

Figure 1a

Parental MAGE-A10 alpha chain extracellular amino acid sequence (SEQ ID No:2)

```
QKEVEQNSGF LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI 50
YNSGDKEDGR FTAQLNKASQ YVSLLLIRDSQ PSDSATYLCA VRGTGRRALT 100
FGSGTRLQVQ PNIQNPDPAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD 150
SDVYITDKTV LDMRSMDFKS NSAVAWSNKS DFACANAFNN SIIPEDTFFP 200
SPSS 206
```

Figure 1b

Parental MAGE-A10 TCR beta chain extracellular amino acid sequence (SEQ ID NO:3)

```
NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
GEGTTAKGEV PDGYNVSRLK KQNFLLGLES AAPQSQTSVYF CASSETDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYPDH 150
VELSWWVNGK EVHSGVSTDP QPLKEQPALN DSRVCLSSRL RVSATFWQNP 200
RNHFERCQVQF YGLSENDEWT QDRAKPVTQI VSAEAWGRAD 240
```

Figure 2a

Reference TCR alpha chain – parental MAGE-A10 alpha chain extracellular amino acid sequence but with cysteine (underlined) substituted for T159 (i.e. T48 of the TRAC constant region) (SEQ ID No:4)

```
QKEVEQNSGP LSVPEGAIAS LNCTYSDRGS QSFFWYRQYS GKSPELIMSI 50
YNSGDKEDGR FTAQLNKASQ YVSLLRDSQ PSDSATYLCA VRGTGRRALT 100
FGSGTRLQVQ PNIQNPDPAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD 150
SDVYITDKCV LDMRSMDFKS NSAVAWSNKS DFACANAFNN SIIPEDTFFP 200
SPSS 206
```

Figure 2b

Reference TCR beta chain – parental beta chain extracellular amino acid sequence but with cysteine substituted for S167 (i.e. S57 of the TRBC2 constant region) and with A185 substituted for C185 and with D199 substituted for N199. All substitutions are underlined. (SEQ ID No:5).

```
NAGVTQTPKF RVLKTGQSM T LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
GEGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVCTDP QPLKEQPALN DSRYALSSRL RVSATFWQDP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTQI VSAEAWGRAD 240
```

Figure 3

Alpha chain amino acid sequences. CDRs are underlined and sequence changes from the parental alpha chain are highlighted

SEQ ID No: 6 (alpha chain has single mutation encoding Gln→Ala at position 5 of alpha CDR1)

```
QKEVEQNSGP LSVPEGAIAS LNCTYSDRGS SSFFWYRQYS GKSPELIMSI 50
YSNGDKEDGR FTAQLNKASQ YVSLLIRDSQ PSDSATYLCA VRGTGRRALT 100
FGSGTRLQVQ PNIQNPDPAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD 150
SDVYITDKTV LDMRSMDFKS NSAVAWSNKS DFACANAFNN SIIPEDTFFP 200
SPESS 206
```

SEQ ID No: 7 (alpha chain has single mutation encoding Gln→ Ser at position 5 of alpha CDR1)

```
QKEVEQNSGP LSVPEGAIAS LNCTYSDRGS SSFFWYRQYS GKSPELIMSI 50
YSNGDKEDGR FTAQLNKASQ YVSLLIRDSQ PSDSATYLCA VRGTGRRALT 100
FGSGTRLQVQ PNIQNPDPAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD 150
SDVYITDKTV LDMRSMDFKS NSAVAWSNKS DFACANAFNN SIIPEDTFFP 200
SPESS 206
```

Figure 4

Beta chain amino acid sequences. CDRs are underlined and sequence changes from the parental alpha chain are highlighted

SEQ ID No: 8 (beta chain has single mutation encoding Gly→Ser at position 3 of beta CDR2)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
EGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVSTDP QPLKEQPALN DSRVCLSSRL RVSATFWQNP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

SEQ ID No: 9 (beta chain has single mutation encoding Gly→Ala at position 3 of beta CDR2)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
EGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVSTDP QPLKEQPALN DSRVCLSSRL RVSATFWQNP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

SEQ ID No: 10 (beta chain has single mutation encoding Gly→Phe at position 3 of beta CDR2)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
EGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVSTDP QPLKEQPALN DSRVCLSSRL RVSATFWQNP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

