

Conditional

Annex A - Defendant's Claim Amendments – EP (UK) 3 130 347 B1

Conditional Amendment D – list of positions

Claims

~~1. A modified PH20 polypeptide, comprising one or more amino acid replacements in an unmodified PH20 polypeptide, wherein:~~

~~the unmodified PH20 polypeptide consists of the sequence of amino acids set forth in SEQ ID NO: 3, 7 or 32-66; the modified PH20 polypeptide exhibits increased hyaluronidase activity that is at least 120% of the hyaluronidase activity compared to the unmodified PH20 polypeptide not containing the amino acid replacement(s); the amino acid replacement is at a position corresponding to a position selected from among 1, 12, 15, 24, 26, 27, 29, 30, 31, 32, 33, 37, 39, 46, 48, 52, 58, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 84, 86, 87, 92, 93, 94, 97, 118, 120, 127, 131, 135, 141, 142, 147, 148, 150, 151, 152, 155, 156, 163, 164, 165, 166, 169, 170, 174, 198, 206, 209, 212, 213, 215, 219, 233, 234, 236, 238, 247, 257, 259, 260, 261, 263, 269, 271, 272, 276, 277, 278, 282, 291, 293, 305, 308, 309, 310, 313, 315, 317, 318, 320, 324, 325, 326, 328, 347, 353, 359, 371, 377, 380, 389, 392, 395, 399, 405, 407, 409, 410, 418, 419, 421, 425, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide having the sequence of amino acids set forth in SEQ ID NO:3, with the proviso that if the modified PH20 polypeptide includes only a single amino acid replacement, the replacement does not correspond to amino acid replacement N219A with reference to amino acid positions set forth in SEQ ID NO:3; and the modified PH20 polypeptide comprises up to 10 amino acid replacement(s) compared to the unmodified PH20 polypeptide not containing the replacement(s);~~

~~2. 1. A modified PH20 polypeptide, comprising one or more amino acid replacements in an unmodified PH20 polypeptide, wherein:~~

~~the unmodified PH20 polypeptide consists of the sequence of amino acids set forth in SEQ ID NO: 3; 7 or 32-66 the modified PH20 polypeptide exhibits increased hyaluronidase activity that is at least 120% of the hyaluronidase activity compared to the unmodified PH20 polypeptide not containing the amino acid replacement(s); the amino acid replacement is at a position corresponding to a position selected from among 1, 12, 15, 24, 26, 27, 29, 30, 31, 32, 33, 37, 39, 46, 48, 52, 58, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 84, 86, 87, 92, 93, 94, 97, 118, 120, 127, 131, 135, 141, 142, 147, 148, 150, 151, 152, 155, 156, 163, 164, 165, 166, 169, 170, 174, 198, 206, 209, 212, 213, 215, 219, 233, 234, 236, 238, 247, 257, 259, 260, 261, 263, 269, 271, 272, 276, 277, 278, 282, 291, 293, 305, 308, 309, 310, 313, 315, 317, 318, 320, 324, 325, 326, 328, 347, 353, 359, 371, 377, 380, 389, 392, 395, 399, 405, 407, 409, 410, 418, 419, 421, 425, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide having the sequence of amino acids set forth in SEQ ID NO:3, with the proviso that if the modified PH20 polypeptide includes only a single amino acid replacement, the replacement does not correspond to amino acid replacement N219A with reference to amino acid positions set forth in SEQ ID NO:3; and the modified PH20 polypeptide comprises up to 10 amino acid replacement(s) compared to the unmodified PH20 polypeptide not containing the replacement(s);~~

~~The modified PH20 polypeptide of claim 1,~~ wherein the amino acid replacement is selected from among:

~~histidine (H) at a position corresponding to position 1; Q at a position corresponding to position 1; V at a position corresponding to position 15; E at a position corresponding to position 24; H at a position corresponding to position 24; K at a position corresponding to position 27; R at a position corresponding to position 27; E at a position corresponding to position 29; I at a position corresponding to position 29; L at a position corresponding to position 29; M at a position corresponding to position 29; P at a position corresponding to position 29; S at a position corresponding to position 29; V at a position corresponding to position 29; G at a position corresponding to position 30; H at a position corresponding to position 30; K at a position corresponding to position 30; M at a position corresponding to position 30; R at a position corresponding to position 30; S at a position corresponding to position 30; A at a position corresponding to position 31; C at a position corresponding to position 31; H at a position corresponding to position 31; I at a position corresponding to position 31; K at a position corresponding to position 31; L at a position corresponding to position 31; P at a position corresponding to position 31; R at a position corresponding to position 31; S at a position corresponding to position 31; T at a position corresponding to position 31; V at a position corresponding to position 31; F at a position corresponding to position 32; G at a position corresponding to position 32; H at a position corresponding to position 32; Wat at a position corresponding to position 33; F at a position corresponding to position 37; N at a position corresponding to position 39; T at a position corresponding to position 39; **F at a position corresponding to position 48;** H at a position corresponding to position 48; N at a position corresponding to position~~

