

Figures

Figure 1: IFN- γ production from CD4+CD25- T cells

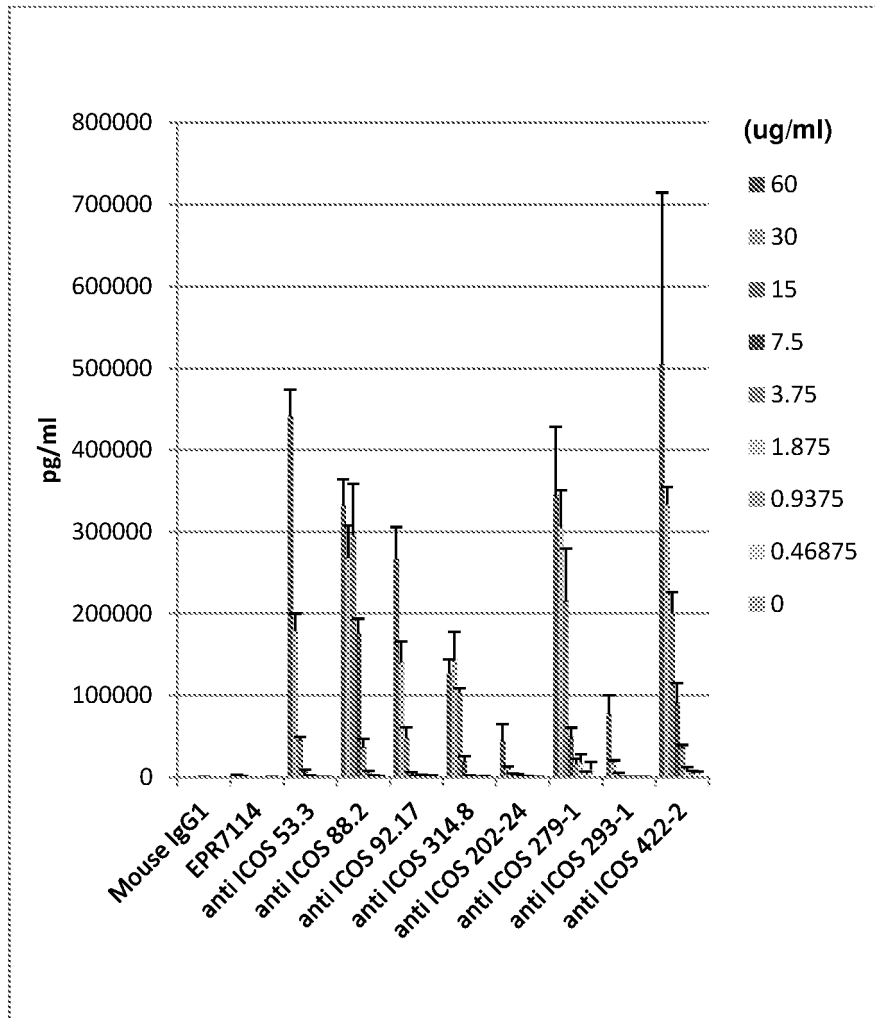


Figure 2: Proliferation in CD4+CD25- T cells

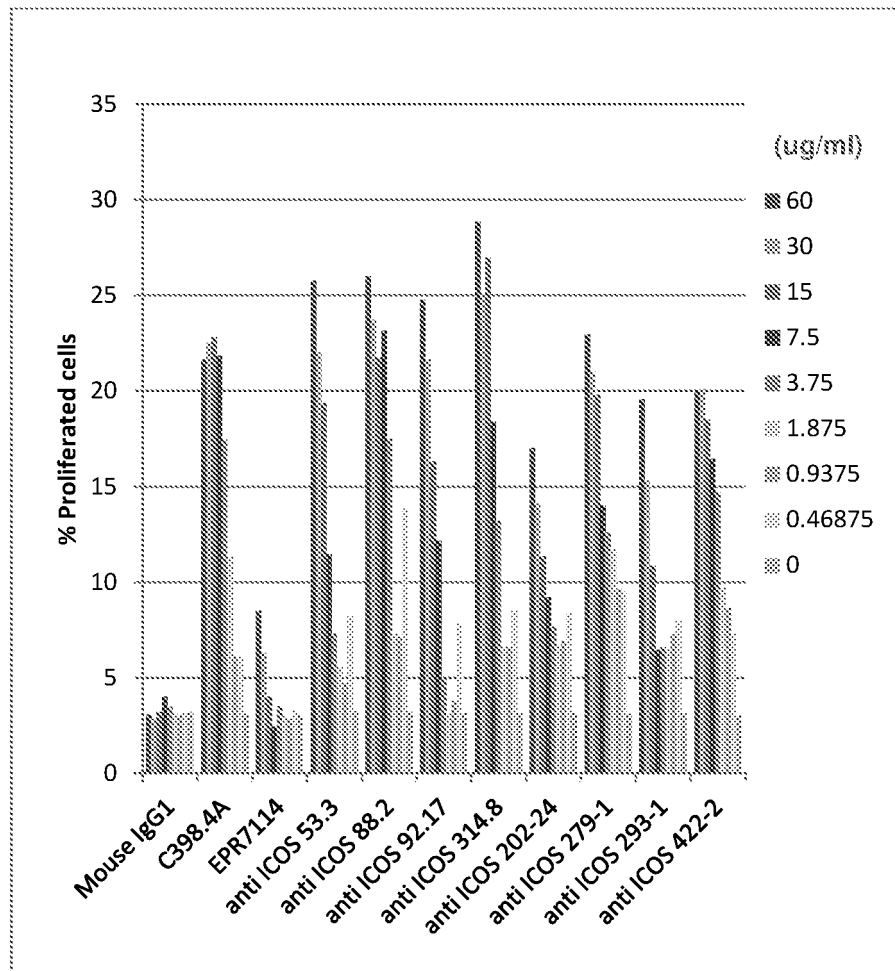
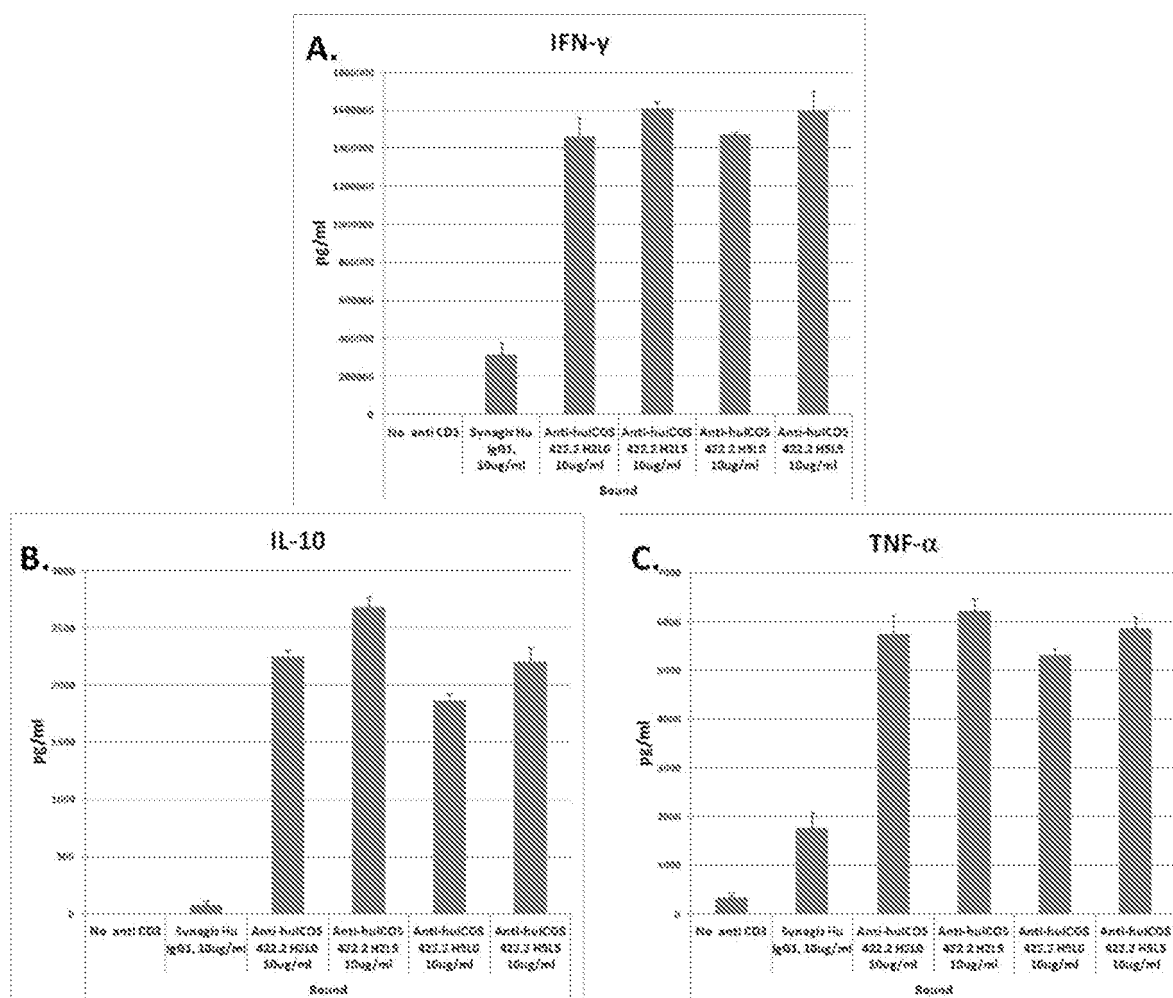
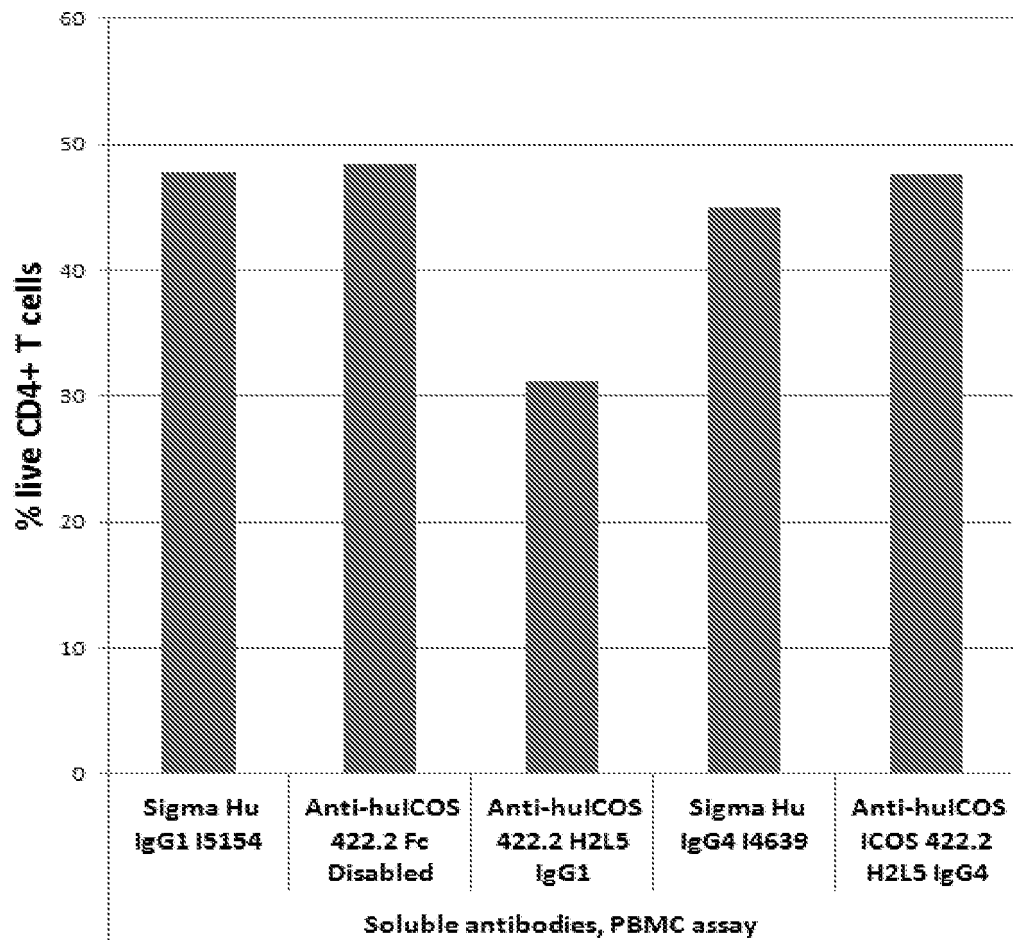


