(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 112912109 A
(43)申请公布日 2021.06.04

(21)申请号 201980061665.4

(22)申请日 2019.09.19

(30)优先权数据
2018-175510 2018.09.20 JP

(85)PCT国际申请进入国家阶段日
2021.03.19

(86)PCT国际申请的申请数据
PCT/JP2019/036668 2019.09.19

(87)PCT国际申请的公布数据
WO2020/059772 JA 2020.03.26

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(51)Int.Cl.
A61K 47/68(2006.01)
A61K 31/437(2006.01)
A61K 39/395(2006.01)
A61K 47/65(2006.01)
A61P 1/00(2006.01)
A61P 11/00(2006.01)
A61P 15/00(2006.01)
A61P 17/00(2006.01)
A61P 25/00(2006.01)
A61P 35/00(2006.01)

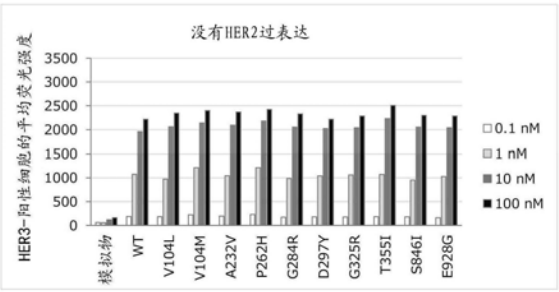
权利要求书4页 说明书41页
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(54)发明名称

通过施用抗HER3抗体-药物缀合物治疗HER3-突变的癌症

(57)摘要

包含抗HER3抗体-药物缀合物作为活性成分的HER3-突变的癌症的治疗剂,和/或癌症的治疗方法,所述方法包括向确定具有HER3-突变的癌症的受试者施用抗HER3抗体-药物缀合物。



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1.HER3-突变的癌症的治疗剂,其包含抗HER3抗体-药物缀合物作为活性成分。

2.根据权利要求1所述的治疗剂,其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种:V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G。

3.根据权利要求1所述的治疗剂,其中所述HER3-突变的癌症中的HER3突变是Q809R。

4.根据权利要求1至3中任一项所述的治疗剂,其中HER2在所述HER3-突变的癌症中过表达。

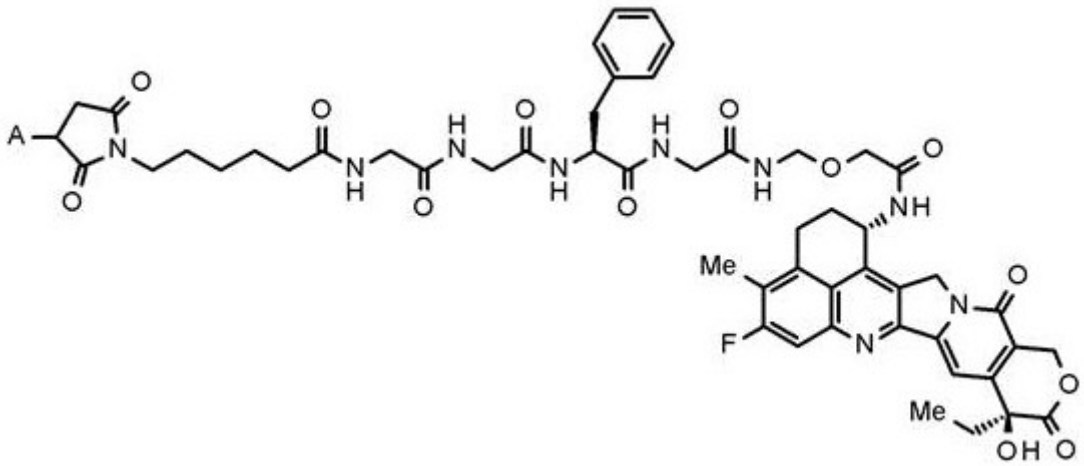
5.根据权利要求1至3中任一项所述的治疗剂,其中HER2在所述HER3-突变的癌症中未过表达。

6.根据权利要求1至5中任一项所述的治疗剂,其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间,所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

7.根据权利要求1至6中任一项所述的治疗剂,其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间,所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

8.根据权利要求1至7中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物,其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合:

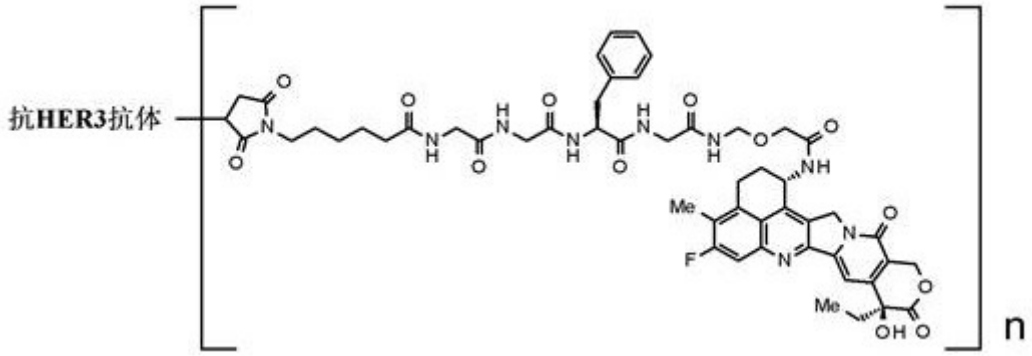
[式1]



其中A代表与抗HER3抗体的连接位置。

9.根据权利要求1至7中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物:

[式2]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合，且n是每个抗体分子缀合的药物-接头的单元的平均数。

10.根据权利要求1至9中任一项所述的治疗剂，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3；且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

11.根据权利要求1至9中任一项所述的治疗剂，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区，且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

12.根据权利要求1至9中任一项所述的治疗剂，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链由SEQ ID NO:9代表的氨基酸序列组成，且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

13.根据权利要求12所述的治疗剂，其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

14.根据权利要求1至13中任一项所述的治疗剂，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

15.根据权利要求1至13中任一项所述的治疗剂，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

16.根据权利要求1至15中任一项所述的治疗剂，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

17.根据权利要求1至15中任一项所述的治疗剂，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

18.癌症的治疗方法，其包括向确定具有HER3-突变的癌症的受试者施用抗HER3抗体-药物缀合物。

19.根据权利要求18所述的治疗方法，其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种：V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和

E928G。

20.根据权利要求18所述的治疗方法，其中所述HER3-突变的癌症中的HER3突变是Q809R。

21.根据权利要求18至20中任一项所述的治疗方法，其中HER2在所述HER3-突变的癌症中过表达。

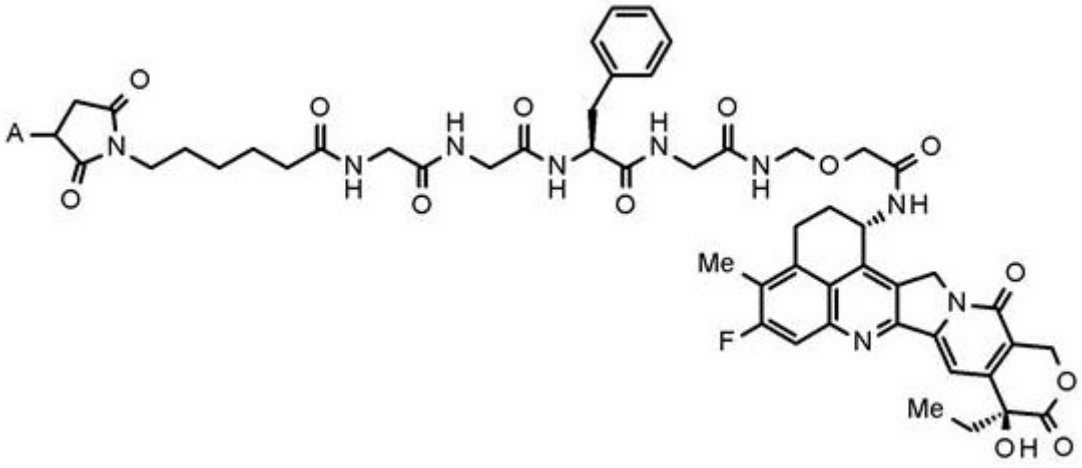
22.根据权利要求18至20中任一项所述的治疗方法，其中HER2在所述HER3-突变的癌症中未过表达。

23.根据权利要求18至22中任一项所述的治疗方法，其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

24.根据权利要求18至23中任一项所述的治疗方法，其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

25.根据权利要求18至24中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物，其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合：

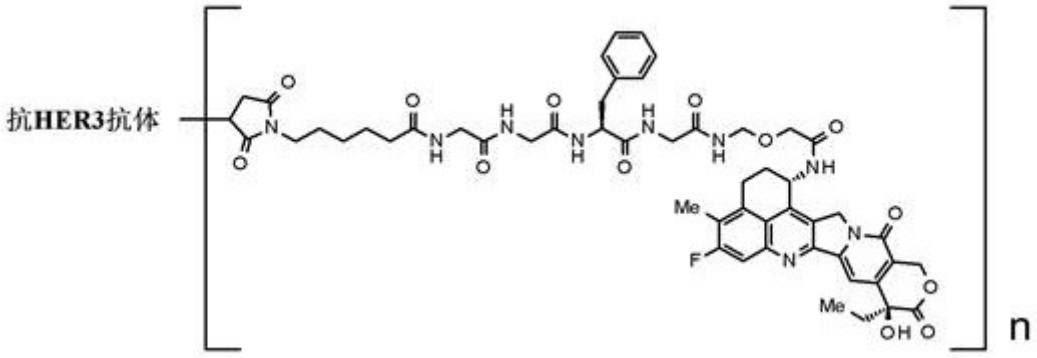
[式3]



其中A代表与抗HER3抗体的连接位置。

26.根据权利要求18至24中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物：

[式4]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合，且n是每个抗体分子缀合的药物-接头的单元的平均数。

27.根据权利要求18至26中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3；且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

28.根据权利要求18至26中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区，且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

29.根据权利要求18至26中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链由SEQ ID NO:9代表的氨基酸序列组成，且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

30.根据权利要求29所述的治疗方法，其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

31.根据权利要求18至30中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

32.根据权利要求18至30中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

33.根据权利要求18至32中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

34.根据权利要求18至32中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

通过施用抗HER3抗体-药物缀合物治疗HER3-突变的癌症

技术领域

[0001] 本发明涉及包含抗HER3抗体-药物缀合物的HER3-突变的癌症的治疗剂和/或癌症的治疗方法，所述方法包括将抗HER3抗体-药物缀合物施用于确定具有HER3-突变的癌症的受试者。

背景技术

[0002] 人表皮生长因子受体3 (HER3；也称为ErbB3)是跨膜受体，其属于受体蛋白酪氨酸激酶的表皮生长因子受体亚家族。已知HER3在各种癌症、诸如乳腺癌、肺癌和结肠直肠癌中表达，并且其与酪氨酸激酶受体、诸如HER2和EGFR一起形成异二聚体，其后，HER3被磷酸化，由此诱导癌细胞生长或凋亡抑制信号(非专利参考文献1至3)。

[0003] 存在HER3的突变体，其被称为癌症驱动突变之一(非专利参考文献4至6)。据报道，这种HER3-突变的癌症存在于例如4%的乳腺癌病例(非专利参考文献7)、10%的胃癌病例(非专利参考文献8)、1%的卵巢癌病例(非专利参考文献9)、1%的结肠直肠癌病例(非专利文献5)和1%的头颈癌病例(非专利文献10)中。

[0004] 已经报道，在HER2过表达的情况下，抗HER2药物、诸如曲妥珠单抗、帕妥珠单抗和拉帕替尼在体外和体内表现出针对HER3-突变的癌症的有效性(非专利参考文献11)。

[0005] 同时，也已经报道在使用作为抗HER2药物的诺拉替尼的临床研究中未显示针对HER3-突变的癌症的有效性(非专利参考文献12)。

[0006] 已经表明，抗HER2药物针对HER3-突变的癌症无效的原因之一是HER3-突变的癌症可以不依赖于HER2的过表达而发挥功能。也就是说，已经报道，甚至在HER2没有过表达的情况下，HER3-突变的癌症也可以诱导癌细胞生长(非专利文献13和14)，且因此据信在HER2未过表达的情况下，抗HER2药物不能发挥对HER3-突变的癌症的抗肿瘤效果。

[0007] 此外，还已知已经实施研究以验证抗HER3抗体针对HER3-突变的癌症的效力(非专利参考文献11)。然而，没有报道：无论是否存在HER2过表达，抗HER3抗体已经表明针对HER3-突变的癌症的明显的效力。另外，通常假设抗HER3抗体与HER3的结合随着HER3的突变而降低，且因此将难以获得持续表现出优异的针对各种HER3突变体的抗肿瘤活性的抗HER3抗体。因此，尚未建立治疗HER3-突变的癌症的有效方法。

[0008] 因此，预期具有与抗体缀合的具有细胞毒性的药物的抗体-药物缀合物(ADC)(其抗原在癌细胞的表面上表达，并且还结合能够细胞内化的抗原，且因此可以将药物选择性递送至癌细胞)引起药物在癌细胞内的累积并杀死癌细胞(非专利参考文献15至19)。

[0009] 作为一种这样的抗体-药物缀合物，包含抗HER3抗体和作为拓扑异构酶I抑制剂的依沙替康的衍生物作为其组分的抗HER3抗体-药物缀合物是已知的(专利参考文献1)。没有关于抗HER3抗体-药物缀合物针对HER3-突变的癌症的效力的报道。

[0010] 引文列表

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非专利参考文献4:Sergina等人, Nature (2007) 445, 437-41.

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非专利参考文献6:Ding等人, Nature (2008) 455, 1069-75.

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非专利参考文献16:Alley等人, Current Opinion in Chemical Biology (2010) 14, 529-537.

非专利参考文献17:Damle, Expert Opin. Biol. Ther. (2004) 4, 1445-1452.

非专利参考文献18:Senter等人, Nature Biotechnology (2012) 30, 631-637.

非专利参考文献19:Howard等人, J Clin Oncol (2011) 29, 398-405。

[0011] 发明概述

技术问题

本发明的一个目的是提供包含抗HER3抗体-药物缀合物的HER3-突变的癌症的治疗剂和/或癌症的治疗方法,所述方法包括将抗HER3抗体-药物缀合物施用于确定具有HER3-突变的癌症的受试者。

[0012] 问题的解决方案

作为为了解决上述问题而进行的认真研究的结果,发明人已经发现抗HER3抗体-药物缀合物表现出优异的针对HER3-突变的癌症的抗肿瘤活性,由此完成了本发明。

[0013] 因此,本发明提供了以下[1]至[85]。

[0014] [1] HER3-突变的癌症的治疗剂,其包含抗HER3抗体-药物缀合物作为活性成分。

[0015] [2]根据[1]所述的治疗剂,其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种:V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G。

[0016] [3]根据[1]所述的治疗剂,其中所述HER3-突变的癌症中的HER3突变是Q809R。

[0017] [4]根据[1]至[3]中任一项所述的治疗剂,其中HER2在所述HER3-突变的癌症中过表达。

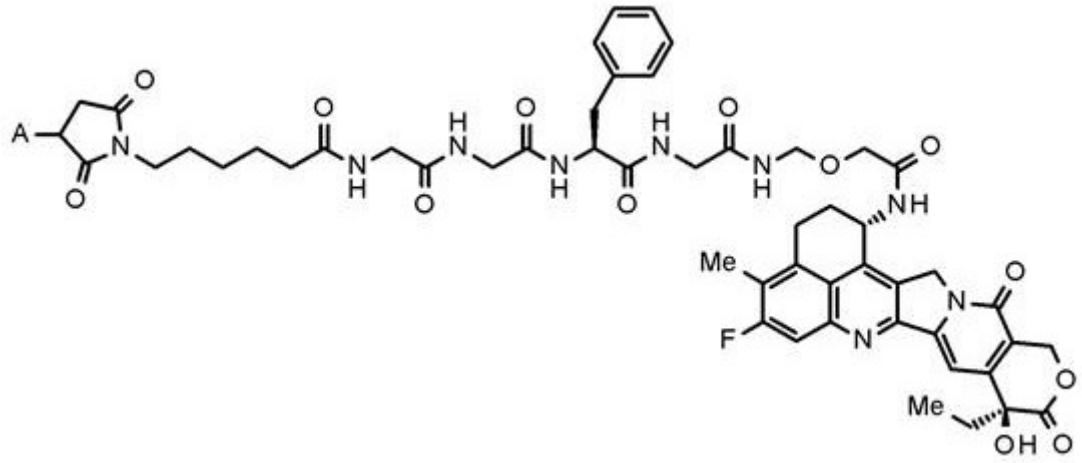
[0018] [5]根据[1]至[3]中任一项所述的治疗剂,其中HER2在所述HER3-突变的癌症中未过表达。

[0019] [6]根据[1]至[5]中任一项所述的治疗剂,其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间,所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0020] [7]根据[1]至[6]中任一项所述的治疗剂,其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间,所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0021] [8]根据[1]至[7]中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物,其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合:

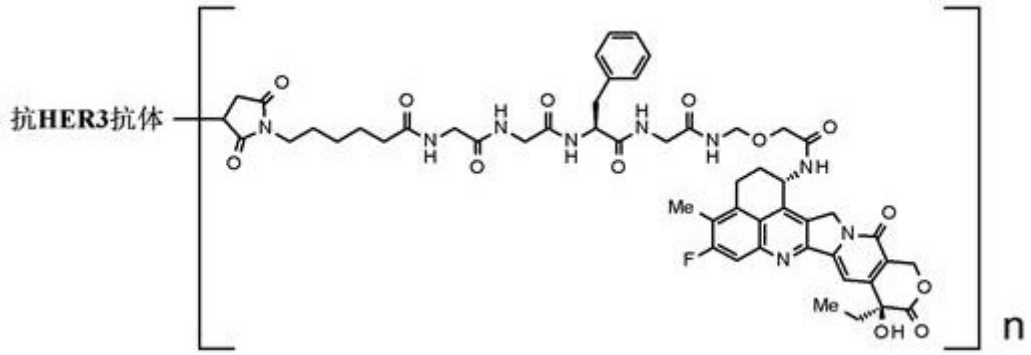
[式1]



其中A代表与抗HER3抗体的连接位置。

[0022] [9]根据[1]至[7]中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物:

[式2]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合,且n是每个抗体分子缀合的药物-接头的单元的平均数。

[0023] [10]根据[1]至[9]中任一项所述的治疗剂,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3;且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0024] [11]根据[1]至[9]中任一项所述的治疗剂,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区,且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0025] [12]根据[1]至[9]中任一项所述的治疗剂,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链由SEQ ID NO:9代表的氨基酸序列组成,且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0026] [13]根据[12]所述的治疗剂,其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0027] [14]根据[1]至[13]中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0028] [15]根据[1]至[13]中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0029] [16]根据[1]至[15]中任一项所述的治疗剂,其中所述癌症是选自以下的至少一种:乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0030] [17]根据[1]至[15]中任一项所述的治疗剂,其中所述癌症是选自以下的至少一种:乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

[0031] [18]癌症的治疗方法,其包括向确定具有HER3-突变的癌症的受试者施用抗HER3抗体-药物缀合物。

[0032] [19]根据[18]所述的治疗方法,其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种:V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G。

[0033] [20]根据[18]所述的治疗方法,其中所述HER3-突变的癌症中的HER3突变是Q809R。

[0034] [21]根据[18]至[20]中任一项所述的治疗方法,其中HER2在所述HER3-突变的癌症中过表达。

[0035] [22]根据[18]至[20]中任一项所述的治疗方法,其中HER2在所述HER3-突变的癌症中未过表达。

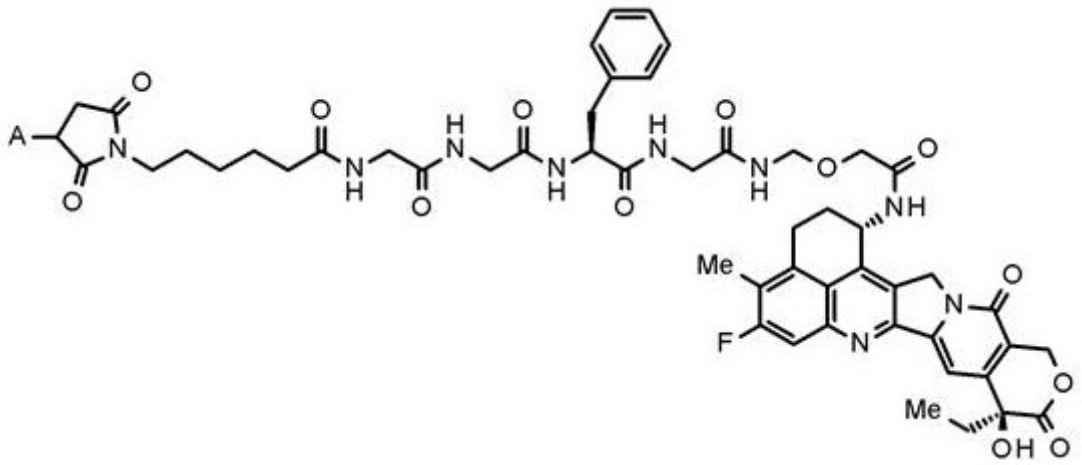
[0036] [23]根据[18]至[22]中任一项所述的治疗方法,其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间,所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0037] [24]根据[18]至[23]中任一项所述的治疗方法,其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间,所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0038] [25]根据[18]至[24]中任一项所述的治疗方法,其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物,其中由下式代表的药物-接头经由硫醚键与抗HER3抗

体缀合：

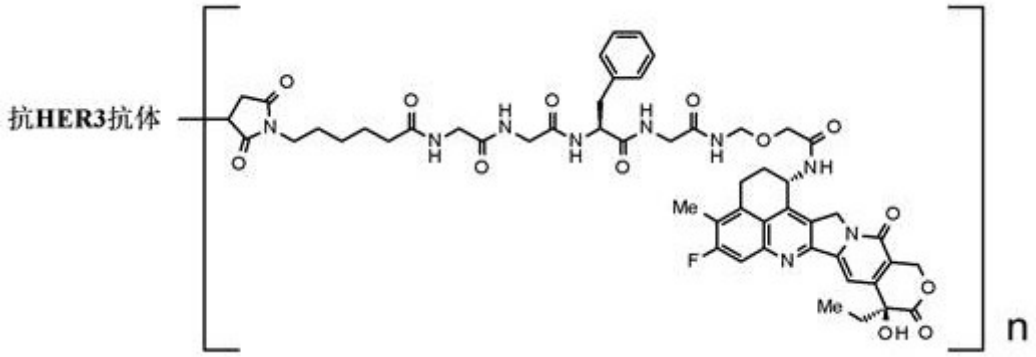
[式3]



其中A代表与抗HER3抗体的连接位置。

[0039] [26]根据[18]至[24]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物：

[式4]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合，且n是每个抗体分子缀合的药物-接头的单元的平均数。

[0040] [27]根据[18]至[26]中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3；且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0041] [28]根据[18]至[26]中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区，且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0042] [29]根据[18]至[26]中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链由SEQ ID NO:9代表的氨基酸序列组成，且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0043] [30]根据[29]所述的治疗方法，其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0044] [31]根据[18]至[30]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0045] [32]根据[18]至[30]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0046] [33]根据[18]至[32]中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0047] [34]根据[18]至[32]中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

[0048] [35]抗HER3抗体-药物缀合物，其用于治疗HER3-突变的癌症。

[0049] [36]根据[35]所述的抗HER3抗体-药物缀合物，其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种：V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G。

[0050] [37]根据[35]所述的抗HER3抗体-药物缀合物，其中所述HER3-突变的癌症中的HER3突变是Q809R。

[0051] [38]根据[35]至[37]中任一项所述的抗HER3抗体-药物缀合物，其中HER2在所述HER3-突变的癌症中过表达。

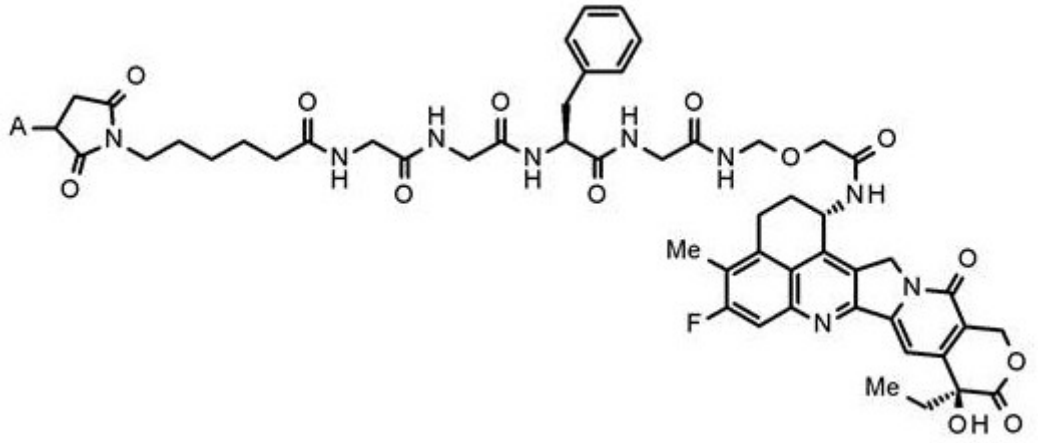
[0052] [39]根据[35]至[37]中任一项所述的抗HER3抗体-药物缀合物，其中HER2在所述HER3-突变的癌症中未过表达。

[0053] [40]根据[35]至[39]中任一项所述的抗HER3抗体-药物缀合物，其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0054] [41]根据[35]至[40]中任一项所述的抗HER3抗体-药物缀合物，其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0055] [42]根据[35]至[41]中任一项所述的抗HER3抗体-药物缀合物，其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物，其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合：

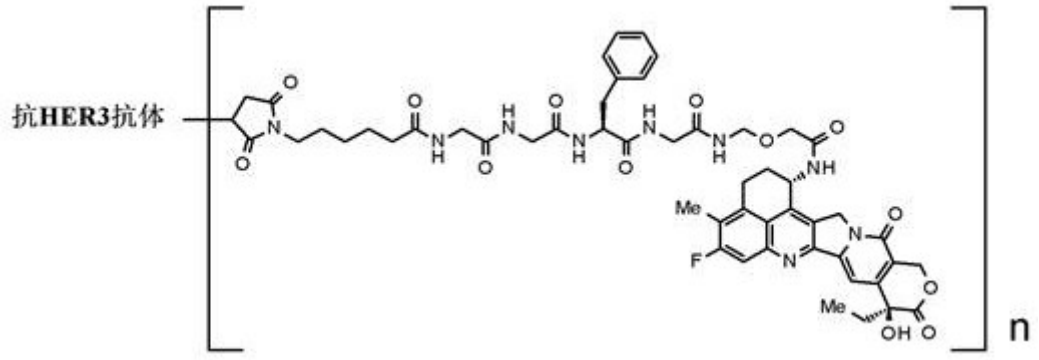
[式5]



其中A代表与抗HER3抗体的连接位置。

[0056] [43]根据[35]至[41]中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物:

[式6]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合,且n是每个抗体分子缀合的药物-接头的单元的平均数。

[0057] [44]根据[35]至[43]中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3;且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0058] [45]根据[35]至[43]中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区,且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0059] [46]根据[35]至[43]中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链由SEQ ID NO:9代表的氨基酸序列组成,且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0060] [47]根据[46]所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0061] [48]根据[35]至[47]中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0062] [49]根据[35]至[47]中任一项所述的抗HER3抗体-药物缀合物，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0063] [50]根据[35]至[49]中任一项所述的抗HER3抗体-药物缀合物，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0064] [51]根据[35]至[49]中任一项所述的抗HER3抗体-药物缀合物，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

[0065] [52]抗HER3抗体-药物缀合物用于制备用于治疗HER3-突变的癌症的药物的用途。

[0066] [53]根据[52]所述的用途，其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种：V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G。

[0067] [54]根据[52]所述的用途，其中所述HER3-突变的癌症中的HER3突变是Q809R。

[0068] [55]根据[52]至[54]中任一项所述的用途，其中HER2在所述HER3-突变的癌症中过表达。

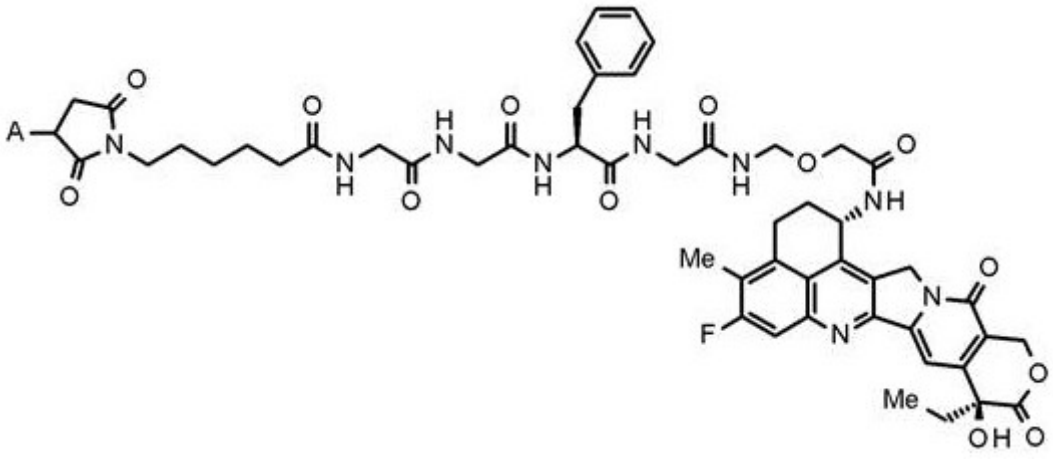
[0069] [56]根据[52]至[54]中任一项所述的用途，其中HER2在所述HER3-突变的癌症中未过表达。

[0070] [57]根据[52]至[56]中任一项所述的用途，其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0071] [58]根据[52]至[57]中任一项所述的用途，其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0072] [59]根据[52]至[58]中任一项所述的用途，其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物，其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合：

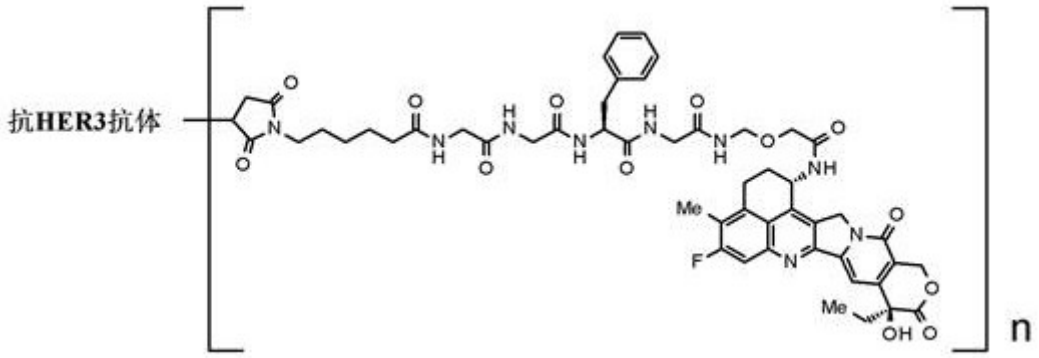
[式7]



其中A代表与抗HER3抗体的连接位置。

[0073] [60]根据[52]至[58]中任一项所述的用途，其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物：

[式8]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合，且n是每个抗体分子缀合的药物-接头的单元的平均数。

[0074] [61]根据[52]至[60]中任一项所述的用途，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3；且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0075] [62]根据[52]至[60]中任一项所述的用途，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区，且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0076] [63]根据[52]至[60]中任一项所述的用途，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链由SEQ ID NO:9代表的氨基酸序列组成，且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0077] [64]根据[63]所述的用途，其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0078] [65]根据[52]至[64]中任一项所述的用途，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0079] [66]根据[52]至[64]中任一项所述的用途，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0080] [67]根据[52]至[66]中任一项所述的用途，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0081] [68]根据[52]至[66]中任一项所述的用途，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

[0082] [69] HER3-突变的癌症的治疗方法，其包括向需要针对HER3-突变的癌症的治疗的受试者施用抗HER3抗体-药物缀合物。

[0083] [70]根据[69]所述的治疗方法，其中所述HER3-突变的癌症中的HER3突变是选自以下的至少一种：V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G。

[0084] [71]根据[69]所述的治疗方法，其中所述HER3-突变的癌症中的HER3突变是Q809R。

[0085] [72]根据[69]至[71]中任一项所述的治疗方法，其中HER2在所述HER3-突变的癌症中过表达。

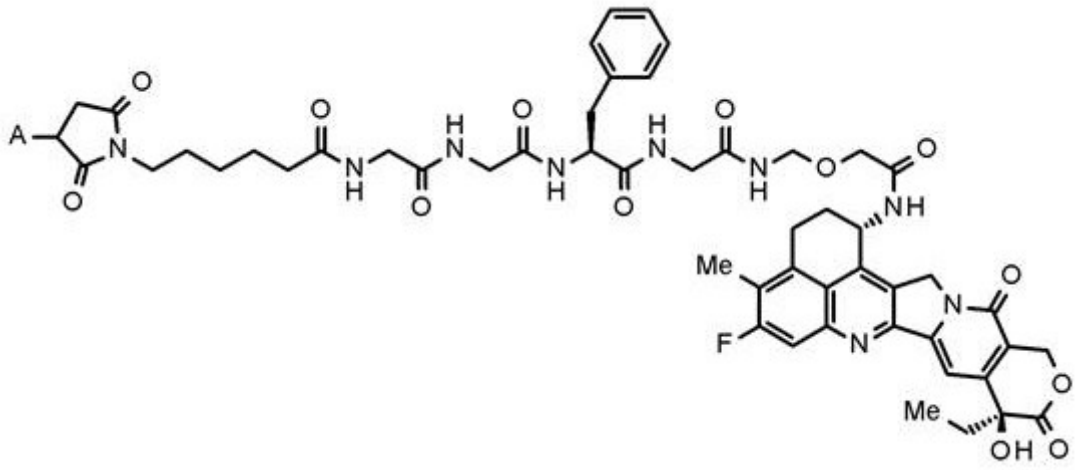
[0086] [73]根据[69]至[71]中任一项所述的治疗方法，其中HER2在所述HER3-突变的癌症中未过表达。

[0087] [74]根据[69]至[73]中任一项所述的治疗方法，其中在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0088] [75]根据[69]至[74]中任一项所述的治疗方法，其中在过表达HER2的HER3-表达细胞中和在不过表达HER2的HER3-表达细胞中之间，所述抗HER3抗体-药物缀合物的溶酶体迁移没有实质性差异。

[0089] [76]根据[69]至[75]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物，其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合：

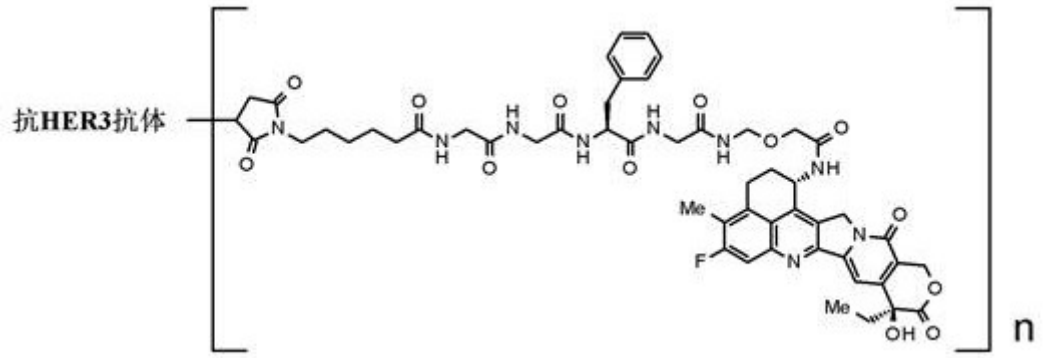
[式9]



