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Award Number: DAMD17-98-1-8172

TITLE: Novel Vector System for Breast Cancer Therapy

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CONTRACTING ORGANIZATION: The University of Alabama at Birmingham
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REPORT DATE: October 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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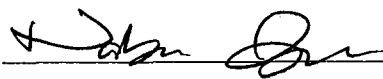
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12/03/02

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

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1. AGENCY USE ONLY (Leave blank)**2. REPORT DATE**

October 2001

3. REPORT TYPE AND DATES COVERED

Final (1 Oct 98 - 30 Sep 01)

4. TITLE AND SUBTITLE

Novel Vector System for Breast Cancer Therapy

5. FUNDING NUMBERS

DAMD17-98-1-8172

6. AUTHOR(S)

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REPORT NUMBER****9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES**

20030122 106

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12b. DISTRIBUTION CODE**13. ABSTRACT (Maximum 200 Words)**

The funds from this proposal have been used to develop a novel, sustained-release delivery system for tumor necrosis factor alpha (TNF α). Coacervate microspheres were made to contain TNF α that was released over 3 days. Efficacy testing by administering a single intratumoral dose of the sustained release preparation showed that it was superior to free-TNF α as either a stand-alone therapy, or in combination with other anti-neoplastic modalities. Additional studies described within suggest that this formulation could also be used as a means of targeting other anti-neoplastic modalities into tumors masses.

14. SUBJECT TERMS

breast cancer, drug delivery, controlled release, monoclonal antibody

15. NUMBER OF PAGES

27

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

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20. LIMITATION OF ABSTRACT

Unlimited

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I. INTRODUCTION

This project was a breast carcinoma experimental therapeutics effort that examined the efficacy of a novel sustained release formulation of human recombinant tumor necrosis factor alpha (TNF α) in combination with adenovirus E1A products delivered by a conditionally replicative adenovirus. Shown here is an abbreviated version of the Statement of Work:

Task #1: Construct and characterize microspheres that contain and release tumor necrosis factor α (TNF α) over an extended period of time

Task #2: Identify a conditionally replicative adenovirus suitable for use in combination with the extended release TNF α formulation

Task #3: Evaluate individual and combined activity of *dl338* virus and TNF α *in vitro*

Task #4: Evaluate the combined activity of *dl338* virus and TNF α *in vivo* by intratumoral injection

Task #5: Evaluate combined activity of *dl338* virus administered systemically and TNF α administered by intratumoral injection

BODY

For this final report, the progress towards completion of each task in the SOW will be described. Please refer to the figure legends that precede the figures themselves in the appendix.

Task 1

We have succeeded in demonstrating that can be encapsulated within a novel coacervate microsphere formulation comprised of human serum albumin and heparin, and released in a bioactive form over 3 days (refer to figs 1-3). The antitumor activity of these microspheres was assessed in human tumor xenografts on the flanks of nude mice by intratumoral injection of the microspheres. In experiments that assessed the dose response and compared tumor growth inhibition to the same doses of unencapsulated TNF α , it was repeatedly shown that the encapsulated TNF α was superior to the free, unencapsulated TNF α (refer to figs 4,5). These experiments completed Task 1.

Task 2

Our original proposal called for the use of an adenovirus partially deleted in the E1B region, *dl338*, as a conditionally replicative adenovirus. However, we established a collaboration with Dr. Paul Reynolds for the use of adenoviruses he had designed that were even more selective in replication within neoplastic tissues as opposed to normal tissue. These adenoviruses used the midkine (MK) promoter region to transcriptionally direct the E1 region, and he had data that indicated the MK promoter was more active in neoplastic tissues with the consequence that replication was better limited to neoplastic tissues as desired. Our earlier work (Garver et al, Cancer 1994) had shown that MK was strongly expressed in breast carcinomas, and this was another rationale for selecting these series of viruses. Three different MK viruses were selected:

"AdMKE1" which contained an intact E1 under transcriptional direction of MK, "AdMKE4/E1" that contained both E1 and E4 under transcriptional control of MK, and "AdMKE1/del19kd" that was similar to the *dl338* virus in that the 19kd E1B was deleted but the remaining E1 was under transcriptional control of MK. By obtaining these viruses for use in this project, Task 2 was completed.

Task 3

Since adenovirus E1A products had been shown in earlier studies to enhance the toxicity of TNF α , we performed in vitro experiments that examined the effects of combining adenovirus infection with TNF α exposure on subsequent tumor cell line growth as quantified by the MTS colorimetric assay. These experiments were disappointing, finding little enhancement of TNF α -mediated killing in the A549 cell line with the AdMKE1, AdMKE4/E1 or the AdMKE1/19kd del. Note that all of these viruses replicated within these cells as evidenced by the marked reduction in viable cell number at higher MOIs, but the addition of TNF α over a wide dose range did not enhance the killing (figs 6-8). We also tried different schedules of virus and TNF α addition (figs 9-11) which failed to elicit any augmentation of killing by the combination of cytokine and virus. We also tried a second cell line, H1299 (fig 12-14) that also failed to show any benefit of combining the TNF α with the three different viruses. These experiments completed Task 3.

Tasks 4 and 5

These Tasks were animal experiments initially intended to confirm the anticipated positive in vitro results of combining TNF α with the conditionally replicative adenoviruses. We felt that the results did not justify the animal experiments as originally planned. Therefore, we modified our animal experiment plans to examine the combination of ionizing radiation with the sustained release TNF α . Following dose ranging pilot experiments that identified the appropriate TNF α intratumoral dose, we performed duplicate experiments on both A549 and H1299 tumor nodules that did show a significant enhancement of tumor nodule growth delay in groups treated with both radiation and TNF α compared with either treatment alone.

II. FIGURE LEGENDS

Fig. 1. Time-dependent release of TNF α from heparin-albumin coacervate microspheres in vitro from one representative lot. Ordinate: release of TNF α from microspheres as a percent of the total amount of cytokine encapsulated, abscissa: incubation time in days.

Fig. 2: Time-dependent release of TNF α from a different lot of heparin-albumin coacervate microspheres than shown in fig. 1.

Fig. 3. Time-dependent release of TNF α from another lot of heparin-albumin coacervate microspheres than shown in figs 1 and 2. In this case, the release is quantified on the ordinate in micrograms.

Fig. 4. Dose-response curve of H1299 tumor growth following intratumoral administration of free or encapsulated TNF α . Shown are the results of one representative experiment (n=6/grp) in which tumors received a single intratumoral administration of TNF α . The formulation and amount administered in micrograms is shown in the legend above.

Fig. 5. Dose-response curve of A549 tumor growth following intratumoral administration of free or encapsulated TNF α . Shown are the results of one representative experiment (n=6/grp) in which tumors received a single intratumoral administration of TNF α . The formulation and amount administered in micrograms is shown in the legend above.

Fig. 6. Effects of combined TNF α and adenovirus AdMKE1 infection on growth of A549 cells. Shown is the relative growth of cells 5 days after the addition of TNF α in the amount in micrograms shown in the legend above and infection with the AdMKE1 adenovirus containing a complete E1 transcription unit under control of the midkine promoter region. Cells with replicating virus would contain the viral E1A proteins that were expected to act synergistically with the TNF α to inhibit carcinoma growth. Data here is the average of two experiments, each data point performed in quadruplicate.

Fig. 7. Effects of combined TNF α and adenovirus AdMKE4/E1 infection on growth of A549 cells. Shown is the relative growth of cells 5 days after the addition of TNF α in the amount in micrograms shown in the legend above and infection with the AdMKE4/E1 adenovirus containing the E1 and E4 transcription units under control of the midkine promoter with a deletion of the E4 region. Data here is the average of two experiments, each data point performed in quadruplicate.

Fig. 8. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection on growth of A549 cells. Shown is the relative growth of cells 5 days after the addition of TNF α in the amount in micrograms shown in the legend above and infection with the AdMKE1/19kd-del adenovirus containing the E1 transcription unit under control of the midkine promoter with a deletion of the 19 kd E1B region. Data here is the average of two experiments, each data point performed in quadruplicate.

Fig. 9. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection when virus infection is followed by the addition of TNF α 24 hrs later ("Seq 1") on growth of A549 cells. Shown is the relative growth of cells 5 days after the addition of TNF α in the amount in micrograms shown in the legend above and infection with the AdMKE1/19kd-del adenovirus containing the E1 transcription unit under control of the midkine promoter with a deletion of the 19 kd E1B region. Data here is the average of two experiments, each data point performed in quadruplicate.

Fig. 10. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection when virus infection is followed by the addition of TNF α 4 hrs later ("Seq 2") on growth of A549 cells. Shown is the relative growth of cells 5 days after the addition of TNF α in the amount in micrograms shown in the legend above and infection with the AdMKE1/19kd-del adenovirus containing the E1 transcription unit under control of the midkine promoter with a deletion of the 19 kd E1B region. Data here is the average of two experiments, each data point performed in quadruplicate.

Fig. 11. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection when TNF α was added 48 hrs prior to virus infection for 4 hrs, followed by the addition of TNF α after infection ("Seq 3") on growth of A549 cells. Shown is the relative growth of cells 5 days after the addition of TNF α (second addition) in the amount in micrograms shown in the legend above and infection with the AdMKE1/19kd-del adenovirus containing the E1 transcription unit under control of the midkine promoter with a deletion of the 19 kd E1B region. Data here is the average of two experiments, each data point performed in quadruplicate.

Fig. 12. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection when virus infection is followed by the addition of TNF α 24 hrs later ("Seq 1") on growth of H1299 cells. This is similar to Fig. 9, except the cell line is changed.

Fig. 13. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection when virus infection is followed by the addition of TNF α 4 hrs later ("Seq 2") on growth of H1299 cells. This is similar to Fig. 10, except the cell line is changed.

Fig. 14. Effects of combined TNF α and adenovirus AdMKE1/19kd-del infection when TNF α was added 48 hrs prior to virus infection for 4 hrs, followed by the addition of TNF α after infection ("Seq 3") on growth of H1299 cells. This is similar to Fig. 11, except the cell line is changed.

Fig. 15. H1299 tumor nodule growth following combined intratumoral TNF α plus radiation therapy. H1299 tumor nodules were treated with radiation only ("H-XRT"), radiation plus free TNF α ("H-XRT+Free"), or radiation plus encapsulated TNF α ("H-XRT-encap"). Both TNF α groups employed 10 μ g of TNF α . Growth is shown on the ordinate as the percentage of the starting tumor volume on day 0 $\pm$ SEM (n=6 mice/grp).

