(12) 按照专利合作条约所公布的国际申请

(19) 世界知识产权组织
国 际 局

(43) 国际公布日
2023 年 7 月 27 日 (27.07.2023)

WIPO | PCT

(10) 国际公布号
WO 2023/137947 A1

(51) 国际专利分类号:
C07K 19/00 (2006.01) *A61K 39/39* (2006.01)
C07K 14/52 (2006.01) *A61P 31/12* (2006.01)
C12N 15/09 (2006.01) *A61P 37/04* (2006.01)

(21) 国际申请号: PCT/CN2022/097092

(22) 国际申请日: 2022 年 6 月 6 日 (06.06.2022)

(25) 申请语言: 中文

(26) 公布语言: 中文

(30) 优先权:
202210076816.7 2022年1月24日 (24.01.2022) CN

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(81) 指定国 (除另有指明，要求每一种可提供的国家保护): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS,

(54) **Title:** USE OF CCL11

(54) 发明名称: CCL11的用途

(57) **Abstract:** The present invention relates to the technical field of vaccine preparation, and in particular to an immune-enhancing delivery system formed by targeted antigen delivery by CCL11. The system further enhances immunogenicity by fusing a chemokine CCL11 with a corresponding antigen molecule, and adding a T2 label at a terminal of the antigen molecule. The system can be a nucleic acid vector or a fusion protein or the like to be applied to prevention and/or treatment of diseases caused by a corresponding antigen. According to the present invention, by utilizing a chemotactic binding capacity of CCL11 with a surface receptor of an immune cell such as a DC, different antigen proteins are transported to the surface of the DC, so that the efficiency of phagocytosis, processing and presentation of the DC on various antigen proteins is improved, and the effect of preventing and treating related diseases is improved. The key point of the present invention is that a T2 sequence added to an antigen can enhance an immune response.

(57) 摘要: 本发明涉及疫苗制备技术领域，尤其涉及CCL11靶向递送抗原形成的免疫增强递送系统。该系统是趋化因子CCL11融合相应抗原分子，并在抗原分子末端添加T2标签，以这种方式进一步增强免疫原性。该系统可以是核酸载体或者是融合蛋白等形式应用于相应抗原所引起的疾病的预防和/或治疗。本发明利用CCL11与DC细胞等免疫细胞表面受体的趋化结合能力将不同的抗原蛋白转运到DC细胞表面，提高了各种抗原蛋白被DC细胞吞噬、加工、呈递的效率，改善了其防治相关疾病的效果。本发明关键点在于给抗原中添加的T2序列可以增强免疫效果。

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IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW。

(84) 指定国(除另有指明，要求每一种可提供的地区保护)：ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), 欧亚 (AM, AZ, BY, KG, KZ, RU, TJ, TM), 欧洲 (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG)。

本国际公布:

— 包括国际检索报告(条约第21条(3))。

— 包括说明书序列表部分(细则5.2(a))。

CCL11 的用途

本申请要求于 2022 年 01 月 24 日提交中国专利局、申请号为 202210076816.7、发明名称为“CCL5 的用途”的中国专利申请的优先权，其全部内容通过引用结合在本申请中。

技术领域

本发明涉及疫苗制备技术领域，尤其涉及 CCL11 靶向递送抗原形成的免疫增强递送系统。

背景技术

人体时刻面对着外界治病微生物的攻击以及自身正常细胞的癌变。免疫系统是人体应对病原微生物以及自身肿瘤细胞的有力武器。免疫系统的应答过程包括识别，反应以及效应三个阶段。每个阶段由人体组成免疫系统的蛋白，细胞，组织和器官协调完成。在整个应答过程中的识别阶段，免疫系统需要对自身抗原和病原进行区分，以免产生对自身的攻击。识别之后会进入针对特定病原的反应阶段，包括产生大量的蛋白以及特异性细胞。这些蛋白和细胞在效应阶段对病原进行清除并形成针对相应病原的免疫记忆。

病毒、细菌、真菌和寄生虫等病原微生物经常会引起大规模流行性疾病。譬如 2019 年底爆发的 SARS-CoV-2 已经在全球范围内传播。病原微生物侵入人体后有的诱发一过性疾病不在人体长期潜伏，但有的病原体会在人体细胞，组织内长期潜伏并引起相应的疾病。因而需要预防性疫苗预防病原微生物的传播，需要治疗性疫苗治疗清除病原微生物所寄宿的细胞。这需要免疫系统产生体液免疫以及细胞免疫反应。而措施正是利用病原微生物的特异性抗原注射人体引起免疫识别。并且既往的经验告诉我们引起的免疫反应越强则疫苗的预防和治疗效果越好。

CCL11 属于 CC 家族趋化因子成员。主要表达在小肠，心脏和肾脏以及胰腺。TNFa 和 IL-4 等促炎症细胞因子可以上调 CCL11 的表达。CCL11 发挥趋化功能主要通过其受体 CCR3。研究表明 CCR3 表达在 DC 细胞以及嗜酸性粒细胞中 DC 细胞是专职抗原提呈细胞主要介导抗原的识别。嗜酸性粒细胞除具有经典抗菌作用外，还可以与增强特异性 T 细胞免疫反应介导抗肿瘤免疫反应。基于以上将抗原分子与 CCL11 相融合会增强抗原的免疫原性，启动更强的免疫应答，为更强效力的疫苗制备提供选择。

树突状细胞（DC）是专职抗原提呈细胞，是组成免疫系统的重要成分。他们在血液中循环同时也驻留在全身各处，主要负责吞噬，加工和呈递抗原。在免疫识别阶段发挥至关重要的作用。DC 细胞对抗原的识别程度决定着免疫反应的强弱。

肿瘤起源于自身组织细胞因而很难被免疫系统所识别，但肿瘤细胞相比于正常组织细胞通常会高表达或者突变某些促癌基因以促进肿瘤细胞的增殖、存活。这些高表

达或者突变的基因产物通常只具有很弱的免疫原性，因而不会引起很强的免疫应答去消除这些肿瘤细胞。因而如何调动免疫系统产生强力的免疫反应成为清除肿瘤的关键。

肿瘤疫苗通过免疫增强已有的抗肿瘤反应或启动初始T细胞来诱导患者的效应T细胞功能，抗原特异性CD8+细胞毒性T淋巴细胞（CTL）在抗肿瘤过程中发挥重要作用。DC细胞是唯一能激活初始CD8+T细胞的专职抗原呈递细胞，对细胞外肿瘤抗原通过MHC-I进行摄取、加工和交叉提呈，对产生有效的CTL至关重要。因此，通过偶联DC细胞表面分子将肿瘤抗原递送至DC细胞是有效诱导CD8+T细胞免疫应答的肿瘤治疗策略。

目前，已经探明的能够对DC细胞的递呈起到增强作用的分子包括XCL-1，但仍亟待探明更多的能够起到促进递呈作用的DC细胞表面分子。

发明内容

有鉴于此，本发明要解决的技术问题在于提供 T2 片段和/或 CCL11 靶向递送抗原形成的免疫增强递送系统。

本发明提供了：

如Ⅰ）~Ⅵ）中的至少一种在提高抗原递呈效果中的应用；

I)、如 SEQ ID NO:4 所示氨基酸序列的 T2 片段；

II)、趋化因子 CCL11；

III)、与Ⅰ）或Ⅱ）具有 80%以上同源性，且具有相同或相似功能的片段；

IV)、编码Ⅰ）或Ⅱ）的核酸分子；

V)、在 IV)的所述核酸分子的核苷酸序列中经取代、缺失或添加一个或多个核苷酸，且能编码相同或相似功能蛋白的核酸分子；

Ⅵ)、与Ⅴ）完全互补或部分互补的核酸分子。

本发明中，T2 片段由 31 个氨基酸组成，其序列为 SEQ ID NO:4。研究表明，经过人工改造后的 T2 片段具有增强人体内免疫的效果。

本发明中，所述 CCL11 为趋化因子 CCL11，其来自于人类或其他动物，其为全长序列，或具有 CCL11 活性的部分片段。本发明发现，CCL11 能够递送抗原到 DC 细胞，从而提高递呈效果，增强免疫反应。

本发明还提供了一种融合蛋白，其包括 CCL11 和抗原。或包括 CCL11、抗原和 T2 片段。

本发明实施例，所述融合蛋白自 N 端→C 端依次包括：IgE 信号肽、CCL11、linker、抗原和 T2 片段。

本发明中，所述抗原来自病毒、病原菌和/或肿瘤。

本发明中，所述 CCL11 为人源 CCL11 序列，所述抗原为 HPV16 的 E6 蛋白和/或 HPV16 的 E7 蛋白。

一些实施例中，所述融合蛋白自 N 端→C 端依次包括：IgE 信号肽、CCL11、linker、

HPV16 的 E6 蛋白、HPV16 的 E7 蛋白和 T2 片段。

一些具体实施例中，所述 IgE 信号肽的氨基酸序列如 SEQ ID NO:5 所示；

所述 CCL11 的氨基酸序列如 SEQ ID NO:3 所示；

所述 linker 为（G_5S）n，其中 n 为 1~10；本发明实施例中，所述 linker 序列为 GGGGGSGGGGG。

所述 HPV16 的 E6 蛋白的氨基酸序列如 SEQ ID NO:1 所示；

所述 HPV16 的 E7 蛋白的氨基酸序列如 SEQ ID NO:2 所示；

所述 T2 片段的氨基酸序列如 SEQ ID NO:4 所示；

一些具体实施例中，所述融合蛋白的 C 端还包括 FLAG 标签序列，其氨基酸序列为 DYKDDDDK，但该序列只为鉴定蛋白表达的标签不影响序列的免疫效果。

本发明还提供了编码所述融合蛋白的核酸。

本发明所述的编码融合蛋白的核苷酸序列如 SEQ ID NO:9 所示。

本发明提供了一种核酸片段，其包括编码本发明所述融合蛋白的核酸、5'-UTR、3'-UTR 和 3'-端 PolyA。其中，所述 5'-UTR 为β-globin-2，所述 3'-UTR 为 2β-globin，所述 3'- 端 PolyA 的长度为 120bp 。所述核酸片段的结构为 5'UTR-CCL11-E6E7-3'UTR-A(120)。

本发明还提供了含有编码所述融合蛋白的转录单元。

所述转录单元中包括启动子和编码所述融合蛋白的核酸。

一些实施例中，所述转录单元还包括终止子。

一些具体实施例中，所述启动子为 CMV 或者 CMV/R 启动子。

本发明还提供了表达载体，其包括骨架载体和编码所述融合蛋白的核酸。

本发明中，所述骨架载体选自 pVAX1 系列载体、或 pVR 系列载体。

本发明还提供了转化或转染所述重组载体的重组宿主。

本发明所述重组宿主的宿主细胞为细菌或哺乳动物细胞。

本发明所述融合蛋白的制备方法，其包括培养本发明所述重组宿主，获得含有所述融合蛋白的培养物。

本发明提供了一种将病毒、细菌、真菌、肿瘤等抗原物质输送到 CCR3 阳性抗原提呈细胞中的递送系统。该系统由趋化因子 CCL11 融合相应抗原分子，并在抗原分子末端添加 T2 标签以进一步增强免疫原性，该系统可以是核酸载体或者是融合蛋白形式用于相应抗原所引起疾病的预防或治疗。

本发明提供了一种抗原递送系统在制备预防或治疗性疫苗中的用途，所述输送系统包括：与 CCR3 结合的配体 CCL11。

本发明所述的融合蛋白、核酸、表达载体、宿主、权所述制备方法制得的融合蛋白，和/或所述制备方法制得的含有所述融合蛋白的培养物在制备防治疾病的产品中的应用。

本发明还提供了一种防治疾病的产品，其包括：所述的融合蛋白、核酸、表达载体、宿主、所述制备方法制得的融合蛋白，和/或所述制备方法制得的含有所述融合

蛋白的培养物。

本发明还提供了一种防治疾病的方法，其为给予本发明所述的防治疾病的产品。

本发明中，所述防治包括预防和/或治疗，具体包括提高血清中抗体水平、预防肿瘤的形成、抑制肿瘤生长，提高机体对肿瘤的免疫反应能力。

本发明中，所述疾病包括由病毒和/或病原菌引起的疾病。或者所述疾病为肿瘤。

本发明中，所述防治疾病的产品包括药物和/或疫苗。本发明中，所述疫苗为 DNA 疫苗、重组蛋白疫苗或 mRNA 疫苗。

本发明中，所述给予的方式包括口服、注射和/或电转。

相关研究和多方实验都显示出并非所有 CC 家族趋化因子成员都可以与抗原分子融合来增强免疫原性；只有部分会与抗原分子相融合去增强抗原的免疫原性，启动更强的免疫应答，为更强效力的疫苗制备提供选择。例如相关研究指出 4-1BBL-S、4-1BBL-Fc、CD80-Fc 等与抗原分子相融合会较大程度增强抗原的免疫原性，但是 GM-CSF、mIL-23、IL-15SAG1 等与抗原分子相融合不具有增强免疫原性的作用。

本发明利用 CCL11 与 DC 细胞等免疫细胞表面受体的趋化结合能力将不同的抗原蛋白转运交叉呈递到 DC 细胞表面，提高了各种抗原蛋白被 DC 细胞吞噬、加工、呈递的效率，改善了其防治相关疾病的效果。本发明中的 T2 序列经实验测定其具有非常强的免疫增强效果，可以在促进抗原呈递的过程中进一步激发体液和细胞免疫反应，最终达到抑制相关肿瘤生长的效果。

附图说明

为了更清楚地说明本发明具体实施方式或现有技术中的技术方案，下面将对具体实施方式或现有技术描述中所需要使用的附图作简单地介绍，显而易见地，下面描述中的附图是本发明的一些实施方式，对于本领域普通技术人员来讲，在不付出创造性劳动的前提下，还可以根据这些附图获得其他的附图：

图 1 示 CCL11 趋化 DC 细胞和嗜酸性粒细胞能力分析；

图 2 示编码融合蛋白核苷酸的载体图谱；其中图 2 中的 A 示 pVR-CCL11-E6E7-T2、图 2 中的 B 示 pVR-CCL11-E6E7、图 2 中的 C 示 pVR-E6E7 的质粒图谱；

图 3 示检测 pVR-CCL11-E6E7-T2、pVR-CCL11-E6E7 和 pVR-E6E7 的三种质粒目的基因的表达情况，利用蛋白免疫印迹技术（Westernblot）检测 C 端带有 Flag 标签的融合蛋白编码核苷酸的表达情况；

图 4 示不同融合基因对小鼠进行预防型免疫用于后期测试特异性 T 细胞反应的时间轴；

图 5 示不同融合基因对小鼠进行免疫后特异性 T 细胞反应的流式检测结果；

图 6 示不同融合基因对小鼠进行免疫后特异性抗体反应的检测结果；

图 7 示不同融合基因、mRNA 和蛋白疫苗分别对小鼠进行治疗型免疫和接瘤时间轴；

图 8 示各组处理的细胞瘤体积。

具体实施方式

本发明提供了 CCL11 靶向递送抗原形成的免疫增强递送系统，本领域技术人员可以借鉴本文内容，适当改进工艺参数实现。特别需要指出的是，所有类似的替换和改动对本领域技术人员来说是显而易见的，它们都被视为包括在本发明。本发明的方法及应用已经通过较佳实施例进行了描述，相关人员明显能在不脱离本发明内容、精神和范围内对本文的方法和应用进行改动或适当变更与组合，来实现和应用本发明技术。

除非另有定义，本文使用的所有科技术语具有本领域普通技术人员所理解的相同含义。关于本领域的定义及术语，专业人员具体可参考 Current Protocols in Molecular Biology（Ausubel）。氨基酸残基的缩写是本领域中所用的指代 20 个常用 L-氨基酸之一的标准 3 字母和/或 1 字母代码。

本发明所述 CCL11 是一种人体内重要的趋化因子，它属于 CC 趋化因子家族（CC chemokines）中的一员，其可以特异性的在人体心脏和肾脏高水平表达。本发明研究表明 CCL11 能够用于递送物质到专职抗原提呈细胞，特别是 DC 细胞和嗜酸性粒细胞从而增强 DC 细胞的递呈效果和 T 细胞的招募效果。

本发明中，所述 CCL11 可为人源片段，也可为其他动物来源的片段，例如鼠源，兔源、猴源、猪源等，其可为完整的 CCL11，也可为其中具有 CCL11 活性的片段或突变体，本申请对此不做限定。本申请实施例中，以人源 CCL11 为实验对象，验证 CCL11 对抗原的递呈效果的提高。所述人源 CCL11 的氨基酸序列为：SEQ ID NO:3。

本发明所述融合蛋白中，所述 T2 序列是由 T4 噬菌体纤维蛋白 C 末端的一段短肽（T4 phageheadfibritin）改造而来，种属来源属于外源序列，该序列在人体内完全没有，不会出现增强免疫后对人体其他蛋白造成杀伤的问题。有报道认为该序列可以在某些情况下促进某些蛋白的三聚体化。本发明中发现 T2 这段作为经过人工改造后的多肽序列具有人体内的免疫增强效果。本发明中，T2 片段由 31 个氨基酸组成，其序列为 SEQ ID NO:4。

本发明所述融合蛋白中的抗原的个数至少为 1 个。例如，可为 1 个、2 个、3 个、4 个、5 个、6 个、7 个、8 个、9 个或 10 个及以上。在本发明的研究中，对 1 个或 2 个抗原的融合效果进行实验，都表现出良好的效果。

本发明所述抗原来自病毒、细菌、真菌、寄生虫和/或肿瘤的蛋白。一些实施例中，所述抗原来自病毒的衣壳蛋白或非结构蛋白、病原菌的膜蛋白、鞭毛蛋白或肿瘤的表面抗原。其可为的完整片段，也可为其抗原决定簇。其可仅含有一个抗原决定簇，也可以由多个抗原决定簇串联而成，也可以为一个抗原决定簇重复串联两次或以上。

本发明中，所述病毒包括但不限于 HPV 病毒、HCMV、EBV、HCV、HIV、HBV、VZV 和/或冠状病毒中至少一种;

