

1/28

MKHLWFFLLLVAAPRWVLSQVQLVQSGAEVKKPGASVK
VSCKASGYTFTTAGMQWVRQAPGQGLEWMGWINTHSGV
PKYAEDFKGRVTISADTSTSTAYLQLSSLKSEDTAVYY
CARSGFGSSYWYFDVWGQGT LVT VSSASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGV
HTFPAVLQSSGLYSLSSV VTPSSSLGTQTYICNVNHK
PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTP E VTCV VVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALH
NHYTQKSLSLSPGK

(SEQ ID No. 1: Amino acid sequence of a
heavy chain of the anti-TROP2 antibody)

FIG. 1

MVLQTQVFISLLLLWISGAYGDIQMTQSPSSLSASVGDR
VTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYRYT
GVPSRFGSGSGSGTDFTLT ISSLQPEDFAVYYCQQHYIT
PLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC

(SEQ ID No. 2: Amino acid sequence of
a light chain of the anti-TROP2 antibody)

FIG. 2

TAGMQ

(SEQ ID No. 3: Amino acid sequence of
CDRH1 of the anti-TROP2 antibody)

FIG. 3

2/28

W I N T H S G V P K Y A E D F K G

(SEQ ID No. 4: Amino acid sequence
of CDRH2 of the anti-TROP2 antibody)

FIG. 4

S G F G S S Y W Y F D V

(SEQ ID No. 5: Amino acid sequence
of CDRH3 of the anti-TROP2 antibody)

FIG. 5

K A S Q D V S T A V A

(SEQ ID No. 6: Amino acid sequence
of CDRL1 of the anti-TROP2 antibody)

FIG. 6

S A S Y R Y T

(SEQ ID No. 7: Amino acid sequence of
CDRL2 of the anti-TROP2 antibody)

FIG. 7

Q Q H Y I T P L T

(SEQ ID No. 8: Amino acid sequence of
CDRL3 of the anti-TROP2 antibody)

FIG. 8

3/28

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTAGMQWVR
QAPGQGLEWMGWINTHSGVPKYAEDFKGRVTISADTST
STAYLQLSSLKSEDTAVYYCARSGFGSSYWFYFDVWGQG
TLVTVSS

(SEQ ID No. 9: Amino acid sequence of a heavy
chain variable region of the anti-TROP2 antibody)

FIG. 9

DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQ
KPGKAPKLLIYSASYRYTGVPSTRFSGSGSGTDFTLTIS
SLQPEDFAVYYCQQHYITPLTFGGGTKLEIKRT

(SEQ ID No. 10: Amino acid sequence of a light
chain variable region of the anti-TROP2 antibody)

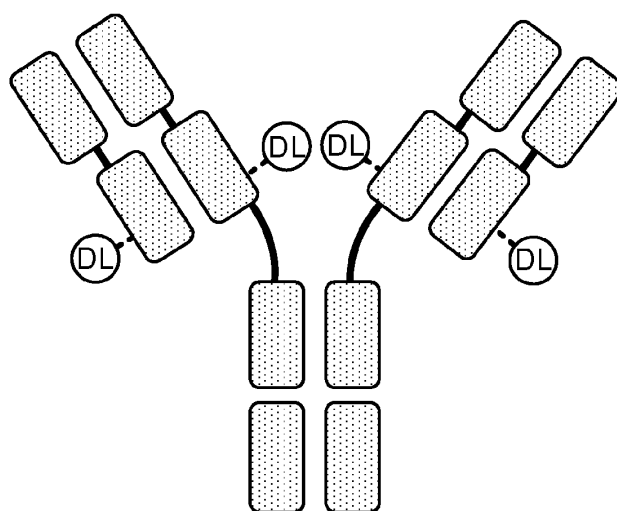
FIG. 10

QVQLVQSGAEVKKPGASVKVSCKASGYTFTTAGMQWVR
QAPGQGLEWMGWINTHSGVPKYAEDFKGRVTISADTST
STAYLQLSSLKSEDTAVYYCARSGFGSSYWFYFDVWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH
TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

(SEQ ID No. 11: Amino acid sequence of a
heavy chain of the anti-TROP2 antibody)

FIG. 11

4/28



(Humanized anti-TROP2 IgG1 mAb³)

FIG. 12

5/28

METHOD FOR
PREDICTING PATIENT
RESPONSE TO AN ANTI-
TROP2 ADC THERAPY

10

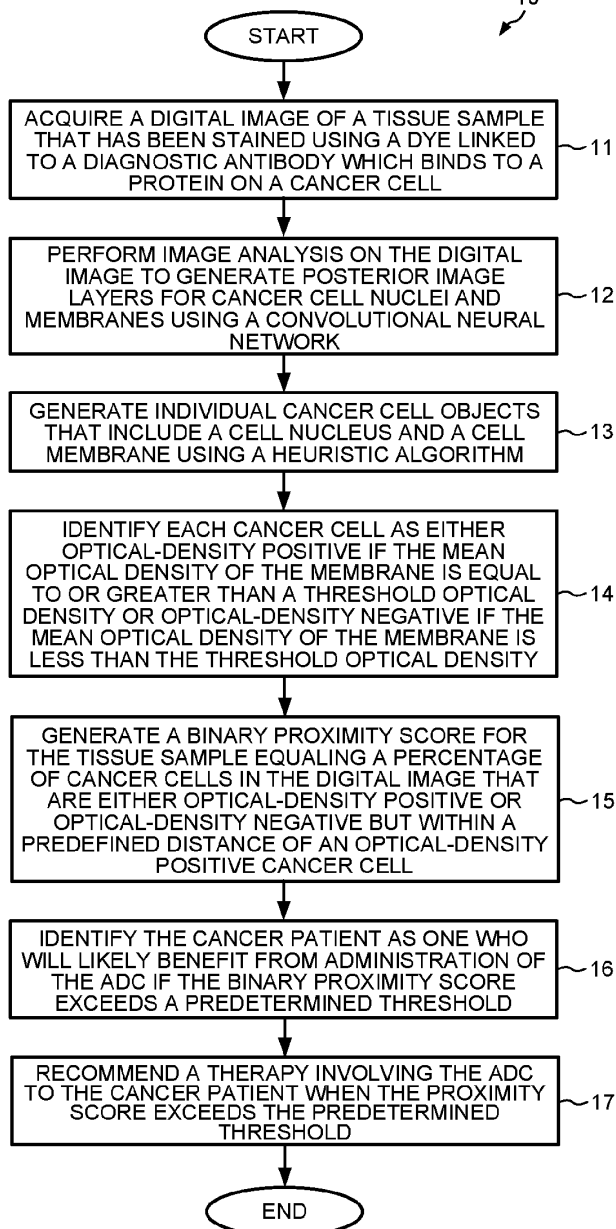
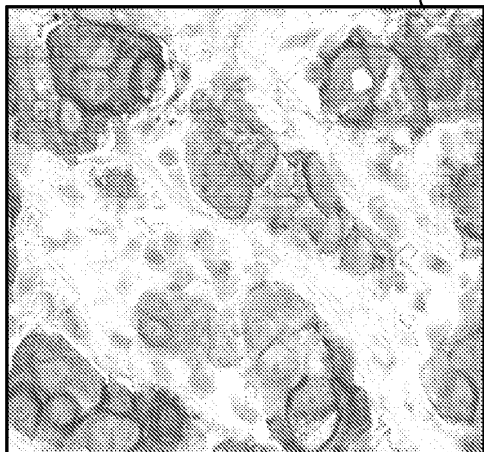


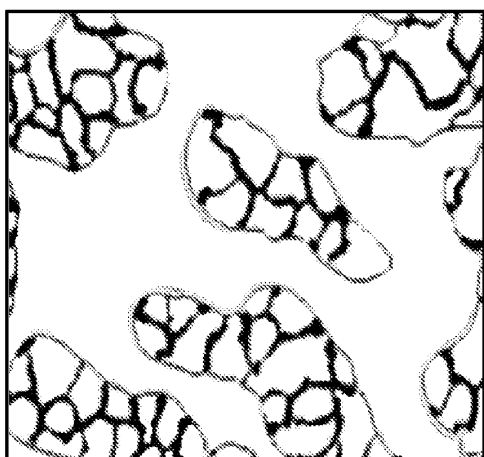
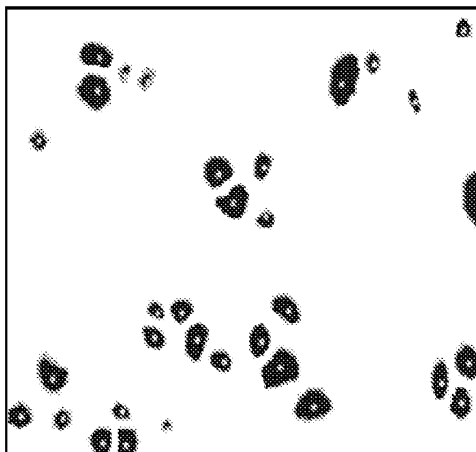
FIG. 13

