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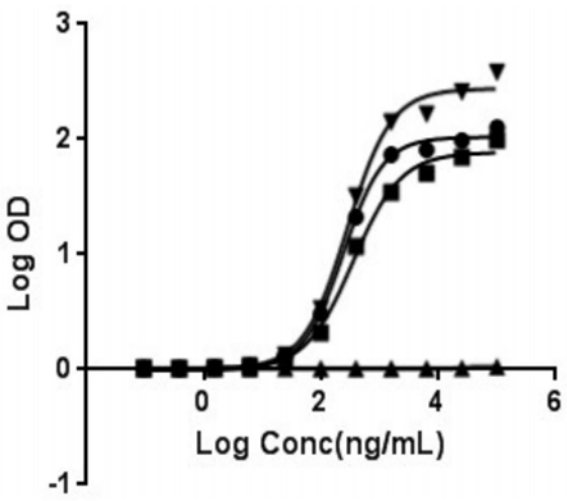
(54)发明名称

针对KN044的单域抗体

(57)摘要

本发明涉及医药生物领域，公开了针对KN044的单域抗体。具体而言，本发明公开了一种衍生自所述单域抗体的针对KN044的结合分子及其用途，特别是在检测KN044、用作KN044的抗药抗体(ADA)检测的阳性抗体、减少由于施用KN044所引发的副作用中的用途。



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1.一种针对KN044的结合分子,其能够结合KN044且包含至少一个免疫球蛋白单一可变结构域,所述至少一个免疫球蛋白单一可变结构域包含选自以下的CDR1、CDR2和CDR3:

(1)SEQ ID NO:1所示的CDR1,SEQ ID NO:2所示的CDR2,SEQ ID NO:3所示的CDR3;

(2)SEQ ID NO:4所示的CDR1,SEQ ID NO:5所示的CDR2,SEQ ID NO:6所示的CDR3;

(3)SEQ ID NO:7所示的CDR1,SEQ ID NO:8所示的CDR2,SEQ ID NO:9所示的CDR3;

(4)SEQ ID NO:10所示的CDR1,SEQ ID NO:11所示的CDR2,SEQ ID NO:12所示的CDR3;

(5)SEQ ID NO:13所示的CDR1,SEQ ID NO:14所示的CDR2,SEQ ID NO:15所示的CDR3;

(6)SEQ ID NO:16所示的CDR1,SEQ ID NO:17所示的CDR2,SEQ ID NO:18所示的CDR3;

(7)SEQ ID NO:19所示的CDR1,SEQ ID NO:20所示的CDR2,SEQ ID NO:21所示的CDR3;

(8)SEQ ID NO:22所示的CDR1,SEQ ID NO:23所示的CDR2,SEQ ID NO:24所示的CDR3;

(9)SEQ ID NO:25所示的CDR1,SEQ ID NO:26所示的CDR2,SEQ ID NO:27所示的CDR3;

(10)SEQ ID NO:28所示的CDR1,SEQ ID NO:29所示的CDR2,SEQ ID NO:30所示的CDR3;

(11)SEQ ID NO:31所示的CDR1,SEQ ID NO:32所示的CDR2,SEQ ID NO:33所示的CDR3;

(12)SEQ ID NO:34所示的CDR1,SEQ ID NO:35所示的CDR2,SEQ ID NO:36所示的CDR3;

(13)SEQ ID NO:37所示的CDR1,SEQ ID NO:38所示的CDR2,SEQ ID NO:39所示的CDR3;

(14)SEQ ID NO:40所示的CDR1,SEQ ID NO:41所示的CDR2,SEQ ID NO:42所示的CDR3;

(15)SEQ ID NO:43所示的CDR1,SEQ ID NO:44所示的CDR2,SEQ ID NO:45所示的CDR3;

(16)SEQ ID NO:46所示的CDR1,SEQ ID NO:47所示的CDR2,SEQ ID NO:48所示的CDR3;和

(17)SEQ ID NO:49所示的CDR1,SEQ ID NO:50所示的CDR2,SEQ ID NO:51所示的CDR3。

2.权利要求1的针对KN044的结合分子,其中所述免疫球蛋白单一可变结构域是VHH。

3.权利要求2的针对KN044的结合分子,其中所述免疫球蛋白单一可变结构域是人源化的VHH。

4.权利要求2的针对KN044的结合分子,其中所述VHH包含SEQ ID NO:52-68中任一氨基酸序列。

5.权利要求1-4任一项的针对KN044的结合分子,其还包含免疫球蛋白Fc区。

6.权利要求5的针对KN044的结合分子,其中所述免疫球蛋白Fc区是人免疫球蛋白Fc区或是鼠免疫球蛋白Fc区。

7.权利要求6的针对KN044的结合分子,其中所述免疫球蛋白Fc区是人IgG1的Fc区或鼠IgG1的Fc区。

8.权利要求6的针对KN044的结合分子,其中所述人免疫球蛋白Fc区的氨基酸序列示于SEQ ID NO:69,所述鼠免疫球蛋白Fc区的氨基酸序列示于SEQ ID NO:70。

9.权利要求1-4中任一项的针对KN044的结合分子,其结合KN044的KD值小于1×10^{-7}M。

10.核酸分子,其编码权利要求1-9中任一项的针对KN044的结合分子。

11.表达载体,其包含与表达调控元件可操作地连接的权利要求10的核酸分子。

12.宿主细胞,其包含权利要求10的核酸分子或以权利要求11的表达载体转化,并能够表达所述针对KN044的结合分子。

13.产生权利要求1-9中任一项的针对KN044的结合分子的方法,包括:

a)在允许所述针对KN044的结合分子表达的条件下培养权利要求12的宿主细胞;

b)从得自步骤a)的培养物回收由所述宿主细胞表达的针对KN044的结合分子;及

c)任选进一步纯化和/或修饰得自步骤b)的针对KN044的结合分子。

14.一种检测靶样品中KN044的存在和/或对靶样品中KN044定量的试剂盒,包含权利要求1-9中任一项的针对KN044的结合分子。

15.权利要求14的试剂盒,其还包含含有预定量的KN044的对照样品。

16.一种检测靶样品中KN044的存在和/或对样品中KN044定量的非诊断方法,所述方法包括:

a)在针对KN044的结合分子与KN044之间能够形成复合物的条件下,使所述靶样品和对照样品分别与权利要求1-9中任一项的针对KN044的结合分子接触;

b)检测复合物的形成,

其中所述靶样品与对照样品之间复合物形成的差异指示靶样品中KN044的存在和/或量。

17.权利要求16的方法,其中所述对照样品含有预定量的KN044。

18.权利要求16或17的方法,其用于KN044的药代动力学检测,所述靶样品为血液样品。

19.权利要求18的方法,其中所述靶样品为血浆或血清。

20.一种用于检测针对KN044的抗药抗体(ADA)的试剂盒,包含权利要求1-9中任一项的针对KN044的结合分子,其中所述针对KN044的结合分子用作所述针对KN044的抗药抗体(ADA)检测的阳性抗体。

21.权利要求20的试剂盒,其还包含KN044。

22.权利要求1-9中任一项的针对KN044的结合分子在制备试剂盒中的用途,所述试剂盒用于检测靶样品中KN044的抗药抗体(ADA)的方法,所述方法包括:

a)使KN044分别与靶样品和权利要求1-9中任一项的针对KN044的结合分子接触;

b)检测复合物的形成,

其中KN044与权利要求1-9中任一项的针对KN044的结合分子所形成的复合物用作靶样品中KN044的抗药抗体(ADA)检测的阳性对照,所述靶样品为施用了KN044的对象的血液样品。

23.权利要求22的用途,其中所述靶样品为血浆或血清。

24.药物组合物,其包含权利要求1-9中任一项的针对KN044的结合分子以及药学上可接受的载体。

25.权利要求1-9中任一项的针对KN044的结合分子或权利要求24的药物组合物在制备药物中的用途,所述药物用于减少由于施用KN044所引发的副作用,所述KN044用于在所述对象中预防和/或治疗与CTLA4相关的疾病。

26.权利要求25的用途,其中所述与CTLA4相关的疾病是癌症或感染性疾病。

27.如权利要求25的用途,其中所述由于施用KN044所引发的副作用为细胞因子风暴。

28.如权利要求25所述的用途,其中所述针对KN044的结合分子或药物组合物用于中和KN044。

针对KN044的单域抗体

技术领域

[0001] 本发明涉及医药生物领域，公开了针对KN044的单域抗体。具体而言，本发明公开了一种衍生自所述单域抗体的针对KN044的结合分子及其用途，特别是在检测KN044、用作KN044的抗药抗体(ADA)检测的阳性抗体、治疗由于施用KN044所引发的副作用中的用途。

背景技术

[0002] KN044是一种针对CTLA4的重组人源化抗CTLA4单域抗体Fc融合蛋白(参见CN201610332590.7)。KN044具有高特异性、高亲和力和高稳定性，用于预防和/或治疗与CTLA4相关的疾病，如癌症或感染性疾病。

[0003] 在KN044的相关研究中，需要能够识别KN044并与KN044高亲和力结合的分子用于KN044的检测。另外，抗药抗体(ADA)反应可能会降低药物疗效或导致严重的不良反应，需要用于KN044的抗药抗体(ADA)检测的阳性抗体，用于临床试验的免疫原性研究。此外，考虑到针对CTLA4的抗体药物KN044有可能引发细胞因子风暴，需要能够快速中和KN044活性的药物，作为应对细胞因子风暴等急性严重副作用的临床应急治疗手段。

[0004] 发明概述

[0005] 在第一方面，本发明提供一种针对KN044的结合分子，其能够结合KN044且包含至少一个免疫球蛋白单一可变结构域，所述至少一个免疫球蛋白单一可变结构域包含选自以下的CDR1、CDR2和CDR3：

[0006] (1)SEQ ID NO:1所示的CDR1，SEQ ID NO:2所示的CDR2，SEQ ID NO:3所示的CDR3；

[0007] (2)SEQ ID NO:4所示的CDR1，SEQ ID NO:5所示的CDR2，SEQ ID NO:6所示的CDR3；

[0008] (3)SEQ ID NO:7所示的CDR1，SEQ ID NO:8所示的CDR2，SEQ ID NO:9所示的CDR3；

[0009] (4)SEQ ID NO:10所示的CDR1，SEQ ID NO:11所示的CDR2，SEQ ID NO:12所示的CDR3；

[0010] (5)SEQ ID NO:13所示的CDR1，SEQ ID NO:14所示的CDR2，SEQ ID NO:15所示的CDR3；

[0011] (6)SEQ ID NO:16所示的CDR1，SEQ ID NO:17所示的CDR2，SEQ ID NO:18所示的CDR3；

[0012] (7)SEQ ID NO:19所示的CDR1，SEQ ID NO:20所示的CDR2，SEQ ID NO:21所示的CDR3；

[0013] (8)SEQ ID NO:22所示的CDR1，SEQ ID NO:23所示的CDR2，SEQ ID NO:24所示的CDR3；

[0014] (9)SEQ ID NO:25所示的CDR1，SEQ ID NO:26所示的CDR2，SEQ ID NO:27所示的CDR3；

[0015] (10)SEQ ID NO:28所示的CDR1，SEQ ID NO:29所示的CDR2，SEQ ID NO:30所示的CDR3；

[0016] (11)SEQ ID NO:31所示的CDR1，SEQ ID NO:32所示的CDR2，SEQ ID NO:33所示的

CDR3；

[0017] (12)SEQ ID NO:34所示的CDR1,SEQ ID NO:35所示的CDR2,SEQ ID NO:36所示的CDR3；

[0018] (13)SEQ ID NO:37所示的CDR1,SEQ ID NO:38所示的CDR2,SEQ ID NO:39所示的CDR3；

[0019] (14)SEQ ID NO:40所示的CDR1,SEQ ID NO:41所示的CDR2,SEQ ID NO:42所示的CDR3；

[0020] (15)SEQ ID NO:43所示的CDR1,SEQ ID NO:44所示的CDR2,SEQ ID NO:45所示的CDR3；

[0021] (16)SEQ ID NO:46所示的CDR1,SEQ ID NO:47所示的CDR2,SEQ ID NO:48所示的CDR3;和

[0022] (17)SEQ ID NO:49所示的CDR1,SEQ ID NO:50所示的CDR2,SEQ ID NO:51所示的CDR3。

[0023] 在一些实施方案中,所述免疫球蛋白单一可变结构域是VHH,例如,人源化的VHH。

[0024] 在一些具体实施方案中,所述VHH包含SEQ ID NO:52-68中任一氨基酸序列。

[0025] 在一些实施方案中,所述针对KN044的结合分子还包含免疫球蛋白Fc区。

[0026] 在一些实施方案中,所述免疫球蛋白Fc区是人免疫球蛋白Fc区,优选是人IgG1的Fc区;或是鼠免疫球蛋白Fc区,优选是鼠IgG1的Fc区。

[0027] 在一些具体实施方案中,所述人免疫球蛋白Fc区的氨基酸序列示于SEQ ID NO:69,所述鼠免疫球蛋白Fc区的氨基酸序列示于SEQ ID NO:70。

[0028] 在一些具体实施方案中,所述针对KN044的结合分子结合KN044的KD值小于1×10^{-7}M,优选小于1×10^{-8}M,更优选小于1×10^{-9}M,更优选小于1×10^{-10}M,尤其更优选小于1×10^{-11}M。

[0029] 在第二方面,本发明提供一种核酸分子,其编码本发明的针对KN044的结合分子。

[0030] 在第三方面,本发明提供一种表达载体,其包含与表达调控元件可操作地连接的本发明第二方面的核酸分子。

[0031] 在第四方面,本发明提供一种宿主细胞,其包含本发明的核酸分子或以本发明的表达载体转化,并能够表达所述针对KN044的结合分子。

[0032] 在第五方面,本发明提供一种产生本发明的针对KN044的结合分子的方法,包括：

[0033] a)在允许所述针对KN044的结合分子表达的条件下培养本发明的宿主细胞；

[0034] b)从得自步骤a)的培养物回收由所述宿主细胞表达的针对KN044的结合分子;及

[0035] c)任选进一步纯化和/或修饰得自步骤b)的针对KN044的结合分子。

[0036] 在第六方面,本发明提供一种检测靶样品中KN044的存在和/或对靶样品中KN044定量的试剂盒,包含本发明的针对KN044的结合分子。

[0037] 在一些实施方案中,所述试剂盒还包含含有预定量的KN044的对照样品。

[0038] 在第七方面,本发明提供一种检测靶样品中KN044的存在和/或对样品中KN044定量的方法,所述方法包括：

[0039] a)在针对KN044的结合分子与KN044之间能够形成复合物的条件下,使所述靶样品和对照样品分别与本发明的针对KN044的结合分子接触；

[0040] b)检测复合物的形成，

[0041] 其中所述靶样品与对照样品之间复合物形成的差异指示靶样品中KN044的存在和/或量，优选地，所述对照样品含有预定量的KN044。

[0042] 在一些实施方案中，所述方法用于KN044的药代动力学检测，所述靶样品为血液样品，例如，血浆或血清。

[0043] 在第八方面，本发明提供一种用于KN044的抗药抗体(ADA)检测的试剂盒，包含本发明的针对KN044的结合分子，其中所述针对KN044的结合分子用作所述KN044的抗药抗体(ADA)检测的阳性抗体。

[0044] 在一些实施方案中，所述试剂盒还包含KN044。

[0045] 在第九方面，本发明提供一种用于靶样品中KN044的抗药抗体(ADA)检测的方法，所述方法包括：

[0046] a)使KN044分别与靶样品和本发明的针对KN044的结合分子接触；

[0047] b)检测复合物的形成，

[0048] 其中KN044与本发明的针对KN044的结合分子所形成的复合物用作靶样品中KN044的抗药抗体(ADA)检测的阳性对照，所述靶样品为施用了KN044的对象的血液样品，例如，血浆或血清。

[0049] 在第十方面，本发明提供一种药物组合物，其包含本发明的针对KN044的结合分子以及药学上可接受的载体。

[0050] 在第十一方面，本发明提供一种在对象中治疗由于施用KN044所引发的副作用的方法，包括给所述对象施用有效量的本发明的针对KN044的结合分子或本发明的药物组合物，所述KN044用于在所述对象中预防和/或治疗与CTLA4相关的疾病，例如癌症或感染性疾病。

[0051] 在一些实施方案中，所述由于施用KN044所引发的副作用为细胞因子风暴。

[0052] 在一些实施方案中，所述针对KN044的结合分子或药物组合物用于中和KN044。

[0053] 在第十二方面，本发明提供本发明的针对KN044的结合分子或本发明的药物组合物在制备药物中的用途，所述药物用于治疗由于施用KN044所引发的副作用，所述KN044用于在所述对象中预防和/或治疗与CTLA4相关的疾病，例如癌症或感染性疾病。

[0054] 在第十三方面，本发明提供本发明的针对KN044的结合分子或本发明的药物组合物，其用于治疗由于施用KN044所引发的副作用，所述KN044用于在所述对象中预防和/或治疗与CTLA4相关的疾病，例如癌症或感染性疾病。

附图说明

[0055] 图1.示出针对KN044的单域抗体的序列。

[0056] 图2.示出针对KN044的带有His标签的候选单域抗体与抗原KN044的结合曲线。

[0057] 图3.示出针对KN044的单域抗体-Fc融合蛋白与抗原KN044的结合曲线。

[0058] 发明详述

[0059] 定义

[0060] 除非另有指示或定义，否则所有所用术语均具有本领域中的通常含义，该含义将为本领域技术人员所了解。参考例如标准手册，如Sambrook et al.，“Molecular Cloning：

A Laboratory Manual";Lewin,"Genes VIII";及Roitt et al.,"Immunology"(第8版),以及本文中引用的一般现有技术;此外,除非另有说明,否则未具体详述的所有方法、步骤、技术及操作均可以且已经以本身已知的方式进行,该方式将为本领域技术人员所了解。亦参考例如标准手册、上述一般现有技术及其中引用的其他参考文献。

[0061] 除非另有说明,否则可互换使用的术语"抗体"或"免疫球蛋白"在本文中无论是指重链抗体还是指常规4链抗体,均用作一般术语以包括全长抗体、其单个的链以及其所有部分、结构域或片段(包括但不限于抗原结合结构域或片段,分别例如VHH结构域或VH/VL结构域)。此外,本文所用的术语"序列"(例如在"免疫球蛋白序列"、"抗体序列"、"单一可变结构域序列"、"VHH序列"或"蛋白序列"等的术语中)一般应理解为既包括相关氨基酸序列,又包括编码所述序列的核酸序列或核苷酸序列,除非本文需要更限定的解释。

[0062] 如本文所用,术语(多肽或蛋白的)"结构域"是指折叠蛋白结构,其能够独立于蛋白的其余部分维持其三级结构。一般而言,结构域负责蛋白的单个的功能性质,且在许多情况下可添加、移除或转移至其他蛋白而不损失蛋白的其余部分和/或结构域的功能。

[0063] 如本文所用的术语"免疫球蛋白结构域"是指抗体链(例如常规4链抗体的链或重链抗体的链)的球形区域,或是指基本上由这类球形区域组成的多肽。免疫球蛋白结构域的特征在于其维持抗体分子的免疫球蛋白折叠特征,其由排列在两个β折叠中任选由保守二硫键稳定的约7个反平行β折叠股的2层夹层组成。

[0064] 如本文所用的术语"免疫球蛋白可变结构域"是指基本上由本领域及下文中分别称为"框架区1"或"FR1"、"框架区2"或"FR2"、"框架区3"或"FR3"、及"框架区4"或"FR4"的四个"框架区"组成的免疫球蛋白结构域,其中所述框架区由本领域及下文中分别称为"互补决定区1"或"CDR1"、"互补决定区2"或"CDR2"、及"互补决定区3"或"CDR3"的三个"互补决定区"或"CDR"间隔开。因此,免疫球蛋白可变结构域的一般结构或序列可如下表示为:FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4。免疫球蛋白可变结构域因具有抗原结合位点而赋予抗体对抗原的特异性。

