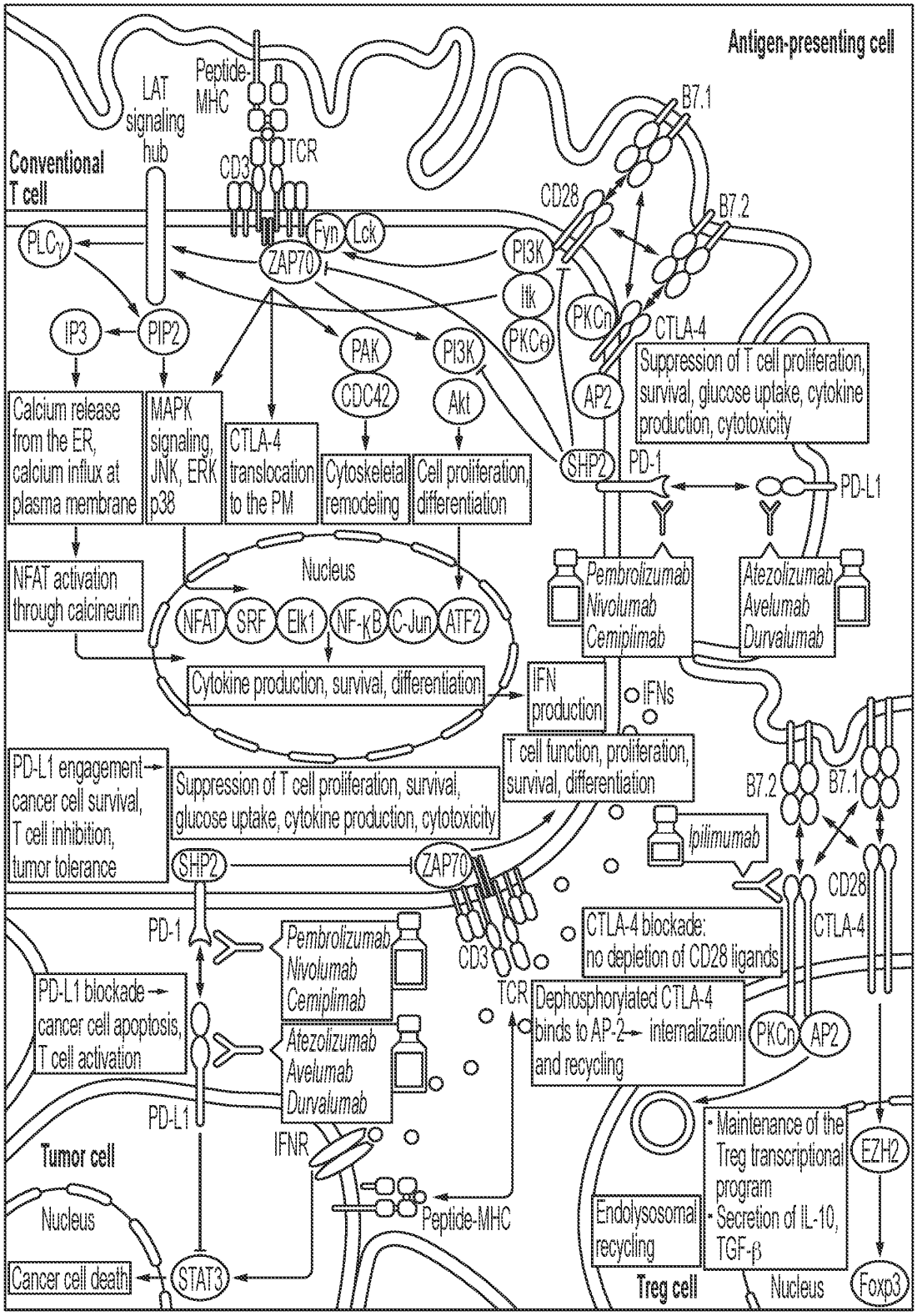

Antigen-presenting cell
Conventional T cell
LAT signaling hub
Peptide-MHC
TCR
CD3
B7.1
CD28
B7.2
PLCγ
Fyn
Lck
ZAP70
PI3K
Itk
PKCθ
PKCη
CTLA-4
IP3
PIP2
PAK
CDC42
PI3K
Akt
AP2
Suppression of T cell proliferation, survival, glucose uptake, cytokine production, cytotoxicity
Calcium release from the ER, calcium influx at plasma membrane
MAPK signaling, JNK, ERK p38
CTLA-4 translocation to the PM
Cytoskeletal remodeling
Cell proliferation, differentiation
SHP2
PD-1
PD-L1
Nucleus
NFAT activation through calcineurin
NFAT
SRF
Elk1
NF-κB
C-Jun
ATF2
Pembrolizumab Nivolumab Cemiplimab
Atezolizumab Avelumab Durvalumab
Cytokine production, survival, differentiation
IFN production
IFNs
T cell function, proliferation, survival, differentiation
PD-L1 engagement → cancer cell survival, T cell inhibition, tumor tolerance
Suppression of T cell proliferation, survival, glucose uptake, cytokine production, cytotoxicity
B7.2
B7.1
Ipilimumab
SHP2
ZAP70
CD28
CTLA-4
PD-1
Pembrolizumab Nivolumab Cemiplimab
CD3
CTLA-4 blockade: no depletion of CD28 ligands
PD-L1 blockade → cancer cell apoptosis, T cell activation
TCR
Dephosphorylated CTLA-4 binds to AP-2 → internalization and recycling
PKCη
AP2
Atezolizumab Avelumab Durvalumab
PD-L1
IFNR
Tumor cell
Maintenance of the Treg transcriptional program
Secretion of IL-10, TGF-β
EZH2
Peptide-MHC
Endolysosomal recycling
Nucleus
Cancer cell death
STAT3
Treg cell
Nucleus
Foxp3

