BIOCHEMICAL ENZYME ASSAY DATA

Compound	B-RafV600E $K_{m(ATP)}$=65 uM		WT B-Raf $K_{m(ATP)}$= 5 uM		WT c-Raf $K_{m(ATP)}$= 3 uM	
	$K_{i(app)}$	Adj. IC50 @ 1mM ATP	$K_{i(app)}$	Adj. IC50 @ 1mM ATP	$K_{i(app)}$	Adj. IC50 @ 1mM ATP
GDC-0879[1]	0.2	3.0	0.2	37	0.54	179
PLX-4720[2]	4.6	75	2.8	562	3.3	1091

<100 nM >100 nM >500 nM

[1]*Cancer Res.69(7):3042-*, [2]*Proc Natl Acad Sci U S A. 105(8):3041-6*

Figure 1. Biochemical enzyme assay data. The enzymatic activity of full length human V600E B-Raf, WT B-Raf, and WT c-Raf was quantified as incorporation of radiolabel from [γ-^{33}P]ATP (4 μM /33 μCi/mL) into FSBA-wtMEK. IC_{50} values were calculated by fitting a standard 4-parameter logistic model to the curve plotted as % of control versus [compound]. K_i's calculated from the estimated IC_{50}'s using a transformation of the Cheng-Prusoff equation were used to predict IC_{50}'s at 1 mM ATP from the equation $IC_{50} = K_i (1 + S/K_m)$.

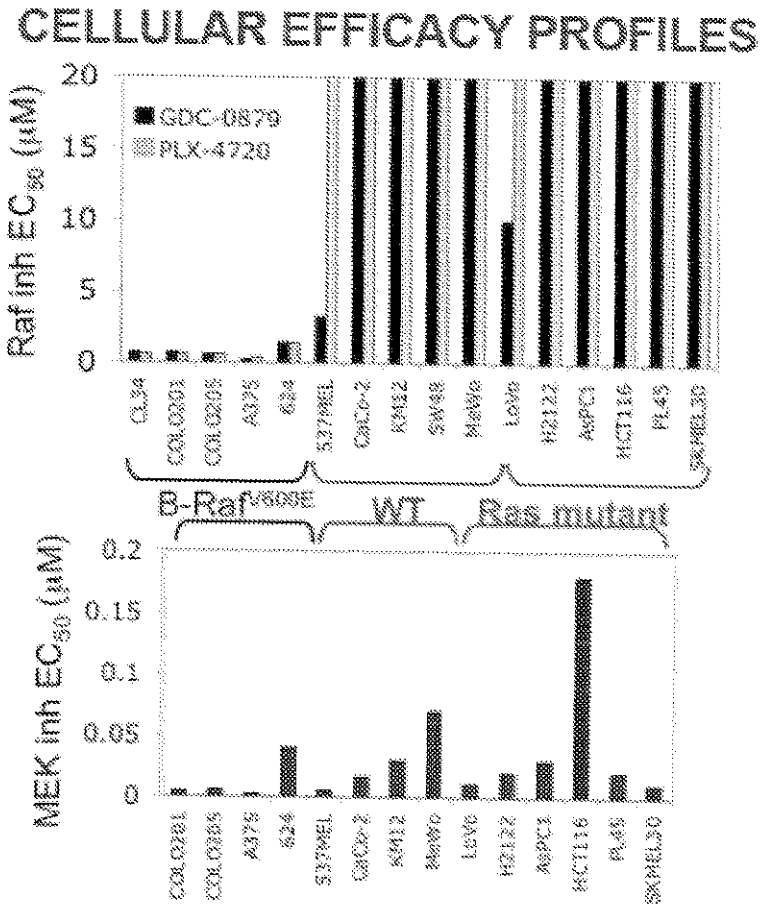


Figure 2. Viability assays in tumor lines of different Raf/Ras mutational status. Compound EC_{50} values measured using B-Raf^{V600E} and WT tumor lines in 4-day viability/proliferation assays using the CellTiter-Glo reagent (Promega)

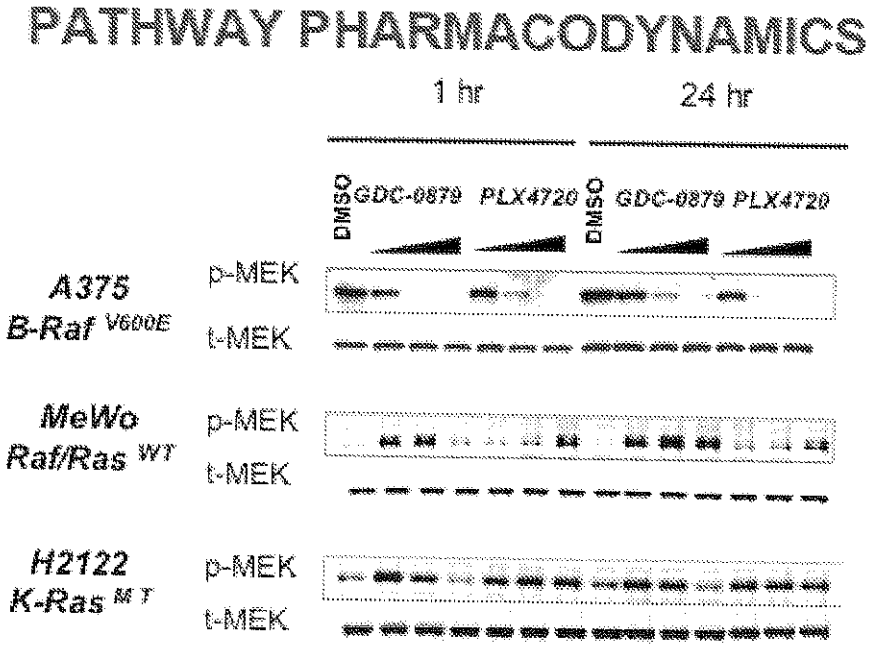


Figure 3. Western blot analysis of pMEK levels in B-Raf^{V600E}, Raf/Ras^{WT} and K-Ras^{MT} lines.
Cells were treated with increasing doses of GDC-0879 and PLX4720 (0.1, 1, 10 μM) for the indicated times.

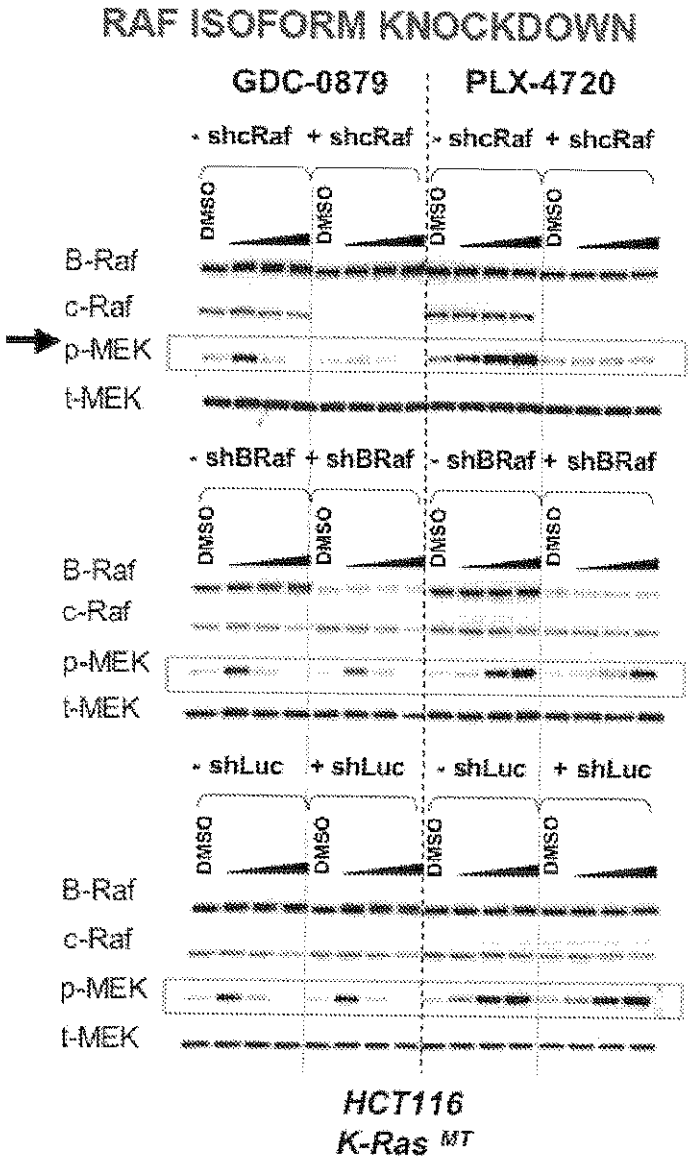


Figure 4. Western blot analysis of pMEK levels upon inducible c- and B-Raf knockdown in HCT116 Ras^{MT} cells. Stable clones were incubated with doxycycline for 24 hours to induce shRNA expression and were subsequently treated for 1 hr with increasing amounts of inhibitors (0.1, 1, 10 μM).

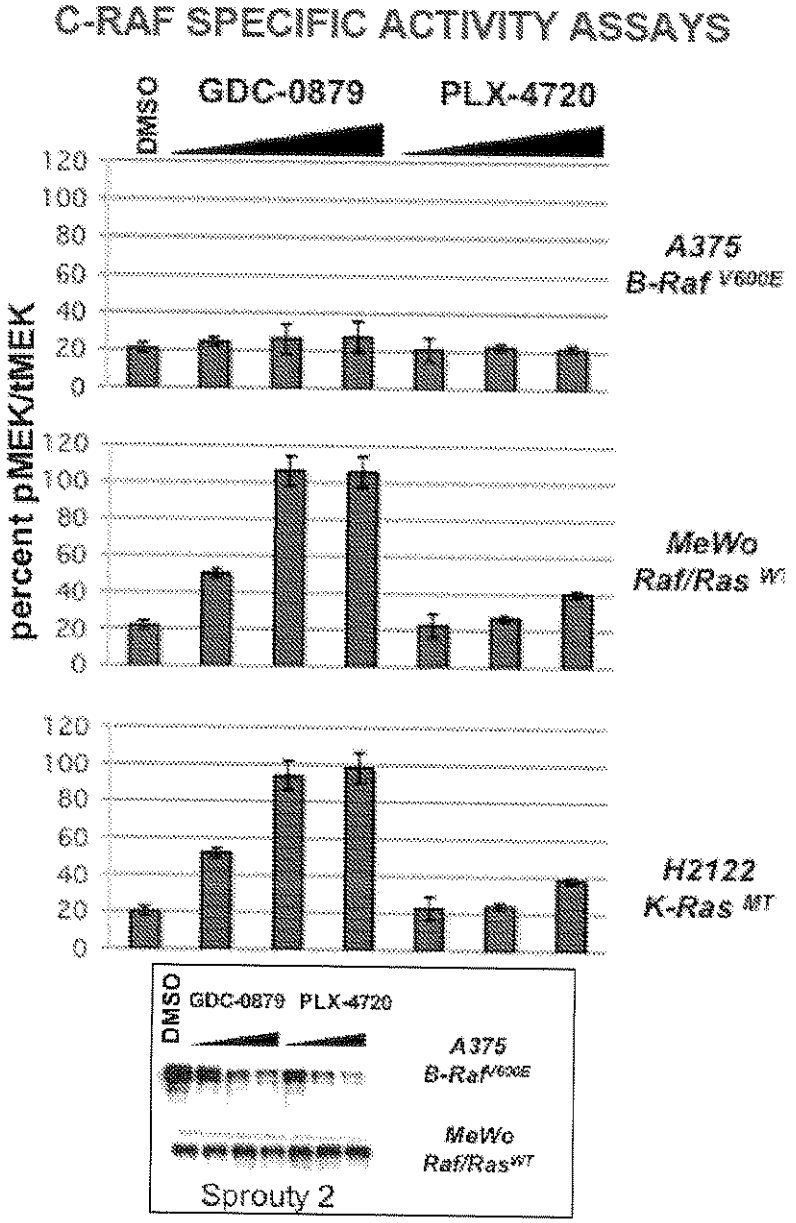
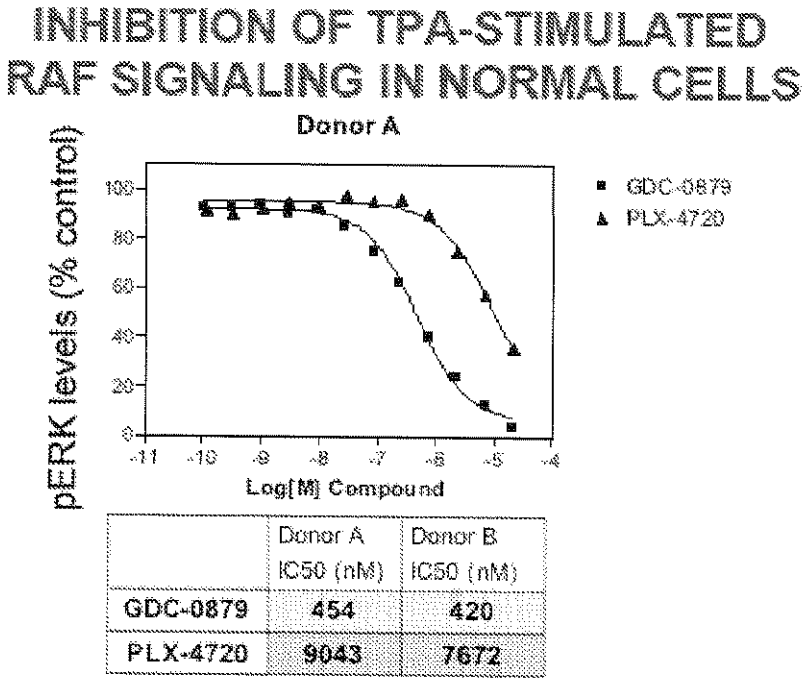


Figure 5. c-Raf specific activity assays after short-term treatment with inhibitors. Cells were treated for 1hr with inhibitors (0.1, 1, 10 μM), washed and lysed. c-Raf was immunoprecipitated and used in an in vitro kinase assay with MEK as substrate.



