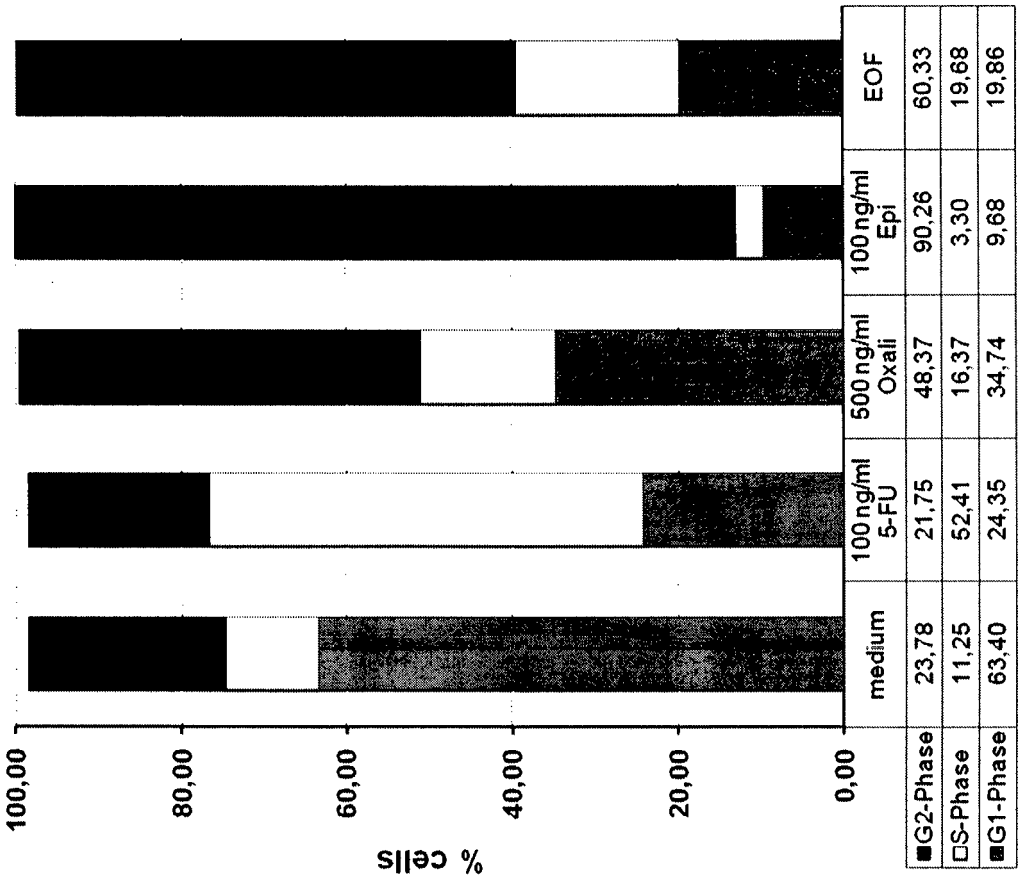
Figure 1

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

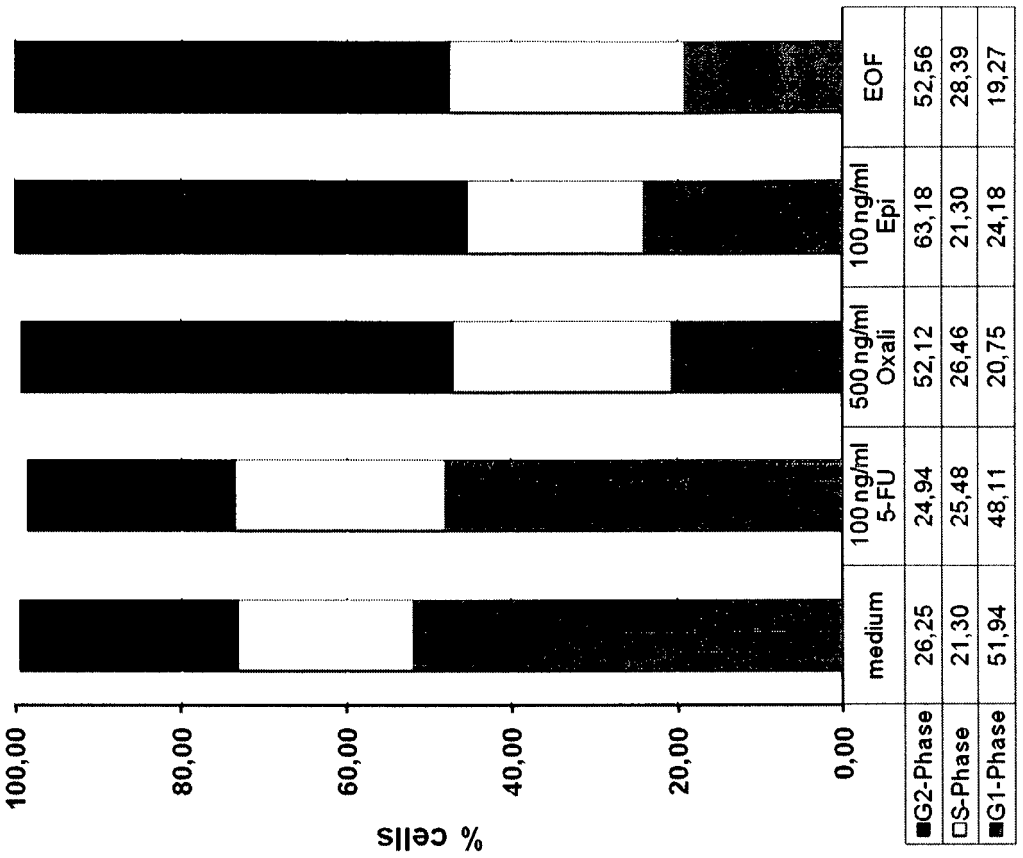
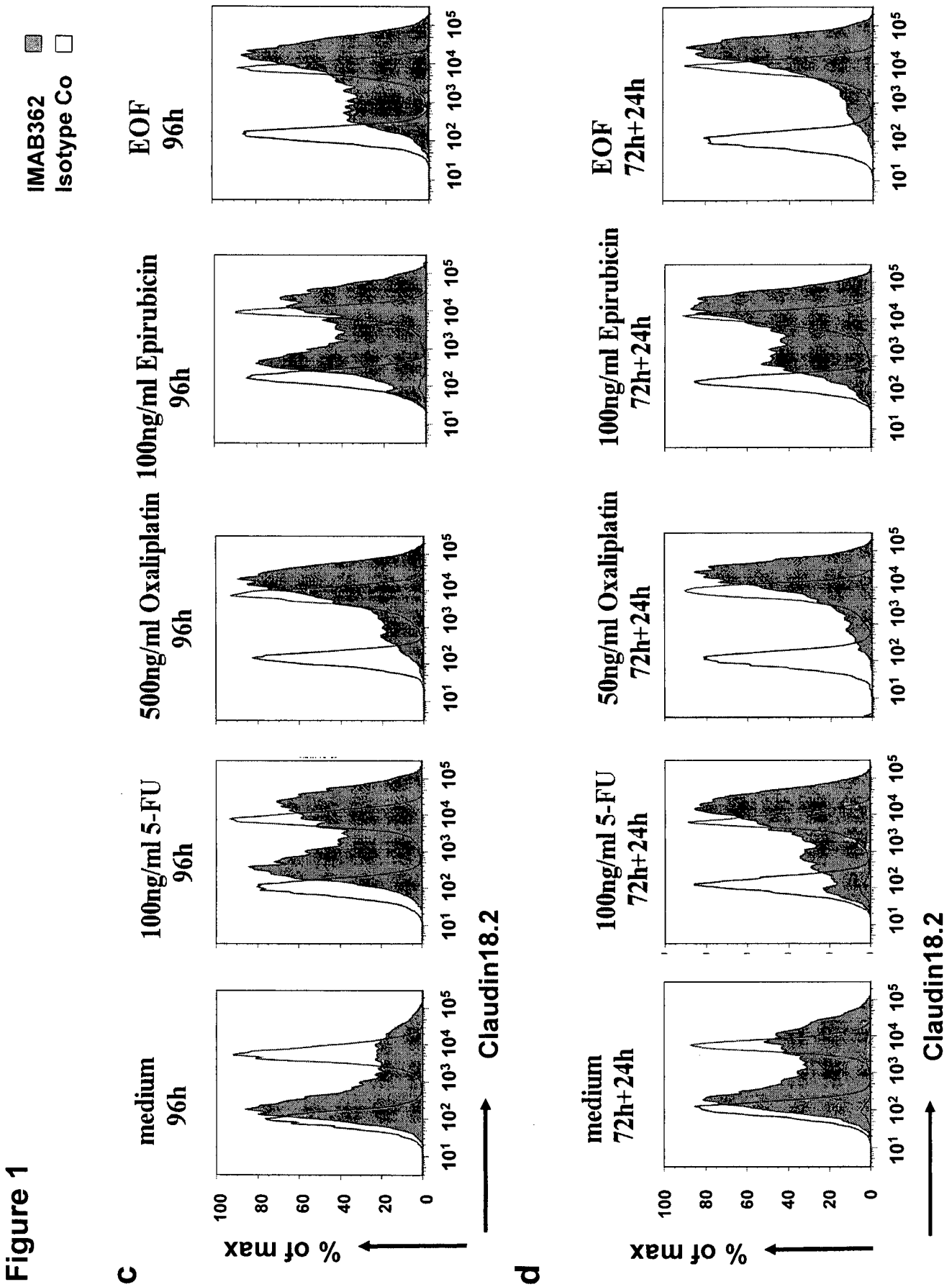

KatoIII 72h CTx+24h med cell cycle
% cells
100,00
80,00
60,00
40,00
20,00
0,00
medium
100 ng/ml 5-FU
500 ng/ml Oxali
100 ng/ml Epi
EOF
G2-Phase 26,25 24,94 52,12 63,18 52,56
S-Phase 21,30 25,48 26,46 21,30 28,39
G1-Phase 51,94 48,11 20,75 24,18 19,27

Figure 1


IMAB362
Isotype Co
c
d
medium 96h
100ng/ml 5-FU 96h
500ng/ml Oxaliplatin 96h
100ng/ml Epirubicin 96h
EOF 96h
medium 72h+24h
100ng/ml 5-FU 72h+24h
50ng/ml Oxaliplatin 72h+24h
100ng/ml Epirubicin 72h+24h
EOF 72h+24h
% of max
Claudin18.2

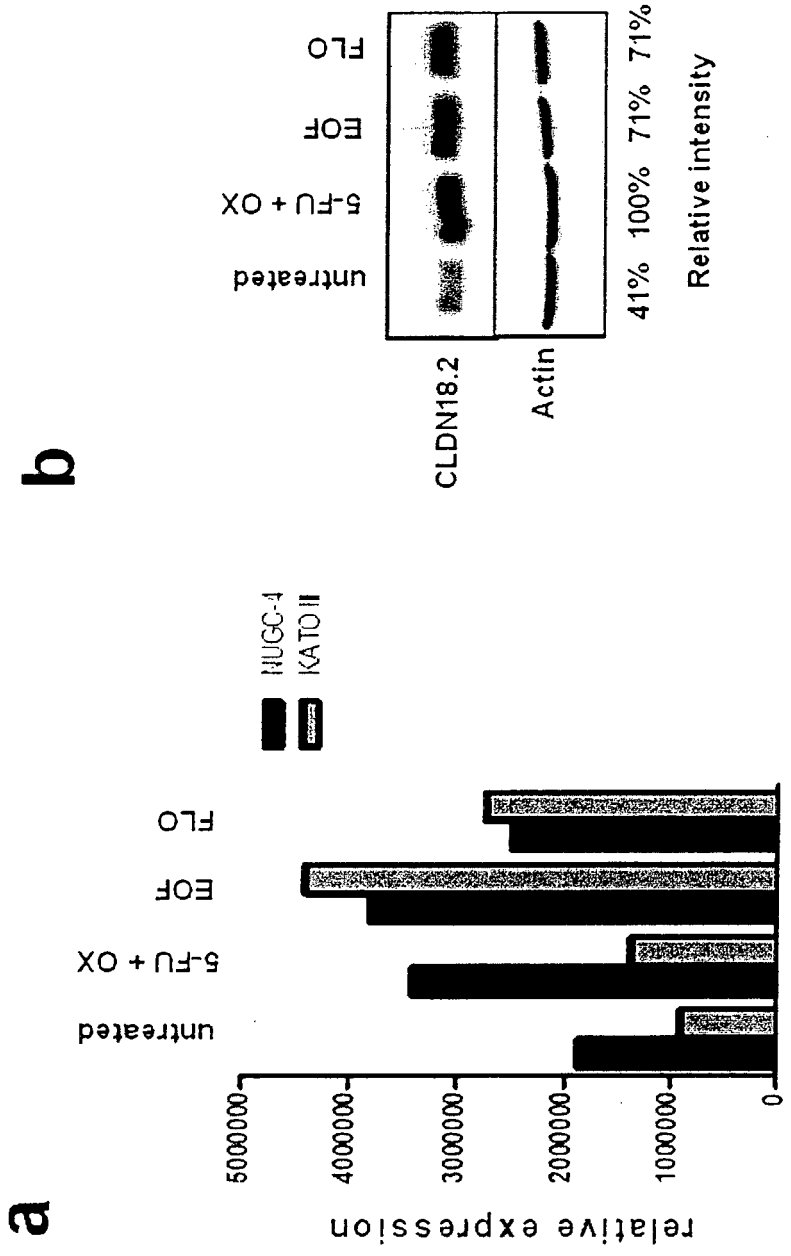
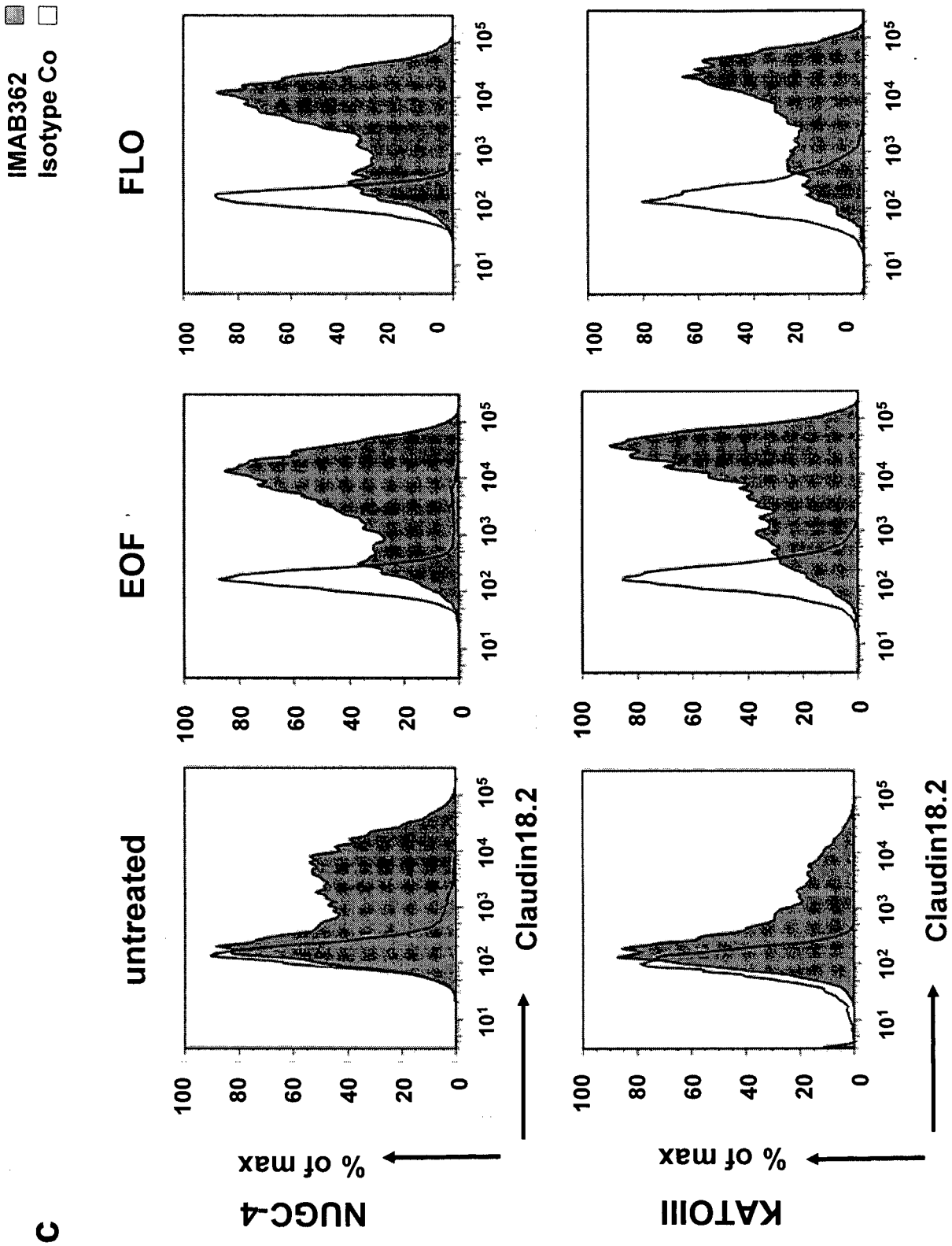