FIG. 1

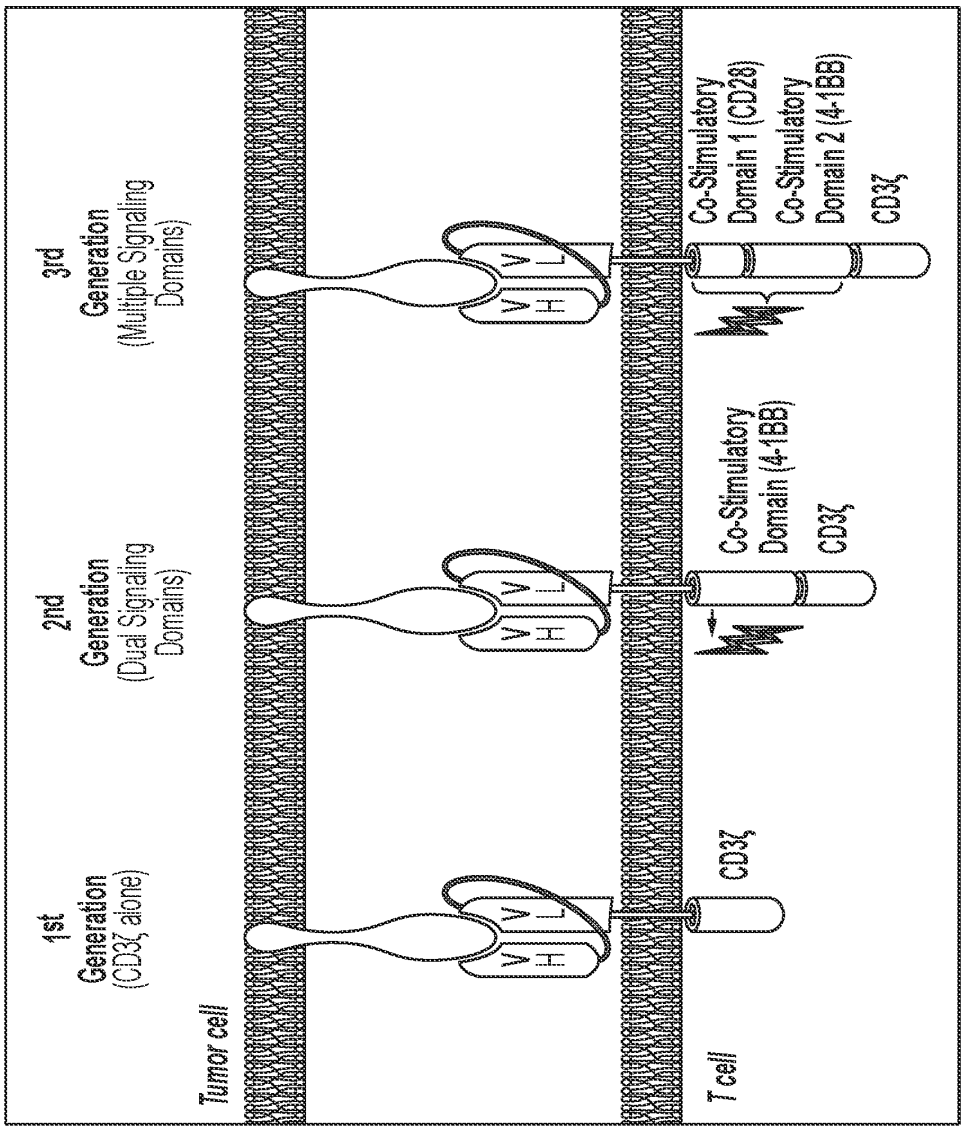

1st Generation (CD3ζ alone)
2nd Generation (Dual Signaling Domains)
3rd Generation (Multiple Signaling Domains)
Tumor cell
T cell
CD3ζ
Co-Stimulatory Domain (4-1BB)
CD3ζ
Co-Stimulatory Domain 1 (CD28)
Co-Stimulatory Domain 2 (4-1BB)
CD3ζ
V
V
H
L

