534470	asasgcugcuAfUfGfauggccgcguL96	557	asdCsgcdGgdCcaucdAuAfgcagcuusgsc	821	GCAAGCUGCUAUGAUGGCCGCGG	1085

雙鏈體名稱	5'至 3'有義股序列	SEQ ID NO：	5'至 3'反義股序列	SEQ ID NO：	5'至 3'mRNA 目標序列	SEQ ID NO：
AD-1534471	asasguacaaAfGfCfugaagagcauL96	558	asUfsgcdTc(Tgn)ucagcuUfuGfuacuusasu	822	AUAAGUACAAAGCUGAAGAGCAC	1086
AD-1534472	asasgugcuuUfGfAfgccucagcuuL96	559	asAfsgcdTg(Agn)ggcucaAfaGfcacuuscsu	823	AGAAGUGCUUUGAGCCUCAGCUU	1087
AD-1534473	asasuaaaguGfCfUfuugaaaauguL96	560	asdCsaudTudTcaaadGcAfcuuuauusgsa	824	UCAAUAAAGUGCUUUGAAAAUGC	1088
AD-1534474	ascsaaauguAfCfCfcacaagggcuL96	561	asdGsccdCudTguggdGuAfcauuugusgsg	825	CCACAAAUGUACCCACAAGGGCC	1089
AD-1534475	ascsacagucGfUfUfcucacugucuL96	562	asGfsacdAg(Tgn)gagaacGfaCfugugusgsc	826	GCACACAGUCGUUCUCACUGUCA	1090
AD-1534476	ascsaccgauGfUfGfgccuacuacuL96	563	asGfsuadGu(Agn)ggccacAfuCfggugusasg	827	CUACACCGAUGUGGCCUACUACC	1091
AD-1534477	ascsacuuucGfAfUfuccaccuuguL96	564	asdCsaadGgdTggaadTcGfaaagugususg	828	CAACACUUUCGAUUCCACCUUGG	1092
AD-1534478	ascsacuuucGfAfUfuccaccuuguL96	565	asCfsaadGg(Tgn)ggaaucGfaAfagugususg	829	CAACACUUUCGAUUCCACCUUGG	1093
AD-1534479	ascsagucguUfCfUfcacugucacuL96	566	asGfsugdAc(Agn)gugagaAfcGfacugusgsu	830	ACACAGUCGUUCUCACUGUCACC	1094
AD-1534480	ascsaucgccGfCfGfcuguacugguL96	567	asdCscadGudAcagcdGcGfgcgaugusasg	831	CUACAUCGCCGCGCUGUACUGGG	1095
AD-1534481	ascscagcacGfAfCfcuggcucuguL96	568	asdCsagdAgdCcaggdTcGfugcuggusasg	832	CUACCAGCACGACCUGGCUCUGU	1096
AD-1534482	ascsccggacAfAfCfgacauccgcuL96	569	asGfscgdGa(Tgn)gucguuGfuCfcgggususc	833	GAACCCGGACAACGACAUCCGCC	1097
AD-1534483	ascsccugcaAfGfGfcaucaucaguL96	570	asdCsugdAudGaugcdCuUfgcagggusgsa	834	UCACCCUGCAAGGCAUCAUCAGC	1098
AD-1534484	ascscgcaacAfAfGfccaggcgucuL96	571	asdGsacdGcdCuggcdTuGfuugcggucsca	835	UGACCGCAACAAGCCAGGCGUCU	1099
AD-1534485	ascscuggcuCfUfGfuugcgccuuuL96	572	asdAsagdGcdGcaacdAgAfgccaggucsg	836	CGACCUGGCUCUGUUGCGCCUUC	1100
AD-1534486	ascsguucagCfCfGfgugugccuguL96	573	asCfsagdGc(Agn)caccggCfuGfaacgusasa	837	UUACGUUCAGCCGGUGUGCCUGC	1101
AD-1534487	ascsuaccugGfCfCfuggauccgguL96	574	asdCscgdGadTccagdGcCfagguagusasg	838	CUACUACCUGGCCUGGAUCCGGG	1102
AD-1534488	ascsucaauaAfAfGfugcuuugaauL96	575	asdTsucdAadAgcacdTuUfauugagususc	839	GAACUCAAUAAAGUGCUUUGAAA	1103
AD-1534489	ascsugagcaAfGfCfagcuguggcuL96	576	asGfscdAc(Agn)gcugcuUfgCfucagususc	840	GAACUGAGCAAGCAGCUGUGGCC	1104
AD-1534490	asgsagaaguGfCfUfuugagccucuL96	577	asGfsagdGc(Tgn)caaagcAfcUfucucususu	841	AAAGAGAAGUGCUUUGAGCCUCA	1105
AD-1534491	asgsagcacaCfAfGfucguucucauL96	578	asUfsgadGa(Agn)cgacugUfgUfgcucususc	842	GAAGAGCACACAGUCGUUCUCAC	1106
AD-1534492	asgsagucugUfCfUfucgaugaccuL96	579	asGfsgudCa(Tgn)cgaagaCfaGfacucususg	843	CAAGAGUCUGUCUUCGAUGACCC	1107
AD-1534493	asgsaugccaGfUfGfcaagggucccuL96	580	asdGsgadCcdCuugcdAcUfggcaucusgsg	844	CCAGAUGCCAGUGCAAGGGUCCU	1108
AD-1534494	asgscacgacCfUfGfgcucuguuguL96	581	asdCsaadCadGagccdAgGfucgugcusgsg	845	CCAGCACGACCUGGCUCUGUUGC	1109
AD-1534495	asgsccaggcGfUfCfuacaccgauuL96	582	asdAsucdGgdTguagdAcGfccuggcususg	846	CAAGCCAGGCGUCUACACCGAUG	1110
AD-1534496	asgsccaggcGfUfCfuacaccgauuL96	583	asAfsucdGg(Tgn)guagacGfcCfuggcususg	847	CAAGCCAGGCGUCUACACCGAUG	1111

雙鏈體名稱	5'至3'有義股序列	SEQ ID NO：	5'至3'反義股序列	SEQ ID NO：	5'至3'mRNA目標序列	SEQ ID NO：
AD-1534497	asgscccaagAfAfAfgugaaagacuL96	584	asGfsucdTu(Tgn)cacuuuCfuUfgggcuscsc	848	GGAGCCCAAGAAAGUGAAAGACC	1112
AD-1534498	asgscugaagAfGfCfacacagucguL96	585	asdCsgadCudGugugdCuCfuucagcususu	849	AAAGCUGAAGAGCACACAGUCGU	1113
AD-1534499	asgscugcuaUfGfAfuggccgcgguL96	586	asdCscgdCgdGccaudCaUfagcagcususg	850	CAAGCUGCUAUGAUGGCCGCGGG	1114
AD-1534500	asgscuguacCfAfCfaaauguaccuL96	587	asGfsgudAc(Agn)uuugugGfuAfcagcusgsc	851	GCAGCUGUACCACAAAUGUACCC	1115
AD-1534501	asgsgagcauAfAfGfuacaaagcuuL96	588	asdAsgcdTudTguacdTuAfugcuccususg	852	CAAGGAGCAUAAGUACAAAGCUG	1116
AD-1534502	asgsgagcauAfAfGfuacaaagcuuL96	589	asAfsgcdTu(Tgn)guacuuAfuGfcuccususg	853	CAAGGAGCAUAAGUACAAAGCUG	1117
AD-1534503	asgsgauggcGfCfAfggaacucaauL96	590	asUfsugdAg(Tgn)uccugcGfcCfauccusgsg	854	CCAGGAUGGCGCAGGAACUCAAU	1118
AD-1534504	asgsgcgucuAfCfAfccgaugugguL96	591	asdCscadCadTcggudGuAfgacgccusgsg	855	CCAGGCGUCUACACCGAUGUGGC	1119
AD-1534505	asgsgcuucaUfGfUfcccacucauuL96	592	asAfsugdAg(Tgn)gggacaUfgAfagccusasg	856	CUAGGCUUCAUGUCCCACUCAUG	1120
AD-1534506	asgsucaacaCfUfUfucgauuccauL96	593	asdTsggdAadTcgaadAgUfguugacuscsc	857	GGAGUCAACACUUUCGAUUCCAC	1121
AD-1534507	asgsucaacaCfUfUfucgauuccauL96	594	asUfsggdAa(Tgn)cgaaagUfgUfugacuscsc	858	GGAGUCAACACUUUCGAUUCCAC	1122
AD-1534508	asusaaagugCfUfUfugaaaaugcuL96	595	asdGscadTudTucaadAgCfacuuuaususg	859	CAAUAAAGUGCUUUGAAAAUGCU	1123
AD-1534509	asuscegcccGfUfGfgugcuucguuL96	596	asdAscgdAadGcaccdAcGfggcggausgsu	860	ACAUCCGCCCGUGGUGCUUCGUG	1124
AD-1534510	asuscgggcuGfUfGfgugaccgcauL96	597	asUfsgcdGg(Tgn)caccacAfgCfccgauscsc	861	GGAUCGGGCUGUGGUGACCGCAA	1125
AD-1534511	asusgccaguGfCfAfaggguccuguL96	598	asdCsagdGadCccuudGcAfcuggcauscsu	862	AGAUGCCAGUGCAAGGGUCCUGA	1126
AD-1534512	asusgcucugCfGfCfaggguuccuuL96	599	asdAsggdAadCccugdCgCfagagcausgsc	863	GCAUGCUCUGCGCAGGGUUCCUC	1127
AD-1534513	asusguacccAfCfAfagggccggcuL96	600	asdGsccdGgdCccuudGuGfgguacaususu	864	AAAUGUACCCACAAGGGCCGGCC	1128
AD-1534514	asusugcucaGfGfGfacucaucuuuL96	601	asdAsagdAudGagucdCcUfgagcaauscsa	865	UGAUUGCUCAGGGACUCAUCUUU	1129
AD-1534515	csasaagcugAfAfGfagcacacaguL96	602	asCfsugdTg(Tgn)gcucuuCfaGfcuuugsusa	866	UACAAAGCUGAAGAGCACACAGU	1130
AD-1534516	csasaauguaCfCfCfacaagggccuL96	603	asGfsgcdCc(Tgn)ugugggUfaCfauuugsusg	867	CACAAAUGUACCCACAAGGGCCG	1131
AD-1534517	csasagaaagUfGfAfaagaccacuuL96	604	asAfsgudGg(Tgn)cuuucaCfuUfucuugsgsg	868	CCCAAGAAAGUGAAAGACCACUG	1132
AD-1534518	csasagagucUfGfUfcuucgaugauL96	605	asdTscadTcdGaagadCaGfacucuugscsg	869	CGCAAGAGUCUGUCUUCGAUGAC	1133
AD-1534519	csasaggagcAfUfAfaguacaaaguL96	606	asCfsuudTg(Tgn)acuuauGfcUfccuugsgsg	870	CCCAAGGAGCAUAAGUACAAAGC	1134
AD-1534520	csascaaaugUfAfCfccacaaggguL96	607	asdCsccdTudGugggdTaCfauuugugsgsu	871	ACCACAAAUGUACCCACAAGGGC	1135
AD-1534521	csascccugcAfAfGfgcaucaucauL96	608	asUfsgadTg(Agn)ugccuuGfcAfgggugsasg	872	CUCACCCUGCAAGGCAUCAUCAG	1136
AD-1534522	csascegaugUfGfGfccuacuaccuL96	609	asGfsgudAg(Tgn)aggccaCfaUfcggugsusa	873	UACACCGAUGUGGCCUACUACCU	1137

雙鏈體名稱	5'至 3'有義股序列	SEQ ID NO：	5'至 3'反義股序列	SEQ ID NO：	5'至 3'mRNA 目標序列	SEQ ID NO：
AD-1534523	csascgaccuGfGfCfucugugggcguL96	610	asdCsgcdAadCagagdCcAfggucgugscsu	874	AGCACGACCUGGCUCUGUUGCGC	1138
AD-1534524	csascugucuCfUfGfuccacaacauL96	611	asUfsgudTg(Tgn)ggacagAfgAfcagugsgsg	875	CCGACUGUCUCUGUCCACAACAG	1139
AD-1534525	csascuuucgAfUfUfccaccuuggauL96	612	asdCscadAgdGuggadAuCfgaaagugsusu	876	AACACUUUCGAUUCCACCUUGGG	1140
AD-1534526	csasgaugccAfGfUfgcaaggucuL96	613	asGfsacdCc(Tgn)ugcacuGfgCfaucugsgsc	877	GCCAGAUGCCAGUGCAAGGGUCC	1141
AD-1534527	csasgcacgaCfCfUfggcucuguuuL96	614	asAfsacdAg(Agn)gccaggUfcGfugcugsgsu	878	ACCAGCACGACCUGGCUCUGUUG	1142
AD-1534528	csasgcuguaCfCfAfcaaauguacuL96	615	asdGsuadCadTuugudGgUfacagcugscsc	879	GGCAGCUGUACCACAAAUGUACC	1143
AD-1534529	csasgcugugGfCfCfagaugccagaL96	616	asCfsugdGc(Agn)ucuggcCfaCfagcugscsu	880	AGCAGCUGUGGCCAGAUGCCAGU	1144
AD-1534530	csasggaacuCfAfAfuaaagugcuuL96	617	asAfsgcdAc(Tgn)uuauugAfgUfuccugscsg	881	CGCAGGAACUCAAUAAAGUGCUU	1145
AD-1534531	csasggauggCfGfUfcaggaacucauL96	618	asUfsgadGu(Tgn)ccugcgCfcAfuccugsgsc	882	GCCAGGAUGGCGCAGGAACUCAA	1146
AD-1534532	csasggcgucUfAfCfaccgauguguL96	619	asdCsacdAudCggugdTaGfacgccugsgsc	883	GCCAGGCGUCUACACCGAUGUGG	1147
AD-1534533	csasggcgacuCfAfUfcuuuccccucuL96	620	asdGsagdGgdAaagadTgAfguccugsasg	884	CUCAGGGACUCAUCUUUCCCUCC	1148
AD-1534534	csasgugcaaGfGfGfcfuccugaugcguL96	621	asGfscadTc(Agn)ggacccUfuGfcacugsgsc	885	GCCAGUGCAAGGGUCCUGAUGCC	1149
AD-1534535	csascacaaauGfUfAfcccacaaggguL96	622	asdCscudTgdTgggudAcAfuuguggsusa	886	UACCACACAAAUGUACCCACAAGGG	1150
AD-1534536	cscsacaaauGfUfAfcccacaaggguL96	623	asCfscudTg(Tgn)ggguacAfuUfuguggsusa	887	UACCACACAAAUGUACCCACAAGGG	1151
AD-1534537	cscsagaugcCfAfGfugcaaggguuL96	624	asdAsccdCudTgcacdTgGfcaucuggcsc	888	GGCCAGAUGCCAGUGCAAGGGUC	1152
AD-1534538	cscsaggcguCfUfAfcaccgauguuL96	625	asdAscadTcdGgugudAgAfcgccuggcsu	889	AGCCAGGCGUCUACACCGAUGUG	1153
AD-1534539	cscscaagaaAfGfUfgaaagaccauL96	626	asUfsggdTc(Tgn)uucacuUfuCfuugggscsu	890	AGCCCAAGAAAGUGAAAGACCAC	1154
AD-1534540	cscscggacaAfCfCfGfacauccgccuL96	627	asdGsgcdGgdAugucdGuUfgucgggsusu	891	AACCCGGACAACGACAUCCGCCC	1155
AD-1534541	cscscggacaAfCfCfGfacauccgccuL96	628	asGfsgcdGg(Agn)ugucguUfgUfccgggsusu	892	AACCCGGACAACGACAUCCGCCC	1156
AD-1534542	cscscugcaaGfGfGfCfaucaucagcuL96	629	asGfscudGa(Tgn)gaugccUfuGfcagggsusg	893	CACCCUGCAAGGGCAUCAUCAGCU	1157
AD-1534543	cscsgaugugGfCfCfCfuacuaccuguL96	630	asdCsagdGudAguagdGcCfacaucggsusg	894	CACCGAUGUGGCCUACUACCUGG	1158
AD-1534544	cscsgcaacaAfGfCfCfcaggcgucuuL96	631	asdAsgadCgdCcuggdCuUfguugcggsusc	895	GACCGCAACAAGCCAGGCGUCUA	1159
AD-1534545	cscsgcaagaGfUfCfCfugucuucgauL96	632	asdTscgdAadGacagdAcUfcuugcggsasg	896	CUCCGCAAGAGGUCUGUCUUCGAU	1160
AD-1534546	cscsggaaccCfGfGfGfacaacgacauL96	633	asdTsgudCgdTugucdCgGfguuccggscsa	897	UGCCGGAACCCGGACAACGACAU	1161
AD-1534547	cscsggaaccCfGfGfGfacaacgacauL96	634	asUfsgudCg(Tgn)uguccgGfgUfuccggscsa	898	UGCCGGAACCCGGACAACGACAU	1162
AD-1534548	cscsugcaggAfGfGfGfcgcagguacuL96	635	asGfsuadCc(Tgn)gcggccuCfcUfgcaggsasa	899	UUCCUGCAGGAGGCGCAGGUACC	1163

