# FIGURES

Figure 1 Number of NK cells in spleen of normal and tumour bearing mice. Summation of three experiments, each with triplicate readings.

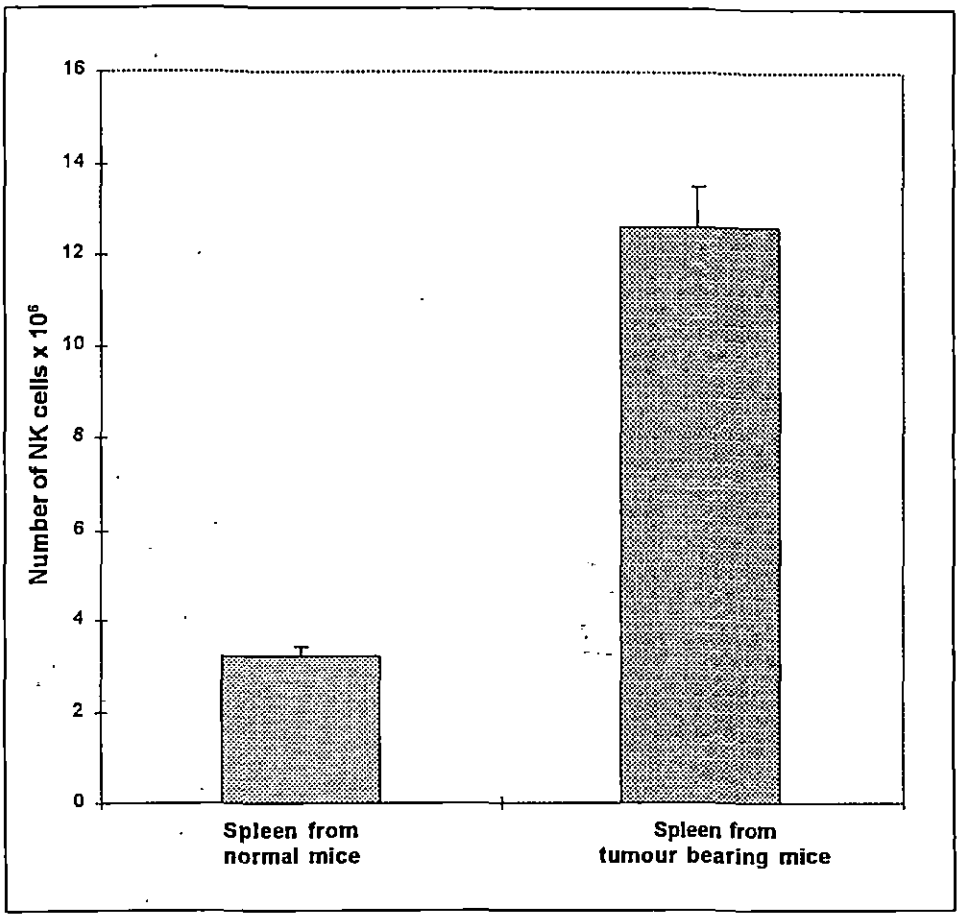
16
14
12
10
8
6
4
2
0
Number of NK cells x 10⁶
Spleen from normal mice
Spleen from tumour bearing mice

Fig. 1

Figure 2 Percentage of blast cells generated at 24 hrs after stimulation with varying doses of rIL-2 and 50 µl of gIL-2 *in vitro*. The lines indicate the mean of the triplicate values of three experiments for each activation schedule.

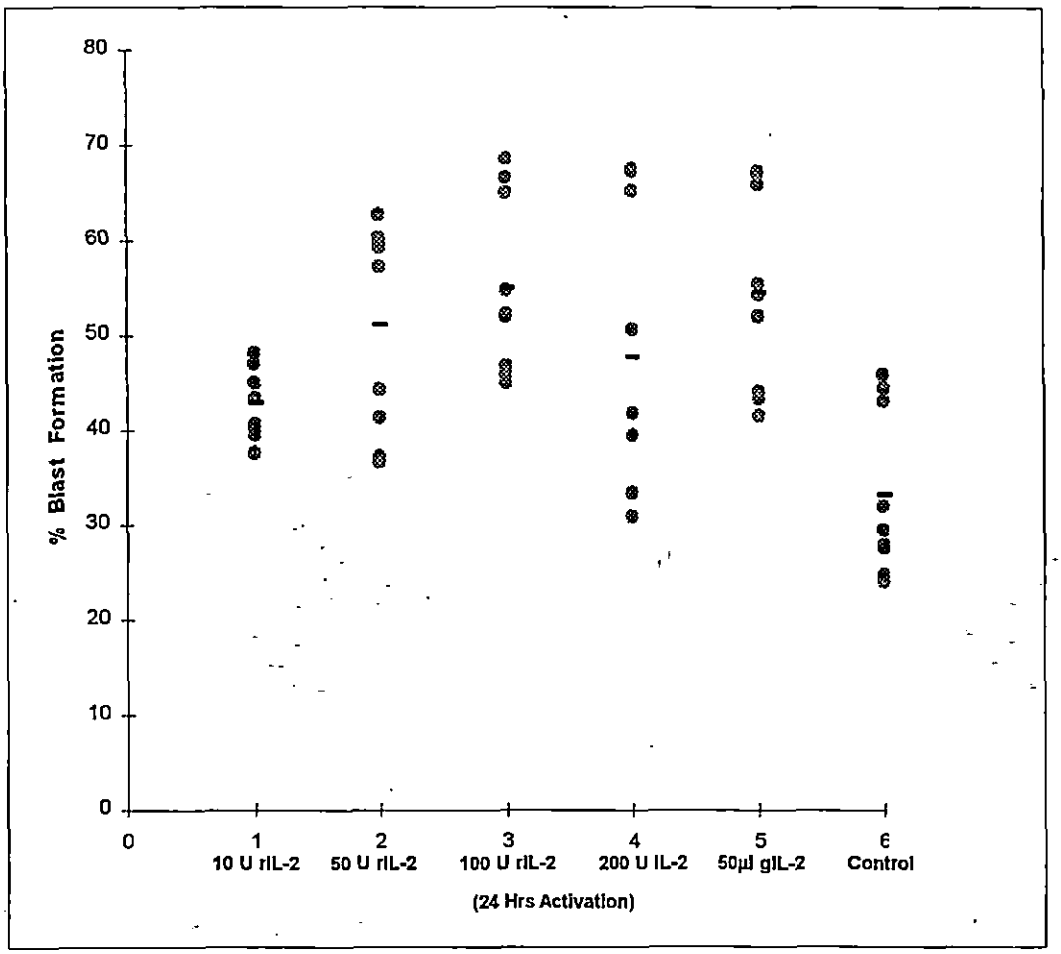
% Blast Formation
80
70
60
50
40
30
20
10
0
0
1
10 U rIL-2
2
50 U rIL-2
3
100 U rIL-2
4
200 U IL-2
5
50µl gIL-2
6
Control
(24 Hrs Activation)

Fig. 2

Figure 3 Percentage of blast cells generated at 48 hrs after stimulation with varying doses of rIL-2 and 50µl of gIL-2 *in vitro*. The lines indicate the mean of the triplicate values of three experiments for each activation schedule.

