Figure 1

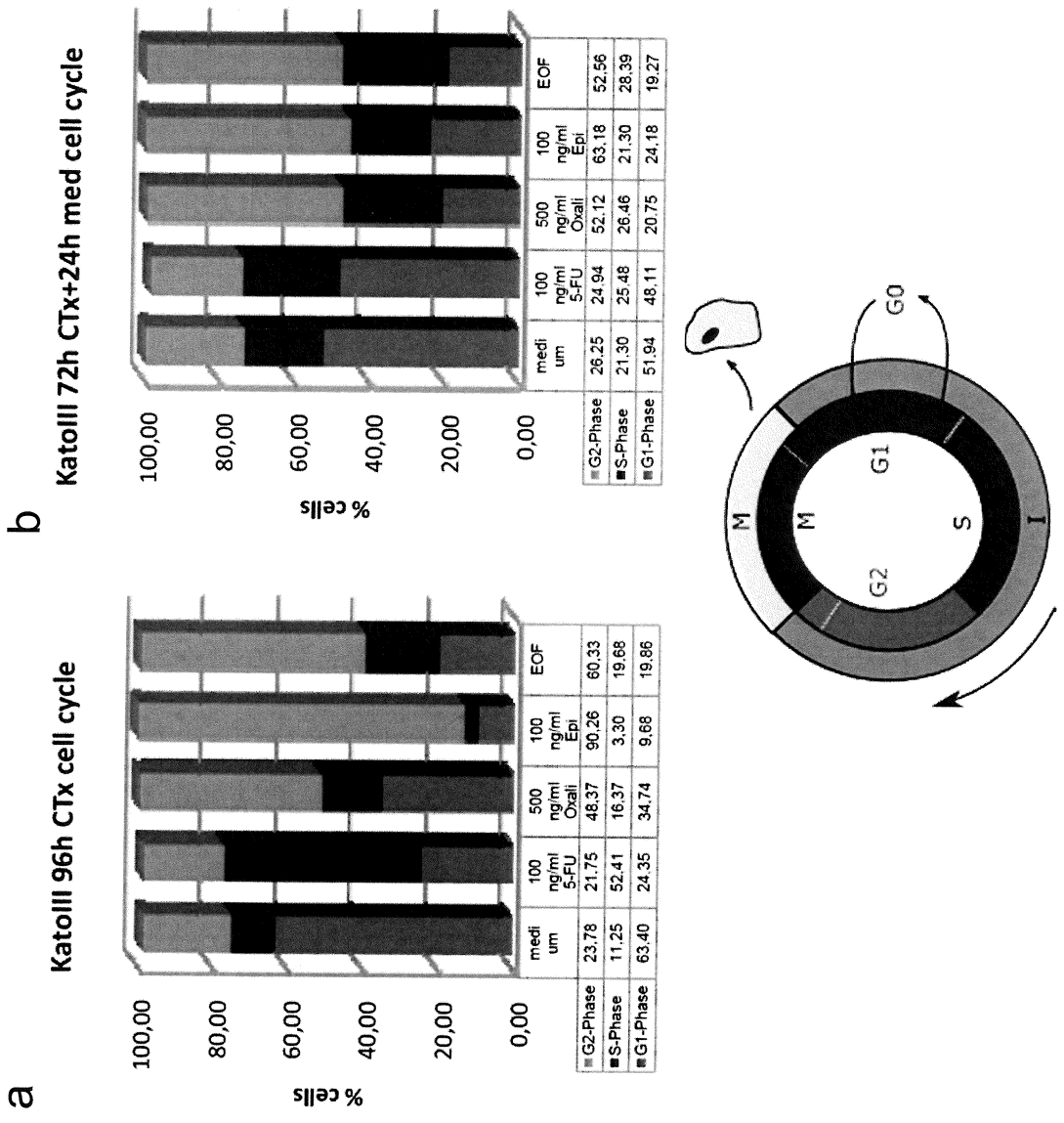

a
KatoIII 96h CTx cell cycle
% cells
100,00
80,00
60,00
40,00
20,00
0,00
medium
100 ng/ml 5-FU
500 ng/ml Oxali
100 ng/ml Epi
EOF
G2-Phase 23.78 21.75 48.37 90.26 60.33
S-Phase 11.25 52.41 16.37 3.30 19.68
G1-Phase 63.40 24.35 34.74 9.68 19.86
b
KatoIII 72h CTx+24h med cell cycle
% cells
100,00
80,00
60,00
40,00
20,00
0,00
medium
100 ng/ml 5-FU
500 ng/ml Oxali
100 ng/ml Epi
EOF
G2-Phase 26.25 24.94 52.12 63.18 52.56
S-Phase 21.30 25.48 26.46 21.30 28.39
G1-Phase 51.94 48.11 20.75 24.18 19.27
M
G2
M
G1
S
G0

Figure 1 continue

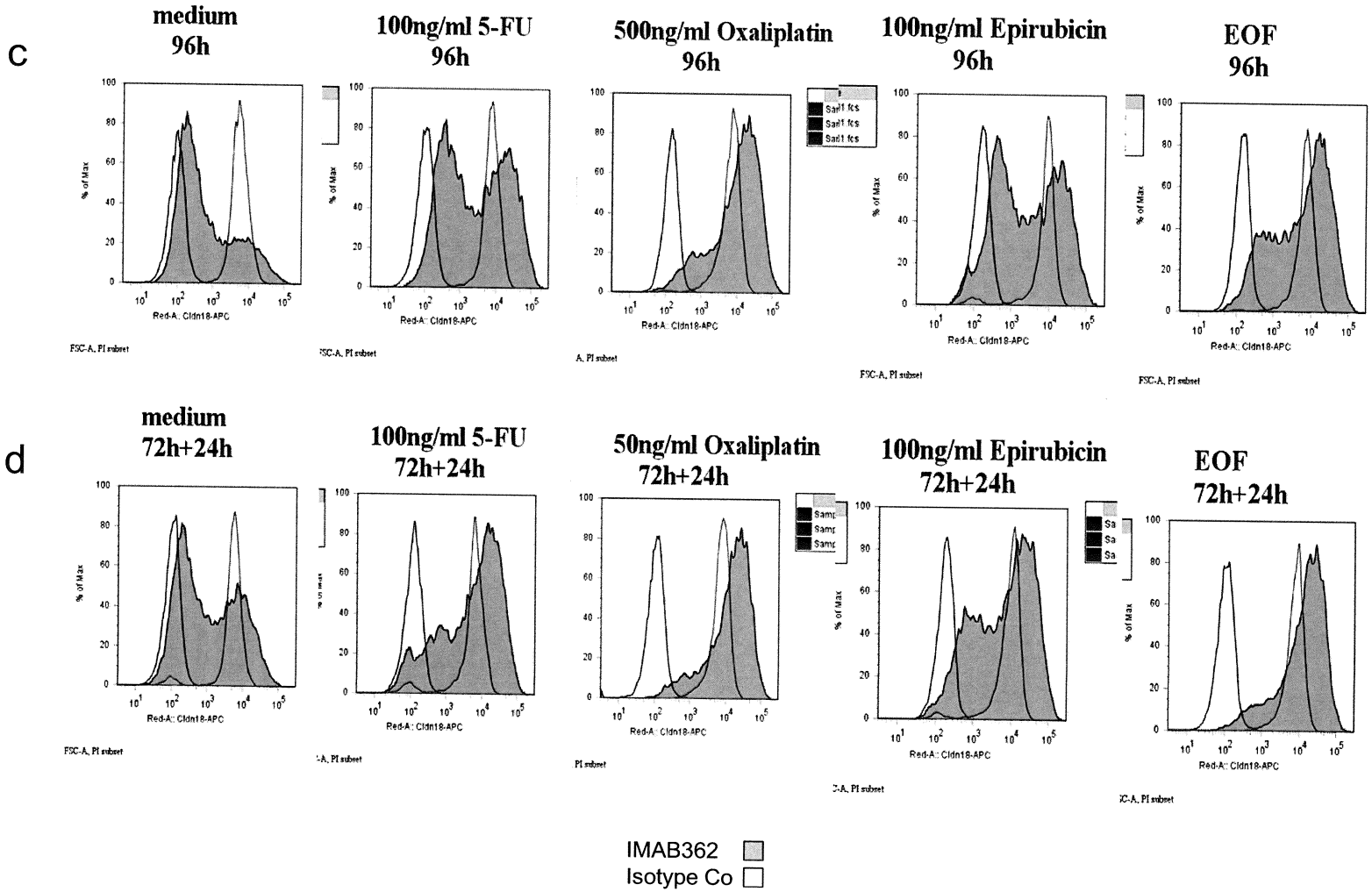


Figure 2

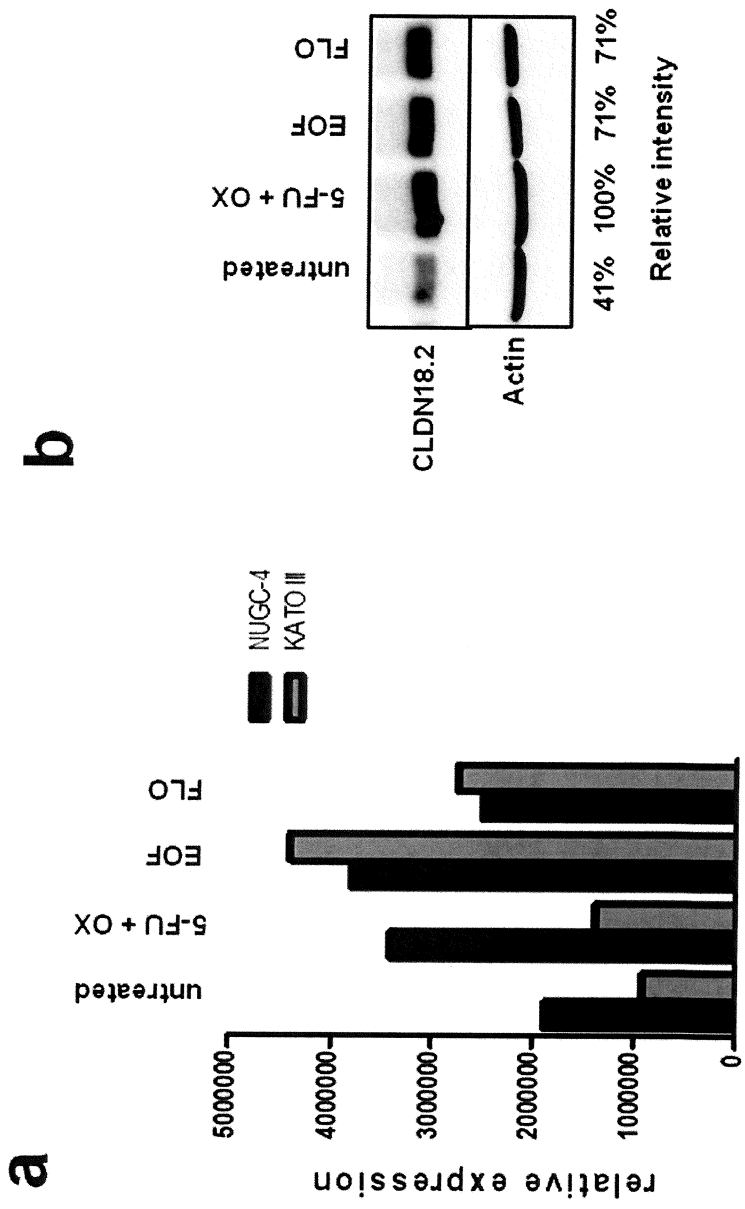

a
relative expression
5000000
4000000
3000000
2000000
1000000
0
untreated
5-FU + OX
EOF
FLO
NUGC-4
KATO III
b
CLDN18.2
Actin
untreated
5-FU + OX
EOF
FLO
41% 100% 71% 71%
Relative intensity