SEQ ID No. 11 (beta chain has single mutation encoding Glu→Asp at position 4 of beta CDR1)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNH YMYWYRQDPG MGLRLIHYSV 50
GEGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVSTDP QPLKEQPALN DSRVCLSSRL RVSATFWQNP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

Figure 5

Alpha chain amino acid sequences of the soluble TCRs. CDRs are underlined and sequence changes from the reference alpha chain are highlighted

SEQ ID No: 12 (alpha chain has single mutation encoding Gln→Ala at position 5 of alpha CDR1)

```
QKEVEQNSGP LSVPEGAIAS LNCTYSDRGS SSFFWYRQYS GKSPELIMSI 50
YSNGDKEDGR FTAQLNKASQ YVSLLIRDSQ PSDSATYLCA VRGTGRRALT 100
FGSGTRLQVQ PNIQNPDPVAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD 150
SDVYITDKCV LDMRSMDFKS NSAVAWSNKS DFACANAFNN SIIPEDTFFP 200
SPSS 206
```

SEQ ID No: 13 (alpha chain has single mutation encoding Gln→Ser at position 5 of alpha CDR1)

```
QKEVEQNSGP LSVPEGAIAS LNCTYSDRGS SSFFWYRQYS GKSPELIMSI 50
YSNGDKEDGR FTAQLNKASQ YVSLLIRDSQ PSDSATYLCA VRGTGRRALT 100
FGSGTRLQVQ PNIQNPDPVAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD 150
SDVYITDKCV LDMRSMDFKS NSAVAWSNKS DFACANAFNN SIIPEDTFFP 200
SPSS 206
```

Figure 6

Beta chain amino acid sequences of the soluble TCRs. CDRs are underlined and sequence changes from the reference beta chain are highlighted.

SEQ ID No: 14 (beta chain has single mutation encoding Gly→Ser at position 3 of beta CDR2)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
EGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVCTDP QPLKEQPALN DSRYLSSRL RVSATFWQNP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

SEQ ID No: 15 (beta chain has single mutation encoding Gly→Ala at position 3 of beta CDR2)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
EGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVCTDP QPLKEQPALN DSRYALSSRL RVSATFWQDP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

SEQ ID No: 16 (beta chain has single mutation encoding Gly→Phe at position 3 of beta CDR2)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNHE YMYWYRQDPG MGLRLIHYSV 50
EGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVCTDP QPLKEQPALN DSRYALSSRL RVSATFWQDP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

SEQ ID No. 17 (beta chain has single mutation encoding Glu→Asp at position 4 of beta CDR1)

```

NAGVTQTPKF RVLKTGQSMT LLCAQDMNH YMYWYRQDPG MGLRLIHYSV 50
GEGTTAKGEV PDGYNVSRK KQNFLLGLES AAPSQTSVYF CASSFTDTQY 100
FGPGTRLTVL EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYDPH 150
VELSWWVNGK EVHSGVCTDP QPLKEQPALN DSRYALSSRL RVSATFWQNP 200
RNHFRCQVQF YGLSENDEWT QDRAKPVTOI VSAEAWGRAD 240

```

Figure 7

DNA sequence for the parental MAGE A10 TCR gene (alpha chain-2A-beta chain construct with the Porcine teschovirus-1 2A sequence)