本发明中，所述肿瘤包括但不限于肝癌、宫颈癌、卵巢癌、肺癌、头颈癌、前列腺癌、乳腺癌、血液肿瘤、黑色素瘤、鼻咽癌和/或结直肠癌中至少一种。

一些实施例中，所述抗原为 HPV 病毒的 E2、E5、E6 和/或 E7 蛋白或其突变表位。

所述 HPV 病毒包括各种亚型的 HPV 病毒，例如， HPV6、HPV11、HPV16、HPV18、HPV31、HPV33、HPV35、HPV39、HPV45、HPV51、HPV52、HPV56 和/或 HPV58 型。

一些实施例中，所述抗原为 EB 病毒的 LMP1、LMP2、EBNA1 或其突变表位。

一些实施例中，所述抗原为冠状病毒的 S 蛋白、N 蛋白、E 蛋白、M 蛋白或其表位。所述冠状病毒为 SARS-CoV、MERS-CoV 和/或 SARS-CoV-2。

一些实施例中，所述抗原为 VEGFR2, Survivin，FAP 等泛癌蛋白

一些实施例中，所述抗原为肝癌的 GPC3 蛋白和/或 AFP 蛋白。

一些实施例中，所述抗原为前列腺癌的 PSA，PSMA，PSCA，PAP 和/或 STEAP1。

一些实施例中，所述抗原为乳腺癌的 Her2/neu 和/或 BCAR3 的优势表位。

一些实施例中，所述抗原为黑色素瘤的 MAGE-A3，ISR2，NY-ESO-1，Melan A，gp100，Tyrosinase，TRP1 和/或 TRP2。

一些实施例中，所述抗原为血癌的 Immunoglobulin idiotype，Immunoglobulinκ-chain 和/或 Immunoglobulin λ-chain。

一些实施例中，所述抗原为结直肠癌的 AIM2，HT001，TAF1B，Micoryx 和/或 TGFβRII。

一些实施例中，所述抗原为卵巢癌的 folate receptor-α。

一些实施例中，所述抗原为多种原癌基因、抑癌基因和/或肿瘤特异性抗原的 P53，IDH1/ 2， BAGE，GAGE1，GAGE2，CAG3，RAGE，CEA，CDK4，CASP-8，KRAS，bcr/abl 和/或 MUC-1。

本发明利用 CCL11 与 DC 细胞等免疫细胞表面受体的趋化结合能力将上述的抗原蛋白转运交叉呈递到 DC 细胞表面，从而提高了各种抗原蛋白被 DC 细胞吞噬、加工、呈递的效率，改善了其防治相关疾病的效果。在此前的预实验中，上述蛋白中包括 HPV16 的 E6 或 E7 的蛋白在内的多个抗原的递呈效率已经被验证能够被 CCL11 提高。本发明实施例中，以 HPV16 的 E6 和 E7 蛋白为案例，证明 CCL11 对抗原蛋白递呈效率的提高效果，其他蛋白与 CCL11 融合后也会起到良好的效果。

本发明中为了保证融合蛋白中各功能片段不受空间位阻影响而顺利地折叠，在片段间添加 linker，其中，CCL11 与 HPV 病毒抗原蛋白之间的 linker 为 GGGGGSGGGGG。不同抗原之间可以通过（G_5S）n 和/或 AGA 相连。

在本发明中，为了提高融合蛋白的表达效果，在 CCL11 的 N 端添加促使融合蛋白分泌到胞外的信号肽。一些实施例中，所述信号肽为 IgE 信号肽。具体的，其氨基酸序列为 SEQ ID NO.5

在本发明中，为了方便融合蛋白的纯化，在融合蛋白的 C 端添加标签。所述标签选自本领域熟知的重组蛋白纯化标签。一些实施例中，所述标签为 DYKDDDDK。

一些具体实施例中，所述融合蛋白的结构为自 N 端→C 端依次包括: IgE 信号肽、人源 CCL11 蛋白序列、linker 序列（GGGGGSGGGGG）、E6E7 蛋白序列、T2 蛋白序列、Flag 标签序列。具体的，其氨基酸序列如 SEQ ID NO.15。

本发明所述的编码蛋白的核酸可以是 DNA、RNA、cDNA 或 PNA。在本发明实施例中，所述核酸为 DNA 或 RNA 形式。所述 DNA 形式包括 cDNA、基因组 DNA 或人工合成的 DNA。所述 DNA 可以是单链的或是双链的。核酸可以包括具有不同功能的核苷酸序列，如编码区和非编码区如调控序列(例如启动子或转录终止子)。核酸在拓扑学上可以是线性或环状的。核酸可以是例如载体(如表达或克隆载体)的一部分，或一个片段。所述核酸可直接从天然来源获得，或者可由重组、酶法或化学技术辅助制备。所述 RNA 形式为由基因转录获得的 mRNA 等。

在本发明中，对表达融合蛋白的 DNA 序列进行了优化，这些优化包括但不限于：密码子使用偏好性，消除不利于表达的二级结构(如发夹结构)，改变 GC 含量，CpG 二核苷酸含量，mRNA 的二级结构，隐蔽剪接位点，早期多聚腺苷化位点，内部核糖体进入位点和结合位点，负 CpG 岛，RNA 不稳定区，重复序列(直接重复、反向重复等)和可能影响克隆的限制性位点。

本发明所述的预防是指在肿瘤发生前给予本发明所述的药物能够起到降低肿瘤发生风险的作用。本发明所述的治疗是指在肿瘤发生之后给予本发明所述的药物，能够抑制肿瘤生长，降低肿瘤体积或延缓肿瘤的生长速度。本发明实施例中，以小鼠移植瘤细胞 TC-1 为实验对象，验证融合蛋白疫苗的效果。

本发明中还提供了融合蛋白的转录单元，所述转录单元是指启动子开始至终止子结束的 DNA 序列。启动子和终止子两侧或之间还可包括调控片段，所述调控片段可以包括与核酸序列可操作地连接的启动子、增强子、转录终止信号、多腺苷酸化序列、复制起点、核酸限制性位点、和同源重组位点，例如启动子的增强子，poly（A）信号等。本发明提供的转录单元中，包括 CMV 或 CMV/R 启动子、CMV 增强子和编码融合蛋白的核酸片段。

本发明所述重组载体，是指重组的核酸载体，是一种重组 DNA 分子，其包含期望的编码序列和对可操作连接的编码基因在具体宿主生物内的表达所必不可少的合适的核酸序列。对原核细胞中的表达必需的核酸序列包括启动子，任选包括操纵基因序列，核糖体结合位点及可能的其它序列。已知原核细胞利用启动子，增强子以及终止和多腺苷酸化信号。一经转化进入合适的宿主，载体可以独立于宿主基因组进行复制和发挥作用，或者，在一些情况下，自己整合进入基因组。在本说明书中，“质粒”和“载体”有时可以交换通用，因为质粒是当前最普遍使用的载体形式。然而，本发明意图包括表达载体的这样的其它形式，其发挥等价作用，其在本领域是已知的或将变为已知的，包括但不限于：质粒，噬菌体颗粒，病毒载体和/或仅为潜在的基因组插入物。具体实施例中，编码本发明提供的融合蛋白的核酸可构建于各种真核表达载体中。例如，其骨架载体可以是 pVAX1 系列载体、或 pVR 系列载体（参见中国专利 ZL 202110624820.8）。

本发明所述的宿主细胞，为含有核酸载体和/或目标基因的原核或真核宿主。用使用重组 DNA 技术构建的载体转化或转染宿主细胞。这样的转化宿主细胞有能力复制编码蛋白质的载体或表达期望蛋白质。

本发明实施例中，所述融合蛋白的制备方法采用诱导重组宿主表达的方式，所述培养物可以为培养获得的菌体、细胞体、培养液、或者有上述培养物中经提取和/或纯化获得的物质。

本发明提供的防治疾病的产品包括：所述的融合蛋白、核酸、表达载体、宿主、所述制备方法制得的融合蛋白，和/或所述制备方法制得的含有所述融合蛋白的培养物。本发明中，所述防治疾病的产品包括药物和/或疫苗。所述疫苗中还包括药学上可接受的运载体、赋形剂和/或佐剂。所述药物中还包括药学上可接受的辅料。

本发明所述的预防是指在疾病发生前给予本发明所述的防治疾病的产品，从而能够起到降低疾病发生风险的作用。本发明所述的治疗是指在疾病发生之后给予本发明所述的防治疾病的产品，能够改善病症，抑制疾病发展，使患者恢复健康。例如，将本发明所述的产品用于防治肿瘤，能够提高血清中抗体水平、抑制肿瘤生长，降低肿瘤体积或延缓肿瘤的生长速度。本发明实施例中，以小鼠移植瘤细胞 TC-1 为实验对象，验证融合蛋白疫苗的效果并获得良好的效果。

本发明实施例中，涉及的片段的氨基酸序列和编码的核酸片段如表 1：

表 1 本发明涉及的氨基酸序列和编码的核酸片段

SEQ ID NO.1	HPV16 的 E6 蛋白的氨基酸序列
SEQ ID NO.2	HPV16 的 E7 蛋白的氨基酸序列
SEQ ID NO.3	CCL11 的氨基酸序列
SEQ ID NO.4	T2 多肽氨基酸序列
SEQ ID NO:5	IgE 信号肽
SEQ ID NO.6	质粒 pVR-CCL11-E6E7-T2 的核苷酸序列
SEQ ID NO.7	质粒 pVR-CCL11-E6E7 的核苷酸序列
SEQ ID NO.8	质粒 pVR-E6E7 的核苷酸序列
SEQ ID NO.9	质粒 pVR-CCL11-E6E7-T2 中编码融合蛋白的 DNA 序列
SEQ ID NO.10	质粒 pVR-CCL11-E6E7 中编码融合蛋白的 DNA 序列
SEQ ID NO.11	质粒 pVR-E6E7 中编码融合蛋白的 DNA 序列
SEQ ID NO.12	质粒 pVR-CCL11-E6E7-T2 中编码融合蛋白的 mRNA 序列
SEQ ID NO.13	质粒 pVR-CCL11-E6E7 中编码融合蛋白的 mRNA 序列
SEQ ID NO.14	质粒 pVR-E6E7 中编码融合蛋白的 mRNA 序列
SEQ ID NO.15	质粒 pVR-CCL11-E6E7-T2 中融合蛋白的氨基酸序列
SEQ ID NO.16	质粒 pVR-CCL11-E6E7 中融合蛋白的氨基酸序列
SEQ ID NO.17	质粒 pVR-E6E7 中融合蛋白的氨基酸序列

下面将结合实施例对本发明的实施方案进行详细描述，但是本领域技术人员将会理解，下列实施例仅用于说明本发明，而不应视为限制本发明的范围。实施例中未注明具体条件者，按照常规条件或制造商建议的条件进行。所用试剂或仪器未注明生产厂商者，均为可以通过市售购买获得的常规产品。

实施例 1

从小鼠淋巴结以及外周血分别分离 DC 细胞和嗜酸性粒细胞后进行趋化实验。趋化小室(碳酸脂膜 Transwell 小室：5μm；Costar，Cat：3422)的上室中放入上述分离的细胞，根据实验室前期工作基础加入细胞数量为 1×10^6/100μl/孔。将趋化小室放入含有 600μL 培养基的下室，培养基中含有趋化因子 CCL11。同时设置自发迁移对照组以及 CCL11 细胞因子组，所加入的细胞数目相同。设置 3 个复孔。CCL11 因子采用 *E.coli* 纯化重组鼠 CCL11 蛋白，根据实验室前期已有的工作基础，100ng/ml 为最佳趋化效率剂量。4 小时后收集趋化下室中的细胞，并进行流式分析 CCL11 对各类免疫细胞的趋化能力。结果显示：CCL11 能有效的从上室中募集各类免疫细胞细胞到下室中 ($P < 0.001$)(图 1)。

实施例 2 融合基因或蛋白疫苗的抗原设计方案及哺乳动物表达质粒的构建与制备

构建 pVR-CCL11-E6E7-T2 质粒：根据人乳头瘤病毒亚型 HPV16 的 E6 和 E7 蛋白，人源 CCL11 蛋白及 T2 多肽序列构建融合蛋白 CCL11-E6E7-T2。在融合蛋白 CCL11-E6E7-T2 的 N 末端连接一个氨基酸序列为 MDWTWILFLVAAATRVHS 的 IgE 信号肽；在融合蛋白 CCL11-E6E7-T2 的 C 末端连接一个 DYKDDDDK 的 8 个氨基酸组成的 Flag 标签。

最终得到的融合蛋白，自 N 端→C 端依次包括：IgE 信号肽、人源 CCL11 蛋白序列、linker 序列（GGGGGSGGGGG）、E6 蛋白序列、E7 蛋白序列、T2 蛋白序列、Flag 标签序列。

将融合蛋白的氨基酸序列进行哺乳动物细胞表达偏好的密码子优化，确定其融合基因序列为 SEQ ID NO:8 将该融合基因序列进行基因合成，之后将其整体构建到 pVR 质粒载体的对应多克隆位点区域，使其能够以正确的密码子翻译顺序表达融合蛋白。最终构建形成的质粒命名为 pVR-CCL11-E6E7-T2 质粒。如图 2 中的 A 所示。

构建质粒 pVR-CCL11-E6E7：同样的令最终构建的融合基因自 N 端→C 端依次包括：IgE 信号肽、人源 CCL11 蛋白序列、linker 序列（GGGGGSGGGGG）、E6E7 蛋白序列、Flag 标签序列。

将融合蛋白的氨基酸序列进行哺乳动物细胞表达偏好的密码子优化，确定其融合基因序列为 SEQ ID NO:10 将该融合基因序列进行基因合成，之后将其整体构建到 pVR 质粒载体的对应多克隆位点区域，使其能够以正确的密码子翻译顺序表达融合蛋白。最终构建形成的质粒命名为 pVR-CCL11-E6E7 质粒。如图 2 中 B 所示。

构建 pVR-E6E7 质粒：在人乳头瘤病毒亚型 HPV16 的 E6 和 E7 蛋白序列之前在连接一个氨基酸序列为 MDWTWILFLVAAATRVHS 的 IgE 信号肽，之后连接 DYKDDDDK 的 8 个氨基酸组成的 Flag 标签。

使得最终得到的融合蛋白自 N 端→C 端依次包括：IgE 信号肽、E6E7 蛋白序列、Flag 标签序列。如图 2 中的 C 所示。

将融合蛋白的氨基酸序列进行哺乳动物细胞表达偏好的密码子优化，确定其融合基因序列为 SEQ ID NO:11 该融合基因序列进行基因合成，之后将其整体构建到 pVR 质粒载体的对应多克隆位点区域，使其能够以正确的密码子翻译顺序表达融合蛋白。最终构建形成的质粒命名为 pVR-E6E7 质粒。

具体的，在本实施例所阐述的实验中构建的质粒模式实际上为：pVR-CCL11-抗原-T2、pVR-CCL11-抗原及其对照质粒 pVR-抗原。在本实施例的实验中抗原使用的是 HPV16 亚型的 E6E7 融合蛋白。

实施例 3

构建质粒的体外细胞转染实验(以实施例 2 构建的抗原为 HPV16 E6 和 E7 蛋白的载体为例，对含有其他抗原的载体转染的方式与此相同):

转染前 24 小时，在 6 孔细胞培养板内接种 1×10^6 个 HEK293T 细胞，待细胞密度长到 80%以上时开始转染试验。转染时提前在 37℃水浴锅中预热细胞培养基、无血清的 Opti-MEM 培养基。转染时将 3 微克空载体（Vector），pVR-CCL11-E6E7-T2 表达载体，pVR-CCL11-E6E7 表达载体，pVR-E6E7 表达载体和 12μL PEI 转染试剂先后加入到 200 μL 无血清的 Opti-MEM 中，混合均匀后，室温下静置 10 分钟。将需要转染的细胞更换新鲜培养基，轻柔加入上述转染体系，轻轻摇匀。将细胞放回细胞培养箱中培养 6 小时后换液。转 48 小时后收取细胞用 Western Blot 检测 E6E7 融合基因质粒 HEK293T 细胞中的表达效果。

将收集细胞，加入 60μL 的含有 PMSF 或 Cocktail 蛋白酶抑制剂的 0.5% NP40 裂解缓冲液。充分重悬细胞，4℃旋转裂解细胞 30 分钟。12000 rpm， 4℃离心裂解液 10 分钟，收集上清至新的 1.5 mL EP 管中，弃掉沉淀。根据样品实际体积加入 5×SDS-PAGE 蛋白上样缓冲液，混合均匀后将样品放在 100℃空气浴中加热 10 分钟，立即进行 Western blot，利用 Flag 标签抗体(Sigma, F3165)进行检测,结果如图 3 显示空载体（Vector）无蛋白表达，pVR-CCL11-E6E7-T2 和 pVR-CCL11-E6E7 表达蛋白大小位置明显高于 pVR-E6E7，说明实验组质粒 pVR-CCL11-E6E7-T2、pVR-CCL11-E6E7 和对照组质粒 pVR-E6E7 都能够顺利正常在哺乳动物细胞中表达。

实施例 4

探索 CCL11 趋化因子和 T2 多肽分别对于融合基因疫苗诱导细胞特异性 T 细胞反应的影响(以抗原为 HPV16 E6 和 E7 蛋白的疫苗为例，对含有其他抗原的疫苗诱导 T 细胞反应的步骤与此相同):

鉴于融合基因在哺乳动物细胞可以正常表达。我们提取单独 pVR-CCL11-E6E7-T2、pVR-CCL11-E6E7、pVR-E6E7 质粒，利用 TERESA 活体基因导入仪对小鼠进行免疫质粒电转，质粒剂量 25μg，加上阴性对照 PBS 组共 4 组，每组 5 只。按照图 4 的时间轴标注的免疫策略对小鼠进行免疫，之后在 D14 对各组小鼠进行采血并加入加了肝素的 PBS 溶液中。将样品整体进行 3000rpm 离心 5min。弃上

