(19)中华人民共和国国家知识产权局

(12)发明专利

(10)授权公告号 CN 106536563 B
(45)授权公告日 2020.02.07

(21)申请号 201580011327.1

(22)申请日 2015.03.06

(65)同一申请的已公布的文献号
申请公布号 CN 106536563 A

(43)申请公布日 2017.03.22

(30)优先权数据
1403972.1 2014.03.06 GB

(85)PCT国际申请进入国家阶段日
2016.08.30

(86)PCT国际申请的申请数据
PCT/GB2015/050649 2015.03.06

(87)PCT国际申请的公布数据
WO2015/132604 EN 2015.09.11

(73)专利权人 UCL商务有限公司
地址 英国伦敦

(72)发明人 M.普莱 J.安德森 S.托马斯

(74)专利代理机构 北京市柳沈律师事务所 11105
代理人 邹宗亮 张文辉

(51)Int.Cl.
C07K 19/00(2006.01)
A61K 39/395(2006.01)
A61P 35/00(2006.01)
C12N 15/13(2006.01)
C07K 16/30(2006.01)
C07K 14/00(2006.01)
C07K 14/705(2006.01)
C07K 14/725(2006.01)

(56)对比文件
WO 2013040557 A2,2013.03.21,
WO 01/23573 A1,2001.04.05,
WO 2013040371 A3,2013.05.23,
CN 103145849 A,2013.06.12,
Ruth Esser et al..NK cells engineered to express a GD2-specific antigen receptordisplay built-in ADCC-like activity against tumour cells of neuroectodermal origin.《J. Cell. Mol. Med.》.2012,第16卷(第3期),第569-581页.
Eric Yvon et al..Immunotherapy of metastatic melanoma using genetically engineered GD2-specific T cells.《Clin Cancer Res.》.2009,第15卷(第18期),第5852-5860页.

审查员 李恩

权利要求书1页 说明书17页
序列表42页 附图10页

(54)发明名称

嵌合抗原受体

(57)摘要

提供包含双唾液酸神经节苷脂(GD2)结合域的嵌合抗原受体(CAR),所述GD2结合域包含a)具有互补决定区(CDR)的重链可变区(VH),所述CDRs具有以下序列;b)具有CDR的轻链可变区(VL),所述CDR具有以下序列:表达此类CAR的T细胞在一些癌症的治疗中有用。

CN 106536563 B

1.包含双唾液酸神经节苷脂(GD2)结合域的嵌合抗原受体(CAR),所述GD2结合域包含

a)重链可变区(VH),其包含具有如SEQ ID No:10所示的序列的VH域;和

b)轻链可变区(VL),其包含具有如SEQ ID No:12所示的序列的VL域。

2.根据权利要求1的CAR,其中所述GD2结合域包含如SEQ ID No.8所示的序列。

3.根据前述权利要求任一项的CAR,其包含跨膜域,所述跨膜域包含如SEQ ID No.13所示的序列。

4.根据前述权利要求任一项的CAR,其中GD2结合域和所述跨膜域通过间隔区连接。

5.根据权利要求4的CAR,其中所述间隔区包含以下的一种:人IgG1 Fc域;IgG1铰链;IgG1铰链-CD8茎部;或CD8茎部。

6.根据权利要求5的CAR,其中所述间隔区包含IgG1铰链-CD8茎部或CD8茎部。

7.根据权利要求5的CAR,其中所述间隔区包含IgG1 Fc域。

8.根据权利要求7的CAR,其中所述间隔区包含IgG1 Fc域,其包含如SEQ ID No.23或SEQ ID No.24所示的序列。

9.前述权利要求任一项的CAR,其还包含细胞内T细胞信号传导域。

10.根据权利要求9的CAR,其中所述细胞内T细胞信号传导域包含一个或多个以下胞内域:CD28胞内域;OX40和CD3-Zeta胞内域。

11.根据权利要求10的CAR,其中所述细胞内T细胞信号传导域包含所有以下胞内域:CD28胞内域;OX40和CD3-Zeta胞内域。

12.根据前述权利要求任一项的CAR,其包含如SEQ ID No.27至35任一项所示的序列。

13.编码根据前述权利要求任一项的CAR的核酸序列。

14.根据权利要求13的核酸序列,其为密码子优化的。

15.根据权利要求13或14的核酸序列,其包含如SEQ ID No.25所示的序列。

16.根据权利要求13至15任一项的核酸,其还编码自杀基因。

17.包含根据权利要求13至16任一项的核酸序列的载体。

18.表达根据权利要求1至12任一项的CAR的T细胞。

19.T细胞,其共表达根据权利要求1至12任一项的CAR和自杀基因。

20.根据权利要求19的T细胞,其中所述自杀基因是iCasp9或RQR8。

21.用于制造根据权利要求18至20任一项的T细胞的方法,其包括将根据权利要求13至16任一项的核酸导入T细胞的步骤。

22.药物组合物,其包含根据权利要求18至20任一项的T细胞以及药学上可接受的载体,稀释剂或赋形剂。

23.根据权利要求18至20任一项的T细胞在制造用于治疗癌症的药物中的用途。

24.根据权利要求23的用途,其中所述癌症为神经母细胞瘤。

嵌合抗原受体

发明领域

[0001] 本发明涉及嵌合抗原受体(CAR),其结合癌抗原双唾液酸神经节苷脂(disialoganglioside)(GD2)。表达此类CAR的T细胞在癌症疾病,例如神经母细胞瘤的治疗中是有用的。

[0002] 发明背景

[0003] 双唾液酸神经节苷脂(GD2,pubchem:6450346)是一种含唾液酸的鞘糖脂,主要在细胞表面上表达。该碳水化合物抗原的功能尚未完全理解;然而,认为其在肿瘤细胞对细胞外基质蛋白的附着中发挥重要作用。GD2在神经母细胞瘤(neuroblastoma)上密集,同质且几乎普遍表达的。在正常组织中,GD2的表达很大程度上限制在皮肤黑色素细胞,和外周疼痛纤维髓鞘。在CNS中,GD2似乎是胚胎抗原,但发现在分散的少突胶质细胞中和在垂体后叶中暗淡地表达。这使得GD2非常适合用于靶向抗肿瘤疗法。

[0004] 抗GD2抗体已经在神经母细胞瘤的治疗中广泛地测试。两个克隆和它们的衍生物是目前临床使用的:3F814和3F8。在同种型转换为IgG2a(14g2a)和最终与人IgG1嵌合以形成ch4.18后,已经测试了作为小鼠IgG3的另一个克隆14.187。后者抗体在随机化研究中已经产生了明确的效力:US Children's Oncology Group报道了在具有高风险神经母细胞瘤的儿童中ch14:18的随机化III期研究,所述儿童在最初治疗后已经实现了放射学缓解(radiological remission)。在这些患者中,在ch14:18组(arm)中,EFS中存在20%的改善,其中平均随访2.1年。重要地,神经毒性是这些试剂的主要剂量限制毒性,所述神经毒性最常见作为慢性疼痛诱导神经病,而不太常见作为眼肌麻痹(ophthalmoplegia)。

[0005] 这些治疗性mAb继续被完善:已经描述了衍生于ch14.18的IL-2免疫细胞因子。这是相当毒性的试剂,对微量残存疾病具有一些效果,但无一针对大体积的疾病(bulky disease)。Ch14.18已经完全人源化且其Fc经突变以抑制补体活化。Ch14.18的这一人源化版本在临床研究中但仅非常有限的数据是可用的。也描述了3F8抗体的人源化。尽管来自GD2血清疗法的临床数据是令人鼓舞的,但是持续的完全缓解仍然有限,且除了在最小程度疾病背景中外,对于抗体在临床上有用的作用没有证据。

[0006] 因此,对于治疗神经母细胞瘤和其它表达GD2的癌症的改进的治疗方法存在需要。

[0007] 嵌合抗原受体(CAR)

[0008] 嵌合抗原受体是常见形式为将单克隆抗体(mAb)的特异性移植到T细胞的效应功能的蛋白质。它们通常的形式是I型跨膜域蛋白的修饰,具有抗原识别氨基末端,间隔区,跨膜域、与它们全部连接的复合胞内域(endodomain),其传输T细胞存活和活化信号(见图1a)。

[0009] 这些分子的最常见形式是识别靶抗原的来源于单克隆抗体的单链可变片段(scFv)通过间隔区和跨膜域融合至信号传导胞内域的融合物。响应scFv对其靶标的识别,此类分子导致T细胞的活化。当T细胞表达此类CAR时,它们识别并杀伤表达所述靶抗原的靶细胞。针对肿瘤相关抗原已经开发了几种CAR,且使用此类表达CAR的T细胞的过继转移方法目前在临床试验中用于多种癌症的治疗。

[0010] 已经描述了针对GD2的嵌合抗原受体,其抗原结合域基于scFv14g2a(WO 2013/040371和Yvon等人(2009,Clin Cancer Res 15:5852-5860))。

[0011] 表达14g2a-CD28-OX40-ζCAR的人T细胞被证明具有一些抗肿瘤活性,但不能完全地根除该疾病(Yvon等人(2009)如上文)。

[0012] 本发明人寻求制造具有改进的特性的备选GD2-靶向CAR。

[0013] 附图简述

[0014] 图1-嵌合抗原受体(CAR)设计。

[0015] (a)CAR的一般性架构:结合域识别抗原;间隔区将结合域从细胞表面升高;跨膜域将蛋白锚定到膜上而胞内域传输信号。(b)至(d):CAR胞内域的不同代和排列:(b)初始设计通过FcεR1-γ或CD3胞内域仅传输ITAM信号,而后来的设计以顺式(in cis)传输额外的(c)一种或(d)两种共刺激信号。

[0016] 图2-构建的抗GD2CARs的变体:(a)使用小鼠KM666抗体作为scFv与人IgG1间隔区和CD28-OX40-Zeta胞内域的抗GD2CAR;(b)使用Nakamura人源化的抗体huKM666与(a)为相同形式的抗GD2CAR;(c)与(b)相同的形式,除了Fc域经修饰以去除Fc受体识别基序;(d)与(c)相同的形式,除了间隔区为IgG1铰链-CD8茎部;(e)与(c)相同的形式,除了间隔区为仅CD8茎部;(f)与(c)相同的形式,除了间隔区为仅IgG1铰链。

[0017] 图3-基于muKM666和huKM666的CAR的比较。(a)来自3个正常供体的外周血T细胞上的表达;(b)这些facs图的平均荧光强度示作柱状图;(c)使用非转导的,经muKM666和经huKM666转导的T细胞作为效应细胞针对A204(GD2阴性),和LAN-1(GD2阳性)靶标的铬释放测定;(d)来自相同激发(challenge)的IL-2产生;(e)来自相同激发的干扰素-gamma产生;和(f)来自相同激发的倍数增殖(fold-proliferation)。

[0018] 图4.(a)允许CD34标志物基因与CAR的1:1共表达的逆转录病毒构建体;(b)相对于CD34标志物基因的CAR表达(HA标签)的流式细胞分析;(c)非转导的T细胞和使用3种不同CAR变体转导的T细胞针对GD2阳性靶标(LAN-1),和GD2阴性靶标(A204)的铬释放测定;(d)干扰素-gamma释放;(e)IL-2释放;和(f)相同靶标和效应细胞的增殖。

[0019] 图5-将FcR结合破坏突变引入Fc间隔区:(a)引入的突变;(b)通过抗Fc染色确认的CAR表达:非转导的,wt和突变的;(c)使用非转导的,wt Fc和突变的Fc抗GD2CAR T细胞对GD2阴性和GD2阳性靶标的杀伤;(d)使用表达FcR的细胞系THP-1对非转导的,wt Fc和突变的Fc抗GD2T细胞的活化;响应非转导的,wt Fc和突变的Fc CAR T细胞由THP-1细胞系的IL-1Beta释放。

[0020] 图6-表达基因盒的优化

[0021] (a)引入以下盒中的图谱优化:SAR或CHS4;(b)具有使用wt或密码子优化的开放读码框的不同修饰的CAR的代表性表达。SAR构建体给出表达的紧密峰(tight peak),其为所需的。(c)来自3个正常供体的该FACS数据的条形图表示。

[0022] 图7-不同胞内域的比较

[0023] 比较了三种不同的嵌合抗原受体。受体都由huK666scFv,突变以去除FcR结合的IgG1的Fc域和CD8跨膜域构成。CAR“28tmZ”具有CD3Zeta胞内域;“28Z”具有复合CD28-CD3Zeta胞内域;“28OXZ”具有包含CD28,OX40和CD3Zeta的复合胞内域。使用这些构建体用相似滴度的逆转录病毒载体转导来正常供体的外周血T细胞。比较了这些不同T细胞系,连

同非转导的T细胞作为对照。使用A204细胞(GD2阴性的横纹肌肉瘤细胞系),和LAN-1细胞(GD2阳性的神经母细胞瘤细胞系)激发T细胞。增殖和细胞因子释放表明受体活性为28tmZ<28Z<28OXZ。

[0024] 图8-与iCasp9自杀基因共表达

[0025] (a)使用FMD-2A序列,iCasp9与抗GD2CAR的共表达;(b)单独和使用CID处理后的NTT细胞,经GD2CAR转导的T细胞和经iCasp9-2A-GD2CART细胞中的CAR表达;(c)使用或不使用CID处理的未转导的,经GD2CAR转导的和经iCasp9-2A-GD2CAR转导的T细胞对GD2阳性(LAN-1)和阴性(A204)靶标的杀伤。5位正常供体T细胞的平均值。

[0026] 图9-与RQR8自杀基因的共表达

[0027] (a)将CAR huK666Fc与RQR8分选自杀基因(sort-suicide gene)在逆转录病毒载体中共表达。(b)使用该逆转录病毒载体转导T细胞并通过使用多克隆抗Fc和单克隆抗体QBend10染色转导的细胞确认CAR和RQR8的共表达。(c)在利妥昔单抗(Rituximab)和补体的存在下,可以将来自这些T细胞的CAR阳性群体耗竭。(d)使用利妥昔单抗耗竭的T细胞不再识别表达GD2的靶标。

[0028] 图10-(a)表达GM3合酶和GD2合酶的双顺反子载体。(b)使用该载体转导的SupT1细胞成为GD2阳性(非转导的空心图;转导的灰色图)。

[0029] 图11-在以下群体(cohort)中小鼠中的单个肿瘤的生长曲线:左上:具有表达GD2的CT26肿瘤的小鼠,接受抗GD2CAR脾细胞;右上:表达GD2的CT26肿瘤,接受假(mock)转导的脾细胞;左下:GD2阴性(wt)CD26肿瘤与抗GD2CAR脾细胞;和右下:表达GD2的CT26肿瘤,不接受脾细胞。

[0030] 图12-氨基酸序列

[0031] A.图2中示作(a)的抗GD2CAR(muKM666-HCH2CH3-CD28OXZ-SEQ ID No.26)

[0032] B.图2中示作(b)的抗GD2CAR(huKM666-HCH2CH3-CD28OXZ-SEQ ID No.27)

[0033] C.图2中示作(c)的抗GD2CAR(huKM666-HCH2CH3pvaa-CD28OXZ-SEQ ID No.28)

[0034] D.图2中示作(d)的抗GD2CAR(huKM666-HSTK-CD28OXZ-SEQ ID No.29)

[0035] E.图2中示作(e)的抗GD2CAR(huKM666-STK-CD28XOXZ-SEQ ID No.30)

[0036] F.图2中示作(e)的抗GD2CAR(huKM666-HNG-CD28OXZ-SEQ ID No.31)

[0037] G.图2(c)中所示的抗GD2CAR但具有第1代胞内域(huKM666-HCH2CH3pvaa-CD28tmZ-SEQ ID No.32)

[0038] H.图2(c)中所示的抗GD2CAR但具有第2代胞内域(huMK666-HCH2CH3pvaa-CD28Z-SEQ ID No.33)

[0039] I.与iCasp9自杀基因共表达的抗GD2CAR-SEQ ID No.34

[0040] J.与RQR8自杀基因共表达的抗GD2CAR-SEQ ID No.35

[0041] 图13-GD2的结构

[0042] 图14-huK666和14g2a CARs的比较。(a)构建体的图谱

[0043] 测试:在原代T细胞中测试了两种构建体。两种都是编码与FMD-2A样序列共表达的RQR8和第2代GD2CAR的逆转录病毒载体。构建体之间仅有的差异为在一个中,scFv为huK666而在另一个中为14g2a。使用这些构建体转导的T细胞使用A204(GD2阴性横纹肌肉瘤细胞系),和LAN-1(GD2阳性细胞系)之任一以1:1激发。(b)在24小时,从上清测量干扰素-gamma。

huK666CAR T细胞产生更多的IF-G。(c)一周后对T细胞计数,huK666表现出更多增殖。

[0044] 图15-基于huK666或14g2a的第2代CAR与神经母细胞瘤细胞系LAN1之间共培养的流式细胞分析。(a)实验的设置。共培养一周后,收获细胞并通过流式细胞术分析。CD45表达允许区分淋巴样细胞和非淋巴样细胞,其中CD45-细胞为LAN-1细胞。使用CD3/QBEND/10进一步染色允许对CAR T细胞的计数。(b)单独T细胞;(c)NT T细胞和LAN-1细胞;(d)huK666-28-Z CAR T细胞和LAN-1细胞;(e)14g2a-28-Z CAR T细胞和LAN-1细胞。在14g2a CAR T细胞共培养中看到了LAN-1残留物。

[0045] 发明简述

[0046] 本发明人已经构建了靶向GD2的新的嵌合抗原受体(CAR),其包含基于K666抗体的GD2结合域。

[0047] 抗GD2抗体14g2a可以视为黄金标准,因为其用作治疗性抗体且是CAR研究中迄今测试的唯一scFv(PMID:18978797)。本发明人比较了基于14g2a和huK666的第二代形式的CAR,因为这是在临床研究中最广泛使用的CAR形式。我们发现huK666CAR T细胞比14g2a等同物释放更多IFN-γ,增殖更好且杀伤更完全。

[0048] 因此,在本发明的第一个方面中提供包含双唾液酸神经节苷脂(GD2)结合域的嵌合抗原受体(CAR),其包含

[0049] a)具有互补决定区(CDRs)的重链可变区(VH),所述CDR具有以下序列:

[0050] CDR1-SYNIH(SEQ ID No.1);

[0051] CDR2-VIWAGGSTNYNSALMS(SEQ ID No.2)

[0052] CDR3-RSDDYSWFAY(SEQ ID No.3);和

[0053] b)具有CDRs的轻链可变区(VH),所述CDR具有以下序列:

[0054] CDR1-RASSSVSSSYLH(SEQ ID No.4);

[0055] CDR2-STSNLAS(SEQ ID No.5)

[0056] CDR3-QQYSGYPIT(SEQ ID No.6)

[0057] GD2结合域可以包含具有如SEQ ID No.9或SEQ ID NO 10所示序列的VH域;或具有如SEQ ID No 11或SEQ ID No.12所示序列或具有至少90%序列同一性的其变体的VL域,,所述变体保留了i)结合GD2和ii)诱导T细胞信号传导的能力。

[0058] GD2结合域可以包含如SEQ ID No 7或SEQ ID No.8所示的序列,或具有至少90%序列同一性的其变体,其保留了i)结合GD2和ii)诱导T细胞信号传导的能力。