Fig. 16. H1299 tumor nodule growth following combined intratumoral TNF α plus radiation therapy. Duplicate experiment of that shown in fig. 15.

Fig. 17. A549 tumor nodule growth following combined intratumoral TNF α plus radiation therapy. Same as fig. 15, except the cell line is changed.

Fig. 18. A549 tumor nodule growth following combined intratumoral TNF α plus radiation therapy. Duplicate experiment of that shown in fig. 17.

III. BIBLIOGRAPHY

1. Abstract presented at Era of Hope Meeting: "Progress Towards Developing a Novel Strategy for Intratumoral Breast Cancer Therapy"
2. Abstract submitted for 2002 AACR meeting: Intratumoral Sustained Release TNF α As a Novel Radiosensitizing Agent"

3. Manuscript in preparation: "Novel Intratumoral Therapy for Non-Small Cell Lung Cancer: Sustained Release TNF α "

IV. PERSONNEL SUPPORTED DURING GRANT DURATION

UAB: R.Garver

JHU: R.J. Song
S.Q. Liu

V. KEY RESEARCH ACCOMPLISHMENTS

- development of novel sustained-release delivery system for TNF α
- demonstrating enhanced efficacy of the sustained release formulation of TNF α compared with free TNF α for the direct inhibition of tumor nodule growth
- demonstrating enhanced efficacy of the sustained release formulation of TNF α compared with free TNF α as a radiosensitization agent
- demonstrating that the sustained release formulation of TNF α can enhance the delivery of therapeutic agents into tumor masses

VI. REPORTABLE OUTCOMES

- a. Era of Hope Abstract Presentation 6/00
- b. AACR abstract submitted 11/01: Administration of Sustained-Release TNF α into Human Lung Cancer Xenografts Radiosensitizes and Enhances Tumor Permeability
- c. Manuscript in preparation based on data used for abstract described in VI.b.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

The pivotal experiments in Task 3 did not substantiate our original hypothesis: that combination of TNF α with adenovirus E1 products would enhance tumoricidal effects of either agent alone. The animal experiments showed that the sustained release TNF α was more efficacious than the free TNF α alone, and was additive when used in combination with external beam radiotherapy.

Since conclusion of this grant, we have extended the TNF α animal experiments, and also performed mechanistic experiments to explore the means by which TNF α and radiotherapy act more effectively. Further funding is being sought to extend these observations.

Figure 1

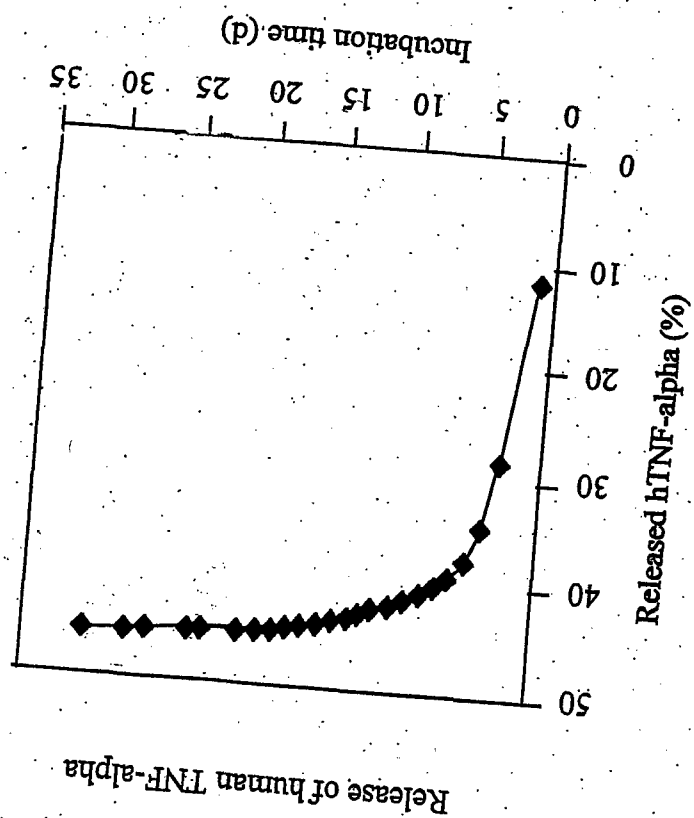
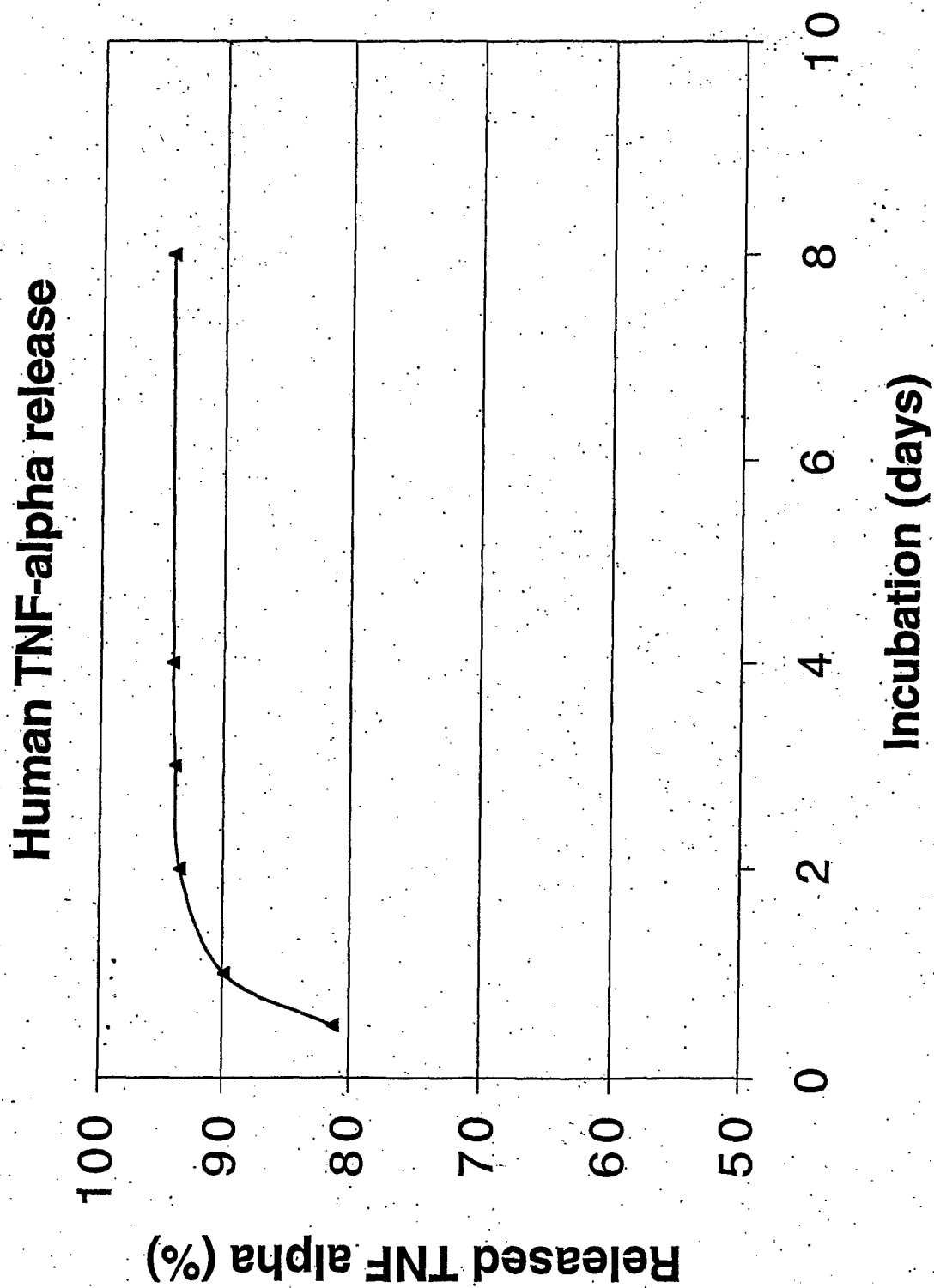