Figure 3: H2L5 humanized variant of anti-ICOS 422.2 shows better cytokine production in PBMC cells



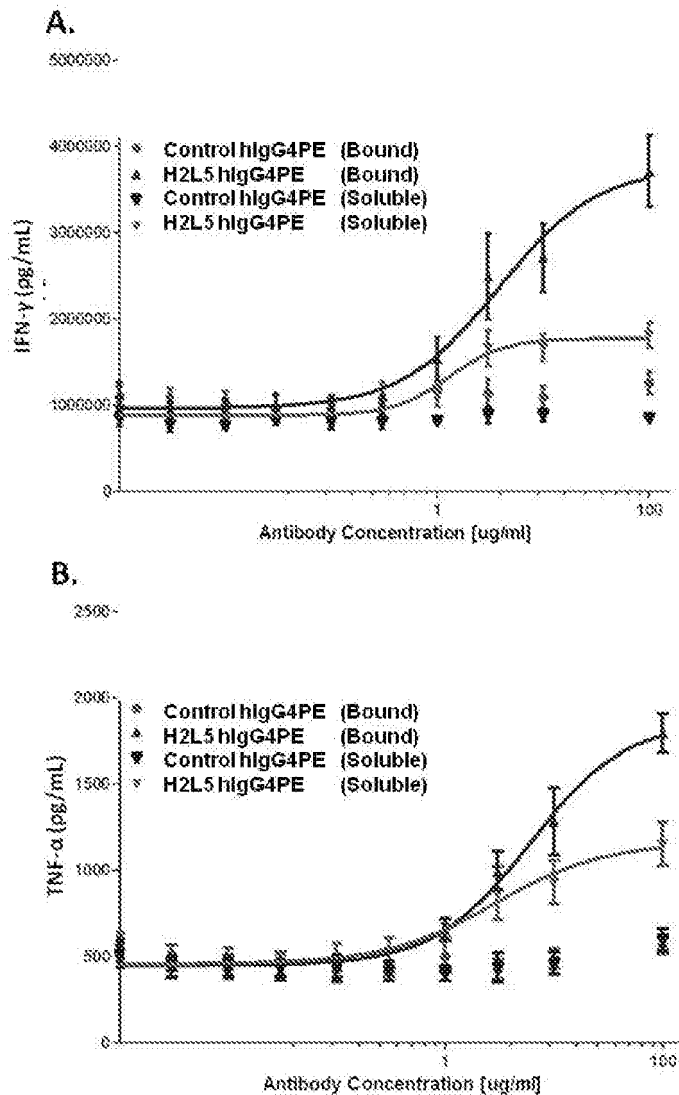
Human PBMCs were freshly isolated from healthy human donor and activated on plates pre-coated with anti-CD3 (OKT3) and each of the four soluble anti ICOS 422.2 humanized variants. After treatment for 2.5 days, the amounts of IFN- γ (A), IL-10 (B) and TNF- α (C) in supernatant were measured by MSD.

Figure 4: 422 H2L5 IgG1 induced decreased T cell viability which was not apparent with Fc-disabled or hIgG4PE isotypes.