Figure 2 continue

C

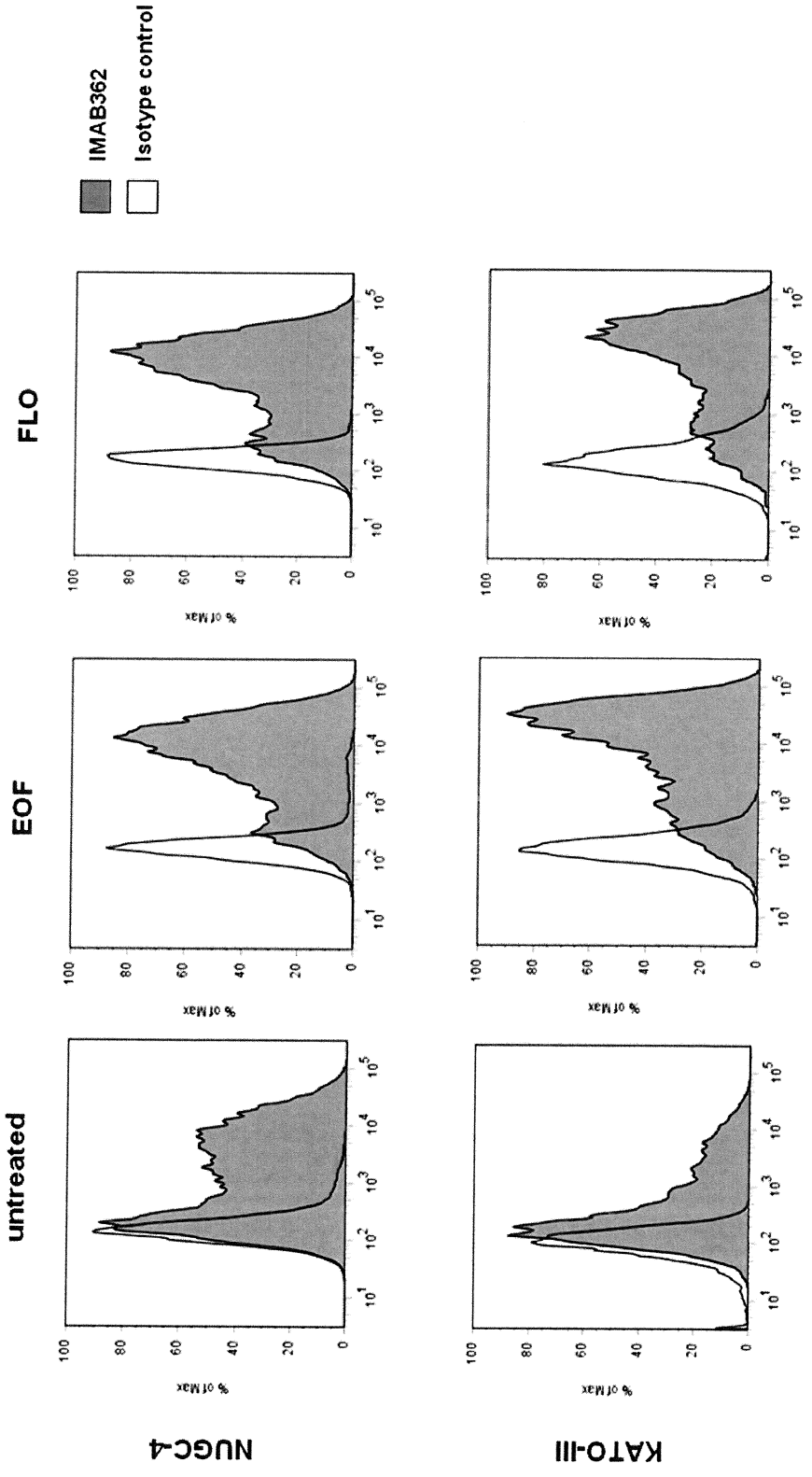


Figure 3

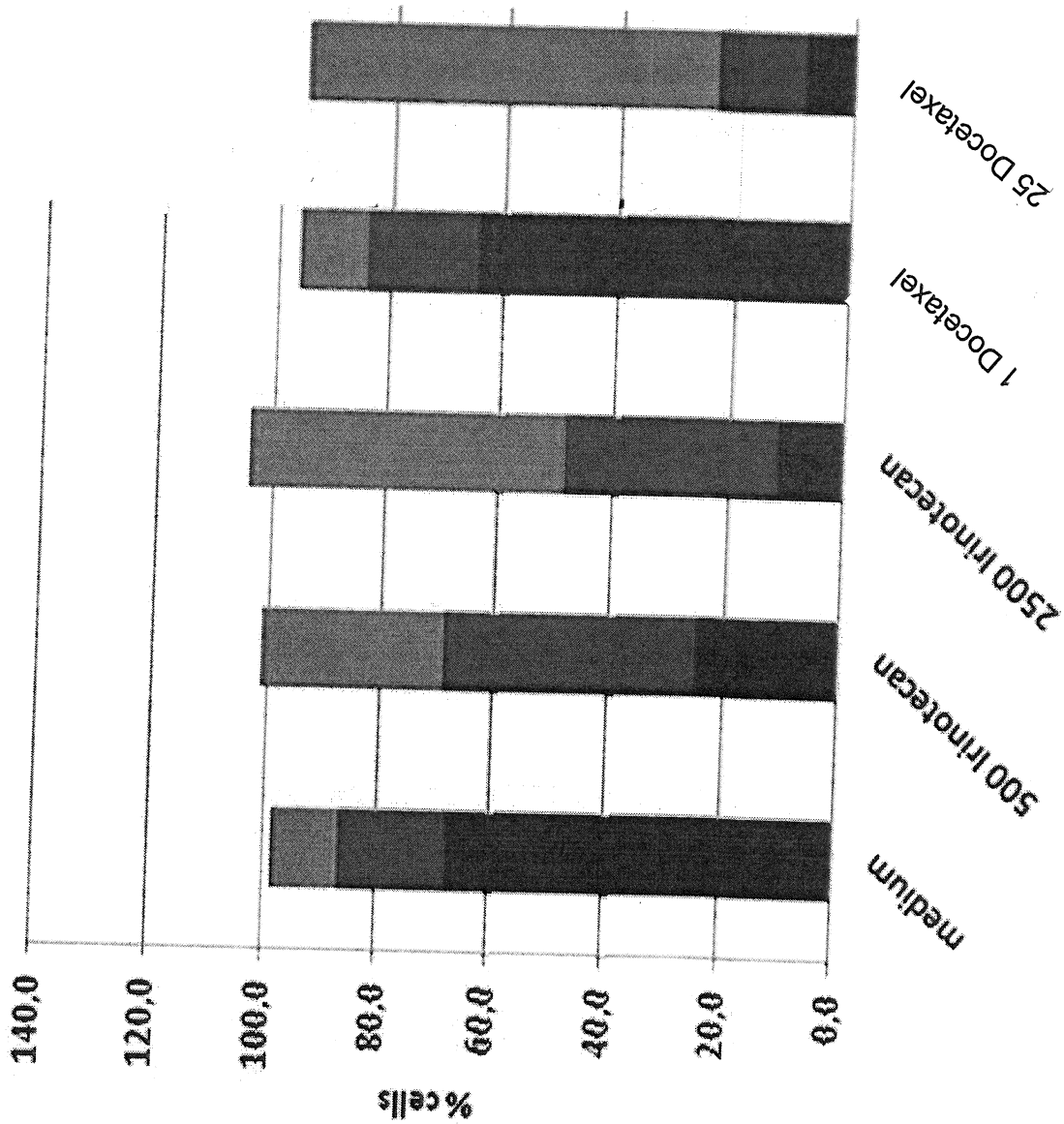

% cells
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
medium
500 Irinotecan
2500 Irinotecan
1 Docetaxel
25 Docetaxel

Figure 4

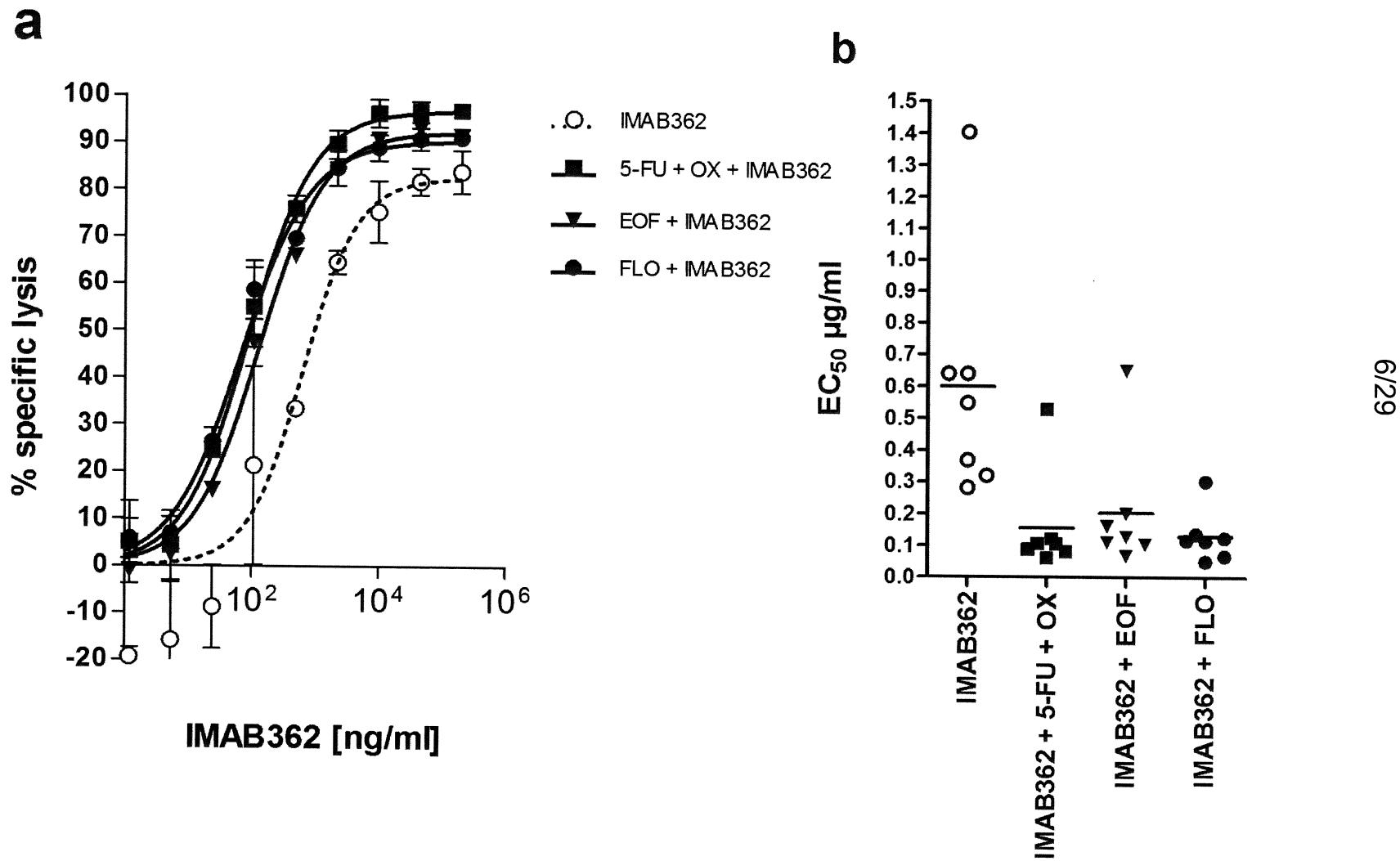

a
% specific lysis
100
90
80
70
60
50
40
30
20
10
0
-10
-20
10^2
10^4
10^6
IMAB362 [ng/ml]
IMAB362
5-FU + OX + IMAB362
EOF + IMAB362
FLO + IMAB362
b
EC_{50} µg/ml
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
IMAB362
IMAB362 + 5-FU + OX
IMAB362 + EOF
IMAB362 + FLO

Figure 5

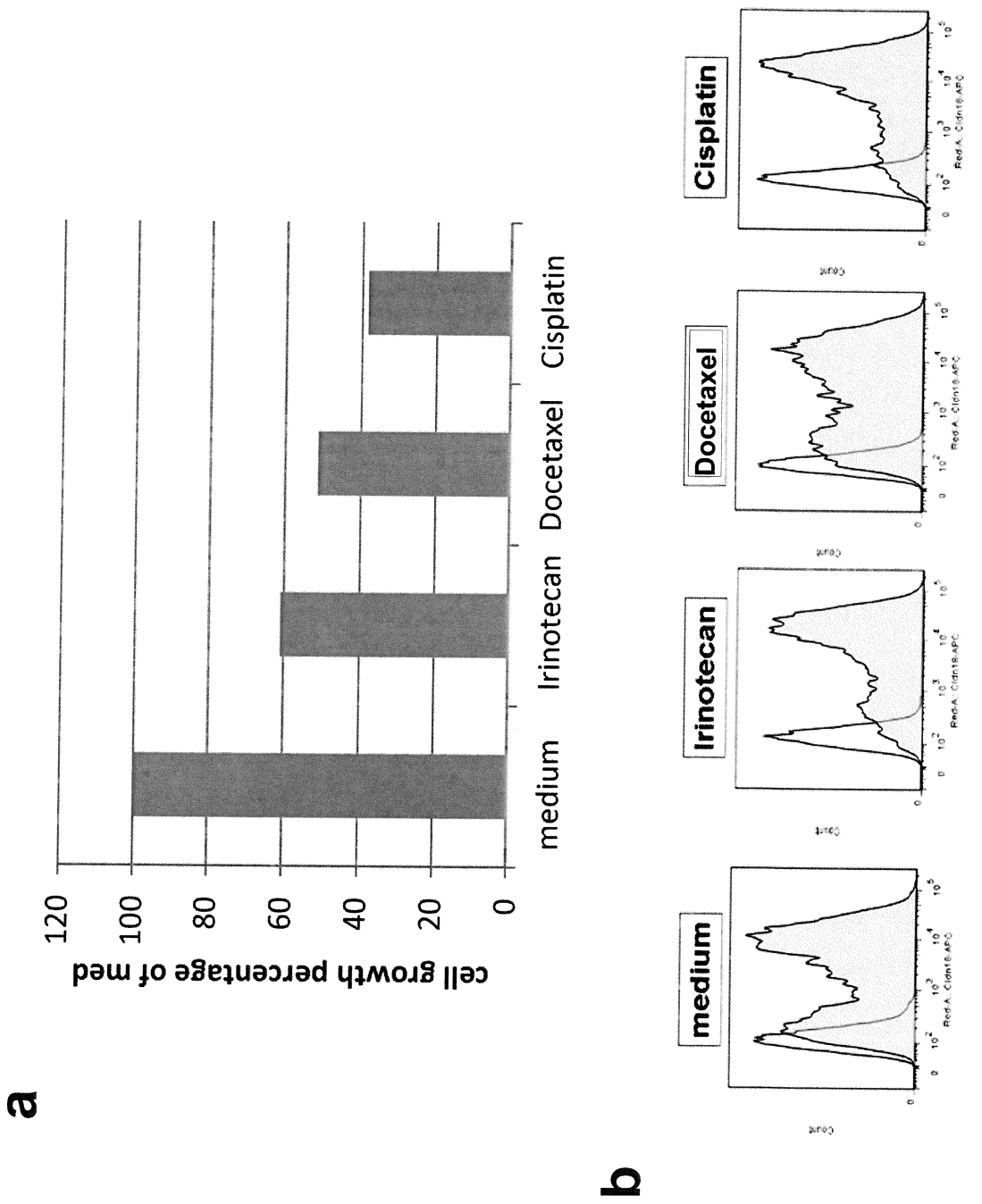

a
cell growth percentage of med
120
100
80
60
40
20
0
medium
Irinotecan
Docetaxel
Cisplatin
b
medium
Irinotecan
Docetaxel
Cisplatin
Count
Red-A: Cldn18-APC