其中A代表与抗HER3抗体的连接位置。

[0090] [77]根据[69]至[75]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物是由下式代表的抗HER3抗体-药物缀合物：

[式10]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合，且n是每个抗体分子缀合的药物-接头的单元的平均数。

[0091] [78]根据[69]至[77]中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3；且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0092] [79]根据[69]至[77]中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区，且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0093] [80]根据[69]至[77]中任一项所述的治疗方法，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链由SEQ ID NO:9代表的氨基酸序列组成，且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0094] [81]根据[80]所述的治疗方法，其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0095] [82]根据[69]至[81]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0096] [83]根据[69]至[81]中任一项所述的治疗方法，其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0097] [84]根据[69]至[83]中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0098] [85]根据[69]至[83]中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

[0099] 此外，本发明还可以表示为以下(1)至(48)。

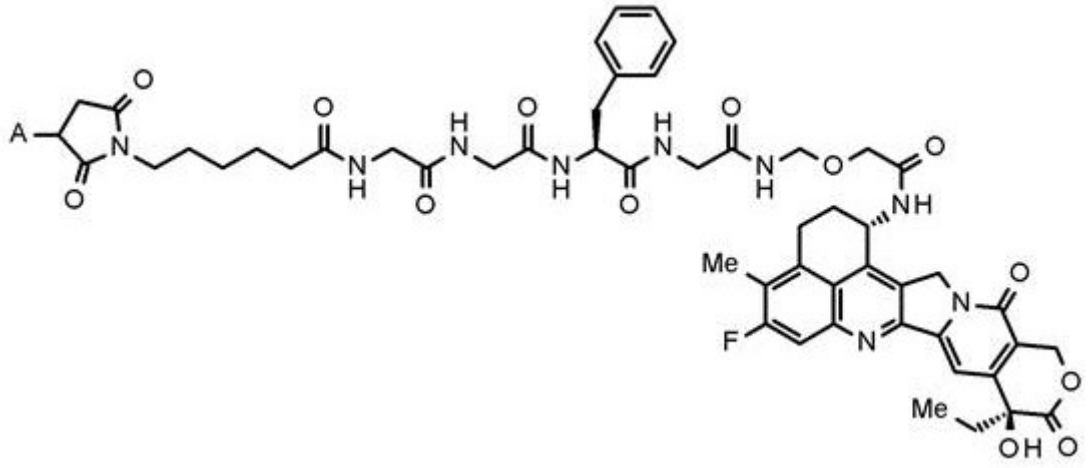
[0100] (1) HER3基因突变的癌症的治疗剂，其包含抗HER3抗体-药物缀合物作为活性成分。

[0101] (2)根据(1)所述的治疗剂，其中HER2在所述HER3基因突变的癌症中过表达。

[0102] (3)根据(1)所述的治疗剂,其中HER2在所述HER3基因突变的癌症中未过表达。

[0103] (4)根据(1)至(3)中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物,其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合:

[式11]



其中A代表与抗HER3抗体的连接位置。

[0104] (5)根据(1)至(4)中任一项所述的治疗剂,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3;且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0105] (6)根据(1)至(5)中任一项所述的治疗剂,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区,且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0106] (7)根据(1)至(6)中任一项所述的治疗剂,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链由SEQ ID NO:9代表的氨基酸序列组成,且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0107] (8)根据(7)所述的治疗剂,其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0108] (9)根据(1)至(8)中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0109] (10)根据(1)至(8)中任一项所述的治疗剂,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0110] (11)根据(1)至(10)中任一项所述的治疗剂,其中所述癌症是选自以下的至少一种:乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0111] (12)根据(1)至(10)中任一项所述的治疗剂,其中所述癌症是选自以下的至少一

种:乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

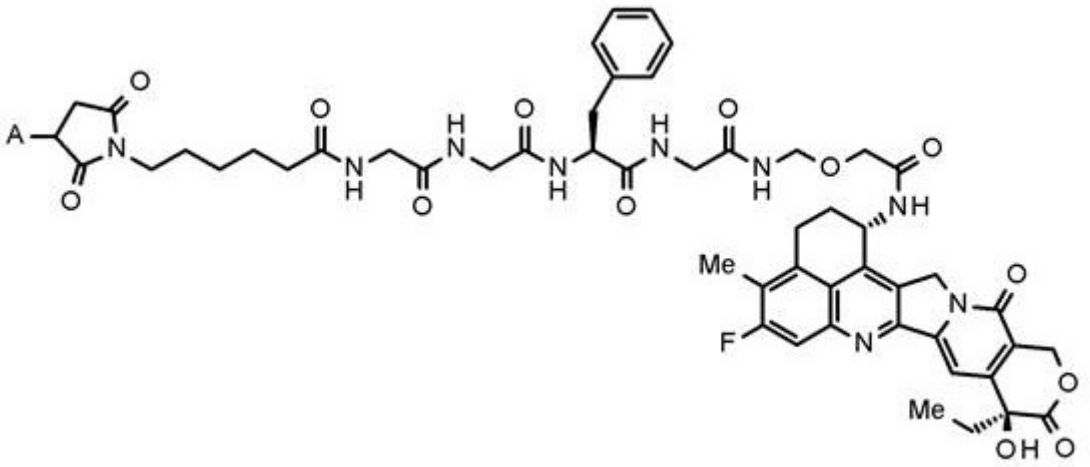
[0112] (13)癌症的治疗方法,其包括向确定具有HER3基因突变的癌症的受试者施用抗HER3抗体-药物缀合物。

[0113] (14)根据(13)所述的治疗方法,其中HER2在所述HER3基因突变的癌症中过表达。

[0114] (15)根据(13)所述的治疗方法,其中HER2在所述HER3基因突变的癌症中未过表达。

[0115] (16)根据(13)至(15)中任一项所述的治疗方法,其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物,其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合:

[式12]



其中A代表与抗HER3抗体的连接位置。

[0116] (17)根据(13)至(16)中任一项所述的治疗方法,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3;且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0117] (18)根据(13)至(17)中任一项所述的治疗方法,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区,且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0118] (19)根据(13)至(18)中任一项所述的治疗方法,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链由SEQ ID NO:9代表的氨基酸序列组成,且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0119] (20)根据(19)所述的治疗方法,其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0120] (21)根据(13)至(20)中任一项所述的治疗方法,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0121] (22)根据(13)至(20)中任一项所述的治疗方法,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0122] (23)根据(13)至(22)中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0123] (24)根据(13)至(22)中任一项所述的治疗方法，其中所述癌症是选自以下的至少一种：乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

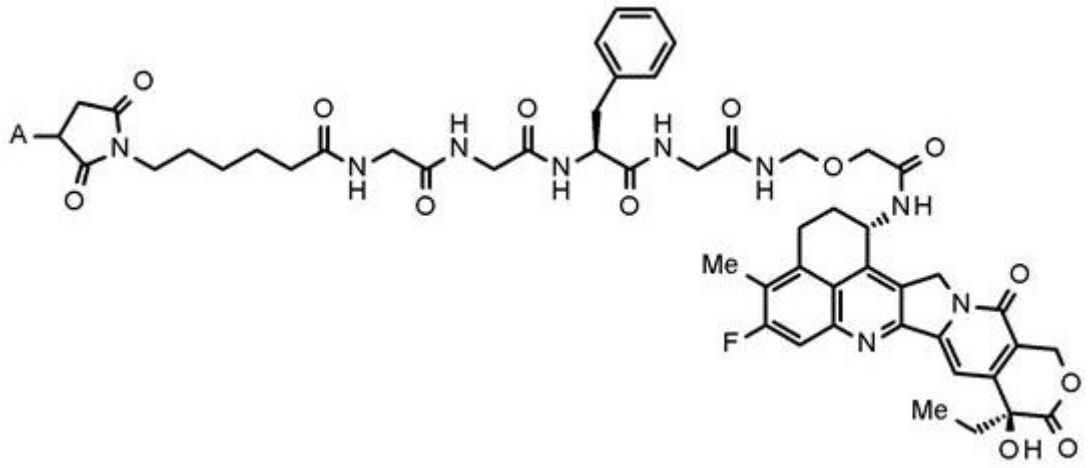
[0124] (25)抗HER3抗体-药物缀合物，其用于治疗HER3基因突变的癌症。

[0125] (26)根据(25)所述的抗HER3抗体-药物缀合物，其中HER2在所述HER3基因突变的癌症中过表达。

[0126] (27)根据(25)所述的抗HER3抗体-药物缀合物，其中HER2在所述HER3基因突变的癌症中未过表达。

[0127] (28)根据(25)至(27)中任一项所述的抗HER3抗体-药物缀合物，其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物，其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合：

[式13]



其中A代表与抗HER3抗体的连接位置。

[0128] (29)根据(25)至(28)中任一项所述的抗HER3抗体-药物缀合物，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3；且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0129] (30)根据(25)至(29)中任一项所述的抗HER3抗体-药物缀合物，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区，且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0130] (31)根据(25)至(30)中任一项所述的抗HER3抗体-药物缀合物，其中所述抗HER3抗体是包含重链和轻链的抗体，所述重链由SEQ ID NO:9代表的氨基酸序列组成，且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0131] (32)根据(31)所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0132] (33)根据(25)至(32)中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0133] (34)根据(25)至(33)中任一项所述的抗HER3抗体-药物缀合物,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0134] (35)根据(25)至(34)中任一项所述的抗HER3抗体-药物缀合物,其中所述癌症是选自以下的至少一种:乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0135] (36)根据(25)至(34)中任一项所述的抗HER3抗体-药物缀合物,其中所述癌症是选自以下的至少一种:乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

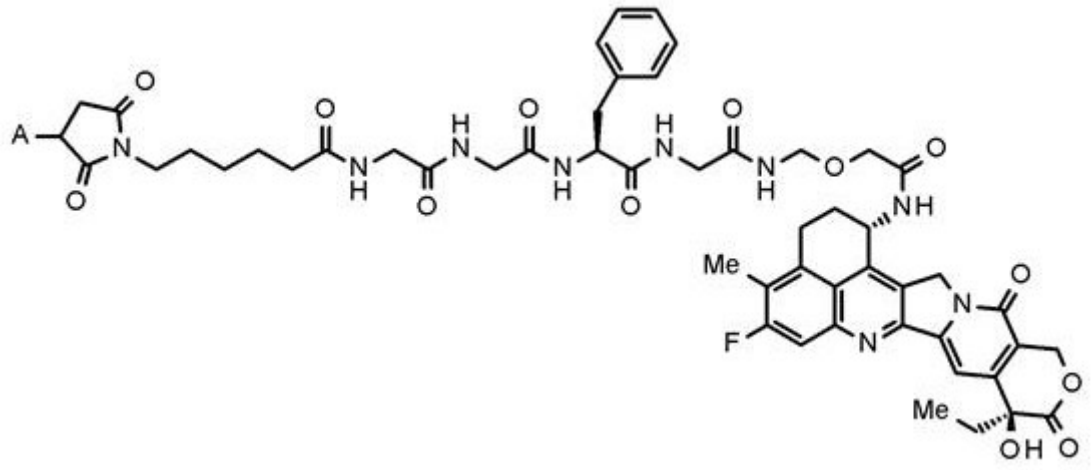
[0136] (37)抗HER3抗体-药物缀合物用于制备用于治疗HER3基因突变的癌症的药物的用途。

[0137] (38)根据(37)所述的用途,其中HER2在所述HER3基因突变的癌症中过表达。

[0138] (39)根据(37)所述的用途,其中HER2在所述HER3基因突变的癌症中未过表达。

[0139] (40)根据(37)至(39)中任一项所述的用途,其中所述抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物,其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合:

[式14]



其中A代表与抗HER3抗体的连接位置。

[0140] (41)根据(37)至(40)中任一项所述的用途,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3;且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3。

[0141] (42)根据(37)至(41)中任一项所述的用途,其中所述抗HER3抗体是包含重链和轻

链的抗体,所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区,且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区。

[0142] (43)根据(37)至(42)中任一项所述的用途,其中所述抗HER3抗体是包含重链和轻链的抗体,所述重链由SEQ ID NO:9代表的氨基酸序列组成,且所述轻链由SEQ ID NO:10代表的氨基酸序列组成。

[0143] (44)根据(43)所述的用途,其中所述抗HER3抗体在重链的羧基末端缺乏赖氨酸残基。

[0144] (45)根据(37)至(44)中任一项所述的用途,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7至8的范围内。

[0145] (46)根据(37)至(44)中任一项所述的用途,其中所述抗HER3抗体-药物缀合物中每个抗体分子缀合的药物-接头的单元的平均数在7.5至8的范围内。

[0146] (47)根据(37)至(46)中任一项所述的用途,其中所述癌症是选自以下的至少一种:乳腺癌、肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤、黑色素瘤、肾癌、尿路上皮癌、前列腺癌、胰腺癌、膀胱癌、胃肠道间质瘤、宫颈癌、食道癌、鳞状细胞癌、腹膜癌、多形胶质母细胞瘤、肝癌、肝细胞癌、子宫内膜癌、子宫癌、唾液腺癌、外阴癌、甲状腺癌、肝癌、肛门癌和阴茎癌。

[0147] (48)根据(37)至(46)中任一项所述的用途,其中所述癌症是选自以下的至少一种:乳腺癌、非小细胞肺癌、结肠直肠癌、胃癌、卵巢癌、头颈癌、多形胶质母细胞瘤和黑色素瘤。

[0148] 本发明的有利效果

本发明可以提供包含抗HER3抗体-药物缀合物的HER3-突变的癌症的治疗剂和/或癌症的治疗方法,所述方法包括将抗HER3抗体-药物缀合物施用于确定具有HER3-突变的癌症的受试者。

[0149] 附图简述

[图1]图1显示抗HER3抗体的重链的氨基酸序列(SEQ ID NO:9)。

[0150] [图2]图2显示抗HER3抗体的轻链的氨基酸序列(SEQ ID NO:10)。

[0151] [图3]图3显示各种HER3-稳定表达细胞的RT-PCR产物的1%琼脂糖凝胶电泳的结果(HER3表达)。

[0152] [图4]图4显示各种HER2-过表达和HER3-稳定表达细胞的RT-PCR产物的1%琼脂糖凝胶电泳的结果(HER3表达)。

[0153] [图5]图5显示各种HER2-过表达和HER3-稳定表达细胞的RT-PCR产物的1%琼脂糖凝胶电泳的结果(HER2表达)。

[0154] [图6]图6显示HER3-ADC (1)在HER3-稳定表达细胞中的结合活性。

[0155] [图7]图7显示HER3-ADC (1)在HER2-过表达和HER3-稳定表达细胞中的结合活性。

[0156] [图8]图8显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对空载体-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0157] [图9]图9显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对野生型HER3-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0158] [图10]图10显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3

(V104L)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0159] [图11]图11显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(V104M)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0160] [图12]图12显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(A232V)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0161] [图13]图13显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(P262H)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0162] [图14]图14显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(G284R)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0163] [图15]图15显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(D297Y)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0164] [图16]图16显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(G325R)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0165] [图17]图17显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(T355I)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0166] [图18]图18显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(S846I)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0167] [图19]图19显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(E928G)-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0168] [图20]图20显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对空载体-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0169] [图21]图21显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对野生型HER3-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0170] [图22]图22显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(V104L)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0171] [图23]图23显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(V104M)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0172] [图24]图24显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(A232V)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0173] [图25]图25显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(P262H)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0174] [图26]图26显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(G284R)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0175] [图27]图27显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(D297Y)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0176] [图28]图28显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(G325R)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0177] [图29]图29显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(T355I)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0178] [图30]图30显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(S846I)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0179] [图31]图31显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(E928G)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0180] [图32]图32显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对空载体-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0181] [图33]图33显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对野生型HER3-引入的细胞(没有HER2过表达)的细胞生长抑制活性。

[0182] [图34]图34显示突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)的RT-PCR产物的1%琼脂糖凝胶电泳的结果(HER3表达)。

[0183] [图35]图35显示突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)的RT-PCR产物的1%琼脂糖凝胶电泳的结果(HER2表达)。

[0184] [图36]图36显示HER3-ADC (1)在突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)中的结合活性。

[0185] [图37]图37显示HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对突变型HER3(Q809R)-引入的细胞(具有HER2过表达)的细胞生长抑制活性。

[0186] [图38]图38显示HER3-ADC (1)针对空载体-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0187] [图39]图39显示HER3-ADC (1)针对野生型HER3-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0188] [图40]图40显示HER3-ADC (1)针对突变型HER3 (V104L)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0189] [图41]图41显示HER3-ADC (1)针对突变型HER3 (V104M)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0190] [图42]图42显示HER3-ADC (1)针对突变型HER3 (A232V)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0191] [图43]图43显示HER3-ADC (1)针对突变型HER3 (P262H)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0192] [图44]图44显示HER3-ADC (1)针对突变型HER3 (G284R)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0193] [图45]图45显示HER3-ADC (1)针对突变型HER3 (D297Y)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0194] [图46]图46显示HER3-ADC (1)针对突变型HER3 (G325R)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0195] [图47]图47显示HER3-ADC (1)针对突变型HER3 (T355I)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0196] [图48]图48显示HER3-ADC (1)针对突变型HER3 (S846I)-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0197] [图49]图49显示HER3-ADC (1)针对突变型HER3 (E928G)-引入的细胞(没有HER2

过表达)的溶酶体迁移。

[0198] [图50]图50显示HER3-ADC (1)针对空载体-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0199] [图51]图51显示HER3-ADC (1)针对野生型HER3-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0200] [图52]图52显示HER3-ADC (1)针对突变型HER3 (V104L)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0201] [图53]图53显示HER3-ADC (1)针对突变型HER3 (V104M)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0202] [图54]图54显示HER3-ADC (1)针对突变型HER3 (A232V)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0203] [图55]图55显示HER3-ADC (1)针对突变型HER3 (P262H)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0204] [图56]图56显示HER3-ADC (1)针对突变型HER3 (G284R)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0205] [图57]图57显示HER3-ADC (1)针对其中引入的突变型HER3 (D297Y)(具有HER2过表达)的溶酶体迁移。

[0206] [图58]图58显示HER3-ADC (1)针对突变型HER3 (G325R)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0207] [图59]图59显示HER3-ADC (1)针对突变型HER3 (T355I)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0208] [图60]图60显示HER3-ADC (1)针对突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0209] [图61]图61显示HER3-ADC (1)针对突变型HER3 (S846I)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0210] [图62]图62显示HER3-ADC (1)针对突变型HER3 (E928G)-引入的细胞(具有HER2过表达)的溶酶体迁移。

[0211] [图63]图63显示HER3-ADC (1)针对空载体-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0212] [图64]图64显示HER3-ADC (1)针对野生型HER3-引入的细胞(没有HER2过表达)的溶酶体迁移。

[0213] [图65]图65显示HER3蛋白的氨基酸序列(SEQ ID NO:69)。

[0214] 实施方案的描述

在下文中,描述了用于实施本发明的优选模式。给出以下描述的实施方案仅用于举例说明本发明的典型实施方案的一个实例,并且不意欲限制本发明的范围。

[0215] [定义]

在本发明中,“HER3”与人表皮生长因子受体3 (HER3;也称为ErbB3)同义,并且是与HER1 (EGFR或ErbB-1)、HER2 (ErbB2)和HER4 (ErbB4)一起属于受体蛋白酪氨酸激酶的表皮生长因子受体亚家族的跨膜受体。已知HER3在各种癌症、诸如乳腺癌、肺癌和结肠直肠

癌中表达，并且其与酪氨酸激酶受体、诸如HER2和EGFR一起形成异二聚体，其后，HER3被磷酸化，由此诱导癌细胞生长或凋亡抑制信号(Alimandi等人，Oncogene (1995) 10，1813-1821，deFazio等人，Int. J. Cancer (2000) 87，487-498，Naidu等人，Br. J. Cancer (1998) 78，1385-1390)。

[0216] 在本发明中，术语“HER3蛋白”以与HER3相同的含义使用。可以使用本领域技术人员众所周知的方法、诸如免疫组织化学(IHC)来检测HER3蛋白的表达。此外，还可以通过将Flag肽引入HER3蛋白并使用抗Flag肽抗体来检测HER3蛋白的表达。

[0217] HER3蛋白的氨基酸序列显示于SEQ ID NO:69 (图65)中。

[0218] 在本发明中，“HER3基因”意指编码HER3蛋白的基因。HER3蛋白是HER3基因的基因产物。

[0219] 在本发明中，“HER3突变”意指在HER3蛋白的氨基酸序列中具有一个或多个突变。

[0220] 在本发明中，“HER3-突变的癌症”意指在HER3蛋白的氨基酸序列中具有一个或多个突变的癌症。此外，在HER3-突变的癌症中包括含有具有HER3突变的癌细胞的癌症，其甚至在整个肿瘤组织中都不具有HER3突变。

[0221] 在本发明中，“HER3基因突变”意指在HER3基因中具有一个或多个突变。

[0222] 在本发明中，“HER3基因突变的癌症”意指在HER3基因中具有一个或多个突变的癌症。此外，在HER3基因突变的癌症中包括含有具有HER3基因突变的癌细胞的癌症，其甚至在整个肿瘤组织中都不具有HER3基因突变。HER3基因突变引起作为基因产物的HER3蛋白的氨基酸序列中的突变，由此引起HER3突变。

[0223] HER3突变的具体实例可以包括，其中作为HER3蛋白的氨基酸残基104的V(缬氨酸)被L(亮氨酸)替代的突变(其也可以称为“V104L”)(参见例如Cancer Cell. 2013 May 13; 23 (5): 603-17, Nat Genet. 2014 Aug; 46 (8): 872-6, Cancer Res. 2014 Nov 1; 74 (21): 6071-81, Cancer. 2016 Sep 1; 122 (17): 2654-62, Nat Med. 2017 Jun; 23 (6): 703-713, 和Nature. 2018 Feb 8; 554 (7691): 189-194)，其中作为HER3蛋白的氨基酸残基104的V(缬氨酸)被M(甲硫氨酸)替代的突变(其也可以称为“V104M”)(参见例如Hum Mutat. 2008 Mar; 29 (3): 441-50, Cancer Cell. 2013 May 13; 23 (5): 603-17, Genome Biol. 2014 Apr 1; 15 (4): R55, Cancer Res. 2014 Jun 15; 74 (12): 3238-47, Nat Genet. 2014 Aug; 46 (8): 872-6, 和Ann Oncol. 2016 Jan; 27(1): 127-33)，其中作为HER3蛋白的氨基酸残基232的A(丙氨酸)被V(缬氨酸)替代的突变(其也可以称为“A232V”)(参见例如Nat Genet. 2013 May; 45 (5): 478-86, Cancer Cell. 2013 May 13; 23 (5): 603-17, Cancer Cell. 2016 Feb 8; 29 (2): 229-40, Cell Rep. 2016 Apr 26; 15 (4): 857-865)，其中作为HER3蛋白的氨基酸残基262的P(脯氨酸)被H(组氨酸)替代的突变(其也可以称为“P262H”)(参见例如Cancer Cell. 2013 May 13; 23 (5): 603-17, Gastroenterology. 2014 Feb; 146 (2): 530-38. e5, 和Nat Med. 2017 Jun; 23 (6): 703-713)，其中作为HER3蛋白的氨基酸残基284的G(甘氨酸)被R(精氨酸)替代的突变(其也可以称为“G284R”)(参见例如Nature. 2008 Oct 23; 455 (7216): 1069-75, Cancer Cell. 2013 May 13; 23 (5): 603-17, Nat Genet. 2014 Jun; 46 (6): 573-82, 和Ann Oncol. 2015 Aug; 26 (8): 1704-9)，其中作为HER3蛋白的氨基酸残基297的D(天冬氨酸)被Y(酪氨酸)替代的突变(其也可以称为“D297Y”)(参见例如PLoS

One. 2014 Mar 5; 9 (3): e90459, Nat Genet. 2014 Jun; 46 (6): 573-82, Nat Genet. 2014 Oct; 46 (10): 1097-102, Ann Oncol. 2016 Jan; 27(1): 127-33, 和Cancer. 2016 Sep 1; 122 (17): 2654-62),其中作为HER3蛋白的氨基酸残基325的G(甘氨酸)被R(精氨酸)替代的突变(其也可以称为"G325R")(参见例如Nat Med. 2017 Jun; 23 (6): 703-713),其中作为HER3蛋白的氨基酸残基355的T(苏氨酸)被I(异亮氨酸)替代的突变(其也可以称为"T355I"))(参见例如Nat Med. 2017 Jun; 23 (6): 703-713, 和Nature. 2018 Feb 8; 554 (7691): 189-194),其中作为HER3蛋白的氨基酸残基809的Q(谷氨酰胺)被R(精氨酸)替代的突变(其也可以称为"Q809R")(参见例如Cancer Cell. 2013 May 13; 23 (5): 603-17, Cell Rep. 2016 Apr 26; 15 (4): 857-865, 和Nat Med. 2017 Jun; 23 (6): 703-713),其中作为HER3蛋白的氨基酸残基846的S(丝氨酸)被I(异亮氨酸)替代的突变(其也可以称为"S846I")(参见例如Int J Cancer. 2006 Dec 15; 119 (12): 2986-7, Nat Genet. 2014 Dec; 46 (12): 1264-6, 和Cell Rep. 2016 Apr 26; 15 (4): 857-865),以及其中作为HER3蛋白的氨基酸残基928的E(谷氨酸)被G(甘氨酸)替代的突变(其也可以称为"E928G")(参见例如Nat Genet. 2011 Oct 30; 43 (12): 1219-23, Clin Cancer Res. 2016 Apr 1; 22 (7): 1583-91, Cancer. 2016 Sep 1; 122 (17): 2654-62, Clin Cancer Res. 2016 Dec 15; 22 (24): 6061-6068, Cancer Res. 2016 Oct 15; 76 (20): 5954-5961, 和PLoS Med. 2016 Dec 27; 13 (12): e1002201)。

[0224] 在上述HER3突变中,已经表明尤其具有Q809R的癌症显示对现有抗HER2药物和抗HER3抗体的强烈抗性(Jaiswal等人, Cancer Cell (2013) 23, 603-17)。

[0225] 本发明中的HER3突变没有特别限制,只要其在HER3蛋白的氨基酸序列中具有一个或多个突变,但其实例可以包括选自以下的至少一者:优选为V104L、V104M、A232V、P262H、G284R、D297Y、G325R、T355I、Q809R、S846I和E928G,更优选Q809R。

[0226] HER3突变的存在或不存在可以例如通过从癌症患者收集肿瘤组织并使福尔马林固定石蜡包埋的样本(FFPE)经受方法、诸如实时定量PCR (qRT-PCR)或微阵列分析来证实。

[0227] 此外,可以通过从癌症患者收集无细胞血液循环肿瘤DNA (ctDNA)并对其进行方法、诸如下一代测序(NGS)来证实HER3突变的存在或不存在(例如Sergina等人, Nature (2007) 445, 437-41, Jeong等人, Int. J. Cancer (2006) 119, 2986-7, Ding等人, Nature (2008) 455, 1069-75, Kan等人, Nature (2010) 466, 869-73, Wang等人, Nat. Genet. (2011) 43, 1219-23, Greenman等人, Nature (2007) 446, 153-8, Stransky等人, Science (2011) 333, 1157-60, Jaiswal等人, Cancer Cell (2013) 23, 603-17, Hyman等人, Cancer Res. (2017) Abstract CT001, Mishra等人, Oncotarget (2017) 69, 114371-114392, Mishra等人, Oncotarget (2018) 45, 27773-27788)。

[0228] 在本发明中,术语"HER3突变"以与HER3基因突变相同的含义使用。

[0229] 在本发明中,"野生型HER3"意指没有HER3突变的HER3蛋白。在本发明中,这种野生型可以被称为"WT"。

[0230] 在本发明中,"突变型HER3"意指具有HER3突变的HER3蛋白。

[0231] 可以通过化学转染方法(通过使用阳离子脂质、阳离子聚合物、磷酸钙等的转染),

物理转染方法(通过电穿孔、显微注射、声波穿孔、激光照射等),生物转染方法(使用病毒载体等),来产生HER3-稳定表达细胞。例如,在生物转染方法中使用慢病毒的情况下,所述细胞可以通过如下产生:将慢病毒蛋白和包膜蛋白的表达质粒以及HER3表达质粒引入包装细胞(诸如Lenti-X 293 T细胞,其后从培养上清液制备慢病毒溶液,并使用该溶液培养肿瘤细胞。在此,可以根据所使用的HER3表达质粒的类型来选择性产生野生型或突变型HER3-表达细胞。突变型HER3表达质粒可以使用HER3诱变引物产生。

[0232] 在本发明中,"抗HER3抗体"意指特异性结合HER3、优选具有通过结合HER3而在HER3-表达细胞中被内化的活性的抗体,换言之,具有结合HER3后迁移至HER3-表达细胞中的活性的抗体。

[0233] 在本发明中,"HER2"与人表皮生长因子受体2(其也可以称为neu或ErbB-2)同义,并且是与HER1、HER3和HER4一起属于受体蛋白酪氨酸激酶的表皮生长因子受体亚家族的跨膜受体。已知HER2通过由于与HER1、HER3或HER4形成异二聚体而被细胞间酪氨酸残基的自磷酸化激活而在正常细胞和肿瘤细胞的细胞生长、分化和存活中发挥重要作用。

[0234] 在本发明中,术语"HER2蛋白"以与HER2相同的含义使用。

[0235] 在本发明中,HER2的"过表达"意指HER2表达被确定为阳性。例如,其表明通过免疫组织化学(IHC)确定HER2表达为3+,或通过免疫组织化学确定HER2表达为2+,并且通过原位杂交(ISH)确定HER2表达为阳性。免疫组织化学和原位杂交可以使用本领域技术人员众所周知的方法进行。例如,可以参考HER2 Test Guide, Breast Cancer Edition,第4版(由Breast Cancer HER2 Laboratory Pathology Subcommittee创建)。

[0236] 可以通过化学转染方法(通过使用阳离子脂质、阳离子聚合物、磷酸钙等的转染),物理转染方法(通过电穿孔、显微注射、声波穿孔、激光照射等),生物转染方法(使用病毒载体等),来产生HER2-过表达细胞。例如,在生物转染方法中使用慢病毒的情况下,所述细胞可以通过如下产生:将慢病毒蛋白和包膜蛋白的表达质粒以及HER2表达质粒引入包装细胞(诸如Lenti-X 293 T细胞,其后从培养上清液制备慢病毒溶液,并使用该溶液培养肿瘤细胞。此外,可以通过使用上述通过引入HER3表达质粒而获得的慢病毒溶液培养HER2-过表达细胞来产生HER2-过表达和HER3-稳定表达细胞。

[0237] [抗HER3抗体-药物缀合物]

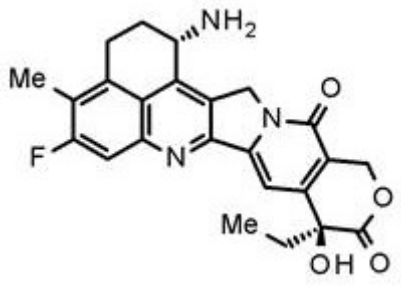
在本发明中,"抗体-药物缀合物"意指其中细胞毒性药物经由接头与抗体结合的复合物。作为此类抗体-药物缀合物,例如,可以例举美国专利号6214345、国际公开号WO 2002/083067、国际公开号WO 2003/026577、国际公开号WO 2004/054622、国际公开号WO 2005/112919、国际公开号WO 2006/135371、国际公开号WO 2007112193、国际公开号WO 2008/033891、国际公开号WO 2009/100194、国际公开号WO 2009/134976、国际公开号WO 2009/134977、国际公开号WO 2010/093395、国际公开号WO 2011/130613、国际公开号WO 2011/130616、国际公开号WO 2013/055993、国际公开号WO 2014/057687、国际公开号WO 2014/061277、国际公开号WO 2014/107024、国际公开号WO 2014/134457和国际公开号WO 2014/145090中描述的那些,可以优选地例举国际公开号WO 2014/057687和国际公开号WO 2014/061277中描述的那些,并且可以更优选地例举国际公开WO 2014/057687中描述的那些。这些抗体-药物缀合物可以通过前面提及的参考文献中描述的方法产生。

[0238] 细胞毒性药物没有特别限制,只要其具有抗肿瘤效果和可以与接头结合的取代基

或部分结构即可。作为此类细胞毒性药物，例如，可以例举喜树碱、加利车霉素、多柔比星、道诺霉素、丝裂霉素C、博来霉素、环胞苷、长春新碱、长春碱、甲氨蝶呤、顺铂、澳瑞他汀E、美登素、紫杉醇、吡咯并苯并二氮杂䓬及其衍生物，可以优选地例举喜树碱衍生物，并且可以更优选地例举依喜替康衍生物。

[0239] 依喜替康(IUPAC名称：(1S,9S)-1-氨基-9-乙基-5-氟-1,2,3,9,12,15-六氢-9-羟基-4-甲基-10H,13H-苯并[de]吡喃并[3',4':6,7]吲哚嗪并[1,2-b]喹啉-10,13-二酮，(其也可以由以下化学名表示：(1S,9S)-1-氨基-9-乙基-5-氟-2,3-二氢-9-羟基-4-甲基-1H,12H-苯并[de]吡喃并[3',4':6,7]吲哚嗪并[1,2-b]喹啉-10,13(9H,15H)-二酮))(其为拓扑异构酶I抑制剂)是由下式代表的化合物。

[0240] [式15]

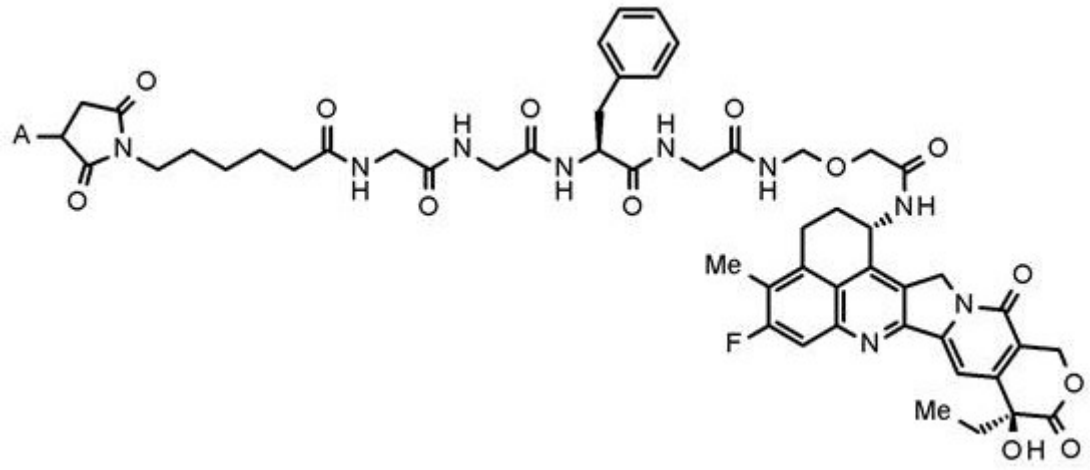


在本发明中，“药物-接头”意指抗体-药物缀合物中的药物和接头部分，换言之，抗体-药物缀合物中除了抗体以外的部分结构。

[0241] 在本发明中，“抗HER3抗体-药物缀合物”意指其中抗体是抗HER3抗体的抗体-药物缀合物。作为此类抗HER3抗体-药物缀合物，例如，可以例举国际公开号WO 2012/019024、国际公开号WO 2012/064733和国际公开号WO 2015/155998中描述的那些，并且可以优选地例举国际公开号WO 2015/155998中描述的那些。这些抗HER3抗体-药物缀合物可以通过前面提及的参考文献中描述的方法产生。

