FIGURE 1

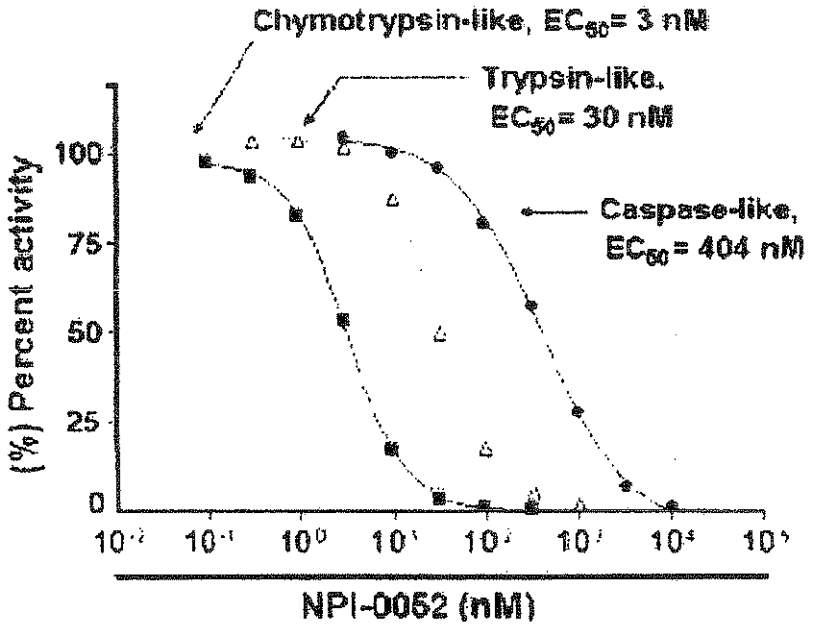

Chymotrypsin-like, EC50= 3 nM
Trypsin-like,
EC50= 30 nM
Caspase-like,
EC50= 404 nM
(%) Percent activity
100
75
50
25
0
NPI-0052 (nM)

FIGURE 2

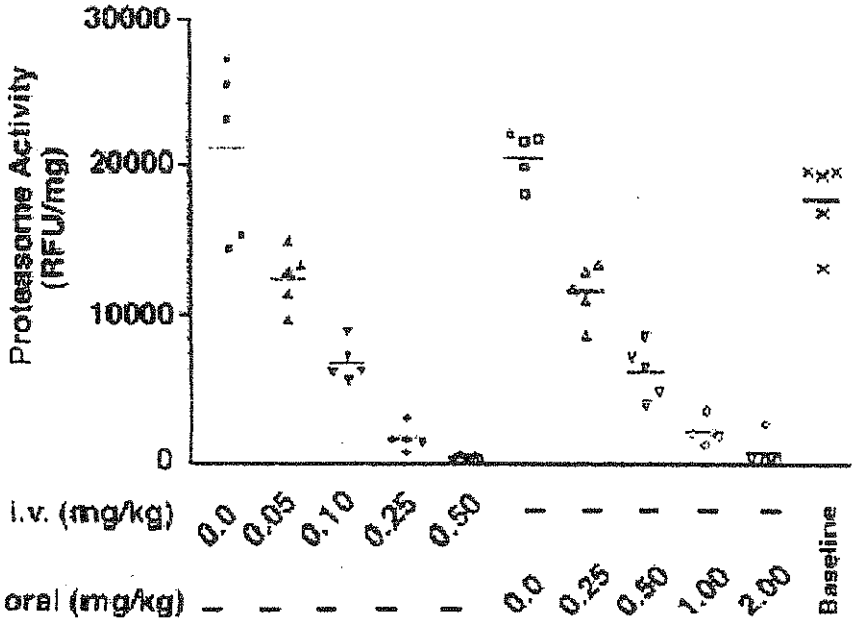

30000
20000
10000
0
Proteasome Activity (RFU/mg)
i.v. (mg/kg)
0.0
0.05
0.10
0.25
0.50
oral (mg/kg)
0.0
0.25
0.50
1.00
2.00
Baseline