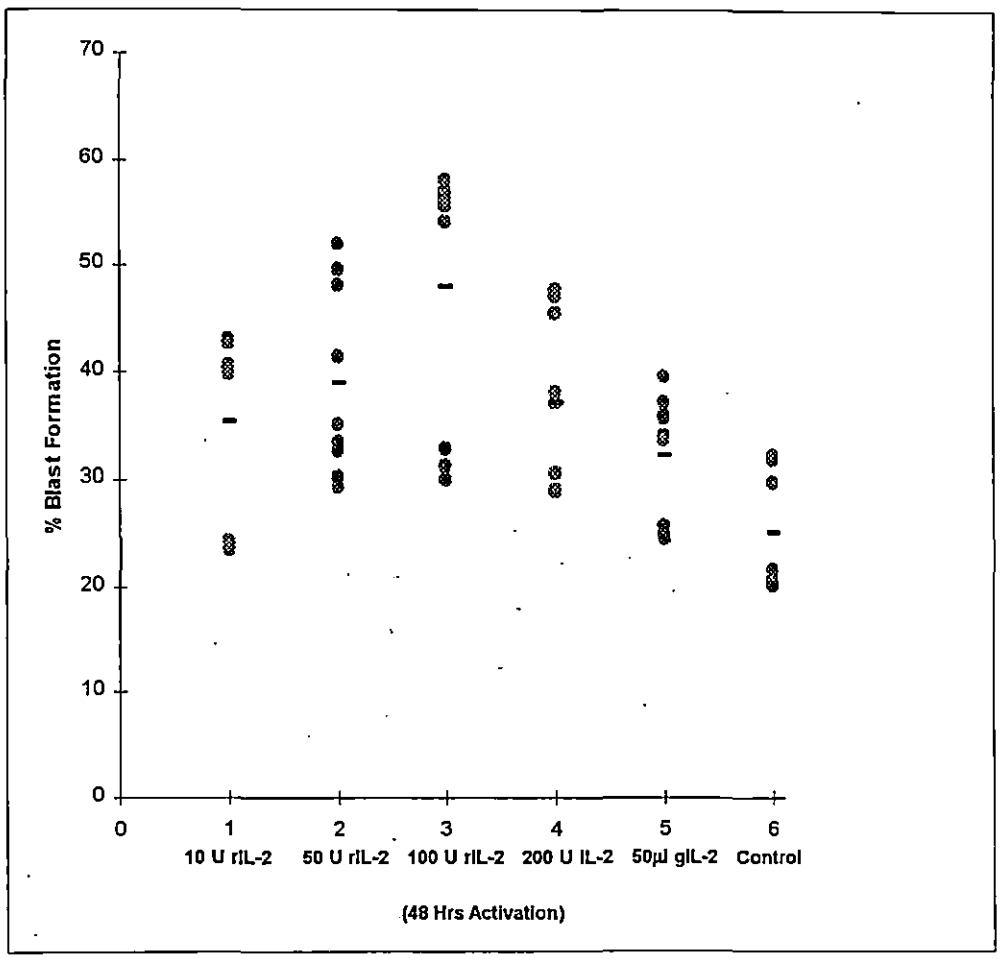
70
60
50
40
30
20
10
0
% Blast Formation
0
1
2
3
4
5
6
10 U rIL-2
50 U rIL-2
100 U rIL-2
200 U IL-2
50µl gIL-2
Control
(48 Hrs Activation)

Fig. 3

Figure 4 Incorporation of $^{3}$H-TdR by $NK^{N}$ cells after treatment with varying doses of rIL-2 and 50 µl of gIL-2 for 24 hrs and 48 hrs.

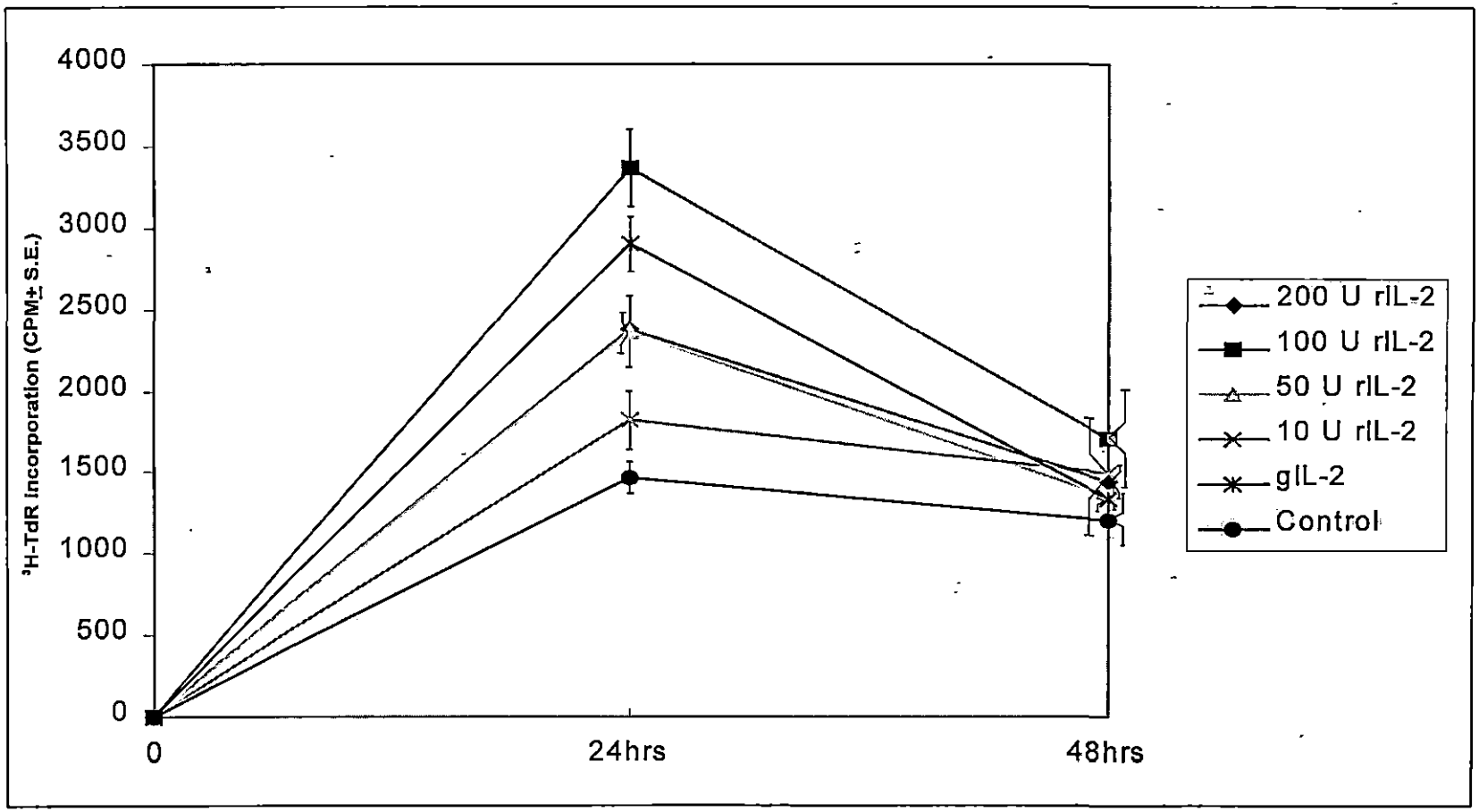
4000
3500
3000
2500
2000
1500
1000
500
0
³H-TdR Incorporation (CPM± S.E.)
0
24hrs
48hrs
200 U rIL-2
100 U rIL-2
50 U rIL-2
10 U rIL-2
gIL-2
Control

Fig. 4

Figure 5 Percentages of spontaneous cytotoxicity and ADCC of NK cells from spleen of normal mice ( ) and tumour bearing mice ( ).

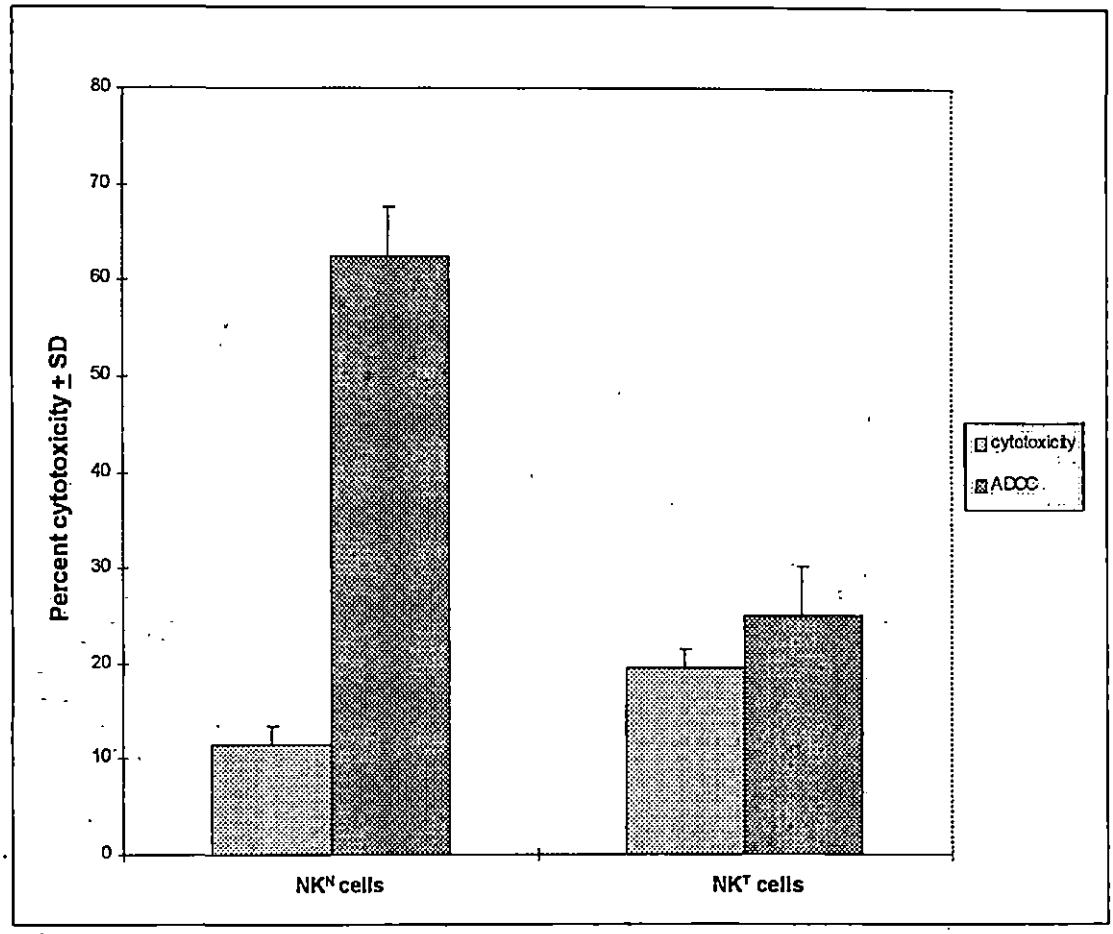
80
70
60
50
40
30
20
10
0
Percent cytotoxicity ± SD
cytotoxicity
ADCC
NK^N cells
NK^T cells