SEQ ID No: 18

gctagccgccaccatgatgaagtcctgcggtgctgctgggtatcctgtggctgcagctgtcctgggtctggtcccagcaga
aagaggtggagcagaacagcggccctctgagcgtgccgagggcgctatgccagcctgaactgcacctacagcgaca
gaggcagccagagcttctgtgtacagacagtacagcggcaagagccccgagctgatcatgagcatctacagcaacggc
gacaaagaggacggcgggttcaccgcccagctgaacaaggccagccagtagctgtccctgctgatccgggacagccagc
ccagcgacagcgccacctacctgtgcccgtgagaggcacaggcagaagggccctgacattggcagcggcaccagact
gcaggtgcagcccaatattcagaacccccgaccccgccgtgtaccagctgcgggacagcaagagcagcgacaagagcgt
gtgcctgttcaccgacttcgacagccagaccaacgtgtcccagagcaaggacagcgacgtgtacatcaccgacaagaccg
tgctggacatgcggagcatggacttcaagagcaacagcgccgtggcctggtccaacaagagcgacttcgctgcgccaac
gccttcaacaacagcatcatccccgaggacacctttttcccgagccccgagagcagctgcgacgtcaaactggtggagaag
tccttcgagacagacaccaacctgaactccagaacctgagcgtgatcggcttcagaatcctgctgctgaaggtggccggctt
caatctgctgatgacctgcggctgtggagcagcggcagccgggccaagagaagcggatccggcgccaccaacttcagc
ctgctgaagcaggccggcgacgtggaggaaaacctgcccctaggtgtctctgggcctgctgtgctgtggcgtgttctccct
gctgtgggcccggacctgtgaatgccggcgtgaccagaccccccaagtccgggtgtgaaaaccggccagagcatgaca
ctgctgtgcgcccaggacatgaaccacgagtacatgtattggtacagacaggaccccgcatgggcctgcggctgatccac
tattctgtgggcgagggcaccaccgccaagggcgaagtgcctgatggctacaacgtgtcccggctgaagaagcagaacttc
ctgctgggcttgaaagcgccgctcctagccagaccagcgtgtacttctgcgccagcagcttcaccgacaccagctacttcg
gccctggcaccagactgaccgtgctggaggacctgaagaacgtgttccccagaggtggccgtgttcgagccctctgagg
ccgagatcagccacaccagaaagccacctggtctgcctggccaccggcttctaccccgaccacgtggaactgtcttggtg
ggtgaacggcaaagaggtgcacagcggcgtcagcaccgacctcagccccctgaaagagcagcccgcctgaacgaca
gccggtactgcctgagcagcagactgcgggtgtccgccaccttctggcagaacccccggaaccacttcagatgccagggtgc
agtctacggcctgagcgagaacgacgagtggaccagagaccgggccaagcctgtgaccagatcgtgtctgccgaagc
atgggggcgcgccgattgcggcttcacaagcgagagctaccagcagggcgtgctgagcgccaccatcctgtacgagatcc
tgctgggcaaggccacctgtacgcgtgctggtgtccgctctggtgctgatggccatggtgaaacggaaggacagccggg
gctaataagtcgac

Figure 8

Amino acid sequence of the parental MAGE A10 TCR for T-cell transduction produced from the DNA sequence of Figure 7. The Porcine teschovirus-1 2A sequence is bold and underlined.

SEQ ID NO: 19

```
MMKSLRVLLV ILWLQLSWVW SQQKEVEQNS GPLSVPEGAI ASLNCTYSDR 50
GSQSFFWYRQ YSGKSPELIM SIYSNGDKED GRFTAQLNKA SQYVSLIIRD 100
SQPSDSATYL CAVRGTGRRA LTFGSGTRLQ VQPNIQNPDP AVYQLRDSKS 150
SDKSVCLFTD FDSQTNVSQS KDSDEVYITDK TVLDMRSMDF KSNSAVAWSN 200
KSDFACANAF NNSIIPEDTF FPSPESSCDV KLVEKSFETD TNLNFQNLSV 250
IGFRILLLLKV AGFNLLMTLR LWSSGSRAGR SGSGATNFSL LKQAGDVEEN 300
PGPRMSLGLL CCGVFSLWA GPVNAGVTQT PKFRVLKTGQ SMTLLCAQDM 350
NHEYMYWYRQ DPGMGLRLIH YSVGEGTTAK GEVPDGYNVS RLKKQNFLLG 400
LESAAPSQTS VYFCASSFTD TQYFGPGTRL TVLEDLKNVF PPEVAVFEPS 450
EAEISHTQKA TLVCLATGFY PDHVELSWWV NGKEVHSGVS TDPQPLKEQP 500
ALNDSRYCLS SRLRVSATFW QNPRNHFRQ VQFYGLSEND EWTQDRAKPV 550
TQIVSAEAWG RADCGFTSES YQQGVLSATI LYEILLGKAT LYAVLVSAIV 600
LMAMVKKRDS RG 612
```

Figure 9

Activation of MAGE-A10 TCR engineered T-cells (IFN γ production)