雙鏈體名稱	5'至3'有義股序列	SEQ ID NO：	5'至3'反義股序列	SEQ ID NO：	5'至3'mRNA目標序列	SEQ ID NO：
AD-1534549	cscsugucgcCfUfUfacguucagcuL96	636	asdGscudGadAcguadAgGfcgacaggsasg	900	CUCCUGUCGCCUUACGUUCAGCC	1164
AD-1534550	cscsugucgcCfUfUfacguucagcuL96	637	asGfscudGa(Agn)cguaagGfcGfacaggsasg	901	CUCCUGUCGCCUUACGUUCAGCC	1165
AD-1534551	cscsuuacguUfCfAfgccgguguguL96	638	asdCsacdAcdCggcudGaAfcguaaggscsg	902	CGCCUUACGUUCAGCCGGUGUGC	1166
AD-1534552	csgscaacaaGfCfCfaggcgucuauL96	639	asdTsagdAcdGccugdGcUfuguugcgsgsu	903	ACCGCAACAAGCCAGGCGUCUAC	1167
AD-1534553	csgscaggaaCfUfCfaauaaaguguL96	640	asdCsacdTudTauugdAgUfuccugcgscsc	904	GGCGCAGGAACUCAAUAAAGUGC	1168
AD-1534554	csgsccuuacGfUfUfcagccgguguL96	641	asdCsacdCgdGcugadAcGfuaaggcgsasc	905	GUCGCCUUACGUUCAGCCGGUGU	1169
AD-1534555	csgscuccugUfCfGfccuuacguuuL96	642	asdAsacdGudAaggcdGaCfaggagcgscsg	906	CGCGCUCCUGUCGCCUUACGUUC	1170
AD-1534556	csgsgaacccGfGfAfcaacgacauuL96	643	asdAsugdTcdGuugudCcGfgguuccgsgsc	907	GCCGGAACCCGGACAACGACAUC	1171
AD-1534557	csgsgcuccgCfAfAfgagucugucuL96	644	asGfsacdAg(Agn)cucuugCfgGfagccgscsu	908	AGCGGCUCCGCAAGAGUCUGUCU	1172
AD-1534558	csgsucuacaCfCfGfauguggccuuL96	645	asAfsggdCc(Agn)caucggUfgUfagacgscsc	909	GGCGUCUACACCGAUGUGGCCUA	1173
AD-1534559	csgsugccagGfGfUfgauuccggauL96	646	asdTsccdGgdAaucadCcCfuggcacgscsa	910	UGCGUGCCAGGGUGAUUCCGGAG	1174
AD-1534560	csusacaucgCfCfGfcgcuguacuuL96	647	asAfsgudAc(Agn)gcgcggCfgAfuguagsgsg	911	CCCUACAUCGCCGCGCUGUACUG	1175
AD-1534561	csusaccuggCfCfUfggauccggguL96	648	asdCsccdGgdAuccadGgCfcagguagsusa	912	UACUACCUGGCCUGGAUCCGGGA	1176
AD-1534562	csuscaauaaAfGfUfgcuuugaaauL96	649	asdTsuudCadAagcadCuUfuauugagsusu	913	AACUCAAUAAAGUGCUUUGAAAA	1177
AD-1534563	csuscaauaaAfGfUfgcuuugaaauL96	650	asUfsuudCa(Agn)agcacuUfuAfuugagsusu	914	AACUCAAUAAAGUGCUUUGAAAA	1178
AD-1534564	csuscagggaCfUfCfaucuuucccuL96	651	asdGsggdAadAgaugdAgUfcccugagscsa	915	UGCUCAGGGACUCAUCUUUCCCU	1179
AD-1534565	csusccugucGfCfCfuuacguucauL96	652	asdTsgadAcdGuaagdGcGfacaggagscsg	916	CGCUCCUGUCGCCUUACGUUCAG	1180
AD-1534566	csuscuguugCfGfCfcuucaggaguL96	653	asdCsucdCudGaaggdCgCfaacagagscsc	917	GGCUCUGUUGCGCCUUCAGGAGG	1181
AD-1534567	csusgaagagCfAfCfacagucguuuL96	654	asdAsacdGadCugugdTgCfucuucagscsu	918	AGCUGAAGAGCACACAGUCGUUC	1182
AD-1534568	csusgauugcUfCfAfgggacucauuL96	655	asAfsugdAg(Tgn)cccugaGfcAfaucagsgsa	919	UCCUGAUUGCUCAGGGACUCAUC	1183
AD-1534569	csusgcaaggCfAfUfcaucagcuguL96	656	asdCsagdCudGaugadTgCfcuugcagsgsg	920	CCCUGCAAGGCAUCAUCAGCUGG	1184
AD-1534570	csusgccggaAfCfCfcggacaacguL96	657	asCfsgudTg(Tgn)ccggguUfcCfggcagsasa	921	UUCUGCCGGAACCCGGACAACGA	1185
AD-1534571	csusguaccaCfAfAfauguacccauL96	658	asUfsggdGu(Agn)cauuugUfgGfuacagscsu	922	AGCUGUACCACAAAUGUACCCAC	1186
AD-1534572	csusgucgccUfUfAfcguucagccuL96	659	asGfsgcdTg(Agn)acguaaGfgCfgacagsgsa	923	UCCUGUCGCCUUACGUUCAGCCG	1187
AD-1534573	csusgucucuGfUfCfcacaacaccuL96	660	asGfsgudGu(Tgn)guggacAfgAfgacagsusg	924	CACUGUCUCUGUCCACAACACCU	1188
AD-1534574	csusguggugAfCfCfgcaacaagcuL96	661	asGfscudTg(Tgn)ugcggcuCfaCfcacagscsc	925	GGCUGUGGUGACCGCAACAAGCC	1189

雙鏈體名稱	5'至 3'有義股序列	SEQ ID NO：	5'至 3'反義股序列	SEQ ID NO：	5'至 3'mRNA 目標序列	SEQ ID NO：
AD-1534575	csusuacguuCfAfGfccggugugcuL96	662	asdGscadCadCcggcdTgAfacguaagsgsc	926	GCCUUACGUUCAGCCGGUGUGCC	1190
AD-1534576	csusucgaugAfCfCfcgcgucguuuL96	663	asdAsacdGadCgcggdGuCfaucgaagsasc	927	GUCUUCGAUGACCCGCGUCGUUG	1191
AD-1534577	csusuggaguCfAfAfcacuuucgauL96	664	asdTscgdAadAgugudTgAfcuccaagscsu	928	AGCUUGGAGUCAACACUUUCGAU	1192
AD-1534578	csusuugagcCfUfCfagcuucuccuL96	665	asGfsgadGa(Agn)gcugagGfcUfcaaagscsa	929	UGCUUUGAGCCUCAGCUUCUCCG	1193
AD-1534579	gsasaaugaAfAfGfaccacugcauL96	666	asUfsgcdAg(Tgn)ggucuuUfcAfcuuucsusu	930	AAGAAAGUGAAAGACCACUGCAG	1194
AD-1534580	gsasacccggAfCfAfacgacauccuL96	667	asGfsgadTg(Tgn)cguuguCfcGfgguucscsg	931	CGGAACCCGGACAACGACAUCCG	1195
AD-1534581	gsasacucaaUfAfAfagugcuuuguL96	668	asdCsaadAgdCacuudTaUfugaguucscsu	932	AGGAACUCAAUAAAGUGCUUUGA	1196
AD-1534582	gsasagagcaCfAfCfagucguucuuL96	669	asdAsgadAcdGacugdTgUfgcucuucsasg	933	CUGAAGAGCACACAGUCGUUCUC	1197
AD-1534583	gsascegcaaCfAfAfgccaggcguuL96	670	asAfscgdCc(Tgn)ggcuugUfuGfcggucsasc	934	GUGACCGCAACAAGCCAGGCGUC	1198
AD-1534584	gsasgcacacAfGfUfcguucucacuL96	671	asdGsugdAgdAacgadCuGfugugcucsusu	935	AAGAGCACACAGUCGUUCUCACU	1199
AD-1534585	gsasgcauaaGfUfAfcaaagcugauL96	672	asUfscadGc(Tgn)uuguacUfuAfugcucscsu	936	AGGAGCAUAAGUACAAAGCUGAA	1200
AD-1534586	gsasgccucaGfCfUfucuccgguuuL96	673	asdAsacdCgdGagaadGcUfgaggcucsasa	937	UUGAGCCUCAGCUUCUCCGGUUU	1201
AD-1534587	gsasgucuguCfUfUfcgaugacccuL96	674	asGfsggdTc(Agn)ucgaagAfcAfgacucsusu	938	AAGAGUCUGUCUUCGAUGACCCG	1202
AD-1534588	gsasugccagUfGfCfaagggucccuuL96	675	asdAsggdAcdCcuugdCaCfuggcaucsusg	939	CAGAUGCCAGUGCAAGGGUCCUG	1203
AD-1534589	gsasuggcgcAfGfGfaacucaauauL96	676	asUfsaudTg(Agn)guuccuGfcGfccaucscsu	940	AGGAUGGCGCAGGAACUCAAUAA	1204
AD-1534590	gsasuugcucAfGfGfgacucaucuuL96	677	asAfsgadTg(Agn)gucccuGfaGfcaaucsasg	941	CUGAUUGCUCAGGGACUCAUCUU	1205
AD-1534591	gscsaagaguCfUfGfucuucgauguL96	678	asdCsaudCgdAagacdAgAfcucuugcsgsg	942	CCGCAAGAGUCUGUCUUCGAUGA	1206
AD-1534592	gscsacacagUfCfGfuucucacuguL96	679	asCfsagdTg(Agn)gaacgaCfuGfugugcsusc	943	GAGCACACAGUCGUUCUCACUGU	1207
AD-1534593	gscsacgaccUfGfGfcucuguugcuL96	680	asdGscadAcdAgagcdCaGfgucgugcsusg	944	CAGCACGACCUGGCUCUGUUGCG	1208
AD-1534594	gscsacgaccUfGfGfcucuguugcuL96	681	asGfscadAc(Agn)gagccaGfgUfcgugcsusg	945	CAGCACGACCUGGCUCUGUUGCG	1209
AD-1534595	gscsagcuguGfGfCfcagaugccauL96	682	asUfsggdCa(Tgn)cuggccAfcAfgcugcsusu	946	AAGCAGCUGUGGCCAGAUGCCAG	1210
AD-1534596	gscsaggaacUfCfAfauaaagugcuL96	683	asdGscadCudTuauudGaGfuuccugcsgsc	947	GCGCAGGAACUCAAUAAAGUGCU	1211
AD-1534597	gscscagaugCfCfAfgugcaaggguL96	684	asdCsccdTudGcacudGgCfaucuggcscsa	948	UGGCCAGAUGCCAGUGCAAGGGU	1212
AD-1534598	gscscaggcgUfCfUfacaccgauguL96	685	asdCsaudCgdGuguadGaCfgccuggcsusu	949	AAGCCAGGCGUCUACACCGAUGU	1213
AD-1534599	gscsccaagaAfAfGfugaaagaccuL96	686	asGfsgudCu(Tgn)ucacuuUfcUfugggcsusc	950	GAGCCCAAGAAAGUGAAAGACCA	1214
AD-1534600	gscscuuacgUfUfCfagccgguguuL96	687	asdAscadCcdGgcugdAaCfguaaggcsgsa	951	UCGCCUUACGUUCAGCCGGUGUG	1215

雙鏈體名稱	5'至3'有義股序列	SEQ ID NO：	5'至3'反義股序列	SEQ ID NO：	5'至3'mRNA目標序列	SEQ ID NO：
AD-1534601	gscsgcaggaAfCfUfcaauaaaguuL96	688	asdAscudTudAuugadGuUfccugcgcscsa	952	UGGCGCAGGAACUCAAUAAAGUG	1216
AD-1534602	gscsgccuucAfGfGfaggaugcgguL96	689	asdCscgdCadTccucdCuGfaaggcgcsasa	953	UUGCGCCUUCAGGAGGAUGCGGA	1217
AD-1534603	gscsggcuccGfCfAfagagucuguuL96	690	asdAscadGadCucuudGcGfgagccgcsusg	954	CAGCGGCUCCGCAAGAGUCUGUC	1218
AD-1534604	gscsucagggAfCfUfcaucuuuccuL96	691	asdGsgadAadGaugadGuCfccugagcsasa	955	UUGCUCAGGGACUCAUCUUUCCC	1219
AD-1534605	gscsuccgcaAfGfAfgucugucuuuL96	692	asAfsagdAc(Agn)gacucuUfgCfggagcscsg	956	CGGCUCCGCAAGAGUCUGUCUUC	1220
AD-1534606	gscsuccuguCfGfCfcuuacguucuL96	693	asdGsaadCgdTaaggdCgAfcaggagcsgsc	957	GCGCUCCUGUCGCCUUACGUUCA	1221
AD-1534607	gscsucugcgCfAfGfgguuccucguL96	694	asdCsgadGgdAacccdTgCfgcagagcsasu	958	AUGCUCUGCGCAGGGUUCCUCGA	1222
AD-1534608	gscsucugcgCfAfGfgguuccucguL96	695	asCfsgadGg(Agn)acccugCfgCfagagcsasu	959	AUGCUCUGCGCAGGGUUCCUCGA	1223
AD-1534609	gscsucuguuGfCfGfccuucaggauL96	696	asdTsccdTgdAaggcdGcAfacagagcscsa	960	UGGCUCUGUUGCGCCUUCAGGAG	1224
AD-1534610	gscsucuguuGfCfGfccuucaggauL96	697	asUfsccdTg(Agn)aggcgcAfaCfagagcscsa	961	UGGCUCUGUUGCGCCUUCAGGAG	1225
AD-1534611	gscsugaagaGfCfAfcacagucguuL96	698	asdAscgdAcdTgugudGcUfcuucagcsusu	962	AAGCUGAAGAGCACACAGUCGUU	1226
AD-1534612	gscsugcuauGfAfUfggccgcggguL96	699	asdCsccdGcdGgccadTcAfuagcagcsusu	963	AAGCUGCUAUGAUGGCCGCGGGC	1227
AD-1534613	gscsugguguGfUfGfaggaccaaguL96	700	asCfsuudGg(Tgn)ccucacAfcAfccagcsgsg	964	CCGCUGGUGUGUGAGGACCAAGC	1228
AD-1534614	gscsugugguGfAfCfcgcaacaaguL96	701	asdCsuudGudTgcggdTcAfccacagcscsc	965	GGGCUGUGGUGACCGCAACAAGC	1229
AD-1534615	gscsugugguGfAfCfcgcaacaaguL96	702	asCfsuudGu(Tgn)gcggucAfcCfacagcscsc	966	GGGCUGUGGUGACCGCAACAAGC	1230
AD-1534616	gscsuucaugUfCfCfcacucaugcuL96	703	asGfscadTg(Agn)gugggaCfaUfgaagcscsu	967	AGGCUUCAUGUCCCACUCAUGCC	1231
AD-1534617	gscsuuugagCfCfUfcagcuucucuL96	704	asdGsagdAadGcugadGgCfucaaagcsasc	968	GUGCUUUGAGCCUCAGCUUCUCC	1232
AD-1534618	gsgsaacucaAfUfAfaagugcuuuuL96	705	asAfsaadGc(Agn)cuuuauUfgAfguuccsusg	969	CAGGAACUCAAUAAAGUGCUUUG	1233
AD-1534619	gsgsagcauaAfGfUfacaaagcuguL96	706	asdCsagdCudTuguadCuUfaugcuccsusu	970	AAGGAGCAUAAGUACAAAGCUGA	1234
AD-1534620	gsgsagcccaAfGfAfaagugaaaguL96	707	asCfsuudTc(Agn)cuuucuUfgGfgcuccsasa	971	UUGGAGCCCAAGAAAGUGAAAGA	1235
AD-1534621	gsgsagucaaCfAfCfuuucgauucuL96	708	asdGsaadTcdGaaagdTgUfugacuccsasa	972	UUGGAGUCAACACUUUCGAUUCC	1236
AD-1534622	gsgscgucuaCfAfCfcgauguggcuL96	709	asdGsccdAcdAucggdTgUfagacgccsusg	973	CAGGCGUCUACACCGAUGUGGCC	1237
AD-1534623	gsgscgucuaCfAfCfcgauguggcuL96	710	asGfsccdAc(Agn)ucggugUfaGfacgccsusg	974	CAGGCGUCUACACCGAUGUGGCC	1238
AD-1534624	gsgscuccgcAfAfGfagucugucuuL96	711	asdAsgadCadGacucdTuGfcggagccsgsc	975	GCGGCUCCGCAAGAGUCUGUCUU	1239
AD-1534625	gsgscucuguUfGfCfgccuucagguL96	712	asdCscudGadAggcgdCaAfcagagccsasg	976	CUGGCUCUGUUGCGCCUUCAGGA	1240
AD-1534626	gsgsguccugAfUfGfcccacugccuL96	713	asGfsgcdAg(Tgn)gggcauCfaGfgacccsusu	977	AAGGGUCCUGAUGCCCACUGCCA	1241