6/28

Digital image of a TROP2
stained tissue section



Posterior layer for cell nuclei,
generated by a CNN



Posterior layer for cell membranes
generated by the CNN



Unique Identifiers for cells, initially
empty

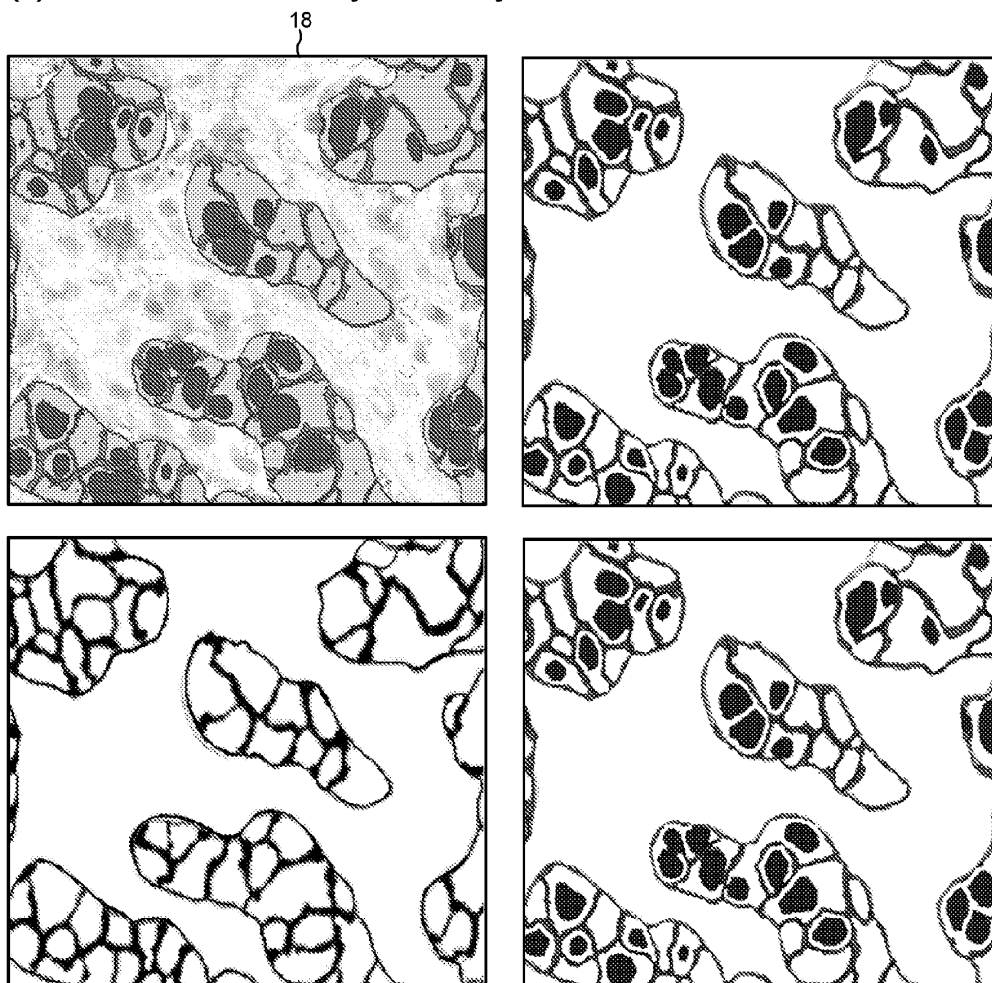
In the images, dark corresponds
to high probability, and white
corresponds to low probability

FIG. 14

7/28

Cell segmentation results by region growing of each nucleus with restrictions:

- (1) not to grow downhill in the membrane posterior,
- (2) and use a minimal object density constraint for the cell border



The unique identifier (UID) for each membrane object is the same as that of the associated nucleus object.

FIG. 15

8/28

Cytoplasm and membrane segmentation results by identification of cell border pixels as membrane, and then region growing of the membrane into pixels with membrane posterior $>20\%$.

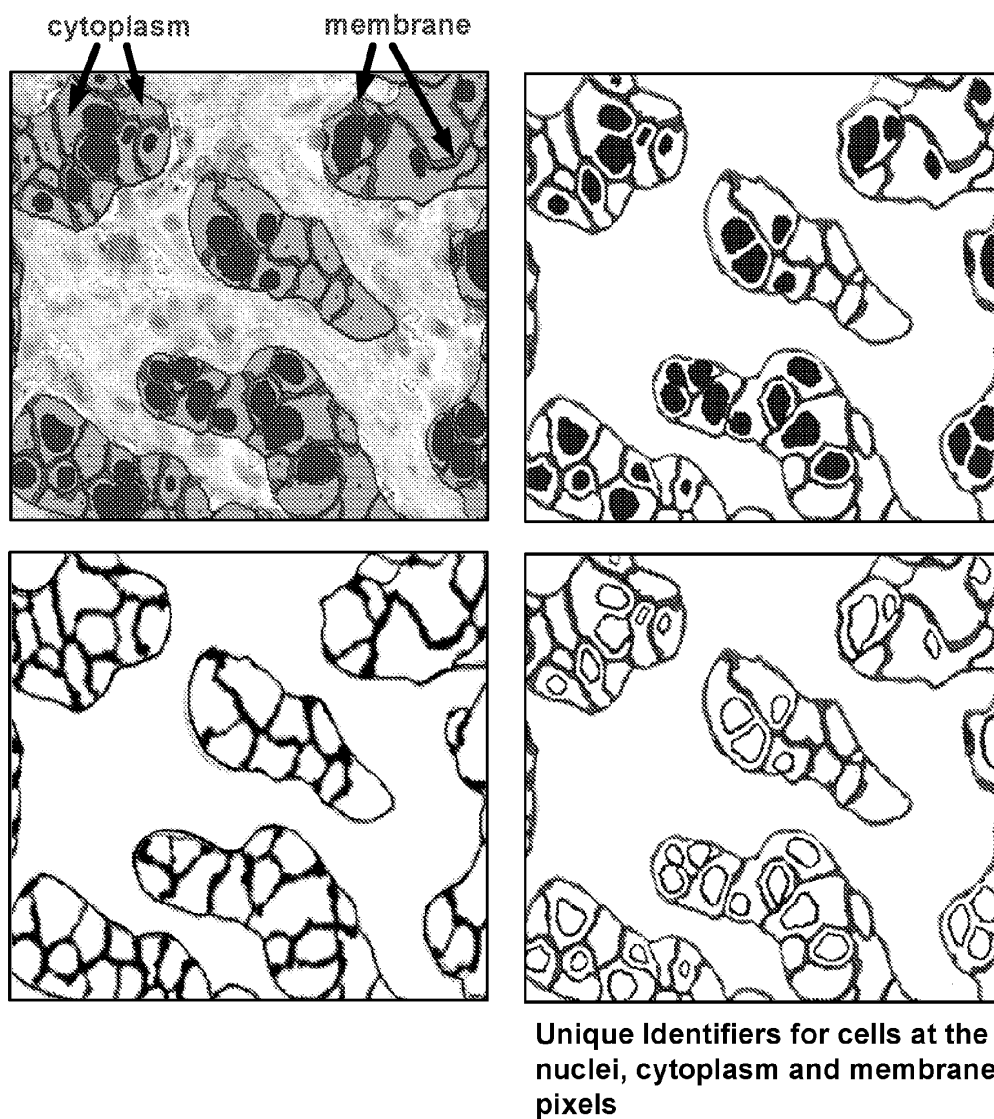
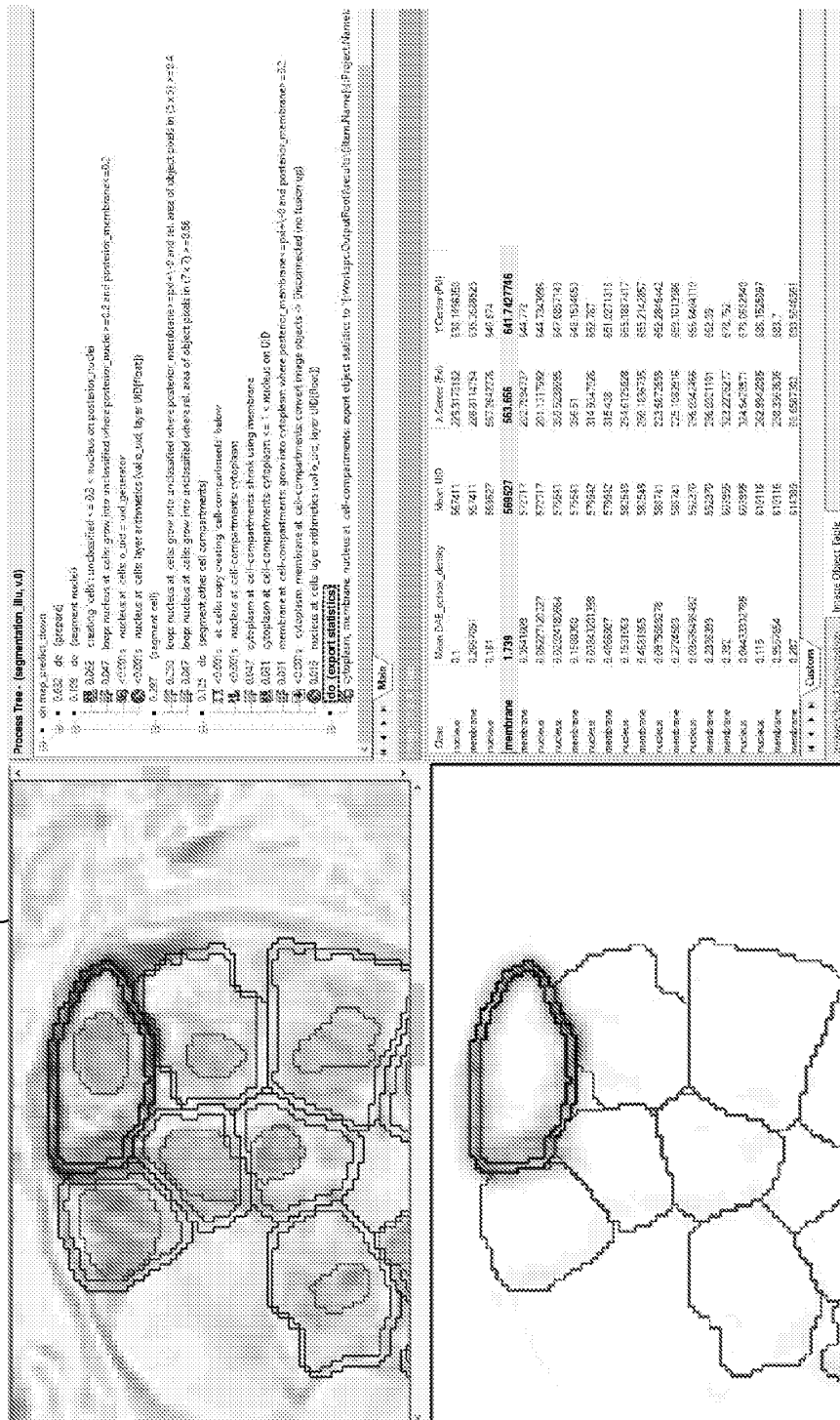