[0059] 跨膜域可以包含如SEQ ID No.13所示的序列或具有至少90%序列同一性的其变体,其保留了i)结合GD2和ii)诱导T细胞信号传导的能力。

[0060] GD2结合域和所述跨膜域可以通过间隔区连接。

[0061] 间隔区可以包含以下之一:人IgG1Fc域;IgG1铰链;IgG1铰链-CD8茎部;或CD8茎部。

[0062] 间隔区可以包含IgG1铰链-CD8茎部;或CD8茎部。

[0063] 间隔区可以包含IgG1Fc域或其变体。

[0064] 间隔区可以包含IgG1Fc域,其包括如SEQ ID No.23或SEQ ID No.24所示的序列或具有至少80%序列同一性的其变体。

[0065] CAR可以包含细胞内T细胞信号传导域或与细胞内T细胞信号传导域相关。

[0066] 细胞内T细胞信号传导域可以包含一个或多个以下胞内域:CD28胞内域;OX40和CD3-Zeta胞内域。

[0067] 细胞内T细胞信号传导域可以包含所有以下胞内域:CD28胞内域;OX40和CD3-Zeta胞内域。

[0068] CAR可以包含SEQ ID No.26至35任一项所示的序列,或具有至少80%序列同一性但保留了i)结合GD2和ii)诱导T细胞信号传导的能力的其变体。

[0069] 在第二个方面中,本发明提供核酸序列,其编码根据本发明的第一个方面的CAR。

[0070] 核酸序列可以是密码子优化的。

[0071] 核酸序列可以包含如SEQ ID No 25所示的序列或具有至少90%序列同一性的其变体。

[0072] 核酸可以也编码自杀基因。

[0073] 在第三个方面中,本发明提供载体,其包含根据本发明的第二个方面的核酸序列。

[0074] 在第四个方面中,本发明提供细胞,其表达根据本发明的第一个方面的CAR。所述细胞可以是细胞溶解性免疫细胞,如T细胞或天然杀伤(NK)细胞。

[0075] 细胞可以共表达根据本发明的第一个方面的CAR和自杀基因。

[0076] 自杀基因可以是,例如iCasp9或RQR8。

[0077] 在第五个方面中,本发明提供用于制造根据本发明的第四个方面的细胞的方法,其包括将根据本发明的第二个方面的核酸导入细胞中。

[0078] 在第六个方面中,本发明提供药物组合物,其包含根据本发明的第三个方面的载体,或根据本发明的第二个方面的细胞,连同药学上可接受的载体,稀释剂或赋形剂。

[0079] 在第七个方面中,本发明提供用于治疗癌症的方法,其包括向受试者施用根据本发明的第三个方面的载体或根据本发明的第四个方面的细胞的步骤。

[0080] 癌症可以是神经母细胞瘤。

[0081] 在第八个方面中,本发明提供根据本发明的第三个方面的载体或根据本发明的第四个方面的细胞,用于治疗癌症中的用途。

[0082] 在第九个方面中,本发明提供根据本发明的第三个方面的载体或根据本发明的第四个方面的细胞在制造用于治疗癌症的药物中的用途。

[0083] 在第十个方面中,本发明提供用于制造表达GD2的细胞的方法,其包括将编码GM3合酶的核酸和编码GD2合酶的核酸导入细胞中。

[0084] 在第十一个方面中,本发明提供表达GD2的细胞,其包含编码GM3合酶的异源性核酸和编码GD2合酶的异源性核酸。

[0085] 在第十二个方面中,本发明提供用于体外刺激根据本发明的第四个方面的细胞的方法,其包括使得所述细胞与根据本发明的第十一个方面的表达GD2的细胞接触的步骤。

[0086] 在第十三个方面,本发明提供表达CAR的表达基因盒,所述CAR包括支架附着区(scaffold attachment region)(SAR)。

[0087] 表达基因盒可以表达根据本发明的第一个方面的CAR。

[0088] 发明详述

[0089] 嵌合抗原受体(CAR)

[0090] 嵌合抗原受体,也称为嵌合T细胞受体,人工T细胞受体和嵌合免疫受体,是工程化

的受体,其将任意特异性嫁接到免疫效应细胞上。在经典的CAR中,将单克隆抗体嫁接到T细胞上。可以使用例如逆转录病毒将编码CAR的核酸转移到T细胞中。以这种方式,可以产生大量癌症特异性的T细胞用于过继细胞转移。该方法的I期临床研究表现出效力。

[0091] CAR的靶抗原结合域通常经由间隔区和跨膜域融合至信号传导胞内域。当所述CAR结合靶抗原时,这导致活化信号传输至它在上面表达的T细胞。

[0092] 本发明的CAR包含GD2结合域,其基于KM666单克隆抗体(Nakamura等人,(2001)Cancer Immunol.Immunother.50:275-284)。

[0093] 本发明的CAR包含GD2结合域,其包含

[0094] a)具有互补决定区(CDRs)的重链可变区(VH),所述CDR具有以下序列:

[0095] CDR1-SYNIH(SEQ ID No.1);

[0096] CDR2-VIWAGGSTNYNSALMS(SEQ ID No.2)

[0097] CDR3-RSDDYSWFAY(SEQ ID No.3);和

[0098] b)具有CDRs的轻链可变区(VH),所述CDR具有以下序列:

[0099] CDR1-RASSSVSSSYLH(SEQ ID No.4);

[0100] CDR2-STSNLAS(SEQ ID No.5)

[0101] CDR3-QQYSGYPIT(SEQ ID No.6)。

[0102] 将一个或多个突变(取代,添加或缺失)引入所述或每个CDR而不负面影响GD2结合活性可以是可能的。每个CDR可以例如具有一个,两个或三个氨基酸突变。

[0103] 本发明的CAR可以包含以下氨基酸序列之一:

[0104] SEQ ID No.7(鼠KM666序列)

[0105] QVQLKESGPVLVAPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLSISKDNSKSQVFLQMNSLQTDDTAMYYCAKRSDDYSWFAYWGQGTLVTVSASGGGGSGGGGSGGGGSENVLTQSPAIMSASPGEKVTMTCRASSSVSSSYLHWYQQKSGASPKVWIYSTSNLASGVPGRFSGSGSGTSYSLTISSVEAEDAATYYCQQYSGYPITFGAGTKVEVKR

[0106] SEQ ID No.8(人源化的KM666序列)

[0107] QVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKR

[0108] 本发明的CAR可以包含以下VH序列之一:

[0109] SEQ ID No.9(鼠KM666VH序列)

[0110] QVQLKESGPVLVAPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLSISKDNSKSQVFLQMNSLQTDDTAMYYCAKRSDDYSWFAYWGQGTLVTVSA

[0111] SEQ ID No.10(人源化的KM666VH序列)

[0112] QVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSS

[0113] 本发明的CAR可以包含以下VL序列之一:

[0114] SEQ ID No.11(鼠KM666VL序列)

[0115] ENVLTQSPAIMSASPGEKVTMTCRASSSVSSSYLHWYQQKSGASPKVWIYSTSNLASGVPGRFSGSGSG

TSYSLTISSVEAEDAATYYCQQYSGYPITFGAGTKVEVK

[0116] SEQ ID No.12(人源化的KM666VH序列)

[0117] ENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSG TDYTLTISSLQPEDFATYYCQQYSGYPITFGQGTKVEIK

[0118] 本发明的CAR可以包含如SEQ ID No.7,8,9,10,11或12所示的序列的变体,具有至少80,85,90,95,98或99％序列同一性,只要所述变体序列保留了结合GD2的能力(当与互补VL或VH域结合时,若适当的话)。

[0119] 两个多肽序列之间的百分比同一性可以通过例如BLAST的程序容易地确定,其在http://blast.ncbi.nlm.nih.gov免费可用。

[0120] 跨膜域

[0121] 本发明的CAR还可以包含跨越膜的跨膜域。其可以包含疏水alpha螺旋。跨膜域可以来源于CD28,其给予良好的受体稳定性。

[0122] 跨膜域可以包含如SEQ ID No.13所示的序列。

[0123] SEQ ID No.13

[0124] FWVLVVVGGVLACYSLLVTVAFIIFWV

[0125] 细胞内T细胞信号传导域(胞内域)

[0126] 胞内域是CAR的信号传输部分。抗原识别后,受体簇集且信号传输至细胞。最普遍使用的胞内域组分是CD3-zeta的,其含有3个ITAMs。在抗原结合后,其传输活化信号至T细胞。CD3-zeta可以不提供完全有能力的活化信号,可以需要另外的共刺激信号。例如,嵌合CD28和OX40可以与CD3-Zeta一起使用以传输增殖/存活信号,或所有三种可以一起使用。

[0127] 本发明的CAR的胞内域可以包含CD28胞内域和OX40及CD3-Zeta胞内域。

[0128] 本发明的CAR的跨膜和细胞内T细胞信号传导域(胞内域)可以包含如SEQ ID No.14,15,16,17或18所示的序列,或具有至少80％序列同一性的其变体。

[0129] SEQ ID No.14(CD28胞内域)

[0130] RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY

[0131] SEQ ID No.15(CD40胞内域)

[0132] RSRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI

[0133] SEQ ID No.16(CD3zeta胞内域)

[0134] RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0135] SEQ ID No.17(CD28Z)

[0136] RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR

[0137] SEQ ID No.18(CD28OXZ)

[0138] RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPGGGSFRTPIQEEQAD AHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0139] 变体序列可以与SEQ ID No.13,14,15,16,17或18具有至少80％,85％,90％,

95％,98％或99％序列同一性,只要所述序列提供有效的跨膜域/细胞内T细胞信号传导域。

[0140] 信号肽

[0141] 本发明的CAR可以包含信号肽,使得当CAR表达在细胞如T细胞内时,初生蛋白被引导至内质网和随后到细胞表面,在那里其被表达。

[0142] 信号肽的核心可以含有一长段疏水性氨基酸,其具有形成单个α螺旋的倾向。信号肽可以以短的,带正电的氨基酸段开始,其帮助转位期间执行多肽的正确拓扑结构。在信号肽的末端,通常是由信号肽酶识别并切割的氨基酸段。信号肽可以在转位期间或转位完成后切割,以生成游离的信号肽和成熟蛋白。接着,游离的信号肽被特定蛋白酶消化。

[0143] 信号肽可以在分子的氨基末端。

[0144] 本发明的CAR可以具有以下通式:

[0145] 信号肽-GD2结合域-间隔域-跨膜域-细胞内T细胞信号传导域

[0146] 信号肽可以包含SEQ ID No.19或具有5,4,3,2或1个氨基酸突变(插入,取代或添加)的其变体,只要信号肽仍然发挥功能以引起CAR的细胞表面表达。

[0147] SEQ ID No.19:METDTLLLWVLLLWVPGSTG

[0148] SEQ ID No.19的信号肽是紧密并高度有效的。预测它在末端甘氨酸之后产生约95％的切割,产生由信号肽酶的有效移除。

[0149] 间隔区

[0150] 本发明的CAR可以包含间隔区序列以连接GD2结合域与跨膜域并在空间上分开GD2结合域与胞内域。柔性间隔区允许GD2结合域在不同方向定向使得能够进行GD2结合。

[0151] 间隔区序列可以例如包含IgG1Fc区,IgG1铰链或CD8茎部,或其组合。或者该接头可以包含备选序列,其具有与IgG1Fc区,IgG1铰链或CD8茎部相似的长度和/或结构域间隔特性。

[0152] 可以改变人IgG1间隔区以去除Fc结合基序。

[0153] 这些间隔区的氨基酸序列的例子在以下给出:

[0154] SEQ ID No.20(人IgG1铰链-CH2CH3)

[0155] AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKD

[0156] SEQ ID No.21(人CD8茎部):

[0157] TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

[0158] SEQ ID No.22(人IgG1铰链):

[0159] AEPKSPDKTHTCPPCPKDPK

[0160] SEQ ID No.23(IgG1铰链-Fc)

[0161] AEPKSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPK

[0162] SEQ ID No.24(IgG1铰链-Fc经修饰以去除Fc结合基序)

[0163] AEPKSPDKTHTCPPCPAPPVA*GPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGKKDPK

[0164] 修饰的残基用下划线标出;*表示缺失。

[0165] GD2

[0166] GD2是在神经外胚层来源的肿瘤(包括人神经母细胞瘤和黑素瘤)上表达的双唾液酸神经节苷脂,在正常组织上具有高度限制的表达,主要在人的小脑和外周神经上。

[0167] GD2的相对肿瘤特异性表达使得其为用于免疫疗法的合适靶标。

[0168] 核酸序列

[0169] 本发明的第二个方面涉及核酸序列,其编码本发明的第一个方面的CAR。

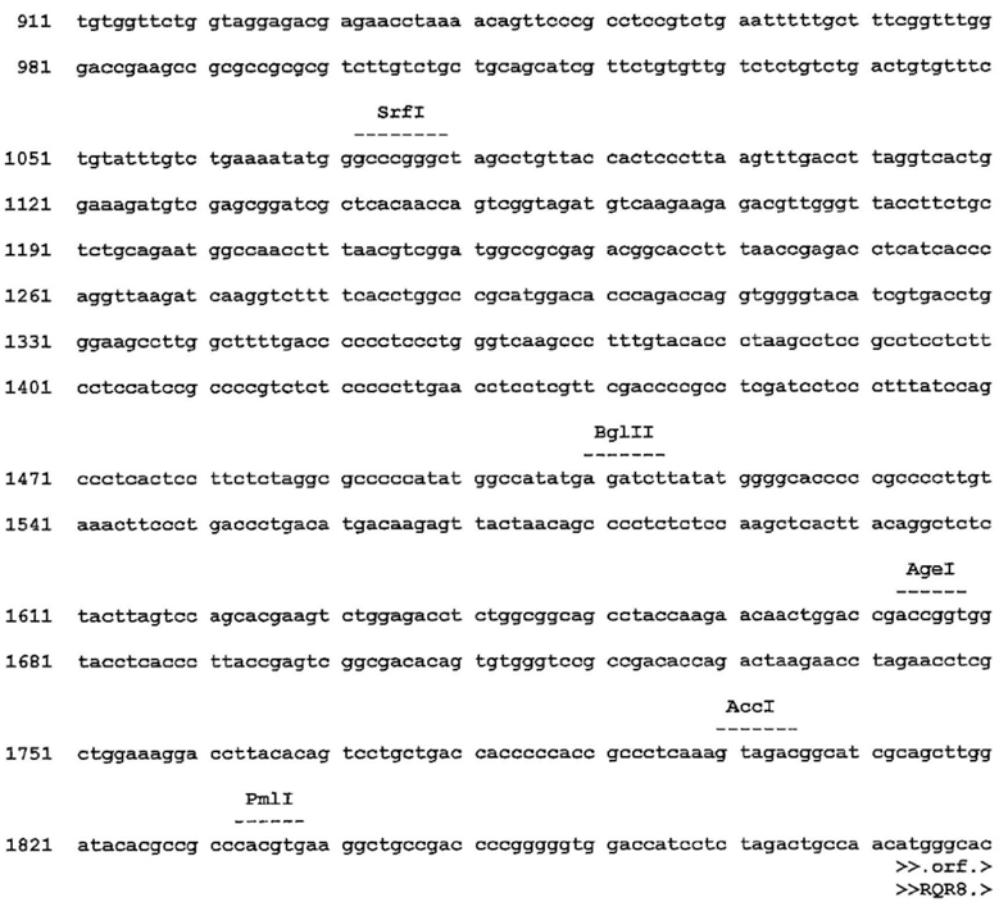
[0170] 核酸序列可以能够编码具有如SEQ ID No.26-35任一项所示的氨基酸序列的CAR。

[0171] 核酸序列可以包含以下序列:

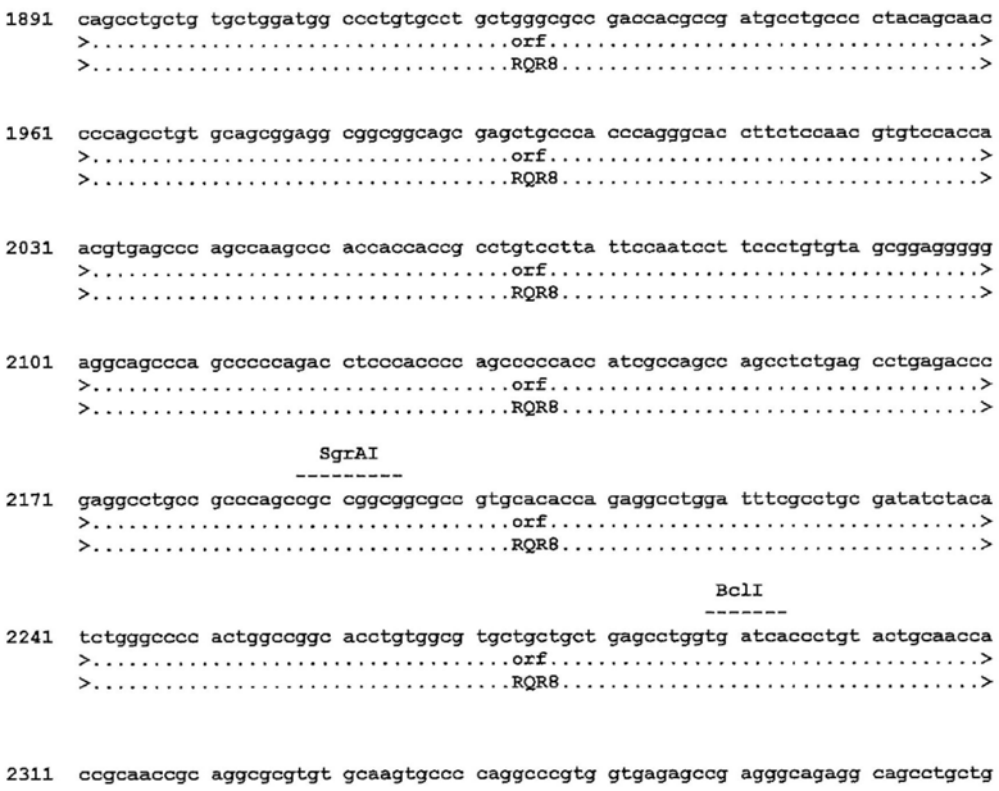
[0172] SEQ ID No.25逆转录病毒基因盒的DNA序列,包括与RQR8自杀基因共表达的抗GD2CAR,具有密码子优化的框和SAR区以增强表达