SEQ ID NO:3.

~~3.~~ 2. A modified PH20 polypeptide, comprising one or more amino acid replacements in an unmodified PH20 polypeptide, wherein:

the unmodified PH20 polypeptide consists of the sequence of amino acids set forth in SEQ ID NO: 3; 7 or 32-66 the modified PH20 polypeptide exhibits increased hyaluronidase activity that is at least 120% of the hyaluronidase activity compared to the unmodified PH20 polypeptide not containing the amino acid replacement(s);
the amino acid replacement is at a position corresponding to a position selected from among 1, 12, 15, 24, 26, 27, 29, 30, 31, 32, 33, 37, 39, 46, 48, 52, 58, 63, 67, 68, 69, 70, 71, 72, 73, 74, 75, 84, 86, 87, 92, 93, 94, 97, 118, 120, 127, 131, 135, 141, 142, 147, 148, 150, 151, 152, 155, 156, 163, 164, 165, 166, 169, 170, 174, 198, 206, 209, 212, 213, 215, 219, 233, 234, 236, 238, 247, 257, 259, 260, 261, 263, 269, 271, 272, 276, 277, 278, 282, 291, 293, 305, 308, 309, 310, 313, 315, 317, 318, 320, 324, 325, 326, 328, 347, 353, 359, 371, 377, 380, 389, 392, 395, 399, 405, 407, 409, 410, 418, 419, 421, 425, 431, 433, 436, 437, 438, 439, 440, 441, 442, 443, 445, 446 and 447 with reference to amino acid positions set forth in SEQ ID NO:3, wherein corresponding amino acid positions are identified by alignment of the PH20 polypeptide with the polypeptide having the sequence of amino acids set forth in SEQ ID NO:3, with the proviso that if the modified PH20 polypeptide includes only a single amino acid replacement, the replacement does not correspond to amino acid replacement N219A with reference to amino acid positions set forth in SEQ ID NO:3; and
the modified PH20 polypeptide comprises up to 10 amino acid replacement(s) compared to the unmodified PH20 polypeptide not containing the replacement(s);

