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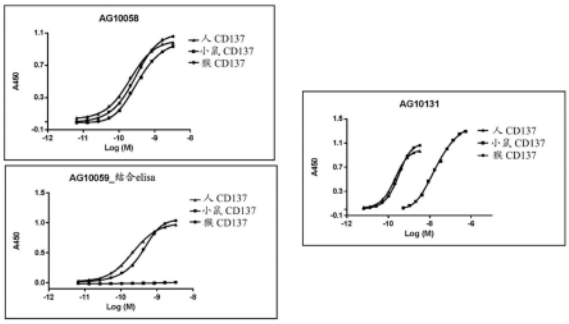
权利要求书2页 说明书49页
序列表314页 附图30页

(54)发明名称

抗CD137分子及其用途

(57)摘要

本公开提供了与人CD137结合的抗体或其抗原结合片段、编码所述抗体或其抗原结合片段的核酸、所述抗体或其抗原结合片段的治疗组合物,以及它们用于增强T细胞功能以上调细胞介导的免疫应答以及用于治疗T细胞功能失调病症,诸如肿瘤免疫,以及用于治疗癌症的用途。



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1.一种与人CD137的细胞外结构域结合的分离抗体,或其抗原结合片段,所述抗体或其抗原结合片段包含重链可变区和轻链可变区,其中:

(i)所述重链可变区包含由氨基酸序列SEQ ID NO:712组成的HVR-H1、由氨基酸序列SEQ ID NO:736组成的HVR-H2和由氨基酸序列SEQ ID NO:760组成的HVR-H3;并且

(ii)所述轻链可变区包含由氨基酸序列SEQ ID NO:784组成的HVR-L1、由氨基酸序列SEQ ID NO:808组成的HVR-L2和由氨基酸序列SEQ ID NO:832组成的HVR-L3。

2.如权利要求1所述的抗体或抗原结合片段,其中所述重链可变区包含氨基酸序列SEQ ID NO:61,并且所述轻链可变区包含氨基酸序列SEQ ID NO:62。

3.如权利要求2所述的抗体或抗原结合片段,其中所述抗体包含重链和轻链,其中所述重链包含氨基酸序列SEQ ID NO:619,并且所述轻链包含氨基酸序列SEQ ID NO:620。

4.如权利要求1-3中任一项所述的抗体或抗原结合片段,其中如通过表面等离子体共振所测量,所述抗体或抗原结合片段以100nM或更小的K_D结合人CD137。

5.如权利要求4所述的抗体或抗原结合片段,其中如通过表面等离子体共振所测量,所述抗体或抗原结合片段以50nM或更小的K_D结合人CD137。

6.如权利要求1-3中任一项所述的抗体或抗原结合片段,其中所述抗体或抗原结合片段与食蟹猴CD137结合。

7.如权利要求1-3中任一项所述的抗体或抗原结合片段,其中当与所述抗体或抗原结合片段接触时,人细胞上表达的人CD137的活性降低。

8.如权利要求1-3中任一项所述的抗体或抗原结合片段,其中对于阻断人CD137与人CD137L的体外结合,所述抗体或抗原结合片段具有100nM或更小的半最大抑制浓度(IC_{50})。

9.如权利要求1-3中任一项所述的抗体或抗原结合片段,其中当所述抗体或抗原结合片段以1μM或更大的浓度提供时,所述抗体或抗原结合片段完全阻断人CD137与人CD137L的体外结合。

10.如权利要求1-3中任一项所述的抗体或抗原结合片段,其中当与所述抗体或抗原结合片段接触时,人细胞上表达的人CD137的活性增加。

11.如权利要求10所述的抗体或抗原结合片段,其中使表达CD137的人细胞与所述抗体或抗原结合片段接触导致增加的NF-κB依赖性转录。

12.如权利要求1或2所述的抗体或抗原结合片段,其中所述抗体包含人IgG2 Fc区。

13.如权利要求1或2所述的抗体或抗原结合片段,其中所述抗体包含人IgG4 Fc区。

14.如权利要求13所述的抗体或抗原结合片段,其中所述人IgG4Fc区包含S241P突变,其中编号是根据Kabat进行的。

15.一种多核苷酸,其编码如权利要求1-14中任一项所述的抗体或抗原结合片段。

16.一种载体,其包含如权利要求15所述的多核苷酸。

17.如权利要求16所述的载体,其中所述载体是表达载体。

18.一种宿主细胞,其包含如权利要求15所述的多核苷酸或如权利要求16或17所述的载体。

19.一种制备抗体或抗原结合片段的方法,其包括在适于产生所述抗体或抗原结合片段的条件下培养如权利要求18所述的宿主细胞。

20.如权利要求19所述的方法,其还包括回收由所述细胞产生的所述抗体或抗原结合

片段。

21.一种与人CD137的细胞外结构域结合的分离抗体或其抗原结合片段,其是通过如权利要求19或20所述的方法制备的。

22.一种药物组合物,其包含如权利要求1-14和21中任一项所述的抗体或抗原结合片段,以及药学上可接受的载剂。

抗CD137分子及其用途

[0001] 对序列表的引用

[0002] ASCII文本文件上的以下提交的内容以引用方式整体并入本文:计算机可读形式(CRF)的序列表(文件名:695402000340seqlist.txt,记录日期:2017年8月3日,大小:537KB)。

技术领域

[0003] 本公开涉及与人CD137结合的抗体或其抗原结合片段、所述抗体或其抗原结合片段的编码核酸、所述抗体或其抗原结合片段的治疗组合物,以及它们的抗肿瘤用途。

背景技术

[0004] CD137(也称为CD137受体、4-1BB、TNFRSF9等)是肿瘤坏死因子受体超家族(TNFRS)的跨膜蛋白。目前对CD137的了解表明,其表达通常是活化依赖性的并且存在于免疫细胞的广泛子集中,所述细胞包括活化的NK和NKT细胞、调节T细胞、树突细胞(DC)、被刺激的肥大细胞、分化中的骨髓细胞、单核细胞、嗜中性粒细胞以及嗜酸性粒细胞(Wang,2009,Immunological Reviews 229:192-215)。CD137表达还已证实处于肿瘤血管上(Broll,2001,Amer.J.Clin.Pathol.115(4):543-549;Seaman,2007,Cancer Cell 11:539-554)和发炎或动脉粥样硬化的内皮部位上(Drenkard,2007FASEB J.21:456-463;Olofsson,2008,Circulation 117:1292-1301)。刺激CD137的配体,即CD137配体(CD137L),在活化的抗原呈递细胞(APC)、骨髓祖细胞和造血干细胞上表达。

[0005] 人CD137是255个氨基酸的蛋白质(GenBank登录号NM_001561;NP_001552;SEQ ID NO.:1)。所述蛋白质包含信号序列(氨基酸残基1-17),接着是细胞外结构域(169个氨基酸)、跨膜区(27个氨基酸),以及细胞内结构域(42个氨基酸)(Cheuk ATC等人2004Cancer Gene Therapy 11:215-226)。受体以单体和二聚体形式在细胞表面表达并且可能与CD137配体三聚化以进行信号传导。

[0006] 鼠和人T细胞的许多研究表明,CD137促进增强的细胞增殖、存活和细胞因子产生(Croft,2009,Nat Rev Immunol 9:271-285)。研究已表明,一些CD137激动剂mAb增加共刺激分子表达并且显著增强溶细胞性T淋巴细胞应答,从而在各种模型中导致抗肿瘤功效。CD137激动剂mAb已展示出在预防和治疗环境中的功效。此外,CD137单一疗法和组合疗法肿瘤模型已经建立了持久的抗肿瘤保护性T细胞记忆应答(Lynch,2008,Immunol Rev.22:277-286)。CD137激动剂还已经显示出在多种本领域公认的自身免疫性模型中抑制自身免疫反应(Vinay,2006,J Mol Med 84:726-736)。CD137的这种双重活性提供了在减少可与破坏免疫耐受性的免疫治疗方法相关的自身免疫副作用的同时提供抗肿瘤活性的潜力。

[0007] 对于结合人CD137,增加CD137介导的应答并从而提供用于治疗各种疾病和病状(包括癌症和自身免疫疾病)的潜在治疗剂的抗体存在长期未满足的需要。此外,对于在不同物种(诸如人和实验动物(小鼠、猴、狗等))之间交叉反应,以使得能够进行动物模型研究并同时提供治疗剂候选物的抗CD137抗体存在需要。

发明内容

[0008] 本公开的一个目标是提供一种与人CD137结合的分离的结合分子,诸如抗体或其结合片段,或其衍生物。本公开的另一个目标是提供一种包含与CD137结合的结合分子的组合物。本公开的又一个目标是提供用于治疗与CD137信号传导相关或由其介导的疾病和/或病状的方法,其通过使用一种或多种本公开的结合分子实现。本公开的这些和其他目标在本文更完全地描述。

[0009] 因此,在一个方面,本文提供了一种或多种抗体(例如,分离抗体)或一种或多种其抗原结合片段,所述抗体或其抗原结合片段与人CD137的细胞外结构域结合,并且包括一个或多个(例如,一个或多个、两个或更多个、三个或更多个、四个或更多个、五个或更多个、六个或更多个、七个或更多个、八个或更多个、九个或更多个,或全部10个)以下功能特征:(a)结合SEQ ID NO:1的氨基酸残基34-108内的一个或多个氨基酸残基;(b)不与SEQ ID NO:1的氨基酸残基109-112、125、126、135-138、150和151内的一个或多个氨基酸残基结合;(c)以100nM或更小的K_D与人CD137结合;(d)在人CD137上具有激动剂活性;(e)在最高至1000nM的浓度下不与人OX40、CD40、GITR和/或CD27受体结合;(f)与猴、小鼠、大鼠和/或狗CD137交叉反应;(g)不引起ADCC效应;(h)能够抑制肿瘤细胞生长;(i)对癌症具有治疗作用;和/或(j)阻断CD137与CD137L之间的结合。

[0010] 因此,在一个方面,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段。在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含HVR-H1、HVR-H2和HVR-H3,其中HVR-H1包含根据选自由以下组成的组的式的氨基酸序列:式(I):$X_1TFX_2X_3YX_4IHWV$(SEQ ID NO:2),其中X1为F或Y,X2为S或T,X3为G、N或S,并且X4为A、G或W;式(II):$YSIX_1SGX_2X_3WX_4WI$(SEQ ID NO:3),其中X1为S或T,X2为H或Y,X3为H或Y,并且X4为A、D、G、N、S或T;以及式(III):$FSLSTX_1GVX_2VX_3WI$(SEQ ID NO:4),其中X1为G或S,X2为A或G,并且X3为A、G、S或T;其中HVR-H2包含根据选自由以下组成的组的式的氨基酸序列:式(IV):LALIDWX1X2DKX3YSX4SLKSRL(SEQ ID NO:5),其中X1为A、D或Y,X2为D或G,X3为R、S或Y,并且X4为P或T;式(V):IGX1IYHSGX2TYYX3PSLKSRV(SEQ ID NO:6),其中X1为D或E,X2为N或S,并且X3为N或S;以及式(VI):VSX1ISGX2GX3X4TYYADSVKGRF(SEQ ID NO:7),其中X1为A、G、S、V或Y,X2为A、D、S或Y,X3为D、G或S,并且X4为S或T;并且其中HVR-H3包含根据下式的氨基酸序列:式(VII):ARX1GX2X3X4VX5GDWFX6Y(SEQ ID NO:8),其中X1为E或G,X2为E或S,X3为D或T,X4为A、T或V,X5为A、I、L、T或V,并且X6为A、D或G。

[0011] 在一些实施方案中,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段,所述抗体或其抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含HVR-H1、HVR-H2和HVR-H3,其中HVR-H1包含根据选自由以下组成的组的式的氨基酸序列:式(XII):X1TFSX2YWIHWV(SEQ ID NO:853),其中X1为F或Y,并且X2为N或S;式(XIII):YSIX1SGX2X3WX4WI(SEQ ID NO:854),其中X1为S或T,X2为H或Y,X3为H或Y,并且X4为A、D、G、N或S;以及式(XIV):FSLSTX1GVX2VX3WI(SEQ ID NO:855),其中X1为G或S,X2为A或G,并且X3为A、G或S;其中HVR-H2包含根据选自由以下组成的组的式的氨基酸序列:式(IV):LALIDWX1X2DKX3YSX4SLKSRL(SEQ ID NO:5),其中X1为A、D或Y,X2为D或G,X3为R、S或Y,并且X4为P或T;以及式(XV):VSX1ISGX2GX3X4TYYADSVKGRF(SEQ ID NO:856),其中X1为

G、S、V或Y,X2为A、D、S或Y,X3为D、G或S,并且X4为S或T;并且其中HVR-H3包含根据下式的氨基酸序列:式(VII):ARX1GX2X3X4VX5GDWFX6Y(SEQ ID NO:8),其中X1为E或G,X2为E或S,X3为D或T,X4为A、T或V,X5为A、I、L、T或V,并且X6为A、D或G。

[0012] 在另一方面,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段。在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中轻链可变区包含HVR-L1、HVR-L2和HVR-L3,其中HVR-L1包含根据下式的氨基酸序列:式(VIII):X1ASQX2X3X4X5X6X7X8(SEQ ID NO:9),其中X1为Q或R,X2为D、G或S,X3为I或V,X4为G、R、S或T,X5为P、R、S或T,X6为A、D、F、S、V或Y,X7为L或V,并且X8为A、G或N;其中HVR-L2包含根据下式的氨基酸序列:式(IX):X1ASX2X3X4X5GX6(SEQ ID NO:10),其中X1为A或D,X2为N、S或T,X3为L或R,X4为A、E或Q,X5为S或T,并且X6为I或V;并且其中HVR-L3包含根据选自由以下组成的组的式的氨基酸序列:式(X):YCQQX1YX2X3X4T(SEQ ID NO:11),其中X1为A、G、S或Y,X2为Q、S或Y,X3为I、L、T或Y,并且X4为I、S、V或W;以及式(XI):YCX1QX2X3X4X5PX6T(SEQ ID NO:12),其中X1为E或Q,X2为P、S或Y,X3为D、L、S、T或Y,X4为D、E、H、S或T,X5为D、L T或W,并且X6为L、P、R或V。

[0013] 在一些实施方案中,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段,所述抗体或其抗原结合片段包含重链可变区和轻链可变区,其中轻链可变区包含HVR-L1、HVR-L2和HVR-L3,其中HVR-L1包含根据下式的氨基酸序列:式(XVI):X1ASQX2X3X4X5X6X7X8(SEQ ID NO:857),其中X1为Q或R,X2为D、G或S,X3为I或V,X4为G、R、S或T,X5为P、R、S或T,X6为A、F、S、V或Y,X7为L或V,并且X8为A或G;其中HVR-L2包含根据下式的氨基酸序列:式(XVII):$X_1ASX_2X_3X_4X_5GX_6$(SEQ ID NO:858),其中X1为A或D,X2为N或S,X3为L或R,X4为A、E或Q,X5为S或T,并且X6为I或V;并且其中HVR-L3包含根据下式的氨基酸序列:式(XVIII):$YCQQX_1YX_2X_3WT$(SEQ ID NO:859),其中X1为A或G,X2为S或Y,并且X3为I、L或T。

[0014] 在另一方面,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段。在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含以下各项的HVR-H1、HVR-H2和HVR-H3:VH1、VH2、VH3、VH4、VH5、VH6、VH7、VH8、VH9、VH10、VH11、VH12、VH13、VH14、VH15、VH16、VH17、VH18、VH19、VH20、VH21、VH22、VH23、VH24、VH25、VH26、VH27、VH28、VH29、VH30、VH31、VH32、VH33、VH34、VH35、VH36、VH37、VH38、VH39、VH40、VH41、VH42、VH43、VH44、VH45、VH46、VH47、VH48、VH49、VH50、VH51、VH52、VH53、VH54、VH55、VH56、VH57、VH58、VH59或VH60;并且/或者轻链可变区包含以下各项的HVR-L1、HVR-L2和HVR-L3:VL1、VL2、VH3、VL4、VH5、VL6、VL7、VL8、VL9、VL10、VL11、VL12、VL13、VL14、VL15、VL16、VL17、VL18、VL19、VL20、VL21、VL22、VL23、VL24、VL25、VL26、VL27、VL28、VL29、VL30、VL31、VL32、VL33、VL34、VL35、VL36、VL37、VL38、VL39、VL40、VL41、VL42、VL43、VL44、VL45、VL46、VL47、VL48、VL49、VL50、VL51、VL52、VL53、VL54、VL55、VL56、VL57、VL58、VL59或VL60(如表1c所示)。在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区和轻链可变区包含以下各项的HVR-H1、HVR-H2、HVR-H3、HVR-L1、HVR-L2和HVR-L3:VH1和VL1、VH2和VL2、VH3和VL3、VH4和VL4、VH5和VL5、VH6和VL6、VH7和VL7、VH8和VL8、VH9和VL9、VH10和VL10、VH11和VL11、VH12和VL12、VH13和VL13、VH14和VL14、VH15和VL15、VH16和VL16、VH17和VL17、VH18和VL18、VH19和VL19、VH20和VL20、VH21和

VL21、VH22和VL22、VH23和VL23、VH24和VL24、VH25和VL25、VH26和VL26、VH27和VL27、VH28和VL28、VH29和VL29、VH30和VL30、VH31和VL31、VH32和VL32、VH33和VL33、VH34和VL34、VH35和VL35、VH36和VL36、VH37和VL37、VH38和VL38、VH39和VL39、VH40和VL40、VH41和VL41、VH42和VL42、VH43和VL43、VH44和VL44、VH45和VL45、VH46和VL46、VH47和VL47、VH48和VL48、VH49和VL49、VH50和VL50、VH51和VL51、VH52和VL52、VH53和VL53、VH54和VL54、VH55和VL55、VH56和VL56、VH57和VL57、VH58和VL58、VH59和VL59,或VH60和VL60(如表1c所示)。

[0015] 在另一方面,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段。在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含以下各项的重链可变区:VH1、VH2、VH3、VH4、VH5、VH6、VH7、VH8、VH9、VH10、VH11、VH12、VH13、VH14、VH15、VH16、VH17、VH18、VH19、VH20、VH21、VH22、VH23、VH24、VH25、VH26、VH27、VH28、VH29、VH30、VH31、VH32、VH33、VH34、VH35、VH36、VH37、VH38、VH39、VH40、VH41、VH42、VH43、VH44、VH45、VH46、VH47、VH48、VH49、VH50、VH51、VH52、VH53、VH54、VH55、VH56、VH57、VH58、VH59或VH60;并且/或者轻链可变区包含以下各项的轻链可变区:VL1、VL2、VH3、VL4、VH5、VL6、VL7、VL8、VL9、VL10、VL11、VL12、VL13、VL14、VL15、VL16、VL17、VL18、VL19、VL20、VL21、VL22、VL23、VL24、VL25、VL26、VL27、VL28、VL29、VL30、VL31、VL32、VL33、VL34、VL35、VL36、VL37、VL38、VL39、VL40、VL41、VL42、VL43、VL44、VL45、VL46、VL47、VL48、VL49、VL50、VL51、VL52、VL53、VL54、VL55、VL56、VL57、VL58、VL59或VL60(如表1c所示)。在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区和轻链可变区包括以下各项的重链可变区和轻链可变区:VH1和VL1、VH2和VL2、VH3和VL3、VH4和VL4、VH5和VL5、VH6和VL6、VH7和VL7、VH8和VL8、VH9和VL9、VH10和VL10、VH11和VL11、VH12和VL12、VH13和VL13、VH14和VL14、VH15和VL15、VH16和VL16、VH17和VL17、VH18和VL18、VH19和VL19、VH20和VL20、VH21和VL21、VH22和VL22、VH23和VL23、VH24和VL24、VH25和VL25、VH26和VL26、VH27和VL27、VH28和VL28、VH29和VL29、VH30和VL30、VH31和VL31、VH32和VL32、VH33和VL33、VH34和VL34、VH35和VL35、VH36和VL36、VH37和VL37、VH38和VL38、VH39和VL39、VH40和VL40、VH41和VL41、VH42和VL42、VH43和VL43、VH44和VL44、VH45和VL45、VH46和VL46、VH47和VL47、VH48和VL48、VH49和VL49、VH50和VL50、VH51和VL51、VH52和VL52、VH53和VL53、VH54和VL54、VH55和VL55、VH56和VL56、VH57和VL57、VH58和VL58、VH59和VL59,或VH60和VL60(如表1c所示)。

[0016] 在另一方面,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体)或其抗原结合片段。在一些实施方案中,抗体或其抗原结合片段与SEQ ID NO:1的氨基酸残基34-108内的一个或多个氨基酸残基结合。在一些实施方案中,抗体或抗原结合片段与SEQ ID NO:1的氨基酸残基34-93内的一个或多个氨基酸残基结合。在一些实施方案中,抗体或抗原结合片段与选自由SEQ ID NO:1的氨基酸残基34-36、53-55和92-93组成的组的一个或多个氨基酸残基结合。在一些实施方案中,抗体或抗原结合片段与SEQ ID NO:1的氨基酸残基34-36中的一个或多个、氨基酸残基53-55中的一个或多个,以及氨基酸残基92-93中的一个或多个结合。在一些实施方案中,抗体或抗原结合片段不与选自由SEQ ID NO:1的氨基酸残基109-112、125、126、135-138、150和151组成的组的氨基酸残基中的一个或多个结合。在一些实施方案中,抗体或抗原结合片段不与SEQ ID NO:1的氨基酸残基109-112、125、126、135-138、150和151结合。在一些实施方案中,抗体或抗原结合片段与来自选

自食蟹猴(cynomolgus monkey)、小鼠、大鼠和/或狗的至少一种非人物种的CD137多肽交叉反应。在一些实施方案中,抗体或抗原结合片段与食蟹猴CD137结合。

[0017] 在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含有包含氨基酸序列SEQ ID NO:711的HVR-H1、包含氨基酸序列SEQ ID NO:735的HVR-H2,以及包含氨基酸序列SEQ ID NO:759的HVR-H3;并且/或者其中轻链可变区包含有包含氨基酸序列SEQ ID NO:783的HVR-L1、包含氨基酸序列SEQ ID NO:807的HVR-L2,以及包含氨基酸序列SEQ ID NO:831的HVR-L3。在一些实施方案中,重链可变区包含氨基酸序列SEQ ID NO:41,并且/或者轻链可变区包含氨基酸序列SEQ ID NO:42。在一些实施方案中,抗体包含重链和轻链,并且其中重链包含氨基酸序列SEQ ID NO:617,并且/或者轻链包含氨基酸序列SEQ ID NO:618。

[0018] 在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含有包含氨基酸序列SEQ ID NO:712的HVR-H1、包含氨基酸序列SEQ ID NO:736的HVR-H2,以及包含氨基酸序列SEQ ID NO:760的HVR-H3;并且/或者其中轻链可变区包含有包含氨基酸序列SEQ ID NO:784的HVR-L1、包含氨基酸序列SEQ ID NO:808的HVR-L2,以及包含氨基酸序列SEQ ID NO:832的HVR-L3。在一些实施方案中,重链可变区包含氨基酸序列SEQ ID NO:61,并且/或者轻链可变区包含氨基酸序列SEQ ID NO:62。在一些实施方案中,抗体包含重链和轻链,其中重链包含氨基酸序列SEQ ID NO:619,并且/或者轻链包含氨基酸序列SEQ ID NO:620。

[0019] 在一些实施方案中,抗体或抗原结合片段包含重链可变区和轻链可变区,其中重链可变区包含有包含氨基酸序列SEQ ID NO:731的HVR-H1、包含氨基酸序列SEQ ID NO:755的HVR-H2,以及包含氨基酸序列SEQ ID NO:779的HVR-H3;并且/或者其中轻链可变区包含有包含氨基酸序列SEQ ID NO:803的HVR-L1、包含氨基酸序列SEQ ID NO:827的HVR-L2,以及包含氨基酸序列SEQ ID NO:851的HVR-L3。在一些实施方案中,重链可变区包含氨基酸序列SEQ ID NO:71,并且/或者轻链可变区包含氨基酸序列SEQ ID NO:72。在一些实施方案中,抗体包含重链和轻链,其中重链包含氨基酸序列SEQ ID NO:657,并且/或者轻链包含氨基酸序列SEQ ID NO:658。

[0020] 在另一方面,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体),其包含重链可变区和轻链可变区,其中重链可变区包含HVR-H1、HVR-H2和HVR-H3,其中HVR-H1包含根据选自由以下组成的组的式的氨基酸序列:式(I)、式(II)和式(III);HVR-H2包含根据选自由以下组成的组的式的氨基酸序列:式(IV)、式(V)和式(VI);并且HVR-H3包含根据式(VII)的氨基酸序列;并且/或者轻链可变区包含HVR-L1、HVR-L2和HVR-L3,其中HVR-L1包含根据式(VIII)的氨基酸序列;HVR-L2包含根据式(IX)的氨基酸序列;并且HVR-L3包含根据选自由以下组成的组的式的氨基酸序列:式(X)和式(XI)。在一些实施方案中,本文提供了一种与人CD137的细胞外结构域结合的抗体(例如,分离抗体),其包含重链可变区和轻链可变区,其中重链可变区包含HVR-H1、HVR-H2和HVR-H3,其中HVR-H1包含根据选自由以下组成的组的式的氨基酸序列:式(XIII)和式(XVI);HVR-H2包含根据选自由以下组成的组的式的氨基酸序列:式(IV)和式(XV);并且HVR-H3包含根据式(VII)的氨基酸序列;并且/或者轻链可变区包含HVR-L1、HVR-L2和HVR-L3,其中HVR-L1包含根据式(XVI)的氨基酸序列;HVR-L2包含根据式(XVII)的氨基酸序列;并且HVR-L3包含根据式(XVIII)的氨基酸序

列。

[0021] 在一些实施方案中,HVR-H1包含选自由SEQ ID NO:253-312组成的组的氨基酸序列,HVR-H2包含选自由SEQ ID NO:313-372组成的组的氨基酸序列,HVR-H3包含选自由SEQ ID NO:373-432组成的组的氨基酸序列,HVR-L1包含选自由SEQ ID NO:433-492组成的组的氨基酸序列,HVR-L2包含选自由SEQ ID NO:493-552组成的组的氨基酸序列,并且/或者HVR-L3包含选自由SEQ ID NO:553-612组成的组的氨基酸序列。在一些实施方案中,重链可变区包含选自由SEQ ID NO:13、15、17、19、21、23、25、27、29、31、33、35、37、39、41、43、45、47、49、51、53、55、57、59、61、63、65、67、69、71、73、75、77、79、81、83、85、87、89、91、93、95、97、99、101、103、105、107、109、111、113、115、117、119、121、123、125、127、129和131组成的组的氨基酸序列,并且/或者轻链可变区包含选自由SEQ ID NO:14、16、18、20、22、24、26、28、30、32、34、36、38、40、42、44、46、48、50、52、54、56、58、60、62、64、66、68、70、72、74、76、78、80、82、84、86、88、90、92、94、96、98、100、102、104、106、108、110、112、114、116、118、120、122、124、126、128、130和132组成的组的氨基酸序列。

[0022] 在一些实施方案中,HVR-H1包含根据选自由式(XII)、式(XIII)和式(XIV)组成的组的式的氨基酸序列;HVR-H2包含根据式(IV)或式(XV)的氨基酸序列;并且HVR-H3包含根据式(VII)的氨基酸序列;并且/或者其中HVR-L1包含根据式(XVI)的氨基酸序列;HVR-L2包含根据式(XVII)的氨基酸序列;并且HVR-L3包含根据式(XVIII)的氨基酸序列。在一些实施方案中,HVR-H1包含选自由SEQ ID NO:709-732组成的组的氨基酸序列,HVR-H2包含选自由SEQ ID NO:733-756组成的组的氨基酸序列,HVR-H3包含选自由SEQ ID NO:757-780组成的组的氨基酸序列,HVR-L1包含选自由SEQ ID NO:781-804组成的组的氨基酸序列,HVR-L2包含选自由SEQ ID NO:805-828组成的组的氨基酸序列,并且HVR-L3包含选自由SEQ ID NO:829-852组成的组的氨基酸序列。在一些实施方案中,重链可变区包含选自由SEQ ID NO:15、17、31、33、35、37、39、41、43、45、47、49、53、61、63、65、67、71、73、75、79、83、85和87组成的组的氨基酸序列,并且/或者轻链可变区包含选自由SEQ ID NO:16、18、32、34、36、38、40、42、44、46、48、50、54、62、64、66、68、72、74、76、80、84、86和88组成的组的氨基酸序列。在一些实施方案中,抗体包含重链和轻链,其中重链包含选自由SEQ ID NO:613、615、617、619、621、623、625、627、629、631、633、635、637、639、641、643、645、647、649、651、653、655、657和659组成的组的氨基酸序列,并且/或者轻链包含选自由SEQ ID NO:614、616、618、620、622、624、626、628、630、632、634、636、638、640、642、644、646、648、650、652、654、656、658和660组成的组的氨基酸序列。

[0023] 在一些实施方案中,HVR-H1包含氨基酸序列SEQ ID NO:711或731;HVR-H2包含氨基酸序列SEQ ID NO:735或755;HVR-H3包含氨基酸序列SEQ ID NO:759或779;HVR-L1包含氨基酸序列SEQ ID NO:783或803;HVR-L2包含氨基酸序列SEQ ID NO:807或827;并且HVR-L3包含氨基酸序列SEQ ID NO:831或851。在一些实施方案中,重链可变区包含氨基酸序列SEQ ID NO:41或71,并且轻链可变区包含氨基酸序列SEQ ID NO:42或72。在一些实施方案中,抗体包含重链和轻链,其中重链包含氨基酸序列SEQ ID NO:617或657,并且轻链包含氨基酸序列SEQ ID NO:618或658。

[0024] 在一些实施方案中,HVR-H1包含氨基酸序列SEQ ID NO:712;HVR-H2包含氨基酸序列SEQ ID NO:736;HVR-H3包含氨基酸序列SEQ ID NO:760;HVR-L1包含氨基酸序列SEQ ID

NO:784;HVR-L2包含氨基酸序列SEQ ID NO:808;并且HVR-L3包含氨基酸序列SEQ ID NO:832。在一些实施方案中,重链可变区包含氨基酸序列SEQ ID NO:61,并且轻链可变区包含氨基酸序列SEQ ID NO:62。在一些实施方案中,抗体包含重链和轻链,其中重链包含氨基酸序列SEQ ID NO:619,并且轻链包含氨基酸序列SEQ ID NO:620。

[0025] 在可与任何前述实施方案组合的一些实施方案中,抗体或抗原结合片段以100nM或更小的K_D结合人CD137(例如,如通过表面等离子体共振所测量)。在一些实施方案中,抗体或抗原结合片段以50nM或更小的K_D结合人CD137(例如,如通过表面等离子体共振所测量)。

[0026] 在可与任何前述实施方案组合的一些实施方案中,抗体或抗原结合片段与来自选自食蟹猴(例如,GenBank Gene ID 102127961)、小鼠(例如,GenBank Gene ID 21942)、大鼠(例如,GenBank Gene ID 500590)和/或狗(例如,GenBank Gene ID 608274)的至少一种非人物种的CD137多肽交叉反应。在一些实施方案中,抗体或抗原结合片段与食蟹猴CD137结合。

[0027] 在可与任何前述实施方案组合的一些实施方案中,当与抗体或抗原结合片段接触时,人CD137(例如,当在诸如人细胞的细胞上表达时)的活性降低。

[0028] 在可与任何前述实施方案组合的一些实施方案中,对于阻断人CD137与人CD137L的体外结合,抗体或抗原结合片段具有约100nM或更小的半最大抑制浓度(IC_{50})。在一些实施方案中,当抗体或抗原结合片段以约1μM或更大的浓度提供时,抗体或抗原结合片段完全阻断人CD137与人CD137L的体外结合。在可与任何前述实施方案组合的一些实施方案中,当与抗体或抗原结合片段接触时,人CD137(例如,当在诸如人细胞的细胞上表达时)的活性增加。在一些实施方案中,使CD137(例如,在人细胞上表达)与抗体或抗原结合片段接触导致NF-κB依赖性转录增加。

[0029] 在可与任何前述实施方案组合的一些实施方案中,抗体包含人IgG2 Fc区。在可与任何前述实施方案组合的一些实施方案中,抗体包含人IgG4 Fc区。在一些实施方案中,人IgG4 Fc区包含S241P突变,其中编号是根据Kabat进行的。在一些实施方案中,抗体或抗原结合片段不引起ADCC效应。

[0030] 在另一方面,本文提供了由包含选自SEQ ID NO:133、135、137、139、141、143、145、147、149、151、153、155、157、159、161、163、165、167、169、171、173、175、177、179、181、183、185、187、189、191、193、195、197、199、201、203、205、207、209、211、213、215、217、219、221、223、225、227、229、231、233、235、237、239、241、243、245、247、249和251的序列的多核苷酸编码的抗体重链可变区,和/或由包含选自SEQ ID NO:134、136、138、140、142、144、146、148、150、152、154、156、158、160、162、164、166、168、170、172、174、176、178、180、182、184、186、188、190、192、194、196、198、200、202、204、206、208、210、212、214、216、218、220、222、224、226、228、230、232、234、236、238、240、242、244、246、248、250和252的序列的多核苷酸编码的抗体轻链可变区。在一些实施方案中,本文提供了由包含选自SEQ ID NO:661、663、665、667、669、671、673、675、677、679、681、683、685、687、689、691、693、695、697、699、701、703、705和707的序列的多核苷酸编码的抗体重链,和/或由包含选自SEQ ID NO:662、664、666、668、670、672、674、676、678、680、682、684、686、688、690、692、694、696、698、700、702、704、706和708的序列的多核苷酸编码的抗体轻链。

[0031] 在另一方面,本文提供了一种多核苷酸,其编码本文所述的抗体或抗原结合片段中的任一个。在一些实施方案中,本文提供了一种多核苷酸,其包含选自SEQ ID NO:133-252的序列。

[0032] 在另一方面,本文提供了一种载体,其包含上文所述的多核苷酸中的任一个。在一些实施方案中,载体是表达载体。

[0033] 在另一方面,本文提供了一种宿主细胞(例如,细菌细胞、酵母细胞、昆虫细胞、哺乳动物细胞(诸如CHO细胞或293T细胞)等),其包含本文所述的多核苷酸或载体中的任一个。在一些实施方案中,本文提供了一种制备抗体或抗原结合片段的方法,其包括在适于产生抗体或抗原结合片段的条件下培养宿主细胞。在一些实施方案中,所述方法还包括回收由宿主细胞产生的抗体或抗原结合片段。

[0034] 在另一方面,本文提供了一种药物组合物,其包含本文所述的抗体或抗原结合片段(或其任何衍生物)中的任一个以及药学上可接受的载剂。

[0035] 在另一方面,本文提供了治疗有需要的受试者中的异常细胞生长(例如,癌症)的方法,其包括向受试者施用治疗有效量的本文所述的抗体、抗原结合片段和/或药物组合物中的任一个。在一些实施方案中,本文提供了减少受试者中的肿瘤细胞转移的方法,其包括向所述受试者施用治疗有效量的本文所述的抗体、抗原结合片段和/或药物组合物中的任一个。在一些实施方案中,所述方法还包括向受试者施用治疗有效量的至少一种(例如,至少一种、至少两种、至少三种、至少四种、至少五种、至少10种等)另外治疗剂。在一些实施方案中,至少一种另外治疗剂选自由以下组成的组:病毒基因疗法、免疫检查点抑制剂、靶疗法、放射疗法以及化学疗法。在一些实施方案中,至少一种另外治疗剂选自由以下组成的组:泊马度胺(pomalyst)、来那度胺(revlimid)、雷利度胺(lenalidomide)、泊马度胺(pomalidomide)、沙利度胺(thalidomide)、DNA-烷基化含铂衍生物、顺铂、5-氟尿嘧啶、环磷酰胺、抗CTLA4抗体、抗PD-1抗体、抗PD-L1抗体、抗CD20抗体、抗CD40抗体、抗DR5抗体、抗CD1d抗体、抗TIM3抗体、抗SLAMF7抗体、抗KIR受体抗体、抗OX40抗体、抗HER2抗体、抗ErbB-2抗体、抗EGFR抗体、西妥昔单抗(cetuximab)、利妥昔单抗(rituximab)、曲妥珠单抗(trastuzumab)、派姆单抗(pembrolizumab)、放射疗法、单剂量放射、分割放射(fractionated radiation)、病灶放射(focal radiation)、全器官放射、IL-12、IFNα、GM-CSF、嵌合抗原受体、过继转移的T细胞、抗癌疫苗以及溶瘤病毒。本文还提供了本文所述的药物组合物、抗体和/或抗原结合片段(或其任何衍生物)中的任一个用于治疗有需要的受试者中的异常细胞生长(例如,癌症)和/或减少肿瘤细胞转移的用途。本文还提供了本文所述的抗体或抗原结合片段(或其任何衍生物)中的任一个用于制造用于治疗有需要的受试者中的异常细胞生长(例如,癌症)和/或减少肿瘤细胞转移的药物的用途。

[0036] 应了解,上文和本文所述的各个实施方案的一种、一些或所有特性可组合以形成本公开的其他实施方案。本公开的这些和其他方面对于本领域技术人员将变得显而易见。本公开的这些和其他实施方案通过随后的具体实施方式进一步描述。

附图说明

[0037] 图1A示出对于示例性重链可变区(VH)(SEQ ID NO:13)和示例性轻链可变区(VL)(SEQ ID NO:14),相比于Kabat CDR定义的高变区(HVR)定义。