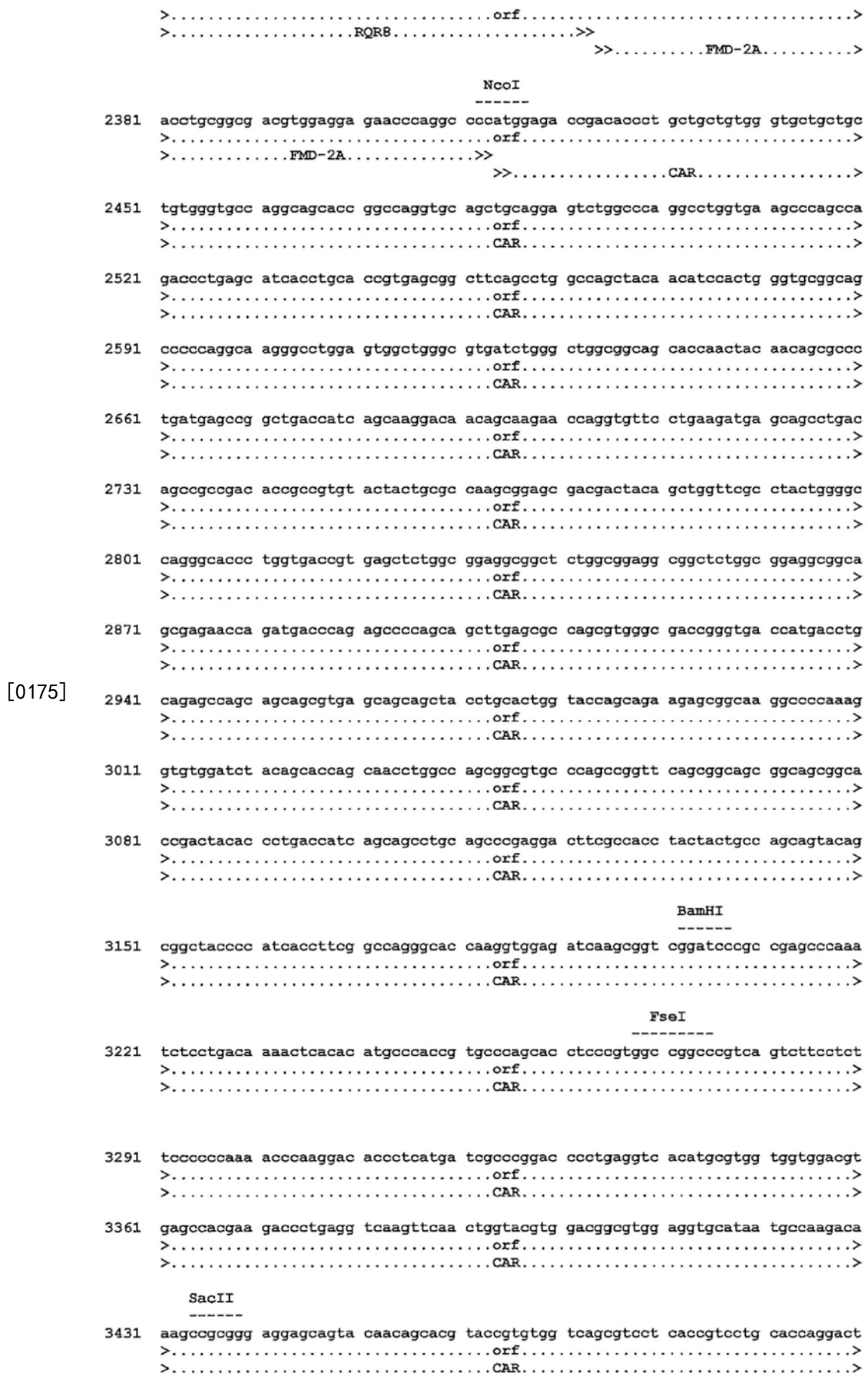
[0173]

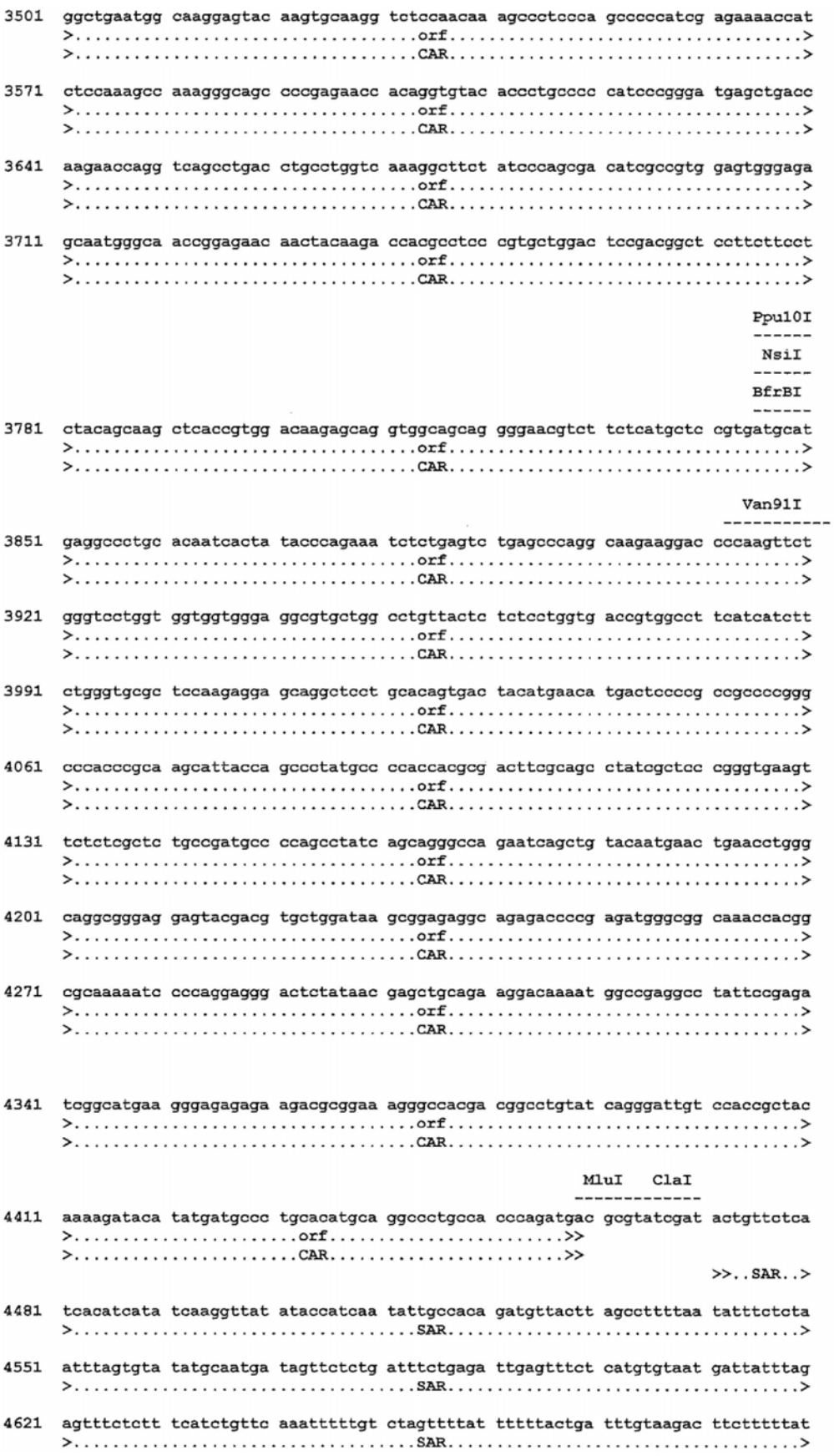
```
   1  tgaaagaccc cacctgtagg tttggcaagc tagcttaagt aacgccattt tgcaaggcat ggaaaaatac
      >>.....................................LTR.....................................>

  71  ataactgaga atagaaaagt tcagatcaag gtcaggaaca gatggaacag ctgaatatgg gccaaacagg
      >.....................................LTR.....................................>

 141  atatctgtgg taagcagttc ctgccccggc tcagggccaa gaacagatgg aacagctgaa tatgggccaa
      >.....................................LTR.....................................>

 211  acaggatatc tgtggtaagc agttcctgcc ccggctcagg gccaagaaca gatggtcccc agatgcggtc
      >.....................................LTR.....................................>

 281  cagccctcag cagtttctag agaaccatca gatgtttcca gggtgcccca aggacctgaa atgaccctgt
      >.....................................LTR.....................................>

 351  gccttatttg aactaaccaa tcagttcgct tctcgcttct gttcgcgcgc ttatgctccc cgagctcaat
      >.....................................LTR.....................................>

 421  aaaagagccc acaacccctc actcggggcg ccagtcctcc gattgactga gtcgcccggg tacccgtgta
      >.....................................LTR.....................................>

 491  tccaataaac cctcttgcag ttgcatccga cttgtggtct cgctgttcct tgggagggtc tcctctgagt
      >.....................................LTR.....................................>

 561  gattgactac ccgtcagcgg gggtctttca tttgggggct cgtccgggat cgggagaccc ctgcccaggg
      >.............LTR.............>>

 631  accaccgacc caccaccggg aggtaagctg gccagcaact tatctgtgtc tgtccgattg tctagtgtct

 701  atgactgatt ttatgcgcct gcgtcggtac tagttagcta actagctctg tatctggcgg acccgtggtg

                           Eco52I
                           ------
 771  gaactgacga gttcggaaca cccggccgca accctgggag acgtcccagg gacttcgggg gccgtttttg

             PshAI
           -----------
 841  tggcccgacc tgagtcctaa aatcccgatc gtttaggact ctttggtgca ccccccttag aggagggata
```


911 tgtggttctg gtaggagacg agaacctaaa acagttcccg cctccgtctg aatttttgct ttcggtttgg
981 gaccgaagcc gcgccgcgcg tcttgtctgc tgcagcatcg ttctgtgttg tctctgtctg actgtgtttc
SrfI
1051 tgtatttgtc tgaaaatatg ggcccgggct agcctgttac cactccctta agtttgacct taggtcactg
1121 gaaagatgtc gagcggatcg ctcacaacca gtcggtagat gtcaagaaga gacgttgggt taccttctgc
1191 tctgcagaat ggccaacctt taacgtcgga tggccgcgag acggcacctt taaccgagac ctcatcaccc
1261 aggttaagat caaggtcttt tcacctggcc cgcatggaca cccagaccag gtggggtaca tcgtgacctg
1331 ggaagccttg gcttttgacc cccctccctg ggtcaagccc tttgtacacc ctaagcctcc gcctcctctt
1401 cctccatccg ccccgtctct ccccccttgaa cctcctcgtt cgaccccgcc tcgatcctcc ctttatccag
BglII
1471 ccctcactcc ttctctaggc gcccccatat ggccatatga gatcttatat ggggcacccc cgccccttgt
1541 aaacttccct gaccctgaca tgacaagagt tactaacagc ccctctctcc aagctcactt acaggctctc
AgeI
1611 tacttagtcc agcacgaagt ctggagacct ctggcggcag cctaccaaga acaactggac cgaccggtgg
1681 tacctcaccc ttaccgagtc ggcgacacag tgtgggtccg ccgacaccag actaagaacc tagaacctcg
AccI
1751 ctggaaagga ccttacacag tcctgctgac cacccccacc gccctcaaag tagacggcat cgcagcttgg
PmlI
1821 atacacgccg cccacgtgaa ggctgccgac cccgggggtg gaccatcctc tagactgcca acatgggcac
>>.orf.>
>>RQR8.>

[0174]


1891 cagcctgctg tgctggatgg ccctgtgcct gctgggcgcc gaccacgccg atgcctgccc ctacagcaac
>.....orf.....>
>.....RQR8.....>
1961 cccagcctgt gcagcggagg cggcggcagc gagctgccca cccagggcac cttctccaac gtgtccacca
>.....orf.....>
>.....RQR8.....>
2031 acgtgagccc agccaagccc accaccaccg cctgtcctta ttccaatcct tccctgtgta gcggaggggg
>.....orf.....>
>.....RQR8.....>
2101 aggcagccca gcccccagac ctcccacccc agcccccacc atcgccagcc agcctctgag cctgagaccc
>.....orf.....>
>.....RQR8.....>
SgrAI
2171 gaggcctgcc gcccagccgc cggcggcgcc gtgcacacca gaggcctgga tttcgcctgc gatatctaca
>.....orf.....>
>.....RQR8.....>
BclI
2241 tctgggcccc actggccggc acctgtggcg tgctgctgct gagcctggtg atcaccctgt actgcaacca
>.....orf.....>
>.....RQR8.....>
2311 ccgcaaccgc aggcgcgtgt gcaagtgccc caggcccgtg gtgagagccg agggcagagg cagcctgctg


orf
RQR8
FMD-2A
NcoI
2381 acctgcggcg acgtggagga gaacccaggc cccatggaga ccgacaccct gctgctgtgg gtgctgctgc
FMD-2A
CAR
2451 tgtgggtgcc aggcagcacc ggccaggtgc agctgcagga gtctggccca ggcctggtga agcccagcca
2521 gaccctgagc atcacctgca ccgtgagcgg cttcagcctg gccagctaca acatccactg ggtgcggcag
2591 cccccaggca agggcctgga gtggctgggc gtgatctggg ctggcggcag caccaactac aacagcgccc
2661 tgatgagccg gctgaccatc agcaaggaca acagcaagaa ccaggtgttc ctgaagatga gcagcctgac
2731 agccgccgac accgccgtgt actactgcgc caagcggagc gacgactaca gctggttcgc ctactggggc
2801 cagggcaccc tggtgaccgt gagctctggc ggaggcggct ctggcggagg cggctctggc ggaggcggca
2871 gcgagaacca gatgacccag agccccagca gcttgagcgc cagcgtgggc gaccgggtga ccatgacctg
[0175]
2941 cagagccagc agcagcgtga gcagcagcta cctgcactgg taccagcaga agagcggcaa ggccccaaag
3011 gtgtggatct acagcaccag caacctggcc agcggcgtgc ccagccggtt cagcggcagc ggcagcggca
3081 ccgactacac cctgaccatc agcagcctgc agcccgagga cttcgccacc tactactgcc agcagtacag
BamHI
3151 cggctacccc atcaccttcg gccagggcac caaggtggag atcaagcggt cggatcccgc cgagcccaaa
FseI
3221 tctcctgaca aaactcacac atgcccaccg tgcccagcac ctcccgtggc cggcccgtca gtcttcctct
3291 tccccccaaa acccaaggac accctcatga tcgcccggac ccctgaggtc acatgcgtgg tggtggacgt
3361 gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca
SacII
3431 aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact


3501 ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca gcccccatcg agaaaaccat
orf
CAR
3571 ctccaaagcc aaagggcagc cccgagaacc acaggtgtac accctgcccc catcccggga tgagctgacc
orf
CAR
3641 aagaaccagg tcagcctgac ctgcctggtc aaaggcttct atcccagcga catcgccgtg gagtgggaga
orf
CAR
3711 gcaatgggca accggagaac aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct
orf
CAR
Ppu10I
NsiI
BfrBI
3781 ctacagcaag ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat
orf
CAR
Van91I
3851 gaggccctgc acaatcacta tacccagaaa tctctgagtc tgagcccagg caagaaggac cccaagttct
orf
CAR
3921 gggtcctggt ggtggtggga ggcgtgctgg cctgttactc tctcctggtg accgtggcct tcatcatctt
orf
CAR
3991 ctgggtgcgc tccaagagga gcaggctcct gcacagtgac tacatgaaca tgactccccg ccgccccggg
orf
CAR
4061 cccacccgca agcattacca gccctatgcc ccaccacgcg acttcgcagc ctatcgctcc cgggtgaagt
orf
CAR
4131 tctctcgctc tgccgatgcc ccagcctatc agcagggcca gaatcagctg tacaatgaac tgaacctggg
orf
CAR
4201 caggcgggag gagtacgacg tgctggataa gcggagaggc agagaccccg agatgggcgg caaaccacgg
orf
CAR
4271 cgcaaaaatc cccaggaggg actctataac gagctgcaga aggacaaaat ggccgaggcc tattccgaga
orf
CAR
4341 tcggcatgaa gggagagaga agacgcggaa agggccacga cggcctgtat cagggattgt ccaccgctac
orf
CAR
MluI ClaI
4411 aaaagataca tatgatgccc tgcacatgca ggccctgcca cccagatgac gcgtatcgat actgttctca
orf >>
CAR >>
>>..SAR..>
4481 tcacatcata tcaaggttat ataccatcaa tattgccaca gatgttactt agccttttaa tatttctcta
SAR
4551 atttagtgta tatgcaatga tagttctctg atttctgaga ttgagtttct catgtgtaat gattatttag
SAR
4621 agtttctctt tcatctgttc aaatttttgt ctagttttat tttttactga tttgtaagac ttctttttat
SAR

[0176]

[0177]

[0178] 传密码子的简并性可以具有不同的核酸序列。核酸序列可以与如SEQ ID No.25所示的序列具有至少80,85,90,95,98或99％同一性，只要其编码如本发明的第一个方面所定义的CAR。

[0179] 自杀基因

[0180] 由于T细胞嫁接且是自主的，在抗GD2CAR T细胞的接受体中选择性删除CAR T细胞的方式是理想的。自杀基因是遗传上可编码的机制，其导致面对不可接受的毒性时对输注的T细胞的选择性破坏。对于自杀基因，最早的临床经验是用疱疹病毒胸苷激酶(HSV-TK)，其使得T细胞对于更昔洛韦(Ganciclovir)易感。HSV-TK是高度有效的自杀基因。然而，预先形成的免疫应答可以将其限于具有相当大的免疫抑制的临床环境，如单倍相合造血干细胞(haploidentical stem cell)移植。可诱导半胱天冬酶(caspase)9(iCasp9)是一种通过使用经修饰的FKBP12置换半胱天冬酶9的活化域构建的自杀基因。iCasp9由在其它方面为惰

性的小分子二聚化化学诱导子(CID)活化。最近,在单倍相合HSCT的环境中测试了iCasp9并可以终止GvHD。iCasp9的最大限制依赖于临床级别的专有CID的可用性。iCasp9和HSV-TK两者都是细胞内蛋白,所以当用作单独的转基因时,它们已经与标志物基因共表达以允许对转导细胞的选择

[0181] iCasp9可以包含如SEQ ID No.36所示的序列或具有至少80,90,95或98%序列同一性的变体。

[0182] SEQ ID No.36

[0183] MLEGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLESGGGSGVDGFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRRRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPGAVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTSAS

[0184] 本发明人最近描述了称为RQR8的新的标志物/自杀基因,其可以使用抗体QBEnd10检测且表达细胞被治疗性抗体利妥昔单抗(Rituximab)裂解。

[0185] RQR8可以包含如SEQ ID No.37所示的序列或具有至少80,90,95或98%序列同一性的变体。

[0186] SEQ ID No.37

[0187] MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVV

[0188] 自杀基因可以与CAR作为单个多肽表达,例如通过使用两个序列之间的自剪切肽。

[0189] 载体

[0190] 本发明还提供载体,其包含根据本发明的核酸序列。此类载体可以用于将核酸序列引入宿主细胞中使得其表达并产生根据本发明的第一个方面的分子。

[0191] 载体可以是例如质粒或病毒载体,如逆转录病毒载体或慢病毒载体。

[0192] 载体可以能够转染或转导T细胞。

[0193] 载体可以包含编码自杀基因如iCasp9或RQR8的核酸序列。

[0194] 宿主细胞

[0195] 本发明还提供宿主细胞,其包含根据本发明的核酸。宿主细胞可以能够表达根据本发明的第一个方面的CAR。

[0196] 宿主细胞可以是溶细胞性免疫细胞,如人T细胞或自然杀伤(NK)细胞。

[0197] 可以通过使用编码CAR的核酸转导或转染T细胞制成能够表达根据本发明的CAR的T细胞。

[0198] 可以离体生成CAR T细胞。T细胞可以来自患者或供体的外周血单核细胞(PBMC)。在使用编码CAR的核酸转导前,可以活化和/或扩增T细胞,例如通过使用抗CD3单克隆抗体处理。

