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(54)发明名称

由于异位骨化的副作用减少而具有增强的治疗效果的骨形态发生蛋白-9和骨形态发生蛋白-10变体以及包含所述变体的药物组合物

(57)摘要

本发明涉及BMP-9变体及其衍生物。所述变体刺激内皮细胞特异性信号传导，但是与野生型BMP-9相比，不刺激骨化相关信号传导，因此具有增强对包括肿瘤、心血管疾病、纤维化疾病、炎性疾病、代谢性疾病和自身免疫性疾病在内的各种疾病的治疗效果并且减少副作用的作用。

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1.一种骨形态发生蛋白-9(BMP-9)变体,所述BMP-9变体由选自SEQ ID NO:2至29的任何一个氨基酸序列表示。

2.一种BMP-9变体-Fc融合蛋白,其中免疫球蛋白的Fc片段与根据权利要求1所述的BMP-9变体连接。

3.根据权利要求2所述的BMP-9变体-Fc融合蛋白,其中所述免疫球蛋白的Fc片段由SEQ ID NO:30的氨基酸序列表示。

4.根据权利要求2所述的BMP-9变体-Fc融合蛋白,其中所述BMP-9变体和所述免疫球蛋白的Fc片段经由接头彼此连接。

5.根据权利要求4所述的BMP-9变体-Fc融合蛋白,其中所述接头由SEQ ID NO:31的氨基酸序列表示。

6.一种用于治疗肿瘤的药物组合物,所述药物组合物包含根据权利要求1所述的BMP-9变体或根据权利要求2至5中任一项所述的BMP-9变体-Fc融合蛋白作为活性成分。

7.根据权利要求6所述的药物组合物,其中所述肿瘤是选自以下的至少一种:乳腺癌、肺癌、结直肠癌、结肠癌、肝癌、胰腺癌、脑瘤、前列腺癌、皮肤癌、骨肉瘤和血癌。

8.一种用于治疗炎性疾病的药物组合物,所述药物组合物包含根据权利要求1所述的BMP-9变体或根据权利要求2至5中任一项所述的BMP-9变体-Fc融合蛋白作为活性成分。

9.根据权利要求8所述的药物组合物,其中所述炎性疾病是选自以下的至少一种:脂肪性肝炎、肝炎和肠炎。

10.一种用于治疗代谢性疾病的药物组合物,所述药物组合物包含根据权利要求1所述的BMP-9变体或根据权利要求2至5中任一项所述的BMP-9变体-Fc融合蛋白作为活性成分。

11.根据权利要求10所述的药物组合物,其中所述代谢性疾病是选自以下的至少一种:肥胖、体重减轻、糖尿病、动脉粥样硬化、动脉硬化、心肺疾病、神经系统疾病、阿尔茨海默病、认知损害、氧化应激、皮肤病、皮肤老化、由紫外线照射引起的损伤、高血压、高胆固醇血症(LDL、HDL、VLDL)、高脂血症(甘油三酯)、免疫缺陷、癌症和代谢综合征。

12.根据权利要求11所述的药物组合物,其中所述心肺疾病是选自以下的至少一种:心肌梗死、高血压、肺动脉高压、心肌纤维化和肺纤维化。

13.一种用于治疗自身免疫性疾病的药物组合物,所述药物组合物包含根据权利要求1所述的BMP-9变体或根据权利要求2至5中任一项所述的BMP-9变体-Fc融合蛋白作为活性成分。

14.根据权利要求13所述的药物组合物,其中所述自身免疫性疾病是选自以下的至少一种:胰岛素依赖型糖尿病、多发性硬化、自身免疫性脑脊髓炎、类风湿性关节炎、骨关节炎、重症肌无力、甲状腺炎、葡萄膜炎、桥本甲状腺炎、甲状腺毒症、恶性贫血、自身免疫性萎缩性胃炎、自身免疫性溶血性贫血、特发性白细胞减少症、原发性硬化性胆管炎、酒精性/非酒精性脂肪性肝炎、炎性肠病、克罗恩病、溃疡性肠病、银屑病、舍格伦综合征、硬皮病、韦氏肉芽肿病、多肌炎、皮肌炎、盘状红斑狼疮和系统性红斑狼疮。

由于异位骨化的副作用减少而具有增强的治疗效果的骨形态发生蛋白-9和骨形态发生蛋白-10变体以及包含所述变体的药物组合物

技术领域

[0001] 本发明涉及BMP-9变体及其衍生物,更具体地涉及这样的BMP-9变体及其衍生物,其中在治疗包括肿瘤、心血管疾病、纤维化疾病和代谢性疾病在内的各种疾病、障碍和病症方面异位骨化的副作用减少并且治疗效果增强。

背景技术

[0002] BMP-9与独特的受体结合,而不是与常规已知的BMP受体结合,并且由于其在多种细胞内过程中的不同作用而与其他类型的BMP区分开来。例如,BMP-9在肝脏中产生,并且能够抑制脂质代谢,控制肝葡萄糖产生,调节心血管系统中内皮细胞的生长和迁移,并抑制心肌细胞纤维化。特别地,在心血管系统中,BMP-9与BMP-10一起与内皮ALK-1受体BMPR-II和内皮联蛋白结合,因此参与血管稳态和血压调节。在许多文献中,已经报道,当将BMP-9和BMP-10应用于由相应受体缺失引起的肺动脉高压或心肌纤维化时,症状得到缓解,表明BMP-9作为治疗材料有高实用性。此外,BMP-9是一种强大的BMP,这样的BMP可以根据肿瘤的类型诱导前列腺癌细胞的死亡,并且可以调节骨组织中的成骨分化。另外,已知BMP-9具有作为新的抗糖尿病或抗肥胖治疗靶标的高潜力,因为它在改善胰岛素敏感性方面是有效的。