Figure 5 continue

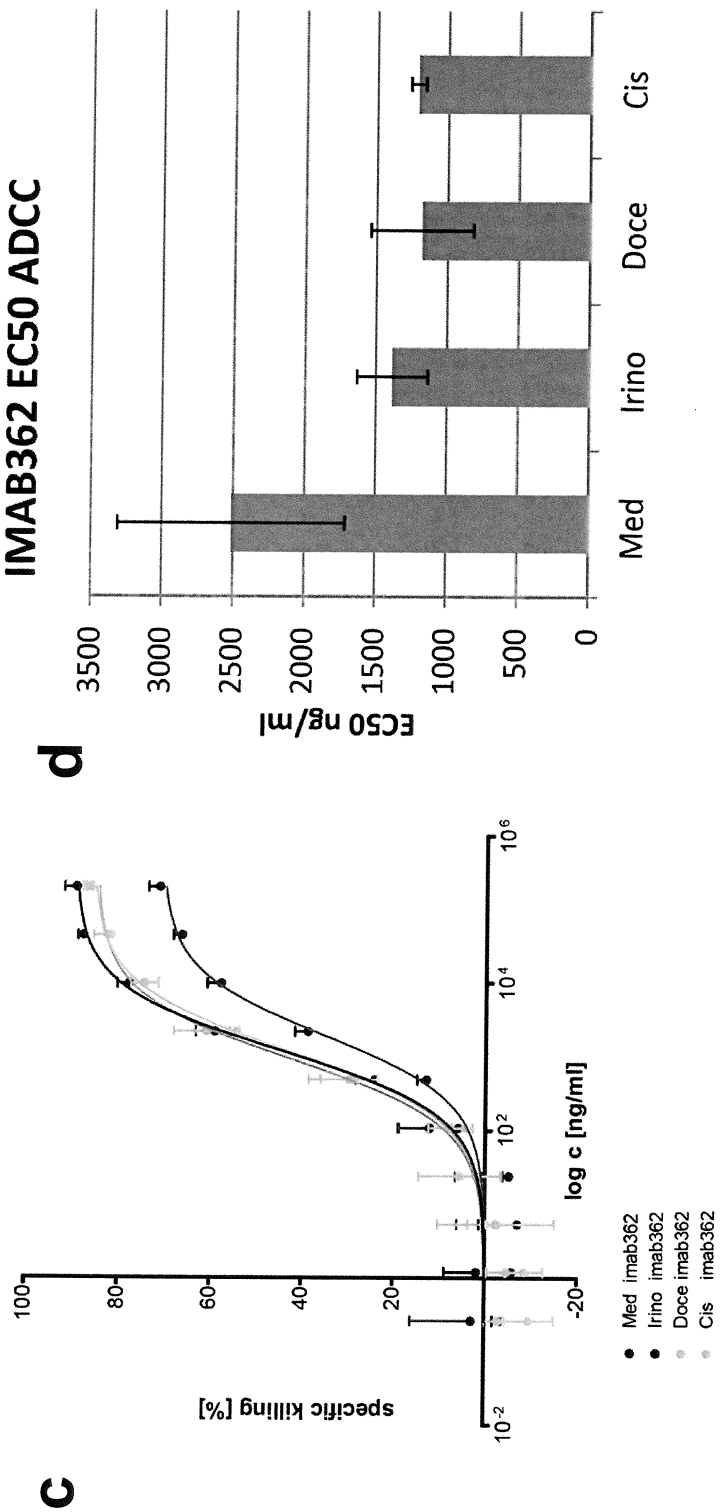


Figure 6

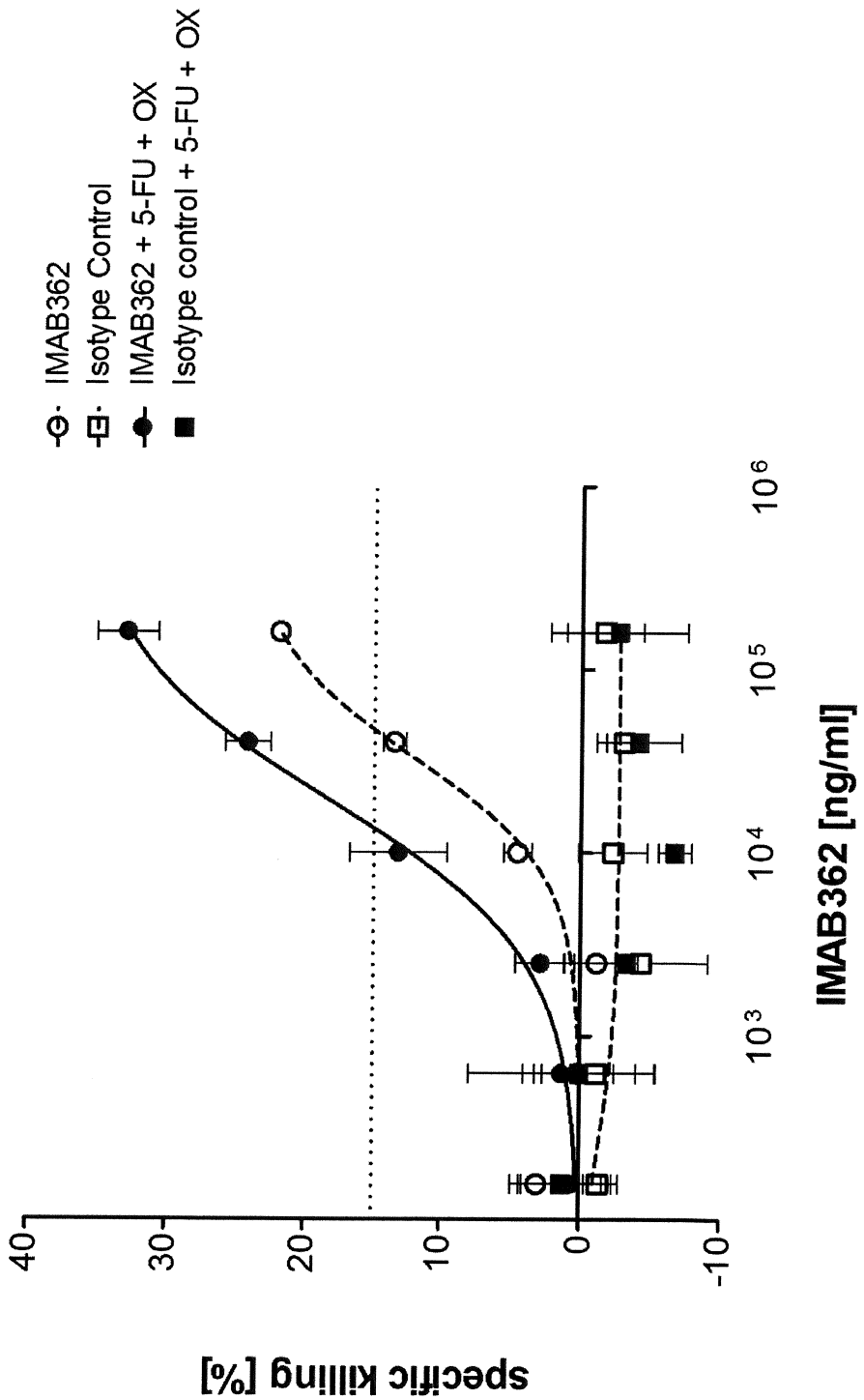

IMAB362
Isotype Control
IMAB362 + 5-FU + OX
Isotype control + 5-FU + OX
specific killing [%]
40
30
20
10
0
-10
10³
10⁴
10⁵
10⁶
IMAB362 [ng/ml]

Figure 7

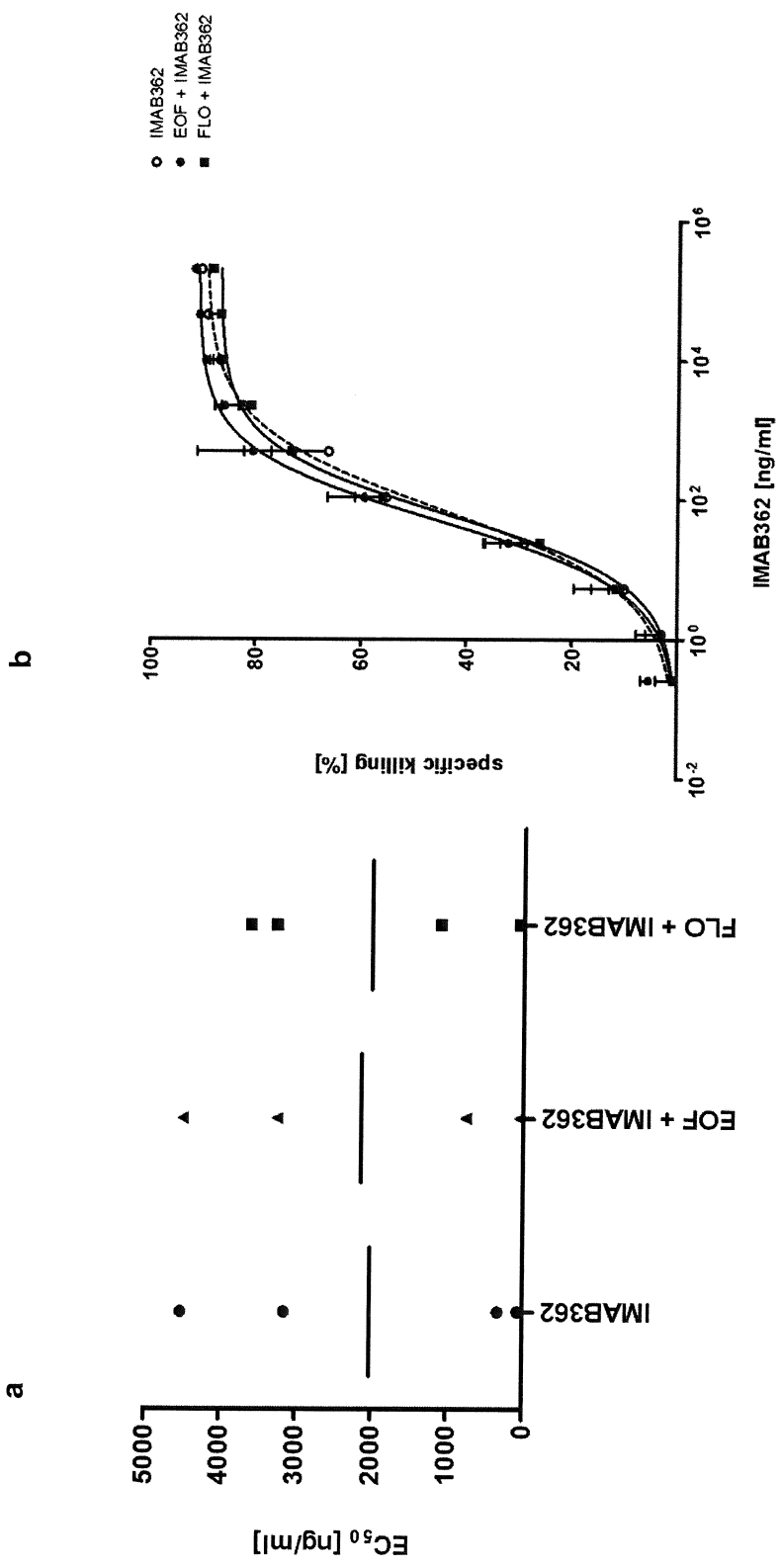

a
EC_{50} [ng/ml]
5000
4000
3000
2000
1000
0
IMAB362
EOF + IMAB362
FLO + IMAB362
b
specific killing [%]
100
80
60
40
20
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
IMAB362 [ng/ml]
IMAB362
EOF + IMAB362
FLO + IMAB362