FIGURE 3

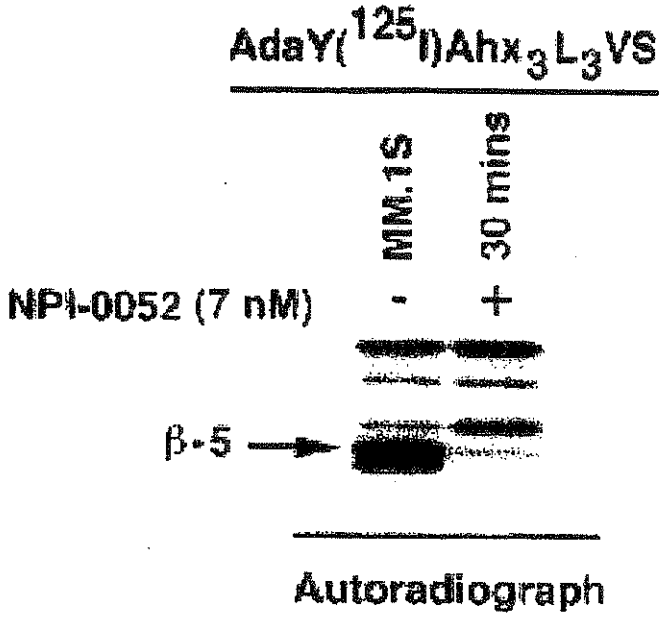

AdaY(125I)Ahx3L3VS
MM.1S
30 mins
NPI-0052 (7 nM)
-
+
β-5
Autoradiograph

FIGURE 4

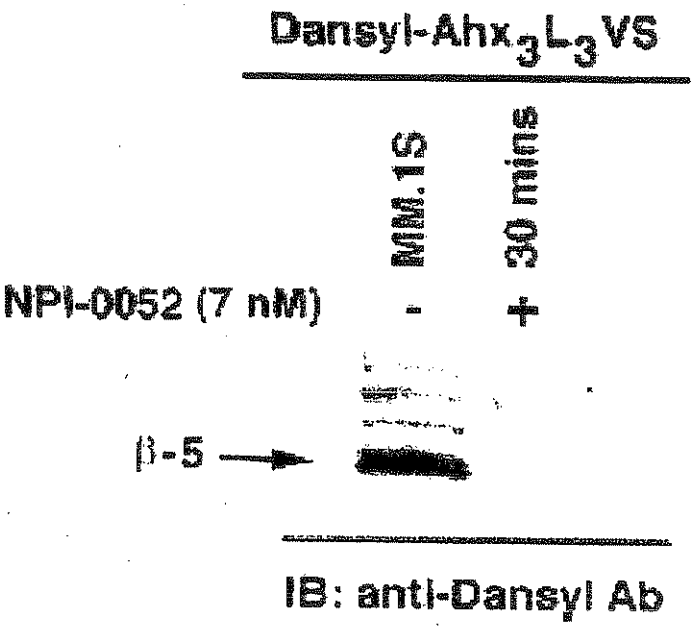

Dansyl-Ahx3L3VS
MM.1S
30 mins
NPI-0052 (7 nM)
-
+
β5
IB: anti-Dansyl Ab

FIGURE 5A

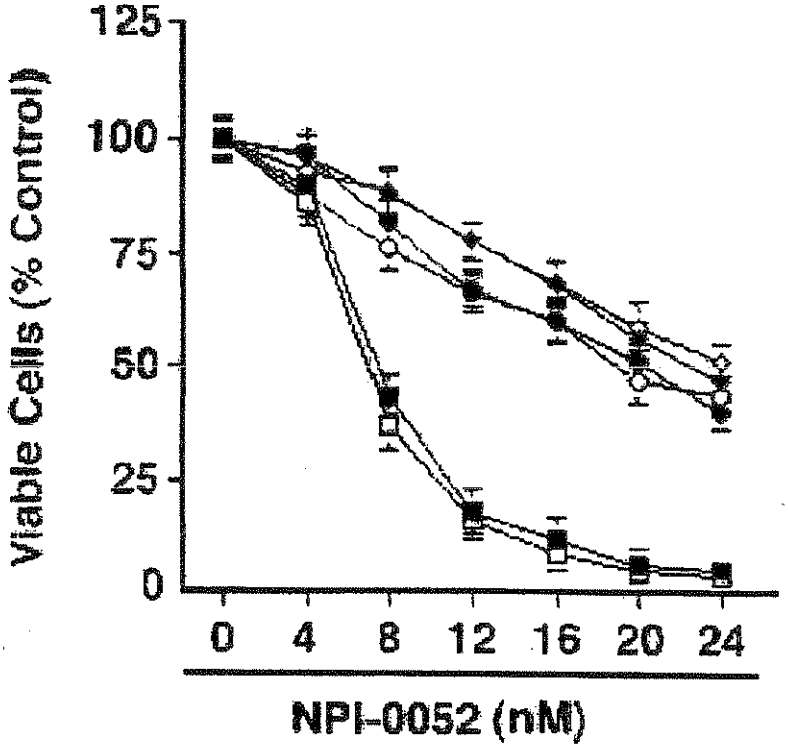

125
100
75
50
25
0
Viable Cells (% Control)
0
4
8
12
16
20
24
NPI-0052 (nM)

FIGURE 5B

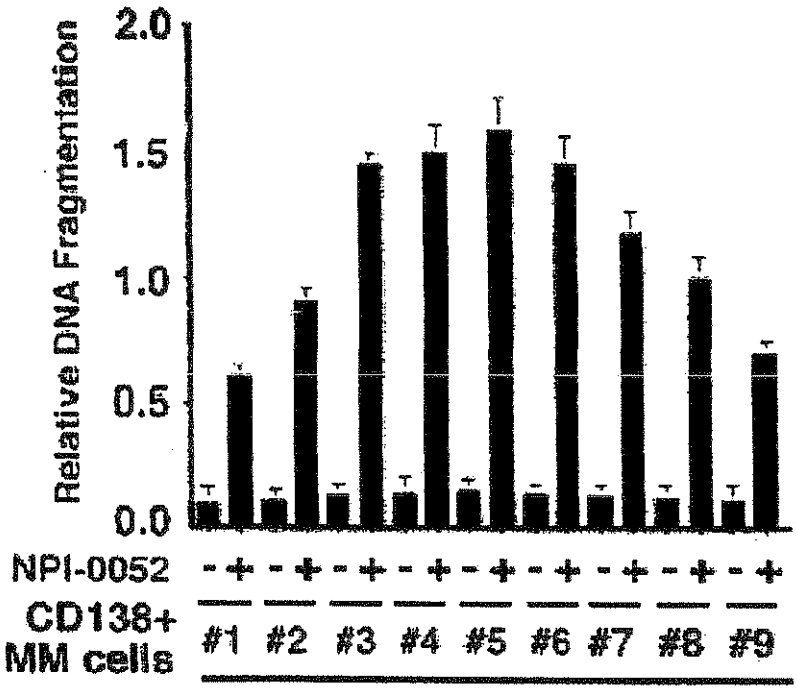

Relative DNA Fragmentation
2.0
1.5
1.0
0.5
0.0
NPI-0052
- + - + - + - + - + - + - + - + - +
CD138+
MM cells
#1 #2 #3 #4 #5 #6 #7 #8 #9

FIGURE 6

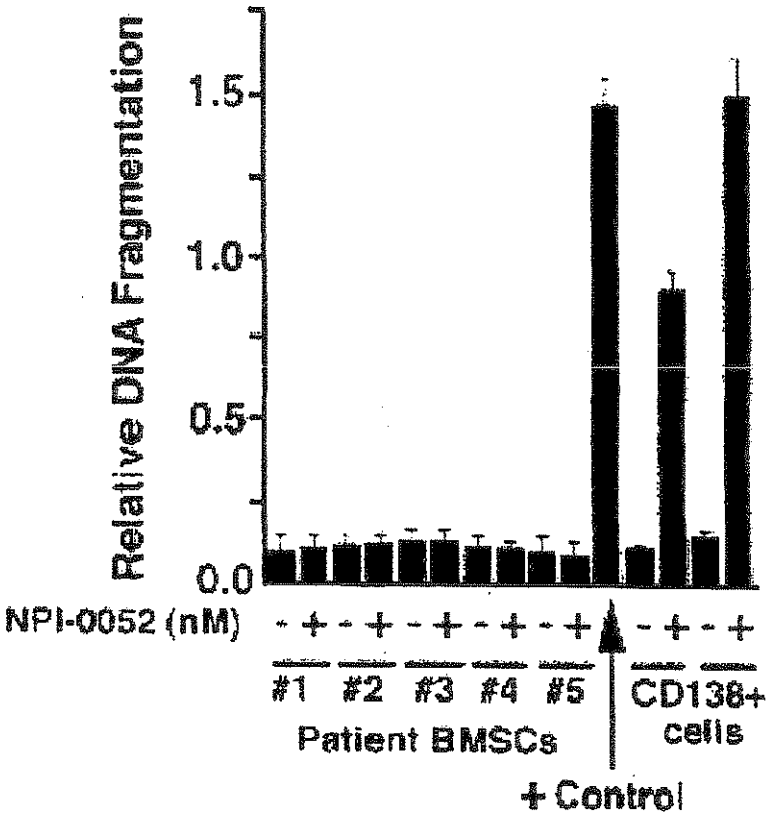

Relative DNA Fragmentation
1.5
1.0
0.5
0.0
NPI-0052 (nM)
- + - + - + - + - + - + - + - +
#1 #2 #3 #4 #5
Patient BMSCs
CD138+ cells
+ Control

FIGURE 7

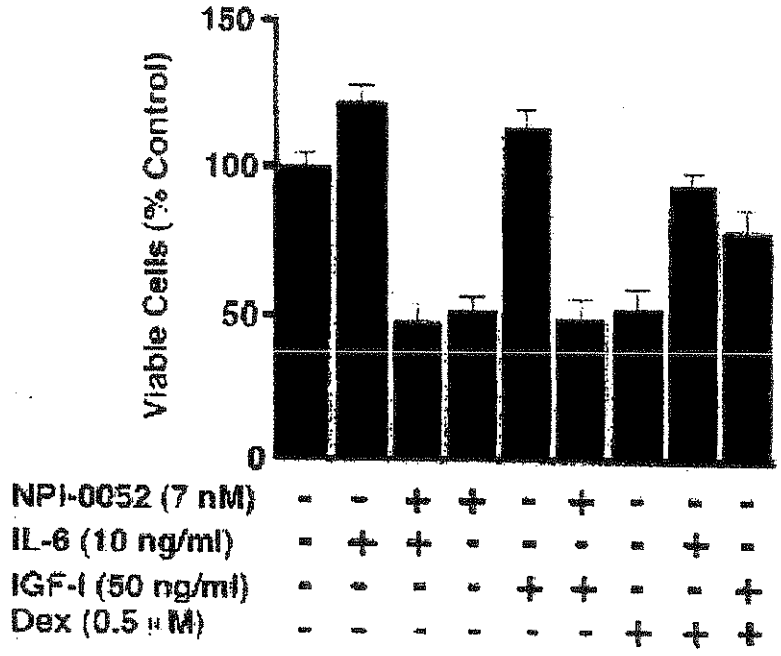

150
100
50
0
Viable Cells (% Control)
NPI-0052 (7 nM) - - + + - + - - -
IL-6 (10 ng/ml) - + + - - - - + -
IGF-I (50 ng/ml) - - - - + + - - +
Dex (0.5 μM) - - - - - - + + +