FIG. 2

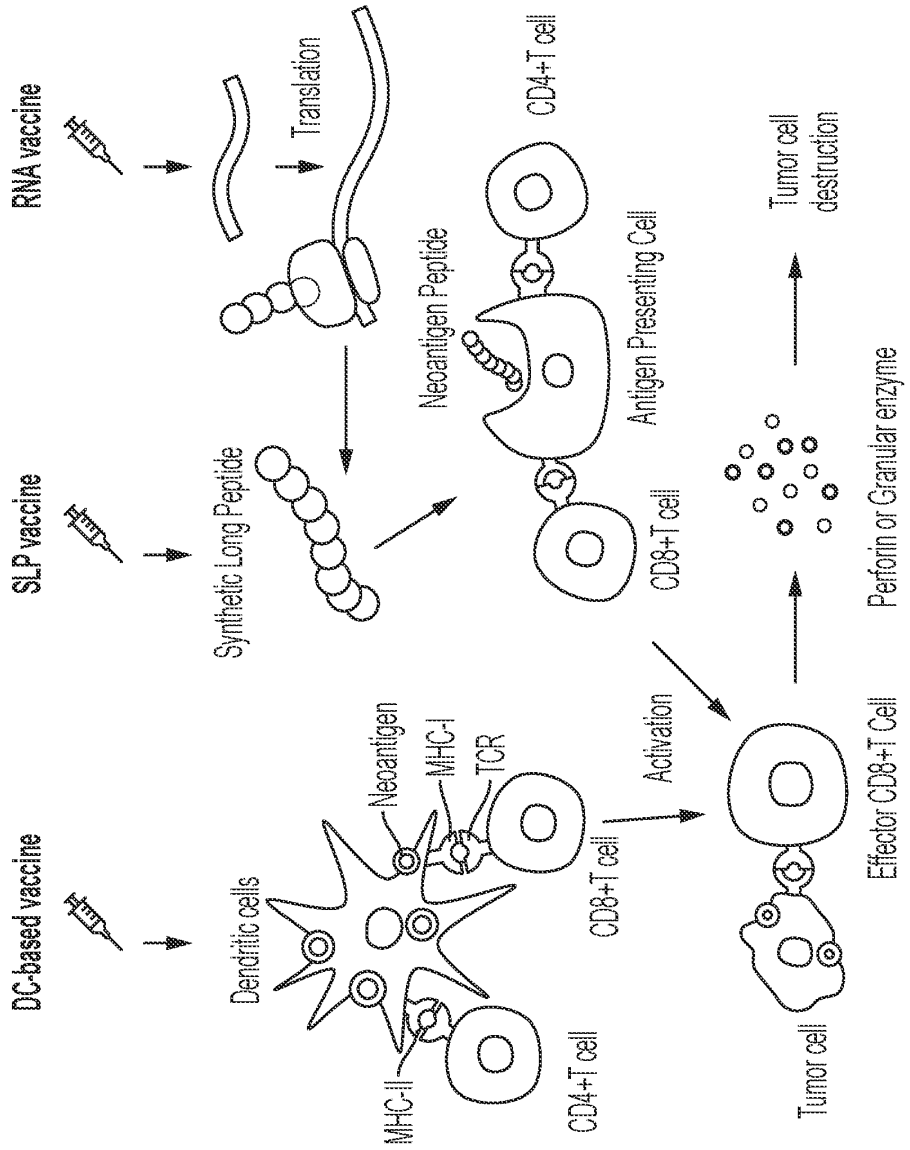

DC-based vaccine
SLP vaccine
RNA vaccine
Dendritic cells
Neoantigen
MHC-I
TCR
MHC-II
CD4+T cell
CD8+T cell
Activation
Synthetic Long Peptide
Translation
Neoantigen Peptide
CD4+T cell
Antigen Presenting Cell
CD8+T cell
Perforin or Granular enzyme
Tumor cell
Effector CD8+T Cell
Tumor cell destruction

FIG. 3

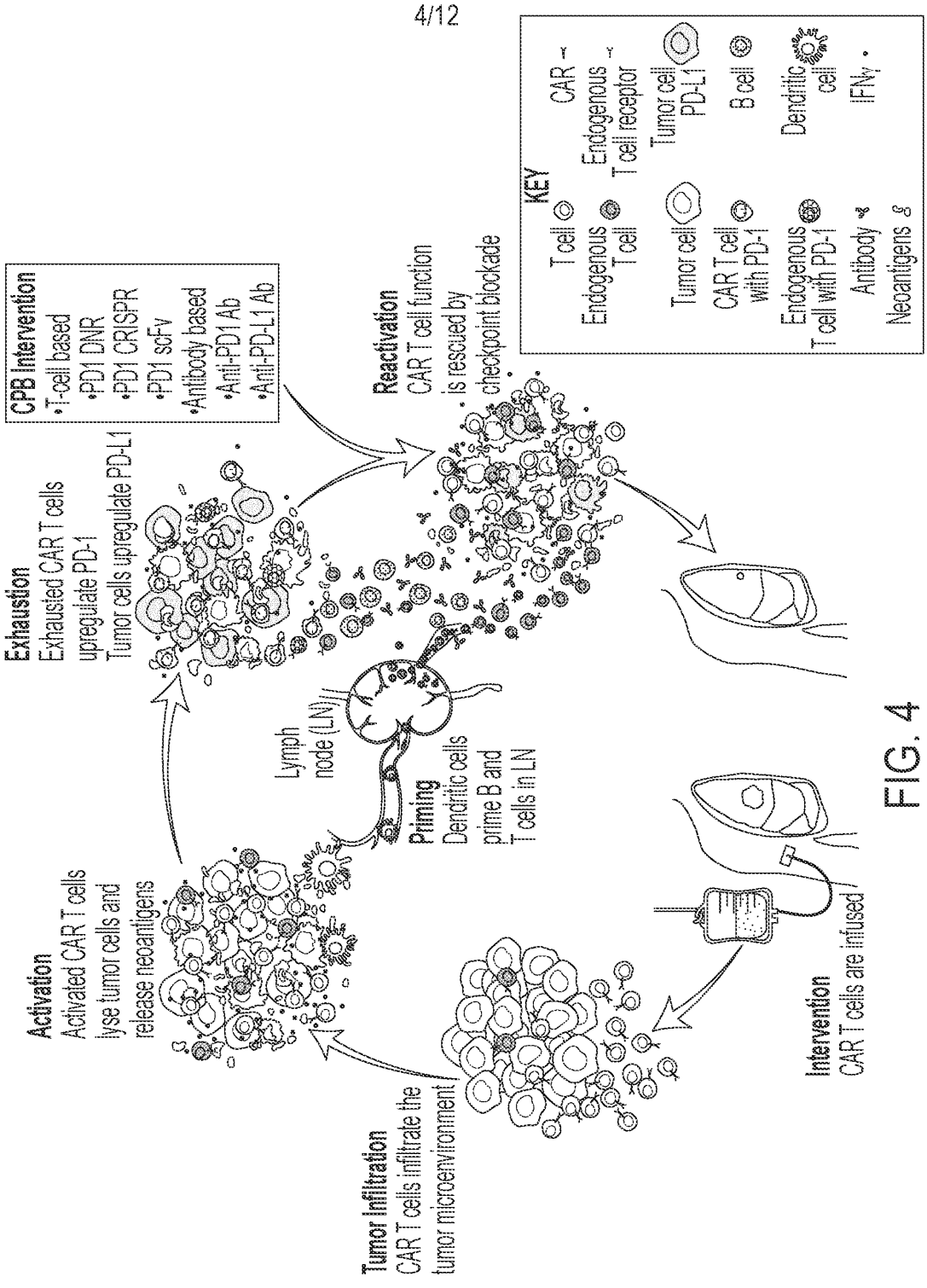

CPB Intervention
•T-cell based
•PD1 DNR
•PD1 CRISPR
•PD1 scFv
•Antibody based
•Anti-PD1 Ab
•Anti-PD-L1 Ab
Exhaustion
Exhausted CAR T cells upregulate PD-1
Tumor cells upregulate PD-L1
Reactivation
CAR T cell function is rescued by checkpoint blockade
KEY
T cell
Endogenous T cell
Tumor cell
CAR T cell with PD-1
Endogenous T cell with PD-1
Antibody
Neoantigens
CAR
Endogenous T cell receptor
Tumor cell PD-L1
B cell
Dendritic cell
IFNγ
Lymph node (LN)
Priming
Dendritic cells prime B and T cells in LN
Activation
Activated CAR T cells lyse tumor cells and release neoantigens
Tumor Infiltration
CAR T cells infiltrate the tumor microenvironment
Intervention
CAR T cells are infused