[0003] 因此,为了开发靶向包括BMP-9在内的生长因子的新治疗剂,已经尝试通过取代、引入和去除野生型生长因子的一些氨基酸以产生突变来增加生长因子的生物活性(WO2010/065439)。然而,与直接使用包括BMP-9在内的生长因子作为生物治疗剂相关的问题在于同时表达异位骨化和畸形生长因子作用。这是由于包括BMP-9在内的生长因子的受体的复杂性,并且仍然很少有缓解与异位骨化有关的副作用的案例。BMP-9和BMP-10的受体主要存在于血管内皮细胞中,并且已知它们在充当Alk-1和BMPR-II的配体的同时参与血管功能调节。具体地,BMPR-II与ALK-1形成复合物,并且选择性地与BMP-9或BMP-10反应。因此,在受体缺失或者BMP-9或BMP-10有缺陷的情况下,最常见的疾病是肺动脉高压和心肌纤维化。特别地,BMP-9直接作用于内皮细胞以增强血管内壁的完整性,从而增加血管稳定性,因而抑制血管细胞死亡或血管生成。此外,与其他类型的BMP(如BMP-2、BMP-4、BMP-6等)不同,已知BMP-9即使在不引起骨化的低浓度下也可促进内皮细胞活性。然而,BMP-9也可能在高浓度下诱导异位骨化,因此,在实际临床实践中需要应用异位骨化功能得到控制的变体。

[0004] 另外,已经指出,由于生长因子的特性,野生型的非常短的体内半衰期使得难易作为治疗剂商业化(Kharitonenkov A.等人,Journal of Clinical Investigation,115:1627-1635,2005)。BMP-9的体内半衰期较短,在小鼠中为10分钟至1小时且在猴中为1.5小时至2小时,并且如果将其开发为治疗剂,则它具有必须每天施用的缺点。迄今为止,已经报道了各种增加重组蛋白的体内半衰期的技术。有这样一个例子,其中蛋白质与作为聚合物材料的聚乙二醇(PEG)连接,使得其分子量增加,从而抑制肾排泄,最终增加体内停留时间

(WO 2012/066075)。还有这样一个例子,其中通过将与人白蛋白结合的脂肪酸与生长因子分子融合来增加半衰期(WO 2012/010553)。此外,有通过合成与单独的人生长因子受体或和β-Klotho的复合物特异性结合的激动性抗体来在展现出与生长因子的作用机制相同的药理活性的同时增加半衰期的例子(WO 2012/170438)。另外,有这样一个例子,其中通过合成长效融合蛋白来增加半衰期,在所述长效融合蛋白中免疫球蛋白IgG的Fc与生长因子分子连接(WO 2013/188181)。

[0005] 因此,诸位发明人在研究基于BMP9的治疗性变体蛋白以抑制异位骨化并延长作用时间方面做出了巨大努力,因此确定一些变体可以抑制异位骨化并具有增加的体内半衰期,从而达到本发明的目的。

[0006] 本背景部分中所述的信息仅用于提高对本发明的背景的理解,并不应被解释为包括形成本发明所属领域的普通技术人员已经知晓的相关技术的信息。

[0007] 现有技术

[0008] 专利文献

[0009] (专利文件1)WO 2010/065439

[0010] (专利文件2)WO 2012/066075

[0011] (专利文件3)WO 2012/010553

[0012] (专利文件4)WO 2012/170438

[0013] (专利文件5)WO 2013/188181

[0014] 非专利文献

[0015] (非专利文件1)Kharitonenkov A.等人,Journal of Clinical Investigation,115:1627-1635,2005

发明内容

[0016] 本发明的一个目的在于提供异位骨化的副作用减少的骨形态发生蛋白-9(BMP-9)及其融合物。

[0017] 本发明的另一个目的在于提供所述骨形态发生蛋白-9(BMP-9)变体及其融合物的各种治疗用途。

[0018] 本发明的仍另一个目的在于提供用于预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的药物组合物,所述药物组合物包含所述BMP-9变体或其融合物。

[0019] 本发明的又另一个目的在于提供预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的方法,所述方法包括施用所述BMP-9变体或其融合物。

[0020] 本发明的仍又另一个目的在于提供所述BMP-9变体及其融合物用于预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的用途。

[0021] 本发明的甚至又另一个目的在于提供所述BMP-9变体及其融合物用于制造用以治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的药物的用途。

[0022] 为了实现以上目的,本发明提供了一种骨形态发生蛋白-9(BMP-9)变体,所述BMP-9变体由选自SEQ ID NO:2至29的任何一个氨基酸序列表示。

[0023] 另外,本发明提供了一种BMP-9变体-Fc融合蛋白,其中免疫球蛋白的Fc片段与所述BMP-9变体连接。

[0024] 另外,本发明提供了一种用于治疗肿瘤的药物组合物,所述药物组合物包含所述BMP-9变体或所述BMP-9变体-Fc融合蛋白作为活性成分。

[0025] 另外,本发明提供了一种用于治疗炎性疾病的药物组合物,所述药物组合物包含所述BMP-9变体或所述BMP-9变体-Fc融合蛋白作为活性成分。

[0026] 另外,本发明提供了一种用于治疗代谢性疾病的药物组合物,所述药物组合物包含所述BMP-9变体或所述BMP-9变体-Fc融合蛋白作为活性成分。

[0027] 另外,本发明提供了一种用于治疗自身免疫性疾病的药物组合物,所述药物组合物包含所述BMP-9变体或所述BMP-9变体-Fc融合蛋白作为活性成分。

[0028] 另外,本发明提供了一种预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的方法,所述方法包括施用所述BMP-9变体或所述BMP-9变体-Fc融合蛋白。

[0029] 另外,本发明提供了所述BMP-9变体或所述BMP-9变体-Fc融合蛋白用于预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的用途。