FIGURE 8

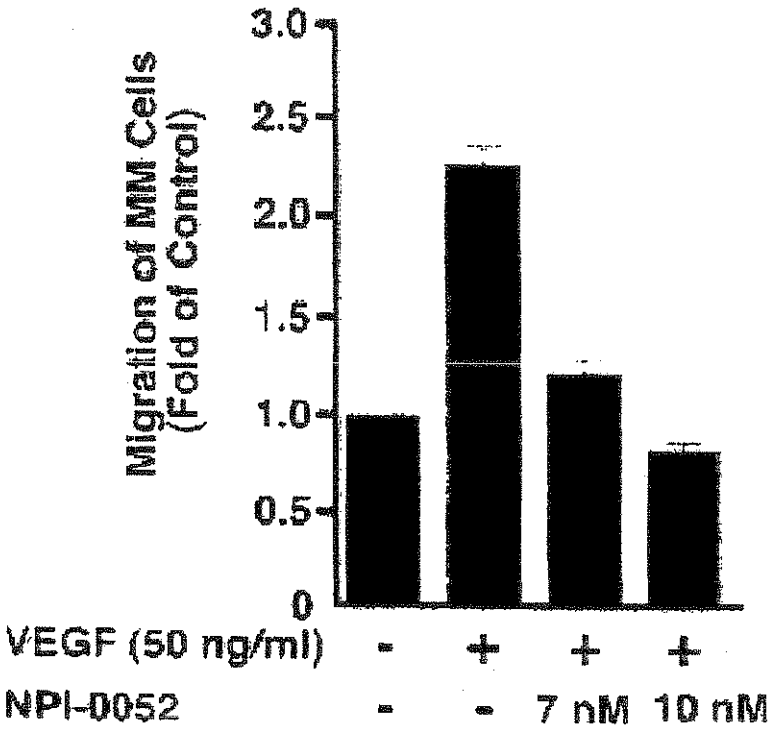

Migration of MM Cells
(Fold of Control)
3.0
2.5
2.0
1.5
1.0
0.5
0
VEGF (50 ng/ml) - + + +
NPI-0052 - - 7 nM 10 nM

FIGURE 9

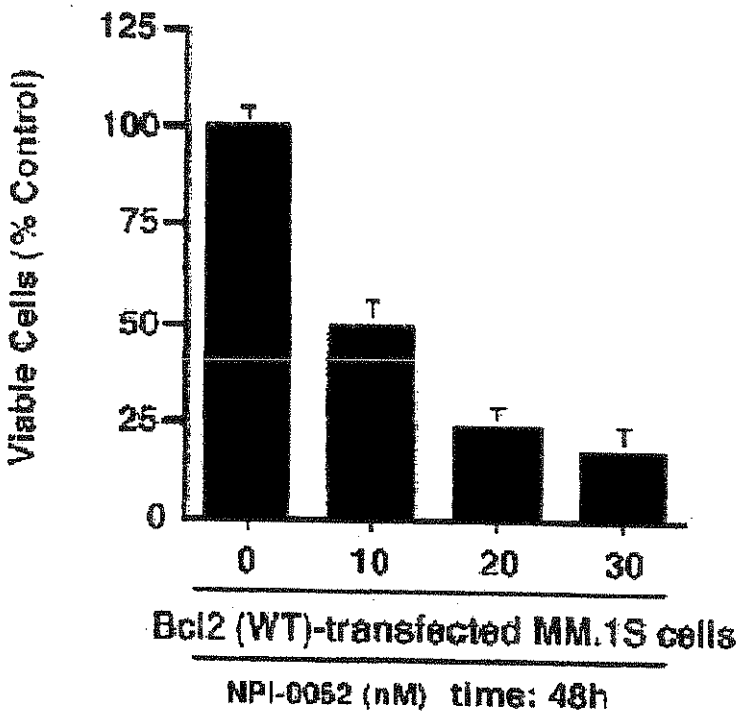

125
100
75
50
25
0
Viable Cells (% Control)
0
10
20
30
Bcl2 (WT)-transfected MM.1S cells
NPI-0052 (nM) time: 48h

FIGURE 10A

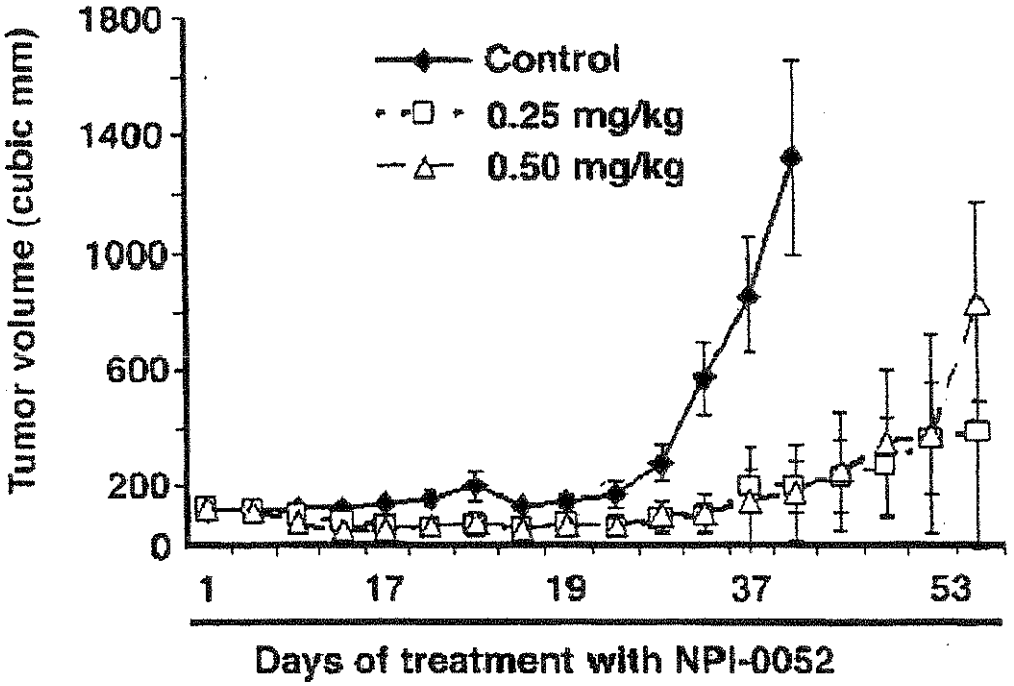

1800
1400
1000
600
200
0
Tumor volume (cubic mm)
Control
0.25 mg/kg
0.50 mg/kg
1
17
19
37
53
Days of treatment with NPI-0052