	Donor A IC50 (nM)	Donor B IC50 (nM)
GDC-0879	454	420
PLX-4720	9043	7672

Figure 6. Inhibition of TPA-stimulated pERK levels in peripheral blood mononuclear cells (PBMC). Human PBMCs were incubated with compounds for 1 hour, then stimulated for 10 minutes with 1 μg/mL TPA. Cells were fixed in formaldehyde, permeabilized in methanol, stained for phospho-ERK (pERK) and analyzed by flow cytometry.

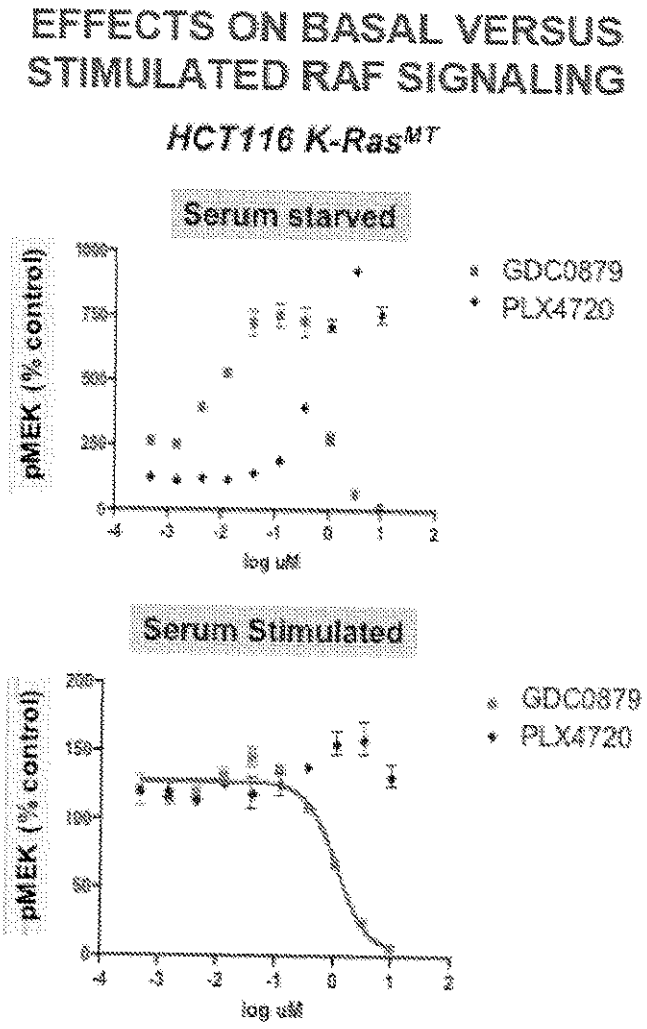


Figure 7. Effects on basal vs. serum stimulated pMEK levels in HCT116 cells. Cells were serum starved overnight and incubated with compounds for 1 hour, then stimulated for 10 minutes with 10% FCS in triplicate for bottom panel. Phospho- and total MEK levels were determined by MSD. pMEK levels were normalized to total MEK and plotted as percent of DMSO control.

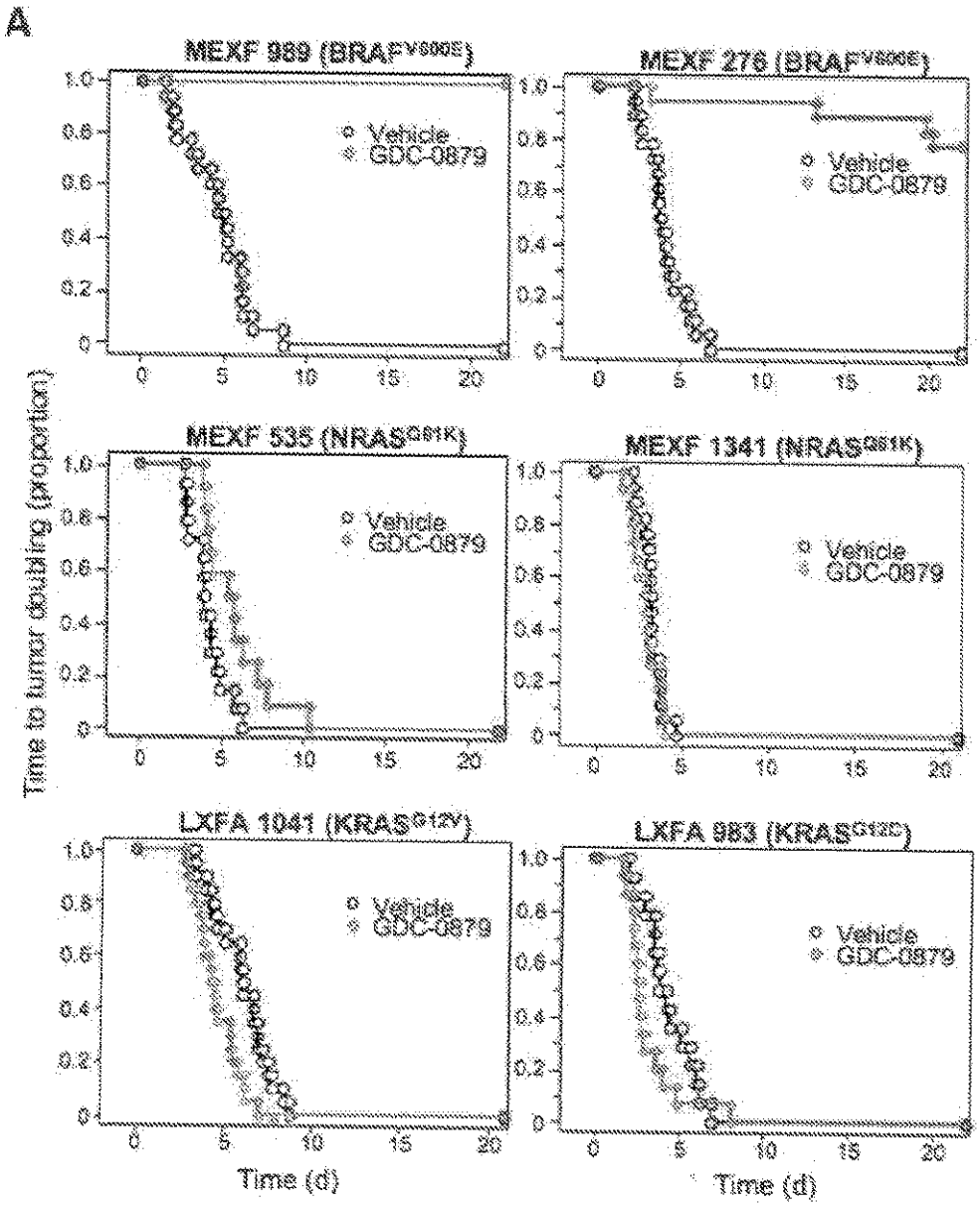

A
MEXF 989 (BRAF V600E)
MEXF 276 (BRAF V600E)
MEXF 535 (NRAS Q61K)
MEXF 1341 (NRAS Q61K)
LXFA 1041 (KRAS G12V)
LXFA 983 (KRAS G12D)
Vehicle
GDC-0879
Time to tumor doubling (proportion)
Time (d)

Fig. 8A

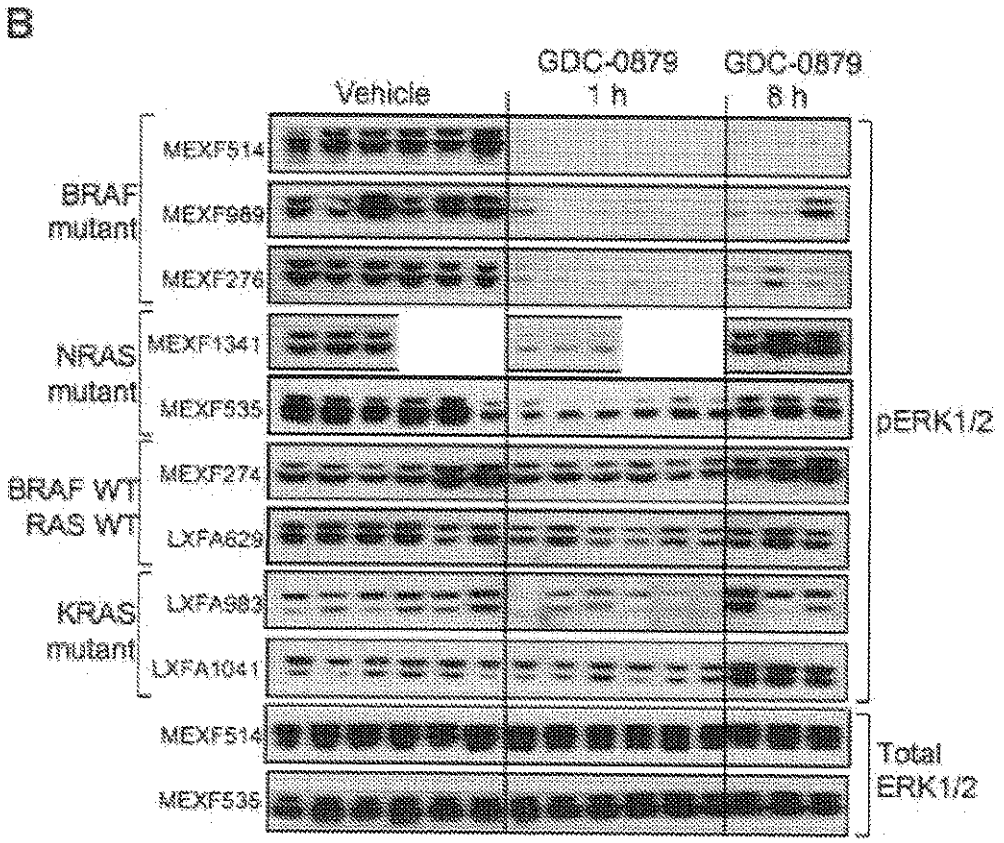

B
Vehicle
GDC-0879 1 h
GDC-0879 8 h
BRAF mutant
NRAS mutant
BRAF WT RAS WT
KRAS mutant
MEXF514
MEXF989
MEXF276
MEXF1341
MEXF535
MEXF274
LXFA629
LXFA983
LXFA1041
pERK1/2
Total ERK1/2

Fig. 8B

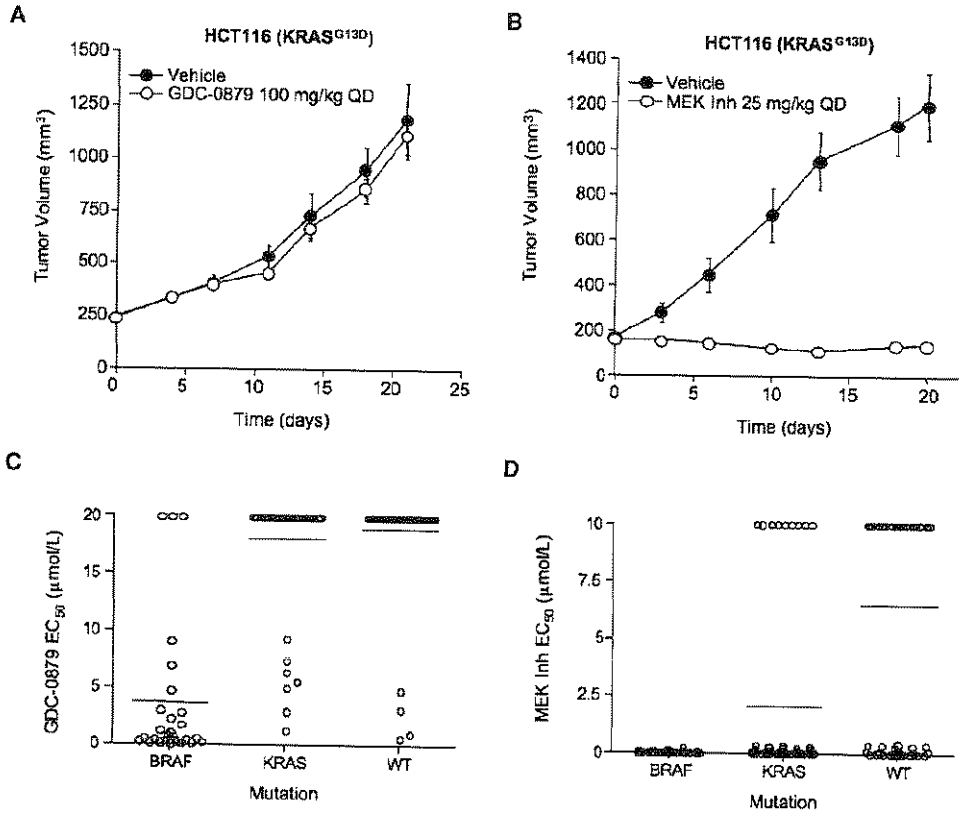

A
HCT116 (KRASG13D)
Vehicle
GDC-0879 100 mg/kg QD
Tumor Volume (mm3)
Time (days)
B
HCT116 (KRASG13D)
Vehicle
MEK Inh 25 mg/kg QD
Tumor Volume (mm3)
Time (days)
C
GDC-0879 EC50 (µmol/L)
BRAF
KRAS
WT
Mutation
D
MEK Inh EC50 (µmol/L)
BRAF
KRAS
WT
Mutation