a
relative expression
5000000
4000000
3000000
2000000
1000000
0
untreated
5-FU + OX
EOF
FLO
NUGC-4
KATO III
b
CLDN18.2
Actin
untreated
5-FU + OX
EOF
FLO
41% 100% 71% 71%
Relative intensity

Figure 2

Figure 2

C


IMAB362
Isotype Co
untreated
EOF
FLO
NUGC-4
KATOIII
% of max
Claudin18.2
0
20
40
60
80
100
10¹
10²
10³
10⁴
10⁵

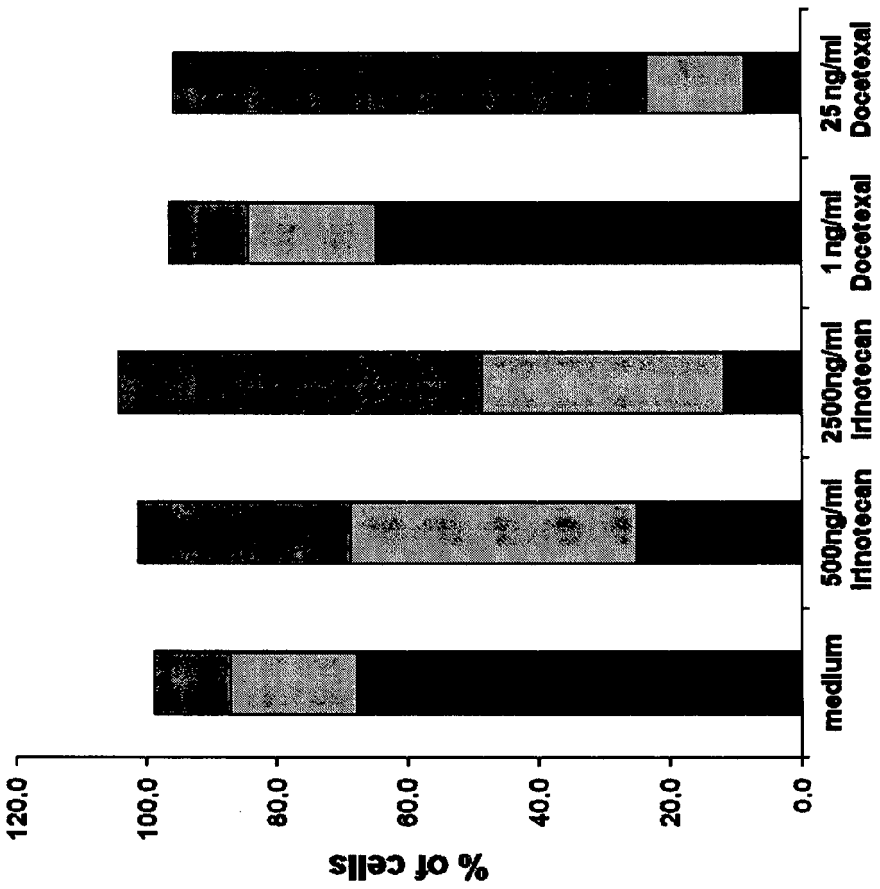
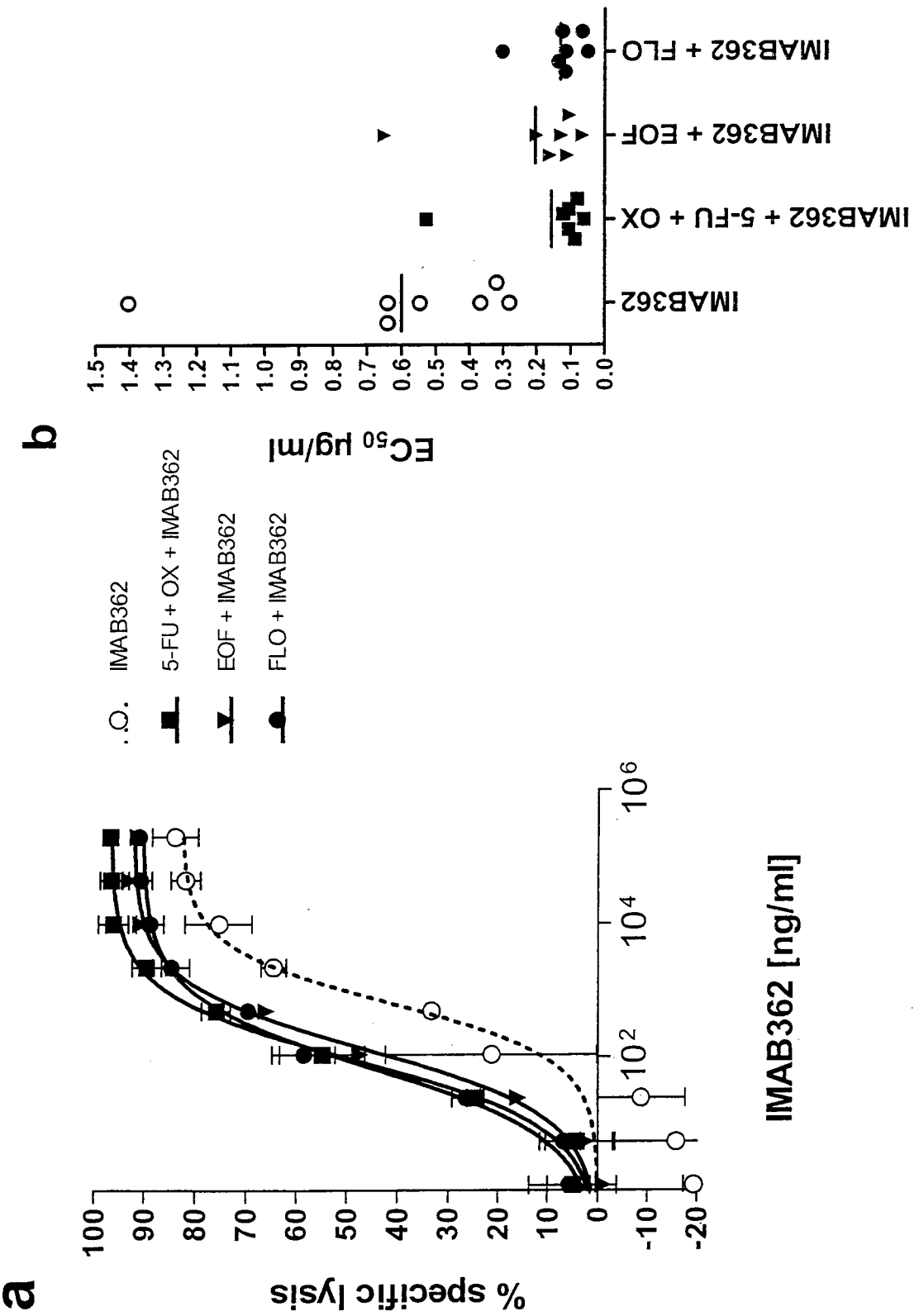

% of cells
120.0
100.0
80.0
60.0
40.0
20.0
0.0
medium
500ng/ml Irinotecan
2500ng/ml Irinotecan
1 ng/ml Docetexal
25 ng/ml Docetexal

Figure 3

Figure 4


a
% specific lysis
IMAB362 [ng/ml]
n.d. IMAB362
5-FU + OX + IMAB362
EOF + IMAB362
FLO + IMAB362
b
EC50 µg/ml
IMAB362
IMAB362 + 5-FU + OX
IMAB362 + EOF
IMAB362 + FLO

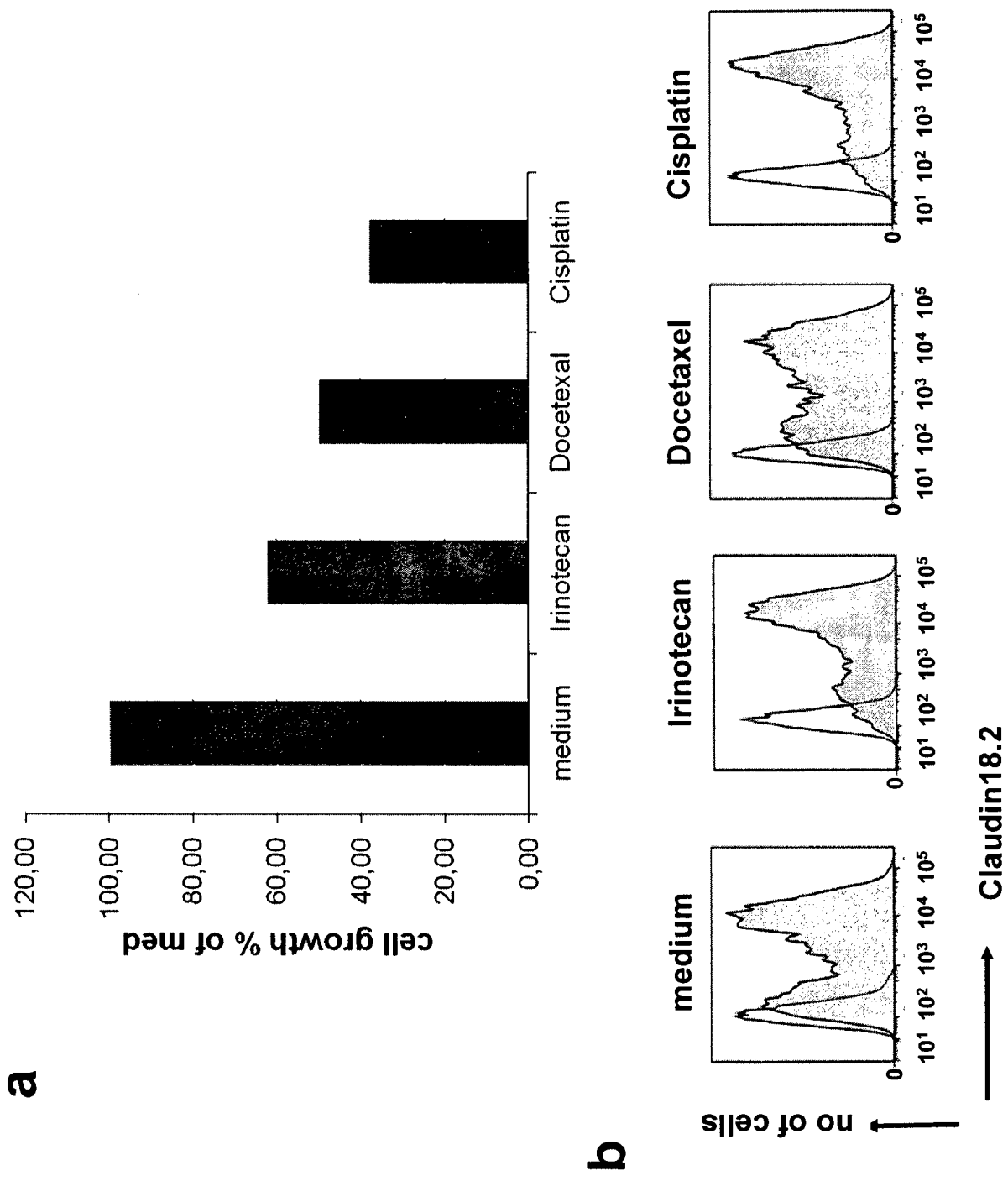
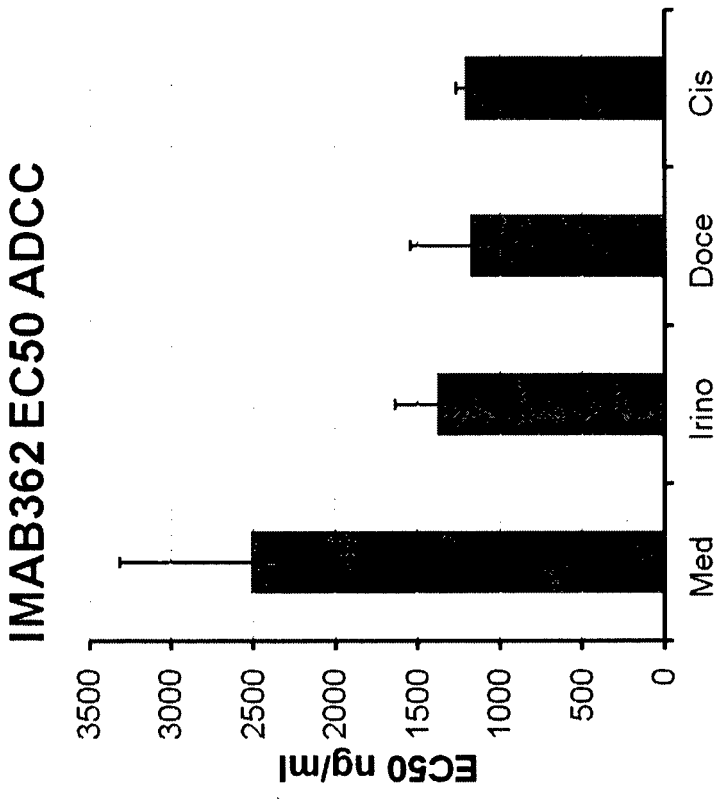