清，剩余沉淀采用震荡法打散后加入 1mL 裂红液于室温裂解 1min。后将全部样品进行 1200rpm 离心 6min。弃去上清，使用 700μL 的 PBS 溶液洗涤一次。第二次 1200rpm 离心 6min。弃去上清后加入 300μL 灭活过的 10%FBS 的 1640 培养基，重悬沉淀后，加入 1μL E7 蛋白 tetramer(E7-tetramer)染色 1h。流式染色: CD8-FITC; E7-tetramer-PE。流式结果如图 5 所示。结果显示，在相同剂量组别的比较中所有 CCL11-E6E7-T2 组的 E7 特异性 T 细胞数量都远远多于 E6E7 组。在相同剂量组别的比较中所有 CCL11-E6E7-T2 组的 E7 特异性 T 细胞数量都多于 CCL11-E6E7 组。在相同剂量组别的比较中所有 CCL11-E6E7 组的 E7 特异性 T 细胞数量都多于 E6E7 组。这说明，CCL11 趋化因子在抗原蛋白的 N 端可以有效诱导抗原分子与特异性免疫细胞的结合，从而大大加强了抗原分子交叉呈递的效果，使得 CCL11 最终能够诱导出抗原分子更强的特异性免疫反应。另外，T2 多肽在抗原蛋白 C 端可以有效加强抗原分子在细胞免疫过程中的免疫强度，大大增加了特异性 T 细胞产生的数量，起到了免疫增强因子的决定性作用。

实施例 5

探索 CCL11 趋化因子和 T2 多肽分别对于融合基因疫苗诱导体液免疫反应的影响(以抗原为 HPV16 E6 和 E7 蛋白的疫苗为例，对含有其他抗原的疫苗诱导 T 细胞反应的步骤与此相同):

接下来我们评价了是否 CCL11 也可以增强体液免疫应答。我们提取单独 pVR-CCL11-E6E7-T2、pVR-CCL11-E6E7、pVR-E6E7 质粒，利用 TERESA 活体基因导入仪对小鼠进行免疫质粒电转，质粒剂量 25μg，加上阴性对照 PBS 组共 4 组，每组 5 只。按照图 4 的时间轴标注的免疫策略对小鼠进行免疫，之后在 D21 对各组小鼠进行采血，不加入抗凝剂。将样品整体进行 3000rpm 离心 20min。取上清。提前一天将实验室自行纯化的 E6E7 融合蛋白用 PBS 稀释，包被到 ELISA 板子上，每孔 200μl 含有蛋白 10μg，放于 4°过夜。第二天洗涤板子并用封闭液封闭室温 2 小时。将采取的小鼠血清 1: 100 稀释后加 100μl 到孔中后放于 4℃过夜。第三天 ELISA 显色。结果如图 6 所示。结果显示，在相同剂量组别的比较中所有 CCL11-E6E7-T2 组的 E7 特异性抗体数量都远远多于 E6E7 组。在相同剂量组别的比较中所有 CCL11-E6E7-T2 组的 E7 特异性抗体数量都多于 CCL11-E6E7 组。在相同剂量组别的比较中所有 CCL11-E6E7 组的 E7 特异性抗体数量都多于 E6E7 组。这说明，CCL11 趋化因子在抗原蛋白的 N 端可以有效诱导抗原分子与特异性免疫细胞的结合，从而大大加强了抗原分子交叉呈递的效果，使得 CCL11 最终能够诱导出抗原分子更强的特异性免疫反应。另外，T2 多肽在抗原蛋白 C 端可以有效加强抗原分子在细胞免疫过程中的免疫强度，大大增加了特异性抗体产生的数量，起到了免疫增强因子的决定性作用。

实施例 6

融合基因疫苗对小鼠移植瘤细胞 TC-1 同种移植瘤的治疗效果(以抗原为 HPV16

E6 和 E7 蛋白的融合基因疫苗为例，对含有其他抗原的疫苗对肿瘤干预效果的验证步骤与此相同):

鉴于在细胞和体液免疫实验中 CCL11-E6E7-T2 等疫苗的效果优良。我们又对融合基因免疫的 TC-1 同种移植瘤的治疗效果进行实验探索。除采用 DNA 疫苗形式外我们还合成了 CCL11-E6E7-T2 的 mRNA 疫苗形式并用纳米颗粒包裹，具体过程为将 5'UTR-CCL11-E6E7-3'UTR-A(120)分别连入克隆载体 pGEM-3Zf(+)（promega）中，完成体外转录表达系统的构建,命名为 pGEM-CCL11-E6E7,体外转录系统的 UTR 序列如下:

5-UTR (β-globin-2)

agagcggccgctttttcagcaagattaagcccagggcagagccatctattgcttacatttgcttctgacacaactgtgttcactagcaacctcaaacagacacc

3-UTR (2β-globin)

agctcgctttcttgctgtccaatttctattaaaggttcctttgttccctaagtccaactactaaactgggggatattatgaagggccttgagcatctggattctgcctaataaaaaacatttattttcattgcagctcgctttcttgctgtccaatttctattaaaggttcctttgttccctaagtccaactactaaactgggggatattatgaagggccttgagcatctggattctgcctaataaaaaacatttattttcattgc

重组质粒线性化，单酶切反应体系如下表：反应条件为 37℃，3h

组成	体积（μL）
Xho I	4
重组质粒	12 μg
10×CutSmart Buffer	5
dd H_2O	补加
Total	50

体外转录反应:

1、选用 T7-Flash Scribe™ Transcription Kit （Cell script）进行体外转录，在体外转录体系配制过程中，将 UTP 替换为 N1-Methylpseudouridine-5'-Triphosphate（Trilink Biotech）。

反应体系如下表所示，反应条件为：第一步反应结束后，35℃，30 min；第二步，反应后，35℃，15 min。

试剂	体积（μL）
Rnase-Free Water	X
线性化 DNA	1 μg
10 ×T7 转录溶液	2
100mM ATP	1.8
100mM CTP	1.8
100mM GTP	1.8
100mM UTP	1.8
100mM DTT	2
Script Cuard Rnase In hibitor	0.5
T7-Flash Scribe Enzyme Solution	2
Total	20
上述产物	20
Rnase-free DnaseI	1
Total	21

mRNA 加帽：选用试剂盒 ScriptCap™ Cap 1 Capping System （Cell script）进行操作。

Cap-mRNA 纯化

选用 MEGAclear™ Kit Purification（Invitrogen）试剂盒进行纯化反应。

脂质体纳米颗粒 LNP-Man 的制备

DOTAP（(2,3-二油酰基-丙基)三甲基氯化铵）、DOPE（二油酰磷脂酰乙醇胺）、DSPE-PEG2000（二硬脂酰基磷脂酰乙醇胺-聚乙二醇 2000）均购自上海艾韦特医药科技有限公司；DSPE-PEG2000-Man 购自西安昊然生物科技有限公司；

采用旋转蒸发法制备纳米颗粒。LNP-Man 制备的具体操作过程如下：

1) 按摩尔比 DOTAP: DOPE: DSPE-PEG2000-Man = 50 : 50 : 1，依次加入圆底烧瓶中，加入 6 mL 三氯甲烷，直至固体充分溶解；

2) 水浴超声 15 min；

3) 将圆底烧瓶装入旋转蒸发仪中，圆底烧瓶内溶解物需没过水面以下。转速为 100 rpm，旋转蒸发 15 min；

4) 取下圆底烧瓶，放置通风橱内，加入 8 mL HEPES 缓冲液，将瓶内壁上的薄膜溶解下来；

5) 水浴超声 30 min；

6) 将超声好的溶液通过 0.22 μm 的滤膜，过滤三次，即得到所需脂质体纳米颗粒 LNP-Man。

LNPs/mRNA 的制备

用制备的阳离子脂质纳米材料 LNP-Man 与 mRNA 按照设定的 N/P = 10:1(摩尔

比）混合，计算所需的 LNPs 和 mRNA 的体积；将 LNPs 与 mRNA 混合前分别加入等体积的 10 mM HEPES 缓冲液；混合后的 LNPs/mRNA 在漩涡振荡器上震荡 1 min，室温静置 30min。 同时我们还纯化了 CCL11-E6E7-T2 蛋白用于疫苗对照。采用如图 7 中的时间轴对小鼠进行接瘤和质粒治疗性免疫。每次免疫接种量为 25μg。在接瘤后开始对小鼠进行免疫两次分别为 D4 和 D11。之后按照相同方法进行量瘤，统计肿瘤体积。绘制肿瘤生长曲线如图 8 所示。结果显示，pVR-CCL11-E6E7-T2 质粒、CCL11-E6E7-T2-mRNA 以及 CCL11-E6E7-T2 蛋白的三组相较 pVR-E6E7 质粒组更早发生肿瘤生长抑制，且 pVR-CCL11-E6E7-T2 质粒、CCL11-E6E7-T2-mRNA 以及 CCL11-E6E7-T2 蛋白的三组在接瘤第 17 天前后移植瘤完全消失，达到了完全的肿瘤治疗效果。

最后应说明的是：以上各实施例仅用以说明本发明的技术方案，而非对其限制；尽管参照前述各实施例对本发明进行了详细的说明，但本领域的普通技术人员应当理解：其依然可以对前述各实施例所记载的技术方案进行修改，或者对其中部分或者全部技术特征进行等同替换；而这些修改或者替换，并不使相应技术方案的本质脱离本发明各实施例技术方案的范围。

权 利 要 求

1、如Ⅰ）~Ⅵ）中的至少一种在提高抗原递呈效果中的应用；

Ⅰ)、如 SEQ ID NO:4 所示氨基酸序列的 T2 片段；

Ⅱ)、趋化因子 CCL11；

Ⅲ)、与Ⅰ）或Ⅱ）具有 80%以上同源性，且具有相同或相似功能的片段；

Ⅳ)、编码Ⅰ）或Ⅱ）的核酸分子；

Ⅴ)、在Ⅳ)的所述核酸分子的核苷酸序列中经取代、缺失或添加一个或多个核苷酸，且能编码相同或相似功能蛋白的核酸分子；

Ⅵ)、与Ⅴ）完全互补或部分互补的核酸分子。

2、融合蛋白，其包括 CCL11 和抗原；或包括 CCL11、抗原和 T2 片段。

3、根据权利要求 2 所述的融合蛋白，其特征在于，自 N 端→C 端依次包括：IgE 信号肽、CCL11、linker、抗原和 T2 片段。

4、根据权利要求 2 或 3 所述的融合蛋白，其特征在于，所述抗原来自病毒、病原菌和/或肿瘤；

所述病毒包括 HPV 病毒、EBV、HCV、HIV、HBV、VZV 或冠状病毒中至少一种；

所述肿瘤包括肝癌、宫颈癌、卵巢癌、肺癌、头颈癌、前列腺癌、乳腺癌、血癌、卵巢癌、结直肠癌中至少一种。

5、编码权利要求 2~4 任一项所述融合蛋白的核酸。

6、核酸片段，其包括权利要求 5 所述的核酸、5’-UTR、3’-UTR 和 3’-端 PolyA。

7、表达载体，其包括骨架载体和权利要求 5 所述的核酸，或者权利要求 6 所述的核酸片段。

8、转化或转染权利要求 7 所述表达载体的宿主。

9、权利要求 2~4 任一项所述融合蛋白的制备方法，其特征在于，培养权利要求 8 所述宿主，获得含有所述融合蛋白的培养物。

10、权利要求 2~4 任一项所述的融合蛋白、权利要求 5 所述的核酸、权利要求 6 所述的核酸片段、权利要求 7 所述的表达载体、权利要求 8 所述的宿主、权利要求 9 所述制备方法制得的融合蛋白，和/或权利要求 9 所述制备方法制得的含有所述融合蛋白的培养物在制备防治疾病的产品中的应用。

11、一种防治疾病的产品，其特征在于，其包括：权利要求 2~4 任一项所述的融合蛋白、权利要求 5 所述的核酸、权利要求 6 所述的核酸片段、权利要求 7 所述的表达载体、权利要求 8 所述的宿主、权利要求 9 所述制备方法制得的融合蛋白，和/或权利要求 9 所述制备方法制得的含有所述融合蛋白的培养物。

12、一种防治疾病的方法，其特征在于，给予权利要求 11 所述的产品。

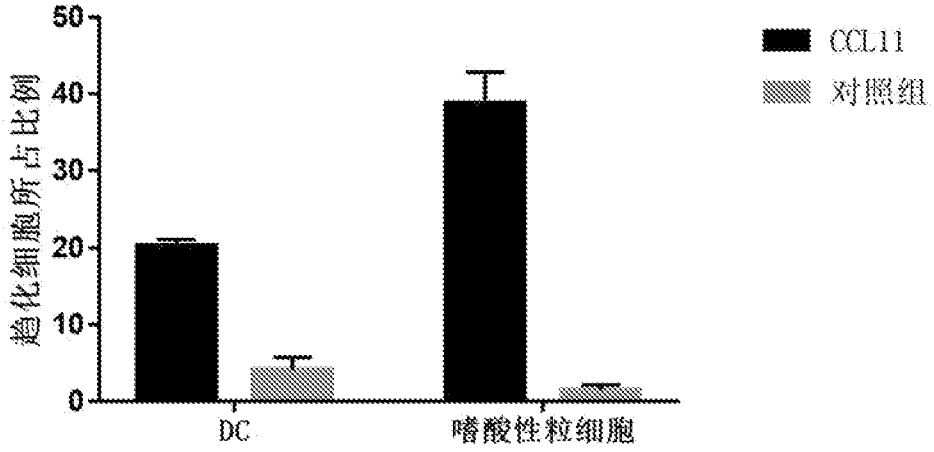
50
40
30
20
10
0
趋化细胞所占比例
CCL11
对照组
DC
嗜酸性粒细胞

图 1

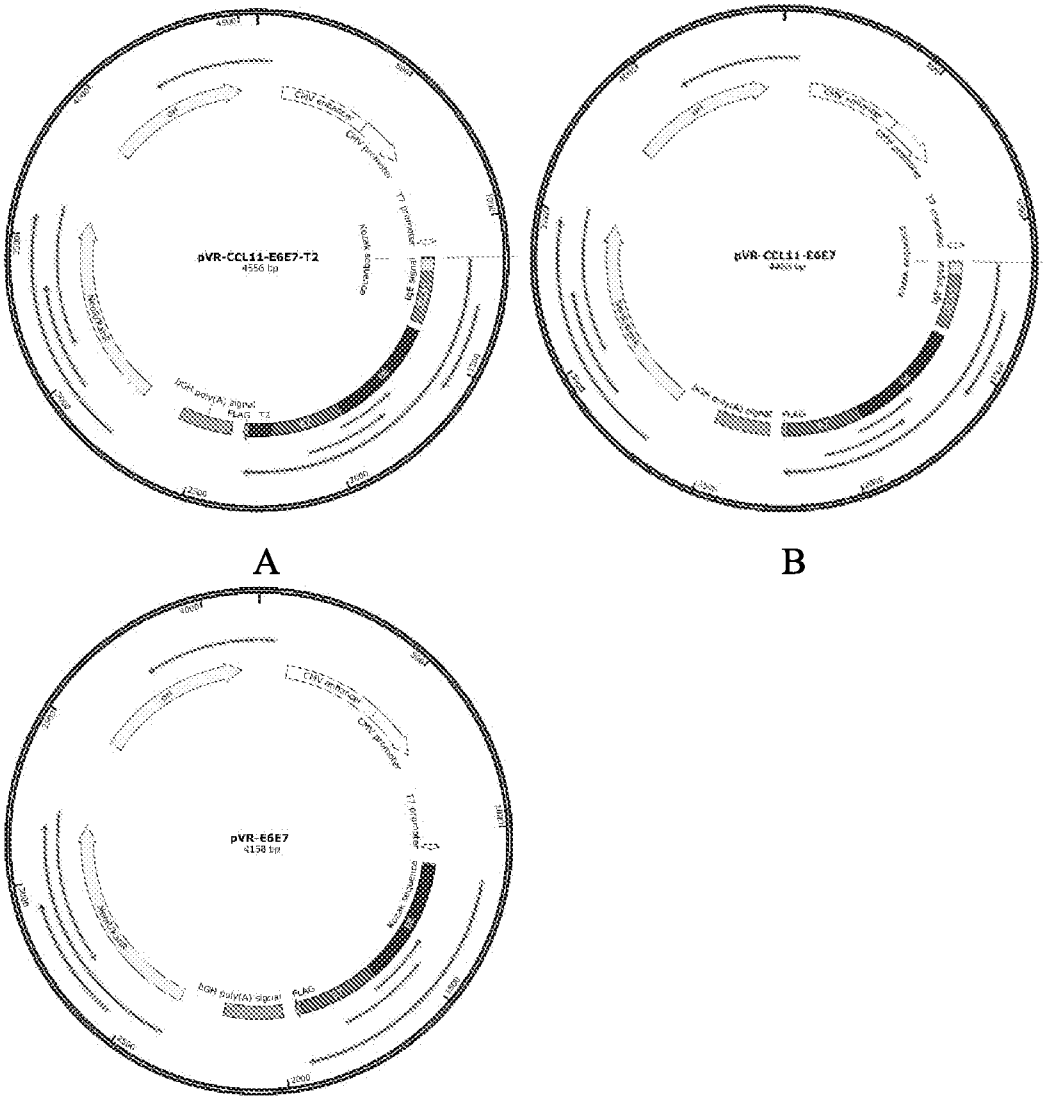
pVR-CCL11-E6E7-T2
4556 bp
pVR-CCL11-E6E7
pVR-E6E7
4158 bp
A
B
C