Figure 8

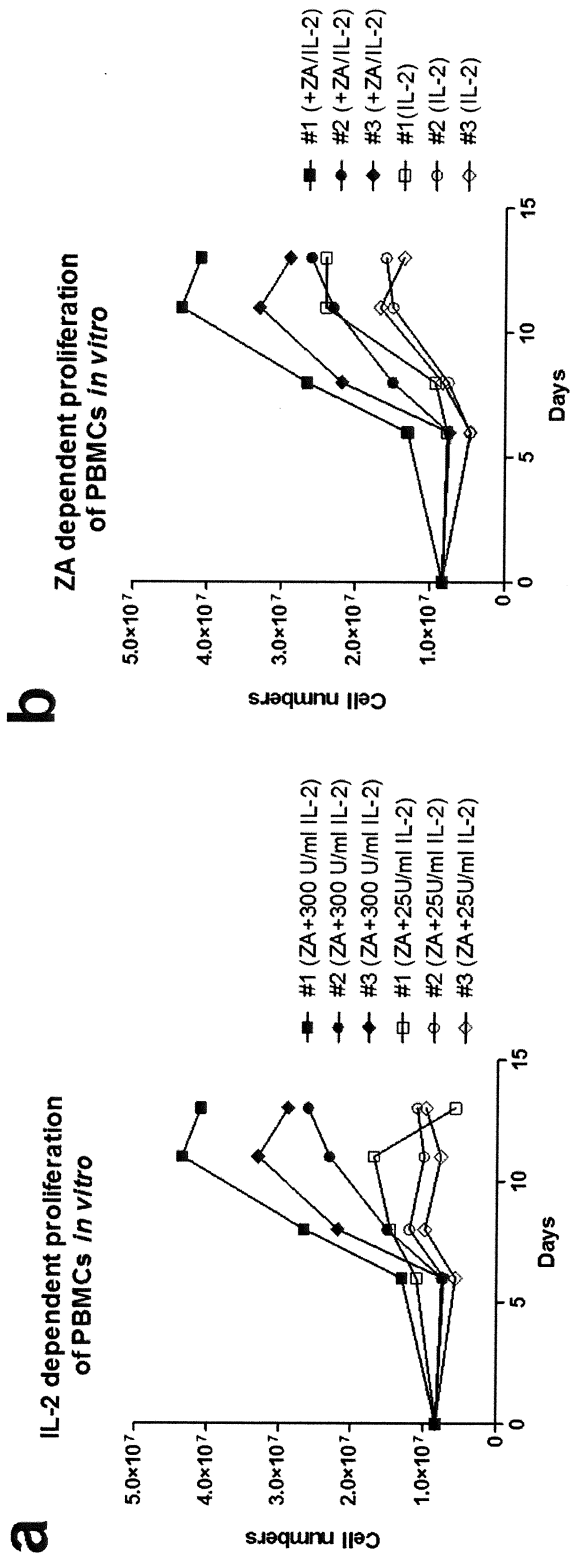

a
IL-2 dependent proliferation of PBMCs in vitro
Cell numbers
5.0×10^7
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0
0
5
10
15
Days
#1 (ZA+300 U/ml IL-2)
#2 (ZA+300 U/ml IL-2)
#3 (ZA+300 U/ml IL-2)
#1 (ZA+25U/ml IL-2)
#2 (ZA+25U/ml IL-2)
#3 (ZA+25U/ml IL-2)
b
ZA dependent proliferation of PBMCs in vitro
Cell numbers
5.0×10^7
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0
0
5
10
15
Days
#1 (+ZA/IL-2)
#2 (+ZA/IL-2)
#3 (+ZA/IL-2)
#1(IL-2)
#2 (IL-2)
#3 (IL-2)

Figure 9

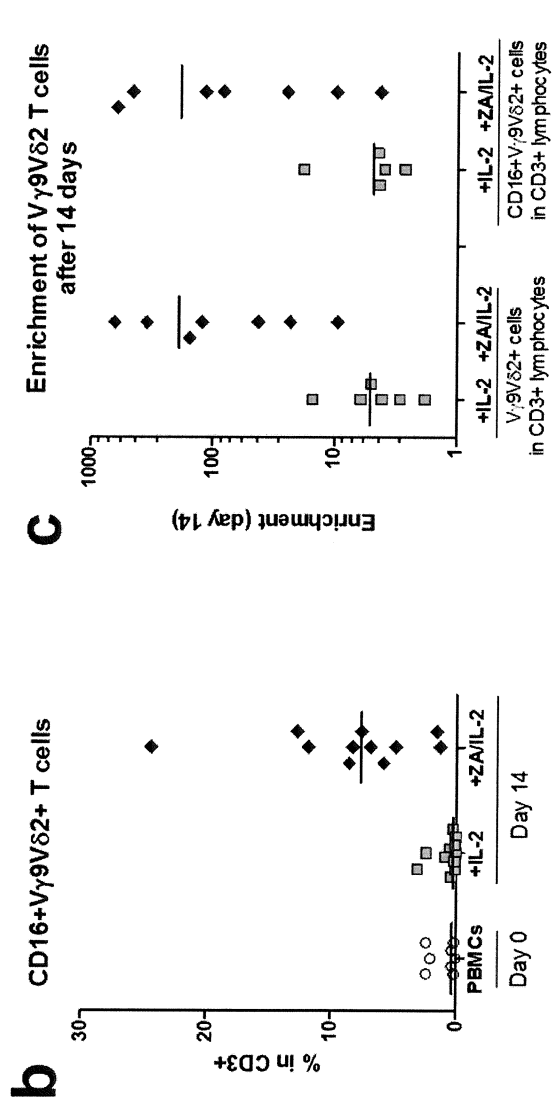
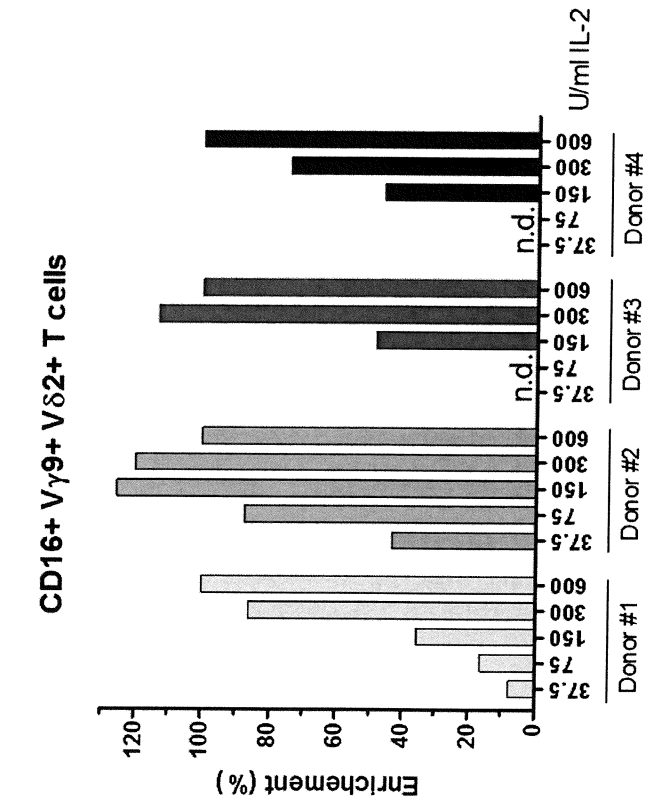

a
Vγ9+Vδ2+ T cells
% in CD3+
100
80
60
40
20
0
PBMCs
+IL-2
+ZA/IL-2
Day 0
Day 14
b
CD16+Vγ9Vδ2+ T cells
% in CD3+
30
20
10
0
PBMCs
+IL-2
+ZA/IL-2
Day 0
Day 14
c
Enrichment of Vγ9Vδ2 T cells after 14 days
Enrichment (day 14)
1000
100
10
1
+IL-2
+ZA/IL-2
+IL-2
+ZA/IL-2
Vγ9Vδ2+ cells in CD3+ lymphocytes
CD16+Vγ9Vδ2+ cells in CD3+ lymphocytes

Figure 10


CD16+ Vγ9+ Vδ2+ T cells
Enrichement (%)
0
20
40
60
80
100
120
Donor #1
Donor #2
Donor #3
Donor #4
37.5
75
150
300
600
n.d.
U/ml IL-2

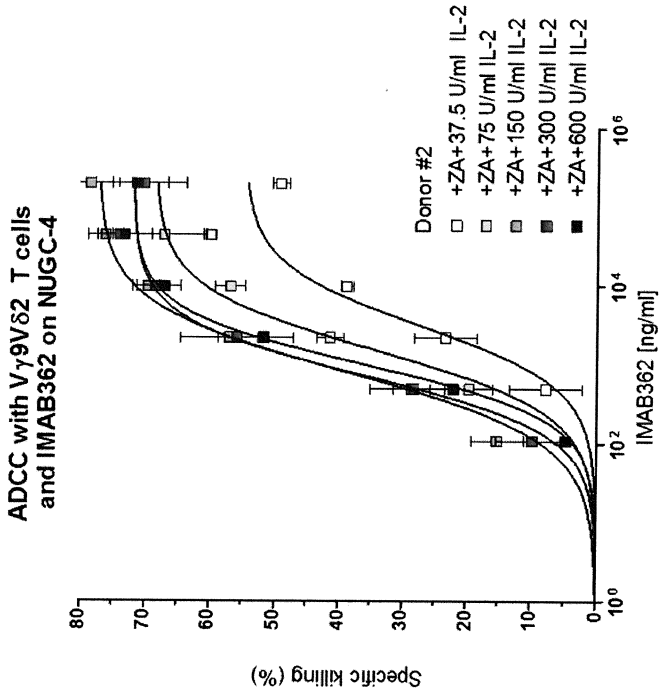

ADCC with Vγ9Vδ2 T cells
and IMAB362 on NUGC-4
Specific killing (%)
IMAB362 [ng/ml]
Donor #2
+ZA+37.5 U/ml IL-2
+ZA+75 U/ml IL-2
+ZA+150 U/ml IL-2
+ZA+300 U/ml IL-2
+ZA+600 U/ml IL-2

Figure 11

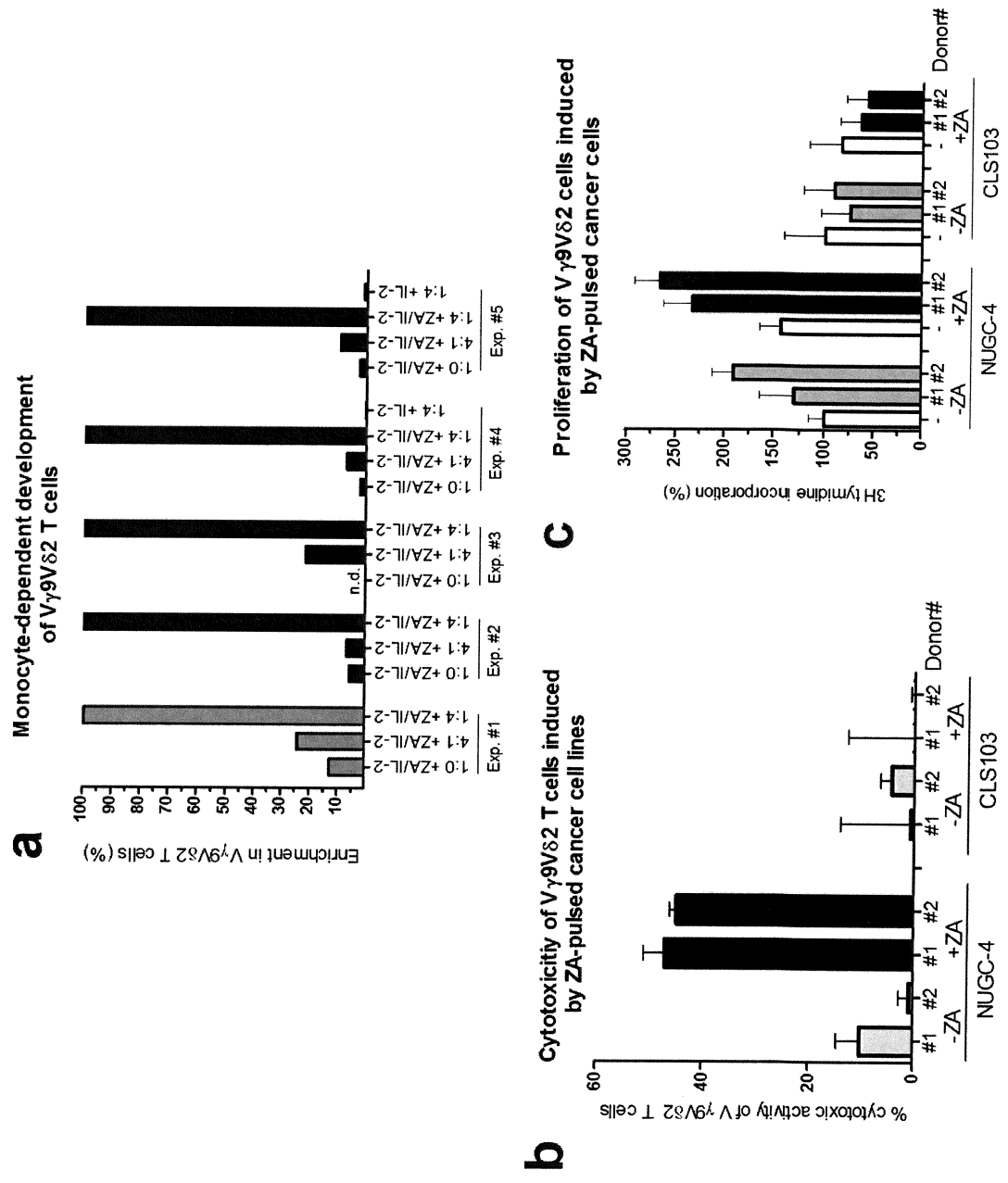

a
Monocyte-dependent development of Vγ9Vδ2 T cells
Enrichment in Vγ9Vδ2 T cells (%)
1:0 +ZA/IL-2
4:1 +ZA/IL-2
1:4 +ZA/IL-2
Exp. #1
Exp. #2
n.d.
Exp. #3
Exp. #4
Exp. #5
1:4 +IL-2
b
Cytotoxicity of Vγ9Vδ2 T cells induced by ZA-pulsed cancer cell lines
% cytotoxic activity of V γ9Vδ2 T cells
NUGC-4
CLS103
-ZA
+ZA
#1
#2
Donor#
c
Proliferation of Vγ9Vδ2 cells induced by ZA-pulsed cancer cells
3H tymidine incorporation (%)