[0038] 图1B示出与小鼠CD137交叉反应的Fab命中物(hits)的选择。

[0039] 图2示出示例性抗体对人、猴和小鼠CD137的ELISA结合测定。每个小图针对如小图顶部所指示的不同抗体。

[0040] 图3A示出示例性抗体对人、猴、小鼠和大鼠CD137的基于FACS的结合测定。每个小图针对如小图顶部所指示的不同抗原。

[0041] 图3B示出示例性抗体和参考抗体之间物种交叉反应性的比较。

[0042] 图4A示出示例性抗体结合活化的人和猴T细胞,但不结合原初(naïve)人T细胞。

[0043] 图4B示出AG10131与活化的人、猴、小鼠和大鼠T细胞的结合。

[0044] 图5示出示例性抗体对CD137,而不是其他TNFR家族成员的结合特异性。

[0045] 图6A和图6B示出示例性抗体阻断CD137与其同源配体CD137L的结合,这通过ELISA(图6A)和流式细胞术测定(图6B)获得。

[0046] 图7A示出通过流式细胞术获得的表位作图结果。

[0047] 图7B示出人(SEQ ID NO:1)、食蟹猴(SEQ ID NO:860)和小鼠(SEQ ID NO:861)CD137的一部分与从表位作图实验中鉴定的目标CD137序列/区域(加注释)的多序列比对。

[0048] 图8示出在NFκB报告基因测定中示例性抗体的激动剂活性。

[0049] 图9示出示例性抗体在CD8+T细胞增殖(顶部小图)和INF-γ分泌(底部小图)中的激动剂活性。

[0050] 图10示出H22小鼠肝癌模型中示例性抗体的抗肿瘤功效,以及$CD4^+$和$CD8^+$T细胞在肿瘤中的浸润。

[0051] 图11示出示例性抗体在CT26小鼠结肠癌模型中的抗肿瘤功效。

[0052] 图12示出示例性抗体在EMT6小鼠乳腺癌模型中的抗肿瘤功效。

[0053] 图13示出用示例性抗体治疗的CT26小鼠在用相同的肿瘤细胞再激发后保持无肿瘤。

[0054] 图14示出利用来自肿瘤排斥性再激发小鼠的脾细胞实现的肿瘤细胞杀伤。

[0055] 图15示出AG10131不显示ADCC效应。

[0056] 图16示出示例性抗体在高浓度下表现出很少聚集。

[0057] 图17示出示例性抗体在加速应激条件(accelerated stress condition)下的稳定性。

[0058] 图18示出热稳定性。

[0059] 图19示出AG10131在最高至每两周100mg/kg(BIW)x 2下在正常小鼠中没有血液学毒性。

[0060] 图20示出AG10131在最高至每两周100mg/kg(BIW)x 2下在正常小鼠中没有组织学肝异常。

[0061] 图21示出AG10131在10mg/kg/周x 4下在食蟹猴中没有血液学毒性。

[0062] 图22示出AG10131在10mg/kg/周x 4下在猴中没有肝毒性。

[0063] 图23示出AG10131在猴中的药代动力学特征。

[0064] 图24示出AG10131在大鼠中的药代动力学特征。

[0065] 图25示出各种抗体在小鼠中的药代动力学特征。

具体实施方式

[0066] A.定义

[0067] 除非本文另外定义,否则与本公开关联使用的科学和技术术语将具有由本领域普通技术人员通常所理解的含义。另外,除非上下文另有要求,否则单数术语应包括复数,并且复数术语应包括单数。一般来说,结合本文所述的抗体工程改造、免疫疗法、细胞和组织培养、分子生物学、免疫学、微生物学、遗传学和蛋白质和核酸化学使用的命名法以及抗体工程改造、免疫疗法、细胞和组织培养、分子生物学、免疫学、微生物学、遗传学和蛋白质和核酸化学的技术是本领域中所熟知且常用的那些。

[0068] 如本文所用,以下各术语在此部分中均具有与它相关联的含义。

[0069] 冠词“一个/种(“a”和“an”)”是指一个/种或多个/种(即指至少一个/种)的所述冠词的语法宾语。通过举例的方式,“元件”意指一个元件或多于一个元件。

[0070] 术语“氨基酸”是指天然存在和合成氨基酸,以及功能类似于天然存在的氨基酸的氨基酸类似物和氨基酸模拟物。天然存在的氨基酸是由遗传密码编码的那些,以及稍后被修饰的那些氨基酸,例如,羟基脯氨酸、γ-羧基谷氨酸盐和O-磷酸丝氨酸。术语“氨基酸类似物”是指具有与天然存在的氨基酸相同的基本化学结构,但是C末端羧基、N末端氨基或侧链官能团已经被化学修饰为另一官能团的化合物。术语“氨基酸模拟物”是指具有与氨基酸的一般化学结构不同的结构,但功能类似于天然存在的氨基酸的化学化合物。

[0071] 术语“抗体”在本文中以最广泛的意义使用并且具体地涵盖单克隆抗体(包括全长单克隆抗体)、多克隆抗体、多特异性抗体(例如,双特异性抗体)以及抗体片段(例如,单链可变片段或scFv),只要它们表现出期望的生物学活性即可。

[0072] 术语“抗体”是本领域公认术语并且可指具有由两条相同的重(H)链和两条相同的轻(L)链组成的基本四多肽链结构的抗原结合蛋白(即,免疫球蛋白)。各L链通过一个共价二硫键连接至H链,同时两条H链取决于H链同种型通过一个或多个二硫键彼此连接。每条重链在N末端具有可变区(在本文缩写为V_H、),接着是恒定区。重链恒定区包含三个结构域:C_{H1}、C_{H2}和C_{H3}。每条轻链在N末端具有可变区(在本文缩写为V_I),接着是在其另一端的恒定区。轻链恒定区包含一个结构域,C_L。将V_L与V_H比对并且将C_L与重链的第一恒定结构域(CH1)比对。V_H和V_L的配对一起形成单一抗原结合位点。IgM抗体由5个基本的异四聚体单元以及被称为J链的另一多肽组成,因此含有10个抗原结合位点;而分泌型IgA抗体可聚合形成包含2-5个基本的4链单元以及J链的多价装配物。

[0073] V_H和V_L区还可以基于结构和序列分析细分成具有高变性的区域,称为高变区(HVR)。HVR散布有更加保守的区域,称为框架区(FW)。为了进行比较,下文列出Yvonne Chen等人(Selection and Analysis of an Optimized Anti-VEGF Antibody:Crystal Structure of an Affinity-matured Fab in Complex with Antigen,J.Mol.Biol.(1999)293,865-881)的Kabat CDR定义(另外参见图1a)。各V_H和V_L由三个HVR和四个FW组成,以下列顺序从氨基末端到羧基末端排列:FW1、HVR1、FW2、HVR2、FW3、HVR3、FW4。在本公开通篇,重链的三个HVR被称为HVR_H1、HVR_H2和HVR_H3。类似地,轻链的三个HVR被称为HVR_L1、HVR_L2和HVR_L3。

[0074] 重链和轻链的可变区含有与抗原相互作用的结合结构域。抗体的恒定区可介导免疫球蛋白与宿主组织或因子的结合,包括免疫系统的各种细胞(例如,效应细胞)和经典补

体系统的第一组分(Clq)。在轻链和重链内,可变区和恒定区通过具有约12个或更多个氨基酸的“J”区连接,其中重链还包括具有约10个或更多个氨基酸的“D”区。一般参见Fundamental Immunology第7章(Paul,W.编,第2版,Raven Press,N.Y.(1989))。

[0075] 来自任何脊椎动物物种的L链可基于其恒定结构域的氨基酸序列指定为两种明显不同类型(称为κ和λ)中的一者。取决于其重链的恒定结构域(CH)的氨基酸序列,抗体可指定为不同的种类或同种型。存在五类抗体:IgA、IgD、IgE、IgG和IgM,其分别具有被命名为α(alpha)、δ(delta)、ε(epsilon)、γ(gamma)和μ(mu)的重链。IgG类抗体可以分别通过γ重链Y1-Y4进一步分类为四个亚类IgG1、IgG2、IgG3和IgG4。

[0076] 术语“抗体衍生物”或抗体的“衍生物”是指能够结合所述抗体所结合的相同抗原(例如,CD137)且包含与另一分子实体连接的所述抗体的氨基酸序列的分子。抗体衍生物中包含的所述抗体的氨基酸序列可以是所述抗体的全长重链、全长轻链、全长重链的任何一个或多个部分、全长轻链的任何一个或多个部分、抗体的任何其他一个或多个片段,或互补抗体。另一分子实体可以是化学或生物分子。另一分子实体的实例包括化学基团、氨基酸、肽、蛋白质(诸如酶、抗体)以及化学化合物。另一分子实体可具有任何实用性,诸如用作检测剂、标记、标志物、药剂或治疗剂。抗体的氨基酸序列可通过化学偶联、基因融合、非共价缔合或其他方式与另一分子实体附接或连接。术语“抗体衍生物”还涵盖嵌合抗体、人源化抗体,以及由CD137抗体的氨基酸序列的修饰,诸如保守氨基酸取代、添加和插入衍生的分子。

[0077] 术语抗体的“抗原结合片段”或“抗原结合部分”是指抗体的保留与所述抗体所结合的抗原(例如,CD137)相结合的能力的一个或多个部分。抗体的“抗原结合片段”的实例包括(i)Fab片段,其为由V_L、V_H、C_L和C_{H1}结构域组成的一价片段;(ii)$F(ab')_2$片段,其为包含由在铰链区的二硫键连接的两个Fab片段的二价片段;(iii)由V_H和C_{H1}结构域组成的Fd片段;(iv)由抗体的单臂的V_L和V_H结构域组成的Fv片段;(v)由V_H结构域组成的dAb片段(Ward等人,Nature 341:544-546(1989));以及(vi)分离的互补决定区(CDR)。

[0078] 术语“结合分子”涵盖(1)抗体,(2)抗体的抗原结合片段,以及(3)抗体的衍生物,各自如本文所定义。

[0079] 术语“结合CD137(binding CD137/binds CD137)”或“与CD137结合(binding to CD137/binds to CD137)”是指如本文所定义的结合分子在体外测定中以100nM或更小的亲和力(K_D)与人CD137的结合,所述体外测定诸如实施例4中所述的Biacore测定。

[0080] 术语“CD137”和“CD137受体”在本申请中可互换使用,并且包括人CD137受体,以及其变体、同种型以及物种同源物。因此,如本文所定义和公开的结合分子也可结合来自除人之外的物种的CD137。在其他情况中,结合分子对于人CD137可以是完全特异性的并且可能不表现出物种或其他类型的交叉反应性。

[0081] 术语“CD137抗体”是指能够与人CD137受体结合的如本文所定义的抗体。

[0082] 术语“嵌合抗体”是指包含衍生自不同动物物种的氨基酸序列的抗体,诸如具有衍生自人抗体的可变区和鼠免疫球蛋白恒定区的那些。

[0083] 术语“竞争结合”是指两种抗体在它们与结合靶标的结合中的相互作用。如果与不存在第二抗体的情况下第一抗体的结合相比,在第二抗体的存在下,第一抗体与其同源表位的结合可检测地减少,那么第一抗体与第二抗体竞争结合。其中在第一抗体的存在下,第

二抗体与其表位的结合也可检测地减少的替代情况可以,但不一定是这样的。也就是说,第一抗体可以抑制第二抗体与其表位的结合,而所述第二抗体不抑制第一抗体与其相应表位的结合。然而,在每种抗体可检测地抑制另一抗体与其同源表位的结合的情况下,无论程度是相同、更大还是更小,所述抗体均被称为彼此“交叉竞争”结合它们的一个或多个相应表位。

[0084] 术语“表位”是指抗原上与抗体(或其抗原结合片段)相结合的部分。可由通过蛋白质的三级折叠来并置的连续氨基酸或不连续氨基酸形成表位。由连续氨基酸形成的表位通常在暴露于变性溶剂时保留,而通过三级折叠形成的表位通常在用变性溶剂处理时丢失。表位可包括处于独特空间构象的不同数量的氨基酸。确定表位的空间构象的方法包括,例如,x射线晶体学、2维核磁共振、与质谱组合的氘氢交换,或定点诱变,或与抗原以及具有其结合抗体及其变体的其复合物结构的计算机建模组合使用的所有方法。参见,例如,Epitope Mapping Protocols,在Methods in Molecular Biology中,第66卷,G.E.Morris编辑,(1996)。一旦确定了抗原的期望表位,就可以例如使用本文所述的技术生成针对所述表位的抗体。抗体的生成和表征也可阐明关于期望表位的信息。由此信息,接着可以竞争性地筛选结合相同表位的抗体。实现这一点的方法是进行交叉竞争研究,以寻找彼此竞争结合的抗体,即竞争结合抗原的抗体。基于其交叉竞争“分拣(binning)”抗体的高通量方法描述于PCT公布号WO 03/48731中。

[0085] 术语“生殖系”是指抗体基因和基因区段的核苷酸序列,因为其经由生殖细胞从亲代传递至后代。生殖系序列不同于编码成熟B细胞中的抗体的核苷酸序列,所述成熟B细胞在B细胞成熟过程中已通过重组和高变事件发生了改变。

[0086] 术语“糖基化位点”是指由真核细胞识别为用于附接糖残基的位置的氨基酸残基。在其中附接碳水化合物(诸如低聚糖)的氨基酸通常为天冬酰胺(N-键)、丝氨酸(O-键)和苏氨酸(O-键)残基。附接的特异性位点通常以在本文称为“糖基化位点序列”的氨基酸序列为标志。用于N-连接糖基化的糖基化位点序列为:-Asn-X-Ser-或-Asn-X-Thr-,其中X可以是除脯氨酸之外的任何常规氨基酸。术语“N-连接的”和“O-连接的”是指在糖分子与氨基酸残基之间用作附接位点的化学基团。N-连接的糖通过氨基附接;O-连接的糖通过羟基附接。术语“聚糖占据(glycan occupancy)”是指存在连接至糖基化位点的碳水化合物部分(即,聚糖位点被占据)。在多肽上存在至少两个潜在糖基化位点的情况下,没有一个(0-聚糖位点占据)、一个(1-聚糖位点占据)或两个(2-聚糖位点占据)位点可以被碳水化合物部分占据。

[0087] 术语“宿主细胞”是指可以被工程改造以产生目标蛋白质、蛋白质片段或肽的细胞系统。宿主细胞包括但不限于培养细胞,例如来源于啮齿动物(大鼠、小鼠、豚鼠或仓鼠)的哺乳动物培养细胞,诸如CHO、BHK、NSO、SP2/0、YB2/0;或人组织或杂交瘤细胞、酵母细胞和昆虫细胞,以及转基因动物或培养组织内包括的细胞。该术语不仅涵盖具体的主题细胞,而且涵盖这种细胞的子代。因为某些修饰可能由于突变或环境影响而在继代中发生,所以所述子代可能不与亲本细胞相同,但仍包括在术语“宿主细胞”的范围内。

[0088] 术语“人抗体”是指其中轻链和重链的整个氨基酸序列均来自于人免疫球蛋白基因的抗体。如果在小鼠、小鼠细胞或衍生自小鼠细胞的杂交瘤中产生的话,人抗体可包含鼠碳水化合物链。人抗体可通过本领域已知的多种方式制备。

[0089] 术语“人源化抗体”是指包含来源于人抗体序列的氨基酸残基的嵌合抗体。人源化

抗体可包含来自非人动物或合成抗体的一些或所有CDR或HVR,而抗体的框架和恒定区包含来源于人抗体序列的氨基酸残基。

[0090] 术语“例示性抗体”是指在本公开描述且被命名为表1a和1b所列的那些的抗体中的任一种。这些抗体可属于任何种类(例如,IgA、IgD、IgE、IgG和IgM)。因此,以上鉴定的各抗体涵盖对于V_L和V_H区具有相同的氨基酸序列的全部五个种类中的抗体。此外,IgG类中的抗体可属于任何亚类(例如,IgG1、IgG2、IgG3和IgG4)。因此,IgG亚类中的以上鉴定的各抗体涵盖对于V_L和V_H区具有相同的氨基酸序列的全部四个亚类中的抗体。五个种类以及四个IgG亚类中的人抗体的重链恒定区的氨基酸序列是本领域已知的。表1b所示的例示性抗体中IgG4亚类中的每一个的全长重链和轻链的氨基酸序列在本公开中提供。

[0091] 术语“分离抗体”或“分离的结合分子”是指如本文所定义的抗体或结合分子,其:(1)不与在其天然状态中与其相邻的天然关联组分相关联;(2)不含来自相同物种的其他蛋白质;(3)由来自不同物种的细胞表达;或(4)在自然界中不存在。分离抗体的实例包括已经使用CD137亲和纯化的CD137抗体、已经通过杂交瘤或其他细胞系在体外生成的CD137抗体,以及来源于转基因动物的CD137抗体。

[0092] 术语“分离核酸”是指与在核酸的天然来源中存在的其他核酸分子分开的基因组、cDNA或合成来源或其组合的核酸分子。例如,关于基因组DNA,术语“分离的”包括与基因组DNA天然相关联的染色体分开的核酸分子。优选地,“分离的”核酸不含天然侧接于所述核酸的序列(即,位于目标核酸的5′端和3′端的序列)。

[0093] 术语“k_a”是指特定抗体-抗原相互作用的缔合速率常数,而术语“k_d”是指特定抗体-抗原相互作用的解离速率常数。

[0094] 术语“K_D”是指特定抗体-抗原相互作用的平衡解离常数。它是由k_d与k_a的比率(即,k_d/k_a)获得的并且表示为摩尔浓度(M)。K_D用作抗体与其结合配偶体的结合亲和力的量度。K_D越小,则抗体的结合就越紧密,或抗体与抗原之间的亲和力越高。例如,具有纳摩尔(nM)解离常数的抗体比具有微摩尔(μM)解离常数的抗体更紧密地与结合至特定抗原。抗体的K_D值可以使用本领域的良好建立的方法确定。确定抗体K_D的一种方法是通过使用表面等离子体共振,通常使用生物传感器系统,诸如Biacore®系统。使用BIACORE™系统进行的测定程序(BIAcore测定)在本公开的实施例部分中描述。

[0095] 术语“哺乳动物”是指哺乳动物种类的任何动物物种。哺乳动物的实例包括:人;实验室动物,诸如大鼠、小鼠、猿和豚鼠;家养动物,诸如猫、狗、兔、牛、绵羊、山羊、马和猪;以及圈养野生动物,诸如狮子、老虎、大象等。

[0096] 关于哺乳动物中的某一疾病病状的术语“预防(prevent或preventing)”是指预防或延迟疾病的发作,或预防其临床或亚临床症状的出现。

[0097] 如本文所用,两个多肽序列之间的“序列同一性”指示在所述序列之间相同的氨基酸的百分比。多肽的氨基酸序列同一性可以使用已知计算机程序诸如Bestfit、FASTA或BLAST常规地确定(参见,例如Pearson,Methods Enzymol.183:63-98(1990);Pearson,Methods Mol.Biol.132:185-219(2000);Altschul等人,J.Mol.Biol.215:403-410(1990);Altschul等人,Nucelic Acids Res.25:3389-3402(1997))。当使用Bestfit或任何其他序列比对程序确定特定序列是否与参考氨基酸序列具有例如95%同一性时,设定参数,使得在参考氨基酸序列的全长上计算同一性的百分比并且允许参考序列中的氨基酸残基的总

数的同源性的间隔最高至5％。确定多肽之间的同一性百分比的这一上述方法适用于本文公开的所有蛋白质、其片段或变体。

[0098] 在提及如本文所定义的结合分子(例如抗体)与其结合配偶体(例如抗原)的相互作用时,术语“特异性结合(specifically binds)”或“与...特异性结合(specifically binds to)”是指在给定的一组条件下,结合分子在来自一个动物物种的目标抗原与来自不同动物物种的抗原直向同源物(antigen orthologue)之间作出区分的能力。如在体外测定中所确定的,如果CD137结合分子以其结合大鼠或小鼠的CD137的EC50的50％以下的EC50结合人CD137的话,那么所述CD137结合分子被称为与人CD137特异性结合。抗体的结合特异性可以使用本领域已知的方法确定。此类方法的实例包括使用PHA刺激的原代细胞的FACS、Western blots、ELISA-、RIA-、ECL-、IRMA-测试和肽扫描。

[0099] 在提及如本文所定义的结合分子(例如抗体)与其结合配偶体(例如抗原)的相互作用时,术语“选择性结合(selectively binds)”或“与...选择性结合(selectively binds to)”是指在给定的一组条件下,结合分子在来自一个动物物种的目标抗原(诸如人CD137)与来自相同动物物种的不同抗原(诸如人CD40)之间作出区分的能力。如在体外测定中所确定的,如果CD137结合分子以其结合人CD40或人CD134的EC50的10％以下的EC50结合人CD137的话,那么所述CD137结合分子被称为与人CD137选择性结合。

[0100] 关于哺乳动物中的某一疾病病状的术语“治疗(treat、treating或treatment)”是指在患有该疾病病状的哺乳动物中导致期望或有益的效应。期望或有益的效应可包括疾病的一种或多种症状(即,肿瘤生长和/或转移,或由免疫细胞的数量和/或活性介导的其他作用等)的频率或严重程度降低,或疾病、病状或病症的进一步发展的停止或抑制。在治疗哺乳动物的癌症的上下文中,期望或有益的效应可包括癌细胞的进一步生长或扩散的抑制、癌细胞的死亡、癌症复发的抑制、癌症相关疼痛的减少,或哺乳动物存活率的提高。所述作用可以是主观或客观的。例如,如果哺乳动物是人,那么人可将精力或活力改善或疼痛减少记录为改善的自觉症状或对疗法的反应。可替代地,临床医生可基于体检、实验室参数、肿瘤标志物或影像学发现注意到肿瘤大小或肿瘤负荷的减小。对于对治疗的反应,临床医生可观察的一些实验室征象包括测试的标准化,诸如白血细胞计数、红血细胞计数、血小板计数、红细胞沉降率以及各种酶水平。另外,临床医生可观察到可检测肿瘤标志物的减少。可替代地,可使用其他测试评估客观改善,诸如声像图、核磁共振测试以及正电子发射测试。

[0101] 术语“载体”是指能够运输外来核酸分子的核酸分子。外来核酸分子通过重组技术诸如连接或重组连接至载体核酸分子。这允许在宿主细胞或生物体中倍增、选择、进一步操纵或表达外来核酸分子。载体可以是质粒、噬菌体、转座子、粘粒、染色体、病毒或病毒体。一类载体可在引入宿主细胞后整合到宿主细胞的基因组中,并且由此与宿主基因组一起复制(例如,非附加型哺乳动物载体)。另一类载体能够在其被引入的宿主细胞中自主复制(例如,具有细菌复制起点的细菌载体和附加型哺乳动物载体)。能够指导其所操作性地连接的可表达外来核酸的表达的另一特定类型的载体通常称为“表达载体”。表达载体通常具有驱动可表达外来核酸的表达的控制序列。已知为“转录载体”的更简单的载体仅能够转录,但不能翻译:它们可以在靶细胞中复制,但不表达。术语“载体”涵盖所有类型的载体,而不考虑其功能。能够指导其所操作性地连接的可表达核酸的表达的载体通常称为“表达载体”。

[0102] 除非另外指示,否则本公开的方法和技术通常根据本领域熟知且如本说明书通篇

引用和论述的各种一般性和更特定参考文献中所描述的方法来执行。此类参考文献包括,例如,Sambrook和Russell,Molecular Cloning,A Laboratory Approach,Cold Spring Harbor Press,Cold Spring Harbor,N.Y.(2001),Ausubel等人,Current Protocols in Molecular Biology,John Wiley&Sons,NY(2002),以及Harlow和Lane Antibodies:A Laboratory Manual,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,N.Y.(1990)。酶促反应和纯化技术是根据制造商说明书,如本领域中通常所达成或如本文所述来执行。与本文所述的分析化学、合成有机化学以及医学和药物化学结合使用的命名法,以及所述分析化学、合成有机化学以及医学和药物化学的实验室程序和技术是本领域熟知和常用的那些。标准技术用于化学合成、化学分析、药物制备、配制和递送以及患者的治疗。

[0103] 如本文所用,20种常规氨基酸及其缩写遵循常规用法。参见Immunology-A Synthesis(第2版,E.S.Golub和D.R.Gren编辑,Sinauer Associates,Sunderland,Mass.(1991))。

[0104] B.与人CD137结合的结合分子

[0105] 本公开提供了与人CD137结合的分离的结合分子,包括CD137抗体、CD137抗体的抗原结合片段,以及CD137抗体的衍生物。在一些实施方案中,结合分子是本文所述的任何抗体,包括关于表位结合所描述的抗体和关于HVR、可变区(VL、VH)和IgG(例如,IgG4)轻链和重链的具体氨基酸序列所描述的抗体。在一些实施方案中,本公开涉及结合分子,所述结合分子与人CD137结合,并且具有以下功能特性中的至少一个(例如,至少一个、至少两个、至少三个、至少四个、至少五个、至少六个、至少七个或全部八个):(a)以500nM或更小的KD与人CD137结合;(b)对人CD137具有激动剂活性;(c)在最高至1000nM的浓度下不与人OX40、CD40、GITR和/或CD27受体结合;(d)与猴、小鼠、大鼠或狗CD137交叉反应;(e)不引起ADCC效应;(f)能够抑制肿瘤细胞生长;(g)对癌症具有治疗作用;以及(h)阻断CD137与CD137L之间的结合。在一些实施方案中,本文公开的抗体还可以阻断,例如完全阻断,CD137与其配体CD137L之间的结合。本文还提供了与如本文所述的抗体或抗原结合片段中的一种或多种交叉竞争结合人CD137的一种或多种抗CD137抗体或抗原结合片段。

[0106] 在一些实施方案中,抗体或其抗原结合片段与SEQ ID NO:1的氨基酸残基34-108内的一个或多个氨基酸残基结合。在一些实施方案中,抗体或抗原结合片段与SEQ ID NO:1的氨基酸残基34-93内的一个或多个氨基酸残基结合。在一些实施方案中,抗体或抗原结合片段与选自由SEQ ID NO:1的氨基酸残基34-36、53-55和92-93组成的组的一个或多个氨基酸残基结合。在一些实施方案中,抗体或抗原结合片段与SEQ ID NO:1的氨基酸残基34-36中的一个或多个、氨基酸残基53-55中的一个或多个,以及氨基酸残基92-93中的一个或多个结合。在一些实施方案中,抗体或抗原结合片段不与选自由SEQ ID NO:1的氨基酸残基109-112、125、126、135-138、150和151组成的组的氨基酸残基中的一个或多个结合。在一些实施方案中,抗体或抗原结合片段不与SEQ ID NO:1的氨基酸残基109-112、125、126、135-138、150和151结合。测量抗体或抗原结合片段结合靶抗原的能力的方法可使用本领域已知的任何方法进行,包括例如,通过表面等离子体共振、ELISA、等温滴定量热法、过滤结合测定、EMSA等。在一些实施方案中,抗体或抗原结合片段结合靶抗原的能力通过表面等离子体共振测量(参见例如,以下实施例1)。

[0107] 在一些实施方案中,抗体或抗原结合片段以约500nM或更小(例如,约500nM或更

小、约400nM或更小、约300nM或更小、约200nM或更小、约150nM或更小、约100nM或更小、约90nM或更小、约80nM或更小、约75nM或更小、约70nM或更小、约60nM或更小、约50nM或更小、约40nM或更小、约30nM或更小、约25nM或更小、约20nM或更小、约10nM或更小、约1nM或更小、约0.1nM或更小等)的KD与人CD137结合。在一些实施方案中,抗体或抗原结合片段以约100nM或更小的KD与人CD137结合。在一些实施方案中,抗体或抗原结合片段以约50nM或更小的KD与人CD137结合。测量抗体或抗原结合片段的KD的方法可使用本领域已知的任何方法进行,包括例如,通过表面等离子体共振、ELISA、等温滴定量热法、过滤结合测定、EMSA等。在一些实施方案中,KD通过表面等离子体共振测量(参见例如,以下实施例1)。

[0108] 抗CD137抗体必须交联,以变得具有激动性。例如,交联通过Fc γ 受体在体内实现,而通常多克隆抗Fc抗体在体外用于基于细胞的实验中。在一些实施方案中,本文所述的抗体或抗原结合片段对人CD137具有激动剂活性。在一些实施方案中,当表达人CD137的细胞(例如,人细胞)接触抗体或抗原结合片段时,抗体或抗原结合片段诱导人CD137的一种或多种(例如,一种或多种、两种或更多种、三种或更多种等)活性。各种CD137活性是本领域已知的并且可包括但不限于NF-κB依赖性转录的诱导、T细胞增殖的诱导、T细胞存活的延长、活化T细胞的共刺激、细胞因子分泌(诸如IL-2)的诱导,以及单核细胞活化的诱导。在一些实施方案中,一种或多种CD137活性不是CD137与其配体的结合。测量CD137活性(例如,NF-κB依赖性转录和/或T细胞增殖的诱导等)的方法是本领域已知的,包括,例如,经由以下实施例8和9中所述的方法。在一些实施方案中,抗体或抗原结合片段增加表达人CD137的细胞(例如,人细胞)中的NF-κB依赖性转录。在一些实施方案中,相对于不与抗体或抗原结合片段接触的对应细胞(例如,不与抗体接触,或与同种型对照抗体接触的对应细胞),在与抗体或抗原结合片段接触的表达CD137的细胞(例如,人细胞)中,NF-κB依赖性转录增加约10%或更多、约20%或更多、约30%或更多、约40%或更多、约50%或更多、约60%或更多、约70%或更多、约80%或更多、约90%或更多,或约99%或更多。在一些实施方案中,相对于不与抗体或抗原结合片段接触的对应细胞(例如,不与抗体接触,或与同种型对照抗体接触的对应细胞),在与抗体或抗原结合片段接触的表达CD137的细胞(例如,人细胞)中,NF-κB依赖性转录增加约2倍、3倍、4倍、5倍、6倍、7倍、8倍、9倍、10倍、100倍、1000倍或更多。

[0109] 在一些实施方案中,抗体或抗原结合片段与猴(例如,食蟹猴)、小鼠、大鼠和/或狗CD137交叉反应。在一些实施方案中,抗体或抗原结合片段与猴CD137交叉反应。在一些实施方案中,抗体或抗原结合片段与小鼠CD137交叉反应。在一些实施方案中,抗体或抗原结合片段与大鼠CD137交叉反应。在一些实施方案中,抗体或抗原结合片段与狗CD137交叉反应。在一些实施方案中,抗体或抗原结合片段与猴和小鼠CD137;猴和大鼠CD137;猴和狗CD137;小鼠和大鼠CD137;小鼠和狗CD137;大鼠和狗CD137;猴、小鼠和大鼠CD137;猴、小鼠和狗CD137;猴、大鼠和狗CD137;小鼠、大鼠和狗CD137;或猴、小鼠、大鼠和狗CD137交叉反应。在一些实施方案中,抗体或抗原结合片段在约100nM下(例如,在约1nM下、在约10nM下、在约25nM下、在约50nM下、在约75nM下、在约100nM下)交叉反应。测量抗体交叉反应性的方法是本领域已知的,包括但不限于表面等离子体共振、ELISA、等温滴定量热法、过滤结合测定、EMSA等。在一些实施方案中,交叉反应性通过ELISA测量(参见例如,以下实施例2)。

[0110] 在一些实施方案中,抗体不引起ADCC效应。测量ADCC效应的方法(例如,体内方法)是本领域已知的,包括但不限于经由以下实施例11中所述的方法。在一些实施方案中,相对

于对照,抗体不引起超过约10％的ADCC效应(不引起超过约10％、超过约5％、超过约1％、超过约0.1％、超过约0.01％的ADCC效应)。

[0111] 在一些实施方案中,抗体或抗原结合片段能够抑制肿瘤细胞生长/增殖。在一些实施方案中,相对于不与抗体或抗原结合片段接触的对应肿瘤细胞,当与抗体或抗原结合片段接触时,肿瘤细胞生长/增殖被抑制至少约5％(例如,至少约5％、至少约10％、至少约20％、至少约30％、至少约40％、至少约50％、至少约60％、至少约70％、至少约80％、至少约90％,或至少约99％)。在一些实施方案中,在将抗体或抗原结合片段施用给受试者时,抗体或抗原结合片段能够减小受试者的肿瘤体积。在一些实施方案中,相对于受试者的初始肿瘤体积(例如,在施用抗体或抗原结合片段之前),抗体或抗原结合片段能够将受试者的肿瘤体积减少至少约5％(例如,至少约5％、至少约10％、至少约20％、至少约30％、至少约40％、至少约50％、至少约60％、至少约70％、至少约80％、至少约90％,或至少约99％)。监测肿瘤细胞生长/增殖、肿瘤体积和/或肿瘤抑制的方法是本领域已知的,包括,例如,经由以下实施例10中所述的方法。

[0112] 在一些实施方案中,抗体或抗原结合片段对癌症具有治疗作用。在一些实施方案中,抗体或抗原结合片段减少癌症的一种或多种体征或症状。在一些实施方案中,当施用抗体或抗原结合片段时,罹患癌症的受试者进入部分或完全缓解。

[0113] 在另一方面,本公开提供了与本公开的例示性抗体中的任一种,诸如AG10058、AG10059和/或AG10131竞争或交叉竞争结合人CD137的分离抗体。在一个特定实施方案中,本公开提供了与本公开的例示性抗体中的任一种竞争或交叉竞争结合人CD137上的相同表位的分离抗体。抗体与另一抗体竞争或交叉竞争结合的能力可以使用本领域已知的标准结合测定确定,诸如BIAcore分析、ELISA测定或流式细胞术。例如,可以允许本公开的例示性抗体在饱和条件下与人CD137结合,然后测量测试抗体结合CD137的能力。如果测试抗体能够与例示性抗体同时结合至CD137,那么测试抗体结合至与例示性抗体不同的表位。然而,如果测试抗体不能同时结合至CD137,那么测试抗体结合至例示性抗体所结合表位的相同表位、重叠表位,或与其紧密相邻的表位。此实验可以使用各种方法执行,诸如ELISA、RIA、FACS或表面等离子体共振。

[0114] 在一些实施方案中,抗体或抗原结合片段阻断CD137与其配体(例如,人CD137与人CD137L)之间的结合。在一些实施方案中,抗体或抗原结合片段在体外阻断CD137与其配体之间的结合。在一些实施方案中,对于阻断CD137与其配体的结合,抗体或抗原结合片段具有约500nM或更小(例如,约500nM或更小、约400nM或更小、约300nM或更小、约200nM或更小、约100nM或更小、约50nM或更小、约25nM或更小、约10nM或更小、约1nM或更小等)的半最大抑制浓度(IC50)。在一些实施方案中,对于阻断CD137与其配体的结合,抗体或抗原结合片段具有约100nM或更小的半最大抑制浓度(IC50)。在一些实施方案中,当以约100nM或更大(例如,约100nM或更大、约500nM或更大、约1μM或更大、约10μM或更大等)的浓度提供时,抗体或抗原结合片段完全阻断人CD137与其配体的结合。如本文所用,术语“完全阻断(complete blocking或completely blocks)”是指抗体或抗原结合片段将第一蛋白质与第二蛋白质之间的结合减少至少约80％(例如,至少约80％、至少约85％、至少约90％、至少约95％、至少约99％等)的能力。测量抗体或抗原结合片段阻断第一蛋白质(例如,CD137)与第二蛋白质(例如,CD137L)的结合的能力的方法是本领域已知的,包括但不限于经由BIAcore分析、

ELISA测定和流式细胞术(参见例如,以下实施例6)。

[0115] B-1.CD137抗体

[0116] 在一些方面,本公开提供了一种分离抗体,其在SEQ ID NO.:1的氨基酸残基34-108或34-93内的表位上结合至人CD137。在一些实施方案中,抗体以50nM或更小的K_D结合人CD137,如通过表面等离子体共振所测量的。在某些实施方案中,抗体可以与选自由食蟹猴、小鼠、大鼠和狗组成的列表的至少一种非人物种交叉反应。

[0117] 在一个方面,本公开提供了一种分离抗体,其包含重链可变区和轻链可变区,a)其中重链可变区包含HVR-H1、HVR-H2和HVR-H3,其中HVR-H1包含根据选自由以下组成的组的式的氨基酸序列:式(I):X1TFX2X3YX4IHWV(SEQ ID NO:2),其中X1为F或Y,X2为S或T,X3为G、N或S,并且X4为A、G或W;式(II):YSIX1SGX2X3WX4WI(SEQ ID NO:3),其中X1为S或T,X2为H或Y,X3为H或Y,并且X4为A、D、G、N、S或T;以及式(III):FSLSTX1GVX2VX3WI(SEQ ID NO:4),其中X1为G或S,X2为A或G,并且X3为A、G、S或T;其中HVR-H2包含根据选自由以下组成的组的式的氨基酸序列:式(IV):LALIDWX1X2DKX3YSX4SLKSRL(SEQ ID NO:5),其中X1为A、D或Y,X2为D或G,X3为R、S或Y,并且X4为P或T;式(V):IGX1IYHSGX2TYYX3PSLKSRV(SEQ ID NO:6),其中X1为D或E,X2为N或S,并且X3为N或S;以及式(VI):VSX1ISGX2GX3X4TYYADSVKGRF(SEQ ID NO:7),其中X1为A、G、S、V或Y,X2为A、D、S或Y,X3为D、G或S,并且X4为S或T;并且其中HVR-H3包含根据下式的氨基酸序列:式(VII):ARX1GX2X3X4VX5GDWFX6Y(SEQ ID NO:8),其中X1为E或G,X2为E或S,X3为D或T,X4为A、T或V,X5为A、I、L、T或V,并且X6为A、D或G;并且/或者b)其中轻链可变区包含HVR-L1、HVR-L2和HVR-L3,其中HVR-L1包含根据下式的氨基酸序列:式(VIII):X1ASQX2X3X4X5X6X7X8(SEQ ID NO:9),其中X1为Q或R,X2为D、G或S,X3为I或V,X4为G、R、S或T,X5为P、R、S或T,X6为A、D、F、S、V或Y,X7为L或V,并且X8为A、G或N;其中HVR-L2包含根据下式的氨基酸序列:式(IX):X1ASX2X3X4X5GX6(SEQ ID NO:10),其中X1为A或D,X2为N、S或T,X3为L或R,X4为A、E或Q,X5为S或T,并且X6为I或V;并且其中HVR-L3包含根据选自由以下组成的组的式的氨基酸序列:式(X):YCQQX1YX2X3X4T(SEQ ID NO:11),其中X1为A、G、S或Y,X2为Q、S或Y,X3为I、L、T或Y,并且X4为I、S、V或W;以及式(XI):YCX1QX2X3X4X5PX6T(SEQ ID NO:12),其中X1为E或Q,X2为P、S或Y,X3为D、L、S、T或Y,X4为D、E、H、S或T,X5为D、L T或W,并且X6为L、P、R或V。