图 2

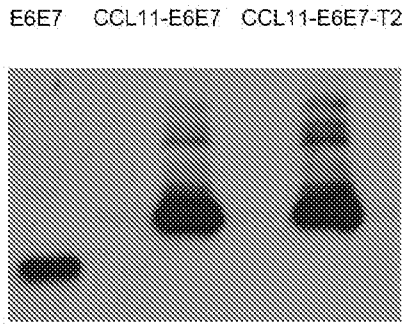
E6E7
CCL11-E6E7
CCL11-E6E7-T2

图 3

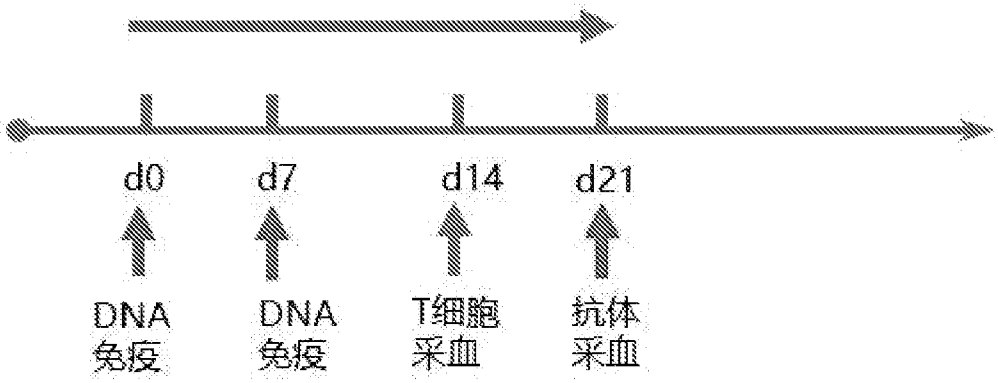
d0
d7
d14
d21
DNA
免疫
DNA
免疫
T细胞
采血
抗体
采血

图 4

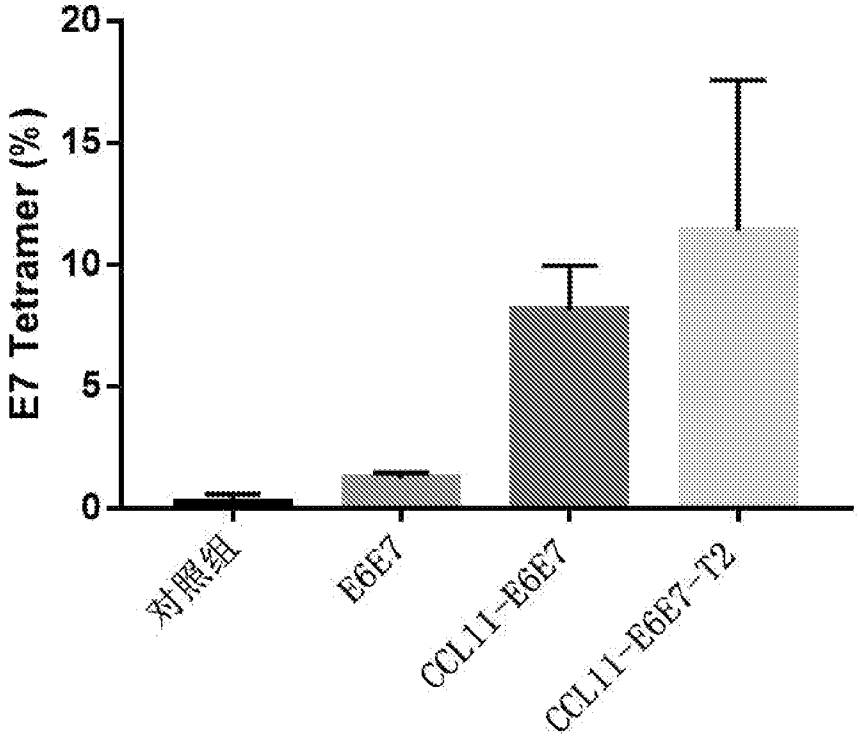

图 5

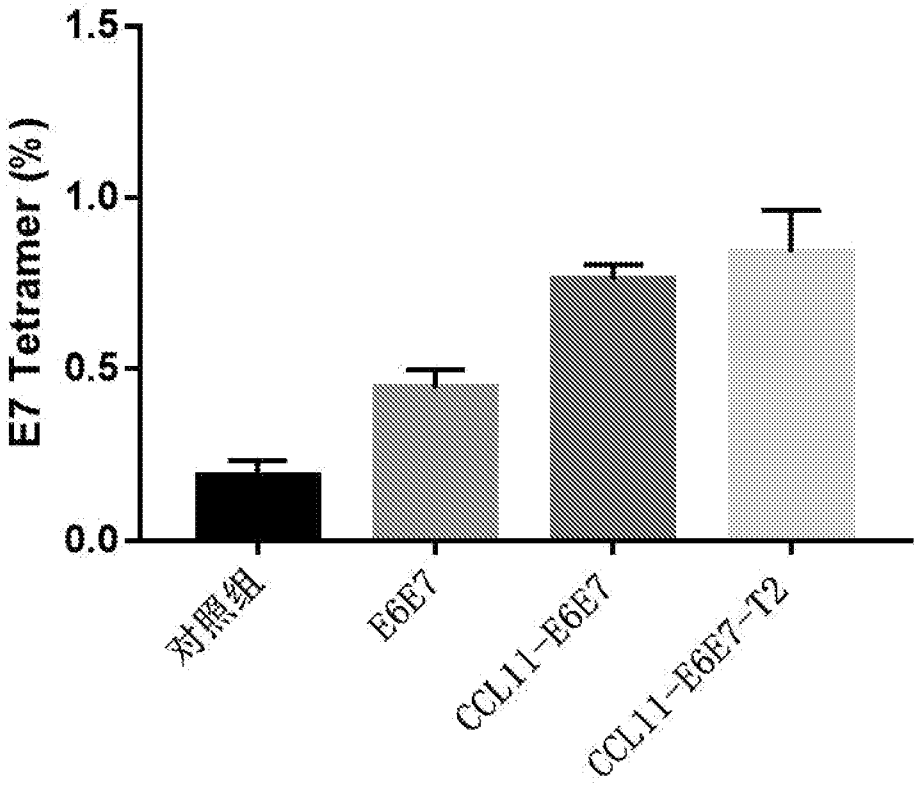
E7 Tetramer (%)
1.5
1.0
0.5
0.0
对照组
E6E7
CCL11-E6E7
CCL11-E6E7-T2

图 6

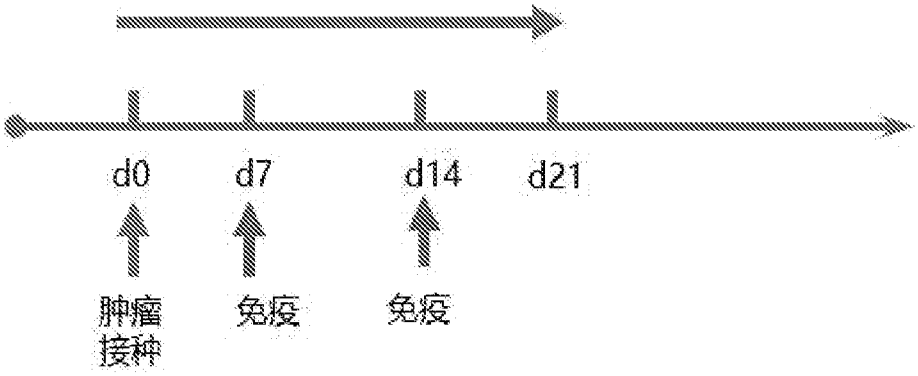
d0
d7
d14
d21
肿瘤
接种
免疫
免疫

图 7

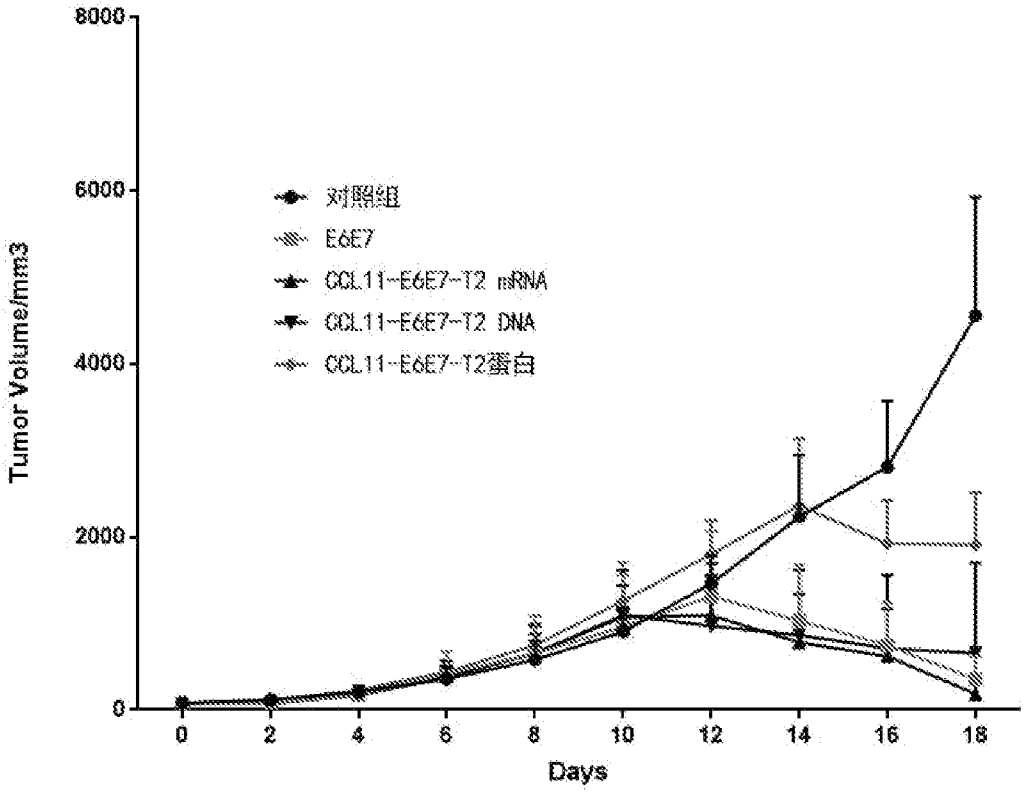
8000
6000
4000
2000
0
Tumor Volume/mm3
0
2
4
6
8
10
12
14
16
18
Days
对照组
E6E7
CCL11-E6E7-T2 mRNA
CCL11-E6E7-T2 DNA
CCL11-E6E7-T2蛋白

图 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/097092

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00(2006.01)i; C07K 14/52(2006.01)i; C12N 15/09(2006.01)i; A61K 39/39(2006.01)i; A61P 31/12(2006.01)i; A61P 37/04(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, DWPI, SIPOABS, CNKI, NCBI, ISI Web of Science: 趋化因子, 配体, 嗜酸性粒细胞, 抗原, 呈, 融合, chemokine, ligand, eotaxin, 11, 1, CCL11, antigen, presenting, fusion, t2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sylvie Beaulieu, et al. "Expression of a Functional Eotaxin (CC Chemokine Ligand 11) Receptor CCR3 by Human Dendritic Cells" *J Immunol September 15, 2002, 169 (6) 2925-2936,* Vol. 169, No. 6, 15 September 2002 (2002-09-15), abstract	1-12
PY	CN 114106207 A (NOXI TECHNOLOGY (BEIJING) LTD. CO.) 01 March 2022 (2022-03-01) entire document, in particular claims and abstract	1-12

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 June 2022	**14 October 2022**
Name and mailing address of the ISA/CN **China National Intellectual Property Administration (ISA/CN)** **No. 6, Xitucheng Road, Jimenqiao, Haidian District, Beijing 100088, China** Facsimile No. **(86-10)62019451**	Authorized officer Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/097092

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/097092

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 114106207 A	01 March 2022	None	

国际检索报告

国际申请号
PCT/CN2022/097092

A. 主题的分类

C07K 19/00(2006.01)i; C07K 14/52(2006.01)i; C12N 15/09(2006.01)i; A61K 39/39(2006.01)i; A61P 31/12(2006.01)i; A61P 37/04(2006.01)i

按照国际专利分类(IPC)或者同时按照国家分类和IPC两种分类

B. 检索领域

检索的最低限度文献(标明分类系统和分类号)

C07K; C12N; A61K; A61P

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称，和使用的检索词（如使用）)

CNABS, DWPI, SIPOABS, CNKI, NCBI, ISI Web of Science: 趋化因子，配体，嗜酸性粒细胞，抗原，呈，融合，chemokine，ligand，eotaxin，11，1，CCL11，antigen，presenting，fusion，t2

C. 相关文件

类 型*	引用文件，必要时，指明相关段落	相关的权利要求
Y	Sylvie Beaulieu，等. "Expression of a Functional Eotaxin (CC Chemokine Ligand 11) Receptor CCR3 by Human Dendritic Cells" J Immunol September 15， 2002， 169 (6) 2925-2936, 第169卷，第6期，2002年9月15日 (2002 - 09 - 15)， 摘要	1-12
PY	CN 114106207 A (诺未科技北京有限公司) 2022年3月1日 (2022 - 03 - 01) 全文，尤其是权利要求书和摘要	1-12

☐ 其余文件在C栏的续页中列出。

☑ 见同族专利附件。

* 引用文件的具体类型：
"A" 认为不特别相关的表示了现有技术一般状态的文件
"E" 在国际申请日的当天或之后公布的在先申请或专利
"L" 可能对优先权要求构成怀疑的文件，或为确定另一篇引用文件的公布日而引用的或者因其他特殊理由而引用的文件（如具体说明的）
"O" 涉及口头公开、使用、展览或其他方式公开的文件
"P" 公布日先于国际申请日但迟于所要求的优先权日的文件
"T" 在申请日或优先权日之后公布，与申请不相抵触，但为了理解发明之理论或原理的在后文件
"X" 特别相关的文件，单独考虑该文件，认定要求保护的发明不是新颖的或不具有创造性
"Y" 特别相关的文件，当该文件与另一篇或者多篇该类文件结合并且这种结合对于本领域技术人员为显而易见时，要求保护的发明不具有创造性
"&" 同族专利的文件

国际检索实际完成的日期	国际检索报告邮寄日期
2022年6月15日	2022年10月14日
ISA/CN的名称和邮寄地址 中国国家知识产权局(ISA/CN) 中国北京市海淀区蓟门桥西土城路6号 100088 传真号 (86-10)62019451	受权官员 吴永庆 电话号码 +86-10-62089161

国际检索报告

国际申请号
PCT/CN2022/097092

第I栏　核苷酸和/或氨基酸序列(续第1页第1.c项)

1. 关于国际申请中所公开的任何核苷酸和/或氨基酸序列,国际检索是基于下列序列表进行的：

a. ☑ 作为国际申请的一部分提交的：
   - ☑ 附件C/ST.25文本文件形式
   - ☐ 纸件或图形文件形式

b. ☐ 根据细则13之三.1(a)仅为国际检索目的以附件C/ST.25文本文件形式与国际申请同时提交的：

c. ☐ 仅为国际检索目的在国际申请日之后提交的：
   - ☐ 附件C/ST.25文本文件形式（细则13之三.1(a)）
   - ☐ 纸件或图形文件形式（细则13之三.1(b)和行政规程第713段）

2. ☐ 另外，在提交/提供了多个版本或副本的序列表的情况下，提供了关于随后提交的或附加的副本中的信息与申请时提交的作为申请一部分的序列表的信息相同或未超出申请时提交的申请中的信息范围（如适用）的所需声明。

3. 补充意见：

国际检索报告
关于同族专利的信息

国际申请号
PCT/CN2022/097092

检索报告引用的专利文件	公布日 (年/月/日)	同族专利	公布日 (年/月/日)
CN 114106207 A	2022年3月1日	无	

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 4 474 396 A1**

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 153(4) EPC

(43) Date of publication:
11.12.2024 Bulletin 2024/50

(21) Application number: **22921378.0**

(22) Date of filing: **06.06.2022**

(51) International Patent Classification (IPC):
C07K 19/00 *(2006.01)* ***C07K 14/52*** *(2006.01)*
C12N 15/09 *(2006.01)* ***A61K 39/39*** *(2006.01)*
A61P 31/12 *(2006.01)* ***A61P 37/04*** *(2006.01)*

(86) International application number:
PCT/CN2022/097092

(87) International publication number:
WO 2023/137947 (27.07.2023 Gazette 2023/30)

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME
Designated Validation States:
KH MA MD TN

(30) Priority: **24.01.2022 CN 202210076816**

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Remarks:
The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

(54) **USE OF CCL11**

(57) The present invention relates to the technical field of vaccine preparation, and in particular to an immune-enhancing delivery system formed by targeted antigen delivery by CCL11. The system further enhances immunogenicity by fusing a chemokine CCL11 with a corresponding antigen molecule, and adding a T2 label at a terminal of the antigen molecule. The system can be a nucleic acid vector or a fusion protein or the like to be applied to prevention and/or treatment of diseases caused by a corresponding antigen. According to the present invention, by utilizing a chemotactic binding capacity of CCL11 with a surface receptor of an immune cell such as a DC, different antigen proteins are transported to the surface of the DC, so that the efficiency of phagocytosis, processing and presentation of the DC on various antigen proteins is improved, and the effect of preventing and treating related diseases is improved. The key point of the present invention is that a T2 sequence added to an antigen can enhance an immune response.

EP 4 474 396 A1

Description

[0001] This application claims the priority of Chinese Patent Application No. 202210076816.7, filed with the China National Intellectual Property Administration on January 24, 2022, and titled with "USE OF CCL5", which is hereby incorporated by reference in its entirety.

FIELD

[0002] The present disclosure relates to the technical field of vaccine preparation, and in particular to an immune enhancement delivery system with targeted delivery of antigen through CCL11.

BACKGROUND

[0003] The human body is always facing the attack of external pathogenic microorganisms and carcinogenesis of its own normal cells. The immune system is a powerful weapon of human body against pathogenic microorganisms as well as its own tumor cells. The immune system response has a process including three stages of recognition, response and effect. Each stage is completed by the coordination of the proteins, cells, tissues and organs that constitute the immune system of human body. During the recognition stage of the overall response process, the immune system needs to distinguish between self-antigens and pathogens to avoid an attack on the body. The recognition stage is followed by the response stage against specific pathogens, which includes the production of a large number of proteins and specific cells. These proteins and cells eliminate pathogens and form immunological memory against corresponding pathogens in the effect stage.