Figure 2



Preparation at Feb. 2001

Figure 3

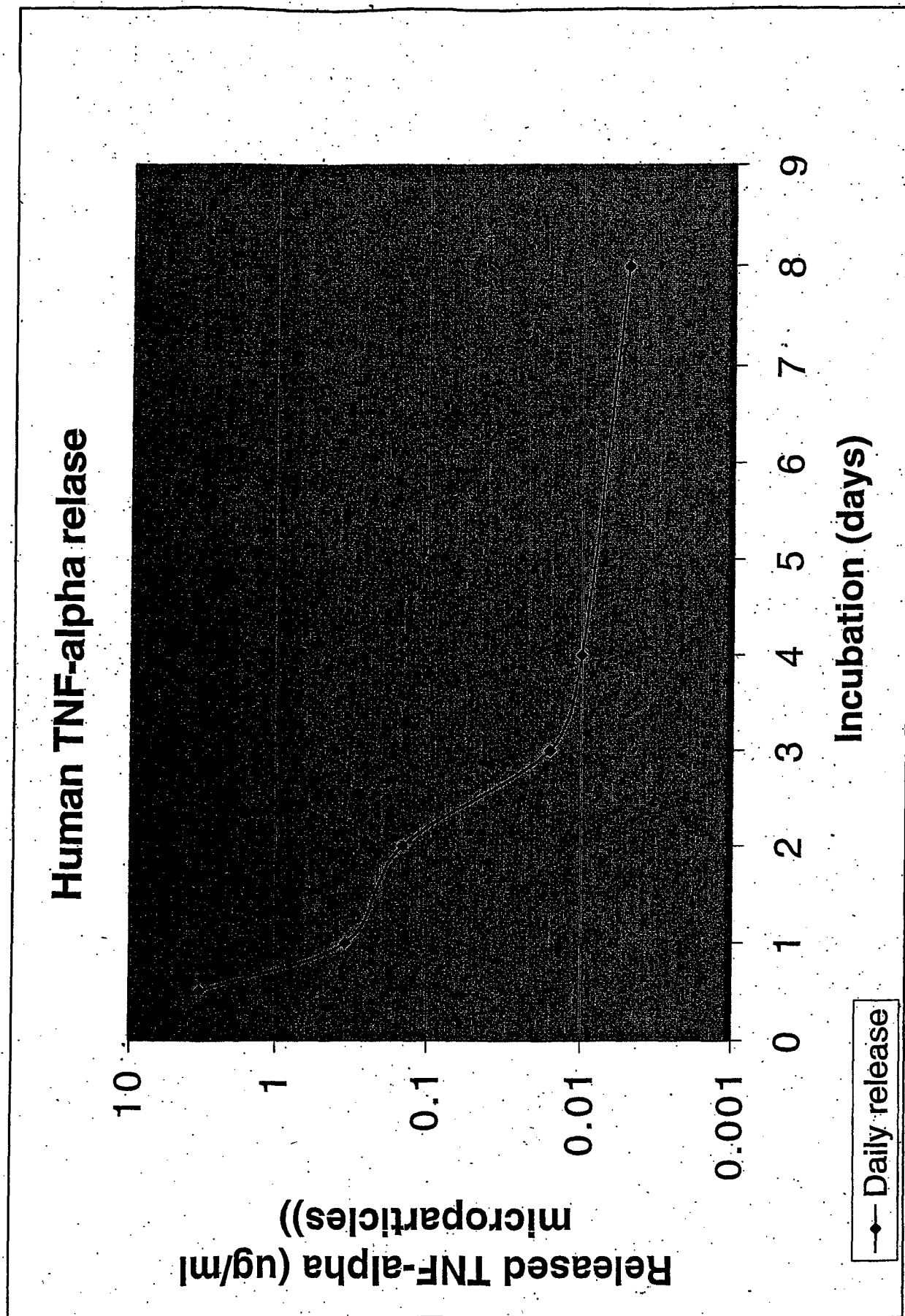


Figure 4

Encap v Free TNF alpha

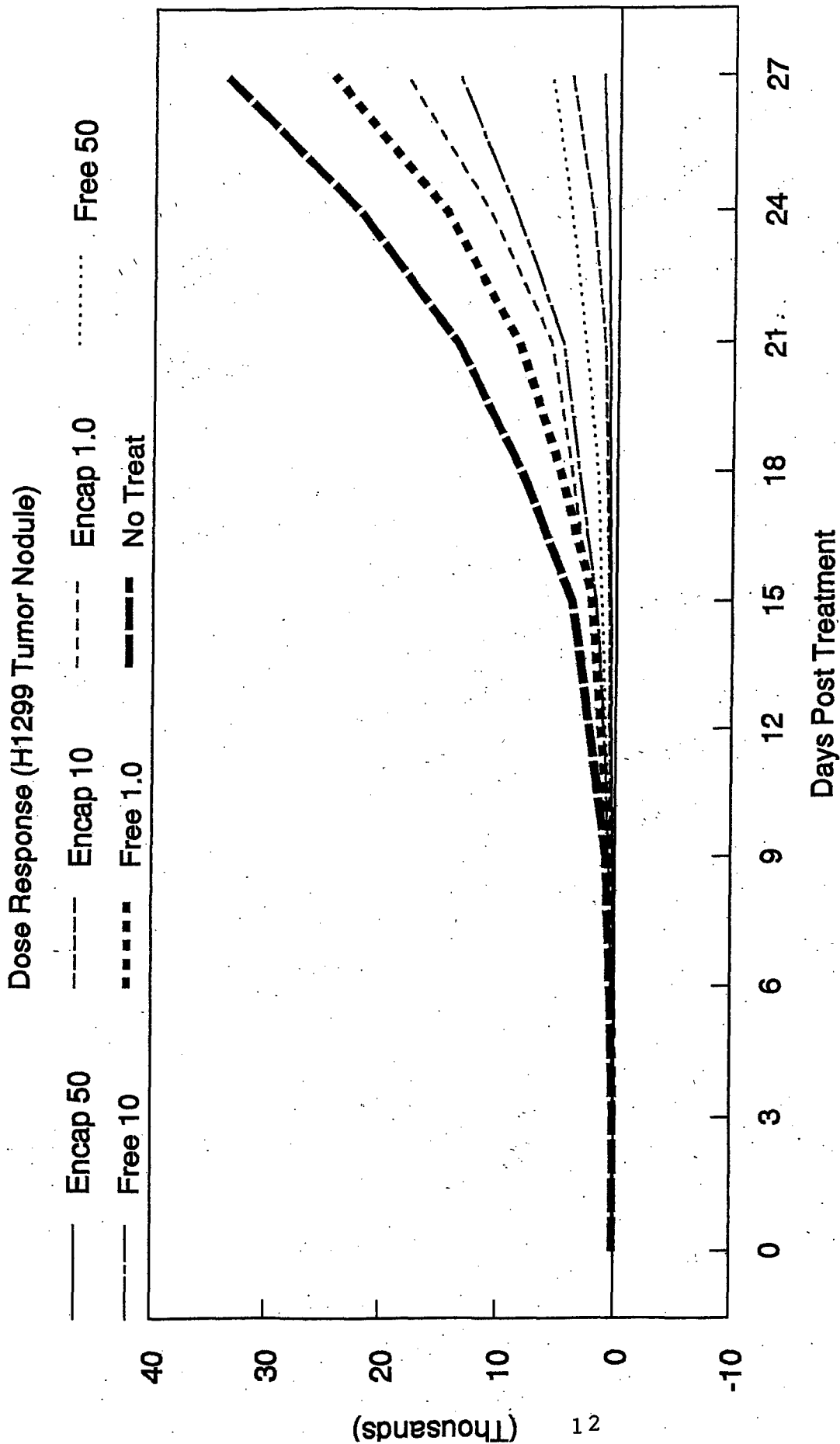
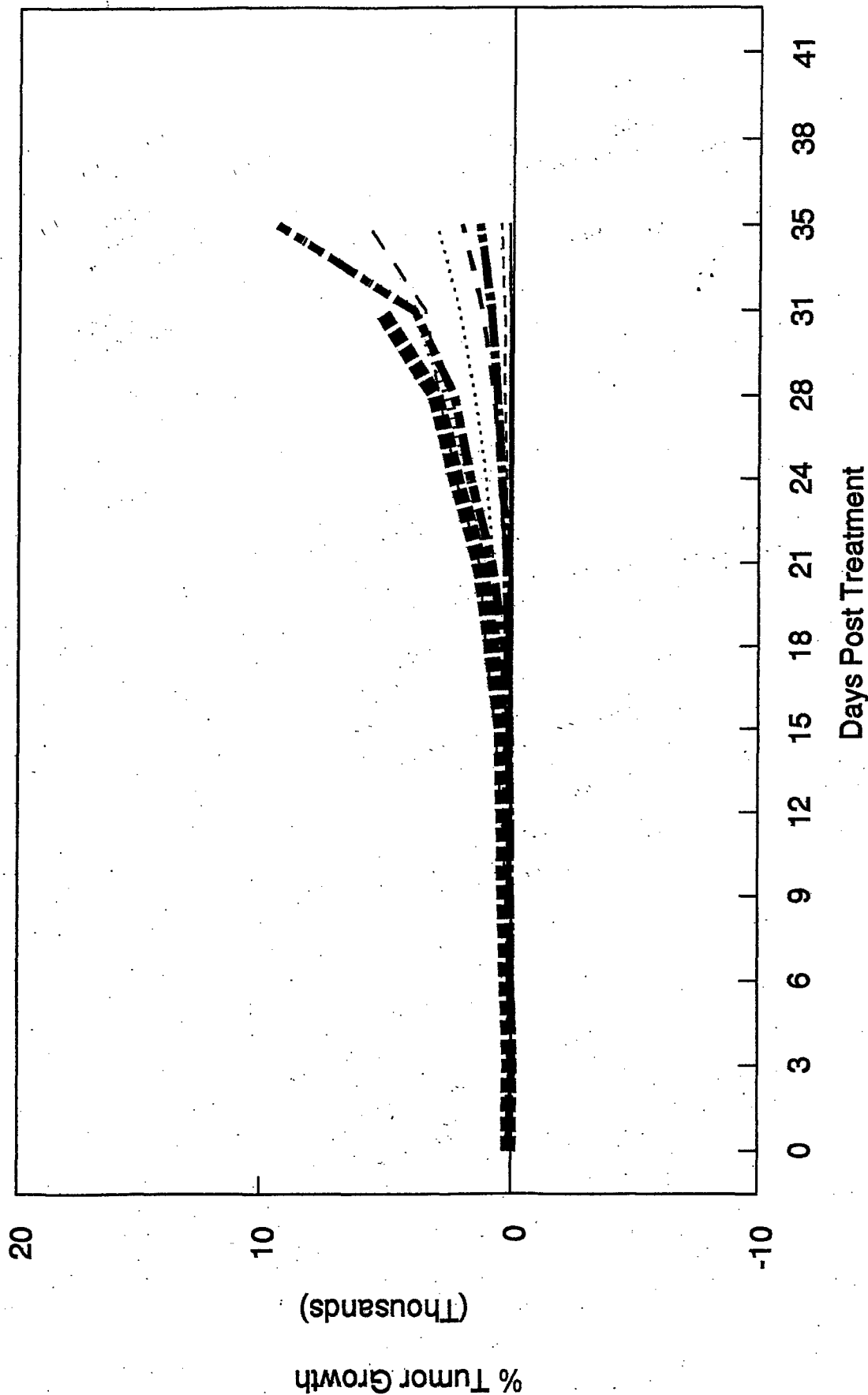
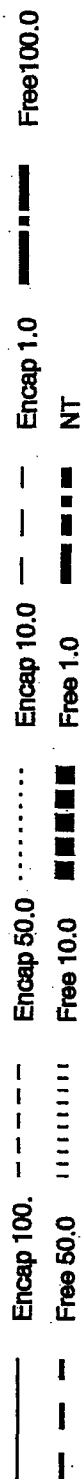


Figure 5

Encap v Free TNF alpha

Dose Response (A549 Tumor Nodule)