Fig. 5

Figure 6 Percentage of homing of $^{3}$H-Thymidine labelled IL-2 activated $NK^{N}$ cells in tumour and in different tissues at 12 hrs, 24hrs and 48 hrs. The result is from a representative experiment consisting of four animals.

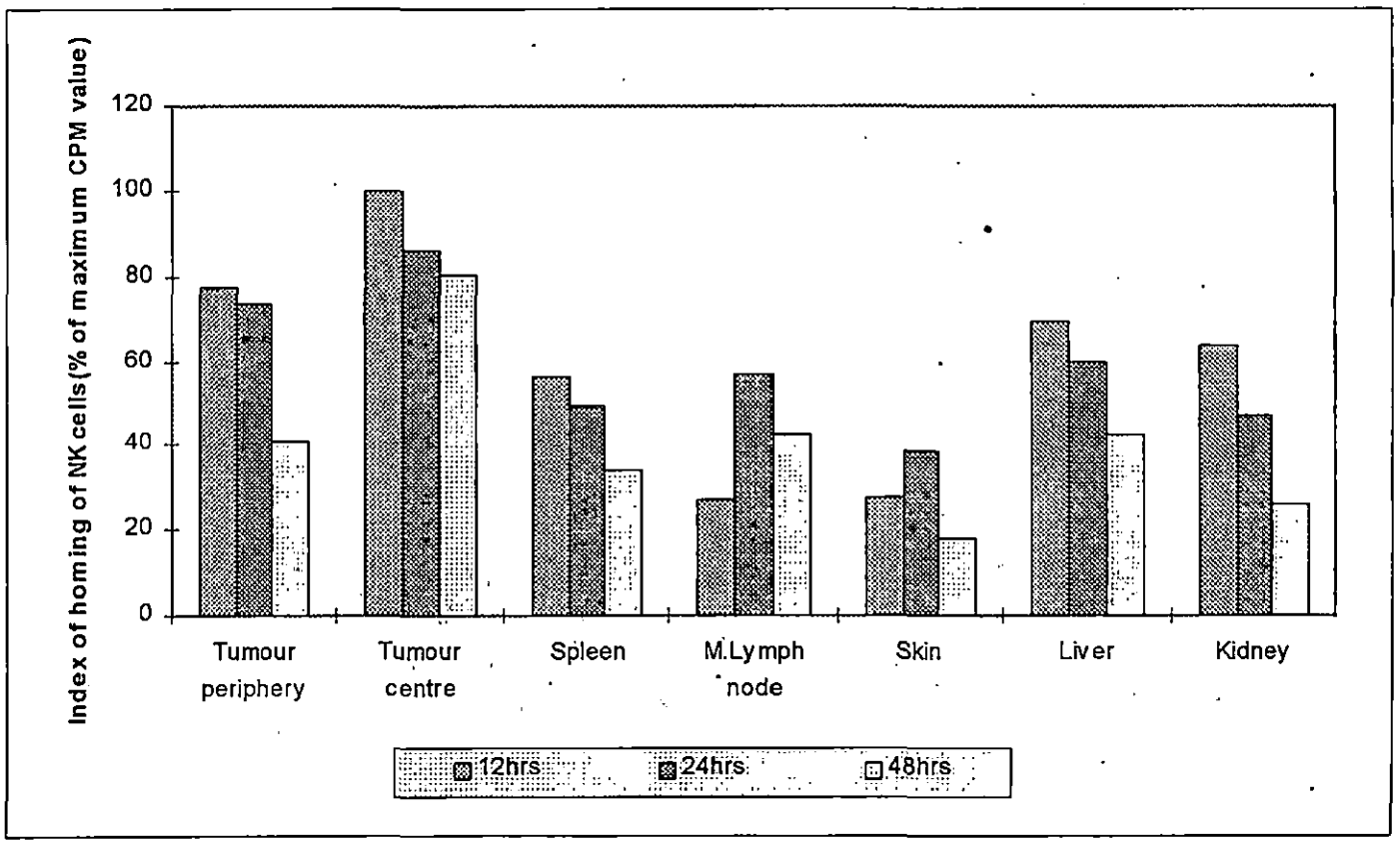
Index of homing of NK cells (% of maximum CPM value)
120
100
80
60
40
20
0
Tumour periphery
Tumour centre
Spleen
M.Lymph node
Skin
Liver
Kidney
12hrs
24hrs
48hrs

Fig. 6

Figure 7 Rate of tumour growth after repeated injections of 50µl of gIL-2 by intravenous route (—■—) and at the tumour site (--Δ--). First injection was made seven days after tumour induction. Control animals were injected with normal saline only (-.-◆.-) The lines are drawn according to least square fit method.

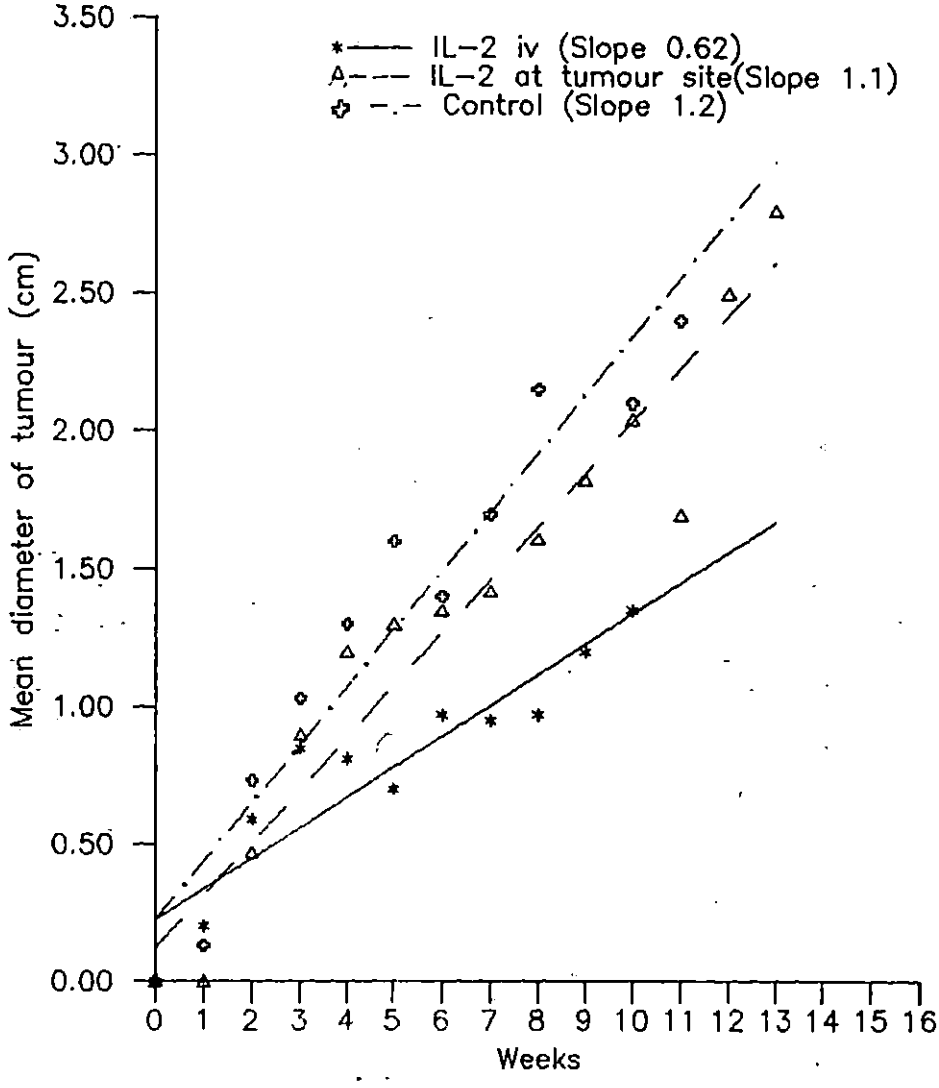
IL-2 iv (Slope 0.62)
IL-2 at tumour site(Slope 1.1)
Control (Slope 1.2)
Mean diameter of tumour (cm)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks

Fig.7

Figure 8 Survivality of tumour bearing mice on different days after repeated injections of 50µl of gIL-2 by intravenous route (_*_) and at the tumour site (-- Δ--). Control animal were injected with normal saline only (-.-✠-.-). Number in parentheses next to the keys indicate total number of mice at the beginning of the experiment; number next to the plotting indicates number of surviving mice.