Figure 12

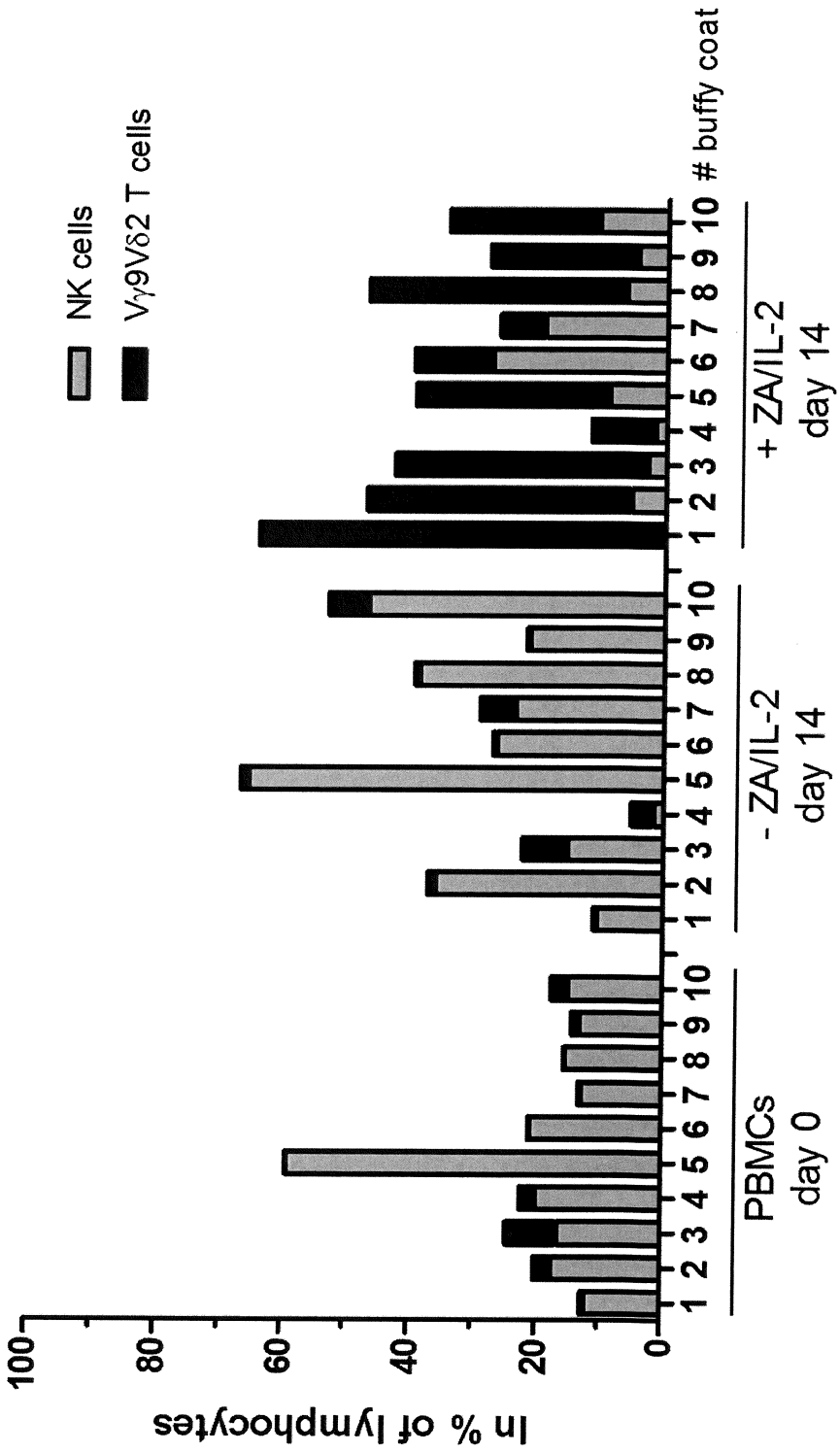

Cell types
NK cells
Vγ9Vδ2 T cells
In % of lymphocytes
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 10 # buffy coat
PBMCs
day 0
- ZA/IL-2
day 14
+ ZA/IL-2
day 14

Figure 13

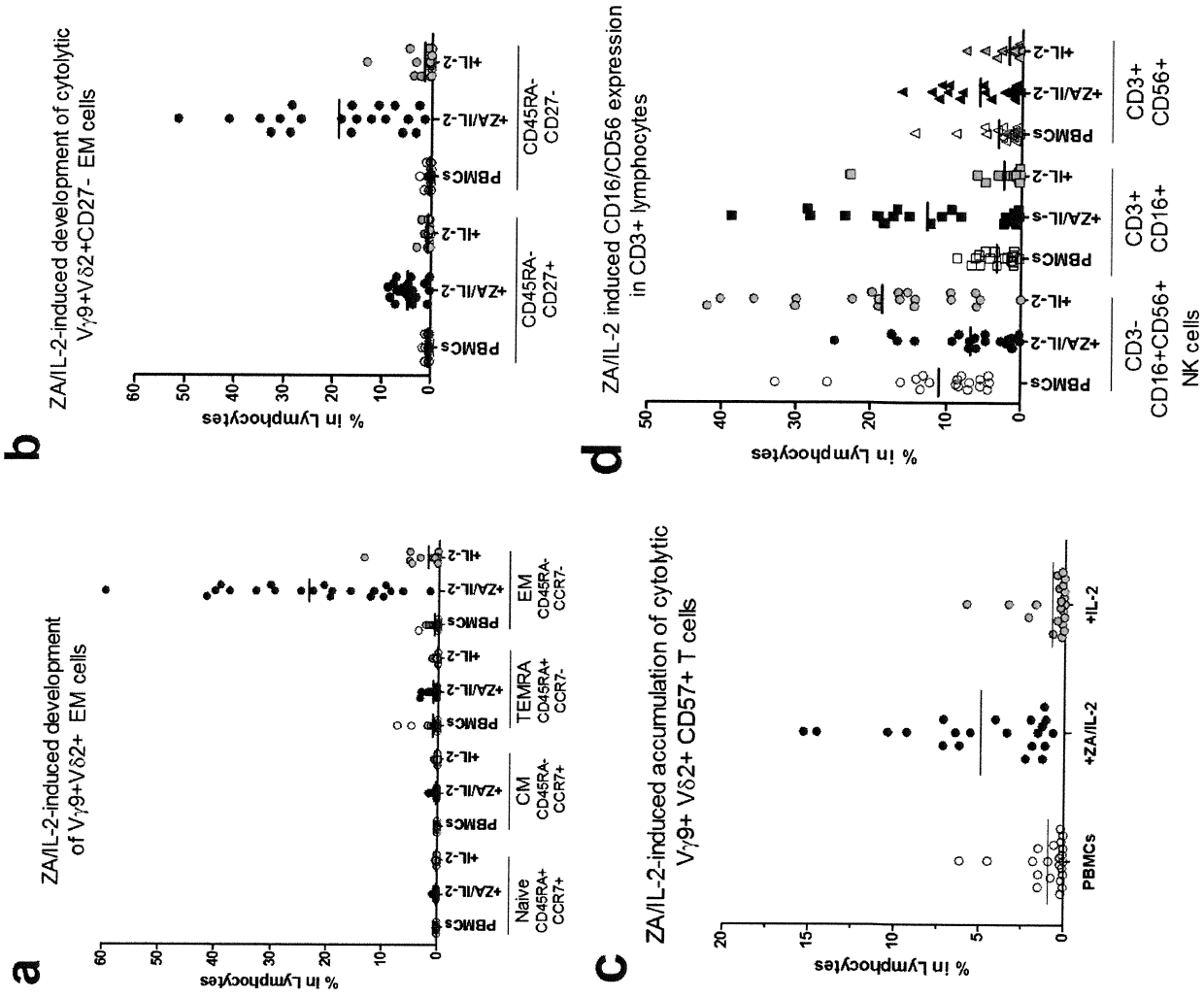

a
ZA/IL-2-induced development of Vγ9+Vδ2+ EM cells
% in Lymphocytes
PBMCs
+ZA/IL-2
+IL-2
Naive CD45RA+ CCR7+
CM CD45RA- CCR7+
TEMRA CD45RA+ CCR7-
EM CD45RA- CCR7-
b
ZA/IL-2-induced development of cytolytic Vγ9+Vδ2+CD27- EM cells
% in Lymphocytes
CD45RA- CD27+
CD45RA- CD27-
c
ZA/IL-2-induced accumulation of cytolytic Vγ9+ Vδ2+ CD57+ T cells
% in Lymphocytes
PBMCs
+ZA/IL-2
+IL-2
d
ZA/IL-2 induced CD16/CD56 expression in CD3+ lymphocytes
% in Lymphocytes
CD3- CD16+CD56+ NK cells
CD3+ CD16+
CD3+ CD56+

Figure 14

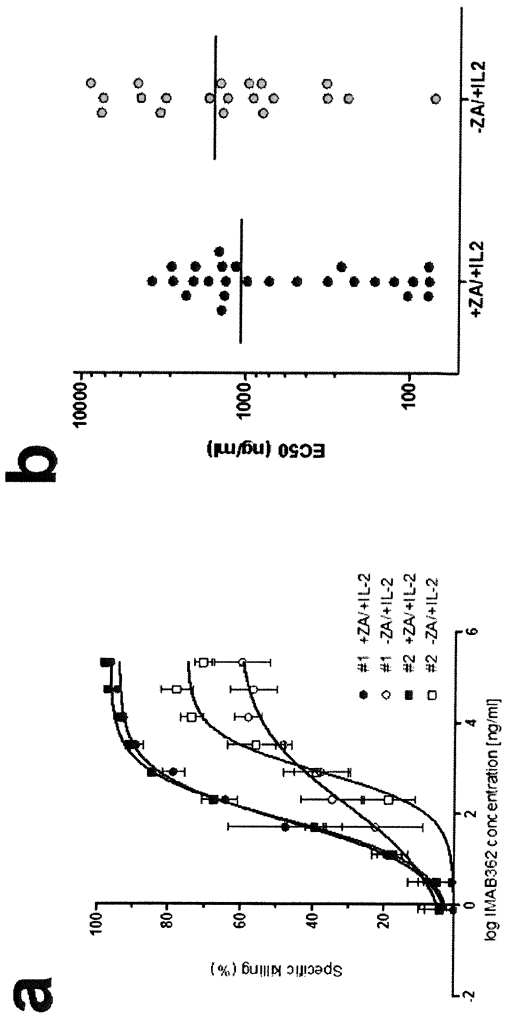

a
Specific killing (%)
log IMAB362 concentration [ng/ml]
#1 +ZA/+IL-2
#1 -ZA/+IL-2
#2 +ZA/+IL-2
#2 -ZA/+IL-2
b
EC50 (ng/ml)
+ZA/+IL2
-ZA/+IL2
c
Max. specific killing (%)
+ZA/+IL2
-ZA/+IL2