AdMKE1 + TNF

Figure 6

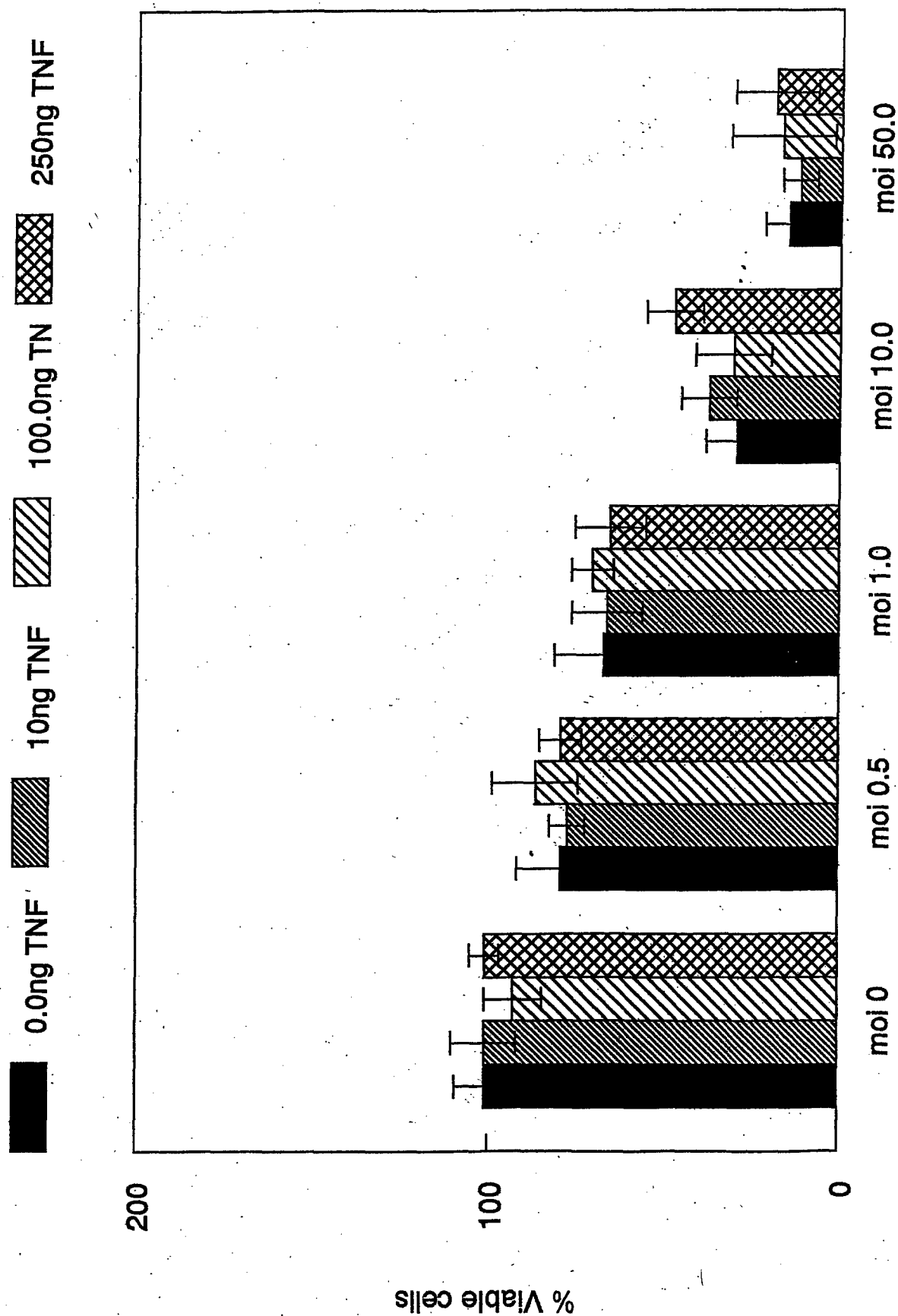


Figure 7

AdMKE4/E1 + TNF

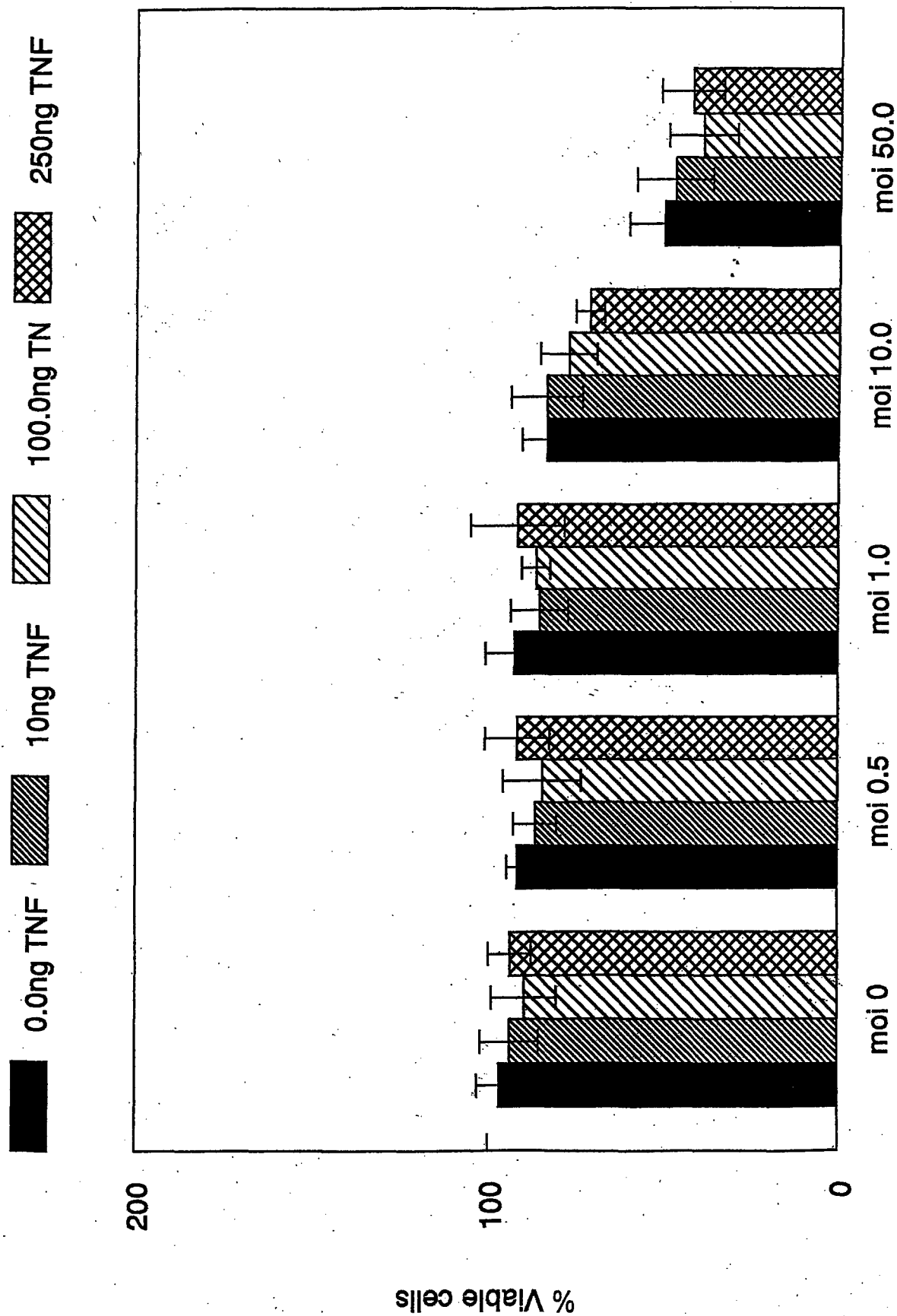


Figure 8

AdMKE1//19kd del+ TNF

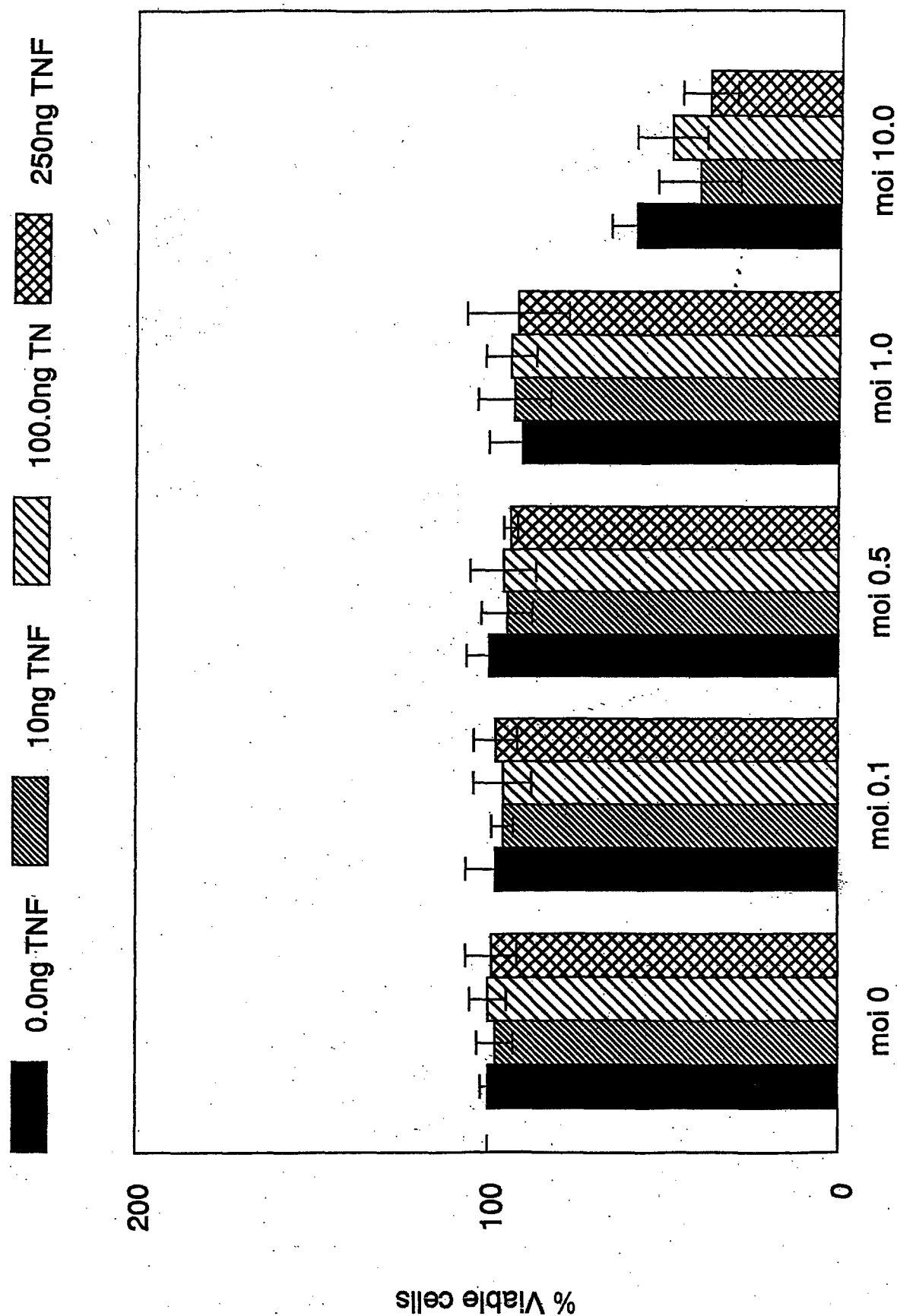


Figure 9

AdMKE1//19kd del+ TNF