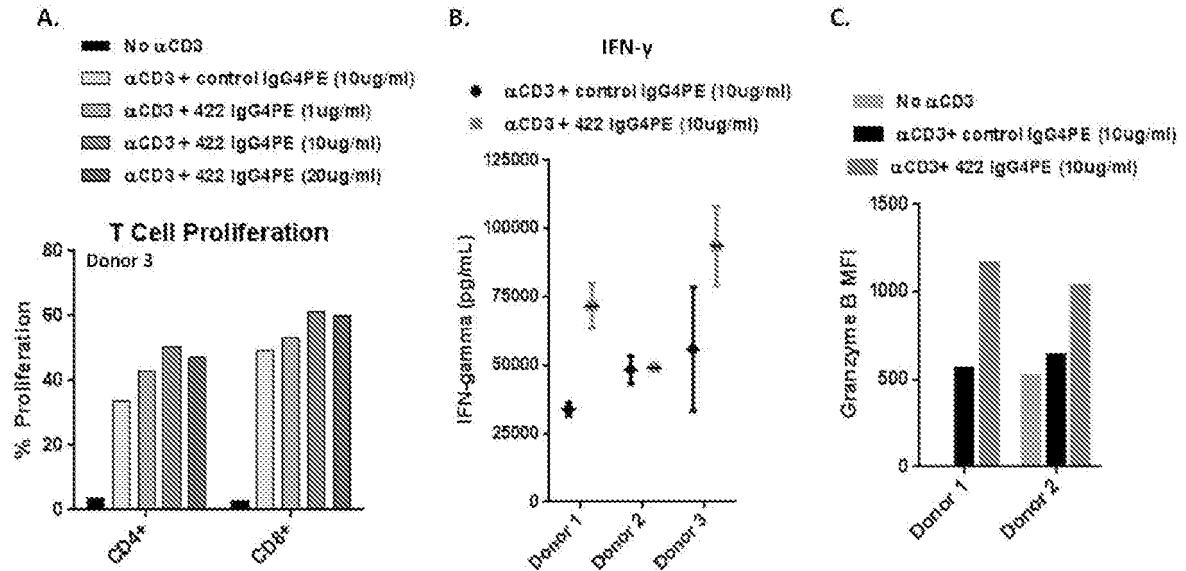
Human PBMCs were freshly activated on plates pre-coated with anti-CD3 (OKT3) and soluble humanized anti ICOS 422.2 with different Fc isotype. After treatment for 2.5 days, the percentages of live CD4+ T cells were measured by Flow cytometry.

Figure 5: Dose response of H2L5 hIgG4PE induced proinflammatory cytokine induction in human CD4+ T cells.



Human healthy donor CD4+ T cells pre-stimulated with anti-CD3/CD28 for 2 days were re-stimulated by anti-CD3 (OKT3) plus serial concentrations of isotype control (Synagis IgG4PE) or H2L5 hIgG4PE mAb as plate bound or soluble format. The concentration of **(A)** IFN- γ and **(B)** TNF- α was measured in the supernatants by MSD after 3.5 days of treatment.

Figure 6: H2L5 hIgG4PE induces proliferation, cytokine production and increased cytotoxic potential in activated PBMCs from healthy human donors



Human PBMC cells pre-stimulated with anti-CD3/CD28 for 2 days were re-stimulated by anti-CD3 (OKT3) plus soluble H2L5 hIgG4PE mAb or isotype control. After 2.5 days of treatment, T cell proliferation was measured by CFSE FACS assay (A). IFN- γ concentration in the supernatants was measured by MSD (B). Intracellular Granzyme B expression of CD4+ T cells was measured by flow cytometry.

Figure 7: Meso Scale Discovery (MSD) assay showing the inhibition of ICOS-L binding to ICOS by H2L5 hIgG4PE, indicating that it binds to the same epitope on ICOS as ICOS-L and competes for binding

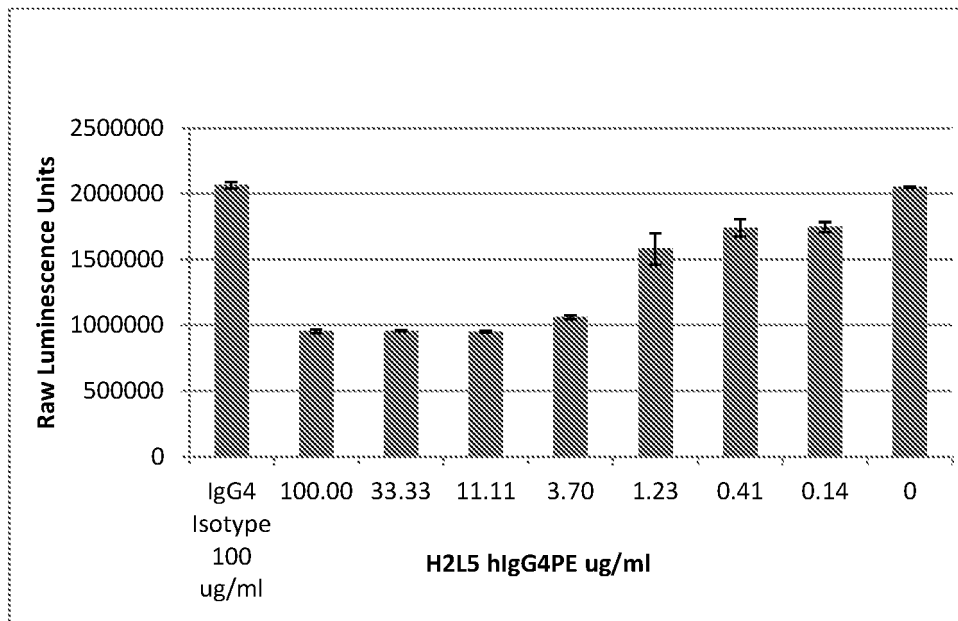


Figure 8: Recovered antibody V_H and V_L genes from RNA of hybridoma clone 422.2

422 HC

QVQLQQSGPELVRPGESVKISCMGSGYTFTDYAMHWVKQSHAKSLEWIGLISIYSDHTNYNQKFQ GKATMTVDKS
SSTAYMELARLTSEDSAIYYCGRNNYGNYGWYFDVWGAGTTVTVSS (SEQ ID NO:19)

422 LC

ENVLTQSPAIMASAPGEKVTMTCSASSSVSYMHWYQQKSITSPKLWIYDTSKLGVPGRFSGSGSGNSYSLTIS
SMEAEDVATYYCFQGS GYPYTFGGGTKLEIKR (SEQ ID NO:20)

Figure 9: Protein sequences of heavy and light chains of H2L5 hIgG4PE with signal sequence

Heavy chain

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYAMHWVRQAPGQGLEWMGLISIIYSD
HTNYNQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCGRNNYGNYGWYFDVWGQTTTVTVSSASTKGPSVFP
 LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTKTYTCNV
 DHKPSNTKVKDKRVESKYGPPCPPEPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVD
 GVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIISKAKGQPREPQVYTLPPSQ
 EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
 ALHNHYTQKSLSLGLK (SEQ ID NO:9)

Light chain

MGWSCIILFLVATATGVHSEIVLTQSPATLSLSPGERATLSCSASSSVSYMHWYQQKPGQAPRLLIYDTSKLASG
IPARFSGSGSGTDYTLTISSELPEDFAVYYCFQSGSYPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV
 VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
 SFNRGEC (SEQ ID NO:12)

Signal Sequence is double underlined

CDR sequences are underlined

S228P, L235E (EU numbering) Substitutions in IgG4

Murine residues incorporated in variable heavy chain framework (Kabat numbering G27Y, S30T, A93G)

Murine residue incorporated in variable light chain framework (Kabat numbering F71Y)

Figure 10: DNA sequence of coding region of H2L5 hIgG4PE heavy chain with signal sequence