Fig. 9A, B, C & D

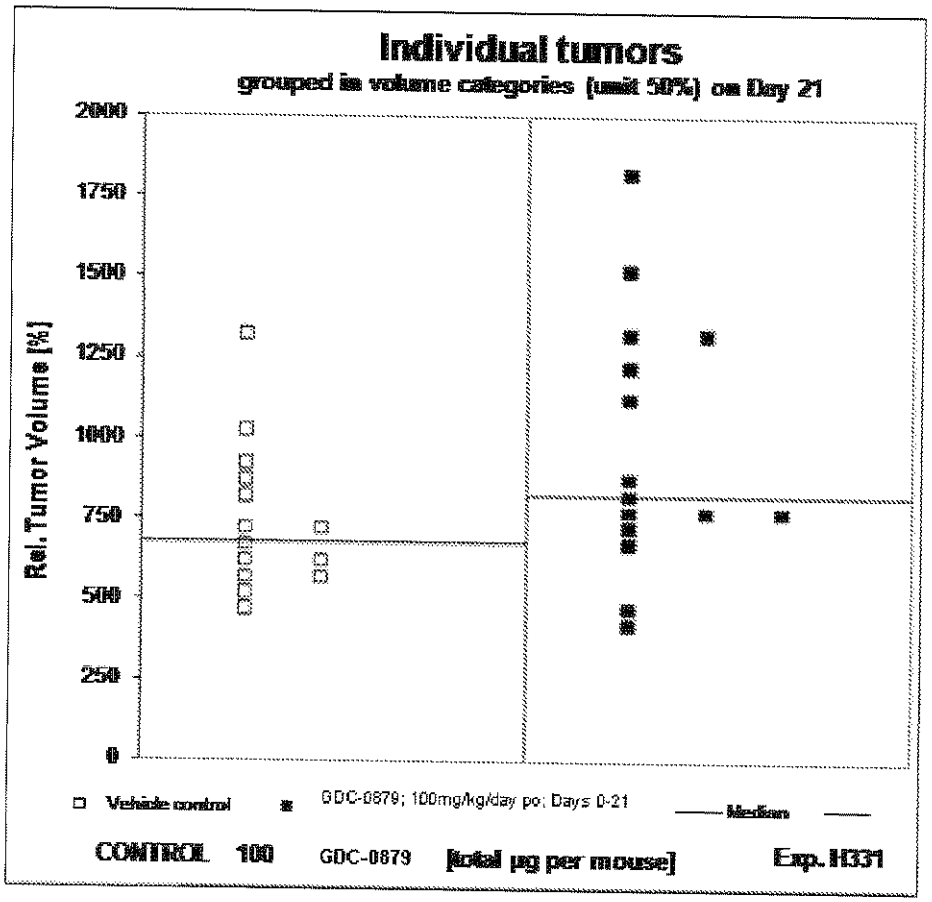

Individual tumors
grouped in volume categories (unit 50%) on Day 21
Rel. Tumor Volume [%]
2000
1750
1500
1250
1000
750
500
250
0
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
Median
CONTROL
100
GDC-0879
[total µg per mouse]
Exp. H331

Fig. 10

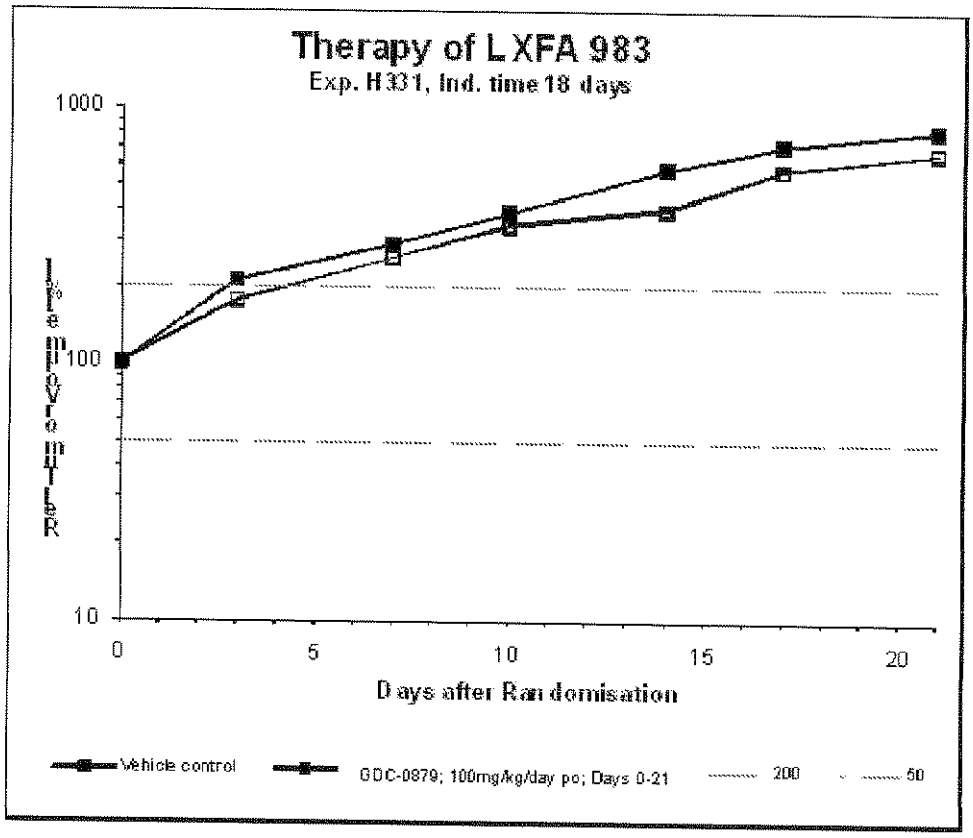

Therapy of LXFA 983
Exp. H331, Ind. time 18 days
1000
100
10
Tumor volume [mm³]
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
200
50

Fig. 11

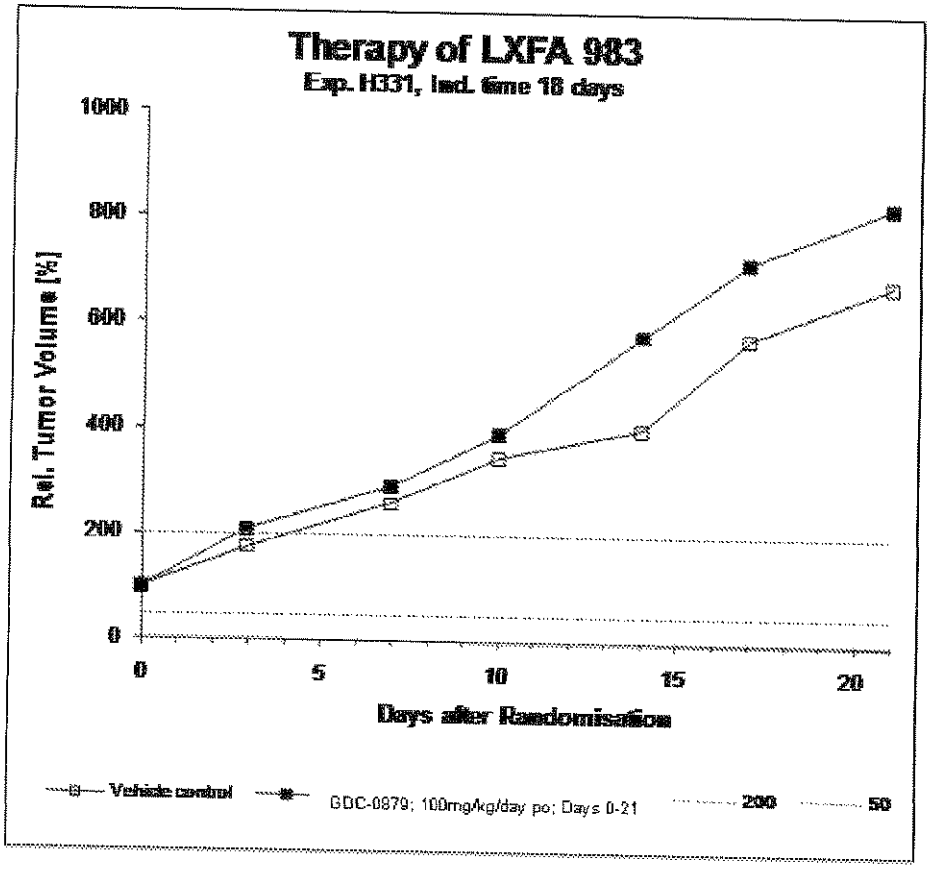

Therapy of LXFA 983
Exp. H331, last time 18 days
Rel. Tumor Volume [%]
1000
800
600
400
200
0
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
200
50

Fig. 12

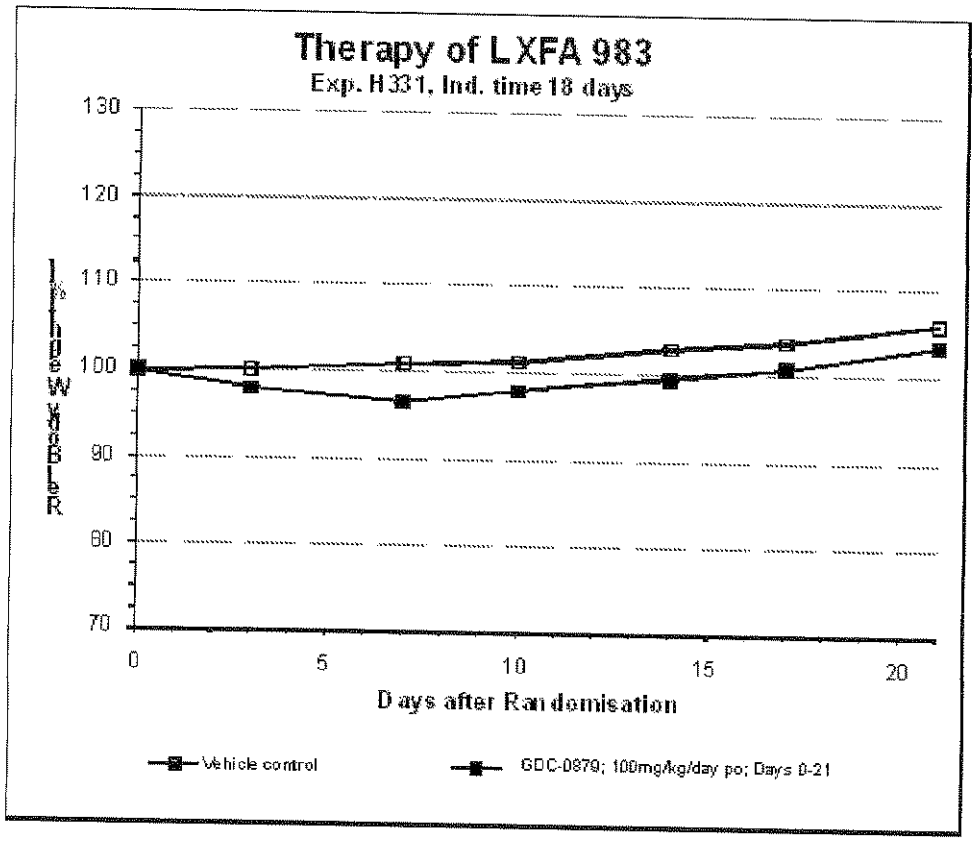

Therapy of LXFA 983
Exp. H331, Ind. time 18 days
130
120
110
100
90
80
70
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21

Fig. 13

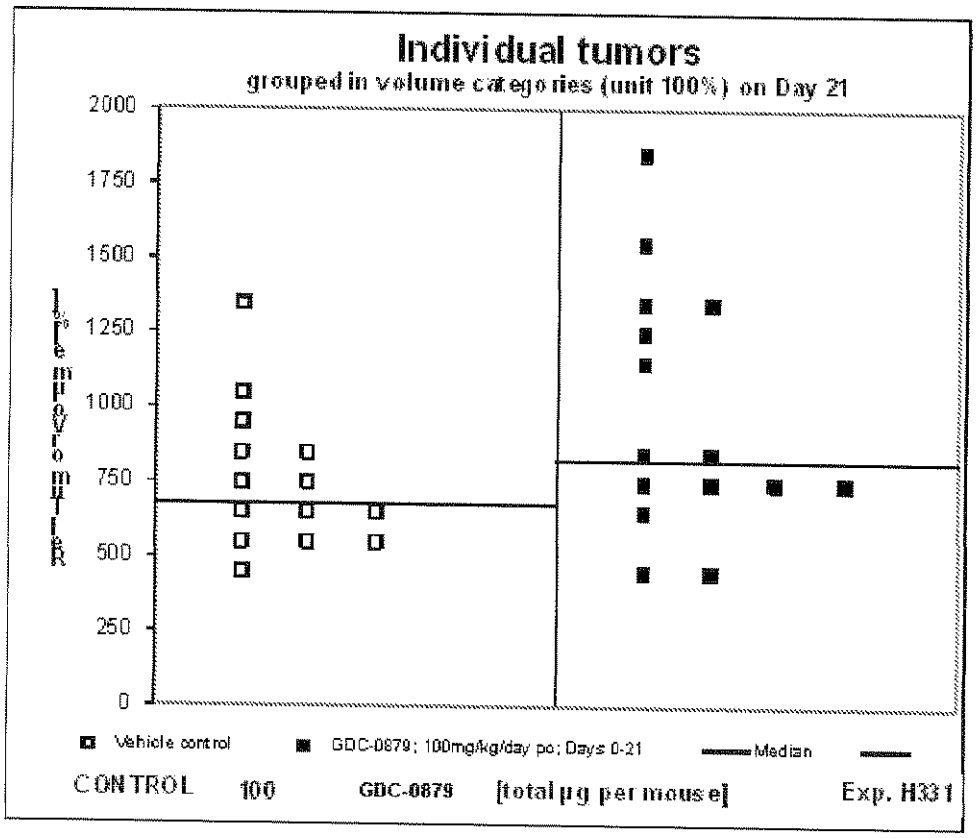

Individual tumors
grouped in volume categories (unit 100%) on Day 21
2000
1750
1500
1250
1000
750
500
250
0
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-21
Median
CONTROL
100
GDC-0879
[total µg per mouse]
Exp. H331