~~A modified PH20 polypeptide of claim 1,~~ wherein the amino acid replacement is selected from among E at a position corresponding to position 12; E at a position corresponding to position 26; K at a position corresponding to position 26; R at a position corresponding to position 46; Q at a position corresponding to position 52; Q at a position corresponding to position 58; H at a position corresponding to position 68; Q at a position corresponding to position 68; A at a position corresponding to position 70; C at a position corresponding to position 70; F at a position corresponding to position 70; G at a position corresponding to position 70; H at a position corresponding to position 70; K at a position corresponding to position 70; L at a position corresponding to position 70; ~~N at a position corresponding to position 70;~~ P at a position corresponding to position 70; R at a position corresponding to position 70; S at a position corresponding to position 70; T at a position corresponding to position 70; ~~A at a position corresponding to position 74;~~ C at a position corresponding to position 74; G at a position corresponding to position 74; N at a position corresponding to position 74; P at a position corresponding to position 74; R at a position corresponding to position 74; S at a position corresponding to position 74; V at a position corresponding to position 74; W at a position corresponding to position 74; G at a position corresponding to position 84; R at a position corresponding to position 84; G at a position corresponding to position 97; H at a position corresponding to position 127; N at a position corresponding to position 127; G at a position corresponding to position 131; V at a position corresponding to position 131; R at a position corresponding to position 142; A at a position corresponding to position 166; F at a position corresponding to position 166; H at a position corresponding to position 166; L at a position corresponding to position 166; R at a position corresponding to position 166; Y at a position corresponding to position 166; ~~K at a position corresponding to position 174;~~ K at a position corresponding to position 206; M at a position corresponding to position 213; N at a position corresponding to position 213; Sat a position corresponding to position 238; N at a position corresponding to position 261; ~~R at a position corresponding to position 277;~~ T at a position corresponding to position 277; ~~L at a position corresponding to position 309;~~ N at a position corresponding to position 309; Q at a position corresponding to position 309; R at a position corresponding to position 309; T at a position corresponding to position 309; A at a position corresponding to position 310; G at a position corresponding to position 310; H at a position corresponding to position 315; I at a position corresponding to position 317; K at a position corresponding to position 317; R at a position corresponding to position 317; M at a position corresponding to position 318; G at a position corresponding to position 347; S at a position corresponding to position 347; M at a position corresponding to position 399; T at a position corresponding to position 399; Wat a position corresponding to position 399; D at a position corresponding to position 407; Q at a position corresponding to position 407; F at a position corresponding to position 419; I at a position corresponding to position 419; R at a position corresponding to position 419; S at a position corresponding to position 419; K at a position corresponding to position 421; N at a position corresponding to position 421; Q at a position corresponding to position 421; R at a position corresponding to position 421; S at a position corresponding to position 421; A at a position corresponding to position 431; H at a position corresponding to position 431; K position corresponding to position 431; Q at a position corresponding to position 431; R at a position corresponding to position 431; S at a position corresponding to position 431; V at a position corresponding to position 431; L at a position corresponding to position 433; R at a position corresponding to position 433; V at a position corresponding to position 439; H at a position corresponding to position 440; R at a position corresponding to position 440; A at a position corresponding to position 443; M at a position corresponding to position 443; M at a position corresponding to position 445; and/or with P at a position corresponding to position 445, with reference to amino acid positions set forth in SEQ ID NO:3.

~~4. A modified PH20 polypeptide of claim 1, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 73, 78, 91, 95, 96, 105, 106, 108, 109, 111, 112, 113, 115, 117, 118, 119, 120, 123-126, 128-136, 139-141, 149, 154, 155, 164, 165, 167,~~

~~191, 192, 196, 197, 199-205, 207, 208, 225, 226, 228, 229, 231, 233, 237, 253, 254, 256, 257, 277, 283, 293, 295, 296, 298, 300, 303, 316, 318, 321, 322, 338-340, 344, 348, 367, 369, 371, 377, 384-388, 394, 398, 399, 401, 406-408, 410, 412, 414, 416, 431, 448, 459, 465, 469, 478, 479, 482, 493, 497, 501, 503, 507, 508, 510-512, 514, 518, 522, 523, 537-543, 545, 558, 559, 561, 563-566, 569, 572, 574, 594, 596, 618-620, 624-634, 637, 640-644, 652, 657, 675, 695, 698, 699, 700, 712, 717, 725, 738, 748-750, 757, 775, 799-801, 807, 822, 825, 844, 847, 851 and 853, or a sequence of amino acids that exhibits at least 96% sequence identity to a sequence of amino acids set forth in any of SEQ ID NOS: 73, 78, 91, 95, 96, 105, 106, 108, 109, 111, 112, 113, 115, 117, 118, 119, 120, 123-126, 128-136, 139-141, 149, 154, 155, 164, 165, 167, 191, 192, 196, 197, 199-205, 207, 208, 225, 226, 228, 229, 231, 233, 237, 253, 254, 256, 257, 277, 283, 293, 295, 296, 298, 300, 303, 316, 318, 321, 322, 338-340, 344, 348, 367, 369, 371, 377, 384-388, 394, 398, 399, 401, 406-408, 410, 412, 414, 416, 431, 448, 459, 465, 469, 478, 479, 482, 493, 497, 501, 503, 507, 508, 510-512, 514, 518, 522, 523, 537-543, 545, 558, 559, 561, 563-566, 569, 572, 574, 594, 596, 618-620, 624-634, 637, 640-644, 652, 657, 675, 695, 698, 699, 700, 712, 717, 725, 738, 748-750, 757, 775, 799-801, 807, 822, 825, 844, 847, 851 and 853 and that contains the amino acid replacement as compared to corresponding positions in a polypeptide of any of SEQ ID NOS: 3, 7 and 32-66.~~