A549 SEQ 1

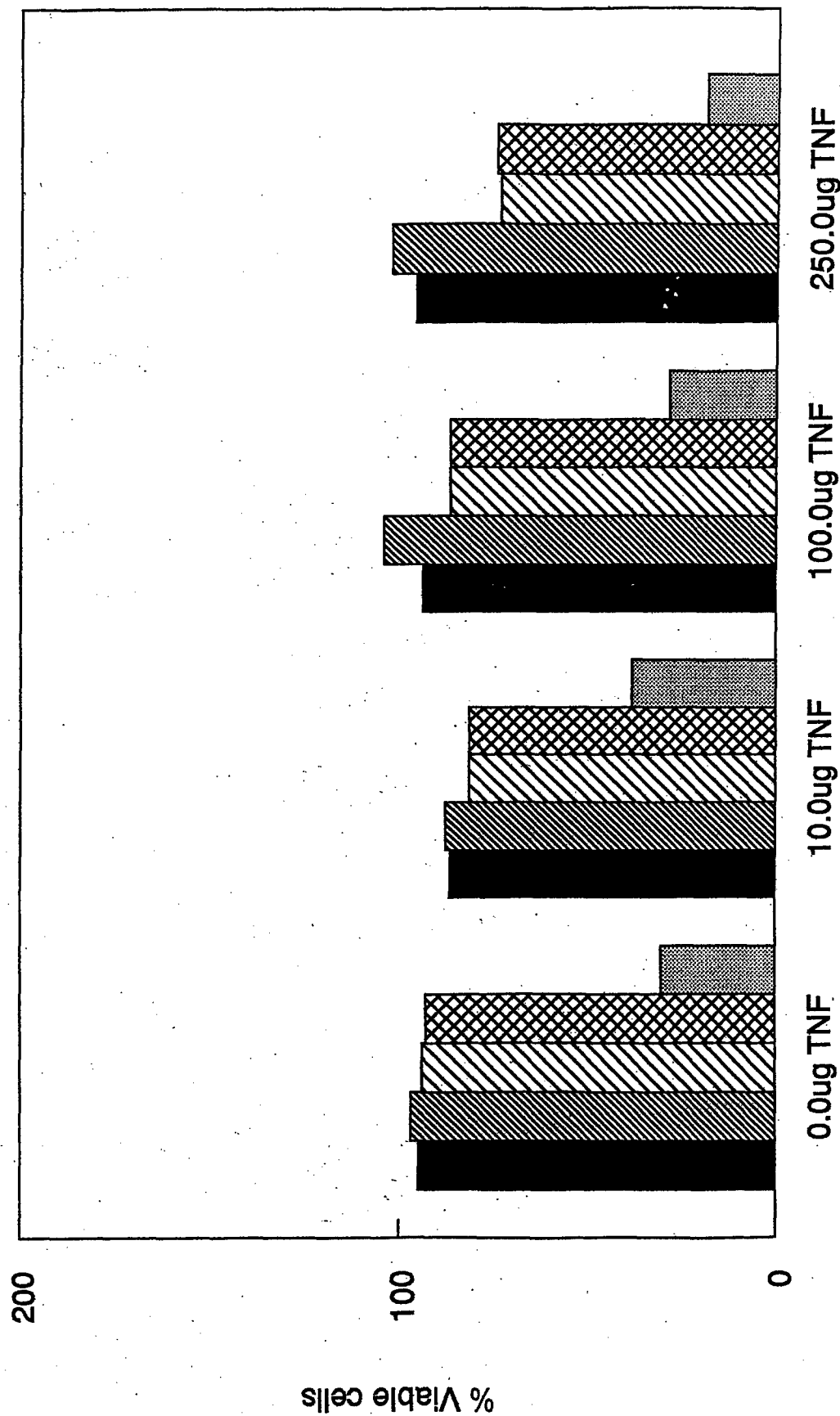


Figure 10

AdMKE1//19kd del+ TNF

A549 SEQ 2

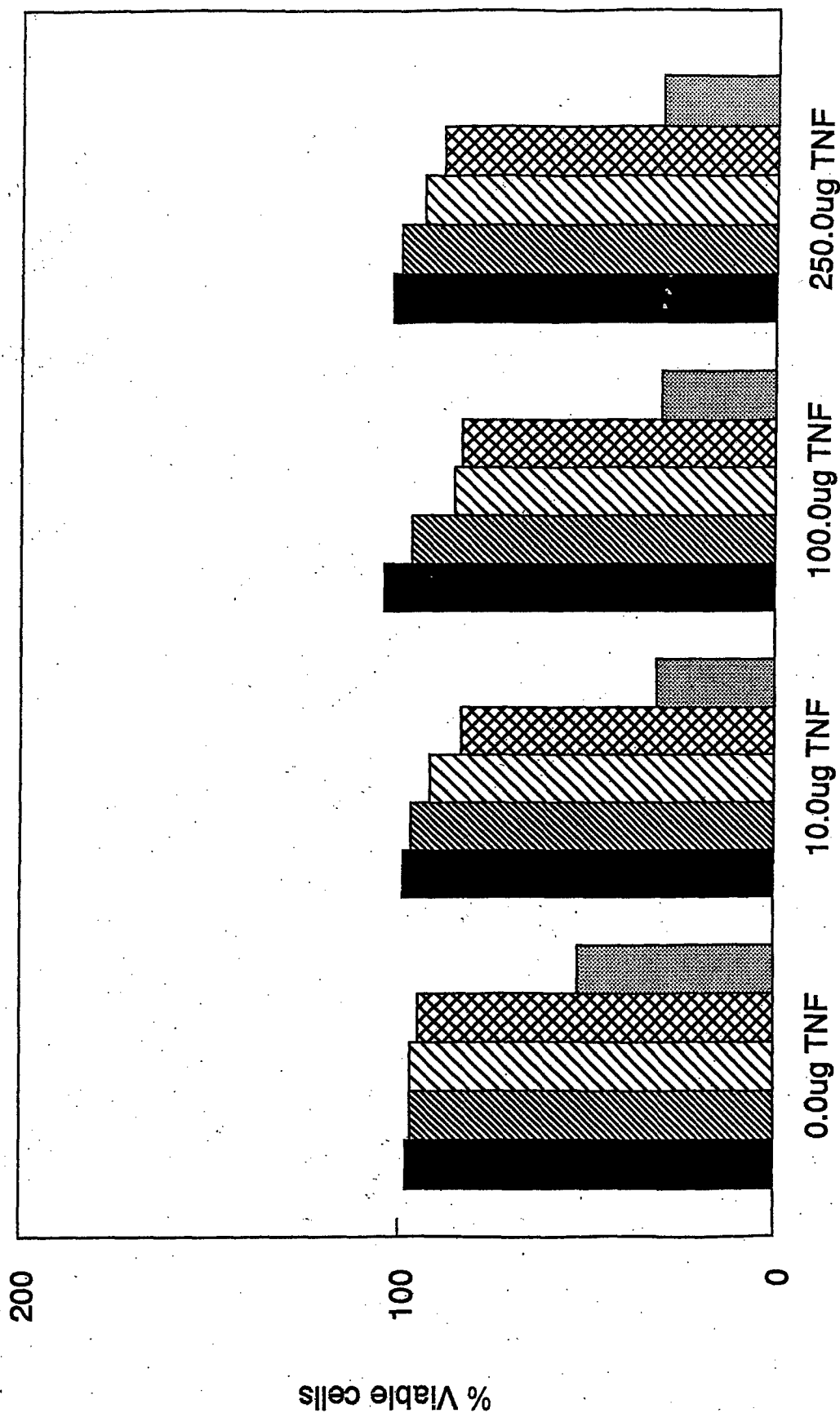
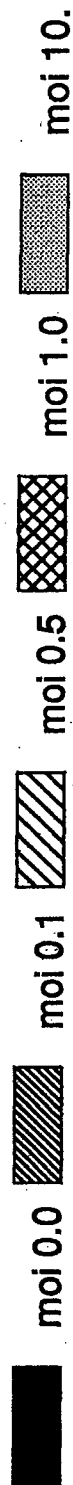
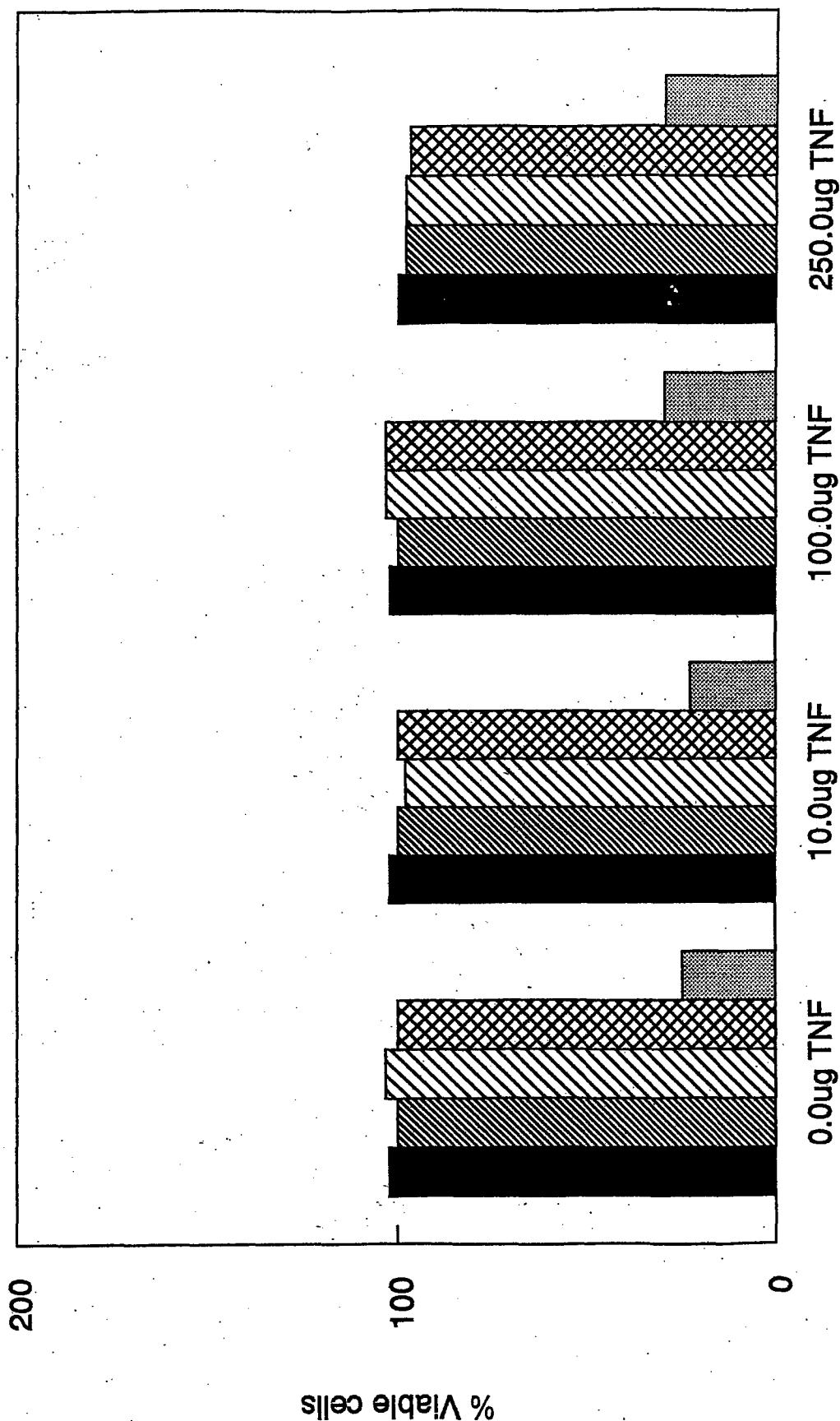