Heavy Chain

ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCCACCGCCACCGGCGTGACAGCCAGGTGCAGCTGGTGCAG
AGCGGAGCCGAGGTGAAAAAGCCCGGCTCAAGCGTGAAGGTGAGCTGCAAGGCCAGCGGCTACACCTTACCGAC
TACGCTATGCACTGGGTGAGGCAGGCCCCCGGCCAGGGCCTGGAGTGGATGGGCCTGATCAGCATCTACAGCGAC
CACACCAACTACAACCAGAAGTTCCAGGGCAGGGTGACCATCACCGCCGATAAGAGCACCAGCACAGCCTACATG
GAGCTGAGCAGCCTGAGGAGCGAAGACACCGCCGTGTACTATTGCGGCAGGAACAACCTACGGCAACTACGGCTGG
TACTTCGACGTGTGGGGCCAGGGAACCACTGTCACCGTGAGCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCCC
CTGGCCCCCTGCAGCAGAAGCACAGCGAGAGCACAGCCGCCCTGGGCTGCCCTGGTGAAGGACTACTTCCCCGAG
CCCGTGACCGTGAGCTGGAACAGCGGAGCCCTGACCAGCGGCGTGACACCTTCCCCGCCGTGCTGCAGAGCAGC
GGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCAGCAGCAGCCTGGGCACCAAGACCTACACCTGCAACGTG
GACCACAAGCCCAGCAACACCAAGGTGGACAAGCGGGTGGAGAGCAAGTACGGCCCTCCCTGCCCCCCTGCCCT
GCCCCCGAGTTTCGAGGGCGGACCCCTCCGTGTTCCCTGTTCCCCCACAAGCCAAGGACACCCTGATGATCAGCCGG
ACCCCCGAGGTGACCTGCGTGGTGGTGACGTGAGCCAGGAAGATCCCGAGGTCCAGTTCAATTGGTACGTGGAC
GGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAACAGTTCAACAGCACCTACCGGGTGGTGTCCGTG
CTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGTCCAACAAGGGCCTGCCCAGC
TCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCATCCCAG
GAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAG
TGGGAGAGCAACGGCCAGCCCCGAGAACAACCTACAAGACCACCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTC
CTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACGTCTTTAGCTGCAGCGTGATGCACGAG
GCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAG (SEQ ID NO:21)

Leader sequence indicated by double underline

Figure 11: DNA sequence of coding region of H2L5 hIg4PE light chain with signal sequence

Light Chain

ATGGGCTGGTCTCTGCATCATCCTGTTTCTGGTGGCCACCGCCACCGGCGTGACACAGCGAGATTGTGCTGACCCAG
AGCCCCGCCACCCTGAGCCTGAGCCCCGGCGAAAGGGCAACCCTCAGCTGCAGCGCCAGCAGCAGCGTGAGCTAC
ATGCACTGGTACCAGCAGAAGCCCCGGCCAGGCCCCCTAGGCTGCTGATCTACGACACCTCCAAGCTGGCCAGCGGC
ATCCCAGCCAGGTTCTCAGGCAGCGGCAGCGGCACCGACTATACTCTGACCATCAGCAGCCTGGAGCCCCGAGGAC
TTCGCCGTGTACTACTGCTTCCAGGGAAGCGGCTACCCCTACACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG
CGTACGGTGGCCGCCCCCAGCGTGTTTCATCTTCCCCCCCAGCGATGAGCAGCTGAAGAGCGGCACCGCCAGCGTG
GTGTGTCTGCTGAACAACCTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGC
AACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGC
AAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGTGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAG
AGCTTCAACCGGGGCGAGTGC (SEQ ID NO:22)

Leader sequence indicated by double underline

12/25

Figure 12: Plasma concentrations of H2L5 hIgG4PE in cynomolgus monkeys. Concentrations were determined after the (A) first or (B) second dose (day 15) of H2L5 hIgG4PE. Animals were sacrificed 48 hours post second dose for tissue sample collection and histopath analysis

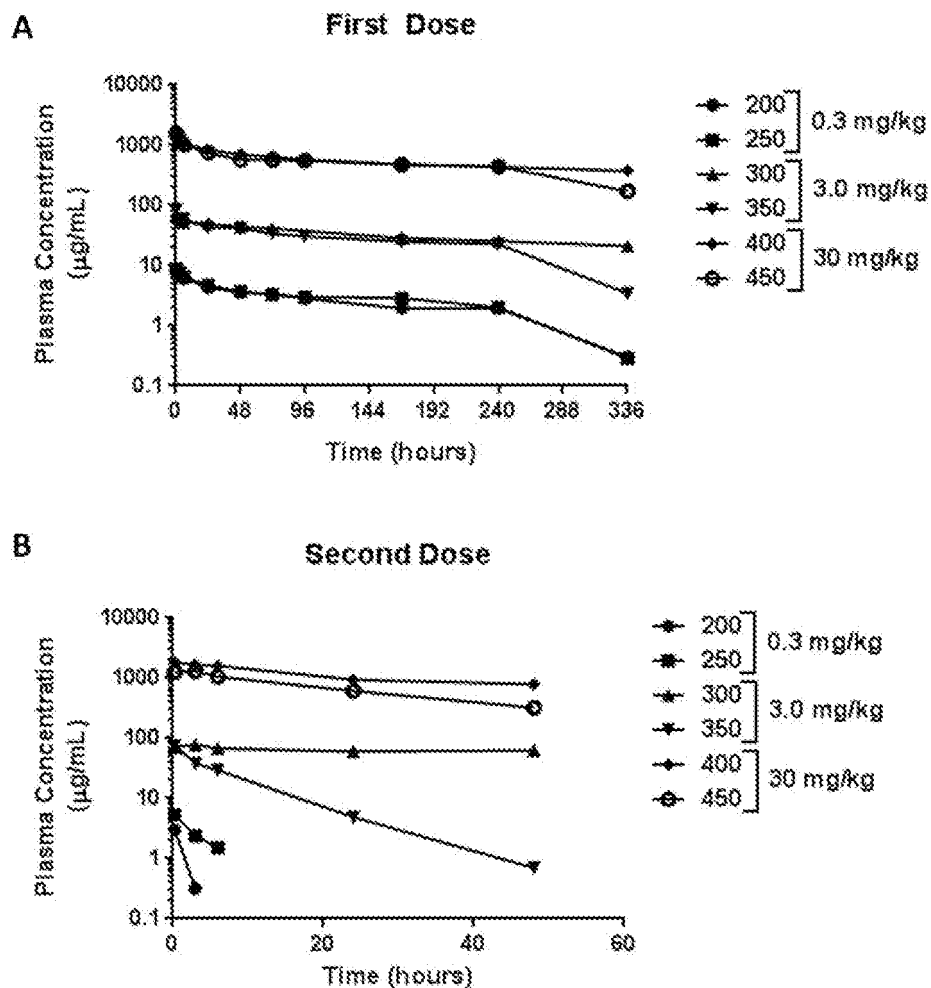


Figure 13: Detection of H2L5 hIgG4PE binding to CD4⁺ T cells from the spleen and axillary lymph nodes of monkeys. Tissue was collected 48 hours post-second dose (Day 17).

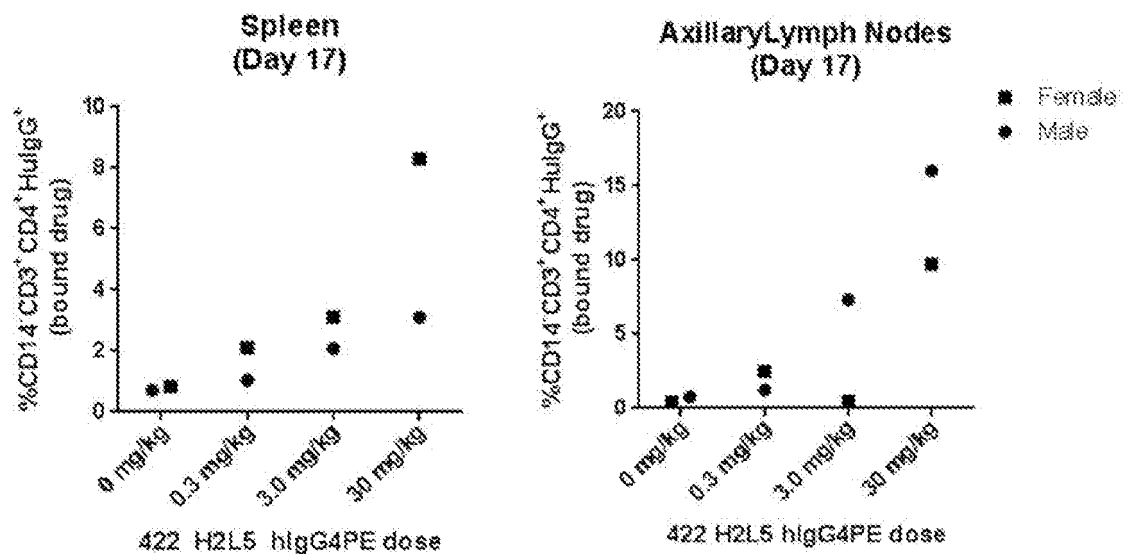


Figure 14: Receptor Occupancy of H2L5 hIgG4PE in blood CD4⁺ T cells from cyno monkeys.

