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(54)名稱

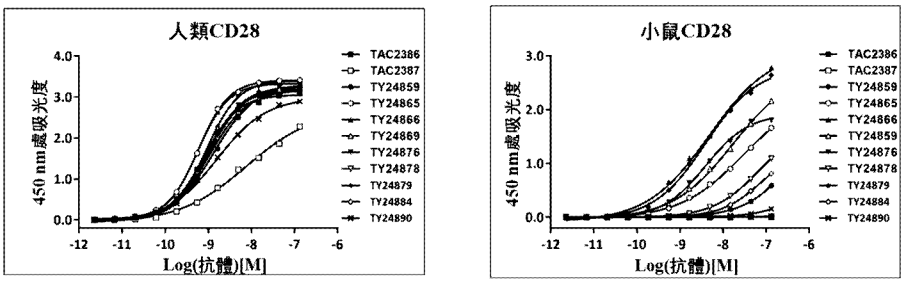
抗ＣＤ２８抗體及其使用方法

(57)摘要

本揭示案為結合至人類 CD28 及其抗原結合片段之抗 CD28 抗體，組成其之組合物，及其在延遲及/或預防腫瘤生長之用途。

The present disclosure relates to anti-CD28 antibodies that bind to human CD28 and antigen-binding fragments thereof, compositions comprising same, and uses thereof in delaying and/or preventing tumor growth.

指定代表圖：



【圖1】

專利案號：112113303
202409082

【發明摘要】

【中文發明名稱】抗CD28抗體及其使用方法

【英文發明名稱】ANTI-CD28 ANTIBODIES AND METHODS OF USE THEREOF

【中文】本揭示案為結合至人類CD28及其抗原結合片段之抗CD28抗體，組成其之組合物，及其在延遲及/或預防腫瘤生長之用途。

【英文】The present disclosure relates to anti-CD28 antibodies that bind to human CD28 and antigen-binding fragments thereof, compositions comprising same, and uses thereof in delaying and/or preventing tumor growth.

【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】抗CD28抗體及其使用方法

【英文發明名稱】ANTI-CD28 ANTIBODIES AND METHODS OF USE THEREOF

【技術領域】

【0001】 [相關申請案之交互參照]

本申請案主張 2022 年 4 月 8 日申請之 PCT/CN2022/085838 之優先權，其全文以引用的方式併入本文中。

【0002】 序列表

本申請案含有以電子方式遞交之 XML 格式序列表且該序列表全文以引用的方式併入本文中。2023 年 4 月 6 日創建並命名為 123687_WO003_SL.xml 之序列表電子拷貝，大小為 488,908 bytes。

【先前技術】

【0003】 CD28 為 T 細胞上之共刺激信號分子，且在其活化、增殖及存活中起關鍵作用。CD28 共刺激信號分子活化具有增強抗腫瘤 T 細胞免疫力之潛力。然而，靶向 CD28 之系統性 T 細胞活化療法已引起有害之細胞介素風暴及多器官衰竭(Suntharalingam 等人，N Engl J Med. (2006) 355(10):1018-28)。因此，有必要改進 CD28 靶向療法，以避免與系統性 CD28 活化相關之嚴重自身免疫不良

事件。

【發明內容】

【0004】 本發明提供靶向腫瘤相關抗原(例如，B7-H3、HER2、TROP2 或 PD-L1)及 CD28 之新穎結合分子，及包含一個或多個此等結合分子之醫藥組合物，以及使用此等結合分子及醫藥組合物治療癌症。相較於當前可獲得之癌症治療(包括抗體治療)，本發明之結合分子預計可提供更優之臨床回應。

【0005】 於一些態樣中，本發明提供抗原結合蛋白(例如抗體或其抗原結合片段)，包含 CD28 結合部分，其中 CD28 結合部分結合人類 CD28 並與食蟹獼猴及小鼠 CD28 交叉反應。於一些實施例中，CD28 結合部分結合至 CD28 抗原表位，其包含人類 CD28 (SEQ ID NO: 1)之 51-122 胺基酸殘基。於特定實施例中，CD28 抗原表位包含 SEQ ID NO: 1 之 51、52、54、55、98-101、110-111、113-114 及 118-122 胺基酸殘基。

【0006】 於一些實施例中，本發明提供抗原結合蛋白(例如抗體或其抗原結合片段)，其包含結合人類 CD28 之 CD28 結合部分，其中 CD28 結合部分包含抗體重鏈可變區(V_H)及抗體輕鏈可變區(V_L)，及其中 V_H 及 V_L 包含所示之重鏈互補決定區(CDRs) 1-3 及輕鏈 CDR1-3，分別為 SEQ ID NOs: 5-10，分別為 SEQ ID NOs: 15、6、16、17-19，分別為 SEQ ID NOs: 24、6、25、26-28，分別為 SEQ ID NOs: 33、6、35-38，分別為 SEQ ID NOs: 43、6、44、45、9 及

46，分別為 SEQ ID NOs: 33、51-53、300 及 10，分別為 SEQ ID NOs: 24、58、59、60、300 及 61，分別為 SEQ ID NOs: 66-69、300 及 70，分別為 SEQ ID NOs: 24、6、75、76、18 及 28，分別為 SEQ ID NOs: 24、58、81、82、27 及 83，分別為 SEQ ID NOs: 88-91、300 及 70，分別為 SEQ ID NOs: 24、96-98、9 及 70，分別為 SEQ ID NOs: 103-106、18 及 83，分別為 SEQ ID NOs: 111、6、112、113、18 及 114，分別為 SEQ ID NOs: 15、6、119、120、9 及 121，分別為 SEQ ID NOs: 126、67、127、128、18 及 129，分別為 SEQ ID NOs: 134、6、135、136、27 及 83，分別為 SEQ ID NOs: 43、58、141、142、300 及 143，分別為 SEQ ID NOs: 148、6、149、150、300 及 83，分別為 SEQ ID NOs: 15、155、16、156、27 及 70，或 SEQ ID NOs: 161、6、162、163、300 及 164。

【0007】 於一些實施例中，CD28 結合部分包含所示之 VH 及 VL，分別為 SEQ ID NOs: 11 及 12，分別為 SEQ ID NOs: 20 及 21，分別為 SEQ ID NOs: 29 及 30，分別為 SEQ ID NOs: 39 及 40，分別為 SEQ ID NOs: 47 及 48，分別為 SEQ ID NOs: 54 及 55，分別為 SEQ ID NOs: 62 及 63，分別為 SEQ ID NOs: 71 及 72，分別為 SEQ ID NOs: 77 及 78，分別為 SEQ ID NOs: 84 及 85，分別為 SEQ ID NOs: 92 及 93，分別為 SEQ ID NOs: 99 及 100，分別為 SEQ ID NOs: 107 及 108，分別為 SEQ ID NOs: 115 及 116，分別為 SEQ ID NOs: 122 及 123，分別為 SEQ ID NOs: 130 及 131，分別為 SEQ ID NOs: 137 及 138，分別為 SEQ ID NOs: 144 及 145，分別為 SEQ ID

NOs: 151 及 152，分別為 SEQ ID NOs: 157 及 158，分別為 SEQ ID NOs: 165 及 166，分別為 SEQ ID NOs: 362 及 363，分別為 SEQ ID NOs: 364 及 365，分別為 SEQ ID NOs: 366 及 367，分別為 SEQ ID NOs: 368 及 369，分別為 SEQ ID NOs: 370 及 371，分別為 SEQ ID NOs: 372 及 373，分別為 SEQ ID NOs: 374 及 375，分別為 SEQ ID NOs: 376 及 12，分別為 SEQ ID NOs: 377 及 378，分別為 SEQ ID NOs: 379 及 380，分別為 SEQ ID NOs: 381 及 12，分別為 SEQ ID NOs: 382 及 12，分別為 SEQ ID NOs: 383 及 12，分別為 SEQ ID NOs: 384 及 12，分別為 SEQ ID NOs: 385 及 12，或分別為 SEQ ID NOs: 386 及 12。

【0008】 於一些實施例中，CD28 結合部分包含所示之重鏈(HC)及輕鏈(LC)，分別為 SEQ ID NOs: 13 及 14，分別為 SEQ ID NOs: 22 及 23，分別為 SEQ ID NOs: 31 及 32，分別為 SEQ ID NOs: 41 及 42，分別為 SEQ ID NOs: 49 及 50，分別為 SEQ ID NOs: 56 及 57，分別為 SEQ ID NOs: 64 及 65，分別為 SEQ ID NOs: 73 及 74，分別為 SEQ ID NOs: 79 及 80，分別為 SEQ ID NOs: 86 及 87，分別為 SEQ ID NOs: 94 及 95，分別為 SEQ ID NOs: 101 及 102，分別為 SEQ ID NOs: 109 及 110，分別為 SEQ ID NOs: 117 及 118，分別為 SEQ ID NOs: 124 及 125，分別為 SEQ ID NOs: 141 及 142，分別為 SEQ ID NOs: 132 及 133，分別為 SEQ ID NOs: 139 及 140，分別為 SEQ ID NOs: 146 及 147，分別為 SEQ ID NOs: 153 及 154，分別為 SEQ ID NOs: 159 及 160，或分別為 SEQ ID NOs: 167 及

168。

【0009】 於一些態樣中，本發明亦提供醫藥組合物，其包含抗原結合蛋白(例如本文所述抗體或其抗原結合片段)及醫藥上可接受之載劑；編碼抗原結合蛋白之核酸分子；包含核酸分子之表現載體；及包含載體之宿主細胞，其中宿主細胞可為原核細胞或真核細胞(例如哺乳動物細胞)。

【0010】 於一些態樣中，本發明亦提供一種產生抗原結合蛋白(例如本文所述抗體或其抗原結合片段)之方法，包括在允許抗原結合蛋白表現之條件下培養宿主細胞，及自培養基中單離抗原結合蛋白。

【0011】 於一些態樣中，本發明亦提供一種治療有需要患者癌症之方法，包括向患者投與治療有效量之抗原結合蛋白，例如本文所述抗體或抗原結合片段。於一些實施例中，方法另包含向患者投與另外抗癌症治療劑。於進一步之實施例中，另外抗癌症治療劑為靶向 CD3 及腫瘤相關抗原(TAA)之雙特異性抗體(bsAb)。於特定實施例中，TAA 為 B7-H3、HER2 或 TROP2。於一些實施例中，另外抗癌症治療劑為免疫檢查點抑制劑，視情況可為抗 PD-1、抗 CTLA4 或抗 PD-L1 抗體。

【0012】 本文亦提供本發明之抗原結合蛋白(例如抗體或其抗原結合片段)，或醫藥組合物，其用於治療有需要患者癌症；使用本發明之抗原結合蛋白(例如抗體或其抗原結合片段)製造用於治療有需要患者癌症之藥物；及製品(例如，套組)，其含有本發明抗原結

合蛋白(例如抗體或其片段)之一個或多個給藥單元。

【0013】 本發明之其它特徵、目的及優點於以下詳細描述中顯而易見。然而，應瞭解，詳細描述在指示本發明之實施例及方面之同時，僅以說明而非限制之方式給出。根據詳細描述，本領域技術人員將清楚地理解本發明範圍內之多種變化及修改。

【圖式簡單說明】

【0014】

圖 1 為一組顯示抗 CD28 抗體與重組 CD28 (人類及小鼠)蛋白結合親和力檢定之圖表。

圖 2 為顯示抗 CD28 IgG 與人類 CD3+ T 細胞結合之圖表。

圖 3 為一組顯示 IgG 與人類 CD28-CD80 (圖表右上及左上)及 CTLA-4 CD80 對(圖表底部)配體阻斷檢定之圖表。

圖 4A 為顯示 T 細胞增殖檢定之圖表，其顯示相較於 CD28 超促效劑 TAC2386 (TGN1412)，IgG 不會誘導系統性 T 細胞活化。

圖 4B 為顯示藉由 CellTiter-Glo® (CTG)讀出 T 細胞活化共刺激檢定-增殖之圖表。

圖 4C 為顯示 T 細胞活化共刺激檢定 IFN-γ 釋放之圖表。

圖 5A 為顯示使用 OKT3 抗 CD28 測試抗體藉由 CTG 讀出 T 細胞活化及增殖檢定-增殖之圖表。

圖 5B 為顯示 T 細胞活化及增殖檢定 IL-2 釋放之圖表。

圖 6 為顯示抗 HER2 抗體及雙特異性抗體(BsAb)與 SK-OV3

細胞結合之圖表。亦顯示包含標繪值之表。

圖 7 為顯示 Jurkat-NFκB 螢光素酶報告基因檢定量測抗 HER2×CD28 BsAb (TY27566 或 TY27807)與固定濃度之抗 HER2×CD3 BsAb (TY25238)組合，及抗 HER2×CD3 BsAb 與固定濃度之抗 HER2×CD28 BsAb 組合時在最大信號值及 EC_{50} 值對 NFκB 信號傳導刺激效應之圖表。亦顯示包含標繪值之表。

圖 8 為顯示具有相同或不同 HER2 抗原表位之 CD28 BsAb 及 CD3 BsAb 之 MCF-7 細胞之殺傷檢定圖表以及包含標繪值之表。

圖 9 為一組顯示於腫瘤細胞株上量測抗 TROP2 雙特異性抗體濃度-依賴性結合活性之檢定圖表。

圖 10A 為顯示 Jurkat-NFκB 螢光素酶報告子檢定於 H292 細胞量測雙特異性抗體在最大信號值及 EC_{50} 值對 NFκB 信號傳導刺激效應之圖表。亦顯示包含標繪值之表。

圖 10B 為顯示 Jurkat-NFκB 螢光素酶報告子檢定於 H292 細胞量測雙特異性抗體在最大信號值及 EC_{50} 值對 NFκB 信號傳導刺激效應之圖表。亦顯示包含標繪值之表。

圖 11 為一組顯示 PD-L1 及 B7H3 於 MDA-MB-231 細胞上共表現之流式細胞術圖。

圖 12 為顯示 B7H3 IgG 及 B7H3xCD28 bsAb 與 MDA-MB-231 細胞之結合檢定圖表。亦顯示包含標繪值之表。

圖 13A 及 13B 為顯示單向 MLR 檢定測試 B7H3xCD28 bsAb 與抗 PD-1 或抗 PD-L1 組合阻斷 mAb 對原代人類 T 細胞活化活性

之圖表，藉由 IL-2 分泌(圖 13A)及 IFN-γ 分泌(圖 13B)量測。

圖 14 為顯示體外檢定之圖表，其於 MCF-7 腫瘤細胞株上量測基於抗 CD3 或基於抗 CD28 靶向 HER2 之 bsAb 或其組合腫瘤殺傷活性。亦顯示包含標繪值之表。

圖 15 為顯示體外檢定之圖表，其於 EMT6-HER2 腫瘤細胞株上量測基於抗 CD3 或基於抗 CD28 靶向 HER2 之 bsAb 或其組合之腫瘤殺傷活性。亦顯示包含標繪值之表。

圖 16 為一組顯示量測 IL-6 及 IFN-γ 全身細胞介素釋放(分別為圖表左上及右上)，及量測 CD3+ T 細胞與總 CD45+ T 細胞之百分比(圖表底部)之檢定圖表，WT 小鼠用 TCE 處理。

圖 17 顯示 SK-OV3+ PBMC 異種移植腫瘤模型中 HER2xCD3 bsAb 及 B7H3xCD28 bsAb 單獨或組合體內功效研究及圖表。

圖 18 為一組顯示 EMT6-HER2 模型中 B7H3xCD28 或 HER2xCD28 bsAb 體內功效研究圖表。

圖 19 為顯示抗 CD28 可活化抗體結合至重組人類 CD28 遮蔽效率之 ELISA 量測值圖表。

圖 20 為顯示抗 CD28 可活化抗體結合至重組人類 CD28 遮蔽效率之 ELISA 量測值圖表。

圖 21 (SEQ ID NOs: 359-361)為顯示人類與小鼠 CD28 不同結合殘基之表。

圖 22A 為顯示抗 PD-L1×CD28 bsAb TY29815 及 TY30413 與人類 CD28 結合之圖表。

圖 22B 為顯示抗 PD-L1×CD28 bsAb TY29815、TY30406、TY30410 及 TY30413 與小鼠 CD28 結合之圖表。

圖 23A 為顯示抗 HER2×CD28 bsAb TY27566、TY28652、TY28653、TY28654、TY28655、TY28656、TY28657、TY28658、TY28659、TY28660 及 TY28661 與人類 CD28 結合之圖表。

圖 23B 為顯示抗 HER2×CD28 bsAb TY29109、TY29306、TY29307、TY29308、TY29309、TY29310、TY29311、TY29312、TY29313、TY29314 及 TY29315 與人類 CD28 結合之圖表。

圖 24A 為顯示抗 B7H3×CD28 bsAb TY29021、TY30120、TY30121 及 TY30123 與人類 CD28 結合之圖表。

圖 24B 為顯示抗 B7H3×CD28 bsAb TY29021、TY30120、TY30121 及 TY30123 與小鼠 CD28 結合之圖表。

圖 25 為顯示小鼠 T 細胞結合檢定量測抗 B7H3×CD28 bsAb TY29021、TY30120、TY30121 及 TY30123 在最大信號值、EC_{50} 值及 AUC 值對 T 細胞結合效應之圖表。亦顯示包含標繪值之表。

【實施方式】

【0015】 I. 定義

在詳細描述本發明之前，應瞭解，本發明揭示內容不限於特定組合物或生物系統，當然其可變化。亦應瞭解，本文中所用之術語係僅出於描述特定實施例之目的，且不旨在限制。

【0016】 如本文中所用，術語「約」系指為此技術領域之熟習者容

易知曉之各自值的通常誤差範圍。本文中提及「約」值或參數包括(且描述)指向該值或參數本身之實施例。

【0017】 應瞭解，本文中所述之本發明之態樣及實施例包括「包含」、「由...組成」及「基本上由...組成」態樣及實施例。

【0018】 如本文中所用，術語「及/或」，諸如「A 及/或 B」之短語意欲包括 A 及 B 二者；A 或 B；A (單獨)；及 B (單獨)。同樣，如本文中所用，術語「及/或」，諸如「A、B 及/或 C」之短語意欲涵蓋下列實施例各者：A、B 及 C；A、B 或 C；A 或 C；A 或 B；B 或 C；A 及 C；A 及 B；B 及 C；A (單獨)；B (單獨)；及 C (單獨)。

【0019】 術語「抗體」涵蓋多種抗體結構，包括但不限於單株抗體(包括全長單株抗體)、多株抗體、經遮蔽抗體(例如，可活化或不可活化抗體)、多特異性抗體(例如，雙特異性抗體，包括經遮蔽雙特異性抗體)及抗體片段(例如，單鏈可變片段或 scFv)，只要其展示所需生物活性(例如，以期望之特異性及親和力結合靶抗原之能力)。

【0020】 術語「抗體」涵蓋多種抗體結構，包括但不限於單株抗體(包括全長單株抗體)、多株抗體、經遮蔽抗體(例如，可活化或不可活化抗體)及多特異性抗體(例如，雙特異性抗體)。術語「抗體」亦包括，但不限於，嵌合抗體、人源化抗體及全長人類抗體。

【0021】 於一些實施例中，術語「抗體」指具有由兩條相同重(H)鏈及兩條相同輕(L)鏈組成之基本四多肽鏈結構之抗原結合蛋白(即免疫球蛋白)。每一 L 鏈藉由一個共價二硫鍵連接至 H 鏈，而兩條 H 鏈藉由一個或多個二硫鍵彼此連接，此端視 H 鏈同型而定。

每一重鏈在 N 末端具有可變區(亦稱為可變結構域) (在本文中縮寫為 V_H)，其後為恆定區。重鏈恆定區包含三個結構域 C_H1、C_H2 及 C_H3。每一輕鏈在 N 末端具有可變區(亦稱為可變結構域) (在本文中縮寫為 V_L)，其後為處於該輕鏈另一端之恆定區。輕鏈恆定區包含一個結構域 C_L。V_L 與 V_H 對準且 C_L 與重鏈之第一恆定結構域(C_H1)對準。V_H 及 V_L 之配對一起形成單一抗原結合位點。

【0022】 V_H 及 V_L 可進一步細分成互補決定區(CDR)及框架區(FW)。CDR 具有最高之序列變異性及/或參與抗原識別。CDR 及 FR 按照 FR1、CDR1、FR2、CDR2、FR3、CDR3 及 FR4 之順序散佈。CDR 亦包含「特異性決定殘基」或「SDR」，其為接觸抗原之殘基。SDR 含於 CDR 中稱為縮短-CDR (abbreviated-CDR)或 a-CDR 之區內。例示性 a-CDR (a-LCDR1、a-LCDR2、a-LCDR3、a-HCDR1、a-HCDR2 及 a-HCDR3)各自出現在輕鏈之 31-34、50-55、89-96，以及重鏈之 31-35、50-58 及 95-102 胺基酸殘基處。參見 Almagro 及 Fransson，Front. Biosci. (2008) 13:1619-33。除非另有指示，否則可變區殘基在本文中係根據 Kabat 等人，J Biol Chem. (1977) 252:6609-16；Kabat 等人，U.S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991)來編號。

【0023】 下表 1 提供根據此項技術中已知之各種演算法之例示性 CDR 定義。

表 1. CDR 定義

	Kabat[1]	Chothia[2]	MacCallum[3]	IMGT[4]	AHo[5]
HCDR1	31-35	26-32	30-35	27-38	25-40
HCDR2	50-65	53-55	47-58	56-65	58-77
HCDR3	95-102	96-101	93-101	105-117	109-137
LCDR1	24-34	26-32	30-36	27-38	25-40
LCDR2	50-56	50-52	46-55	56-65	58-77
LCDR3	89-97	91-96	89-96	105-117	109-137

[1] 殘基編號遵循 Kabat 等人，參見上文之命名法。

[2] 殘基編號遵循 Chothia 等人，J. Mol. Biol. (1987) 196:901-917；Al-Lazikani B.等人，J. Mol. Biol., (1997) 273: 927-948 之命名法。

[3] 殘基編號遵循 MacCallum 等人，J. Mol. Biol. (1996) 262:732-745；Abhinandan 及 Martin，Mol. Immunol., (2008) 45: 3832-3839 之命名法。

[4] 殘基編號遵循 Lefranc M.P.等人，Dev. Comp. Immunol., (2003) 27:55-77；及 Honegger 及 Plückthun，J. Mol. Biol., (2001) 309:657-670 之命名法。

[5] 殘基編號遵循 Honegger 及 Plückthun，J. Mol. Biol., (2001) 309:657-670 之命名法。

【0024】 來自任一脊椎動物物種之 L 鏈基於其恆定結構域之胺基酸序列可分配至兩種明顯不同之類型(稱為 κ 及 λ)中之一者。端視抗體重鏈(C_H)之恆定結構域之胺基酸序列，該等抗體可分配至不同之類別或同型。存在五類抗體：IgA、IgD、IgE、IgG 及 IgM，其重鏈分別命名為 α (alpha)、δ (delta)、ε (epsilon)、γ (gamma)及 μ (mu)。藉由 γ 重鏈 Y1-Y4，IgG 類抗體可進一步分成四個子類，分別為 IgG_1、IgG_2、IgG_3 及 IgG_4。

【0025】 術語「抗原結合片段」或「抗原結合部分」於本文中可交換使用，係指保留結合至抗體抗原之能力之抗體之部分。抗體之「抗原結合片段」之實例包括但不限於，(i) Fab 片段，由 V_L、V_H、C_L 及 C_{H1} 結構域組成之單價片段，藉由木瓜蛋白酶消化獲得；(ii) $F(ab')_2$ 片段，包含由鉸鏈區之二硫橋連接之兩個 Fab 片段之二價片段，藉由胃蛋白酶消化獲得；(iii)由抗體之單臂之 V_L 及 V_H 結構域組成之 Fv 片段，(iv)單鏈 Fv (scFv)片段，其包含彼此融合之抗體之 V_H 及 V_L 結構域；及(v)單鏈 Fab (scFab)片段，其包含含有 V_L、V_H、C_L 及 C_{H1} 結構域之單鏈多肽。

【0026】 術語「經遮蔽抗體」係指包含遮蔽性肽之抗體(包括多特異性抗體)或其抗原結合片段幹預、阻止、降低(能力)、防止、抑制結合至其靶，或與抗體之抗原結合域競爭結合至其靶。經遮蔽抗體可以藉由將遮蔽性肽連接至抗體之抗原結合域來產生。於一些實施例中，經遮蔽抗體或其抗原結合片段在未活化狀態下(例如，被遮蔽性肽抑制或遮蔽)對靶表現出之第一結合親和力，並在活化狀態下(例如，未被遮蔽性肽抑制或遮蔽(例如，從抗體上裂解之遮蔽性肽))對靶表現出第二結合親和力，其中第二結合親和力大於第一結合親和力。經遮蔽抗體可以藉由將包含可活化部分(例如，連接單元之可裂解位點或「LU」)之遮蔽性肽連接至抗體之抗原結合域來產生。於一些實施例中，經遮蔽抗體，或其經遮蔽抗原結合片段為多特異性抗體，其包含對 T 細胞表面分子(例如，CD28、CD3)及腫瘤細胞表面抗原(例如，HER2、B7H3、TROP2 等)具有特異性之

結合域。於一些實施例中，經遮蔽抗體為二價，且在兩個結合域中的一個上具有單價遮蔽。於一些實施例中，經遮蔽抗體為二價，且在兩個結合域中的每一個上都具有遮蔽。例如，對於單價經遮蔽抗體，抗體的一個結合域被融合或綴合遮蔽性肽遮蔽。對於雙特異性抗體，一個或兩個結合域可被具有特異性但不同的遮蔽性肽遮蔽。同時靶向癌細胞及 T 細胞之未經活化雙特異性抗體可具有遮蔽兩個結合域之結合位點，以抑制(或最小化)與抗原表現癌症細胞及 T 細胞之結合。然而，在活化狀態下，遮蔽性部分被裂解以允許抗體於腫瘤微環境(TME)中與腫瘤抗原及 T 細胞表面分子(例如，CD28)結合。於此種情況下，經活化雙特異性抗體選擇性地接合 T 細胞，殺死靶向腫瘤抗原表現之癌症細胞。

【0027】 術語「遮蔽性肽」係指抑制抗原結合域與其靶抗原結合之肽，並且通常自 N 端至 C 端包含遮蔽性單元(MU)及連接單元(LU)。遮蔽性肽之 C 端通常與抗原結合域之 V_H 或 V_L 之 N 端相連。於一些實施例中，遮蔽性肽或其一部分幹擾或抑制抗原結合域與其靶之結合如此有效使得抗原結合域與其靶結合極低及/或在檢測限以下(例如，於 ELISA 或流式細胞術檢定中不可檢測結合)。本文所述經遮蔽抗體或多肽可包含一個或多個連接體，例如，在 LU 內、置於 MU 及 LU 之間、LU 及 V_H 或 V_L 之間或 V_H 及 Fc 鉸鏈區之間。

【0028】 遮蔽性肽之 LU 可以包含至少一個可裂解位點。該裂解位點一般包含可裂解之胺基酸序列，例如，用作能形成可還原二硫鍵

之酵素及/或半胱胺酸-半胱胺酸對之受質。因而，當術語「裂解」、「可裂解」、「經裂解」及類似者結合裂解位點使用時，該等術語涵蓋例如藉由蛋白酶之酶促裂解，以及半胱胺酸-半胱胺酸對之間之二硫鍵經由可由暴露於還原劑產生之二硫鍵之還原之中斷。該裂解位點之胺基酸序列可與該 MU 重迭或包含於該 MU 內。經遮蔽抗體或經遮蔽多肽可以包含配置為介導抗體或多肽活化之裂解位點。例如，當可活化抗體之裂解位點為完整時(例如，未藉由對應酵素裂解，及/或含有未經還原之半胱胺酸-半胱胺酸二硫鍵)，該遮蔽性肽或其一部分可能幹預或抑制抗原結合域結合至其靶。於一些實施例中，遮蔽性肽之 LU 不包含可裂解位點。

【0029】 術語「遮蔽效率」係指遮蔽性肽抑制抗原結合域與靶抗原結合之效率。遮蔽效率可以藉由包含抗原結合域之經遮蔽抗體或經遮蔽多肽之結合親和力相對於包含抗原結合域(例如，從抗體上裂解之遮蔽性肽)之未經遮蔽抗體或未經遮蔽多肽之結合親和力之差異或比率來量測。例如，遮蔽效率可以藉由經遮蔽抗體之未活化狀態(例如，抑制、遮蔽及/或未裂解)與靶抗原結合之 EC_{50} 或 K_D 除以未經遮蔽抗體之活化狀態(例如，未抑制、未遮蔽及/或裂解)與靶抗原結合之 EC_{50} 或 K_D，或親本抗體(例如，未連接遮蔽性肽)與靶抗原結合之 EC_{50} 或 K_D 來量測。可以藉由 ELISA 量測或 Jurkat NFAT 報告子分析測定 EC_{50} 值，例如美國專利公開第 US20210207126 A1 號所述。K_D 值可以藉由，例如，使用表面電漿子共振量測。

【0030】 術語「抗原表位」係指抗體(或其抗原結合片段)結合之抗原之部分。抗原表位可自鄰接胺基酸或藉由蛋白質之三級折疊並置之非鄰接胺基酸二者形成。自鄰接胺基酸形成之抗原表位通常保留暴露於變性劑，然而藉由三級折疊形成之抗原表位通常在用變性溶劑處理中喪失。抗原表位可包含各種數目之胺基酸於獨特空間構形中。測定抗原表位之空間構形之方法包括例如 x-射線結晶學、2 維核磁共振、氘及氫交換與質譜法組合、或定點誘變、或與抗原及其複合結構與其結合抗體及其變異體之電腦建模組合使用之所有方法(參見例如，Epitope Mapping Protocols in Methods in Molecular Biology，第 66 卷，G. E. Morris 編輯.(1996))。一旦確定抗原之所需抗原表位，可例如使用本文中所述技術產生該抗原表位之抗體。抗體之產生及表徵亦可闡明關於所需抗原表位之資訊。自此資訊，然後可競爭篩選結合至相同抗原表位之抗體。達成此之一種方法為進行交叉競爭研究以找到彼此競爭結合之抗體，即，競爭結合至抗原之抗體。基於其交叉競爭「分類」抗體之高通量方法描述於 PCT 公開案第 WO 03/48731 號中。

【0031】 術語「生殖系」係指抗體基因及基因片段之核苷酸序列，因為其經由生殖細胞自親本傳給後代。生殖系序列不同於成熟 B 細胞中編碼抗體之核苷酸序列，該等核苷酸序列已在 B 細胞成熟過程期間藉由重組及高突變事件改變。

【0032】 術語「糖基化位點」係指藉由真核細胞識別為糖殘基之連接位置之胺基酸殘基。在碳水化合物(諸如寡糖)之情況下連接之胺

基酸通常為天冬醯胺(N-鍵聯)、絲胺酸(O-鍵聯)及蘇胺酸(O-鍵聯)殘基。特定連接位點通常藉由胺基酸序列(本文中稱作「糖基化位點序列」)信號傳導。用於 N-連接之糖基化之糖基化位點序列為：-Asn-X-Ser-或-Asn-X-Thr-，其中 X 可為習知胺基酸中之任一者，除了脯胺酸。術語「N-連接」及「O-連接」係指用作糖分子與胺基酸殘基之間之連接位點之化學基團。N-連接之糖通過胺基連接；O-連接之糖通過羥基連接。術語「多醣佔用」係指連接至糖基化位元元點之碳水化合物部分之存在(即，佔用多醣位點)。在多肽上存在至少兩個潛在糖基化位點之情況下，無(0-多醣位點佔用)、一個(1-多醣位點佔用)或兩個(2-多醣位點佔用)位元點可由碳水化合物部分佔用。

【0033】 術語「宿主細胞」係指可工程改造以產生所關注蛋白質、蛋白質片段或肽之細胞系統。宿主細胞包括(不限於)經培養細胞，例如，源自嚙齒動物(大鼠、小鼠、豚鼠或倉鼠)之哺乳動物培養細胞，諸如 CHO、BHK、NSO、SP2/0、YB2/0；人類細胞(例如，HEK293F 細胞、HEK293T 細胞)；或人類組織或雜交瘤細胞、酵母細胞、昆蟲細胞(例如，S2 細胞)、細菌細胞(例如，大腸桿菌(E. coli)細胞)及包含於轉殖基因動物或經培養組織內之細胞。術語不僅涵蓋特定受試者細胞，而且涵蓋此細胞之後代。因為某些修飾可發生於後代中，由於突變或環境影響，此後代可不與親本細胞相同，但是仍包含於術語「宿主細胞」之範圍內。

【0034】 「人類抗體」為具有胺基酸序列者，該胺基酸序列對應於

藉由人類或人類細胞產生或源自利用人類抗體庫或其他人類抗體編碼序列之非人類來源之抗體之胺基酸序列。人類抗體之此定義明確排除包含非人類抗原結合殘基之人源化抗體。

【0035】 術語「人源化抗體」係指含有源自人類抗體序列之胺基酸殘基之嵌合抗體。人源化抗體可含有來自非人類動物或合成抗體之 CDR 中之一些或所有，而抗體之框架區及恒定區含有源自人類抗體序列之胺基酸殘基。

【0036】 術語「例示性抗體」係指本文所述抗體中之任一者。此等抗體可為任何類別(例如，IgA、IgD、IgE、IgG 及 IgM)。因此，以上所識別之各抗體涵蓋具有 V_L 及 V_H 區之相同胺基酸序列之所有五類抗體。此外，IgG 類別之抗體可為任何子類別(例如，IgG_1、IgG_2、IgG_3 及 IgG_4)。因此，以上於 IgG 子類別中所識別之各抗體涵蓋具有 V_L 及 V_H 區之相同胺基酸序列之所有四種子類別之抗體。五類以及四種 IgG 子類別之人類抗體之重鏈恆定區的胺基酸序列係此項技術中已知。

【0037】 「經單離」抗體或結合分子為已自其自然環境之組分分離者。於一些實施例中，抗體經純化為大於 95%或 99%純度，如藉由例如電泳(例如，SDS-PAGE、等電聚焦(IEF)、毛細管電泳)或層析(例如，離子交換或逆相 HPLC)所測定。評論用於評估抗體純度之方法，參見例如，Flatman 等人，J. Chromatogr. B 848:79-87 (2007)。術語「k_a」係指特定抗體-抗原相互作用之締合速率常數，其中術語「k_d」係指特定抗體-抗原相互作用之解離速率常數。

【0038】 術語「K_D」係指特定抗體-抗原相互作用之平衡解離常數。其自 k_d 與 k_a 之比率(即，k_d/k_a)獲得且表示為莫耳濃度(M)。K_D 係用作抗體結合至其結合搭檔之親和力之量度。K_D 越小，抗體結合越緊密，或抗體與抗原之間之親和力越高。例如，具有奈莫耳(nM)解離常數之抗體較具有微莫耳(μM)解離常數之抗體與特定抗原結合更緊密。可使用此項技術中良好建立之方法測定抗體之 K_D 值。一種測定抗體之 K_D 之方法為藉由使用 ELISA。例如，使用 ELISA 之檢定程式。

【0039】 術語「哺乳動物」係指哺乳綱類別之任何動物物種。哺乳動物之實例包括：人類；實驗動物，諸如大鼠、小鼠、倉鼠、兔、非人類靈長類動物及豚鼠；家養動物，諸如貓、狗、牛、綿羊、山羊、馬及豬；及被俘野生動物，諸如獅子、老虎、大象及類似者。