[0030] 另外,本发明提供了所述BMP-9变体或所述BMP-9变体-Fc融合蛋白用于制造用以治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的药物的用途。

附图说明

[0031] 图1示意性地示出了本发明的概要。

[0032] 图2示出了向其中引入了BMP-9、BMP-9变体和Fc融合的BMP-9的表达载体。

[0033] 图3a示出了表达和纯化的BMP-9变体的SDS-PAGE和蛋白质印迹的结果,并且图3b示出了表达和纯化的Fc融合的BMP-9的SDS-PAGE和蛋白质印迹的结果。

[0034] 图4示出了表达和纯化的BMP-9和变体在血管内皮细胞上的信号传导活性(图4a)和骨化信号传导活性(图4b)。

[0035] 图5示出了BMP-9变体对抑制成肌细胞中的纤维化相关标志的作用。

[0036] 图6是示出了纯化的BMP-9和Fc融合的BMP-9的血液浓度的测量结果的图。

具体实施方式

[0037] 除非另外定义,否则本文使用的所有技术和科学术语都具有与本发明所属领域的技术人员通常所理解的含义相同的含义。通常,本文使用的术语和下面描述的测试方法是本领域熟知的,并且是典型的。

[0038] 在本发明中,克隆BMP-9基因并产生其各种变体,然后在哺乳动物细胞(CHO细胞和人胚肾细胞系)中进行表达和纯化,从而选择对肿瘤、炎性疾病、心血管疾病、代谢性疾病和自身免疫性疾病有效的BMP-9变体,并且将其与Fc融合,因而增加其血液半衰期。具体地,在本发明中,BMP-9被设计成通过经由取代与其中的腕部表位和指节表位相对应的氨基酸序列中的一个或几个氨基酸而调节与受体结合的能力来诱导最佳治疗效果。本发明旨在选择在维持内皮细胞特异性信号传导的同时能够将异位骨化抑制至十分之一或更低的变体,并且通过碱性磷酸酶活性和茜素红染色证实,与野生型相比,根据本发明的变体大大减少了小鼠成肌细胞(C2C12)中的异位骨化。

[0039] 因此,本发明的一个方面涉及由选自SEQ ID NO:1至29的任何一个氨基酸序列表示的骨形态发生蛋白-9(BMP-9)或其变体,并且本发明的另一个方面涉及这样的BMP-9变

体-Fc融合蛋白,其中免疫球蛋白的Fc片段与骨形态发生蛋白-9(BMP-9)或其变体连接。

[0040] SEQ ID NO:1的氨基酸序列表示野生型BMP-9,其既包含粗体的信号序列,又包含带下划线的Pro-BMP-9序列,如下所示。其中,FFPLADDVTPTKHAIVQTLVHLKF的序列是与ALK-1结合的区域且从整体BMP-9结构来看属于腕部表位,并且KVGKACCVPTKLSPISVLYK的序列属于与BMP-2受体结合的指节表位。

[0041] **MAWVWTLLFLMAAAQSIQA**KPLQSWGRGSAGGNAHSPLGVPGGGLPEHTFNLKMFLENVKVDFLRSLN
LSGVPSQDKTRVEPPQYMIDLYNRYTSDKSTTPASNIVRSFSMEDAISITATEDFPFQKHILLFNISIPRHEQIT
RAELRLYVSCQNHVDPSHDLKGSVVIYDVLDGTDAWDSATETKTFLVSQDIQDEGWETLEVSSAVKRWVRSDSTK
SKNKLEVTVESHRKGCDTLDISVPPGSRNLPFFVVFSNDHSSGTKETRLELREMISHEQESVLKKLSKDGSTEAG
ESSHEEDTDGHVAAGSTLARRKRSAGAGSHCQKTSLRVNFEDIGWDSWIIAPKEYEAYECKGGCFFPLADDVTPT
KHAIVQTLVHLKFPTKVGKACCVPTKLSPISVLYKDDMGVPTLKYHYEGMSVAECGCR

[0042] 衍生自野生型BMP-9序列的变体序列可以由选自SEQ ID NO:2至29的任何一个氨基酸序列表示,并且这些变体被配置为使得一些氨基酸序列在野生型BMP-9序列中被取代。然而,根据本发明的变体不限于由序列ID号表示的特定氨基酸序列,此外,可以被认为等同于相应氨基酸序列的氨基酸序列也落入本发明的范围,正如对于本领域技术人员而言将是清楚的那样。例如,当与野生型相比,SEQ ID NO:2中的第335个氨基酸被Ala而不是Asp取代,只要除了变体的核心构型之外的一些序列被突变以不影响BMP-9的蛋白质功能和结构时,相应的变体也落入本发明的范围,正如对于本领域技术人员而言将是清楚的那样。因此,除了作为变体核心的氨基酸的取代以外,与选自SEQ ID NO:2至29的任何一个氨基酸序列中的剩余序列具有至少95%、至少90%、至少80%或至少70%同源性的变体应被理解为落入本发明的范围。

[0043] 此外,本发明的变体可以应用于其他亚型的骨形态发生蛋白,例如BMP-7或BMP-10。

[0044] 在本发明中,为了增加野生型或变体BMP-9的血液半衰期,可以将免疫球蛋白的Fc片段与其融合。免疫球蛋白的Fc片段可以由SEQ ID NO:30的氨基酸序列表示,但不限于此。除了免疫球蛋白的Fc片段外,还可以将各种肽或蛋白质(如白蛋白)与其融合,以增加蛋白质的血液半衰期。