[0004] Pathogenic microorganisms such as viruses, bacteria, fungi, and parasites often cause large-scale epidemic diseases. For example, SARS-CoV-2, which broke out at the end of 2019, has spread globally. After pathogenic microorganisms invade the human body, some induce transient diseases without long-term incubation in the human body, but some pathogens can incubate in human cells and tissues for a long time and cause corresponding diseases. Therefore, prophylactic vaccines are required to prevent the spread of pathogenic microorganisms, and therapeutic vaccines are required to treat and eliminate the cells that are host to pathogenic microorganisms. This requires the immune system to generate humoral as well as cellular immune response, which is realized by the measure of injecting the human body with specific antigens of pathogenic microorganisms to cause immune recognition. Besides, it can be known from the past experience that the stronger the immune response, the better the prevention and treatment effect of the vaccine.

[0005] CCL11, as a member of the CC family of chemokines, is mainly expressed in small intestine, heart, kidneys and pancreas. Pro-inflammatory cytokines such as TNFa and IL-4 can upregulate the expression of CCL11. CCL11 plays a chemotactic function mainly through its receptor CCR3. Studies have shown that CCR3 is expressed on DCs and eosinophils. DCs are professional antigen-presenting cells, and mainly mediate antigen recognition. Eosinophils, in addition to the classic antibacterial effect, can also mediate anti-tumor immune responses by enhancing specific T cell immune responses. Based on the above, the fusion of antigen molecules and CCL11 will enhance the immunogenicity of antigen, which initiates a stronger immune response and provides options for the preparation of more effective vaccines.

[0006] Dendritic cells (DCs) are professional antigen-presenting cells and an important component of the immune system. They circulate in the blood and reside throughout the body, where they are primarily responsible for phagocytosis, processing and presentation of antigens, and play a crucial role in the immune recognition stage. The recognition degree of antigens by DCs determines the strength of the immune response.

[0007] Tumors originate from autologous tissue cells and are thus difficult to be recognized by the immune system. Compared with normal tissue cells, however, tumor cells usually have certain highly expressed or mutated oncogenes for the proliferation and survival of tumor cells. These highly expressed or mutated gene products usually have only weak immunogenicity, which will not cause a strong immune response to eliminate the tumor cells. Therefore, how to mobilize the immune system to generate a strong immune response has become the key to eliminating tumors.

[0008] Tumor vaccines induce the effector T cells in patients to exert functions by potentiating the existing anti-tumor response or activating naive T cells. Antigen-specific CD8+ cytotoxic T lymphocytes (CTL) play an important role in the anti-tumor process. DCs are the only professional antigen-presenting cells that can activate naive CD8+ T cells. They uptake, process and cross-present extracellular tumor antigens through MHC-I, and are crucial for the production of effective CTLs. Therefore, delivery of tumor antigens to DCs by conjugating DC cell surface molecules is an effective tumor therapy strategy for inducing CD8+ T cell immune responses.

[0009] At present, the proven molecules that can enhance the presentation of DCs include XCL-1, but it is still urgent to find out more DC cell surface molecules that can promote the presentation.

SUMMARY

[0010] In view of this, the technical problem to be solved by the present disclosure is to provide an immune enhancement delivery system formed by a T2 fragment and/or CCL11 targeting delivering antigen.

[0011] The present disclosure provides:

Use of at least one of I)-VI) in improving antigen presentation effect;

I) a T2 fragment with an amino acid sequence set forth in SEQ ID NO: 4;

II) chemokine CCL11;

III) a fragment being more than 80% homologous with and functionally identical or similar to I) or II);

IV) a nucleic acid molecule encoding I) or II);

V) a nucleic acid molecule that is derived from the nucleotide sequence of the nucleic acid molecule in IV) by deletion, addition or substitution of one or more nucleotides and encode a protein functionally identical or similar to the nucleic acid molecule in IV);

VI) a nucleic acid molecule fully or partially complementary to V).

[0012] In the present disclosure, the T2 fragment consists of 31 amino acids, and has a sequence set forth in SEQ ID NO: 4. Studies have shown that the artificially modified T2 fragment has the effect of enhancing immunity in the human body.

[0013] In the present disclosure, the CCL11 is a chemokine CCL11 derived from humans or other animals, and is a full-length sequence or a fragment with CCL11 activity. In the present disclosure, it is found that CCL11 can deliver antigens to DCs, thereby improving the presentation effect and enhancing immune response.

[0014] The present disclosure also provides a fusion protein comprising CCL11 and an antigen or comprising CCL11, an antigen and a T2 fragment.

[0015] In an embodiment of the present disclosure, the fusion protein, from N-terminal to C-terminal, sequentially comprises an IgE signal peptide, CCL11, a linker, the antigen and the T2 fragment.

[0016] In the present disclosure, the antigen is derived from a virus, pathogenic microbe and/or tumor.

[0017] In the present disclosure, the CCL11 is a humanized CCL11 sequence, and the antigen is E6 protein of HPV16 and/or E7 protein of HPV16.

[0018] In some embodiments, the fusion protein, from N-terminal to C-terminal, sequentially comprises an IgE signal peptide, CCL11, a linker, E6 protein of HPV16, E7 protein of HPV16, and the T2 fragment.

[0019] In some specific embodiments, the IgE signal peptide has an amino acid sequence set forth in SEQ ID NO: 5;

the CCL11 has an amino acid sequence set forth in SEQ ID NO: 3;

the linker is (GsS)n, wherein n is 1-10; in the embodiments of the present disclosure, the linker has a sequence of GGGGGSGGGGG.

[0020] The E6 protein of HPV16 has an amino acid sequence set forth in SEQ ID NO: 1.

[0021] The E7 protein of HPV16 has an amino acid sequence set forth in SEQ ID NO: 2.

[0022] The T2 fragment has an amino acid sequence set forth in SEQ ID NO: 4.

[0023] In some specific embodiments, the fusion protein further comprises a FLAG tag sequence at the C-terminal with an amino acid sequence of DYKDDDDK, which is only a tag for identifying protein expression and does not affect the immune effect of the sequence.

[0024] The present disclosure further provides a nucleic acid encoding the fusion protein.

[0025] The nucleic acid encoding the fusion protein of the present disclosure has a nucleotide sequence set forth in SEQ ID NO: 9.

[0026] The present disclosure provides a nucleic acid fragment, which comprises the nucleic acid encoding the fusion protein of the present disclosure, 5'-UTR, 3'-UTR and 3'-end polyA, wherein the 5'-UTR is 0-globin-2, the 3'-UTR is 20-globin, and the 3'-end polyA has a length of 120 bp. The nucleic acid fragment has a structure of 5'UTR-CCL11-E6E7-3'UTR-A(120).

[0027] The present disclosure also provides a transcription unit encoding the fusion protein.

[0028] The transcription unit comprises a promoter and the nucleic acid encoding the fusion protein.

[0029] In some embodiments, the transcription unit further comprises a terminator.

[0030] In some specific embodiments, the promoter is a CMV or CMV/R promoter.

[0031] The present disclosure further provides an expression vector, comprising a vector backbone and the nucleic acid encoding the fusion protein.

[0032] In the present disclosure, the vector backbone is selected from the group consisting of pVAX1 series vectors and pVR series vectors.

[0033] The present disclosure further provides a recombinant host transformed or transfected with the expression vector.

[0034] In the present disclosure, the host cells of the recombinant host are bacteria or mammalian cells.

[0035] The present disclosure provides a method for producing the fusion protein, comprising culturing the recombinant host of the present disclosure and collecting a culture containing the fusion protein.

[0036] The present disclosure provides a delivery system for delivering antigenic substances such as viruses, bacteria, fungi and tumors to CCR3-positive antigen-presenting cells. In the system, the antigen molecule is fused with chemokine CCL11, and a T2 tag is added at the end of the antigen molecule to further enhance the immunogenicity. The system may be in the form of a nucleic acid vector or fusion protein for the prevention or treatment of diseases caused by the antigen.

[0037] The present disclosure provides use of an antigen delivery system in the manufacture of a preventive or therapeutic vaccine, wherein the delivery system comprises a ligand CCL11 to bind to CCR3.

[0038] The present disclosure provides use of the fusion protein, nucleic acid, expression vector, host, fusion protein prepared by the method, and/or the culture containing the fusion protein prepared by the method of the present disclosure in the manufacture of a product for preventing and/or treating a disease.

[0039] The present disclosure further provides a product for preventing and/or treating a disease, comprising the fusion protein, nucleic acid, expression vector, host, fusion protein prepared by the method, and/or the culture containing the fusion protein prepared by the method.

[0040] The present disclosure also provides a method for preventing and/or treating a disease, comprising administering the product for preventing and/or treating a disease of the present disclosure.

[0041] In the present disclosure, the preventing and/or treating specifically includes increasing antibody level in serum, preventing tumor formation, inhibiting tumor growth, and improving immune response ability of the body against tumors.

[0042] In the present disclosure, the disease includes diseases caused by viruses and/or pathogenic microbes, or the disease is a tumor.

[0043] In the present disclosure, the product for preventing and/or treating a disease includes a medicine and/or a vaccine. In the present disclosure, the vaccine is selected from the group consisting of a DNA vaccine, a recombinant protein vaccine and an mRNA vaccine.

[0044] In the present disclosure, the administering includes oral administration, injection and/or electroporation.

[0045] Relevant studies and multiple experiments have shown that not all CC family chemokine members fused with antigen molecules can enhance immunogenicity. Only some are used to be fused with antigen molecules to enhance the immunogenicity of antigens, initiate a stronger immune response, and provide options for more potent vaccine preparations. For example, relevant studies have indicated that the fusion of 4-1BBL-S, 4-1BBL-Fc or CD80-Fc with antigen molecules can greatly enhance the immunogenicity of antigens, but the fusion of GM-CSF, mIL-23, or IL-15SAG1 with antigen molecules does not have the effect of enhancing immunogenicity.

[0046] In the present disclosure, the chemotactic binding ability of CCL11 to surface receptors on immune cells such as DCs is utilized to transport and cross-present various antigenic proteins to the surface of DCs, which improves the efficiency of phagocytosis, processing and presentation of various antigenic proteins by DCs, and improves the effect of preventing and/or treating related diseases. The T2 fragment of the present disclosure has been determined to have an extremely strong immune enhancement effect, and can further stimulate humoral and cellular immune responses in the process of promoting antigen presentation, finally achieving the effect of inhibiting the growth of related tumors.

BRIEF DESCRIPTION OF DRAWINGS

[0047] In order to more clearly illustrate the specific embodiments of the present disclosure or the technical solutions in the prior art, the following will briefly introduce the drawings used in the description of the specific embodiments or the prior art. Apparently, the drawings in the following description are some embodiments of the present disclosure. For those of ordinary skill in the art, other drawings can also be obtained according to these drawings without making creative effort:

FIG. 1 shows the analysis of the ability of CCL11 to chemoattract DCs and eosinophils.

FIG. 2 shows the vector maps of nucleotides encoding the fusion proteins; wherein A shows the plasmid map of pVR-CCL11-E6E7-T2, B shows the plasmid map of pVR-CCL11-E6E7, and C shows the plasmid map of pVR-E6E7.

FIG. 3 shows the expression of the target genes of the three plasmids pVR-CCL11-E6E7-T2, pVR-CCL11-E6E7 and

pVR-E6E7, which is obtained by detecting the expression of the nucleotide encoding the fusion protein with a Flag tag at the C-terminal using Western blot.

FIG. 4 shows the timeline of the prophylactic immunization performed on mice with different fusion genes for later testing of specific T cell responses.

FIG. 5 shows the results of flow cytometric detection of specific T cell responses after immunization of mice with different fusion genes.

FIG. 6 shows the results of detection of specific antibody responses after immunization of mice with different fusion genes.

FIG. 7 shows the timeline of tumor inoculation in mice with different fusion genes, mRNA and protein vaccines for therapeutic immunization.

FIG. 8 shows the volume of cell tumors treated by each group.

DETAILED DESCRIPTION

[0048] The present disclosure provides an immune enhancement delivery system with targeted delivery of antigen through CCL11. Those skilled in the art can learn from the content herein and appropriately improve the process parameters for realization. It should be particularly pointed out that all similar replacements and modifications are apparent to those skilled in the art, and they are all considered to be included in the present disclosure. The method and use of the present disclosure have been described through preferred embodiments, and it is apparent that relevant persons can make changes or appropriate modifications and combinations of the methods and use herein without departing from the content, spirit and scope of the present disclosure to implement and apply the present disclosure.

[0049] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art. For definitions and terms in the art, those skilled can refer to Current Protocols in Molecular Biology (Ausubel). The standard three- and/or one-letter code used for expressing one of 20 common L-amino acids in the art is adopted as the abbreviation of an amino acid residue.

[0050] The CCL11 of the present disclosure is an important chemokine in the human body, belonging to the CC chemokine family, and can be specifically expressed at a high level in human heart and kidneys. The present disclosure shows that CCL11 can be used to deliver substances to professional antigen-presenting cells, especially DCs and eosinophils, so as to enhance the presentation effect of DCs and the recruitment effect of T cells.

[0051] In the present disclosure, the CCL11 can be a humanized fragment, or fragments from other animals, such as murine, rabbit's, simian and porcine fragments. It can be a complete CCL11, or a fragment or mutant with CCL11 activity, which is not limited by the present application. In the examples of the present application, humanized CCL11 was used as the experimental object to demonstrate the improvement of the antigen presentation effect of CCL11. The humanized CCL11 has an amino acid sequence set forth in SEQ ID NO: 3.

[0052] In the fusion protein of the present disclosure, the T2 fragment is modified from a short peptide at the C-terminal of bacteriophage T4 fibrin, and is an exogenous sequence in terms of species source. This sequence is completely absent in the human body, and will not cause problems of killing other human proteins after immune enhancement. It has been reported that this sequence can promote the trimerization of certain proteins under certain conditions. In the present disclosure, it is found that the T2 fragment, as an artificially modified polypeptide sequence, has an immune enhancing effect in the human body. In the present disclosure, the T2 fragment consists of 31 amino acids, and has a sequence set forth in SEQ ID NO: 4.

[0053] The fusion protein of the present disclosure comprises at least one antigen. For example, it may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more antigens. In the present disclosure, experiments were conducted on the fusion effect of one or two antigens, which all showed good results.

[0054] The antigen of the present disclosure is derived from proteins derived from viruses, bacteria, fungi, parasites and/or tumors. In some embodiments, the antigen is derived from capsid proteins or non-structural proteins of viruses, membrane proteins or flagellin of pathogenic microbes, or surface antigens of tumors. It may be a complete fragment or an antigenic determinant thereof. It may contain only one antigenic determinant, or may be composed of multiple antigenic determinants in series, or may contain two or more repeats in series of one antigenic determinant.

[0055] In the present disclosure, the virus is selected from the group consisting of HPV, HCMV, EBV, HCV, HIV, HBV, VZV, a coronavirus, and a combination thereof.

[0056] In the present disclosure, the tumor is selected from the group consisting of liver cancer, cervical cancer, ovarian cancer, lung cancer, head and neck cancer, prostate cancer, breast cancer, blood cancer, melanoma, nasopharyngeal

carcinoma, and a combination thereof.

[0057] In some embodiments, the antigen is E2, E5, E6 and/or E7 protein of HPV or a mutant epitope thereof. The HPV includes viruses of various HPV subtypes, for example, HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56 and/or HPV58.

[0058] In some embodiments, the antigen is LMP1, LMP2, EBNA1 of EB virus or a mutant epitope thereof.

[0059] In some embodiments, the antigen is S protein, N protein, E protein, M protein of a coronavirus or an epitope thereof. The coronavirus is SARS-CoV, MERS-CoV and/or SARS-CoV-2.

[0060] In some embodiments, the antigen is pan-oncoproteins such as VEGFR2, Survivin, and FAP.

[0061] In some embodiments, the antigen is GPC3 protein and/or AFP protein of liver cancer.

[0062] In some embodiments, the antigen is PSA, PSMA, PSCA, PAP and/or STEAP1 of prostate cancer.

[0063] In some embodiments, the antigen is a dominant epitope of Her2/neu and/or BCAR3 of breast cancer.

[0064] In some embodiments, the antigen is MAGE-A3, ISR2, NY-ESO-1, Melan A, gp100, Tyrosinase, TRP1 and/or TRP2 of melanoma.

[0065] In some embodiments, the antigen is immunoglobulin idiotype, immunoglobulin κ-chain and/or immunoglobulin λ-chain of blood cancer.

[0066] In some embodiments, the antigen is AIM2, HT001, TAF1B, Micoryx and/or TGFβRII of colorectal cancer.

[0067] In some embodiments, the antigen is folate receptor-α of ovarian cancer.

[0068] In some embodiments, the antigen is P53, IDH1/2, BAGE, GAGE1, GAGE2, CAG3, RAGE, CEA, CDK4, CASP-8, KRAS, bcr/abl and/or MUC-1 of various proto-oncogenes, antioncogenes and/or tumor-specific antigens.

[0069] In the present disclosure, the chemotactic binding ability of CCL11 to surface receptors on immune cells such as DCs is utilized to transport and cross-present the above antigenic proteins to the surface of DCs, which improves the efficiency of phagocytosis, processing and presentation of various antigenic proteins by DCs, and improves the effect of preventing and/or treating related diseases. In previous preliminary experiments, the presentation efficiency of multiple antigens of the above proteins including E6 or E7 protein of HPV16 has been demonstrated to be improved by CCL11. In the embodiments of the present disclosure, E6 and E7 proteins of HPV16 are taken as examples to prove that CCL11 can improve the efficiency of antigen protein presentation, and other proteins fused with CCL11 can also have a good effect.

[0070] In the present disclosure, in order to ensure smooth folding of each functional fragment in the fusion protein without being affected by steric hindrance, a linker is added between the fragments, wherein the linker between CCL11 and the antigenic protein of HPV is GGGGGSGGGGG. Different antigens can be linked by $(G_5S)_n$ and/or AGA.

[0071] In the present disclosure, in order to improve the expression effect of the fusion protein, a signal peptide that promotes the secretion of the fusion protein to the extracellular space is added to the N-terminal of CCL11. In some embodiments, the signal peptide is an IgE signal peptide. Specifically, it has an amino acid sequence set forth in SEQ ID NO: 5.