雙鏈體名稱	5'至3'有義股序列	SEQ ID NO：	5'至3'反義股序列	SEQ ID NO：	5'至3'mRNA目標序列	SEQ ID NO：
AD-1534627	gsuscaacacUfUfUfcgauuccacuL96	714	asdGsugdGadAucgadAaGfuguugacsusc	978	GAGUCAACACUUUCGAUUCCACC	1242
AD-1534628	gsuscaacacUfUfUfcgauuccacuL96	715	asGfsugdGa(Agn)ucgaaaGfuGfuugacsusc	979	GAGUCAACACUUUCGAUUCCACC	1243
AD-1534629	gsuscgccuuAfCfGfuucagccgguL96	716	asdCscgdGcdTgaacdGuAfaggcgacsasg	980	CUGUCGCCUUACGUUCAGCCGGU	1244
AD-1534630	gsuscgccuuAfCfGfuucagccgguL96	717	asCfscgdGc(Tgn)gaacguAfaGfgcgacsasg	981	CUGUCGCCUUACGUUCAGCCGGU	1245
AD-1534631	gsuscguucuCfAfCfugucaccgguL96	718	asdCscgdGudGacagdTgAfgaacgacsusg	982	CAGUCGUUCUCACUGUCACCGGG	1246
AD-1534632	gsuscugucuUfCfGfaugacccgcuL96	719	asGfscgdGg(Tgn)caucgaAfgAfcagacsusc	983	GAGUCUGUCUUCGAUGACCCGCG	1247
AD-1534633	gsuscuucgaUfGfAfcccgcgucguL96	720	asdCsgadCgdCgggudCaUfcgaagacsasg	984	CUGUCUUCGAUGACCCGCGUCGU	1248
AD-1534634	gsusgcaaggGfUfCfcugaugcccuL96	721	asGfsggdCa(Tgn)caggacCfcUfugcacsusg	985	CAGUGCAAGGGUCCUGAUGCCCA	1249
AD-1534635	gsusggugacCfGfCfaacaagccauL96	722	asUfsggdCu(Tgn)guugcgGfuCfaccacsasg	986	CUGUGGUGACCGCAACAAGCCAG	1250
AD-1534636	gsusugcgccUfUfCfaggaggauguL96	723	asdCsaudCcdTccugdAaGfgcgcaacsasg	987	CUGUUGCGCCUUCAGGAGGAUGC	1251
AD-1534637	gsusugcgccUfUfCfaggaggauguL96	724	asCfsaudCc(Tgn)ccugaaGfgCfgcaacsasg	988	CUGUUGCGCCUUCAGGAGGAUGC	1252
AD-1534638	usasaguacaAfAfGfcugaagagcuL96	725	asdGscudCudTcagcdTuUfguacuuasusg	989	CAUAAGUACAAAGCUGAAGAGCA	1253
AD-1534639	usasaguacaAfAfGfcugaagagcuL96	726	asGfscudCu(Tgn)cagcuuUfgUfacuuasusg	990	CAUAAGUACAAAGCUGAAGAGCA	1254
AD-1534640	usascaucgcCfGfCfgcuguacuguL96	727	asdCsagdTadCagcgdCgGfcgauguasgsg	991	CCUACAUCGCCGCGCUGUACUGG	1255
AD-1534641	usasccuggcCfUfGfgauccgggauL96	728	asdTsccdCgdGauccdAgGfccagguasgsu	992	ACUACCUGGCCUGGAUCCGGGAG	1256
AD-1534642	usascuaccuGfGfCfcuggauccguL96	729	asdCsggdAudCcaggdCcAfgguaguasgsg	993	CCUACUACCUGGCCUGGAUCCGG	1257
AD-1534643	uscsaacacuUfUfCfgauuccaccuL96	730	asGfsgudGg(Agn)aucgaaAfgUfguugascsu	994	AGUCAACACUUUCGAUUCCACCU	1258
AD-1534644	uscsacccugCfAfAfggcaucaucuL96	731	asGfsaudGa(Tgn)gccuugCfaGfggugasgsc	995	GCUCACCCUGCAAGGCAUCAUCA	1259
AD-1534645	uscsagccggUfGfUfgccugccaauL96	732	asUfsugdGc(Agn)ggcacaCfcGfgcugasasc	996	GUUCAGCCGGUGUGCCUGCCAAG	1260
AD-1534646	uscsaggaggAfUfGfcggacggcauL96	733	asUfsgcdCg(Tgn)ccgcauCfcUfccugasasg	997	CUUCAGGAGGAUGCGGACGGCAG	1261
AD-1534647	uscsagggacUfCfAfucuuucccuuL96	734	asdAsggdGadAagaudGaGfucccugasgsc	998	GCUCAGGGACUCAUCUUUCCCUC	1262
AD-1534648	uscscgcaagAfGfUfcugucuucguL96	735	asdCsgadAgdAcagadCuCfuugcggasgsc	999	GCUCCGCAAGAGUCUGUCUUCGA	1263
AD-1534649	uscscugucgCfCfUfuacguucaguL96	736	asdCsugdAadCguaadGgCfgacaggasgsc	1000	GCUCCUGUCGCCUUACGUUCAGC	1264
AD-1534650	uscsgccuuaCfGfUfucagccgguuL96	737	asdAscdGgdCugaadCgUfaaggcgascsa	1001	UGUCGCCUUACGUUCAGCCGGUG	1265
AD-1534651	uscsucugucCfAfCfaacaccucauL96	738	asUfsgadGg(Tgn)guugugGfaCfagagascsa	1002	UGUCUCUGUCCACAACACCUCAC	1266
AD-1534652	uscsuguugcGfCfCfuucaggagguL96	739	asdCscudCcdTgaagdGcGfcaacagasgsc	1003	GCUCUGUUGCGCCUUCAGGAGGA	1267

雙鏈體名稱	5'至 3'有義股序列	SEQ ID NO：	5'至 3'反義股序列	SEQ ID NO：	5'至 3'mRNA 目標序列	SEQ ID NO：
AD-1534653	usgsaagagcAfCfAfcagucguucuL96	740	asdGsaadCgdAcugudGuGfcucuucasgsc	1004	GCUGAAGAGCACACAGUCGUUCU	1268
AD-1534654	usgsagccucAfGfCfUfuucuccggguuL96	741	asdAscdGgdAgaagdCuGfaggcucasasa	1005	UUUGAGCCUCAGCUUCUCCGGUU	1269
AD-1534655	usgsagcuggGfAfGfuacugcgacuL96	742	asGfsucdGc(Agn)guacucCfcAfgcucasgsc	1006	GCUGAGCUGGGAGUACUGCGACC	1270
AD-1534656	usgsauggccGfCfGfggcucagcuuL96	743	asAfsgcdTg(Agn)gccgcGfgCfcaucasusa	1007	UAUGAUGGCCGCGGGCUCAGCUA	1271
AD-1534657	usgscaaggcAfUfCfaucagcuggguL96	744	asdCscadGcdTgaugdAuGfccugcasgsg	1008	CCUGCAAGGCAUCAUCAGCUGGG	1272
AD-1534658	usgscaaggcAfUfCfaucagcuggguL96	745	asCfscadGc(Tgn)gaugauGfcCfuugcasgsg	1009	CCUGCAAGGCAUCAUCAGCUGGG	1273
AD-1534659	usgscaaggUfCfCfugaugcccauL96	746	asUfsggdGc(Agn)ucaggaCfcCfuugcascsu	1010	AGUGCAAGGGUCCUGAUGCCCAC	1274
AD-1534660	usgsccagugCfAfAfggguccugauL96	747	asUfscadGg(Agn)cccuugCfaCfuggcasusc	1011	GAUGCCAGUGCAAGGGUCCUGAU	1275
AD-1534661	usgsccaugaGfGfGfcucugcugcuL96	748	asGfscadGc(Agn)gagcccUfcAfuggcasusc	1012	GAUGCCAUGAGGGCUCUGCUGCU	1276
AD-1534662	usgscgccuuCfAfGfgaggaugcguL96	749	asdCsgcdAudCcuccdTgAfaggcgcasasc	1013	GUUGCGCCUUCAGGAGGAUGCGG	1277
AD-1534663	usgscucaggGfAfCfucaucuuucuL96	750	asdGsaadAgdAugagdTcCfcugagcasasu	1014	AUUGCUCAGGGACUCAUCUUUCC	1278
AD-1534664	usgscucaggGfAfCfucaucuuucuL96	751	asGfsaadAg(Agn)ugagucCfcUfgagcasasu	1015	AUUGCUCAGGGACUCAUCUUUCC	1279
AD-1534665	usgsgagucaAfCfAfcuuucgauuuL96	752	asdAsaudCgdAaagudGuUfgacuccasasg	1016	CUUGGAGUCAACACUUUCGAUUC	1280
AD-1534666	usgsgccagaUfGfCfcagugcaaguL96	753	asCfsuudGc(Agn)cuggcaUfcUfggccascsa	1017	UGUGGCCAGAUGCCAGUGCAAGG	1281
AD-1534667	usgsgccuacUfAfCfcuggccuggguL96	754	asdCscadGgdCcaggdTaGfuaggccascsa	1018	UGUGGCCUACUACCUGGCCUGGA	1282
AD-1534668	usgsgcucugUfUfGfcgccuucaguL96	755	asdCsugdAadGgcgcdAaCfagagccasgsg	1019	CCUGGCUCUGUUGCGCCUUCAGG	1283
AD-1534669	usgsgugaccGfCfAfacaagccaguL96	756	asCfsugdGc(Tgn)ugugccGfgUfcaccascsa	1020	UGUGGUGACCGCAACACAAGCCAGG	1284
AD-1534670	usgsuaccacAfAfAfuguacccacuL96	757	asGfsugdGg(Tgn)acauuuGfuGfguacasgsc	1021	GCUGUACCACACAAAUGUACCCACA	1285
AD-1534671	usgsucucugUfCfCfacaacaccuuL96	758	asAfsggdTg(Tgn)uguggaCfaGfagacasgsu	1022	ACUGUCUCUGUCCACAACACCUC	1286
AD-1534672	usgsuggccaGfAfUfgccagugcauL96	759	asUfsgcdAc(Tgn)ggcaucUfgGfccacasgsc	1023	GCUGUGGCCAGAUGCCAGUGCAA	1287
AD-1534673	ususacguucAfGfCfcgguguggccuL96	760	asGfsgcdAc(Agn)ccggcuGfaAfcguaasgsg	1024	CCUUACGUUCAGCCGGUGUGCCU	1288
AD-1534674	ususcaugucCfCfAfcucaugcccuL96	761	asGfsggdCa(Tgn)gaguggGfaCfaugaasgsc	1025	GCUUCAUGUCCCACUCAUGCCCG	1289
AD-1534675	ususcgaugaCfCfCfgcgucguuguL96	762	asdCsaadCgdAcgcgdGgUfcaucgaasgsa	1026	UCUUCGAUGACCCGCGUCGUUGG	1290
AD-1534676	ususgagccuCfAfGfcuucuccgguL96	763	asdCscgdGadGaagcdTgAfggcucaasasg	1027	CUUUGAGCCUCAGCUUCUCCGGU	1291
AD-1534677	ususggagccCfAfAfgaaagugaauL96	764	asUfsucdAc(Tgn)uucuugGfgCfuccaasasc	1028	GUUUGGAGCCCAAGAAAGUGAAA	1292
AD-1534678	ususggagucAfAfCfacuuucgauuL96	765	asdAsucdGadAagugdTuGfacuccaasgsc	1029	GCUUGGAGUCAACACUUUCGAUU	1293