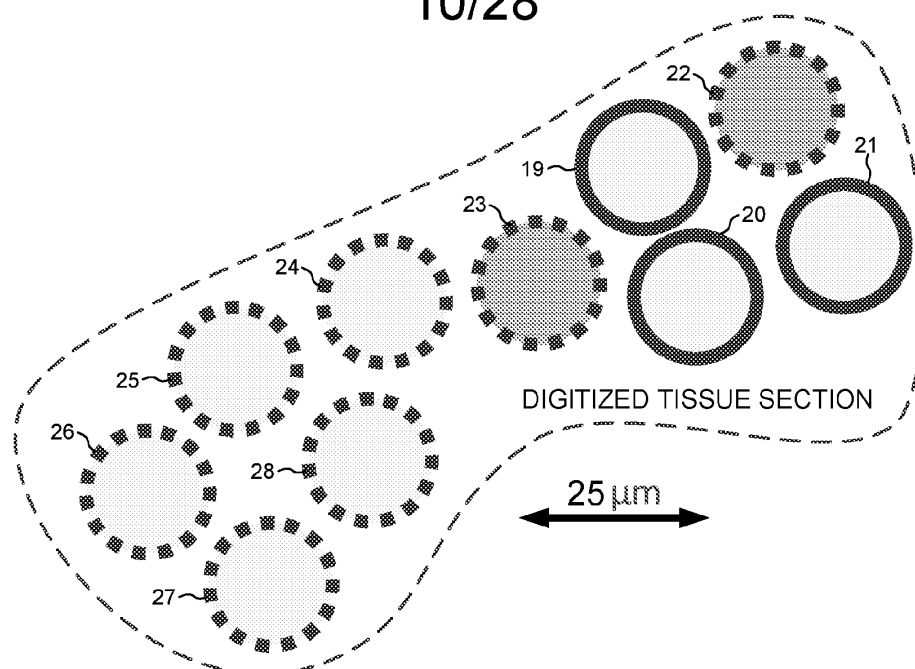
FIG. 16

9/28



In the lower image, dark corresponds to high optical density, and bright corresponds to low optical density

10/28



TUMOR CELLS IN WHICH THE MEAN OF THE OPTICAL DENSITY OF THE MEMBRANE PIXELS IS ≥ 12 OF A MAXIMUM SCALE OF 220.



TUMOR CELLS FOR WHICH BOTH THE MEAN OF THE OPTICAL DENSITY OF THE MEMBRANE PIXELS IS < 12 AND THE CELL IS WITHIN $25 \mu\text{m}$ OF A TUMOR CELL FOR WHICH THE MEAN OPTICAL DENSITY OF THE MEMBRANE PIXELS IS ≥ 12 .



TUMOR CELLS FOR WHICH THE MEAN OF THE OPTICAL DENSITY OF THE MEMBRANE PIXELS IS < 12

$$\text{bSPS}(r, \text{THRESHOLD}) = ((\# \text{ TUMOR CELLS HAVING SINGLE CELL OPTICAL DENSITY SCORE } \geq \text{THRESHOLD}) + (\# \text{ TUMOR CELLS HAVING BOTH SINGLE CELL OPTICAL DENSITY SCORE } < \text{THRESHOLD AND BEING WITHIN RADIUS } r \text{ OF A TUMOR CELL WITH SINGLE CELL OPTICAL DENSITY SCORE } \geq \text{THRESHOLD})) / (\text{NUMBER OF ALL TUMOR CELLS})$$

FOR EXAMPLE ILLUSTRATED ABOVE:

$$\text{bSPS}(r=25\mu\text{m}, \text{membrane OD}_{\text{cut}}=12) = (3+2)/10 = 0.5 = 50\%$$

**Binary Spatial Proximity Score (bSPS)
to estimate ADC bystander activity**

FIG. 18

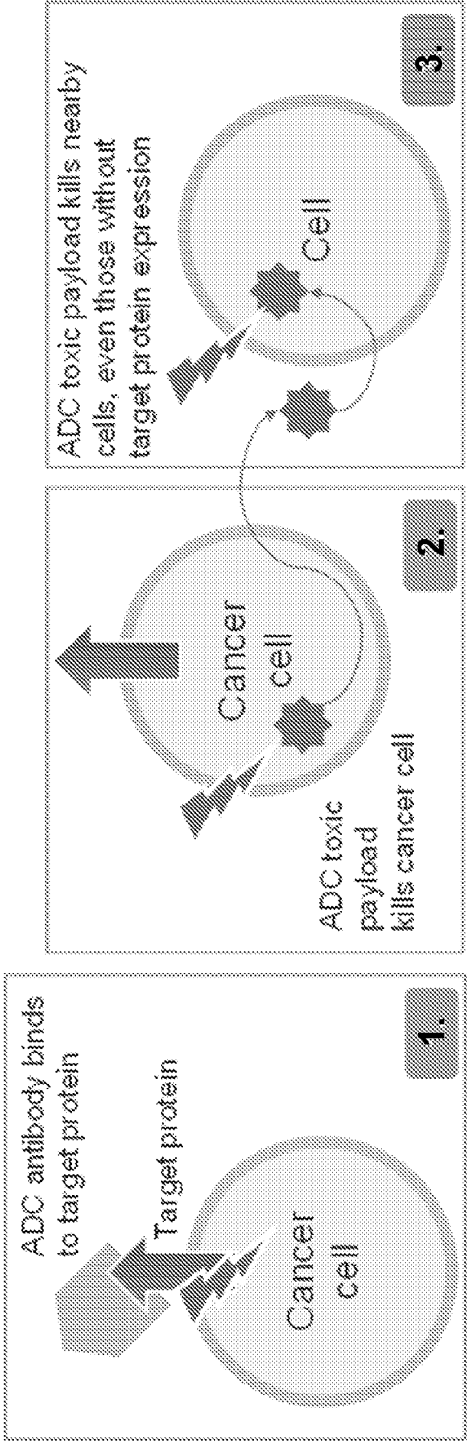


FIG. 19

12/28

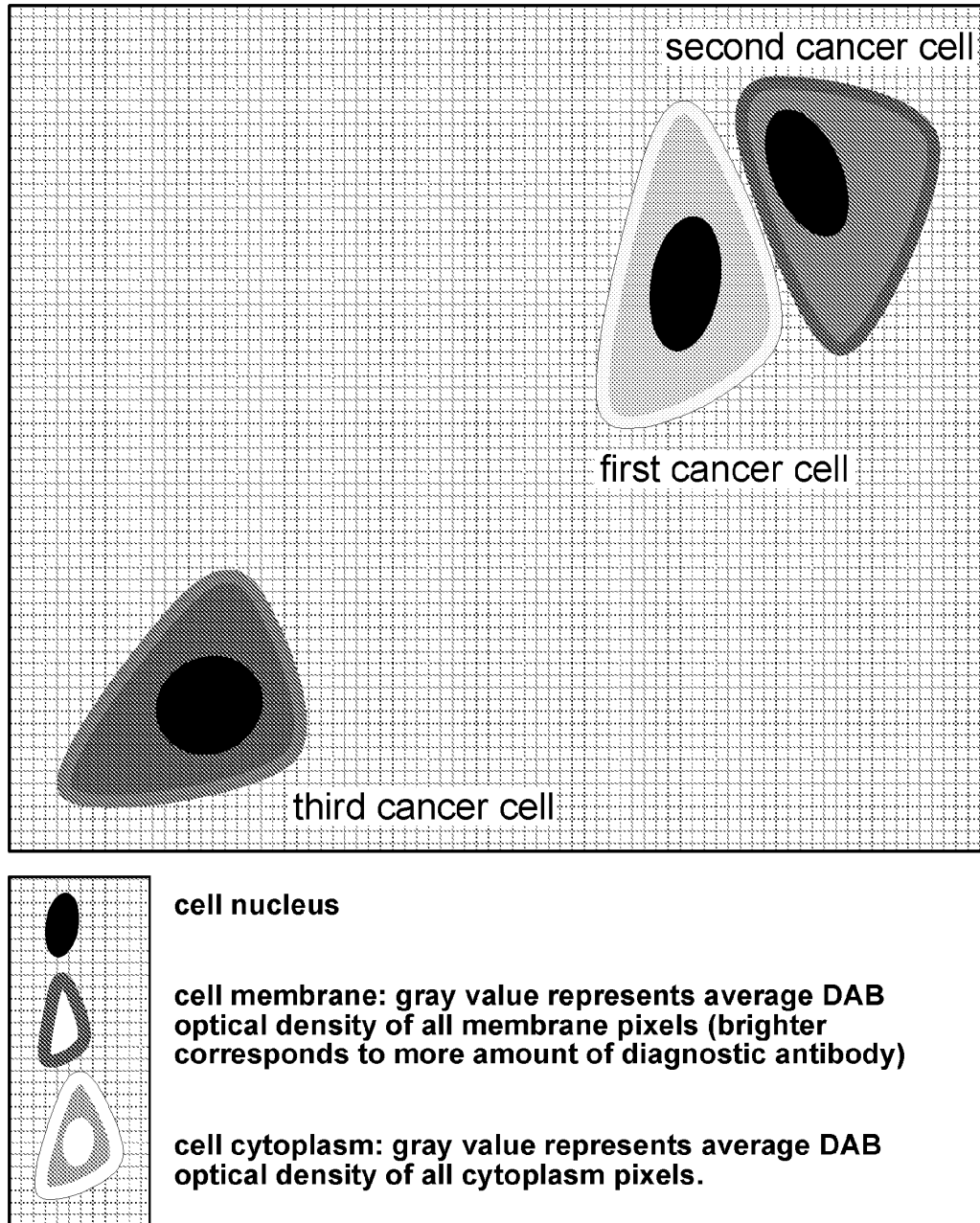
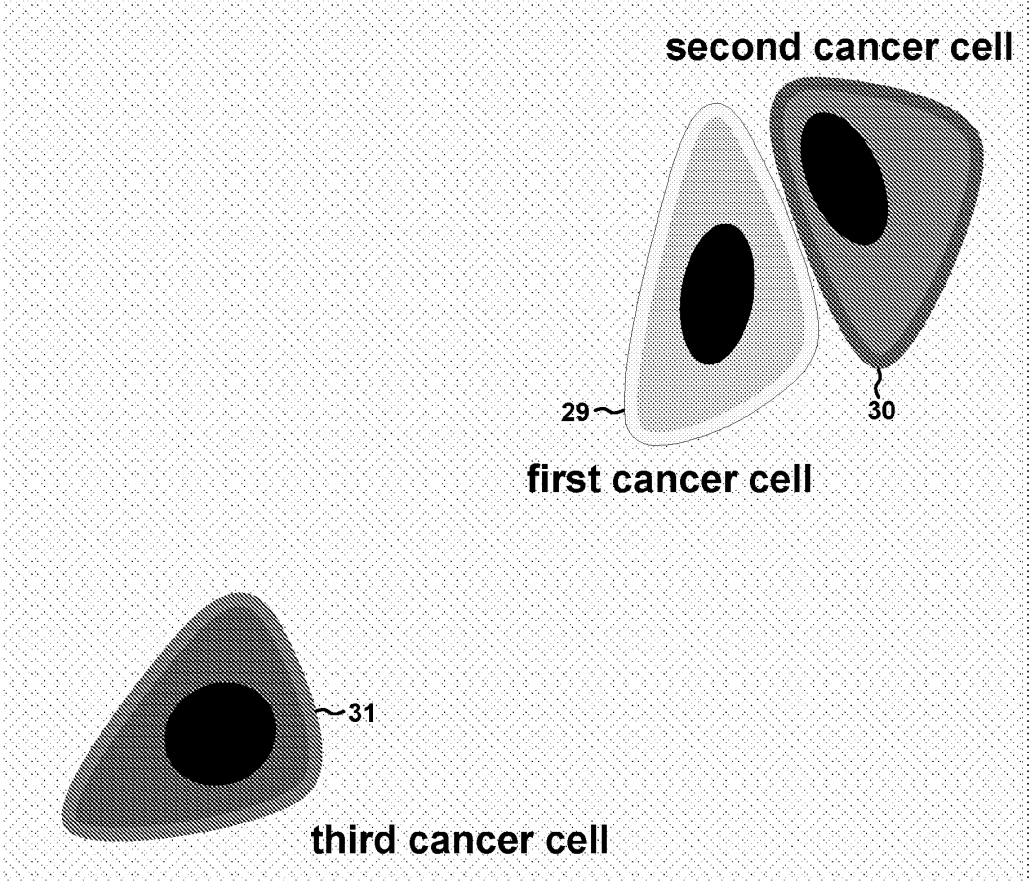