[0045] 在本发明中,免疫球蛋白的Fc片段可以与骨形态发生蛋白-9(BMP-9)或其变体的N末端或C末端连接。

[0046] 在本发明中,骨形态发生蛋白-9(BMP-9)或其变体和免疫球蛋白的Fc片段可以经由接头彼此连接,但是本发明不限于此。

[0047] 在本发明中,接头可以由SEQ ID NO:31的氨基酸序列表示,但不限于此。

[0048] 在本发明中,BMP-9变体-Fc融合蛋白可以由SEQ ID NO:32或SEQ ID NO:33的氨基酸序列表示,但不限于此。

[0049] 根据本发明的BMP-9或其变体或其融合蛋白可以用作治疗试剂、诊断试剂或研究试剂,但是最优选地用于治疗目的。

[0050] 具体地,本发明提供了用于治疗肿瘤的药物组合物,所述药物组合物包含骨形态发生蛋白-9(BMP-9)或其变体或BMP-9变体-Fc融合蛋白作为活性成分。

[0051] 在本发明中,肿瘤可以是选自以下的至少一种:乳腺癌、肺癌、结直肠癌、结肠癌、

肝癌、胰腺癌、脑瘤、前列腺癌、皮肤癌、骨肉瘤和血癌，但不限于此。

[0052] 本发明还提供了用于治疗炎性疾病的药物组合物，所述药物组合物包含骨形态发生蛋白-9(BMP-9)或其变体或BMP-9变体-Fc融合蛋白作为活性成分。

[0053] 在本发明中，炎性疾病可以是选自以下的至少一种：脂肪性肝炎、肝炎和肠炎，但不限于此。

[0054] 本发明还提供了用于治疗代谢性疾病的药物组合物，所述药物组合物包含骨形态发生蛋白-9(BMP-9)或其变体或BMP-9变体-Fc融合蛋白作为活性成分。

[0055] 在本发明中，代谢性疾病可以是选自以下的至少一种：肥胖、体重减轻、糖尿病、动脉粥样硬化、动脉硬化、心肺疾病、神经系统疾病、阿尔茨海默病、认知损害、氧化应激、皮肤病、皮肤老化、由紫外线照射引起的损伤、高血压、高胆固醇血症(LDL、HDL、VLDL)、高脂血症(甘油三酯)、免疫缺陷、癌症和代谢综合征，但不限于此。

[0056] 在本发明中，心肺疾病可以是选自以下的至少一种：心肌梗死、高血压、肺动脉高压、心肌纤维化和肺纤维化，但不限于此。

[0057] 本发明还提供了用于治疗自身免疫性疾病的药物组合物，所述药物组合物包含骨形态发生蛋白-9(BMP-9)或其变体或BMP-9变体-Fc融合蛋白作为活性成分。

[0058] 在本发明中，自身免疫性疾病可以是选自以下的至少一种：胰岛素依赖型糖尿病、多发性硬化、自身免疫性脑脊髓炎、类风湿性关节炎、骨关节炎、重症肌无力、甲状腺炎、葡萄膜炎、桥本甲状腺炎、甲状腺毒症、恶性贫血、自身免疫性萎缩性胃炎、自身免疫性溶血性贫血、特发性白细胞减少症、原发性硬化性胆管炎、酒精性/非酒精性脂肪性肝炎、炎性肠病、克罗恩病、溃疡性肠病、银屑病、舍格伦综合征、硬皮病、韦氏肉芽肿病、多肌炎、皮肌炎、盘状红斑狼疮和系统性红斑狼疮，但不限于此。

[0059] 为了治疗目的，根据本发明的活性BMP-9变体或其衍生物可以单独施用，但是优选以药物组合物(配制品)、更优选无菌配制品的形式施用。

[0060] 在本发明中，药物组合物可以被配制成选自以下的任何一种剂型：可注射制剂、口服制剂、液体(例如用于注射)(如水性溶液、混悬剂、乳剂等)、胶囊剂、颗粒剂、片剂和粘膜施用制剂，但是本发明不限于此。这些配制品可以通过本领域用于配制的典型方法或Remington's Pharmaceutical Science(最新版),Mack Publishing Company,Easton PA中披露的方法来制备，并且可以根据个体疾病或组分制备成各种配制品。

[0061] 此外，除了治疗性BMP-9变体或其衍生物外，本发明的药物组合物还可以进一步包含至少一种药学上可接受的载体。药学上可接受的载体可以选自盐水、无菌水、林格氏溶液、缓冲盐水、葡萄糖溶液、麦芽糖糊精溶液、甘油、乙醇以及其中一种或多种的混合物。

[0062] 根据需要，本发明的药物组合物可以进一步包含药学上可接受的佐剂。佐剂可以是选自以下的至少一种：赋形剂、稀释剂、分散剂、缓冲剂、抗微生物防腐剂、抑菌剂、表面活性剂、粘合剂、润滑剂、抗氧化剂、增稠剂和粘度调节剂，但不限于此。

[0063] 根据本发明的药物组合物可以根据所需方法口服或肠胃外(例如静脉内、皮下、肌内、腹膜内或局部)施用，并且其剂量可以按照专家的意见根据患者的体重、年龄、性别、健康状况和饮食、施用时间、施用方法、排泄速率和疾病的严重程度而不同地改变和应用。

[0064] 在本发明的一个实施方案中，蛋白质的单次剂量可以是1μg/kg至100mg/kg、优选5μg/kg至50mg/kg，并且蛋白质可以每天施用一次或每周施用1-3次，但是剂量和施用间隔不

限于此。

[0065] 本发明的仍另一个方面涉及预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的方法，所述方法包括施用BMP-9变体或BMP-9变体-Fc融合蛋白。

[0066] 本发明的又另一个方面涉及BMP-9变体或BMP-9变体-Fc融合蛋白用于预防或治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的用途。