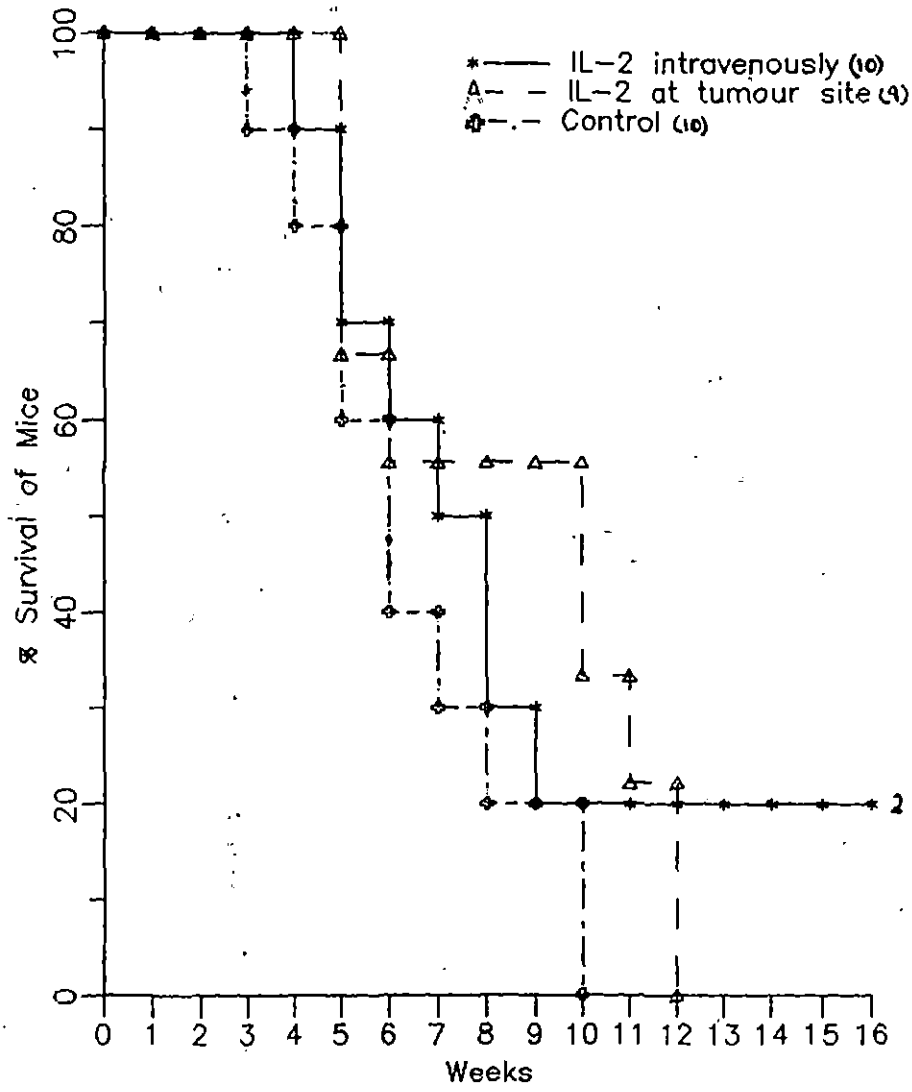
IL-2 intravenously (10)
IL-2 at tumour site (9)
Control (10)
% Survival of Mice
Weeks
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
2

Fig. 8

Figure 9 Rate of tumour growth after one time adoptive transfer of $0.5x10^6$ $NK^N$ cells at the tumour site. Adoptive transfer was made on day 7 after tumour induction.

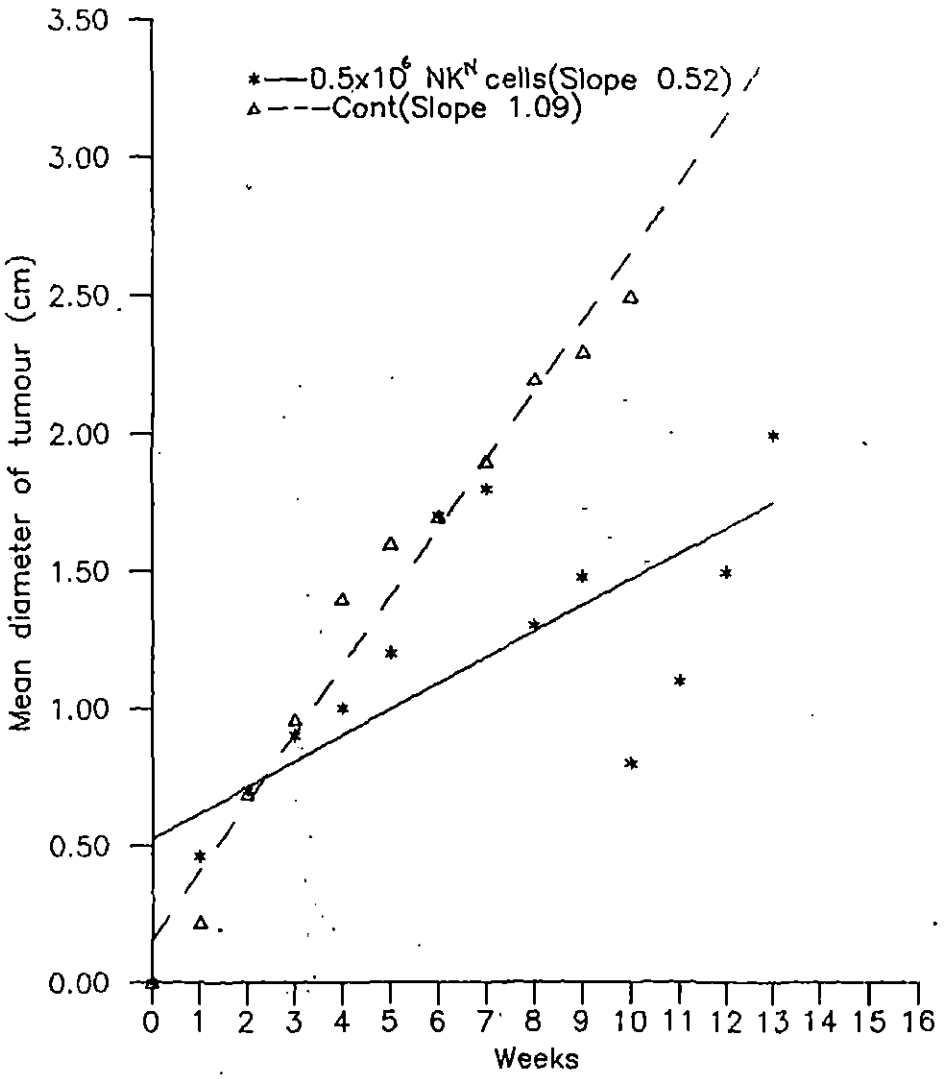
*—0.5x10^6 NK^N cells(Slope 0.52)
Δ---Cont(Slope 1.09)
Mean diameter of tumour (cm)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks

Fig. 9

Figure 10 Percentage of survival of tumour bearing mice on different days after adoptive transfer of $0.5x10^6$ $NK^N$ cells at tumour site (-*-). Control animals were injected with normal saline only (--Δ--).The number in the parenthesis indicates the total number of mice at the beginning of the experiment.