[0242] 本发明中优选使用的抗HER3抗体-药物缀合物是这样的抗HER3抗体-药物缀合物，其中由下式代表的药物-接头经由硫醚键与所述抗体缀合：

[式16]

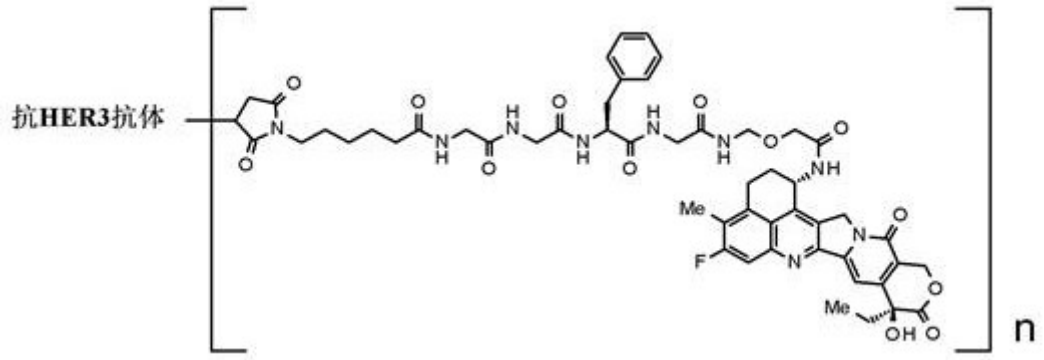


其中A代表与抗体的连接位置。所述药物-接头与在抗体的链间二硫键位点(重链之间的两个位点，以及重链和轻链之间的两个位点)处形成的硫醇基团(换言之，半胱氨酸

残基的硫原子)连接。

[0243] 本发明中优选使用的抗HER3抗体-药物缀合物也可以由下式代表。

[0244] [式17]



其中所述药物-接头经由硫醚键与抗HER3抗体缀合。n的含义与所谓缀合的药物分子的平均数(DAR;药物:抗体比)的含义相同,并且指示每个抗体分子缀合的药物-接头的单元的平均数。

[0245] 本发明中优选使用的抗HER3抗体-药物缀合物的每个抗体分子缀合的药物-接头的单元的平均数目优选为2至8,更优选为3至8,甚至更优选为7至8,甚至更优选为7.5至8,且甚至更优选为约8。

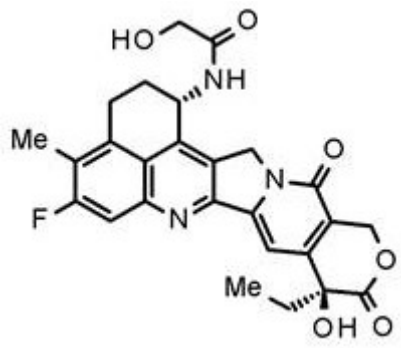
[0246] 本发明中使用的抗HER3抗体-药物缀合物的抗HER3抗体部分优选为包含重链和轻链的抗体,所述重链包含由SEQ ID NO:1代表的氨基酸序列组成的CDRH1、由SEQ ID NO:2代表的氨基酸序列组成的CDRH2和由SEQ ID NO:3代表的氨基酸序列组成的CDRH3;且所述轻链包含由SEQ ID NO:4代表的氨基酸序列组成的CDRL1、由SEQ ID NO:5代表的氨基酸序列组成的CDRL2和由SEQ ID NO:6代表的氨基酸序列组成的CDRL3,

更优选地,包含重链和轻链的抗体,所述重链包含由SEQ ID NO:7代表的氨基酸序列组成的重链可变区,且所述轻链包含由SEQ ID NO:8代表的氨基酸序列组成的轻链可变区,和,

甚至更优选地,包含重链和轻链的抗体,所述重链由SEQ ID NO:9代表的氨基酸序列组成,且所述轻链由SEQ ID NO:10代表的氨基酸序列组成,或其中重链的羧基末端的赖氨酸残基缺失的抗体的变体。

[0247] 在迁移至癌细胞中之后,本发明中优选使用的抗HER3抗体-药物缀合物通过释放由下式代表的化合物发挥抗肿瘤效果:

[式18]

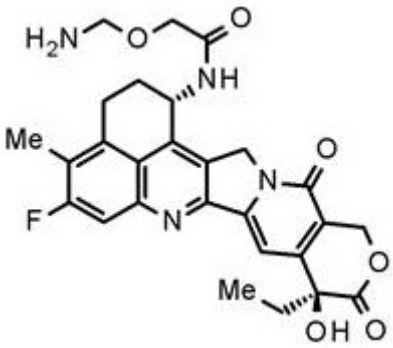


。

[0248] 推断上面提及的化合物是本发明中优选使用的抗HER3抗体-药物缀合物的抗肿瘤活性的原始来源，并且已经证实其具有拓扑异构酶I抑制作用(Ogitani Y. 等人，Clinical Cancer Research, 2016, Oct15; 22 (20): 5097-5108, Epub 2016 Mar 29)。

[0249] 推断上面提及的化合物通过由下式代表的化合物的氨基结构的分解而形成：

[式19]



推断其通过抗HER3抗体-药物缀合物的接头部分的切割而形成。

[0250] [抗HER3抗体的产生]

本发明中使用的HER3蛋白可以直接从人HER3-表达细胞纯化，使用此类细胞的细胞膜级分制备为HER3蛋白(在用作抗原的情况下)，或者通过在体外合成HER3或通过基因工程改造在宿主细胞中产生HER3来获得。在基因工程改造中，HER3可以通过如下具体合成：将HER3 cDNA整合至可表达的载体中，且其后在含有转录和翻译所需的酶、底物和能量物质的溶液中孵育该载体。或者，可以通过用表达HER3的载体转化另一原核或真核宿主细胞来获得蛋白。此外，还可能使用通过基因工程改造的HER3-表达细胞或表达HER3的细胞系作为HER3蛋白抗原。

[0251] HER3的RNA序列、cDNA序列和氨基酸序列公开于公共数据库中，并且可以通过登录号、诸如AAA35979(在氨基末端含有由19个氨基酸残基组成的信号序列的前体)和M34309(NCBI)进行指代。

[0252] 此外，在HER3中还包括由在HER3的氨基酸序列中1至10个氨基酸被取代、缺失、添加和/或插入的氨基酸序列组成且具有与所述蛋白等效的生物活性的蛋白。

[0253] 本发明中使用的抗HER3抗体可以通过已知方法获得。例如，所述抗体可以通过如下获得：使用本领域中通常实施的方法，用选自充当抗原的HER3或HER3的氨基酸序列的任何多肽免疫动物，收集体内产生的抗体并纯化所述抗体。抗原的来源不限于人，并且还可能用源自除了人以外的动物、诸如小鼠和大鼠的抗原免疫动物。在这种情况下，可以通过测试结合获得的异源抗原的抗体和人抗原之间的交叉反应性来选择适用于人疾病的抗HER3抗体。

[0254] 此外，根据已知方法(例如，Kohler和Milstein, Nature (1975) 256, p. 495-497; Kennet, R. 编, Monoclonal Antibodies, p.365-367, Plenum Press, N.Y. (1980))，也可能通过经由将产生针对抗原的抗体的产生抗体的细胞与骨髓瘤细胞融合来建立杂交瘤而获得单克隆抗体。

[0255] 可以通过经由基因工程改造在宿主细胞中产生编码抗原蛋白的基因来获得抗原。具体地，可以产生能够表达抗原基因的载体，使得将所述载体引入宿主细胞以表达该基因，

随后纯化表达的抗原。还可以通过使用通过基因工程改造获得的抗原表达细胞或表达所述抗原的细胞系对动物进行免疫的方法来获得抗体。

[0256] 本发明中使用的抗HER3抗体优选为人工修饰(例如,为了降低对人的异源抗原性的目的)的重组抗体,诸如嵌合抗体和人源化抗体,或优选为仅具有源自人的抗体基因序列的抗体,即,人抗体。可以使用已知方法产生此类抗体。

[0257] 作为嵌合抗体,可以例举其中抗体的可变区和恒定区彼此异质的抗体,诸如其中小鼠或大鼠来源的抗体的可变区与人来源的恒定区缀合的嵌合抗体(Proc. Natl. Acad. Sci. U.S.A., 81, 6851-6855, (1984))。

[0258] 作为人源化抗体,可以例举其中仅将异源抗体的互补决定区(CDR;互补决定区)并入人来源的抗体中的抗体(Nature (1986) 321, p. 522-525),其中除了异源抗体的CDR序列以外还通过CDR移植(WO 90/07861)将异源抗体的一部分框架中的氨基酸残基移植至人抗体中的抗体,以及使用基因转化诱变策略人源化的抗体(美国专利号5821337)。

[0259] 作为人抗体,可以例举使用具有含有人抗体的重链和轻链基因的人染色体片段的人抗体产生小鼠制备的抗体(参见例如Tomizuka, K. 等人, Nature Genetics (1997) 16, p. 133-143; Kuroiwa, Y. 等人, Nucl. Acids Res. (1998) 26, p. 3447-3448; Yoshida, H. 等人, Animal Cell Technology: Basic and Applied Aspects vol.10, p. 69-73 (Kitagawa, Y., Matsuda, T. 和Iijima, S. 编), Kluwer Academic Publishers, 1999; 和Tomizuka, K. 等人, Proc. Natl. Acad. Sci. USA (2000) 97, p. 722-727)。作为一个替代方案,可以例举通过选自人抗体文库的噬菌体展示获得的抗体(参见例如Wormstone, I. M.等人, Investigative Ophthalmology & Visual Science. (2002) 43 (7), p. 2301-2308; Carmen, S.等人, Briefings in Functional Genomics and Proteomics (2002), 1 (2), p. 189-203; 和Siriwardena, D. 等人, Ophthalmology (2002) 109 (3), p. 427-431)。

[0260] 本发明中使用的抗HER3抗体也包括抗体的修饰变体。根据本发明的修饰的变体意指通过化学或生物修饰根据本发明的抗体获得的那些。化学修饰的变体包括具有附接至氨基酸骨架或N-连接或O-连接的碳水化合物链的化学部分的化学修饰的变体。生物修饰的变体包括翻译后修饰(诸如添加N-连接或O-连接的糖链,N-末端或C-末端加工,脱酰胺基,天冬氨酸的异构化和甲硫氨酸的氧化)的那些,以及具有通过使用原核宿主细胞表达在N-末端添加的甲硫氨酸残基的那些。此外,修饰的变体的含义还包括标记为使得能够检测或分离本发明中使用的抗HER3抗体或抗原的那些,诸如酶标记体、荧光标记体和亲和力标记体。本发明中使用的抗HER3抗体的此类修饰变体可用于提高抗体的稳定性和血液保留,降低抗原性以及检测或分离抗体或抗原。

[0261] 此外,可以通过调节与本发明中使用的抗HER3抗体结合的糖链的修饰(诸如糖基化和去岩藻糖基化)来增强抗体依赖性细胞毒性活性。作为用于调节抗体的糖链的修饰的技术,国际公开号WO 99/54342、国际公开号WO 00/61739、国际公开号WO 02/31140、国际公开号WO 2007/133855和国际公开号WO 2013/120066等是已知的,但对这些实例没有限制。本发明中使用的抗HER3抗体还包括此类抗体,其具有调节的糖链修饰。

[0262] 已知在培养的哺乳动物细胞中产生的抗体的重链的羧基末端的赖氨酸残基被缺失(Journal of Chromatography A, 705: 129-134 (1995))。此外,还已知重链的羧基末

端的两个氨基酸残基甘氨酸和赖氨酸也被缺失，并且新位于羧基末端的脯氨酸残基被酰胺化(Analytical Biochemistry, 360: 75-83 (2007))。然而，重链序列的此类缺失和修饰对抗体的抗原结合能力和效应子功能(诸如补体激活和抗体-依赖性细胞毒性)没有影响。因此，本发明中使用的抗HER3抗体包括如上修饰的抗体和抗体的功能片段，并且包括在重链羧基末端缺失一个或两个氨基酸的缺失变体和酰胺化的缺失变体(诸如羧基末端的脯氨酸残基被酰胺化的重链)。然而，本发明中使用的抗HER3抗体的重链的羧基末端的缺失变体不限于上面提及的类型，只要维持抗原结合能力和效应子功能即可。构成本发明中使用的抗HER3抗体的两条重链可以由任何一条重链构成，或者可以是选自全长和上述缺失变体的任何两条重链的组合。缺失变体的定量比可以受到培养的哺乳动物细胞的类型和用于产生本发明中使用的抗HER3抗体的培养条件的影响，但本发明中使用的抗HER3抗体的实例可以优选包括在两条重链各自的羧基末端缺失一个氨基酸残基的那些。

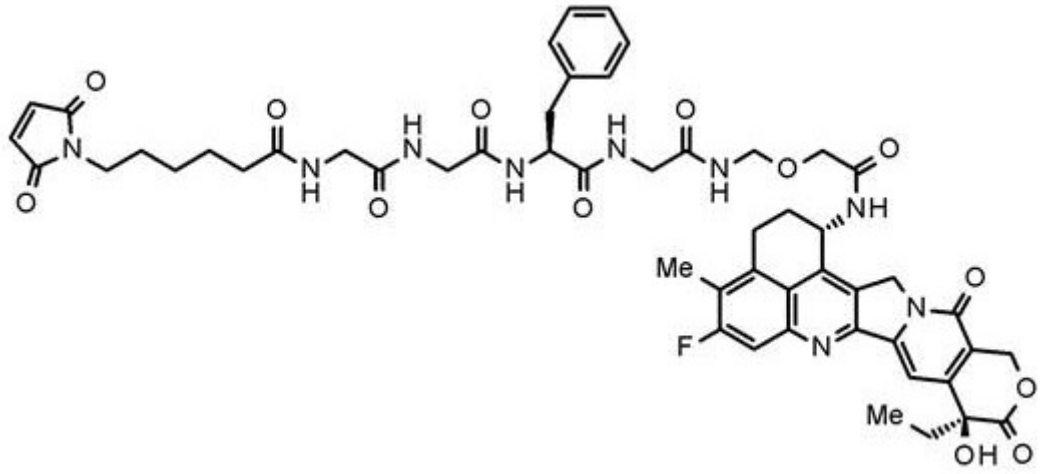
[0263] 作为本发明中使用的抗HER3抗体的同种型，可以例举IgG (IgG1、IgG2、IgG3和IgG4)，并且可以优选地例举IgG1或IgG2。此外，它们的变体也可以用作根据本发明的抗HER3抗体。

[0264] 本发明中可以使用的抗HER3抗体的实例包括patritumab (U3-1287)，U1-59(国际公开号WO 2007/077028)，AV-203 (国际公开号WO 2011/136911)，LJM-716 (国际公开号WO 2012/022814)，duligotumab (MEHD-7945A)(国际公开号WO 2010/108127)，istiratumab (MM-141) (国际公开号WO 2011/047180)，lumretuzumab (RG-7116) (国际公开号WO 2014/108484)，setibantumab (MM-121) (国际公开号WO 2008/100624)，REGN-1400 (国际公开号WO 2013/048883)，ZW-9 (国际公开号WO 2013/063702)及其变体、活性片段和修饰的变体。优选地，可以例举patritumab和U1-59。这些抗HER3抗体可以通过上面提及的参考文献中描述的方法产生。

[0265] [抗HER3抗体-药物缀合物的产生]

用于产生根据本发明的抗HER3抗体-药物缀合物的药物-接头中间体由下式代表。

[0266] [式20]



所述药物-接头中间体可以表示为化学名N- [6-(2,5-二氧代-2,5-二氢-1H-吡咯-1-基)己酰基]甘氨酰基甘氨酰基-L-苯丙氨酰基-N-[(2-{[(1S,9S)-9-乙基-5-氟-9-羟基-4-甲基-10,13-二氧代-2,3,9,10,13,15-六氢-1H,12H-苯并[de]吡喃并[3',4':6,7]吲哚嗪并[1,2-b]喹啉-1-基]氨基}-2-氧代乙氧基)甲基]甘氨酰胺，并且可以参考国际公开

号WO 2014/057687、国际公开号WO 2015/155998、国际公开号WO 2019/044947等的描述来产生。

[0267] 本发明中使用的抗HER3抗体-药物缀合物可以通过使上述药物-接头中间体与具有硫醇基团(或者称为巯基基团)的抗HER3抗体反应来产生。

[0268] 具有巯基基团的抗HER3抗体可通过本领域技术人员众所周知的方法获得(Hermanson, G.T, Bioconjugate Techniques, pp. 56-136, pp. 456-493, Academic Press (1996))。例如,通过在抗体内每个链间二硫化物使用0.3至3摩尔当量的还原剂诸如三(2-羧乙基)膦盐酸盐(TCEP)并在含有螯合剂诸如乙二胺四乙酸(EDTA)的缓冲液中与抗HER3抗体反应,可以获得在抗体内具有部分或完全还原的链间二硫化物的具有巯基基团的抗HER3抗体。

[0269] 此外,通过每个具有巯基基团的抗HER3抗体使用2至20摩尔当量的药物-接头中间体,可以产生其中每个抗体分子缀合2至8个药物分子的抗HER3抗体-药物缀合物。

[0270] 产生的抗HER3抗体-药物缀合物的每个抗体分子缀合的药物分子的平均数目可以例如通过如下进行确定:基于在两个波长280 nm和370 nm处测量抗HER3抗体-药物缀合物及其缀合前体的UV吸光度的计算方法(UV方法),或基于通过HPLC测量定量通过用还原剂处理抗体-药物缀合物获得的片段的计算方法(HPLC方法)。

[0271] 抗HER3抗体和药物-接头中间体之间的缀合,以及抗HER3抗体-药物缀合物的每个抗体分子缀合的药物分子的平均数的计算,可以参考国际公开号WO 2015/155998等等中的描述来进行。

[0272] [治疗剂和/或治疗方法]

本发明的治疗剂和/或治疗方法包括施用抗HER3抗体-药物缀合物,并且可以用于治疗HER3-突变的癌症。

[0273] 可以使用本发明的治疗剂和/或治疗方法的HER3-突变的癌症优选为选自以下的至少一种:乳腺癌,肺癌(包括小细胞肺癌和非小细胞肺癌),结肠直肠癌(其也可以称为结肠直肠癌,并且包括结肠癌和直肠癌),胃癌(其也可以称为胃腺癌),卵巢癌,头颈癌,多形胶质母细胞瘤,黑色素瘤,肾癌,尿路上皮癌,前列腺癌,胰腺癌,膀胱癌,胃肠道间质瘤,宫颈癌,食道癌,鳞状细胞癌,腹膜癌,多形胶质母细胞瘤,肝癌,肝细胞癌,子宫内膜癌,子宫癌,唾液腺癌,外阴癌,甲状腺癌,肝癌,肛门癌和阴茎癌,并且更优选为选自以下的至少一种:乳腺癌,非小细胞肺癌,结肠直肠癌,胃癌,卵巢癌,头颈癌,多形性胶质母细胞瘤和黑色素瘤。

[0274] 本发明的治疗剂和治疗方法可以优选用于哺乳动物,并且可以更优选用于人。

[0275] 本发明的治疗剂和治疗方法的抗肿瘤效果可以例如通过如下来证实:使肿瘤细胞表达突变型HER3并且在没有HER2过表达和/或具有HER2过表达的情况下测量抗HER3抗体-药物缀合物的细胞生长抑制活性。此外,也可能通过如下来证实该效果:通过将表达突变型HER3的肿瘤移植至测试动物中来产生模型,并向动物应用本发明的治疗剂或治疗方法。

[0276] 此外,还可以在临床研究中证实本发明的治疗剂和治疗方法的抗肿瘤效果。也就是说,该效果可以通过如下来证实:将本发明的治疗剂或治疗方法应用于确定为具有HER3突变的癌症患者并使用实体瘤的应答评估标准(RECIST)评估方法、WHO评估方法、Macdonald评估方法、重量测量和其他技术。该效果可以通过指数、诸如完全应答(CR)、部分

应答(PR)、进行性疾病(PD)、客观应答率(ORR)、应答的持续时间(DoR)、无进展存活(PFS)和总体存活(OS)来确定。

[0277] 对于针对HER3-突变的癌症的抗肿瘤效果,可以通过上面提及的方法证实本发明的治疗剂和治疗方法相对于现有抗癌剂的优势。

[0278] 本发明的治疗剂和治疗方法减慢癌细胞的生长,抑制生长,和甚至破坏癌细胞。这些作用可以使癌症患者摆脱癌症症状并改善QOL,由此达到治疗效果,同时维持癌症患者的生命。即使不破坏癌细胞,也可以通过抑制或控制癌细胞的增殖,癌症患者可以存活更久,同时实现更高的QOL。

[0279] 可以预期本发明的治疗剂通过局部应用于癌组织而不是作为全身疗法应用于患者而具有治疗作用。

[0280] 本发明的治疗剂可以作为含有至少一种药学上合适的成分的药物组合物施用。考虑到本发明中使用的抗HER3抗体-药物缀合物的剂量、施用浓度等,药学上合适的成分可以从本领域中通常使用的制剂添加剂等中适当地选择和应用。例如,本发明的治疗剂可以作为含有缓冲剂、诸如组氨酸缓冲剂、赋形剂、诸如蔗糖或海藻糖和表面活性剂、诸如聚山梨酯80或20的药物组合物(下文中称为“本发明的药物组合物”)施用。本发明的药物组合物可以优选地用作注射剂,可以更优选地用作水性注射剂或冻干注射剂,并且甚至可以更优选地用作冻干注射剂。

[0281] 在本发明的药物组合物是水性注射剂的情况下,其可以优选地用合适的稀释剂稀释,且然后作为静脉内输注给予。对于稀释剂,可以例举葡萄糖溶液、生理盐水等,并且可以优选地例举葡萄糖溶液,并且可以更优选地例举5%葡萄糖溶液。

[0282] 在本发明的药物组合物是冻干注射剂的情况下,可以优选地将其溶解于注射用水中,随后可以将所需量用合适的稀释剂稀释,且然后作为静脉内输注给予。对于稀释剂,可以例举葡萄糖溶液、生理盐水等,并且可以优选地例举葡萄糖溶液,并且可以更优选地例举5%葡萄糖溶液。

[0283] 可用于施用本发明的药物组合物的施用途径的实例包括静脉内、皮内、皮下、肌内和腹膜内途径,并且优选地包括静脉内途径。

[0284] 本发明中使用的抗HER3抗体-药物缀合物可以优选地每周一次、每2周一次、每3周一次或每4周一次施用于人,并且甚至可以更优选地每3周一次施用。

[0285] 另外,本发明中使用的抗HER3抗体-药物缀合物可以优选地以1.6 mg/kg至12.8 mg/kg的剂量施用于人,并且可以更优选地以1.6 mg/kg、3.2 mg/kg、4.8 mg/kg、5.6 mg/kg、6.4 mg/kg、8.0 mg/kg、9.6 mg/kg或12.8 mg/kg的剂量施用,并且可以甚至更优选以4.8 mg/kg、5.6 mg/kg或6.4 mg/kg的剂量施用。

[0286] 本发明的治疗剂还可以与除了本发明中使用的抗HER3抗体-药物缀合物以外的癌症治疗剂组合施用,由此增强抗肿瘤效果。用于这种目的的其他癌症治疗剂可以与本发明的治疗剂同时、分开或随后施用于受试者,并且可以在改变各自的施用间隔的同时进行施用。此类癌症治疗剂不受限制,只要其为具有抗肿瘤活性的试剂即可,并且可以通过选自以下的至少一种进行例举:伊立替康(CPT-11)、顺铂、卡铂、奥沙利铂、氟尿嘧啶(5-FU)、吉西他滨、卡培他滨、紫杉醇、多西他赛、多柔比星、表柔比星、环磷酰胺、丝裂霉素C、替加氟-吉美拉西汀-奥替拉西制剂、西妥昔单抗、帕尼单抗、贝伐单抗、雷莫西单抗、瑞戈非尼、曲氟胸

苷-地匹福林制剂、吉非替尼、厄洛替尼、阿法替尼、奥希替尼、甲氨蝶呤、培美曲塞、他莫昔芬、托瑞米芬、氟维司群、亮丙瑞林、戈舍瑞林、来曲唑、阿那曲唑、孕酮制剂、曲妥珠单抗emtansin、曲妥珠单抗、帕妥珠单抗、拉帕替尼、尼沃单抗、派姆单抗、阿特珠单抗、杜瓦鲁单抗、阿维单抗、伊匹单抗和曲美单抗。

[0287] 本发明的治疗剂也可以与放射疗法组合使用。例如，癌症患者在用本发明的治疗剂治疗之前和/或之后或与其同时进行放射疗法。

[0288] 本发明的治疗剂还可以与外科手术组合用作辅助化学疗法。外科手术通过例如除去脑肿瘤的全部或部分来实施。本发明的治疗剂可以为了在外科手术之前减小脑肿瘤的大小的目的进行施用(称为手术前辅助化学疗法或新辅助疗法)，或者可以在外科手术后为了预防脑肿瘤的复发的目的进行施用(称为手术后辅助化学疗法或辅助治疗)。

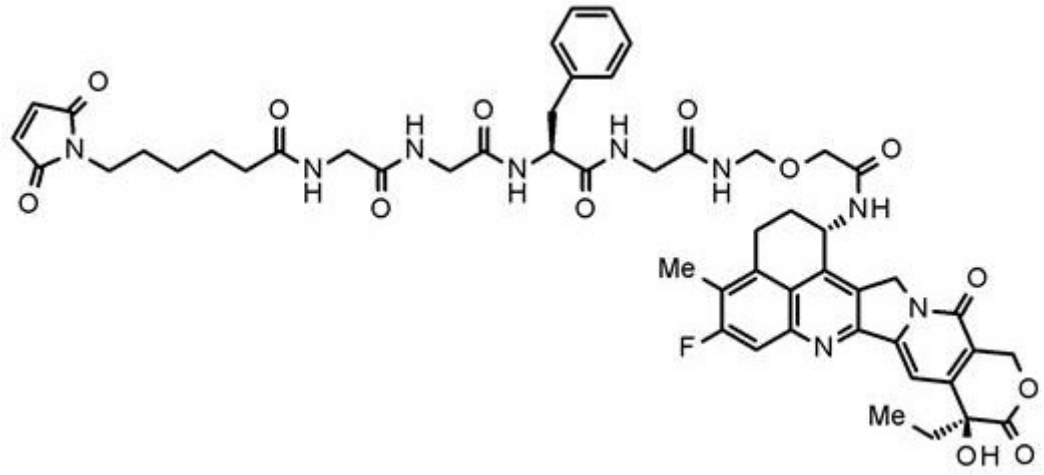
实施例

[0289] 鉴于以下所示的实施例具体描述了本发明。然而，本发明不限于这些。此外，其决不应以限制的方式进行解释。

[0290] [实施例1]抗HER3抗体-药物缀合物的产生

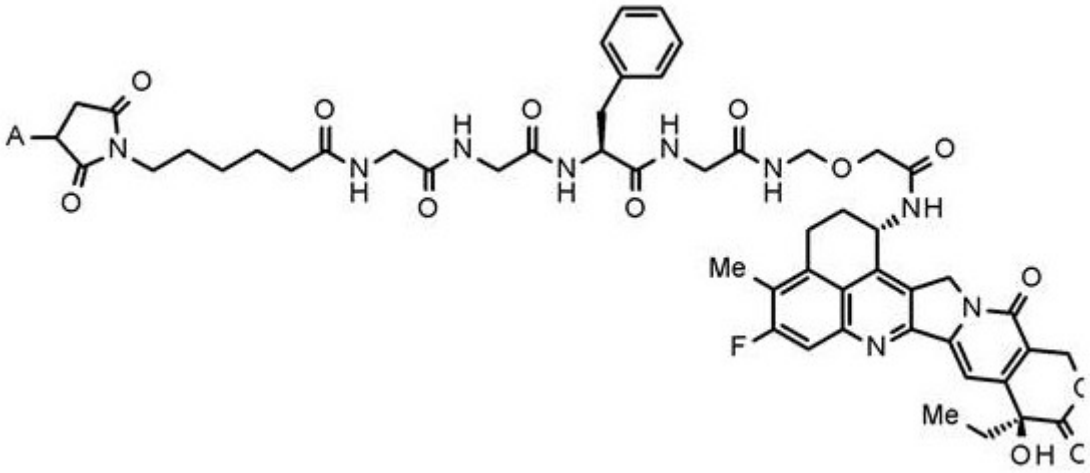
根据国际公开号WO 2015/155998中描述的产生方法，使用包含由SEQ ID NO:9代表的氨基酸序列组成的重链和由SEQ ID NO:10代表的氨基酸序列组成的轻链的抗HER3抗体(在本发明中称为"HER3-Ab (1)")，且使其与由下式代表的药物-接头中间体(下文中，称为"药物-接头中间体(1)")反应：

[式21]



产生抗HER3抗体-药物缀合物(在本发明中称为"HER3-ADC (1)")，其中由下式代表的药物-接头经由硫醚键与抗HER3抗体缀合：

[式22]



其中A代表与抗体的连接位置。每个HER3-ADC (1)的抗体分子的缀合的药物分子的平均数在7至8的范围内。

[0291] [实施例2] HER3-稳定表达细胞的产生

实施例2-1:用于表达HER3突变的慢病毒的载体的产生

1. pLVSIN EF1α HER3 (WT) Pur的产生

使用HER3 cDNA作为模板,将DNA用由SEQ ID NO:11代表的HER3 IF引物(Fw)和由SEQ ID NO:12代表的HER3 IF引物(Rev)扩增,并使用InFusion System (可得自Clontech Laboratories, Inc.)插入pLVSIN EF1α Pur (#6186, 可得自Takara Bio Inc.)的XbaI位点(pLVSIN EF1α HER3 (WT) Pur)。通过DNA测序证实插入序列。

[0292] 2. pLVSIN EF1α flag-HER3 (WT) Pur的产生

使用pLVSIN EF1α HER3 (WT) Pur作为模板,将质粒的全长用由SEQ ID NO:13代表的Flag IF引物(Fw)和由SEQ ID NO:14代表的Flag IF引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得pLVSIN EF1α flag-HER3 (WT) Pur。通过DNA测序证实序列。编码Flag-HER3 (WT)的cDNA的核苷酸序列显示于SEQ ID NO:15中,且Flag-HER3 (WT)的氨基酸序列显示于SEQ ID NO:16中。

[0293] 3. 用于表达各种突变型HER3的每种质粒的产生

i) pLVSIN EF1α flag-HER3 (V104L) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:17代表的HER3诱变引物(Fw)和由SEQ ID NO:18代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (V104L) Pur)。通过DNA测序证实序列。编码Flag-HER3 (V104L)的cDNA的核苷酸序列显示于SEQ ID NO:19中,且Flag-HER3 (V104L)的氨基酸序列显示于SEQ ID NO:20中。

[0294] ii) pLVSIN EF1α flag-HER3 (V104M) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:21代表的HER3诱变引物(Fw)和由SEQ ID NO:22代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (V104M) Pur)。通过DNA测序证实序列。编码Flag-HER3 (V104M)的cDNA的核苷酸序列显示于SEQ ID NO:23中,且Flag-HER3 (V104M)的氨基

酸序列显示于SEQ ID NO:24中。

[0295] iii) pLVSIN EF1α flag-HER3 (A232V) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:25代表的HER3诱变引物(Fw)和由SEQ ID NO:26代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (A232V) Pur)。通过DNA测序证实序列。编码Flag-HER3 (A232V)的cDNA的核苷酸序列显示于SEQ ID NO:27中,且Flag-HER3 (A232V)的氨基酸序列显示于SEQ ID NO:28中。

[0296] iv) pLVSIN EF1α flag-HER3 (P262H) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:29代表的HER3诱变引物(Fw)和由SEQ ID NO:30代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (P262H) Pur)。通过DNA测序证实序列。编码Flag-HER3 (P262H)的cDNA的核苷酸序列显示于SEQ ID NO:31中,且Flag-HER3 (P262H)的氨基酸序列显示于SEQ ID NO:32中。

[0297] v) pLVSIN EF1α flag-HER3 (G284R) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:33代表的HER3诱变引物(Fw)和由SEQ ID NO:34代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (G284R) Pur)。通过DNA测序证实序列。编码Flag-HER3 (G284R)的cDNA的核苷酸序列显示于SEQ ID NO:35中,且Flag-HER3 (G284R)的氨基酸序列显示于SEQ ID NO:36中。

[0298] vi) pLVSIN EF1α flag-HER3 (D297Y) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:37代表的HER3诱变引物(Fw)和由SEQ ID NO:38代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (D297Y) Pur)。通过DNA测序证实序列。编码Flag-HER3 (D297Y)的cDNA的核苷酸序列显示于SEQ ID NO:39中,且Flag-HER3 (D297Y)的氨基酸序列显示于SEQ ID NO:40中。

[0299] vii) pLVSIN EF1α flag-HER3 (G325R) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:41代表的HER3诱变引物(Fw)和由SEQ ID NO:42代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (G325R) Pur)。通过DNA测序证实序列。编码Flag-HER3 (G325R)的cDNA的核苷酸序列显示于SEQ ID NO:43中,且Flag-HER3 (G325R)的氨基酸序列显示于SEQ ID NO:44中。

[0300] viii) pLVSIN EF1α flag-HER3 (T355I) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:45代表的HER3诱变引物(Fw)和由SEQ ID NO:46代表的HER3诱变引物(Rev)扩增,以通过使用

InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (T355I) Pur)。通过DNA测序证实序列。编码Flag-HER3 (T355I)的cDNA的核苷酸序列显示于SEQ ID NO:47中,且Flag-HER3 (T355I)的氨基酸序列显示于SEQ ID NO:48中。

[0301] ix) pLVSIN EF1α flag-HER3 (S846I) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:49代表的HER3诱变引物(Fw)和由SEQ ID NO:50代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (S846I) Pur)。通过DNA测序证实序列。编码Flag-HER3 (S846I)的cDNA的核苷酸序列显示于SEQ ID NO:51中,且Flag-HER3 (S846I)的氨基酸序列显示于SEQ ID NO:52中。

[0302] x) pLVSIN EF1α flag-HER3 (E928G) Pur

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:53代表的HER3诱变引物(Fw)和由SEQ ID NO:54代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (E928G) Pur)。通过DNA测序证实序列。编码Flag-HER3 (E928G)的cDNA的核苷酸序列显示于SEQ ID NO:55中,且Flag-HER3 (E928G)的氨基酸序列显示于SEQ ID NO:56中。