FIG. 4

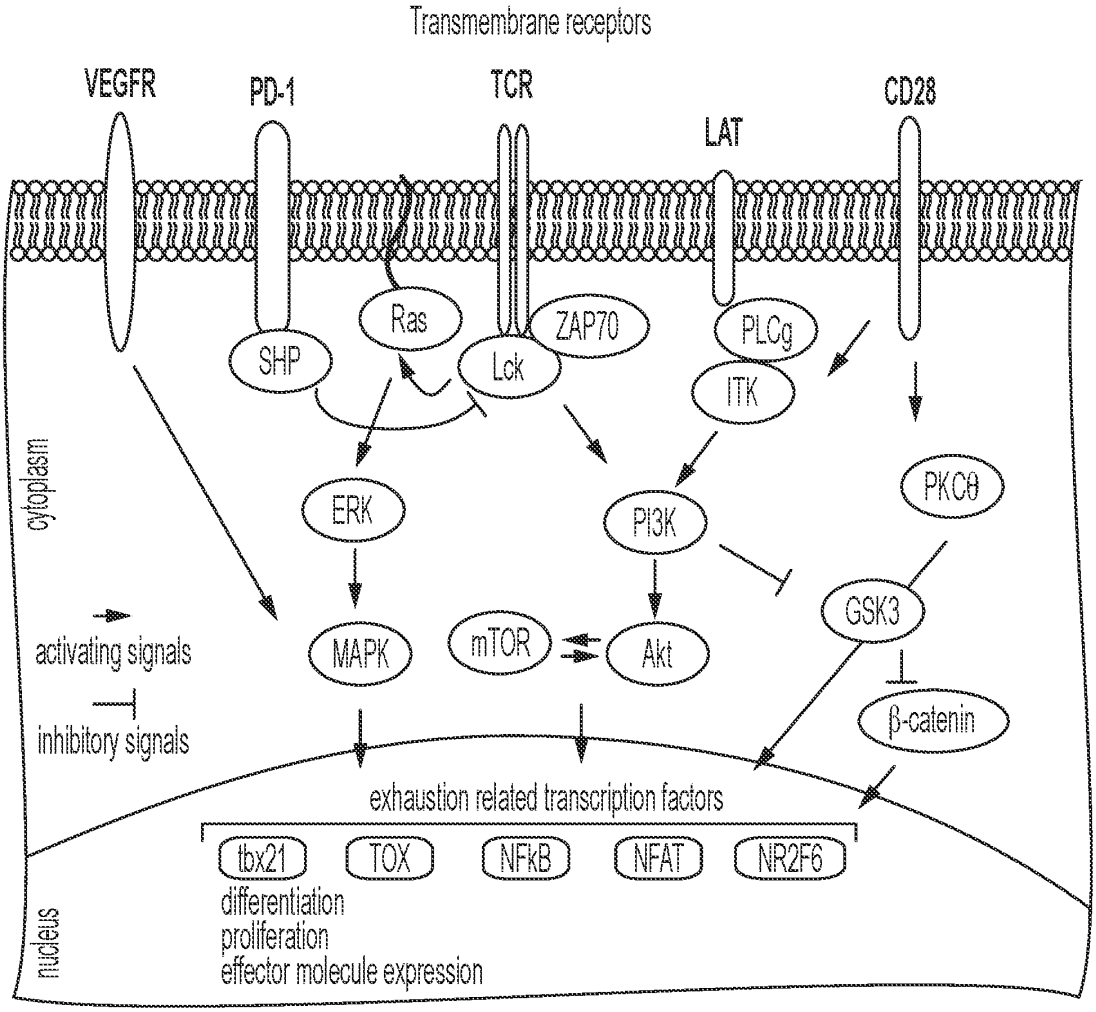

Transmembrane receptors
VEGFR
PD-1
TCR
LAT
CD28
Ras
ZAP70
PLCg
SHP
Lck
ITK
cytoplasm
ERK
PI3K
PKCθ
activating signals
MAPK
mTOR
Akt
GSK3
inhibitory signals
β-catenin
exhaustion related transcription factors
tbx21
TOX
NFkB
NFAT
NR2F6
differentiation
proliferation
effector molecule expression
nucleus