[0065] 如本文所用的术语"免疫球蛋白单一可变结构域"是指能够在不与其他免疫球蛋白可变结构域配对的情况下特异性结合抗原表位的免疫球蛋白可变结构域。本发明含义中的免疫球蛋白单一可变结构域的一个实例为"结构域抗体",例如免疫球蛋白单一可变结构域VH及VL(VH结构域及VL结构域)。免疫球蛋白单一可变结构域的另一实例为如下文定义的骆驼科的"VHH结构域"(或简称为"VHH")。

[0066] "VHH结构域",亦称为重链单域抗体、VHH、V_HH结构域、VHH抗体片段和VHH抗体,是称为"重链抗体"(即"缺乏轻链的抗体")的抗原结合免疫球蛋白的可变结构域(Hamers-Casterman C,Atarhouch T,Muyldermans S,Robinson G,Hamers C,Songa EB,Bendahman N,Hamers R.:"Naturally occurring antibodies devoid of light chains";Nature 363,446-448(1993))。使用术语"VHH结构域"以将所述可变结构域与存在于常规4链抗体中的重链可变结构域(其在本文中称为"VH结构域")以及存在于常规4链抗体中的轻链可变结构域(其在本文中称为"VL结构域")进行区分。VHH结构域特异性结合表位而无需其他抗原结合结构域(此与常规4链抗体中的VH或VL结构域相反,在该情况下表位由VL结构域与VH结构域一起识别)。VHH结构域为由单一免疫球蛋白结构域形成的小型稳定及高效的抗原识别单元。

[0067] 在本发明的上下文中，术语“重链单域抗体”、“VHH结构域”、“VHH”、“V_HH结构域”、“VHH抗体片段”、“VHH抗体”以及“Nanobody®”及“Nanobody®结构域”(“Nanobody”为Ablynx N.V.公司，Ghent，Belgium的商标)可互换使用。

[0068] 例如Riechmann及Muyldermans，J.Immunol.Methods 231，25-38(1999)的图2中所示，对于骆驼科的VHH结构域所应用的氨基酸残基，根据Kabat等人给出的VH结构域的一般编号法来编号(“Sequence of proteins of immunological interest”，US Public Health Services，NIH Bethesda，MD，公开案第91号)。根据该编号法，

[0069] -FR1包含在位置1-30处的氨基酸残基，

[0070] -CDR1包含在位置31-35处的氨基酸残基，

[0071] -FR2包含在位置36-49处的氨基酸，

[0072] -CDR2包含在位置50-65处的氨基酸残基，

[0073] -FR3包含在位置66-94处的氨基酸残基，

[0074] -CDR3包含在位置95-102处的氨基酸残基，且

[0075] -FR4包含在位置103-113处的氨基酸残基。

[0076] 然而应注意，如本领域中对于VH结构域及VHH结构域所公知的，各CDR中的氨基酸残基的总数可能不同，且可能不对应于由Kabat编号指示的氨基酸残基的总数(即根据Kabat编号的一个或多个位置可能在实际序列中未被占据，或实际序列可能含有多于Kabat编号所允许数目的氨基酸残基)。这意味着一般而言，根据Kabat的编号可能对应或可能不对应于实际序列中氨基酸残基的实际编号。

[0077] 本领域中已知还有对VH结构域的氨基酸残基进行编号的其他系统，如Chothia编号系统。Chothia的氨基酸编号与Kabat相同，但其将对CDR区划分是基于抗体可变区结构中的环(loop)区来进行的，因此在CDR区所包含的氨基酸区域上会与Kabat的有所不同。此外还有AbM编码系统等。所述其他编码系统也可以类似地应用于VHH结构域。然而，除非另有说明，否则在本说明书、权利要求书及附图中，将遵循如上所述的根据Kabat且适于VHH结构域的编号，或者综合Kabat与Chothia的编号。

[0078] VHH结构域中的氨基酸残基的总数将通常在110至120范围内，常常介于112与115之间。然而应注意较小及较长序列也可适于本文所述的目的。

[0079] VHH结构域及含有其的多肽的其他结构特性及功能性质可总结如下：

[0080] VHH结构域(其已经天然“设计”以在不存在轻链可变结构域且不与轻链可变结构域相互作用的情况下与抗原功能性结合)可用作单一且相对较小的功能性抗原结合结构单元、结构域或多肽。此区分VHH结构域与常规4链抗体的VH及VL结构域，这些VH及VL结构域自身通常不适于作为单一抗原结合蛋白或免疫球蛋白单一可变结构域进行实际应用，但需要以某种形式或另一形式组合以提供功能性抗原结合单元(如以诸如Fab片段等常规抗体片段的形式；或以由与VL结构域共价连接的VH结构域组成的scFv的形式)。

[0081] 由于这些独特性质，使用VHH结构域一单独或作为较大多肽的一部分一提供许多优于使用常规VH及VL结构域、scFv或常规抗体片段(例如Fab-或$F(ab')_2$-片段)的显著优势：仅需要单一结构域以高亲和力及高选择性结合抗原，从而使得既不需要存在两个单独结构域，也不需要确保该两个结构域以适当空间构象及构型存在(例如scFv一般需要使用经特别设计的接头)；VHH结构域可自单一基因表达且不需要翻译后折叠或修饰；VHH结构域

可容易地改造成多价及多特异性格式(格式化);VHH结构域高度可溶且无聚集趋势;VHH结构域对热、pH、蛋白酶及其他变性剂或条件高度稳定,且因此可在制备、储存或运输中不使用冷冻设备,从而达成节约成本、时间及环境;VHH结构域易于制备且相对廉价,甚至在生产所需的规模上亦如此;VHH结构域与常规4链抗体及其抗原结合片段相比相对较小(大约15kDa或大小为常规IgG的1/10),因此相比于常规4链抗体及其抗原结合片段,显示较高的组织渗透性且可以较高剂量给药;VHH结构域可显示所谓腔结合性质(尤其由于与常规VH结构域相比其延长的CDR3环),从而可到达常规4链抗体及其抗原结合片段不可到达的靶及表位。

[0082] 获得结合特定抗原或表位的VHH的方法,先前已公开于以下文献中:R.van der Linden et al.,Journal of Immunological Methods,240(2000)185-195;Li et al.,J Biol Chem.,287(2012)13713-13721;Deffar et al.,African Journal ofBiotechnology Vol.8(12),pp.2645-2652,17June,2009;WO94/04678;US7790367,2006-09-14,METHOD FOR SCREENING A LIBRARY OF VHH POLYPEPTIDES,Casterman,Cecile,Hamers,Raymond;和US 7786047,2006-02-10,IMMUNOGLOBULINS DEVOID OF LIGHTCHAINS,Casterman,Cecile,Hamers,Raymond。

[0083] 此外,本领域技术人员还将了解,有可能将一个或多个上述CDR“移植”于其他“支架”(包括但不限于人支架或非免疫球蛋白支架)上。适于所述CDR移植的支架及技术在本领域中是已知的。

[0084] 如本文所用,术语“表位”或可互换使用的术语“抗原决定簇”指抗体的互补位所结合的抗原上的任何抗原决定簇。抗原决定簇通常包含分子的化学活性表面基团,例如氨基酸或糖侧链,并且通常具有特定的三维结构特征以及特定的电荷特征。例如,表位通常以独特的空间构象包括至少3、4、5、6、7、8、9、10、11、12、13、14或15个连续或非连续的氨基酸,其可以是“线性”表位或“构象”表位。参见,例如,Epitope Mapping Protocols in Methods in Molecular Biology,第66卷,G.E.Morris,Ed.(1996)。在线性表位中,蛋白质与相互作用分子(例如抗体)之间的所有相互作用的点沿着蛋白质的一级氨基酸序列线性存在。在构象表位中,相互作用的点跨越彼此分开的蛋白质氨基酸残基而存在。

[0085] 可使用本领域中熟知的许多表位定位技术鉴别给定抗原的表位。参见例如Epitope Mapping Protocols in Methods in Molecular Biology,第66卷,G.E.Morris,Ed.(1996)。举例而言,线性表位可通过例如以下方法来确定:在固体支持物上同时合成大量肽,其中这些肽对应于蛋白质分子的各部分,且使这些肽在仍然与支持物连接的情况下与抗体反应。这些技术在本领域中为已知的且描述于例如美国专利第4,708,871号;Geysen等人(1984)Proc.Natl.Acad.Sci.USA 81:3998-4002;Geysen等人(1986)Molec.Immunol.23:709-715中。类似地,构象表位可通过诸如通过例如x射线结晶学及2维核磁共振确定氨基酸的空间构形加以鉴别。参见例如Epitope Mapping Protocols(同上)。

[0086] 可使用本领域技术人员已知的常规技术,就与相同表位的结合竞争性筛选抗体。例如,可进行竞争和交叉竞争研究,以获得彼此竞争或交叉竞争与抗原结合的抗体。基于它们的交叉竞争来获得结合相同表位的抗体的高通量方法描述于国际专利申请WO03/48731中。因此,可使用本领域技术人员已知的常规技术,获得与本发明的抗体分子竞争结合KN044上的相同表位的抗体及其抗原结合片段。

[0087] 一般而言,术语“特异性”是指特定抗原结合分子或抗原结合蛋白(例如本发明的免疫球蛋白单一可变结构域)分子可结合的不同类型抗原或表位的数目。可基于抗原结合分子的亲和力和/或亲合力确定其特异性。由抗原与抗原结合蛋白的解离平衡常数(KD)所表示的亲和力,是表位与抗原结合蛋白上抗原结合位点之间结合强度的量度:KD值越小,表位与抗原结合分子之间的结合强度越强(或者,亲和力也可表示为缔合常数(KA),其为1/KD)。如本领域技术人员将了解,取决于具体感兴趣的抗原,可以以已知方式测定亲和力。亲合力为抗原结合分子(例如免疫球蛋白、抗体、免疫球蛋白单一可变结构域或含有其的多肽)与相关抗原之间结合强度的量度。亲合力与以下两者有关:与其抗原结合分子上的抗原结合位点之间的亲和力,以及存在于抗原结合分子上的相关结合位点的数目。

[0088] 如本文所用,术语“针对KN044的结合分子”意指任何能够以高亲和力结合KN044的分子。针对KN044的结合分子可以包括针对KN044的如本文定义的抗体或其缀合物。针对KN044的结合分子还涵盖免疫球蛋白超家族抗体(IgSF)或CDR移植分子。

[0089] “针对KN044的结合分子”或者可以指结合KN044的单价分子(即与KN044的一个表位结合的分子),以及二价或多价结合分子(即结合一个以上表位的结合分子)。本发明的“针对KN044的结合分子”可以包含至少一个结合KN044的免疫球蛋白单一可变结构域如VHH。在一些实施方案中,本发明的“针对KN044的结合分子”可以包含两个结合KN044的免疫球蛋白单一可变结构域如VHH。

[0090] 通常,本发明的针对KN044的结合分子将以如通过Biacore或KinExA或生物膜干涉技术(Biolayerinterferometry,BLI)测量的优选10^{-7}至10^{-11}摩尔/升(M)、更优选10^{-8}至10^{-11}摩尔/升、甚至更优选10^{-9}至10^{-11}、甚至更优选10^{-10}至10^{-11}或更低的解离常数(KD),和/或以至少$10^{7}M^{-1}$、优选至少$10^{8}M^{-1}$、更优选至少$10^{9}M^{-1}$,更优选至少$10^{10}M^{-1}$、例如至少$10^{11}M^{-1}$的缔合常数(KA)结合所要结合的抗原(即KN044)。抗原结合蛋白对抗原或表位的结合可以以已知的任何适合方式来测定,包括例如表面等离子体共振术(SPR)测定、Scatchard测定、生物膜干涉(Bio-Layer Interferometry,BLI)检测、和/或竞争性结合测定(例如放射免疫测定(RIA)、酶免疫测定(EIA)及夹心式竞争性测定。

[0091] 相比于其天然生物来源和/或获得该多肽或核酸分子的反应介质或培养基,当其已与至少一种在该来源或介质(培养基)中通常与之相关的其他组分(例如另一蛋白/多肽、另一核酸、另一生物组分或大分子或至少一种污染物、杂质或微量组分)分离时,多肽或核酸分子视为“基本上分离的”。特别地,多肽或核酸分子在其已纯化至少2倍、特别是至少10倍、更特别是至少100倍且多达1000倍或1000倍以上时被视为“基本上分离的”。经适合的技术(例如适合色谱技术,如聚丙烯酰胺凝胶电泳)确定,“基本上分离的”多肽或核酸分子优选基本上为均质的。

[0092] “亲和力成熟”的针对KN044的抗体,特别是VHH或结构域抗体,在一个或多个CDR中具有一个或多个变化,所述变化导致对KN044的亲和力相比于其各自的亲本针对KN044的抗体有所增加。亲和力成熟的针对KN044的抗体可通过例如由以下所述的本领域中已知的方法来制备:Marks等人,1992,Biotechnology 10:779-783或Barbas等人,1994,Proc.Nat.Acad.Sci,USA 91:3809-3813.;Shier等人,1995,Gene 169:147-155;Yelton等人,1995,Immunol.155:1994-2004;Jackson等人,1995,J.Immunol.154(7):3310-9;及Hawkins等人,1992,J.MoI.Biol.226(3):889896;KS Johnson及RE Hawkins,“Affinity

maturation of antibodies using phage display",Oxford University Press 1996。

[0093] 如本文所用的术语"对象"意指哺乳动物,尤其灵长类动物,尤其是人。

[0094] 如本文所用的术语"抗药抗体"或"ADA"指与药物抗体的抗原性区域结合的抗体。这种抗原性区域可以是药物抗体的可变区、CDR、恒定区或糖结构。这种抗药抗体可在药物疗法过程中由于对象的免疫原性反应发生,这样的免疫原性反应可能会降低药物疗效或导致严重的不良反应。术语"药物抗体"指可施用至对象用于治疗疾病的抗体,例如,在本发明的实施方案中,药物抗体为KN044。

[0095] 本发明的针对KN044的结合分子

[0096] 在第一方面,本发明提供了一种针对KN044的结合分子,其包含至少一个能够结合KN044的免疫球蛋白单一可变结构域。在一些实施方案中,所述针对KN044的结合分子包含一个结合KN044的免疫球蛋白单一可变结构域。在一些实施方案中,所述针对KN044的结合分子包含两个或更多个结合KN044的免疫球蛋白单一可变结构域。在一些实施方式中,本发明所述的KN044包含示于SEQ ID NO:88的氨基酸序列。

[0097] 在一些实施方案中,所述至少一个免疫球蛋白单一可变结构域包含选自以下的CDR1、CDR2和CDR3:

[0098] (1)SEQ ID NO:1所示的CDR1,SEQ ID NO:2所示的CDR2,SEQ ID NO:3所示的CDR3(对应于抗体株nKN044-19-3的CDR);

[0099] (2)SEQ ID NO:4所示的CDR1,SEQ ID NO:5所示的CDR2,SEQ ID NO:6所示的CDR3(对应于抗体株nKN044-97的CDR);

[0100] (3)SEQ ID NO:7所示的CDR1,SEQ ID NO:8所示的CDR2,SEQ ID NO:9所示的CDR3(对应于抗体株nKN044-125的CDR);

[0101] (4)SEQ ID NO:10所示的CDR1,SEQ ID NO:11所示的CDR2,SEQ ID NO:12所示的CDR3(对应于抗体株nKN044-194的CDR);

[0102] (5)SEQ ID NO:13所示的CDR1,SEQ ID NO:14所示的CDR2,SEQ ID NO:15所示的CDR3(对应于抗体株nKN044-200的CDR);

[0103] (6)SEQ ID NO:16所示的CDR1,SEQ ID NO:17所示的CDR2,SEQ ID NO:18所示的CDR3(对应于抗体株nKN044-216的CDR);

[0104] (7)SEQ ID NO:19所示的CDR1,SEQ ID NO:20所示的CDR2,SEQ ID NO:21所示的CDR3(对应于抗体株nKN044-220的CDR);

[0105] (8)SEQ ID NO:22所示的CDR1,SEQ ID NO:23所示的CDR2,SEQ ID NO:24所示的CDR3(对应于抗体株nKN044-227的CDR);

[0106] (9)SEQ ID NO:25所示的CDR1,SEQ ID NO:26所示的CDR2,SEQ ID NO:27所示的CDR3(对应于抗体株nKN044-235的CDR);

[0107] (10)SEQ ID NO:28所示的CDR1,SEQ ID NO:29所示的CDR2,SEQ ID NO:30所示的CDR3(对应于抗体株nKN044-244的CDR);

[0108] (11)SEQ ID NO:31所示的CDR1,SEQ ID NO:32所示的CDR2,SEQ ID NO:33所示的CDR3(对应于抗体株nKN044-249的CDR);

[0109] (12)SEQ ID NO:34所示的CDR1,SEQ ID NO:35所示的CDR2,SEQ ID NO:36所示的CDR3(对应于抗体株nKN044-252的CDR);

[0110] (13)SEQ ID NO:37所示的CDR1,SEQ ID NO:38所示的CDR2,SEQ ID NO:39所示的CDR3(对应于抗体株nKN044-264的CDR);

[0111] (14)SEQ ID NO:40所示的CDR1,SEQ ID NO:41所示的CDR2,SEQ ID NO:42所示的CDR3(对应于抗体株nKN044-266的CDR);

[0112] (15)SEQ ID NO:43所示的CDR1,SEQ ID NO:44所示的CDR2,SEQ ID NO:45所示的CDR3(对应于抗体株nKN044-268的CDR);

[0113] (16)SEQ ID NO:46所示的CDR1,SEQ ID NO:47所示的CDR2,SEQ ID NO:48所示的CDR3(对应于抗体株nKN044-275的CDR);和

[0114] (17)SEQ ID NO:49所示的CDR1,SEQ ID NO:50所示的CDR2,SEQ ID NO:51所示的CDR3(对应于抗体株nKN044-280的CDR)。

[0115] 在一些实施方案中,本发明的针对KN044的结合分子中的至少一个免疫球蛋白单一可变结构域是VHH,例如,人源化的VHH。在一些具体实施方案中,所述VHH包含SEQ ID NO:52-68中任一的氨基酸序列。在另一些实施方案中,本发明的针对KN044的结合分子中的VHH包含与SEQ ID NO:52-68中任一具有至少80%、优选地至少90%、更优选地至少95%、甚至更优选地至少99%的序列相同性的氨基酸序列。或者,所述VHH的氨基酸序列与SEQ ID NO:52-68中任一相比包含一或多个氨基酸取代,优选保守氨基酸取代。例如,包含1、2、3、4、5、6、7、8、9或10个保守氨基酸取代。

[0116] 在一些实施方案中,本发明的针对KN044的结合分子是经过亲和力成熟获得的。经亲和力成熟的针对KN044的结合分子可以在一个或多个CDR中具有一个或多个变化,所述变化导致对KN044的亲和力相比于亲本针对KN044的结合分子有所增加。

[0117] 在一些实施方案中,本发明的针对KN044的结合分子,除了至少一个能够特异性结合KN044的免疫球蛋白单一可变结构域外,还包含免疫球蛋白Fc区。在本发明的针对KN044的结合分子中包含免疫球蛋白Fc区可以使所述结合分子形成二聚体。可用于本发明的Fc区可以来自不同亚型的免疫球蛋白,例如,IgG(例如,IgG1、IgG2、IgG3或IgG4亚型)、IgA1、IgA2、IgD、IgE或IgM。

[0118] 在一些实施方案中,所述免疫球蛋白Fc区优选是人免疫球蛋白Fc区,更优选是人IgG1的Fc区。在一些具体实施方案中,所述免疫球蛋白Fc区的氨基酸序列示于SEQ ID NO:69。

