Eur J Cancer Clin Oncol, Vol. 21, No. 10, pp. 1147-1151, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Tumor Dependent Growth Kinetics of Human Tumor Xenografts Using the Subrenal Capsule Assay*

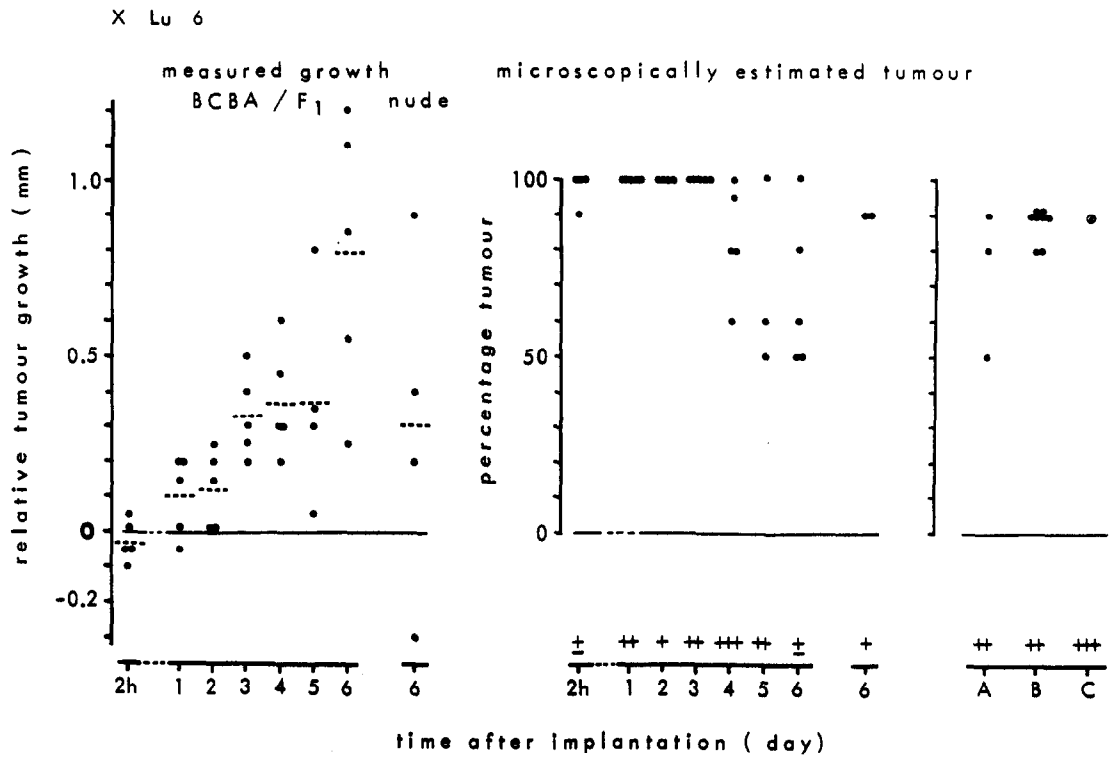
MARK B. EDELSTEIN,†‡ TRUUS SMINK,§ DEREK RUITER† and L. M. VAN PUTTEN
†*Rijks University, Leiden and* §*Radiobiological Institute, TNO,* 151 *Lange Kleiweg,* 2280 *HV Rijswijk, The Netherlands*

Abstract—*Using the subrenal capsule assay modified by us in order to decrease the ingrowth of host cells, evaluation of the growth kinetics of three human ovarian tumors and one human lung tumor were made by multiple measurements of the tumor implantation site over the 6-day growth period. For all tumors, a lag period of 2-3 days was noticed before growth occurred in the subrenal location. In general, estimation of the composition of the fragments growing under the renal capsule did not change greatly in terms of percentage tumor of which they consisted but by day 6 most had shown a significant degree of host cell infiltration despite the effects of pre-implantation immunosuppression. We would suggest that only certain human tumors are suitable for implantation using this technique. Further, it appears that within even this group of selected tumors only some are suitable for drug studies, those having established exponential growth early enough so that a measurable endpoint can be reached within the 6-day time limit of the assay.*