FIG. 5

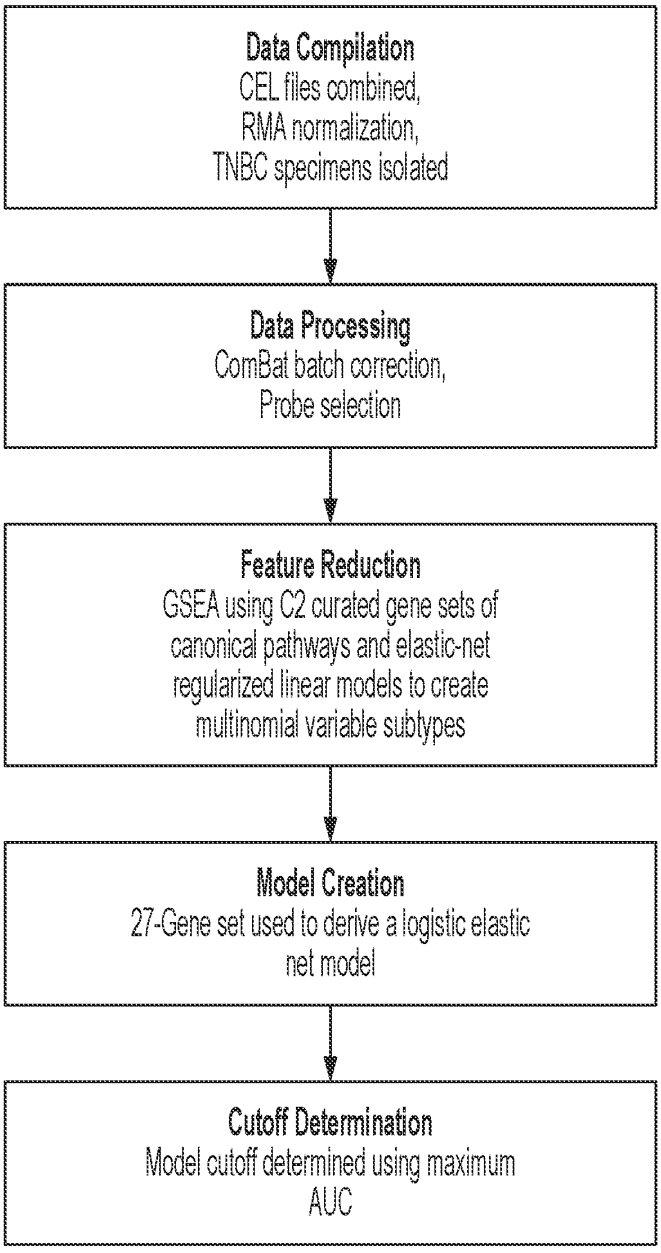

Data Compilation
CEL files combined,
RMA normalization,
TNBC specimens isolated
Data Processing
ComBat batch correction,
Probe selection
Feature Reduction
GSEA using C2 curated gene sets of
canonical pathways and elastic-net
regularized linear models to create
multinomial variable subtypes
Model Creation
27-Gene set used to derive a logistic elastic
net model
Cutoff Determination
Model cutoff determined using maximum
AUC

FIG. 6

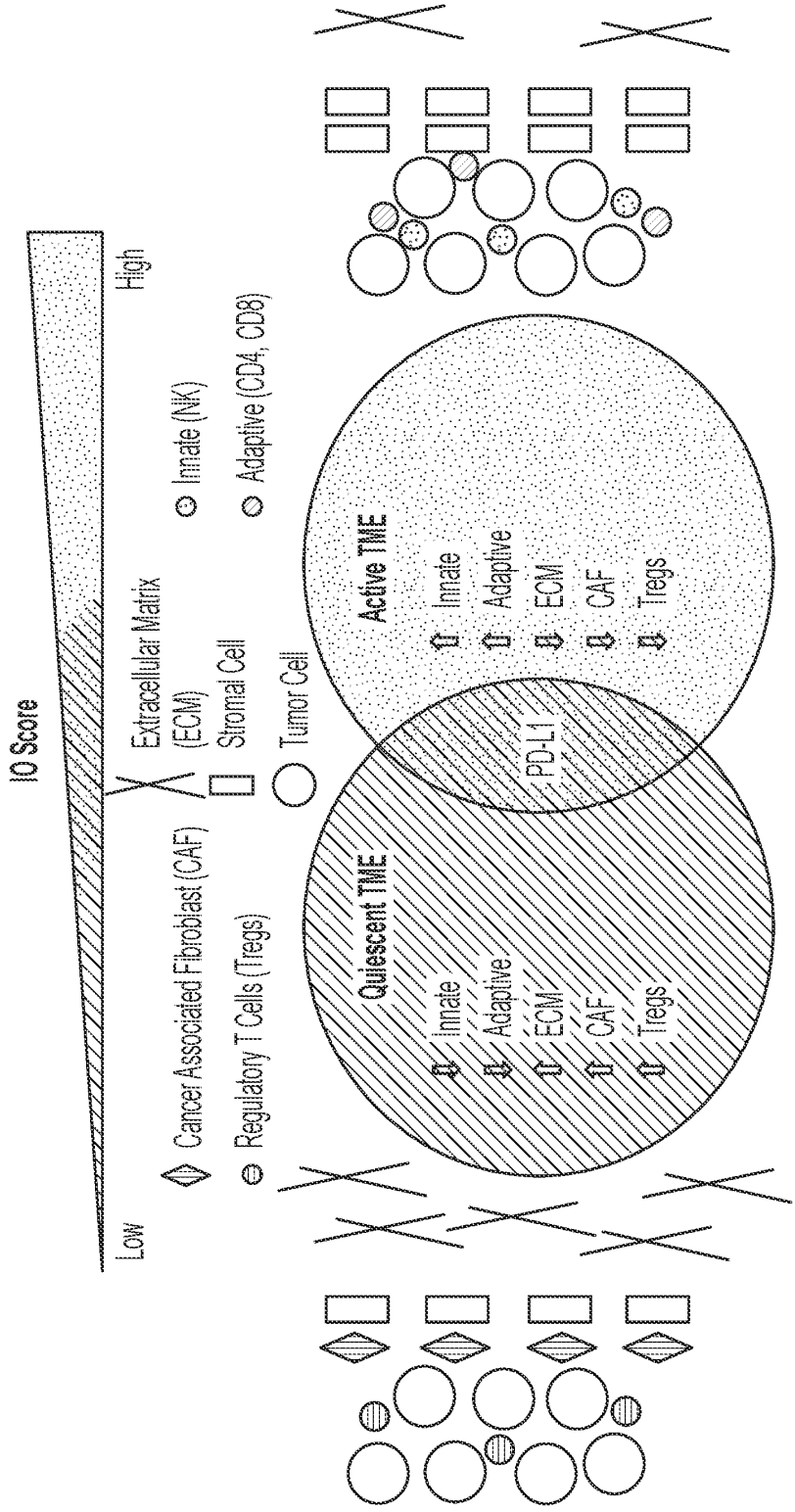

IO Score
High
Low
Extracellular Matrix (ECM)
Stromal Cell
Tumor Cell
Innate (NK)
Adaptive (CD4, CD8)
Cancer Associated Fibroblast (CAF)
Regulatory T Cells (Tregs)
Active TME
Innate
Adaptive
ECM
CAF
Tregs
Quiescent TME
PD-L1