[0118] 在一些实施方案中,所述抗体可以包含具有选自由SEQ ID NO:253-312组成的组的氨基酸序列的HVR_H1、具有选自由SEQ ID NO:313-372组成的组的氨基酸序列的HVR_H2、具有选自由SEQ ID NO:373-432组成的组的氨基酸序列的HVR_H3、具有选自由SEQ ID NO:433-492组成的组的氨基酸序列的HVR_L1、具有选自由SEQ ID NO:493-552组成的组的氨基酸序列的HVR_L2,和/或具有选自由SEQ ID NO:553-612组成的组的氨基酸序列的HVR_L3。

[0119] 在某些实施方案中,抗体可以包含具有选自由SEQ ID NO:13-132组成的组的氨基酸序列的VL和/或VH,其可以优选地分别由选自由SEQ ID NO:133-252组成的组的DNA序列编码。

[0120] 在一些实施方案中,所述抗体可以包含具有选自由SEQ ID NO:709-732组成的组的氨基酸序列的HVR_H1、具有选自由SEQ ID NO:733-756组成的组的氨基酸序列的HVR_H2、具有选自由SEQ ID NO:757-780组成的组的氨基酸序列的HVR_H3、具有选自由SEQ ID NO:781-804组成的组的氨基酸序列的HVR_L1、具有选自由SEQ ID NO:805-828组成的组的氨基

酸序列的HVR_L2,和/或具有选自由SEQ ID NO:829-852组成的组的氨基酸序列的HVR_L3。

[0121] 在某些实施方案中,抗体可以包含具有选自由SEQ ID NO:613-660组成的组的氨基酸序列的轻链和/或重链(例如,IgG诸如IgG4的那些),其可以优选地分别由选自由SEQ ID NO:661-708组成的组的DNA序列编码。

[0122] 本文所述的CD137抗体可以属于任何种类,诸如IgG、IgM、IgE、IgA或IgD。优选的是,CD137抗体属于IgG类,诸如IgG1、IgG2、IgG3或IgG4亚类。可以使用本领域已知的方法将CD137抗体从一个种类或亚类转换为另一种类或亚类。用于产生属于期望种类或亚类的抗体的示例性方法包括以下步骤:分离编码CD137抗体的重链的核酸和编码CD137抗体的轻链的核酸;分离编码V_H区的序列;将V_H序列与编码期望种类或亚类的重链恒定区的序列连接;在细胞中表达轻链基因和重链构建体;以及收集CD137抗体。

[0123] 此外,本公开提供的抗体可以是单克隆或多克隆,但优选为单克隆。

[0124] 本公开提供的具体分离抗体的实例包括表1a和1b中列出的那些。这些抗体的重链可变区、IgG2和IgG4亚类的全长重链、轻链可变区以及全长轻链的核苷酸和氨基酸序列也在下文提供。

[0125] 本公开的抗体可以通过本领域已知的技术产生,包括常规单克隆抗体方法,例如,标准体细胞杂交技术(参见例如,Kohler和Milstein,Nature 256:495(1975)、B淋巴细胞的病毒或致癌转化,或重组抗体技术,如下文详细描述。

[0126] 杂交瘤产生是非常完善的程序。用于制备杂交瘤的常见动物系统是鼠系统。用于分离供融合用的免疫的脾细胞的免疫方案和技术是本领域已知的。融合配偶体(例如,鼠骨髓瘤细胞)和融合程序也是已知的。可用于制备本公开提供的人CD137抗体的一种众所周知的方法涉及XenoMouse™动物系统的使用。XenoMouse™小鼠是工程改造的小鼠品系,其包含大片段的人免疫球蛋白重链和轻链基因座并且在小鼠抗体产生中有缺陷。参见,例如,Green等人,Nature Genetics 7:13-21(1994)和WO2003/040170。将动物用CD137抗原免疫。CD137抗原是分离和/或纯化的CD137,优选地CD137。它可以是CD137的片段,诸如CD137的细胞外结构域,尤其是包含SEQ ID NO:1的氨基酸残基34-108或34-93的CD137细胞外结构域片段。动物的免疫可以通过本领域已知的任何方法进行。参见,例如,Harlow和Lane,Antibodies:A Laboratory Manual,New York:Cold Spring Harbor Press,1990。用于对非人动物诸如小鼠、大鼠、绵羊、山羊、猪、牛和马免疫的方法在本领域中是众所周知的。参见,例如,Harlow和Lane,同上以及美国专利号5,994,619。CD137抗原可与佐剂一起施用以刺激免疫应答。示例性佐剂包括完全或不完全弗氏佐剂、RIBI(胞壁酰二肽)或ISCOM(免疫刺激复合物)。在用CD137抗原对动物免疫之后,由分离自免疫动物的细胞制备产生抗体的永生化细胞系。在免疫之后,将动物处死并且将淋巴结和/或脾B细胞永生化。将细胞永生化的方法包括但不限于用致癌基因转染所述细胞,用致癌病毒感染所述细胞,在针对永生化细胞选择的条件下培养所述细胞,使所述细胞经受致癌或突变化合物,将所述细胞与永生化细胞(例如,骨髓瘤细胞)融合,以及使肿瘤抑制基因失活。参见,例如,Harlow和Lane,同上。如果使用具有骨髓瘤细胞的融合体,骨髓瘤细胞优选地不分泌免疫球蛋白多肽(非分泌细胞系)。永生化细胞使用CD137、其部分或表达CD137的细胞筛选。选择产生CD137抗体的细胞,例如杂交瘤,克隆并针对期望特征进一步筛选,所述特征包括稳健的生长、高抗体产量和期望的抗体特征,如下文进一步讨论。杂交瘤可以在体内在同系动物中,在缺乏免疫系统

的动物(例如,裸小鼠)中,或在体外在细胞培养物中扩展。选择、克隆和扩展杂交瘤的方法是本领域普通技术人员所熟知的。

[0127] 本公开的抗体还可以使用噬菌体展示或酵母展示方法制备。在本领域建立了用于分离人抗体的此类展示方法,诸如Achim 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;以及Michael J.Feldhaus等人,"Flow-cytometric isolation of human antibodies from a non-immune Saccharomyces cerevisiae surface display library"Nat Biotechnol (2003)21:163-170。

[0128] B-2.抗原结合片段

[0129] 在一些其他方面,本公开提供了本公开提供的任何CD137抗体的抗原结合片段。

[0130] 抗原结合片段可包含抗体的任何序列。在一些实施方案中,抗原结合片段包含以下的氨基酸序列:(1)CD137抗体的轻链;(2)CD137抗体的重链;(3)来自CD137抗体的轻链的可变区;(4)来自CD137抗体的重链的可变区;(5)CD137抗体的一个或多个HVR(两个、三个、四个、五个或六个HRV);或(6)来自CD137抗体的轻链的三个HVR和来自所述CD137抗体的重链的三个HVR。

[0131] 在一些特定实施方案中,本公开提供了选自表1a和1b中列出的那些的抗体的抗原结合片段。

[0132] 在一些其他特定实施方案中,CD137抗体的抗原结合片段包括:(i)Fab片段,其为由V_L、V_H、C_L和C_H1结构域组成的一价片段;(ii)F(ab′)$_2$片段,其为包含由在铰链区的二硫键连接的两个Fab片段的二价片段;(iii)由V_H和C_H1结构域组成的Fd片段;(iv)由抗体的单臂的V_L和V_H结构域组成的Fv片段;(v)由V_H结构域组成的dAb片段(Ward等人,(1989)Nature 341:544-546);(vi)分离的CDR,以及(vii)单链抗体(scFv),其为包含与抗体的V_H区连接的抗体的V_L区的多肽。Bird等人,(1988)Science 242:423-426和Huston等人,(1988)Proc.Natl.Acad.Sci.USA 85:5879-5883。

[0133] 在一些特定实施方案中,抗原结合片段是选自表1a中列出的那些的Fab片段。

[0134] B-3.抗体衍生物

[0135] 在一些另外的方面,本公开提供了本公开提供的任何CD137抗体的衍生物。

[0136] 在一个方面,抗体衍生物衍生自本公开的例示性抗体("亲本抗体")的氨基酸序列的修饰,同时保留亲本抗体氨基酸序列的总体分子结构。亲本抗体链的任何区域的氨基酸序列可被修饰,诸如框架区、HVR区或恒定区。修饰的类型包括亲本抗体的一个或多个氨基酸的取代、插入、缺失或其组合。

[0137] 在一些实施方案中,抗体衍生物包含V_L或V_H区,其与如SEQ ID NO:13-132中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_H1氨基酸序列区域,其与如SEQ ID NO:253-312中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_H2氨基酸序列区域,其与如SEQ ID NO:313-372中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_H3氨基酸序列区域,其与如SEQ ID NO:373-432中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含表1a所示的所有Fab命中物的HVR_L1氨基酸序列,其可见于SEQ ID NO:433-492。在一些实施方案中,抗体衍生物包含表1a所示的所有Fab命中物的HVR_L2氨基酸序列,其可见于SEQ ID NO:493-552。

[0138] 在一些实施方案中,抗体衍生物包含表1a所示的所有Fab命中物的HVR_L3氨基酸序列,其可见于SEQ ID NO:553-612。在一些特定实施方案中,所述衍生物包含对如SEQ ID NO:13-132和253-612中的任一个中所示的氨基酸序列的1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个保守或非保守取代,和/或1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个添加和/或缺失。

[0139] 在一些实施方案中,抗体衍生物包含与如SEQ ID NO:613-660中的任一个中所示的氨基酸序列具有至少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％同一性的轻链或重链。

[0140] 在一些实施方案中,抗体衍生物包含HVR_H1氨基酸序列区域,其与如SEQ ID NO:709-732中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_H2氨基酸序列区域,其与如SEQ ID NO:733-756中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_H3氨基酸序列区域,其与如SEQ ID NO:757-780中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_L1氨基酸序列区域,其与如SEQ ID NO:781-804中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_L2氨基酸序列区域,其与如SEQ ID NO:805-828中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,抗体衍生物包含HVR_L3氨基酸序列区域,其与如SEQ ID NO:829-852中的任一个中所示的氨基酸序列具有至少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。在一些实施方案中,所述衍生物包含对如SEQ ID NO:613-660和709-852中的任一个中所示的氨基酸序列的1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个保守或非保守取代,和/或1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个添加和/或缺失。

[0141] 氨基酸取代涵盖保守取代和非保守取代。术语“保守氨基酸取代”是指一个氨基酸替换为另一个氨基酸,其中两个氨基酸在某些物理-化学特性上具有相似性,诸如所涉及残基的极性、电荷、溶解度、疏水性、亲水性、和/或两亲性质。例如,取代通常可以在以下组中的每一个内进行:(a)非极性(疏水性)氨基酸,诸如丙氨酸、亮氨酸、异亮氨酸、缬氨酸、脯氨酸、苯丙氨酸、色氨酸以及甲硫氨酸;(b)极性中性氨基酸,诸如甘氨酸、丝氨酸、苏氨酸、半胱氨酸、酪氨酸、天冬酰胺以及谷氨酰胺;(c)带正电的(碱性)氨基酸,诸如精氨酸、赖氨酸

以及组氨酸;以及(d)带负电的(酸性)氨基酸,诸如天冬氨酸和谷氨酸。

[0142] 修饰可在抗体的氨基酸序列的任何位置进行,包括HVR、框架区或恒定区。在--个实施方案中,本公开提供了一种抗体衍生物,其包含本公开的例示性抗体的V_H和V_L HVR序列,还包含与例示性抗体的那些不同的框架序列。此类框架序列可以从包括生殖系抗体基因序列的公共DNA数据库或公布的参考文献获得。例如,人重链和轻链可变区基因的生殖系DNA序列可以在Genbank数据库或"VBase"人生殖系序列数据库中找到(Kabat,E.A.等人,Sequences of Proteins of Immunological Interest,第5版,美国卫生与公共服务部(U.S.Department of Health and Human Services),NIH出版号91-3242(1991);Tomlinson,I.M.等人,J.Mol.Biol.227:776-798(1992);以及Cox,J.P.L.等人,Eur.J.Immunol.24:827-836(1994))。可用于构建抗体衍生物的框架序列包括在结构上类似于本公开的例示性抗体所用的框架序列,例如,类似于本公开的例示性抗体所用的V_H3-23框架序列和/或V_Lλ3或λ1-13框架序列的那些。例如,可以将例示性抗体的HVR_H1、HVR_H2和HVR_H3序列,以及HVR_L1、HVR_L2和HVR_L3序列移植到具有与框架序列所来源的生殖系免疫球蛋白基因中所发现的相同序列的框架区上,或可以将HVR序列移植到与生殖系序列相比包含一个或多个突变的框架区上。

[0143] 在一个特定实施方案中,抗体衍生物是包含本公开的例示性抗体的氨基酸序列的嵌合抗体。在一个实例中,来自一种或多种例示性人抗体的一个或多个HVR与来自非人动物(诸如小鼠或大鼠)的抗体的HVR组合。在另一个实例中,嵌合抗体的所有HVR来源于一种或多种例示性抗体。在一些特定实施方案中,嵌合抗体包含来自例示性抗体的重链可变区或轻链可变区的一个、两个或三个HVR。可以使用本领域已知的常规方法生成嵌合抗体。

[0144] 另一类修饰是使V_H和/或V_L链的HRV区内的氨基酸残基突变。可以执行定点诱变或PCR介导的诱变以引入一个或多个突变,并且可以在本领域已知的体外或体内测定中评估对抗体结合或其他感兴趣的功能特性的影响。通常,引入保守取代。突变可以是氨基酸添加和/或缺失。此外,通常改变HVR区内的不超过一个、两个、三个、四个或五个残基。在一些实施方案中,抗体衍生物在重链HVR和/或轻链HVR中包含1、2、3或4个氨基酸取代。在另一个实施方案中,氨基酸取代是将抗体中的一个或多个半胱氨酸变为另一残基,诸如但不限于丙氨酸或丝氨酸。半胱氨酸可以是经典或非经典半胱氨酸。在一个实施方案中,相对于例示性抗体的氨基酸序列,抗体衍生物在重链HVR区中具有1、2、3或4个保守氨基酸取代。

[0145] 还可对V_H和/或V_L区内的框架残基进行修饰。通常,制备此类框架变体以降低抗体的免疫原性。一种方法是将一个或多个框架残基"回复突变"为对应的生殖系序列。已经进行了体细胞突变的抗体可包含不同于抗体所来源的生殖系序列的框架残基。可以通过将抗体框架序列与抗体所来源的生殖系序列相比较来鉴定此类残基。为了将框架区序列恢复至其生殖系构造,可以通过例如定点诱变或PCR介导的诱变将体细胞突变"回复突变"为生殖系序列。

[0146] 此外,还可在例示性抗体的Fc区内进行修饰,通常以改变抗体的一种或多种功能特性,诸如血清半衰期、补体固定、Fc受体结合,和/或抗原依赖性细胞毒性。在一个实例中,CH1的铰链区经过修饰,使得铰链区中的半胱氨酸残基数量改变,例如增加或减少。此方法在美国专利号5,677,425中进一步描述。改变CH1的铰链区中的半胱氨酸残基数量,例如,以有利于轻链和重链的装配,或增加或降低抗体的稳定性。在另一情况下,使抗体的Fc铰链区

突变,以减少抗体的生物学半衰期。

[0147] 此外,可根据本领域已知的常规实验对本公开的抗体进行修饰,以改变其潜在的糖基化位点或模式。在另一方面,本公开提供了本公开的CD137抗体的衍生物,其在轻链或重链的可变区中包含至少一个突变,所述突变改变可变区中的糖基化模式。对于抗原的结合,这种抗体衍生物可具有增大的亲和力和/或改变的特异性。突变可在V区中增添新的糖基化位点,改变一个或多个V区糖基化位点的位置,或除去先前存在的V区糖基化位点。在一个实施方案中,本公开提供了CD137抗体的衍生物,其在重链可变区中的天冬酰胺处具有潜在的N-连接糖基化位点,其中一个重链可变区中的潜在的N-连接糖基化位点被除去。在另一个实施方案中,本公开提供了CD137抗体的衍生物,其在重链可变区中的天冬酰胺处具有潜在的N-连接糖基化位点,其中两个重链可变区中的潜在的N-连接糖基化位点均被除去。改变抗体的糖基化模式的方法是本领域已知的,诸如美国专利号6,933,368中描述的那些,所述专利的公开内容以引用方式并入本文。

[0148] 在另一方面,本公开提供了一种抗体衍生物,其包含与另一分子实体连接的如本文所述的CD137抗体,或其抗原结合片段。另一分子实体的实例包括药剂、肽或蛋白质、检测剂或标记,以及抗体。

[0149] 在一些实施方案中,抗体衍生物包含与药剂连接的本公开的抗体。药剂的实例包括细胞毒性剂或其他癌症治疗剂,以及放射性同位素。细胞毒性剂的具体实例包括紫杉醇、细胞松弛素B、短杆菌肽D、溴化乙锭、依米丁(emetine)、丝裂霉素、依托泊苷(etoposide)、替尼泊苷(tenoposide)、长春新碱、长春花碱、秋水仙碱、多柔比星、柔红霉素、二羟基炭疽菌素二酮、米托蒽醌、光神霉素、放线菌素D、1-去氢睾酮、糖皮质激素、普鲁卡因、丁卡因、利多卡因、普萘洛尔和嘌呤霉素以及其类似物或同源物。治疗剂还包括,例如,抗代谢物(例如,甲氨蝶呤、6-巯基嘌呤、6-硫鸟嘌呤、阿糖胞苷、5-氟尿嘧啶氮烯咪胺)、烷化剂(例如,二氯甲基二乙胺、塞替派苯丁酸氮芥(thioepa chlorambucil)、美法仑(melphalan)、卡莫司汀(carmustine)(BSNU)和洛莫司汀(lomustine)(CCNU)、环磷酰胺、白消安(busulfan)、二溴甘露醇、链脲霉素、丝裂霉素C和顺式二氯二胺铂(II)(DDP)顺铂)、蒽环霉素(例如,柔红霉素(以前称为道诺霉素)和多柔比星)、抗生素(例如,更生霉素(以前称为放线菌素)、博来霉素、光神霉素和安曲霉素(AMC))和抗有丝分裂剂(例如,长春新碱和长春花碱)。可以与抗体缀合以用于诊断或治疗性用途的放射性同位素的实例包括但不限于碘^{131}、铟^{111}、钇^{90}和镥^{177}。用于将抗体与药剂连接的方法是本领域已知的,诸如使用各种接头技术。接头类型的实例包括含有腙、硫醚、酯、二硫化物和肽的接头。对于用于将治疗剂与抗体连接的接头和方法的进一步讨论,另外参见Saito等人,Adv.Drug Deliv.Rev.55:199-215(2003);Trail等人,Cancer Immunol.Immunother.52:328-337(2003);Payne,Cancer Cell 3:207-212(2003);Allen,Nat.Rev.Cancer 2:750-763(2002);Pastan,I.和Kreitman,Curr.Opin.Investig.Drugs 3:1089-1091(2002);Senter,P.D.和Springer,C.J.(2001)Adv.Drug Deliv.Rev.53:247-264。

[0150] 在一个特定实施方案中,抗体衍生物是CD137抗体多聚体,其为CD137抗体的多聚体形式,诸如单体抗体的抗体二聚体、三聚体或高阶多聚体(higher-order multimer)。抗体多聚体内的各个单体可以是相同或不同的。此外,多聚体内的各个单体可具有相同或不同的结合特异性。抗体的多聚化可通过抗体的天然聚集实现。例如,一定百分比的纯化抗体

制剂(例如,纯化的IgG4分子)自发形成包含抗体同型二聚体和其他高阶抗体多聚体的蛋白质聚集物。可替代地,抗体同型二聚体可通过本领域已知的化学连接技术形成,诸如通过使用交联剂。合适的交联剂包括为异源双功能、具有由适当的间隔基分开的两个不同的反应性基团(诸如间马来酰亚胺基苯甲酰基-N-羟基琥珀酰亚胺酯、4-(马来酰亚胺基甲基)环己烷-1-羧酸琥珀酰亚胺基酯和S-乙酰基硫代-乙酸N-琥珀酰亚胺基酯)或为同源双功能(诸如双琥珀酰亚胺辛二酸酯)的那些。此类接头可从例如Pierce Chemical Company,Rockford,IL商购获得。还可以通过本领域已知的重组DNA技术使抗体多聚化。

[0151] 本公开提供的其他抗体衍生物的实例包括单链抗体、双抗体、结构域抗体、纳米抗体和迷你抗体(unibodies)。“单链抗体”(scFv)由包含与V_H结构域连接的V_L结构域的单个多肽链组成,其中V_L结构域和V_H结构域配对形成一价分子。单链抗体可以根据本领域已知的方法制备(参见,例如,Bird等人,(1988)Science 242:423-426和Huston等人,(1988)Proc.Natl.Acad.Sci.USA 85:5879-5883)。“双抗体”由两条链组成,每条链包含通过短肽接头连接的同一多肽链上与轻链可变区连接的重链可变区,其中同一条链上的两个区域不彼此配对,但是与另一条链上的互补结构域配对形成双特异性分子。制备双抗体的方法是本领域已知的(参见,例如,HolligerP.等人,(1993)Proc.Natl.Acad.Sci.USA 90:6444-6448和Poljak R.J.等人,(1994)Structure 2:1121-1123)。结构域抗体(dAb)是抗体的小功能结合单元,其对应于抗体的重链或轻链的可变区。结构域抗体在细菌、酵母和哺乳动物细胞系统中良好表达。结构域抗体及其产生方法的另外细节是本领域已知的(参见,例如,美国专利号6,291,158;6,582,915;6,593,081;6,172,197;6,696,245;欧洲专利0368684&0616640;WO05/035572、WO04/101790、WO04/081026、WO04/058821、WO04/003019和WO03/002609)。纳米抗体衍生自抗体的重链。纳米抗体通常包含单个可变结构域和两个恒定结构域(CH2和CH3)并且保持原始抗体的抗原结合能力。可以通过本领域已知的方法制备纳米抗体(参见例如,美国专利号6,765,087、美国专利号6,838,254、WO 06/079372)。迷你抗体由IgG4抗体的一条轻链和一条重链组成。可通过除去IgG4抗体的铰链区制备迷你抗体。迷你抗体及其制备方法的另外细节可见于WO2007/059782。

[0152] C.产生CD137抗体的核酸、载体、宿主细胞和重组方法

[0153] 本公开的另一方面提供一种分离的核酸分子,其包含编码本公开提供的结合分子的氨基酸序列的核苷酸序列。由所述核苷酸序列编码的氨基酸序列可以是抗体的任何部分,诸如HVR,包含一个、两个或三个HVR的序列,重链的可变区,轻链的可变区,或可以是全长重链或全长轻链。本公开的核酸可以是,例如,DNA或RNA,并且可包含或可不包含内含子序列。通常,核酸是cDNA分子。

[0154] 在一些实施方案中,本公开提供了一种分离的核酸分子,其包含编码氨基酸序列的核苷酸序列或由其组成,所述氨基酸序列选自由以下组成的组:(1)例示性抗体的HVR_H3或HVR_L3的氨基酸序列;(2)例示性抗体的重链的可变区或轻链的可变区;或(3)例示性抗体的全长重链或全长轻链。

[0155] 在其他实施方案中,核酸分子包含核苷酸序列或由其组成,所述核苷酸序列编码如SEQ ID NO:13-132、253-612、613-660和709-852中的任一个中所示的氨基酸序列。

[0156] 在其他实施方案中,核酸分子包含选自由SEQ ID NO:133-252和661-708组成的组的核苷酸序列或由其组成。

[0157] 本公开的核酸可以使用任何合适的分子生物学技术获得。对于杂交瘤表达的抗体,编码通过杂交瘤制备的抗体的轻链和重链的cDNA可以通过PCR扩增或cDNA克隆技术获得。对于从免疫球蛋白基因文库获得的抗体(例如,使用噬菌体展示技术),可以从文库中回收编码所述抗体的核酸。

[0158] 可以通过将编码V_H的DNA操作性地连接至编码重链恒定区(CH1、CH2和CH3)的另一DNA分子而将编码V_H区的分离DNA转换成全长重链基因。人重链恒定区基因的序列是本领域已知的(参见例如,Kabat等人(1991)Sequences of Proteins of Immunological Interest,第5版,美国卫生与公共服务部,NIH出版号91-3242)并且涵盖这些区域的DNA片段可以通过标准PCR扩增获得。重链恒定区可以是IgG1、IgG2、IgG3、IgG4、IgA、IgE、IgM或IgD恒定区,但最优选的是不具有ADCC效应的IgG4或IgG2恒定区。IgG4恒定区序列可以是已知存在于不同个体中的各种等位基因或同种异型中的任一个。这些同种异型代表IgG4恒定区中天然存在的氨基酸取代。对于Fab片段重链基因,可以将编码V_H的DNA操作性地连接至仅编码重链CH1恒定区的另一DNA分子。

[0159] 可以通过将编码V_L的DNA操作性地连接至编码轻链恒定区CL的另一DNA分子而将编码V_L区的分离DNA转换成全长轻链基因(以及Fab轻链基因)。人轻链恒定区基因的序列是本领域已知的(参见例如,Kabat等人(1991)Sequences of Proteins of Immunological Interest,第5版,美国卫生与公共服务部,NIH出版号91-3242)并且涵盖这些区域的DNA片段可以通过标准PCR扩增获得。轻链恒定区可以是κ或λ恒定区。

[0160] 为了产生scFv基因,将编码V_H和V_L的DNA片段操作性地连接至编码柔性接头,例如编码氨基酸序列$(Gly_4\text{-}Ser)_3$的另一片段,使得V_H和V_L序列可以表达为具有通过柔性接头连接的V_L和V_H区的连续的单链蛋白质(参见例如,Bird等人,Science 242:423-426(1988);Huston等人,Proc.Natl.Acad.Sci.USA 85:5879-5883(1988);以及McCafferty等人,Nature 348:552-554(1990))。

[0161] 本公开还提供了一种载体,其包含本公开提供的核酸分子。所述核酸分子可编码轻链或重链的一部分(诸如CDR或HVR)、全长轻链或重链、包含重链或轻链的一部分或全长的多肽,或抗体衍生物或抗原结合片段的氨基酸序列。在一些实施方案中,载体是可用于表达结合分子诸如抗体或其抗原结合片段的表达载体。在一些实施方案中,本文提供了载体,其中第一载体包含编码如本文所述的重链可变区的多核苷酸序列,并且第二载体包含编码如本文所述的轻链可变区的多核苷酸序列。在一些实施方案中,单个载体包含编码如本文所述的重链可变区和如本文所述的轻链可变区的多核苷酸。

[0162] 为了表达本公开的结合分子,将编码部分或全长轻链和重链的DNA插入表达载体中,使得DNA分子操作性地连接至转录和翻译控制序列。在此上下文中,术语“操作性地连接”是指抗体基因连接到载体中,使得载体内的转录和翻译控制序列发挥其调控DNA分子的转录和翻译的预期功能。所选择的表达载体和表达控制序列应可与所使用的表达宿主细胞相容。抗体轻链基因和抗体重链基因可插入独立载体中,或者更通常两个基因插入同一表达载体中。抗体基因通过任何合适的方法插入表达载体中(例如,连接抗体基因片段和载体上的互补限制性位点,或基于同源重组的DNA连接)。本文所述抗体的轻链和重链可变区可以用于产生任何抗体同种型和亚类的全长抗体基因,这通过将它们插入已经编码期望的同种型和亚类的重链恒定区和轻链恒定区的表达载体中,使得V_H区段操作性地连接至载体内

的一个或多个C_H区段并且V_L区段操作性地连接至载体内的C_L区段来实现。另外或可替代地，重组表达载体可以编码有助于宿主细胞分泌抗体链的信号肽。可将抗体链基因克隆到载体中，使得信号肽与抗体链基因的氨基末端同框连接。信号肽可以是免疫球蛋白信号肽或异源信号肽(即来自非免疫球蛋白的信号肽)。

[0163] 除抗体链基因外，本公开的表达载体通常还携带控制抗体链基因在宿主细胞中的表达的调控序列。术语“调控序列”旨在包括启动子、增强子和控制抗体链基因的转录或翻译的其他表达控制元件(例如，聚腺苷酸化信号)。此类调控序列描述于，例如，Goeddel(Gene Expression Technology.Methods in Enzymology 185,Academic Press,San Diego,Calif.(1990))中。本领域技术人员将认识到，表达载体的设计，包括调控序列的选择，可取决于诸如待转化的宿主细胞的选择、期望蛋白质的表达水平等因素。用于哺乳动物宿主细胞表达的调控序列的实例包括指导哺乳动物细胞中的高水平蛋白质表达的病毒元件，诸如来源于巨细胞病毒(CMV)、猿猴病毒40(SV40)、腺病毒(例如，腺病毒主要晚期启动子(AdMLP))和多瘤病毒的启动子和/或增强子。可替代地，可使用非病毒调控序列，诸如遍在蛋白启动子或β-球蛋白启动子。此外，调控元件包含来自不同来源的序列，诸如SR启动子系统，其包含来自SV40早期启动子和人T细胞白血病1型病毒的长末端重复序列的序列(Takebe,Y.等人(1988)Mol.Cell.Biol.8:466-472)。

[0164] 除抗体链基因和调控序列外，表达载体还可携带另外的序列，诸如调控载体在宿主细胞中的复制的序列(例如，复制起点)和选择性标志物基因。选择性标志物基因有助于载体已经引入其中的宿主细胞的选择(参见例如，美国专利号4,399,216、4,634,665和5,179,017，所有均由Axel等人申请)。举例来说，通常选择性标志物基因将对于药物诸如G418、潮霉素或甲氨喋呤的抗性赋予其中已经引入载体的宿主细胞。选择性标志物基因包括二氢叶酸还原酶(DHFR)基因(用于使用甲氨蝶呤选择/扩增的dhfr-宿主细胞中)和neo基因(用于G418选择)。

[0165] 为表达轻链和重链，通过任何合适的技术将编码重链和轻链的一种或多种表达载体转染到宿主细胞中。各种形式的术语“转染”旨在涵盖通常用于将外源DNA引入原核或真核宿主细胞中的广泛多种技术，例如电穿孔、磷酸钙沉淀、DEAE-葡聚糖转染等。尽管可以在原核或真核宿主细胞中表达本公开的抗体，但是最通常在真核细胞且通常在哺乳动物宿主细胞中表达抗体。

[0166] 本公开还提供了一种宿主细胞，其包含本公开提供的核酸分子。宿主细胞实际上可以是表达载体适用的任何细胞。所述细胞可以是，例如，高级真核宿主细胞，诸如哺乳动物细胞；低级真核宿主细胞，诸如酵母细胞；并且可以是原核细胞，诸如细菌细胞。将重组核酸构建体引入宿主细胞中可以通过磷酸钙转染、DEAE、葡聚糖介导的转染、电穿孔或噬菌体感染实现。

[0167] 适用于转化的真核宿主包括大肠杆菌(E.coli)、枯草芽孢杆菌(Bacillus subtilis)、鼠伤寒沙门氏菌(Salmonella typhimurium)以及假单胞菌属(Pseudomonas)、链霉菌属(Streptomyces)和葡萄球菌属(Staphylococcus)内的各菌种。

[0168] 用于表达本公开的结合分子的哺乳动物宿主细胞包括，例如，中国仓鼠卵巢(CHO)细胞(包括dhfr-CHO细胞，描述于Urlaub和Chasin,Proc.Natl.Acad.Sci.USA 77:4216-4220(1980)中，其与DHFR选择性标志物一起使用，例如，如Kaufman和Sharp，

J.Mol.Biol.159:601-621(1982)中所述)、NS0骨髓瘤细胞、COS细胞和Sp2细胞。具体地讲,对于与NS0骨髓瘤或CHO细胞一起使用,另一表达系统是WO 87/04462、WO 89/01036和EP 338,841中公开的GS(谷氨酰胺合成酶)基因表达系统。当将编码抗体基因的表达载体引入到哺乳动物宿主细胞中时,通过将宿主细胞培养一段足以允许抗体在宿主细胞中表达或使抗体分泌到宿主细胞所生长的培养基中的时间来产生抗体。抗体可以使用任何合适的蛋白质纯化方法从培养基中回收。

[0169] D.组合物

[0170] 在其他方面,本公开提供了一种组合物,其包含本公开提供的结合分子。在一个方面,所述组合物是包含结合分子和药学上可接受的载剂的药物组合物。所述组合物可以通过本领域已知的常规方法制备。

[0171] 在一些实施方案中,本公开提供了一种组合物,其包含本公开提供的抗体或其抗原结合片段,以及药学上可接受的载剂,其中所述抗体包含有包含本文公开的HVR氨基酸序列的可变结构域,并且其中所述组合物包含与所述组合物中存在的抗体或其抗原结合部分的总量相比不超过约11%、10%、8%、5%、3%或2%的在所述氨基酸序列的天冬酰胺处糖基化的所述抗体或抗原结合部分。在另一个实施方案中,所述组合物包含与所述组合物中存在的抗体或其抗原结合部分的总量相比至少约2%的在所述氨基酸序列的天冬酰胺处糖基化的所述抗体或抗原结合部分。

[0172] 术语“药学上可接受的载剂”是指适于在用于递送结合分子的制剂中使用的任何非活性物质。载剂可以是抗粘剂、粘结剂、包衣、崩解剂、填料或稀释剂、防腐剂(诸如抗氧化剂、抗细菌剂或抗真菌剂)、甜味剂、吸收延迟剂、湿润剂、乳化剂、缓冲剂等。合适的药学上可接受的载剂的实例包括水、乙醇、多元醇(诸如甘油、丙二醇、聚乙二醇等)右旋糖、植物油(诸如橄榄油)、盐水、缓冲液、缓冲盐水,以及等渗剂,诸如糖、聚醇、山梨醇和氯化钠。

[0173] 组合物可处于任何合适的形式,诸如液体、半固体和固体剂型。液体剂型的实例包括溶液(例如,可注射和可输注溶液)、微乳液、脂质体、分散体或混悬液。固体剂型的实例包括片剂、丸剂、胶囊、微胶囊和粉剂。适用于递送结合分子的组合物的特定形式是无菌液体,诸如溶液、悬浮液或分散液,以用于注射或输注。无菌溶液可以通过将所需量的抗体掺入适当的载剂中,然后进行灭菌微过滤来制备。一般来说,通过将抗体掺入无菌媒介物中来制备分散液,所述无菌媒介物包含基本分散介质和其他载剂。对于用于制备无菌液体的无菌粉末来说,制备方法包括真空干燥和冷冻干燥(冻干),以产生活性成分加上来自其先前经过无菌过滤的溶液的任何另外的期望成分的粉末。组合物的各种剂型可以通过本领域已知的常规技术制备。

[0174] 组合物中包含的结合分子的相对量将取决于多种因素而变化,诸如所用的具体结合分子和载剂、剂型以及期望的释放和药效动力学特征。单一剂型中结合分子的量将通常为产生治疗效果的量,但也可以是较少的量。一般来说,此量将在相对于剂型的总重量约0.01%至约99%、约0.1%至约70%,或约1%至约30%的范围内。

[0175] 除结合分子外,一种或多种另外的治疗剂可包含在组合物中。另外治疗剂的实例在下文描述。待包含在组合物中的另外治疗剂的合适量可以由本领域技术人员容易地选择,并且将取决于多种因素而变化,诸如所用的特定药剂和载剂、剂型,以及期望的释放和药效动力学特征。包括在单一剂型中的另外治疗剂的量将通常为产生治疗效果的药剂的

量,但也可以是较少的量。

[0176] E.结合分子和药物组合物的用途

[0177] 本公开提供的结合分子和药物组合物可用于治疗、诊断或其他目的,诸如调节免疫应答、治疗癌症、增强其他癌症疗法的功效、增强疫苗功效,或治疗自身免疫疾病。因此,在其他方面,本公开提供了使用结合分子或药物组合物的方法。在一个方面,本公开提供了一种治疗哺乳动物的病症的方法,其包括向需要治疗的哺乳动物施用治疗有效量的本公开提供的结合分子。结合分子可以是CD137激动剂或拮抗剂。在一些实施方案中,结合分子是CD137激动剂。在一些实施方案中,哺乳动物是人。