Fig. 14

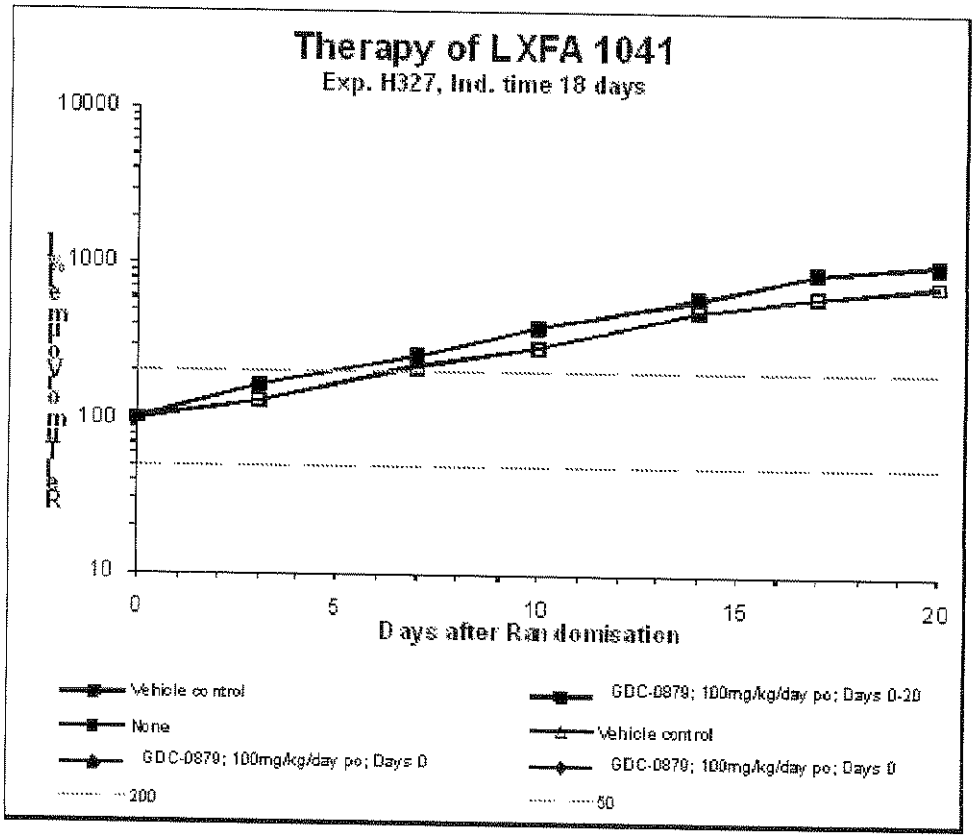

Therapy of LXFA 1041
Exp. H327, Ind. time 18 days
10000
1000
100
10
Tumor Volume [mm3]
0
5
10
15
20
Days after Randomisation
Vehicle control
None
GDC-0879; 100mg/kg/day po; Days 0
200
GDC-0879; 100mg/kg/day po; Days 0-20
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0
50

Fig. 15

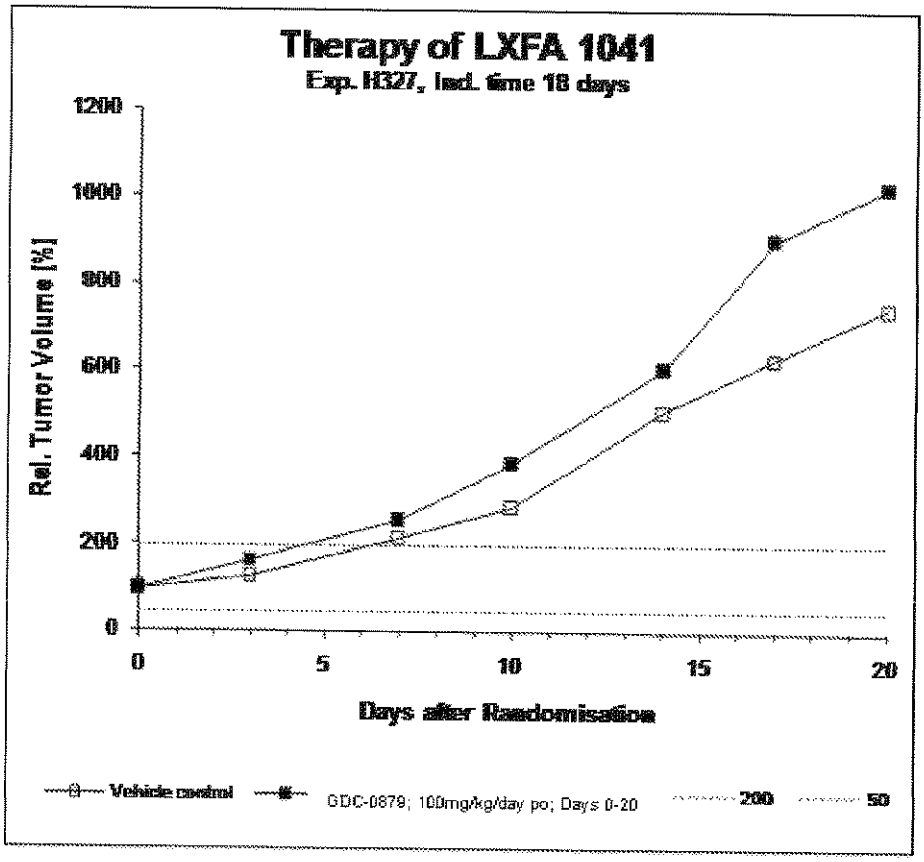

Therapy of LXFA 1041
Exp. H327, Incl. time 18 days
Rel. Tumor Volume [%]
1200
1000
800
600
400
200
0
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-20
200
50

Fig. 16

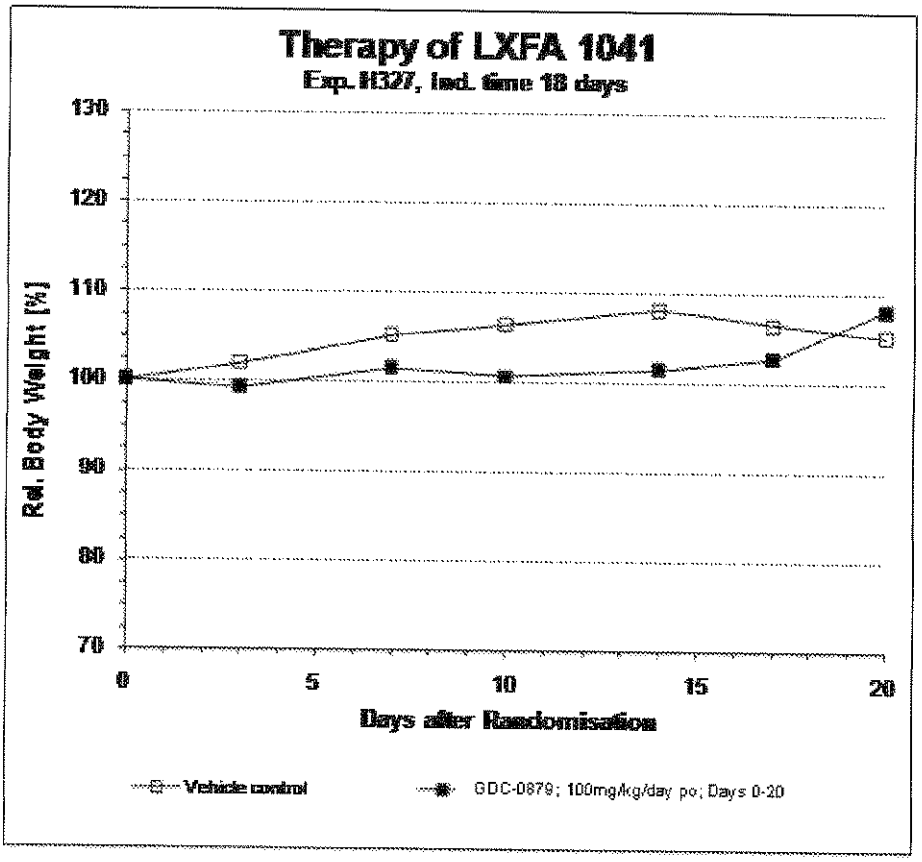

Therapy of LXFA 1041
Exp. H327, Ind. time 18 days
Rel. Body Weight [%]
130
120
110
100
90
80
70
0
5
10
15
20
Days after Randomisation
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-20

Fig. 17

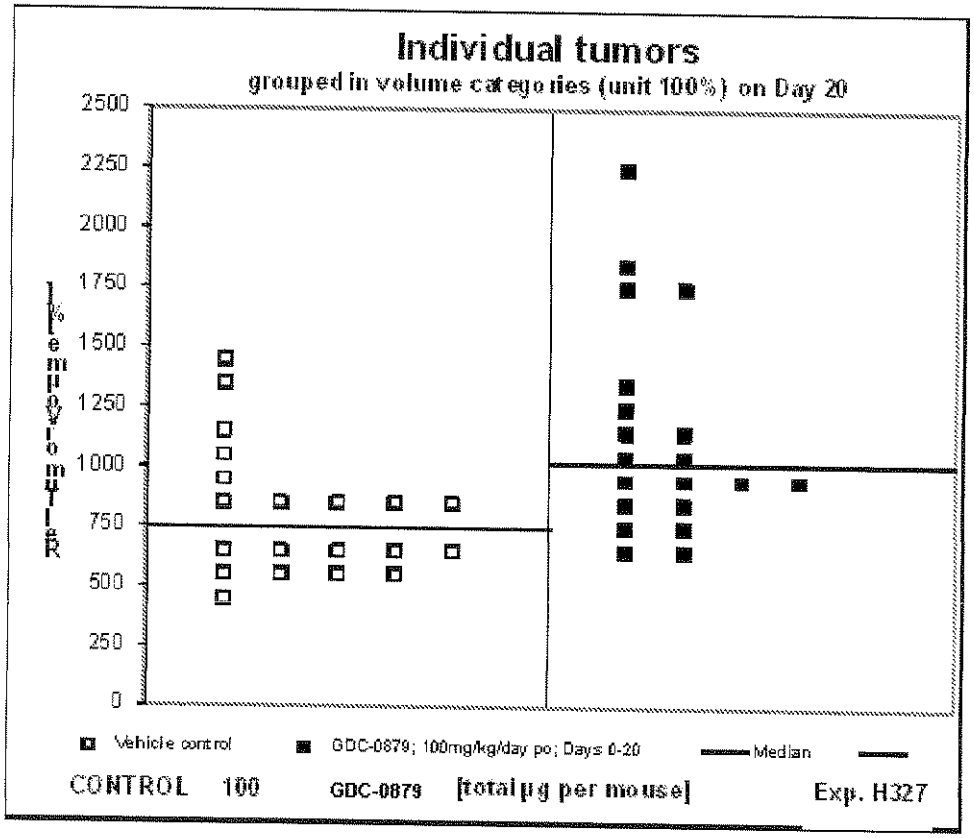

Individual tumors
grouped in volume categories (unit 100%) on Day 20
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Vehicle control
GDC-0879; 100mg/kg/day po; Days 0-20
Median
CONTROL 100
GDC-0879
[total µg per mouse]
Exp. H327