【0040】 關於哺乳動物之某種疾病病狀之術語「預防(prevent/preventing)」係指預防或延遲該疾病之發作，或預防其臨床或亞臨床症狀之表現。

【0041】 如本文所用之兩條多肽序列之間之「序列一致性」指示序列之間一致之胺基酸之百分比。多肽之胺基酸序列一致性可按慣例使用已知電腦程式(例如 Bestfit、FASTA 或 BLAST)來確定(參見例如 Pearson, Methods Enzymol. (1990) 183:63-98；Pearson, Methods Mol. Biol. (2000) 132:185-219；Altschul 等人，J. Mol. Biol. (1990) 215:403-410；Altschul 等人，Nucleic Acids Res. (1997) 25:3389-3402)。在使用 Bestfit 或任何其他序列比對程式來確定例

如特定序列是否與參考胺基酸序列 95%一致時，設定參數，使得在參考胺基酸序列之全長內計算一致性百分比且允許參考序列中高達 5%之胺基酸殘基總數之同源性差異。此上文所提及確定多肽之間之一致性百分比之方法適用於本文所揭示之所有蛋白質、其片段或變異體。

【0042】 如本文中所用，術語「結合」、「結合至」、「特異性結合」、「特異性結合至」或「對...特異性」係指可量測且可再生相互作用，諸如靶與抗體之間之結合，其確定在分子(包括生物分子)異質群之存在下靶之存在。例如，結合至或特異性結合至靶(其可為抗原表位)之抗體為結合此靶之抗體，結合此靶較其結合至其他靶具有更高親和力、抗體親抗原性(avidity)、更便利及/或更多持續時間。於一實施例中，抗體與不相關靶之結合程度係小於抗體與該靶之結合之約 10%，如例如藉由放射性免疫檢定(RIA)所量測。於某些實施例中，特異性結合至靶之抗體具有≤ 1 μM、≤ 100 nM、≤ 10 nM、≤ 1 nM 或≤ 0.1 nM 之解離常數(Kd)。於某些實施例中，抗體特異性結合至蛋白質上之抗原表位，該蛋白質在來自不同物種之蛋白質中係保守的。於另一實施例中，特異性結合可包括但不需要專一結合。例如，若本文所述經遮蔽抗 CD28 抗體結合至人類 CD28 之 EC_{50} 低於如在活體外分析中其結合不同抗原之 EC_{50} 的 10%，則稱本文所述經遮蔽抗 CD28 抗體選擇性結合至人類 CD28。

【0043】 提及哺乳動物之某一疾病病狀之術語「治療(treat)」、「治療(treating)」或「治療(treatment)」係指在患有疾病病狀之哺乳動

物中產生期望或有益效應。期望或有益效應可包括減小疾病之一種或多種症狀(即腫瘤生長及/或轉移，或由免疫細胞之數量及/或活性介導之其他效應及諸如此類)之頻率或嚴重程度、或阻止或抑制疾病、疾患或病症之進一步發展。在治療哺乳動物之癌症之上下文中，期望或有益效應可包括抑制癌細胞之進一步生長或擴散、癌細胞之死亡、抑制癌症之再發、減輕與癌症相關之疼痛或改良哺乳動物之存活。效應可為主觀或客觀的。舉例而言，若哺乳動物係人類，則人類可注意到改良之精力或活力或減輕的疼痛作為對療法之改良或反應之主觀症狀。替代地，臨床醫師可基於體檢、實驗室參數、腫瘤標記物或放射學發現注意到腫瘤大小或腫瘤負荷之減小。臨床醫師可觀察到治療反應之一些實驗室跡象包括測試之正規化，例如白血球計數、紅血球計數、血小板計數、成熟紅血球沉降速率及各個酶水準。另外，臨床醫師可觀察到可偵測腫瘤標記物之減少。替代地，可使用其他測試來評估客觀改良，例如音波圖、核磁共振測試及正電子發射測試。

【0044】 術語「載體」係指能轉運外來核酸分子之核酸分子。外來核酸分子藉由重組技術(諸如連接或重組)連接至載體核酸分子。此允許外來核酸分子被操作，選擇，進一步操作或於宿主細胞或生物體中表現。載體可為質粒、噬菌體、轉位子、黏粒、染色體、病毒或病毒子。可在引入宿主細胞後將一種載體融合至宿主細胞之基因組，及從而連同宿主基因組(例如，非表小體哺乳動物載體)複製。另一種載體能於引入其之宿主細胞中自發複製(例如，具有複製之

細菌來源之細菌載體及表小體哺乳動物載體)。能指導以可操作方式連接其之可表現外來核酸之表現之另一特定類型之載體通常被稱作「表現載體」。表現載體一般具有驅動可表現外來核酸之表現之控制序列。稱作「轉錄載體」之更簡單載體僅能被轉錄但是不能被轉譯：其可於靶細胞中複製但是不可表現。術語「載體」涵蓋所有類型之載體，不管其功能。能指導以可操作方式連接其之可表現核酸之表現之載體通常被稱作「表現載體」。「載體」之其他實例可包括顯示載體(例如，指導編碼多肽在病毒或細胞(諸如細菌細胞、酵母細胞、昆蟲細胞及/或哺乳動物細胞)表面上表現及顯示之載體)。

【0045】 如本文中所用，「受試者」、「患者」或「個體」可係指人類或非人類動物。「非人類動物」可係指不歸類為人類之任何動物，諸如家養動物、農場動物或動物園動物、競技動物、寵物(諸如狗、馬、貓、牛等)，以及用於研究之動物。研究動物可係指(不限於)線蟲類動物、節肢動物、脊椎動物、哺乳動物、青蛙、囓齒動物(例如，小鼠或大鼠)、魚(例如，斑馬魚或尖鼻鮭)、禽(例如，雞)、狗、貓及非人類靈長類動物(例如，恆河猴、食蟹獼猴、黑猩猩等)。於一些實施例中，受試者、患者或個體為人類。

【0046】 「有效量」係指至少在必要之劑量及時間段內達到一種或多種預期或訓示效果之有效量，包括治療或預防效果。可在一次或多次施用中提供有效量。為了本公開之目的，有效量之抗體、藥物、化合物或藥物組合物是足以直接或間接完成預防性或治療性

治療之量。如在臨床上下文中所理解，藥物、化合物或醫藥組合物之有效量可或可不結合另一藥物、化合物或醫藥組合物達成(例如，作為單藥療法或組合療法施用之有效量)。因此，若結合一種或多種其他劑可達成或達成期望結果，則「有效量」可視為在投與一種或多種治療劑之上下文中，且單一劑可視為以有效量給予。

【0047】 本文引用之所有參考文獻，包括專利申請及出版物，其全文以引用的方式併入本文中。

II. 抗體

【0048】 本發明之某些態樣係關於單特異性抗體(例如，傳統、非遮蔽單特異性抗體)、多特異性抗體(例如，非遮蔽多特異性抗體)、經遮蔽抗體(例如，可活化單特異性或多特異性抗體)、其抗原結合片段、或此等抗體之衍生物。

A. Fc 區及 C_H3 結構域

【0049】 於一些實施例中，本文所述抗體(例如，多特異性抗體)包含一個或多個抗體恒定區，例如人類重鏈恒定區及/或人類輕鏈恒定區。於一些實施例中，人類重鏈恆定區屬於選自 IgA、IgG 及 IgD 之同型。在一些實施例中，人類輕鏈恆定區屬於選自 κ 及 λ 之同型。在一些實施例中，抗體包含人類 IgG 恆定區。在一些實施例中，抗體包含人類 IgG_4 重鏈恆定區。在一些實施例中，抗體包含人類 IgG_1 重鏈恆定區。在一些此類實施例中，抗體在人類 IgG_4 恆定區中包含 S228P 突變。

【0050】 效應功能是否為所需的可視意欲用於抗體之特定處理方

法而定。在一些實施例中，當效應功能為所需的時，選擇包含人類 IgG_1 重鏈恆定區或人類 IgG_3 重鏈恆定區之抗體。在一些實施例中，當效應功能不為所需的時，選擇包含人類 IgG_4 或 IgG_2 重鏈恆定區之抗體。在一些實施例中，抗體包含含有降低效應功能之一個或多個突變之人類 IgG_1 重鏈恆定區。在一些實施例中，抗體包含含有 N297A 取代之 IgG_1 重鏈恆定區。

【0051】 本文所描述之多特異性抗體(包括可活化多特異性抗體)可包含 C_H3 結構域，其具有一個或多個經工程改造之二硫鍵、一個或多個經工程改造(例如重排或逆轉)之鹽橋或其組合。除非另外說明，否則本文之所有胺基酸殘基編號係基於 Eu 編號，且胺基酸取代係在野生型(或天然存在之) C_H3 結構域序列中之對應胺基酸位置相對於野生型(或天然存在之)序列。應瞭解，本文所描述之突變或取代適用於所有 IgG 亞類及同種異型。IgG 同種異型已描述於例如 Jefferis 及 Lefranc mAbs (2009) 1:4, 1-7 中，該參考文獻以全文引用的方式併入本文中。在一些實施例中，本文所描述之胺基酸突變或取代係相對於 IgG1 之野生型 C_H3 結構域序列，諸如 IgG1 同種異型 G1m、1 (a)、2 (x)、3 (f)或 17 (z)。在一些實施例中，本文所描述之胺基酸突變或取代係相對於 IgG_4 之野生型 C_H3 結構域序列。舉例而言，相對於一種人類 IgG_1 同種異型之野生型 C_H3 結構域(Uniprot 編號 P01857)的 D356K 取代等效於相對於第二人類 IgG_1 同種異型之野生型 C_H3 結構域或人類 IgG_4 之野生型 C_H3 結構域的 E356K 取代。示例性 C_H3 結構域突變顯示於表 2 及 3 中。在

一些實施例中，本文所描述之胺基酸突變或取代係相對於野生型Fc區序列，例如 IgG_1 Fc 區或 IgG_4 Fc 區。具有突變之 C_H3 序列描述於 WO 2021/148006 中，其全文以引用的方式併入本文中。於下表及說明書之其它部分，C_H3 突變注釋中撇號表示第二 C_H3 結構域中之殘基。例如，於 N390C-S400'C 中，S400C 突變位於第二 C_H3 結構域。

表 2. Fc 突變

設計	突變(第一 C_H3 結構域-第二 C_H3 結構域)
二硫鍵	N390C-S400'C
	S400C-N390'C
	K392C-V397'C
	V397C-K392'C
	K392C-S400'C
	S400C-K392'C
電荷設計	E357K:T411K-L351'D:K370'D
	E357K:S364K-L351'D:K370'D
	D356K:E357K:S364K-L351'D:K370'D:K439'D
電荷+二硫鍵設計	E357K:S364K:N390C-L351'D:K370'D:S400'C
	E357K:S364K:S400C-L351'D:K370'D:N390'C
	D356K:E357K:S364K:N390C-L351'D:K370'D:S400'C:K439'D
	D356K:E357K:S364K:S400C-L351'D:K370'D:N390'C:K439'D

表 3. Fc 突變 ID

突變 ID	突變(第一 C_H3 結構域-第二 C_H3 結構域)
TRF01	T366S, L368A, Y407V, Y349C-T366'W, S354'C
TRF02	T350V, L351Y, F405A, Y407V-T350'V, T366'L, K392'L, T394'W
TRF03	K196Q, S228P, F296Y, E356K, R409K, H435R, L445P-K196'Q, S228'P, F296'Y, R409'K, K439'E, L445'P
TYM01	T366S, L368A, Y407V, N390C-T366'W, S400'C
TYM02	T366S, L368A, Y407V, S400C-T366'W, N390'C
TYM03	Y349C, L368V, Y407V-S354'C, T366'W
TYM04	L368V, Y407V-T366'W
TYM05	L368V, Y407V, N390C-T366'W, S400'C
TYM06	L368V, Y407V, S400C-T366'W, N390'C

突變 ID	突變(第一 C_H3 結構域-第二 C_H3 結構域)
TYM07	E357K:T411K-L351’D:K370’D
TYM08	E357K:S364K-L351’D:K370’D
TYM09	D or E356K:E357K:S364K-L351’D:K370’D:K439’D
TYM10	E357K:S364K:N390C-L351’D:K370’D:S400’C
TYM11	E357K:S364K:S400C-L351’D:K370’D:N390’C
TYM12	D or E356K:E357K:S364K:N390C-L351’D:K370’D:S400’C:K439’D
TYM13	D or E356K:E357K:S364K:S400C-L351’D:K370’D:N390’C:K439’D

【0052】 在一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含第一 C_H3 結構域中之 C390 與第二 C_H3 結構域中之 C400 之間、第一 C_H3 結構域中之 C392 與第二 C_H3 結構域中之 C397 之間或第一 C_H3 結構域中之 C392 與第二 C_H3 結構域中之 C400 之間的經工程改造之二硫鍵。在一些實施例中，與野生型 C_H3 結構域相比，多特異性抗體(例如可活化多特異性抗體)包含例如在第一 C_H3 結構域中之位置 357 及 411 與第二 C_H3 結構域中之位置 351 及 370 間(例如 E357K:T411K-L351'D:K370'D)，或在第一 C_H3 結構域中之位置 357 及 364 與第二 C_H3 結構域中之位置 351 及 370 間(例如 E357K:S364K-L351'D:K370'D)的重排鹽橋網狀結構。在一些實施例中，與野生型 C_H3 結構域相比，多特異性抗體包含在第一 C_H3 結構域中之位置 356 與第二 C_H3 結構域中之位置 439 之間的逆轉鹽橋(例如 D356-K439')。具有 C_H3 突變之抗體可具有高產率、優良穩定性(例如，在高溫下或凍融迴圈下抗聚集和沉澱)及有效活性。

【0053】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含 C_H3 結構域，其具有一個或多個經工程改造之殘基，該等經工程改造之殘基如本文所述促進異二聚體形成。本文亦涵蓋包

含多個由包含第一經工程改造之 C_H3 結構域之第一多肽及包含第二經工程改造之 C_H3 結構域之第二多肽形成的異二聚體之異多聚體。

【0054】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：i)第一 C_H3 結構域包含在位置 390 之半胱胺酸(C)殘基且第二 C_H3 結構域包含在位置 400 之半胱胺酸殘基，或第一 C_H3 結構域包含在位置 400 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 390 之半胱胺酸殘基；或 ii)第一 C_H3 結構域包含在位置 392 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 397 之半胱胺酸殘基，或第一 C_H3 結構域包含在位置 397 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 392 之半胱胺酸殘基；或 iii)第一 C_H3 結構域包含在位置 392 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 400 之半胱胺酸殘基，或第一 C_H3 結構域包含在位置 400 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 392 之半胱胺酸殘基；且其中胺基酸殘基編號係基於 EU 編號。

【0055】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中：i)第一 C_H3 結構域另包含在位置 357 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 351 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 351 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 357 之帶正電荷之殘基；或 ii)第一 C_H3 結構域另包含在位置 411 之帶正電荷之殘基且第二 C_H3 結構域另

包含在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 370 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 411 之帶正電荷之殘基；或 iii)第一 C_H3 結構域另包含在位置 364 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 370 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 364 之帶正電荷之殘基；或 i)與 ii)之組合，或 i)與 iii)之組合，其中胺基酸殘基編號係基於 EU 編號。

【0056】 在一些實施例中，第一 C_H3 結構域另包含在位置 356 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 439 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 439 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 356 之帶正電荷之殘基；且其中胺基酸殘基編號係基於 EU 編號。

【0057】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中：i)第一 C_H3 結構域包含在位置 390 之半胱胺酸(C)殘基且第二 C_H3 結構域包含在位置 400 之半胱胺酸殘基，或第一 C_H3 結構域包含在位置 400 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 390 之半胱胺酸殘基；或 ii)第一 C_H3 結構域包含在位置 392 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 397 之半胱胺酸殘基，或第一 C_H3 結構域包含在位置 397 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 392 之半胱胺酸殘基；或 iii)第一 C_H3 結構域包含在位置 392 之半胱胺酸殘基且第二 C_H3 結構域包含在

位置 400 之半胱胺酸殘基，或第一 C_H3 結構域包含在位置 400 之半胱胺酸殘基且第二 C_H3 結構域包含在位置 392 之半胱胺酸殘基；且其中：a)第一 C_H3 結構域另包含在位置 357 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 351 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 351 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 357 之帶正電荷之殘基；或 b)第一 C_H3 結構域另包含在位置 411 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 370 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 411 之帶正電荷之殘基；或 c)第一 C_H3 結構域另包含在位置 364 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 370 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 364 之帶正電荷之殘基；或 a)與 b)之組合，或 a)與 c)之組合；其中胺基酸殘基編號係基於 EU 編號。

【0058】 在一些實施例中，第一 C_H3 結構域另包含在位置 356 之帶正電荷之殘基且第二 C_H3 結構域另包含在位置 439 之帶負電荷之殘基，或第一 C_H3 結構域另包含在位置 439 之帶負電荷之殘基且第二 C_H3 結構域另包含在位置 356 之帶正電荷之殘基，且其中胺基酸殘基編號係基於 EU 編號。

【0059】 C_H3 結構域可衍生自任何天然存在之免疫球蛋白分子。在一些實施例中，C_H3 結構域衍生自 IgG_1 分子、IgG_2 分子、IgG_3 分子或 IgG_4 分子。在一些實施例中，C_H3 結構域為人類 C_H3 結構域。

在一些實施例中，C_H3 結構域衍生自人類 IgG_1 分子。

【0060】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中：i)第一 C_H3 結構域包含 N390C 取代且第二 C_H3 結構域包含 S400C 取代，或第一 C_H3 結構域包含 S400C 取代且第二 C_H3 結構域包含 N390C 取代；或 ii)第一 C_H3 結構域包含 K392C 取代且第二 C_H3 結構域包含 V397C 取代，或第一 C_H3 結構域包含 V397C 取代且第二 C_H3 結構域包含 K392C 取代；或 iii)第一 C_H3 結構域包含 K392C 取代且第二 C_H3 結構域包含 S400C 取代，或第一 C_H3 結構域包含 S400C 取代且第二 C_H3 結構域包含 K392C 取代。

【0061】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中：i)第一 C_H3 結構域包含 E357K 及 T411K 取代且第二 C_H3 結構域包含 L351D 及 K370D 取代，或第一 C_H3 結構域包含 L351D 及 K370D 取代且第二 C_H3 結構域包含 E357K 及 T411K 取代；或 ii)第一 C_H3 結構域包含 E357K 及 S364K 取代且第二 C_H3 結構域包含 L351D 及 K370D 取代，或第一 C_H3 結構域包含 L351D 及 K370D 取代且第二 C_H3 結構域包含 E357K 及 S364K 取代；或 iii)第一 C_H3 結構域包含 D356K、E357K 及 S364K 取代且第二 C_H3 結構域包含 L351D、K370D 及 K439D 取代，或第一 C_H3 結構域包含 L351D、K370D 及 K439D 取代且第二 C_H3 結構域

包含 D356K、E357K 及 S364K 取代。

【0062】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中第一 C_H3 結構域包含 E357K、S364K 及 N390C 取代且第二 C_H3 結構域包含 L351D、K370D 及 S400C 取代，或第一 C_H3 結構域包含 L351D、K370D 及 S400C 取代且第二 C_H3 結構域包含 E357K、S364K 及 N390C 取代。

【0063】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中第一 C_H3 結構域包含 E357K、S364K 及 S400C 取代且第二 C_H3 結構域包含 L351D、K370D 及 N390C 取代，或第一 C_H3 結構域包含 L351D、K370D 及 N390C 取代且第二 C_H3 結構域包含 E357K、S364K 及 S400C 取代。

【0064】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中第一 C_H3 結構域包含 D356K、E357K、S364K 及 S400C 取代且第二 C_H3 結構域包含 L351D、K370D、N390C 及 K439D 取代，或第一 C_H3 結構域包含 L351D、K370D、N390C 及 K439D 取代且第二 C_H3 結構域包含 D356K、E357K、S364K 及 S400C 取代。

【0065】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含：含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域

之第二多肽，其中第一 C_H3 結構域包含 D356K、E357K、S364K 及 N390C 取代且第二 C_H3 結構域包含 L351D、K370D、K439D 及 S400C 取代，或第一 C_H3 結構域包含 L351D、K370D、K439D 及 S400C 取代且第二 C_H3 結構域包含 D356K、E357K、S364K 及 N390C 取代。

【0066】 於一些實施例中，多特異性抗體(例如可活化多特異性抗體)包含含有經工程改造之 C_H3 結構域之 IgG Fc 區。Fc 區可衍生自任何適合之 Fc 亞類，包括但不限於 IgG_1、IgG_2、IgG_3 及 IgG_4 亞類。

B. 半胱胺酸突變

【0067】 在一些實施例中，本文所描述之多特異性抗體(例如本文所描述之可活化多特異性抗體)包含含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中第一 C_H3 結構域包含第一經工程改造之半胱胺酸殘基且第二 C_H3 結構域包含第二經工程改造之半胱胺酸殘基，其中第一經工程改造之半胱胺酸殘基及第二半胱胺酸殘基形成二硫鍵。

【0068】 在一些實施例中，第一 C_H3 結構域包含在位置 390 之 C 且第二 C_H3 結構域包含在位置 400 之 C，或第一 C_H3 結構域包含在位置 400 之 C 且第二 C_H3 結構域包含在位置 390 之 C。在一些實施例中，第一 C_H3 結構域包含 N390C 取代且第二 C_H3 結構域包含 S400C 取代，或第一 C_H3 結構域包含 S400C 取代且第二 C_H3 結構域包含 N390C 取代。

【0069】 在一些實施例中，第一 C_H3 結構域包含在位置 392 之 C 且第二 C_H3 結構域包含在位置 397 之 C，或第一 C_H3 結構域包含在位置 397 之 C 且第二 C_H3 結構域包含在位置 392 之 C。在一些實施例中，第一 C_H3 結構域包含 K392C 取代且第二 C_H3 結構域包含 V397C 取代，或第一 C_H3 結構域包含 V397C 取代且第二 C_H3 結構域包含 K392C 取代。

【0070】 在一些實施例中，第一 C_H3 結構域包含在位置 392 之 C 且第二 C_H3 結構域包含在位置 400 之 C，或第一 C_H3 結構域包含在位置 400 之 C 且第二 C_H3 結構域包含在位置 392 之 C。在一些實施例中，第一 C_H3 結構域包含 K392C 取代且第二 C_H3 結構域包含 S400C 取代，或第一 C_H3 結構域包含 S400C 取代且第二 C_H3 結構域包含 K392C 取代。

C. 鹽橋突變

【0071】 在一些實施例中，本文所描述之多特異性抗體(例如本文所描述之可活化多特異性抗體)包含含有第一 C_H3 結構域之第一多肽及含有第二 C_H3 結構域之第二多肽，其中第一 C_H3 結構域包含經工程改造之帶正電荷之殘基且第二 C_H3 結構域包含經工程改造之帶負電荷之殘基，其中經工程改造之帶正電荷之殘基及經工程改造之帶負電荷之殘基形成鹽橋。相對於野生型 C_H3 結構域，經工程改造之鹽橋可在 C_H3 結構域之間引入新的鹽橋，使兩個或更多個胺基酸殘基間之鹽橋網狀結構重排，或逆轉形成鹽橋之胺基酸殘基上之電荷(亦即，「逆轉」鹽橋)。在一些實施例中，經工程

改造之帶正電荷之殘基將野生型 C_H3 結構域中之帶負電荷之殘基用帶正電荷之殘基取代。在一些實施例中，經工程改造之帶正電荷之殘基將野生型 C_H3 結構域中之帶正電荷之殘基用帶負電荷之殘基取代。重排及逆轉鹽橋可使得包含經工程改造之 C_H3 結構域的異二聚體及均二聚體之等電點(PI)改變，由此允許在純化製程中更好地分離異二聚體與均二聚體。

【0072】 在一些實施例中，第一 C_H3 結構域包含在位置 357 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 351 之帶負電荷之殘基，或第一 C_H3 結構域包含在位置 351 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 357 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含在位置 357 之 K 且第二 C_H3 結構域包含在位置 351 之 D，或第一 C_H3 結構域包含在位置 351 之 D 且第二 C_H3 結構域包含在位置 357 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 357 之 K 且第二 C_H3 結構域包含在位置 351 之 E，或第一 C_H3 結構域包含在位置 351 之 E 且第二 C_H3 結構域包含在位置 357 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 357 之 R 且第二 C_H3 結構域包含在位置 351 之 D，或第一 C_H3 結構域包含在位置 351 之 D 且第二 C_H3 結構域包含在位置 357 之 R。在一些實施例中，第一 C_H3 結構域包含在位置 357 之 R 且第二 C_H3 結構域包含在位置 351 之 E，或第一 C_H3 結構域包含在位置 351 之 E 且第二 C_H3 結構域包含在位置 357 之 R。在一些實施例中，第一 C_H3 結構域包含 E357K 取代且第二 C_H3 結構域包含 L351D

取代，或第一 C_H3 結構域包含 L351D 取代且第二 C_H3 結構域包含 E357K 取代。

【0073】 在一些實施例中，第一 C_H3 結構域包含在位置 411 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域包含在位置 370 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 411 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含在位置 411 之 K 且第二 C_H3 結構域包含在位置 370 之 D，或第一 C_H3 結構域包含在位置 370 之 D 且第二 C_H3 結構域包含在位置 411 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 411 之 K 且第二 C_H3 結構域包含在位置 370 之 E，或第一 C_H3 結構域包含在位置 370 之 E 且第二 C_H3 結構域包含在位置 411 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 411 之 R 且第二 C_H3 結構域包含在位置 370 之 D，或第一 C_H3 結構域包含在位置 370 之 D 且第二 C_H3 結構域包含在位置 411 之 R。在一些實施例中，第一 C_H3 結構域包含在位置 411 之 R 且第二 C_H3 結構域包含在位置 370 之 E，或第一 C_H3 結構域包含在位置 370 之 E 且第二 C_H3 結構域包含在位置 411 之 R。在一些實施例中，第一 C_H3 結構域包含 T411K 取代且第二 C_H3 結構域包含 K370D 取代，或第一 C_H3 結構域包含 K370D 取代且第二 C_H3 結構域包含 T411K 取代。

【0074】 在一些實施例中，第一 C_H3 結構域包含在位置 364 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 370 之帶負電荷之殘

基，或第一 C_H3 結構域包含在位置 370 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 364 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含在位置 364 之 K 且第二 C_H3 結構域包含在位置 370 之 D，或第一 C_H3 結構域包含在位置 370 之 D 且第二 C_H3 結構域包含在位置 364 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 364 之 K 且第二 C_H3 結構域包含在位置 370 之 E，或第一 C_H3 結構域包含在位置 370 之 E 且第二 C_H3 結構域包含在位置 364 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 364 之 R 且第二 C_H3 結構域包含在位置 370 之 D，或第一 C_H3 結構域包含在位置 370 之 D 且第二 C_H3 結構域包含在位置 364 之 R。在一些實施例中，第一 C_H3 結構域包含在位置 364 之 R 且第二 C_H3 結構域包含在位置 370 之 E，或第一 C_H3 結構域包含在位置 370 之 E 且第二 C_H3 結構域包含在位置 364 之 R。在一些實施例中，第一 C_H3 結構域包含 S364K 取代且第二 C_H3 結構域包含 K370D 取代，或第一 C_H3 結構域包含 K370D 取代且第二 C_H3 結構域包含 S364K 取代。

【0075】 在一些實施例中，第一 C_H3 結構域包含在位置 356 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 439 之帶負電荷之殘基，或第一 C_H3 結構域包含在位置 439 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 356 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含在位置 356 之 K 且第二 C_H3 結構域包含在位置 439 之 D，或第一 C_H3 結構域包含在位置 439 之 D 且第二 C_H3

結構域包含在位置 356 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 356 之 K 且第二 C_H3 結構域包含在位置 439 之 E，或第一 C_H3 結構域包含在位置 439 之 E 且第二 C_H3 結構域包含在位置 356 之 K。在一些實施例中，第一 C_H3 結構域包含在位置 356 之 R 且第二 C_H3 結構域包含在位置 439 之 D，或第一 C_H3 結構域包含在位置 439 之 D 且第二 C_H3 結構域包含在位置 356 之 R。在一些實施例中，第一 C_H3 結構域包含在位置 356 之 R 且第二 C_H3 結構域包含在位置 439 之 E，或第一 C_H3 結構域包含在位置 439 之 E 且第二 C_H3 結構域包含在位置 356 之 R。在一些實施例中，第一 C_H3 結構域包含 D356K 取代且第二 C_H3 結構域包含 K439D 取代，或第一 C_H3 結構域包含 K439D 取代且第二 C_H3 結構域包含 D356K 取代。

【0076】 本文所描述之經工程改造之鹽橋中的任一者可彼此組合。在一些實施例中，第一 C_H3 結構域包含在位置 357 之帶正電荷之殘基及在位置 411 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 351 之帶負電荷之殘基及在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域包含在位置 351 之帶負電荷之殘基及在位置 370 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 357 之帶正電荷之殘基及在位置 411 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含 E357K 及 T411K 取代且第二 C_H3 結構域包含 L351D 及 K370D 取代，或第一 C_H3 結構域包含 L351D 及 K370D 取代且第二 C_H3 結構域包含 E357K 及 T411K 取代。

【0077】 在一些實施例中，第一 C_H3 結構域包含在位置 357 之帶正電荷之殘基及在位置 364 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 351 之帶負電荷之殘基及在位置 370 之帶負電荷之殘基，或第一 C_H3 結構域包含在位置 351 之帶負電荷之殘基及在位置 370 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 357 之帶正電荷之殘基及在位置 364 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含 E357K 及 S364K 取代且第二 C_H3 結構域包含 L351D 及 K370D 取代，或第一 C_H3 結構域包含 L351D 及 K370D 取代且第二 C_H3 結構域包含 E357K 及 S364K 取代。

【0078】 在一些實施例中，第一 C_H3 結構域包含在位置 356 之帶正電荷之殘基、在位置 357 之帶正電荷之殘基及在位置 364 之帶正電荷之殘基且第二 C_H3 結構域包含在位置 351 之帶負電荷之殘基、在位置 370 之帶負電荷之殘基及在位置 439 之帶負電荷之殘基，或第一 C_H3 結構域包含在位置 351 之帶負電荷之殘基、在位置 370 之帶負電荷之殘基及在位置 439 之帶負電荷之殘基且第二 C_H3 結構域包含在位置 356 之帶正電荷之殘基、在位置 357 之帶正電荷之殘基及在位置 364 之帶正電荷之殘基。在一些實施例中，第一 C_H3 結構域包含 D356K、E357K 及 S364K 取代且第二 C_H3 結構域包含 L351D、K370D 及 K439D 取代，或第一 C_H3 結構域包含 L351D、K370D 及 K439D 取代且第二 C_H3 結構域包含 D356K、E357K 及 S364K 取代。

D. 其他突變

【0079】 本文所描述之 C_H3 結構域或 Fc 區可另包含下表 4 中所列經工程改造之二硫鍵及/或鹽橋。

表 4. 示例性 Fc 突變

第一多肽鏈中之突變	第二多肽鏈中之突變
F405L	K409R
S364H、F405A	Y349T、T394F
S364K、E357Q	L368D、K370S
T366W	T366S、L368A、Y407V
S354C、T366W	Y349C、T366S、L368A、Y407V
T350、L351、F405、Y407 T350 為 T350V、T350I、T350L 或 T350M L351 為 L351Y F450 為 F450A、F450V、F450T 或 F450S Y407 為 Y407V、Y407A 或 Y407I	T350、T366、K392、T394 T350 為 T350V、T350I、T350L 或 T350M T366 為 T366L、T366I、T366V 或 T366M K392 為 K392F、K392L 或 K392M T394 為 T394W
D399K、E356K	K409D、K392D
D221E、P228E、L368E	D221R、P228R、K409R
C223E、E225E、P228E、L368E	C223R、E225R、P228R、K409R
H435R	無
K196Q、S228P、F296Y、E356K、R409K、H435R、L445P	K196Q、S228P、F296Y、R409K、K439E、L445P
K409W	D399V/F405T
K360E	Q347R
Y349C/K360E/K409W	Q347R/S354C/D399V/F405T
K360E/K409W	Q347R/D399V/F405T
Y349S/K409W	E357W/D399V/F405T
Y349S/S354C/K409W	Y349C/E357W/D399V/F405T
T366K	L351D
Y349E 或 D 及 L368E	L351D
Y349C/T366W	D356C/T366S/L368A/Y407V/F405K
Y349C/T366W/F405K	D356C/T366S/L368A/Y407V
Y349C/T366W/K409E	D356C/T366S/L368A/Y407V/F405K
Y349C/T366W/K409A	D356C/T366S/L368A/Y407V/F405K
S364K	L368D
S364K	K370S
F405K	K409F

第一多肽鏈中之突變	第二多肽鏈中之突變
F405R	K409F
S364K/K409F	L368D/F405R
S364K/K409F	K370S/F405R
S364K/K409W	K370S/F405R
K370E 或 E356K 及 K409R	E357K 及 K409R 或 K370E
S354I/S364L/Y407V	Q347V/Y349I/T350K/L351Q/T366W/L368I/Y407V
Y349I	S354I/E357Q/M/G/S364L/T366S/L368A/Y407V
P395K/P396K/V397K	T394D/P395D/P396D
F405E/Y407E/K409E	F405K/Y407K
M428S/N434S/Y436H	H435R

【0080】 在一些實施例中，第一 C_H3 結構域另包含在位置 392 之 C 且第二 C_H3 結構域包含在位置 399 之 C，或第一 C_H3 結構域包含在位置 399 之 C 且第二 C_H3 結構域包含在位置 392 之 C。在一些實施例中，第一 C_H3 結構域另包含 K392C 取代且第二 C_H3 結構域另包含 D399C 取代，或第一 C_H3 結構域另包含 D399C 取代且第二 C_H3 結構域另包含 K392C 取代。

【0081】 在一些實施例中，第一 C_H3 結構域另包含在位置 394 之 C 且第二 C_H3 結構域包含在位置 354 之 C，或第一 C_H3 結構域包含在位置 354 之 C 且第二 C_H3 結構域包含在位置 394 之 C。在一些實施例中，第一 C_H3 結構域另包含 Y394C 取代且第二 C_H3 結構域另包含 S354C 取代，或第一 C_H3 結構域另包含 S354C 取代且第二 C_H3 結構域另包含 Y394C 取代。

【0082】 在一些實施例中，第一 C_H3 結構域另包含在位置 356 之 C 且第二 C_H3 結構域包含在位置 349 之 C，或第一 C_H3 結構域包含在位置 349 之 C 且第二 C_H3 結構域包含在位置 356 之 C。在一些實施例中，第一 C_H3 結構域另包含 D356C 取代且第二 C_H3 結構

域另包含 Y349C 取代，或第一 C_H3 結構域另包含 Y349C 取代且第二 C_H3 結構域另包含 D356C 取代。

【0083】 在一些實施例中，第一 C_H3 結構域另包含 K392D 及 K409D 取代且第二 C_H3 結構域另包含 D356K 及 D399K 取代，或第一 C_H3 結構域另包含 D356K 及 D399K 取代且第二 C_H3 結構域另包含 K392D 及 K409D 取代。

【0084】 在一些實施例中，第一 C_H3 結構域另包含 L368D 及 K370S 取代且第二 C_H3 結構域另包含 E357Q 及 S364K 取代，或第一 C_H3 結構域另包含 E357Q 及 S364K 取代且第二 C_H3 結構域另包含 L368D 及 K370S 取代。

【0085】 在一些實施例中，第一 C_H3 結構域另包含 L351K 及 T366K 取代且第二 C_H3 結構域另包含 L351D 及 L368E 取代，或第一 C_H3 結構域另包含 L351D 及 L368E 取代且第二 C_H3 結構域另包含 L351K 及 T366K 取代。

【0086】 在一些實施例中，第一 C_H3 結構域另包含 P395K、P396K 及 V397K 取代且第二 C_H3 結構域包含 T394D、P395D 及 P396D 取代，或第一 C_H3 結構域另包含 T394D、P395D 及 P396D 取代且第二 C_H3 結構域另包含 P395K、P396K 及 V397K 取代。