[0067] 本发明的仍又另一个方面涉及BMP-9变体或BMP-9变体-Fc融合蛋白用于制造用以治疗肿瘤、炎性疾病、代谢性疾病或自身免疫性疾病的药物的用途。

[0068] 实施例

[0069] 通过以下实施例可以获得对本发明的更好理解。这些实施例仅仅是为了说明本发明而提出的，而不应被解释为限制本发明的范围，正如对于本领域技术人员而言将是清楚的那样。

[0070] 实施例1.重组人proBMP-9和proBMP-10的构建

[0071] 将包括人前proBMP9的开放阅读框在内的整个范围的cDNA插入并克隆到pcDNA3.4载体中(图2)，通过DNA测序对其进行确认。使用QuickChange定点诱变试剂盒获得proBMP9的变体，也通过DNA测序对其进行确认。

[0072] 在本发明中使用的BMP-9野生型和变体序列如下。

[0073] SEQ ID NO:1.野生型潜伏期BMP-9(NIBEC-J)

[0074]
MAWVWTLLFLMAAAQSIQAKPLQSWGRGSAGGNAHSPLGVPGGGLPEHTFNLKMFLENVKVDFLRSLN
LSGVPSQDKTRVEPPQYMIDLYNRYTSDKSTTPASNIVRSFSMEDAISITATEDFPFQKHILLFNISIPRHEQIT
RAELRLYVSCQNHVDPSHDLKGSVVIYDVLDGTDAWDSATETKTFLVSQDIQDEGWETLEVSSAVKRWVRSDSTK
SKNKLEVTVESHRKGCDTLDISVPPGSRNLPFFVVFSNDHSSGTKETRLELREMISHEQESVLKKLSKDGSTEAG
ESSHEEDTDGHVAAGSTLARRKRSAGAGSHCQKTSLRVNFEDIGWDSWIIAPKEYEAYECKGGCFFPLADDVTPT
KHAIVQTLVHLKFPTKVGKACCVPTKLSPISVLYKDDMGVPTLKYHYEGMSVAECGCR

[0075] 在野生型序列中，信号肽序列用粗体表示，Pro-BMP序列加下划线，并且BMP-9序列未加标记。

[0076] 将含有前proBMP9的质粒用聚乙烯亚胺转染到CHO-S细胞系中，然后向其中添加增强剂，然后培养8天。通过蛋白质印迹方法使用抗BMP9抗体和抗BMP10抗体测量表达的proBMP9和proBMP10。

[0077] 通过已经使用1-5L条件培养基作为缓冲液达到平衡的Q-Sepharose柱分离表达的蛋白质。通过氯化钠梯度对附着在柱上的靶蛋白进行分级分离，并且将分级分离的样品浓缩，然后对其进行凝胶色谱，以获得靶分子量。确认如此获得的蛋白质具有95％或更高的纯度。

[0078] 实施例2.重组人proBMP-9变体的构建

[0079] BMP-9或BMP-10与血管内皮细胞中的ALK1受体选择性地结合，因此有望作为心血管疾病的治疗剂。然而，它们仍然具有通过刺激间充质细胞或成肌细胞来刺激骨化的潜力。在开发治疗剂时，必须使这种潜力丧失能力。迄今为止，尚不知道哪些受体参与BMP-9或BMP-10的成骨分化能力。本发明的研究人员已经鉴定出BMP-9的结构中的腕部表位和指节表位充当介导主要信号传导的配体，证实通过调节这些序列，可以在维持内皮细胞信号传导的同时抑制成骨分化能力(SEQ ID NO:1-29，图4)。这些变体被确定为在体内具有相似的

生理活性,并且预期会提供在最小化对副作用的担忧的同时增强治疗效果的优点。

[0080] 实施例3.在内皮细胞中通过BMP-9变体和衍生物进行的信号传导

[0081] 通过ELISA测量每种proBMP9变体的量,并且用它们处理细胞。在无血清状态下,将上面得到的变体以指定的浓度范围添加到HUVEC细胞中。在处理8小时后,收获细胞,从中提取mRNA,并且通过定量PCR测量ID1和BMPR-II的表达。另外,对于pSmad1/5/8的表达,在无血清状态下用BMP-9变体处理细胞,并且在1小时后,观察其结果。通过针对抗pSmad1/5/8抗体的免疫印迹测量在裂解缓冲液中处理细胞后获得的蛋白质的表达水平。基于观察结果(图4),证实变体显著增加ID1和BMPR-II的表达,并且增加的量与野生型BMP-9相比没有显示出很大的差异。如在先前的报道中所见,证实BMP-9或BMP-10充当血管内皮细胞中的ALK1受体的配体。根据文献,已知BMP-9抑制循环系统中的内皮细胞迁移、增殖和通过内皮细胞的血管生成。

[0082] 实施例4.在C2C12细胞培养物中通过BMP-9变体进行的成骨分化信号传导

[0083] 选择小鼠成肌细胞C2C12来观察成骨分化能力,因为它具有与间充质干细胞相似的分化能力。将C2C12细胞在含有0.25% FBS的DMEM中培养16小时,然后用BMP-9变体处理。在进一步培养72小时后,用1% Triton X-100/PBS裂解细胞,并且测量所得蛋白质的ALP酶活性。通过测量经由与作为酶底物的4-硝基苯基磷酸酯二钠盐反应而产生的水溶性材料在405nm处的吸光度来确定ALP酶活性,并且购买BMP-9作为标准材料并与之进行比较。基于观察结果,证实与标准材料相比,变体具有大大降低的成骨分化能力(图4)。通过在实施例3中在维持血管内皮细胞信号传导的同时使骨化丧失能力,判断如此形成的变体提供治疗效果并且也没有异位骨化的副作用。