Fig. 18

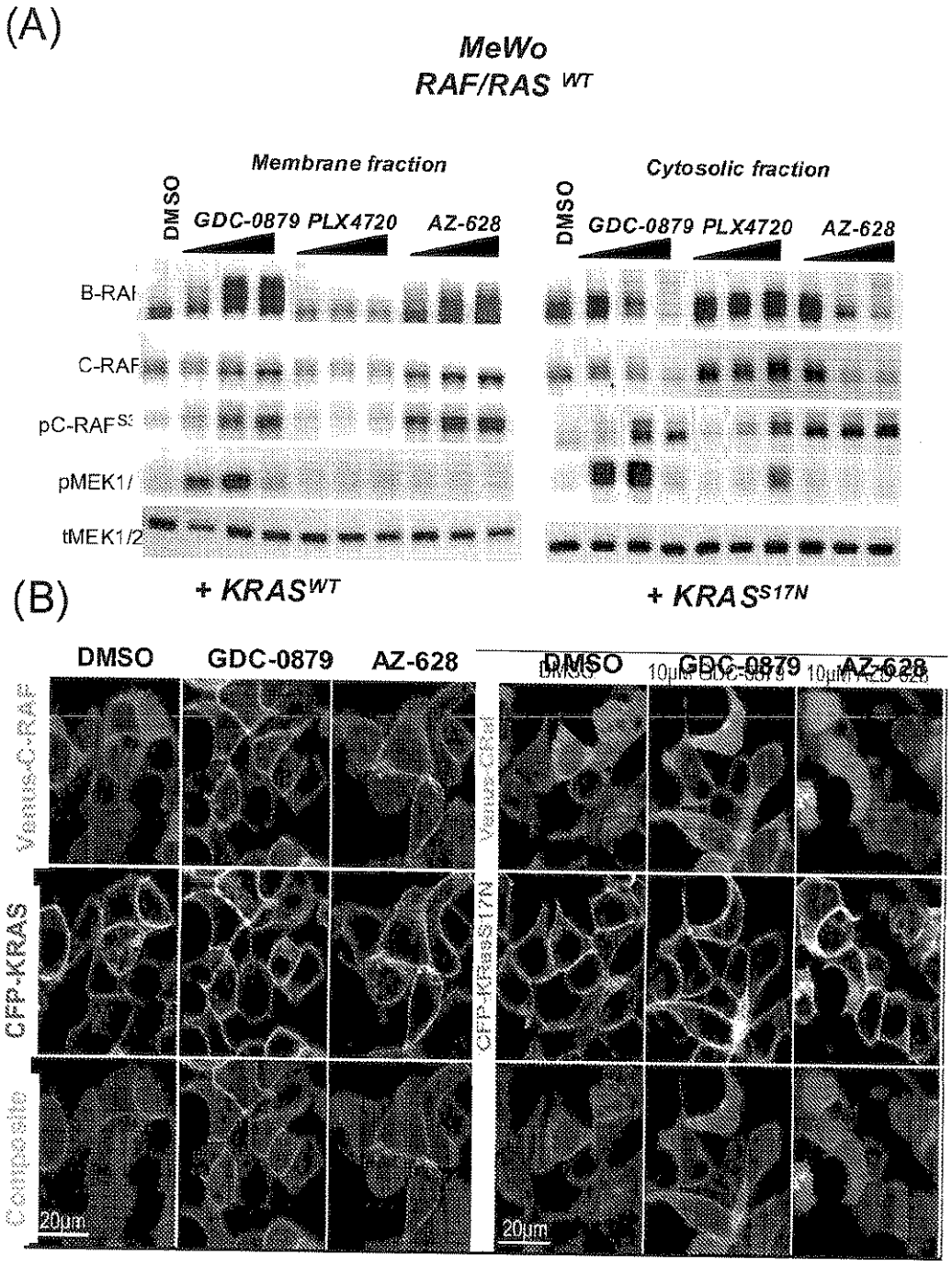
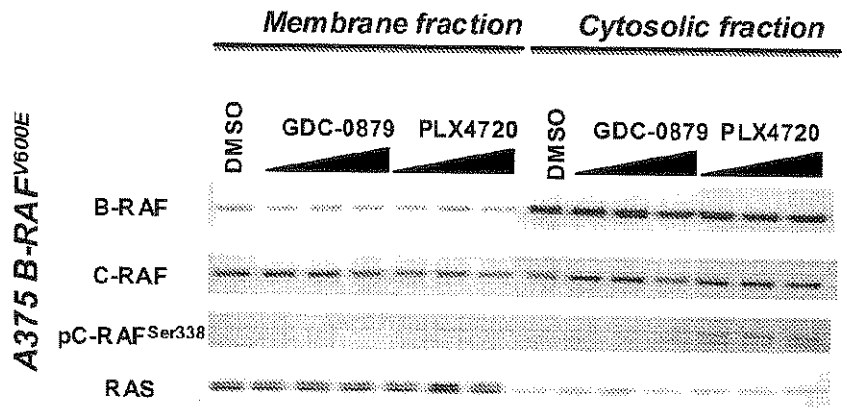
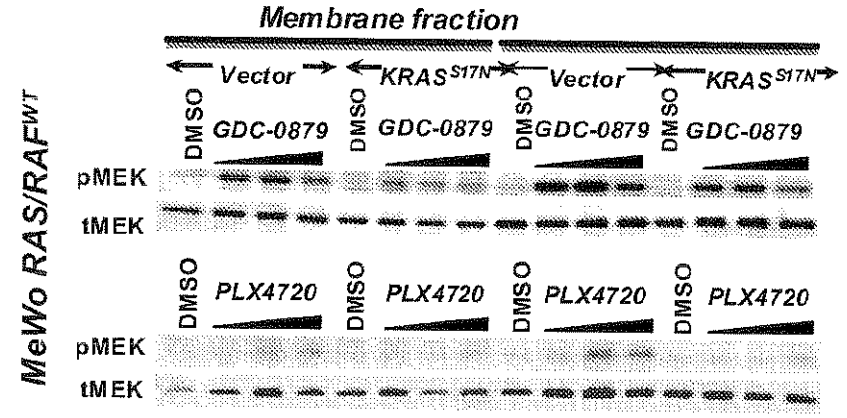

(A)
MeWo
RAF/RAS WT
Membrane fraction
Cytosolic fraction
DMSO
GDC-0879 PLX4720 AZ-628
B-RAF
C-RAF
pC-RAF
pMEK1/
tMEK1/2
(B)
+ KRASWT
+ KRASS17N
DMSO GDC-0879 AZ-628
Venus-C-RAF
CFP-KRAS
Composite
20μm

Fig. 19A & B

(A)

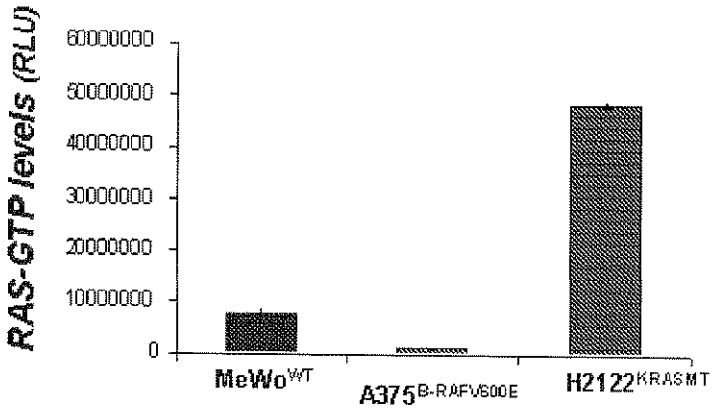

Membrane fraction
Cytosolic fraction
DMSO
GDC-0879
PLX4720
DMSO
GDC-0879
PLX4720
A375 B-RAF V600E
B-RAF
C-RAF
pC-RAF Ser338
RAS

(B)

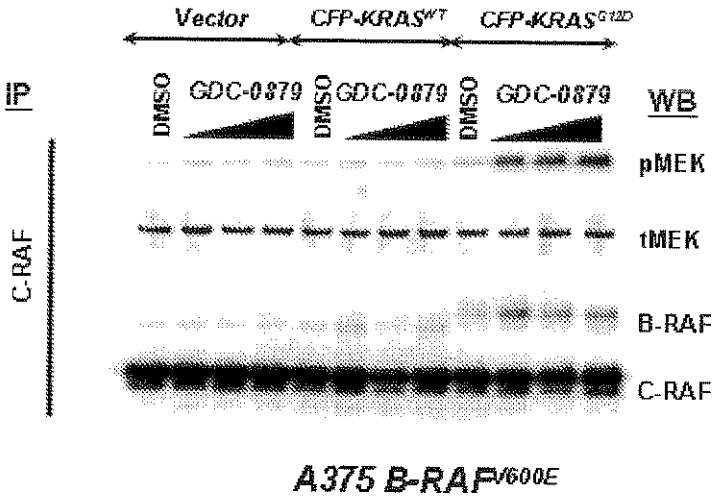

Membrane fraction
Vector
KRAS S17N
Vector
KRAS S17N
DMSO
GDC-0879
DMSO
GDC-0879
DMSO
GDC-0879
DMSO
GDC-0879
MeWo RAS/RAF WT
pMEK
tMEK
DMSO
PLX4720
DMSO
PLX4720
DMSO
PLX4720
DMSO
PLX4720
pMEK
tMEK

Figs. 20A & B

(C)


RAS-GTP levels (RLU)
60000000
50000000
40000000
30000000
20000000
10000000
0
MeWo WT
A375 B-RAFV600E
H2122 KRASMT

(D)

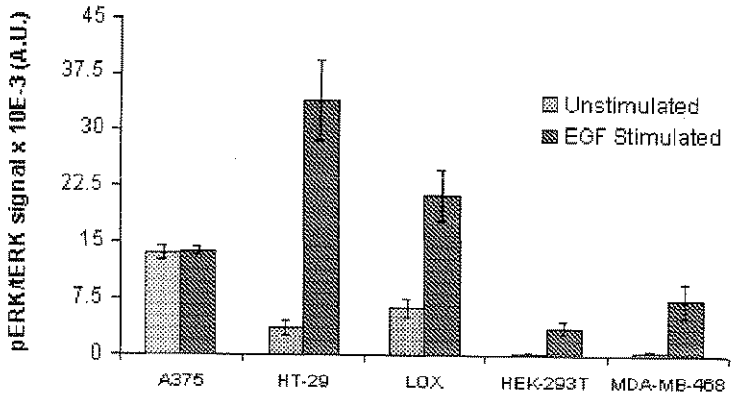

Vector
CFP-KRAS WT
CFP-KRAS G12D
IP
DMSO
GDC-0879
DMSO
GDC-0879
DMSO
GDC-0879
WB
C-RAF
pMEK
tMEK
B-RAF
C-RAF
A375 B-RAF V600E

Figs. 20C & D

(A)

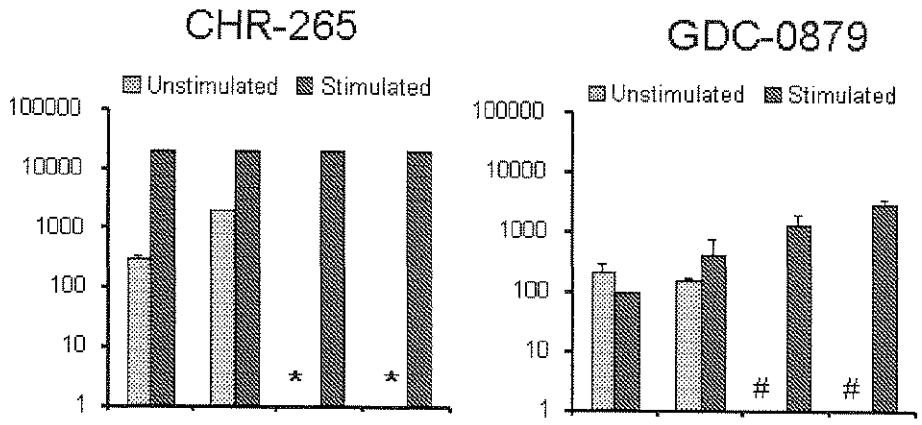
Cell Line	Tissue type	B-Raf status	Surface EGFR Staining
A375	Melanoma	V600E mutant	57
HT29	Colon	V600E mutant	81
LOX IMVI	Melanoma	V600E mutant	168
HEK-293T	Kidney	Wildtype	ND
MDA-MD-468	Breast	Wildtype	ND

(B)

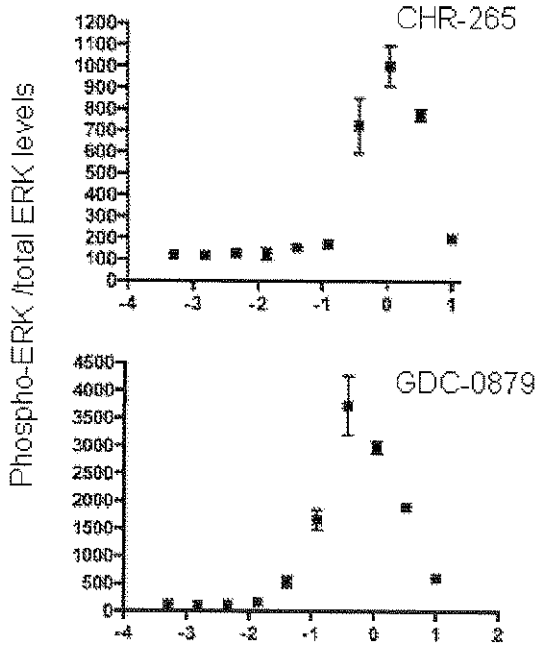


Figs. 21 A & B

(C)


CHR-265
GDC-0879
Unstimulated
Stimulated
100000
10000
1000
100
10
1
*
#

(D)