INTRODUCTION

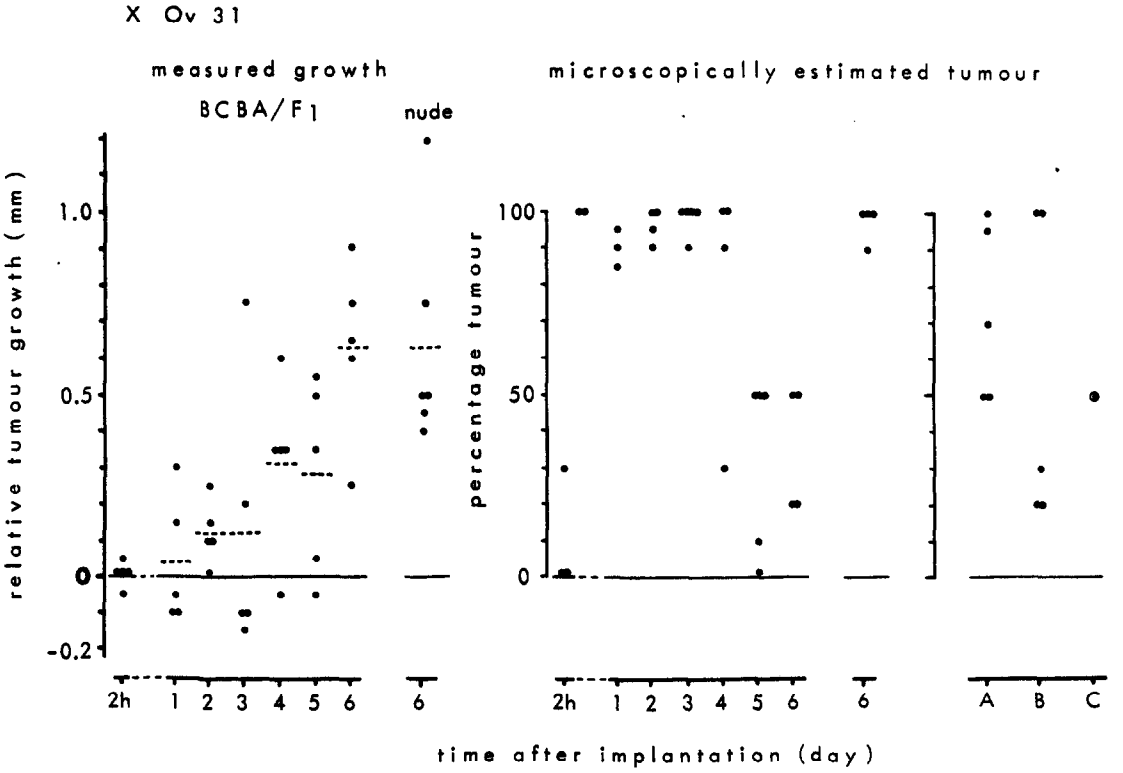
THE SUBRENAL location for implantation of human tumor material was initially reported and popularized by Bogden *et al.* and has been used both for the screening of new drugs and for prospective tumor sensitivity testing [1, 2]. Its reported advantages include a high success rate [3, 4], a short 6-day delay before a result can be given and a possibly more realistic pharmockinetic profile, possibly more similar to the clinical situation. The initial technique was developed for use with nude mice, and 12 days were allowed for tumor growth and treatment. Later publications have stressed that a 6-day time period and normal immunocompetent mice could also be used [5]. We have shown [6] that the use of normal mice is fraught with problems in that there are tumor- and drug-dependent variations in regard to ingrowth of normal host response cells with some drugs predisposing to increased, and others to a decreased, immune response. An ingrowth of normal host response cells can mean that rather than an antitumor response being measured, the degree of immunosuppression produced by a given agent is the true measure being taken. We were then able to show that pretreatment of normal mice with a variety of immunosuppressive techniques was capable of largely preventing the host cell infiltration, although not abolishing the problem entirely [7]. The degree of xenografted tumor infiltration with host cells was also found to be a function of the infiltration with non-tumor cells in the initial tumor in the sense that stromal cells as well as host response cells could not be scored as 'tumors' and that many human tumors prepared for the renal capsule assay by creation of 1 × 1 × 1-mm portions apparently contained very few histologically verified tumor cells. This was especially apparent from primary human tumors and less of a problem with tumors that have been transplanted in nude mice for several generations. In this paper we report on the growth of some of these very