[0199] 药物组合物

[0200] 本发明还涉及药物组合物,其含有本发明的载体或表达CAR的T细胞连同药学上可

接受的载体,稀释剂或赋形剂,以及可选地一种或多种另外的药学上活性的多肽和/或化合物。此类配制剂可以是例如为适合用于静脉内输注的形式。

[0201] 治疗方法

[0202] 表达本发明的CAR分子的T细胞能够杀伤癌细胞,例如神经母细胞瘤细胞。可以离体或从患者自身的外周血(第一方),或在来自供体外周血的造血干细胞移植物(第二方)的背景中,或来自无关联供体的外周血(第三方)中创建表达CAR的T细胞。或者,CAR T细胞可以来源于可诱导祖细胞或胚胎祖细胞离体向T细胞的分化。在这些情况下,通过许多方法的一种引入编码CAR的DNA或RNA生成CAR T细胞,所述方法包括使用病毒载体转导,使用DNA或RNA转染。

[0203] 表达本发明的CAR分子的T细胞可以用于癌症疾病的治疗,特别是GD2表达相关的癌症疾病。

[0204] 癌症可以是外胚层肿瘤(ectodermal tumour)。

[0205] 与升高的GD2表达水平相关的癌症的例子为:神经母细胞瘤,黑色瘤,成神经管细胞瘤,软组织肉瘤,骨肉瘤,和小细胞肺癌例如NSCLC。

[0206] 用于疾病的治疗的方法涉及本发明的载体或T细胞的治疗性用途。在这方面,可以将载体或T细胞施用至患有存在的疾病或状况的受试者,以减轻,降低或改善与疾病相关的至少一种症状和/或减慢,降低或阻断疾病的进展。本发明的方法可以导致或促进T细胞介导的对表达GD2细胞,如癌细胞的杀伤。

[0207] 表达GD2的细胞

[0208] 本发明还提供用于制造表达GD2的细胞的方法,其包括将编码GM3合酶的核酸和编码GD2合酶的核酸引入细胞中的步骤。

[0209] 例如,可以通过使用载体,如质粒或病毒载体转染或转导引入核酸。

[0210] 本发明还涉及表达GD2的细胞,其包含编码GM3合酶的异源性核酸和编码GD2合酶的异源性核酸。

[0211] 核酸可以是“异源性”,就其通常不存在于细胞中而言。其为人工引入的重组核酸序列。

[0212] 细胞可以来自细胞系。

[0213] 细胞可以用于刺激培养中的GD2CAR T细胞,如本发明的T细胞。

[0214] 本发明将通过实施例的方式进一步描述,其是为了起到帮助本领域的普通技术人员实施本发明,而不意图以任何方式限制本发明的范围。

实施例

[0215] 实施例1-使用人源化的抗体huK666作为结合物

[0216] 使用如Nakamura等人(2001-如上文)所描述的来自小鼠抗体KM666或其人源化版本hu666的序列,使用scFv构建了CAR(以上图2中变体(a)和(b))。比较这些受体的表达/稳定性并发现对于两种受体相等。接着,测试了当由不表达或表达GD2的靶细胞激发时,使用这些受体转导的T细胞的杀伤,细胞因子分泌和增殖。得出的结论是两种受体的杀伤是相似的,但基于人源化scFc的受体导致更好的IL2产生和增殖(图3)。

[0217] 实施例2-测试不同间隔区形式对表达和功能的影响

[0218] 生成了具有Fc间隔区，铰链，铰链-CD8茎部和CD8茎部的抗GD2CAR(分别为图2(b)，(d)，(e)和(f))。将这些CARs与标志物基因，截短的CD34共表达与2A口蹄疫自剪切肽强制1:1的形式以允许精确比较(图4a)。此外，使用氨基末端HA标签标记huK666scFv以允许转基因相对于CAR表达的比较。

[0219] 对使用这些构建体转导的正常供体T细胞的流式细胞分析表明了以下顺序的较明亮的CAR表达:Fc>铰链-茎部＝茎部>铰链(图4b)。

[0220] 使用铬释放测定比较了对GD2阳性靶标相对于GD2阴性靶标的杀伤。这允许以下顺序的杀伤效率:Fc>铰链-茎部＝茎部>铰链(图4c)。

[0221] 比较了当使用GD2阳性或阴性靶标激发CAR T细胞时，干扰素-gamma释放和IL-2释放。在具有Fc，铰链-茎部和茎部的CARs中，干扰素-gamma释放相似，但在铰链变体中较少。检测到IL2释放为以下顺序:Fc，茎部，铰链-茎部，铰链(图4d和e)。

[0222] 实施例3-FcR突变消除了非特异性活性

[0223] 来自以上实施例的整体数据提示Fc间隔区总体表现最好。然而，Fc域在体内可以引起来自表达Fc受体的细胞的非特异性活性。为了消除该效果，如图5(a)中所示将突变引入Fc区中。这些突变对CAR表达没有不利影响，如图5(b)中所示。

[0224] 另外，证明了这些突变对CAR杀伤功能没有影响(图5(c))。最后，证明了在对表达FcR的靶标(称为THP1的单核细胞样系)的非特异性杀伤，和由这些单核细胞的IL-1Beta释放方面，这些突变具有期望的效果(图5e)。

[0225] 实施例4-表达基因盒的优化

[0226] 考虑到受体表达的优化，测试了以下项:(a)将支架附接区(SAR)纳入盒中;(b)将鸡beta血红蛋白染色质绝缘子(CHS4)纳入3'LTR中和(c)对开放阅读框的密码子优化(图6a)。表明了SAR的纳入改善表达的性质，密码子优化亦然，而CHS4影响不大(图6b)。将SAR和密码子优化组合叠加地改进了表达(图6c)。

[0227] 实施例5-不同胞内域的比较

[0228] 生成了具有三种不同胞内域的构建体:具有CD3-zeta胞内域的CD28跨膜域(CD28tmZ);具有CD28胞内域和CD3-zeta胞内域的CD28跨膜域(CD28Z)，和CD28跨膜域，CD28胞内域，OX30胞内域以及CD3-zeta胞内域(CD28OXZ)，其中CAR以Fc间隔区的形式。注意到增殖，IFNγ释放和IL2释放以CD28tmZ<CD28Z<CD28OXZ的顺序增加(图7)。

[0229] 实施例6-与iCasp9自杀基因共表达

[0230] 将iCasp9自杀基因与抗GD2CAR共表达(图8a-CAR以Fc间隔区的形式，随意选择CD28OXZ以证明功能)。尽管与iCasp9共表达，CAR可以良好表达(图8b)。使用小分子二聚化剂(dimerizer)活化iCasp9导致CAR阳性T细胞的删除(图8b)。暴露于该二聚化剂的iCasp9-GD2CAR T细胞当暴露于二聚化剂时，失去了它们的GD2特异性(图8c)。

[0231] 实施例7-与RQR8自杀基因共表达

[0232] 将抗GD2CAR与RQR8分选自杀基因(sort-suicide gene)共表达(图9a-CAR以Fc间隔区的形式，随意选择CD28Z以证明功能)。共表达受体和CAR是可能的(图9b)。使用利妥昔单抗和补体对RQR8自杀基因功能的活化导致转导的T细胞的删除和GD2识别的丧失(图9c和d)。

[0233] 实施例8-GD2合酶和GM3合酶的表达导致任意细胞系中GD2表达

[0234] 为了刺激培养中的GD2CAR T细胞，为了具有理想的GD2-或GD2+靶标，且为了能够生成同基因细胞用于小动物模型，期望的是能够在细胞系上转基因表达GD2。GD2不是蛋白质，并且需要由一组复杂的酶合成。这里表明了仅两种酶：GM3合酶和GM2合酶的转基因表达导致在至今转导的所有细胞系中明亮的GD2表达(图10)。

[0235] 实施例9-抗GD2CAR的体内功能

[0236] 如上文所述CT26细胞系经工程化以表达GD2(命名为CT26克隆#7或缩写为CT25#7)。将$2x10^5$个野生型(wt)或GD2阳性细胞接种到C57BL/6小鼠(与CT26同基因)体侧。肿瘤攻击后10天，制备假转导的和抗GD2CAR转导的同基因脾细胞。将小鼠分为以下4个群体：具有表达GD2的CT26肿瘤的小鼠，接受抗GD2CAR脾细胞；表达GD2的CT26肿瘤，接受假转导的脾细胞；GD2阴性(wt)CD26肿瘤与抗GD2CAR脾细胞；和表达GD2的CT26肿瘤，不接受脾细胞。使用数显卡尺以3维测量肿瘤并以此估算体积。图11示出了肿瘤的生长曲线。仅接受抗GD2CAR T细胞小鼠中的GD2阳性肿瘤具有很少的生长或没有生长。

[0237] 实施例10-比较包含基于huK666和14g2a的抗原结合域的CAR的功能

[0238] CAR的抗原结合域可以影响其功能。在本研究中，与具有基于14g2a的抗原结合域的等同CAR比较具有基于huK666的抗原结合域的本发明的CAR的功能。

[0239] 抗体14g2a可以视为针对GD2的黄金标准抗体，因为其用作治疗性mAb且是在CAR研究中测试的唯一scFv。

[0240] 基于huK666或14g2a构建并表达了第二代CAR。它们的结构示于图14a中。

[0241] 通过使用编码GD2CAR、gag/pol和包膜蛋白RD114的质粒瞬时转染293T细胞产生了逆转录病毒。3天后收获上清并用于在Retronectin包被的平板上与相同滴度的逆转录病毒转导PHA/IL2活化的PBMCs。CAR仅在它们的抗原结合域中不同。在两种情况下，都使用IgG Fc片段将结合域连接到膜，并含有来自CD28和CD3-zeta的细胞内活化基序。转导后六天，通过流式细胞术确认CAR表达，并将PBMCs与GD2阳性Lan1细胞(GD2阳性细胞系)或GD2阴性A204细胞(GD2阴性横纹肌肉瘤细胞系)以1:1比率培养。一天后，来自这些共培养的上清通过ELISA测定干扰素-γ水平，并在6天后通过流式细胞术评估T细胞增殖。

[0242] 结果示于图14和图15中。24小时后，从上清测量干扰素-gamma。证明了huK666CAR产生更多的IFN-γ(图14b)。一周后对T细胞计数，且证明了huK666CAR具有更多的增殖(图14c)。

[0243] 与神经母细胞瘤细胞系LAN1共培养一周后，收获细胞并通过流式细胞术分析。CD45表达允许区分淋巴样细胞和非淋巴样细胞，其中CD45-细胞为LAN-1细胞。使用CD3/QBEND/10进一步染色允许对CAR T细胞计数。发现huK666CAR T细胞比14g2a等同物增殖更好且杀伤更完全(图15)。

[0244] 以上说明书中提及的所有出版物通过提述并入本文。本发明描述的方法和系统的各种修改和变化对于本领域的技术人员将是显而易见的而不脱离本发明的范围和精神。尽管已经与特定的优选实施方案相关联描述本发明，应当理解作为要求保护的本发明不应当过度限于这些特定实施方案。实际上，对于实现本发明所描述的模式的各种修改对于分子生物学，细胞免疫学或相关领域的技术人员是显而易见的，并意图在所附权利要求书的范围内。

序列表

<110> UCL商务股份有限公司

<120> 嵌合抗原受体

<130> P103293PCT

<150> 1403972.1
<151> 2014-03-06

<160> 37

<170> PatentIn version 3.5

<210> 1
<211> 5
<212> PRT
<213> 人工序列

<220>
<223> 重链可变区 CDR1

<400> 1

Ser Tyr Asn Ile His
1 5

<210> 2
<211> 16
<212> PRT
<213> 人工序列

<220>
<223> 重链可变区 CDR2

[0001] <400> 2

Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met Ser
1 5 10 15

<210> 3
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> 重链可变区 CDR3

<400> 3

Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr
1 5 10

<210> 4
<211> 12
<212> PRT
<213> 人工序列

<220>
<223> 轻链可变区 CDR1

<400> 4

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His
1 5 10

<210> 5
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> 轻链可变区 CDR2

<400> 5

Ser Thr Ser Asn Leu Ala Ser
1 5

<210> 6
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> 轻链可变区 CDR3

<400> 6

Gln Gln Tyr Ser Gly Tyr Pro Ile Thr
1 5

<210> 7
<211> 243
<212> PRT
<213> 人工序列

<220>
<223> 鼠KM666序列

<400> 7

Gln Val Gln Leu Lys Glu Ser Gly Pro Val Leu Val Ala Pro Ser Gln
1 5 10 15

[0002] Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ala Ser Tyr
20 25 30

Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Gln Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Glu Asn Val Leu Thr Gln Ser Pro Ala Ile
130 135 140

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
145 150 155 160

Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln Lys Ser Gly
165 170 175

```
Ala Ser Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly
            180                 185                 190

Val Pro Gly Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
        195                 200                 205

Thr Ile Ser Ser Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
    210                 215                 220

Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Ala Gly Thr Lys Val Glu
225                 230                 235                 240

Val Lys Arg
```

<210> 8
<211> 242
<212> PRT
<213> 人工序列

<220>
<223> 人源化的KM666序列

<400> 8

```
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1               5                   10                  15

Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ala Ser Tyr
            20                  25                  30
```

[0003]

```
Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
    50                  55                  60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65                  70                  75                  80

Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
                85                  90                  95

Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125

Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser Pro Ser Ser Leu
    130                 135                 140

Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Arg Ala Ser Ser
145                 150                 155                 160

Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln Lys Ser Gly Lys
                165                 170                 175

Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val
```

180 185 190

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr
195 200 205

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
210 215 220

Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
225 230 235 240

Lys Arg

<210> 9
<211> 118
<212> PRT
<213> 人工序列

<220>
<223> 鼠KM666 VH（重链可变区）序列

<400> 9

Gln Val Gln Leu Lys Glu Ser Gly Pro Val Leu Val Ala Pro Ser Gln
1 5 10 15

Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ala Ser Tyr
20 25 30

[0004] Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
65 70 75 80

Gln Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Tyr Cys Ala
85 90 95

Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 10
<211> 118
<212> PRT
<213> 人工序列

<220>
<223> 人源化的 KM666 VH 序列

<400> 10

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Ala Ser Tyr

20 25 30

Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met
50 55 60

Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
65 70 75 80

Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 11
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 鼠 KM666 VL (轻链可变区) 序列

<400> 11

[0005]

Glu Asn Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Ser Gly Ala Ser Pro Lys Val Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Gly Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Val Glu
65 70 75 80

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro
85 90 95

Ile Thr Phe Gly Ala Gly Thr Lys Val Glu Val Lys
100 105

<210> 12
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 人源化的 KM666 VL 序列

<400> 12

Glu Asn Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly

1 5 10 15

Asp Arg Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Ser Gly Lys Ala Pro Lys Val Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro
85 90 95

Ile Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 13
<211> 27
<212> PRT
<213> 人工序列

<220>
<223> 跨膜区

<400> 13

[0006]

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
20 25

<210> 14
<211> 39
<212> PRT
<213> 人工序列

<220>
<223> CD28 胞内域

<400> 14

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr
35

<210> 15
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> CD40 胞内域

<400> 15

Arg Ser Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly
1 5 10 15

Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His
20 25 30

Ser Thr Leu Ala Lys Ile
35

<210> 16
<211> 114
<212> PRT
<213> 人工序列

<220>
<223> CD3 zeta 胞内域

<400> 16

Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
1 5 10 15

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
20 25 30

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
35 40 45

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
50 55 60

[0007]

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
65 70 75 80

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
85 90 95

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
100 105 110

Pro Arg

<210> 17
<211> 153
<212> PRT
<213> 人工序列

<220>
<223> CD28Z

<400> 17

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser
35 40 45

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
50 55 60

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
65 70 75 80

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
85 90 95

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
100 105 110

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
115 120 125

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
130 135 140

Leu His Met Gln Ala Leu Pro Pro Arg
145 150

<210> 18
<211> 189
<212> PRT
<213> 人工序列

<220>
<223> CD28OXZ

<400> 18

[0008]

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro Pro
35 40 45

Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile Gln
50 55 60

Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg Val Lys
65 70 75 80

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
85 90 95

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
100 105 110

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
115 120 125

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
130 135 140

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly

145 150 155 160

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
165 170 175

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
180 185

<210> 19
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> 信号肽

<400> 19

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly
20

<210> 20
<211> 234
<212> PRT
<213> 人工序列

<220>
<223> 人IgG1铰链-CH2CH3间隔区

[0009] <400> 20

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser

145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp
225 230

<210> 21
<211> 46
<212> PRT
<213> 人工序列

<220>
<223> 人CD8茎部间隔区

<400> 21

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1 5 10 15

[0010] Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
35 40 45

<210> 22
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> 人IgG1铰链间隔区

<400> 22

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

Lys Asp Pro Lys
20

<210> 23
<211> 237
<212> PRT
<213> 人工序列

<220>
<223> IgG1 铰链-Fc间隔区

<400> 23

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
20 25 30

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
35 40 45

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
50 55 60

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
65 70 75 80

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
85 90 95

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
100 105 110

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
115 120 125

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
130 135 140

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
145 150 155 160

[0011] Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
165 170 175

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
180 185 190

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
195 200 205

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
210 215 220

Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys
225 230 235

<210> 24
<211> 236
<212> PRT
<213> 人工序列

<220>
<223> IgG1 铰链间隔区 - Fc经修饰以去除Fc受体识别基序

<400> 24

Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val

35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

[0012]