[0178] 在一些实施方案中,病症是癌症。CD137涉及其中的多种癌症,无论是恶性还是良性且无论是原发性还是继发性癌症,都可使用本公开提供的方法进行治疗或预防。此类癌症的实例包括肺癌诸如支气管癌(例如,鳞状细胞癌、小细胞癌、大细胞癌和腺癌)、肺泡细胞癌、支气管腺瘤、软骨瘤性错构瘤(非癌性)以及肉瘤(癌性);心脏癌症,诸如粘液瘤、纤维瘤和横纹肌瘤;骨癌,诸如骨软骨瘤、软骨瘤(condromas)、成软骨细胞瘤、软骨粘液样纤维瘤、骨样骨瘤、巨细胞肿瘤、软骨肉瘤、多发性骨髓瘤、骨肉瘤、纤维肉瘤、恶性纤维组织细胞瘤、尤文氏瘤(尤文氏肉瘤)和网状细胞肉瘤;脑癌,诸如神经胶质瘤(例如,多形性成胶质细胞瘤)、间变型星形细胞瘤、星形细胞瘤、少突神经胶质瘤、成神经管细胞瘤、脊索瘤、神经鞘瘤、室管膜瘤、脑膜瘤、垂体腺瘤、松果体瘤、骨瘤、成血管细胞瘤、颅咽管瘤、脊索瘤、生殖细胞瘤、畸胎瘤、皮样囊肿和血管瘤;消化系统中的癌症,诸如平滑肌瘤、表皮样癌、腺癌、平滑肌肉瘤、胃腺癌、肠道脂肪瘤、肠道神经纤维瘤、肠道纤维瘤、大肠中的息肉以及结肠直肠癌;肝癌,诸如肝细胞腺瘤、血管瘤、肝细胞癌、纤维板层癌、胆管癌、肝胚细胞瘤和血管肉瘤;肾癌,诸如肾腺癌、肾细胞癌、肾上腺样瘤和肾盂的移行细胞癌;膀胱癌;血癌,诸如急性淋巴细胞性(成淋巴细胞性)白血病、急性髓性(髓细胞性、骨髓性、成髓细胞性、髓单核细胞性)白血病、慢性淋巴细胞性白血病(例如,塞扎里综合征(Sezary syndrome)和毛细胞白血病)、慢性髓细胞性(髓样、骨髓性、粒细胞性)白血病、霍奇金氏淋巴瘤、非霍奇金氏淋巴瘤、B细胞淋巴瘤、蕈样肉芽肿病和骨髓增生病(包括诸如真性红细胞增多、骨髓纤维化、血小板增多症和慢性粒细胞性白血病的骨髓增生病);皮肤癌,诸如基底细胞癌、鳞状细胞癌、黑素瘤、卡波西肉瘤和佩吉特病;头颈癌;眼相关癌症,诸如视网膜母细胞瘤和眼内黑素癌;男性生殖系统癌症,诸如良性前列腺肥大、前列腺癌和睾丸癌(例如,精原细胞瘤、畸胎瘤、胚胎癌和绒毛膜癌);乳腺癌;女性生殖系统癌症,诸如子宫癌(子宫内膜癌)、宫颈癌(宫颈癌)、卵巢癌症(卵巢癌)、外阴癌、阴道癌、输卵管癌和水泡状胎块;甲状腺癌(包括乳突癌、滤泡癌、退行发育性癌或髓样癌);嗜铬细胞瘤(肾上腺);甲状旁腺的非癌性生长;胰腺癌;以及血癌,诸如白血病、骨髓瘤、非霍奇金氏淋巴瘤和霍奇金氏淋巴瘤。

[0179] 在一些其他实施方案中,病症是自身免疫疾病。可用结合分子治疗的自身免疫疾病的实例包括自身免疫性脑脊髓炎、红斑狼疮和类风湿性关节炎。结合分子还可用于治疗炎症(诸如过敏性哮喘)和慢性移植物抗宿主病,

[0180] 在另一方面,本公开提供了一种在哺乳动物中增强免疫应答的方法,其包括向哺乳动物施用治疗有效量的本公开提供的结合分子。在一些实施方案中,结合分子是CD137抗体或其抗原结合片段并且哺乳动物是人。在另一个实施方案中,结合分子是CD137激动剂抗体或其抗原结合片段。术语“增强免疫应答”或其语法变型是指刺激、激起、增加、提高或加

强哺乳动物的免疫系统的任何应答。免疫应答可以是细胞应答(即,细胞介导的,诸如细胞毒性T淋巴细胞介导的)或体液应答(即,抗体介导的应答),并且可以是初次免疫应答或二次免疫应答。免疫应答增强的实例包括CD4+辅助T细胞活性的增加和溶细胞性T细胞的产生。免疫应答的增强可以使用本领域技术人员已知的多种体外或体内测量进行评估,包括但不限于细胞毒性T淋巴细胞测定、细胞因子的释放(例如IL-2产生)、肿瘤的消退、荷瘤动物的存活、抗体产生、免疫细胞增殖、细胞表面标志物的表达,以及细胞毒性。通常,当与未治疗哺乳动物或未使用所要求保护的方法治疗的哺乳动物的免疫应答相比时,本公开的方法增强哺乳动物的免疫应答。在一个实施方案中,结合分子用于增强人对微生物病原体(诸如病毒)的免疫应答。在另一个实施方案中,结合分子用于增强人对疫苗的免疫应答。结合分子可以是CD137激动剂或拮抗剂。在一些实施方案中,结合分子是CD137激动剂。在一个实施方案中,所述方法增强细胞免疫应答,尤其是细胞毒性T细胞应答。在另一个实施方案中,细胞免疫应答是T辅助细胞应答。在另一个实施方案中,免疫应答是细胞因子产生,尤其是IL-2产生。结合分子可用于增强人对微生物病原体(诸如病毒)或对疫苗的免疫应答。结合分子可以是CD137激动剂或拮抗剂。在一些实施方案中,结合分子是CD137激动剂。

[0181] 在实践治疗方法时,结合分子可作为单一疗法单独施用,或与一种或多种另外的治疗剂或疗法组合施用。因此,在另一方面,本公开提供了一种组合疗法,其包括与一种或多种另外的疗法或治疗剂组合的结合分子,以用于分开、顺序或同时施用。术语“另外的疗法”是指不采用本公开提供的结合分子作为治疗剂的疗法。术语“另外的治疗剂”是指除本公开提供的结合分子之外的任何治疗剂。在一个特定方面,本公开提供了一种用于在哺乳动物中治疗癌症的组合疗法,其包括向哺乳动物施用治疗有效量的与一种或多种另外的治疗剂组合的本公开提供的结合分子。在另一个实施方案中,哺乳动物是人。

[0182] 广泛多种癌症治疗剂可与本公开提供的结合分子组合使用。本领域普通技术人员将认识到可与本公开的方法和结合分子组合使用的其他癌症疗法的存在和发展,并且将不局限于本文所述疗法的那些形式。可在用于治疗癌症的组合疗法中使用的另外的治疗剂的类别的实例包括(1)化学治疗剂,(2)免疫治疗剂,以及(3)激素治疗剂。

[0183] 术语“化学治疗剂”是指可以导致癌细胞死亡或干扰癌细胞的生长、分裂、修复和/或功能的化学或生物学物质。化学治疗剂的实例包括在WO 2006/129163和US 20060153808中公开的那些,所述专利的公开内容以引用方式并入本文。特定的化学治疗剂的实例包括:(1)烷化剂,诸如瘤可宁(chlorambucil)(LEUKERAN)、环磷酰胺(mcyclophosphamide)(CYTOXAN)、异环磷酰胺(IFEX)、盐酸氮芥(MUSTARGEN)、塞替派(THIOPLEX)、链脲霉素(ZANOSAR)、卡莫司汀(BICNU、GLIADELWAFER)、洛莫司汀(CEENU)和达卡巴嗪(DTIC-DOME);(2)生物碱或植物长春花生物碱,包括细胞毒性抗生素,诸如多柔比星(ADRIAMYCIN)、表柔比星(ELLENCE、PHARMORUBICIN)、柔红霉素(CERUBIDINE、DAUNOXOME)、奈莫柔比星、伊达比星(IDAMYCIN PFS、ZAVEDOS)、米托蒽醌(DHAD,NOVANTRONE)、更生霉素(放线菌素D、COSMEGEN)、普卡霉素(MITHRACIN)、丝裂霉素(MUTAMYCIN)和博来霉素(BLENOXANE)、酒石酸长春瑞滨(NAVELBINE))、长春碱(VELBAN)、长春新碱(ONCOVIN)和长春地辛(ELDISINE);(3)抗代谢药,诸如卡培他滨(XELODA)、阿糖胞苷(CYTOSAR-U)、氟达拉滨(FLUDARA)、吉西他滨(GEMZAR)、羟基脲(HYDRA)、甲氨蝶呤(FOLEX、MEXATE、TREXALL)、奈拉滨(ARRANON)、三甲曲沙(NEUTREXIN)和培美曲塞(ALIMTA);(4)嘧啶拮抗剂,诸如5-氟尿嘧啶(5-FU)、卡培他滨

(XELODA)、雷替曲塞(TOMUDEX)、替加氟-尿嘧啶(UFTORAL)和吉西他滨(GEMZAR)；(5)紫杉烷,诸如多西他赛(TAXOTERE)、紫杉醇(TAXOL)；(6)铂类药物,诸如顺铂(PLATINOL)和卡铂(PARAPLATIN)以及奥沙利铂(ELOXATIN)；(7)拓扑异构酶抑制剂,诸如伊立替康(CAMPTOSAR)、拓扑替康(HYCAMTIN)、依托泊苷(ETOPOPHOS、VEPESSID、TOPOSAR)和替尼泊苷(VUMON)；(8)表鬼臼毒素(鬼臼毒素衍生物),诸如依托泊苷(ETOPOPHOS、VEPESSID、TOPOSAR)；(9)叶酸衍生物,诸如亚叶酸(WELLCOVORIN)；(10)亚硝基脲,诸如卡莫司汀(BiCNU)、洛莫司汀(CeeNU)；(11)受体酪氨酸激酶,包括表皮生长因子受体(EGFR)、血管内皮生长因子(VEGF)、胰岛素受体、胰岛素样生长因子受体(IGFR)、肝细胞生长因子受体(HGFR)和血小板源性生长因子受体(PDGFR)的抑制剂,诸如吉非替尼(IRESSA)、厄洛替尼(TARCEVA)、硼替佐米(VELCADE)、甲磺酸伊马替尼(GLEEVEC)、吉非替尼(genefitinib)、拉帕替尼、索拉非尼、沙利度胺、舒尼替尼(SUTENT)、阿西替尼、利妥昔单抗(RITUXAN、MABTHERA)、曲妥珠单抗(HERCEPTIN)、西妥昔单抗(ERBITUX)、贝伐珠单抗(AVASTIN)和来尼珠单抗(ranibizumab,LUCENTIS)、lym-1(ONCOLYM)、WO2002/053596中公开的胰岛素样生长因子-1受体(IGF-1R)的抗体)；(12)血管生成抑制剂,诸如贝伐珠单抗(AVASTIN)、苏拉明(GERMANIN)、血管抑素、SU5416、沙利度胺和基质金属蛋白酶抑制剂(诸如巴马司他(batimastat)和马立马司他(marimastat)),和WO2002055106中公开的那些;以及(13)蛋白酶体抑制剂,诸如硼替佐米(VELCADE)。

[0184] 术语“免疫治疗剂”是指可增强哺乳动物的免疫应答的化学或生物学物质。免疫治疗剂的实例包括:卡介苗(BCG);细胞因子,诸如干扰素;疫苗,诸如MyVax个性化免疫疗法、Onyvax-P、Oncophage、GRNVAC1、Favld、Provenge、GVAX、Lovaxin C、BiovaxID、GMXX和NeuVax;以及抗体,诸如阿仑单抗(CAMPATH)、贝伐单抗(AVASTIN)、西妥昔单抗(ERBITUX)、吉妥珠单抗奥唑米星(gemtuzunab ozogamicin)(MYLOTARG)、替伊莫单抗(ibritumomab tiuxetan)(ZEVALIN)、帕尼单抗(VECTIBIX)、利妥昔单抗(RITUXAN、MABTHERA)、曲妥珠单抗(HERCEPTIN)、托西莫单抗(BEXXAR)、伊匹木单抗(YERVOY)、曲美利木单抗(tremelimumab)、CAT-3888、针对OX40受体的激动剂抗体(诸如WO2009/079335中公开的那些)、针对CD40受体的激动剂抗体(诸如WO2003/040170中公开的那些,以及TLR-9激动剂(诸如WO2003/015711、WO2004/016805和WO2009/022215中公开的那些)。

[0185] 术语“激素治疗剂”是指抑制或消除激素的产生,或抑制或抵消激素对癌细胞的生长和/或存活的作用的化学或生物学物质。适用于本文中的方法的此类药剂的实例包括US20070117809中公开的那些。特定的激素治疗剂的实例包括他莫昔芬(NOLVADEX)、托瑞米芬(Fareston)、氟维司群(FASLODEX)、阿那曲唑(ARIMIDEX)、依西美坦(AROMASIN)、来曲唑(FEMARA)、醋酸甲地孕酮(MEGACE)、戈舍瑞林(ZOLADEX)和亮丙立德(LUPRON)。本公开的结合分子还可与非药物激素疗法组合使用,所述非药物激素疗法诸如(1)除去全部或部分的参与激素产生的器官或腺体诸如卵巢、睾丸、肾上腺和垂体的手术方法,以及(2)放射治疗,其中使患者的器官或腺体经受量足以抑制或消除靶向激素的产生的放射。

[0186] 用于治疗癌症的组合疗法还涵盖结合分子与除去肿瘤的手术的组合。可在手术之前、期间或之后向哺乳动物施用结合分子。

[0187] 用于治疗癌症的组合疗法还涵盖结合分子与放射疗法诸如电离(电磁)放射疗法(例如,X射线或γ射线)和粒子束放射疗法(例如,高线性能放射(high linear energy

radiation))的组合。放射源对于哺乳动物可以是外部的或内部的。可在放射疗法之前、期间或之后向哺乳动物施用结合分子。

[0188] 可以通过任何合适的施用的肠途径或肠胃外途径施用本公开提供的结合分子和组合物。术语施用的“肠途径”是指经由胃肠道的任何部分的施用。肠途径的实例包括口腔、粘膜、颊面和直肠途径,或胃内途径。施用的“肠胃外途径”是指除了肠途径以外的施用途径。施用的肠胃外途径的实例包括静脉内、肌内、真皮内、腹膜内、瘤内、膀胱内、动脉内、鞘内、囊内、眶内、心脏内、经气管、关节内、被膜下、蛛网膜下、脊柱内、硬膜外和胸骨内、皮下或局部施用。可使用任何合适的方法施用本公开的抗体和组合物,诸如通过口服摄取、鼻胃管、胃造口管(gastrostomy tube)、注射、输注、可植入输注泵,以及渗透泵。合适的施用途径和方法可取决于多种因素而变化,诸如所用的具体抗体、期望的吸收速率、所用的具体制剂或剂型、所治疗病症的类型或严重程度、具体的作用部位,以及患者的状况,并且可以由本领域技术人员容易地选择。

[0189] 术语结合分子的“治疗有效量”是指对于预期治疗目的有效的量。例如,在增强免疫应答的上下文中,“治疗有效量”是在刺激、激起、增加、提高或加强哺乳动物免疫系统的任何应答中有效的任何量。在治疗疾病的上下文中,“治疗有效量”是足以在所治疗的哺乳动物中引起任何期望或有益的效应的任何量。具体地,在癌症的治疗中,期望或有益的效应的实例包括癌细胞的进一步生长或扩散的抑制、癌细胞的死亡、癌症复发的抑制、癌症相关疼痛的减少,或哺乳动物存活率的提高。CD137抗体的治疗有效量的范围通常为约0.001至约500mg/kg,且更通常地为约0.01至约100mg/kg的哺乳动物体重。例如,所述量可以是约0.3mg/kg、1mg/kg、3mg/kg、5mg/kg、10mg/kg、50mg/kg或100mg/kg的哺乳动物体重。在一些实施方案中,CD 137抗体的治疗有效量在约0.01-30mg/kg的哺乳动物体重的范围内。在一些其他实施方案中,CD137抗体的治疗有效量在约0.05-15mg/kg的哺乳动物体重的范围内。待施用的精确剂量水平可由本领域技术人员容易地确定,并且将取决于多种因素,诸如待治疗病症的类型和严重程度、所使用的特定结合分子、施用途径、施用时间、治疗的持续时间、所使用的特定的另外的疗法、所治疗的患者的年龄、性别、体重、状况、一般健康和先前的医疗史,以及医学领域中熟知的类似因素。

[0190] 通常在多个情况下施用结合分子或组合物。单一剂量之间的间隔可以是,例如,每周1次、每月1次、每3个月1次或每年1次。示例性治疗方案使施用为每周1次、每2周1次、每3周1次、每4周1次、每月1次、每3个月1次或每3至6个月1次。CD137抗体的典型剂量方案包括静脉内施用1mg/kg体重或3mg/kg体重,使用下列给药方案中的一个:(i)每4周1次,进行6次剂量,然后每3个月1次;(ii)每3周1次;(iii)3mg/kg体重1次,然后1mg/kg体重,每3周1次。

[0191] 通过参考以下实施例将更完全地理解本公开。然而,实施例不应解释为限制本公开的范围。应了解,本文所述的实施例和实施方案仅用于例示性目的,并且将建议本领域的技术人员根据其进行各种修改或变化,并且它们将包括在本申请的精神和权限以及所附权利要求书的范围内。本公开通篇引用的所有附图以及所有参考文献、专利和公布的专利申请的内容明确地以引用方式整体并入本文。

[0192] 实施例

[0193] 实施例1

[0194] 特异性结合人CD137的初级Fab的生成

[0195] 使用专有质粒文库(参见在代理人案卷号69540-2000140下与此同时提交的标题为“Dynamic Human Antibody Light Chain Libraries”的PCT国际申请,其以引用方式整体并入本文;另外参见在代理人案卷号69540-2000240下与此同时提交的标题为“Dynamic Human Heavy Chain Antibody Libraries”的PCT国际申请,其以引用方式整体并入本文)淘选(pan against)人CD137抗原。进行共计三轮或四轮淘选。在最后一轮淘选之后,进行单菌落上清液ELISA,以鉴定特异性识别人CD137的初级命中物。初级命中物定义为其ELISA信号为背景的至少两倍的那些。对它们进行测序,将独特克隆表达并通过ForteBio和Biacore针对亲和力测量进行纯化。将列表精细调整为Fab中具有ELISA阳性命中物和独特序列两者的124个。根据K_D应答信号R＞0.1,R^2＞0.9和亲和力K_D＜100nM的标准,将列表进一步精细调整为60个命中物(表1a)。接着将其中的24个转换成IgG(表1b),以用于详细的生物物理和功能表征。

[0196] 将与独特命中物相对应的Fab在大肠杆菌中表达并纯化。通过ForteBio Octet RED96系统测量它们对人CD137的亲和力。简而言之,使用AHC传感器(Anti-Human IgG Fc Capture Dip and Read Biosensors)捕获CD137-hisFc融合蛋白(Sino Biological目录号10041-H03H),并且浸插到含有用动力学缓冲液(kinetic buffer)(10mM HEPES、150mM NaCl、3mM EDTA、0.005％体积/体积表面活性剂P20,pH 7.4、)稀释至5-10μg/ml的纯化Fab的孔中。获取的ForteBio数据用Data Acquisition软件7.1处理,并且将动力学数据拟合于1:1朗格缪尔(Langmuir)结合模型。亲和力和动力学参数(已减除背景)在表1a中列出。它们对应的IgG对人CD137的亲和力通过Biacore测量并且在表1b中示出。

[0197] 表1a:所选Fab对人CD137的亲和力和对应的氨基酸序列(在SEQ ID NO.中)

[0198]

命中物 ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (上行 VH；下行 VL)
3760	1.26E-08	3.52E+05	4.44E-03	13
				14
4072	6.95E-09	1.54E+05	1.07E-03	15
				16
4074	1.95E-08	4.80E+04	9.37E-04	17
				18
4076	7.44E-09	6.38E+04	4.75E-04	19
				20
4079	3.15E-08	6.93E+04	2.19E-03	21
				22
4134	1.30E-08	5.93E+04	7.69E-04	23
				24
4137	<1.0E-12	8.56E+04	<1.0E-07	25
				26
4139	1.65E-09	4.96E+04	8.17E-05	27
				28
4140	<1.0E-12	2.57E+04	<1.0E-07	29
				30

[0199]

命中物 ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (上行 VH；下行 VL)
4217	9.67E-08	5.64E+05	5.45E-02	31
				32
5299	1.37E-08	5.55E+05	7.60E-03	33
				34
5300	1.53E-08	5.96E+05	9.10E-03	35
				36
5302	1.21E-09	3.54E+05	4.26E-04	37
				38
5303	5.12E-09	9.95E+05	5.09E-03	39
				40
5310	5.72E-09	8.13E+05	4.65E-03	41
				42
5314	8.39E-09	2.10E+05	1.77E-03	43
				44
5316	1.14E-08	140600	0.001605	45
				46
5318	1.90E-08	1.41E+05	2.69E-03	47
				48
5323	1.04E-08	7.82E+05	8.12E-03	49
				50
5341	2.93E-08	6.42E+04	1.88E-03	51
				52
5342	3.89E-08	1.57E+05	6.12E-03	53
				54
5346	1.61E-08	6.05E+05	9.77E-03	55
				56
5348	1.02E-08	1.31E+06	1.33E-02	57
				58
5349	6.20E-09	1.62E+05	1.01E-03	59
				60
5351	7.29E-09	4.66E+05	3.40E-03	61
				62
5353	1.61E-08	3.70E+05	5.97E-03	63
				64
5359	7.10E-10	4.64E+05	3.30E-04	65
				66
5360	2.41E-08	1.20E+05	2.89E-03	67
				68

[0200]

命中物 ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (上行 VH；下行 VL)
5363	9.87E-09	8.37E+04	8.26E-04	69
				70
5365	2.56E-09	7.01E+05	1.79E-03	71
				72
5367	1.49E-08	4.07E+05	6.08E-03	73
				74
5370	1.91E-09	5.24E+05	1.00E-03	75
				76
5371	3.97E-09	1.21E+06	4.79E-03	77
				78
5404	3.30E-09	3.95E+05	1.30E-03	79
				80
5407	1.76E-09	2.48E+05	4.37E-04	81
				82
5408	2.36E-08	3.18E+05	7.50E-03	83
				84
5409	1.70E-08	2.51E+05	4.27E-03	85
				86
5413	9.93E-10	5.55E+05	5.51E-04	87
				88
5417	4.04E-08	5.72E+04	2.31E-03	89
				90
7077	1.88E-08	4.98E+05	9.34E-03	91
				92
7078	2.52E-08	3.45E+05	8.70E-03	93
				94
7079	2.99E-08	1.00E+05	3.00E-03	95
				96
7080	2.44E-08	3.06E+05	7.46E-03	97
				98
7081	4.31E-08	2.87E+05	1.23E-02	99
				100
7087	6.96E-08	1.23E+05	8.55E-03	101
				102
7088	4.36E-08	2.55E+05	1.11E-02	103
				104
7090	5.55E-08	3.12E+05	1.73E-02	105
				106

[0201]

命中物 ID	KD (nM)	kon(1/Ms)	koff(1/s)	SEQ ID NO. (上行 VH；下行 VL)
7092	4.57E-08	4.31E+05	1.97E-02	107
				108
7097	2.43E-08	5.42E+05	1.32E-02	109
				110
7100	3.50E-08	4.62E+05	1.62E-02	111
				112
7105	3.33E-08	3.30E+05	1.10E-02	113
				114
7109	3.20E-08	1.73E+05	5.55E-03	115
				116
7120	3.45E-08	2.64E+05	9.11E-03	117
				118
7128	3.97E-08	3.09E+05	1.23E-02	119
				120
7131	3.04E-08	2.66E+05	8.10E-03	121
				122
7133	4.03E-08	1.01E+05	4.05E-03	123
				124
7135	3.17E-08	1.02E+05	3.22E-03	125
				126
7159	3.79E-08	1.06E+05	4.03E-03	127
				128
7163	1.26E-08	2.99E+05	3.78E-03	129
				130
7166	1.24E-08	3.45E+05	4.29E-03	131
				132

[0202] 编码氨基酸序列SEQ ID NO:13-132的对应DNA序列分别可见于SEQ ID NO:133-252。表1a所示的所有Fab命中物的HVR_H1氨基酸序列分别可见于SEQ ID NO:253-312。表1a所示的所有Fab命中物的HVR_H2氨基酸序列分别可见于SEQ ID NO:313-372。表1a所示的所有Fab命中物的HVR_H3氨基酸序列分别可见于SEQ ID NO:373-432。表1a所示的所有Fab命中物的HVR_L1氨基酸序列分别可见于SEQ ID NO:433-492。表1a所示的所有Fab命中物的HVR_L2氨基酸序列分别可见于SEQ ID NO:493-552。表1a所示的所有Fab命中物的HVR_L3氨基酸序列分别可见于SEQ ID NO:553-612(另外参见表1c)。

[0203] 表1b:Fab和对应IgG对人CD137的亲和力

[0204]

命中物 ID	Fab			IgG ID	IgG			IgG SEQ ID NO.(上行重链;下行轻链)
	KD (M)	ka(1/Ms)	kd(1/s)		KD (M)	Ka(1/Ms)	kd (1/s)	
4072	7.0E-09	1.5E+05	1.1E-03	AG10054	1.3E-08	1.4E+05	1.9E-03	613 614
5303	5.1E-09	1.0 E+06	5.1E-03	AG10057	7.9E-09	7.7E+05	6.1E-03	615 616
5310	5.7E-09	8.1E+05	4.7E-03	AG10058	5.9E-09	4.1E+05	2.4E-03	617 618
5351	7.3E-09	4.7E+05	3.4E-03	AG10059	3.8E-08	1.6E+05	6.3E-03	619 620
5359	7.1E-10	4.6E+05	3.3E-04	AG10060	1.1E-09	2.2E+05	2.5E-04	621 622
5370	1.9E-09	5.2E+05	1.0 E-03	AG10061	3.6E-09	2.2E+05	7.8E-04	623 624
5404	3.3E-09	4.0E+05	1.3E-03	AG10062	5.9E-09	1.6E+05	9.4E-04	625 626
5413	9.9E-10	5.6 E+05	5.5E-04	AG10063	9.9E-10	3.9E+05	3.9E-04	627 628
4074	2.0E-08	4.8E+04	9.4E-04	AG10079	1.4E-09	1.9E+05	2.7E-04	629 630
4217	9.7E-08	5.6 E+05	5.5E-02	AG10080	1.0 E-08	1.2E+06	1.2E-02	631 632
5299	1.4E-08	5.6 E+05	7.6E-03	AG10081	6.9E-09	2.4E+05	1.7E-03	633 634
5300	1.5E-08	6.0E+05	9.1E-03	AG10082	1.3E-08	5.6 E+05	7.2E-03	635 636
5323	1.0 E-08	7.8E+05	8.1E-03	AG10083	1.2E-08	5.7E+05	6.9E-03	637 638
5360	2.4E-08	1.2E+05	2.9E-03	AG10084	4.3E-08	6.6E+04	2.8E-03	639 640
5367	1.5E-08	4.1E+05	6.1E-03	AG10085	5.4E-08	1.5E+05	7.9E-03	641 642
5409	1.7E-08	2.5E+05	4.3E-03	AG10086	4.6E-08	1.0 E+05	4.5E-03	643 644
5302	1.2E-09	3.5E+05	4.3E-04	AG10124	6.0E-09	5.0 E+05	3.0E-03	645 646
5314	8.4E-09	2.1E+05	1.8E-03	AG10125	1.5E-08	1.1E+05	1.7E-03	647 648

[0205]

5316	1.1E-08	1.4E+05	1.6E-03	AG10126	1.4E-08	5.4E+09	7.3E+01	649 650
5318	1.9E-08	1.4E+05	2.7E-03	AG10127	9.6E-09	3.0E+05	2.9E-03	651 652
5342	3.9E-08	1.6E+05	6.1E-03	AG10128	3.0E-09	1.2E+05	3.7E-04	653 654
5353	1.6E-08	3.7E+05	6.0E-03	AG10129	1.9E-08	3.1E+05	6.0E-03	655 656
5365	2.6E-09	7.0E+05	1.8E-03	AG10131	3.7E-09	5.1E+05	1.9E-03	657 658
5408	2.4E-08	3.2E+05	7.5E-03	AG10132	6.9E-08	2.0E+05	1.4E-02	659 660

[0206] 编码氨基酸序列SEQ ID NO:613-660的对应DNA序列分别可见于SEQ ID NO:661-708。表1b所示的所有IgG序列的HVR_H1氨基酸序列分别可见于SEQ ID NO:709-732。表1b所示的所有IgG序列的HVR_H2氨基酸序列分别可见于SEQ ID NO:733-756。表1b所示的所有IgG序列的HVR_H3氨基酸序列分别可见于SEQ ID NO:757-780。表1b所示的所有IgG序列的HVR_L1氨基酸序列分别可见于SEQ ID NO:781-804。表1b所示的所有IgG序列的HVR_L2氨基酸序列分别可见于SEQ ID NO:805-828。表1b所示的所有IgG序列的HVR_L3氨基酸序列分别可见于SEQ ID NO:829-852。

[0207] 表1c:Fab的CDR序列

[0208]

命中物 ID	VH/VL	HVR-H1 SEQ ID NO.	HVR-H2 SEQ ID NO.	HVR-H3 SEQ ID NO.	HVR-L1 SEQ ID NO.	HVR-L2 SEQ ID NO.	HVR-L31 SEQ ID NO.
3760	VH1/VL1	253	313	373	433	493	553
4072	VH2/VL2	254	314	374	434	494	554
7074	VH3/VL3	255	315	375	435	495	555
4076	VH4/VL4	256	316	376	436	496	556
4079	VH5/VL5	257	317	377	437	497	557
4134	VH6/VL6	258	318	378	438	498	558
4137	VH7/VL7	259	319	379	439	499	559
4139	VH8/VL8	260	320	380	440	500	560
4140	VH9/VL9	261	321	381	441	501	561
4217	VH10/VL10	262	322	382	442	502	562
5299	VH11/VL11	263	323	383	443	503	563
5300	VH12/VL12	264	324	384	444	504	564

[0209]

5302	VH13/VL13	265	325	385	445	505	565
5303	VH14/VL14	266	326	386	446	506	566
5310	VH15/VL15	267	327	387	447	507	567
5314	VH16/VL16	268	328	388	448	508	568
5316	VH17/VL17	269	329	389	449	509	569
5318	VH18/VL18	270	330	390	450	510	570
5323	VH19/VL19	271	331	391	451	511	571
5341	VH20/VL20	272	332	392	452	512	572
5342	VH21/VL21	273	333	393	453	513	573
5346	VH22/VL22	274	334	394	454	514	574
5348	VH23/VL23	275	335	395	455	515	575
5349	VH24/VL24	276	336	396	456	516	576
5351	VH25/VL25	277	337	397	457	517	577
5353	VH26/VL26	278	338	398	458	518	578
5359	VH27/VL27	279	339	399	459	519	579
5360	VH28/VL28	280	340	400	460	520	580
5363	VH29/VL29	281	341	401	461	521	581
5365	VH30/VL30	282	342	402	462	522	582
5367	VH31/VL31	283	343	403	463	523	583
5370	VH32/VL32	284	344	404	464	524	584
5371	VH33/VL33	285	345	405	465	525	585
5404	VH34/VL34	286	346	406	466	526	586
5407	VH35/VL35	287	347	407	467	527	587
5408	VH36/VL36	288	348	408	468	528	588
5409	VH37/VL37	289	349	409	469	529	589
5413	VH38/VL38	290	350	410	470	530	590
5417	VH39/VL39	291	351	411	471	531	591
7077	VH40/VL40	292	352	412	472	532	592
7078	VH41/VL41	293	353	413	473	533	593
7079	VH42/VL42	294	354	414	474	534	594
7080	VH43/VL43	295	355	415	475	535	595
7081	VH44/VL44	296	356	416	476	536	596
7087	VH45/VL45	297	357	417	477	537	597
7088	VH46/VL46	298	358	418	478	538	598
7090	VH47/VL47	299	359	419	479	539	599
7092	VH48/VL48	300	360	420	480	540	600
7097	VH49/VL49	301	361	421	481	541	601
7100	VH50/VL50	302	362	422	482	542	602
7105	VH51/VL51	303	363	423	483	543	603
7109	VH52/VL52	304	364	424	484	544	604
7120	VH53/VL53	305	365	425	485	545	605
7128	VH54/VL54	306	366	426	486	546	606
7131	VH55/VL55	307	367	427	487	547	607
7133	VH56/VL56	308	368	428	488	548	608
7135	VH57/VL57	309	369	429	489	549	609
7159	VH58/VL58	310	370	430	490	550	610
7163	VH59/VL59	311	371	431	491	551	611
7166	VH60/VL60	312	372	432	492	552	612

[0210] 实施例2

[0211] 与小鼠CD137交叉反应的Fab命中物的选择

[0212] Fab命中物的物种交叉反应性使用ELISA确定。简而言之,将200μL的5μg/mL抗人IgG(Fab特异性)(Sigma#I5260)涂布在Maxisorp微板(Thermo Scientific 446469)上,在4℃下过夜。封闭之后,添加100μL Fab 5310(5μg/mL)、5351(2.8μg/mL)和5365(5μg/mL)并孵育1hr。洗涤三次之后,添加与人FC片段融合的人或小鼠CD137抗原的系列稀释液并孵育1hr。洗涤之后,将HRP标记的山羊抗人FC用PBS 1:2000稀释,并添加到每个孔中,孵育1hr。将板洗涤三次并且与TMB底物一起在室温下孵育20min。在反应停止后,测量450nm下的吸光度。结果呈现于图1b中,下部小图示出Fab 5310和5365结合人和小鼠CD137两者,而Fab 5351结合人CD137,但不结合小鼠CD137。

[0213] 实施例3

[0214] IgG转换和表达:AG10058、AG10059和AG10131

[0215] 将Fab 5310、5351和5365的重链和轻链单独克隆到具有S241P突变的IgG4同种型中的哺乳动物表达载体pCDNA3.3(Thermo Fisher Scientific)中。另外分别将两个参考抗体的重链和轻链克隆到IgG4和IgG2同种型中的pCDNA3.3中。

[0216] 参考抗体AC1097中使用的重链可变区包含序列EVQLVQSGAEVKKPGESLRISCKGSGYSFSTYWISWVRQMPGKGLEWMGKIYPGDSYTNYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGYGIFDYWGQGTLVTVSS(SEQ ID NO:862),并且参考抗体AC1097中的轻链可变区包含序列SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSPVLVIYQDKNRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCATYTGFGSLAVFGGGTKLTVL(SEQ ID NO:863)。参考抗体AC1121中使用的重链可变区包含序列QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPEKGLEWIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDYGPGNYDWYFDLWGRGTLVTVSS(SEQ ID NO:864),并且参考抗体AC1121中的轻链可变区包含序列EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPALTFGGGTKVEIK(SEQ ID NO:865)。本文使用的IgG在表2中示出。

[0217] 表2:IgG的列表

[0218]

IgG	Fab	同种型	描述
AC1097	参考1	IgG2	参考Ab
AC1121	参考2	IgG4(S241P)	参考Ab
AG10058	5310	IgG4(S241P)	Adagene mAb
AG10059	5351	IgG4(S241P)	Adagene mAb
AG10131	5365	IgG4(S241P)	Adagene mAb
AG10154		IgG4(S241P)	同种型对照

[0219] 按照制造商的说明书,将质粒对瞬时转染到HEK293F细胞中。收获上清液,通过离心和过滤使其澄清,并且利用标准蛋白A亲和色谱(MabSelect SuRe,GE Healthcare)纯化IgG。将蛋白质洗脱并中和,并且缓冲液交换到PB缓冲液(20mM磷酸钠、150mM NaCl,pH 7.0)中。通过紫外分光光度法(UV-spectrophotometry)确定蛋白质浓度,并且通过SDS-PAGE或SEC-HPLC,在变性、还原和非还原条件下分析IgG纯度。

[0220] 实施例4

[0221] 对人、猴和小鼠CD137的结合亲和力

[0222] 通过BIAcore、ELISA和流式细胞术测量IgG对人、猴和小鼠CD137的结合亲和力。结

果汇总在表3中。

[0223] 表3:抗体对人、猴和小鼠CD137的结合亲和力

[0224]

KD (nM)	Biacore			ELISA			HEK293F 细胞表面		
	人	食蟹猴 (Cyno)	小鼠	人	食蟹猴	小鼠	人	食蟹猴	小鼠
AG10131	3.7	12.5	64.5	0.2	0.3	23.9	1.3	1.2	49.4
AG10058	5.9	9.3	15.2	0.2	0.3	0.3	1.8	2	10.1
AG10059	24.2	23.1	NC	0.8	0.4	NC	5	2.6	NC
AC1097	20.9	37.6	NC	0.2	0.4	NC	1.9	2.9	NC
AC1121	9.6	NC	NC	0.2		NC	3.3	NC	NC