[0303] 实施例2-2:用于表达HER2的慢病毒的载体的产生

1. pLVSIN EF1α HER2 Neo的产生

使用HER2 cDNA作为模板,将DNA用由SEQ ID NO:57代表的HER2 IF引物(Fw)和由SEQ ID NO:58代表的HER2 IF引物(Rev)扩增,并使用InFusion System (可得自Clontech Laboratories, Inc.)插入pLVSIN EF1α Neo (Takara Bio Inc., #6184)的NotI位点(pLVSIN EF1α HER2 Neo)。通过DNA测序证实插入序列。编码HER2的cDNA的核苷酸序列显示于SEQ ID NO:59中,且HER2的氨基酸序列显示于SEQ ID NO:60中。

[0304] 实施例2-3:HER3-稳定表达细胞的产生

将Lenti-X 293 T细胞(可得自Clontech Laboratories, Inc.)以1 x 10^6个细胞/孔接种至6-孔板中,并培养过夜。将ViraPower (可得自Thermo Fisher SCIENTIFIC K.K.)和实施例2-1中产生的用于表达各种HER3的质粒(pLVSIN EF1α flag-HER3 (WT) Pur、pLVSIN EF1α flag-HER3 (V104L) Pur、pLVSIN EF1α flag-HER3 (V104M) Pur、pLVSIN EF1α flag-HER3 (A232V) Pur、pLVSIN EF1α flag-HER3 (P262H) Pur、pLVSIN EF1α flag-HER3 (G284R) Pur、pLVSIN EF1α flag-HER3 (D297Y) Pur、pLVSIN EF1α flag-HER3 (G325R) Pur、pLVSIN EF1α flag-HER3 (T355I) Pur、pLVSIN EF1α flag-HER3 (S846I) Pur和pLVSIN EF1α flag-HER3 (E928G) Pur)各自使用Lipofectamine 2000 (可得自Thermo Fisher SCIENTIFIC K.K)引入Lenti-X 293 T细胞。此外,以与上述相同的方式将质粒(pLVSIN EF1α Pur)引入Lenti-X 293 T细胞,以产生空载体-引入的细胞。在引入后两天,收集培养上清液,并使其通过MILLEX-HP 0.45 UM过滤器(可得自Merck KGaA)以制备慢病毒溶液。将一半量的制备的慢病毒溶液转移至RetroNectin包被板(12孔板)中,并在37℃下静置过夜。用PBS洗涤后,将MDA-MB-231细胞以1 x 10^5个细胞/孔接种并培养三天。

在培养完成后,分离细胞,并将细胞总量接种至6-孔板中,以使用在0.5 μg/mL嘌呤霉素(可得自Thermo Fisher SCIENTIFIC K.K.)存在的情况下的药物抗性作为指标获得具有稳定表达的细胞(多克隆)。从获得的细胞提取总RNA,并使用600 ng总RNA进行逆转录反应。然后,使用1/20体积的逆转录反应产物作为模板,使用由SEQ ID NO:61代表的HER3引物(Fw)和由SEQ ID NO:62代表的HER3引物(Rev)进行PCR反应。PCR在25个循环的条件下进行,每个循环包括在94℃下加热2分钟,然后在98℃下加热10秒,且在68℃下加热1分钟。将RT-PCR产物进行1%琼脂糖凝胶电泳,以证实HER3基因表达。

[0305] 实施例2-4:HER2-过表达和HER3-稳定表达细胞系的产生

1. HER2-过表达细胞的产生

将Lenti-X 293 T细胞(可得自Clontech Laboratories, Inc.)以1 x 10^6个细胞/孔接种至6-孔板中,并培养过夜。将ViraPower (可得自Thermo Fisher SCIENTIFIC K.K.)和实施例2-2中产生的用于表达HER2的质粒(pLVSIN EF1α HER2 Neo)使用Lipofectamine 2000 (可得自Thermo Fisher SCIENTIFIC K.K)引入Lenti-X 293 T细胞。在引入后两天,收集培养上清液,并使其通过MILLEX-HP 0.45 UM过滤器(可得自Merck KGaA)以制备慢病毒溶液。将制备的慢病毒溶液转移至用RetroNextin (TAKARA)包被的板(6-孔板)中,并在37℃下静置过夜。用PBS洗涤后,将MDA-MB-231细胞以4x10^5个细胞/孔接种并培养三天。在培养完成后,分离细胞,并将细胞总量接种至T25烧瓶中,以使用在800 μg/mL遗传霉素(可得自Thermo Fisher SCIENTIFIC K.K.)存在的情况下的药物抗性作为指标获得HER2-稳定表达细胞(多克隆)。从获得的细胞提取总RNA,并使用600 ng总RNA进行逆转录反应。然后,使用1/20体积的逆转录反应产物作为模板,使用由SEQ ID NO:63代表的HER2引物(Fw)和由SEQ ID NO:64代表的HER2引物(Rev)进行PCR反应。PCR在23个循环的条件下进行,每个循环包括在94℃下加热2分钟,然后在98℃下加热10秒,且在68℃下加热1分钟。将RT-PCR产物进行1%琼脂糖凝胶电泳,以证实HER2基因表达。

[0306] 2. HER2-过表达和HER3-稳定表达细胞的产生

将Lenti-X 293 T细胞(可得自Clontech Laboratories, Inc.)以1 x 10^6个细胞/孔接种至6-孔板中,并培养过夜。将ViraPower (可得自Thermo Fisher SCIENTIFIC K.K.)和实施例2-1中产生的用于表达各种HER3的质粒(pLVSIN EF1α flag-HER3 (WT) Pur、pLVSIN EF1α flag-HER3 (V104L) Pur、pLVSIN EF1α flag-HER3 (V104M) Pur、pLVSIN EF1α flag-HER3 (A232V) Pur、pLVSIN EF1α flag-HER3 (P262H) Pur、pLVSIN EF1α flag-HER3 (G284R) Pur、pLVSIN EF1α flag-HER3 (D297Y) Pur、pLVSIN EF1α flag-HER3 (G325R) Pur、pLVSIN EF1α flag-HER3 (T355I) Pur、pLVSIN EF1α flag-HER3 (S846I) Pur和pLVSIN EF1α flag-HER3 (E928G) Pur)各自使用Lipofectamine 2000 (可得自Thermo Fisher SCIENTIFIC K.K)引入Lenti-X 293 T细胞。此外,以与上述相同的方式将质粒(pLVSIN EF1α Pur)引入Lenti-X 293 T细胞,以产生空载体-引入的细胞。在引入后两天,收集培养上清液,并使其通过MILLEX-HP 0.45 UM过滤器(可得自Merck KGaA)以制备慢病毒溶液。将一半量的制备的慢病毒溶液转移至用RetroNextin (TAKARA)包被的板(12孔板)中,并在37℃下静置过夜。用PBS洗涤后,将过表达HER2的MDA-MB-231细胞以1 x 10^5个细胞/孔接种并培养三天。在培养完成后,分离细胞,并将细胞总量接种至6-孔板中,以使用在0.5 μg/mL嘌呤霉素(可得自Thermo Fisher SCIENTIFIC K.K.)存在的情况下的

药物抗性作为指标获得具有稳定表达的细胞(多克隆)。从获得的细胞提取总RNA,并使用600 ng总RNA进行逆转录反应。然后,使用1/20体积的逆转录反应产物作为模板,使用由SEQ ID NO:61代表的HER3引物(Fw)和由SEQ ID NO:62代表的HER3引物(Rev)进行PCR反应。PCR在25个循环的条件下进行,每个循环包括在94℃下加热2分钟,然后在98℃下加热10秒,且在68℃下加热1分钟。将RT-PCR产物进行1%琼脂糖凝胶电泳,以证实HER3基因表达。此外,还通过上面提及的方法证实HER2基因表达。

[0307] 图3至图5显示结果。图3显示各种HER3-稳定表达细胞的RT-PCR产物的1%琼脂糖凝胶电泳的结果,且图4和图5显示各种HER2-过表达和HER3-稳定表达细胞的RT-PCR产物的1%琼脂糖凝胶电泳的结果。

[0308] 在HER3-稳定表达细胞中,在野生型和突变型HER3两者中均观察到HER3基因表达。在HER2-过表达和HER2-稳定表达细胞中,观察到HER2过表达,并且在HER3野生型和突变型HER3两者中均观察到HER3基因表达。

[0309] 实施例2-5:细胞表面上的HER3表达阳性率的证实

将HER3-稳定表达MDA-MB-231细胞用含有10% FBS(可得自Hyclone Laboratories, Inc)的RPMI1640培养基(可得自Thermo Fisher SCIENTIFIC K.K.)培养。用TrypLE (R) Express Enzyme (可得自Thermo Fisher SCIENTIFIC K.K.)将细胞与培养板分离,以通过台盼蓝处理测量活细胞的数目。将相同数目的活细胞添加至96-孔U-型底板,通过离心沉淀细胞,并用Stain Buffer(可得自Becton, Dickinson and Company)替换培养基。再次,通过离心沉淀细胞,并通过添加100 μL稀释至1 μg/mL的PE抗DYKDDDDK标签抗体(BioLegend, #637310)进行悬浮。将用未添加PE抗DYKDDDDK标签抗体的Stain Buffer处理的细胞用作对照组。使细胞在黑暗中在冰上反应60分钟,且然后用Stain Buffer洗涤。再次,将细胞悬浮于100 μL Stain Buffer中,然后向其中添加等量的4%多聚甲醛磷酸盐缓冲液(Wako Pure Chemical Industries, Ltd.),并使混合物在黑暗中在冰上反应20分钟。用Stain Buffer洗涤后,使用Attune NxT流式细胞仪(可得自Thermo Fisher SCIENTIFIC K.K.)测量荧光信号,并使用FlowJo软件(版本10.5.0,可得自Becton, Dickinson and Company)分析测量结果。表1显示用PE抗DYKDDDDK标签抗体处理的各种突变型HER3-引入的细胞的HER3表达阳性率。

[表 1]

细胞系	细胞表面上的 HER3 表达阳性率(%)	
	没有 HER2 过表达	有 HER2 过表达
模拟物	-	-
WT	65.4	76.3
V104L	79.3	75.8
V104M	67.2	61.8
A232V	76.8	70.7
P262H	79.0	65.0
G284R	82.6	79.7
D297Y	79.8	83.9
G325R	76.8	79.0
T355I	75.9	72.8
S846I	81.6	80.3
E928G	81.8	73.3

[0310] 细胞表面上的HER3表达比例为65.0%至83.9%,并且确定比较细胞之间的抗HER3抗体-药物缀合物的特性没有问题。

[0311] [实施例3]HER3-ADC (1)与野生型和各种突变型HER3-引入的细胞系各自的结合活性的证实。

[0312] 将野生型和各种突变型HER3-引入的MDA-MB-231细胞用含有10% FBS(可得自Hyclone Laboratories, Inc)的RPMI1640培养基(可得自Thermo Fisher SCIENTIFIC K.K.)培养。用TrypLE (R) Express Enzyme (可得自Thermo Fisher SCIENTIFIC K.K.)将细胞与培养板分离,以通过台盼蓝处理测量活细胞的数目。将相同数目的活细胞添加至96-孔U-型底板,然后通过离心沉淀细胞,并用Stain Buffer(可得自Becton, Dickinson and Company)替换培养基。再次,通过离心沉淀细胞,并将其悬浮于用冰冷却的100 μL HER3-ADC (1)稀释液(通过使用Stain Buffer进行1/10系列稀释,制备为100 nM至0.1 nM)中。将用未添加HER3-ADC (1)的Stain Buffer处理的细胞用作对照组。使细胞在冰上反应60分钟,且然后用Stain Buffer洗涤。此外,向其中添加100 μL稀释至10 μg/mL的Stain Buffer或二抗(山羊抗人IgG (H+L)交叉吸附的二抗, Alexa Fluor647, Thermo Fisher SCIENTIFIC K.K., #A-21445),并将细胞悬浮于其中。使细胞在黑暗中在冰上反应60分钟之后,且然后用Stain Buffer洗涤。将细胞悬浮于100 μL Stain Buffer中之后,向其中添加等量的4%多聚甲醛磷酸盐缓冲液(Wako Pure Chemical Industries, Ltd.),并使混合物在黑暗中在冰上反应20分钟。用Stain Buffer洗涤后,使用Attune NxT流式细胞仪(可得自Thermo Fisher SCIENTIFIC K.K.)测量荧光信号,并使用FlowJo软件(版本10.5.0,可得

自Becton, Dickinson and Company)分析测量结果。为了定量源自细胞中的HER3-ADC (1)的荧光信号,使用通过减去用Stain Buffer处理的对照组的信号而获得的值。

[0313] 图6和图7显示结果。纵轴显示在每种浓度下HER3-ADC (1)与野生型和各种突变型HER3-引入的细胞系各自的结合活性(HER3-阳性细胞的平均荧光强度)。

[0314] HER3-ADC (1)与各种突变型HER3-稳定表达细胞的结合以浓度依赖性方式增加,表现出与野生型HER3中相同的结合活性(图6)。HER3-ADC (1)在HER2-过表达和HER3-稳定表达细胞中的结合也以浓度依赖性方式增加,表现出与野生型HER3中相同的结合活性(图7)。在该实验中,使用没有HER2过表达的细胞用于比较,再次测量结合活性,以证实HER3-ADC (1)的结合活性没有差异,这取决于HER2表达水平(图7)。

[0315] [实施例4]通过HER3-ADC (1)抑制野生型和各种突变型HER3-引入的细胞系各自中的体外细胞生长。

[0316] 在含有10% FBS(可得自Hyclone Laboratories, Inc)的RPM1640培养基(可得自Thermo Fisher SCIENTIFIC K.K.)存在的情况下,测量HER3-ADC (1)针对野生型和各种突变型HER3-引入的MDA-MB-231细胞各自的生长抑制活性。通过测量未处理组、HER3-ADC (1)-处理组和HER3-Ab (1)-处理组中的三磷酸腺苷(ATP)活性来评估细胞生长。作为阴性对照,使用通过IgG抗体和药物-接头中间体(1)之间的缀合反应产生的IgG抗体-药物缀合物(在本发明中称为“IgG-ADC (1)”)。

[0317] 实施例4-1:细胞的处理

将野生型和各种突变型HER3-引入的MDA-MB-231细胞系各自作为500个细胞/100 μL/孔的细胞悬浮液接种至96-微孔板(Corning,#3904,黑色壁和透明底部)中。第二天,用含有10% FBS的RPMI1640培养基制备HER3-ADC (1)、HER3-Ab (1)和阴性对照IgG-ADC (1)的10-倍浓缩液,各自添加10 μL,并将混合物在37℃、5% CO_2下培养6天。仅将10 μL培养基添加至孔中,充当对照。每种条件在3孔至5孔中进行测量。

[0318] 实施例4-2:确定细胞生长抑制作用

基于ATP活性,对于具有代谢活性的活细胞,评估在培养6天期间各种试剂的细胞生长抑制活性。将100 μL CellTiter-Glo (R)试剂(Promega, #G7573)或100 μL ATPlite 1-步Luminescence Assay System (PerkinElmer, #6016739)添加至96-微孔板的每个孔中,以使用EnVision Workstation (超灵敏发光测量方案,h = 0.9)测量活性。为了测量ATP活性的降低,测定每种条件下3孔或5孔的平均发光值。通过与未处理组中的细胞比较来确定发光残余比例(%),并将该值解释为细胞活力(%)。

[0319] 图8至图33显示结果。图8至图19是显示各种试剂针对野生型和各种没有HER2过表达的突变型HER3-引入的细胞各自的细胞生长抑制活性的图。图20至图31是显示各种试剂针对野生型和各种具有HER2过表达的突变型HER3-引入的细胞各自的细胞生长抑制活性的图。发现HER3-ADC (1)表现出针对各种突变型HER3-引入的细胞各自以及针对野生型HER3-引入的细胞的细胞生长抑制活性(图8至图19)。另外,在HER2-过表达和HER3-稳定表达细胞中,发现HER3-ADC (1)表现出针对各种突变型HER3-引入的细胞各自以及针对野生型HER3-引入的细胞的细胞生长抑制活性(图20至图31)。在该实验中,使用没有HER2过表达的细胞,再次测量各种试剂的细胞生长抑制活性用于比较(图32和33),以证实HER3-ADC (1)的细胞生长抑制活性没有差异,这取决于HER2表达水平。

[0320] [实施例5]突变型HER3 (Q809R)-引入的细胞的产生及其药理评估

实施例5-1:用于表达突变型HER3的质粒的产生

使用pLVSIN EF1α flag-HER3 (WT)作为模板,将质粒的全长用由SEQ ID NO:65代表的HER3诱变引物(Fw)和由SEQ ID NO:66代表的HER3诱变引物(Rev)扩增,以通过使用InFusion System (可得自Clontech Laboratories, Inc)自连接获得用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (Q809R) Pur)。通过DNA测序证实序列。编码Flag-HER3 (Q809R)的cDNA的核苷酸序列显示于SEQ ID NO:67中,且Flag-HER3 (Q809R)的氨基酸序列显示于SEQ ID NO:68中。

[0321] 实施例5-2:突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)的产生

使用实施例5-1中产生的用于表达突变型HER3的质粒(pLVSIN EF1α flag-HER3 (Q809R) Pur),通过与实施例2-4中相同的方法获得突变型HER3 (Q809R)-引入的细胞,作为HER2-过表达和HER3-稳定表达细胞(多克隆)。以与实施例2-4中相同的方式,从获得的细胞提取总RNA,并对RT-PCR产物进行1%琼脂糖凝胶电泳,以证实HER3基因表达(图34)和HER2基因表达(图35)。

[0322] 实施例5-3:细胞表面上的HER3表达阳性率的证实

对于实施例5-2中产生的突变型HER3 (Q809R)-引入的细胞(具有HER2过表达),通过与实施例2-5中相同的方法证实细胞表面上的HER3表达阳性率。细胞表面上的HER3表达比例为58.8%,并且确定比较抗HER3抗体-药物缀合物的特性没有问题。

[0323] 实施例5-4:证实与突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)的结合活性

对于实施例5-2中产生的突变型HER3 (Q809R)-引入的细胞(具有HER2过表达),通过与实施例3中相同的方法评估HER3-ADC (1)与野生型和突变型HER3 (Q809R)-引入的细胞系各自在每种浓度的结合活性。HER3-ADC (1)与突变型HER3 (Q809R)引入的细胞系的结合以浓度依赖的方式增加,表现出与野生型HER3中相同的结合活性(图36)。

[0324] 实施例5-5:抑制突变型HER3 (Q809R)-引入的细胞(具有HER2过表达)中的体外细胞生长

对于实施例5-2中产生的突变型HER3 (Q809R)-引入的细胞(具有HER2过表达),通过与实施例4中相同的方法,评估HER3-ADC (1)、HER3-Ab (1)和IgG-ADC (1)针对野生型和突变型HER3 (Q809R)-引入的细胞系各自的体外细胞生长的抑制。发现HER3-ADC (1)表现出针对突变型HER3 (Q809R)-引入的细胞以及针对野生型HER3-引入的细胞的细胞生长抑制活性(图37)。

[0325] [实施例6]证实HER3-ADC (1)在野生型和各种突变型HER3-引入的细胞各自中的溶酶体迁移

在含有10% FBS(可得自Hyclone Laboratories, Inc)的RPMI1640培养基(可得自Thermo Fisher SCIENTIFIC K.K.)存在的情况下,测量HER3-ADC (1)在野生型和各种突变型HER3-引入的细胞各自中的溶酶体迁移。HER3-ADC (1)用具有pH敏感性的荧光染料pHrodo标记,并通过测量通过溶酶体迁移发射的荧光信号进行评估。

[0326] 实施例6-1:pHrodo-标记的HER3-ADC (1)的制备

对于pHrodo标记,使用pHrodo (R) iFL Microscale Protein Labeling Kit。使

用10 mM乙酸盐/5%山梨糖醇(pH5.5, NACALAI TESQUE, INC),将HER3-ADC (1)制备为1 mg/mL。将其100 μL(等于100μg)添加至反应管中。将pHrodo (R) iFL Red STP酯溶解于DMSO中,并将对应于HER3-ADC (1)的蛋白的摩尔数10倍的量添加至反应管中并混合,且然后使混合物在室温下反应60分钟。将反应溶液使用Zeba-spin (R)脱盐柱(可得自Thermo Fisher SCIENTIFIC K.K.)和Amicon Ultra-0.5 (Merck Millipore Corporation)进行离心,以纯化pHrodo-标记的HER3-ADC (1)。使用NanoDrop (R) 8000分光光度计测量280 nm和566 nm处的吸光度,以测定标记效率。

[0327] 实施例6-2:在培养细胞中的溶酶体迁移

将野生型和各种突变型HER3-引入的细胞各自作为$5x10^4$个细胞/100 μL/孔的细胞悬浮液接种至96-微孔板(Cellcarrier-96 Ultra,可得自Thermo Fisher SCIENTIFIC K.K.)中。第二天,在吸取培养基后,向其中添加50 μL用含有10% FBS的RPMI1640培养基稀释至100 ng/mL的Hoechst 33342(可得自Life Technologies Corporation)。在30分钟后,向其中添加50 μL使用含有100 ng/mL的Hoechst 33342的含有10% FBS的RPMI1640培养基制备的pHrodo-标记的HER3-ADC (1)的2倍浓缩液,同时向阴性对照添加培养基替代pHrodo-标记的HER3-ADC (1)。对于荧光信号,使用Opera Phenix(R)高通量/高含量成像系统(可得自PerkinElmer, Inc)在10个小时内每30分钟获得活细胞成像图像。每种条件以三个斑点/孔进行测量。使用图像分析软件Harmony (R) (可得自PerkinElmer, Inc)定量分析获得的图像。定量值显示为通过将每个细胞的荧光点数乘以每个点的荧光强度而获得的值(运输指数)。

[0328] 图38至64显示结果。图38至49显示HER3-稳定表达细胞(没有HER2过表达)的结果,且图50至62显示HER3-稳定表达细胞(具有HER2过表达)的结果。

[0329] pHrodo-标记的HER3-ADC (1)在各种突变型HER3-引入的细胞各自以及野生型HER3-引入的细胞中以浓度依赖的方式表现出溶酶体迁移(图38至图49)。由此发现,在野生型HER3-表达细胞中和在突变型HER3-表达细胞中之间,HER3-ADC (1)的溶酶体迁移没有实质性差异。

[0330] 还发现pHrodo-标记的HER3-ADC (1)在HER2-过表达和HER3-稳定表达细胞中也以浓度依赖的方式表现出溶酶体迁移(图50至图62)。在该实验中,再次证实HER3-ADC (1)在没有HER2过表达的细胞中的溶酶体迁移用于比较(图63和64)。由此发现,在具有HER2的过表达的HER3-表达细胞中和没有HER2的过表达的HER3-表达细胞中之间,HER3-ADC (1)的溶酶体迁移没有实质性差异。

[0331] 前面提及的结果证实,无论是否存在HER2过表达,HER3-ADC (1)表现出针对各种突变型HER3-引入的细胞各自的细胞生长抑制活性。

[0332] 当前,已经临床研究HER3-ADC (1),并且已经表明,对于突变型HER3情况以及对于野生型HER3情况,可以预期临床效力。

[0333] 序列表的自由文本

SEQ ID NO:1 - 抗HER3抗体的CDRH1的氨基酸序列

SEQ ID NO:2 - 抗HER3抗体的CDRH2的氨基酸序列

SEQ ID NO:3 - 抗HER3抗体的CDRH3的氨基酸序列

SEQ ID NO:4 - 抗HER3抗体的CDRL1的氨基酸序列

SEQ ID NO:5 - 抗HER3抗体的CDRL2的氨基酸序列
SEQ ID NO:6 - 抗HER3抗体的CDRL3的氨基酸序列
SEQ ID NO:7 - 抗HER3抗体的重链可变区的氨基酸序列
SEQ ID NO:8 - 抗HER3抗体的轻链可变区的氨基酸序列
SEQ ID NO:9 - 抗HER3抗体的重链的氨基酸序列
SEQ ID NO:10 - 抗HER3抗体的轻链的氨基酸序列
SEQ ID NO:11 - HER3 IF引物(Fw)的核苷酸序列
SEQ ID NO:12 - HER3 IF引物(Rev)的核苷酸序列
SEQ ID NO:13 - Flag IF引物(Fw)的核苷酸序列
SEQ ID NO:14 - Flag IF引物(Rev)的核苷酸序列
SEQ ID NO:15 - 编码Flag-HER3 (WT)的cDNA的核苷酸序列
SEQ ID NO:16 - Flag-HER3 (WT)的氨基酸序列
SEQ ID NO:17 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:18 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:19 - 编码Flag-HER3 (V104L)的cDNA的核苷酸序列
SEQ ID NO:20 - Flag-HER3 (V104L)的氨基酸序列
SEQ ID NO:21 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:22 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:23 - 编码Flag-HER3 (V104M)的cDNA的核苷酸序列
SEQ ID NO:24 - Flag-HER3 (V104M)的氨基酸序列
SEQ ID NO:25 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:26 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:27 - 编码Flag-HER3 (A232V)的cDNA的核苷酸序列
SEQ ID NO:28 - Flag-HER3 (A232V)的氨基酸序列
SEQ ID NO:29 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:30 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:31 - 编码Flag-HER3 (P262H)的cDNA的核苷酸序列
SEQ ID NO:32 - Flag-HER3 (P262H)的氨基酸序列
SEQ ID NO:33 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:34 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:35 - 编码Flag-HER3 (G284R)的cDNA的核苷酸序列
SEQ ID NO:36 - Flag-HER3 (G284R)的氨基酸序列
SEQ ID NO:37 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:38 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:39 - 编码Flag-HER3 (D297Y)的cDNA的核苷酸序列
SEQ ID NO:40 - Flag-HER3 (D297Y)的氨基酸序列
SEQ ID NO:41 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:42 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:43 - 编码Flag-HER3 (G325R)的cDNA的核苷酸序列

SEQ ID NO:44 - Flag-HER3 (G325R)的氨基酸序列
SEQ ID NO:45 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:46 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:47 - 编码Flag-HER3 (T355I)的cDNA的核苷酸序列
SEQ ID NO:48 - Flag-HER3 (T355I)的氨基酸序列
SEQ ID NO:49 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:50 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:51 - 编码Flag-HER3 (S846I)的cDNA的核苷酸序列
SEQ ID NO:52 - Flag-HER3 (S846I)的氨基酸序列
SEQ ID NO:53 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:54 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:55 - 编码Flag-HER3 (E928G)的cDNA的核苷酸序列
SEQ ID NO:56 - Flag-HER3 (E928G)的氨基酸序列
SEQ ID NO:57 - HER2 IF引物(Fw)的核苷酸序列
SEQ ID NO:58 - HER2 IF引物(Rev)的核苷酸序列
SEQ ID NO:59 - 编码HER2的cDNA的核苷酸序列
SEQ ID NO:60 - HER2的氨基酸序列
SEQ ID NO:61 - HER3引物(Fw)的核苷酸序列
SEQ ID NO:62 - HER3引物(Rev)的核苷酸序列
SEQ ID NO:63 - HER2引物(Fw)的核苷酸序列
SEQ ID NO:64 - HER2引物(Rev)的核苷酸序列
SEQ ID NO:65 - HER3诱变引物(Fw)的核苷酸序列
SEQ ID NO:66 - HER3诱变引物(Rev)的核苷酸序列
SEQ ID NO:67 - 编码Flag-HER3 (Q809R)的cDNA的核苷酸序列
SEQ ID NO:68 - Flag-HER3 (Q809R)的氨基酸序列
SEQ ID NO:69 - HER3蛋白的氨基酸序列

序列表

<110> DAIICHI SANKYO COMPANY, LIMITED

<120> 通过施用抗HER3抗体-药物缀合物治疗HER3-突变的癌症

<130> DSPCT-FP1926

<150> JP2018-175510
<151> 2018-09-20

<160> 69

<170> PatentIn version 3.5

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<213> 人工序列

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[0001]

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<212> PRT
<213> 人工序列

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<210> 3
<211> 9
<212> PRT
<213> 人工序列

<220>
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<210> 4
<211> 16
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<213> 人工序列

<220>
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<213> 人工序列

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[0002]

<210> 6
<211> 9
<212> PRT
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<220>
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1 5

<210> 7
<211> 117
<212> PRT
<213> 人工序列

<220>
<223> 抗HER3抗体的重链可变区

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1 5 10 15

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

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

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

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

Arg Asp Lys Trp Thr Trp Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

[0003]

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<211> 113
<212> PRT
<213> 人工序列

<220>
<223> 抗HER3抗体的轻链可变区

<400> 8

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

Glu Arg Ala Thr Ile Asn Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Ser Asn Arg Asn Tyr Leu Ala Trp Tyr Gln Gln Asn Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 9
<211> 447
<212> PRT
<213> 人工序列

<220>
<223> 抗HER3抗体的重链

<400> 9

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

[0004]

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

Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

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

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

Arg Asp Lys Trp Thr Trp Tyr Phe Asp Leu Trp Gly Arg Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225 230 235 240

[0005]

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

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

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

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

<210> 10
<211> 220
<212> PRT
[0006] <213> 人工序列

<220>
<223> 抗HER3抗体的轻链

<400> 10

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

Glu Arg Ala Thr Ile Asn Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Ser Asn Arg Asn Tyr Leu Ala Trp Tyr Gln Gln Asn Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln

85 90 95

Tyr Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
145 150 155 160

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
165 170 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
180 185 190

[0007] Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
195 200 205

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215 220

<210> 11
<211> 39
<212> DNA
<213> 人工序列

<220>
<223> HER3 IF引物(Fw)

<400> 11
ggatttaaat tctagagcca ccatgagagc caacgatgc 39

<210> 12
<211> 38
<212> DNA
<213> 人工序列

<220>
<223> HER3 IF引物(Rev)

<400> 12

ccgcggccgc tctagattat gttctctgag cgttggcc 38

<210> 13
<211> 43
<212> DNA
<213> 人工序列

<220>
<223> Flag IF引物(Fw)

<400> 13
acaaggatga cgacgataag tctgaagtgg gcaacagcca ggc 43

<210> 14
<211> 43
<212> DNA
<213> 人工序列

<220>
<223> Flag IF引物(Rev)

<400> 14
cgtcgtcatc cttgtaatcg cctctagcca gagagaacag cag 43

[0008] <210> 15
<211> 4053
<212> DNA
<213> 人工序列

<220>
<223> 编码Flag-HER3(WT)的cDNA

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atgagagcca acgatgctct gcaggttctg ggcctgctgt tctctctggc tagaggcgat 60

tacaaggatg acgacgataa gtctgaagtg ggcaacagcc aggctgtgtg tcccggaaca 120

ctgaacggct tgtctgtgac aggcgacgcc gagaaccagt accagacact gtacaagctg 180

tacgagagat gcgaggtggt catgggcaac ctggaaatcg tgctgacagg ccacaacgcc 240

gacctgtctt tcctgcagtg gatcagagaa gtgaccggct acgtgctggt ggccatgaac 300

gagttcagca ccctgcctct gcctaacctg agagtcgtca gaggcaccca ggtgtacgat 360

ggcaagttcg ccatcttcgt gatgctgaac tacaacacca acagctctca cgccctgaga 420

cagctgagac tgacccagct gaccgagatc ctgtctggcg gagtgtacat cgagaagaac 480

gacaagctgt gccacatgga caccatcgac tggcgggata tcgtgcggga tagagatgcc 540

gagatcgtgg tcaaggacaa cggcagaagc tgccctcctt gccacgaagt gtgcaagggc 600

[0009]

agatgttggg gccctggctc tgaggactgt cagaccctga ccaagaccat ctgcgcccct 660

cagtgtaacg gccactgctt cggccctaat cctaaccagt gctgccacga cgaatgcgct 720

ggcggatgtt ctggacctca ggacacagac tgcttcgcct gcagacactt caacgactct 780

ggcgcctgcg tgccaagatg tcctcagcct ctggtgtaca acaagctgac ctttcagctg 840

gaacccaatc ctcacaccaa gtaccagtac ggcggcgtgt gtgtggccag ctgtcctcac 900

aacttcgtgg tggaccagac cagctgtgtg cgggcttgtc ctcctgacaa gatggaagtg 960

gacaagaacg gcctgaagat gtgcgagcct tgcggcggac tgtgtcctaa ggcttgtgaa 1020

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aactgcacca agatcctggg aaacctggac ttcctgatca ccggcctgaa cggcgatccc 1140

tggcataaga tccctgctct ggaccccgag aagctgaacg tgttcagaac cgtgcgcgag 1200

atcacaggct acctgaacat ccagagctgg cctccacaca tgcacaactt cagcgtgttc 1260

tccaacctga ccaccatcgg cggcagatcc ctgtacaaca ggggcttcag cctgctgatc 1320

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acactggtgc tgatcggcaa gacccacctg acaatggccc tgacagtgat cgctggcctg 1980

gtggtcatct ttatgatgct cggcggcacc ttcctgtatt ggagaggcag aagaatccag 2040

aacaagaggg ccatgcggag atacctggaa agaggcgaga gcatcgagcc actggaccct 2100

agcgagaagg ccaacaaggt gctggccaga atcttcaaag agacagagct gcggaagctg 2160

aaggtgctcg gatctggcgt gttcggcaca gtgcacaaag gcgtgtggat ccctgagggc 2220

gagtccatca agatccccgt gtgcatcaaa gtgatcgagg acaagtccgg caggcagagc 2280

[0010]

ttccaggccg tgacagatca catgctggct atcggcagcc tggatcacgc ccacatcgtt	2340
agactgctgg gactgtgccc tggcagctct ctgcagctcg tgacacagta tctgcctctg	2400
ggatctctgc tggaccacgt gcgacaacat agaggcgctc tgggacctca gctgctgctg	2460
aattggggag tgcagatcgc caagggcatg tactacctgg aagaacacgg catggtgcac	2520
agaaacctgg ccgccagaaa cgtgctgctc aagtctccta gccaggtgca ggtcgccgat	2580
ttcggagttg ctgatctcct gccacctgac gacaaacagc tgctgtactc cgaggccaag	2640
acacccatca agtggatggc cctggaatct atccacttcg gcaagtacac ccaccagagc	2700
gacgtgtggt cttacggcgt gacagtgtgg gagctgatga ccttcggagc cgagccttac	2760
gctggactga gactggctga agtgcccgac ctgctggaaa agggcgaaag acttgcccag	2820
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aacatcagac ccacattcaa agagctggct aacgagttta cccggatggc cagagatcct	2940
cctagatacc tcgtgatcaa gagagagagc ggccctggca ttgctcctgg acctgaacct	3000
cacggactga ccaacaagaa gctggaagag gtcgaactgg aacccgagct ggacctggat	3060
ctcgacctgg aagccgaaga ggataacctg gccaccacaa cactgggctc tgctctgagt	3120
ctgcctgtgg gcacactgaa cagaccaaga ggaagccaga gcctgctgtc tccaagcagc	3180
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gcttctgagt ctagcgaggg acacgtgaca ggatccgagg ccgaactgca agagaaagtg	3360
tctatgtgca gaagccgcag cagaagcaga agccctagac ctagaggcga cagcgcctac	3420
cactctcaga gacactcact gctgacccct gtgacacctc tgtctccacc tggactcgag	3480
gaagaggacg tcaacggata cgtgatgccc gacacacacc tgaagggcac ccctagctct	3540
agagagggaa ccctgagtag cgtgggcctg agttctgtgc tgggcaccga agaggaagat	3600
gaggacgagg aatacgagta catgaacaga agaaggcggc acagccctcc acatcctcca	3660
agacctagca gcctcgagga actgggctac gagtatatgg acgtgggcag cgatctgagc	3720
gctagcctgg gatctacaca gtcttgccca ctgcaccctg tgcctatcat gcctacagcc	3780
ggcaccacac cagacgagga ctatgagtat atgaatcggc agagagatgg cggcggacct	3840
ggcggagatt atgctgctat gggagcctgt cctgctagcg agcagggcta cgaagagatg	3900
agagcctttc aaggccctgg ccatcaggct cctcacgtgc actatgccag actgaaaacc	3960

ctgagatctc tggaagccac cgactccgcc ttcgacaacc ctgactactg gcacagcaga 4020

ctgttcccca aggccaacgc tcagagaaca taa 4053

<210> 16
<211> 1350
<212> PRT
<213> 人工序列

<220>
<223> Flag-HER3(WT)