[0119] 在一些实施方案中,所述免疫球蛋白Fc区优选是鼠免疫球蛋白Fc区,更优选是鼠IgG1的Fc区。在一些具体实施方案中,所述免疫球蛋白Fc区的氨基酸序列示于SEQ ID NO:70。

[0120] 在一些具体实施方案中,本发明的针对KN044的结合分子中,所述免疫球蛋白Fc区(例如人IgG1的Fc区或鼠IgG1的Fc区)直接或通过接头(如肽接头)间接连接至所述免疫球蛋白单一可变结构域(如VHH)的C端。

[0121] 在另一方面,本发明的针对KN044的结合分子还涵盖能够与由SEQ ID NO:52-68中任一的氨基酸序列组成的VHH结合KN044上的相同表位的针对KN044的抗体分子。

[0122] 本发明的所述针对KN044的结合分子结合KN044的KD值可以小于1×10^{-7}M,优选小于1×10^{-8}M、更优选小于1×10^{-9}M、更优选小于1×10^{-10}M、尤其更优选小于1×10^{-11}M。

[0123] 在一些实施方案中,本发明的针对KN044的结合分子不阻断KN044与靶点(例如

CTLA4)之间的相互作用。

[0124] 在一些实施方案中,本发明的针对KN044的结合分子可阻断KN044与靶点(例如CTLA4)之间的相互作用。在一些实施方案中,本发明的针对KN044的结合分子用于作为KN044抗药抗体(ADA)检测的阳性抗体。

[0125] 在一些实施方案中,本发明的针对KN044的结合分子用于KN044的药代动力学检测。

[0126] 核酸、载体、宿主细胞

[0127] 在另一方面中,本发明涉及编码本发明的针对KN044的结合分子的核酸分子。本发明的核酸可为RNA、DNA或cDNA。根据本发明的一个实施方案,本发明的核酸是基本上分离的核酸。在一些具体实施方式中,编码本发明的针对KN044的结合分子的核酸分子包含SEQ ID NO:71-87中任一的核苷酸序列。

[0128] 本发明的核酸也可呈载体形式,可存在于载体中和/或可为载体的一部分,该载体例如质粒、粘端质粒或YAC。载体可尤其为表达载体,即可提供针对KN044的结合分子体外和/或体内(即在适合宿主细胞、宿主有机体和/或表达系统中)表达的载体。该表达载体通常包含至少一种本发明的核酸,其可操作地连接至一个或多个适合的表达调控元件(例如启动子、增强子、终止子等)。针对在特定宿主中的表达对所述元件及其序列进行选择为本领域技术人员的常识。对本发明的针对KN044的结合分子的表达有用或必需的调控元件及其他元件的具体实例,例如启动子、增强子、终止子、整合因子、选择标记物、前导序列、报告基因。

[0129] 本发明的核酸可基于关于本文给出的本发明的多肽的氨基酸序列的信息通过已知的方式(例如通过自动DNA合成和/或重组DNA技术)制备或获得,和/或可从适合的天然来源加以分离。

[0130] 在另一方面中,本发明涉及表达或能够表达一种或多种本发明的针对KN044的结合分子和/或含有本发明的核酸或载体的宿主细胞。本发明的优选宿主细胞为细菌细胞、真菌细胞或哺乳动物细胞。

[0131] 适合的细菌细胞包括革兰氏阴性细菌菌株(例如大肠杆菌(Escherichia coli)菌株、变形杆菌属(Proteus)菌株及假单胞菌属(Pseudomonas)菌株)及革兰氏阳性细菌菌株(例如芽孢杆菌属(Bacillus)菌株、链霉菌属(Streptomyces)菌株、葡萄球菌属(Staphylococcus)菌株及乳球菌属(Lactococcus)菌株)的细胞。

[0132] 适合的真菌细胞包括木霉属(Trichoderma)、脉孢菌属(Neurospora)及曲菌属(Aspergillus)的物种的细胞;或者包括酵母属(Saccharomyces)(例如酿酒酵母(Saccharomyces cerevisiae))、裂殖酵母属(Schizosaccharomyces)(例如粟酒裂殖酵母(Schizosaccharomyces pombe))、毕赤酵母属(Pichia)(例如巴斯德毕赤酵母(Pichia pastoris)及嗜甲醇毕赤酵母(Pichia methanolica))及汉森酵母属(Hansenula)的物种的细胞。

[0133] 适合的哺乳动物细胞包括例如HEK293细胞、CHO细胞、BHK细胞、HeLa细胞、COS细胞等。

[0134] 然而,本发明也可使用两栖类细胞、昆虫细胞、植物细胞及本领域中用于表达异源蛋白的任何其他细胞。

[0135] 本发明还提供产生本发明的针对KN044的结合分子的方法，所述方法通常包含以下步骤：

[0136] -在允许表达本发明的针对KN044的结合分子的条件下培养本发明的宿主细胞；及

[0137] -从培养物回收由所述宿主细胞表达的针对KN044的结合分子；及

[0138] -任选进一步纯化和/或修饰本发明的针对KN044的结合分子。

[0139] 在一个优选的实施方案中，本发明的针对KN044的结合分子使用哺乳动物细胞产生。本发明的针对KN044的结合分子可以在哺乳动物细胞中获得高表达。例如，表达水平可达大约100mg/L、优选大约150mg/L、优选大约200mg/L、优选大约300mg/L、更优选大约400mg/L或更优选大约500mg/L或者更高。

[0140] 本发明的针对KN044的结合分子可在如上所述细胞中以细胞内方式(例如在细胞质中、在周质中或在包涵体中)产生，接着从宿主细胞分离且任选进一步纯化；或其可以细胞外方式(例如在培养宿主细胞的培养基中)产生，接着自培养基分离且任选进一步纯化。

[0141] 用于重组产生多肽的方法及试剂，例如特定适合表达载体、转化或转染方法、选择标记物、诱导蛋白表达的方法、培养条件等在本领域中是已知的。类似地，适用于制造本发明的针对KN044的结合分子的方法中的蛋白分离及纯化技术为本领域技术人员所公知。

[0142] 然而，本发明的针对KN044的结合分子也可以通过本领域已知的其它产生蛋白质的方法获得，例如化学合成，包括固相或液相合成。

[0143] 检测

[0144] 在另一方面，本发明提供了一种检测靶样品中KN044的存在和/或对样品中KN044定量的方法，所述方法包括：

[0145] a)在针对KN044的结合分子与KN044之间能够形成复合物的条件下，使所述靶样品和对照样品分别与本发明的针对KN044的结合分子接触；

[0146] b)检测复合物的形成，

[0147] 其中所述靶样品与对照样品之间复合物形成的差异指示靶样品中KN044的存在，优选地，所述对照样品含有预定量的KN044。

[0148] 在一些实施方案中，所述方法用于KN044的药代动力学检测，所述靶样品为血液样品，例如，血浆或血清。

[0149] 在一些实施方案中，本发明的针对KN044的结合分子可缀合有可用于检测或可被其他试剂检测到的荧光染料、化学物质、多肽、酶、同位素、标签等。在一个实施方案中，所述检测通过本领域已知的用于免疫检测的方法进行，如蛋白印迹或ELISA。

[0150] 在一些实施方案中，所述靶样品是通过不同方法制备的KN044产品。

[0151] 在另一方面，本发明提供一种用于靶样品中KN044的抗药抗体(ADA)检测的方法，所述方法包括：

[0152] a)使KN044分别与靶样品和本发明的针对KN044的结合分子接触；

[0153] b)检测复合物的形成，

[0154] 其中KN044与本发明的针对KN044的结合分子所形成的复合物用作靶样品中KN044的抗药抗体(ADA)检测的阳性对照，所述靶样品为施用了KN044的对象的血液样品，例如，血浆或血清。

[0155] 在一些实施方案中，本发明的KN044可缀合有可用于检测或可被其他试剂检测到

的荧光染料、化学物质、多肽、酶、同位素、标签等。在一个实施方案中，所述检测通过本领域已知的用于免疫检测的方法进行，如蛋白印迹或ELISA。

[0156] 在一些实施方案中，本发明的针对KN044的结合分子识别KN044中的VHH的框架区，因此可以广泛地识别与KN044的VHH的框架区相同、CDR不同的其他VHH，由此可以用于广泛的VHH的检测以及亲和纯化。

[0157] 试剂盒

[0158] 本发明的范围内还包括一种试剂盒，该试剂盒包括本发明的针对KN044的结合分子。试剂盒一般包括表明试剂盒内容物的预期用途(例如，用于检测KN044或其活性)的标签。术语标签包括在试剂盒上或与试剂盒一起提供的或以其他方式随试剂盒提供的任何书面的或记录的材料。

[0159] 另一方面，本发明的范围内还包括一种试剂盒，该试剂盒包括本发明的针对KN044的结合分子。试剂盒一般包括表明试剂盒内容物的预期用途(例如，用于KN044的抗药抗体(ADA)检测)的标签。术语标签包括在试剂盒上或与试剂盒一起提供的或以其他方式随试剂盒提供的任何书面的或记录的材料。

[0160] 药物组合物

[0161] 另一方面，本发明提供一种组合物，例如药物组合物，其含有与药学上可接受的载体配制在一起的一种或组合的本发明的针对KN044的结合分子。这样的组合物可以包含一种或组合的(例如两种或多种不同的)本发明的针对KN044的结合分子。例如，本发明的药物组合物可以含有结合靶抗原上的不同表位的抗体分子组合。

[0162] 本文使用的“药学上可接受的载体”包括生理学相容的任何和所有的溶剂、分散介质、包衣、抗细菌剂和抗真菌剂、等渗剂和吸收延迟剂等。优选地，该载体适合于静脉内、肌内、皮下、肠胃外、脊柱或表皮施用(如通过注射或输注)。根据施用途径，可将活性化合物即抗体分子包裹于一种材料中，以保护该化合物免受可使该化合物失活的酸和其他天然条件的作用。

[0163] 疾病预防和治疗

[0164] 在另一方面，本发明提供了本发明所述针对KN044的结合分子、核酸分子、宿主细胞及药物组合物在治疗与KN044相关的疾病中的用途和方法。可用本发明的针对KN044的结合分子治疗的KN044相关的疾病为由于施用KN044所引发的副作用，如细胞因子风暴，所述KN044用于在所述对象中预防和/或治疗与CTLA4相关的疾病，例如癌症或感染性疾病。本发明的针对KN044的结合分子或药物组合物用于中和KN044。

实施例

[0165] 下面将通过实施例的方式进一步说明本发明，但并不因此将本发明限制在所描述的实施例范围中。

[0166] 实施例1:针对KN044的重链单域抗体的筛选及亲和力检测

[0167] 1.1文库的构建：

[0168] 选取未经免疫过的14只骆驼分离其淋巴细胞，并摘取了5只骆驼的脾脏和8只骆驼的淋巴结，使用QIAGEN公司提供的RNA提取试剂盒提取淋巴细胞和组织总RNA，使用Super-Script III FIRST STRANDSUPERMIX试剂盒按照说明书将提取的RNA全部反转录为cDNA，用

巢式PCR扩增编码重链抗体的可变区的核酸片段。

[0169] 回收目标重链单域抗体核酸片段，并使用限制性内切酶(购自NEB)PstI及NotI将其克隆进入噬菌体展示用载体pMECS中。产物随后电转化至大肠杆菌电转感受态细胞TG1中，构建非免疫单域抗体噬菌体展示文库并对文库进行检定。通过梯度稀释铺板，计算库容的大小为1.4×10^{9}。为检测文库的插入率，随机挑选100个克隆测序检测，具有正确的外源片段插入的克隆为99个，正确率为99％。通过对测序克隆的DNA和氨基酸序列分别分析比对，证实所有序列完全不同，均为预期的骆驼VHH序列，其多样性为100％。

[0170] 1.2针对KN044的重链单域抗体淘选：

[0171] 以2μg/孔的量用重组蛋白KN044包被平板，4℃放置过夜。第二天用2％脱脂奶室温封闭2小时后，每孔加入100μl噬菌体(约10^{10}-10^{13}pfu，来自1.1双峰驼非免疫单域抗体展示文库)，在室温下作用2小时。之后用PBST(PBS中含有0.05％吐温20)洗25遍，以洗掉不结合的噬菌体。最后用甘氨酸(100mM，pH 2.0)将与KN044特异性结合的噬菌体解离，并感染处于生长对数期的大肠杆菌TG1，产生并纯化噬菌体用于下一轮的筛选。上述筛选过程重复3轮，第4轮降低重组蛋白KN044的包被浓度至0.5μg/孔，其余操作同上。由此，富集阳性的克隆，从而利用噬菌体展示技术筛选抗体库中KN044特异抗体。

[0172] 1.3用酶联免疫方法(ELISA)筛选特异性单个阳性克隆：

[0173] 将以上淘选后获得的KN044结合阳性的噬菌体感染空白大肠杆菌并铺板。随后随机挑选单菌落，分别接种至2TY-AG，培养至OD600约0.8时，加入IPTG终浓度约1mM，25℃过夜诱导表达。单域抗体表达于大肠杆菌周质中，次日收获菌体，并加入TES用渗透压冲击法裂解菌体，上清液用于ELISA检测。用KN044包被平板4℃过夜，将获得的样品裂解上清液(对照组为空白大肠杆菌裂解上清液)加入，室温下反应2小时。洗涤之后加入二抗山羊抗HA标签HRP(购自abcam)，室温反应2小时。洗涤之后加入TMB显色液，读取450nm和650nm波长吸光度值，450nm波长的吸光度值减去650nm波长的吸光度值为最终的吸光度值。当样品孔OD值大于对照孔OD值2倍以上时，判定为阳性克隆孔。将阳性克隆测序。

[0174] 表1.用酶联免疫方法(ELISA)筛选特异性单个阳性克隆结果

[0175]

克隆名称	OD
nKN044-19-3	0.159
nKN044-97	0.678
nKN044-125	1.172
nKN044-194	0.754
nKN044-200	0.981
nKN044-216	0.886
nKN044-220	1.591
nKN044-227	0.917
nKN044-235	1.268

[0176]

nKN044-244	1.056
nKN044-249	0.534
nKN044-252	0.76
nKN044-264	1.111
nKN044-266	0.968
nKN044-268	1.027
nKN044-275	0.791
nKN044-280	1.298
空白	0.03

[0177] 根据序列比对软件BioEdit分析各个克隆的氨基酸序列。把CDR1、CDR2、CDR3序列同源性＞90％的克隆视为同一抗体株。最终共获得17株不同的抗体，结合检测结果见表1，序列见图1，并根据Kabat和Chothia的规则用框标出CDR区。

[0178] 1.4阳性克隆的原核表达以及纯化

[0179] 将1.3中筛选获得的阳性克隆单菌落在2TY-AG中培养过夜的种子液转接到50mL2TY-AG培养基中，37℃培养至OD600约0.8时，加入IPTG终浓度约1mM，25℃过夜诱导表达。次日收获菌体，并用Tris缓冲液重悬并超声破碎菌体，使用IMAC层析利用单域抗体上的His标签对上清液中的单域抗体进行纯化，得到相应的目标蛋白。

[0180] 1.5原核表达的针对KN044的重链单域抗体对KN044的亲和力检测

[0181] 以0.5μg/孔的量用KN044包被平板，4℃放置过夜。加入1.4中获得的、带有His标签的候选单域抗体的梯度稀释系列，同时设空白对照组，室温下反应2小时。洗涤之后加入辣根过氧化物酶标记的兔抗His标签的二抗(streptavidin-HRP，SIGMA)，室温反应1.5小时。洗涤之后加入显色液，读取450nm和650nm波长的吸光度值，450nm波长的吸光度值减去650nm波长的吸光度值为最终的吸光度值。应用软件SotfMax Pro v5.4进行数据处理和作图分析，通过四参数拟合，得到针对KN044的候选单域抗体与KN044的结合曲线及EC50值，以反映这些候选单域抗体对KN044的亲和能力。

[0182] 结果见图2A和2B，其中纵坐标为OD值，横坐标为针对KN044的候选单域抗体的浓度(单位ng/mL)。结果表明这些针对KN044的候选单域抗体都能有效的结合KN044。

[0183] 实施例2：通过在哺乳动物细胞中表达制备针对KN044的单域抗体-Fc融合蛋白

[0184] 2.1制备表达针对KN044的单域抗体-Fc融合蛋白的载体

[0185] 设计引物进行PCR扩增KN044单域抗体VHH片段，与编码人和鼠IgG1-Fc的DNA片段融合(其中nKN044-19-3扩增的KN044单域抗体VHH片段分别与编码人和鼠IgG1-Fc的DNA片段融合，nKN044-97扩增的KN044单域抗体VHH片段与编码人IgG1-Fc的DNA片段融合)，并克隆至常规哺乳动物表达载体，获得用于在哺乳动物中表达针对KN044的单域抗体-Fc融合蛋白和/或针对KN044的单域抗体-muFc融合蛋白的重组质粒。其中扩增不同VHH片段使用通用引物，与人IgG1-Fc的DNA片段融合使用的通用引物如下：

[0186] 上游引物cccACCGGTCAGGTGCAGCTGCAGGAGTC(SEQ ID NO:89)

[0187] 下游引物cccGGATCCTGAGGAGACGGTGACCTGG(SEQ ID NO:90)

[0188] 与鼠IgG1-Fc的DNA片段融合使用的通用引物如下：

[0189] 上游引物cccACCGGTCAGGTGCAGCTGCAGGAGTC(SEQ ID NO:91)

[0190] 下游引物cccGGATCCATGCTGCCTGAGGAGACGGTGACCTGG(SEQ ID NO:92)

[0191] 2.2制备针对KN044的单域抗体-Fc融合蛋白

[0192] 将2.1构建获得载体转染至HEK293细胞进行抗体的瞬时表达。将重组表达质粒用Freestyle293培养基稀释并加入转化所需聚乙烯亚胺(PEI)溶液，将每组质粒/PEI混合物分别加入HEK293细胞悬液中，放置在37℃，5％CO_2，130rpm培养。四小时后补加EX-CELL293培养基，130rpm悬浮培养。24小时后加3.8mM VPA，72小时后加入4g/L葡萄糖。培养5～6天后，收集瞬时表达培养上清液，通过Protein A亲和层析法，纯化目标针对KN044的单域抗体-Fc融合蛋白和/或针对KN044的单域抗体-muFc融合蛋白。通过SDS-PAGE以及SEC-HPLC检测所获得的蛋白质的纯度。各蛋白质表达情况和纯度分析见下表2：

[0193] 表2:所获得的针对KN044的单域抗体-F_C融合蛋白瞬转后一步纯化结果

[0194]

抗体	表达量(mg/L)	SDS-PAGE纯度%	单体比例%
nKN044-19-3-Fc	358.5	＞95%	98.97
nKN044-19-3-muFc	384	＞95%	96.47
nKN044-97-Fc	93.6	＞95%	95.69

[0195] 可见,针对KN044的单域抗体-Fc融合蛋白nKN044-19-3-Fc和nKN044-19-3-muFc表达量均在300mg/L以上,且经过Protein A亲和层析柱一步纯化后,获得了具有稳定浓度以及高纯度的目的蛋白质;nKN044-97-Fc表达量相对低一些,但纯度很高,满足实验需求。

[0196] 实施例3:鉴定针对KN044的单域抗体-Fc融合蛋白的功能

[0197] 3.1针对KN044的单域抗体-Fc融合蛋白与抗原KN044的结合曲线

[0198] 以0.5μg/孔的量用实施例2所得的针对KN044的单域抗体-Fc融合蛋白包被平板,同时设置阳性对照,4℃过夜。加入KN044-Biotin的梯度稀释系列,室温下反应2小时。洗涤之后加入辣根过氧化物酶标记链霉亲和素(streptavidin-HRP,SIGMA),室温反应1.5小时。洗涤之后加入显色液,读取450nm和650nm波长的吸光度值,450nm波长的吸光度值减去650nm波长的吸光度值为最终的吸光度值。应用软件SotfMax Pro v5.4进行数据处理和作图分析,通过四参数拟合,得到针对KN044的单域抗体-Fc融合蛋白与KN044的结合曲线及EC50值,以反映抗体对KN044的亲和能力。