[0084] 实施例5.延长BMP-9-Fc衍生物的体内半衰期的作用

[0085] 通过小鼠的尾静脉以1mg/kg的剂量注射BMP-9和Fc融合的BMP-9衍生物中的每一种,并且以规则的时间间隔收集血液,并且使用BMP-9定量试剂盒测量血液中BMP-9的量。基于测量结果(图5),结合Fc的BMP-9衍生物(SEQ ID NO:32-33)显示出显著增加的血液浓度曲线。

[0086] 尽管已经在上面详细地公开了本发明的具体实施方案,但对于本领域技术人员而言将清楚的是,描述仅仅是优选的示例性实施方案的描述,而不应被解释为限制本发明的范围。因此,本发明的实质范围将由所附权利要求及其等同方案限定。

[0087] 工业实用性

[0088] 根据本发明,变体被设计以最大化BMP-9的治疗效果并大大降低其副作用,并且在哺乳动物细胞中高效地表达和纯化。此外,为了延长BMP-9的血液半衰期,另外构建了与免疫球蛋白的Fc区融合的融合蛋白。这种变体和融合蛋白在体外和通过动物实验可以对诸如肿瘤、纤维化、心肺血管疾病、肥胖和脂肪肝的各种疾病展现出优异的治疗效果,因此可以预期作为治疗这些疾病的新型治疗剂是有用的。

[0089] 序列表自由文本

[0090] 附有电子文件。

<110> 纳米智能生物医学工程有限公司

<120> 由于异位骨化的副作用减少而具有增强的治疗效果的骨形态发生蛋白-9和骨形态发生蛋白-10变体以及包含所述变体的药物组合物

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Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
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Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
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His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
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325 330 335
Glu Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 4
<211> 426
<212> PRT
<213> Artificial Sequence
<220>

<223> BMP-9 varient

<400> 4

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His

290 295 300

Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320

Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335

Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350

Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Glu Val Thr Pro Thr
355 360 365

Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380

Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400

Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415

Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 5

<211> 426

<212> PRT

<213> Artificial Sequence

<220>

<223> BMP-9 varient

<400> 5

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30

Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45

Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60

Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80

Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95

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

Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His

115 120 125

Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg

130 135 140

Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser

145 150 155 160

His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr

165 170 175

Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln

180 185 190

Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val

195 200 205

Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu

210 215 220

Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser

225 230 235 240

Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn

245 250 255

Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met

260 265 270

Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly

275 280 285

Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His

290 295 300

Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala

305 310 315 320

Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile

325 330 335

Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu

340 345 350

Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr

355 360 365

Lys His Asp Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys

370 375 380

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

385 390 395 400

Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu

405 410 415

Gly Met Ser Val Ala Glu Cys Gly Cys Arg

420 425

<210> 6
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 6

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255

Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Glu Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 7
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 7

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80

Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val

385 390 395 400
Leu Tyr Lys Asp Asn Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 8
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 8

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu

210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Gln Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 9
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 9
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His

35 40 45

Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60

Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80

Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95

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

Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125

Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140

Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160

His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175

Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190

Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205

Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220

Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240

Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255

Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270

Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285

Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300

Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320

Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335

Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350

Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Trp Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 10
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 10

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175

Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Lys Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 11
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 11

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala

305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asn Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 12
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 12
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg

130 135 140

Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Gln Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 13

<211> 426

<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 13

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270

Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Pro Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 14
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 14

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95

Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Arg Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu

405 410 415

Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 15
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 15

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser

225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Arg Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 16
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 16

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu

50 55 60

Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80

Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95

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

Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125

Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140

Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160

His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175

Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190

Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205

Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220

Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240

Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255

Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270

Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285

Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300

Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320

Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335

Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350

Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365

Lys His His Lys Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 17
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 17

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190

Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys Arg Arg Lys Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 18
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 18
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile

325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Arg Lys Val Arg Thr Arg Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 19
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 19
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser

145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys Pro Arg Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 20
<211> 426
<212> PRT
<213> Artificial Sequence

<220>

<223> BMP-9 varient

<400> 20

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285

Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Trp Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Tyr Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 21
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 21

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110

Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Cys Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Cys Ile Asp Gln Thr Leu Asp His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg

420 425

<210> 22
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 22

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn

245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Cys Gly Gly Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 23
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 23
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val

65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Cys Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Cys Ile Asp Gln Thr Leu Asp His Leu Lys Phe Pro Thr Lys
370 375 380

Val Gly Lys Asp Cys Cys Val Pro Thr Lys Ile Ser Pro Ile Ser Val
385 390 395 400
Leu Trp Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 24
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 24

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205

Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Cys Gly Gly Lys
370 375 380
Val Gly Lys Ala Cys Cys Ala Pro Thr Pro Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Trp Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 25
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 25

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30

Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu

340 345 350

Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Ala Asp Val Thr Pro Thr
355 360 365

Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380

Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400

Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415

Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 26

<211> 426

<212> PRT

<213> Artificial Sequence

<220>

<223> BMP-9 varient

<400> 26

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30

Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45

Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60

Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80

Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95

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

Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125

Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140

Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160

His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr

165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Ala Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 27
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient

<400> 27

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300

Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Glu Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 28
<211> 426
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 28

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125

Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala
305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Ala Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Ala Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg
420 425

<210> 29

<211> 24
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-9 varient
<400> 29

Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr Lys His Ala Ile Val
1 5 10 15
Gln Thr Leu Val His Leu Lys Phe
20

<210> 30
<211> 243
<212> PRT
<213> Artificial Sequence
<220>
<223> Fc
<400> 30

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
20 25 30
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
35 40 45
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
50 55 60
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
65 70 75 80
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
85 90 95
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
100 105 110
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
115 120 125
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
130 135 140
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
145 150 155 160
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
165 170 175

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
180 185 190
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
195 200 205
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
210 215 220
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
225 230 235 240
Pro Gly Lys

<210> 31
<211> 15
<212> PRT
<213> Artificial Sequence
<220>
<223> Linker
<400> 31
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 32
<211> 368
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-Fc fusion
<400> 32
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
20 25 30
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
35 40 45
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
50 55 60
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
65 70 75 80
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
85 90 95
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
100 105 110