[0072] In the present disclosure, in order to facilitate the purification of the fusion protein, a tag is added to the C-terminal of the fusion protein. The tag is selected from recombinant protein purification tags well known in the art. In some embodiments, the tag is DYKDDDDK.

[0073] In some specific embodiments, the fusion protein has a structure which, from N-terminal to C-terminal, sequentially comprises an IgE signal peptide, a humanized CCL11 protein sequence, a linker sequence (GGGGGSGGGGG), an E6 E7 protein sequence, a T2 fragment sequence, and a Flag tag sequence. Specifically, it has an amino acid sequence set forth in SEQ ID NO: 15.

[0074] In the present disclosure, the nucleic acid encoding the protein may be DNA, RNA, cDNA or PNA. In an embodiment of the present disclosure, the nucleic acid is in the form of DNA or RNA. The DNA form includes cDNA, genomic DNA or synthetic DNA. The DNA may be single-stranded or double-stranded. The nucleic acid can comprise nucleotide sequences that have different functions, for example, a coding region and a non-coding region such as a regulatory sequence (e.g., a promoter or a transcription terminator). The nucleic acid can be linear or circular in topology. The nucleic acid can be, for example, a portion or a fragment of a vector, such as an expression or cloning vector. The nucleic acid may be obtained directly from natural sources, or may be prepared with the aid of recombinant, enzymatic or chemical techniques. The RNA form is mRNA obtained by gene transcription or the like.

[0075] In the present disclosure, the DNA sequence for expressing the fusion protein is optimized, and such optimization includes but is not limited to: codon usage bias, elimination of secondary structures (such as hairpin structures) that are not conducive to expression, changes in GC content, CpG dinucleotide content, secondary structure of mRNA, cryptic splice sites, early polyadenylation sites, internal ribosome entry and binding sites, negative CpG islands, RNA instability region, repeats (direct repeats, reverse repeats, etc.) and restriction sites that may affect cloning.

[0076] The prevention of the present disclosure refers to reducing the risk of tumor occurrence by administering the drug of the present disclosure before the tumor occurrence. The treatment of the present disclosure refers to inhibiting tumor growth, reducing tumor volume or delaying tumor growth by administering the drug of the present disclosure after tumor occurrence. In the examples of the present disclosure, the mouse transplanted tumor cell TC-1 is used as the experimental object to demonstrate the effect of the fusion protein vaccine.

[0077] The present disclosure further provides a transcription unit of the fusion protein, and the transcription unit refers to the DNA sequence beginning from the promoter and ending at the terminator. Regulatory fragments may also be comprised on either side of or between the promoter and terminator, and may include promoters, enhancers, transcription termination signals, polyadenylation sequences, origins of replication, nucleic acid restriction sites, and homologous recombination sites, such as promoter enhancers and poly(A) signals, which are operably linked to the nucleic acid sequence. The transcription unit provided by the present disclosure comprises a CMV or CMV/R promoter, a CMV enhancer and a nucleic acid fragment encoding the fusion protein.

[0078] The expression vector of the present disclosure refers to a recombinant nucleic acid vector, which is a recombinant DNA molecule that comprises a desired coding sequence and suitable nucleic acid sequences necessary for the expression of an operably linked coding gene in a specific host organism. Nucleic acid sequences necessary for expression in prokaryotic cells include a promoter with an optional operator sequence, a ribosome binding site and possibly other sequences. It is known that a promoter, enhancer, terminator and polyadenylation signals are used in prokaryotic cells. Once transformed into a suitable host, the vector can replicate and function independently of the host genome, or, in some cases, it can be integrated into the genome. In this specification, "plasmid" and "vector" are sometimes used interchangeably because plasmid is currently the most commonly used form of vector. However, the present disclosure is intended to include such other forms of expression vectors, which serve equivalent functions, and are or will become known in the art, including but not limited to: plasmids, phage particles, viral vectors and/or potential genomic insertions. In a specific embodiment, the nucleic acid encoding the fusion protein provided by the present disclosure can be constructed in various eukaryotic expression vectors. For example, the vector backbone can be a pVAX1 series vector, or a pVR series vector (see Chinese patent ZL 202110624820.8).

[0079] The host cell of the present disclosure is a prokaryotic or eukaryotic host containing a nucleic acid vector and/or a target gene. The host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of replicating the vector encoding the protein or expressing the desired protein.

[0080] In the embodiments of the present disclosure, the fusion protein is prepared by a method of inducing the expression of the recombinant host, and the obtained culture can be bacteria, cells and culture solution obtained from culture, or substances obtained by extraction and/or purification from the above cultures.

[0081] The product for preventing and/or treating a disease provided by the present disclosure comprises the fusion protein, nucleic acid, expression vector, host, the fusion protein prepared by the method, and/or the culture containing the fusion protein prepared by the method. In the present disclosure, the product for preventing and/or treating a disease includes a medicine and/or a vaccine. The vaccine further comprises a pharmaceutically acceptable carrier, an excipient and/or an adjuvant. The medicine further comprises a pharmaceutically acceptable auxiliary material.

[0082] The prevention of the present disclosure refers to reducing the risk of disease occurrence by administering the product for preventing and/or treating a disease of the present disclosure before the disease occurrence. The treatment of the present disclosure refers to improving a disease, inhibiting the development of a disease, and restoring patients to health by administering the product for preventing and/or treating a disease of the present disclosure after the disease occurrence. For example, the use of the product of the present disclosure in preventing and/or treating tumors can increase the level of antibodies in serum, inhibit tumor growth, reduce tumor volume or delay tumor growth. In the examples of the present disclosure, the mouse transplanted tumor cell TC-1 is used as the experimental object to demonstrate the effect of the fusion protein vaccine, and good effects were obtained.

[0083] In the examples of the present disclosure, the amino acid sequences of the fragments involved and the coding nucleic acid fragments are shown in Table 1:

Table 1 Amino acid sequences and coding nucleic acid fragments involved in the present disclosure

SEQ ID NO: 1	Amino acid sequence of E6 protein of HPV16
SEQ ID NO: 2	Amino acid sequence of E7 protein of HPV16
SEQ ID NO: 3	Amino acid sequence of CCL11
SEQ ID NO: 4	Amino acid sequence of T2 fragment
SEQ ID NO: 5	IgE signal peptide
SEQ ID NO: 6	Nucleotide sequence of plasmid pVR-CCL11-E6E7-T2
SEQ ID NO: 7	Nucleotide sequence of plasmid pVR-CCL11-E6E7
SEQ ID NO: 8	Nucleotide sequence of plasmid pVR-E6E7
SEQ ID NO: 9	DNA sequence encoding the fusion protein in plasmid pVR-CCL11-E6E7-T2
SEQ ID NO: 10	DNA sequence encoding the fusion protein in plasmid pVR-CCL11-E6E7

(continued)

SEQ ID NO: 11	DNA sequence encoding the fusion protein in plasmid pVR-E6E7
SEQ ID NO: 12	mRNA sequence encoding the fusion protein in plasmid pVR-CCL11-E6E7-T2
SEQ ID NO: 13	mRNA sequence encoding the fusion protein in plasmid pVR-CCL11-E6E7
SEQ ID NO: 14	mRNA sequence encoding the fusion protein in plasmid pVR-E6E7
SEQ ID NO: 15	Amino acid sequence of the fusion protein in plasmid pVR-CCL11-E6E7-T2
SEQ ID NO: 16	Amino acid sequence of the fusion protein in plasmid pVR-CCL11-E6E7
SEQ ID NO: 17	Amino acid sequence of the fusion protein in plasmid pVR-E6E7

[0084] The embodiments of the present disclosure will be described in detail below in conjunction with examples, but those skilled in the art will understand that the following examples are only for illustrating the present disclosure, and should not be considered as limiting the scope of the present disclosure. The examples without specific conditions indicated were carried out according to the conventional conditions or the conditions recommended by the manufacturers. The reagents or instruments used without manufacturer indicated were all conventional commercially available products.

Example 1

[0085] DCs and eosinophils were isolated from mouse lymph nodes and peripheral blood for chemotaxis experiments. The above isolated cells were placed in the upper chamber of a chemotaxis chamber (Transwell carbonate membrane chamber: 5 μm; Costar, Cat: 3422), and the cells were added at 1×10^6 cells/100 μL/well based on the previous work in the laboratory. The chemotaxis chamber was put into the lower chamber containing 600 μL of medium containing chemokine CCL11. A spontaneous migration control group and a CCL11 cytokine group were set up simultaneously, and added with the same number of cells. 3 replicate wells were set up. The purified recombinant (*E.coli*) murine CCL11 protein was used as chemokine CCL11. According to the previous work in the laboratory, 100 ng/mL was the optimal dose for chemotactic efficiency. After 4 h, the cells in the lower chemotaxis chamber were collected, and the chemotactic ability of CCL11 to various immune cells was analyzed by flow cytometry. The results show that CCL11 could effectively recruit various immune cells from the upper chamber to the lower chamber ($P<0.001$) (FIG. 1).

Example 2 Antigen design scheme of fusion gene or protein vaccine and construction and preparation of mammalian expression plasmid

[0086] Construction of plasmid pVR-CCL11-E6E7-T2: A fusion protein CCL11-E6E7-T2 was constructed based on E6 and E7 proteins of human papillomavirus subtype HPV16, humanized CCL11 protein and T2 fragment. An IgE signal peptide with an amino acid sequence of MDWTWILFLVAAATRVHS was connected to the N-terminal of the fusion protein CCL11-E6E7-T2, and a Flag tag consisting of 8 amino acids of DYKDDDDK was connected to the C-terminal of the fusion protein CCL11-E6E7-T2.

[0087] The finally obtained fusion protein, from N-terminal to C-terminal, sequentially comprised an IgE signal peptide, a humanized CCL11 protein sequence, a linker sequence (GGGGGSGGGGG), an E6 protein sequence, an E7 protein sequence, a T2 fragment sequence and a Flag tag sequence.

[0088] The amino acid sequence of the fusion protein was subjected to codon optimization for expression preference of mammalian cells. The gene sequence of the fusion protein, determined as SEQ ID NO: 8, was then synthesized and inserted into the corresponding polyclonal sites region of pVR plasmid vector so as to express the fusion protein in the correct codon translation sequence. The finally constructed plasmid was named plasmid pVR-CCL11-E6E7-T2, as shown in A in FIG. 2.

[0089] Construction of plasmid pVR-CCL11-E6E7: Similarly, the finally constructed fusion gene, from N-terminal to C-terminal, sequentially comprised an IgE signal peptide, a humanized CCL11 protein sequence, a linker sequence (GGGGGSGGGGG), an E6E7 protein sequence, and a Flag tag sequence.

[0090] The amino acid sequence of the fusion protein was subjected to codon optimization for expression preference of mammalian cells. The gene sequence of the fusion protein, determined as SEQ ID NO: 10, was then synthesized and inserted into the corresponding polyclonal sites region of pVR plasmid vector so as to express the fusion protein in the correct codon translation sequence. The finally constructed plasmid was named plasmid pVR-CCL11-E6E7, as shown in B in FIG. 2.

[0091] Construction of plasmid pVR-E6E7: The E6 and E7 protein sequences of human papillomavirus subtype HPV16 were preceded by an IgE signal peptide with an amino acid sequence of MDWTWILFLVAAATRVHS, and followed by a

Flag tag consisting of 8 amino acids of DYKDDDDK.

[0092] The finally obtained fusion protein, from N-terminal to C-terminal, sequentially comprised an IgE signal peptide, an E6E7 protein sequence, and a Flag tag sequence, as shown in C in FIG. 2.

[0093] The amino acid sequence of the fusion protein was subjected to codon optimization for expression preference of mammalian cells. The gene sequence of the fusion protein, determined as SEQ ID NO: 11, was then synthesized and inserted into the corresponding polyclonal sites region of pVR plasmid vector so as to express the fusion protein in the correct codon translation sequence. The finally constructed plasmid was named plasmid pVR-E6E7.

[0094] Specifically, the plasmid patterns constructed in the experiments in this example are actually: pVR-CCL11-antigen-T2, pVR-CCL11-antigen and a control plasmid pVR-antigen. In the experiment of this example, the E6E7 fusion protein of HPV 16 subtype was used as the antigen.

Example 3

[0095] *In vitro* cell transfection experiments with the constructed plasmids were performed. The vectors comprising antigens of HPV16 E6 and E7 proteins constructed in Example 2 were taken as examples, and the vectors comprising other antigens were transfected by the same method.

[0096] 24 h before transfection, 1×10^6 HEK293T cells were plated in a 6-well cell culture plate. The transfection experiment was started when the cell density reached more than 80%. Before transfection, cell culture medium and serum-free Opti-MEM medium were pre-warmed in a 37°C water bath. During transfection, 3 μg of control vector, pVR-CCL11-E6E7-T2 expression vector, pVR-CCL11-E6E7 expression vector, and pVR-E6E7 expression vector and 12 μL of PEI transfection reagent were added to 200 μL of serum-free Opti-MEM, mixed evenly, and then left to stand at room temperature for 10 min. The cells to be transfected were fed with fresh medium, gently added to the above transfection system, and shaken gently. The cells were then placed in the cell culture incubator again for 6 h of culture and then fed with fresh medium. After 48 h of transfection, the cells were collected, and the expression effect of the E6E7 fusion gene plasmid in HEK293T cells was then detected by Western Blot.

[0097] The collected cells were added with 60 μL of 0.5% NP40 lysis buffer containing PMSF or Cocktail protease inhibitor, resuspended fully and lysed by rotating at 4°C for 30 min. The lysate was centrifuged at 12,000 rpm at 4°C for 10 min. The supernatant was collected into a new 1.5 mL EP tube, and the precipitate was discarded. 5×SDS-PAGE protein loading buffer was added according to the actual volume of the sample and mixed well. Then the sample was heated in an air bath at 100°C for 10 min, subjected to Western blot immediately, and detected using Flag tag antibody (Sigma, F3165). The results are shown in FIG. 3 that the control vector had no protein expression, and the position showing the size of the expressed proteins of pVR-CCL11-E6E7-T2 and pVR-CCL11-E6E7 was significantly higher than that of pVR-E6E7, indicating that the plasmids pVR-CCL11-E6E7-T2 and pVR-CCL11-E6E7 of the experimental group and the plasmid pVR-E6E7 of the control group can all be smoothly and normally expressed in mammalian cells.

Example 4

[0098] The effects of chemokine CCL11 and T2 fragment on cell-specific T cell responses induced by the fusion gene vaccine were explored. The vaccines comprising antigens of HPV16 E6 and E7 proteins were taken as an example and the steps for inducing T cell responses by vaccines comprising other antigens were the same.

[0099] Given that the fusion gene can be normally expressed in mammalian cells, plasmids pVR-CCL11-E6E7-T2, pVR-CCL11-E6E7, and pVR-E6E7 were extracted separately, and the mice were immunized with the plasmids of 25 μg by an in vivo gene transfer instrument from TERESA. There were four groups including a negative control PBS group, 5 mice in each group. The mice were immunized according to the immunization strategy marked in the timeline in FIG. 4, and blood was collected from the mice in each group on D14 and added to the PBS solution containing heparin. All the samples were centrifuged at 3000 rpm for 5 min. The supernatant was discarded, and the remaining pellets were dispersed by shaking, and then added with 1 mL of red blood cell lysis solution for 1 min of lysis at room temperature. Then all the samples were centrifuged at 1200 rpm for 6 min. The supernatant was discarded, and the remaining pellets were washed once with 700 μL of PBS solution and centrifuged for the second time at 1200 rpm for 6 min. After the supernatant was discarded, the pellets were resuspended with 300 μL of inactivated 1640 medium containing 10% FBS, and added with 1 μL of E7-tetramer for 1 h of staining. Flow staining: CD8-FITC; E7-tetramer-PE. The flow results are shown in FIG. 5. The results show that in the comparison between groups at the same dose, the number of E7-specific T cells in CCL11-E6E7-T2 group was far more than that in the E6E7 group. In the comparison between groups at the same dose, the number of E7-specific T cells in CCL11-E6E7-T2 group was more than that in the CCL11-E6E7 group. In the comparison between groups at the same dose, the number of E7-specific T cells in CCL11-E6E7 group was more than that in the E6E7 group. This shows that chemokine CCL11 at the N-terminal of the antigen protein can effectively induce the binding of antigen molecules and specific immune cells, thereby greatly enhancing the effect of crosspresentation of antigen molecules, so that CCL11 can finally induce a stronger specific immune response to antigen molecules. In addition, the T2 fragment added at the C-

terminal of the antigenic protein can effectively enhance the immune strength against the antigen molecule in the process of cellular immune, which greatly increases the number of specific T cells, playing a decisive role as an immune enhancement factor.

Example 5

[0100] The effects of chemokine CCL11 and T2 fragment on the humoral immune responses induced by fusion gene vaccine were explored. The vaccines comprising antigens of HPV16 E6 and E7 proteins were taken as an example, and the steps for inducing T cell responses by vaccines comprising other antigens were the same.

[0101] Whether CCL11 can also enhance humoral immune responses was next evaluated. Plasmids pVR-CCL11-E6E7-T2, pVR-CCL11-E6E7, and pVR-E6E7 were extracted separately, and the mice were immunized with the plasmids of 25 μg by an in vivo gene transfer instrument from TERESA. There were four groups including a negative control PBS group, 5 mice in each group. The mice were immunized according to the immunization strategy marked in the timeline in FIG. 4, and blood was collected from the mice in each group on D21 with no anticoagulant added. All the samples were centrifuged at 3000 rpm for 20 min. The supernatant was collected. On the first day, the E6E7 fusion protein purified in the laboratory was diluted with PBS, and coated onto an ELISA plate at 200 μL per well containing 10 μg of protein. The plate was then kept at 4°C overnight. On the second day, the plate was washed and blocked with blocking solution for 2 h at room temperature. The collected mouse serum was diluted by 1:100, and added into wells at 100 μL per well. The plate was then kept at 4°C overnight. On the third day, the color development was performed by ELISA. The results are shown in FIG. 6. The results show that in the comparison between groups at the same dose, the number of E7-specific antibodies in CCL11-E6E7-T2 group was far more than that in the E6E7 group. In the comparison between groups at the same dose, the number of E7-specific antibodies in CCL11-E6E7-T2 group was more than that in the CCL11-E6E7 group. In the comparison between groups at the same dose, the number of E7-specific antibodies in CCL11-E6E7 group was more than that in the E6E7 group. This shows that chemokine CCL11 at the N-terminal of the antigen protein can effectively induce the binding of antigen molecules and specific immune cells, thereby greatly enhancing the effect of crosspresentation of antigen molecules, so that CCL11 can finally induce a stronger specific immune response to antigen molecules. In addition, the T2 fragment added at the C-terminal of the antigenic protein can effectively enhance the immune strength against the antigen molecule in the process of cellular immune, which greatly increases the number of specific antibodies produced, playing a decisive role as an immune enhancement factor.