a
cell growth % of med
120,00
100,00
80,00
60,00
40,00
20,00
0,00
medium
Irinotecan
Docetexal
Cisplatin
b
no of cells
medium
Irinotecan
Docetaxel
Cisplatin
0
10^1 10^2 10^3 10^4 10^5
Claudin18.2

Figure 5


d
IMAB362 EC50 ADCC
EC50 ng/ml
3500
3000
2500
2000
1500
1000
500
0
Med
Irino
Doce
Cis

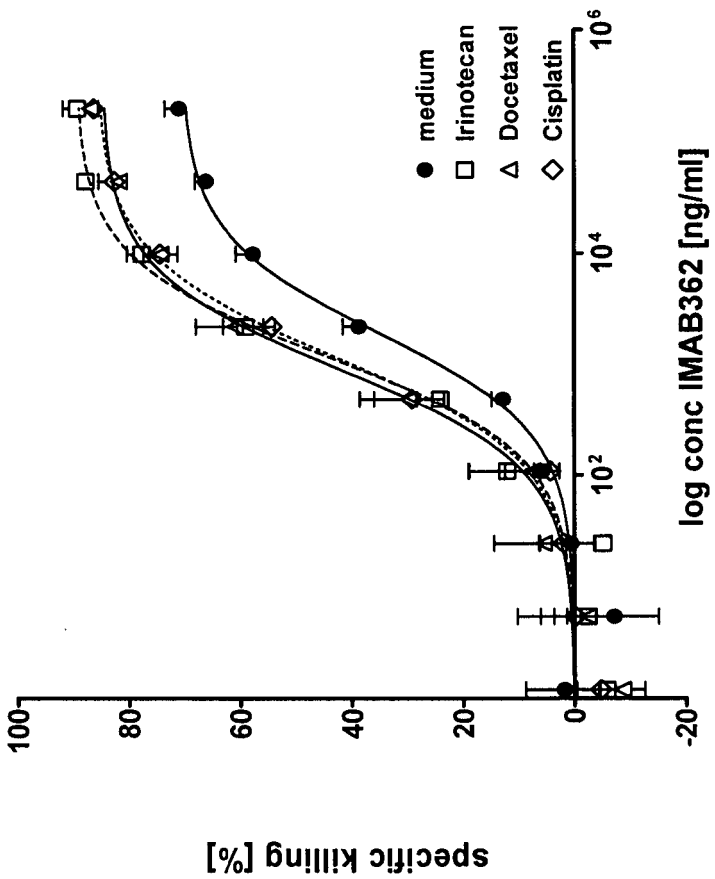

c
specific killing [%]
100
80
60
40
20
0
-20
medium
Irinotecan
Docetaxel
Cisplatin
10^2
10^4
10^6
log conc IMAB362 [ng/ml]

Figure 5

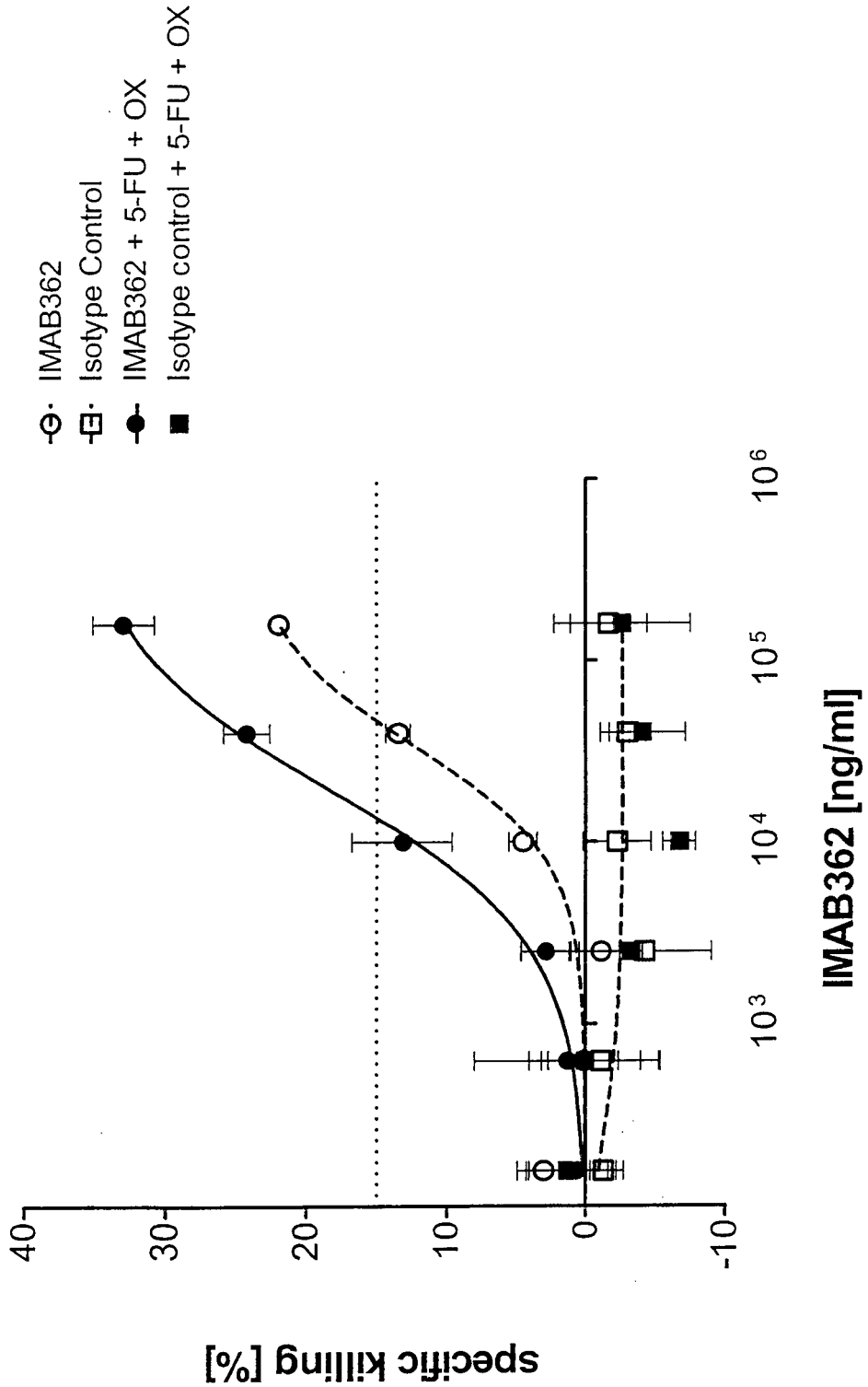

IMAB362
Isotype Control
IMAB362 + 5-FU + OX
Isotype control + 5-FU + OX
specific killing [%]
40
30
20
10
0
-10
10^3
10^4
10^5
10^6
IMAB362 [ng/ml]

Figure 6

Figure 7

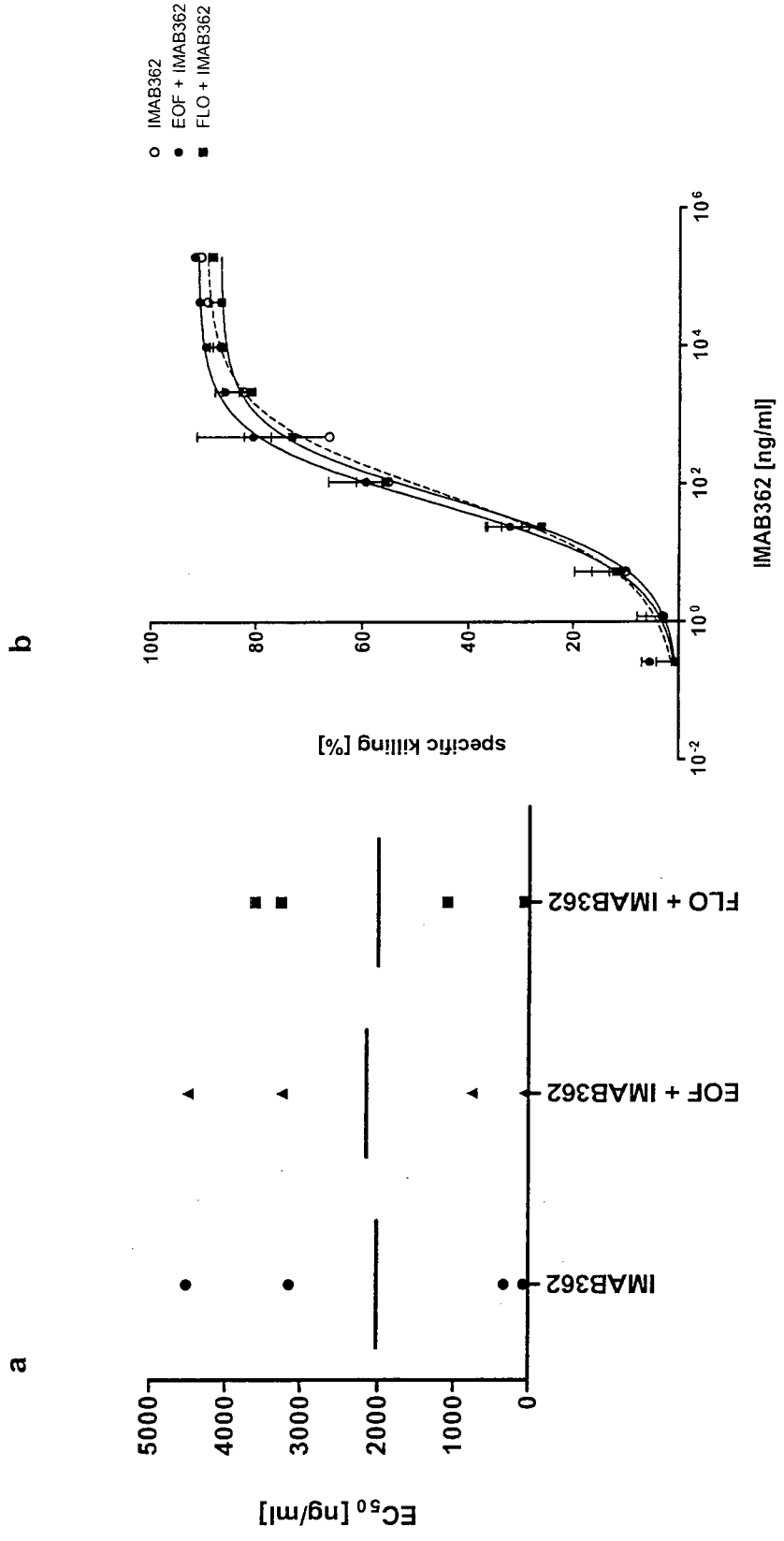

a
b
IMAB362
EOF + IMAB362
FLO + IMAB362
EC50 [ng/ml]
5000
4000
3000
2000
1000
0
specific killing [%]
100
80
60
40
20
10-2
100
102
104
106
IMAB362 [ng/ml]