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
115 120 125
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
130 135 140
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
145 150 155 160
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
165 170 175
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
180 185 190
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
195 200 205
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
210 215 220
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
225 230 235 240
Pro Gly Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
245 250 255
Gly Ser Ser Ala Gly Ala Gly Ser His Cys Gln Lys Thr Ser Leu Arg
260 265 270
Val Asn Phe Glu Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys
275 280 285
Glu Tyr Glu Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala
290 295 300
Asp Asp Val Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His
305 310 315 320
Leu Lys Phe Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys
325 330 335
Leu Ser Pro Ile Ser Val Leu Tyr Lys Asp Asp Met Gly Val Pro Thr
340 345 350
Leu Lys Tyr His Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg
355 360 365

<210> 33
<211> 665
<212> PRT
<213> Artificial Sequence
<220>
<223> BMP-Fc fusion
<400> 33

Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15
Ile Gln Ala Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser Ala Gly Gly
20 25 30
Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu Pro Glu His
35 40 45
Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val Asp Phe Leu
50 55 60
Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys Thr Arg Val
65 70 75 80
Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr Thr Ser Asp
85 90 95
Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe Ser Met Glu
100 105 110
Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe Gln Lys His
115 120 125
Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln Ile Thr Arg
130 135 140
Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val Asp Pro Ser
145 150 155 160
His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu Asp Gly Thr
165 170 175
Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu Val Ser Gln
180 185 190
Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser Ser Ala Val
195 200 205
Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn Lys Leu Glu
210 215 220
Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu Asp Ile Ser
225 230 235 240
Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val Phe Ser Asn
245 250 255
Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu Arg Glu Met
260 265 270
Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser Lys Asp Gly
275 280 285
Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr Asp Gly His
290 295 300
Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser Ala Gly Ala

305 310 315 320
Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe Glu Asp Ile
325 330 335
Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu Ala Tyr Glu
340 345 350
Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val Thr Pro Thr
355 360 365
Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe Pro Thr Lys
370 375 380
Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Val
385 390 395 400
Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr His Tyr Glu
405 410 415
Gly Met Ser Val Ala Glu Cys Gly Cys Arg Gly Gly Gly Gly Ser Gly
420 425 430
Gly Gly Gly Ser Gly Gly Gly Gly Ser His Thr Cys Pro Pro Cys Pro
435 440 445
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
450 455 460
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
465 470 475 480
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
485 490 495
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
500 505 510
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
515 520 525
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
530 535 540
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
545 550 555 560
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
565 570 575
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
580 585 590
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
595 600 605
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
610 615 620

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
625 630 635 640
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
645 650 655
Lys Ser Leu Ser Leu Ser Pro Gly Lys
660 665

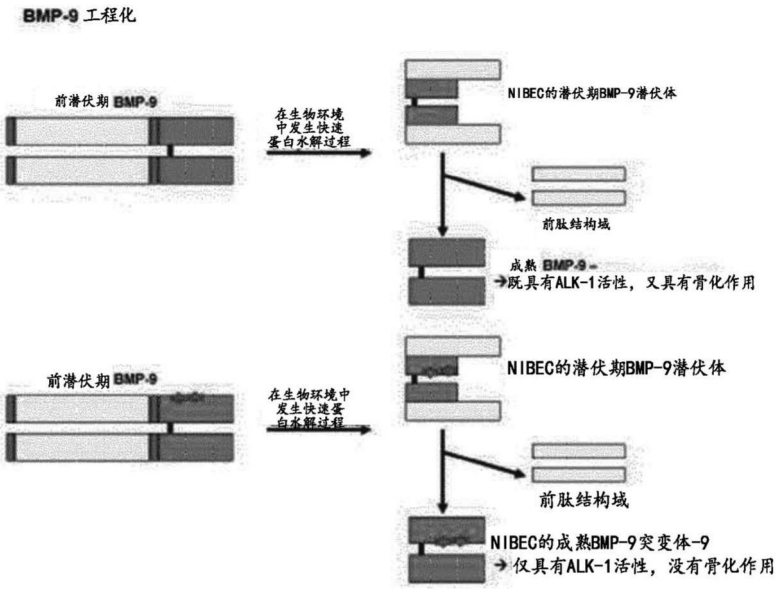
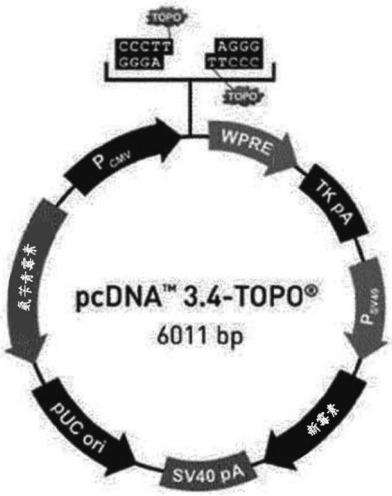


图1



pcDNA™ 3.4-TOPO®的特征

6011个核苷酸

CMV 启动子 (P_{CMV}):	47-726
CMV正向引物结合位点:	584-604
TOPO® 克隆位点:	741
WPRE:	782-1379
pcDNA™ 3.4 反向引物结合位点:	822-844 (c)*
TK 多腺苷酸化信号 (TK pA):	1384-1655
SV40 早期启动子 (P_{SV40}):	2124-2493
新霉素抗性基因:	2529-3323
SV40 多腺苷酸化位点 (SV40 pA):	3499-3629
pUC 复制起点:	4012-4685 (c)
氨苄青霉素抗性基因:	4830-5690 (c)
bla 启动子:	5691-5789 (c)