Example 6

[0102] The therapeutic effects of fusion gene vaccines on mouse xenograft tumor cell TC-1 allograft tumor were explored. The fusion gene vaccines comprising antigens of HPV16 E6 and E7 proteins were taken as an example, and the steps for the demonstration of tumor intervention effect of vaccines comprising other antigens were the same.

[0103] In view of the excellent effects of vaccines such as CCL11-E6E7-T2 in the cellular and humoral immunity experiments, the therapeutic effects on the TC-1 allograft tumor after immunization with the fusion genes were further explored by experiments. In addition to the DNA vaccine form, the mRNA vaccine form of CCL11-E6E7-T2 was also synthesized, which was encapsulated with nanoparticles. The specific process is as follows: 5'UTR-CCL11-E6E7-3'UTR-A(120) was connected into the cloning vector pGEM-3Zf(+) (promega) to complete the construction of an *in vitro* transcription expression system, which was named pGEM -CCL 11- E6E7. The *in vitro* transcription system had a UTR sequence as follows:

5-UTR (β-globin-2)

agagcggccgcttttcagcaagattaagcccagggcagagccatctattgcttacatttgcttctgacacaactgtgttcactagcaacctca aacagacacc

3-UTR (2β-globin)

agctcgctttcttgctgtccaatttctattaaaggttcctttgttccctaagtccaactactaaactgggggatattatgaagggccttgagcatctg gattctgcctaataaaaaacatttattttcattgcagctcgctttcttgctgtccaatttctattaaaggttcctttgttccctaagtccaactactaaac tgggggatattatgaagggccttgagcatctggattctgcctaataaaaaacatttattttcattgc

[0104] The recombinant plasmid was linearized. The single enzyme digestion reaction system is shown in the table

below, and the reaction was conducted at 37°C for 3 h.

Component	Volume (μL)
Xho I	4
Recombinant plasmid	12 μg
10× CutSmart Buffer	5
dd H_2O	Balance
Total	50

[0105] *In vitro* transcription reaction:

1. T7-Flash Scribe™ Transcription Kit (Cell script) was use for *in vitro* transcription, and UTP was replaced with N1-Methylpseudouridine-5'-Triphosphate (Trilink Biotech) during the preparation of *in vitro* transcription system.

[0106] The reaction system is shown in the table below. After the first step of reaction was completed, the reaction system was kept at 35°C for 30 min, and after the second step of reaction was completed, the reaction system was kept at 35°C for 15 min.

Regents	Volume (μL)
Rnase-free Water	X
Linearized DNA	1 μg
10 ×T7 Transcription Solution	2
100 mM ATP	1.8
100 mM CTP	1.8
100 mM GTP	1.8
100 mM UTP	1.8
100 mM DTT	2
Script Cuard Rnase Inhibitor	0.5
T7-Flash Scribe Enzyme Solution	2
Total	20
The above products	20
Rnase-free Dnase I	1
Total	21

[0107] mRNA capping: The kit ScriptCap™ Cap 1 Capping System (Cell script) was used.

Cap-mRNA purification

[0108] Purification was performed using the kit MEGAclear™ Kit Purification (Invitrogen).

Preparation of liposome nanoparticles LNP-Man

[0109] DOTAP ((2,3-dioleoyl-propyl)trimethylammonium chloride), DOPE (dioleoylphosphatidylethanolamine), and DSPE-PEG2000 (distearoylphosphatidylethanolaminepolyethylene glycol 2000) were all purchased from Shanghai Advanced Vehicle Technology Co., Ltd.. DSPE-PEG2000-Man was purchased from Xi'an Haoran Biotechnology Co., Ltd.;
Nanoparticles were prepared by rotary evaporation method. The specific operation process of preparation of LNP-Man is as follows:

1) DOTAP, DOPE, and DSPE-PEG2000-Man were sequentially added to a round bottom flask according to the molar ratio of 50:50:1. 6 mL of chloroform was added until the solid was fully dissolved.

2) Ultrasonication in a water bath was performed for 15 min.

3) The round-bottom flask was put into a rotary evaporator, and the dissolved matter in the round-bottom flask should be submerged below the water surface. Then rotary evaporation was performed at a speed of 100 rpm for 15 min.

4) The round bottom flask was detached, placed in a fume hood, and added with 8 mL of HEPES buffer to dissolve the film on the inner wall of the bottle.

5) Ultrasonication in a water bath was performed for 30 min.

6) The ultrasonicated solution was filtered with a 0.22 μm filter membrane three times to obtain the desired liposome nanoparticles LNP-Man.

Preparation of LNPs/mRNA

[0110] The prepared cationic lipid nanomaterial LNP-Man and mRNA were mixed according to the set N/P = 10:1 (molar ratio), and the required volume of LNPs and mRNA was calculated. LNPs and mRNA were respectively added with equal volumes of 10 mM HEPES buffer solution before mixing. The mixed LNPs/mRNA was shaken on a vortex shaker for 1 min, and left to stand at room temperature for 30 min. Moreover, CCL11-E6E7-T2 protein was also purified as a vaccine control. Mice were subjected to tumor inoculation and plasmid therapeutic immunization according to the timeline shown in FIG. 7, with an amount of each immunization of 25 μg. After tumor inoculation, the mice were immunized twice on D4 and D11, respectively. Afterwards, the tumors were measured in the same way, and the tumor volume was recorded. The tumor growth curve was drawn as shown in FIG. 8. The results show that the three groups pVR-CCL11-E6E7-T2 plasmid, CCL11-E6E7-T2-mRNA and CCL11-E6E7-T2 protein exhibited earlier tumor growth inhibition than the group pVR-E6E7 plasmid, and in the three groups pVR-CCL11-E6E7-T2 plasmid, CCL11-E6E7-T2-mRNA and CCL11-E6E7-T2 protein, the transplanted tumors completely disappeared around the 17th day after tumor inoculation, achieving a complete tumor treatment effect.

[0111] Finally, it should be stated that: the above embodiments are only intended for illustrating the technical solutions of the present disclosure rather than limiting the present disclosure. Although the present disclosure is illustrated in detail with reference to the embodiments described above, it should be understood by those skilled in the art that, modifications can still be made to the technical solutions recited in the embodiments described above, or equivalent substitutions can be made onto a part or all of the technical features of the technical solution. While such modifications or substitutions will not cause the essence of corresponding technical solutions to depart from the scope of the technical solutions of the embodiments of the present disclosure.

Claims

1. Use of at least one of I)-VI) in improving antigen presentation effect, wherein

I) a T2 fragment with an amino acid sequence set forth in SEQ ID NO: 4;
II) chemokine CCL11;
III) a fragment being more than 80% homologous with and functionally identical or similar to I) or II);
IV) a nucleic acid molecule encoding I) or II);
V) a nucleic acid molecule that is derived from the nucleotide sequence of the nucleic acid molecule in IV) by deletion, addition or substitution of one or more nucleotides and encode a protein functionally identical or similar to the nucleic acid molecule in IV); and
VI) a nucleic acid molecule fully or partially complementary to V).

2. A fusion protein, comprising CCL11 and an antigen or comprising CCL11, an antigen and a T2 fragment.

3. The fusion protein according to claim 2, from N-terminal to C-terminal, sequentially comprising an IgE signal peptide, CCL11, a linker, the antigen and the T2 fragment.

4. The fusion protein according to claim 2 or 3, wherein the antigen is derived from a virus, pathogenic microbe and/or

tumor;

the virus is selected from the group consisting of HPV, EBV, HCV, HIV, HBV, VZV, a coronavirus, and a combination thereof; and

the tumor is selected from the group consisting of liver cancer, cervical cancer, ovarian cancer, lung cancer, head and neck cancer, prostate cancer, breast cancer, blood cancer, ovarian cancer, colorectal cancer, and a combination thereof.

5. A nucleic acid molecule encoding the fusion protein according to any one of claims 2 to 4.

6. A nucleic acid fragment comprising the nucleic acid molecule according to claim 5, 5'-UTR, 3'-UTR and 3'-end polyA.

7. An expression vector comprising a vector backbone and the nucleic acid molecule according to claim 5 or the nucleic acid fragment according to claim 6.

8. A host transformed or transfected with the expression vector according to claim 7.

9. A method for producing the fusion protein according to any one of claims 2 to 4, comprising culturing the host according to claim 8 and collecting a culture containing the fusion protein.

10. Use of the fusion protein according to any one of claims 2 to 4, the nucleic acid molecule according to claim 5, the nucleic acid fragment according to claim 6, the expression vector according to claim 7, the host according to claim 8, the fusion protein prepared by the method according to claim 9, and/or the culture containing the fusion protein prepared by the method according to claim 9 in the manufacture of a product for preventing and/or treating a disease.

11. A product for preventing and/or treating a disease, comprising the fusion protein according to any one of claims 2 to 4, the nucleic acid molecule according to claim 5, the nucleic acid fragment according to claim 6, the expression vector according to claim 7, the host according to claim 8, the fusion protein prepared by the method according to claim 9, and/or the culture containing the fusion protein prepared by the method according to claim 9.

12. A method for preventing and/or treating a disease, comprising administering to a subject in need thereof the product according to claim 11.

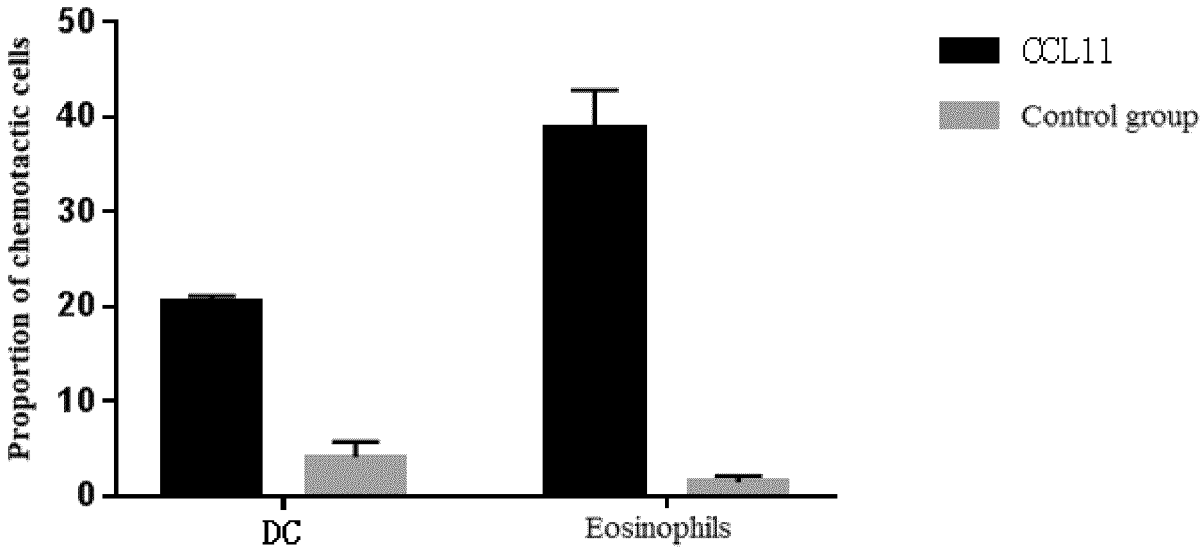
Proportion of chemotactic cells
50
40
30
20
10
0
DC
Eosinophils
CCL11
Control group

FIG. 1

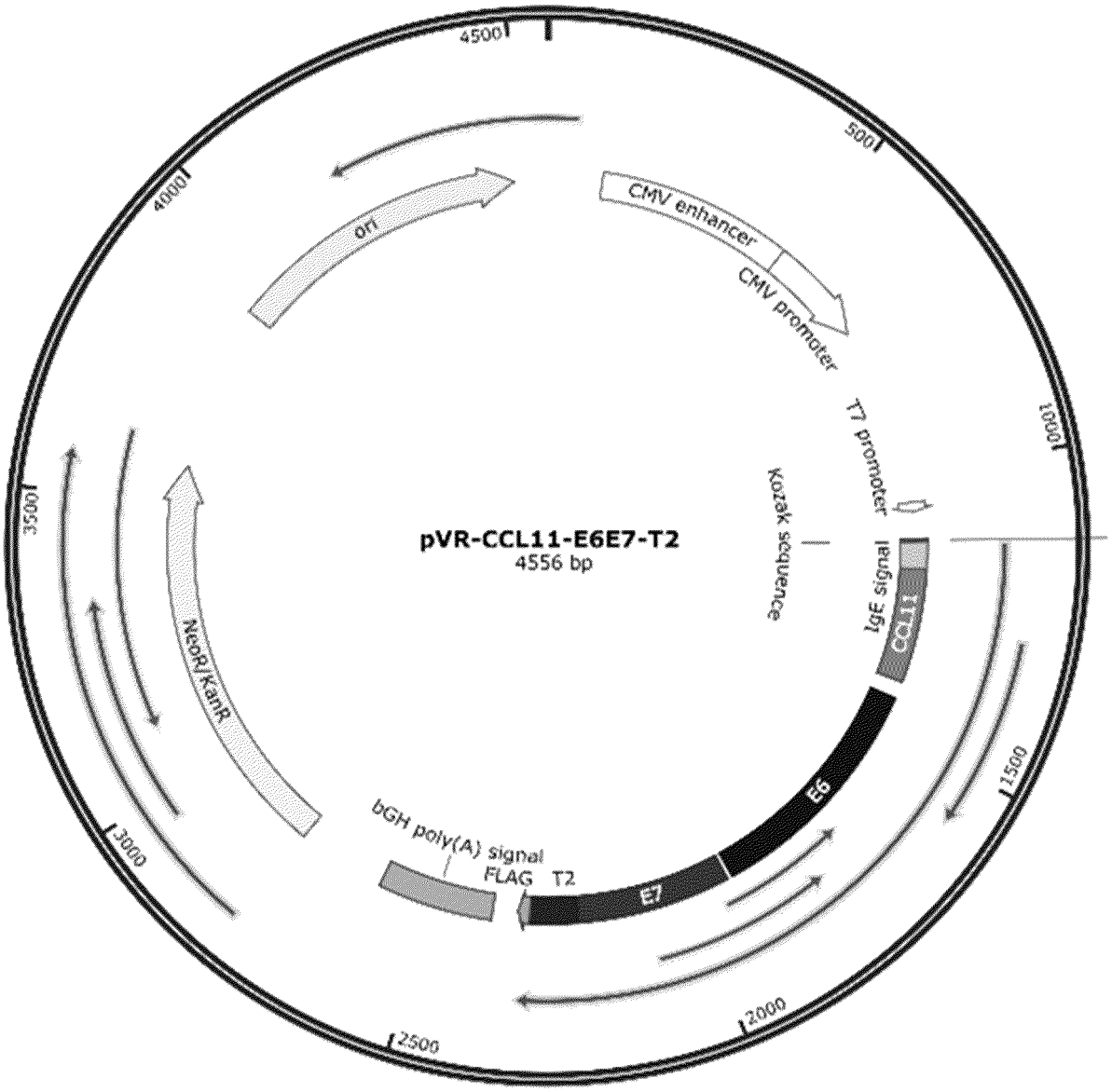
4500
500
1000
1500
2000
2500
3000
3500
4000
ori
CMV enhancer
CMV promoter
T7 promoter
Kozak sequence
IgE signal
CCL11
E6
E7
FLAG
T2
bGH poly(A) signal
NeoR/KanR
pVR-CCL11-E6E7-T2
4556 bp