FIGURE 10B

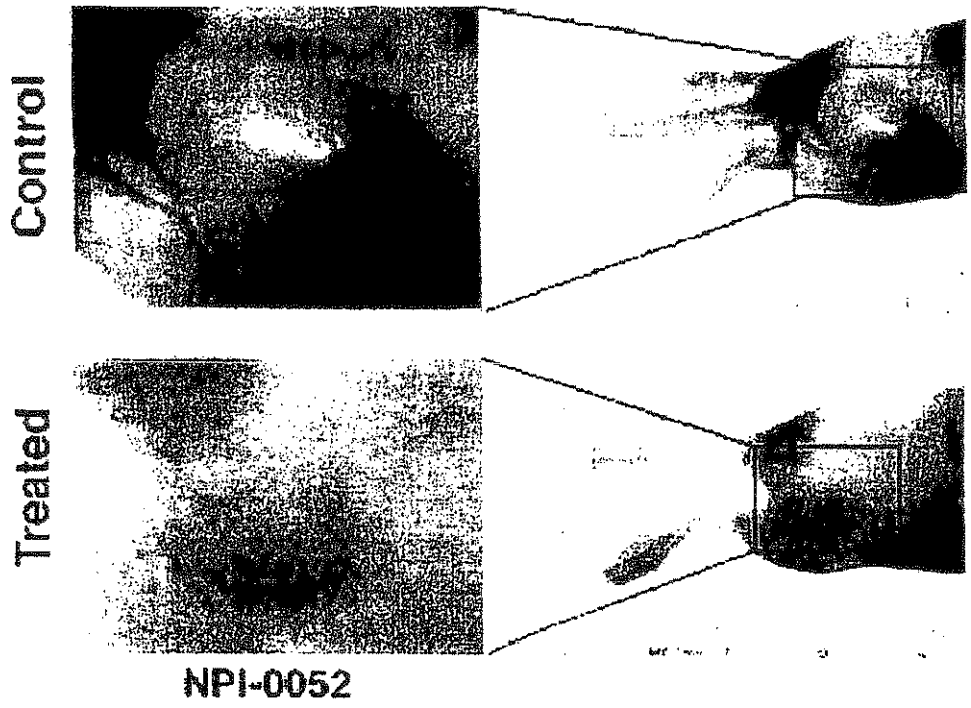


FIGURE 10C

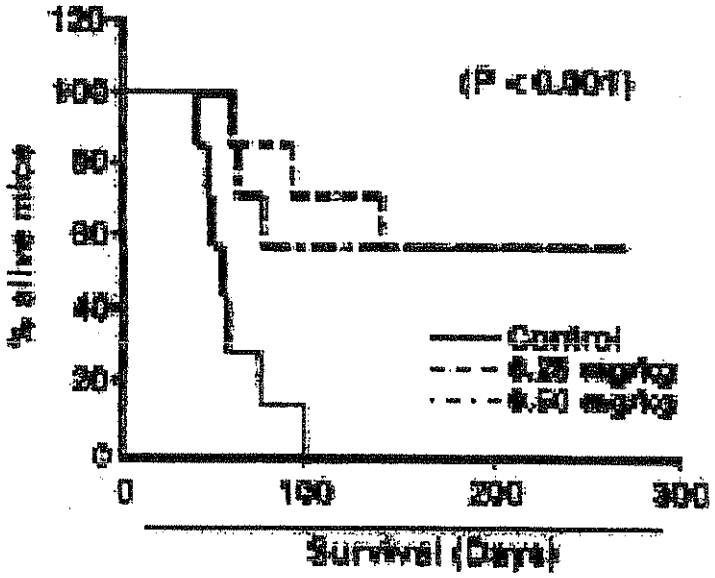


FIGURE 10D

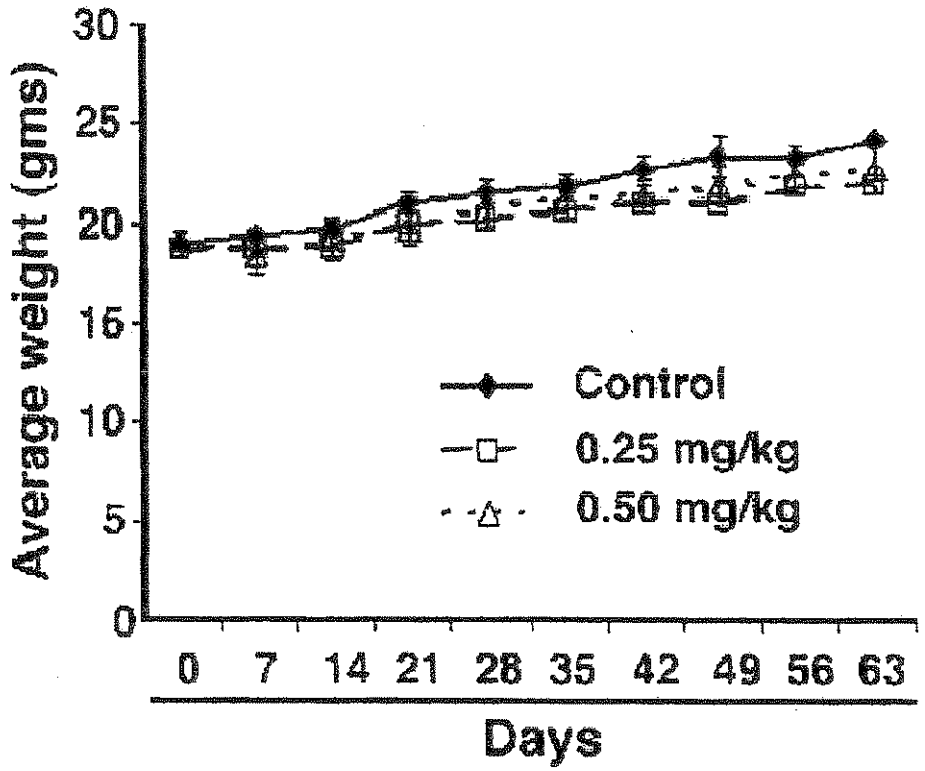

Average weight (gms)
30
25
20
15
10
5
0
0
7
14
21
28
35
42
49
56
63
Days
Control
0.25 mg/kg
0.50 mg/kg