Figure 8

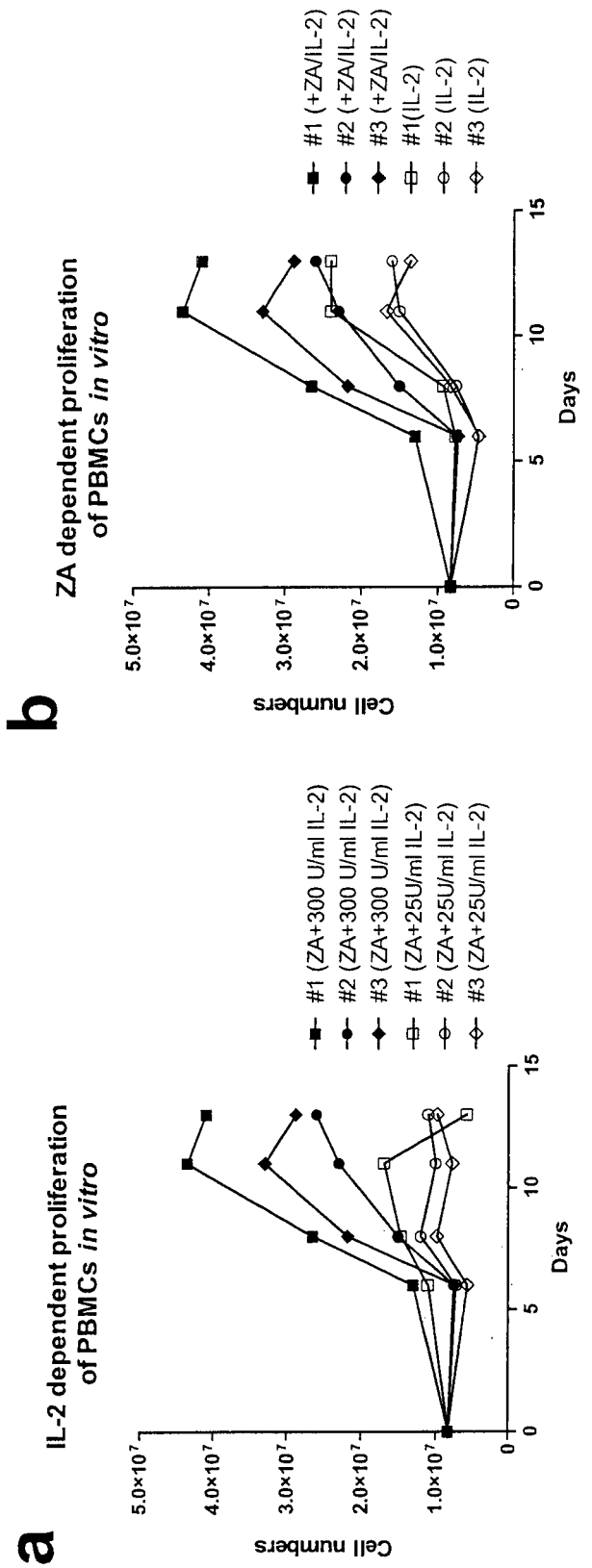

a
IL-2 dependent proliferation of PBMCs in vitro
Cell numbers
5.0×10^7
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0
5
10
15
Days
#1 (ZA+300 U/ml IL-2)
#2 (ZA+300 U/ml IL-2)
#3 (ZA+300 U/ml IL-2)
#1 (ZA+25U/ml IL-2)
#2 (ZA+25U/ml IL-2)
#3 (ZA+25U/ml IL-2)
b
ZA dependent proliferation of PBMCs in vitro
Cell numbers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

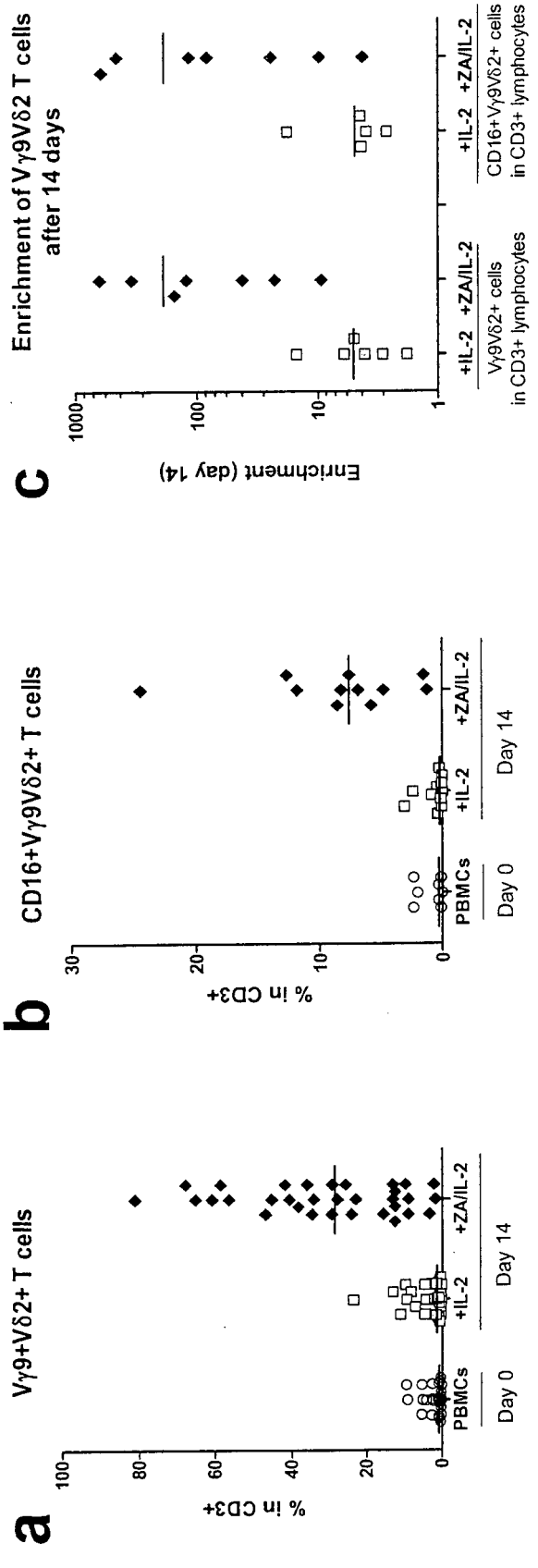

a
Vγ9+Vδ2+ T cells
% in CD3+
100
80
60
40
20
0
PBMCs
+IL-2
+ZA/IL-2
Day 0
Day 14
b
CD16+Vγ9Vδ2+ T cells
% in CD3+
30
20
10
0
PBMCs
+IL-2
+ZA/IL-2
Day 0
Day 14
c
Enrichment of Vγ9Vδ2 T cells after 14 days
Enrichment (day 14)
1000
100
10
1
+IL-2
+ZA/IL-2
Vγ9Vδ2+ cells in CD3+ lymphocytes
CD16+Vγ9Vδ2+ cells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

Figure 10

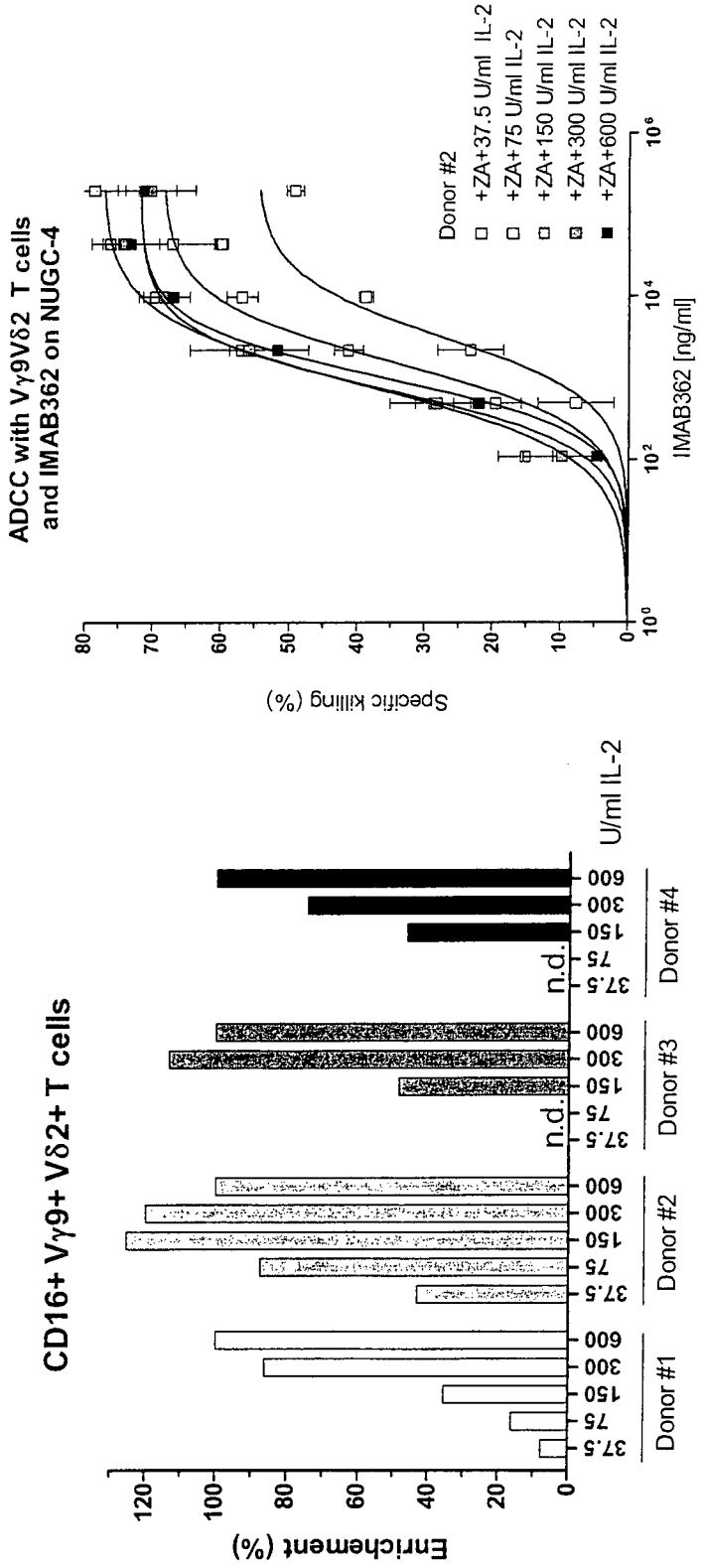
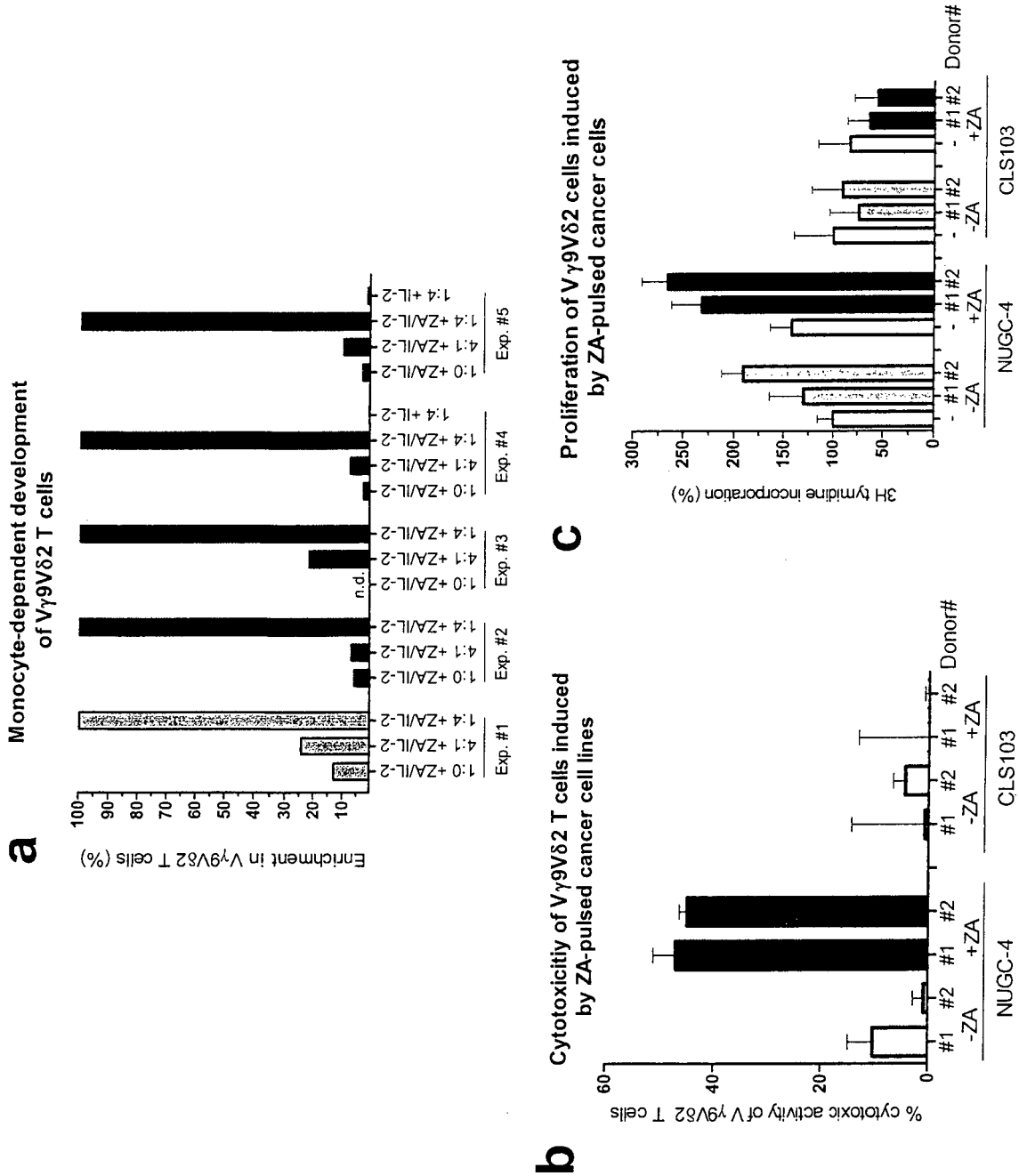

ADCC with Vγ9Vδ2 T cells
and IMAB362 on NUGC-4
Specific killing (%)
IMAB362 [ng/ml]
Donor #2
+ZA+37.5 U/ml IL-2
+ZA+75 U/ml IL-2
+ZA+150 U/ml IL-2
+ZA+300 U/ml IL-2
+ZA+600 U/ml IL-2
CD16+ Vγ9+ Vδ2+ T cells
Enrichement (%)
U/ml IL-2
Donor #1
Donor #2
Donor #3
Donor #4
n.d.
n.d.