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys
225 230 235

<210> 25
<211> 6014
<212> DNA
<213> 人工序列

<220>
<223> 逆转录病毒基因盒

<400> 25
tgaaagaccc cacctgtagg tttggcaagc tagcttaagt aacgccattt tgcaaggcat 60

ggaaaaatac ataactgaga atagaaaagt tcagatcaag gtcaggaaca gatggaacag 120

ctgaatatgg gccaaacagg atatctgtgg taagcagttc ctgccccggc tcagggccaa 180

gaacagatgg aacagctgaa tatgggccaa acaggatatc tgtggtaagc agttcctgcc 240

ccggctcagg gccaagaaca gatggtcccc agatgcggtc cagccctcag cagtttctag 300

agaaccatca gatgtttcca gggtgcccca aggacctgaa atgaccctgt gccttatttg 360

aactaaccaa tcagttcgct tctcgcttct gttcgcgcgc ttatgctccc cgagctcaat 420

aaaagagccc acaacccctc actcggggcg ccagtcctcc gattgactga gtcgcccggg 480

[0013]

tacccgtgta tccaataaac cctcttgcag ttgcatccga cttgtggtct cgctgttcct 540

tgggagggtc tcctctgagt gattgactac ccgtcagcgg gggtctttca tttgggggct 600

cgtccgggat cgggagaccc ctgcccaggg accaccgacc caccaccggg aggtaagctg 660

gccagcaact tatctgtgtc tgtccgattg tctagtgtct atgactgatt ttatgcgcct 720

gcgtcggtac tagttagcta actagctctg tatctggcgg acccgtggtg gaactgacga 780

gttcggaaca cccggccgca accctgggag acgtcccagg gacttcgggg gccgtttttg 840

tggcccgacc tgagtcctaa aatcccgatc gtttaggact ctttggtgca ccccccttag 900

aggagggata tgtggttctg gtaggagacg agaacctaaa acagttcccg cctccgtctg 960

aatttttgct ttcggtttgg gaccgaagcc gcgccgcgcg tcttgtctgc tgcagcatcg 1020

ttctgtgttg tctctgtctg actgtgtttc tgtatttgtc tgaaaatatg ggcccgggct 1080

agcctgttac cactccctta agtttgacct taggtcactg gaaagatgtc gagcggatcg 1140

ctcacaacca gtcggtagat gtcaagaaga gacgttgggt taccttctgc tctgcagaat 1200

ggccaacctt taacgtcgga tggccgcgag acggcacctt taaccgagac ctcatcaccc 1260

aggttaagat caaggtcttt tcacctggcc cgcatggaca cccagaccag gtggggtaca 1320

tcgtgacctg ggaagccttg gcttttgacc cccctccctg ggtcaagccc tttgtacacc 1380

ctaagcctcc gcctcctctt cctccatccg ccccgtctct ccccccttgaa cctcctcgtt 1440

cgaccccgcc tcgatcctcc ctttatccag ccctcactcc ttctctaggc gcccccatat 1500

ggccatatga gatcttatat ggggcacccc cgccccttgt aaacttccct gaccctgaca 1560

tgacaagagt tactaacagc ccctctctcc aagctcactt acaggctctc tacttagtcc 1620

agcacgaagt ctggagacct ctggcggcag cctaccaaga acaactggac cgaccggtgg 1680

tacctcaccc ttaccgagtc ggcgacacag tgtgggtccg ccgacaccag actaagaacc 1740

tagaacctcg ctggaaagga ccttacacag tcctgctgac cacccccacc gccctcaaag 1800

tagacggcat cgcagcttgg atacacgccg cccacgtgaa ggctgccgac cccgggggtg 1860

gaccatcctc tagactgcca acatgggcac cagcctgctg tgctggatgg ccctgtgcct 1920

gctgggcgcc gaccacgccg atgcctgccc ctacagcaac cccagcctgt gcagcggagg 1980

cggcggcagc gagctgccca cccagggcac cttctccaac gtgtccacca acgtgagccc 2040

agccaagccc accaccaccg cctgtcctta ttccaatcct tccctgtgta gcggaggggg 2100

aggcagccca gcccccagac ctcccacccc agcccccacc atcgccagcc agcctctgag 2160

cctgagaccc gaggcctgcc gcccagccgc cggcggcgcc gtgcacacca gaggcctgga 2220

tttcgcctgc gatatctaca tctgggcccc actggccggc acctgtggcg tgctgctgct 2280

gagcctggtg atcaccctgt actgcaacca ccgcaaccgc aggcgcgtgt gcaagtgccc 2340

caggcccgtg gtgagagccg agggcagagg cagcctgctg acctgcggcg acgtggagga 2400

gaacccaggc cccatggaga ccgacaccct gctgctgtgg gtgctgctgc tgtgggtgcc 2460

aggcagcacc ggccaggtgc agctgcagga gtctggccca ggcctggtga agcccagcca 2520

gaccctgagc atcacctgca ccgtgagcgg cttcagcctg gccagctaca acatccactg 2580

ggtgcggcag cccccaggca agggcctgga gtggctgggc gtgatctggg ctggcggcag 2640

caccaactac aacagcgccc tgatgagccg gctgaccatc agcaaggaca acagcaagaa 2700

ccaggtgttc ctgaagatga gcagcctgac agccgccgac accgccgtgt actactgcgc 2760

[0014]

caagcggagc gacgactaca gctggttcgc ctactggggc cagggcaccc tggtgaccgt 2820

gagctctggc ggaggcggct ctggcggagg cggctctggc ggaggcggca gcgagaacca 2880

gatgacccag agccccagca gcttgagcgc cagcgtgggc gaccgggtga ccatgacctg 2940

cagagccagc agcagcgtga gcagcagcta cctgcactgg taccagcaga agagcggcaa 3000

ggccccaaag gtgtggatct acagcaccag caacctggcc agcggcgtgc ccagccggtt 3060

cagcggcagc ggcagcggca ccgactacac cctgaccatc agcagcctgc agcccgagga 3120

cttcgccacc tactactgcc agcagtacag cggctacccc atcaccttcg gccagggcac 3180

caaggtggag atcaagcggt cggatcccgc cgagcccaaa tctcctgaca aaactcacac 3240

atgcccaccg tgcccagcac ctcccgtggc cggcccgtca gtcttcctct tccccccaaa 3300

acccaaggac accctcatga tcgcccggac ccctgaggtc acatgcgtgg tggtggacgt 3360

gagccacgaa gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa 3420

tgccaagaca aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct 3480

caccgtcctg caccaggact ggctgaatgg caaggagtac aagtgcaagg tctccaacaa 3540

agccctccca gcccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc 3600

acaggtgtac accctgcccc catcccggga tgagctgacc aagaaccagg tcagcctgac 3660

ctgcctggtc aaaggcttct atcccagcga catcgccgtg gagtgggaga gcaatgggca 3720

accggagaac aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct 3780

ctacagcaag ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc 3840

cgtgatgcat gaggccctgc acaatcacta tacccagaaa tctctgagtc tgagcccagg 3900

caagaaggac cccaagttct gggtcctggt ggtggtggga ggcgtgctgg cctgttactc 3960

tctcctggtg accgtggcct tcatcatctt ctgggtgcgc tccaagagga gcaggctcct 4020

gcacagtgac tacatgaaca tgactccccg ccgccccggg cccacccgca agcattacca 4080

gccctatgcc ccaccacgcg acttcgcagc ctatcgctcc cgggtgaagt tctctcgctc 4140

tgccgatgcc ccagcctatc agcagggcca gaatcagctg tacaatgaac tgaacctggg 4200

caggcgggag gagtacgacg tgctggataa gcggagaggc agagaccccg agatgggcgg 4260

caaaccacgg cgcaaaaatc cccaggaggg actctataac gagctgcaga aggacaaaat 4320

ggccgaggcc tattccgaga tcggcatgaa gggagagaga agacgcggaa agggccacga 4380

cggcctgtat cagggattgt ccaccgctac aaaagataca tatgatgccc tgcacatgca 4440

ggccctgcca cccagatgac gcgtatcgat actgttctca tcacatcata tcaaggttat 4500

ataccatcaa tattgccaca gatgttactt agccttttaa tatttctcta atttagtgta 4560

tatgcaatga tagttctctg atttctgaga ttgagtttct catgtgtaat gattatttag 4620

agtttctctt tcatctgttc aaatttttgt ctagttttat tttttactga tttgtaagac 4680

ttctttttat aatctgcata ttacaattct ctttactggg gtgttgcaaa tattttctgt 4740

cattctatgg cctgactttt cttaatggtt ttttaatttt aaaaataagt cttaatattc 4800

atgcaatcta attaacaatc ttttctttgt ggttaggact ttgagtcata agaaattttt 4860

ctctacactg aagtcatgat ggcatgcttc tatattattt tctaaaagat ttaaagtttt 4920

gccttctcca tttagactta taattcactg gaatttttttt gtgtgtatgg tatgacatat 4980

gggttccctt ttatttttta catataaata tatttccctg tttttctaaa aaagaaaaag 5040

atcatcattt tcccattgta aaatgccata ttttttttcat aggtcactta catatatcaa 5100

tgggtctgtt tctgagctct actctatttt atcagcctca ctgtctatcc ccacacatct 5160

catgctttgc tctaaatctt gatatttagt ggaacattct ttcccatttt gttctacaag 5220

aatatttttg ttattgtctt tgggctttct atatacattt tgaaatgagg ttgacaagtt 5280

cggattagtc caatttgtta aagacaggat atcagtggtc caggctctag ttttgactca 5340

acaatatcac cagctgaagc ctatagagta cgagccatag ataaaataaa agattttatt 5400

tagtctccag aaaaaggggg gaatgaaaga ccccacctgt aggtttggca agctagctta 5460

agtaacgcca ttttgcaagg catggaaaaa tacataactg agaatagaga agttcagatc 5520

aaggtcagga acagatggaa cagctgaata tgggccaaac aggatatctg tggtaagcag 5580

ttcctgcccc ggctcagggc caagaacaga tggaacagct gaatatgggc caaacaggat 5640

atctgtggta agcagttcct gccccggctc agggccaaga acagatggtc cccagatgcg 5700

gtccagccct cagcagtttc tagagaacca tcagatgttt ccagggtgcc ccaaggacct 5760

gaaatgaccc tgtgccttat ttgaactaac caatcagttc gcttctcgct tctgttcgcg 5820

cgcttctgct ccccgagctc aataaaagag cccacaaccc ctcactcggg gcgccagtcc 5880

tccgattgac tgagtcgccc gggtacccgt gtatccaata aaccctcttg cagttgcatc 5940

cgacttgtgg tctcgctgtt ccttgggagg gtctcctctg agtgattgac tacccgtcag 6000

cgggggtctt tcac 6014

[0015]

<210> 26
<211> 719
<212> PRT
<213> 人工序列

<220>
<223> 抗GD2 CAR, muKM666-HCH2CH3-CD28OXZ

<400> 26

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Lys Glu Ser Gly Pro Val Leu Val
20 25 30

Ala Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
35 40 45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
50 55 60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65 70 75 80

Ser Ala Leu Met Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser
85 90 95

Gln Val Phe Leu Gln Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met
100 105 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
115 120 125

```
Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ser Gly Gly Gly Gly Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Val Leu Thr Gln
145                 150                 155                 160

Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr
                165                 170                 175

Cys Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln
            180                 185                 190

Gln Lys Ser Gly Ala Ser Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn
        195                 200                 205

Leu Ala Ser Gly Val Pro Gly Arg Phe Ser Gly Ser Gly Ser Gly Thr
    210                 215                 220

Ser Tyr Ser Leu Thr Ile Ser Ser Val Glu Ala Glu Asp Ala Ala Thr
225                 230                 235                 240

Tyr Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Ala Gly
                245                 250                 255

Thr Lys Val Glu Val Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro
            260                 265                 270
```

[0016]

```
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
        275                 280                 285

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
    290                 295                 300

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
305                 310                 315                 320

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
                325                 330                 335

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
            340                 345                 350

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
        355                 360                 365

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
    370                 375                 380

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
385                 390                 395                 400

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
                405                 410                 415

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
            420                 425                 430
```

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
435 440 445

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
450 455 460

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
465 470 475 480

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
485 490 495

Pro Gly Lys Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly
500 505 510

Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe
515 520 525

Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn
530 535 540

Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr
545 550 555 560

Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu
565 570 575

[0017]

Pro Pro Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro
580 585 590

Ile Gln Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg
595 600 605

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln
610 615 620

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
625 630 635 640

Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro
645 650 655

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
660 665 670

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
675 680 685

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
690 695 700

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
705 710 715

<210> 27
<211> 718
<212> PRT

<213> 人工序列

<220>
<223> 抗GD2 CAR, huKM666-HCH2CH3-CD28OXZ

<400> 27

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
20 25 30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
35 40 45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
50 55 60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65 70 75 80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
85 90 95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
115 120 125

[0018]

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
165 170 175

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
180 185 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
195 200 205

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
210 215 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225 230 235 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
245 250 255

Lys Val Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp
260 265 270

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly

275 280 285

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
290 295 300

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
305 310 315 320

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
325 330 335

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
340 345 350

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
355 360 365

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
370 375 380

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
385 390 395 400

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
405 410 415

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
420 425 430

[0019]

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
435 440 445

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
450 455 460

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
465 470 475 480

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
485 490 495

Gly Lys Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly Val
500 505 510

Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp
515 520 525

Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met
530 535 540

Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala
545 550 555 560

Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro
565 570 575

Pro Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile

580 585 590

Gln Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg Val
595 600 605

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
610 615 620

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
625 630 635 640

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg
645 650 655

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
660 665 670

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg
675 680 685

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys
690 695 700

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
705 710 715

[0020]

<210> 28
<211> 717
<212> PRT
<213> 人工序列

<220>
<223> 抗GD2 CAR, huKM666-HCH2CH3pvaa-CD28OXZ

<400> 28

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
20 25 30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
35 40 45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
50 55 60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65 70 75 80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
85 90 95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
115 120 125

```
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145                 150                 155                 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
                165                 170                 175

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
            180                 185                 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
        195                 200                 205

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
    210                 215                 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225                 230                 235                 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
                245                 250                 255

Lys Val Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp
            260                 265                 270
```

[0021]

```
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
        275                 280                 285

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala
    290                 295                 300

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
305                 310                 315                 320

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
                325                 330                 335

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
            340                 345                 350

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
        355                 360                 365

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
    370                 375                 380

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
385                 390                 395                 400

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
                405                 410                 415

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
            420                 425                 430
```

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
435 440 445

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
450 455 460

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
465 470 475 480

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
485 490 495

Lys Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly Val Leu
500 505 510

Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
515 520 525

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
530 535 540

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
545 550 555 560

Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu Pro Pro
565 570 575

[0022] Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro Ile Gln
580 585 590

Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg Val Lys
595 600 605

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
610 615 620

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
625 630 635 640

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
645 650 655

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
660 665 670

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
675 680 685

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
690 695 700

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
705 710 715

<210> 29
<211> 527
<212> PRT

<213> 人工序列

<220>
<223> 抗GD2 CAR, huKM666-HSTK-CD28OXZ

<400> 29

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
20 25 30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
35 40 45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
50 55 60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65 70 75 80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
85 90 95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
115 120 125

[0023]

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
165 170 175

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
180 185 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
195 200 205

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
210 215 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225 230 235 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
245 250 255

Lys Val Glu Ile Lys Arg Ser Asp Pro Thr Thr Thr Pro Ala Pro Arg
260 265 270

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg

275 280 285

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
290 295 300

Leu Asp Phe Ala Cys Asp Ile Phe Trp Val Leu Val Val Val Gly Gly
305 310 315 320

Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe
325 330 335

Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn
340 345 350

Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr
355 360 365

Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu
370 375 380

Pro Pro Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro
385 390 395 400

Ile Gln Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg
405 410 415

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln
420 425 430

[0024]

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
435 440 445

Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro
450 455 460

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
465 470 475 480

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
485 490 495

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
500 505 510

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
515 520 525

<210> 30
<211> 527
<212> PRT
<213> 人工序列

<220>
<223> 抗GD2 CAR, huKM666-STK-CD28XOXZ

<400> 30

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

```
Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
            20                  25                  30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
        35                  40                  45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
    50                  55                  60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65                  70                  75                  80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
                85                  90                  95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145                 150                 155                 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
                165                 170                 175

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
            180                 185                 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
        195                 200                 205

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
    210                 215                 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225                 230                 235                 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
                245                 250                 255

Lys Val Glu Ile Lys Arg Ser Asp Pro Thr Thr Thr Pro Ala Pro Arg
            260                 265                 270

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg
        275                 280                 285

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
    290                 295                 300

Leu Asp Phe Ala Cys Asp Ile Phe Trp Val Leu Val Val Val Gly Gly
305                 310                 315                 320
```

[0025]

Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe
325 330 335

Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn
340 345 350

Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr
355 360 365

Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Asp Gln Arg Leu
370 375 380

Pro Pro Asp Ala His Lys Pro Pro Gly Gly Gly Ser Phe Arg Thr Pro
385 390 395 400

Ile Gln Glu Glu Gln Ala Asp Ala His Ser Thr Leu Ala Lys Ile Arg
405 410 415

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln
420 425 430

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
435 440 445

Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro
450 455 460

[0026]

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
465 470 475 480

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
485 490 495

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
500 505 510

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
515 520 525

<210> 31
<211> 501
<212> PRT
<213> 人工序列

<220>
<223> 抗GD2 CAR, huKM666-HNG-CD28OXZ

<400> 31

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
20 25 30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
35 40 45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly

50 55 60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65 70 75 80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
85 90 95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
165 170 175

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
180 185 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
195 200 205

[0027]

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
210 215 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225 230 235 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
245 250 255

Lys Val Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp
260 265 270

Lys Thr His Thr Cys Pro Pro Cys Pro Lys Asp Pro Lys Phe Trp Val
275 280 285

Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr
290 295 300

Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu
305 310 315 320

His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg
325 330 335

Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg
340 345 350

Ser Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly

355 360 365

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
370 375 380

Thr Leu Ala Lys Ile Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
385 390 395 400

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
405 410 415

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
420 425 430

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
435 440 445

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
450 455 460

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
465 470 475 480

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
485 490 495

Ala Leu Pro Pro Arg
500

[0028]

<210> 32
<211> 642
<212> PRT
<213> 人工序列

<220>
<223> 抗GD2 CAR, huKM666-HCH2CH3pvaa-CD28tmZ

<400> 32

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
20 25 30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
35 40 45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
50 55 60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65 70 75 80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
85 90 95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
100 105 110

```
Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145                 150                 155                 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
                165                 170                 175

Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
            180                 185                 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
        195                 200                 205

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
    210                 215                 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225                 230                 235                 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
                245                 250                 255
```

[0029]

```
Lys Val Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp
            260                 265                 270

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
        275                 280                 285

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala
    290                 295                 300

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
305                 310                 315                 320

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
                325                 330                 335

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
            340                 345                 350

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
        355                 360                 365

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
    370                 375                 380

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
385                 390                 395                 400

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
                405                 410                 415
```

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
420 425 430

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
435 440 445

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
450 455 460

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
465 470 475 480

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
485 490 495

Lys Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly Val Leu
500 505 510

Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
515 520 525

Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
530 535 540

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
545 550 555 560

[0030]

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
565 570 575

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
580 585 590

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
595 600 605

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
610 615 620

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
625 630 635 640

Pro Arg

<210> 33
<211> 681
<212> PRT
<213> 人工序列

<220>
<223> 抗GD2 CAR, huMK666-HCH2CH3pvaa-CD28Z

<400> 33

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val

```
                20                  25                  30

Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser
        35                  40                  45

Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys Gly
    50                  55                  60

Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr Asn
65                  70                  75                  80

Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys Asn
                85                  90                  95

Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln Ser
145                 150                 155                 160

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
                165                 170                 175
```

[0031]

```
Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln Gln
            180                 185                 190

Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn Leu
        195                 200                 205

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
    210                 215                 220

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
225                 230                 235                 240

Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly Thr
                245                 250                 255

Lys Val Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro Asp
            260                 265                 270

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
        275                 280                 285

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala
    290                 295                 300

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
305                 310                 315                 320

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
```

325 330 335

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
340 345 350

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
355 360 365

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
370 375 380

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
385 390 395 400

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
405 410 415

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
420 425 430

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
435 440 445

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
450 455 460

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
465 470 475 480

[0032]

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
485 490 495

Lys Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly Val Leu
500 505 510

Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
515 520 525

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
530 535 540

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
545 550 555 560

Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser
565 570 575

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
580 585 590

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
595 600 605

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
610 615 620

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr

625 630 635 640

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
645 650 655

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
660 665 670

Leu His Met Gln Ala Leu Pro Pro Arg
675 680

<210> 34
<211> 1103
<212> PRT
<213> 人工序列

<220>
<223> 与iCasp9自杀基因共表达的抗GD2 CAR

<400> 34

Met Leu Glu Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg
1 5 10 15

Thr Phe Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met
20 25 30

Leu Glu Asp Gly Lys Lys Val Asp Ser Ser Arg Asp Arg Asn Lys Pro
35 40 45

[0033] Phe Lys Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu
50 55 60

Gly Val Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser
65 70 75 80

Pro Asp Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro
85 90 95

His Ala Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu Ser Gly
100 105 110