FIGURE 10E

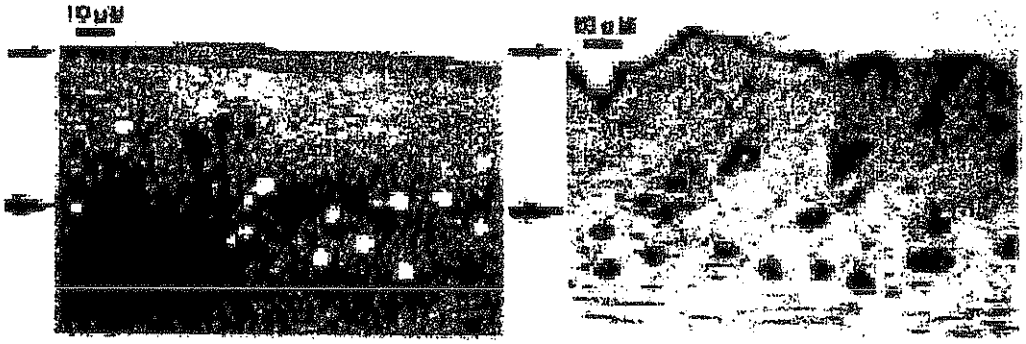


FIGURE 10F

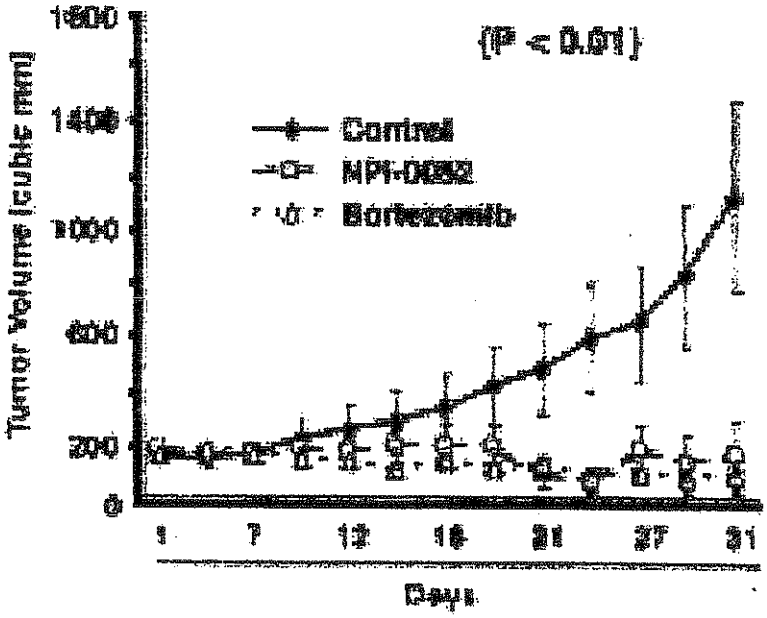


FIGURE 10G

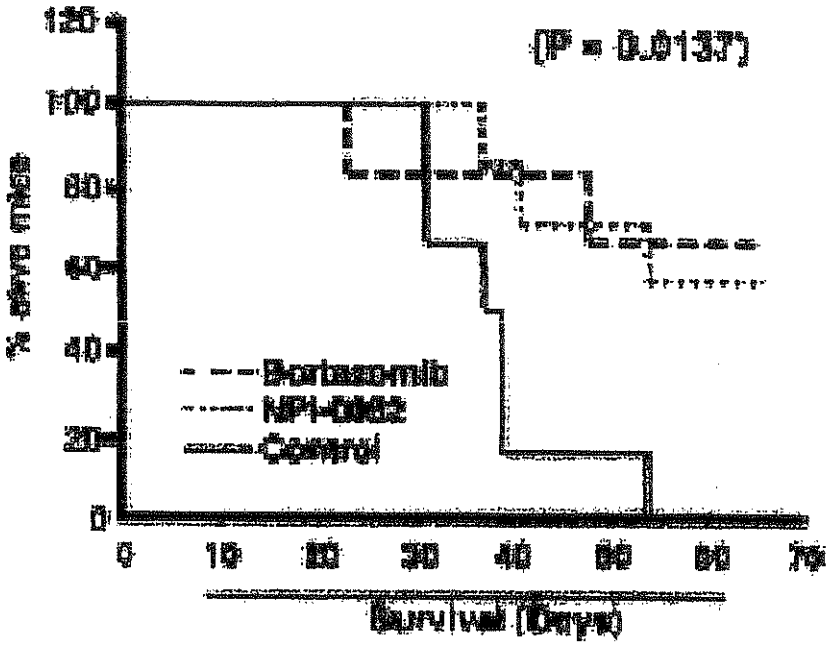

(P = 0.01337)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Bortezomib
NPI-0052
Control

FIGURE 11A

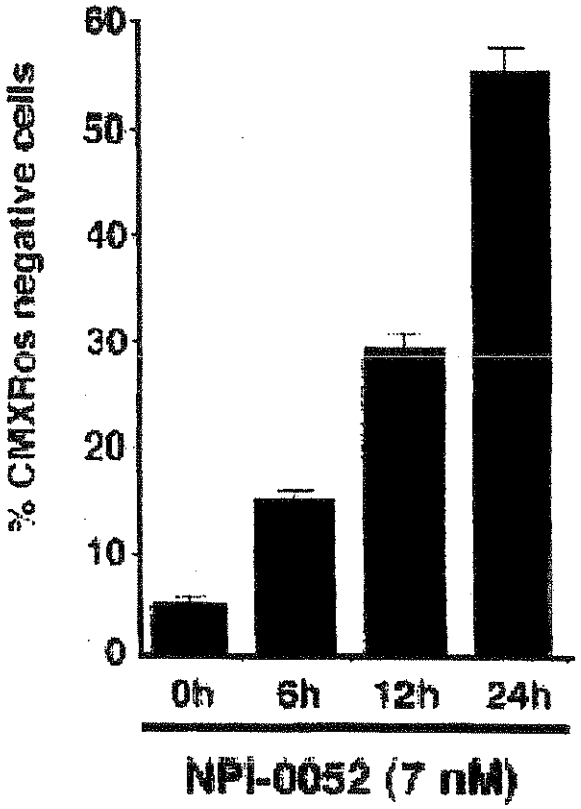

% CMXRos negative cells
60
50
40
30
20
10
0
0h
6h
12h
24h
NPI-0052 (7 nM)

FIGURE 11B

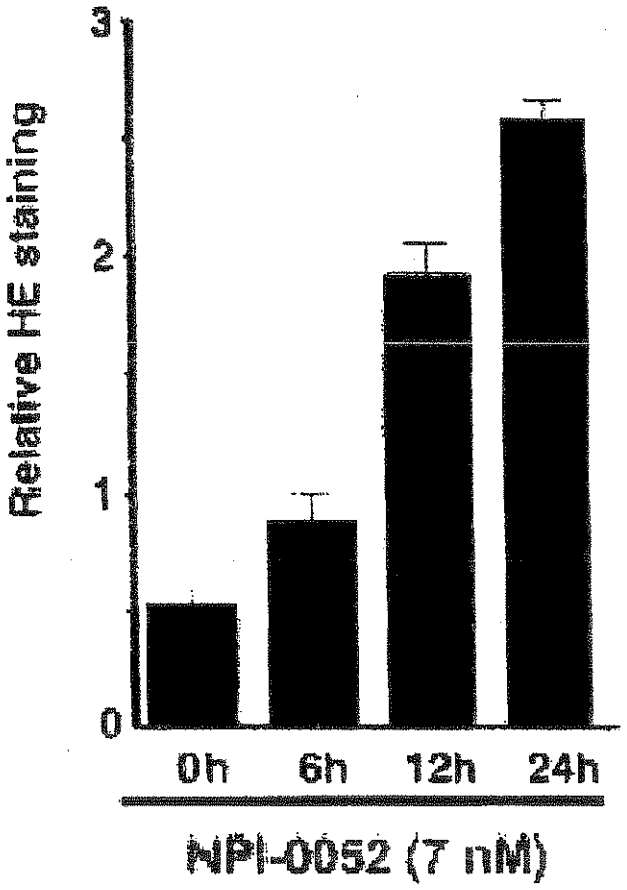


FIGURE 11C

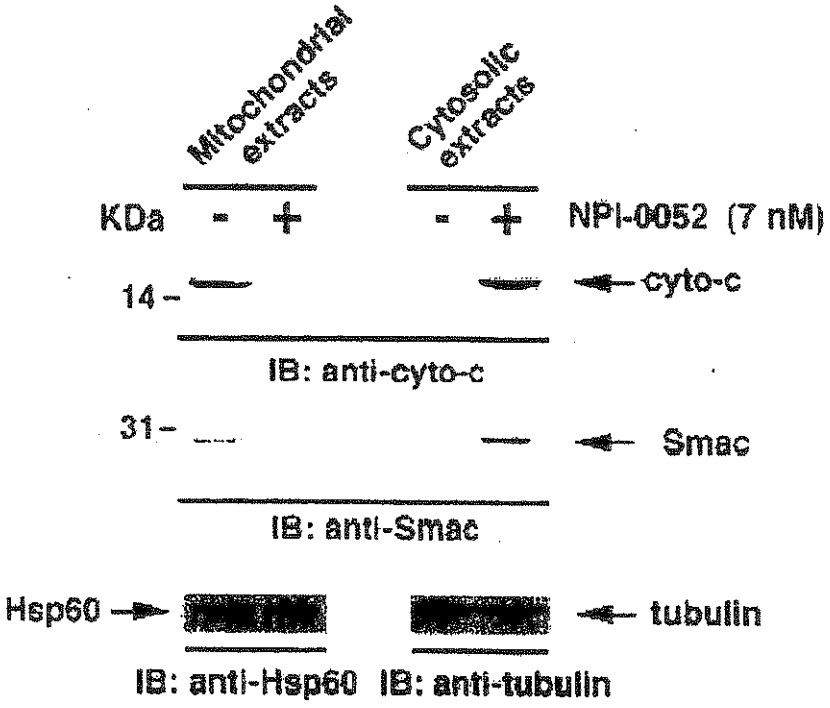

Mitochondrial extracts
Cytosolic extracts
KDa
-
+
-
+
NPI-0052 (7 nM)
14 –
cyto-c
IB: anti-cyto-c
31 –
Smac
IB: anti-Smac
Hsp60
tubulin
IB: anti-Hsp60
IB: anti-tubulin