Figure 11

AdMKE1//19kd del+ TNF

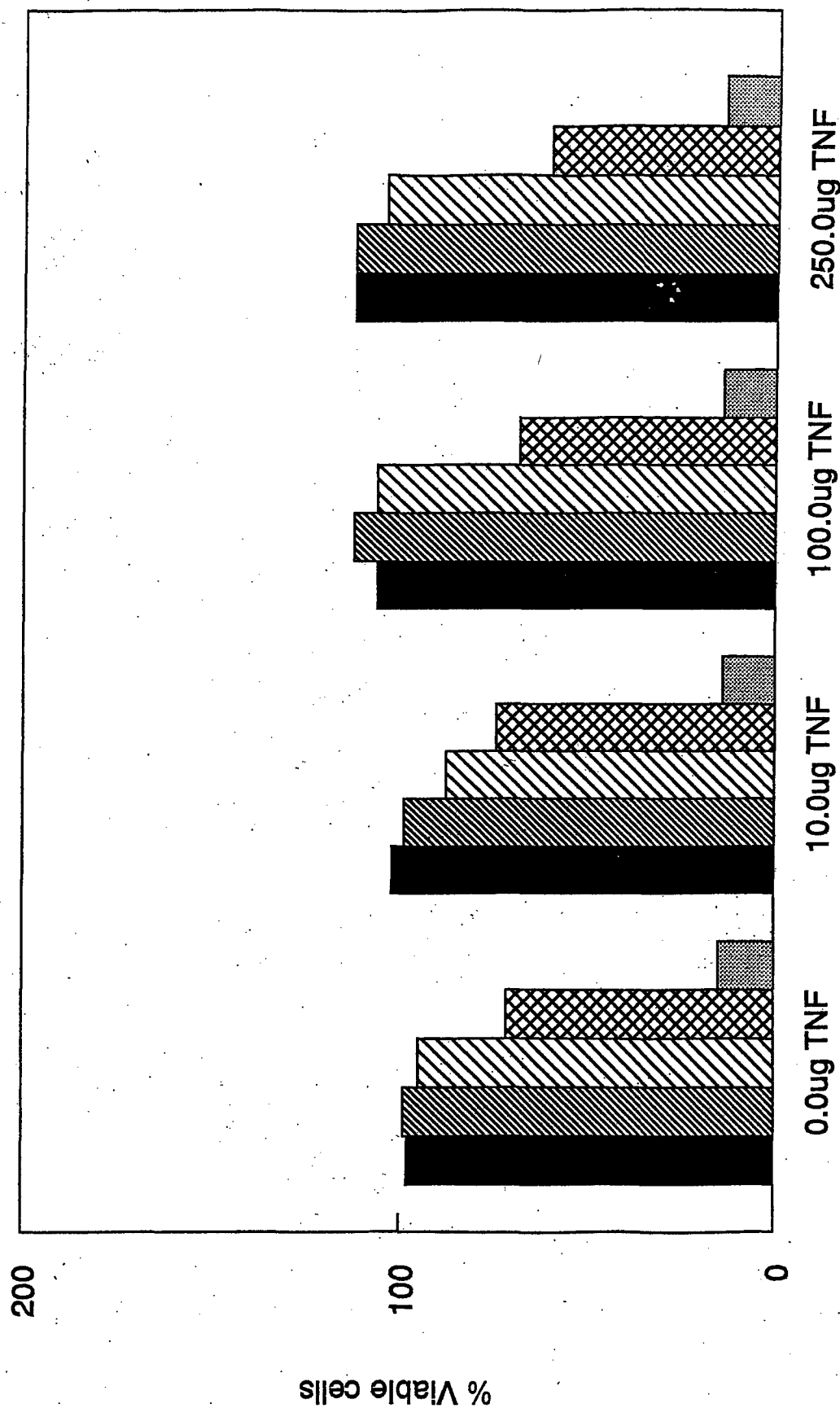
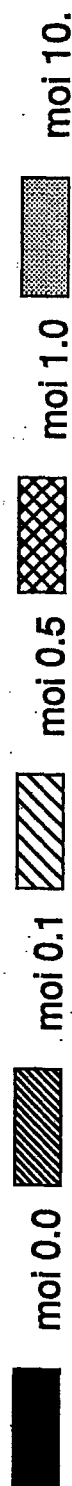
A549 SEQ 3