雙鏈體名稱	5'至 3'有義股序列	SEQ ID NO：	5'至 3'反義股序列	SEQ ID NO：	5'至 3'mRNA 目標序列	SEQ ID NO：
AD-1534679	ususugagccUfCfAfgcuucuccguL96	766	asdCsggdAgdAagcudGaGfgcucaaasgsc	1030	GCUUUGAGCCUCAGCUUCUCCGG	1294
AD-1631272	asasgagcacAfCfAfgucguucucuL96	767	asdGsagdAadCgacudGuGfugcucuuscsu	1031	UGAAGAGCACACAGUCGUUCUCA	1295
AD-1631273	asasuaaaguGfCfUfuugaaaauguL96	768	asdCsaudTudTcaaadGcAfcuuuauuscsu	1032	UCAAUAAAGUGCUUUGAAAAUGC	1296
AD-1631274	ascsaucgccGfCfGfcuguacugguL96	769	asdCscadGudAcagcdGcGfgcgaugucsu	1033	CUACAUCGCCGCGCUGUACUGGG	1297
AD-1631275	ascscagcacGfAfCfcuggcucuguL96	770	asdCsagdAgdCcaggdTcGfugcuggucsu	1034	CUACCAGCACGACCUGGCUCUGU	1298
AD-1631276	ascsccugcaAfGfGfcaucaucaguL96	771	asdCsugdAudGaugcdCuUfgcagggucsu	1035	UCACCCUGCAAGGCAUCAUCAGC	1299
AD-1631277	ascscgcaacAfAfGfccaggcgucuL96	772	asdGsacdGcdCuggcdTuGfuugcggucsu	1036	UGACCGCAACAAGCCAGGCGUCU	1300
AD-1631278	ascsuaccugGfCfCfuggauccgguL96	773	asdCscgdGadTccagdGcCfagguagucsu	1037	CUACUACCUGGCCUGGAUCCGGG	1301
AD-1631279	asusugcucaGfGfGfacucaucuuuL96	774	asdAsagdAudGagucdCcUfgagcaaucsu	1038	UGAUUGCUCAGGGACUCAUCUUU	1302
AD-1631280	csasgggacuCfAfUfcuuucccucuL96	775	asdGsagdGgdAaagadTgAfgucccugscsu	1039	CUCAGGGACUCAUCUUUCCCUCC	1303
AD-1631281	cscsacaaauGfUfAfcccacaagguL96	776	asdCscudTgdTgggudAcAfuuuguggscsu	1040	UACCACAAAUGUACCCACAAGGG	1304
AD-1631282	cscsgcaagaGfUfCfugucuucgauL96	777	asdTscgdAadGacagdAcUfcuugcggscsu	1041	CUCCGCAAGAGUCUGUCUUCGAU	1305
AD-1631283	cscsggaaccCfGfGfacaacgacauL96	778	asdTsgudCgdTugucdCgGfguuccggscsu	1042	UGCCGGAACCCGGACAACGACAU	1306
AD-1631284	cscsugucgcCfUfUfacguucagcuL96	779	asdGscudGadAcguadAgGfcgacaggscsu	1043	CUCCUGUCGCCUUACGUUCAGCC	1307
AD-1631285	csgsccuuacGfUfUfcagccgguguL96	780	asdCsacdCgdGcugadAcGfuaaggcgscsu	1044	GUCGCCUUACGUUCAGCCGGUGU	1308
AD-1631286	csgsugccagGfGfUfgauuccggauL96	781	asdTsccdGgdAaucadCcCfuggcacgscsu	1045	UGCGUGCCAGGGUGAUUCCGGAG	1309
AD-1631287	csusaccuggCfCfUfggauccggguL96	782	asdCsccdGgdAuccadGgCfcagguagscsu	1046	UACUACCUGGCCUGGAUCCGGGA	1310
AD-1631288	csuscagggaCfUfCfaucuuucccuL96	783	asdGsggdAadAgaugdAgUfcccugagscsu	1047	UGCUCAGGGACUCAUCUUUCCCU	1311
AD-1631289	csusucgaugAfCfCfcgcgucguuuL96	784	asdAsacdGadCgcggdGuCfaucgaagscsu	1048	GUCUUCGAUGACCCGCGUCGUUG	1312
AD-1631290	gsasagagcaCfAfCfagucguucuuL96	785	asdAsgadAcdGacugdTgUfgcucuucscsu	1049	CUGAAGAGCACACAGUCGUUCUC	1313
AD-1631291	gsasgccucaGfCfUfucuccgguuuL96	786	asdAsacdCgdGagaadGcUfgaggcucscsu	1050	UUGAGCCUCAGCUUCUCCGGUUU	1314
AD-1631292	gscscagaugCfCfAfgugcaaggguL96	787	asdCsccdTudGcacudGgCfaucuggcscsu	1051	UGGCCAGAUGCCAGUGCAAGGGU	1315
AD-1631293	gscscuuacgUfUfCfagccgguguuL96	788	asdAscadCcdGgcugdAaCfguaaggcscsu	1052	UCGCCUUACGUUCAGCCGGUGUG	1316
AD-1631294	gscsgcaggaAfCfUfcaauaaaguuL96	789	asdAscudTudAuugadGuUfccugcgcscsu	1053	UGGCGCAGGAACUCAAUAAAGUG	1317
AD-1631295	gscsgccuucAfGfGfaggaugcgguL96	790	asdCscgdCadTccucdCuGfaaggcgcscsu	1054	UUGCGCCUUCAGGAGGAUGCGGA	1318
AD-1631296	gscsucagggAfCfUfcaucuuuccuL96	791	asdGsgadAadGaugadGuCfccugagcscsu	1055	UUGCUCAGGGACUCAUCUUUCCC	1319

雙鏈體名稱	5'至3'有義股序列	SEQ ID NO：	5'至3'反義股序列	SEQ ID NO：	5'至3'mRNA目標序列	SEQ ID NO：
AD-1631297	gscsucugcgCfAfGfgguuccucguL96	792	asdCsgadGgdAacccdTgCfgcagagcscsu	1056	AUGCUCUGCGCAGGGUUCCUCGA	1320
AD-1631298	gscsucuguuGfCfGfccuucaggauL96	793	asdTsccdTgdAaggcdGcAfacagagcscsu	1057	UGGCUCUGUUGCGCCUUCAGGAG	1321
AD-1631299	gscsuuugagCfCfUfcagcuucucuL96	794	asdGsagdAadGcugadGgCfucaaagcscsu	1058	GUGCUUUGAGCCUCAGCUUCUCC	1322
AD-1631300	gsgsagucaaCfAfCfuuucgauucuL96	795	asdGsaadTcdGaaagdTgUfugacuccscsu	1059	UUGGAGUCAACACUUUCGAUUCC	1323
AD-1631301	gsgscucuguUfGfCfgccuucagguL96	796	asdCscudGadAggcgdCaAfcagagccscsu	1060	CUGGCUCUGUUGCGCCUUCAGGA	1324
AD-1631302	gsuscgccuuAfCfGfuucagccgguL96	797	asdCscgdGcdTgaacdGuAfaggcgacscsu	1061	CUGUCGCCUUACGUUCAGCCGGU	1325
AD-1631303	gsuscuucgaUfGfAfcccgcgucguL96	798	asdCsgadCgdCgggudCaUfcgaagacscsu	1062	CUGUCUUCGAUGACCCGCGUCGU	1326
AD-1631304	gsusugcgccUfUfCfaggaggauguL96	799	asdCsaudCcdTccugdAaGfgcgcaacscsu	1063	CUGUUGCGCCUUCAGGAGGAUGC	1327
AD-1631305	uscsgccuuaCfGfUfucagccgguuL96	800	asdAsccdGgdCugaadCgUfaaggcgascsu	1064	UGUCGCCUUACGUUCAGCCGGUG	1328
AD-1631306	usgsagccucAfGfCfuucuccgguuL96	801	asdAsccdGgdAgaagdCuGfaggcucascsu	1065	UUUGAGCCUCAGCUUCUCCGGUU	1329
AD-1631307	usgscgccuuCfAfGfgaggaugcguL96	802	asdCsgcdAudCcuccdTgAfaggcgcascsu	1066	GUUGCGCCUUCAGGAGGAUGCGG	1330
AD-1631308	usgscucaggGfAfCfucaucuuucuL96	803	asdGsaadAgdAugagdTcCfcugagcascsu	1067	AUUGCUCAGGGACUCAUCUUUCC	1331
AD-1631309	usgsgagucaAfCfAfcuuucgauuuL96	804	asdAsaudCgdAaagudGuUfgacuccascsu	1068	CUUGGAGUCAACACUUUCGAUUC	1332
AD-1631310	usgsgccuacUfAfCfcuggccugguL96	805	asdCscadGgdCcaggdTaGfuaggccascsu	1069	UGUGGCCUACUACCUGGCCUGGA	1333
AD-1631311	ususcgaugaCfCfCfgcgucguuguL96	806	asdCsaadCgdAcgcgdGgUfcaucgaascsu	1070	UCUUCGAUGACCCGCGUCGUUGG	1334
AD-1631312	ususgagccuCfAfGfcuucuccgguL96	807	asdCscgdGadGaagcdTgAfggcucaascsu	1071	CUUUGAGCCUCAGCUUCUCCGGU	1335
AD-85525	ascsucaaUfaAfAfGfugcuuugaaaL96	808	usUfsucaaagcacuuUfaUfugagususc	1072	GAACUCAAUAAAGUGCUUUGAAA	1336
AD-85531	uscsaauaAfaGfUfGfcuuugaaaauL96	809	asUfsuuucaaagcacUfuUfauugasgsu	1073	ACUCAAUAAAGUGCUUUGAAAAU	1337
AD-85537	csuscaauAfaAfGfUfgcuuugaaaaL96	810	usUfsuucaaagcacuUfuAfuugagsusu	1074	AACUCAAUAAAGUGCUUUGAAAA	1338
AD-85541	gsasacucAfaUfAfAfagugcuuugaL96	811	usCfsaaagcacuuuaUfuGfaguucscsu	1075	AGGAACUCAAUAAAGUGCUUUGA	1339
AD-67246	asasuaaaGfuGfCfUfuugaaaacguL96	812	asCfsguuUfuCfAfaagcAfcUfuuauusgsa	1076	UCAATAAAGTGCTTTGAAAA	1340

表 4. PHH 細胞中的單一劑量篩查

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534661.1	44.05	4.93	69.18	4.10	85.65	8.36
AD-1534577.1	12.11	1.61	24.85	1.11	28.45	0.69
AD-1534678.1	10.93	1.19	22.47	2.79	26.15	3.02
AD-1534665.1	5.73	0.32	14.01	0.67	21.39	1.31
AD-1631309.1	7.03	0.66	14.67	0.88	21.63	2.28
AD-1534621.1	6.70	0.50	17.96	4.44	17.38	0.96
AD-1631300.1	10.06	1.77	17.60	1.48	24.79	2.03
AD-1534506.1	6.24	0.92	13.38	1.17	20.59	0.68
AD-1534507.1	10.74	0.98	15.73	1.15	24.57	3.14
AD-1534627.1	25.35	2.73	43.05	3.27	60.05	4.14
AD-1534628.1	41.16	3.47	63.30	4.02	73.10	3.82
AD-1534643.1	59.52	1.86	79.54	3.61	74.34	4.06
AD-1534477.1	9.74	1.31	21.58	1.03	33.46	3.63
AD-1534478.1	49.87	2.04	63.36	2.03	85.92	4.39
AD-1534525.1	25.71	2.68	48.60	7.18	60.80	3.95
AD-1534519.1	67.15	5.73	75.69	8.82	88.37	5.21
AD-1534501.1	7.52	1.75	15.11	1.03	22.92	1.42
AD-1534502.1	8.00	0.87	15.90	1.77	26.14	2.65
AD-1534619.1	11.28	0.97	25.24	2.79	30.78	3.45
AD-1534585.1	11.34	0.38	21.88	1.41	27.59	3.05
AD-1534638.1	17.72	1.81	27.67	0.63	33.57	2.93
AD-1534639.1	20.54	3.14	29.81	1.93	37.26	6.55
AD-1534471.1	6.51	0.69	12.63	1.00	22.48	2.27
AD-1534515.1	16.51	1.81	32.66	10.43	32.77	3.57
AD-1534498.1	10.26	1.63	19.40	2.23	32.84	4.14
AD-1534611.1	5.59	0.50	14.54	2.13	20.10	1.53
AD-1534567.1	3.99	0.33	11.49	2.11	13.54	1.29
AD-1534653.1	12.81	1.20	21.70	1.26	32.04	0.85
AD-1534582.1	4.41	0.68	15.00	3.15	18.57	2.10
AD-1631290.1	7.40	0.51	16.85	0.91	24.34	1.01
AD-1534468.1	12.06	1.11	25.04	1.77	34.73	3.53
AD-1631272.1	21.96	0.94	33.00	3.29	37.18	2.22
AD-1534491.1	5.19	0.37	13.84	1.96	21.60	1.19
AD-1534584.1	10.01	0.57	26.72	4.03	34.05	4.78
AD-1534475.1	34.84	2.54	55.91	4.77	80.78	1.98
AD-1534479.1	5.79	0.57	12.45	0.83	20.10	1.83
AD-1534631.1	9.40	1.46	18.99	0.61	29.08	2.89
AD-1534528.1	5.96	0.99	11.36	0.79	18.28	1.72
AD-1534500.1	54.63	8.31	61.60	0.80	78.36	3.61
AD-1534571.1	7.52	1.01	17.48	3.54	20.30	1.81
AD-1534670.1	34.73	3.06	44.58	6.06	52.88	1.88
AD-1534535.1	28.24	0.99	43.88	2.96	63.88	3.07
AD-1631281.1	49.87	6.19	69.66	4.09	82.29	8.76

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534536.1	85.33	11.49	81.33	3.08	90.47	3.99
AD-1534520.1	54.45	4.62	64.37	4.70	78.68	15.12
AD-1534474.1	47.54	4.63	75.38	3.44	88.71	13.21
AD-1534516.1	39.65	4.68	59.02	2.12	75.27	2.71
AD-1534463.1	69.01	3.89	53.48	2.10	63.99	6.25
AD-1534513.1	83.25	15.32	77.18	16.79	81.45	9.40
AD-1534677.1	88.76	4.69	76.94	2.75	86.09	3.80
AD-1534620.1	40.56	4.37	52.67	3.28	61.12	8.69
AD-1534497.1	20.56	1.70	39.51	2.12	61.93	3.33
AD-1534599.1	22.24	1.26	33.58	1.07	35.83	1.27
AD-1534539.1	15.85	1.61	28.76	6.80	35.78	2.40
AD-1534517.1	17.72	1.90	23.72	2.15	33.03	0.61
AD-1534579.1	16.26	2.41	24.17	3.87	28.24	3.91
AD-1534524.1	10.59	0.91	16.19	2.05	30.88	3.69
AD-1534573.1	31.99	3.60	47.90	1.48	46.22	5.02
AD-1534671.1	43.31	2.22	53.10	5.75	71.83	10.58
AD-1534651.1	70.56	7.43	64.51	4.91	67.31	4.94
AD-85525.11	14.68	2.89	25.29	0.71	29.25	2.79
AD-1534462.1	14.29	0.98	26.48	2.15	37.09	3.81
AD-1534490.1	11.99	0.21	25.71	1.39	39.22	2.55
AD-1534472.1	17.33	0.82	27.11	1.94	46.36	2.38
AD-1534617.1	23.19	1.41	42.01	11.05	54.20	5.66
AD-1631299.1	24.00	2.03	33.21	3.53	44.13	2.46
AD-1534578.1	76.95	9.07	96.34	10.63	86.34	4.96
AD-1534679.1	48.99	2.74	70.38	4.13	83.54	2.17
AD-1631312.1	32.27	2.68	45.09	5.43	57.26	1.81
AD-1534676.1	33.71	2.91	45.46	5.28	56.88	5.20
AD-1631306.1	23.23	0.77	32.22	2.00	42.10	5.65
AD-1534654.1	24.93	2.14	40.86	4.67	51.55	3.84
AD-1534586.1	9.45	1.12	20.64	3.59	27.92	1.46
AD-1631291.1	11.51	1.14	20.31	2.19	28.60	2.23
AD-1534467.1	5.30	0.63	12.78	0.40	20.64	1.07
AD-1534466.1	26.06	0.15	45.65	3.89	60.77	6.19
AD-1534489.1	33.87	3.66	66.95	5.67	87.84	9.58
AD-1534469.1	55.57	4.56	68.07	7.11	89.20	6.12
AD-1534595.1	55.23	2.75	69.66	7.29	70.81	6.57
AD-1534529.1	101.12	7.09	87.33	3.56	102.13	4.30
AD-1534672.1	72.47	5.94	64.77	3.99	83.59	3.66
AD-1534666.1	63.91	6.67	76.11	4.79	86.79	5.64
AD-1534597.1	49.56	3.29	89.70	12.87	96.87	8.59
AD-1631292.1	51.62	7.41	71.35	3.78	86.68	3.55
AD-1534537.1	29.30	2.87	40.25	4.60	62.01	2.78
AD-1534526.1	60.24	4.43	95.64	5.54	110.26	5.46
AD-1534493.1	21.43	1.40	34.04	3.79	56.91	5.89
AD-1534588.1	44.54	5.12	70.83	12.48	67.02	3.48