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
Cytotoxicitiy of Vγ9Vδ2 T cells induced by ZA-pulsed cancer cell lines
% cytotoxic activity of Vγ9Vδ2 T cells
-ZA
+ZA
NUGC-4
CLS103
Donor#
c
Proliferation of Vγ9Vδ2 cells induced by ZA-pulsed cancer cells
3H tymidine incorporation (%)

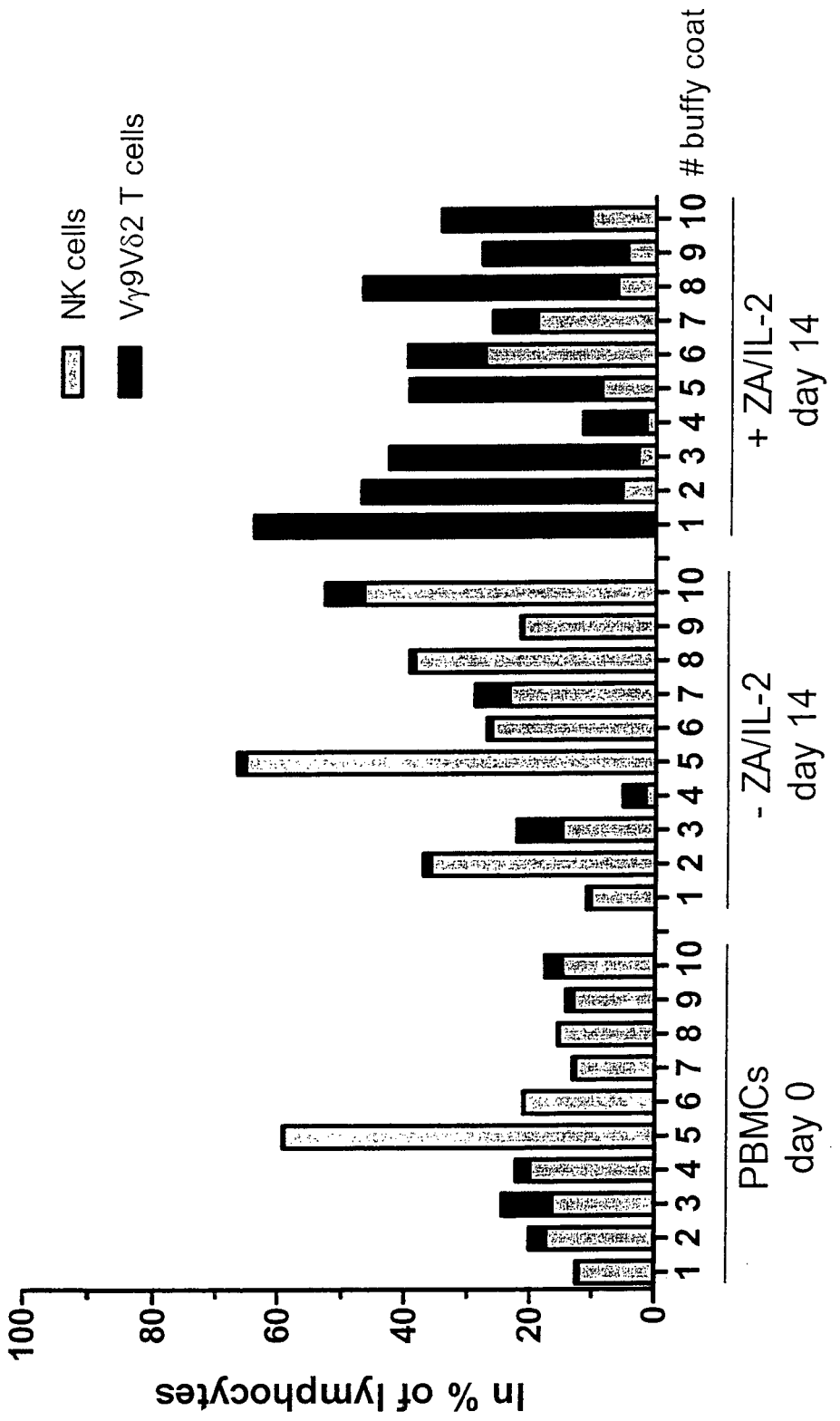

Cell types
NK cells
Vγ9Vδ2 T cells
In % of lymphocytes
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 10
buffy coat
PBMCs
day 0
- ZA/IL-2
day 14
+ ZA/IL-2
day 14

Figure 12

Figure 13

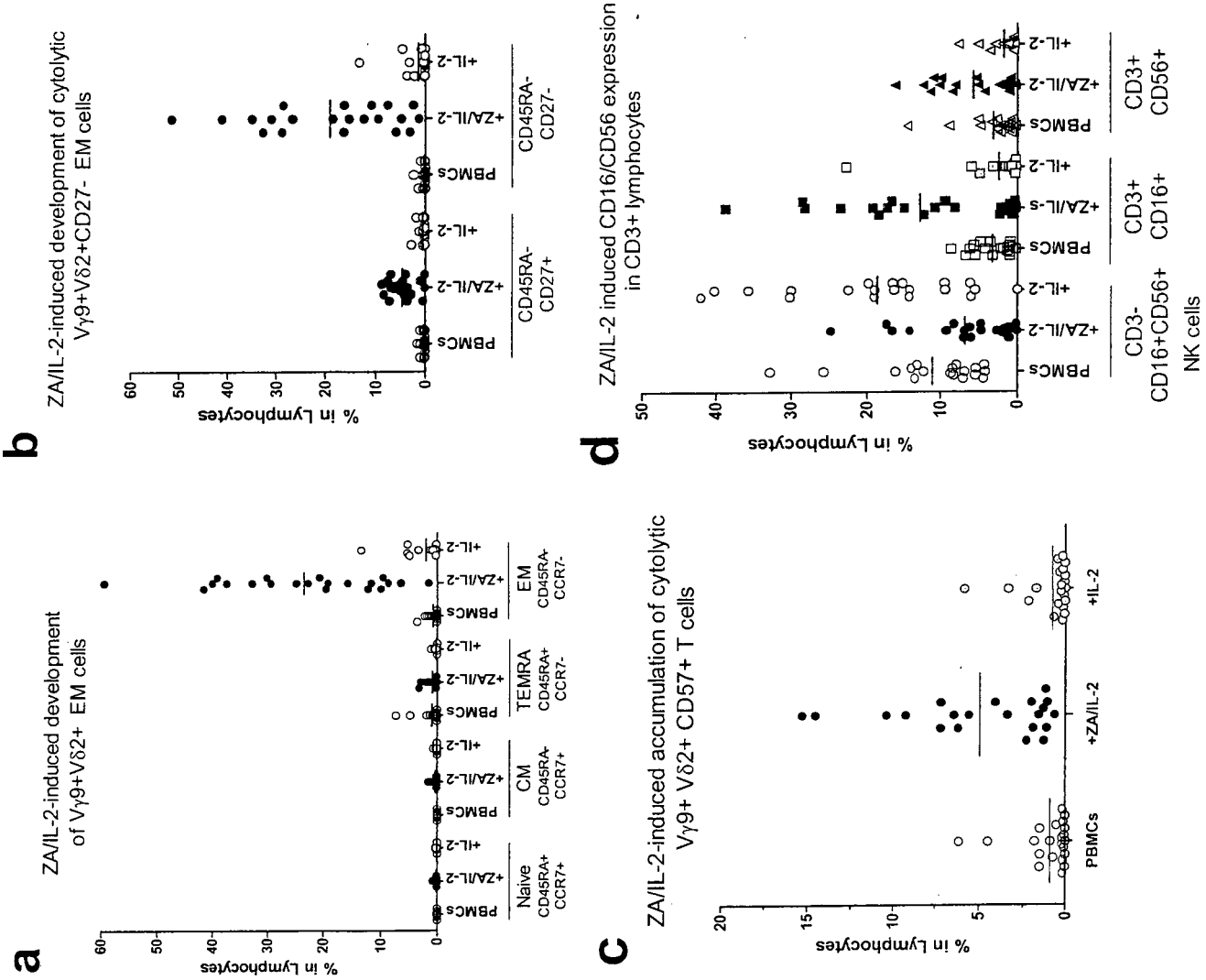

a
ZA/IL-2-induced development of Vγ9+Vδ2+ EM cells
% in Lymphocytes
Naive CD45RA+ CCR7+
CM CD45RA- CCR7+
TEMRA CD45RA+ CCR7-
EM CD45RA- CCR7-
PBMCs
+ZA/IL-2
+IL-2
b
ZA/IL-2-induced development of cytolytic Vγ9+Vδ2+CD27- EM cells
% in Lymphocytes
CD45RA- CD27+
CD45RA- CD27-
c
ZA/IL-2-induced accumulation 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

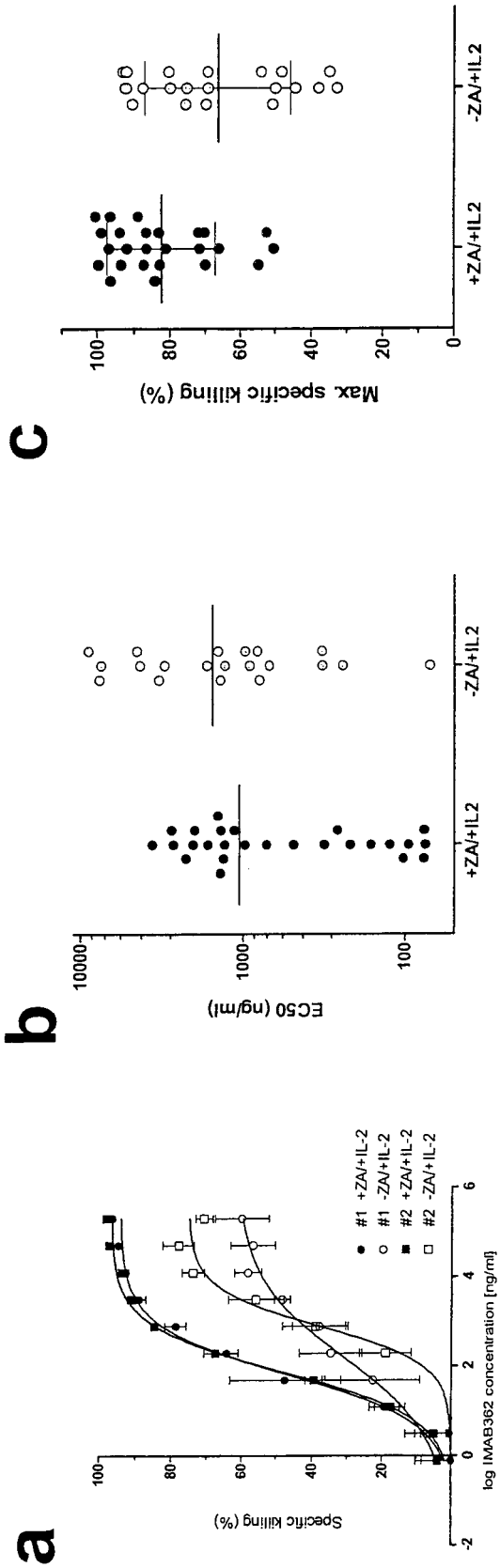
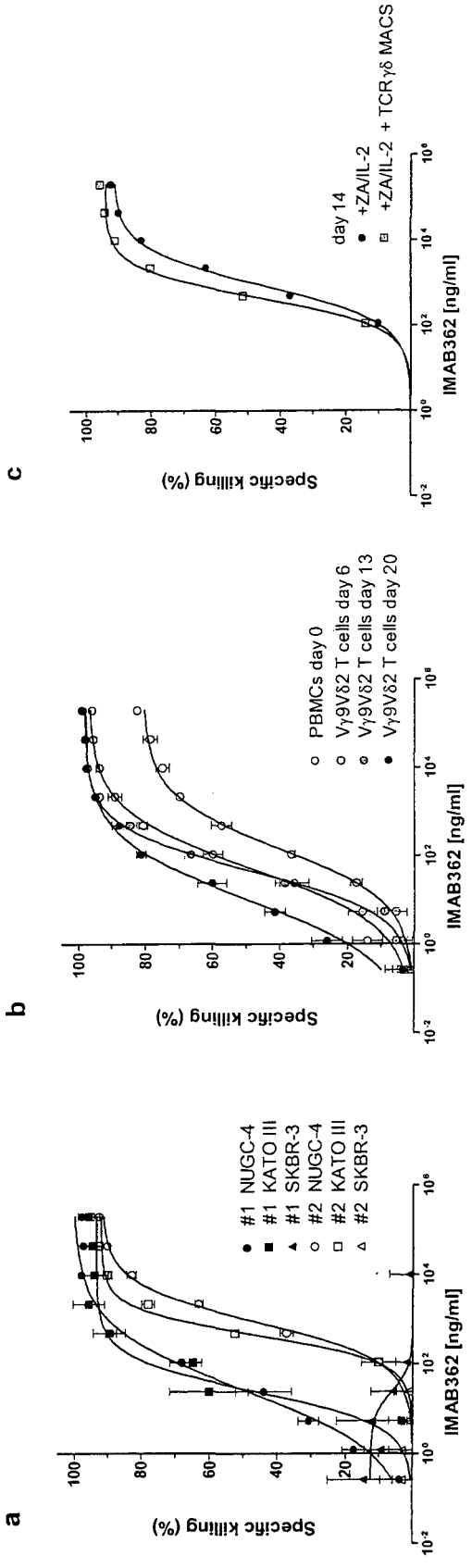

a
Specific killing (%)
log IMAB362 concentration [ng/ml]
#1 +ZA/+IL-2
#1 -ZA/+IL-2
#2 +ZA/+IL-2
#2 -ZA/+IL-2
b
EC50 (ng/ml)
+ZA/+IL2
-ZA/+IL2
c
Max. specific killing (%)
+ZA/+IL2
-ZA/+IL2