<400> 16

Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
1 5 10 15

Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

[0011]

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg

165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

[0012] Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu

385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

[0013]

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn

610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

[0014]

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val

835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

[0015] Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly

1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

[0016]

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly

1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

<210> 17
<211> 31
<212> DNA
<213> 人工序列

[0017]

<220>
<223> HER3诱变引物(Fw)

<400> 17
ctgagactgg tcagaggcac ccaggtgtac g 31

<210> 18
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Rev)

<400> 18
tctgaccagt ctcaggttag gcagaggcag g 31

<210> 19
<211> 4053
<212> DNA
<213> 人工序列

<220>
<223> 编码Flag-HER3(V104L)的cDNA

<400> 19

[0018]

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tacaaggatg acgacgataa gtctgaagtg ggcaacagcc aggctgtgtg tcccggaaca 120

ctgaacggct tgtctgtgac aggcgacgcc gagaaccagt accagacact gtacaagctg 180

tacgagagat gcgaggtggt catgggcaac ctggaaatcg tgctgacagg ccacaacgcc 240

gacctgtctt tcctgcagtg gatcagagaa gtgaccggct acgtgctggt ggccatgaac 300

gagttcagca ccctgcctct gcctaacctg agactggtca gaggcaccca ggtgtacgat 360

ggcaagttcg ccatcttcgt gatgctgaac tacaacacca acagctctca cgccctgaga 420

cagctgagac tgacccagct gaccgagatc ctgtctggcg gagtgtacat cgagaagaac 480

gacaagctgt gccacatgga caccatcgac tggcgggata tcgtgcggga tagagatgcc 540

gagatcgtgg tcaaggacaa cggcagaagc tgccctcctt gccacgaagt gtgcaagggc 600

agatgttggg gccctggctc tgaggactgt cagaccctga ccaagaccat ctgcgccccc 660

cagtgtaacg gccactgctt cggccctaat cctaaccagt gctgccacga cgaatgcgct 720

ggcggatgtt ctggacctca ggacacagac tgcttcgcct gcagacactt caacgactct 780

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aactgcacca agatcctggg aaacctggac ttcctgatca ccggcctgaa cggcgatccc 1140

tggcataaga tccctgctct ggaccccgag aagctgaacg tgttcagaac cgtgcgcgag 1200

atcacaggct acctgaacat ccagagctgg cctccacaca tgcacaactt cagcgtgttc 1260

tccaacctga ccaccatcgg cggcagatcc ctgtacaaca ggggcttcag cctgctgatc 1320

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tgcgtggccg agggcaaagt gtgcgaccct ctttgttcta gcggcggctg ttggggacca 1560

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[0020]

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Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu

85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Leu
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

[0021]

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val

305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

[0022] Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu

530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

[0023]

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met

755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

[0024] Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro

980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

[0025] Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu

1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

[0026]

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

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<223> HER3诱变引物(Fw)

<400> 21
ctgagaatgg tcagaggcac ccaggtgtac g 31

<210> 22

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<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Rev)

<400> 22
tctgaccatt ctcaggttag gcagaggcag g 31

<210> 23
<211> 4053
<212> DNA
<213> 人工序列

<220>
<223> 编码Flag-HER3(V104M)的cDNA

<400> 23

[0027]

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ctgaacggct tgtctgtgac aggcgacgcc gagaaccagt accagacact gtacaagctg 180

tacgagagat gcgaggtggt catgggcaac ctggaaatcg tgctgacagg ccacaacgcc 240

gacctgtctt tcctgcagtg gatcagagaa gtgaccggct acgtgctggt ggccatgaac 300

gagttcagca ccctgcctct gcctaacctg agaatggtca gaggcaccca ggtgtacgat 360

ggcaagttcg ccatcttcgt gatgctgaac tacaacacca acagctctca cgccctgaga 420

cagctgagac tgacccagct gaccgagatc ctgtctggcg gagtgtacat cgagaagaac 480

gacaagctgt gccacatgga caccatcgac tggcgggata tcgtgcggga tagagatgcc 540

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gacaagaacg gcctgaagat gtgcgagcct tgcggcggac tgtgtcctaa ggcttgtgaa 1020

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[0028]

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ggctacatgc ctatgaacca gggcaatctg ggcgagagct gtcaagagtc tgccgtgtct 3240

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gctagcctgg gatctacaca gtcttgccca ctgcaccctg tgcctatcat gcctacagcc 3780

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ggcggagatt atgctgctat gggagcctgt cctgctagcg agcagggcta cgaagagatg 3900

agagcctttc aaggccctgg ccatcaggct cctcacgtgc actatgccag actgaaaacc 3960

ctgagatctc tggaagccac cgactccgcc ttcgacaacc ctgactactg gcacagcaga 4020

ctgttcccca aggccaacgc tcagagaaca taa 4053

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<213> 人工序列

<220>
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Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Met
100 105 110

[0030] Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala

225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

[0031] Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile

450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

[0032] His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr

675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

[0033] Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu

900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

[0034]

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg

1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

[0035]

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His

1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

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<210> 26
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<213> 人工序列

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tccgccgacg cattcgtcgt ggcagcactg g 31

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ctgttcccca aggccaacgc tcagagaaca taa 4053

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Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

[0039] Ser Gln Ala Val Cys Pro Gly Thr Leu Asn Gly Leu Ser Val Thr Gly
35 40 45

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

Thr Gln Leu Thr Glu Ile Leu Ser Gly Gly Val Tyr Ile Glu Lys Asn

145 150 155 160

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Val
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

[0040] Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile

370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

[0041] Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile

595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

[0042]

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr

820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

[0043] Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln

1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

[0044]

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr

1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

[0045] <210> 29
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<223> HER3诱变引物(Rev)

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caccaggtgc tgaggacatc ttggcacgca g 31

<210> 31
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<223> 编码Flag-HER3(P262H)的cDNA

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aacatcagac ccacattcaa agagctggct aacgagttta cccggatggc cagagatcct 2940

cctagatacc tcgtgatcaa gagagagagc ggccctggca ttgctcctgg acctgaacct 3000

cacggactga ccaacaagaa gctggaagag gtcgaactgg aacccgagct ggacctggat 3060

ctcgacctgg aagccgaaga ggataacctg gccaccacaa cactgggctc tgctctgagt 3120

ctgcctgtgg gcacactgaa cagaccaaga ggaagccaga gcctgctgtc tccaagcagc 3180

ggctacatgc ctatgaacca gggcaatctg ggcgagagct gtcaagagtc tgccgtgtct 3240

ggcagcagcg agagatgtcc tagacctgtg tctctgcacc ccatgcctag aggctgtctg 3300

gcttctgagt ctagcgaggg acacgtgaca ggatccgagg ccgaactgca agagaaagtg 3360

tctatgtgca gaagccgcag cagaagcaga agccctagac ctagaggcga cagcgcctac 3420

cactctcaga gacactcact gctgacccct gtgacacctc tgtctccacc tggactcgag 3480

gaagaggacg tcaacggata cgtgatgccc gacacacacc tgaagggcac ccctagctct 3540

agagagggaa ccctgagtag cgtgggcctg agttctgtgc tgggcaccga agaggaagat 3600

gaggacgagg aatacgagta catgaacaga agaaggcggc acagccctcc acatcctcca 3660

agacctagca gcctcgagga actgggctac gagtatatgg acgtgggcag cgatctgagc 3720

gctagcctgg gatctacaca gtcttgccca ctgcaccctg tgcctatcat gcctacagcc 3780

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ggcggagatt atgctgctat gggagcctgt cctgctagcg agcagggcta cgaagagatg 3900

agagcctttc aaggccctgg ccatcaggct cctcacgtgc actatgccag actgaaaacc 3960

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ctgttcccca aggccaacgc tcagagaaca taa 4053

[0048]

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Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
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Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala

65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

[0049] Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln His Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val

290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

[0050] Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys

515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

[0051] Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile

740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

[0052] Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met

965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

[0053]

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val

1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

[0054]

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

<210> 33
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Fw)

<400> 33

tacggcagag tgtgtgtggc cagctgtcct c 31

<210> 34
<211> 32
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Rev)

<400> 34
acacactctg ccgtactggt acttggtgtg ag 32

<210> 35
<211> 4053
<212> DNA
<213> 人工序列

<220>
<223> 编码Flag-HER3(G284R)的cDNA

<400> 35
atgagagcca acgatgctct gcaggttctg ggcctgctgt tctctctggc tagaggcgat 60

tacaaggatg acgacgataa gtctgaagtg ggcaacagcc aggctgtgtg tcccggaaca 120

[0055]

ctgaacggct tgtctgtgac aggcgacgcc gagaaccagt accagacact gtacaagctg 180

tacgagagat gcgaggtggt catgggcaac ctggaaatcg tgctgacagg ccacaacgcc 240

gacctgtctt tcctgcagtg gatcagagaa gtgaccggct acgtgctggt ggccatgaac 300

gagttcagca ccctgcctct gcctaacctg agagtcgtca gaggcaccca ggtgtacgat 360

ggcaagttcg ccatcttcgt gatgctgaac tacaacacca acagctctca cgccctgaga 420

cagctgagac tgacccagct gaccgagatc ctgtctggcg gagtgtacat cgagaagaac 480

gacaagctgt gccacatgga caccatcgac tggcgggata tcgtgcggga tagagatgcc 540

gagatcgtgg tcaaggacaa cggcagaagc tgccctcctt gccacgaagt gtgcaagggc 600

agatgttggg gccctggctc tgaggactgt cagaccctga ccaagaccat ctgcgccct 660

cagtgtaacg gccactgctt cggccctaat cctaaccagt gctgccacga cgaatgcgct 720

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ggcgcctgcg tgccaagatg tcctcagcct ctggtgtaca acaagctgac ctttcagctg 840

gaacccaatc ctcacaccaa gtaccagtac ggcagagtgt gtgtggccag ctgtcctcac 900

aacttcgtgg tggaccagac cagctgtgtg cgggcttgtc ctcctgacaa gatggaagtg 960

[0056]

gacaagaacg gcctgaagat gtgcgagcct tgcggcggac tgtgtcctaa ggcttgtgaa 1020

ggcacaggca gcggcagcag attccagaca gtggacagca gcaacatcga cggcttcgtg 1080

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tggcataaga tccctgctct ggaccccgag aagctgaacg tgttcagaac cgtgcgcgag 1200

atcacaggct acctgaacat ccagagctgg cctccacaca tgcacaactt cagcgtgttc 1260

tccaacctga ccaccatcgg cggcagatcc ctgtacaaca ggggcttcag cctgctgatc 1320

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agaatctaca tcagcgccaa cagacagctg tgctaccacc acagcctgaa ctggacaaag 1440

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tgcgtggccg agggcaaagt gtgcgaccct ctttgttcta gcggcggctg ttggggacca 1560

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acactggtgc tgatcggcaa gacccacctg acaatggccc tgacagtgat cgctggcctg 1980

gtggtcatct ttatgatgct cggcggcacc ttcctgtatt ggagaggcag aagaatccag 2040

aacaagaggg ccatgcggag atacctggaa agaggcgaga gcatcgagcc actggaccct 2100

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ctgcctgtgg gcacactgaa cagaccaaga ggaagccaga gcctgctgtc tccaagcagc 3180

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agagagggaa ccctgagtag cgtgggcctg agttctgtgc tgggcaccga agaggaagat 3600

gaggacgagg aatacgagta catgaacaga agaaggcggc acagccctcc acatcctcca 3660

agacctagca gcctcgagga actgggctac gagtatatgg acgtgggcag cgatctgagc 3720

gctagcctgg gatctacaca gtcttgccca ctgcaccctg tgcctatcat gcctacagcc 3780

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ggcggagatt atgctgctat gggagcctgt cctgctagcg agcagggcta cgaagagatg 3900

agagcctttc aaggccctgg ccatcaggct cctcacgtgc actatgccag actgaaaacc 3960

ctgagatctc tggaagccac cgactccgcc ttcgacaacc ctgactactg gcacagcaga 4020

ctgttcccca aggccaacgc tcagagaaca taa 4053

<210> 36
<211> 1350
<212> PRT
<213> 人工序列

<220>
<223> Flag-HER3(G284R)

〈400〉 36

Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
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Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
[0058] 100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly

210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Arg Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

[0059] Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser

435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

[0060] Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu

660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

[0061] Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr

885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

[0062] Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu

1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

[0063]

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser

1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

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<213> 人工序列

<220>
<223> HER3诱变引物(Fw)

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<210> 38
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[0064]

<220>
<223> HER3诱变引物(Rev)

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[0067]

Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
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Ser Gln Ala Val Cys Pro Gly Thr Leu Asn Gly Leu Ser Val Thr Gly
35 40 45

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu

130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

[0068] Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Tyr Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn

355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

[0069] Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

Gly Ser Asp Thr Cys Ala Gln Cys Ala His Phe Arg Asp Gly Pro His

580 585 590

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

[0070] Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

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

805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

[0071] Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala

1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

[0072]

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser

1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

[0073] Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

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<220>
<223> HER3诱变引物(Rev)

<400> 42
acacagtctg ccgcaaggct cgcacatctt c 31

<210> 43

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<400> 43

[0074]

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ctgttcccca aggccaacgc tcagagaaca taa 4053

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<212> PRT
<213> 人工序列

<220>
<223> Flag-HER3(G325R)

<400> 44

Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
1 5 10 15

Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys

50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

[0077]

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr

275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Arg Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

[0078] Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys

500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

[0079] Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp

725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

[0080] Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu

945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

[0081] Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His

1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

[0082] Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

<210> 45
<211> 30
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Fw)

<400> 45
aactgcatca agatcctggg aaacctggac 30

<210> 46
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Rev)

<400> 46
gatcttgatg cagttcacga agccgtcgat g 31

<210> 47
<211> 4053
<212> DNA
<213> 人工序列

<220>
<223> 编码Flag-HER3(T355I)的cDNA

[0083] <400> 47
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tacaaggatg acgacgataa gtctgaagtg ggcaacagcc aggctgtgtg tcccggaaca 120

ctgaacggct tgtctgtgac aggcgacgcc gagaaccagt accagacact gtacaagctg 180

tacgagagat gcgaggtggt catgggcaac ctggaaatcg tgctgacagg ccacaacgcc 240

gacctgtctt tcctgcagtg gatcagagaa gtgaccggct acgtgctggt ggccatgaac 300

gagttcagca ccctgcctct gcctaacctg agagtcgtca gaggcaccca ggtgtacgat 360

ggcaagttcg ccatcttcgt gatgctgaac tacaacacca acagctctca cgccctgaga 420

cagctgagac tgacccagct gaccgagatc ctgtctggcg gagtgtacat cgagaagaac 480

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gagatcgtgg tcaaggacaa cggcagaagc tgccctcctt gccacgaagt gtgcaagggc 600

agatgttggg gccctggctc tgaggactgt cagaccctga ccaagaccat ctgcgccccc 660

cagtgtaacg gccactgctt cggccctaat cctaaccagt gctgccacga cgaatgcgct 720

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ggcgcctgcg tgccaagatg tcctcagcct ctggtgtaca acaagctgac ctttcagctg 840

[0084]

gaacccaatc ctcacaccaa gtaccagtac ggcggcgtgt gtgtggccag ctgtcctcac 900

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gacaagaacg gcctgaagat gtgcgagcct tgcggcggac tgtgtcctaa ggcttgtgaa 1020

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aactgcatca agatcctggg aaacctggac ttcctgatca ccggcctgaa cggcgatccc 1140

tggcataaga tccctgctct ggaccccgag aagctgaacg tgttcagaac cgtgcgcgag 1200

atcacaggct acctgaacat ccagagctgg cctccacaca tgcacaactt cagcgtgttc 1260

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aacaagaggg ccatgcggag atacctggaa agaggcgaga gcatcgagcc actggaccct 2100

agcgagaagg ccaacaaggt gctggccaga atcttcaaag agacagagct gcggaagctg 2160

aaggtgctcg gatctggcgt gttcggcaca gtgcacaaag gcgtgtggat ccctgagggc 2220

gagtccatca agatccccgt gtgcatcaaa gtgatcgagg acaagtccgg caggcagagc 2280

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gacgtgtggt cttacggcgt gacagtgtgg gagctgatga ccttcggagc cgagccttac 2760

gctggactga gactggctga agtgcccgac ctgctggaaa agggcgaaag acttgcccag 2820

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cctagatacc tcgtgatcaa gagagagagc ggccctggca ttgctcctgg acctgaacct 3000

cacggactga ccaacaagaa gctggaagag gtcgaactgg aacccgagct ggacctggat 3060

ctcgacctgg aagccgaaga ggataacctg gccaccacaa cactgggctc tgctctgagt 3120

ctgcctgtgg gcacactgaa cagaccaaga ggaagccaga gcctgctgtc tccaagcagc 3180

ggctacatgc ctatgaacca gggcaatctg ggcgagagct gtcaagagtc tgccgtgtct 3240

ggcagcagcg agagatgtcc tagacctgtg tctctgcacc ccatgcctag aggctgtctg 3300

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[0085] tctatgtgca gaagccgcag cagaagcaga agccctagac ctagaggcga cagcgcctac 3420

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gaggacgagg aatacgagta catgaacaga agaaggcggc acagccctcc acatcctcca 3660

agacctagca gcctcgagga actgggctac gagtatatgg acgtgggcag cgatctgagc 3720

gctagcctgg gatctacaca gtcttgccca ctgcaccctg tgcctatcat gcctacagcc 3780

ggcaccacac cagacgagga ctatgagtat atgaatcggc agagagatgg cggcggacct 3840

ggcggagatt atgctgctat gggagcctgt cctgctagcg agcagggcta cgaagagatg 3900

agagcctttc aaggccctgg ccatcaggct cctcacgtgc actatgccag actgaaaacc 3960

ctgagatctc tggaagccac cgactccgcc ttcgacaacc ctgactactg gcacagcaga 4020

ctgttcccca aggccaacgc tcagagaaca taa 4053

<210> 48
<211> 1350
<212> PRT

〈213〉 人工序列

〈220〉
〈223〉 Flag-HER3(T355I)

〈400〉 48

Met Arg Ala Asn Asp Ala Leu Gln Val Leu Gly Leu Leu Phe Ser Leu
1 5 10 15

Ala Arg Gly Asp Tyr Lys Asp Asp Asp Asp Lys Ser Glu Val Gly Asn
20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

[0086] Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu

195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

[0087]

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Ile Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr

420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

[0088] Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val

645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

[0089] Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys

865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

[0090] Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly

1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

[0091]

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His

1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

<210> 49
<211> 31
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Fw)

<400> 49
tctcctatcc aggtgcaggt cgccgatttc g 31

[0092]

<210> 50
<211> 32
<212> DNA
<213> 人工序列

<220>
<223> HER3诱变引物(Rev)

<400> 50
cacctggata ggagacttga gcagcacgtt tc 32

<210> 51
<211> 4053
<212> DNA
<213> 人工序列

<220>
<223> 编码Flag-HER3(S846I)的cDNA

<400> 51
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ctgaacggct tgtctgtgac aggcgacgcc gagaaccagt accagacact gtacaagctg 180

tacgagagat gcgaggtggt catgggcaac ctggaaatcg tgctgacagg ccacaacgcc 240

[0093]

gacctgtctt tcctgcagtg gatcagagaa gtgaccggct acgtgctggt ggccatgaac 300

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ggcaagttcg ccatcttcgt gatgctgaac tacaacacca acagctctca cgccctgaga 420

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<400> 52

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met

115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

[0096] His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp

340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

[0097] Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser

565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

[0098] Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu

785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ile Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

[0099] Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu

1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

[0100]

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met

1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

[0101]

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
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Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
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<223> HER3诱变引物(Rev)

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35 40 45

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala
65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

[0105] Thr Gln Leu Thr Glu Ile Leu Ser Gly Gly Val Tyr Ile Glu Lys Asn
145 150 155 160

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val

260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val
290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

[0106] Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg

485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys
515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

[0107] Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu

705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile
740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

[0108] Gln Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Gly Arg Leu Ala Gln Pro Gln Ile Cys

930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met
965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

[0109]

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
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Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly

1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val
1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

[0110] Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
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caccagagtg atgtgtggag ttatggtgtg actgtgtggg agctgatgac ttttggggcc 2760

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tattactggg accaggaccc accagagcgg ggggctccac ccagcacctt caaagggaca 3720

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Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
35 40 45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
50 55 60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65 70 75 80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
85 90 95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
100 105 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
115 120 125

[0114] Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
130 135 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145 150 155 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
165 170 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
180 185 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
195 200 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
210 215 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225 230 235 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu

245 250 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
260 265 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
275 280 285

Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
290 295 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305 310 315 320

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
325 330 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
340 345 350

[0115] Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
355 360 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
370 375 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385 390 395 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
405 410 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
420 425 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
435 440 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
450 455 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val

465 470 475 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
485 490 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
500 505 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
515 520 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
530 535 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545 550 555 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
565 570 575

[0116] Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
580 585 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
595 600 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
610 615 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625 630 635 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
645 650 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
660 665 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
675 680 685

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

690 695 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705 710 715 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
725 730 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
740 745 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
755 760 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
770 775 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785 790 795 800

[0117] Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
805 810 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
820 825 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
835 840 845

Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
850 855 860

Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865 870 875 880

Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
885 890 895

Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
900 905 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala

915 920 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
930 935 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945 950 955 960

Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
965 970 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
980 985 990

Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
995 1000 1005

Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr
1010 1015 1020

[0118]

Leu Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly
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Ala Gly Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg
1040 1045 1050

Ser Gly Gly Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu
1055 1060 1065

Glu Ala Pro Arg Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser
1070 1075 1080

Asp Val Phe Asp Gly Asp Leu Gly Met Gly Ala Ala Lys Gly Leu
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Gln Ser Leu Pro Thr His Asp Pro Ser Pro Leu Gln Arg Tyr Ser
1100 1105 1110

Glu Asp Pro Thr Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val
1115 1120 1125

Ala Pro Leu Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro

1130 1135 1140

Asp Val Arg Pro Gln Pro Pro Ser Pro Arg Glu Gly Pro Leu Pro
1145 1150 1155

Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu Arg Pro Lys Thr Leu
1160 1165 1170

Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val Phe Ala Phe Gly
1175 1180 1185

Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln Gly Gly Ala
1190 1195 1200

Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala Phe Asp
1205 1210 1215

Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala Pro
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[0119] Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
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Leu Gly Leu Asp Val Pro Val
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[0120] <210> 65
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<223> HER3诱变引物(Rev)

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tgcgctcagt gcgcccactt tagagatggc cctcactgcg tgtcctcttg tcctcatggt 1800

gtcctgggcg ctaagggccc catctacaag taccctgacg tgcagaacga gtgcaggccc 1860

tgccacgaga actgtacaca gggatgcaag ggccctgagc tgcaggattg tctgggacag 1920

acactggtgc tgatcggcaa gacccacctg acaatggccc tgacagtgat cgctggcctg 1980

gtggtcatct ttatgatgct cggcggcacc ttcctgtatt ggagaggcag aagaatccag 2040

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agcgagaagg ccaacaaggt gctggccaga atcttcaaag agacagagct gcggaagctg 2160

aaggtgctcg gatctggcgt gttcggcaca gtgcacaaag gcgtgtggat ccctgagggc 2220

gagtccatca agatccccgt gtgcatcaaa gtgatcgagg acaagtccgg caggcagagc 2280

ttccaggccg tgacagatca catgctggct atcggcagcc tggatcacgc ccacatcgtt 2340

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ggatctctgc tggaccacgt gcgacaacat agaggcgctc tgggacctag actgctgctg 2460

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gacgtgtggt cttacggcgt gacagtgtgg gagctgatga ccttcggagc cgagccttac 2760

gctggactga gactggctga agtgcccgac ctgctggaaa agggcgaaag acttgcccag 2820

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cctagatacc tcgtgatcaa gagagagagc ggccctggca ttgctcctgg acctgaacct 3000

cacggactga ccaacaagaa gctggaagag gtcgaactgg aacccgagct ggacctggat 3060

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ggctacatgc ctatgaacca gggcaatctg ggcgagagct gtcaagagtc tgccgtgtct 3240

ggcagcagcg agagatgtcc tagacctgtg tctctgcacc ccatgcctag aggctgtctg 3300

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[0123]

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20 25 30

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

Asp Ala Glu Asn Gln Tyr Gln Thr Leu Tyr Lys Leu Tyr Glu Arg Cys
50 55 60

Glu Val Val Met Gly Asn Leu Glu Ile Val Leu Thr Gly His Asn Ala

65 70 75 80

Asp Leu Ser Phe Leu Gln Trp Ile Arg Glu Val Thr Gly Tyr Val Leu
85 90 95

Val Ala Met Asn Glu Phe Ser Thr Leu Pro Leu Pro Asn Leu Arg Val
100 105 110

Val Arg Gly Thr Gln Val Tyr Asp Gly Lys Phe Ala Ile Phe Val Met
115 120 125

Leu Asn Tyr Asn Thr Asn Ser Ser His Ala Leu Arg Gln Leu Arg Leu
130 135 140

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

Asp Lys Leu Cys His Met Asp Thr Ile Asp Trp Arg Asp Ile Val Arg
165 170 175

[0124] Asp Arg Asp Ala Glu Ile Val Val Lys Asp Asn Gly Arg Ser Cys Pro
180 185 190

Pro Cys His Glu Val Cys Lys Gly Arg Cys Trp Gly Pro Gly Ser Glu
195 200 205

Asp Cys Gln Thr Leu Thr Lys Thr Ile Cys Ala Pro Gln Cys Asn Gly
210 215 220

His Cys Phe Gly Pro Asn Pro Asn Gln Cys Cys His Asp Glu Cys Ala
225 230 235 240

Gly Gly Cys Ser Gly Pro Gln Asp Thr Asp Cys Phe Ala Cys Arg His
245 250 255

Phe Asn Asp Ser Gly Ala Cys Val Pro Arg Cys Pro Gln Pro Leu Val
260 265 270

Tyr Asn Lys Leu Thr Phe Gln Leu Glu Pro Asn Pro His Thr Lys Tyr
275 280 285

Gln Tyr Gly Gly Val Cys Val Ala Ser Cys Pro His Asn Phe Val Val

290 295 300

Asp Gln Thr Ser Cys Val Arg Ala Cys Pro Pro Asp Lys Met Glu Val
305 310 315 320

Asp Lys Asn Gly Leu Lys Met Cys Glu Pro Cys Gly Gly Leu Cys Pro
325 330 335

Lys Ala Cys Glu Gly Thr Gly Ser Gly Ser Arg Phe Gln Thr Val Asp
340 345 350

Ser Ser Asn Ile Asp Gly Phe Val Asn Cys Thr Lys Ile Leu Gly Asn
355 360 365

Leu Asp Phe Leu Ile Thr Gly Leu Asn Gly Asp Pro Trp His Lys Ile
370 375 380

Pro Ala Leu Asp Pro Glu Lys Leu Asn Val Phe Arg Thr Val Arg Glu
385 390 395 400

[0125] Ile Thr Gly Tyr Leu Asn Ile Gln Ser Trp Pro Pro His Met His Asn
405 410 415

Phe Ser Val Phe Ser Asn Leu Thr Thr Ile Gly Gly Arg Ser Leu Tyr
420 425 430

Asn Arg Gly Phe Ser Leu Leu Ile Met Lys Asn Leu Asn Val Thr Ser
435 440 445

Leu Gly Phe Arg Ser Leu Lys Glu Ile Ser Ala Gly Arg Ile Tyr Ile
450 455 460

Ser Ala Asn Arg Gln Leu Cys Tyr His His Ser Leu Asn Trp Thr Lys
465 470 475 480

Val Leu Arg Gly Pro Thr Glu Glu Arg Leu Asp Ile Lys His Asn Arg
485 490 495

Pro Arg Arg Asp Cys Val Ala Glu Gly Lys Val Cys Asp Pro Leu Cys
500 505 510

Ser Ser Gly Gly Cys Trp Gly Pro Gly Pro Gly Gln Cys Leu Ser Cys

515 520 525

Arg Asn Tyr Ser Arg Gly Gly Val Cys Val Thr His Cys Asn Phe Leu
530 535 540

Asn Gly Glu Pro Arg Glu Phe Ala His Glu Ala Glu Cys Phe Ser Cys
545 550 555 560

His Pro Glu Cys Gln Pro Met Glu Gly Thr Ala Thr Cys Asn Gly Ser
565 570 575

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

Cys Val Ser Ser Cys Pro His Gly Val Leu Gly Ala Lys Gly Pro Ile
595 600 605

Tyr Lys Tyr Pro Asp Val Gln Asn Glu Cys Arg Pro Cys His Glu Asn
610 615 620

[0126] Cys Thr Gln Gly Cys Lys Gly Pro Glu Leu Gln Asp Cys Leu Gly Gln
625 630 635 640

Thr Leu Val Leu Ile Gly Lys Thr His Leu Thr Met Ala Leu Thr Val
645 650 655

Ile Ala Gly Leu Val Val Ile Phe Met Met Leu Gly Gly Thr Phe Leu
660 665 670

Tyr Trp Arg Gly Arg Arg Ile Gln Asn Lys Arg Ala Met Arg Arg Tyr
675 680 685

Leu Glu Arg Gly Glu Ser Ile Glu Pro Leu Asp Pro Ser Glu Lys Ala
690 695 700

Asn Lys Val Leu Ala Arg Ile Phe Lys Glu Thr Glu Leu Arg Lys Leu
705 710 715 720

Lys Val Leu Gly Ser Gly Val Phe Gly Thr Val His Lys Gly Val Trp
725 730 735

Ile Pro Glu Gly Glu Ser Ile Lys Ile Pro Val Cys Ile Lys Val Ile

740 745 750

Glu Asp Lys Ser Gly Arg Gln Ser Phe Gln Ala Val Thr Asp His Met
755 760 765

Leu Ala Ile Gly Ser Leu Asp His Ala His Ile Val Arg Leu Leu Gly
770 775 780

Leu Cys Pro Gly Ser Ser Leu Gln Leu Val Thr Gln Tyr Leu Pro Leu
785 790 795 800

Gly Ser Leu Leu Asp His Val Arg Gln His Arg Gly Ala Leu Gly Pro
805 810 815

Arg Leu Leu Leu Asn Trp Gly Val Gln Ile Ala Lys Gly Met Tyr Tyr
820 825 830

Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala Arg Asn Val
835 840 845

[0127] Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly Val Ala
850 855 860

Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala Lys
865 870 875 880

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser Ile His Phe Gly Lys Tyr
885 890 895

Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp Glu Leu
900 905 910

Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
915 920 925

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys
930 935 940

Thr Ile Asp Val Tyr Met Val Met Val Lys Cys Trp Met Ile Asp Glu
945 950 955 960

Asn Ile Arg Pro Thr Phe Lys Glu Leu Ala Asn Glu Phe Thr Arg Met

965 970 975

Ala Arg Asp Pro Pro Arg Tyr Leu Val Ile Lys Arg Glu Ser Gly Pro
980 985 990

Gly Ile Ala Pro Gly Pro Glu Pro His Gly Leu Thr Asn Lys Lys Leu
995 1000 1005

Glu Glu Val Glu Leu Glu Pro Glu Leu Asp Leu Asp Leu Asp Leu
1010 1015 1020

Glu Ala Glu Glu Asp Asn Leu Ala Thr Thr Thr Leu Gly Ser Ala
1025 1030 1035

Leu Ser Leu Pro Val Gly Thr Leu Asn Arg Pro Arg Gly Ser Gln
1040 1045 1050

Ser Leu Leu Ser Pro Ser Ser Gly Tyr Met Pro Met Asn Gln Gly
1055 1060 1065

[0128]

Asn Leu Gly Glu Ser Cys Gln Glu Ser Ala Val Ser Gly Ser Ser
1070 1075 1080

Glu Arg Cys Pro Arg Pro Val Ser Leu His Pro Met Pro Arg Gly
1085 1090 1095

Cys Leu Ala Ser Glu Ser Ser Glu Gly His Val Thr Gly Ser Glu
1100 1105 1110

Ala Glu Leu Gln Glu Lys Val Ser Met Cys Arg Ser Arg Ser Arg
1115 1120 1125

Ser Arg Ser Pro Arg Pro Arg Gly Asp Ser Ala Tyr His Ser Gln
1130 1135 1140

Arg His Ser Leu Leu Thr Pro Val Thr Pro Leu Ser Pro Pro Gly
1145 1150 1155

Leu Glu Glu Glu Asp Val Asn Gly Tyr Val Met Pro Asp Thr His
1160 1165 1170

Leu Lys Gly Thr Pro Ser Ser Arg Glu Gly Thr Leu Ser Ser Val

1175 1180 1185

Gly Leu Ser Ser Val Leu Gly Thr Glu Glu Glu Asp Glu Asp Glu
1190 1195 1200

Glu Tyr Glu Tyr Met Asn Arg Arg Arg Arg His Ser Pro Pro His
1205 1210 1215

Pro Pro Arg Pro Ser Ser Leu Glu Glu Leu Gly Tyr Glu Tyr Met
1220 1225 1230

Asp Val Gly Ser Asp Leu Ser Ala Ser Leu Gly Ser Thr Gln Ser
1235 1240 1245

Cys Pro Leu His Pro Val Pro Ile Met Pro Thr Ala Gly Thr Thr
1250 1255 1260

Pro Asp Glu Asp Tyr Glu Tyr Met Asn Arg Gln Arg Asp Gly Gly
1265 1270 1275

[0129]

Gly Pro Gly Gly Asp Tyr Ala Ala Met Gly Ala Cys Pro Ala Ser
1280 1285 1290

Glu Gln Gly Tyr Glu Glu Met Arg Ala Phe Gln Gly Pro Gly His
1295 1300 1305

Gln Ala Pro His Val His Tyr Ala Arg Leu Lys Thr Leu Arg Ser
1310 1315 1320

Leu Glu Ala Thr Asp Ser Ala Phe Asp Asn Pro Asp Tyr Trp His
1325 1330 1335

Ser Arg Leu Phe Pro Lys Ala Asn Ala Gln Arg Thr
1340 1345 1350

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Leu Asn Gly Leu Ser Val Thr Gly Asp Ala Glu Asn Gln Tyr Gln Thr
35 40 45