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534511.1	22.85	1.44	44.49	8.24	61.39	4.77
AD-1534660.1	74.45	5.23	80.92	3.39	94.10	9.13
AD-1534534.1	22.93	0.28	39.25	2.24	61.05	2.64
AD-1534634.1	93.70	4.94	96.32	8.44	111.83	41.06
AD-1534659.1	37.46	5.93	53.76	6.38	64.44	4.47
AD-1534626.1	111.67	28.84	122.51	2.77	92.76	14.98
AD-1534470.1	41.78	7.90	44.45	4.38	62.80	6.14
AD-1534499.1	69.72	3.78	62.83	11.66	82.50	8.57
AD-1534612.1	59.51	6.06	102.44	11.86	109.07	8.44
AD-1534656.1	95.76	9.13	102.62	7.53	110.72	12.77
AD-1534570.1	45.17	2.30	61.43	4.73	66.64	4.62
AD-1534546.1	30.42	2.00	44.54	2.58	63.28	2.03
AD-1631283.1	53.77	7.68	66.21	5.15	81.68	2.33
AD-1534547.1	73.39	6.72	69.85	3.20	83.71	6.41
AD-1534556.1	28.70	2.96	36.69	2.47	44.55	3.73
AD-1534580.1	46.19	5.80	70.43	6.94	79.09	5.28
AD-1534464.1	14.54	0.91	23.22	3.19	36.06	2.74
AD-1534482.1	105.24	4.35	99.89	10.63	113.69	7.62
AD-1534540.1	56.22	2.62	71.10	5.57	95.48	9.44
AD-1534541.1	58.76	1.47	78.48	10.61	87.69	3.78
AD-1534509.1	111.13	12.91	98.75	3.75	120.57	7.80
AD-1534655.1	75.99	10.47	82.03	2.89	94.00	6.37
AD-1534505.1	50.27	4.39	53.63	1.20	67.81	1.73
AD-1534616.1	63.20	4.69	90.91	9.30	87.50	6.44
AD-1534674.1	46.60	5.31	61.18	5.37	77.75	4.04
AD-1534603.1	57.38	3.04	71.07	8.17	76.83	11.06
AD-1534557.1	49.28	10.63	61.58	6.28	61.57	3.16
AD-1534624.1	37.25	4.11	46.55	3.61	58.11	11.13
AD-1534605.1	38.22	2.61	52.90	2.33	57.25	5.66
AD-1534648.1	23.28	0.90	36.75	0.48	43.91	5.12
AD-1534545.1	8.12	0.79	18.64	3.89	39.39	11.63
AD-1631282.1	17.87	1.46	25.82	1.70	33.53	1.13
AD-1534591.1	11.90	2.88	25.25	7.53	27.05	2.76
AD-1534518.1	14.37	0.54	23.94	2.38	39.01	3.34
AD-1534492.1	22.26	1.86	42.88	5.64	64.28	4.43
AD-1534587.1	31.44	6.31	64.88	7.94	67.09	4.43
AD-1534632.1	31.89	5.60	51.77	5.88	61.51	7.49
AD-1534633.1	25.37	1.39	39.39	1.51	50.81	7.80
AD-1631303.1	33.26	2.26	41.62	3.79	57.71	7.24
AD-1534576.1	29.90	4.81	48.65	5.92	56.26	2.84
AD-1631289.1	30.28	4.57	40.69	2.13	51.59	3.03
AD-1631311.1	33.11	1.82	45.93	3.49	57.99	2.57
AD-1534675.1	39.66	4.13	41.94	1.18	50.88	3.23
AD-1534560.1	52.00	5.20	80.40	16.47	64.60	2.84
AD-1534640.1	55.00	5.33	66.23	6.74	77.75	7.61

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534480.1	51.85	0.44	71.51	1.59	90.26	2.48
AD-1631274.1	87.27	7.50	109.28	6.04	108.52	11.21
AD-1534481.1	67.88	8.36	72.24	9.31	98.31	4.10
AD-1631275.1	101.48	8.70	97.91	9.77	107.97	6.90
AD-1534527.1	30.30	1.72	34.31	2.33	59.43	6.24
AD-1534494.1	50.99	8.88	64.25	2.23	77.92	2.68
AD-1534593.1	42.30	4.13	70.83	4.22	84.34	2.97
AD-1534594.1	95.11	15.44	120.35	13.47	96.58	5.41
AD-1534523.1	20.73	2.38	35.28	4.19	52.64	4.37
AD-1534485.1	11.58	2.09	21.66	4.21	30.97	4.40
AD-1534668.1	62.38	4.22	69.11	4.86	78.53	3.23
AD-1631301.1	55.40	2.99	76.96	3.34	93.86	6.65
AD-1534625.1	62.75	4.49	95.80	8.19	89.76	22.82
AD-1534609.1	31.94	6.22	49.22	5.02	81.17	11.88
AD-1631298.1	32.28	0.71	49.57	2.43	67.87	2.30
AD-1534610.1	66.61	10.90	85.27	7.31	83.15	7.74
AD-1534566.1	30.57	1.26	50.48	4.59	61.07	7.08
AD-1534652.1	56.37	4.36	80.74	2.00	101.95	2.26
AD-1534636.1	39.83	3.63	47.37	6.03	66.01	2.11
AD-1631304.1	58.79	5.17	71.58	5.61	89.88	6.49
AD-1534637.1	78.43	10.72	81.46	2.87	98.09	7.91
AD-1631307.1	32.14	4.23	40.25	4.31	53.35	2.05
AD-1534662.1	39.36	1.75	56.21	4.92	65.12	2.72
AD-1534602.1	71.84	2.85	87.49	7.15	96.69	21.16
AD-1631295.1	75.13	9.72	82.13	8.33	98.16	10.17
AD-1534646.1	74.39	8.40	79.91	2.36	88.50	8.43
AD-1534555.1	14.19	2.04	21.78	2.32	24.72	4.67
AD-1534606.1	22.38	2.44	41.68	6.48	48.18	5.88
AD-1534565.1	15.15	1.05	27.81	3.52	36.49	4.85
AD-1534649.1	32.03	2.33	42.71	2.10	55.30	1.28
AD-1534549.1	37.16	3.08	53.88	1.01	56.37	5.70
AD-1631284.1	58.27	4.92	73.77	4.58	89.86	12.68
AD-1534550.1	64.40	2.40	76.19	4.60	67.48	3.22
AD-1534572.1	70.78	8.37	101.43	14.08	82.58	5.38
AD-1534630.1	27.27	1.95	55.63	5.09	66.82	5.46
AD-1534629.1	30.89	2.17	44.31	4.68	53.39	2.22
AD-1631302.1	42.51	4.16	65.86	6.21	84.55	1.75
AD-1534650.1	24.92	3.62	44.74	3.33	49.68	1.53
AD-1631305.1	26.38	1.33	38.75	1.67	48.40	1.43
AD-1534554.1	36.30	3.47	49.15	4.29	54.07	2.93
AD-1631285.1	45.99	4.16	53.92	4.59	71.46	1.05
AD-1534600.1	21.88	1.47	36.18	6.87	40.92	0.57
AD-1631293.1	32.31	3.00	40.47	3.64	60.50	6.29
AD-1534551.1	27.52	1.50	51.79	5.47	50.75	4.67
AD-1534575.1	30.21	1.40	59.31	4.42	66.58	6.66

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534673.1	88.45	7.15	96.79	5.22	97.59	5.35
AD-1534486.1	97.44	8.32	83.05	7.44	91.03	5.44
AD-1534645.1	92.81	4.34	97.75	7.27	94.46	1.87
AD-1534548.1	54.84	3.66	61.01	4.40	72.69	6.52
AD-1534512.1	41.38	4.05	49.95	5.89	72.18	11.00
AD-1534607.1	22.41	3.51	41.77	6.21	50.84	4.56
AD-1631297.1	34.17	0.45	45.39	2.50	65.66	1.88
AD-1534608.1	71.59	2.79	93.51	12.94	88.83	6.00
AD-1534559.1	67.09	14.06	88.92	9.40	67.72	9.79
AD-1631286.1	85.82	7.69	101.72	9.46	104.03	5.31
AD-1534613.1	48.97	1.67	81.30	3.49	89.54	4.96
AD-1534644.1	71.22	12.37	74.84	2.89	82.63	6.72
AD-1534521.1	95.41	5.25	91.24	10.80	87.94	7.81
AD-1534483.1	18.60	0.72	29.29	2.89	48.24	2.38
AD-1631276.1	28.16	3.11	38.22	4.72	46.80	4.46
AD-1534542.1	32.16	4.33	42.12	3.99	76.81	4.74
AD-1534569.1	24.90	1.57	37.58	2.47	37.74	4.56
AD-1534657.1	68.88	9.23	83.63	3.46	86.67	3.28
AD-1534658.1	87.50	6.51	92.64	4.12	103.85	12.76
AD-1534510.1	66.95	12.63	76.26	6.81	109.70	8.76
AD-1534614.1	22.17	1.33	35.69	1.44	39.16	1.94
AD-1534615.1	48.68	5.86	58.64	0.34	56.03	5.90
AD-1534574.1	88.89	3.27	110.60	9.42	90.50	12.65
AD-1534635.1	97.55	11.72	99.79	11.64	79.32	6.76
AD-1534669.1	100.48	6.95	96.71	3.35	105.96	3.90
AD-1534583.1	40.12	6.39	53.52	6.93	74.22	3.31
AD-1534484.1	29.17	1.79	45.44	3.20	74.06	9.81
AD-1631277.1	45.53	6.63	51.88	3.05	64.54	4.20
AD-1534544.1	9.80	0.90	16.59	2.33	26.79	2.32
AD-1534552.1	11.79	0.84	18.76	1.61	21.40	1.21
AD-1534495.1	27.51	0.57	34.36	2.18	49.08	4.64
AD-1534496.1	59.63	5.55	71.87	4.27	85.96	9.83
AD-1534598.1	27.36	0.93	46.88	3.64	60.96	0.21
AD-1534538.1	16.64	0.97	28.96	7.93	36.95	3.66
AD-1534532.1	37.38	7.38	53.22	5.16	79.64	4.48
AD-1534504.1	33.37	2.43	63.89	10.64	78.46	2.75
AD-1534622.1	24.91	1.70	37.38	2.39	46.48	4.99
AD-1534623.1	38.76	3.45	59.33	3.08	63.85	6.29
AD-1534558.1	21.15	5.51	38.81	8.73	35.65	4.91
AD-1534476.1	100.42	3.72	103.32	7.53	112.28	15.47
AD-1534522.1	97.52	9.13	90.32	9.05	103.24	11.99
AD-1534543.1	23.40	3.30	31.89	5.31	62.63	5.62
AD-1631310.1	42.04	3.06	69.53	5.72	77.18	5.51
AD-1534667.1	79.92	5.34	85.29	6.91	89.36	1.42
AD-1534642.1	56.02	4.92	59.10	1.21	64.92	5.08

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534487.1	57.71	2.85	82.81	4.42	101.67	8.02
AD-1631278.1	65.24	7.94	87.91	5.94	92.85	5.90
AD-1534561.1	21.42	0.17	45.34	4.28	52.27	6.74
AD-1631287.1	58.56	3.21	82.99	4.93	100.17	5.77
AD-1534641.1	73.86	8.50	84.35	7.79	86.67	8.05
AD-1534568.1	28.20	3.72	37.09	3.62	36.20	6.63
AD-1534590.1	45.61	4.96	65.21	2.09	64.08	8.72
AD-1534514.1	9.15	0.81	20.79	7.75	21.40	3.78
AD-1631279.1	15.09	1.83	24.40	1.84	29.12	3.14
AD-1534663.1	17.43	2.55	33.15	2.30	38.35	3.26
AD-1631308.1	18.85	2.28	26.52	1.35	32.51	3.61
AD-1534664.1	45.68	5.11	69.10	4.79	72.27	3.37
AD-1534604.1	22.49	2.74	37.81	4.80	43.88	1.36
AD-1631296.1	28.83	3.32	41.33	4.52	56.38	4.98
AD-1534564.1	11.94	0.63	26.23	3.96	30.49	2.09
AD-1631288.1	25.02	0.26	31.89	4.09	44.86	1.53
AD-1534647.1	24.19	3.90	36.83	2.48	47.37	4.84
AD-1534533.1	13.64	1.69	23.78	1.15	34.73	2.94
AD-1631280.1	22.71	3.94	33.78	1.19	40.60	5.98
AD-1534531.1	56.59	2.28	56.07	2.84	77.08	1.45
AD-1534503.1	101.70	5.18	99.76	6.60	104.76	9.28
AD-1534589.1	31.00	2.96	39.68	4.48	33.08	4.27
AD-1534601.1	22.70	4.11	34.40	5.02	32.23	5.36
AD-1631294.1	29.61	4.32	31.06	5.07	41.95	4.70
AD-1534553.1	11.13	0.74	17.12	2.96	21.57	2.04
AD-1534596.1	37.73	2.95	59.86	3.33	70.31	2.58
AD-1534530.1	7.36	0.65	16.61	1.33	23.87	2.58
AD-1534618.1	12.56	1.50	27.00	2.97	29.22	2.76
AD-1534581.1	9.22	1.37	15.93	2.05	24.66	3.16
AD-85541.7	13.70	3.06	23.70	1.93	34.59	4.55
AD-85541.8	21.00	1.70	34.00	4.71	39.06	4.06
AD-85541.9	24.54	2.01	30.34	1.23	36.28	2.05
AD-1534465.1	10.13	1.67	21.35	3.86	27.79	2.47
AD-1534488.1	14.81	2.55	25.05	2.54	39.86	4.32
AD-1534562.1	12.89	0.79	23.06	1.66	30.15	2.35
AD-85537.26	16.08	2.94	30.40	7.02	31.69	0.91
AD-1534563.1	19.54	1.26	28.43	5.85	32.33	3.44
AD-85531.33	9.59	1.14	16.37	1.69	27.63	2.32
AD-85531.34	11.63	1.13	25.34	3.07	25.90	1.56
AD-85531.35	14.41	0.76	21.61	0.97	25.76	5.03
AD-1534473.1	8.34	1.93	19.25	1.09	24.74	3.16
AD-1631273.1	14.39	1.20	24.40	1.84	30.21	2.02
AD-67246.37	17.29	1.51	32.62	4.37	48.98	6.48
AD-67246.39	26.36	0.60	36.55	3.64	41.01	1.88
AD-67246.38	27.36	3.49	36.80	3.65	41.07	4.79

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1 nM	ST DEVP
AD-1534508.1	16.93	2.35	29.16	1.21	37.18	2.49