Figure 15

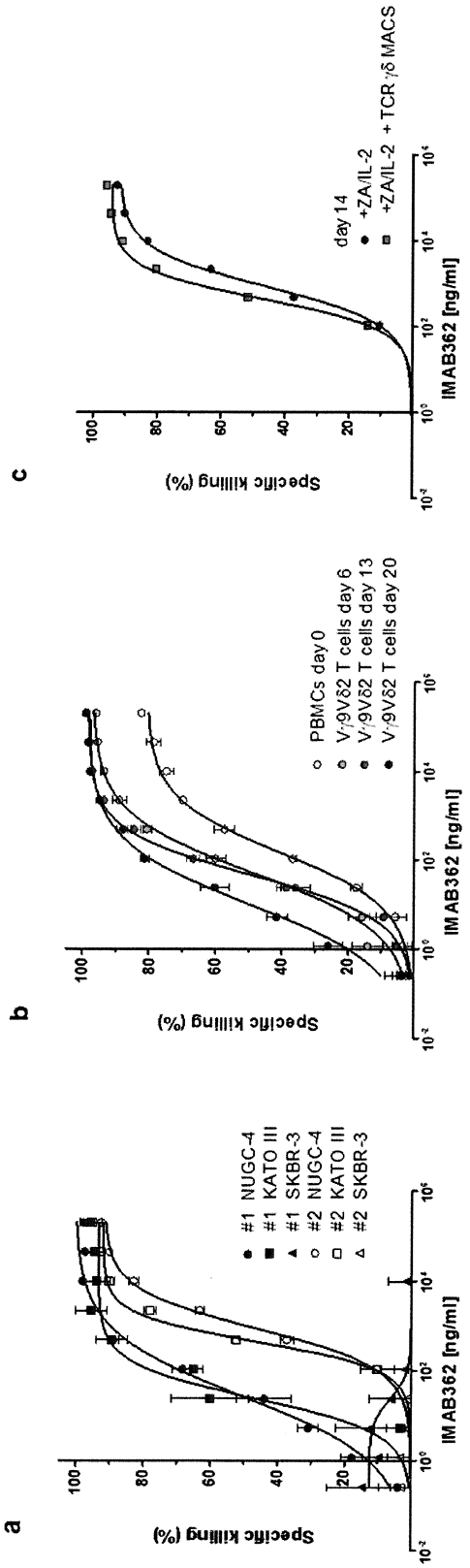
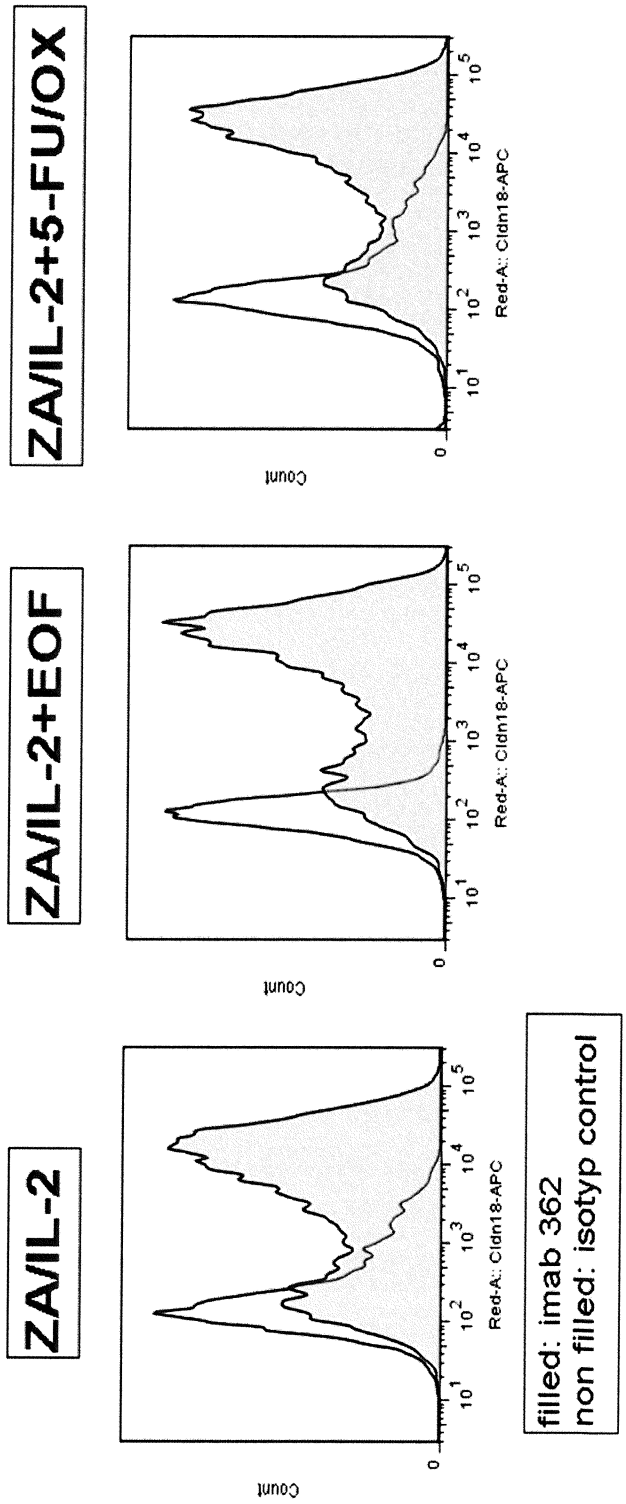

a
Specific killing (%)
IMAB362 [ng/ml]
#1 NUGC-4
#1 KATO III
#1 SKBR-3
#2 NUGC-4
#2 KATO III
#2 SKBR-3
b
Specific killing (%)
IMAB362 [ng/ml]
PBMCs day 0
Vγ9Vδ2 T cells day 6
Vγ9Vδ2 T cells day 13
Vγ9Vδ2 T cells day 20
c
Specific killing (%)
IMAB362 [ng/ml]
day 14
+ZA/IL-2
+ZA/IL-2 + TCR γδ MACS

Figure 16


ZA/IL-2
ZA/IL-2+EOF
ZA/IL-2+5-FU/OX
Count
Red-A:: Cldn18-APC
filled: imab 362
non filled: isotyp control

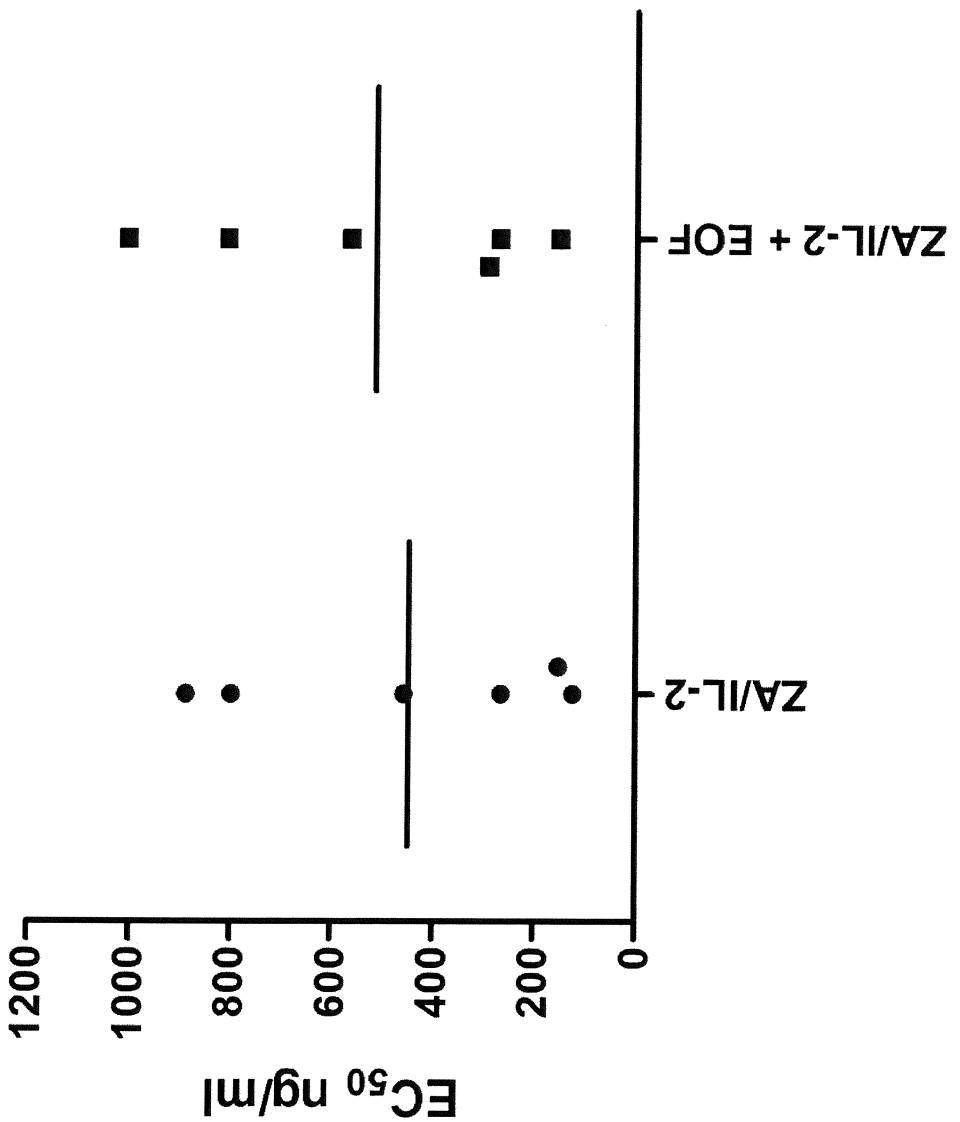
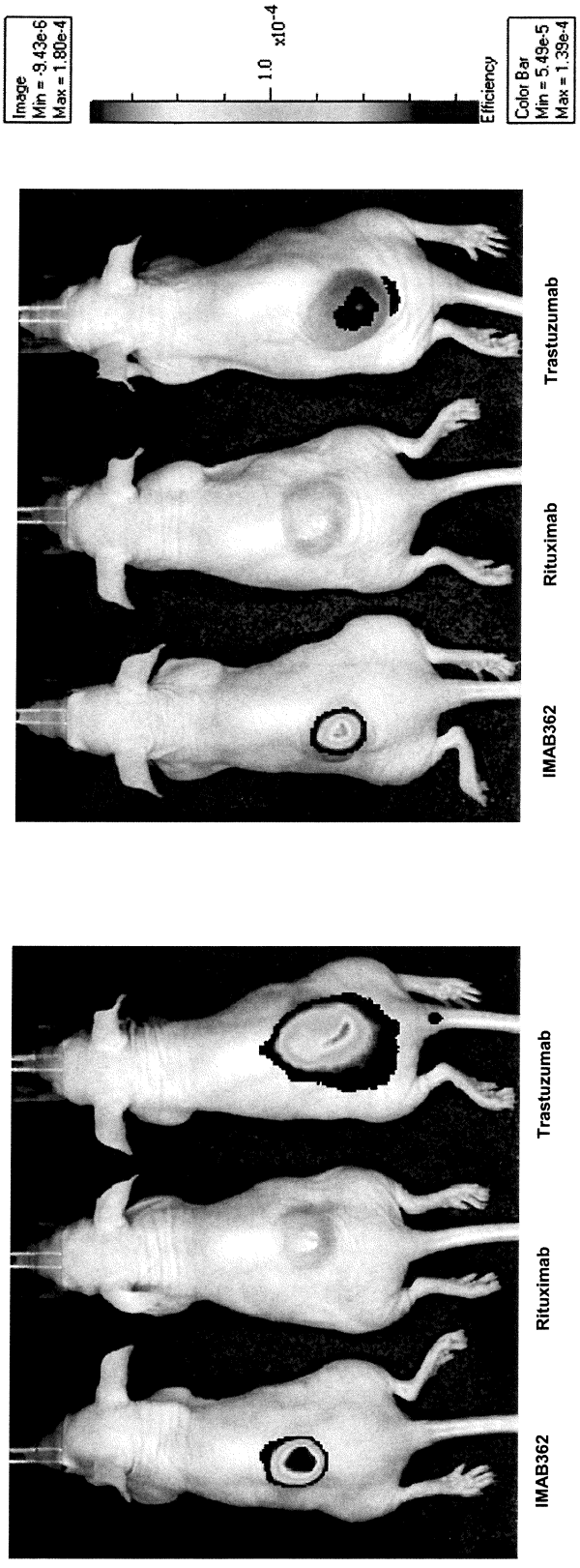


Figure 17

Figure 18


24 h
120 h
IMAB362
Rituximab
Trastuzumab
IMAB362
Rituximab
Trastuzumab
Image
Min = -9.43e-6
Max = 1.80e-4
1.0 x10-4
Efficiency
Color Bar
Min = 5.49e-5
Max = 1.39e-4