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 + TCRγδ MACS

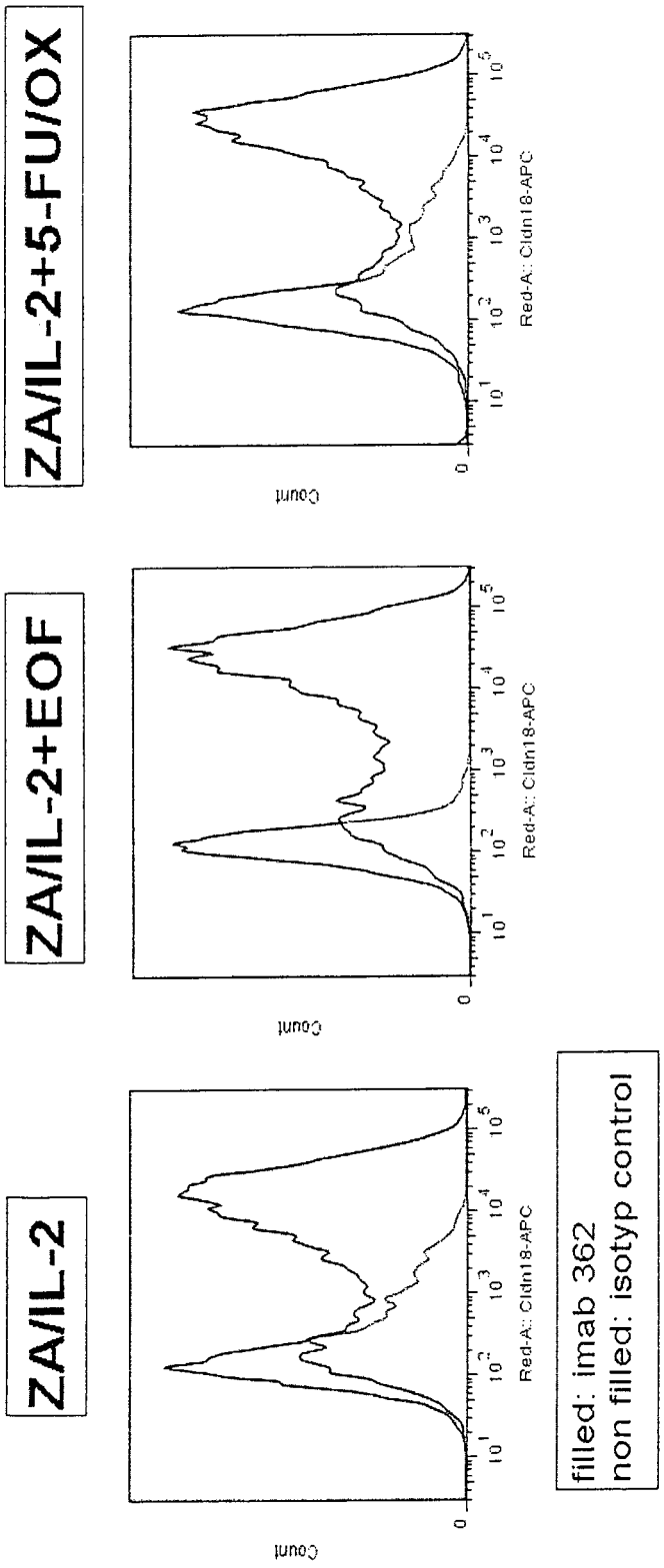

ZA/IL-2
ZA/IL-2+EOF
ZA/IL-2+5-FU/OX
Count
Red-A:: Cldn18-APC
filled: imab 362
non filled: isotyp control

Figure 16

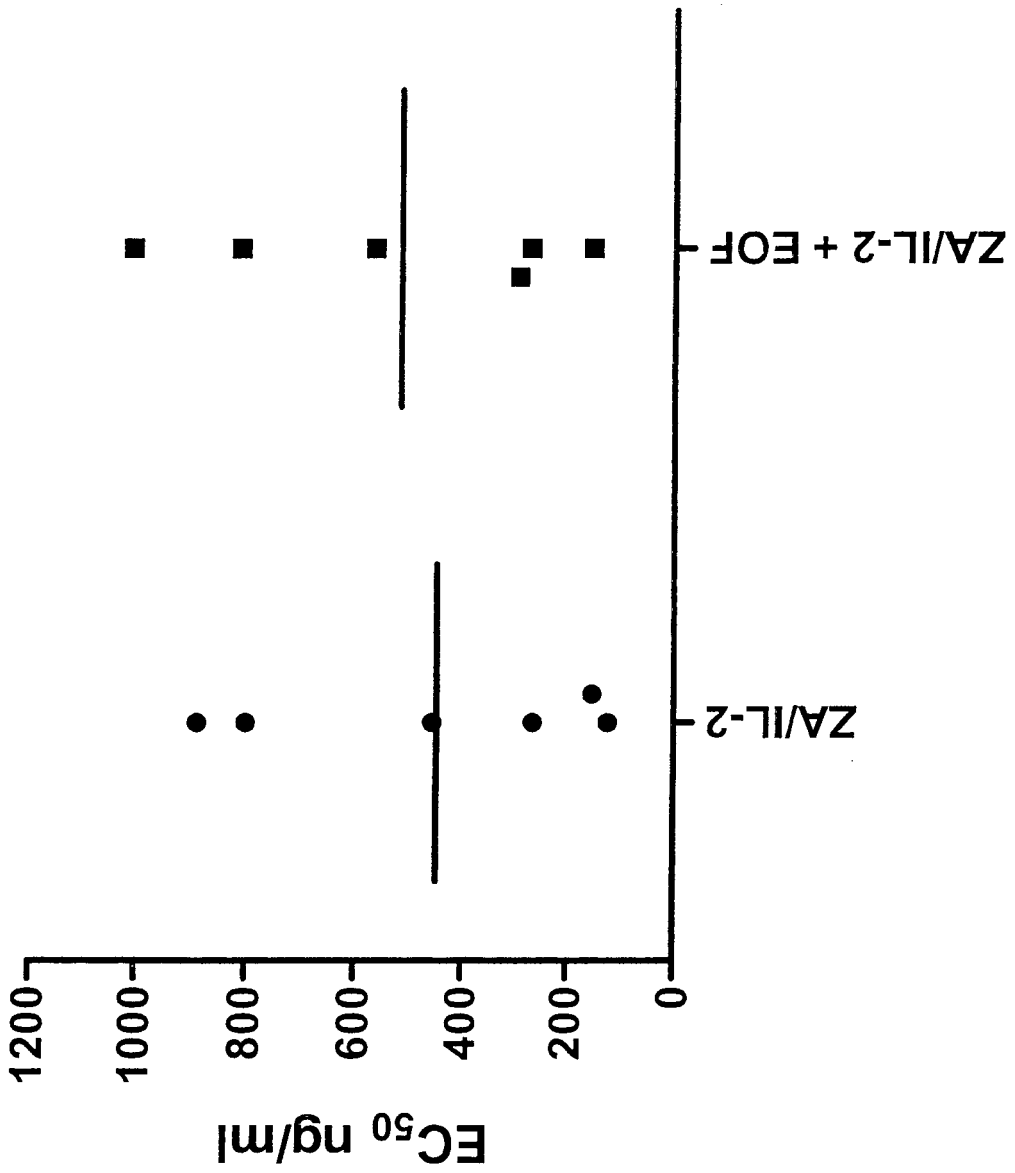


Figure 17

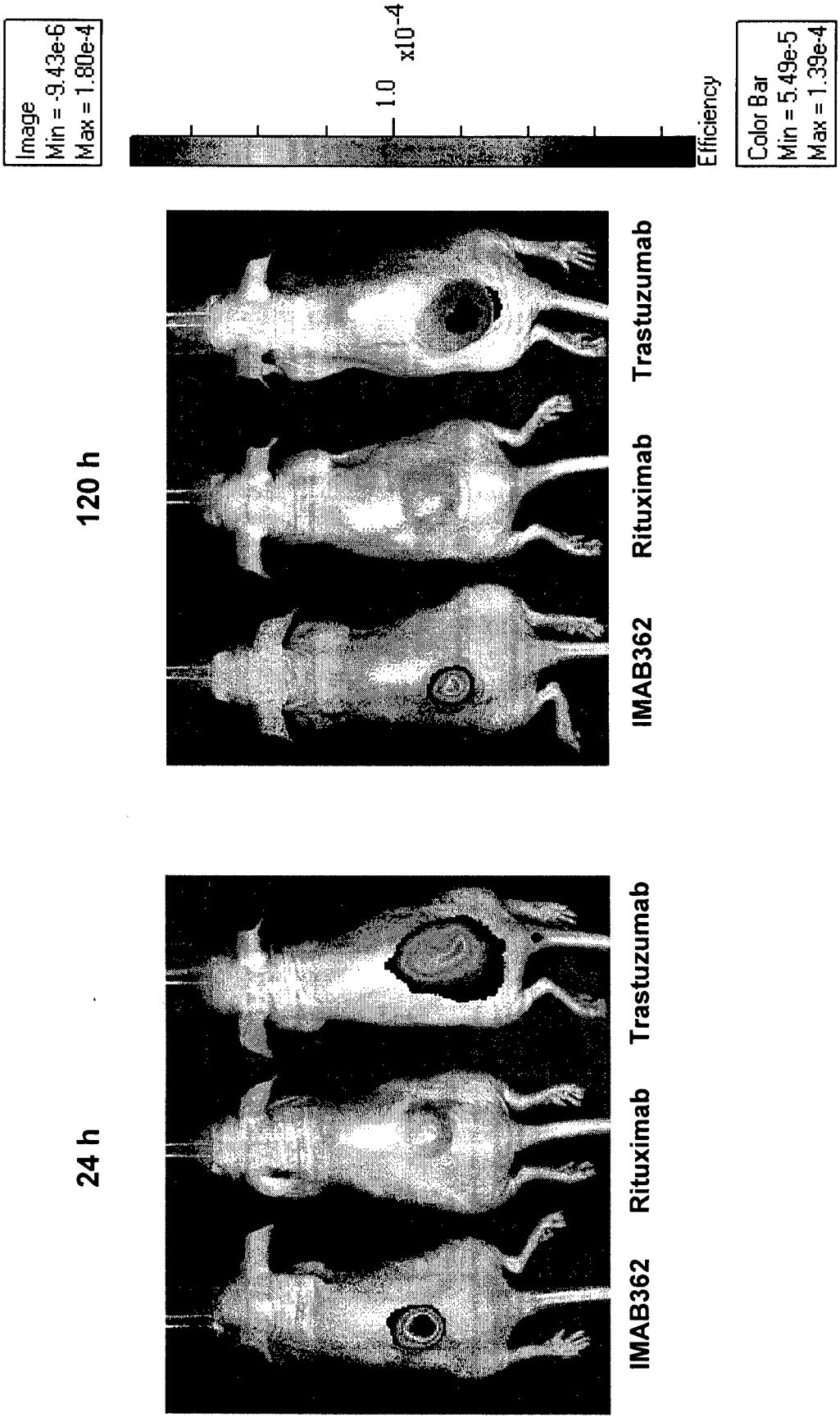

Image
Min = -9.43e-6
Max = 1.80e-4
1.0
x10-4
Efficiency
Color Bar
Min = 5.49e-5
Max = 1.39e-4
120 h
IMAB362
Rituximab
Trastuzumab
24 h
IMAB362
Rituximab
Trastuzumab

Figure 18

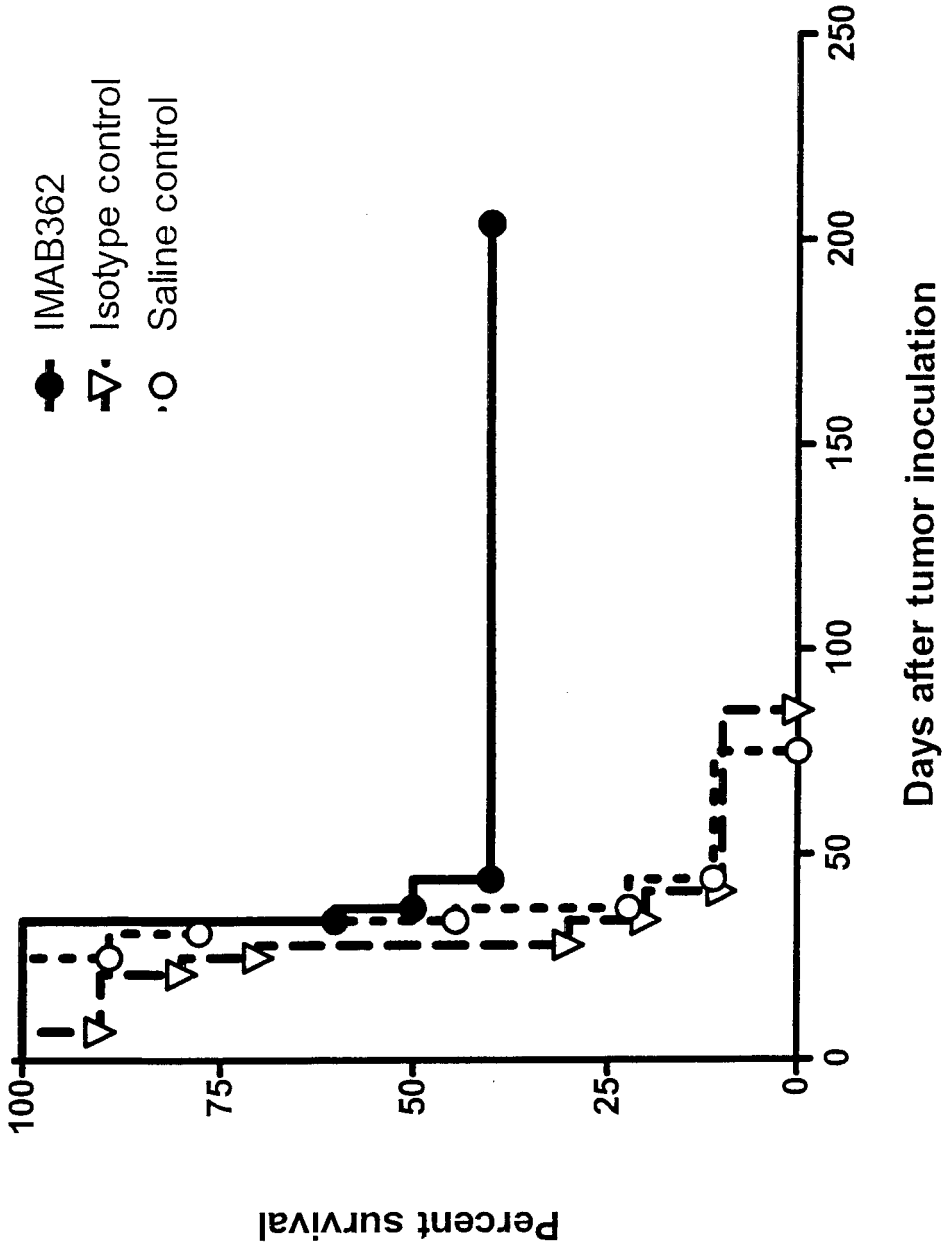
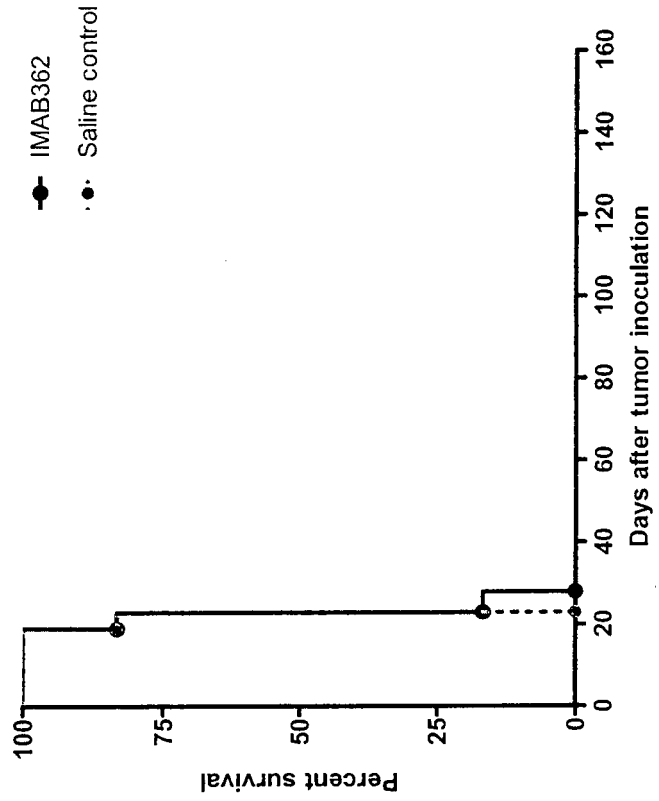