[0225] NC:不交叉反应

[0226] 4a.通过SPR测量结合亲和力和动力学

[0227] 使用Biacore™ T200仪器(Biacore AB,Uppsala,Sweden),根据制造商的指南,通过表面等离子体共振(SPR)分析检查抗体对人、猴和小鼠CD137蛋白的结合亲和力和动力学。根据胺偶联试剂盒(GE Biacore#BR-1000-50)的说明书,通过将来自人抗体捕获试剂盒(Human Antibody Capture Kit)(GE BR-1008-39)的抗人IgG(Fc)抗体的氨基偶联到传感器芯片的羧基化表面上而将其固定在CM5芯片上。使用固定的抗人IgG(Fc)抗体捕获AG10058、AG10059、AG10131、AC1121和AC1097。最后,将六种浓度(3.13、6.25、12.5、25、50、100)(nM)(在运行缓冲液中稀释)的人CD137-His6(Sino Biological#10041-H08H)以30μl/min的流速注射300秒,并且解离时间为300秒。所用的运行缓冲液为1×HBS-EP(10mM HEPES、150mM NaCl、3mM EDTA、0.005%体积/体积表面活性剂P20,pH 7.4,25℃)。使用不具有固定蛋白质的空白流动池在各情况下进行对应的对照,以用于"背景"减除。使用Biacore T200评估软件(Biacore AB,Uppsala,Sweden),根据制造商的指南将缔合和解离曲线拟合于1:1朗格缪尔结合模型。如表3所示,所有抗体均与人CD137结合。AG10058和AG10059显示出比两种参考抗体更高的亲和力。除了AC1121参考mAb之外,所有抗体均结合猴CD137。只有AG10058和AG10131结合小鼠和大鼠CD137。AG10058具有比AG10131(64.5nM)更高的亲和力(15.2nM)。

[0228] 4b.使用ELISA测定测量对可溶性CD137的结合亲和力

[0229] 制备与人FC片段融合的人、猴或小鼠CD137的系列稀释液并用于涂布ELISA板,在37℃下1hr。封闭之后,添加100μLIgG(5μg/mL)并在37℃下孵育1hr。将板洗涤三次,然后与HRP缀合的蛋白质L(1:2000稀释液)一起在37℃下孵育1hr。再次将板洗涤三次并且与TMB底物一起在室温下孵育20min。在反应停止后,测量450nm下的吸光度。通过Graphpad Prism 6利用非线性拟合分析数据。如图2所示,所有抗体均以类似的sub nM亲和力结合人CD137(FC融合蛋白)。除了AC1121参考mAb之外,所有抗体均以类似的sub nM亲和力结合猴CD137。与来自Biacore的结果一致,只有AG10058和AG10131结合小鼠CD137。AG10058具有比AG10131(23.9nM)更高的亲和力(0.3nM)。

[0230] 4c.通过流式细胞术测量对在细胞表面过表达的CD137的结合亲和力

[0231] 还评估了抗体对在HEK293F细胞的表面上瞬时表达的人、猴和小鼠CD137的亲和力。简而言之,用来自双顺反子IRES载体的表达全长人、猴或小鼠CD137的质粒转染HEK293F细胞,使用EGFP鉴定转染细胞。48hr后,收获转染细胞并且接着用冷的FACS缓冲液(补充有1%BSA的PBS)洗涤一次。然后将细胞与各种IgG(各自为100nM)一起在冰上孵育1hr,用预先冷却的FACS缓冲液洗涤两次,并且与Alexa Fluor® 647缀合的小鼠抗人FC抗体一起在冰上孵育30min。将细胞洗涤一次,然后通过流式细胞术(Beckman® CytoFlex)进行分析。如图3a所示,所有抗体均以低nM亲和力与在细胞表面上表达的人CD137结合。AG10058、AG10059和AG10131略优于两种参考抗体。除了AC1121参考mAb之外,所有抗体均以低nM亲和力结合猴CD137,AG10058、AG10059和AG10131略优于AC1097参考抗体。与来自Biacore和ELISA的结果一致,只有AG10058和AG10131结合小鼠和大鼠CD137。AG10058对小鼠CD137的亲和力高于AG10131。另外,AG10058和AG10131(各自为100nM)还结合在HEK293F细胞表面上过表达的大鼠和犬CD137(图3b)。

[0232] 4d.IgG与活化的人、猴、小鼠和大鼠T细胞的结合。

[0233] 使用人、猴、小鼠和大鼠的PMA和离子霉素刺激的PBMC或T细胞进一步确认示例性抗体的物种交叉反应性。通过Ficoll-密度梯度离心分离人和食蟹猴PBMC。简而言之,将来自健康供体或食蟹猴的新鲜全血用等体积的PBS稀释并且仔细地加载到Histopaque 1077(14ml,在50ml离心管中)的顶部。利用无闸减速(brake off),在室温下以1,200×g离心30分钟。离心之后,用移液管将上层仔细抽吸至0.5cm的含有单核细胞的不透明界面之内。弃去上层。用移液管将不透明界面(约3-5ml)仔细转移到干净的50ml锥形离心管中。用20ml的PBS洗涤细胞,通过在400×g下离心5分钟收集细胞,并且将细胞重悬于20ml的PBS中。用血球计对细胞计数,并且通过在400×g下离心5分钟再次收集细胞。通过以下方式分离小鼠或大鼠脾细胞:使脾穿过附接至50-mL锥形管的45μm细胞筛网以得到单细胞悬浮液,并且用PBS将细胞洗涤穿过筛网。以1600rpm离心5min并弃去上清液。将细胞沉淀物重悬于2ml红血细胞裂解溶液中,持续2min。过量添加10倍体积的PBS并且以1600rpm离心5min来收集细胞。弃去上清液并且将脾细胞重悬于RPMI 1640/10%FBS中。分别利用对人、猴、小鼠和大鼠具有特异性的商业试剂盒(Stemcell Technologies)中的磁珠,通过阴性选择从PBMC(人/猴)或脾细胞(小鼠/大鼠)中富集泛T细胞(Pan-T cell)。人/猴PBMC,或小鼠/大鼠脾细胞的活化通过将细胞与50ng/ml PMA+1μM离子霉素一起在37℃、5%CO2下孵育过夜来进行。

[0234] 将活化细胞(~$2x10^5$个细胞/管)在预先冷却的染色缓冲液(补充有2%FBS的PBS)中洗涤并且与100nM测试抗体一起在冰上孵育1hr。然后将细胞用1mL染色缓冲液洗涤两次并且重悬于包含Alexa Fluor® 647缀合的小鼠抗人FC抗体和物种特异性T细胞标志物抗体的100μL染色缓冲液中。T细胞标志物抗体使用如下:CD3、CD4或CD8。在黑暗中孵育30分钟后,用染色缓冲液洗涤细胞两次。最后,将细胞重悬于300μL染色缓冲液中并通过Beckman CytoFlex进行分析。使用Flowjo 10软件进行数据分析。如图4a所示,所有测试抗体均结合活化的人和猴T细胞,但不结合原初人T细胞。进一步评估AG10131对活化的小鼠和大鼠T细胞的结合亲和力(图4b)。AG10131结合活化的小鼠和大鼠T细胞。

[0235] 概括地说,AG10058和AG10131抗体对人和猴CD137显示出更高的亲和力。它们表现出广泛的物种交叉反应性,对于AG10131而言包括人、食蟹猴、小鼠、大鼠和狗,但是对于

AG10058而言包括人、食蟹猴、小鼠和狗,从而允许快速评估在小鼠同系模型中的体内功效。

[0236] 实施例5

[0237] 抗体对CD137的结合选择性

[0238] 抗体对CD137的选择性使用其对TNFR超家族成员的结合亲和力的流式细胞术分析进行评估。将包括CD 137、OX40、CD40、GITR和CD27的TNFRSF受体在HEK293F细胞的表面上瞬时过表达。将转染细胞在预先冷却的染色缓冲液(补充有2%FBS的PBS)中洗涤并且与100nM测试抗体一起在冰上孵育1hr。用染色缓冲液洗涤细胞两次,添加Alexa Fluor® 647缀合的小鼠抗人FC抗体并在冰上孵育30min。将样品用染色缓冲液洗涤一次,然后通过流式细胞术进行分析。如图5所示,AG10058、AG10059和AG10131与CD137特异性结合,但是不特异性结合任何其他所测试的家族成员或用空载体转染的亲本细胞。

[0239] 实施例6

[0240] 使用ELISA和流式细胞术测试配体竞争

[0241] 通过ELISA和流式细胞术测定,针对抗体阻断CD137与其同源配体CD137L结合的能力对所述抗体进行测试。如图6a和6b所示,所有测试抗体均阻断CD137和CD137L的结合。

[0242] 6a.通过ELISA测试配体竞争结合

[0243] 将重组人CD137(与人Fc和His标签融合)在PBS中稀释至1μg/mL并涂布在Maxisorp板上,在4℃下过夜。将板用补充有3%脱脂奶的PBS在37℃下封闭1hr。洗涤之后,向每个孔中添加50uL生物素化的CD137L(4μg/mL)和各种浓度的测试抗体(在500μg/mL至2μg/mL范围内的八份1:2系列稀释液)的总体积100uL混合物并在37℃下孵育1hr。将板洗涤三次并且向每个孔中添加100μLHRP缀合的中性亲和素(neutravidin)(1:1000)并在37℃下孵育1hr。如先前所述将板洗涤,添加50μLTMB底物溶液并在室温下孵育20分钟,然后通过50μLH_2SO_4停止反应。如图6a所示,所有测试抗体AG10058、AG10059和AG10131均阻断CD137与CD137L的结合。AG10131表现出最强或完全阻断能力,在大约uM范围,接着是>uM的显著阻断的AG10058;以及处于uM范围的有效阻断的AG10059。这些数据表明,在所测试的条件下且利用所用的试剂,广泛物种交叉反应性抗体AG10131和AG10058是CD137与其配体CD137L之间的相互作用的高度有效抑制剂,而AG10059仅显示出对CD137与其配体CD137L之间的相互作用的中等有效阻断。应注意,与人和猴CD137交叉反应的参考抗体AC1097,而仅与人CD137反应的AC1121几乎根本不显示出阻断。

[0244] 6b.通过流式细胞术测试配体竞争结合

[0245] 将编码全长人CD137的质粒在HEK293F细胞中瞬时表达。将细胞用染色缓冲液(补充有1%BSA的PBS)洗涤并重悬于含有100nM测试抗体的染色缓冲液中。在冰上孵育30min后,向每个孔中添加33nM生物素化的CD137L并且再在冰上孵育1hr。将细胞用染色缓冲液洗涤两次,添加50μL含有Alexa fluor 647缀合的链霉亲和素的染色缓冲液并在冰上孵育30min。将细胞洗涤一次并通过CytoFlex流式细胞术进行分析。如图6b所示,所有三种测试抗体均可以浓度依赖性方式阻断CD137与CD137L之间的结合。AG10131显示出最强阻断能力,接着是具有显著阻断的AG10058,以及具有不太有效阻断的AG10059。这些数据表明,广泛物种交叉反应性抗体AG10131和AG10058高度有效地阻断CD137与其配体CD137L之间的相互作用,而AG10059显示出对CD137与其配体CD137L之间的相互作用的部分阻断。相比之下,与人和猴CD137交叉反应的AC1097参考抗体仅显示出部分阻断,而仅与人CD137反应的

AC1121参考抗体不显示出阻断。

[0246] 实施例7

[0247] 表位作图

[0248] 为了在氨基酸残基水平上确定测试抗体的结合区域,在人CD137的细胞外结构域上进行一系列突变(表5)。使用这些CD137突变质粒转染HEK293F细胞。抗体与人CD137突变体的结合通过流式细胞术分析进行评估,如先前在实施例5中描述且在图7A中示出。结果汇总于表5中,连同这些抗体与处于感兴趣的差异(differentiation)的人、猴、小鼠和大鼠CD137的交叉反应性,指示了来自衍生自Adagene文库的命中物的精细表位。AG10131与全部4个物种结合,而AG10058结合全部3种CD137,但不结合大鼠CD137。AG10058、AG10059和AG10131丧失对GFT34AAA、FSS53AAA和FH92AA突变的结合能力,表明其结合表位在这些区域内,例如SEQ ID NO.:1的氨基酸残基34-93或34-108(另外参见图7B)。AG10058和AG10131可结合相同或高度相似的表位,而AG10059可结合与AG10058和AG10131不同的表位。

[0249] 突变构建体意在将AG10058、AG10059和AG10131所结合的表位与参考抗体AC1121和AC1097区分开。清楚的是,所有三种抗体AG10058、AG10059和AG10131靶向与AC1121和AC1097非常不同的表位。AG10058、AG10059和AG10131在由突变体Hu_FH92AA和Hu_FSS53AAA以及可能的Hu_GTF34AAA限定的区域中不同于AC1121,而AG10058、AG10059和AG10131在由大多数所用突变体限定的区域中不同于AC1097,Hu_FH92AA以及它们与猴的物种交叉反应性除外,但是在其他物种交叉反应性诸如小鼠、大鼠和狗CD137中不同。在一些实施方案中,AG10058、AG10059和AG10131或本文公开的其他抗体不结合位于SEQ ID NO.:1的氨基酸残基115-156内的表位。图7A和表5中还示出,人CD137配体与野生型人CD137相对突变型人CD137的结合与测试抗体的结合模式良好匹配,这与这些抗体阻断CD137配体与其受体的结合的观察是一致的。

[0250] 表5:表位作图

[0251]

突变	AG10058	AG10059	AG10131	AC1121	AC1097	**HuCD137L**
Hu_WT	+	+	+	+	+	+
Cyno_WT	+	+	+	-	+	
小鼠_WT	+	-	+	-	-	
大鼠_WT	-	-	+	-	-	
Hu_GTF34AAA	-	-	-	-/+	+	-
Hu_FSS53AAA	-	-	-	+	+	-
Hu_FH92AA	-	-	-	+	-	-
Hu_GQ109AA	+	+	+	+	-	+
Hu_EL111AA	+	+	+	+	-	+
Hu_F125A	+	+	+	+	-	+
Hu_FN125AA	+	+	+	+	-	+

Hu_PW135AA	+	+	+	+	-	+
Hu_TN137AA	+	+	+	+	-	+
Hu_GT150AA	+	+	+	+	-	+

[0252] 实施例8

[0253] NFκB荧光素酶报告基因测定中抗体的激动剂活性

[0254] 使用NFκB报告基因测定评估抗体的激动剂活性。将293T细胞用表达人、猴或小鼠CD137的质粒以及NFκB光素酶报告基因质粒转染。4h后,以0.4x 10^6/mL的密度将50μL细胞涂板到96孔板的每个测定孔中。添加含有测试抗体和3:1比率的交联抗体(Fab'山羊抗人IgG FC)的总体积50μL抗体混合物并孵育18h。除去培养基后,添加50μL Passive裂解缓冲液(Promega E1980)并且在37℃下孵育30min。将20μL裂解物转移到白板中并添加荧光素酶底物。测量萤火虫和肾素(Renina)的发光信号并且它们的比率用于通过GraphPad Prism 6.0软件进行数据分析。如图8所示,相比于同种型对照抗体,当人和猴CD137表达时,所有测试抗体均激活NFκB报告基因表达。当小鼠CD137表达时,AG10058和AG10131,但不是AG10059,激活NFκB报告基因表达。这与AG10058和AG10131结合小鼠CD137,而AG10059则不如此的之前观察一致。

[0255] 实施例9

[0256] T细胞活化测定中抗体的激动剂活性

[0257] 在T细胞活化测定中进一步确认抗体的激动剂活性。用单独的或连同50μL在1×PBS中的测试抗体(60μg/mL、20μg/mL、6μg/mL、2μg/mL和0μg/mL)一起的50μL抗CD3抗体(2μg/ml)涂布96孔细胞培养板,在4℃下过夜。使用根据制造商的说明书的方案分离CD8+T细胞。以1×10^7个细胞/mL的密度在补充有10%FBS的RPMI 1640培养基中制备细胞。将200μL细胞涂板到每个测定孔中并在37℃、5%CO2培养箱中孵育4天。每天在显微镜下检查细胞的增殖。孵育96hr后,将100μL上清液转移到新的96孔板中以进行IFN-γ检测。T细胞增殖使用Cell Titer Glow试剂盒(Promega)测定。如图9所示,相比于同种型对照抗体,所有测试抗体均以剂量依赖性方式诱导CD8+T细胞增殖和IFN-γ分泌。

[0258] 实施例10

[0259] 在小鼠同系模型中的抗肿瘤活性

[0260] 与小鼠CD137的物种交叉反应性允许快速体内功能评估。AG10058和AG10131已经在多个小鼠同系模型中进行了测试。对BALB/c小鼠(n=8/组)皮下移植2×10^6个H22肝癌细胞(Xiao等人,Soluble PD-1 facilitates 4-1BBL-triggered antitumor immunity against murine H22 hepatocarcinoma in vivo.Clin Cancer Res.2007;13(6):1823-30.)、5×10^5个CT26结肠癌细胞,或5×10^5个EMT6乳腺癌细胞。当肿瘤建立(>50mm^3)时,开始每周两次通过腹膜内注射用同种型对照抗体、AG10058或AG10131进行治疗,持续最多至3周。每周两次监测肿瘤生长并报告为随时间推移的平均肿瘤体积±s.e.m.。如图10-13所示,相比于同种型对照抗体,AG10058和AG10131在这些不同的同系小鼠肿瘤模型中表现出有效的体内抗肿瘤活性。

[0261] 10a.CD137激动剂抗体在H22小鼠肝癌模型中表现出抗肿瘤功效

[0262] 首先,以50mg/kg的剂量持续3周每周两次施用AG10058或AG10131。两种分子均显示出几乎100%TGI(肿瘤生长抑制)(图10,小图a)。CD4和CD8标志物的免疫组织化学染色显

示,AG10131显著增加CD4+和CD8+T细胞在H22肿瘤微环境中的浸润(Xiao等人,Soluble PD-1 facilitates 4-1BBL-triggered antitumor immunity against murine H22 hepatocarcinoma in vivo.Clin Cancer Res.2007;13(6):1823-30.)(图10,小图b)。降低至3mg/kg的进一步剂量滴定仍然显示出~100%TGI,表明两种分子均具有有效的抗肿瘤活性(图10,小图c和d)。降低至1和0.1mg/kg的AG10131的进一步剂量滴定在0.1mg/kg和1mg/kg下显示出大于50%TGI(图10,小图e)。

[0263] 10b.CD137激动剂抗体在CT26小鼠j结肠癌模型中表现出抗肿瘤功效

[0264] 如图10所示,AG10058和AG10131在CT26小鼠结肠癌模型中在50mg/kg的剂量下显示出几乎100%TGI(肿瘤生长抑制)(图11,小图a)(Martinez-Forero等人,T cell costimulation with anti-CD137monoclonal antibodies is mediated by K63-polyubiquitin-dependent signals from endosomes.J Immunol.2013;190(12):6694-706)。AG10131的进一步剂量滴定(图11,小图b)在5mg/kg和1mg/kg的剂量下显示出几乎100%TGI。在0.1mg/kg剂量下,实现大约40%TGI,表明了剂量依赖性抗肿瘤活性。

[0265] 10c.EMT6乳腺癌模型

[0266] 在EMT6小鼠乳腺癌同系模型中进一步评估抗肿瘤活性(Shi和Siemann,Augmented antitumor effects of radiation therapy by 4-1BB antibody(BMS-469492) treatment.Anticancer Res.2006;26:3445-53)(图12)。AG10058和AG10131均表现出几乎~100%肿瘤生长抑制。

[0267] 10d.对CD137激动剂抗体治疗具有完全应答的小鼠在用新肿瘤细胞再激发后保持无肿瘤

[0268] 在CT26肿瘤模型中用AG10058或AG10131治疗3周后,具有完全肿瘤消退的小鼠保持一个多月不进行治疗。然后在第62天对保持完全应答的小鼠在相对侧腹部用5×10^5个CT26肿瘤细胞进行皮下再激发,并监测肿瘤生长。同时利用接种了相同数量的CT26肿瘤细胞的原初小鼠建立再激发对照组。如图13所示,用AG10131治疗(在1和5mg/kg下,参见图13,分别为顶部小图和底部小图)在CT26肿瘤模型中表现出有效的抗肿瘤活性,AG10131(1mg/kg组)中的5/8、AG10131(5mg/kg组)中的6/8在再次用CT26肿瘤细胞再激发之前在60天中显示出完全应答。此外,这些小鼠在用相同的肿瘤细胞再激发之后保持无肿瘤,表明在这些小鼠中发展了特定的抗肿瘤记忆。

[0269] 为了证明这一假设,从这些肿瘤排斥性再激发小鼠和对照小鼠中收集脾细胞并且将其与丝裂霉素C阻滞的(arrested)CT26肿瘤细胞一起在体外共培养7天以扩增肿瘤特异性记忆T细胞。然后回收这些脾细胞并且与荧光标记的活CT26肿瘤细胞以不同的E/T比率混合4h,并且通过活/死染色和FACS分析检测肿瘤细胞杀伤。如图14所示,利用来自之前用AG10058和AG10131治疗的肿瘤排斥性再激发小鼠的脾细胞观察到显著增加的肿瘤细胞杀伤。

[0270] 实施例11

[0271] AG10131-IgG4不引起ADCC效应

[0272] 利用EasySep人$CD8^+$T细胞富集试剂盒(StemCell Technologies)从来自健康供体的外周血中分离人$CD8^+$T细胞,接着用PMA(50ng/ml)+离子霉素(1uM)体外刺激18小时。然后用钙黄绿素-AM标记这些活化的$CD8^+$T细胞并用作靶细胞。利用人NK分离试剂盒(StemCell

Technologies)分离来自不同健康供体的NK细胞,并用作效应细胞。对于抗体依赖性细胞毒性(ADCC)测定,在不存在和存在系列稀释抗体的情况下,将效应细胞(NK)和靶细胞(活化的$CD8^+$T细胞)以5:1比率在96孔板中混合,在培养条件下持续4小时。然后收集来自每个孔的上清液,并且通过读板器SpectraMax i3x(Ex 488nm,Em 520nm)检测荧光信号。使用同种型hIgG4 mAb作为阴性对照,而人源化的OKT3(来自Novoprotein的抗CD3 hIgG1)用作阳性对照。接着使用下式计算%裂解:%裂解=[(实验释放)-Ave(靶标+NK)]/[Ave(靶标Max)-Ave(仅靶标)]×100%(图15)。

[0273] 实施例12

[0274] 抗体的开发性特征

[0275] 对于开发性评估,将纯化的AG10058、AG10059、AG10131和AC1097交换到PB缓冲液(20mM PB、150mM NaCl,pH 7.0)中。所有实验,包括过滤、离心、加速应激测试,均在PB缓冲液中进行。对于所有SEC-HPLC分析,使用TSKgel柱(Tosoh Bioscience G3000SWxl)。

[0276] 12a.溶解度

[0277] 所有三种抗体可以在没有明显沉淀的情况下在PB缓冲液中浓缩至高于100mg/ml(表6)。然后将抗体在PB缓冲液中调节至20mg/ml。然后通过SEC-HPLC对样品(各自10μg)进行测定,以检测高分子量(HMW)聚集物。如色谱图(图16)中所示,对于所有测试抗体,在高浓度(20mg/ml)下均未观察到HMW聚集物的增加。

[0278] 表6:抗体的溶解度

[0279]

样品	浓度(mg/ml)	聚集度(HMW %)
AG10058	108	1.0
AG10059	134	1.4
AG10131	110	2.0

[0280] 12b.在加速应激条件下的抗体溶解度

[0281] 还在加速应激条件下检查抗体溶解度,结果汇总于表7中。所有抗体在六个冷冻(-80℃)和融化(室温)循环之后仍保持稳定(图17)。在50℃下七天后,HMW聚集物或LMW片段的变化很小(图17)。在更长期时间过程的实验中(40℃持续最多至28天),所有抗体保持稳定,并且HMW聚集物或LMW片段没有显著增加(图17)。

[0282] 表7:HMW在加速条件下的变化

[0283]

	AG10058	AG10059	AG10131
冷冻-融化 6X	4.6%	1.2%	0.4%
50℃, 7 d	0.7%	1.2%	0%
40℃, 28 d	0.9%	0%	0%

[0284] 此外,通过差示扫描量热法(DSC)测得的热稳定性显示,AG10131和AG10058在高达至少约59℃时是稳定的。转变中点Tm(几乎所有蛋白质结构域发生去折叠转变时的特征温度)在图18和以下表8中示出。

[0285] 表8:通过DSC测得的热稳定性

[0286]

	Tm开始点(℃)	Tm1(℃)	Tm2(℃)
AG10058	61.5	67.3	76.9
AG10131	59.3	67.6	81.5

[0287] 此外,AG10131和AG10058在离心之后的最高可实现浓度分别超过180mg/mL和220mg/mL。

[0288] 实施例13

[0289] 在相关物种中的安全性特征:小鼠和食蟹猴

[0290] 13a.AG10131在正常C57BL/6小鼠中的重复给药毒性研究。

[0291] AG10131的重复给药毒性在正常C57BL/6小鼠中进行。在第1天、第4天、第8天和第11天腹膜内(i.p.)(10mL/kg)施用媒介物、AG10131(100mg/kg)。每组中包括五只雌性小鼠(7-8周龄)。每天监测小鼠的异常行为和症状,并且每天测量食物摄取和体重。在第14天,对动物实施安乐死以进行尸检和其他分析。从每只动物中收集血液,其中每组2份血液样品用于血液学分析(RBC、血小板、WBC、WBC分类)并且该组中的另外3份血液样品用于血液生物化学分析(AL、AST、ALB、GLB、A/G、TBIL、ALP、GGT和LDH)。收集来自每只小鼠的以下器官并保存在FFPE中:心脏、肺、胸腺、肝、脾和肾。制备肝组织的FFPE块、切片并H&E染色以用于组织病理学分析。

[0292] 在整个研究的生命阶段(in-life period)期间,未观察到异常行为或计划外的动物死亡。与媒介物治疗相比,AG10131不影响食物摄取和体重。在使用AG10131的治疗组的小鼠中,尸检也未显示出任何明显的病变。血液学分析未显示出任何显著变化,在用AG10131治疗的小鼠中测试的血液生物化学参数同样如此(图19)。在所有这些小鼠的肝的组织病理学切片中均未发现明显异常(图20)。总体而言,AG10131在此研究中耐受良好并且在小鼠中未观察到显著毒性。

[0293] 13b.AG10131在食蟹猴中的重复给药研究

[0294] 在正常的食蟹猴中进行AG10131的重复给药研究。在第0天、第7天、第14天和第22天,静脉内(i.v.)(1mL/kg)施用人IgG4同种型对照(10mg/kg)、AG10131(0.5和10mg/kg)。每组中包括一只雄性和一只雌性食蟹猴(3~5岁)。每天监测动物的异常行为和临床体征,并且每天测量食物摄取。在给药前第(-15)天、第(-5)天和给药后第6天、第13天、第18天和第26天测量体重。在给药前第(-12)天、第(-5)天和给药后第7天、第14天、第19天和第27天(仅10mg/kg组)测量血液学和血液化学参数,在给药前第(-12)天、第(-5)天和给药后第6天、第13天、第18天进行尿液分析。在第27天对10mg/kg组中的动物实施安乐死,以进行尸检和其他分析。切除主要器官并称重。制备FFPE肝组织块、切片并H&E染色以进行组织病理学分析。

[0295] 在整个研究的生命阶段(in-life period)期间,在所有组中均未观察到异常行为或计划外的动物死亡。与媒介物治疗相比,10mg/kg的AG10131治疗不影响食物摄取和体重。未注意到临床体征,也包括注射部位反应。在用10mg/kg的AG10131治疗的食蟹猴中检查的所有器官中,尸检未显示出任何明显病变和重量异常。在所有治疗组中,血液学、血液化学和尿液参数也在正常范围内(图21)。在以10mg/kg重复给药AG10131后,肝的组织病理学分析未显示出任何明显异常,包括淋巴细胞浸润(图22)。总体而言,AG10131在最高至10mg/kg周剂量下在食蟹猴中耐受良好并且未检测到明显毒性。

[0296] 实施例14

[0297] AG10131在食蟹猴中的药代动力学

[0298] 14a.AG10131在食蟹猴中的药代动力学

[0299] 在原初食蟹猴中进行AG10131的药代动力学研究。将三种剂量水平的AG10131(10mg/kg、30mg/kg和100mg/kg)静脉内推注施用给三组猴。每组合有3只雄性和3只雌性。在给药前、给药后0.083、0.25、0.5、1、2、6、12、24、36、48、72、96、120、144、168、240、336、408、504、672和840小时收集血清样品。通过ELISA确定AG10131的血清浓度。

[0300] 在16只动物中的12只中,AG10131在第14天(336hr)快速清除,即来自低剂量和中等剂量组的所有动物,以及来自高剂量组的6只动物中的2只。在第21天,又多2只来自高剂量组的动物显示出快速清除。这14只动物中的血清浓度低或低于量化的极限。这与在这些动物中产生抗药抗体的观察是一致的。将来自高剂量组的具有可能不受影响的药代动力学的两只动物的数据拟合,以预测药代动力学参数(图23)。AG10131的半衰期在7.3至8.8天的范围内。

[0301] 14b.AG10131在大鼠中的药代动力学

[0302] 在原初SD大鼠中进行AG10131的药代动力学研究。将三种剂量水平的AG10131(10mg/kg、30mg/kg和100mg/kg)静脉内推注施用给三组动物。每组合有15只雄性和15只雌性。在各时间点从3只动物中收集血清样品:给药前、给药后0.083、0.25、0.5、1、2、6、12、24、36、48、72、96、120、144、168、240、336、408、504、672和840小时。通过ELISA确定AG10131的血清浓度并且通过Phoenix Professional V6.3分析数据。

[0303] 结果:来自低剂量、中等剂量和高剂量的PK参数相似(图24)。AG10131的清除速率为约0.004ml/kg/min。AG10131的半衰期在11.5至14.6天的范围内。

[0304] 14c.AG10131在小鼠中的药代动力学

[0305] 在约8周龄的BALB/c小鼠中进行AG10131的药代动力学研究。通过尾静脉以1mg/kg向每个给药组的3只雌性BALB/c小鼠静脉内注射包括AG10131的测试抗体。在给药后1h、8、48、168和336小时收集血液样品(每份样品~100u1)。从没有施用抗体的3只原初雌性小鼠中收集空白对照血液。包括AG10131的每种测试抗体的血清浓度通过ELISA确定,其中抗人IgG(Fc特异性)抗体用于捕获并且HRP标记的抗人IgG(Fab特异性)抗体用于检测。

[0306] 所有测试抗体,包括同种型对照(AG10154)、两种基准抗体(AC1020和AC1021)以及三种Adagene抗体(AG10131、AG10058和AG10059)在小鼠中表现出相当的药代动力学(图25)。

[0001] 序列表

[0002] <110> 天演药业(Adagene Inc.)

[0003] <120> 抗CD137分子及其用途

[0004] <130> 695402000340

[0005] <140> 尚未分配

[0006] <141> 与此同时

[0007] <160> 865

[0008] <170> PatentIn 3.5版

[0009] <210> 1

[0010] <211> 255

[0011] <212> PRT

[0012] <213> 智人(Homo sapiens)

[0013] <400> 1

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[0016] Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro

[0017] 20 25 30

[0018] Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys

[0019] 35 40 45

[0020] Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile

[0021] 50 55 60

[0022] Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser

[0023] 65 70 75 80

[0024] Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly

[0025] 85 90 95

[0026] Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu

[0027] 100 105 110

[0028] Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln

[0029] 115 120 125

[0030] Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys

[0031] 130 135 140

[0032] Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro

[0033] 145 150 155 160

[0034] Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala

[0035] 165 170 175

[0036] Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu

[0037] 180 185 190

[0038] Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu

[0039] 195 200 205

[0040] Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe

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[0070] 1 5 10
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[0358] 1 5 10
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[0371] 35 40 45
[0372] Ser Gly Ile Ser Gly Ala Gly Asp Thr Thr Tyr Tyr Ala Asp Ser Val
[0373] 50 55 60
[0374] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[0376] Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
[0377] 85 90 95

[0378] Ala Arg Glu Arg Asp Tyr Asp Phe Asp Tyr Trp Gly Gln Gly Thr Leu
[0379] 100 105 110
[0380] Val Thr Val Ser Ser
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[0392] 20 25 30
[0393] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[0394] 35 40 45
[0395] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[0396] 50 55 60
[0397] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[0399] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Ser His
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[0401] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[0415] 35 40 45
[0416] Trp Ile Gly Ile Ile Asn Pro Asn Phe Gly Asp Thr Asn Tyr Ala Gln
[0417] 50 55 60
[0418] Lys Phe Gln Gly Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
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[0422] Tyr Cys Ala Arg Asp Glu Tyr Tyr Gly Gly Ser Tyr Tyr Phe Asp Tyr
[0423] 100 105 110
[0424] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[0425] 115 120
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[0438] 35 40 45
[0439] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[0440] 50 55 60
[0441] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[0443] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Asp Trp Pro Pro
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[0445] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[0457] 20 25 30
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[0459] 35 40 45
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[0462] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
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[0466] Tyr Cys Ala Arg Asp Asp Leu Tyr Ser Trp Tyr Phe Asp Val Trp Gly
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[0468] Gln Gly Thr Leu Val Thr Val Ser Ser
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[0482] 35 40 45
[0483] Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[0484] 50 55 60
[0485] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[0486] 65 70 75 80
[0487] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Tyr Thr Trp Thr
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[0504] Val Ser Ala Ile Ser Gly Ala Gly Gly Ser Thr Tyr Tyr Ala Asp Ser
[0505] 50 55 60
[0506] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
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[0508] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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[0510] Cys Ala Arg Asp Gly Tyr Gly Gly Ser Tyr Phe Asp Tyr Trp Gly Gln
[0511] 100 105 110
[0512] Gly Thr Leu Val Thr Val Ser Ser
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[0527] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
[0528] 50 55 60
[0529] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[0531] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Tyr Ser Ile Trp Thr
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[0533] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[0552] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
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[0554] Tyr Cys Ala Arg Leu Gly Gly His Trp Tyr Phe Asp Val Trp Gly Gln
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[0570] 35 40 45
[0571] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[0572] 50 55 60
[0573] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[0575] Glu Asp Phe Ala Thr Tyr Tyr Cys Glu Gln Pro Leu Glu Leu Pro Arg
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[0577] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[0578] 100 105
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[0592] Val Ser Gly Ile Ser Gly Tyr Gly Asp Thr Thr Tyr Tyr Ala Asp Ser
[0593] 50 55 60
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[0596] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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[0598] Cys Ala Arg Asp Pro Tyr Ser Ser Gly Ser Gly Tyr Phe Asp Tyr Trp
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[0600] Gly Gln Gly Thr Leu Val Thr Val Ser Ser
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[0614] 35 40 45
[0615] Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[0616] 50 55 60
[0617] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[0619] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Tyr Thr Trp Thr
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[0621] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[0634] Tyr Tyr Trp Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
[0635] 35 40 45
[0636] Val Ser Gly Ile Ser Gly Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
[0637] 50 55 60
[0638] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[0639] 65 70 75 80
[0640] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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[0642] Cys Ala Arg Gly Thr Tyr Ser Phe Asp Val Trp Gly Gln Gly Thr Leu
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[0659] Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[0660] 50 55 60
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[0677] 20 25 30

[0678] Ala Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

[0679] 35 40 45

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[0682] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

[0683] 65 70 75 80

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[0685] 85 90 95

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[0703] Ile Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser

[0704] 50 55 60

[0705] Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln

[0706] 65 70 75 80

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[0721] 20 25 30
[0722] Tyr His Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
[0723] 35 40 45
[0724] Val Ser Ser Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser
[0725] 50 55 60
[0726] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[0727] 65 70 75 80
[0728] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[0729] 85 90 95
[0730] Cys Ala Arg Asp Pro Asn Tyr Tyr Ser Ser Gly Ser Tyr Phe Asp Tyr
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[0732] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[0733] 115 120
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[0744] 20 25 30
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[0746] 35 40 45
[0747] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly
[0748] 50 55 60
[0749] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[0750] 65 70 75 80
[0751] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Leu Trp Thr
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[0753] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[0765] 20 25 30
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[0767] 35 40 45
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[0769] 50 55 60
[0770] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
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[0772] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[0773] 85 90 95
[0774] Cys Ala Arg Glu Tyr Tyr Gly Tyr Gly Tyr Ala Leu Asp Tyr Trp Gly
[0775] 100 105 110
[0776] Gln Gly Thr Leu Val Thr Val Ser Ser
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[0790] 35 40 45
[0791] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
[0792] 50 55 60
[0793] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[0794] 65 70 75 80
[0795] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[0798] 100 105

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[0810] His Tyr Trp Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp

[0811] 35 40 45

[0812] Leu Ala Leu Ile Asp Trp Tyr Gly Asp Lys Tyr Tyr Ser Thr Ser Leu

[0813] 50 55 60

[0814] Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

[0815] 65 70 75 80

[0816] Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

[0817] 85 90 95

[0818] Ala Arg Ser Asp Tyr Tyr Gly Ser His Phe Asp Tyr Trp Gly Gln Gly

[0819] 100 105 110

[0820] Thr Leu Val Thr Val Ser Ser

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[0831] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Asp Phe Asp

