

SEQUENCE LISTING

<110> Xbrane Biopharma AB

<120> NOVEL COMBINATION OF TIS SEQUENCE AND SIGNAL PEPTIDE SEQUENCE FOR EXPRESSING A RECOMBINANT PROTEIN

<130> 2020Q105-2

<160> 14

<170> BiSSAP 1.3.6

<210> 1

<211> 11

<212> DNA

<213> Artificial Sequence

<220>

<223> Kozak sequence - DNA

<400> 1

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<210> 2

<211> 78

<212> DNA

<213> Artificial Sequence

<220>

<223> Signal peptide - DNA sequence

<400> 2

atggctctgc tgcacaagaa catgaagcac ctgtggttct ttctgctgct ggtggccgct 60

cctagatggg tgctgtct 78

<210> 3

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Signal peptide aa sequence

<400> 3

Met	Ala	Leu	Leu	His	Lys	Asn	Met	Lys	His	Leu	Trp	Phe	Phe	Leu	Leu
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Leu	Val	Ala	Ala	Pro	Arg	Trp	Val	Leu	Ser						
		20				25									

<210> 4

<211> 1326

<212> DNA

<213> Artificial Sequence

<220>

<223> Nivolumab - heavy chain - DNA sequence

<400> 4

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gatt	gc	aagg	cctc	cg	cat	cac	tt	ct	cc	aact	ct	gg	ca	tg	cact	gg	gt	ccg	ac	aggcc	120					
cctg	gaaa	aag	gact	gg	aatg	gg	tc	gcc	gtg	att	tg	tac	g	ac	gg	ct	ctaa	gc	gg	tact	ac	180				
gcc	gact	ccg	tga	agg	gcag	att	cac	cat	c	tct	cg	gg	aca	act	ca	aaga	a	cac	ct	gtt		240				
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ctgg	tca	agg	att	act	ttc	tg	ag	ct	gtg	acc	gt	gt	ctt	gga	act	ct	gg	tg	ct	g	acc				480	
tcc	gg	cg	tg	c	ac	att	tc	ag	ct	gt	g	ctg	cag	tc	ct	ccg	gc	ct	g	tact	c	tct	g	ct	ct	540
gtc	gt	g	acc	g	tg	c	tt	ct	ag	ct	ct	g	gg	c	acca	ag	ac	ct	g	taa	cg	tg	g	acc	ac	600
aag	ct	tcca	ac	acca	agg	tg	g	aca	ag	cg	c	gt	g	aat	cta	ag	ta	c	gg	ccc	tc	ct	g	tc	ct	660
ccat	g	tc	ctg	ct	caga	aatt	c	ct	cg	gc	gga	c	ct	tc	cg	gt	t	ct	g	ttt	cc	tc	caa	ag	cct	720

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<210> 5

<211> 440

<212> PRT

<213> Artificial Sequence

<220>

<223> Nivolumab - heavy chain - protein sequence

<400> 5

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

Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
 100 105 110
 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
 115 120 125
 Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
 130 135 140
 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
 145 150 155 160
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
 165 170 175
 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
 180 185 190
 Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
 195 200 205
 Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
 210 215 220
 Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 225 230 235 240
 Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 245 250 255
 Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
 260 265 270
 Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 275 280 285
 Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 290 295 300
 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
 305 310 315 320
 Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 325 330 335
 Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
 340 345 350
 Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 355 360 365
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 370 375 380
 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 385 390 395 400
 Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
 405 410 415
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 420 425 430
 Ser Leu Ser Leu Ser Leu Gly Lys
 435 440

<210> 6
<211> 648
<212> DNA
<213> Artificial Sequence

<220>
<223> Nivolumab - light chain - DNA sequence

<400> 6
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ggacaggccc ctgggtgct gatctacgat gcctctaata gagccacagg catccccgcc 180
agattctctg gctctggatc tggcaccgac ttcacctga ccatctctag cctggaacct 240
gaggacttcg ccgtgtacta ctgccagcag tctccaact ggcttagaac cttggccag 300
ggcaccaagg tggaaatcaa gagaaccgtg gctgccctt ccgtgttcat ctcccacca 360
tctgacgagc agctgaagtc cggcacagct tctgtctgt gctgctgaa caacttctac 420
cctcggaag ccaaggtgca gtggaagggtg gacaatgcc tgagtcagg caactccaa 480
gagtctgtga ccgagcagga ctccaaggac tctacctaca gcctgtctc cacactgacc 540
ctgtctaagg ccgactacga gaagcacaag gtgtacgcct gtgaagtga ccaccaggga 600
ctgtctagcc ccgtgaccaa gtcttcaac agaggcgagt gctgatag 648

<210> 7
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Nivolumab - light chain - protein sequence