*(c): 互补链

图2

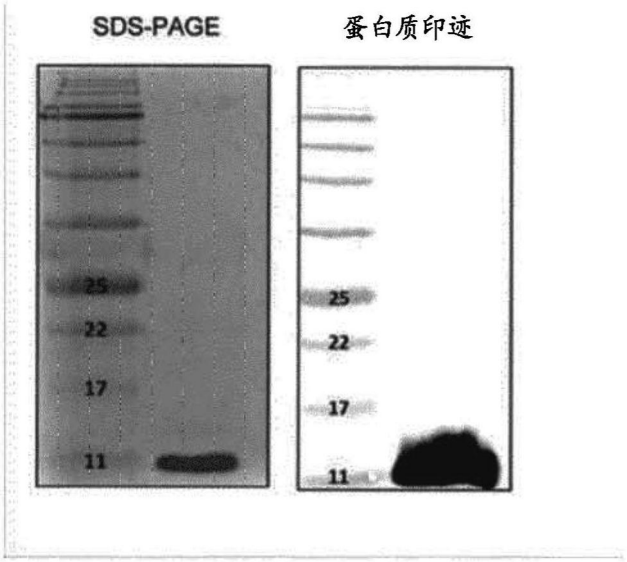

SDS-PAGE
蛋白质印迹
25
22
17
11
25
22
17
11

图3a

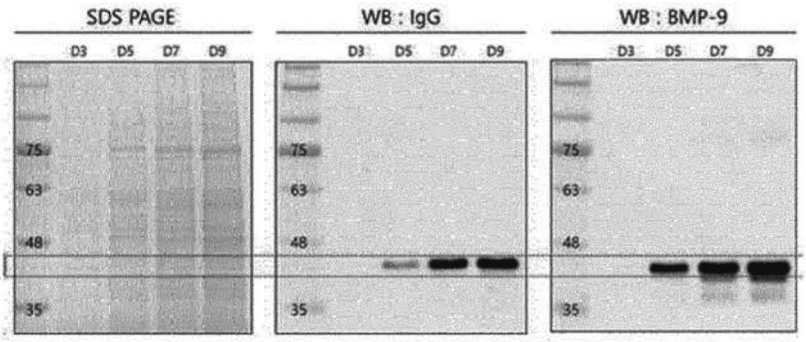

SDS PAGE
WB : IgG
WB : BMP-9
D3 D5 D7 D9
D3 D5 D7 D9
D3 D5 D7 D9
75
63
48
35
75
63
48
35
75
63
48
35

图3b

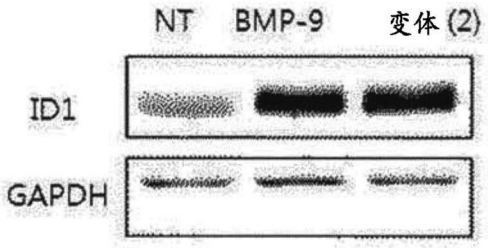

NT
BMP-9
变体 (2)
ID1
GAPDH

图4a

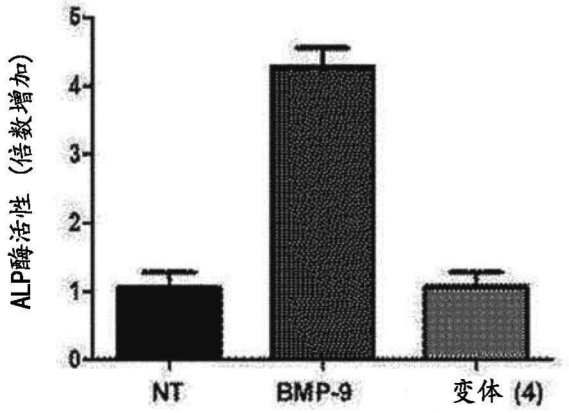
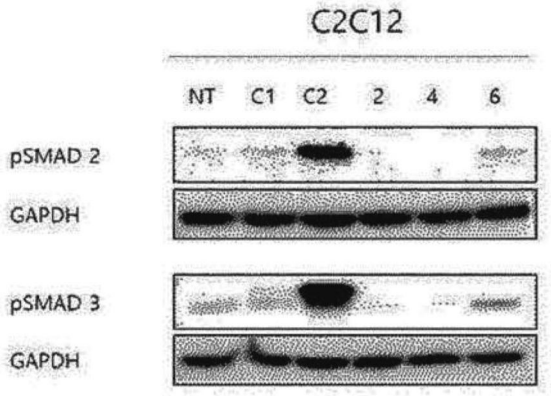


图4b



NT：不做处理
C1：阴性对照
C2： 野生型 BMP9
2, 4, 6： 变体 BMP9（点突变的，SEQ ID NO）

图5

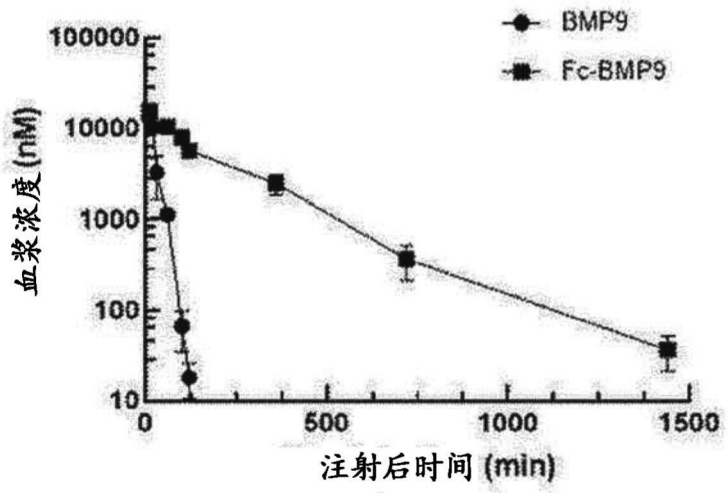

BMP9
Fc-BMP9
100000
10000
1000
100
10
血浆浓度(nM)
0
500
1000
1500
注射后时间 (min)

图6