表 5. PCH 細胞(轉染)中的單一劑量篩查

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1nM	ST DEVP
AD-1534465	18.28	2.59	15.45	4.14	45.78	2.05
AD-1534467	12.07	3.10	6.05	2.30	30.98	4.37
AD-1534471	16.33	1.87	26.32	5.91	33.02	5.65
AD-1534473	21.00	7.01	12.36	2.50	30.99	8.28
AD-1534479	14.34	2.96	17.49	2.45	23.12	4.90
AD-1534485	18.04	3.77	18.63	3.07	39.26	5.50
AD-1534491	15.63	3.10	14.30	0.62	27.53	3.54
AD-1534501	19.41	4.66	23.53	9.64	37.27	5.39
AD-1534502	22.47	5.56	24.66	3.53	42.18	6.77
AD-1534506	15.83	4.58	17.68	2.11	31.63	2.75
AD-1534507	22.94	2.67	18.38	1.38	47.06	11.33
AD-1534514	13.19	2.76	12.59	2.91	34.32	9.35
AD-1534528	9.97	0.51	7.92	1.46	32.13	3.41
AD-1534530	14.33	2.80	11.59	1.97	39.65	8.17
AD-1534544	22.56	1.46	21.96	5.92	40.59	5.23
AD-1534552	20.31	6.06	20.69	2.53	44.00	7.98
AD-1534553	19.61	6.51	14.53	2.92	37.40	3.63
AD-1534555	25.83	5.93	20.51	6.09	41.03	7.75
AD-1534562	23.80	5.39	21.31	3.20	43.33	7.54
AD-1534564	31.11	6.46	16.14	2.26	50.02	2.49
AD-1534567	13.12	2.13	14.23	2.49	32.72	1.77
AD-1534571	11.59	2.16	12.09	2.09	27.03	5.12
AD-1534577	23.92	4.59	25.94	6.11	35.58	6.86
AD-1534579	25.22	1.77	23.60	2.32	51.23	17.17
AD-1534581	16.65	3.40	20.77	1.77	36.46	8.19
AD-1534582	15.20	1.36	11.39	2.15	24.75	5.88
AD-1534585	27.97	5.53	21.50	5.56	41.44	5.78
AD-1534586	21.38	2.25	15.24	3.56	35.81	5.46
AD-1534591	22.21	4.44	11.47	2.83	39.13	14.38
AD-1534611	21.54	5.60	16.92	5.02	26.32	3.67
AD-1534618	12.24	1.49	17.18	1.28	23.62	3.38
AD-1534619	28.85	4.69	23.37	7.36	48.40	6.21
AD-1534621	19.40	3.69	18.97	3.45	34.18	10.17
AD-1534631	21.33	5.30	18.94	6.73	35.68	7.14
AD-1534653	19.84	2.61	10.18	1.16	37.95	7.51
AD-1534665	18.23	3.25	18.42	2.70	23.38	2.41
AD-1534678	18.35	3.52	21.08	2.66	53.65	27.11
AD-1631290	11.66	1.99	11.86	2.45	20.87	4.45
AD-1631291	20.88	6.70	14.34	3.15	32.19	7.09
AD-1631300	15.46	2.62	15.49	2.16	27.01	4.53
AD-1631309	22.31	5.30	16.15	2.09	29.91	6.11

雙鏈體 ID	10 nM	ST DEVP	1 nM	ST DEVP	0.1nM	ST DEVP
AD-85525	20.67	1.13	18.92	2.46	31.87	7.27
AD-85531	18.32	4.78	14.89	2.49	35.11	5.44

表 6. PCH 細胞(自由攝取)中的單一劑量篩查

雙鏈體 ID	250 nM	ST DEVP	100 nM	ST DEVP	10 nM	ST DEVP	1 nM	ST DEVP
AD-1534465	15.49	4.60	11.75	0.66	23.94	3.21	72.39	5.65
AD-1534467	20.24	4.16	13.59	1.17	23.53	9.72	42.28	6.59
AD-1534471	12.66	5.63	7.71	0.77	19.57	2.37	39.78	1.01
AD-1534473	12.65	2.06	14.85	4.29	29.38	5.61	65.25	5.21
AD-1534479	8.80	2.22	7.78	1.45	19.10	3.76	33.74	3.53
AD-1534485	11.34	0.85	17.18	3.88	28.81	7.44	60.10	13.66
AD-1534491	15.43	5.04	9.75	1.79	27.15	7.17	54.43	9.67
AD-1534501	11.68	3.23	16.81	3.56	30.38	9.35	63.22	2.82
AD-1534502	18.95	4.92	19.95	3.55	35.53	5.83	72.11	10.93
AD-1534506	10.74	4.18	13.59	4.49	19.73	5.44	27.82	2.19
AD-1534507	17.45	3.38	17.10	2.07	26.46	4.47	90.06	17.23
AD-1534514	23.71	3.55	30.52	8.84	41.85	11.92	77.66	10.23
AD-1534528	11.03	1.87	11.12	0.94	25.24	6.79	36.92	2.42
AD-1534530	9.76	4.12	12.56	1.14	18.21	3.97	49.47	6.22
AD-1534544	32.95	12.99	21.42	1.92	34.90	6.86	66.88	6.19
AD-1534552	13.18	1.31	18.30	2.30	32.02	2.70	96.85	27.65
AD-1534553	13.62	1.42	14.67	1.69	28.77	4.34	64.03	6.70
AD-1534555	14.18	0.94	20.63	4.16	27.78	0.59	100.52	17.63
AD-1534562	27.51	5.92	15.55	1.93	35.28	7.24	87.15	23.59
AD-1534564	21.25	3.97	22.68	5.10	73.82	13.07	120.81	18.30
AD-1534567	8.54	1.44	8.92	2.37	13.74	2.39	43.37	3.72
AD-1534571	13.45	5.58	10.94	3.01	18.93	1.57	35.38	1.55
AD-1534577	19.69	5.02	15.07	5.24	33.22	8.93	55.75	10.30
AD-1534579	42.43	8.99	41.99	10.32	41.21	3.06	94.19	22.09
AD-1534581	18.28	5.74	17.64	2.32	20.20	4.12	47.15	5.06
AD-1534582	7.21	1.33	8.39	3.39	12.99	1.61	38.17	6.33
AD-1534585	20.27	4.42	19.78	3.81	31.81	4.61	74.16	8.03
AD-1534586	15.25	4.94	11.47	1.52	20.78	2.67	59.00	4.49
AD-1534591	17.17	5.83	16.29	5.06	25.53	2.44	75.89	9.01
AD-1534611	11.01	4.84	9.49	2.77	13.67	4.40	40.93	8.96
AD-1534618	10.02	0.99	12.89	3.09	21.95	2.61	47.87	7.74
AD-1534619	12.75	5.93	10.30	1.34	25.26	4.53	89.16	25.96
AD-1534621	7.79	1.98	8.33	2.89	13.57	1.24	41.04	10.75
AD-1534631	9.40	2.12	7.47	0.88	22.42	2.80	57.95	9.29
AD-1534653	9.46	1.32	18.66	7.80	18.66	7.90	75.10	4.95
AD-1534665	6.48	0.67	7.45	1.98	12.71	4.79	32.19	1.28
AD-1534678	10.38	2.80	16.52	8.97	27.79	8.28	117.53	7.03
AD-1631290	10.18	5.40	9.24	3.50	11.56	2.35	29.98	1.78
AD-1631291	17.98	5.19	19.35	5.09	26.38	3.97	57.54	12.13
AD-1631300	6.32	0.57	7.06	0.52	12.87	0.74	40.73	5.64
AD-1631309	8.01	1.29	6.06	1.13	11.91	2.02	39.44	10.42
AD-85525	28.43	7.95	27.87	9.26	30.92	7.75	84.19	18.23
AD-85531	18.70	9.18	15.06	5.36	28.70	5.06	73.62	7.07

【0617】 實施例 3. 人源化小鼠中的單一劑量篩查

在 1 X PBS 中，上述體外研究中識別的感興趣雙鏈體(AD-67244、AD-1534528、AD-1534582、AD-1534611、AD-1534479、AD-1534506、AD-1631309、AD-1534471、AD-1534555、或 AD-1534678)以 0.2 mg/kg 之單一劑量經尾靜脈注射至表現人類 F12 mRNA 的 36 週大小鼠(人源化 F12 小鼠)(n=4)。

【0618】 AD-67244 之有義和反義股的未修飾核苷酸序列為：

5'-AACUCAAUAAAGUGCUUUGAA-3'(SEQ ID NO：13)和 5'-UUCAAAGCACUUUAUUGAGUUUC-3'(SEQ ID NO：18)；以及 AD-67244 之有義和反義股之經修飾之核苷酸序列為：

5'-asascucaAfuAfAfAfgugcuuugaa-3'(SEQ ID NO：19)和 5'-usUfscaaAfgCfAfcuuuAfuUfgaguususc-3'(SEQ ID NO：20)。

【0619】 血液樣本以 3.8%檸檬酸鈉(使用檸檬酸鹽與血液比例為 1：9)在投予後六周內的每週間收集。在收集後的 45 分鐘內，將血液處理為血漿，且在-80℃下冷凍該血漿樣本直到使用 Gryolab xP(BioAgilytix)定量人類 FXII(hFXII)蛋白質。生物素的捕獲和 Gyrolab 平台使用的 Alexa647 標記檢測單株抗 hFXII 抗體分別為 H4H26029P 和 H4H26064P。在血漿樣本中人類 FXII 蛋白質量以 hFXII (HFXII 1212, Enzyme Research Laboratories)產生的標準曲線校正，並且使用 GraphPad Prism(Version 9.1.0)繪製各受測雙鏈體中的各動物作圖隨時間的變化情況。

【0620】 這些測定結果列於下表和圖 1 中，證明雙鏈體有效且持久地抑制人類第十二因子蛋白表現。

	AD-67244				AD-1534528				AD-1534582			
投予後週數	1	2	3	4	1	2	3	4	1	2	3	4
0	100	100	100	100	100	100	100	100	100	100	100	100
1	65.34895	20.50654	17.29683	27.21547	11.64461	3.554502	10.1083	14.98929	6.960784	7.714844	5.288673	9.657948
2	47.48658	15.84967	14.64581	14.79982	10.69943	8.395396	11.2515	12.95503	7.303922	5.615234	4.936095	7.243461
3	56.56418	21.07843	29.42199	21.1426	9.754253	7.989167	15.94465	15.63169	8.529412	8.886719	5.773468	7.696177
4	51.73255	39.29739	35.54976	28.78992	10.88847	13.30399	17.38869	21.19914	19.11765	19.33594	10.57735	14.78873
5	89.26305	56.0049	51.0213	45.56905	18.14745	56.93974	47.50301	45.61028	39.7549	37.64648	17.40855	29.67807
6	99.51196	67.81046	78.92221	77.10301	26.61626	24.64455	41.06498	44.48608	54.5098	63.08594	37.85809	45.57344

	AD-1534611				AD-1534479				AD-1534506			
投予後週數	1	2	3	4	1	2	3	4	1	2	3	4
0	100	100	100	100	100	100	100	100	100	100	100	100
1	6.979543	7.963937	11.20084	18.43778	6.711409	18.90204	8.954584	12.01629	29.11985	45.15939	37.0155	27.28698
2	7.380666	8.226897	13.58848	17.93824	4.32047	19.00066	5.869752	8.044807	27.99625	52.89256	31.87984	35.18035
3	9.466506	11.26972	15.66011	27.65668	5.411074	14.49704	8.997429	11.0998	42.69663	63.10508	52.13178	62.25823
4	18.21099	21.93839	30.58287	47.00273	7.214765	14.2998	11.39674	13.30618	60.44007	87.7804	78.34302	99.94773
5	33.213	48.98573	74.92978	98.09264	12.41611	33.79356	19.66581	23.0482	107.912	159.3861	106.5891	134.2394
6	52.1059	68.67017	84.33989	97.32062	20.05034	29.91453	21.33676	29.9389	109.7846	174.144	109.5446	114.7412

	AD-1631309				AD-1534471				AD-1534555			
投予後週數	1	2	3	4	1	2	3	4	1	2	3	4
0	100	100	100	100	100	100	100	100	100	100	100	100
1	50.47089	60.5	49.63441	39.58333	12.29314	17.51859	11.31222	17.60363	41.53754	37.8902	32.23109	39.03743
2	63.69863	65.6	71.91398	40.14757	12.19858	25.23234	16.20732	14.99148	45.29201	36.84966	33.86545	34.46767
3	75.21404	91.9	84.34409	69.57465	13.38061	25.78996	16.90662	17.54685	54.64839	41.15536	41.27708	44.57948
4	100.7705	100	82.92473	79.60069	17.68322	36.01301	23.44714	24.41794	77.89035	60.24399	55.72026	70.92854
5	133.476	137.7	91.69892	120.8333	23.92435	23.9777	20.19745	26.17831	138.4982	96.77072	88.93957	124.0156
6	135.8733	141.3	95.87097	108.7674	31.15839	38.61524	26.0798	51.50483	142.789	90.70685	102.8886	126.1546

AD-1534678				
投予後週數	1	2	3	4
0	100	100	100	100
1	44.48113	58.29255	32.05556	45.29486
2	43.06604	61.12017	34.05556	49.49812
3	47.0283	70.85372	49.88889	61.29235
4	67.59434	94.72539	69.38889	87.32748
5	105.8491	151.4954	122.3333	119.5734
6	125.9434	142.9581	139.3889	133.8143

【0621】 均等物

所屬領域具通常知識者不需經過度常規實驗而能夠識別及查明本文所述特定實施例和方法的均等物。該均等物欲涵蓋於以下申請專利範圍中。

非正式序列表

SEQ ID NO: 1

>NM_000505.4 Homo sapiens coagulation factor XII(F12)，mRNA

ACTCCTGGATAGGCAGCTGGACCAACGGACGGATGCCATGAGGGCTCTGCTGCTCCTGGGGTTCCTGCTG
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AGCACACAGTCGTTCTCACTGTCACCGGGGAGCCCTGCCACTTCCCCTTCCAGTACCACCGGCAGCTGTA
CCACAAATGTACCCACAAGGGCCGGCCAGGCCCTCAGCCCTGGTGTGCTACCACCCCCAACTTTGATCAG
GACCAGCGATGGGGATACTGTTTGGAGCCCAAGAAAGTGAAAGACCACTGCAGCAAACACAGCCCCTGCC
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CCACTGCCAGAAAGAGAAGTGCTTTGAGCCTCAGCTTCTCCGGTTTTTCCACAAGAATGAGATATGGTAT
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CCTACATCGCCGCGCTGTACTGGGGCCACAGTTTCTGCGCCGGCAGCCTCATCGCCCCCTGCTGGGTGCT
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CCGTCAGCTACCAGCACGACCTGGCTCTGTTGCGCCTTCAGGAGGATGCGGACGGCAGCTGCGCGCTCCT
GTCGCCTTACGTTCAGCCGGTGTGCCTGCCAAGCGGCGCCGCGCGACCCTCCGAGACCACGCTCTGCCAG
GTGGCCGGCTGGGGCCACCAGTTCGAGGGGGCGGAGGAATATGCCAGCTTCCTGCAGGAGGCGCAGGTAC
CGTTCCTCTCCCTGGAGCGCTGCTCAGCCCCGGACGTGCACGGATCCTCCATCCTCCCCGGCATGCTCTG
CGCAGGGTTCCTCGAGGGCGGCACCGATGCGTGCCAGGGTGATTCCGGAGGCCCGCTGGTGTGTGAGGAC
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CAGGGACTCATCTTTCCCTCCTTGGTGATTCCGCAGTGAGAGAGTGGCTGGGGCATGGAAGGCAAGATTG
TGTCCCATTCCCCCAGTGCGGCCAGCTCCGCGCCAGGATGGCGCAGGAACTCAATAAAGTGCTTTGAAAA
TGCTGA

SEQ ID NO: 2 SEQ ID NO: 1 的反向互補體

TCAGCATTTTCAAAGCACTTTATTGAGTTCCTGCGCCATCCTGGCGCGGAGCTGGCCGCACTGGGGGAATGGGACACAAT
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GGTGTGCTCCCGGATCCAGGCCAGGTAGTAGGCCACATCGGTGTAGACGCCTGGCTTGTTGCGGTCACCACAGCCCGATC
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GGCTGCGCGGGCATGAGTGGGACATGAAGCCTAGGGGACACCGGGGTCGGAGGCGCCGCCTGGGTTGGGGTCTGGCACTG
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SEQ ID NO: 3

>NM_021489.3 小鼠凝血因子XII(哈格曼因子)(F12)，mRNA

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SEQ ID NO: 4 SEQ ID NO: 3 的反向互補體

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SEQ ID NO: 5

>NM_001014006.1 褐家鼠凝血因子 XII(F12)， mRNA

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SEQ ID NO: 6 SEQ ID NO: 5 的反向補體

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SEQ ID NO: 7

>XM_015452310.1 預測：食蟹猴凝血因子 XII(F12)，mRNA

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SEQ ID NO: 8 SEQ ID NO: 7 的反向互補體

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SEQ ID NO: 9

>XM_028849951.1 預測：獼猴凝血因子 XII(F12)，轉錄變體 X2，mRNA

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SEQ ID NO: 10 SEQ ID NO: 9 的反向互補體