【0087】 在一些實施例中，第一 C_H3 結構域另包含 F405E、Y407E 及 K409E 取代且第二 C_H3 結構域包含 F405K 及 Y407K 取代，或第一 C_H3 結構域另包含 F405K 及 Y407K 取代且第二 C_H3 結構域另包含 F405E、Y407E 及 K409E 取代。

【0088】 在一些實施例中，第一 C_H3 結構域另包含 T336S、L368A 及 Y407V 取代且第二 C_H3 結構域另包含 T366W 取代，或第一 C_H3 結構域另包含 T366W 取代且第二 C_H3 結構域另包含 T336S、L368A 及 Y407V 取代。

【0089】 在一些實施例中，第一 C_H3 結構域包含 L368V 及 Y407V 取代且第二 C_H3 結構域包含 T366W 取代，或第一 C_H3 結構域包含 T366W 取代且第二 C_H3 結構域包含 L368V 及 Y407V 取代。

III. CD28 結合分子

【0090】 本揭示案提供結合至人類 CD28 之經單離結合分子，包括抗 CD28 抗體及其抗 CD28 抗原結合片段。於一些實施例中，結合分子包括參照抗原表位結合所描述之抗體以及關於互補決定區(CDR)、可變區(V_L、V_H)以及 IgG (例如，IgG_4)輕鏈及重鏈的具體胺基酸序列所描述之抗體。

【0091】 於一些實施例中，抗體或其抗原結合片段與 SEQ ID NO: 1 之胺基酸殘基 34-108 內之一個或多個胺基酸殘基結合。於一些實施例中，抗體或抗原結合片段與 SEQ ID NO: 1 之胺基酸殘基 51-122 內之一個或多個胺基酸殘基結合。於一些實施例中，抗體或抗原結合片段與選自由 SEQ ID NO: 1 之胺基酸殘基 51、52、54、55、98-101、110-111、113-114 及 118-122 組成之群之一個或多個胺基酸殘基結合。量測抗體或抗原結合片段結合靶抗原之能力之方法可使用此項技術中已知之任何方法來進行，包括例如，藉由表面等離子體共振、ELISA、等溫滴定量熱法、過濾結合測定、EMSA 等。

於一些實施例中，抗體或抗原結合片段結合靶抗原之能力藉由ELISA 或 Octet® RED96 量測(參見例如，下述實例 3)。

【0092】 於一些實施例中，抗體或抗原結合片段以約 500 nM 或更低(例如，約 500 nM 或更低、約 400 nM 或更低、約 300 nM 或更低、約 200 nM 或更低、約 150 nM 或更低、約 100 nM 或更低、約 90 nM 或更低、約 80 nM 或更低、約 75 nM 或更低、約 70 nM 或更低、約 60 nM 或更低、約 50 nM 或更低、約 40 nM 或更低、約 30 nM 或更低、約 25 nM 或更低、約 20 nM 或更低、約 10 nM 或更低、約 1 nM 或更低、約 0.1 nM 或更低等)之 KD 結合至人類 CD28。於一些實施例中，抗體或抗原結合片段以約 100 nM 或更低之 KD 結合至人類 CD28。於一些實施例中，抗體或抗原結合片段以約 50 nM 或更低之 KD 結合至人類 CD28。量測抗體或抗原結合片段之 K_D 之方法可使用此項技術中已知之任何方法來進行，包含例如藉由表面電漿子共振、ELISA、等溫滴定量熱法、篩檢程式結合檢定法、EMSA 等。於一些實施例中，KD 藉由 Octet® RED96 系統來量測(參見例如，下述實例 3)。

A. 抗 CD28 抗體

【0093】 於一些實施例中，本公開提供一種經單離單克隆抗體，其在 SEQ ID NO: 1 之胺基酸殘基 33-37、80-83、92-96 及 100-104 之抗原表位上與人類 CD28 結合。於特定實施例中，本公開提供一種經單離抗體，其在 SEQ ID NO: 1 之胺基酸殘基 33、34、36 及 37、80-83、92 及 93、95 及 96 及 100-104 代表之抗原表位上與人類

CD28 結合。於一些實施例中，抗體以 10 nM 或更小之 KD 結合人類 CD28，如藉由 Octet® RED96 系統量測。於某些實施例中，除結合人類抗原表位外，本公開之抗體可與選自食蟹獼猴、小鼠、大鼠及狗的至少一種非人類物種交叉反應。於某些實施例中，本公開之抗體具有跨物種結合小鼠、人類及猴子之優點，然而本公開之基準對照 TAC2386 及 TAC2387 不具有此範圍之物種交叉反應性。於特定實施例中，基準對照 TAC2386 及 TAC2387 結合人類抗原表位但不結合小鼠抗原表位(參見本文表 7)。本公開抗體之物種交叉反應性亦能提供使用小鼠模擬抗體安全性、活性及功能之額外優勢。因此，相較於本公開之 TAC2386 及 TAC2387，使用本公開之抗體更易進行動物建模。

【0094】 於特定實施例中，經單離抗 CD28 單克隆抗體包含 SEQ ID NO: 5 之 HCDR1、SEQ ID NO: 6 之 HCDR2 及 SEQ ID NO: 7 之 HCDR3，以及 SEQ NO ID: 8 之 LCDR1、SEQ ID NO: 9 之 LCDR2 及 SEQ ID NO: 10 之 LCDR3。於特定實施例中，經單離單克隆抗體包含 SEQ ID NO: 11 之重鏈可變區及 SEQ ID NO: 12 之輕鏈可變區。於特定實施例中，經單離單克隆抗體包含 SEQ ID NO: 13 之重鏈及 SEQ ID NO: 14 之輕鏈。

【0095】 本文所述 CD28 抗體可為任一類別，例如 IgG、IgM、IgE、IgA 或 IgD。CD28 抗體較佳地系 IgG 類別，例如 IgG_1、IgG_2、IgG_3 或 IgG_4 子類。抗 CD28 抗體可使用此項技術中已知之方法自一個類別或子類轉化成另一類別或子類。產生期望類別或子類之抗體

之例示性方法包括以下步驟：分離編碼抗 CD28 抗體重鏈之核酸及編碼 CD28 抗體輕鏈之核酸，分離編碼 V_H 區之序列，將 V_H 序列聯結至編碼期望類別或子類之重鏈恒定區之序列，在細胞中表現輕鏈基因及重鏈構築物，及收集 CD28 抗體。

【0096】 本文所述抗 CD28 抗體可為任一類別，例如 IgG、IgM、IgE、IgA 或 IgD。CD28 抗體較佳地系 IgG 類別，例如 IgG_1、IgG_2、IgG_3 或 IgG_4 子類。抗 CD28 抗體可使用此項技術中已知之方法自一個類別或子類轉化成另一類別或子類。產生期望類別或子類之抗體之例示性方法包括以下步驟：分離編碼抗 CD28 抗體重鏈之核酸及編碼抗 CD28 抗體輕鏈之核酸，分離編碼 V_H 區之序列，將 V_H 序列聯結至編碼期望類別或子類之重鏈恒定區之序列，在細胞中表現輕鏈基因及重鏈構築物，及收集 CD28 抗體。

【0097】 此外，本公開提供之抗體可為單克隆或多克隆，但優選為單克隆。

【0098】 本發明之抗體可藉由此項技術中已知之技術產生，包括習知單株抗體方法學，例如，標準體細胞雜交技術(參見例如，Kohler 及 Milstein，Nature (1975) 256:495)、B 淋巴細胞之病毒或致癌轉形、或如下文中詳細所述之重組抗體技術。

【0099】 雜交瘤產生為極完善建立之程式。用於製備雜交瘤之常見動物系統為鼠科系統。用於單離經免疫脾細胞用於融合之免疫方案及技術係此項技術中已知。融合搭檔(例如，鼠科骨髓瘤細胞)及融合程式亦已知。可用於製備本發明提供之人類 CD28 抗體之

一種熟知方法涉及使用 XenoMouse™動物系統。XenoMouse™小鼠經小鼠株工程改造，該等小鼠株包含人類免疫球蛋白重鏈及輕鏈基因座之大片段且於小鼠抗體產生中有缺陷。參見例如，Green 等人，Nature Genetics (1994) 7:13-21 及 WO2003/040170。將動物用 CD28 抗原免疫。該 CD28 抗原為經單離及/或經純化之 CD28，較佳地 CD28。其可為 CD28 之片段，諸如 CD28 之細胞外域，具體而言包含 SEQ ID NO: 1 之胺基酸殘基 34、34、36 及 37、80-83、92 及 93、95 及 96、以及 100-104 之 CD28 細胞外結構域片段。動物之免疫可藉由此項技術中已知之任一方法來實施。參見例如 Harlow 及 Lane, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Press, 1990。用於免疫非人類動物(例如小鼠、大鼠、綿羊、山羊、豬、牛及馬)之方法為此項技術中所熟知。參見例如 Harlow 及 Lane，參見上文及美國專利第 5,994,619 號。CD28 抗原可與佐劑一起投與來刺激免疫反應。例示性佐劑包括完全或不完全弗氏佐劑(Freund's adjuvant)、RIBI (胞壁醯二肽)或 ISCOM (免疫刺激複合物)。用 CD28 抗原免疫動物後，自經免疫動物單離之細胞製備產生抗體之永生化細胞株。免疫後，將動物殺死且使淋巴結及/或脾 B 細胞永生化。使細胞永生化之方法包括(但不限於)將其用致癌基因轉移、將其用致癌病毒感染、將其在選擇永生化細胞之條件下培養、使其經歷致癌或突變化合物、將其與永生化細胞(例如骨髓瘤細胞)融合及不活化腫瘤阻抑基因。參見例如 Harlow 及 Lane，參見上文。若使用與骨髓瘤細胞之融合物，則骨髓瘤細

胞較佳地不會分泌免疫球蛋白多肽(非分泌性細胞株)。使用 CD28、其部分或表現 CD28 之細胞來篩選永生化細胞。針對期望特徵選擇、選殖且進一步篩選產生 CD28 抗體之細胞(例如雜交瘤)，包括穩健生長、高抗體產生及期望抗體特徵，如下文進一步論述。可在同基因動物活體內、在缺乏免疫系統之動物(例如裸小鼠)中或在細胞培養物活體外擴增雜交瘤。選擇、選殖及擴增雜交瘤之方法為熟習此項技術者所熟知。

【0100】 本揭示案之抗體亦可使用噬菌體展示或酵母菌展示方法製備。此項技術中已建立用於分離人類抗體之此等展示方法，例如 Knappik 等人，“Fully Synthetic Human Combinatorial Antibody Libraries (HuCAL) Based on Modular Consensus Frameworks and CDRs Randomized with Trinucleotides.” J. Mol. Biol. (2000) 296, 57-86；及 Feldhaus 等人，“Flow-cytometric isolation of human antibodies from a non-immune Saccharomyces cerevisiae surface display library” Nat Biotechnol (2003) 21:163-170。

B. 抗原結合片段

【0101】 於一些其他態樣中，本公開提供由本公開提供之 CD28 抗體中之任一者之抗原結合片段。

【0102】 抗原結合片段可包含任何抗體序列。於一些實施例中，該抗原結合片段包含以下之胺基酸序列：(1)抗 CD28 抗體之輕鏈；(2) CD28 抗體之重鏈；(3)來自抗 CD28 抗體輕鏈之可變區；(4)來自 CD28 抗體重鏈之可變區；(5) CD28 抗體之一個或多個 CDR (2、

3、4、5 或 6 個 CDR)；或(6)來自抗 CD28 抗體之輕鏈之三個 CDR 及來自 CD28 抗體之重鏈之三個 CDR。

【0103】 於一些其它特定實施例中，抗 CD28 抗體之抗原結合片段包括：(i) Fab 片段，其為由 V_L、V_H、C_L 及 C_H1 結構域組成之單價片段；(ii) $F(ab')_2$ 片段，其為包含由鉸鏈區處之二硫橋連接之兩個 Fab 片段之二價片段；(iii)由 V_H 及 C_H1 結構域組成之 Fd 片段；(iv)由抗體之單臂之 V_L 及 V_H 結構域組成之 Fv 片段；(v) dAb 片段(Ward 等人，Nature (1989) 341:544-546)，其由 V_H 結構域組成；(vi)經單離 CDR，及(vii)單鏈抗體(scFv)，其為包含連接至抗體之 V_H 區之抗體之 V_L 區的多肽。Bird 等人，Science (1988) 242:423-426，及 Huston 等人，Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883)。

【0104】 於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 11、20、29、39、47、54、62、71、77、84、92、99、107、115、122、130、137、144、151、157 及 165 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之 V_H 區。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 12、21、30、40、48、55、63、72、78、85、93、100、108、116、123、131、138、145、152、158 及 166 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%

同一性之 V_L 區。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 5、15、24、33、43、66、88、103、111、126、134、148 及 161 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之 HCDR1 胺基酸序列。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 6、51、58、67、89、96、104 及 155 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之 HCDR2 胺基酸序列。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 7、16、25、35、44、52、59、81、90、97、105、112、119、127、135、141、149 及 162 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之 HCDR3 胺基酸序列。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 8、17、26、36、45、53、60、76、82、91、98、106、113、120、128、136、142、150、156 及 163 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之 LCDR1 胺基酸序列。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 9、18、27、37 及 300 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、

至少 96%、至少 97%、至少 98%、或至少 99%同一性之 LCDR2 胺基酸序列。於一些實施例中，本公開之抗 CD28 抗體或抗體片段包含與如 SEQ ID NO: 10、19、28、38、46、61、70、83、114、121、129、143 及 164 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之 LCDR3 胺基酸序列。

【0105】 於一些實施例中，本公開之抗體包含與如 SEQ ID NO: 13、22、31、41、49、56、64、73、79、86、94、101、109、117、124、141、132、139、146、153、159 及 167 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之重鏈。

【0106】 於一些實施例中，本公開之抗體包含與如 SEQ ID NO: 14、23、32、42、50、57、65、74、80、87、95、102、110、118、125、142、133、140、147、154、160 及 168 中之任一者中所述之胺基酸序列至少 65%、至少 75%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%、或至少 99%同一性之輕鏈。

IV. 多特異性抗體

【0107】 亦提供多特異性抗體，其對應本文所述可活化多特異性抗體或經遮蔽多特異性抗體。本申請案之一個態樣提供可活化之多特異性抗體(包括可活化雙特異性 T 細胞銜接體(TCE)分子)，其能夠結合至 T 細胞及靶細胞(諸如腫瘤細胞)二者。由於其中靶脫腫瘤效應，傳統 TCE 分子與高細胞毒性相關，包括對中樞神經系

統(CNS)之毒性及細胞介素風暴。因此，業內需要能結合 T 細胞及靶細胞(例如具有增強之特異性及降低之副作用之腫瘤細胞)之抗體。

【0108】 於一些實施例中，多特異性抗體為雙特異性(bsAb)。於一些實施例中，多特異性抗體為三特異性(tsAb)。

【0109】 於特定實施例中，本公開之雙特異性抗體對 T 細胞表面 CD28 具有特異性。於一些實施例中，多特異性抗體為特異性結合 TAA 及 CD28 之腫瘤相關抗原(TAA)xCD28 雙特異性抗體。於一些實施例中，本公開之抗體為 IgG 抗體，例如，包含 IgG Fc 區(例如，人類 IgG Fc 區)。

【0110】 於一些實施例中，多特異性抗體結合至 T 細胞表面之 CD28。於一些實施例中，多特異性抗體為特異性結合至 TAA 及 CD28 之腫瘤相關抗原(TAA)xCD28 雙特異性抗體。於一些實施例中，多特異性抗體不包含任何遮蔽性部分或可裂解部分。於一些實施例中，多特異性抗體為經可裂解部分或可裂解部分裂解獲得。

【0111】 於一些實施例中，多特異性抗體結合至 T 細胞表面之 CD3。於一些實施例中，多特異性抗體為特異性結合至 TAA 及 CD3 之腫瘤相關抗原(TAA)xCD3 雙特異性抗體。於一些實施例中，多特異性抗體以弱親和力特異性結合 CD3，例如藉由 ELISA 檢定測定之 EC50 至少為 10 nM (例如，至少 100 nM)，及/或 Kd 至少為 50 nM。於一些實施例中，多特異性抗體不包含任何遮蔽性部分或可裂解部分。於一些實施例中，多特異性抗體為經可裂解部分或可裂解部

分裂解獲得。

【0112】 於一些實施例中，提供一種多特異性抗體包含：a)第一抗原結合片段，其包含特異性結合靶抗原(例如，腫瘤抗原，如 B7-H3、HER2 或 TROP2)之抗體之 VH1 及 V_L1；及 b)第二抗原結合片段，其包含特異性結合 CD3 之抗 CD3 抗體之 VH2 及 V_L2，其中第一及/或第二抗原結合片段融合至第一及/或第二遮蔽性肽(MP1/MP2)。於一些實施例中，提供一種多特異性抗體包含：a)第一抗原結合片段，其包含特異性結合靶抗原(例如，腫瘤抗原，如 B7-H3、HER2 或 TROP2)之抗體之 VH1 及 V_L1；及 b)第二抗原結合片段，其包含特異性結合 CD28 之抗 CD28 抗體之 VH2 及 V_L2，其中第一及/或第二抗原結合片段融合至第一及/或第二遮蔽性肽(MP1/MP2)。

【0113】 於一些實施例中，第一抗原結合片段選自由 Fab、Fv、scFab 及 scFv 組成之群。於一些實施例中，第一抗原結合片段為 Fab。於一些實施例中，第二抗原結合片段選自由 Fab、Fv、scFab 及 scFv 組成之群。於一些實施例中，第二抗原結合片段為 scFv，其自 N 端至 C 端包含 V_L2、視情況選用連接子及 VH2。於一些實施例中，第一抗原結合片段為 Fab 及第二抗原結合片段為 Fab。於一些實施例中，第一抗原結合片段為 Fab 及第二抗原結合片段為 scFv。

【0114】 本文所描述之包含經工程改造之 C_H3 結構域二硫鍵及/或鹽橋的抗體可另包含一個或多個杵入臼殘基。「杵入臼」或「KIH」

係指此項技術中已知用於製備雙特異性抗體之方法，亦稱為「突起入空腔」法(參見例如美國專利第 5,731,168 號)。在此方法中，兩個免疫球蛋白多肽(例如重鏈多肽)各包含一介面。一個免疫球蛋白多肽之介面與另一免疫球蛋白多肽上之對應介面相互作用，由此允許兩個免疫球蛋白多肽相締合。此等介面可經工程改造，使得定位於一個免疫球蛋白多肽之介面中的「杵」或「突起」(此等術語在本文中可互換使用)與定位於另一免疫球蛋白多肽之介面中的「臼」或「空腔」(此等術語在本文中可互換使用)對應。在一些實施例中，臼之尺寸與杵相同或類似且適當定位，使得當兩個介面相互作用時，一個介面之杵可安置於另一介面之對應臼中。在不希望受理論約束之情況下，此被認為穩定異多聚體且相較於其他物質(例如均多聚體)有利於形成異多聚體。在一些實施例中，KIH 法與本文所描述之經工程改造之二硫鍵及/或鹽橋組合使用，以促進兩種不同免疫球蛋白多肽之異多聚化，從而形成包含對不同抗原表位具有結合特異性之兩個免疫球蛋白多肽的雙特異性抗體。在一些實施例中，本文所描述之可活化多特異性抗體之 C_H3 結構域不包含 KIH 殘基。

【0115】 於一些實施例中，提供靶向 CD28 及腫瘤抗原(例如，B7-H3、HER2、TROP2 或 PD-L1)之雙特異性抗體，其包含第一多肽、第二多肽及第三多肽，其中：

(i)第一多肽包含由下式表示之結構：

V_H1-C_H1-鉸鏈-C_H2-第一 C_H3；

(ii)第二多肽包含由下式表示之結構：

scFv-鉸鏈-C_H2-第二 CH3；及

(iii)第三多肽包含由下式表示之結構：

V_L1-C_L；

其中：

V_L1 為第一免疫球蛋白輕鏈可變結構域；

V_H1 為第一免疫球蛋白重鏈可變結構域；

scFv 為單鏈可變片段，包含第二免疫球蛋白輕鏈可變結構域(V_L2)及第二免疫球蛋白重鏈可變結構域(V_H2)；

C_L 為免疫球蛋白輕鏈恆定結構域；

C_H1 為免疫球蛋白重鏈恆定結構域 1；

C_H2 為免疫球蛋白重鏈恆定結構域 2；及

鉸鏈為連接 C_H1 結構域及 C_H2 結構域之免疫球蛋白鉸鏈區；

其中 V_L1 與 VH1 相締合以形成特異性結合腫瘤抗原(例如，B7-H3、HER2、TROP2 或 PD-L1)之第一 Fv；及其中 scFv 特異性結合 CD28。於一些實施例中，scFv 藉由 ELISA 測定，以 0.1 nM 至 1000 nM 範圍之半最大結合抗體濃度(EC_{50})結合 CD28 (例如，如實施例 3 所述)。於一些實施例中，scFv 藉由 Octet® RED96 檢定測定，以低於 10 nM (例如，在 1 nM 至 0.1 pM 之間)之半最大結合抗體濃度(EC_{50})結合 CD28 (例如，如實施例 3 所述)。於特定實施例中，scFv 以低於 10 nM 之解離常數(K*d*)結合 CD28。

【0116】 於一些實施例中，提供靶向 CD28 及腫瘤抗原(例如，B7-

H3、HER2 或 TROP2)之雙特異性抗體，其包含第一多肽、第二多肽、第三多肽及第四多肽，其中：

(i)第一多肽包含由下式表示之結構：

V_H1-C_H1-鉸鏈-C_H2-第一 CH3；

(ii)第二多肽包含由下式表示之結構：

V_H2-C_H1-鉸鏈-C_H2-第二 CH3；

(iii)第三多肽包含由下式表示之結構：

V_L1-C_L；及

(iv)第四多肽包含由下式表示之結構：

V_L2-C_L；

其中：

V_L1 為第一免疫球蛋白輕鏈可變結構域；

V_H1 為第一免疫球蛋白重鏈可變結構域；

V_L2 為第二免疫球蛋白輕鏈可變結構域；

V_H2 為第二免疫球蛋白重鏈可變結構域；

C_L 為免疫球蛋白輕鏈恆定結構域；

C_H1 為免疫球蛋白重鏈恆定結構域 1；

C_H2 為免疫球蛋白重鏈恆定結構域 2；及

鉸鏈為連接 C_H1 結構域及 C_H2 結構域之免疫球蛋白鉸鏈區；

其中 V_L1 與 V_H1 相締合以形成特異性結合腫瘤抗原(例如，B7-H3、HER2、TROP2 或 PD-L1)之第一 Fv；及其中 V_L2 與 V_H2 相締合以形成特異性結合 CD28 之第二 Fv。於一些實施例中，scFv

藉由 ELISA 檢定測定，以 0.1 nM 至 1000 nM 範圍之半最大結合抗體濃度(EC_{50})結合 CD28 (例如，如實施例 3 所述)。於一些實施例中，第二 Fv 藉由 Octet® RED96 檢定測定，以低於 10 nM (例如，在 1 nM 至 0.1 pM 之間)之半最大結合抗體濃度(EC_{50})結合 CD28 (例如，如實施例 3 所述)。於特定實施例中，第二 Fv 以低於 10 nM 之解離常數(K*d*)結合 CD28。

【0117】 於一些實施例中，提供多特異性抗體，其包含：

a)第一抗原結合片段，其包含特異性結合靶抗原(例如，腫瘤抗原，如 B7-H3、HER2 或 TROP2)之抗體之 V_H1 及 V_L1；及

b)第二抗原結合片段，其包含特異性結合 CD28 之抗 CD28 抗體之 V_H2 及 V_L2，其中第二抗原結合片段融合至第一遮蔽性肽(MP1)。

【0118】 於一些實施例中，提供多特異性抗體，其包含：

a)第一抗原結合片段，其包含特異性結合靶抗原(例如，腫瘤抗原，如 B7-H3、HER2 或 TROP2)之抗體之 V_H1 及 V_L1，其中第一抗原結合片段融合至第一遮蔽性肽(MP1)；及

b)第二抗原結合片段，其包含特異性結合 CD28 之抗 CD28 抗體之 V_H2 及 V_L2，其中第二抗原結合片段融合至第二遮蔽性肽(MP2)。

【0119】 於一些實施例中，第一抗原結合片段選自由 Fab、Fv、scFab 及 scFv 組成之群。於一些實施例中，第一抗原結合片段為 Fab。於一些實施例中，第二抗原結合片段選自由 Fab、Fv、scFab

及 scFv 組成之群。於一些實施例中，第二抗原結合片段為 scFv，其自 N 端至 C 端包含 V_L2、視情況選用連接子及 V_H2。

【0120】 於一些實施例中，提供多特異性抗體，其包含第一多肽、第二多肽及第三多肽，其中：

(i)第一多肽包含由下式表示之結構：

V_H1-C_H1-鉸鏈-C_H2-第一 C_H3；

(ii)第二多肽包含由下式表示之結構：

MP1-V_L2-V_H2-鉸鏈-C_H2-第二 C_H3；及

(iii)第三多肽包含由下式表示之結構：

V_L1-C_L；

其中：

V_L1 為第一免疫球蛋白輕鏈可變結構域；

V_H1 為第一免疫球蛋白重鏈可變結構域；

V_L2 為第二免疫球蛋白輕鏈可變結構域；

V_H2 為第二免疫球蛋白重鏈可變結構域；

C_L 為免疫球蛋白輕鏈恆定結構域；

C_H1 為免疫球蛋白重鏈恆定結構域 1；

C_H2 為免疫球蛋白重鏈恆定結構域 2；

第一 C_H3 為第一免疫球蛋白重鏈恆定結構域 3；

第二 C_H3 為第二免疫球蛋白重鏈恆定結構域 3；

鉸鏈為連接 C_H1 結構域及 C_H2 結構域之免疫球蛋白鉸鏈區；

MP1 為第一遮蔽性肽；MP1 自 N 端至 C 端包含 N 端單元

(NU)、遮蔽性單元(MU)及連接單元(LU)；該遮蔽性肽之 LU 可不包含裂解位點，或包含至少一個裂解位點。

【0121】 於一些實施例中，提供多特異性抗體，其包含第一多肽、第二多肽及第三多肽，其中：

(i)第一多肽包含由下式表示之結構：

V_H1-C_H1-鉸鏈-C_H2-第一 C_H3；

(ii)第二多肽包含由下式表示之結構：

MP2-V_L2-V_H2-鉸鏈-C_H2-第二 C_H3；及

(iii)第三多肽包含由下式表示之結構：

MP1-V_L1-C_L；

其中：

V_L1 為第一免疫球蛋白輕鏈可變結構域；

V_H1 為第一免疫球蛋白重鏈可變結構域；

V_L2 為第二免疫球蛋白輕鏈可變結構域；

V_H2 為第二免疫球蛋白重鏈可變結構域；

C_L 為免疫球蛋白輕鏈恆定結構域；

C_H1 為免疫球蛋白重鏈恆定結構域 1；

C_H2 為免疫球蛋白重鏈恆定結構域 2；

第一 C_H3 為第一免疫球蛋白重鏈恆定結構域 3；

第二 C_H3 為第二免疫球蛋白重鏈恆定結構域 3；

鉸鏈為連接 C_H1 結構域及 C_H2 結構域之免疫球蛋白鉸鏈區；

MP1 為遮蔽性肽；MP1 自 N 端至 C 端包含 N 端單元(NU)、

遮蔽性單元(MU)及連接單元(LU)；該遮蔽性肽之 LU 可不包含、包含至少一個或多個裂解位點。

MP2 為遮蔽性肽；MP2 自 N 端至 C 端包含 N 端單元(NU)、遮蔽性單元(MU)及連接單元(LU)；該遮蔽性肽之 LU 可不包含裂解位點，或包含至少一個裂解位點。

【0122】 於一些實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28，及該雙特異性抗體包含包含選自由 SEQ ID NOs: 5、15、24、33、43、66、88、103、111、126、134、148 及 161 組成之群之胺基酸序列之 HCDR1；包含選自由 SEQ ID NOs: 6、51、58、67、89、96、104 及 155 組成之群之胺基酸序列之 HCDR2；及包含選自由 SEQ ID NOs: 7、16、25、35、44、52、59、81、90、97、105、112、119、127、135、141、149 及 162 之胺基酸序列之 HCDR3；以及包含選自由 SEQ ID NOs: 8、17、26、36、45、53、60、76、82、91、98、106、113、120、128、136、142、150、156 及 163 組成之群之胺基酸序列之 LCDR1；包含選自由 SEQ ID NOs: 9、18、27、37 及 300 組成之群之胺基酸序列之 LCDR2；及包含選自由 SEQ ID NOs: 10、19、28、38、46、61、70、83、114、121、129、143 及 164 組成之群之胺基酸序列之 LCDR3。於一些實施方案中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為人類 CD28，及其中雙特異性抗體包含包含選自由 SEQ ID NOs: 11、20、29、39、47、54、62、71、77、84、92、99、107、115、122、130、137、144、151、157 及 165 組成之群之胺基酸序列之重鏈可變區，

及包含選自由 SEQ ID NOs: 12、21、30、40、48、55、63、72、78、85、93、100、108、116、123、131、138、145、152、158 及 166 組成之群之胺基酸序列之輕鏈可變區。

【0123】 於一些實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 B7H3，其中雙特異性抗體包含 CD28 結合部分，其包含選自由 SEQ ID NOs: 11、12 及 171 組成之群之胺基酸序列，及其中雙特異性抗體包含含有 SEQ ID NO: 176 中所示之胺基酸序列之重鏈，及含有 SEQ ID NO: 175 中所示之胺基酸序列之輕鏈。於特定實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 B7H3，其中雙特異性抗體包含含有 SEQ ID NO: 171 中所示之胺基酸序列之重鏈，含有 SEQ ID NO: 176 中所示之胺基酸序列之第二重鏈，及含有 SEQ ID NO: 175 中所示之胺基酸序列之輕鏈。

【0124】 於一些實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 HER2，其中雙特異性抗體包含 CD28 結合部分，其包含 SEQ ID NO: 172 中所示之胺基酸序列，及其中雙特異性抗體包含含有 SEQ ID NO: 170 中所示之胺基酸序列之重鏈，及含有 SEQ ID NO: 169 中所示之胺基酸序列之輕鏈。於特定實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 HER2，其中雙特異性抗體包含含有 SEQ ID NO: 172 中所示之胺基酸序列之重鏈，含有 SEQ ID NO: 170 中所示之胺基酸序列之第二重鏈，及含有 SEQ ID NO: 169 中

所示之胺基酸序列之輕鏈。

【0125】 於一些實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 HER2，其中雙特異性抗體包含 CD28 結合部分，其包含 SEQ ID NO: 11、12、171 中所示之胺基酸序列，含有 SEQ ID NO: 170 中所示之胺基酸序列之重鏈，及含有 SEQ ID NO: 169 中所示之胺基酸序列之輕鏈。於特定實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 HER2，其中雙特異性抗體包含含有 SEQ ID NO: 171 中所示之胺基酸序列之重鏈，含有 SEQ ID NO: 170 中所示之胺基酸序列之第二重鏈，及含有 SEQ ID NO: 169 中所示之胺基酸序列之輕鏈。

【0126】 於一些實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為人類 CD28 及第二靶點為 TROP2，其中雙特異性抗體包含 CD28 結合部分，其包含選自由 SEQ ID NOs: 13、14 及 171 組成之群之胺基酸序列，及其中雙特異性抗體包含含有 SEQ ID NO: 174 中所示之胺基酸序列之重鏈，及含有 SEQ ID NO: 173 中所示之胺基酸序列之輕鏈。於特定實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD28 及第二靶點為 TROP2，其中雙特異性抗體包含含有 SEQ ID NO: 171 中所示之胺基酸序列之重鏈，含有 SEQ ID NO: 174 中所示之胺基酸序列之第二重鏈，及含有 SEQ ID NO: 173 中所示之胺基酸序列之輕鏈。

【0127】 於特定實施例中，雙特異性抗體結合至第一及第二靶點，

其中第一靶點為 CD3 及第二靶點為 B7-H3，及其中雙特異性抗體包含含有 SEQ ID NO: 177 中所示之胺基酸序列之重鏈或 SEQ ID NO: 299 中所示之 scFv 融合多肽，含有 SEQ ID NO: 176 中所示之胺基酸序列之重鏈，及含有 SEQ ID NO: 175 中所示之胺基酸序列之輕鏈。

【0128】 於特定實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為 CD3 及第二靶點為 TROP2。

【0129】 於特定實施例中，雙特異性抗體結合至第一及第二靶點，其中第一靶點為人類 CD28，及其中雙特異性抗體包含選自由 SEQ ID NOs: 13、22、31、41、49、56、64、73、79、86、94、101、109、117、124、141、132、139、146、153、159 及 167 組成之群之重鏈胺基酸序列，及選自由 SEQ ID NOs: 14、23、32、42、50、57、65、74、80、87、95、102、110、118、125、142、133、140、147、154、160 及 168 組成之群之輕鏈胺基酸序列。

V. 經遮蔽抗體

【0130】 於一些實施例中，本公開提供經遮蔽抗體，其可為針對特定靶點之經遮蔽單克隆抗體或多特異性(例如，雙特異性)抗體。於一些實施例中，本文提供之經遮蔽抗體包含全長抗體輕鏈，例如，κ 或 λ 輕鏈。此外或者，於一些實施例中，抗體包含全長抗體重鏈。抗體之重鏈可為任何類別，諸如 IgG、IgM、IgE、IgA 或 IgD。於一些實施例中，抗體重鏈為 IgG 類別，諸如 IgG_1、IgG_2、IgG_3 或 IgG_4 子類別。可使用此項技術中已知之方法將抗體重鏈自一種類

別或子類別轉變成另一種類別或子類別。於一些實施例中，經遮蔽抗體係或包含含有 Fc 區(例如，人類 Fc 區或其變體)之全長抗體。於一些實施例中，人類 Fc 區係人類 IgG_1 Fc 區，人類 IgG_2 Fc 區，人類 IgG_4 Fc 區，或前述任一項之變體。於一些實施例中，相對於衍生變體之野生型人類 Fc 區，Fc 區變體包含一個或多個胺基酸取代、插入或刪除。於一些實施例中，經遮蔽抗體包含人類 IgG_1 Fc 區變體。於一些實施例中，IgG_1 Fc 變體包含增加 Fc 變體與 FcγRIIb 親和力之一個或多個胺基酸取代。於一些實施例中，人類 IgG_1 Fc 區變體包含選自由 G236D；L328F；S239D；S267E；G236D 及 S267E；S239D 及 S267E；S267E 及 L328S；以及 S267E 及 L328F 組成之群之取代，其中胺基酸編號根據 EU 索引(參見，例如 Edelman 等人，Proc Natl Acad Sci USA （1969) 63: 78-85)。前述取代於 Chu 等人 Mol Immunol. (2008) 45(15):3926-33 中描述。此外或者，於一些實施例中，人類 IgG_1 Fc 區變體包含選自由 E233D 及 P238D；G237D 及 P238D；H268D 及 P238D；P271G 及 P238D；A330R 及 P238D；E233D，P238D 及 A330R；E233D，P231G，P238D 及 A330R；G237D，H268D，P238D 及 P271G；G237D，P238D，P271G 及 A330R；E233D，H268D，P238D，P271G 及 A330R；G237D，H268D，P238D，P271G 及 A330R；以及 E233D，G237D，P238D，H268D，P271G 及 A330R 組成之群之取代，其中胺基酸編號根據 EU 索引。前述取代於 Mimoto 等人 Protein Eng Des Sel. (2013) 26(10):589-98 中描述。此外或者，於一些實施例中，人類 IgG_1 Fc 區變體包含 S2657A