Accepted 24 April 1985.
*This study was supported by the Koningin Wilhelmina Fonds (Netherlands Cancer Foundation) and the Wayne State University Ben Kasle Trust for Cancer Research.
‡Present address: Division of Medical Oncology, Wayne State University, P.O. Box 01288, Detroit, MI 48201, U.S.A.

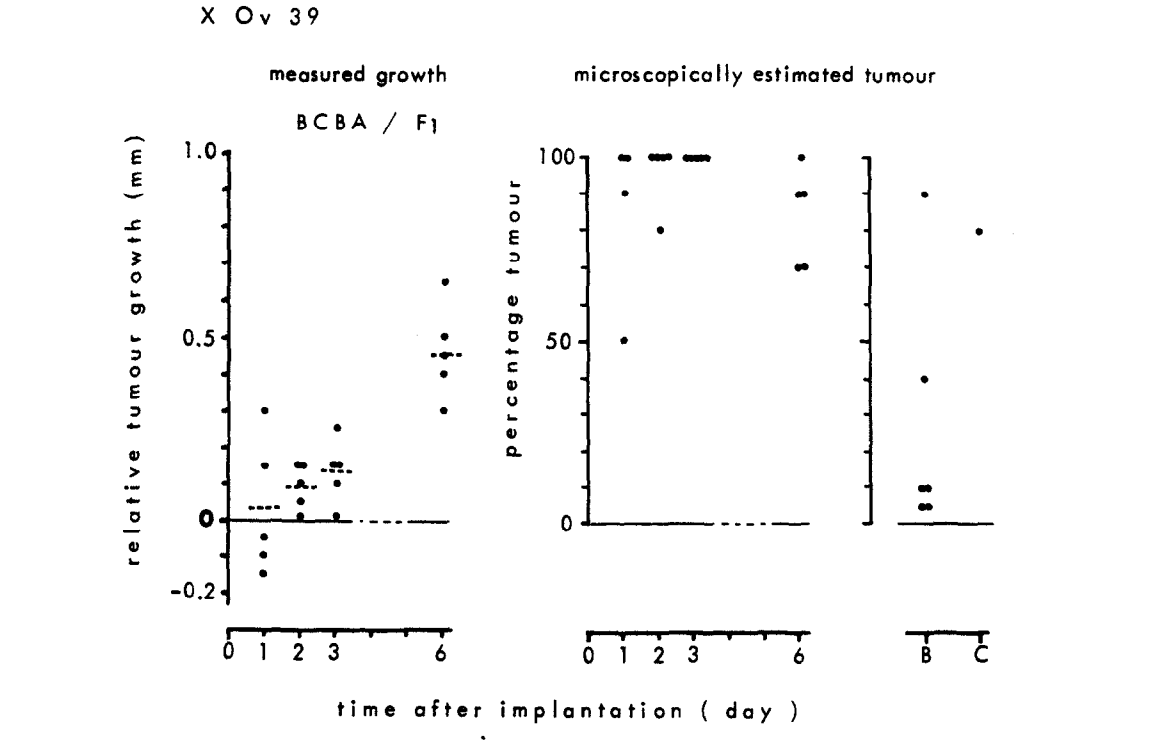


± = <5 mitosis ++ = 10 - 20 mitosis
+ = 5 - 10 ,, +++ = > 20 ,,

A : tumour piece before implantation
B : tumour piece after implantation
C : initial tumour specimen