Leu Tyr Lys Leu Tyr Glu Arg Cys Glu Val Val Met Gly Asn Leu Glu
50 55 60

Ile Val Leu Thr Gly His Asn Ala Asp Leu Ser Phe Leu Gln Trp Ile
65 70 75 80

Arg Glu Val Thr Gly Tyr Val Leu Val Ala Met Asn Glu Phe Ser Thr
85 90 95

Leu Pro Leu Pro Asn Leu Arg Val Val Arg Gly Thr Gln Val Tyr Asp
100 105 110

Gly Lys Phe Ala Ile Phe Val Met Leu Asn Tyr Asn Thr Asn Ser Ser
115 120 125

[0130]

His Ala Leu Arg Gln Leu Arg Leu Thr Gln Leu Thr Glu Ile Leu Ser
130 135 140

Gly Gly Val Tyr Ile Glu Lys Asn Asp Lys Leu Cys His Met Asp Thr
145 150 155 160

Ile Asp Trp Arg Asp Ile Val Arg Asp Arg Asp Ala Glu Ile Val Val
165 170 175

Lys Asp Asn Gly Arg Ser Cys Pro Pro Cys His Glu Val Cys Lys Gly
180 185 190

Arg Cys Trp Gly Pro Gly Ser Glu Asp Cys Gln Thr Leu Thr Lys Thr
195 200 205

Ile Cys Ala Pro Gln Cys Asn Gly His Cys Phe Gly Pro Asn Pro Asn
210 215 220

Gln Cys Cys His Asp Glu Cys Ala Gly Gly Cys Ser Gly Pro Gln Asp
225 230 235 240

Thr Asp Cys Phe Ala Cys Arg His Phe Asn Asp Ser Gly Ala Cys Val
245 250 255

Pro Arg Cys Pro Gln Pro Leu Val Tyr Asn Lys Leu Thr Phe Gln Leu
260 265 270

Glu Pro Asn Pro His Thr Lys Tyr Gln Tyr Gly Gly Val Cys Val Ala
275 280 285

Ser Cys Pro His Asn Phe Val Val Asp Gln Thr Ser Cys Val Arg Ala
290 295 300

Cys Pro Pro Asp Lys Met Glu Val Asp Lys Asn Gly Leu Lys Met Cys
305 310 315 320

Glu Pro Cys Gly Gly Leu Cys Pro Lys Ala Cys Glu Gly Thr Gly Ser
325 330 335

Gly Ser Arg Phe Gln Thr Val Asp Ser Ser Asn Ile Asp Gly Phe Val
340 345 350

[0131]

Asn Cys Thr Lys Ile Leu Gly Asn Leu Asp Phe Leu Ile Thr Gly Leu
355 360 365

Asn Gly Asp Pro Trp His Lys Ile Pro Ala Leu Asp Pro Glu Lys Leu
370 375 380

Asn Val Phe Arg Thr Val Arg Glu Ile Thr Gly Tyr Leu Asn Ile Gln
385 390 395 400

Ser Trp Pro Pro His Met His Asn Phe Ser Val Phe Ser Asn Leu Thr
405 410 415

Thr Ile Gly Gly Arg Ser Leu Tyr Asn Arg Gly Phe Ser Leu Leu Ile
420 425 430

Met Lys Asn Leu Asn Val Thr Ser Leu Gly Phe Arg Ser Leu Lys Glu
435 440 445

Ile Ser Ala Gly Arg Ile Tyr Ile Ser Ala Asn Arg Gln Leu Cys Tyr
450 455 460

His His Ser Leu Asn Trp Thr Lys Val Leu Arg Gly Pro Thr Glu Glu
465 470 475 480

Arg Leu Asp Ile Lys His Asn Arg Pro Arg Arg Asp Cys Val Ala Glu
485 490 495

Gly Lys Val Cys Asp Pro Leu Cys Ser Ser Gly Gly Cys Trp Gly Pro
500 505 510

Gly Pro Gly Gln Cys Leu Ser Cys Arg Asn Tyr Ser Arg Gly Gly Val
515 520 525

Cys Val Thr His Cys Asn Phe Leu Asn Gly Glu Pro Arg Glu Phe Ala
530 535 540

His Glu Ala Glu Cys Phe Ser Cys His Pro Glu Cys Gln Pro Met Glu
545 550 555 560

Gly Thr Ala Thr Cys Asn Gly Ser Gly Ser Asp Thr Cys Ala Gln Cys
565 570 575

[0132]

Ala His Phe Arg Asp Gly Pro His Cys Val Ser Ser Cys Pro His Gly
580 585 590

Val Leu Gly Ala Lys Gly Pro Ile Tyr Lys Tyr Pro Asp Val Gln Asn
595 600 605

Glu Cys Arg Pro Cys His Glu Asn Cys Thr Gln Gly Cys Lys Gly Pro
610 615 620

Glu Leu Gln Asp Cys Leu Gly Gln Thr Leu Val Leu Ile Gly Lys Thr
625 630 635 640

His Leu Thr Met Ala Leu Thr Val Ile Ala Gly Leu Val Val Ile Phe
645 650 655

Met Met Leu Gly Gly Thr Phe Leu Tyr Trp Arg Gly Arg Arg Ile Gln
660 665 670

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

```
Pro Leu Asp Pro Ser Glu Lys Ala Asn Lys Val Leu Ala Arg Ile Phe
690                 695                 700

Lys Glu Thr Glu Leu Arg Lys Leu Lys Val Leu Gly Ser Gly Val Phe
705                 710                 715                 720

Gly Thr Val His Lys Gly Val Trp Ile Pro Glu Gly Glu Ser Ile Lys
                725                 730                 735

Ile Pro Val Cys Ile Lys Val Ile Glu Asp Lys Ser Gly Arg Gln Ser
            740                 745                 750

Phe Gln Ala Val Thr Asp His Met Leu Ala Ile Gly Ser Leu Asp His
        755                 760                 765

Ala His Ile Val Arg Leu Leu Gly Leu Cys Pro Gly Ser Ser Leu Gln
    770                 775                 780

Leu Val Thr Gln Tyr Leu Pro Leu Gly Ser Leu Leu Asp His Val Arg
785                 790                 795                 800
```

[0133]

```
Gln His Arg Gly Ala Leu Gly Pro Gln Leu Leu Leu Asn Trp Gly Val
                805                 810                 815

Gln Ile Ala Lys Gly Met Tyr Tyr Leu Glu Glu His Gly Met Val His
            820                 825                 830

Arg Asn Leu Ala Ala Arg Asn Val Leu Leu Lys Ser Pro Ser Gln Val
        835                 840                 845

Gln Val Ala Asp Phe Gly Val Ala Asp Leu Leu Pro Pro Asp Asp Lys
    850                 855                 860

Gln Leu Leu Tyr Ser Glu Ala Lys Thr Pro Ile Lys Trp Met Ala Leu
865                 870                 875                 880

Glu Ser Ile His Phe Gly Lys Tyr Thr His Gln Ser Asp Val Trp Ser
                885                 890                 895

Tyr Gly Val Thr Val Trp Glu Leu Met Thr Phe Gly Ala Glu Pro Tyr
            900                 905                 910
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Ala Gly Leu Arg Leu Ala Glu Val Pro Asp Leu Leu Glu Lys Gly Glu
        915                 920                 925

Arg Leu Ala Gln Pro Gln Ile Cys Thr Ile Asp Val Tyr Met Val Met
    930                 935                 940

Val Lys Cys Trp Met Ile Asp Glu Asn Ile Arg Pro Thr Phe Lys Glu
945                 950                 955                 960

Leu Ala Asn Glu Phe Thr Arg Met Ala Arg Asp Pro Pro Arg Tyr Leu
                965                 970                 975

Val Ile Lys Arg Glu Ser Gly Pro Gly Ile Ala Pro Gly Pro Glu Pro
            980                 985                 990

His Gly Leu Thr Asn Lys Lys Leu  Glu Glu Val Glu Leu  Glu Pro Glu
        995                 1000                1005

Leu Asp  Leu Asp Leu Asp Leu  Glu Ala Glu Glu Asp  Asn Leu Ala
    1010                1015                1020
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[0134]

```
Thr Thr  Thr Leu Gly Ser Ala  Leu Ser Leu Pro Val  Gly Thr Leu
    1025                1030                1035

Asn Arg  Pro Arg Gly Ser Gln  Ser Leu Leu Ser Pro  Ser Ser Gly
    1040                1045                1050

Tyr Met  Pro Met Asn Gln Gly  Asn Leu Gly Glu Ser  Cys Gln Glu
    1055                1060                1065

Ser Ala  Val Ser Gly Ser Ser  Glu Arg Cys Pro Arg  Pro Val Ser
    1070                1075                1080

Leu His  Pro Met Pro Arg Gly  Cys Leu Ala Ser Glu  Ser Ser Glu
    1085                1090                1095

Gly His  Val Thr Gly Ser Glu  Ala Glu Leu Gln Glu  Lys Val Ser
    1100                1105                1110

Met Cys  Arg Ser Arg Ser Arg  Ser Arg Ser Pro Arg  Pro Arg Gly
    1115                1120                1125
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Asp Ser Ala Tyr His Ser Gln Arg His Ser Leu Leu Thr Pro Val
1130 1135 1140

Thr Pro Leu Ser Pro Pro Gly Leu Glu Glu Glu Asp Val Asn Gly
1145 1150 1155

Tyr Val Met Pro Asp Thr His Leu Lys Gly Thr Pro Ser Ser Arg
1160 1165 1170

Glu Gly Thr Leu Ser Ser Val Gly Leu Ser Ser Val Leu Gly Thr
1175 1180 1185

Glu Glu Glu Asp Glu Asp Glu Glu Tyr Glu Tyr Met Asn Arg Arg
1190 1195 1200

Arg Arg His Ser Pro Pro His Pro Pro Arg Pro Ser Ser Leu Glu
1205 1210 1215

Glu Leu Gly Tyr Glu Tyr Met Asp Val Gly Ser Asp Leu Ser Ala
1220 1225 1230

[0135]

Ser Leu Gly Ser Thr Gln Ser Cys Pro Leu His Pro Val Pro Ile
1235 1240 1245

Met Pro Thr Ala Gly Thr Thr Pro Asp Glu Asp Tyr Glu Tyr Met
1250 1255 1260

Asn Arg Gln Arg Asp Gly Gly Gly Pro Gly Gly Asp Tyr Ala Ala
1265 1270 1275

Met Gly Ala Cys Pro Ala Ser Glu Gln Gly Tyr Glu Glu Met Arg
1280 1285 1290

Ala Phe Gln Gly Pro Gly His Gln Ala Pro His Val His Tyr Ala
1295 1300 1305

Arg Leu Lys Thr Leu Arg Ser Leu Glu Ala Thr Asp Ser Ala Phe
1310 1315 1320

Asp Asn Pro Asp Tyr Trp His Ser Arg Leu Phe Pro Lys Ala Asn
1325 1330 1335

[0136] Ala Gln Arg Thr
1340

SEQ ID NO: 1 - 抗HER3抗体的重链的氨基酸序列

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIR
QPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVETSKN
QFSLKLSSVTAADTAVYYCARDKWTWYFDLWGRGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

图 1

SEQ ID NO: 2 - 抗HER3抗体的轻链的氨基酸序列

DIEMTQSPDSLAVSLGERATINCRSSQSVLYSSSNRNY
LAWYQQNPGQPPKLLIYWASTRESGVPDRFSGSGSGTD
FTLTISSLQAEDVAVYYCQQYYSTPRTFGQGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

图 2



图 3

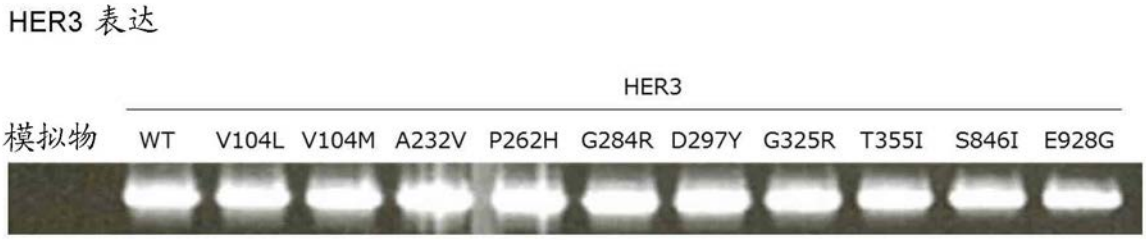

HER3 表达
HER3
模拟物
WT
V104L
V104M
A232V
P262H
G284R
D297Y
G325R
T355I
S846I
E928G

图 4

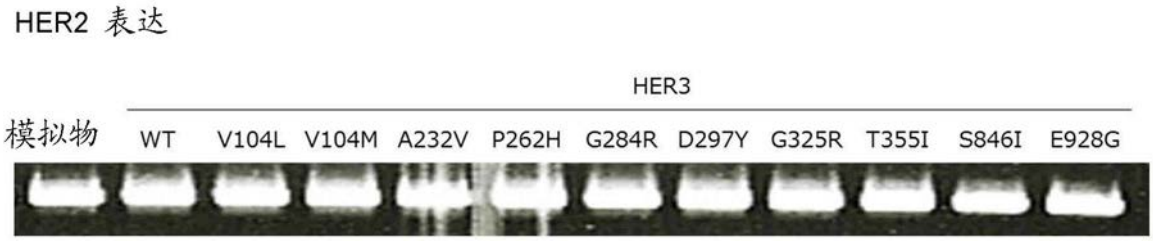

HER2 表达
HER3
模拟物
WT
V104L
V104M
A232V
P262H
G284R
D297Y
G325R
T355I
S846I
E928G

图 5

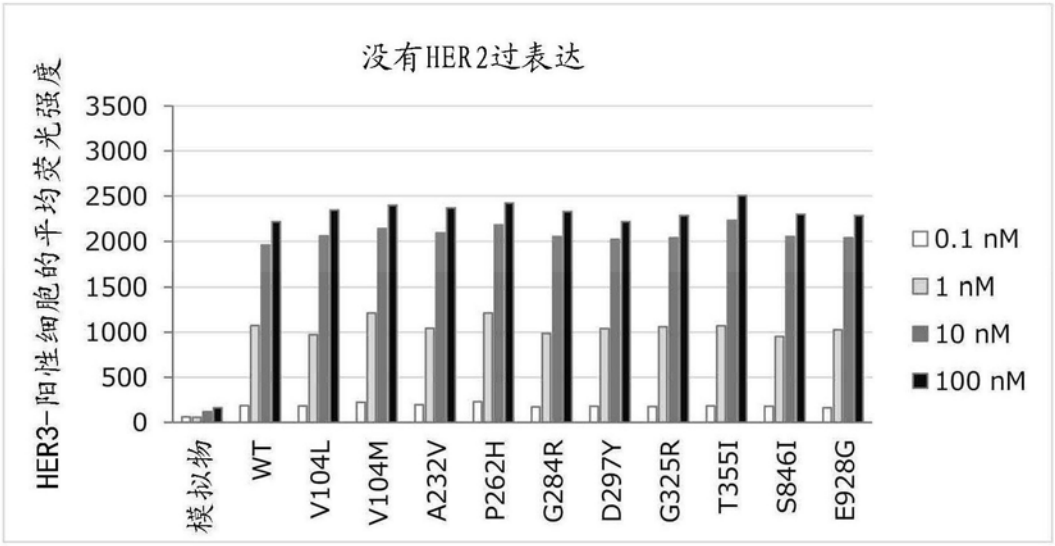

没有HER2过表达
HER3-阳性细胞的平均荧光强度
3500
3000
2500
2000
1500
1000
500
0
模拟物
WT
V104L
V104M
A232V
P262H
G284R
D297Y
G325R
T355I
S846I
E928G
0.1 nM
1 nM
10 nM
100 nM

图 6

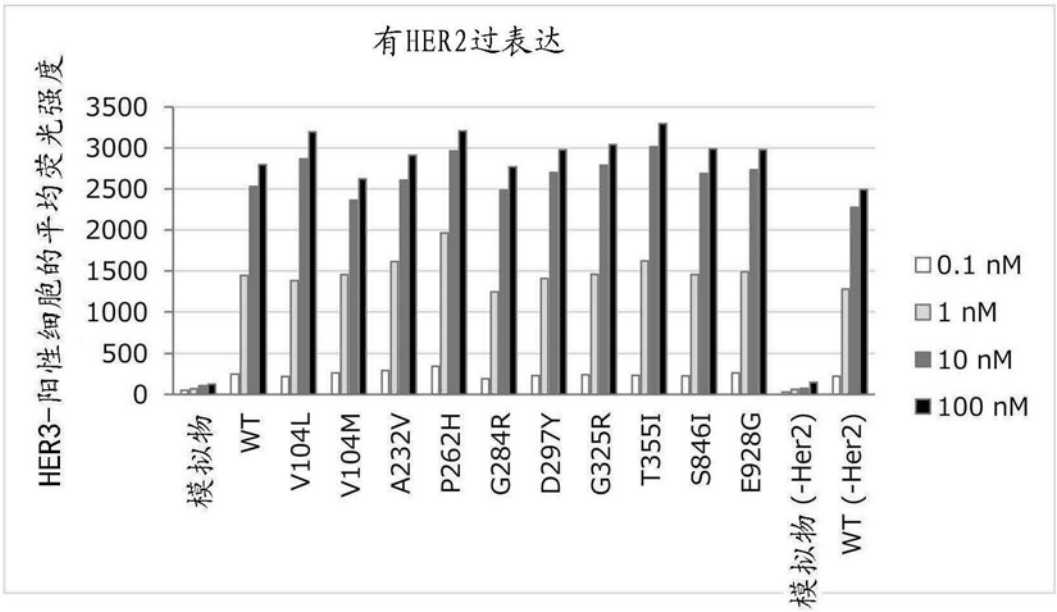

有HER2过表达
HER3-阳性细胞的平均荧光强度
3500
3000
2500
2000
1500
1000
500
0
模拟物
WT
V104L
V104M
A232V
P262H
G284R
D297Y
G325R
T355I
S846I
E928G
模拟物 (-Her2)
WT (-Her2)
0.1 nM
1 nM
10 nM
100 nM

图 7

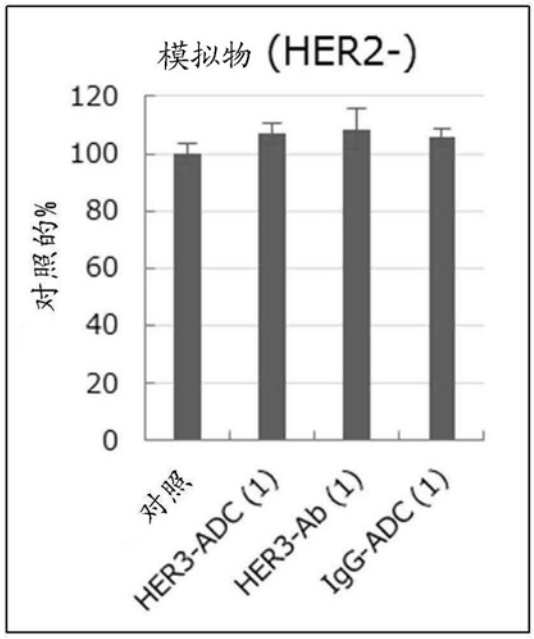

模拟物 (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 8

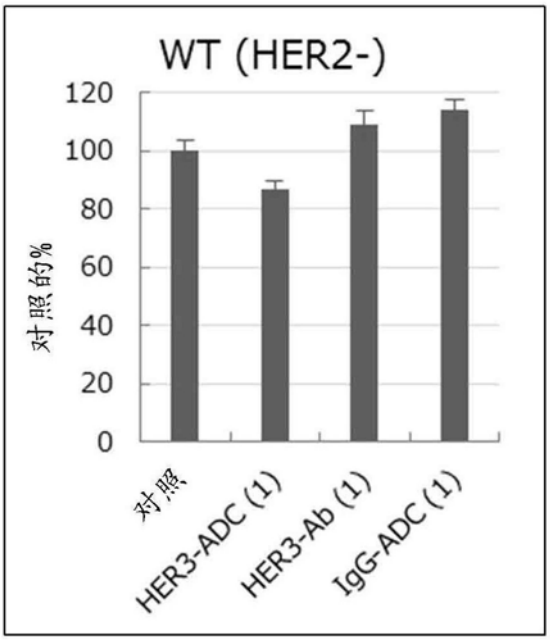

WT (HER2-)
120
100
80
60
40
20
0
对照的%
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 9

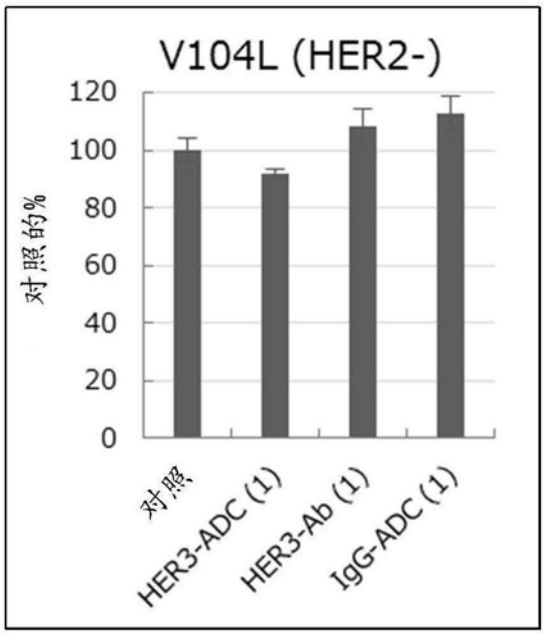

V104L (HER2-)
120
100
80
60
40
20
0
对照的%
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 10

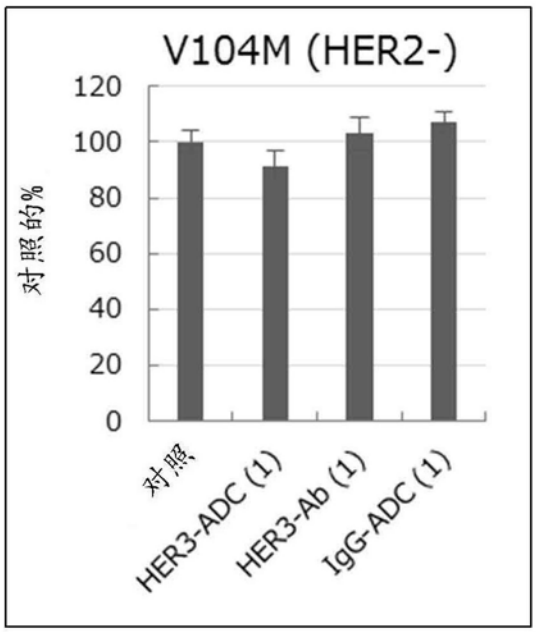

V104M (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 11

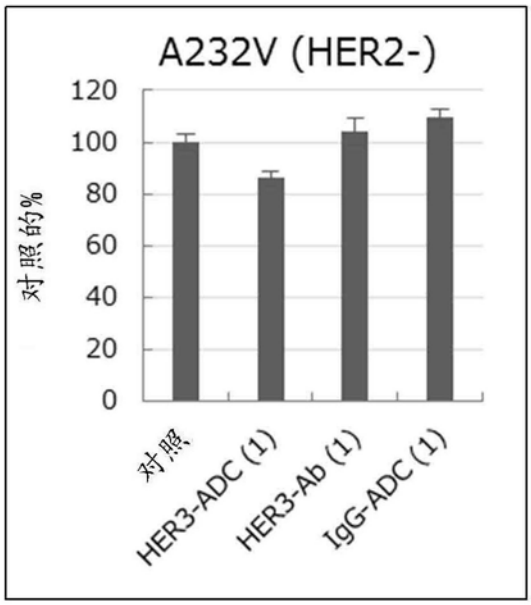

A232V (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 12

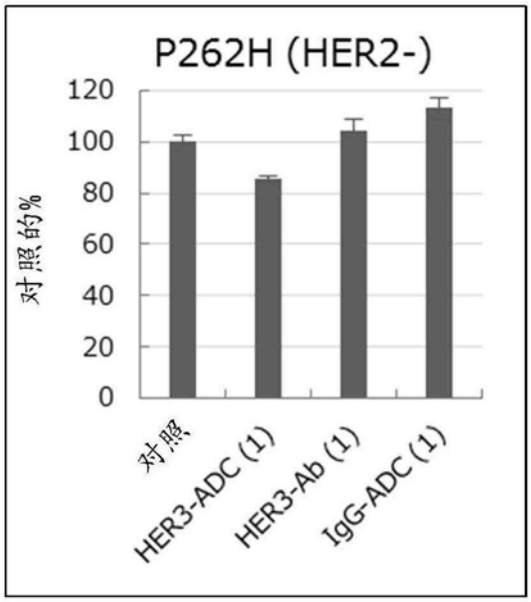

P262H (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 13

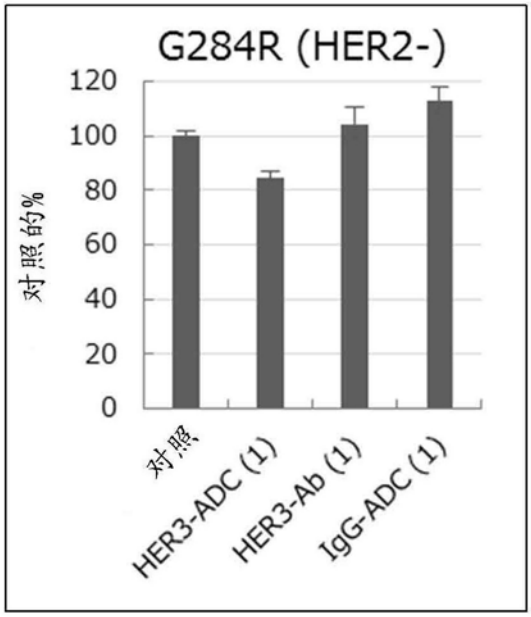

G284R (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 14

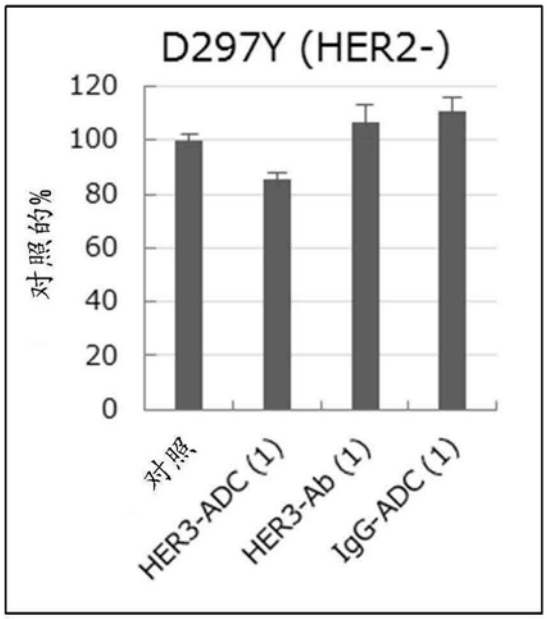

D297Y (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 15

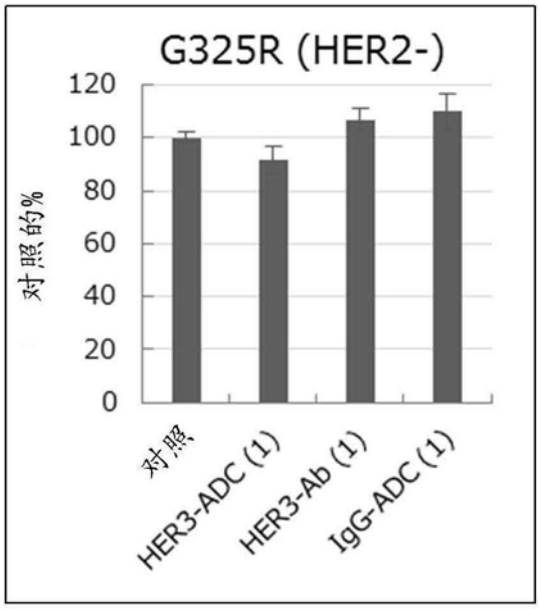

G325R (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 16

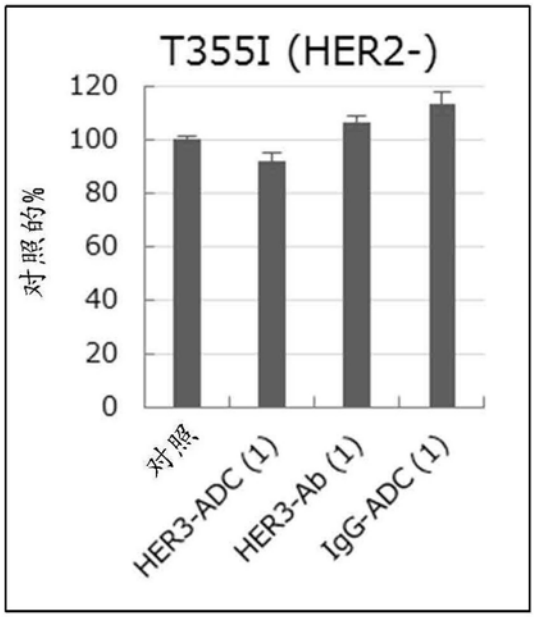

T355I (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 17

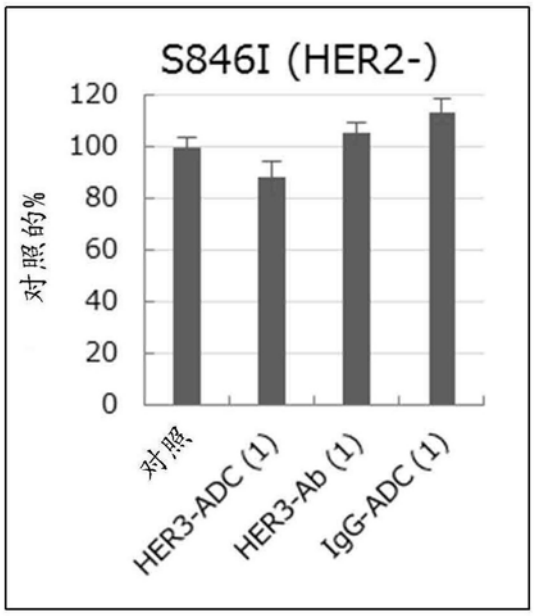

S846I (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 18

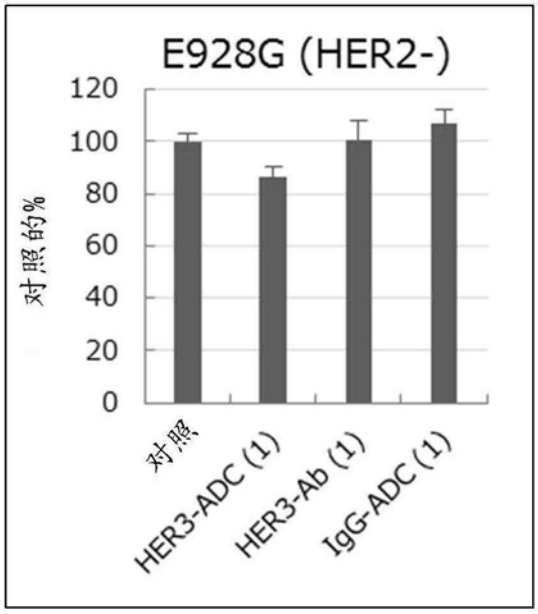

E928G (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 19

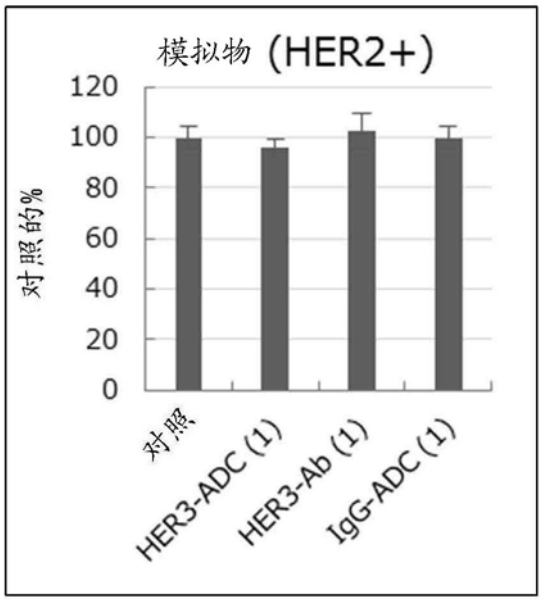

模拟物 (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 20

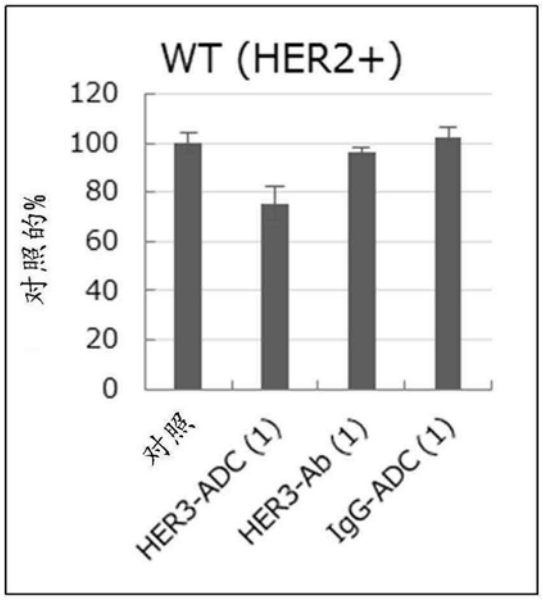

WT (HER2+)
120
100
80
60
40
20
0
对照的%
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 21

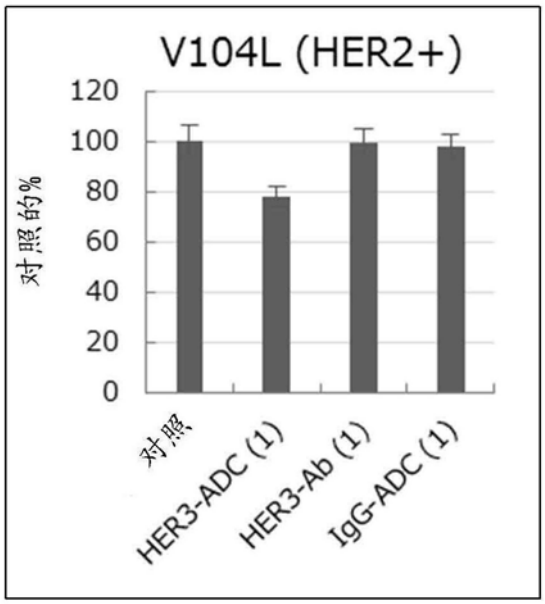

V104L (HER2+)
120
100
80
60
40
20
0
对照的%
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 22