取代(參見 Buschor 等人 Int Arch Allergy Immunol. (2014) 163(3):206-14)，其中胺基酸編號根據 EU 索引。此外或者，於一些實施例中，人類 IgG_1 Fc 區變體包含 T437R 及/或 K248E 取代(參見 Zhang 等人 MAbs. (2017) 9(7):1129-1142)，其中胺基酸編號根據 EU 索引。於一些實施例中，經遮蔽抗體包含人類 IgG_4 Fc 區變體。於一些實施例中，IgG_4 Fc 變體包含增加 Fc 變體與 FcγRIIb 親和力之一個或多個胺基酸取代。於一些實施例中，人類 IgG_4 Fc 區變體包含選自由 G236D；L328F；S239D；S267E；G236D 及 S267E；S239D 及 S267E；S267E 及 L328S；以及 S267E 及 L328F 組成之群之取代，其中胺基酸編號根據 EU 索引。此外或者，於一些實施例中，人類 IgG_4 Fc 區變體包含選自由 E233D 及 P238D；G237D 及 P238D；H268D 及 P238D；P271G 及 P238D；A330R 及 P238D；E233D，P238D 及 A330R；E233D，P231G，P238D 及 A330R；G237D，H268D，P238D 及 P271G；G237D，P238D，P271G 及 A330R；E233D，H268D，P238D，P271G 及 A330R；G237D，H268D，P238D，P271G 及 A330R；以及 E233D，G237D，P238D，H268D，P271G 及 A330R 組成之群之取代，其中胺基酸編號根據 EU 索引。此外或者，於一些實施例中，人類 IgG_4 Fc 區變體包含 S2657A 取代，其中胺基酸編號根據 EU 索引。此外或者，於一些實施例中，人類 IgG_1 Fc 區變體包含 T437R 及/或 K248E 取代，其中胺基酸編號根據 EU 索引。

【0131】 於一些實施例中，本文所述經遮蔽抗體另包含人類 IgG_1

結構域或其變體含有一個或多個取代突變。於一些實施例中，IgG_1變體包含選自由 G236D；L328F；S239D；S267E；G236D 及 S267E；S239D 及 S267E；S267E 及 L328S；以及 S267E 及 L328F；E233D 及 P238D；G237D 及 P238D；H268D 及 P238D；P271G 及 P238D；A330R 及 P238D；E233D，P238D 及 A330R；E233D，P231G，P238D 及 A330R；G237D，H268D，P238D 及 P271G；G237D，P238D，P271G 及 A330R；E233D，H268D，P238D，P271G 及 A330R；G237D，H268D，P238D，P271G 及 A330R；以及 E233D，G237D，P238D，H268D，P271G 及 A330R；S2657A；T437R；K248E；以及 T437R 及 K248E 組成之群之取代，其中胺基酸編號根據 EU 索引。於一些實施例中，本文所述經遮蔽抗體另包含人類 IgG_4 結構域或其變體含有一個或多個取代突變。於一些實施例中，IgG_4 變體包含選自由 G236D；L328F；S239D；S267E；G236D 及 S267E；S239D 及 S267E；S267E 及 L328S；以及 S267E 及 L328F；E233D 及 P238D；G237D 及 P238D；H268D 及 P238D；P271G 及 P238D；A330R 及 P238D；E233D，P238D 及 A330R；E233D，P231G，P238D 及 A330R；G237D，H268D，P238D 及 P271G；G237D，P238D，P271G 及 A330R；E233D，H268D，P238D，P271G 及 A330R；G237D，H268D，P238D，P271G 及 A330R；以及 E233D，G237D，P238D，H268D，P271G 及 A330R；S2657A；T437R；K248E；以及 T437R 及 K248E 組成之群之取代，其中胺基酸編號根據 EU 索引。

【0132】 於一些實施例中，術語「經遮蔽抗體」係指抗體片段，例如，經遮蔽抗 CD28 抗體之經遮蔽抗原結合片段。於一些實施例中，該抗體片段係或包含 Fab、Fab'、Fab'-SH、F(ab')2、Fv、scFv (參見 Bird 等人(1988) Science 242:423-426 及 Huston 等人(1988) Proc. Natl. Acad. Sci. USA 85:5879-5883)、$(scFv)_2$、線性抗體、單鏈抗體、微型抗體(minibody)或雙特異抗體(diabody)。

【0133】 於一些實施例中，本文所述經遮蔽抗 CD28 抗體與來自不同物種之 CD28 交叉反應，從而允許經遮蔽抗 CD28 抗體用於臨床前及臨床研究。於一些實施例中，本文所述經遮蔽抗 CD28 抗體活化後(例如，經遮蔽抗體藉由裂解(例如，蛋白酶裂解)活化後)結合至人類 CD28、食蟹獼猴 CD28、鼠類(小鼠) CD28 及/或大鼠 CD28 中之兩種或更多種。於一些實施例中，經遮蔽抗 CD28 抗體活化後(例如，經遮蔽抗體藉由裂解(例如，蛋白酶裂解)活化後)結合人類 CD28、食蟹獼猴 CD28、鼠類(小鼠) CD28 及大鼠 CD28。

【0134】 於一些實施例中，本文所述經遮蔽抗 CD28 抗體具有背景依賴性(例如，於某些背景下(諸如於蛋白酶濃化腫瘤微環境中)經活化(僅能結合其靶))。於一些實施例中，本文所述經遮蔽抗 CD28 抗體提供超過更多傳統非遮蔽抗體之提高之安全性(例如，顯示降低之毒性，不誘導許多器官之重量之顯著改變，不改變肝臟組織病理學、血液學及/或血液生物化學等)。於一些實施例中，本文所述經遮蔽抗 CD28 抗體展示出與傳統非遮蔽抗 CD28 抗體類似之藥物動力學性質(例如，具有類似之活體內半衰期)。於一些實施例中，

本文所述經遮蔽抗 CD28 抗體相較於更多傳統非遮蔽抗 CD28 抗體展示改善之藥物動力學性質(例如，具有更長活體內半衰期)。

【0135】 於一些實施例中，本文所述經遮蔽抗 CD28 抗體之抗體重鏈可變區(V_H)及抗體輕鏈可變區(V_L)形成結合 hCD28 之抗原結合域(ABD)。於一些實施例中，本文所述經遮蔽抗 CD28 抗體之遮蔽性單元(MU)結合至 ABD 及相較於缺少 MU 之對應抗 CD28 抗體結合至 hCD28 及/或相較於 ABD 結合至 hCD28 減少或抑制經遮蔽抗 CD28 抗體結合至 hCD28。

【0136】 於一些實施例中，在從本文提供之經遮蔽抗體中去除 MU 之前，遮蔽性單元具有至少約 2.0 之遮蔽效率(例如，至少約 2.0，至少約 3.0，至少約 4.0，至少約 5.0，至少約 6.0，至少約 7.0，至少約 8.0，至少約 9.0，至少約 10，至少約 25，至少約 50，至少約 75，至少約 100，至少約 150，至少約 200，至少約 300，至少約 400，至少約 500，至少約 600，至少約 700，至少約 800，至少約 900，至少約 1000，至少約 1100，至少約 1200，至少約 1300，至少約 1400，至少約 1500 等，包括這些值之間之任何範圍)。例如，於一些實施例中，經遮蔽抗 CD28 抗體之遮蔽效率量測為包含遮蔽性單元(MU)之經遮蔽抗 CD28 抗體與 hCD28 結合(例如，在經遮蔽抗體活化前)相對於缺少 MU 之抗 CD28 抗體結合 hCD28 之親和力差異。於另一實例中，遮蔽效率量測為包含 MU 之經遮蔽抗 CD28 抗體對 hCD28 之親和力(例如，在經由裂解(例如，蛋白酶裂解)活化經遮蔽抗體前)相對於未遮蔽抗 CD28 抗體(例如，經由裂解(例

如，蛋白酶裂解)活化經遮蔽抗體後)對 hCD28 之親和力差異。於一些實施例中，遮蔽效率可以藉由包含 MU 之經遮蔽抗體(例如，在活化前)之 EC_{50} 除以對相同靶點特異性缺少遮蔽性肽或經遮蔽單元之相應抗體之 EC_{50} 來量測。於一些實施例中，EC_{50} 藉由 ELISA 來量測。於一些實施例中，經遮蔽抗體之遮蔽性單元(MU)結合至 ABD，及預防經遮蔽多肽結合至其靶點。於特定實施例中，靶點為 CD28。於其它實施例中，靶點為 CD3、B7-H3、HER2、或 TROP2。

【0137】 於一些實施例中，相較於同一靶標但缺少遮蔽性肽或遮蔽性單元之相應特異性抗體，當遮蔽性單元從抗體中去除時(例如，藉由一種或多種連接單元內裂解之蛋白酶處理活化後)，本揭示案之經遮蔽抗體之親和力增加至少約 2 倍(例如，至少約 2 倍、至少約 2.5 倍、至少約 3 倍、至少約 3.5 倍、至少約 4 倍、至少約 4.5 倍、至少約 5 倍、至少約 5.5 倍、至少約 6 倍、至少約 6.5 倍、至少約 7 倍、至少約 7.5 倍、至少約 8 倍、至少約 8.5 倍、至少約 9 倍、至少約 9.5 倍、至少約 10 倍、至少約 25 倍、至少約 50 倍、至少約 75 倍、至少約 100 倍、至少約 250 倍、至少約 500 倍、至少約 750 倍、或至少約 1000 倍、或更多，包括前述值之間之任何範圍)。於一些實施例中，本文所述經遮蔽抗體之 EC_{50} 藉由一種或多種連接單元內裂解之蛋白酶處理活化後減少至少約 2 倍(例如，至少約 2 倍、至少約 2.5 倍、至少約 3 倍、至少約 3.5 倍、至少約 4 倍、至少約 4.5 倍、至少約 5 倍、至少約 5.5 倍、至少約 6 倍、至少約 6.5 倍、至少約 7 倍、至少約 7.5 倍、至少約 8 倍、至少約

8.5 倍、至少約 9 倍、至少約 9.5 倍、至少約 10 倍、至少約 25 倍、至少約 50 倍、至少約 75 倍、至少約 100 倍、至少約 250 倍、至少約 500 倍、至少約 750 倍、或至少約 1000 倍、或更多，包括前述值之間之任何範圍) (例如，藉由 ELISA 或 FACS 檢定量測)。

【0138】 於一些實施例中，當遮蔽性單元結合至本文所述經遮蔽抗體之 ABD 時，抗體對其靶之 K_D 約為遮蔽性單元從經遮蔽抗 CD28 抗體之 ABD 去除時(例如經連接單元內裂解之蛋白酶處理後)抗體之 K_D 之 2 倍(例如，約 2，約 2.5，約 3，約 3.5，約 4，約 4.5，約 5，約 5.5，約 6，約 6.5，約 7，約 7.5，約 8，約 8.5，約 9，約 9.5，約 10，約 25，約 50，約 75，約 100，約 250，約 500，約 750，或約 1000 或更多，包括前述值之間之任何範圍)。於一些實施例中，當遮蔽性單元結合至本文所述經遮蔽抗體之 ABD 時，抗體對其靶之 K_D 約為對相同靶點特異性缺少遮蔽性肽或遮蔽性單元之相應抗體之 K_D 之 2 倍(例如，約 2，約 2.5，約 3，約 3.5，約 4，約 4.5，約 5，約 5.5，約 6，約 6.5，約 7，約 7.5，約 8，約 8.5，約 9，約 9.5，約 10，約 25，約 50，約 75，約 100，約 250，約 500，約 750，或約 1000 或更多，包括前述值之間之任何範圍)。

【0139】 於一些實施例中，該遮蔽性單元空間阻礙經遮蔽結合多肽結合至其靶及/或變構阻礙經遮蔽結合多肽結合至其靶。

【0140】 於一些實施例中，遮蔽性單元對本文所述經遮蔽抗體(例如，抗 CD28)對 ABD 之解離常數高於經遮蔽抗體對其靶點之解離常數(例如，hCD28，當經遮蔽抗體處於活化形式，例如經由蛋白

酶處理後)。於一些實施例中，遮蔽性單元對本文所述經遮蔽抗體之 ABD 之解離常數高於經遮蔽抗體(例如，抗 CD28)對其靶點之解離常數(例如，hCD28，當經遮蔽抗體處於活化形式，例如經由蛋白酶處理後)約 2 (例如，約 2、約 2.5、約 3、約 3.5、約 4、約 4.5、約 5、約 5.5、約 6、約 6.5、約 7、約 7.5、約 8、約 8.5、約 9、約 9.5、約 10、約 25、約 50、約 75、約 100、約 250、約 500、約 750、或約 1000 或更多，包括前述值之間之任何範圍)倍。於一些實施例中，遮蔽性單元對本文所述經遮蔽抗體(例如，抗 CD28)對 ABD 之解離常數約等於經遮蔽抗體對其靶點之解離常數(例如，hCD28，當經遮蔽抗體處於活化形式，例如經由蛋白酶處理後)。於一些實施例中，當經遮蔽抗體尚未被活化(例如，藉由一種或多種在連接單元內裂解之蛋白酶處理後)時，遮蔽性單元(MU)結合至本文所述經遮蔽抗體(例如，抗 CD28)之 ABD 及預防抗體結合至其靶點(例如，hCD28)。於一些實施例中，活化誘導裂解位點內之多肽之裂解。於一些實施例中，活化誘導多肽之構形變化(例如，遮蔽性單元(MU)之置換)，其導致遮蔽性肽不再防止多肽結合至其靶。

A. 單臂經遮蔽之抗體

【0141】 於一些實施例中，提供經遮蔽單克隆抗體，其包含遮蔽性肽(MP)及結合 CD28 之抗體，其中抗體包含重鏈可變區(V_H)及輕鏈可變區(V_L)，其中 MP 連接至 V_L 之 N 端，其中 MP 自 N 端至 C 端包含遮蔽性單元(MU)，及連接單元(LU)，其中 MP 包含選自由 SEQ

ID NOs: 215-248 組成之群之胺基酸序列；及其中抗體 V_H 區包含選自由 SEQ ID NOs: 5、15、24、33、43、66、88、103、111、126、134、148 及 161 組成之群之 HCDR1 胺基酸序列；選自由 SEQ ID NOs: 6、51、58、67、89、96、104 及 155 組成之群之 HCDR2 胺基酸序列；及選自由 SEQ ID NOs: 7、16、25、35、44、52、59、81、90、97、105、112、119、127、135、141、149 及 162 組成之群之 HCDR3 胺基酸序列;以及抗體 V_L 區包含選自由 SEQ ID NOs: 8、17、26、36、45、53、60、76、82、91、98、106、113、120、128、136、142、150、156 及 163 組成之群之 LCDR1 胺基酸序列；選自由 SEQ ID NOs: 9、18、27、37 及 300 組成之群之 LCDR2 胺基酸序列；及選自由 SEQ ID NOs: 10、19、28、38、46、61、70、83、114、121、129、143 及 164 組成之群之 LCDR3 胺基酸序列。

【0142】 於一些實施例中，提供經遮蔽單克隆抗體，其包含遮蔽性肽(MP)及結合 CD28 之抗體，其中抗體包含重鏈可變區(V_H)及輕鏈可變區(V_L)，其中 MP 連接至 V_L 之 N 端，其中 MP 自 N 端至 C 端包含遮蔽性單元(MU)，及連接單元(LU)，其中 MP 包含選自由 SEQ ID NOs: 215-248 組成之群之胺基酸序列；及其中抗體重鏈可變區包含選自由 SEQ ID NOs: 11、20、29、39、47、54、62、71、77、84、92、99、107、115、122、130、137、144、151、157 及 165 組成之群之胺基酸序列；及輕鏈可變區包含選自由 SEQ ID NOs: 12、21、30、40、48、55、63、72、78、85、93、100、108、116、123、131、138、145、152、158 及 166 組成之群之胺基酸序列。於一些

實施例中，提供經遮蔽單克隆抗體，其包含遮蔽性肽(MP)及結合人類 CD28 之抗體，其中抗體包含重鏈及輕鏈，其中 MP 連接至 LC 之 N 端，其中 MP 自 N 端至 C 端包含遮蔽性單元(MU)，及連接單元(LU)，其中 MP 包含選自由 SEQ ID NOs: 215-248 組成之群之胺基酸序列，其中抗體 HC 包含選自由 SEQ ID NOs: 13、22、31、41、49、56、64、73、79、86、94、101、109、117、124、141、132、139、146、153、159 及 167 組成之群之胺基酸序列，及其中抗體 LC 包含選自由 SEQ ID NOs: 14、23、32、42、50、57、65、74、80、87、95、102、110、118、125、142、133、140、147、154、160 及 168 組成之群之胺基酸序列。

B. 單臂經遮蔽之多特異性抗體

【0143】 於一些實施例中，亦提供對第一及第二靶點特異性之經遮蔽雙特異性單克隆抗體，其中第一靶點為 CD28，其中抗體包含遮蔽性肽(MP)及 CD28 結合部分，其中抗體包含重鏈可變區(V_H)及輕鏈可變區(V_L)，其中 MP 連接至 V_L 之 N 端，其中 MP 自 N 端至 C 端包含遮蔽性單元(MU)及連接單元(LU)，其中 MP 包含選自由 SEQ ID NOs: 215-248 組成之群之胺基酸序列，其中 CD28 結合部分包含 V_H 區，其含有 SEQ ID NO: 5 之 HCDR1、SEQ ID NO: 6 之 HCDR2 及 SEQ ID NO: 7 之 HCDR3，以及 V_L 區，其含有 SEQ ID NO: 8 之 LDR1、SEQ ID NO: 9 之 LCDR2 及 SEQ ID NO: 10 之 LCDR3，及其中第二靶點為 B7-H3、HER2 或 TROP2 蛋白。

【0144】 於一些實施例中，亦提供對第一及第二靶點特異性之經

遮蔽雙特異性單克隆抗體，其中第一靶點為 CD28，其中抗體包含遮蔽性肽(MP)及 CD28 結合部分，其中抗體包含重鏈可變區(V_H)及輕鏈可變區(V_L)，其中 MP 連接至 V_L 之 N 端，其中 MP 自 N 端至 C 端包含遮蔽性單元(MU)及連接單元(LU)，其中 MP 包含選自由 SEQ ID NOs: 215-248 組成之群之胺基酸序列，及其中 CD28 結合部分包含包含選自由 SEQ ID NOs: 11、20、29、39、47、54、62、71、77、84、92、99、107、115、122、130、137、144、151、157 及 165 組成之群之胺基酸序列之重鏈可變區；及包含選自由 SEQ ID NOs: 12、21、30、40、48、55、63、72、78、85、93、100、108、116、123、131、138、145、152、158 及 166 組成之群之胺基酸序列之輕鏈可變區，及其中第二靶點為 B7-H3、HER2 或 TROP2 蛋白。

C. 雙臂經遮蔽之多特異性抗體

【0145】 於特定實施例中，亦提供對第一及第二靶點特異性之經遮蔽雙特異性單克隆抗體，其中第一靶點為 CD28 及第二靶點為選自 HER2、B7-H3 及 TROP2 之腫瘤相關抗原，其中抗體包含兩個遮蔽性肽(MP)。

VI. 遮蔽性肽

【0146】 於一些實施例中，MP 另包含 N 端單元。於一些實施例中，N 端單元長度在約 1 至 10 個胺基酸之間。於一些實施例中，N 端單元包含 SEQ ID NO: 210。於一些實施例中，LU 包含至少一個第一裂解位點(CS_1) (例如第一蛋白酶裂解位點)。於一些實施例

中，LU 另包含第二裂解位點(CS_2)。於一些實施例中，第一及/或第二裂解位點為蛋白酶裂解位點。於一些實施例中，第一及第二裂解位點相同。於一些實施例中，第一及第二裂解位點不同。可使用藉由此項技術中已知之任何蛋白酶(例如，已知與包含裂解位點之可活化抗體之靶共定位之蛋白酶)識別及/或裂解之任何適宜蛋白酶裂解位點，包含例如，藉由以下識別及/或裂解之蛋白酶裂解位點：尿激酶型纖維蛋白溶酶原活化因數(uPA)、基質金屬蛋白酶(例如，MMP-1、MMP-2、MMP-3、MMP-7、MMP-8、MMP-9、MMP-10、MMP-11、MMP-12、MMP-13、MMP-14、MMP-15、MMP-16、MMP-17、MMP-19、MMP-20、MMP-23、MMP-24、MMP-26 及/或 MMP-27)、煙草蝕紋病毒(TEV)蛋白酶、胞漿素、凝血酶、PSA、PSMA、ADAMS/ADAMTS (例如，ADAM 8、ADAM 9、ADAM 10、ADAM 12、ADAM 15、ADAM 17/TACE、ADAMDEC1、ADAMTS1、ADAMTS4 及/或 ADAMTS5)、卡斯蛋白酶(例如，卡斯蛋白酶-1、卡斯蛋白酶-2、卡斯蛋白酶-3、卡斯蛋白酶-4、卡斯蛋白酶-5、卡斯蛋白酶-6、卡斯蛋白酶-7、卡斯蛋白酶-8、卡斯蛋白酶-9、卡斯蛋白酶-10、卡斯蛋白酶-11、卡斯蛋白酶-12、卡斯蛋白酶-13 及/或卡斯蛋白酶-14)、天冬胺酸蛋白酶(例如，RACE 及/或腎素(Renin))；天冬胺酸組織蛋白酶(例如，組織蛋白酶 D 及/或組織蛋白酶 E)、半胱胺酸組織蛋白酶(例如，組織蛋白酶 B、組織蛋白酶 C、組織蛋白酶 K、組織蛋白酶 L、組織蛋白酶 S、組織蛋白酶 V/L2 及/或組織蛋白酶 X/Z/P)、半胱胺酸蛋白酶(例如，Cruzipain、Legumain

及/或 Otubain-2)、KLK (例如，KLK4、KLK5、KLK6、KLK7、KLK8、KLK10、KLK11、KLK13 及/或 KLK14)、金屬蛋白酶(例如，Meprin、奈溶酶(Neprilysin)、PSMA 及/或 BMP-1)、絲胺酸蛋白酶(例如，經活化之蛋白 C、組織蛋白酶 A、組織蛋白酶 G、胃促胰酶(Chymase)及/或凝血因數蛋白酶(諸如 FVIIa、FIXa、Fxa、FXIa、FXIIa))、彈性蛋白酶、粒酶 B、胍基苯甲酸酶(guanidino-benzoatase)、HtrA1、人類嗜中性白血球彈性蛋白酶、乳鐵蛋白(lactoferrin)、marapsin、NS3/4A、PACE4、tPA、類胰蛋白酶(tryptase)、II 型跨膜絲胺酸蛋白酶(TTSP) (例如，DESC1、DPP-4、FAP、第二型穿膜絲胺酸蛋白酶(Hepsin)、間質蛋白酶(Matriptase)-2、MT-SP1/間質蛋白酶、TMPRSS2、TMPRSS3 及/或 TMPRSS4)等。於一些實施例中，第一蛋白酶裂解位點為選自 uPA、MMP-1、MMP-2、MMP-3、MMP-8、MMP-9、MMP-14、TEV 蛋白酶、胞漿素、凝血酶、因數 X、PSA、PSMA、組織蛋白酶 D、組織蛋白酶 K、組織蛋白酶 S、ADAM10、ADAM12、ADAMTS、卡斯蛋白酶-1、卡斯蛋白酶-2、卡斯蛋白酶-3、卡斯蛋白酶-4、卡斯蛋白酶-5、卡斯蛋白酶-6、卡斯蛋白酶-7、卡斯蛋白酶-8、卡斯蛋白酶-9、卡斯蛋白酶-10、卡斯蛋白酶-11、卡斯蛋白酶-12、卡斯蛋白酶-13、卡斯蛋白酶-14 及 TACE 蛋白酶之裂解位點。於一些實施例中，第一蛋白酶裂解位點選自 uPA、MMP-2、MMP-9 及/或 TEV 蛋白酶之裂解位點。

【0147】 於一些實施例中，LU 另包含第一連接子(L_1)。於一些實施例中，第一連接子(L_1)為第一裂解位點(CS_1) (例如，第一蛋白酶

裂解位點)之 C 端。於一些實施例中，LU 自 N 端至 C 端包含(CS_1)-L1 之結構。於一些實施例中，LU 另包含第二連接子(L_2)。於一些實施例中，L_2 為第二裂解位點之 C 端。於一些實施例中，LU 自 N 端至 C 端包含(CS_1)-L_1-(CS_2)-L_2 之結構。於一些實施例中，L_1 及 L_2 為此項技術中已知之任何適宜連接子(例如，可撓性連接子)，包括但不限於例如，甘胺酸聚合物(G)n，其中 n 為至少 1 (例如，至少 1、至少 2、至少 3、至少 4、至少 5、至少 6、至少 7、至少 8、至少 9、至少 10 等)之整數；甘胺酸-絲胺酸聚合物(GS)n，其中 n 為至少 1 (例如，至少 1、至少 2、至少 3、至少 4、至少 5、至少 6、至少 7、至少 8、至少 9、至少 10 等)之整數，諸如 SEQ ID NOs: 249-257;甘胺酸-丙胺酸聚合物;丙胺酸-絲胺酸聚合物;及類似者。連接子序列可為任何長度，諸如約 1 個胺基酸(例如，甘胺酸或絲胺酸)至約 20 個胺基酸(例如，20 個胺基酸甘胺酸聚合物或甘胺酸-絲胺酸聚合物)、約 1 個胺基酸至約 15 個胺基酸、約 3 個胺基酸至約 12 個胺基酸、約 4 個胺基酸至約 10 個胺基酸、約 5 個胺基酸至約 9 個胺基酸、約 6 個胺基酸至約 8 個胺基酸等。於一些實施例中，連接子為長度約 1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、或 20 個胺基酸中之任一者。

【0148】 於一些實施例中，LU 包含選自由 SEQ ID NOs: 213 及 214 組成之群之胺基酸序列。於一些實施例中，遮蔽性肽(MP)自 N 端至 C 端包含(MU)-(LU)之結構，其中 LU 包含(CS_1)-L_1 或(CS_1)-L_1-(CS_2)-L_2 之結構。於一些實施例中，本揭示案之遮蔽性肽包含選自

由 SEQ ID NOs: 215-248 組成之群之胺基酸序列。

【0149】 於一些實施例中，遮蔽性肽(MP)包含 SEQ ID NOs: 178-211 中任一者所示之 MU 及 SEQ ID NO: 213 或 214 中所示之 LU。於一些實施例中，MP 包含 SEQ ID NOs: 215-248 中任一者所示之序列。

VII. 抗體產生

【0150】 本發明之另一態樣提供一種或多種經單離核酸分子，其包含編碼本文中所描述之經遮蔽抗 CD28 抗體之胺基酸序列之核苷酸序列，包括經遮蔽抗 CD28 抗體。於一些實施例中，提供一種或多種經單離核酸分子，其包含編碼本文中所描述之多特異性抗體之胺基酸序列之核苷酸序列，包括經遮蔽多特異性抗體。藉由核苷酸序列編碼之胺基酸序列可為抗體之任何部分(諸如 CDR、包含 1、2 或 3 個 CDR 之序列、重鏈可變區、輕鏈可變區)，或可為全長重鏈或全長輕鏈。本發明之核酸可為例如 DNA 或 RNA，且可含或可不含內含子序列。通常，核酸為 cDNA 分子。

【0151】 於一些實施例中，本發明提供經單離核酸分子，其包含編碼胺基酸序列之核苷酸序列或由該核苷酸序列組成，例如，本文中所述抗體之重鏈可變區及/或輕鏈可變區，或例如，本文中所述抗體之全長重鏈或全長輕鏈。

【0152】 可使用任何適宜分子生物技術(例如，PCR 擴增或 cDNA 選殖技術)獲得本發明之核酸。針對獲自文庫篩選之本文所述抗體，編碼該抗體之核酸可自該庫恢復。

【0153】 編碼 V_H 區之經單離 DNA 可藉由將編碼 V_H 之 DNA 可操作連接至編碼重鏈恆定區(C_H1、C_H2 及 C_H3)之另一 DNA 分子轉化成全長重鏈基因。人類重鏈恆定區基因之序列為此項技術中已知(參見例如 Kabat 等人(1991) Sequences of Proteins of Immunological Interest，第 5 版，美國衛生與公眾服務部，NIH 出版物第 91-3242 號)，且涵蓋該等區域之 DNA 片段可藉由標準 PCR 擴增獲得。重鏈恆定區可為 IgG_1、IgG_2、IgG_3、IgG_4、IgA、IgE、IgM 或 IgD 恆定區，但最佳地為不具 ADCC 效應之 IgG_4 或 IgG_2 恆定區。IgG_4 恆定區序列可為已知出現在不同個體中之多種對偶基因或異型中之任一者。該等異型代表 IgG_4 恆定區中之天然胺基酸取代。對於 Fab 片段重鏈基因，編碼 V_H 之 DNA 可操作連接至僅編碼重鏈 C_H1 恆定區之另一 DNA 分子。

【0154】 編碼 V_L 區之經單離 DNA 可藉由將編碼 V_L 之 DNA 可操作連接至編碼輕鏈恆定區 C_L 之另一 DNA 分子轉化成全長輕鏈基因(以及 Fab 輕鏈基因)。人類輕鏈恆定區基因之序列為此項技術中已知(參見例如 Kabat 等人(1991) Sequences of Proteins of Immunological Interest，第 5 版，美國衛生與公眾服務部，NIH 出版物第 91-3242 號)，且涵蓋該等區域之 DNA 片段可藉由標準 PCR 擴增獲得。輕鏈恆定區可為 κ 或 λ 恆定區。

【0155】 為產生 scFv 基因，將編碼 V_H 及 V_L 之 DNA 片段可操作連接至編碼撓性連接體(例如編碼胺基酸序列$(Gly_4\text{-}Ser)_3$)之另一片段，使得 V_H 及 V_L 序列可表現為連續單鏈蛋白，且 V_L 區及 V_H 區

藉由撓性連接體連結(參見例如 Bird 等人，Science (1988) 242:423-426；Huston 等人，Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883；及 McCafferty 等人，Nature (1990) 348:552-554)。

【0156】 本揭示案另提供包含本文中所述之一種或多種核酸分子之載體。於一些實施例中，載體係可用於表現本文所述抗體或此種抗體之抗原結合片段之表現載體。於一些實施例中，本文提供載體，其中第一載體包含編碼如本文所述重鏈可變區之多核苷酸序列，且第二載體包含編碼如本文所述輕鏈可變區之多核苷酸序列。於一些實施例中，單一載體包含編碼如本文所述重鏈可變區及如本文所述輕鏈可變區之多核苷酸。

【0157】 為表現本揭示案之結合分子，將編碼部分或全長輕鏈及重鏈之 DNA 插入表現載體中，使得 DNA 分子可操作連接至轉錄及轉譯控制序列。在此上下文中，術語「可操作連接」意指抗體基因聯結至載體中，使得載體內之轉錄及轉譯控制序列發揮其調控 DNA 分子之轉錄及轉譯之預期功能。表現載體及表現控制序列經選擇以與所用表現宿主細胞相容。抗體輕鏈基因及抗體重鏈基因可插入單獨載體中，或更通常，將兩種基因插入同一表現載體中。抗體基因係藉由任何適宜方法(例如聯結抗體基因片段及載體上之互補限制位點或基於同源重組之 DNA 聯結)插入表現載體中。本文所述抗體之輕鏈及重鏈可變區可藉由將其插入已編碼期望同型及子類之重鏈恆定區及輕鏈恆定區之表現載體中，使得 V_H 區段可操作連接至載體內之 C_H 區段且 V_L 區段可操作連接至載體內之

C_L 區段，用於產生任一抗體同型及子類之全長抗體基因。另外或替代地，重組表現載體可編碼促進抗體鏈自宿主細胞分泌之信號肽。可將抗體鏈基因選殖至載體中，使得信號肽框內連接至抗體鏈基因之胺基末端。信號肽可為免疫球蛋白信號肽或異源信號肽(即來自非免疫球蛋白蛋白之信號肽)。

【0158】 除抗體鏈基因外，本揭示案之表現載體通常攜帶控制抗體鏈基因在宿主細胞中之表現之調控序列。術語「調控序列」意欲包括啟動子、增強子及控制抗體鏈基因之轉錄或轉譯之其他表現控制元件(例如多腺苷酸化信號)。此等調控序列闡述於例如Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990))中。熟習此項技術者應瞭解，表現載體之設計(包括調控序列之選擇)可端視諸如欲轉型宿主細胞之選擇、期望蛋白質之表現水準等因素而定。用於哺乳動物宿主細胞表現之調控序列之實例包括引導哺乳動物細胞中之高蛋白質表現水準之病毒元件，例如衍生自巨細胞病毒(CMV)、猿猴病毒40 (SV40)、腺病毒(例如腺病毒主要晚期啟動子(AdMLP))及多瘤病毒之啟動子及/或增強子。替代地，可使用非病毒調控序列，例如泛素啟動子或 β-球蛋白啟動子。另外，調控元件由不同來源之序列構成，例如 SR 啟動子系統，其含有來自 SV40 早期啟動子及 1 型人類 T 細胞白血病病毒之長末端重複之序列(Takebe, Y.等人 Mol. Cell. Biol. (1988) 8:466-472)。

【0159】 除抗體鏈基因及調控序列外，表現載體可攜帶其他序列，

例如增強子元件，轉錄終止序列，調控載體在宿主細胞中之複製之序列(例如複製起點)及可選擇標記基因。可選擇標記基因促進已引入載體之宿主細胞之選擇(參見例如美國專利第 4,399,216 號、美國專利第 4,634,665 號及美國專利第 5,179,017 號，其皆來自 Axel 等人)。舉例而言，可選擇標記基因通常賦予其中已引入載體之宿主細胞對藥物(例如 G418、潮黴素(hygromycin)或胺甲喋呤(methotrexate))之抗性。可選擇標記基因包括二氫葉酸還原酶(DHFR)基因(用於利用胺甲喋呤選擇/擴增之 dhfr-宿主細胞中)及 neo 基因(用於 G418 選擇)。

【0160】 對於輕鏈及重鏈之表現，藉由任何適宜技術將編碼重鏈及輕鏈之表現載體轉染至宿主細胞中。術語「轉染」之各種形式意欲涵蓋常用於將外源 DNA 引入原核或真核宿主細胞中之眾多種技術，例如電穿孔、磷酸鈣沈澱、DEAE-葡聚糖轉染及諸如此類。儘管可在原核或真核宿主細胞中表現本文所述抗體，但在真核細胞，例如哺乳動物宿主細胞中表現抗體係最典型。

【0161】 本揭示案進一步提供含有本揭示案所提供之核酸分子或載體之宿主細胞。宿主細胞實質上可為表現載體可用之任何細胞。其可為例如較高等真核宿主細胞(例如哺乳動物細胞)、較低等真核宿主細胞(例如酵母菌細胞)，且可為原核細胞(例如細菌細胞)。將重組核酸構築物引入宿主細胞中可藉由磷酸鈣轉染、DEAE、葡聚糖介導之轉染、電穿孔或噬菌體感染來實現。

【0162】 適用於轉型之原核宿主包括大腸桿菌、枯草桿菌(*Bacillus*

subtilis)、鼠傷寒沙氏桿菌(*Salmonella typhimurium*)及假單胞菌屬(*Pseudomonas*)、鏈黴菌屬(*Streptomyces*)及葡萄球菌屬(*Staphylococcus*)內之各個物種。

【0163】 用於表現本揭示案之結合分子之哺乳動物宿主細胞包括例如中國倉鼠卵巢(CHO)細胞(包括 dhfr-CHO 細胞，闡述於 Urlaub 及 Chasin, Proc. Natl. Acad. Sci. USA (1980) 77:4216-4220 中，與 DHFR 可選擇標記物一起使用，例如如 Kaufman 及 Sharp, J. Mol. Biol. (1982) 159:601-621 中所述)、NS0 骨髓瘤細胞、COS 細胞及 Sp2 細胞。具體而言，為與 NS0 骨髓瘤或 CHO 細胞一起使用，另一表現系統係 WO 87/04462、WO 89/01036 及 EP 338,841 中所揭示之 GS (麩醯胺酸合成酶)基因表現系統。