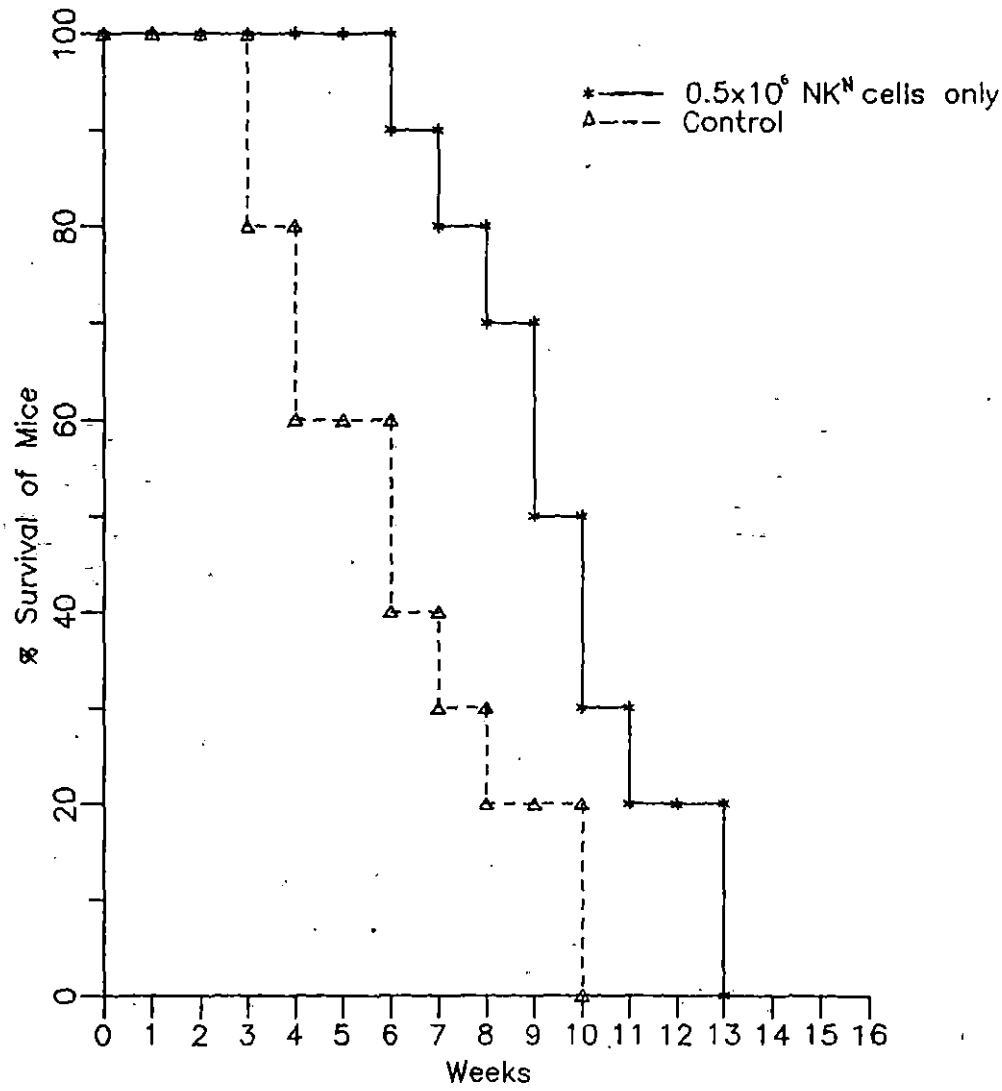
0.5x10⁶ NK^N cells only
Control
% Survival of Mice
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks

Fig.10

Figure 11 Rate of tumour growth after injections of $0.5x10^6$ $NK^N$ cells activated with 50µl of gIL-2 at the tumour site (-*-) and intravenously(--Δ--). Both sets of animals received weekly IL-2 injections for five times through respective routes. Control animals were injected with normal saline only(-.-◊-.-). Tumour was induced on day 0 and adoptive transfer made on day 7.

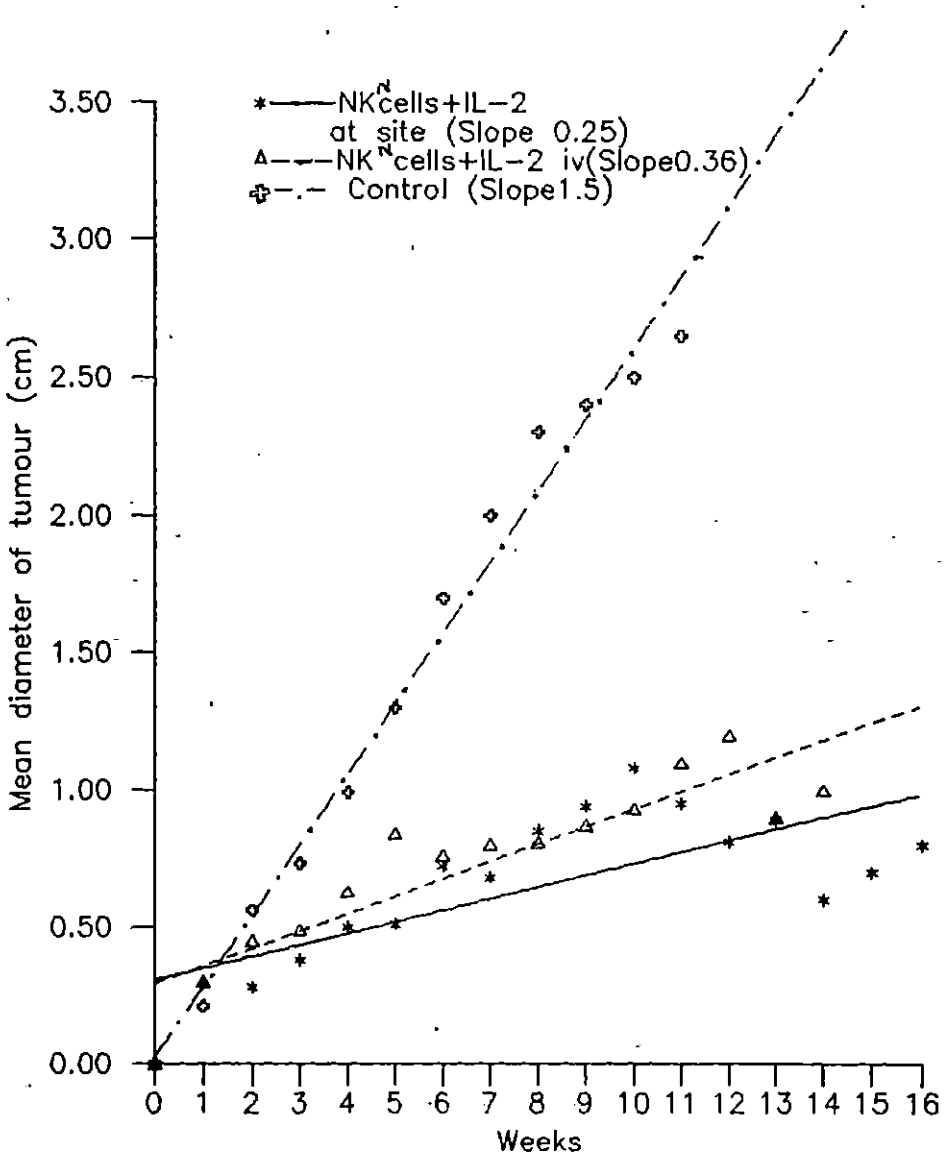
*——NK^N cells+IL-2
at site (Slope 0.25)
Δ– –NK^N cells+IL-2 iv(Slope0.36)
⊕–.– Control (Slope1.5)
Mean diameter of tumour (cm)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks

Fig. 11

Figure 12 Percentage of survival of tumour bearing mice on different days after adoptive transfer of of $0.5x10^6$ $NK^N$ cells activated with 50µl of gIL-2 at tumour site (-*-) and intravenously (-·Δ--). Rest of the protocol as in the legend of Fig.11.