FIG. 7

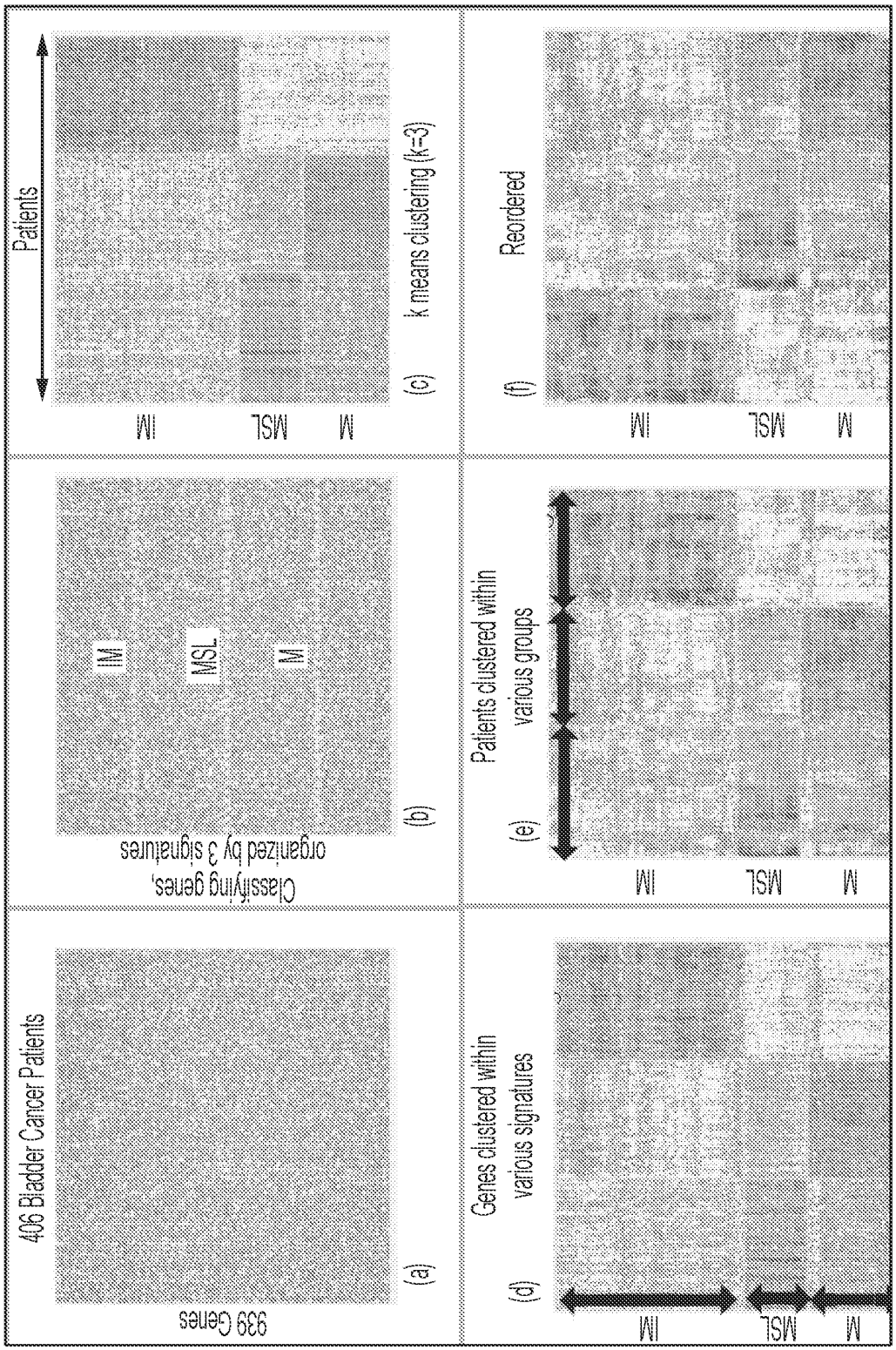

406 Bladder Cancer Patients
939 Genes
(a)
Classifying genes, organized by 3 signatures
IM
MSL
M
(b)
Patients
IM
MSL
M
(c) k means clustering (k=3)
Genes clustered within various signatures
(d)
Patients clustered within various groups
(e)
Reordered
(f)