[0199] 结果见图3,其中纵坐标为OD值,横坐标为KN044-Biotin蛋白的浓度(单位ng/mL);圆形、方形、正三角、倒三角分别代表针对KN044的单域抗体-Fc融合蛋白:nKN044-19-3-Fc、nKN044-19-3-muFc、nKN044-97-Fc、阳性对照。抗体nKN044-19-3-Fc和nKN044-19-3-muFc对KN044均有很高的亲和力,且与阳性对照相差不大;抗体nKN044-97-Fc对KN044没有结合。

[0200] 3.2竞争ELISA考察针对KN044的单域抗体-Fc融合蛋白对CTLA4和KN044相互作用的阻断效果

[0201] 以0.5μg/孔的量用CTLA4-muFc融合蛋白包被平板,同时设置阴性对照,4℃过夜。加入实施例2所得的融合蛋白nKN044-19-3-muFc的梯度稀释系列(对照组只是缓冲液)以及20ng/孔KN044(空白组不加入任何抗体或蛋白,只加入等体积缓冲液),室温下反应2小时。之后加入山羊抗人IgG(Fc特异性)-HRP(购自Sigma公司),室温反应2小时。洗涤之后加入显色液,读取450nm和650nm波长吸光度值,450nm波长的吸光度值减去650nm波长的吸光度值为最终的吸光度值。当样品OD值比对照OD值＜0.8时,则认为抗体有阻断效果。结果显示,抗体nKN044-19-3-muFc对CTLA4和KN044相互作用无阻断效应。

[0202] 3.3 Bridging ELISA验证针对KN044的单域抗体-Fc融合蛋白作为KN044抗药抗体(ADA)检测中的阳性抗体

[0203] 用KN044包被平板,4℃过夜。加入实施例2所得的融合蛋白nKN044-19-3-muFc的梯度稀释系列,稀释液分别为1%BSA-0.05%PBST、1X人空白血浆、1X食蟹猴空白血浆,同时设空白对照组,室温下反应2小时。之后加入KN044-Biotin,室温反应2小时。洗涤之后加入辣根过氧化物酶标记链霉亲和素(streptavidin-HRP,SIGMA),室温反应1.5小时。洗涤之后加入显色液,读取450nm和650nm波长吸光度值,450nm波长的吸光度值减去650nm波长的吸光度值为最终的吸光度值。应用软件SotfMax Pro v5.4进行数据处理和作图分析,通过四参数拟合,得到不同稀释液稀释后的抗体与KN044的结合曲线及EC50值,以反映不同稀释液中

成分对抗体的中和作用。

[0204] 结果见表3。由结果可知，nKN044-19-3-Fc能够通过ADA bridging ELISA的方法检出，且保持较好的灵敏度。而其在未稀释的人空白血浆和食蟹猴空白血浆中仍然能被检出，且其灵敏度与在PBST稀释液中时基本一致。因此，抗体nKN044-19-3-Fc可以作为KN044抗药抗体(ADA)检测中的阳性抗体。

[0205] 表3.Bridging ELISA验证针对KN044的单域抗体-Fc融合蛋白作为KN044抗药抗体(ADA)检测中的阳性抗体的结果

[0206]

样品浓度(ng/mL)	nKN044-19-3-muFc(1%BSA+PBST20)	nKN044-19-3-muFc(1X 人空白血浆)	nKN044-19-3-muFc(1X 猴空白血清)
12800	3.578	2.013	1.471
6400	3.503	1.266	0.8
3200	2.717	0.727	0.477

[0207]

1600	1.534	0.436	0.275
800	0.607	0.272	0.159
400	0.24	0.196	0.104
200	0.122	0.167	0.08
100	0.098	0.153	0.07
50	0.083	0.154	0.072
0	0.077	0.146	0.068

[0208] 序列表：

[0209] >SEQ ID NO:1

[0210] GYTYTNYIMA

[0211] >SEQ ID NO:2

[0212] SIYIGSGSTNYADSVKG

[0213] >SEQ ID NO:3

[0214] TRDGRWY

[0215] >SEQ ID NO:4

[0216] GYTYRRRSMG

[0217] >SEQ ID NO:5

[0218] SISTDGTTNYADSVKG

[0219] >SEQ ID NO:6

[0220] RSGLAASLLGSWYRY

[0221] >SEQ ID NO:7

[0222] GYSYRRRCMG

[0223] >SEQ ID NO:8

[0224] TINSGGGSTYYADSVKG

[0225] >SEQ ID NO:9

[0226] VRTMTLRYGNLTLRPDY

[0227] >SEQ ID NO:10

[0228] RYTIRSICMA

[0229] >SEQ ID NO:11

[0230] DIDRYGTTHVADSVKD

[0231] >SEQ ID NO:12

[0232] DSSRWCGAWWSPSSYNY

[0233] >SEQ ID NO:13

[0234] RYTPRMA

[0235] >SEQ ID NO:14

[0236] ELNFFGTATYADSVKG

[0237] ＞SEQ ID NO:15

[0238] GLRPGWWSLRLEPGAYNY

[0239] ＞SEQ ID NO:16

[0240] GFPFSWSSMN

[0241] ＞SEQ ID NO:17

[0242] SINRRGTVTVYADSVKG

[0243] >SEQ ID NO:18

[0244] ARRPETWYTDIWTPALFGT

[0245] >SEQ ID NO:19

[0246] GFTFAAPYYIS

[0247] >SEQ ID NO:20

[0248] SINTYNSVTYYADSVKG

[0249] >SEQ ID NO:21

[0250] GWLFRGSWTGPRNFRY

[0251] >SEQ ID NO:22

[0252] GYTNSISKMG

[0253] >SEQ ID NO:23

[0254] TIFTAGGSTYYADSVKG

[0255] >SEQ ID NO:24

[0256] ARPGWIWPTIKTMTRYEYNY

[0257] >SEQ ID NO:25

[0258] GYTYRRYCMG

[0259] >SEQ ID NO:26

[0260] RIGTYGTTWYADSVKG

[0261] >SEQ ID NO:27

[0262] DPGRYCRGDLLRTTLFAK

[0263] >SEQ ID NO:28

[0264] RNTYRNRWMG

[0265] >SEQ ID NO:29

[0266] RINIRSGRAYYADSVKG

[0267] >SEQ ID NO:30

[0268] SQSGGFFYGVLDTRSYHY

[0269] >SEQ ID NO:31

[0270] GYTYSSNCIG

[0271] >SEQ ID NO:32

[0272] LTSSGNGRTWVADSVKG

[0273] >SEQ ID NO:33

[0274] GPACSGVYWKWALRG

[0275] >SEQ ID NO:34

[0276] GYIYSRNWMG

[0277] >SEQ ID NO:35

[0278] SISVNGDNTHYADSVKG

[0279] >SEQ ID NO:36

[0280] YWPGGGGSAAWSFWGRIFNF

[0281] >SEQ ID NO:37

[0282] GYIYSNCMG

[0283] >SEQ ID NO:38

[0284] AIDRYGRATYADSVKG

[0285] >SEQ ID NO:39

[0286] ARWRASCVTLVRFTS

[0287] >SEQ ID NO:40

[0288] GYTLRTNYIG

[0289] >SEQ ID NO:41

[0290] AIYRGGGSTYYGSTYYADSVKG

[0291] >SEQ ID NO:42

[0292] GRSPFPVAFGGAWYSAGRYPY

[0293] >SEQ ID NO:43

[0294] GYTYSTKRVA

[0295] >SEQ ID NO:44

[0296] TISATMGIPIYADSVKG

[0297] >SEQ ID NO:45

[0298] GRPSRAAFLGYLRAAAYDY

[0299] >SEQ ID NO:46

[0300] SEYTRRSKRMG

[0301] >SEQ ID NO:47

[0302] AISSSGAFTYYADSVKG

[0303] >SEQ ID NO:48

[0304] GLRPGWWSLRLEPGAYNY

[0305] >SEQ ID NO:49

[0306] GSTYTNNYIA

[0307] >SEQ ID NO:50

[0308] TIDRRLGSTYYADSVRG

[0309] >SEQ ID NO:51

[0310] GRGRAWLSRVWYNY

[0311] >SEQ ID NO:52 nKN044-19-3

[0312] QVQLQESGGASVQAGGSLRLSCAASGYTYTNYIMAWFRQYPGKEREGVASIYIGSGSTNYADSVKGRFTISQDNAKNTLY LQMNNLKPEDTAMYYCAATRDGRWYFGQGTQVTVSS

[0313] >SEQ ID NO:53 nKN044-97

[0314] QVQLQESGGGSVQAGGSLRLSCAASGYTYRRRSMGWFRQAPGKEREFVSSISTDGTTNYADSVKGRFTIYRDNAKNTVY LQMNSLKPEDTAVYYCASRSGLAASLLGSWYRYWGQGTQVTVSS

[0315] >SEQ ID NO:54 nKN044-125

[0316] QVQLQESGGGSVQTGGSLRLSCVASGYSYRRRCMGWFRQAPGKGLEWVSTINSGGGSTYYADSVKGRFTISHDSATSTV YLQMNNLKPEDTAMYHCAVVRTMTLRYGNLTLRPDYWGQGTQVTVSS

[0317] >SEQ ID NO:55 nKN044-194

[0318] QVQLQESGGGSVQAGGSLRLSCAASRYTIRSICMAWFRQAPGKERERVADIDRYGTTHVADSVKDRFSISTDSAKNTLYL QMNNLKPEDAGMYYCAADSSRWCGAWWSPSSYNYWGQGTQVTVSSAAA

[0319] >SEQ ID NO:56 nKN044-200

[0320] QVQLQESGGGPVQAGGSLRLSCAASRYTPRMAWFRQGPGKEREVVGELNFFGTATYADSVKGRFTISKDNTNNTLYLQ MNALKPEDTAMYYCAAGLRPGWWSLRLEPGAYNYWGQGTQVTVSS

[0321] >SEQ ID NO:57 nKN044-216

[0322] QVQLQESGGGLVQPGGSLRLSCAASGFPFSWSSMNWVRQAPGKGMEWVSSINRRGTVTVYADSVKGRFTISRDNAKN TVALQMNNLQPEDTAVYYCARARRPETWYTDIWTPALFGTRGQGTQVTVSS

[0323] >SEQ ID NO:58 nKN044-220

[0324] QVQLQESGGGLVQPGGSLTLSCEASGFTFAAPYYISWVRQAPGKGLDWLSSINTYNSVTYYADSVKGRFTITRQNGGRTW NLQMNYLEPEDSGIYYCAAGWLFRGSWTGPRNFRYWGQGTQVTVSS

[0325] >SEQ ID NO:59 nKN044-227

[0326] QVQLQESGGGSVQAGGSLRLSCTASGYTNSISKMGWFRQAPGKGRTEVATIFTAGGSTYYADSVKGRFTISQDNAKNTLY LQMNNLKPEDTAMYYCAVARPGWIWPTIKTMTRYEYNYWGQGTQVTVSS

[0327] >SEQ ID NO:60nKN044-235

[0328] QVQLQESGGGSVQAGGSLRLSCTASGYTYRRYCMGWFRQAPGKEREGVARIGTYGTTWYADSVKGRFTISRDNAKNTV YLQMNSLKPEDTAMYYCAADPGRYCRGDLLRTTLFAKWGQGTQVTVSS

[0329] >SEQ ID NO:61 nKN044-244

[0330] QVQLQESGGGSVQPGGSLRLSCAASRNTYRNRWMGWFRQAPGKEREGVARINIRSGRAYYADSVKGRFTISRDNAKNT LYLQMNSLKPEDTAMYYCAASQSGGFFYGVLDTRSYHYWGPGTQVTVSS

[0331] >SEQ ID NO:62 nKN044-249

[0332] QVQLQESGGGSVQAGGSLRLSCAASGYTYSSNCIGWFRQAPGKGRNWVALTSSGNGRTWVADSVKGRFTISQDNAKN TVYLQMNSLKPEDTGAYYCAAGPACSGVYWKWALRGWGQGTQVTVSS

[0333] >SEQ ID NO:63 nKN044-252

[0334] QVQLQESGGGSVQAGGSLRLSCAASGYIYSRNWMGWFRQAPGKEREWVASISVNGDNTHYADSVKGRFTISQDAAKN TVYLQMTSLKPEDTAMYYCAAYWPGGGGSAAWSFWGRIFNFRGQGTQVTVSS

[0335] >SEQ ID NO:64 nKN044-264

[0336] QVQLQESGGGSVQAGGSLRLSCVASGYIYSNCMGWFRQAPGKEREGLAAIDRYGRATYADSVKGRFTISRDNLKNTVSL QMNSLKPEDTATYYCAAARWRASCVTLVRFTSWGQGTQVTVSS

[0337] >SEQ ID NO:65 nKN044-266

[0338] QVQLQESGGGSVQAGGSLRLSCVAAGYTLRTNYIGWFRQAPGKEREGVAAIYRGGGSTYYGSTYYADSVKGRFTISRDNA KNTVYLEMNSLKPEDTAMYYCAAGRSPFPVAFGGAWYSAGRYPYWGQGTQVTVSS

[0339] >SEQ ID NO:66 nKN044-268

[0340] QVQLQESGGGSVQAGGSLRLSCAASGYTYSTKRVAWFRQAPGKEREGVATISATMGIPIYADSVKGRFTISRDNAKNTVYL QMNNLKPEDTAMYYCAAGRPSRAAFLGYLRAAAYDYWGQGTQVTVSS

[0341] >SEQ ID NO:67 nKN044-275

[0342] QVQLQESGGGSVQAGGSLRLSCAASEYTRRSKRMGWFRQAPGKEREGVAAISSSGAFTYYADSVKGRFTMSQDNTKNT VYLQMNSLKPEDSAMYYCAAGLRPGWWSLRLEPGAYNYWGQGTQVTVSS

[0343] >SEQ ID NO:68 nKN044-280

[0344] QVQLQESGGGLVQPGGSLRLSCTASGSTYTNNYIAWFRQAPGKEREGVATIDRRLGSTYYADSVRGRFTISQDKAKNTVYL QMNSLKPEDTAMYYCAAGRGRAWLSRVWYNYWGQGTQVTVSS

[0345] >SEQ ID NO:69 IgG1-Fc

[0346] EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0347] >SEQ ID NO:70 IgG1-muFc

[0348] MDPKSSDKTHTCPPCPAPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNST FRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEW QWNGQPAENYKNTQPIMNTNGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

[0349] >SEQ ID NO:71 nKN044-19-3

[0350] CAGGTGCAGCTGCAGGAGTCTGGGGGAGCCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTGTGCAGCCTCTG GATACACCTACACAAATTACATCATGGCCTGGTTCCGCCAGTATCCAGGGAAGGAGCGCGAGGGGGTCGCATCTATTTA TATTGGTAGTGGTAGCACAAACTATGCCGACTCCGTGAAGGGCCGATTCACCATCTCCCAAGACAACGCCAAGAACAC CCTTTATCTGCAAATGAACAATCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCGACGCGGGATGGTCGATGG TACTTCGGCCAGGGGACCCAGGTCACCGTCTCCTCA

[0351] >SEQ ID NO:72 nKN044-97

[0352] CAGGTGCAGCTGCAGGAGTCTGGAGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTGTGCAGCCTCTG GATACACCTACAGGCGCAGGTCTATGGGGTGGTTCCGCCAGGCTCCAGGGAAGGAGCGCGAGTTCGTCTCATCTATTA GCACTGATGGTACCACAAACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTACCGAGACAACGCAAAGAACACGG TGTATCTGCAGATGAACAGCCTGAAACCTGAGGACACTGCCGTGTACTACTGTGCGTCGAGATCAGGACTTGCTGCCA GCCTGTTGGGATCGTGGTATAGGTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

[0353] >SEQ ID NO:73 nKN044-125

[0354] CAGGTGCAGCTGCAGGAGTCTGGAGGAGGCTCGGTGCAGACTGGAGGGTCTCTGAGACTCTCCTGTGTAGCCTCTG
GGTACTCGTATAGACGCCGCTGCATGGGCTGGTTCCGACAGGCTCCAGGGAAGGGGCTCGAGTGGGTCTCAACTATTA
ATAGTGGTGGTGGTAGCACATACTATGCCGACTCCGTGAAGGGCCGATTCACCATCAGCCATGACAGCGCCACGAGCA
CGGTCTATCTGCAAATGAATAATCTGAAACCTGAGGACACCGCTATGTACCACTGTGCGGTAGTTCGGACAATGACCCTT
AGGTACGGAAACCTTACGCTTCGACCCGACTACTGGGGCCAGGGAACCCAGGTCACCGTCTCCTCA

[0355] >SEQ ID NO:74 nKN044-194

[0356] CAGGTGCAGCTGCAGGAGTCTGGAGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTGTGCAGCCTCCA
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[0336] <400> 35

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[0338] 1 5 10 15

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[0396] Ala Asp Ser Val Lys Gly

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[0406] 1 5 10 15

[0407] Gly Arg Tyr Pro Tyr

[0408] 20

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[0429] <211> 19

[0430] <212> PRT

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[0433] <223> Artificial Sequence

[0434] <400> 45

[0435] Gly Arg Pro Ser Arg Ala Ala Phe Leu Gly Tyr Leu Arg Ala Ala Ala

[0436] 1 5 10 15

[0437] Tyr Asp Tyr

[0438] <210> 46

[0439] <211> 11

[0440] <212> PRT

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[0443] <223> Artificial Sequence

[0444] <400> 46

[0445] Ser Glu Tyr Thr Arg Arg Ser Lys Arg Met Gly

[0446] 1 5 10

[0447] <210> 47

[0448] <211> 17

[0449] <212> PRT

[0450] <213> Artificial Sequence

[0451] <220>

[0452] <223> Artificial Sequence

[0453] <400> 47

[0454] Ala Ile Ser Ser Ser Gly Ala Phe Thr Tyr Tyr Ala Asp Ser Val Lys

[0455] 1 5 10 15

[0456] Gly

[0457] <210> 48

[0458] <211> 18

[0459] <212> PRT

[0460] <213> Artificial Sequence

[0461] <220>

[0462] <223> Artificial Sequence

[0463] <400> 48

[0464] Gly Leu Arg Pro Gly Trp Trp Ser Leu Arg Leu Glu Pro Gly Ala Tyr

[0465] 1 5 10 15

[0466] Asn Tyr

[0467] <210> 49

[0468] <211> 10

[0469] <212> PRT

[0470] <213> Artificial Sequence

[0471] <220>

[0472] <223> Artificial Sequence

[0473] <400> 49

[0474] Gly Ser Thr Tyr Thr Asn Asn Tyr Ile Ala

[0475] 1 5 10

[0476] <210> 50

[0477] <211> 17

[0478] <212> PRT

[0479] <213> Artificial Sequence

[0480] <220>

[0481] <223> Artificial Sequence

[0482] <400> 50

[0483] Thr Ile Asp Arg Arg Leu Gly Ser Thr Tyr Tyr Ala Asp Ser Val Arg

[0484] 1 5 10 15

[0485] Gly

[0486] <210> 51

[0487] <211> 14

[0488] <212> PRT

[0489] <213> Artificial Sequence

[0490] <220>

[0491] <223> Artificial Sequence

[0492] <400> 51

[0493] Gly Arg Gly Arg Ala Trp Leu Ser Arg Val Trp Tyr Asn Tyr

[0494] 1 5 10

[0495] <210> 52

[0496] <211> 116

[0497] <212> PRT

[0498] <213> Artificial Sequence

[0499] <220>

[0500] <223> nKN044-19-3

[0501] <400> 52

[0502] Gln Val Gln Leu Gln Glu Ser Gly Gly Ala Ser Val Gln Ala Gly Gly

[0503] 1 5 10 15

[0504] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Tyr Thr Asn Tyr

[0505] 20 25 30

[0506] Ile Met Ala Trp Phe Arg Gln Tyr Pro Gly Lys Glu Arg Glu Gly Val

[0507] 35 40 45

[0508] Ala Ser Ile Tyr Ile Gly Ser Gly Ser Thr Asn Tyr Ala Asp Ser Val

[0509] 50 55 60

[0510] Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Thr Leu Tyr

[0511] 65 70 75 80

[0512] Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys

[0513] 85 90 95

[0514] Ala Ala Thr Arg Asp Gly Arg Trp Tyr Phe Gly Gln Gly Thr Gln Val

[0515] 100 105 110

[0516] Thr Val Ser Ser

[0517] 115

[0518] <210> 53

[0519] <211> 123

[0520] <212> PRT

[0521] <213> Artificial Sequence

[0522] <220>

[0523] <223> nKN044-97

[0524] <400> 53

[0525] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly

[0526] 1 5 10 15

[0527] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Tyr Arg Arg Arg

[0528] 20 25 30

[0529] Ser Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val

[0530] 35 40 45

[0531] Ser Ser Ile Ser Thr Asp Gly Thr Thr Asn Tyr Ala Asp Ser Val Lys

[0532] 50 55 60

[0533] Gly Arg Phe Thr Ile Tyr Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu

[0534] 65 70 75 80

[0535] Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala

[0536] 85 90 95

[0537] Ser Arg Ser Gly Leu Ala Ala Ser Leu Leu Gly Ser Trp Tyr Arg Tyr

[0538] 100 105 110

[0539] Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0540] 115 120

[0541] <210> 54

[0542] <211> 126

[0543] <212> PRT

[0544] <213> Artificial Sequence

[0545] <220>

[0546] <223> nKN044-125

[0547] <400> 54

[0548] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Thr Gly Gly

[0549] 1 5 10 15

[0550] Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Tyr Ser Tyr Arg Arg Arg

[0551] 20 25 30

[0552] Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

[0553] 35 40 45

[0554] Ser Thr Ile Asn Ser Gly Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val

[0555] 50 55 60

[0556] Lys Gly Arg Phe Thr Ile Ser His Asp Ser Ala Thr Ser Thr Val Tyr

[0557] 65 70 75 80

[0558] Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Met Tyr His Cys

[0559] 85 90 95

[0560] Ala Val Val Arg Thr Met Thr Leu Arg Tyr Gly Asn Leu Thr Leu Arg

[0561] 100 105 110

[0562] Pro Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0563] 115 120 125

[0564] <210> 55

[0565] <211> 128

[0566] <212> PRT

[0567] <213> Artificial Sequence

[0568] <220>

[0569] <223> nKN044-194

[0570] <400> 55

[0571] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly

[0572] 1 5 10 15

[0573] Ser Leu Arg Leu Ser Cys Ala Ala Ser Arg Tyr Thr Ile Arg Ser Ile

[0574] 20 25 30

[0575] Cys Met Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Arg Val

[0576] 35 40 45

[0577] Ala Asp Ile Asp Arg Tyr Gly Thr Thr His Val Ala Asp Ser Val Lys

[0578] 50 55 60

[0579] Asp Arg Phe Ser Ile Ser Thr Asp Ser Ala Lys Asn Thr Leu Tyr Leu

[0580] 65 70 75 80

[0581] Gln Met Asn Asn Leu Lys Pro Glu Asp Ala Gly Met Tyr Tyr Cys Ala

[0582] 85 90 95

[0583] Ala Asp Ser Ser Arg Trp Cys Gly Ala Trp Trp Ser Pro Ser Ser Tyr

[0584] 100 105 110

[0585] Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Ala Ala Ala
[0586] 115 120 125
[0587] <210> 56
[0588] <211> 123
[0589] <212> PRT
[0590] <213> Artificial Sequence
[0591] <220>
[0592] <223> nKN044-200
[0593] <400> 56
[0594] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Pro Val Gln Ala Gly Gly
[0595] 1 5 10 15
[0596] Ser Leu Arg Leu Ser Cys Ala Ala Ser Arg Tyr Thr Pro Arg Met Ala
[0597] 20 25 30
[0598] Trp Phe Arg Gln Gly Pro Gly Lys Glu Arg Glu Val Val Gly Glu Leu
[0599] 35 40 45
[0600] Asn Phe Phe Gly Thr Ala Thr Tyr Ala Asp Ser Val Lys Gly Arg Phe
[0601] 50 55 60
[0602] Thr Ile Ser Lys Asp Asn Thr Asn Asn Thr Leu Tyr Leu Gln Met Asn
[0603] 65 70 75 80
[0604] Ala Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala Ala Gly Leu
[0605] 85 90 95
[0606] Arg Pro Gly Trp Trp Ser Leu Arg Leu Glu Pro Gly Ala Tyr Asn Tyr
[0607] 100 105 110
[0608] Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
[0609] 115 120
[0610] <210> 57
[0611] <211> 128
[0612] <212> PRT
[0613] <213> Artificial Sequence
[0614] <220>
[0615] <223> nKN044-216
[0616] <400> 57
[0617] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
[0618] 1 5 10 15
[0619] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Pro Phe Ser Trp Ser
[0620] 20 25 30
[0621] Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Met Glu Trp Val
[0622] 35 40 45
[0623] Ser Ser Ile Asn Arg Arg Gly Thr Val Thr Val Tyr Ala Asp Ser Val

[0624] 50 55 60

[0625] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Ala

[0626] 65 70 75 80

[0627] Leu Gln Met Asn Asn Leu Gln Pro Glu Asp Thr Ala Val Tyr Tyr Cys

[0628] 85 90 95

[0629] Ala Arg Ala Arg Arg Pro Glu Thr Trp Tyr Thr Asp Ile Trp Thr Pro

[0630] 100 105 110

[0631] Ala Leu Phe Gly Thr Arg Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0632] 115 120 125

[0633] <210> 58

[0634] <211> 126

[0635] <212> PRT

[0636] <213> Artificial Sequence

[0637] <220>

[0638] <223> nKN044-220

[0639] <400> 58

[0640] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0641] 1 5 10 15

[0642] Ser Leu Thr Leu Ser Cys Glu Ala Ser Gly Phe Thr Phe Ala Ala Pro

[0643] 20 25 30

[0644] Tyr Tyr Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Asp Trp

[0645] 35 40 45

[0646] Leu Ser Ser Ile Asn Thr Tyr Asn Ser Val Thr Tyr Tyr Ala Asp Ser

[0647] 50 55 60

[0648] Val Lys Gly Arg Phe Thr Ile Thr Arg Gln Asn Gly Gly Arg Thr Trp

[0649] 65 70 75 80

[0650] Asn Leu Gln Met Asn Tyr Leu Glu Pro Glu Asp Ser Gly Ile Tyr Tyr

[0651] 85 90 95

[0652] Cys Ala Ala Gly Trp Leu Phe Arg Gly Ser Trp Thr Gly Pro Arg Asn

[0653] 100 105 110

[0654] Phe Arg Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0655] 115 120 125

[0656] <210> 59

[0657] <211> 129

[0658] <212> PRT

[0659] <213> Artificial Sequence

[0660] <220>

[0661] <223> nKN044-227

[0662] <400> 59

[0663] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0664] 1 5 10 15
[0665] Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Tyr Thr Asn Ser Ile Ser
[0666] 20 25 30
[0667] Lys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Thr Glu Val
[0668] 35 40 45
[0669] Ala Thr Ile Phe Thr Ala Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
[0670] 50 55 60
[0671] Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Thr Leu Tyr
[0672] 65 70 75 80
[0673] Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
[0674] 85 90 95
[0675] Ala Val Ala Arg Pro Gly Trp Ile Trp Pro Thr Ile Lys Thr Met Thr
[0676] 100 105 110
[0677] Arg Tyr Glu Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
[0678] 115 120 125
[0679] Ser
[0680] <210> 60
[0681] <211> 126
[0682] <212> PRT
[0683] <213> Artificial Sequence
[0684] <220>
[0685] <223> nKN044-235
[0686] <400> 60
[0687] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0688] 1 5 10 15
[0689] Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Tyr Thr Tyr Arg Arg Tyr
[0690] 20 25 30
[0691] Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
[0692] 35 40 45
[0693] Ala Arg Ile Gly Thr Tyr Gly Thr Thr Trp Tyr Ala Asp Ser Val Lys
[0694] 50 55 60
[0695] Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
[0696] 65 70 75 80
[0697] Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala
[0698] 85 90 95
[0699] Ala Asp Pro Gly Arg Tyr Cys Arg Gly Asp Leu Leu Arg Thr Thr Leu
[0700] 100 105 110
[0701] Phe Ala Lys Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0702] 115 120 125

[0703] <210> 61

[0704] <211> 127

[0705] <212> PRT

[0706] <213> Artificial Sequence

[0707] <220>

[0708] <223> nKN044-244

[0709] <400> 61

[0710] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Pro Gly Gly

[0711] 1 5 10 15

[0712] Ser Leu Arg Leu Ser Cys Ala Ala Ser Arg Asn Thr Tyr Arg Asn Arg

[0713] 20 25 30

[0714] Trp Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val

[0715] 35 40 45

[0716] Ala Arg Ile Asn Ile Arg Ser Gly Arg Ala Tyr Tyr Ala Asp Ser Val

[0717] 50 55 60

[0718] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr

[0719] 65 70 75 80

[0720] Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys

[0721] 85 90 95

[0722] Ala Ala Ser Gln Ser Gly Gly Phe Phe Tyr Gly Val Leu Asp Thr Arg

[0723] 100 105 110

[0724] Ser Tyr His Tyr Trp Gly Pro Gly Thr Gln Val Thr Val Ser Ser

[0725] 115 120 125

[0726] <210> 62

[0727] <211> 124

[0728] <212> PRT

[0729] <213> Artificial Sequence

[0730] <220>

[0731] <223> nKN044-249

[0732] <400> 62

[0733] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly

[0734] 1 5 10 15

[0735] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Tyr Ser Ser Asn

[0736] 20 25 30

[0737] Cys Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Arg Asn Trp Val

[0738] 35 40 45

[0739] Ala Leu Thr Ser Ser Gly Asn Gly Arg Thr Trp Val Ala Asp Ser Val

[0740] 50 55 60

[0741] Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Thr Val Tyr
[0742] 65 70 75 80
[0743] Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly Ala Tyr Tyr Cys
[0744] 85 90 95
[0745] Ala Ala Gly Pro Ala Cys Ser Gly Val Tyr Trp Lys Trp Ala Leu Arg
[0746] 100 105 110
[0747] Gly Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
[0748] 115 120
[0749] <210> 63
[0750] <211> 129
[0751] <212> PRT
[0752] <213> Artificial Sequence
[0753] <220>
[0754] <223> nKN044-252
[0755] <400> 63
[0756] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0757] 1 5 10 15
[0758] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ile Tyr Ser Arg Asn
[0759] 20 25 30
[0760] Trp Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Trp Val
[0761] 35 40 45
[0762] Ala Ser Ile Ser Val Asn Gly Asp Asn Thr His Tyr Ala Asp Ser Val
[0763] 50 55 60
[0764] Lys Gly Arg Phe Thr Ile Ser Gln Asp Ala Ala Lys Asn Thr Val Tyr
[0765] 65 70 75 80
[0766] Leu Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
[0767] 85 90 95
[0768] Ala Ala Tyr Trp Pro Gly Gly Gly Gly Ser Ala Ala Trp Ser Phe Trp
[0769] 100 105 110
[0770] Gly Arg Ile Phe Asn Phe Arg Gly Gln Gly Thr Gln Val Thr Val Ser
[0771] 115 120 125
[0772] Ser
[0773] <210> 64
[0774] <211> 122
[0775] <212> PRT
[0776] <213> Artificial Sequence
[0777] <220>
[0778] <223> nKN044-264
[0779] <400> 64

[0780] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0781] 1 5 10 15
[0782] Ser Leu Arg Leu Ser Cys Val Ala Ser Gly Tyr Ile Tyr Ser Asn Cys
[0783] 20 25 30
[0784] Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Leu Ala
[0785] 35 40 45
[0786] Ala Ile Asp Arg Tyr Gly Arg Ala Thr Tyr Ala Asp Ser Val Lys Gly
[0787] 50 55 60
[0788] Arg Phe Thr Ile Ser Arg Asp Asn Leu Lys Asn Thr Val Ser Leu Gln
[0789] 65 70 75 80
[0790] Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala
[0791] 85 90 95
[0792] Ala Arg Trp Arg Ala Ser Cys Val Thr Leu Val Arg Phe Thr Ser Trp
[0793] 100 105 110
[0794] Gly Gln Gly Thr Gln Val Thr Val Ser Ser
[0795] 115 120
[0796] <210> 65
[0797] <211> 135
[0798] <212> PRT
[0799] <213> Artificial Sequence
[0800] <220>
[0801] <223> nKN044-266
[0802] <400> 65
[0803] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0804] 1 5 10 15
[0805] Ser Leu Arg Leu Ser Cys Val Ala Ala Gly Tyr Thr Leu Arg Thr Asn
[0806] 20 25 30
[0807] Tyr Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
[0808] 35 40 45
[0809] Ala Ala Ile Tyr Arg Gly Gly Gly Ser Thr Tyr Tyr Gly Ser Thr Tyr
[0810] 50 55 60
[0811] Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
[0812] 65 70 75 80
[0813] Lys Asn Thr Val Tyr Leu Glu Met Asn Ser Leu Lys Pro Glu Asp Thr
[0814] 85 90 95
[0815] Ala Met Tyr Tyr Cys Ala Ala Gly Arg Ser Pro Phe Pro Val Ala Phe
[0816] 100 105 110
[0817] Gly Gly Ala Trp Tyr Ser Ala Gly Arg Tyr Pro Tyr Trp Gly Gln Gly
[0818] 115 120 125

[0819] Thr Gln Val Thr Val Ser Ser
[0820] 130 135
[0821] <210> 66
[0822] <211> 128
[0823] <212> PRT
[0824] <213> Artificial Sequence
[0825] <220>
[0826] <223> nKN044-268
[0827] <400> 66
[0828] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0829] 1 5 10 15
[0830] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Tyr Ser Thr Lys
[0831] 20 25 30
[0832] Arg Val Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
[0833] 35 40 45
[0834] Ala Thr Ile Ser Ala Thr Met Gly Ile Pro Ile Tyr Ala Asp Ser Val
[0835] 50 55 60
[0836] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
[0837] 65 70 75 80
[0838] Leu Gln Met Asn Asn Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys
[0839] 85 90 95
[0840] Ala Ala Gly Arg Pro Ser Arg Ala Ala Phe Leu Gly Tyr Leu Arg Ala
[0841] 100 105 110
[0842] Ala Ala Tyr Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
[0843] 115 120 125
[0844] <210> 67
[0845] <211> 127
[0846] <212> PRT
[0847] <213> Artificial Sequence
[0848] <220>
[0849] <223> nKN044-275
[0850] <400> 67
[0851] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
[0852] 1 5 10 15
[0853] Ser Leu Arg Leu Ser Cys Ala Ala Ser Glu Tyr Thr Arg Arg Ser Lys
[0854] 20 25 30
[0855] Arg Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
[0856] 35 40 45
[0857] Ala Ala Ile Ser Ser Ser Gly Ala Phe Thr Tyr Tyr Ala Asp Ser Val

[0858] 50 55 60

[0859] Lys Gly Arg Phe Thr Met Ser Gln Asp Asn Thr Lys Asn Thr Val Tyr

[0860] 65 70 75 80

[0861] Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Ser Ala Met Tyr Tyr Cys

[0862] 85 90 95

[0863] Ala Ala Gly Leu Arg Pro Gly Trp Trp Ser Leu Arg Leu Glu Pro Gly

[0864] 100 105 110

[0865] Ala Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0866] 115 120 125

[0867] <210> 68

[0868] <211> 123

[0869] <212> PRT

[0870] <213> Artificial Sequence

[0871] <220>

[0872] <223> nKN044-280

[0873] <400> 68

[0874] Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

[0875] 1 5 10 15

[0876] Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Ser Thr Tyr Thr Asn Asn

[0877] 20 25 30

[0878] Tyr Ile Ala Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val

[0879] 35 40 45

[0880] Ala Thr Ile Asp Arg Arg Leu Gly Ser Thr Tyr Tyr Ala Asp Ser Val

[0881] 50 55 60

[0882] Arg Gly Arg Phe Thr Ile Ser Gln Asp Lys Ala Lys Asn Thr Val Tyr

[0883] 65 70 75 80

[0884] Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys

[0885] 85 90 95

[0886] Ala Ala Gly Arg Gly Arg Ala Trp Leu Ser Arg Val Trp Tyr Asn Tyr

[0887] 100 105 110

[0888] Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser

[0889] 115 120

[0890] <210> 69

[0891] <211> 232

[0892] <212> PRT

[0893] <213> Artificial Sequence

[0894] <220>

[0895] <223> IgG1-Fc

[0896] <400> 69

[0897] Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
[0898] 1 5 10 15
[0899] Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
[0900] 20 25 30
[0901] Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
[0902] 35 40 45
[0903] Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
[0904] 50 55 60
[0905] Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
[0906] 65 70 75 80
[0907] Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
[0908] 85 90 95
[0909] Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
[0910] 100 105 110
[0911] Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
[0912] 115 120 125
[0913] Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
[0914] 130 135 140
[0915] Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
[0916] 145 150 155 160
[0917] Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
[0918] 165 170 175
[0919] Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
[0920] 180 185 190
[0921] Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
[0922] 195 200 205
[0923] Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
[0924] 210 215 220
[0925] Ser Leu Ser Leu Ser Pro Gly Lys
[0926] 225 230
[0927] <210> 70
[0928] <211> 230
[0929] <212> PRT
[0930] <213> Artificial Sequence
[0931] <220>
[0932] <223> IgG1-muF
[0933] <400> 70
[0934] Met Asp Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
[0935] 1 5 10 15

[0936] Ala Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp
[0937] 20 25 30
[0938] Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
[0939] 35 40 45
[0940] Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp
[0941] 50 55 60
[0942] Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn
[0943] 65 70 75 80
[0944] Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp
[0945] 85 90 95
[0946] Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro
[0947] 100 105 110
[0948] Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala
[0949] 115 120 125
[0950] Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp
[0951] 130 135 140
[0952] Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile
[0953] 145 150 155 160
[0954] Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn
[0955] 165 170 175
[0956] Thr Gln Pro Ile Met Asn Thr Asn Gly Ser Tyr Phe Val Tyr Ser Lys
[0957] 180 185 190
[0958] Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys
[0959] 195 200 205
[0960] Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu
[0961] 210 215 220
[0962] Ser His Ser Pro Gly Lys
[0963] 225 230
[0964] <210> 71
[0965] <211> 348
[0966] <212> PRT
[0967] <213> Artificial Sequence
[0968] <220>
[0969] <223> nKN044-19-3
[0970] <400> 71
[0971] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[0972] 1 5 10 15
[0973] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Cys Cys Thr Cys
[0974] 20 25 30