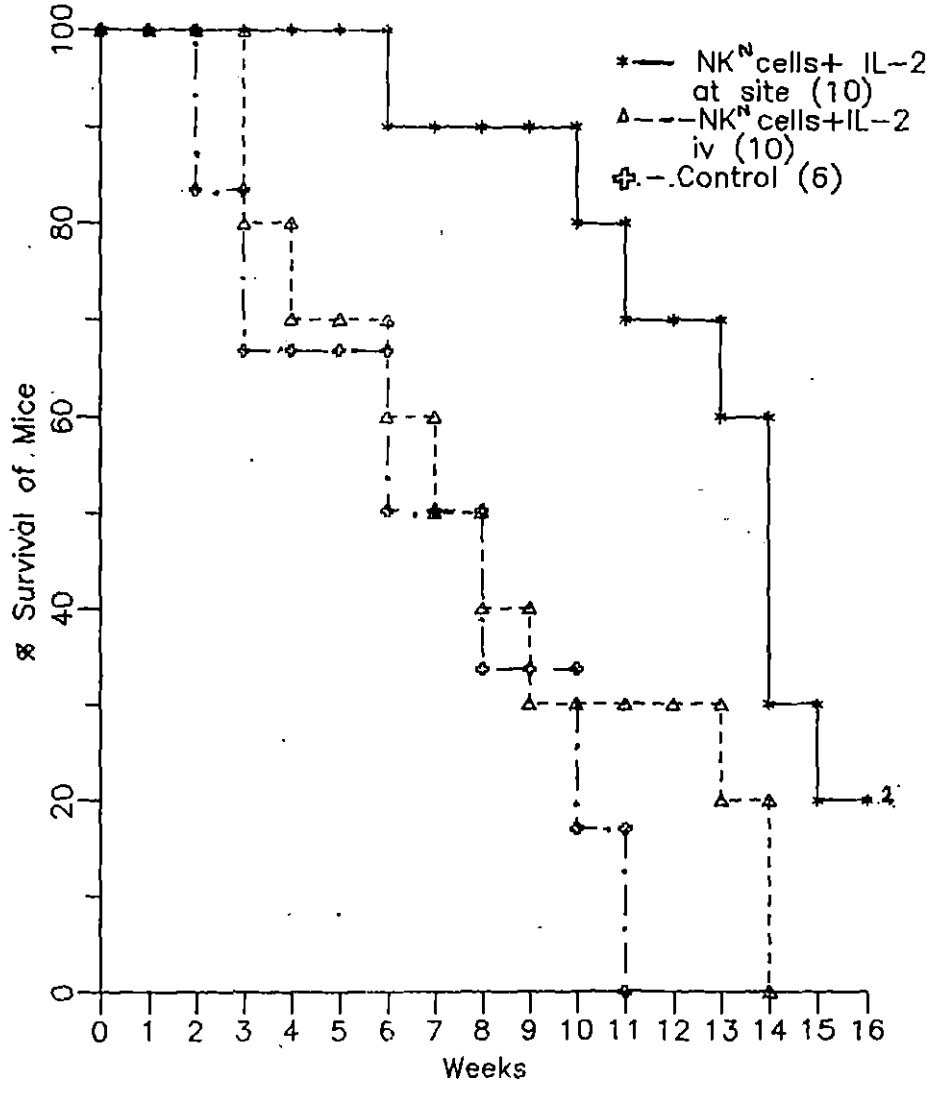
NK^N cells+ IL-2 at site (10)
NK^N cells+IL-2 iv (10)
Control (6)
% Survival of Mice
Weeks
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig.12

Figure 13 Rate of tumour growth after one time adoptive transfer of IL-2 activated $10^6$ $NK^N$ cells at the tumour site (-*-). Rest of the protocol as before in case of Fig.11.

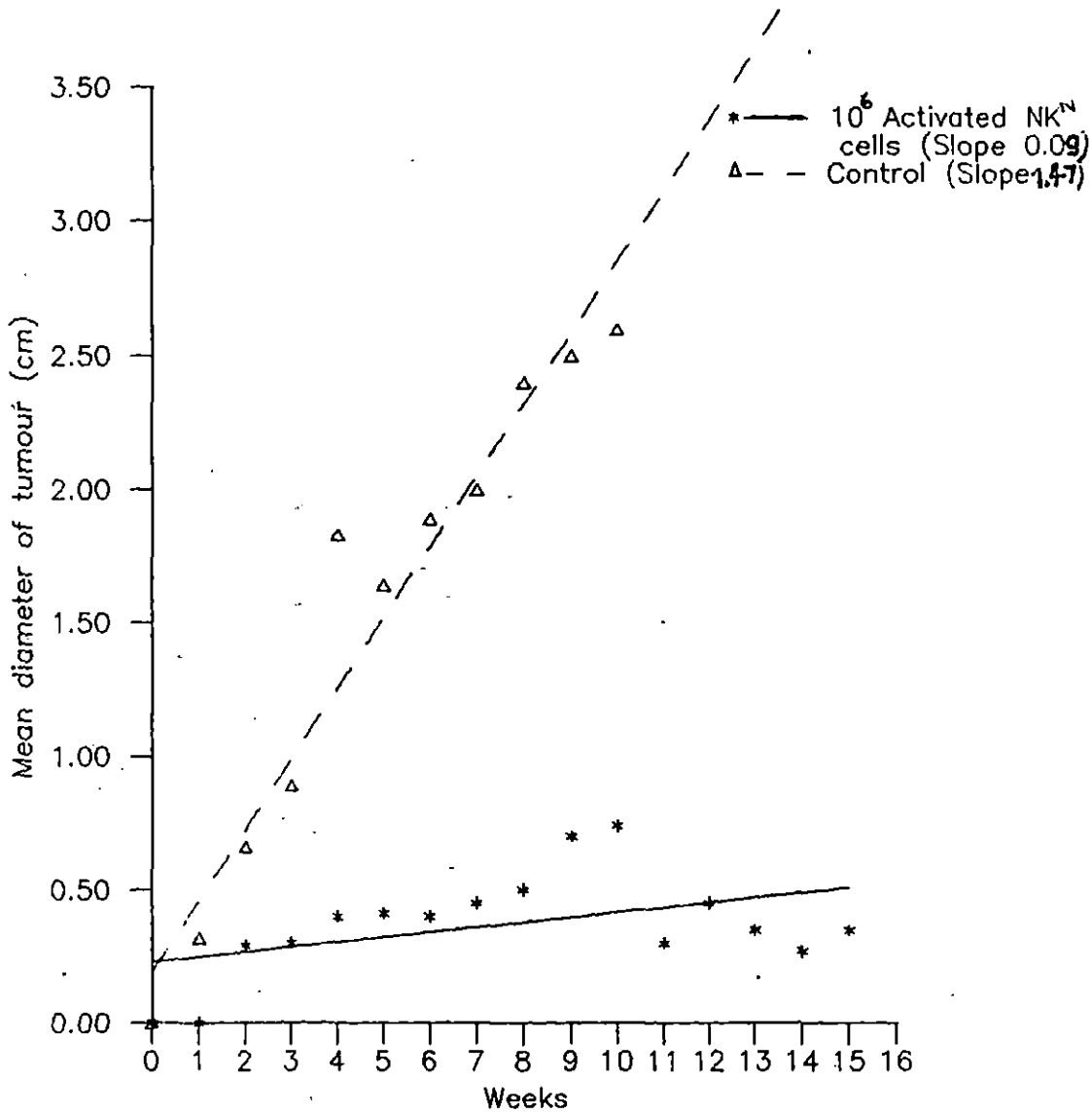
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Mean diameter of tumour (cm)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks
10^6 Activated NK^N cells (Slope 0.09)
Control (Slope 1.17)

Fig.13

Figure 14 Percentage of survival of tumour bearing mice on different days after adoptive transfer of IL-2 activated $10^6$ $NK^N$ cells (-*-). The protocol same as in case of Fig 13.