IMAB362
Isotype control
Saline control
Percent survival
100
75
50
25
0
0
50
100
150
200
250
Days after tumor inoculation

Figure 19

Figure 20


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

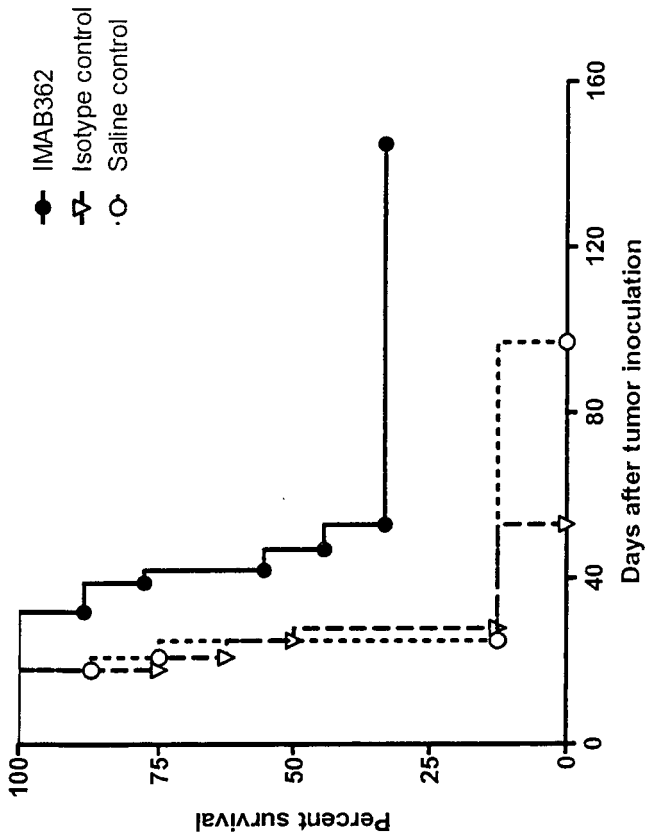
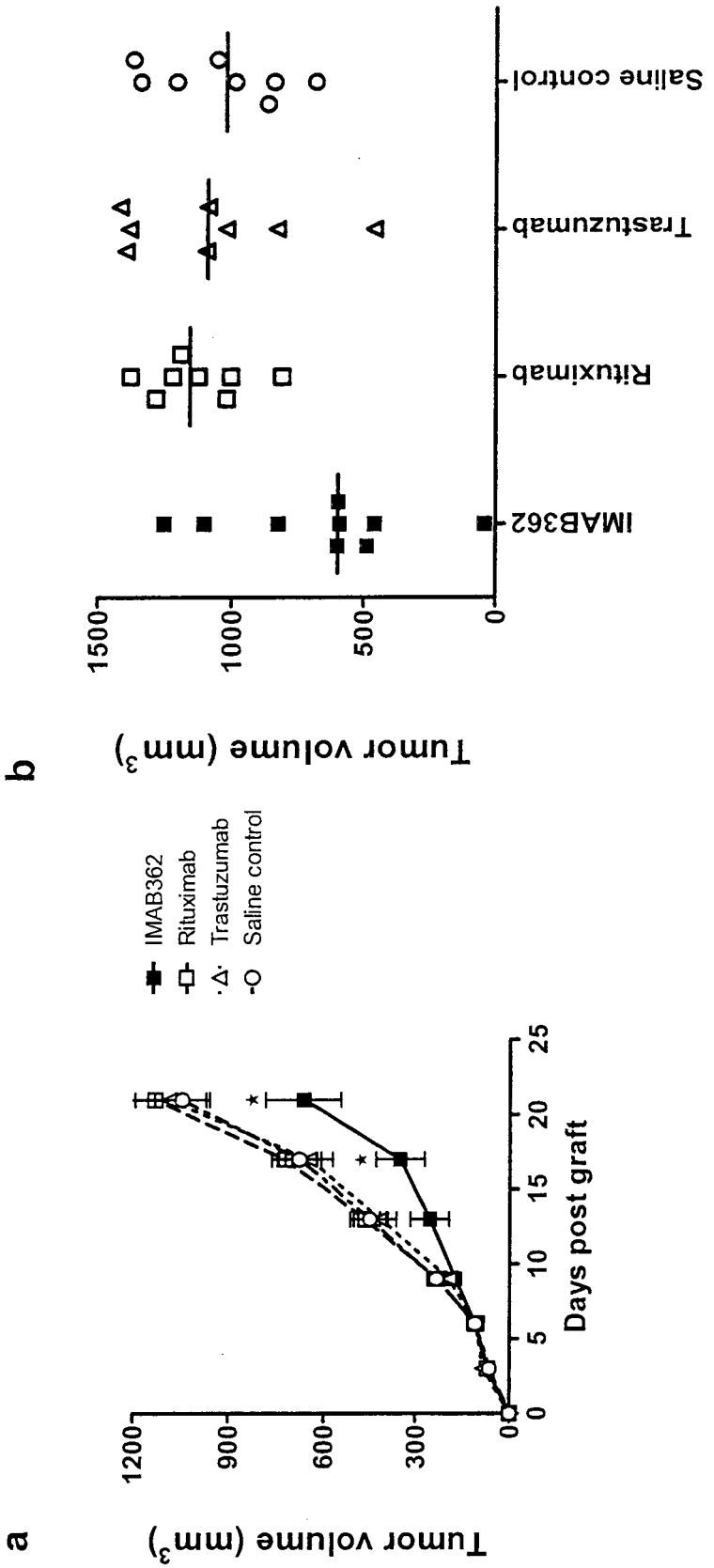

IMAB362
Isotype control
Saline control
Percent survival
Days after tumor inoculation
0
25
50
75
100
0
40
80
120
160

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

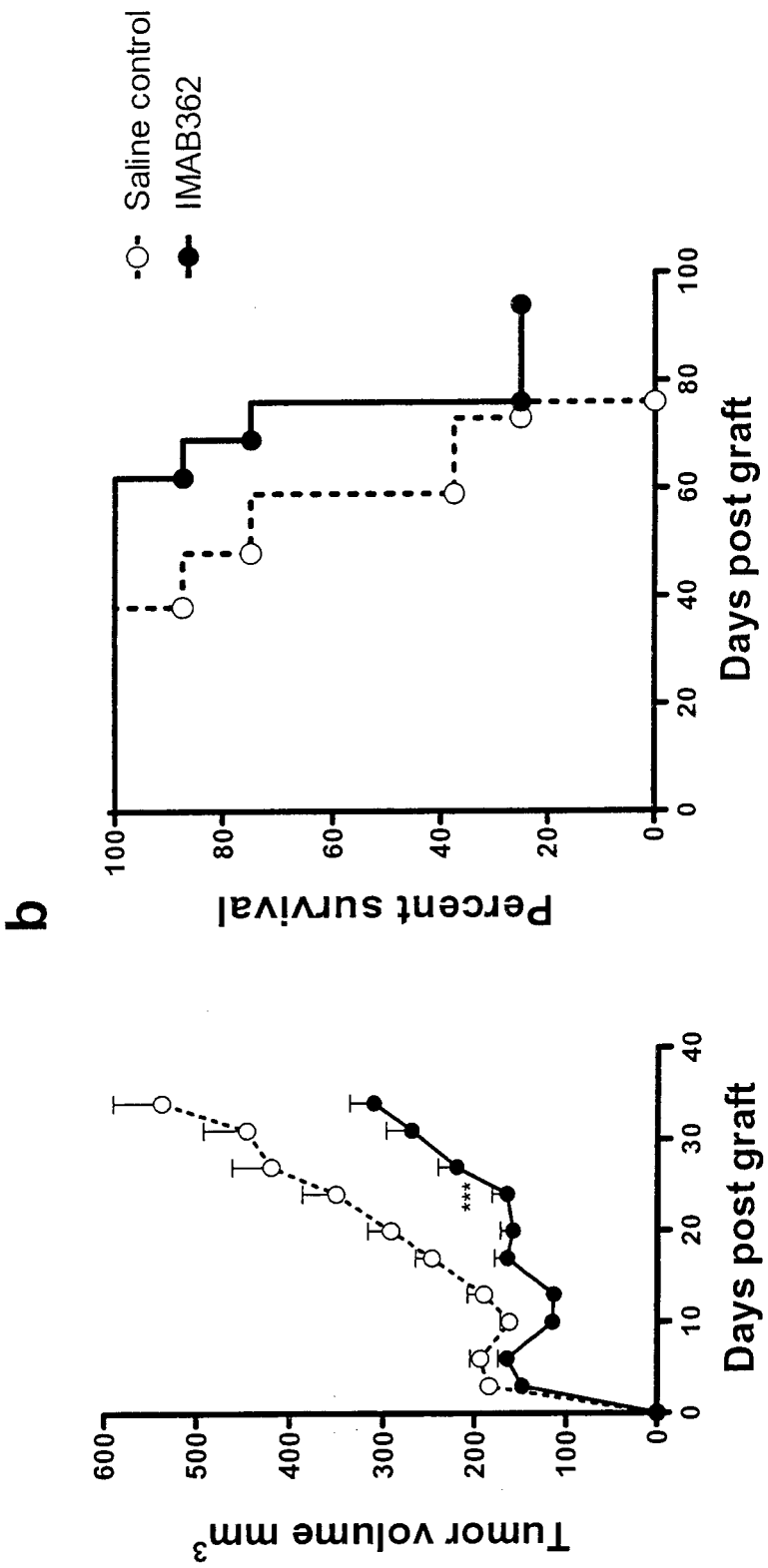

a
Tumor volume mm³
600
500
400
300
200
100
0
0
10
20
30
40
Days post graft
***
b
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days post graft
Saline control
IMAB362