AdMKE1//19kd del+ TNF

Figure 12

H1299 SEQ 1



AdMKE1//19kd del+ TNF

Figure 13

H1299 SEQ 2

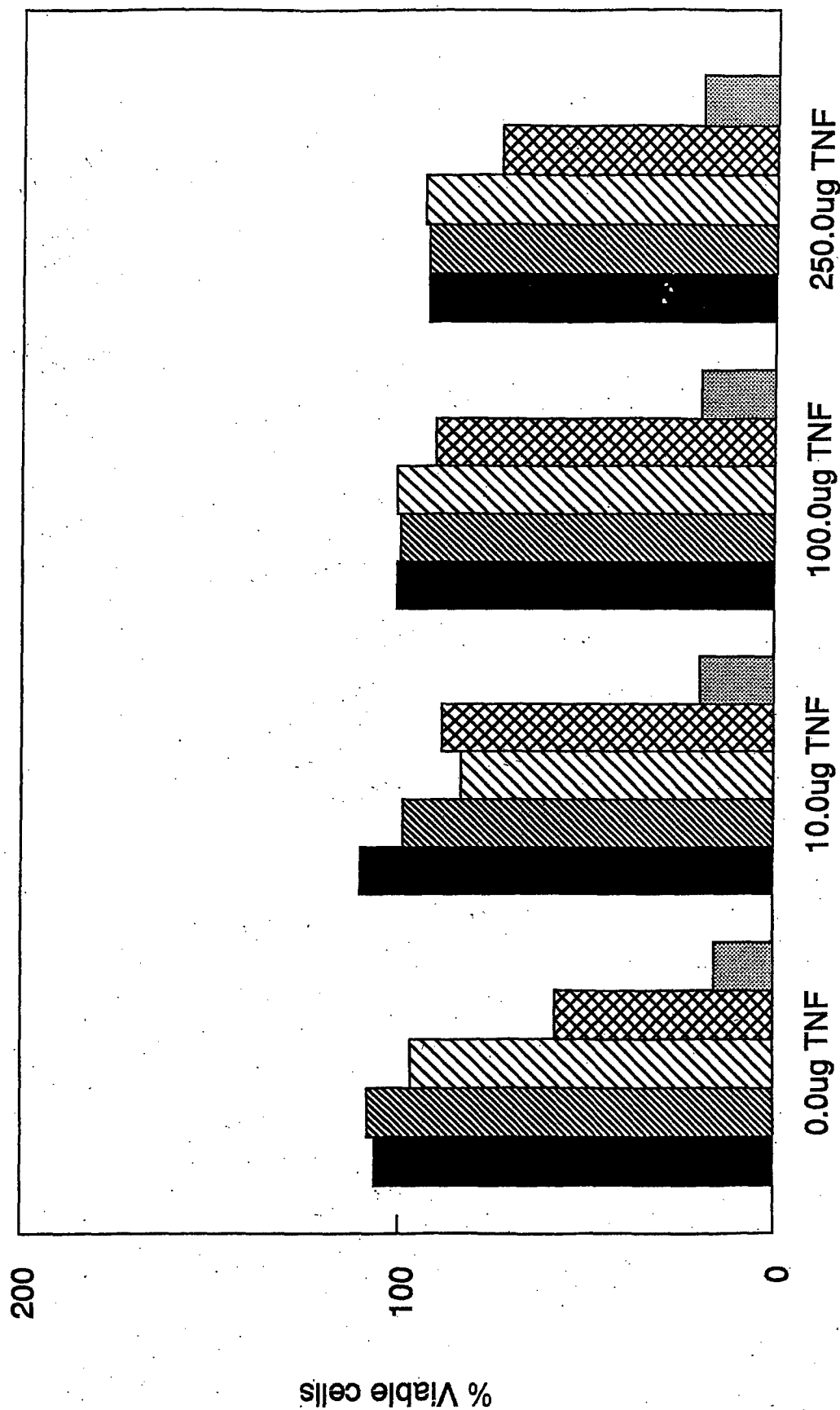
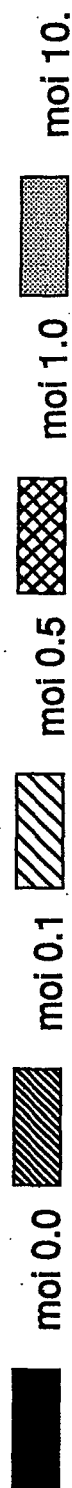
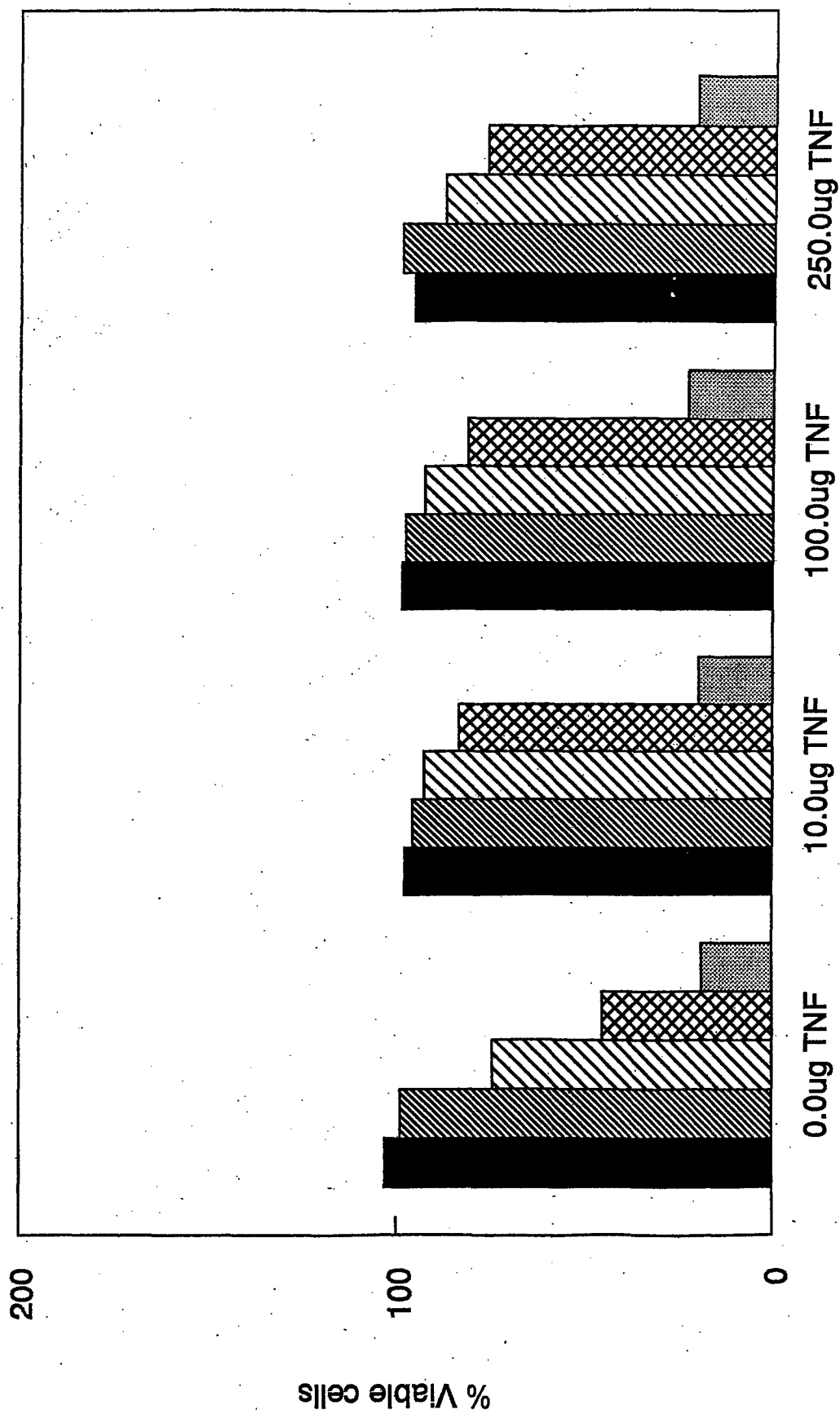
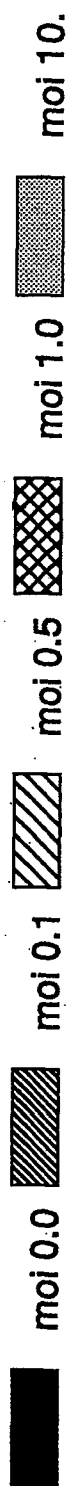


Figure 14

AdMKE1//19kd del+ TNF

H1299 SEQ 3



TNF alpha +/- XRT #1

H1299 Tumor Nodule (minus NT grp)

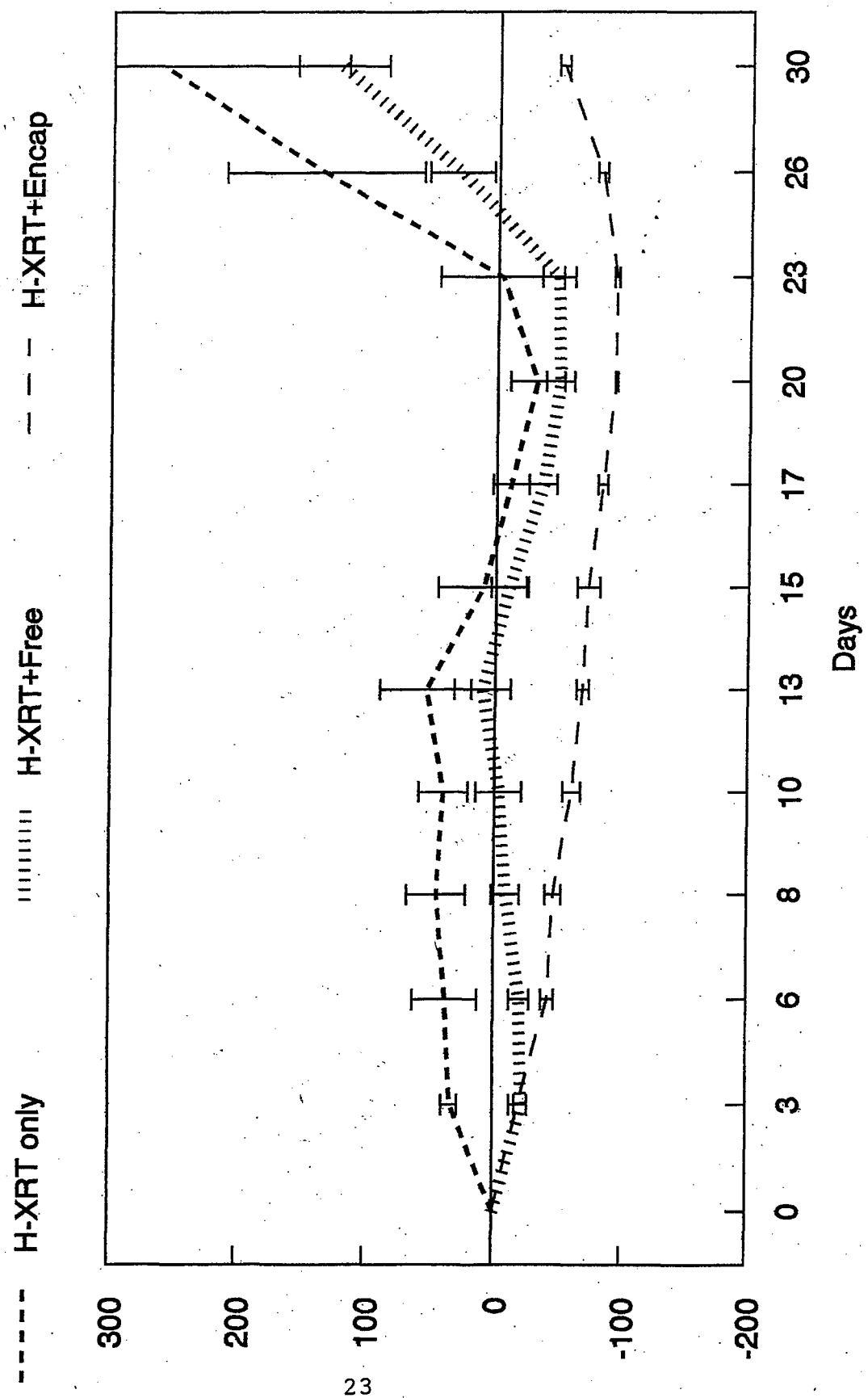
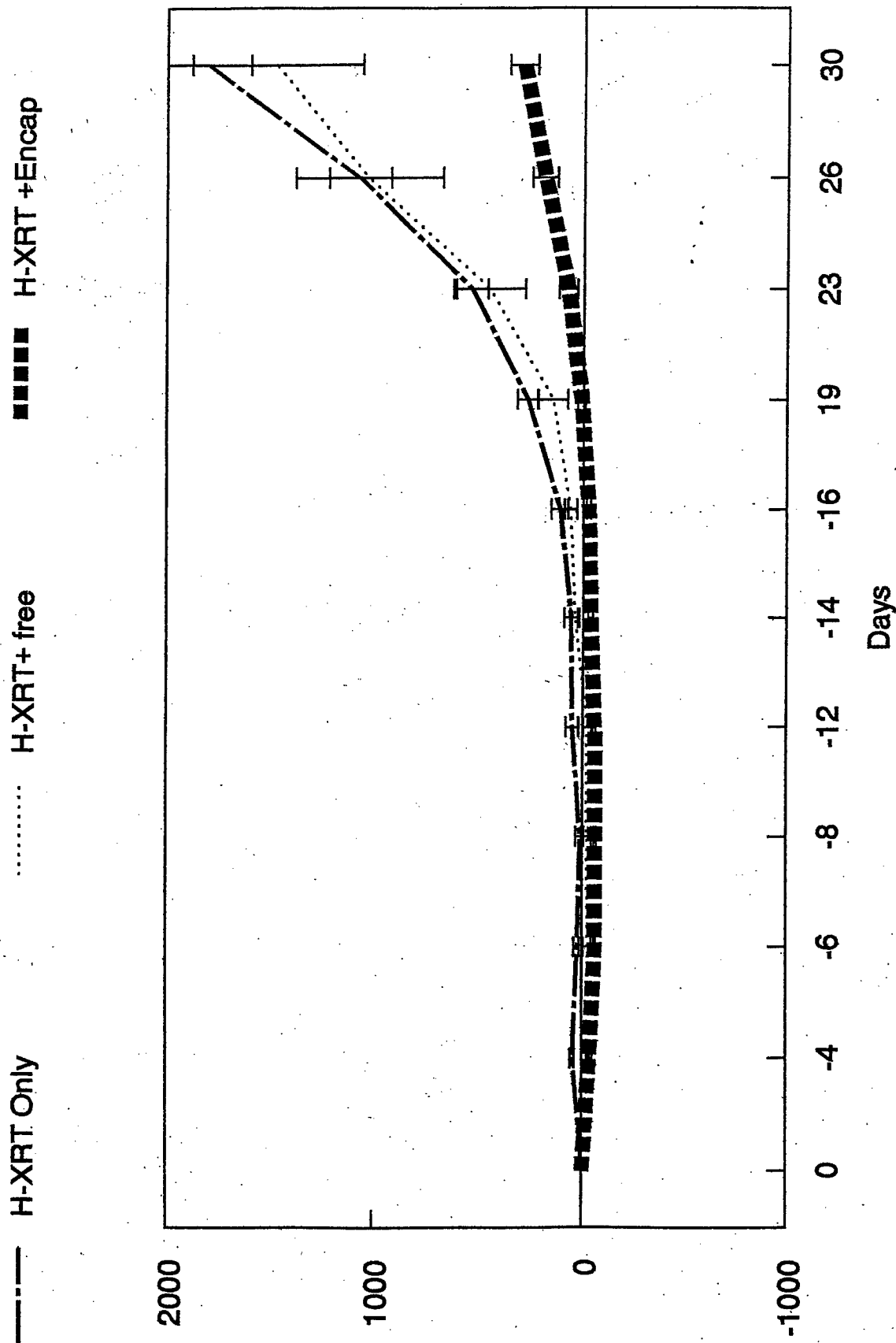