FIG. 8

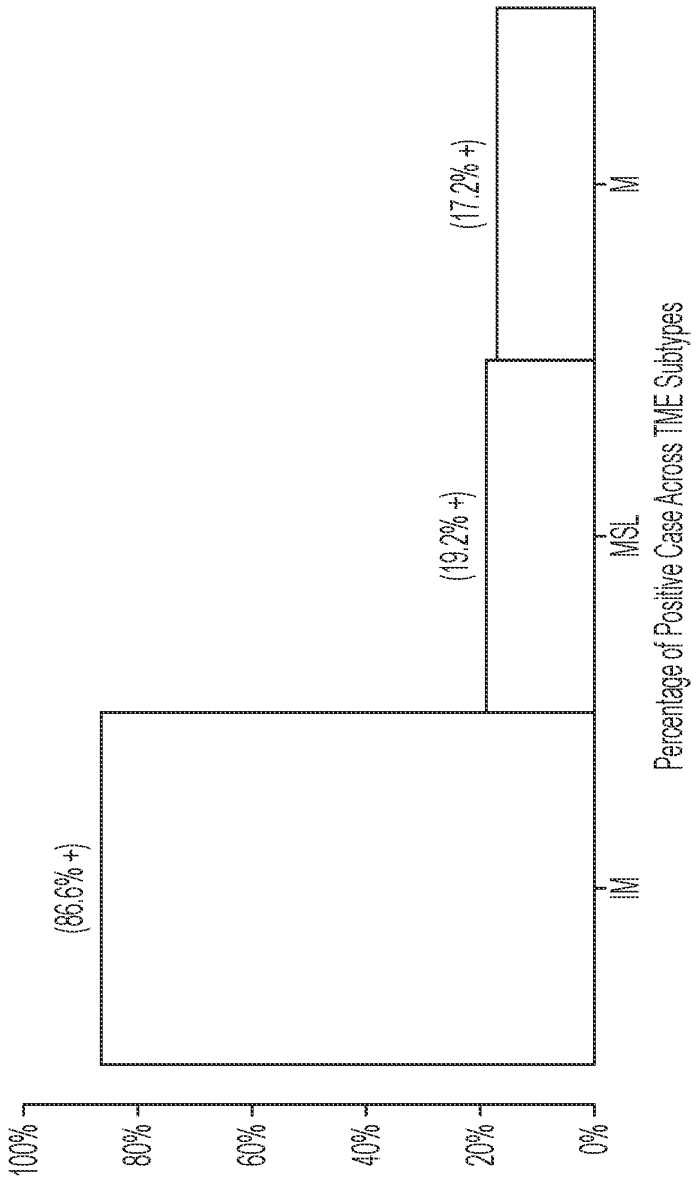

27 Gene Predictor Across Three Subtypes
Percentage of Positive Case Across TME Subtypes
100%
80%
60%
40%
20%
0%
(86.6% +)
(19.2% +)
(17.2% +)
IM
MSL
M

FIG. 9

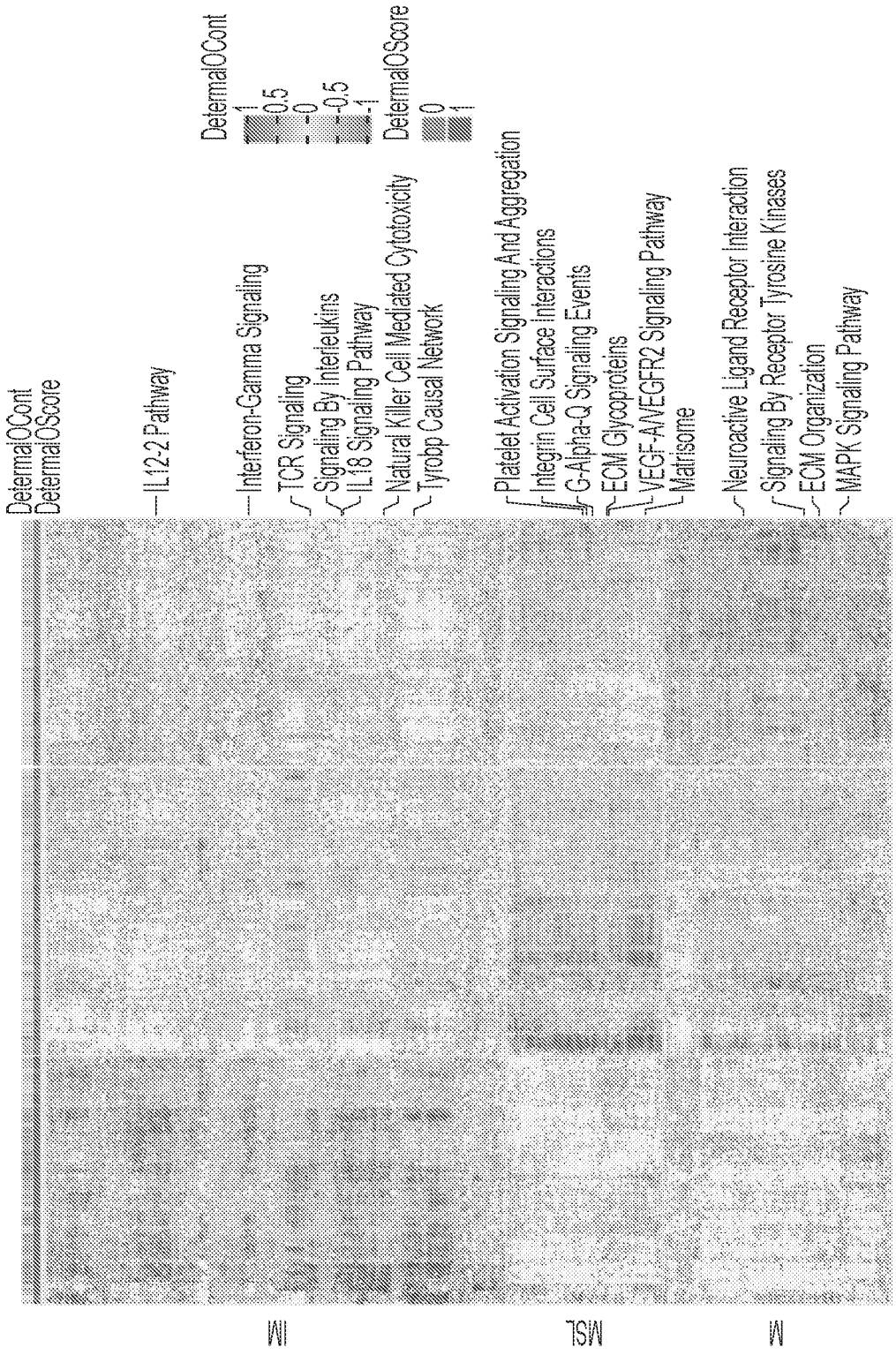

DetermalOCont
DetermalOScore
IL12-2 Pathway
Interferon-Gamma Signaling
TCR Signaling
Signaling By Interleukins
IL18 Signaling Pathway
Natural Killer Cell Mediated Cytotoxicity
Tyrobp Causal Network
Platelet Activation Signaling And Aggregation
Integrin Cell Surface Interactions
G-Alpha-Q Signaling Events
ECM Glycoproteins
VEGF-A/VEGFR2 Signaling Pathway
Matrisome
Neuroactive Ligand Receptor Interaction
Signaling By Receptor Tyrosine Kinases
ECM Organization
MAPK Signaling Pathway
DetermalOCont
1
0.5
0
-0.5
-1
DetermalOScore
0
1
IM
MSL
M
TCGA Bladder Cancer