(A) ICOS “free receptor” as measured by positive binding of the anti-ICOS fluorescently labeled antibody used for flow cytometry, which binds only when H2L5 hIgG4PE is not present.

(B) Receptor bound H2L5 hIgG4PE on peripheral blood CD4⁺ cells as measured by fluorescently labeled anti-Human IgG.

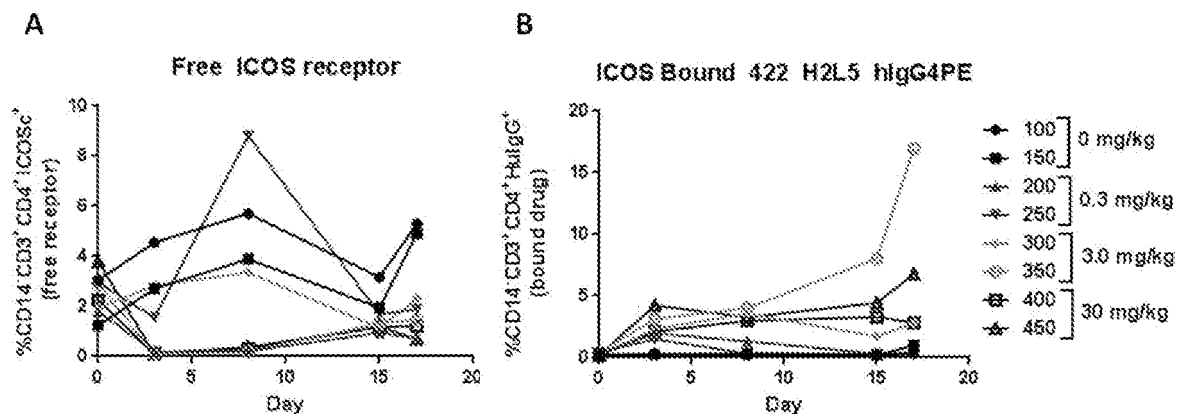


Figure 15(a) Phospho-AKT (T308) expression levels in Ba/F3-ICOS cells treated with H2L5 hIgG4PE - Intracellular signalling antibody array

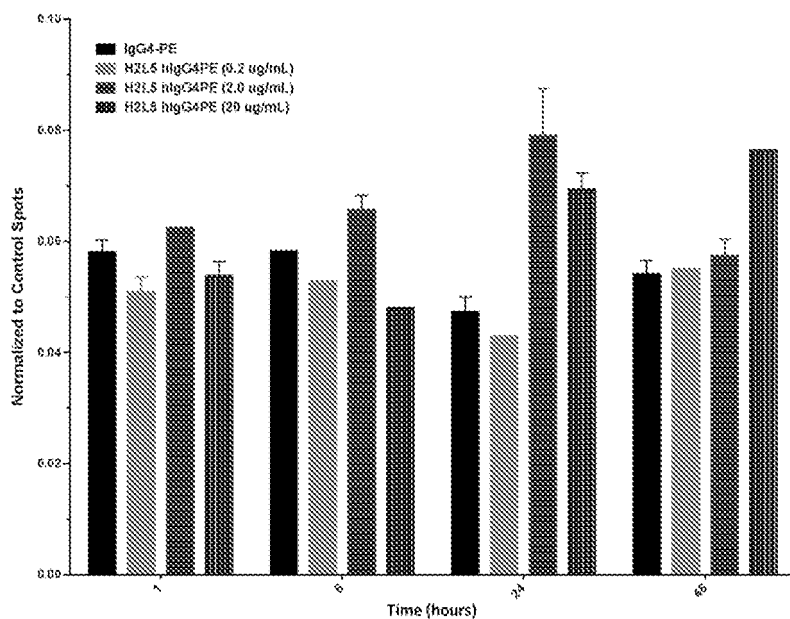


Figure 15 (b) Phospho-AKT (S473) expression levels in Ba/F3-ICOS cells treated with H2L5 hIgG4PE - Intracellular signalling antibody array

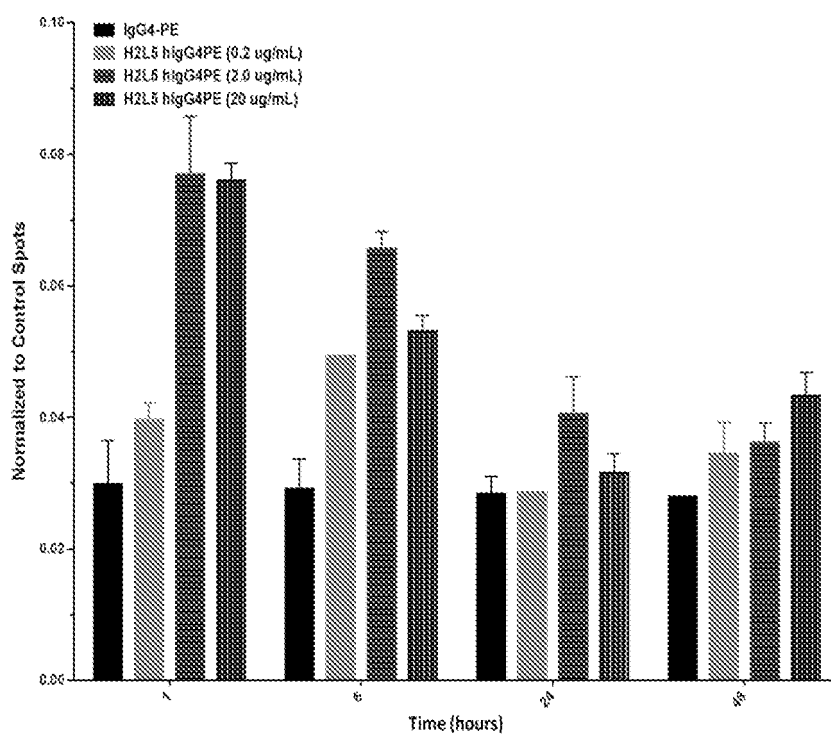


Figure 16: H2L5 hIgG4PE in combination with ipilimumab results increased proinflammatory cytokine production as compared to single antibody treatment in PBMC pre-stimulation assay

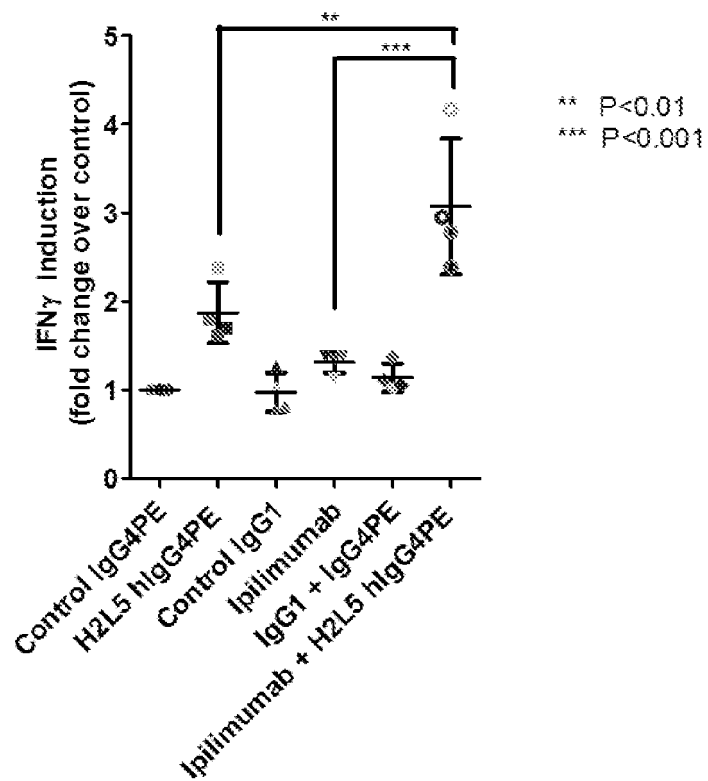


Figure 17: H2L5 hIgG4PE in combination with pembrolizumab results increased proinflammatory cytokine production as compared to single antibody treatment in PBMC pre-stimulation assay