Figure 16

TNF alpha +/- XRT #2

H1299 Tumor Nodule (minus NT grp)



TNF alpha +/- XRT #1

FIGURE 17

A549 Tumor Nodule (minus NT grp)

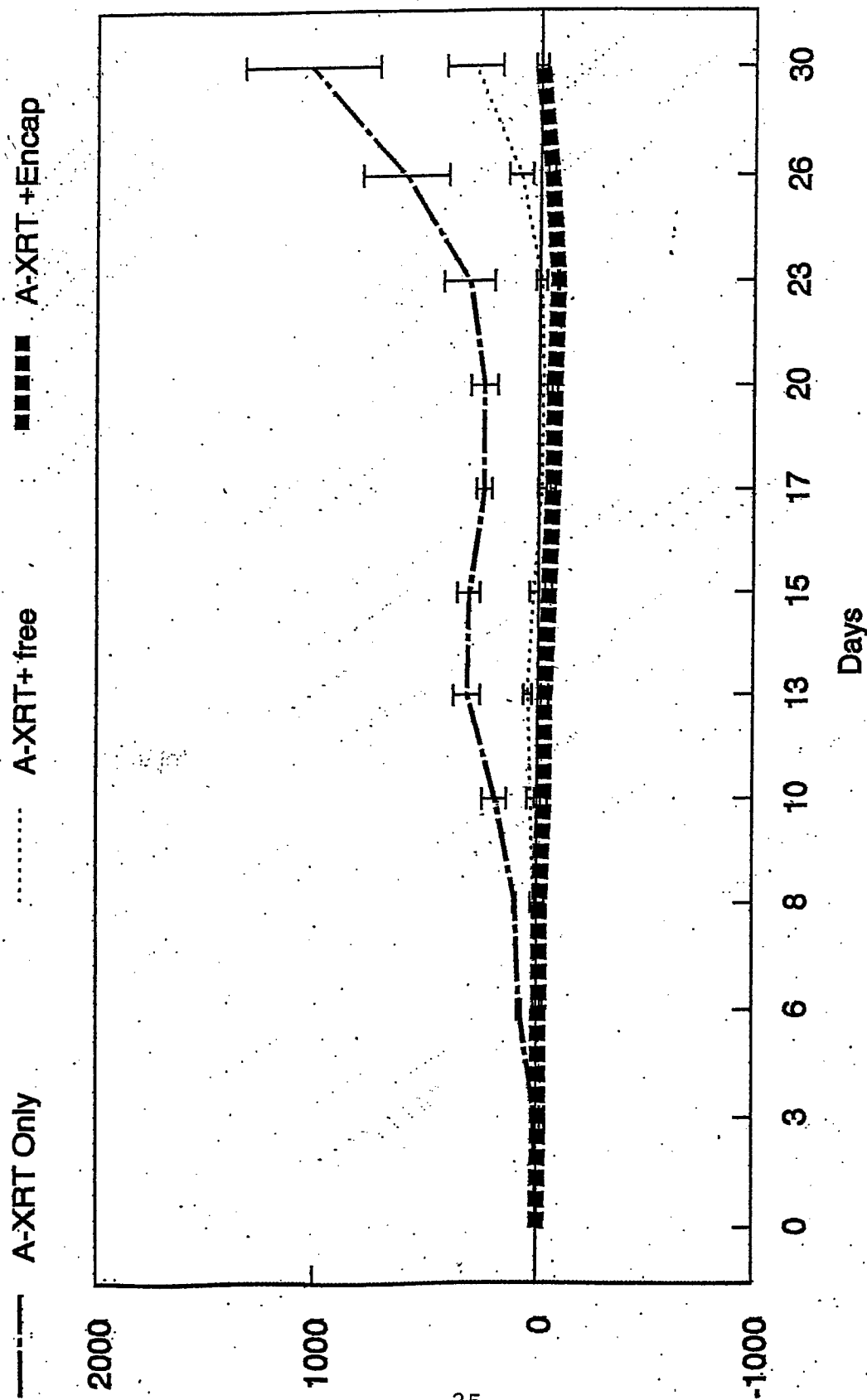
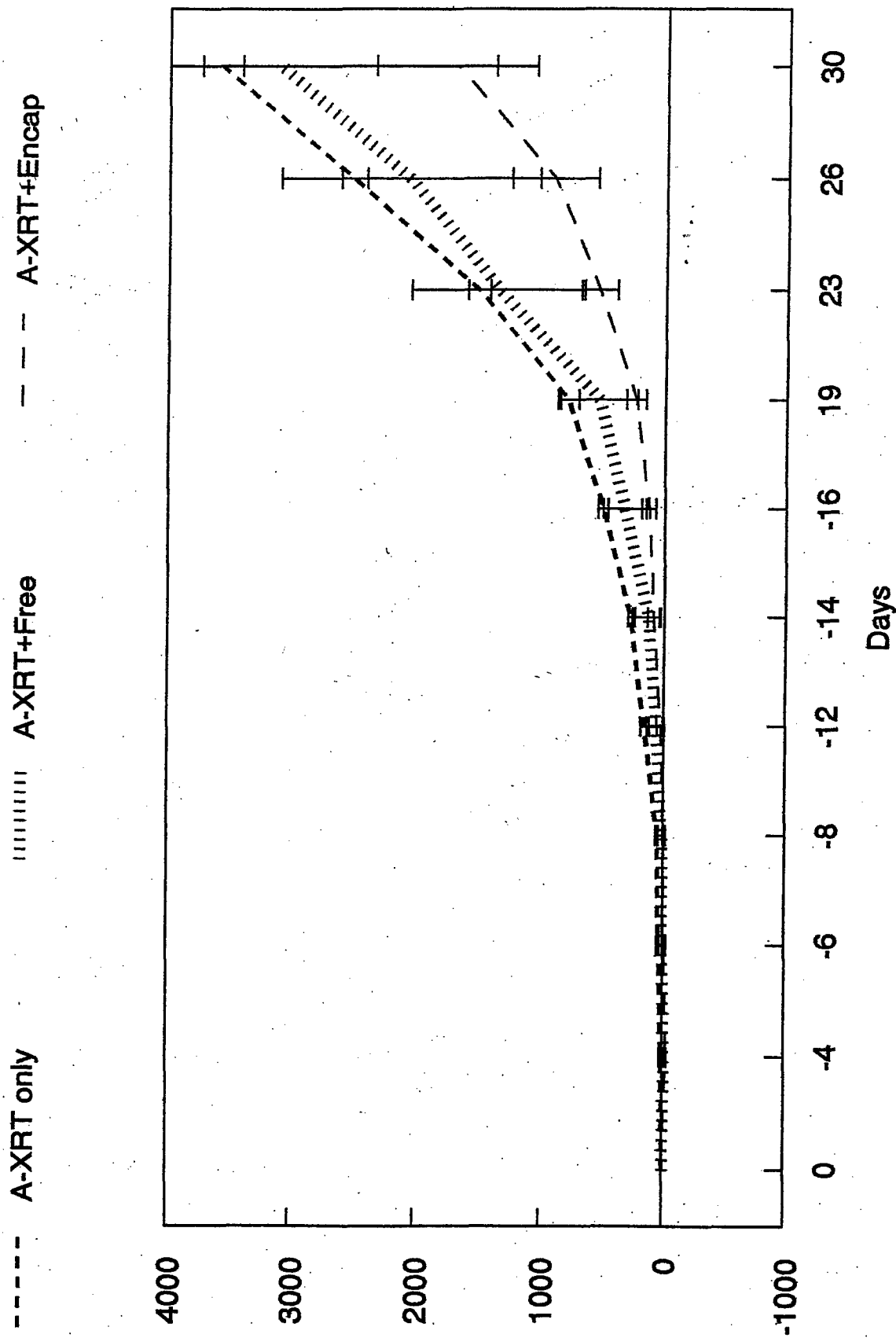


Figure 18

TNF alpha +/- XRT #2

A549 Tumor Nodule (minus NT grp)





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REPLY TO
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