[0975] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[0976] 35 40 45
[0977] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[0978] 50 55 60
[0979] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Ala
[0980] 65 70 75 80
[0981] Cys Ala Cys Cys Thr Ala Cys Ala Cys Ala Ala Ala Thr Thr Ala Cys
[0982] 85 90 95
[0983] Ala Thr Cys Ala Thr Gly Gly Cys Cys Thr Gly Gly Thr Thr Cys Cys
[0984] 100 105 110
[0985] Gly Cys Cys Ala Gly Thr Ala Thr Cys Cys Ala Gly Gly Gly Ala Ala
[0986] 115 120 125
[0987] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Gly Thr Cys
[0988] 130 135 140
[0989] Gly Cys Ala Thr Cys Thr Ala Thr Thr Thr Ala Thr Ala Thr Thr Gly
[0990] 145 150 155 160
[0991] Gly Thr Ala Gly Thr Gly Gly Thr Ala Gly Cys Ala Cys Ala Ala Ala
[0992] 165 170 175
[0993] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[0994] 180 185 190
[0995] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[0996] 195 200 205
[0997] Thr Cys Thr Cys Cys Cys Ala Ala Gly Ala Cys Ala Ala Cys Gly Cys
[0998] 210 215 220
[0999] Cys Ala Ala Gly Ala Ala Cys Ala Cys Cys Cys Thr Thr Thr Ala Thr
[1000] 225 230 235 240
[1001] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Ala Thr Cys
[1002] 245 250 255
[1003] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1004] 260 265 270
[1005] Thr Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr
[1006] 275 280 285
[1007] Gly Cys Gly Gly Cys Gly Ala Cys Gly Cys Gly Gly Gly Ala Thr Gly
[1008] 290 295 300
[1009] Gly Thr Cys Gly Ala Thr Gly Gly Thr Ala Cys Thr Thr Cys Gly Gly
[1010] 305 310 315 320
[1011] Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala Gly Gly Thr Cys
[1012] 325 330 335
[1013] Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala

[1014] 340 345

[1015] <210> 72

[1016] <211> 369

[1017] <212> PRT

[1018] <213> Artificial Sequence

[1019] <220>

[1020] <223> nKN044-97

[1021] <400> 72

[1022] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly

[1023] 1 5 10 15

[1024] Ala Gly Thr Cys Thr Gly Gly Ala Gly Gly Ala Gly Gly Cys Thr Cys

[1025] 20 25 30

[1026] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly

[1027] 35 40 45

[1028] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr

[1029] 50 55 60

[1030] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Ala

[1031] 65 70 75 80

[1032] Cys Ala Cys Cys Thr Ala Cys Ala Gly Gly Cys Gly Cys Ala Gly Gly

[1033] 85 90 95

[1034] Thr Cys Thr Ala Thr Gly Gly Gly Gly Thr Gly Gly Thr Thr Cys Cys

[1035] 100 105 110

[1036] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala

[1037] 115 120 125

[1038] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Thr Thr Cys Gly Thr Cys

[1039] 130 135 140

[1040] Thr Cys Ala Thr Cys Thr Ala Thr Thr Ala Gly Cys Ala Cys Thr Gly

[1041] 145 150 155 160

[1042] Ala Thr Gly Gly Thr Ala Cys Cys Ala Cys Ala Ala Ala Cys Thr Ala

[1043] 165 170 175

[1044] Thr Gly Cys Ala Gly Ala Cys Thr Cys Cys Gly Thr Gly Ala Ala Gly

[1045] 180 185 190

[1046] Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala Thr Cys Thr

[1047] 195 200 205

[1048] Ala Cys Cys Gly Ala Gly Ala Cys Ala Ala Cys Gly Cys Ala Ala Ala

[1049] 210 215 220

[1050] Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr Cys Thr Gly

[1051] 225 230 235 240

[1052] Cys Ala Gly Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys Thr Gly Ala

[1053] 245 250 255

[1054] Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys Thr Gly Cys

[1055] 260 265 270

[1056] Cys Gly Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr Gly Cys Gly

[1057] 275 280 285

[1058] Thr Cys Gly Ala Gly Ala Thr Cys Ala Gly Gly Ala Cys Thr Thr Gly

[1059] 290 295 300

[1060] Cys Thr Gly Cys Cys Ala Gly Cys Cys Thr Gly Thr Thr Gly Gly Gly

[1061] 305 310 315 320

[1062] Ala Thr Cys Gly Thr Gly Gly Thr Ala Thr Ala Gly Gly Thr Ala Cys

[1063] 325 330 335

[1064] Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys

[1065] 340 345 350

[1066] Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys

[1067] 355 360 365

[1068] Ala

[1069] <210> 73

[1070] <211> 378

[1071] <212> PRT

[1072] <213> Artificial Sequence

[1073] <220>

[1074] <223> nKN044-125

[1075] <400> 73

[1076] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly

[1077] 1 5 10 15

[1078] Ala Gly Thr Cys Thr Gly Gly Ala Gly Gly Ala Gly Gly Cys Thr Cys

[1079] 20 25 30

[1080] Gly Gly Thr Gly Cys Ala Gly Ala Cys Thr Gly Gly Ala Gly Gly Gly

[1081] 35 40 45

[1082] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr

[1083] 50 55 60

[1084] Gly Thr Gly Thr Ala Gly Cys Cys Thr Cys Thr Gly Gly Gly Thr Ala

[1085] 65 70 75 80

[1086] Cys Thr Cys Gly Thr Ala Thr Ala Gly Ala Cys Gly Cys Cys Gly Cys

[1087] 85 90 95

[1088] Thr Gly Cys Ala Thr Gly Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys

[1089] 100 105 110

[1090] Gly Ala Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala

[1091] 115 120 125

[1092] Gly Gly Gly Gly Cys Thr Cys Gly Ala Gly Thr Gly Gly Gly Thr Cys
[1093] 130 135 140
[1094] Thr Cys Ala Ala Cys Thr Ala Thr Thr Ala Ala Thr Ala Gly Thr Gly
[1095] 145 150 155 160
[1096] Gly Thr Gly Gly Thr Gly Gly Thr Ala Gly Cys Ala Cys Ala Thr Ala
[1097] 165 170 175
[1098] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1099] 180 185 190
[1100] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1101] 195 200 205
[1102] Thr Cys Ala Gly Cys Cys Ala Thr Gly Ala Cys Ala Gly Cys Gly Cys
[1103] 210 215 220
[1104] Cys Ala Cys Gly Ala Gly Cys Ala Cys Gly Gly Thr Cys Thr Ala Thr
[1105] 225 230 235 240
[1106] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Thr Ala Ala Thr Cys
[1107] 245 250 255
[1108] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1109] 260 265 270
[1110] Cys Gly Cys Thr Ala Thr Gly Thr Ala Cys Cys Ala Cys Thr Gly Thr
[1111] 275 280 285
[1112] Gly Cys Gly Gly Thr Ala Gly Thr Thr Cys Gly Gly Ala Cys Ala Ala
[1113] 290 295 300
[1114] Thr Gly Ala Cys Cys Cys Thr Thr Ala Gly Gly Thr Ala Cys Gly Gly
[1115] 305 310 315 320
[1116] Ala Ala Ala Cys Cys Thr Thr Ala Cys Gly Cys Thr Thr Cys Gly Ala
[1117] 325 330 335
[1118] Cys Cys Cys Gly Ala Cys Thr Ala Cys Thr Gly Gly Gly Gly Cys Cys
[1119] 340 345 350
[1120] Ala Gly Gly Gly Ala Ala Cys Cys Cys Ala Gly Gly Thr Cys Ala Cys
[1121] 355 360 365
[1122] Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala
[1123] 370 375
[1124] <210> 74
[1125] <211> 375
[1126] <212> PRT
[1127] <213> Artificial Sequence
[1128] <220>
[1129] <223> nKN044-194
[1130] <400> 74

[1131] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1132] 1 5 10 15
[1133] Ala Gly Thr Cys Thr Gly Gly Ala Gly Gly Ala Gly Gly Cys Thr Cys
[1134] 20 25 30
[1135] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1136] 35 40 45
[1137] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1138] 50 55 60
[1139] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Cys Ala Gly Ala Thr Ala
[1140] 65 70 75 80
[1141] Cys Ala Cys Thr Ala Thr Thr Ala Gly Gly Ala Gly Thr Ala Thr Cys
[1142] 85 90 95
[1143] Thr Gly Cys Ala Thr Gly Gly Cys Cys Thr Gly Gly Thr Thr Cys Cys
[1144] 100 105 110
[1145] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala
[1146] 115 120 125
[1147] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Ala Gly Gly Gly Thr Thr
[1148] 130 135 140
[1149] Gly Cys Ala Gly Ala Thr Ala Thr Thr Gly Ala Thr Ala Gly Ala Thr
[1150] 145 150 155 160
[1151] Ala Thr Gly Gly Ala Ala Cys Cys Ala Cys Thr Cys Ala Cys Gly Thr
[1152] 165 170 175
[1153] Cys Gly Cys Ala Gly Ala Cys Thr Cys Cys Gly Thr Gly Ala Ala Gly
[1154] 180 185 190
[1155] Gly Ala Cys Cys Gly Ala Thr Thr Cys Ala Gly Cys Ala Thr Cys Thr
[1156] 195 200 205
[1157] Cys Cys Ala Cys Ala Gly Ala Cys Ala Gly Cys Gly Cys Cys Ala Ala
[1158] 210 215 220
[1159] Gly Ala Ala Cys Ala Cys Thr Cys Thr Gly Thr Ala Thr Cys Thr Thr
[1160] 225 230 235 240
[1161] Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Ala Cys Cys Thr Gly Ala
[1162] 245 250 255
[1163] Ala Ala Cys Cys Gly Gly Ala Gly Gly Ala Cys Gly Cys Thr Gly Gly
[1164] 260 265 270
[1165] Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr Gly Cys Gly
[1166] 275 280 285
[1167] Gly Cys Ala Gly Ala Cys Thr Cys Thr Thr Cys Thr Cys Gly Gly Thr
[1168] 290 295 300
[1169] Gly Gly Thr Gly Cys Gly Gly Thr Gly Cys Cys Thr Gly Gly Thr Gly

[1170] 305 310 315 320
[1171] Gly Thr Cys Thr Cys Cys Cys Thr Cys Thr Ala Gly Thr Thr Ala Thr
[1172] 325 330 335
[1173] Ala Ala Thr Thr Ala Thr Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly
[1174] 340 345 350
[1175] Gly Gly Ala Cys Cys Cys Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr
[1176] 355 360 365
[1177] Cys Thr Cys Cys Thr Cys Ala
[1178] 370 375
[1179] <210> 75
[1180] <211> 369
[1181] <212> PRT
[1182] <213> Artificial Sequence
[1183] <220>
[1184] <223> nKN044-200
[1185] <400> 75
[1186] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1187] 1 5 10 15
[1188] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Cys Cys
[1189] 20 25 30
[1190] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1191] 35 40 45
[1192] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1193] 50 55 60
[1194] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Ala Gly Ala Thr Ala
[1195] 65 70 75 80
[1196] Cys Ala Cys Cys Cys Cys Cys Cys Gly Cys Ala Thr Gly Gly Cys Cys
[1197] 85 90 95
[1198] Thr Gly Gly Thr Thr Cys Cys Gly Cys Cys Ala Gly Gly Gly Thr Cys
[1199] 100 105 110
[1200] Cys Ala Gly Gly Cys Ala Ala Gly Gly Ala Gly Cys Gly Cys Gly Ala
[1201] 115 120 125
[1202] Gly Gly Thr Gly Gly Thr Gly Gly Gly Thr Gly Ala Gly Cys Thr Cys
[1203] 130 135 140
[1204] Ala Ala Thr Thr Thr Thr Thr Thr Thr Gly Gly Cys Ala Cys Cys Gly
[1205] 145 150 155 160
[1206] Cys Ala Ala Cys Cys Thr Ala Cys Gly Cys Ala Gly Ala Cys Thr Cys
[1207] 165 170 175
[1208] Cys Gly Thr Gly Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys

[1209] 180 185 190
[1210] Ala Cys Cys Ala Thr Cys Thr Cys Cys Ala Ala Ala Gly Ala Cys Ala
[1211] 195 200 205
[1212] Ala Cys Ala Cys Cys Ala Ala Cys Ala Ala Cys Ala Cys Thr Cys Thr
[1213] 210 215 220
[1214] Ala Thr Ala Thr Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys
[1215] 225 230 235 240
[1216] Gly Cys Thr Cys Thr Gly Ala Ala Gly Cys Cys Thr Gly Ala Gly Gly
[1217] 245 250 255
[1218] Ala Cys Ala Cys Gly Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala
[1219] 260 265 270
[1220] Cys Thr Gly Thr Gly Cys Gly Gly Cys Gly Gly Gly Thr Cys Thr Thr
[1221] 275 280 285
[1222] Cys Gly Ala Cys Cys Ala Gly Gly Gly Thr Gly Gly Thr Gly Gly Thr
[1223] 290 295 300
[1224] Cys Ala Cys Thr Gly Cys Gly Cys Cys Thr Ala Gly Ala Ala Cys Cys
[1225] 305 310 315 320
[1226] Thr Gly Gly Cys Gly Cys Gly Thr Ala Thr Ala Ala Cys Thr Ala Cys
[1227] 325 330 335
[1228] Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys
[1229] 340 345 350
[1230] Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys
[1231] 355 360 365
[1232] Ala
[1233] <210> 76
[1234] <211> 384
[1235] <212> PRT
[1236] <213> Artificial Sequence
[1237] <220>
[1238] <223> nKN044-216
[1239] <400> 76
[1240] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1241] 1 5 10 15
[1242] Ala Gly Thr Cys Thr Gly Gly Ala Gly Gly Ala Gly Gly Cys Thr Thr
[1243] 20 25 30
[1244] Gly Gly Thr Thr Cys Ala Ala Cys Cys Thr Gly Gly Gly Gly Gly Gly
[1245] 35 40 45
[1246] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1247] 50 55 60

[1248] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Thr
[1249] 65 70 75 80
[1250] Thr Cys Cys Cys Thr Thr Cys Ala Gly Thr Thr Gly Gly Thr Cys Gly
[1251] 85 90 95
[1252] Thr Cys Cys Ala Thr Gly Ala Ala Cys Thr Gly Gly Gly Thr Cys Cys
[1253] 100 105 110
[1254] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala
[1255] 115 120 125
[1256] Gly Gly Gly Gly Ala Thr Gly Gly Ala Gly Thr Gly Gly Gly Thr Cys
[1257] 130 135 140
[1258] Thr Cys Ala Thr Cys Thr Ala Thr Thr Ala Ala Cys Cys Gly Gly Cys
[1259] 145 150 155 160
[1260] Gly Gly Gly Gly Ala Ala Cys Thr Gly Thr Cys Ala Cys Ala Gly Thr
[1261] 165 170 175
[1262] Gly Thr Ala Thr Gly Cys Ala Gly Ala Cys Thr Cys Cys Gly Thr Ala
[1263] 180 185 190
[1264] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1265] 195 200 205
[1266] Thr Cys Thr Cys Cys Ala Gly Ala Gly Ala Cys Ala Ala Cys Gly Cys
[1267] 210 215 220
[1268] Cys Ala Ala Gly Ala Ala Cys Ala Cys Ala Gly Thr Gly Gly Cys Thr
[1269] 225 230 235 240
[1270] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Ala Cys Cys
[1271] 245 250 255
[1272] Thr Gly Cys Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1273] 260 265 270
[1274] Gly Gly Cys Cys Gly Thr Gly Thr Ala Thr Thr Ala Thr Thr Gly Thr
[1275] 275 280 285
[1276] Gly Cys Cys Ala Gly Ala Gly Cys Cys Cys Gly Cys Cys Gly Gly Cys
[1277] 290 295 300
[1278] Cys Gly Gly Ala Ala Ala Cys Thr Thr Gly Gly Thr Ala Cys Ala Cys
[1279] 305 310 315 320
[1280] Thr Gly Ala Thr Ala Thr Ala Thr Gly Gly Ala Cys Thr Cys Cys Cys
[1281] 325 330 335
[1282] Gly Cys Ala Cys Thr Cys Thr Thr Cys Gly Gly Thr Ala Cys Ala Ala
[1283] 340 345 350
[1284] Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala
[1285] 355 360 365
[1286] Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala

[1287] 370 375 380

[1288] <210> 77

[1289] <211> 378

[1290] <212> PRT

[1291] <213> Artificial Sequence

[1292] <220>

[1293] <223> nKN044-220

[1294] <400> 77

[1295] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly

[1296] 1 5 10 15

[1297] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Thr

[1298] 20 25 30

[1299] Gly Gly Thr Gly Cys Ala Ala Cys Cys Thr Gly Gly Gly Gly Gly Gly

[1300] 35 40 45

[1301] Thr Cys Thr Cys Thr Gly Ala Cys Thr Cys Thr Cys Thr Cys Cys Thr

[1302] 50 55 60

[1303] Gly Cys Gly Ala Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Thr

[1304] 65 70 75 80

[1305] Cys Ala Cys Cys Thr Thr Cys Gly Cys Cys Gly Cys Thr Cys Cys Cys

[1306] 85 90 95

[1307] Thr Ala Cys Thr Ala Cys Ala Thr Cys Ala Gly Cys Thr Gly Gly Gly

[1308] 100 105 110

[1309] Thr Cys Cys Gly Cys Cys Ala Gly Gly Cys Gly Cys Cys Ala Gly Gly

[1310] 115 120 125

[1311] Gly Ala Ala Gly Gly Gly Cys Cys Thr Gly Gly Ala Cys Thr Gly Gly

[1312] 130 135 140

[1313] Cys Thr Ala Thr Cys Cys Ala Gly Cys Ala Thr Thr Ala Ala Thr Ala

[1314] 145 150 155 160

[1315] Cys Thr Thr Ala Cys Ala Ala Thr Ala Gly Thr Gly Thr Cys Ala Cys

[1316] 165 170 175

[1317] Ala Thr Ala Cys Thr Ala Thr Gly Cys Ala Gly Ala Cys Thr Cys Cys

[1318] 180 185 190

[1319] Gly Thr Gly Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala

[1320] 195 200 205

[1321] Cys Cys Ala Thr Cys Ala Cys Gly Cys Gly Ala Cys Ala Ala Ala Ala

[1322] 210 215 220

[1323] Thr Gly Gly Cys Gly Gly Thr Cys Gly Cys Ala Cys Gly Thr Gly Gly

[1324] 225 230 235 240

[1325] Ala Ala Cys Thr Thr Ala Cys Ala Gly Ala Thr Gly Ala Ala Thr Thr

[1326] 245 250 255
[1327] Ala Cys Thr Thr Gly Gly Ala Gly Cys Cys Thr Gly Ala Ala Gly Ala
[1328] 260 265 270
[1329] Cys Thr Cys Thr Gly Gly Thr Ala Thr Thr Thr Ala Cys Thr Ala Cys
[1330] 275 280 285
[1331] Thr Gly Thr Gly Cys Ala Gly Cys Ala Gly Gly Gly Thr Gly Gly Cys
[1332] 290 295 300
[1333] Thr Gly Thr Thr Cys Cys Gly Thr Gly Gly Thr Ala Gly Cys Thr Gly
[1334] 305 310 315 320
[1335] Gly Ala Cys Cys Gly Gly Gly Cys Cys Cys Cys Gly Ala Ala Ala Thr
[1336] 325 330 335
[1337] Thr Thr Thr Cys Gly Thr Thr Ala Cys Thr Gly Gly Gly Gly Cys Cys
[1338] 340 345 350
[1339] Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala Gly Gly Thr Cys Ala Cys
[1340] 355 360 365
[1341] Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala
[1342] 370 375
[1343] <210> 78
[1344] <211> 387
[1345] <212> PRT
[1346] <213> Artificial Sequence
[1347] <220>
[1348] <223> nKN044-227
[1349] <400> 78
[1350] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1351] 1 5 10 15
[1352] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys
[1353] 20 25 30
[1354] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1355] 35 40 45
[1356] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1357] 50 55 60
[1358] Gly Thr Ala Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Gly Thr Ala
[1359] 65 70 75 80
[1360] Cys Ala Cys Cys Ala Ala Cys Ala Gly Cys Ala Thr Cys Ala Gly Thr
[1361] 85 90 95
[1362] Ala Ala Ala Ala Thr Gly Gly Gly Thr Thr Gly Gly Thr Thr Cys Cys
[1363] 100 105 110
[1364] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala

[1365] 115 120 125

[1366] Gly Gly Gly Gly Cys Gly Cys Ala Cys Gly Gly Ala Gly Gly Thr Cys

[1367] 130 135 140

[1368] Gly Cys Ala Ala Cys Thr Ala Thr Thr Thr Thr Thr Ala Cys Thr Gly

[1369] 145 150 155 160

[1370] Cys Gly Gly Gly Thr Gly Gly Cys Ala Gly Cys Ala Cys Ala Thr Ala

[1371] 165 170 175

[1372] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly

[1373] 180 185 190

[1374] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala

[1375] 195 200 205

[1376] Thr Cys Thr Cys Cys Cys Ala Ala Gly Ala Cys Ala Ala Cys Gly Cys

[1377] 210 215 220

[1378] Cys Ala Ala Gly Ala Ala Cys Ala Cys Gly Thr Thr Gly Thr Ala Thr

[1379] 225 230 235 240

[1380] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Ala Cys Cys

[1381] 245 250 255

[1382] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys

[1383] 260 265 270

[1384] Thr Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr

[1385] 275 280 285

[1386] Gly Cys Gly Gly Thr Ala Gly Cys Thr Ala Gly Gly Cys Cys Gly Gly

[1387] 290 295 300

[1388] Gly Cys Thr Gly Gly Ala Thr Ala Thr Gly Gly Cys Cys Thr Ala Cys

[1389] 305 310 315 320

[1390] Thr Ala Thr Ala Ala Ala Gly Ala Cys Thr Ala Thr Gly Ala Cys Cys

[1391] 325 330 335

[1392] Cys Gly Cys Thr Ala Thr Gly Ala Gly Thr Ala Thr Ala Ala Cys Thr

[1393] 340 345 350

[1394] Ala Cys Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys

[1395] 355 360 365

[1396] Cys Cys Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys

[1397] 370 375 380

[1398] Thr Cys Ala

[1399] 385

[1400] <210> 79

[1401] <211> 378

[1402] <212> PRT

[1403] <213> Artificial Sequence

[1404] <220>

[1405] <223> nKN044-235

[1406] <400> 79

[1407] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly

[1408] 1 5 10 15

[1409] Ala Gly Thr Cys Thr Gly Gly Ala Gly Gly Ala Gly Gly Cys Thr Cys

[1410] 20 25 30

[1411] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly

[1412] 35 40 45

[1413] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr

[1414] 50 55 60

[1415] Gly Thr Ala Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Ala

[1416] 65 70 75 80

[1417] Thr Ala Cys Cys Thr Ala Cys Ala Gly Ala Ala Gly Ala Thr Ala Cys

[1418] 85 90 95

[1419] Thr Gly Cys Ala Thr Gly Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys

[1420] 100 105 110

[1421] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala

[1422] 115 120 125

[1423] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Gly Thr Cys

[1424] 130 135 140

[1425] Gly Cys Ala Cys Gly Thr Ala Thr Thr Gly Gly Thr Ala Cys Cys Thr

[1426] 145 150 155 160

[1427] Ala Thr Gly Gly Thr Ala Cys Cys Ala Cys Ala Thr Gly Gly Thr Ala

[1428] 165 170 175

[1429] Cys Gly Cys Ala Gly Ala Thr Thr Cys Cys Gly Thr Gly Ala Ala Gly

[1430] 180 185 190

[1431] Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala Thr Cys Thr

[1432] 195 200 205

[1433] Cys Cys Cys Gly Cys Gly Ala Cys Ala Ala Cys Gly Cys Cys Ala Ala

[1434] 210 215 220

[1435] Gly Ala Ala Thr Ala Cys Gly Gly Thr Gly Thr Ala Thr Cys Thr Gly

[1436] 225 230 235 240

[1437] Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys Thr Gly Ala

[1438] 245 250 255

[1439] Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys Cys Gly Cys

[1440] 260 265 270

[1441] Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr Gly Cys Gly

[1442] 275 280 285

[1443] Gly Cys Ala Gly Ala Thr Cys Cys Ala Gly Gly Gly Ala Gly Ala Thr
[1444] 290 295 300
[1445] Ala Thr Thr Gly Thr Ala Gly Gly Gly Gly Thr Gly Ala Cys Thr Thr
[1446] 305 310 315 320
[1447] Ala Cys Thr Thr Cys Gly Cys Ala Cys Thr Ala Cys Cys Cys Thr Cys
[1448] 325 330 335
[1449] Thr Thr Cys Gly Cys Thr Ala Ala Gly Thr Gly Gly Gly Gly Cys Cys
[1450] 340 345 350
[1451] Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala Gly Gly Thr Cys Ala Cys
[1452] 355 360 365
[1453] Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala
[1454] 370 375
[1455] <210> 80
[1456] <211> 381
[1457] <212> PRT
[1458] <213> Artificial Sequence
[1459] <220>
[1460] <223> nKN044-244
[1461] <400> 80
[1462] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1463] 1 5 10 15
[1464] Ala Gly Thr Cys Thr Gly Gly Ala Gly Gly Ala Gly Gly Cys Thr Cys
[1465] 20 25 30
[1466] Gly Gly Thr Gly Cys Ala Gly Cys Cys Thr Gly Gly Ala Gly Gly Gly
[1467] 35 40 45
[1468] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1469] 50 55 60
[1470] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Ala Gly Ala Ala Ala
[1471] 65 70 75 80
[1472] Cys Ala Cys Cys Thr Ala Cys Ala Gly Ala Ala Ala Thr Cys Gly Thr
[1473] 85 90 95
[1474] Thr Gly Gly Ala Thr Gly Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys
[1475] 100 105 110
[1476] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala
[1477] 115 120 125
[1478] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Gly Thr Cys
[1479] 130 135 140
[1480] Gly Cys Gly Cys Gly Thr Ala Thr Thr Ala Ala Thr Ala Thr Ala Cys
[1481] 145 150 155 160

[1482] Gly Cys Ala Gly Thr Gly Gly Ala Cys Gly Gly Gly Cys Ala Thr Ala
[1483] 165 170 175
[1484] Cys Thr Ala Thr Gly Cys Ala Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1485] 180 185 190
[1486] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1487] 195 200 205
[1488] Thr Cys Thr Cys Cys Cys Gly Ala Gly Ala Cys Ala Ala Cys Gly Cys
[1489] 210 215 220
[1490] Cys Ala Ala Gly Ala Ala Cys Ala Cys Ala Cys Thr Gly Thr Ala Thr
[1491] 225 230 235 240
[1492] Cys Thr Cys Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys
[1493] 245 250 255
[1494] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1495] 260 265 270
[1496] Gly Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr
[1497] 275 280 285
[1498] Gly Cys Gly Gly Cys Gly Thr Cys Cys Cys Ala Ala Thr Cys Ala Gly
[1499] 290 295 300
[1500] Gly Gly Gly Gly Ala Thr Thr Thr Thr Thr Thr Thr Ala Cys Gly Gly
[1501] 305 310 315 320
[1502] Cys Gly Thr Ala Cys Thr Thr Gly Ala Cys Ala Cys Gly Cys Gly Ala
[1503] 325 330 335
[1504] Thr Cys Gly Thr Ala Thr Cys Ala Thr Thr Ala Cys Thr Gly Gly Gly
[1505] 340 345 350
[1506] Gly Cys Cys Cys Gly Gly Gly Gly Ala Cys Cys Cys Ala Gly Gly Thr
[1507] 355 360 365
[1508] Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala
[1509] 370 375 380
[1510] <210> 81
[1511] <211> 372
[1512] <212> PRT
[1513] <213> Artificial Sequence
[1514] <220>
[1515] <223> nKN044-249
[1516] <400> 81
[1517] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1518] 1 5 10 15
[1519] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys
[1520] 20 25 30

[1521] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1522] 35 40 45
[1523] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1524] 50 55 60
[1525] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Cys Thr Ala
[1526] 65 70 75 80
[1527] Cys Ala Cys Cys Thr Ala Cys Ala Gly Thr Ala Gly Cys Ala Ala Cys
[1528] 85 90 95
[1529] Thr Gly Cys Ala Thr Ala Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys
[1530] 100 105 110
[1531] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Ala Ala Ala
[1532] 115 120 125
[1533] Gly Gly Gly Gly Cys Gly Cys Ala Ala Cys Thr Gly Gly Gly Thr Cys
[1534] 130 135 140
[1535] Gly Cys Gly Cys Thr Thr Ala Cys Thr Thr Cys Thr Ala Gly Thr Gly
[1536] 145 150 155 160
[1537] Gly Cys Ala Ala Thr Gly Gly Ala Cys Gly Cys Ala Cys Ala Thr Gly
[1538] 165 170 175
[1539] Gly Gly Thr Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1540] 180 185 190
[1541] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1542] 195 200 205
[1543] Thr Cys Thr Cys Cys Cys Ala Ala Gly Ala Cys Ala Ala Cys Gly Cys
[1544] 210 215 220
[1545] Cys Ala Ala Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr
[1546] 225 230 235 240
[1547] Cys Thr Gly Cys Ala Gly Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys
[1548] 245 250 255
[1549] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1550] 260 265 270
[1551] Thr Gly Gly Cys Gly Cys Gly Thr Ala Cys Thr Ala Thr Thr Gly Thr
[1552] 275 280 285
[1553] Gly Cys Gly Gly Cys Ala Gly Gly Cys Cys Cys Gly Gly Cys Thr Thr
[1554] 290 295 300
[1555] Gly Thr Ala Gly Thr Gly Gly Thr Gly Thr Thr Thr Ala Cys Thr Gly
[1556] 305 310 315 320
[1557] Gly Ala Ala Ala Thr Gly Gly Gly Cys Cys Cys Thr Thr Ala Gly Ala
[1558] 325 330 335
[1559] Gly Gly Cys Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala

[1560] 340 345 350
[1561] Cys Cys Cys Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys
[1562] 355 360 365
[1563] Cys Thr Cys Ala
[1564] 370
[1565] <210> 82
[1566] <211> 387
[1567] <212> PRT
[1568] <213> Artificial Sequence
[1569] <220>
[1570] <223> nKN044-252
[1571] <400> 82
[1572] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1573] 1 5 10 15
[1574] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys
[1575] 20 25 30
[1576] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1577] 35 40 45
[1578] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1579] 50 55 60
[1580] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Ala
[1581] 65 70 75 80
[1582] Cys Ala Thr Cys Thr Ala Cys Ala Gly Thr Cys Gly Cys Ala Ala Cys
[1583] 85 90 95
[1584] Thr Gly Gly Ala Thr Gly Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys
[1585] 100 105 110
[1586] Gly Cys Cys Ala Ala Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala
[1587] 115 120 125
[1588] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Thr Gly Gly Gly Thr Cys
[1589] 130 135 140
[1590] Gly Cys Ala Thr Cys Thr Ala Thr Thr Ala Gly Thr Gly Thr Thr Ala
[1591] 145 150 155 160
[1592] Ala Thr Gly Gly Thr Gly Ala Cys Ala Ala Cys Ala Cys Ala Cys Ala
[1593] 165 170 175
[1594] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1595] 180 185 190
[1596] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1597] 195 200 205
[1598] Thr Cys Thr Cys Cys Cys Ala Ala Gly Ala Cys Gly Cys Ala Gly Cys

[1599] 210 215 220

[1600] Cys Ala Ala Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr

[1601] 225 230 235 240

[1602] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Cys Cys Ala Gly Cys Cys

[1603] 245 250 255

[1604] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys

[1605] 260 265 270

[1606] Thr Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr

[1607] 275 280 285

[1608] Gly Cys Gly Gly Cys Ala Thr Ala Thr Thr Gly Gly Cys Cys Cys Gly

[1609] 290 295 300

[1610] Gly Ala Gly Gly Gly Gly Gly Gly Gly Gly Gly Ala Gly Thr Gly Cys

[1611] 305 310 315 320

[1612] Thr Gly Cys Cys Thr Gly Gly Thr Cys Thr Thr Thr Thr Thr Gly Gly

[1613] 325 330 335

[1614] Gly Gly Gly Cys Gly Gly Ala Thr Cys Thr Thr Thr Ala Ala Cys Thr

[1615] 340 345 350

[1616] Thr Cys Cys Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys

[1617] 355 360 365

[1618] Cys Cys Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys

[1619] 370 375 380

[1620] Thr Cys Ala

[1621] 385

[1622] <210> 83

[1623] <211> 366

[1624] <212> PRT

[1625] <213> Artificial Sequence

[1626] <220>

[1627] <223> nKN044-264

[1628] <400> 83

[1629] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly

[1630] 1 5 10 15

[1631] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys

[1632] 20 25 30

[1633] Gly Gly Thr Ala Cys Ala Gly Gly Cys Gly Gly Gly Gly Gly Gly Gly

[1634] 35 40 45

[1635] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr

[1636] 50 55 60

[1637] Gly Thr Gly Thr Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Ala

[1638] 65 70 75 80
[1639] Cys Ala Thr Cys Thr Ala Cys Ala Gly Thr Ala Ala Cys Thr Gly Cys
[1640] 85 90 95
[1641] Ala Thr Gly Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys Gly Cys Cys
[1642] 100 105 110
[1643] Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala Gly Gly Ala
[1644] 115 120 125
[1645] Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Cys Thr Cys Gly Cys Thr
[1646] 130 135 140
[1647] Gly Cys Thr Ala Thr Thr Gly Ala Thr Cys Gly Thr Thr Ala Thr Gly
[1648] 145 150 155 160
[1649] Gly Thr Cys Gly Thr Gly Cys Gly Ala Cys Gly Thr Ala Cys Gly Cys
[1650] 165 170 175
[1651] Gly Gly Ala Cys Thr Cys Cys Gly Thr Gly Ala Ala Gly Gly Gly Ala
[1652] 180 185 190
[1653] Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala Thr Cys Thr Cys Cys Ala
[1654] 195 200 205
[1655] Gly Ala Gly Ala Cys Ala Ala Cys Cys Thr Cys Ala Ala Gly Ala Ala
[1656] 210 215 220
[1657] Cys Ala Cys Gly Gly Thr Gly Thr Cys Thr Cys Thr Gly Cys Ala Ala
[1658] 225 230 235 240
[1659] Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys Thr Gly Ala Ala Ala Cys
[1660] 245 250 255
[1661] Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys Thr Gly Cys Cys Ala Cys
[1662] 260 265 270
[1663] Thr Thr Ala Cys Thr Ala Cys Thr Gly Thr Gly Cys Gly Gly Cys Ala
[1664] 275 280 285
[1665] Gly Cys Cys Cys Gly Ala Thr Gly Gly Cys Gly Thr Gly Cys Thr Ala
[1666] 290 295 300
[1667] Gly Cys Thr Gly Cys Gly Thr Ala Ala Cys Cys Thr Thr Gly Gly Thr
[1668] 305 310 315 320
[1669] Thr Cys Gly Cys Thr Thr Thr Ala Cys Thr Thr Cys Thr Thr Gly Gly
[1670] 325 330 335
[1671] Gly Gly Thr Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala Gly Gly
[1672] 340 345 350
[1673] Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala
[1674] 355 360 365
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[1677] <212> PRT

[1678] <213> Artificial Sequence

[1679] <220>

[1680] <223> nKN044-266

[1681] <400> 84

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[1683] 1 5 10 15

[1684] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys

[1685] 20 25 30

[1686] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly

[1687] 35 40 45

[1688] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr

[1689] 50 55 60

[1690] Gly Thr Gly Thr Ala Gly Cys Cys Gly Cys Thr Gly Gly Ala Thr Ala

[1691] 65 70 75 80

[1692] Cys Ala Cys Cys Cys Thr Cys Cys Gly Thr Ala Cys Cys Ala Ala Cys

[1693] 85 90 95

[1694] Thr Ala Cys Ala Thr Cys Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys

[1695] 100 105 110

[1696] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala

[1697] 115 120 125

[1698] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Gly Thr Cys

[1699] 130 135 140

[1700] Gly Cys Ala Gly Cys Thr Ala Thr Thr Thr Ala Cys Cys Gly Thr Gly

[1701] 145 150 155 160

[1702] Gly Thr Gly Gly Thr Gly Gly Thr Ala Gly Thr Ala Cys Ala Thr Ala

[1703] 165 170 175

[1704] Cys Thr Ala Thr Gly Gly Thr Ala Gly Thr Ala Cys Ala Thr Ala Cys

[1705] 180 185 190

[1706] Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly Ala

[1707] 195 200 205

[1708] Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala Thr

[1709] 210 215 220

[1710] Cys Thr Cys Cys Cys Gly Ala Gly Ala Cys Ala Ala Cys Gly Cys Cys

[1711] 225 230 235 240

[1712] Ala Ala Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr Cys

[1713] 245 250 255

[1714] Thr Gly Gly Ala Ala Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys Thr

[1715] 260 265 270

[1716] Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys Thr
[1717] 275 280 285
[1718] Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr Gly
[1719] 290 295 300
[1720] Cys Gly Gly Cys Ala Gly Gly Thr Cys Gly Ala Thr Cys Cys Cys Cys
[1721] 305 310 315 320
[1722] Thr Thr Thr Cys Cys Cys Cys Gly Thr Gly Gly Cys Gly Thr Thr Cys
[1723] 325 330 335
[1724] Gly Gly Thr Gly Gly Thr Gly Cys Cys Thr Gly Gly Thr Ala Thr Thr
[1725] 340 345 350
[1726] Cys Gly Gly Cys Gly Gly Gly Gly Ala Gly Ala Thr Ala Thr Cys Cys
[1727] 355 360 365
[1728] Cys Thr Ala Cys Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly
[1729] 370 375 380
[1730] Ala Cys Cys Cys Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr
[1731] 385 390 395 400
[1732] Cys Cys Thr Cys Ala
[1733] 405
[1734] <210> 85
[1735] <211> 384
[1736] <212> PRT
[1737] <213> Artificial Sequence
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[1740] <400> 85
[1741] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1742] 1 5 10 15
[1743] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys
[1744] 20 25 30
[1745] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1746] 35 40 45
[1747] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1748] 50 55 60
[1749] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Ala
[1750] 65 70 75 80
[1751] Cys Ala Cys Cys Thr Ala Cys Ala Gly Thr Ala Cys Cys Ala Ala Ala
[1752] 85 90 95
[1753] Ala Gly Gly Gly Thr Gly Gly Cys Cys Thr Gly Gly Thr Thr Cys Cys
[1754] 100 105 110

[1755] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Ala Gly Gly Gly Ala Ala
[1756] 115 120 125
[1757] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Ala Gly Thr Cys
[1758] 130 135 140
[1759] Gly Cys Ala Ala Cys Thr Ala Thr Thr Thr Cys Thr Gly Cys Thr Ala
[1760] 145 150 155 160
[1761] Cys Thr Ala Thr Gly Gly Gly Thr Ala Thr Cys Cys Cys Gly Ala Thr
[1762] 165 170 175
[1763] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1764] 180 185 190
[1765] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1766] 195 200 205
[1767] Thr Cys Thr Cys Cys Cys Gly Ala Gly Ala Cys Ala Ala Cys Gly Cys
[1768] 210 215 220
[1769] Cys Ala Ala Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr
[1770] 225 230 235 240
[1771] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Ala Cys Cys
[1772] 245 250 255
[1773] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1774] 260 265 270
[1775] Thr Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr
[1776] 275 280 285
[1777] Gly Cys Ala Gly Cys Ala Gly Gly Cys Cys Gly Thr Cys Cys Gly Thr
[1778] 290 295 300
[1779] Cys Ala Cys Gly Cys Gly Cys Cys Gly Cys Thr Thr Thr Thr Cys Thr
[1780] 305 310 315 320
[1781] Thr Gly Gly Thr Thr Ala Cys Cys Thr Cys Ala Gly Ala Gly Cys Cys
[1782] 325 330 335
[1783] Gly Cys Ala Gly Cys Ala Thr Ala Thr Gly Ala Cys Thr Ala Cys Thr
[1784] 340 345 350
[1785] Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala
[1786] 355 360 365
[1787] Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala
[1788] 370 375 380
[1789] <210> 86
[1790] <211> 381
[1791] <212> PRT
[1792] <213> Artificial Sequence
[1793] <220>