Gly Gly Ser Gly Val Asp Gly Phe Gly Asp Val Gly Ala Leu Glu Ser
115 120 125

Leu Arg Gly Asn Ala Asp Leu Ala Tyr Ile Leu Ser Met Glu Pro Cys
130 135 140

Gly His Cys Leu Ile Ile Asn Asn Val Asn Phe Cys Arg Glu Ser Gly
145 150 155 160

Leu Arg Thr Arg Thr Gly Ser Asn Ile Asp Cys Glu Lys Leu Arg Arg
165 170 175

Arg Phe Ser Ser Leu His Phe Met Val Glu Val Lys Gly Asp Leu Thr
180 185 190

Ala Lys Lys Met Val Leu Ala Leu Leu Glu Leu Ala Gln Gln Asp His
195 200 205

```
Gly Ala Leu Asp Cys Cys Val Val Val Ile Leu Ser His Gly Cys Gln
    210                 215                 220

Ala Ser His Leu Gln Phe Pro Gly Ala Val Tyr Gly Thr Asp Gly Cys
225                 230                 235                 240

Pro Val Ser Val Glu Lys Ile Val Asn Ile Phe Asn Gly Thr Ser Cys
                245                 250                 255

Pro Ser Leu Gly Gly Lys Pro Lys Leu Phe Phe Ile Gln Ala Cys Gly
            260                 265                 270

Gly Glu Gln Lys Asp His Gly Phe Glu Val Ala Ser Thr Ser Pro Glu
        275                 280                 285

Asp Glu Ser Pro Gly Ser Asn Pro Glu Pro Asp Ala Thr Pro Phe Gln
    290                 295                 300

Glu Gly Leu Arg Thr Phe Asp Gln Leu Asp Ala Ile Ser Ser Leu Pro
305                 310                 315                 320

Thr Pro Ser Asp Ile Phe Val Ser Tyr Ser Thr Phe Pro Gly Phe Val
                325                 330                 335

Ser Trp Arg Asp Pro Lys Ser Gly Ser Trp Tyr Val Glu Thr Leu Asp
            340                 345                 350
```

[0034]

```
Asp Ile Phe Glu Gln Trp Ala His Ser Glu Asp Leu Gln Ser Leu Leu
        355                 360                 365

Leu Arg Val Ala Asn Ala Val Ser Val Lys Gly Ile Tyr Lys Gln Met
    370                 375                 380

Pro Gly Cys Phe Asn Phe Leu Arg Lys Lys Leu Phe Phe Lys Thr Ser
385                 390                 395                 400

Ala Ser Arg Ala Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val
                405                 410                 415

Glu Glu Asn Pro Gly Pro Met Glu Thr Asp Thr Leu Leu Leu Trp Val
            420                 425                 430

Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Gln Val Gln Leu Gln Glu
        435                 440                 445

Ser Gly Pro Gly Leu Val Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys
    450                 455                 460

Thr Val Ser Gly Phe Ser Leu Ala Ser Tyr Asn Ile His Trp Val Arg
465                 470                 475                 480

Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Ala Gly
                485                 490                 495

Gly Ser Thr Asn Tyr Asn Ser Ala Leu Met Ser Arg Leu Thr Ile Ser
            500                 505                 510
```

Lys Asp Asn Ser Lys Asn Gln Val Phe Leu Lys Met Ser Ser Leu Thr
515 520 525

Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr
530 535 540

Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
545 550 555 560

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
565 570 575

Asn Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
580 585 590

Arg Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr
595 600 605

Leu His Trp Tyr Gln Gln Lys Ser Gly Lys Ala Pro Lys Val Trp Ile
610 615 620

Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
625 630 635 640

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
645 650 655

[0035] Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile
660 665 670

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Ser Asp Pro Ala
675 680 685

Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
690 695 700

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
705 710 715 720

Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val
725 730 735

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
740 745 750

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
755 760 765

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
770 775 780

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
785 790 795 800

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
805 810 815

```
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
            820                 825                 830

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        835                 840                 845

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
    850                 855                 860

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
865                 870                 875                 880

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                885                 890                 895

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
            900                 905                 910

Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe Trp Val Leu Val
        915                 920                 925

Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala
    930                 935                 940

Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser
945                 950                 955                 960
```

[0036]

```
Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His
                965                 970                 975

Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg
            980                 985                 990

Val Lys Phe Ser Arg Ser Ala Asp  Ala Pro Ala Tyr Gln  Gln Gly Gln
        995                 1000                1005

Asn Gln  Leu Tyr Asn Glu Leu  Asn Leu Gly Arg Arg  Glu Glu Tyr
    1010                1015                1020

Asp Val  Leu Asp Lys Arg Arg  Gly Arg Asp Pro Glu  Met Gly Gly
    1025                1030                1035

Lys Pro  Arg Arg Lys Asn Pro  Gln Glu Gly Leu Tyr  Asn Glu Leu
    1040                1045                1050

Gln Lys  Asp Lys Met Ala Glu  Ala Tyr Ser Glu Ile  Gly Met Lys
    1055                1060                1065

Gly Glu  Arg Arg Arg Gly Lys  Gly His Asp Gly Leu  Tyr Gln Gly
    1070                1075                1080

Leu Ser  Thr Ala Thr Lys Asp  Thr Tyr Asp Ala Leu  His Met Gln
    1085                1090                1095

Ala Leu  Pro Pro Arg
    1100
```

<210> 35
<211> 858
<212> PRT
<213> 人工序列

<220>
<223> 与RQR8自杀基因共表达的抗GD2 CAR

<400> 35

```
Met Gly Thr Ser Leu Leu Cys Trp Met Ala Leu Cys Leu Leu Gly Ala
1               5                  10                  15

Asp His Ala Asp Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly
            20                  25                  30

Gly Gly Gly Ser Glu Leu Pro Thr Gln Gly Thr Phe Ser Asn Val Ser
        35                  40                  45

Thr Asn Val Ser Pro Ala Lys Pro Thr Thr Thr Ala Cys Pro Tyr Ser
    50                  55                  60

Asn Pro Ser Leu Cys Ser Gly Gly Gly Gly Ser Pro Ala Pro Arg Pro
65                  70                  75                  80

Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro
                85                  90                  95

Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu
            100                 105                 110
```

[0037]

```
Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys
        115                 120                 125

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His Arg
    130                 135                 140

Asn Arg Arg Arg Val Cys Lys Cys Pro Arg Pro Val Val Arg Ala Glu
145                 150                 155                 160

Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu Asn Pro Gly
                165                 170                 175

Pro Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val
            180                 185                 190

Pro Gly Ser Thr Gly Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu
        195                 200                 205

Val Lys Pro Ser Gln Thr Leu Ser Ile Thr Cys Thr Val Ser Gly Phe
    210                 215                 220

Ser Leu Ala Ser Tyr Asn Ile His Trp Val Arg Gln Pro Pro Gly Lys
225                 230                 235                 240

Gly Leu Glu Trp Leu Gly Val Ile Trp Ala Gly Gly Ser Thr Asn Tyr
                245                 250                 255

Asn Ser Ala Leu Met Ser Arg Leu Thr Ile Ser Lys Asp Asn Ser Lys
```

260 265 270

Asn Gln Val Phe Leu Lys Met Ser Ser Leu Thr Ala Ala Asp Thr Ala
275 280 285

Val Tyr Tyr Cys Ala Lys Arg Ser Asp Asp Tyr Ser Trp Phe Ala Tyr
290 295 300

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
305 310 315 320

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn Gln Met Thr Gln
325 330 335

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
340 345 350

Cys Arg Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His Trp Tyr Gln
355 360 365

Gln Lys Ser Gly Lys Ala Pro Lys Val Trp Ile Tyr Ser Thr Ser Asn
370 375 380

Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
385 390 395 400

Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
405 410 415

[0038]

Tyr Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro Ile Thr Phe Gly Gln Gly
420 425 430

Thr Lys Val Glu Ile Lys Arg Ser Asp Pro Ala Glu Pro Lys Ser Pro
435 440 445

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
450 455 460

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
465 470 475 480

Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
485 490 495

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
500 505 510

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
515 520 525

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
530 535 540

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
545 550 555 560

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr

565 570 575

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
580 585 590

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
595 600 605

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
610 615 620

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
625 630 635 640

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
645 650 655

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
660 665 670

Gly Lys Lys Asp Pro Lys Phe Trp Val Leu Val Val Val Gly Gly Val
675 680 685

Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp
690 695 700

Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met
705 710 715 720

[0039]

Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala
725 730 735

Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg
740 745 750

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
755 760 765

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
770 775 780

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
785 790 795 800

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
805 810 815

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
820 825 830

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
835 840 845

Ala Leu His Met Gln Ala Leu Pro Pro Arg
850 855

<210> 36

<211> 402
<212> PRT
<213> 人工序列

<220>
<223> 可诱导半胱天冬酶9 (iCasp9) 序列

<400> 36

Met Leu Glu Gly Val Gln Val Glu Thr Ile Ser Pro Gly Asp Gly Arg
1 5 10 15

Thr Phe Pro Lys Arg Gly Gln Thr Cys Val Val His Tyr Thr Gly Met
20 25 30

Leu Glu Asp Gly Lys Lys Val Asp Ser Ser Arg Asp Arg Asn Lys Pro
35 40 45

Phe Lys Phe Met Leu Gly Lys Gln Glu Val Ile Arg Gly Trp Glu Glu
50 55 60

Gly Val Ala Gln Met Ser Val Gly Gln Arg Ala Lys Leu Thr Ile Ser
65 70 75 80

Pro Asp Tyr Ala Tyr Gly Ala Thr Gly His Pro Gly Ile Ile Pro Pro
85 90 95

His Ala Thr Leu Val Phe Asp Val Glu Leu Leu Lys Leu Glu Ser Gly
100 105 110

[0040] Gly Gly Ser Gly Val Asp Gly Phe Gly Asp Val Gly Ala Leu Glu Ser
115 120 125

Leu Arg Gly Asn Ala Asp Leu Ala Tyr Ile Leu Ser Met Glu Pro Cys
130 135 140

Gly His Cys Leu Ile Ile Asn Asn Val Asn Phe Cys Arg Glu Ser Gly
145 150 155 160

Leu Arg Thr Arg Thr Gly Ser Asn Ile Asp Cys Glu Lys Leu Arg Arg
165 170 175

Arg Phe Ser Ser Leu His Phe Met Val Glu Val Lys Gly Asp Leu Thr
180 185 190

Ala Lys Lys Met Val Leu Ala Leu Leu Glu Leu Ala Gln Gln Asp His
195 200 205

Gly Ala Leu Asp Cys Cys Val Val Val Ile Leu Ser His Gly Cys Gln
210 215 220

Ala Ser His Leu Gln Phe Pro Gly Ala Val Tyr Gly Thr Asp Gly Cys
225 230 235 240

Pro Val Ser Val Glu Lys Ile Val Asn Ile Phe Asn Gly Thr Ser Cys
245 250 255

Pro Ser Leu Gly Gly Lys Pro Lys Leu Phe Phe Ile Gln Ala Cys Gly
260 265 270

Gly Glu Gln Lys Asp His Gly Phe Glu Val Ala Ser Thr Ser Pro Glu
275 280 285

Asp Glu Ser Pro Gly Ser Asn Pro Glu Pro Asp Ala Thr Pro Phe Gln
290 295 300

Glu Gly Leu Arg Thr Phe Asp Gln Leu Asp Ala Ile Ser Ser Leu Pro
305 310 315 320

Thr Pro Ser Asp Ile Phe Val Ser Tyr Ser Thr Phe Pro Gly Phe Val
325 330 335

Ser Trp Arg Asp Pro Lys Ser Gly Ser Trp Tyr Val Glu Thr Leu Asp
340 345 350

Asp Ile Phe Glu Gln Trp Ala His Ser Glu Asp Leu Gln Ser Leu Leu
355 360 365

Leu Arg Val Ala Asn Ala Val Ser Val Lys Gly Ile Tyr Lys Gln Met
370 375 380

Pro Gly Cys Phe Asn Phe Leu Arg Lys Lys Leu Phe Phe Lys Thr Ser
385 390 395 400

Ala Ser

[0041]

<210> 37
<211> 157
<212> PRT
<213> 人工序列

<220>
<223> 新的标志物/自杀基因RQR8序列

<400> 37

Met Gly Thr Ser Leu Leu Cys Trp Met Ala Leu Cys Leu Leu Gly Ala
1 5 10 15

Asp His Ala Asp Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly
20 25 30

Gly Gly Gly Ser Glu Leu Pro Thr Gln Gly Thr Phe Ser Asn Val Ser
35 40 45

Thr Asn Val Ser Pro Ala Lys Pro Thr Thr Thr Ala Cys Pro Tyr Ser
50 55 60

Asn Pro Ser Leu Cys Ser Gly Gly Gly Gly Ser Pro Ala Pro Arg Pro
65 70 75 80

Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro
85 90 95

Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu
100 105 110

Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys

115 120 125

[0042]

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Asn His Arg
130 135 140

Asn Arg Arg Arg Val Cys Lys Cys Pro Arg Pro Val Val
145 150 155

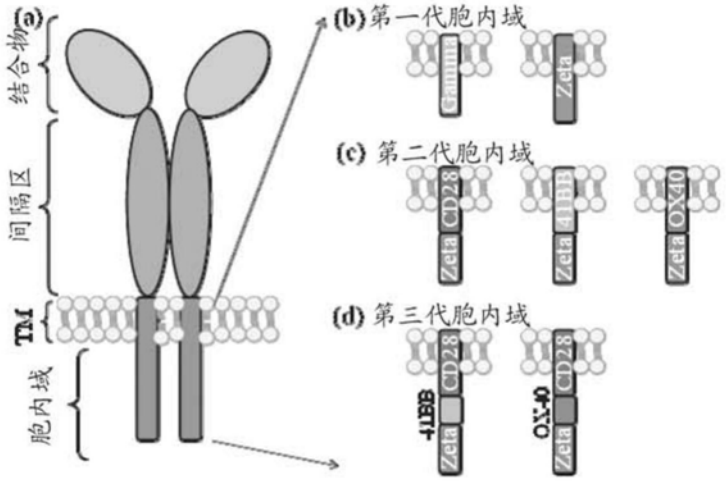

(a)
结合物
间隔区
TM
胞内域
(b)第一代胞内域
Zeta
(c) 第二代胞内域
Zeta CD28
Zeta 4-1BB
Zeta OX40
(d) 第三代胞内域
4-1BB+
OX40
Zeta
CD28

图1

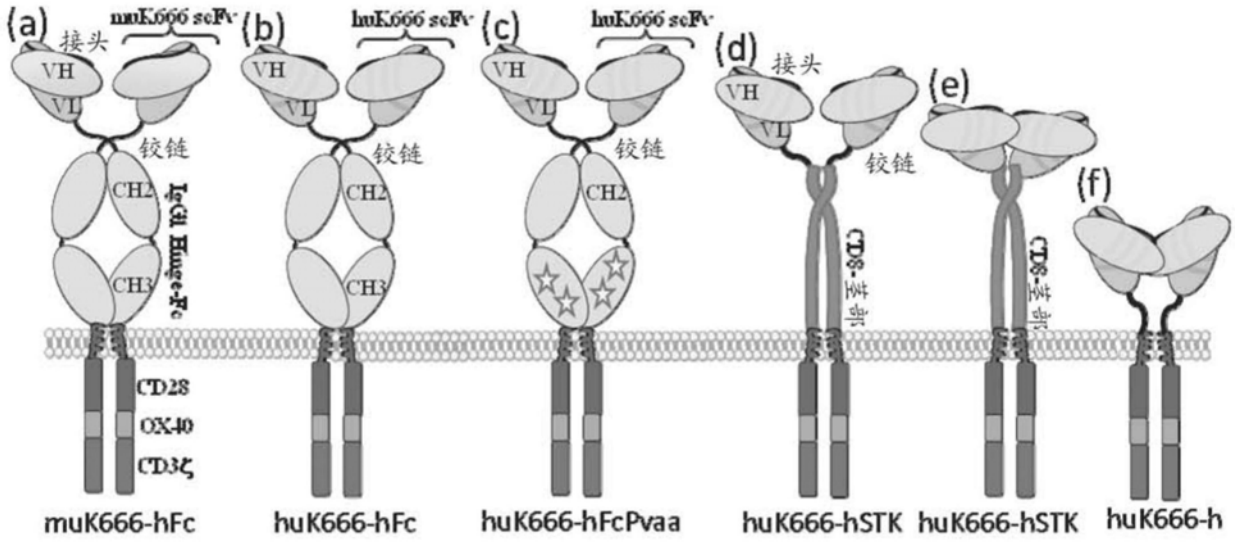

(a)
muK666 scFv
接头
VH
VL
铰链
CH2
CH3
IgG1 Fc
CD28
OX40
CD3ζ
muK666-hFc
(b)
huK666 scFv
huK666-hFc
(c)
huK666-hFcPvaa
(d)
huK666-hSTK
(e)
huK666-hSTK
(f)
huK666-h

图2

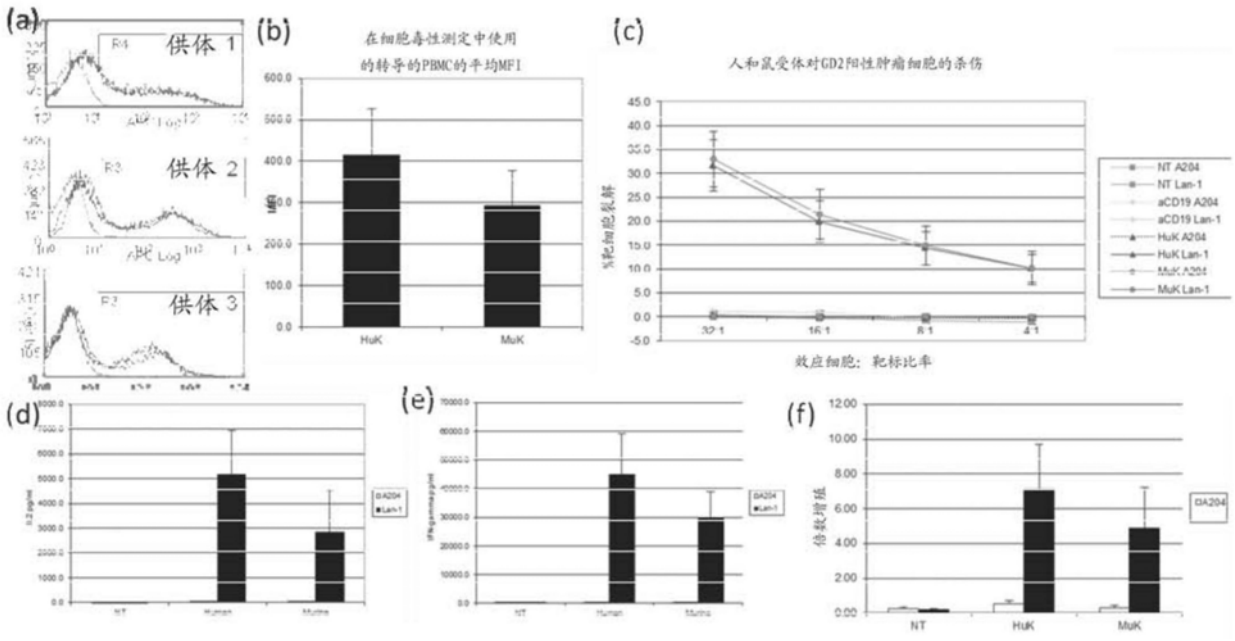

(a)
供体 1
供体 2
供体 3
(b)
在细胞毒性测定中使用
的转导的PBMC的平均MFI
HuK
MuK
(c)
人和鼠受体对GD2阳性肿瘤细胞的杀伤
效应细胞：靶标比率
NT A204
NT Lan-1
aCD19 A204
aCD19 Lan-1
HuK A204
HuK Lan-1
MuK A204
MuK Lan-1
(d)
(e)
(f)
NT
HuK
MuK