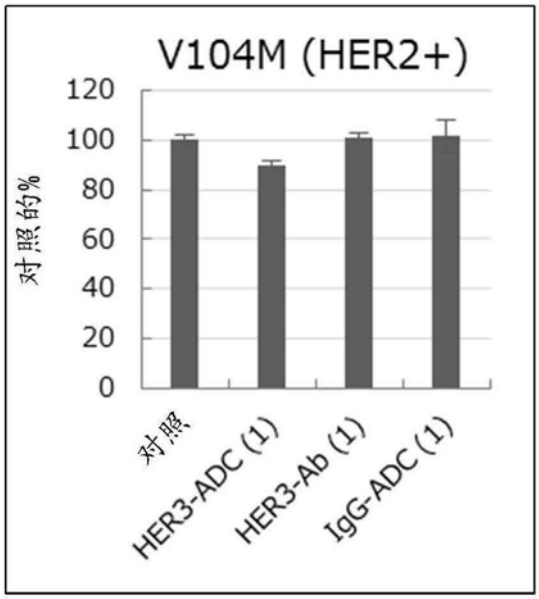

V104M (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 23

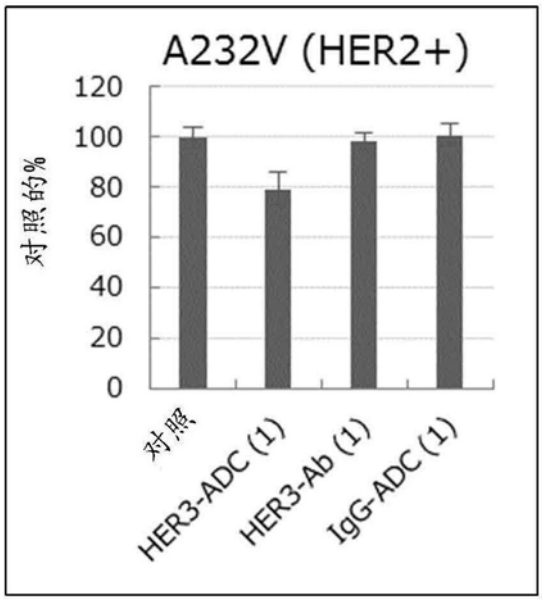

A232V (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 24

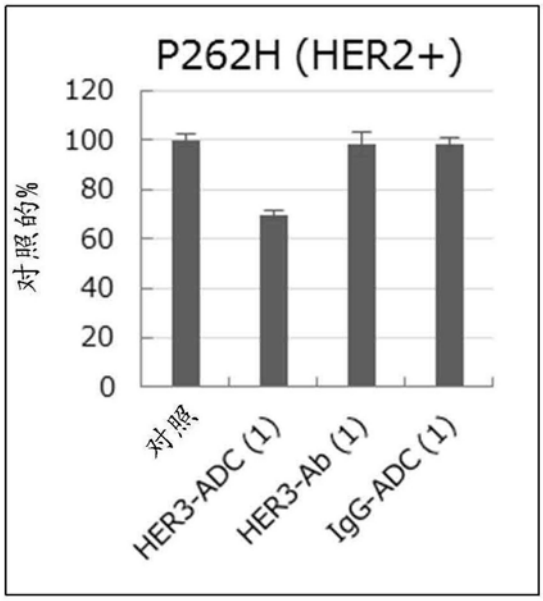

P262H (HER2+)
120
100
80
60
40
20
0
对照的%
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 25

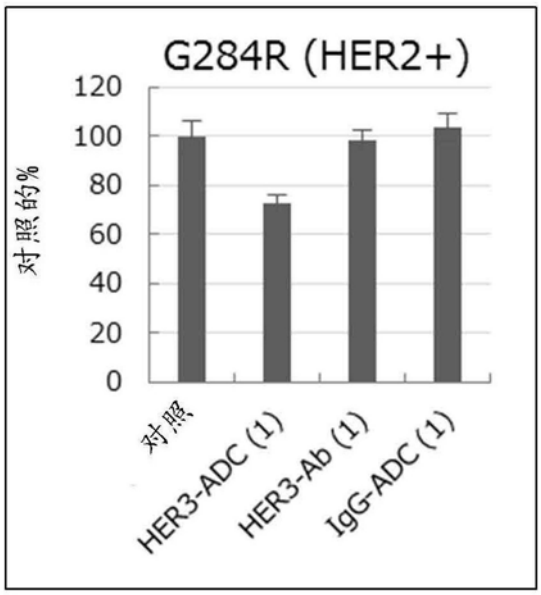

G284R (HER2+)
120
100
80
60
40
20
0
对照的%
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 26

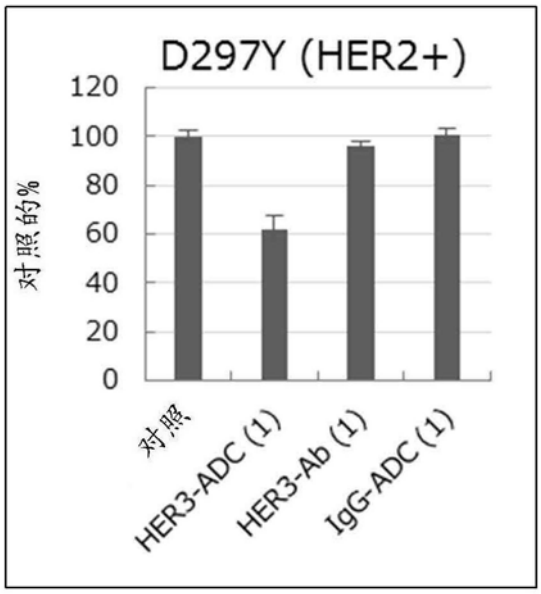

D297Y (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 27

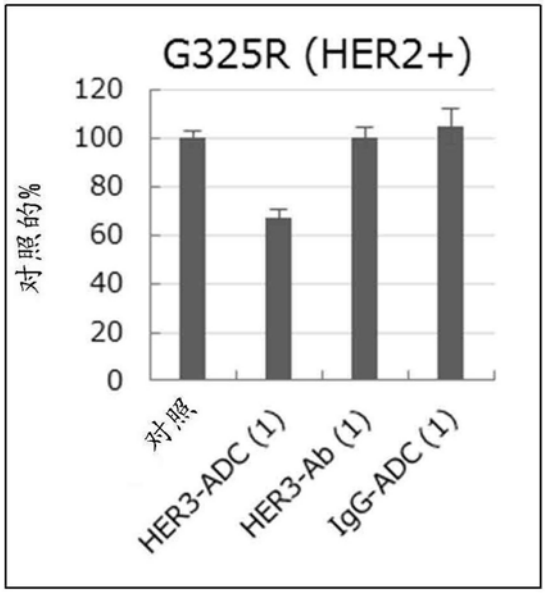

G325R (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 28

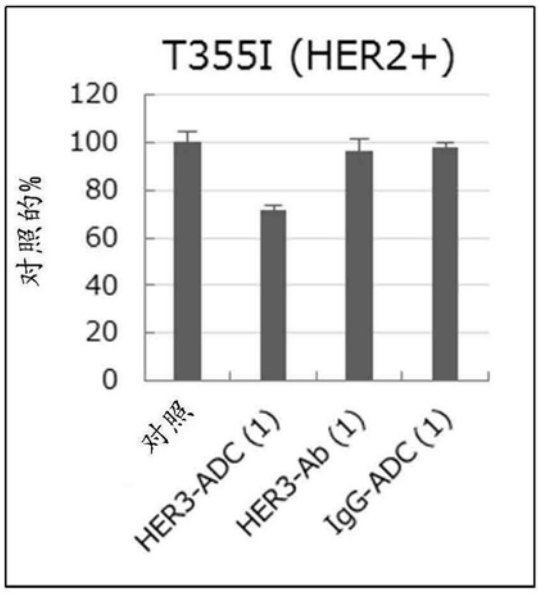

T355I (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 29

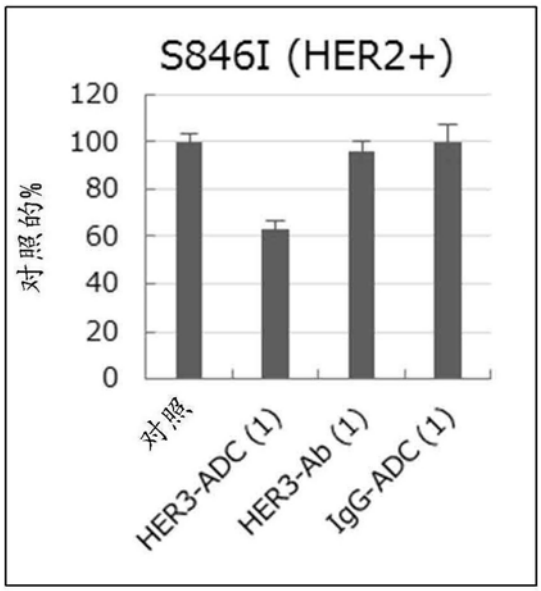

S846I (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 30

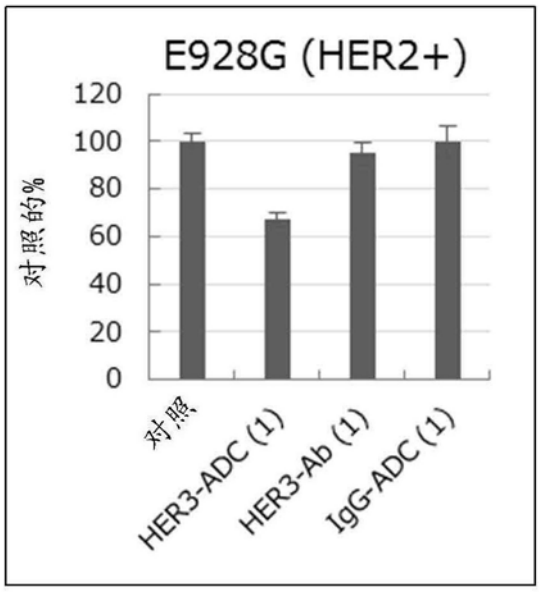

E928G (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 31

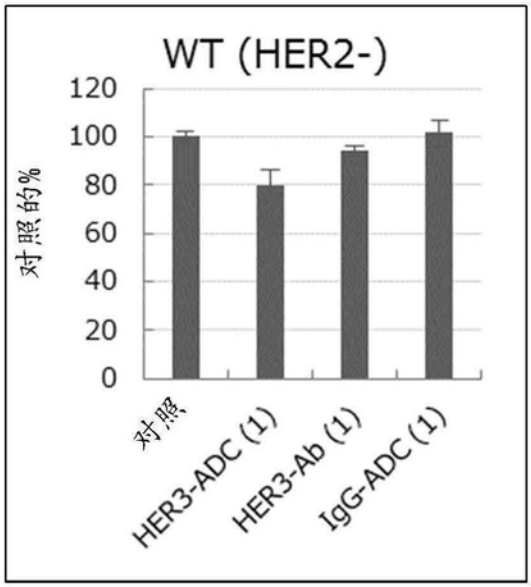

WT (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 32

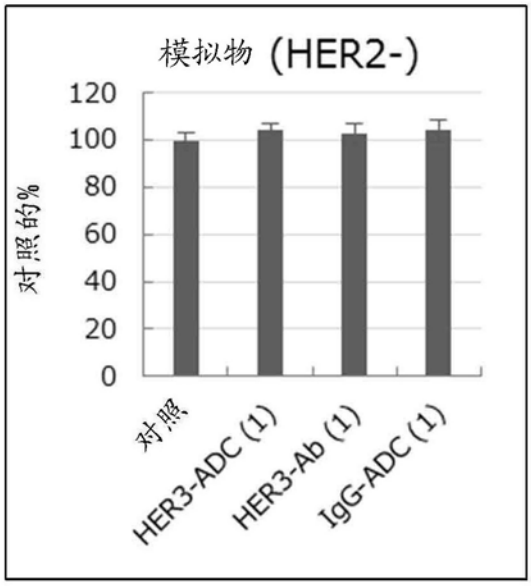

模拟物 (HER2-)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 33

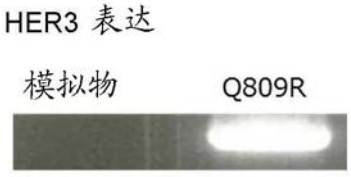

HER3 表达
模拟物
Q809R

图 34

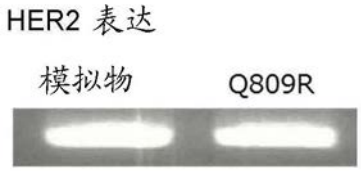

HER2 表达
模拟物
Q809R

图 35

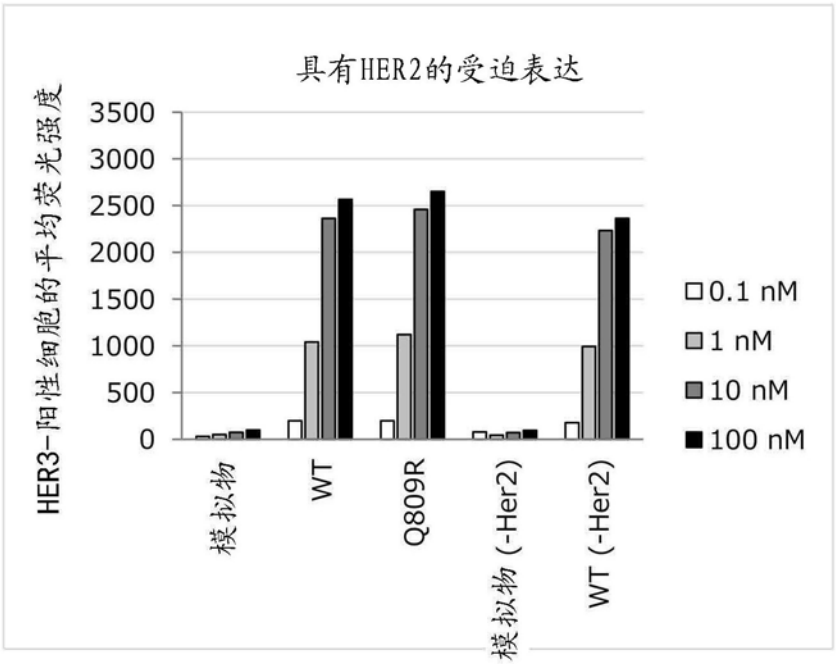

具有HER2的受迫表达
HER3-阳性细胞的平均荧光强度
3500
3000
2500
2000
1500
1000
500
0
模拟物
WT
Q809R
模拟物 (-Her2)
WT (-Her2)
0.1 nM
1 nM
10 nM
100 nM

图 36

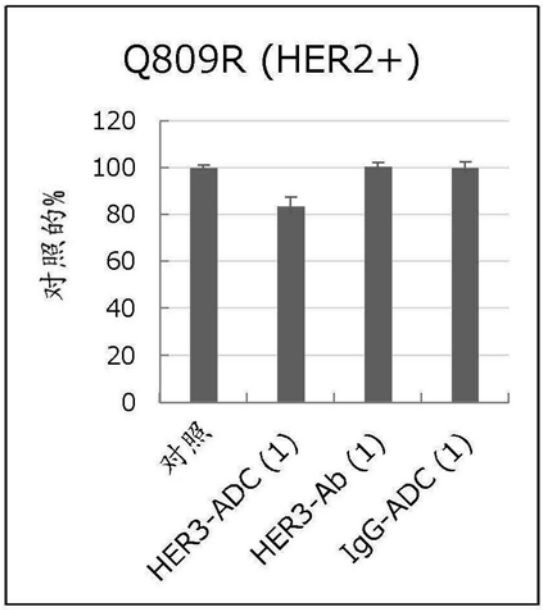

Q809R (HER2+)
对照的%
120
100
80
60
40
20
0
对照
HER3-ADC (1)
HER3-Ab (1)
IgG-ADC (1)

图 37

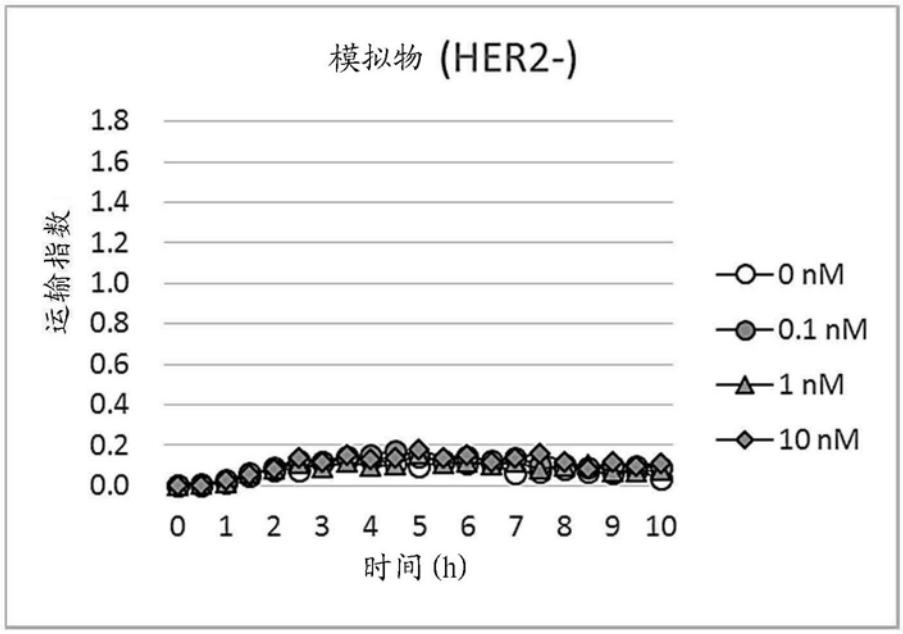

模拟物 (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 38

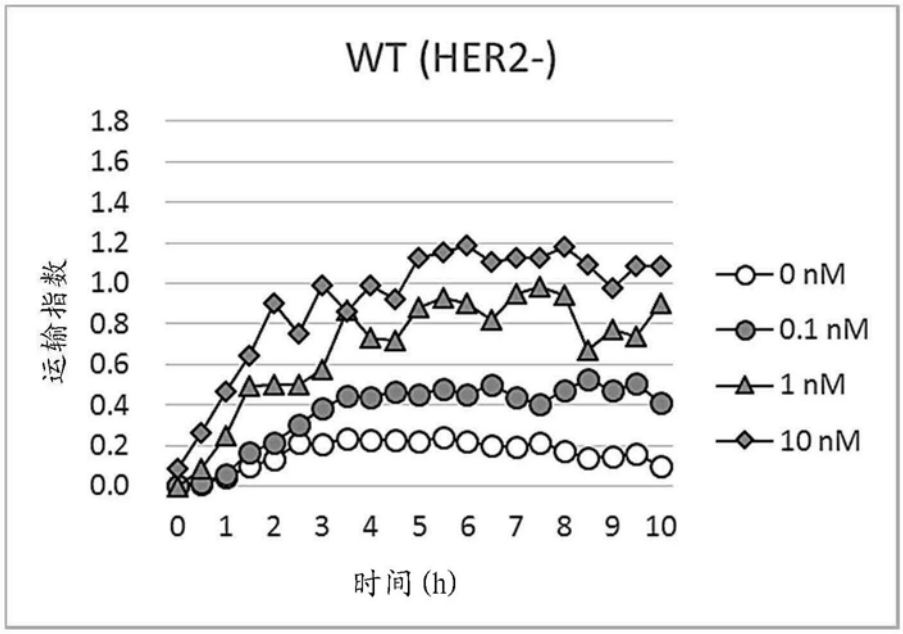

WT (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 39

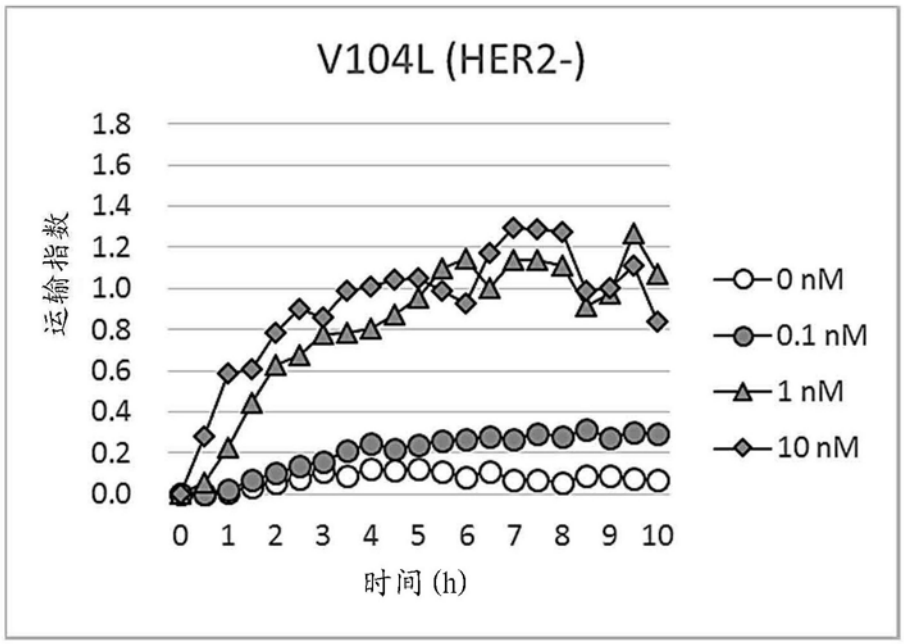

V104L (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 40

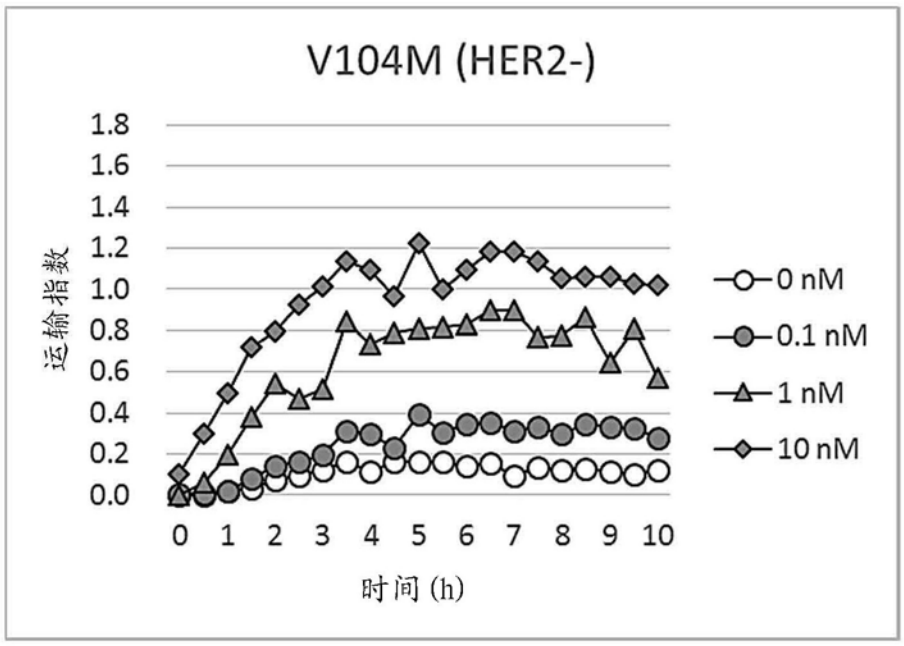

V104M (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 41

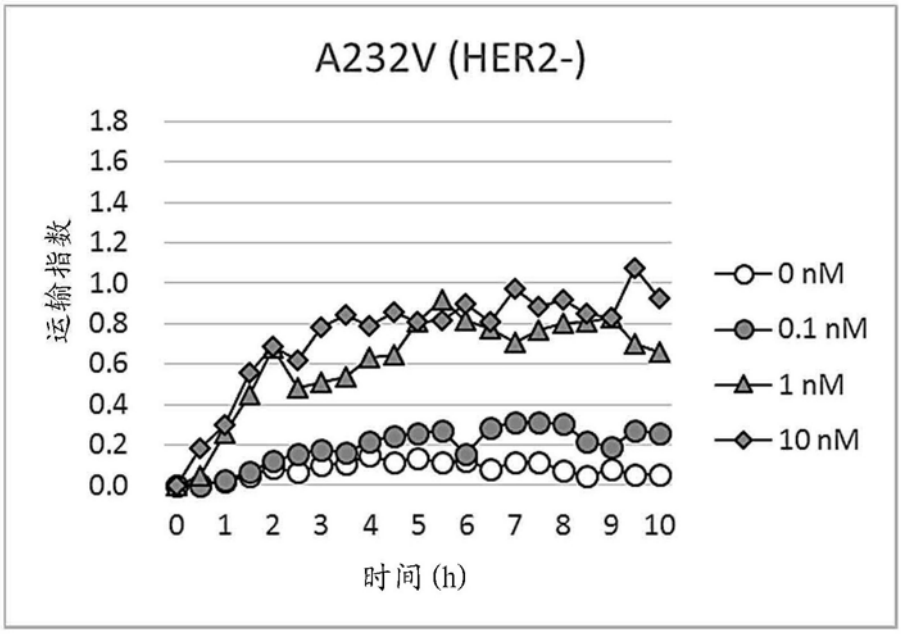

A232V (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 42

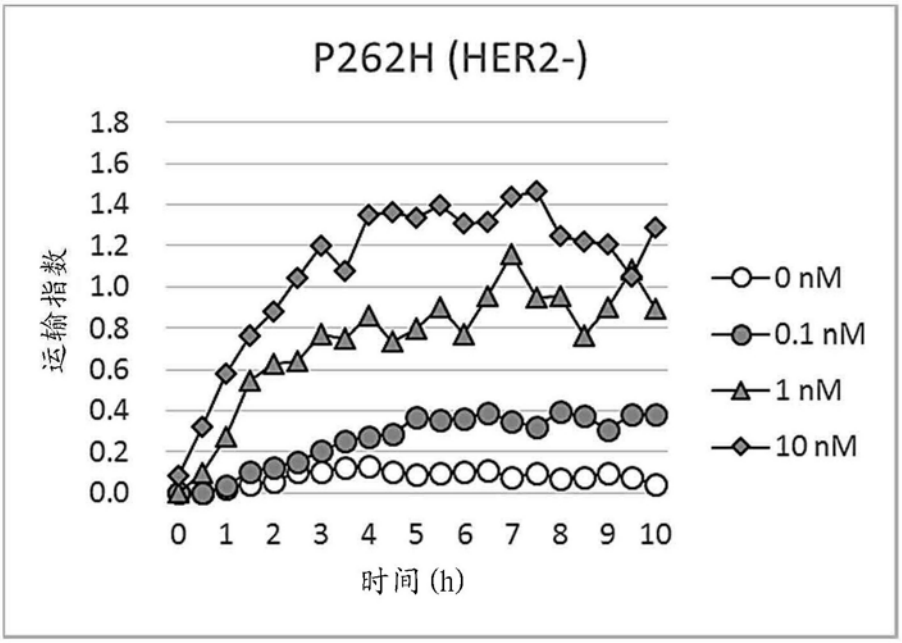

P262H (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 43

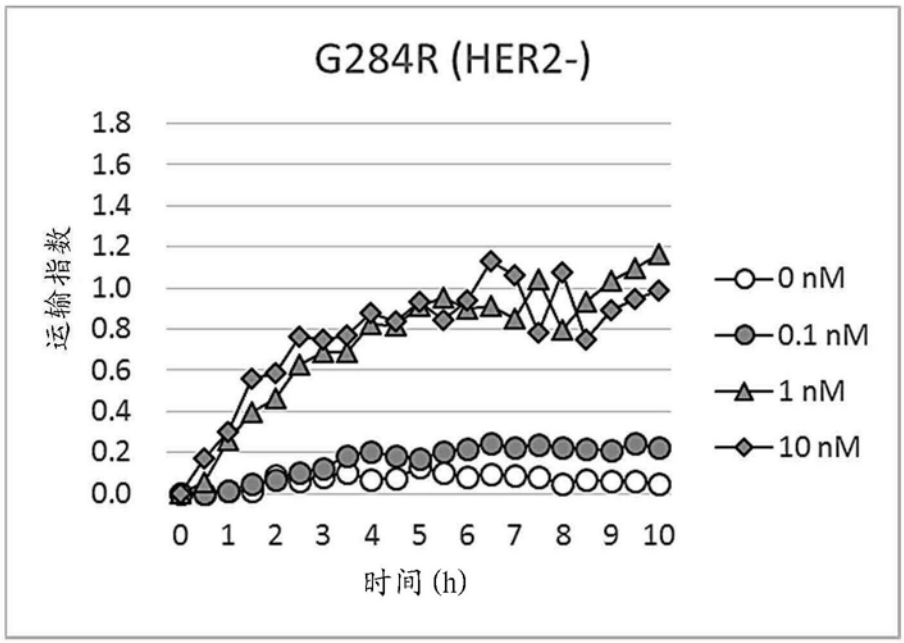

G284R (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 44

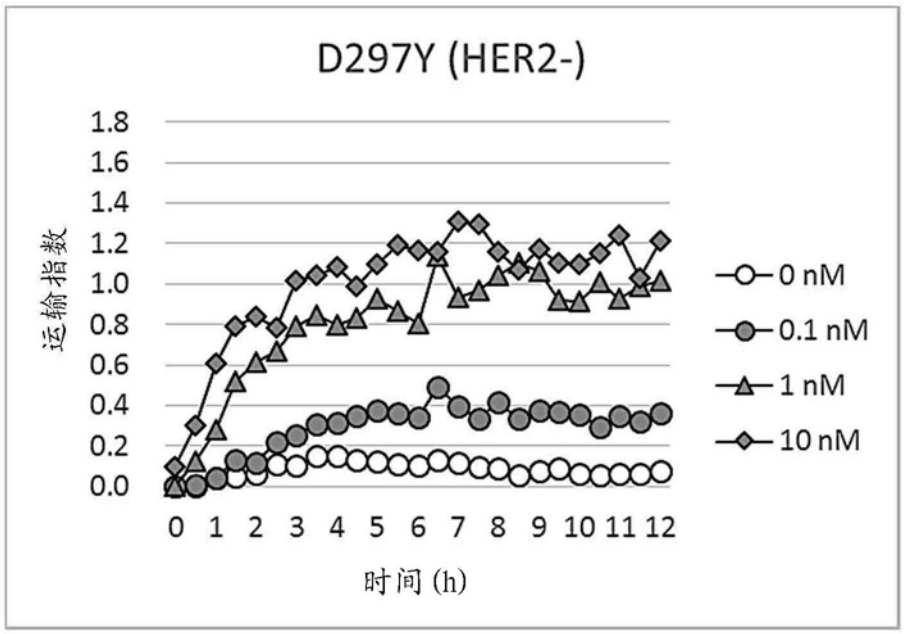

D297Y (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 45

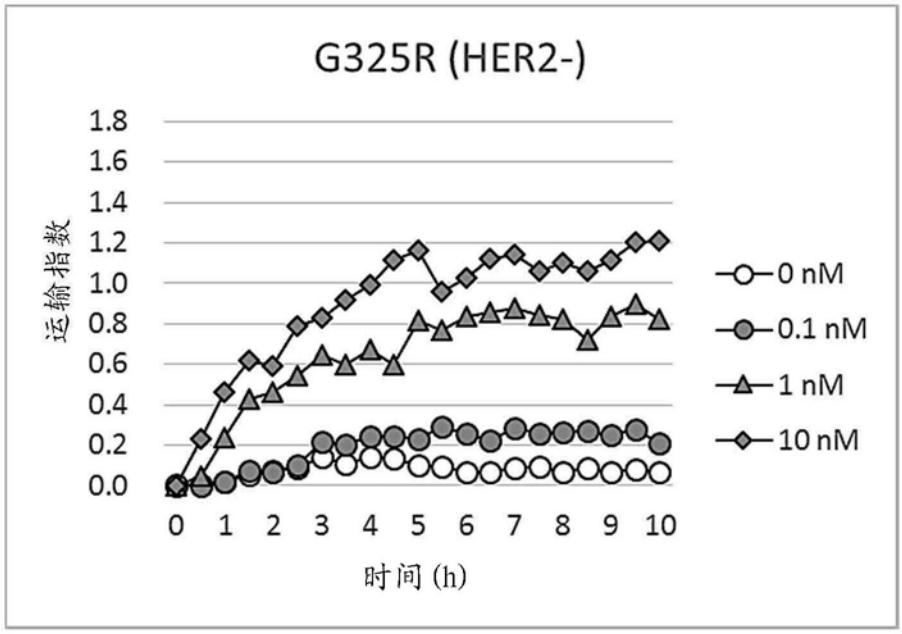

G325R (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 46

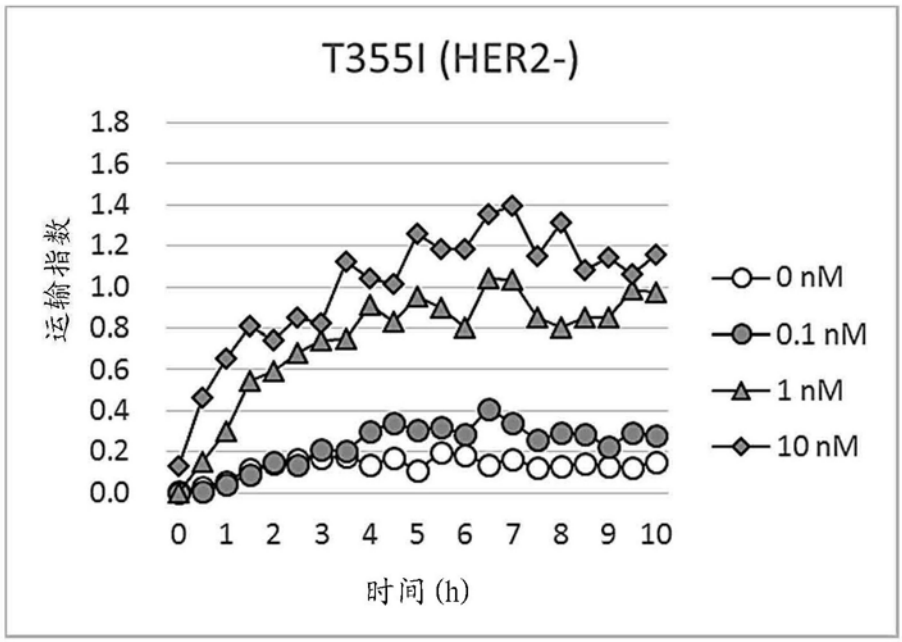

T355I (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 47

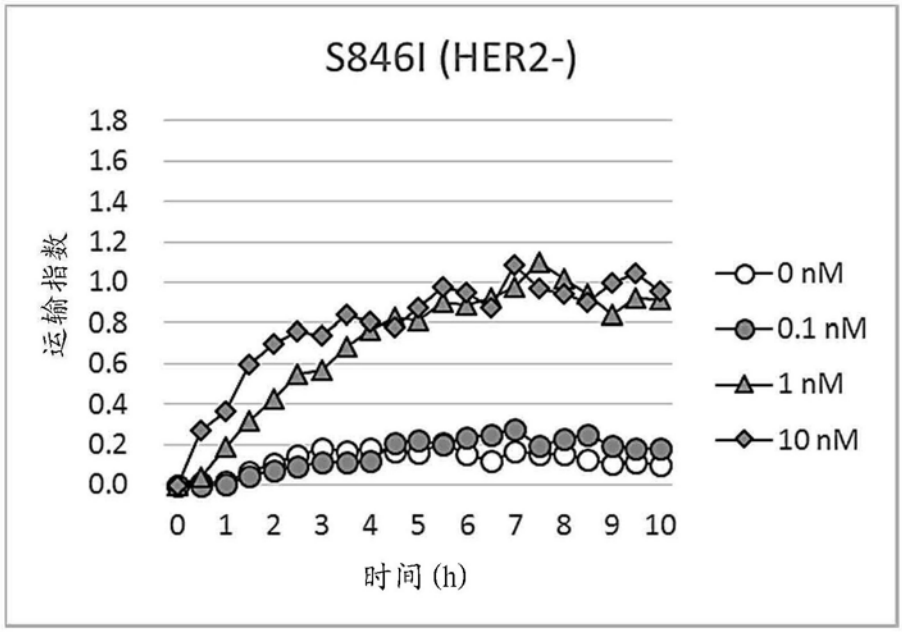

S846I (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 48

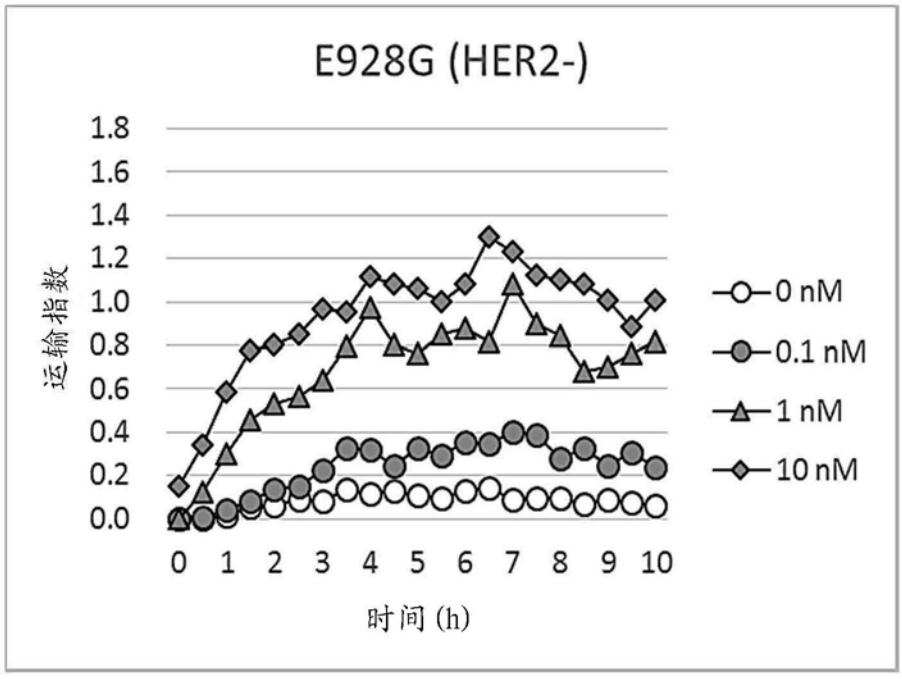

E928G (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
时间 (h)
0 nM
0.1 nM
1 nM
10 nM