FIG. 20

13/28



UID	X [pixels]	Y [pixels]	Optical density membrane (ODM)	Optical density cytoplasm (ODC)
1	480	190	$242/255 = .949$	$203/255 = .796$
2	590	130	$94/255 = .369$	$136/255 = .533$
3	140	500	$127/255 = .498$	$94/255 = .369$

FIG. 21

Cell statistics with single cell spatial proximity scoring

$r_1 - r_i$: euclidean distance from center of gravity of the cell with UID 1 to the cell with UID i.

$w_{ij} = \exp (- |r_i - r_j| / r_{norm})$: exponential weighting factor to include ADC payload uptake from neighboring cells

Single cell ADC score for cell i = min (K1 , Sum_j with $w_{ij} < 15\%$ { $w_{ij} \times \max (0, ODM_j \times (K2 + ODM_j - ODC_j))$ }

14/28

UID	X [pixels]	Y [pixels]	Optical density membrane (ODM)	Optical density cytoplasm (ODC)	max(0, ODM x(ODM-ODC))	$r_1 - r_i$	$r_2 - r_i$	$r_3 - r_i$	$\exp(-(r_1 - r_i)/50)$	$\exp(-(r_2 - r_i)/50)$	$\exp(-(r_3 - r_i)/50)$	Single-Cell continuous SPS
1	480	190	0.949019608	0.796078431	0.145144175	0	125.2996	460.1087	1	0.08159455	0.00010082	0.145144175
2	590	130	0.368627451	0.533333333	0	125.2996	0	582.5805	0.08159455	1	8.705E-06	0.011842974
3	140	500	0.498039216	0.368627451	0.064452134	460.1087	582.5805	0	0.00010082	8.705E-06	1	0.064452134

r_norm=50

FIG. 22

15/28

GENERATING A SCORE FOR EACH CELL WHICH REFLECTS THE
LIKELIHOOD THAT A CANCER CELL IS DAMAGED IN FUNCTION OR KILLED

Single Cell ADC score = Weighted sum of this cell and neighboring cells of a bilinear combination of the amounts of Target Protein on the cells membrane and cytoplasm, the weights depend on the distance of the neighboring cells to this cell.

Mathematics for cell i with position r_i on the slide and average
membrane (cytoplasm) optical density values ODM_i (ODC_i)

$$\text{single_cell_score}_i = \text{Sum}_j \text{ with } |r_j - r_i| < d \{ a_{20}(|r_j - r_i|) \times \text{ODM}_j^2 + a_{11}(|r_j - r_i|) \times \text{ODM}_j \times \text{ODC}_j + a_{02}(|r_j - r_i|) \times \text{ODC}_j^2 + a_{00}(|r_j - r_i|) \}$$

with the functions a_{kl} depending on the distance $|r_j - r_i|$ of the cell j to the cell i,
e.g., $a_{kl}(|r_j - r_i|) = A_{kl} \times \exp(-|r_j - r_i|/r_{\text{norm}})$ with A_{kl} and r_0 constants determined by
optimized therapy success prediction.

FIG. 23

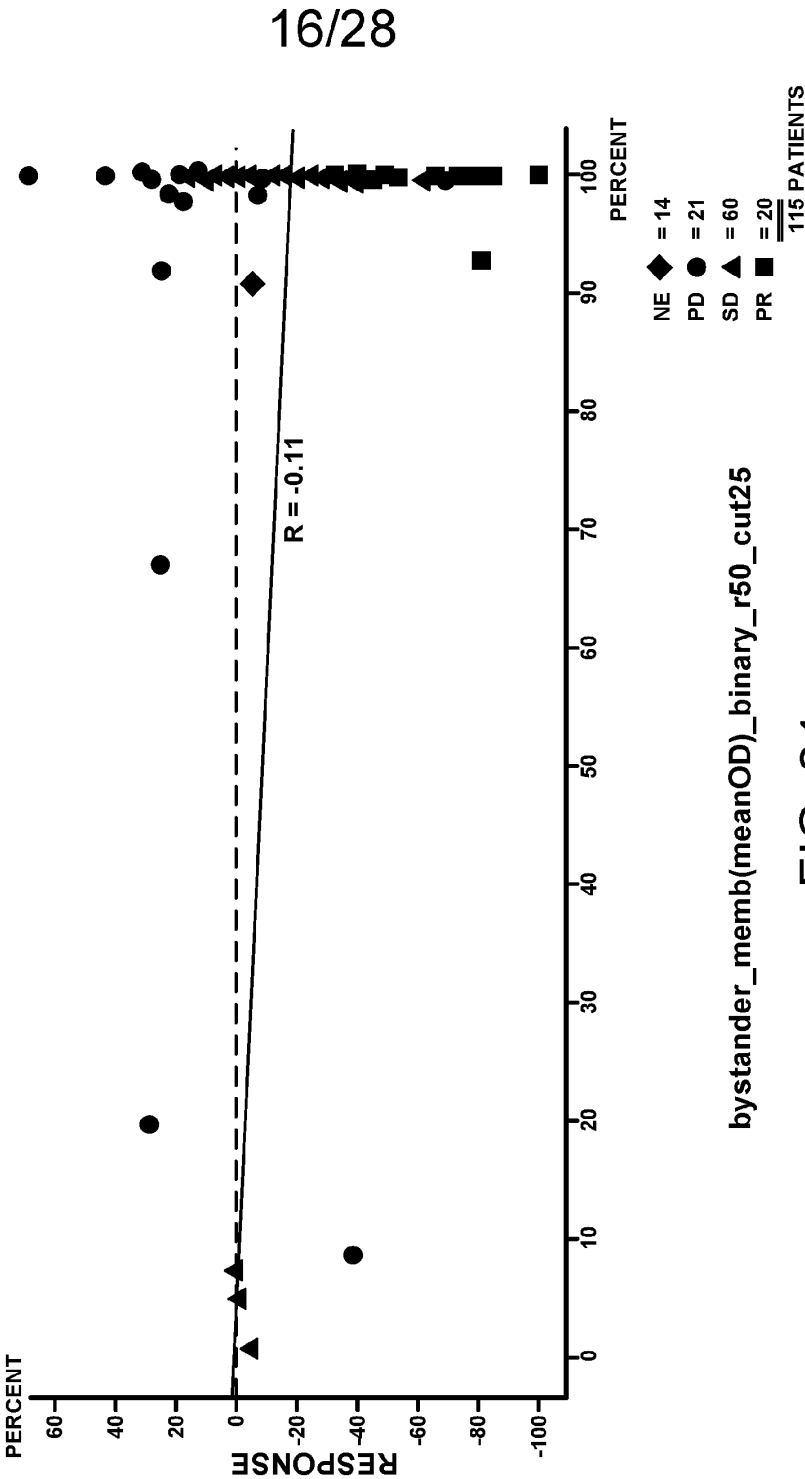
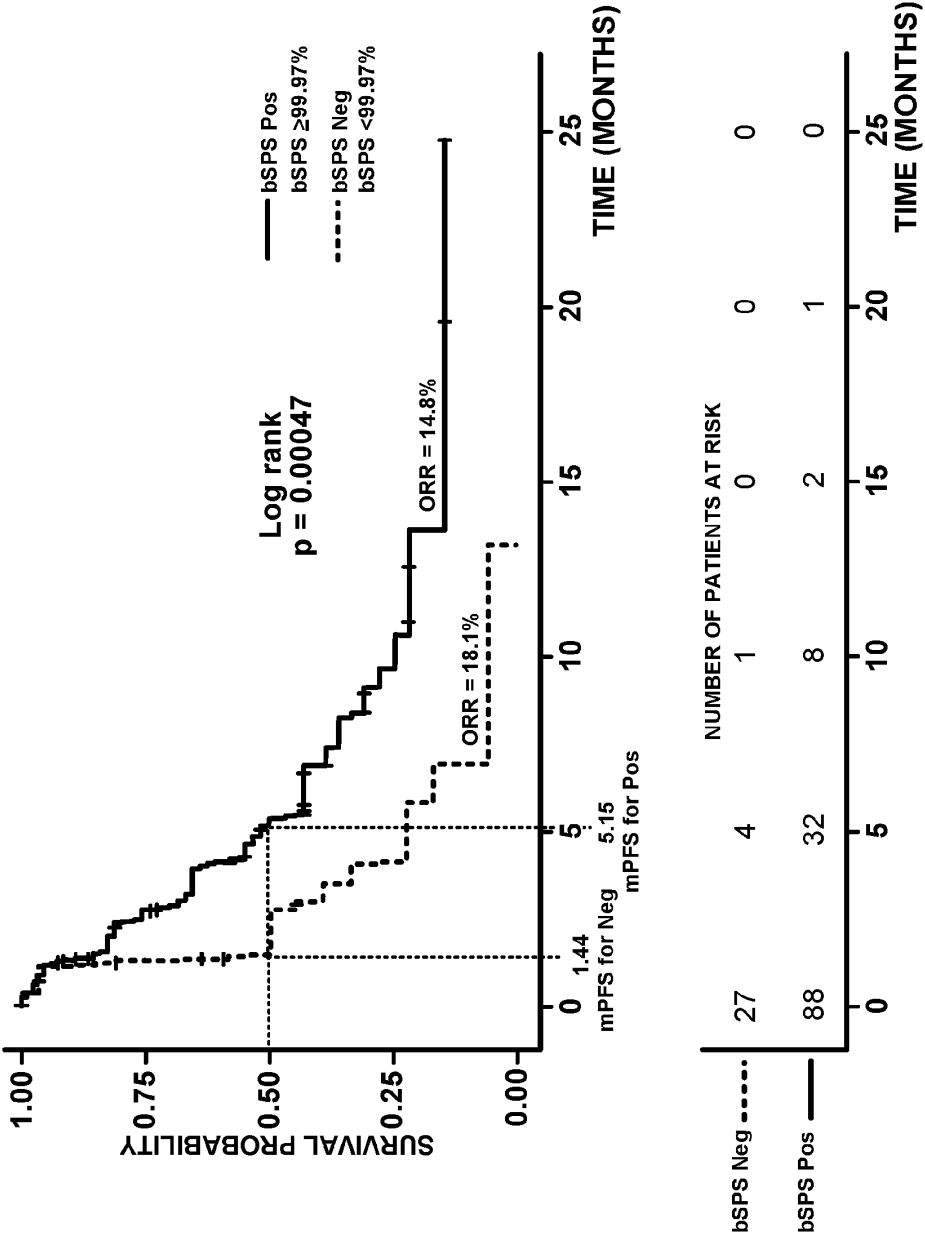