~~5. The modified PH20 polypeptide of claim 3, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 86, 89, 99, 100, 159, 173, 181, 193, 195, 209-220, 238, 239, 242, 247-252, 267, 269, 277, 308, 324, 325, 330, 335, 355, 419, 421-423, 425, 428, 435, 455, 456, 462, 463, 484, 490, 506, 531, 533, 579, 581-583, 585, 587, 588, 602, 605, 606, 609, 613, 648, 731, 732, 734, 742, 746, 760, 762, 764, 765, 769-773, 779, 782, 783, 786-789, 794, 795, 814, 816, 819, 826, 830, 836 and 838, or a sequence of amino acids that exhibits at least 96% sequence identity to a sequence of amino acids set forth in any of 86, 89, 99, 100, 159, 173, 181, 193, 195, 209-220, 238, 239, 242, 247-252, 267, 269, 277, 308, 324, 325, 330, 335, 355, 419, 421-423, 425, 428, 435, 455, 456, 462, 463, 484, 490, 506, 531, 533, 579, 581-583, 585, 587, 588, 602, 605, 606, 609, 613, 648, 731, 732, 734, 742, 746, 760, 762, 764, 765, 769-773, 779, 782, 783, 786-789, 794, 795, 814, 816, 819, 826, 830, 836 and 838 and that contains the amino acid replacement as compared to corresponding positions in a polypeptide of any of SEQ ID NOS: 3, 7 and 32-66.~~

~~6. 3.~~ The modified PH20 polypeptide of any of claims 1-~~5~~ 2 that exhibits at least 2.0-fold of the hyaluronidase activity compared to the PH20 polypeptide not containing the amino acid replacement(s).

~~7. 4.~~ The modified PH20 polypeptide of any of claims 1-~~6~~ 3 that comprises one or more of a modification selected from among glycosylation, sialation, albumination, farnesylation, carboxylation, hydroxylation and phosphorylation.

~~8. 5.~~ The modified PH20 polypeptide of any of claims 1-~~7~~ 4 that comprises at least an N-acetylglucosamine moiety linked to each of at least three asparagine (N) residues.

~~9. 6.~~ The modified PH20 polypeptide of claim ~~8~~ 5, wherein the three asparagine residues correspond to amino acid residues 200, 333 and 358 of SEQ ID NO: 3.

~~10. 7.~~ The modified PH20 polypeptide of any of claims 1-~~9~~ 6 that is conjugated to a polymer.

~~11. 8.~~ The modified PH20 polypeptide of claim ~~10~~ 7, wherein the polymer is dextran or PEG.

~~12. 9.~~ A nucleic acid molecule, encoding a modified PH20 polypeptide of any of claims 1-~~11~~ 8.

~~13. 10.~~ A vector, comprising the nucleic acid molecule of claim ~~12~~ 9.

~~14. 11.~~ An isolated cell, comprising the vector of claim ~~13~~ 10.

~~15. 12.~~ A method of producing a modified PH20 polypeptide, comprising:

introducing the nucleic acid molecule of claim ~~12~~ 9 into a cell capable of incorporating N-linked sugar moieties into the polypeptide;

culturing the cell under conditions whereby an encoded modified PH20 polypeptide is produced and secreted by the cell; and

recovering the expressed polypeptide.

~~16. 13.~~ A pharmaceutical composition, comprising a modified PH20 polypeptide of any of claims 1-~~14~~ 8.

~~17. 14.~~ The pharmaceutical composition of Claim ~~16~~ 13 for use for treating a disease or disorder selected from one involving glycosaminoglycan accumulation in the brain; a cardiovascular disorder; an ophthalmic disorder; and pulmonary disease.

~~18-15.~~ The pharmaceutical composition of Claim ~~46~~ 13 for use for treating a tumor.

~~19-16.~~ The pharmaceutical composition of Claim ~~46~~ 13 for delivering a therapeutic agent for use in treating a tumor; for use in treating glycosaminoglycan accumulation in the brain; for use in treating a cardiovascular disorder; for use in treating an ophthalmic disorder; or for use in treating pulmonary disease.

~~20-17.~~ The pharmaceutical composition of Claim ~~46~~ 13 for increasing penetration of therapeutic agents into solid tumors or for increasing bioavailability of therapeutic agents for use in treating a tumor; for use in treating glycosaminoglycan accumulation in the brain; for use in treating a cardiovascular disorder; for use in treating an ophthalmic disorder; or for use in treating pulmonary disease.

~~24-18.~~ The modified polypeptide of claim ~~44~~ 8 for use for treating a tumor, wherein the modified PH20 polypeptide is conjugated to PEG.