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【發明申請專利範圍】

【請求項1】 一種用於在細胞內抑制第 XII 因子(F12)表現的雙股核糖核酸(dsRNA)劑，其中，該 dsRNA 劑包括形成雙股區域的有義股和反義股，其中，該反義股包括編碼 F12 的 mRNA 互補區域，以及其中，該互補區域包括與表 2 至表 3 任一者中的任一反義股核苷酸序列相差不超過 3 個核苷酸的至少 15 個連續核苷酸。

【請求項2】 如請求項 1 所述之 dsRNA 劑，其中，該 dsRNA 劑包括有義股，其包括與表 2 至表 3 任一者中的任一有義股核苷酸序列相差不超過 3 個核苷酸的至少 15 個連續核苷酸，以及反義股，其包括與表 2 至表 3 任一者中的任一反義股核苷酸序列相差不超過 3 個核苷酸的至少 15 個連續核苷酸。

【請求項3】 如請求項 1 所述之 dsRNA 劑，其中，該 dsRNA 劑包括有義股，其包括與表 2 至表 3 任一者中的任一有義股核苷酸序列相差不超過 2 個核苷酸的至少 15 個連續核苷酸，以及反義股，其包括與表 2 至 3 任一者中的任一反義股核苷酸序列相差不超過 2 個核苷酸的至少 15 個連續核苷酸。

【請求項4】 如請求項 1 所述之 dsRNA 劑，其中，該 dsRNA 劑包括有義股，其包括與表 2 至表 3 任一者中的任一有義股核苷酸序列相差不超過 1 個核苷酸的至少 15 個連續核苷酸，以及反義股，其包括與表 2 至表 3 任一者中的任一反義股核苷酸序列相差不超過 1 個核苷酸的至少 15 個連續核苷酸。

【請求項5】 如請求項 1 所述之 dsRNA 劑，其中，該 dsRNA 劑包括有義股，其包括選自由表 2 至表 3 任一者中之任一有義股核苷酸序列組成之組；以及反義股，其包括選自由表 2 至表 3 任一者中之任一反義股核苷酸序列組成之組。

【請求項6】 一種用於在細胞內抑制第 XII 因子(F12)表現的雙股核糖核酸(dsRNA)劑，其中，該 dsRNA 劑包括形成雙股區域的有義股和反義股，其中，該有義股包括與 SEQ ID NO：1 中核苷酸 75 至 97、76 至 98、79 至 101、115 至 137、117 至 139、123 至 145、132 至 154、135 至 157、137 至 159、144 至 166、201 至 223 或 1462 至 1484 之任一核苷酸序列相差不超過 3 個核苷酸之至少 15 個連續核苷酸，以及該反義股包括與 SEQ ID NO：2 中相對應的核苷酸序列相差不超過 3 個核苷酸之至少 15 個連續核苷酸。

【請求項7】 如請求項 1 至 6 任一項所述之 dsRNA 劑，其中，該有義股包括與 SEQ ID NO： 1 中核苷酸 135 至 157、144 至 166 或 201 至 223 之任一核苷酸序列相差不超過 3 個不同的核苷酸之至少 15 個連續核苷酸，以及其中，該反義股包括與 SEQ ID NO：2 中相對應的核苷酸序列不超過 3 個核苷酸之至少 15 個連續核苷酸。

【請求項8】 如請求項 1 至 7 任一項所述之 dsRNA 劑，其中，該反義股包括與選自由 AD-1534528、AD-1534479、AD-1534582、AD-1534611、AD-1534506、AD-1631309、AD-1534471、AD-1534555、AD-1534678、AD-1534585、AD-1534502 以及 AD-1534491 之雙鏈體組成之組的任一反義股核苷酸序列相差不超過 3 個不同的核苷酸之至少 15 個連續核苷酸。

【請求項9】 如請求項 1 至 8 任一項所述之 dsRNA 劑，其中，該反義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一反義股核苷酸序列相差不超過 3 個核苷酸之至少 15 個連續核苷酸。

【請求項10】 如請求項 1 至 9 任一項所述之 dsRNA 劑，其中，該有義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任

一有義股核苷酸序列相差不超過 3 個核苷酸之至少 15 個連續核苷酸，以及該反義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一反義股核苷酸序列相差不超過 3 個核苷酸之至少 15 個連續核苷酸。

【請求項11】 如請求項 1 至 10 任一項所述之 dsRNA 劑，其中，該有義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一有義股核苷酸序列相差不超過 2 個核苷酸之至少 15 個連續核苷酸，以及該反義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一反義股核苷酸序列之群組不超過 2 個不同的核苷酸之至少 15 個連續核苷酸。

【請求項12】 如請求項 1 至 11 任一項所述之 dsRNA 劑，其中，該有義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一有義股核苷酸序列相差不超過 1 個核苷酸之至少 15 個連續核苷酸，以及該反義股包括與選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一反義股核苷酸序列相差不超過 1 個核苷酸之至少 15 個連續核苷酸。

【請求項13】 如請求項 1 至 12 任一項所述之 dsRNA 劑，其中，該有義股包括選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一有義股核苷酸序列，以及該反義股包括選自由 AD-1534528、AD-1534479 以及 AD-1534582 之雙鏈體組成之組的任一反義股核苷酸序列。

【請求項14】 如請求項 1 至 13 中任一項所述之 dsRNA 劑，其中，該 dsRNA 劑包括至少一個經修飾之核苷酸。

【請求項15】 如請求項 1 至 14 中任一項所述之 dsRNA 劑，其中，實質上所有有義股的核苷酸是經修飾之核苷酸；實質上所有反義股的核苷酸是經修飾之

核苷酸；或是實質上所有有義股的核苷酸及實質上所有反義股的核苷酸是經修飾之核苷酸。

【請求項16】如請求項 1 至 15 中任一項所述之 dsRNA 劑，其中，所有有義股的核苷酸是經修飾之核苷酸；所有反義股的核苷酸是經修飾之核苷酸；或是所有有義股的核苷酸及實質上所有反義股的核苷酸是經修飾之核苷酸

【請求項17】如請求項 14 至 16 中任一項所述之 dsRNA 劑，其中，至少一個經修飾之核苷酸是選自由去氧核苷酸、3'-末端去氧胸腺嘧啶核苷酸、2'-O-甲基修飾之核苷酸、2'-氟修飾之核苷酸、2'-去氧-修飾之核苷酸、2'-5'-連接的核糖核苷酸(3'-RNA)、鎖核苷酸、未鎖核苷酸、構形限定核苷酸、約束性乙基核苷酸、無鹼基核苷酸、2'-胺-修飾之核苷酸、2'-O-烯丙基修飾之核苷酸、2'-C 烷基修飾之核苷酸、2'-羥基修飾之核苷酸、2'-甲氧基乙基修飾之核苷酸、2'-O-烷基修飾之核苷酸、N-嗎啉基核苷酸、磷醯胺酯、含非天然鹼基之核苷酸、四氫吡喃修飾之核苷酸、1,5-脫水己糖醇修飾之核苷酸、環己烯基修飾之核苷酸、包含硫代磷酸酯基之核苷酸、包含甲基磷酸酯基之核苷酸、包含 5'-磷酸酯之核苷酸、包含 5'-磷酸酯之核苷酸模擬物、乙烯基膦酸核苷酸、熱去穩定核苷酸、乙二醇修飾之核苷酸(GNA)、乙二醇核酸 S-異構物(S-GNA)、包含磷酸酯之核苷酸和 2-O-(N-甲基乙醯胺)修飾之核苷酸；及其組合所組成之組。

【請求項18】如請求項 14 至 16 中任一項所述之 dsRNA 劑，其中，該至少一個經修飾之核苷酸是選自由 LNA、HNA、CeNA、2′-甲氧乙氧、2′-O-烷基、2′-O-丙烯基、2′-C- 丙烯基、2′-氟、2′-去氧、2'-羥基和乙二醇；及其組合所組成之組。

【請求項19】 如請求項 14 至 16 中任一項所述之 dsRNA 劑，其中，該至少一個經修飾之核苷酸是選自由去氧核苷酸、2'-O-甲基修飾之核苷酸、2'-氟修飾之核苷酸、2'-去氧-修飾之核苷酸、2'-5'-連接的核糖核苷酸(3'-RNA)、乙二醇修飾之核苷酸(GNA)、乙二醇核酸 S-異構物(S-GNA)、包含磷酸酯之核苷酸和包含硫代磷酸酯基之核苷酸；及其組合所組成之組。

【請求項20】 如請求項 14 至 16 中任一項所述之 dsRNA 劑，其中，該至少一個經修飾之核苷酸具有熱去穩定核苷酸修飾之核苷酸修飾。

【請求項21】 如請求項 20 所述之 dsRNA 劑，其中，該熱去穩定核苷酸修飾是選自由無鹼基修飾、在雙股中具有互補的核苷酸之錯配、不穩定的糖修飾、2'-去氧修飾、無環核苷酸、未鎖核酸(UNA)、甘油核酸(GNA)和乙二醇核酸 S-異構物(S-GNA)組成之組。

【請求項22】 如請求項 1 至 21 中任一項所述之 dsRNA 劑，其中，該雙股區域長度為 19 至 30 個核苷酸對。

【請求項23】 如請求項 22 所述之 dsRNA 劑，其中，該雙股區域長度為 19 至 25 個核苷酸對。

【請求項24】 如請求項 22 所述之 dsRNA 劑，其中，該雙股區域長度為 19 至 23 個核苷酸對。

【請求項25】 如請求項 22 所述之 dsRNA 劑，其中，該雙股區域長度為 23 至 27 個核苷酸對。

【請求項26】 如請求項 22 所述之 dsRNA 劑，其中，該雙股區域長度為 21 至 23 個核苷酸對。

【請求項27】 如請求項 1 至 26 中任一項所述之 dsRNA 劑，其中，該各股長度為獨立地不超過 30 個核苷酸。

【請求項28】 如請求項 1 至 27 中任一項所述之 dsRNA 劑，其中，該有義股長度為 21 個核苷酸以及該反義股長度為 23 個核苷酸。

【請求項29】 如請求項 1 至 28 中任一項所述之 dsRNA 劑，其中，該互補區域的長度為至少 17 個核苷酸。

【請求項30】 如請求項 1 至 29 中任一項所述之 dsRNA 劑，其中，該互補區域的長度為 19 和 23 個核苷酸之間。

【請求項31】 如請求項 1 至 30 中任一項所述之 dsRNA 劑，其中，該互補區域的長度為 19 個核苷酸。

【請求項32】 如請求項 1 至 31 中任一項所述之 dsRNA 劑，其中，該至少一股包括至少 1 個核苷酸之 3’突出。

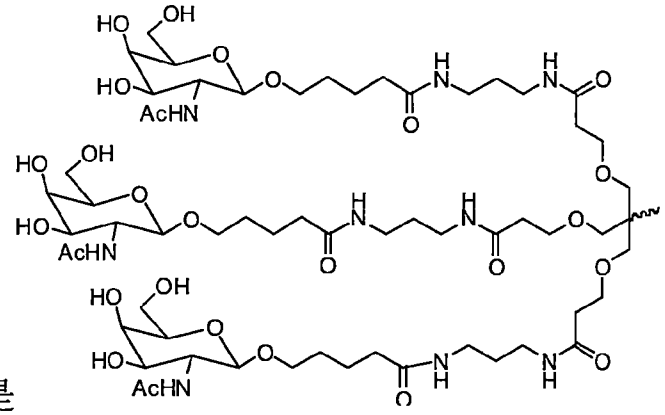
【請求項33】 如請求項 1 至 31 中任一項所述之 dsRNA 劑，其中，該至少一股包括至少 2 個核苷酸之 3’突出。

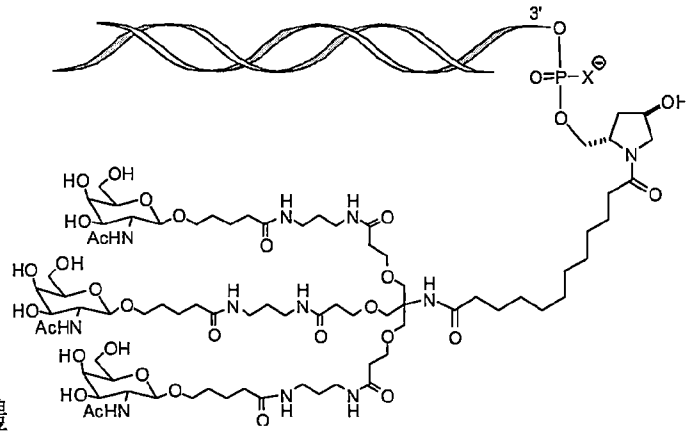
【請求項34】 如請求項 1 至 33 中任一項所述之 dsRNA 劑，進一步包括配體。

【請求項35】 如請求項 34 所述之 dsRNA 劑，其中，該配體接合至 dsRNA 劑的有義股之 3’末端。

【請求項36】 如請求項 34 或 35 所述之 dsRNA 劑，其中，該配體為 N-乙醯半乳胺糖(GalNAc)衍生物。

【請求項37】 如請求項 34 至 36 中任一項所述之 dsRNA 劑，其中，該配體藉由經由單價、雙價或三價分支的連接子連結至一個或多個 GalNAc 衍生物。

【請求項38】 如請求項 34 至 37 中任一項所述之 dsRNA 劑，其中，該配體是  。

【請求項39】 如請求項 38 所述之 dsRNA 劑，其中，該 dsRNA 劑係如下所示接合至配體  ，且其中，X 是 O 或 S。

【請求項40】 如請求項 39 所述之 dsRNA 劑，其中，X 是 O。

【請求項41】 如請求項 1 至 40 中任一項所述之 dsRNA 劑，其中，該 dsRNA 劑進一步包括至少一個硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯。

【請求項42】 如請求項 41 所述之 dsRNA 劑，其中，該硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯位在一股的 3’末端。

【請求項43】 如請求項 42 所述之 dsRNA 劑，其中，該股是反義股。

【請求項44】 如請求項 42 所述之 dsRNA 劑，其中，該股是有義股。

【請求項45】 如請求項 41 所述之 dsRNA 劑，其中，該硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯位在一股的 5’末端。

【請求項46】 如請求項 45 所述之 dsRNA 劑，其中，該股是反義股。

【請求項47】 如請求項 45 所述之 dsRNA 劑，其中，該股是有義股。

【請求項48】 如請求項 41 所述之 dsRNA 劑，其中，該硫代磷酸酯或甲基磷酸酯核苷酸間鍵聯位在一股的 5’和 3’末端。

【請求項49】 如請求項 48 所述之 dsRNA 劑，其中，該股是反義股。

【請求項50】 如請求項 1 至 49 中任一項所述之 dsRNA 劑，其中，該位在雙股的反義股 5’末端的位置 1 是 AU 鹼基對。

【請求項51】 一種包含如請求項 1 至 50 中任一項所述之 dsRNA 劑的細胞。

【請求項52】 一種用於抑制編碼第 XII 因子(F12)之基因表現的醫藥組成物，包括請求項 1 至 50 中任一項所述之 dsRNA 劑及藥學上可接受的載劑。

【請求項53】 如請求項 52 所述之醫藥組成物，其中，該 dsRNA 劑是未緩衝溶液。

【請求項54】 如請求項 53 所述之醫藥組成物，其中，該未緩衝溶液是生理實驗水或水。

【請求項55】 如請求項 52 所述之醫藥組成物，其中，該 dsRNA 劑位於緩衝溶液中。

【請求項56】 如請求項 55 之醫藥組成物，其中，該緩衝溶液包括醋酸鹽、檸檬酸鹽、醇溶蛋白、碳酸鹽或磷酸酯或其任意之組合。

【請求項57】 如請求項 56 所述之醫藥組成物，其中，該緩衝溶液為磷酸酯緩衝生理食鹽水(PBS)。

【請求項58】 一種抑制細胞內第 XII 因子(F12)之基因表現的方法，該方法包括藉由將請求項 1 至 50 中任一項所述之 dsRNA 劑或請求項 52 至 57 中任一項所述之醫藥組成物與細胞接觸，從而抑制細胞內 F12 之基因表現。