[1794] <223> nKN044-275

[1795] <400> 86

[1796] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly
[1797] 1 5 10 15
[1798] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Cys
[1799] 20 25 30
[1800] Gly Gly Thr Gly Cys Ala Gly Gly Cys Thr Gly Gly Ala Gly Gly Gly
[1801] 35 40 45
[1802] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr
[1803] 50 55 60
[1804] Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Ala Ala Thr Ala
[1805] 65 70 75 80
[1806] Cys Ala Cys Cys Cys Gly Cys Ala Gly Ala Ala Gly Thr Ala Ala Gly
[1807] 85 90 95
[1808] Cys Gly Cys Ala Thr Gly Gly Gly Cys Thr Gly Gly Thr Thr Cys Cys
[1809] 100 105 110
[1810] Gly Cys Cys Ala Gly Gly Cys Thr Cys Cys Cys Gly Gly Gly Ala Ala
[1811] 115 120 125
[1812] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Gly Thr Cys
[1813] 130 135 140
[1814] Gly Cys Ala Gly Cys Ala Ala Thr Thr Thr Cys Gly Ala Gly Cys Ala
[1815] 145 150 155 160
[1816] Gly Cys Gly Gly Thr Gly Cys Ala Thr Thr Cys Ala Cys Ala Thr Ala
[1817] 165 170 175
[1818] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1819] 180 185 190
[1820] Ala Ala Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1821] 195 200 205
[1822] Thr Gly Thr Cys Cys Cys Ala Ala Gly Ala Cys Ala Ala Cys Ala Cys
[1823] 210 215 220
[1824] Cys Ala Ala Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr
[1825] 225 230 235 240
[1826] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys
[1827] 245 250 255
[1828] Thr Gly Ala Ala Ala Cys Cys Cys Gly Ala Gly Gly Ala Cys Ala Gly
[1829] 260 265 270
[1830] Thr Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr
[1831] 275 280 285
[1832] Gly Cys Gly Gly Cys Gly Gly Gly Thr Cys Thr Thr Cys Gly Ala Cys

[1833] 290 295 300

[1834] Cys Ala Gly Gly Gly Thr Gly Gly Thr Gly Gly Thr Cys Ala Cys Thr

[1835] 305 310 315 320

[1836] Gly Cys Gly Cys Cys Thr Ala Gly Ala Ala Cys Cys Thr Gly Gly Cys

[1837] 325 330 335

[1838] Gly Cys Gly Thr Ala Thr Ala Ala Cys Thr Ala Cys Thr Gly Gly Gly

[1839] 340 345 350

[1840] Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys Ala Gly Gly Thr

[1841] 355 360 365

[1842] Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala

[1843] 370 375 380

[1844] <210> 87

[1845] <211> 369

[1846] <212> PRT

[1847] <213> Artificial Sequence

[1848] <220>

[1849] <223> nKN044-280

[1850] <400> 87

[1851] Cys Ala Gly Gly Thr Gly Cys Ala Gly Cys Thr Gly Cys Ala Gly Gly

[1852] 1 5 10 15

[1853] Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Thr

[1854] 20 25 30

[1855] Gly Gly Thr Gly Cys Ala Gly Cys Cys Thr Gly Gly Gly Gly Gly Gly

[1856] 35 40 45

[1857] Thr Cys Thr Cys Thr Gly Ala Gly Ala Cys Thr Cys Thr Cys Cys Thr

[1858] 50 55 60

[1859] Gly Thr Ala Cys Thr Gly Cys Cys Thr Cys Thr Gly Gly Ala Ala Gly

[1860] 65 70 75 80

[1861] Cys Ala Cys Cys Thr Ala Thr Ala Cys Thr Ala Ala Cys Ala Ala Thr

[1862] 85 90 95

[1863] Thr Ala Cys Ala Thr Cys Gly Cys Cys Thr Gly Gly Thr Thr Cys Cys

[1864] 100 105 110

[1865] Gly Cys Cys Ala Gly Gly Cys Gly Cys Cys Ala Gly Gly Ala Ala Ala

[1866] 115 120 125

[1867] Gly Gly Ala Gly Cys Gly Cys Gly Ala Gly Gly Gly Gly Gly Thr Cys

[1868] 130 135 140

[1869] Gly Cys Ala Ala Cys Gly Ala Thr Thr Gly Ala Thr Cys Gly Thr Cys

[1870] 145 150 155 160

[1871] Gly Thr Cys Thr Thr Gly Gly Cys Ala Gly Cys Ala Cys Gly Thr Ala

[1872] 165 170 175
[1873] Cys Thr Ala Thr Gly Cys Cys Gly Ala Cys Thr Cys Cys Gly Thr Gly
[1874] 180 185 190
[1875] Ala Gly Gly Gly Gly Cys Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
[1876] 195 200 205
[1877] Thr Cys Thr Cys Cys Cys Ala Ala Gly Ala Cys Ala Ala Gly Gly Cys
[1878] 210 215 220
[1879] Cys Ala Ala Gly Ala Ala Cys Ala Cys Gly Gly Thr Gly Thr Ala Thr
[1880] 225 230 235 240
[1881] Cys Thr Gly Cys Ala Ala Ala Thr Gly Ala Ala Cys Ala Gly Cys Cys
[1882] 245 250 255
[1883] Thr Gly Ala Ala Ala Cys Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
[1884] 260 265 270
[1885] Thr Gly Cys Cys Ala Thr Gly Thr Ala Cys Thr Ala Cys Thr Gly Thr
[1886] 275 280 285
[1887] Gly Cys Gly Gly Cys Cys Gly Gly Gly Cys Gly Cys Gly Gly Thr Cys
[1888] 290 295 300
[1889] Gly Thr Gly Cys Cys Thr Gly Gly Cys Thr Thr Thr Cys Gly Cys Gly
[1890] 305 310 315 320
[1891] Thr Gly Thr Thr Thr Gly Gly Thr Ala Thr Ala Ala Thr Thr Ala Cys
[1892] 325 330 335
[1893] Thr Gly Gly Gly Gly Cys Cys Ala Gly Gly Gly Gly Ala Cys Cys Cys
[1894] 340 345 350
[1895] Ala Gly Gly Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys
[1896] 355 360 365
[1897] Ala
[1898] <210> 88
[1899] <211> 493
[1900] <212> PRT
[1901] <213> Artificial Sequence
[1902] <220>
[1903] <223> KN044
[1904] <400> 88
[1905] Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
[1906] 1 5 10 15
[1907] Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ile Tyr Ser Ala Tyr
[1908] 20 25 30
[1909] Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Gly Val
[1910] 35 40 45

[1911] Ala Ala Ile Tyr Ile Gly Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
[1912] 50 55 60
[1913] Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[1914] 65 70 75 80
[1915] Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
[1916] 85 90 95
[1917] Ala Ala Asp Val Ile Pro Thr Glu Thr Cys Leu Gly Gly Ser Trp Ser
[1918] 100 105 110
[1919] Gly Pro Phe Gly Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
[1920] 115 120 125
[1921] Gly Ala Pro Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
[1922] 130 135 140
[1923] Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ile Tyr
[1924] 145 150 155 160
[1925] Ser Ala Tyr Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu
[1926] 165 170 175
[1927] Glu Gly Val Ala Ala Ile Tyr Ile Gly Gly Gly Ser Thr Tyr Tyr Ala
[1928] 180 185 190
[1929] Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
[1930] 195 200 205
[1931] Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
[1932] 210 215 220
[1933] Tyr Tyr Cys Ala Ala Asp Val Ile Pro Thr Glu Thr Cys Leu Gly Gly
[1934] 225 230 235 240
[1935] Ser Trp Ser Gly Pro Phe Gly Tyr Trp Gly Gln Gly Thr Leu Val Thr
[1936] 245 250 255
[1937] Val Ser Ser Gly Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys
[1938] 260 265 270
[1939] Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
[1940] 275 280 285
[1941] Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
[1942] 290 295 300
[1943] Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
[1944] 305 310 315 320
[1945] Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
[1946] 325 330 335
[1947] Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
[1948] 340 345 350
[1949] Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys

[1950] 355 360 365

[1951] Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys

[1952] 370 375 380

[1953] Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser

[1954] 385 390 395 400

[1955] Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys

[1956] 405 410 415

[1957] Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln

[1958] 420 425 430

[1959] Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly

[1960] 435 440 445

[1961] Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln

[1962] 450 455 460

[1963] Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn

[1964] 465 470 475 480

[1965] His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

[1966] 485 490

[1967] <210> 89

[1968] <211> 29

[1969] <212> DNA

[1970] <213> Artificial Sequence

[1971] <220>

[1972] <223> Artificial Sequence

[1973] <400> 89

[1974] cccaccggtc aggtgcagct gcaggagtc 29

[1975] <210> 90

[1976] <211> 28

[1977] <212> DNA

[1978] <213> Artificial Sequence

[1979] <220>

[1980] <223> Artificial Sequence

[1981] <400> 90

[1982] cccggatcct gaggagacgg tgacctgg 28

[1983] <210> 91

[1984] <211> 29

[1985] <212> DNA

[1986] <213> Artificial Sequence

[1987] <220>

[1988] <223> Artificial Sequence

[1989] <400> 91

[1990] cccaccggtc aggtgcagct gcaggagtc 29

[1991] <210> 92

[1992] <211> 36

[1993] <212> DNA

[1994] <213> Artificial Sequence

[1995] <220>

[1996] <223> Artificial Sequence

[1997] <400> 92

[1998] cccggatcca tgctgcctga ggagacggtg acctgg 36

17 个 dAb 的氨基酸序列（根据 Kabat 和 Chothia 的规则用框标出 CDR）

>nKN044-97

QVQLQESGGGSVQAGGSLRLSCAASGYTYRRRSMGWFRQAPGKEREFVSSISTDGTTNYADSVKGRFTIYRDNAKNTVY
LQMNSLKPEDTAVYYCASRSGLAASLLGSWYRYWGQGTQVTVSS

>nKN044-125

QVQLQESGGGSVQTGGSLRLSCVASGYSYRRRCMGWFRQAPGKGLEWVSTINSGGGSTYYADSVKGRFTISHDSATSTV
YLQMNNLKPEDTAMYHCAVVRTMTLRYGNLTLRPDYWGQGTQVTVSS

>nKN044-19-3

QVQLQESGGASVQAGGSLRLSCAASGYTYTNYIMAWFRQYPGKEREGVASIYIGSGSTNYADSVKGRFTISQDNAKNTLY
LQMNNLKPEDTAMYYCAATRDGRWYFGQGTQVTVSS

>nKN044-266

QVQLQESGGGSVQAGGSLRLSCVAAGYTLRTNYIGWFRQAPGKEREGVAAIYRGGGSTYYGSTYYADSVKGRFTISRDNA
KNTVYLEMNSLKPEDTAMYYCAAGRSPFPVAFGGAWYSAGRYPYWGQGTQVTVSS

>nKN044-200

QVQLQESGGGPVQAGGSLRLSCAASRYTPRMAWFRQGPGKEREVVGELNFFGTATYADSVKGRFTISKDNTNNTLYLQ
MNALKPEDTAMYYCAAGLRPGWWSLRLEPGAYNYWGQGTQVTVSS

>nKN044-275

QVQLQESGGGSVQAGGSLRLSCAASEYTRRSKRMGWFRQAPGKEREGVAAISSSGAFTYYADSVKGRFTMSQDNTKNT
VYLQMNSLKPEDSAMYYCAAGLRPGWWSLRLEPGAYNYWGQGTQVTVSS

>nKN044-280

QVQLQESGGGLVQPGGSLRLSCTASGSTYTNNYIAWFRQAPGKEREGVATIDRRLGSTYYADSVRGRFTISQDKAKNTVYL
QMNSLKPEDTAMYYCAAGRGRAWLSRVWYNYWGQGTQVTVSS

>nKN044-244

QVQLQESGGGSVQPGGSLRLSCAASRNTYRNRWMGWFRQAPGKEREGVARINIRSGRAYYADSVKGRFTISRDNAKNT
LYLQMNSLKPEDTAMYYCAASQSGGFFYGVLDTRSYHYWGPGTQVTVSS

>nKN044-268

QVQLQESGGGSVQAGGSLRLSCAASGYTYSTKRVAWFRQAPGKEREGVATISATMGIPIYADSVKGRFTISRDNAKNTVYL
QMNNLKPEDTAMYYCAAGRPSRAAFLGYLRAAAYDYWGQGTQVTVSS

>nKN044-227

QVQLQESGGGSVQAGGSLRLSCTASGYTNSISKMGWFRQAPGKGRTEVATIFTAGGSTYYADSVKGRFTISQDNAKNTLY
LQMNNLKPEDTAMYYCAVARPGWIWPTIKTMTRYEYNYWGQGTQVTVSS

图1

>nKN044-235

QVQLQESGGGSVQAGGSLRLSCTASGYTYRRYCMGWFRQAPGKEREGVARIGTYGTTWYADSVKGRFTISRDNAKNTV

YLQMNSLKPEDTAMYYCAADPGRYCRGDLLRTTLFAKWGQGTQVTVSS

>nKN044-194

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QMNNLKPEDAGMYYCAADSSRWCGAWWSPSSYNYWGQGTQVTVSSAAA

>nKN044-264

QVQLQESGGGSVQAGGSLRLSCVASGYIYSNCMGWFRQAPGKEREGLAAIDRYGRATYADSVKGRFTISRDNLKNTVSL

QMNSLKPEDTATYYCAAARWRASCVTLVRFTSWGQGTQVTVSS

>nKN044-252

QVQLQESGGGSVQAGGSLRLSCAASGYIYSRNWMGWFRQAPGKEREWVASISVNGDNTHYADSVKGRFTISQDAAKN

TVYLQMTSLKPEDTAMYYCAAYWPGGGGSAAWSFWGRIFNFRGQGTQVTVSS

>nKN044-249

QVQLQESGGGSVQAGGSLRLSCAASGYTYSSNCIGWFRQAPGKGRNWVALTSSGNGRTWVADSVKGRFTISQDNAKN

TVYLQMNSLKPEDTGAYYCAAGPACSGVYWKWALRGWGQGTQVTVSS

>nKN044-216

QVQLQESGGGLVQPGGSLRLSCAASGFPFSWSSMNWVRQAPGKGMEWVSSINRRGTVTVYADSVKGRFTISRDNAKN

TVALQMNNLQPEDTAVYYCARARRPETWYTDIWTPALFGTRGQGTQVTVSS

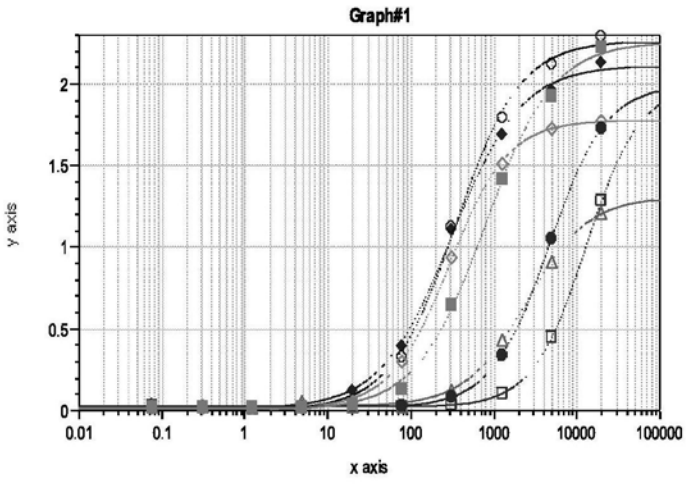
>nKN044-220

QVQLQESGGGLVQPGGSLTLSCEASGFTFAAPYYISWVRQAPGKGLDWLSSINTYNSVTYYADSVKGRFTITRQNGGRTW

NLQMNYLEPEDSGIYYCAAGWLFRGSWTGPRNFRYWGQGTQVTVSS

图1(续)

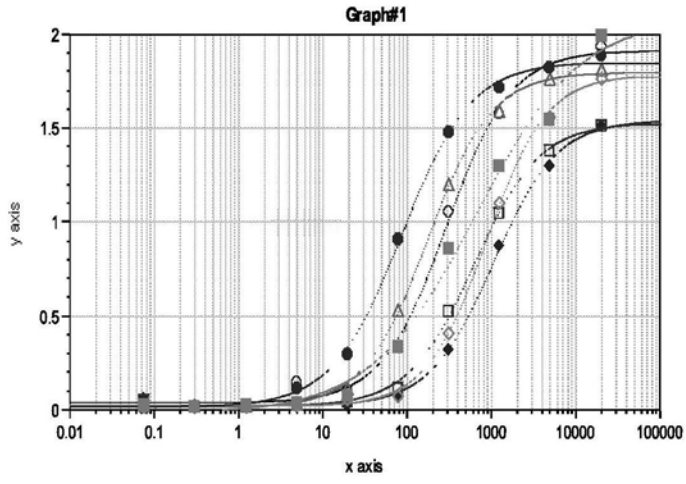
A



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
◆ Plot#1 (nKN044-216: Concentration vs OD)	0.0118	1.03	310	2.12	0.999
○ Plot#2 (nKN044-220: Concentration vs OD)	0.00315	1.11	346	2.27	0.999
□ Plot#3 (nKN044-227: Concentration vs OD)	0.0219	1.35	1.3e+04	1.99	1
△ Plot#4 (nKN044-249: Concentration vs OD)	0.0269	1.17	2.51e+03	1.31	0.999
◇ Plot#5 (nKN044-244: Concentration vs OD)	0.0124	1.23	294	1.78	1
● Plot#6 (nKN044-194: Concentration vs OD)	0.019	1.26	4.65e+03	2	1
■ Plot#7 (nKN044-200: Concentration vs OD)	0.00506	1.05	786	2.26	0.999

Weighting: Fixed

B



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
◆ Plot#1 (nKN044-268: Concentration vs OD)	0.0209	1.15	1.06e+03	1.54	0.999
○ Plot#2 (nKN044-266: Concentration vs OD)	0.0373	1.14	293	1.91	0.997
□ Plot#3 (nKN044-264: Concentration vs OD)	0.0181	1.12	627	1.53	0.999
△ Plot#4 (nKN044-235: Concentration vs OD)	0.0111	1.14	176	1.8	0.999
◇ Plot#5 (nKN044-252: Concentration vs OD)	0.0177	1.21	877	1.78	0.999
● Plot#6 (nKN044-280: Concentration vs OD)	0.0149	1.06	87	1.85	0.999
■ Plot#7 (nKN044-275: Concentration vs OD)	-0.017	0.696	657	2.09	0.991

Weighting: Fixed

图2

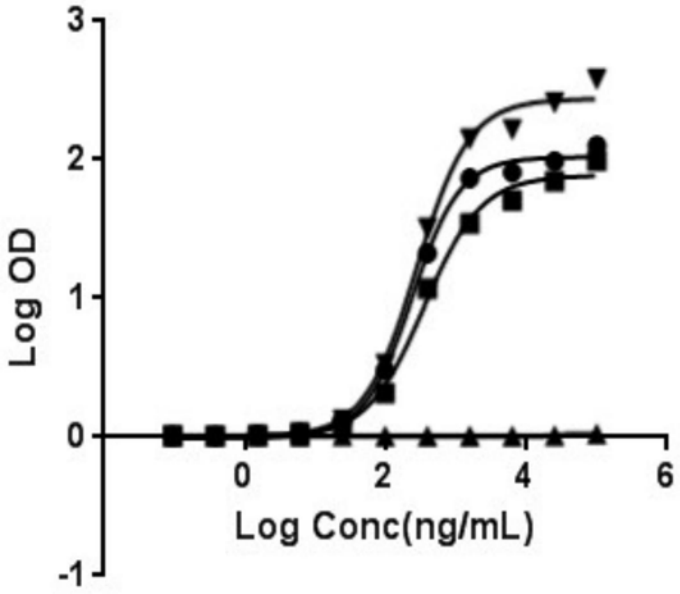


图3