FIG. 2A

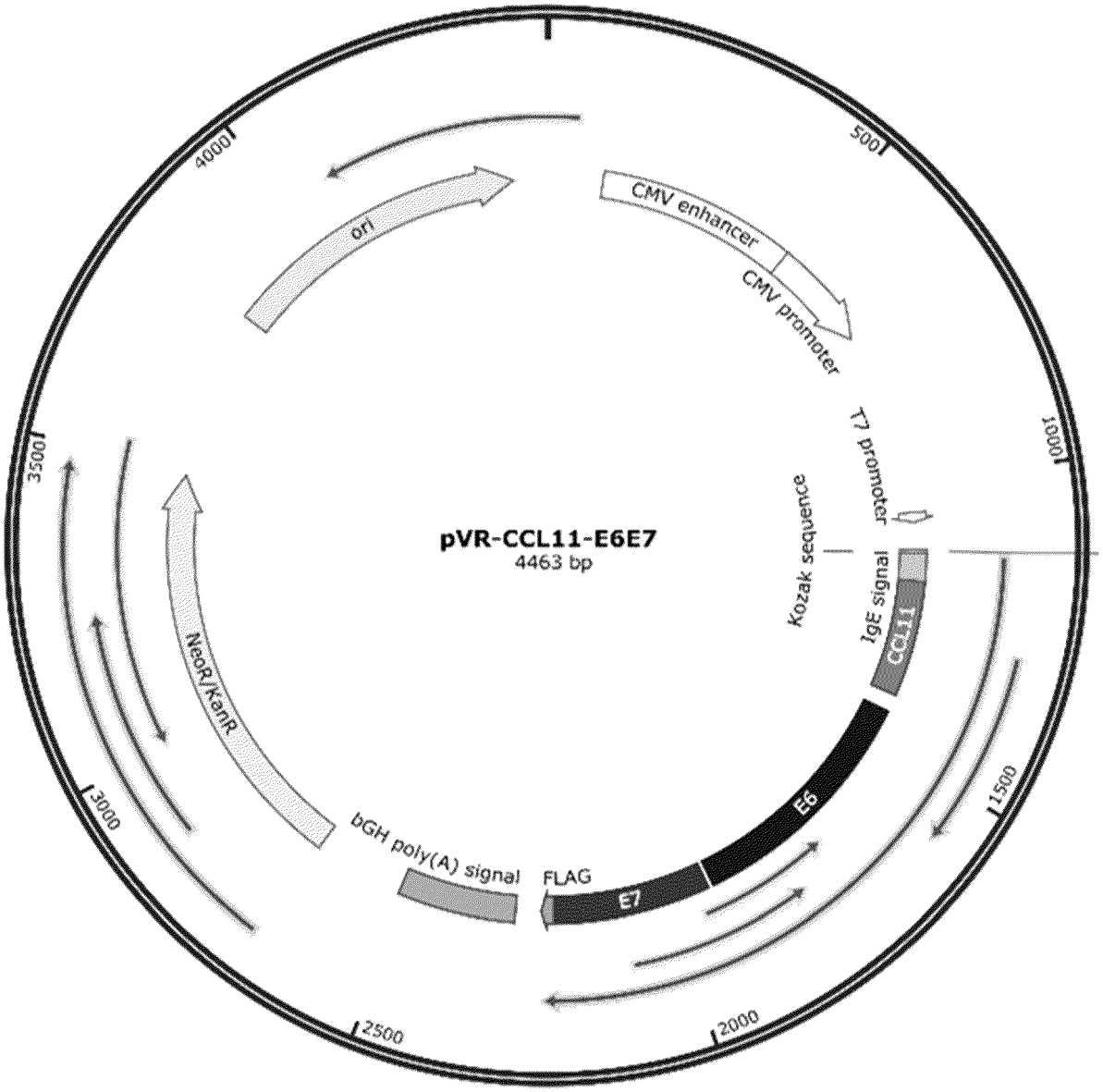
pVR-CCL11-E6E7
4463 bp
ori
CMV enhancer
CMV promoter
T7 promoter
Kozak sequence
IgE signal
CCL11
E6
E7
FLAG
bGH poly(A) signal
NeoR/KanR
500
1000
1500
2000
2500
3000
3500
4000

FIG 2B

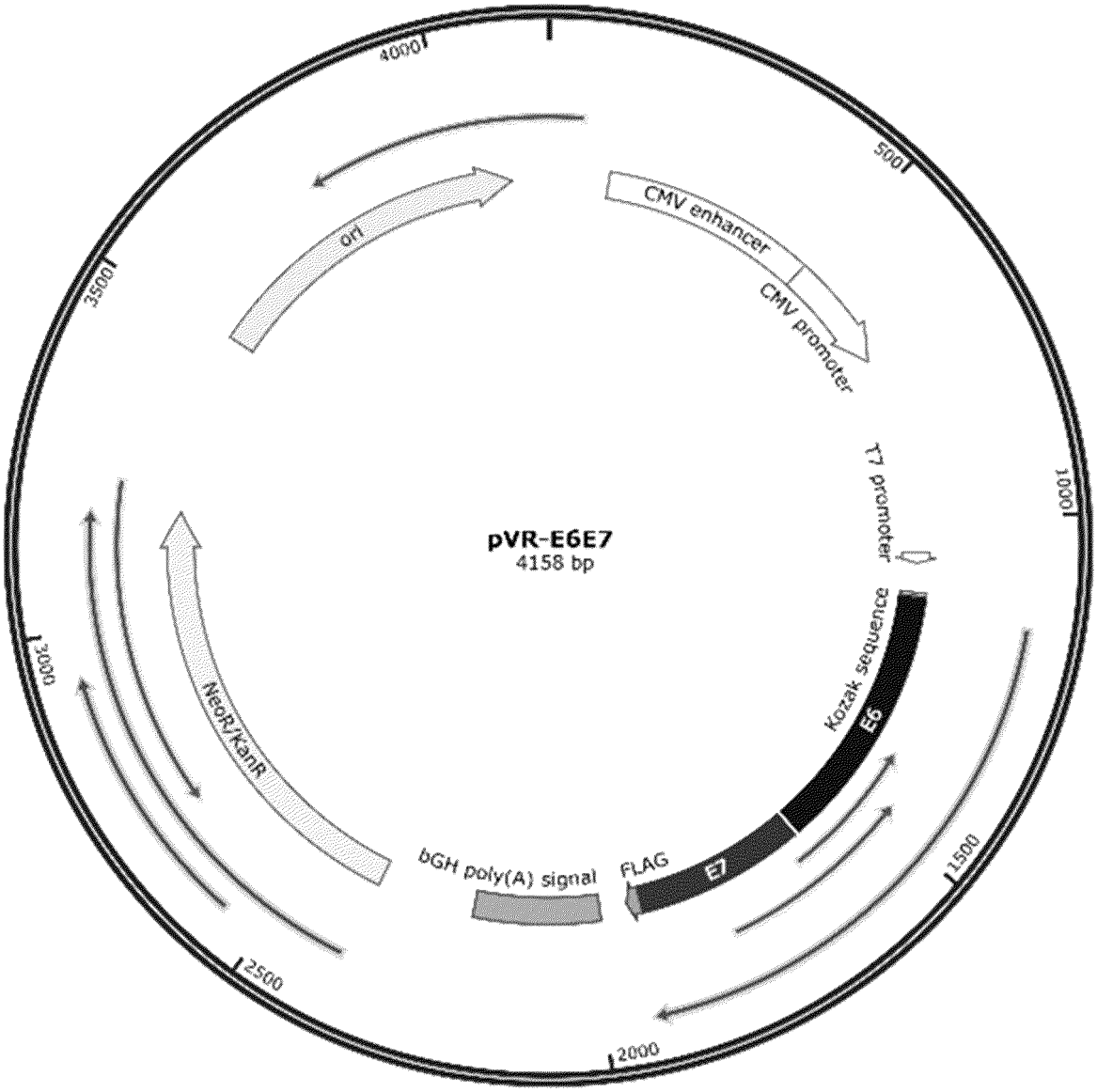
4000
500
3500
1000
3000
1500
2500
2000
ori
CMV enhancer
CMV promoter
T7 promoter
pVR-E6E7
4158 bp
Kozak sequence
E6
FLAG
E7
bGH poly(A) signal
NeoR/KanR

FIG. 2C

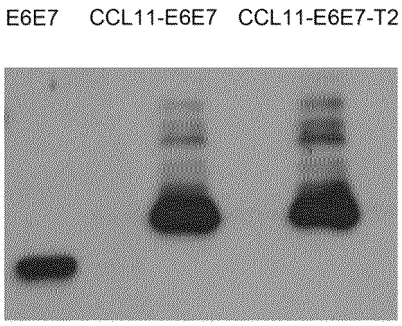
E6E7
CCL11-E6E7
CCL11-E6E7-T2

FIG. 3

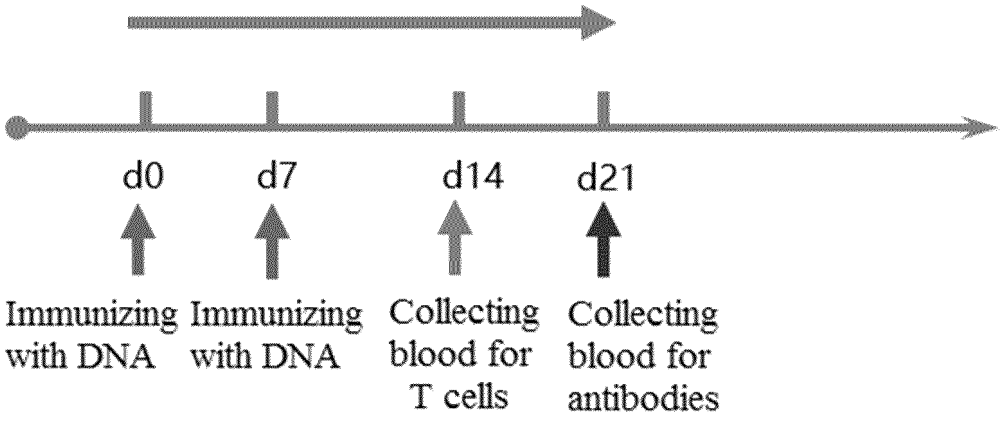
d0
d7
d14
d21
Immunizing with DNA
Immunizing with DNA
Collecting blood for T cells
Collecting blood for antibodies

FIG. 4

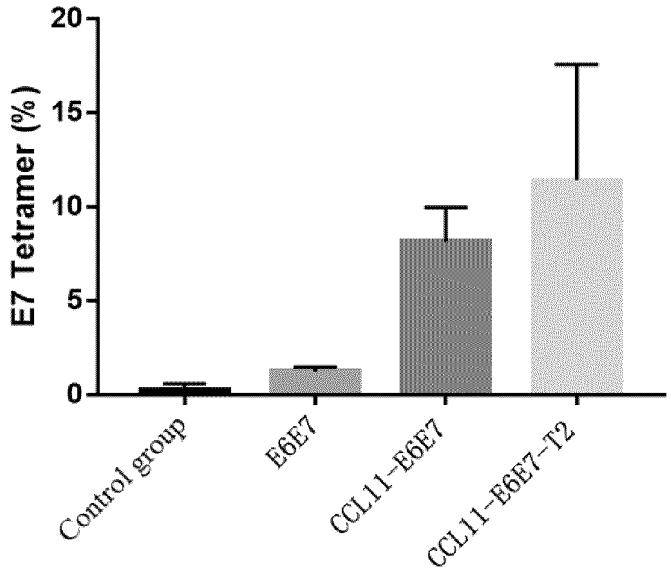
20
15
10
5
0
E7 Tetramer (%)
Control group
E6E7
CCL11-E6E7
CCL11-E6E7-T2

FIG. 5

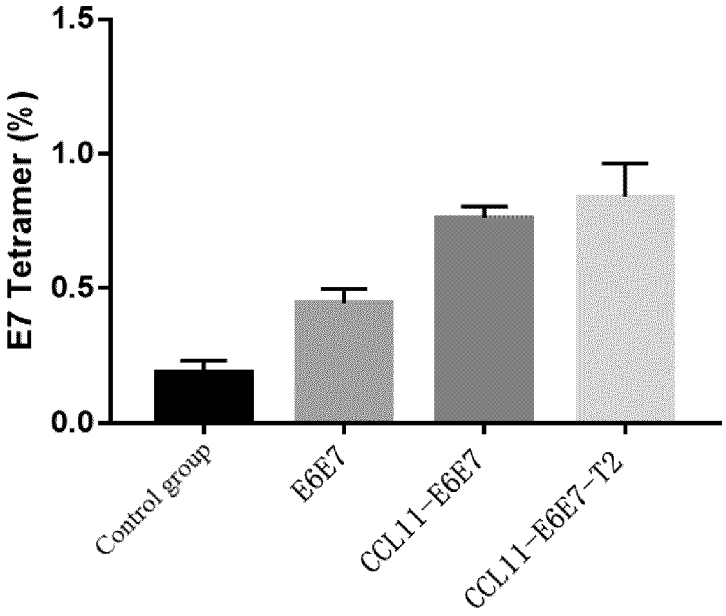
1.5
1.0
0.5
0.0
E7 Tetramer (%)
Control group
E6E7
CCL11-E6E7
CCL11-E6E7-T2

FIG. 6

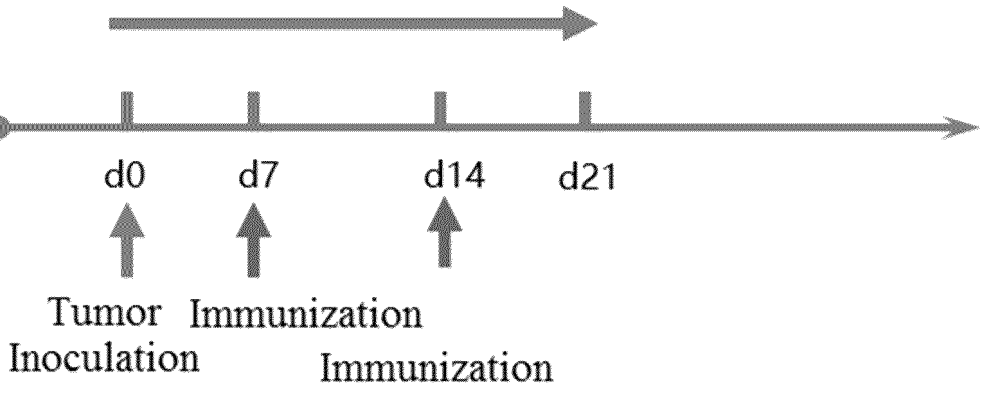
d0
d7
d14
d21
Tumor Inoculation
Immunization
Immunization

FIG. 7

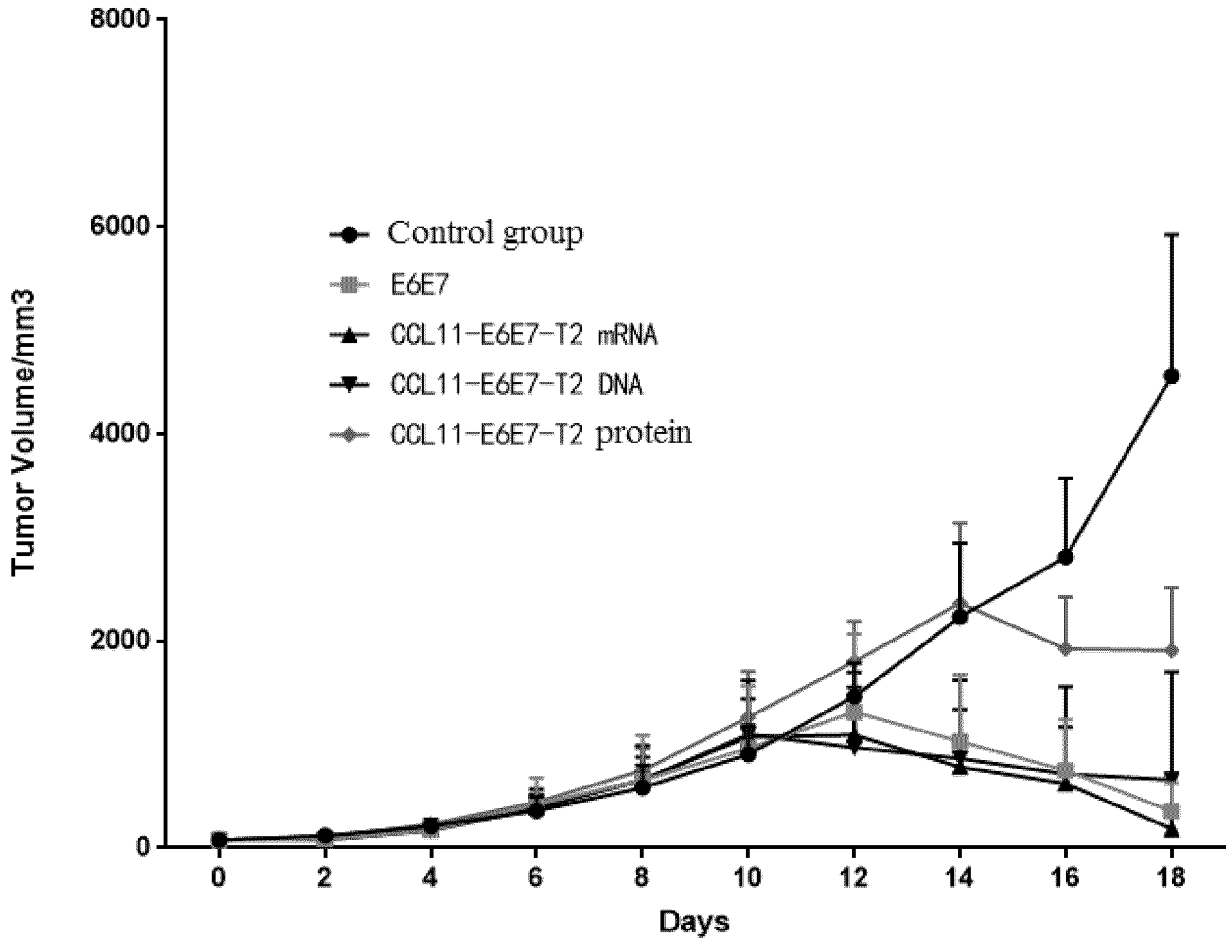
8000
6000
4000
2000
0
Tumor Volume/mm3
Control group
E6E7
CCL11-E6E7-T2 mRNA
CCL11-E6E7-T2 DNA
CCL11-E6E7-T2 protein
0
2
4
6
8
10
12
14
16
18
Days

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/097092

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00(2006.01)i; C07K 14/52(2006.01)i; C12N 15/09(2006.01)i; A61K 39/39(2006.01)i; A61P 31/12(2006.01)i; A61P 37/04(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, DWPI, SIPOABS, CNKI, NCBI, ISI Web of Science: 趋化因子, 配体, 嗜酸性粒细胞, 抗原, 呈, 融合, chemokine, ligand, eotaxin, 11, 1, CCL11, antigen, presenting, fusion, t2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sylvie Beaulieu, et al. "Expression of a Functional Eotaxin (CC Chemokine Ligand 11) Receptor CCR3 by Human Dendritic Cells" *J Immunol September 15, 2002, 169 (6) 2925-2936,* Vol. 169, No. 6, 15 September 2002 (2002-09-15), abstract	1-12
PY	CN 114106207 A (NOXI TECHNOLOGY (BEIJING) LTD. CO.) 01 March 2022 (2022-03-01) entire document, in particular claims and abstract	1-12

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 June 2022	**14 October 2022**
Name and mailing address of the ISA/CN **China National Intellectual Property Administration (ISA/CN)** **No. 6, Xitucheng Road, Jimenqiao, Haidian District, Beijing 100088, China** Facsimile No. **(86-10)62019451**	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/097092

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/097092

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 114106207 A	01 March 2022	None	

Form PCT/ISA/210 (patent family annex) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- CN 202210076816 **[0001]**