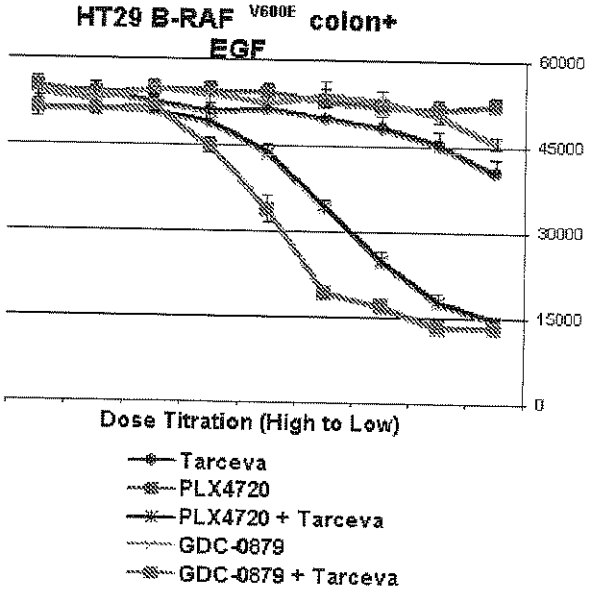

Phospho-ERK /total ERK levels
CHR-265
GDC-0879

Figs. 21C & D

(A)

Compound	LOX (B-Raf^{V600E}) pMEK IC_{50} (nM)		HT-29 (B-Raf^{V600E}) pMEK IC_{50} (nM)	
	-EGF	+EGF	-EGF	+EGF
GDC-0879				
Expt 1	25	27	10	25
Expt 2	14	17	19	20
PLX-4720				
Expt 1	16	>1000	34	>1000
Expt 2	14	>1000	34	>1000

(B)



Figs. 22A & B

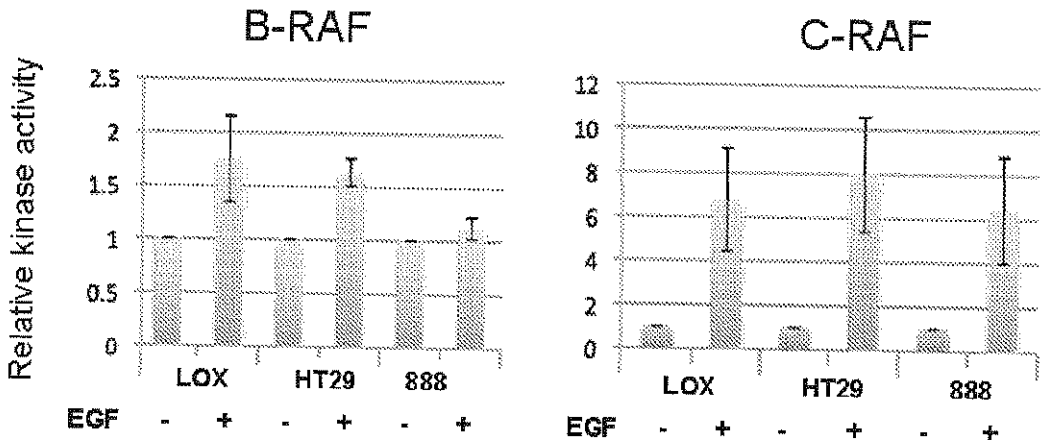

B-RAF
C-RAF
Relative kinase activity
2.5
2
1.5
1
0.5
0
12
10
8
6
4
2
0
LOX
HT29
888
EGF - + - + - +

Fig. 23

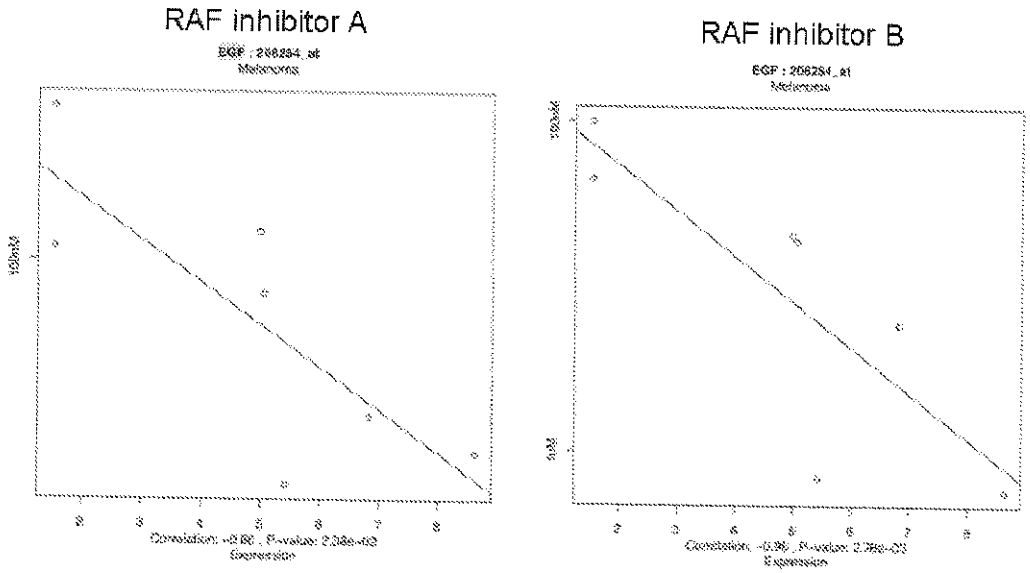


Fig. 24

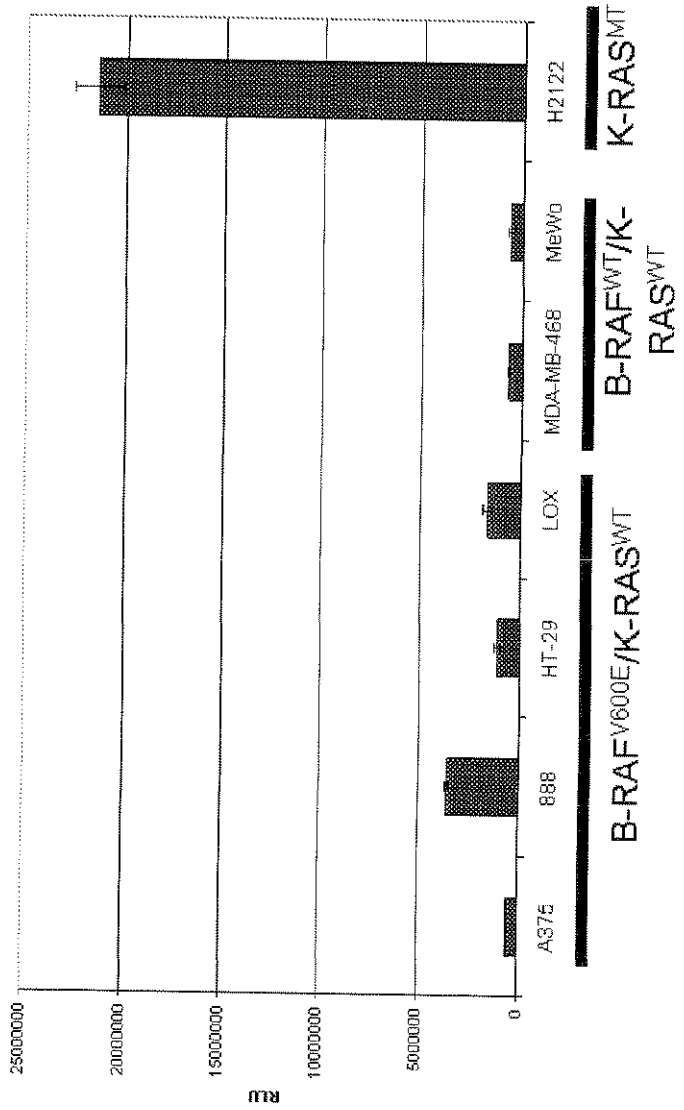

Basal Ras-GTP Levels
RLU
25000000
20000000
15000000
10000000
5000000
0
A375
888
HT-29
LOX
MDA-MB-468
MeWo
H2122
B-RAF^{V600E}/K-RAS^{WT}
B-RAF^{WT}/K-RAS^{WT}
K-RAS^{MT}

Fig. 25

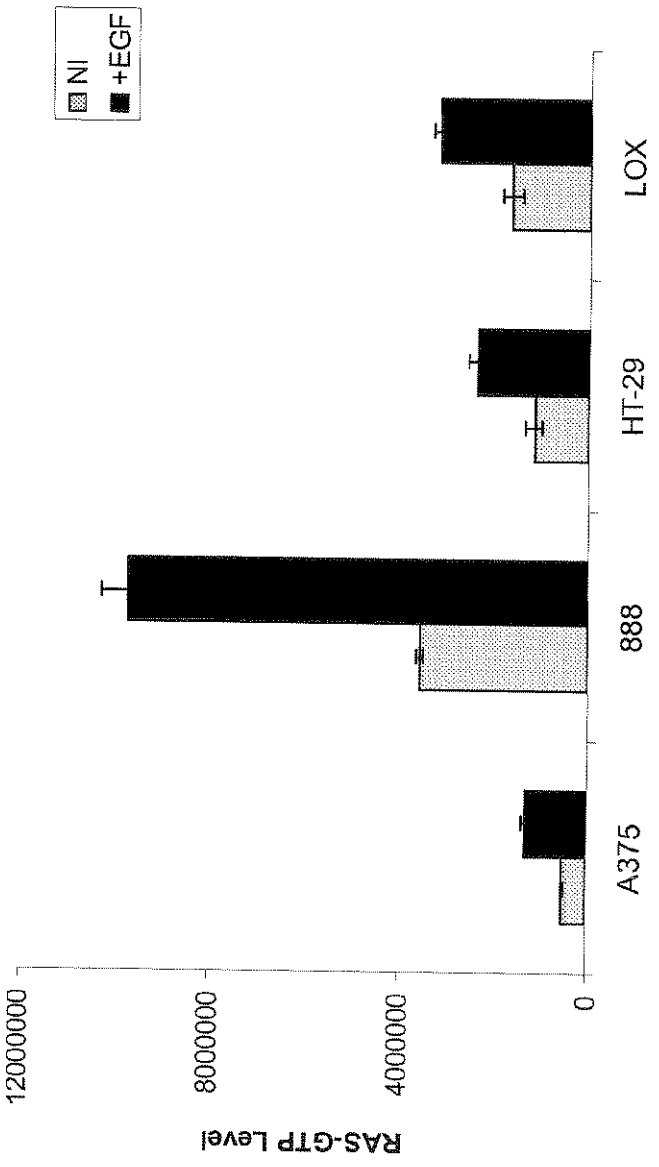

NI
+EGF
RAS-GTP Level
12000000
8000000
4000000
0
A375
888
HT-29
LOX

Fig. 26

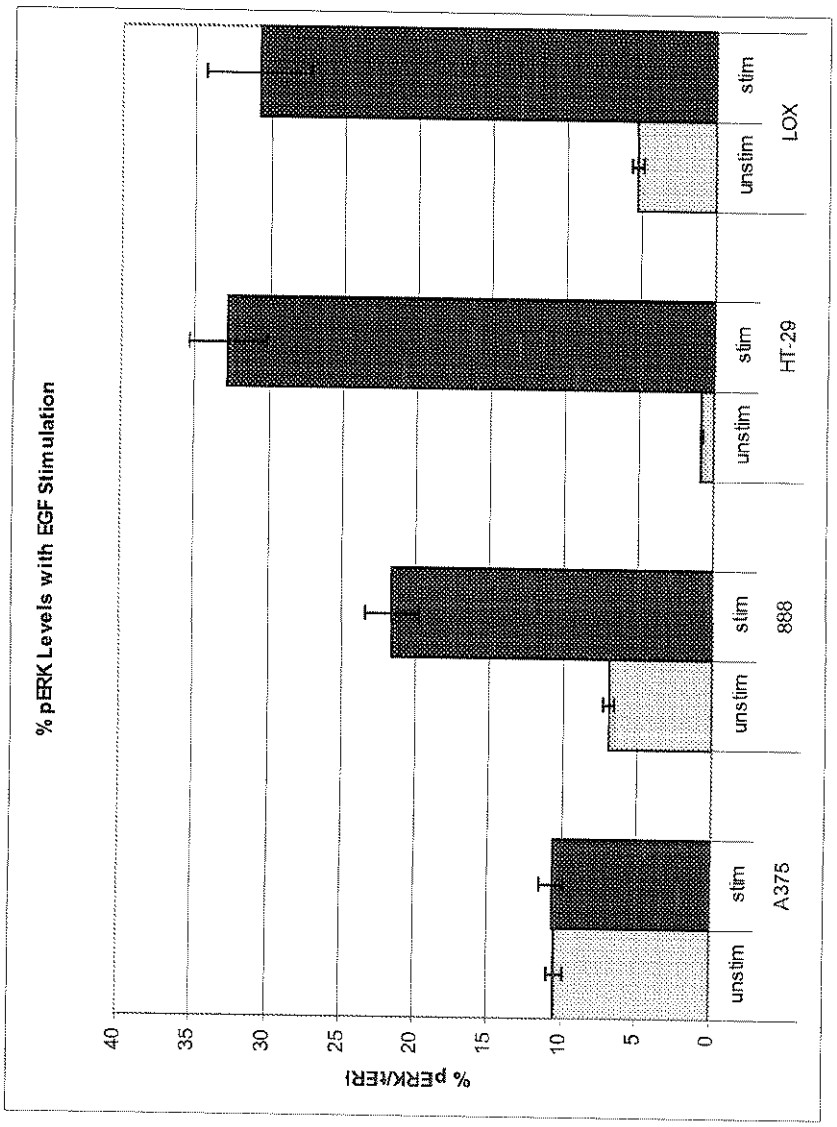

% pERK Levels with EGF Stimulation
% pERK/tERK
40
35
30
25
20
15
10
5
0
unstim
stim
A375
888
HT-29
LOX