图3

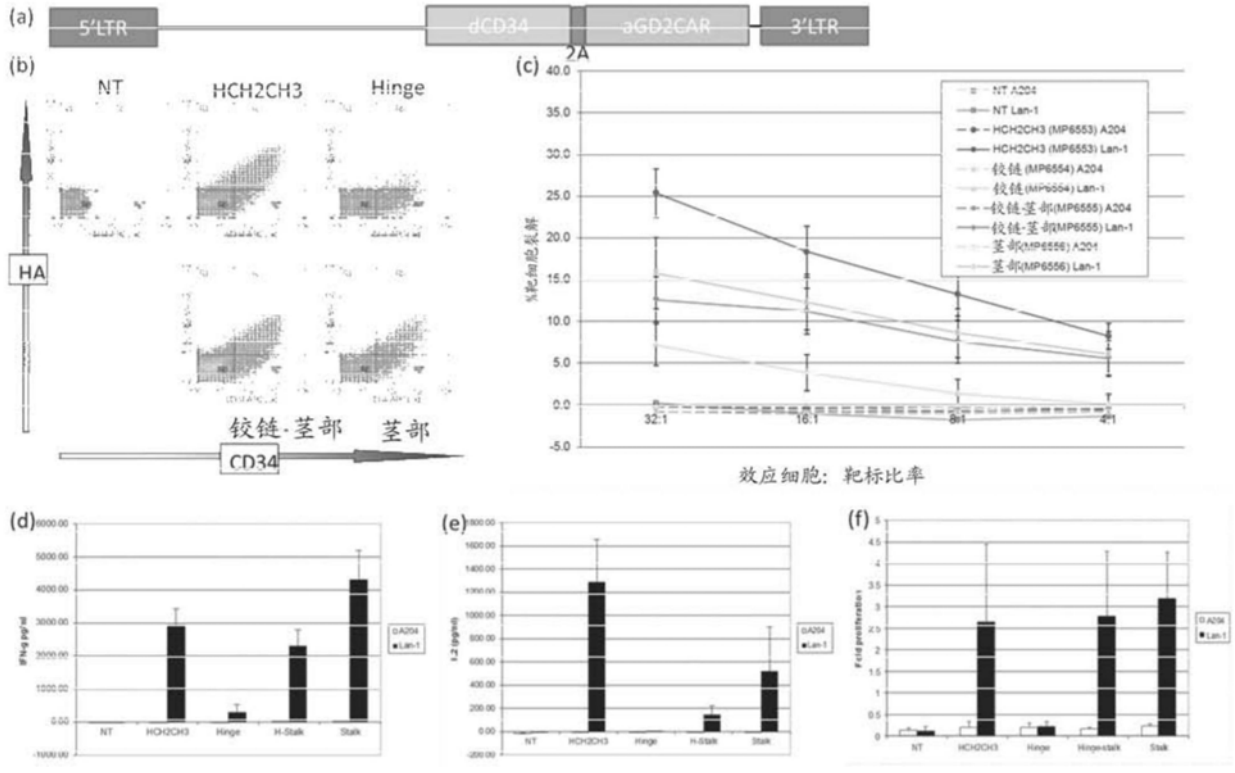

(a)
5'LTR
dCD34
aGD2CAR
3'LTR
2A
(b)
NT
HCH2CH3
Hinge
HA
铰链-茎部
茎部
CD34
(c)
效应细胞：靶标比率
NT A204
NT Lan-1
HCH2CH3 (MP6553) A204
HCH2CH3 (MP6553) Lan-1
铰链(MP6554) A204
铰链(MP6554) Lan-1
铰链-茎部(MP6555) A204
铰链-茎部(MP6555) Lan-1
茎部(MP6556) A201
茎部(MP6556) Lan-1
(d)
(e)
(f)

图4

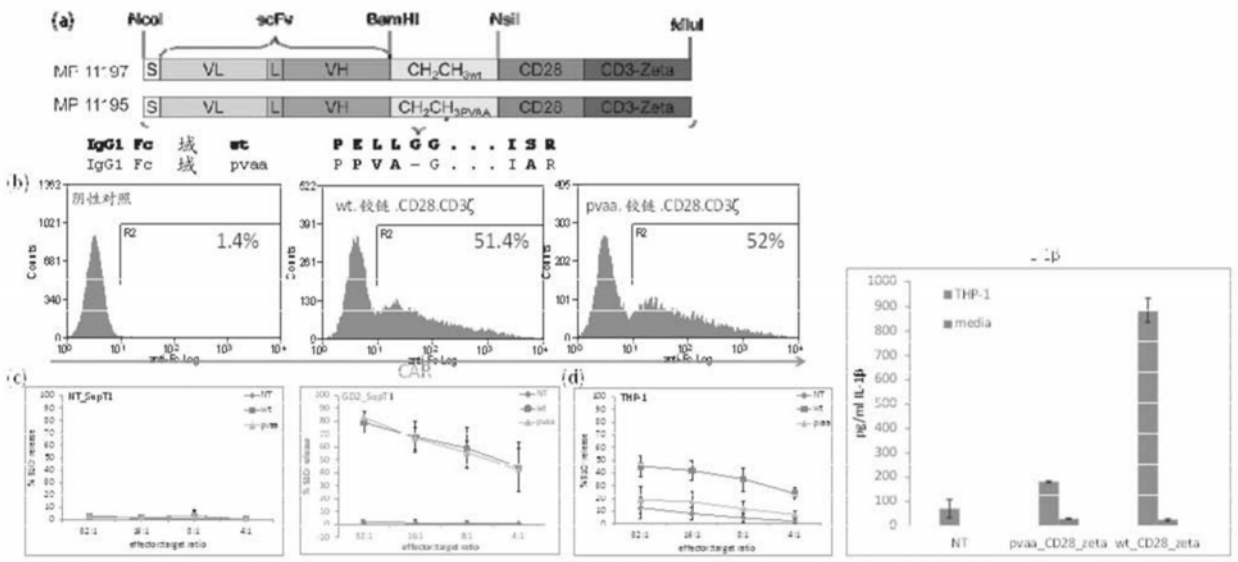

(a)
NcoI
scFv
BamHI
NsiI
MluI
MP 11-97
MP 11-95
S
VL
L
VH
CD28
CD3-Zeta
IgG1 Fc 域 wt
IgG1 Fc 域 pvaa
P E L L G G . . . I S R
P P V A - G . . . I A R
(b)
阴性对照
1.4%
wt.铰链.CD28.CD3ζ
51.4%
pvaa.铰链.CD28.CD3ζ
52%
Counts
CAR
(c)
(d)
THP-1
media
pg/ml IL-1β
NT
pvaa_CD28_zeta
wt_CD28_zeta

图5

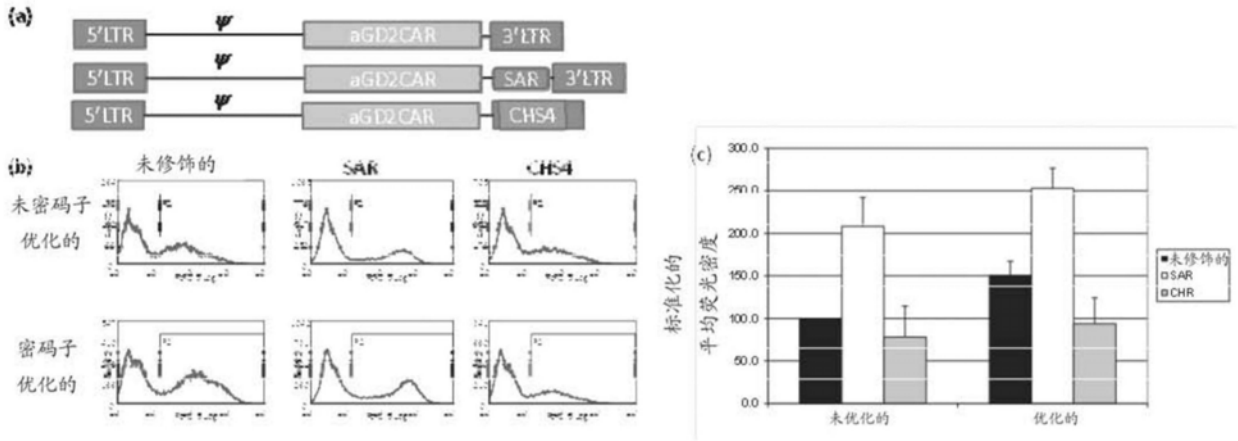

(a)
5'LTR
ψ
aGD2CAR
3'LTR
SAR
CHS4
(b)
未修饰的
未密码子优化的
密码子优化的
(c)
标准化的平均荧光密度
未修饰的
SAR
CHR
未优化的
优化的

图6

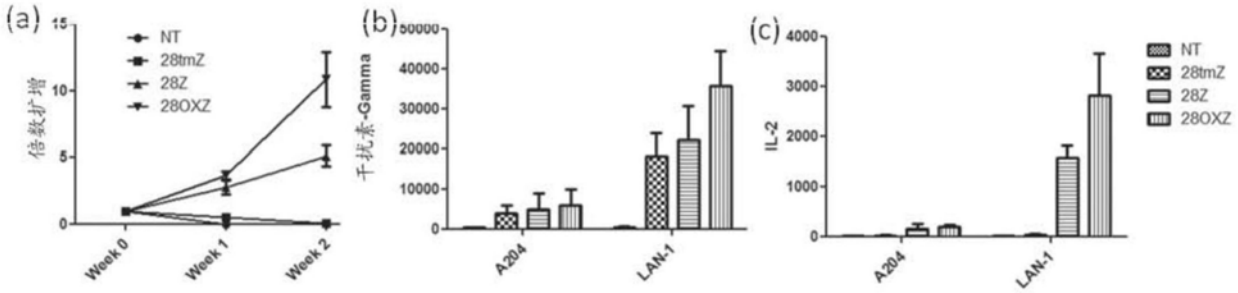

(a)
倍数扩增
NT
28tmZ
28Z
28OXZ
Week 0
Week 1
Week 2
(b)
干扰素-Gamma
A204
LAN-1
(c)
IL-2
A204
LAN-1
NT
28tmZ
28Z
28OXZ

图7

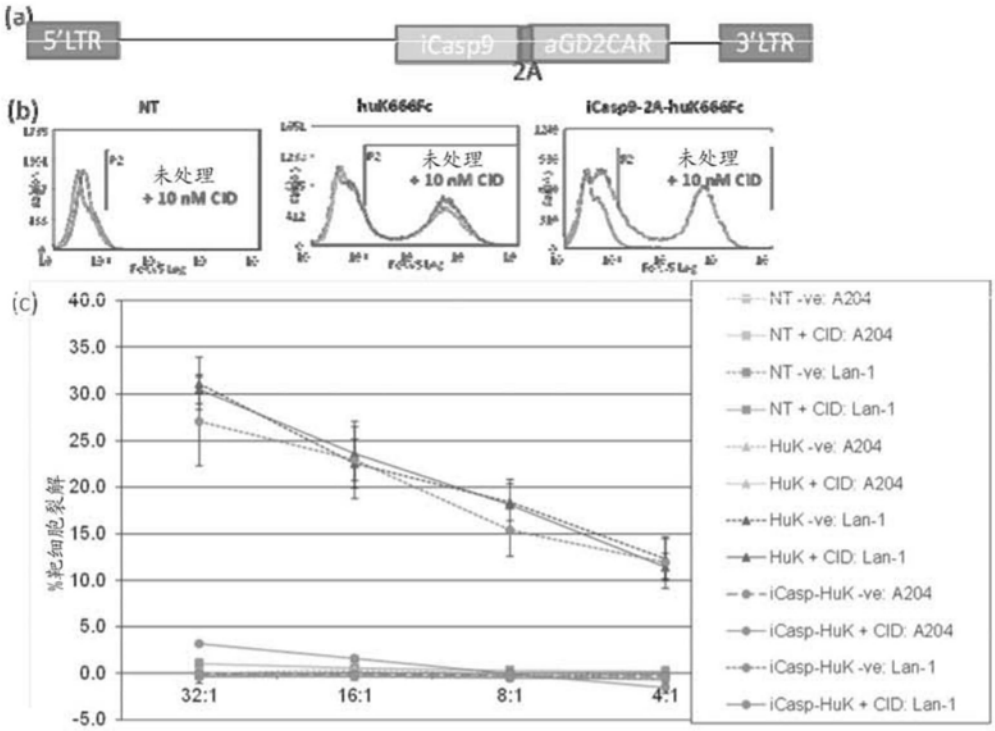

(a)
5'LTR
iCasp9
aGD2CAR
3'LTR
2A
(b)
NT
huK666Fc
iCasp9-2A-huK666Fc
未处理
+ 10 nM CID
(c)
%靶细胞裂解
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
32:1
16:1
8:1
4:1
NT -ve: A204
NT + CID: A204
NT -ve: Lan-1
NT + CID: Lan-1
HuK -ve: A204
HuK + CID: A204
HuK -ve: Lan-1
HuK + CID: Lan-1
iCasp-HuK -ve: A204
iCasp-HuK + CID: A204
iCasp-HuK -ve: Lan-1
iCasp-HuK + CID: Lan-1

图8

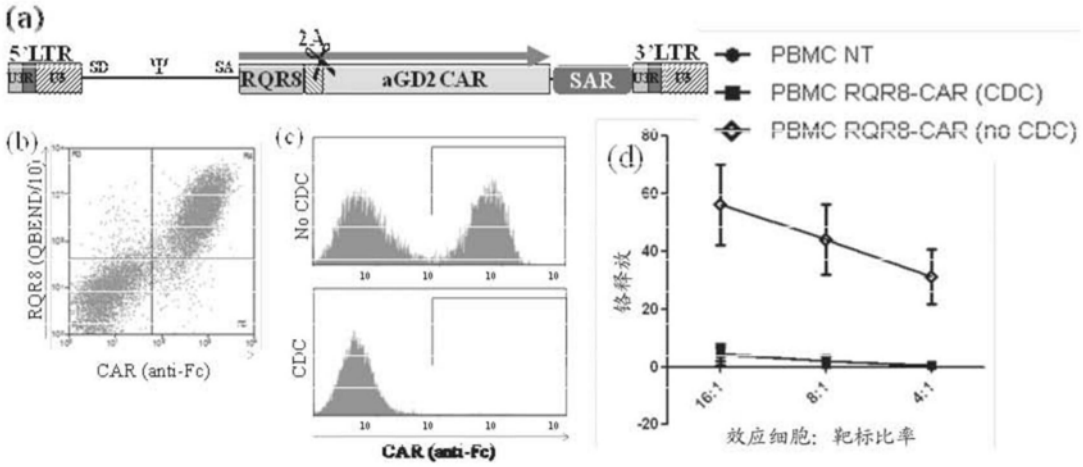

(a)
5'LTR
SD
SA
RQR8
aGD2 CAR
SAR
3'LTR
PBMC NT
PBMC RQR8-CAR (CDC)
PBMC RQR8-CAR (no CDC)
(b)
RQR8 (QBEND/10)
CAR (anti-Fc)
(c)
No CDC
CDC
CAR (anti-Fc)
(d)
特异放
16:1
8:1
4:1
效应细胞：靶标比率

图9

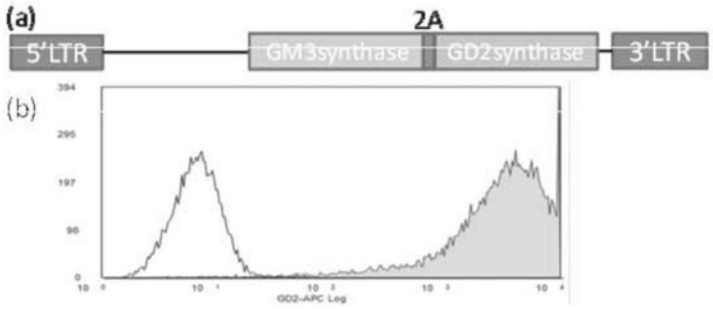

(a)
2A
5'LTR
GM3synthase
GD2synthase
3'LTR
(b)
394
295
197
98
0
10 0
10 1
10 2
10 3
10 4
GD2-APC Log

图10

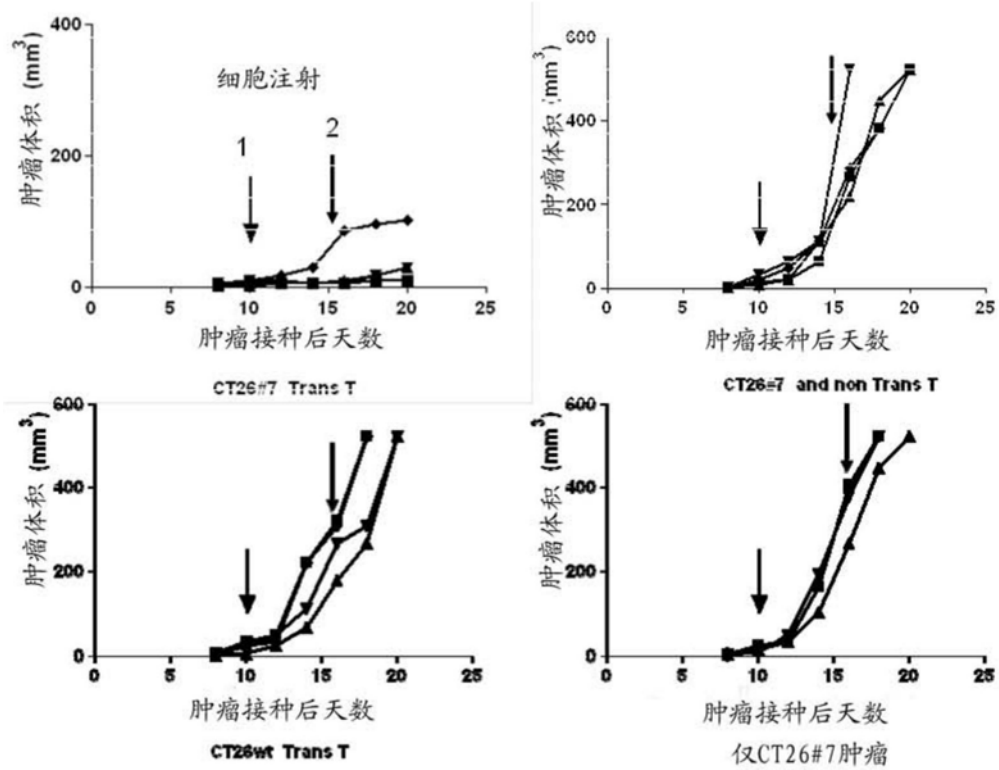

细胞注射
1
2
肿瘤体积 (mm3)
肿瘤接种后天数
CT26#7 Trans T
CT26wt Trans T
CT26#7 and non Trans T
仅CT26#7肿瘤