【0164】 本揭示案之抗體(或其抗原結合片段)可藉由此項技術中任何已知方法來產生。抗體產生之例示性技術見美國專利第 4,816,567 號；然而僅出於說明目的提供此例示性技術，且不旨在限制。當將編碼本文所述抗體之核酸或表現載體引入到宿主細胞中時，通過將宿主細胞培養一段足以允許抗體在宿主細胞中表現或使抗體分泌到宿主細胞所生長的培養基中的時間來產生抗體。因此，於一些實施例中，提供一種產生本文所述抗體之方法，此方法包括在適宜抗體表現之條件下培養包含一種或多種編碼抗體(例如，上文中提供)之核酸或載體之宿主細胞。於一些實施例中，該方法另包含從宿主細胞(或宿主細胞培養基)中回收抗體。可使用任何適宜蛋白質純化之方法自培養基中回收抗體。

VIII. 藥物組合物

【0165】 於一些實施例中，本發明提供含有本文所述一種或多種抗體之組合物。於一些實施例中，該組合物為包含本文所述抗體及醫藥上可接受之載劑之醫藥組合物。該等組合物可藉由此項技術中已知之習知方法製備。

【0166】 術語「醫藥學上可接受之載劑」係指適用於調配物中以遞送多肽(例如本文所述抗體)之任何無活性物質。載劑可為抗黏劑、黏合劑、包衣、崩解劑、填充劑或稀釋劑、防腐劑(例如抗氧化劑、抗細菌劑或抗真菌劑)、甜味劑、吸收延遲劑、潤濕劑、乳化劑、緩衝劑及諸如此類。適宜醫藥學上可接受之載劑之實例包括水、乙醇、多元醇(例如乙二醇、丙二醇、聚乙二醇及諸如此類)、右旋糖、植物油(例如橄欖油)、鹽水、緩衝劑、緩衝鹽水及等滲劑(例如糖、多元醇、山梨醇及氯化鈉)。

【0167】 組合物可呈任何適宜形式，例如液體、半固體及固體劑量形式。液體劑量形式之實例包括溶液(例如可注射及可輸注溶液)、微乳液、脂質體、分散液或懸浮液。固體劑量形式之實例包括錠劑、丸劑、膠囊、微膠囊及粉末。適用於遞送本文所述抗體之組合物之特定形式係注射或輸注用無菌液體，例如溶液、懸浮液或分散液。無菌溶液可藉由將所需量之抗體納入適當載劑中、然後無菌微過濾來製備。分散液可藉由將抗體納入含有鹼性分散介質及其他載劑之無菌媒劑中來製備。在用於製備無菌液體之無菌粉末之情形下，製備方法包括真空乾燥及冷凍乾燥(凍乾)，以產生活性成分加

來自其先前無菌過濾溶液之任何其他期望成分的粉末。組合物之各種劑量形式可藉由此項技術中已知之習用技術來製備。

【0168】 組合物中所包括之抗體之相對量將端視多種因素而變化，例如所用之具體多肽及載劑、劑量形式及期望釋放及藥效學特徵。單一劑量形式中抗體之量通常為產生治療效應之量，但亦可為更小量。通常，相對於劑量形式之總重量，此量將介於約 0.01%至約 99%、約 0.1%至約 70%、或約 1%至約 30%範圍內。

【0169】 除了本文所述抗體外，一種或多種另外治療劑可包含於組合物中。於一些實施例中，該至少一種另外治療劑係選自由病毒基因療法、免疫檢查點抑制劑、靶療法、放射療法及化療組成之群。於一些實施例中，該至少一種另外治療劑係選自由以下組成之群：鉑美特(pomalyst)、瑞復美(revlimid)、來那度胺(lenalidomide)、泊馬度胺(pomalidomide)、沙利度胺(thalidomide)、DNA 烷基化含鉑衍生物、順鉑(cisplatin)、5-氟尿嘧啶、環磷醯胺(cyclophosphamide)、抗 CTLA4 抗體、抗 PD-1 抗體、抗 PD-L1 抗體、抗 CD20 抗體、抗 CD40 抗體、抗 DR5 抗體、抗 CD1d 抗體、抗 TIM3 抗體、抗 SLAMF7 抗體、抗 KIR 受體抗體、抗 OX40 抗體、抗 HER2 抗體、抗 ErbB-2 抗體、抗 EGFR 抗體、西妥昔單抗(cetuximab)、利妥昔單抗(rituximab)、曲妥珠單抗(trastuzumab)、派姆單抗(pembrolizumab)、放射療法、單劑量輻射、分次輻射、焦點輻射、全器官輻射、IL-12、IFNα、GM-CSF、嵌合抗原受體、授受性轉移之 T 細胞、抗癌疫苗及溶瘤病毒。待包含於組合物中之另外治療

劑之適宜量可由熟習此項技術者容易選擇，且將取決於許多因素(諸如使用之特定劑及載劑、劑型及所需釋放及藥效動力學特性)變化。包含於單一劑型中之另外治療劑之量一般將為產生治療效應之劑之量，但是亦可為更少量。

【0170】 本文中所述之抗體可經進一步修飾。於一些實施例中，該等抗體連接至另外分子實體。另外分子實體之實例包括醫藥劑、肽或蛋白質、檢測劑或標籤及抗體。

【0171】 於一些實施例中，本文所述抗體連接至醫藥劑。醫藥劑之實例包括細胞毒性劑或其他癌症治療劑及放射性同位素。細胞毒性劑之特定實例包括紫杉醇、細胞鬆弛素 B、短桿菌肽 D、溴化乙錠、依米丁、絲裂黴素、依託泊苷、替尼泊苷、長春新鹼、長春鹼、秋水仙素、多柔比星、柔紅黴素、二羥基蒽醌二酮、米托蒽醌、光神黴素、放線菌素 D、1-去氫睾酮、糖皮質激素、普魯卡因、丁卡因、利多卡因、普萘洛爾及嘌呤黴素及其類似物或同系物。治療劑亦包括例如抗代謝劑(例如，胺甲喋呤、6-巰基嘌呤、6-硫鳥嘌呤、阿糖胞苷、5-氟尿嘧啶、達卡巴嗪)、烷基化劑(例如，二氯甲基二乙胺、噻替派、苯丁酸氮芥、美法侖、卡莫司汀(BSNU)及洛莫司汀(CCNU)、環磷醯胺、白消安、二溴甘露醇、鏈脲佐菌素、絲裂黴素 C 及順-二氯二胺鉑(II) (DDP)順鉑)、蒽環黴素(例如，柔紅黴素(先前稱作道諾黴素)及多柔比星)、抗生素(例如，更生黴素(先前稱作放線菌素)、博來黴素、光神黴素及安麯黴素(AMC))及抗有絲分裂劑(例如，長春新鹼及長春鹼)。可與抗體共軛用於診斷或治療

使用之放射性同位素之實例包括(但不限於)碘 ^{131}、銦 ^{111}、釔 ^{90} 及鐿 ^{177}。用於將多肽連接至醫藥劑之方法係此項技術中已知，諸如使用各種連接子技術。連接子類型之實例包括腙、硫醚、酯、二硫醚及含肽連接子。將治療劑連接至抗體之連接子及方法之進一步討論參見例如，Saito 等人，Adv. Drug Deliv. Rev. (2003) 55:199-15；Trail 等人，Cancer Immunol. Immunother. (2003) 52:328-37；Payne, Cancer Cell (2003) 3:207-12；Allen, Nat. Rev. Cancer (2002) 2:750-63；Pastan 及 Kreitman, Curr. Opin. Investig. Drugs (2002) 3:1089-91；Senter 及 Springer Adv. Drug Deliv. Rev. (2001) 53:247-64。

【0172】 本文中所述之抗體及/或組合物(例如，醫藥組合物)中之任一者可用於製備藥劑(例如，用於治療有需要受試者之癌症或延遲該癌症進展之藥劑)。

IX. 治療方法

【0173】 本文所述抗體及醫藥組合物可用於治療目的，諸如治療癌症或增強其他癌症療法之功效。因此，於一些實施例中，本發明提供使用本文所述抗體或醫藥組合物之方法。於一些實施例中，本發明提供一種治療受試者(例如，人類受試者)癌症之方法，包括對受試者投與有效量之本文所述抗體。於一些實施例中，該癌症為乳癌、肝癌、或結腸直腸癌、胃癌、卵巢癌、肺癌、胰腺癌、或腎癌。

【0174】 於實踐治療方法中，本文所述經遮蔽抗 CD28 抗體可單獨投與，例如，作為單藥療法或與一種或多種另外治療劑或療法組合

投與。因此，於另一態樣中，本發明提供一種組合療法，其包含結合分子與一種或多種另外療法或治療劑組合用於分開、依序或同時投與。於一些實施例中，術語「附加療法」是指不採用本文所述抗體作為治療劑之療法。於一些實施例中，術語「另外的治療劑」係指除了本文所述抗體外之任何治療劑。於一些實施例中，本發明提供一種治療受試者(例如，人類受試者)癌症之方法，其包含對該受試者投與有效量之本文中所述之抗體(例如，抗 CD28 抗體或靶向 CD28 及一個或多個其它靶點之多特異性抗體)及有效量之抗 PD-1 抗體。於一些實施例中，本發明提供一種治療受試者(例如，人類受試者)癌症之方法，其包含對該受試者投與有效量之本文中所述之抗體(例如，抗 CD28 抗體或靶向 CD28 及一個或多個其它靶點之多特異性抗體)及有效量之抗 CTLA4 抗體。於一些實施例中，該抗 CTLA4 抗體為經遮蔽抗 CTLA4 抗體。

X. 套組及製品

【0175】 於一些實施例中，提供一種套組，其包含一個或多個本文所述抗體(例如，抗 CD28 抗體或靶向 CD28 及一個或多個其它靶點之多特異性抗體)。於一些實施例中，該套組另包含一種包裝插入物，其包含本文所述抗體之使用說明。於一些實施例中，製品或套組包含一種容器，其包含一個或多個本文所述經遮蔽抗體或組合物。於某些實施例中，製品或套組包含一種容器，其包含編碼一個(或多個)本文所述經遮蔽抗體之核酸。於一些實施例中，套組包括生產本文所述抗體之細胞株之細胞。於一些實施例中，套組包括

一個或多個 CD28 (例如人類 CD28、食蟹獼猴 CD28、小鼠 CD28、大鼠 CD28 或前述任一片段)或 CD28+細胞之陽性對照。於一些實施例中，套組包括陰性對照，例如幾乎不含 CD28 之表面或溶液，或不表現 CD28 之細胞。

【0176】 於某些實施例中，製品或套組包含容器及在容器上或與容器相關聯之標籤或包裝插頁。適宜容器包括例如瓶、小瓶、注射器、IV 溶液袋、試管等。容器可由多種材料形成，例如玻璃或塑膠。容器容納本文所用之抗體(或包含此抗體之組合物)，該抗體本身或與另一組合物組合投與，可有效治療，延緩進程，及/或預防受試者(例如，人類受試者)癌症。容器可具有無菌進入口(例如，容器可為靜脈內溶液袋或具有可藉由皮下注射針頭刺穿之塞子的小瓶)。於一些實施例中，所述標籤或包裝插頁指示所述組合物用於治療受試者(例如，人類受試者)乳癌、肝癌、或結腸直腸癌。

【0177】 另外，製品或套組可包含(a)含有組合物在內之第一容器，其中組合物包含本文所述抗體(或其免疫活性片段)；及(b)含有組合物在內之第二容器，其中組合物包含另外之細胞毒性劑或其他治療劑。於一些實施例中，第二容器含有包含抗 PD-1 抗體之組合物，及包含指示抗體及抗 PD-L1 用於治療有需要之受試者(例如，人類受試者)結腸癌之標籤或包裝插頁之製品，例如，根據本文提供之方法。於一些實施例中，第二容器含有包含抗 CTLA4 抗體(例如，經遮蔽抗 CTLA4 抗體)之組合物，及包含指示本文所述抗體及抗 CTLA4 抗體(例如，經遮蔽抗 CTLA-4 抗體)用於治療有需要之

受試者(例如,人類受試者)結腸癌之標籤或包裝插頁之製品,例如,根據本文提供之方法。

【0178】 另外,製品可另包含其他容器,其包含醫藥學上可接受之緩衝劑,例如抑菌性注射用水(BWFI)、磷酸鹽緩衝鹽水、林格氏溶液(Ringer's solution)及右旋糖溶液。其可進一步包括自商業及使用者角度來看期望之其他材料,包括其他緩衝劑、稀釋劑、過濾器、針及注射器。

【0179】 認為上述書面描述足以使熟習此項技術者能實踐本發明。僅出於說明目的提供下列實例,且不意欲以任何方式限制本發明之範圍。的確,除了本文中所示及所述之彼等外之本發明之各種修改將自上述描述對熟習此項技術者變得顯而易見且落於隨附申請專利範圍之範圍內。

【0180】 除非本文另外定義,否則與本揭示案結合使用之科學及技術術語應具有普通熟習此項技術者通常理解之含義。例示性方法及材料描述如下,儘管與本文所述方法及材料相似或等效之方法及材料亦可用於本公開之實踐或測試。如有衝突,以本說明書(包括定義)為准。此外,除非上下文另外要求,否則單數術語應包括複數,且複數術語應包括單數。於本說明書及實施例中,字語「具有(have)」及「包含(comprise)」,或變體諸如「具有(has)」、「具有(having)」、「包含(comprises)」或「包含(comprising)」應被理解為包含所述整數或整數組,但不包含任何其它整數或整數組。本文中所引用之所有參考文獻(包括專利申請案、專利及非專利公開案

及 UniProtKB/Swiss-Prot 寄存編號)之全文以引用的方式併入本文中，如同各個別參考文獻明確且個別地指示為以整體引用的方式併入般。僅管本文引用了許多文獻，但該引用並不構成承認此等文獻中之任何一個構成本領域公知常識的一部分。如本文中所用，術語「近似」或「大約」被應用於一個或多個感興趣之值是指類似於所述參考值的值。於某些實施例中，除非另有說明或從上下文中看出，術語係指在所述參考值任一方向(大於或小於)，落在 10%、9%、8%、7%、6%、5%、4%、3%、2%、1%或更小範圍內之值。

【0181】 根據本公開，從屬權利要求中之反向引用係指對反向引用所指示之每一項權利要求組合進行直接及明確披露之速記。本文公開之任何化合物皆可用於此處之任何治療方法，其中待治療個體如本文任何地方所定義。此外，本文標題為便於組織而創建，並不旨在以任何方式限制要求保護之發明之範圍。

【0182】 為更好地理解本發明，列舉下述實施例。僅出於說明目的提供此等實例，且不意欲以任何方式限制本發明之範圍。

實例

實例 1：特異性結合至人類 CD28 之初級 Fab 之產生

【0183】 採用專有噬菌粒庫篩選人類 CD28 抗原。進行總共 3 或 4 輪篩選。於最後一輪篩選後，進行個別純系培養上清液 ELISA 以識別特異性識別人類 CD28 (例如，主要命中)。當 ELISA 信號為背景至少兩倍時，純系被定義為陽性。陽性純系被挑選定序，及將獨特命中相應之 Fab 於大腸桿菌中表現及親和純化。其對人類 CD28

之親和力藉由 Octet® RED96 系統(ForteBio)量測。簡言之，使用浸及讀取抗人類 IgG Fc 捕獲(AHC)生物感測器(ForteBio)捕獲人類或小鼠 CD28-Fc 融合蛋白，及浸入含有經純化 Fab 之孔中，該等 Fab 於 ForteBio 動態緩衝液(10 mM HEPES，150 mM NaCl，3 mM EDTA，0.005% v/v Surfactant P20，pH 7.4)中稀釋 5 至 10 倍。將獲取之資料用 Octet 資料分析軟體 7.1 (ForteBio)處理，及將動力學資料擬合至 1:1 朗繆爾(Langmuir)結合模型。

【0184】 藉由 ELISA 進一步表徵人類及小鼠 CD28 物種交叉反應之主要 Fab 命中，且自該等主要命中，將一組 46 個獨特序列 Fab 轉化成具有核心鉸鏈突變 S241P (Kabat 編號方案)之 IgG4 同型 mAb，以進行詳細之生物物理及功能表徵(表 5)。

實例 2：IgG_4 轉變及表現

【0185】 將表 5 所列經工程改造之人類抗 CD28 IgG_4 同型 mAb 之重鏈及輕鏈選殖至哺乳動物表現載體 pcDNA3.3 (ThermoFisher Scientific)中。根據製造商之方案，將攜帶一條重鏈及一條輕鏈之質粒對短暫轉染至 HEK293 細胞。孵育後，收集上清液，離心及過濾澄清，及將 IgG_4 同型 mAb 藉由 MabSelect™ SuRe™ 蛋白 A 親和層析法(GE Healthcare)捕獲。

【0186】 將 mAb 溶離及中和，及洗脫緩衝液交換至恢復緩衝液(20 mM 組胺酸，pH 5.5)中。藉由 UV-分光光度法測定蛋白質濃度，及在變性、還原及非還原條件下藉由 SDS-PAGE 或 SEC-HPLC 分析 IgG 純度。

表 5. 人類抗 CD28 IgG_4 單克隆抗體列表

序號	IgG ID	同型/突變
1	TY24853	hIgG4/ S241P
2	TY24854	hIgG4/ S241P
3	TY24855	hIgG4/ S241P
4	TY24856	hIgG4/ S241P
5	TY24857	hIgG4/ S241P
6	TY24858	hIgG4/ S241P
7	TY24859	hIgG4/ S241P
8	TY24860	hIgG4/ S241P
9	TY24861	hIgG4/ S241P
10	TY24862	hIgG4/ S241P
11	TY24863	hIgG4/ S241P
12	TY24864	hIgG4/ S241P
13	TY24865	hIgG4/ S241P
14	TY24866	hIgG4/ S241P
15	TY24867	hIgG4/ S241P
16	TY24868	hIgG4/ S241P
17	TY24869	hIgG4/ S241P
18	TY24870	hIgG4/ S241P
19	TY24871	hIgG4/ S241P
20	TY24872	hIgG4/ S241P
21	TY24873	hIgG4/ S241P
22	TY24874	hIgG4/ S241P
23	TY24875	hIgG4/ S241P
24	TY24876	hIgG4/ S241P
25	TY24877	hIgG4/ S241P
26	TY24878	hIgG4/ S241P
27	TY24879	hIgG4/ S241P
28	TY24880	hIgG4/ S241P
29	TY24881	hIgG4/ S241P
30	TY24882	hIgG4/ S241P
31	TY24883	hIgG4/ S241P
32	TY24884	hIgG4/ S241P
33	TY24885	hIgG4/ S241P
34	TY24886	hIgG4/ S241P
35	TY24887	hIgG4/ S241P
36	TY24888	hIgG4/ S241P
37	TY24889	hIgG4/ S241P

序號	IgG ID	同型/突變
38	TY24890	hIgG4/ S241P
39	TY24891	hIgG4/ S241P
40	TY24892	hIgG4/ S241P
41	TY24772	hIgG4/ S241P
42	TY24773	hIgG4/ S241P
43	TY24774	hIgG4/ S241P
44	TY24775	hIgG4/ S241P
45	TY24776	hIgG4/ S241P
46	TY24777	hIgG4/ S241P

實例 3：抗 CD28 抗體之結合特性

【0187】 藉由 Octet® RED96 系統(ForteBio)、ELISA 及 CytoFlex 流式細胞儀(Beckman)量測抗 CD28 mAb 組與人類、食蟹獼猴及小鼠 CD28 以及人類 CTLA4 之結合親和力。抗 CD28 抗體 TAC2386 (亦稱為 TGN1412，如 WO2006/050949A2 專利所述)及 TAC2387 (如 WO2019/246514A2 專利所述)被用作基準對照。

藉由 Octet RED96 系統與人類 CD28 及 CTLA4 之結合親和力

【0188】 使用 Octet® RED96 系統(ForteBio)評估抗 CD28 mAb 組與人類 CD28 及 CTLA4 之結合動力學。簡而言之，將 mAb 在動力學緩衝液(補充有 0.02% Tween 20 及 0.1% BSA 之 PBS)中稀釋至 15 μg/mL，及藉由浸及讀取 AHC 生物感測器(ForteBio)平行捕獲。然後允許將感測器與 His 標記之人類 CD28 及 CTLA4 蛋白質(100 nM)締合 300 秒，及於動力學緩衝液中再締合 300 秒。使用 Octet® 資料分析軟體 7.1 (ForteBio)將締合及解離曲線擬合至 1:1 朗繆爾結合模型。

【0189】 如表 6 所示，藉由 Octet® RED96 系統量測，抗 CD28 測試組及基準 mAb 對人類 CD28 展示出高結合親和力(<10 nM)。此

外，測試 mAb TY24773、TY24853、TY24854、TY24860、TY24865 及 TY24871 以及基準對照 mAb 未對人類 CTLA4 展示出可偵測之親和力。

表 6. 與人類 CD28 及 CTLA4 之結合親和力

mAb ID	人類 CD28			人類 CTLA4		
	KD (nM)	Kon (1/Ms)	Koff (1/s)	KD (nM)	Kon (1/Ms)	Koff (1/s)
TAC2386	<0.001	9.625E+04	<1.0E-07	ND		
TAC2387	4.873	7.071E+04	3.446E-04			
TY24773	8.285	3.848E+04	3.188E-04			
TY24853	3.955	4.159E+04	1.645E-04			
TY24854	5.434	6.488E+04	3.526E-04			
TY24855	7.927	7.918E+04	6.277E-04	4.022	7.596E+05	3.055E-03
TY24856	7.805	6.887E+04	5.376E-04	5.127	3.036E+05	1.557E-03
TY24857	8.639	7.294E+04	6.301E-04	5.442	6.515E+05	3.546E-03
TY24858	9.882	7.101E+04	7.017E-04	7.904	3.055E+05	2.415E-03
TY24859	2.684	7.081E+04	1.900E-04	6.631	4.687E+05	3.108E-03
TY24860	6.664	6.323E+04	4.213E-04	ND		
TY24864	4.413	9.418E+04	4.157E-04	5.501	5.168E+05	2.843E-03
TY24865	<0.001	5.958E+04	<1.0E-07	ND		
TY24866	<0.001	5.804E+04	<1.0E-07	7.645	5.238E+05	4.004E-03
TY24869	2.733	4.676E+04	1.278E-04	1.198	3.793E+05	4.544E-04
TY24871	4.993	7.011E+04	3.501E-04	ND		

TY24876	<0.001	3.251E+04	<1.0E-07	4.280	1.366E+05	5.848E-04
TY24877	7.191	2.686E+04	1.931E-04	13.20	2.353E+05	3.105E-03
TY24878	4.214	9.747E+04	4.108E-04	0.9686	4.233E+05	4.100E-04
TY24879	<0.001	5.939E+04	<1.0E-07	7.165	4.369E+05	3.130E-03
TY24881	5.246	1.262E+05	6.618E-04	2.353	1.114E+06	2.622E-03
TY24884	2.420	6.836E+04	1.655E-04	6.359	6.409E+05	4.076E-03
TY24890	4.440	9.846E+04	4.371E-04	3.133	1.099E+06	3.444E-03

ND: 未偵測到

使用 ELISA 量測與人類及小鼠 CD28 之親和力

【0190】 將重組人類及小鼠 CD28-Fc 於 PBS 中稀釋至 2 μg/mL 及在 4°C 下塗覆在 Nunc MaxiSorp™ 高蛋白結合能力 96 孔 ELISA 板(ThermoFisher Scientific)上過夜。在 37°C 下將板用補充有 3%脫脂乳之 PBS 阻斷 1 小時。於洗滌後，將 50 μL 抗 CD28 測試 mAb 組之 3 倍連續稀釋添加至各孔。於 37°C 下培育 1 小時後，將板洗滌 4 次，及將 100 μL 辣根過氧化物酶(HRP)-結合之抗人類 IgG (Fab 特異性) (1：6000 稀釋)二抗添加至各孔。將板在 37℃下培育 1 小時，洗滌四次，及然後將 50 μL TMB 受質(3,3',5,5'-四甲基聯苯胺)溶液添加至各孔，及將板在室溫下培育。向每孔中加入 50 μL 硫酸終止液停止反應後，量測 450 nm 處之吸亮度。藉由 GraphPad Prism 7 (Windows 版本，GraphPad Software，La Jolla California USA，www.graphpad.com)之非對稱 S 形(四參數邏輯方程)模型擬合 ELISA 資料評價 EC_{50}。

【0191】 如表 7 及圖 1 所示，抗 CD28 測試 mAb 組與兩個基準對照對人類 CD28 展示出相似之親和力。此外，測試 mAb (除 TY24890 外)與小鼠 CD28 結合，而兩個基準對照不與小鼠 CD28 結合。

表 7. 藉由 ELISA 量測與重組 CD28 之結合親和力

mAb ID	EC_{50}(nM)	
	人類 CD28	小鼠 CD28
TAC2386	0.78	ND
TAC2387	>1.65	ND
TY24859	1.34	>44.44
TY24865	0.58	>4.94
TY24866	0.57	>1.65
TY24869	0.87	>14.81
TY24876	1.02	3.93
TY24878	1.06	>14.81
TY24879	0.9	>1.65
TY24884	1.02	>44.44
TY24890	1.55	ND

ND: 未偵測到

抗 CD28 抗體於 Jurkat 細胞之結合活性

【0192】 將 Jurkat (克隆 E6-1)細胞以 1.0×10^5 (50 µL/孔)接種於 96 孔板中，與連續稀釋之基準陽性對照、同型陰性對照抗體及一組抗 CD28 測試 mAb (100、20、4、0.8、0.16 及 0.032 nM)於 2%胎牛血清/Dulbecco 之 PBS (FBS/DPBS)中在 4°C 下培育 30 分鐘。接下來，將細胞用 DPBS 洗滌兩次並在 4°C 下與 APC-抗人類 IgG Fc 二抗 (1 µg/mL，100 µL/孔，Biolegend)進一步培育 30 分鐘。最後，將細胞用 DPBS 洗滌兩次並懸浮於 FACS 緩衝液中用於流式細胞術分析。為進行分析，使用 FlowJo 10 軟體(FlowJo LLC)繪製平均螢光強度(MFI)值與濃度圖，並藉由 GraphPad Prism 7 (Windows 版本，

GraphPad Software，La Jolla California USA，www.graphpad.com）之四參數非線性回歸進一步擬合資料以獲取 EC_{50} 值。

【0193】 如表 8 所示，除 TY24775 及 TY24867 外，於 Jurkat 細胞上，其他所有抗 CD28 測試 mAb 均與 CD28 分子展示出高結合活性(EC_{50} 值範圍為 0.38 至 10.42 nM)。此外，量測之結合活性與兩個基準對照相當。

表 8. 藉由流式細胞術量測與表現於 Jurkat 細胞之 CD28 之結合親和力

Ab ID	EC_{50}(nM)	Ab ID	EC_{50}(nM)	Ab ID	EC_{50}(nM)
TAC2386	1.10	TY24863	2.60	TY24881	0.38
TAC2387	0.81	TY24864	0.54	TY24882	2.24
TY24772	0.74	TY24865	0.46	TY24883	5.92
TY24773	0.71	TY24866	0.70	TY24884	1.09
TY24774	3.35	TY24867	ND	TY24885	5.77
TY24775	ND	TY24868	2.08	TY24886	10.42
TY24776	1.83	TY24869	1.07	TY24887	3.17
TY24777	1.50	TY24870	3.95	TY24888	0.58
TY24853	0.43	TY24871	0.89	TY24889	2.32
TY24854	0.60	TY24872	1.07	TY24890	0.59
TY24855	0.61	TY24873	2.08	TY24891	0.62
TY24856	0.76	TY24874	1.65	TY24892	1.30
TY24857	0.75	TY24875	1.72	同型對照	ND
TY24858	0.70	TY24876	0.38		
TY24859	0.97	TY24877	0.79		
TY24860	0.78	TY24878	0.39		
TY24861	2.25	TY24879	1.17		
TY24862	2.82	TY24880	1.62		

ND：未偵測到

【0194】 藉由流式細胞術量測抗 CD28 抗體與人類 T 細胞之體外結合使用 EasySep™人類初始總 T 細胞單離套組(STEMCELL Technologies)將人類 $CD3^+$ T 細胞自冷凍保存之外周血單核細胞

(PBMC)中單離。將經單離人類 T 細胞以 1.0×10^5 個細胞/孔添加至 96 孔板，並在冰上將 100 nM 基準對照、同型陰性對照抗體及一組抗 CD28 測試 mAb 於 FACS 緩衝液中培育 30 分鐘。接下來，將細胞用 PBS 洗滌三次並在冰上與 PE 標記之二抗進一步培育 30 分鐘。最後，將細胞用 PBS 洗滌三次並重懸浮於 FACS 緩衝液中用於流式細胞術分析。為進行分析，使用 FlowJo 10 軟體(FlowJo LLC)計算 MFI 值，並藉由 GraphPad Prism 7 (Windows 版本，GraphPad Software，La Jolla California USA，www.graphpad.com)繪製幾何平均 MFI 值與 mAb 圖。

【0195】 如圖 2 所示，相較於基準對照 TAC2386 及 TAC2387，除 TY24772 外，所有測試 mAb 均展示相當之 MFI 值。TY24772 結合約為基準對照之兩倍。

實例 4： 使用 Elisa 之配體競爭

【0196】 藉由 ELISA 測試抗體阻斷 CD28 或 CTLA4 結合至其天然配體 CD80 之能力。

【0197】 如圖 3 及表 9 所示，相較於對照抗 CTLA4 抗體 TY21580，基準對照 TAC2386 及 TAC2387 以及所有經測試抗 CD28 測試 mAb 均阻斷 CD28 與 CD80 之結合，而不阻斷 CTLA4 與 CD80 之結合。

表 9. 抗 CD28 或抗 CTLA4 抗體對人類 CD28-CD80 及 CTLA4-CD80 對之 IC_{50}

樣品 ID	IC_{50}(nM)	
	CD28/CD80	CTLA4/CD80
TAC2386	0.48	-

樣品 ID	IC_{50}(nM)	
	CD28/CD80	CTLA4/CD80
TAC2387	0.12	-
TY24859	0.42	-
TY24865	0.68	-
TY24866	0.27	-
TY24890	0.21	-
TY24869	0.55	ND
TY24876	0.54	ND
TY24878	0.35	ND
TY24879	0.42	ND
TY24884	0.28	ND
TY21580	ND	37.24

實例 5：幹式塗覆人類 T 細胞增殖檢定中超促效劑之評估

【0198】 藉由 CellTiter-Glo®螢光細胞活力檢定(Promega)量測基準對照 TAC2386 及 TAC2387、同型陰性對照抗體、以及一組抗 CD28 測試 mAb 誘導淋巴細胞增殖之水準。於此測定中，將 100 nM 測試 mAb 以 50 μL/孔一式三份添加至 96 孔微孔板，並直接風乾到孔壁上。接下來，將微孔板用 PBS 洗滌兩次。將極冷保藏之 PBMC 恢復，並用 10% FBS/RPMI1640 將細胞密度調節至 5.0×10^5 個細胞/mL，並將 1.0×10^5 PBMC (200 μL/孔)添加至預包被微孔板。將細胞於 37°C、5% CO_2 中培育 72 小時，及然後使用 CellTiter-Glo®檢定評估淋巴細胞增殖。

【0199】 如圖 4A 所示，固定之基準對照 TAC2386 (一種已知的 CD28 超促效劑)誘導淋巴細胞增殖之水準明顯高於所有測試 mAb。相較於 TAC2386，基準對照 TAC2387 及抗 CD28 測試 mAb (包括 TAC2387、TY24865、TY24866、TY24876、TY24878、TY24879 及 TY24884)於體外展示出較弱之超促效活性。

實例 6：抗 CD28 抗體對體外人類 T 細胞活化之影響

T 細胞活化共刺激檢定：IFN-Γ 釋放

【0200】 藉由 ELISA 檢測 IFN-γ 細胞介素分泌量測抗 CD28 mAb 作為促效型 T 細胞共刺激劑在體外活化人類 T 細胞之生物活性。Ultra-LEAF™ 純化抗人類 CD28 抗體(Biolegend)用作陽性對照。

【0201】 使用 EasySep™人類初始總 T 細胞單離套組(STEMCELL Technologies)將人類 $CD3^+$ T 細胞自冷凍保存之 PBMC 中單離。將經單離細胞在連續稀釋之基準對照、同型陰性對照抗體、商業化抗人類 CD28 陽性對照抗體及一組抗 CD28 測試 mAb (0.1、1、10 及 100 nM)之存在下於預包被次優濃度(10 nM，50 μL/孔)抗人類 CD3 抗體(OKT3)之 96 孔組織培養板(1.0×10^5/孔)中培養。將細胞於 37°C、5% CO_2 中培育 120 小時，及然後收集細胞上清液藉由 ELISA 進行 IFN-γ 細胞介素分析，藉由 CellTiter-Glo®量測 T 細胞增殖。

【0202】 如圖 4B 及 4C 所示，相較於同型對照抗體，抗 CD28 mAb 顯示出濃度依賴性生物活性，包括 T 細胞增殖及 IFN-γ 細胞介素分泌。通常，抗 CD28 mAb 對人類 T 細胞活化之影響與兩種基準對照抗體相當或更有效。

T 細胞活化共刺激檢定：IL-2 釋放

【0203】 藉由 CellTiter-Glo®螢光細胞活力檢定(Promega)量測 T 細胞增殖及 ELISA 量測 IL-2 細胞介素分泌量測抗 CD28 mAb 作為促效型 T 細胞共刺激劑在體外活化人類 T 細胞之生物活性。Ultra-LEAF™ 純化抗人類 CD28 抗體(Biolegend)用作陽性對照。

【0204】 使用 EasySep™ 人類初始總 T 細胞單離套組(STEMCELL Technologies)將人類 T 細胞自亞洲供體之新鮮 PBMC 中單離。將細胞在連續稀釋之基準對照 TAC2387、同型陰性對照抗體、商業化抗人類 CD28 陽性對照抗體及一組抗 CD28 測試 mAb 之存在下於預包被次優濃度(5 nM)抗人類 CD3 抗體(OKT3)之 96 孔組織培養板(1.0×10^5 個細胞/孔)中培養。將細胞於 37°C、5% CO_2 中培育 72 小時，及然後收集細胞上清液藉由 ELISA 進行 IL-2 細胞介素分析，並使用 CellTiter-Glo®檢定量測 T 細胞增殖水準。

【0205】 如圖 5A 至 B 所示，相較於同型對照抗體，抗 CD28 抗體顯示出濃度依賴性生物活性，包括 T 細胞增殖及 IL-2 細胞介素分泌。TY24859、TY24865、TY24866 及 TY24890 對人類 T 細胞活化之影響與基準對照 TAC2387 相當。未預包被之抗 CD3 陰性對照組未顯示出可偵測之 T 細胞增殖及 IL-2 細胞介素分泌。

實例 7：抗 HER2×CD28 雙特異性抗體構築及功能表征

抗 HER2×CD28 雙特異性抗體之產生

【0206】 使用 TYM13 Fc 突變(D 或 E356K：E357K：S364K：S400C L351'D：K370'D：N390'C：K439'D；根據 IgG1 C_H3 結構域之 Kabat 編號方案)對異二聚雙特異性框架進行工程改造。將輕鏈-重鏈半抗體及單鏈可變片段(scFv)-Fc 鏈組合形成具有 TYM13 突變之異-Fc 結構域之雙特異性抗體(BsAb)。

【0207】 將編碼 BsAb 重鏈、輕鏈及 scFv-Fc 鏈之質粒短暫轉染至哺乳動物細胞中。轉染 7 天後，於 14000 g 下離心 30 分鐘收穫含

有雙特異性抗體之細胞，培養上清液且經無菌篩檢程式(0.22 μm)過濾。使用 MabSelect™ SuRe™ 預填充管柱(GE Healthcare)藉由蛋白 A 親和層析將抗體純化且隨後於 20 mM 組胺酸(pH 5.5)緩衝液中進行緩衝液交換。