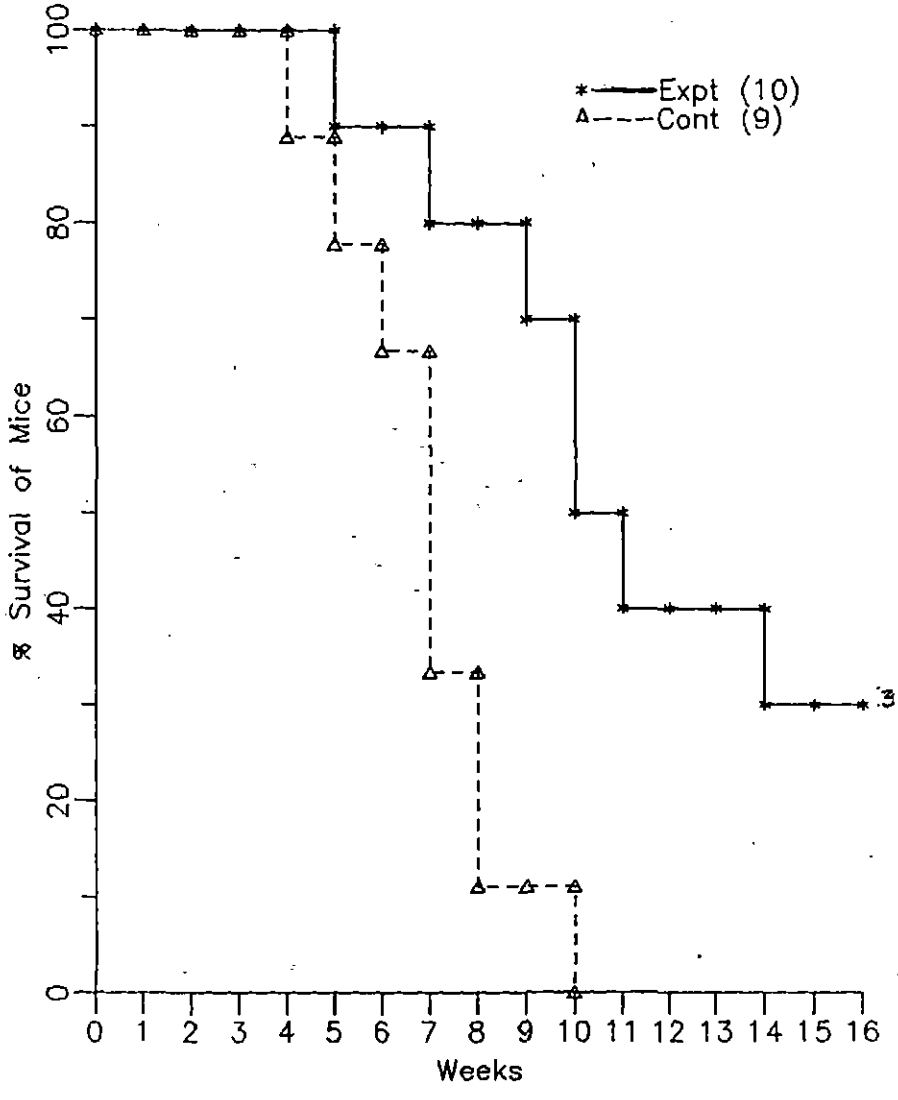
% Survival of Mice
100
80
60
40
20
0
Expt (10)
Cont (9)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks

Fig. 14

Figure 15 Efficacy of NK cells from tumour bearing mice:

Study of tumour growth after one time adoptive transfer of $0.5x10^6$ $NK^T$cells activated with 50µl of gIL-2 at tumour site (-*-) in one set of animals and intravenously in another set of animals (--Δ--).Both sets of animals received weekly IL-2 injections for five times through respective routes. Tumour was induced on day 0 and adoptive transfer made on day 7.

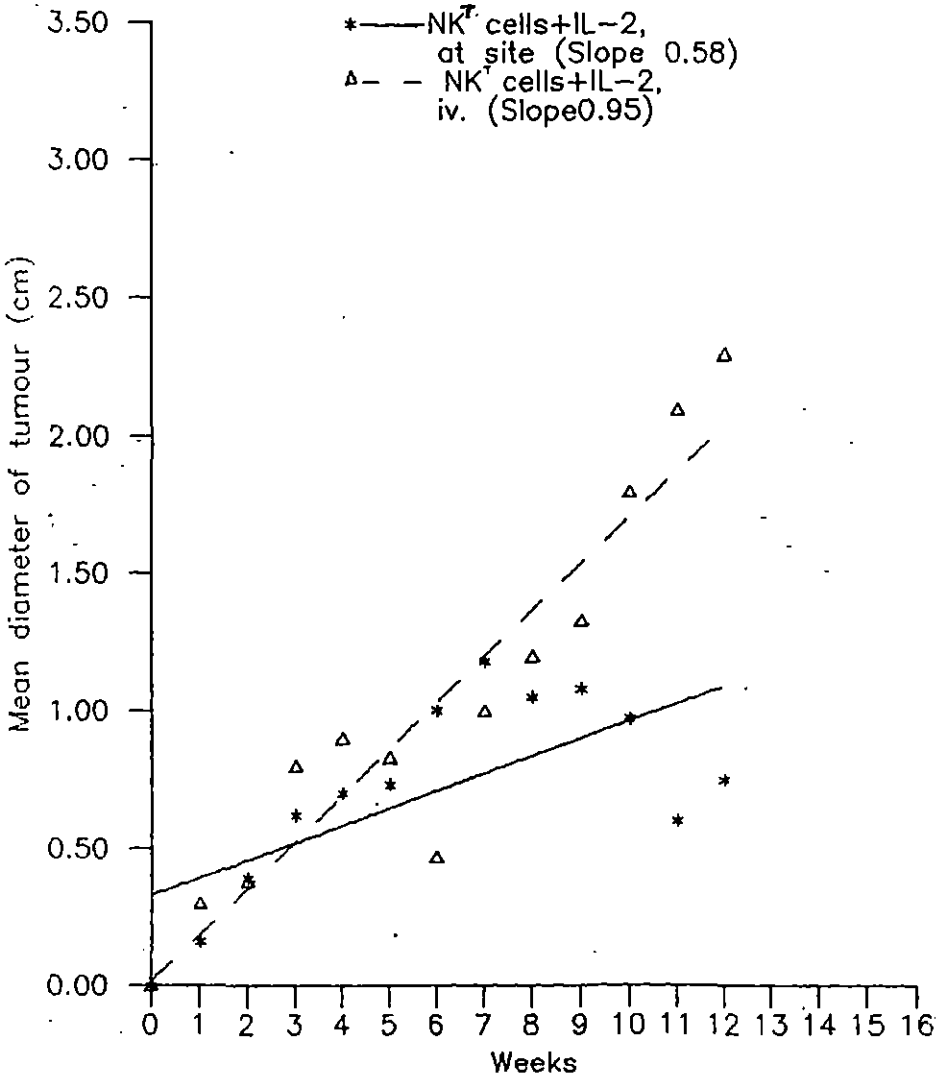
*——NK$^T$ cells+IL-2, at site (Slope 0.58)
Δ– – NK$^T$ cells+IL-2, iv. (Slope0.95)
Mean diameter of tumour (cm)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks

Fig. 15

Figure 16 Survivality of the tumour bearing mice on different days after adoptive transfer of IL-2 activated $0.5x10^6$ $NK^T$ cells as per specifications in the legend of Fig. 15.

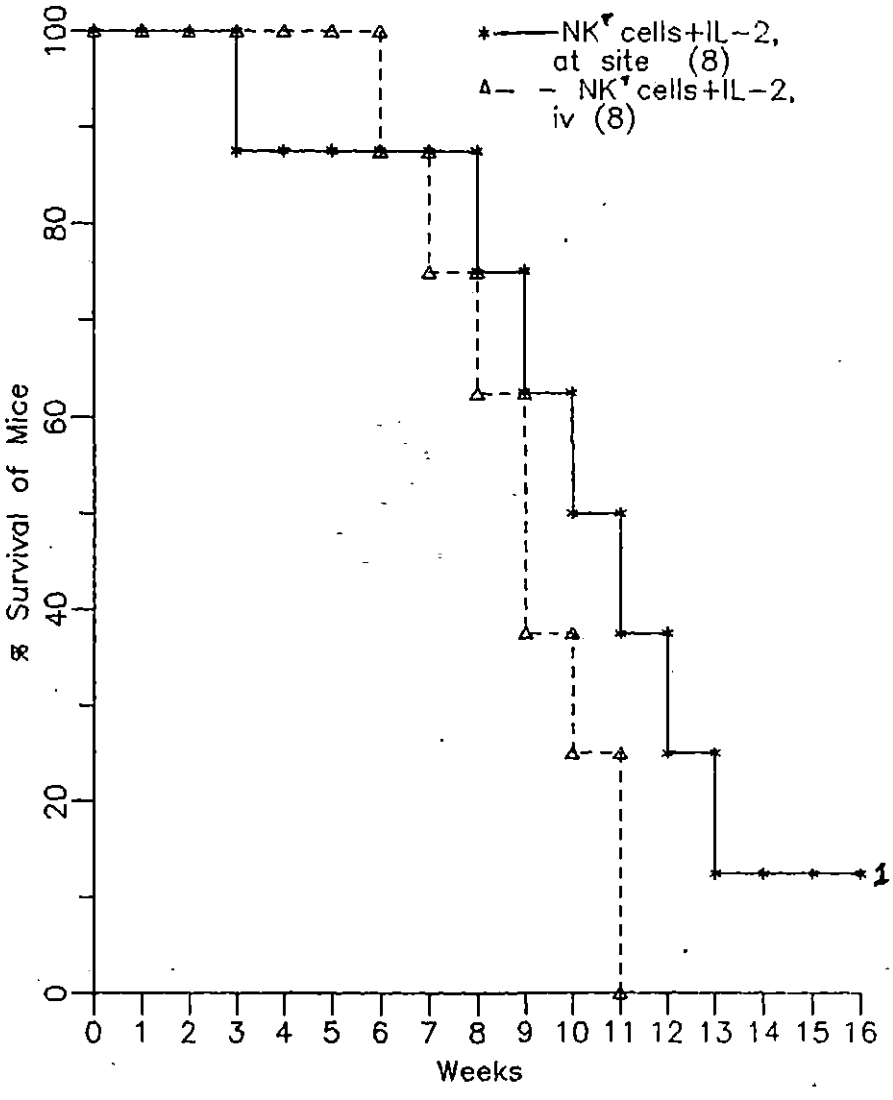
NK cells+IL-2, at site (8)
NK cells+IL-2, iv (8)
% Survival of Mice
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Weeks
1

Fig.16

Figure17 Percentage of recurrence and non-recurrence of tumours after their surgical removal and followed by adoptive transfer of $0.5x10^6$ $NK^N$ cells activated with 50µl of gIL-2 or 50µl of gIL-2 alone at the tumour site. Both sets of animals received weekly IL-2 injections for five times. Control animals received same volume of normal saline after surgery. (Difference in results of both experimental groups with control was significant at $p<0.001$ by Student's t test ).