FIG. 24



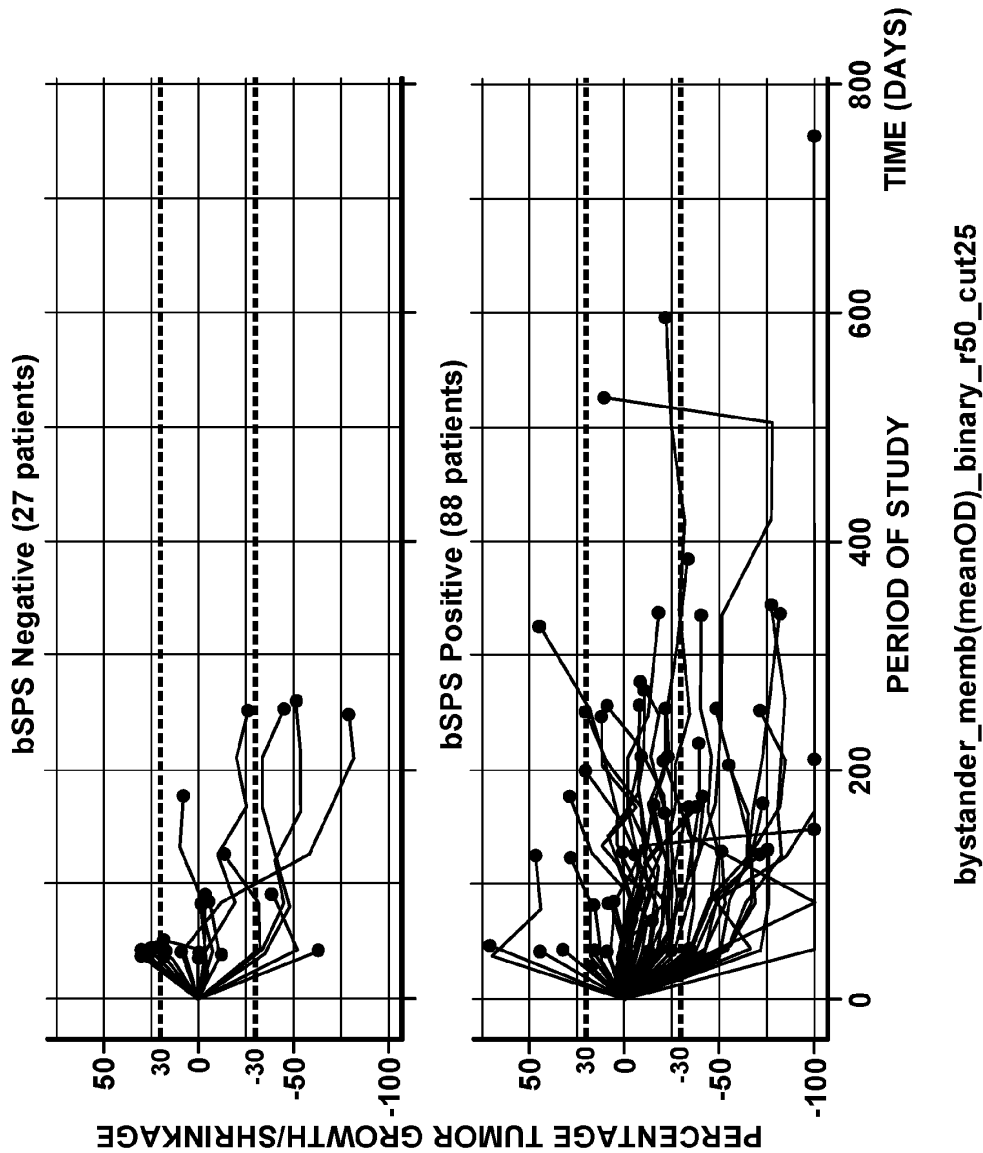


FIG. 26

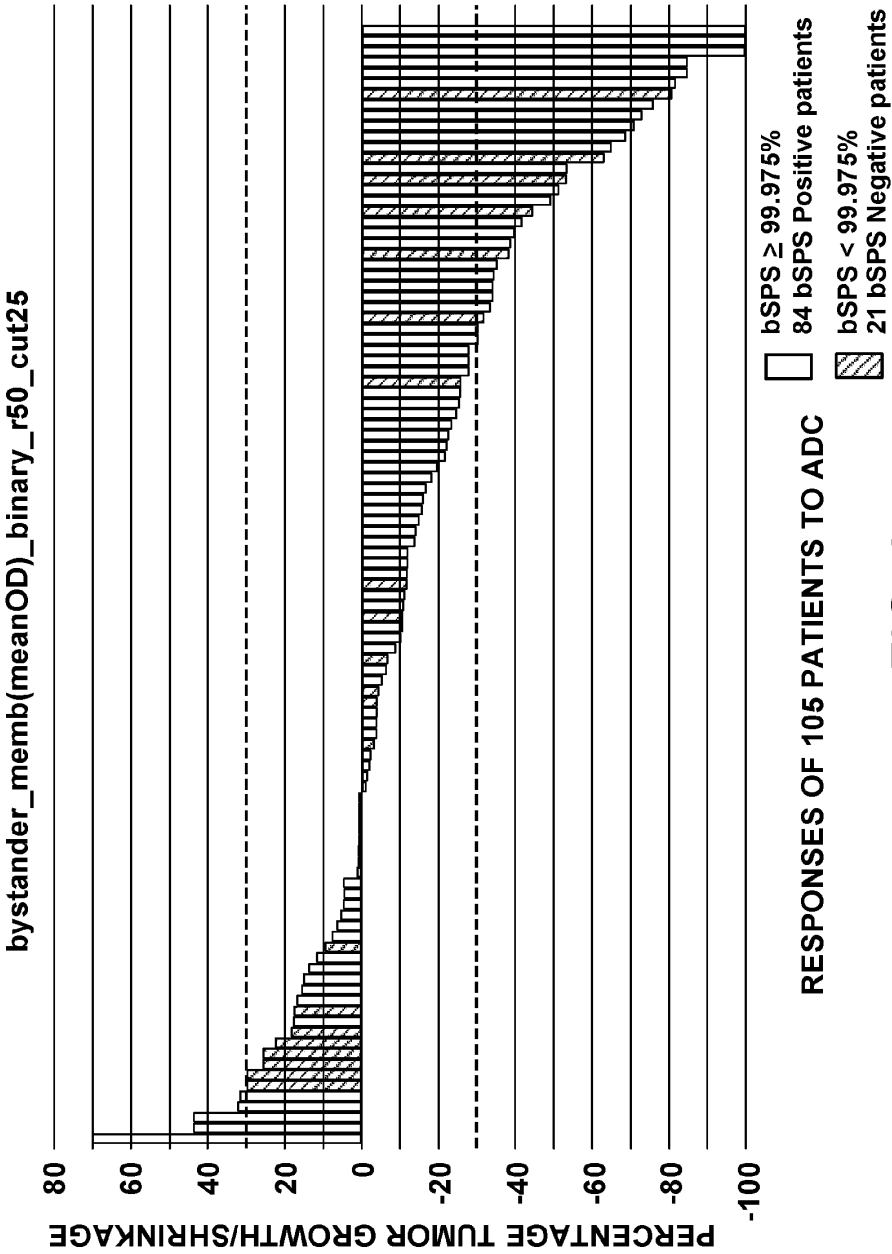


FIG. 27

20/28

METHOD OF
PREDICTING THE
EFFICACY OF AN ANTI-
TROP2 ADC THERAPY
32