FIGURE 11D

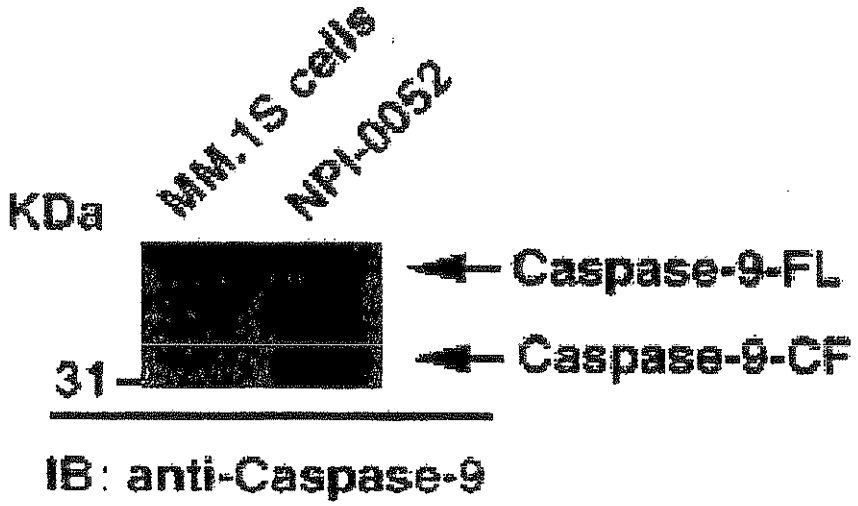

KDa
MM.1S cells
NPI-0052
Caspase-9-FL
Caspase-9-CF
31
IB: anti-Caspase-9

FIGURE 11E

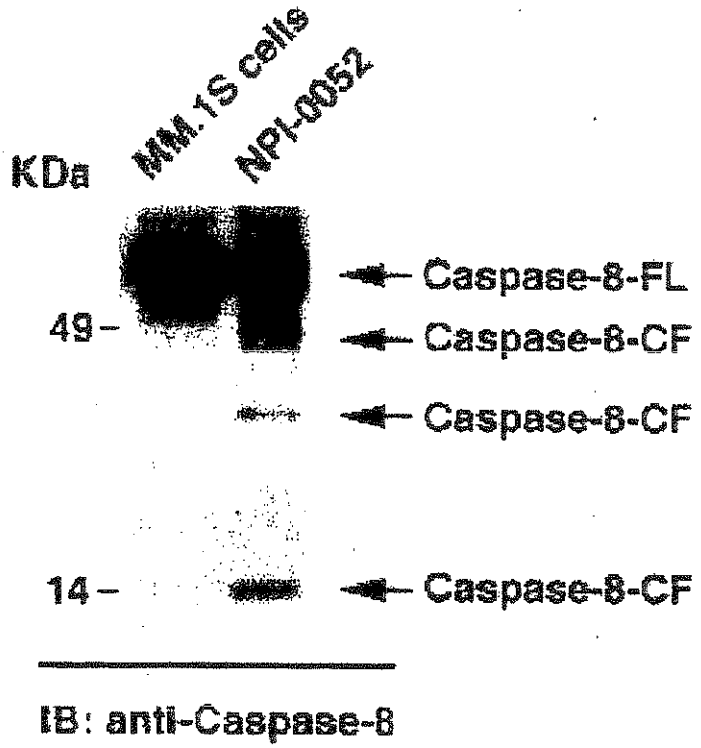

KDa
MM.1S cells
NPI-0052
49 –
14 –
Caspase-8-FL
Caspase-8-CF
Caspase-8-CF
Caspase-8-CF
IB: anti-Caspase-8

FIGURE 11F

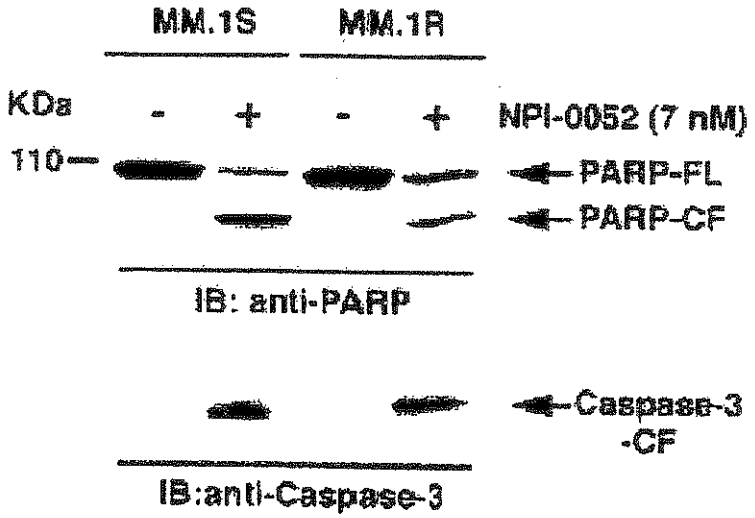

MM.1S
MM.1R
KDa
-
+
-
+
NPI-0052 (7 nM)
110
PARP-FL
PARP-CF
IB: anti-PARP
Caspase-3
-CF
IB:anti-Caspase-3

FIGURE 12A

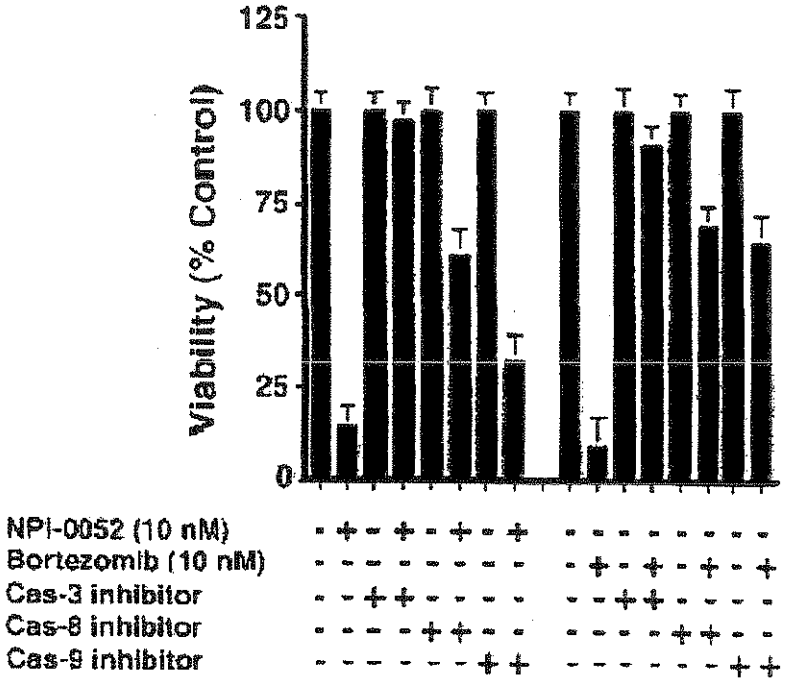

125
100
75
50
25
0
Viability (% Control)
NPI-0052 (10 nM)
Bortezomib (10 nM)
Cas-3 inhibitor
Cas-8 inhibitor
Cas-9 inhibitor