【0208】 選擇使用 TY24865(高親和力 CD28)及 TY24865 突變體(低親和力 CD28)框架構築 CD28 BsAb。構築體描述於表 10 中。

表 10. 抗 HER2×CD28 雙特異性抗體設計

BsAb ID	Fab 臂(抗 HER2)	scFv 臂(抗 CD28)
TY27566	Perjeta®	TY24865
TY27881	Perjeta®	TY24865 突變體
TY27807	Herceptin®	TY24865

藉由 FACS 量測與 SK-OV-3 細胞之結合

【0209】 使用流式細胞術量測抗 HER2×CD28 BsAb (TY27566)、抗 HER2×CD3 BsAb (TY25238，亦描述於 PCT/CN2021/076626 中，其全文以引用的方式併入本文中)及抗 HER2 mAb 對 Perjeta® (TAC2319)或 Herceptin® (TAC2320)之濃度依賴性結合活性。Perjeta®(TAC2319)與 Herceptin®(TAC2320)結合至不同 HER2 二聚化結構域之抗原表位。

【0210】 培養 SK-OV-3 細胞並以 8.0×10^4 個細胞/孔添加至 96 孔板中，並在 4°C 下與連續稀釋之測試 BsAb 於 2% FBS/RPMI1640 緩衝液中培育 60 分鐘。接下來，將細胞用 DPBS 洗滌兩次，並在 4°C 下與二級 APC-抗人類 IgG Fc 抗體(1：400 稀釋)進一步培育 30 分鐘。最後，將細胞用 DPBS 洗滌兩次並重懸浮於 FACS 緩衝液中用於流式細胞術分析。為進行分析，使用 FlowJo 10 軟體(FlowJo

LLC)繪製 MFI 值與濃度圖，並藉由 GraphPad Prism 7 (Windows 版本，GraphPad Software，La Jolla California USA，www.graphpad.com)之四參數非線性回歸進一步擬合資料以獲取 EC_{50} 值。同型抗體用作陰性對照。

【0211】 如圖 6 所示，相較於抗 HER2 mAb 與 Perjeta®(TAC2319)或 Herceptin®(TAC2320)，抗 HER2×CD28 或抗 HER2×CD3 BsAb 與 SK-OV-3 細胞展示出非常相似之結合親和力。

T 細胞受體及 CD28 受體信號傳導之刺激活性

【0212】 藉由抗 HER2×CD3 BsAb、抗 HER2×CD28 BsAb 或其組合評價 T 細胞受體(TCR)及 CD28 受體信號傳導之刺激活性。於此測定中，TCR 及 CD28 同時活化導致 NFkB 之轉錄活性增強，進而誘導報告基因之產生。同型抗體作為陰性對照。

【0213】 將 Jurkat-NFkB-Nluc 效應(E)報告細胞(5×10^4 個細胞/孔)與 SK-OV-3 靶(T)細胞(1×10^4 個細胞/孔，E：T=5：1)共培養，內源性表現 HER2 抗原。將連續稀釋之抗 HER2×CD3 BsAb 或具有固定濃度之抗 HER2×CD28 BsAb (10 nM)之同型對照抗體，或相反，將連續稀釋之抗 HER2xCD28 BsAb 或具有固定濃度之抗 HER2×CD3 BsAb (0.01 nM)之同型對照抗體，添加至報告細胞系統中，以評估其在刺激下游螢光素酶活性方面之綜合效應。將共培養細胞於 37°C、5% CO_2 中培育 6 小時。然後，將 100 µL Nano-Glo® 螢光素酶檢定系統(Promega)試劑添加至細胞中，並將細胞裂解 10 分鐘。收集上清液(100 µL)用於使用 SpectraMax® i3x 多模式微孔

板讀取器(分子元件)之發光量測。

【0214】 如圖 7 所示，抗 HER2×CD28 BsAb (TY27566)與固定濃度之抗 HER2×CD3 BsAb (TY25238)組合，及抗 HER2×CD3 BsAb 與固定濃度之抗 HER2×CD28 BsAb 組合，在最大信號值及 EC_{50} 值方面展示出協同或增強刺激效應。

具有相同或不同 TAA 抗原表位之抗 HER2×CD28 BsAb 及抗 HER2×CD3 BsAb 之細胞毒性

【0215】 使用乳酸脫氫酶(LDH)釋放檢定法量測 MCF-7 腫瘤細胞株上抗 HER2×CD3 BsAb (TY25238)單獨或與抗 HER2×CD28 BsAb (TY27566 或 TY27807)組合之體外細胞毒性活性。TY25238 與 TY27566 結合至不同 HER2 二聚化結構域之腫瘤相關抗原(TAA)抗原表位，但與 TY27807 結合至相同之 TAA 抗原表位。

【0216】 將人類 T 細胞自冷凍保存之 PBMC 中單離。在 37℃下將培養之 MCF-7 腫瘤靶細胞(1×10^4 個細胞/孔)與連續稀釋之抗 CD3 BsAb FG14127 單獨或與固定濃度(1 μg/mL)之結合不同 TAA 抗原表位之兩種抗 CD28 BsAb (TY27566 或 TY27807)組合培育 30 分鐘。然後，添加經單離人類 T 效應細胞(2×10^4 個細胞/孔)，並在 37°C、5% CO_2 下培育 72 小時(E：T=2：1)。藉由 CytoTox 96®非放射性細胞毒性檢定(Promega)定量基於殺傷 MCF-7 靶細胞釋放 LDH 至上清液中之細胞毒性。

【0217】 如圖 8 所示，作為單藥劑，抗 HER2×CD3 BsAb (TY25238)在 MCF-7 靶細胞上引發有效濃度依賴性細胞毒性。針對不同 TAA

抗原表位之抗 HER2×CD28 BsAb (TY27566)及抗 HER2×CD3 BsAb (TY25238)之組合，進一步增強體外腫瘤殺傷活性之 EC_{50}。然而，相較於單獨使用抗 HER2×CD3 BsAb (TY25238)處理，添加具有相同 TAA 抗原表位之抗 HER2×CD28 BsAb (TY27807)顯著降低 MCF-7 腫瘤細胞之 EC_{50} 及最大裂解。同型抗體作為單藥檢定之陰性對照。

實例 8：抗 TROP2×CD28 或抗 TROP2×CD3 BsAb 構築及功能表征

【0218】 使用如實例 7 所述之 TYM13 Fc 突變體對抗腫瘤相關鈣信號轉導蛋白 2 (TROP2)×CD28 或抗 TROP2×CD3 異二聚雙特異性框架進行設計。抗 TROP2×CD28 BsAb 之構築體描述於表 11 中。

【0219】 抗 TROP2×CD3 BsAb 之構築體描述於表 12 中。

表 11. 抗 TROP2×CD28 雙特異性抗體設計

BsAb ID	Fab 臂(抗 TROP2)	scFv 臂(抗 CD28)
TY27571	TY25616	TY24865

表 12. 抗 TROP2×CD3 雙特異性抗體設計

BsAb ID	Fab 臂(抗 TROP2)	scFv 臂(抗 CD3)
TY25839	TY25616	TY24742

基於 CD3 或 CD28 之雙特異性與具有高、中或低 TROP2 表現之腫瘤細胞株之結合

【0220】 使用流式細胞術量測抗 TROP2 BsAb 在具有高、中或低 TROP2 表現之不同腫瘤細胞株上之濃度依賴性結合活性。同型抗體作為陰性對照。

【0221】 將 H292、NCI-N87 或 HT29 細胞培養並以 1.0×10^5 個細

胞/孔接種於 96 孔板中，並在 4°C 下與連續稀釋之測試抗 TROP2 BsAb 於 2% FBS/RPMI1640 緩衝液中培育 30 分鐘。接下來，將細胞用 DPBS 洗滌兩次，並在 4°C 下與二級抗人類 IgG 抗體(APC-抗人類 IgG Fc 抗體，H292 細胞 1:300 稀釋；APC-F(ab')2 片段山羊抗人類 IgG (H+L)，NCI-N87 及 HT29 細胞 1:500 稀釋)進一步培育 30 分鐘。最後，將細胞用 DPBS 洗滌兩次並重懸浮於 FACS 緩衝液中用於流式細胞術分析。為進行分析，使用 FlowJo 10 軟體(FlowJo LLC)繪製 MFI 值與濃度圖，並藉由 GraphPad Prism 7 (Windows 版本)之四參數非線性回歸進一步擬合資料以獲取 EC_{50} 值。

【0222】 如圖 9 及表 13 所示，TY25839 及 TY27571 於具有高、中或低 TROP2 表現之三種不同腫瘤細胞株上 TROP2 臂結合活性相似。

表 13. 抗 TROP2 雙特異性抗體於腫瘤細胞株上之 EC_{50} 值

BsAb ID	H292			NCI-N87			HT-29		
	EC_{50} (nM)	範圍	AUC	EC_{50} (nM)	範圍	AUC	EC_{50} (nM)	範圍	AUC
TY25839	0.813	149008	418398	0.901	612873	1715321	0.693	52548	156975
TY27571	0.822	156304	465058	0.911	589022	1644423	0.559	52992	162739

TCR 及 CD28 受體信號傳導之刺激活性

【0223】 藉由抗 TROP2×CD3 BsAb、抗 TROP2×CD28 BsAb 或其組合評價 TCR 及 CD28 受體信號傳導之刺激活性。TCR 及 CD28

同時活化導致 NFkB 之轉錄活性增強，進而誘導報告基因之產生。

【0224】 將 Jurkat-NFkB-Nluc 效應報告細胞(5×10^4 個細胞/孔)與 H292 靶細胞(E:T=5:1)共培養。將連續稀釋之抗 TROP2×CD3 BsAb 與固定濃度之抗 TROP2×CD28 BsAb (5 nM)，或相反，將連續稀釋之抗 TROP2xCD28 BsAb 與固定濃度之抗 TROP2×CD3 BsAb (0.01 nM)添加至報告細胞系統中，以評估其在刺激下游螢光素酶活性方面之綜合活性。將共培養細胞於 37°C、5% CO_2 中培育 6 小時。然後，將 100 μL Nano-Glo 螢光素酶檢定系統(Promega)試劑添加至細胞中，並將細胞裂解 10 分鐘。收集上清液(100 μL)用於使用 SpectraMax i3x 多模式微孔板讀取器(分子元件)之發光量測。

【0225】 如圖 10 所示，基於 CD28 BsAb 之單藥劑展示出非常弱之報告基因活性。然而，抗 TROP2×CD3 及抗 TROP2×CD28 BsAb 之組合在最大信號值或 EC_{50} 值方面展示出協同或增強刺激效應。

實例 9：B7H3×CD28 雙特異性抗體構築及功能表征

【0226】 構築了 B7H3×CD28 BsAb。構築體描述於表 14 中。

【0227】 B7H3×CD3 BsAb 構築體描述於表 15 中。

表 14. B7H3×CD28 雙特異性抗體設計

BsAb ID	Fab 臂(抗 B7H3)	scFv 臂(抗 CD28)
TY27556	TY21601	TY24865

表 15. B7H3×CD3 雙特異性抗體設計

BsAb ID	Fab 臂(抗 B7H3)	scFv 臂(抗 CD3)
TY26999	TY21601	中等親和力 CD3

藉由 FACS 量測與 MDA-MB-231 細胞之結合

【0228】 使用流式細胞術(圖 11)量測抗 B7H3×CD28 BsAb TY27556 及其親本抗 B7H3 mAb TY21601 於 MDA-MB-231 細胞之濃度依賴性結合活性。同型抗體作為陰性對照。

【0229】 將 MDA-MB-231 細胞培養並以 1.0×10^5 個細胞/孔接種於 96 孔板中，並在 4°C 下與連續稀釋之測試 BsAb 於 2% FBS/RPMI1640 緩衝液中培育 60 分鐘。接下來，將細胞用 DPBS 洗滌兩次，並在 4°C 下與二級 APC-抗人類 IgG Fc 抗體(1：400 稀釋)進一步培育 30 分鐘。最後，將細胞用 DPBS 洗滌兩次並重懸浮於 FACS 緩衝液中用於流式細胞術分析。為進行分析，使用 FlowJo 10 軟體(FlowJo LLC)繪製 MFI 值與濃度圖，並藉由 GraphPad Prism 7 (Windows 版本)之四參數非線性回歸進一步擬合資料以獲取 EC_{50} 值。

【0230】 如圖 12 所示，抗 B7H3 mAb TY21601 對 MDA-MB-231 靶細胞顯示出次-nM (0.4525 nM)之結合親和力，且 BsAb TY27556 對靶細胞之結合活性降低了約 28 倍(12.84 nM)。

B7H3xCD28 雙特異性抗體增強 PD-1 或 PD-L1 阻斷體外誘導 T 細胞活化之能力

【0231】 抗 PD-1 阻斷 mAb 與 B7H3xCD28 雙特異性 Ab 組合對體外原代人類 T 細胞活化之影響藉由 ELISA 量測 IFN-γ 及 IL-2 之分泌。本文使用改良之混合淋巴細胞反應(MLR)模擬生理 PD-L1 表現及 TCR/CD3 刺激。為產生單向 MLR 檢定，將來自健康供體(D#XC11147W)之人類 T 細胞(1×10^5 個細胞/孔)在單獨存在不同測

試抗體(B7H3xCD28 或同型對照)或與抗 PD-1 (pembrolizumab，Keytruda®)或抗 PD-L1 (atezolizumab，Tecentriq®)組合之情況下與異基因 MDA-MB-231 細胞(E：T=5：1)培育。然後將細胞於 37°C、5% CO_2 培養箱中共培養 120 小時。用 Elisa 套組定量由經活化 T 細胞上清液中分泌之 IL-2 (72 小時)及 IFN-γ (120 小時)細胞介素。

【0232】 如圖 13A 至 13B 所示，於該 MLR 檢定中，添加 100 nM PD-1/PD-L1 mAb 或滴定 B7H3xCD28 導致無或僅有輕微細胞介素釋放。然而，相較於單藥療法，B7H3xCD28 與 100 nM PD-1/PD-L1 mAb 組合顯著增加 T 細胞活化。此等結果證明，B7H3xCD28 雙特異性在腫瘤細胞存在下能協同結合 PD-1/PD-L1 阻斷劑，以促進 T 細胞活化，內源性表現 PD-L1 及 B7H3。

共刺激雙特異性抗體在雙向結合時在體外增強對 MCF-7 細胞之 T 細胞細胞毒性

【0233】 使用 LDH 釋放細胞毒性檢定法量測基於抗 CD3，或基於抗 CD28 BsAb 或其組合在 MCF-7 腫瘤細胞株上之體外腫瘤細胞殺傷活性。

【0234】 將人類 T 細胞自冷凍保存之 PBMC 中單離。在 37℃下將 MCF-7 細胞(1×10^4 個細胞/孔)與連續稀釋之抗 HER2×CD3 BsAb TY25238 或與固定濃度之高親和力 CD28 臂抗 HER2×CD28 BsAb TY27566、抗 CD28×B7H3 TY27556、或低親和力 CD28 臂抗 HER2×CD28 BsAb TY27881 (1 ug/mL 或 10 ug/mL)組合培育 30 分鐘，並加入人類 T 細胞(2×10^4 個細胞/孔)於 37°C、5% CO_2 中培育

72 小時(E：T=2：1)。使用 CytoTox 96®非放射性細胞毒性檢定(Promega)定量基於殺傷 MCF-7 靶細胞上清液中釋放 LDH 之細胞毒性。

【0235】 如圖 14 所示，作為單藥劑，基於 CD28 BsAb 在 MCF-7 靶細胞上未引起細胞毒性。相反，作為單藥劑，抗 CD3 BsAb TY25238 顯示出濃度依賴性效應，且針對 HER2 之 BsAb 引起最高細胞毒性，伴隨 54.47%之 MCF-7 細胞裂解。抗 CD3 及抗 CD28 BsAb 之組合進一步增強了體外腫瘤細胞殺傷活性，表現為 EC_{50} 降低。相較於單獨使用抗 CD3 BsAb TY25238 處理，添加具有高親和力 CD28 臂之抗 CD28 BsAb TY27566 顯著增強約 10 倍(EC_{50})之腫瘤細胞裂解。然而，當與具有低親和力 CD28 臂之抗 CD28 BsAb TY27881 組合時，未偵測到增強之細胞毒性。

共刺激雙特異性抗體在雙向結合時在體外增強對 EMT-6-HER2 細胞之 T 細胞細胞毒性

【0236】 使用 LDH 釋放細胞毒性檢定法量測基於抗 CD3 或基於抗 CD28 BsAb 或其組合在 EMT-6-HER2 腫瘤細胞株上之體外腫瘤細胞殺傷活性。

【0237】 使用 EasySep™ 人類初始總 T 細胞單離套組(STEMCELL Technologies)將人類 T 細胞自新鮮 PBMC 中單離。在 37℃下將 EMT-6-HER2 (5×10^3 個細胞/孔)靶細胞與連續稀釋之抗 HER2×CD3 BsAb (TY25238)或與固定濃度之抗 B7H3×CD3 BsAb TY27556 (8 nM)組合培育 30 分鐘，或相反，在 37℃下將 EMT-6-HER2 (5×10^3

個細胞/孔)細胞與連續稀釋之抗 B7H3×CD3 BsAb (TY26999)或與固定濃度之抗 HER2×CD28 BsAb TY27566 (8 或 0.8 nM)組合培育 30 分鐘。接下來，加入經單離人類 T 效應細胞(2×10^4 個細胞/孔)並於 37°C、5% CO_2 中培育 72 小時(E：T=2：1)。使用 CytoTox 96® 非放射性細胞毒性檢定(Promega)定量基於殺傷 EMT-6-HER2 靶細胞上清液中釋放 LDH 之細胞毒性。同型抗體作為陰性對照用於單藥劑檢定。

【0238】 如圖 15 所示，作為單藥劑，TY27556 (抗 B7H3×CD28)或 TY27566 (抗 HER2×CD28)未對 EMT-6-HER2 細胞展示出殺傷效能。相較於 TY25238，TY25238 與 TY27556 組合導致 EC_{50} 降低約 2 倍，及最大殺傷增加約 3 倍(從 17%增加至 50%)。

【0239】 相較於 TY26999，TY26999 與 TY27566 組合導致 EC_{50} 降低約 1.3 倍，及最大殺傷增加約 2 倍(從 23%增加至 47%)。

基於抗 CD3 或抗 CD28 之雙特異性抗體或其組合之體內全身細胞介素釋放風險

【0240】 使用 ELISA 量測基於抗小鼠 CD3 或抗 CD28 BsAb 單獨或組合在 BALB/c 小鼠模型中體內全身細胞介素釋放風險(IL-6 及 IFN-γ)。

【0241】 將 BALB/c 小鼠隨機分為四組(每組 3 隻小鼠)，並分別注射抗小鼠 CD3 mAb (145-2C11 克隆，1 mg/kg)、抗 B7H3×CD28 (TY27556，2 mg/kg)、抗 B7H3×CD3 (TY27042，其中 CD3 臂衍生自小鼠特異性 145-2C11 克隆，2 mg/kg)或其組合(TY27556，2 mg/kg

與 TY27042，2 mg/kg)。在注射前及注射後之不同時間點(給藥前 3.5 及 24 小時)收集小鼠血清及全血。藉由 ELISA 經 IL-6 及 IFN-γ 評估全身細胞介素釋放風險。藉由流式細胞術於每個時間點測定 $CD3^+$ T 細胞與總外周 T 細胞之百分比。

【0242】 如圖 16 所示，測試抗體處理 3.5 小時後，小鼠投與抗 mCD3 或抗 B7H3×CD3 (TY27042)導致細胞介素釋放(IL-6 及 IFN-γ)顯著降低。測試抗體處理後，抗 B7H3×CD28 (TY27556)處理之小鼠未顯示出可偵測之細胞介素釋放。此外，與單藥劑抗 B7H3×CD3 (TY27042)相比，抗 B7H3×CD3 (TY27042)與抗 B7H3×CD28 (TY27556)組合未顯示出增強之細胞介素釋放風險。在單藥劑抗小鼠 CD3、抗 B7H3×CD3 (TY27042)及抗 B7H3×CD28 (TY27556)組以及抗 B7H3×CD3 (TY27042)與抗 B7H3×CD28 (TY27556)組合組中，外周 $CD3^+$ T 細胞急劇減少至幾乎為零。注射後 3.5 小時，僅有約 2%至 3%之 $CD3^+$ T 細胞保留在外周血中。

實例 10： 體內功效研究

SK-OV3 模型中 B7H3xCD28 BsAb 單藥療法及與 HER2xCD3 BsAb 組合療法體內功效研究

【0243】 藉由腹膜注射向免疫缺陷 M-NSG 小鼠(n=8 隻/組，雌性，7 至 8 周齡)移植 5×10^6 個 PBMC。7 天后，向小鼠皮下接種 2×10^6 個 SK-OV3 細胞。當腫瘤接種後第 8 天平均腫瘤體積達到約 90 mm^3 時，開始治療。藉由腹膜注射向小鼠投與 5 mg/kg $hIgG_1$ 同型對照、0.2 mg/kg 抗 HER2×CD3 雙特異性雙鏈經遮蔽抗體、5 mg/kg

抗 B7H3×CD28 雙特異性抗體 TY27556 或 0.2 mg/kg TY27151 與 5 mg/kg TY27556 之組合。TY27151 先前於 PCT/CN2021/076626 中描述。每週兩次向小鼠投與該 Ab，達總計五個劑量。一周兩次監測腫瘤生長，並報告為隨時間之平均腫瘤體積±s.e.m。

【0244】 如圖 17 所示，雙鏈經遮蔽抗 HER2×CD3 雙特異性抗體 TY27151 與抗 B7H3×CD28 雙特異性抗體 TY27556 於該模型中顯示出較強之協同抗腫瘤效應。

EMT6-HER2 小鼠乳癌同源模型中 CD28 BsAb 體內功效研究

【0245】 向 BALB/c 小鼠(n=5 隻/組，雌性，8 至 9 周齡)皮下接種 5×10^5 個 EMT-HER2 細胞。當腫瘤接種後第 7 天平均腫瘤體積達到約 110 mm^3 時，開始治療。藉由腹膜注射向小鼠投與媒劑、0.5 mg/kg 及 0.05 mg/kg 抗 B7H3×CD28 BsAb TY27556，或 0.2 mg/kg 抗 HER2×CD28 雙特異性抗體 TY27566。每週兩次向小鼠投與該 Ab，達總計四個劑量。一周兩次監測腫瘤生長，並報告為隨時間之平均腫瘤體積±s.e.m。

【0246】 如圖 18 所示，抗 B7H3×CD28 雙特異性抗體 TY27556 於該模型中顯示出劑量依賴性抗腫瘤效應。如圖 18 所示，抗 HER2×CD28 雙特異性抗體 TY27566 顯示出較強之抗腫瘤效應。

實例 11：識別經遮蔽抗 CD28 抗體之自阻斷肽的方法

經遮蔽抗 CD28 抗體之產生

【0247】 已設計並執行了一種篩選系統，其用於有效發現具有良好可展性之可有效遮蔽未經遮蔽親本抗 CD28 抗體之遮蔽性部分。

於此系統中，靶抗 CD28 scFv 首先在酵母表面展示，及經證實功能結合至其 CD28 抗原。然後將來自改良 MP 肽庫之遮蔽性肽(MP)直接融合至靶抗 CD28 scFv 之輕鏈之 N 端，及構築在酵母表面上展示融合蛋白之酵母庫。然後該酵母庫經歷若干輪基於 FACS 之篩選：1)將具有與抗原低結合之酵母純系濃化，2)將經濃化酵母純系用蛋白酶處理以移除 N 端 MP，及 3)選擇展示與抗原高結合之純系。於 5 至 6 輪分選後，自此等純系提取質粒及通過 DNA 定序證實 MP 序列。在 MP 之存在下，呈 scFv 形式之選定經遮蔽抗 CD28 抗體純系展示與抗原之少結合。然而，當將酵母細胞用煙草蝕紋病毒核酸內含物——一種內肽酶(TEV)處理以移除 MP 時，與抗原之結合急劇增加。將作為裂解位點之 TEV 識別位點併入 MP 中，與應用 TEV 蛋白酶組合驗證選定純系顯著增加 MP 選擇之成功率。

【0248】 為識別 MP 序列，使用質粒提取套組(Generay)自選定酵母純系提取穿梭質粒，及轉形至活性大腸桿菌細胞。製備質粒，及將編碼 MP 之區域定序及比對。如所預期，可將此等序列分成若干組，指示通過分選輪之清除濃化。選定 MP 之遮蔽效率如下表 16 所示，及每個 MP 之序列如下述序列部分所示。

【0249】 將經遮蔽抗 CD28 scFv 蛋白轉變成 IgG_4 同型 mAb。經遮蔽 IgG_4 mAb 被改造為包括 MP，該 MP 具有單個不變基質金屬蛋白酶(MMP)裂解位點，其以與在酵母表面上展示相同之方式融合至輕鏈之 N 端。將重鏈及輕鏈分開選殖至哺乳動物表現載體

pCDNA3.3(Thermo Fisher Scientific)。親本抗 CD28 抗體(TY24865)之 V_H 及 V_L 序列列於以下序列部分。

【0250】 將質粒對短暫轉染至 HEK293F 細胞中。於 6 天后，收集上清液，藉由離心及過濾澄清，及將 IgG 用標準蛋白 A 親和層析法(MabSelect SuRe, GE Healthcare)純化。將 IgG 溶離及中和，及緩衝交換至 20 mM 組胺酸，pH 5.5 緩衝液中。藉由 UV-分光亮度法測定蛋白質濃度，及在變性、還原及非還原條件下藉由 SDS-PAGE 或 EC-HPLC 分析 IgG 純度。重要的是，經遮蔽抗體於 HEK293 細胞中之表現水準類似於或低於其親本抗體，且其於蛋白 A 樹脂後之純化產率亦相似，表明遮蔽性肽及裂解肽之存在對哺乳動物細胞中之抗體表現不具有顯著負面影響。

表 16. 遮蔽性肽

IgG ID	ELISA 遮蔽效率	IgG ID	ELISA 遮蔽效率	IgG ID	ELISA 遮蔽效率
TY26142	3062	TY26152	1867	TY26162	ND
TY26143	ND	TY26153	1825	TY26163	ND
TY26144	ND	TY26154	ND	TY26164	ND
TY26145	1430	TY26155	368	TY26165	ND
TY26146	2179	TY26156	670	TY26166	ND
TY26147	4882	TY26157	ND	TY26167	1427
TY26148	ND	TY26158	887	TY26168	2567
TY26149	3841	TY26159	468	TY26169	1818
TY26150	1027	TY26160	1461	TY26170	ND
TY26151	2476	TY26161	1484	TY26171	406

遮蔽效率之量測

【0251】 當藉由 ELISA 量測遮蔽效率時，將重組人類 CD28-Fc 於 PBS 中稀釋至 2 μg/mL 及在 4°C 下塗覆在 MaxiSorp™ 高蛋白結合能力 96 孔 ELISA 板(ThermoFisher Scientific)上過夜。在 37°C 下

將板用補充有 3%脫脂乳之 PBS 阻斷 1 小時。於洗滌後，將 100 μL 抗 CD28 測試 mAb 之 3 倍連續稀釋添加至各孔。於 37°C 下培育 1 小時後，將板洗滌四次，及將 100 μL 辣根過氧化物酶(HRP)結合之抗人類 IgG (Fab 特異性) (1：6000 稀釋)二抗添加至個孔。將板在 37°C 下培育 1 小時，洗滌四次，及然後將 50 μL TMB 受質(3,3',5,5'-四甲基聯苯胺)溶液添加至各孔，及將板在室溫下培育。向每孔中加入 50 μL 硫酸終止液停止反應後，量測 450 nm 處之吸亮度。使用 GraphPad Prism 6 (Windows 版本，GraphPad Software，La Jolla California USA，www.graphpad.com)之 S 形(四參數邏輯方程)模型擬合 ELISA 資料來評價 EC_{50}。

【0252】 選定經遮蔽抗 CD28 測試 mAb 之遮蔽效率藉由經遮蔽 mAb 之結合之 EC_{50} 除以未經遮蔽親本 mAb (TY24865)之 EC_{50} 來計算，並於表 17 中列出。如圖 19 所示，相較於親本 mAb，所有可活化 mAb 顯示與其抗原之急劇減少之結合，及經計算之遮蔽效率範圍自 368 至高於 4000。此等結果指示當於哺乳動物細胞中表現時，自改良之 MP 肽庫識別之多個 MP 維持其遮蔽效率且作為全 IgG 分子之部分。

表 17. 經遮蔽抗 CD28 抗體之 SEC 純度及遮蔽效率

IgG ID	SEC 純度		遮蔽效率
	HMW(%)	LMW(%)	
TY26142	2	0	3062
TY26143	>2.0	0	ND
TY26144	24.6	0	ND
TY26145	4.5	0	1430
TY26146	3.6	0.2	2179

TY26147	1.9	0	4882
TY26148	1.9	0.1	ND
TY26149	1.4	0.1	3841
TY26150	1.9	0	1027
TY26151	1.7	0	2476
TY26152	2.1	0	1867
TY26153	1.7	0	1825
TY26154	14.2	0	ND
TY26155	1.8	0	368
TY26156	2.7	0	670
TY26157	無主峰		ND
TY26158	低峰		887
TY26159	36.6	0	468
TY26160	1.4	0	1461
TY26161	1.8	0	1484
TY26162	0.9	0	ND
TY26163	1.1	0	ND
TY26164	2.5	0	ND
TY26165	2.8	0	ND
TY26166	58.5	0	ND
TY26167	2.2	0.3	1427
TY26168	20.8	0	2567
TY26169	非對稱主峰		1818
TY26170	3.9	0.8	ND
TY26171	5.9	0	406

選定抗 CD28 可活化抗體之優化

【0253】對於兩個先導經遮蔽抗 CD28 mAb (TY26149 及 TY26152)，在其 MP 上進行了修飾，包括去除一些 N 端殘基，並在 TY26149 序列之「D」及「G」殘基之間添加「S」胺基酸殘基(表 18 中 TY26149 之粗體及底線殘基)。如圖 20 所示，新型經遮蔽抗體之表現及遮蔽效率未受到顯著影響。

表 18. 遮蔽性肽序列之修飾

IgG ID	遮蔽性肽+裂解肽序列：	SEQ ID NO:
TY26149	EVGSYAATYSDCYSDPYACHDGGGPLGLAGSGGS	301

TY28338	EAATYSDCYSDPYACHDSGGGPLGLAGSGGS	302
TY28339	EYSDCYSDPYACHDSGGGPLGLAGSGGS	303
TY26152	EVGSYAAFSPACYTDPYSCHAGGGPLGLAGSGGS	304
TY28341	EAAFSPACYTDPYSCHAGGGPLGLAGSGGS	305
TY28342	ESPACYTDPYSCHAGGGPLGLAGSGGS	306

實例 12：表位作圖

【0254】 為了確定測試抗體在胺基酸殘基水準上之結合區域，製備了一系列之人類 CD28 細胞外結構域之突變體(表 19)。使用這些 CD28 突變質粒轉染 HEK293F 細胞。抗體與人類 CD28 突變體之結合藉由流式細胞術分析進行評估。結果匯總於表 19 中，連同這些抗體與處於感興趣之差異(differentiation)之人類、猴及小鼠 CD28 之交叉反應性。TY24865 與人類、猴及小鼠 CD28 交叉反應，然而 TAC2386 及 TAC2387 不結合至小鼠 CD28。突變構建體意在將 TY24863 所結合之表位與參考抗體 TAC2386 及 TAC2387 區分開。清楚地是，TY24865 保持與 RE49AA、VY68AA、YS79AA 及 KT81AA 之結合能力，其指示 TY24865 不結合至 RE49、VY68、YS79 及 KT81 殘基，且該等殘基位於人類及小鼠 CD28 非保守區域。然而 TY24865 喪失對 FR51AA、SL54AA、YL98AA、QN100AA、YF110AA、KI113AA、YP118AA、PPP119AAA、PP120AA、PY121AA 及 Y122A 突變之結合能力，表明其結合抗原表位在這些區域內，例如 SEQ ID NO.: 1 之胺基酸殘基 51、52、54、55、98-101、110-111、113-114、118-122。

表 19. 表位作圖

突變體	TY24865	TAC2386	TAC2387

Hu_WT	+	+	+
Cyno_WT	+	+	+
小鼠_WT	+	-	-
Hu_RE49AA	+	+	-
Hu_FR51AA	-	-	-
Hu_SL54AA	-	-	-
Hu_VY68AA	+	+	-
Hu_Y69A	+	+	-
Hu_YS79AA	+	-	+
Hu_KT81AA	+	-	+
Hu_YL98AA	-	-	-
Hu_QN100AA	-	-	-
Hu_YF110AA	-	-	-
Hu_KI113AA	-	-	-
Hu_M117A	+	+	-
Hu_YP118AA	-	+	-
Hu_PPP119AAA	-	+	-
Hu_PP120AA	-	+	-
Hu_PY121AA	-	+	-
Hu_Y122A	-	+	+

實例 13：TY24865 變體

【0255】 採用兩種方法產生 TY24865 變體。將不同點突變引入 TY24865 scFv 之 YYYY 序列中，及通過基於酵母之成熟文庫篩選發現新批次 TY24865 scFv 變體。以 Fab-scFv-Fc 形式構建抗 TAA×CD28 雙特異性抗體，並使用 ELISA 量測該等 bsAb 之 CD28 親和力。

【0256】 以抗 PD-L1 TY21421 Fab (見 WO 2019/185035) 及 TY24865 scFv (VH-VL 形式)或其變體之 Fab-scFv-Fc 形式構建抗 PD-L1×CD28 雙特異性抗體。該等 bsAb 中部分與 CD28 之親和力匯總於表 20 以及圖 22A 及 22B 中；TY29815 為野生型 bsAb，且 TY30413 與 TY29815 具有相似之親和力，然而其他 bsAb 具有更

高或更低之親和力。

表 20. 具有 TY24865 變體之抗 PD-L1×CD28 BsAb 之構築及表徵

BsAb	CD28 scFv	表現量 (mg/L)	SEC 純度		單點 ELISA	ELISA EC_{50}(nM)
			HMW(%)	LMW(%)		
TY29815	VH-VL(YYYY)	54.1	6.4	0.6		22.26
TY30406	VH-VL(YDYY)	37.5	7.2	1.6	更高	
TY30410	VH-VL(YYEY)	23.2	9.5	1.7	更低	
TY30413	VH-VL(YYHY)	60.2	2.8	0.5	未改變	26.81

[0257] 使用 VH-VL 或 VL-VH 形式之 TY24865 scFv 變體構築抗 HER2×CD28 bsAb 或抗 B7-H3×CD28 bsAb。如表 21 所示，該等 bsAb 顯示出正常表現量及 SEC 純度。如圖 23A 及 23B 所示，藉由 ELISA 量測該等 bsAb 之 CD28 親和力。該等 scFv 變體顯示出多變之親和力，範圍自 0.954 至 417 nM，且 VL-VH 形式之 scFv 比 VH-VL 形式之 scFv 具有更高之親和力。表 22 以及圖 24A 及 24B 中，一典變體顯示出對人類及小鼠高親和力，且該親和力於小鼠 T 細胞結合檢定中得到進一步證實(圖 25)。

表 21. 具有 TY24865 變體之抗 HER2×CD28 BsAb 之構築及表徵

BsAb ID	CD28 scFv (VL-VH)	表現量 (mg/L)	SEC 純度		EC_{50}(nM)
			HMW(%)	LMW(%)	
TY27566	WT	61.7	4.4	0.0	3.89
TY28652	BC8227	60.5	NA	NAA	3.68
TY28653	BC8228	42.2	6.8	0.1	7.30
TY28654	BC8230	59.6	3	0	68.41
TY28655	BC8233	55.2	3.4	0.2	8.14
TY28656	BC8235	70.6	2.7	0	127.7