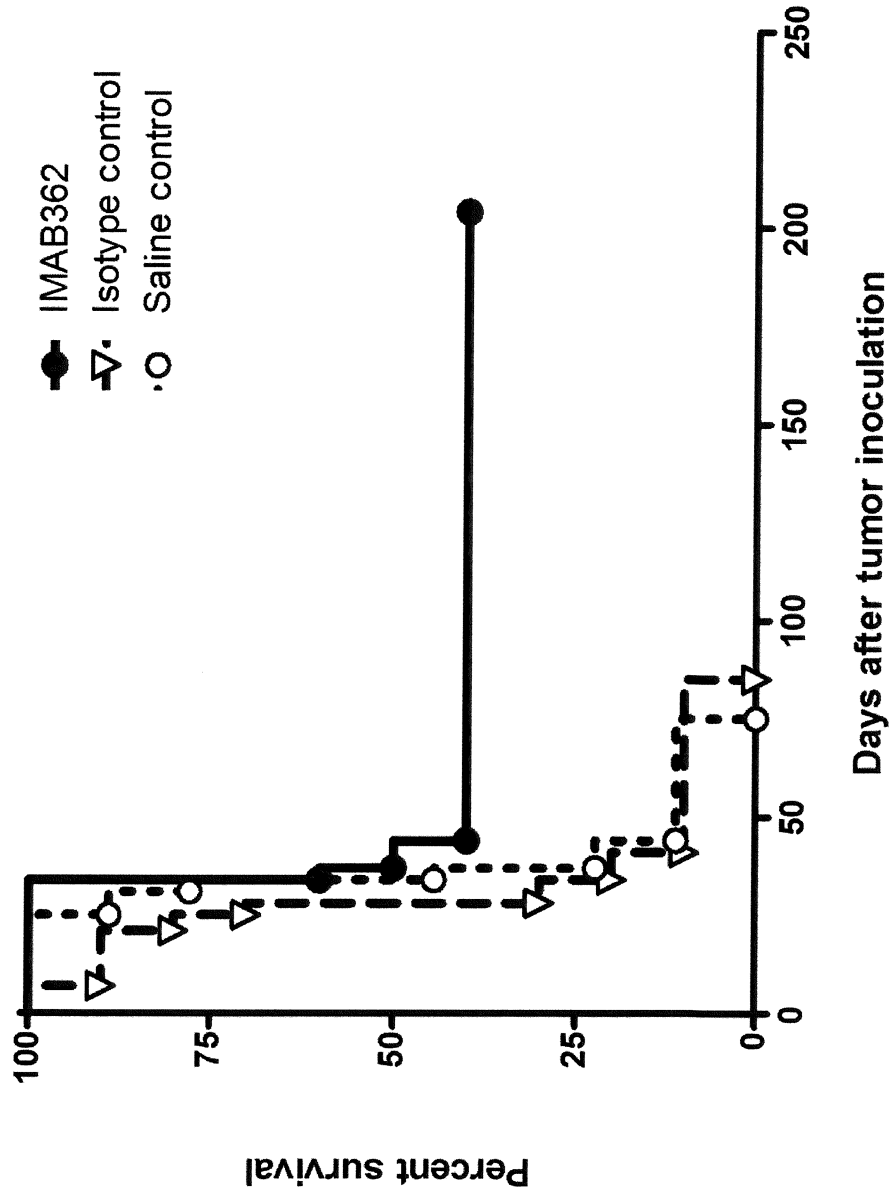

IMAB362
Isotype control
Saline control
Percent survival
100
75
50
25
0
0
50
100
150
200
250
Days after tumor inoculation

Figure 19

Figure 20

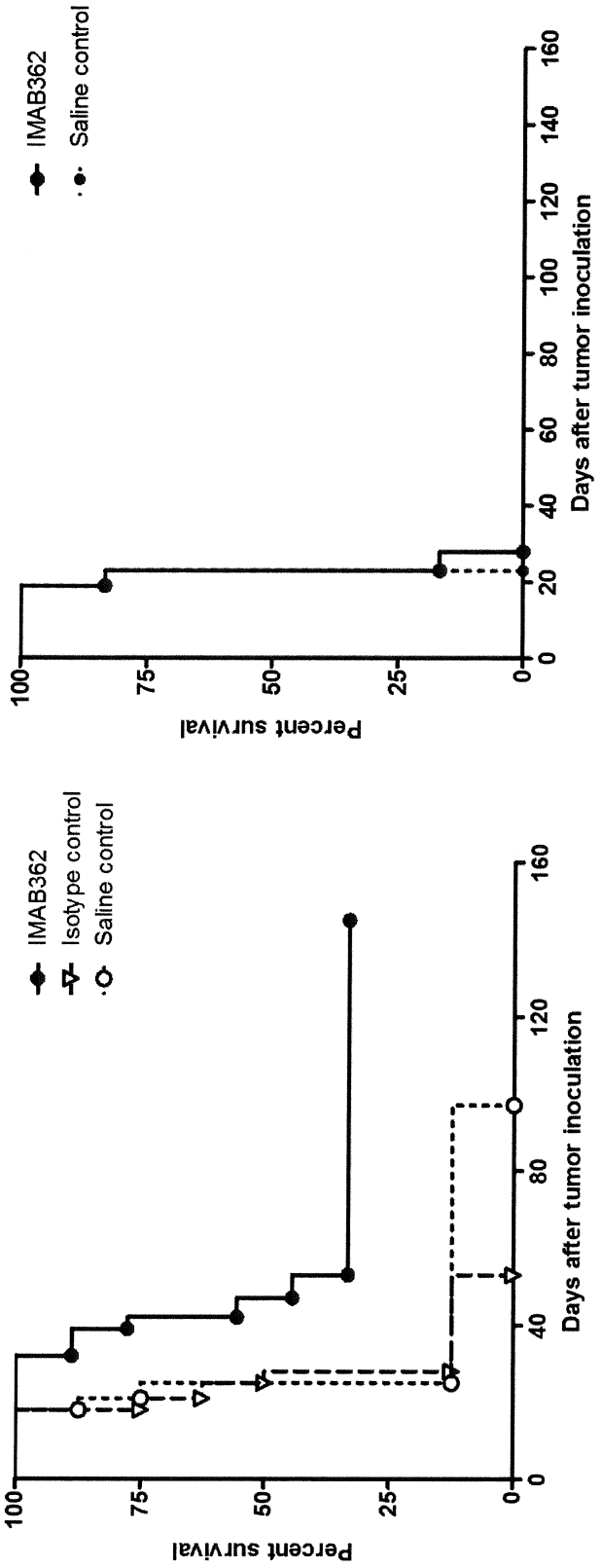

IMAB362
Isotype control
Saline control
Percent survival
100
75
50
25
0
0
40
80
120
160
Days after tumor inoculation
IMAB362
Saline control
Percent survival
100
75
50
25
0
0
20
40
60
80
100
120
140
160
Days after tumor inoculation

Figure 21

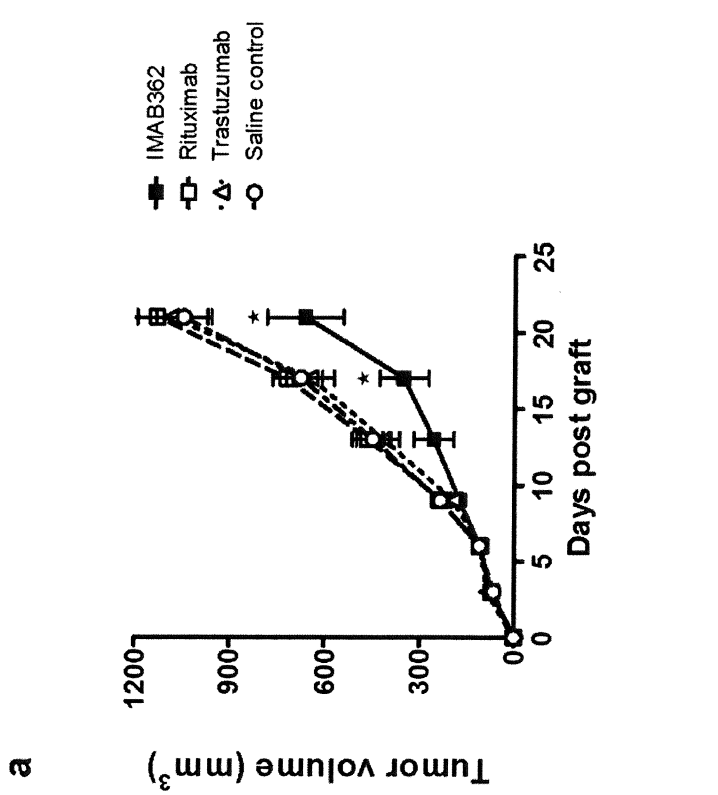

a
Tumor volume (mm³)
1200
900
600
300
0
0
5
10
15
20
25
Days post graft
IMAB362
Rituximab
Trastuzumab
Saline control
b
Tumor volume (mm³)
1500
1000
500
0
IMAB362
Rituximab
Trastuzumab
Saline control

Figure 22

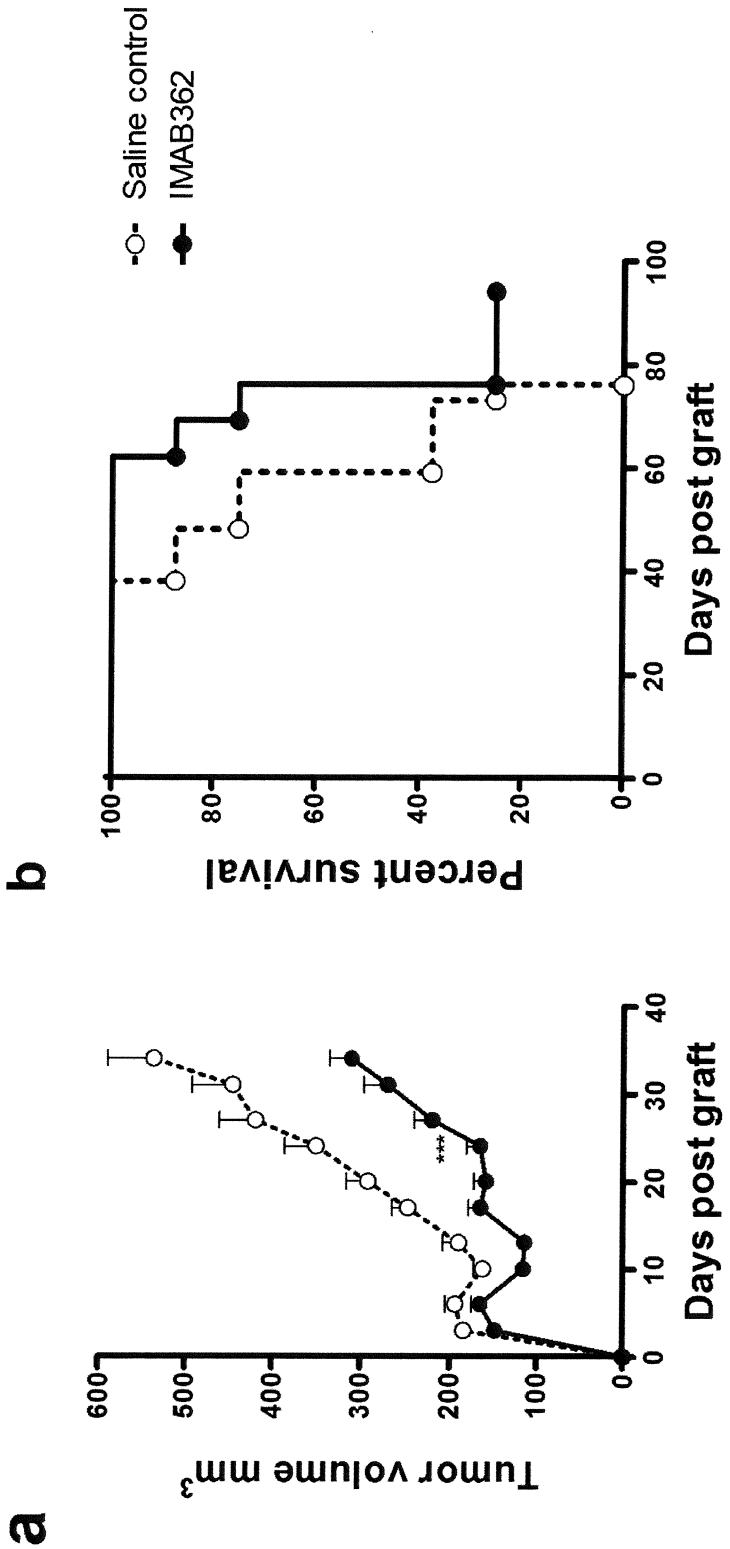

a
Tumor volume mm3
600
500
400
300
200
100
0
0
10
20
30
40
Days post graft
***
b
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days post graft
Saline control
IMAB362