FIGURE 12B

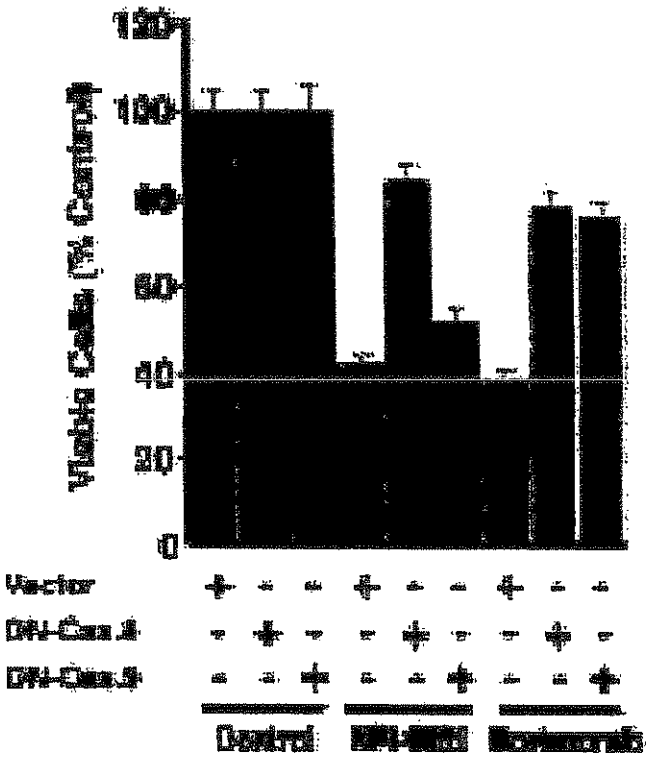


FIGURE 12C

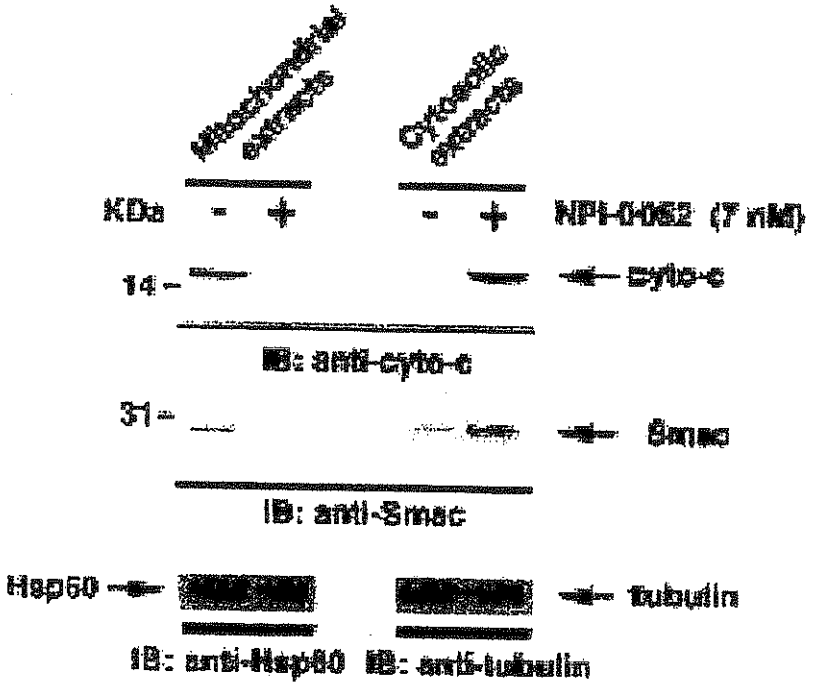

Mitochondrial extracts
Cytosolic extracts
KDa
-
+
-
+
NPI-0052 (7 nM)
14 –
Cyto-c
IB: anti-cyto-c
31 –
Smac
IB: anti-Smac
Hsp60
tubulin
IB: anti-Hsp60
IB: anti-tubulin

FIGURE 12D

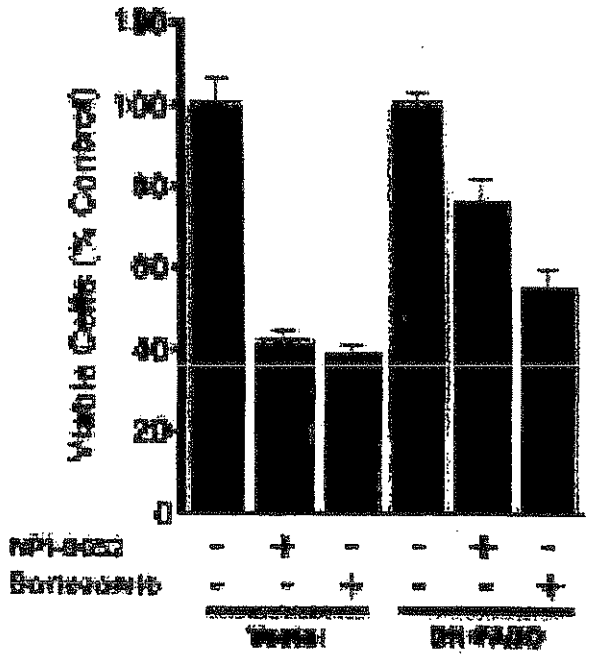


FIGURE 12E

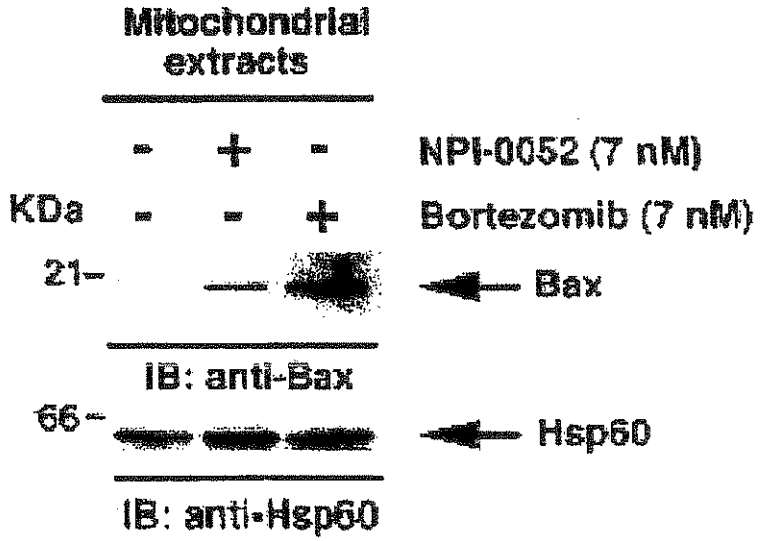

Mitochondrial extracts
- + -
NPI-0052 (7 nM)
KDa - - +
Bortezomib (7 nM)
21-
Bax
IB: anti-Bax
66-
Hsp60
IB: anti-Hsp60

FIGURE 12F

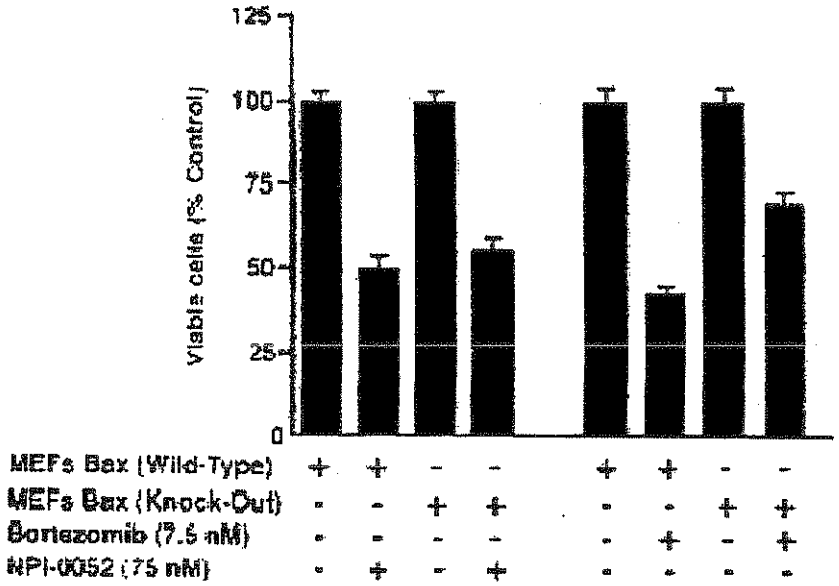

125
100
75
50
25
0
Viable cells (% Control)
MEFs Bax (Wild-Type) + + - - + + - -
MEFs Bax (Knock-Out) - - + + - - + +
Bortezomib (7.5 nM) - - - - - + - +
NPI-0052 (75 nM) - + - + - - - -