[0832] 20 25 30

[0833] Gly Phe Ser Phe Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro

[0834] 35 40 45

[0835] Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser

[0836] 50 55 60

[0837] Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser

[0838] 65 70 75 80

[0839] Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp

[0840] 85 90 95

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[0853] 20 25 30

[0854] Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

[0855] 35 40 45

[0856] Ser Tyr Ile Ser Gly Asp Gly Asp Thr Thr Tyr Tyr Ala Asp Ser Val

[0857] 50 55 60

[0858] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

[0859] 65 70 75 80

[0860] Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

[0861] 85 90 95

[0862] Ala Arg Glu Gly Ser Thr Thr Val Ala Gly Asp Trp Phe Ala Tyr Trp

[0863] 100 105 110

[0864] Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[0865] 115 120

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[0874] 1 5 10 15

[0875] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Ser Phe

[0876] 20 25 30

[0877] Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[0878] 35 40 45

[0879] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly

[0880] 50 55 60

[0881] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[0882] 65 70 75 80
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[0884] 85 90 95
[0885] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[0886] 100 105
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[0895] 1 5 10 15
[0896] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[0897] 20 25 30
[0898] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[0899] 35 40 45
[0900] Trp Val Ser Ser Ile Ser Gly Tyr Gly Ser Thr Thr Tyr Tyr Ala Asp
[0901] 50 55 60
[0902] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[0903] 65 70 75 80
[0904] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[0905] 85 90 95
[0906] Tyr Cys Ala Arg Glu Gly Ser Asp Ala Val Leu Gly Asp Trp Phe Gly
[0907] 100 105 110
[0908] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[0909] 115 120
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[0918] 1 5 10 15
[0919] Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Thr Thr Tyr
[0920] 20 25 30
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[0924] 50 55 60

[0925] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[0926] 65 70 75 80

[0927] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Tyr Leu Trp Thr

[0928] 85 90 95

[0929] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[0930] 100 105

[0931] <210> 39

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[0936] <223> 合成序列

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[0939] 1 5 10 15

[0940] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser

[0941] 20 25 30

[0942] Gly Val Ala Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[0943] 35 40 45

[0944] Trp Leu Ala Leu Ile Asp Trp Ala Asp Asp Lys Tyr Tyr Ser Pro Ser

[0945] 50 55 60

[0946] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

[0947] 65 70 75 80

[0948] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr

[0949] 85 90 95

[0950] Cys Ala Arg Glu Gly Ser Asp Ala Val Leu Gly Asp Trp Phe Ala Tyr

[0951] 100 105 110

[0952] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[0953] 115 120

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[0956] <212> PRT

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[0962] 1 5 10 15

[0963] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Thr Phe

[0964] 20 25 30

[0965] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[0966] 35 40 45

[0967] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly

[0968] 50 55 60

[0969] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[0970] 65 70 75 80

[0971] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Tyr Ser Ile Trp Thr

[0972] 85 90 95

[0973] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[0974] 100 105

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[0979] <220>

[0980] <223> 合成序列

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[0983] 1 5 10 15

[0984] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser

[0985] 20 25 30

[0986] Gly Val Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[0987] 35 40 45

[0988] Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Tyr Tyr Ser Pro Ser

[0989] 50 55 60

[0990] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

[0991] 65 70 75 80

[0992] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr

[0993] 85 90 95

[0994] Cys Ala Arg Gly Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala Tyr

[0995] 100 105 110

[0996] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[0997] 115 120

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[1002] <220>

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[1006] 1 5 10 15

[1007] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Ser Pro Tyr

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[1009] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[1010] 35 40 45
[1011] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
[1012] 50 55 60
[1013] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1014] 65 70 75 80
[1015] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Leu Trp Thr
[1016] 85 90 95
[1017] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1018] 100 105
[1019] <210> 43
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[1027] 1 5 10 15
[1028] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[1029] 20 25 30
[1030] Gly Val Ala Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[1031] 35 40 45
[1032] Trp Leu Ala Leu Ile Asp Trp Ala Gly Asp Lys Ser Tyr Ser Thr Ser
[1033] 50 55 60
[1034] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[1035] 65 70 75 80
[1036] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[1037] 85 90 95
[1038] Cys Ala Arg Gly Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala Tyr
[1039] 100 105 110
[1040] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1041] 115 120
[1042] <210> 44
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[1050] 1 5 10 15
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[1053] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[1054] 35 40 45
[1055] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly
[1056] 50 55 60
[1057] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1058] 65 70 75 80
[1059] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Thr Trp Thr
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[1061] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1062] 100 105
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[1072] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Ile Ser Ser Gly
[1073] 20 25 30
[1074] Tyr His Trp Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
[1075] 35 40 45
[1076] Val Ser Ser Ile Ser Gly Asp Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
[1077] 50 55 60
[1078] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[1079] 65 70 75 80
[1080] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[1081] 85 90 95
[1082] Cys Ala Arg Glu Gly Ser Asp Ala Val Thr Gly Asp Trp Phe Ala Tyr
[1083] 100 105 110
[1084] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1085] 115 120
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[1094] 1 5 10 15

[1095] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Val Gly Thr Tyr

[1096] 20 25 30

[1097] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[1098] 35 40 45

[1099] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly

[1100] 50 55 60

[1101] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[1102] 65 70 75 80

[1103] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Leu Trp Thr

[1104] 85 90 95

[1105] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[1106] 100 105

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[1112] <223> 合成序列

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[1116] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser

[1117] 20 25 30

[1118] Gly Val Ala Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[1119] 35 40 45

[1120] Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Tyr Tyr Ser Thr Ser

[1121] 50 55 60

[1122] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

[1123] 65 70 75 80

[1124] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr

[1125] 85 90 95

[1126] Cys Ala Arg Glu Gly Ser Thr Ala Val Ala Gly Asp Trp Phe Ala Tyr

[1127] 100 105 110

[1128] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[1129] 115 120

[1130] <210> 48

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[1132] <212> PRT

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[1138] 1 5 10 15
[1139] Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Arg Thr Phe
[1140] 20 25 30
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[1142] 35 40 45
[1143] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly
[1144] 50 55 60
[1145] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1146] 65 70 75 80
[1147] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Thr Trp Thr
[1148] 85 90 95
[1149] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1150] 100 105
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[1161] 20 25 30
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[1163] 35 40 45
[1164] Trp Ile Gly Glu Ile Tyr His Ser Gly Ser Thr Tyr Tyr Ser Pro Ser
[1165] 50 55 60
[1166] Leu Lys Ser Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[1167] 65 70 75 80
[1168] Tyr Leu Gln Leu Asn Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr
[1169] 85 90 95
[1170] Cys Ala Arg Glu Gly Ser Asp Val Val Thr Gly Asp Trp Phe Ala Tyr
[1171] 100 105 110
[1172] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1173] 115 120
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[1179] <223> 合成序列
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[1182] 1 5 10 15
[1183] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Val
[1184] 20 25 30
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[1337] 20 25 30
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[1339] 35 40 45
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[1341] 50 55 60
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[1343] 65 70 75 80

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[1383] 35 40 45
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[1386] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[1387] 65 70 75 80
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[1408] 50 55 60
[1409] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[1452] 50 55 60
[1453] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1454] 65 70 75 80
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[1496] 50 55 60
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[1515] 35 40 45
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[1517] 50 55 60
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[1605] 50 55 60
[1606] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
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[1647] 35 40 45
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[1649] 50 55 60
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[1672] 50 55 60
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[1698] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
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[1711] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Arg Ser Tyr
[1712] 20 25 30
[1713] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[1714] 35 40 45
[1715] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly
[1716] 50 55 60
[1717] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1718] 65 70 75 80
[1719] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Ile Trp Thr
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[1721] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[1722] 100 105

[1723] <210> 75

[1724] <211> 123

[1725] <212> PRT

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[1732] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser

[1733] 20 25 30

[1734] Gly Val Ala Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[1735] 35 40 45

[1736] Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Arg Tyr Ser Thr Ser

[1737] 50 55 60

[1738] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

[1739] 65 70 75 80

[1740] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr

[1741] 85 90 95

[1742] Cys Ala Arg Glu Gly Ser Thr Thr Val Val Gly Asp Trp Phe Asp Tyr

[1743] 100 105 110

[1744] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[1745] 115 120

[1746] <210> 76

[1747] <211> 107

[1748] <212> PRT

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[1750] <220>

[1751] <223> 合成序列

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[1754] 1 5 10 15

[1755] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Thr Tyr

[1756] 20 25 30

[1757] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[1758] 35 40 45

[1759] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly

[1760] 50 55 60

[1761] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[1762] 65 70 75 80

[1763] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Leu Trp Thr

[1764] 85 90 95
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[1766] 100 105
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[1775] 1 5 10 15
[1776] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser
[1777] 20 25 30
[1778] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[1779] 35 40 45
[1780] Trp Val Ser Tyr Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr Ala Asp
[1781] 50 55 60
[1782] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[1783] 65 70 75 80
[1784] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[1785] 85 90 95
[1786] Tyr Cys Ala Arg Glu Gly Ser Asp Val Val Ala Gly Asp Trp Phe Ala
[1787] 100 105 110
[1788] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1789] 115 120
[1790] <210> 78
[1791] <211> 107
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[1798] 1 5 10 15
[1799] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Ser Val
[1800] 20 25 30
[1801] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[1802] 35 40 45
[1803] Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
[1804] 50 55 60
[1805] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[1806] 65 70 75 80
[1807] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Gln Ile Trp Thr
[1808] 85 90 95
[1809] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1810] 100 105
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[1812] <211> 119
[1813] <212> PRT
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[1819] 1 5 10 15
[1820] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Ile Ser Ser Gly
[1821] 20 25 30
[1822] Tyr His Trp Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
[1823] 35 40 45
[1824] Ile Gly Glu Ile Tyr His Ser Gly Ser Thr Tyr Tyr Ser Pro Ser Leu
[1825] 50 55 60
[1826] Lys Ser Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[1827] 65 70 75 80
[1828] Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
[1829] 85 90 95
[1830] Ala Arg Ser Pro Tyr Tyr Tyr Gly Val Phe Asp Tyr Trp Gly Gln Gly
[1831] 100 105 110
[1832] Thr Leu Val Thr Val Ser Ser
[1833] 115
[1834] <210> 80
[1835] <211> 107
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[1842] 1 5 10 15
[1843] Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Ser Val
[1844] 20 25 30
[1845] Tyr Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
[1846] 35 40 45
[1847] Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser

[1848] 50 55 60
[1849] Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
[1850] 65 70 75 80
[1851] Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Thr His Asp Pro Val Thr
[1852] 85 90 95
[1853] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1854] 100 105
[1855] <210> 81
[1856] <211> 124
[1857] <212> PRT
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[1860] <223> 合成序列
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[1862] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Ala
[1863] 1 5 10 15
[1864] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[1865] 20 25 30
[1866] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[1867] 35 40 45
[1868] Trp Val Ser Ser Ile Ser Gly Tyr Gly Ser Thr Thr Tyr Tyr Ala Asp
[1869] 50 55 60
[1870] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[1871] 65 70 75 80
[1872] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[1873] 85 90 95
[1874] Tyr Cys Ala Arg Glu Gly Ser Asp Ala Val Leu Gly Asp Trp Phe Gly
[1875] 100 105 110
[1876] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1877] 115 120
[1878] <210> 82
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[1880] <212> PRT
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[1882] <220>
[1883] <223> 合成序列
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[1885] Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
[1886] 1 5 10 15
[1887] Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Thr Thr Tyr
[1888] 20 25 30
[1889] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[1890] 35 40 45

[1891] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly

[1892] 50 55 60

[1893] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[1894] 65 70 75 80

[1895] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Tyr Leu Trp Thr

[1896] 85 90 95

[1897] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[1898] 100 105

[1899] <210> 83

[1900] <211> 123

[1901] <212> PRT

[1902] <213> 人工生物体(Artificial organism)

[1903] <220>

[1904] <223> 合成序列

[1905] <400> 83

[1906] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[1907] 1 5 10 15

[1908] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Ile Thr Ser Gly

[1909] 20 25 30

[1910] Tyr His Trp Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp

[1911] 35 40 45

[1912] Val Ser Ser Ile Ser Gly Tyr Gly Asp Thr Thr Tyr Tyr Ala Asp Ser

[1913] 50 55 60

[1914] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

[1915] 65 70 75 80

[1916] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr

[1917] 85 90 95

[1918] Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala Tyr

[1919] 100 105 110

[1920] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[1921] 115 120

[1922] <210> 84

[1923] <211> 107

[1924] <212> PRT

[1925] <213> 人工生物体(Artificial organism)

[1926] <220>

[1927] <223> 合成序列

[1928] <400> 84

[1929] Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly

[1930] 1 5 10 15

[1931] Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Arg Thr Tyr

[1932] 20 25 30
[1933] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[1934] 35 40 45
[1935] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[1936] 50 55 60
[1937] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1938] 65 70 75 80
[1939] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Ile Trp Thr
[1940] 85 90 95
[1941] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1942] 100 105
[1943] <210> 85
[1944] <211> 123
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[1947] <220>
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[1951] 1 5 10 15
[1952] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Ile Ser Ser Gly
[1953] 20 25 30
[1954] His His Trp Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
[1955] 35 40 45
[1956] Val Ser Val Ile Ser Gly Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser
[1957] 50 55 60
[1958] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[1959] 65 70 75 80
[1960] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[1961] 85 90 95
[1962] Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala Tyr
[1963] 100 105 110
[1964] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1965] 115 120
[1966] <210> 86
[1967] <211> 107
[1968] <212> PRT
[1969] <213> 人工生物体(Artificial organism)
[1970] <220>
[1971] <223> 合成序列
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[1974] 1 5 10 15
[1975] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
[1976] 20 25 30
[1977] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[1978] 35 40 45
[1979] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[1980] 50 55 60
[1981] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[1982] 65 70 75 80
[1983] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Thr Trp Thr
[1984] 85 90 95
[1985] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[1986] 100 105
[1987] <210> 87
[1988] <211> 123
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[1995] 1 5 10 15
[1996] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[1997] 20 25 30
[1998] Gly Val Ala Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[1999] 35 40 45
[2000] Trp Leu Ala Leu Ile Asp Trp Asp Gly Asp Lys Ser Tyr Ser Thr Ser
[2001] 50 55 60
[2002] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[2003] 65 70 75 80
[2004] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[2005] 85 90 95
[2006] Cys Ala Arg Glu Gly Ser Thr Ala Val Val Gly Asp Trp Phe Asp Tyr
[2007] 100 105 110
[2008] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2009] 115 120
[2010] <210> 88
[2011] <211> 107
[2012] <212> PRT
[2013] <213> 人工生物体(Artificial organism)
[2014] <220>
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[2016] <400> 88

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[2018] 1 5 10 15

[2019] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Arg Tyr

[2020] 20 25 30

[2021] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[2022] 35 40 45

[2023] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly

[2024] 50 55 60

[2025] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[2026] 65 70 75 80

[2027] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Leu Trp Thr

[2028] 85 90 95

[2029] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[2030] 100 105

[2031] <210> 89

[2032] <211> 124

[2033] <212> PRT

[2034] <213> 人工生物体(Artificial organism)

[2035] <220>

[2036] <223> 合成序列

[2037] <400> 89

[2038] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[2039] 1 5 10 15

[2040] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser

[2041] 20 25 30

[2042] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[2043] 35 40 45

[2044] Trp Val Ser Ser Ile Ser Gly Ala Gly Gly Thr Thr Tyr Tyr Ala Asp

[2045] 50 55 60

[2046] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr

[2047] 65 70 75 80

[2048] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr

[2049] 85 90 95

[2050] Tyr Cys Ala Arg Gly Gly Ser Thr Ala Val Thr Gly Asp Trp Phe Asp

[2051] 100 105 110

[2052] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[2053] 115 120

[2054] <210> 90

[2055] <211> 107

[2056] <212> PRT

[2057] <213> 人工生物体(Artificial organism)

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[2062] 1 5 10 15
[2063] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Pro Tyr
[2064] 20 25 30
[2065] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2066] 35 40 45
[2067] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly
[2068] 50 55 60
[2069] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2070] 65 70 75 80
[2071] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Ser Leu Trp Thr
[2072] 85 90 95
[2073] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2074] 100 105
[2075] <210> 91
[2076] <211> 124
[2077] <212> PRT
[2078] <213> 人工生物体(Artificial organism)
[2079] <220>
[2080] <223> 合成序列
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[2082] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
[2083] 1 5 10 15
[2084] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[2085] 20 25 30
[2086] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[2087] 35 40 45
[2088] Trp Val Ser Tyr Ile Ser Gly Ser Gly Asp Thr Thr Tyr Tyr Ala Asp
[2089] 50 55 60
[2090] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2091] 65 70 75 80
[2092] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2093] 85 90 95
[2094] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Val Gly Asp Trp Phe Ala
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[2096] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2097] 115 120
[2098] <210> 92
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[2100] <212> PRT
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[2102] <220>
[2103] <223> 合成序列
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[2106] 1 5 10 15
[2107] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
[2108] 20 25 30
[2109] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2110] 35 40 45
[2111] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2112] 50 55 60
[2113] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2114] 65 70 75 80
[2115] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
[2116] 85 90 95
[2117] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2118] 100 105
[2119] <210> 93
[2120] <211> 124
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[2124] <223> 合成序列
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[2126] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
[2127] 1 5 10 15
[2128] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[2129] 20 25 30
[2130] Gly Val Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[2131] 35 40 45
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[2133] 50 55 60
[2134] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2135] 65 70 75 80
[2136] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2137] 85 90 95
[2138] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Ala Gly Asp Trp Phe Ala
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[2141] 115 120

[2142] <210> 94
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[2150] 1 5 10 15
[2151] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
[2152] 20 25 30
[2153] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2154] 35 40 45
[2155] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2156] 50 55 60
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[2158] 65 70 75 80
[2159] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
[2160] 85 90 95
[2161] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2162] 100 105
[2163] <210> 95
[2164] <211> 124
[2165] <212> PRT
[2166] <213> 人工生物体(Artificial organism)
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[2168] <223> 合成序列
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[2171] 1 5 10 15
[2172] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser
[2173] 20 25 30
[2174] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[2175] 35 40 45
[2176] Trp Val Ser Ser Ile Ser Gly Tyr Gly Gly Thr Thr Tyr Tyr Ala Asp
[2177] 50 55 60
[2178] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2179] 65 70 75 80
[2180] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2181] 85 90 95
[2182] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
[2183] 100 105 110

[2184] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2185] 115 120
[2186] <210> 96
[2187] <211> 108
[2188] <212> PRT
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[2194] 1 5 10 15
[2195] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
[2196] 20 25 30
[2197] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2198] 35 40 45
[2199] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2200] 50 55 60
[2201] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2202] 65 70 75 80
[2203] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
[2204] 85 90 95
[2205] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2206] 100 105
[2207] <210> 97
[2208] <211> 124
[2209] <212> PRT
[2210] <213> 人工生物体(Artificial organism)
[2211] <220>
[2212] <223> 合成序列
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[2214] Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
[2215] 1 5 10 15
[2216] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[2217] 20 25 30
[2218] Gly Val Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[2219] 35 40 45
[2220] Trp Val Ser Tyr Ile Ser Gly Ala Gly Gly Thr Thr Tyr Tyr Ala Asp
[2221] 50 55 60
[2222] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2223] 65 70 75 80
[2224] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2225] 85 90 95

[2226] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
[2227] 100 105 110
[2228] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2229] 115 120
[2230] <210> 98
[2231] <211> 108
[2232] <212> PRT
[2233] <213> 人工生物体(Artificial organism)
[2234] <220>
[2235] <223> 合成序列
[2236] <400> 98
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[2238] 1 5 10 15
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[2243] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
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[2247] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
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[2263] 35 40 45
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[2265] 50 55 60
[2266] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2267] 65 70 75 80

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[2284] 20 25 30
[2285] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2286] 35 40 45
[2287] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2288] 50 55 60
[2289] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[2291] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
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[2304] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Gly
[2305] 20 25 30
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[2307] 35 40 45
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[2309] 50 55 60

[2310] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
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[2313] 85 90 95
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[2330] 35 40 45
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[2332] 50 55 60
[2333] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[2349] 20 25 30
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[2351] 35 40 45

[2352] Trp Val Ser Tyr Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr Ala Asp
[2353] 50 55 60
[2354] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2355] 65 70 75 80
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[2373] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2374] 35 40 45
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[2376] 50 55 60
[2377] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2378] 65 70 75 80
[2379] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
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[2393] 20 25 30

[2394] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[2395] 35 40 45
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[2397] 50 55 60
[2398] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
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[2402] Tyr Cys Ala Arg Glu Gly Ser Thr Ala Val Leu Gly Asp Trp Phe Ala
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[2418] 35 40 45
[2419] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2420] 50 55 60
[2421] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2422] 65 70 75 80
[2423] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[2425] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[2437] 20 25 30
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[2439] 35 40 45
[2440] Trp Val Ser Tyr Ile Ser Gly Tyr Gly Gly Thr Thr Tyr Tyr Ala Asp
[2441] 50 55 60
[2442] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2443] 65 70 75 80
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[2462] 35 40 45
[2463] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2464] 50 55 60
[2465] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2466] 65 70 75 80
[2467] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[2483] 35 40 45
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[2485] 50 55 60
[2486] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[2487] 65 70 75 80
[2488] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[2489] 85 90 95
[2490] Cys Ala Arg Glu Gly Ser Asp Ala Val Leu Gly Asp Trp Phe Ala Tyr
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[2492] Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2493] 115 120
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[2502] 1 5 10 15
[2503] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
[2504] 20 25 30
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[2506] 35 40 45
[2507] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2508] 50 55 60
[2509] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2510] 65 70 75 80
[2511] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
[2512] 85 90 95
[2513] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2514] 100 105
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[2525] 20 25 30

[2526] Gly Val Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[2527] 35 40 45

[2528] Trp Val Ser Tyr Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr Ala Asp

[2529] 50 55 60

[2530] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr

[2531] 65 70 75 80

[2532] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr

[2533] 85 90 95

[2534] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala

[2535] 100 105 110

[2536] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

[2537] 115 120

[2538] <210> 112

[2539] <211> 108

[2540] <212> PRT

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[2543] <223> 合成序列

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[2546] 1 5 10 15

[2547] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr

[2548] 20 25 30

[2549] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[2550] 35 40 45

[2551] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly

[2552] 50 55 60

[2553] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[2554] 65 70 75 80

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[2557] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[2558] 100 105

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[2569] 20 25 30
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[2571] 35 40 45
[2572] Trp Val Ser Tyr Ile Ser Gly Ala Gly Ser Thr Thr Tyr Tyr Ala Asp
[2573] 50 55 60
[2574] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2575] 65 70 75 80
[2576] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2577] 85 90 95
[2578] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
[2579] 100 105 110
[2580] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2581] 115 120
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[2592] 20 25 30
[2593] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2594] 35 40 45
[2595] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2596] 50 55 60
[2597] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2598] 65 70 75 80
[2599] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[2601] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[2611] 1 5 10 15
[2612] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser
[2613] 20 25 30
[2614] Gly Val Gly Val Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
[2615] 35 40 45
[2616] Trp Val Ser Tyr Ile Ser Gly Ser Gly Asp Thr Thr Tyr Tyr Ala Asp
[2617] 50 55 60
[2618] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2619] 65 70 75 80
[2620] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2621] 85 90 95
[2622] Tyr Cys Ala Arg Glu Gly Ser Asp Ala Val Leu Gly Asp Trp Phe Ala
[2623] 100 105 110
[2624] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2625] 115 120
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[2635] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
[2636] 20 25 30
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[2638] 35 40 45
[2639] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2640] 50 55 60
[2641] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2642] 65 70 75 80
[2643] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[2646] 100 105

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[2652] <223> 合成序列

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[2656] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Thr Ser

[2657] 20 25 30

[2658] Gly Val Gly Val Gly Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[2659] 35 40 45

[2660] Trp Val Ser Tyr Ile Ser Gly Asp Gly Asp Thr Thr Tyr Tyr Ala Asp

[2661] 50 55 60

[2662] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr

[2663] 65 70 75 80

[2664] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr

[2665] 85 90 95

[2666] Tyr Cys Ala Arg Glu Gly Ser Thr Thr Val Leu Gly Asp Trp Phe Ala

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[2669] 115 120

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[2672] <212> PRT

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[2678] 1 5 10 15

[2679] Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr

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[2681] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[2682] 35 40 45

[2683] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly

[2684] 50 55 60

[2685] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[2686] 65 70 75 80

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[2688] 85 90 95
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[2701] 20 25 30
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[2703] 35 40 45
[2704] Trp Val Ser Tyr Ile Ser Gly Asp Gly Gly Thr Thr Tyr Tyr Ala Asp
[2705] 50 55 60
[2706] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2707] 65 70 75 80
[2708] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2709] 85 90 95
[2710] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
[2711] 100 105 110
[2712] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2713] 115 120
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[2726] 35 40 45
[2727] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2728] 50 55 60
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[2730] 65 70 75 80
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[2733] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2734] 100 105
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[2743] 1 5 10 15
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[2745] 20 25 30
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[2747] 35 40 45
[2748] Trp Val Ser Tyr Ile Ser Gly Ala Gly Asp Ser Thr Tyr Tyr Ala Asp
[2749] 50 55 60
[2750] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
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[2752] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
[2753] 85 90 95
[2754] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
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[2756] Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[2757] 115 120
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[2769] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2770] 35 40 45
[2771] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly

[2772] 50 55 60

[2773] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[2774] 65 70 75 80

[2775] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu

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[2777] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

[2778] 100 105

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[2789] 20 25 30

[2790] Gly Val Gly Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu

[2791] 35 40 45

[2792] Trp Val Ser Ser Ile Ser Gly Tyr Gly Gly Thr Thr Tyr Tyr Ala Asp

[2793] 50 55 60

[2794] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Arg

[2795] 65 70 75 80

[2796] Leu Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr

[2797] 85 90 95

[2798] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala

[2799] 100 105 110

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[2813] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

[2814] 35 40 45
[2815] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2816] 50 55 60
[2817] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2818] 65 70 75 80
[2819] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
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[2821] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
[2822] 100 105
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[2835] 35 40 45
[2836] Trp Val Ser Ser Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp
[2837] 50 55 60
[2838] Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
[2839] 65 70 75 80
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[2842] Tyr Cys Ala Arg Glu Gly Ser Asp Thr Val Leu Gly Asp Trp Phe Ala
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[2858] 35 40 45
[2859] Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
[2860] 50 55 60
[2861] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2862] 65 70 75 80
[2863] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Thr Thr Pro Leu
[2864] 85 90 95
[2865] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[2881] 50 55 60
[2882] Leu Lys Ser Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[2883] 65 70 75 80
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[2902] 35 40 45
[2903] Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[2904] 50 55 60
[2905] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2906] 65 70 75 80
[2907] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
[2908] 85 90 95
[2909] Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[2923] 35 40 45
[2924] Trp Ile Gly Asp Ile Tyr His Ser Gly Ser Thr Tyr Tyr Ser Pro Ser
[2925] 50 55 60
[2926] Leu Lys Ser Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[2927] 65 70 75 80
[2928] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
[2929] 85 90 95
[2930] Cys Ala Arg Glu Gly Ser Asp Ala Val Ala Gly Asp Trp Phe Asp Tyr
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[2945] Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
[2946] 35 40 45
[2947] Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[2948] 50 55 60
[2949] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
[2950] 65 70 75 80
[2951] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[2965] 20 25 30
[2966] Gly Val Ala Val Ala Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu
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[2968] Trp Ile Gly Asp Ile Tyr His Ser Gly Asn Thr Tyr Tyr Asn Pro Ser
[2969] 50 55 60
[2970] Leu Lys Ser Arg Val Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
[2971] 65 70 75 80
[2972] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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[2977] 115 120

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[2988] 20 25 30
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[2990] 35 40 45
[2991] Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
[2992] 50 55 60
[2993] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[2995] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr Pro Leu
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[3024] ggtaccaagg tggagatcaa acga 324

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[3029] <220>

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[3033] tcctgtgcag cttccggatt ctctctgtct accagcggtg tggctgtgag ctggattcgt 120

[3034] caggccccgg gtaagggcct cgagtggatc ggtatcatca acccaaactt cggtgatact 180

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[3043] <220>

[3044] <223> 合成序列

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[3049] cgcttctctg gatccggttc cgggacggat ttcactctga ccatcagcag tctgcagccg 240

[3050] gaagacttcg caacttatta ctgccagcag tcctacgact ggcctccgac cttcggacag 300

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[3056] <220>

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[3060] tcctgtgcag cttccggatt ctctctgtct accggcggtg tgggtgtgag ctggattcgt 120

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[3062] tactacgccg actctgtcaa gggccgtttc actataagtc gcgacaattc gaaaaacaca 240

[3063] ctgtacctac aactgaacag cttaagagct gaggacactg ccgtctatta ttgcgccaga 300

[3064] gacgacctat actcctggta cttcgacgtg tggggtcaag gaacactagt caccgtctcc 360

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[3078] accaaggtgg agatcaaacg a 321
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[3154] ggaaaagctc cgaagcttct gatctacgcc gcctcttctt tgcagtctgg tgtgccatct 180

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[3167] gccccgggta agggcctcga gtgggtgtct ggtatctctg gttccggttc ttctacctac 180

[3168] tacgccgact ctgtcaaggg ccgtttcact ataagtcgcg acaattcgaa aaacacactg 240

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[3171] <210> 146

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[3184] <210> 147

[3185] <211> 351

[3186] <212> DNA

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[8995] Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro

[8996] 195 200 205

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[9042] Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
[9043] 65 70 75 80
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[9046] Thr Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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[9048] Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
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[9253] Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
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[9319] 405 410 415
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[9352] 145 150 155 160
[9353] Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
[9354] 165 170 175
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[9356] 180 185 190
[9357] Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
[9358] 195 200 205
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[9375] 50 55 60
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[9387] 145 150 155 160
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[9389] 165 170 175
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[9395] 210 215 220
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[9440] 50 55 60
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[9447] Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
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[9450] 130 135 140

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[9452] 145 150 155 160

[9453] Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser

[9454] 165 170 175

[9455] Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala

[9456] 180 185 190

[9457] Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe

[9458] 195 200 205

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[9460] 210

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[9475] 50 55 60

[9476] Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

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[9490] Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val

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[9501] 260 265 270
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[9538] 35 40 45
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[9540] 50 55 60
[9541] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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[9593] 195 200 205
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[9609] 325 330 335
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[9620] Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met
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[9639] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ser Arg Phe Ser Gly
[9640] 50 55 60
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[9643] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Tyr Tyr Leu Trp Thr
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[9645] Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
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[9653] Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
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[9656] 180 185 190
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[9674] Trp Leu Ala Leu Ile Asp Trp Ala Gly Asp Lys Ser Tyr Ser Thr Ser

[9675] 50 55 60

[9676] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

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[9701] 260 265 270

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[9719] 405 410 415
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[9740] 50 55 60
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[9775] 50 55 60
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[9779] 85 90 95
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[9819] 405 410 415
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[10040] 50 55 60
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[10108] Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
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[10138] 50 55 60
[10139] Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln
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[10141] Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Tyr Trp Pro
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[10143] Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val
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[10154] 180 185 190
[10155] Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
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[10173] 50 55 60

[10174] Leu Lys Ser Arg Leu Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu

[10175] 65 70 75 80

[10176] Tyr Leu Gln Leu Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr

[10177] 85 90 95

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[10201] 275 280 285

[10202] Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg

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[10238] 50 55 60
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[10252] 165 170 175
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[10254] 180 185 190
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[10273] 50 55 60
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[12746] 1 5 10
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[13043] <213> 食蟹猴(Macaca fascicularis)
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[13064] 145 150 155
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[13068] <213> 小家鼠(Mus musculus)
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[13078] Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys Phe Cys Ser Ser Thr
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[13080] His Asn Ala Glu Cys Glu Cys Ile Glu Gly Phe His Cys Leu Gly Pro
[13081] 85 90 95
[13082] Gln Cys Thr Arg Cys Glu Lys Asp Cys Arg Pro Gly Gln Glu Leu Thr
[13083] 100 105 110
[13084] Lys Gln Gly Cys Lys Thr Cys Ser Leu Gly Thr Phe Asn Asp Gln Asn
[13085] 115 120 125
[13086] Gly Thr Gly Val Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Arg
[13087] 130 135 140
[13088] Ser Val Leu Lys Thr Gly Thr Thr Glu Lys Asp Val Val Cys Gly Pro
[13089] 145 150 155 160
[13090] Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu
[13091] 165 170 175
[13092] Gly Gly
[13093] <210> 862
[13094] <211> 116
[13095] <212> PRT
[13096] <213> 人工序列(Artificial Sequence)
[13097] <220>
[13098] <223> 合成序列
[13099] <400> 862
[13100] Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
[13101] 1 5 10 15
[13102] Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Ser Thr Tyr
[13103] 20 25 30

[13104] Trp Ile Ser Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
[13105] 35 40 45
[13106] Gly Lys Ile Tyr Pro Gly Asp Ser Tyr Thr Asn Tyr Ser Pro Ser Phe
[13107] 50 55 60
[13108] Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
[13109] 65 70 75 80
[13110] Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
[13111] 85 90 95
[13112] Ala Arg Gly Tyr Gly Ile Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
[13113] 100 105 110
[13114] Thr Val Ser Ser
[13115] 115
[13116] <210> 863
[13117] <211> 108
[13118] <212> PRT
[13119] <213> 人工序列(Artificial Sequence)
[13120] <220>
[13121] <223> 合成序列
[13122] <400> 863
[13123] Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
[13124] 1 5 10 15
[13125] Thr Ala Ser Ile Thr Cys Ser Gly Asp Asn Ile Gly Asp Gln Tyr Ala
[13126] 20 25 30
[13127] His Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Tyr
[13128] 35 40 45
[13129] Gln Asp Lys Asn Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
[13130] 50 55 60
[13131] Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
[13132] 65 70 75 80
[13133] Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Tyr Thr Gly Phe Gly Ser Leu
[13134] 85 90 95
[13135] Ala Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
[13136] 100 105
[13137] <210> 864
[13138] <211> 121
[13139] <212> PRT
[13140] <213> 人工序列(Artificial Sequence)
[13141] <220>
[13142] <223> 合成序列
[13143] <400> 864
[13144] Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
[13145] 1 5 10 15

[13146] Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
[13147] 20 25 30
[13148] Tyr Trp Ser Trp Ile Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Ile
[13149] 35 40 45
[13150] Gly Glu Ile Asn His Gly Gly Tyr Val Thr Tyr Asn Pro Ser Leu Glu
[13151] 50 55 60
[13152] Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
[13153] 65 70 75 80
[13154] Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
[13155] 85 90 95
[13156] Arg Asp Tyr Gly Pro Gly Asn Tyr Asp Trp Tyr Phe Asp Leu Trp Gly
[13157] 100 105 110
[13158] Arg Gly Thr Leu Val Thr Val Ser Ser
[13159] 115 120
[13160] <210> 865
[13161] <211> 109
[13162] <212> PRT
[13163] <213> 人工序列(Artificial Sequence)
[13164] <220>
[13165] <223> 合成序列
[13166] <400> 865
[13167] Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
[13168] 1 5 10 15
[13169] Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
[13170] 20 25 30
[13171] Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
[13172] 35 40 45
[13173] Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
[13174] 50 55 60
[13175] Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
[13176] 65 70 75 80
[13177] Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
[13178] 85 90 95
[13179] Ala Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
[13180] 100 105


VH EVQLVESGGGLVQPGGSLRLSCAASGFTFSYGIHWVRQAPGKGLEWVSGIDGADTTYYADSVKGRFTISRDNSKNTLYLQLNSLRAEDTAVYYCARERDYDFDYWGQGTLVTVSS
ADG FW1 HVR_H1 FW2 HVR_H2 FW3 HVR_H3 FW4
VH EVQLVESGGGLVQPGGSLRLSCAASGFTFSYGIHWVRQAPGKGLEWVSGISGAGDTTYYADSVKGRFTISRDNSKNTLYLQLNSLRAEDTAVYYCARERDYDFDYWGQGTLVTVSS
Kabat FW1 CDR1 FW2 CDR2 FW3 CDR3 FW4
VL DIQLTQSPSSLSASVGDRVTITCRASQSVSSYLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTSHTFGQGTKVEIKR
ADG FW1 HVR_L1 FW2 HVR_L2 FW3 HVR_L3 FW4
VL DIQLTQSPSSLSASVGDRVTITCRASQSVSSYLAWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTSHTFGQGTKVEIKR
Kabat FW1 CDR1 FW2 CDR2 FW3 CDR3 FW4