Figure 23

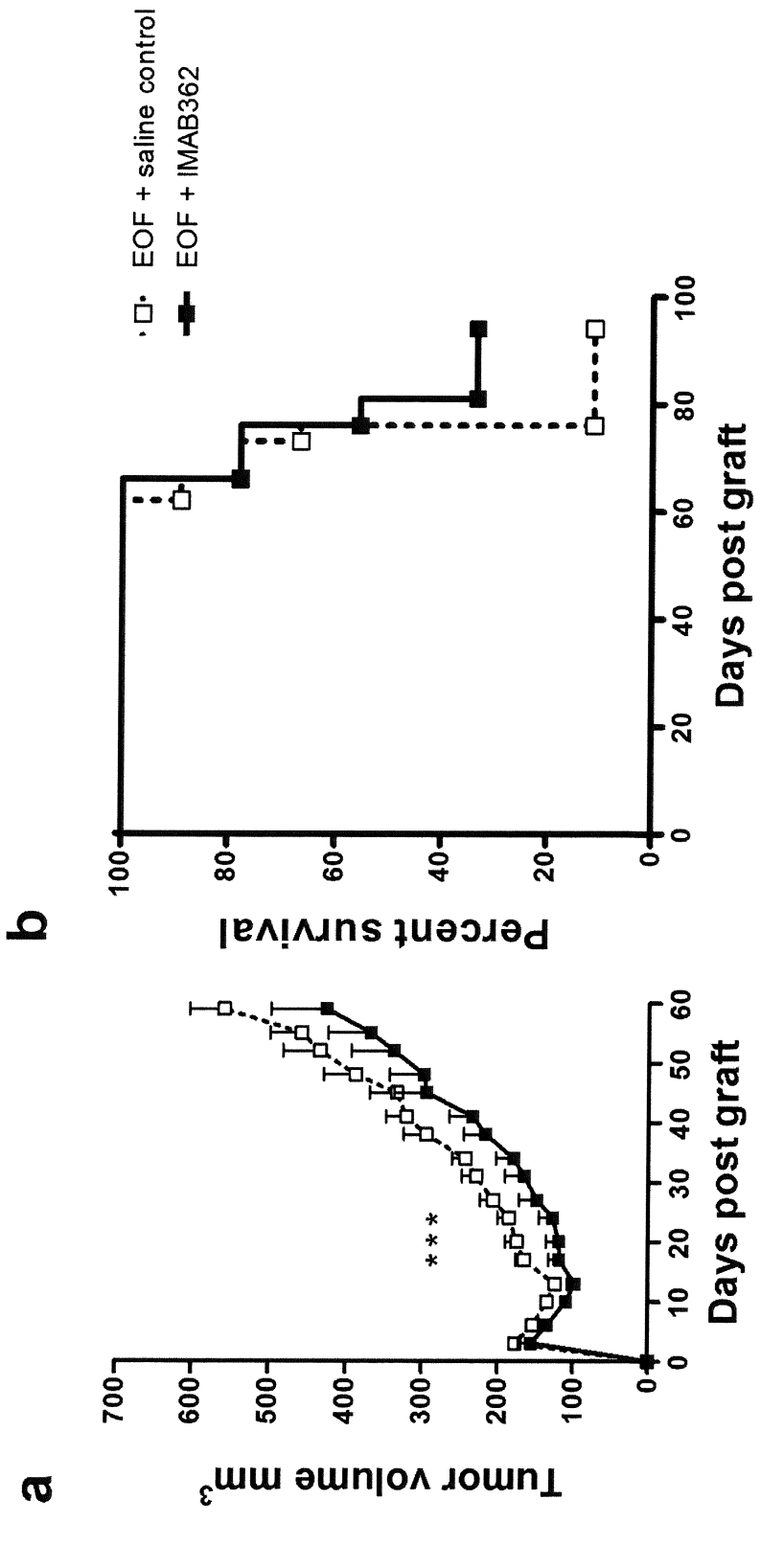
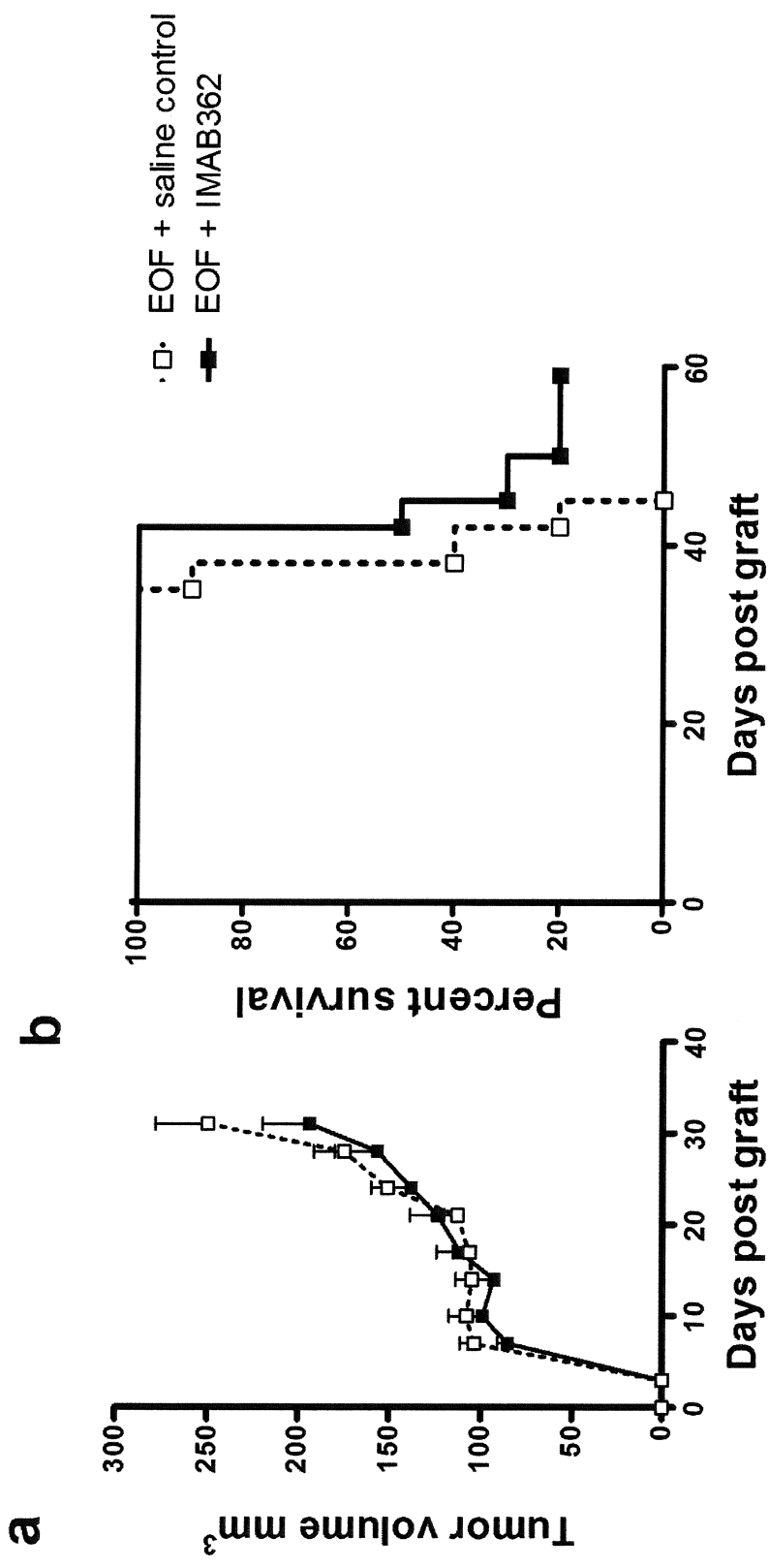

a
Tumor volume mm3
700
600
500
400
300
200
100
0
***
0
10
20
30
40
50
60
Days post graft
b
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days post graft
EOF + saline control
EOF + IMAB362

Figure 24


a
Tumor volume mm3
300
250
200
150
100
50
0
0
10
20
30
40
Days post graft
b
Percent survival
100
80
60
40
20
0
0
20
40
60
Days post graft
EOF + saline control
EOF + IMAB362

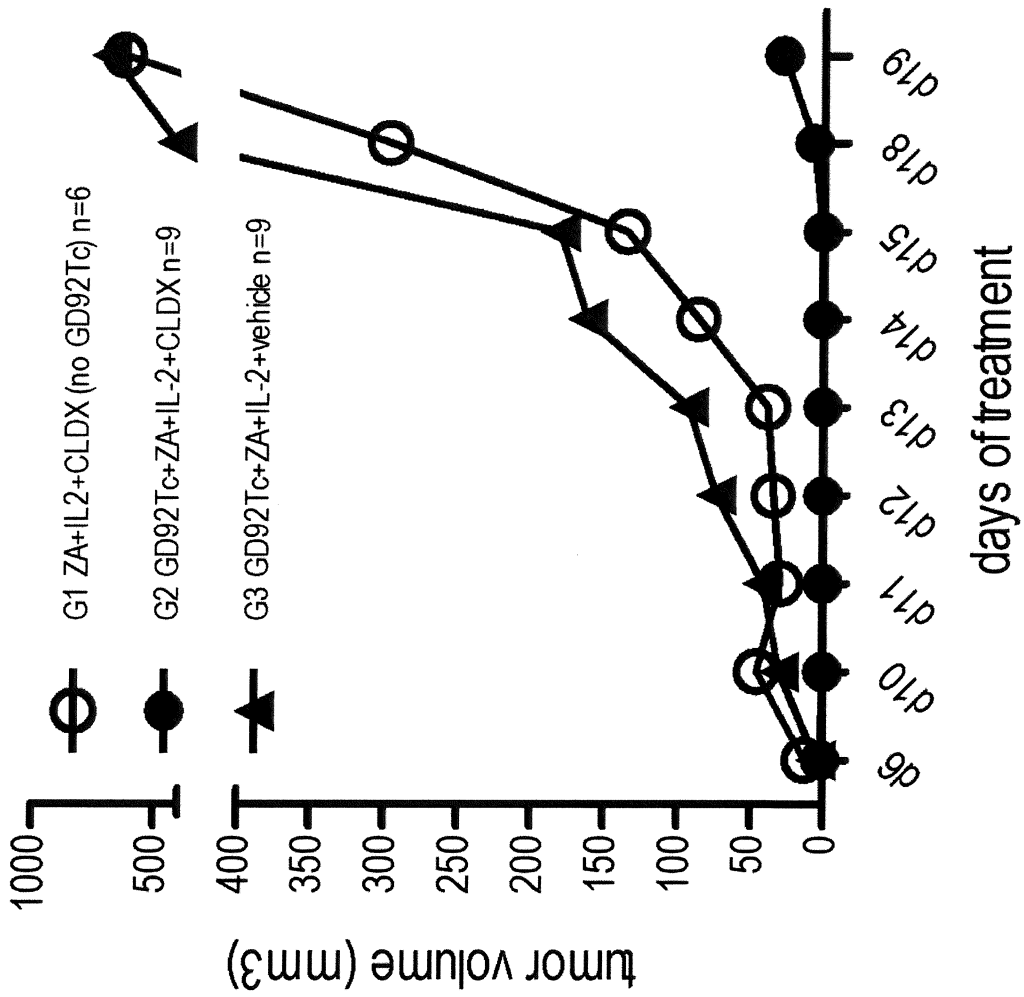
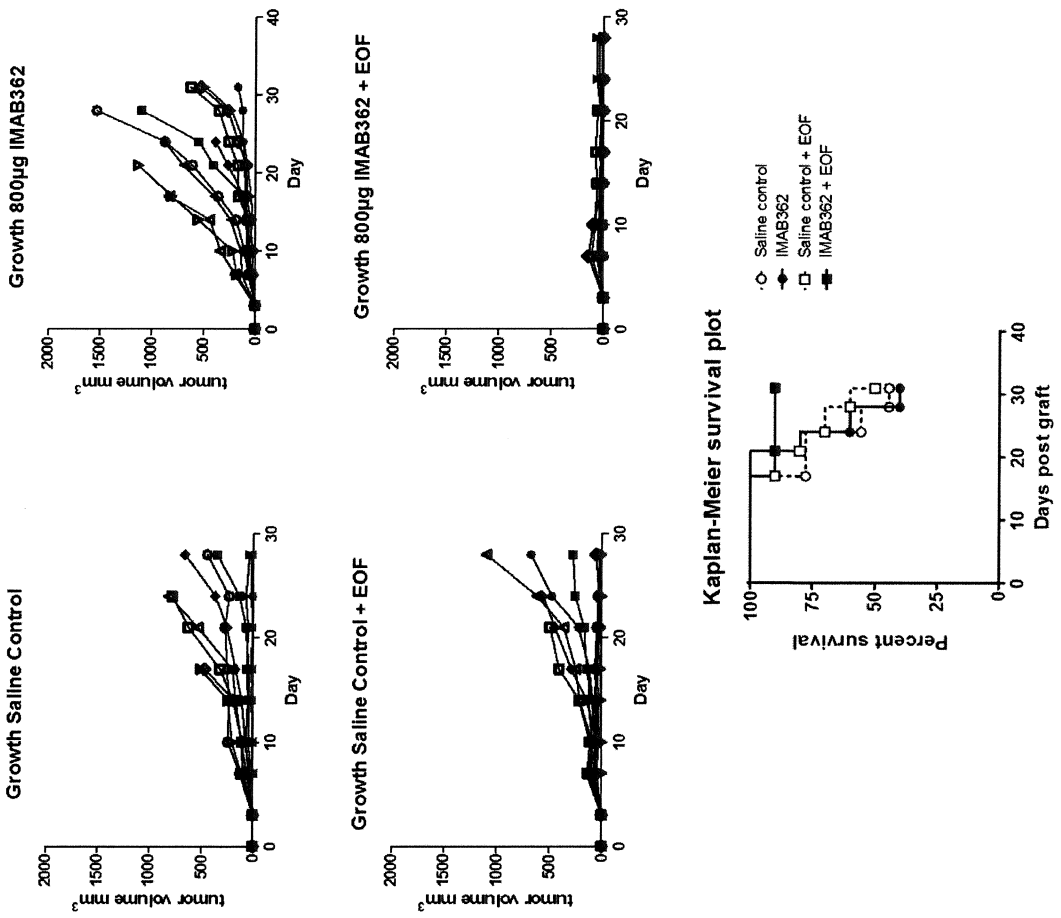

G1 ZA+IL2+CLDX (no GD92Tc) n=6
G2 GD92Tc+ZA+IL-2+CLDX n=9
G3 GD92Tc+ZA+IL-2+vehicle n=9
tumor volume (mm3)
1000
500
400
350
300
250
200
150
100
50
0
d6
d10
d11
d12
d13
d14
d15
d18
d19
days of treatment

Figure 25

Figure 26


Growth 800µg IMAB362
Growth 800µg IMAB362 + EOF
Growth Saline Control
Growth Saline Control + EOF
tumor volume mm3
Day
Kaplan-Meier survival plot
Saline control
IMAB362
Saline control + EOF
IMAB362 + EOF
Percent survival
Days post graft