TY28657	BC8237	67.1	3.3	0	5.29
TY28658	BC8240	67.3	4.9	0	78.02
TY28659	BC8242	64	3.4	0.3	0.954
TY28660	BC8257	67.5	4.9	0.4	3.77
TY28661	BC8263	62.8	4.9	0	52.57

BsAb ID	CD28 scFv (VH-VL)	表現量 (mg/L)	SEC 純度		EC_{50}(nM)
			HMW(%)	LMW(%)	
TY29109	WT	73.4	5.1	0.0	30.33
TY29306	BC8227	63.7	1.4	0.6	9.27
TY29307	BC8228	69.3	1.4	0	27.79
TY29308	BC8230	34.1	2.4	0	417.3
TY29309	BC8233	38.6	2	0	27.7
TY29310	BC8235	50.4	2.7	0	348.9
TY29311	BC8237	25.4	3	2.1	91.28
TY29312	BC8240	43.0	2.6	0	624.1
TY29313	BC8242	36.0	2.4	1.8	6.97
TY29314	BC8257	96.5	4	0.9	27.51
TY29315	BC8263	60.5	1.3	0	291.2

表 22. 具有 TY24865 變體之抗 B7-H3×CD28 BsAb 之構築及表徵

BsAb ID	CD28 scFv (VH-VL)	人類 CD28 EC_{50}(nM)	小鼠 CD28 EC_{50}(nM)
TY29021	WT	36.39	NM
TY30120	BC10599	3.026	74.18
TY30121	BC10601	5.731	108.3
TY30123	BC10621	3.588	96.41

[0258] 僅出於說明目的提供上述非限制性實例，以便更全面地理解所公開之主題。該等實例不應被解釋為限制本說明書中描述之任何實施例，包括那些涉及抗體、醫藥組合物或用於治療癌症、神經退行性疾病或傳染病之方法及用途。

序列

[0259] 本文公開之序列於下表中列出及 SEQ ID NOs (SEQ)於左

欄中顯示。

SEQ	描述	序列
1	人類 CD28	MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSC KYSYNLFSREFRASLHKGLDSAVEVCVVYGNYSQQLQV YSKTGFNCDGKLGNESVTFYLQNLYVNQTDIYFCKIEVM YPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLV VVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTP RRPGPTRKHYQPYAPPRDFAAYRS
2	恆河猴 CD28	MLRLLLALNLLPSIQVTGNKILVKQSPMLVAYDNAVNLSC KYSYNLFSREFRASLHKGLDSAVEVCVVYGNYSQQLQV YSKTGFNCDGKLGNESVTFYLQNLYVNQTDIYFCKIEVM YPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWALVV VGGVLACYSLLVTVAFCIFWMRSKRSRLLHSDYMNMTP RRPGPTRKHYQPYAPPRDFAAYRS
3	食蟹獼猴 CD28	MLRLLLALNLLPSIQVTGNKILVKQSPMLVAYDNAVNLSC KYSYNLFSREFRASLHKGLDSAVEVCVVYGNYSQQLQV YSKTGFNCDGKLGNESVTFYLQNLYVNQTDIYFCKIEVM YPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWALVV VGGVLACYSLLVTVAFCIFWMRSKRSRLLHSDYMNMTP RRPGPTRKHYQPYAPPRDFAAYRS
4	小鼠 CD28	MTLRLLFLALNFFSVQVTENKILVKQSPLLVVDSNEVSLS CRYSYNLLAKEFRASLYKGVNSDVEVCVGNGNFTYQPQF RSNAEFNCDGDFDNETVTFRLWNLHVNHTDIYFCKIEFM YPPPYLDNERSNGTIIHIKEKHLCHTQSSPKLFWALVVVA GVLFCYGLLVTVALCVIWTNSRRNRLLQSDYMNMTPRRP GLTRKPYQPYAPARDFAAYRP
未經遮蔽抗 CD28 抗體		
抗 CD28 mAb (TY24865)		
5	HCDR1	TGGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
7	HCDR3	YEYYYYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
11	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWI RQAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARYEYYYYDFDYWGQGTLVT VSS

12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPRTFGQGTKVEIK
13	HC	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWI RQAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARYEYYYYDFDYWGQGTLVT VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGK
14	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPRTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24853)		
15	HCDR1	SGHHWS
6	HCDR2	EIYHSGSTYYSPSLKS
16	HCDR3	KDDYYAFDY
17	LCDR1	RASESVDFYGISFLH
18	LCDR2	AASTLQS
19	LCDR3	QQSYRTPIT
20	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSITSGHHWSWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKDDYYAFDYWGQGTLVTVS S
21	VL	DIQLTQSPSSLSASVGDRVTITCRASESVDFYGISFLHWYQ QKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRTPITFGQGTKVEIK
22	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSITSGHHWSWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKDDYYAFDYWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK

		EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
23	LC	DIQLTQSPSSLSASVGDRVTITCRASESVDFYGISFLHWYQ QKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRTPITFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24854)		
24	HCDR1	SGYHWG
6	HCDR2	EIYHSGSTYYSPSLKS
25	HCDR3	RRGDGYFDF
26	LCDR1	RASQSVDFLGISFLH
27	LCDR2	DASNRAT
28	LCDR3	QQSYRTPLT
29	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSITSGYHWGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRRGDGYFDFWGQGTLVTVS S
30	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFLGISFLHWYQ QKPGKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQSYRTPLTFGQGTKVEIK
31	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSITSGYHWGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRRGDGYFDFWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
32	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVDFLGISFLHWYQ QKPGKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQSYRTPLTFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24859)		

33	HCDR1	TGGVGVG
6	HCDR2	EIYHSGSTYYSPSLKS
35	HCDR3	YGYSYYALDY
36	LCDR1	SASSSVGYVQ
37	LCDR2	DASNLET
38	LCDR3	QQSYTSPRT
39	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWI RQAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARYGYSYYALDYWGQGTLVT VSS
40	VL	DIQLTQSPSSLSASVGDRVTITCSASSSVGYVQWYQQKPG KAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSYTSPRTFGQGTKVEIK
41	HC	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWI RQAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARYGYSYYALDYWGQGTLVT VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGK
42	LC	DIQLTQSPSSLSASVGDRVTITCSASSSVGYVQWYQQKPG KAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSYTSPRTFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
		抗 CD28 mAb (TY24860)
43	HCDR1	TSGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
44	HCDR3	KGYYYYAFDY
45	LCDR1	RASQDIGSFLA
9	LCDR2	AASSLQS
46	LCDR3	QQSYRSPPT
47	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKGYYYYAFDYWGPGTLVTV SS
48	VL	DIQLTQSPSSLSASVGDRVTITCRASQDIGSFLAWYQQKPG

		KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYRSPPTFGQGTKVEIK
49	HC	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKGYYYYAFDYWGPGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
50	LC	DIQLTQSPSSLSASVGDRVTITCRASQDIGSFLAWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYRSPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
		抗 CD28 mAb (TY24866)
33	HCDR1	TGGVGVG
51	HCDR2	EIYHSGSTYYPPSLKS
52	HCDR3	YEYSSYGLDY
53	LCDR1	RASQDIRKFLA
300	LCDR2	DASSLES
10	LCDR3	QQSYRSPRT
54	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWI RQAPGKGLEWIGEIYHSGSTYYPPSLKSRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARYEYSSYGLDYWGQGTLVT VSS
55	VL	DIQLTQSPSSLSASVGDRVTITCRASQDIRKFLAWYQQKP GKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSYRSPRTFGQGTKVEIK
56	HC	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWI RQAPGKGLEWIGEIYHSGSTYYPPSLKSRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARYEYSSYGLDYWGQGTLVT VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ

		EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH NHYTQKSLSLSLGK
57	LC	DIQLTQSPSSLSASVGDRVTITCRASQDIRKFLAWYQQKP GKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSYRSPRTFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
抗 CD28 mAb (TY24869)		
24	HCDR1	SGYHWG
58	HCDR2	AIYHSGSTYYSPSLKS
59	HCDR3	SGLVYYAFDY
60	LCDR1	RASQGIGSYLA
300	LCDR2	DASSLES
61	LCDR3	QQYSTSPLT
62	VH	QVQLVQSGAEVKKPGSSVKVSCKASGYSISSGYHWGWIR QAPGQGLEWIGAIYHSGSTYYSPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARSGLVYYAFDYWGQGTLVTVSS
63	VL	DIQLTQSPSSLSASVGDRVTITCRASQGIGSYLAWYQQKP GKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYSTSPLTFGQGTKVEIK
64	HC	QVQLVQSGAEVKKPGSSVKVSCKASGYSISSGYHWGWIR QAPGQGLEWIGAIYHSGSTYYSPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARSGLVYYAFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
65	LC	DIQLTQSPSSLSASVGDRVTITCRASQGIGSYLAWYQQKP GKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYSTSPLTFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
抗 CD28 mAb (TY24871)		
66	HCDR1	SYAIH
67	HCDR2	SIYHSGSTYYSPSLKS

68	HCDR3	DVYGSYALDV
69	LCDR1	RASQSVDFDGISFLH
300	LCDR2	DASSLES
70	LCDR3	QQSYRSPIT
71	VH	QVQLVQSGAEVKKPGSSVKVSCKASGFTFSSYAIHWVRQ APGQGLEWIGSIYHSGSTYYSPSLKSRVTITADKSTSTAYM ELSSLRSEDTAVYYCARDVYGSYALDVWGQGTLVTVSS
72	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFDGISFLHWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPITFGQGTKVEIK
73	HC	QVQLVQSGAEVKKPGSSVKVSCKASGFTFSSYAIHWVRQ APGQGLEWIGSIYHSGSTYYSPSLKSRVTITADKSTSTAYM ELSSLRSEDTAVYYCARDVYGSYALDVWGQGTLVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT QKSLSLSLGK
74	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVDFDGISFLHWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPITFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24773)		
24	HCDR1	SGYHWG
6	HCDR2	EIYHSGSTYYSPSLKS
75	HCDR3	KDYYYALDY
76	LCDR1	RASQSVDFYGKSFLH
18	LCDR2	AASTLQS
28	LCDR3	QQSYRTPLT
77	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGYHWGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKDYYYALDYWGQGTLVTVS S
78	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWY QQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQSYRTPLTFGQGTKVEIK
79	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGYHWGWIR

		QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKDYYYALDYWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
80	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWY QQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQSYRTPLTFGQGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24876)		
24	HCDR1	SGYHWG
58	HCDR2	AIYHSGSTYYSPSLKS
81	HCDR3	GPLSYYAFDY
82	LCDR1	RASQSVSRYLA
27	LCDR2	DASNRAT
83	LCDR3	QQYYRSPIT
84	VH	QVQLVQSGAEVKKPGSSVKVSCKASGYSITSGYHWGWIR QAPGQGLEWIGAIYHSGSTYYSPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARGPLSYYAFDYWGQGTLVTVSS
85	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVSRYLAWYQQKP GKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYYRSPITFGQGTKVEIK
86	HC	QVQLVQSGAEVKKPGSSVKVSCKASGYSITSGYHWGWIR QAPGQGLEWIGAIYHSGSTYYSPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARGPLSYYAFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
87	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVSRYLAWYQQKP

		GKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYYRSPITFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
抗 CD28 mAb (TY24878)		
88	HCDR1	TGGVAVA
89	HCDR2	QIYYSGSTYYNPSLKS
90	HCDR3	SSAHYYFDV
91	LCDR1	RASESVDFQGISFLD
300	LCDR2	DASSLES
70	LCDR3	QQSYRSPIT
92	VH	QVQLVQSGAEVKKPGSSVKVSCKASGFSLSTGGVAVAWI RQAPGQGLEWIGQIYYSGSTYYNPSLKSRVTITADKSTST AYMELSSLRSEDTAVYYCARSSAHYYFDVWGQGTLVTVS S
93	VL	DIQLTQSPSSLSASVGDRVTITCRASESVDFQGISFLDWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPITFGQGTKVEIK
94	HC	QVQLVQSGAEVKKPGSSVKVSCKASGFSLSTGGVAVAWI RQAPGQGLEWIGQIYYSGSTYYNPSLKSRVTITADKSTST AYMELSSLRSEDTAVYYCARSSAHYYFDVWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
95	LC	DIQLTQSPSSLSASVGDRVTITCRASESVDFQGISFLDWYQ QKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPITFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24879)		
24	HCDR1	SGYHWG
96	HCDR2	SIYHSGSTYYNPSLKS
97	HCDR3	SGLRYYAFDV
98	LCDR1	AASQDVSTAVA
9	LCDR2	AASSLQS

70	LCDR3	QQSYRSPIT
99	VH	QVQLVQSGAEVKKPGSSVKVSCKASGYSISSGYHWGWIR QAPGQGLEWIGSIYHSGSTYYNPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARSGLRYYAFDVWGQGTLVTVSS
100	VL	DIQLTQSPSSLSASVGDRVTITCAASQDVSTAVAWYQQKP GKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSYRSPITFGQGTKVEIK
101	HC	QVQLVQSGAEVKKPGSSVKVSCKASGYSISSGYHWGWIR QAPGQGLEWIGSIYHSGSTYYNPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARSGLRYYAFDVWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
102	LC	DIQLTQSPSSLSASVGDRVTITCAASQDVSTAVAWYQQKP GKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQSYRSPITFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
		抗 CD28 mAb (TY24884)
103	HCDR1	GYAIH
104	HCDR2	QIYHSGSTYYNPSLKS
105	HCDR3	SGYGGYDFDY
106	LCDR1	RASQSVPRRYLA
18	LCDR2	AASTLQS
83	LCDR3	QQYYRSPIT
107	VH	QVQLVQSGAEVKKPGSSVKVSCKASGFTFSGYAIHWVRQ APGQGLEWIGQIYHSGSTYYNPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARSGYGGYDFDYWGQGTLVTVS S
108	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVPRRYLAWYQQK PGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYYRSPITFGQGTKVEIK
109	HC	QVQLVQSGAEVKKPGSSVKVSCKASGFTFSGYAIHWVRQ APGQGLEWIGQIYHSGSTYYNPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARSGYGGYDFDYWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS

		WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
110	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVPRRYLAWYQQK PGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYYRSPITFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
		抗 CD28 mAb (TY24890)
111	HCDR1	SGHHWG
6	HCDR2	EIYHSGSTYYSPSLKS
112	HCDR3	RELGYAYFDY
113	LCDR1	RASQDIRKYLA
18	LCDR2	AASTLQS
114	LCDR3	QQYYRTPLT
115	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGHHWGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRELGYAYFDYWGQGTLVTV SS
116	VL	EIVLTQSPATLSLSPGERATLSCRASQDIRKYLAWYQQKPG QAPRLLIYAASTLQSGVPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQYYRTPLTFGQGTKVEIK
117	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGHHWGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRELGYAYFDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
118	LC	EIVLTQSPATLSLSPGERATLSCRASQDIRKYLAWYQQKPG QAPRLLIYAASTLQSGVPARFSGSGSGTDFTLTISSLEPEDF AVYYCQQYYRTPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQ

		LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
抗 CD28 mAb (TY24856)		
15	HCDR1	SGHHWS
6	HCDR2	EIYHSGSTYYSPSLKS
119	HCDR3	RDFGASYFDY
120	LCDR1	RASQGVGPWLA
9	LCDR2	AASSLQS
121	LCDR3	QQYYSYST
122	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGHHWSWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRDFGASYFDYWGQGTLVTV SS
123	VL	DIQLTQSPSSLSASVGDRVTITCRASQGVGPWLAWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYYSYSTFGQGTKVEIK
124	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGHHWSWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRDFGASYFDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
125	LC	DIQLTQSPSSLSASVGDRVTITCRASQGVGPWLAWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYYSYSTFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
抗 CD28 mAb (TY24881)		
126	HCDR1	TGGVGVT
67	HCDR2	SIYHSGSTYYSPSLKS
127	HCDR3	YDYGYYSFDV
128	LCDR1	RASQSVDFDAFSFLA
18	LCDR2	AASTLQS
129	LCDR3	QQSYSAST
130	VH	QVQLVQSGAEVKKPGSSVKVSCKASGFSLSTGGVGVTWI

		RQAPGQGLEWIGSIYHSGSTYYSPSLKSRVTITADKSTSTA YMELSSLRSEDTAVYYCARYDYGYYSFDVWGQGTLVTV SS
131	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFDAFSFLAWY QQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQSYSASTFGQGTKVEIK
132	HC	QVQLVQSGAEVKKPGSSVKVSCKASGFSLSTGGVGVTWI RQAPGQGLEWIGSIYHSGSTYYSPSLKSRVTITADKSTSTA YMELSSLRSEDTAVYYCARYDYGYYSFDVWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
133	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVDFDAFSFLAWY QQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQSYSASTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
抗 CD28 mAb (TY24858)		
134	HCDR1	SGHHWT
6	HCDR2	EIYHSGSTYYSPSLKS
135	HCDR3	RDYGSSYFDY
136	LCDR1	RASQSVSRSFLA
27	LCDR2	DASNRAT
83	LCDR3	QQYYRSPIT
137	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGHHWTWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRDYGSSYFDYWGQGTLVTV SS
138	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVSRSFLAWYQQKP GKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYYRSPITFGQGTKVEIK
139	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGHHWTWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARRDYGSSYFDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK

		TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
140	LC	DIQLTQSPSSLSASVGDRVTITCRASQSVSRSFLAWYQQKP GKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYYRSPITFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
		抗 CD28 mAb (TY24864)
43	HCDR1	TSGVGVS
58	HCDR2	AIYHSGSTYYSPSLKS
141	HCDR3	YGYSYYAFDY
142	LCDR1	RASQTIGSFLN
300	LCDR2	DASSLES
143	LCDR3	QQSYSYQT
144	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIR QAPGKGLEWIGAIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARYGYSYYAFDYWGQGTLVTV SS
145	VL	DIQLTQSPSSLSASVGDRVTITCRASQTIGSFLNWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSYQTFGQGTKVEIK
146	HC	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIR QAPGKGLEWIGAIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARYGYSYYAFDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
147	LC	DIQLTQSPSSLSASVGDRVTITCRASQTIGSFLNWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSYSYQTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES

		VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
抗 CD28 mAb (TY24877)		
148	HCDR1	NYGIH
6	HCDR2	EIYHSGSTYYSPSLKS
149	HCDR3	GPLSYYALDY
150	LCDR1	RASQSIRRFLN
300	LCDR2	DASSLES
83	LCDR3	QQYYRSPIT
151	VH	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYGIHWVR QAPGQGLEWIGEIYHSGSTYYSPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARGPLSYYALDYWGQGTLVTVSS
152	VL	DIQLTQSPSSLSASVGDRVTITCRASQSIRRFLNWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQYYRSPITFGQGTKVEIK
153	HC	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYGIHWVR QAPGQGLEWIGEIYHSGSTYYSPSLKSRVTITADKSTSTAY MELSSLRSEDTAVYYCARGPLSYYALDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
154	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIRRFLNWYQQKPG KAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQYYRSPITFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
抗 CD28 mAb (TY24855)		
15	HCDR1	SGHHWS
155	HCDR2	DIYHSGSTYYNPSLKS
16	HCDR3	KDDYYAFDY
156	LCDR1	RAPQSVDFHGKSFLH
27	LCDR2	DASNRAT
70	LCDR3	QQSYRSPIT
157	VH	EVQLVESGGGLVQPGGSLRLSCAASGYSITSGHHWSWIR QAPGKGLEWIGDIYHSGSTYYNPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKDDYYAFDYWGQGTLVTVS

		S
158	VL	DIQLTQSPSSLSASVGDRVTITCRAPQSVDFHGKSFLHWY QQKPGKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPITFGQGTKVEIK
159	HC	EVQLVESGGGLVQPGGSLRLSCAASGYSITSGHHWSWIR QAPGKGLEWIGDIYHSGSTYYNPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARKDDYYAFDYWGQGTLVTVS SASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH YTQKSLSLSLGK
160	LC	DIQLTQSPSSLSASVGDRVTITCRAPQSVDFHGKSFLHWY QQKPGKAPKLLIYDASNRATGIPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPITFGQGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN SQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
		抗 CD28 mAb (TY24857)
161	HCDR1	TSGVAVG
6	HCDR2	EIYHSGSTYYSPSLKS
162	HCDR3	HGYHYYALDY
163	LCDR1	PASQDVGTAVA
300	LCDR2	DASSLES
164	LCDR3	QQYYSSPPT
165	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVAVGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARHGYHYYALDYWGQGTLVTV SS
166	VL	DIQLTQSPSSLSASVGDRVTITCPASQDVGTAVAWYQQKP GKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYYSSPPTFGQGTKVEIK
167	HC	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVAVGWIR QAPGKGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTL YLQLNSLRAEDTAVYYCARHGYHYYALDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTK TYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY

		VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK
168	LC	DIQLTQSPSSLSASVGDRVTITCPASQDVGTAVAWYQQKP GKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED FATYYCQQYYSSPPTFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
未經遮蔽雙特異性抗體		
HER2×CD28 雙特異性抗體—(親本—TY24865)		
TY27566		
169	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKP GKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
170	HC-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVR QAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKN TLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRKKLTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
171	HC-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPRTFGQGTKVEIKGGGGSGGGGS GGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSL STGGVGVSWIRQAPGKGLEWIGEIYHSGSTYYSPSLKSRV TISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYDFD YWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTDPPSRDELTK NQVSLTCLVDGFYPSDIAVEWESNGQPENCYKTTPPVLDS

		DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ DSLSLSPGK
TY27881		
169	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKP GKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
170	HC-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVR QAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKN TLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLV TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRKKLTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
172	HC-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQSYRSPLTFGQGTKVEIKGGGGSGGGG SGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFS LSTSGVGVSWIRQAPGKGLEWIGEIYHSGSTYYSPSLKSR VTISRDNSKNTLYLQLNSLRVEDTAVYYCARDEYDYYAF DYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTDPPSRDELT KNQVSLTCLVDGFYPSDIAVEWESNGQPENCYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QDSLSLSPGK
TROP2×CD28 雙特異性抗體		
TY27571		
173	LC	DIQLTQSPSSLSASVGDRVTITCRASQDVSIAVAWYQQKPG KAPKLLIYSASYRYTGVPARFSGSGSGTDFTLTISSLQPED FATYYCQQHYITPLTFGQGTKVEIKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGEC
174	HC-A	QVQLLQSGSELKKPGASVKVSCKASGYTFTNYGINWVR

		QAPGQGLEWLGWIHTYTGEPTYTDDFKGRFVFSLDTSVS TAYLQISSLKADDTAVYYCARGGFGSSYWYFDVWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRKKLTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
171	HC-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPRTFGQGTKVEIKGGGGSGGGGS GGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSL STGGVGVSWIRQAPGKGLEWIGEIYHSGSTYYSPSLKSRV TISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYDFD YWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTDPPSRDELTK NQVSLTCLVDGFYPSDIAVEWESNGQPENCYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ DSLSLSPGK
B7H3×CD28 雙特異性抗體		
TY27556		
175	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIRKYLNWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYFCSQGTHVPFTFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
176	HC-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGYGIHWVRQ APGKGLEWIGWISPSGGDTKYAQKFQGRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARGGTVVYFDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRKKLTKNQVKLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVFSCSVMH

		EALHNHYTQKSLSLSPGK
171	HC-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQ QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQQSYRSPRTFGQGTKVEIKGGGGSGGGGS GGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFSL STGGVGVSWIRQAPGKGLEWIGEIYHSGSTYYSPSLKSRV TISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYDFD YWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTDPPSRDELTK NQVSLTCLVDGFYPSDIAVEWESNGQPENCYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ DSLSLSPGK
TY26999		
175	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIRKYLNWYQQKP GKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYFCSQGTHVPFTFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
176	HC-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGYGIHWVRQ APGKGLEWIGWISPSGGDTKYAQKFQGRVTISRDNSKNT LYLQLNSLRAEDTAVYYCARGGTVVYFDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSRKKLTKNQVKLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
177	HC-B	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYPNWVQ QKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGKAALTLS GAQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSGG GGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASG FTFNTYAINWVRQAPGKGLEWVGRIRSKYNNYATYYAES VKGRFTISRDDSKNTLYLQINSLRAEDTAVYYCVRHGNFG TSYVSWFAYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTD

		PPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENCY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQDSLSLSPGK

遮蔽性單元(MU)	
SEQ ID NO:	序列
178	ASDLYVCAHDPYFCHS
179	PNVFDTCYVDPYSCTI
180	YAAAPSCPPANYYCAD
181	HHADPLCFAQHSACNT
182	YDTHDYCLIHYHSCDT
183	ADFAPYCYADPYYCHD
184	DIASDSCYYHPNHCDT
185	AATYSDCYSDPYACHD
186	PTYDHDCAVYNNFCNA
187	HNHALTCYTDPYACHS
188	AAFSPACYTDPYSCHA
189	TPHPDACFVDPYHCHT
190	APSDAYCYADPYACHT
191	AVDDDPCYTDPYQCTA
192	THSDAVCAPDHAYCLY
193	PYIYVDCYAPPFFCAY
194	YFCAEGVPIVLCIG
195	DYCEGPPAFFYCAK
196	FPCEPLADPFLCRQ
197	LVCEDVPFPAACRA
198	ALCEFYAFPYFCAR
199	IVCKYFDDPFLCRR
200	YFCPVRVPLAVCLR
201	PSCPDYVPFPVCAQ
202	VACAKAFAYVYCLV
203	VLCGDSVPFLLCEP
204	ILCPEGVPVVFCVL
205	SDCGLGVPLLVCLK
206	TDCGIPFPVDDCLS
207	HIPDHYCAADHHYCYY
208	AATYSDCYSDPYACHD
209	YSDCYSDPYACHD
210	AAFSPACYTDPYSCHA
211	SPACYTDPYSCHA

遮蔽性肽組分		
SEQ ID NO:	描述	序列
212	N-terminal Unit	EVGSY
213	Linkage Unit(LU)	GGGPLGLAGSGGS
214	Linkage Unit(LU)	SGGGPLGLAGSGGS

SEQ ID NO:	樣本ID名稱	遮蔽性肽+裂解肽序列：:	SEQ ID NO			
			LC	HC	MU	LU
215	TY26142	*EVGSY*ASDLYVCAHDPYFCHSGGGPLGLAGSGGS	14	13	171	213
216	TY26143	*EVGSY*PNVFDTCYVDPYSCTIGGGPLGLAGSGGS	14	13	172	213
217	TY26144	*EVGSY*YAAAPSCPPANYYCADGGGPLGLAGSGGS	14	13	173	213
218	TY26145	*EVGSY*HHADPLCFAQHSACNTGGGPLGLAGSGGS	14	13	174	213
219	TY26146	*EVGSY*YDTHDYCLIHYHSCDTGGGPLGLAGSGGS	14	13	175	213
220	TY26147	*EVGSY*ADFAPYCYADPYYCHDGGGPLGLAGSGGS	14	13	176	213
221	TY26148	*EVGSY*DIASDSCYYHPNHCDTGGGPLGLAGSGGS	14	13	177	213
222	TY26149	*EVGSY*AATYSDCYSDPYACHDGGGPLGLAGSGGS	14	13	178	213
223	TY26150	*EVGSY*PTYDHDCAVYNNFCNAGGGPLGLAGSGGS	14	13	179	213
224	TY26151	*EVGSY*HNHALTCYTDPYACHSGGGPLGLAGSGGS	14	13	180	213
225	TY26152	*EVGSY*AAFSPACYTDPYSCHAGGGPLGLAGSGGS	14	13	181	213
226	TY26153	*EVGSY*TPHPDACFVDPYHCHTGGGPLGLAGSGGS	14	13	182	213
227	TY26154	*EVGSY*APSDAYCYADPYACHTGGGPLGLAGSGGS	14	13	183	213
228	TY26155	*EVGSY*AVDDDPCYTDPYQCTAGGGPLGLAGSGGS	14	13	184	213
229	TY26156	*EVGSY*THSDAVCAPDHAYCLYGGGPLGLAGSGGS	14	13	185	213

SEQ ID NO:	樣本ID名稱	遮蔽性肽+裂解肽序列：:	SEQ ID NO			
230	TY26157	*EVGSY*PYIYVDCYAPPFFCAYGGGPLGLAGSGGS	14	13	186	213
231	TY26158	*EVGSY*YFCAEGVPIVLCIGGGGPLGLAGSGGS	14	13	187	213
232	TY26159	*EVGSY*DYCEGPPAFFYCAKGGGPLGLAGSGGS	14	13	188	213
233	TY26160	*EVGSY*FPCEPLADPFLCRQGGGPLGLAGSGGS	14	13	189	213
234	TY26161	*EVGSY*LVCEDVPFPAACRAGGGPLGLAGSGGS	14	13	190	213
235	TY26162	*EVGSY*ALCEFYAFPYFCARGGGPLGLAGSGGS	14	13	191	213
236	TY26163	*EVGSY*IVCKYFDDPFLCRRGGGPLGLAGSGGS	14	13	192	213
237	TY26164	*EVGSY*YFCPVRVPLAVCLRGGGPLGLAGSGGS	14	13	193	213
238	TY26165	*EVGSY*PSCPDYVPFPVCAQGGGPLGLAGSGGS	14	13	194	213
239	TY26166	*EVGSY*VACAKAFAYVYCLVGGGPLGLAGSGGS	14	13	195	213
240	TY26167	*EVGSY*VLCGDSVPFLLCEPGGGPLGLAGSGGS	14	13	196	213
241	TY26168	*EVGSY*ILCPEGVPVVFCVLGGGPLGLAGSGGS	14	13	197	213
242	TY26169	*EVGSY*SDCGLGVPLLVCLKGGGPLGLAGSGGS	14	13	198	213
243	TY26170	*EVGSY*TDCGIPFPVDDCLSGGGPLGLAGSGGS	14	13	199	213
244	TY26171	*EVGSY*HIPDHYCAADHHYCYYGGGPLGLAGSGGS	14	13	200	213
245	TY28338	*E*AATYSDCYSDPYACHDSGGGPLGLAGSGGS	14	13	201	214
246	TY28339	*E*YSDCYSDPYACHDSGGGPLGLAGSGGS	14	13	202	214
247	TY28341	*E*AAFSPACYTDPYSCHAGGGPLGLAGSGGS	14	13	203	213
248	TY28342	*E*SPACYTDPYSCHAGGGPLGLAGSGGS	14	13	204	213

SEQ	撓性連接子

249	GGGGS
250	GGGGT
251	SGGS
252	GGSG
253	GGSGG
254	GSGSG
255	GSGGG
256	GGGSG
257	GSSSG

經遮蔽雙特異性抗體		
HER2×CD28 雙特異性抗體—(親本—TY24865)		
TY27566		
258	LCDR1	KASQDVSIGVA
259	LCDR2	SASYRYT
260	LCDR3	QQYYIYPYT
261	VL	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIK
262	HCDR1-A	DYTMD
263	HCDR2-A	DVNPNSGGSIYNQRFKG
264	HCDR3-A	NLGPSFYFDY
265	VH-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSS
5	HCDR1-B	TGGVGVS
6	HCDR2-B	EIYHSGSTYYSPSLKS
7	HCDR3-B	YEYYYYDFDY
8	LCDR1-B	RASQSVDFHGISFLH
9	LCDR2-B	AASSLQS
10	LCDR3-B	QQSYRSPRT
266	scFv-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYD FDYWGQGTLVTVSS
TY27807		
267	LCDR1	RASQDVNTAVA
268	LCDR2	SASFLYS
269	LCDR3	QQHYTTPPT
270	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLL

		IYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPT FGQGTKVEIK
271	LC	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLL IYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC
272	HCDR1-A	DTYIH
273	HCDR2-A	RIYPTNGYTRYADSVKG
274	HCDR3-A	WGGDGFYAMDY
275	VH-A	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV YYCSRWGGDGFYAMDYWGQGTLVTVSS
276	HC-A	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLE WVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV YYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
5	HCDR1-B	TGGVGVS
6	HCDR2-B	EIYHSGSTYYSPSLKS
7	HCDR3-B	YEYYYYDFDY
8	LCDR1-B	RASQSVDFHGISFLH
9	LCDR2-B	AASSLQS
10	LCDR3-B	QQSYRSPRT
266	scFv-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYD FDYWGQGTLVTVSS
171	HC-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYD FDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

		QPREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPE NCYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQDSLSLSPGK
TROP2×CD28 雙特異性抗體		
TY27571		
277	LCDR1-A	RASQDVSIAVA
259	LCDR2-A	SASYRYT
278	LCDR3-A	QQHYITPLT
279	VL-A	DIQLTQSPSSLSASVGDRVTITCRASQDVSIAVAWYQQKPGKAPKLLI YSASYRYTGVPARFSGSGSGTDFTLTISSLQPEDFATYYCQQHYITPLT FGQGTKVEIK
280	HCDR1-A	NYGIN
281	HCDR2-A	WIHTYTGEPTYTDDFKG
282	HCDR3-A	GGFGSSYWYFDV
283	VH-A	QVQLLQSGSELKKPGASVKVSCKASGYTFTNYGINWVRQAPGQGLE WLGWIHTYTGEPTYTDDFKGRFVFSLDTSVSTAYLQISSLKADDTAV YYCARGGFGSSYWYFDVWGQGTLVTVSS
5	HCDR1-B	TGGVGVS
6	HCDR2-B	EIYHSGSTYYSPSLKS
7	HCDR3-B	YEYYYYDFDY
8	LCDR1-B	RASQSVDFHGISFLH
9	LCDR2-B	AASSLQS
10	LCDR3-B	QQSYRSPRT
266	scFv-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYD FDYWGQGTLVTVSS
B7H3×CD28 雙特異性抗體		
TY27556		
287	LCDR1-A	RASQSIRKYLN
18	LCDR2-A	AASTLQS
288	LCDR3-A	QGTHVPFT
289	VL-A	DIQLTQSPSSLSASVGDRVTITCRASQSIRKYLNWYQQKPGKAPKLLI YAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQGTHVPFT FGQGTKVEIK
290	HCDR1-A	GYGIH
291	HCDR2-A	WISPSGGDTKYAQKFQG
292	HCDR3-A	GGTVVYFDY
293	VH-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGYGIHWVRQAPGKGLE WIGWISPSGGDTKYAQKFQGRVTISRDNSKNTLYLQLNSLRAEDTAV YYCARGGTVVYFDYWGQGTLVTVSS

5	HCDR1-B	TGGVGVS
6	HCDR2-B	EIYHSGSTYYSPSLKS
7	HCDR3-B	YEYYYYDFDY
8	LCDR1-B	RASQSVDFHGISFLH
9	LCDR2-B	AASSLQS
10	LCDR3-B	QQSYRSPRT
266	scFv-B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYD FDYWGQGTLVTVSS
B7H3×CD3 雙特異性抗體		
TY26999		
287	LCDR1-A	RASQSIRKYLN
18	LCDR2-A	AASTLQS
288	LCDR3-A	QGTHVPFT
289	VL-A	DIQLTQSPSSLSASVGDRVTITCRASQSIRKYLNWYQQKPGKAPKLLI YAASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCSQGTHVPFT FGQGTKVEIK
290	HCDR1-A	GYGIH
291	HCDR2-A	WISPSGGDTKYAQKFQG
292	HCDR3-A	GGTVVYFDY
293	VH-A	EVQLVESGGGLVQPGGSLRLSCAASGFTFSGYGIHWVRQAPGKGLE WIGWISPSGGDTKYAQKFQGRVTISRDNSKNTLYLQLNSLRAEDTAV YYCARGGTVVYFDYWGQGTLVTVSS
5	HCDR1-B	TYAIN
294	HCDR2-B	RIRSKYNNYATYYAESVKG
295	HCDR3-B	HGNFGTSYVSWFAY
296	LCDR1-B	GSSTGAVTTSNYPN
297	LCDR2-B	GTNKRAP
298	LCDR3-B	ALWYSNLWV
299	scFv-B	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYPNWVQQKPGQAPR GLIGGTNKRAPGVPARFSGSLLGGKAALTLSGAQPEDEAEYYCALW YSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSEVQLVESGG GLVQPGGSLRLSCAASGFTFNTYAINWVRQAPGKGLEWVGRIRSKY NNYATYYAESVKGRFTISRDDSKNTLYLQINSLRAEDTAVYYCVRHG NFGTSYVSWFAYWGQGTLVTVSS