图1A

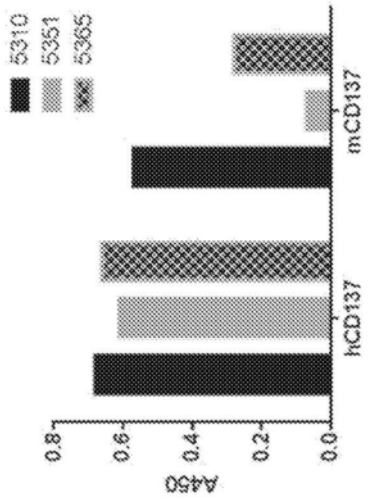


图1B

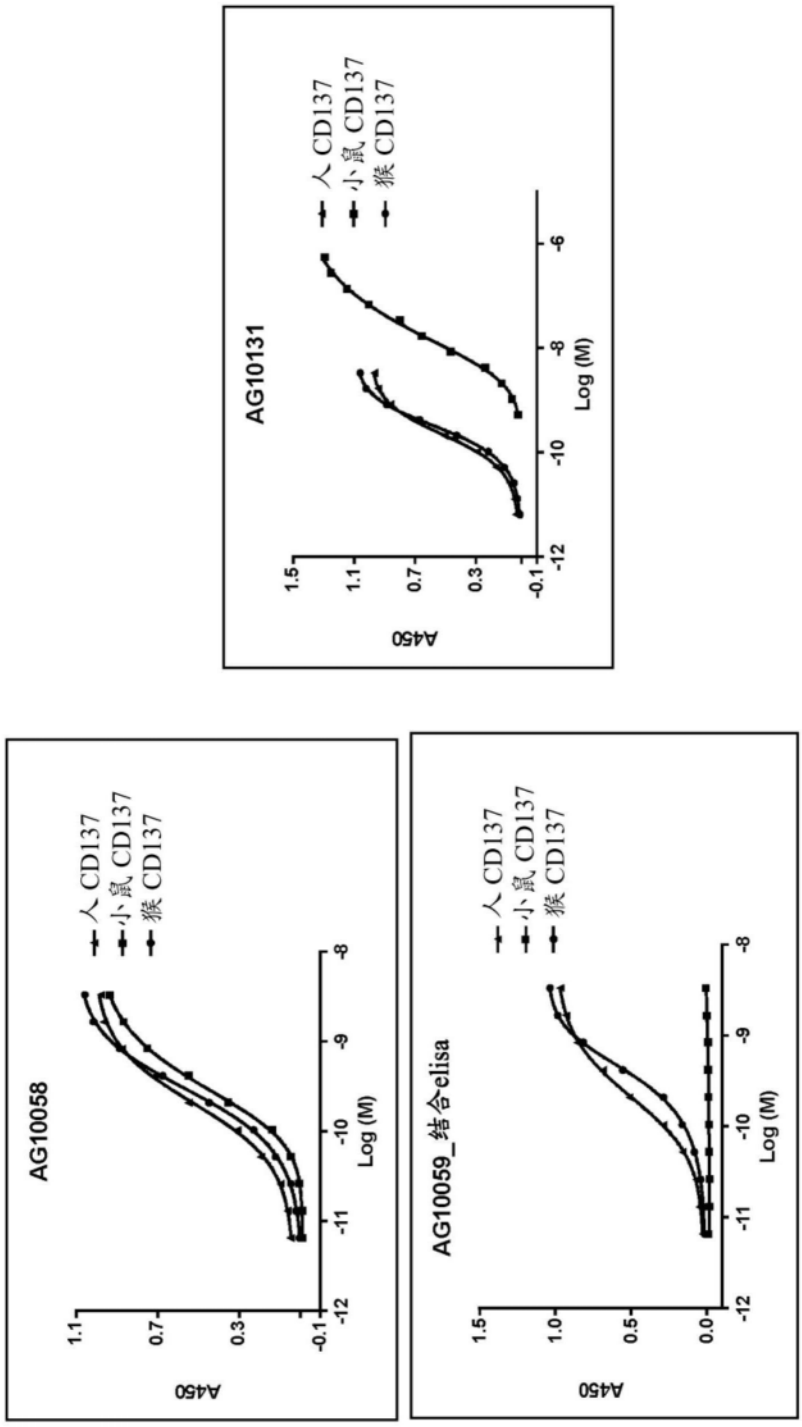

AG10131
人 CD137
小鼠 CD137
猴 CD137
A450
Log (M)
AG10058
AG10059_结合elisa

图2

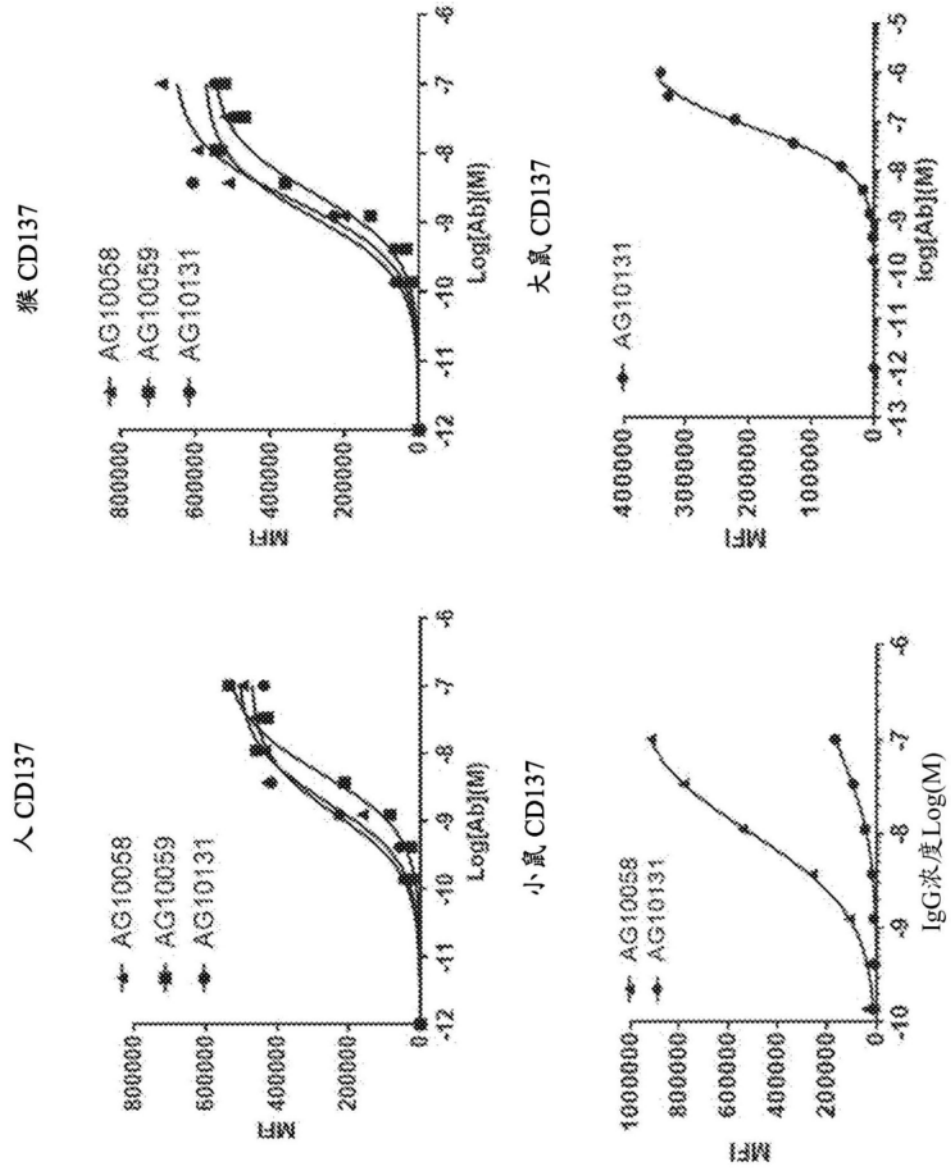

猴 CD137
大鼠 CD137
人 CD137
小鼠 CD137
AG10058
AG10059
AG10131
MFI
Log[Ab](M)
IgG浓度Log(M)

图3A

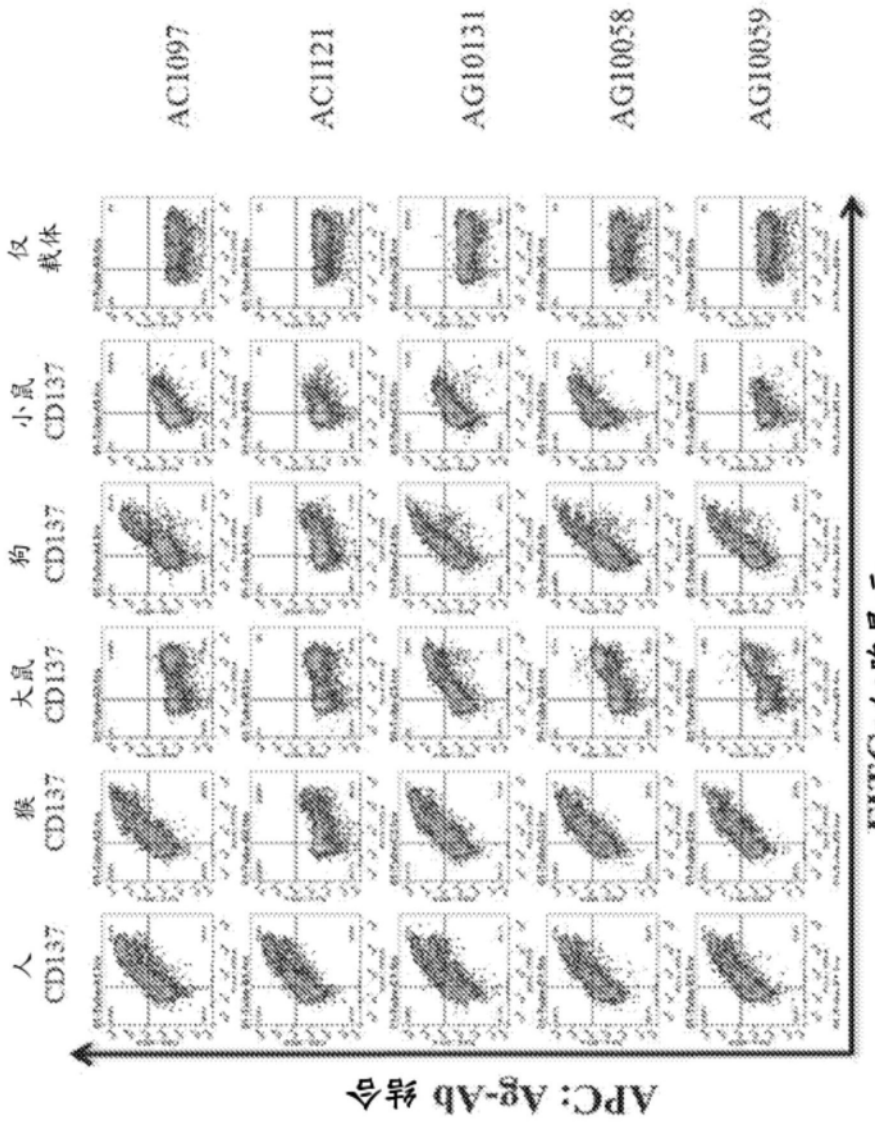


图3B.物种交叉反应性

图3B

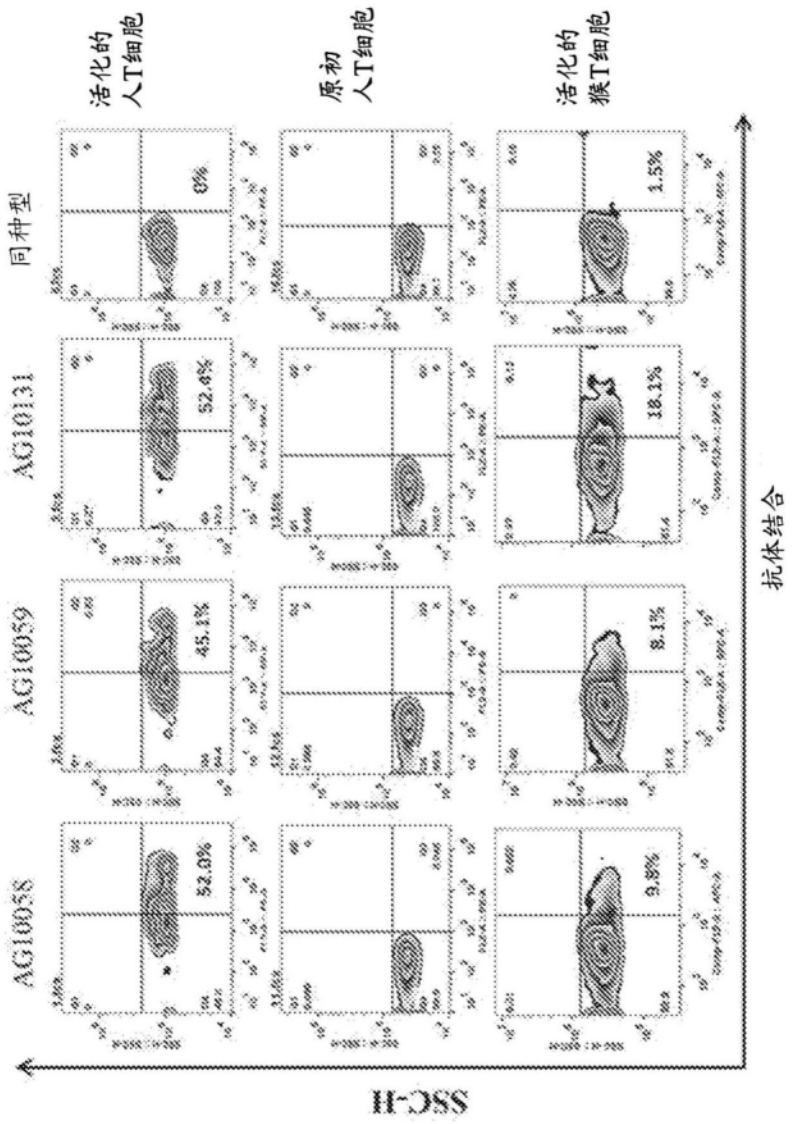

活化的
人T细胞
原初
人T细胞
活化的
猴T细胞
同种型
AG10131
AG10059
AG10058
0%
52.6%
45.1%
52.0%
1.5%
18.1%
8.1%
9.8%
抗体结合
SSC-H

图4A

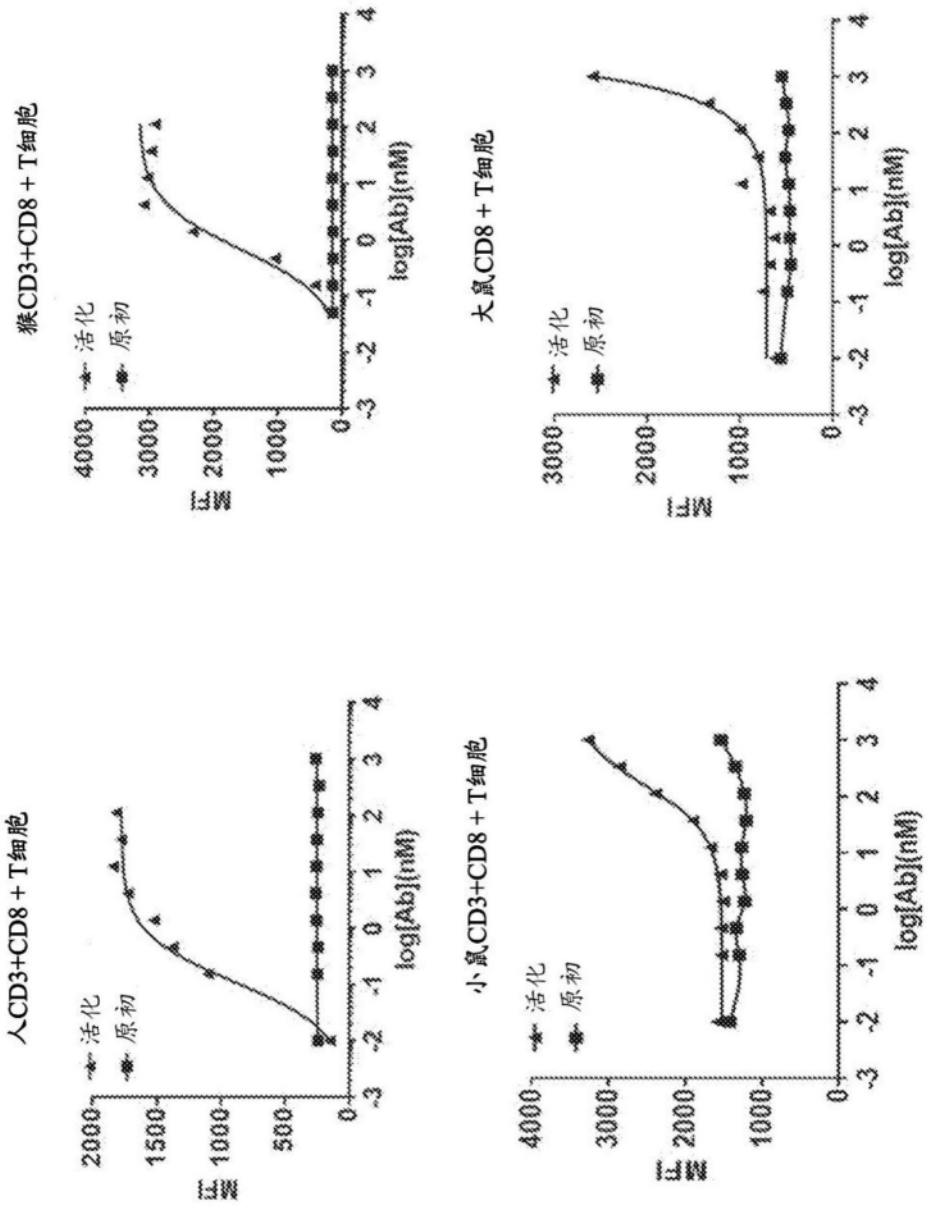

猴CD3+CD8 + T细胞
活化
原初
MFI
log[Ab](nM)
大鼠CD8 + T细胞
人CD3+CD8 + T细胞
小鼠CD3+CD8 + T细胞

图4B

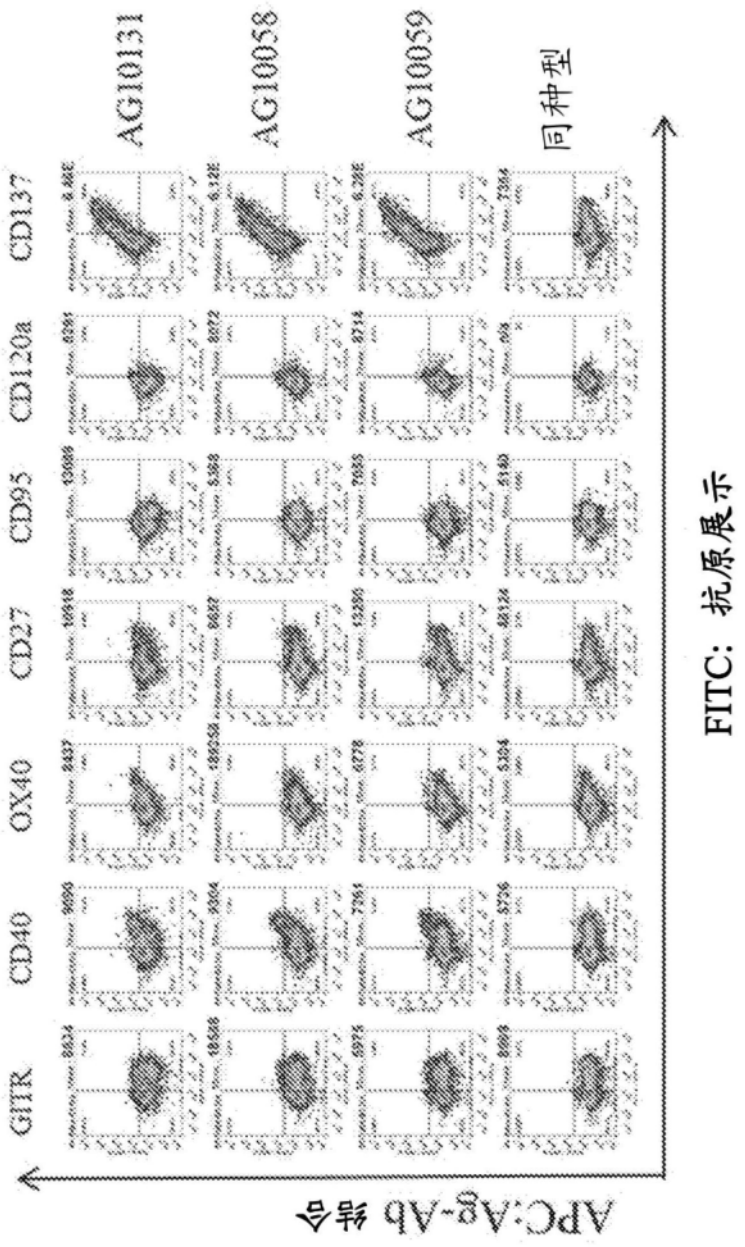

AG10131
AG10058
AG10059
同种型
CD137
CD120a
CD95
CD27
OX40
CD40
GITR
FITC：抗原展示
APC:Ag-Ab 结合

图5

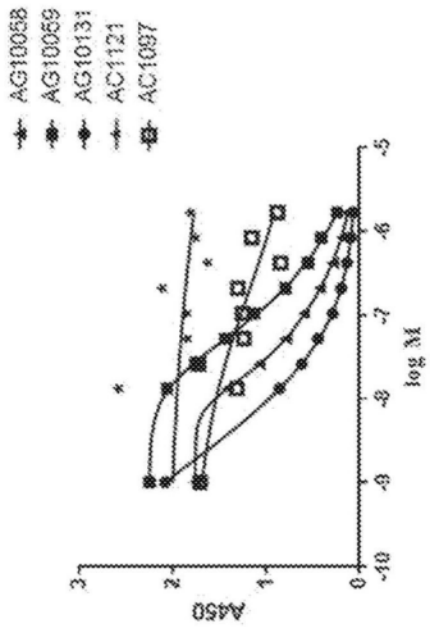


图6A

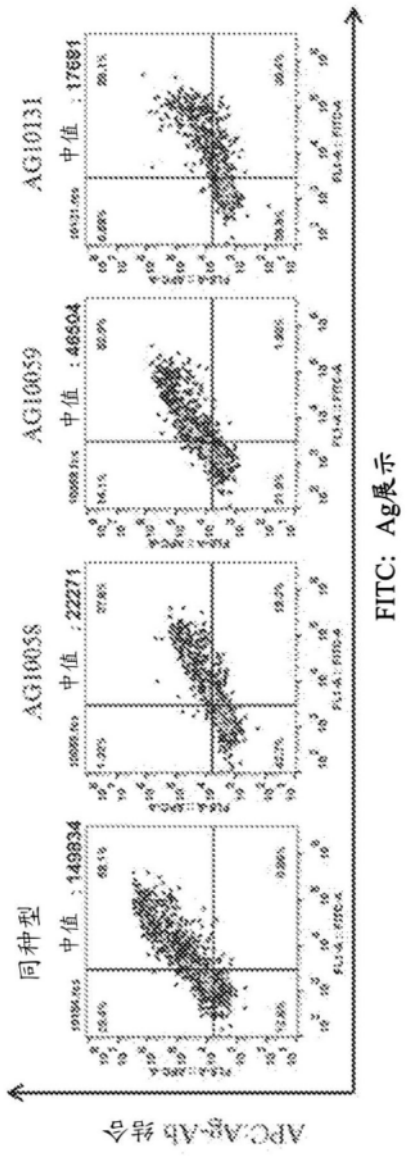

同种型
AG10038
AG10039
AG10131
中值：149834
中值：222271
中值：46504
中值：17681
APC:Ag-Ab 结合
FITC: Ag展示

图6B

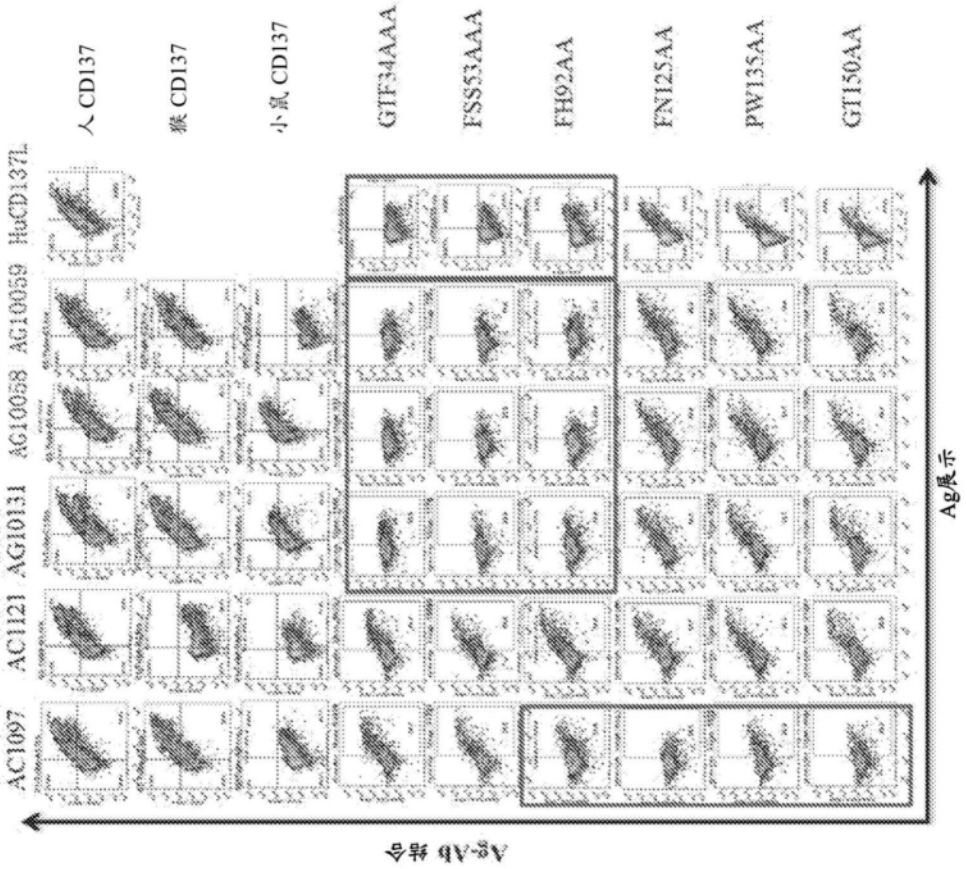

人 CD137
猴 CD137
小鼠 CD137
GTF34AAA
FSS53AAA
FH92AA
FN125AA
PW135AA
GT150AA
HuCD137L
AG10059
AG10058
AG10131
AC1121
AC1097
Ag展示
Ag-Ab 结合

图7A

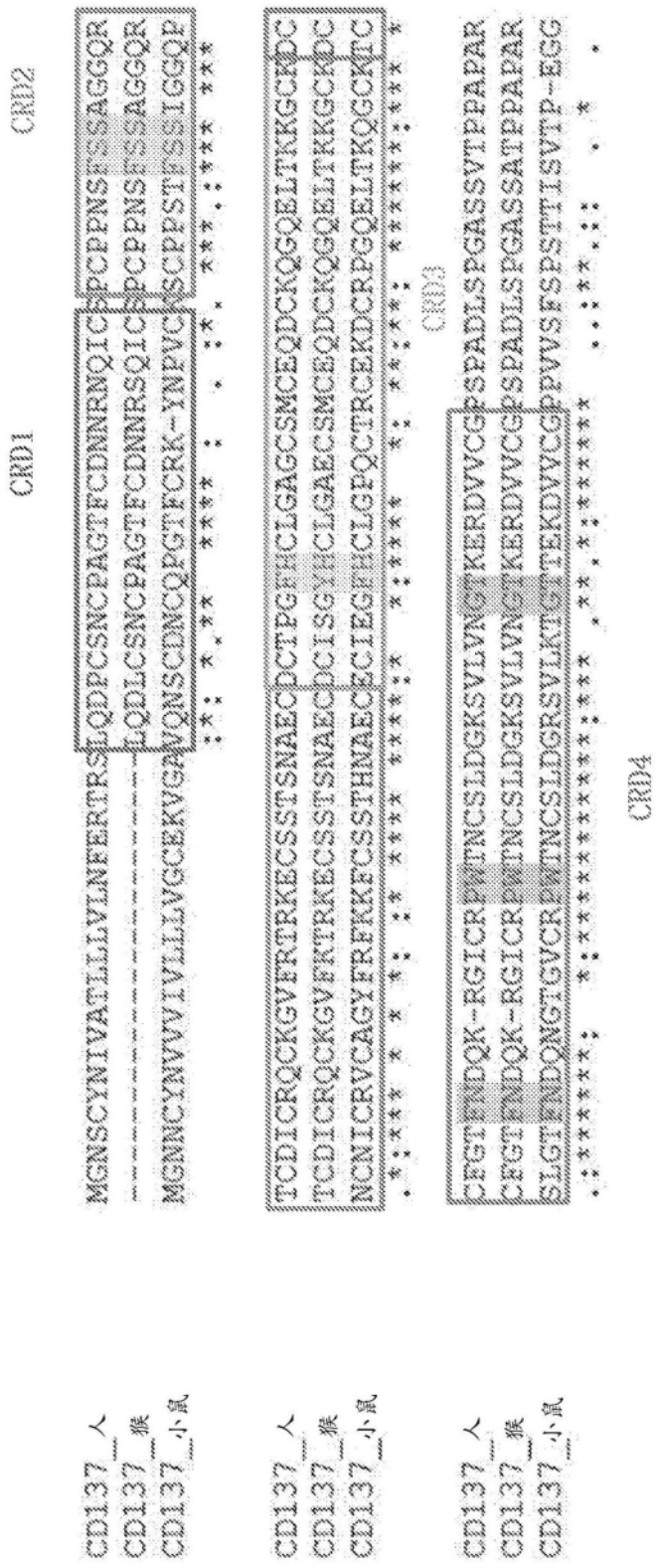


图7B

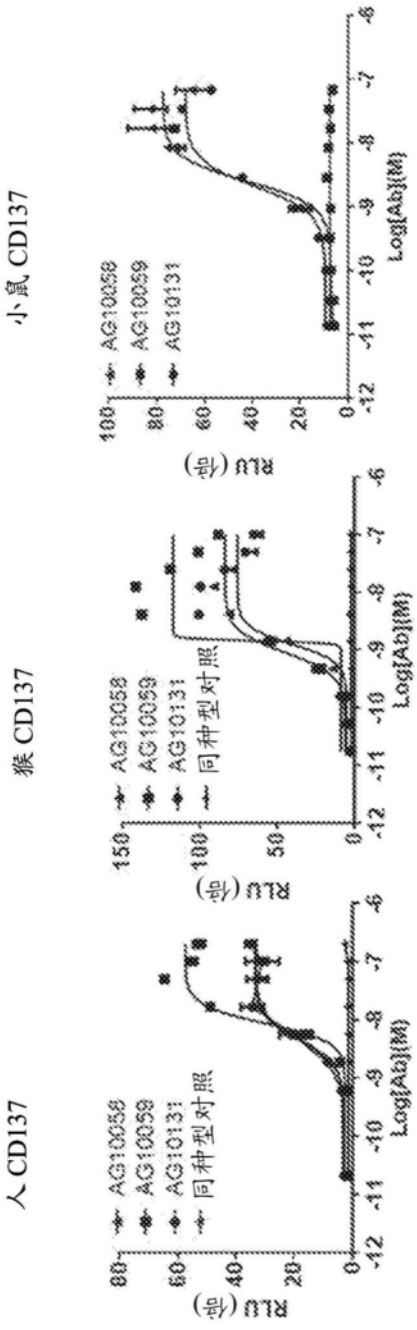

人 CD137
猴 CD137
小鼠 CD137
AG10058
AG10059
AG10131
同种型对照
RLU (倍)
Log[Ab](M)

图8

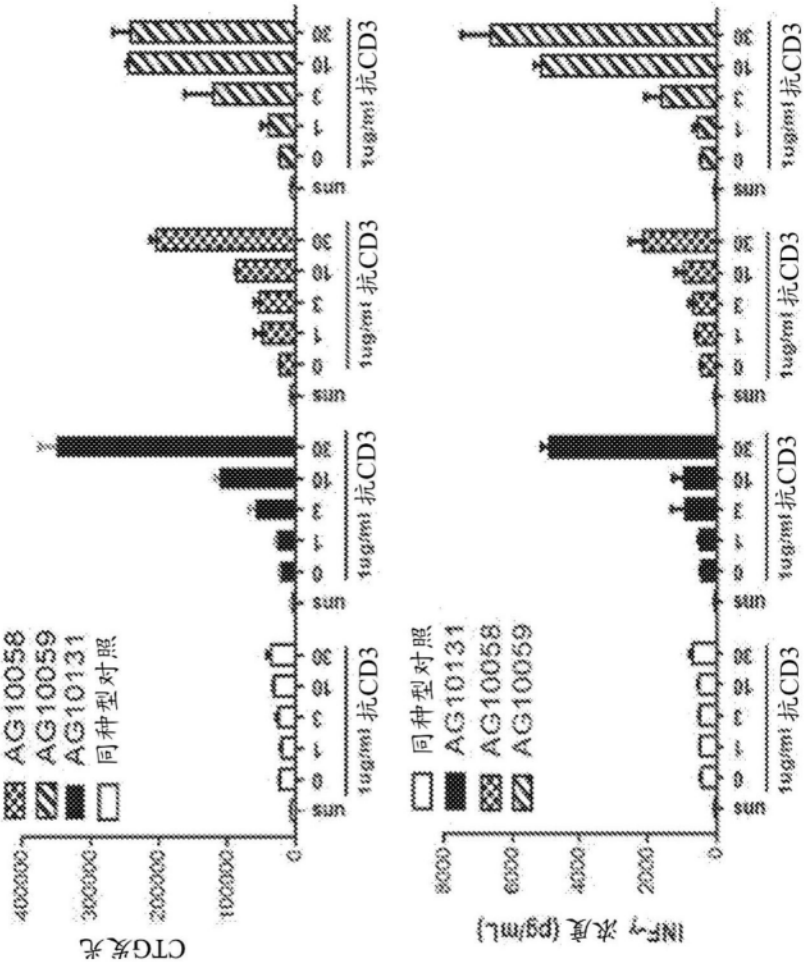

CTG关光
4000000
3000000
2000000
1000000
0
AG10058
AG10059
AG101131
同种型对照
1ug/ml 抗CD3
IFNγ 浓度 (pg/mL)
8000
6000
4000
2000
0
同种型对照
AG101131
AG10058
AG10059
1ug/ml 抗CD3

图9

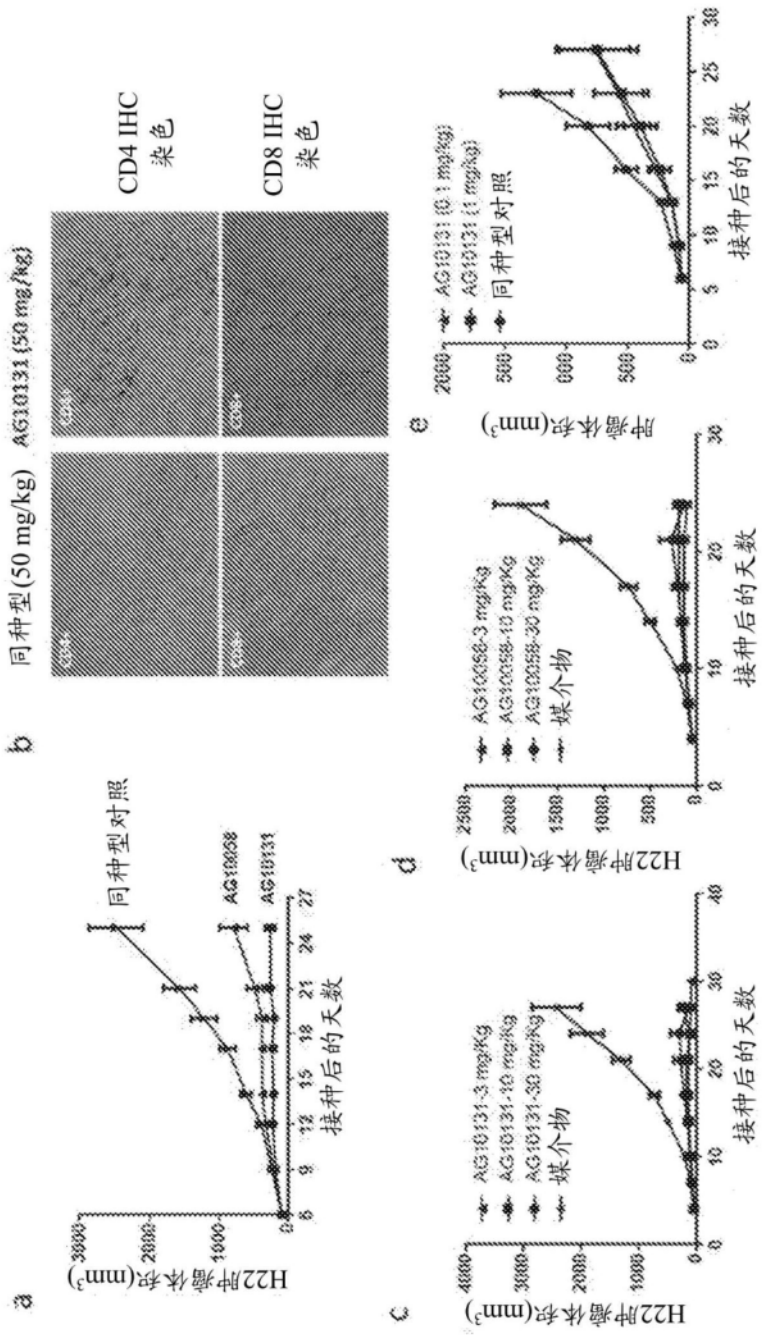

a
H22肿瘤体积(mm³)
同种型对照
AG10058
AG10131
接种后的天数
b
同种型(50 mg/kg)
AG10131(50 mg/kg)
CD4 IHC 染色
CD8 IHC 染色
c
H22肿瘤体积(mm³)
AG10131-3 mg/kg
AG10131-10 mg/kg
AG10131-30 mg/kg
媒介物
接种后的天数
d
H22肿瘤体积(mm³)
AG10058-3 mg/kg
AG10058-10 mg/kg
AG10058-30 mg/kg
媒介物
接种后的天数
e
肿瘤体积(mm³)
同种型对照
接种后的天数