Fig. 27

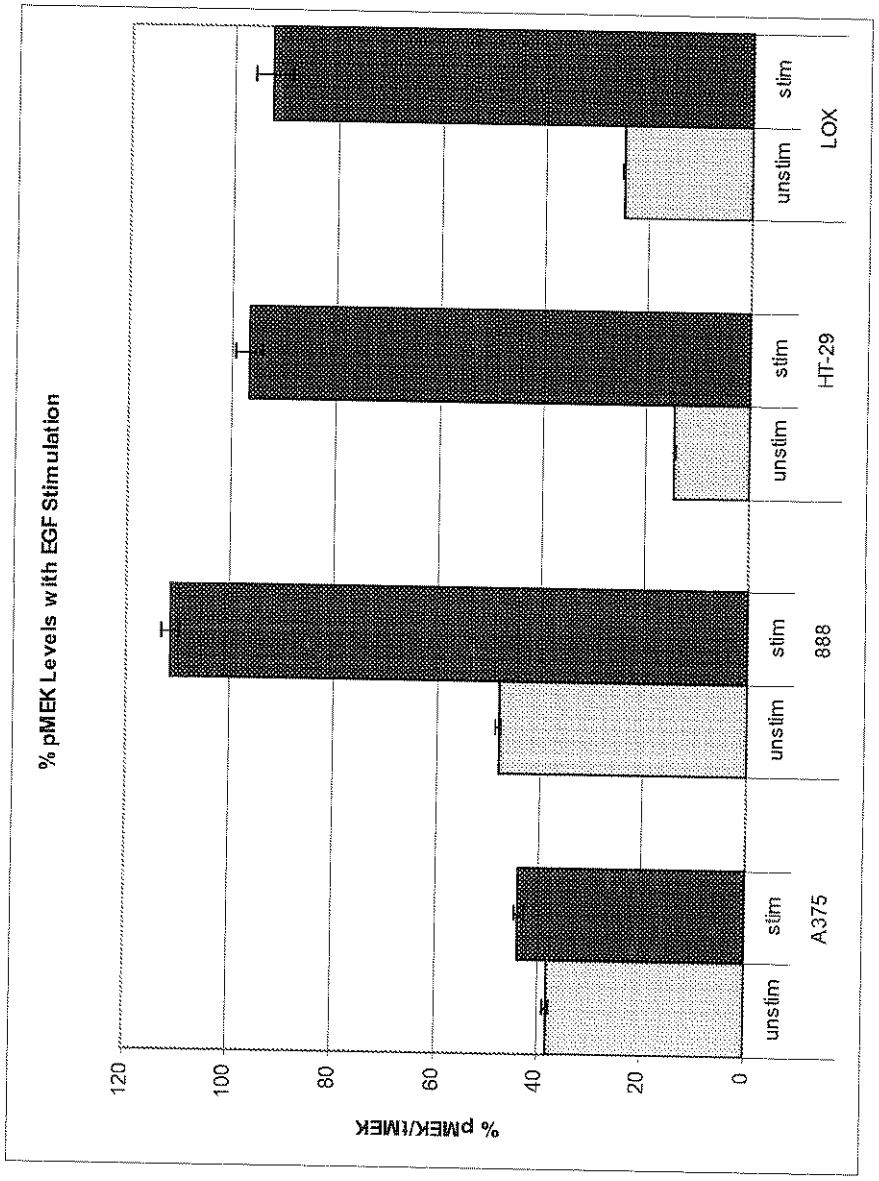

% pMEK Levels with EGF Stimulation
% pMEK/tMEK
120
100
80
60
40
20
0
unstim
stim
A375
unstim
stim
888
unstim
stim
HT-29
unstim
stim
LOX

Fig. 28

pERK IC50 (nM)	888 (B-RAFV600E)		HT-29 (B-RAFV600E)		LOX (B-RAFV600E)	
	-EGF	+EGF	-EGF	+EGF	-EGF	+EGF
GDC-0879	14	599	15	136	75	231
RAF inhibitor a	16	>1000	49	>1000	99	>1000
PLX-4720	10	>1000	6	>1000	28	>1000

pERK IC50 (nM)	624 (B-RAFV600E)	
	-HGF	+HGF
RAF inhibitor a	6.4	>10000

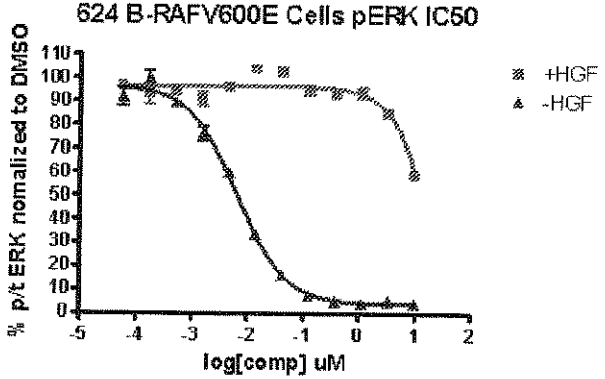


Fig. 29

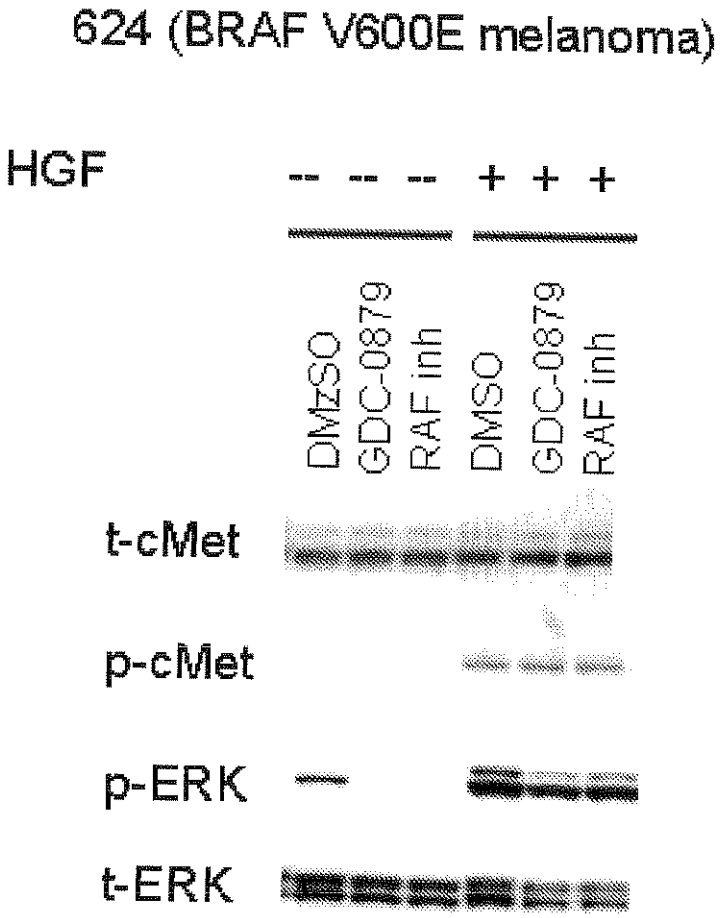

624 (BRAF V600E melanoma)
HGF
-- -- -- + + +
DMzSO
GDC-0879
RAF inh
DMSO
GDC-0879
RAF inh
t-cMet
p-cMet
p-ERK
t-ERK

Fig. 30

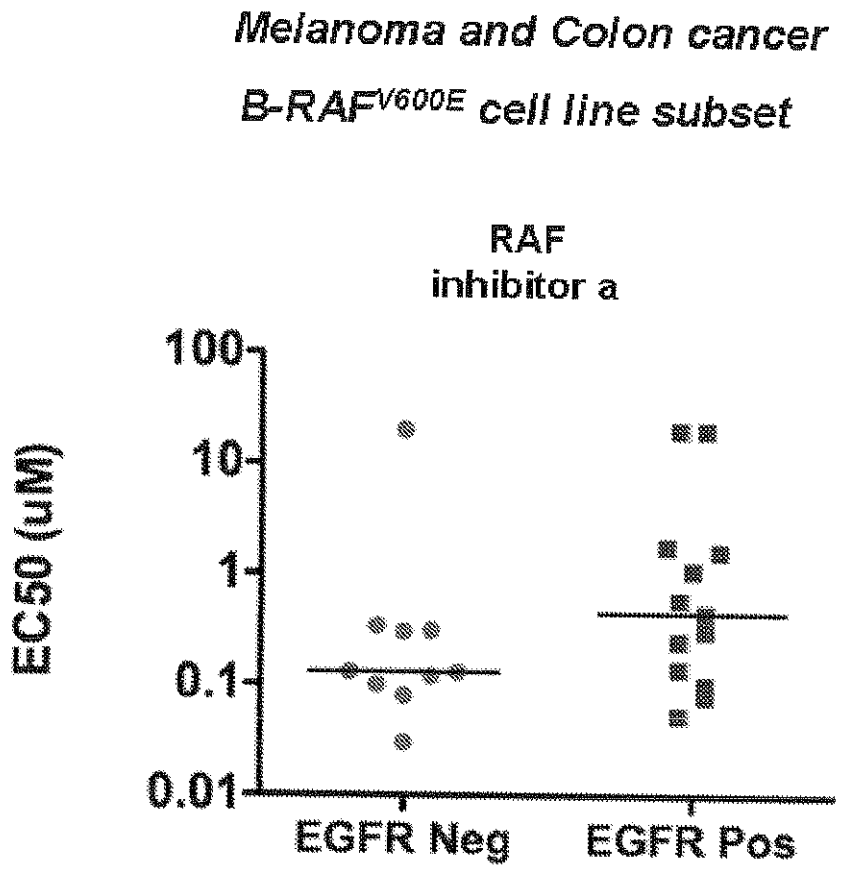
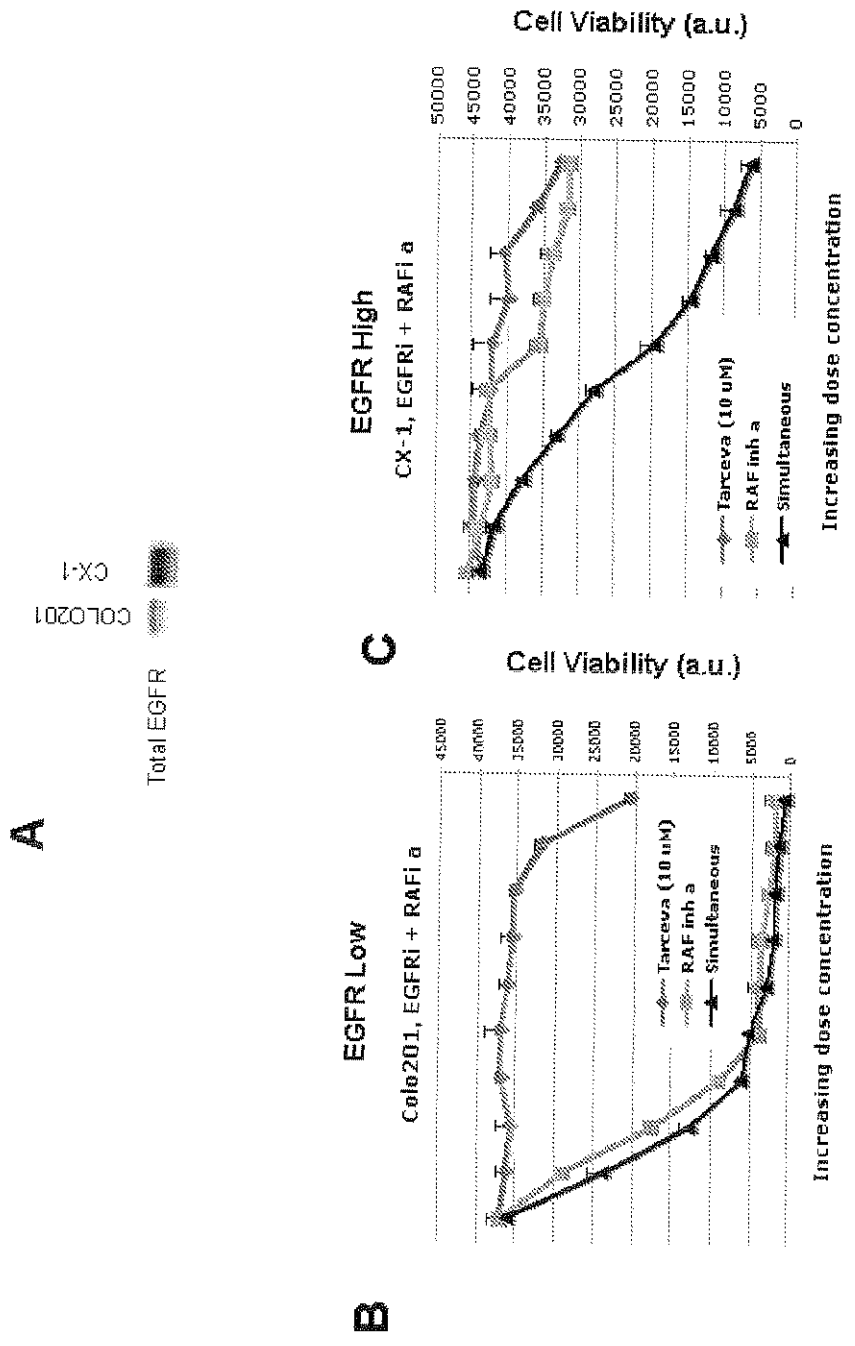