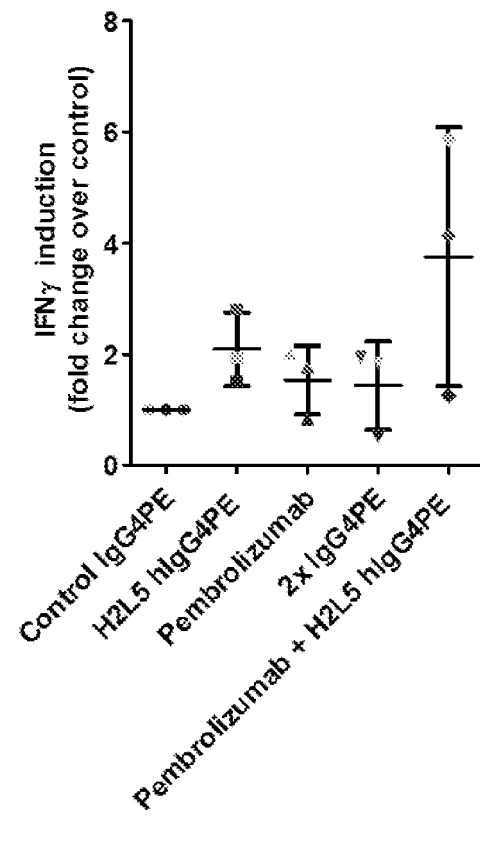


Figure 18 H2L5 hIgG4PE plus ipilimumab combination induces increased proinflammatory cytokine production in a modified MLR assay with CEFT peptide and pre-incubation

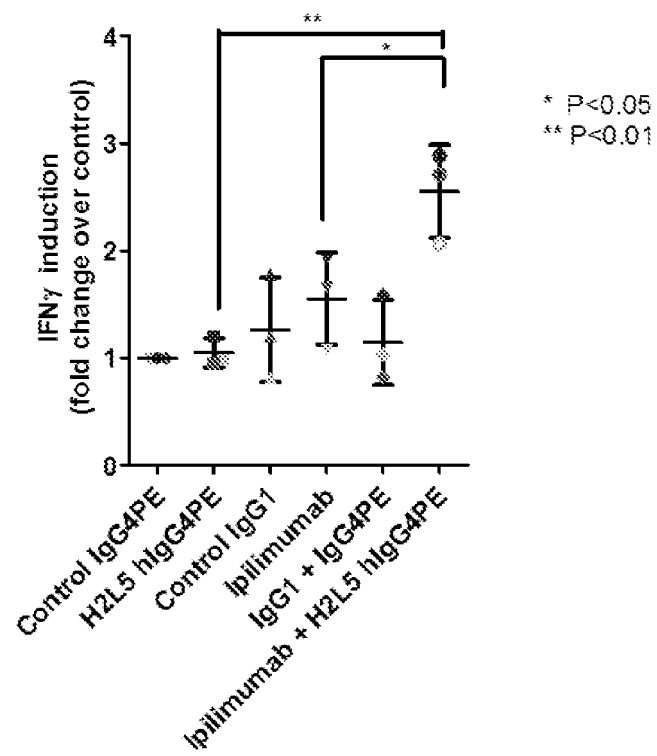


Figure 19 H2L5 hIgG4PE plus pembrolizumab combination induces increased proinflammatory cytokine production in a modified MLR assay with CEFT peptide and pre-incubation

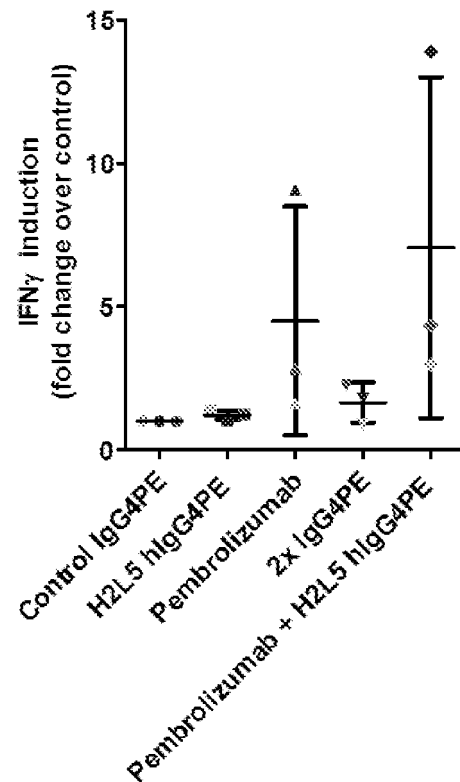


Figure 20: H2L5 hIgG4PE anti-ICOS agonist mAb alone and in combination with pembrolizumab results in tumor growth inhibition in a human PBMC A2058 Melanoma mouse tumor model

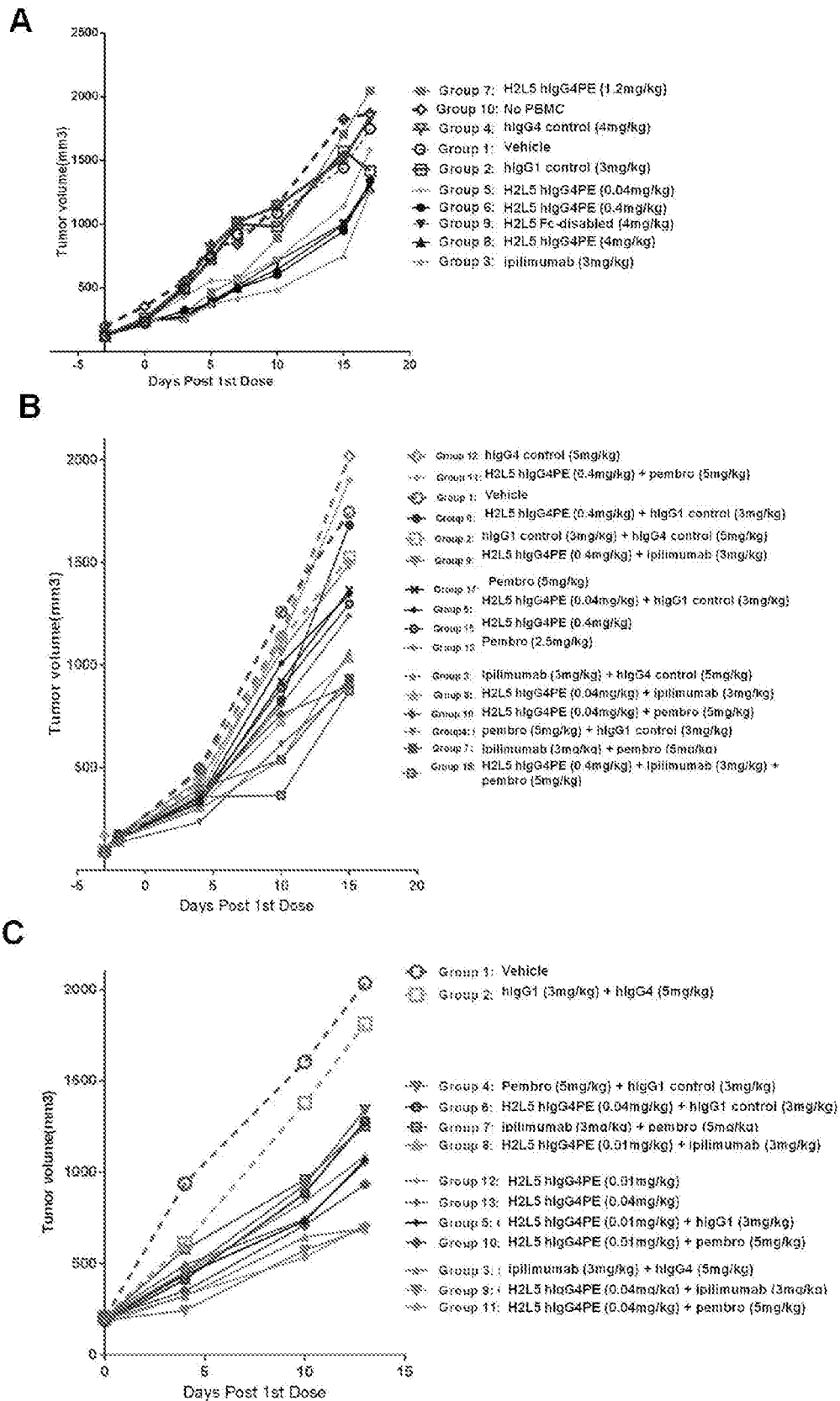


Figure 21: anti-ICOS murine surrogate mAb results in significant tumor growth inhibition and increased survival in combination with an anti-PD1 murine surrogate mAb in the CT26 mouse tumor model