<400> 7
Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

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

<210> 8

<211> 1410

<212> DNA

<213> Artificial Sequence

<220>

<223> Kozak - SP - protein heavy chain - DNA

<400> 8

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 gccgctccta gatgggtggt gtctcaggtg cagctgggtg aatctggtgg cggagtgggt 120
 cagcctggca gatctctgag actggattgc aaggcctccg gcatcacctt ctccaactct 180
 ggcatgcact ggggtccgaca ggcccctgga aaaggactgg aatgggtcgc cgtgatttgg 240

tacgacggct ctaagcggta ctacgccgac tccgtgaagg gcagattcac catctctcgg 300
 gacaactcca agaacaccct gtttctgcag atgaactccc tgagagccga ggacaccgcc 360
 gtgtactact gtgccaccaa cgatgattat tggggccagg gcacactggc caccgtgtcc 420
 tctgcttcta ccaagggacc ctctgtgttc cctctggctc ctgctccag atccacctct 480
 gagtctaccg ctgctctggg ctgcctgggc aaggattact ttctgagcc tgtgaccgtg 540
 tcttggaaact ctggtgctct gacctccggc gtgcacacat ttccagctgt gctgcagtcc 600
 tccggcctgt actctctgtc ctctgtcgtg accgtgcctt ctagctctct gggcaccaag 660
 acctacacct gtaacgtgga ccacaagcct tccaacacca aggtggacaa gcgcgtggaa 720
 tctaagtacg gccctccttg tctccatgt cctgctccag aattctcgg cggaccttcc 780
 gtgttctgtt ttctccaaa gcctaaggac accctgatga tctctcggac ccctgaagtg 840
 acctgcgtgg tggatgatgt gtctcaagag gaccccgagg tgcagttcaa ttggtacgtg 900
 gacggcgtgg aagtgcacaa cgccaagacc aagcctagag aggaacagtt caactccacc 960
 tacagagtgg tgtccgtgct gaccgtgctg caccaggatt ggctgaacgg caaagagtac 1020
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<210> 9

<211> 732

<212> DNA

<213> Artificial Sequence

<220>

<223> Kozak - SP - protein light chain - DNA

<400> 9

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gccgctccta gatgggtgct gtctgagatc gtgctgaccc agtctcctgc cacattgtct    120
ctgagtcctg gcgagagagc taccctgtct tgcagagctt cccagtcctg gtcctcctac    180
ctggcctggt atcagcagaa acctggacag gccctcggc tgctgatcta cgatgcctct    240
aatagagcca caggcatccc cgccagattc tctggctctg gatctggcac cgacttcacc    300
ctgaccatct ctgcctgga acctgaggac ttgccgtgt actactgcca gcagtcctcc    360
aactggccta gaaccttgg ccagggcacc aaggtggaaa tcaagagaac cgtggctgcc    420
ccttcctgt tcatcttccc accatctgac gagcagctga agtcggcac agcttctgtc    480
gtgtgcctgc tgaacaactt ctaccctcg gaagccaagg tgcagtggaa ggtggacaat    540
gccctgcagt ccggcaactc ccaagagtct gtgaccgagc aggactcaa ggactctacc    600
tacagcctgt cctccacact gaccctgtct aaggccgact acgagaagca caaggtgtac    660
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gagtgtgat ag                                         732
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<210> 10

<211> 466

<212> PRT

<213> Artificial Sequence

<220>

<223> SP - protein heavy chain - protein

<400> 10

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Met Ala Leu Leu His Lys Asn Met Lys His Leu Trp Phe Phe Leu Leu
1           5           10          15
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Leu Val Ala Ala Pro Arg Trp Val Leu Ser Gln Val Gln Leu Val Glu
 20 25 30
 Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Asp Cys
 35 40 45
 Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser Gly Met His Trp Val Arg
 50 55 60
 Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val Ile Trp Tyr Asp
 65 70 75 80
 Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile
 85 90 95
 Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asn Ser Leu
 100 105 110
 Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Thr Asn Asp Asp Tyr
 115 120 125
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 130 135 140
 Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
 145 150 155 160
 Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 165 170 175
 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 180 185 190
 Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 195 200 205
 Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
 210 215 220
 Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys
 225 230 235 240
 Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly
 245 250 255
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
 260 265 270
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
 275 280 285
 Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
 290 295 300
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
 305 310 315 320
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
 325 330 335
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
 340 345 350
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 355 360 365
 Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu

370 375 380
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 385 390 395 400
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 405 410 415
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp
 420 425 430
 Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
 435 440 445
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
 450 455 460
 Gly Lys
 465

<210> 11

<211> 240

<212> PRT

<213> Artificial Sequence

<220>

<223> SP - protein light chain - protein

<400> 11

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

165 170 175
 Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
 180 185 190
 Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
 195 200 205
 Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
 210 215 220
 Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235 240

<210> 12

<211> 1422

<212> DNA

<213> Artificial Sequence

$\langle 220 \rangle$

<223> Restriction site - SP - protein heavy chain - DNA

<400> 12

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gtggtgcagc ctggcagatc tctgagactg gattgcaagg cctccggcat caccttctcc	180
aactctggca tgactgggt ccgacaggcc cctggaaaag gactggaatg ggtcgccgtg	240
atttggtacg acggctctaa gcggtactac gccgactccg tgaagggcag attcaccatc	300
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cagtctccg gcctgtactc tctgtcctct gtcgtgaccg tgccttctag ctctctgggc	660
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 gccgtggaat gggagtcaa tggccagcct gagaacaact acaagacaac ccctcctgtg 1260
 ctggactccg acggctcctt ctttctgtat tcccgctga ccgtggacaa gtctagatgg 1320
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<210> 13

<211> 744

<212> DNA

<213> Artificial Sequence

<220>

<223> Restriction site - SP - light chain - DNA

<400> 13

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 tcctacctgg cctggtatca gcagaaacct ggacaggccc ctgggtgct gatctacgat 240
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 tctacctaca gcctgtcctc cacttgacc ctgtctaagg ccgactacga gaagcacaag 660
 gtgtacgcct gtgaagtac ccaccaggga ctgtctagcc ccgtgaccaa gtctttcaac 720
 agaggcgagt gctgatagct cgag 744

<210> 14
 <211> 11
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> TIS RNA

<400> 14
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