图 49

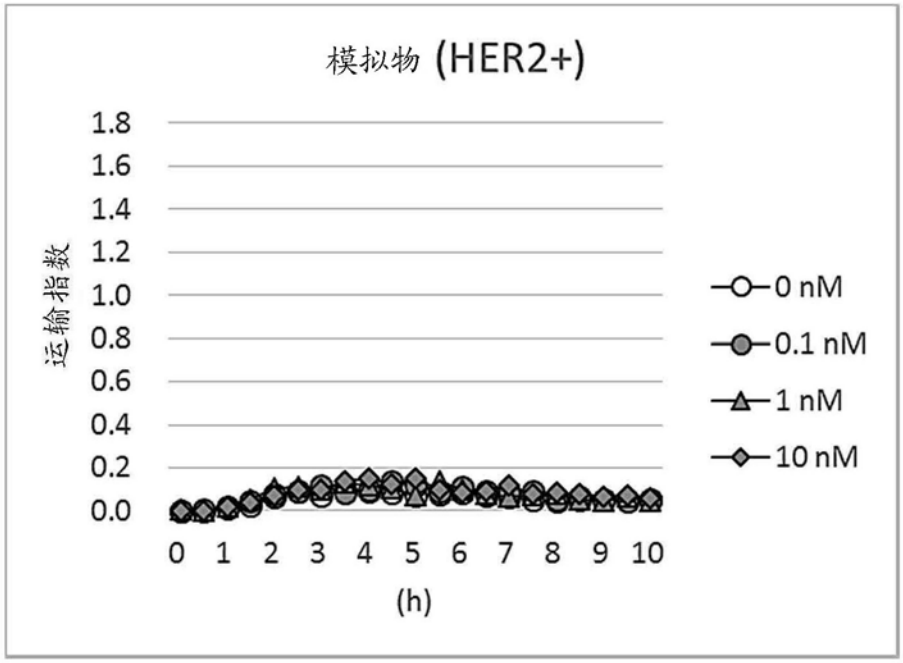

模拟物 (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 50

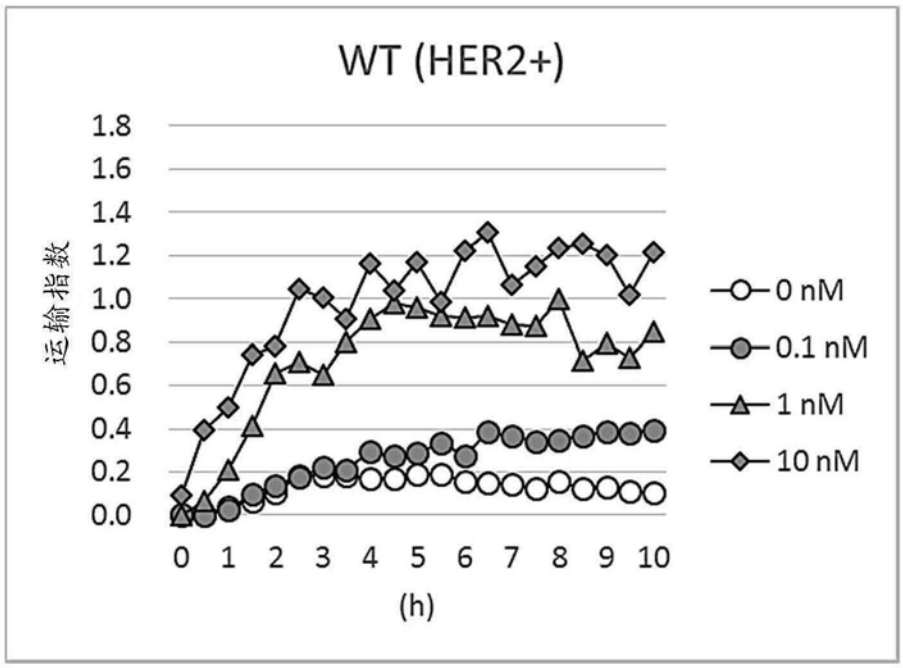

WT (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 51

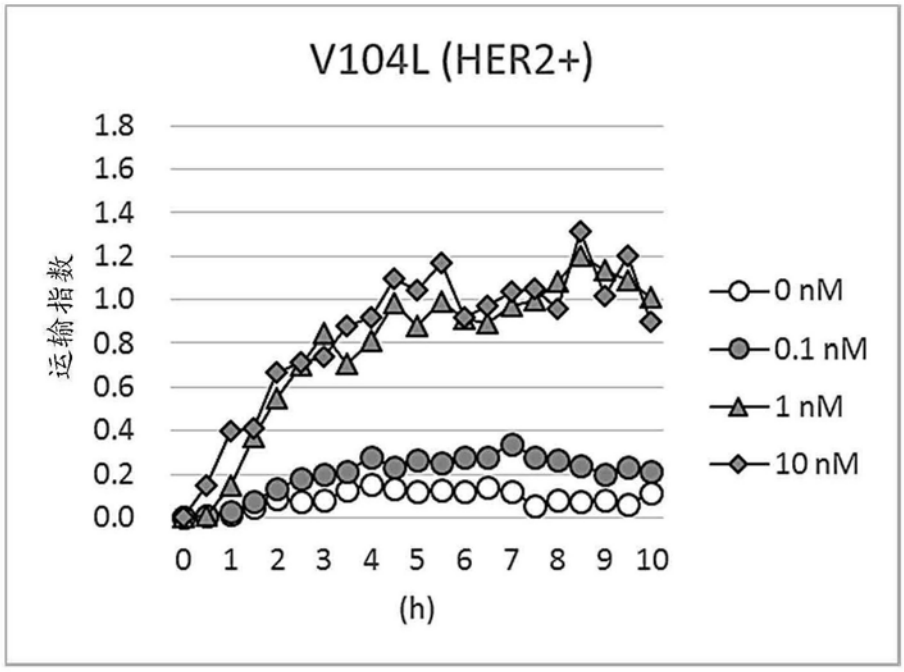

V104L (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 52

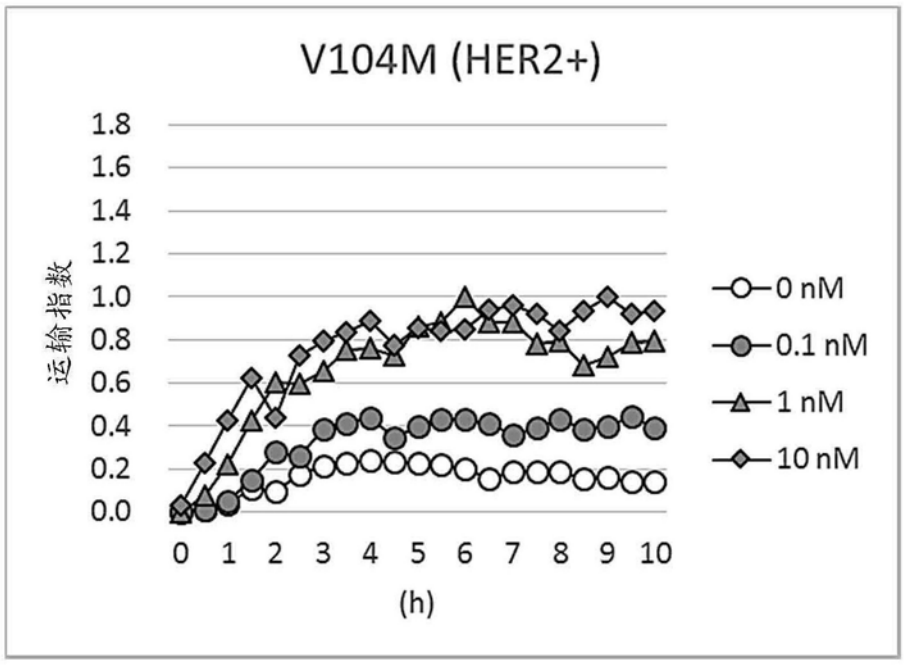

V104M (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 53

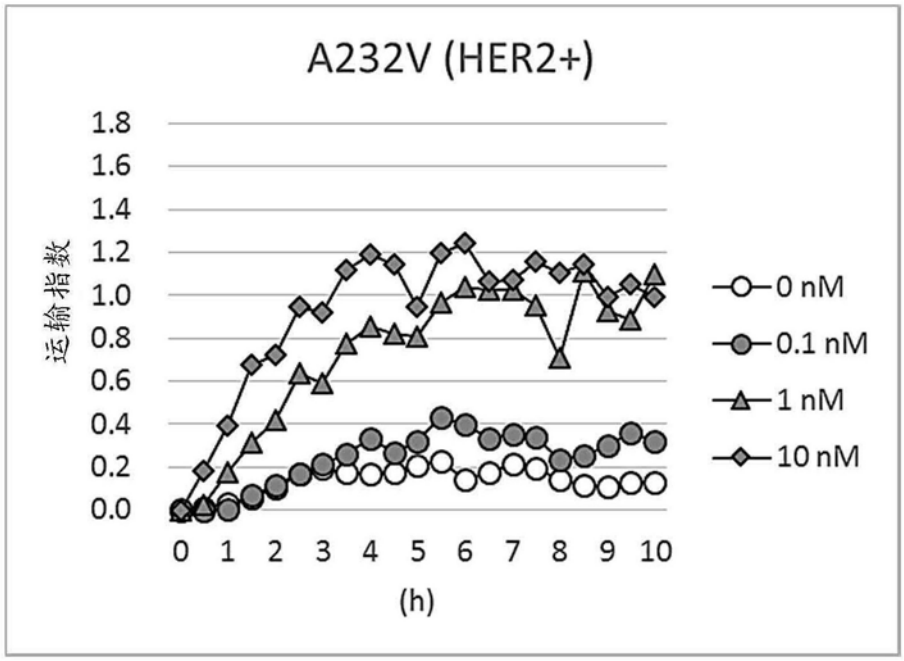

A232V (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 54

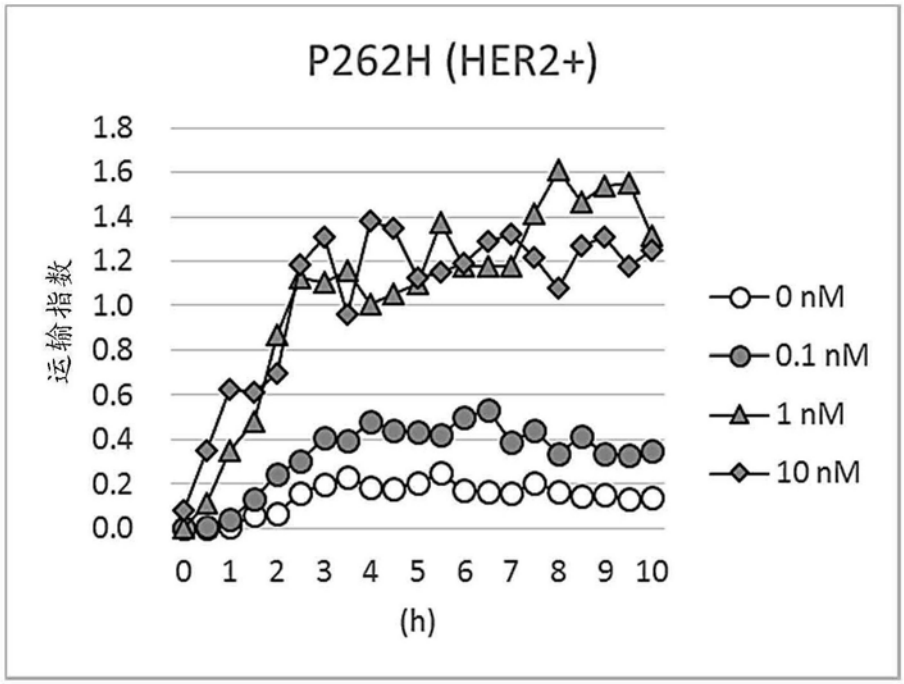

P262H (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 55

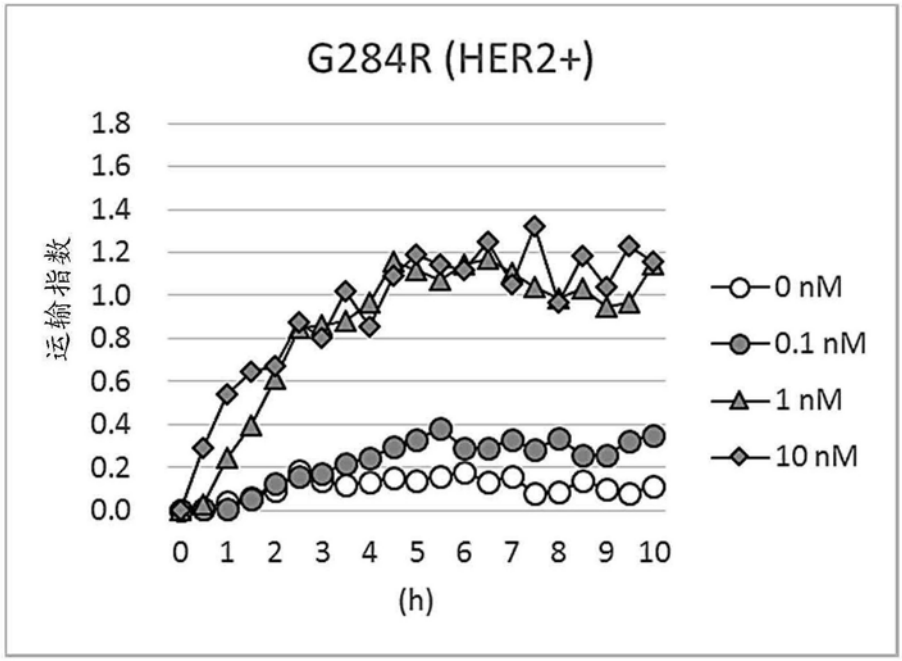

G284R (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 56

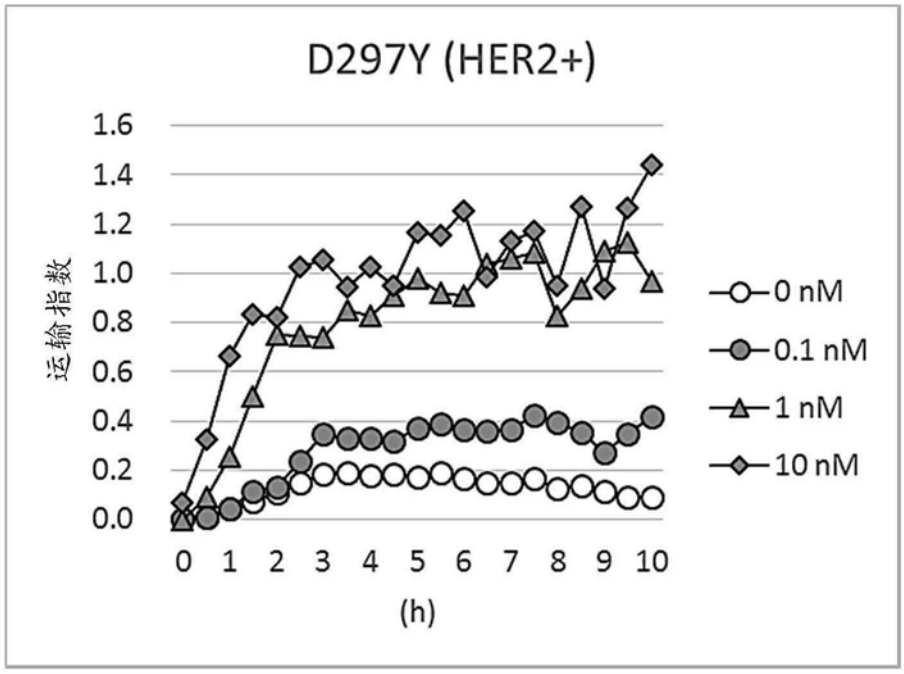

D297Y (HER2+)
运输指数
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 57

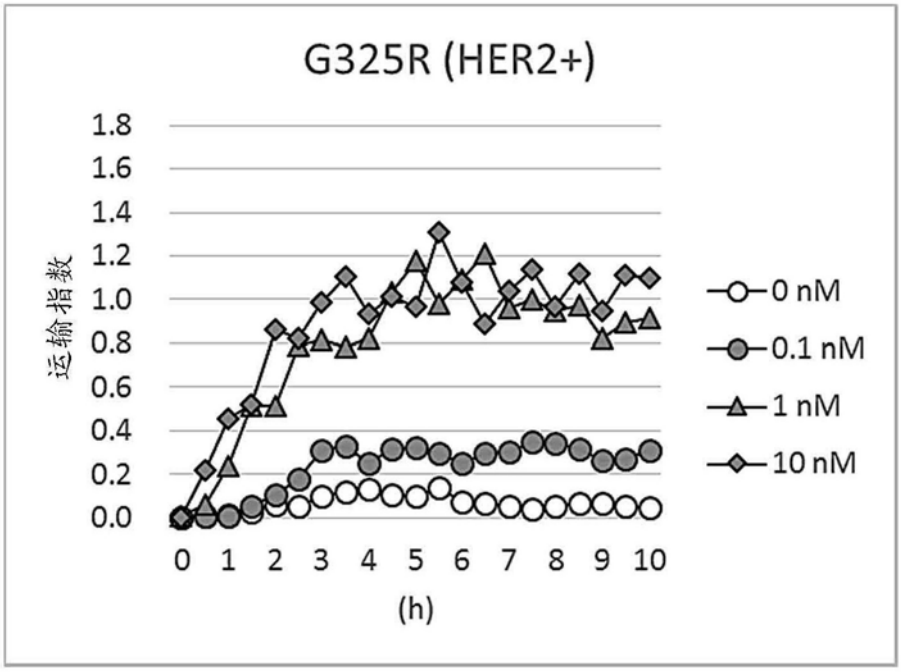

G325R (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 58

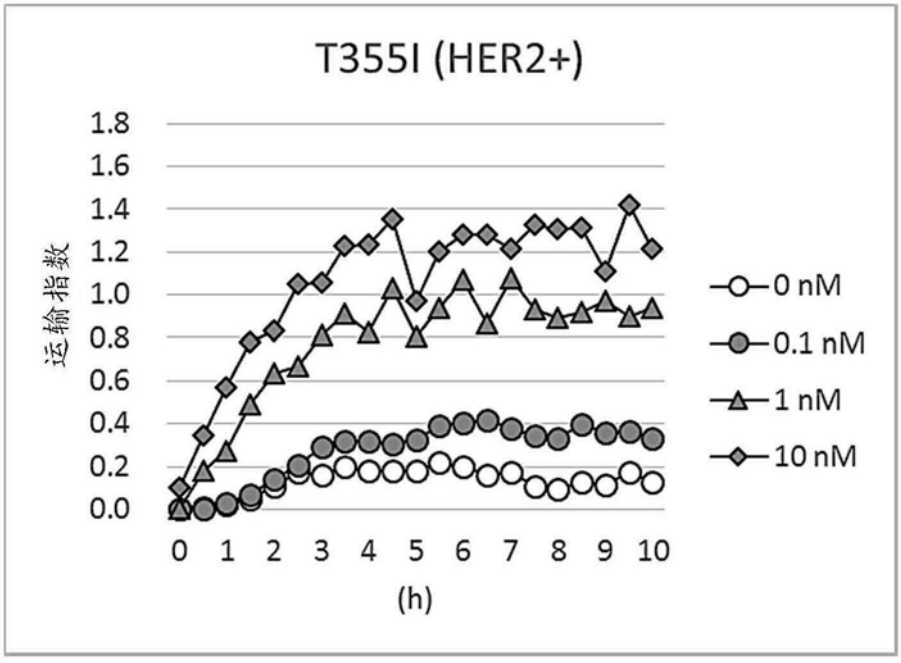

T355I (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 59

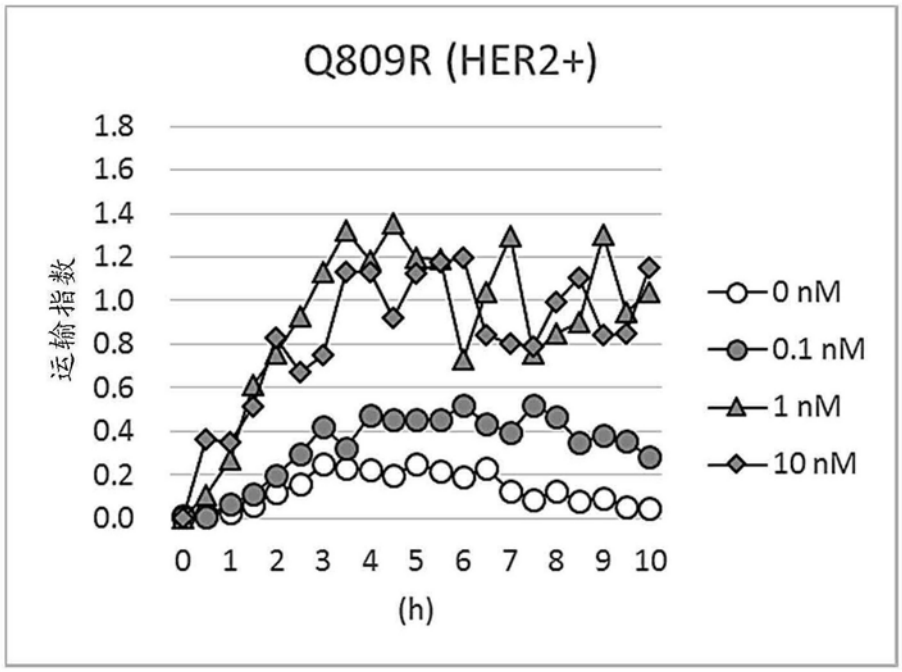

Q809R (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 60

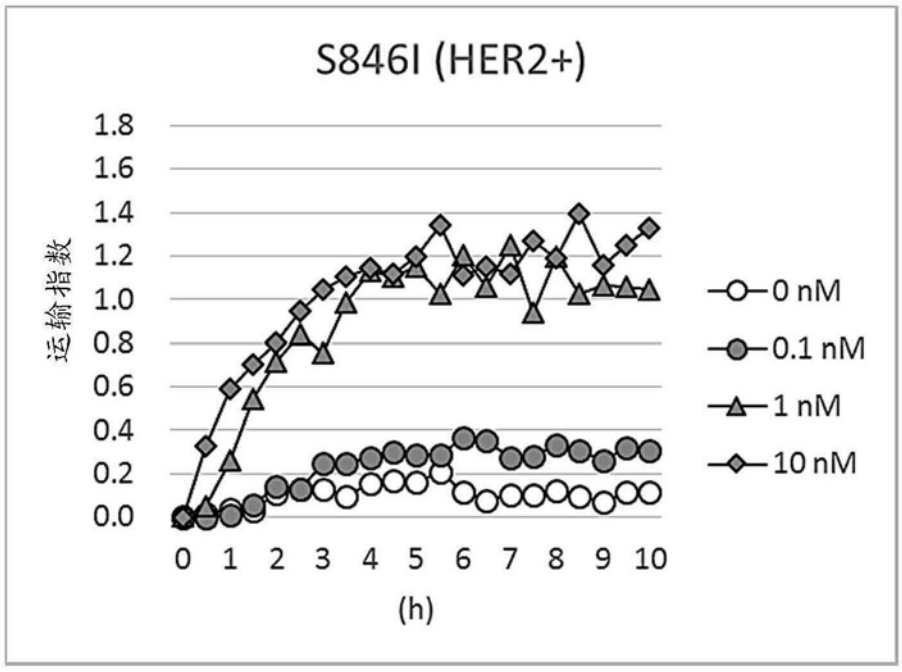

S846I (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 61

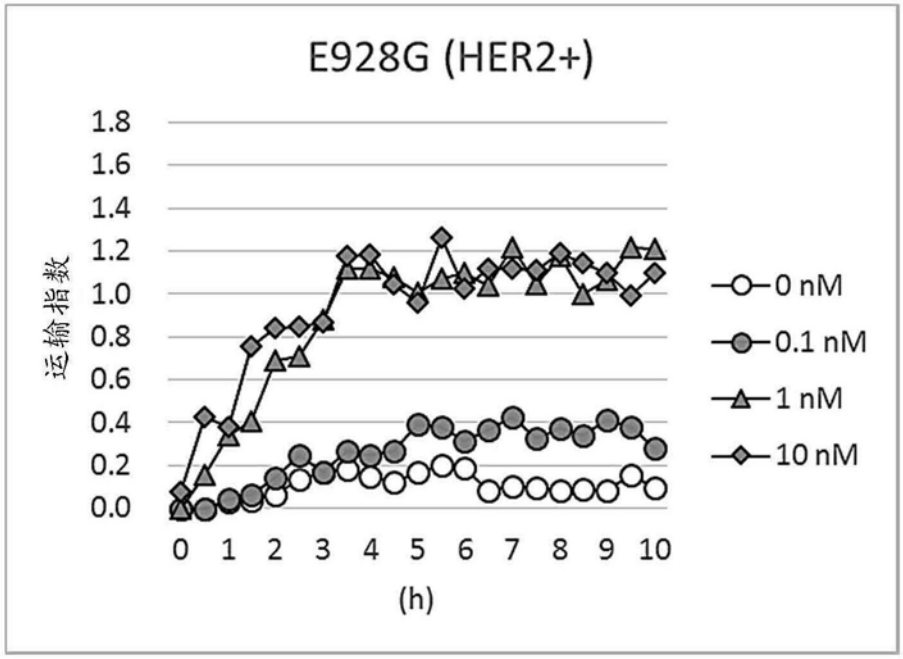

E928G (HER2+)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 62

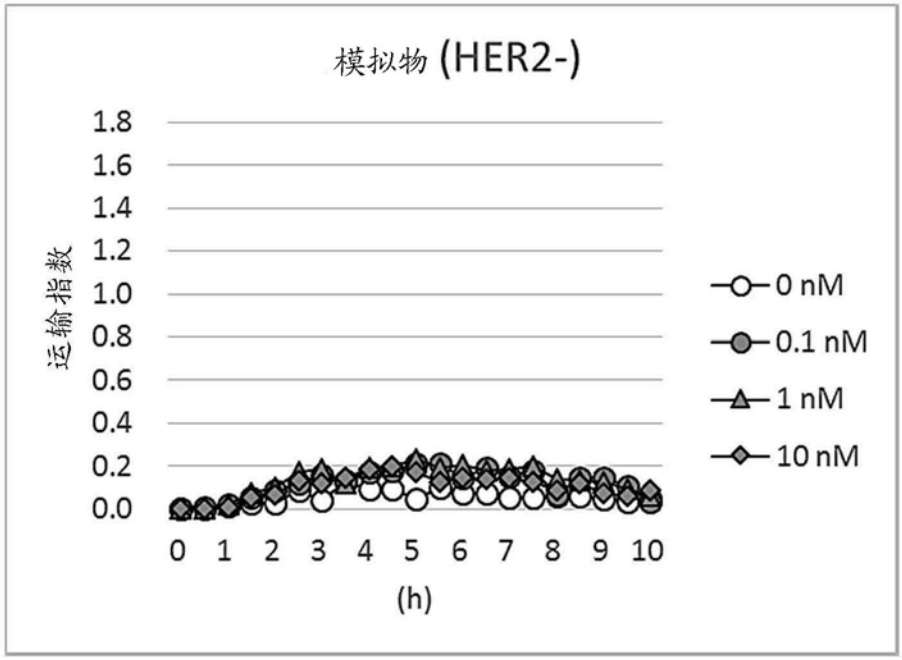

模拟物 (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 63

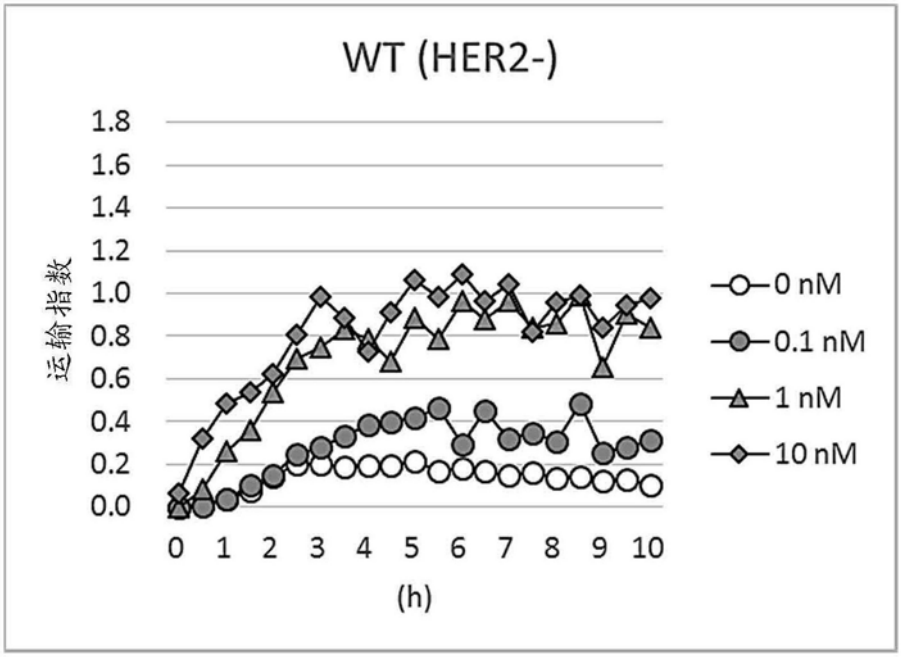

WT (HER2-)
运输指数
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 1 2 3 4 5 6 7 8 9 10
(h)
0 nM
0.1 nM
1 nM
10 nM

图 64

SEQ ID NO: 69 - HER3蛋白的氨基酸序列

MRANDALQVLGLLFSLARGSEVGNSQAVCPGTLNGLSVTG
DAENQYQTLYKLYERCEVVMGNLEIVLTGHNADLSFLQWI
REVTGYVLVAMNEFSTLPLPNLRVVRGTQVYDGKFAIFVM
LNYNTNSSHALRQLRLTQLTEILSGGVYIEKNDKLCHMDT
IDWRDIVRDRDAEIVVKDNGRSCPPCHEVCKGRCWGPGSE
DCQTLTKTICAPQCNGHCFGPNPNQCCHDECAGGCSGPQD
TDCFACRHFNDSGACVPRCPQPLVYNKLTFQLEPNPHTKY
QYGGVCVASCPHNFVVDQTSCVRACPPDKMEVDKNGLKMC
EPCGGLCPKACEGTGSGSRFQTVDSSNIDGFVNCTKILGN
LDFLITGLNGDPWHKIPALDPEKLNVFRTVREITGYLNIQ
SWPPHMHNFSVFSNLTTIGGRSLYNRGFSLLIMKNLNVTS
LGFRSLKEISAGRIYISANRQLCYHHSLNWTKVLRGPTEE
RLDIKHNRPRRDCVAEGKVCDPLCSSGGCWGPGPGQCLSC
RNYSRGGVCVTHCNFLNGEPREFAHEAECFSCHPECQPME
GTATCNGSGSDTCAQCAHFRDGPHCVSSCPHGVLGAKGPI
YKYPDVQNECRPCHENCTQGCKGPELQDCLGQTLVLIGKT
HLTMALTVIAGLVVIFMMLGGTFLYWRGRRIQNKRAMRRY
LERGESIEPLDPSEKANKVLARIFKETELRKLKVLGSGVF
GTVHKGVWIPEGESIKIPVCIKVIEDKSGRQSFQAVTDHM
LAIGSLDHAHIVRLLGLCPGSSLQLVTQYLPLGSLLDHVR
QHRGALGPQLLLNWGVQIAKGMYYLEEHGMVHRNLAARNV
LLKSPSQVQVADFGVADLLPPDDKQLLYSEAKTPIKWMAL
ESIHFGKYTHQSDVWSYGVTVWELMTFGAEPYAGLRLAEV
PDLLEKGERLAQPQICTIDVYMVMVKCWMIDENIRPTFKE
LANEFTRMARDPPRYLVIKRESGPGIAPGPEPHGLTNKKL
EEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNR
PRGSQSLLSPSSGYMPMNQGNLGESCQESAVSGSSERCPR
PVSLHPMPRGCLASESSEGHVTGSEAELQEKVSMCRSRSR
SRSPRPRGDSAYHSQRHSLLTPVTPLSPPGLEEEDVNGYV
MPDTHLKGTPSSREGTLSSVGLSSVLGTEEEDEDEEYEYM
NRRRRHSPPHPPRPSSLEELGYEYMDVGSDLSASLGSTQS
CPLHPVPIMPTAGTTPDEDYEYMNRQRDGGGPGGDYAAMG
ACPASEQGYEEMRAFQGPGHQAPHVHYARLKTLRSLEATD
SAFDNPDYWHSRLFPKANAQRT

图 65