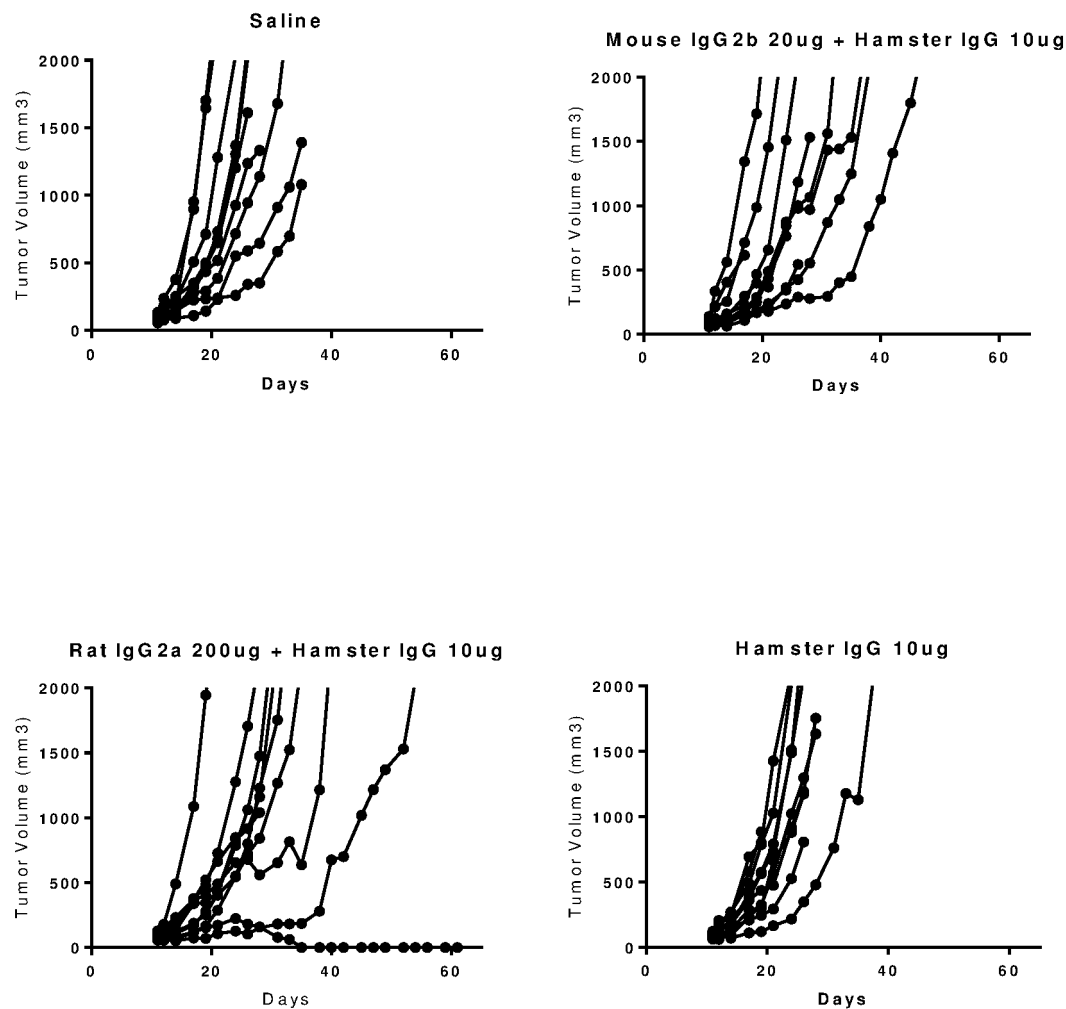


Figure 21 (Continued)

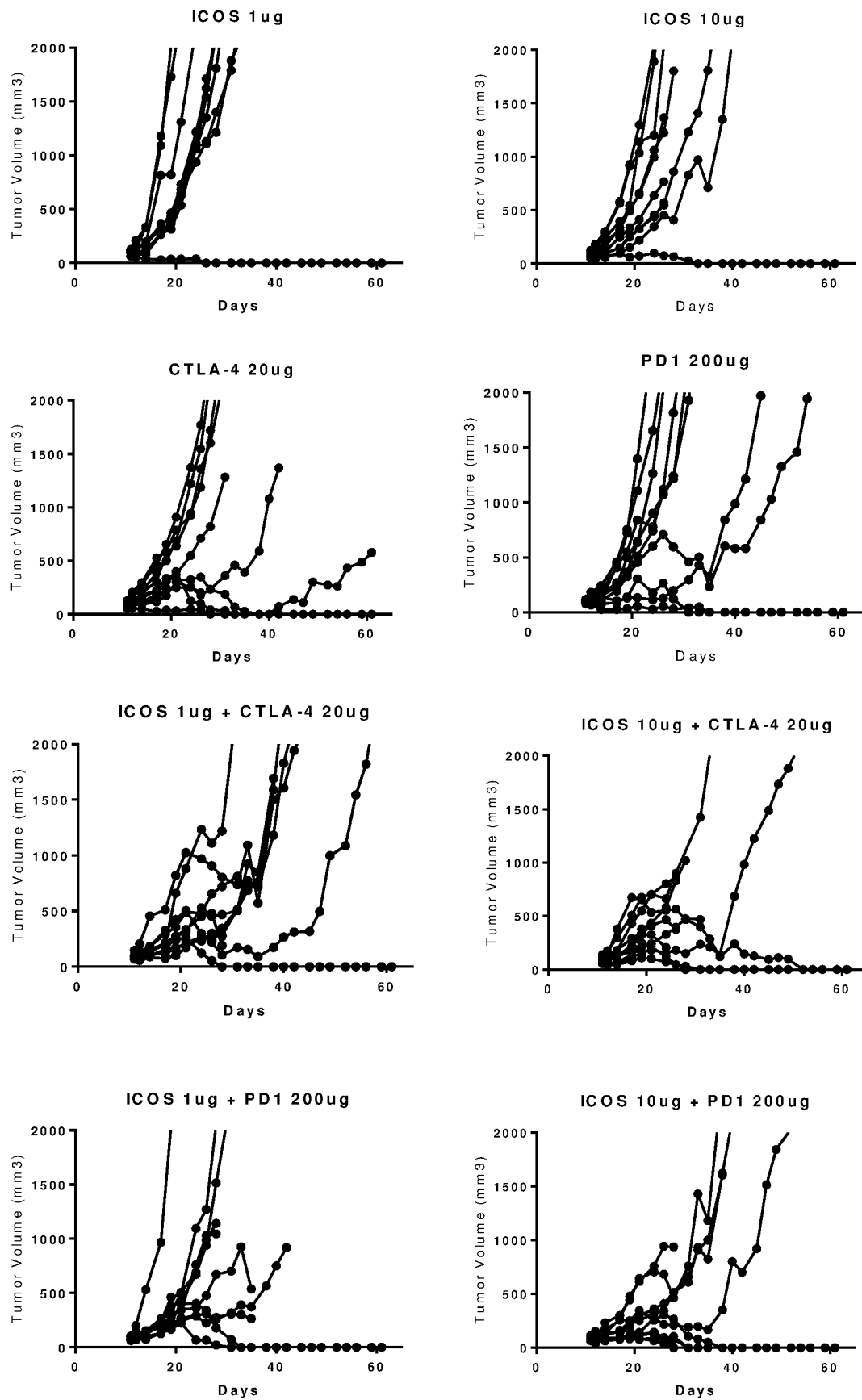


Figure 22: anti-ICOS murine surrogate mAb results in significant tumor growth inhibition and increased survival in combination with an anti-PD1 murine surrogate mAb in the EMT6 mouse tumor model