A : tumour piece before implantation
B : tumour piece after implantation
C : initial tumour specimen



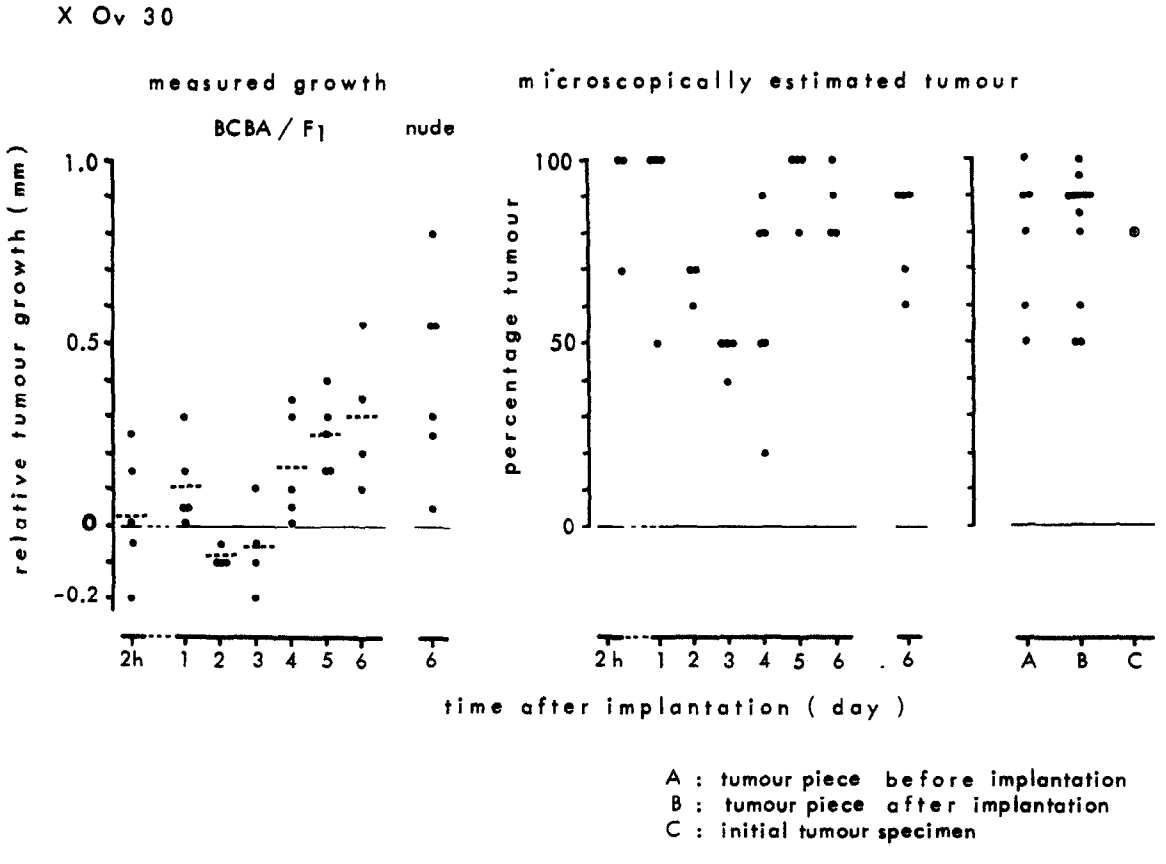


Fig. 1 (a-d). Graphic representation of results obtained using the subrenal capsule assay in three ovarian tumors and one lung tumor. Plotted are the individual data points, with the measurement of each of the five mean diameters per experimental point for animals assayed at the times indicated on the abscissa. The middle and right panels indicate histologically estimated tumor percentages for individual xenografted tumors and either the fragments at a time before and after implantation, with the circular dot indicating the percentage of tumor cells in the original tumor specimen.