Figure 22

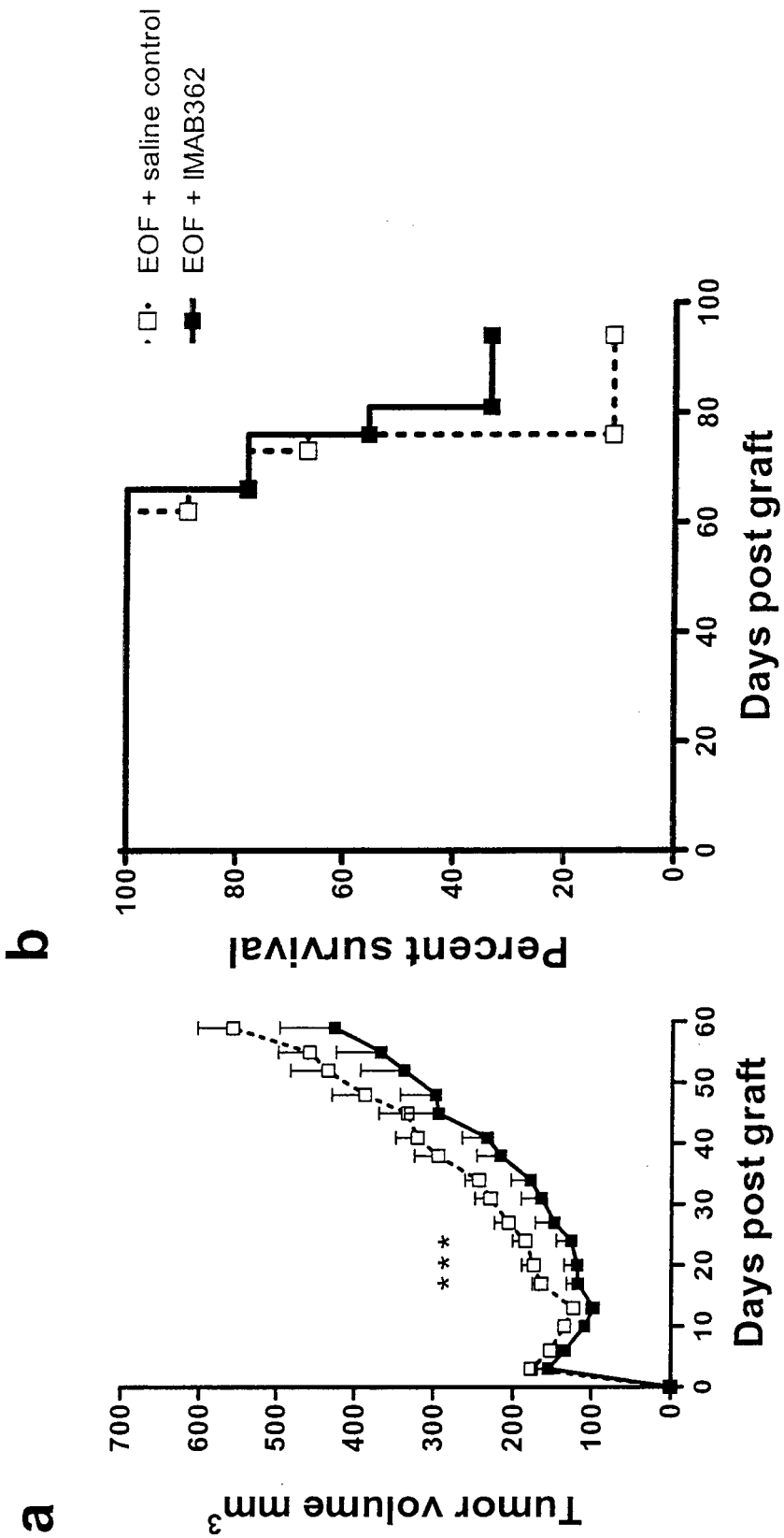

a
Tumor volume mm3
700
600
500
400
300
200
100
0
0 10 20 30 40 50 60
Days post graft
***
b
Percent survival
100
80
60
40
20
0
0 20 40 60 80 100
Days post graft
EOF + saline control
EOF + IMAB362

Figure 23

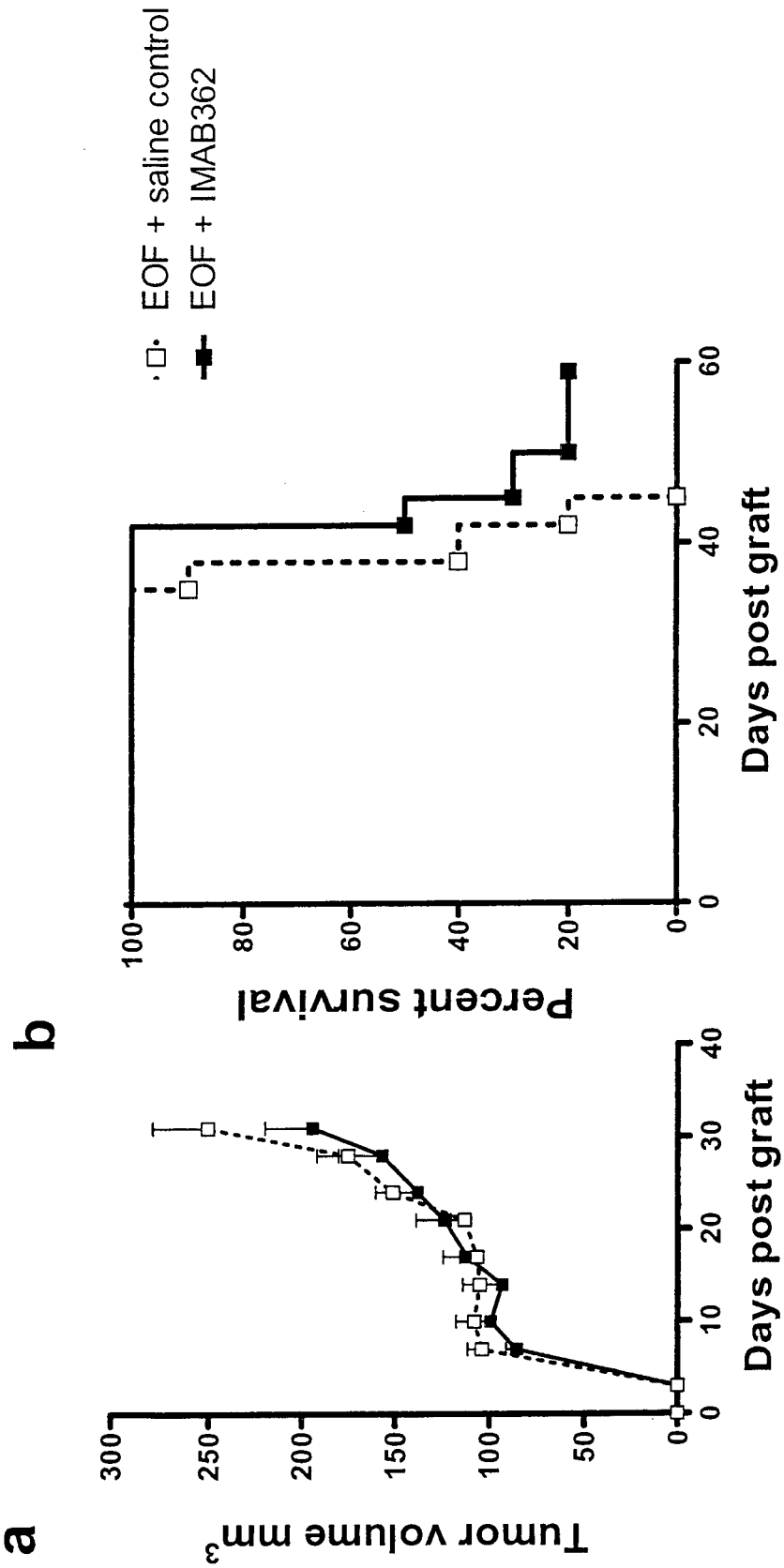

a
Tumor volume mm³
300
250
200
150
100
50
0
0
10
20
30
40
Days post graft
b
Percent survival
100
80
60
40
20
0
0
20
40
60
Days post graft
EOF + saline control
EOF + IMAB362

Figure 24

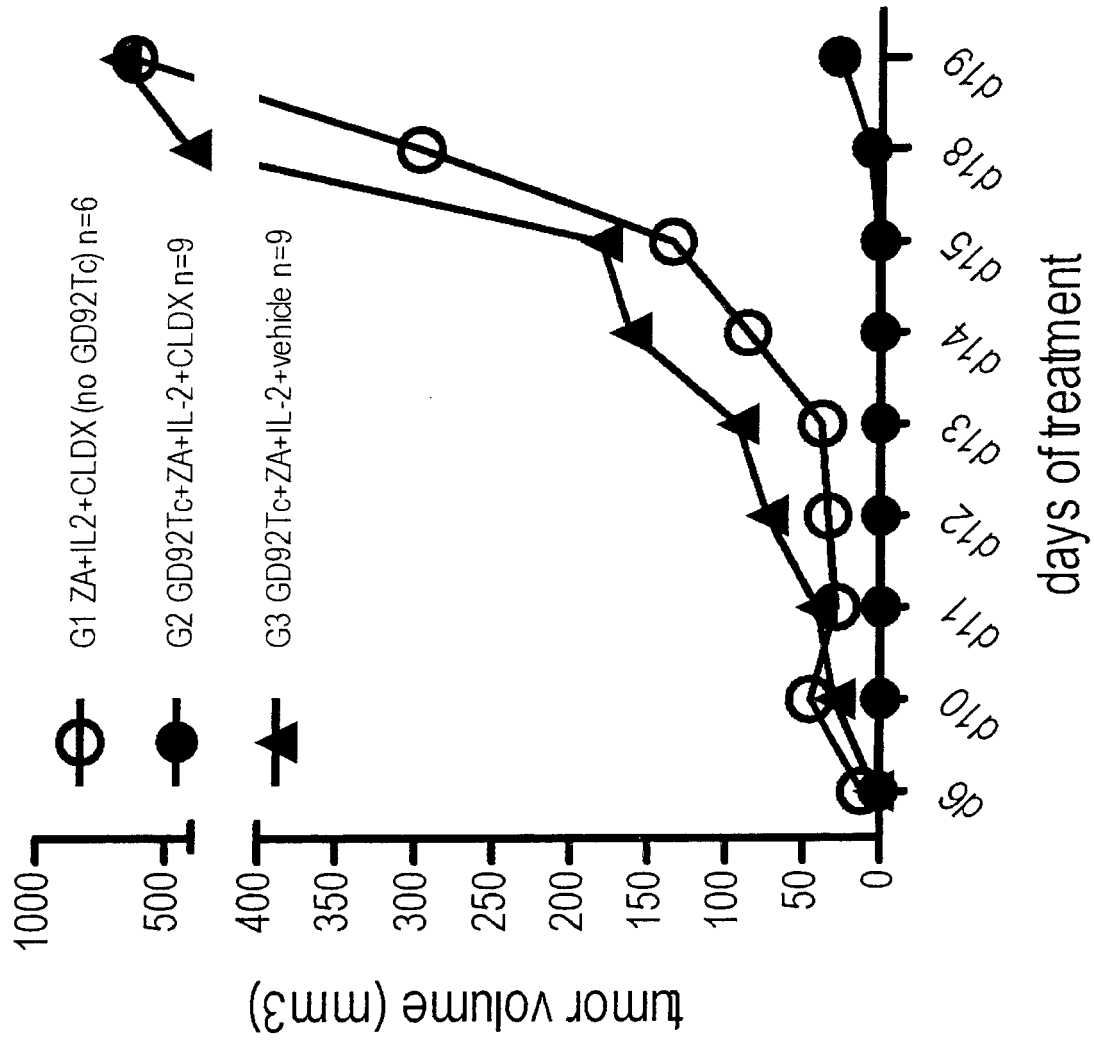

G1 ZA+IL2+CLDX (no GD92Tc) n=6
G2 GD92Tc+ZA+IL-2+CLDX n=9
G3 GD92Tc+ZA+IL-2+vehicle n=9
tumor volume (mm3)
1000
500
400
350
300
250
200
150
100
50
0
d6
d10
d11
d12
d13
d14
d15
d18
d19
days of treatment

Figure 25

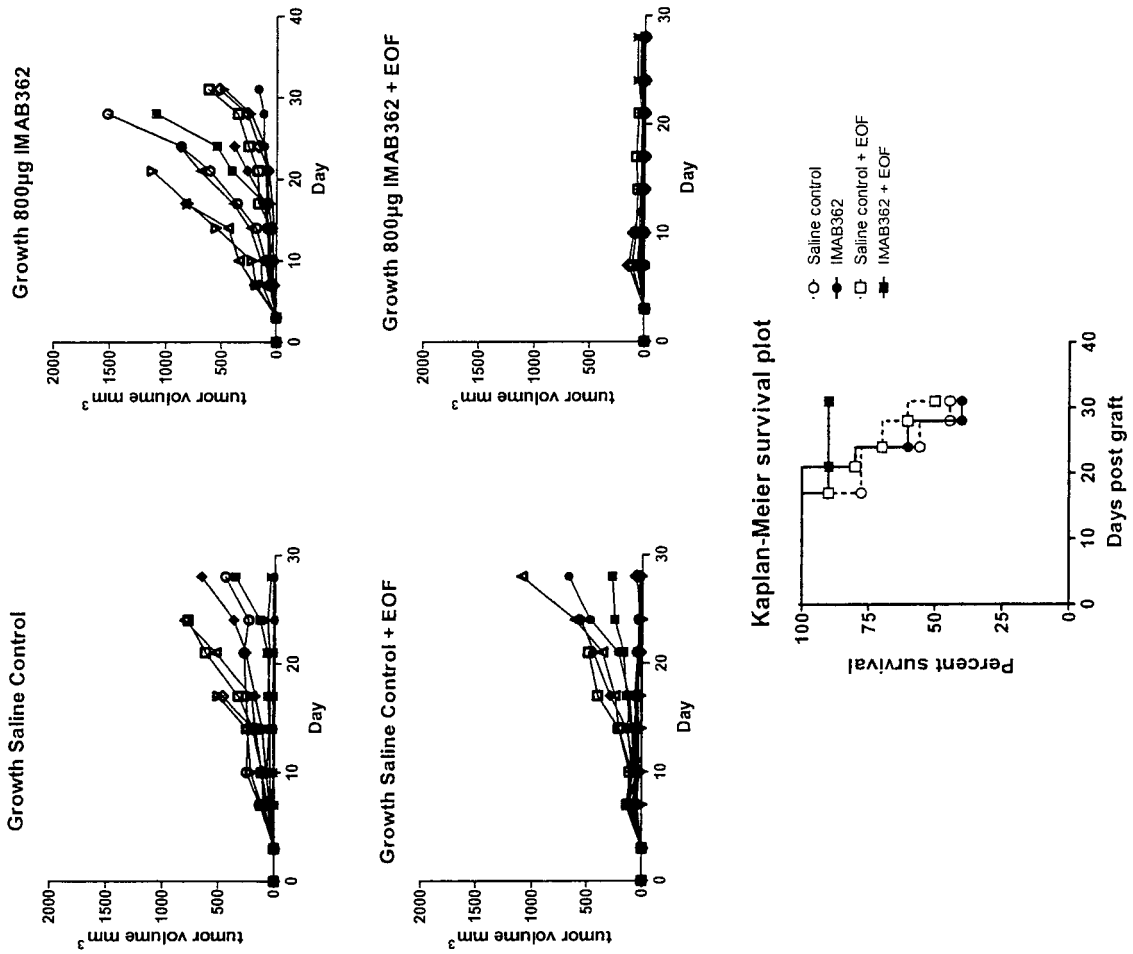

Growth 800µg IMAB362
Growth 800µg IMAB362 + EOF
Growth Saline Control
Growth Saline Control + EOF
tumor volume mm3
Day
Kaplan-Meier survival plot
Saline control
IMAB362
Saline control + EOF
IMAB362 + EOF
Percent survival
Days post graft

Figure 26