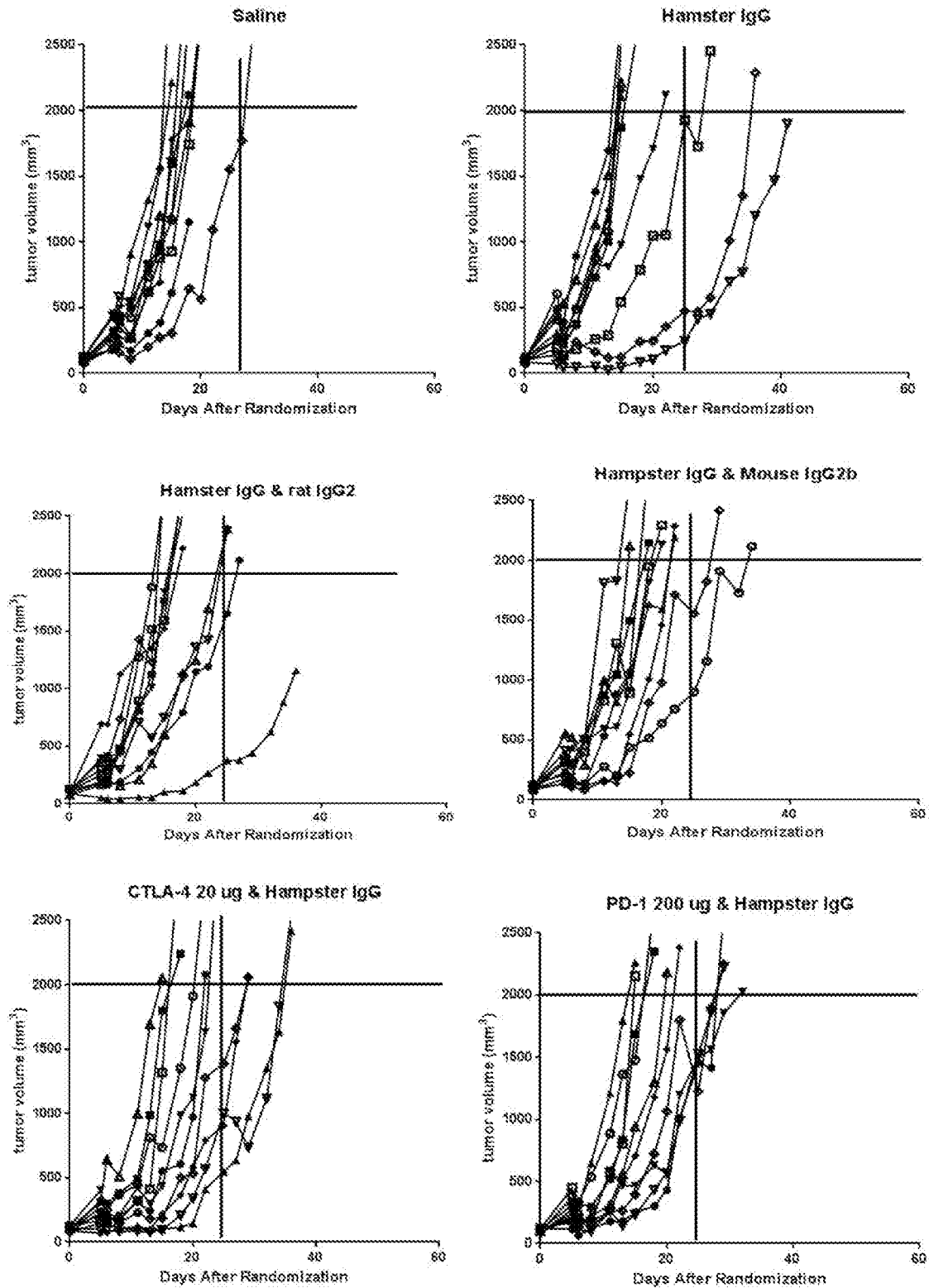


Figure 22 (continued)

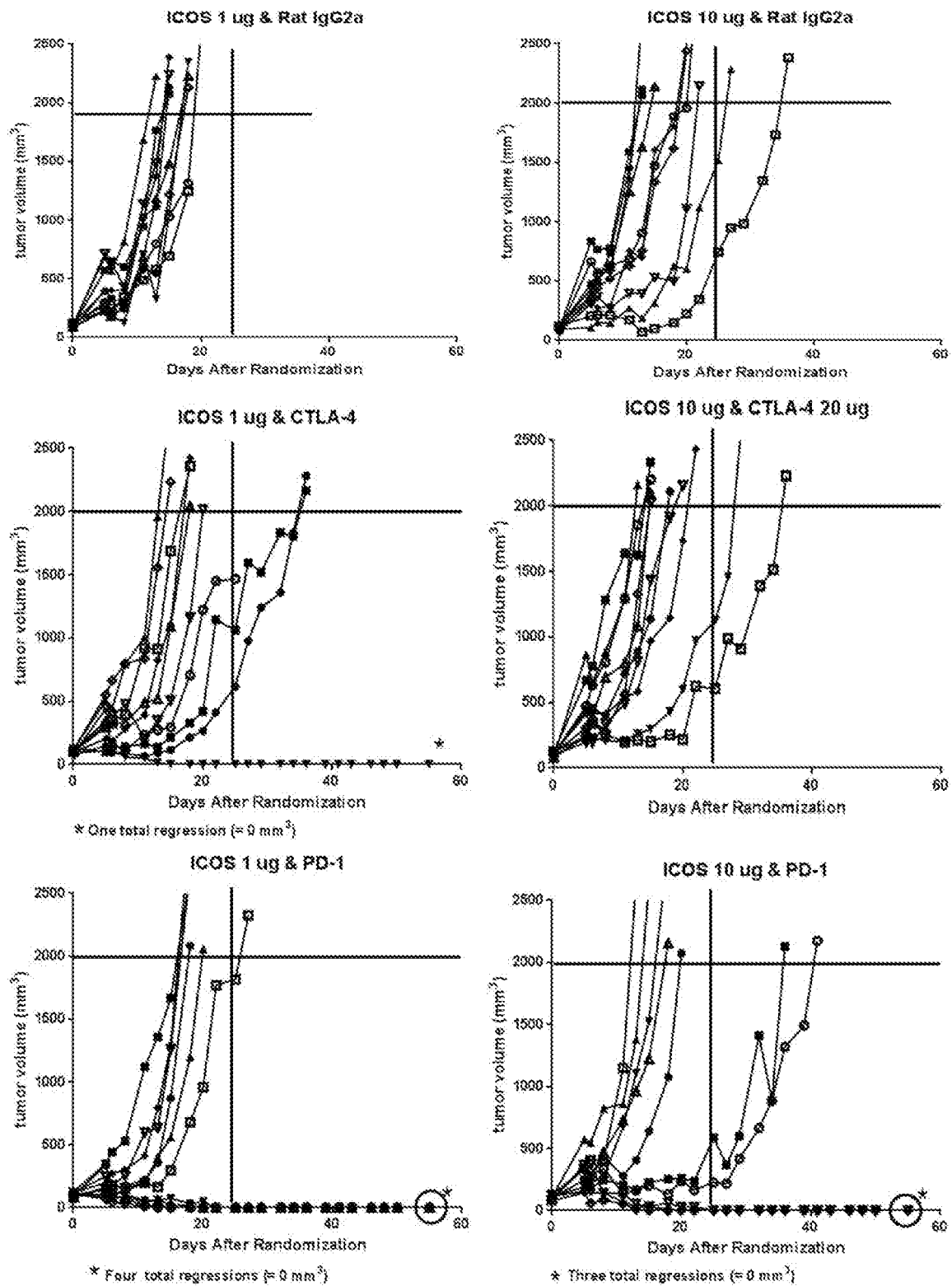


Figure 22 (continued)