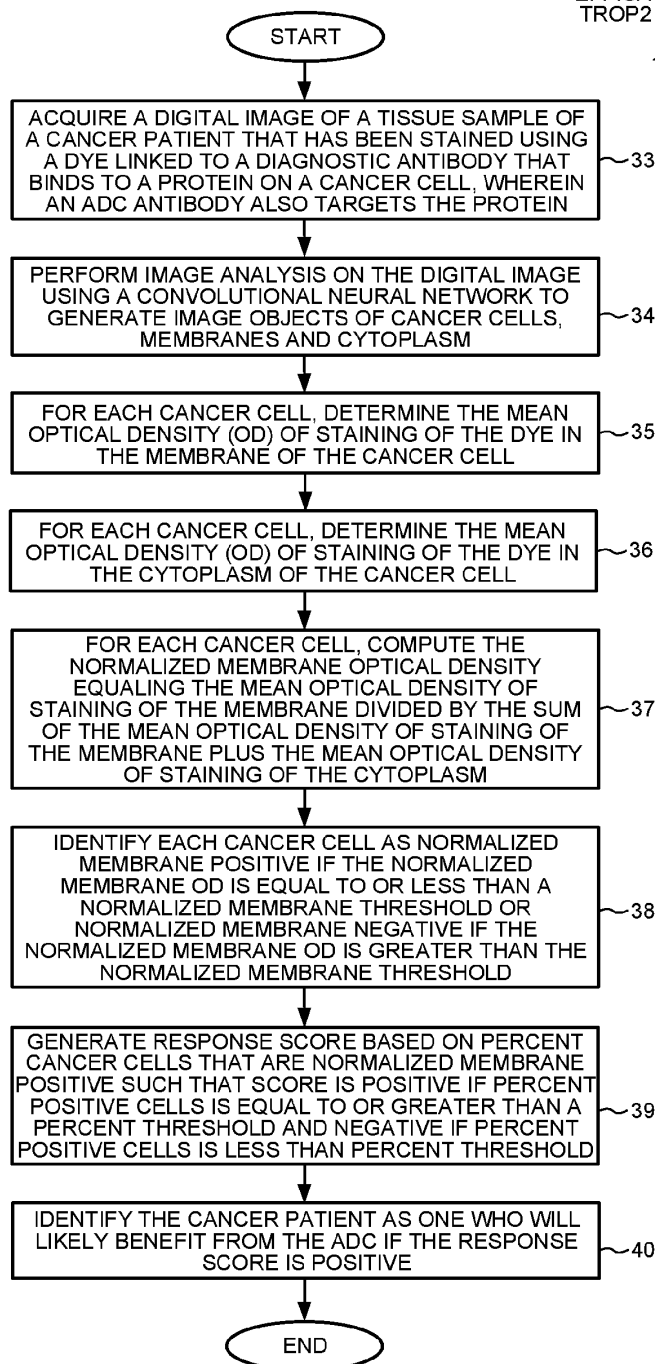


FIG. 28

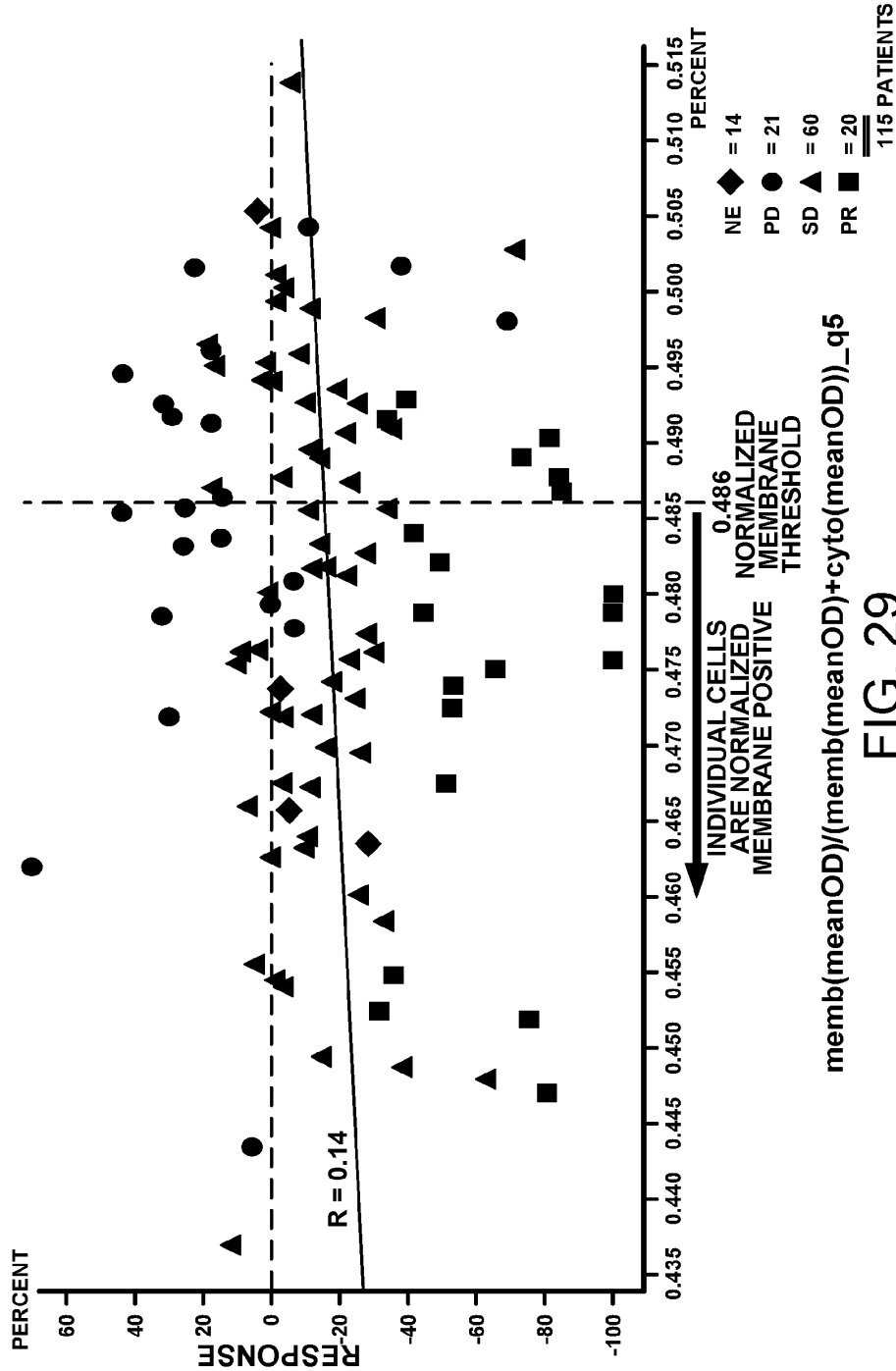


FIG. 29

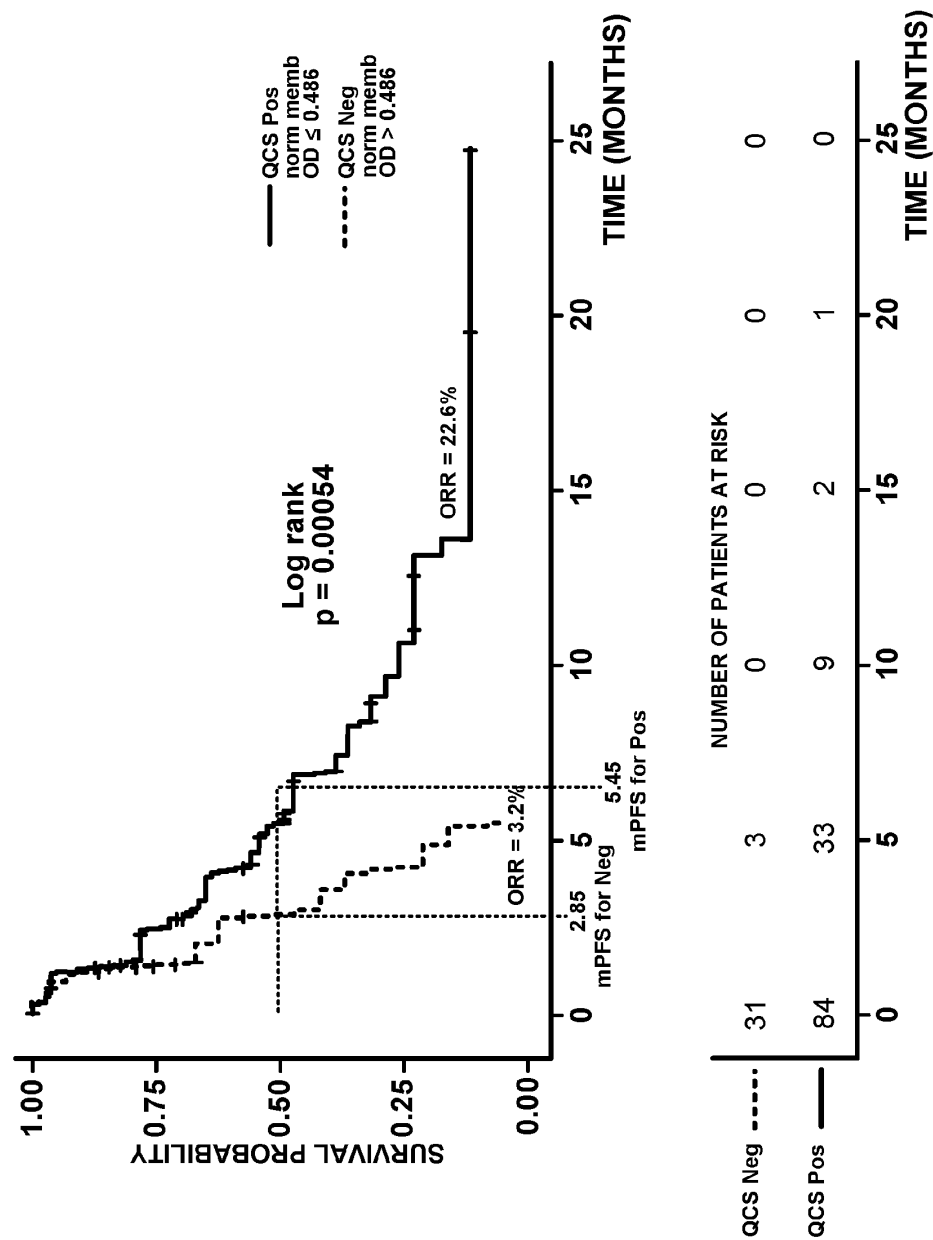
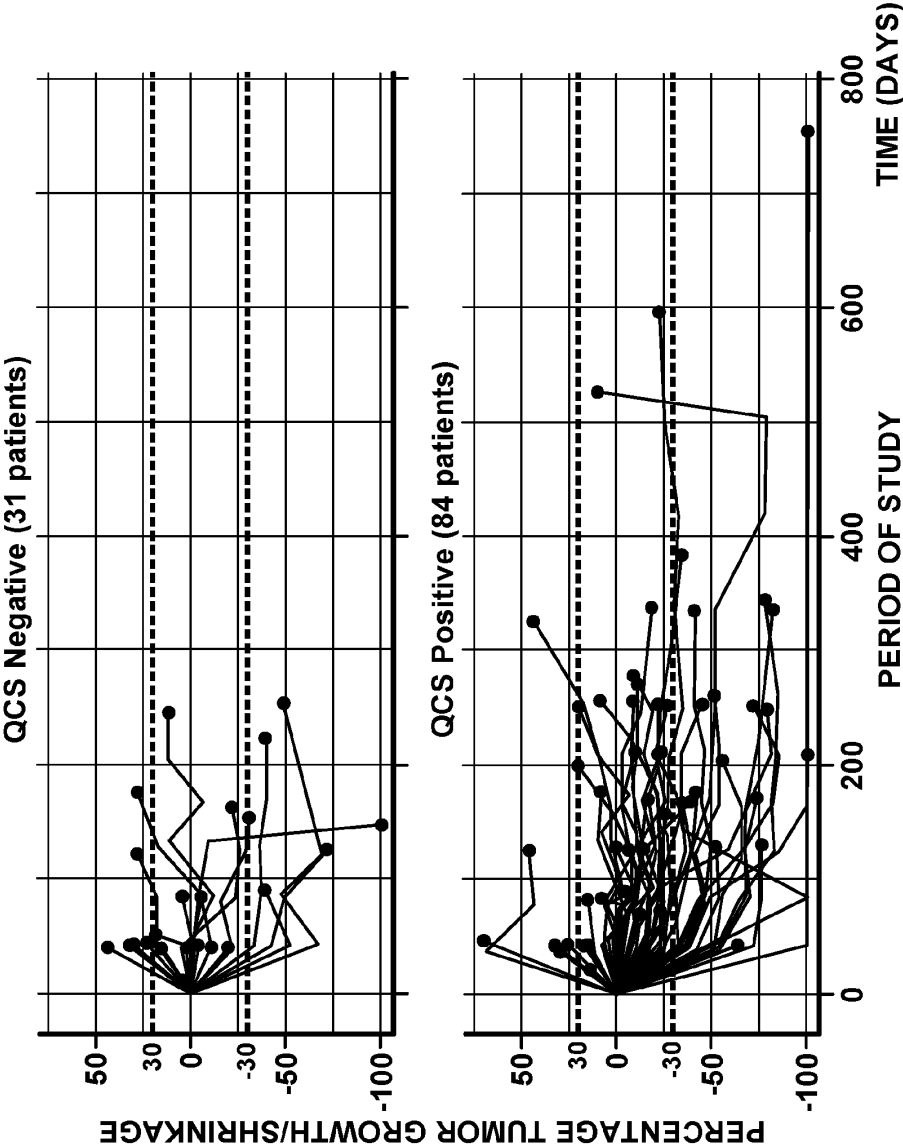