select tumors as a function of time after drug implantation. In an ideal test of tumor sensitivity, tumor growth would be well established before the initiation of treatment in order to avoid non-specific drug-host-tumor interactions, preventing establishment of the tumor in the implantation site. Histological analysis suggested that because of the nature of implantation the tumor fragment may not have time within the first day or two after implantation to have fully implanted in the site chosen and that as a consequence, treatment schedules which included day 1 or 2 therapy might be measuring an anti-implantation rather than an antiproliferation effect of a given drug; that this is the case is reported here.

MATERIALS AND METHODS

Mice

Only male mice were used. They were of the strain C57BL/Rij × CBA/Rij F_1 hybrids, and were 6 weeks of age when used. Nude mice used in this study were bred on a BALB/c background and used when they reached the age of 12 weeks. Nude mice were kept in laminar flow stalls, normal mice in our mouse colonies. All mice received acidified water and processed food *ad libitum*.

Renal capsule technique

The technique that we used has been described [6]. Briefly, after receipt of tumor material in sterile Hanks' BSS medium, 1 × 1 × 1-mm specimens were prepared using sharp dissection under a dissecting microscope. A sample of the starting tumor was also placed in formalin for histologic evaluation. In addition, of the tumor fragments prepared, randomly chosen fragments from before implantation and those remaining at the end of implantation of the test animals were submitted for histologic evaluation. Normal or nude mice were then anesthetized with Avertine (2,2,2-tribromoethanol) i.p. using approximately 0.3 ml of a 25-mg/ml solution. The normal animals were then shaved on one flank (this not being necessary with the nude animals), the skin prepared with alcohol (70%) and an incision made over the kidney. After division of the skin and subcutaneous tissues, the kidney was gently externalized. With a No. 10 scalpel blade a 5-mm-long incision was made in the capsule of the kidney, and using a trocar, a single tumor specimen was then placed underneath the capsule. The presenting tumor was then measured in two directions using a microscopic grating marked in 0.1-mm intervals and the abdominal incision closed with surgical staples. Two hours, and 1, 2, 3, 4, 5 and 6 days after tumor implantation groups of five mice were killed, the kidney was again gently brought outside the body, and measurement of the tumor length and width repeated. Five mice were used for each time point; and for each drug and treatment experiment 5–10 mice were used as untreated controls. All normal mice were pre-irradiated with 450 rad (X-ray) total-body irradiation within 4 hr prior to tumor implantation.

Subcutaneous inoculation in nude mice

One flank of the nude mouse was cleaned with alcohol and using a forceps and scissors, the skin divided. Blocks of tumor of 1–5 mm^3 were implanted subcutaneously in both flanks. The sides were palpated weekly for 6 months or until obvious tumor growth was noted and in this way tumor specimens were carried for evaluation. The subrenal capsule assay was performed on nude mice in the same manner as with normal mice.

Histologic procedures

Histologic examination was done from the initial tumor material and from the tumor fragments as described. Sections of kidneys with the xenografted tumor were investigated after the last tumor measurement. We routinely evaluated one section when we demonstrated homogeneity. Hematoxylin/phloxine/safran staining was routinely performed on these materials which were reviewed by two of us.