图11

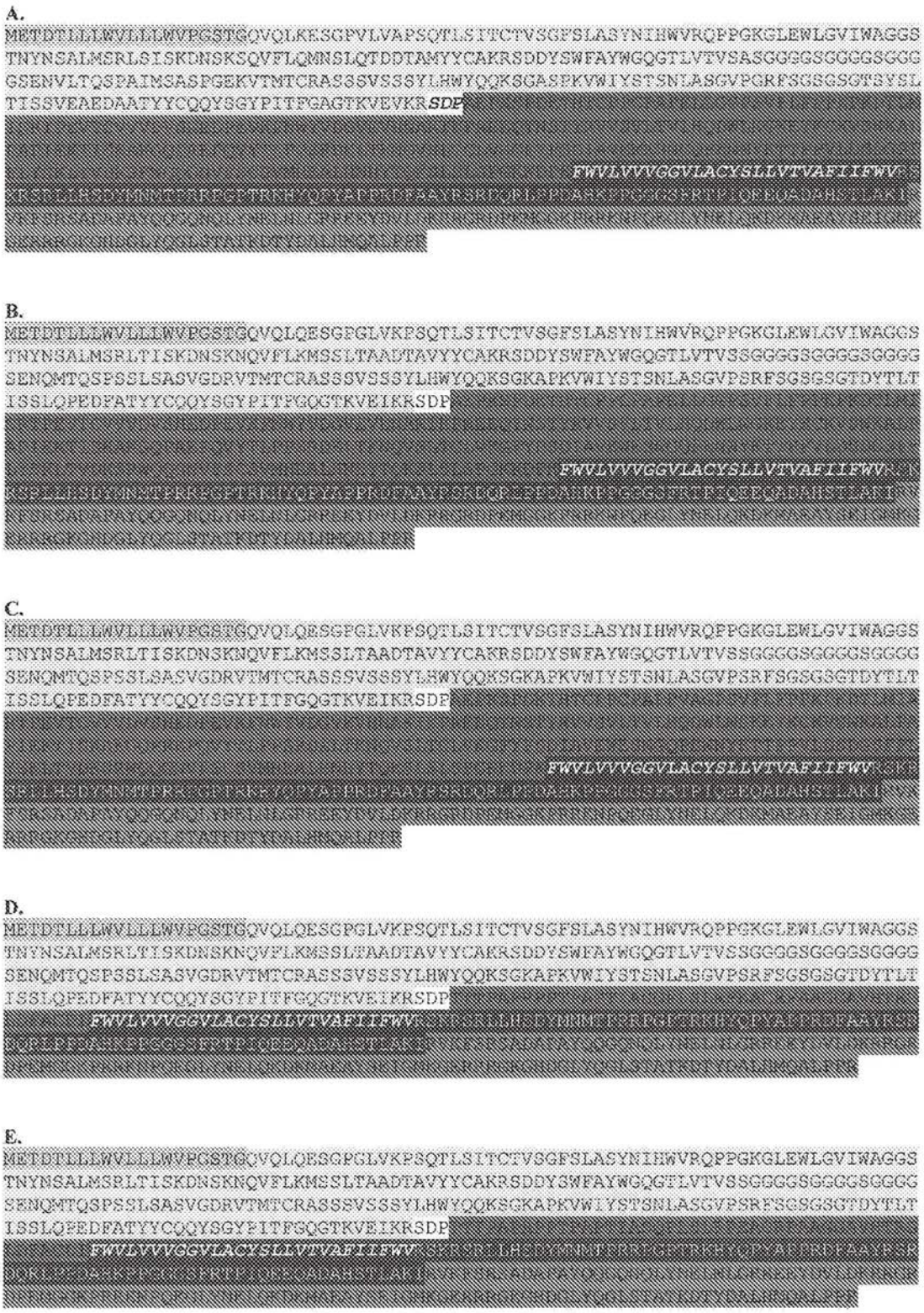

A.
METDTLLLWVLLLWVPGSTGQVQLKESGPVLVAPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLSISKDNSKSQVFLQMNSLQTDDTAMYYCAKRSDDYSWFAYWGQGTLVTVSASGGGGSGGGGSGGG
GSENVLTQSPAIMSASPGEKVTMTCRASSSVSSSYLHWYQQKSGASPKVWIYSTSNLASGVPGRFSGSGSGTSYSL
TISSVEAEDAATYYCQQYSGYPITFGAGTKVEVKR
FWVLVVVGGVLACYSLLVTVAFIIFWV
B.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVTVAFIIFWV
C.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVTVAFIIFWV
D.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVTVAFIIFWV
E.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVTVAFIIFWV

图12

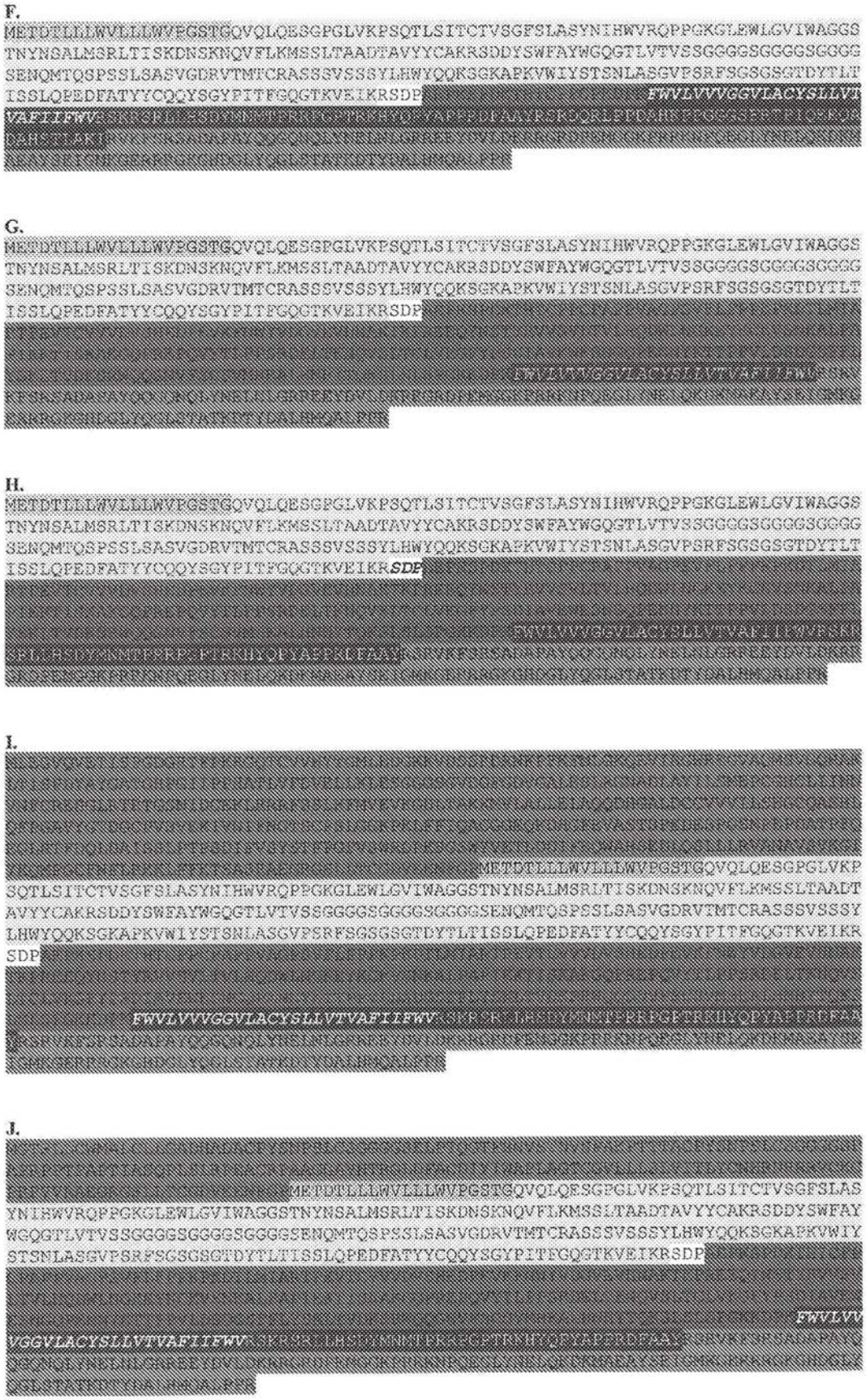

F.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVT
VAFIIFWV
G.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVTVAFIIFWV
H.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGS
TNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGG
SENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLT
ISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLVVVGGVLACYSLLVTVAFIIFWV
I.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKP
SQTLSITCTVSGFSLASYNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLTISKDNSKNQVFLKMSSLTAADT
AVYYCAKRSDDYSWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSENQMTQSPSSLSASVGDRVTMTCRASSSVSSSY
LHWYQQKSGKAPKVWIYSTSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKR
SDP
FWVLVVVGGVLACYSLLVTVAFIIFWV
J.
METDTLLLWVLLLWVPGSTGQVQLQESGPGLVKPSQTLSITCTVSGFSLAS
YNIHWVRQPPGKGLEWLGVIWAGGSTNYNSALMSRLTISKDNSKNQVFLKMSSLTAADTAVYYCAKRSDDYSWFAY
WGQGTLVTVSSGGGGSGGGGSGGGGSENQMTQSPSSLSASVGDRVTMTCRASSSVSSSYLHWYQQKSGKAPKVWIY
STSNLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSGYPITFGQGTKVEIKRSDP
FWVLV
VGGVLACYSLLVTVAFIIFWV

图12续

区域	描述
自杀基因	iCasp9或RQR8
FMD-2A	口蹄疫2A肽
信号	信号肽
scFv1	scFv（muKM666或huKM666）
SDP	接头和链断裂
间隔区	CD8alpha茎部
CD28 TM	CD28跨膜域
CD28 endo	CD28胞内域
OX40 endo	OX40胞内域
CD3Z endo	CD3 Zeta胞内域

图12续

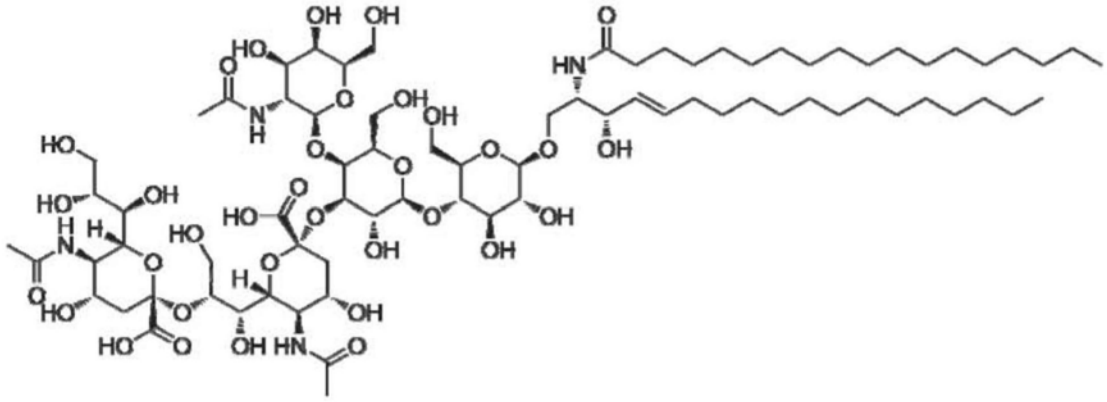


图13

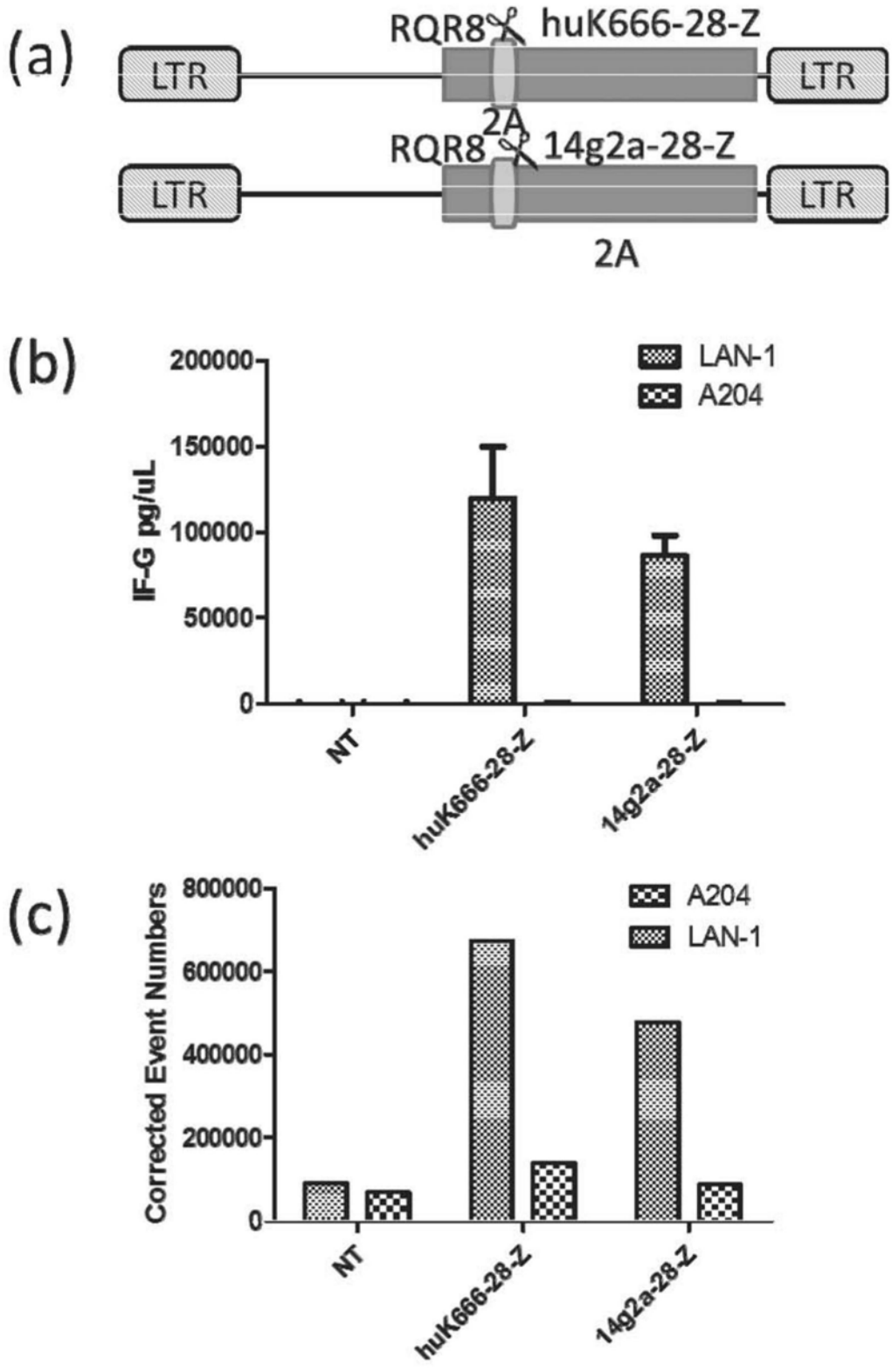

(a)
RQR8
huK666-28-Z
LTR
LTR
2A
RQR8
14g2a-28-Z
LTR
LTR
2A
(b)
LAN-1
A204
IF-G pg/uL
200000
150000
100000
50000
0
NT
huK666-28-Z
14g2a-28-Z
(c)
A204
LAN-1
Corrected Event Numbers
800000
600000
400000
200000
0
NT
huK666-28-Z
14g2a-28-Z

图14

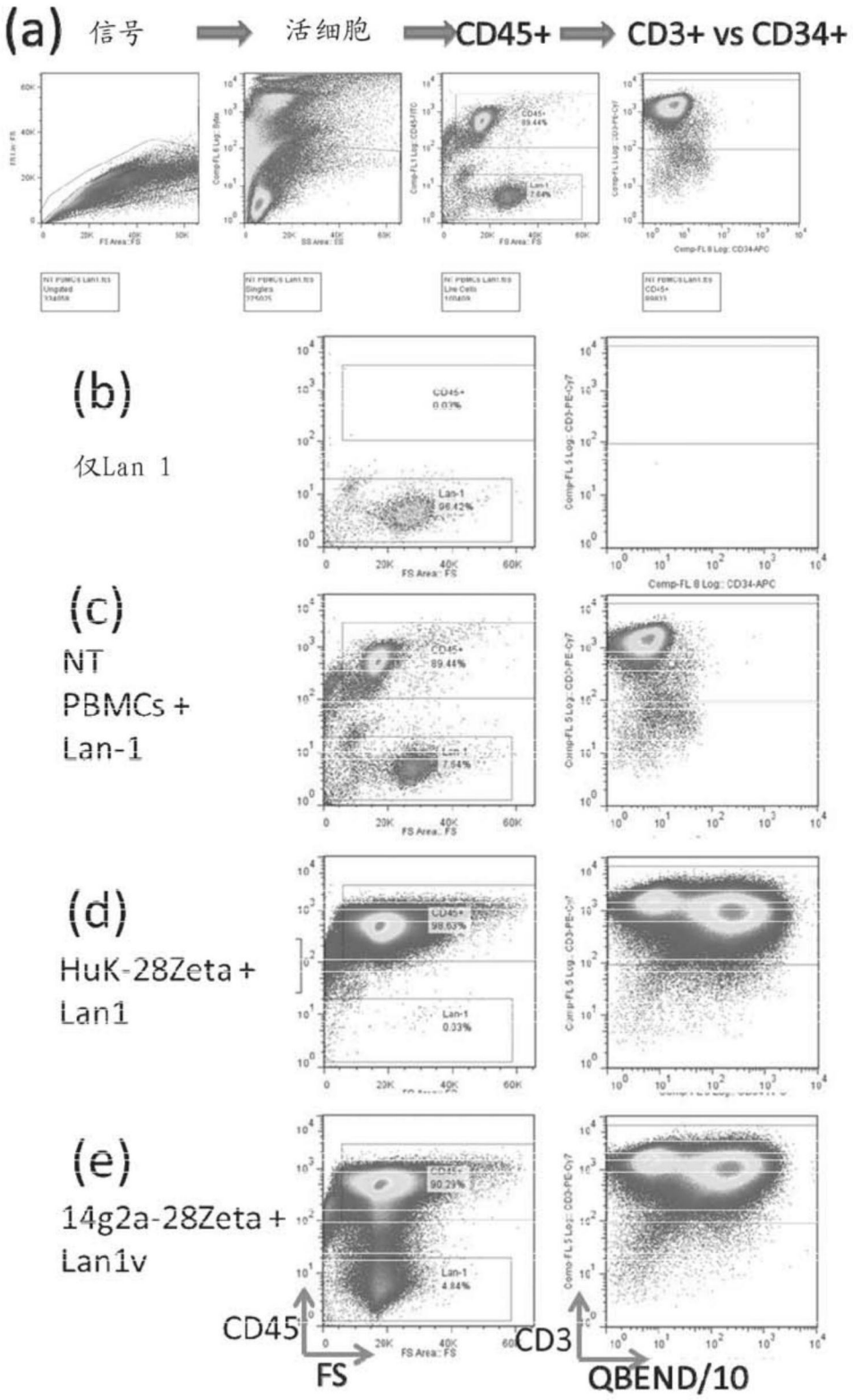

(a) 信号 活细胞 CD45+ CD3+ vs CD34+
(b)
仅Lan 1
(c)
NT
PBMCs +
Lan-1
(d)
HuK-28Zeta +
Lan1
(e)
14g2a-28Zeta +
Lan1v
CD45
FS
CD3
QBEND/10

图15