FIGURE 13

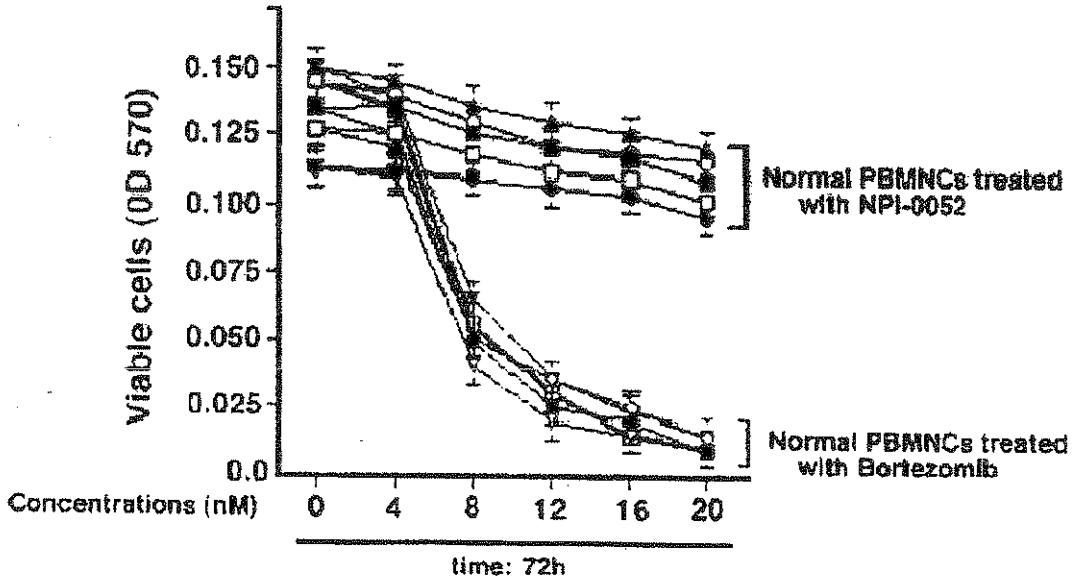

Viable cells (OD 570)
0.150
0.125
0.100
0.075
0.050
0.025
0.0
Concentrations (nM)
0
4
8
12
16
20
time: 72h
Normal PBMNCs treated with NPI-0052
Normal PBMNCs treated with Bortezomib

FIGURE 14A

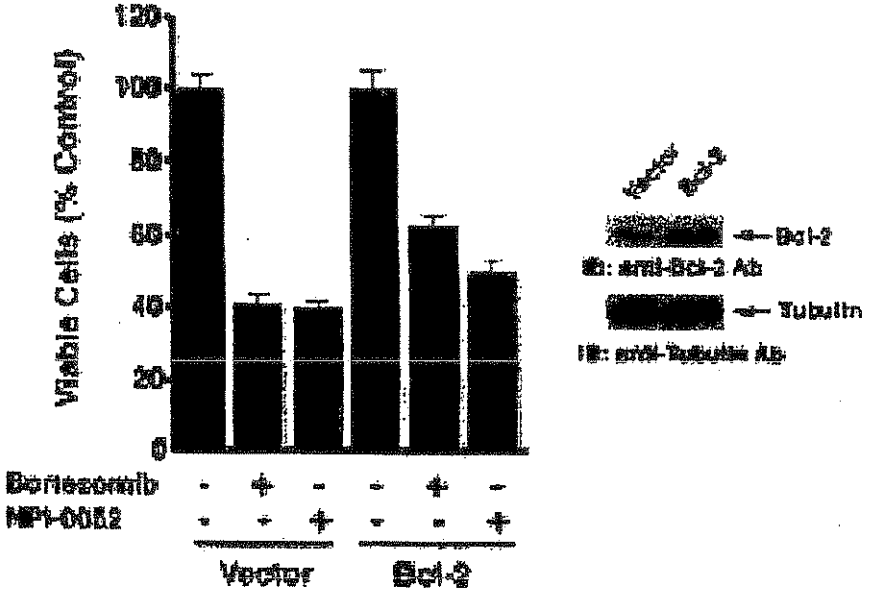

120
100
80
60
40
20
0
Viable Cells (% Control)
Bortezomib
MP1-0052
Vector
Bcl-2
Bcl-2
IB: anti-Bcl-2 Ab
Tubulin

FIGURE 14B

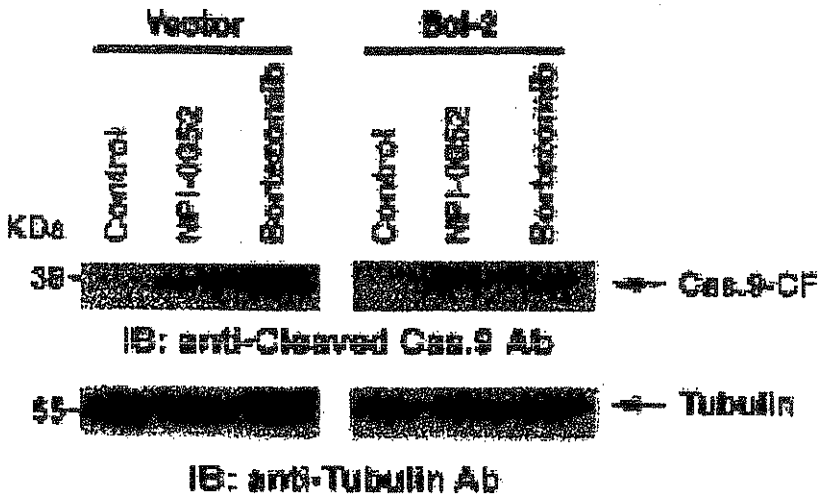
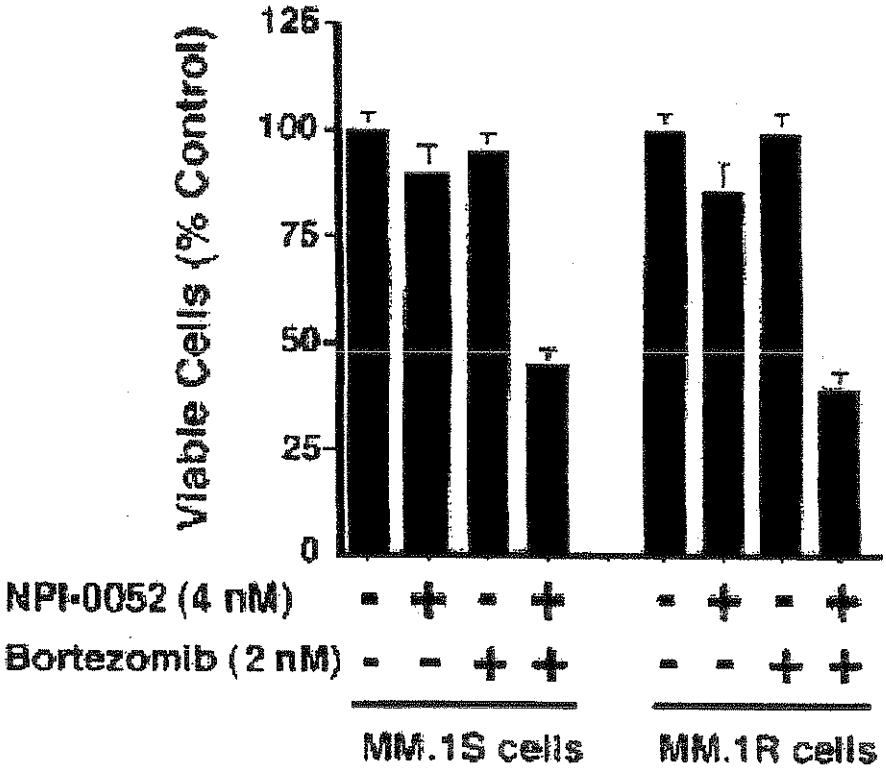

Vector
Bcl-2
Control
NPI-0052
Bortezomib
Control
NPI-0052
Bortezomib
KDa
38
Cas.9-CF
IB: anti-Cleaved Cas.9 Ab
55
Tubulin
IB: anti-Tubulin Ab

FIGURE 15


Viable Cells (% Control)
125
100
75
50
25
0
NPI-0052 (4 nM) - + - + - + - +
Bortezomib (2 nM) - - + + - - + +
MM.1S cells
MM.1R cells