FIG. 10

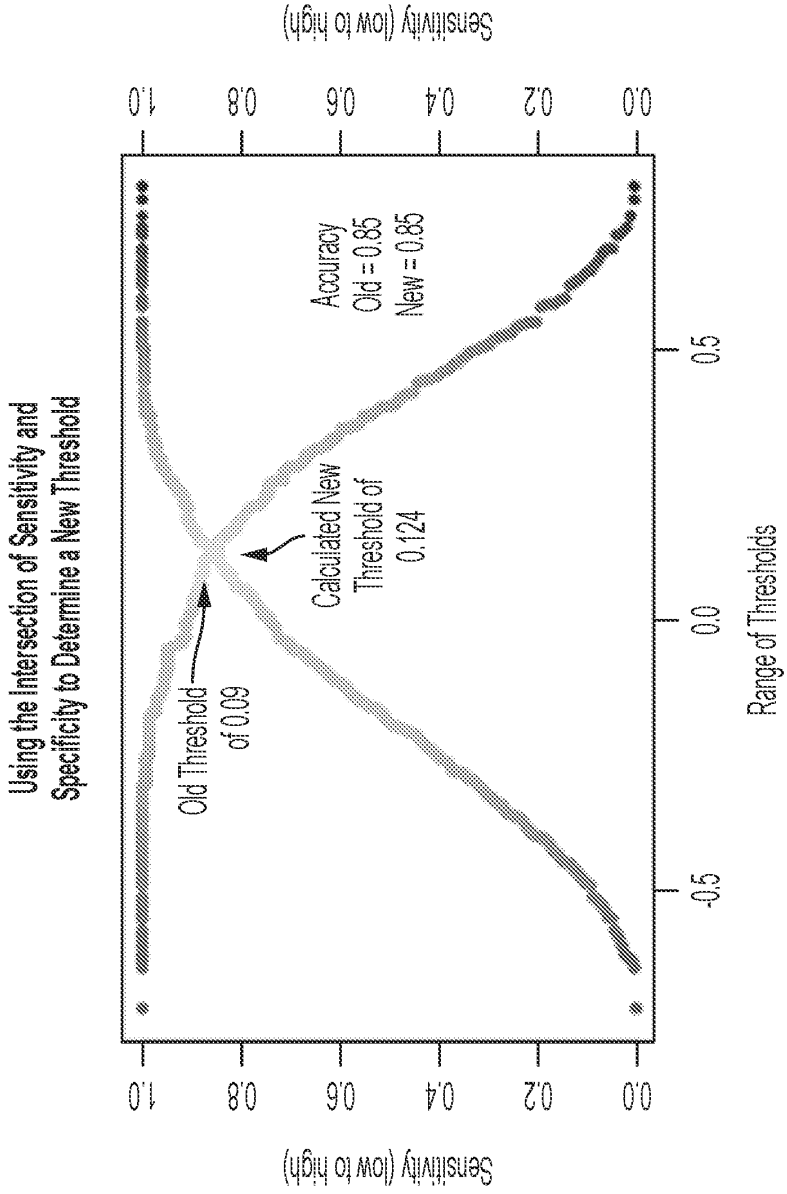
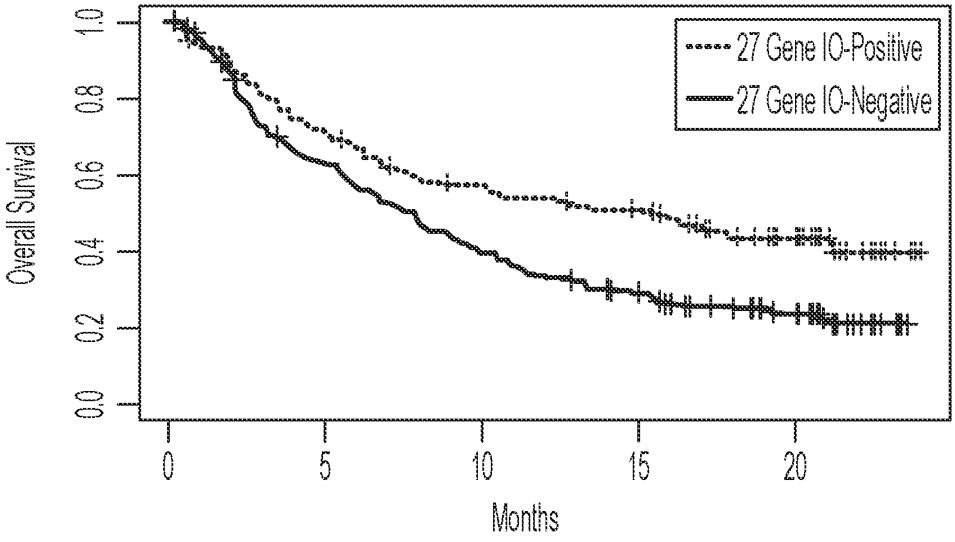

Using the Intersection of Sensitivity and Specificity to Determine a New Threshold
Sensitivity (low to high)
0.0
0.2
0.4
0.6
0.8
1.0
Accuracy
Old = 0.85
New = 0.85
Calculated New Threshold of 0.124
Old Threshold of 0.09
-0.5
0.0
0.5
Range of Thresholds

FIG. 11



	Median OS (mos)	2 Year OS
27 Gene IO-Positive	15.4	39.6%
27 Gene IO-Negative	7.9	20.9%

FIG. 12