FIG. 30



memb(meanOD)/(memb(meanOD)+cyto(meanOD))_q5

FIG. 31

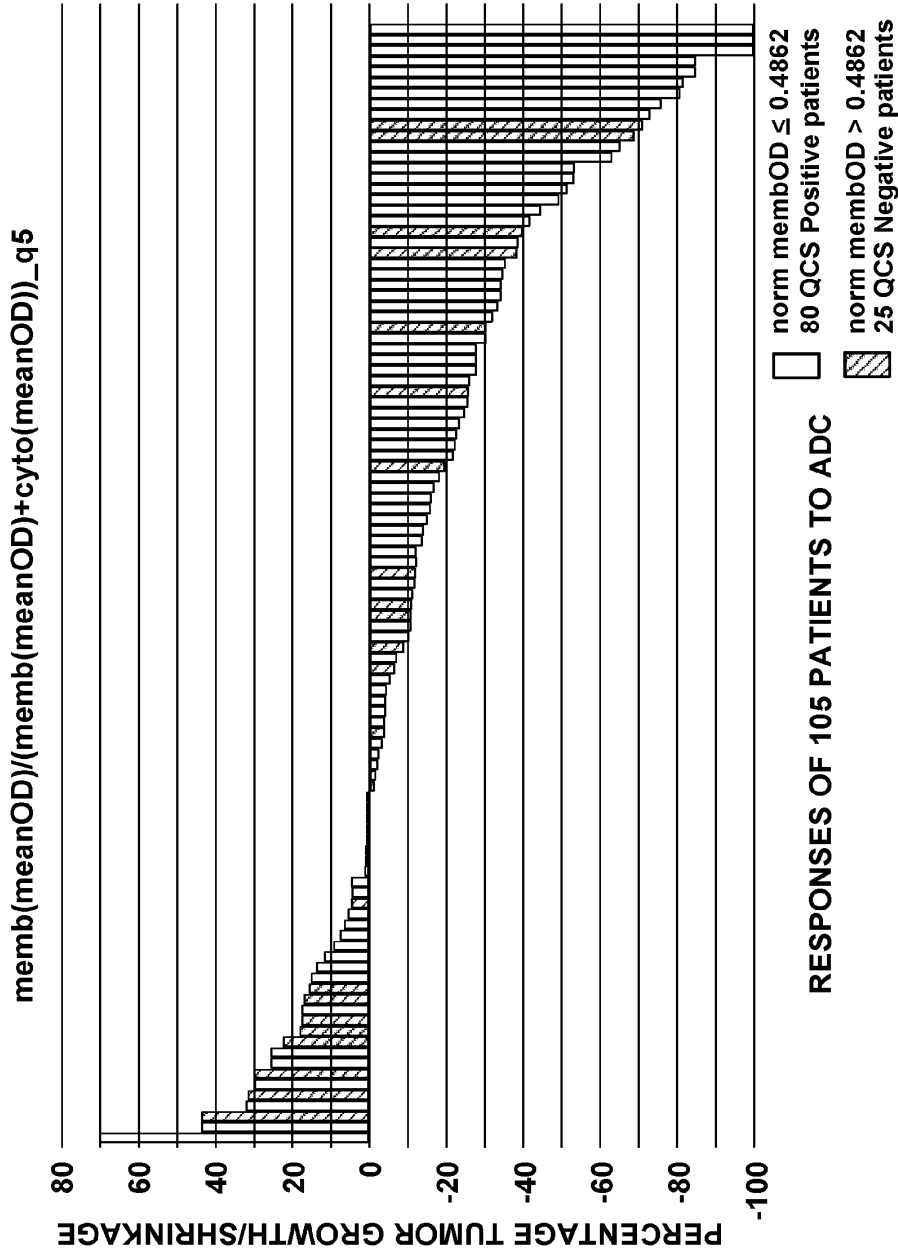


FIG. 32

25/28

METHOD FOR
PREDICTING PATIENT
RESPONSE TO AN ANTI-
TROP2 ADC THERAPY

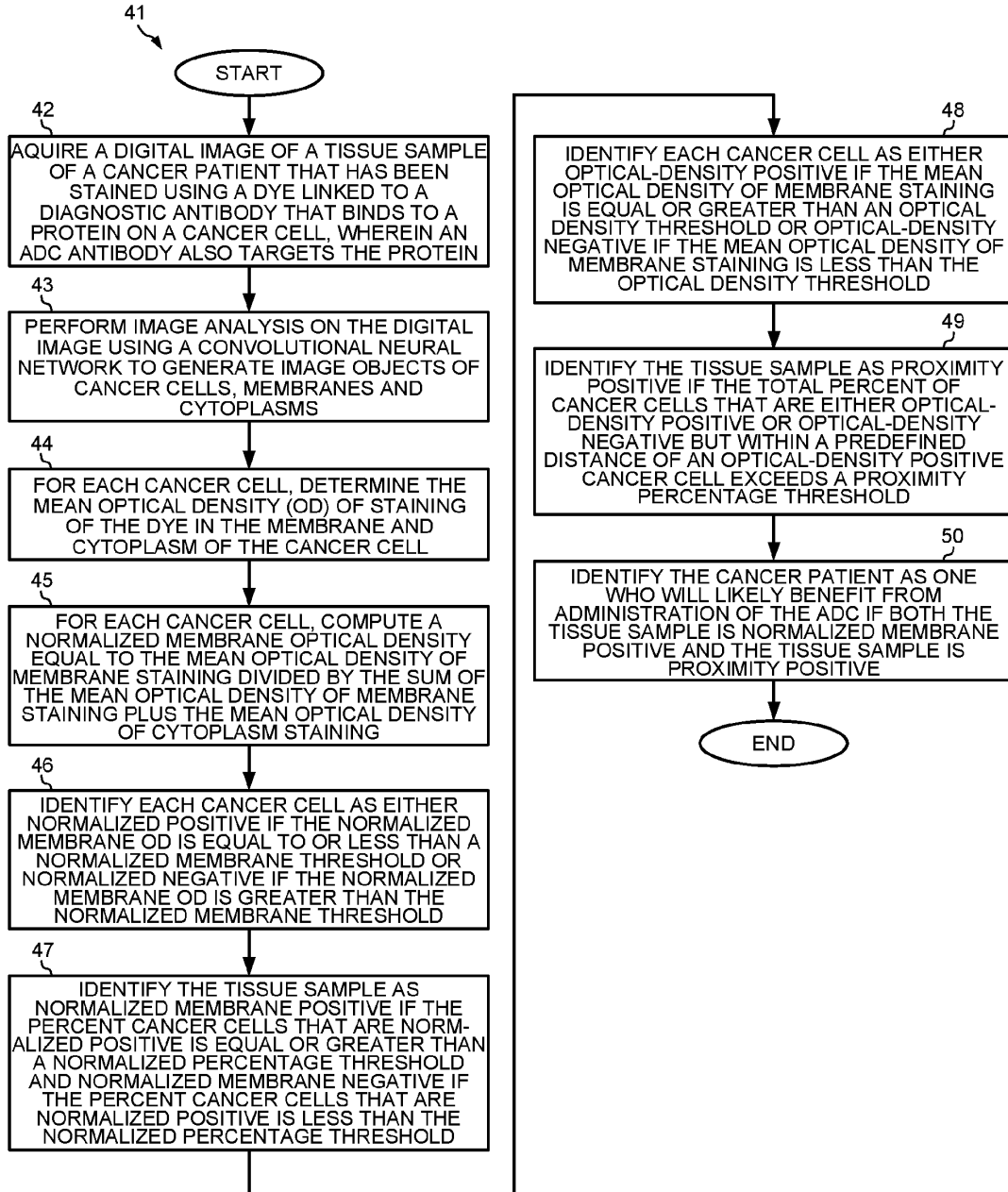


FIG. 33