Melanoma and Colon cancer
B-RAF V600E cell line subset
RAF
inhibitor a
EC50 (uM)
100
10
1
0.1
0.01
EGFR Neg
EGFR Pos

Fig. 31


A
Total EGFR
COLO201
CX-1
B
EGFR Low
Colo201, EGFRi + RAFi a
Cell Viability (a.u.)
Increasing dose concentration
Tarceva (10 uM)
RAF inh a
Simultaneous
C
EGFR High
CX-1, EGFRi + RAFi a
Cell Viability (a.u.)
Increasing dose concentration
Tarceva (10 uM)
RAF inh a
Simultaneous

Figs. 32A – C

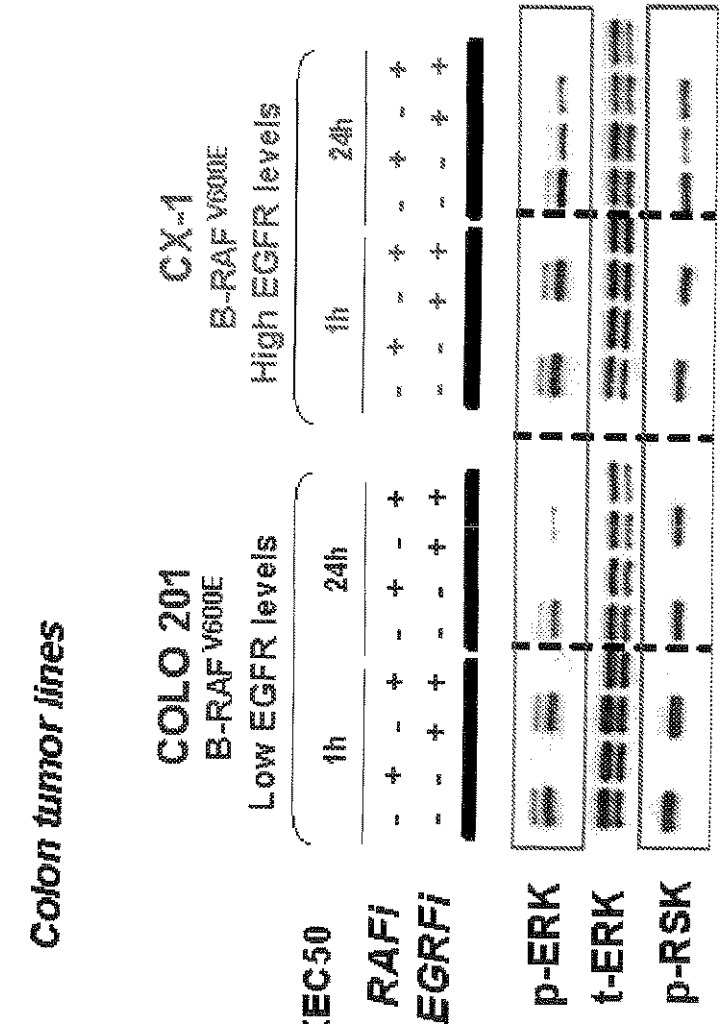

Colon tumor lines
COLO 201
B-RAF V600E
Low EGFR levels
CX-1
B-RAF V600E
High EGFR levels
1XEC50
1h
24h
RAFi
EGRFi
p-ERK
t-ERK
p-RSK

Fig. 33

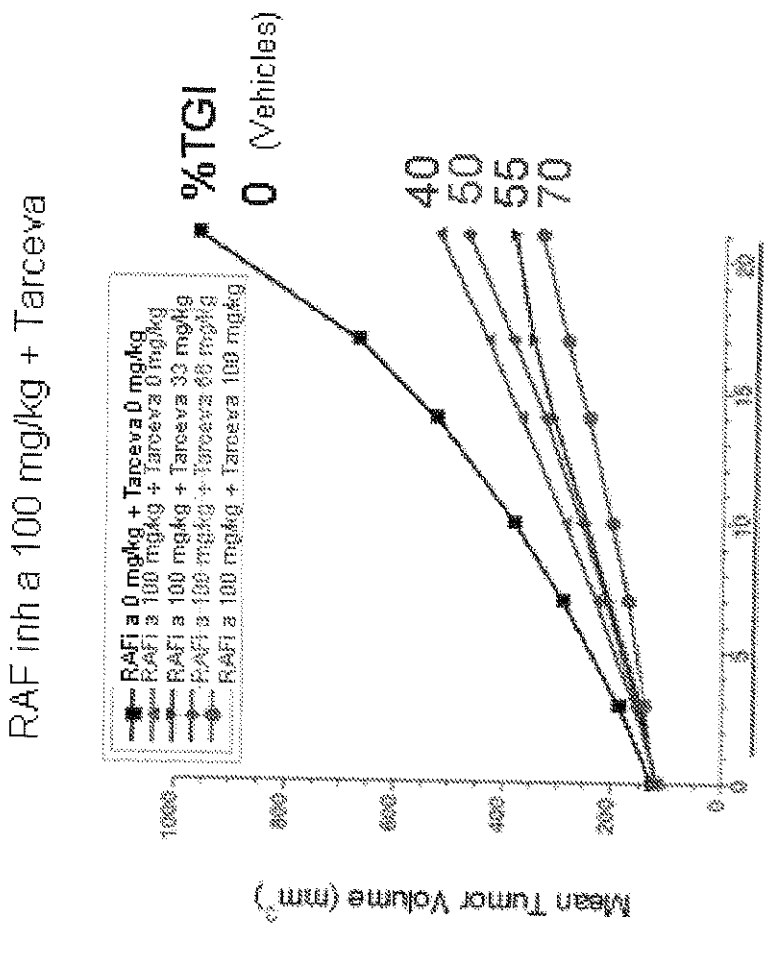

RAF inh a 100 mg/kg + Tarceva
RAFi a 0 mg/kg + Tarceva 0 mg/kg
RAFi a 100 mg/kg + Tarceva 0 mg/kg
RAFi a 100 mg/kg + Tarceva 33 mg/kg
RAFi a 100 mg/kg + Tarceva 66 mg/kg
RAFi a 100 mg/kg + Tarceva 100 mg/kg
%TGI
0 (Vehicles)
40
50
55
70
Mean Tumor Volume (mm3)
1000
800
600
400
200
0
0
5
10
15
20
Day
Tarceva= qdX21
RAF inh a=qdX21

Fig. 34A

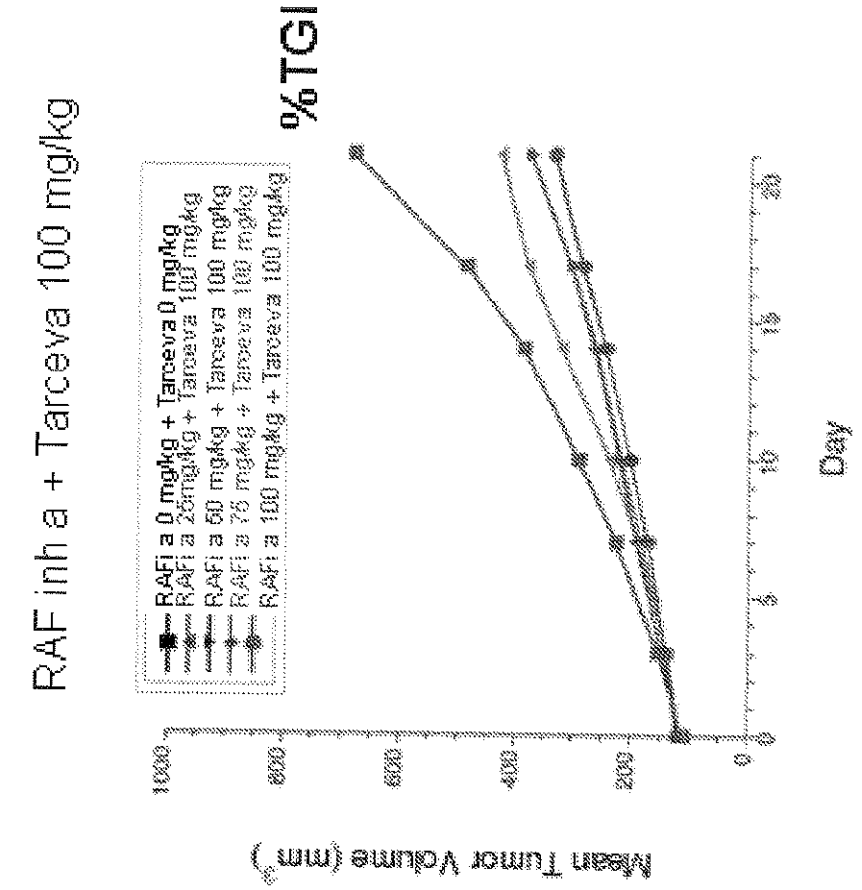

RAF inh a + Tarceva 100 mg/kg
%TGI
RAFi a 0 mg/kg + Tarceva 0 mg/kg
RAFi a 25mg/kg + Tarceva 100 mg/kg
RAFi a 50 mg/kg + Tarceva 100 mg/kg
RAFi a 75 mg/kg + Tarceva 100 mg/kg
RAFi a 100 mg/kg + Tarceva 100 mg/kg
Mean Tumor Volume (mm³)
1000
800
600
400
200
0
0
5
10
15
20
Day

Fig. 34B

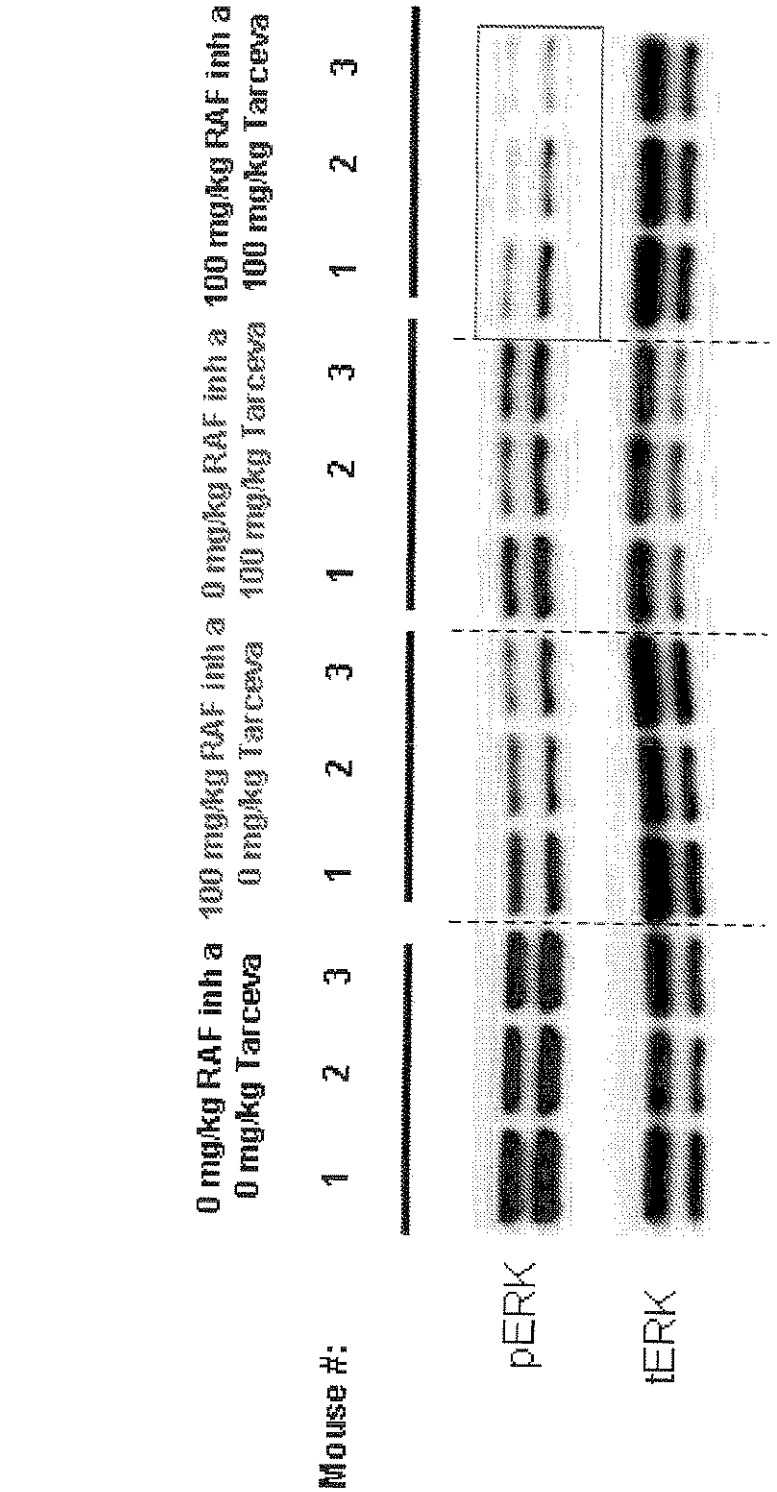

0 mg/kg RAF inh a
0 mg/kg Tarceva
100 mg/kg RAF inh a
0 mg/kg Tarceva
0 mg/kg RAF inh a
100 mg/kg Tarceva
100 mg/kg RAF inh a
100 mg/kg Tarceva
Mouse #:
1 2 3 1 2 3 1 2 3 1 2 3
pERK
tERK

Fig. 34C