【請求項59】 如請求項 58 所述之方法，其中，該細胞在個體體內。

【請求項60】 如請求項 59 所述之方法，其中，該個體為人類。

【請求項61】 如請求項 59 或 60 所述之方法，其中，該個體罹患 F12 相關疾病。

【請求項62】 如請求項 61 所述之方法，其中，該 F12 相關疾病選自由遺傳性血管水腫(HAE)、前激肽釋放酶缺乏症、惡性原發性高血壓、高血壓、末期腎臟疾病以及夫列契因子缺乏症組成之組。

【請求項63】 如請求項 62 所述之方法，其中，該 F12 相關疾病為遺傳性血管水腫(HAE)。

【請求項64】 如請求項 62 所述之方法，其中，該 F12 相關疾病為前激肽釋放酶缺乏症。

【請求項65】 如請求項 62 所述之方法，其中，該 F12 相關疾病為惡性原發性高血壓。

【請求項66】 如請求項 62 所述之方法，其中，該 F12 相關疾病為末期腎臟疾病。

【請求項67】 如請求項 62 所述之方法，其中，該 F12 相關疾病為夫列契因子缺乏症。

【請求項68】 如請求項 58 至 67 中任一項所述之方法，其中，藉由藉由該 dsRNA 劑與細胞接觸抑制至少 50%、60%、70%、80%、90%或 95%的 F12 之基因表現。

【請求項69】 如請求項 58 至 68 中任一項所述之方法，其中，該抑制 F12 表現降低個體血清內至少 50%、60%、70%、80%、90%或 95%的 F12 蛋白含量。

【請求項70】 一種治療罹患能受惠於第 XII(F12)因子表現降低之疾病的個體之方法，包括投予該個體如請求項 1 至 50 中任一項所述之治療有效量的 dsRNA 劑或如請求項 52 至 57 中任一項所述之醫藥組成物，從而治療罹患能受惠於 F12 表現降低之個體。

【請求項71】 一種預防罹患能受惠於第 XII(F12)因子表現降低之疾病的個體中至少一種症狀之方法，包括投予該個體預防有效量的如請求項 1 至 50 中任一項所述之 dsRNA 劑或如請求項 52 至 57 中任一項所述之醫藥組成物，從而預防罹患能受惠於 F12 表現降低之疾病的該個體中的至少一種症狀。

【請求項72】 如請求項 70 或 71 所述之方法，其中，該疾病是 F12 相關疾病。

【請求項73】 如請求項 72 所述之方法，其中，該 F12 相關疾病選自由遺傳性血管水腫(HAE)、前激肽釋放酶缺乏症、惡性原發性高血壓、高血壓、末期腎臟疾病和夫列契因子缺乏症組成之組。

【請求項74】 如請求項 73 所述之方法，其中，該 F12 相關疾病為遺傳性血管水腫(HAE)。

【請求項75】 如請求項 73 所述之方法，其中，該 F12 相關疾病為前激肽釋放酶缺乏症。

【請求項76】 如請求項 73 所述之方法，其中，該 F12 相關疾病為惡性原發性高血壓。

【請求項77】 如請求項 73 所述之方法，其中，該 F12 相關疾病為末期腎臟疾病。

【請求項78】 如請求項 73 所述之方法，其中，該 F12 相關疾病為夫列契因子缺乏症。

【請求項79】 如請求項 70 至 78 中任一項所述之方法，其中，該個體為人類。

【請求項80】 如請求項 70 至 79 中任一項所述之方法，其中，投予該 dsRNA 劑至該個體降低該個體體內之 F12 蛋白堆積。

【請求項81】 如請求項 70 至 80 中任一項所述之方法，其中，投予該 dsRNA 劑至該個體使以下降低：一種或多種升高的舒緩肽、前激肽釋放酶缺乏症、惡性原發性高血壓、高血壓、末期腎臟疾病、夫列契因子缺乏症；四肢、臉部、咽喉、上呼吸道、腹部、軀幹及生殖器水腫；前驅症候(prodrome)；喉水腫、非搔癢性皮疹、噁心、嘔吐或腹部疼痛。

【請求項82】 如請求項 70 至 81 中任一項所述之方法，其中，投予該個體大約 0.01 mg/kg 至大約 50 mg/kg 之該 dsRNA 劑劑量。

【請求項83】 如請求項 70 至 82 中任一項所述之方法，其中，以皮下注射投予該個體該 dsRNA 劑。

【請求項84】 如請求項 70 至 83 中任一項所述之方法，進一步包括判定取自該個體的樣本中之 F12 量。

【請求項85】 如請求項 84 所述之方法，其中，在該個體的樣本中的 F12 量為在血液或血清或肝臟組織樣本的 F12 蛋白質量。

【請求項86】 如請求項 70 至 85 中任一項所述之方法，進一步包括判定該個體中的舒緩肽量、前激肽釋放酶量或血壓。

【請求項87】 如請求項 70 至 86 中任一項所述之方法，進一步包括投予該個體附加治療劑以治療 F12 相關疾病。

【請求項88】 如請求項 87 所述之方法，其中，該附加治療劑選自由雄性激素、達那唑(danazol)或氧雄龍(oxandrolone)、Berinert®、Cinryze™、Rhuconest®、伊卡蘭肽(Ecallantide)、Firazyr®、Kalbitor®或其任意組合組成之組。

【請求項89】 一種包括如請求項 1 至 45 中任一項所述之 dsRNA 劑或請求項 52 至 57 中任一項所述之醫藥組成物的套組。

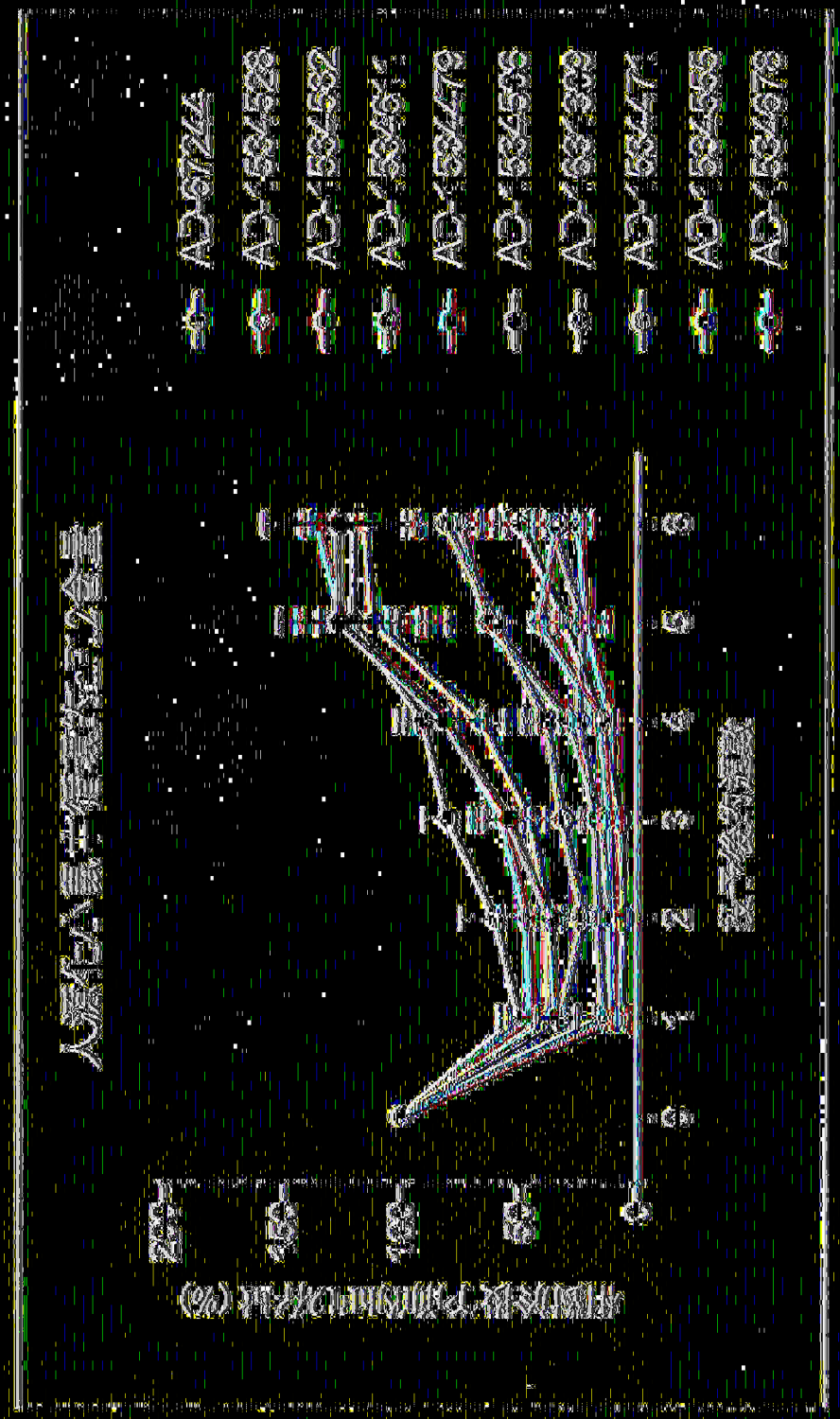
【請求項90】 一種包括如請求項 1 至 45 中任一項所述之 dsRNA 劑或請求項 52 至 57 中任一項所述之醫藥組成物的小瓶。

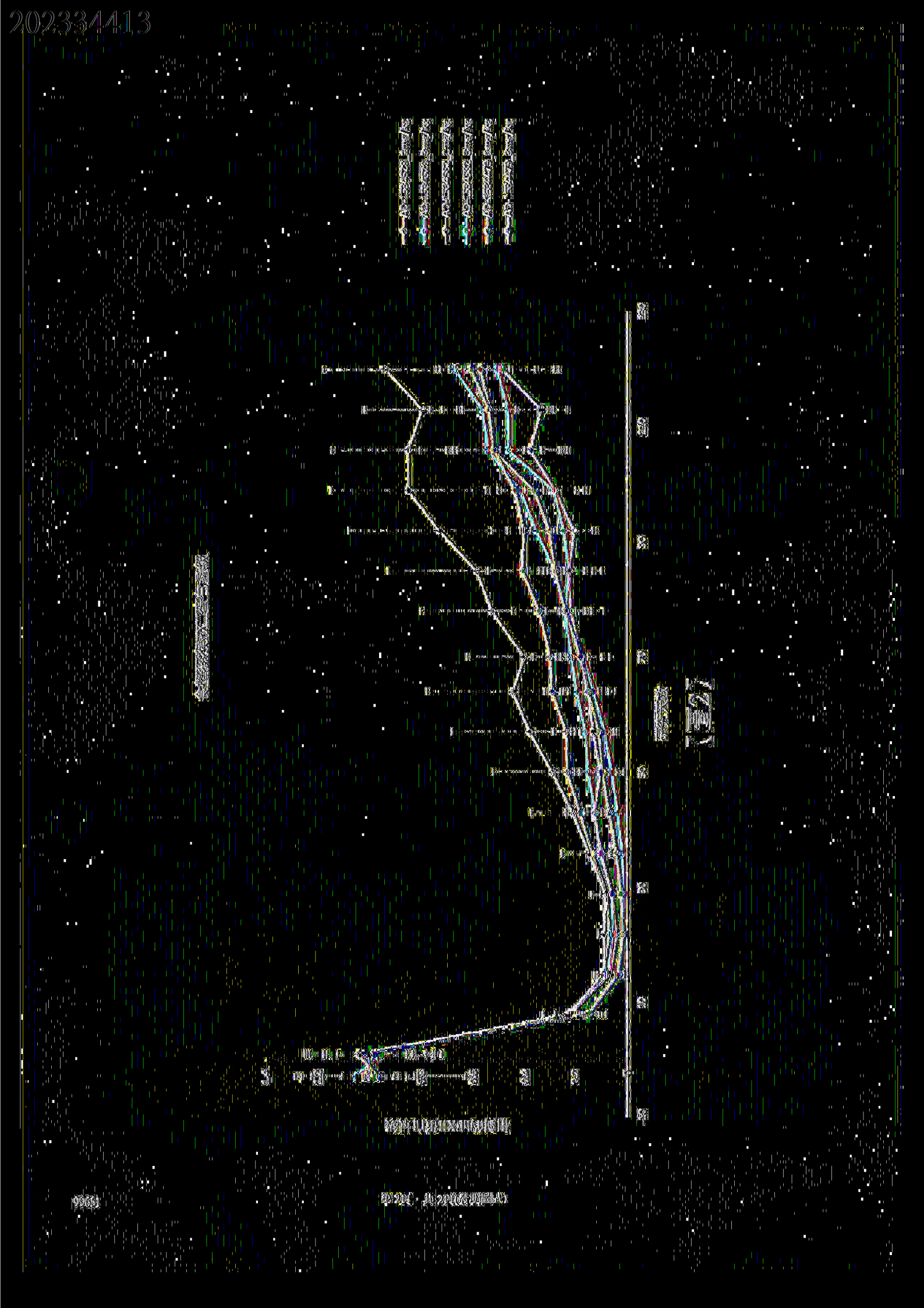
【請求項91】 一種包括如請求項 1 至 45 中任一項所述之 dsRNA 劑或請求項 52 至 57 中任一項所述之醫藥組成物的注射器。

【請求項92】 一種包括如請求項 1 至 50 中任一項所述之 dsRNA 劑的反義股之 RNA 誘導沉默複合物(RISC)。

202334413

【発明の詳細な説明】





【0082】 圖 1 描述每週注射單一 0.2g/kg 劑量的所示雙鏈體後，人源化第十二因子小鼠血清中殘餘的人類第十二因子蛋白百分比。殘餘的人類第十二因子蛋白百分比係相對於給藥前個體中人類第十二因子的量。

圖 2 描述投予 1mg/kg 或 3mg/kg 劑量的所示雙鏈體後，人源化第十二因子食蟹獼猴肝細胞中殘餘的人類第十二因子蛋白量級。殘餘的人類第十二因子蛋白量級係相對於給藥前個體中人類第十二因子的量。

【實施方式】

【0083】 本發明提供 iRNA 組成物，其會影響第十二因子(F12)基因的 RNA 轉錄本之 RNA 誘導沉默複合物(RISC)介導的裂解。基因可位在細胞內，例如個體(如人類)體內的細胞。使用這些 iRNA 可靶向降解哺乳動物中相應基因(F12)的 mRNA。

【0084】 本發明的 iRNA 設計成靶向人類第十二因子(F12)基因，包括在其他哺乳動物物種的 F12 直系同源物中保守的基因部分。不欲受縛理論，據信，上述特性和特定靶位點的組合或子組合或此類 iRNA 中的特定修飾賦予本發明的 iRNA 改進的功效、穩定性、效力、耐久性和安全性。

【0085】 據此，本發明提供治療和預防第十二因子(F12)相關的疾病之方法，例如遺傳性血管水腫(例如第一型遺傳性血管水腫、第二型遺傳性血管水腫、第三型遺傳性血管水腫、或任何因升高的舒緩肽量所致的遺傳性血管水腫)、前激肽釋放酶缺乏、高血壓如惡性原發性高血壓、末期腎臟疾病及夫列契因子缺乏症。該方法使用影響 F12 基因的 RNA 轉錄本之 RNA 誘導沉默複合物(RISC)介導的裂解之 iRNA 組成物。

【0086】 本發明之 iRNA 包含具有長度高達或少於 30 個核苷酸的 RNA 股，例如長度為 19 至 30、19 至 29、19 至 28、19 至 27、19 至 26、19 至 25、19 至 24、19 至 23、19 至 22、19 至 21、19 至 20、20 至 30、20 至 29、20 至 28、20