SEQ ID NO:	IgG ID	遮蔽性肽+裂解肽序列：
301	TY26149	EVGSYAATYSDCYSDPYACHDGGGPLGLAGSGGS
302	TY28338	EAATYSDCYSDPYACHDSGGGPLGLAGSGGS
303	TY28339	EYSDCYSDPYACHDSGGGPLGLAGSGGS
304	TY26152	EVGSYAAFSPACYTDPYSCHAGGGPLGLAGSGGS
305	TY28341	EAAFSPACYTDPYSCHAGGGPLGLAGSGGS
306	TY28342	ESPACYTDPYSCHAGGGPLGLAGSGGS

TY24865 變體		
SEQ ID NO:	描述	序列
TY28652		
5	HCDR1	TGGVGVS
96	HCDR2	SIYHSGSTYYNPSLKS
307	HCDR3	YEYDYYAFDY
76	LCDR1	RASQSVDFYGKSFLH
308	LCDR2	AASSLES
46	LCDR3	QQSYRSPPT
362	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPG KGLEWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYDYYAFDYWGQGTLVTVSS
363	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPG KAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPPTFGQGTKVEIK
TY28653		
5	HCDR1	TGGVGVS
96	HCDR2	SIYHSGSTYYNPSLKS
309	HCDR3	YEYGYYALDY
310	LCDR1	RASQSVDFYGISFLH
9	LCDR2	AASSLQS
311	LCDR3	QQSYRTPPT
364	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPG KGLEWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYGYYALDYWGQGTLVTVSS
365	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRTPPTFGQGTKVEIK
TY28654		
43	HCDR1	TSGVGVS
96	HCDR2	SIYHSGSTYYNPSLKS
312	HCDR3	YEYGYYAFDY

76	LCDR1	RASQSVDFYGKSFLH
9	LCDR2	AASSLQS
28	LCDR3	QQSYRTPLT
366	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPG KGLEWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYGYYAFDYWGQGTLVTVSS
367	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRTPLTFGQGTKVEIK
TY28655		
43	HCDR1	TSGVGVS
96	HCDR2	SIYHSGSTYYNPSLKS
307	HCDR3	YEYDYYAFDY
310	LCDR1	RASQSVDFYGISFLH
9	LCDR2	AASSLQS
313	LCDR3	QQSYRTPRT
368	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPG KGLEWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYDYYAFDYWGQGTLVTVSS
369	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRTPRTFGQGTKVEIK
TY28656		
314	HCDR1	TSGVGVG
315	HCDR2	EIYHSGSTNYNPSLKS
309	HCDR3	YEYGYYALDY
76	LCDR1	RASQSVDFYGKSFLH
9	LCDR2	AASSLQS
313	LCDR3	QQSYRTPRT
370	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPG KGLEWIGEIYHSGSTNYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYGYYALDYWGQGTLVTVSS
371	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRTPRTFGQGTKVEIK
TY28657		
33	HCDR1	TGGVGVG
316	HCDR2	EIYHSGSTYYNPSLKS
312	HCDR3	YEYGYYAFDY
8	LCDR1	RASQSVDFHGISFLH
308	LCDR2	AASSLES
313	LCDR3	QQSYRTPRT
372	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWIRQAPG

		KGLEWIGEIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYGYYAFDYWGQGTLVTVSS
373	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRTPRTFGQGTKVEIK
TY28658		
314	HCDR1	TSGVGVG
96	HCDR2	SIYHSGSTYYNPSLKS
317	HCDR3	YEYDYYDFDY
76	LCDR1	RASQSVDFYGKSFLH
308	LCDR2	AASSLES
318	LCDR3	QQSYRSPLT
374	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPG KGLEWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYDYYDFDYWGQGTLVTVSS
375	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPG KAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPLTFGQGTKVEIK
TY28659		
43	HCDR1	TSGVGVS
316	HCDR2	EIYHSGSTYYNPSLKS
312	HCDR3	YEYGYYAFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
376	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPG KGLEWIGEIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYEYGYYAFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK
TY28660		
43	HCDR1	TSGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
319	HCDR3	YEYYSYAFDY
320	LCDR1	RASQSVDFHGKSFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
377	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPG KGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYEYYSYAFDYWGQGTLVTVSS
378	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGKSFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY

		CQQSYRSPRTFGQGTKVEIK
TY28661		
5	HCDR1	TGGVGVS
67	HCDR2	SIYHSGSTYYSPSLKS
307	HCDR3	YEYDYYAFDY
310	LCDR1	RASQSVDFYGISFLH
308	LCDR2	AASSLES
28	LCDR3	QQSYRTPLT
379	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPG KGLEWIGSIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYEYDYYAFDYWGQGTLVTVSS
380	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPG KAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRTPLTFGQGTKVEIK
TY30120		
43	HCDR1	TSGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
321	HCDR3	YGYYYYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
381	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPG KGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYGYYYYDFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK
TY30121		
314	HCDR1	TSGVGVG
316	HCDR2	EIYHSGSTYYNPSLKS
322	HCDR3	YKYDYYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
382	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPG KGLEWIGEIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRA EDTAVYYCARYKYDYYDFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK
TY30123		
33	HCDR1	TGGVGVG
6	HCDR2	EIYHSGSTYYSPSLKS

321	HCDR3	YGYYYYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
383	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWIRQAPG KGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYGYYYYDFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK
TY30406		
5	HCDR1	TGGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
317	HCDR3	YEYDYYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
384	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPG KGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYEYDYYDFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK
TY30410		
5	HCDR1	TGGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
323	HCDR3	YEYYEYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT
385	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPG KGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYEYYEYDFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK
TY30413		
5	HCDR1	TGGVGVS
6	HCDR2	EIYHSGSTYYSPSLKS
324	HCDR3	YEYYHYDFDY
8	LCDR1	RASQSVDFHGISFLH
9	LCDR2	AASSLQS
10	LCDR3	QQSYRSPRT

386	VH	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPG KGLEWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAE DTAVYYCARYEYYHYDFDYWGQGTLVTVSS
12	VL	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQSYRSPRTFGQGTKVEIK

SEQ ID NO:	PD-L1xCD28 雙特異性抗體(VH-VL)	
	TY29815(WT)	
325	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIPSFLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHYISWPRQ FTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC*
326	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGYYWGWIRQAPGKGLE WIGIIYPSGGGTNYAQKFQGRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARGGGLGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK*
327	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYYYYDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY30406		
325	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIPSFLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHYISWPRQF TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC*
326	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGYYWGWIRQAPGKGLE

		WIGIIYPSGGGTNYAQKFQGRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARGGGLGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK*
328	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYDYYDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY30410		
325	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIPSFLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHYISWPRQF TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC*
326	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGYYWGWIRQAPGKGLE WIGIIYPSGGGTNYAQKFQGRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARGGGLGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK*
329	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYYEYDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY

		ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY30413		
325	LC	DIQLTQSPSSLSASVGDRVTITCRASQSIPSFLNWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHYISWPRQF TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC*
326	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGYSISSGYYWGWIRQAPGKGLE WIGIIYPSGGGTNYAQKFQGRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARGGGLGFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK*
330	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYYHYDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
HER2xCD28 雙特異性抗體(VL-VH)		
TY27566(WT)		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP

		ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
333	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYYYD FDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28652		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
334	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPPTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGSIYHSGST YYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYDYYA FDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*

TY28653		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
335	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR TPPTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGSIYHSGST YYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYGYYA LDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28654		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*

336	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY RTPLTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPGKGLEWIGSIYHSGST YYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYGYYA FDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28655		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
337	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR TPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTSGVGVSWIRQAPGKGLEWIGSIYHSGSTY YNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYDYYAF DYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28656		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV

		YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
338	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY RTPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPGKGLEWIGEIYHSGS TNYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYGYY ALDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPEN CYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQDSLSLSPGK*
TY28657		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
339	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRT PRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQ PGGSLRLSCAASGFSLSTGGVGVGWIRQAPGKGLEWIGEIYHSGSTY YNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYGYYAF

		DYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28658		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
340	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPLTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTSGVGVGWIRQAPGKGLEWIGSIYHSGST YYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYDYYD FDYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28659		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV

		VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
341	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLV QPGGSLRLSCAASGFSLSTSGVGVSWIRQAPGKGLEWIGEIYHSGSTY YNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYGYYAF DYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY28660		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
342	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGKSFLHWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY RSPRTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGL VQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPGKGLEWIGEIYHSGST YYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYYSYAF DYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY

		TQDSLSLSPGK*
TY28661		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
343	HC_B	DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPGKAP KLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRT PLTFGQGTKVEIKGGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQ PGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGLEWIGSIYHSGSTY YSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVYYCARYEYDYYAF DYWGQGTLVTVSSEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
HER2XCD28 雙特異性抗體(VH-VL)		
TY29109(WT)		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF

		YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
327	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGEIYHSGSTYYSPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYYYYDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29306		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
344	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYDYYAFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPPTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29307		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT

		FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
345	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVSWIRQAPGKGL EWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYGYYALDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR TPPTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29308		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
346	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPGKGL EWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYGYYAFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS

		DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY RTPLTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29309		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
347	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVSWIRQAPGKGL EWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYDYYAFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGISFLHWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR TPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29310		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA

		VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
348	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPGKGL EWIGEIYHSGSTNYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYGYYALDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY RTPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29311		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
349	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTGGVGVGWIRQAPGKG LEWIGEIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAV YYCARYEYGYYAFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGG SDIQLTQSPSSLSASVGDRVTITCRASQSVDFHGISFLHWYQQKPGKA PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR TPRTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP

		REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29312		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
350	HC_B	EVQLVESGGGLVQPGGSLRLSCAASGFSLSTSGVGVGWIRQAPGKGL EWIGSIYHSGSTYYNPSLKSRVTISRDNSKNTLYLQLNSLRAEDTAVY YCARYEYDYYDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS DIQLTQSPSSLSASVGDRVTITCRASQSVDFYGKSFLHWYQQKPGKA PKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYR SPLTFGQGTKVEIKEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTDPPSRDELTKNQVSLTCLVDGFYPSDIAVEWESNGQPENC YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQDSLSLSPGK*
TY29313		
331	LC	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLI YSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC*
332	HC_A	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLE WVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTA VYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS

		NKALPAPIEKTISKAKGQPREPQVYTLPPSRKKLTKNQVKLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDCDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
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【符號說明】

【0260】

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【發明申請專利範圍】

【請求項1】 一種抗原結合蛋白或其抗原結合片段，包含CD28結合部分，其中CD28結合部分結合人類CD28並與食蟹獼猴及小鼠CD28交叉反應。

【請求項2】 如請求項1之抗原結合蛋白或其片段，其中CD28結合部分結合至CD28抗原表位，其包含人類CD28 (SEQ ID NO: 1)之51-122胺基酸殘基。

【請求項3】 如請求項1之抗原結合蛋白或其片段，其中CD28抗原表位包含SEQ ID NO: 1之51、52、54、55、98-101、110-111、113-114及118-122胺基酸殘基。

【請求項4】 如請求項1至3中任一項之抗原結合蛋白或其片段，其中CD28結合部分包含抗體重鏈可變區(V_H)及抗體輕鏈可變區(V_L)，及其中V_H及V_L包含所示之重鏈互補決定區(CDRs) 1-3及輕鏈CDR1-3，

分別為 SEQ ID NOs: 5-10，

分別為 SEQ ID NOs: 15、6、16、17-19，

分別為 SEQ ID NOs: 24、6、25、26-28，

分別為 SEQ ID NOs: 33、6、35-38，

分別為 SEQ ID NOs: 43、6、44、45、9 及 46，

分別為 SEQ ID NOs: 33、51-53、300 及 10，

分別為 SEQ ID NOs: 24、58、59、60、300 及 61，

分別為 SEQ ID NOs: 66-69、300 及 70，

分別為 SEQ ID NOs: 24、6、75、76、18 及 28，

分別為 SEQ ID NOs: 24、58、81、82、27 及 83，

分別為 SEQ ID NOs: 88-91、300 及 70，

分別為 SEQ ID NOs: 24、96-98、9 及 70，

分別為 SEQ ID NOs: 103-106、18 及 83，

分別為 SEQ ID NOs: 111、6、112、113、18 及 114，

分別為 SEQ ID NOs: 15、6、119、120、9 及 121，

分別為 SEQ ID NOs: 126、67、127、128、18 及 129，

分別為 SEQ ID NOs: 134、6、135、136、27 及 83，

分別為 SEQ ID NOs: 43、58、141、142、300 及 143，

分別為 SEQ ID NOs: 148、6、149、150、300 及 83，

分別為 SEQ ID NOs: 15、155、16、156、27 及 70，

分別為 SEQ ID NOs: 161、6、162、163、300 及 164，

分別為 SEQ ID NOs: 5、96、307、76、308 及 46，

分別為 SEQ ID NOs: 5、96、309、310、9 及 311，

分別為 SEQ ID NOs: 43、96、312、76、9 及 28，

分別為 SEQ ID NOs: 43、96、307、310、9 及 313，

分別為 SEQ ID NOs: 314、315、309、76、9 及 313，

分別為 SEQ ID NOs: 33、316、312、8、308 及 313，

分別為 SEQ ID NOs: 314、96、317、76、308 及 318，

分別為 SEQ ID NOs: 43、316、312、8、9 及 10，

分別為 SEQ ID NOs: 43、6、319、320、9 及 10，

分別為 SEQ ID NOs: 5、67、307、310、308 及 28，

分別為 SEQ ID NOs: 43、6、321、8、9 及 10，

分別為 SEQ ID NOs: 314、316、322、8、9 及 10，

分別為 SEQ ID NOs: 33、6、321、8、9 及 10，

分別為 SEQ ID NOs: 5、6、317、8、9 及 10，

分別為 SEQ ID NOs: 5、6、323、8、9 及 10，或

分別為 SEQ ID NOs: 5、6、324、8、9 及 10。

【請求項5】 抗原結合蛋白或其抗原結合片段，包含結合人類CD28之CD28結合部分，其中CD28結合部分包含抗體重鏈可變區(V_H)及抗體輕鏈可變區(V_L)，及其中V_H及V_L包含所示之重鏈互補決定區(CDRs) 1-3及輕鏈CDR1-3，

分別為 SEQ ID NOs: 5-10，

分別為 SEQ ID NOs: 15、6、16、17-19，

分別為 SEQ ID NOs: 24、6、25、26-28，

分別為 SEQ ID NOs: 33、6、35-38，

分別為 SEQ ID NOs: 43、6、44、45、9 及 46，

分別為 SEQ ID NOs: 33、51-53、300 及 10，

分別為 SEQ ID NOs: 24、58、59、60、300 及 61，

分別為 SEQ ID NOs: 66-69、300 及 70，

分別為 SEQ ID NOs: 24、6、75、76、18 及 28，

分別為 SEQ ID NOs: 24、58、81、82、27 及 83，

分別為 SEQ ID NOs: 88-91、300 及 70，

分別為 SEQ ID NOs: 24、96-98、9 及 70，

分別為 SEQ ID NOs: 103-106、18 及 83，

分別為 SEQ ID NOs: 111、6、112、113、18 及 114，

分別為 SEQ ID NOs: 15、6、119、120、9 及 121，

分別為 SEQ ID NOs: 126、67、127、128、18 及 129，

分別為 SEQ ID NOs: 134、6、135、136、27 及 83，

分別為 SEQ ID NOs: 43、58、141、142、300 及 143，

分別為 SEQ ID NOs: 148、6、149、150、300 及 83，

分別為 SEQ ID NOs: 15、155、16、156、27 及 70，

分別為 SEQ ID NOs: 161、6、162、163、300 及 164，

分別為 SEQ ID NOs: 5、96、307、76、308 及 46，

分別為 SEQ ID NOs: 5、96、309、310、9 及 311，

分別為 SEQ ID NOs: 43、96、312、76、9 及 28，

分別為 SEQ ID NOs: 43、96、307、310、9 及 313，

分別為 SEQ ID NOs: 314、315、309、76、9 及 313，

分別為 SEQ ID NOs: 33、316、312、8、308 及 313，

分別為 SEQ ID NOs: 314、96、317、76、308 及 318，

分別為 SEQ ID NOs: 43、316、312、8、9 及 10，

分別為 SEQ ID NOs: 43、6、319、320、9 及 10，

分別為 SEQ ID NOs: 5、67、307、310、308 及 28，

分別為 SEQ ID NOs: 43、6、321、8、9 及 10，

分別為 SEQ ID NOs: 314、316、322、8、9 及 10，

分別為 SEQ ID NOs: 33、6、321、8、9 及 10，

分別為 SEQ ID NOs: 5、6、317、8、9 及 10，

分別為 SEQ ID NOs: 5、6、323、8、9 及 10，或

分別為 SEQ ID NOs: 5、6、324、8、9 及 10。

【請求項6】 如請求項4或5之抗原結合蛋白或其片段，其中CD28結合部分包含所示之V_H及V_L，

分別為 SEQ ID NOs: 11 及 12，

分別為 SEQ ID NOs: 20 及 21，

分別為 SEQ ID NOs: 29 及 30，

分別為 SEQ ID NOs: 39 及 40，

分別為 SEQ ID NOs: 47 及 48，

分別為 SEQ ID NOs: 54 及 55，

分別為 SEQ ID NOs: 62 及 63，

分別為 SEQ ID NOs: 71 及 72，

分別為 SEQ ID NOs: 77 及 78，

分別為 SEQ ID NOs: 84 及 85，

分別為 SEQ ID NOs: 92 及 93，

分別為 SEQ ID NOs: 99 及 100，

分別為 SEQ ID NOs: 107 及 108，

分別為 SEQ ID NOs: 115 及 116，

分別為 SEQ ID NOs: 122 及 123，

分別為 SEQ ID NOs: 130 及 131，

分別為 SEQ ID NOs: 137 及 138，

分別為 SEQ ID NOs: 144 及 145，

分別為 SEQ ID NOs: 151 及 152，

分別為 SEQ ID NOs: 157 及 158，

分別為 SEQ ID NOs: 165 及 166，

分別為 SEQ ID NOs: 362 及 363，

分別為 SEQ ID NOs: 364 及 365，

分別為 SEQ ID NOs: 366 及 367，

分別為 SEQ ID NOs: 368 及 369，

分別為 SEQ ID NOs: 370 及 371，

分別為 SEQ ID NOs: 372 及 373，

分別為 SEQ ID NOs: 374 及 375，

分別為 SEQ ID NOs: 376 及 12，

分別為 SEQ ID NOs: 377 及 378，

分別為 SEQ ID NOs: 379 及 380，

分別為 SEQ ID NOs: 381 及 12，

分別為 SEQ ID NOs: 382 及 12，

分別為 SEQ ID NOs: 383 及 12，

分別為 SEQ ID NOs: 384 及 12，

分別為 SEQ ID NOs: 385 及 12，或

分別為 SEQ ID NOs: 386 及 12。

【請求項7】 如請求項4或5之抗原結合蛋白，包含所示之抗體重鏈及抗體輕鏈，

分別為 SEQ ID NOs: 13 及 14，

分別為 SEQ ID NOs: 22 及 23，

分別為 SEQ ID NOs: 31 及 32，

分別為 SEQ ID NOs: 41 及 42，

分別為 SEQ ID NOs: 49 及 50，

分別為 SEQ ID NOs: 56 及 57，

分別為 SEQ ID NOs: 64 及 65，

分別為 SEQ ID NOs: 73 及 74，

分別為 SEQ ID NOs: 79 及 80，

分別為 SEQ ID NOs: 86 及 87，

分別為 SEQ ID NOs: 94 及 95，

分別為 SEQ ID NOs: 101 及 102，

分別為 SEQ ID NOs: 109 及 110，

分別為 SEQ ID NOs: 117 及 118，

分別為 SEQ ID NOs: 124 及 125，

分別為 SEQ ID NOs: 141 及 142，

分別為 SEQ ID NOs: 132 及 133，

分別為 SEQ ID NOs: 139 及 140，

分別為 SEQ ID NOs: 146 及 147，

分別為 SEQ ID NOs: 153 及 154，

分別為 SEQ ID NOs: 159 及 160，或

分別為SEQ ID NOs: 167及168。

【請求項8】 如請求項1至7中任一項之抗原結合蛋白或其片段，其中抗原結合部分或其片段無超促效活性。

【請求項9】 如請求項1至8中任一項之抗原結合蛋白或其片段，另包含靶向腫瘤相關抗原(TAA)之第二抗原結合部分。

【請求項10】 如請求項9之抗原結合蛋白或其片段，其中TAA為HER2、B7-H3或TROP-2。

【請求項11】 如請求項10之抗原結合蛋白或其片段，包含HER2結合部分，其含有

分別如 SEQ ID NOs: 262-264 及 258-260 中所示之 HCDR1-3 及 LCDR1-3，或

分別如SEQ ID NOs: 265及261中所示之VH及VL。

【請求項12】 如請求項10之抗原結合蛋白或其片段，包含B7-H3結合部分，其含有

分別如 SEQ ID NOs: 290-292 以及 287、18 及 288 中所示之 HCDR1-3 及 LCDR1-3，或

分別如SEQ ID NOs: 293及289中所示之VH及VL。

【請求項13】 如請求項10之抗原結合蛋白或其片段，包含TROP-2結合部分，其含有

分別如 SEQ ID NOs: 280-282 以及 277、259 及 278 中所示之 HCDR1-3 及 LCDR1-3，或

分別如SEQ ID NOs: 283及279中所示之VH及VL。

【請求項14】 如請求項1至13中任一項之抗原結合蛋白或其片段，其中CD28結合部分及TAA結合部分之任意一個或兩個為單鏈Fv (scFv)、Fv、 scFab或Fab。

【請求項15】 如請求項11之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 169及170之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 171之重鏈或含有SEQ ID NO: 266之scFv融合多肽。

【請求項16】 如請求項11之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 169及170之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 172之重鏈或含有SEQ ID NO: 266之scFv融合多肽。

【請求項17】 如請求項11之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 271及276之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 171之重鏈或含有SEQ ID NO: 266之scFv融合多肽。

【請求項18】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 327之重鏈。

【請求項19】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 333之重鏈。

【請求項20】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 334之重鏈。

【請求項21】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 335之重鏈。

【請求項22】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 336之重鏈。

【請求項23】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 337之重鏈。

【請求項24】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 338之重鏈。

【請求項25】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 339之重鏈。

【請求項26】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 340之重鏈。

【請求項27】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 341之重鏈。

【請求項28】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 342之重鏈。

【請求項29】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 343之重鏈。

【請求項30】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 344之重鏈。

【請求項31】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 345之重鏈。

【請求項32】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 346之重鏈。

【請求項33】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 347之重鏈。

【請求項34】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 348之重鏈。

【請求項35】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 349之重鏈。

【請求項36】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 350之重鏈。

【請求項37】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 351之重鏈。

【請求項38】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 352之重鏈。

【請求項39】 如請求項10之抗原結合蛋白或其片段，其中HER2結合部分包含分別含有SEQ ID NOs: 331及332之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 353之重鏈。

【請求項40】 如請求項12之抗原結合蛋白或其片段，其中B7-H3結合部分包含分別含有SEQ ID NOs: 175及176之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 171之重鏈或含有SEQ ID NO: 266之scFv融合多肽。

【請求項41】 如請求項10之抗原結合蛋白或其片段，其中B7-H3結合部分包含分別含有SEQ ID NOs: 354及355之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 327之重鏈。

【請求項42】 如請求項10之抗原結合蛋白或其片段，其中B7-H3結合部分包含分別含有SEQ ID NOs: 354及355之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 356之重鏈。

【請求項43】 如請求項10之抗原結合蛋白或其片段，其中B7-H3結合部分包含分別含有SEQ ID NOs: 354及355之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 357之重鏈。

【請求項44】 如請求項10之抗原結合蛋白或其片段，其中B7-H3結合部分包含分別含有SEQ ID NOs: 354及355之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 358之重鏈。

【請求項45】 如請求項13之抗原結合蛋白或其片段，其中TROP2結合部分包含分別含有SEQ ID NOs: 173及174之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 171之重鏈或含有SEQ ID NO: 266之scFv融合多肽。

【請求項46】 如請求項1至45中任一項之抗原結合蛋白或其片段，另包含靶向免疫檢查點抑制劑之另一抗原結合部分。

【請求項47】 如請求項46之抗原結合蛋白，其中免疫檢查點抑制劑為PD-L1。

【請求項48】 如請求項47之抗原結合蛋白或其片段，其中PD-L1結合部分包含分別含有SEQ ID NOs: 325及326之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 327之重鏈。

【請求項49】 如請求項47之抗原結合蛋白或其片段，其中PD-L1結合部分包含分別含有SEQ ID NOs: 325及326之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 328之重鏈。

【請求項50】 如請求項47之抗原結合蛋白或其片段，其中PD-L1結合部分包含分別含有SEQ ID NOs: 325及326之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 329之重鏈。

【請求項51】 如請求項47之抗原結合蛋白或其片段，其中PD-L1結合部分包含分別含有SEQ ID NOs: 325及326之抗體輕鏈及抗體重鏈，及其中CD28結合部分包含含有SEQ ID NO: 330之重鏈。

【請求項52】 如請求項1至51中任一項之抗原結合蛋白或其片段，另包含Fc區。

【請求項53】 如請求項52之抗原結合蛋白或其片段，其中Fc區為人類IgG1子類。

【請求項54】 如請求項52之抗原結合蛋白或其片段，其中Fc區為人類IgG4子類。

【請求項55】 如請求項52至54中任一項之抗原結合蛋白或其片段，其中Fc區具有降低或無效應功能、降低或無抗體依賴性細胞毒性(ADCC)效應及/或降低或無交聯效應。

【請求項56】 如請求項1至55中任一項之抗原結合蛋白或其片段，包含第一CH3結構域及第二CH3結構域，其中

i)第一CH3結構域包含在位置390之半胱胺酸(C)殘基且第二CH3結構域包含在位置400之半胱胺酸殘基，或第一CH3結構域包含在位置400之半胱胺酸殘基且第二CH3結構域包含在位置390之半胱胺酸殘基；或

ii)第一CH3結構域包含在位置392之半胱胺酸殘基且第二CH3結構域包含在位置397之半胱胺酸殘基，或第一CH3結構域包含在位置397之半胱胺酸殘基且第二CH3結構域包含在位置392之半胱胺酸殘基；或

iii)第一CH3結構域包含在位置392之半胱胺酸殘基且第二CH3結構域包含在位置400之半胱胺酸殘基，或第一CH3結構域包含在位置400之半胱胺酸殘基且第二CH3結構域包含在位置392之半胱胺酸殘基；且其中胺基酸殘基編號係基於EU編號。

【請求項57】 如請求項56之抗原結合蛋白或其片段，其中

i)第一CH3結構域另包含在位置357之帶正電荷之殘基且第二CH3結構域另包含在位置351之帶負電荷之殘基，或第一CH3結構域另包含在位置351之帶負電荷之殘基且第二CH3結構域另包含在位置357之帶正電荷之殘基；或

ii)第一CH3結構域另包含在位置411之帶正電荷之殘基且第二CH3結構域另包含在位置370之帶負電荷之殘基，或第一CH3結

構域另包含在位置370之帶負電荷之殘基且第二CH3結構域另包含在位置411之帶正電荷之殘基；或

iii)第一CH3結構域另包含在位置364之帶正電荷之殘基且第二CH3結構域另包含在位置370之帶負電荷之殘基，或第一CH3結構域另包含在位置370之帶負電荷之殘基且第二CH3結構域另包含在位置364之帶正電荷之殘基；或i)與ii)之組合，或i)與iii)之組合；且其中胺基酸殘基編號係基於EU編號。

【請求項58】 如請求項57之抗原結合蛋白或其片段，其中第一CH3結構域包含D/E356K、E357K、S364K及S400C取代以及第二CH3結構域包含L351D、K370D、N390C及K439D取代，或第一CH3結構域包含L351D、K370D、N390C及K439D取代以及第二CH3結構域包含D/E356K、E357K、S364K及S400C取代(Eu編號)。

【請求項59】 如請求項58之抗原結合蛋白或其片段，其中CH3結構域另包含N297A取代(Eu編號)。

【請求項60】 如請求項1至60中任一項之抗原結合蛋白或其片段，另包含至少一個遮蔽性肽，其中遮蔽性肽(MP)連接至VL之N端，其中MP自N端至C端包含遮蔽性單元(MU)及具有或不具有裂解位點之連接單元(LU)。

【請求項61】 如請求項60之抗原結合蛋白或其片段，及其中MU包含選自由SEQ ID NOs: 173-206組成之群之序列。

【請求項62】 如請求項61之抗原結合蛋白或其片段，其中MP另包含與MU之N端連接之N端單元(NU)。

【請求項63】 如請求項62之抗原結合蛋白或其片段，其中N端單元長約1-10個胺基酸殘基。

【請求項64】 如請求項63之抗原結合蛋白或其片段，其中N端單元包含E或EVGSY。

【請求項65】 如請求項60至64中任一項之抗原結合蛋白或其片段，其中LU包含裂解位點。

【請求項66】 如請求項65之抗原結合蛋白或其片段，其中第一裂解位點為選自由尿激酶型纖維蛋白溶酶原活化因數/uPA、基質金屬蛋白酶-1/MMP-1、MMP-2、MMP-3、MMP-8、MMP-9、MMP-14、煙草蝕紋病毒蛋白酶/TEV蛋白酶、胞漿素、凝血酶、因數X、PSA、PSMA、組織蛋白酶D、組織蛋白酶K、組織蛋白酶S、ADAM10、ADAM12、ADAMTS、卡斯蛋白酶-1、卡斯蛋白酶-2、卡斯蛋白酶-3、卡斯蛋白酶-4、卡斯蛋白酶-5、卡斯蛋白酶-6、卡斯蛋白酶-7、卡斯蛋白酶-8、卡斯蛋白酶-9、卡斯蛋白酶-10、卡斯蛋白酶-11、卡斯蛋白酶-12、卡斯蛋白酶-13、卡斯蛋白酶-14及TACE蛋白酶組成之群之蛋白酶裂解位點。

【請求項67】 如請求項60至66中任一項之抗原結合蛋白或其片段，其中至少一個遮蔽性肽連接至B7-H3、HER2或TROP2結合部分或CD28結合部分，其中抗原結合蛋白或其片段視情況包含分別

結合B7-H3、HER2或TROP2結合部分及CD28結合部分之兩個遮蔽性肽。

【請求項68】 如請求項1至67中任一項之抗原結合蛋白或其片段，另包含綴合治療劑部分，其中治療劑部分視情況為放射性部分或細胞毒性部分。

【請求項69】 醫藥組合物包含如請求項1至68中任一項之抗原結合蛋白或其片段及醫藥上可接受之載劑。

【請求項70】 核酸分子或編碼如請求項1至68中任一項之抗原結合蛋白或其片段之核酸分子。

【請求項71】 一種宿主細胞包含如請求項70之核苷酸序列。

【請求項72】 一種產生抗原結合蛋白或其抗原結合片段之方法，包括：

在允許抗原結合蛋白或其片段表現之條件下培養如請求項71之宿主細胞，及

自培養基中單離抗原結合蛋白或其片段。

【請求項73】 一種治療有需要患者癌症之方法，包括向患者投與治療有效量之如請求項1至68中任一項之抗原結合蛋白或其片段或如請求項69之醫藥組合物。

【請求項74】 如請求項73之方法，另包括向患者投與另外抗癌症治療劑。

【請求項75】 如請求項74之方法，其中另外抗癌症治療劑為靶向CD3及腫瘤抗原之雙特異性抗體，其中腫瘤抗原視情況與TAA相同或不同。

【請求項76】 如請求項75之抗原結合蛋白或其片段，其中TAA為B7-H3、HER2或TROP2。

【請求項77】 如請求項74之方法，其中另外抗癌症治療劑為免疫檢查點抑制劑，視情況為抗PD-1、抗CTLA-4或抗PD-L1抗體。

【請求項78】 如請求項73至77中任一項之方法，其中患者具有實體瘤或血液惡性腫瘤，視情況選自乳癌、胃癌、肺癌、卵巢癌、腎癌、胰腺癌及結腸癌。

【請求項79】 使用如請求項1至68中任一項之抗原結合蛋白或其片段製造用於治療癌症之藥物，視情況如請求項73至78中任一項之方法。

【請求項80】 如請求項1至68中任一項之抗原結合蛋白或其片段，或如請求項69之醫藥組合物，用於治療癌症，視情況如請求項73至78中任一項之方法。

【發明圖式】

圖1

圖 3

圖4A

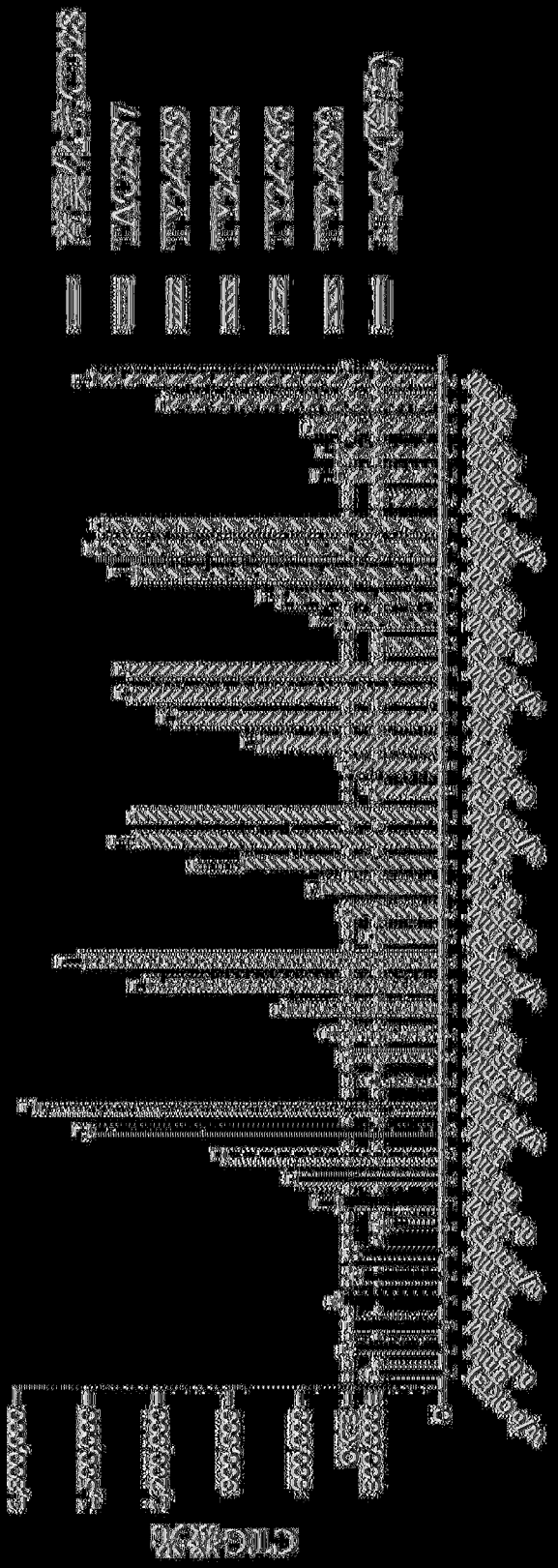
圖5A

圖 1

圖 3-1

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