RESULTS

Three human ovarian tumors (X-Ovarian 30, X-Ovarian 31 and X-Ovarian 39) and one lung tumor (X-Lung 6) were implanted as described and measured at the 2-hr point after implantation and daily thereafter for 6 days (days 4 and 5 data were not obtained for X-Ovarian 39). In addition, for XOV30, XOV31 and XLU6 tumor specimens were also implanted subrenally in the nude mouse and results of these measurements are also indicated. Results are seen for the four tumors in Fig. 1, where tumor growth calculated as $(l+w)/2$ in mm is plotted for each of five kidneys per experimental point, with mean level being given by the dotted horizontal line. In addition, the microscopically estimated tumor at those time points is plotted in the middle portion of each graph with, finally, the right hand portion of the graph representing the histologic estimation of tumor in tumor pieces either before implantation, in those fragments left over after implantation of the experimental animals and, when available, of the initial tumor specimen. For none of the tumors presented is there a straight line in the growth curve seen between time of implantation and the 6-day point. For all tumors there appears to be a 2- to 3-day delay before which the tumor

appears to grow relatively linearly. In addition, there is marked variability of the different fragments at a given time point. In evaluating the middle panel of the figures, this measured variability is paralleled by marked variability in the percentage of tumor cells in a given fragment implanted under the renal capsule, with, in general, much more heterogeneity of the percentage tumor specimens seen on days 5 and 6, with decreases in the percentage tumor in the histologic specimen noted for all four of these tumors. This does not necessarily correlate with the percentage tumor in the primary tumor, nor does it correlate with the range of tumor percentages found in the fragments prior to or after implantation. It is clear that the choice of fragment for implantation itself leads to a great deal of variability. For example, for X-Ovarian 30 the percentage of tumor in the implanted fragments was rather homogeneous between 40 and 100%, whereas for X-Lung 6 it appeared that there was more variation of preselected fragments than those left over after implantation.

DISCUSSION

The experiments performed here demonstrate a tumor-dependent variation in the time after implantation before growth occurs. However, independent of the percentage of tumor variations seen in the control tumor and the individual variability in the time before growth occurs, certainly the data suggest that days 1 and 2 are not suitable for treatment since anti-implantation rather than antiproliferative effects are the only things that can be effected. These data plus the histologic data suggesting that by day 6 the immunosuppressive effectiveness of our preoperative irradiation was diminishing suggest that for screening purposes the subrenal assay should be used with treatment schedules designed such that antiproliferative effects are truly measured. That this forms another limitation to the usefulness of the subrenal capsule technique is unavoidable. Our general conclusion is, as previously, that it is a technique with possibly limited usefulness for screening and certainly very little usefulness for prospective testing.

REFERENCES

1. Bogden AE, Kelton DE, Cobb WE *et al.* A rapid screening method for testing chemotherapeutic agents against human tumor xenografts. In: Houchens DP, Ovejera AA, eds. *Proceedings of the Symposium on the Use of Athymic (Nude) Mice in Cancer Research.* New York, Gustav Fisher, 1978, 231-250.
2. Griswold DP, Dykes DJ, Corbett TH *et al.* The subrenal capsule assay as a sensitivity test for determination of the response of individual tumors to specific cancer chemotherapeutic agents. *Proc AACR* 1982, **23**, 221.
3. Bodgen AE, Cobb WB, LePage DJ *et al.* Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse. *Cancer* 1981, **48**, 10-20.
4. Bogden AE. Chemotherapy sensitivity of human breast tumors in the 6-day subrenal capsule (src) assay. Proceedings of the 4th Annual San Antonio Breast Cancer Symposium, 1981, Abstr. 6.
5. Bogden AE, Haskell PM, LePage DJ *et al.* Growth of human tumor xenografts implanted under the renal capsule of normal mice. *Exp Cell Biol* 1979, **47**, 281-293.
6. Edelstein MB, Fiebig HH, Smink T *et al.* Comparison between macroscopic and microscopic evaluation of tumor responsiveness using the subrenal capsule assay. *Eur J Cancer Clin Oncol* 1983, **19**, 995-1009.
7. Edelstein MB, Smink T, Ruiter DJ *et al.* Improvements and limitations of the subrenal capsule assay for determining tumour sensitivity to cytostatic drugs. *Eur J Cancer Clin Oncol* 1983, **20**, 1549-1556.