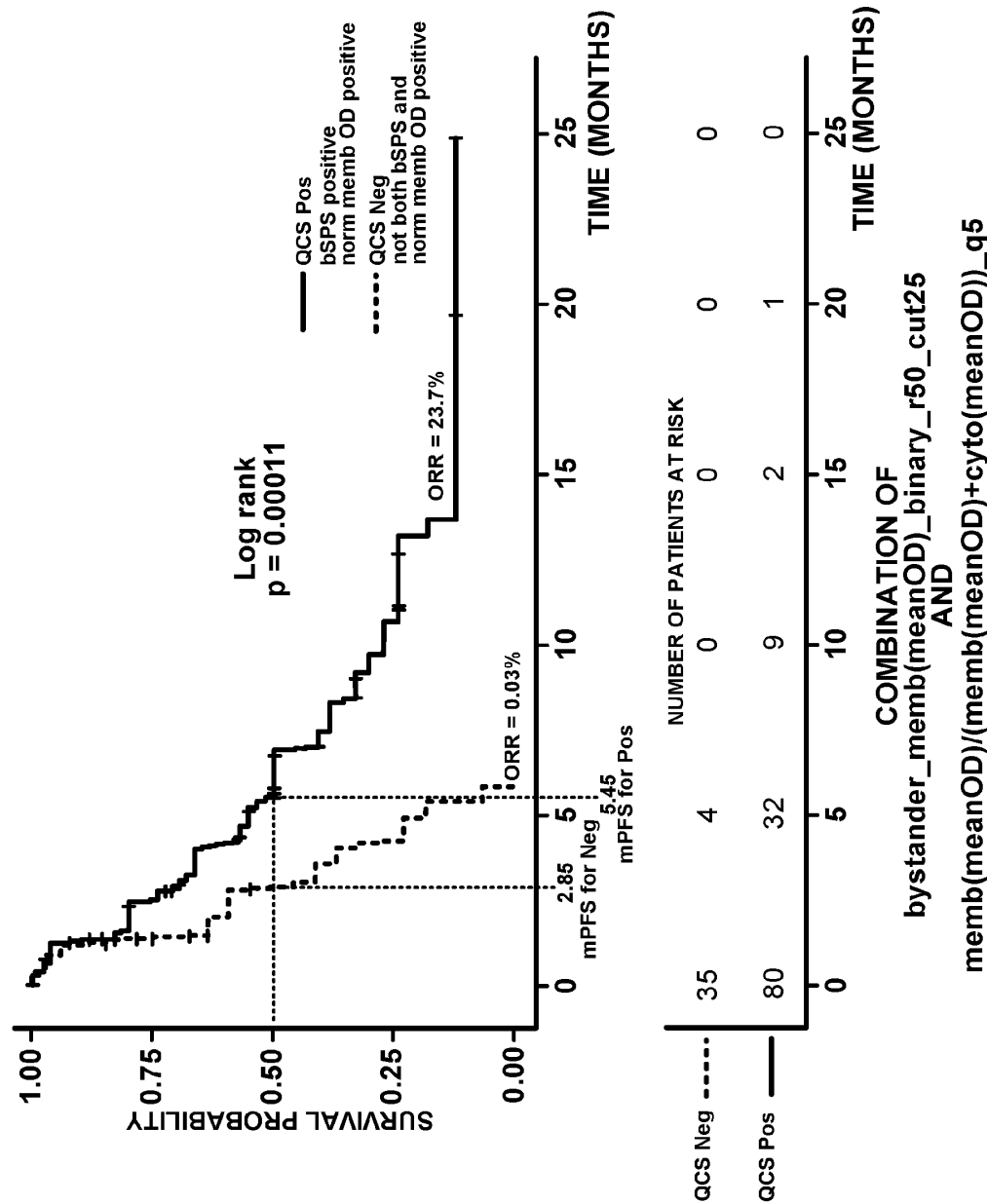
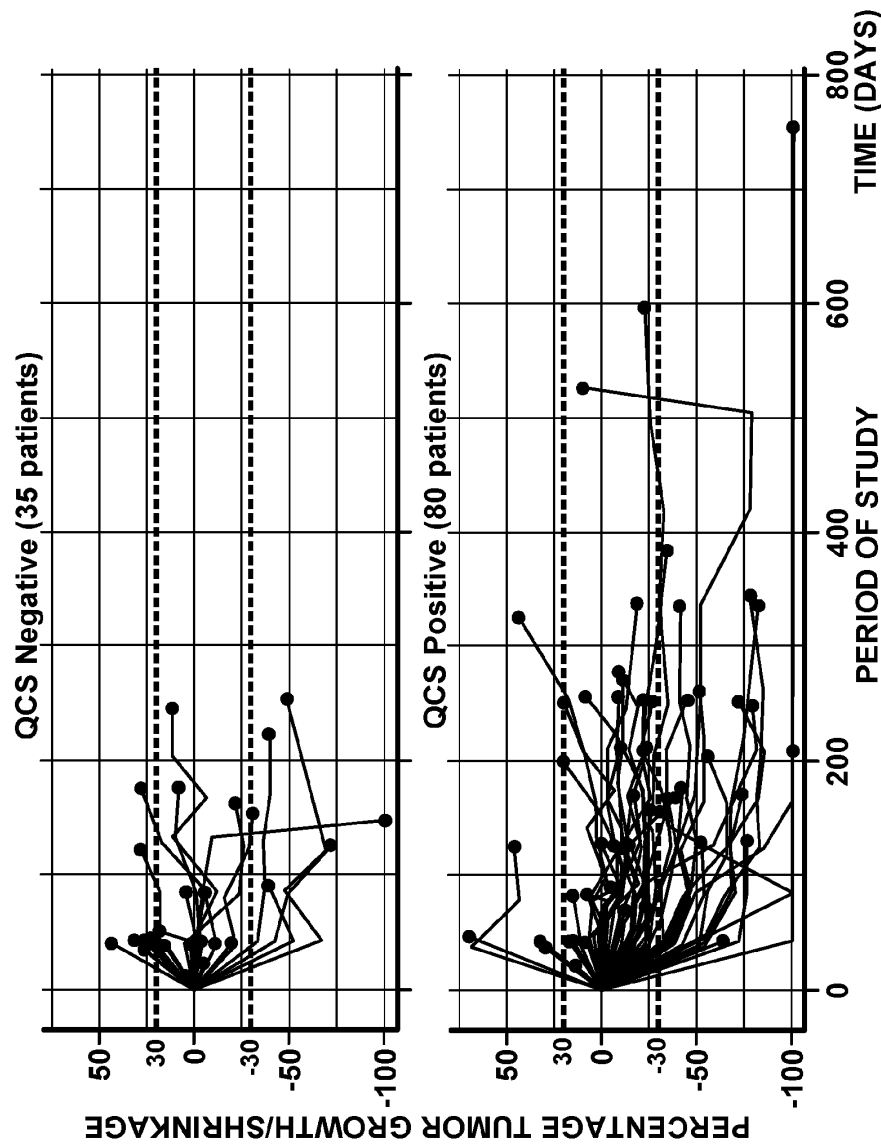


FIG. 34



COMBINATION OF bystander_memb(meanOD)_binary_r50_cut25
AND memb(meanOD)/(memb(meanOD)+cyto(meanOD))_q5
FIG. 35

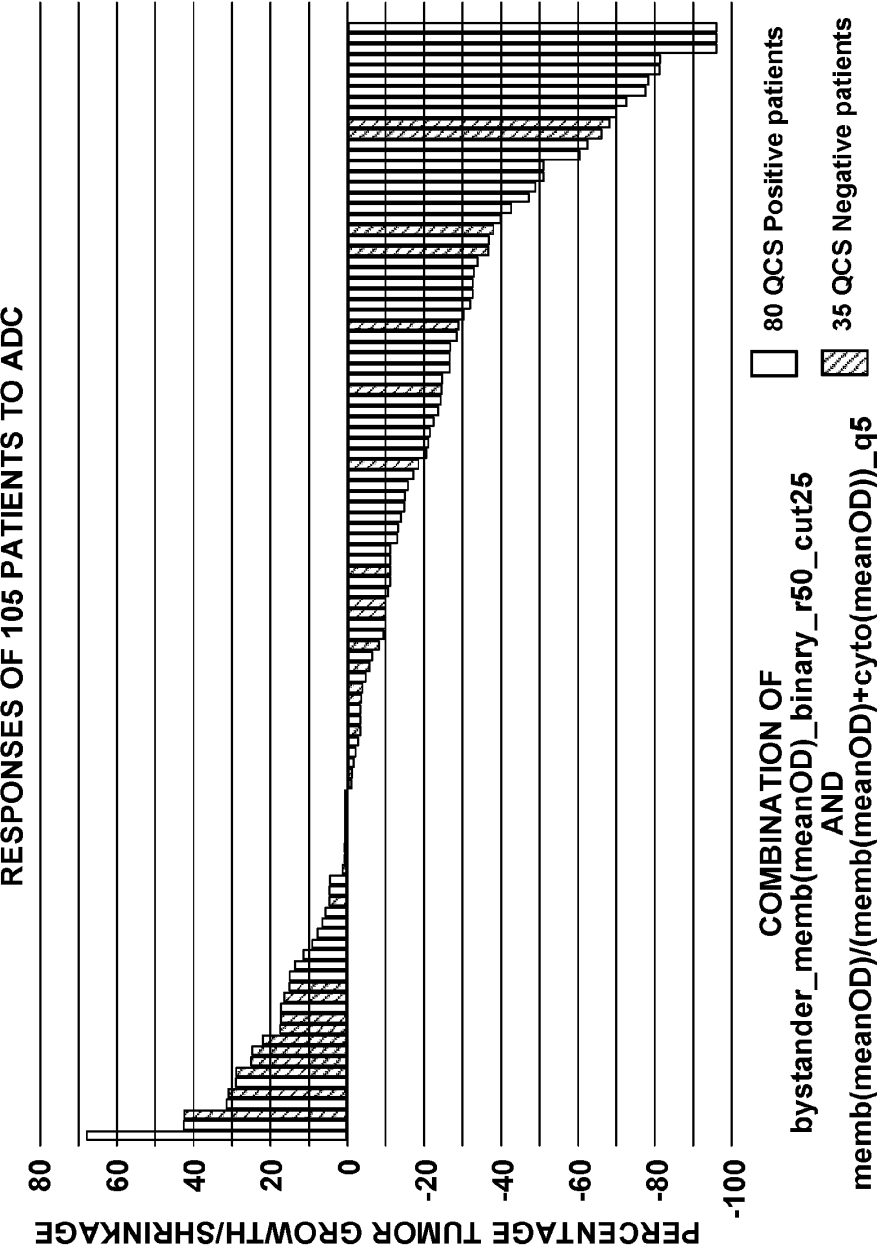


FIG. 36