图10

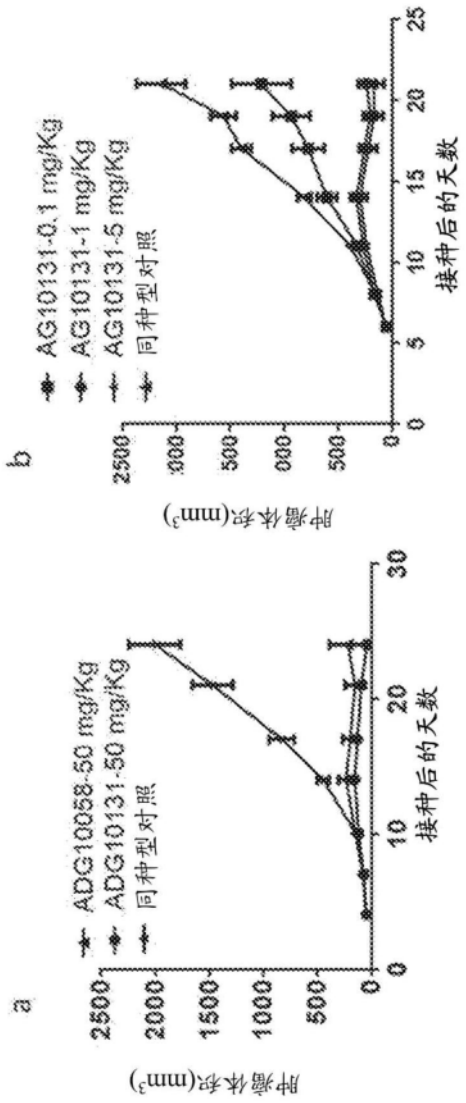

a
肿瘤体积(mm³)
2500
2000
1500
1000
500
0
0
10
20
30
接种后的天数
ADG10058-50 mg/Kg
ADG10131-50 mg/Kg
同种型对照
b
肿瘤体积(mm³)
2500
000
500
000
500
0
0
5
10
15
20
25
接种后的天数
AG10131-0.1 mg/Kg
AG10131-1 mg/Kg
AG10131-5 mg/Kg
同种型对照

图11

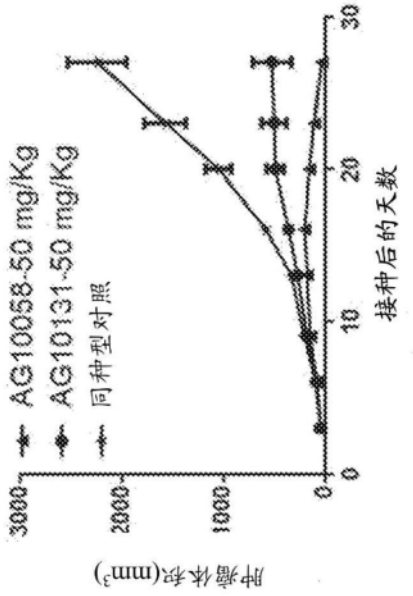

AG10058-50 mg/Kg
AG10131-50 mg/Kg
同种型对照
肿瘤体积(mm³)
3000
2000
1000
0
0
10
20
30
接种后的天数

图12

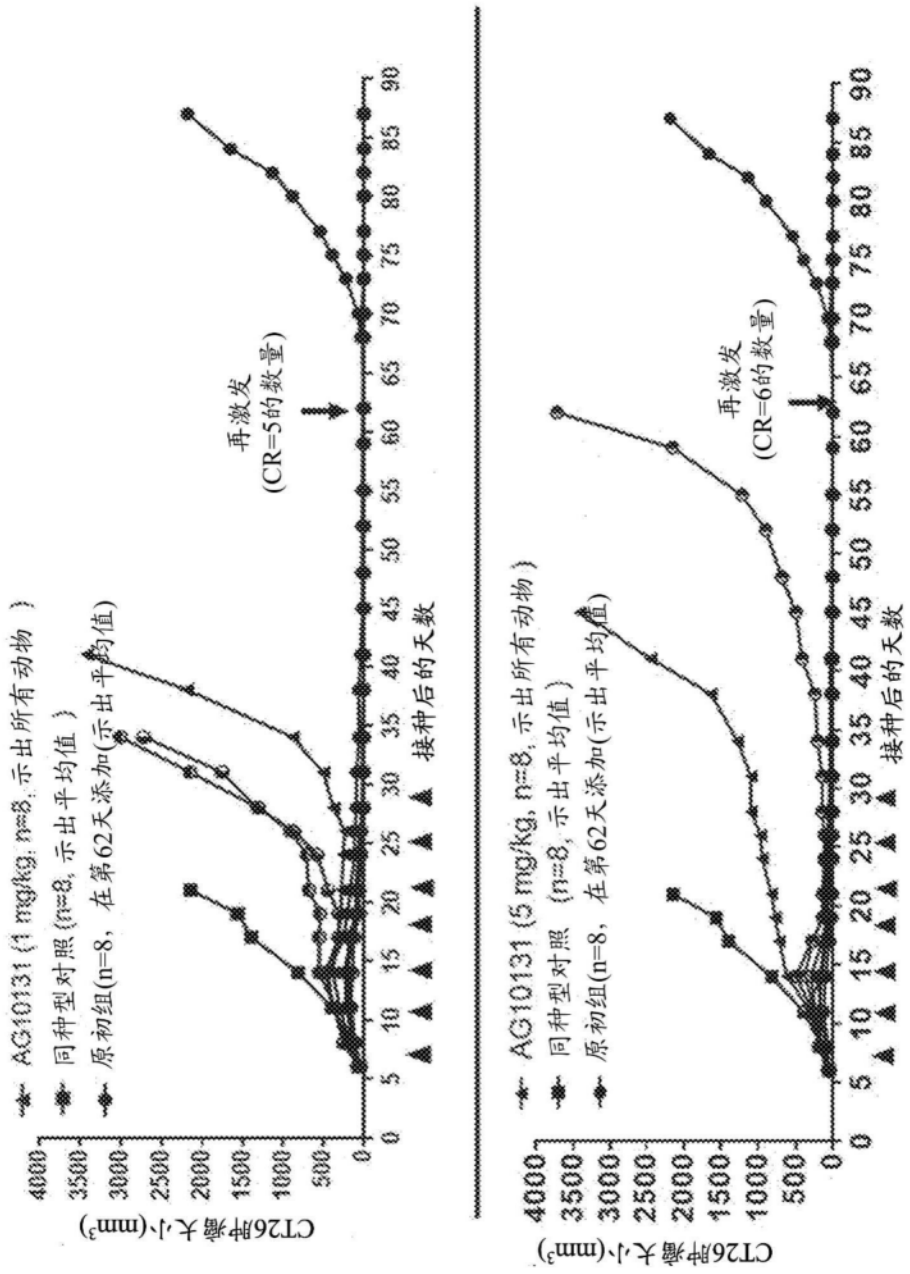

AG101131 (1 mg/kg, n=8, 示出所有动物)
同种型对照 (n=8, 示出平均值)
原初组(n=8，在第62天添加(示出平均值)
再激发
(CR=5的数量)
CT26肿瘤大小(mm³)
接种后的天数
AG101131 (5 mg/kg, n=8, 示出所有动物)
同种型对照 (n=8, 示出平均值)
原初组(n=8，在第62天添加(示出平均值)
再激发
(CR=6的数量)
CT26肿瘤大小(mm³)
接种后的天数

图13

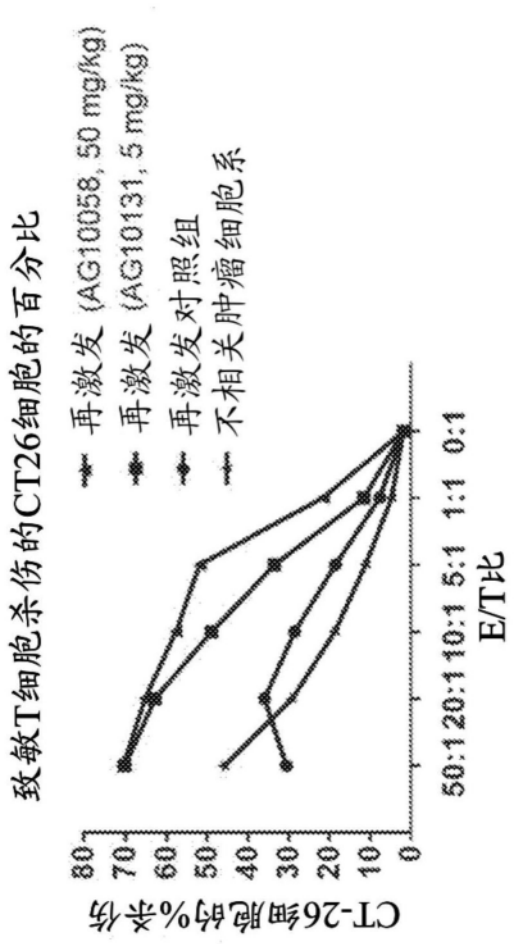

致敏T细胞杀伤的CT26细胞的百分比
再激发 (AG10058, 50 mg/kg)
再激发 (AG10131, 5 mg/kg)
再激发对照组
不相关肿瘤细胞系
CT-26细胞的%杀伤
80
70
60
50
40
30
20
10
0
50:1 20:1 10:1 5:1 1:1 0:1
E/T比

图14

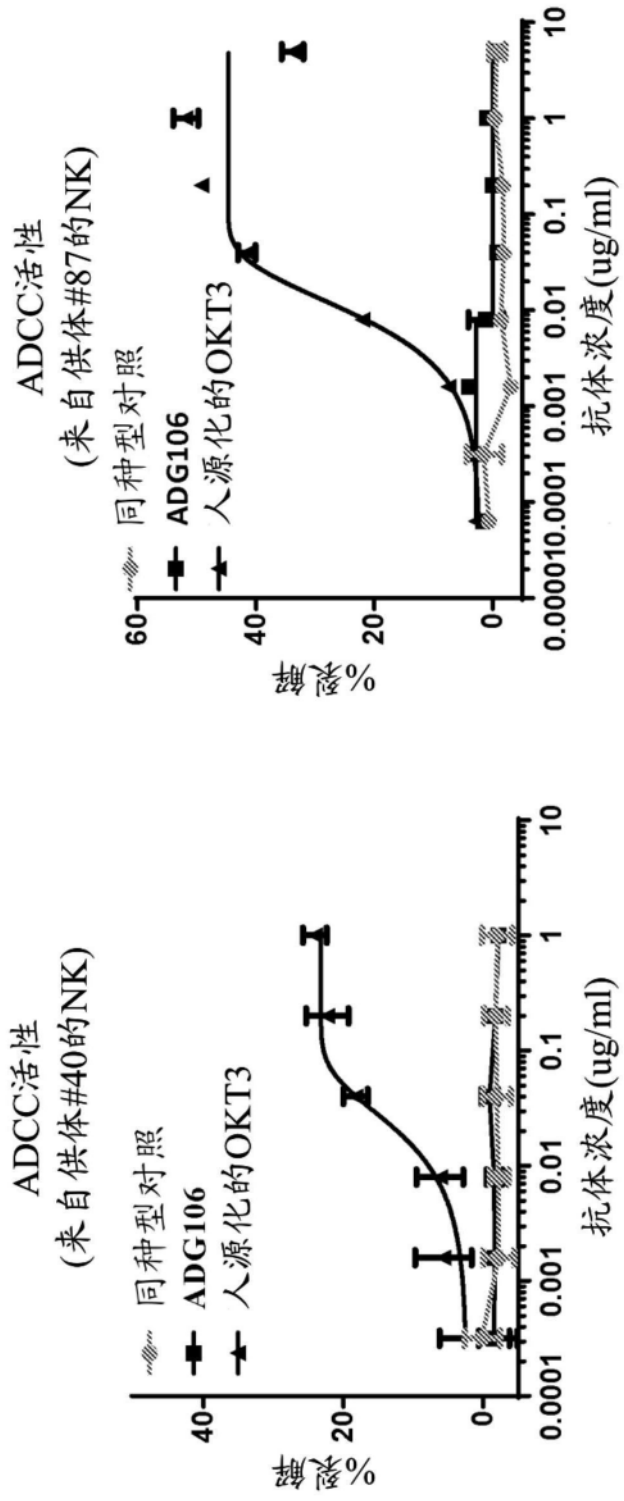

ADCC活性
(来自供体#87的NK)
同种型对照
ADG106
人源化的OKT3
%裂解
60
40
20
0
0.00001
0.0001
0.001
0.01
0.1
1
10
抗体浓度(ug/ml)
ADCC活性
(来自供体#40的NK)
同种型对照
ADG106
人源化的OKT3
%裂解
40
20
0
0.0001
0.001
0.01
0.1
1
10
抗体浓度(ug/ml)

图15

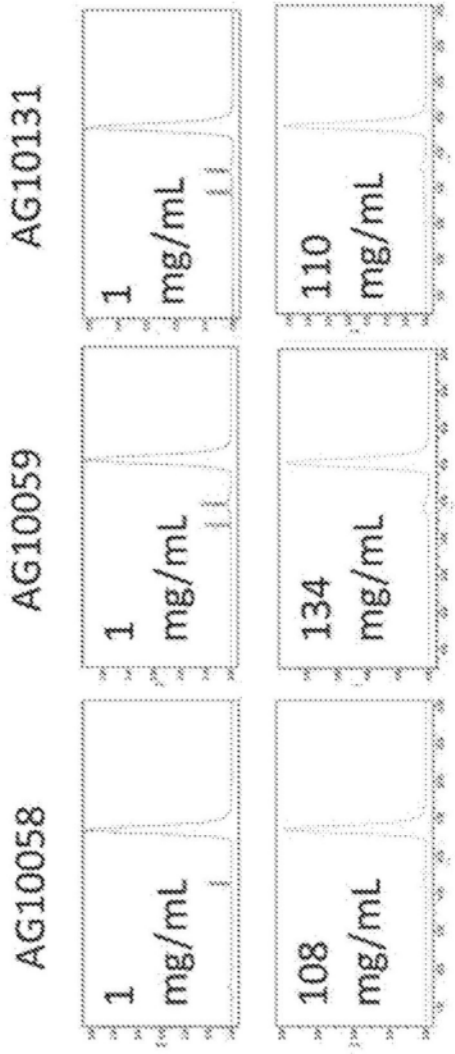

AG10058
AG10059
AG10131
1 mg/mL
1 mg/mL
1 mg/mL
108 mg/mL
134 mg/mL
110 mg/mL

图16

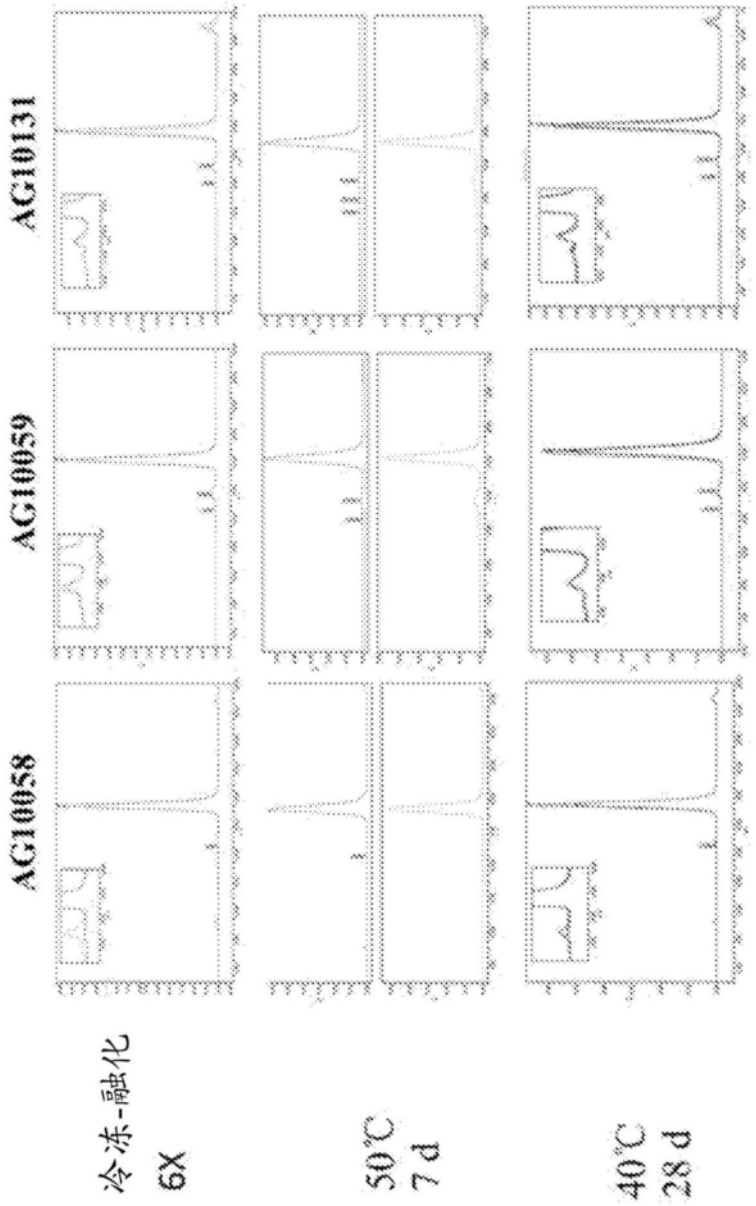


图17

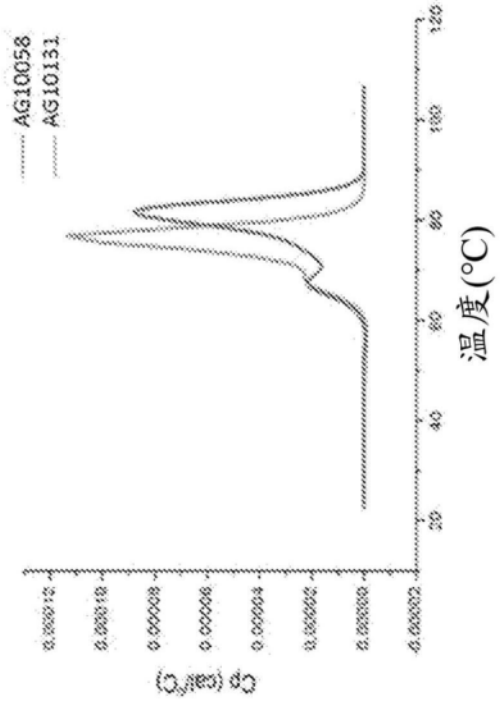


图18

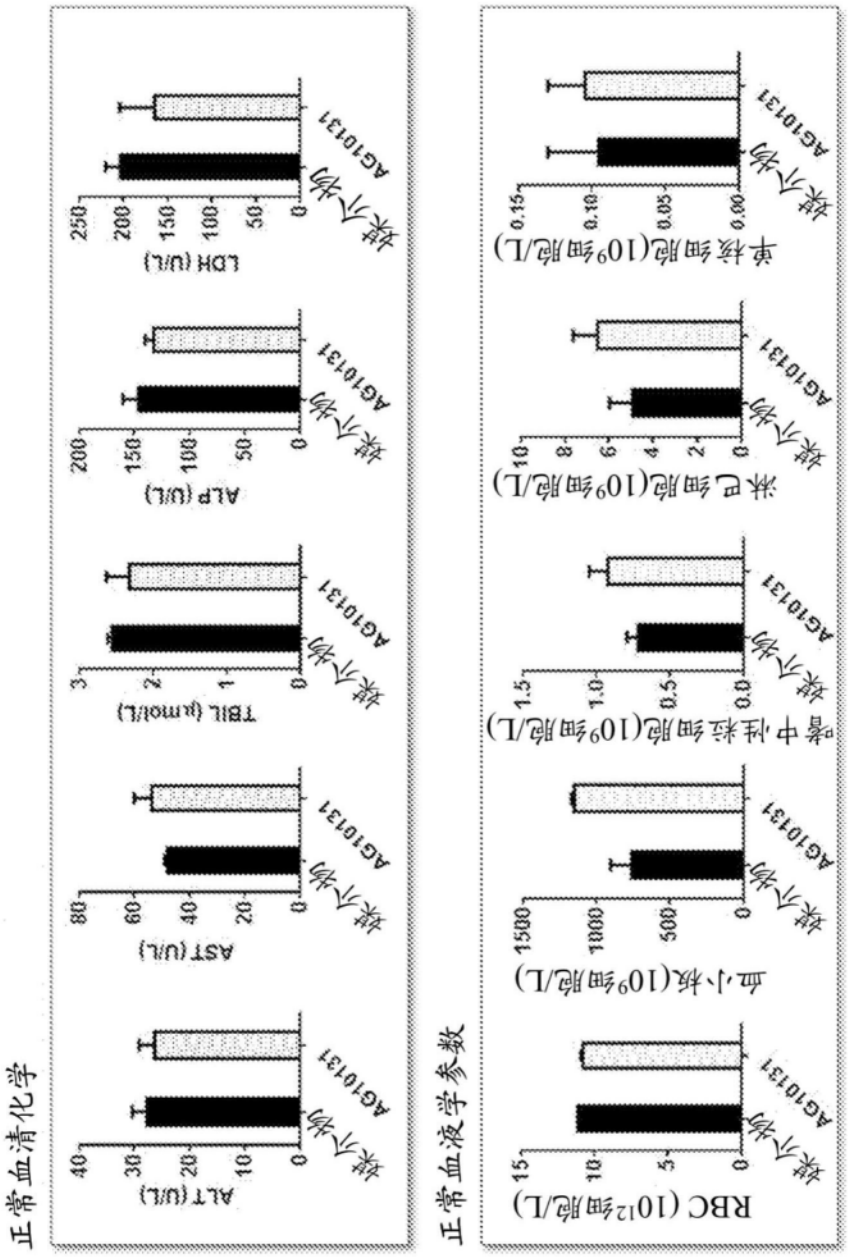

正常血液学参数
RBC (10¹²细胞/L)
血小板(10⁹细胞/L)
嗜中性粒细胞(10⁹细胞/L)
淋巴细胞(10⁹细胞/L)
单核细胞(10⁹细胞/L)
正常血清化学
ALT (U/L)
AST (U/L)
TBIL (μmol/L)
ALP (U/L)
LDH (U/L)
媒介物
AG101131

图19

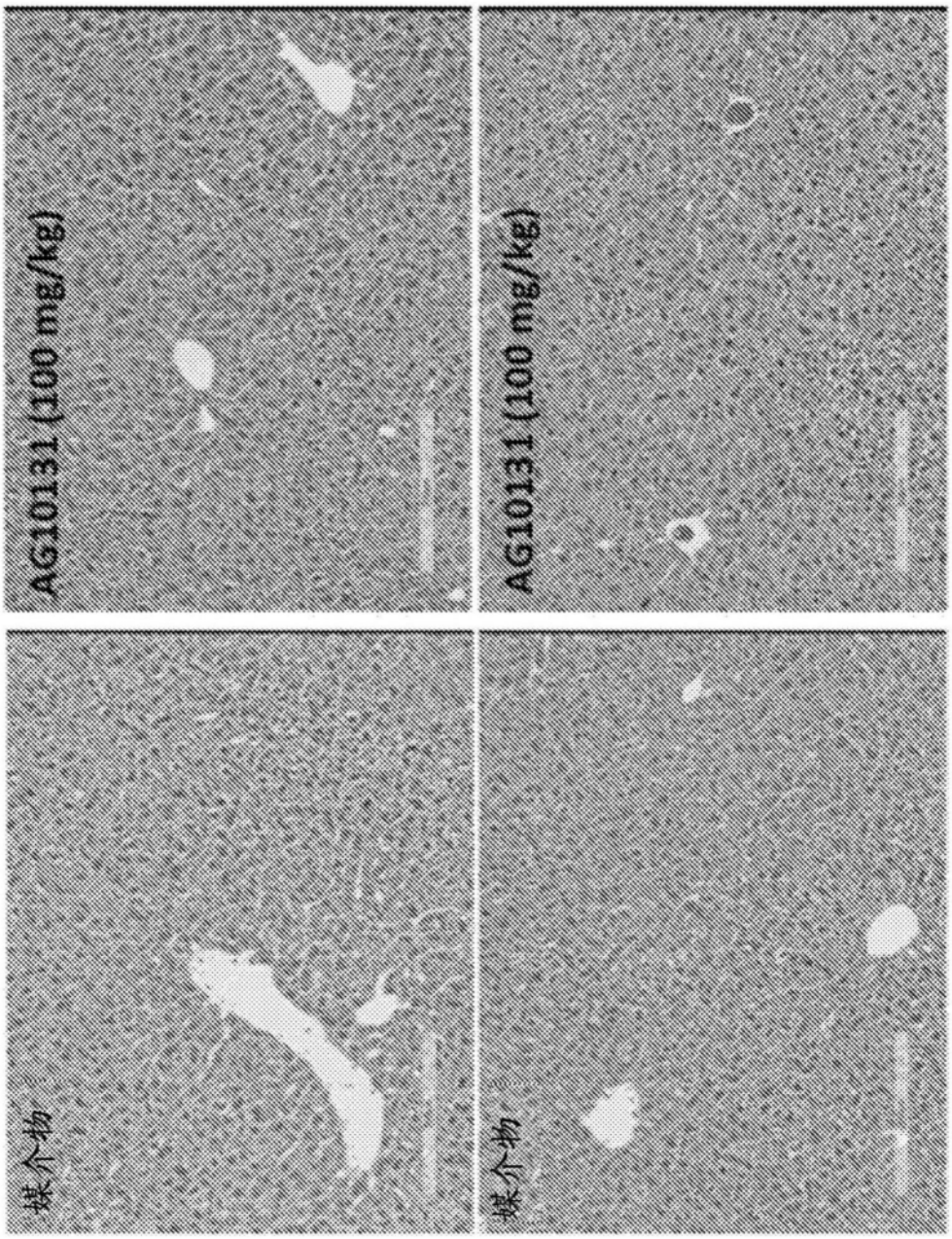


图20

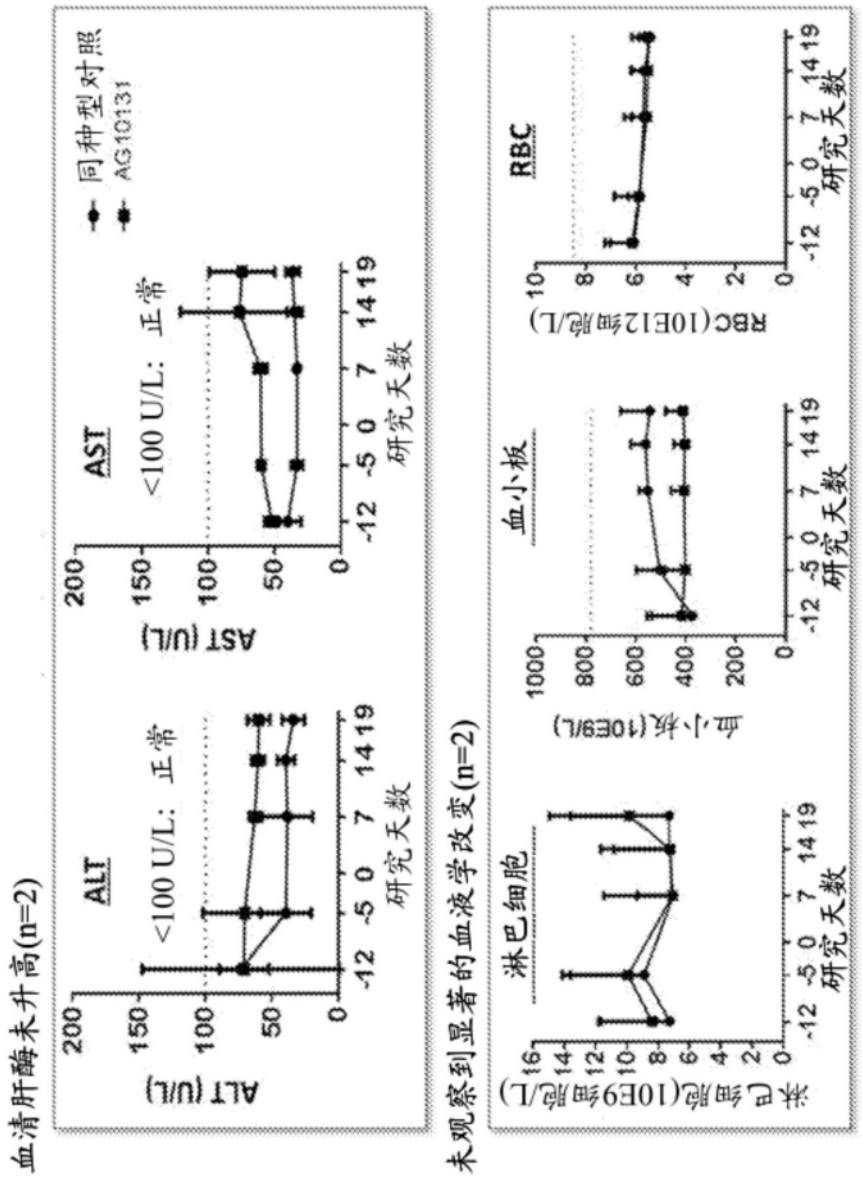

血清肝酶未升高(n=2)
ALT
<100 U/L: 正常
ALT (U/L)
AST
<100 U/L: 正常
AST (U/L)
同种型对照
AG10131
研究天数
未观察到显著的血液学改变(n=2)
淋巴细胞
嗜酸性细胞
中性粒细胞
血小板
血小板(10E9/L)
RBC
RBC(10E12细胞/L)

图21

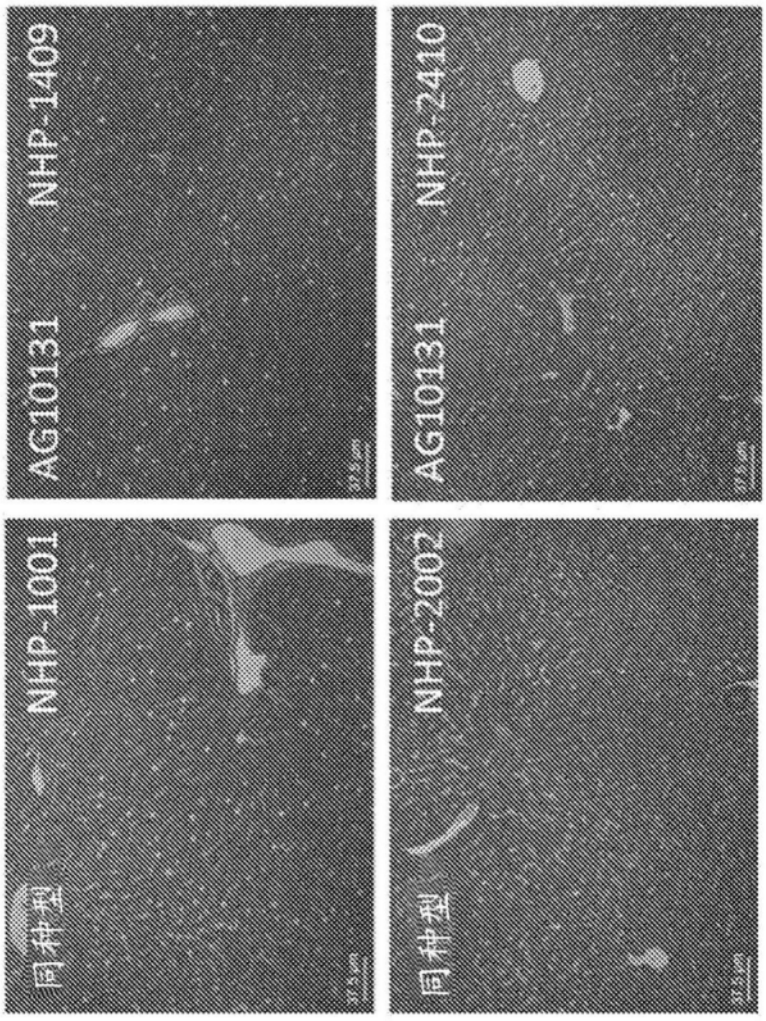
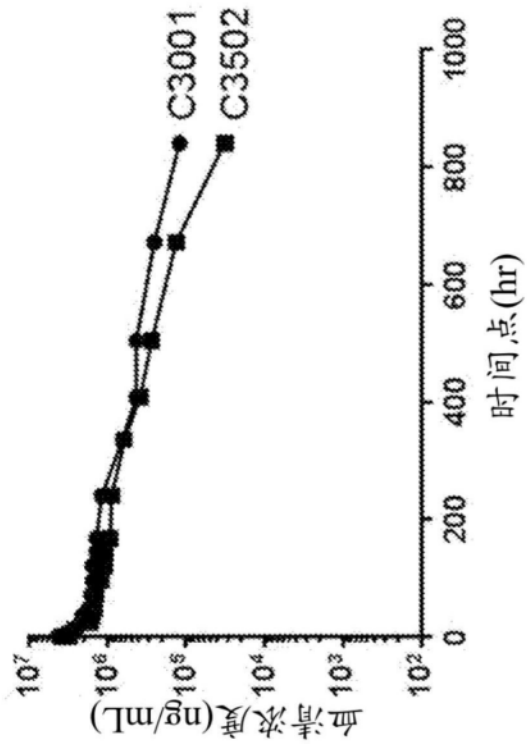


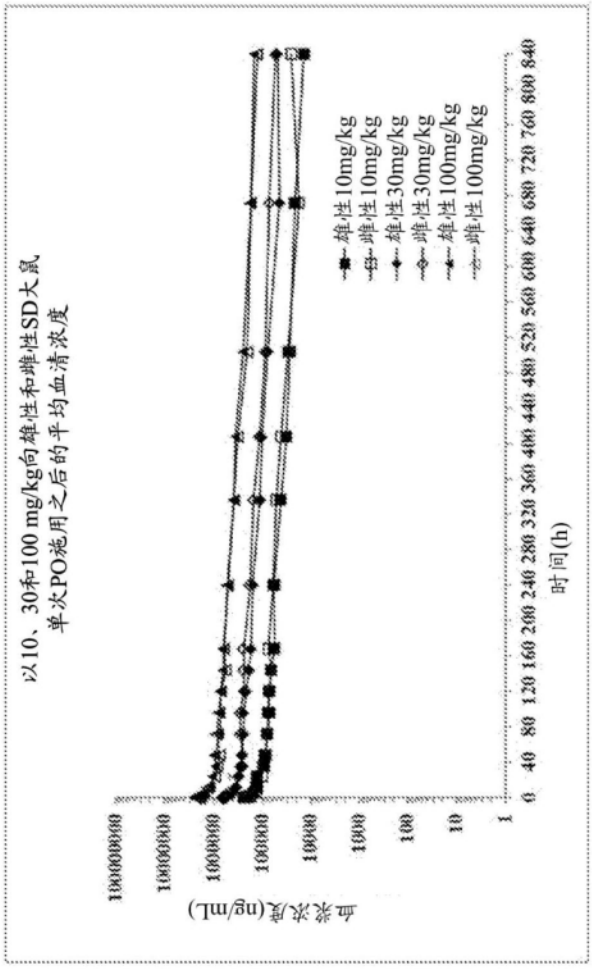
图22



剂量水平 (mg/kg)	$T_{1/2}$ (天)	C_{max} (μg/mL)	AUC_{inf} (天*μg/ml)
C3001(100)	7.3	4410	27099
C3502(100)	8.8	3770	22639

注：使用来自具有可能未受影响的血清浓度的动物的所有实际数据进行的双室分析

图23



PK参数								
强度	10 mg/kg		30 mg/kg		100 mg/kg	
性别	雄性	雌性	雄性	雌性	雄性	雌性
用于$T_{1/2}$计算的时间范围	48-840	24-840	24-840	24-840	72-840	24-840
C_0 (ng/mL)	248000	194000	645000	618000	2250000	2580000
$T_{1/2}$ (h)	304	351	287	311	300	277
Vd_{ss} (L/kg)	0.102	0.109	0.11	0.0949	0.108	0.106
Cl (mL/min/kg)	0.00399	0.00326	0.00419	0.0035	0.00419	0.00344
T_{last} (h)	840	840	840	840	840	840
$AUC_{0\text{-}last}$ (ng.h/mL)	35700000	38300000	99500000	119000000	336000000	325000000
$AUC_{0\text{-}inf}$ (ng.h/mL)	41700000	51200000	119000000	143000000	398000000	376000000

图24

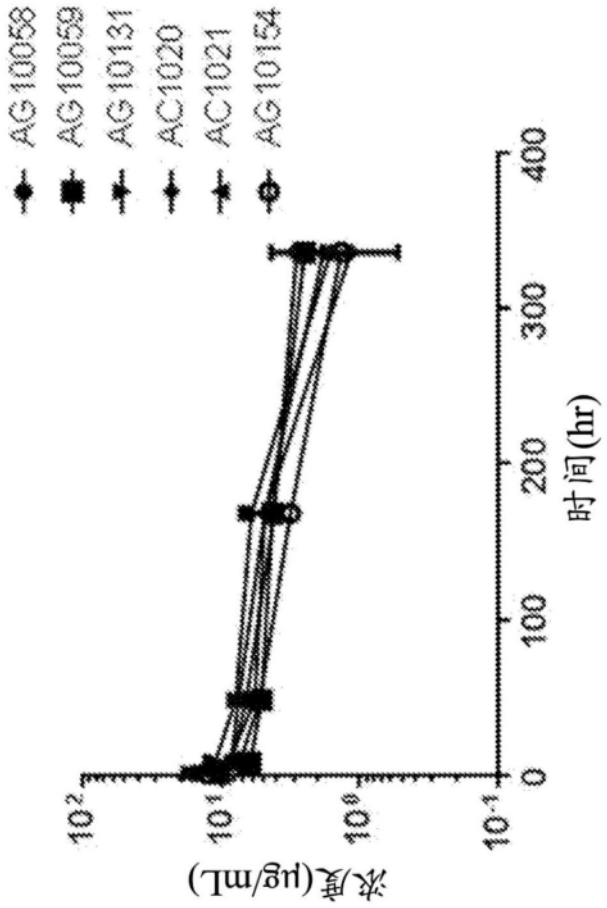

AG10058
AG10059
AG10131
AC1020
AC1021
AG10154
浓度(μg/mL)
10^{2}
10^{1}
10^{0}
10^{-1}
0
100
200
300
400
时间(hr)

图25