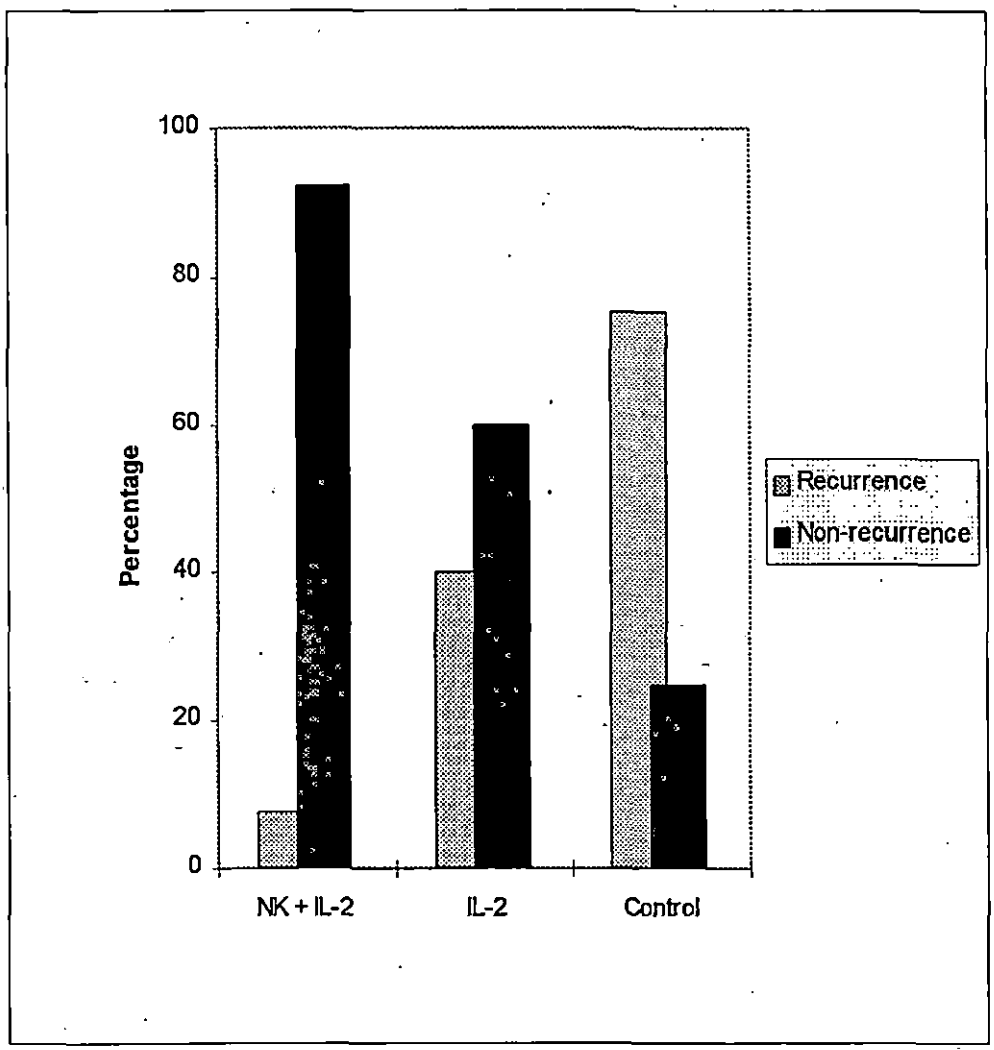
100
80
60
40
20
0
Percentage
NK + IL-2
IL-2
Control
Recurrence
Non-recurrence

Fig. 17

Figure 18 Survivality of the different groups of mice as in Fig. 17.

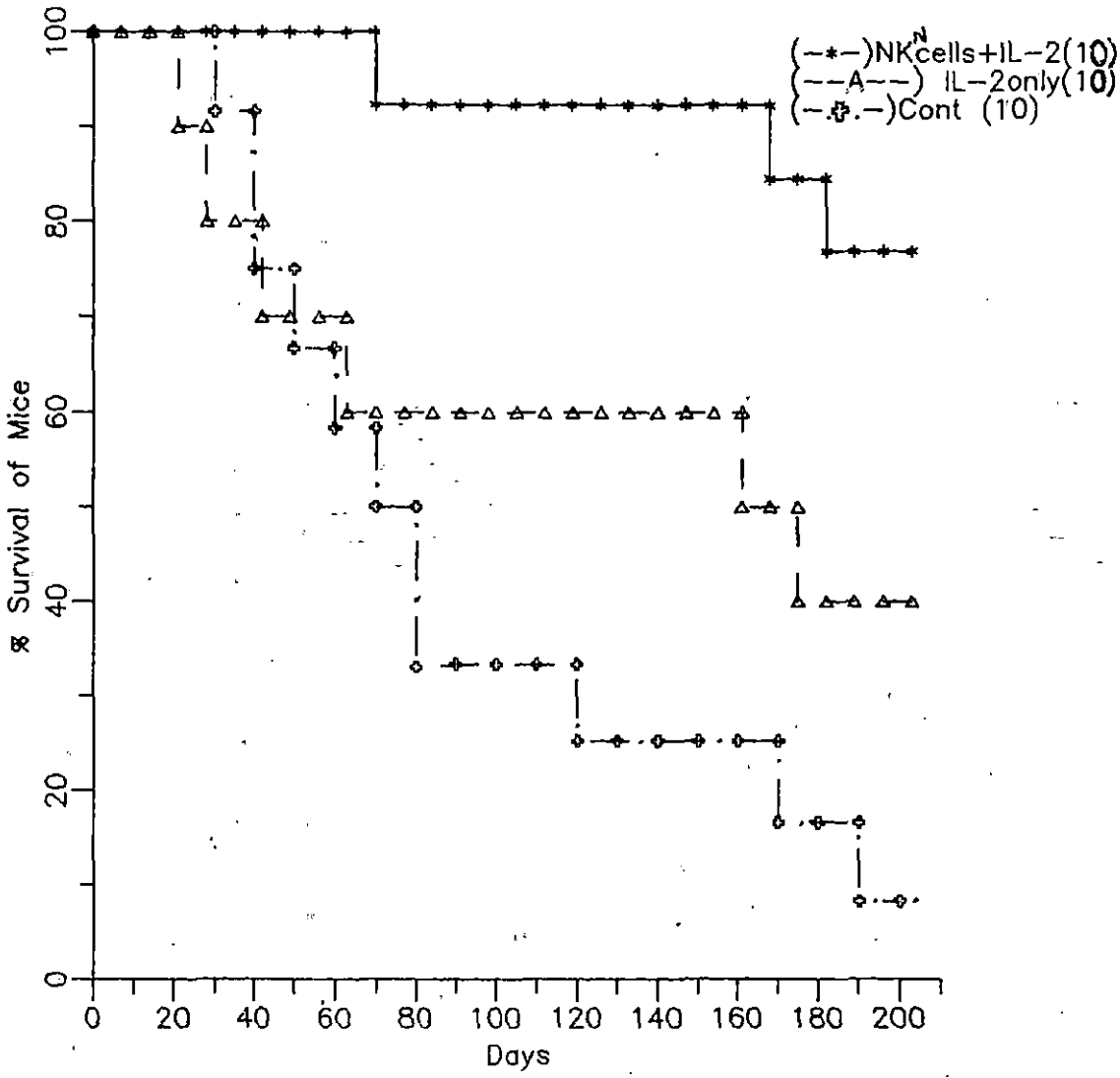
(-*-)NK cells+IL-2(10)
(--A--) IL-2only(10)
(-.◇.-)Cont (10)
% Survival of Mice
0
20
40
60
80
100
0
20
40
60
80
100
120
140
160
180
200
Days

Fig. 18